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Light and electron microscopic study of the pathology of a species of didymozoid (Trematoda, Digenea) infecting the gill arches of *Scomber australasicus* (Teleostei, Scombridae)

K. M. L. Perera

Department of Zoology, University of New England, Armidale, New South Wales 2351, Australia

ABSTRACT: The response of the host, slimy mackerel *Scomber australasicus*, to infection with a didymozoid parasite consists of (1) the formation of a capsule around the worms by proliferation of cells of the epidermis and dermis, (2) infiltration of leucocytes into the capsule wall, (3) high vascularisation of the capsule wall and (4) formation of additional filaments in the cytoplasm of the capsule epidermal cells. Neither host connective tissue, amoeboid cells nor blood vessels were observed between worm sections in the lumen of the capsule.

KEY WORDS: Ultrastructure · Pathology · Didymozoid · Trematoda · *Scomber australasicus* · Teleostei

INTRODUCTION

The histopathology of didymozoid parasites of fishes has been studied by only a few authors (e.g. Lester 1979, 1980, Eiras & Rego 1987). This is one of a series of papers describing the pathological effects on the host of didymozoid parasites living in different tissues of slimy mackerel *Scomber australasicus*. In order to understand the pathology, it is important to establish the exact nature of the normal uninfected tissue. The light and electron microscopic structure of the normal primary gill lamellae of *S. australasicus* was described by Perera (1992a, 1993). The light and electron microscopic pathology of the didymozoid species infecting the gill filaments of the same host was described by Perera (1992a, b). Perera (unpubl.) described the structure and ultrastructure of the normal uninfected skin of the gill arch. In this paper, I present light and electron microscopic data on the pathology due to a didymozoid parasite (subfamily Nematobothriinae) living in the tissue of the gill arch.

MATERIALS AND METHODS

Slimy mackerel were caught in Jervis Bay, New South Wales, Australia. Parts of gill arches with parasites were processed using the methods employed by Perera (1992a, b). A series of wax sections of the same parasite species, stained with haematoxylin and eosin or Masson's trichrome, was also used to study the pathological effects on the host.

RESULTS

The didymozoid worms usually lived in pairs encapsulated by the host tissue. The parasite capsule was attached to the gill arch by a short and narrow stalk. The capsules were found on either the third or fourth gill arch, on both external and internal sides. The size of the capsule was large compared with the capsules of didymozoids infecting gill filaments (Perera 1992a, b). The species decribed in the present paper is not as abundant as the other species infecting gill filaments (Perera 1992a, b). No developing stages of the didymozoids were observed. The scanning electron micrograph (Fig. 1A) shows the external morphology of the parasite capsule.

Worms were covered by a thin-walled capsule (Fig. 1C). According to light and electron microscopic observations, the structure of the capsule wall is similar to that of skin of the normal gill arch (Perera unpubl.). The capsule wall consists of epidermis and dermis.

Worm sections can be observed inside the dermis (Fig. 1D, E & F). In some areas, the worm tegument is close to the dermis of the capsule (Fig. 1D, F), and in other areas there is a space between the worm tegument and the dermis of the capsule. The space is possibly filled with a liquid (Fig. 1E). No host connective tissue, cells or blood capillaries were observed between worm sections. The thickness of the capsule wall varied depending on the site (Figs. 1D, E, 2A-i, A-ii, C).

The epidermis of the capsule wall was about 4 cells thick around the stalk (Fig. 1E, F) and about 13 cells thick in the lobes of the capsule (Fig. 1D). More intercellular spaces were observed in the capsule epidermis than in uninfected tissue. Many intercellular spaces were observed around the stalk (Fig. 1E, F). These spaces were usually occupied by amoeboid cells (Fig. 3B).

Epidermal cells are the most common cell type in the capsule epidermis, as in uninfected epidermis. The plasma membrane of the epidermal cells does not show extensive interdigitations as in uninfected tissue (Fig. 3A-iii, B, C), but some cells show pseudopodia-like cytoplasmic processes, extending into intercellular spaces, as in the epithelial cells of the capsule wall of the didymozoid-infected gill filaments (Perera 1992b). Also, the plasma membrane at the base of some basal epidermal cells shows frequent indentations (Fig. 3A-iii, C) which are not common in the basal epidermal cells of the uninfected tissue. The ultrastructure of the basal and middle epidermal cells in the capsule is similar to that in the uninfected gill arch described by Perera (unpubl.), except that more cytoplasmic filaments were observed in capsule cells (Fig. 3D).

