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The biology of British marine hemiuridae

Matthews, Brenda Fay

THE BIOLOGY OF BRITISH MARINE HEMIURIDAE

Brenda Fay Matthews B.Sc., M.Sc., A.R.C.S.

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DECLARATION

This work has not been accepted in substance for any other degree, and is not concurrently being submitted for any other degree.

Signed .....
Candidate

Date 12.3.82

This is to certify that the work here submitted was carried out by the candidate herself.

Signed .....
Candidate

Date 12.3.82

Signed .....
Supervisor of Studies

Date 12.3.82

Signed R.P. Harris
Supervisor of Studies

Date 12.3.82

PUBLICATIONS

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Offprints of papers 1-3 are included within the thesis.

ABSTRACT

The Biology of British Marine Hemiuridae

Brenda Fay Matthews

The cystophorous hemiurid cercariae, Cercaria vaullegeardi and C. calliostomae, are recorded from the digestive gland of Gibbula umbilicalis and the gonad of Calliostoma ziziphinum respectively. The encysted forms are described at ultrastructural level for the first time, and developmental stages redescribed. Specialisation of the anterior region and birth pore of the daughter sporocyst in C. vaullegeardi, and migration of the redia in C. calliostomae, ensure that in neither species does the cercaria, hindered by a bulky immotile cystophorous tail, itself have to migrate through blood vessels or tissues to the site of emergence from the molluscan host.

The inoculative mechanism whereby hemiurid cercariae infect the copepod second intermediate host is described for the first time in C. vaullegeardi. Experimental infections of the harpacticoid copepod Tigriopus brevicornis are recorded, and the infection process is related to the ultrastructure of the cercaria and to the feeding mechanics of harpacticoids. The cystophorous tail of C. vaullegeardi is shown to be a device whose shape and construction ensure that the cercarial body is neither damaged by copepod mouthparts nor swallowed, but reaches the host haemocoel during the initial stages of feeding. The metacercaria grows rapidly within T. brevicornis, virtually filling the haemocoel 21 days after infection when maintained at 17°C. The development of the ecsoma from the eversible excretory vesicle is described. Post-larval Gobius paganellus have been experimentally infected by feeding 21 day-old metacercariae of C. vaullegeardi raised in T. brevicornis.

Encapsulated metacercariae and adults are recorded from 7 out of 12 species of fish examined for natural hemiuroid infections. The function of the hemiurid ecsoma is discussed in relation both to habitat of the adult within the host pyloric stomach, and to ultrastructural and autoradiographic studies of metacercarial and adult stages.

CONTENTS

INTRODUCTION

Our knowledge of the developmental stages and life cycles of hemiurid trematodes is "meagre and fragmentary, often faulty and sometimes erroneous" (Stunkard, 1973). It is surprising that the group has not received greater attention in view of the uniqueness of the Hemiuridae both in being the only Digenea to exploit the stomach as a habitat, and in their remarkable cystophorous cercariae, considered by Rothschild (1938) to be "amongst the most extraordinary and beautiful of all known cercariae". As is the case with most digenean parasites, the adults of many species have been exhaustively described and documented, and at present the greatest advances to be made may be gained from an experimental approach. Knowledge of life cycles is fundamental to the establishment of experimental systems in the laboratory, when host/parasite relationships can be studied under controlled conditions. The present work developed from a basic interest in infection processes, two objectives being to study the inoculative mechanism whereby the copepod second intermediate host becomes infected in relation to the ultrastructure of the cystophorous tail, and the involvement of rockpool fishes in a four-host life cycle. In addition to investigating other aspects of the biology, particular emphasis is given to the retractile ecsoma - its development and function, and possible relationship to the site of infection within the host stomach.

LITERATURE REVIEW

1. Miracidia, Sporocyst and Redial Stages

The miracidia of Halipegus eccentricus and Derogenes varicus have been described by Thomas (1939) and Køie (1980) respectively. The early sporocyst stages of two species of Halipegus and of Tubulovesicula pinguis were recorded in experimental infections of the molluscan host by Krull (1935), Thomas (1939), Stunkard (1980), and Ameel, Cort & Van der Woude (1949), the latter workers giving an account of germinal development within both the mother sporocyst and the redia. Stunkard (1980) recorded that in T. pinguis there are both small spherical and large oval, immotile sporocysts, and successive generations of rediae. Apart from the investigations of Krull (1935) who found sporocysts and rediae of H. occidualis in experimentally-infected Helisoma antrosa within the "proximal end of the digestive gland", no information is available concerning the location of early developmental stages within the molluscan host, this being omitted from the accounts of Thomas (1939), Ameel et al. (1949) and Stunkard (1980). It would be unusual if the mother sporocysts of H. eccentricus and T. pinguis occurred within the digestive gland, as those of other digenea generally develop in a different site to that occupied by the daughter sporocysts and/or rediae, either attached to the intestinal wall or kidney or within blood sinuses (Erasmus, 1972).

Pelseneer (1906), Gaillard (1953), Chabaud & Campana-Rouget (1958), Arvy (1963), James (1973) and Popiel (1976 a,b; 1978) have described the daughter sporocyst of Cercaria vaullegeardi. The germinal sac in which Cercaria A is produced has been considered to be a sporocyst by Miller (1925) and a redia by Ching (1960). All

other cystophorous cercariae so far described develop within rediae, the latter in C. calliostomae, C. sinitzini, D. varicus and T. pinguis being characterised by anterior birth pores (Dollfus, 1923, 1950; Rothschild, 1938; Køie, 1979; Stunkard, 1980).

2. Cystophorous Cercariae

The views of Stunkard (1973), Rothschild (1938) and Cable (1974) that cystophorous cercariae are characteristic of the Hemiuridae are modified by the reclassification of the group by Gibson & Bray (1979), under which cystophorous cercariae occur in the families Bunocotylidae, Derogenidae, Hemiuridae and Lecithasteridae within the Hemiuroidea. Dollfus (1950) lists 40 species grouped according to the systematic position of the molluscan host, emphasising both the wide range of groups parasitised, including prosobranchs, opisthobranchs, pulmonates and scaphopods, and their global distribution.

At least 27 freshwater species have been recorded. These include eight from prosobranchs collected in China, Japan, India and Egypt (Sonsino, 1892; Cort & Nichols, 1920; Sewell, 1922; Faust, 1924), 18 in prosobranchs and opisthobranchs from N. and S. America and southern Africa (Cort & Nichols, 1920; Faust, 1924, 1926; Willey, 1930; Krull, 1933; Thomas, 1934, 1939; Porter, 1938; Rankin, 1944; Dollfus, 1950; Ruiz, 1952; Fain, 1953) and one from Germany (Wagener, 1866).

Marine cystophorous cercariae have so far only been recorded from the northern hemisphere, in the Channel and Mediterranean, the North and Baltic Seas, and the Atlantic and Pacific Oceans. Eight species have been described in prosobranchs collected off the

Channel, Atlantic and Mediterranean coasts of France. Four of these occur in Hydrobia spp.; Deblock (1980) described C. Bunocotyle progenetica and C. cystocerque apanache 2, and redescribed C. sinitzini Rothschild, 1938. Chabaud & Biguet (1954) linked an unnamed cercaria from H. stagnalis with the metacercaria of Bunocotyle cingulata on morphological grounds, this species being also found in H. ventrosa and H. acuta by Rebecq & Aguesse (1960). Dollfus (1923, 1950) described C. calliostomae from Calliostoma ziziphinum and Arvy (1952 a,b) C. tregouboffi from Columbella rustica. C. vaullegeardi, first described from Gibbula cineraria by Pelseneer (1906) from Wimereux, has since been recorded only from G. umbilicalis, by Gaillard (1953), Chabaud & Campana-Rouget (1958), and Arvy (1963) at Iles Chausey, Banyuls and Roscoff respectively. The cercaria of Derogenes varicus (= C. appendiculata) was first examined by Pelseneer (1906) from Natica spp. collected off Finistere; it has since been redescribed by Chubrik (1952) and by Køie (1979), the latter worker elucidating the life cycle using specimens shed from Natica spp. collected off Denmark and W. Greenland.

C. rothschildi and C. sagittarius Sinitzin, 1911 were recorded in Tricolia speciosa and Cerithium spp. from the Italian Mediterranean by Palombi (1940).

In addition to the cercaria of D. varicus, three further species are recorded from prosobranchs collected in the Baltic; C. laqueator was described by Sinitzin (1911), and C. octocauda Chubrik, 1952 and C. saccocaudata Chubrik, 1966 by Timofeeva (1976).

Five species of cystophorous cercariae have been investigated from prosobranchs collected off the east and west coasts of N. America.

Hunninen & Cable (1943) showed experimentally that a cercaria from Odostomia trifida is that of Lecithaster confusus, and Cable & Nahhas (1963) linked a cercaria from Zebina browniana with Dichadena acuta. Ching (1960) suggested that both Cercaria A Miller, 1925 and Cercaria B Miller, 1925 in Thais spp. from Washington harbour may be species of Lecithaster. Stunkard (1980) redescribed the cercaria of Tubulovesicula pinguis in Nassarius trivitattatus from Massachusetts.

The four species of cystophorous cercariae recorded from British waters are all parasitic in marine prosobranchs. C. sinitzini was described from Hydrobia ulvae by Rothschild (1938), and Dollfus (1950) included unpublished personal communications from Rothschild concerning Cercaria K and Cercaria sp. W.J. Rees. C. vaullegeardi was found in G. umbilicalis collected off S. Wales by James (1973) and Popiel (1976 a,b).

Three species are described from opisthobranch hosts in Europe. Pelseneer (1906) recorded a cercaria from Philine aperta at Fouras, Charente (Atlantic coast of France), Arvy (1951) C. dollfusi from P. aperta collected in the Channel, and Graeffe (1860) and Monticelli (1888) C. cymbuliae from Cymbulia peroni and Gleba spp. off Nice and Naples respectively.

The scaphopods Dentalium entalis and D. dalli are hosts for C. prenanti described by Arvy (1949) and Ching (1960) from Dinard, France and Washington harbour, U.S.A.

3. The infection of the second intermediate host

In almost all cystophorous cercariae so far described, the body retracts into the tail cavity, the "encysted" form being then eaten by

a crustacean second intermediate host (Stunkard, 1973). Sinitzin (1911) and Cort & Nichols (1920) however recorded that in C. laqueator, C. californiensis and the cercaria of H. eccentricus the body does not "encyst" within the tail.

Willey (1930), Hunninen & Cable (1943), Chabaud & Biguet (1954), Timofeeva (1976), Køie (1979) and Stunkard (1980) observed the passage of the cercarial body into the everted delivery tube at encystment. Krull (1935) first established that the function of the delivery tube is to inoculate the cercarial body through the intestinal wall and into the haemocoel of the copepod host. Further observations concerning the infection process have been made by Chabaud & Biguet (1954) and Køie (1979).

Although it is considered that in the Hemiuridae the second intermediate host is generally a copepod (Stunkard, 1973), metacercariae have been recorded from a wide variety of other invertebrates including ctenophores, chaetognaths, ostracods, barnacles and hermit crabs (Dollfus, 1923, 1960; Macy & DeMott, 1957; Stunkard, 1973; Combes & Kechemir, 1978; Køie, 1979). Copepods have been experimentally infected with cystophorous cercariae by Krull (1935), Thomas (1939), Hunninen & Cable (1943), Rankin (1944), Chabaud & Biguet (1954), Macy & DeMott (1957), Ching (1960), Combes & Kechemir (1978) and Stunkard (1980).

4. Encapsulated Metacercariae

According to Stunkard (1973) and Cable (1974) cystogenous glands do not occur in cystophorous cercariae, and records confirm that metacercariae lie free within the haemocoel of their invertebrate hosts. Hemiurid metacercariae have been recorded from the body cavity

of a large number of fish species (Szidat, Angelescu & Siccardi, 1950; Chabaud & Campana-Rouget, 1958; Sinclair, Smith & Sullivan, 1972; Mhaisen, 1977; Stunkard, 1980). In these cases the metacercariae are enclosed within a capsule which Dollfus (1954) suggested is of host origin. Mhaisen (1977) studied the incidence of infection of Blennius pholis collected at Aberystwyth and Milford Haven with an encapsulated metacercaria morphologically linked with Lecithochirium gravidum Looss, 1907 (syn. L. rufoviride (Rudolphi, 1819) Lühe, 1901).

5. Progenesis

References to progenesis in hemiurid metacercariae are common in the literature, the phenomenon being recorded by Markowski (1936), Chabaud & Biguet (1954), Szidat (1956), Rebecq (1964) and Deblock (1974). Stunkard (1973) drew attention to the work of Chabaud & Buttner (1959) who showed that in the genus Bunocotyle, B. progenetica becomes progenetic in the molluscan host Hydrobia spp., B. meridionalis in the copepod second intermediate host, and B. cingulata in the fish host. Mhaisen (1977) recorded progenetic lecithochiriine metacercariae encapsulated in Blennius pholis.

6. Adults

The recent publication concerning the taxonomy of the Hemiuroidea by Gibson & Bray (1979) considerably changes earlier proposals by Dawes (1947) and revises the classifications of La Rue (1957), Skrjabin & Guschanskaja (1956, 1958, 1960), Manter & Pritchard (1960), Mehra (1962) and Yamaguti (1971). Gibson & Bray (1979) consider that "neither the gross morphology of the adult, due to its variability, nor the use of life-cycle patterns and cercarial morphology, due to a

lack of knowledge and understanding with regard to their significance" are able to provide a satisfactory classification, and therefore base their revision upon functional adult morphology. Their classification is outlined in Table 1. The family Hemiuridae sensu Gibson & Bray (1979) is restricted to ecsomate forms; the genera Sterrhurus and Ceratotrema are considered synonyms of Lecithochirium.

The unusual habitat of most adult Hemiuridae, parasitic within the pyloric stomach of marine teleosts, an environment subject to great variation in pH and osmolarity (MacKenzie & Gibson, 1970) has prompted Gibson & Bray (1979) to suggest that the ecsoma may act as a "feeding organ which is extended during periods when the pH or osmolarity of the stomach contents is at a tolerable level", and Kryvi (1972) to investigate the tegument of Hemiurus communis at the ultrastructural level.

Table 1. Classification of the Hemiuroidea, after Gibson and Bray, 1979.

Superfamily Hemiuroidea Looss, 1899

Family	Sub-family
Accacoeliidae	Accacoeliinae, Paraccacladiinae
Azygiidae	Azygiinae, Leuceruthrinae
Bathycotylidae	
Bunocotylidae	Bunocotylinae, Aphanurinae, Opisthadeninae, Thelotrinae
Derogenidae	Derogeninae, Gonocercinae, Halopeginae
Dictysarcidae	Dictysarcinae, Albulatrematinae, Cylindrorchiinae sub-fam. inq.
Hemiuridae	Hemiurinae, Dinurinae, Elytrophallinae, Glomericirrinae, Hypohepaticolinae, Lecithochiriinae, Lethadeninae, Plerurinae, Pulmoverminae
Hirudinellidae	
Isoparorchiidae	
Lecithasteridae	Lecithasterinae, Hysterolecithinae, Macradeninae, Prolecithinae, Quadrifoliovariinae, Trifoliovariinae
Ptychogonimidae	
Sclerodistomidae	Sclerodistominae, Prosogonotrematinae, Prosorchiinae
Sclerodistomoididae	
Syncoeliidae	Syncoeliinae, Otiotrematinae

MATERIALS AND METHODS

1. Collection and examination of molluscan, crustacean and fish hosts

Material was collected from coastal and offshore waters at each of the 13 sites listed in Table 2.

Table 2. Collection sites

Location	Grid reference number	Station number
Aberystwyth, Dyfed, Wales	SN 5881	1
Barbican, Plymouth, Devon	SX 483542	2
Bovisand, Plymouth, Devon	SX 492506	3
Brixham, Devon	SX 925564	4
Broadsands, Torquay, Devon	SX 896575	5
Fowey Estuary, Golant, Cornwall	SX 122548	6
Heybrook Bay, Plymouth, Devon	SX 497488	7
Mothercombe Estuary, Plymouth, Devon	SX 6147	8
Ringmore (Toby's Point), Plymouth, Devon	SX 642451	9
St. John's Lake, Cornwall	SX 4254	10
Wembury Bay, Devon	SX 5147	11
Widemouth, Bude, Cornwall	SS 201024	12
Yealm Estuary, Newton Ferrers, Devon	SX 541479	13

a. Mollusca

Prosobranch molluscs, listed in Table 3, were collected at low tide from stations 1, 3, 9, 10, 11, 12 and 13. Calliostoma ziziphinum

Table 3. Prosobranch molluscs examined for hemiurid larvae 1974-1981

Mollusc species	Station number	Date	Number examined
Calliostoma ziziphinum L.	11	1976-81	428
	13	1976-81	97
Gibbula cineraria L.	11	1974-76	162
	13	September 1976	137
Gibbula umbilicalis (Da Costa)	1	December 1981	200
	3	April 1976	425
	9	May 1976	234
	11	1974-80	7000+
	12	July 1976	348
Hydrobia ulvae (Pennant)	10	1976-80	553
Monodonta lineata (Da Costa)	11	1979	1000+
Nassarius reticulatus L.	13	December 1978	8
Odostomia plicata (Montagu)	11	1976-79	4
Rissoa guerini (Récluz)	11	September 1976	10

were collected only at low spring tide, from overhanging rock faces at Station 11 and from Fucus holdfasts at Station 13. Molluscs were either examined directly on return to the laboratory, or were screened for living infected specimens as follows: groups of 3-5 snails were placed in seawater in covered 150 ml beakers and maintained at room temperature (approximately 20°C) for 4-8 hours. A binocular microscope was used to detect the tiny, immotile cystophorous cercariae on the bottom of the beakers. Infected snails were isolated by sub-screening in fresh seawater.

b. Crustacea

(i) Copepods. Tigriopus brevicornis were collected with the aid of a hand plankton net at Stations 2 and 11. Tisbe furcata were collected from Enteromorpha gathered at Station 11. Both species were screened for natural infections of metacercariae by the following method: large numbers of copepods (200 +) were fixed in 70% alcohol, stained in Gower's carmine, and dehydrated. After clearing in xylene, they were examined in a watchglass with a binocular microscope, when specimens infected with hemiurid metacercariae were easily distinguished.

(ii) Amphipods. Amphithöe rubricata and Gammarus spp. were collected at Station 11 and examined for natural infections of metacercariae either by the method for screening large numbers of copepods described above, or by teasing apart under seawater using a binocular microscope.

c. Fish

Information concerning the species and number of fish examined, and the collection sites is given in Table 4.

Table 4. Fish species examined for hemiurid parasites 1974-1981

Fish species	Number examined	Collecting station
Blennius pholis (shanny)	138	5, 11, 12, 13
Ciliata mustela (five-bearded rockling)	56	1, 3, 4, 6, 11, 12
Conger conger (conger eel)	3	offshore waters, Plymouth
Crenilabrus melops (corkwing wrasse)	6	11
Gaidropsarus mediterraneus (three-bearded shore rockling)	8	7, 11, 12
Gobius niger (black goby)	1	6
Gobius paganellus (rock goby)	139	5, 11
Lophius piscatorius (angler)	14	offshore waters, Plymouth
Pholis gunnellus (butterfish)	2	6, 12
Pomatoschistus microps (sand goby)	113	5, 8
Scophthalmus maximus (turbot)	26	12
Taurulus bubalis (cottus)	23	3, 7, 11, 12

Quinaldine (20% in acetone) was used to temporarily anaesthetise rockpool fish so that they might be more easily seen and collected with a hand net. '0' group turbot were collected with the aid of a Riley Pushnet, sand gobies with a hand net. Conger eels and angler fish were supplied by courtesy of the Marine Biological Association (M.B.A.), The Citadel, Plymouth. Post-mortems were performed on fish killed by a lethal concentration of MS222 (Ethyl-m-aminobenzoate; Sigma Chemicals) in seawater.

2. Maintenance of molluscs, crustacea and fish

a. Mollusca

Screened, infected Gibbula umbilicalis were maintained for periods of up to two months in covered crystallising bowls (19 cm diameter) in aerated seawater at 14°C, at a density of approximately 2/litre. The bowls were kept in a Psycrotherm Controlled Environment Incubator Shaker (New Brunswick Scientific Company Ltd.), with constant illumination. Snails were supplied with rocks naturally encrusted with algae including Fucus, Enteromorpha and Ulva. Rocks and water were changed at weekly intervals.

Infected Calliostoma ziziphinum were maintained for periods of up to 10 days in shallow trays of seawater at either 14°C or room temperature (± 20°C). Water depth did not exceed 3 cm., and trays were loosely covered with black polythene. Unsuccessful attempts were made to maintain C. ziziphinum in balanced aquaria with plentiful supplies of rocks encrusted with red calcareous algae.

b. Crustacea

(i) Copepods. Tigriopus brevicornis Müller and Tisbe furcata were

reared in the laboratory by the following methods:

1. Dense cultures of mixed age were obtained by adding 25 paired male and female copepods, or 25 females carrying egg sacs, to covered perspex tanks (30 x 19 x 19 cm) of aerated filtered seawater. Tanks were maintained in a controlled environment incubator at 17°C with constant illumination. Cultures were fed with the alga Isochrysis twice weekly, and the water changed once every four weeks.

2. Copepods of known age were obtained by adding female T. brevicornis, with egg sacs, to covered crystallising bowls (8 cm diameter) of filtered sea water, one copepod per bowl. When nauplii were observed, usually within seven days at 17°C, the adult females were removed. Cultures were maintained as described in (1), the copepods being removed to fresh bowls at weekly intervals.

3. Infected copepods were maintained singly in crystallising bowls as described in (2).

(ii) Amphipods. Amphithöe rubricata and Gammarus spp. were reared using method (1) described above, harvesting and rearing offspring from wild females.

c. Fish

Stocks of rockpool fish were maintained in polypropylene tanks (60 x 40 x 30 cm), aerated seawater being circulated through an Eheim biological filter unit and Paxman cooler. Rocks were provided to afford shelter. Fish were fed twice weekly with the following

mixture, recommended by the White Fish Authority.

minced trash fish	100 parts
synthetic binder	3-4 "
vitamin pre-mix "Beta" animal food supplement	1-2 "
red shrimp meal	10 "

Seawater was obtained from the M.B.A., where it had been monitored to ensure high quality. Partial water changes were made at four week intervals and complete changes of water and filter units made every 12 weeks.

Post-larval fish (under 3 cm in total length) used in infection experiments were maintained in perspex tanks (30 x 19 x 19 cm), with shell gravel filter systems. Partial water changes were made at four week intervals and the shell gravel was removed, washed and sterilised every 12 weeks. Fish were fed as above, the food being frozen before use.

3. Light microscopy

Morphological details of cercariae and metacercariae were best seen by examining living specimens using phase contrast microscopy. All measurements were made on live specimens without application of pressure. Values given are averages of 20 specimens unless otherwise stated.

Permanent preparations of all stages were made by fixing in 10% neutral buffered formalin or 3% glacial acetic acid in 70% alcohol, and staining in Gower's carmine (Pantin, 1962) or Kirkpatrick's carmalum (Drury & Wallington, 1967). For routine histological and histochemical investigations, material was fixed in 10% neutral buffered

formalin, Bouin's fluid or Baker's formol-calcium, dehydrated in a graded series of alcohols, cleared in xylene or chloroform and embedded in Fibrowax. Sections were cut at 7 μm, and stained with Heidenhain's Azan (Pantin, 1962), Mallory's Triple Stain or Erhlich's haematoxylin and eosin to show general structure. For the morphological study of germinal sacs and developing cercarial stages, methylene-blue 1 μm resin sections, fixed and embedded for electron microscopy as described below, were found to be superior to paraffin wax embedded material.

For the study of cercarial emergence, screened, infected molluscs were killed whilst shedding large numbers of cercariae; the shell was removed by crushing carefully in a vice, the body then being fixed in Bouin's fluid, double-embedded in celloidin and paraffin wax (Drury & Wallington, 1967), serially-sectioned at 4-8 μm and stained in Heidenhain's Azan (Pantin, 1962).

Staining techniques for carbohydrates, mucosubstances and lipids followed standard procedures given by Pearse (1968) and Chayen, Bitensky & Butcher (1973). Acetylcholinesterase and non-specific esterases were located using the acetylthiocholine iodide and bromoindoxyl acetate methods respectively, as described by Jennings & LeFlore (1972). In control experiments, material was held at 60 or 90^{o}C for 30 or 5 minutes respectively prior to incubation, or incubated in media from which the specific substrate (acetylthiocholine iodide or O-acetyl-5-bromoindoxyl) had been omitted. Further controls were performed by adding eserine, which inhibits cholinesterase activity, at final concentrations of 10^{-3}M or 10^{-4}M, to the incubation media.

4. Electron microscopy

a. Transmission electron microscopy

Material was fixed for 2.5 hours at 0.4°C in either 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), or 4% paraformaldehyde in 3% glutaraldehyde in the same buffer. It was then rinsed overnight in three changes of cacodylate buffer, post-fixed for 1.5 hours in 1% osmium tetroxide, rinsed and dehydrated through a graded series of alcohols. Propylene oxide was used as an intermediate fluid before infiltration in Taab Araldite, Epon or Spurr's resin (Spurr, 1969). Sections showing silver or light gold interference colours were cut using a Porter-Blum MT2B ultramicrotome, collected on uncoated copper grids, and stained with saturated aqueous uranyl acetate followed by Reynold's lead citrate (Reynolds, 1963). Sections were examined with a Philips 300 transmission electron microscope.

b. Scanning electron microscopy

Material was washed in three changes of filtered seawater or Young's teleost saline (Y.T.S.) (Young, 1933), fixed for five days in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), rinsed and dehydrated through a graded series of alcohols. Parasites were critical point dried in a Tousimis Samdri PVT-3 with CO_2 as transitional fluid, gold sputter-coated in a Polaron E5100 SEM coating unit, and examined in a Hitachi 550 or a Jeol 35 scanning electron microscope.

5. Infection experiments

a. Experimental hosts

Attempts were made to infect crustacea, annelids and fish with various hemiurid larval stages, with a view to investigating the life cycles and infection mechanisms involved. Potential experimental hosts were selected on the basis of data obtained from the survey of infections, availability of laboratory-reared or uninfected specimens, and ease of maintenance.

Experimental hosts and hemiurid larval stages used are listed in Table 5. Crustacea were laboratory-reared, and thus known to be free from infection. Fish were from one of three sources:

(i) Locations shown by the survey of prosobranchs to be free from hemiurid cercariae.

(ii) Post-larval fish, under 3 cm in total length, collected from splash zone pools at Station 11. A sample of 100 controls was found to be free from infection with digenean metacercariae by microscopic examination after squashing between two slides.

(iii) Young rockpool fish, previously maintained in aquaria for more than eight months before use, when encapsulated hemiurid metacercariae already present were fully mature or dead.

b. Techniques

(i) Crustacea: The infection of Tigriopus brevicornis with Cercaria vaullegeardi.

Naturally-emerged cercariae from freshly-collected, screened Gibbula umbilicalis were counted into the individual chambers of haemagglutination plates, and a single T. brevicornis added to each

Table 5. Host species and hemiurid larval stages used in infection experiments

Experimental host species	Source	Parasite stage used	Source
Tigriopus brevicornis	LR	Encysted Cercaria vaullegeardi	Natural emergence from Gibbula umbilicalis collected at Station 11
Tisbe furcata	LR		
Amphithöe rubricata	LR		
Gammarus spp.	LR		
Nereis diversicolor	Station 10		
Gobius paganellus	PL, Y		
T. brevicornis	LR	Encysted Cercaria calliostomae	Natural emergence from Calliostoma ziziphinum collected at Station 11
T. furcata	LR		
G. paganellus	PL	Metacercariae of C. vaullegeardi	LR in T. brevicornis
Blennius pholis	PL		
Taurulus bubalis	Y		
Ciliata mustela	Y		
Crenilabrus melops	Y		
Pomatoschistus microps	Station 8		
Scophthalmus maximus	" 12		
Scophthalmus maximus	Station 12	Encapsulated hemiurid metacercariae	Mesenteries of G. paganellus collected at Station 11

LR laboratory reared

PL post larval fish

Y fish previously maintained in aquaria for 8-12 months

chamber. The plate was covered with a glass sheet, supported by plasticine to allow circulation of air, and placed in a Psycrotherm Controlled Environment Incubator Shaker at 17°C, with constant illumination. Observations were made concerning cercarial viability and infection rates. After infection, copepods were maintained as described above. Infections were checked, and the growth rate of individual metacercariae measured, by the following method: each T. brevicornis was removed with a Pasteur pipette and placed in a drop of seawater on a glass slide. A 19 x 19 mm coverslip was lowered gently, restraining but not crushing the copepod. After microscopic examination a further drop of seawater was added, raising the coverslip sufficiently to allow the copepod to swim out from under it. The slide was then dipped gently into the culture dish, when the copepod swam freely away. This method proved useful in allowing frequent microscopic examination without damage to the T. brevicornis, or without causing it to release its egg sac.

The technique described above was also used in attempts to infect other invertebrates (listed in Table 5) with C. vaullegeardi and C. calliostomae.

(ii) Fish

Attempts were made to infect fish using the following methods:

1. Both living Tigriopus brevicornis with metacercariae of C. vaullegeardi and encapsulated metacercariae, were readily ingested by fish isolated in crystallising bowls of aerated seawater.

2. Fish were placed in a solution of MS222 in seawater (1:1000) until anaesthetised. Infective material was inserted directly into the

stomach using a hypodermic syringe and cannula tubing. The procedure was carried out as rapidly as possible, and the fish immediately returned to a well-aerated aquarium.

6. Nutrient Uptake

a. Electron dense tracers

The following experiments were performed in order to investigate the uptake of particulate material by the metacercariae of C. vaullegeardi. T. brevicornis were experimentally infected with C. vaullegeardi as described above, and maintained at 20°C for 21 days in a controlled environment incubator. Metacercariae were removed from the copepod haemocoel by tearing off the extremity of either the prosoma or metasoma, when gentle needle pressure caused extrusion of the metacercaria. The parasites were incubated for 30 minutes at room temperature (20°C) in seawater in which were suspended either 2% ruthenium red (sonicated for more efficient dispersal) or 2% ferritin (twice recrystallised from horse spleen). Control metacercariae were in each case either fixed in 3% glutaraldehyde before incubation, or incubated in seawater alone. Metacercariae were also incubated for 30 minutes with ferritin in seawater at 37°C. Metacercariae were checked for activity before, during and after incubation with both tracers.

Following treatment, metacercariae were prepared for transmission electron microscopy as described above.

b. Autoradiography

Autoradiographic studies, using tritiated glucose and amino acids obtained from the Radiochemical Centre, Amersham, were made in

order to investigate nutrient uptake in hemiurid metacercariae and adults. The experiments are described separately according to the parasite used.

(i) Metacercariae of C. vaullegeardi

21-day old metacercariae raised singly in T. brevicornis at 17°C, were removed from the copepod haemocoels as described above and placed in seawater. They were then incubated at room temperature (20°C) for periods of 2, 5, 15 or 30 minutes in filtered seawater containing either L-[3,5-^{3}H] tyrosine (40-60 Ci/m mol) or D[2-^{3}H] glucose (5-12 Ci/m mol) at a final concentration of 100 µCi/ml, and rinsed in four changes of filtered seawater. Metacercariae incubated with ^{3}H-glucose were fixed in 3% glutaraldehyde, then prepared for transmission electron microscopy as described above. Metacercariae incubated with ^{3}H-tyrosine were fixed in 4% paraformaldehyde in cacodylate buffer pH 7.4 at 0-4°C for 2.5 hours, rinsed overnight in buffer, and post-fixed in 1% osmium tetroxide in cacodylate for 1.5 hours followed by further rinses. They were then dehydrated using a graded ethylene glycol series and passed through propylene oxide (three changes over four hours) before embedding in Spurr's resin. The substitution of paraformaldehyde for glutaraldehyde, and ethylene glycol for alcohol, is recommended by Hanna (1975) to overcome fixation artefacts and protein extraction respectively during amino-acid autoradiography.

Control metacercariae were prepared as follows:

1. The above procedures were carried out, the incubation with ^{3}H-tyrosine or ^{3}H-glucose being at 38°C and not at room temperature.

2. Metacercariae were pre-fixed in the appropriate fixative for 30 minutes, rinsed thoroughly, then incubated as above.

3. Metacercariae were prepared as above but the labelled glucose and tyrosine were omitted from the incubation media.

4. Further controls were set up using excysted metacercariae of Bucephalus haimeanus (removed from the livers of Pomatoschistus microps collected at Station 10) shown to take up ^{3}H-tyrosine through the tegument by Higgins (1977). These metacercariae were incubated for periods of 2 or 30 minutes in Y.T.S. containing ^{3}H-tyrosine at a final concentration of 100 µCi/ml.

The activity of experimental and control metacercariae was checked before, during and after treatment.

(ii) Hemiurid metacercariae encapsulated on the peritoneal membranes of Gobius paganellus and Blennius pholis collected at Station 11.

Active metacercariae were excapsulated using fine mounted needles and a binocular microscope. Experimental and control metacercariae were treated and embedded in Spurr's resin as described above in (i), except that Y.T.S. was substituted for seawater in the rinses and incubation media.

(iii) Adult Lecithochirium rufoviride and L. fusiforme removed from the stomach of Conger conger.

The parasites were rinsed three times in Y.T.S. and incubated for periods of 5 and 15 minutes in either D[2-^{3}H] glucose (5-12 Ci/m mol) or L[3,5-^{3}H] tyrosine (40-60 ci/m mol) in saline at a final concentration of 100 µCi/ml, at pH 2, 5 or 8. After rinsing three times in Y.T.S., each

worm was placed in the appropriate fixative and cut transversely into three sections (oral sucker, ventral sucker and ecsomal regions) before processing and embedding in Spurr's resin as described above in (i). Control worms were treated as described in (i) 2 and (i) 3.

(iv) Adult Lecithochirium furcolabiata, removed from the body cavity of Ciliata mustela collected at Station 11.

The parasites were incubated with tritiated alanine and methionine following the pulse-chase technique developed by Hanna (1975) in order to investigate protein synthesis in the gut cells of Fasciola hepatica. Worms were placed for periods of 2, 5, 10, 15, 30, 60 or 120 minutes at room temperature (20°C) in Y.T.S. containing either L-[2,3-^{3}H] alanine (35 Ci/m mol) or L-[2(n)-^{3}H] methionine (9.4 Ci/m mol) at final concentrations of 50, 100 or 200 µCi/ml. They were then either chase-rinsed in Y.T.S. for 10 minutes before fixing in 4% paraformaldehyde, or rinsed in three changes of Y.T.S. (totalling 1 minute) before fixing. The worms were then osmicated, dehydrated in a graded series of alcohols, and embedded in Spurr's resin.

Sections of the above were all cut at 1 µm and dried onto alcohol-cleaned slides at 60°C. They were then coated with Ilford K2 nuclear emulsion and exposed at 0-4°C in light proof black plastic boxes with silicon gel for 3-4 weeks, when developed test sections showed sufficient silver grains. All the sections were then developed in Kodak D-19 at 20°C for four minutes, fixed in Kodak acid fixer for 10 minutes, washed in running water and dried. Sections were examined unstained and stained at 60°C in 0.5% methylene blue in 1% borax, filtered immediately before use.

OBSERVATIONS AND RESULTS

1. Germinal sacs

a. The daughter sporocyst of Cercaria vaullegeardi Pelseneer, 1906

As shown in Table 6, out of over 7,000 specimens of Gibbula umbilicalis collected at Station 11, 1.13% were found to be infected with the daughter sporocysts of C. vaullegeardi. Infected specimens were recognised by careful removal of the shell, when parts of the sporocysts were visible within the digestive gland. To show the distribution of the daughter sporocysts, the mantle cavity was opened along the left side, the gill being displaced towards the cut edge of the mantle (Fig. 1).

(i) General account and distribution of the daughter sporocyst

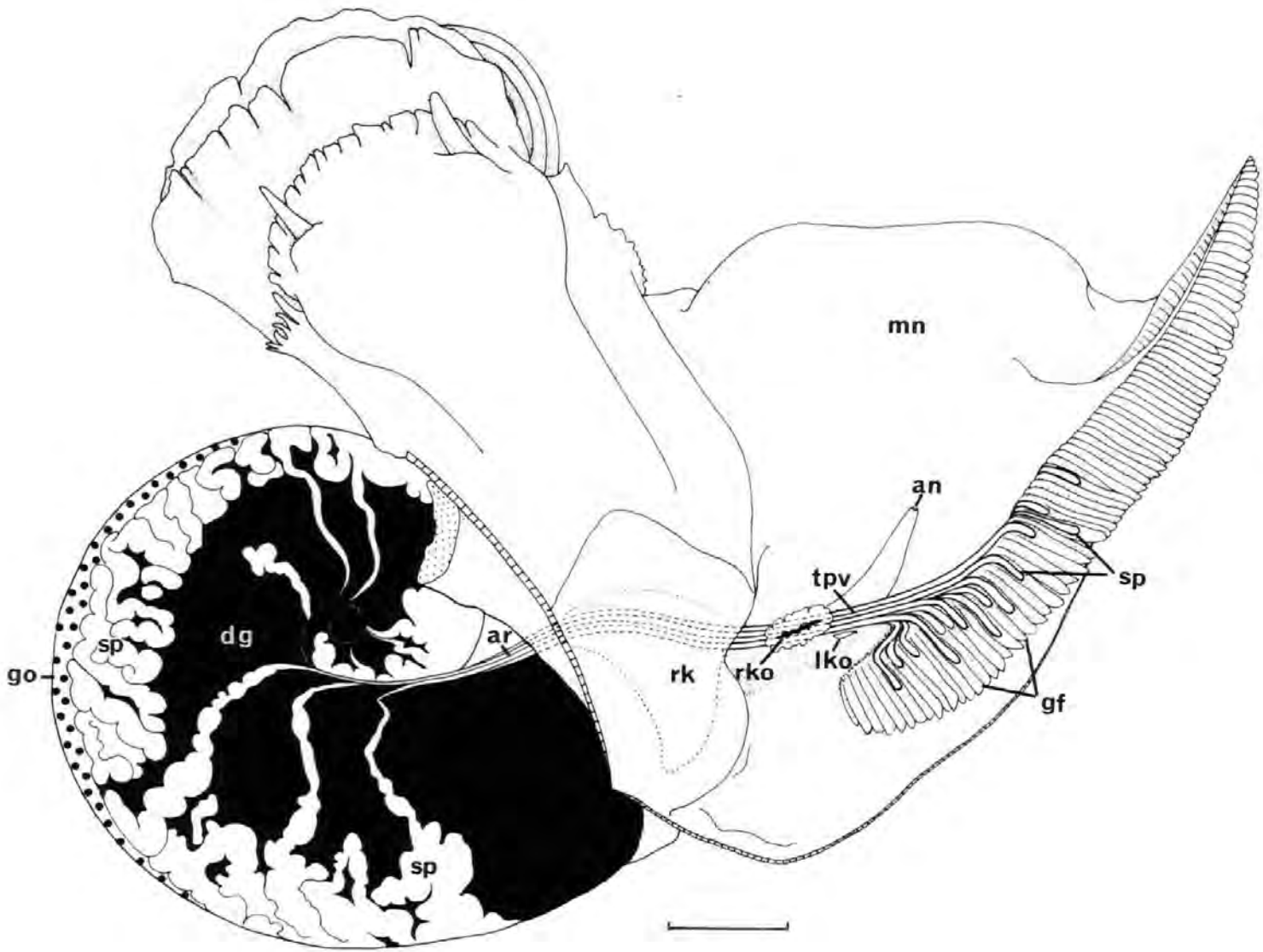
The daughter sporocyst (Fig. 2A) is unbranched and filamentous, the anterior region (Region A, Fig. 2A) comprising approximately half the body length, being narrow and white in colour, the wider posterior region (Region B, Fig. 2A) being orange with white bulges corresponding to the position of groups of developing cercariae. The posterior region only is visible in the digestive gland on removal of the host shell.

The living daughter sporocyst is highly elastic and sticky, a feature which makes removal from the host extremely difficult. Intact specimens undergo slow writhing movements in sea-water. In length they measure 6-26 mm, in maximum breadth an average of 0.1 mm anteriorly and 0.25 mm posteriorly. The posterior region is the site of cercarial production, germinal cells being proliferated near the posterior extremity (Fig. 2A). Groups of developing cercariae occur

Table 6. Results of the examination of prosobranchs for hemiurid cercariae

Mollusc species	Station number	Number examined	% positive for hemiurid larvae	Cystophorous cercariae
Calliostoma ziziphinum	11	428	3	Cercaria calliostomae Dollfus, 1923
	13	97	2	"
Gibbula cineraria	11	162	0	
	13	137	0	
Gibbula umbilicalis	1	200	0	
	3	425	0	
	9	234	1.5	Cercaria vaullegeardi Pelseneer, 1906
	11	7000+	1.13	"
	12	348	0	
Hydrobia ulvae	10	553	0	
Monodonta lineata	11	1000+	0	
Nassarius reticulatus	13	8	0	
Odostomia plicata	11	4	0	
Rissoa guerini	11	10	0	

Fig. 1. Gibbula umbilicalis displayed to show the distribution of daughter sporocysts of Cercaria vaullegeardi within digestive gland haemocoel, afferent renal vein, right kidney haemocoel, transverse pallial vessel and blood channel of gill filaments. Host shell removed; mantle cut along left side. Scale line represents 2 mm.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at irregular intervals along the entire length of the posterior region. The anterior region contains only fully developed cercariae, moving actively forwards, head to tail in a single line, to enter the muscular birth canal from which they emerge through the terminal birth pore. In situ the sporocyst lies with the posterior region within the haemocoel of the host digestive gland; the anterior region passes forwards along the afferent renal vein through the haemocoel of the right kidney into the transverse pallial vessel which crosses the mantle wall to the gill (Fig. 1, Pl. 5B). The extreme anterior end of the sporocyst lies within the blood channel of a single gill filament (Pls. 5C,D; 6).

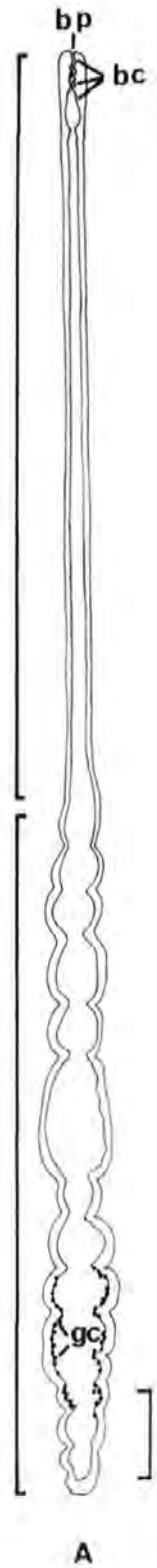
(ii) Structure of the daughter sporocyst

1. Body wall

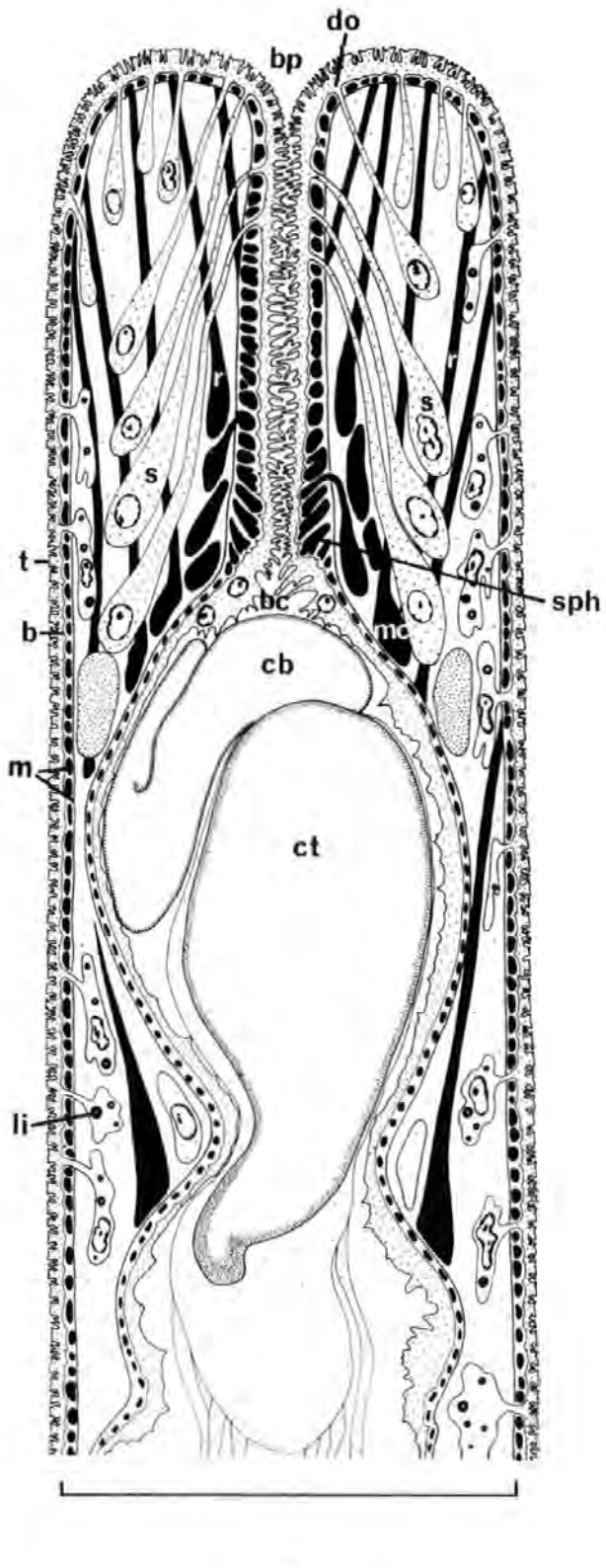
Anterior region (Region A, Fig. 2A). The entire surface is covered with an anucleate, syncytial microvillous tegument. In the vicinity of the birth canal the microvilli average 1.64 µm in length and may be single and narrow, or branched with proximal or distal swellings (Pl. IA). These swellings may anastomose distally to form an incomplete outer membranous sheet in contact with the host, perforated by irregular channels passing deep into the tegument. Electron-lucent vesicles, measuring 34 nm in average diameter, mitochondria and free ribosomes have been observed within the tegument. Outer circular and inner longitudinal muscle layers lie under the basement lamina. Sub-tegumental cells in the region of the birth pore are of two types: muscle cells with clear cytoplasm, scattered aggregations of ribosomes, mitochondria and vesicles, and cells containing prominent lipid bodies and vesicles (Pl. IA).

Fig. 2A. Daughter sporocyst of Cercaria vaullegeardi to show narrow anterior region A with birth canal, and posterior region B. Scale line represents 0.4 mm.

Fig. 2B. Anterior end of daughter sporocyst of C. vaullegeardi. A cercaria has entered the posterior region of the canal prior to emergence through the birth pore. Scale line represents 0.1 mm.



A



B

Germinal cells were not observed within the anterior region of the sporocyst.

Posterior region (Region B, Fig. 2A). The ultrastructure of the body wall of the posterior region within the host digestive gland has been described by Popiel (1976a,c). The present study confirms these descriptions, however germinal cells are here described for the first time. These measure 3.8 µm in average diameter, have large, indented nuclei 3.4 µm average diameter, and sparse dense cytoplasm with rough endoplasmic reticulum (RER) and free ribosomes (Pl. 8C), being similar in appearance to those found in the redia of Cryptocotyle lingua by Rees & Day (1976). The germinal cells have been observed within nucleated cells (Pls. 8A,B; 9) lying adjacent to the sporocyst lumen, there being no limiting membrane as described in this position in the sporocyst of Cercaria stunkardi by James (1973). The cytoplasm of these cells (Pls. 7B; 8A,B; 9) is drawn out into a thin layer parallel to the body wall, increasing in thickness at the sites of germinal cell accumulation (Pl. 9). It is dense and highly vacuolated, with mitochondria, RER, free ribosomes, α and β glycogen, and electron-lucent vesicles often with granular or flocculent contents (Pls. 8A; 9). The germinal cells lie within cavities in the cytoplasm, from which they are liberated into the lumen of the sporocyst (Pl. 8B). The cavities may contain a single cell (Pl. 8A,B) or may be enlarged accommodating masses of 30 or more germinal cells.

A column of approximately 15 cells of unknown function has been observed within one of these enlarged cavities (Pl. 9). The column is attached by one end to the sporocyst cytoplasm; folds of dense fibrous tissue surround the point of attachment (Pl. 10A), and a thin layer of fibres lies around the periphery of the cells (Pl. 10B).

The effect of the sporocyst upon adjacent cells of digestive gland tubules is shown in Pl. 7A. The cytoplasm of affected cells becomes highly vacuolated, the membranes of the nuclear envelope more widely spaced, and nuclear contents sparse. A zone of cellular debris separates the microvilli from the necrotic host cells.

2. Birth pore and associated structures

A sinuous canal measuring 0.35 mm in average length (Fig. 2B), connects the terminal birth pore with the sporocyst lumen. The canal has three distinct regions; a short anterior region around the birth pore itself, a middle muscular region terminating in a prominent sphincter and a posterior region which widens and merges with the lumen. The anterior and middle regions are lined by tegument similar to that covering the body. At the posterior end of the birth canal the tegument consists of a single layer of large cuboidal cells whose free surface is usually extended into long anastomosing microvilli which partially occlude the lumen of the canal. Each cell has a large rounded nucleus and dense cytoplasm with RER, Golgi, free ribosomes, membrane-bound, electron-lucent vesicles and α and β glycogen (Pls. IB, 4A).

The muscle coats of the general body wall pass inwards to line the three regions of the birth canal, forming its outer longitudinal and inner circular layers. These muscles are thickened in the middle region, especially at the sphincter. Radial muscles pass from the middle and posterior canal regions obliquely forwards to the body wall (Fig. 2B; Pl. 3A).

Numerous large secretory cells lying around the middle and posterior canal regions have ducts which pass forwards to open into the

tegument lining the middle and anterior canal and that covering external areas adjacent to the birth pore (Fig. 2B; Pl. 2A). Each cell has a large indented nucleus with several nucleoli and dense cytoplasm rich in electron-lucent vesicles, RER, Golgi, α and β glycogen, mitochondria, and occasional protein whorls and lipid bodies (Pl. 3A).

The nervous system is as described for the daughter sporocyst of Cercaria stunkardi by James (1973); the cerebral ganglia, situated posterior to the birth canal sphincter, being a site of acetylcholinesterase (Pl. 5A) and non-specific esterase production.

(iii) Emergence of the cercaria

In the living sporocyst cercariae may be seen throughout most of the length of the anterior region, moving in single file towards the birth pore. Progression is achieved by a combination of muscular action of the cercarial body and peristaltic movements of the sporocyst wall. The tail at this stage is incapable of active movement and is dragged behind the body of the cercaria with all appendages trailing, except the delivery tube which has now been withdrawn into the tail cavity.

During periods of cercarial release, the anterior end of the sporocyst actively extends along the blood channel of the gill filament and penetrates the epithelium to protrude approximately 1 mm into the mantle cavity. The columnar epithelium of the gill filament is disrupted and torn (Pl. 5C,D), suggestive of muscular and lytic activity on the part of the parasite. Cercariae emerge through the sporocyst birth pore directly onto the surface of the gill within the host mantle cavity. Within seconds of emergence the cercarial body retracts into the cystophorous tail, the sphincter muscle surrounding

the tail aperture (Fig. 4B) is closed to seal the point of entry, and the 'encysted' cercaria is dislodged by the exhalant water current and swept out of the mantle cavity.

b. The redia of Cercaria calliostomae Dollfus, 1923

As shown in Table 6, 3% of 428 specimens of Calliostoma ziziphinum collected at Station 11 were found to be infected with the rediae of C. calliostomae. Rediae containing fully-developed cercariae are orange, measure 3.8 x 0.6 mm, and occur throughout the gonad haemocoel. In heavily-infected snails the gonad may be completely destroyed. The redia has been described by Dollfus (1950). The present investigation confirms that the birth pore opens beside the mouth at the anterior extremity of the body. Numerous sub-tegumental secretory cells open into the anucleate, syncytial, microvillous tegument around the anterior end.

Cercarial emergence

Dissection of five C. ziziphinum, opened whilst shedding C. calliostomae, showed active rediae within the gonad, afferent renal vein and right kidney haemocoel, but not within the blood vessels of the mantle or gill. Examination of serial sections of two entire C. ziziphinum, fixed whilst shedding large numbers of cercariae, confirmed the redial distribution described above. No free cercariae, easily visible in sections due to differential staining of the collagenous caudal cyst, were observed in any tissue or blood vessel throughout the molluscs. In both snails, the pharynx and adjacent birth pore of several rediae were applied to the disrupted kidney tubule wall. Cercariae were not observed in sections within the

genital ducts, kidney sac or urinogenital papilla.

2. Cercariae

a. Cercaria vaullegeardi Pelseneer, 1906

Although cercarial development within the filamentous daughter sporocyst is continuous, it is convenient for description to refer to three stages, namely pre-migratory, migratory and encysted cercariae. Pre-migratory (Fig. 3A-I) and migratory (Fig. 4A) cercariae are stages which occur within the posterior and anterior region of the sporocyst respectively. The encysted cercaria (Fig. 4B,C) is the stage at which the retracted body is coiled within the cavity of the cystophorous tail.

(i) Cercarial body

The cercarial body (Fig. 4A) has a well-defined oral sucker and pharynx, digestive caeca, nervous and excretory systems. The ventral sucker, although defined early in development (Fig. 3F) remains comparatively rudimentary. The posterior end of the body is inserted through the aperture of the caudal cyst (Fig. 4A) onto the inner surface of its dorsal wall.

The tegument resembles that of other digeneans in that it consists of an outer syncytial layer connected to sub-tegumental cell bodies by cytoplasmic bridges (Pl. 11B). The sub-tegumental cell cytoplasm contains secretory vesicles, measuring 0.33 µm in average diameter, with flocculent and electron-dense neutral mucopolysaccharide contents (Pl. 11C). The vesicles pass into the tegument at encystment (Pl. 16), some being discharged at its surface (Pl. 16B).

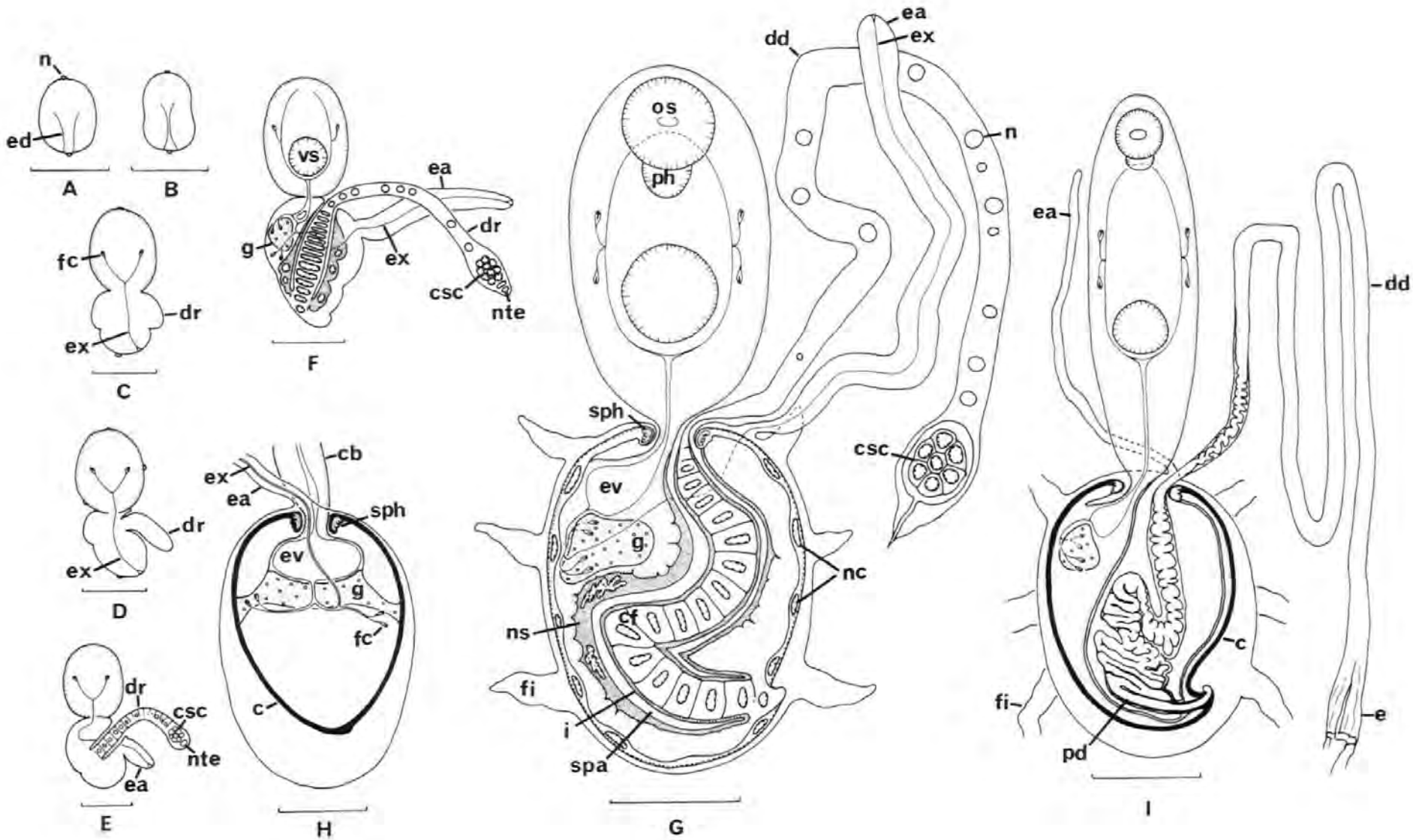
Fig. 3. Pre-migratory Cercaria vaullegeardi removed from posterior region of daughter sporocyst.

Figs. 3 A-F. Early stages, showing development of delivery tube rudiment and excretory appendage. Ventro-lateral view. Scale lines represent 5 µm.

Fig. 3G. Later stage showing folding of proximal delivery tube within developing caudal cyst, formation of space between outer nucleated and inner investing syncytial layers, and caudal filament buds. Ventro-lateral view of tail, body twisted to ventral view. Scale line represents 50 µm.

Fig. 3H. Dorsal view of cystophorous tail showing caudal gland and excretory system. The delivery tube and caudal filaments are not shown in this diagram to increase clarity. Scale line represents 50 µm.

Fig. 3I. Delivery tube retraction into caudal cyst. Ventro-lateral view of tail, body twisted to ventral view. Scale line represents 50 µm.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The loosely packed parenchyma cells (Pls. 11, 16) have ill-defined cell boundaries. Their cytoplasm, with free ribosomes and mitochondria, is chiefly confined within deep indentations at the nuclear surface (Pl. 16B).

