STUDIES ON THE LIFE-CYCLE OF THE DIGENETIC TREMATODE RHIPIDOCOTYLE CAMPANULA (DUJARDIN, 1845) (GASTEROSTOMATA : BUCEPHALIDAE) WITH PARTICULAR REFERENCE TO THE LARVAL STAGES

SHANTHINI DEVI RICHARDSON B.Sc.

A thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy

The University of Leeds
Department of Pure and Applied Biology

September 1990

Abstract

The life-cycle of *Rhipidocotyle campanula* (Dujardin,1845) has been experimentally demonstrated and the species identity confirmed.

Sporocysts were recovered from digestive glands of the freshwater mussel *Anodonta anatina*, and in heavily infected hosts from the reproductive system. Both cercariae and glochidia are released simultaneously in mussels where the reproductive system is partly invaded by sporocyst tubules. The development of the cercariae in the sporocyst tubules has been studied briefly using histological and histochemical methods.

The liberation of cercariae varied between >1000/mussel/day to none, and is intermittent. Behaviour of the cercariae including swimming, response to light and gravity, survival and entry into the secondary host, is described. The morphology of the cercaria has been studied in detail using electron microscopy, histochemistry and histological methods and its significance analysed in relation to free-living existence. This is the first attempt to study the cercaria of *R. campanula* in any detail.

Cercariae enter the secondary host passively and encyst in the subcutaneous fatty tissue beneath the lining of the pharynx, and in some cases in the gill arches. This is the only species of bucephalids where cercariae enter the secondary host passively. Encysted

metacercariae attain maximum development after 5-6 weeks and survive nearly 200 days, but spontaneous excystation takes place only in cysts 80-90 days old.

Adult flukes were recovered from the posterior intestine and rectum of the perch (*Perca fluviatilis*) six weeks after feeding fully developed metacercariae. Spermatogenesis and oogenesis were studied in the adults until egg formation. Miracidial development could not be observed.

External morphology using SEM of cercaria, metacercaria and adult has been studied and compared. This is the first report of SEM study of these stages of *R. campanula*.

A brief review of the literature is given and the problems of systematics and taxonomy of the family Bucephalidae the taxonomic position of *R. campanula* are discussed. Tabular summaries are given for the previous life-cycle studies of bucephalids, synopses of bucephalid trematodes and all previous reports of bucephalid cercariae.

ACKNOWLEDGEMENTS

I would like to thank Professor R. McNeil Alexander of the Department of pure and Applied Biology for providing all the necessary facilities for carrying out this research.

Especially I wish to record my indebtedness to the late Dr. Wynne Owen for his sound advice, valuable assistance and his constant encouragement as my supervisor during the first year of my research, also his help in obtaining financial assistance towards this research. I also thank Mrs. Gwynneth Owen, his widow, for her continued kindness, encouragement and support during the completion of this work.

I gratefully acknowledge Dr. P. J. Evennett and Dr. J. B. Jennings who agreed to become joint supervisors following the sudden and untimely death of Dr. Owen, and who have given helpful guidence and advice on my work; also Professor D. L. Lee for his valuable time spent discussing the work and Dr. P. Harris for his criticism and advice.

This work could not have been undertaken without the technical staff of the department, especially Mr. Stewart Pickersgill and Mr. Peter Broughton who helped to collect fish and mussels from the River Aire and maintain them in the aquarium, Mr. A. Holliday who helped with photography, Mr. G. Standley who helped with electron microscopy and Mr. A. J. Hick who assisted with both electron microscopy and photographic processing.

My thanks are due to the University of Leeds and to the Committee of Vice-Chancellors and Principles of the Universities of the United Kingdom, both of whom granted Overseas Students' Research awards to help with the work.

I express gratitude to my mother without whose financial help this work could not have commenced, and without whose encouragement and support it would certainly never have been completed. Thanks also to my sisters for their endless support, to Roger my husband for his encouragement and support, to other members of my family, and many colleagues and friends who helped in various ways.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

a.d.n ... anterior dorsal nerve

a.g ... anterior gland

a.l ... anterior lips

a.l.n ... anterior lateral nerve

a.o ... anterior organ

a.s ... anterior sucker

a.v.n ... anterior ventral nerve

b.a ... body attachment

b.c ... brood chamber

b.l ... basement layer

b.ts.c ... body/tail-stem commissure

c.b ... cercarial body

c.e.d ... caudal excretory duct

c.g ... cerebral ganglia

c.h.o ... cyst wall of host origin

c.p ... cytoplasmic projections

c.p.o ... cyst wall of parasite origin

c.l ... cytoplasmic layer

cy ... cyst

cy.p ... cyst wall plug

e ... eggs

e.c ... epithelial cell

e.d ... excretory duct

e.p ... excretory pore

e.t ... excretory tubule

e.v ... excretory vesicle

f ... foot

f.c ... flame cell

g ... gut

g.a ... genital anlagen

gi ... gill

g.p ... genital pore

g.s ... glycogen storage cells

h.s ... hood of sucker

i.l ... inner layer of cyst wall

int ... intestine

l.m ... longitudinal muscle

m ... mouth

m.c ... mantle cavity

m.ce ... matured cercaria

m.cy ... metacercarial cyst

me.c ... metacercaria

mit ... mitochondria

m.l ... middle layer of cyst wall

m.s ... mucus secreting cells

m.e.d ... median excretory duct

mu ... muscles

n ... nervous system

n.b ... new branches

o ... ovary

o.l ... outer layer of cyst wall

p ... pharynx

pa ... papillae

p.c ... primordium of cirrus pouch

p.d.n ... posterior dorsal nerve

ph.s ... pharyngeal nerve supply

p.l.n ... posterior lateral nerve

po ... pore

p.te ... primordium of testes

p_1 ... papillae with a bulbous base and a tegumentary ring and a cilium

p_2 ... papillae with a bulbous base and a pore in the middle

p_3 ... papillae with a single cilium surrounded by a tegumental ring

p_4 ... dome shaped papillae without cilium

p_5 ... papillae with a long cilium surrounded by a tegumental ring

r.a.s ... rudimentary anterior sucker

r.c ... resting cercaria

r.c.d ... right collecting duct

s ... shell

s.m ... surface membrane

sp_1 ... anterior spines on the lips

sp_2 ... spines along the body

s.po ... secretory pore

sps ... sporocyst

s.v ... secretory vesicle

t ... tail

t.a ... tail attachment

te ... testis

teg ... tegument

t.n ... tail nerve

t.p ... tail pore

ts ... tail-stem

v ... vitelline cells

v_1 ... oval or elongated vesicles with finely granular electron dense matrix with an electron opaque periphery

v_2 ... smaller elongated or rounded vesicles with homogenous electron dense matrix

v_3 ... condensed granular matrix

v_4 ... electron dense rod shaped vesicles

v_5 ... electron opaque vesicles

CHAPTER ONE

INTRODUCTION

Introduction

The family Bucephalidae, within the order Gasterostomata, is an aberrant group of digenetic trematodes which in their adult stage infect the pyloric caeca, intestine, and rarely the stomach of marine and freshwater teleosts. The uniqueness of bucephalids among trematodes is the ventral rather than terminal mouth, and the saccate rather than bifid intestine, like that of rhabdocoel turbellarians. These differences were first noted by von Siebold (1848).

The taxonomic position of the group has given rise to much speculation and controversy and many of its structural characters have greatly puzzled investigators. The presence of a ventral mouth and sac-like gut have been interpreted as links with the Turbellaria but on the other hand the method of formation of the excretory bladder, the type of cercaria and its method of penetration into the secondary intermediate host led LaRue (1957) to believe that the family has close relationships with four other families of Digenea: the Schistosomatidae, Clinostomatidae, Brachylaemidae, and Strigeidae. Dawes (1946) stated that "the phylogeny of Trematoda is a problem which cannot be solved in the present state of our knowledge" and this still holds true today especially in the case of the Gasterostomata.

Problems of classification relating to the gasterostome trematodes have been reviewed by Kniskern (1952a), Hopkins (1956), Yamaguti (1971 , 1975) and Stunkard (1974d, 1975, 1976b). Kniskern (1952a) listed only 2 subfamilies and 7 genera while Yamaguti (1971) recognised six subfamilies and 22 genera with 226 species. Since Yamaguti's (1971) work three new genera and ten new species have been introduced into the family Bucephalidae: *Pararhipidocotyle* and *Chabaudtrema* have been introduced by Kohn (1971), and the genus *Rhudolphius* by Stunkard (1974d)

In the classification of the family most of these species are known only in the adult stage while others have been described only from metacercarial and cercarial stages, in marine and freshwater fishes from widely separate parts of the world. All are parasites of the intestine except species of *Paurorhynchus* Dickerman, 1954, which occur in the gall-bladder. The subfamilies are distinguished by differences in the structure of the anterior adhesive organ and location of the internal organs, particularly in the relationship of the ovary to the vitellaria and testes. The genera are not clearly delineated; generic concepts are indefinite, indeterminate, and often overlapping, and a definitive systematic arrangement of the gasterostomes requires further information on life-cycles, developmental stages and detailed morphology, especially of the excretory system. At present there is a growing interest to adopt

chaetotaxy in taxonomy and phylogeny of trematodes (Richard 1968, 1971 and Bayssade-Defour 1979). Ulmer and Rhode (1981) stated that studies on cercariae and metacercariae belonging to different families of Digenea show that such studies of papillae are taxonomically significant at specific, generic or suprageneric levels. But the lack of information on chaetotaxy among bucephalids demands further studies in this field along with the excretory system, as flame-cell formula (arrangement of the flame cells) is known for only few species, and the lack of correlation between excretory pattern and other features complicates the situation. So at present, a challenging problem in the family Bucephalidae is the acquisition of detailed information on chaetotaxy, excretory system and life-cycles to compile a definitive form of classification.

The life-cycle of gasterostomes is complex, involving three parasitic and two free-living phases. The adults are generally intestinal parasites of predatory fish. They produce eggs which hatch in water or possibly within the rectum of the host, to liberate free-swimming miracidia. These are small pear-shaped organisms, unique among trematode miracidia in that their cilia are borne on rod-like appendages. If they penetrate a suitable bivalve mollusc they develop into long, highly branched germinal sacs or sporocysts in the digestive glands and gonads and eventually cause parasitic castration of the bivalve. Germinal cells in the sporocyst wall proliferate into the sporocyst lumen

and give rise to gasterostomate furcocercous (fork tailed) cercariae characterised by a long, forked tail. After liberation from the sporocyst the cercariae commence a short free-living phase during which they either penetrate the skin of small fishes and encyst as metacercariae, or enter passively through the respiratory or food currents to encyst in the cephalic region. When infected fish are eaten by predatory fish the metacercariae excyst and develop into sexually mature adults. Although these outlines of the life-cycle are clear, correlations between larval and adult stages are known for only six fresh-water and twelve marine species. In two of the marine species the primary intermediate hosts are marine mussels and their secondary and final hosts are estuarine fish. (Table 1.1) Lack of information on successive stages of particular species has led inevitably to confusion in systematic and taxonomic determinations.

The status of the freshwater bucephalids in Europe appears reasonably clear now but the situation with regard to marine species is still confused. The first-ever reports of furcocercariae from undesignated species of the European freshwater clams *Anodonta* and *Unio* were by von Baer (1827) as *Bucephalus polymorphus*. A furcocercaria similar to *B. polymorphus* was later reported from the marine oyster *Ostrea edulis* and the cockle *Cardium rustricum* by Lacaze-Duthiers(1854). The first report of a North American bucephalid was by McCrady (1874), who described branching sporocysts and

Note: The reference to Manter, 1954 in line 15 of this page is as an authority for the species B. longicornulus and not as a bibliographic citation for this thesis.

cercariae infecting the oyster, *Ostrea virginica*, taken at Charleston, South Carolina, and recognised this as a new species, *Bucephalus cuculus*. Woodhead(1929) was the first to describe a bucephalid life-cycle, using *Bucephalus papillosus* from the caecal pouches of the small-mouthed black bass *Micropterus dolomieu* and gave the first detailed account of the miracidium. The sporocysts and cercarial stages were found in *Elliptio dilatatus* from the Huron River, Michigan. He also described a second species, *Bucephalus elegans* from the caecal pouches of the red-eyed bass or rockbass, *Ambloplites rupestris*, from the same river (Woodhead, 1938). Howell (1966) demonstrated experimentally, for the first time, the life-cycle of the marine bucephalid *Bucephalus longicornutus* (Manter,1954) from the New Zealand mud-oyster, *Ostrea lutaria*. In Europe the first marine bucephalid life-cycle was described by Matthews (1973) using *Prosorhynchus squamatus* Odhner,1905 and *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905. Sporocysts and cercariae were found in *Mytilus edulis* from the coast of Wales. So far, no miracidial stages have been obtained naturally or experimentally for any marine species except in China for the first time when experimentally demonstrating the life cycle of *Parabucephalopsis prosthorchis* (Tang & Tang, 1963) by Tang and Tang (1976). Miracidia are known from the figures by Woodhead (1929,1930) of certain freshwater species whose generic status is uncertain. It is therefore an interesting challenge for the parasitologist

to obtain miracidia in order to demonstrate the complete life-cycle.

Only two freshwater species of the family Bucephalidae are known from Europe, namely *Bucephalus polymorphus* Baer 1827 and *Rhipidocotyle campanula* (Dujardin, 1845). Koval (1949) in the Soviet Union and Vejnar (1956) in Czechoslovakia recognised two species of bucephalids in freshwater fishes, but did not designate them under correct generic and specific names. Even though parasites with slight morphological differences were observed, they were either incorrectly identified or the differences were ignored and designated as *Bucephalus polymorphus* Baer,1827, until Kozika (1959) recognised the two parasites as two different species. He then for the first time designated the parasites under the correct generic and specific names as *Bucephalus polymorphus* Baer, 1827 and *Rhipidocotyle illense* (Ziegler,1883). Stunkard (1976) and Baturo (1977, 1979) on comparison of *Rhipidocotyle illense* (Ziegler, 1883) with the descriptions of *Distoma campanula* (Dujardin, 1945) agreed that they were identical and on the basis of priority changed the name of the species to *R. campanula* (Dujardin, 1845) new combination.

In the present work, the origin and the history of the family Bucephalidae are discussed along with the problems relating to the taxonomy and systematics of the family. A synopsis of the bucephalid trematodes and all the previous reports of the cercariae known to the author are given in Tables 2.1 and 2.2 to show clearly, the

problems relating to the taxonomy of the family. In addition a list of all the experimentally demonstrated life-cycles of the bucephalids is given in Table 1.1 to complete the historical study of the family. The confusion relating to the identity of the two freshwater bucephalids, *Bucephalus polymorphus* Baer,1827 and *Rhipidocotyle campanula* (Dujardin,1845), have been discussed in chapter two and the identity of the present species as *Rhipidocotyle campanula* (Dujardin,1845) is confirmed. This is the only species found in the River Aire where the survey reported in this thesis was carried out.

The life-cycle of *R. campanula* has been studied in the laboratory in order to obtain cercariae of known origin which could be characterised as fully as possible. The development of the cercaria within the sporocyst in the mussel and the effects of parasitism to the digestive glands of the mussel have been studied. The shedding pattern of the cercariae in relation to the activity of its mussel host, *Anodonta anatina*, the behaviour of the freeliving cercariae, and the entry into the secondary intermediate host have been studied. The host-parasite relationships are also discussed in relation to the cercaria and its mussel host and the secondary intermediate host. The morphology of the metacercaria and the adult parasite has also been discussed, to complete the study of the life-cycle.

Table 1.1

Previous reports of the Bucephalid life-cycles

Date	Author	Name of the species	Synonym	Location	Remarks
1848 1858 1874 1883	von Siebold Wagener Giard Ziegler	*Bucephalus-polymorphus* Baer,1827	*Distoma-campanula* Dujardin,1845 *Gasterostomum fimbriatum* Siebold,1848 *Gasterostomum lacinatum* Molin,1859	EUROPE (FW)	Pieced together; experimentally not demonstrated.
1977	Baturo	* *Bucephalus polymorphus* Baer,1827	*Distoma-campanula* Dujardin,1845 *Gasterostomum fimbriatum* Siebold,1848 *Gasterostomum lacinatum* Molin,1859		Experimentally demonstrated; no miracidia obtained.
1929	Woodhead	** *Bucephalus-elegans* Woodhead,1929	*Bucephalus polymorphus* Nagaty,1937	NORTH AMERICA (FW)	Experimentally demonstrated; miracidium described for the first time in history.
1929 1930	Woodhead _____	** *Rhipidocotyle papillosa* (Woodhead,1929) Eckmann,1932	*Bucephalus pusillus* Cooper,1915 *Bucephalus papillosus* Woodhead,1929	NORTH AMERICA (FW)	Experimentally demonstrated; miracidium described.
1934 1950 1952b	Krull Kniskern _____	* *Rhipidocotyle septpapillata* Krull,1934	*Cercaria basi* Woodhead,1936	NORTH AMERICA (FW)	Experimentally demonstrated;no miracidium described.
1905 1906 1907 1911	Tennant _____ Lebour _____	*Bucephalopsis haimeanus* (Lacaze-Duthiers 1854)	*Bucephalus minimum* Stossich,1887 *Bucephalus cuculus* Ziegler,1883	EUROPE (E)	Speculation on the life-cycle; experimentally not demonstrated; metacercaria and adult stages have

			Bucephalus cuculus McCrady,1874 *Gasterostomum gracilescens* Linton,1906 nec Rudolphi,1819 *Bucephalus haimeanus* Lacaze-Duthiers 1854		not been described.
		****			
1973	Matthews	*Bucephalopsis haimeanus* (Lacaze-Duthiers 1854)	*Bucephalus minimum* Stossich,1887 *Bucephalus cuculus* Ziegler,1883 *Bucephalus cuculus* McCrady,1874 *Gasterostomum gracilescens* Linton,1906 nec Rudolphi,1819 *Bucephalus haimeanus* Lacaze-Duthiers 1854	EUROPE (E)	Part of the life - cycle experimentally demonstrated; metacercaria described for the first time; metacercaria linked with the adult on the basis of comparative morphology.
1952 1966	Chubrik _____	*Prosorhynchus squamatus* Odhner,1905	*Bucephalus mytili* Cole,1935	ARCTIC (S) (White sea)	Part of the life - cycle demonstrated; Cercaria included on assumption; no miracidium obtained.
		***			
1972	Matthews	*Prosorhynchus squamatus* Odhner,1905	_____	U.K (Cardi- gan Bay)	Experimentally demonstrated; cercaria described.
		****			
1954	Ozaki	*Prosorhynchus uniporus* Ozaki,1924	*Prosorhynchus aculeatus* Manter,1940 *Skrjabiniella uniporus* (Ozaki,1924) Issaitschikow, 1928	JAPAN (SW)	Adult obtained experimentally; no miracidium obtained; first attempt for a marine sp.
		***			
1966	Howell	*Bucephalus- longicornutus* (Manter,1954) Howell,1966	_____	NEW- ZEALAND (SW)	Experimentally demonstrated for the first time; no miracidium obtained.

1972 Matthews	*** *Prosorhynchus crucibulum* Odhner,1905	*Monostomum crucibulum* Rudolphi,1819 *Gasterostomum armatum* Molin,1859 *Bucephalus crux* Levinson,1881 *P.grandis* Lebour,1908 *P.triglae* Nicoll,1914 *P.costae* Travassos,1928 *P.scalpellus* McFarlane,1936	EUROPE (SW)	Experimentally demonstrated; no miracidium obtained.
1974 Matthews & 1979 Halton	*** *Bucephaloides gracilescens* (Rudolphi,1819) Hopkins,1954	*Bucephalopsis gracilescens* (Rudolphi,1819)	EUROPE (SW)	Experimentally demonstrated; no miracidium obtained.
1976 Stunkard	*** *Rhipidocotyle lintoni* Hopkins,1954	_____	AMERICA (SW)	First report of an American marine sp. life-cycle; life starts in marine mussel and ends in estuarine fish; no miracidium obtained.
1976 Stunkard	*** *Rhipidocotyle transversale* Chandler,1935	_____	AMERICA (SW)	First report of an American marine sp. life - cycle; life starts in marine mussel and ends in estuarine fish; no miracidium obtained.
1976 Tang and Tang	*Parabucephalopsis prosthorchis* Tang et Tang, 1963	_____	CHINA (SW)	First report of a bucephalid lifecycle in China; miracidium obtained.
	Dollfustrema foochowensis Tang et Tang, 1963	_____		First report of a bucephalid lifecycle in China; no

					miracidium obtained.
1977 Baturo	*Rhipidocotyle illense* (Dujardin,1845)	*Distoma campanula* Dujardin,1845 *Gasterostomum fimbriatum* Zieg.,1883 *Bucephalus polymorphus* Luhe,1909 *B. polymorphus* Kowalevski,1949		EUROPE (FW)	Experimentally demonstrated; no miracidium obtained; cercarial stage confirmed.
1981 Millard & Saad Fares	**** *Bucephalus baeri*		———	MEDITER-RANEAN (Italian &French Coasts)	Part of the life-cycle experimentally demonstrated; no miracidium obtained.
1985 Lushchiva	**** *Baucephalus marinum*		———	USSR (Black-sea)	Part of the life - cycle experimentally demonstrated; no miracidium obtained.

* Experimentally demonstrated; no miracidia obtained; freshwater species.

** Experimentally demonstrated; miracidia obtained; freshwater species.

*** Experimentally demonstrated; no miracidia obtained; marine species.

**** Part of the life-cycle experimentally demonstrated; no miracidia obtained; marine species.

FW - Freshwater ES - Estuarine water SW - Seawater

CHAPTER TWO

A REVIEW OF THE BUCEPHALID TREMATODES, THEIR SYSTEMATICS AND TAXONOMY

2.1 Introduction

The historical review of the Bucephalid trematodes and the systematic review of the parasite *Rhipidocotyle campanula* (Dujardin, 1845) are discussed briefly in this chapter.

The first record of the bucephalid trematode was described by Rudolphi (1819) of the adult parasite obtained from the intestine of marine fishes from Naples and Trieste. Later the cercariae were found in different tissues of European freshwater mussels by Von Baer (1827). In the period of 1890 and 1900, encysted stages of gasterostome (the metacercarial stage) were found in marine fishes, *Gadus* spp., *Phyais blennioides*, *Pleuronectes platessa*, *Belone belone*, and lampreys. Early in the 19th century, the complete life-cycle of the bucephalid trematode was first described, with a detailed account of the miracidium by Woodhead (1929) using *Bucephalus papillous* from the caecal pouches of the small-mouthed black bass *Micropterus dolomieu* obtained from the Huron River, Michigan.

Most studies of bucephalids have been limited to descriptions and illustrations of the morphological features concerned with the anterior attachment organ, and to the topography of the larger internal organs of the adults. As the above information is not sufficient for the identification of the animal, the systematic

arrangement of the bucephalids underwent repeated and extensive revisions. Complete life-cycle studies have been made for only six fresh-water and twelve marine species from the 236 species so-far described from the adult, metacercarial and cercarial stages (Table 1.1).

The significance of the excretory system in the systematic arrangement and taxonomy of the digenetic trematodes has been considered by many investigators. [Cort (1917); Faust (1919; 1924, 1932); Hopkins (1954, 1956); LaRue (1957); Stunkard (1974d, 1975)]. Stunkard (1975) gave a record of flame-cell formulae for 20 species of bucephalid trematodes, which helped in the identification of the species.

The complicated problem of taxonomy and synonymy of the two European freshwater bucephalids, *Bucephalus polymorphus* Baer,1827 and *Rhipidocotyle campanula* (Dujardin,1845) have been discussed in this chapter. Due to misinterpreted identity, what was considered to be the only freshwater bucephalid in Europe was described as *Bucephalus polymorphus* by several workers, until Kozika (1959) distinguished two forms of bucephalids in Lake Druzno, Poland from the differences in the anterior sucker and the size. He named them as *Bucephalus polymorphus* Baer,1827 and *Rhipidocotyle illense* (Ziegler,1883). Baturo (1977), after her experimental demonstration of part of the life-cycle, determined the identity of the two parasites and requested the International Commission on Zoological

Nomenclature to maintain the name of *Bucephalus polymorphus* Baer,1827 for the species commonly known under that name and to replace the specific name *Rhipidocotyle illense* (Ziegler, 1883), Vejnar, 1956 by *Rhipidocotyle campanula* (Dujardin, 1845), Dollfus,1968.

2.2 Brief historical review of the Bucephalid trematodes

The history of the family Bucephalidae begins with Rudolphi's (1819) description of the adult forms of *Monostomum crucibulum*, from *Muraena* spp. from Naples; *Monostomum galeatum* from *Centronotus glaucus* from Naples; and *Distoma gracilescens* from *Lophius piscatorius* from Trieste. All three bucephalids parasitize marine fishes and have subsequently been transferred to the genus *Gasterostomum*.

Von Baer (1827) was the first to introduce the generic name *Bucephalus*, naming the larval trematodes found in different tissues of freshwater mussels, *Unio pictorum* and several species of *Anodonta* in a lake near Konigsberg as *Bucephalus polymorphus*. Von Baer gave them the generic name of "*Bucephalus*" on account of their resemblance to the head of an ox, and the specific name "*polymorphus*" on account of the instability in form of the germ-tubes and in the variation in shape of the tail process. Further light was thrown upon these cercariae by Badcock (1875) and Stewart (1875) from their observations of similar animals released in Badcock's aquarium during summer 1874 from a freshwater mussel. He expressed the cercaria as "tranparent creatures flying like eagles through the water". Since Von Baer's introduction, the name *Bucephalus* has been erroneously given to any larval Bucephalid, until its validity is recognised.

Von Siebold (1848) erected the genus

Gasterostomum to accommodate an adult trematode from the intestine of *Perca* sp. and *Lucioperca* sp. which he named *Gasterostomum fimbriatum*.This generic name is derived from the location of the mouth on the ventral surface. From morphological agreement Von Siebold himself suggested that *G.fimbriatum* represented the adult stage of *B. polymorphus* Baer, 1827 and was the first to suggest a possible relationship between the cercaria and the adult. Wagener (1858) discussed the probable life cycle as the cercaria from freshwater clams was to encyst on the gills of *Cyprinus* sp. and to develop into mature *G. fimbriatum* in perch and pike. This view was supported by Giard (1874), and Ziegler (1883) based on morphological similarity without any experimental evidence. Wagener (1858) also indicated that the generic name *Bucephalus* had proper priority over *Gasterostomum* in this case, and discussed the probable life-cycle of *B. polymorphus* Baer,1827. Poche (1907) formally declared *Gasterostomum* a synonym of *Bucephalus* and set up the family Bucephalidae with *Bucephalus* Baer, 1827, as type genus.

Another digenetic trematode was described by Dujardin (1845) from the intestine of *Esox lucius* as *Distoma campanula*. This was redescribed and figured by Wedl (1858) and his work had clearly established the validity of the specimen. Wagener (1858) suppresed[5] *D. campanula* as a synonym of *G. fimbriatum* Siebold,1848 and recognised the identity of the latter species and *B. polymorphus* Baer,1827 purely on morphological similarity

without any experimental evidence. However, later authors accepted the identity of the two forms and recognised *B. polymorphus* as the single bucephalid freshwater species in Europe, until Kozika (1959) recognised two species in the freshwaters of Europe. His recognition was further supported by Baturo (1977) and other workers. Stunkard (1976a) suggested that if *D. campanula* and *G. fimriatum* are identical, the valid name is *campanula* and not *fimbriatum*.

A second furcocercous cercaria similar to *B. polymorphus* was identified by Lacaze-Duthiers (1854) in the marine environment. He described the specimen as *Bucephalus haimeanus* from the oyster, *Ostrea edulis* and cockle, *Cardium rusticum* in the Balearic Islands. Giard (1874) stated in his paper that Claparede in 1863 discovered similar cercaria on the coast of Normandy, when fishing with fine nets. He himself found it encysted in the liver, genital glands and other organs of the gar fish (*Belone vulgaris*) and an adult in the intestinal cavity of *Cydippe pileus*. But he regarded the adult specimen as an accidental infection. He also mentioned that Claparede found the *Cercaria haimeanus* several times fixed on to *Sarsia* and *Oceania*. From the above observations Badcock (1875) stated "it is therefore reasonable to support that *B. haimeanus* encysted in *Belone vulgaris* metamorphoses itself into some species of *Gasterostomum* in the intestine of some big fish which feeds upon the Belone". But there was no experimental

evidence to support the probable life-cycle.

Since the introduction of *B. haimeanus* to a marine cercaria by Lacaze-Duthiers (1854), the name has been given to cercariae recovered from 14 different species of marine bivalve molluscs without adequate description. Of the 42 bucephalid cercariae reported in Table 2.2, only for 18 of the species has the adult stage been determined experimentally. The remainder cannot be assigned their adult status under the present system of classification since adult characters are not exhibited by cercariae. Also as stated by Hopkins (1954) it seems most probable that each of the bucephalid cercariae reported from a different molluscan host will eventually be found to be a distinct species.

The first attempt in buchephalid history to demonstrate experimentally the connections between the various stages in the life history was by Tennent (1905, 1906, 1909) but was not successful in completing the life cycle of any one species. His belief that all forms of bucephalids were merely "physiological varieties" of the one species, *B. haimeanus* Lacaze-Duthiers, 1854, and the assumption of *B. cuculus* McCrady, 1874 and *B. haimeanus* as identical, led him to produce erroneous results. But he obtained adult worms in the intestine of predaceous fishes by feeding encysted larvae from *Menidia menidia* and observing heavy infection in the oyster by injecting faeces into the mantle cavity of oyster from a gar, *Lepisosteus osseus*, which contained eggs of an

unknown bucephalid species (1909). Since his attempt none of the American marine gasterostomes were demonstrated experimentally until Stunkard (1974b) experimentally demonstrated the life cycle of *R. transversale* Chandler,1935 and *R. lintoni* Hopkins,1954. But the life cycles of three North American freshwater bucephalids have been elucidated before the marine species. They are *Rhipidocotyle papillosa* (Woodhead,1929) Eckmann, 1932 by Woodhead (1929); *Bucephalus elegans* Woodhead, 1929 by Woodhead (1930); and *Rhipidocotyle septpapillata* Krull, 1934 by Krull (1934) and Kniskern (1952b). The other experimentally demonstrated lifecycles have been tabulated in Table 1.1.

Another generic name was erected by Diesing (1845) as *Rhipidocotyle* to accommodate *Gasterostomum minimum* Wagener, 1852 and *Gasterostomum gracilescens* Wagener, 1852 (syn. *Distoma gracilescens* Rudolphi, 1819). (Stiles and Hassall, 1908). The generic name *Rhipidocotyle* indicates a fan-shaped hood above the anterior sucker.

McCrady (1874) made the first report of an American bucephalid from the oyster, *Crassostrea virginica* at Charleston, South Carolina. This was recognised as a new species, *Bucephalus cuculus*. Tennent (1906) considered this to be identical with *Bucephalus haimeanus* because of his belief that all forms of bucephalids were merely "physiological varieties" of the one species *B. haimeanus*.

Odhner (1905) erected a new genus, *Prosorhynchus* and included specimens obtained from *Cottus scorpius* and *Cottus vulgaris* from the Arctic region as *P. squamatus* and *P. aculeatus*, and from *Muraena conger* from the Mediterranean Sea, as *P. crucibulum* (syn. *Monostomum crucibulum* Rud.,1819) Eckmann (1932) and Nagaty (1937) considered the following species to be identical with *P. crucibulum* (Rud.,1819): *Gasterostomum armatum* Molin, 1859; *Prosorhynchus squamatus* Odhner,1905; *Prosorhynchus grandis* Lebour,1908; *Prosorhynchus triglae* Nicoll,1914; *Prosorhynchus costae* Travassos et al, 1928; *Prosorhynchus scalpellus* McFarlane,1936. There is no agreement on the validity of these proposed identities as the conclusions are drawn from adult morphology, such as the adhesive organ and location of the internal organs, which are without phylogenetic significance.

MacCallum (1917) erected the genus *Alcicornis* to accommodate *A. carangis* from *Caranx ruber*. Later Nagaty (1937) found similar specimen of *A. carangis* from *Caranx* sp. and established the validity of the genus. He proposed to place this genus under the subfamily Prosorhynchinae Nicoll,1914, since it possesses a rhynchus and no sucker; he regarded this genus as a link between the subfamily Bucephalinae and the subfamily Prosorhynchinae as this genus possess tentacles and a rhynchus at the anterior end.

In the 1920s and 1930s eight more genera were introduced to accommodate different species of

bucephalids (Table 2.1). Nagaty (1937) listed *Gotonius* Ozaki,1924; *Skrjabiniella* Issaitschikow,1928; *Mordvilkovia* Pigulewsky, 1931; *Dollfustrema* Eckmann,1934; and *Psedoprosorhynchus* Yamaguti,1938 as synonyms of *Prosorhynchus* Odhner,1905. Manter (1953) recognised two distinct groups of species in the genus *Prosorhynchus*, one group of species with oval or lenticular rhynchus and the other group with conical rhynchus. He also discussed generic and specific criteria in the subfamily Prosorhynchinae and the status of the named species in the genus *Prosorhynchus*. In the subfamily he accepted the genera: *Dollfustrema* Eckmann, 1934, *Mordvilkovia* Pigulewsky, 1931; *Neidhartia* Nagaty,1937 (syn. *Psedoprosorhynchus* Yamaguti, 1938); *Telorhynchus* Crowcroft, 1947, and *Alcicornis* McCallum, 1917. Yamaguti (1971) considered all the other above mentioned except *Mordvilkovia* Piguleswsky,1931, as valid genera, but accepted them under different subfamilies. According to him *Mordvilkovia* is still a synonym of *Prosorhynchus* Odhner,1905. Stunkard (1974d) stated that Yamaguti (1971) listed 42 species in the genus *Prosorhynchus*, but as the generic concept was so loose and the morphology of the included species so varied, the generic integrity is lost. Even though the various species have been assigned to different new genera, there is no agreement on the validity of certain of these proposed genera since no information concerning their developmental stages and life-cycles are available.

Nagaty (1937) erected the genus *Neidhartia* to accommodate *N. neidharti* and *N.ghardagae* from *Serranus* sp. *Neidhartia* is the only member of the subfamily to have the ovary on the left side of the body.

Dayal (1948) erected two new genera *Neobucephalopsis* and *Neoprosorhynchus* to accomodate *Neobucephalopsis bagarius* from *Bagarius yarrelli* and *Neoprosorhynchus purius* from *Epinephelus lanceolatus*, both from Puri, India. In the *Neoprosorhynchus* the excretory vesicle is Y-shaped, and its arms reach to the intestine, one of the most important features of the genus and subfamily. More light was thrown by Verma (1936 a, b), Srivastava (1938, 1963), Srivastava and Chauhan (1972), Bilquees (1976a) and Gupta et al (1976, 1983a,b) on the contribution of genus and species of the bucephalids in the Indian subcontinent.

Chu (1950) described a new species, *Bucephalopsis kweiyangensis*, from a giant salamander, *Megalobatrachus japonicus* in China. This is the first report of a bucephalid from any host other than a fish; it may purely be accidental, but is of interest.

Hopkins (1954) proposed a new genus *Bucephaloides* for *Bucephalopsis* Nicoll,1914 nec Diesing,1855 with *Gasterostomum gracilescens* (Rud.,1819) as type species restricting the generic name *Bucephalopsis* to a cercaria, *B. haimeanus* Lacaze-Duthiers,1854, the adult of which is unknown. He included all the other species formerly included in the genus

Bucephalopsis to *Bucephaloides*. Later authors, Velasquez (1959) and Nahhas and Cable (1964) and Overstreet (1969) have adopted the proposal of Hopkins and recognised *Bucephaloides* with *Bucephaloides gracilescens* (Rud.,1819) as type species of the genus. Yamaguti (1971) stated that Hopkins had erected the genus without giving evidence to show that Nicoll's *Bucephalopsis* is different from Diesing's *Bucephalopsis*.

Dickermann (1954) erected the new genus *Paurorhynchus* with *Paurorhynchus hiodontis* from the body cavity of the mooneye fish, *Hiodon tergiscis*, from Maumee river at Grand Rapids, Ohio, as type species. This was placed in the newly created subfamily Paurorhynchinae by Dickermann (1954). The new trematode differs from all other described species of bucephalids in being considerably larger, spineless, having lobed gonads and living in the body cavity of the host.

Another new genus *Myorrhynchus* similar to *Prosorhynchus* was erected by Durio and Manter (1968) from a serranid "commonly called 'leche'" to accommodate *M. prichardae*. The new genus is separated from *Prosorhynchus* on the complex structure of the rhynchus, the position of the ovary in front of the vitellaria, and in having spiny eggs.

Kohn erected three new genera and species of bucephalids found in Brazilian waters. *Paraprosorhynchus* was erected (1967) to accommodate *P. jupe* from the intestine and stomach of *Promicrops guttatus* from

Conceicao Island, Victoria Bay. *Chabaudtrema* and *Pararhipidocotyle* were erected (1970a,b) to accommodate *C. rarus* from the intestine of *Garrupa* sp. from Gaunabara Bay and *P. jeffersoni* from the intestine of *Salminus maxillosus* from the river Mogi-Guacu, Sao Paulo State. *Pararhipidocotyle* is similar to *Rhipidocotyle* but the ovary is posterior to the testis, and *Chabaudtrema* is nearest to *Bellumcorpus* but has a simple, muscular funnel-shaped rhynchus.

Stunkard (1974d) erected a new genus *Rudolphius* to accommodate *P. crucibulum* (Rud.,1819) Odhner,1905, on the basis of the differences between the larval and the adult stages of *P. squamatus* and *P. crucibulum*.

The problems of the systematics of the gasterostome trematodes were reviewed by Kniskern (1952a), Hopkins (1956), Yamaguti (1971) and Stunkard (1974d, 1975, 1976b). Yamaguti (1971) recognised six subfamilies and twenty-two genera. All are parasites of the intestine except species of *Paurorhynchus*, Dickerman,1954, which occurs in the gallbladder. The synopsis of the family to the present knowledge is given on Table 2.1. The subfamilies and genera are distinguished principally on differences in the structure of the anterior adhesive organ and location of the internal organs, particularly the relations of the ovary to the vitellaria and testes, which are not the same for the larval stages. Stunkard (1974d) stated that adhesive organs have long been regarded as cenogenetic, adaptive

structures in parasitic species and without phylogenetic significance. In the digenetic trematodes, the excretory system has acquired increased importance on the most conservative, fundamental and the significant feature for taxonomic determination. However from the studies of Woodhead (1929, 1930, 1936), Kniskern (1950, 1952a,b), Hopkins (1954, 1956) and Stunkard (1974d, 1975) it became evident that the flame cell formula is known for only a few species, and the lack of correlation between the excretory pattern and other features prevents the significant use of the excretory system in buchephalid taxonomy. At the same time, study of the excretory system is difficult and tedious and can be made only on a living specimen. Long and repeated observations may be required to complete the pattern in any species.

At present there is a growing interest to adopt chaetotaxy in taxonomy and phylogeny of trematodes (Richard 1968, 1971 and Bayssade-Defour 1979). Ulmer and Rhode (1981) stated that studies on cercariae and metacercariae belonging to different families of Digenea show that such studies of papillae are taxonomically significant at specific, generic or suprageneric levels. But the lack of information on chaetotaxy among bucephalids demands further studies in this field.

The systematic arrangement of the bucephalid trematodes has undergone repeated and extensive revisions because of the confusion in identification, and

unsupported and unwarranted presumptions concerning relation between the larval and adult stages. Until more information on life-cycle developmental stages and detailed morphology especially of the excretory system is available, taxonomic treatment cannot be definitive.

Table 2.1

Synopsis of Bucephalid Trematodes

Subfamily	Genera	Synonym	Remarks
BUCEPHALINAE Nicoll,1914	*Bucephalus* Baer,1827	*Gasterostomum* Siebold,1848 *Eubucephalus* Diesing,1855	Includes 59 spp.of marine & fresh-water fishes; life cycle known for some.
	Alcicornis MacCallum, 1917.	*Bucephalus* Eckmann,(1932)	Includes 11 spp.of marine fishes; larval stages not known.
	Dollfustrema Eckmann,1934	*Dollfusina* Eckmann,1932 *Neodollfustrema* Long and Lee, 1964 *Prosorhynchus* Odhner,1905 *Mordvilkovia* Pigulewsky, 1931	Includes 7 spp.of marine & fresh-water fishes; miracidia & cercariae not known.
	Pseudorhipido -cotyle Long and Lee, 1964.	————	Includes 1 sp.of freshwater fish; larval stages not known.
	Rhipidocotyle Diesing,1858	*Nannoenterum* Ozaki,1924. *Bucephalus* Baer,1827.	Includes 40 spp.of Marine & fresh-water fishes; larval stages known for some, not miracidium.
	Rhipidocoty -loides Long and Lee, 1964.	————	Includes 1 sp.of freshwater fish; larval stages not known.
	Telorhynchus Crowcroft, 1947	————	Includes 1 sp.of marine fish; larval stages not known.
	Pararhipidocotyle Kohn,1970		Includes 1 sp.of freshwater fish;

			larval stages not known.
DOLICHOENTE -RINAE Yamaguti,1958	*Dolichoenterum* Ozaki,1924	———	Includes 7 spp.of marine fish; miracidia & cer. not known.
	Bellumcorpus Kohn,1962	———	Includes 1 sp.of freshwater fish; larval stages not known.
	Pseudodolicho -enterum Yamaguti,1971	———	Includes 1 sp.of marine fish; larval stages not known.
	Chabaudtrema Kohn,1970	———	Includes 1 sp.of marine fish; larval stages not known.
NEIDHARTIINAE Yamaguti,1958	*Neidhartia* Nagaty,1937	———	Includes 5 spp.of marine fish; larval stages not known.
	Pseudoproso -rhynchus Yamaguti,1938	———	Includes 1 sp. of marine fish; larval stages not known.
NEOPROSORHYN- -CHINAE Yamaguti,1958	*Neoprosorhyn -chus* Dayal,1948	———	Includes 1 sp.of marine fish; larval stages not known.
PAURORHYNCHINAE Dickerman,1954	*Paurorhynchus* Dickerman,1954	———	Includes 2 spp.of freshwater fish; miracidia & cer. not known.
PROSORHYNCHINAE Nicoll,1914	*Prosorhynchus* Odhner,1905	*Gotonius* Ozaki,1924 *Mordvilkovia* Pigulewsky,1932 *Skrjabiniella* Issaitschikow, 1928 *Dollfusia* Eckmann,1932 *Dollfustrema* Eckmann,1934	Includes 53 spp. of freshwater & marine fishes; miracidia not known.
	Bucephalopsis (Diesing,1855)	*Bucephaloides* Hopkins,1954	Includes 59 spp. of freshwater &

		Prosorhynchoides Dollfus,1929	marine fishes; miracidia not known.
	Myorhynchus Durio and Manter,1968	———	Includes 1 sp.of marine fish; larval stages not known.
	Neobucepha-lopsis Dayal,1948	———	Includes 4 spp.of freshwater fish; larval stages not known.
	Parabucepha-lopsis Tang and Tang, 1963	———	Includes 2 sp.of freshwater fish; larval stages not known.
	Paraproso-rhynchus Kohn,1967	———	Includes 1 sp.of marine fish; larval stages not known.
	Pseudobucepha-lopsis Long and Lee, 1964	———	Includes 1 sp.of fresh/marine fishes; larval stages not known.
	Rudolphius Stunkard,1974	———	Includes 1 sp.of marine fish; larval stages not known.

Table 2.2

Previous reports of Bucephalid cercariae from Bivalve Molluscs

Date	Author	Host	Name given to species	Locality
1827	von Baer	*Unio pictorum*	*Bucephalus-polymorphus*	Europe (F)
1827	von Baer	*Anodonta - anatina*	*B.polymorphus*	Europe (F)
1827	von Baer	*A.mutabilis*	*B.polymorphus*	Europe (F)
1848	von Siebold	*A.cellensis*	*B.polymorphus*	Europe (F)
1854	Lacaze-Duthiers	*Ostrea edulis* (European-oyster)	*B.haimeanus*	Mediterranean (S)
1854	Lacaze-Duthiers	*Cardium edulis* (Cockle)	*B.haimeanus*	Mediterranean (S)
1857	Pagenstecher	*A.anatina*	*B.polymorphus*	Europe (F)
1863	Claparede	Found free swimming cercaria	*B.haimeanus*	Normandy (S)
1874	McCrady	*Crassostrea-virginica* (American-oyster)	*B.cuculus*	Carolina (S)
1878	Ulicny	*A.cellensis*	*B.intermedius* (Syn.*B.polymorphus*)	Europe (F)
1881	Ercolani	*U.pictorum*	*Cercaria-bucephalus* (Syn.*B.polymorphus*)	Europe (F)
1881	Levinsen	*Mediolaria-discors*	*B.crux*	Egedesminde Greenland (S)
1882	Ercolani	*A.anatina* & *Unio* sp.	*C.bucephalus* (Syn.*B.polymorphus*)	Europe (F)

1883	Ziegler	_____	*B.polymorphus*	Europe (F)
1888	Huet	*Cardium edulis*	*B.haimeanus*	Normandy (S)
1888	Huet	*Spisula solida*	*B.haimeanus*	Europe (S)
1893	Huet	*Mactra solida*	*B.haimeanus*	Normandy (S)
1894	Vaullegeard	*Tapes-pullastra*	*B.haimeanus*	Europe (S)
1894	Vaullegeard	*Tapes-decussatus*	*B.haimeanus*	Europe (S)
1899	Kelly	Unionidae	*B.polymorphus*	Mississippi-river (F)
1903	Haswell	*Mytilus latus*	*Bucephalus sp.*	New Zealand (S)
1904	Johnstone	*Cardium edule*	*B.haimeanus*	Lancashire, England (S)
1905, 06,09	Tennent	*Crossostrea-virginica*	*B.haimeanus* (Syn.*B.cuculus*)	North Carolina (S)
1906	Pelseneer	*Abra alba*	*B.haimeanus*	Europe (S)
1906	Pelseneer	*Cardium edule*	*B.haimeanus*	Europe (S)
1906	Pelseneer	*Mactra-subtruncata*	*B.haimeanus*	Europe (S)
1909	Sinitzin	*Dreissensia-polymorpha*	*B.polymorphus*	Warsaw (F)
1909	Sinitzin	*A.mutabilis*	*B.polymorphus*	Warsaw (F)
1909	Sinitzin	*Tapes rugatus*	*B.haimaenus*	Black Sea (S)
1911	Sinitzin	*Tapes rugatus*	*C.hydriformis* (=*B.haimeanus*)	Black Sea (S)
1911	Lebour	*Syndosmya alba*	*C.syndosmyae* (=*B.haimeanus*)	Millport, U.K(S)
1911	Lebour	*Cardium edule*	*B.haimeanus*	North-umberland (S)
1924	Wunder	_____	*B.polymorphus*	Europe (F)
1925	Miller	*Pinna cornea*	Cercaria N	Tortugas (S)
1926	Faust	*Modiola-capensis*	*Bucephalopsis-modiolae*	S.Africa (F)

1929	Woodhead	*Elliptio-dilatatus*	*Cercaria-papillosum* (=*B.papillosum*) (syn.*R.papillo-sum*)	Michigan (F)
1930	Woodhead	*Micromya sp*	*C.elegans* (=*B.elegans*)	Michigan (F)
1933	Roughley	*Ostrea-commercialis*	*B.haimeanus*	N.S.W (S)
1933	Roughley	*Ostrea angasi*	*B.haimeanus*	N.S.W (S)
1934	Ozaki & Ishibashi	*Pictada-martensi* (Pearl oyster)	*B.margaritae* (syn.*B.varicus*)	Japan (S)
1934	Palombi	*Tapes-descussatus*	*B.haimeanus*	Naples (S)
1934	Wesenberg-Lund	_____	*B.polymorphus*	Europe (F)
1935	Cole	*Mytilus - edulis*	*B.mytili*	N.Wales (S)
1935	Cole	*Cardium edule*	*B.mytili*	N.Wales (S)
1936	Woodhead	*A.grandis*	*C.argi* (=*Bucephalopsis-pusilla*)	Michigan (F)
1936	Woodhead	*Lampsilis-siliquoidea*	*C.basi* (*R.septpa-pillata*)	Michigan (F)
1936	Woodhead	*Micromya iris* (=*Eurynia iris*)	*C.scioti*	Michigan (F)
1949	Andrev	*Venerupois aurea*	*B.haimeanus*	Spain (S)
1952	Chubrik	*M.latus*	*Prosorhynchus-squamatus*	Arctic (S)
1952	Kniskern	*L.siliquoidea*	*R.septpapillata*	Michigan (F)
1954	Ozaki	*Ostrea-denselamellosa*	*Bucephalus-itabo* (=*Prosorhynchus uniporus*)	Japan (S)
1954	Hopkins	*Crassostrea-virginica*	*B.cuculus*	U.S.A (S)
1956	Cable	*Donax-denticulata*	*C.caribea XL11*	Jamaica (S)

1956	Cable	*Tellina-lintea*	*C.caribea* XL11	Jamaica (S)
1958	Hopkins	*Donax-variabilis*	*B.loeschi*	Texas (S)
1960	Ozaki	*Caecella chinensis*	*P.caecellae*	Japan (S)
1960	Ozaki	*Gryphaea-gigas*	*P.magakii*	Japan (S)
1961	Angel	*Velesunionis-ambiguus*	*C.velesunionis* (=*Prosorhynchinae* sp.)	Australia (F)
1961	Holliman	*Mulinia-lateralis*	*C.apalachiensis*	Florida (S)
1961	Laird	*O.belcheri*	Unidentified sp.	Pakistan (S)
1961	Laird	*Crassostrea-virginica*	*B.cuculus*	Canada (S)
1965	Singh & Rai	*Corbicula-striatella*	*Cercaria-katangii*	India (F)
1965	Chubrik	*M.edulis*	*Prosorhynchus-crucibulum* & *P.squamatus*	Arctic (S)
1966	Sanders	*Pecten alba*	*Cercaria* sp.	Australia (S)
1966	Howell	*O.lutaria*	*Bucephalus-longicornutus*	New Zealand (S)
1966	James et al	*C.edule*	*B.haimeanus*	Europe (S)
1970	Breton	*M.edulis*	*Bucephalus-mytili*	France (S)
1973	Matthews	*M.edulis*	*P.crucibulum*	Cardigan Bay U.K.(S)
1974	Matthews	*Abra alba*	*Bucephaloida-gracilescens*	Scotland
1974	Chun	*Crassostrea-gigas*	*Bucephalus* sp. (=*B.varicus*)	Korea (S)
1974	Stunkard	*Lyonsia-hyalina*	*R.transversale* & *R.lintoni*	U.S.A. (S)

1976	Tang & Tang	*Limnoperca-lacustris*	*Parabucepha-lopsis prosthorchis*	China (S)
1976	Tang & Tang	*Limnoperca-lacustris*	*Dollfustrema-foochowensis*	China (S)
1977	Sannia & James	*M.edulis*	*P.squamatus*	Iceland (S)
1977	Koubek	*Unio timidus* and *Unio pictorum*	*B.polymorphus*	Czechoslo-vakia (F)
1977	Baturo	*Driessena-polymorpha*	*B.polymorphus*	Poland (F)
1977	Baturo	*Unio pictorum*	*R.campanula*	Poland (F)
1978	Joseph	*Crassostrea-madrasensis*	*Bucephalus* sp.	India (E)
1978	Samuel	*Crassostrea-madrasensis*	*Bucephalopsis-haimeanus*	India (S)
1978	Tuffery	*Driessena-polymorpha*	*B.polymorphus*	France (F)
1981	Maillard & Saad Fares	*Tapes-aureus*	*B.baeri*	Mediter-ranean (S)

F - Freshwater; S - Seawater

2.3 Systematic review of *Rhipidocotyle campanula* (Duj.,1845)

The taxonomy of bucephalid trematodes is complicated because of unsupported and unwarranted presumptions concerning relations between larval and adult stages; this causes a problem in the recognition of the freshwater bucephalids of Europe.

Von Siebold (1848) was the first to suggest a possible relationship between the first ever described European freshwater cercaria, *Bucephalus polymorphus* Baer,1827 from *Anodonta mutabilis* and *Unio pictorum*, and the adult trematode *Gasterostomum fimbriatum* Siebold,1848, from the intestine of European freshwater predaceous fish,*Perca fluviatilis* and *Lucioperca* spp., on the basis of the ventral mouth and sac-like intestine. The probable identity was supported by Wagener (1858), Giard (1874), and Ziegler(1883) and until recently all bucephalid parasites from Europe freshwater hosts were referred to a single species.

Dujardin(1845) described a new digenetic trematode from the intestine of *Esox lucius*, which he named as *Distoma campanula*, and believed that the small helminths found encysted on the gills of *Cyprinus idus* and possessing the same sucker were immature forms of *Distoma campanula*. Thirteen years later Wedl (1858) redescribed and figured this species not given in Dujardin's work, clearly establishing the validity of the species. Kozika(1959) stated that from the

description of the parasite concerning the size, the outgrowths on the sides of the anterior sucker, and the position of the pharynx behind the middle of the body, the author was dealing with *Rhipidocotyle* sp.

Wagener (1852) reported *Gasterostomum fimbriatum* Siebold,1848 from the intestine of *Esox lucius* from Berlin. He characterised the trematode by five tentacles on the anterior organ, and the extension and retraction of the tentacles. In 1858 after more work on the species, he considered *Gasterostomum fimbriatum* Siebold and *Distoma campanula* Dujardin to be synonyms of *Bucephalus polymorphus* Baer, and recognised the identity of the former species and *B.polymophus*. He regarded *G.fimbriatum* Siebold as a sexually mature, tailless *B.polymophus* Baer. The identity of *B.polymorphus* and *G.fimbriatum* was based on morphological similarity and the belief that there is only one species of bucephalid trematode in the freshwater fishes of Europe. Ever since his papers, even though there was no experimental evidence to support such a belief, a view has been adopted that the cercariae described under the name *Bucephalus polymorphus* develop into the adult trematode *G.fimbriatum* Siebold, characterised by the presence of long tentacles on the anterior sucker. Luhe 1909, Eckmann 1932, and Hopkins 1954 accepted the identity of the two forms and recognised *B.polymorphus* Baer,1827 as the single bucephalid species in Europe. Stunkard(1976) stated that if *D.campanula* Dujardin and *G.fimbriatum* Siebold are

identical, the valid name of the species should be *campanula* and not *fimbriatum*.