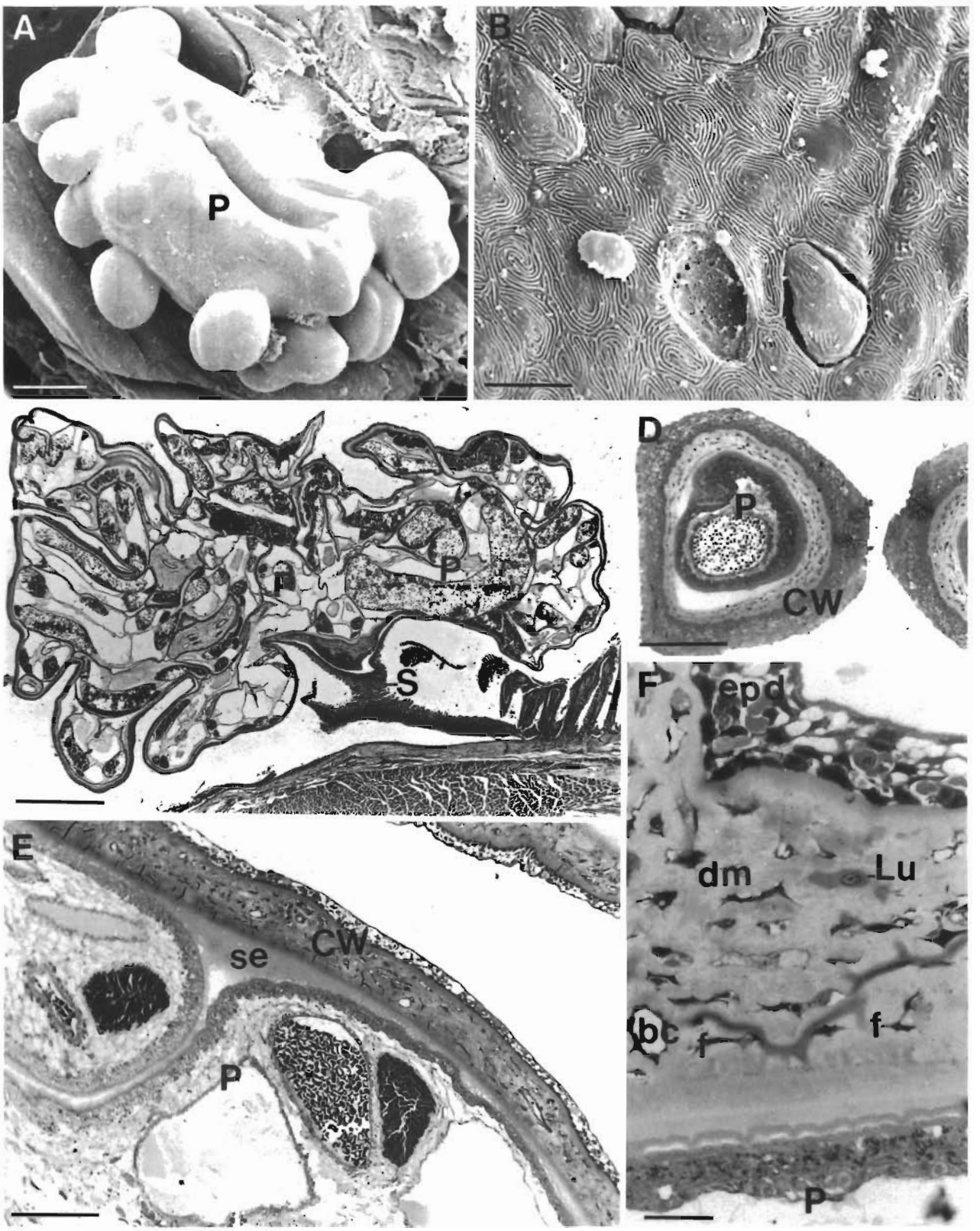
The surface structure of the capsule observed under high magnification of the scanning electron microscope is shown in Fig. 1B. There are characteristic micro-ridges and channels on the surface as in the normal gill arch. Sections of the surface epidermal cells of the capsule possessed short denticular outgrowths of the plasma membrane which are the profiles of microridges (Fig. 3A-i, B). There were cell junctions observed between microridges of the surface epithelial cells. The average size of normal surface epidermal cells in the uninfected gill arch is similar to those of the didymozoid capsule. The ultrastructure of the surface epidermal cells in the capsule is similar to the epidermal cells in the uninfected gill arch described by Perera (unpubl.), except that more cytoplasmic filaments were observed in capsule cells. Unidentified small acellular bodies were observed on the surface of the capsule (Fig. 1B). They could be the product of secretory cells in the epidermis.