(ii) Cystophorous tail

The fully formed cystophorous tail (Fig. 4A), as in all hemiurid cercariae so far described, consists essentially of a hollow vesicle or caudal cyst into which the body is withdrawn at encystment, and an elongated appendage, the delivery tube, through which the cercarial body is inoculated into the host haemocoel during the infection process. The caudal cyst is covered by tegumental membranes which extend to form six slender caudal filaments (Figs. 3G, 4C). A vestigial excretory appendage is attached to the anterior end of the caudal cyst.

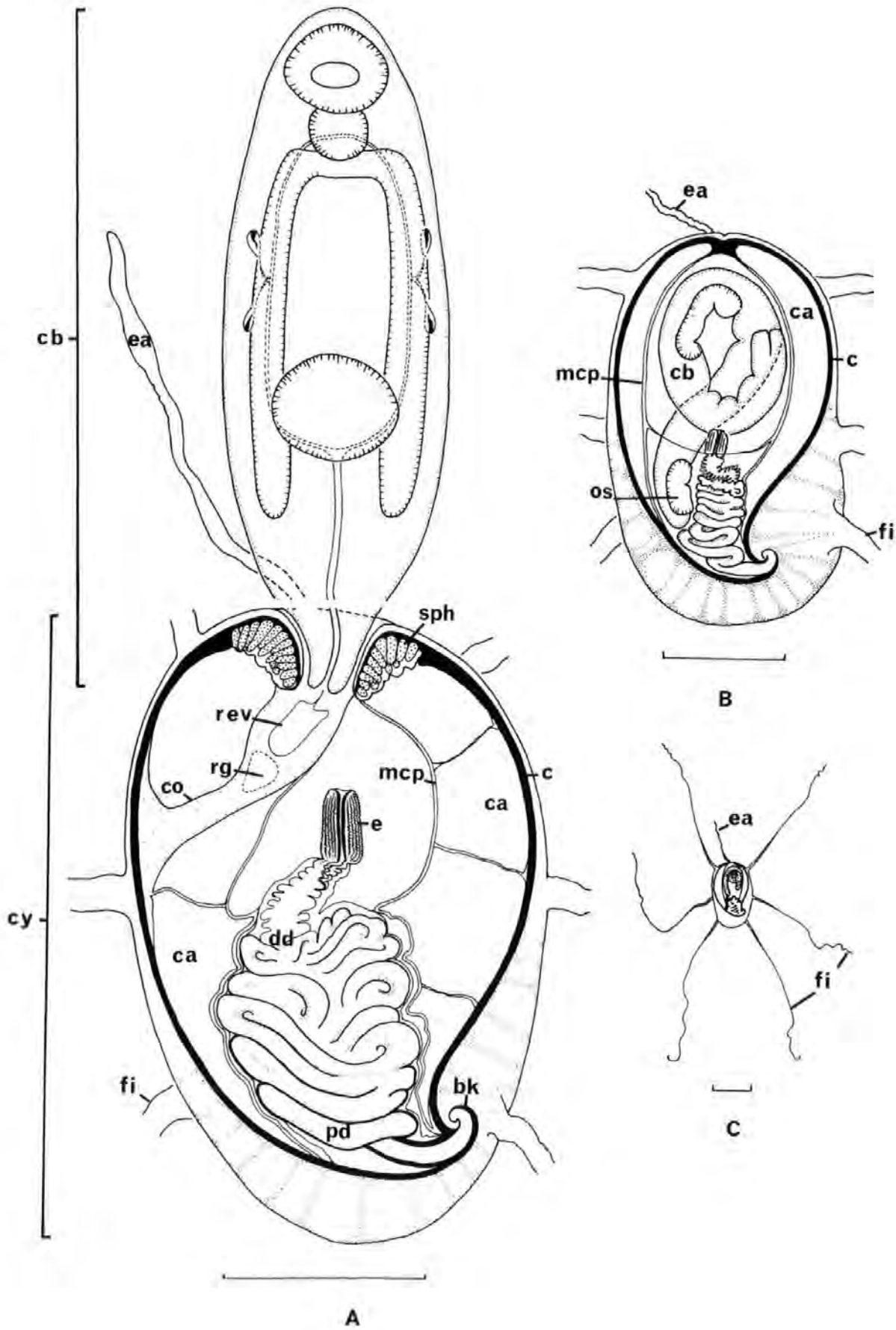
Tegument

The tail of the earliest stages is covered by syncytial tegument, measuring 0.92 µm in average thickness, with a smooth outer bounding membrane (Pl. 12A) and sparse contents including microtubules and mitochondria. The tegument is connected by a branched duct (Pl. 13A) to the antero-dorsal, nucleated syncytial caudal gland (Fig. 3G,H; Pls. 12B, 13). The latter produces large numbers of electron-lucent vesicles, 0.21 µm in average diameter, which pass into the tegument at a stage corresponding to that shown in Fig. 3G. The vesicles accumulate in a single continuous layer (Pl. 15A) before discharging their contents at the tegumental surface membrane. The walls of the collapsed vesicles remain, causing the surface to be completely covered with shallow

Fig. 4A. Migratory Cercaria vaullegeardi removed from anterior region of daughter sporocyst. Ventral and ventro-lateral views of body and tail respectively. Scale line represents 50 µm.

Fig. 4B. Encysted C. vaullegeardi. Ventro-lateral view showing cercarial body and delivery tube coiled within membranous capsule. Scale line represents 50 µm.

Fig. 4C. Ventro-lateral view of encysted C. vaullegeardi showing caudal filaments and excretory appendage. Scale line represents 100 µm.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indentations measuring 0.21 µm in average diameter which are clearly seen in scanning micrographs of encysted cercariae (Pl. 20B).

Cytoplasmic contents of the tegument beneath the vesicular layer break down leaving a cavity, except at the rounded posterior end of the tail where strands of cellular debris pass inwards to the curved caudal cyst beak (Pl. 15C). Following secretion of the vesicles the caudal gland degenerates (Figs. 3I, 4A), being no longer present in the encysted cercaria.

Several cells with flattened nuclei and dense cytoplasm containing RER, cisternae, free ribosomes and mitochondria have been observed between the tegument and the developing caudal cyst (Pl. 12B). Their cytoplasm becomes vacuolated and the cells break down, leaving a homogenous layer of medium electron density measuring 0.2 µm in average thickness (Pl. 15A) which persists until the cercariae leave the posterior region of the sporocyst.

Caudal cyst

The caudal cyst (Fig. 4A) is rounded anteriorly, narrowing sharply at the ventro-laterally curved posterior end, termed the 'beak' by Popiel (1976a). A prominent sphincter muscle passes around the anterior aperture (Fig. 4A; Pl. 14). The caudal cyst wall consists of three occasionally four, layers of curved fibres embedded in a homogenous matrix (Pl. 15B). The fibrous layers are not continuous around the tip of the beak (Pl. 15C). The loosely-packed fibres of the first-formed outer layer are arranged haphazardly (Pl. 15A). In addition, two or three layers of parallel, curved fibres are laid down successively inside the first, the completed caudal cyst (Pl. 15B) measuring 1 µm in average thickness. It stains intensely with aniline blue in Heidenhain's Azan (Pantin, 1962), a

reaction characteristic of collagenous material (Pearse, 1968).

During the development of the caudal cyst, the fibres appear to originate from a single layer of sub-tegumental cells (Pl. 15A), being laid down immediately adjacent to their outer border. That these are fibre-forming cells is further suggested by the progressive thickening of the caudal cyst wall from within. The cells have large nuclei and dense cytoplasm with RER, cisternae, free ribosomes and mitochondria (Pl. 15A). Following completion of the caudal cyst, the fibre-forming cells degenerate. The caudal cyst cavity of encysted forms contains scattered granular material and an irregular homogeneous deposit (Pls. 15B, 16A). Both substances are weakly PAS-positive and negative with Alcian blue (pH 1.0 and 2.5).

Table 7. Measurements of C. vaullegeardi (in µm)

		Migratory stage	Encysted stage
Body	Contracted	117 x 47	-
	Extended	250 x 23	300 x 20
	Oral sucker	16 x 21	(distorted)
	Ventral sucker	18 x 21	(distorted)
Tail	Length x maximum breadth	130 x 100	165 x 104
	Caudal filaments (length x breadth at base)	465 x 14	465 x 14
	Excretory appendage (length x maximum breadth)	115 x 7	115 x 7
	Caudal cyst (length x maximum breadth)	104 x 78	134 x 90
	End-piece	13 x 9	13 x 9

Delivery tube

The delivery tube comprises a short proximal region, measuring 0.2 mm in average length, attached to the internal surface of the caudal cyst beak, and an elongated distal region, 0.7 mm in average length, which terminates in a thickened structure here termed the end-piece (Fig. 4A). The wall of the distal delivery tube consists of an outer anucleate syncytial tegument with microtubules, ribosomes, mitochondria and RER, and an inner fibrous layer measuring 0.2 µm in average thickness (Pl. 18A). The thicker, less flexible wall of the proximal delivery tube in the migratory cercaria has no tegument, but consists of a double layer of curved fibres (0.4 µm in average thickness) (Pl. 17) similar to those described in the caudal cyst.

The rudiment of the fibrous wall of the delivery tube differentiates as a column of 10-12 cells, running along an oblique antero-posterior axis (Fig. 3E,F; Pl. 12A). The cells divide, forming an exterior outgrowth; internal and external portions of the column give rise to the proximal and distal delivery tube respectively. The cells are of similar ultrastructure (Pl. 17A) to the fibre-forming cells of the developing caudal cyst, laying down the fibrous layers which strengthen the delivery tube wall. Each cell elongates, greatly increasing the total length of the column. The external portion develops without hindrance, lying freely within the sporocyst lumen, whilst the restricted internal portion folds within the caudal cyst (Fig. 3G). When the fibrous wall is complete, the fibre-forming cells degenerate and break down, leaving a continuous cavity along the centre of the column.

In pre-migratory cercariae the developing proximal delivery

tube is covered by dense, nucleated syncytial cytoplasm with numerous mitochondria, free ribosomes, microtubules and RER (Pls. 12B, 17A). As the appendage folds within the tail, a cavity develops around it (Fig. 3G); sections now show the syncytium to comprise both the investing layer of the tube and an outer nucleated sleeve (Pl. 12). Cytoplasmic breakdown occurs in both syncytial layers leaving three membranes which together comprise the membranous capsule (Fig. 4A,B).

Differentiation of the end-piece is shown in Figs. 3E-G,I,J. A sub-terminal cell divides to produce a swelling containing a cluster of 10-15 cells with large, rounded nuclei and dense cytoplasm with mitochondria, microtubules, RER and free ribosomes (Pl. 19A). A fibrous layer is laid down by flattened cells of similar ultrastructure to the fibre-forming cells described above. Several terminal cells with cytoplasm containing elongated mitochondria, ribosomes, microtubules and cisternae, extend distally and laterally to form an incomplete tube, or collar-shaped extension. Electron-dense deposits accumulate around the periphery of the terminal cells on the inside of the fibrous layer (Pl. 19B,C). Cellular shrinkage and breakdown, leaving a cavity continuous with the distal delivery tube lumen, is followed by contraction of the fibrous wall producing the heavily folded mature end-piece of the migratory cercaria (Fig. 4A; Pl. 19D). The latter gave a positive reaction for acetylcholinesterase (Pl. 20C,D) and non-specific esterases (Table 8).

The fully formed delivery tube is gradually retracted into the posterior half of the membranous capsule (Fig. 3I; Pls. 16A, 17B, 18B,C), retraction being completed before the cercaria migrates forward into the anterior region of the sporocyst.

Table 8. Histochemical tests on Cercaria vaullegeardi and C. calliostomae

Test		C. vaullegeardi	C. calliostomae	Inference
Indoxyl acetate (Jennings & LeFlore, 1972)	Experimental	+++ cerebral ganglia, nerves & delivery tube endpiece	+++ cerebral ganglia, nerves & tail ribbon	Non-specific esterases, inhibited by 10^{-3}M eserine
	Control 1. No substrate	-	-	
	" 2. Preheated 60°C/30 mins	+ cerebral ganglia & endpiece	++ tail ribbon	
	" 3. Preheated 90°C/5 mins	-	-	
	" 4. 10^{-3}M eserine	-	-	
	" 5. 10^{-4}M eserine	+ cerebral ganglia & endpiece	-	
Acetylthiocholine iodide (Jennings & LeFlore, 1972)	Experimental	+++ cerebral ganglia & endpiece ++ nerves	+++ cerebral ganglia & tail ribbon ++ nerves	Acetylcholinesterase in cerebral ganglia & nerves, also in delivery tube endpiece of C. vaullegeardi and tail ribbon of C. calliostomae (Pl. 20C,D,E). Inhibited by eserine
	Control 1. No substrate	-	-	
	" 2. Preheated 60°C/30 mins	+ cerebral ganglia & endpiece	+++ cerebral ganglia & tail ribbon	
	" 3. Preheated 90°C/5 mins	-	-	
	" 4. 10^{-3}M eserine	-	-	
	" 5. 10^{-4}M eserine	-	-	

-, no reaction; +, faint reaction; ++, average reaction; +++, strong reaction.

Excretory appendage

The posterior median lobe of the embryonic tail with fused caudal excretory ducts (Fig. 3C), narrows and lengthens (Fig. 3D-G) becoming the excretory appendage described diagrammatically by Pelseneer (1906). The appendage moves into an anterior position by the differential growth of the rest of the tail, coming to lie at the dorsal rim of the caudal cyst aperature (Fig. 3G,H). The appendage is at first capable of restricted movement, occasionally undergoing slow contractions. The excretory duct degenerates and cellular breakdown occurs; the shrivelled remains of the excretory appendage are, however, still present in the encysted cercaria (Fig. 4A,C).

(iii) Excretory system

The excretory system is most extensive in pre-migratory forms (Fig. 3G,H). The caudal ducts and four flame cells subsequently break down (Figs. 3I, 4A), only those parts of the excretory system within the cercarial body remaining in encysted cercariae.

(iv) Encystment

At encystment, the retractor muscles within the cercarial body (Pl. 14A) contract, and the strand connecting the body to the caudal cyst (Fig. 4A) breaks. Contraction of the tubular caudal sphincter muscle (Pl. 14) commences whilst the body slips backwards into the tail, the aperture being sealed as retraction is completed. The body moves slowly within the tail, undergoing periodic stretching movements. It increases in length to 0.3 mm, becoming correspondingly slender, 0.02 mm in average diameter (Fig. 4B).

Retraction of the body into the caudal cyst normally occurs on

the surface of the host gill within seconds of emergence from the sporocyst birth pore into the host mantle cavity. If migratory cercariae are obtained by dissection, normal encystment seldom occurs. In these cases cercariae may either not encyst, floating at the water surface, or the body may become trapped by the contracted sphincter muscle in a partially withdrawn state.

b. Cercaria calliostomae Dollfus, 1923

As in C. vaullegeardi, the tail is modified to form a hollow vesicle or caudal cyst, into which the body is retracted at 'encystment', and an inoculative appendage, the delivery tube. Intraredial stages are shown in Fig. 5A-J; encysted forms (Fig. 6A,B), with the detached body coiled within the tail, have been obtained here only by natural emergence from screened, infected hosts. Cercariae removed by dissection of rediae failed to encyst, dying within several hours in sea water at 14°C.

(i) Cercarial body

The intraredial cercarial body (Fig. 5J) measures 156 x 84 µm when contracted and 477 x 28 µm when extended. Oral and ventral suckers measure 34 x 35 µm and 44 x 48 µm respectively. In encysted forms the coiled body maintains an elongated slender shape, measuring approximately 362 x 34 µm, lying with oral sucker posteriad, adjacent to the retracted delivery tube (Fig. 6A). As in C. vaullegeardi, the parenchyma cells are loosely packed and ill-defined, and there are no cystogenous or penetration glands.

Fig. 5. Cercaria calliostomae: intraredial stages.

Fig. 5 A-C. Early stages; the embryonic tail develops two lateral lobes, the caudal excretory ducts opening to the exterior through the median lobe. The delivery tube rudiment differentiates from a lateral lobe.

Fig. 5D. The fused caudal excretory ducts now open near the rim of the developing caudal cyst. Delivery tube rudiment lengthens as a single column of cells, a cluster of specialised cells at its swollen tip.

Fig. 5E. Delivery tube lengthens; its lumen is formed by breakdown of the column of fibre-forming cells.

Fig. 5 F-I. Further stages in endpiece development.

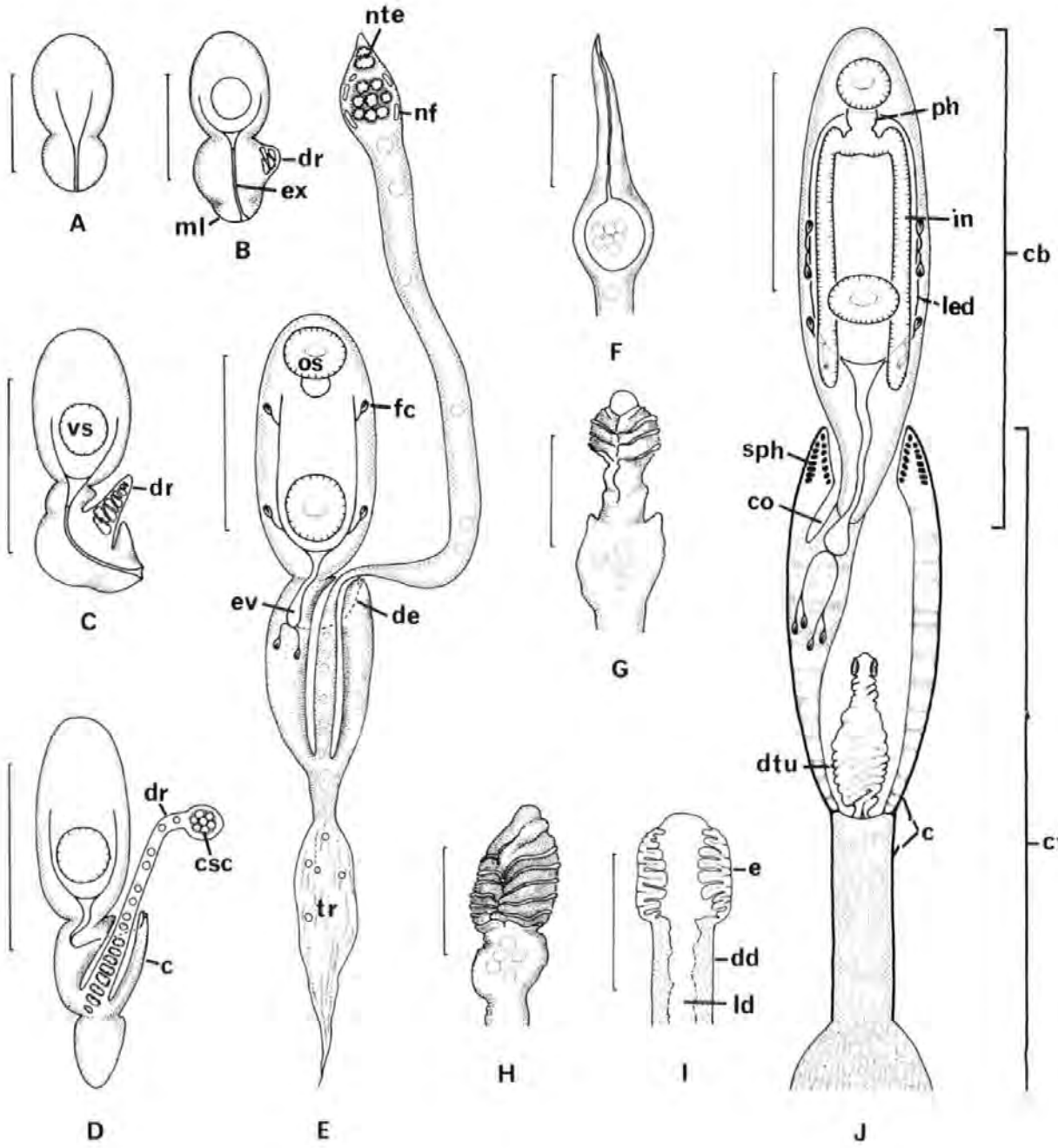
F - Terminal region extends as cells break down. Cluster of specialised cells shrinks leaving a cavity.

G, H - Terminal region contracts from tip, folding the fibrous wall.

I - Mature endpiece.

Fig. 5J. Fully-formed cercaria ready for emergence. The strand connecting the cercarial body to the caudal cyst shrinks and weakens. The delivery tube is fully retracted into the caudal cyst.

Fig. 5A-E, scale lines represent 100 µm; F-I, 25 µm; J, 150 µm.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(ii) Cercarial tail

The fully developed immotile cystophorous tail (Fig. 5J), which measures 1.3 mm in length and 101 µm in maximum breadth, comprises the anterior caudal cyst and the posterior flattened, sticky, membranous ribbon. In intraredial forms, the posterior end of the body is inserted through the anterior aperture of the caudal cyst onto the inner surface of its dorsolateral wall. The elongated caudal cyst is flask-shaped, with wide anterior and narrow posterior sections measuring 270 µm in length and 108 µm in maximum breadth, and 135 x 37 µm respectively. A well-developed tubular sphincter muscle surrounds the anterior aperture, and smooth tegumental membranes cover both wide and narrow sections of the caudal cyst. The latter are separated by a transverse membrane, to the anterior surface of which the posterior end of the retracted, coiled delivery tube is attached (Fig. 6A).

Caudal development (Fig. 5A-J) and ultrastructure (Pls. 21-23) basically resemble that described in C. vaullegeardi. The laminated caudal cyst wall (Pl. 23) comprises four or five layers of curved fibres embedded in a homogenous matrix, and an inner zone, 0.23 µm thick, seen in oblique section as a honeycomb-like lattice (Pl. 23B). Cellular breakdown within the caudal cyst is incomplete, the remains of dividing cell walls being still clearly visible in the encysted cercaria (Fig. 6A). The caudal gland and associated tegumental indentations described in C. vaullegeardi have not been observed in developmental stages of C. calliostomae studied here.

Delivery tube development is shown in Fig. 5. As in C. vaullegeardi, the modified tip, or endpiece, at first contains a cluster of specialised cells with large, rounded nuclei and sparse,

Fig. 6. *Cercaria calliostomae*

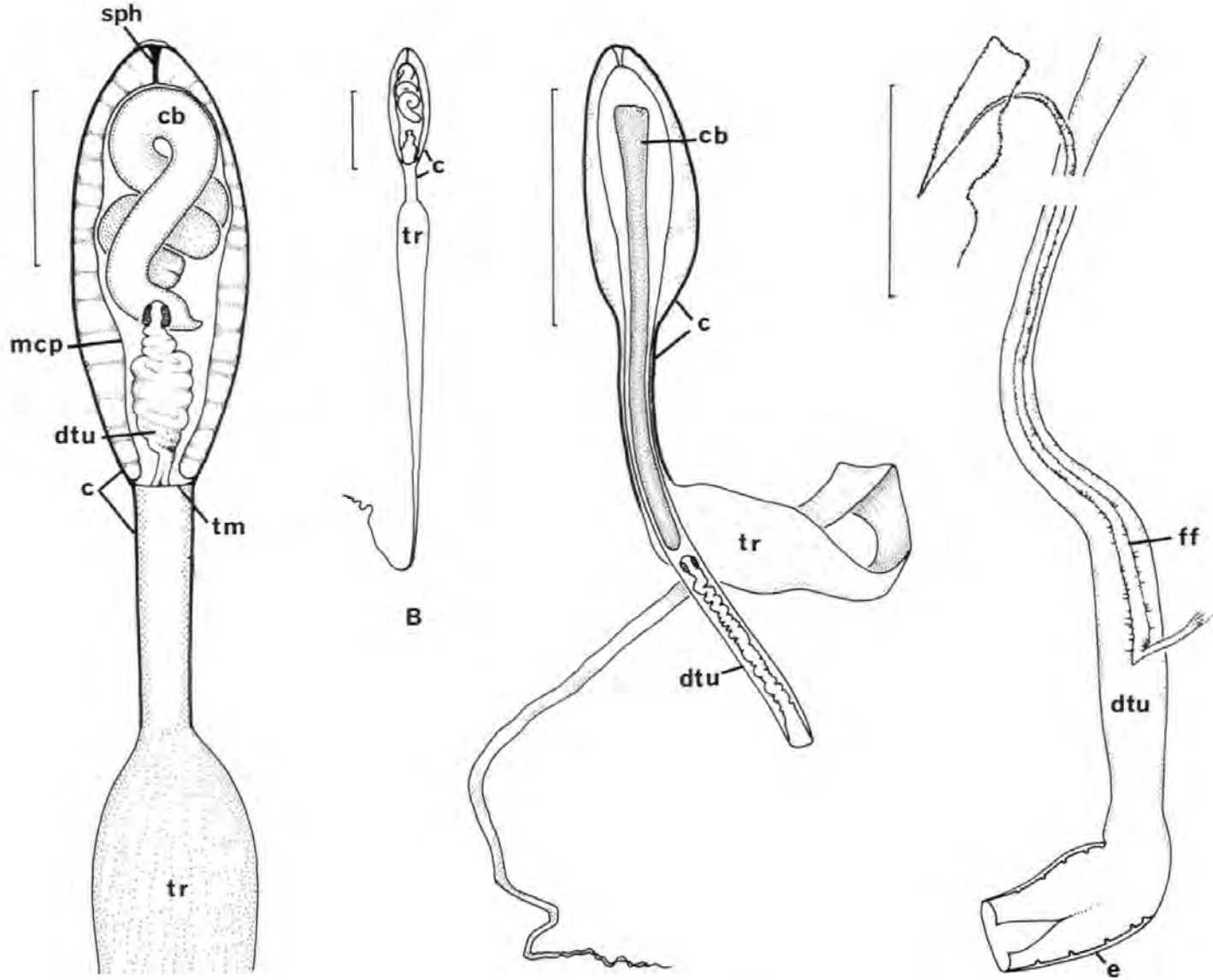
Fig. 6A,B. The encysted form of *C. calliostomae*, as released from the host with the exhalant water current. The complete cercaria is shown in B, its anterior region enlarged in A.

Fig. 6C,D. *Camera lucida* drawings showing *in vitro* delivery tube eversal in *C. calliostomae*.

C. The cercarial body has entered the partially-everted delivery tube.

D. Everted endpiece showing thickened ridges and torn terminal membrane following cercarial body extrusion.

Scale line represents 100 µm in A and D, 200 µm in B and C.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dense cytoplasm, two terminal cells, and several flattened peripheral fibre-forming cells (Fig. 5D,E), cellular breakdown and contraction (Fig. 5F-I) producing the heavily folded mature endpiece. The delivery tube is fully retracted into the caudal cyst before cercarial emergence.

In both C. calliostomae and C. vaullegeardi, differential growth results in movement of the median lobe of the embryonic tail (Fig. 5B) from a posterior to an anterodorsal position. In the present species, however, the median lobe does not extend to form an excretory appendage (Fig. 5C-E), and the four caudal flame cells and their ducts are retained in the fully formed tail (Fig. 5J).

3. Infection of the second intermediate host

The results of attempts to infect various second intermediate hosts with C. vaullegeardi and C. calliostomae are shown in Table 9.

a. C. vaullegeardi

(i) Experimental infections of Tigriopus brevicornis

Naturally emerged encysted C. vaullegeardi lie on the bottom of the container, either singly or entangled with each other in cottonwool-like masses. Tigriopus brevicornis is a raptorial feeder, which scavenges through debris and vegetation and readily selects cystophorous cercariae as food. Repeated observations have shown that the behaviour of T. brevicornis when eating C. vaullegeardi always follows the same pattern; the copepod seizes the cercaria with the second antennae, second maxillae and maxillipeds, turns it so that its long axis lies parallel to that of its own body, and pressing it

Table 9. Results of infection experiments using freshly-emerged, encysted cystophorous cercariae (1974-1980)

Experimental host species	Numbers exposed	Parasite	Number cercariae/host	Result
Tigriopus brevicornis	700+	*C. vaullegeardi*	1-12	100% infection rate with 1-7 metacercariae may be obtained
Tisbe furcata	23	"	1-5	Some cercariae eaten, but no metacercariae obtained
Amphithöe rubricata	300	"	10-20	Cercariae readily eaten, but metacercariae obtained within haemocoels of only 2 amphipods
Gammarus spp.	30	"	10-20	Cercariae readily eaten but no metacercariae obtained
Nereis diversicolor	6	"	50+	Cercariae readily eaten but no metacercariae obtained. Guts became filled with undischarged cercariae which were subsequently digested
Gobius paganellus (post-larval)	3	"	100+	Cercariae were both actively eaten and entered mouth with respiratory water current. Fish were killed 5 mins., 1 hour and 3 hours after cercariae were swallowed. No metacercariae present in body cavity or gut wall; undischarged cercariae in various stages of digestion observed within the gut of all 3 fish
T. brevicornis	78	*C. calliostomae*	1-6	Cercariae eaten. Metacercariae obtained within haemocoel of only 2 copepods
T. furcata	12	"	1-3	Some cercariae eaten. No metacercariae obtained

against the mandibles attempts to bite into its surface. Mandibular purchase cannot be gained against the rounded anterior end of the rigid fibrous caudal cyst and the copepod therefore turns the cercaria until the biting mouthparts can be used to better advantage on the posterior end (Pl. 24B,C). The tegumental membranes eaten, the narrow curved posterior region of the caudal cyst, or beak (Pl. 24B) is pressed into the mouth (Fig. 7A) and crushed by the mandibles. When the tip of the beak, unprotected by fibres, is severed by mandibular action, the delivery tube everts into the copepod's oesophagus. The thickened proximal section emerges first, forcibly penetrating the dorsal wall of the midgut (Fig. 7B), followed by the more flexible thin-walled distal tube which everts within the haemocoel (Fig. 7C). The cercarial body simultaneously passes through the delivery tube lumen into the host haemocoel (Fig. 7D). The collapsed caudal cyst is crushed and eaten.

(ii) Viability of C. vaullegeardi

In freshly emerged cercariae the coiled, transparent body stretches intermittently within the tail, maintaining an elongated slender form averaging 0.2 x 0.002 mm. Movements gradually become less frequent and the body becomes shorter and wider. Movement ceases and the cercarial body, now measuring 0.1 x 0.08 mm, becomes opaque after five days at 14°C and 10 days at 8°C. The viability of cercariae obtained during March 1977 from a single infected G. umbilicalis was found to decrease correspondingly with age. Infection rates of 50 T. brevicornis individually fed with C. vaullegeardi previously maintained for 3 h, 24 h and 3 days at 14°C being 100%, 50% and 6% respectively.

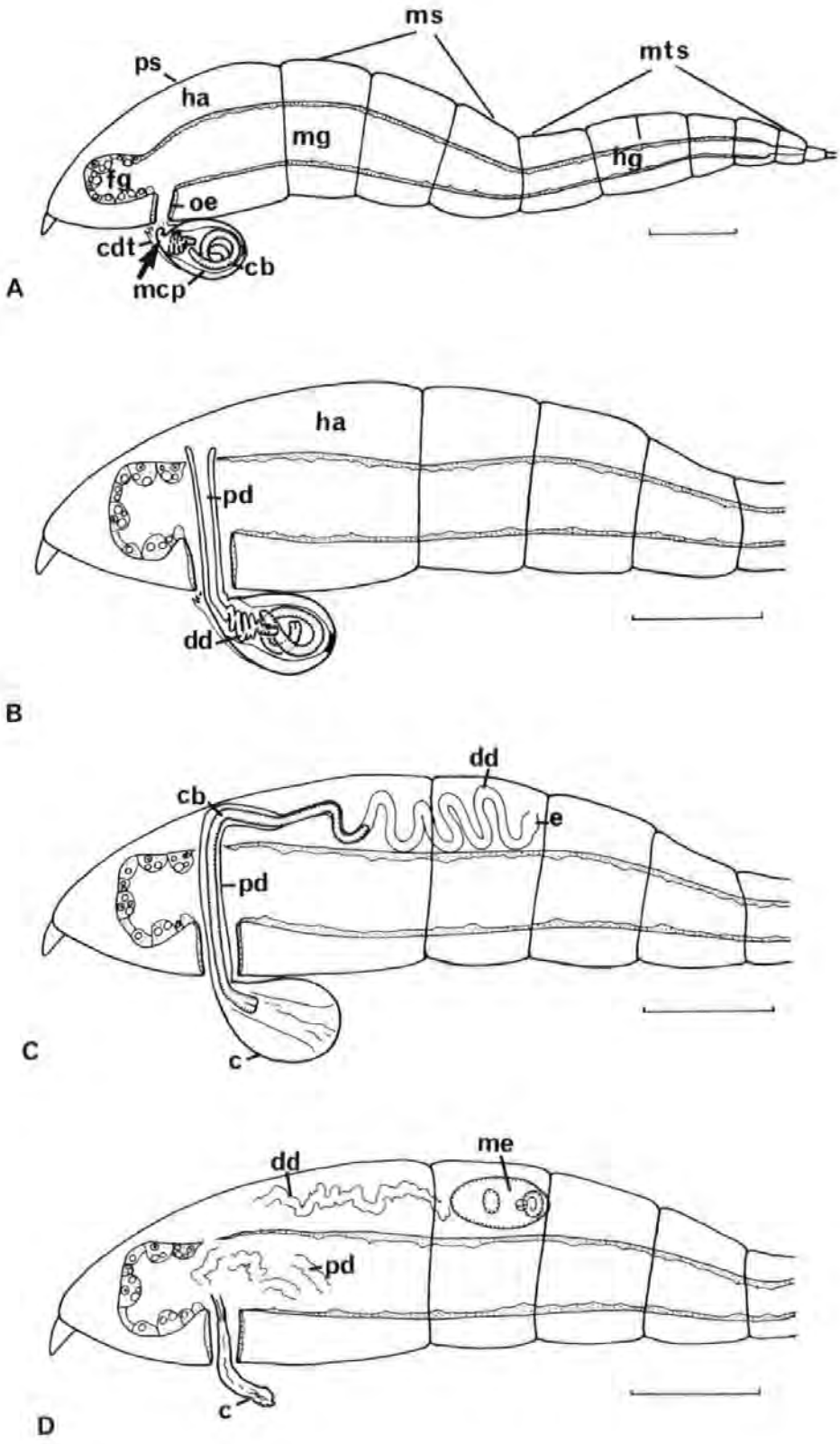
Fig. 7. Diagrams to illustrate stages in the infection of Tigriopus brevicornis with Cercaria vaullegeardi. (The copepod appendages are not shown to increase clarity)

Fig. 7A. The copepod orientates the cercaria so that its longitudinal axis lies parallel to that of its own body. The tegumental membranes at the posterior end eaten, the narrow caudal cyst beak (arrowed) is pressed into the mouth and sheared by the mandibles.

Fig. 7B. The proximal delivery tube everts up the copepod's oesophagus, piercing the dorsal wall of the midgut.

Fig. 7C. The distal delivery tube everts within the host haemocoel; cercarial body passes into the tube.

Fig. 7D. The metacercaria now lies within the host haemocoel. The collapsed caudal cyst and the proximal delivery tube are eaten. Scale lines represent 0.1 mm.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A seasonal effect on viability was noted, cercariae released in spring producing consistently higher infection rates than those shed in late summer and autumn. Infection rates of 100% were obtained in 200 T. brevicornis individually exposed at 14°C to 3-5 C. vaullegeardi newly emerged from freshly collected G. umbilicalis in March and April of 1977 and 1978. In September of the same years similar experimental procedures produced infection rates below 25%.

Shedding of C. vaullegeardi has not here been observed between November and January 1974-1980, although samples of G. umbilicalis opened throughout the winter have shown the usual 1.13% infection rate with the daughter sporocysts. The latter at this time extend into the host gill filaments but their anterior transporting regions contain few, if any, migratory cercariae. The latter, and developing stages within the posterior region of the sporocyst, are completely motionless except for flame cell action. It is not known if cercarial production is resumed by these hosts in the spring, coinciding with increased harpacticoid populations in the rock pools.

(iii) Infection experiments using other invertebrates

Amphithoe rubricata, Gammarus spp. and Nereis diversicolor and Tisbe furcata readily devoured C. vaullegeardi. Metacercariae were obtained within the haemocoels of only 2 out of 300 A. rubricata fed with the cercariae, and the large numbers of Gammarus spp., N. diversicolor and T. furcata remained uninfected. In N. diversicolor the gut frequently became filled with undischarged encysted cercariae which were subsequently digested.

(iv) Delivery tube eversion in vitro

Attempts to induce delivery tube eversion by application of cover-slip pressure resulted in rupture at the caudal sphincter muscle and expulsion of the body through the anterior aperture. Gentle rolling of the cover-slip, however, initiated delivery tube eversion (Pl. 25) by mechanical stress on the asymmetrical caudal break.

The wall of the retracted, coiled distal delivery tube consists of an inner fibrous layer covered by tegument. After eversion, the fibrous layer is seen as torn strips adhering to the outside of the distal delivery tube and end-piece (Pl. 25A,C). The everted end-piece is pear-shaped, covered with refractile granules, and measures 54 µm in length x 20 µm in maximum breadth. The narrow distal end is supported by a thickened collar (Pl. 25B) measuring 7.23 µm in length, with an aperture diameter of 7.23 - 9.64 µm.

Discharge of the delivery tube was associated with simultaneous expansion of the caudal cyst cavity to occupy the space previously taken by the coiled cercarial body and delivery tube.

b. C. calliostomae

(i) Experimental infection experiments

As shown in Table 9, attempts to infect T. brevicornis and T. furcata with C. calliostomae proved unsuccessful. Although many cercariae were eaten only two T. brevicornis became infected each with a single metacercaria of C. calliostomae, out of over 70 exposed.

(ii) In vitro delivery tube eversal

Coverslip pressure resulted in delivery tube eversal and the simultaneous through-passage of the body to the exterior. The delivery

tube everts first through the narrow posterior section of the caudal cyst, before emerging at the junction of the latter with the tail ribbon (Fig. 6C). As described in C. vaullegeardi, the fibrous layer of the delivery tube wall is torn, fragments adhering to the outside of the everted tube near its junction with the endpiece (Fig. 6D).

4. Metacercarial development within the copepod host

a. Experimental infections

(i) Development of the metacercaria of C. vaullegeardi within T. brevicornis at 17°C.

Immediately on entry into the haemocoel, the metacercaria shortens and widens (Pl. 24A) then measuring 0.08 x 0.05 mm in average length and maximum breadth. The parasite occasionally extends and contracts its body, but does not change its position within the haemocoel, remaining near the site of emergence from the distal delivery tube.

Metacercarial tegument fixed in situ within the copepod immediately after infection (Pl. 26A,B) measures 1.5 µm in average thickness, and is sparsely covered with microvilli. The electron-dense vesicles characteristic of the tegument of encysted cercariae have discharged their contents.

Maximum development is reached at 21 days after infection, when metacercariae measure 570 - 852 µm x 104 - 124 µm, average 759 x 118 µm. The oral and ventral suckers then measure 62 x 55 µm and 99 x 95 µm respectively, the anterior margin of the ventral sucker being situated 103 µm behind the anterior extremity of the body. The intestinal caeca are filled with orange material, probably ingested host cellular debris. Although some development of the reproductive system

occurs (Fig. 8C) maturation of the vitellaria is insufficient to allow identification of species using adult criteria.

21 days after infection, tegumental contents include mitochondria, microtubules, pinocytotic vesicles, and vesicles 0.15 µm in average diameter with granular contents (Pl. 29B). Fibres are formed in the interstitial material separating the basement lamina and the outer circular muscle layer (Pl. 29B). At 28 days, large irregularly-shaped, electron-lucent vesicles occur in the tegument; cytoplasmic contents of the sub-tegumental cells include mitochondria, RER, free ribosomes, electron-lucent vesicles and large numbers of biconcave electron-dense secretory bodies (Pls. 28C, 29B).

28 days after infection, metacercariae have well-developed ecsomas (Fig. 8C,D) which when extended measure up to 230 µm in length. The ecsoma is formed initially by the progressive thickening of the wall of the excretory vesicle. By approximately 21 days after infection, the latter comprises circular and longitudinal muscle layers and a nucleated lining 10 µm in average thickness, whose free surface is closely covered with branched microvilli (Pls. 27A; 28A,B; 29A; 30). The dense cytoplasmic contents of the lining include mitochondria, RER, free ribosomes, Golgi and pinocytotic vesicles (Pl. 30). Folds of the basement membrane extend throughout the lining between the large, irregularly-shaped nuclei (Pl. 30); it was not possible to determine, however, whether the lining is an incomplete syncytium as described in the cercaria of Podocotyle staffordi by Gibson (1974).

The mechanism by which eversion of the metacercarial ecsoma is achieved is not known, but may involve contraction of the muscle layers in the excretory vesicle wall (Pl. 30). Splits are formed in the parenchyma between the latter and the general body wall (Pl. 28B).

Fig. 8A-E. Metacercariae of Cercaria vaullegeardi raised in Tigriopus brevicornis at 17°C.

Fig. 8A. 14 day-old metacercaria; single infection.

Fig. 8B. 14 day-old metacercaria; double infection. Growth has been reduced by competition for food and space within host haemocoel.

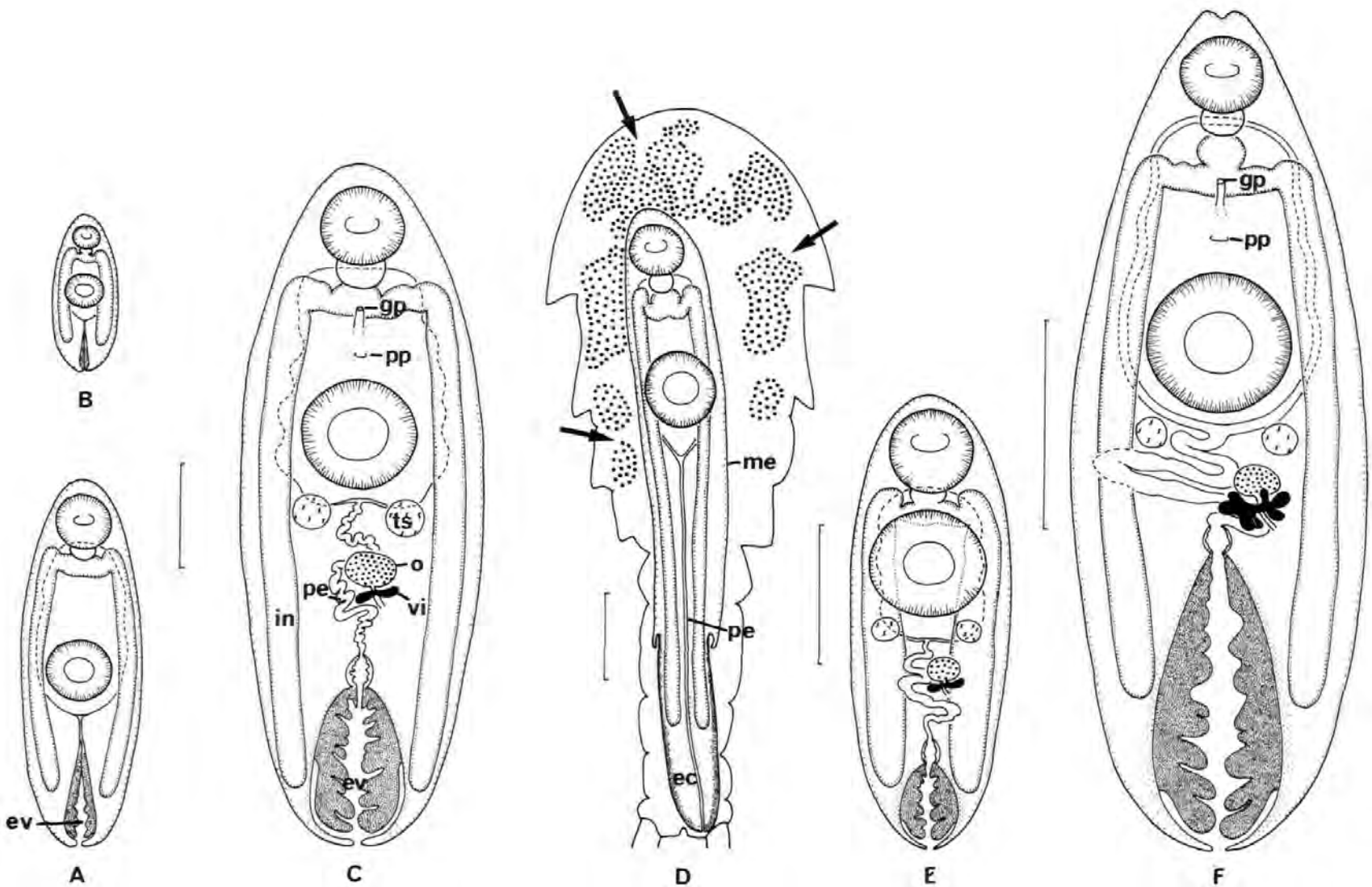
Fig. 8C. 21 day-old metacercaria, single infection.

Scale line represents 150 µm in A, B and C.

Fig. 8D. Camera lucida drawing of 21 day-old metacercaria with fully extended ecsoma, in situ within the haemocoel of a female T. brevicornis. The copepod appendages are not shown to increase clarity; note disruption of ovary and oviducts (arrowed). Scale line represents 0.1 mm.

Fig. 8E. Metacercaria of C. vaullegeardi removed from the body cavity of Gobius paganellus; experimental infection, 24 hours after ingestion of living T. brevicornis containing 21 day-old metacercariae. Scale line represents 0.1 mm.

Fig. 8F. Excapsulated hemiurid metacercaria from the body cavity of G. paganellus; natural infection. Scale line represents 0.5 mm.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21-28 days after infection, the excretory vesicle may be everted through the terminal pore, the microvillous lining then lying in contact with the host haemolymph (Fig. 8D; Pl. 28A). The terminal dilation of the unthickened posterior excretory vessel (Fig. 8C) lies at the posterior extremity of the extended ecsoma.

(ii) Multiple infections of T. brevicornis with metacercariae of C. vaullegeardi

Although T. brevicornis may be experimentally infected with up to seven metacercariae of C. vaullegeardi, maximum growth rate and development are reached when a single parasite only is present within an individual copepod, the metacercaria then virtually filling the haemocoel at 28 days at 17°C (Fig. 8D). In infections where 2 or 3 metacercariae occur within a single T. brevicornis, the growth rate and maximum size reached by each parasite is reduced more or less evenly. In multiple infections of 4-7 metacercariae, however, the latter do not all develop at an equal rate, growth being apparently determined by position within the body of the copepod. The parasite situated most posteriorly within the metasoma, or anteriorly within the prosoma, rounds up and dies approximately seven days after infection, whilst the remaining metacercariae continue to develop at an unequal rate.

(iii) The effect of metacercariae of C. vaullegeardi on T. brevicornis

T. brevicornis infected with 21-28 day-old metacercariae of C. vaullegeardi are inactive, swimming with difficulty only when disturbed. The copepods may become orange in colour; the oil sac and gonads are disrupted, and cellular debris occurs throughout the haemocoel. Fewer egg sacs, each of which contains a reduced number of

eggs, are produced by infected females; no egg sacs are formed by copepods harbouring more than three metacercariae.

b. Natural infections

(i) Natural infections of crustacea with hemiurid metacercariae

Of over 300 T. brevicornis collected from Station 11 during August of 1976 and 1977, only three were found to be naturally infected with hemiurid metacercariae. These were insufficiently developed to allow further identification.

In an examination of over 500 various unidentified copepods, and 200 Amphithöe rubricata and Gammarus spp. collected at Station 11 during August 1977, no hemiurid metacercariae could be demonstrated.

(ii) Natural infections of T. brevicornis with a parasitic yeast, Metschnikowia spp.

During the summers of 1976 and 1977, T. brevicornis cultures became infected with a parasitic yeast, probably by use of seawater contaminated with the spores. The yeast was identified as Metschnikowia spp. according to generic data given by Kreger-van Riz (1969) and by Miller & van Uden (1970). This parasite is of interest here as the number of copepods in infected cultures rapidly declined, adversely affecting material for hemiurid infection experiments. In addition, it was found that although copepods harbouring early infections of Metschnikowia could be hyperinfected with C. vaullegeardi, the metacercariae remained small and development was abnormal.

The fully-formed ascospores, each of which contains a single spore, occur throughout the haemocoel of the body and appendages;

they are slender and elongate, with bluntly-pointed ends, and measure 45 x 2 µm in average length and maximum breadth (Pl. 31). Single (3 x 3 - 7 x 2 µm) and 2-bud (11 x 4 µm) developing stages have been observed (Pl. 31A,B). Affected copepods become inactive, pinkish in colour, and die within approximately 14 days at 17°C.

5. Metacercarial development within the fish host

a. Experimental infections

Living T. brevicornis containing well-developed 21 day old metacercariae of C. vaullegeardi were fed to seven species of fish, listed in Tables 5 and 10. The fish were killed and examined one hour - 14 days after feeding. Only seven out of 32 Gobius paganellus became infected, metacercariae being recovered from the gut lumen and wall, and the body cavity (Table 10). The other six fish species remained uninfected.

Metacercariae removed from the body cavity of G. paganellus killed 24 hours after infection (Fig. 8E) measured 915 x 364 µm; oral and ventral suckers measured 104 x 120 µm and 156 x 156 µm respectively.

b. Natural infections

Hemiurid metacercariae were recorded in five out of 12 species of fish collected at Stations 1 and 3-13 between 1974 and 1981 (Table 11; Appendix). Infection of the fish host probably occurs during late summer and autumn at Station 11, newly-formed, thin-walled capsules being observed in Gobius paganellus collected at this time (Appendix).

(i) Capsule

The parasites were almost always found to be enclosed by a

Table 10. Results of attempts to infect fish by ingestion of living Tigriopus brevicornis harbouring 21-day old metacercariae of Cercaria vaullegeardi

Experimental host species	Body length (cm)	Number of fish used	Number of metacercariae ingested/fish	Method of examination used	Time after ingestion of copepods	Fish number:-	Results		
							Number of metacercariae recovered		
							Gut lumen	Body cavity	Elsewhere
Gobius paganellus (post-larval)	2-2.5	32	4-10	whole fish squashed between 2 glass slides	1 hour	1	2 (stomach)	0	0
					"	2	1 (")	1	1 (rectal wall)
					2 hours	3	2 (")	0	1 (stomach ")
					24 "	4	0	2	1 (" ")
					24 "	5	0	0	1 (intestinal wall)
					48 "	6	1 (intestine)	0	0
					3 days	7	1 (")	1	0
					7 "	8-20	0	0	0
					14 "	21-32	0	0	0
Blennius pholis (post-larval)	2-2.5	7	12-20	"	3 days		0	0	0
Taurulus bubalis	3.3-3.5	4	10-15	"	7 "		0	0	0
Crenilabrus melops	5-7	2	12-20	Complete fish examined by squashing in sections	7 "		0	0	0
Pomatoschistus microps	3-5	5	20+	"	7-14 "		0	0	0
Ciliata mustela	2.9-3.5	5	10-20	"	7-14 "		0	0	0
Scophthalmus maximus	3	1	20+	Complete fish examined by squashing skinned sections	7 "		0	0	0

Table 11. Results of postmortems on fish examined for hemiurid parasites 1974-1981

Fish species	Number examined	Number of fish infected with encapsulated hemiurid metacercariae	Number of fish infected with adult hemiurids
Blennius pholis	138	22	4
Ciliata mustela	56	0	33
Conger conger	3	0	3
Crenilabrus melops	6	5	0
Gaidropsarus mediterraneus	8	6	2
Gobius niger	1	0	0
G. paganellus	139	14	0
Lophius piscatorius	14	0	3
Pholis gunnellus	2	0	0
Pomatoschistus microps	113	0	0
Scophthalmus maximus	26	0	1
Taurulus bubalis	23	14	5

fibrous capsule attached to the mesenteric membranes of liver, stomach, intestine and rectum within the hosts' body cavity (Pls. 32, 33A). Histological study of the cyst wall at light and ultrastructural levels (Pls. 33A, 34) shows it to consist largely of flattened nucleated cells with cisternae, RER, mitochondria, lipid droplets, and desmosomes (Pl. 34). The capsule wall is irregularly thickened, especially at the anterior end around the oral sucker of the uncoiled metacercaria; cellular debris and macrophages occur at the inner face of the capsule. A cyst of parasitic origin has not been observed at any stage.

The degree of capsule pigmentation varies with the age of the infection and the host species. In Gobius paganellus the capsules, which measure 1.2 x 0.6 mm in average length and maximum breadth, usually remain translucent white or pale yellow in colour until the death of the parasite, whilst in Taurulus bubalis (Pl. 32) yellow patches accumulate within the fibrous layer of the wall. In Blennius pholis, however, the capsules rapidly become heavily melanised, darkening from light to dark brown during spring and summer months (Appendix).

(ii) Metacercaria

The metacercaria (Fig. 8F) removed from G. paganellus collected at Station 11 measures 1.6 x 0.8 mm in average length and maximum breadth. The oral and ventral suckers measure 138 x 170 µm and 243 x 263 µm respectively, the posterior margin of the latter being situated 680 µm from the posterior end of the body. The oral lobe is usually rounded, but may be square or even slightly bi-lobed when fully extended in living specimens. The intestinal caeca are filled with cellular debris, the parasite ingesting material removed from the inner surface of the

capsule. A distinct presomatic pit is present on the ventral surface between the genital pore and the anterior margin of the ventral sucker (Fig. 8F). The ecsoma is not extended either within the capsule or after excapsulation into Y.T.S. The reproductive system is well-developed, the vitellaria comprising seven short, rounded lobes (Fig. 8F), but the metacercariae do not become progenetic in this host. Parasites removed from other species of rockpool fish collected at Station 11 appear morphologically similar to those from G. paganellus, both in body and sucker dimensions and in the shape of the vitellaria. Metacercariae in B. pholis, however, usually become progenetic, with oval, greenish-yellow egg capsules; each egg bears a posterior knob, and measures 18 x 10.4 µm.

Ultrastructural study of excapsulated metacercariae from G. paganellus and B. pholis shows the tegument covering the soma and the ecsoma (Pl. 33B,C,D) to be closely similar to that of the adult hemiurids described below.

6. Adults

Adults of five species of Hemiuroidea, namely Lecithochirium rufoviride, L. fusiforme, L. furcolabiata, Hemiurus communis and Lecithaster gibbosus have been recorded from seven out of 12 species of fish collected between 1974 and 1980 (Tables 4, 11; Appendix). The morphology of these parasites is already well-documented at the light microscope level. Ultrastructural aspects of the adult hemiuroid tegument, parenchyma and oral lobe, and the gross pathological effects of high levels of infection of Ciliata mustela with L. furcolabiata have not, however, been previously described.

a. Lecithochirium furcolabiata (syn. Ceratotrema furcolabiata Jones, 1933)

L. furcolabiata is recorded from the body cavity of 84% of 32 Ciliata mustela collected at Station 11, less commonly at Station 12. The parasite is predominantly found upon the surface of the ventral lobe of the liver, where it feeds on hepatic cells and blood (Pl. 38), but may also occur on the intestinal caeca and adjacent body wall in heavily-infected fish (30-100 parasites/host). In these cases the liver is deeply eroded, with haemorrhages and brown or black deposits; the peritoneal membranes between the intestinal caeca are fibritic and brown, and adhesions occur commonly between the organs. The greenish-yellow egg capsules, each of which has a posterior knob (Pl. 37G) and measures approximately 21 x 13 µm, have been recorded from the gall bladder and rectal contents probably entering the bile ducts at the lesions on the eroded liver surface.

b. Ultrastructure

(i) Tegument

The somatic tegument in Hemiurus communis, Lecithochirium fusiforme, L. furcolabiata and Lecithaster gibbosus resembles that of other digenea in that it consists of an anucleate syncytium whose dense matrix contains mitochondria and biconcave electron-dense secretory bodies. The electron-dense peripheral layer, 0.15 µm in average thickness, which occurs in H. communis (Pl. 37A) and L. fusiforme (Pl. 35A) is thin or absent in L. furcolabiata (Pl. 37D) and L. gibbosus (Pl. 37C). Although nucleated tegumental cell bodies, whose cytoplasm is filled with biconcave electron-dense secretory

vesicles, Golgi and RER, occur frequently in the parenchyma, cytoplasmic bridges connecting these to the outer syncytium have not here been observed. As described in H. communis by Kryvi (1972), the basement membrane is folded into the tegument in L. fusiforme (Pl. 35A), L. furcolabiata (Pl. 37D) and L. gibbosus. Fibrous, desmosome-like structures connect the tegumental basement membrane to the muscle layers (Pl. 37C).

The ecsomatic surface in H. communis (Pl. 37A) and L. fusiforme (Pl. 35B,C) is papillate or deeply folded, that in L. furcolabiata being ridged (Pl. 39C). In all three species the ecsomatic tegument differs from that covering the soma in that there is no dense peripheral layer and the bounding membrane bears a prominent glycocalyx; no mitochondria have been observed (Pls. 35B, 37A) although clusters of these organelles occur in pseudopodial extensions of the parenchyma cells which pass into the fibrous interstitial material between the muscle fibres (Pl. 37A).

(ii) Parenchyma

The ultrastructure of the parenchyma cells in H. communis, L. fusiforme, L. furcolabiata and L. gibbosus basically resembles that described in Fasciola hepatica by Threadgold and Gallagher (1966) and Gallagher and Threadgold (1967), each cell having a rounded nucleus and cytoplasm divided into three zones including a narrow perinuclear area with RER and electron-lucent vesicles, a granular central zone with glycogen and mitochondria, and a clear peripheral area (Pl. 40A,B). As in F. hepatica the spherical, oval or elongated mitochondria are concentrated in large numbers in small areas of the cell, especially in the ecsoma, where dense patches of oval mitochondria occur around the posterior excretory vessel

(Pl. 37B). In the adult hemiurid, however, the bounding membrane of each parenchyma cell is infolded into narrow channels, 0.2 µm in average length, thereby increasing the surface area in contact with the densely fibrous interstitial material (Pl. 36C,D). Desmosomes (Pl. 36C) occur at irregular intervals connecting the adjacent boundaries of the pseudopodial extensions of the parenchyma cells.