Ziegler (1883) obtained metacercariae corresponding to *Distoma campanula* Dujardin,1845, by experiment, exposing *Leuciscus erythrophthalmus* to the cercariae released from *Anodonta mutabilis* from the Ill river near Strasburg. Even though the experiments lacked proper controls, he established a possible life-cycle for the species studied, and was the first to obtain metacercariae experimentally. He found no tentacled fluke in the intestine of *Esox lucius* from the Ill river, and the cercariae he used were smaller than those described by Baer in 1827. When comparing his adult specimen with the description given by Wagener(1852,1858) for *G.fimbriatum*, he mentioned that all other findings of Wagener's coincide with his except for the fimbriae; Ziegler believed that the fimbriae form under abnormal conditions and if the tentacles of *Gasterostomum* were a normal and permanent phenomenon, the forms found in the river Ill ought to be regarded as a new species which could be called as *Gasterostomum illense*. Yet, because of his indecision, the representative of the genus *Rhipidocotyle* he described as *Gasterostomum fimbriatum* was ignored.

Following Ziegler's work, it was recently demonstrated (Vejnar 1956, Kozika 1959, Baturo 1977) that in addition to *B.polymorphus* Baer,1827 one more species without tentacles and with fimbriae on the dorsal side of

the anterior sucker, exists in the European inland waters

Luhe(1909) described *Rhipidocotyle illense*, erroneously listed as *B.polymorphus* Baer,1827, from *Esox lucius*, *Perca fluviatilis*, *Lucioperca lucioperca* and *Lota lota*. He considered *G.fimbriatum* Siebold,1848 to be a synonym for *B.polymorphus* Baer,1827, and added that it was the only representative of Bucephalidae in the freshwaters of Germany. As mentioned by Stunkard (1976a) Luhes figure of *B.polymorphus* was clearly the species described by Dujardin(1845), Wedl(1858) and Ziegler(1883), and cannot be included in *Bucephalus*, and definitely is a member of the genus *Rhipidocotyle*.

Eckmann (1932) acknowledged the existence of only one species in the fresh waters of Europe, and this was accepted by Nagaty (1937), Dawes (1946) and Hopkins (1954). Even though Eckmann (1932) assigned the form described by Ziegler as a synonym of *B.polymorphus* Baer,1827, he doubted whether the form obtained by Ziegler was a separate species.

Koval (1949) found two species in the Dnieper, Soviet Union, which he designated to the same genus *Bucephalus* Baer,1827, and described one of them as *Bucephalus markewitschi*, leaving the other with the generally accepted but erroneous name of *Bucephalus polymorphus* Baer, 1827.

Vejnar (1956) identified the parasites which he found in Czechoslovakia, and denoted the tentacled parasite as *B. polymorphus* Baer,1827; he correctly named

the species without the tentacles as *Rhipidocotyle illense* (Ziegler,1883) but presented no drawings or dimensions of the parasite.

Kozika distinguished two forms of bucephalids in Lake Druzno, Poland, and identified (1959) the larger one with extensive tentacles before the oral sucker as *B.polymorphus* Baer,1827, and the smaller with fimbriae instead of tentacles which covered the dorsal part of the oral sucker as *Rhipidocotyle illense* (Ziegler,1883); he gave a full description of the parasites with drawings. His description of *B.polymorphus* coincided with *B.markewitschi* by Koval, and that of *R.illense* coincided with Luhe's (1909) *R.illense*, Koval's (1949) *B.polymorphus* and Vejnar's (1956) *R.illense*. He was the first to state that two species of Bucephalidae, *B.polymorphus* and *R.illense*, have so far been found in Europe's inland waters. He also admitted (1959) the identity of *Distoma campanula* Dujardin,1845 and *R.illense* (Ziegler,1883) Vejnar,1956. Both above parasites have been long known in Europe's fresh waters, but always as one and the same species, *B.polymorphus* (Wagener, Luhe, Eckmann, Ziegler). He stated (1959) that when the tentacles characteristic of the genus *Bucephalus* are hidden in the sucker and the fimbriae characteristic of *Rhipidocotyle* do not spread, the two parasites cannot be designated at first sight, as they look so similar.

Dollfus (1968) proposed a new combination of *Rhipidocotyle campanula* (Dujardin, 1845) after

transferring the identity of Dujardin's *Distoma campanula* to *Rhipidocotyle illense*. His proposal was further supported by Yamaguti's (1971) declaration of synonymy of *R.illense* (Ziegler,1883) Dyke,1954 with *Distoma campanula* Dujardin,1845 as well as Stunkard's (1976a) and Baturo's (1977 & 1979) agreement on the new combination of *R.campanula* (Dujardin,1845) for the species without tentacles.

Baturo (1977) declared from her experimental life-cycle studies of both the above parasites in Poland, that the cercariae from *Unio pictorum*, identical with the cercariae described by Baer,1827 were found to develop into the metacercariae of *R.illense*, this corresponds with that of Ziegler's (1883) experiments; and the cercariae released from *Dreissena polymorpha* and not the cercariae described by Baer were to develop into the metacercariae of *B.polymorphus* which she confirmed with the picture of the cercaria from *D.polymorpha* by Kinkelin et al (1968). From her experimental evidence she requested the International Commission on Zoological Nomenclature to maintain the name of *Bucephalus polymorphus* Baer,1827 for the species commonly known under this name with tentacles, and to replace the specific name *Rhipidocotyle illense* (Ziegler,1883) Vejnar,1956 by *Rhipidocotyle campanula* (Dujardin,1845) Dollfus,1968 for the species without tentacles. After all the confusion in identification, it is now confirmed that there are two members of the family Bucephalidae

represented in the fresh waters of Europe, namely *Bucephalus polymorphus* Baer,1827 and *Rhipidocotyle campanula* (Dujardin,1845) Dollfus,1968. The miracidial stages have yet to be demonstrated for the completion of the life-cycles.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and maintenance of animals

(a) Minnows (*Phoxinus phoxinus*)

Minnows were obtained from the River Aire at Keighley, West Yorkshire (GRID REF:SE 055 433) as suggested by previous records for the metacercarial cysts of *Rhipidocotyle campanula* Dujardin,1854. For a comparative study a few minnows were obtained from the River Wharfe (GRID REF: SE 257 459); these were not infected with the metacercarial cysts of *R. campanula*. More samples were collected, but as the numbers were too low, further experimental studies using non- infected minnows were prevented. These minnows were mainly used to complete the life cycle of the parasite experimentally, to confirm the identity of the species.

Minnows were collected monthly from the Aire by electrofishing, using a generator producing 240V DC, and also by hand nets, from March 1985 to November 1986 except for some winter months (December to February). Samples were collected in 1987 for laboratory experiments.

In summer minnows are pelagic and shoal near the surface but in winter they are found in deep waters and individually under stones. Hand nets were used during summer to catch the shoaling minnows on the surface but electrofishing gear was most successful in collecting deepwater minnows as well as the shoaling minnows. During

electrofishing, as soon as the stunned fish floated to the surface, they were collected by hand nets and transferred to collecting tanks.

The fish were transported to the laboratory in covered buckets and immediately transferred into deep fibreglass tanks (91cm x 61cm x 56cm) in the aquarium with fine wire mesh to prevent them leaping out. The tanks were continually aerated and the temperature maintained between 9° C to 11° C. Every few days fish were fed with "Tropical-fish Flakes" on which they thrived well for more than 10 months. The numbers of minnows caught varied for each season but in summer more than 100 fish were caught in each sample.

Every month the fish remaining after laboratory studies were kept in smaller glass tanks (90cm x 30cm x 45cm) in the same conditions.

Along with minnows when using the electrofishing technique, fishes such as gudgeon (*Gobio gobio*), bullheads (*Cottus gobio*) sticklebacks (*Gasterosteus aculeatus*) and stone loaches (*Noemachulus barbatum*) were caught. They too were brought to the laboratory and kept separately in glass tanks (91cm x 61cm x 56cm) in the same conditions. These fishes were used for a general study of the parasites and for further experimental infection of *R.campanula*.

(b) Perch and Trout

Samples of trout (*Salmo trutta* L,1758) within the total length of 15cm to 20cm were obtained from Grassington trout farm and trout fry from Humberside fisheries, Driffield. Perch (*Perca fluviatilis* L, 1758) were obtained from a pond at Scarcroft, where they are stocked by the Yorkshire Water Authority.

Perch suffered more stress during transport and change of habitat than trout. The mortality rate of perch was fairly high, between 25% and 30% compared to trout (between 3% to 5%). They were kept in the aquarium separately in continually aerated deep fibreglass tanks (91cm x 61cm x 56cm) at temperatures between 9° C to 11°C. Adult trout were fed on pelleted fish food and perch on live *Tubifex* worms until they were experimentally fed on infected minnows with metacercarial cysts to study the development of the adult parasite inside the alimentary canal of the fish. The trout fry were fed on "Tropical fish flakes" and kept in the same conditions.

(c) Mussels

Bivalve molluscs were taken from the River Aire, Keighley (GRID REF: SE 059 434) for investigation as possible intermediate hosts of *R.campanula*. Normally mussels prefer firm mud at the bottom of slow rivers,

canals and ponds, so dredges were used to collect the mussels along the banks and 3 to 4 metres away from the banks. Collection of mussels was quite tedious and normally 15 to 30 individuals of different sizes were collected each month from March 1985 to November 1986, except for the heavy winter months (December to February). Some samples were collected in 1987 for laboratory experiments. All the mussels were identified as *Anodonta anatina*.

The mussels were placed singly in continuously aerated transparent boxes (17.5cm x 11.5cm x 5.5cm) containing fresh water between 15°C to 18°C in the laboratory to observe the release of cercariae. The undersides of the containers were covered with black paper to enable the cercariae to be more easily seen. The mussels were totally immersed in 5cm water in the containers and fed every three days with "Liquifry" - baby fish food. Every two weeks the mussels were transferred into clean containers of water in similar conditions.

In the laboratory, mussels were observed daily to detect the emerging cercariae. If no cercariae were shed within a month, mussels were returned to larger photographic trays (34cm x 43cm x 6cm) in the aquarium to await possible further development and emergence of cercariae.

Fair success was realised in the maintenance of mussels. Some loss occurred, but a large number remained

alive for more than 10 months. Dead mussels were removed and, where possible, examined for infection and included in computing the percentage of infection.

3.2 Examination of the animals

(a) Minnows

The fish were examined for parasites with the minimum of delay following collection. Twenty five minnows were killed each month from March 1985 to November 1986, by decapitation, for the examination of the metacercarial cysts. External examination for infection is not possible; the fish were dissected since the metacercarial cysts are found in the sub-oesophageal region buried in the mucosa of the upper and lower bucco-pharyngeal cavity and also along the gill arches. The length and sex of the fish were recorded. The age of minnows was determined from the opercular bones and scales, but the annular markings were often found to be indistinct. For this reason fish have been grouped into arbitrary length groups for the purposes of analysis of the metacercarial cyst distribution.

The dissected cysts were counted and measured. Then the metacercariae were released from the cysts and the measurements were taken on them. These metacercariae were used for further histological, histochemical and electron microscopical preparations.

All potential sites were also examined for other parasites to give a general record of the parasites in minnows.

As almost all the minnows caught in the River Aire, were infected with the metacercarial cysts of *R.campanula*, they could not be used for further experimental infection by cercariae. The few minnows caught in the River Wharfe were not infected and were used for the experimental infection by cercariae.

(b)Mussels

In the laboratory mussels were observed daily to detect the emerging cercariae. If no cercariae were shed within a month, mussels were returned to large photographic trays (34cm x 43cm x 6cm) in the aquarium at 9°C to 11°C, to await possible further development and cercarial emergence. Some of them were opened and a superficial examination was made of their visceral mass for the presence of sporocysts. If sporocysts were absent, the mussels were dissected or sectioned for deep-seated infection. The infected mussels were sectioned for further study of the sporocysts. The strands of sporocysts were dissected and studied separately.

A total of 433 specimens of mussels were collected from the River Aire, Keighley from March 1985 to November 1986. Of these only 8% were infected

with the sporocysts of *R.campanula*. All the mussels collected were examined carefully and identified as *Anodonta anatina* L. and confirmed by Mr Adrian Morris from Leeds City Museum.

(c)Other fish

All the other fish caught - sticklebacks (*Gasterosteus aculeatus*), Gudgeon (*Gobio gobio*), bullheads (*Cottus gobio*), and stone loaches (*Noemachulus barbatulum*) - were dissected with a minimum of delay following collection and examined in all potential sites for parasites. A general record of all the parasites in River Aire is given in Chapter 1V.

The above species of fishes were also used in experiments to determine the degree of specificity of the *Rhipidocotyle campanula* cercariae to the second intermediate host.

3.3 Life history studies

(a) Recovery of cercariae

Live bivalve molluscs for experimental purposes were obtained from the River Aire, Keighley from March 1985 to November 1986. The mussels were placed singly in continually aerated containers and examined daily for liberated cercariae.

Mussels showing infection were kept in the laboratory at 15°C to 18°C, individually in transparent boxes (17.5cm x 11.5cm x 5.5cm) and the others were transferred to larger trays (34cm x 43cm x 6cm) in the aquarium to await possible further development and emergence of cercariae. Freshly released cercariae were pipetted out carefully as the tail easily detaches from the body.

The maintenance and the examinations of the mussels are not given in detail as they are described under the previous heading in this chapter.

(b) Infection experiments

The term "infection experiments", used throughout the text, denotes an experiment in which cercariae were used to infect a second intermediate host to observe the entry and development of the cercariae as well as to obtain the metacercariae.

Infection experiments were carried out with sticklebacks, bullheads, trout fry, minnows and stoneloach. Random samples were dissected prior to the experiment to ensure that the above species were free from infection.

Fish were singled out in individual plastic boxes (17.5 x 11.5cm x 5.5cm); 100 freshly released cercariae were introduced and the behaviour of the fish and the cercariae at room temperature between 15°C to

18°C were observed closely. The plastic boxes were aerated and left undisturbed for 4h. After this the fish were transferred into bigger tanks (90cm x 30cm x 45cm) and kept at the same temperature. The tanks were continually aerated and the fish fed with tropical fish flakes. The remaining cercariae in the box together with the detached tails after the penetration of the cercarial body, were counted to compute the number of cercariae which had entered the fish. The same experiment was repeated five times, and two days after the experiment the fish were dissected for examination.

Another experiment was carried out by introducing the cercariae into a tank of mixed fish consisting of three of each of the above species. The experimental conditions were the same as above. The behaviour of the cercariae was observed very closely to find whether there is a preferential selection of the fish.

The above two experimental set-ups were slightly modified by introducing infected mussels into the tanks with three fishes of the same species and leaving them for 2 days without any disturbances. After 2 days the mussels were removed and the fish dissected to study the rate of infection and species specificity.

The cercariae were pipetted out very carefully and dropped on to dead minnows and other species of fish to observe penetration and to demonstrate any chemotactic reaction.

The cercariae were introduced only to minnows after confirming the suitability of the host to study the encystment and development of the metacercariae.

(c) Feeding Experiments

The term "feeding experiments", as used here, denotes an attempt to obtain the adults of the metacercariae by feeding naturally infected minnows and also experimentally infected fish, to larger fish.

The larger fish selected as experimental hosts for feeding experiments were perch (*Perca fluviatilis* L) and trout (*Salmo trutta* L) within the total length of 15cm to 20cm. The fish were kept individually in tanks and left for 24h without feeding prior to the experiment. Naturally infected minnows were introduced and after 3h all uneaten minnows were removed from the tank. Feeding live fish was successful as both perch and trout readily ate live infected fish placed in the tanks. Fish were dissected every few days to observe the development of the eggs and miracidium and also the percentage of establishment of the metacercariae within the intestine.

Random sampling of the experimentally infected fish for metacercariae revealed that only minnows were infected with the parasite. As the result of this, the few available experimentally infected minnows were used to feed the perch and trout to complete the life cycle experimentally and to confirm the identity of the parasite.

(d) Attempts to obtain miracidia

Eggs were recovered from gravid adult specimens of *Rhipidocotyle campanula* that had been established experimentally in *Perca fluviatilis* L by keeping the adult worms in freshwater and Tyrode's solution. Some worms were teased with a fine needle, the uterus ruptured and the eggs were released. None of the eggs thus obtained released any miracidia.

The mussels were kept in gently aerated water and the eggs were dropped above the inhalent siphon to infect them; if the tentacles around the siphons were stimulated they closed immediately and the eggs were lost in the excurrent streams from the exhalent siphons. Another set of mussels was left for four weeks in the bottom of a tank which contained experimentally infected *Perca fluviatilis* L. Eggs or miracidia were not observed when the water was examined. Also no primary sporocysts were found when the mussels were examined after two or three weeks. So even the attempts to infect *A.anatina* by dropping eggs into the exhalent siphons were not successful.

3.4 Behavioural experiments of cercariae

(a) Periodicity and diurnal rhythm of emergence of cercariae

As a preliminary experiment 15 infected mussels were monitored individually from March to August 1985 for the liberation of cercariae.

The mussels were placed singly in continually aerated transparent boxes (17.5x11.5x5.5cm) containing fresh water between 15°C to 18°C in the laboratory to observe the release of cercariae. The light and dark periods were not controlled and the mussels were left in containers near the windows for the natural light source. The undersides of the containers were covered with black paper to enable the cercariae to be more easily seen. The mussels were totally immersed in 5cm water and fed every three days with " Liquifry". Every week the mussels were transferred into clean water in similar conditions.

The emerging cercariae were carefully pipetted out at 9am and 5pm each day until the mussels ceased to liberate cercariae.

The diurnal rhythm of the emission of cercariae was studied by keeping the mussels individually in transparent boxes as mentioned above and counting the cercariae every two hours. The mussels were kept in 12h dark and 12h light periods during the experiment as the natural spring condition. Prior to the experiment the

mussels were kept for 2 days in the similar experimental condition. Every two hours the cercariae were counted by carefully transferring the mussels to another container in similar conditions. The readings were taken on six infected mussels.

The experiment was repeated by giving 16h light and 8h dark periods as natural summer condition. The temperature was maintained in both the above experiments at 18°C. The readings were taken continually for four days.

(b) Analysis of cercarial activity patterns

Ten freshly released cercariae were pipetted carefully in a flat bottom transparent culture tube (9.5 cm high x 2.5 cm diameter) filled with freshwater to observe the activity patterns. The relatively large size of the cercariae allowed their activity patterns to be followed without a microscope. A tube when filled with water provided a column of 8cm high and allowed the cercariae to move with minimal restraint.

The tubes were mounted on polystyrene foam pads and fixed to a rigid shelf to minimise vibration and kept in a constant temperature room (18°C). Prior to each experiment the mussels were kept for three days at the temperature at which cercariae were subsequently examined. Different activity patterns were observed carefully and drawings were made to illustrate the movements of the cercarial body and tail.

Some of the cercariae were placed individually in a cavity watchglass and the behaviour was monitored on a television screen connected to a high power microscope. The activity of the cercariae was recorded on a video and later photographic records were made from the video.

The survival of the cercariae was studied by observing each cercaria individually, every hour, under the binocular microscope. Ten freshly released cercariae were placed in petri-dishes (medium size) in different solutions (saline - 0.15 % , 0.30 % , 0.45 % and 0.65 % ; tyrode solution ; freshwater). Each experimental set up was maintained at a different temperatures (15°C , 20°C and 25°C) in the incubator. Each experiment was repeated five times.

(c) Response of cercariae to light and gravity

As a preliminary observation, ten cercariae were placed in flat-bottomed transparent culture tubes (9.5 cm high x 2.5 cm diameter) filled with freshwater. Light from a microscope lamp was shone on the tubes from various angles, and the responses of the cercariae were recorded.

Responses of cercariae to light were tested by using the method as Kennedy (1979). Four glass tubes (9.5 cm high x 2.5 cm diameter) with various portions covered with light-proof tape were marked in thirds: A represents the top, B the middle, and C the bottom third. The first tube was completely covered, so that no light could enter

(test condition (TC) 1), the second was left uncovered (TC 2), the third had only the top 2/3 covered (TC 3) and the last the bottom 2/3 covered (TC 4).Tubes were filled with freshwater and 15 cercariae were pipetted into the middle portion of each tube. The four tubes placed vertically, 30 cm from the light source, were exposed to horizontal light supplied by two microscope lamps (Olympus Tokyo - HT lamp). Each experiment lasted 30mins and was conducted at 18°C. At the end of the experiment the water from each third of a tube was pipetted off, placed in separate dishes, and the numbers of cercariae in each area were counted.

3.5 Histological methods

Whenever possible, studies were made on live parasites with the aid of the light microscope. Intra vital stains, namely brilliant crystal blue and neutral red were found useful in studying some details of morphology. The successful study of the flame cell patterns was achieved by staining the freshly released cercariae in 0.5% neutral red for 5 minutes and observing the flame cells under slight coverslip pressure. As the beating of flame cells ceases quickly, the distribution of the flame cells was observed in only a few specimens.

All measurements were made on live specimens, length and breadth being measured without application of pressure. Egg albumen was used to slow down the movement

of the parasites. Measurements given represent the average of 50 unless otherwise stated. Bismark brown (0.5%) and brilliant crystal blue intra vital stains found useful to study the penetration glands since they stain these glands a deeper hue in contrast to the rest of the body.

The argyrophilic structures in the cercariae were studied by incubating the freshly released cercariae in 0.3% $AgNO_3$ at 7°C for 3h and exposing afterwards to sunlight for 20 minutes.

The general internal morphology of the different stages of the parasites was studied by fixing individual specimen under slight coverslip pressure, mounting in saline and withdrawing the saline with absorbent paper, while irrigating with a variety of fixatives: Bouin's, Gilson's, standard preparation of formal-acetic alcohol (FAA) and 10% formaldehyde solution. The specimen were stained with Mayer's carmalum (Cowdry,1952), and Delafield's haematoxylin and alcoholic eosin. Portions of sporocysts were also fixed and stained as mentioned above. Some sporocysts were completely teased apart for a study of the sporocyst wall and the cercarial embryos.

Infected and non-infected mussels were removed from the shell and the digestive glands and gonads were fixed in Bouin's, 4% neutral formaldehyde and Carnoy's (6:1:1) fixatives. Fixed specimens were embedded in paraffin wax (m.p.41°C) and sectioned at 5µm, 8µm and

10µm. Sections were stained in Delafield's haematoxylin and eosin, Heidenhein iron haematoxylin (Gray,1954), Mallory Heidenhein stain (Rapid - one - step method, Cason, 1950), Mallory's triple connective tissue stain (Krichesky, 1931) and Masson's modified method. (Ponceau Fuchsin 1% in 1% acetic acid and fast green 1% in 1% acetic acid with 1% aquous phosphomolybdic acid as mordant). All the above techniques are described by Humason (1972).

Freshly released cercariae and metacercarial cysts were fixed in 3% glutaraldehyde in 0.13M phosphate buffer (pH = 7.3) and after the buffer rinse post-fixed in 2% osmium tetroxide in 0.7% $K_2Cr_2O_7$. After another buffer rinse, specimens were dehydrated in a series of alcohols. Finally the specimen were embedded in fresh araldite and polymerised at 60°C for 24h. The sections were cut at 0.5 µm thickness with a glass knife and transferred to slides with an aluminium Marrinozzi ring (diameter = 3.5mm). They were stained in 0.1% toluidine blue in 1% borax and mounted in Histomount.

3.6 Histochemistry

Histochemical methods were carried out on whole mounts and sections to reveal the morphology by positive reactions to certain substances on different morphological structures. Techniques used were as described in Pearse (1972) and Culling (1974).

Whole mounts of cercariae and metacercariae were studied histochemically to reveal the nervous, alimentary, excretory and reproductive systems. For these studies freshly released cercariae and metacercariae released from the cyst were placed in a drop of water and fixed by irrigation with 10% neutral buffered formalin (pH =7) and Pearson's formalin at 1°C for 1h. Gentle pressure was used during the process of irrigation to flatten the worm, so that the internal structures would be clearly defined. The following methods were used to demonstrate the presence of a variety of enzymes on different structures of the cercariae - the indoxyl acetate method for non-specific esterase (Holt and Withers,1952), the acetylthiocholine iodide method for acetylcholine esterase (Gomori, 1952) and the Naphthol AS-BI phosphate method for acid phosphatase (Burstone, 1958). The optimum result for non-specific esterase was obtained after 2h incubation of specimens fixed in Pearson's formalin at 20°C, rather than the buffered formalin fixed specimens. For the acetylcholine esterase reaction the best results were obtained after 3h of incubation at 24°C. For this reaction both fixatives showed the same results in terms of density and localization of the end product of the reaction.

For the acid phosphatase reaction, lipids were removed by irrigating the cercariae progressively with 50%, 90% and 100% acetone at 1°C in order to prevent

staining of lipid droplets by diazonium salt. This process was then reversed and the specimen returned to water and incubated. Optimum results were obtained after 4h of incubation of the specimen fixed in both fixatives.

After incubation in the appropriate medium the cercariae were counterstained in nuclear fast red (1%) except for the demonstration of acid phosphatase in cercariae, where this step was found to be unnecessary. After counterstaining, the cercariae were mounted in glycerine jelly. Photographic records were made immediately as some of the reactions fade with age.

Control experiments were carried out in order to eliminate possible errors of interpretation. The cercariae were held at 90°C for 5 minutes prior to incubation, and in addition incubation were made omitting the specific substrate. Also eserine at 10^{-4} M concentration was added to indoxyl acetate and acetylthiocholine iodide incubation media.

Experimentally developed adult parasites from perch were dissected out after one, two, four, six and eight weeks of development and tested for polyphenol oxidases by the catechol technique (Smyth, 1954), phenolic substances by the diazo technique (Johri and Smyth, 1956) and the basic proteins by malachite green method (Johri and Smyth,1956). The specimens were fixed in Bouin's, 10% formalin and 70% alcohol under slight coverslip pressure. Whole mounts and sections were

prepared to study the development of the reproductive system and the production and maturity of the eggs.

To study the distribution of carbohydrates, proteins and lipids in the daughter sporocysts, infected and non-infected mussels were removed from the shell and the digestive glands and gonads were fixed in Carnoy's (6:1:1), Bouin's and 4% neutral formaldehyde fixatives. Fixed specimens were embedded in paraffin wax (mp=41°C). 5 um to 10 um sections were obtained, stained, washed in water and mounted in canada balsam or glycerine jelly.

Tests for carbohydrates were made on materials fixed in all three fixatives. Periodic Acid Schiff (PAS) reaction (McManus, 1946) with the associated salivary enzyme digestive technique was used, mainly on formal fixed material. A positive reaction with PAS indicates such complexes as neutral polysaccharides, muco- and glyco-proteins. Steedman's (1950) Alcian blue method was employed to determine the acid mucopolysaccharides. A combination of Alcian blue (1% in 70% alcohol + 2% HCl), Periodic Acid Schiff and Orange G (2% in 1% aq. Phosphotungstic acid) was employed to distinguish the sites of acid mucopolysaccharides from the other mucopolysaccharides. For the above demonstration the dewaxed sections were hydrated and oxidised in fresh $KMnO_4$ - H_2SO_4 mixture for 11/2 mins. and bleached in sodium metabisulphite (3% aq.) before the staining.

Best's Carmine stain (Best, 1903) was used on sections fixed in Carnoy's and Bouin's to determine the

sites of glycogen deposits. Mercuric bromo-phenol method (Maize et al. 1953) was used for protein after fixation in Bouin's and 4% neutral formaldehyde. As suggested by Dixon (1965), carbohydrate protein complexes are determined by PAS and protein reactions.

Mucopolysaccharides can be separated into different types by the variety of metachromasia produced with toluidine blue. Pearse (1961) recognised two varieties. Sections fixed in Bouin's and 4% neutral formaldehyde were stained in 0.5% aquous toluidine blue for 5h (Lillie, 1929) and mounted in glycerine jelly to demonstrate the presence of mucin (metachromatic substances).

The Oil red O method, following fixation in 4% formaldehyde, was used for detecting lipids, with 1% CaCl added to make the phospholipids insoluble. Glycerine jelly was used as the mounting medium to prevent the removal of fats and fatty acids by the solvents insoluble in the use of Canada balsam and other similar mounting media.

Sudan black B after McManus (1946) was used for detecting lipids in sections fixed in 4% neutral formaldehyde. The material was not dehydrated but mounted in glycerine jelly.

Heads and dissected submucosa of the upper pharyngeal cavity of the minnows were fixed and sectioned, as for the mussels, to study the distribution of the metacercarial cysts. These sections were stained

with the Alcian blue PAS technique and a combination of these two stains together with Orange G as mentioned above.

3.7 Electron microscopy

Freshly released cercariae were fixed in 3% glutaraldehyde in 0.13M phosphate buffer (pH = 7.3) for 2h at 4°C, washed a few times in buffer rinse, and post-fixed in 2% osmium tetroxide for 1h at room temperature (18°C). The specimens were washed in the same buffer solution and dehydrated at room temperature through a graded series of ethanol. They were then transferred using a pipette on to a 13mm diameter Millipore filter (0.45um pore size) for the last rinse in the alcohol. The entire disc with the specimen was then critical point dried in Polaron Jumbo Critical Point Dryer using liquid carbon dioxide, then mounted on the stub and coated with gold at a thickness of about 50nm using Polaron Sputter Coater E 5300 and examined with a CamScan 3 - 30 BM operated at 10kV.

Another set of cercariae after post-fixing was dehydrated through a graded series of ethanol, and after washing in 70% alcohol transferred on to a specimen holder with double-sided adhesive sellotape and the alcohol allowed to evaporate. (Hockley, 1968).

During fixation, post fixation and dehydration the cercariae were kept in a solid watch glass and the

reagents carefully pipetted out at each change rather than transferring the specimen from one container to another, since the tail of the cercaria is easily detached.

For some of the cercariae, after mounting on the stub and before coating , the tail and the body were separated very carefully using a fine needle to show the attachment of these parts. Care was taken as the specimens were very brittle.

Metacercariae released from the cyst, and adult worms, were prepared for scanning electron microscopy in the same way as for the cercariae.

Specimens for transmission electron microscopy were fixed and post-fixed in the same way as for scanning electron microscopy. After post-fixing the specimens were dehydrated through a series of ethanol and embedded in Araldite polymerised at 60°C for 24h. Semithin and ultra-thin sections were cut with a glass knife on a Reichert OmU3 thermal advance microtome. The ultrathin sections were mounted on formvar-coated copper grids (100 and 200 mesh). These were then stained with 5% aqueous uranyl acetate followed by lead citrate. Sections were examined with a JEOL 1200EX electron microscope at 100kV.

CHAPTER FOUR

ECOLOGICAL AND LIFE-CYCLE STUDIES OF *RHIPIDOCOTYLE CAMPANULA* IN THE RIVER AIRE

4.1 Introduction

Rhipidocotyle campanula was described for the first time by Dujardin, 1845 as *Distoma campanula* from the intestine of *Esox lucius* taken at Rennes, France and the metacercaria corresponding to this was experimentally obtained by Ziegler 1883, who conditionally proposed for it the name *Gasterostomum illense*. Later Baturo (1977) reported the connections between the larval stages and the adult for the first time from the specimen obtained in Goslawickie and Slesinskie lakes, Poland. These worms have been known for nearly one hundred and fifty years, but initially the species was confused with the second freshwater bucephalid from Europe - *Bucephalus polymorphus*, the descriptions were deficient and the taxonomic allocations were erroneous.

Studies by Johnstone (1904), Lebour (1907), Nicoll (1914), Crofton and Fraser (1955), Shotter (1972), Matthews (1972, 1973, 1974) and Johnstone and Halton (1981) provide an extensive record of gasterostome parasites of marine fish in the British coastal waters, but the studies on the freshwater bucephalids are very limited in British waters. The adult worms in both pike (*Esox lucius*) and perch (*Perca fluviatilis*) and the metacercariae in minnows (*Phoxinus phoxinus*), roach (*Rutilus rutilus*) and dace (*Leucius leucius*) have been recorded by Chappell,1967 and Shillcock,1972.

Later Mellors and Owen (1980) reported the metacercariae in minnows and cercaria in duck mussels (*Anodonta anatina*) from the R. Aire, Keighley though Chappell (1967) was the first to recognise this species in British waters. Although this species is a fairly common parasite of freshwater fish, the life cycle is not known in detail and the known descriptions of the larval stages are rather poor. Therefore in order to bridge the existing gaps, detailed studies were made on the lifecycle and the ecology of the parasite.

There are several publications on the distribution of parasites of British freshwater fishes [Nicoll, 1924; Rawson, 1952; Aderounmu, 1966; Orr, 1967; Chubb, 1965, 1970, 1975; Chappell & Owen, 1969; Bibby, 1972; Kennedy, 1966, 1974 and Campbell, 1974] but as the information on the parasites of fishes in the River Aire is lacking, a brief survey of parasites in four species of fish was carried out to add to the existing knowledge of the distribution of freshwater fish parasites in the United Kingdom.

4.2 Results

4.2.1 Primary Intermediate Host

(a) Incidence and intensity of infection

Collection of species of mussels known to carry *R. campanula* were carried out from March 1985 to November 1986 to study the incidence and intensity of infection. The species of mussels harbouring the sporocysts was identified as *Anodonta anatina* and was found to be the only species in the area sampled.

The results showed that only about 8% of the mussels were infected and the incidence of infection increased with age. But none of the mussels less than 3 years old showed any sign of harbouring the infection, though they were present in large numbers. (Table 4.1). Greater percentage of infection was observed in the mussels caught in July and August compared with the rest of the months.

Data concerning the number of cercariae liberated from 15 infected mussels caught between March and August, 1985 are given in Table 4.2. There is a general tendency for peak liberation of more than 500 cercariae per day to be followed by lulls with few or no liberated cercariae. The duration of peaks and lulls in liberation and the number of days on which liberation occured, vary considerably in the individual mussels

depending on the state of infection. The mean number of cercariae liberated per hour / day from four individual mussels on a continuous period of more than 60 days are demonstrated by the graphs. (Fig.4.1). Some of the mussels continue to emit cercariae for more than hundred days but the numbers drop drastically to 5-10 / day and sometimes to none. Towards the end of the emission of cercariae there is a marked tendency for peaks in liberation to become less frequent. However, the net results suggest that liberation of cercariae is neither cyclic nor continuous but essentially an intermittent phenomenon. This is discussed further in Chapter 6.

The temperature of water and particularly its fluctuation affect greatly the emergence of cercariae. Each abrupt thermic change from lower to higher (11°C to 18°C) is immediately followed by a great activity of emission of cercariae and whilst transfer of mussels to cold water (temperature between 9°C to 11°C) completely stops the emission. It is difficult to adjudge any conclusion to the production of cercariae within the sporocysts but the effect of temperature on liberation of cercariae is clearly demonstrated.

(b) Brief description of sporocyst

The sporocyst is of the typical gasterostome form, being composed of a mass of dichotomously branched tubules which invade the digestive gland and, in heavy

infection, the gonad. As the sporocysts are highly branched and interwoven, the tracing of the course of any single tubule is difficult.

The bodies of infected mussels removed from the valves, exhibit a characteristic white colour in the region of the digestive glands. During heavy infection the sporocyst tubules protrude into the mantle cavity, but no traces of the tubules were found either in the gills or muscular foot at any stage of the infection (Fig.4.2).

In old and heavily infected *A. anatina*, the gonads are completely invaded and destroyed. In light or moderately infected mussels, the gonadial tissue remains active and simultaneously produces glochidia and cercaria (Fig.4.5 & Fig.4.6). The state of infection is determined by opening individual mussels after they have ceased to liberate cercariae.

The sporocyst tubules have at irregular intervals dilated portions, which serve as brood chambers for developing cercariae. More brood chambers with developing and mature cercariae are observed in mussels collected in summer than in winter, in which period most of the tubules are empty and of a uniform diameter. Generally the brood chambers range from 120µm to 500µm in diameter and the narrower regions separating the brood chambers range between 45µm to 100µm in length and occassionally exhibit a beaded appearance.

The morphology of the sporocysts and the

development of the cercariae from the germ - cell stage are described in Chapter 5.

(c) Release of cercariae

The cercariae are forcibly discharged in small groups, depending on the state of infection of the mussels from tens to hundreds, through the exhalent siphon. As they are not active swimmers, the force created by the respiratory current through the exhalent siphon assists in their dispersal. Once they are released from the mussel, they normally swim upwards. The cercarial swimming behaviour is mainly controlled by external stimuli, as the furcae are extremely sensitive to sudden movements of water and touch. In undisturbed circumstances the cercariae are found in a resting position on the bottom of the container, with the furcae contracted, encircling the body, almost meeting in front of the anterior extremities. (Fig.4.3). The movement and behaviour of the cercariae are discussed in Chapter 6.

There are postulations regarding the release of cercariae from the sporocyst but there are no previous records of a sporocyst birthpore or any other means of escape. Two possible means of escape were observed on the tubules extended from the brood chambers with mature cercariae. A terminal pore and a broken sporocyst tubule adjacent to the brood chambers were noted and it is certain that they are the passageways through which the

mature cercariae escape from the tubules. (Fig.4.7 & 4.8) As postulated by Matthews (1973) at the dilated region of the sporocyst the wall is under considerable tension due to the pressure of the fluid in the lumen and rupture would most likely to occur in those positions of the sporocyst and thus becomes the passage for the escape of cercariae.

Table 4.1

Incidence of *Rhipidocotyle campanula* sporocysts in River Aire in 1985 and 1986

Host species *A.anatina*	Total examined	Age group				Incidence of infection				
		<+2.	+3.	+4.	>+5	<+2.	+3.	+4.	>+5.	Total
						No (%)	No (%)	No (%)	No (%)	No (%)
1985	226	54	61	49	62	---	---	5 10.2%	15 24.2%	20 8.84%
1986	207	30	48	70	59	---	---	6 8.6%	10 16.9%	16 7.72%
TOTAL	433	84	109	119	121	---	---	11 9.2%	25 20.7%	36 8.3%

Table 4.2

Cercarial liberation from infected mussels

(Samples from March to August 1985)

Duck mussels	Samples	Age	No of days of cercarial liberation	Total No liberated	Max. No of cer./ 24h	State of infection
1	March	+ 5	107	12,623	628	Moderately heavy
2	March	+ 4	30	5,288	312	Relatively light
3	April	+ 5	66	9,450	790	Relatively light
4	April	+ 5	61	6,100	384	Relatively light
5	May	+ 5	68	26,140	1,680	Heavy
6	May	+ 6	96	15,680	593	Moderately heavy.
7	June	+ 4	72	10,548	523	Moderately heavy
8	June.	+ 4	79	16,580	820	Moderately heavy
9	June	+ 6	115	22,280	1,240	Heavy
10	July	+ 5	58	12,750	998	Moderately heavy
11	July	+ 5	56	9,174	820	Relatively heavy
12	July	+ 4	67	6,500	421	Relatively heavy
13	August	+ 5	88	12,580	1,080	Moderately heavy
14	August	+ 5	58	28,586	1,598	Heavy
15	August	+ 4	32	4,800	284	Relatively light

* State of infection is determined by opening individual mussels after completing the above observations.

Fig 4.1

LIBERATION OF CERCARIAE FROM INFECTED MUSSELS

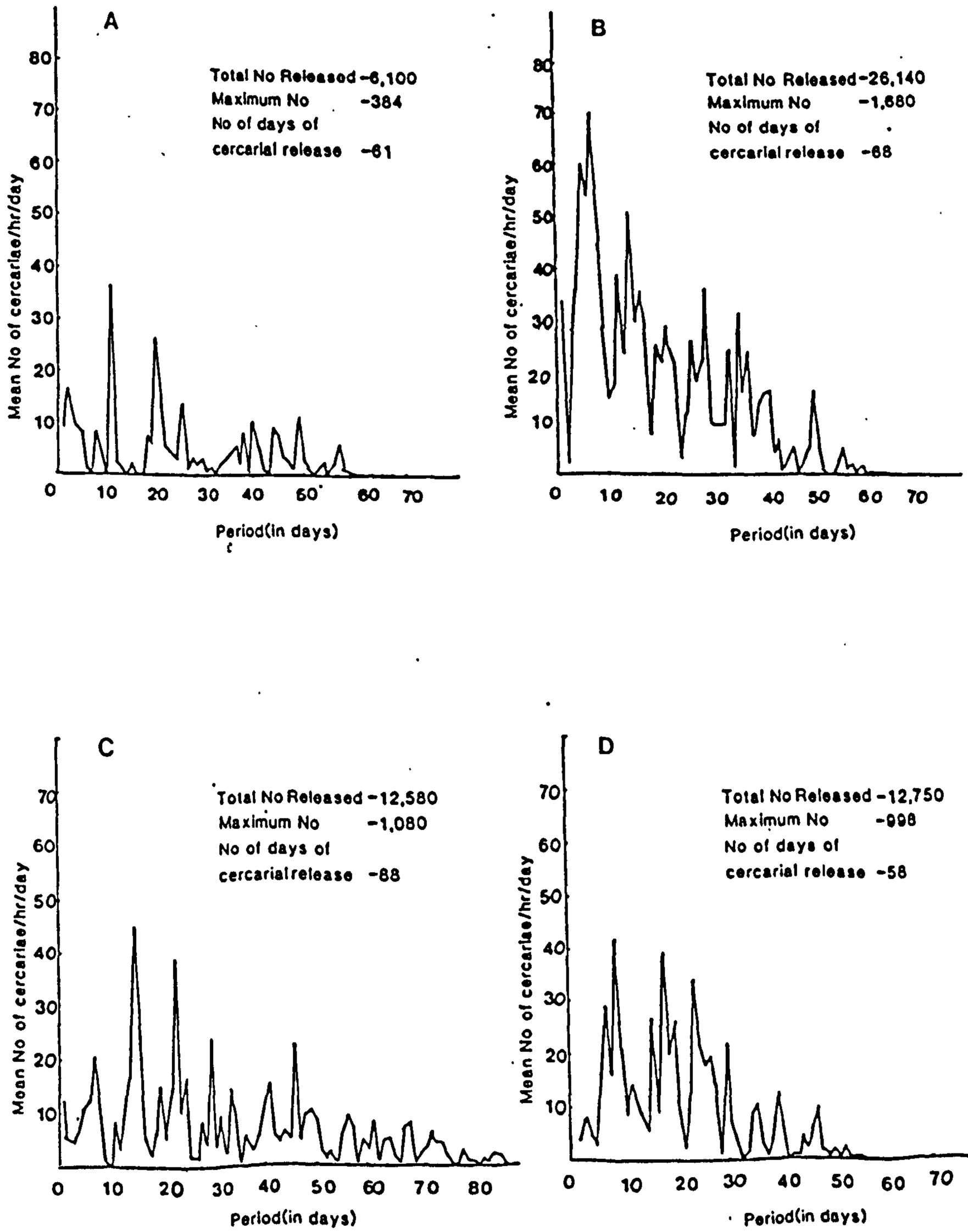


Fig 4.2 Heavily infected mussel with sporocysts (sps) of *R. campanula* in the region of the digestive gland, protruding into the mantle cavity (m.c)

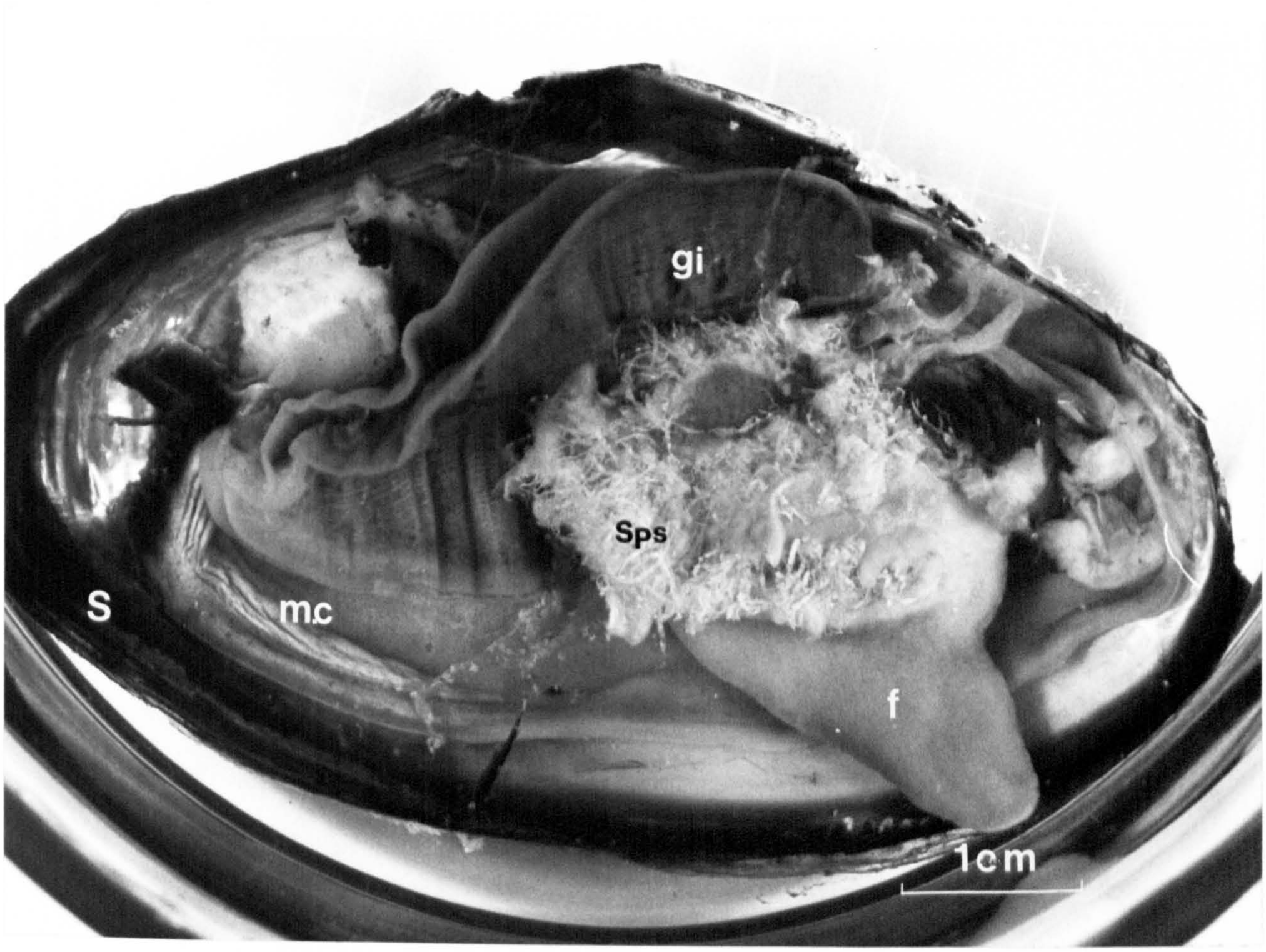
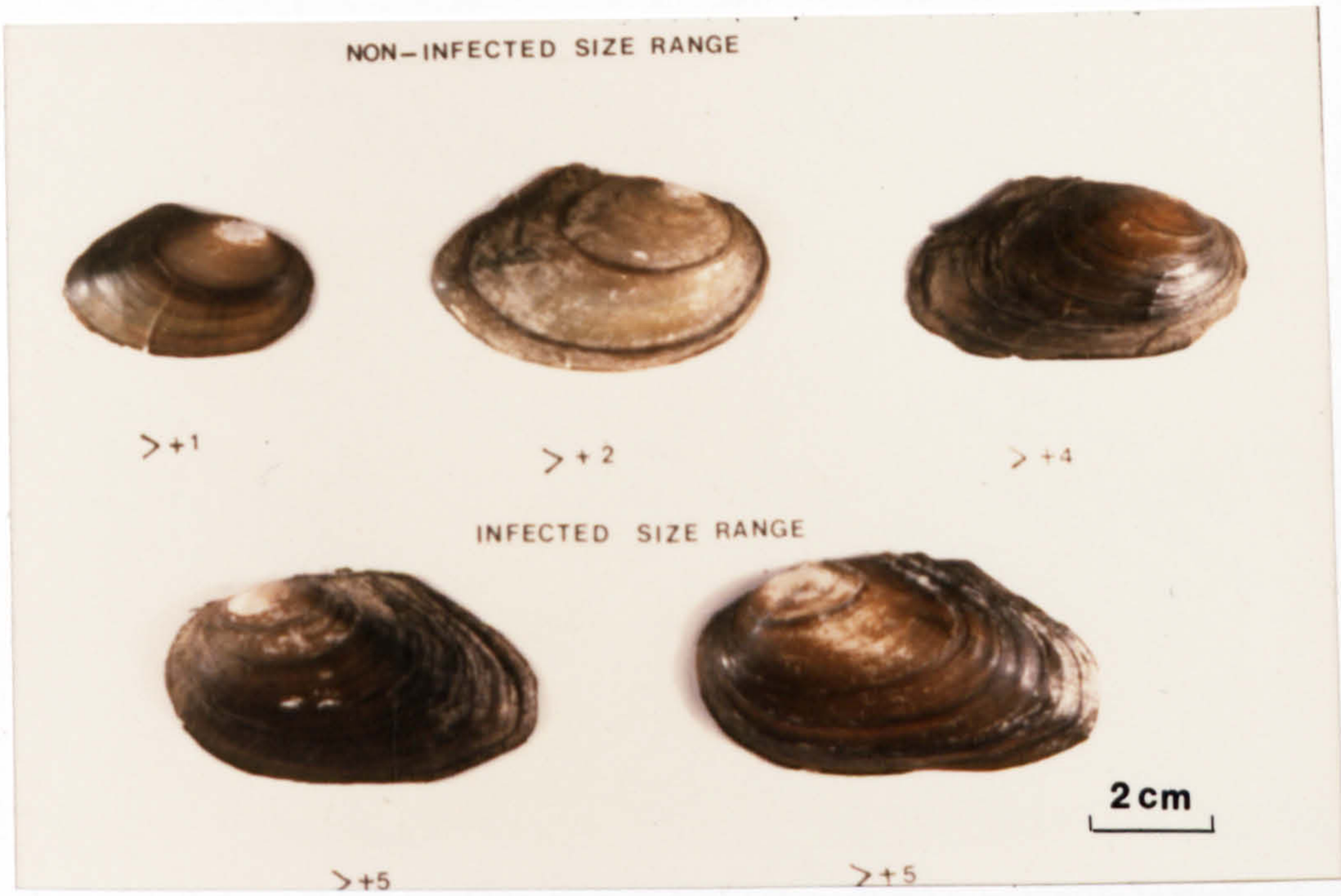

gi
Sps
S
mc
f
1cm

Fig 4.3 Photograph showing the infected and non-infected size range of mussels with sporocysts of *R. campanula*.

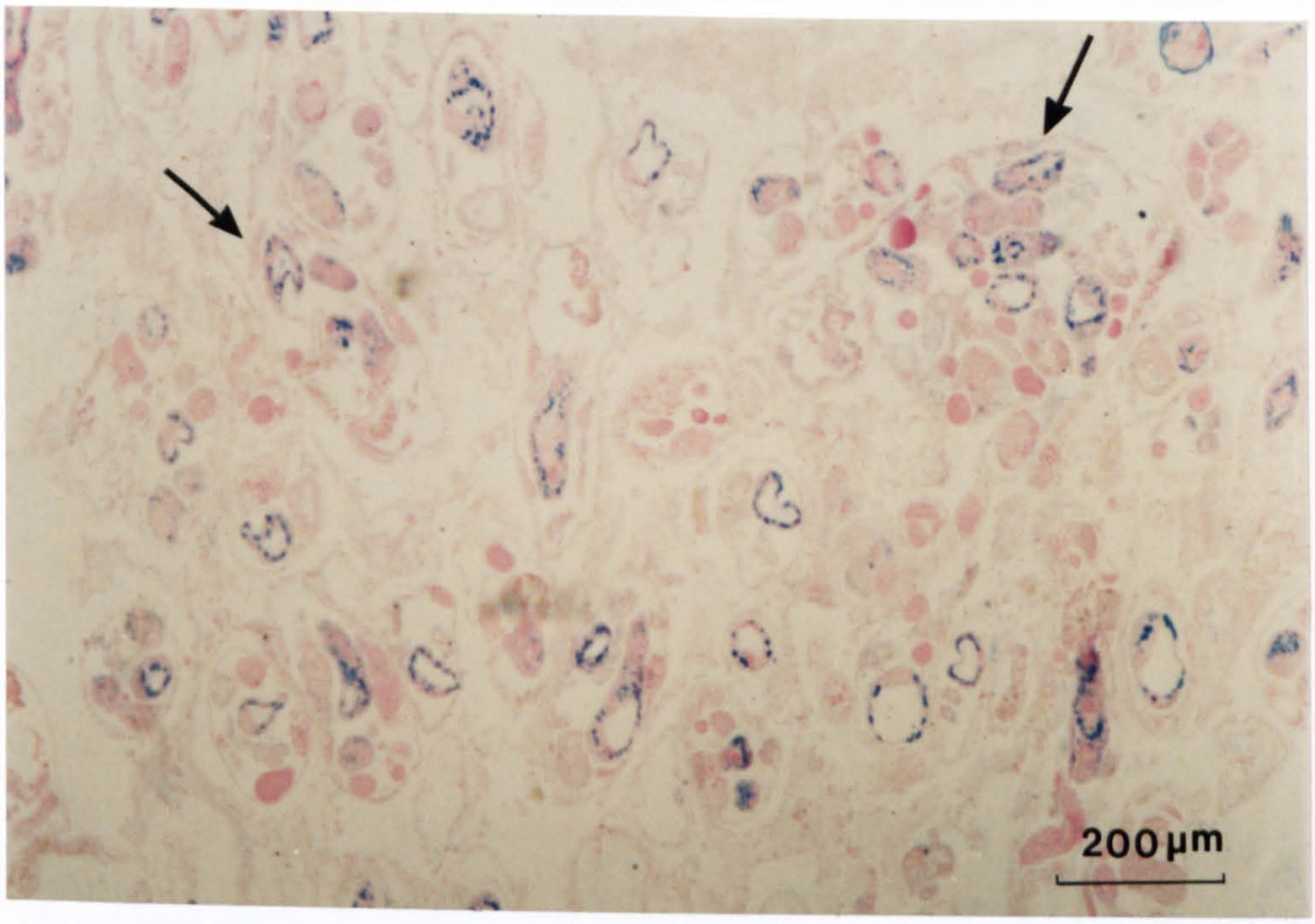
Fig 4.4 Photograph of freshly released unstained cercariae showing the resting cercaria with the furcae folded over the body (r.c), and swimming cercaria with both extended tail (c_1) and contracted tail (c_2).