Mucus cells are present in the middle zone and the surface of the capsule epidermis (Figs. 2A-i, B & 3A-i). Chloride cells were observed in some parts ot the capsule epidermis (not illustrated) and rodlet cells were observed in the surface layers of the capsule epidermis (Fig. 2B). The ultrastructures of the mucus cells, chloride cells and rodlet cells in the capsule epidermis are similar to those of the gill arch epidermis described by Perera (unpubl.). There are no changes in the structure and number of mucus cells, chloride cells and rodlet cells in the capsule epidermis due to infection by the parasite. Acidophilic cells were rarely observed in the capsule epidermis. Dark nucleated cells (probably lymphocytes) and type 1 cells were observed in the intercellular spaces of the capsule epidermis, mostly around the stalk (Fig. 3B). Type 1 cells have a similar structure to those in the capsule of the didymozoids infecting gill filaments and in normal gill filaments (see Perera 1992b, 1993). However, the fibrillar structure of the granules was rarely observed in the present study. A few type 2 cells which have large, highly electron-dense granules in the cytoplasm were also observed in the intercellular spaces of the capsule epidermis.

The thickness of the capsule dermis varies. The dermis of the capsule wall consists mainly of collagen fibres, with no particular pattern in their arrangement. There are a large number of cells in the dermis compared with uninfected tissue (Fig. 2A-ii, C, D). These are fibroblasts, lymphocytes, type 1 cells, type 2 cells, Type 4 cells, neutrophils and unidentified lightly-stained cells. In contrast, only fibroblasts and lymphocytes were present in the dermis of the uninfected gill arch.

Fibroblasts are common and scattered in the capsule dermis, between loose collagen fibres (Fig. 2A-ii). Ultrastructurally they are similar to those in uninfected tissue (Perera unpubl.). There is no obvious increase or