(iii) Oral lobe

The oral lobe in Lecithochirium rufoviride is generally rounded or square, although specimens with bilobed lips with knob-like protuberances have been recorded from the stomach of both Conger conger and Anguilla anguilla (Gibson & Bray; personal communication). These knobs have been considered to be a diagnostic feature of L. furcolabiata (Pl. 39A) and occur in all specimens, both gravid and immature, examined in this study. The function of the oral lobe in L. furcolabiata may be sensory; in living worms, the lobe and knobs are fully extended and applied to the host liver surface as the parasite feeds (Pl. 38). Scanning micrographs show the surface of the tegument covering the knobs to bear a reticulate pattern of grooves separating cushion-like areas. Circular pores occur at intervals (Pl. 39B); the latter have not, however, been located in sections, and may be a preparation artefact. The lobe is not especially muscular, although slender, oblique muscle fibres have been observed. Pseudopodial extensions of the parenchyma cells and neurons (Pl. 41C) extend outwards through the basement lamina to the tegumental basement membrane. Several large cells, distinct from the surrounding parenchyma, occur within the oral lobe. These are characterised by the deeply-divided cytoplasm, radiating processes arising close to the perimeter of the large

rounded nucleus with prominent nucleolus (Pl. 41A,B). Vacuoles, 0.05 µm in average diameter with electron-lucent contents, Golgi and elongated mitochondria occur in patches within their cytoplasm.

7. Nutrient uptake

a. Electron-dense tracers

Ruthenium red and ferritin were not taken up by either the somatic tegument or the lining of the excretory vesicle in the metacercaria of C. vaullegeardi.

b. Autoradiography

The results of autoradiographic experiments at light microscope level, in which hemiurid metacercariae and adults were incubated with tritiated tyrosine or glucose, are shown in Table 12. In the metacercaria of C. vaullegeardi from the haemocoel of T. brevicornis and in the adult ecsoma, tyrosine was taken up by the lining of the excretory vesicle (Pl. 42A) and of the posterior excretory duct (Pl. 42C,D) respectively. Smaller amounts occurred within the sub-tegumental muscles and parenchyma; uptake within the adult soma was negligible. Tyrosine uptake was not affected by pH in the present study. Slight uptake of labelled glucose was observed in the intestinal caeca of metacercariae from both copepod (Pl. 42B) and fish hosts. ^{3}H-tyrosine was taken up in significant amounts by the tegument of the metacercariae of Bucephalus haimeanus; all other controls were negative. Neither ^{3}H-methionine nor ^{3}H-alanine were taken up by the somatic or ecsomatic teguments, or the intestinal caeca, of Lecithochirium furcolabiata.

Table 12. Autoradiography : uptake of ^{3}H-glucose and ^{3}H-tyrosine by hemiurid metacercariae and adults

	Incubation time (minutes)	Metacercariae of *C. vaullegeardi* ex copepod haemocoel	Excapsulated metacercariae ex fish body cavity		Adult hemiurids ex fish stomach	
			soma	ecsoma	soma	ecsoma
^{3}H-glucose (100 µCi/ml)	2	-	-	+ intestinal caeca	-	-
	5	+ intestinal caeca	-	-	-	-
	15	"	-	-	-	-
	30	"	-	-	-	-
^{3}H-tyrosine (100 µCi/ml)	2	-	-	-	-	-
	5	+++ lining of excretory vesicle ++ parenchyma + intestinal caeca	-	-	-	-
	15	"	-	-	+ oral and ventral suckers (pH 2, 5 & 8)	+++ parenchyma around posterior excretory vessel ++ parenchyma (pH 2 & 5)
	30	"	-	-	not done	not done

-, no uptake; +, slight uptake; ++, average uptake; +++, strong uptake, as indicated by comparative density of silver grain over sections.

DISCUSSION

1. Germinal sacs and cercarial emergence

Although many workers (Gaillard, 1953; Chabaud & Campana-Rouget, 1958; Arvy, 1963; James, 1973; Popiel, 1976a,b) have described the daughter sporocyst of C. vaullegeardi from the digestive gland of G. umbilicalis, until now the anterior region of the sporocyst, extending via the right kidney into the host gill, has not been recorded. The technique generally employed for the removal of larval digeneans for examination involves shell removal and the subsequent teasing apart of the visceral mass of the infected mollusc. The fragile sporocyst of C. vaullegeardi is broken by this method, and only its posterior region containing germ cells and developing cercariae is removed. The present investigation shows that dissection and the cutting of serial sections of the entire infected mollusc are essential for the study of filamentous sporocysts.

The anterior region of the sporocyst of C. vaullegeardi is here shown to extend through blood vessels connecting the haemocoels of the host digestive gland, right kidney and gill. The sporocyst is structurally adapted for active penetration of the host gill tissue, with well-developed musculature and nervous system at the anterior end. During periods of cercarial release, the anterior extremity of the sporocyst actively penetrates the gill epithelium using muscular thrusting actions probably assisted by lytic secretions from glands around the birth canal. The specialized anterior sporocyst region therefore serves to transport mature cercariae from the deep-seated digestive gland to the exterior. The cystophorous cercariae emerge from the terminal birth pore directly into the host mantle cavity,

where they 'encyst' prior to passing out into the sea with the exhalant water current. The specialization of the sporocyst in C. vaullegeardi therefore provides a means whereby the cercariae, hindered by a bulky, immotile cystophorous tail, does not itself have to migrate through the molluscan host tissues at any stage.

Results indicate that in C. calliostomae the rediae migrate via the afferent renal vein from the haemocoel of the host gonad to that of the right kidney. The muscular pharynx, probably aided by secretions of the pharyngeal glands, may then be used to penetrate the tubules, allowing cercariae to emerge through the terminal birth pore directly to the exterior through the kidney sac and communicating mantle cavity. The ability of rediae to migrate is well-documented (Najarian, 1953; Dinnik & Dinnik, 1957; Erasmus, 1972) although records have so far described movement from the mother sporocyst towards the digestive gland or gonad, where development occurs. The involvement of rediae in the transport of cercariae to the site of emergence from the host does not appear to have been previously described. It is of interest that despite their obvious ability to break down host tissues and pursue a more direct route, the daughter sporocyst and the redia of C. vaullegeardi and C. calliostomae respectively utilise the molluscan circulatory system in order to gain access to the exterior, as do the freed cercariae of more motile forms (Duke, 1952; Pearson, 1961; Probert & Erasmus, 1965). There is no evidence to suggest that these cystophorous cercariae escape passively via the gut or genital ducts, although considerable damage to the digestive gland and gonad occurs.

A similarly direct method of cercarial emergence has been described in Bucephaloides gracilescens by Matthews (1974). In this

cercaria, as in C. vaullegeardi and C. calliostomae, the bulky tail complex would impede migration through host tissues from the site of development to the exterior. The branched filamentous sporocyst is not, however, differentiated into specialized regions as in C. vaullegeardi, and cercarial release is by rupture of its wall into the exhalant chamber of the bivalve host, Abra alba. In species where the cercariae are immotile and tail-less, the sporocyst itself may migrate to the exterior, as described by Dobrovolny (1939) in Plagioporus sinitsini. Kagan (1952) describes the division of the sporocyst of Neoleucochloridium problematicum into functionally distinct portions, mature cercariae collecting within conspicuous brood pouches in the tentacles of the snail host. In both P. sinitsini and N. problematicum, infection of the final host is by ingestion of metacercariae retained within all or part of the sporocyst respectively. The involvement of a functionally distinct region of the sporocyst in the transport of cercariae to the exterior, as in C. vaullegeardi, does not appear to have been previously described.

The birth canal of the daughter sporocyst of C. vaullegeardi is shown here to consist of three regions. The most anterior of these, surrounding the birth pore, is lined by anucleate, microvillous syncytial tegument identical to that covering the general body surface. The middle canal region is highly innervated, muscular and surrounded by secretory cells, and the posterior region is characterized by a nucleated tegumental lining. This structure is essentially similar to that considered by Imohiosen (1969), James (1973), Popiel (1976a), and Popiel & James (1978) to be characteristic of the mouth, pharynx and caecum of the embryonic gut in rediae of various marine digeneans.

Comparative ultrastructural studies by these workers on the birth pore and embryonic gut in sporocysts and rediae have led them to suggest that the daughter sporocyst is a paedogenetic redia and that its birth canal is homologous to the embryonic redial gut, the least retarded species retaining areas equivalent to mouth, pharynx and caecum (Popiel & James, 1978). The structure of the birth canal of the daughter sporocyst of C. vaullegeardi as described here indicates that in this species the anterior, middle and posterior canal regions may also be considered to be homologous to the mouth, pharynx and caecum, respectively, of an embryonic redial gut. All the cercariae, with the exception of C. vaullegeardi and Cercaria 'A' Miller, 1925, of hemiurids so far investigated develop within rediae. The sporocyst of C. vaullegeardi may then be regarded as corresponding with the rediae of other hemiurids, and such correspondence would be consistent with the views of Popiel & James (1978) on the origin of daughter sporocysts from rediae.

In C. vaullegeardi germinal cells have here been observed within cavities in the cytoplasm of the sporocyst sub-tegumental cells, being liberated into the lumen before further development. The significance of the column of cells described within an enlarged cytoplasmic cavity (Pl. 9) is not known. Serial sections of the posterior ends of several daughter sporocysts failed to locate other such structures, and it is possible that the column could represent the abortive remains of a cercaria retained within the sporocyst wall; this hypothesis is supported by the fibrous nature of the folds surrounding the proximal end and the periphery of the column, suggestive of the developing cercarial delivery tube. The occurrence of germinal balls within the sub-tegumental cytoplasm has been previously described in

the redia of Tubulovesicula pinguis by Stunkard (1980) as "clusters of deeply-staining cells embedded in, or attached to, the parenchymal layer of the body wall, or free in the body cavity", and in that of C. calliostomae by Dollfus (1950). A similar situation occurs in the daughter sporocyst of Cercaria bucephalopsis haimeana, where germinal cells migrate through the sub-tegument to become enclosed by folds of one or several sub-tegumental cells in a germinal cyst (James & Bowers, 1967). The enclosure of germinal cells within vacuoles in the glycogen and lipid rich sub-tegumental cell cytoplasm in C. vaullegeardi may serve a nutritive function. It is possible that the sporocyst cytoplasm may contribute directly to the nucleated primitive epithelium of early cercarial stages in C. vaullegeardi and other hemiurids (Rothschild, 1938), as described by Meuleman & Holzmann (1975) in Schistosoma mansoni.

2. Cercariae

The cystophorous tail in both C. vaullegeardi and C. calliostomae, as in all other hemiurid cercariae so far described, consists essentially of a caudal cyst and a delivery tube. A laminated fibrous structure as seen here in the caudal cyst wall has not been previously described in any other digenean, but a similar arrangement of curved fibres has been reported from such diverse examples throughout the animal kingdom as teleost eggs, crustacean cuticle and peridinean chromosomes (Bouligand, 1965a,b, 1972; Dalingwater, 1975) where strength and flexibility are of prime importance. The delivery tube contains similar fibres, laid down around the periphery of a single column of cells in the appendage rudiment, which subsequently degenerate to form the tube lumen. The fibrous structure of the developing

caudal cyst and delivery tube in C. vaullegeardi were noted by Popiel (1976b); these studies were of immature forms, however, and did not detect the cyst cavity or the lumen of the tube and its function in the delivery of the cercarial body.

The terminal nuclei and the cluster of small, rounded cells at the tip of the developing delivery tube were described in C. calliostomae by Dollfus (1950) and in C. sinitzini by Rothschild (1938). In the present study of C. vaullegeardi, accumulations of electron-dense material were noted within the endpiece peripheral to the terminal cells, and it is of interest that acetylcholinesterase was detected in this region at the light microscope level, in addition to its expected location within cerebral ganglia and longitudinal nerves of the cercarial body. Although neurons have been observed within the tail in connection with the sphincter muscle, they have not been noted in stages studied here within the delivery tube, and their presence in this position seems most unlikely in view of the development of this appendage from a single column of fibre-forming cells and its acellular nature when mature.

At retraction of the delivery tube into the caudal cyst, considerable shortening of the appendage occurs, associated with folding of the fibrous layer within the tegument. Popiel (1976b) also recorded the folded fibrous layer, the absence of muscle fibres in the delivery tube leading her to the opinion that no active contractile process could be involved. Rothschild (1938) similarly noted folding within the delivery tube of C. sinitzini, and suggested a passive withdrawal due to pressure changes set up by the enlargement of the internal cyst cavities. The absence of muscle fibres need not necessarily, however, rule out an active process, as contraction could be a function of

the fibrous layer. The occurrence of contractile properties in many types of non-muscle cell is well documented (Allison, Davies & dePetris, 1971; Bettex-Galland & Hughes, 1973; Bray, 1973; Pollard & Korn, 1973; Schliwa & Bereiter-Hahn, 1975; Saleuddin & Jones, 1976).

Of interest is the dissemination of membrane-bound secretory products from the caudal gland to the tegument of caudal cyst and filaments, and the subsequent persistent indentations at the point of discharge on the surface in C. vaullegeardi. Popiel (1976b) noted electron-lucent vesicles within the caudal tegument and suggested that they might increase buoyancy in the external environment. Studies here show that the contents of the vesicles are discharged prior to emergence from the sporocyst, and although increased surface area resulting from the indentations might also have been considered to increase buoyancy, naturally emerged encysted cercariae rarely float but lie on the bottom. In view of the need to be selected as food by the next intermediate host it might be supposed that gustatory or adhesive properties might be attributed to the secretions of the caudal gland. Following discharge of the vesicles the remaining indentations would assist by affording grip for the copepod appendages, as the cercariae have to be manipulated by the latter during feeding.

The excretory appendage of C. vaullegeardi develops from the median posterior lobe of the embryonic tail which carries the fused, caudal excretory ducts to the exterior, and may therefore on grounds of comparative morphology be considered homologous to the locomotory tail of non-hemiurid cercariae. The term "excretory appendage" was first applied to this structure by Pelseneer (1906) who described diagrammatically its development and regression relative to the rest of the tail. Observations here have shown that the appendage retains

a limited capacity for movement in early stages, undergoing slow sustained contractions, but regresses to a vestigial condition in the fully-formed cercaria. In C. calliostomae the median lobe of the embryonic tail does not extend at any stage, although by differential growth the caudal excretory ducts become curved to open in an anterior position. Comparative study of the early developmental stages of other cystophorous cercariae indicates an evolutionary trend in Hemiuridae towards loss of caudal locomotory function. In the encysted cercaria of Derogenes varicus, the forked excretory appendage is used for active swimming movements (Pelseneer, 1906; Køie, 1979). In the cercaria of Dichadena acuta, the excretory appendage elongates distal to the excretory pores, becoming a tangled, non-contractile thread (Cable & Nahhas, 1963). The excretory appendage of C. vaullegeardi is here considered to be homologous to Appendage II of C. sinitzini as described by Rothschild (1938). Both appendages undergo total regression, the encysted cercaria being immotile in both species. C. calliostomae would seem to represent a final stage in the series, with complete suppression of the median caudal lobe.

Although in C. calliostomae the four caudal flame cells and their ducts are retained in the fully-formed tail, in C. vaullegeardi the excretory system is less extensive in mature than in developing stages. This is as might be expected in view of the more complete breakdown of cellular elements throughout the tail of the latter species. No data are available concerning the caudal excretory system in other hemiurid cercariae, but results here indicate that flame cell formulae (La Rue, 1957) may be misleading in this group of Digenea unless applied to both mature and developing stages.

3. Infection of the second intermediate host

Observations have shown that in C. vaullegeardi the function of the cystophorous tail is two-fold, serving for the protection of the cercarial body against damage by copepod mouthparts, and for its inoculation into the host haemocoel during the initial stages of feeding, before ingestion can occur.

As far as could be seen by direct observation, the feeding behaviour of T. brevicornis closely resembles that of the harpacticoid Tisbe furcata Baird, 1837, the raptorial feeding mechanism of which has been described in detail by Marcotte (1977) using slow-motion videotape. Results here indicate that both host food-particle orientation and mandibular action are vital to the infection mechanism of C. vaullegeardi. The shape and construction of the caudal cyst ensure that the unstrengthened tip of the beak is sheared by the cutting edges of the copepod mandibles, triggering delivery tube eversion and therefore allowing infection to occur without ingestion of, and damage to, the cercarial body. That the process is not random is emphasized by the 100% infection rate achieved when T. brevicornis were exposed to cercariae within 3 h of their emergence. Results here indicate that in C. vaullegeardi pressure applied by mouthparts does not trigger delivery tube eversion, as shown by in vitro excystation in C. calliostomae and described by Køie (1979) in the cercaria of D. varicus; in the latter species, however, a less precise mechanism might account for its wide distribution in both demersal and pelagic fishes.

The delivery tube of C. vaullegeardi is shown to have a thick-walled proximal section which penetrates the copepod midgut wall, allowing eversion of the thinner-walled distal section into the haemocoel. It is

of interest that lateral projections have been recorded in the delivery tube of Lecithaster confusus by Hunninen & Cable (1943), in an unnamed cystophorous cercaria by Chabaud & Biguet (1954) and in Derogenes varicus by Køie (1979), corresponding in position to the end of the proximal section of the appendage described here in C. vaullegeardi. Hunninen & Cable (1943) suggested that the projections may hold the delivery tube in position in the gut wall until the cercarial body has passed through into the haemocoel. Infection experiments described here have shown a degree of specificity to the second intermediate host in C. vaullegeardi; this may be attributed to both the relative sizes of caudal cyst and host mouthparts, and the length of the proximal delivery tube and host oesophagus. The relevance of the respective lengths of delivery tube and host oesophagus were discussed by Krull (1935), who recorded that when fully mature Cyclops fed upon the cercariae of Halipegus occidualis undischarged cercariae only were observed within the gut, no metacercariae reaching the haemocoel. Copepodids of the same species, however, and adults of a smaller species became infected. Krull (1935) suggested that the longer appendages of the larger Cyclops prevented the delivery tube from reaching the gut wall when discharged. Not all cystophorous cercariae appear to show the same degree of specificity to copepods, unidentified metacercariae having been reported from a wide variety of organisms including ostracods, barnacles, ctenophores and chaetognaths (Macy & DeMott, 1957; Stunkard, 1973; Combes & Kechemir, 1978; Køie, 1979).

On delivery tube eversion in C. vaullegeardi the end-piece everts last into the host haemocoel, and can therefore have no penetrating or holding function as suggested for H. occidualis by Krull (1935). The significance of the acetylcholinesterase detected in the

end-piece is not known. The association of this enzyme with non-nervous tissue is well documented, being recorded in mammalian erythrocytes (Froede & Wilson, 1971) and the anterior glands of parasitic nematodes (Lee, 1970; Ogilvie, Rothwell, Bremner, Schnitzerling, Nolan & Keith, 1973; McLaren, Burt & Ogilvie, 1974; Burt & Ogilvie, 1975). Lee (1970) suggested that a possible function of the enzyme in Nippostrongylus brasiliensis may be to cause a localized temporary suppression of host peristalsis and therefore help the nematode to maintain its position within the gut lumen. It is tempting to speculate that the enzyme within the end-piece of C. vaullegeardi may serve a similar function during the infection process, ensuring a momentary paralysis of host muscles and thereby aiding introduction of the cercarial body into the haemocoel. In support of this hypothesis, Krull (1935) reported that when Cyclops spp. eat the cercariae of H. occidualis they frequently lie motionless, with appendages widely separated for as long as a minute before recovery.

In C. vaullegeardi, passage of the cercarial body through the delivery tube was associated with the release of vesicles from the tegument, the contents of which are assumed to have a lubricatory function. The relatively undifferentiated condition of the loosely-packed parenchyma cells and ventral sucker in both C. vaullegeardi and C. calliostomae may be significant in enabling the cercariae to assume the elongated shape necessary for inoculation. The temporarily assumed slender shape is considered to be the result of muscular action; it is of interest that Wilson, Draskau, Miller & Lawson (1978) described a similar situation in Schistosoma mansoni, in which the schistosomules assume an elongated shape prior to capillary migration within the lungs.

The forcible ejection of the delivery tube and cercarial body following triggering of the infection mechanism is suggestive of a pressure mechanism which might be explained in one of two ways. Ejection in C. vaullegeardi was associated with the simultaneous enlargement of the caudal cyst cavity, and it may be that the contained material becomes instantly hydrated on entry of water at cyst rupture, exerting the necessary pressure. Køie (1979) reported that in D. varicus the 'large, hyaline dead cells which form the wall of the caudal vesicle swell and fill the lumen' following in vitro delivery tube eversion. Alternatively, the necessary pressure may be mechanically generated on retraction of the cercarial body through the well-developed and closely applied tubular caudal sphincter muscle, the latter being tightly closed at completion of encystment when a significant increase in caudal cyst size occurred.

Successful infection of an experimental second intermediate host was not achieved in C. calliostomae, a single metacercaria developing in only one out of 78 copepods exposed. Coverslip pressure caused the in vitro eversal of the delivery tube, however, suggesting a similar infection mechanism to that described in C. vaullegeardi. It is possible that the attention of a potential host is drawn first to the fleshy, sticky caudal ribbon, attached to the posterior end of the narrow tubular section of the caudal cyst; the latter may then direct the emerging delivery tube into the host oesophagus.

4. Metacercaria

The present study corroborates the suggestion made by Pratt (1898) that the hemiurid ecsoma is derived from the metacercarial excretory vesicle. Study of the metacercaria of C. vaullegeardi

within the haemocoel of T. brevicornis has shown that the excretory vesicle wall thickens and develops a microvillous lining. By approximately three weeks after infection, the vesicle is evaginated posteriorly through the excretory pore for extended periods, during which time the microvilli lie bathed by host haemolymph. Both ultrastructural and autoradiographic studies indicate that nutrient uptake is a function of the excretory vesicle, or developing ecsoma, at this stage in the life cycle. Whilst the amino acid tyrosine was absorbed here, and to a lesser extent by the general somatic tegument, glucose was only taken up within the gut. Erasmus (1972), in summarising the possible functions of the digenean excretory system, commented that these are probably more varied than has been previously supposed. Some evidence of selective reabsorption of substances from the fluid within cercarial excretory tubules has been put forward by Cardell (1962) in Himasthla quissetus, and by Krupa et al. (1969) in Podocotyle staffordi, and the translocation of dissolved nutrients may be a function of the strigeid reserve bladder system (Erasmus, 1972). The wall of the cercarial excretory vesicle in C. lingua shows ultrastructural characteristics of protein-synthesising cells (Krupa et al., 1969) and the metacercarial excretory vesicle in Stictodora lari synthesises the final parasitic contribution to the cyst wall (Leong & Howell, 1971). The latter workers suggested that the necessary metabolites may enter the excretory vesicle via the collecting ducts, and that the synthesised protein is passed out through the excretory pore. In the present instance, the eversion of the excretory vesicle in the metacercaria of C. vaullegeardi allows the uptake of amino acid directly from the host haemolymph. That the digenean excretory vesicle may assume a

nutritional role does not appear to have been previously discussed.

It is of interest that Ching (1960) described villus-like extensions of the excretory vesicle which protruded through the terminal pore in the metacercaria of Cercaria A Miller, 1925, as did Thomas (1939) in Halipegus eccentricus. Neither worker, however, recorded complete vesicular evagination. A combination of morphological factors led Ching (1960) to postulate that Cercaria A may be a species of Lecithaster; both Lecithasteridae and Halipegidae are partly characterised by the lack of an ecsoma. In Tubulovesicula pinguis, however, Stunkard (1980) described the metacercariae within Acartia tonsa as having well-developed ecsomas, shown extended in a figure, but did not comment on their origin and development. It is possible that during development within the copepod host the excretory vesicle assumes an absorptive function in many hemiuroids, this being carried further in ecsomate forms by vesicular evagination and subsequent growth of the organ and surrounding tissues into a protrusible "appendix". Results here show that the tegument characteristic of the adult ecsoma first appears in metacercarial forms removed from the body cavity of rockpool fish. Although biconcave electron-dense secretory bodies appear in the sub-tegumental cells approximately 21 days after infection in C. vaullegeardi, they do not pass to the surface of either the soma or the developing ecsoma, and replacement of the microvillous excretory vesicle lining may only be initiated after release from the copepod host within the fish stomach or during migration through the fish gut wall.

5. The adult

One of the most characteristic features of the Hemiuridae, the

ecsoma, has received surprisingly little attention in the past, although Gibson & Bray (1979) have commented that this feature is associated with the unusual habitat of this group, within the pyloric stomach of the host. The present study indicates that the tegument covering the soma and the ecsoma is fundamentally similar, but differs in that the outer surface of the former has a thin, electron-dense layer, a feature which was not evident in Lecithaster gibbosus or Lecithochirium furcolabiata, non-stomach dwelling forms. Although it is tempting to speculate that the protrusible ecsoma serves as an absorptive organ, being extended during feeding, there is no evidence from ultrastructural studies or from autoradiographic experiments for the active uptake of glucose or tyrosine through the tegument. The absence of mitochondria in the ecsomatic tegument would support this view. That the ecsoma is selectively more permeable than the soma was indicated by the rapid uptake of neutral red in aqueous solution. Of particular note was the uptake of tyrosine in whole, living worms by the lining of the posterior excretory duct, which extends throughout the length of the protruded ecsoma. It would appear therefore that in the adult, as in the copepod-metacercarial stage, the terminal section of the excretory system has assumed the function of amino acid absorption. The mechanism by which nutrients enter the excretory pore is not known, but could possibly be associated with the pumping action created by ecsomal protrusion and retraction.

In the pyloric stomach, hemiurids are exposed to strong acid proteolytic digestion, at pH 1-2. As far as the writer is aware, there is no other group of parasites which has exploited a habitat within the true stomach of its host. Although digeneans in other parts of the digestive tract have developed mechanisms for evading

destruction by host enzymes, the mucopolysaccharides secreted as a glycocalyx possibly serving a protective role (Bogitsh & Aldridge, 1967; Erasmus, 1967, 1972; Halton & Dermott, 1967), it might be expected that in view of the extreme conditions within the pyloric stomach this group might have developed structural and functional differences. The dense, fibrous, thick tegument covering the soma, and the absence of organellae normally associated with active uptake, suggest that the primary function of this layer is protection. Gibson & Bray (1979) have suggested that the transverse tegumental ridges, or plicae, which occur in ecsomate hemiurids may allow thickening of the somatic tegument during periods of low pH or high osmolarity, when the ecsoma is retracted. That the ecsoma might play a more active role is suggested by the increased permeability of its tegument, its papillate surface protected at extension by a glycocalyx, and its folded basement membrane, together with the accumulation of the amino acid tyrosine within the posterior excretory duct.

6. Life-cycle

Results suggest that in C. vaullegeardi the life-cycle may involve four hosts, including G. umbilicalis, a copepod, and at least two fish. That a period of development within a copepod host is essential is supported by the present investigation, cercariae being uninfective to fish whereas overall growth and ecsomal differentiation occurred in experimental infections of T. brevicornis. In natural infections of rockpool fish examined here, hemiurid metacercariae occurred within the body cavity, each generally enclosed in a host capsule. It follows therefore that upon release from the ingested

copepod within the fish digestive tract, the metacercaria must penetrate the gut wall. A low success rate was achieved in attempts to infect post-larval rockpool fish by feeding T. brevicornis harbouring 21-day old metacercariae of C. vaullegeardi; it is probable that the degree of development reached within this host is insufficient to ensure infectivity to the third intermediate host. It is considered unlikely that host specificity is involved in this respect as on the basis of comparative morphology, hemiurid metacercariae recorded here were found in five species of rockpool fish. According to Hunninen & Cable (1943) and Køie (1979), the hemiuroids Lecithaster confusus and Derogenes varicus develop directly within the gut of the fish which ingests copepods containing the metacercariae. Unlike hemiurids, neither of these parasites matures in the host pyloric stomach, the former being recorded from the intestine, and the latter from the cardiac stomach or oesophagus (Gibson & Bray, 1979; R.A. Matthews, personal communication). In D. varicus several fish may be involved in the life-cycle as paratenic hosts, the first of these being a species in whose diet copepods are included, the others being predatory (Køie, 1979). The occurrence of encapsulated metacercariae in the life cycle of ecsomate hemiurids may have a similar significance, allowing the parasite to fully exploit the food chain of which its various hosts form a part. That the metacercaria, feeding upon material ingested from the inner surface of the host capsule, may survive up to 12 months must further increase the likelihood of ingestion of the rockpool fish by the final host. The identity of the latter in C. vaullegeardi remains a matter for speculation. Chabaud & Campana-Rouget (1958), Popiel (1976a) and Mhaisen (1977) considered that C. vaullegeardi and metacercariae progenetic in

Blennius pholis are developmental stages of Lecithochirium rufoviride (syn. L. gravidum). Gibson & Bray (personal communication) have suggested that L. rufoviride and L. furcolabiata are synonymous, that is, that adult stages from the stomach of Conger conger and the liver of Ciliata mustela are forms of the same species, the prominent oral lobe "knobs" in L. furcolabiata being associated with the specific habitat within the body cavity. As for C. vaullegeardi being the cercarial stage of L. rufoviride, this problem will only be solved by further experimentation. Current investigations are concerned with the attempted infection of G. umbilicalis with eggs both from L. rufoviride and from L. furcolabiata. It will be interesting to see if either or both of these prove to be synonymous with C. vaullegeardi.

KEY TO LETTERING OF

FIGURES AND PLATES

a	ascospore
an	anus
ar	afferent renal vein
aw	ascospore wall
b	basement membrane and lamina
bc	birth canal
bd	cytoplasmic bridge
bk	caudal cyst beak
bp	birth pore
c	caudal cyst wall
ca	caudal cyst cavity
cav	breakdown cavities
cb	cercarial body
cbc	cytoplasm of bordering cell
cbt	cercarial body tegument
cdt	caudal tegument
cf	fibre-forming cells
cg	cerebral ganglion
ch	sub-tegumental cell
ci	cisternae
cm	copepod muscle fibres
co	connection of body to caudal cyst
col	collar
csc	cluster of specialised cells
ct	cercarial tail

cy	cystophorous tail
d	duct of secretory cell
db	cellular debris
dd	distal delivery tube
de	degenerating fused caudal excretory ducts
dg	digestive gland
dr	delivery tube rudiment
ds	desmosome
dt	delivery tube tegument
dtu	delivery tube
du	caudal gland duct
dv	vesicles with electron-dense contents
e	endpiece
ea	excretory appendage
ec	ecsoma
ect	ecsomatic tegument
ed	excretory duct
ep	gill epithelium
ev	excretory vesicle
ex	caudal excretory duct
f	fibrous wall
fc	flame cell
ff	remains of fibrous layer of delivery tube wall
fg	fore-gut
fi	caudal filaments
fsh	fibrous sheath
ft	fleshy tegument

g	caudal gland
gc	germinal cell
gf	gill filament
go	Golgi
gon	gonad
gp	genital pore
gx	glycocalyx
ha	copepod haemocoel
hg	hind-gut
hcp	host capsule
i	investing layer of developing delivery tube
ij	intercellular junction
im	interstitial material
in	intestinal caecum
l	sporocyst lumen
ld	delivery tube lumen
led	lateral excretory duct
lev	lining of excretory vesicle
li	lipid body
lko	left kidney opening
ln	longitudinal nerve
lv	vesicle with electron-lucent contents
m	muscle layers
mc	muscle cell cytoplasm
mcp	membranous capsule
me	metacercaria
mg	mid-gut

mit	mitochondrion
ml	median lobe
mn	host mantle
ms	mesosoma
mt	metacercarial tegument
mtb	microtubule
mtc	host mantle cavity
mts	metasoma
mv	microvilli
n	nucleus
nbc	nucleus of bordering cell
nc	nuclei of fibre-forming cells of caudal cyst
ne	neuron
nf	nuclei of peripheral fibre-forming cells
np	neuropile
ns	outer nucleated portion of syncytium
nt	nucleated tegument lining the posterior birth canal region
nte	nuclei of terminal cells
o	ovary
od	opening of secretory cell duct
oe	oesophagus
os	oral sucker
p	pre-oral lobe
pa	parenchyma cell
pd	proximal delivery tube
pe	posterior excretory duct

ph	pharynx
pin v	pinocytotic vesicle
pf	proximal fold
pp	presomatic pit
ps	prosoma
r	radial muscle
rg	remains of caudal gland
rer	rough endoplasmic reticulum (RER)
rev	remains of caudal section of excretory vesicle
rk	right kidney
rko	right kidney opening
s	secretory cell
sc	sub-tegumental secretory cell
sp	sporocyst
spa	space surrounding elongating proximal delivery tube
sph	sphincter muscle
st	somatic tegument
t	tegument
tg	tegumental membrane
tm	transverse membrane
tpv	transverse pallial vessel
tr	tail ribbon
ts	testis
v	vesicle
vi	vitellaria
vs	ventral sucker

PLATE 1

Cercaria vaullegeardi : electron micrographs of the anterior region of the daughter sporocyst in longitudinal section.

A. General morphology of the body wall.

B. Middle and posterior birth canal regions.

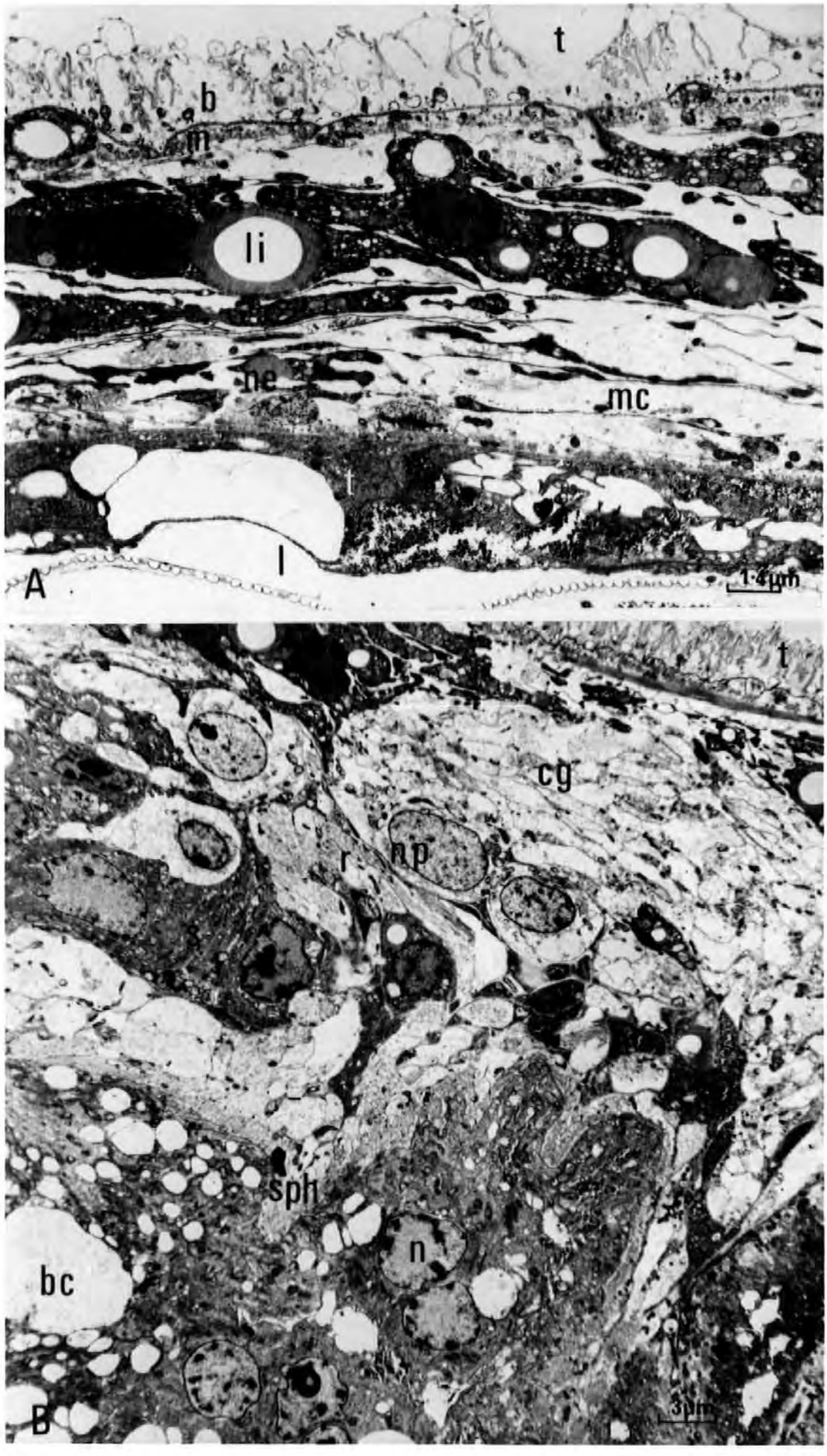

t
b
m
li
ne
mc
t
l
A
1·4µm
t
cg
np
r
sph
n
bc
B
3µm

PLATE 2

Cercaria vaullegeardi : electron micrographs of the anterior region of the daughter sporocyst in longitudinal section.

A. Middle birth canal region showing sphincter muscle, anucleate tegumental lining with long branching microvilli, neurons, and ducts from secretory cells opening into canal.

B. Sphincter muscle enlarged.

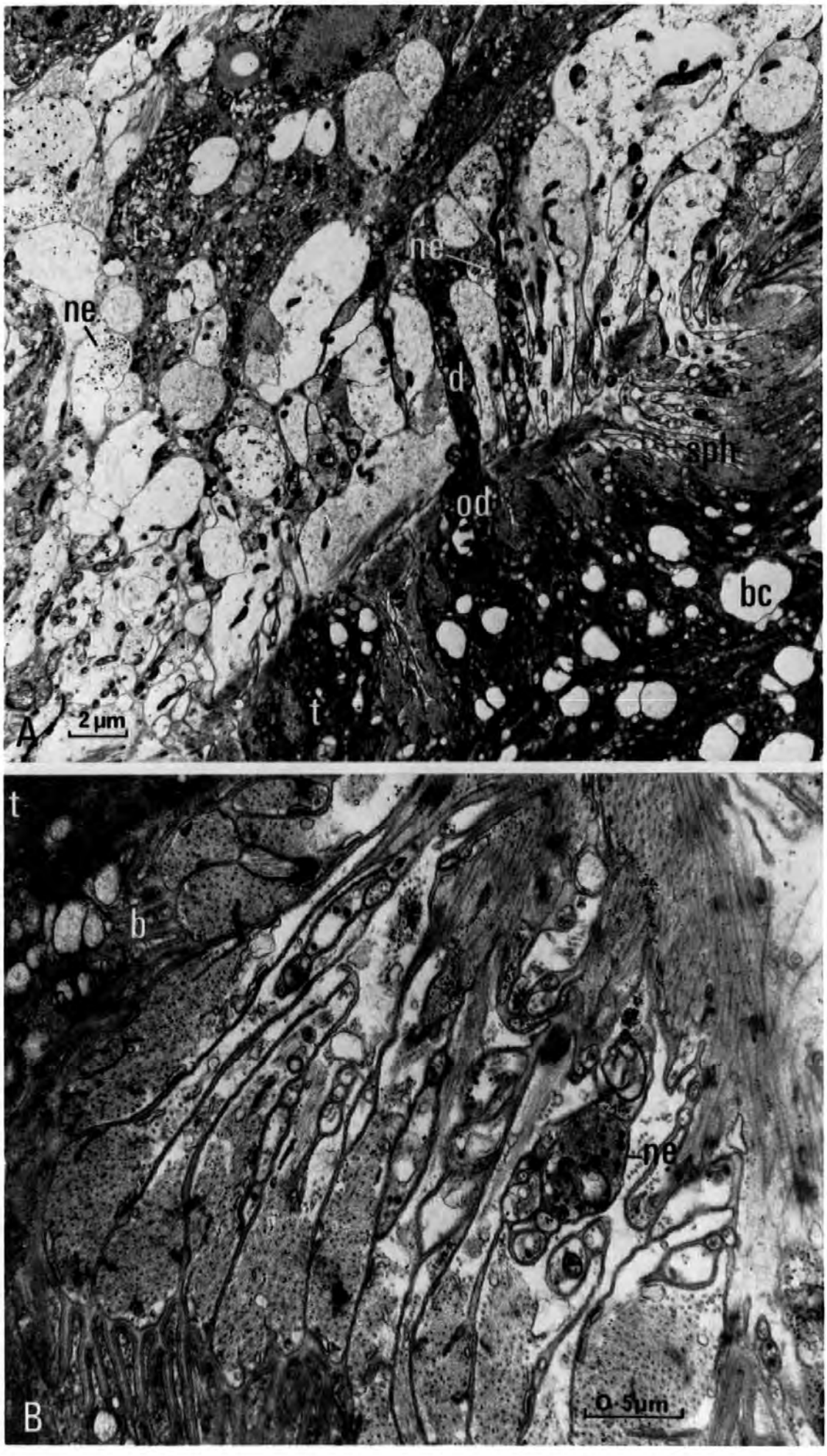

ne
ne
d
sph
od
bc
t
2 µm
A
t
b
ne
0·5µm
B

PLATE 3

Cercaria vaullegeardi : electron micrographs of the anterior region of the daughter sporocyst in longitudinal section.

A. Middle birth canal region, showing secretory cell, radial muscle, and nucleated muscle cell body with associated neurons.

B. Tegumental lining of region shown in A, enlarged to show RER, free ribosomes and mitochondria. Adjacent cytoplasm with α and β glycogen (arrowed), and mitochondria.

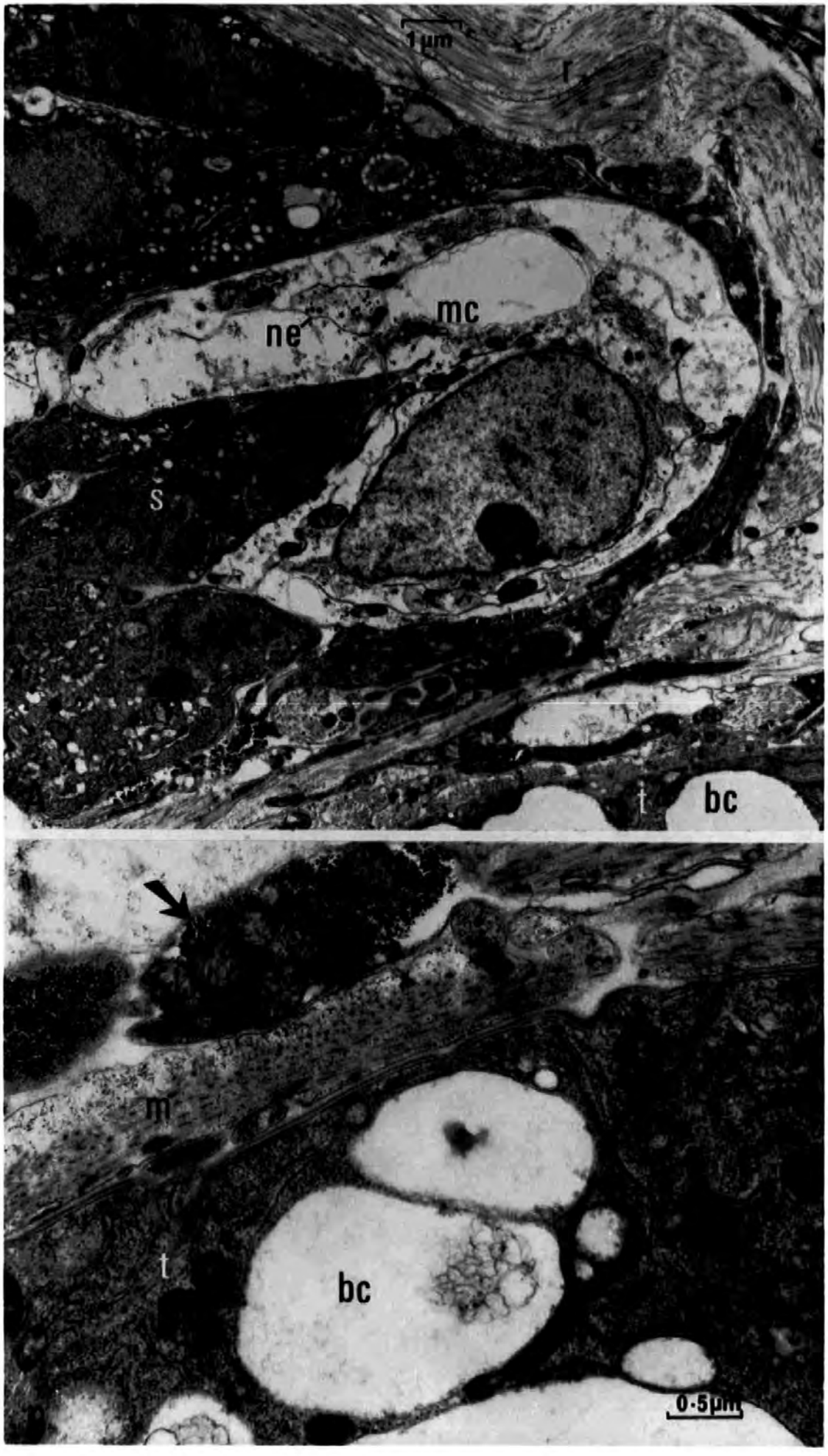

1 µm
r
mc
ne
s
t
bc
m
t
bc
0·5µm

PLATE 4

Cercaria vaullegeardi : electron micrographs of the birth canal of the daughter sporocyst.

A. Transverse section through nucleated tegument lining the posterior region of the birth canal.

B. Oblique section through neuron showing vesicles with electron-lucent and electron-dense contents, glycogen (arrowed), mitochondria and microtubules.

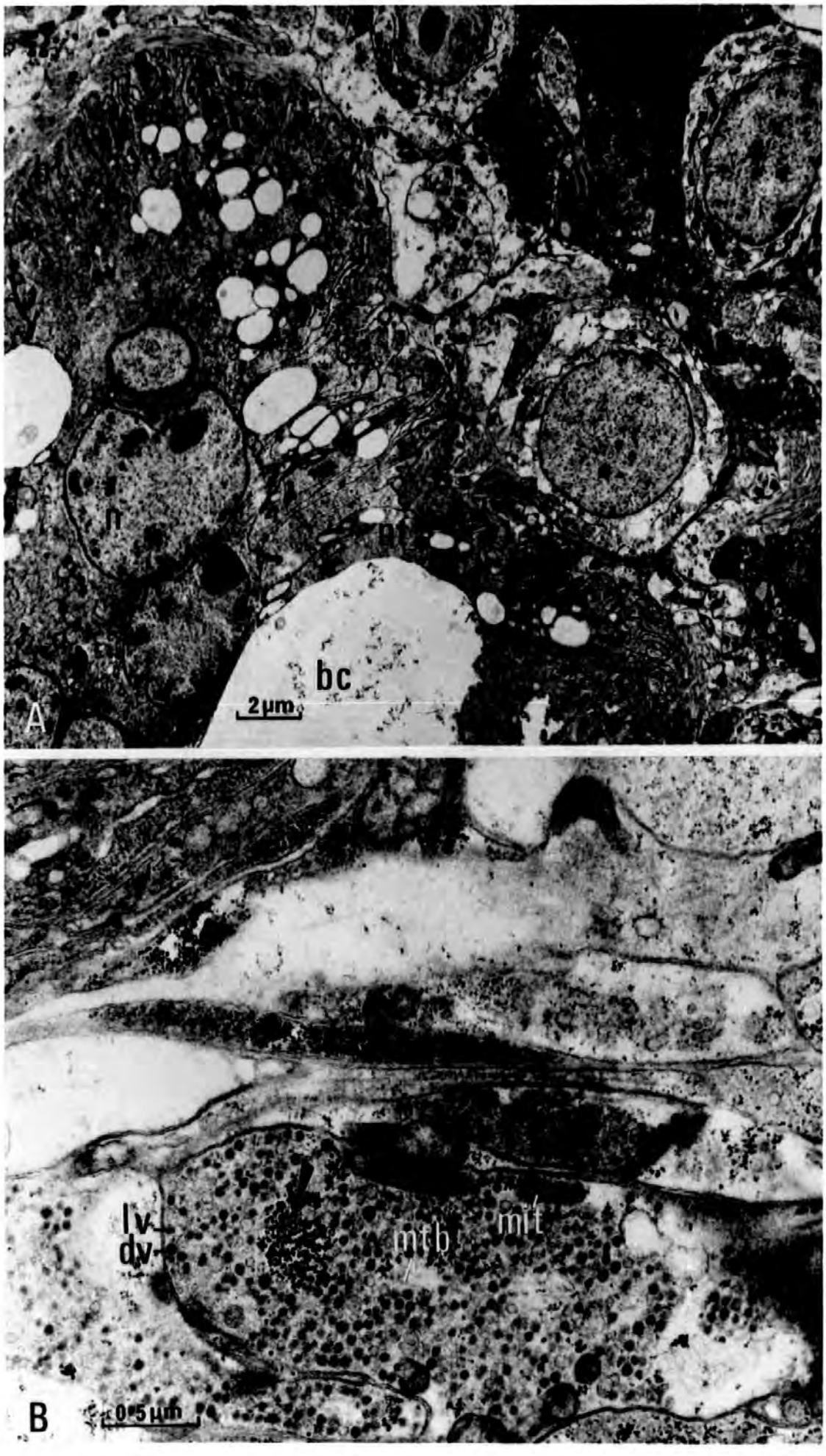

bc
2µm
A
lv
dv
mtb
mit
0·5µm
B

PLATE 5

A. Anterior end of daughter sporocyst of Cercaria vaullegeardi showing acetylcholinesterase located within nervous system. Strongly positive reaction within cerebral ganglia and longitudinal nerves.

B. Micrograph of transverse section through the transverse pallial blood vessel of Gibbula umbilicalis showing 23 sporocysts of C. vaullegeardi within its lumen.

C. Micrograph of longitudinal section through anterior end of the daughter sporocyst of C. vaullegeardi within the blood channel of a host gill filament, showing anterior, middle and posterior regions of the birth canal. The gill epithelium is torn at the site where the sporocyst tip has penetrated for cercarial release.

D. Micrograph of host gill filaments in transverse section showing disrupted epithelium and sporocysts of C. vaullegeardi within blood channels.

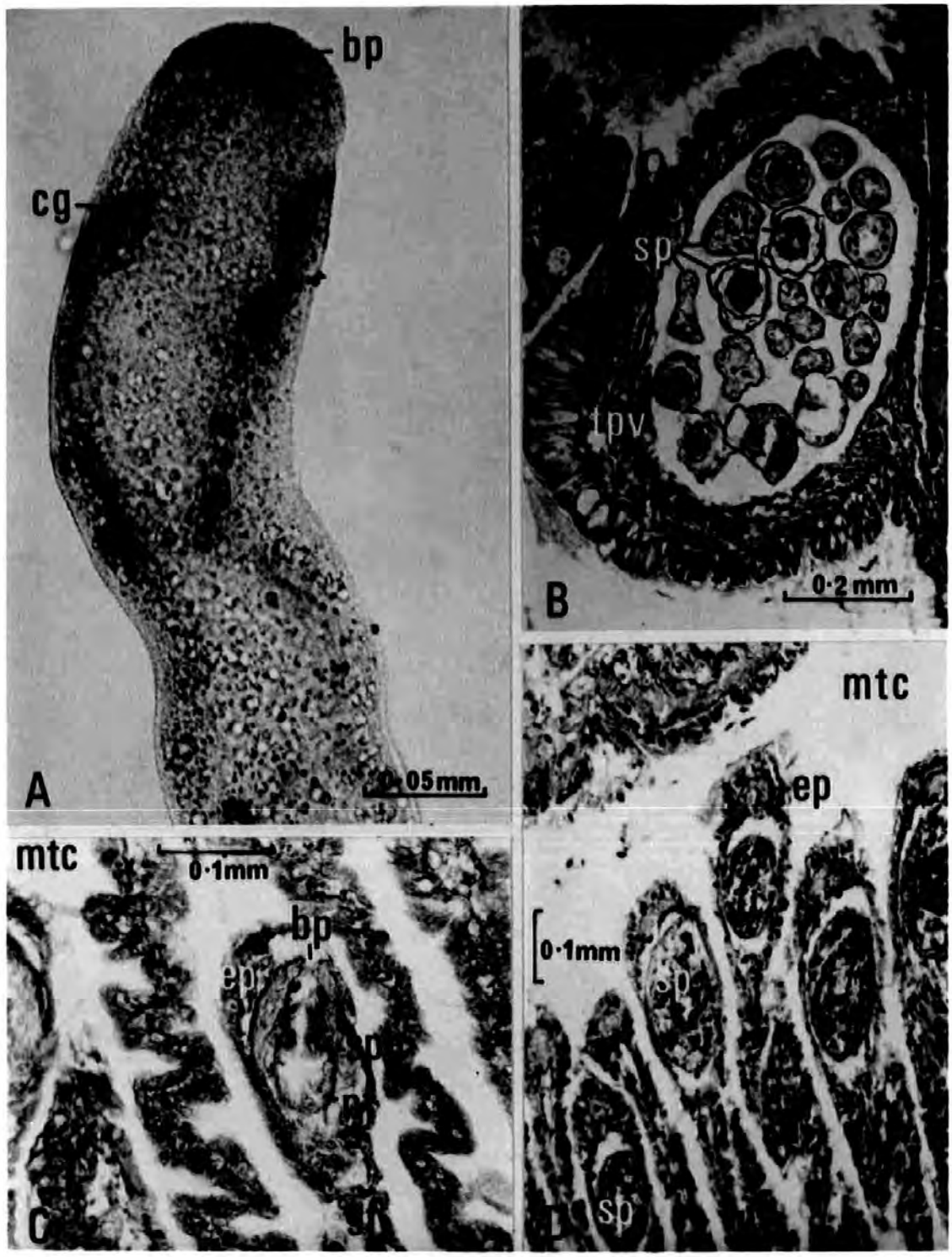

bp
cg
A
0·05mm
sp
tpv
B
0·2mm
mtc
ep
0·1mm
mtc
0·1mm
bp
ep
C
sp

PLATE 6

Cercaria vaullegeardi : electron micrographs of the anterior region of the daughter sporocyst within the gill filament of Gibbula umbilicalis.

A. Oblique section showing sporocyst in situ within gill blood channel (arrowed).

B. Transverse section of sporocyst showing vesicles with electron-dense contents passing into tegument from sub-tegumental secretory cell.

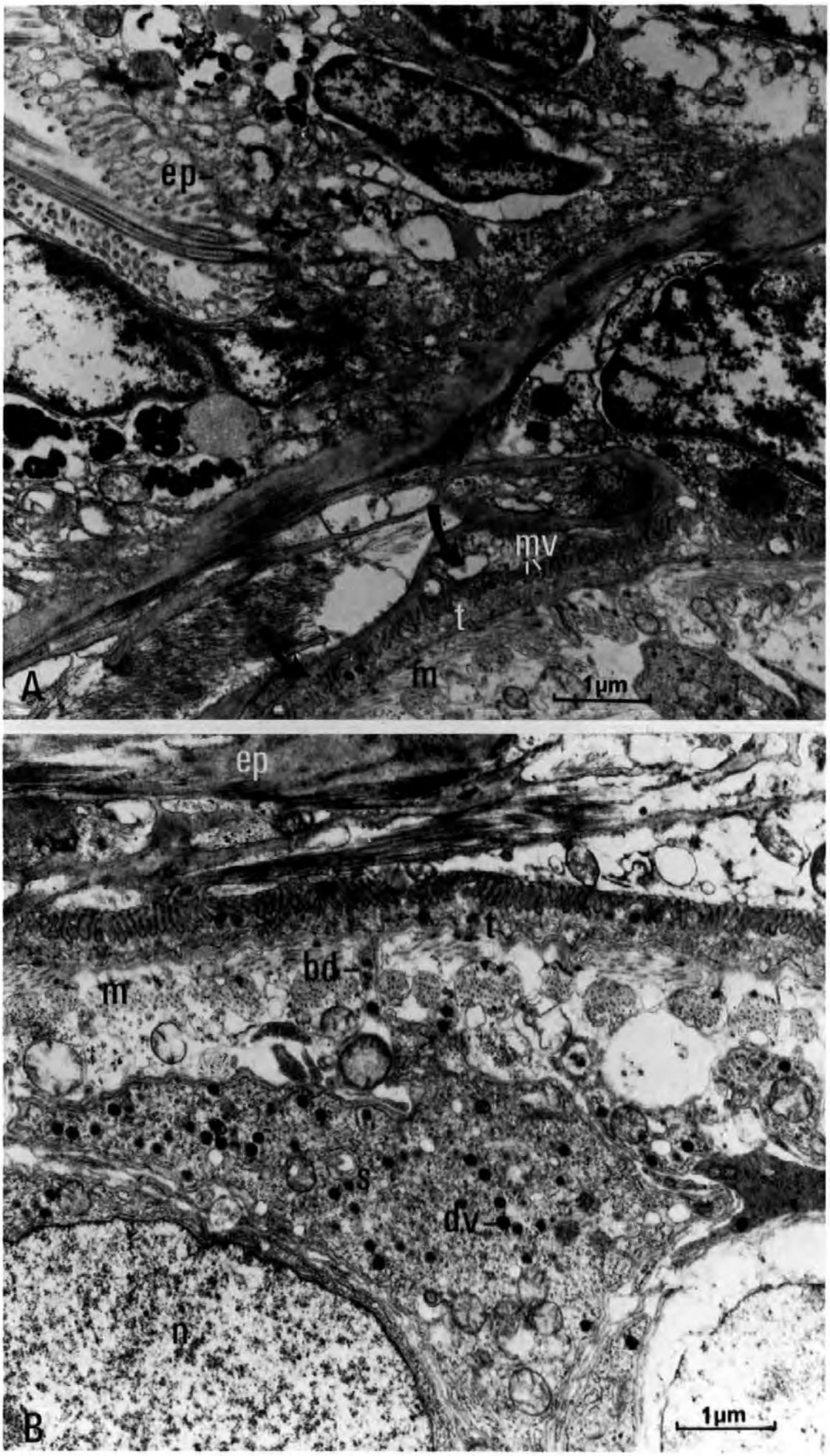

ep
mv
t
m
A
1µm
ep
t
bd
m
s
dv
n
B
1µm

PLATE 7

Cercaria vaullegeardi : electron micrographs of the daughter sporocyst.

A. Transverse section through posterior region of sporocyst within host digestive gland haemocoel, showing necrotic host cells. A zone of cellular debris separates host tissue from the highly branched microvilli covering the anucleate syncytial sporocyst tegument.