NON-INFECTED SIZE RANGE
>+1
>+2
>+4
INFECTED SIZE RANGE
2 cm
>+5
>+5


rc
c1
c2
300 µm

Fig 4.5 Cross-section of the digestive gland of heavily infected mussel, *A. anatina*, showing digestive tissues completely destroyed with the invading sporocyst tubules (arrowed). Stained with Alcian blue and neutral red.

Fig 4.6 Cross-section of the digestive gland of a mildly infected mussel, *A. anatina* showing the digestive tissues partly invaded by sporocyst tubules (arrowed). Stained with Alcian blue and Neutral red.


200μm

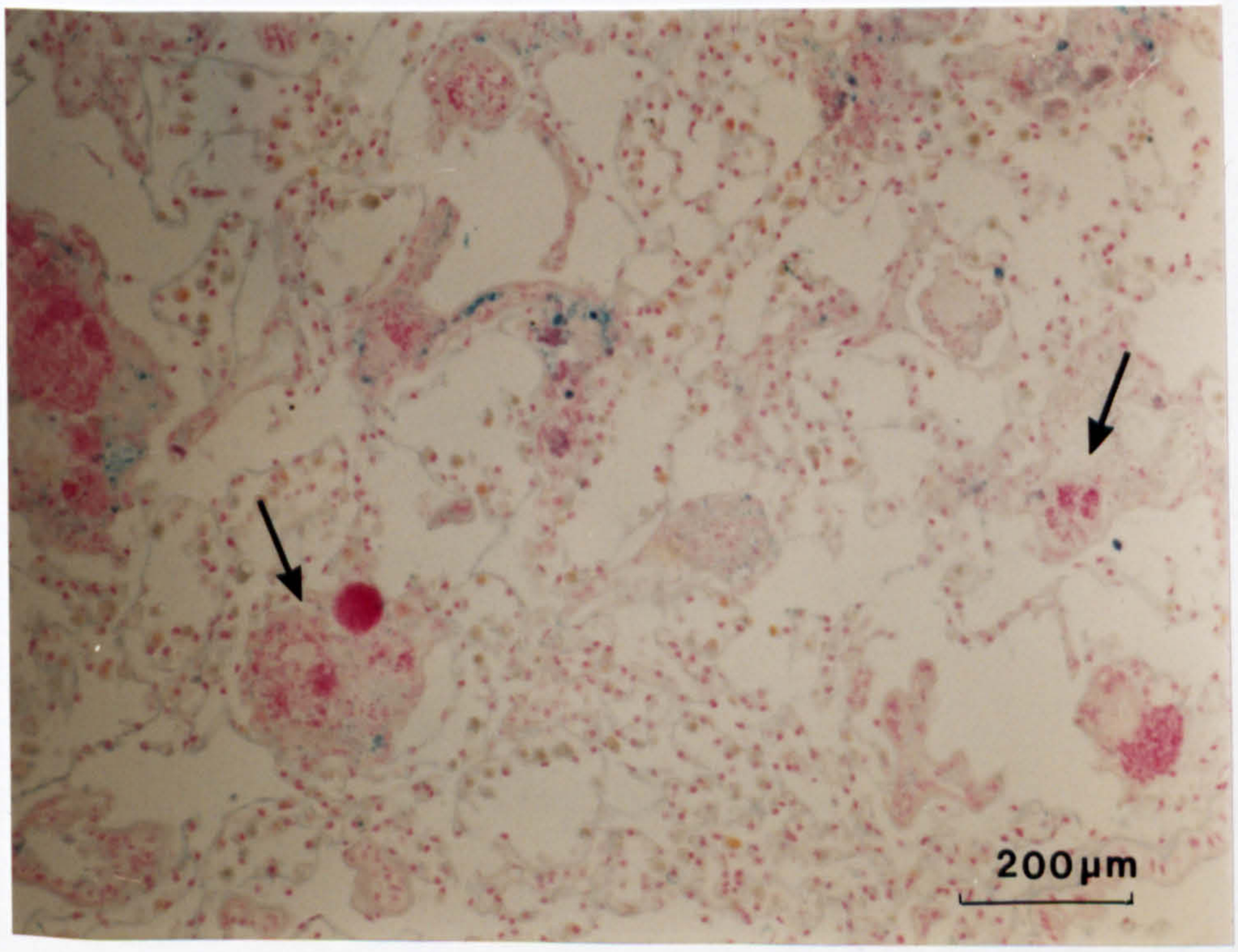
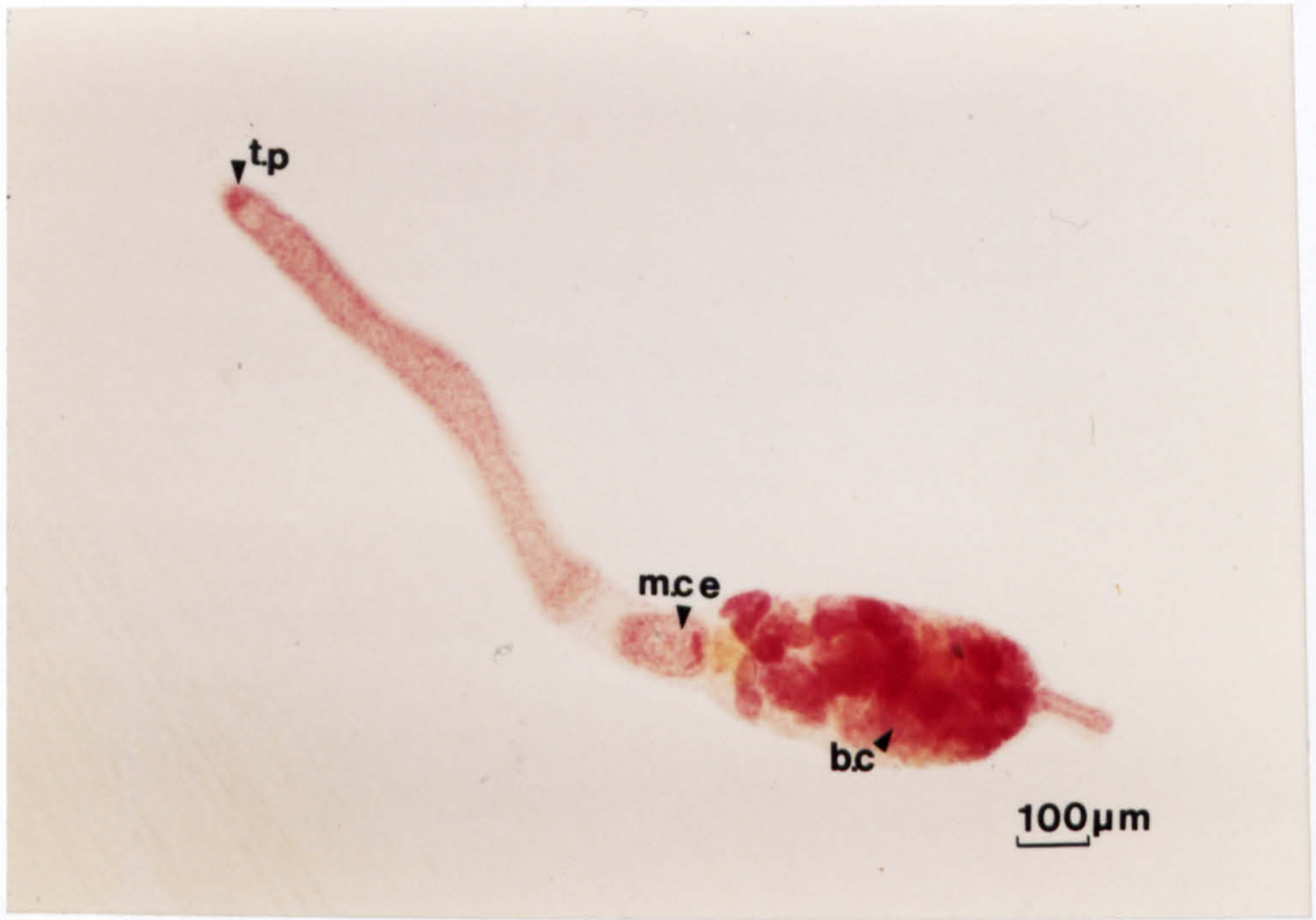
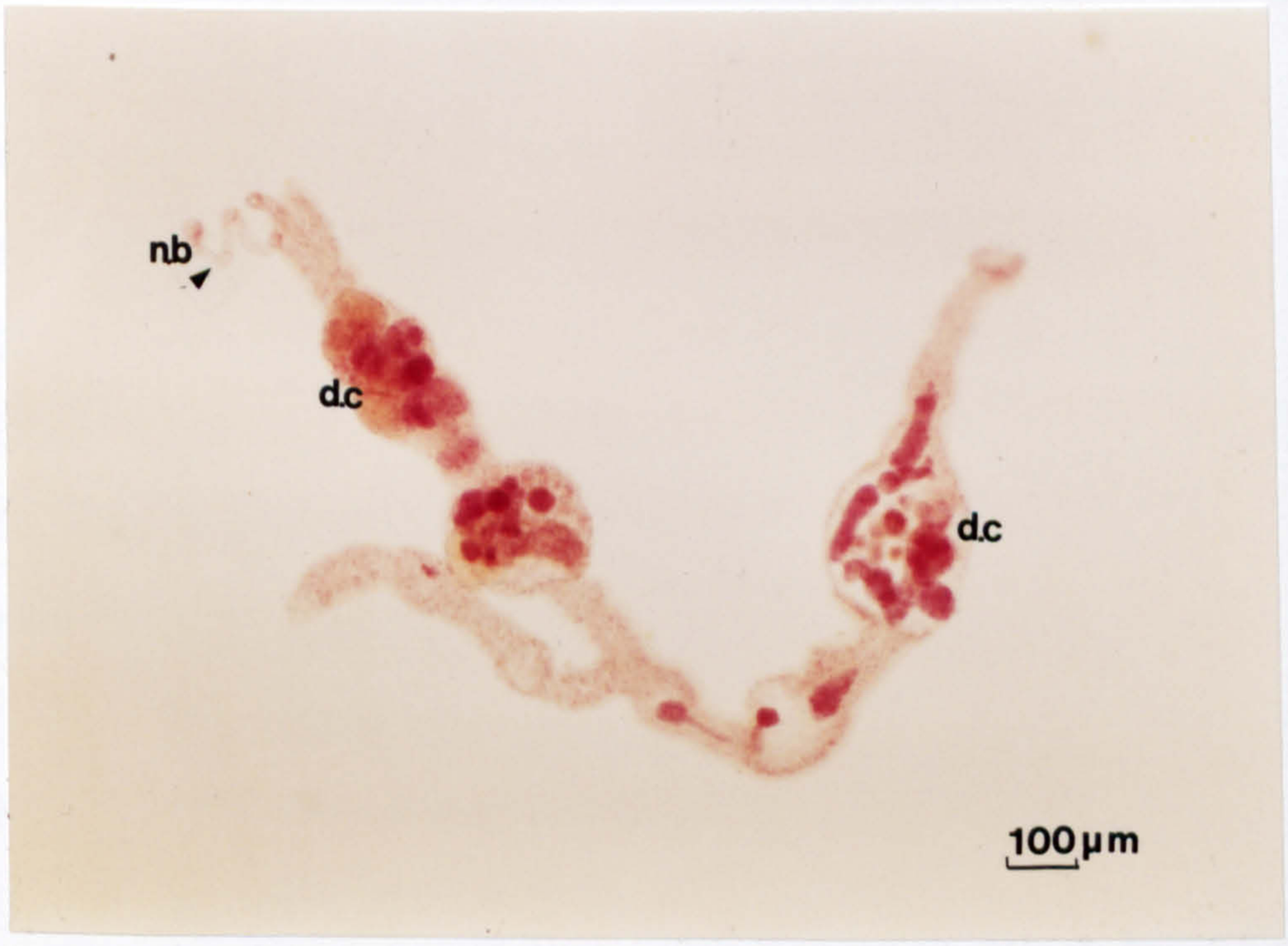

200μm

Fig 4.7 Terminal branch of sporocyst with the brood chamber (b.c) showing the matured cercaria (m.ce) moving towards the terminal pore (t.p). Stained with Mayer's carmalum.

Fig 4.8 Sporocyst tubules with developing cercariae (d.c) inside brood chambers showing formation of new branches (n.b) from the broken end of the tubule.
Stained with Mayer's carmalum.


t.p
m.c e
b.c
100µm


n.b
d.c
d.c
100µm

4.2.2 Secondary Intermediate Host

(a) Occurence and distribution

The only natural intermediate hosts to harbour the metacercarial cysts of the above parasite in River Aire, Keighley are minnows (*Phoxinus phoxinus*), even though there are records of twelve other species of fish which harbour the parasite in Europe. (Table 4.3).

The samples of minnows were collected from March 1985 to November 1986 to study the distribution of the metacercarial cysts. Almost all the fish above 2cm in length were infected with the cysts. The cysts are found in the subcutaneous fatty tissue beneath the lining of the pharynx and in some in the gill-arches, only rarely are newly encysted metacercariae found embedded in the snout muscles, and inside the operculum. (Fig.4.9 & 4.10). Normally a single metacercaria is found enclosed in a cyst except in few cases two metacercariae are found enclosed in a single cyst. (Fig. 4.9). The numbers of cysts / fish ranged generally from 1-100 with an occa$sional increase to more than a hundred cysts / fish.

The monthly prevalence of mean number of infection and the mean intensity of infection with the age are shown on Fig.4.11. Although there is a steady increase in the mean number of cysts with the age, there is a tendency for unequal distribution of cysts within each length groups and is prominent in higher length

groups. The distribution of cysts among 4-5 cm length ranges between 3-39 and in 5-6 cm group between 3-110. (Fig. 4.12). There is no clearly defined trend for the monthly variation of incidence but the numbers of fish carrying more than 30 cysts are greater during May, June, July and August. Also in these months, the newly acquired light infections of the younger fish are clearly distinguishable from those in the older fish.

(b) Experimental infection and host selection of cercariae

During the study of infecting secondary intermediate hosts described in the previous Chapter, the cercariae encysted in all the minnows and in two of the fifteen trout fry used in the experiment. None of the sticklebacks, stoneloaches and bullheads were infected. Since the encystment is internal, dissection of the specimen is essential to study the infection and the non-availability of enough uninfected minnows prevented the detailed study of the metacercarial development.

The entry into the secondary host is passive. The cercariae enter the fish through food and respiratory currents. None of the cercariae were seen to enter actively through the surface either on dead or living fish, and were only rarely observed to settle on the surface of the fish. As soon as the cercariae enter through the mouth of the minnows with the food current, detatched tails and on a few occasions cercarial bodies

were spat out but this peculiar behaviour was not observed in other species of fish. As the cercariae are not very active swimmers, the agitation of the water caused by the swimming of the fishes, enabled the cercariae to swim actively and increased the chances of entry. Normally in an undisturbed environment they are found, settled on the bottom of the container with their furcae folded over their body. (Fig. 4.4)

Free - swimming cercariae remain active for 15 - 20 h at 15°C but 50% of the cercariae remain alive for 30-35 h in the same temperature without being very active. Almost all the cercariae died after 50 h of release except a very few occasional survivors.

(c) Development of metacercaria

Development of metacercariae in the minnows was observed only briefly, due to the non-availability of uninfected minnows. Ten of the experimentally infected minnows were fed to the final host to confirm the identity of the parasite. The other six minnows were used for the study of the development of the metacercariae.

Only 15-20 % of the cercariae entered the minnows within 4h of introduction, and were found tailless with only 3-5 cysts enclosed in transparent thin wall cysts. The encysted cercariae were noted in the subcutaneous fatty tissue beneath the lining of the pharynx and in the gill arches. In two of the five

fishes, 3-4 non encysted cercariae were found just beneath the skin on the base of the tail, in the lower jaw region and operculum. Except for the loss of the tail and tail stem, no size increase was noted, though in the encysted cercariae and few non encysted ones, enlargement of the intestine was observed (Fig. 4.13).

After 3 days, 20-25 % of the entered cercariae were found alive and active enclosed in a thin, membranous, flexible cyst. None of the cercariae found along the caudal region and snout survived. Another 15-20 % of the cercariae though encysted, were found to be dead. Among the encysted cercariae few were found alive but not active. By now the cysts measured approximately 260-290 µm by 198-240 µm and the released metacercariae of 280 -312 µm in length. At this stage the metacercaria fills the cyst and is densely granular except for the distinct, translucent, fluid filled intestine, which occupies the central region of the body. The cystogenous glands near the anterior organ are prominent with their ducts, compared to the cercariae.

Within three weeks, remarkable changes were noticed in the development of the metacercariae. The rhynchus which develops from the anterior organ appears conical in shape with the cone being prominent. The excretory vesicle is more extensive than in the cercaria, reaching to the anterior level of the intestine. Both the excretory vesicle and the intestine are almost completely filled with granules which give them an opaque

appearance. The intestine gradually diminishes in diameter. The cyst increased approximately to 278-320 µm long by 228-266 µm in width with an excysted metacercaria of 412-464 µm long and 121-168 µm wide (Fig 4.14).

Between five to six weeks the metacercariae attained the full size with well developed anterior sucker and spination. The reproductive system which has formed is not functional. Few vitelline cells have formed but are not as fully developed as in the adult (Fig. 4.15). At this stage the cysts excyst spontaneously in tap water, Tyrode solution and saline solution (0.65%), but not the cysts of 3 weeks old. No differences in growth were observed in the 8 weeks old metacercariae. Comparative measurements of the metacercarial cysts and the released metacercariae are given in Table 4.4.

Matured metacercarial cysts survived approximately for 200 days though the spontaneous excystation occured in those cysts of 80-90 days old. As the results were obtained from minnows caught in the wild, the samples are of unknown age, the results are an approximate guide line for the survival of the cysts. (Table 4.5). Excystation occured spontaneously in saline 0.65% and Tyrode at room temperature 15°C - 17°C within 6-10 h and in tap water at a slightly longer time, 12-14 h. As Kniskern (1952b) stated, the ability to excyst coincides closely with the age at which metacercariae are infective and may be used as an index of infectivity.

Table 4.3

Host Records of *Rhipidocotyle campanula*

Place	Primary Host	Secondary Host	Final Host
POLAND by Kozika (1959) & Baturo (1977)	*Unio pictorum*	*Abramis brama* (L) (Bronze bream) *Rutilus rutilus*(L) (Roach) *Blicca bjoerena* (L) (Silver bream) *Scardinius-erythrophthalmus*(L) (Rudd) *Carassius carassius*(L) (Crucian carp) *Gobio gobio*(L) (Gudgeon) *Alburnus alburnus*(L) (Bleak) *Abramis ballerus* **Esox lucius*(L) (Pike) **Perca fluviatilis*(L) (Prech) **Lucioperca lucioperca*(L) (Pike perch) **Acerina cernua*(L) (Ruffe)	*Esox lucius*(L) (Pike) *Perca fluviatilis* (L) (Perch) *Acerina cernua* (L) (Ruffe)
SOVIET UNION by Ivantsiv & Chernego-renko (1984)	*Anodonta-piscinalis* *A.ponderosa* *A.subcircu-laris* *Unio pictorum* *U.longirostris*	*Abramis brama*(L) (Bronze bream) *Blicca bjornicka*(L) (Silver bream) *Leucaspius delineatus*(L) *Scardinius erythrophthalmus* (Rudd) *Carassius carassius*(L) (Crucian carp)	*Perca fluviatilis* (L) (Perch)
UNITED KINGDOM (R.Roding Essex) by Shillcock (1972)	-----	*Phoxinus phoxinus* (Minnow)	*Perca fluviatilis* (L) (Perch)
(R.Bain S.Linconshire) by Chappell (1967)	-----	*Rutilus rutilus* (Roach) *Leucius leucius* (Dace)	*Perca fluviatilis* (L) (Perch) *Esox lucius* (L) (Pike)
(R.Aire Keighley) by Mellors & Owen (1980)	*Anodonta anatina*	*Phoxinuxs phoxinus* (Minnow)	--------

Table 4.4

Comparative measurements of the metacercarial cysts (in µm)

Authors	Metacercarial cysts		Released metacercariae		Anterior organ		Pharynx
	Length	Breadth	Length	Width	Length	Width	Diameter
River Aire,U.K (Richardson,1986) (N=50; before fixation)	298-428	216-292	680-930	155-178	163-190	156-180	61-72
Lower Dnieper, U.S.S.R (Ivantsiv & Chernogorenko, 1984)	(378 - 575)		1185-1207	316-372	(104 - 128)		70
Slesinskie lake Poland (Baturo,1977) (N=60; before fixation)	333-440	207-384	33-?799	140-261	74-178	74-163	28-56
River Bain,U.K (Chappell,1967)	390-520	300-400	---	---	---	---	----
U.S.S.R (Bykhovskaya-Pavlovskaya, 1964)	270-370	270-370	---	---	---	---	----
Druzno lake, Poland (Kozika,1959)	270-510	270-370	---	---	---	---	----

Table 4.5

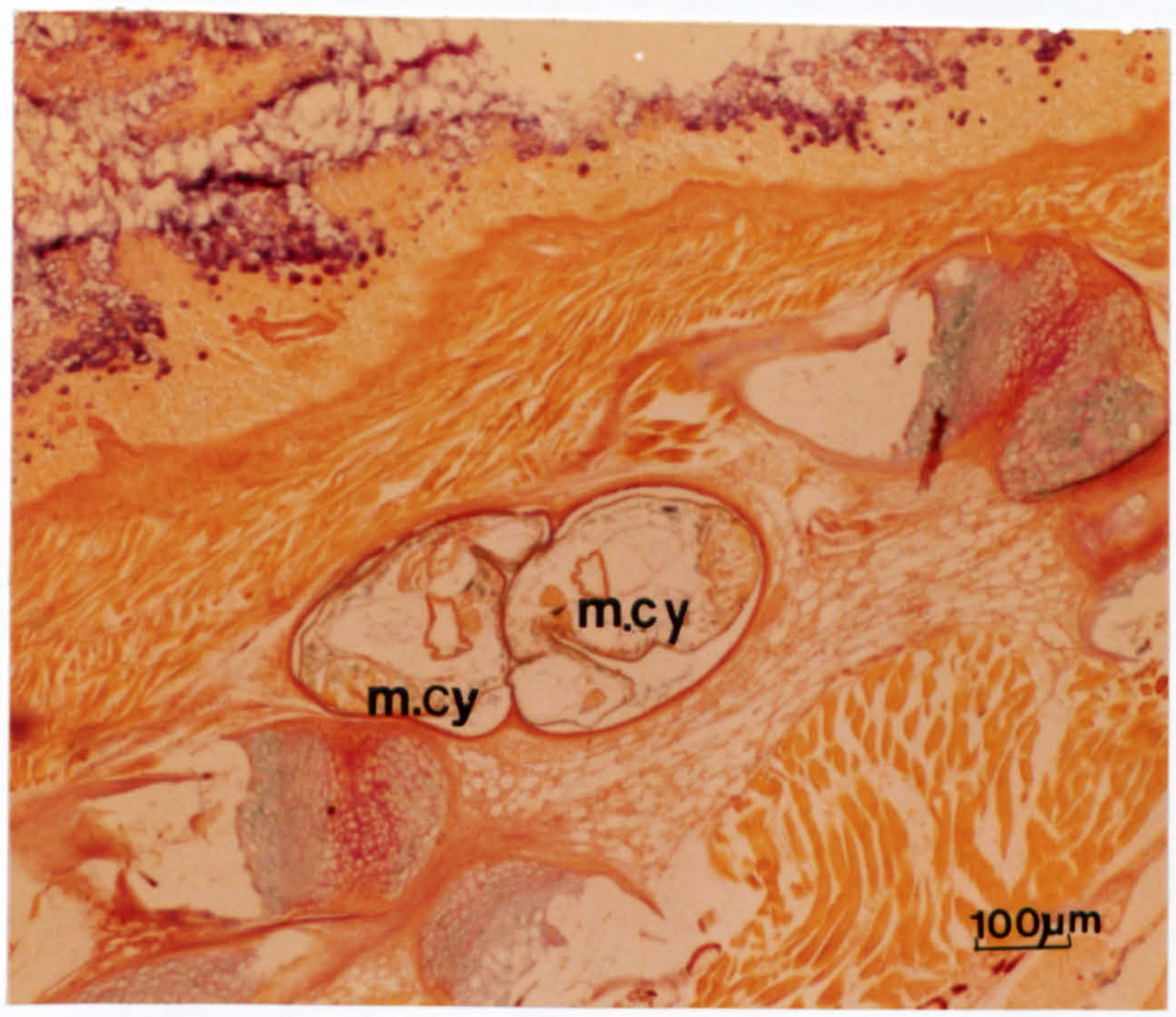
Survival of metacercarial cysts obtained from minnows caught in May 1985

(Temp. 15°C - 17°C)

Days after dissection	No of cysts alive	No of cysts dead	Remarks
19	46	-----	All alive & active; Spontaneous excystation.
20	17	-----	" " " "
31	15	-----	" " " "
39	43	-----	" " " "
54	11	-----	" " " "
82	13	-----	" " " "
82	17	-----	" " " "
82	11	-----	" " " "
84	11	-----	" " " "
119	24	-----	All alive but only 8 excysted spontaneously
149	22	-----	All alive but only 4 excysted spontaneously
180	16	3 dead	Two excysted spontaneously
201	27	10 dead	Two excysted spontaneously
261	08	2 dead	No spontaneous excystation
261	33	8 dead	No spontaneous excystation; metacercariae inactive.
261	09	All alive	No spontaneous excystation; metacercariae inactive.

Fig 4.9 A portion of the L.S through the head of minnow showing the embedded metacercarial cysts (m.cy) in the subcutaneous fatty tissue of the pharyngeal region.
Stained with Schiff's reagent, Alcian blue and Orange G.

Fig 4.10 Surface view of metacercarial cysts (m.cy) embedded in the subcutaneous fatty tissue.
Stained with Neutral red.


m.cy
m.cy
100µm

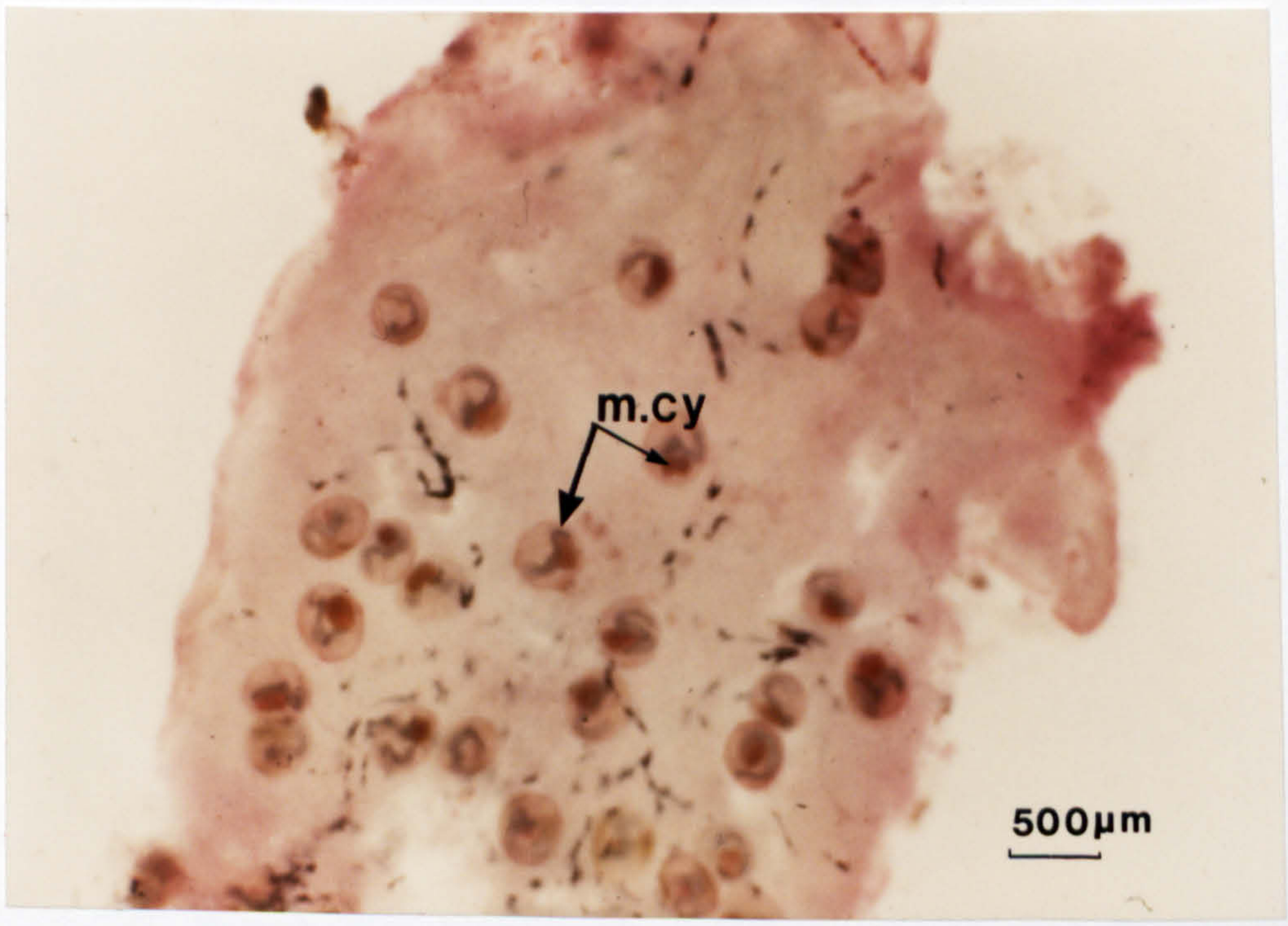
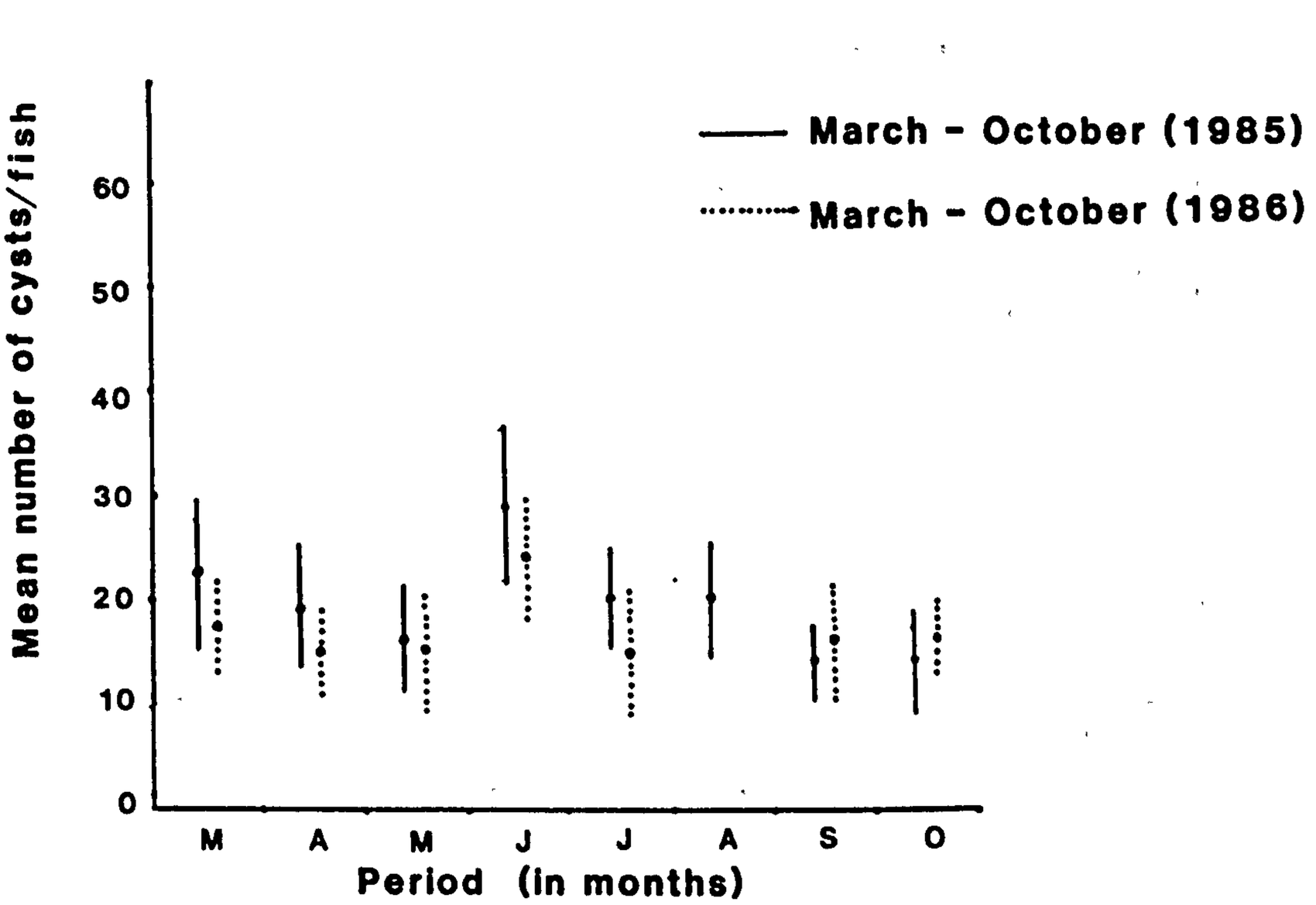
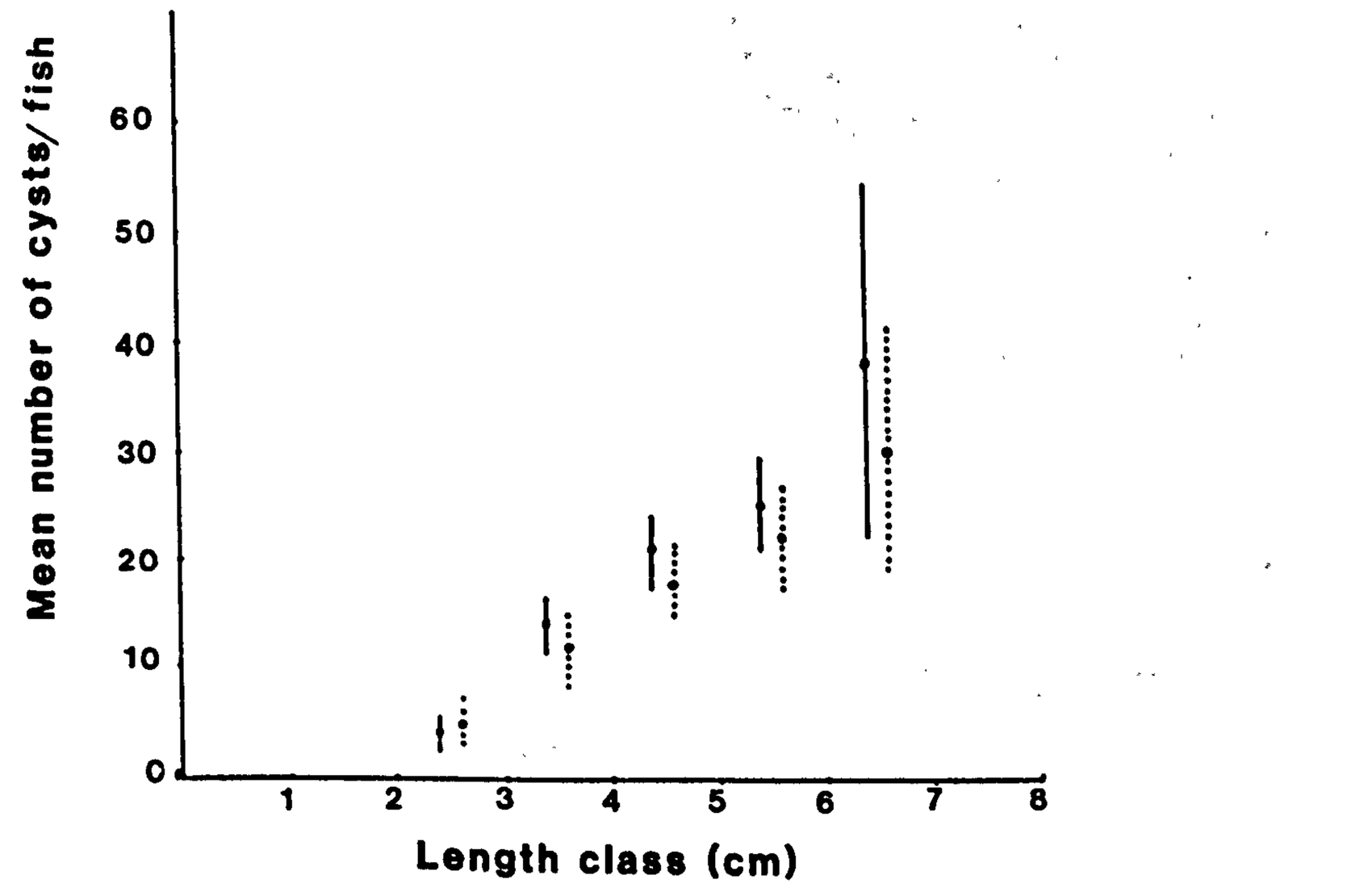

m.cy
500µm

Fig 4.11


MONTHLY PREVALENCE OF METACERCARIAL INFECTION IN MINNOWS
March - October (1985)
March - October (1986)
Mean number of cysts/fish
0
10
20
30
40
50
60
M
A
M
J
J
A
S
O
Period (in months)


METACERCARIAL INFECTION IN DIFFERENT SIZES OF MINNOWS
Mean number of cysts/ fish
0
10
20
30
40
50
60
1
2
3
4
5
6
7
8
Length class (cm)

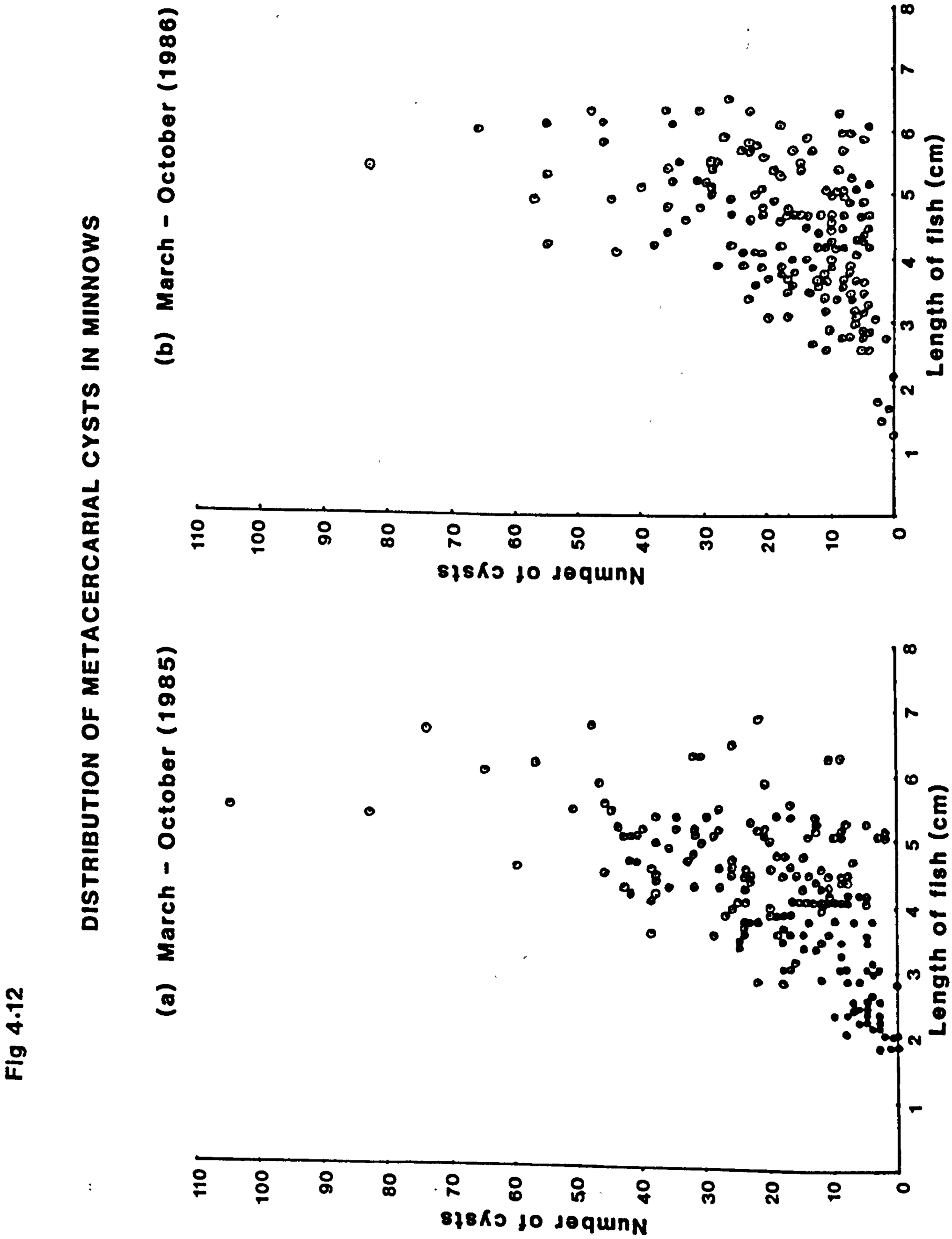

DISTRIBUTION OF METACERCARIAL CYSTS IN MINNOWS
(a) March – October (1985)
(b) March – October (1986)
Number of cysts
Length of fish (cm)
0
10
20
30
40
50
60
70
80
90
100
110
1
2
3
4
5
6
7
8

Fig 4.12

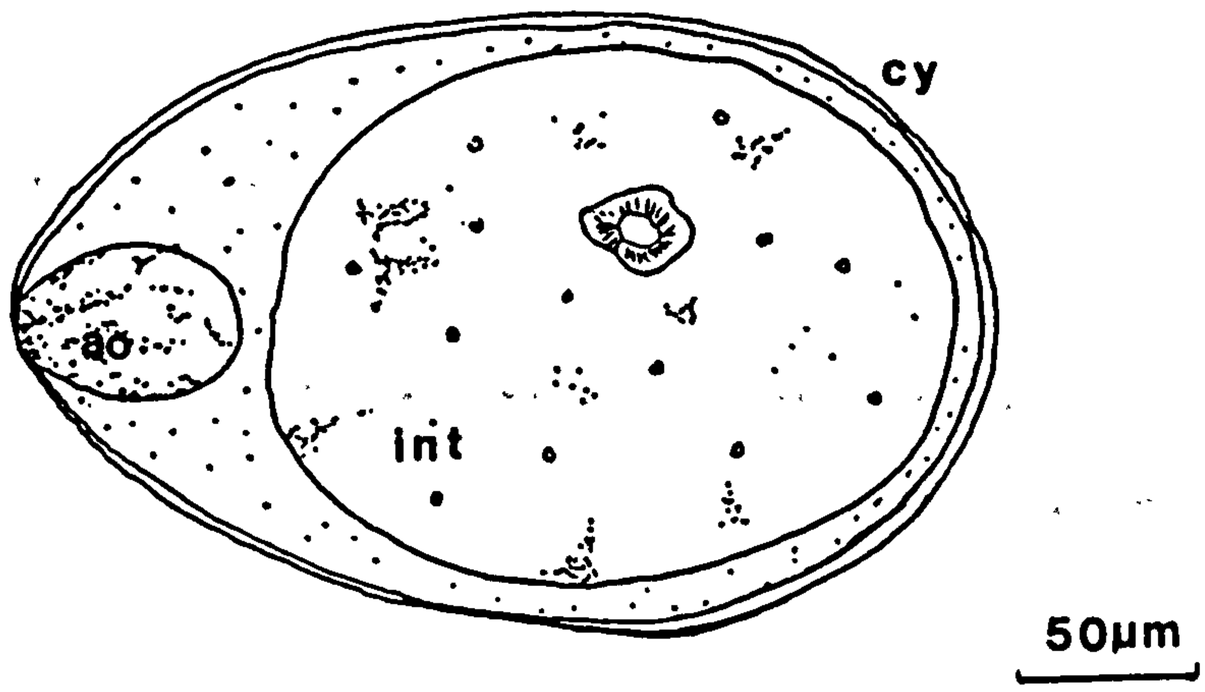


Fig 4.13. Semi-schematic drawing of the ventral view of 4h old metacercaria enclosed in a membraneous thin cyst(cy), showing the enlarged intestine (int) and the anterior organ (ao).

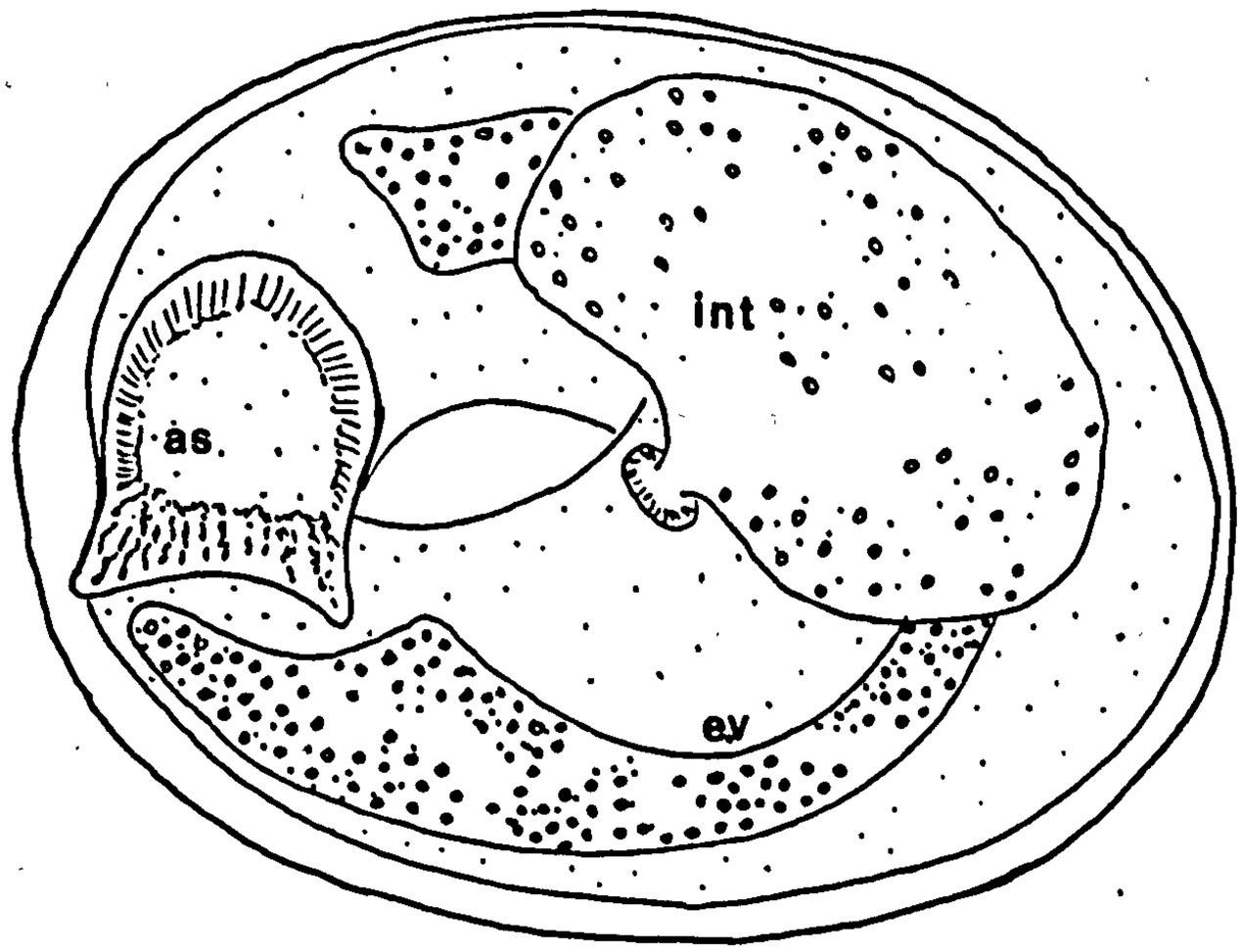


Fig 4.14. Semi-schematic drawing of the 3 weeks old metacercaria enclosed in a fully formed cyst showing the developed anterior sucker (as), excretory vesicle (ev) and intestine (int).

4.2.3. Final Host

(a) Suitability of host

The final hosts of the parasites were known for a long time to be carnivorous fish and have been reported in different parts of Europe as perch (*Perca fluviatilis*), pike (*Esox lucius*) and ruffe (*Acerina cernua*), though in Britain only twice reported as perch and pike by Chappell, 1967 and Shillcock, 1972. (Table 4.3). In the present study due to fishing regulations , unfortunately the natural hosts could not be studied in the environment for the parasite. From the experimental study, trout (*Salmo trutta*) a new host was identified as susceptible to infection, but only a very low percentage of fish (10%-15%) were infected compared to perch, in which all the fish used in the experiment were infected. Also the number of worms developed in perch ranged between 14-199 compared to trout (3-10). This reveals perch is a more suitable host for the parasite as the metacercariae established more abundantly and easily than in trout.

A comparative study of the morphological characters of the adults, obtained by feeding naturally infected and experimentally infected minnows, confirmed the identity of the parasite as *Rhipidocotyle campanula* (Duj., 1845).

(b) Development of the adult

Experimental adults of *R. campanula* were recovered from the intestine and rectum of the perch at intervals of one day to ten weeks after feeding (Table 4.6).

The flukes recovered from the stomach within 24h of entry were all excysted. A gradual migration of worms within the intestine was noted in the next 24h and in two weeks all tha adults recovered were from the middle 1/3rd of the intestine. None of the flukes attached firmly to the walls of the intestine but were found embedded in the mucus of the intestine. In six weeks, except for a few, others were found to be attached to the walls of the posterior 1/4 of the intestine and rectum. This downward movement of the flukes can be linked with maturity.

The effect of temperature on migration of worms was noted in the perch maintained between 9°C to 11°C and 14°C to 15°C. In higher temperature the downward movement of the flukes occured in lesser number of days and the flukes matured in shorter periods. This shows that temperature has an influence over development of adults within the final host and also the migration is influenced by the maturity of the flukes.

The growth rate of adults in terms of body length, width, ovary, testes and pharynx is shown in Table 4.7. A sudden increase in the growth of the flukes

was noted within the first week of development and then a gradual increase in the growth.

The adult represents the metacercariae in all morphological characters except the reproductive structures. (Fig. 4.15 & 4.16). Spermatogenesis and oogenesis were observed in the flukes of one week old but no spermatozoa were present in the seminal vesicle, cirrus pouch, uterus or seminal receptacle.

Following two weeks development spermatozoa were seen in the seminal receptacle of living flukes and in the proximal part of the uterus. The development of the eggs in the ovary were noted but no fully formed eggs were observed in the uterus. Vitelleria were fully developed. The chromosome number is noted as 16 with a similarity of *R. septpapillata* of 16 (Kniskern, 1952) but not of *Bucephalus elegans* of 12 (Woodhead, 1931a).

In four weeks old flukes few eggs were seen in the uterus but more were observed in six weeks old flukes.None of the eggs were hatched when placed in water, saline and Tyrode solution.