Fig. 1. (A) A scanning electron micrograph of the parasite (P). Scale bar = 1 mm. (B) High power scanning electron micrograph of the surface of the didymozoid capsule. Note micro-ridges and channels on the surface of the epidermal cells, a pit of a detached cell and detaching epidermal cells. There are white blobs on the surface that could be secretions from secretory cells. Scale bar = 0.01 µm. (C) Transverse thick section of the didymozoid capsule. Note compactly arranged parasite (P) sections in the thin-walled capsule. The short and narrow stalk (S) connects the capsule to the gill arch. Scale bar = 1 mm. (D) Transverse semi-thin section of a small lobe of the didymozoid capsule. Note darkly stained thick epidermis of the capsule wall. CW: capsule wall. Scale bar = 0.2 mm. (E) Transverse semi-thin section of the capsule near the stalk. Note thin epidermis of the capsule wall and secretion (se) (?) between parasite (P) and capsule wall (CW). Scale bar = 0.1 mm. (F) Enlarged transverse semi-thin section of the capsule near the stalk. Note 4 to 5 cells thick epidermis (epd) with a number of intercellular spaces and dermis (dm) with fibroblasts (f), blood capillaries (bc) and leucocytes (Lu). The tegument of the parasite (P) is seen near to the capsule wall. Scale bar = 15 µm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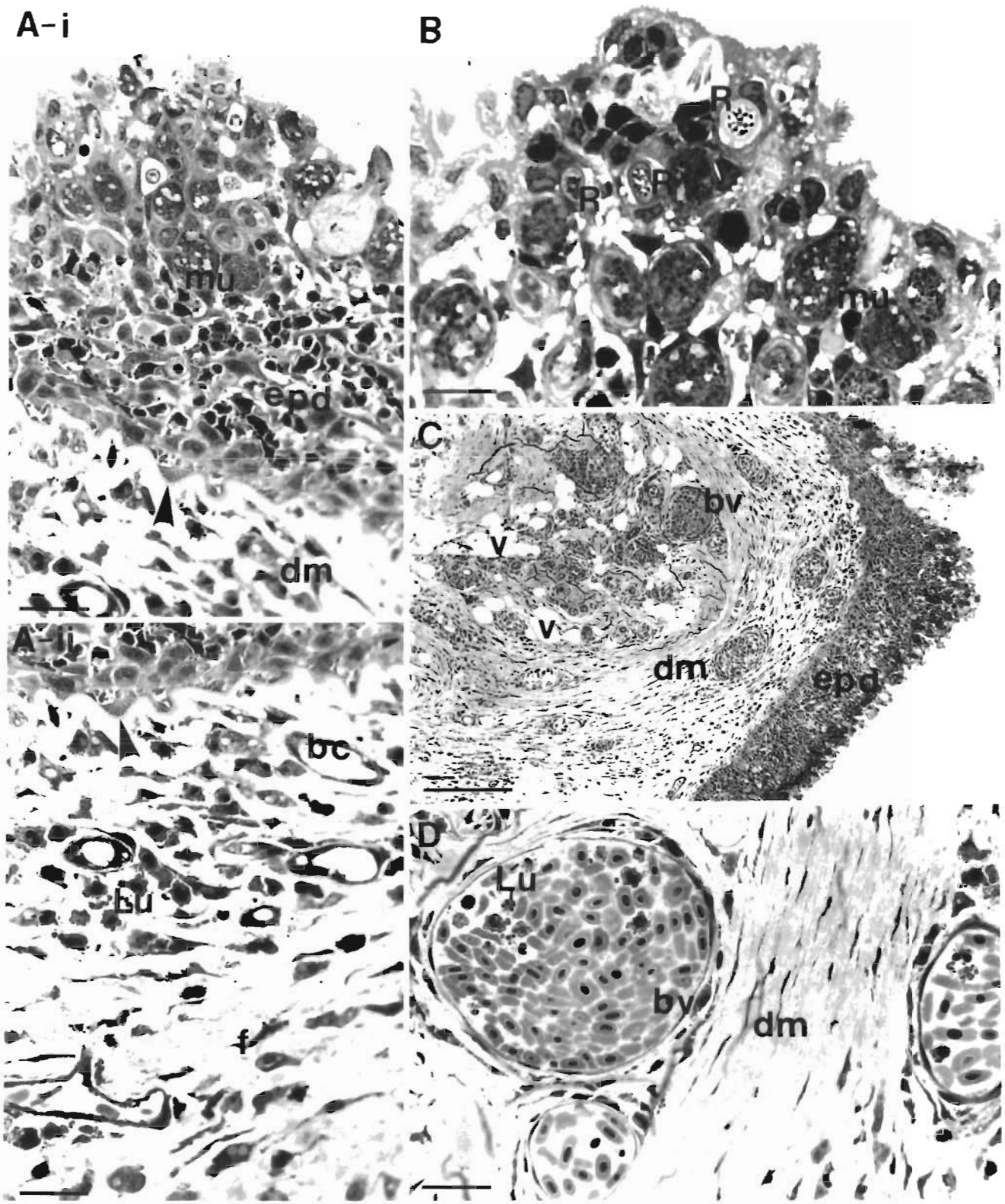


Fig. 2. (A-i) Enlarged transverse semi-thin section of the epidermis of the capsule. Note several cells thick epidermis (epd) and part of the dermis (dm). A mucus cell is secreting mucus. mu: mucus cells; arrowhead: basal lamina. Scale bar = 15 µm. (A-ii) Enlarged transverse semi-thin section of the dermis of the capsule. Note a number of leucocytes (Lu), fibroblasts (f) and blood capillaries (bc). Scale bar = 15 µm. (B) Surface area of the capsule epidermis enlarged. Note a few rodlet cells (R) and a number of mucus cells (mu). Scale bar = 10 µm. (C) Transverse semi-thin section of mid dorsal area of the capsule. Note very thick dermis (dm) containing several blood vessels (bv) and vacuoles (v). The epidermis (epd) is also several cells thick. Scale bar = 0.1 mm. (D) Enlarged semi-thin section of blood vessels of the mid dorsal area of the capsule dermis (dm). Note a number of leucocytes (Lu) in the blood vessels (bv) which seem to be very active. Scale bar = 15 µm