B. Posterior region of sporocyst showing flattened sub-tegumental cells bordering the lumen.

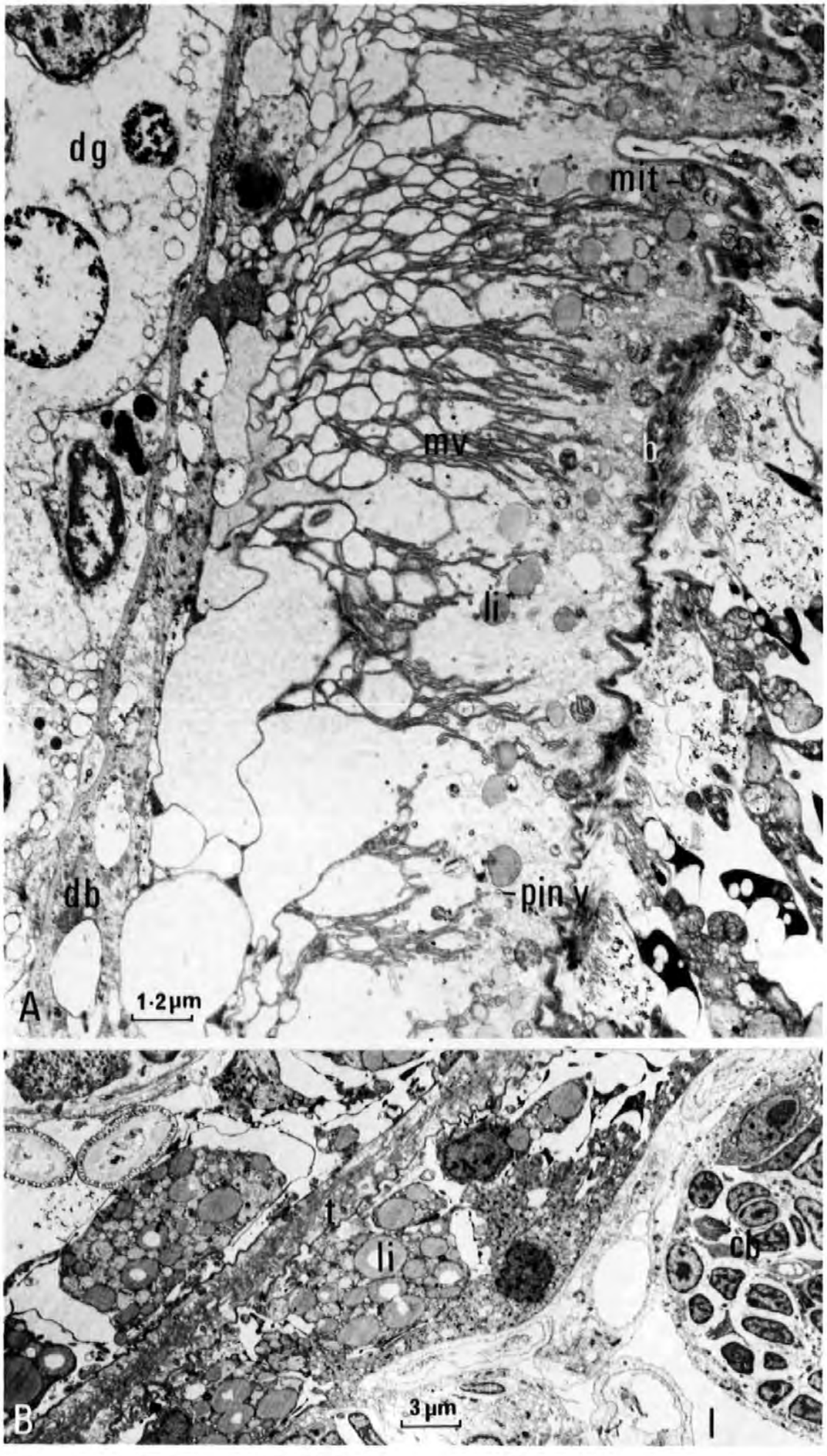

dg
mit
mv
li
db
pin v
1·2µm
A
t
li
cb
3µm
B

PLATE 8

Cercaria vaullegeardi : electron micrographs of the posterior region of the daughter sporocyst.

A,B. Germinal cells enclosed within cavities in flattened sub-tegumental cells bordering the sporocyst lumen.

C. Germinal cell enlarged.

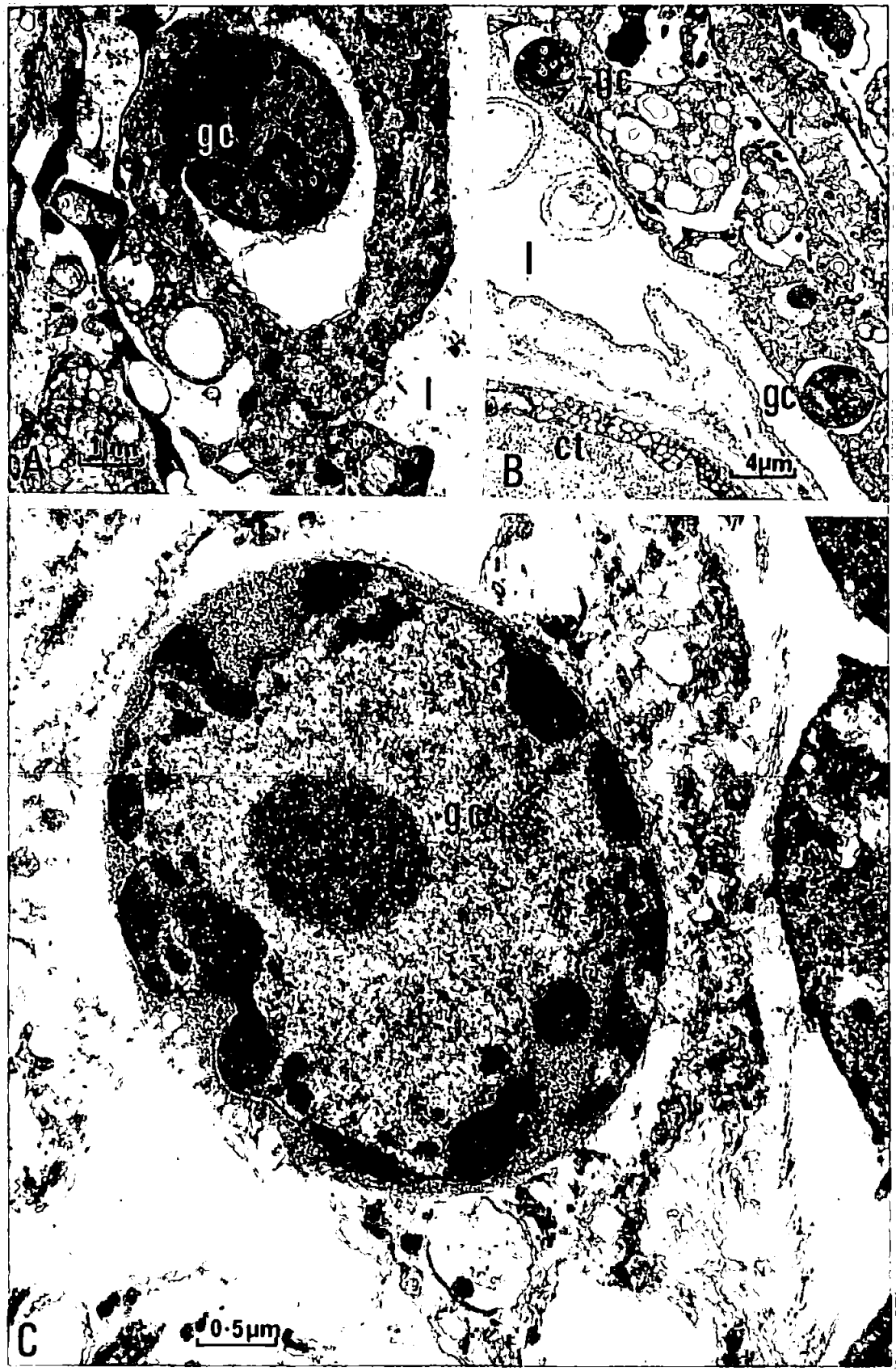

gc
l
A
1μm
gc
l
gc
B
ct
4μm
gc
C
0·5μm

PLATE 9

Cercaria vaullegeardi : electron micrographs of the posterior region of the daughter sporocyst. Montage showing enlarged cavity within flattened bordering cell, containing germinal cells and a column of cells of unknown function.

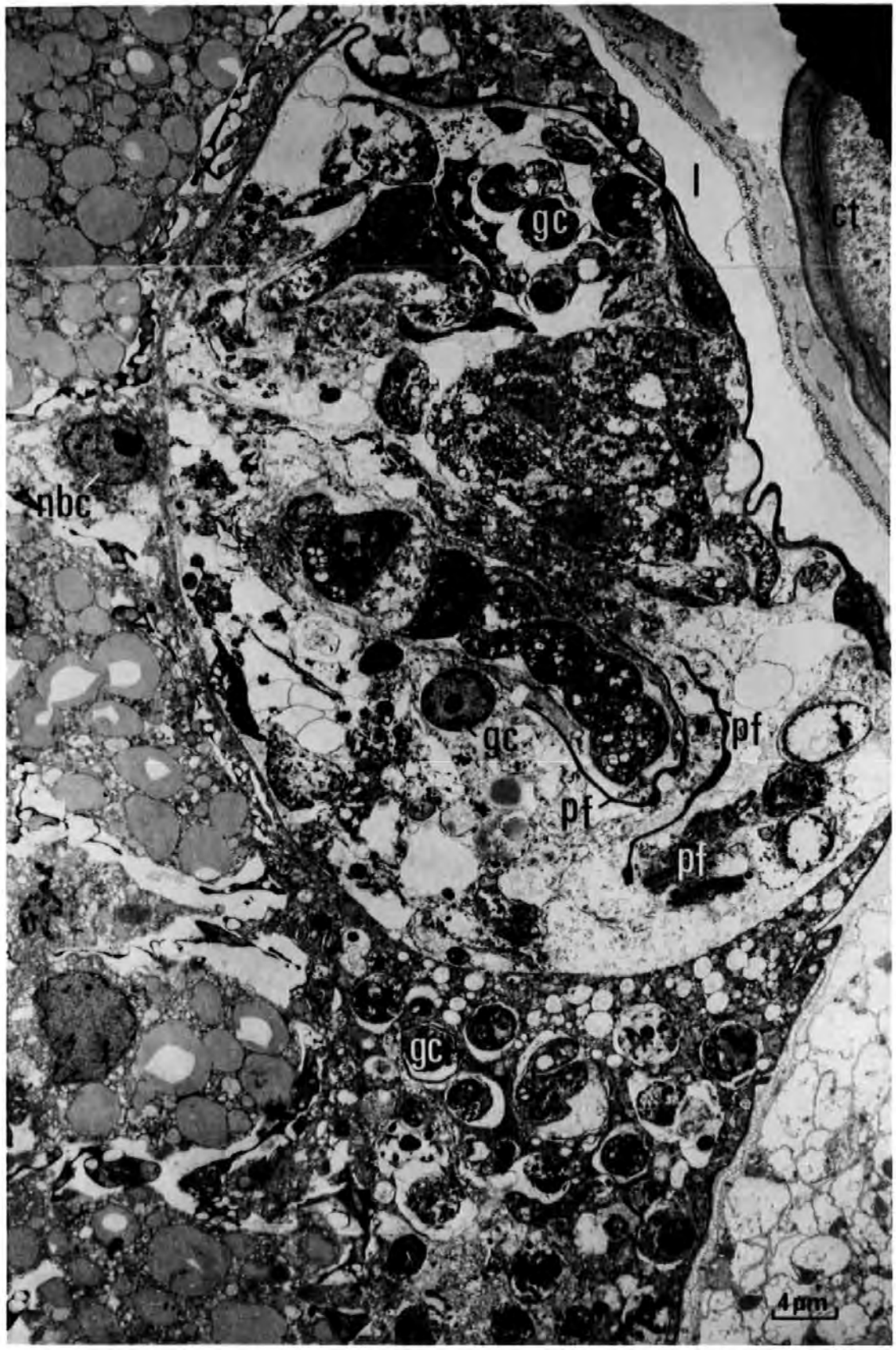

gc
l
ct
nbc
gc
pf
pf
pf
gc
4 µm

PLATE 10

Cercaria vaullegeardi : electron micrographs of the posterior region of the daughter sporocyst. Serial sections to those shown in Plate 9.

A. Showing attachment (arrowed) of the column of cells to the side of the cavity within the bordering cell.

B. Serial section to A, showing proximal cell with possibly hypertrophied nucleus (arrowed).

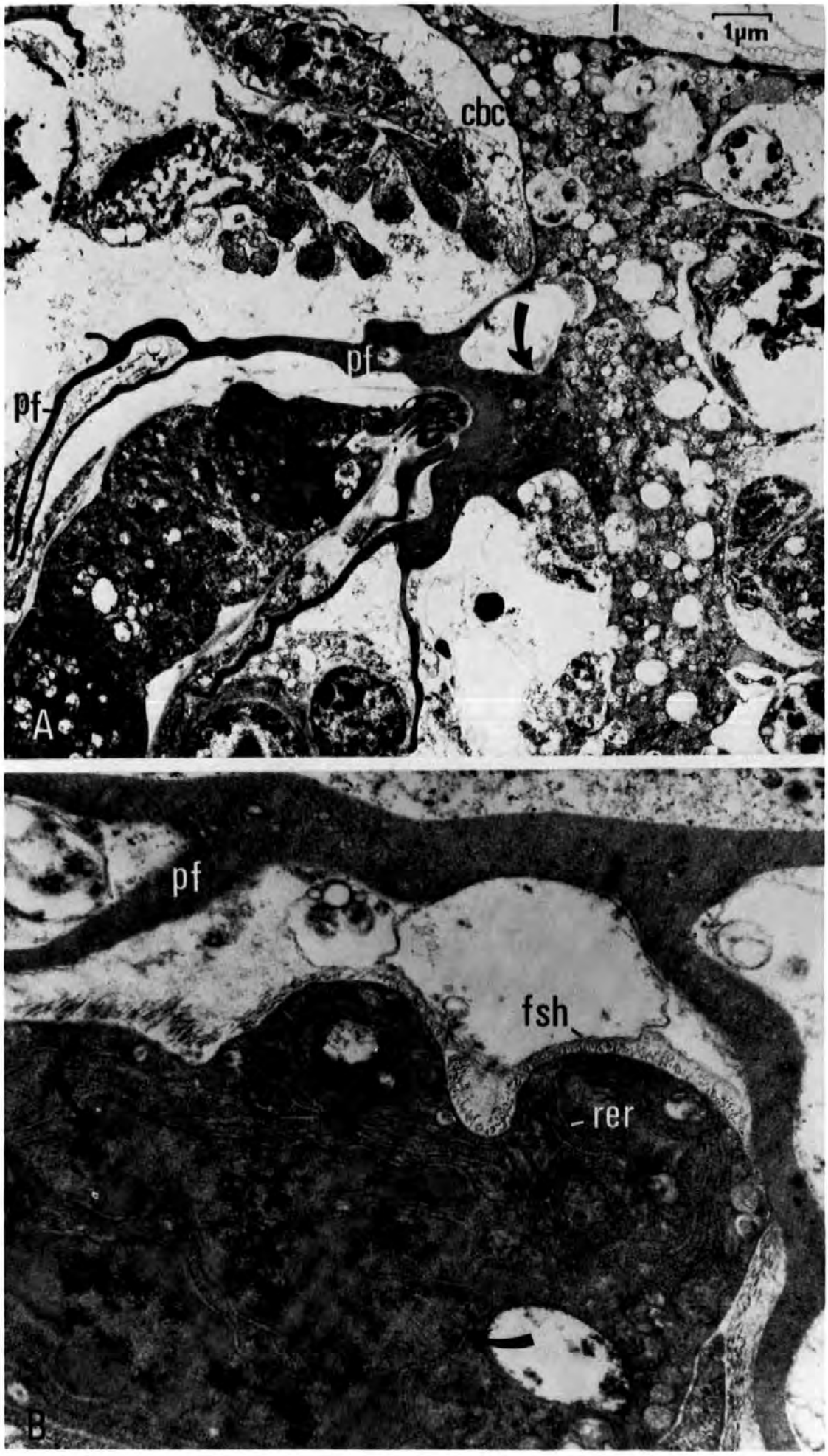

1µm
cbc
pf
pf
A
pf
fsh
rer
B

PLATE 11

Cercaria vaullegeardi : electron micrographs of pre-migratory stage.

A. Cercarial body.

B. Early developmental stage; sub-tegumental cell with cytoplasmic bridge.

C. Later developmental stage, showing vesicles within secretory cell.

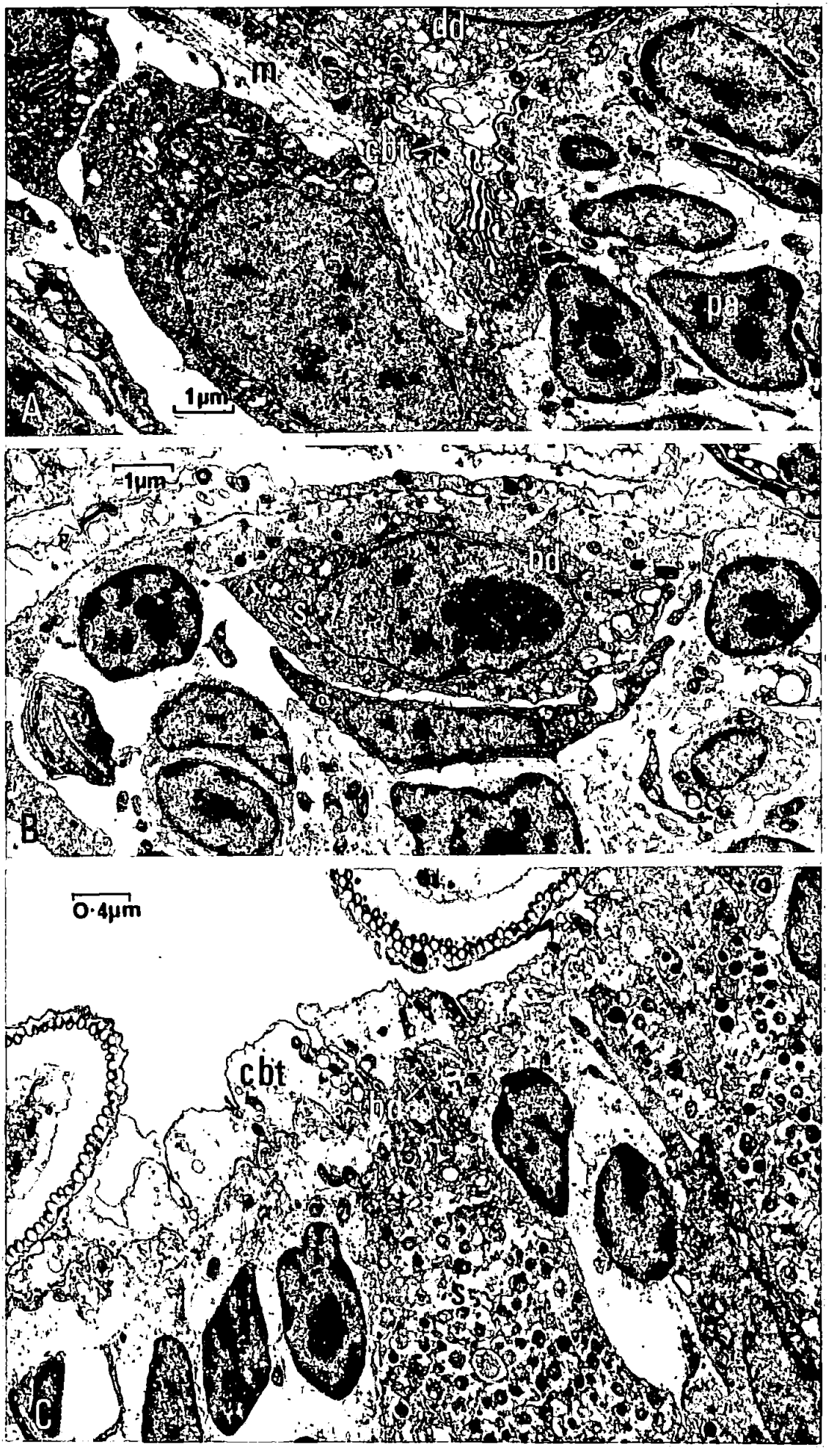

dd
m
cbt
pa
1μm
A
1μm
bd
s
B
0·4μm
cbt
bd
s
C

PLATE 12

Cercaria vaullegeardi : electron micrographs of the tail of the pre-migratory stage.

A. Longitudinal section through posterior end of delivery tube rudiment. Early developmental stage corresponding to Fig. 3E. Note investing layer of developing proximal delivery tube and outer nucleated portion of syncytium, separated by a narrow space (arrowed).

B. Transverse section through caudal gland with Golgi and electron-lucent vesicles, and delivery tube rudiment. Later stage, corresponding to Fig. 5F.

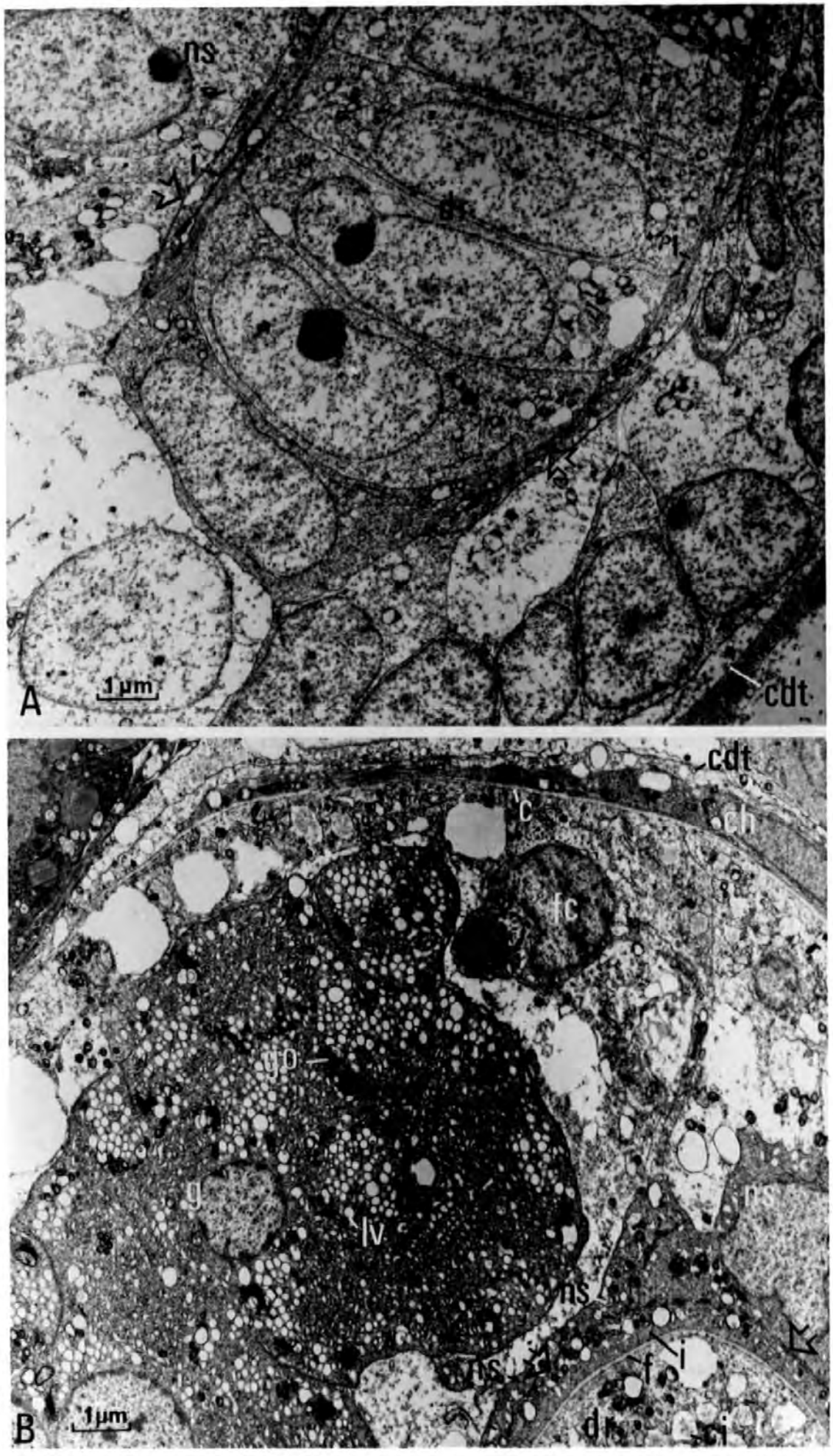

ns
cdt
A
1 µm
cdt
ch
c
fc
gd
g
lv
ns
ns
i
fo
dr
B
1 µm

PLATE 13

Cercaria vaullegeardi : electron micrographs of the tail of the pre-migratory cercarial tail.

A. Longitudinal section of caudal cyst showing branched duct passing from the caudal gland to the tegument.

B. Caudal flame cells.

C,D. Caudal gland showing Golgi and electron-lucent vesicles.

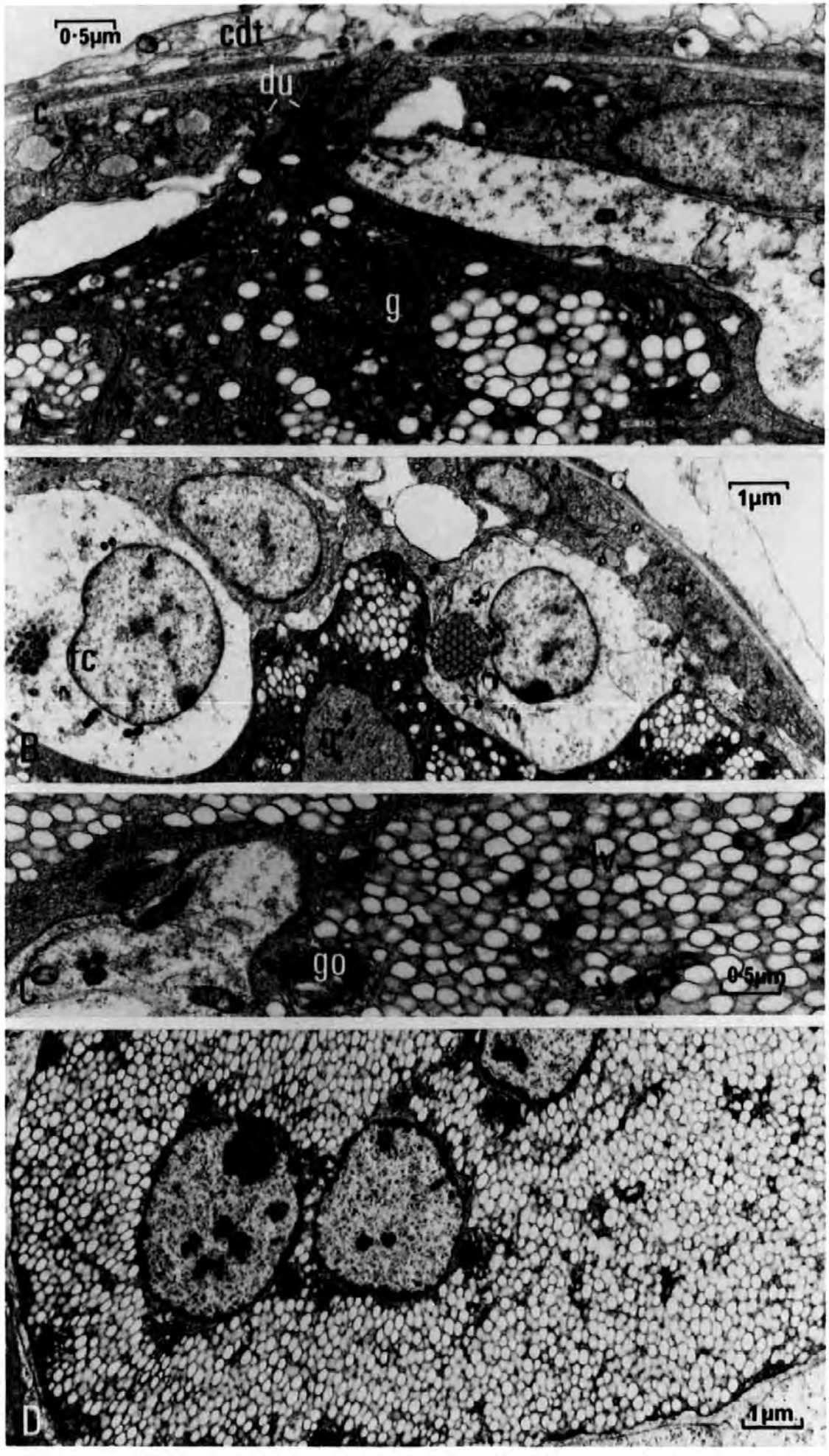

0·5µm
cdt
du
c
g
A
1µm
fc
B
w
go
0·5µm
C
1µm
D

PLATE 14

Cercaria vaullegeardi : electron micrographs of pre-migratory stage.

A. Longitudinal section of caudal sphincter muscle. Note the electron-lucent vesicles which have accumulated in a single layer within the caudal tegument.

B. Caudal sphincter muscle enlarged.

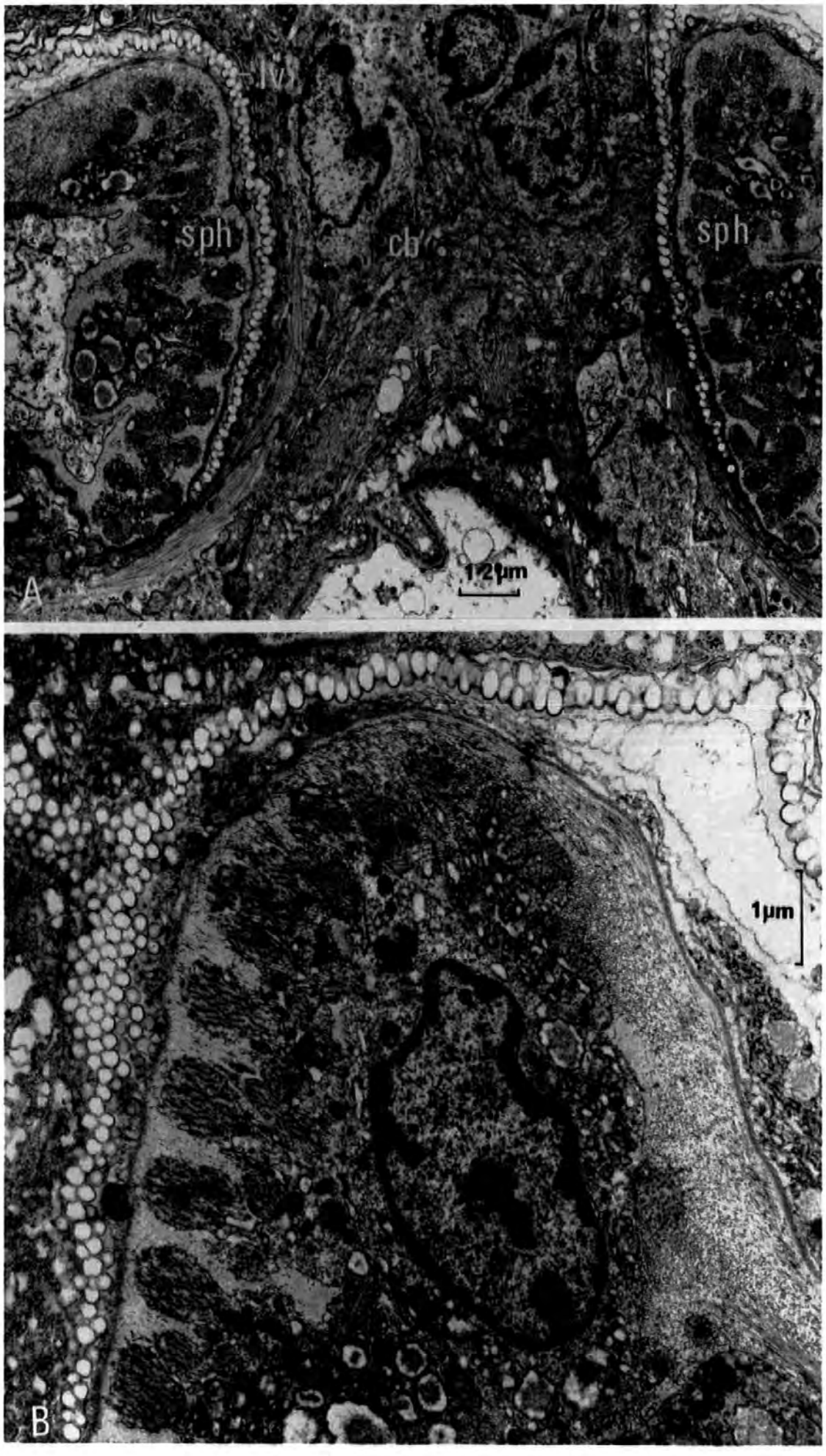

lv
sph
cb
sph
r
1·2µm
A
1µm
B

PLATE 15

Cercaria vaullegeardi : electron micrographs of the cystophorous tail.

A. Pre-migratory stage. Transverse section through developing caudal cyst and fibre-forming cells. A homogenous layer (arrowed) lies between the tegument and the caudal cyst.

B. Encysted stage. Longitudinal section through caudal cyst; note breakdown of the fibre-forming cells contributing to the caudal cyst cavity which contains scattered electron-dense granular material (arrowed).

C. Encysted stage. Longitudinal section through caudal cyst beak; note the discontinuity of the fibrous layers around the tip, and the lumen of the delivery tube.

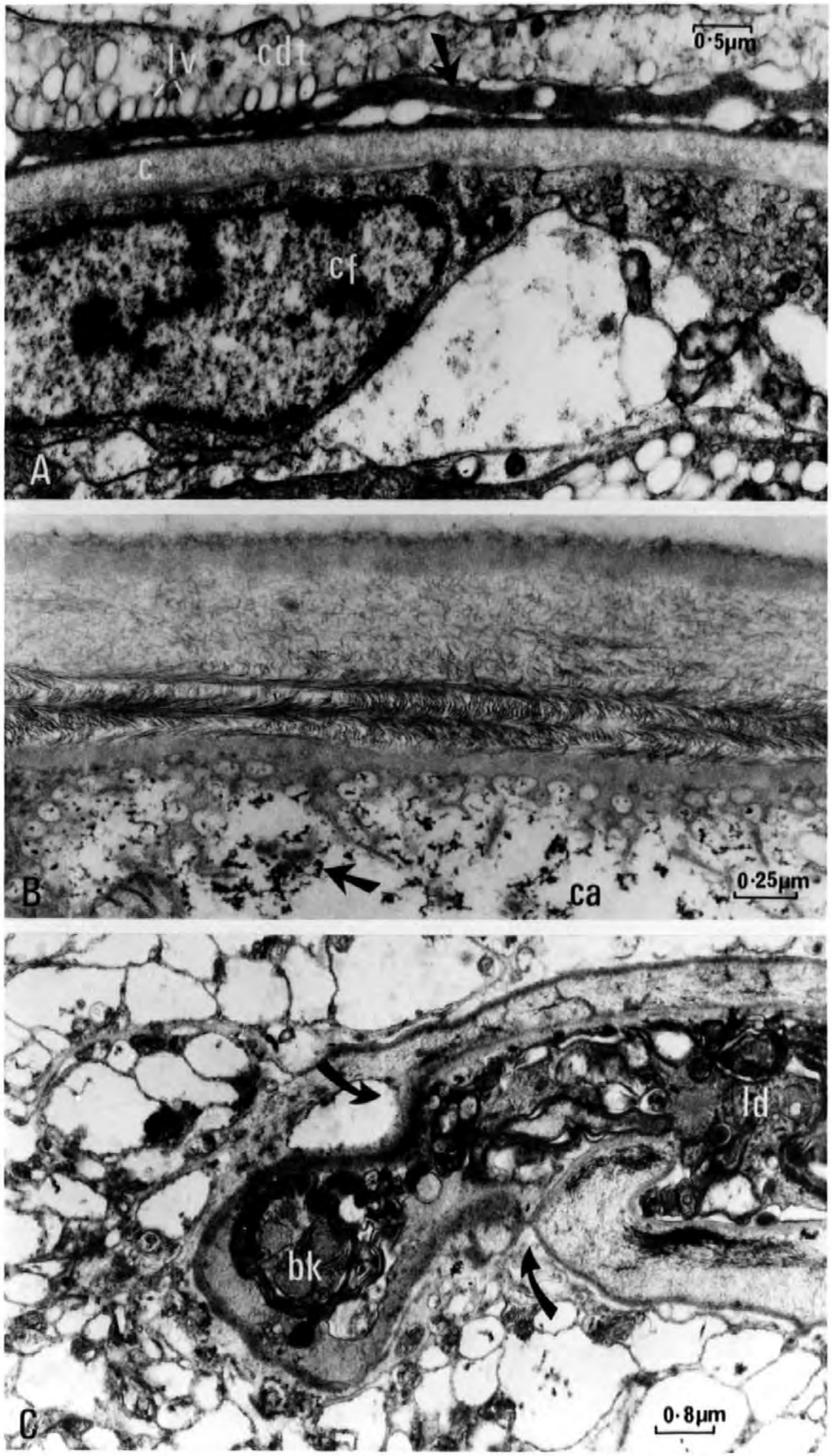
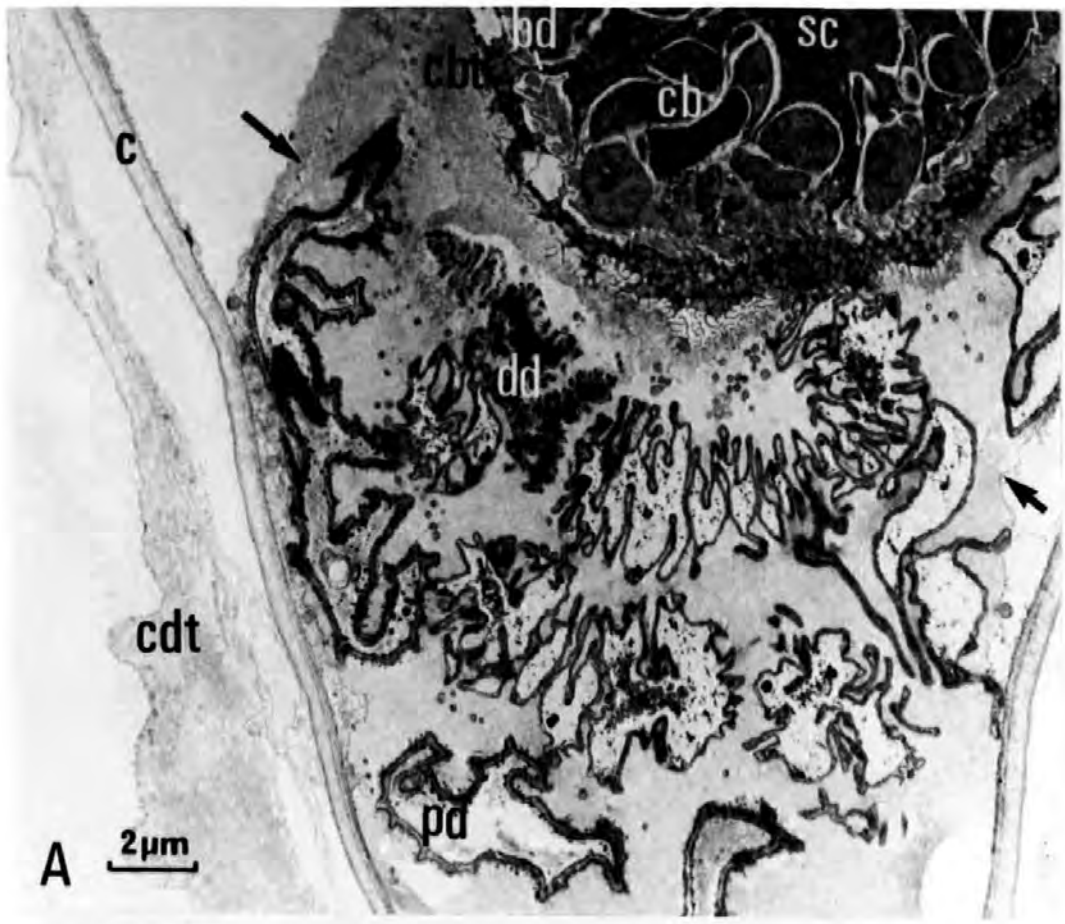

lv
cdt
c
cf
A
0·5µm
B
ca
0·25µm
ld
bk
C
0·8µm

PLATE 16

Cercaria vaullegeardi : electron micrographs of encysted stage.

A. Tangential section showing cercarial body with proximal and distal sections of delivery tube within the membranous capsule (arrowed). Note secretory vesicles within the cercarial body tegument, sub-tegumental secretory cell with cytoplasmic bridge, and nuclei of parenchyma cells.

B. Transverse section through cercarial body within membranous capsule (arrowed). Vesicles with flocculent and electron-dense contents within a sub-tegumental secretory cell have passed into the tegument, some being discharged at the surface into the cavity of the membranous capsule. Note parenchyma cells with cytoplasm containing mitochondria and ribosomes confined to surface indentations of nucleus.


bd
sc
cbt
cb
c
dd
cdt
pd
2µm
A

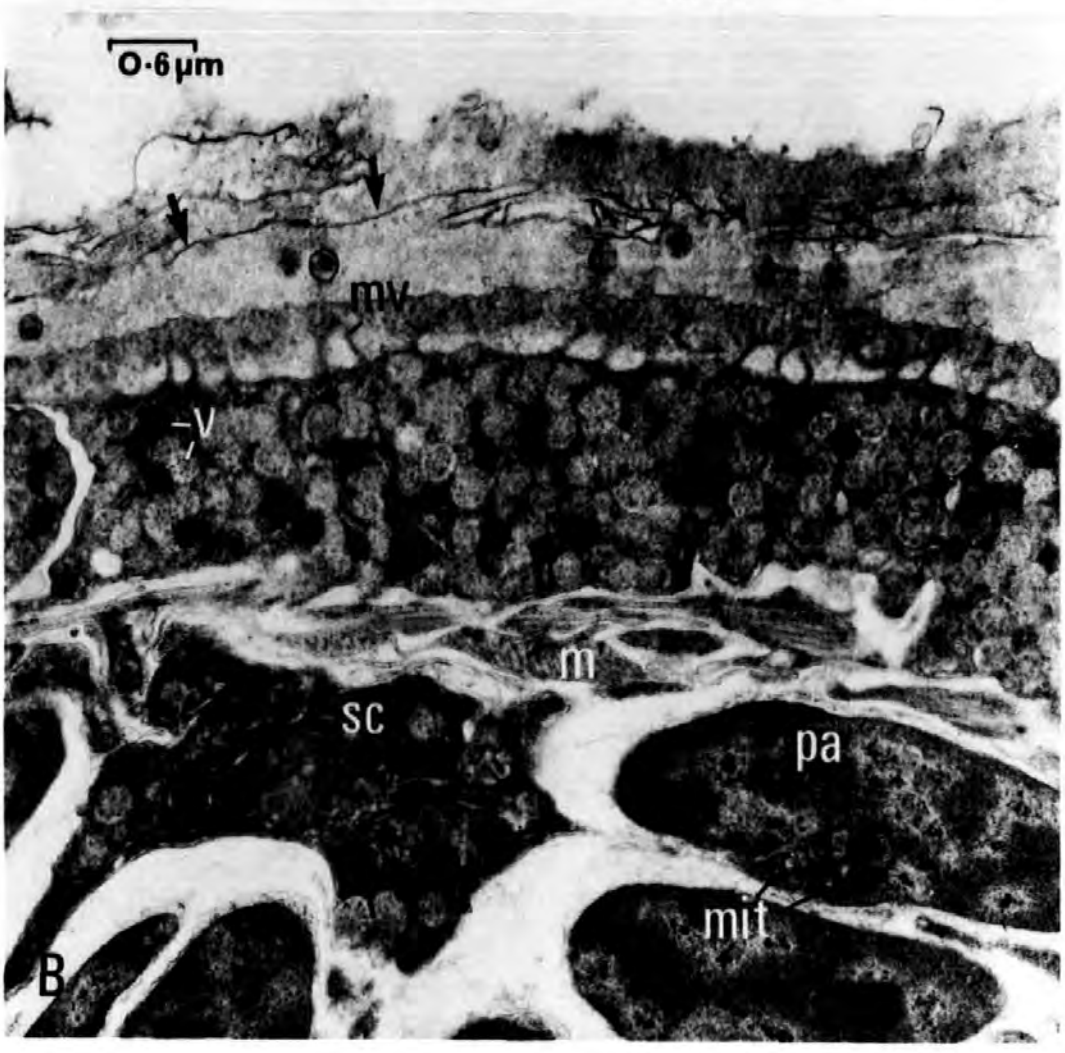

0·6µm
mv
V
m
sc
pa
mit
B

PLATE 17

Cercaria vaullegeardi : electron micrographs of the proximal delivery tube.

A. Pre-migratory cercaria. Oblique section through delivery tube rudiment. Note fibrous wall and narrow space (arrowed) between investing syncytium of adjacent folds of developing proximal delivery tube. Fibre-forming cell cytoplasm containing cisternae is undergoing breakdown to form cavities which will coalesce to form the tube lumen.

B. Encysted cercaria; adjacent folds of proximal delivery tube. Note the folded basement membrane (arrowed) of disintegrated investing syncytium.

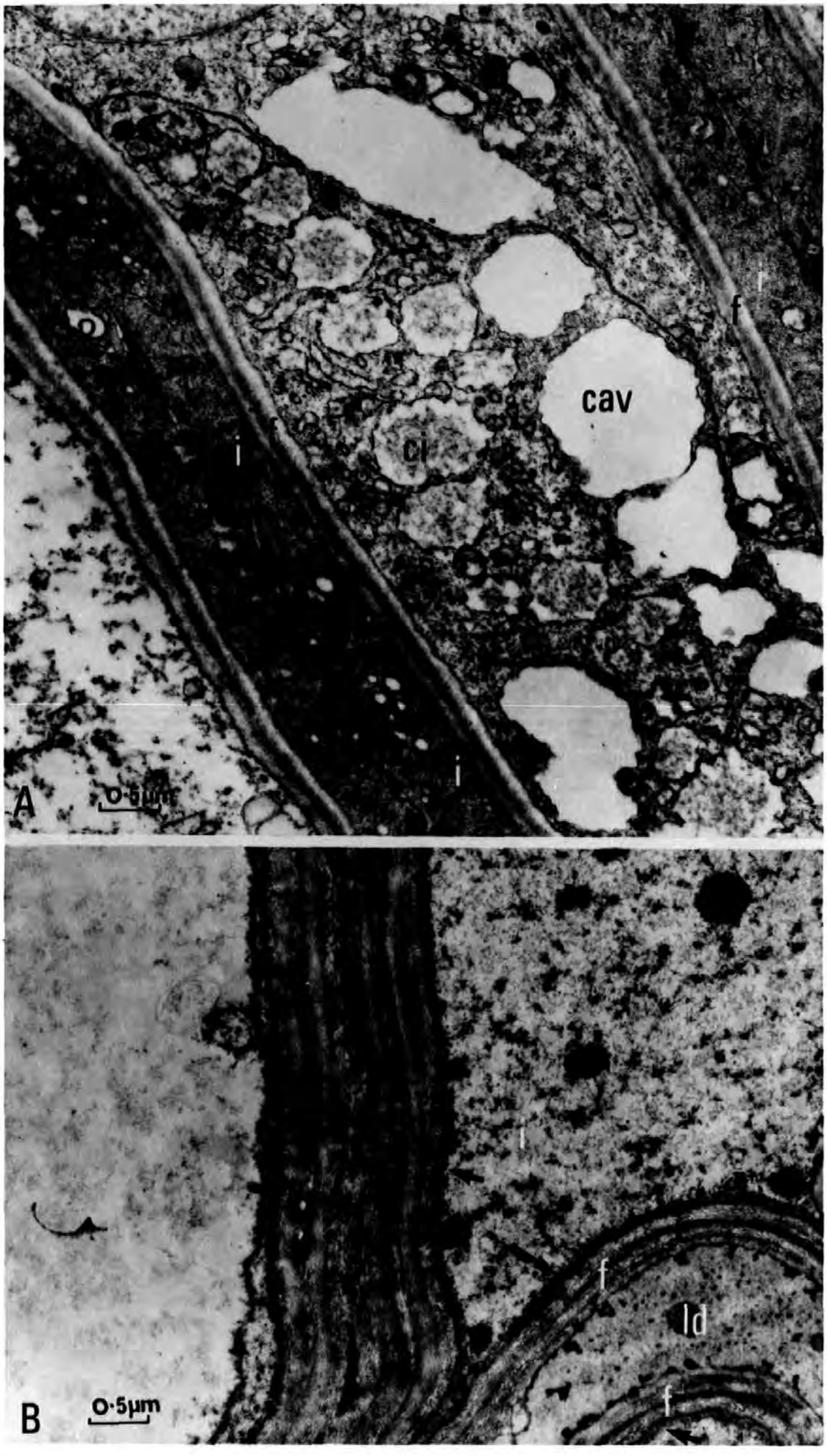

o
i
cav
ci
f
i
A
0·5µm
i
f
ld
f
B
0·5µm

PLATE 18

Cercaria vaullegeardi : pre-migratory cercaria. Electron micrographs of the distal delivery tube.

A. Longitudinal section of distal delivery tube wall before retraction into the caudal cyst.

B. Section through folds of distal delivery tube after retraction into the caudal cyst. Note folded tegumental basement membrane (arrowed).

C. Oblique section through distal delivery tube wall near endpiece, after retraction into the caudal cyst. Note electron-dense deposits (arrowed) within tube lumen.

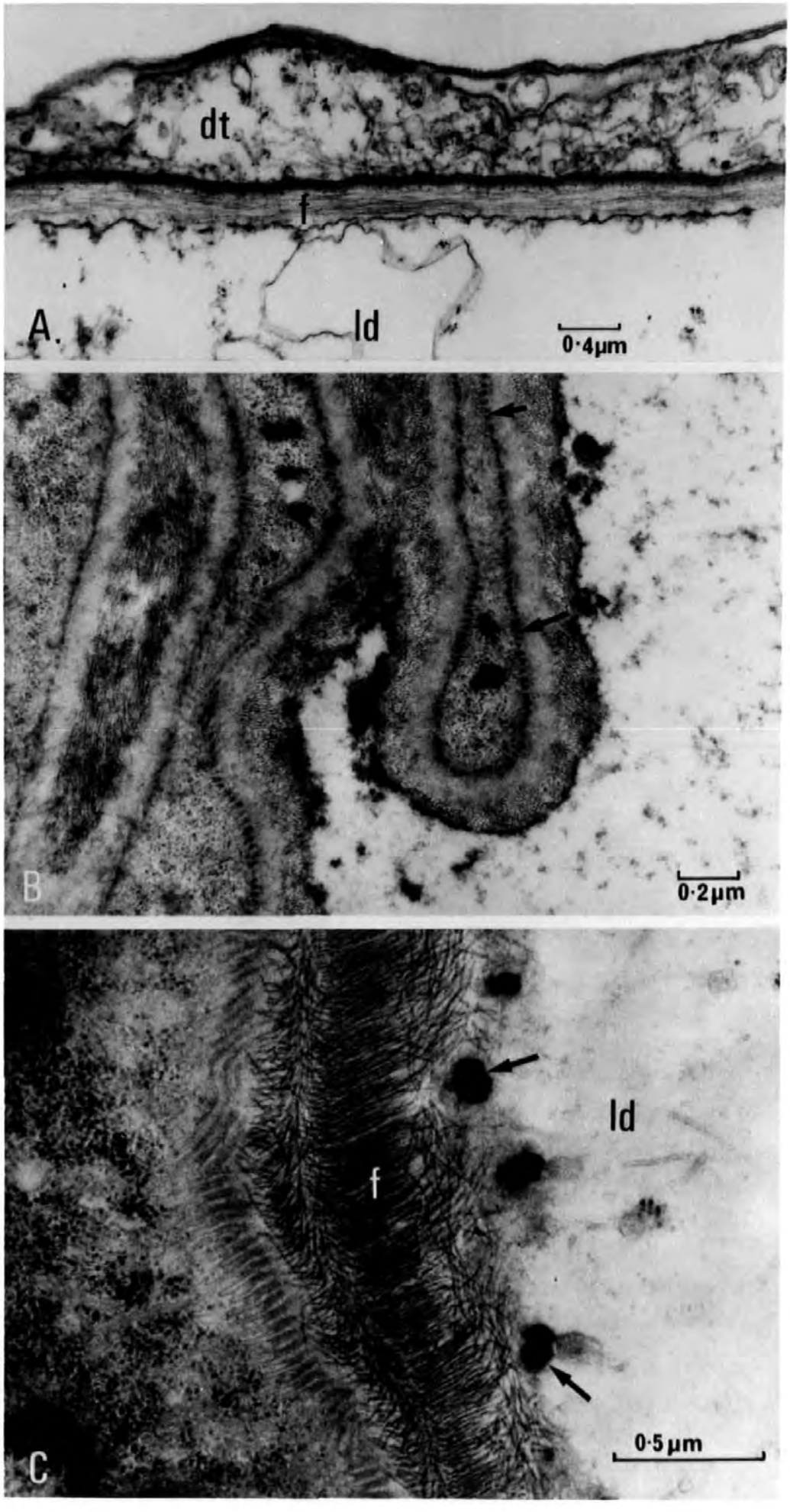

dt
f
A.
ld
0·4µm
B
0·2µm
ld
f
C
0·5µm

PLATE 19

Cercaria vaullegeardi : electron micrographs of the delivery tube endpiece.

A-C. Pre-migratory cercaria showing developing endpiece.

A. Tangential section; note cavity (arrowed) caused by cellular shrinkage and breakdown within the developing endpiece.

B. Oblique section through terminal cell; note deposits of electron-dense material (arrowed).

C. Oblique section through part of endpiece, fixed at a stage corresponding to Fig. 5I. Note electron-dense deposits.

D. Encysted cercaria. Longitudinal section through fully-formed endpiece.

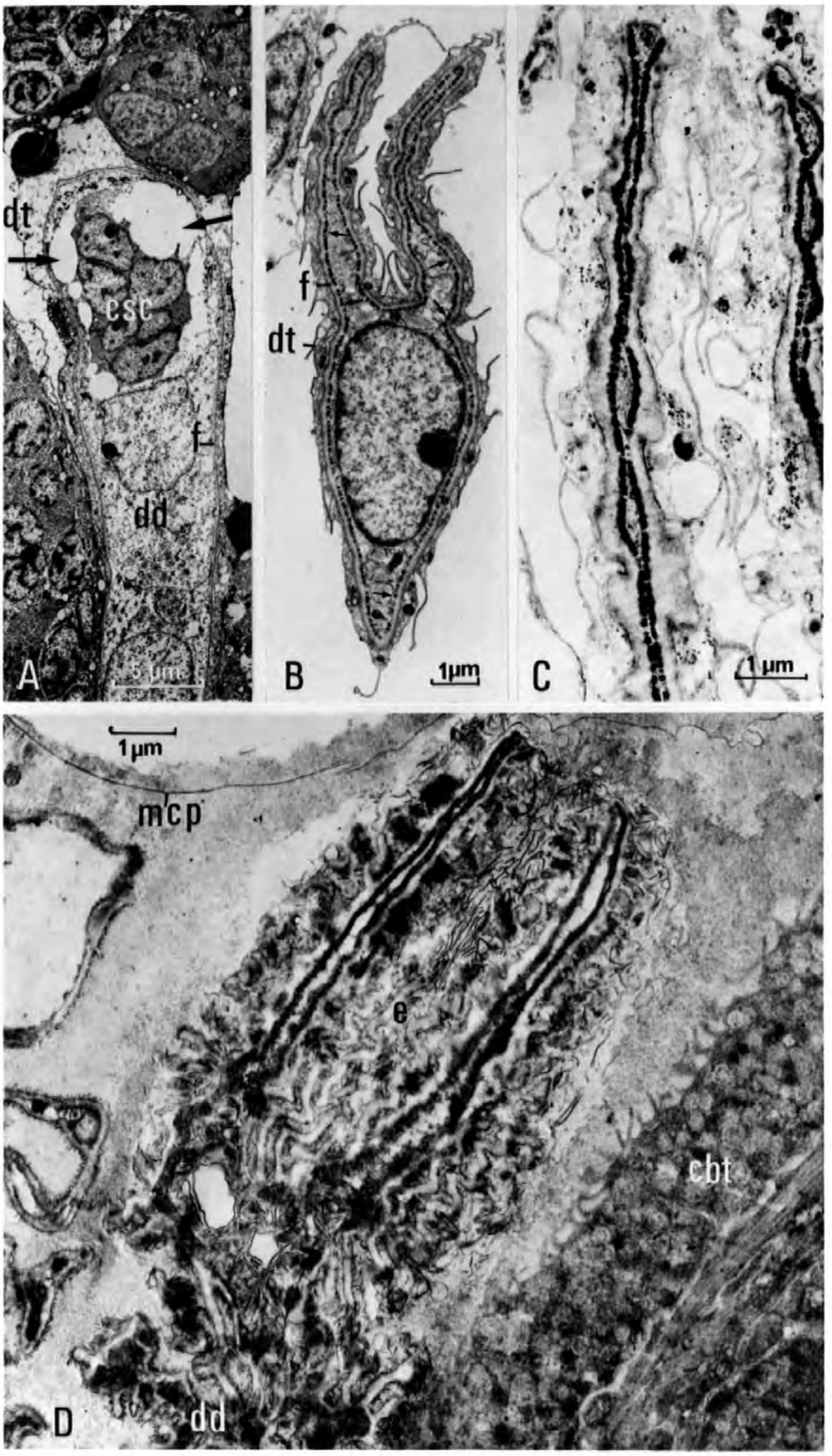

dt
csc
f
dd
A
5 µm
f
dt
B
1µm
C
1 µm
1µm
mcp
e
cbt
D
dd

PLATE 20

A,B. Cercaria vaullegeardi; scanning electron micrographs showing the caudal surface indentations.

A. Migratory cercaria.

B. Encysted cercaria; passage through the sporocyst birth canal has removed the secretions and cellular debris from the surface of the indentations.

C,D. Cercaria vaullegeardi; light micrographs showing the location of acetylcholinesterase as indicated by a positive reaction using the method of Jennings & LeFlore (1972).

C. Two migratory cercariae and the anterior extremity of a daughter sporocyst. Coverslip pressure has caused the expulsion of part of the distal delivery tube through the anterior aperture of the caudal cyst. Positive reaction within the delivery tube endpiece in addition to cerebral ganglia and longitudinal nerves.

D. Delivery tube endpiece (enlargement of C).

E. Cercaria calliostomae : Light micrograph of intraredial cercaria,

PLATE 20 (CONTD.)

showing location of acetylcholinesterase as indicated by a positive reaction using the method of Jennings & LeFlore (1972). Note the intensely positive reaction within the tail ribbon in addition to the cerebral ganglia and nerves.