Table 4.6

Establishment of Adult parasites in Perch

(Temp. 9°C - 11°C)

Host	Std. length (cm)	No of worms	Development
1	15.8	20	24h; flukes in the stomach; all excysted
2	17.5	18	2 days; gradual migration of flukes downwards.
3	14.2	15	3 days; flukes in the anterior 1/3 rd of the intestine.
4	12.9	18	7 days; flukes in the anterior 1/3 rd of the intestine.
5	10.5	14	2 weeks) flukes in the middle 1/3 rd of the intestine.
6	21.0	165	2 weeks)
7	24.0	126	2 weeks
8	22.5	100	4 weeks) flukes in the posterior region of the middle 1/3 rd and posterior intestine.
9	20.0	199	5 weeks)
10	22.5	171	6 weeks) posterior 1/4 th of the intestine and rectum.
11	24.0	65	6 weeks)
12	23.5	117	10 weeks

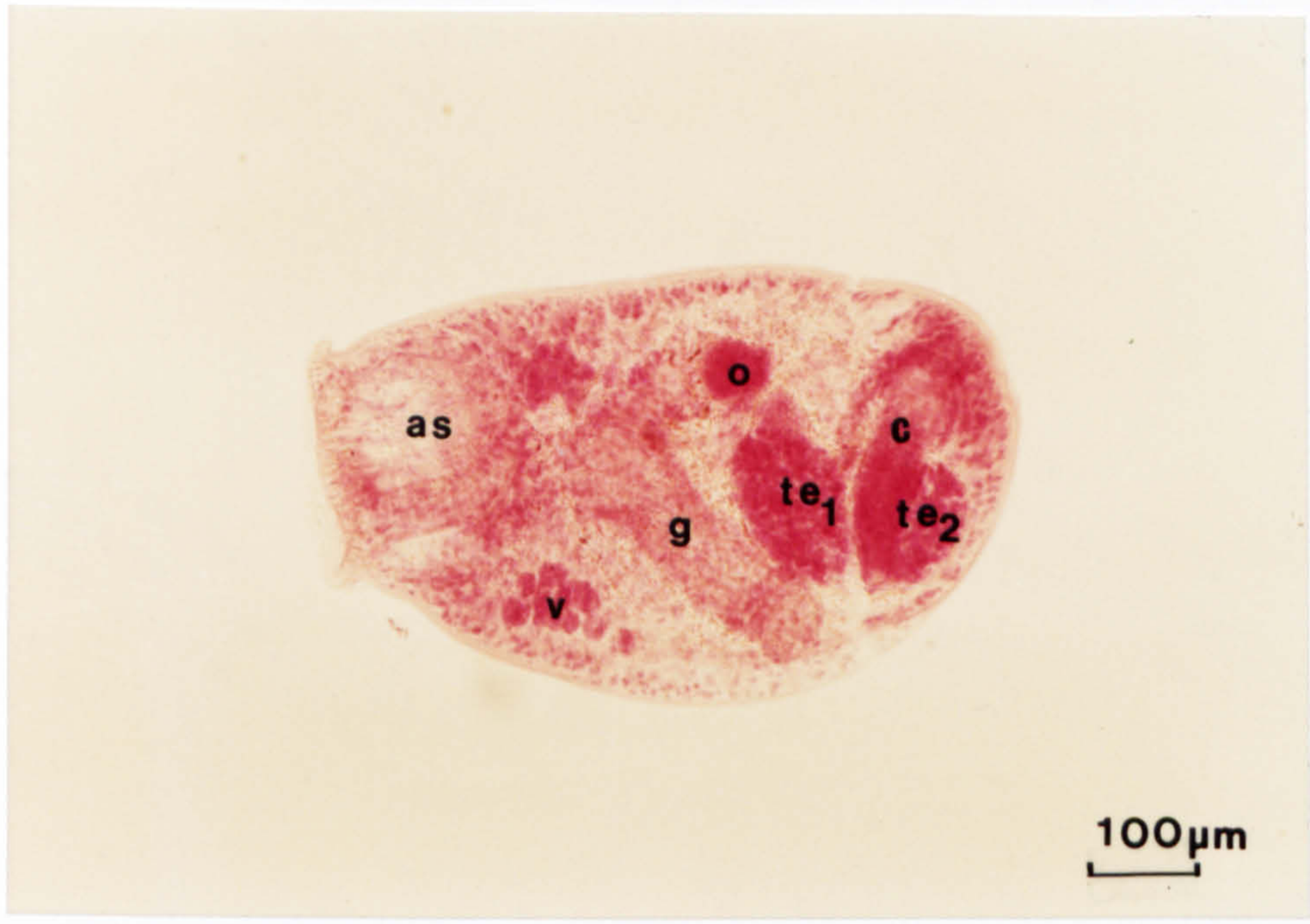
Table 4.7

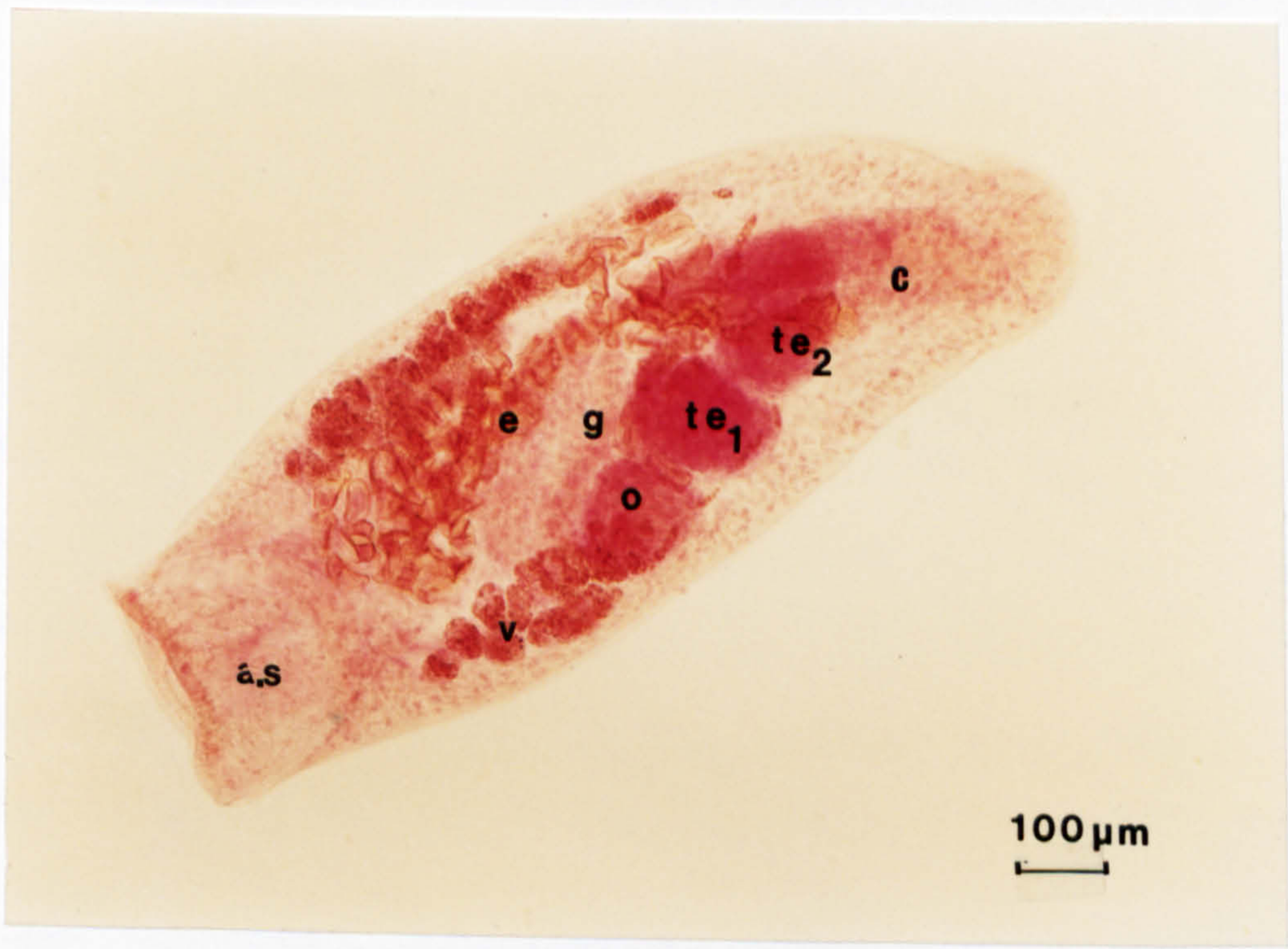
Age and growth of adult *R. campanula* in perch

Measurements (um)	1 day	3 days	1 week	2 weeks	4 weeks	6 weeks
Total length						
Max.	930.0	1100.8	1102.5	1228.2	1508.2	1575.0
Ave.	822.0	981.2	1006.4	1108.9	1291.6	1321.3
Min.	682.0	772.5	969.2	1008.6	1110.2	1104.0
Width						
Max.	178.0	192.0	210.0	272.8	293.5	298.0
Ave.	171.6	184.5	191.1	222.6	241.3	259.5
Min.	155.0	161.8	165.0	181.0	192.6	195.8
Ant. sucker length						
Max.	152.0	187.6	202.5	253.4	263.5	263.3
Ave.	144.8	173.4	182.6	218.2	230.4	226.7
Min.	106.0	139.2	163.3	189.2	204.9	206.6
Width						
Max.	138.0	172.4	190.0	241.2	254.3	256.6
Ave.	121.5	161.9	172.5	198.6	221.9	222.0
Min.	98.0	126.5	156.7	179.5	191.5	186.6
Diam. of pharynx						
Max.	58.2	68.7	72.5	84.6	87.6	90.0
Ave.	54.7	62.5	68.6	71.4	72.6	73.5
Min.	51.6	59.2	61.7	64.5	66.7	66.7
Testis 1 (Diam)						
Max.	64.9	87.8	111.7	143.5	179.6	188.3
Ave.	62.4	82.4	104.4	125.9	141.6	153.2
Min.	61.2	76.4	98.3	119.2	126.5	136.7
Testis 2 (Diam)						
Max.	62.5	87.2	116.7	139.2	179.2	181.7
Ave.	61.8	81.5	96.5	121.6	137.6	141.2
Min.	59.6	74.9	88.3	108.9	121.6	126.7
Ovary (Diam)						
Max.	52.8	79.3	93.3	142.1	160.4	165.0
Ave.	50.9	76.9	82.9	112.6	124.9	132.5
Min.	49.2	61.5	71.7	86.5	97.9	106.7
Intestine length						
Max.	162.3	181.3	191.7	241.6	279.2	280.8
Ave.	159.2	178.9	180.0	196.5	206.9	207.6
Min.	146.2	168.5	173.3	176.5	179.2	178.0

Fig 4.15 Fully developed excysted metacercaria of *R. campanula* showing the developed testes (te_1 & te_2), ovary (o) and partially developed vitelline glands (v).
Stained with Mayer's carmalum.

Fig 4.16 Fully matured adult of *R. campanula* with eggs (e) in the uterus.
Stained with Mayer's carmalum.


o
as
c
te1
te2
g
v
100µm


c
te2
e
g
te1
o
v
á,S
100µm

4.2.4 Records of other parasites in four species of fish in River Aire

The list of the parasites observed in minnows (*Phoxinus phoxinus*), Bullheads (*Cottus gobio*), stone loach (*Noemachilus barbatulus*) and sticklebacks (*Gasterosteous aculeatus*) are given in Table 4.8. The survey was carried out for only three months - April, May and June 1985 and the numbers of fish examined for the parasites were between 15-20 on each of the four species.

All the parasites observed in minnows and sticklebacks have been recorded previously in British waters except *Triaenophorus nodulosus* in minnows, which is the first record to the writer's knowledge. This was found encysted as plerocercoid stage, in only one minnow caught in April.

Previous parasitic records on stoneloaches and bullheads are vary scarce, and the present study noted four new parasites in stoneloaches and four new parasites in bullheads which are not recorded in Britain. Unidentified encysted metacercarial cysts, in almost all the bullheads and stoneloaches above 3cm were found in body cavity, braincase, muscles in the head region and gonads. The cysts are egg shaped with a translucent and fibrous cyst capsule. Unfortunately the feeding experiments with chicks and mice were not successful and hence the parasite could not be identified. However, there is a possibility that it could be *Apatemon*

gracilis (Rudolphi,1819) Szidat,1928 as this cyst resembles of Blair's (1976) report of an identical parasite in stoneloaches in Scotland.

Another non-encysted metacercariae of *Diplostomum* sp. in the body cavity of bullheads and stoneloaches was observed. The number was very high in bullheads, with the highest number of 116 metacercariae recorded in April, but the numbers were low in stoneloaches, infecting only 20% of the fish examined. This *Diplostomum* sp. is the first record in stoneloaches and bullheads in Britain. Also *Phyllodistomum folium* and *Spherostoma bramae* in stoneloaches and *Rhaphidascaris* sp. and *Diplostomum spathaceum* in bullheads which were observed in the present study are not recorded previously.

Diplostomum phoxini and *Rhipidocotyle campanula* which are recorded previously in minnows in River Aire, are very common and almost all the fish above 2cm are infected with these two parasites.

Table 4.8

List of Parasites of Fishes in River Aire

Name of parasites	State and site of infection	Incidence of infection			
		Minnows	Bullheads	Stoneloach	Three spined sticklebacks
PROTOZA					
Trichodina sp.	Fins & skin	Yes	No	No	Yes
Ichthyop--thirius sp.	Fins & skin	Yes	No	No	Yes
Myxobolus sp.	Intestine wall	Yes	No	No	No
MONOGENEA					
Gyrodac-tylus sp.	Fins, gills & skin	Yes	No	No	Yes
DIGENEA					
Diplostomum phoxini	Unencysted metacercariae in the brain & cranial cavity	100-2000	No	No	No
Diplostomum spathaceum	Unencysted metacercariae in the lens & vitreous humour of the eyes	No	2 - 4	2 - 5	5 - 8
Diplostomum gasterostei	Unencysted metacercariae in the vitreous humour & retina of the eye.	No	No	No	6 - 8
Diplostomum sp.	Unencysted metacercariae in the body-cavity.	No	14 - 116	1 - 15	No

Diplostomum sp.	Encysted metacercariae in the body-cavity.	1 - 8	No	No	No
Unidentified cyst.	Body cavity, brain case, muscles in the head region & gonad.	No	8 - 85	8 - 34	No
Rhipidocotyle campanula	Encysted metacercariae in the sub-mucosa of the buccopharyngeal region & gills.	2 - 100	No	No	No
Phyllodis -tomum folium	Adult in the urinary bladder.	No	1 - 10	1 - 2	1 - 2
Sphaero --stoma -bramae	Adult in the posterior intestine & rectum.	1 - 10	No	3 - 6	No
CESTODES					
Trieno -phorus -nodulosus	Encysted plerocercoid in liver.	1 - 2 (2-3)%	1 (6)%	1 (30)%	No
Proteo -cephalus sp.	Adult in the intestine.	No	No	1 - 4	No
NEMATODE					
Raphid -ascaris sp.	Encysted in the liver & intestine.	2 - 3 (6)%	1 - 2 (9)%	3 -18 (80)%	2 - 5 (20)%

4.3 Discussion

There are records of two freshwater bucephalids in Europe, *Rhipidocotyle campanula* (Dujardin, 1845) and *Bucephalus polymorphus* Baer, 1827, in the same environment having the same intermediate hosts but with varying degree of infection (Kozicka, 1959; Baturo, 1977; Chernogorenko, 1983). In the River Aire, Keighley only one species occurs , *Rhipidocotyle campanula*. But the other bucephalid - *B. polymorphus* - has been reported mainly as metacercariae and adults in Lincolnshire and Lancashire rivers (Kennedy, 1974).

The only primary host species known in the United Kingdom for *R. campanula* is *Anodonta anatina* (the Duck mussel), in the River Aire, Keighley, with seven other host species in Europe, all belonging to the same family Unionidae. (Table 4.3). The incidence of infection of the mussel by the sporocysts, increases with age, showing no sign of infection in the mussels of less than 3 years. Matthews (1974) observed similar results with the infection of *Abra abra*, an estuarine bivalve, with *Bucephalopsis gracilescens*. Also, the infections are seemingly of long standing, persisting for the life of the mussel host.

Kelly (1899) stated that infection with a bucephalid results in parasitic castration of the mussel. In the present study this has been found only in heavy and old infection of mussels. In mild

infection, both cercariae and glochidia are produced simultaneously. Similar observations are reported by Woodhead (1930) on *Eurynia iris* harbouring *Bucephalus elegans* and Kniskern (1952b) on *Lampsilis siliquoidea* harbouring *Rhipidocotyle septpapillata*, both freshwater mussels of U.S.A.

Escape of mature cercariae from the sporocyst is either through the birth pore or by breaking the sporocyst tubules. This is the first record of a birth pore for the bucephalids, although pores have been observed in other digenetic trematodes. As the sporocyst tubules are very long and interwoven, tracing of a single strand is very difficult. This may have prevented many workers from observing the means of escape in bucephalids. Breaking of the sporocyst tubules can be attributed to different factors. In the regions of the brood chambers, fluid pressure is increased by the developing cercariae causing the walls to become weak and thus increasing the chances of rupture (Matthews, 1973). Alternatively the anterior glands and penetration organ which serve to gain entrance into the second intermediate host may help in rupturing the wall which is already under increased pressure (Kniskern, 1952b). The cercariae can either be released into the lumen of the gonads and then pass through the genital ducts, or released into the mantle cavity if the sporocysts are protruding into the mantle, and be ejected by the exhalent siphon.

Liberation of cercariae from the mussel is influenced by the activity of the mussel and abrupt thermic changes, though it is not clear how they influence the release of cercariae from the sporocyst tubules. If the release of cercariae from the tubules is unaffected by the above two factors, then the cercariae must be collected and stored in the mantle cavity until their release, as observed on *Gymnocephala* sp. by Chernogorenko (1980). In his observations cercariae ready for release are being concentrated in the blood vessels of the molluscs' mantle and held for a long period of time in water until the optimal temperature is reached for release. In general the liberation of cercariae from *A. anatina* is neither cyclic nor continuous but essentially an intermittent phenomenon.

Chernogorenko (1980) stated that there is a definite relationship between the rate of release of cercariae, the metabolic rate of the host mollusc and environmental conditions, but it must be noted that in *R. campanula* only prolonged long term exposure to high temperatures (15°C - 17°C) influences the development and production of cercariae in the mussels; brief effects (sudden temperature change, water agitation and silt) influence the immediate response of the mussels (withdrawal of the siphons and closure of the shells) and temporarily prevent the release of the cercariae.

The released cercariae enter the second intermediate host passively through the food and respiratory current and encyst along the cephalic region. The entry into the secondary intermediate host by the cercariae is an important identification character for *R. campanula* as this is the only bucephalid known to the writer's knowledge which enters the host passively and encysts in the cephalic region. The only other report is by Matthews (1973), observing *Bucephalus haimeanus* cercariae, passively entering through the mouth of plaice and gobbies, but later found them in various stages of digestion. The passive entry of *R. campanula* cercariae clearly separates it from the second freshwater bucephalid, *B. polymorphus*, which enters actively through the external surface of the fish. (Baturo, 1977).

Cercarial entry and encystment into the secondary host is very rapid and encystment in some of the cercariae is completed within 4h of entry. By comparison the time taken for attachment, penetration and encystment by *B. polymorphus* is 48 - 72h and by *R. campanula* 3-4 days (Baturo, 1977), by *B. longicornutus* within 1/2h - 1h (Howell, 1966), by *B. elegans* a few minutes (Woodhead, 1930) and by *R. septpapillata* 24h (Kniskern, 1952b). All the above mentioned examples penetrate the hosts actively through the surface of the skin except *R. campanula*. Although Baturo (1977) mentioned of 3-4 days for encystment in *R. campanula*, penetration of the cercariae must be rapid to

avoid being washed away by the food and respiratory currents. Supporting this view, only rarely were cercariae noted in the stomach. Also very few cercariae were seen to be spat out immediately after entering the mouth of the fish. Rapid cyst formation and easy release of the cyst from the host tissue clearly explains another feature mentioned by Kniskern (1952b), namely that the cyst wall is of parasitic origin. Later reports by Halton and Johnson, 1982, confirm this view. They mentioned that the inner layer of the metacercaria is contributed by the cercaria, probably from cystogenous cells in the outer tegument.

After 6 weeks the metacercaria has more or less attained its full size, but the reproductive system is not as advanced in development as that of the metacercarial stages of many other species of gasterostomes. The vitellaria are not fully developed, uterine coils and Mehlis's glands are not present, nor is there evidence of gametogenesis. Similar metacercarial stages with less developed reproductive systems were observed by Matthews (1973) on *B. haimeanus* after 6-8 weeks of complete development and by Howell (1966) on *B. longicornutus* after 80 days of complete development. In contrast, fully developed metacercariae similar to adults except for the functional reproductive system were observed by Matthews (1972) on *Prosorhynchus crucibulum* 2 months after complete development, and by Baturo (1977) on *B. polymorphus* and *R. campanula* after 15 days

development. It is clear that sexual maturity is attained only in the digestive system of the final host but that development of the reproductive system in metacercaria may be influenced by the second intermediate host as seen in the variations of development of *R. campanula* in minnows in British waters and in *Scardinus erythrophthalmus* (rudd), *Rutilus rutilus* (roach) and *Blica bjoercna* (silver bream), (Baturo,1977) in Slesinskie lake , Poland.

Fully developed metacercarial cysts survived for approximately 200 days, but only 80 - 90 days old cysts excysted spontaneously (temp.15°C - 17°C), and the number decreased with the increase of age. Howell (1966) observed only 10% excystation spontaneously in *Bucephalus longicornutus* which were 75 - 95 days old. Kniskern (1952b) reported that 12 days or older cysts excysted spontaneously but by twelve weeks or more they have shown degeneration of the metacercariae. Baturo (1977) did not mention excystment but reported of the death of the metacercariae *R. campanula* after 5 months of entry. But none of the observations mentioned the effect of temperature on the survival of the cysts. As stated by Kniskern (1952) the ability to excyst coincides closely with the age at which metacercariae are infective and may be used as an index of infectivity.

In the secondary intermediate hosts, metacercariae appeared to show a high degree of specificity to cyprinid fishes, having been previously

recorded from three species in U.K and eight species in Europe. (Table 4.3). Kozicka (1959) recorded four more species of fish which are carnivorous and known as final hosts, [*Esox lucius* (pike); *Perca fluviatilis* (perch); *Lucioperca lucioperca* (pike perch) and *Acerina cernua* (ruffe)], to be susceptible to infection with the cercariae. From the experimental studies, encysted cercariae were observed in trout fry (*Salmo trutta*) but died within 2 days of entry. As none of the cercariae survived to maturity, the writer feels the infection in carnivorous fish are accidental.

The infection of minnows with cysts were noted in almost all fish above 2 cm in length, with an increase in number in older fish. Mellors (1985) obtained a similar significant relationship between the number of cysts and the length and weight of the fish. The sites of *A. anatina* are the spawning grounds of minnows, as plenty of fry were observed in these sites from time to time from May to August. Although the sites of minnows obtained for the study were a few yards upstream, as the minnows generally mature in the first year (Wheeler, 1969 ; Muus and Dahlstrom, 1978), there is a seasonal contact with the cercariae and thus explains the minnows above 2cm to be infected. Also , the larger the fish, the more surface area in the bucco - pharyngeal region for the cercariae to be encysted.

Infection of minnows was observed from March to October though the relationship between the intensity

of infection and the seasonal effect is not really prominent. Unfortunately, due to difficulties in obtaining samples during the winter months (November to February), the precise seasonal effects on the intensity of infection could not be studied. Mellors (1985) reported obtaining samples in November and January and observed infection. Newly acquired infections were noted during May to August but this is dependent on the release of cercariae from the mussels, as it is controlled mainly by the activity of the mussel and water temperature. Ultimately the intensity of infection is dependent on the availability of the cercariae and survival of the infected metacercarial cysts.

The cysts of *R. campanula* occur in the subcutaneous fatty tissue beneath the lining of the pharynx and in some cases in the gill arches. Similar observations are reported by Chappell (1967), Baturo(1977) and Mellors and Owen (1980). Kozicka (1959) reported cysts along the caudal, dorsal, pectoral and ventral fins, gills, muscles (usually at the base of the tail), in the eyes, brain and the subcutaneous tissue. Similarly Chernogorenko (1983) and Ivantsiv and Chernogorenko (1984) reported encysted cercariae in the gill tissues, fins and tail fin. They also reported intense concentration on the fins, generally the sites of encystment of *B. polymorphus* (Baturo, 1977). Passive entry of *R. campanula* cercariae through the mouth and encystment along the cephalic region and the active

penetration of the *B. polymorphus* cercariae through the external surface of the fish clearly differentiates the sites of encystment. There are reports by Kozicka (1959), Baturo (1977) and Ivantsiv and Chernogorenko (1984) that the above two parasites occupy the same host. Also the external appearance of the metacercarial cysts of both species are similar. Therefore the similarities between the two species and the common hosts for the metacercarial stage of development, is possibly the reason for earlier confusion between the two species.

Unfortunately the final hosts in the River Aire could not be studied but in the experimental studies adult parasites were obtained from perch (*Perca fluviatilis*) and trout (*Salmo trutta*), though perch seems to be a more suitable host than trout. The number of adults obtained from perch varied between 14 - 199 per fish, but all the experimental hosts were found to be infected. In nature Kozicka (1959) observed a maximum infection of over 10,000 in *Esox lucius* and 79 - 719 in perch while Chappell (1967) noted up to 1,575 in *E. lucius*. As mentioned by Wierzbicka (1977) the differences in infestation with intestinal parasites are unquestionably connected with their food requirements.

In five weeks, fully matured adults with eggs were noted in the posterior intestine and rectum (temp. 9°C-11°C) In perch maintained in higher temperatures (14°C-15°C) fully matured adults were noted in 3 weeks.

Similar temperature effects on maturity of the adults were observed by Kniskern (1952) during the development of *R. septpapillata*. The worms matured in 5 days in summer and 10-15 days in colder winter months. As stated by Krull (1934) and Kniskern (1952), temperature is one of the factors determining the life span in the final host, either affecting the parasite directly or reducing the metabolic rate of the hosts and thereby retarding the development of the parasites.

The other factor which affects the development and duration within the final hosts is the age of the metacercariae fed to the final host. (Kniskern,1952). As the metacercariae fed were of unknown age in the present study, except for a few to confirm the identity of the parasite, the duration of the development is not certain.

Migration of the worms in relation to their maturity is seen in the intestine of the final host. The matured worms with eggs are found attached to the walls of the posterior intestine and rectum. Nearly 5 weeks have been taken for the worms to move downwards in the intestine to the final site of development. As mentioned earlier the time taken for the downward migration of the worms may be affected by the metabolic rate of the hosts or the low temperature in which the final hosts are maintained.

No embryos have been observed in eggs within the adult worm or following their deposition. This is the link not found by previous workers nor, unfortunately,

by the present worker. Only a few workers have described the miracidium in other bucephalids namely, Woodhead (1929) on *B. elegans* and in 1930 on *R. papillosum*, Kniskern (1952) on *R. septpapillata* all of which are parasites of American freshwater fish. Later Tang and Tang (1976) briefly described the miracidium of *Parabucephalopsis prosthorchis* in China. The miracidium of *R. septpapillata* resembles those of *R. papillosum* in the shape and possessing of an anterior knob and four anterior ciliated appendages. As *R. campanula* closely resembles the other stages of development with *R. septpapillata*, the appearence of the miracidium may be similar to the above species. Gametogenesis is discussed in Chapter 8.

The miracidium is presumed to enter the first intermediate host via the inhalent siphon. Attempts to infect the primary host by dropping eggs in front of the inhalent siphon of *A. anatina* were not successful. Similar attempts by Tennent (1906) and later by Stunkard (1976b) by dropping eggs in front of the inhalent siphon of the mussels failed, none of these experiments being successful to date.

CHAPTER FIVE

SPOROCYST GENERATION IN THE PRIMARY HOST

5.1 Introduction

Infection of bivalve mussels by the sporocyst generation of bucephalid trematodes can be basically divided into two categories. In the first group development of the sporocysts starts in the gonadal tissues (*Prosorhynchus crucibulum* and *Bucephaloides gracilescens*, Matthews, 1973; *Rhipidocotyle tranversale*, Stunkard, 1976b) and during progressive development invades the digestive glands. In the second group development starts in the digestive glands (*Bucephalus longicornutus*, Howell, 1966 and *Bucephalus polymorphus*, Baturo, 1977) and during progressive development invades the gonadal tissues.

The sporocysts of the present species of bucephalid trematode are found, in mildly infected *Anodonta anatina*, in the digestive glands; in heavily infected mussels they almost destroy the digestive gland cells, completely invade the gonadal tissues and ultimately protrude from them into the mantle cavity. However, neither the gills nor the foot of the mussel are infected with the tubules, unlike some other bucephalid infections.

In the present study the morphology of the sporocyst tubule of *R. campanula*, reproduction in the daughter sporocyst and development of cercariae, the effect of parasitism on the digestive tubules and the

occurrence of some food reserves in the daughter sporocysts have all been examined using histochemical and histological methods.

5.2 Results

5.2.1 The morphology of the sporocyst

The sporocyst is that of a typical gasterostome, being composed of a mass of dichotomously branched tubules, dilated at intervals into chambers which contain developing cercariae. The diameter of the tubules is extremely variable, sections of narrow width of 45 µm may alternate with short enlarged, distended sections as much as 199 µm in diameter. Often large sections of 800µm long and 400µm diameter filled with developing cercariae, are followed by narrow stretches, containing a single row of small germ-balls.

The wall of the sporocyst is composed of an outer covering of tegument, underneath a thin layer of circular and longitudinal muscle bands which can not be clearly differentiated with the light microscope. The parenchymatous layer below the muscle bands contains large cells which became flattened in distended regions of the sporocyst (Fig 6.8).

The germinal cells are situated in the parenchyma below the muscles at dilated regions of the sporocyst and branch endings. The nuclei of the germinal cells stain more intensely with haematoxylin than do the parenchyma cell nuclei, and more or less fill the germinal cells, as the cytoplasm is exceedingly sparse.

5.2.2 Reproduction in the daughter sporocyst

The development of the cercaria of *R. campanula* from the germinal cells was studied by following the sequence of stages in the sectioned sporocysts; the miracidial stage could not be obtained for *R. campanula*, and the sequence of development from this stage, therefore, could not be followed. In the sporocyst tubule of *R. campanula*, often in the same portion, germinal cells, germinal masses, cercarial embryos and mature cercariae could be found. (Fig 5.2a).

Loosely aggregated cells surrounded by a thin membrane are found in the lumen of the sporocyst tubules and are referred to by Ciordia (1956), and James and Bowers (1967c) as germ balls, which develop into the cercaria (Fig 5.1a). The scattered groups of two to five cells in the parenchymatous layer, surrounded by the parenchyma cells are referred to as "germinal cysts". These would eventually be released into the sporocyst lumen and form germ balls by further divisions. The germinal cysts of the five cell stage contain four small cells known as somatic cells and one big cell, the germinal cell. The somatic cells continue to divide, forming more cells, and the germinal cells divide later when forming the cercarial embryo. The germinal ball cells are of different sizes due to mitotic divisions. To follow the developmental stages more easily the terminology of Woodhead (1929) describing the

developmental stages of *R. papillosum* is adopted. This terminology is based upon the number of flame cells and the development of tail furcae but in the present instance the development of the tail furcae is given priority.

In the germ ball stage as development proceeds, the embryo becomes more spherical in shape and a pair of flame cells is visible in the upper half of living germ balls, one on the right side and the other on the left (Fig 5.3a).

In further development a clear constriction between the body and the furcae is noted and also two tail buds are present. This stage is referred to as the rudimentary furcae stage and measured 130µm in length. The two short, rounded furcae converge towards each other along the long axis of the body of the cercaria. The penetration organ is visible as a mass of cells. (Fig 5.3b).

The next stage observed is the crossed furcae stage measuring 180µm in length and is very clear in development. The characteristic feature of this stage is the inwardly curved, crossed tail furcae. The gut is visible and is formed into a small sac connected to the pharynx (Fig 5.3c).

The furcae now spread from each other forming an angle of about 90° and more or less straight, referred to as spread furcae stage (Fig 5.2b). At this stage the excretory vesicle, the anterior tubules and the flame

cells are all clear. The exact number of flame cells is not certain but up to twenty four cells have been counted in different specimens. The spread furcae stage develops into the fully developed cercariae. The movement of cercariae is not seen within the sporocyst lumen, but the matured cercariae ready to escape from the sporocysts are observed pointing towards the terminal pore, thus indicating the cercarial movement within the brood chambers of the sporocysts (Fig 4.7).

The cercarial development was observed only briefly and the flame cell development could not be clearly followed.

5.2.3 The effect of parasitism and the food reserves in the daughter sporocyst

The progressive development of the sporocyst generation in the mussel, *Anodonta anatina*, and the effect on the digestive system and reproductive system have not been observed but the effect of the parasite in the mildly infected stage and heavily infected stage can be briefly described.

In mildly infected mussels, the sporocyst tubules invade the digestive system but most of the digestive tubules contain food particles and no change in the structure of their cells is noticeable. The digestive cells are columnar and have ciliated distal borders adjoining the tubule lumen. Each cell has a large nucleus situated near the basal margin (Fig 5.4a). A dense concentration of epithelial mucin is present on the distal border, demonstrated by the PAS technique as a purplish red lining; this reaction persists even after treatment with diastase. The lining also stains blue with alcian blue demonstrating the presence of acid mucopolysaccharide. Therefore the epithelial lining may probably consist of acid and neutral mucupolysaccharide and muco-protein complex. Alcoholic PAS and fast green demonstrated the presence of glycogen (greenish blue) in the digestive gland cells and in the visceral haemocoel; negative results followed diastase treatment.

In heavily infected mussels the digestive tubules away from the infected sporocyst tubules appeared to be healthy, but close to the sporocyst tubules the digestive tubules were noted to be squashed by the growing sporocysts and in some cases partially disintegrated. In very heavy infections the majority of the digestive gland tubules are destroyed and completely invaded by sporocyst tubules (Fig 5.1 & 5.4b). The digestive tubules close to the sporocyst branches contain fewer food storage globules of glycogen and glycoprotein. Also the visceral haemocoel contained less glycogen.

In the developing cercaria, acid muco-polysaccharides staining a blue colour with alcian blue were observed in the germ ball stage, from advanced cleavage to all stages of development. The tegument and certain parenchymal cells and most likely the glandular cells are positive for acid mucopolysaccharide in the developing cercaria (Fig 5.1).

Glycogen was not observed in the germ balls and only appeared in the cross furcae stage. It was mainly found in the later development of the cercaria, in the tail-stem as bluish-green and scattered along the tail reacting with non-diastase treated alcoholic periodic acid and fast green.

Even after complete invasion of the mussels with the sporocyst tubules, cercariae are released for a few weeks until the death of the mussel. Completely invaded sporocyst tubules, at different stages

of disintegration, and empty tubules were noted a few times in dead mussels. From the external appearance it is very difficult to differentiate a healthy mussel from a very heavily infected mussel unless the activity of the mussel is very closely monitored.

5.3 Discussion

The complex branching of the tubules and their complete penetration of the digestive cells, makes the dissection of a complete sporocyst impossible. For this reason it is very difficult to determine whether any one large sporocyst mass has developed from a single miracidium.

The empty sporocyst tubules observed in a few very heavily infected mussels are most likely the result of nutrient deficiency which prevented further development. These "spent sporocyst tubules" are definitely different from Woodhead's (1930) "spent tubules" observed in *R. papillosum* and also Kniskern's (1952b) "nutritive branches" observed in *R. septpapillata* which are devoid of celluar contents. Woodhead (1930) interpreted the spent tubules as the sporocyst tubules of an infection in a previous year, devoid of reproductive stages. However, the present observations of two types of sporocyst tubules are clearly not the polymorphism mentioned by Woodhead (1930) and Kniskern (1952b).

The new growth from old reproductive branches observed in the present study are similar to the branches mentioned in *R. septpapillata* by Kniskern (1952b) (Fig 4.8), but old spent tubules of sporocyst were not seen to act as chambers for cercarial development as mentioned by this author.

The structure of the sporocyst wall resembles that of *Prosorhynchus crucibulum* as described by Matthews (1973) and both lack the outer region of nucleated tegument described by James et al, (1966) on the sporocyst wall of *Cercaria bucephalopsis haimeana*.

The germinal cyst is produced in *R. campanula* by division of a single germinal cell producing five cells of which four are somatic cells and one is a germinal cell as mentioned by James and Bowers (1967c) in *Cercaria bucephalopsis haimeana*. This is later released into the lumen of the sporocyst tubule and develops into a germ ball and ultimately into cercaria. This is the only method observed in *R. campanula*.

There are two further ways of production of germ balls mentioned by James and Bowers (1967c) in *Cercaria bucephalopsis haimeana*. In one of these, a single germinal cell divides mitotically to produce several germinal cells and thus forms a germinal mass sorrounded by a cyst wall instead of germinal cyst with somatic cells. These germinal cells within the mass then divide mitotically and produce germinal cysts, as mentioned in *R. campanula*. These escape individually into the sporocyst lumen from the main germinal mass and develop further as germinal balls. A similar method was described by Ciordia (1956) for the development of germ balls in *R. papilloaum*.

In the second method mentioned by James and Bowers (1967c) the entire germinal mass surrounded by a

cyst wall, as described above, escapes into the sporocyst lumen and develop further into germ balls. Ciordia (1956) described a method similar to the latter way of production of germ balls but instead of the entire cyst of germinal mass escaping, he mentioned germinal cells releasing individually into the sporocyst lumen and developing into germ balls.

Single germinal cells have not been observed in the sporocyst lumen of *R. campanula*. This agrees with James and Bowers (1967c) report of their absence in *Cercaria bucephalopsis haimeana*. These differences in the development of germ balls are probably due to specific differences within the family Bucephalidae.

Although a redial generation was reported by Woodhead (1931a,b) in *R. papillosum* and *B. elegans*, rediae were not observed in the sporocysts of *R. campanula* similar to *R. papillosum*, (Ciordia 1956), *R. septpapillata*, (Kniskern 1952b), *B. longicornutus*, (Howell 1966) and *Cercaria bucephalopsis haimeana*, (James and Bowers 1967c). With such an effective cercariae producing mechanism as that exhibited by *R. campanula*, it appears possible that the redial generation is unnecessary.

The development stages of cercaria from the germ ball stage in *R. campanula* are basically similar to those in *R. papillosum* as described by Woodhead (1929) and Ciordia (1956). There is no evidence from the present study, or from these previous studies, to determine

whether the continuous production of cercariae is from a single miracidial infection or from consecutive multiple infections.

However the present observations reveal that there are two types of effects caused in *Anodonta anatina* by the infection of the *R. campanula* sporocyst generation. One is the physiological effect of parasitism resulting from the utilisation of the host's food resources and most probably the accumulation of the toxic excretory products of the parasite (Rees,1934 and Cheng and Snyder 1962). The latter is not proven in *R. campanula* due to lack of definite evidence.

The second effect is most often observed in very heavily infected *Anodonta anatina* and can be referred to as a "mechanical effect". This is due to the developing germinal sacs closing the tubule lumen and thus preventing food from passing into the ultimate branches of the digestive gland tubules, causing starvation. In very heavy infections completely compressed columnar cells result from this effect. Similar mechanical damage and starvation autolysis was clearly demonstrated by James (1965) in *Littorina saxatilis*.

A third effect of the collapsing of the digestive cells and tubules, mentioned by James and Bowers (1967a), is the result of cellular disaggregation and depolymerization of hyaluronic acid, causing release of bound water from the tissue. Further, the acid mucopolysaccharide in the visceral haemocoel, which may

be secreted by the daughter sporocyst, probably replaces the host's destroyed hyaluronic acid and chondroitin sulphate, and functions as an adhesive to hold the branches of the daughter sporocyst and host tissue together (James and Bowers, 1967a). In the present study acid mucopoly- saccharide was observed in the sporocyst walls and in the visceral haemocoel of the digestive glands of infected mussels. As suggested by James and Bowers (1967b) itmay be secreted by the parasite. Tests for hyaluronic acid have not been done in either healthy or infected mussels with sporocysts. It is therefore not possible to comment on the above suggestion.

The reduction in glycogen in the digestive gland cells and in the visceral haemocoel near to the sporocyst tubules in the parasitised mussels may be caused by the sporocysts absorbing through their tegument (Cheng, 1963).

James (1965) studying damage done to the digestive glands of *Littorina saxatilis* (Olivi) subsp. *tenebrosa* by five parasite species, stated that the extent of the damage would depend on the rate of development of the germinal sacs in relation to the life span of the host and the time of initial infection, by the mobility of the germinal sacs and finally by the "resistance" of the host or "specificity" of the parasite.

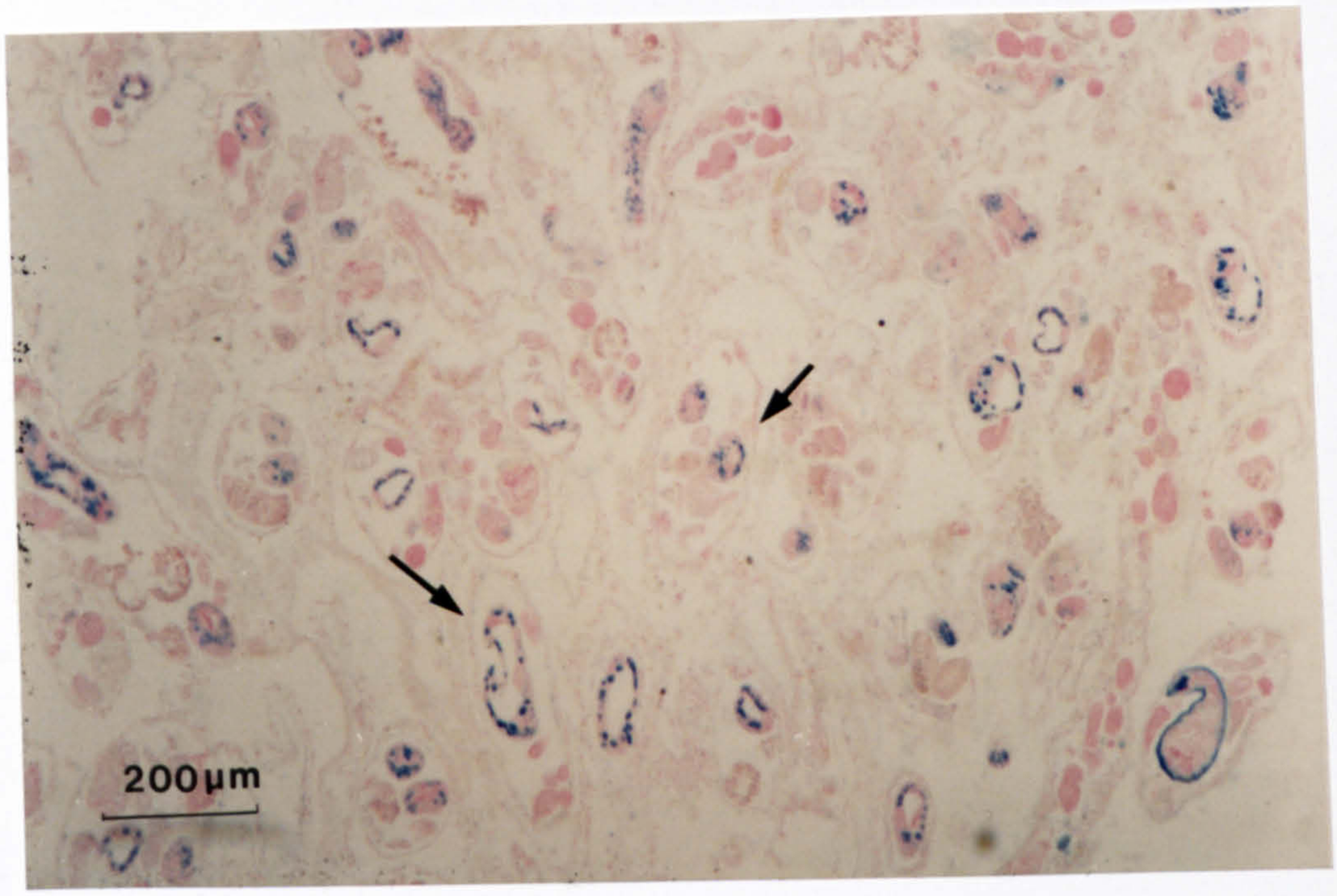
The effect of parasitism on *Anodonta anatina* is a gradual effect rather than the immediate killing of the

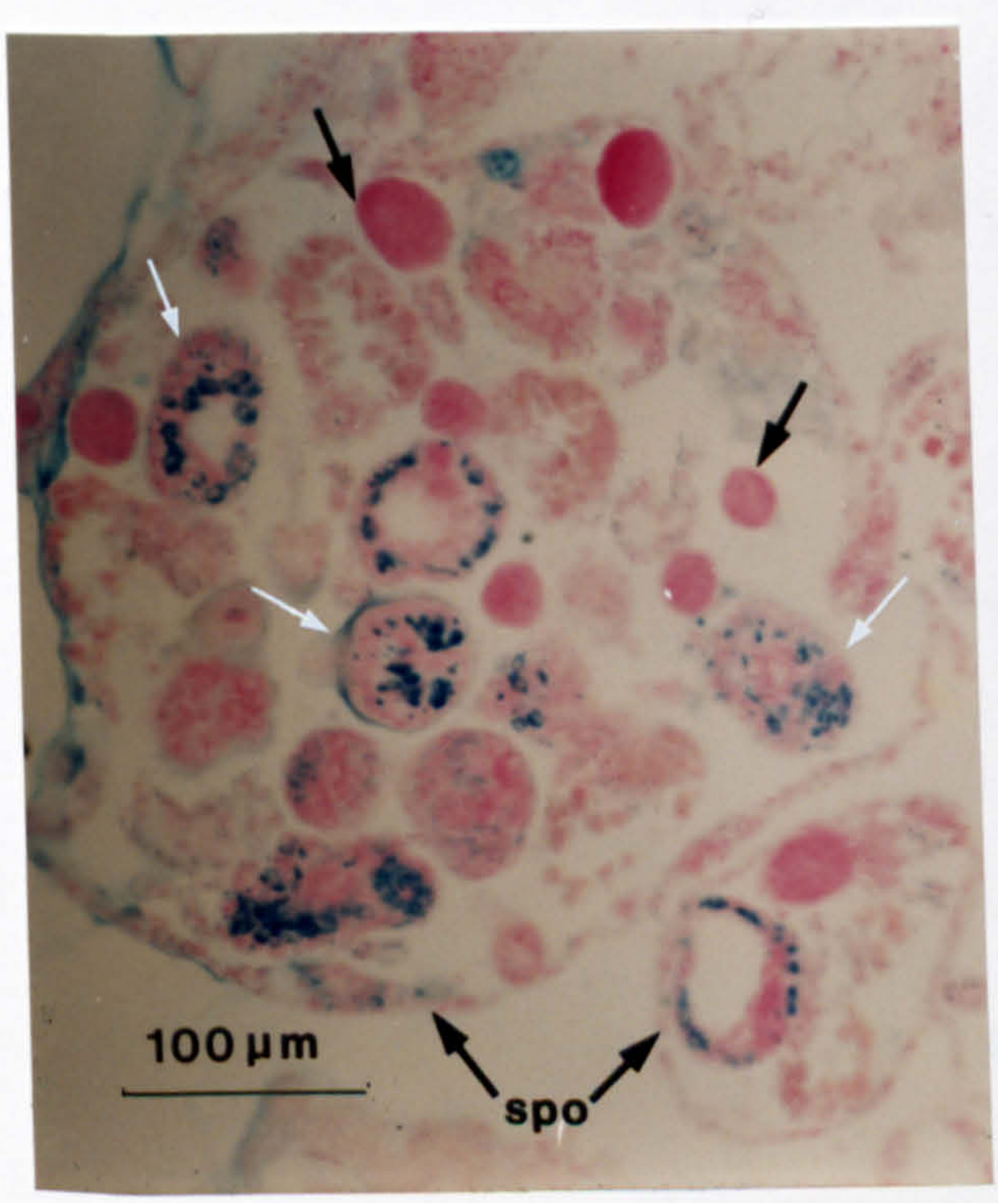
host. In partly invaded mussels the release of cercariae and glochidia happens simultaneously. Also, heavily infected mussels produce cercariae in abundance for a few weeks before they have been completely killed. In order that the relationship between the parasite and host be understood, more knowledge of the normal metabolism of the host, the influence and metabolism of the parasite and the reaction of the host to the metabolic products of the parasites need to be obtained.

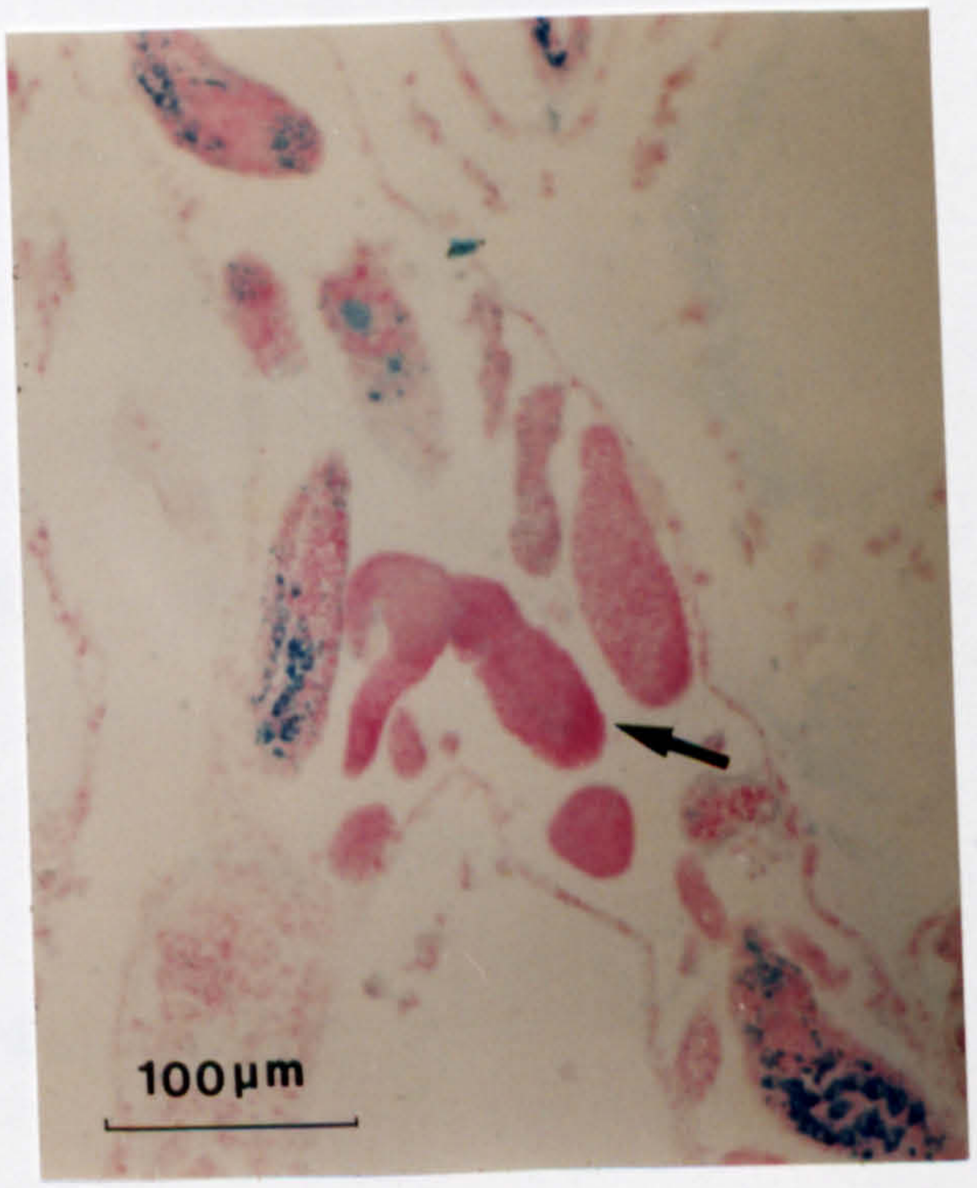
Fig 5.1 Section through the digestive gland of infected mussel, *A. anatina* showing digestive tubules completely destroyed with the invading sporocyst tubules (arrowed). Note the acid mucopolysaccharide reaction (blue) in the developing cercariae at different stages of development.
Stained with alcian blue and neutral red.

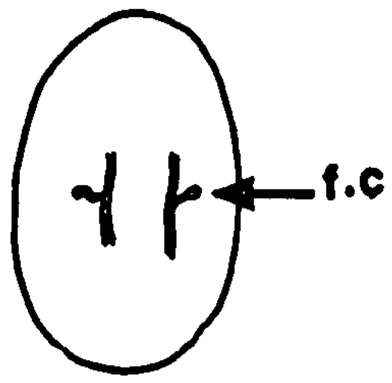
Fig 5.2a Cross-section of a sporocyst tubule showing germinal masses (black arrow) and cercarial embryos at different stages of development (white arrow). Also note the different diameter of the two sporocyst tubules (spo).
Stained with alcian blue and neutral red.

5.2b. The spread furcae stage in section inside the sporocyst tubule with their furcae held parellel to each other (arrowed).
Stained with alcian blue and neutral red.

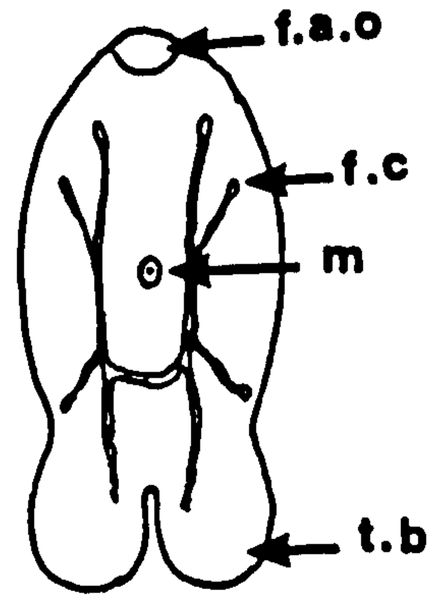

200µm


100µm
spo


100µm

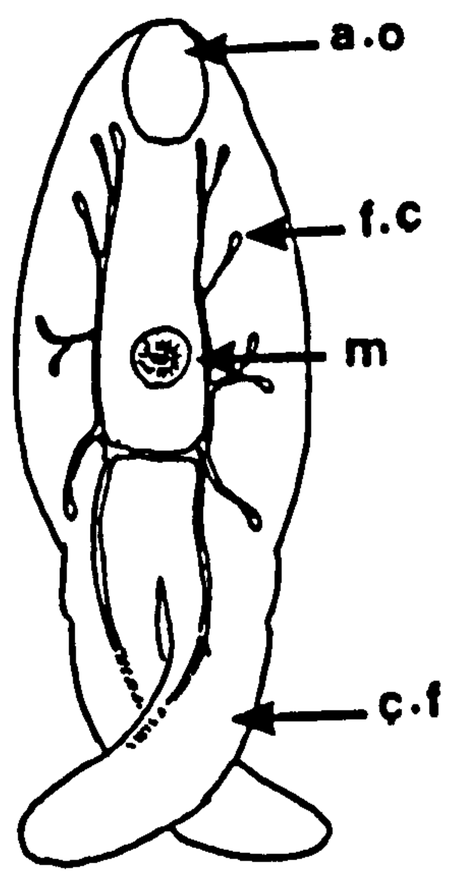


(a) Germ ball stage with a pair of flame cells.



(b) Rudimentary furcae stage.

30µm



(c) Crossed furcae stage.