decrease in their number due to the didymozoid infection. However, more lymphocytes were observed in the capsule dermis than in uninfected tissue. A number of type 1 cells, neutrophils and a few type 2 cells were observed in the capsule dermis but not in the normal tissue. Type 4 cells are quite common in the capsule dermis and were rarely observed in uninfected tissue. The cells are filled with large round or elongated, moderately electron-dense granules, without any substructure (Fig. 4A, B, D). The cytoplasm contains mitochondria and a few profiles of rough endoplasmic reticulum (rER); the nucleus has a large amount of heterochromatin. Type 4 cells were also observed in the blood vessels (Fig. 4D, G). A number of unidentified low electron-dense cells (electron density similar to collagen fibres) were observed in the dermis (Fig. 4C). These cells contain long profiles of rER, large mitochondria, a few moderately electron-dense granules, a nucleus with a thin broken peripheral band of heterochromatin, and small chromocentres. The infiltration of leucocytes in the capsule dermis may be due to the parasite.

Some parts of the capsule dermis are more vascularised than uninfected dermis. Highly vascularised, very thick dermis was observed on the top centre of the capsule (Fig. 2C). There are large blood vessels filled with blood cells, mostly erythrocytes (Fig. 2C, D). Neutrophils (Fig. 4D), type 4 cells (Fig. 4D, G) and highly lobulate (very active) cells (Fig. 4F) were also observed. In contrast, large blood vessels were not seen in the normal dermis. The extra supply of blood in the dermis of the capsule is probably due to the parasite. The structure of the wall of the blood vessel appears to be normal (Fig. 4E). However, on a few occasions lightly stained (probably degenerating) endothelial cells were observed in the wall of some blood capillaries (not illustrated).

On several occasions, degenerating cells were observed in the dermis (Fig. 4H, I). They do not have distinct cell walls. Cytoplasmic organelles such as profiles of rER, moderately electron-dence small granules, and degenerating mitochondria are placed between the dermal collagen (Fig. 4H, I). Most probably they are unidentified low electron-dense cells. Possible indications of phagocytosis were observed in some parts of the capsule dermis. Fig. 4H shows a part of another cell (with highly electron-dense granules) in a degenerating cell and Fig. 4I shows a part of another cell (probably type 4 cell) in a degenerating cell.