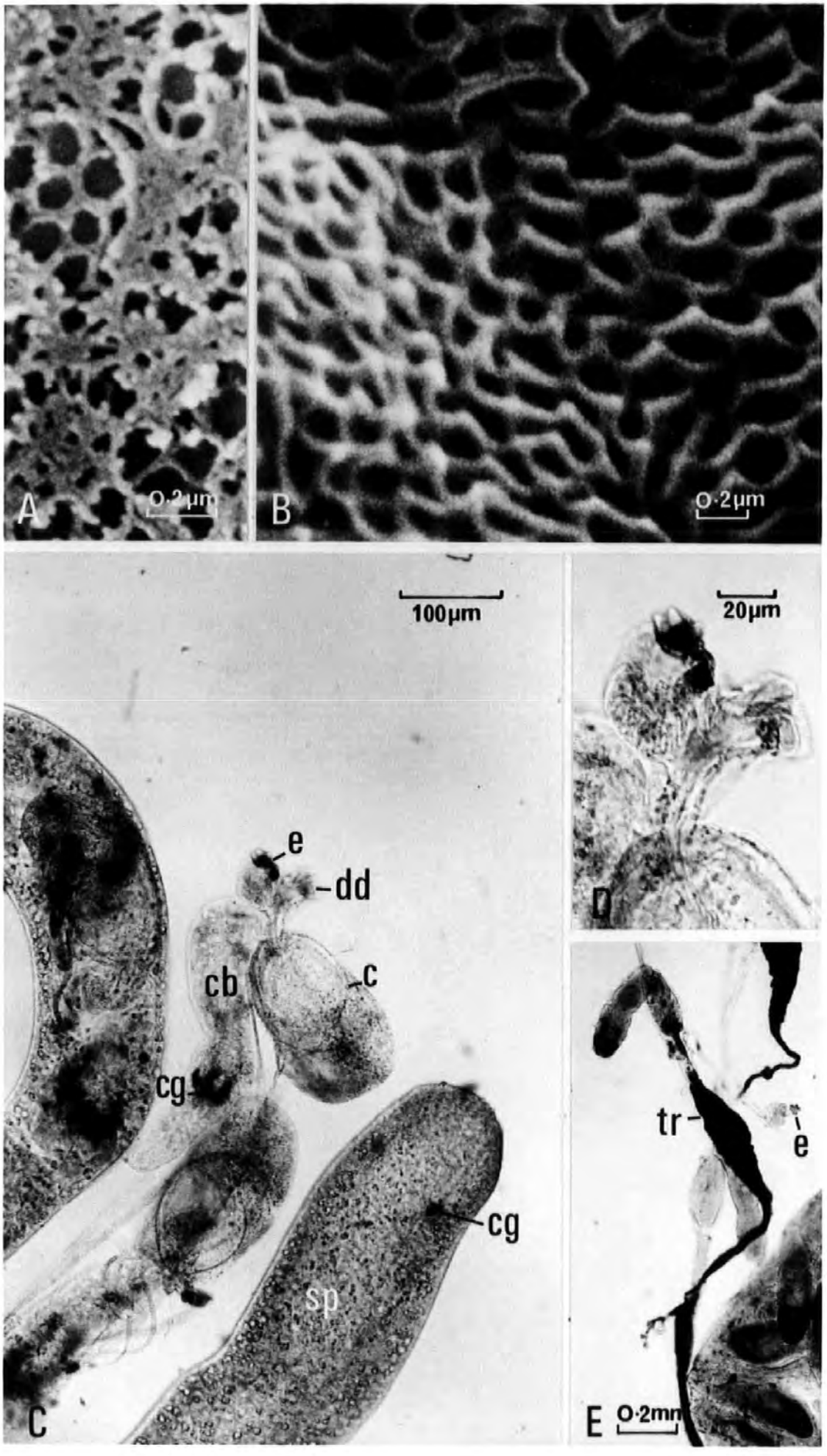

A
0·2µm
B
0·2µm
100µm
e
dd
cb
c
cg
cg
sp
C
20µm
D
tr
e
E
0·2mm

PLATE 21

Cercaria calliostomae : electron micrographs of the intraredial cercaria.

A. Longitudinal section through the cystophorous tail in the region of the retracted delivery tube. Note electron-dense deposits (arrowed) within distal delivery tube lumen.

B. Tail ribbon.

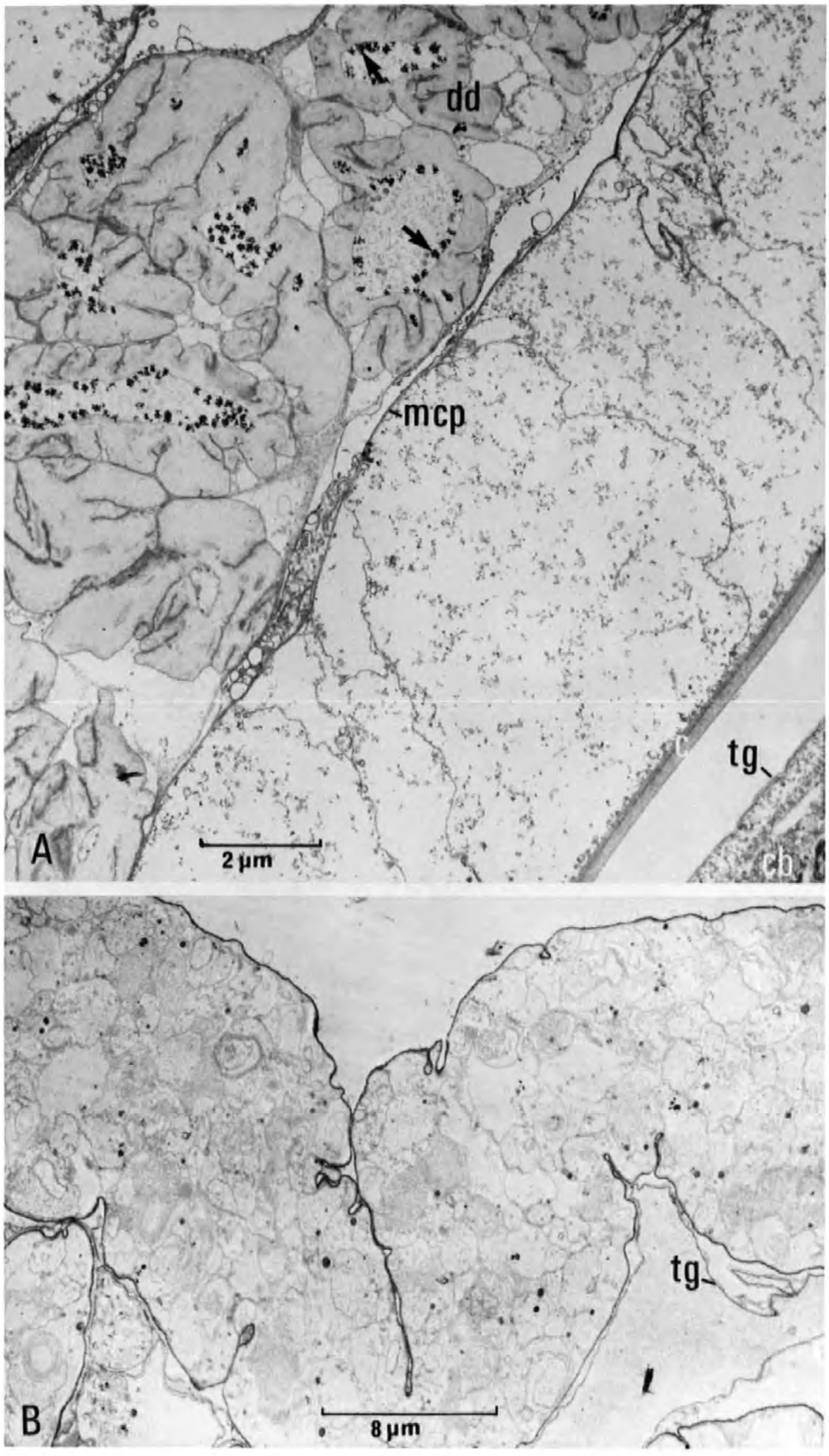

dd
mcp
tg
cb
A
2 µm
tg
B
8 µm

PLATE 22

Cercaria calliostomae : electron micrographs of the intraredial cercaria.

A. Section through folds of retracted proximal delivery tube near to its point of attachment to the transverse membrane.

B. Longitudinal section through fully-formed endpiece, after retraction into caudal cyst.

C. Section through folds of developing delivery tube. Note the curved fibres within the wall, and the folded tegumental basement membrane (arrowed).

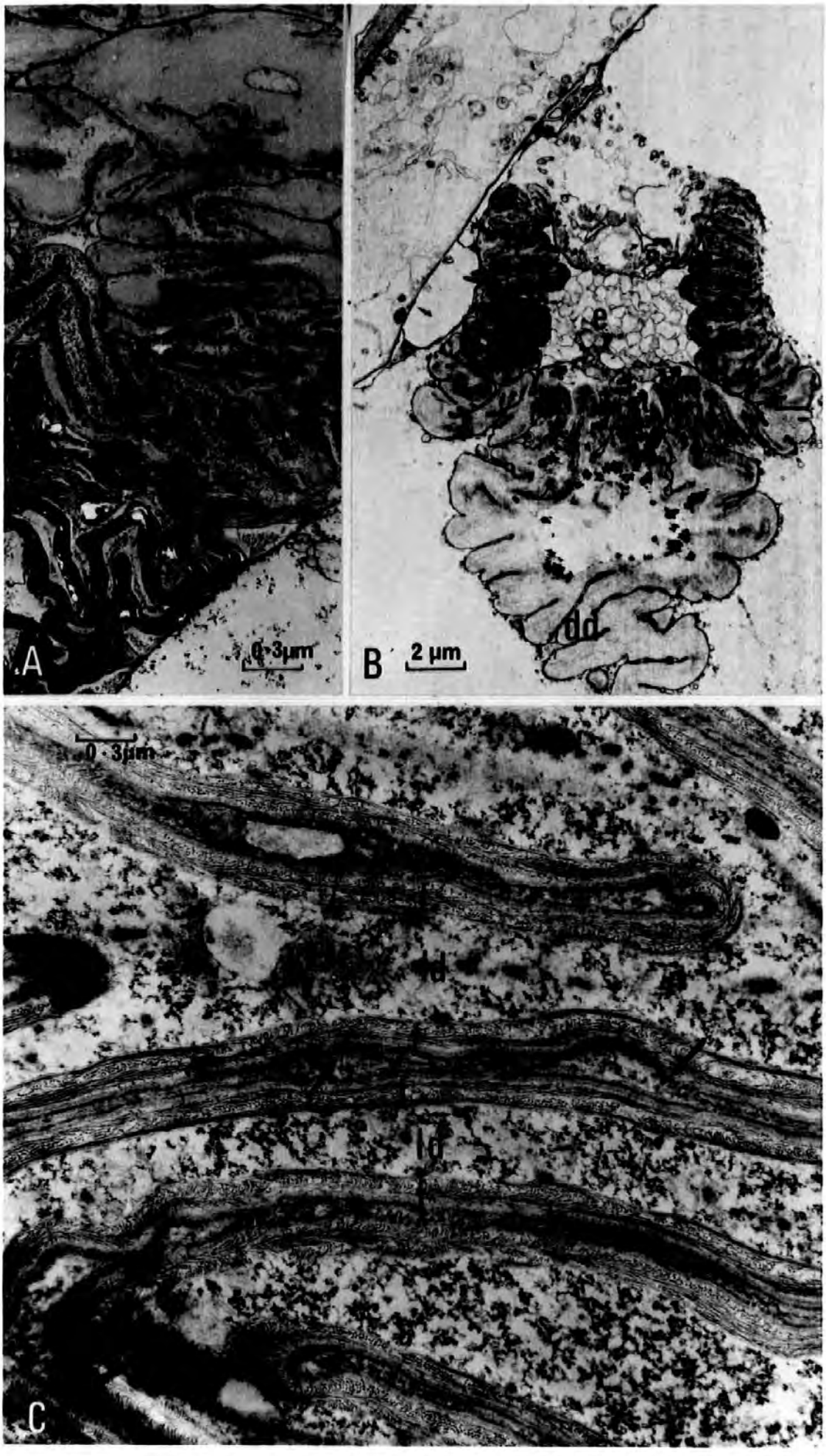

A
0·3μm
B
2 μm
e
dd
0·3μm
d
ld
C

PLATE 23

Cercaria calliostomae : electron micrographs of the caudal cyst of the intraredial cercaria.

A. Longitudinal section showing 5 outer fibrous layers and an inner zone (arrowed).

B. Oblique section through the narrow posterior region of the caudal cyst; inner zone arrowed.

C. Section through the folded developing caudal cyst, showing fibre-forming cells.

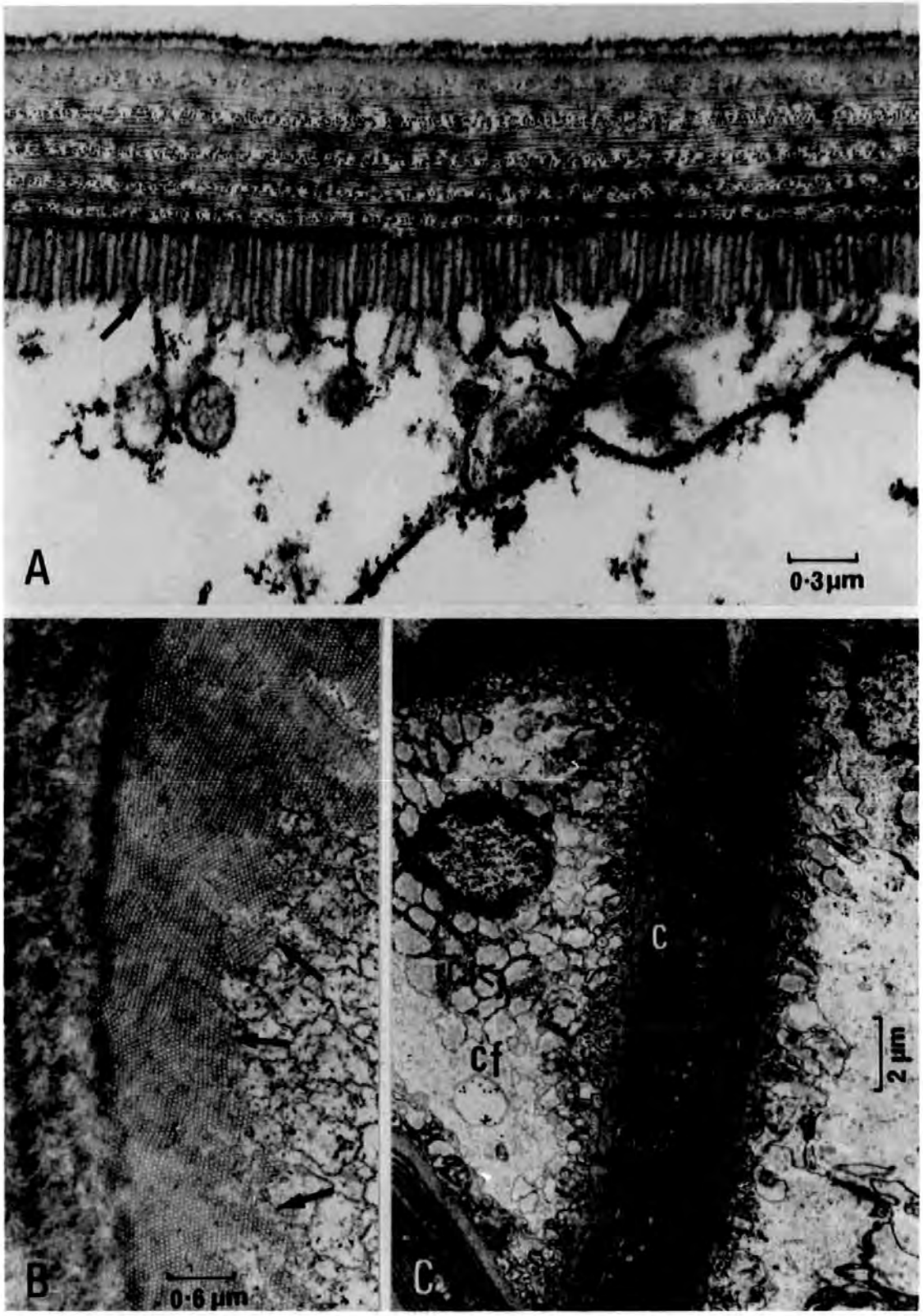

A
0·3µm
B
0·6 µm
C
c
cf
2 µm

PLATE 24

Cercaria vaullegeardi : light micrographs.

A. Metacercaria (arrowed) within the mesosoma of Tigriopus brevicornis, 5 minutes after infection.

B. Naturally-emerged encysted cercariae; note the narrow, curved caudal cyst beak (arrowed) and coiled cercarial body.

C. Encysted cercariae showing the fleshy tegument surrounding the narrow caudal cyst beak, and rounded anterior end of caudal cyst with closed sphincter muscle (arrowed). Both the delivery tube and the cercarial body are coiled within the membranous capsule. Note the entangled caudal filaments.

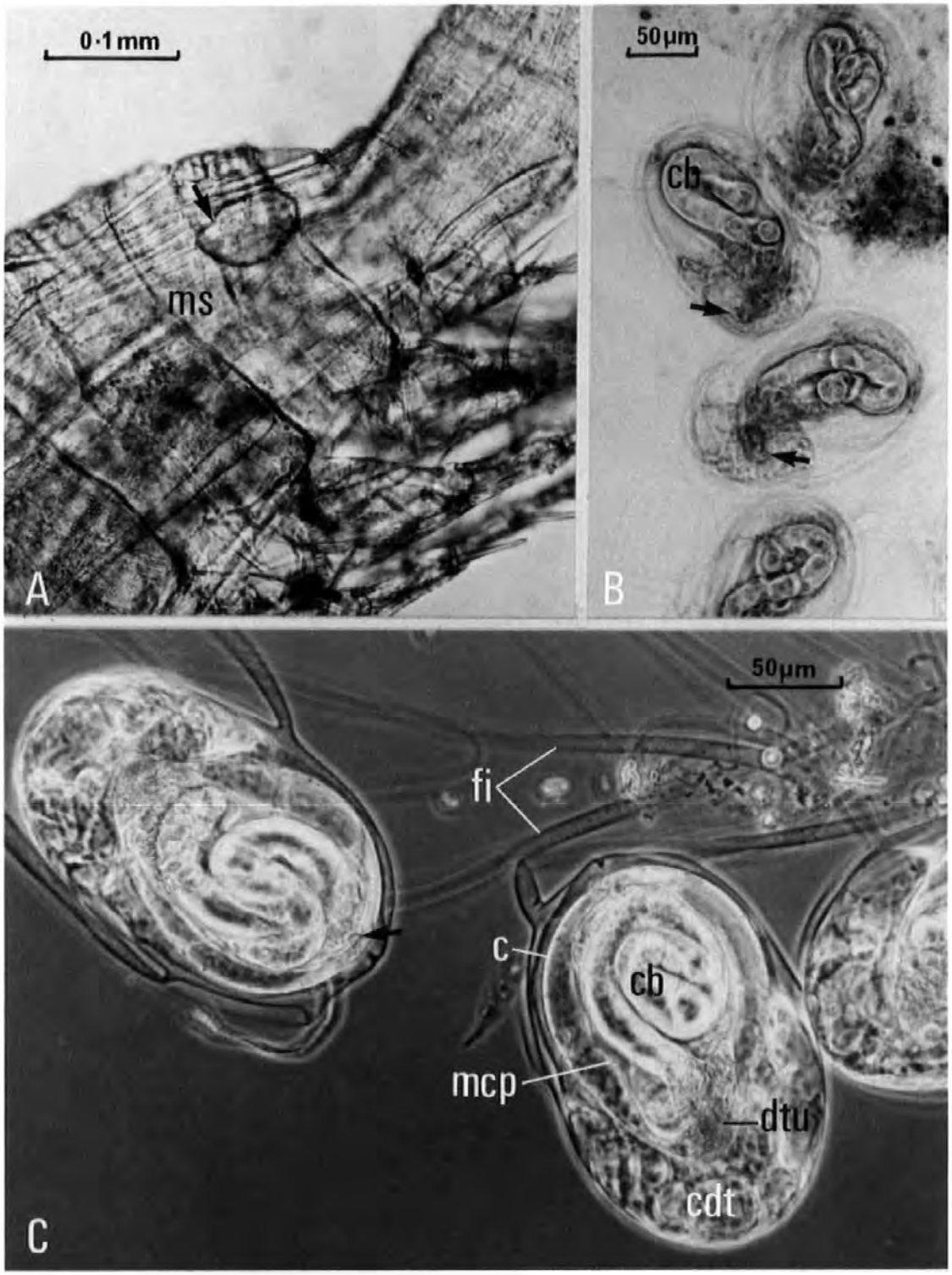

0·1 mm
ms
A
50µm
cb
B
50µm
fi
c
cb
mcp
dtu
cdt
C

PLATE 25

Cercaria vaullegeardi : light micrographs to show in vitro eversal of delivery tube.

A. Distal delivery tube; note torn scraps of fibrous material (arrowed) adhering to the outside of the everted tube.

B. Everted end-piece; note thickened collar supporting aperture.

C. Everted end-piece; note torn fibrous material (arrowed) at junction of end-piece and distal delivery tube.

D. Cercarial body (arrowed) immediately after passage through delivery tube. Shorter cercarial body shows shape assumed within 30 seconds of delivery.

E. Caudal cyst and delivery tube everted by gentle rolling of the coverslip upon a temporary preparation; note wide proximal and narrow distal sections of the tube.

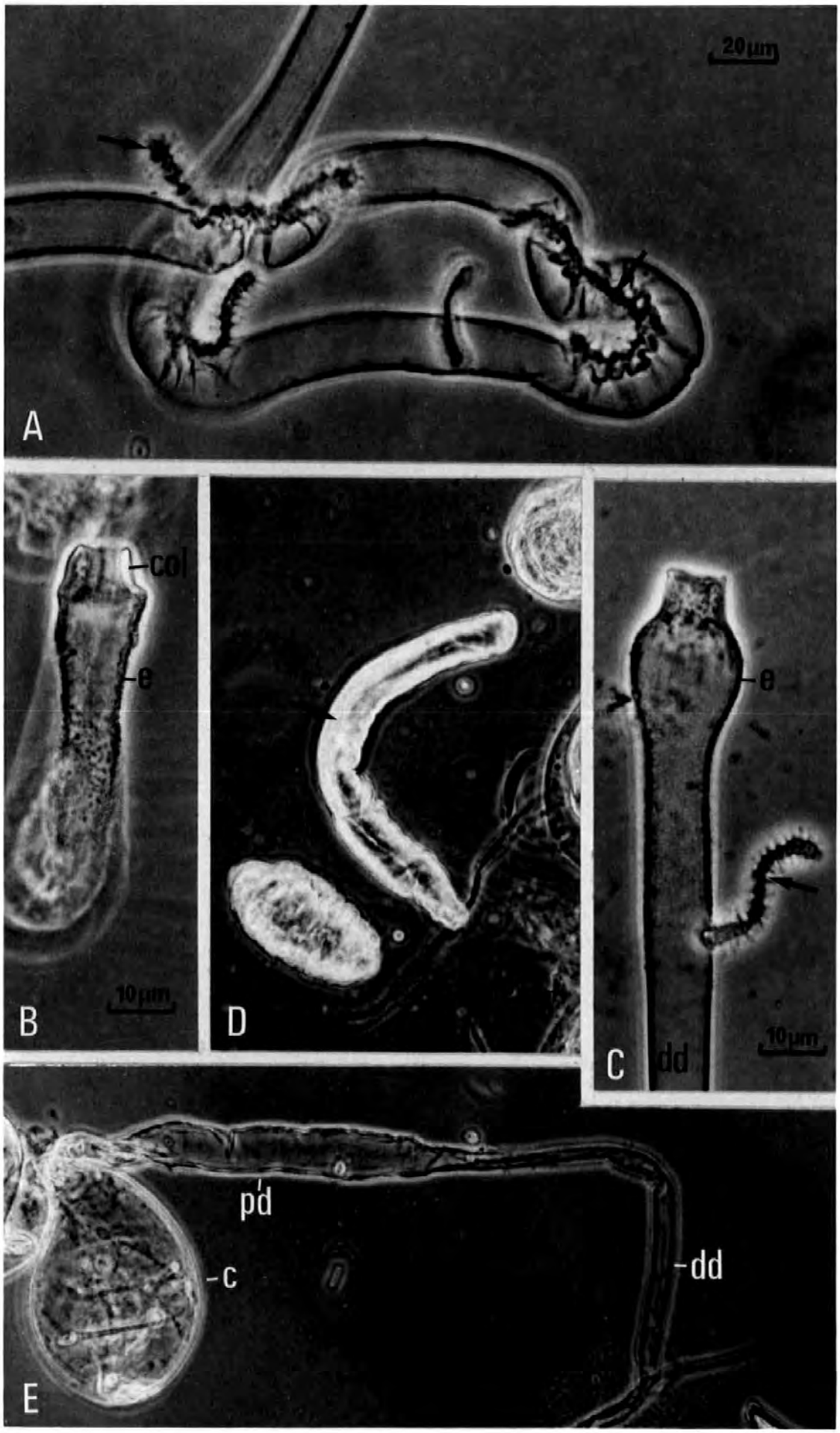

20µm
A
col
e
10µm
B
D
e
dd
10µm
C
pd
c
dd
E

PLATE 26

Cercaria vaullegeardi : electron micrographs of the metacercaria within the haemocoel of Tigriopus brevicornis.

A. Metacercarial tegument; fixed in situ less than 1 minute after infection. Note disrupted copepod sarcolemma (arrowed), muscle fibres and mitochondria.

B. Metacercarial tegument, enlargement of A. Secretory vesicles have been discharged at the surface membrane (arrowed).

C,D. Metacercarial tegument; fixed in situ 7 days after infection. Note pinocytotic vesicles within metacercarial tegument (arrowed) and accumulation of glycogen.

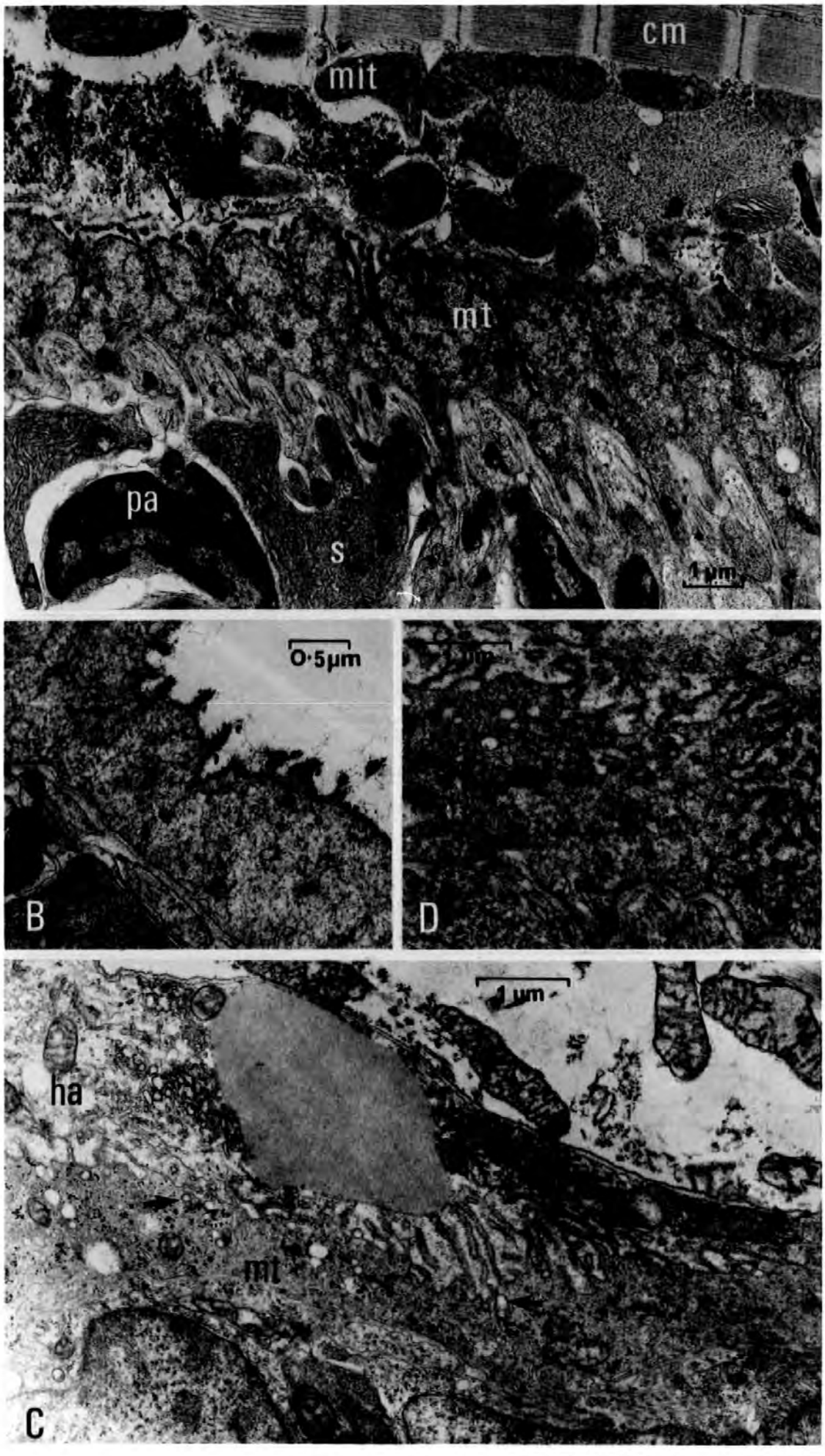

cm
mit
mt
pa
s
1 µm
0·5µm
B
D
1 µm
ha
mt
C

PLATE 27

Cercaria vaullegeardi. Electron micrographs of the metacercaria raised in Tigriopus brevicornis; 21 days after infection/17°C.

A. Oblique section through posterior region of the body, near the junction of the posterior excretory vessel with the excretory vesicle.

B. Transverse section through posterior excretory duct wall.

C. Oblique section through wall of the intestinal caecum.

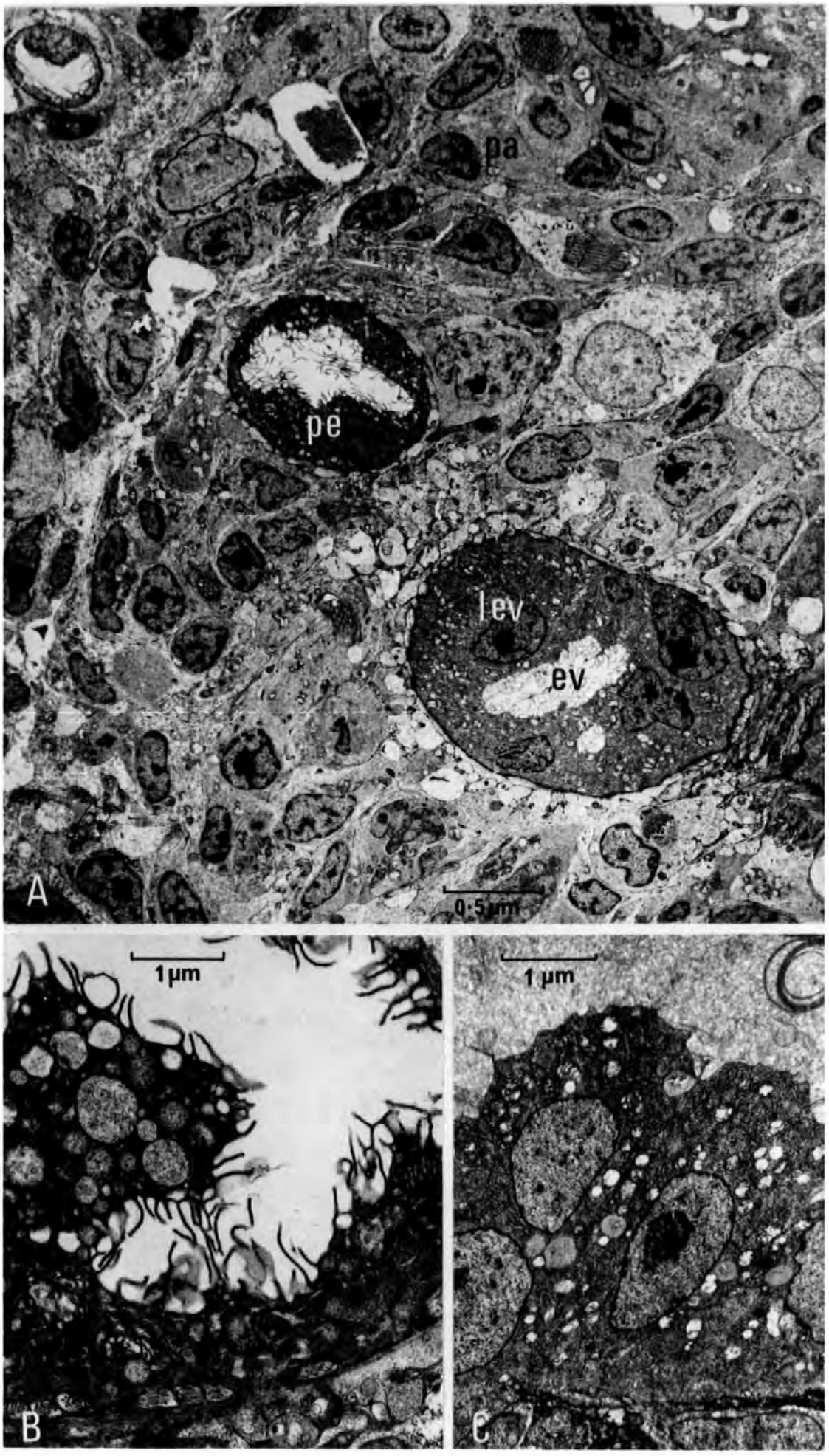

pa
pe
lev
ev
A
0·5 µm
1µm
B
1 µm
C

PLATE 28

Cercaria vaullegeardi. Electron micrographs of the metacercaria in situ within Tigriopus brevicornis; 21 days after infection/17°C.

A. Oblique longitudinal section through posterior region showing the everted lining of the excretory vesicle.

B. Longitudinal section through posterior region of the metacercaria, showing splits (arrowed) in the parenchyma which allow complete eversion of the excretory vesicle, or developing ecsoma, to the exterior.

C. Sub-tegumental cells showing biconcave electron-dense secretory bodies (arrowed) and RER.

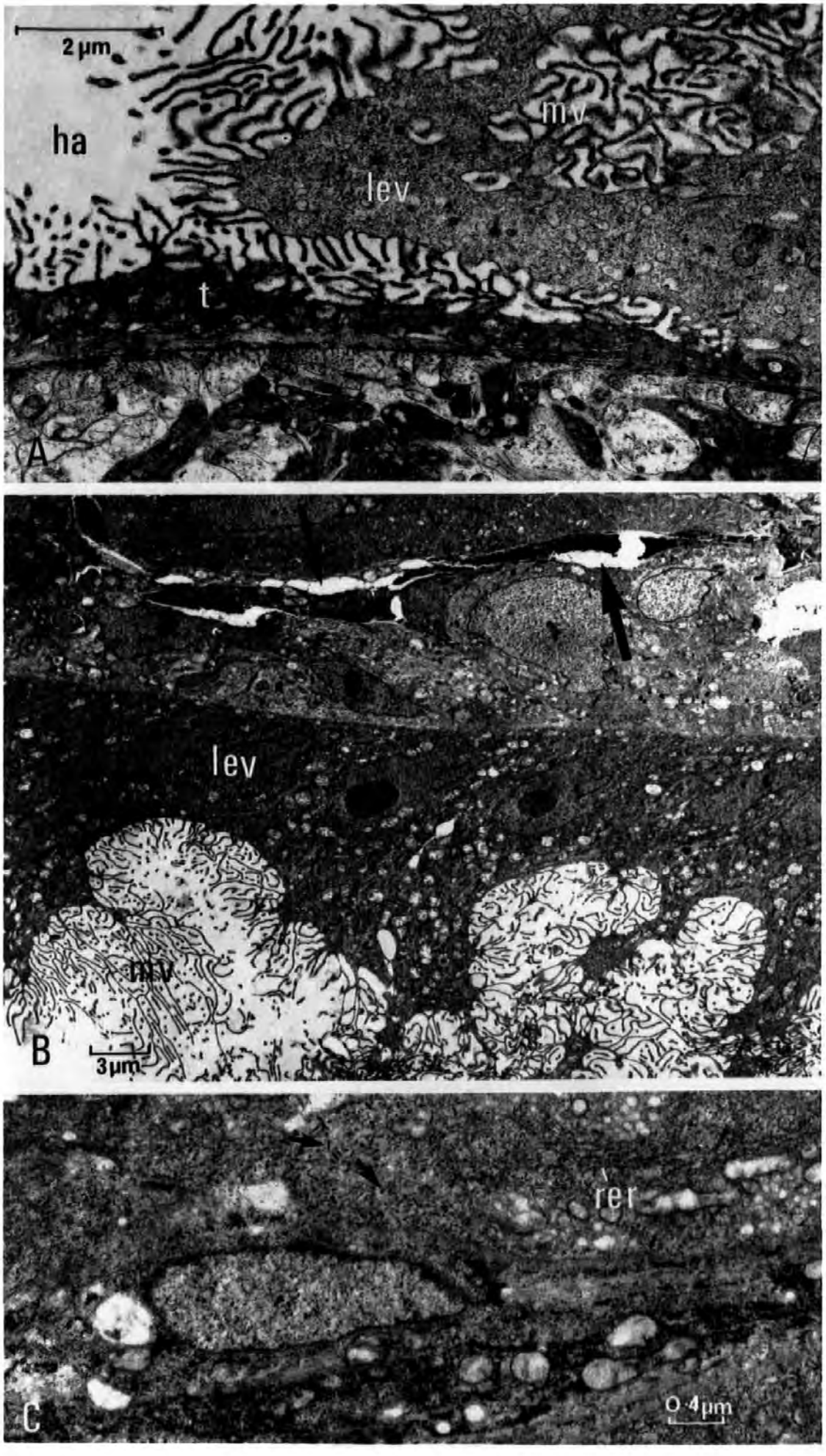
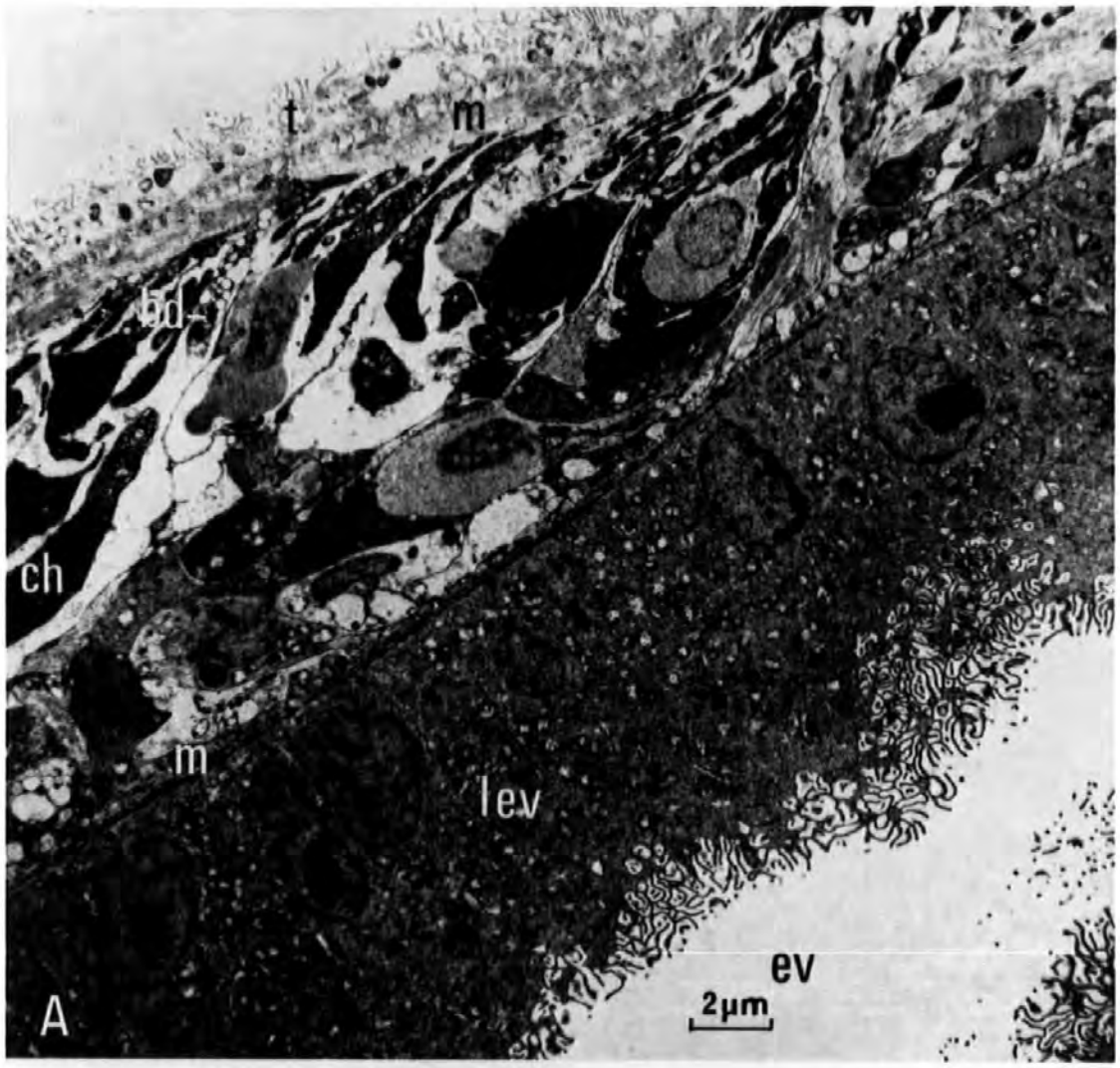

2 µm
ha
mv
lev
t
A
lev
mv
B
3 µm
rer
C
0·4 µm

PLATE 29

Cercaria vaullegeardi. Electron micrographs of the metacercaria raised in Tigriopus brevicornis; 21 days after infection/17°C.

A. Longitudinal section through the posterior region of the metacercaria; note the disparity in development between the somatic tegument and the thickened lining of the excretory vesicle.

B. Somatic tegument, longitudinal section. Note pinocytotic vesicles (arrowed), and unbranched microvilli; biconcave electron-dense secretory bodies within sub-tegumental cells.


t
m
bd
ch
m
lev
ev
2μm
A

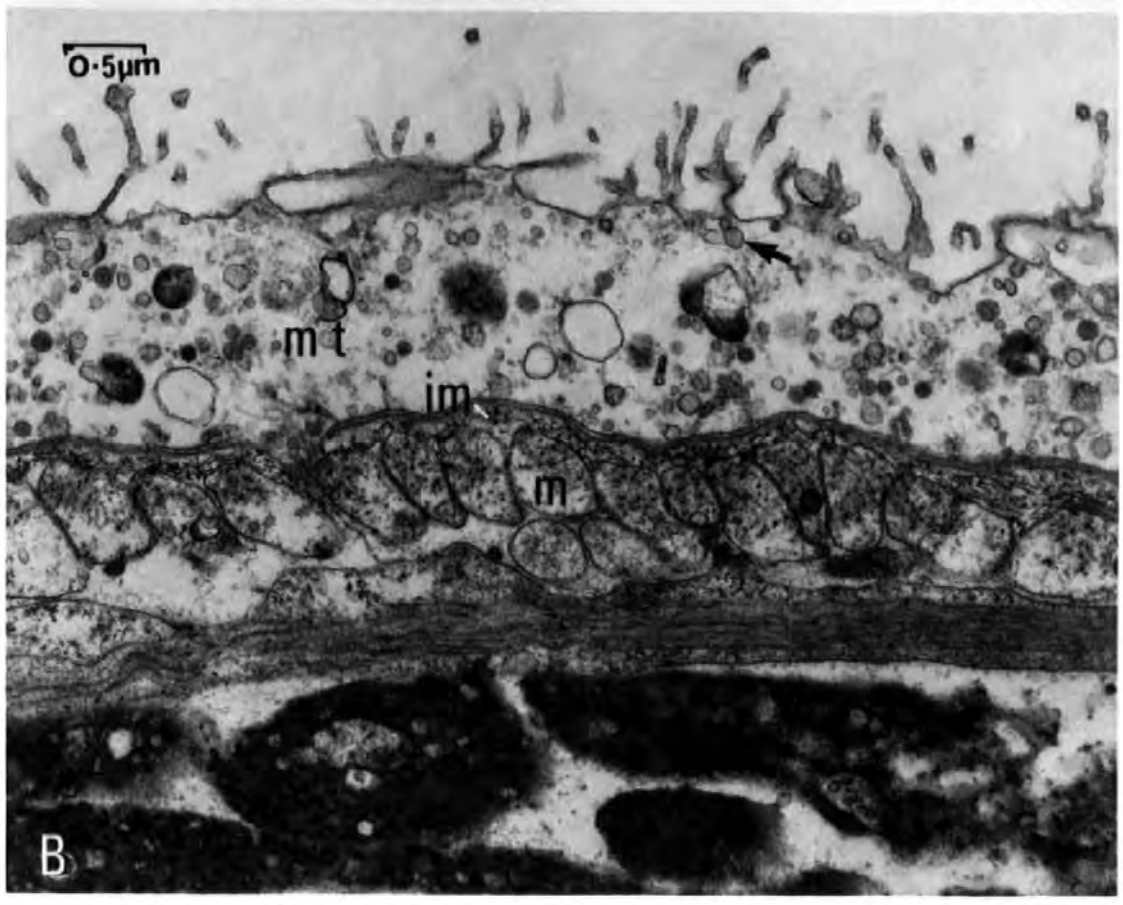

0·5μm
mt
im
m
B

PLATE 30

Cercaria vaullegeardi : electron micrograph of longitudinal section through metacercarial excretory vesicle wall. Metacercaria raised in Tigriopus brevicornis; 21 days after infection/17°C. Note branched microvilli and folded basement membrane (arrowed).

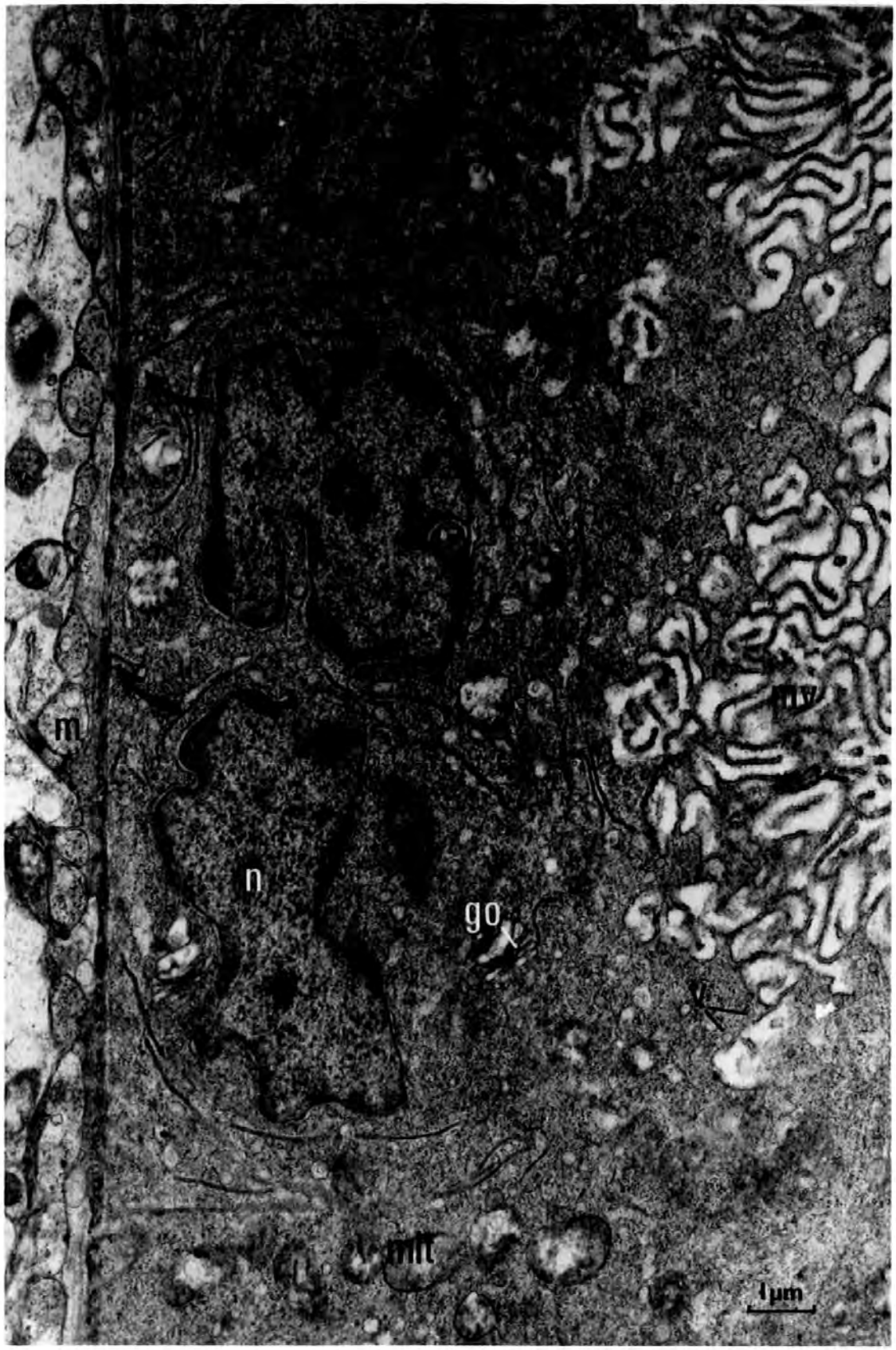

m
n
go
mv
mit
1 µm

PLATE 31

Metschnikowia spp; a parasitic yeast. Natural infections of *Tigriopus brevicornis*.

A,B. Light micrographs of single bud (arrowed in B) and 2-bud (arrowed in A) stages and mature ascospores, removed from the haemocoel of an infected copepod.

C-E. Electron micrographs of ascospores *in situ* within the haemocoel of *T. brevicornis*.

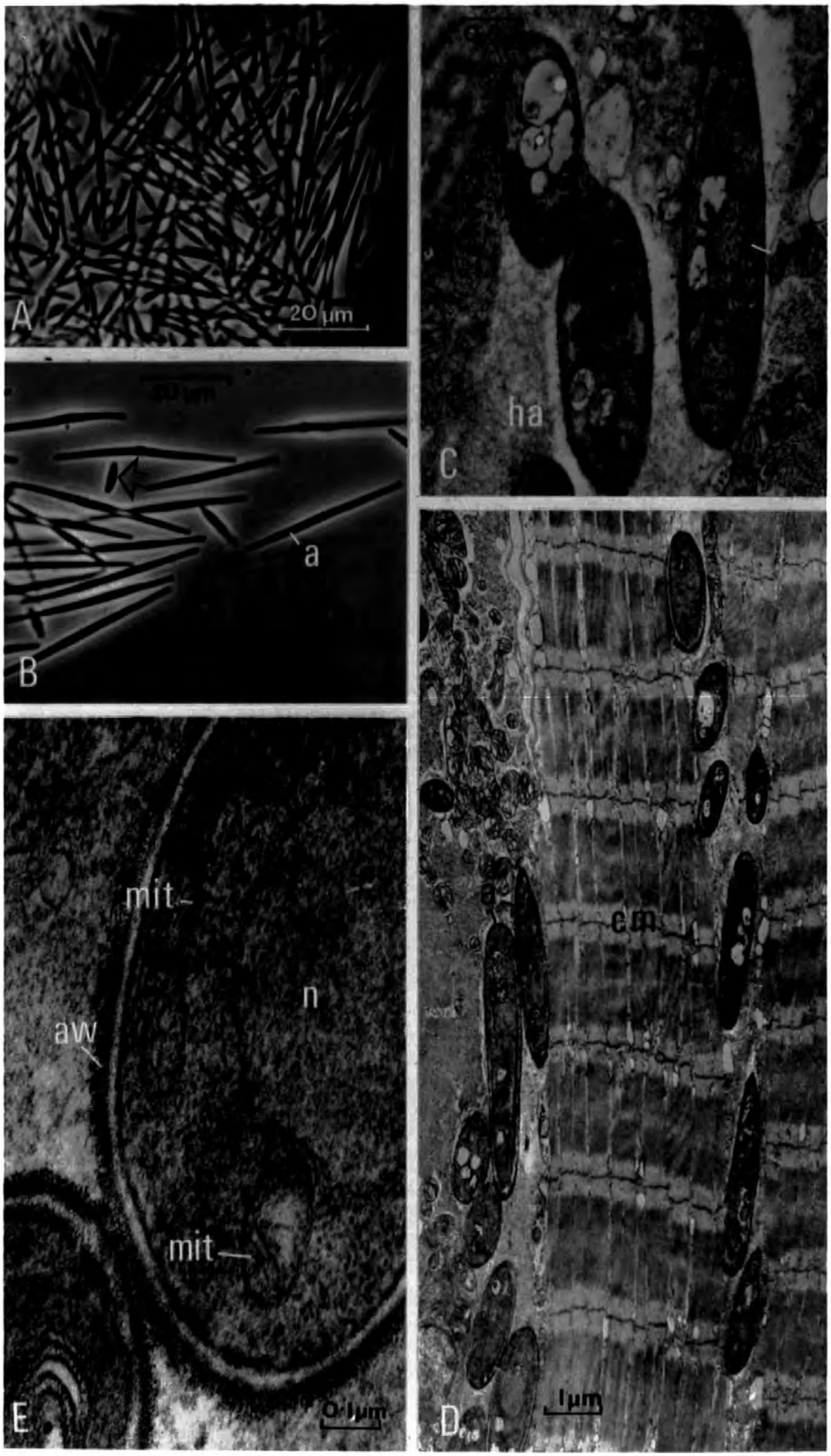
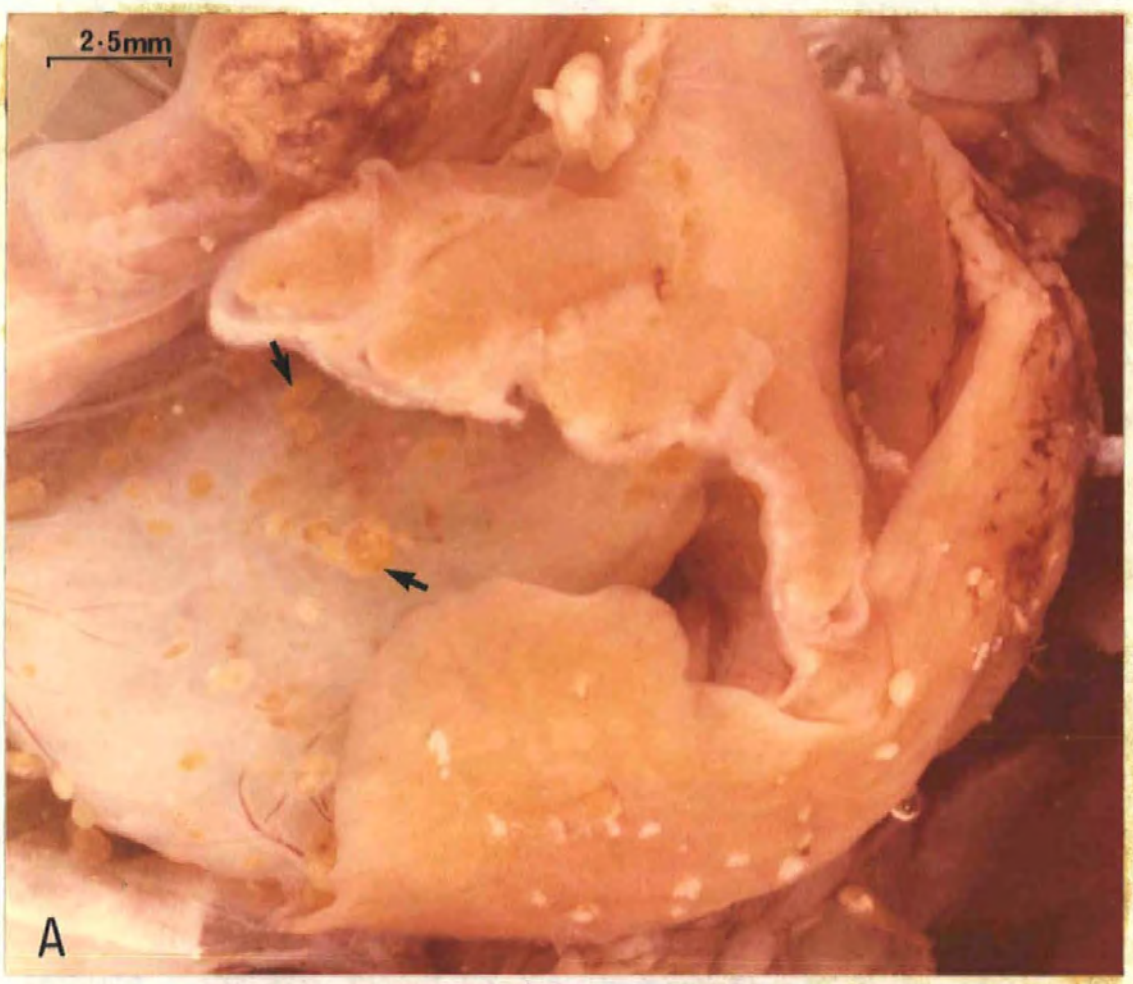

A
20 µm
B
a
C
ha
D
cm
1µm
E
mit
n
aw
mit
0·1µm

PLATE 32

Encapsulated hemiurid metacercariae within the body cavity of Taurulus bubalis; natural infection.

A. Distribution; the parasites (arrowed) are attached to the membranes covering the stomach, the liver and intestine being unaffected.

B. Enlargement of the stomach, showing the majority of the metacercariae to be attached to the outer surface, some being more deeply embedded within the muscle layers (arrowed).