30µm

Fig 5.3. Early stages in the development of cercaria of *R. campanula*.

a.o .. anterior sucker

f.a.o .. formation of anterior sucker

f.c .. flame cell

m .. mouth

t.b .. tail bud

Fig 5.4. Diagramatic representation of transverse section through the digestive cells of *A. anatina* parasitised with sporocysts of *R. campanula* showing the effects of parasitism.

c.t ... connective tissue

ep.m ... epithelial mucin containg acid mucopolysaccharide

f.v ... food vacuole

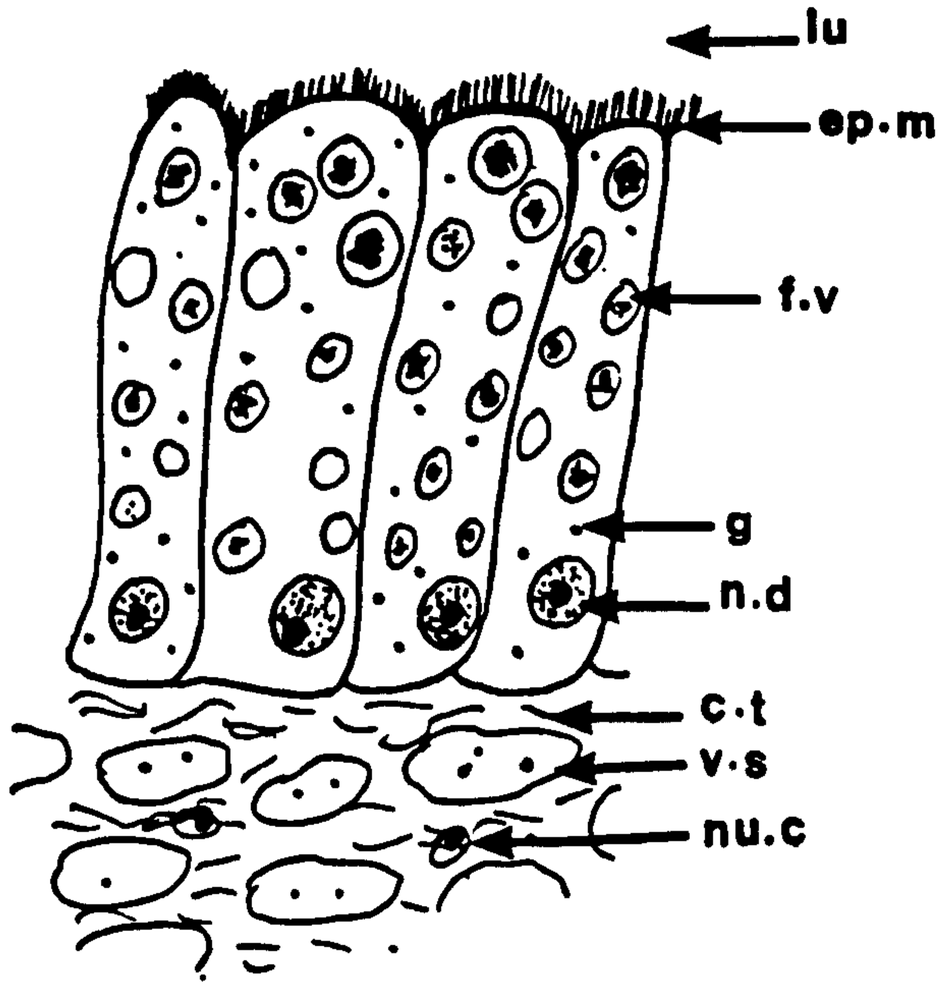
g ... glycogen

lu ... tubule lumen

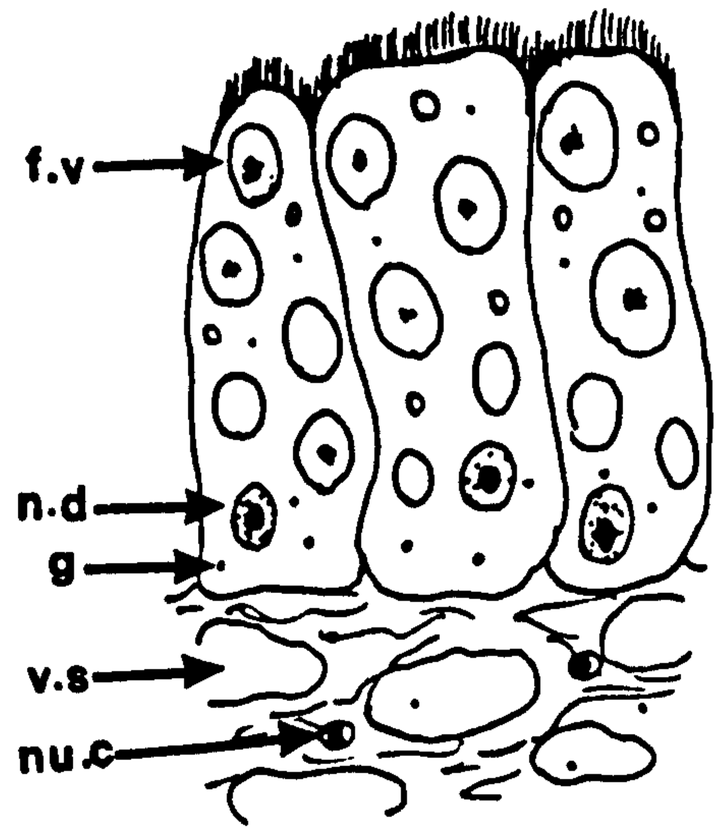
n.d ... nucleus of digestive cell

nu.c ... nucleus of connective tissue

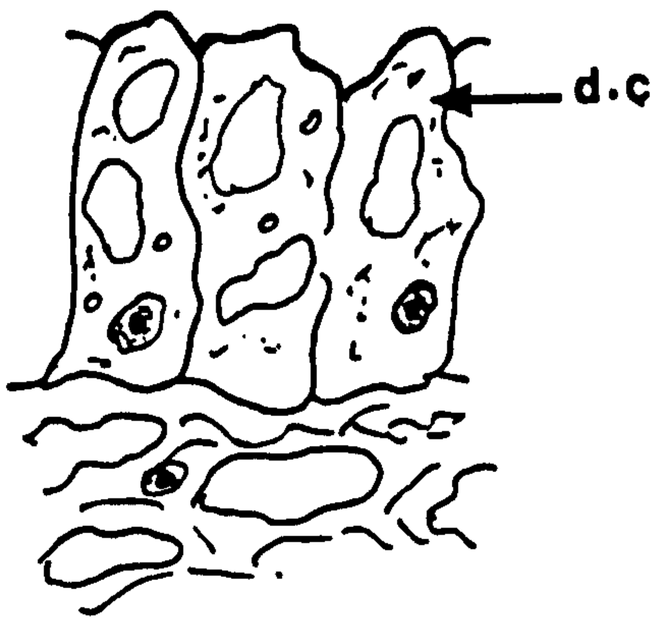
v.s ... visceral haemocoelic spacescreen



(a) Healthy digestive cells



(b) Digestive cells showing physiological effect.



(i) Break-down of lateral and distal cell walls and loss of cilia (d.c).

10 µm

(ii) Complete break down of digestive cells (c.d.c).

(c) Digestive cells showing mechanical damage.

CHAPTER SIX

THE FURCOCERCARIA - SOME ASPECTS OF MORPHOLOGY, BIOLOGY AND BEHAVIOUR

6.1 Introduction

The morphology of bucephalid cercariae has been studied only by the light microscope (Woodhead 1929, 1930; Kniskern 1952b; Howell 1966; Matthews 1972, 1973, 1974; and others). Many are inadequately described for comparative purposes and wrongly identified, as the adult characters are not exhibited by the cercariae except for the structure of the excretory system which was brought to general attention by Stunkard (1974d) in bucephalid taxonomy. Also the biology and behaviour of cercariae have been studied by only a few workers.

Until the mid-19th century all the bucephalid cercariae from marine mussels were being identified as *Bucephalopsis haimeanus* (Lacaze-Duthiers, 1854) and from freshwater mussels as *Bucephalus polymorphus* (Baer, 1927) Many authors placed the cercariae in adult genera without any experimental evidence to substantiate their views. Also it became apparent that the information concerning the bucephalid cercariae was widely scattered in the literature. In support of this view, all the known previous reports of bucephalid cercariae are listed in Table 2.2.

The cercariae of *Rhipidocotyle campanula* was correctly identified for the first time by Baturo (1977) from *Unio pictorum* by completing experimentally the life- cycle of the parasite, but the descriptions were brief and inadequate to characterise the species.

Similarly Ivantsiv and Chernogorenko (1984) described the cercariae briefly without any detailed illustrations. Therefore, a detailed study on the external and internal morphology of cercariae using histological, histochemical and electron microscopical methods was carried out, to characterise the species as fully as possible. Also the behaviour of the cercariae in relation to photo - and geo-responses, swimming activity and shedding pattern of cercariae from the primary host - *Anodonta anatina* have been studied. This was to understand fully the significance of the free-living existence and the transmission of the cercarial stage, which have not previously been fully understood in this species.

6.2 Results

6.2.1 Morphology of the cercaria

(a) General description of the external features

The cercaria of *Rhipidocotyle campanula* is a typical gasterostome cercaria having a tail with two contractile furcae attached to the tail stem. The body is fairly large, white, opaque and just visible to the unaided eye. The body tapers anteriorly, being pear shaped and depressed when contracted, and elongated and cylindrical when expanded (Fig 4.3 & 6.1). The furcae are highly contractile, and extend nearly eight times the length of the body, measuring 0.3- 0.4 mm in length when retracted and 2.0-3.0mm when fully extended. They are inserted on the antero-lateral surface of the tail stem. The furcae when relaxed are club shaped, tapering towards the free end. Generally the cercariae are in a resting position with the furcae contracted, encircling the body and almost meeting in front of the anterior extremities (Fig 4.3). Comparative measurements of cercariae with the previous observations are given in Table 6.1.

The entire surface of the body is covered with a thick tegument which has a rough, finely wrinkled appearance superficially in the anterior region and becomes coarser towards the posterior region. The tegument along the body of the cercaria has been demonstrated to

contain acid mucopolysccharide (Fig 6.8). The tail stem which is bilobed, measuring 106-209 µm in length and 91-189 µm in width superficially resembles honeycomb. (Fig 6.1). Furcae are highly wrinkled when compared to the rest of the body, with transversely running ridges. (Fig 6.2)

The tegument of the body of the cercaria is similar to the basic structure of the trematode tegument. This can be divided into three layers: a thin unit membrane on the surface, a wide cytoplasmic layer containing many vesicles and mitochondria and an inner basement layer (Fig 6.3). Beneath the basement layer are two layers of muscles, an outer circular and an inner longitudinal layer. In the cytoplasmic layer, few mitochondria are scattered proximal to the basement layer and these are small in comparison with the mitochondria found in the parenchyma.

The matrix of the cytoplasmic layer is finely granular and of median electron density. The vesicles contained within it may be divided into four types according to the appearance of the matrix. (Fig 6.3). (1) Oval or elongated vesicles with finely granular electron dense matrix with an electron-opaque periphery (V_1). (2) Smaller elongated or rounded vesicles. with homogenous electron-dense matrix (V_2). (3) Smaller than the above two vesicles with condensed granular matrix (V_3). (4) Very few electron-dense rod shaped bodies along the upper layers of the matrix (V_4). Few electron-opaque vesicles are found scattered along the lower region of

the matrix nearer to the basement lamella (V_5). All these vesicles are randomly scattered in the matrix and not in specific regions as mentioned in *Cryptocotyle lingua*, (Rees, 1974 & Rees and Day, 1976). Also shape and electron density vary compareed with *C. ligula*, (Rees, 1974) and *Acanthatrium cercariae*, (Belton & Harris, 1967). The inner plasma membrane which is raised into digitiform processes extend into the basal cytoplasm. The inner basement layer, which is of a uniform thickness, contains loosely fibrous interstitial material.

Stereoscan observations revealed small, oval, regularly arranged, elevated circular plates, with posteriorly directed single pointed spines (2µm), nearly to 1/3rd of the anterior body; and more irregularly shaped plates, with scattered, smaller spines than the anterior region, progressively fewer in number towards the posterior of the body (Fig 6.4 & 6.5). Most of the posterior spines appear to be buried in the tegument. The demarcation of the spines and plates depends on the state of contraction of the body. There are no spines immediately around the mouth nor on the tail stem or along the furcae.(Fig 6.2 & 6.5).

The anterior end of the cercaria is slightly raised from the rest of the body and bears a seven.lobed anterior organ or penetration organ, measuring 56µm in length and 41µm in greatest breadth. This opens to the surface by a subterminal pore. The lobes or lips of the organ are covered with outwardly directed dagger shaped spines (1.5µm by 0.5µm). (Fig. 6.4).

A small oval, slightly elevated spinose area is situated on the anterior and ventral part of the head organ with an inconspicuous opening at the posterior end. (Fig. 6.4). This may be the rudimentary anterior sucker. On either sides of this organ groups of sensory structures are present. Each receptor consists of a bulb-like base with a raised tegumental ring from which protrudes a ciliuum approximately 1.5-2.0µm long (p_1). Some of the receptors found here are without cilium with a pore in the middle (p_2) (Fig 6.4). Similar pit-like papillae have been observed in *Schistosoma mekongi* adults in the anterior region on the ventral side (Vongpaybal et al 1982) and in the metacercariae of three species of Japanese lung flukes along the dorsal and dorsal-lateral side of the oral sucker (Higo & Ishii 1987). Scattered on the dorsal and ventral surfaces are found another type of receptor (p_3) with a single cilium approximately 2.0-2.5µm long surrounded by a tegumental ring. (Fig 6.6).

The ventral mouth is situated nearly two thirds of the length from the anterior end or nearer its centre as a small transverse slit, 7um to 10um wide, surrounded by inwardly directed irregularly shaped, rectangular plates (Fig 6.5). A rudimentary genital pore is situated 10um from the posterior extremity. (Fig 6.2) The positions of the openings depend on the state of contraction of the body. Two excretory pores open at the antero-lateral position of the tail-stem.

Table 6.1

Dimensions of *Rhipidocotyle campanula* cercariae (µm).

Characters	R.Aire Keighly,U.K. (1984/85) N=50			Goslanickie & Slesinskie Lakes,Poland. (1972/73)N=60	Reservoirs of Lower Dnieper, U.S.S.R. (1982)		
	Min	Max	Aver		Min	Max	Aver
Body length	194	266	220	340-580	430	650	620
Body width	94	141	110	111-160	120	200	185
Diameter of pharynx	30	50	36	42-67	43	52	47
Head organ							
Length	42	72	56	74-104	70	75	72
Width	32	48	41	55-88	--	--	--
Tail stem							
Length	106	209	144	------	--	--	--
Width	91	189	127	------	--	--	--

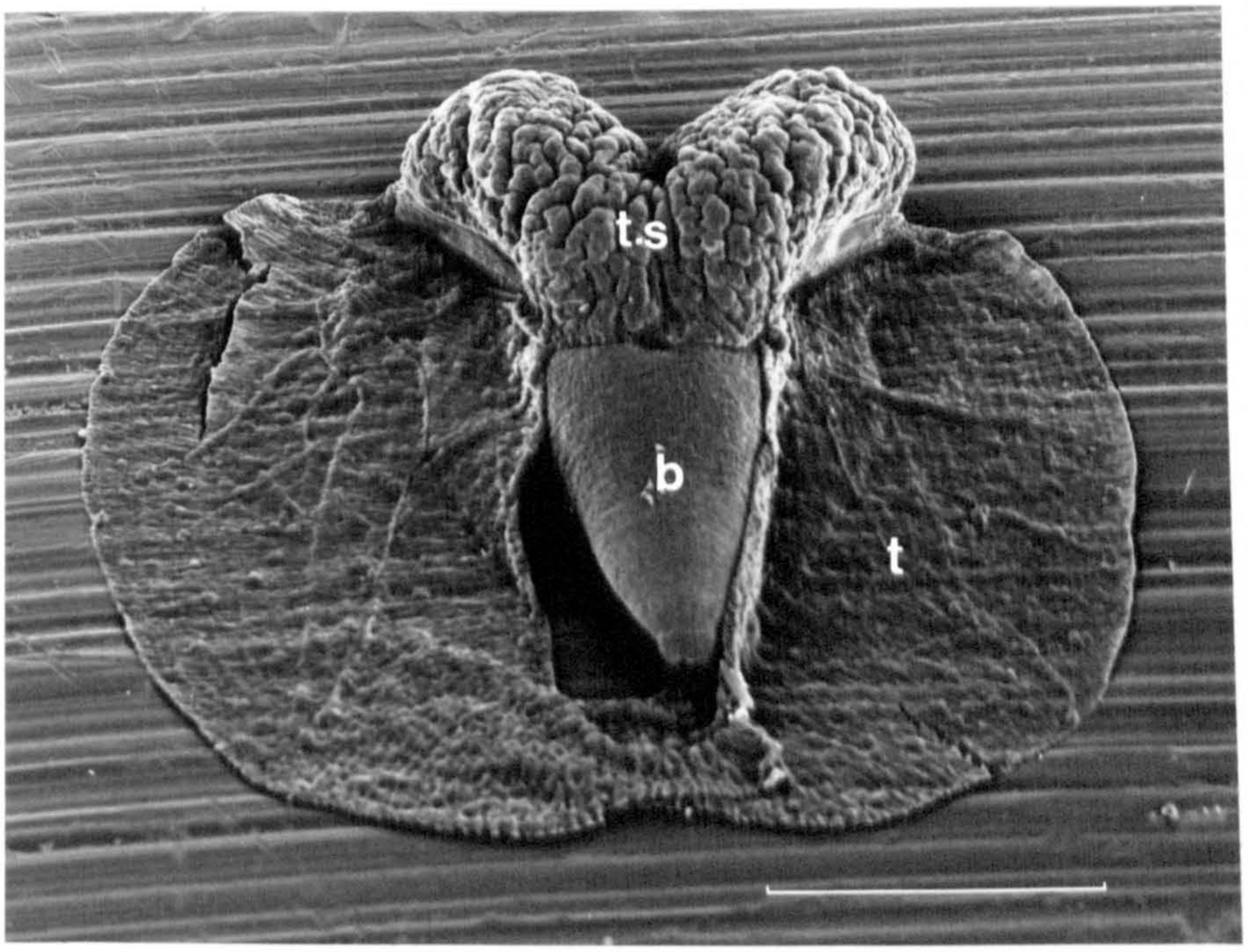
N = Number of measured specimens.

Fig 6.1 Scanning electron micrograph of the cercaria showing the body (b), tail-stem (t.s) & tail (t)

Scale bar = 100µm

Fig 6.2 Scanning electron micrograph of the posterior region of the cercarial body (b),tail-stem (t.s) and tail (t) showing remarkable variations in the external appearance. Note the elevated plates in the body (b), honey comb tail-stem (t.s) and highly wrinkled tail (t) with transversely running ridges; mouth (m) and genital pore (g.p)

Scale bar = 30µm


t.s
b
t

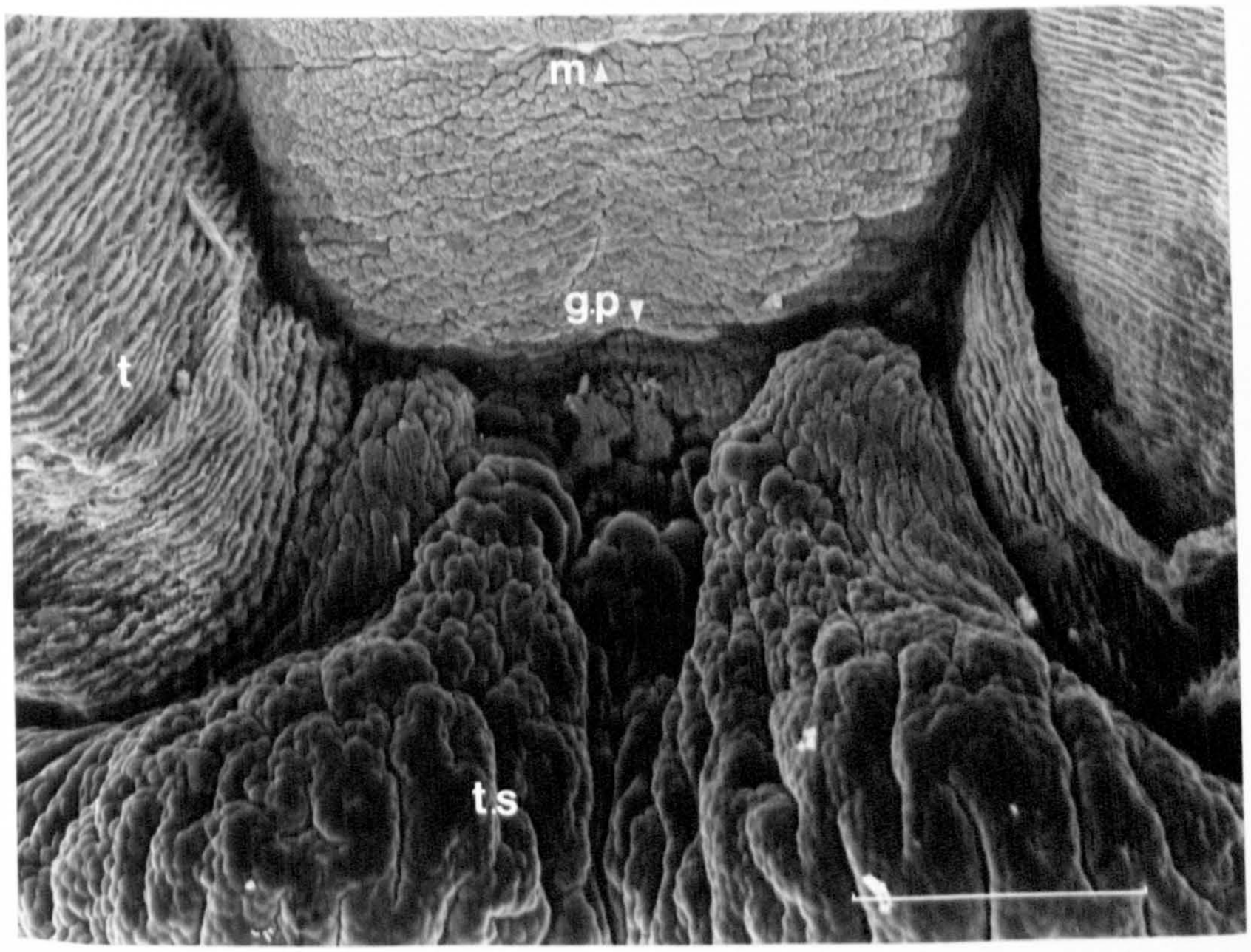

m
g.p
t
t.s

Fig 6.3 Transmission electron micrograph showing the epidermal layer along the mid-ventral region of the cercarial body. Note the three layers: thin outer surface membrane (s.m), thick cytoplasmic layer (c.l) with five distinct types of vesicles (V_1-V_5) and mitochondria (arrowed) and an inner basement layer (b.l) with underlying layer of muscles (mu).

Scale bar = 200nm

Vesicles V_1 - V_5 are as follows:

V_1 .. oval or elongated vesicles with finely granular electron-dense matrix with an electron-opaque periphery

V_2 .. smaller elongated or rounded vesicles with homogenous electron-dense matrix

V_3 .. Condensed granular matrix

V_4 .. electron-dense rod shaped vesicles

V_5 .. electron-opaque vesicles

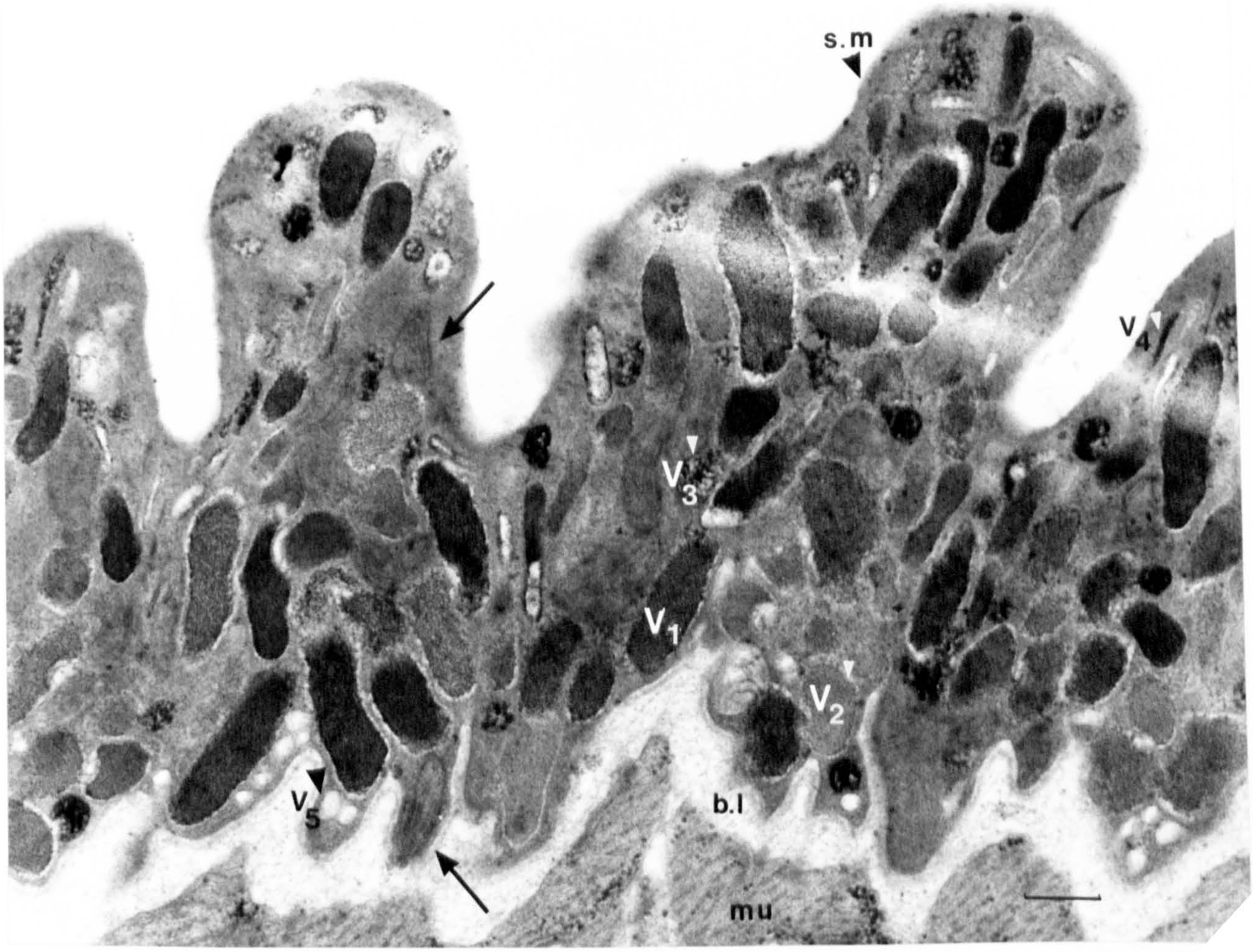
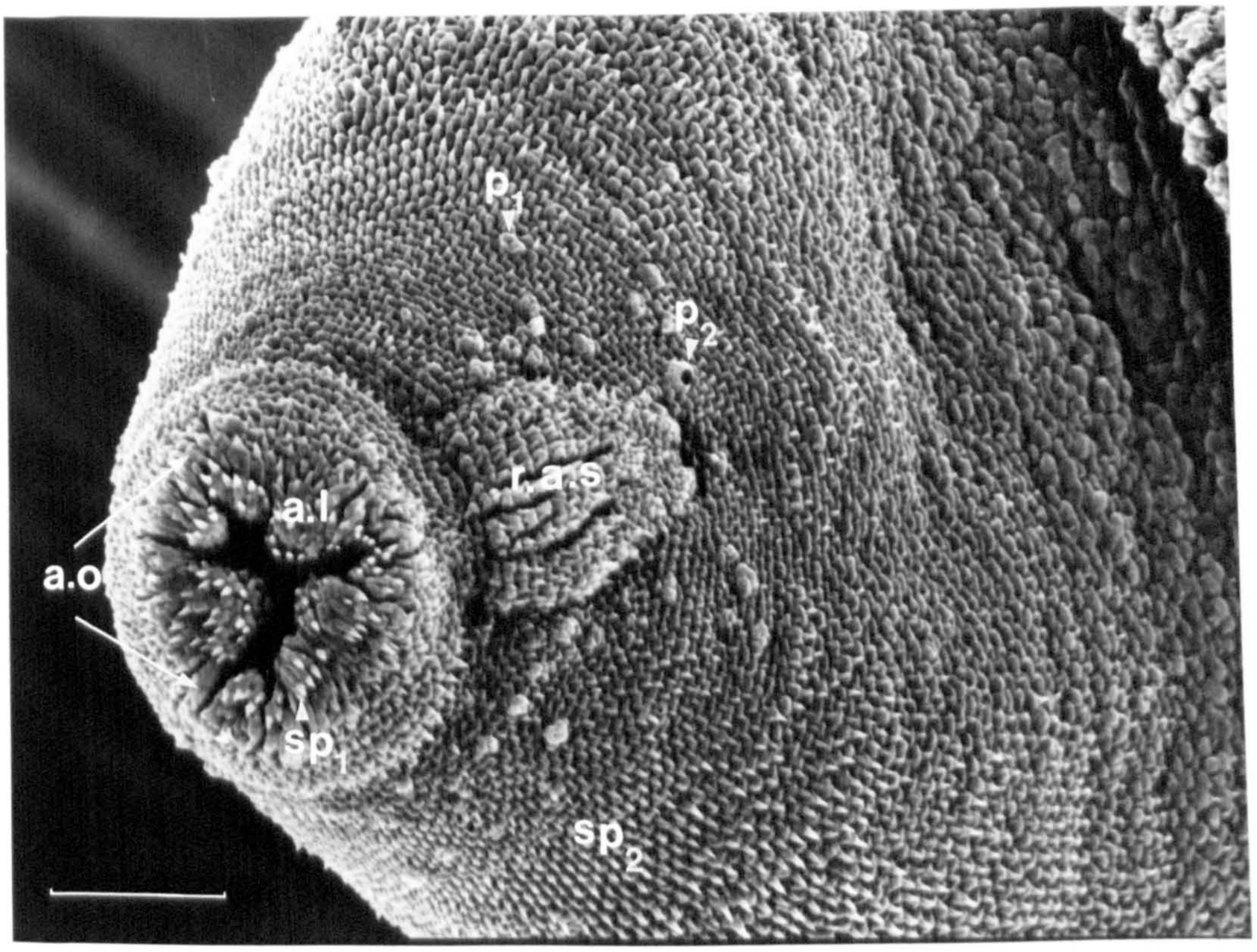

s.m
V4
V3
V1
V2
V5
b.l
mu

Fig 6.4 Scanning electron micrograph of the anterior ventral region of the cercaria showing the opening of the anterior organ (a.o) surrounded by seven anterior lips (a.l) , the rudimentary anterior sucker (r.a.s). Also note the anterior dagger like spines on the lips (sp_1) and the posteriorly directed spines (sp_2) along the body; and the two types of papillae: p_1- with a bulbous base and a tegumental ring from which protrudes a cilium, and , p_2- without cilium and a pore in the middle.

Scale bar = 10μm

Fig 6.5 Scanning electron micrograph of the mid-ventral region of the cercaria showing the mouth (m) and the elevated plates without any spines below the mouth and very few spines (sp) just above the mouth.


p1
p2
r.a.s
a.l
a.o
sp1
sp2

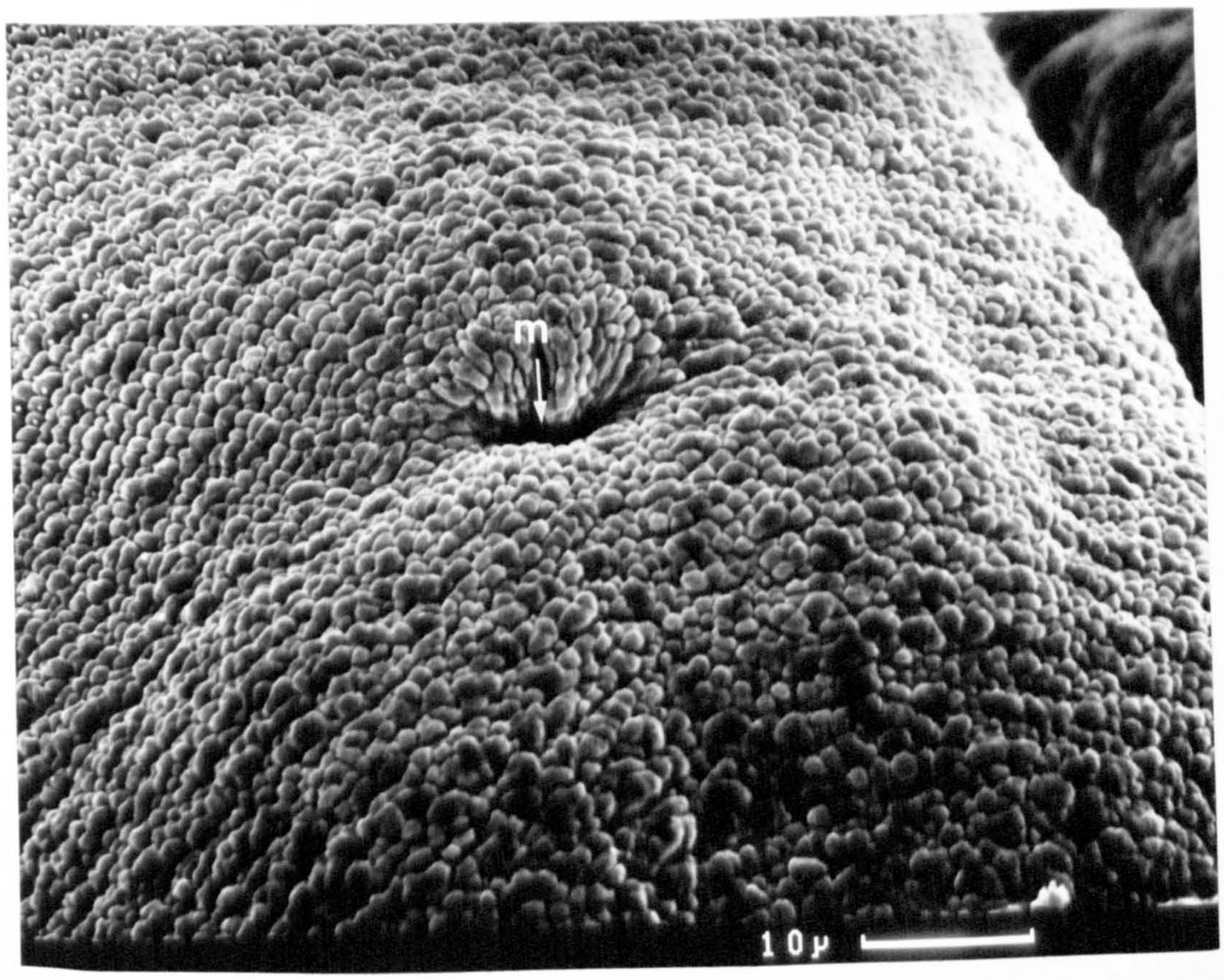
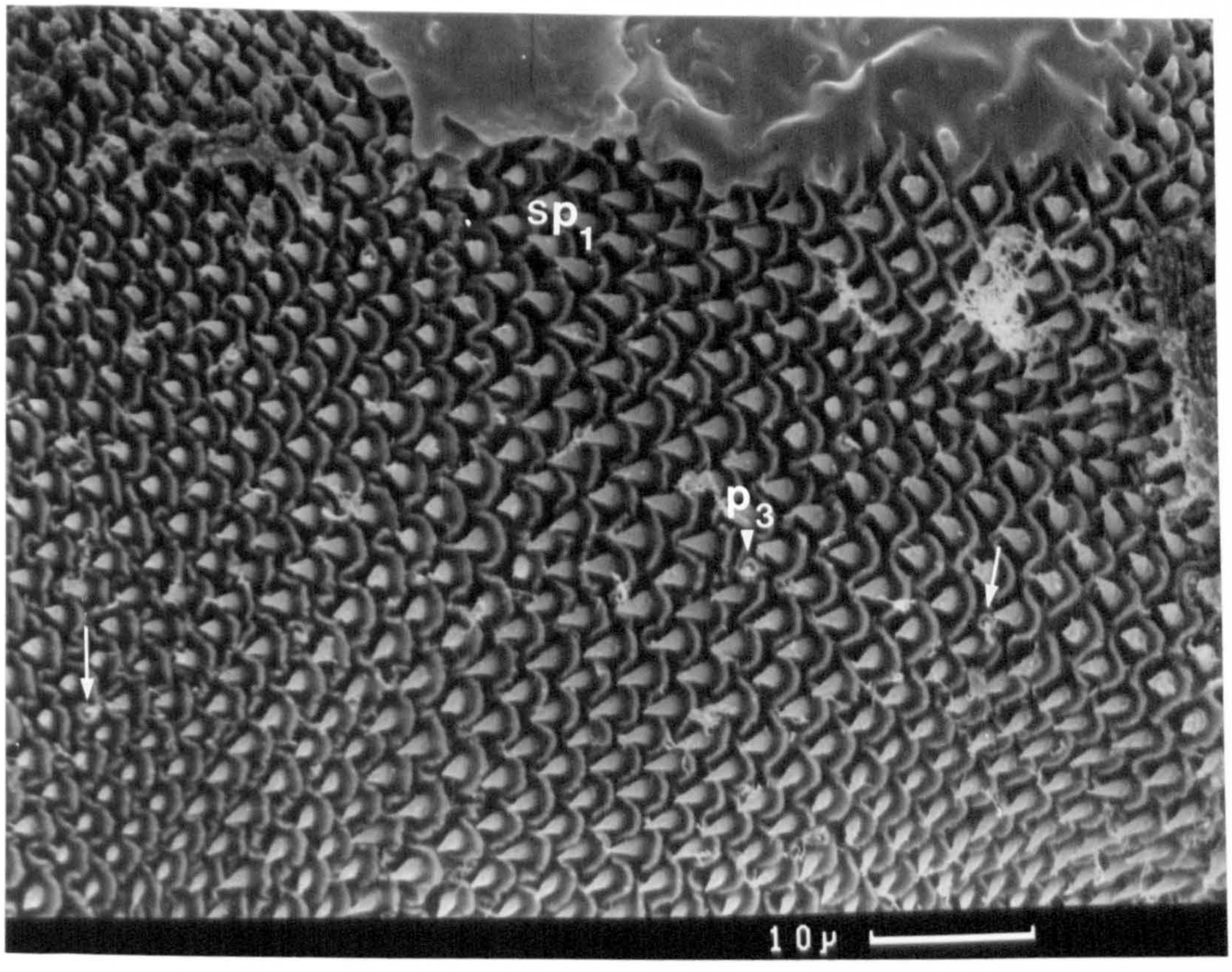

m
10μ

Fig 6.6 Scanning electron micrograph of the anterior ventral region of the cercaria showing the backwardly directed spines (sp_1) and papillae with a single cilium surrounded by a tegumental ring (p_3) (arrowed).

Fig 6.7 Scanning electron micrograph of the posterior region of the tail-stem (adhesive zone) showing the scattered domed shaped sensory papillae without cilium (p_4) and secretory pores on raised cells (s.po).


sp1
p3
10µ

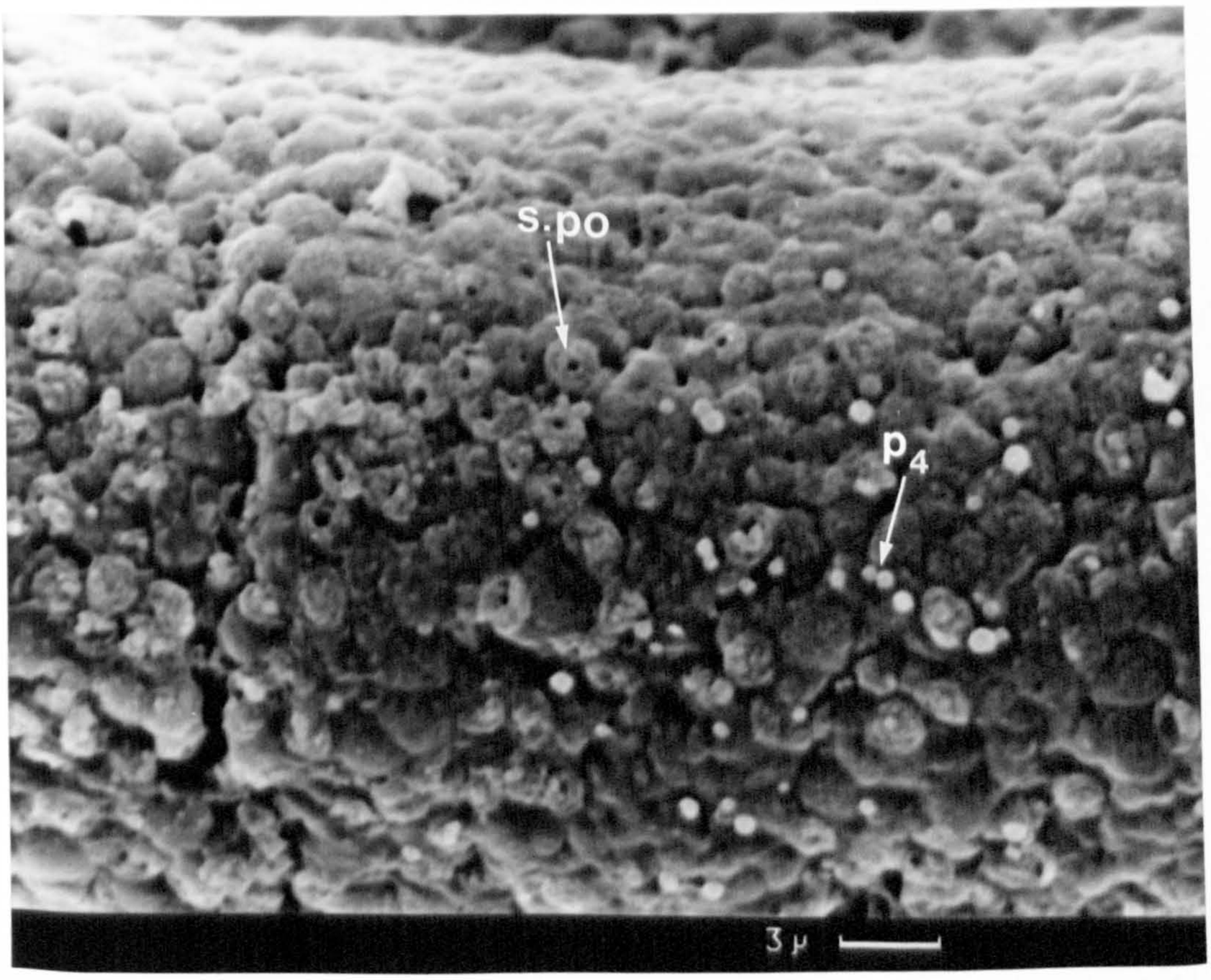
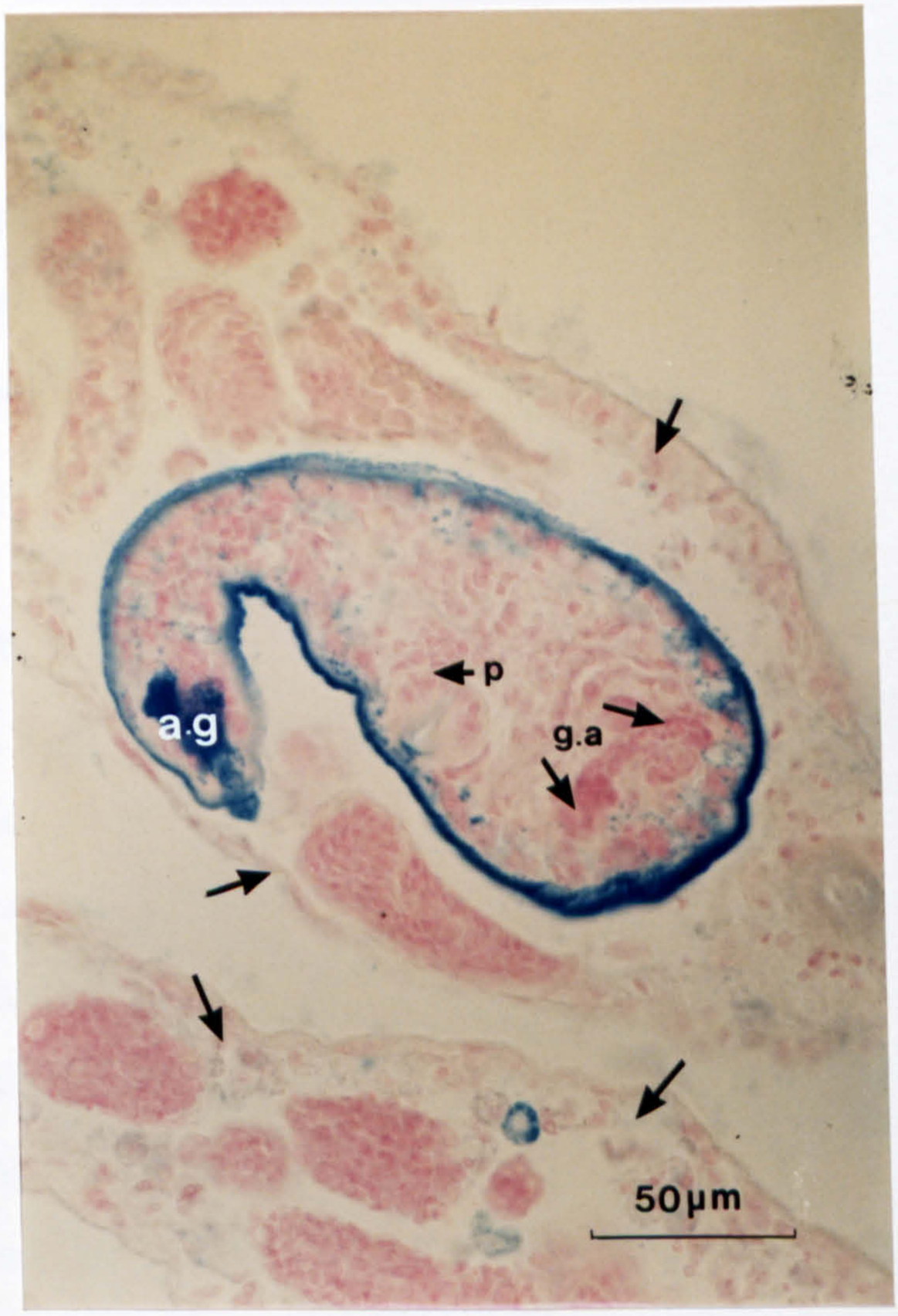

s.po
p4
3µ

Fig 6.8 Transverse section through digestive gland of *A. anatina*, showing a fully developed cercaria in the sporocyst tubule demonstrating the presence of an acid mucopolysaccharide surface coat (blue) with alcian blue method. Note the anterior glands (a.g) stained blue demonstrating the presence of acid mucopolysaccharide; muscular pharynx (p), genital anlagen (g.a) and the varying thickness of the sporocyst tubule walls (arrowed).
Stained in 1% alcian blue in 3% acetic acid and counter stained in 1% neutral red.


a.g
p
g.a
50µm

(b) Excretory system

The excretory vesicle is S-shaped, measuring 64-88 x 5-7µm depending on the state of contraction, and extends from the posterior end of the body to the left and just to the level of mid-pharynx. The most posterior part is dorsal in position, but after a curve to the right, extends ventrally and to the left. Posteriorly a caudal duct passes into the tail-stem where it bifurcates and open at the antero - lateral position of the tail - stem. Immediately before opening each bifurcation receives a caudal duct from each furca. The loss of the tail-stem leaves a terminal excretory pore.

The right collecting duct, which is formed laterally and posteriorly to the pharynx by the union of an anterior and a posterior collecting tubule, opens to the left of the mid-line on the anterior surface of the flexure in the bladder. The left collecting duct opens to the right of the mid-line, posterior to the right collecting duct, receiving anterior and posterior collecting tubules. Anterior ducts divide further into four canals and posterior into three, each of them being terminated with a group of five flame cells totalling seventy flame cells. The flame cell formula is as follows: 2 [(5 + 5 + 5 + 5) + (5 + 5 + 5)] = 70. (Fig 6.9). The collecting ducts and the tubules are convoluted, and are highly dilated in freshly released cercariae. Staining in 5% neutral red increases the

visibility of the flame cells. Thus the freshly released cercariae stained with 5% neutral red were found to be more suitable for the study of the excretory system.

Acid phosphatase and non-specific esterases were observed along the excretory vesicle and a very faint reaction of acid phosphatase along parts of the excretory tubules. (Fig 6.10 & 6.11). Few crystalline bodies composed of concentric lamellae and soluble in HCl were observed in the excretory bladder and tubules. Similar crystalline bodies in digenetic cercariae have been reported by Bennette & Threadgold (1973) and Bennette (1977) in *Fasciola hepatica*.

(c) Digestive system

The digestive system is simple with a slit like mouth, leading almost immediately into a more or less spherical muscular pharynx. This opens through a short oesophagus into a large saccate intestine.

The mouth opens ventrally, measuring 7µm to 10µm wide, and is situated nearly two thirds along from the anterior end or nearer its centre depending on the state of contraction of the body. It is surrounded by inwardly directed irregularly shaped rectangular spineless plates (Fig 6.5). The pharynx measures 30-50µm in diameter. The cavity of the pharynx is surrounded by conspicuous circular muscles outside of which there is a layer of longitudinal muscles (Fig 6.8). The intestine

extends both anteriorly and posteriorly to the pharynx. In living cercariae the intestine is filled with a fluid and was observed to contract and relax continually but no solid particles were noted inside the intestine.

Histochemically positive reactions along the alimentary system were observed for acid phosphatase as dark pink microcrystals and for non - specific esterase as deep blue microcrystals. (Fig 6.10 & 6.11). The functions of the digestive systems are not fully understood but the probable functions will be discussed later in this chapter.