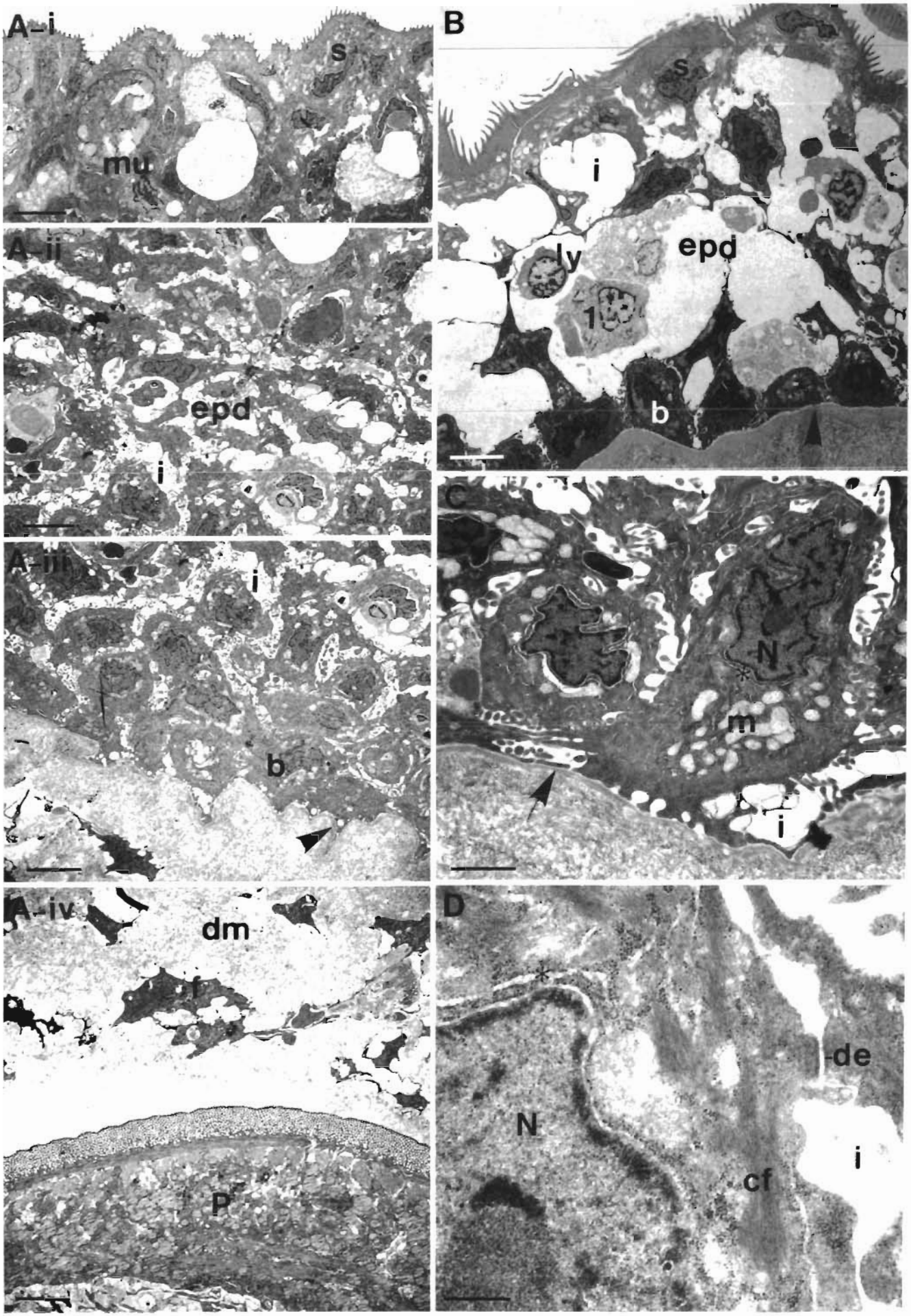
DISCUSSION

Didymozoids infecting the gill arches of several teleosts have been recorded by some authors [e.g. Ishii (1935) noted *Didymocystis semiglobularis* infecting the base of the gill rakers of *Seriola quinqueradiata* and *Thunnus orientalis*, and Lester (1979, 1980) noted *Neometadidymozoon helicis* infecting *Platycephalus fuscus*]. However, in both of these cases the capsules were smaller and oval shaped, and structure of the worms was different to those of the present study.

The response of slimy mackerel consists mainly in the formation of a capsule around the worms. The measurements of normal epidermal cells at the surface of the gill arch are similar to those in the surface of the parasite capsule. It seems that proliferation (hyperplasia) may occur in the host tissue during infection but no developing stages of the parasites (developing capsules) were observed. The structure of the capsule in the present study is very different from that of the didymozoid species infecting gill filaments (Perera 1992a, b). In primary gill filaments, the parasite pair is encapsulated by primary lateral epithelium and the efferent artery; when the parasites become larger the capsule wall (lateral epithelium) stretches to accommodate them. In the present study, however, the parasites were completely surrounded by a thin-walled capsule connected to the gill arch by a short and narrow stalk. The structure of the didymozoid capsule in the present study seems to be similar to the capsule of *Köllikeria filicollis* described by Williams (1959) as a 'cyst', where worms were surrounded by host tissue and attached to the epithelium inside the operculum near the posterior gill of *Brama raii* (Bloch).

According to Williams (1959), capsules are a reaction of the host tissue against secretions from some of the subcuticular gland cells of the female worm, and the inner layer of the capsule is of parasitic origin, derived from discharge products of subcuticular gland cells. The trigger for the development of the didymozoid capsule in the present study is not known. The space between worms in the capsule is possibly filled with a liquid but its origin is not known. The only didymozoid found within a true capsule ('cyst'), according to Lester (1980), is the highly modified *Köllikeria filicollis* described by Williams (1959). The didymozoids in the present study also seem to live in a true capsule. There was 1 female and between 1 and 5 males in the capsules described by Williams (1959). However, only 2 worms were usually observed in the capsule in the present study, and observation of whole mounts indicated that they are hermaphroditic.

Perera (1992b) observed distinct electron-dense filamentous bands in the cytoplasm of the epithelial cells in the capsule wall of didymozoid-infected gill filaments, but they were not observed in the epidermal cells of the capsule wall in the present study. However, filaments not arranged in such dense bands were observed, and more of them were in the cytoplasm of the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epidermal cells of the capsule wall than in those of the normal gill arch. The formation of extra filaments in the cytoplasm of the epidermal cells appears to be due to the tissue reaction of the host to the parasite. The cytoplasmic filaments may give additional strength to the epidermal cells, and the capsule wall may thus resist the pressure produced by the parasites.