2·5mm
A

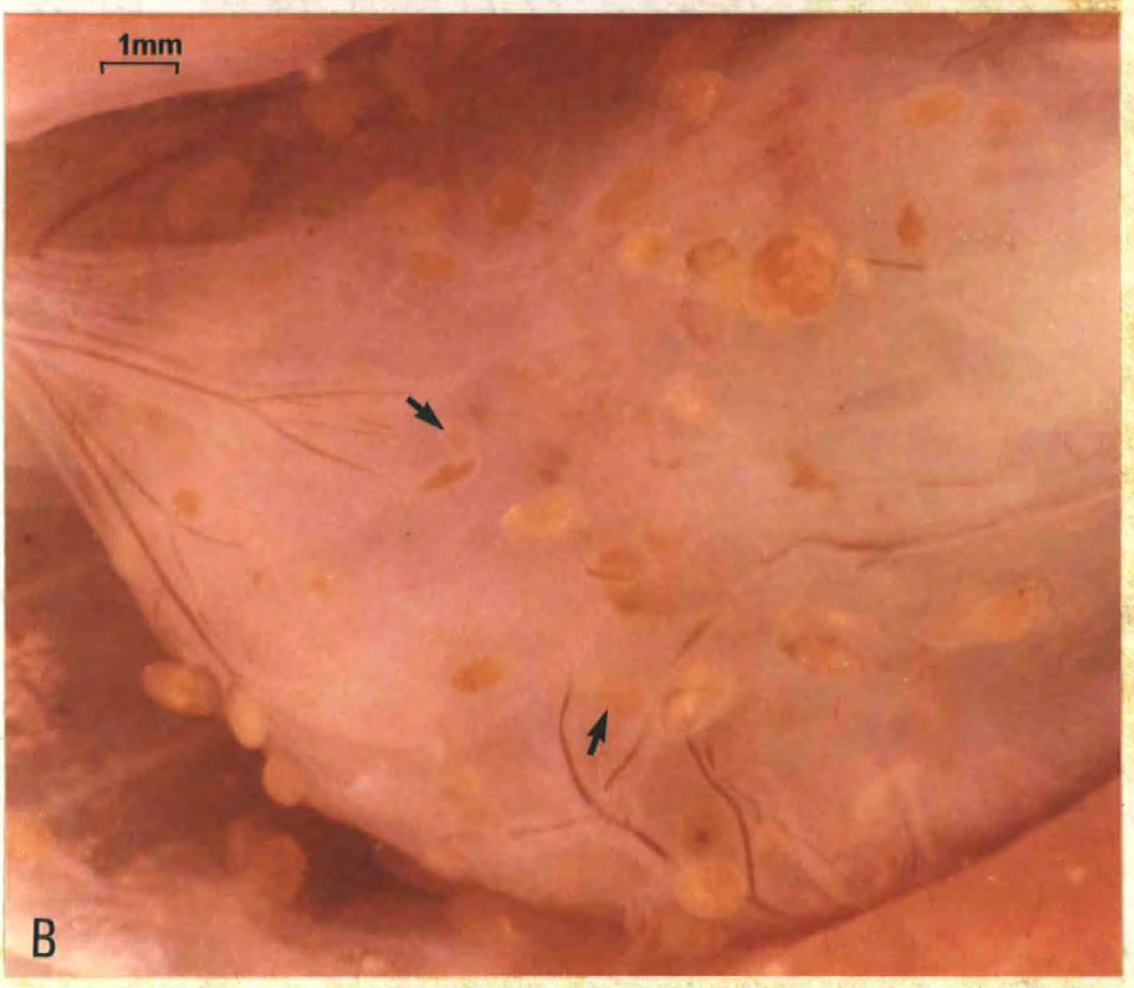

1mm
B

PLATE 33

Encapsulated hemiurid metacercariae within the body cavity of Gobius paganellus. Natural infections.

A. Light micrograph of longitudinal section through encapsulated metacercaria.

B. Electron micrograph of somatic tegument showing biconcave electron-dense secretory bodies.

C,D. Electron micrographs of ecsomatic tegument.

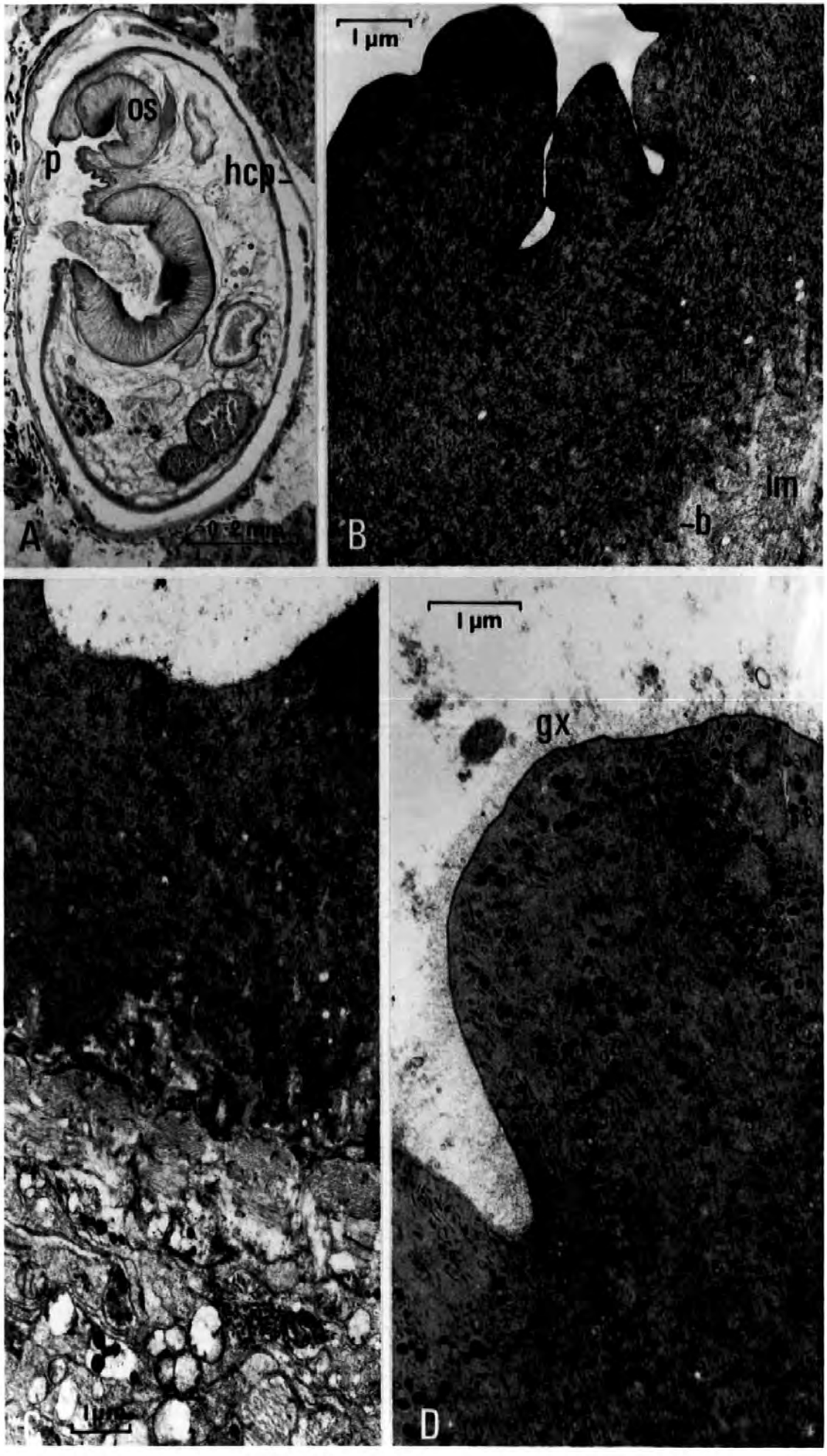

os
p
hcp
A
0·2 mm
1 µm
B
lm
b
1 µm
gx
D

PLATE 34

Electron micrographs of a recently-formed host capsule surrounding a hemiurid metacercaria within the body cavity of Gobius paganellus. Natural infection.

A. Outer surface; note flattened cells with RER, and lipid droplets (arrowed).

B. Inner surface. The innermost cells have been broken down, probably by the action of the metacercaria during feeding. Note fibres (arrowed) and desmosomes.

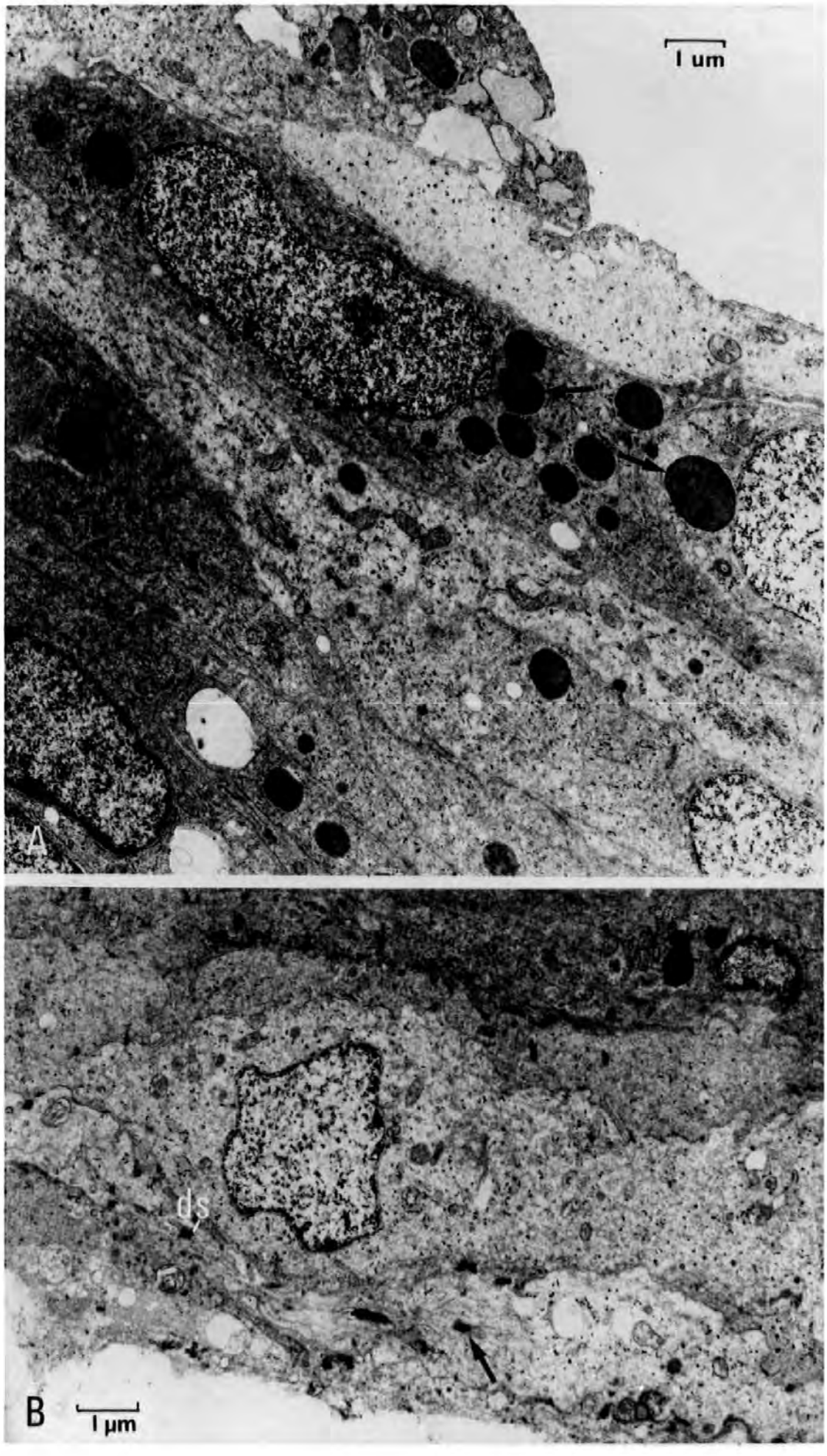

1 um
A
ds
B
1 µm

PLATE 35

Lecithochirium fusiforme : electron micrographs of the adult tegument.

A. Somatic tegument; note electron-dense surface layer, and intensely-folded basement membrane (arrowed).

B,C. Ecsomatic tegument; note papillate surface with prominent glycocalyx of droplet appearance, and the absence of mitochondria and an electron-dense surface layer.

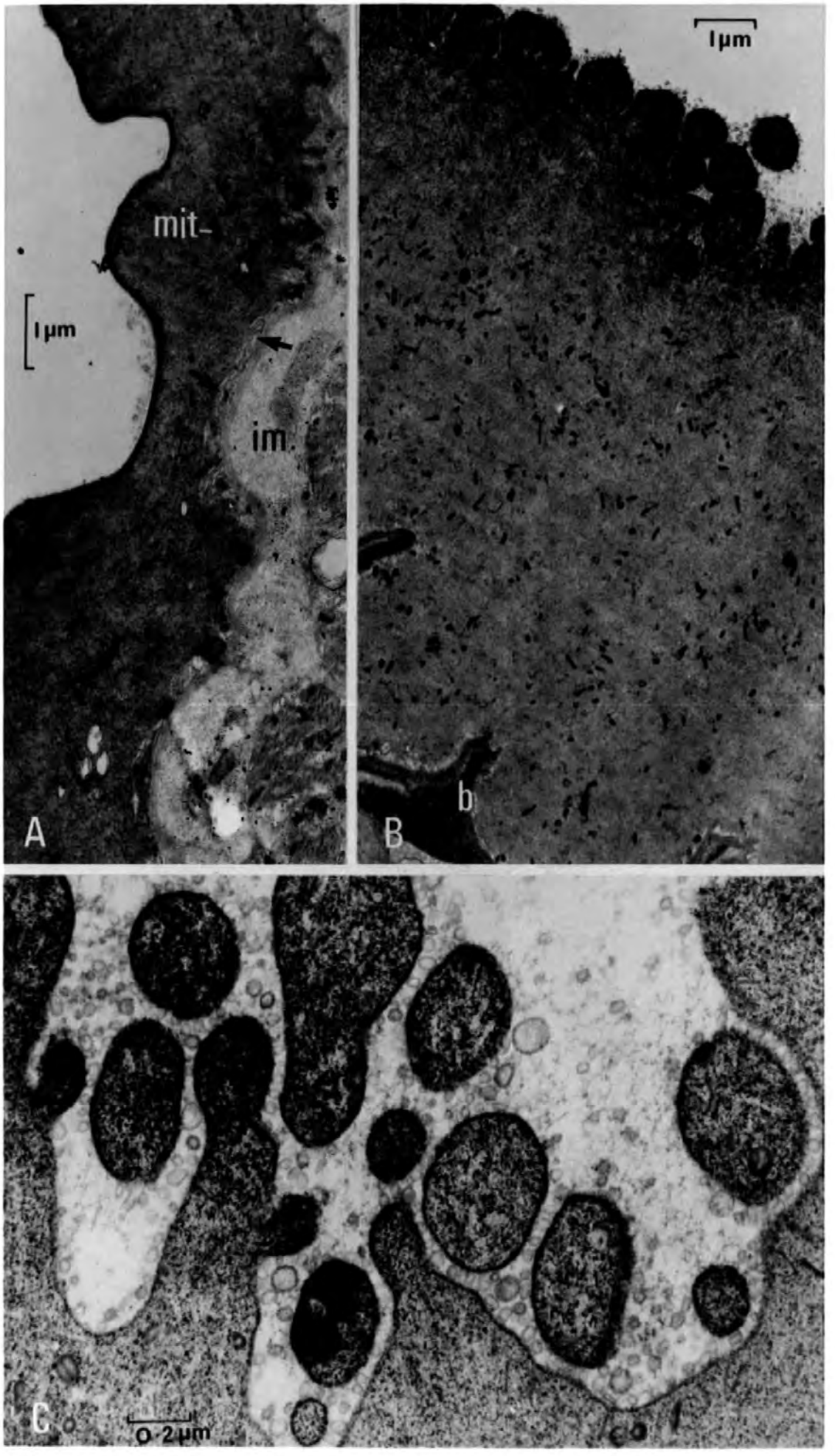

mit-
1µm
im
A
1µm
b
B
C
0·2µm

PLATE 36

Lecithochirium fusiforme : electron micrographs of the adult ecsomatic parenchyma.

A. Sub-tegumental cells with biconcave electron-dense secretory bodies, neurons, and general parenchyma.

B. Enlargement of Golgi and secretory bodies within sub-tegumental cells.

C. Parenchyma; note intercellular junctions, and tubular invaginations of bounding cell membranes.

D. Enlargement of tubular invaginations (arrowed) of bounding cell membranes.

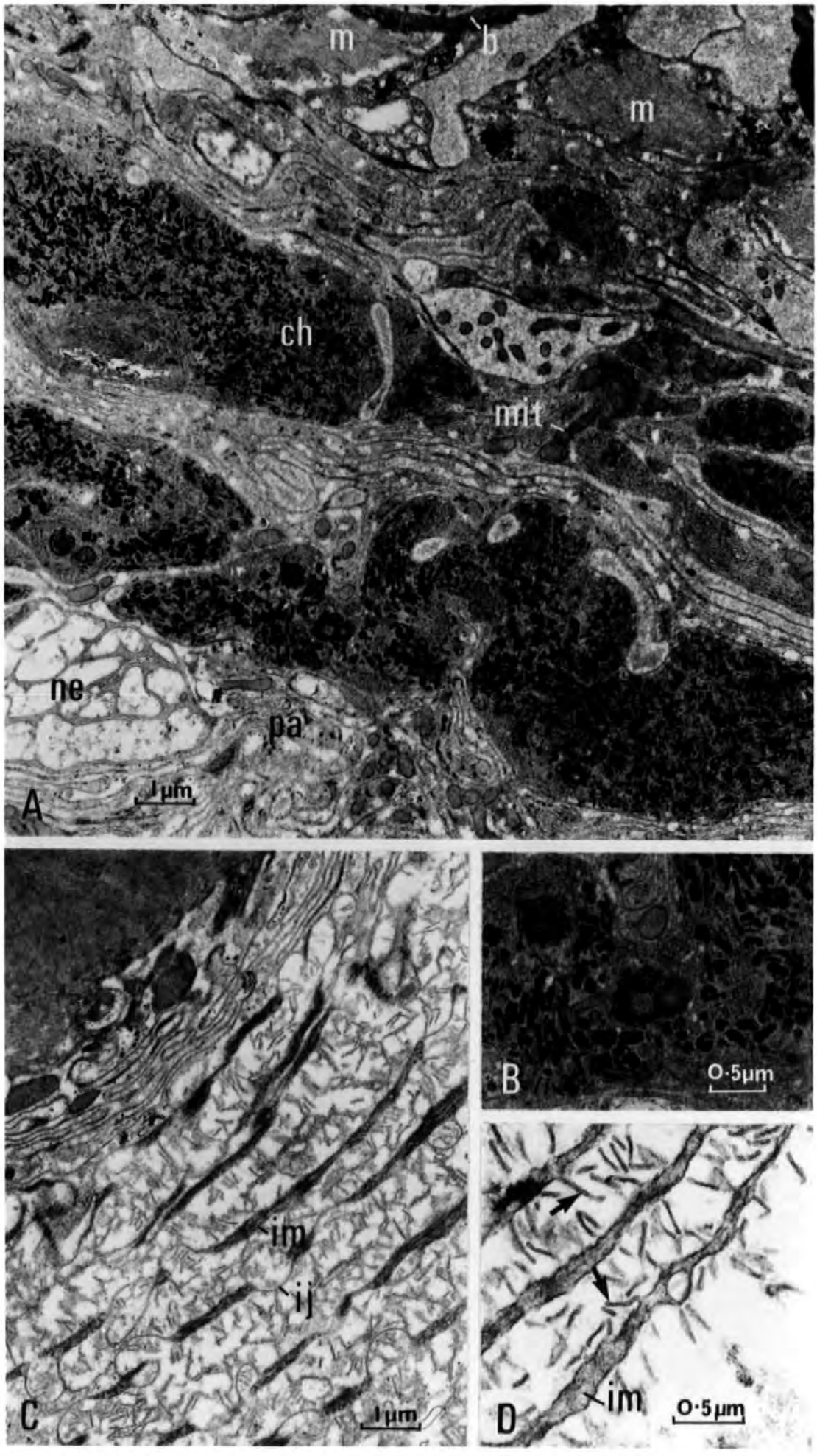

m
b
m
ch
mit
ne
pa
1 µm
A
B
0·5µm
im
ij
1 µm
C
D
im
0·5µm

PLATE 37

A,B. Hemiurus communis : electron micrographs of adult.

A. Somatic and ecsomatic tegument. Note papillate surface of the latter, and lack of mitochondria. Somatic tegument has an electron-dense surface layer, and mitochondria.

B. Oblique section through ecsoma showing lining of central excretory duct (arrowed), and parenchyma with numerous large mitochondria.

C. Lecithaster gibbosus : electron micrograph of longitudinal section through adult tegument. Note dense fibrous desmosome-like structure (arrowed) extending from basement membrane to muscle layer.

D,E. Lecithochirium furcolabiata : electron micrographs of adult.

D. Somatic tegument.

E. Tegument of retracted ecsoma. Note intensely folded basement membrane and lamina (arrowed).

F. Hemiurus communis : electron micrographs of sections through egg capsules showing them to be covered with tufts of hair-like structures (arrowed).

PLATE 37 (CONTD.)

G. Lecithochirium furcolabiata : light micrograph of egg capsule showing posterior knob.

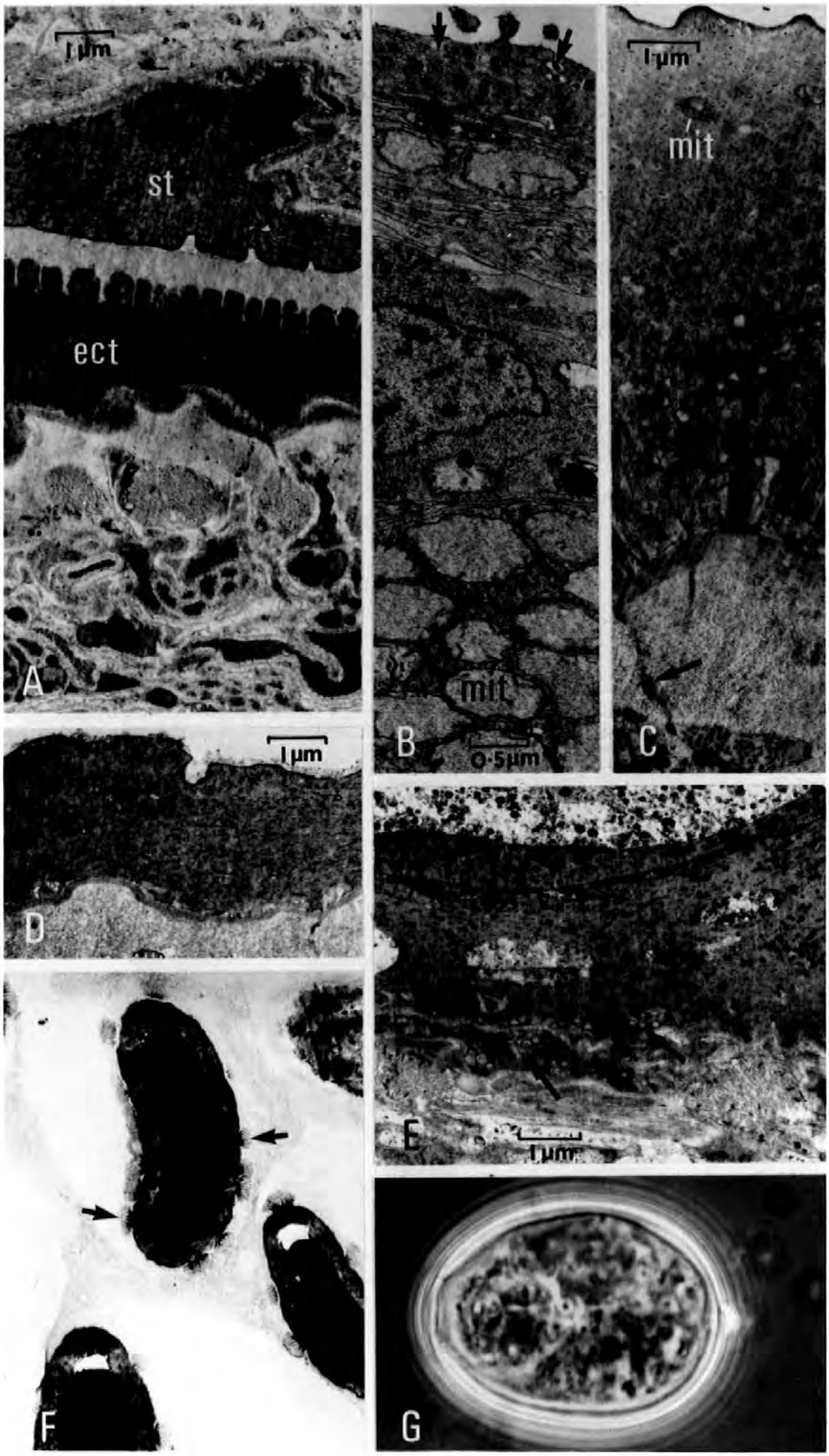

1 µm
st
ect
A
mit
B
0·5µm
1 µm
mit
C
1 µm
D
E
1 µm
F
G

PLATE 38

Lecithochirium furcolabiata : Living adults upon the liver and pyloric caeca within the body cavity of Ciliata mustela.

A. General distribution within the body cavity.

B. Enlargement to show haemorrhaged liver surface, and pigment deposits upon the peritoneal membranes between the pyloric caeca.

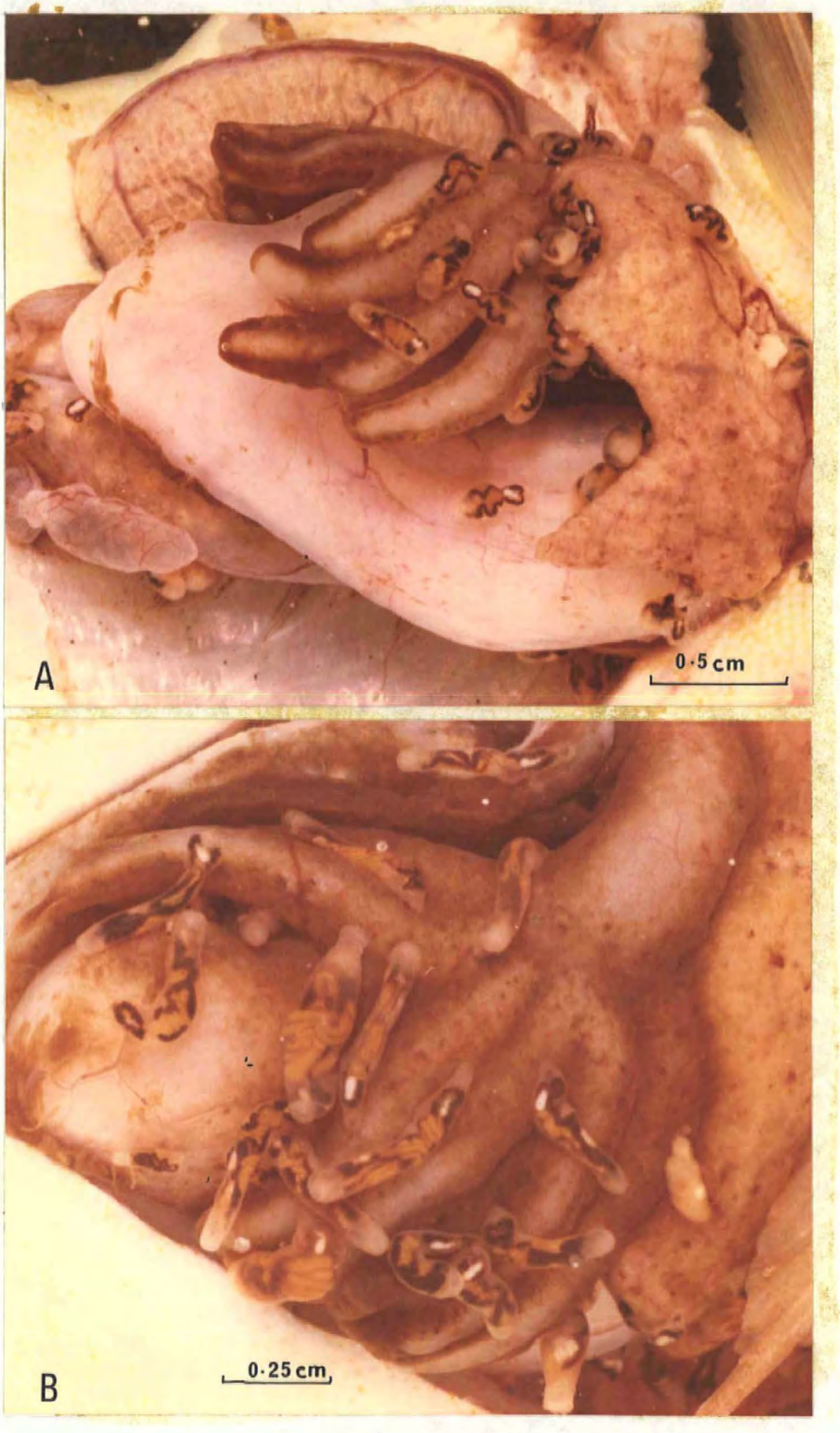

A
0·5 cm
B
0·25 cm

PLATE 39

Lecithochirium furcolabiata : scanning electron micrographs of the adult.

A. Anterior end showing oral sucker with papillae, and pre-oral lobe with prominent "knobs".

B. Surface of pre-oral lobe "knob", enlarged; tegumental pores (arrowed) may be artefacts.

C. Posterior end of parasite showing fully-extended ecsoma.

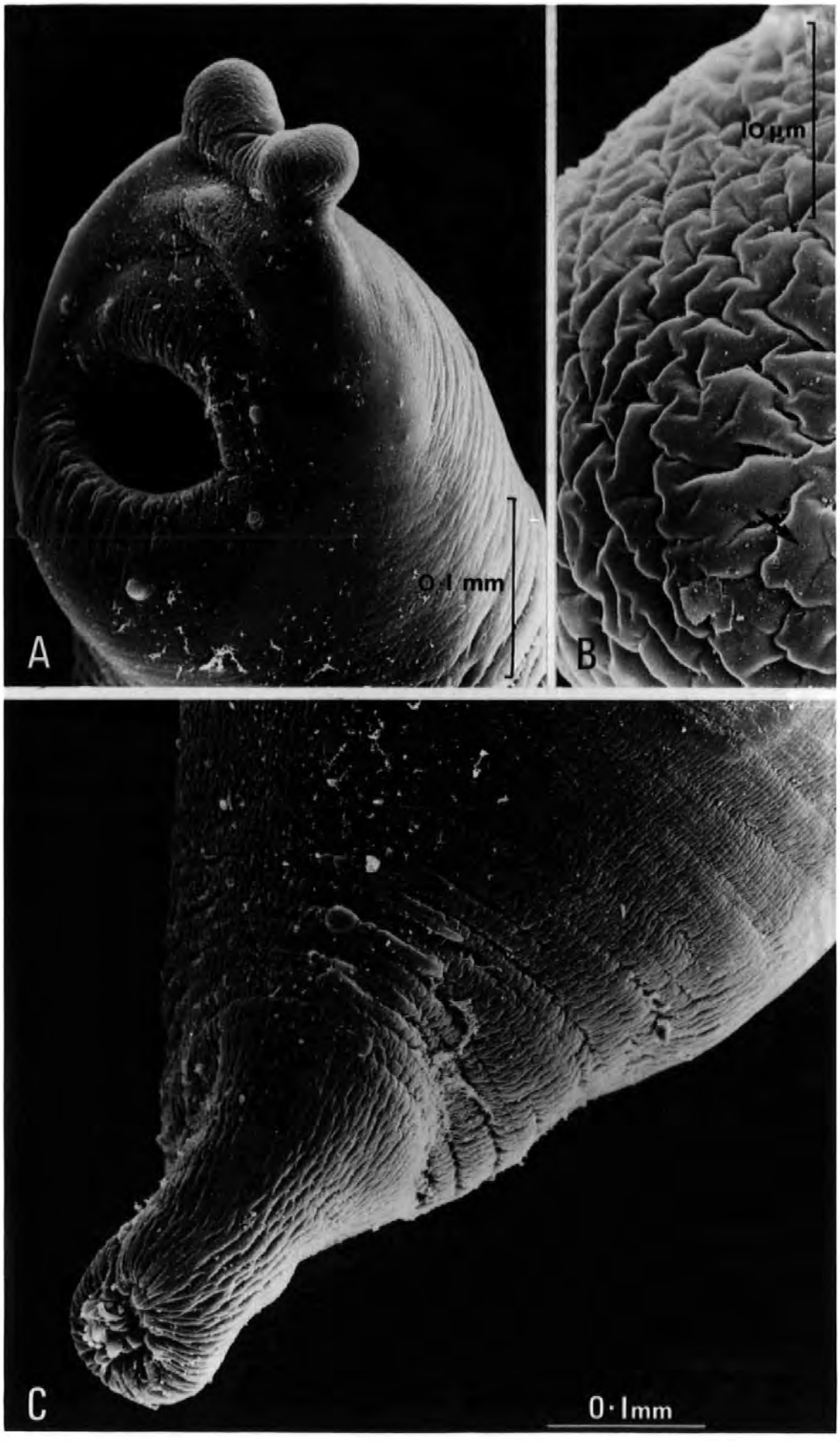

A
0·1 mm
B
10 µm
C
0·1mm

PLATE 40

Lecithochirium furcolabiata : electron micrographs of adult pre-oral lobe.

A. Parenchyma cell showing the cytoplasm divided into peri-nuclear zone with electron-lucent vesicles, wide granular central zone with mitochondria, and narrow clear outer zone with infolded cell bounding membrane.

B. Enlargement of the junction between peri-nuclear and middle zones to show concentric whorl of RER.

C. Parenchyma cell containing crystalline material (arrowed).

D. Enlargement of crystalline material shown in C.

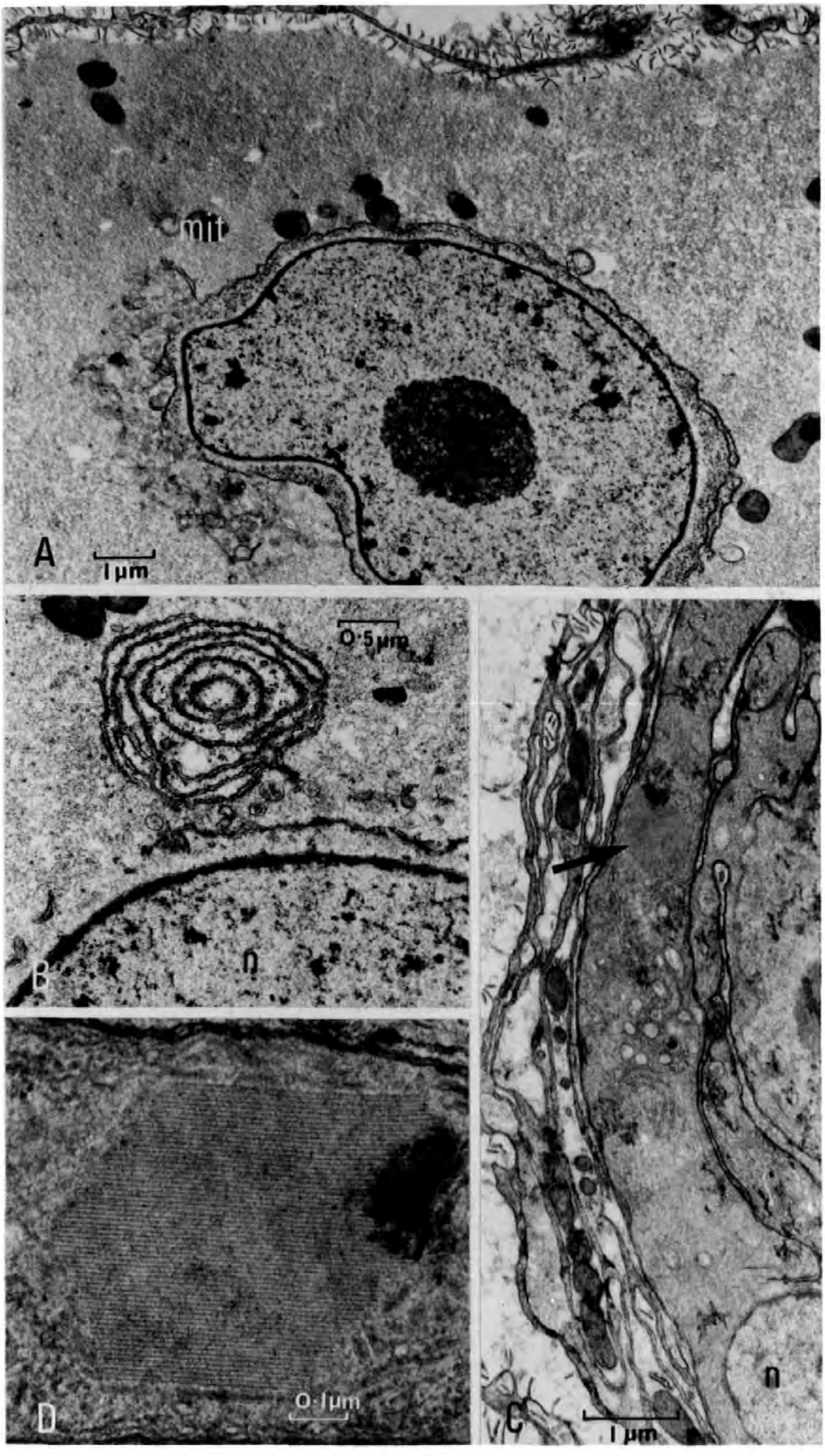

mit
A
1 µm
0·5 µm
n
B
C
1 µm
n
D
0·1 µm

PLATE 41

Lecithochirium furcolabiata : electron micrographs of adult pre-oral lobe.

A. Cell of unknown function, possibly neurosecretory. The cytoplasm is divided (arrowed) close to the nuclear membrane into radiating processes.

B. Edge of the nucleus of the cell shown in A, enlarged; note dense connections to the interstitial material at the cytoplasmic divisions (arrowed).

C. Branching neurons with electron-lucent and electron-dense vesicles.

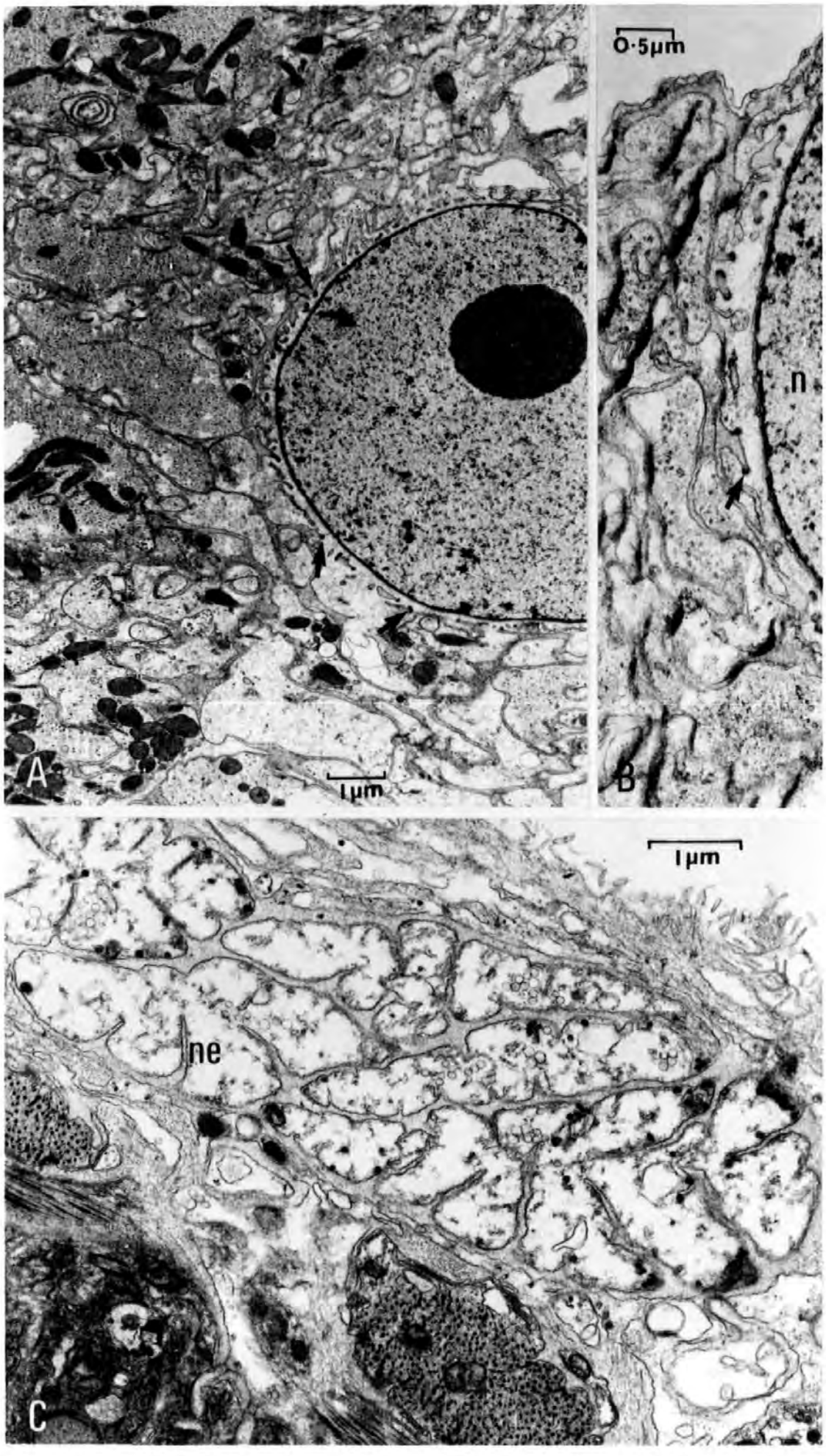

A
1μm
0·5μm
n
B
1μm
ne
C

PLATE 42

A,B. Cercaria vaullegeardi. Metacercariae raised in Tigriopus brevicornis (21 days/17°C). Light autoradiographs; 1 µm resin longitudinal sections (methylene blue stained).

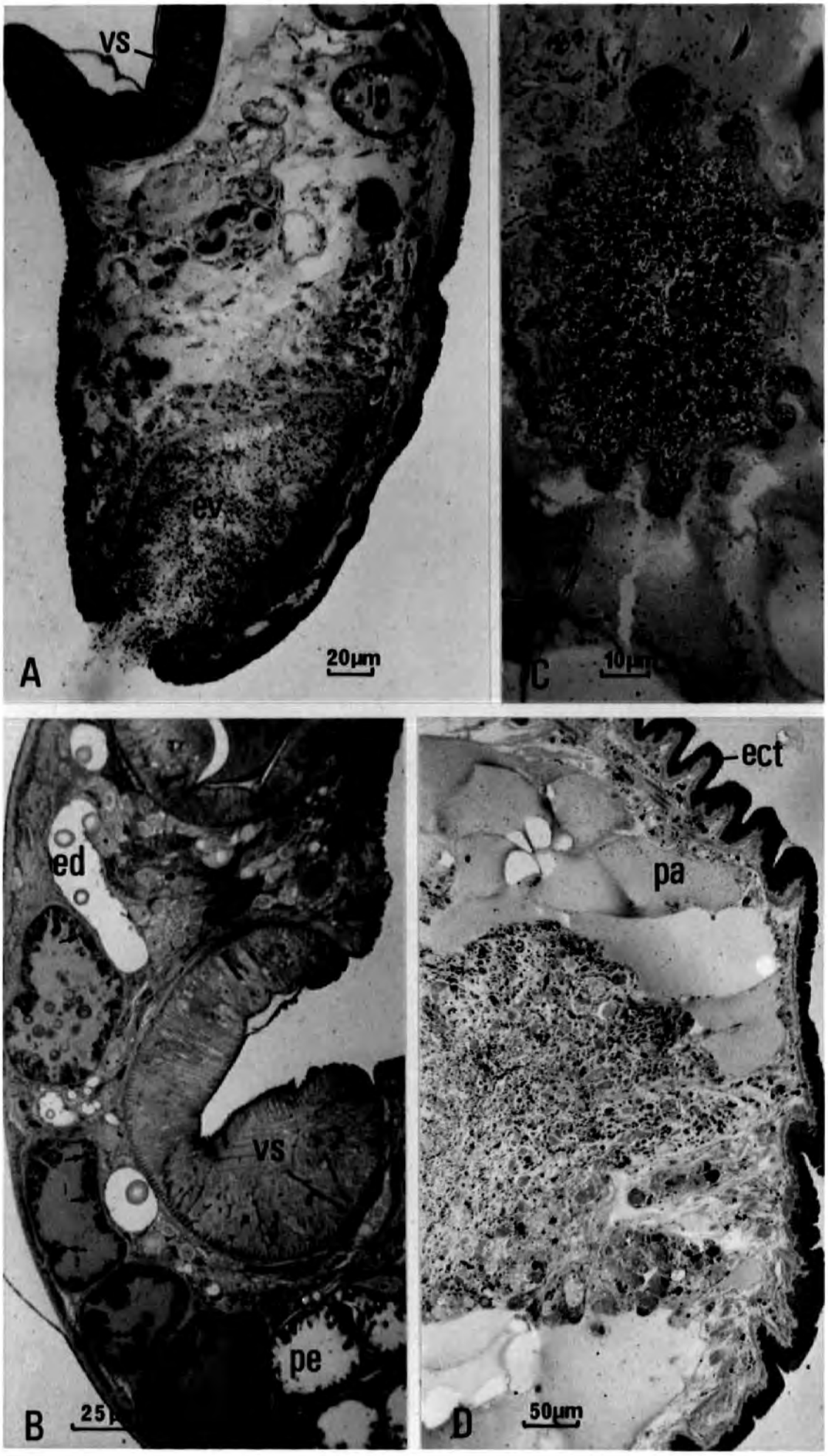
A. L[3,5 - ^{3}H] tyrosine; incubation for 5 minutes at 20°C. Note strong, average and slight uptake of the amino acid within the excretory vesicle, the sub-tegumental parenchyma, and the intestinal caeca respectively, as indicated by the comparative density of silver grains over the section.

B. D[2 - ^{3}H] glucose; incubation for 5 minutes at 20°C. Slight uptake within intestinal caeca (arrowed).

C,D. Lecithochirium fusiforme, adult ecsoma. Whole living worms incubated for 15 minutes at 20°C in Y.T.S. containing L[3,5 - ^{3}H] tyrosine (pH 5.0). Light autoradiographs; 1 µm resin sections (methylene blue stained).

C. Transverse section through posterior excretory duct showing silver grains over lining and within lumen.

D. Oblique section; note scarcity of silver grains over sub-tegumental muscle layers, their absence over parenchyma cells, and relative abundance over the central area adjacent to the posterior excretory duct.


vs
ev
A
20µm
C
10µm
ed
vs
pe
B
25µm
ect
pa
D
50µm

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APPENDIX

Results of the examination for hemiurid parasites of the species of fish listed in Table 4

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
Blennius pholis	1	11.2	12	May	0	0	0	1	dark brown, flat, irregular shape	d (calcified)
	1	10.2	"	"	0	0	0	3	dark brown	2d; 1 ℓ/p
	1	8.8	11	June	0	0	0	3	1 dark brown, flattened; 2 pale brown	1d; 1ℓ
	1	14.0	"	July	0	0	0	0		
	1	9.0	"	"	0	0	0	30+	brown	ℓ/p
	1	6.8	"	"	0	0	0	2	"	ℓ/p
	1	8.5	"	"	0	0	0	2	"	ℓ/p
	1	8.0	"	"	0	0	0	0		
	1	7.7	"	"	0	0	0	0		
	1	13.0	"	"	0	0	0	6	dark brown	3d; 2ℓ; 1 ℓ/p
	100	2.0-3.0	"	"	0	0	0	0		
	1	6.6	"	"	0	0	0	5	dark brown	3d; 2ℓ/p
	1	7.3	12	"	0	0	0	20+	" "	ℓ/p

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
	1	6.9	"	"	0	0	0	0		
	1	7.0	"	"	0	0	0	0		
	1	9.5	"	"	0	0	0	0		
	1	8.9	"	"	1 Hemiurus communis	1 Lecithaster gibbosus	0	0		
	1	10.4	"	"	0	0	0	1	dark brown	ℓ/p
	1	9.5	"	"	0	0	0	1	" "	ℓ/p
	1	13.3	"	"	0	2 L. gibbosus	0	2	" "	1 ℓ/p; 1d (calcified)
	1	12.4	"	"	2 H. communis	0	0	3	" "	d/p
	1	not recorded	13	"	0	0	0	2	brown	ℓ/p
	1	7.0	11	August	0	0	0	1	pale brown	ℓ/p
	1	6.8	"	"	0	0	0	0		
	1	8.7	"	"	0	0	0	0		
	1	7.8	"	"	0	0	0	0		
	1	6.2	5	"	0	0	0	0		

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
								Encapsulated		
					Stomach	Intestine	Free	Number	State of capsule	Condition of metacercaria
	1	6.9	"	"	0	0	0	0		
	1	7.6	"	"	0	0	0	0		
	1	12.5	11	September	0	0	0	14	13 dark brown 1 brown	ℓ/p; d (calcified)
	1	16.0	"	"	0	0	0	100+	dark brown, flattened, irregular shape	4 ℓ/p; remainder dead
	1	13.2	"	"	0	0	0	50+	"	d/p; most calcified
	1	11.6	"	"	0	0	0	30+	"	1 ℓ/p; remainder dead
	1	9.0	12	"	0	0	0	3	brown	ℓ/p
	1	9.7	"	"	0	0	0	1	"	ℓ/p
	1	9.4	11	October	0	0	0	5	dark brown	ℓ
	1	6.9	"	"	0	0	1 ℓ/p	0		
	1	3.2	"	November	0	0	0	0		
	1	7.0	12	"	0	0	1 gravid *Lecithochirium furcolabiata* (massive adhesions in body cavity)	0		

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
Conger conger	1	Not recorded	M.B.A.	September	3 Lecithochirium rufoviride; 14 L. fusiforme	0	0	0		
	1	"	M.B.A.	"	4 L. rufoviride; 7 L. fusiforme	0	0	0		
	1	"	Sutton Harbour, Plymouth	November	11 L. rufoviride; 5 L. fusiforme	0	0	0		
Crenilabrus melops	1	12.3	11	February	0	0	0	90+	20 pale brown 70+ dark brown, flattened	20 ℓ; remainder dead
	1	Not recorded	"	July	0	0	0	30+	dark brown	d
	1	"	"	"	0	0	0	0		
	1	4.5	"	November	0	0	0	6	brown	ℓ
	1	5.1	"	"	0	0	0	20	"	ℓ
	1	3.8	"	"	0	0	0	1	"	ℓ
Gaidropsarus mediterraneus	1	9.0	"	May	Not examined	Not examined	6 (very immature)	0		
	1	9.5	"	"	"	"	0	0		

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
	1	16.6	12	"	12 Hemiurus communis	0	0	2	brown	ℓ
	1	16.5	"	"	1 H. communis	0	0	3	"	ℓ
	1	10.0	11	June	0	2 (very immature)	0	2	"	ℓ
	1	16.0	"	"	0	0	0	50+	"	ℓ
	1	12.2	"	"	0	0	0	50+	"	ℓ
	1	14.8	7	December	0	0	0	30+	dark brown, flattened	ℓ
Gobius niger	1	Not recorded	6	July	0	0	0	0		
G. paganellus	1	5.0	11	May	0	0	1 (immature)	0		
	1	5.0	"	"	0	0	1 (immature)	0		
	1	10.0	"	June	0	0	0	11	yellow	ℓ
	100	2.0-3.5	"	"	0	0	0	0		
	1	17.7	"	August	0	0	0	7	yellowish-thick fibrous wall	6d; 1ℓ
	1	Not recorded	"	"	0	0	0	1	"	ℓ

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
	1	"	"	"	0	0	0	1	"	ℓ
	1	6.6	"	"	0	0	0	9	translucent white	ℓ
	1	17.0	"	"	0	0	0	1	white, with 3 yellow patches in outer fibres	ℓ
	9	5.8-7.7	5	"	0	0	0	0		
	1	6.9	11	September	0	0	0	1	translucent white; very thin	ℓ
	1	7.9	"	"	0	0	0	1	"	ℓ
	1	6.5	"	"	1/immature	0	0	0		
	6	2-2.5	"	"	0	0	0	0		
	1	Not recorded	"	October	0	0	3 (immature)	5	translucent white; thin	ℓ
	1	"	"	"	0	0	0	0		
	1	3.6	"	"	0	0	0	0		
	1	3.8	"	"	0	0	0	1	translucent; thin	ℓ
	1	3.0	"	"	0	0	1 (very immature)	0		

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
	1	4.0	"	"	0	0	0	15	whitish; thin	ℓ
	2	3.8;4.0	"	"	0	0	0	0		
	1	4.0	"	November	0	0	5 (very immature)	2	pale; thin	ℓ
	1	4.0	"	"	0	0	0	1	"	ℓ
	2	3.2;4.5	"	"	0	0	0	0		
	1	3.0	"	December	0	0	0	10	whitish; thin	ℓ
Lophius piscatorius	1	Not recorded	M.B.A.	June	1 Lecithochirium fusiforme	0	0	0		
	1	"	"	"	2 L. rufoviride 2 L. fusiforme	0	0	0		
	1	"	"	"	2 Hemiurus communis	0	0	0		
	11	"	"	"	0	0	0	0		
Pholis gunnellus	1	13.6	6	July	0	0	0	0		
	1	Not recorded	12	September	0	0	0	0		

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
								Encapsulated		
					Stomach	Intestine	Free	Number	State of capsule	Condition of metacercaria
Pomatoschistus microps	1	4.0	8	May	0	0	1 (very immature)	0		
	12	4.0-6.0	"	July	0	0	0	0		
	100	3.0-5.0	5, 10	March-September	0	0	0	0		
Scophthalmus maximus	25	3.0-5.0 ('0' group)	12	August	0	0	0	0		
	1	"	"	"	0	1 Lecithaster gibbosus	0	0		
Taurulus bubalis	1	8.0	11	March	0	0	1	0		
	1	13.0	12	April	1 Hemiurus communis	1 Lecithaster gibbosus	0	0		
	1	10.0	"	May	25+ H. communis	0	0	7	not recorded	ℓ
	1	8.6	11	June	0	0	0	30+	yellow; thick-walled	50% d
	1	9.3	"	"	0	0	0	20+	"	"
	1	9.8	"	"	0	0	0	50+	"	"
	1	8.0	"	"	0	0	0	60+	"	ℓ
	1	8.0	"	"	0	0	0	50+	"	ℓ

Host species	Number of fish	Body length (cms)	Collecting station	Month	Hemiurid parasites					
					Gut		Body cavity			
					Stomach	Intestine	Free	Encapsulated		
								Number	State of capsule	Condition of metacercaria
	1	8.2	"	"	0	0	0	70+	"	50% d
	1	11.0	7	"	0	0	0	0		
	1	13.0	12	July	1 H. communis	0	0	0		
	1	9.5	"	"	10 H. communis	0	0	0		
	1	8.6	"	"	0	0	0	0		
	1	12.0	3	"	0	0	0	50+	yellowish; thick-walled	d
	1	5.0	11	August	0	0	0	10	pale brown; thick-walled	8d; 2 ℓ/p
	1	8.0	"	October	0	0	0	50+	yellowish; thick-walled	d
	1	7.3	"	"	0	0	0	0		
	1	5.7	12	"	2 H. communis	0	0	0		
	1	6.5	11	November	0	0	1	12	pale yellow	ℓ
	1	6.5	"	"	0	0	1	1	"	ℓ
	1	5.0	"	"	0	0	0	1	"	ℓ
	1	6.2	"	"	0	0	0	53	"	50ℓ; 3d

Key: d dead
ℓ living
p progenetic

Host species	Number of fish	Body length (cms)	Collecting station	Month	Gut		Body cavity						
							Free						Encapsulated
					Stomach	Intestine	Number	Species	Approximate age of parasite	Comments: State of host liver	Comments: Eggs in bile	Comments: Eggs in rectal contents	
Ciliata mustela	1	12.8	11	March	Not examined	Not examined	25+	Lecithochirium furcolabiata	mature	e	+	+	0
	1	13.0	"	"	"	"	8	"	"	se	-	-	0
	1	10.0	"	"	"	"	0						0
	1	14.0	"	"	"	"	14	L. furcolabiata	13 mature; 1 dead	se	-	-	0
	1	14.0	"	"	"	"	60+	"	mature	me	+	-	0
	1	11.5	"	"	"	"	26	"	23 mature; 3 immature	e	-	-	0
	2	20.0; 21.3	4	"	0	0	0						0
	1	13.0	11	April	0	0	8	L. furcolabiata	7 mature; 1 immature	se	-	-	0
	1	13.5	"	"	Not examined	Not examined	5	"	immature	h	-	-	0
	1	14.0	"	"	"	"	3	"	"	h	-	-	0
	1	14.2	"	"	0	0	0						0

Host species	Number of fish	Body length (cms)	Collecting station	Month	Gut		Body cavity						
							Free						Encapsulated
					Stomach	Intestine	Number	Species	Approximate age of parasite	Comments			
										State of host liver	Eggs in bile	Eggs in rectal contents	
	1	10.0	"	"	0	0	40+	L. furcolabiata	mature	me	+	-	0
	1	14.0	1	May	0	0	0						0
	1	11.5	11	June	0	0	26	L. furcolabiata	mature	se	-	-	0
	1	3.3	"	"	0	0	0						0
	11	3.6-4.9	12	"	0	0	0						0
	7	12-14	11	July	Not examined	Not examined	50-90+	L. furcolabiata	mature & immature	e	+	-	0
	1	2.9	12	"	2 Hemiurus communis	0	0						0
	1	3.4	"	"	2 "	0	0						0
	1	4.4	"	"	2 "	0	0						0
	1	4.0	"	"	2 "	0	0						0
	1	3.6	"	"	9 "	0	0						0
	1	6.6	6	"	0	0	0						0
	4	14.0-16.0	11	August	0	0	60-100	L. furcolabiata	mature & immature	e	+	+	0
	1	12.0	12	"	0	10 H. communis	3	probably not L. furcolabiata	immature	h	-	-	0

Host species	Number of fish	Body length (cms)	Collecting station	Month	Gut		Body cavity						
							Free						Encapsulated
					Stomach	Intestine	Number	Species	Approximate age of parasite	Comments			
										State of host liver	Eggs in bile	Eggs in rectal contents	
	1	11.5	11	September	0	0	23	L. furcolabiata	mature	se	-	-	0
	1	Not recorded	"	"	0	0	1	?	very immature	h	-	-	0
	1	14.0	"	"	0	0	1	unidentified; not L. furcolabiata	mature	h	-	-	0
	1	5.0	"	"	0	0	0						
	1	7.0	"	"	0	0	1	L. furcolabiata	mature	h	-	-	0
	1	12.5	"	"	0	0	30+	"	mature; 1 dead	e	+	-	0
	1	14.3	"	"	0	0	20+	"	mature; several immature	e	-	-	0
	1	14.1	"	"	0	0	60+	"	mature	me	+	-	0
	1	14.3	12	"	0	0	8	"	6 mature; 1 immature	h	-	-	0
	2	Not recorded	3	November	0	0	0						0

Key:
- h apparently healthy
- se slightly eroded
- e eroded
- me heavily eroded

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Cercaria vaullegeardi Pelseneer, 1906 (Digenea: Hemiuridae); the daughter sporocyst and emergence of the cercaria

B. F. MATTHEWS

Department of Biological Sciences, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA, Devon

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SUMMARY

The daughter sporocyst of *Cercaria vaullegeardi* has hitherto been regarded as occurring within the haemocoel of the digestive gland of *Gibbula umbilicalis*. A re-examination has shown that an important part of the sporocyst, previously overlooked, extends from the digestive gland, through the afferent renal vein, the right kidney haemocoel and the transverse pallial vessel into the blood channel within a gill filament, thus providing a means of exit from the host directly into the mantle cavity. The sporocyst is described at light and electron microscope levels, and the emergence of the cystophorous cercariae is discussed.