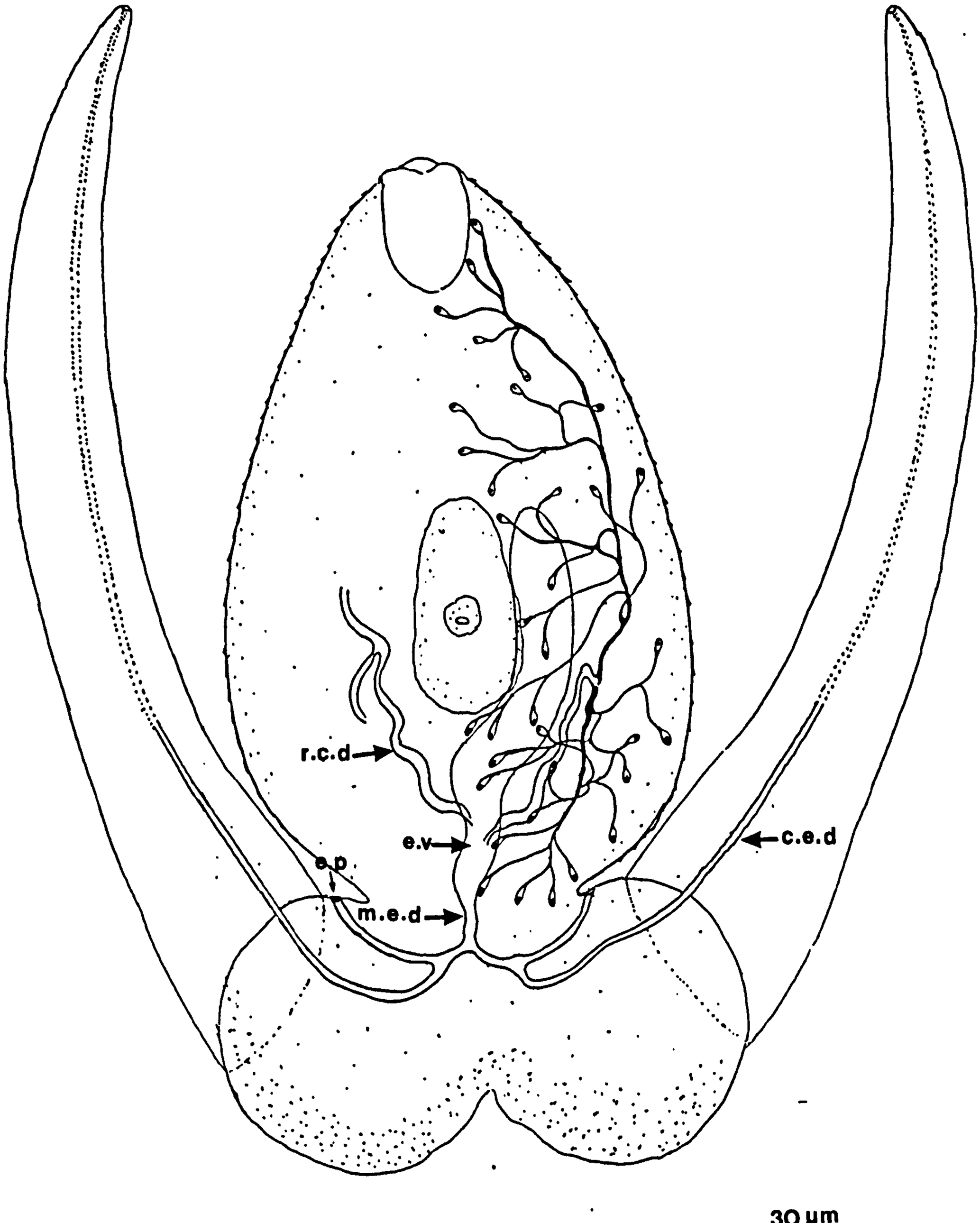
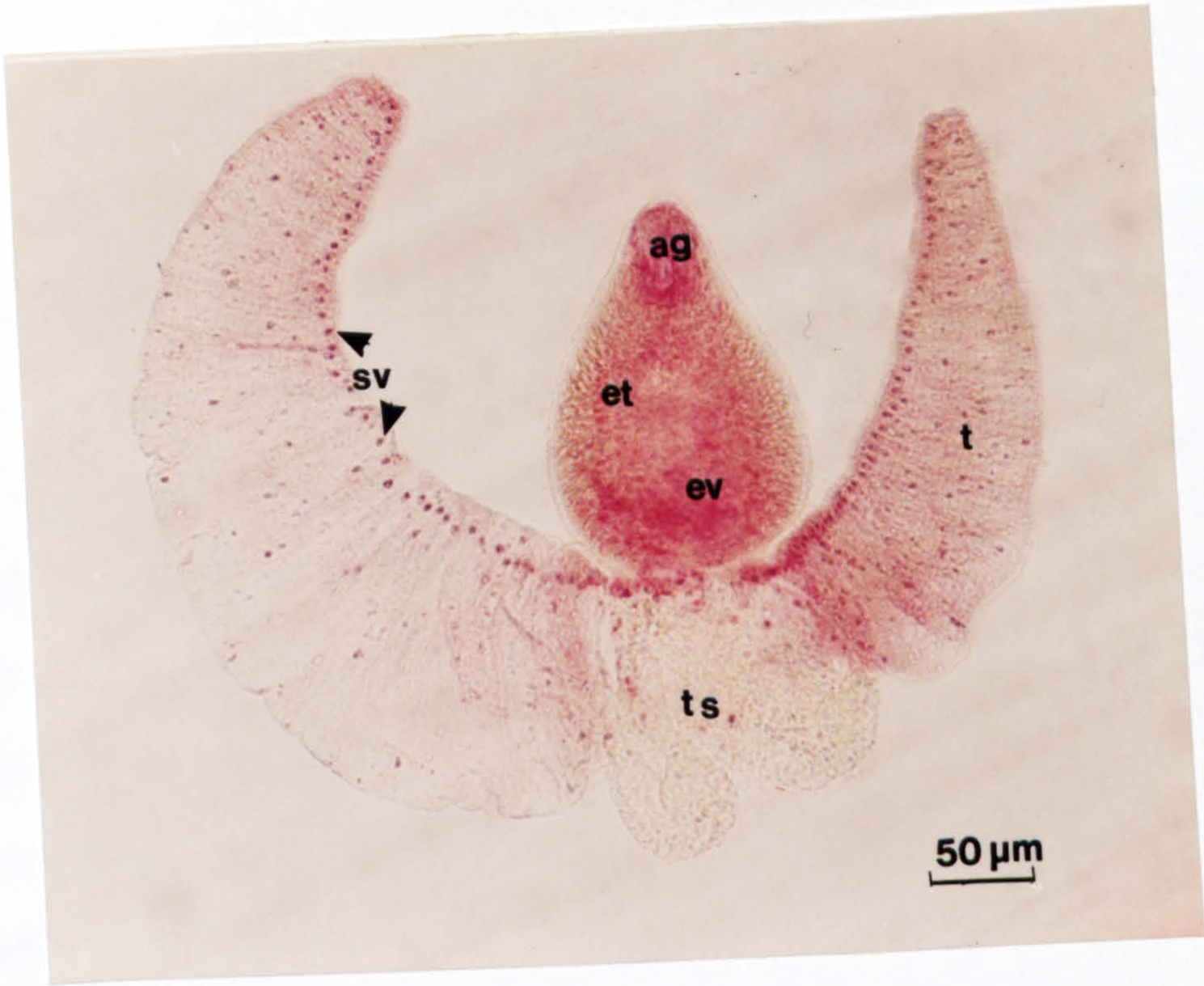
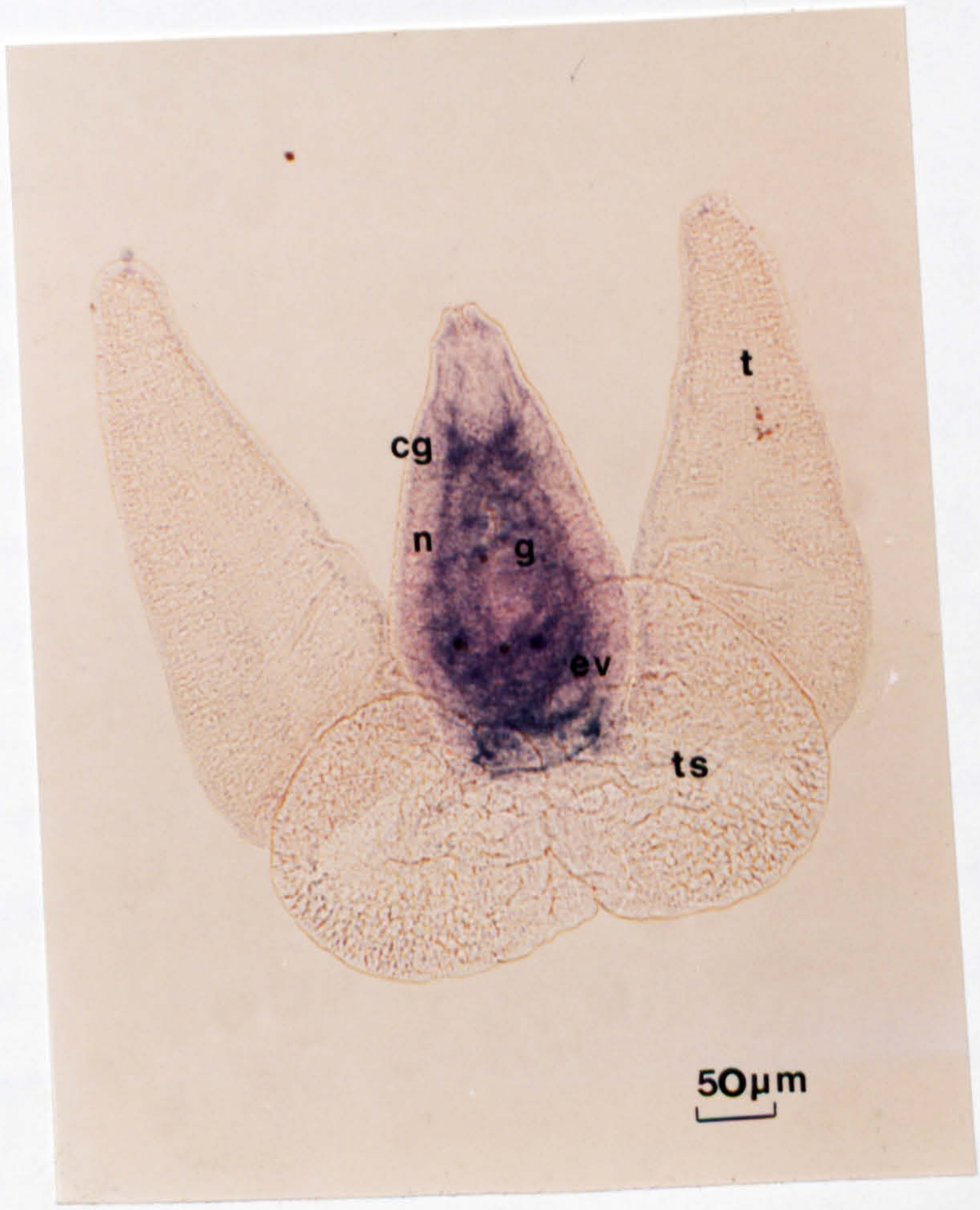


Fig 6.9. Semi-schematic drawing of the excretory system of *R. campanula* cercaria.

Fig 6.10 Cercaria after incubation in naphthol AS-BI phosphate medium demonstrating the positive reaction for acid phosphatase along the anterior gland (a.g), excretory vesicle (e.v) excretory tubule (e.t) and secretory vesicles (s.v) along the adjacent surfaces of the tail and a small number scattered in the tail.

Fig 6.11 Cercaria after incubation in Indoxyl acetate medium demonstrating positive reaction for non-specific esterase along the nervous system (n), gut (g) and excretory vesicle (e.v).


ag
sv
et
ev
t
ts
50 µm


cg
n
g
ev
t
ts
50µm

(d) Nervous system and " Chaetotaxy "

A pair of cerebral ganglia are situated immediately behind the penetration gland, connected by a median commissure. Each ganglion gives off three nerve cords anteriorly and three posteriorly. The antero - ventral, antero - lateral and antero - dorsal nerve cords diverge and supply the penetration organ and body wall. The posterior ventral, lateral and dorsal nerve cords diverge towards the body wall and pass to the posterior extremities of the body. The ventral pair is the best developed among the posterior pairs of nerves.

The pharynx is supplied with two ring nerves at its proximal and distal ends. These are connected by a pair of pharyngeal nerves branching off from the ventral trunks to the proximal ring nerve and distal ring nerve. The posterior end of the cercariae at the place of attachment to the tail-stem is supplied with one ring nerve from which nerves are supplied to the tail and tail-stem. A fine nerve runs along the dorso- lateral position of the tail, ending at the tip of the tail.

The anterior and posterior major nerves are joined by connective nerve strands. In the whole length of the cercarial body transverse commissures join the nerve strands in twelve places. The first commissure joins the anterior nerve strands at a short distance from the anterior openings. The second commissure is situated near the posterior border of the anterior organ and the

third joins the connectives at the level of the nerve ganglia. Three more commissures connect the posterior nerves before the proximal pharyngeal nerve ring near the ventral mouth. Another two nerve commissures connect the posterior nerves between the distal pharyngeal nerve ring and the posterior nerve ring at the base of the tail-stem.

Nucleated cell bodies or neurones which were described in *Diplostomum pseudospathaceum* by Niewiadomska and Moczon (1982) and in *Haplometra cylindracea* cercaria by Grabda- Kazubska and Moczon (1981); and subtegumental nerve plexuses which were described in *Schistosoma mansoni* cercaria by Bruckner and Voge (1974) and in *H. cylindracea* by Grabda-Kazubska and Moczon (1981) have not been observed in *R. campanula*.

Histochemically very clear positive reactions along the longitudinal strands, transverse commissures, ganglia and fine nerve fibres in the body, and along the margin of the furca of the cercaria, were observed for acetyl-cholinesterase (AChE) as dark brown microcrystals and for non-specific esterases as deep blue fine granules. (Fig 6.11 & 6.13). Further, it was observed that the use of inhibitor (10^{-4} M eserine) in the incubation medium eliminated the activity of cholinesterases along the entire nervous system in the control cercaria but that the cerebral ganglia continued to show a positive reaction to the indoxyl acetate method for non-specific esterases. This demonstrates that the

cerebral ganglia contain at least two types of esterases. All cholinesterase activity, however was abolished by exposure to 70°C for 5 minutes and other non - specific esterases at 90°C for 5 minutes.

The drawings of the nervous system are semi-schematised compilations of a number of fragments and were made with the aid of a drawing tube fixed to the eye-piece of the microscope. (Fig 6.12).

The number and position of the papillae were here described using conventional silver impregnation method (Fig. 6.15a,b) and compared with the papillae obtained by the scanning electronmicrographs.

(e) Reproductive system

The genital anlagen are clearly defined. A large oval mass of cells observed on the left side of the posterior end of the body probably gives rise to the cirrus pouch. Two pear shaped structures observed, one to the right of the primordium of the cirrus pouch, and the other anterior and to the right of the flexure of the excretory bladder, connected by a duct or cord of cells probably give rise to the testes (Fig 6.8 & 6.14). A rudimentary genital pore is situated 10µm from the posterior extremity. (Fig 6.5).

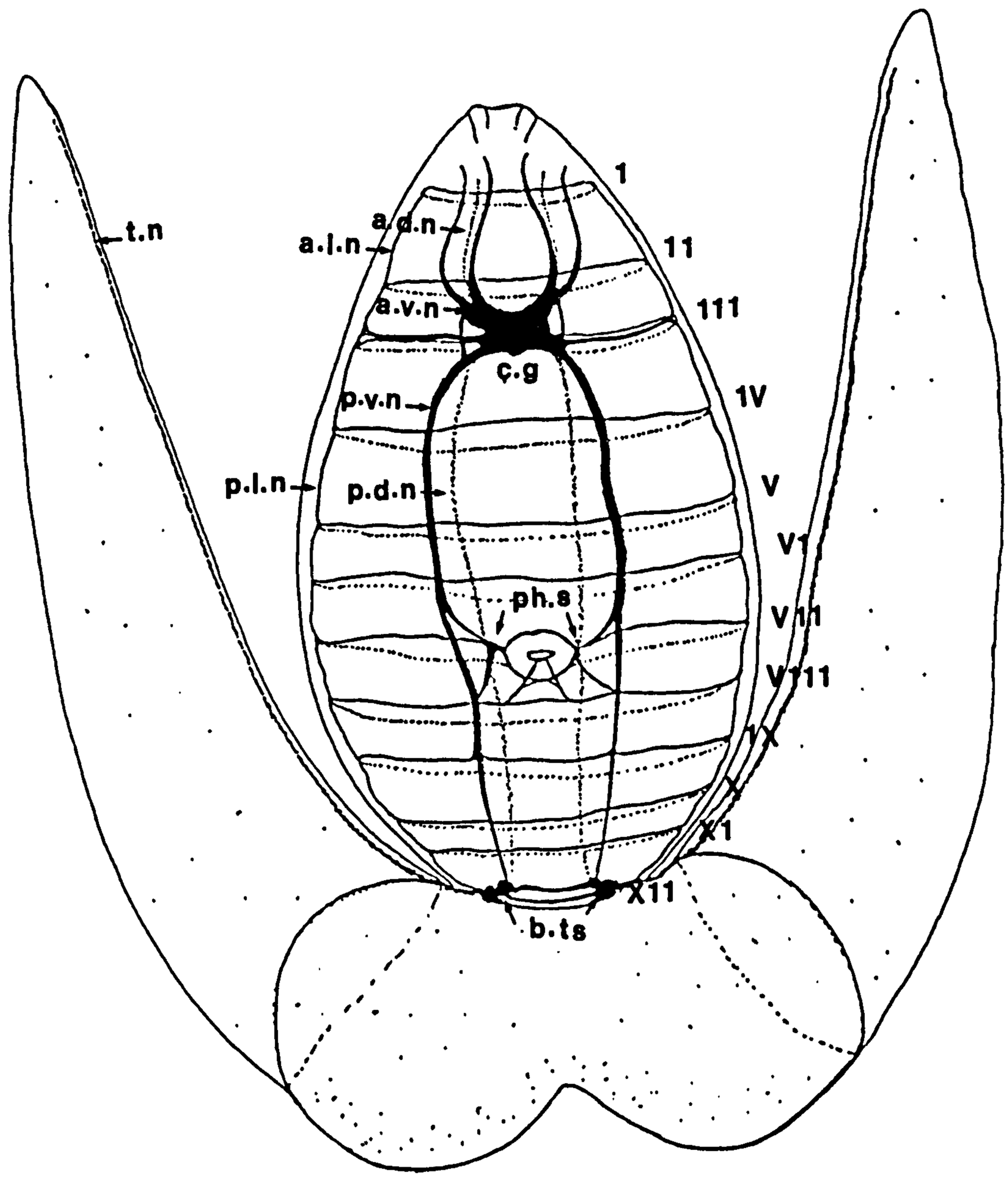


Fig 6.12. Semi-schematic drawing of the nervous system of *R. campanula* cercaria, ventral view.

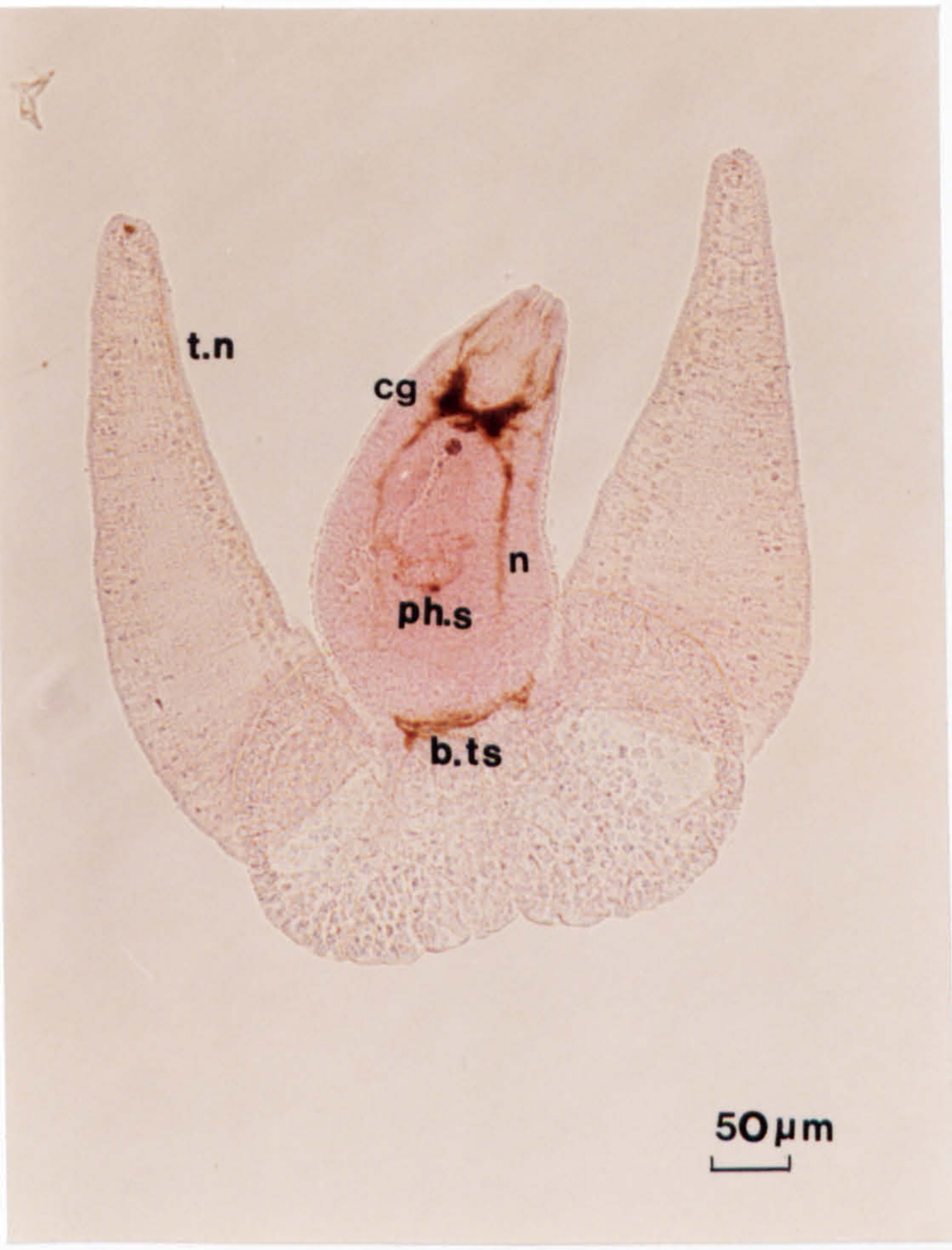
1 - X11 ... ring commissures.

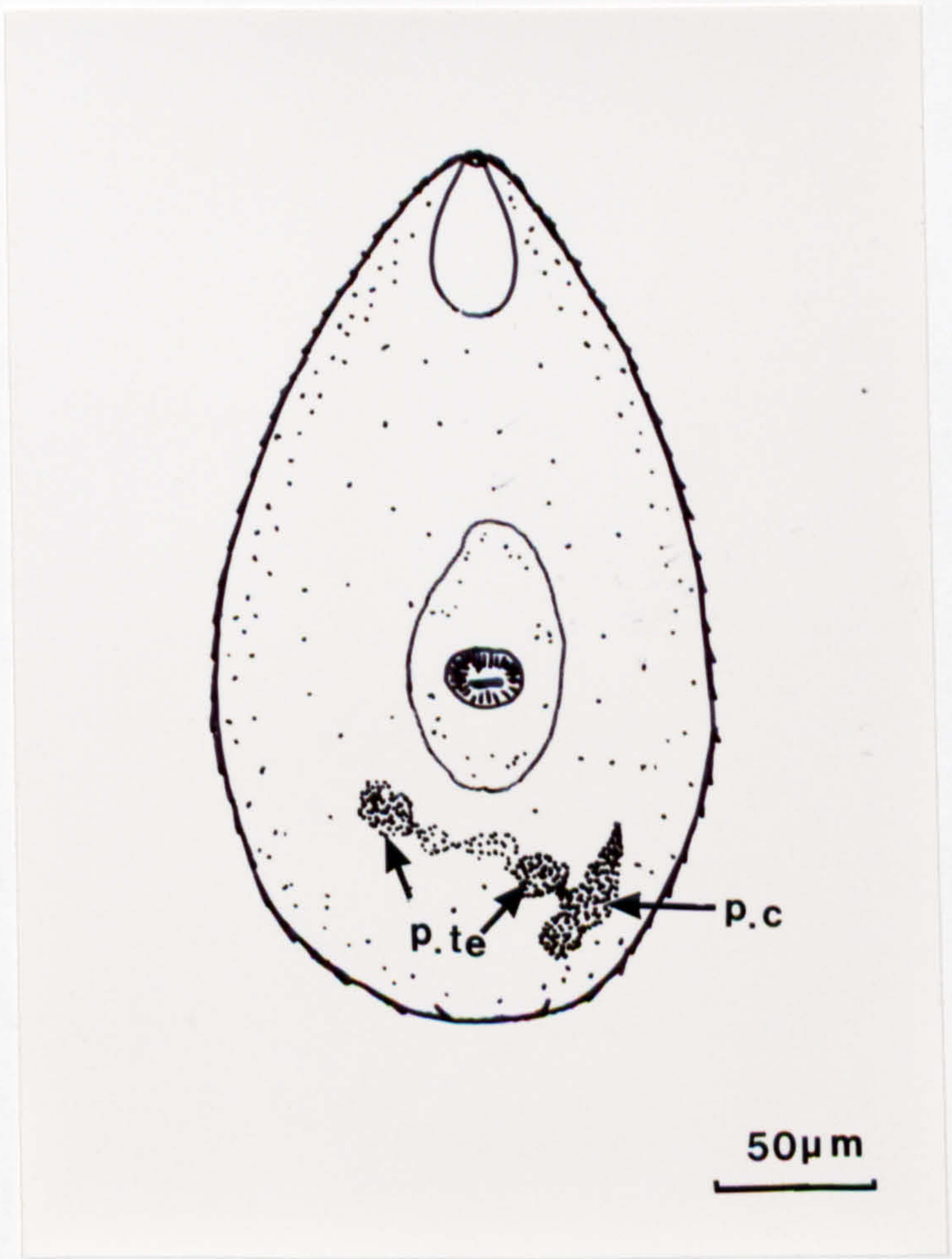
Fig 6.13 Cercaria after incubation in acetylthio-choline-iodide medium demonstrating the positive reaction for cholinesterase along the nervous system of cercaria. Note the cerebral ganglia (c.g), pharyngeal nerve supply (ph.s), body/tail-stem commissure (b.ts) and tail nerve cord (t.n).

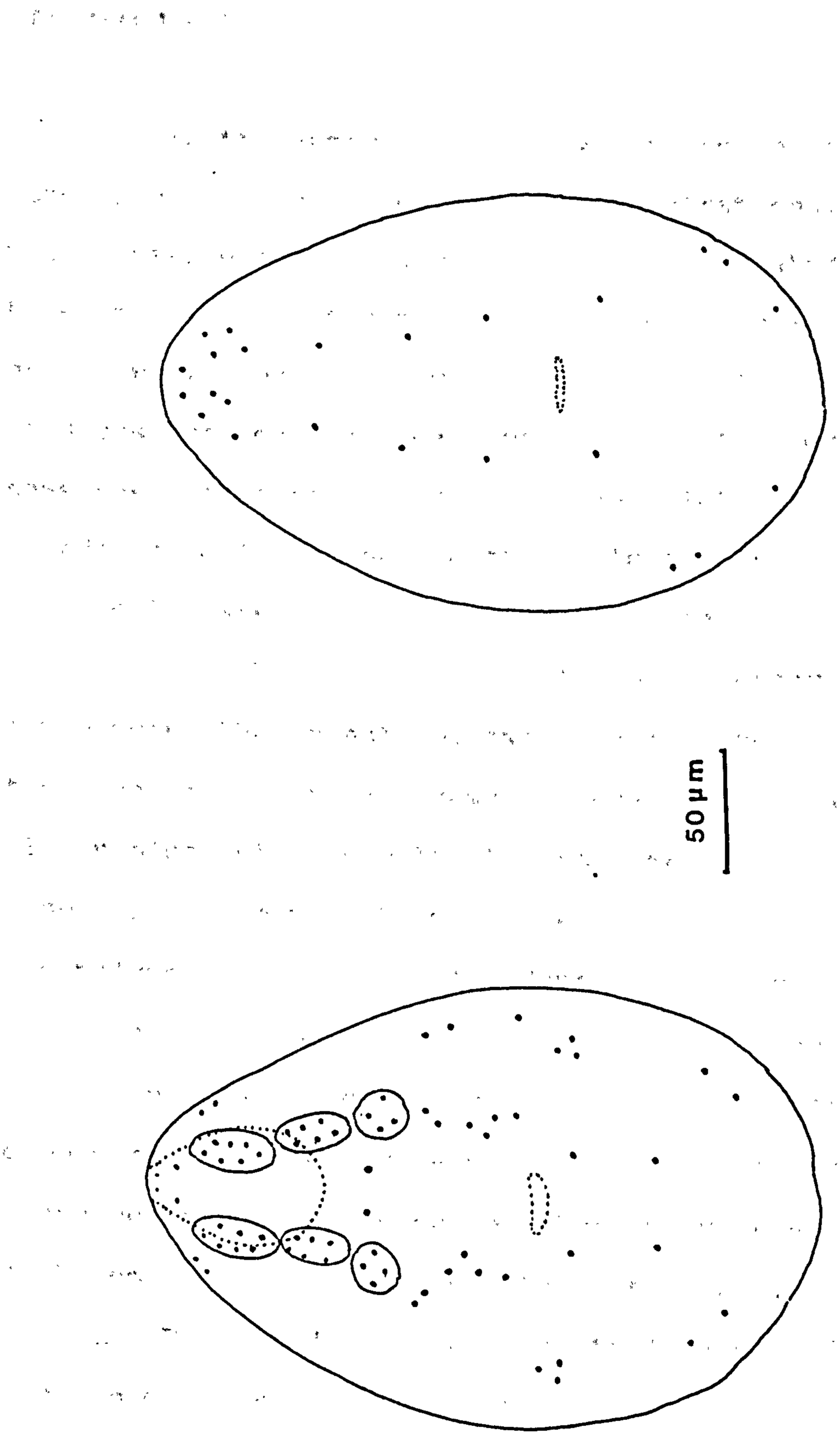
Fig 6.14 Semi-schematic drawing of the genital anlagen of cercaria.

p.c - primordium of cirrus pouch

p.te - primordium of testes


t.n
cg
n
ph.s
b.ts
50µm


p.te
p.c
50µm



(a) ventral view

(b) dorsal view

Fig 6.15. Distribution of papillae on the body of *R. campanula* cercaria

(f) Gland cells

In the anterior organ two types of gland cells were revealed by histochemical demonstrations. Acid-muco -polysccharide secreting cells were demonstrated by Alcian blue (blue) and mucin by alcoholic periodic acid and fast green (red) and Mallory 1 & 11 (faint blue). Two groups of ducts open outside, each consists of five ducts, which pass the secretions from fourteen laterally arranged glands, totalling twenty eight cells at different levels. Gland cells are unicellular and club-shaped.

In the parenchyma of the cercarial body, surrounding the intestine, two lateral, two dorsal and two ventral vertical rows of oval cells staining with alcian blue (blue) similar to the anterior glands were observed demonstrating the presence of acid mucopolysccharide (Fig 5.1). Some of these cells showed a positive reaction to alcoholic periodic acid and fast green (purple) and Mallory 1 & 11 (faint blue) demonstrating the presence of mucus. The openings of these cells were not observed clearly but are most likely to be opening individually outside the body.

The gland cells of the tail and tail-stem are described later in this chapter.

(g) Tail and Tail-stem

The furcae are highly contractile and are inserted on the antero-lateral surface of the tail-stem. These are easily detachable from the tail-stem. (Fig 6.15). The furcae are asymmetrical in cross-section and contain longitudinal muscles surrounded by a series of circular muscles.

The bilobed tail-stem attaches to the body of the cercariae by two sets of retractor muscles. They arise in the tegument at the distal end of the body, being radially arranged about the caudal excretory duct, and pass into the lobes of the tail-stem where they are inserted into the basement membrane. At this point the body of the cercaria detaches easily during the entry into the secondary host (Fig 6.17). The tail-stem is highly lobed and appears superficially as honey comb with many domed-shaped sensory papillae and secretory pores scattered on the adhesive zone (Fig 6.7).

The ultrastructural studies revealed that the outer epimerdal layer of the tail is continuous with that of the body but more highly convoluted near the tail-stem junction than along the tail and is distinct from those of the body. (Fig 6.18 & 6.20). The epidermal layer contains very few mitochondria, few round and oval shaped dense secretory bodies (V_2) and a number of elongated electron-opaque vesicles (V_5) in the region adjacent to the basement membrane. These opaque vesicles are however

more elongated and more numerous than the vesicles observed along the cytoplasmic layer of the body. (Fig 6.20).

A layer of circular muscles lies below the basement lamina at right angles to the longitudinal axis of the tail. These muscles occupy the folds of the epidermal layer and superficially these folds are seen as ridges running circumferentially around the tail. The longitudinal muscles of the tail are seen not to continue into the tail-stem. In the region of the tail and tail-stem attachment, many mitochondria with different sizes are present. The circular muscles continue into the tail-stem region (Fig 6.19 & 6.20). An array of dense bodies is found at the tail root (Fig 6.21). Below the longitudinal muscles and in the centre of the tail, parenchymatous cells with elongated cytoplasmic projections are arranged in a network. Also the oily droplets observed under the light microscope are likely to be lipid droplets. (Fig 6.20).

At the base of the tail-stem, groups of large cells with two types of contents, granular and agranular (Fig 6.16 & 6.18) are demonstrated by histochemical reactions. Alcoholic periodic acid and fast green demonstrated glycogen (greenish blue) and mucin (purplish red). Alcian blue demonstrated scattered acid mucopoly-saccharide (blue) and Mallory-Heidenhain's reaction showed mucus-secreting cells with nucleus (blue cells with red nucleus).

Secretory vesicles which show positive reactions to acid phosphatase are found in a single row in the innerside of the tail and a few are scattered along the furcae but there are none on the tail-stem (Fig 6.10). Mucus-secreting cells are found along the inner side of the tail in similar places to the acid phosphatase granules. Electron microscope studies revealed conical shaped cells in the inner side of the tail and most probably the mucus-secreting cells and acid phosphatase secreting area are along the anterior of these cells. This separation of the two regions is clearly visible on Fig.6.10 as the acid phosphatase reaction can be seen on the upper part of the cell.

These histochemical reactions for gland cells are observed on fully developed cercariae sectioned in the sporocyst tubules but not in the free-swimming cercariae. There may therefore be variations in the concentrations of the glandular contents and storage cells.

The centre of the tail-stem is fluid filled and during contraction and relaxation of the furcae, the tail is pushed in and out of the tail-stem. The space in the tail-stem acts as a reservoir for this peculiar movement of the tail. The function of the tail and tail-stem during swimming is discussed later in this chapter.

Both tail and tail-stem lack spines and have numerous pores opening outside with excretory pores at the tip of the tail. (Fig 6.22).

Fig 6.16 Scanning electron micrograph after removal of the tail showing the place of attachment of tail on the tail-stem (arrowed). Note the mucus-secreting (m.s) and glycogen - storage cells (g.s) inside the tail-stem.

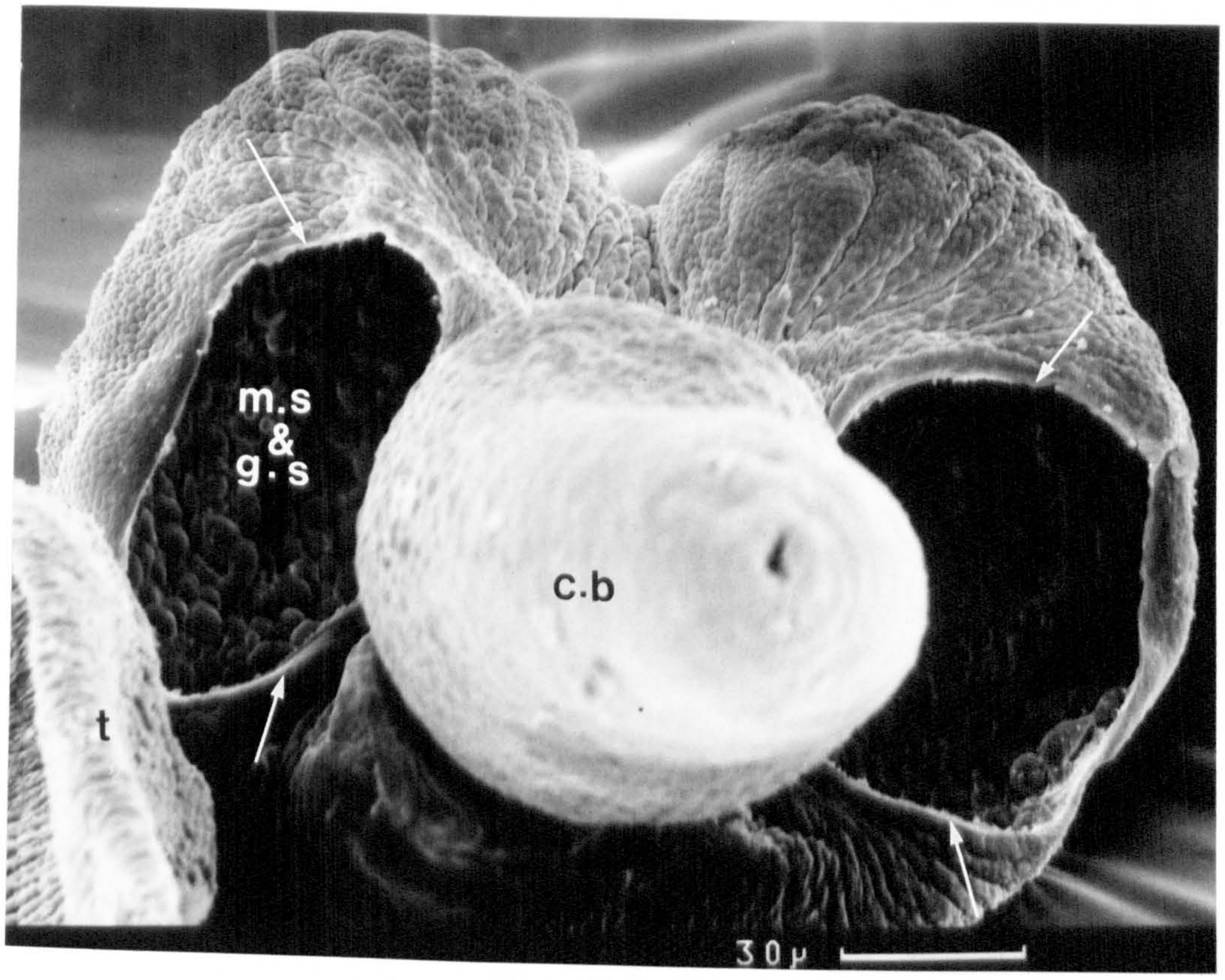
c.b .. cercarial body

Fig 6.17 Scanning electron micrograph of the cercarial tail-stem after removal of tail and body of the cercaria showing the place of attachment of these structures.

t.a ... tail attachment

b.a ... body attachment

c.b ... cercarial body


m.s
&
g.s
c.b
t
30μ

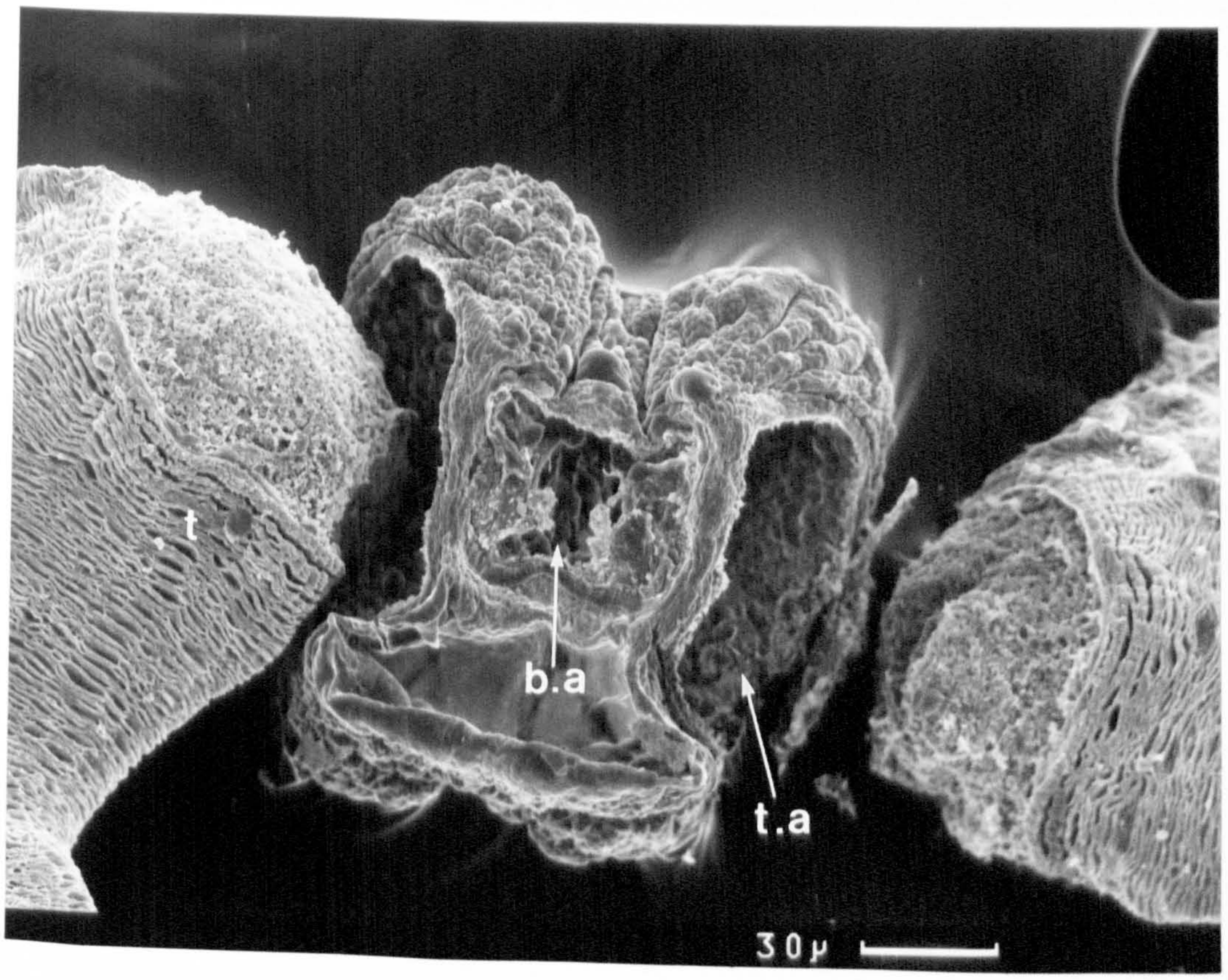

t
b.a
t.a
30μ

Fig 6.18 L.S of the cercaria stained in Toluidine blue showing the attachment of the tail (black arrow) and body of the cercaria (crossed black arrow) to the tail-stem (t.s). Also note the mucus-secreting (m.s) & glycogen-storage cells (g.s) lining the posterior region of the tail-stem and lack of cellular contents in the middle region of the tail-stem; distribution of cell bodies along the tail and the density variation along the proximal and distal surfaces of the tail (white arrow); thickness of the muscle layers along the body (crossed white arrow) of the cercaria and pharynx (p); highly convoluted tegument of the tail in the proximal end of the tail-stem and body.

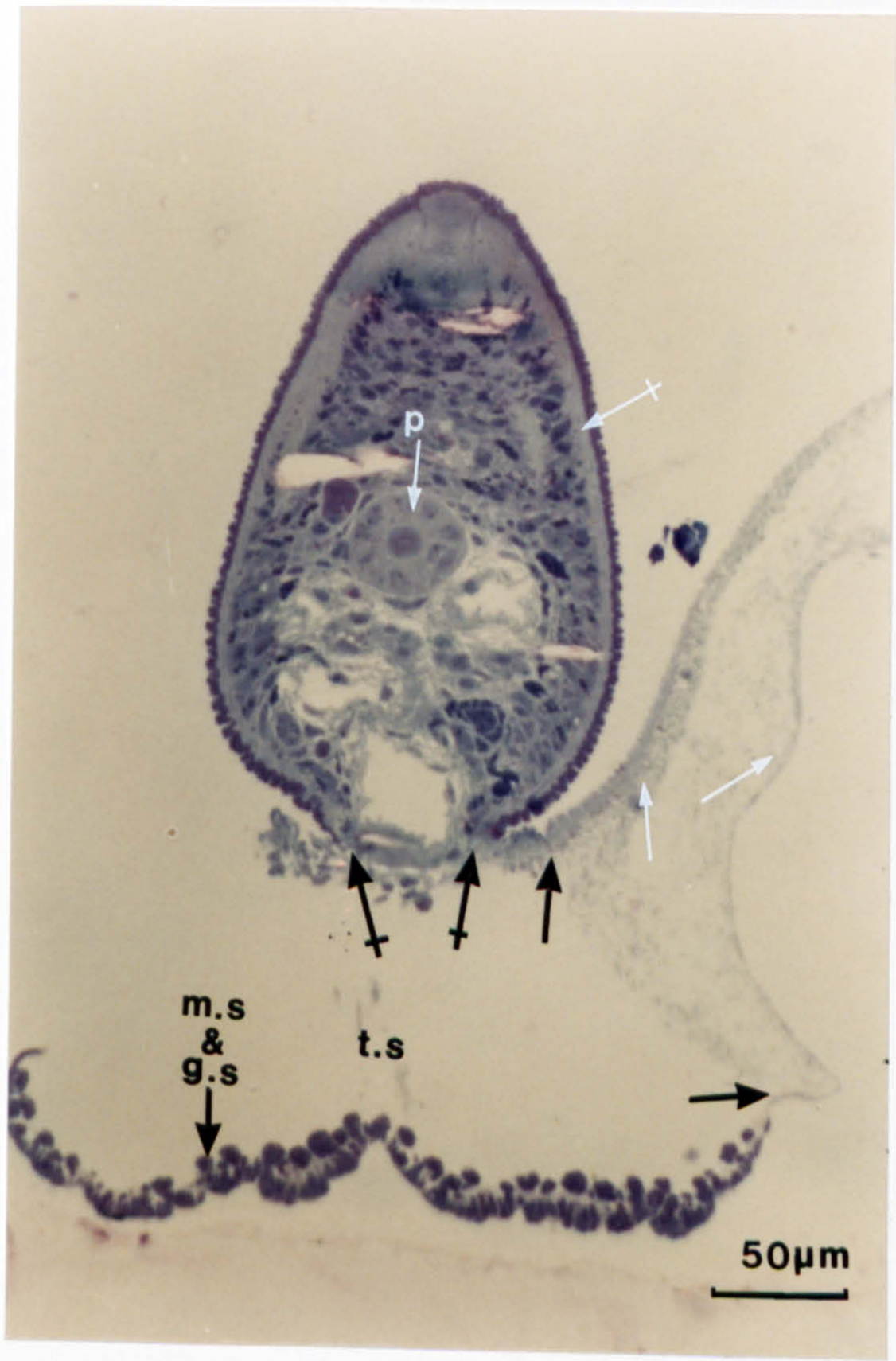

p
m.s
&
g.s
t.s
50µm

Fig 6.19 Transmission electron micrograph through the tail region near the tail-stem showing the highly folded epidermal layer (e.l) of the tail the net-work of cytoplasmic projections (c.p) in the centre of the tail, and the secretory vesicles (s.v) in the adjacent surface of the tail to the body of the cercaria.

l.m .. longitudinal muscle

Scale bar = 2µm

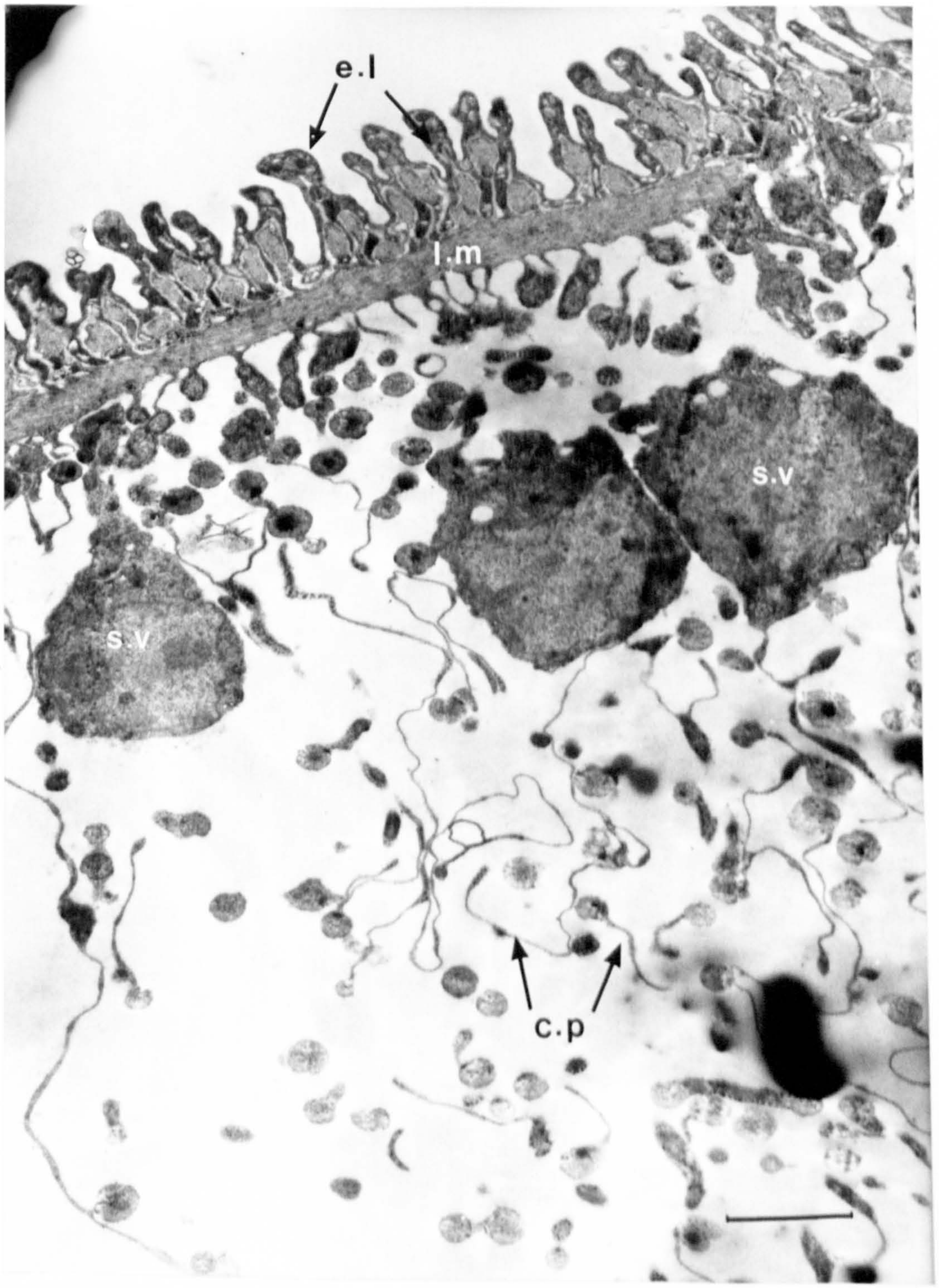

e.l
l.m
s.v
s.v
c.p

Fig 6.20 Transmission electron micrograph of the tail, tail-stem attachment showing the highly folded tegument (teg) with circular muscle blocks inside the folds (c.m). Note the non-continuity of the longitudinal muscle (l.m) into the tail-stem (arrowed); the high concentration of mitochondria (mit) in the muscle layer and below the muscle layers (crossed arrow) and variation in size and shape; more electron-opaque vesicles (V_5) in the cytoplasmic layer.

Scale bar = 500nm

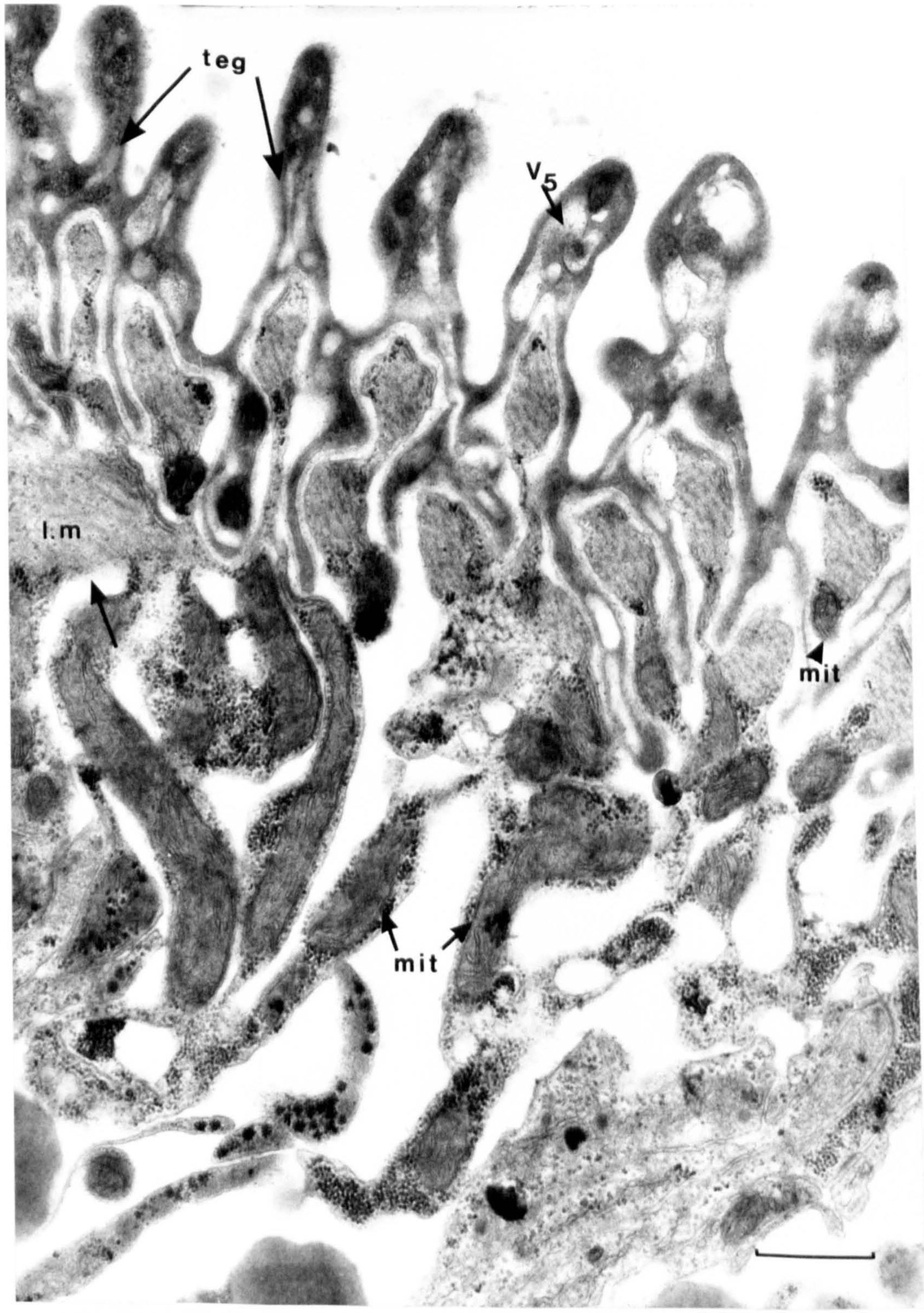
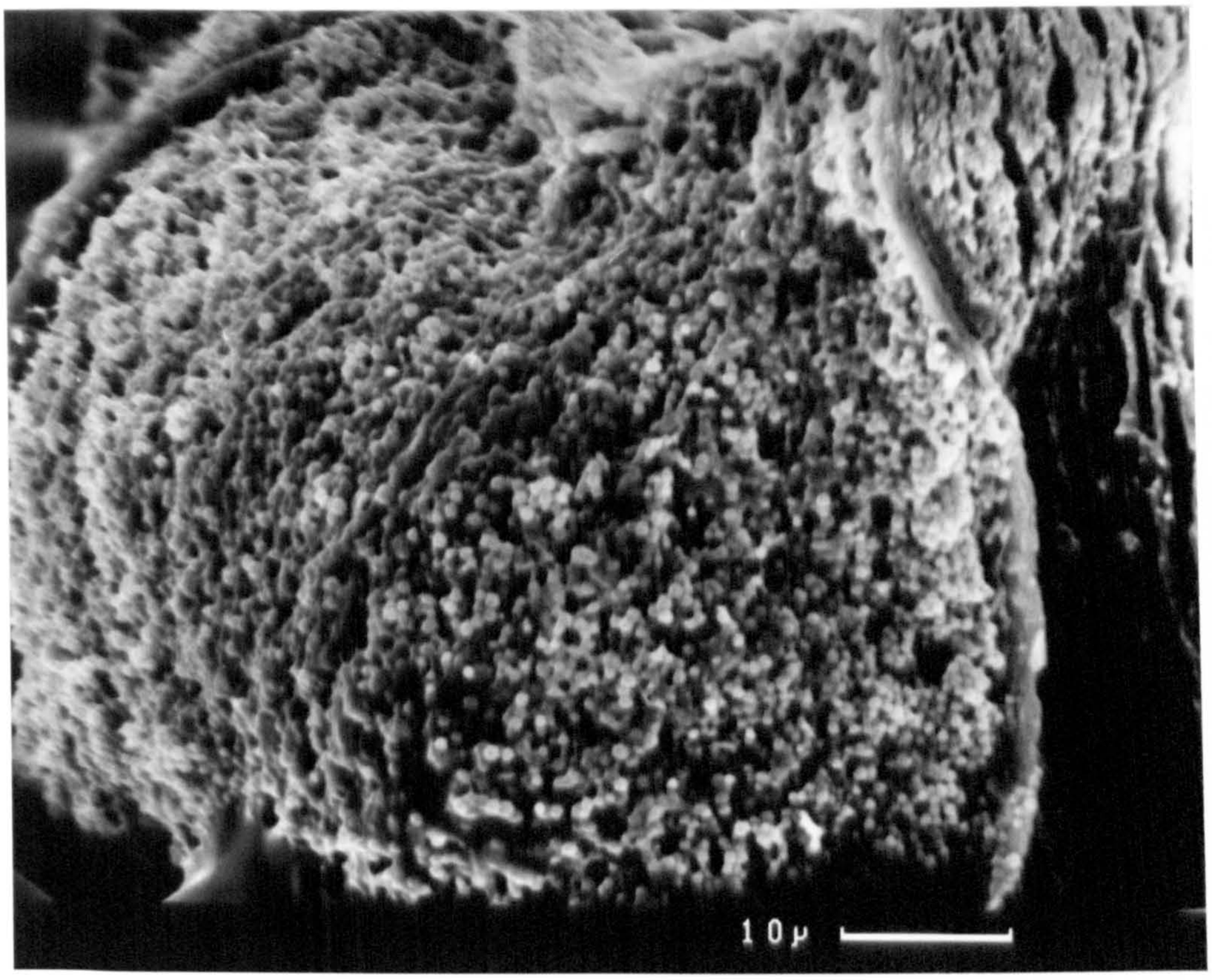
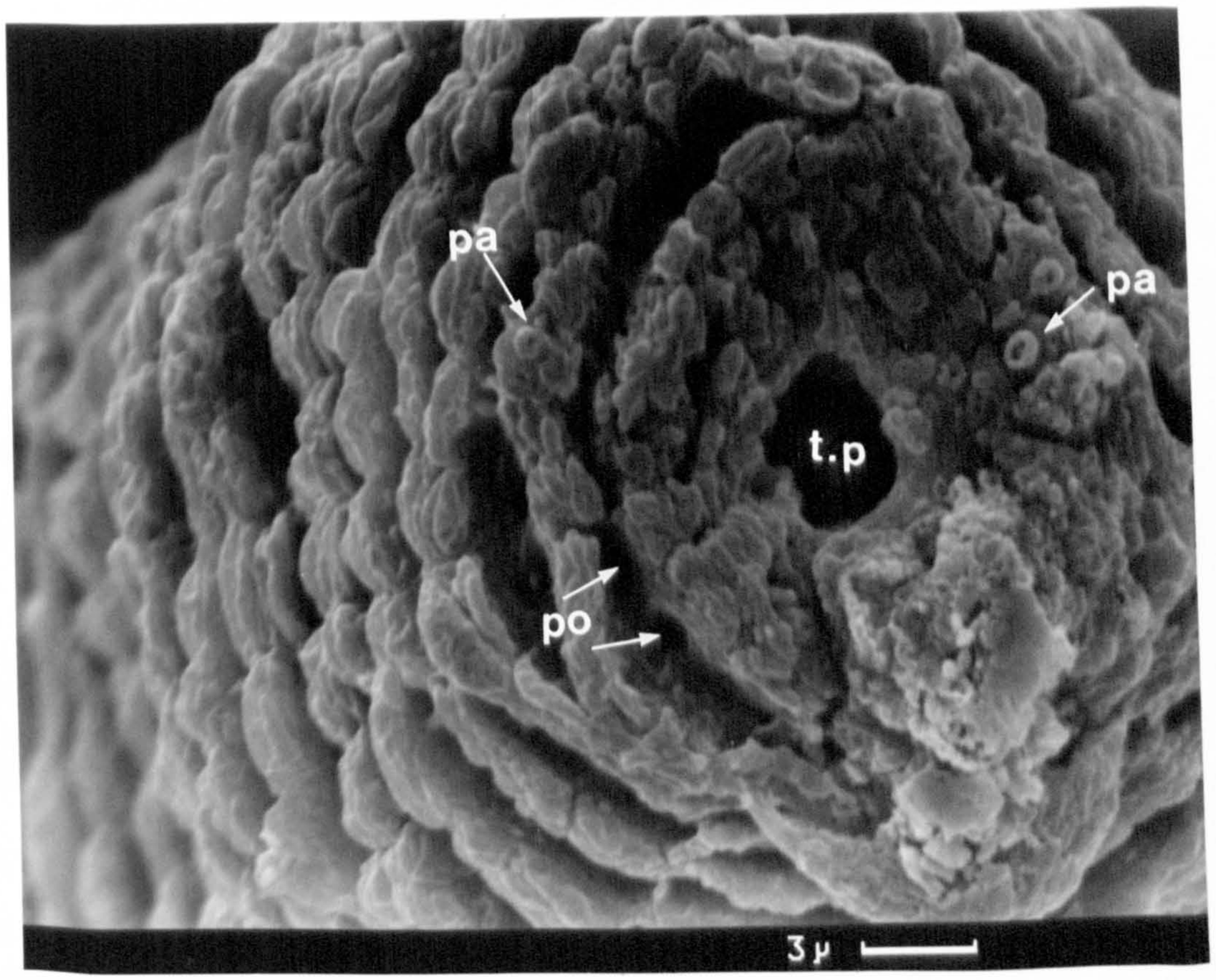

teg
teg
V5
l.m
mit
mit

Fig 6.21 Scanning electron micrograph of posterior end (fixing end to the tail-stem) of tail showing the array of dense bodies.