Several pathological changes due to didymozoids noted in host tissue by other authors were not found in the present study. For example, Lester (1980) and Eiras & Rego (1987) observed haemorrhages in the didymozoid capsules but no haemorrhages were observed by Perera (1992a, b) or in the present study. Patches of cytoplasm were found between connective tissue fibrils of the capsule wall of didymozoid-infected gill filaments (Perera 1992b), but they were not observed in the capsule wall of the present study. Also, the inner margin of the capsule wall in the present study had a more defined edge than that in the previous study by Perera (1992b).

Phagocytosis in infected tissues of teleosts has been observed by some authors (eg. Hoole & Arme 1982, Stehr & Whitaker 1986), and on 2 occasions possible indications of phagocytosis were observed in the capsule dermis of slimy mackerel. Leucocytes have been previously reported in piscine inflammatory responses to infection by parasites (Boxshall 1977, Hayunga 1979, Hawkins et al. 1981, Hoole & Arme 1982, Roubal 1986, 1989, Wanstall et al. 1988, Morrison & Poynton 1989, Perera 1992b, Sharp et al. 1992). The present study is also consistent with this observation, as large numbers of leucocytes (lymphocytes, type 1 cells, type 2 cells, type 4 cells, neutrophils) were observed in the capsule wall. Relatively few leucocytes were observed in normal skin of the gill arch (Perera unpubl.). Additional leucocytes present in the capsule wall could be an inflammatory response to the didymozoid parasites. Perera (1992b) observed amoeboid cells between worm sections but inflammatory cells of the host had never penetrated the tegument of the didymozoid worms. However, amoeboid cells were not observed in the didymozoid capsule, nor did host inflammatory cells penetrate the tegument of the didymozoid worms in the present study. There was no sign of damage to the parasite by the host. This suggests that although the slimy mackerel reacts to the infection of didymozoids by an inflammatory response, it is not capable of rejecting the parasites, at least once they have reached an advanced stage of development.

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Fig. 3. (A-i) Transverse ultra-thin section of the surface area of the capsule epidermis. mu: mucus cell; s: surface epidermal cell (note micro-ridges on the surface). Scale bar = 5 µm. (A-ii) Transverse ultra-thin section of the middle area of the capsule epidermis (epd). i: intercellular spaces. Scale bar = 5 µm. (A-iii) Transverse ultra-thin section of the basal area of the capsule epidermis and part of the dermis. Note indented nuclear membranes of the basal epidermal cells (b). Arrowhead: basal lamina. Scale bar = 5 µm. (A-iv) Transverse ultra-thin section of part of the capsule dermis (dm) and part of the parasite section (P). f: fibroblasts. Scale bar = 5 µm. (B) Transverse ultra-thin section of the capsule epidermis (epd) near stalk. Note large intercellular spaces and leucocytes. ly: lymphocyte; 1: type 1 cell. Scale bar = 4 µm. (C) Basal epidermal cell. Note indentations of the plasma membrane near basal lamina and desmosomes between epidermal cells. N: nucleus; m: mitochondria; •· rough endoplasmic reticulum. Scale bar = 2 µm. (D) Part of a basal epidermal cell enlarged. Note cytoplasmic filaments (cf) in the cytoplasm, a few profiles of rER (•) and free ribosomes. de: desmosome. Scale bar = 0.4 µm