INTRODUCTION

Cercaria vaullegeardi, a cystophorous cercaria first described from *Gibbula cineraria* (L) by Pelseneer (1906) from Wimereux, has since been recorded only from *G. umbilicalis* (da Costa) (Gaillard, 1953; Chabaud & Campana-Rouget, 1958; Arvy, 1963), at Iles Chausey, Banyuls and Roscoff respectively. Apart from the present work, the parasite has been recorded from *G. umbilicalis* in British waters on 2 occasions, namely by James (1973) and Popiel (1976*a*, *b*). Whilst examining specimens of *G. umbilicalis* infected with *C. vaullegeardi*, it was noted that the daughter sporocysts had what appeared to be a novel feature in the form of a specialized anterior region, provided with a birth pore, which carried the immotile cercariae directly to the site of emergence from the host. A detailed investigation was made, therefore, of the daughter sporocyst of *C. vaullegeardi*, and its involvement in an unusual method of cercarial release is described here for the first time.

MATERIALS AND METHODS

A total of 4334 *G. umbilicalis* was collected at monthly intervals from February 1974 to June 1977 from middle and low spring tide levels on sheltered rocky shores around Plymouth, Devon. Infected specimens were recognized by careful removal of the shell, when parts of the daughter sporocysts were visible within the digestive gland. To show the distribution of the daughter sporocyst, the mantle cavity was opened along the left side, the gill being displaced towards the cut edge of the mantle (Fig. 1).

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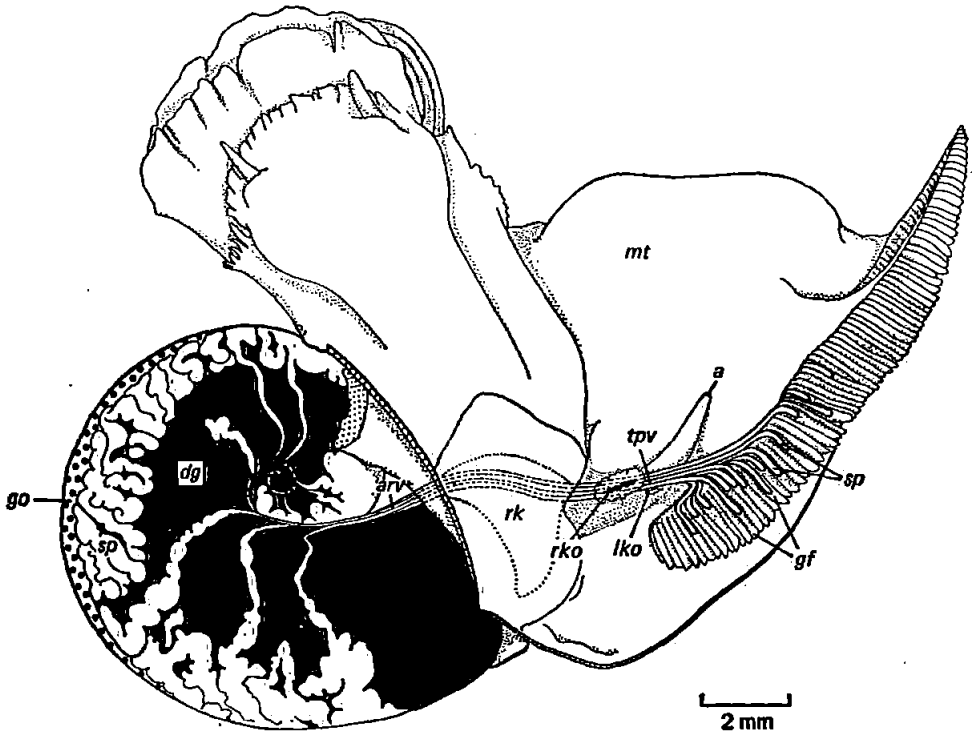

Fig. 1. *Gibbula umbilicalis* displayed to show the distribution of daughter sporocysts of *Cercaria vaullegeardi* within digestive gland haemocoel, afferent renal vein, right kidney haemocoel, transverse pallial vessel and blood channel of gill filaments. Host shell removed; mantle cut along left side. *a*, Anus; *arv*, afferent renal vein; *dg*, host digestive gland; *gf*, gill filament; *go*, gonad; *lko*, left kidney opening; *mt*, host mantle; *rk*, right kidney; *rko*, right kidney opening; *sp*, sporocyst; *tpv*, transverse pallial vessel.

For light microscopy studies, infected *G. umbilicalis* were fixed whole in Bouin's fluid or Baker's formol–calcium, double-embedded in celloidin and Paraffin wax (Pantin, 1962), serial sectioned at a thickness of 5–7 μm, and stained with Heidenhain's Azan (Pantin, 1962). The nervous system was demonstrated by staining for acetylcholinesterase and non-specific esterases using the acetylthiocholine iodide and indoxyl acetate methods respectively (Jennings & Leflore, 1972).

Daughter sporocysts *in situ* within digestive gland, right kidney and gill, and specimens carefully teased from host tissues were prepared for transmission electron microscopy as follows. The material was fixed for 2 h in 3% glutaraldehyde in 0·1 M cacodylate buffer, pH 7·2, at 0–4 °C, then washed overnight in buffer before post-fixing in 1% osmium tetroxide for 1·5 h, rinsing, dehydrating through an alcohol series and embedding in TAAB Araldite or Epon resins. Sections showing gold or silver interference colours were cut using a Porter-Blum MT2B ultramicrotome, collected on uncoated copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Philips 300 transmission electron microscope at 60 and 80 kV.

OBSERVATIONS AND RESULTS

Of the 4334 specimens of *G. umbilicalis* collected 1·13% were found to be infected with daughter sporocysts of *C. vaullegeardi*.

General account and distribution of the daughter sporocyst

The daughter sporocyst (Fig. 2) is unbranched and filamentous, the anterior region (Region A, Fig. 2), comprising approximately half the body length, being narrow and white in colour, the wider posterior region (Region B, Fig. 2) being orange with white bulges corresponding to the position of groups of developing cercariae. The posterior region only is visible in the digestive gland on removal of the host shell.

The living daughter sporocyst is highly elastic and sticky, a feature which makes removal from the host extremely difficult. Intact specimens undergo slow writhing movements in sea-water. Inlength they measure 6–26 mm, in maximum breadth an average of 0·1 mm anteriorly and 0·25 mm posteriorly. The posterior region is the site of cercarial production, germinal cells being proliferated near the posterior extremity (Fig. 2). Groups of developing cercariae occur at irregular intervals along the entire length of the posterior region. The anterior region contains only fully developed cercariae, moving actively forwards, head to tail in a single line, to enter the muscular birth canal from which they emerge through the terminal birth pore. *In situ* the sporocyst lies with the posterior region within the haemocoel of the host digestive gland; the anterior region passes forwards along the afferent renal vein through the haemocoel of the right kidney into the transverse pallial vessel which crosses the mantle wall to the gill (Fig. 1, Pl. 3B). The extreme anterior end of the sporocyst lies within the blood channel of a single gill filament (Pl. 3C, D).

Structure of the daughter sporocyst

Body wall

Anterior region (Region A, Fig. 2). The entire surface is covered with an anucleate, syncytial microvillous tegument. In the vicinity of the birth canal the microvilli average 1·64 μm in length and may be single and narrow, or branched with proximal or distal swellings (Pl. 1A). These swellings may anastomose distally to form an incomplete outer membranous sheet in contact with the host, perforated by irregular channels passing deep into the tegument. Electron-lucent vesicles, measuring 34 nm in average diameter, mitochondria and free ribosomes have been observed within the tegument. Outer circular and inner longitudinal muscle layers lie under the basement lamina. Sub-tegumental cells in the region of the birth pore are of 2 types: muscle cells with clear cytoplasm, scattered aggregations of ribosomes, mitochondria and vesicles, and cells containing prominent lipid bodies and vesicles (Pl. 1A, B).

Germinal cells were not observed within the anterior region of the sporocyst.

Posterior region (Region B, Fig. 2). The ultrastructure of the body wall of the posterior region within the host digestive gland has been described by Popiel

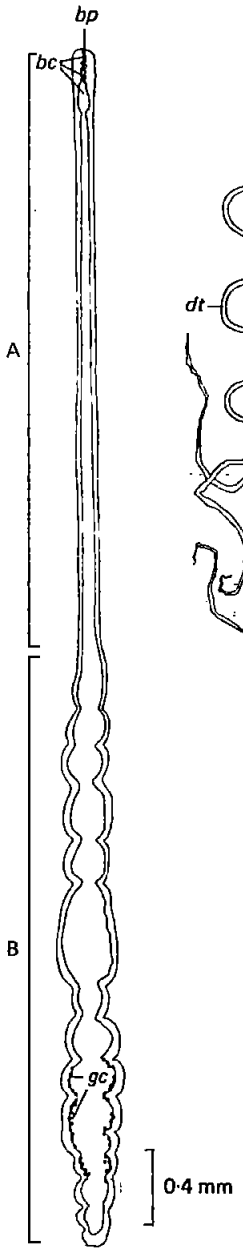

Fig. 2

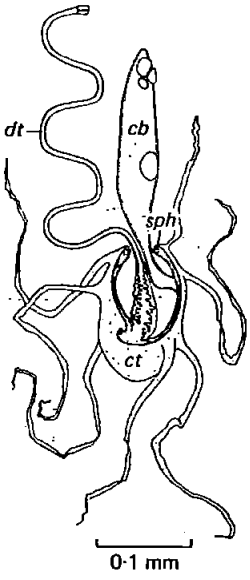

Fig. 3

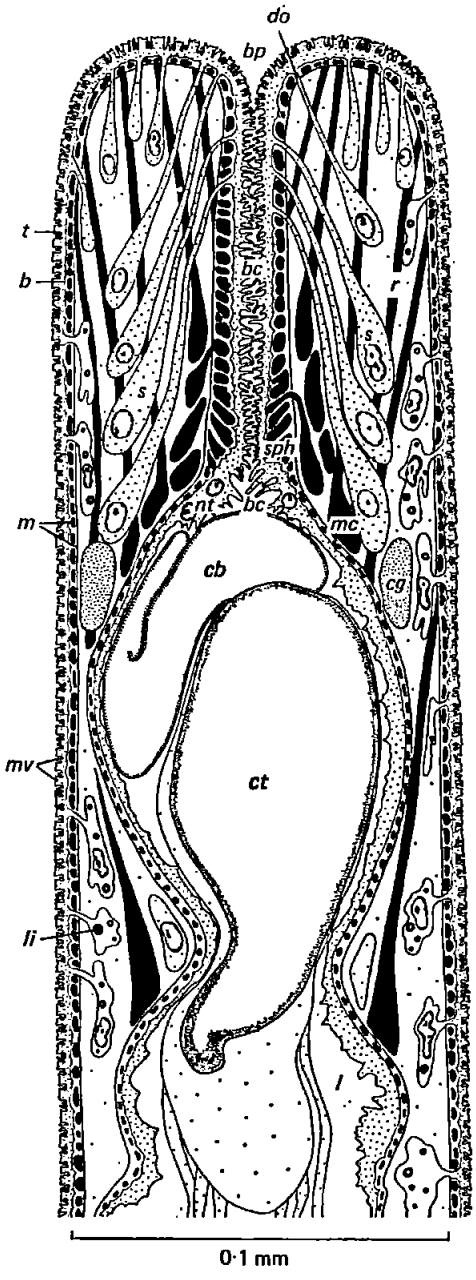

Fig. 4

(1976*a*). Germinal cells observed near the posterior end (Fig. 2) are the subject of a special study presently in progress.

The effect of the sporocyst upon adjacent cells of digestive gland tubules is shown in Pl. 2C. The cytoplasm of affected cells becomes highly vacuolated, the membranes of the nuclear envelope more widely spaced, and nuclear contents sparse. A zone of cellular debris separates the microvilli from the necrotic host cells.

Birth pore and associated structures

A sinuous canal measuring 0·35 mm in average length (Fig. 4), connects the terminal birth pore with the sporocyst lumen. The canal has 3 distinct regions; a short anterior region around the birth pore itself, a middle muscular region terminating in a prominent sphincter and a posterior region which widens and merges with the lumen. The anterior and middle regions are lined by tegument similar to that covering the body. At the posterior end of the birth canal the tegument consists of a single layer of large cuboidal cells whose free surface is usually extended into long anastomosing microvilli which partially occlude the lumen of the canal. Each cell has a large rounded nucleus and dense cytoplasm with granular endoplasmic reticulum, Golgi, free ribosomes, membrane-bound, electron-lucent vesicles and α and β glycogen (Pls 1C and 2B).

The muscle coats of the general body wall pass inwards to line the 3 regions of the birth canal, forming its outer longitudinal and inner circular layers. These muscles are thickened in the middle region, especially at the sphincter. Radial muscles pass from the middle and posterior canal regions obliquely forwards to the body wall (Fig. 4).

Numerous large secretory cells lying around the middle and posterior canal regions have ducts which pass forwards to open into the tegument lining the middle and anterior canal and that covering external areas adjacent to the birth pore (Fig. 4; Pl. 1B). Each cell has a large indented nucleus with several nucleoli and dense cytoplasm rich in electron-lucent vesicles, granular endoplasmic reticulum, Golgi, α and β glycogen, mitochondria, and occasional protein whorls and lipid bodies (Pl. 2A).

Fig. 2. Daughter sporocyst of *Cercaria vaullegeardi* to show narrow anterior region A with birth canal, and posterior region B. *bc*, Birth canal; *bp*, birth pore; *gc*, germinal cell.

Fig. 3. *Cercaria vaullegeardi* showing cystophorous tail (*ct*) with 8 appendages including delivery tube (*dt*), excretory appendage and caudal filaments. *cb*, Cercarial body; *sph*, sphincter muscle.

Fig. 4. Anterior end of daughter sporocyst of *Cercaria vaullegeardi*. A cercaria has entered the posterior region of the canal prior to emergence through the birth pore. *b*, Basement membrane and lamina; *bc*, birth canal; *bp*, birth pore; *cb*, cercarial body; *cg*, cerebral ganglion; *ct*, cystophorous tail; *do*, opening of secretory cell duct; *l*, sporocyst lumen; *li*, lipid body; *m*, muscle coats; *mc*, muscle cell cytoplasm; *mv*, microvilli; *nt*, nucleated tegument lining posterior birth canal region; *r*, radial muscle; *s*, secretory cell; *sph*, sphincter muscle; *t*, tegument.

The nervous system is as described for the daughter sporocyst of *Cercaria stunkardi* by James (1973); the cerebral ganglia, situated posterior to the birth canal sphincter, being a site of acetylcholinesterase (Pl. 3A) and non-specific esterase production.

Emergence of the cercaria

Several workers (Pelseneer, 1906; Arvy, 1963; Popiel, 1976*a*, *b*) have briefly described the cercaria. It suffices here to mention that it has a cystophorous tail with 8 appendages, including a delivery tube (Fig. 3).

In the living sporocyst cercariae may be seen throughout most of the length of the anterior region, moving in single file towards the birth pore. Progression is achieved by a combination of muscular action of the cercarial body and peristaltic movements of the sporocyst wall. The tail at this stage is incapable of active movement and is dragged behind the body of the cercaria with all appendages trailing, except the delivery tube which has now been withdrawn into the tail cavity.

During periods of cercarial release, the anterior end of the sporocyst actively extends along the blood channel of the gill filament and penetrates the epithelium to protrude approximately 1 mm into the mantle cavity. The columnar epithelium of the gill filament is disrupted and torn (Pl. 3C, D), suggestive of muscular and lytic activity on the part of the parasite. Cercariae emerge through the sporocyst birth pore directly onto the surface of the gill within the host mantle cavity. Within seconds of emergence the cercarial body retracts into the cystophorous tail, the sphincter muscle surrounding the tail aperture (Fig. 3) is closed to seal the point of entry, and the 'encysted' cercaria is dislodged by the exhalent water current and swept out of the mantle cavity.

DISCUSSION

Although many workers (Gaillard, 1953; Chabaud & Campana-Rouget, 1958; Arvy, 1963; James, 1973; Popiel, 1976*a*, *b*) have described the daughter sporocyst of *C. vaullegeardi* from the digestive gland of *G. umbilicalis*, until now the anterior region of the sporocyst, extending via the right kidney into the host gill, has not been recorded. The technique generally employed for the removal of larval digeneans for examination involves shell removal and the subsequent teasing apart of the visceral mass of the infected mollusc. The fragile sporocyst of *C. vaullegeardi* is broken by this method, and only its posterior region containing germ cells and developing cercariae is removed. The present investigation shows that dissection and the cutting of serial sections of the entire infected mollusc are essential for the study of filamentous sporocysts.

The anterior region of the sporocyst is here shown to extend through blood vessels connecting the haemocoels of the host digestive gland, right kidney and gill. The sporocyst is structurally adapted for active penetration of the host gill tissue, with well-developed musculature and nervous system at the anterior end. During periods of cercarial release, the anterior extremity of the sporocyst actively penetrates the gill epithelium using muscular thrusting actions probably assisted

by lytic secretions from glands around the birth canal. The specialized anterior sporocyst region therefore serves to transport mature cercariae from the deep-seated digestive gland to the exterior. The cystophorous cercariae emerge from the terminal birth pore directly into the host mantle cavity, where they 'encyst' prior to passing out into the sea with the exhalent water current. The specialization of the sporocyst in *C. vaullegeardi* therefore provides a means whereby the cercaria, hindered by a bulky, immotile cystophorous tail, does not itself have to migrate through the molluscan host tissues at any stage. A study of the emergence of other hemiurid cercariae, developing in rediae with birth pores within the host digestive gland haemocoel, would be of interest from a comparative viewpoint, but at present no data are available.

A similarly direct method of cercarial emergence has been described in *Bucephaloides gracilescens* by Matthews (1974). In this cercaria, as in *C. vaullegeardi*, the bulky tail complex would impede migration through host tissues from the site of development to the exterior. The branched filamentous sporocyst is not, however, differentiated into specialized regions as in *C. vaullegeardi*, and cercarial release is by rupture of its wall into the exhalent chamber of the bivalve host, *Abra alba*. The site of release from redia or sporocyst, and the extent of migration through host tissues to the exterior, appear therefore to be related to the motility of the cercaria. The body of *C. vaullegeardi*, prior to 'encystment', retains some degree of motility, although peristaltic movements of the sporocyst wall are here shown to assist its forward progression. Similarly, the muscular tail complex and body of *B. gracilescens* allow limited movement within the sporocyst. In species where the cercariae are immotile and tail-less, the sporocyst itself may migrate to the exterior, as described by Dobrovolny (1939) in *Plagioporus sinitsini*. Kagan (1952) describes the division of the sporocyst of *Neoleucochloridium problematicum* into functionally distinct portions, mature cercariae collecting within conspicuous brood pouches in the tentacles of the snail host. In both *P. sinitsini* and *N. problematicum*, infection of the final host is by ingestion of metacercariae retained within all or part of the sporocyst respectively. The involvement of a functionally distinct region of the sporocyst in the transport of cercariae to the exterior, as in *C. vaullegeardi*, does not appear to have been previously described.

The birth canal of the daughter sporocyst of *C. vaullegeardi* is shown here to consist of 3 regions. The most anterior of these, surrounding the birth pore, is lined by anucleate, microvillous syncytial tegument identical to that covering the general body surface. The middle canal region is highly innervated, muscular and surrounded by secretory cells, and the posterior region is characterized by a nucleated tegumental lining. This structure is essentially similar to that considered by Imohiosen (1969), James (1973), Popiel (1976*a*), and Popiel & James (1978) to be characteristic of the mouth, pharynx and caecum of the embryonic gut in rediae of various marine digeneans. Comparative ultrastructural studies by these workers on the birth pore and embryonic gut in sporocysts and rediae have led them to suggest that the daughter sporocyst is a paedogenetic redia and that its birth canal is homologous to the embryonic redial gut, the least retarded species retaining areas equivalent to mouth, pharynx and caecum (Popiel & James, 1978).

The structure of the birth canal of the daughter sporocyst of *C. vaullegeardi* as described here indicates that in this species the anterior, middle and posterior canal regions may also be considered to be homologous to the mouth, pharynx and caecum, respectively, of an embryonic redial gut. All the cercariae, with the exception of *C. vaullegeardi* and *Cercaria 'A'* Miller, 1925, of hemiurids so far investigated develop within rediae. The sporocyst of *C. vaullegeardi* may then be regarded as corresponding with the rediae of other hemiurids, and such correspondence would be consistent with the views of Popiel & James (1978) on the origin of daughter sporocysts from rediae.

I would like to express my gratitude to my husband, Dr R. A. Matthews, for his constructive criticism of the manuscript, to Professor F. G. Rees of University College of Wales, Aberystwyth for her helpful comments and to Dr L. Heath for the use of facilities within his Department.

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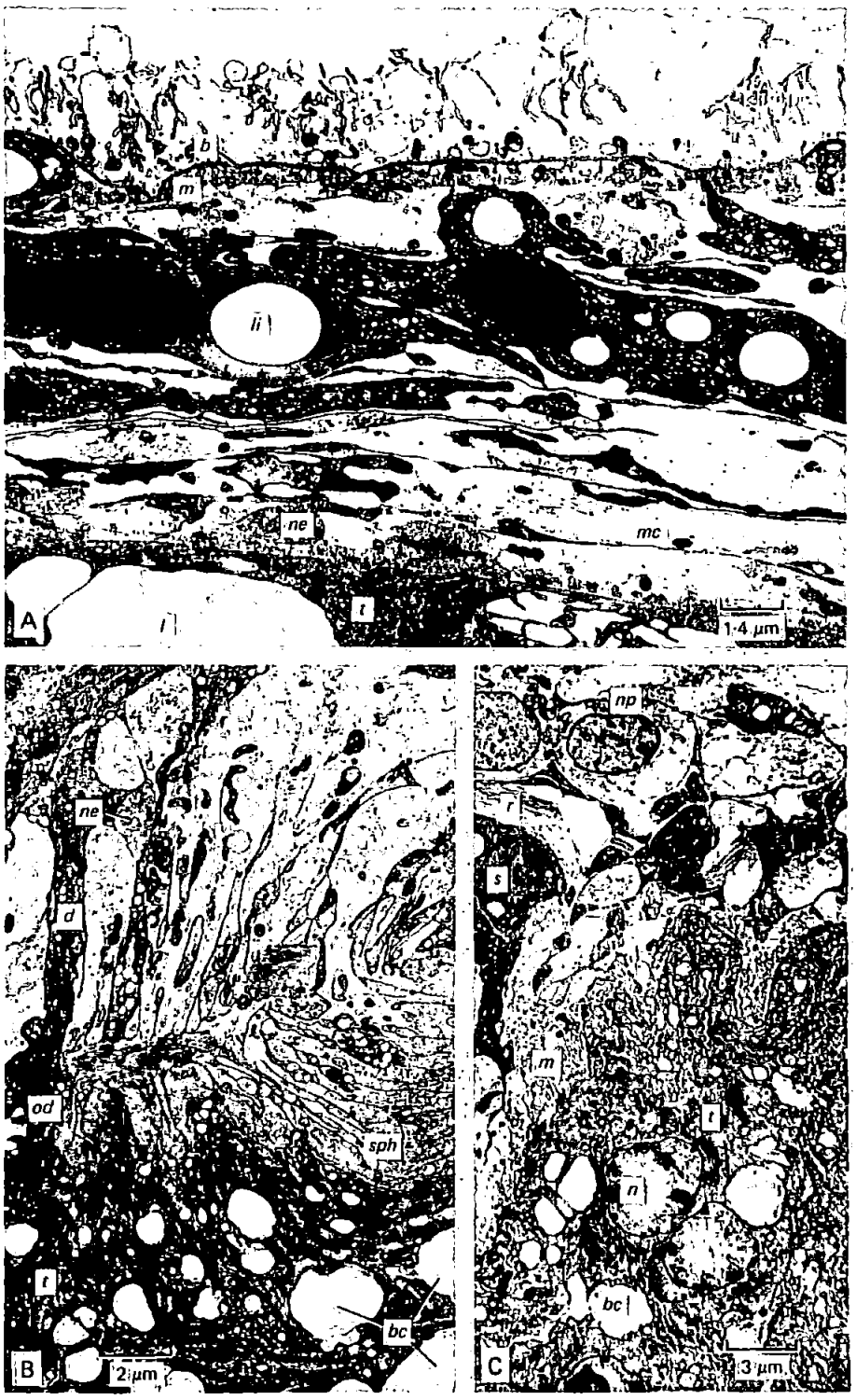

B. F. MATTHEWS

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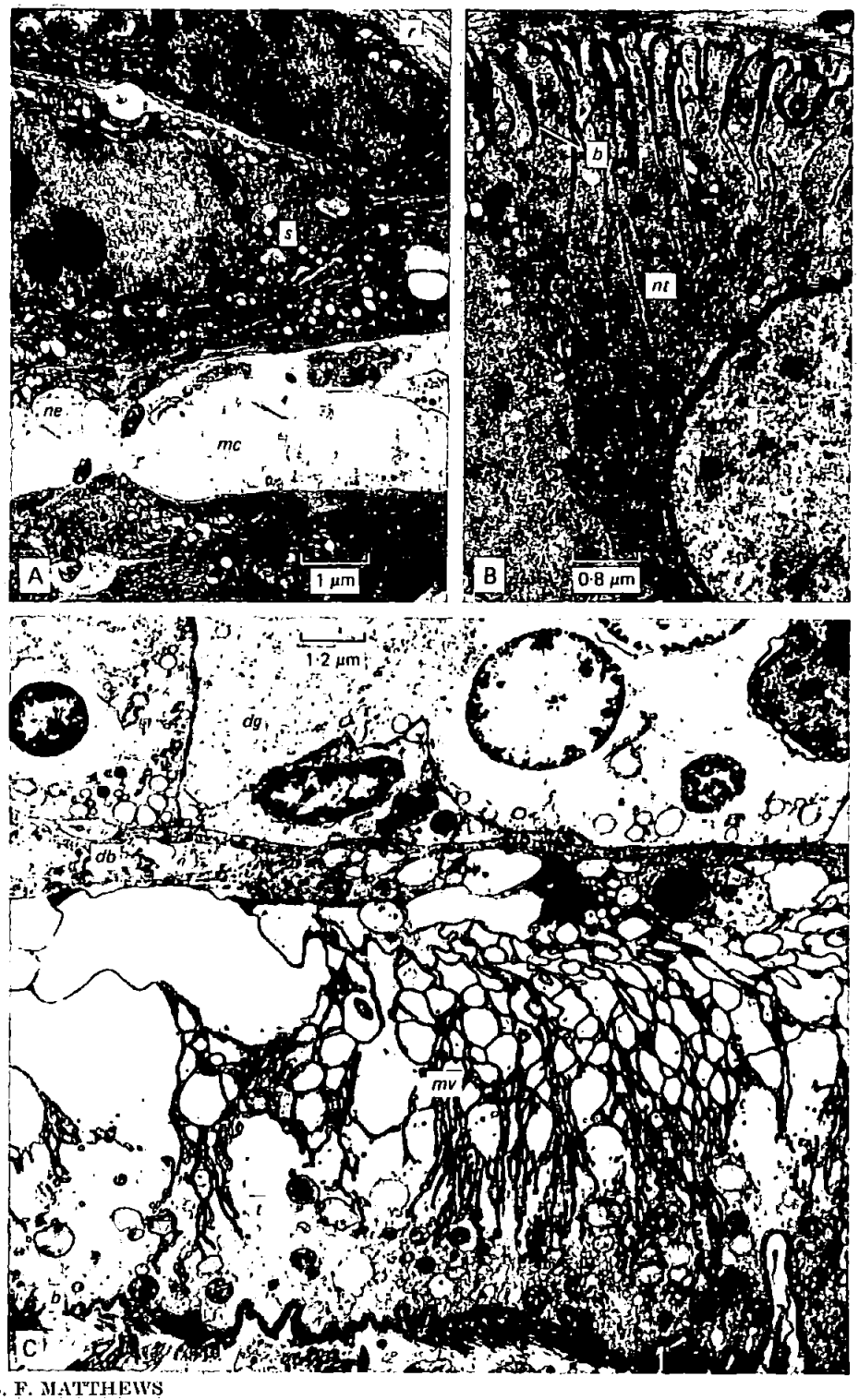

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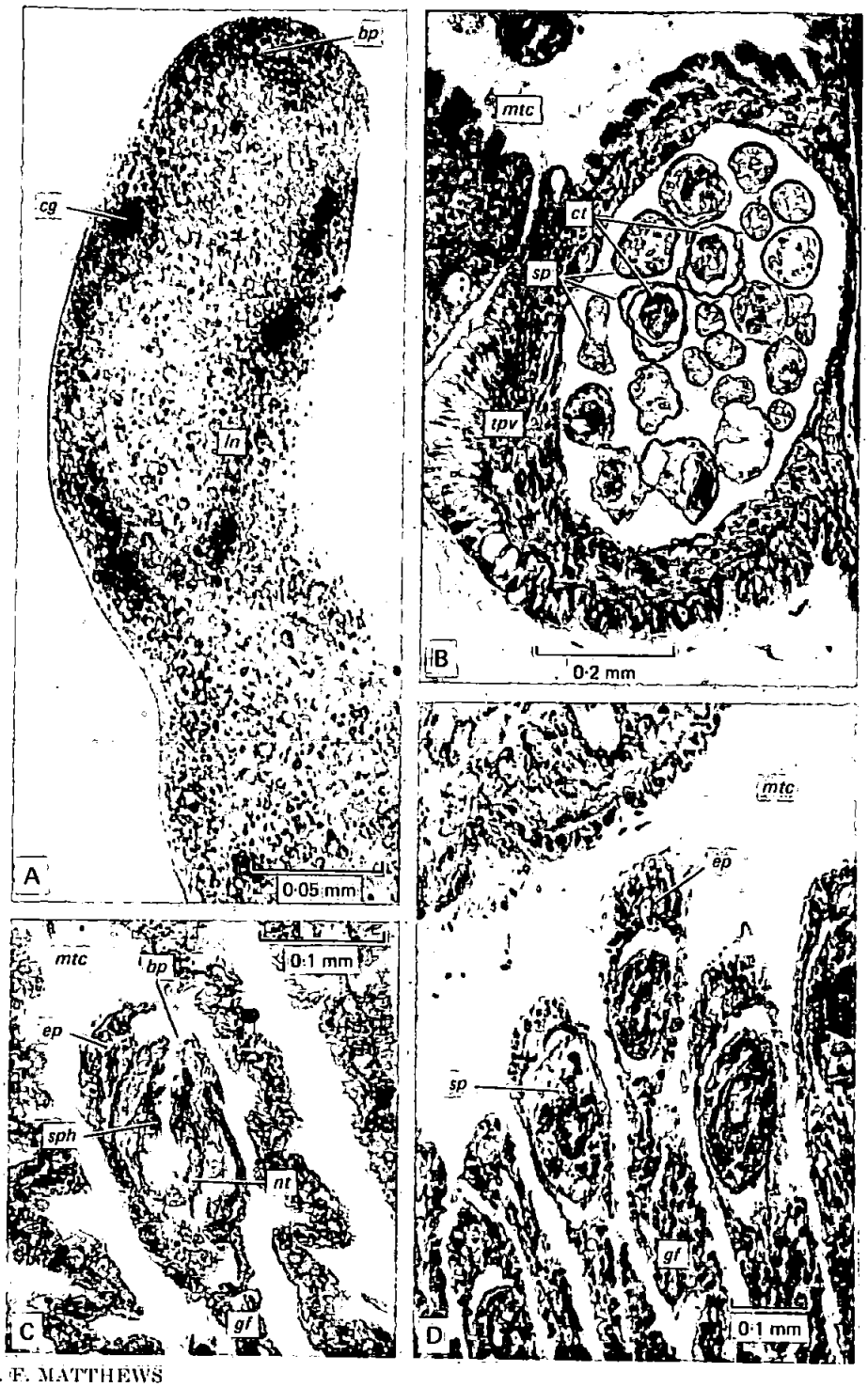
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KEY TO LETTERING OF PLATES

b	basement membrane and lamina	*mc*	muscle cell cytoplasm
bc	birth canal	*mtc*	host mantle cavity
bp	birth pore	*mv*	microvilli
cg	cerebral ganglion	*n*	nucleus
ct	cercarial tail	*ne*	neuron
d	duct of secretory cell	*np*	neuropile
db	cellular debris	*nt*	nucleated tegument lining posterior birth canal region
dg	digestive gland		
ep	gill epithelium	*od*	opening of secretory cell duct
gf	gill filament	*r*	radial muscle
l	sporocyst lumen	*s*	secretory cell
li	lipid body	*sp*	sporocyst
ln	longitudinal nerve	*sph*	sphincter muscle
m	muscle coats	*t*	tegument
		tpv	transverse pallial vessel

EXPLANATION OF PLATES

Plate 1

Electron micrographs of the body wall of the daughter sporocyst of *Cercaria vaullegeardi* in longitudinal section.

A. General morphology of the body wall of the anterior region of the sporocyst.

B. Middle birth canal region, showing sphincter muscle, anucleate tegumental lining with long branching microvilli, neurons, and ducts from secretory cells carrying electron-lucent vesicles opening into canal.

C. Nucleated tegument lining posterior region of birth canal.

Plate 2

Electron micrographs of the daughter sporocyst of *Cercaria vaullegeardi*.

A. Longitudinal section through middle region of birth canal showing secretory cell cytoplasm with granular endoplasmic reticulum, mitochondria, vesicles with electron-lucent contents and α and β glycogen.

B. Transverse section through nucleated tegument lining posterior region of birth canal. Dense cytoplasm with mitochondria, ribosomes, glycogen and vesicles with electron-lucent contents.

C. Transverse section through posterior region of sporocyst within host digestive gland haemocoel, showing necrotic host cells. A zone of cellular debris separates host tissue from the highly branched microvilli covering the anucleate syncytial sporocyst tegument.

Plate 3

A. Anterior end of daughter sporocyst of *Cercaria vaullegeardi* showing acetylcholinesterase located within nervous system. Strongly positive reaction within cerebral ganglia and longitudinal nerves.

B. Micrograph of transverse section through the transverse pallial blood vessel of *Gibbula umbilicalis* showing 23 sporocysts of *C. vaullegeardi* within its lumen.

C. Micrograph of longitudinal section through anterior end of the daughter sporocyst of *C. vaullegeardi* within the blood channel of a host gill filament, showing anterior, middle and posterior regions of the birth canal. The gill epithelium is torn at the site where the sporocyst tip has penetrated for cercarial release.

D. Micrograph of host gill filaments in transverse section showing disrupted epithelium and sporocysts of *C. vaullegeardi* within blood channels.

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Cercaria vaullegeardi Pelseneer, 1906 (Digenea: Hemiuridae); development and ultrastructure

B. F. MATTHEWS

Department of Biology, Plymouth Polytechnic, Drake Circus, Plymouth, Devon PL4 8AA

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SUMMARY

Cercaria vaullegeardi develops in daughter sporocysts within the digestive gland of the prosobranch *Gibbula umbilicalis*. On emergence into the host mantle cavity the cercarial body retracts into the cystophorous tail, the 'encysted' cercaria being then shed into the sea. The encysted cercaria is described at the ultrastructural level for the first time and developmental stages are redescribed. The cercarial body has no penetration or cystogenous glands: a single type of sub-tegumental secretory cell produces vesicles containing neutral mucopolysaccharides which pass into the tegument at encystment. The immotile cystophorous tail consists essentially of a fibrous caudal cyst and 8 appendages including a delivery tube. Scanning electron micrographs show the surface of tegumental membranes enveloping the caudal cyst to be covered with a honeycomb pattern of indentations derived from electron-lucent vesicles produced by the caudal gland. The fibrous-walled delivery tube comprises proximal and distal sections and a modified terminal end-piece.

INTRODUCTION

In hemiurid cercariae the tail is profoundly modified to bring about the infection of the next host in the life-cycle by a unique inoculation mechanism (Stunkard, 1973; Køie, 1979). At present little data is available concerning the structure of the complex cystophorous tail. *Cercaria vaullegeardi* has been studied at the light microscope level by Pelseneer (1906), Chabaud & Campana-Rouget (1958), Arvy (1963) and Popiel (1976*a*), the latter worker also describing ultrastructural aspects of developmental stages of the parasite within the posterior region of the sporocyst (Popiel, 1976*b*). Matthews (1980) described the anterior region and birth pore of the daughter sporocyst of *C. vaullegeardi* through which mature cercariae are transported to the host mantle cavity. The present paper describes at the ultrastructural level further stages in cercarial development and the naturally-emerged encysted form for the first time, thus providing a basis for interpretation of the mode of infection of an experimental 2nd intermediate host in the life-cycle, described by Matthews (1981).

MATERIALS AND METHODS

Developing and naturally-emerged *C. vaullegeardi* were obtained from screened, infected *Gibbula umbilicalis* (Da Costa) collected from sheltered rocky shores around

Plymouth, Devon, between 1974 and 1978. Wherever possible, studies were made of living cercariae with the aid of phase-contrast microscopy. All measurements were made on live specimens without application of pressure; values given in Table 1 are averages of 20 specimens. Material for light microscopy was fixed in neutral buffered-formol saline, Bouin's fluid or Baker's formol-calcium, double-embedded in celloidin and paraffin wax, serial-sectioned at a thickness of 4–7 μm and stained with Heidenhain's Azan (Pantin, 1962) or Cole's haematoxylin and eosin. Staining techniques for differentiation of carbohydrates and mucosubstances followed standard procedures given by Pearse (1968). Acetylcholinesterase and non-specific esterases were located using acetylthiocholine iodide and indoxyl acetate methods respectively (Jennings & LeFlore, 1972).

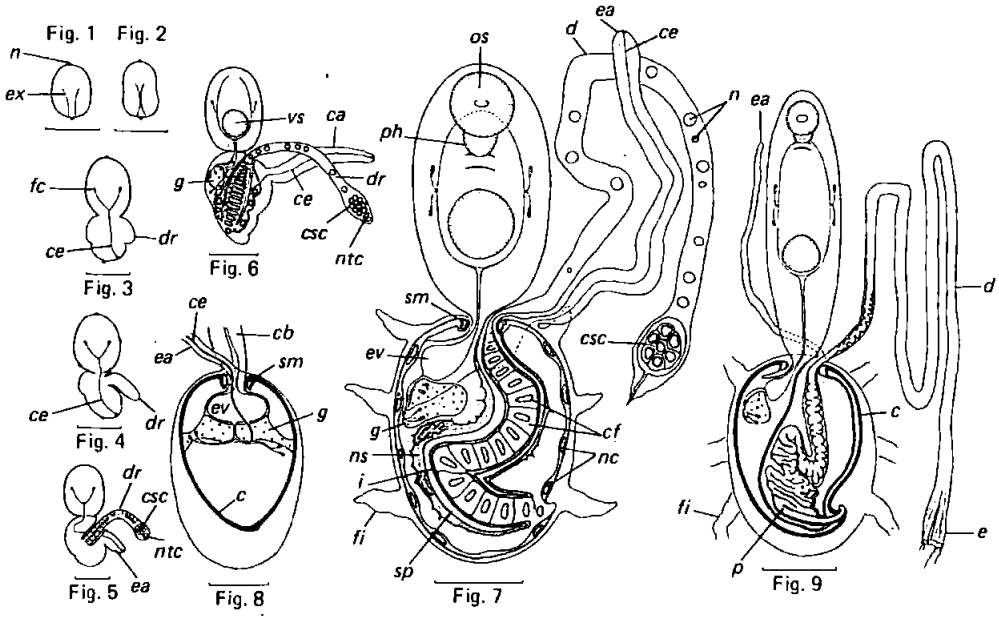
Daughter sporocysts with developmental stages and naturally emerged encysted cercariae were prepared for transmission electron microscopy as follows. The material was fixed for 2 h in 3% glutaraldehyde in 0·1 M cacodylate buffer, pH 7·2, at 0–4 °C, then washed overnight in buffer before post-fixing in 1% osmium tetroxide for 1·5 h, rinsing, dehydrating through an alcohol series and embedding in TAAB Araldite or Epon resins. Sections showing gold or silver interference colours were cut using a Porter-Blum MT2B ultramicrotome, collected on uncoated copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Philips 300 transmission electron microscope at 60 and 80 kv. Specimens for scanning electron microscopy were washed twice in filtered sea water, fixed in 3% glutaraldehyde in 0·1 M cacodylate buffer, pH 7·2, at 0–4 °C for 7 days, rinsed in buffer and dehydrated in an alcohol series. They were then passed through amyl acetate and critical-point dried in a Tousimis Samdri PVT3, gold sputter coated in a Polaron E5100 S.E.M. coating unit, and examined with a Hitachi 550 scanning electron microscope.

OBSERVATIONS AND RESULTS

Of 6143 *G. umbilicalis*, 1·13% were found to be infected with the daughter sporocysts of *C. vaullegeardi*. The unbranched filamentous sporocyst has a posterior region containing developing cercariae within the host digestive gland haemocoel, and a narrow anterior region along which cercariae migrate to the terminal birth pore located within the host gill (Matthews, 1980). Although cercarial development is continuous it is convenient for description to refer to 3 stages, namely pre-migratory, migratory and encysted cercariae. Pre-migratory (Figs 1–9) and migratory (Fig. 10) cercariae are stages which occur within the posterior and anterior region of the sporocyst respectively. The encysted cercaria (Figs 11 and 12) is the stage at which the retracted body is coiled within the cavity of the cystophorous tail. Encystment occurs immediately after emergence from the sporocyst birth pore onto the surface of the host gill, the encysted cercaria being shed into the sea from the mantle cavity with the exhalent water current.

(i) *Cercarial body*

The cercarial body (Fig. 10) has a well-defined oral sucker and pharynx, digestive caeca, nervous and excretory systems. The ventral sucker, although defined early



Figs 1–9. Pre-migratory *Cercaria vaullegeardi* removed from posterior region of daughter sporocyst.

Figs 1–6. Early stages, showing development of delivery tube rudiment and excretory appendage. Ventro–lateral view. Scale lines represent 5 μm.

Fig. 7. Later stage showing folding of proximal delivery tube within developing caudal cyst, formation of space between outer nucleated and inner investing syncytial layers, and caudal filament buds. Ventro–lateral view of tail, body twisted to ventral view. Scale line represents 50 μm.

Fig. 8. Dorsal view of cystophorous tail showing caudal gland and excretory system. The delivery tube and caudal filaments are not shown in this diagram to increase clarity. Scale line represents 50 μm.

Fig. 9. Delivery tube retraction into caudal cyst. Ventro–lateral view of tail, body twisted to ventral view. Scale line represents 50 μm. *c*, caudal cyst; *cb*, cercarial body; *ce*, caudal excretory duct; *cf*, fibre-forming cells of delivery tube rudiment; *csc*, cluster of cells with rounded nuclei within developing endpiece; *d*, distal delivery tube; *dr*, delivery tube rudiment; *e*, end-piece; *ea*, excretory appendage; *ev*, excretory vesicle; *ex*, excretory duct; *fc*, flame cell; *fi*, caudal filament; *g*, caudal gland; *i*, investing layer of developing proximal delivery tube; *n*, nucleus; *nc*, nuclei of fibre-forming cells of caudal cyst; *ns*, outer nucleated portion of syncytium; *ntc*, nuclei of terminal cells within developing end-piece; *os*, oral sucker; *p*, proximal delivery tube; *ph*, pharynx; *sm*, sphincter muscle; *sp*, space surrounding elongating proximal delivery tube; *vs*, ventral sucker.

in development (Fig. 6) remains comparatively rudimentary. The posterior end of the body is inserted through the aperture of the caudal cyst (Fig. 9) onto the inner surface of its dorsal wall.

The tegument resembles that of other digeneans in that it consists of an outer syncytial layer connected to sub-tegumental cell bodies by cytoplasmic bridges (Pl. 5B). The sub-tegumental cell cytoplasm contains secretory vesicles, measuring

0·33 μm in average diameter, with flocculent and electron-dense (ED) neutral mucopolysaccharide contents (Pl. 4B). The vesicles pass into the tegument at encystment (Pls 4B, 5B), some being discharged at its surface (Pl. 4B).

The loosely packed parenchyma cells (Pls 3C, 4B, 5B) have ill-defined cell boundaries. Their cytoplasm, with free ribosomes and mitochondria, is chiefly confined within deep indentations at the nuclear surface (Pl. 4B).

(ii) *Cystophorous tail*

The fully formed cystophorous tail (Fig. 10), as in all hemiurid cercariae so far described, consists essentially of a hollow vesicle or caudal cyst into which the body is withdrawn at encystment, and an elongated appendage, the delivery tube, through which the cercarial body is inoculated into the host haemocoel during the infection process. The caudal cyst is covered by tegumental membranes which extend to form 6 slender caudal filaments (Figs 7 and 12). A vestigial excretory appendage is attached to the anterior end of the caudal cyst.

Tegument

The tail of the earliest stages is covered by syncytial tegument, measuring 0·92 μm in average thickness, with a smooth outer bounding membrane (Pl. 1A) and sparse contents including microtubules and mitochondria. The tegument is connected by a branched duct (Pl. 2C) to the antero–dorsal, nucleated syncytial caudal gland (Figs 7 and 8; Pls 1B, 2C, D). The latter produces large numbers of electron-lucent (EL) vesicles, 0·21 μm in average diameter, which pass into the tegument at a stage corresponding to that shown in Fig. 7. The vesicles accumulate in a single continuous layer (Pl. 3A) before discharging their contents at the tegumental surface membrane. The walls of the collapsed vesicles remain, causing the surface to be completely covered with shallow indentations measuring 0·21 μm in average diameter which are clearly seen in scanning micrographs of encysted cercariae (Pl. 4A).

Cytoplasmic contents of the tegument beneath the vesicular layer break down leaving a cavity, except at the rounded posterior end of the tail where strands of cellular debris pass inwards to the curved caudal cyst beak (Pl. 5A). Following secretion of the vesicles the caudal gland degenerates (Figs 9 and 10), being no longer present in the encysted cercaria.

Several cells with flattened nuclei and dense cytoplasm containing RER, cisternae, free ribosomes and mitochondria have been observed between the tegument and the developing caudal cyst (Pl. 1B). Their cytoplasm becomes vacuolated and the cells break down, leaving a homogenous layer of medium electron density measuring 0·2 μm in average thickness (Pl. 2A) which persists until the cercariae leave the posterior region of the sporocyst.

Caudal cyst

The caudal cyst (Fig. 10) is rounded anteriorly, narrowing sharply at the ventro–laterally curved posterior end, termed the 'beak' by Popiel (1976*a*). A prominent sphincter muscle passes around the anterior aperture (Fig. 10; Pl. 3A, B). The caudal cyst wall consists of 3, occasionally 4, layers of curved fibres embedded in a homogenous matrix (Pl. 4C). The fibrous layers are not continuous

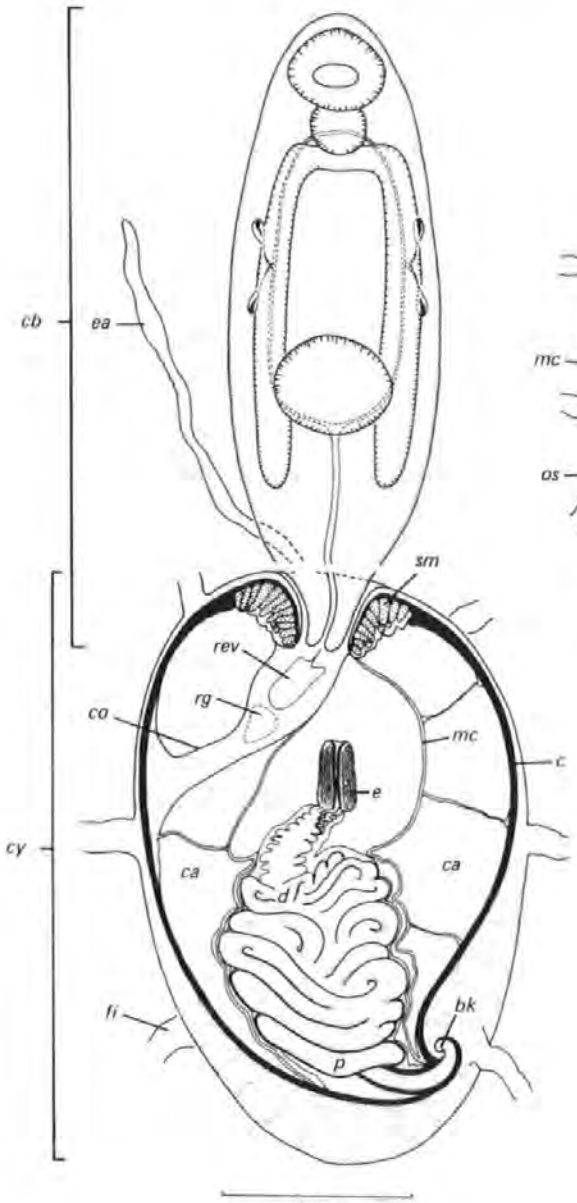


Fig. 10

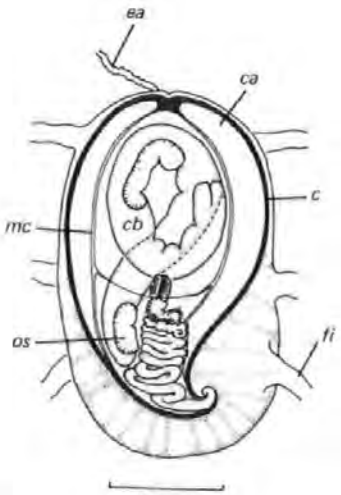


Fig. 11

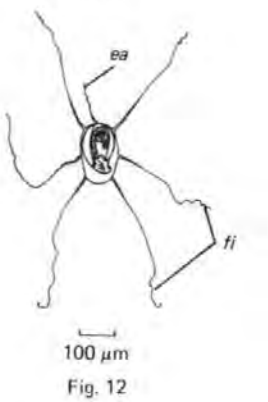


Fig. 12

Fig. 10. Migratory *Cercaria vaullegeardi* removed from anterior region of daughter sporocyst. Ventral and ventro–lateral views of body and tail respectively.

Fig. 11. Encysted *C. vaullegeardi*. Ventro–lateral view showing cercarial body and delivery tube coiled within membranous capsule.

Fig. 12. Ventro–lateral view of encysted *C. vaullegeardi* showing caudal filaments and excretory appendage. *bk*, caudal cyst beak; *c*, caudal cyst; *ca*, caudal cyst cavity; *cb*, cercarial body; *co*, connection of body to caudal cyst; *cy*, cystophorous tail; *d*, distal delivery tube; *e*, end-piece; *ea*, excretory appendage; *fi*, caudal filament; *mc*, membranous capsule; *os*, oral sucker; *p*, proximal delivery tube; *rg*, remains of caudal gland; *rev*, remains of caudal section of excretory vesicle; *sm*, sphincter muscle.

Table 1. *Measurements of* Cercaria vaullegeardi *(in μm)*

		Migratory stage	Encysted stage
Body	Contracted	117 × 47	—
	Extended	250 × 23	300 × 20
	Oral sucker	16 × 21	(distorted)
	Ventral sucker	18 × 21	(distorted)
Tail	Length × maximum breadth	130 × 100	165 × 104
	Caudal filaments (length × breadth at base)	465 × 14	465 × 14
	Excretory appendage (length × maximum breadth)	115 × 7	115 × 7
	Caudal cyst (length × maximum breadth)	104 × 78	134 × 90
	End-piece	13 × 9	13 × 9

around the tip of the beak (Pl. 5A). The loosely-packed fibres of the first-formed outer layer are arranged haphazardly (Pl. 2A). In addition, 2 or 3 layers of parallel, curved fibres are laid down successively inside the first, the completed caudal cyst (Pl. 4C) measuring 1 μm in average thickness. It stains intensely with aniline blue in Heidenhain's Azan (Pantin, 1962), a reaction characteristic of collagenous material (Pearse, 1968).

During the development of the caudal cyst, the fibres appear to originate from a single layer of sub-tegumental cells (Pl. 2A), being laid down immediately adjacent to their outer border. That these are fibre-forming cells is further suggested by the progressive thickening of the caudal cyst wall from within. The cells have large nuclei and dense cytoplasm with rough endoplasmic reticulum (RER), cisternae, free ribosomes and mitochondria (Pl. 2A). Following completion of the caudal cyst, the fibre-forming cells degenerate. The caudal cyst cavity of encysted forms contains scattered granular material and an irregular homogeneous deposit (Pls 4C, 5B). Both substances are weakly PAS-positive and negative with Alcian blue (pH 1·0 and 2·5).

Delivery tube

The delivery tube comprises a short proximal region, measuring 0·2 mm in average length, attached to the internal surface of the caudal cyst beak, and an elongated distal region, 0·7 mm in average length, which terminates in a thickened structure here termed the end-piece (Fig. 10). The wall of the distal delivery tube consists of an outer anucleate syncytial tegument with microtubules, ribosomes, mitochondria and RER, and an inner fibrous layer measuring 0·2 μm in average thickness (Pl. 3C, D). The thicker, less flexible wall of the proximal delivery tube in the migratory cercaria has no tegument, but consists of a double layer of curved fibres (0·4 μm in average thickness) (Pl. 4D) similar to those described in the caudal cyst.

The rudiment of the fibrous wall of the delivery tube differentiates as a column

of 10–12 cells, running along an oblique antero–posterior axis (Figs 5 and 6; Pl. 1A). The cells divide, forming an exterior outgrowth; internal and external portions of the column give rise to the proximal and distal delivery tube respectively. The cells are of similar ultrastructure (Pl. 2B) to the fibre-forming cells of the developing caudal cyst, laying down the fibrous layers which strengthen the delivery tube wall. Each cell elongates, greatly increasing the total length of the column. The external portion develops without hindrance, lying freely within the sporocyst lumen, whilst the restricted internal portion folds within the caudal cyst (Fig. 7). When the fibrous wall is complete, the fibre-forming cells degenerate and break down, leaving a continuous cavity along the centre of the column.

In pre-migratory cercariae the developing proximal delivery tube is covered by dense, nucleated syncytial cytoplasm with numerous mitochondria, free ribosomes, microtubules and RER (Pls 1B, 2B). As the appendage folds within the tail, a cavity develops around it (Fig. 7); sections now show the syncytium to comprise both the investing layer of the tube and an outer nucleated sleeve (Pl. 1A, B). Cytoplasmic breakdown occurs in both syncytial layers leaving 3 membranes which together comprise the membranous capsule (Figs 10 and 11).

Differentiation of the end-piece is shown in Figs 5–7, 9 and 10. A sub-terminal cell divides to produce a swelling containing a cluster of 10–15 cells with large, rounded nuclei and dense cytoplasm with mitochondria, microtubules, RER and free ribosomes (Pl. 6C). A fibrous layer is laid down by flattened cells of similar ultrastructure to the fibre-forming cells described above. Several terminal cells with cytoplasm containing elongated mitochondria, ribosomes, microtubules and cisternae, extend distally and laterally to form an incomplete tube, or collar-shaped extension. Electron-dense deposits accumulate around the periphery of the terminal cells on the inside of the fibrous layer (Pl. 6D). Cellular shrinkage and breakdown, leaving a cavity continuous with the distal delivery tube lumen, is followed by contraction of the fibrous wall producing the heavily folded mature end-piece of the migratory cercaria (Fig. 10). The latter gave a positive reaction for acetylcholinesterase (Pl. 6A, B) and non-specific esterases.

The fully formed delivery tube is gradually retracted into the posterior half of the membranous capsule (Figs 9 and 10; Pls 4D, 5B), retraction being completed before the cercaria migrates forward into the anterior region of the sporocyst (Matthews, 1980).

Excretory appendage

The posterior median lobe of the embryonic tail with fused caudal excretory ducts (Fig. 3), narrows and lengthens (Figs 4–7) becoming the excretory appendage described diagrammatically by Pelseneer (1906). The appendage moves into an anterior position by the differential growth of the rest of the tail, coming to lie at the dorsal rim of the caudal cyst aperture (Figs 8 and 9). The appendage is at first capable of restricted movement, occasionally undergoing slow contractions. The excretory duct degenerates and cellular breakdown occurs; the shrivelled remains of the excretory appendage are, however, still present in the encysted cercaria (Figs 11 and 12).

(iii) *Excretory system*

The excretory system is most extensive in pre-migratory forms (Figs 7 and 8). The caudal ducts and 4 flame cells subsequently break down (Figs 9 and 10), only those parts of the excretory system within the cercarial body remaining in encysted cercariae.

(iv) *Encystment*

At encystment, the retractor muscles within the cercarial body (Pl. 3A) contract, and the strand connecting the body to the caudal cyst (Fig. 10) breaks. Contraction of the tubular caudal sphincter muscle (Pl. 3A, B) commences whilst the body slips backwards into the tail, the aperture being sealed as retraction is completed. The body moves slowly within the tail, undergoing periodic stretching movements. It increases in length to 0·3 mm, becoming correspondingly slender, 0·02 mm in average diameter (Fig. 11).

Retraction of the body into the caudal cyst normally occurs on the surface of the host gill within seconds of emergence from the sporocyst birth pore into the host mantle cavity (Matthews, 1980). If migratory cercariae are obtained by dissection, normal encystment seldom occurs. In these cases cercariae may either not encyst, floating at the water surface, or the body may become trapped by the contracted sphincter muscle in a partially withdrawn state.

DISCUSSION

The cystophorous tail of *C. vaullegeardi*, as in all other hemiurid cercariae so far described, consists essentially of a caudal cyst and various appendages including a delivery tube. A laminated fibrous structure as seen here in the caudal cyst wall has not been previously described in any other digenean, but a similar arrangement of curved fibres has been reported from such diverse examples throughout the animal kingdom as teleost eggs, crustacean cuticle, and peridinian and bacterial chromosomes (Bouligand, 1965*a*, *b*, 1972; Dalingwater, 1975) where strength and flexibility are of prime importance. The delivery tube wall contains similar fibres, laid down around the periphery of a single column of cells in the appendage rudiment which subsequently degenerate to form the tube lumen. The fibrous structure of the developing caudal cyst and delivery tube were noted by Popiel (1976*b*); these studies were of immature forms, however, and did not detect the cyst cavity or the lumen of the tube and its function in the delivery of the cercarial body.