Fig 6.22 Scanning electron micrograph showing the excretory pore (e.p) at the tip of the tail. Note the papillae (pa) and pores (po) in the region.


10μ


pa
pa
t.p
po
3μ

6.2.2 Shedding pattern of the cercariae from the molluscan host

The cercariae are forcibly discharged in small groups, depending on the state of infection of the mussels from tens to hundreds, through the exhalent siphon. Liberation of cercariae from the mussels kept in a natural light source has been discussed in Chapter 4, and the data concerning the number of cercariae liberated from 15 infected mussels caught between March and August, 1985 is given in Table 4.2. There is a general tendency for peak liberation of more than 500 cercariae per day to be followed by troughs with few or no liberated cercariae. The duration of peaks and troughs in the rate of liberation and the number of days on which liberation occurs, both vary considerably in the individual mussels depending upon the state of infection. The mean number of cercariae liberated per hour/day from four individual mussels during a continuous period of more than 60 days are shown on the graphs in Fig 4.1.

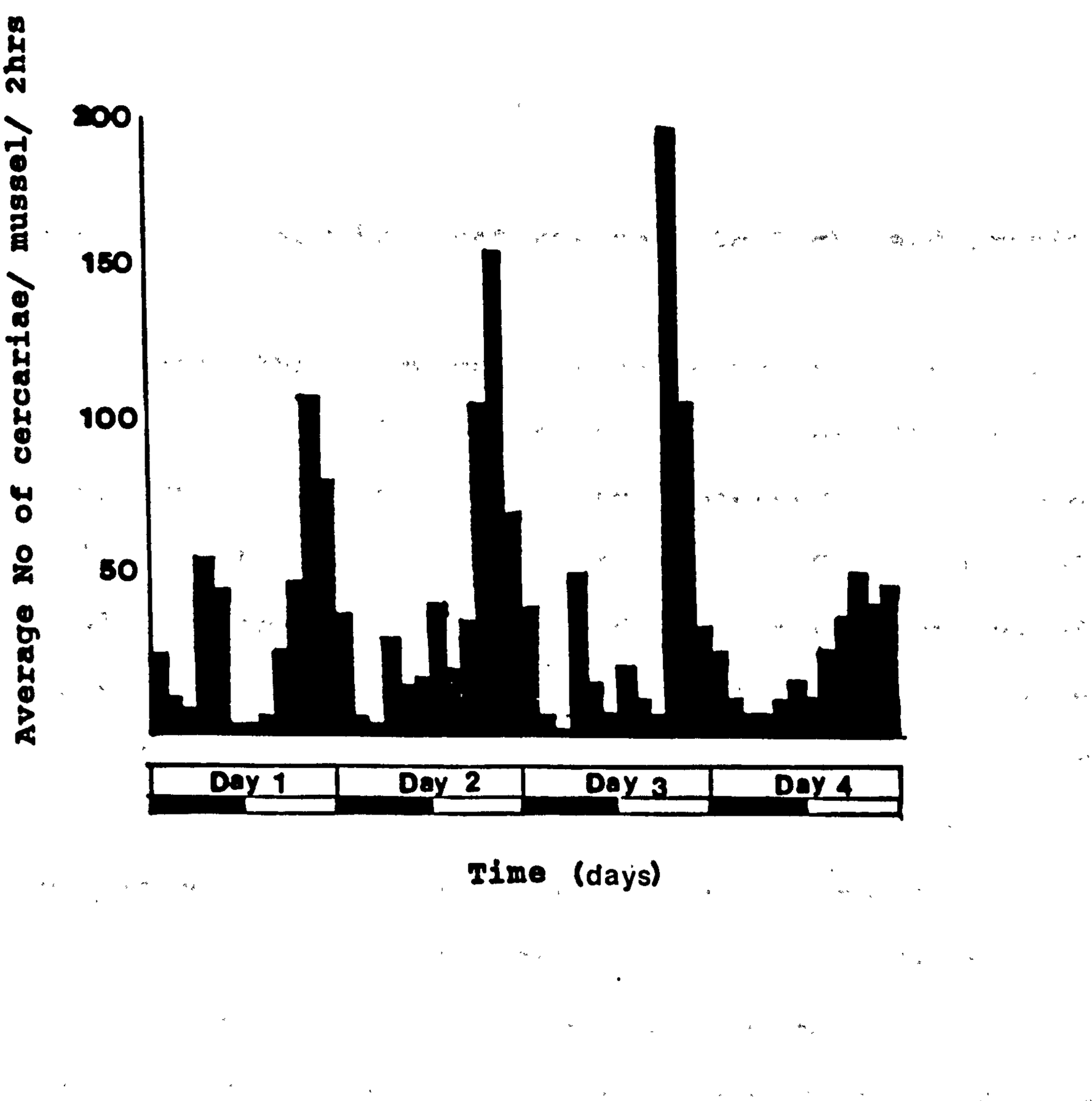
The emergence of cercariae from mussels kept in spring conditions (12L:12D) and summer conditions (16L:8D) have been illustrated by the histograms (Fig 6.23 & 6.24). The peak emergence of cercariae from the mussels kept in summer conditions (16L:8D) was noted after 4-6h of light and the infrequent second peak after 2-4h of darkness. The peak emergence of cercariae during spring conditions was noted after 6-8h of light except

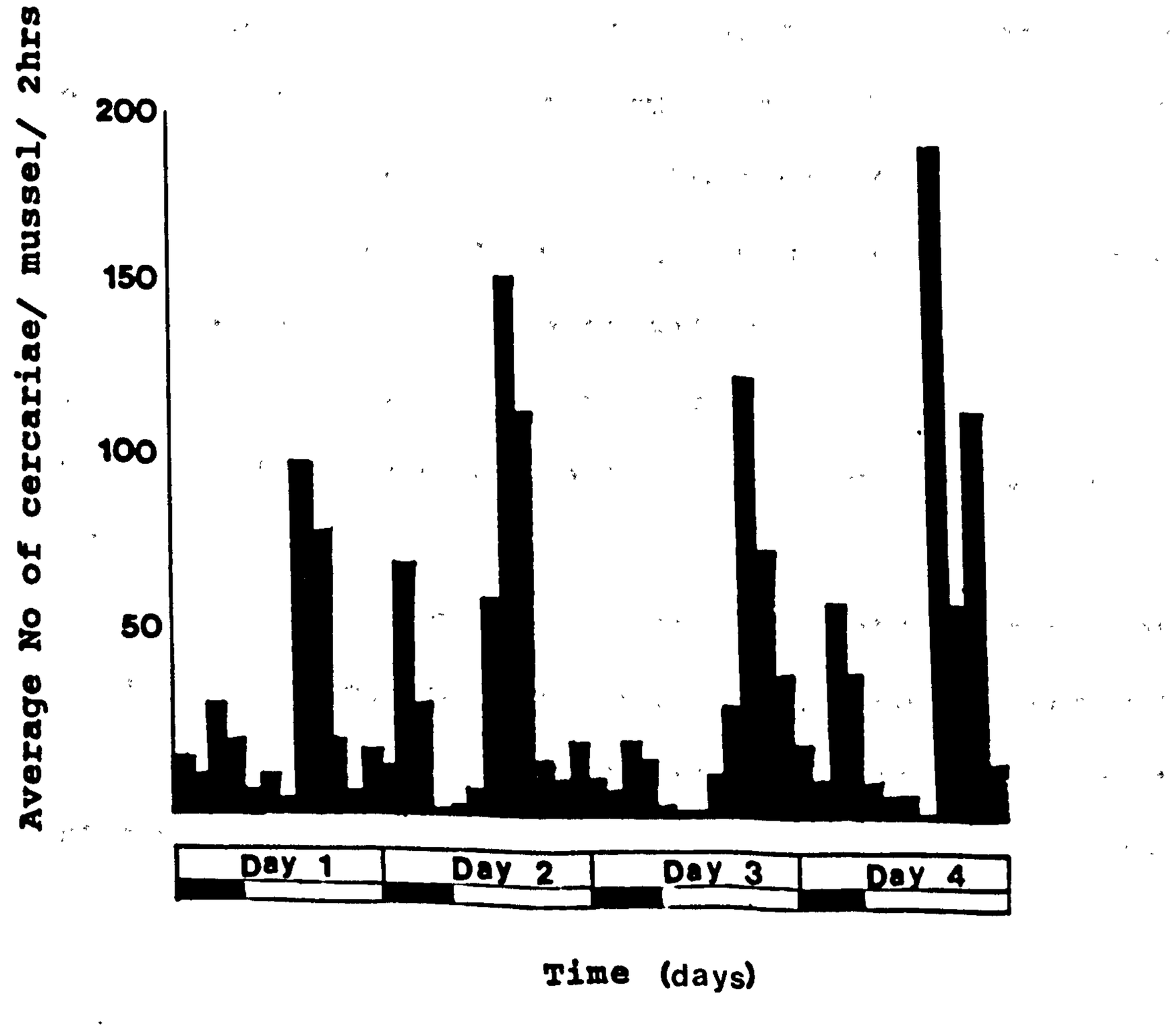
for a few small peak emergences during dark periods after 6h of darkness. The readings were taken continuously for 4 days and repeated for six individual infected mussels. In both the experiments the mussels were kept at constant temperature and in a constant intensity of light throughout the period of illumination.

The effect of temperature and its fluctuations on the mussels has also been clearly observed. Each abrupt rise in temperature (11°C-18°C) is immediately followed by a very active emission of cercariae whilst transfer of mussels to cold water (9°C-11°C) completely stops the emission.

Fig 6.23 Average number of cercariae emerged per mussel at 2h interval for 4 days. Days are from 1800h with 12h light and 12h dark (spring condition).

Fig 6.24 Average number of cercariae emerged per mussel at 2h interval for 4 days. Days are from 1800h to 1800h with 16h light and 8h dark. (summer condition)


Average No of cercariae/ mussel/ 2hrs
200
150
100
50
Day 1
Day 2
Day 3
Day 4
Time (days)


Average No of cercariae/ mussel/ 2hrs
200
150
100
50
Day 1
Day 2
Day 3
Day 4
Time (days)

6.2.3 Cercarial survival and response to light and gravity

From the survival experiments 50% of the cercariae survived in water at 15°C for 45 - 50 h. The maximum survival time was observed in those cercariae kept in 0.15% saline solution. The times of survival of the cercariae in different percentages of saline solution, Tyrode solution and water are given in Table 6.2 for three different temperatures. The time of survival decreased with increase in temperature. Even though some of the cercariae survived more than the specified time, their activity was drastically reduced and most of them were found in the resting phase at the bottom of the container. However, some of the cercariae died within a few hours of their release from the mussels.

In this experimental design, two factors which may possibly invalidate the results are the accumulation of toxic waste products and the shortage of oxygen in the water, as they were kept in containers for a long period of time. However the volume of water used was relatively large, with a considerable area exposed to the air, and it is felt that these factors are unlikely to be limiting.

Cercariae showed both photokinetic and phototactic behaviour when observed in culture tubes with light shining at different angles. When light was shone on resting cercariae they began an active swimming phase

and when light was shone on active swimming cercariae the swimming phase was prolonged. Conversely when the light intensity was decreased on resting cercariae, it did not initiate any swimming activity and when decreased on swimming cercariae it tended to inhibit swimming motions.

The experiments using test-tubes covered in different regions revealed no definite results of positive photoresponse or positive georesponse. Cercariae were observed to distribute more or less evenly along the tube in all three regions in a fully covered test-tube and higher percentages in the lower one third of the tube in TC 11 (not covered) and TC 111 (lower two thirds covered). (Table 6.3). These results of phototactic and photokinetic behaviour and neutral response to uniform light and gravity reveal that cercariae are activated by light when released from darkness. This could be clearly demonstrated by the tendency of cercariae to swim upwards as soon as they are released from the mussel. The later behaviour of neutral response to light and gravity enables the cercariae to maintain their position in the mid and bottom waters of the river, thus increasing the probability of entry into the secondary host, the minnows, which occupy these waters during their visit to the mussel beds in the spawing season.

Table 6.2

Cercarial survival in different solutions

(time in hours)

Temp.	Water	Tyrode sol.	Saline			
			0.15%	0.30%	0.45%	0.65%
15°C	45-50	60-70	>95	75-80	50-55	50-55
20°C	30-35	55-60	>95	75-80	40-50	40-50
25°C	<25	35-45	30-40	30-40	30-40	25-30

* Times measured in different solutions were for 50% survival of the cercariae.

Table 6.3

Photo- and geo-responses of cercariae

Test condition	Location	% Distribution
1 (Fully covered)	A	35.60
	B	32.90
	C	31.50
11 (Not covered)	A	28.00
	B	28.00
	C	44.00
111 (Lower 2/3rds covered)	A	16.00
	B	24.00
	C	60.00
1V (Upper 2/3rds covered)	A	41.90
	B	24.30
	C	33.80

6.2.4 Cercarial swimming behaviour

The pattern of cercarial movement is illustrated diagramatically in Fig 6.25 and actual photographic records are given in Fig 6.26 and Fig 6.27 for comparison. Due to technical difficulties, the photographs could not be taken during vertical movements. They show cercariae swimming diagonally upwards and downwards. The photographic record presented here is based on a series of successive movements rather than the continuous sequence of a single cercaria.

For descriptive purposes, cercarial activity is divided into active swimming, dropping, creeping and resting phases. The cercariae start swimming towards the surface of the water, immediately following liberation from the mussel. The most striking feature in their locomotion is the enormous contraction and expansion of the tail furcae. During active swimming the furcae can be extended to nearly eight times the length of the body. (Fig 6.26 (7,8)).

During upward active swimming the cercaria swims tail-first with the body hanging downward. (Fig 6.26). The angle between the tail and tail-stem (usually varying between 90° to 180°) determines the speed of swimming. When the cercaria moves faster, the tail furcae are held almost parallel to each other with the tip of the furcae extended outward in the form of a sigmoid curve (Fig 6.26 (9); 6.25 (1)a). The smaller the

angle between the tail and tail-stem, the faster the cercaria moves through the water. Gradually, as it reaches the surface, the furcae are stretched, probably increasing the buoyancy of the cercaria (Fig 6.26 (10); 6.25 (1)c). In swimming, the long axis of the body usually assumes a vertical position and the upward swimming paths are generally vertical in direction but can follow paths in any upward direction.

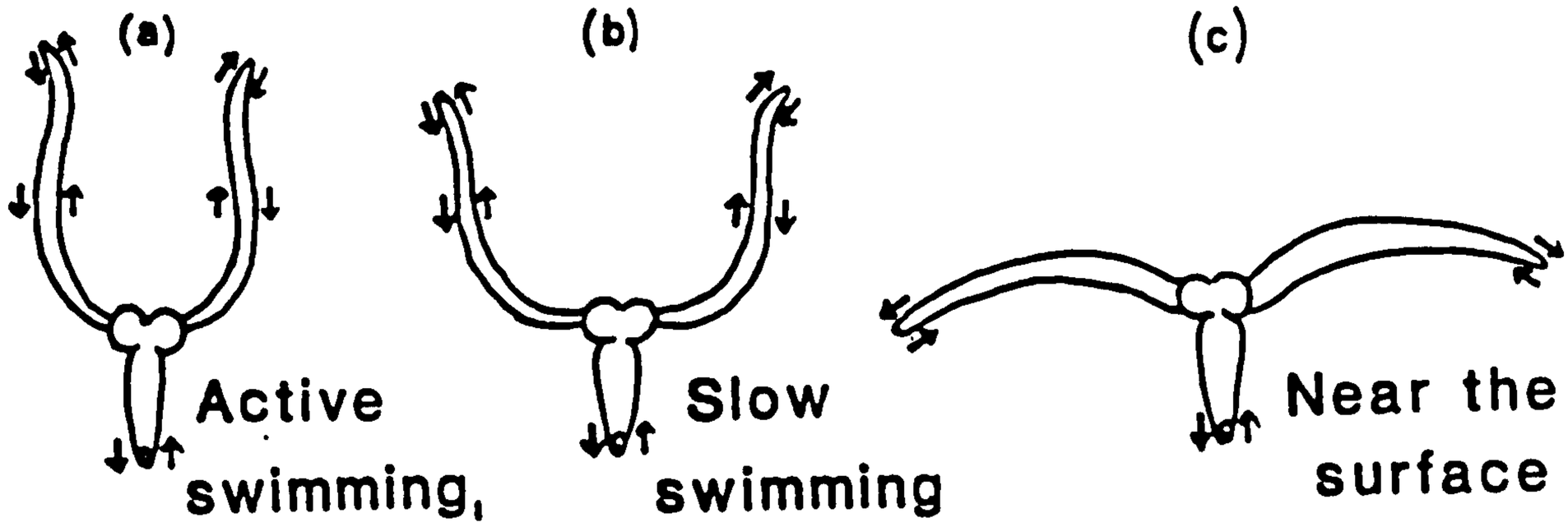
A burst of swimming is often followed by a period of passive dropping or active downward swimming (Fig 6.25 (11)a-c). During passive dropping, the cercaria sinks head-first through the water with the two tail furcae folded in a circle (Fig 6.25 (11)a). Sometimes the cercaria gradually drops tail-stem first through the water with the body and furcae held upwards (Fig 6.25 (11)b). In both these circumstances gradual tail and body movements are noticeable. It can also actively swim downwards either keeping the two tail furcae and body pointing downwards (Fig 6.27) or keeping them upwards (Fig 6.25(11)c). The two tail furcae are spread out laterally as it reaches the bottom and are thought to retard the rate of fall of the descending cercaria. Swimming activity can be renewed when the cercaria hits the bottom of its habitat, after a motionless pause on the habitat bottom (the resting phase) or actually during a dropping phase. During the active swimming the body undergoes an undulating movement extending to nearly twice the size of the body.

The tail-stem is bilobed and structurally different from the tail, lined inside with mucus-secreting and glycogen-storage cells with a central lumen (Fig 6.16 & 6.18). During contraction and extension of the tail, the central lumen of the tail is pumped in and out of the tail-stem and the central space presumably acts as a reservoir. The tail-stem undergoes a slight contraction and relaxation but this is barely noticeable (Fig 6.28).

DIAGRAMATIC REPRESENTATION OF CERCARIAL SWIMMING BEHAVIOUR

Vertical swimming

(1) Upward movement



(11) Downward movement

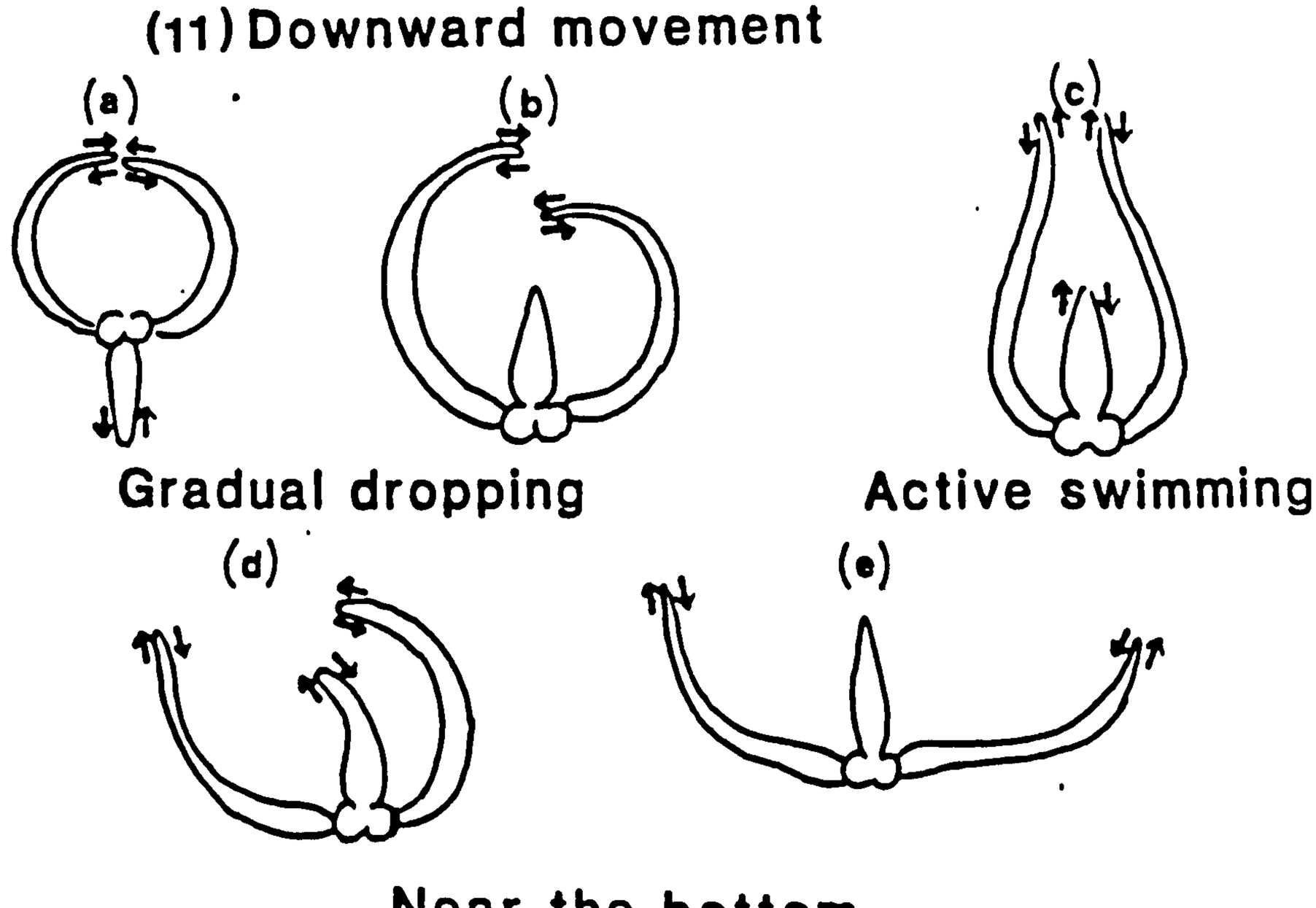
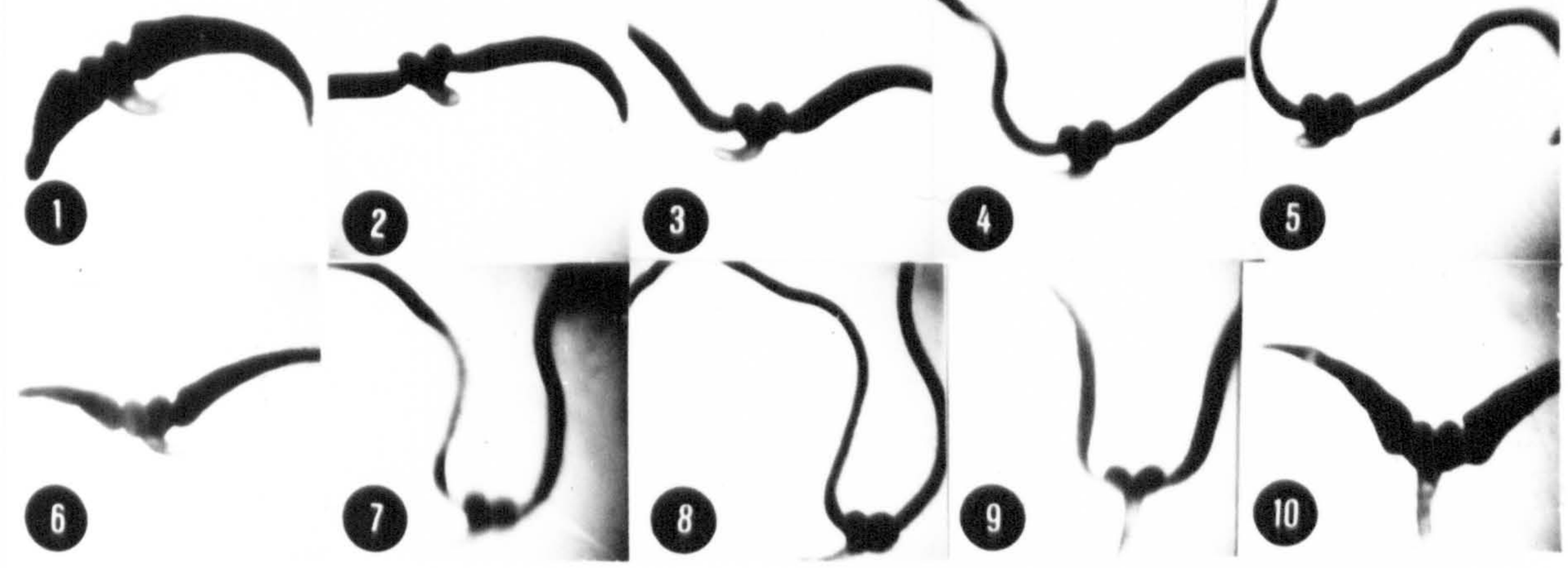


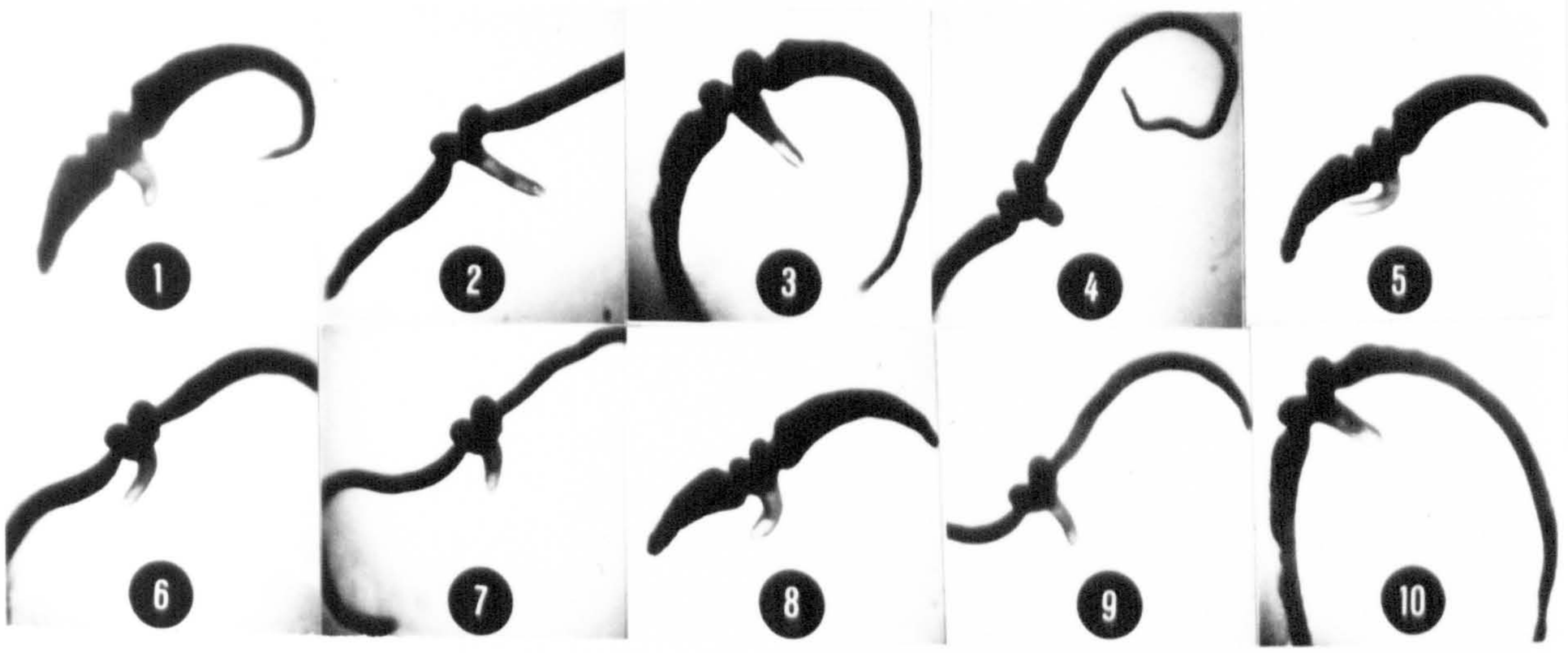
Fig 6.25. The small arrows indicate the direction of movement of the tail and body of the cercaria.

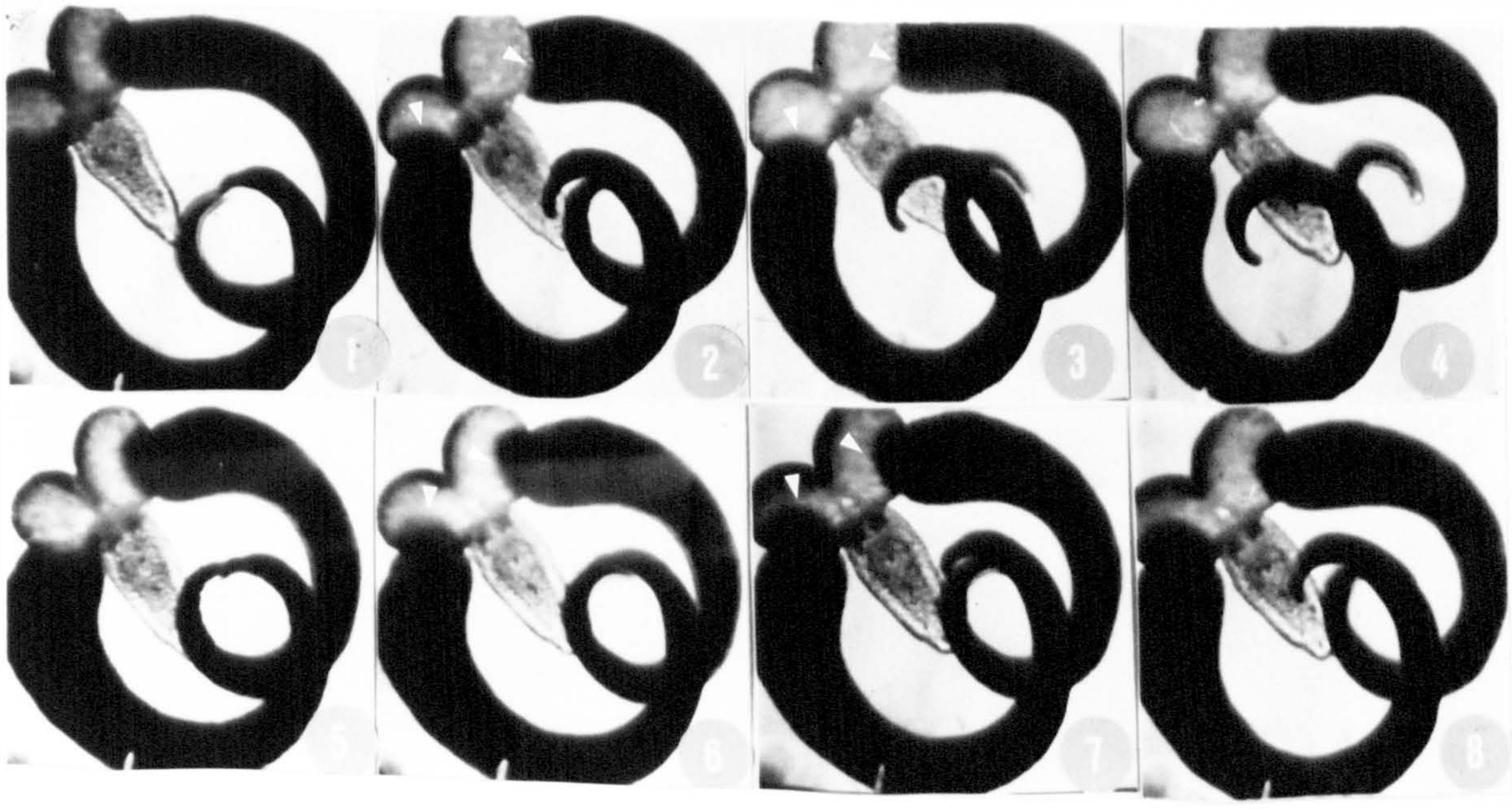
Fig 6.26 Photographic records of upward swimming cercaria

Fig 6.27 Photographic records of downward swimming cercaria

Fig 6.28 Photographs showing the participation of tail-stem during cercarial tail activity.
(The arrows indicate the posterior end of cercarial tail pushing into the tail-stem during contraction of the tail).


1
2
3
4
5
6
7
8
9
10


1
2
3
4
5
6
7
8
9
10


1
2
3
4
5
6
7
8

6.3 Discussion

The study of the surface structures of cercariae provides valuable data for the understanding of their functional morphology and supplements the data obtained by the light microscope necessary for the solution of taxonomic problems (Busta 1985). Investigations by the author has revealed that the surface structures of cercariae of the family Bucephalidae have not been described in the literature except for brief descriptions using light microscopy (Woodhead 1929, 1930; Kniskern, 1952b; Howell, 1966; Matthews 1972, 1973, 1974; Baturo 1977; Chernogorenko 1983).

The epidermis in different regions of the body of the cercaria of *R. campanula* reveals striking differences between the anterior and posterior of body, tail and tail-stem, which explain the significant role of the epidermis in the free-living stage of the cerariae. Similar descriptions of regional variations in the epidermis in different species of cercariae have been published by Erasmus (1967b), Threadgold (1968), Rees (1975), Rees & Day (1976) and Ogba (1982).

Although the epidermis of emerged cercariae is fundamentally the same as in all other species which have been examined, there are variations at the structural level. The main body of the cercaria of *R. campanula* contains four types of tegumental secretory bodies and the tail contains only one type. The number of types of

secretory bodies and their distributions is noted to vary in different species and also in different regions of the body. The cercaria of *Acanthatrium oregonense* has three types of secretory bodies (Belton & Harris 1967), the cercaria of *Fasciola hepatica* (Dixon 1966) and *Parochis acanthus* (Rees 1967) four and cercaria of *Cryptocotyle lingua* (Rees & Day 1976) five.

These epidermal secretory bodies are formed in specialised cells in the parenchyma and are discharged into the outer cytoplasmic layer. Each specialised cell contains either one or more types of secretory bodies. The passage of secretory bodies into the outer cytoplasmic layer of the epidermis has been observed in various cercariae (Stirewalt 1963; Mercer & Dixon 1967; Matricon- Gondran 1971; Rees & Day 1976).

According to Rees and Day (1976) the variations of the secretory bodies in number and distribution are due to the functions they perform in the cercarial life as well as in other stages of development. They suggest that the secretions from these bodies in the cercariae give protection whilst burrowing through the fish tissues to the site of encystment and also play some part in the formation of the cyst wall. Also the sequential appearance of these secretory bodies in the tegument and the relative frequency of their numbers seems to be in response to specific environmental changes and may be of considerable immunological significance (Smyth & Halton 1983). Although tegumental secretory bodies are universal

in trematodes, their functional significance is largely undetermined.

A glycocalyx (surface coat) on the cercaria of *Schistosoma mansoni* (Morris 1971) and on the juvenile *Fasciola hepatica* (Hanna 1980) is reported to be contributed by the secretory bodies in the epidermis. In the cercaria of *R. campanula* as in *Cryptocotyle ligua* (Rees 1974, Rees & Day 1976), no surface coat was observed in the free swimming cercariae but in the electron micrographs release of the contents to the surface layer is noted in a few places in *R. campanula*. It may possibly become evenly distributed and form a protective barrier against enzyme activity as the cercariae enter through the mouth and encyst in the cephalic region of the fish.

Acid mucopolysaccharides have been recorded from the epidermis of different cercariae (Dixon 1966; Belton & Harris 1967) and adult digenea (Lee 1966; Erasmus 1967) where they also contribute to a surface coat. In the present study, in *R. campanula* an acid mucopolysaccharide coat is noted in the developed cercariae in the molluscan host (Fig 6.8) and may be performing a protective function when cercariae are released into the molluscan haemocoel. The cercariae of *R. campanula* are released from *A. anatina* the molluscan host, in batches depending on the activity of the mussel and environmental conditions. Therefore, it is most likely that the cercariae released from the sporocyst tubules have to be

stored temporarily in the haemocoel and thus need a protective coat.

Unlike the body of the cercaria of *R. campanula* the tail contains only one type of epidermal secretory body similar to *Cercaria pectinata* (Matricon-Gondran 1971) and *Cryptocotyle lingua* (Rees 1977). The tail is exposed only for a short time to the enzymes in the molluscan host but there is no evidence of the discharge of these vesicular bodies or of a protective coat. Also more of the elongated clear vesicular secretory bodies are present in the cercarial tail than in the body of the free swimming cercariae. Rees (1977) noted that these clear vesicular bodies move between the outer cytoplasmic epidermis and basement lamina forming the caudal dilation in the developing cercaria of *C. lingua* in the mollusc. However, in the cercaria of *R. campanula* the clear vesicular bodies were scattered in the epidermis rather than forming a layer between the basal lamina and the outer cytoplasmic epidermis.

The variation in size and number in the distribution of mitochondria in different parts of the body of the cercaria clearly corresponds to the energy requirements and related functions of different regions of the tegument. Robinson and Halton (1983) in the trematode of *Corrigia vitta* and Hamajima et al (1982) in the different stages of *Paragonimus* sp. have documented the regional specialisation in the form and function of mitochondria and suggested that they may even be

functioning as anaerobic and aerobic mitochondria depending on the availability of oxygen.

Only a few small mitochondria are found scattered in the epidermis of the body and in the tail. Similarly, mitochondria in the epidermis have been reported on cercariae (Hamajima et al 1982) and adult parasitic trematodes (Robinson & Halton 1983). Large numbers in different sizes are found in the circular and longitudinal muscles on the body and especially in the tail and tail-stem junction. But Cardell (1962) and Rees (1971a, b) reported the absence of mitochondria along the epidermis of the cercariae they investigated.

The epidermal mitochondria in the body probably provide energy for transmembranosis (Rees 1974) and for absorption of nutrients during the release of cercariae from the sporocyst tubules into the hemocoel. Even though the cercarial life is very short, it passes from the sporocyst into the haemocoel and into the freshwater and finally enters through the mouth of the fish and penetrates into the cephalic region. During this short period of migration and free life, the cercaria has to adjust itself to different osmotic and ionic environments. So it is probable that the epidermis, with the energy provided by the mitochondria, functions in ionic and osmoregulation. There are no definitive studies on this subject, but from those on the adult *Fasciola hepatica* (Siddiqui et al 1966) and on five other species of parasitic digenetic trematodes (Siddiqi et

al 1975), it is believed that these species adjust osmotically and ionically, as a result of rapid water influx and outward ionic flux by simple diffusion within the limit of tolerance. From the present observations the cercaria of *R. campanula* has been noted surviving in various dilutions of saline and Tyrode solution which supports the view of osmoregulation through the epidermis

The cercarial stage is a very active stage especially the movement of the tail whose main function is locomotion. This is an occurrence of high energy usage and it is evident that the energy needed is supplied by the large amounts of mitochondria found in the circular and longitudinal muscles. Also at the junction of the tail and tail-stem elongated mitochondria with more cisternae are noted and they would probably supply immediate energy for the breaking of the tail during the penetration by the cercariae.

SEM studies of cerariae have shown that the uniciliated papillae are the most commonly occuring type of sensory organelle located along the body and tail of cercariae (Bibby & Rees 1971; Nuttman 1971; Lo et al 1975; Sobhon et al 1988). Page et al (1980) found five different types of ciliary papillae along the body of the cercariae and Parisella & Matricon-Gondron (1985) reported six different ciliary papillae in the cercaria of *Nicolla gallica* of which the receptors, ensheathed by a collar of tegumentary origin and covered with villi, have not previously been described in Platyhelminthes. In

R. campanula cercaria only three types of receptors are present along the body with a fourth type along the cercarial tail-stem. As mentioned by Page et al (1980) the functions of the various sensory structures located on the cercariae depend on the types of stimuli to which the cercariae respond.

The exact stimuli used by the cercariae of *R. campanula* to locate a suitable fish host is not known but the cercariae were observed to enter and encyst only in cyprinid fishes. This is the only bucephalid known to enter passively through the respiratory and food currents into the secondary host. It is probable that some of the anterior receptors function as chemoreceptors to sense specifically the cyprinid fish and having entered the fish the receptors with longer cilia may act as tangoreceptors to locate the sites for encystment - the cephalic region. From the present study it is noted that the majority of cercariae which enter through the water currents, penetrate the fish but rarely eaten by the fish. This indicates that the sensory structures of the cercariae respond as soon as they have entered the fish before they can be washed away by the respiratory currents. The ciliated receptors along the dorsal, ventral surfaces and the tail also may function as mechanoreceptors to sense the water currents created by the passing fish. These receptors along with those near to the tip of the tail may help in the direction of movement of the cercariae.

The domed-shaped pit papillae may be secretory organs (Edwards et al 1977) but this has not been reported, to the author's knowledge, in any other cercariae except for the metacercariae of *F. hepatica* (Bennett 1975), *C. sinensis* (Fujino et al 1979), and Japanese lung flukes (Higo et al 1987) and adults of *S. mekongi* (Vongpaybal et al 1982). The domed papillae on the posterior end of the tail-stem are most likely to be tangoreceptors.

From observations on the parasitic helminths it is noted that the ciliary receptors are of the same functional type: an apical bulb-like structure which possesses a dorsal cilium and is connected to a nerve trunk or dendron (Dixer & Mercer 1965; Halton & Morris 1969; Nuttman 1971).

The arrangement and distribution of these papillae on cercariae have led Richard (1968, 1971) and Bayssade-Defour (1979) to analyse in detail the importance of the arrangements of sensillae (=chaetotaxy) on cercariae, in taxonomy and phylogeny of trematodes. So far, "chaetotaxy" has been studied in nearly 190 cercariae of which 172 cercariae are tabulated to show the significance of chaetotaxy by Bayssade-defour (1979).

The present study has revealed that the positions of individual papillae vary slightly from specimen to specimen. Also when compared with the distribution of papillae using conventional silver

impregnation method and scanning electron-microscopy, variations were noted especially along the tail region and tail-stem. This may be due to the gland duct openings staining with silver nitrate (Smyth & Halton 1983). However the present study reveals the distribution of the papillae in the anterior region (in front of the rudimentary anterior sucker) and shows a considerable variation from the other bucephalid - *B. polymorphus* whose chaetotaxy has been studied (Wallet & Lambert 1984). In the ventral buccal region (C), of *R. campanula* more papillae (52) have been noted than in *B. polymorphus* (42). More observations should be made to confirm the definitive arrangement of papillae on *R. campanula*.

The dense surface array of backwardly directed, single pointed spines would assist the cercaria in maintaining the position and penetration into the secondary host. They may also assist during the emergence of cercariae from the molluscan intermediate host. There are reports of multipointed spines with each spine bearing 3-25 points in adults of *Urogonimus macrostomum* (Bakke 1978) and *Leucochloridium variae* (Bakke 1982). Variations in shapes and distributions of spines on the surface of the body is highly significant in the type of host the parasite penetrates.

The anterior lips, and the dagger-shaped spines on them, together may form a piercing apparatus as in *Cryptocotyle lingua* (Rees 1974). The anterior lips are controlled by muscles and can be withdrawn or extended

and thus help the spines on the lips to efficiently pierce the cephalic region of the secondary host. The anterior gland ducts open through the opening in the middle of the lips as the mouth is ventral in bucephalids unlike other families. As they penetrate the cephalic region, the lips around the opening of the gland ducts may help to reduce the spread of the enzymatically active secretions in a similar way to the tegumentary folds surrounding the penetration glands in *S. mansoni* (Robson & Erasmus 1970) and in *S. japonicum* (Sobhon et al 1988). The actual mechanism of penetration is not known in *R. campanula* because cercariae enter the host passively through the respiratory and food currents and enter internally through the cephalic region, but it can be assumed that it follows the pattern of other cercariae entering the host. According to the present observations the tail does not seem to take as active a part in fixing the cercariae to the fish as occurs in other bucephalids (Woodhead 1930; Howell 1966; Matthews 1973).

The internal morphology of the cercariae, especially the digestive system, excretory system and nervous system has been studied using histochemical and histological methods and the significance of these structures to the free-living existence of the cercariae have been analysed.

The nervous system of cercariae in trematodes based on the detection of cholinesterase and non-specific esterases, have been demonstrated by several workers

(Dixon & Mercer 1965; Jennings & LeFlore 1972; Bruckner & Voge 1974; Leflore 1979; Grabda-Kazubska & Moczon 1981; Niewiadomska & Moczon 1982 and Choubisa 1986), but the detailed anatomy used for comparative purposes with the present species were given only by Leflore, 1979 (*Plagiorchis elegans*), Grabda-Kazubska & Moczon, 1981 (*Haplometra cylindracea* - Plagiorchidae) and Niewiadomska & Moczon, 1982 (*Diplostomum pseudospathaceum*) - Diplostomatidae).

As mentioned by the above workers, the nervous system of the present cercaria is found to be similar in the basic pattern having a pair of cerebral ganglia and three major pairs of anterior and posterior pairs of longitudinal nerve cords (ventral, lateral & dorsal). In *D. pseudospathaseum* an anterior innermost 4th pair has been mentioned, running along the pharynx towards the buccal cavity (Niewiadomska & Moczon, 1982) and in *H. cylindracea* two more pairs have been mentioned, the innermost anterior 4th pair supplying the oral sucker and the corresponding posterior pair innervating the pharynx (Grabda-Kazubska & Moczon, 1981). As mentioned by Choubisa (1986) in his comparative study of neuroanatomy in four different trematode groups (Monostome, Amphistome, Xiphidiostome & Echinostome) on eight cercarial species, the arrangement and number of nerves in a particular region of the cercariae were found to differ between species. This is correlated with the activity of particular regions such as oral and ventral suckers and tails of certain cercariae.

The other highly developed feature in the present cercaria is the presence of the two nerve ganglia joined by a bridge of three nerve strands at the base of the cercarial body joining the tail-stem. A posterior nerve mass in *P. elegans* (LeFlore, 1979), a strong cholinesterase activity at the body-tail junction in the cercariae of Schistomes (Fripp, 1967) and a mass of nerve fibres forming numerous anastomoses at the base of the furca on *D. Pseudospathaseum* (Niewiadomska & Moczon, 1982) have all been reported but the development of the nerves and the ganglia are not as prominent as in the present species. As mentioned by LeFlore (1979) this posterior nerve commissure may be involved in the co-ordination of the tail movements and can be related to the observations of two sets of retractor muscles connecting the tail-stem to the body of the cercaria at these points of the two ganglia.

In the tail of *R. campanula* cercaria only one nerve trunk could be seen. This arises from the posterior nerve ganglia, one on each side and is not a prolongation of any nerve trunk of the body as is mentioned in *H. cylindrea* (Grabda-Kazubska & Moczon, 1981). However, LeFlore (1979) enumerates three pairs of longitudinal nerves in the tail of *P. elegans* cercaria and Niewiadomska & Moczon (1982) mention two strands along the ventral and dorsal margins of the furca of *D. pseudospathaseum*. The nerve supply to the tail could be related to the activity of the tail. In the present

species the tail is mainly involved in swimming but not in the attachment of the cercaria during penetration into the secondary host. Also, not many sensory papillae are present along the tail in the present species and it may be that one nerve strand is adequate to supply the tail muscles and the few papillae present.

The cercaria is a short-lived, non-feeding stage and the morphology of the digestive system is very simple. The alimentary system persists in cercaria so that there is a developing continuity through cercarial, metacercarial and adult stages (Erasmus 1972). The function of the cercarial gut is not clear, except for a few histochemical demonstrations for the presence of hydrolytic and dehydrogenating enzymes in other digenetic cercariae (Stirewalt & Walters, 1964; Koie 1971; Jennings & LeFlore,1972; LeFlore 1978, 1979 and LeFlore et al 1980).

The presence of acid phosphatase and non-specific esterases in *R. campanula* cercaria along the pharynx and gut, similar to *H. quissentensis* (Jennings & LeFlore 1972) can be correlated with carbohydrate metabolism and energy transfer mechanisms (LeFlore 1979). Halton (1967a), from his observations on the distribution of non-specific esterases in the gut caeca of adult digenetic parasite, mentioned that the enzyme functions in extracellular digestion. Also dehydrogenase enzymes have been demonstrated along the musculature of the acetabulum and oral sucker in *P. elegans* (LeFlore 1978)

and *C. michiganensis* (LeFlore et al 1980). These enzymes were correlated with several energy producing sequences such as the glycolytic pathway and Krebs cycle. Even though the enzymes have been demonstrated histochemically there is no evidence for the presence of any functional digestive cells, or for the location of these enzymes in the alimentary system. Halton & McCrae (1985) when observing the development of the gut in *Fellodistomum fellis*, noted functional digestive cells only in metacercaria and adult.

The cercarial stage is generally very active, exhibiting considerable free-swimming activity which involves utilization of energy. From the observations there is a good evidence to suggest that the cercaria is able to utilize endogenous carbohydrate during this period but the role of the gut is not so clear.