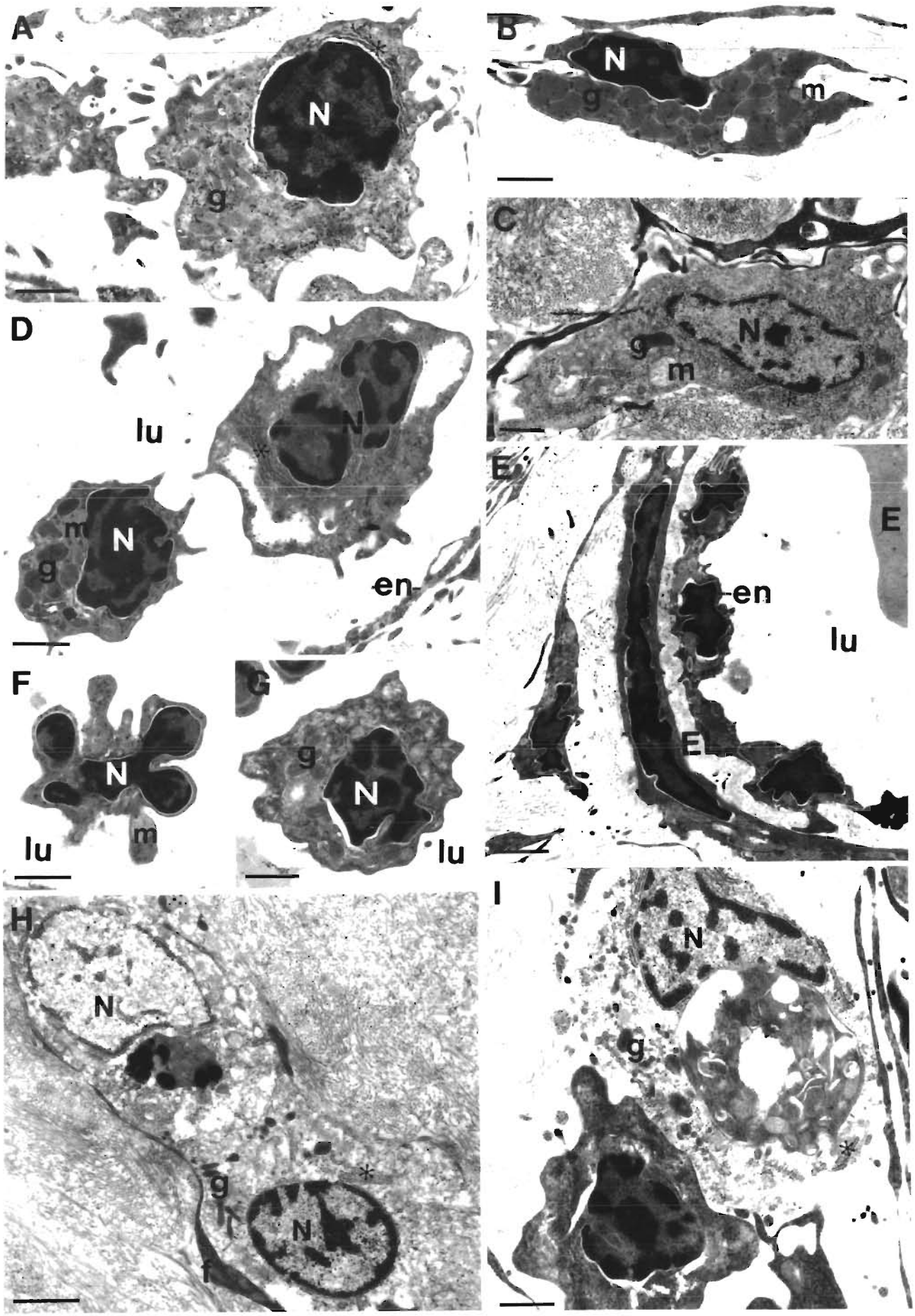
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Fig. 4. (A) Type 4 cell in the capsule dermis. Note a number of low electron-dense round to oval granules (g) without any substructure, eccentric electron-dense nucleus (N) and few profiles of rough endoplasmic reticulum (•). Scale bar = 1 µm. (B) Another type 4 cell in the capsule dermis. Note larger low electron-dense round to oval granules (g) than those in Fig. 4A (possibly a different developmental stage). m: mitochondria. Scale bar = 1 µm. (C) Unidentified low electron-dense cell in the capsule dermis. Note similar electron-density as collagen fibres. Scale bar = 1 µm. (D) Leucocytes in a blood vessel. Note type 4 cell (smaller) with large granules (g) and neutrophil (larger), in which no granules were seen. en: endothelium of blood vessel; lu: lumen of blood vessel. Scale bar = 1 µm. (E) Wall of a blood vessel. EL: elastic layer; E: part of an erythrocyte. Scale bar = 1 µm. (F) Active leucocyte in a blood vessel. Note large pseudopodia and lobed nucleus. Scale bar = 1 µm. (G) Type 4 cell in a blood vessel. Note the size of the granules which are similar to those of Fig. 4A. Scale bar = 1 µm. (H) Degenerating cells in the dermis (probably unidentified low electron-dense cells). The plasma membranes were not clearly seen. Note a part of another cell (?) in the upper degenerating cell. f: fibroblast. Scale bar = 2 µm. (I) Another degenerating cell in the dermis (probably unidentified low electron-dense cells). Note a part of type 4 cell (?) in the degenerating cell. Scale bar = 1 µm

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