The development of the modified tip of the delivery tube, here termed the end-piece, resembles Appendage I described by Rothschild (1938) in *C. sinitzini*, although several terminal cells occur in *C. vaullegeardi* in addition to the group of numerous small cells with rounded nuclei. The present study shows the accumulation of electron-dense material peripheral to the terminal cells and it is of interest that acetylcholinesterase was detected in this region at the light microscope level, in addition to its expected location within cerebral ganglia and longitudinal nerves of the cercarial body. Although neurons have been observed within the tail in connection with the sphincter muscle, they have not been noted

in stages studied here within the delivery tube, and their presence in this position seems most unlikely in view of the development of this appendage from a single column of fibre-forming cells and its acellular nature when mature. The presence of acetylcholinesterase in association with non-nervous tissue has been reported in mammalian erythrocytes (Froede & Wilson, 1971) and the enzyme is secreted by the exo-digestive glands of the nematode *Nippostrongylus brasiliensis* (Lee, 1970).

At retraction of the delivery tube into the caudal cyst, considerable shortening of the appendage occurs, associated with folding of the fibrous layer within the tegument. Popiel (1976*b*) also recorded the folded fibrous layer, the absence of muscle fibres in the delivery tube leading her to the opinion that no active contractile process could be involved. Rothschild (1938) similarly noted folding within the delivery tube of *C. sinitzini*, and suggested a passive withdrawal due to pressure changes set up by the enlargement of the internal cyst cavities. The absence of muscle fibres need not necessarily, however, rule out an active process, as contraction could be a function of the fibrous layer. The occurrence of contractile properties in many types of non-muscle cell is well documented (Allison, Davies & dePetris, 1971; Bettex-Galland & Hughes, 1973; Bray, 1973; Pollard & Korn, 1973; Schliwa & Bereiter-Hahn, 1975; Saleuddin & Jones, 1976).

Of interest is the dissemination of membrane-bound secretory products from the caudal gland to the tegument of caudal cyst and filaments, and the subsequent persistent indentations at the point of discharge on the surface. Popiel (1976*b*) noted electron-lucent vesicles within the caudal tegument and suggested that they might increase buoyancy in the external environment. Studies here show that the contents of the vesicles are discharged prior to emergence from the sporocyst, and although increased surface area resulting from the indentations might also have been considered to increase buoyancy, naturally emerged encysted cercariae rarely float but lie on the bottom. In view of the need to be selected as food by the next intermediate host (Matthews, 1981) it might be supposed that gustatory or adhesive properties might be attributed to the secretions of the caudal gland. Following discharge of the vesicles the remaining indentations would assist by affording grip for the copepod appendages, as the cercariae have to be manipulated by the latter during feeding (Matthews, 1981).

The excretory appendage of *C. vaullegeardi* develops from the median posterior lobe of the embryonic tail which carries the fused caudal excretory ducts to the exterior, and may therefore on grounds of comparative morphology be considered homologous to the locomotory tail of other cercariae. The term 'excretory appendage' was first applied to this structure by Pelseneer (1906) who described diagrammatically its development and regression relative to the rest of the tail. Observations here have shown that the appendage retains a limited capacity for movement in early stages, undergoing slow sustained contractions, but regresses to a vestigial condition in the fully-formed cercaria. Available information concerning the early development of other hemiurid cercariae indicates that the degree of reduction of that part of the embryonic tail carrying the caudal excretory ducts to the exterior determines the final degree of motility retained. The excretory appendage of *C. vaullegeardi* is here considered to be homologous to Appendage II of *C. sinitzini* as described by Rothschild (1938). Both appendages undergo total

regression, the encysted cercaria being immotile in both species. In the encysted cercaria of *Derogenes varicus*, however, the forked excretory appendage is used for active swimming movements (Pelseneer, 1906; Køie, 1979).

Observations have shown that in *C. vaullegeardi* the excretory system is less extensive in mature than in developing stages. This is as might be expected in view of the breakdown of cellular elements throughout the cystophorous tail. No data are available concerning the caudal excretory system in other hemiurid cercariae, but results here indicate that flame cell formulae (La Rue, 1957) may be misleading in this group of Digenea unless applied to both mature and developing stages.

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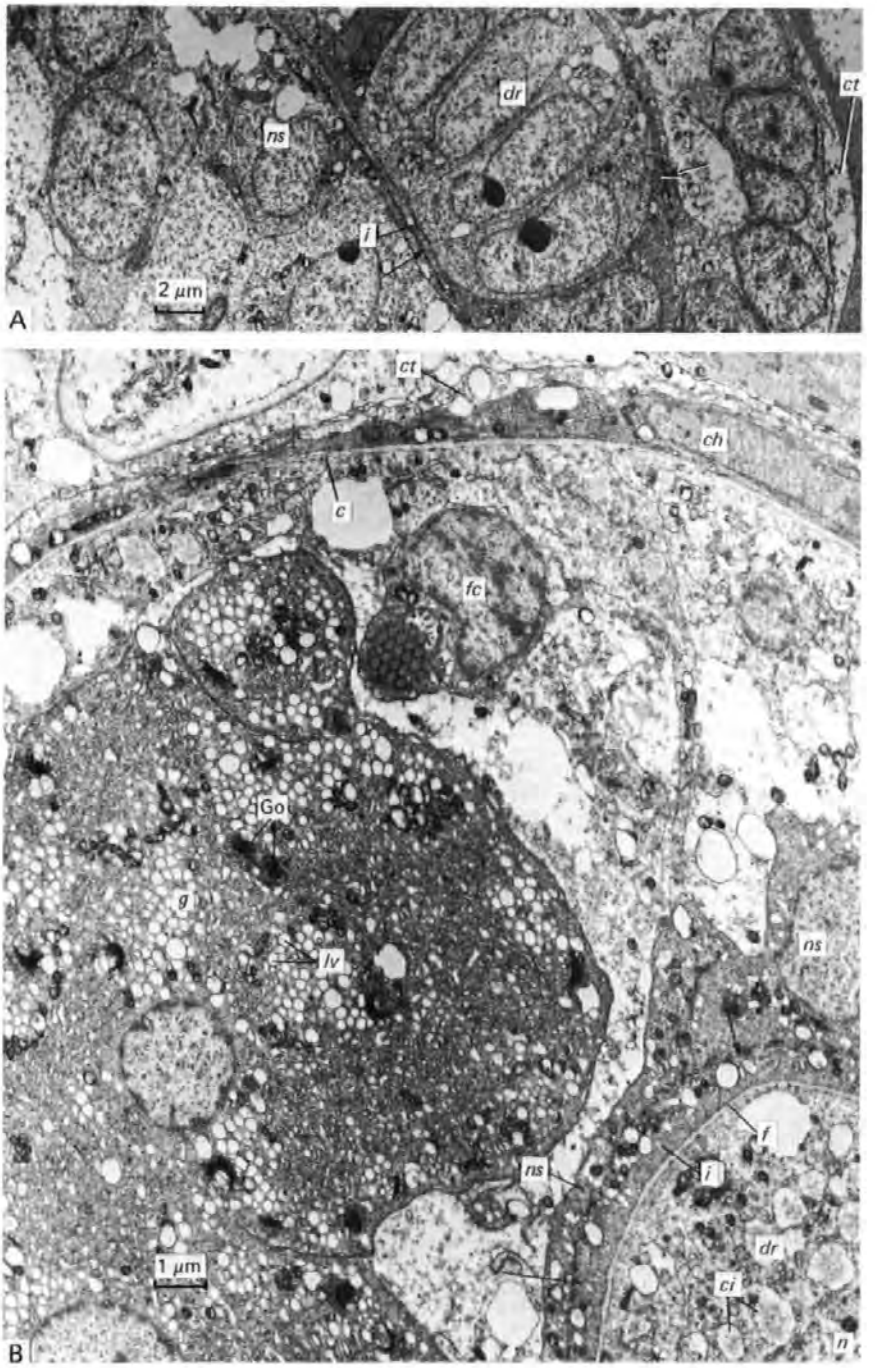
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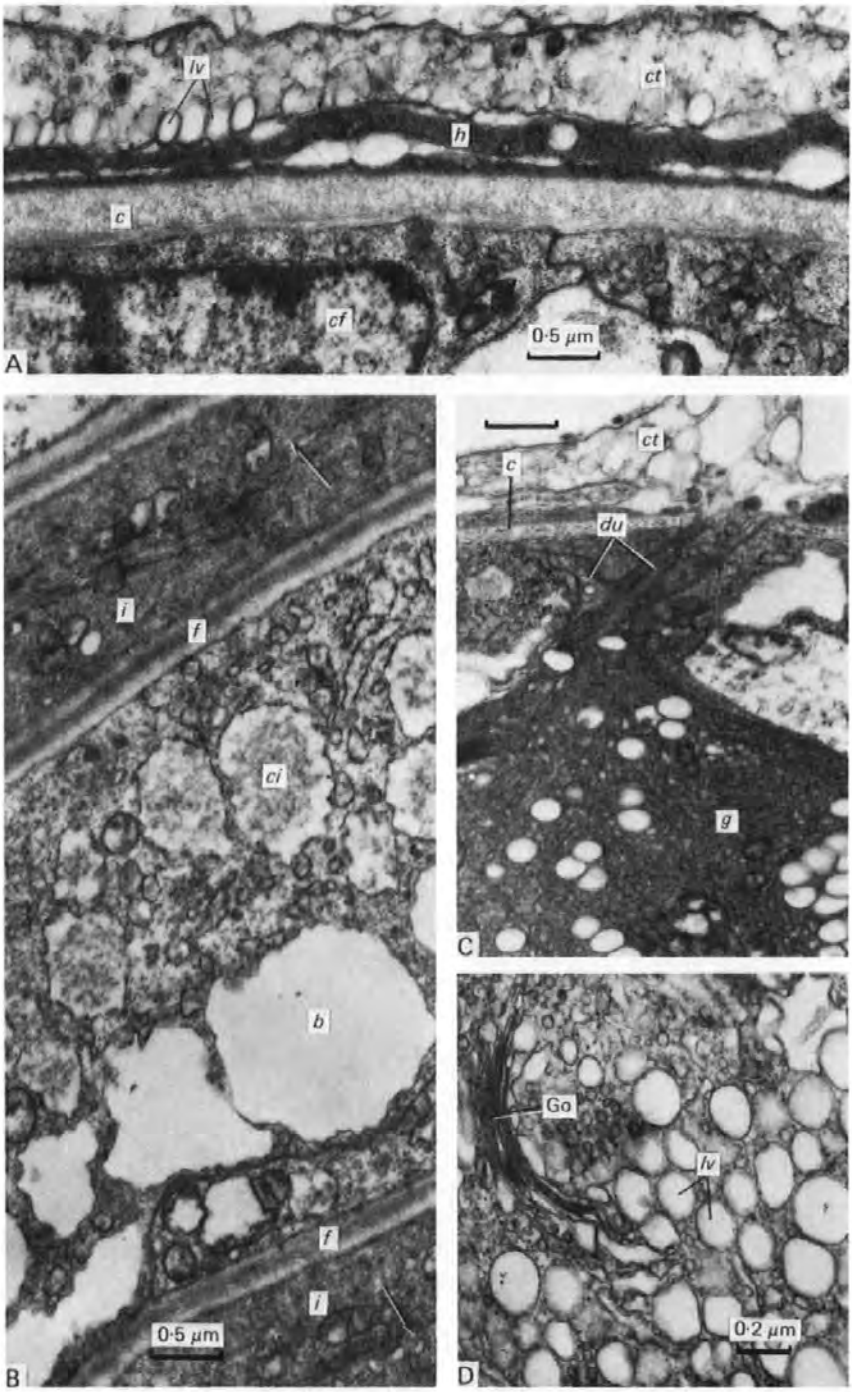

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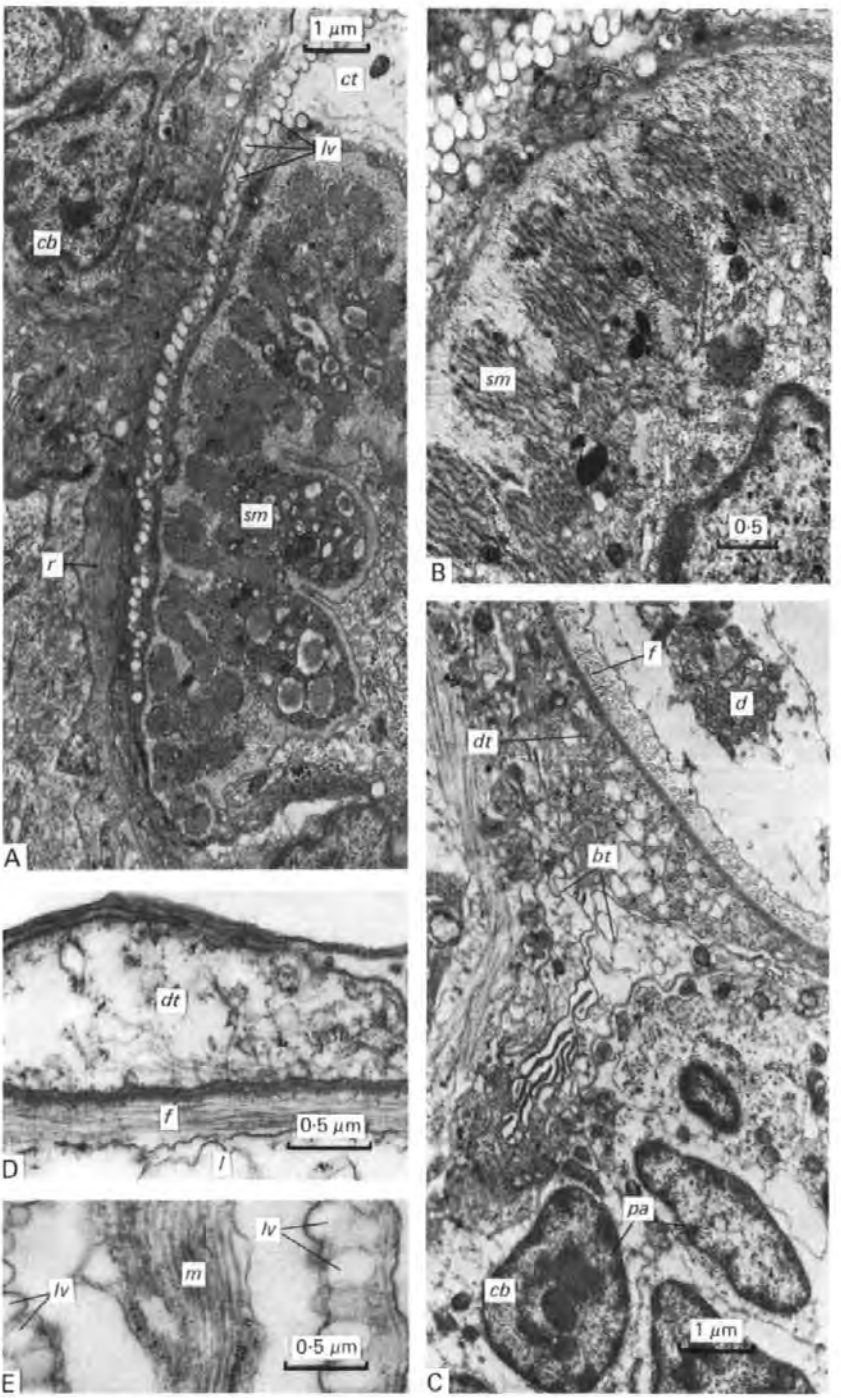
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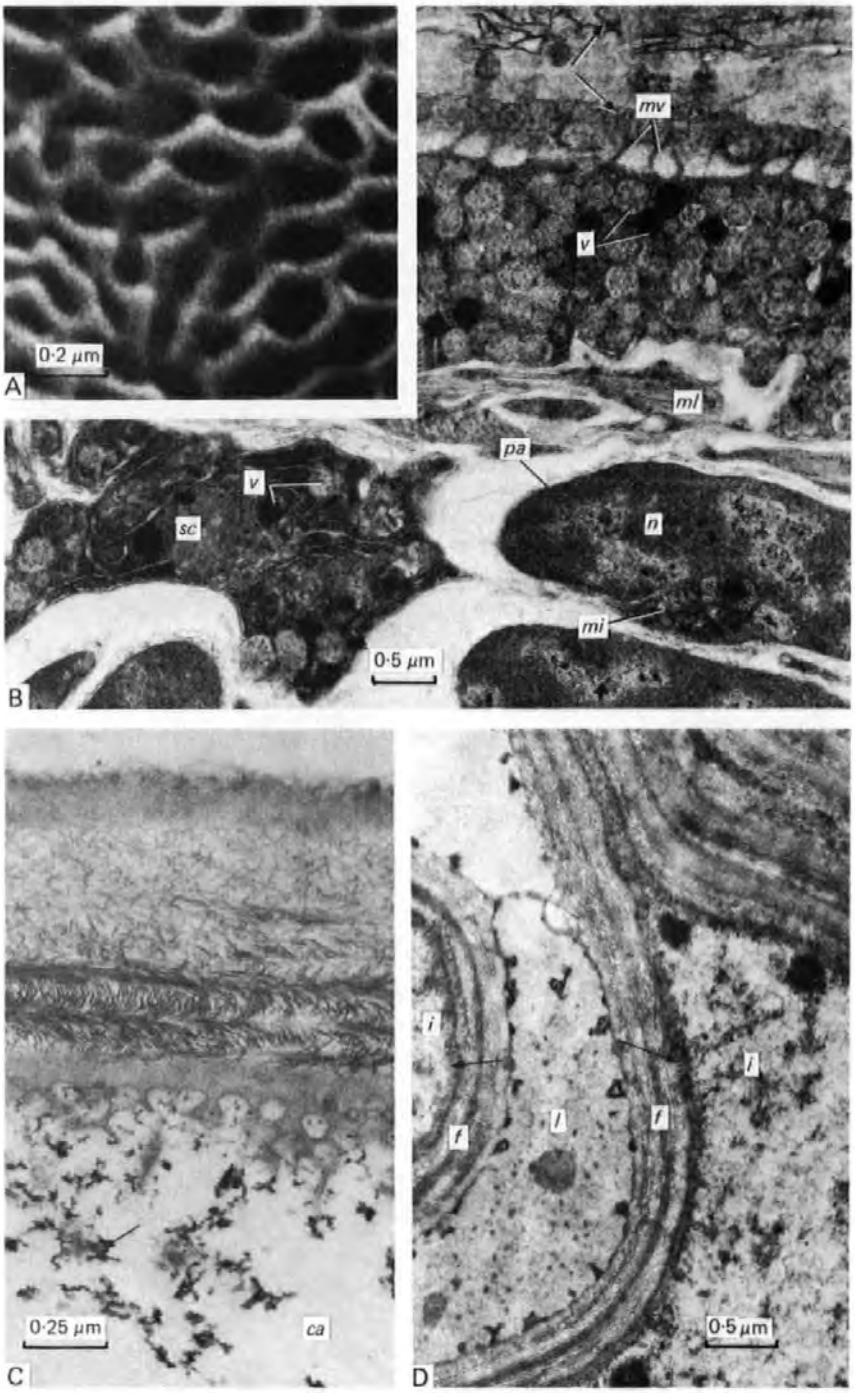

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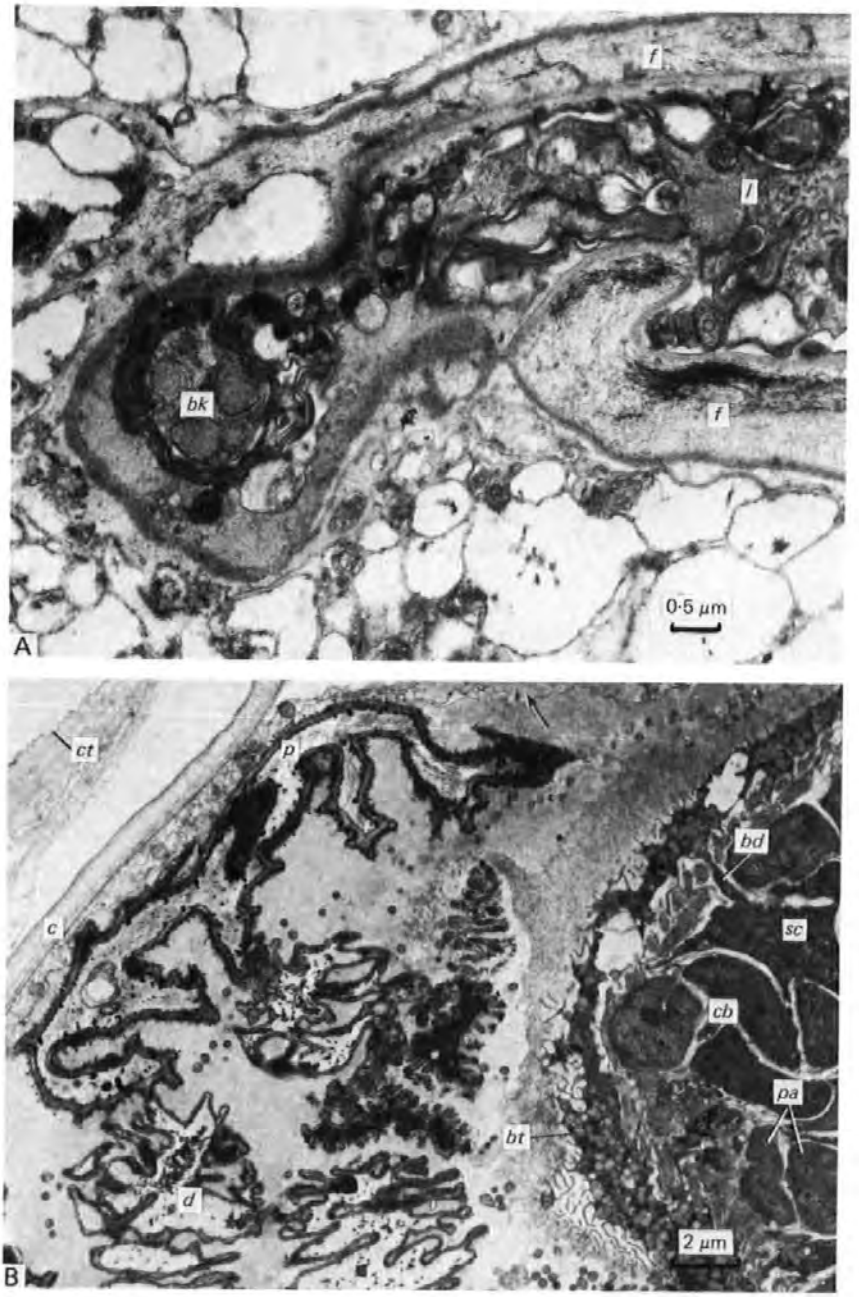

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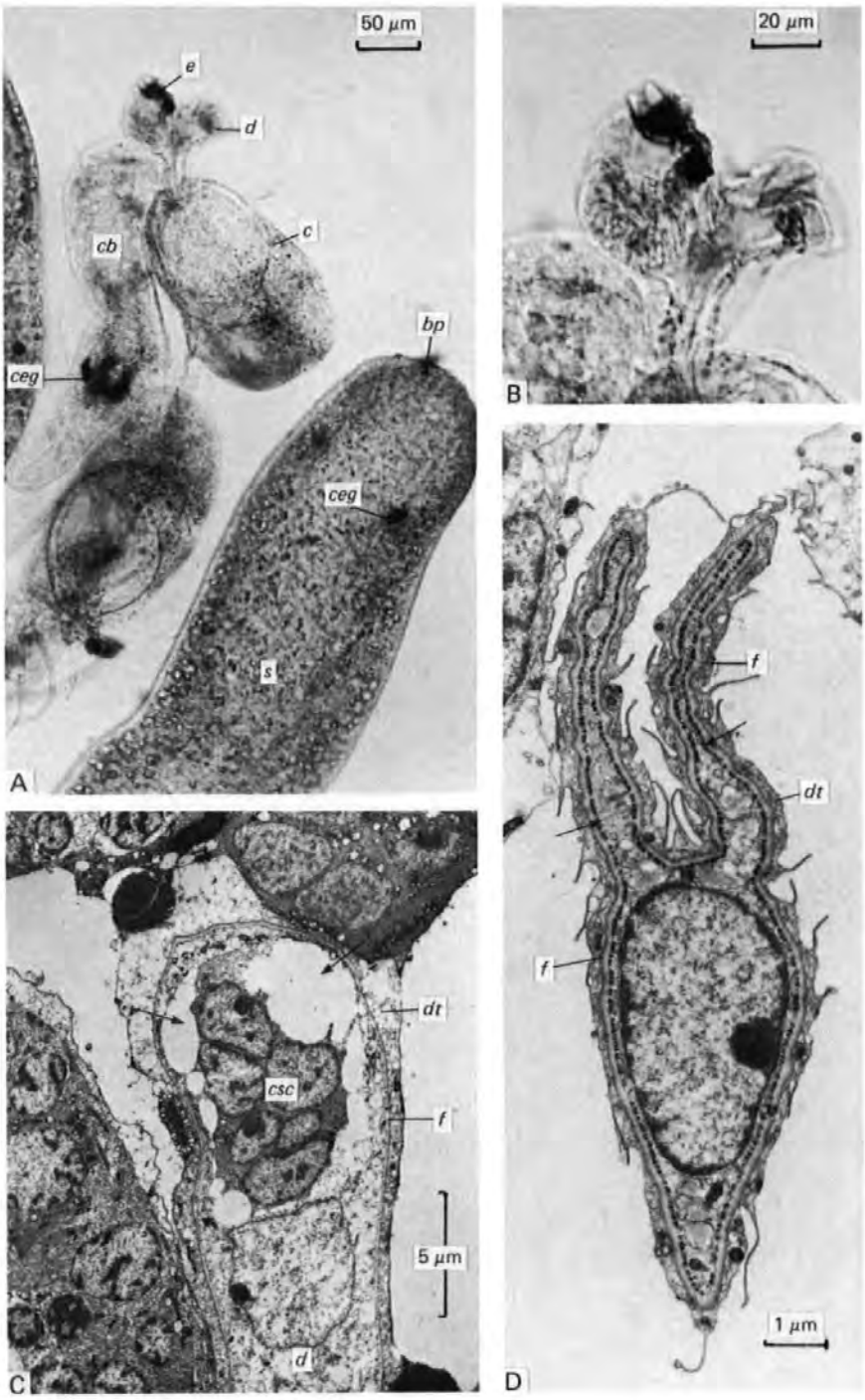

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EXPLANATION OF PLATES

PLATE 1

Electron micrographs of the tail of pre-migratory *Cercaria vaullegeardi*. Note investing layer (*i*) of developing proximal delivery tube (*dr*) and outer nucleated portion of syncytium (*ns*) separated by a narrow space (arrowed); *ct*, caudal tegument.

A. Longitudinal section through posterior end of delivery tube rudiment. Early developmental stage corresponding to Fig. 5.

B. Transverse section through caudal gland (*g*) with Golgi (*Go*) and electron-lucent vesicles (*lv*) and delivery tube rudiment. Later stage, corresponding to Fig. 6. Note nucleus (*n*) and cisternae (*ci*) of fibre-forming cell of delivery tube rudiment; developing fibrous layer (*f*). Sub-tegumental cell (*ch*) lying between tegument and developing caudal cyst (*c*). *fc*, Flame cell.

PLATE 2

Electron micrographs of the tail of pre-migratory *Cercaria vaullegeardi*.

A. Transverse section through developing caudal cyst wall (*c*) and fibre-forming cells (*cf*). The tegument (*ct*) contains electron-lucent vesicles (*lv*); a homogenous layer (*h*) lies between the tegument and the caudal cyst.

B. Oblique section through the delivery tube rudiment. Note fibrous wall (*f*) and narrow space (arrowed) between investing syncytium (*i*) of adjacent folds of developing proximal delivery tube. Fibre-forming cell cytoplasm containing cisternae (*ci*) is undergoing breakdown to form cavities (*b*) which will coalesce to form the tube lumen.

C. Longitudinal section of caudal cyst (*c*) showing branched duct (*du*) passing from the caudal gland (*g*) to the tegument (*ct*).

D. Caudal gland cytoplasm with Golgi (*Go*) and electron-lucent vesicles (*lv*).

PLATE 3

Electron micrographs of pre-migratory *Cercaria vaullegeardi*.

A. Longitudinal section showing one side only of caudal sphincter muscle (*sm*). Note the electron-lucent vesicles (*lv*) which have accumulated in a single layer within the caudal tegument (*ct*); cercarial body (*cb*) with retractor muscle (*r*).

B. Longitudinal section of caudal sphincter muscle (*sm*).

C. Cercarial body (*cb*) (longitudinal section) and distal delivery tube (*d*) (transverse section) within daughter sporocyst lumen. Note cercarial body tegument (*bt*) and nuclei of parenchyma cells (*pa*). *dt*, Tegument of delivery tube; *f*, fibrous wall.

D. Longitudinal section of distal delivery tube wall before retraction into caudal cyst. *dt*, Tegument; *f*, fibrous layer; *l*, tube lumen.

E. Longitudinal section of caudal filament. Note layer of electron-lucent vesicles (*lv*) within the tegument and the microtubules (*m*).

PLATE 4

Electron micrographs of encysted *Cercaria vaullegeardi*.

A. Scanning micrograph showing the indentations which cover the caudal tegument.

B. Transverse section through cercarial body within membranous capsule (arrowed). Vesicles with flocculent and electron-dense contents (*v*) within a sub-tegumental secretory cell (*sc*) have passed into the tegument, some being discharged at the surface into the cavity of the membranous capsule. Note parenchyma cells (*pa*) with cytoplasm containing mitochondria (*mi*) and ribosomes confined to surface indentation of nucleus (*n*). *mv*, Tegumental microvilli; *ml*, muscle layers.

C. Longitudinal section through caudal cyst. Note breakdown of the fibre-forming cells contributing to the caudal cyst cavity (*ca*) which contains scattered electron-dense granular material (arrowed).

D. Adjacent folds of proximal delivery tube; fibrous layers of wall, (*f*) and lumen (*l*). Note the folded basement membrane (arrowed) of disintegrated investing syncytium (*i*).

PLATE 5

Electron micrographs of encysted *Cercaria vaullegeardi*.

A. Longitudinal section through caudal cyst beak (*bk*). Note the discontinuity of fibrous layers (*f*) around the tip and lumen (*l*) of the proximal delivery tube.

B. Tangential section showing cercarial body (*cb*) with proximal (*p*) and distal (*d*) sections of delivery tube within the membranous capsule (arrowed). Note secretory vesicles within the cercarial body tegument (*bt*), sub-tegumental secretory cell (*sc*) with cytoplasmic bridge (*bd*) and nuclei of parenchyma cells (*pa*). *c*, Caudal cyst; *ct*, caudal tegument.

PLATE 6

A and B. Light micrographs of *Cercaria vaullegeardi* showing location of acetylcholinesterase as indicated by a positive reaction using the method of Jennings & LeFlore (1972).

A. Two migratory cercariae and the anterior extremity of a daughter sporocyst (*s*) with birth pore (*bp*). Cover-slip pressure has caused the expulsion of part of the distal delivery tube through the anterior aperture of the caudal cyst. Positive reaction within the delivery tube end-piece (*e*) in addition to cerebral ganglia (*ceg*) and longitudinal nerves. *c*, Cercarial caudal cyst; *cb*, cercarial body; *d*, distal delivery tube.

B. Delivery tube end-piece (enlargement of A).

C and D. Electron micrographs of pre-migratory *C. vaullegeardi* showing developing delivery tube end-piece.

C. Tangential section through cluster of specialized cells (*csc*), distal delivery tube (*d*) with tegument (*dt*) and fibrous layer (*f*). Note cavity (arrowed) caused by cellular shrinkage and breakdown within the developing end-piece.

D. Oblique section through terminal cell: note deposits of electron-dense material (arrowed). *dt*, Tegument; *f*, fibrous layer.

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Cercaria vaullegeardi Pelseneer, 1906 (Digenea: Hemiuridae); the infection mechanism

B. F. MATTHEWS

Department of Biology, Plymouth Polytechnic, Drake Circus, Plymouth, Devon PL4 8AA

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SUMMARY

The inoculative mechanism whereby cystophorous cercariae infect the copepod 2nd intermediate host is described for the first time in *Cercaria vaullegeardi*. Experimental infections of the harpacticoid copepod *Tigriopus brevicornis* are recorded and the infection process is related to the ultrastructure of the cercaria and to the feeding mechanics of harpacticoids. The cystophorous tail of *C. vaullegeardi* is shown to be a device whose shape and construction ensure that the cercarial body is neither damaged by copepod mouthparts nor swallowed, but reaches the host haemocoel during the initial stages of feeding. The cystophorous tail consists essentially of a caudal cyst, into which the cercarial body retracts at encystment, and various appendages including a delivery tube. The infection mechanism is triggered when the copepod mandibles bite the narrow posterior caudal cyst beak, when the delivery tube everts into the mouth and penetrates the dorsal mid-gut wall. The cercarial body, lubricated by tegumental secretions, passes simultaneously through the delivery tube and is inoculated into the host haemocoel.

INTRODUCTION

Stunkard (1973) has drawn attention to the 'meagre and fragmentary' knowledge of the life-history and developmental stages of hemiurid trematodes, which has given rise to a wide divergence of opinion concerning their systematics and classification. The life-cycles of 4 species of the freshwater genus *Halipegus* have been elucidated by Leuckart (1899), Krull (1935), Thomas (1939), Rankin (1944), and Macy, Cook & DeMott (1960), but at present the only marine life-cycles determined experimentally are those of *Lecithaster confusus* by Hunninen & Cable (1943), and *Derogenes varicus* by Køie (1979).

It has been known for some time that in cystophorous cercariae, characteristic of Hemiuridae, the body retracts into a cavity within the tail on emergence from the snail host, and the 'encysted' cercaria is then eaten by the crustacean 2nd intermediate host, generally a copepod (Stunkard, 1973). The cercarial body, containing neither penetration nor cystogenous glands, reaches the host haemocoel by a unique mechanism whereby it is inoculated by a specialized caudal appendage, the delivery tube, through the gut wall. Little information is available concerning the infection process, although some observations have been made by Krull (1935) in *Halipegus occidualis*, Chabaud & Biguet (1955) in an unnamed cystophorous

cercaria, and by Køie (1979) in *D. varicus*. During the course of life-cycle studies here currently in progress, experimental infections of the harpacticoid copepod *Tigriopus brevicornis* with *Cercaria vaullegeardi* were obtained for the first time. The present investigation relates observations on the infection mechanism to the ultrastructure of the cercaria described in a previous paper (Matthews, 1981) and to the mouthparts and feeding behaviour of harpacticoids.

MATERIALS AND METHODS

Naturally emerged *C. vaullegeardi* were obtained from screened infected *Gibbula umbilicalis* Da Costa collected from mid-tide level of sheltered rocky shores around Plymouth, Devon between March and September, 1974–78. A binocular microscope was used for screening the snails and recovery of the tiny immotile cercariae from the bottom of the containers. Laboratory-reared *T. brevicornis* O. F. Müller, maintained in bowls of filtered seawater at 17 °C and fed on *Isochrysis*, were infected with *C. vaullegeardi* as follows: naturally-emerged cercariae from freshly collected infected *G. umbilicalis* were counted into individual chambers of haem-agglutination plates and a single *T. brevicornis* added to each chamber. Observations were made concerning infection rates and viability of cercariae. Infected copepods were removed immediately after infection and prepared for transmission electron microscopy (TEM) as previously described (Matthews, 1981). The copepod cuticle was perforated in both prosomal and metasomal regions to improve penetration of reagents. The mouthparts and internal anatomy of infected and control *T. brevicornis* were studied using live specimens and methylene blue-stained, 2–4 μm serial sections of material fixed for TEM. Numerous unsuccessful attempts were made to infect other invertebrates with *C. vaullegeardi* including *Amphithöe rubricata*, *Gammarus* spp. and *Nereis diversicolor*.

OBSERVATIONS AND RESULTS

Experimental infections of T. brevicornis

Naturally emerged encysted *C. vaullegeardi* lie on the bottom of the container, either singly or entangled with each other in cottonwool-like masses. *T. brevicornis* is a raptorial feeder, which scavenges through debris and vegetation and readily selects cystophorous cercariae as food. Repeated observations have shown that the behaviour of *T. brevicornis* when eating *C. vaullegeardi* always follows the same pattern; the copepod seizes the cercaria with the second antennae, second maxillae and maxillipeds, turns it so that its long axis lies parallel to that of its own body, and pressing it against the mandibles attempts to bite into its surface. Mandibular purchase cannot be gained against the rounded anterior end of the rigid fibrous caudal cyst (Matthews, 1981) and the copepod therefore turns the cercaria until the biting mouthparts can be used to better advantage on the posterior end (Pl. 1C). The tegumental membranes eaten, the narrow curved posterior region of the caudal cyst, or beak (Pl. 1B) is pressed into the mouth (Fig. 1) and crushed by the mandibles. When the tip of the beak, unprotected by fibres (Matthews, 1981) is severed by mandibular action, the delivery tube everts into the copepod's oesophagus. The thickened proximal section emerges first, forcibly penetrating the

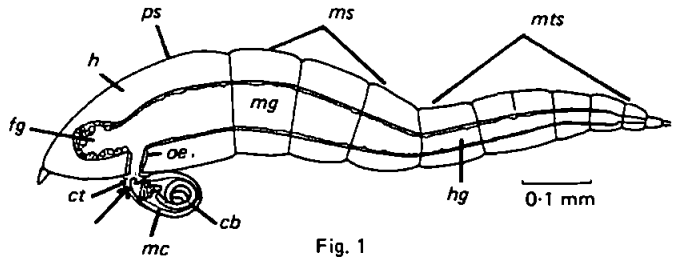
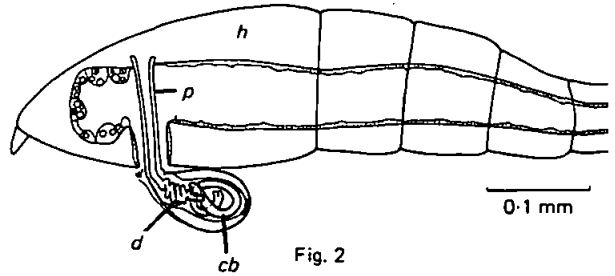


Fig. 1



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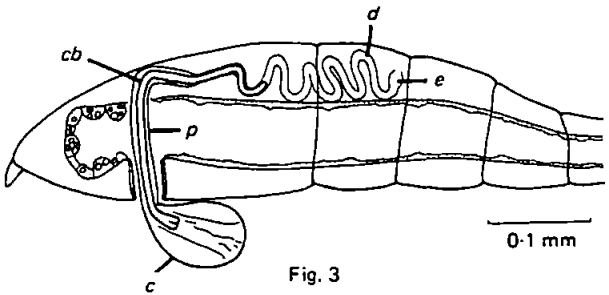

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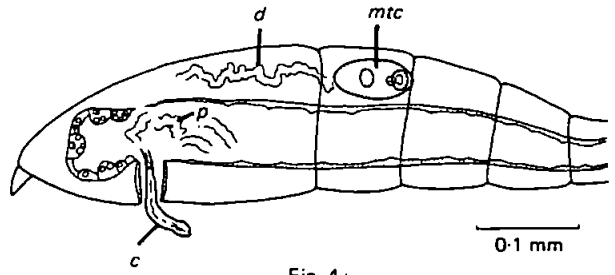

Fig. 4

Figs 1–4. Diagrams to illustrate stages in the infection of *Tigriopus brevicornis* with *Cercaria vaullegeardi*. (The copepod appendages are not shown to increase clarity.)

Fig. 1. The copepod orientates the cercaria so that its longitudinal axis lies parallel to that of its own body. The tegumental membranes at the posterior end eaten, the narrow caudal cyst beak (arrowed) is pressed into the mouth and sheared by the mandibles.

Fig. 2. The proximal delivery tube everts up the copepod oesophagus, piercing the dorsal wall of the midgut.

Fig. 3. The distal delivery tube everts within the copepod haemocoel: the cercarial body passes into the tube.

Fig. 4. The metacercaria now lies within the host haemocoel. The collapsed caudal cyst and the proximal delivery tube are eaten.

c, Caudal cyst; *cb*, cercarial body; *ct*, caudal tegument; *d*, distal delivery tube; *e*, end-piece; *fg*, fore-gut; *h*, haemocoel; *hg*, hind-gut; *mc*, membranous capsule; *mg*, mid-gut; *ms*, mesosoma; *mtc*, metacercaria; *mts*, metasoma; *oe*, oesophagus; *p*, proximal delivery tube; *ps*, prosoma.

dorsal wall of the midgut (Fig. 2), followed by the more flexible thin-walled distal tube which everts within the haemocoel (Fig. 3). The cercarial body simultaneously passes through the delivery tube lumen into the host haemocoel (Fig. 4). The collapsed caudal cyst is crushed and eaten.

Up to 7 metacercariae (measuring 0·08 × 0·05 mm in average length and maximum breadth) may be obtained within the haemocoel of a single *T. brevicornis* (Pl. 1 A). The metacercaria occasionally extends and contracts its body, but does not change its position within the haemocoel, remaining near the site of emergence from the distal delivery tube.

Metacercarial tegument (Pl. 2) fixed *in situ* within the copepod immediately after infection, measures 1·5 μm in average thickness, and is sparsely covered with microvilli. The electron-dense vesicles characteristic of the tegument of encysted cercariae (Matthews, 1981) have discharged their contents.

Viability of C. vaullegeardi

In freshly emerged cercariae the coiled transparent body stretches intermittently within the tail, maintaining an elongated slender form averaging 0·2 × 0·002 mm. Movements gradually become less frequent and the body becomes shorter and wider. Movement ceases and the cercarial body, now measuring 0·1 × 0·08 mm, becomes opaque after 5 days at 14 °C and 10 days at 8 °C. The viability of cercariae obtained during March 1977 from a single infected *G. umbilicalis* was found to decrease correspondingly with age. Infection rates of 50 *T. brevicornis* individually fed with *C. vaullegeardi* previously maintained for 3 h, 24 h and 3 days at 14 °C being 100%, 50% and 6% respectively.

A seasonal effect on viability was noted, cercariae released in spring producing consistently higher infection rates than those shed in late summer and autumn. Infection rates of 100% were obtained in 200 *T. brevicornis* individually exposed at 14 °C to 3–5 *C. vaullegeardi* newly emerged from freshly collected *G. umbilicalis* in March and April of 1977 and 1978. In September of the same years similar experimental procedures produced infection rates below 25%.

Shedding of *C. vaullegeardi* has not here been observed between November and January 1974–1980, although samples of *G. umbilicalis* opened throughout the winter have shown the usual 1·13% infection rate with the daughter sporocysts. The latter at this time extend into the host gill filaments (Matthews, 1980) but their anterior transporting regions contain few, if any, migratory cercariae. The latter, and developing stages within the posterior region of the sporocyst, are completely motionless except for flame cell action. It is not known if cercarial production is resumed by these hosts in the spring, coinciding with increased harpacticoid populations in the rock pools.

Infection experiments using other invertebrates

Amphithöe rubricata, *Gammarus* spp. and *Nereis diversicolor* readily devoured *C. vaullegeardi*. Metacercariae were obtained within the haemocoels of only 2 out of 300 *A. rubricata* fed with the cercariae, and the large numbers of *Gammarus* spp. and *N. diversicolor* remained uninfected. In *N. diversicolor* the gut frequently became filled with undischarged encysted cercariae which were subsequently digested.

Delivery tube eversion in vitro

Attempts to induce delivery tube eversion by application of cover-slip pressure resulted in rupture at the caudal sphincter muscle and expulsion of the body through the anterior aperture. Gentle rolling of the cover-slip, however, initiated delivery tube eversion (Pl. 3 E), by mechanical stress on the asymmetrical caudal beak.

The wall of the retracted, coiled distal delivery tube consists of an inner fibrous layer covered by tegument (Matthews, 1981). After eversion, the fibrous layer is seen as torn strips adhering to the outside of the distal delivery tube and end-piece (Pl. 3 A–C). The everted end-piece is pear-shaped, covered with refractile granules, and measures 54 μm in length × 20 μm in maximum breadth. The narrow distal end is supported by a thickened collar (Pl. 3 B) measuring 7·23 μm in length, with an aperture diameter of 7·23–9·64 μm.

Discharge of the delivery tube was associated with simultaneous expansion of the caudal cyst cavity to occupy the space previously taken by the coiled cercarial body and delivery tube.

DISCUSSION

Observations have shown that in *C. vaullegeardi* the function of the cystophorous tail is two-fold, serving for the protection of the cercarial body against damage by copepod mouthparts, and for its inoculation into the host haemocoel during the initial stages of feeding, before ingestion can occur.

As far as could be seen by direct observation, the feeding behaviour of *T. brevicornis* closely resembles that of the harpacticoid *Tisbe furcata* Baird, 1837, the raptorial feeding mechanism of which has been described in detail by Marcotte (1977) using slow-motion videotape. Results here indicate that both host food-particle orientation and mandibular action are vital to the infection mechanism of *C. vaullegeardi*. The shape and construction of the caudal cyst ensure that the unstrengthened tip of the beak is sheared by the cutting edges of the copepod mandibles, triggering delivery tube eversion and therefore allowing infection to occur without ingestion of, and damage to, the cercarial body. That the process is not random is emphasized by the 100% infection rate achieved when *T. brevicornis* were exposed to cercariae within 3 h of their emergence. Results here indicate that in *C. vaullegeardi* pressure applied by mouthparts does not trigger delivery tube eversion, as described by Køie (1979) in the cercaria of *D. varicus*; in the latter species, however, a less precise mechanism might account for its wide distribution in both demersal and pelagic fishes.

On delivery tube eversion in *C. vaullegeardi* the end-piece everts last into the host haemocoel, and therefore has no penetrating or holding function as suggested for *H. occidualis* by Krull (1935). The significance of the acetylcholin-esterase detected in the end-piece (Matthews, 1981) is not known. The association of this enzyme with non-nervous tissue is well documented, being recorded in mammalian erythrocytes (Froede & Wilson, 1971) and the anterior glands of parasitic nematodes (Lee, 1970; Ogilvie, Rothwell, Bremner, Schnitzerling, Nolan & Keith, 1973; McLaren, Burt & Ogilvie, 1974; Burt & Ogilvie, 1975). Lee (1970)

suggested that a possible function of the enzyme in *Nippostrongylus brasiliensis* may be to cause a localized temporary suppression of host peristalsis and therefore help the nematode to maintain its position within the gut lumen. It is tempting to speculate that the enzyme within the end-piece of *C. vaullegeardi* may serve a similar function during the infection process, ensuring a momentary paralysis of host muscles and thereby aiding introduction of the cercarial body into the haemocoel. In support of this hypothesis, Krull (1935) reported that when *Cyclops* spp. eat the cercariae of *H. occidualis* they frequently lie motionless, with appendages widely separated for as long as a minute before recovery.

Passage of the cercarial body through the delivery tube was associated with the release of vesicles from the tegument (Matthews, 1981), the contents of which are assumed to have a lubricatory function. The relatively undifferentiated condition of the loosely-packed parenchyma cells and ventral sucker (Matthews, 1981) may be significant in enabling the cercaria to assume the elongated shape necessary for inoculation. The temporarily assumed slender shape is considered to be the result of muscular action; it is of interest that Wilson, Draskau, Miller & Lawson (1978) described a similar situation in *Schistosoma mansoni*, in which the schistosomules assume an elongated shape prior to capillary migration within the lungs.

The forcible ejection of the delivery tube and cercarial body following triggering of the infection mechanism is suggestive of a pressure mechanism which might be explained in one of two ways. Ejection was associated with the simultaneous enlargement of the caudal cyst cavity, and it may be that the contained material (Matthews, 1981) becomes instantly hydrated on entry of water at cyst rupture, exerting the necessary pressure. Køie (1979) reported that in *D. varicus* the 'large, hyaline dead cells which form the wall of the caudal vesicle swell and fill the lumen' following *in vitro* delivery tube eversion. Alternatively, the necessary pressure may be mechanically generated on retraction of the cercarial body through the well-developed and closely applied tubular caudal sphincter muscle, the latter being tightly closed at completion of encystment when a significant increase in caudal cyst size occurred (Matthews, 1981).

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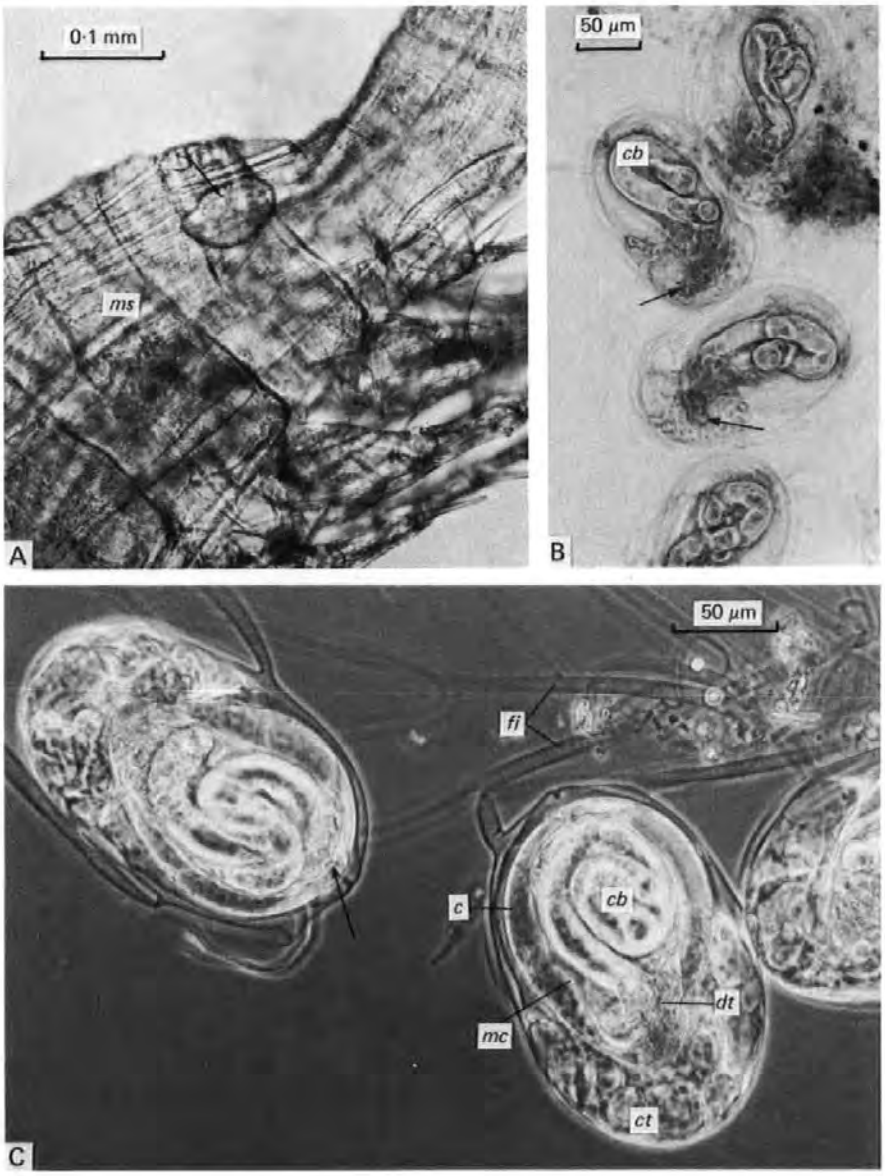
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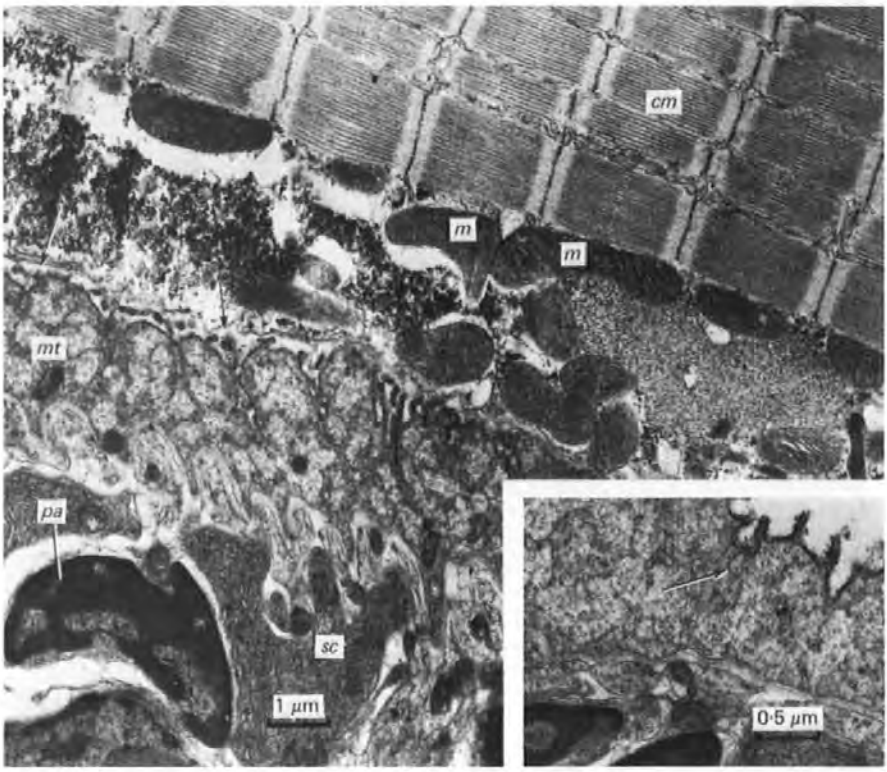
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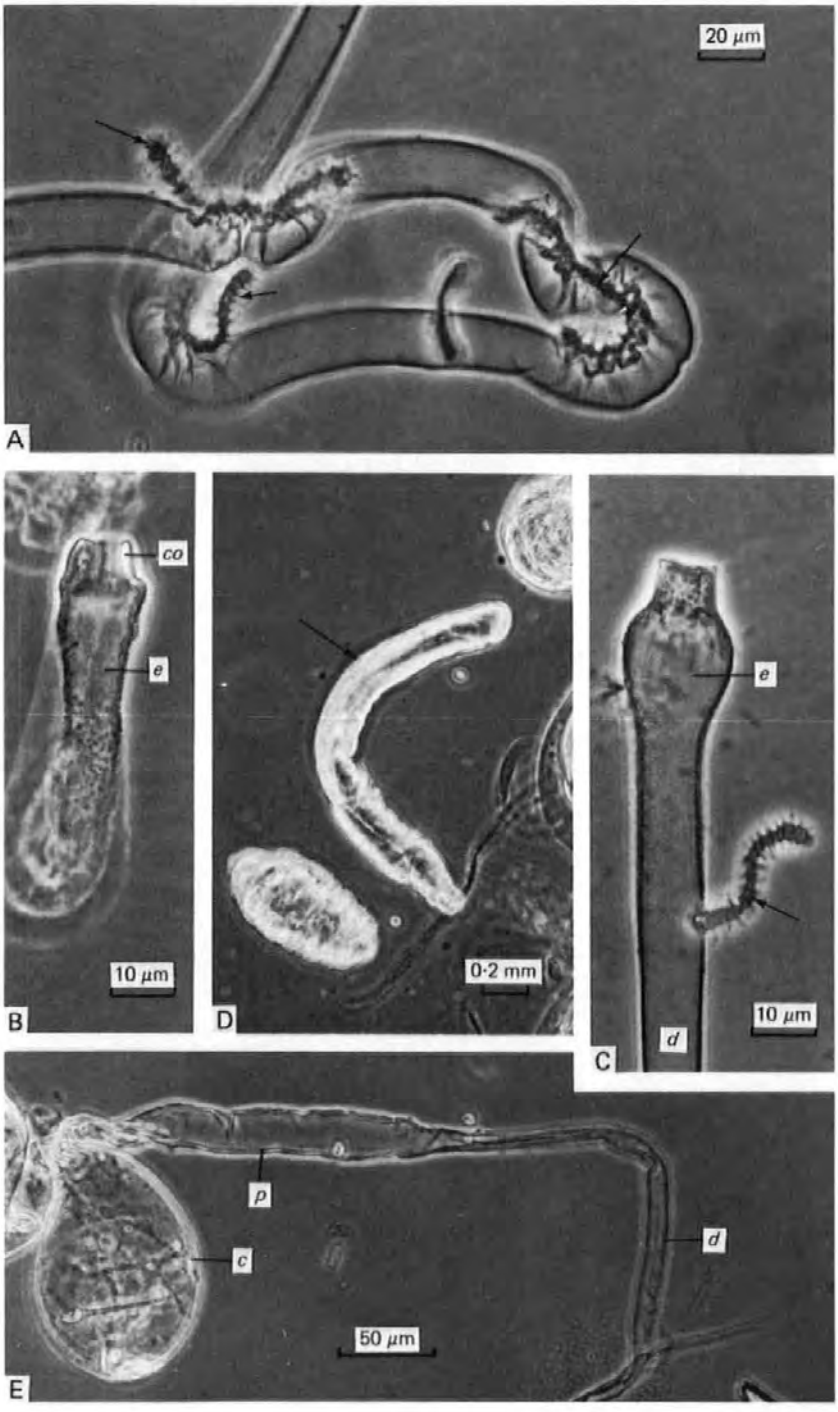
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EXPLANATION OF PLATES

PLATE 1

Light micrographs of *Cercaria vaullegeardi*.

A. Metacercaria (arrowed) within mesosoma (*ms*) of *Tigriopus brevicornis*, 5 min after infection.

B. Naturally emerged encysted cercariae: note narrow curved caudal cyst beak (arrowed) and coiled cercarial body (*cb*).

C. Encysted cercariae showing fleshy tegument (*ct*) surrounding narrow caudal cyst beak, and rounded anterior end of caudal cyst (*c*) with closed sphincter muscle (arrowed). The delivery tube (*dt*) and cercarial body (*cb*) are coiled within a membranous capsule (*mc*). *fi*, Entangled caudal filaments.

PLATE 2

Electron micrographs of metacercaria of *Cercaria vaullegeardi* within haemocoel of *Tigriopus brevicornis*, fixed *in situ* less than 1 min after infection. Note disrupted copepod sarcolemma (arrowed), muscle fibres (*cm*) and mitochondria (*m*). *mt*, Metacercarial tegument; *pa*, parenchyma cell; *sc*, secretory cell. Inset shows metacercarial tegument: secretory vesicles have been discharged at the surface membrane (arrowed).

PLATE 3

Light micrographs of *Cercaria vaullegeardi*: *in vitro* eversal of delivery tube.

A. Distal delivery tube: note torn scraps of fibrous material (arrowed) adhering to the outside of the everted tube.

B. Everted end-piece (*e*): note thickened collar (*co*) supporting aperture.

C. Everted end-piece: note torn fibrous material (arrowed) at junction of end-piece (*e*) and distal delivery tube (*d*).

D. Cercarial body (arrowed) immediately after passage through delivery tube. Shorter cercarial body shows shape assumed within 30 sec of delivery.

E. Caudal cyst (*c*) and delivery tube everted by gentle rolling of the cover-slip upon a temporary preparation: note wide proximal (*p*) and narrow distal (*d*) sections of the tube.

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