The excretory system is one of the most distinctive features of the cercarial stage and one much considered in the taxonomy, as this is the only one common system carried over from cercaria to the adult. The possible significance to the adult taxonomy was commented by Cort (1917), Faust (1919, 1924, 1932), Hopkins (1954, 1956), LaRue (1957) and Stunkard (1974d, 1975). However, according to Hopkins (1956) and Stunkard (1974d, 1975) the lack of correlation between excretory systems and other morphological characters constitutes one of the major problems in the taxonomy of the Bucephalidae. So far, the flame cell formulae have been

known for only 25 species of bucephalids. The present study of the excretory system on *R. campanula* is contributing to the knowledge of excretory systems in the family Bucephalidae. As more work is done in the excretory system, the more indicative it is of possessing value as a natural basis of classification. Even though it is a distinctive feature in the taxonomy, the study of the excretory system is difficult and tedious and can be made only on living specimens. The flame cells may not beat continuously and when quiescent, a flame cell is virtually invisible. Therefore, long and repeated observations may be required to complete the pattern in any species.

The development of the excretory system in bucephalids follows more or less a typical pattern (Woodhead 1929, 1930; Hussey 1943; Kniskern 1952a; Matthews 1973, 1974 and Stunkard 1976b). As the flame cell number increases and the system elaborates with the development of the cercaria, the morphological descriptions of the cercaria must be based on free-swimming fully matured cercaria.

The terminal pore present at the tip of the cercarial tail is more likely to be an excretory pore but in the living cercariae stained in neutral red, the continuity of the excretory tubule in the furca could not be traced. No such pores are reported in any other bucephalid cercariae but there are reports of similar pores on the tip of the furcae as excretory pores in

schistosome cercariae (Wagner, 1961; Hockley, 1968; Sobhon et al 1988). There are reports on the loss of terminal excretory pores from the tail of *R. septpapillata* during the spread furcae stage in the sporocysts (Kniskern 1952b) and the degeneration and loss of caudal excretory tubules in *B. elegans* (Hussey 1943). Matthews (1974) mentioned that the paired ducts which open ventro-laterally and proximally on the tail-stem of *Bucephaloides gracilescens* are a secondary development.

Although the flame cells and collecting ducts together are referred to as the excretory system, their true function has hardly been investigated in the cercariae or in the adults. Histochemical demonstrations, on digenetic cercariae in general, have been reported on the presence of phosphatases (Coil 1958; Dusanic 1959; Cheng 1964; Probert 1966; Porter & Hall 1970; Jennings & LeFlore 1972; LeFlore 1979), and dehydrogenases (Conde-del Pino et al 1966, 1968; LeFlore 1978; LeFlore et al 1980) in the excretory system including the bladder.

The presence of alkaline phosphatase in the walls of the excretory tubules of a gorgoderid cercaria, (Coil 1958), both acid and alkaline phosphatases in the reserve bladder of a strigeid trematode (Erasmus 1967) and the present observation of acid phosphatase along the excretory bladder all may indicate a secretory or absorptive function (Coil 1958 and Erasmus 1967). Halton (1967b) suggested the involvment of phosphatases in the

carbohydrate mechanism and phosphorylated transfer mechanism in adult trematodes. LeFlore demonstrated the presence of dehydrogenases (1978) and phosphatases (1979) in the same sites along the excretory bladder of *P. elegans* cercariae, further supporting the interpretations of the functions of the excretory bladder.

The collapsing of the excretory tubules when the beating of the flame cells has ceased led to a conclusion that these flame cells create a hydrostatic pressure in the excretory system (Wilson 1967). Siddiqi and Lutz (1966) experimenting with *F. gigantica* for osmotic and ionic regulation commented that the excretory system appears to play some role in the osmoregulation of the trematode but Wilson and Webster (1974) commented that definitive evidence for or against osmoregulation by the protonephridia is lacking.

From all the above observations the precise locations of the enzymes along the digestive system and excretory system are not clear as all the observations are made on whole mount preparations. Also from the experiments no definitive conclusions are drawn to the true functions of these systems. More work is needed to relate the functions and structure of the digestive and excretory systems.

The crystalline "calcareous corpuscles" observed in the excretory bladder and in major ducts of the digenetic cercaria and metacercaria are thought to be similar to the calcareous corpuscles of cestodes (von

Brand et al 1965; Chowdhury et al 1962). They have been reported in the developing cercaria of *F. hepatica* (Bennett & Threadgold 1973; Bennett 1977), and in the metacercaria of *Posthodiplostomum minimum* (Mitchell & Crang 1976). No other reports of calcareous corpuscles in any other bucephalids are published except for the present observations on *R. campanula*.

Bennett (1977) from his observations on *F. hepatica* mentioned that the calareous corpuscles disappear once the parasites enter the final host. In the present study these calcareous corpuscles were not observed in the metacercaria or the adult. Elemental analysis of the excretory concretions in *Postho-diplostomum* metacercariae has revealed an abundance of calcium and in some cases magnesium (Mitchell & Crang 1976). However, the exact role of concretions in the cercariae and metacercariae is uncertain but they have been implicated, in the buffering of acids in the cestode excretory systems (von Brand et al 1965) and in carbondioxide fixation (Martin & Bils 1964). Bennett (1977) from his studies on the metacercariae of *F. hepatica* suggested that these corpuscles may immobolize metabolic wastes which would otherwise adversely effect the enclosed juveniles. The cercaria is a free-living stage and the waste products can be eliminated easily through the excretory system, but as mentioned by von Brand et al (1965) on cestode corpuscles, this may be serving in the cercaria to buffer acids when entering the

secondary host. As mentioned by Smyth and Halton (1983) the role of these calcareous corpuscles has yet to be determined.

The fundamental role of cercarial life is to enable completion of the life-cycle by infecting efficiently and effectively the secondary intermediate host, during its short life-span. As mentioned by Erasmus (1958) age or time dependence is likely to be particularly significant in non-feeding larval parasites, since the death rate often increases with time due to the progressive utilization of the non-replaceable food reserves, which are often in the form of glycogen and lipids.

In *R. campanula* the maximum 50% survival time is noted in 0.15% saline at 15° C and 20° C as >95h but the survival time decreases with temperature (Table 6.2). Similarly temperature effects on the survival time of larval digeneans have been mentioned by Farley (1962); Dutt and Srivastava (1962) and Anderson and Whitfield (1975). Also noted is that the activity of the cercariae of *R. campanula* is not directly linked to its survival, since the cercariae remain alive for few hours after active, spontaneous swimming has ceased. As mentioned by Anderson and Whitfield (1975) the rate of change in activity can be directly proportional to the quantity of glycogen present in the cercaria. Similarly, the rate of change in infectivity can be proportional to activity.

The glycogen reserves are observed in the tail-stem and scattered in the tail of *R. campanula* cercaria but the utilization of glycogen in the free-swimming cercariae has not been studied and the histochemical tests were done on the cercariae in the sporocysts before being released into the water. The existence of glycogen in the cercariae and its constant depletion with age have been reported on *S. mansoni* and *Transversotrema patialensis* (Anderson and Whitfield, 1975).

The behaviour of cercariae of *R. campanula* to light and gravity must be regarded as adaptations to increase the opportunity of entry into the secondary host the minnow. The phototactic and photokinetic behaviour of the cercariae clearly demonstrates that the cercariae must be activated when released from a dark environment (inside the mussel) to swim upwards and then maintain their position in the mid and bottom waters by neutral response to light and gravity, in order to increase the possibility of infection of their intermediate hosts. The higher rate of infection is more likely during the spawning season when the minnows are present in the area around the mussel beds in the mid and bottom waters.

In furcocercous cercariae (mainly studied on schistosome cercariae) swimming is usually achieved by keeping the tail first with the furcal rami extended and closely apposed and the propulsion is generally the result of rapid propellor-like vibrations of the tail

(Chapman and Wilson, 1973). In *R. campanula* cercaria, even though it belongs to the same group of furcocercous cercariae, the structure of the tail-stem and tail and their attachment to each other is totally different (Fig 6.16). The tail-stem is round and does not take an active part except for a slight contraction and relaxation during swimming, mainly providing energy and mucus secretions. The inner side of the tail-stem is covered with glycogen-storage cells and mucus-secreting cells. The central lumen of the tail-stem acts as a reservoir for the pumping of the tail during the contraction and relaxation (Fig 6.28).

Among bucephalids, there have been no detailed reports about the swimming behaviour of cercariae except for the present observations on *R. campanula*. Woodhead (1930) and Bevelander (1933) on *Bucephalus elegans* mentioned briefly about the position of furcae during swimming as upward and outward sigmoid curves similar to *R. campanula* cercaria but no detailed diagrams or illustrations were given for comparison. The tail movement of *B. longicornutus* is totally different as the furcae expand to greater lengths than in the present species and coil (Howell, 1966). Characteristically each bucephalid species performs with slight variations in tail contractions and resting phases, and may be used as a generic identity.

In *S. mansoni* two modes of swimming were described. In the forward progression a wave of

contraction passes down the tail-stem and the furcal ramii is closely apposed and functions like a fish fin (Chapman & Wilson, 1973). In *R. campanula* cercaria, no such tail movement is observed and furcae are held upward and outward in a sigmoid curve. During fast swimming the furcae are held parallel to each other. The angle between the tail and tail-stem determines the speed of swimming. Nearing the surface, the furcae are stretched apart and held perpendicular to the body increasing the buoyancy and reducing the resistence.

During the downward movement, the cercaria descends keeping the furcae folded over the body and gradually dropping tail-stem first (Fig 6.25 (ii)b) or actively swimming by keeping the furcae in a sigmoid curve (Fig 6.25 (ii)c), or gradually dropping head first (Fig 6.25 (ii)a). In contrast the backward progression in *S. mansoni* cercaria is effected by oscillation of the tail-stem about two fixed points, one near the junction of the tail with the tail-stem and the other near the body and tail-stem. During backward progression the furcae are spread out and perform "rowing" action (Chapman & Wilson, 1973).

The movement of the tail and body is slow in *R.campanula* cercariae as could be observed with the unaided eye. This is in comparison with *S. mansoni* cercariae in which the movement is rapid (Samuelson et al 1984). The longitudinal muscles along the long axis of the tail in *R. campanula* may function in rapid

contraction and elongation. The circular muscle bands lying perpendicular to the long axis would facilitate the stiffening of the tail either during gradual descent, or when suspended in water with the furcae stretched apart, similar to *S. mansoni* cercaria on which the circular muscles stiffen the furcae during backwards swimming movement (Reger, 1976).

In cercaria of *Schistosoma* sp (Reger, 1976); *Cryptocotyle lingua* (Rees,1975); *Hamasthla quissetensis* (Cardell & Philpott, 1960) and in *C. lingua* and *H. secunda* (Chapman, 1973) the elongation of the tail has not been mentioned and it is likely to be very small compared with that of the present species in which the tail extends nearly five to six times its contracted length. So, definitely the longitudinal muscles have to function in elongation and contraction of the tail to a greater extent than in the above mentioned cercariae. In *S. mansoni*, Reger (1976) mentioned that longitudinal muscles function in rapid tail oscillations. Chapman (1973) mentioned in *H. secunda*, the contractions of the dorsal and ventral longitudinal muscle "blocks" on each side resulting in the two dimensional undulations of the tail referring to fast contractions and in *C. lingua* (Rees, 1975) in three dimensional form, the body proceeding forewards along a helical or a figure of eight pathway. So, detailed study on the ultrastructural level of the tail of *R. campanula* cercaria is needed to explain clearly the role of musculature in the tail movement.

The final event in the cercarial life is the loss of the tail when its function of propelling the cercaria to the secondary intermediate host, the fish, is accomplished. As *R. campanula* cercaria passively enters the fish through the food and water currents, the actual penetration into the subcutaneous tissue in the pharyngeal region was not observed but the furcae were spat out as soon as the cercariae entered the fish mouth. This clearly demonstrates that the attachment of tail to the tail-stem is not firm and as mainly being around the tegumental region it could very easily be broken off. It is likely that the abundance of mitochondria around that region provides sufficient energy for the breakdown of the tail, but the actual mechanism is not known. Howells et al (1974) showed experimentally that enzymes are not involved in the tail loss in *S. mansoni*. Rees (1977) suggested that once the cercarial body is fixed to the fish, or to the substrate, and the tail continues its movements, the stress would be considerable and a break would occur at the weakest point. In *C. lingua* the tail and the body are connected by radial muscles and caudal muscles which are not observed in the tail/tail-stem junction in *R. campanula* cercaria, which make the detachment of the tail much easier.

The cercarial body is attached to the tail-stem by two sets of retractor muscles arising in the tegument at the distal end of the body and inserted into the basement membrane of the tail-stem. The detachment of the

tail-stem may be difficult and could be due to stress caused by the movement of the body or as mentioned by Rees (1977) by neurosecretions. The latter possibility is the more likely as highly developed nerve commissure and ganglia on either side have been observed at the body/ tail-stem junction in the present cercaria (Fig 6.13).

Once the tail-stem is broken off from the cercarial body, the healing of the "wound area" is important but this has not been observed in *R. campanula*. Kuntz (1950) described a membraneous septum between the body and tail, in *S. mansoni* and *S. japonicum*, which forms the posterior part of the body proper after the tail has been shed and will contribute to a sphincter around the excretory pore. In *C. lingua* a layer of outer cytoplasmic epidermis, covers the "wound area" and eventually the terminal part of the body, in the metacercaria. This becomes evenly rounded with the excretory pore opening at the terminal part of the body (Rees, 1975).

CHAPTER SEVEN

BRIEF MORPHOLOGY OF METACERCARIAL STAGE

7.1 INTRODUCTION

The metacercarial stage in digenetic trematodes is often described as "resting" phase of the life-cycle of the parasite. This does not imply a phase of total physiological inactivity, a cessation of metabolic process, nor the absence of development. The advantage of this stage, unlike the cercarial and miracidial stages which are short-lived, is that the metacercaria represents a phase where the potentiality to continue the life-cycle and the ability to infect hosts is retained and extended over a relatively long period.

The cercaria of *R. campanula* passively enters the secondary intermediate host, the fish (minnows), and encysts in the cephalic region of the subcutaneous fatty tissue beneath the lining of the pharynx. Although the presence of cysts has been reported previously by Baturo (1977) and Ivantsiv & Chernogorenko (1984), the morphology of the excysted metacercaria has been described only briefly using the light microscope. Therefore, in the present study the external morphology has been studied using scanning electron microscopy, and the transitional variations from cercarial to metacercarial stage have been analysed. Also the cyst wall of the metacercaria has been studied briefly using histochemical and histological methods.

The infection of cercariae and the development of metcercariae in the intermediate host and the intensity of infection have been analysed in Chapter 4.

7.2.1 Structure of metacercarial cyst

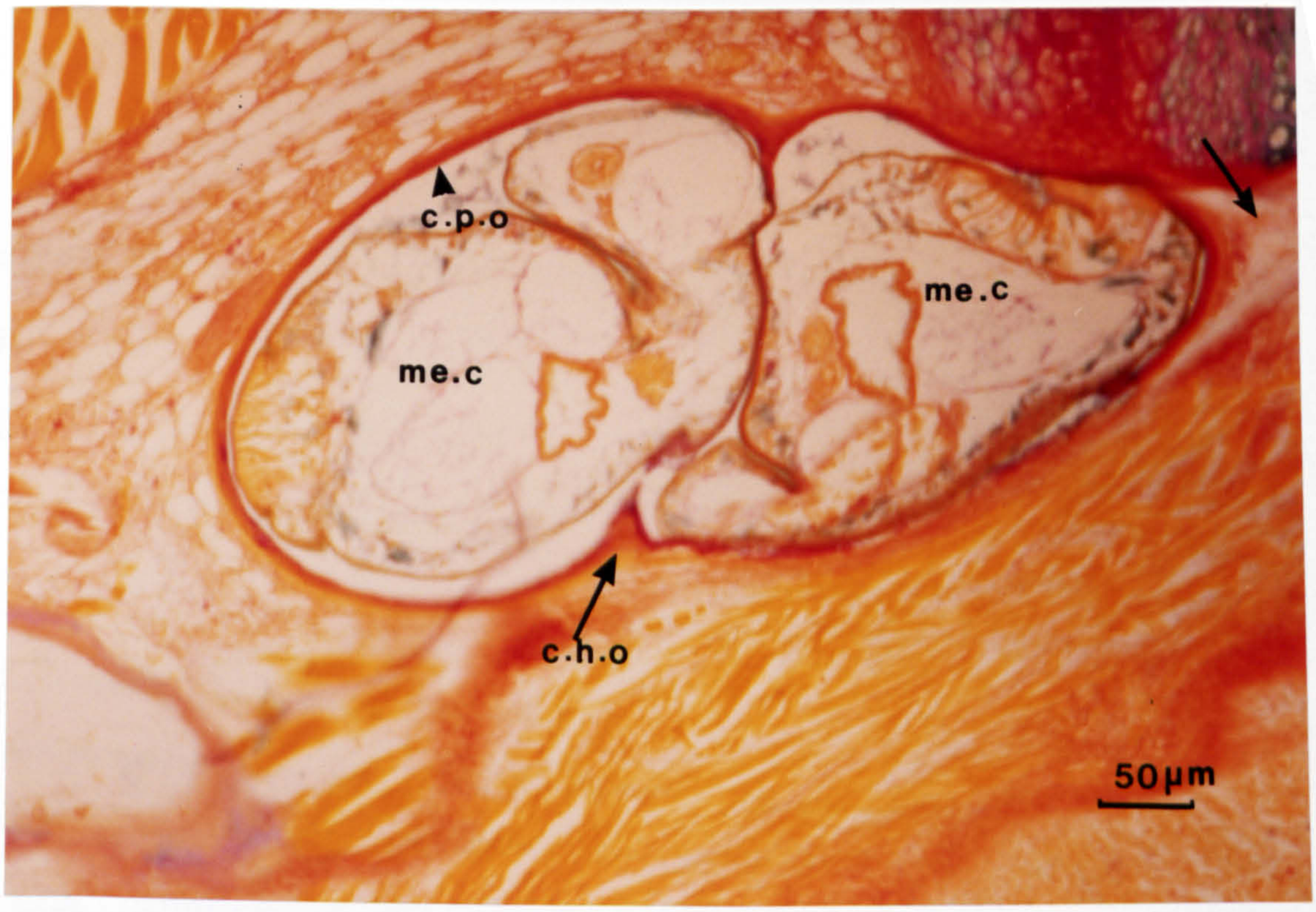
Cysts containing the metacercariae of *R. campanula* are oval in shape, have dimensions of 298-428 x 216-292µm The bent larva with its S-shaped excretory vesicle and yellowish-brown intestine is visible through the transparent cyst wall.

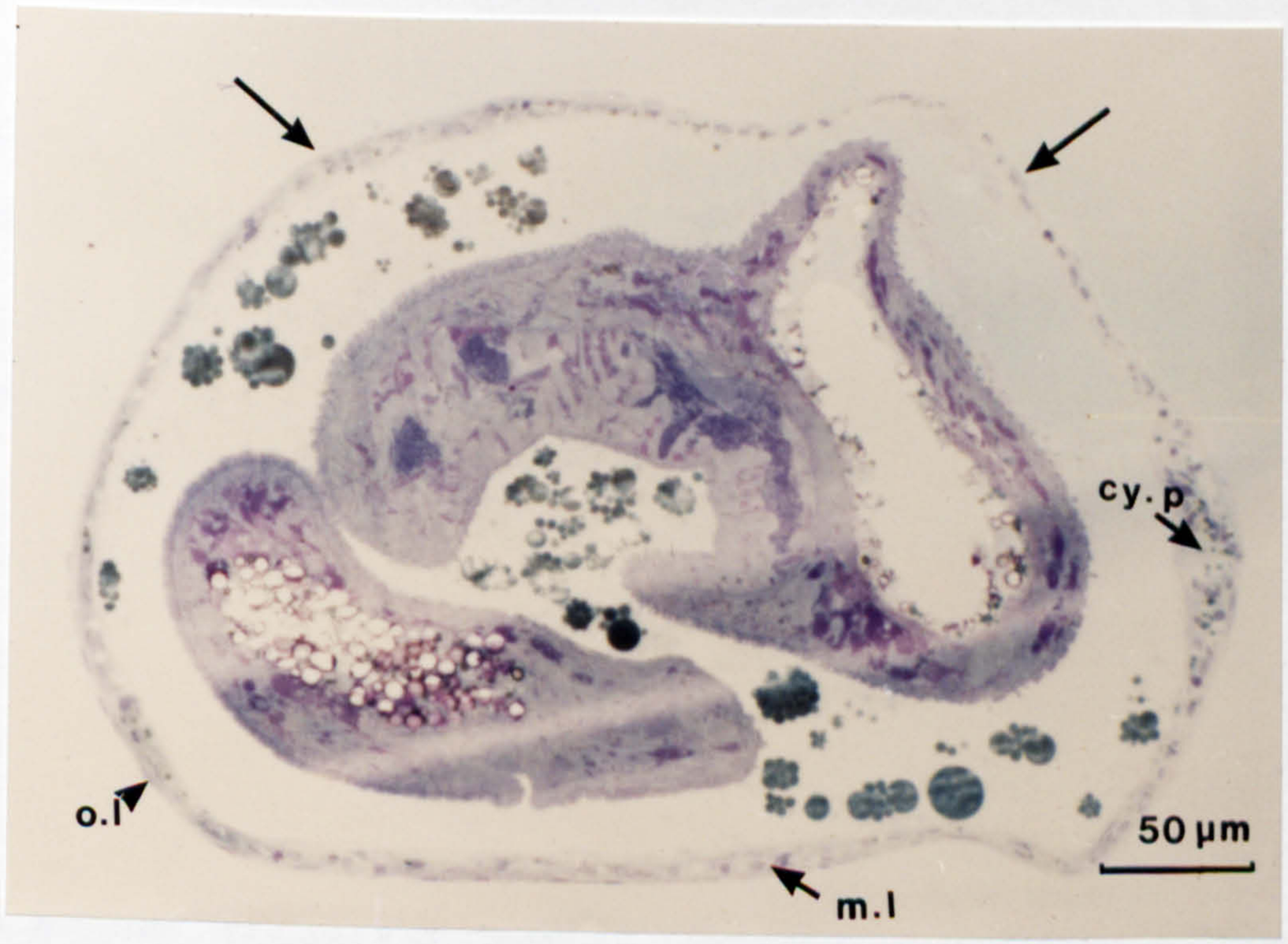
The cyst is composed of an inner thin layer of parasite origin, and an outer fibrous layer of host origin (Fig 7.1). The fibrous capsule is the only visible evidence of a host reaction to the presence of the parasite. However, some degeneration of host tissues is evident along the path of the cercaria as it progresses through the tissues to the site of encystment (Fig 7.1). No degeneration of host tissues observed around the cyst.

The inner cyst wall of parasite origin is PAS positive and demonstrates the presence of mucopoly-saccharides. This layer is composed of three individual layers: a very thin non-cellular inner layer, a considerably thick and vacuolated middle layer, and an outer nucleated layer where the cells are considerably flattened. The thickness of the cyst wall varies at different regions with an anterior plug of considerable thickness.

Fig 7.1 A portion of the L.S through the head of a minnow showing the metacercaria (me.c) inside the cyst embedded in the subcutaneous fatty tissue.Note the cyst wall of host origin (c.h.o) & parasite origin (c.p.o); the degenerated host tissue showing the path of the cercaria to the site of encystment (arrowed).
Stained with Schiff's reagent, Alcian blue and Orange G.

Fig 7.2 Semi-thin section of metacercarial cyst showing the cyst wall consisting of three layers; outer thin layer (o.l), middle thick layer (m.l) and inner thin non-cellular layer (i.l). Note the anterior cyst wall plug (cy.p) and variation in thickness of the cyst wall (arrowed).
Stained with Toluidine blue.


c.p.o
me.c
me.c
c.h.o
50µm


cy.p
o.l
m.l
50 µm

7.2.2 Morphology of the excysted metacercaria

The fully developed metacercaria resembles the adult in the general appearance, though there are characteristic differences between the two stages. The body of the metacercaria is bluntly ovoid. Its anterior margin is truncate, usually with lateral outgrowths, (Fig 4.15) and become elongated and rounded anteriorly during expansion (Fig 7.3). Dimensions are 950-1100 x 165-200µm with the greatest width occuring just behind the pharynx.

The mouth is mid ventral and forms by inwardly directed tegumental plates. The excretory pore is present in the posterior extremity, and 20µm in front of the excretory pore is the genital opening. In the anterior, ventral region is the presence of sucker, which is not as fully developed as in the adult (Fig 7.3).

The entire metacercarial surface is covered with posteriorly directed single pointed spines, which are not uniformly distributed. In the antero-ventral region, in front of the mouth, the spines are regularly arranged between the tegumental plates, as in the cercaria. The number of spines reduces progressively towards the posterior of the body, where they are mostly buried in the tegument. There are no spines surrounding the tegumental plates around the mouth, around the excretory and genital pores, and in the anterior extremity in front of the sucker (Fig7.4). Though the distribution of these spines is similar to the cercaria, the arrangement of

tegumental plates differs from that of the cercaria, especially in the posterior region (Fig 6.5 & Fig 7.3).

In the anterior extremity in front of the sucker the tegument is highly folded, forming a charecteristic longitudinal and lateral ridges. This anterior region with the sucker is significantly different from the cercaria's anterior organ, which has seven lips (Fig 6.4). The development of the sucker in the metacercaria from the anterior organ of the cercaria is a significant structural change from the free-living cercaria to the parasitic adult stage, through the metacercarial stage. The inner roof of the sucker in the ventral region, is lined with posteriorly directed spines. The anterior hood of the sucker is devoid of spines and has pit papillae (p_2) and also ciliary papillae which have bulbous base and a tegumentary ring arranged in a semi-circle.

Three types of papillae are present in the body of the metacercaria: the pit papillae (p'_2 & p''_2), ciliary papillae (p_1), and short ciliary papillae surrounded by a tegumental ring (p_3). The ciliary papillae are found scattered on the dorsal and ventral regions of the body. Two types of pit papillae are observed in metacercariae, which are not clearly differentiable in the cercariae. One is the single collar pit papilla (p'_2), and the other is the double collar pit papilla (p''_2). These papillae are distributed bilaterally and symmetrically in the anterior extremity of the sucker.

The papillae present on both sides of the rudimentary sucker in the cercaria are observed in the anterior extremity of the sucker in the metacercaria (Fig6.4 & Fig 7.4). This indicates that the rudimentary anterior sucker is likely to develop into the true sucker in the metacercaria, and move towards the anterior extremity. More studies on the progressive development of the anterior sucker of the metacercaria are necessary to confirm this since the observations were made on fully developed cercaria and metacercaria.

The digestive system of metacercariae of *R. campanula* is similar to that of the cercaria. The pharynx measures 61-72 µm in diameter. The intestine is greatly dilated and filled with particles. The excretory system resembles that of the cercaria. The flame cell formula remains 2[(5+5+5+5) + (5+5+5)] = 70, the excretory vesicle and the ducts are enlarged and the excretory vesicle reaches the anterior level of the intestine.

The reproductive system is not developed as in many other bucephalid metacercariae. The ovary, which measures 40-60µm in diameter, is situated dorsally on the right of the intestine in the posterior half of the body. Partially developed vitelline glands are situated laterally on either side, anterior to the pharynx. The uterine coils and Mehlis's glands are not developed. The testes measure 50-80µm in diameter and are found to the left of the ovary and behind the pharynx. A large elongated cirrus pouch, situated on the left side of the

body, reaches the level of the anterior testis (Fig 4.15).

The nervous system is similar to that of the cercaria with a pair of nerve ganglia situated beneath the anterior sucker. Each ganglion gives off three nerve cords anteriorly, and three posteriorly. The antero-ventral, antero-dorsal and antero-lateral nerves supply the anterior sucker and body wall. The posterior-ventral, posterior-dorsal and posterior-lateral pass to the posterior extremity, supplying the pharyngeal region and the body of the metacercaria. No nerves to the reproductive structures were visible. The prominent posterior commissure and ganglia at the body/tail-stem junction of the cercaria are replaced by a nerve commissure alone, and a strong positive reaction for non-specific esterase is observed in this region (Fig 7.6). These three pairs of nerves (ventral, dorsal, and lateral) are connected by twelve commissures: two in front of the anterior cerebral ganglia, nine commissures posterior to the cerebral ganglia and one in the region of the cerebral ganglia. The pharyngeal nerves are more clearly developed in the metacercaria than in the cercaria.

Fig 7.3 Scanning electron micrograph of the ventral region of the metacercaria of *R. campanula* showing the anterior sucker (a.s) with extended "hood" (arrowed), mouth (m), genital pore (g.p) and excretory pore (e.p).

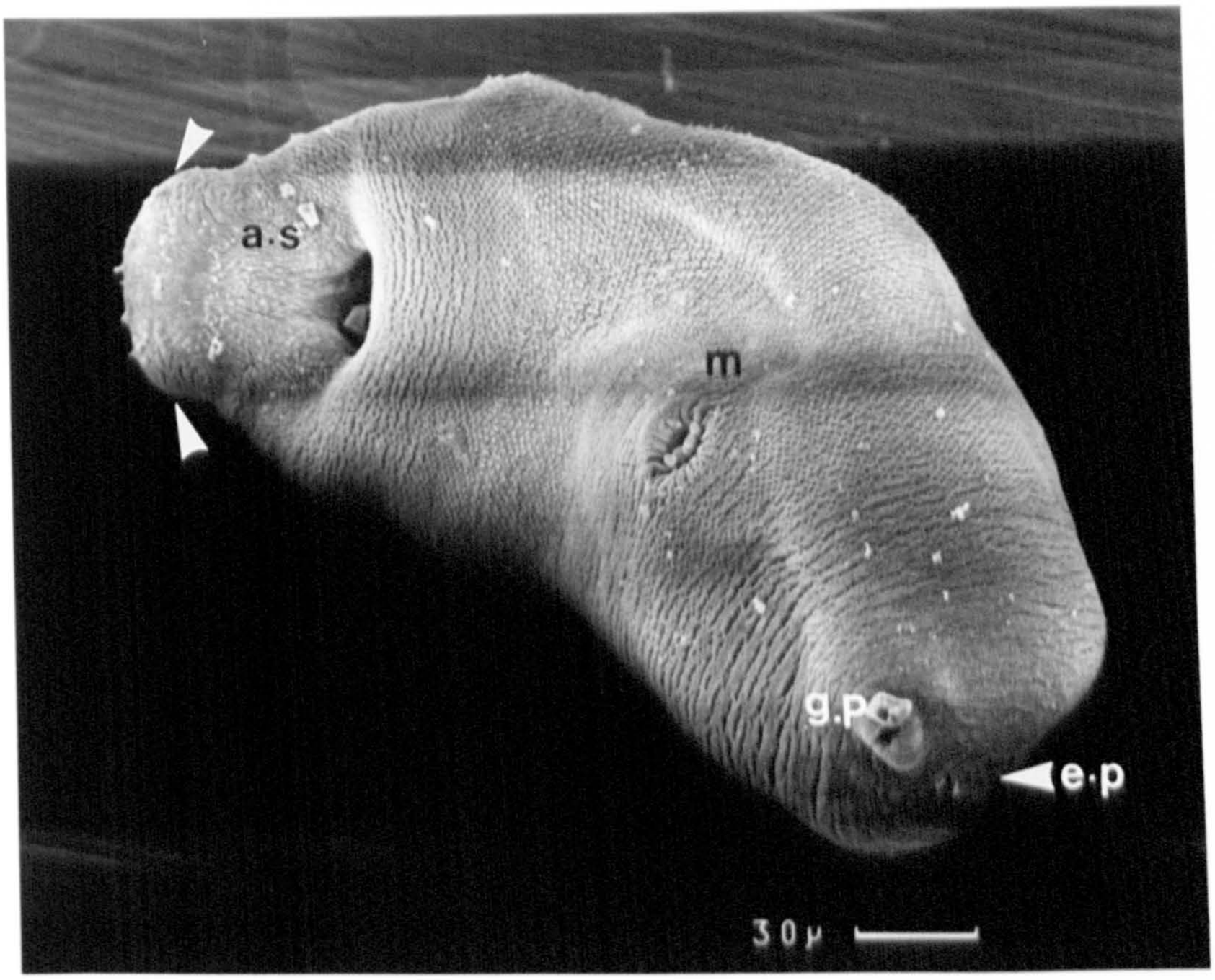
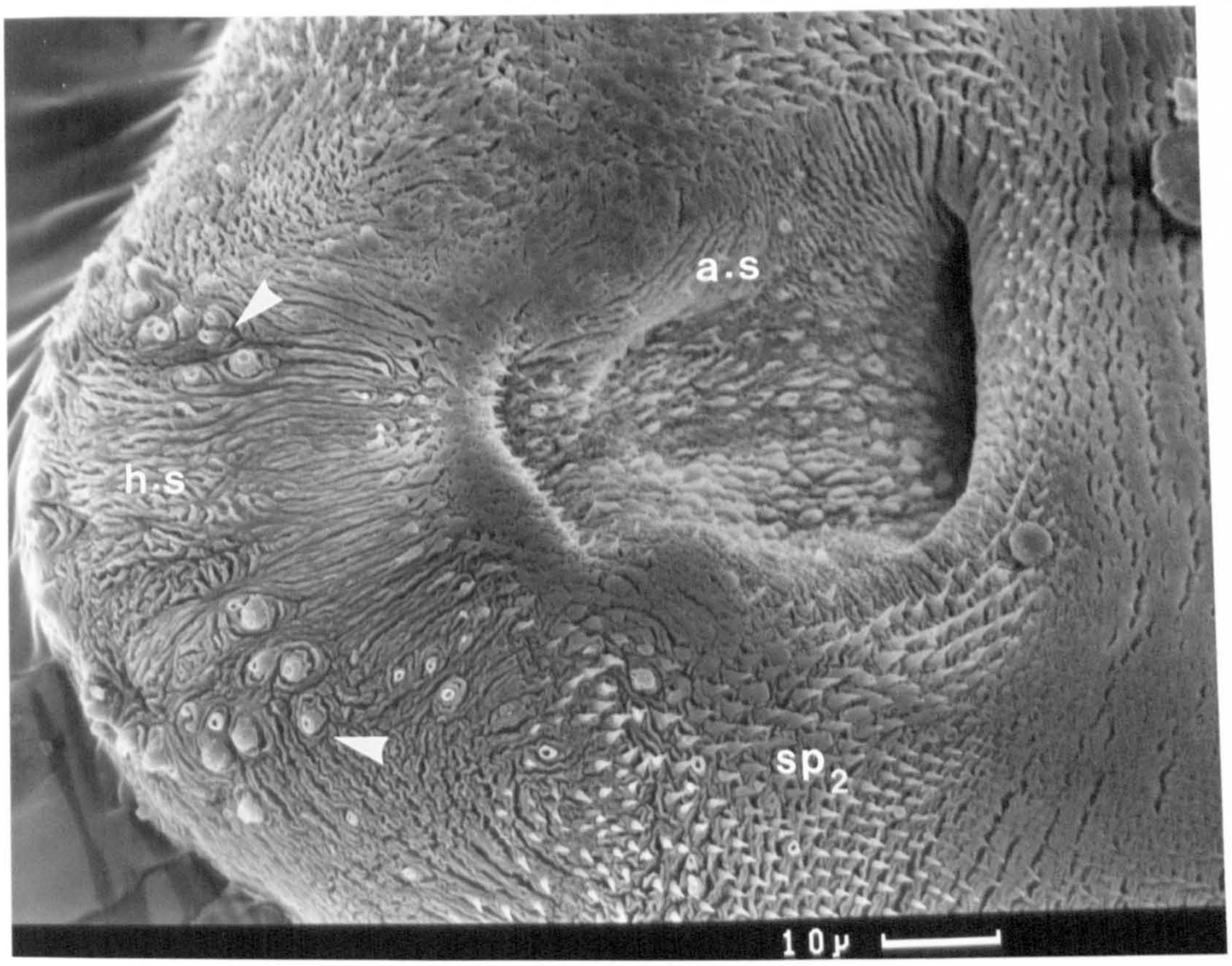

a.s
m
g.p
e.p
30μ

Fig 7.4 Scanning electron micrograph of the anterior ventral region of the metacercaria showing the anterior sucker (a.s) and the "hood" (h.s) with papillae (arrowed). Note the posteriorly directed spines in the innerside of the sucker, also the presence of spines (sp_2) outside the sucker but not in the anterior extremity of the "hood" (h.s).

Fig 7.5 Scanning electron micrograph of the anterior sucker showing the anterior extremity of the "hood" of the sucker with papillae arranged in a semi-circle. Note the clearly visible pit papillae (p_2) and tegumental ridges in this region (arrowed).


a.s
h.s
sp2
10μ

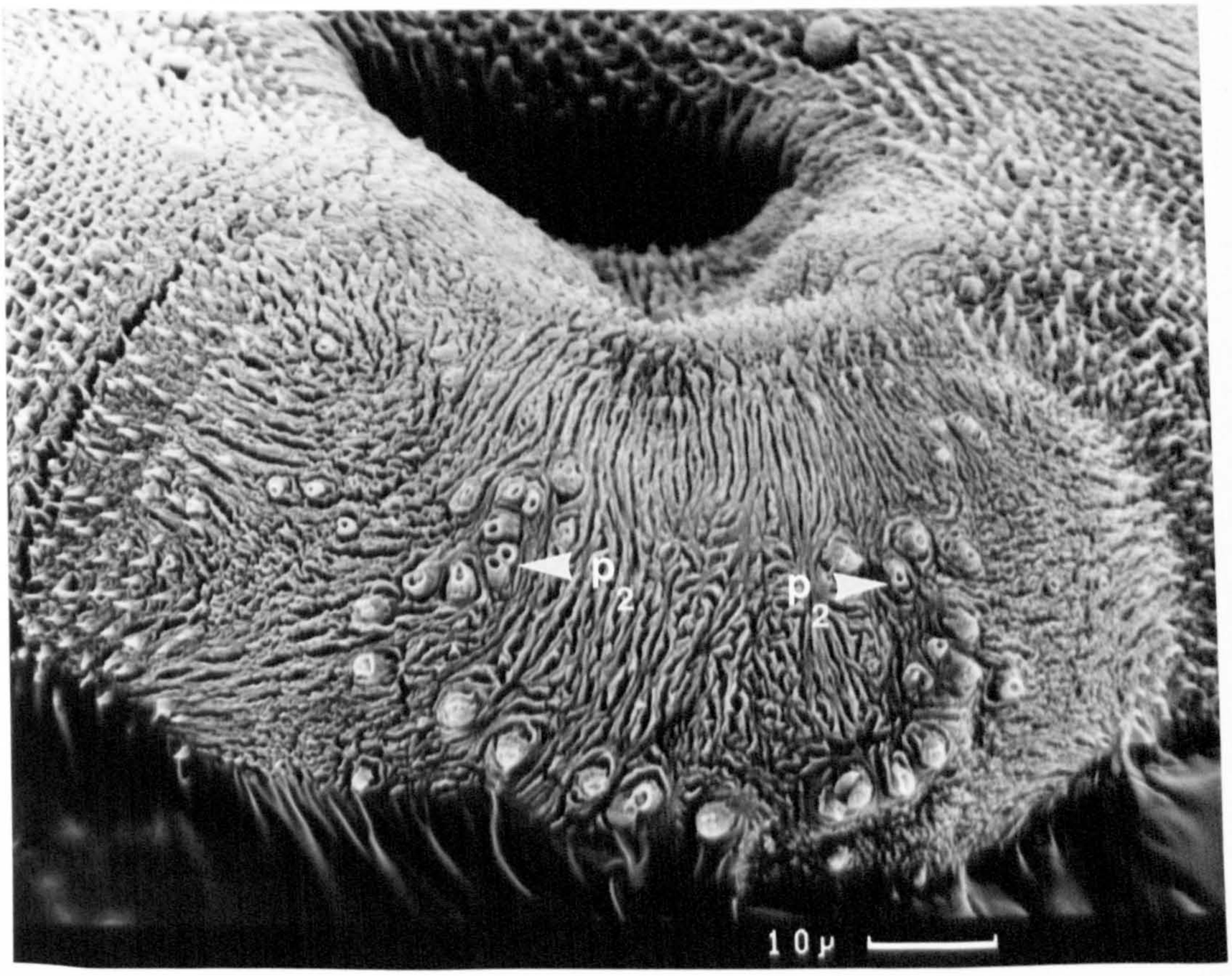
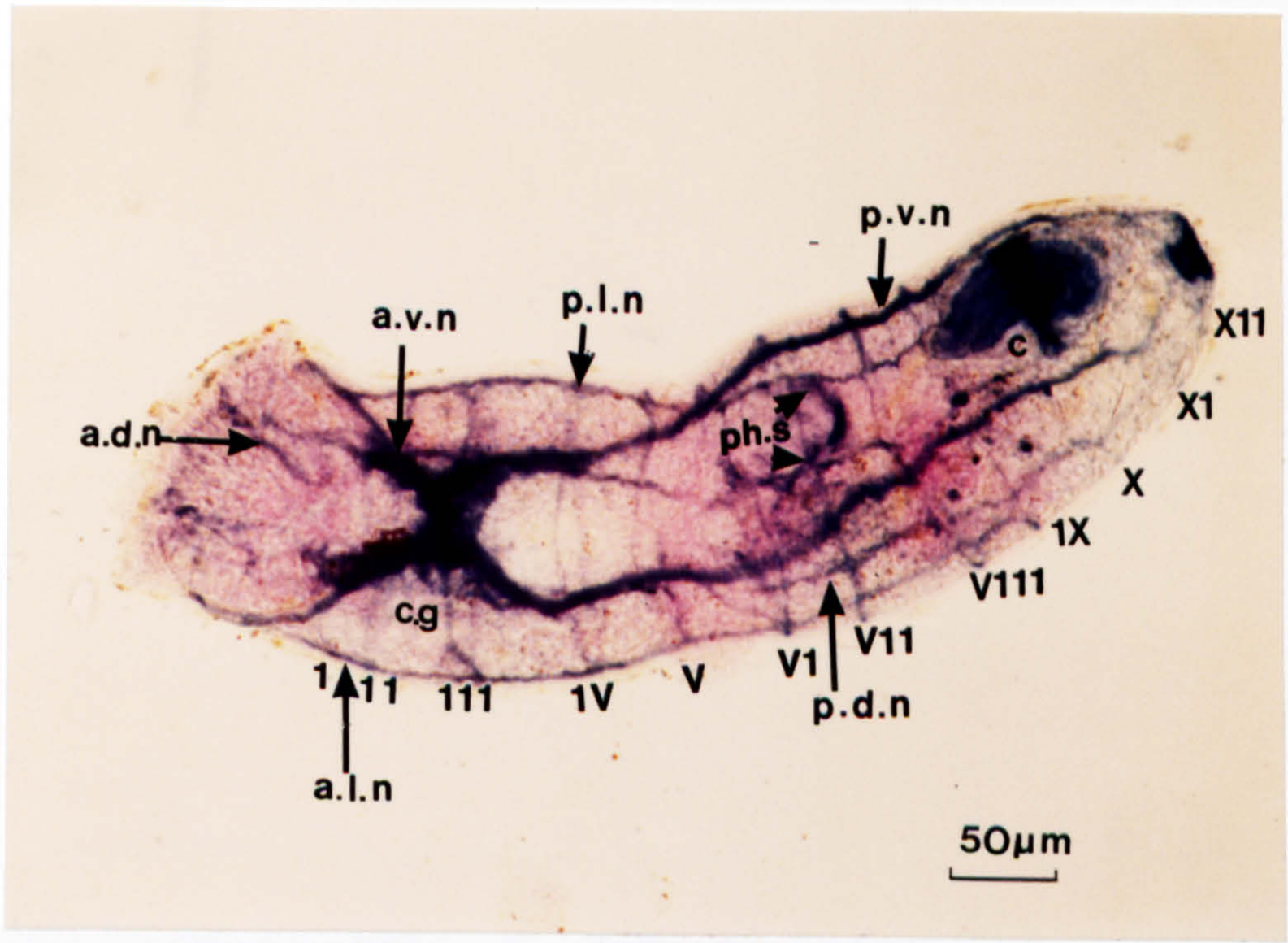

p2
p2
10μ

Fig 7.6 Metacercaria after incubation in Indoxyl acetate medium demonstrating positive reaction for non-specific esterase along the nervous system and cirrus sac (c).

1-X11 are the nerve commissures.


p.v.n
p.l.n
a.v.n
a.d.n
ph.s
c
X11
X1
X
1X
V111
V11
V1
V
1V
111
11
1
c.g
a.l.n
p.d.n
50µm

7.3 Discussion

The cyst wall of *R. campanula* consists of two layers: the outer layer of host origin, and the inner layer of parasite origin, itself composed of three layers. Similar descriptions have been given for *Bucephaloides gracilescens* (Johnston & Halton, 1981 a,b), and *Bucephalopsis haimeanus* (Matthews, 1973 & Higgins et al, 1977).

The cysts of *R. campanula* are easily removed from the host tissue and it is likely that the connections between the cyst layers of host and parasite origin are not firmly integrated. This is evident from the semi-thin sections of the metacercarial cysts, which demonstrated only the cyst wall of parasite origin. Electron microscopical studies by Halton & Johnston (1982) of *Bucephaloides gracilescens* demonstrated that the inner cyst wall is not firmly integrated with the outer fibrous capsule, and is separated from a zone of cellular degeneration. A similar interface zone between the primary cyst wall and the outer capsule has been described for *Posthodiplostomum*, (Mitchell, 1974).

Although the present study provides no evidence on the source of development of the inner cyst wall of parasite origin, gland cells found in the body of cercariae are believed to contribute to the formation of the cyst wall, as described for *B. gracilescens* (Matthews, 1974 and Halton & Johnston, 1982). It is also

possible that the membrane-bound secretions of the tegument itself contribute to the development and maintenance of the inner cyst wall, as has been described in *Cryptocotyle lingua* (Rees & Day, 1976), and *B. haimeanus*, (Higgins, 1980).

The cysts of *R. campanula* are easily excysted in Tyrode solution, saline or water. Experiments demonstrated that spontaneous excystation depends on the maturity of the worms, but the structure of the capsule determines the region on the cyst wall where the metacercaria emerges. The anterior plug observed in the cyst wall of *R. campanula* may be the original site of emergence as described in *F. hepatica*, (Dixon, 1965) and in *Psilotrema oligoon*, (Pike & Erasmus, 1967). Dixon (1965) and Pike & Erasmus (1967) stated that these cyst wall plugs (ventrally placed) are sites of enzyme digestion which assist in the emergence of metacercariae. There is no evidence of the presence of cyst wall plugs in any other bucephalid metacercarial cysts described in the literature.

The variety and complexity of the metacercarial cyst walls are related to the environmental conditions under which the wall has been formed (Asanji & Williams, 1973). However, the cyst wall of *R. campanula* showed fewer layers than they reported.

It is apparent that considerable differences exist between the cercarial body and that of the metacercaria in *R. campanula*. The charecteristic

differences were observed mainly in the anterior sucker of the metacercaria, the types and distributions of papillae, and the development of the reproductive structures. The alimentary tract, excretory system and the external spination of metacercariae are basically similar to that of cercaria, except for an increase in size.

The characteristic differences in the external appearance of the cercaria and metacercaria are the formation of the anterior sucker in the metacercaria in place of the anterior organ with seven lips at the anterior end of the body of cercaria, and the disappearance of the rudimentary sucker of the cercaria. Scanning electron microscopy clearly demonstrated papillae on both sides of the rudimentary sucker of the cercariae, later in front of the anterior sucker of the metacercariae. This could be the result of growth of the rudimentary sucker towards the anterior region, to form the anterior sucker in the metacercariae. There is no clear evidence from the present observations, but a definite indication that the anterior sucker in the metacercaria is formed from the rudimentary anterior sucker of the cercaria of *R. campanula*. Information on the anterior sucker of bucephalid cercariae is scarce.

The types of papillae present in cercariae and metacercariae of *R. campanula* are similar, but their distributions are different in the above two stages. The papillae with a tegumental ring and single cilium (p_3)

are found on the surface of the body of the metacercaria in no definite pattern of distributions. The uniciliated papillae are common in other digenetic trematodes and have been reported by several workers (Robson & Erasmus 1970; Page et al, 1979 and Higo & Ishii , 1987).

The pit papillae (p_2), observed on the cercariae of *R. campanula* on both sides of the rudimentary sucker, were seen in front of the anterior sucker of the metacercariae. The pit papillae present in the anterior region of the metacercariae representd two different types: single collar papillae (p'_2), and double collar papillae (p''_2). These collars were not clear in the cercariae. The pit papillae in the metacercariae may represent an advanced stage of development. Reports of pit papillae of five species of newly excysted metacercariae of Japanese lung flukes (Higo & Ishii, 1987), of the adult *S. mekongi* (Vongpayabal et al, 1982), and the cercaria of *S. mansoni* (Robson & Erasmus, 1970), resemble the pit papillae observed on the cercaria of *R. campanula*. The collared pit papillae observed on *R. campanula* metacercariae and adults have not been reported to the writer's knowledge. Vongpayabal et al (1982) stated that pit papillae they observed may be the result of tearing off or shedding of cilia, or could be an artifact resulting from the preparative techniques. The present observations on *R. campanula* cercariae, metacercariae and adults (discussed in chapter 8) clearly indicate a structural development from a papilla with an

opening on a raised bulbous base in the cercaria to an advanced single and double collar pit papilla in the metacercaria and adults. Unless sections of these papillae are studied by electron microscopy, their exact nature is difficult to confirm.

Even though, the distribution of papillae in the metacercarial stage may have no functional significance within the cyst, it is believed to be essential for the metacercariae to select a suitable site in the final host.

The relationships of the cercarial and metacercarial excretory systems have been grouped into three catogories according Erasmus (1972). Observations on *R. campanula* represent the first group of cercariae (includes families Allocreadiidae, Opecoelidae, Fasciolidae, Echinostomatidae, Microphallidae, & Cryptogonimidae) in which the flame cell formula remains the same on development from cercaria to metacercaria. Similarly Howell (1966) on *B. longicornutus* and Matthews (1973) on *B. haimeanus* reported the same flame cell formula in metacercaria as in the cercaria. These observations indicate that the family Bucephalidae could be included in the first category of classification, in which the flame cell formula remains the same in cercaria and metacercaria.

The basic patterns of nervous system in cercaria and metacercaria of *R. campanula* are similar, and also resemble the nervous system of adult reported by

Mellors (1985), though the nerves and commissures of metacercariae and adults are clearly defined. These observations indicate a single pattern of nervous system in all three stages of *R. campanula*. Studies on the superfamily Strigeoidea revealed a development of the nervous system from cercaria to metacercaria and adult, with different characteristic patterns in each stage (Niewiadomska & Moczon, 1984, 1987). There are no other reports of comparative studies on the nervous system of cercaria, metacercaria and adult of bucephalids, apart from the nervous systems of adults of *P. crucibulum* (Matthews, 1973) and of *P. squamatus* (Kotikova et al, 1983). According to Kotikova et al (1983), from a comparative study of the nervous system of *P. squamatus* and that of the other trematodes, the nervous system of *P. squamatus* is of a relatively primitive type. These observations indicate the necessity for further studies on the nervous system of larval and adult stages of the family Bucephalidae.

The reproductive system which has formed in the metacercaria of *R. campanula* is not as advanced in development as that of the metacercarial stages of many other species of gasterostomes, and it is not functional at this stage. Similar metacercarial stages with less-developed reproductive systems were observed in *B. haimeanus* (Matthews, 1973) and *B. longicornutus* (Howell, 1966). In contrast, fully developed systems were observed in *P. crucibulum* (Matthews, 1972) and *B. polymorphus* and

R. campanula (Baturo, 1977). It is clear that sexual maturity is attained only when the adult is within the digestive system of the final host, though development of the reproductive system in metacercariae may be influenced also by the second intermediate host. This is clearly evident from the development of *R. campanula* in minnows in British waters, and in *S. erythrophthalmus* (rudd), R. *rutilus* (roach), and *B. bjoercna* (silver bream) in Sleisinskie lake, Poland (Baturo, 1977).

CHAPTER EIGHT

BRIEF MORPHOLOGY AND MATURATION OF ADULT STAGE

8.1 Introduction

The adult stage of *Rhipidocotyle campanula* lives in the posterior intestine and rectum of carnivorous fish. There are reports of pike (*Esox lucius*) and perch (*Perca fluviatilis*) (Chappell, 1967; Shillcock, 1972) as final hosts in British waters, pike (*E. lucius*), perch (*P. fluviatilis*) and ruffe (*Acerina cernua*) (Kozicka, 1959; Baturo, 1977; Ivantsiv & Chernogorenko, 1984) as final hosts in other parts of Europe. It was not possible to investigate specimens of the natural fish host from the River Aire at Keighley, because a licence to fish could not be obtained. The experimental final hosts were perch (*P. fluviatilis*) and trout (*Salmo trutta*). This is the first record of the latter species being susceptible to infection in Britain or in Europe.

The general morphology of the adult stage of *R. campanula* has been described previously by Kozicka, 1959; Baturo, 1977; Chernogorenko, 1983. Therefore, the general morphology of the adult was studied using light microscopy, mainly to confirm the identity of the parasite being investigated, following its life-cycle. The external surface of the adult was studied in detail using scanning electronmicroscopy as it had never been investigated in *R. campanula* or in any other bucephalids. The significance of their structural variations has been analysed on a comparative basis with the cercarial and metacercarial stages.

The maturation of the adult of *R. campanula*, including the spermatogenesis and oogenesis, up to egg formation, has been studied using histochemical and histological methods. Unfortunately, the miracidial stage could not be observed.

8.2 Results

8.2.1 External morphology of the adult

The fully matured specimens measure 1104-1575 µm in length and 195-298 µm in width. Typically, the worms are oval to ovate in shape, wider anteriorly and are often attenuated posteriorly (Fig 8.1). The anterior sucker measures 206-263 µm in length, 186-256 µm in width and opens on the ventral side. The length to width ratio of the sucker varies continuosuly depending on the high contractibility of the organ. Above the opening of the sucker, the anterior end spreads into a fan with seven finger-like projections, this being a characteristic feature of the species (Fig 4.14), which is difficult to observe. When this fan-shaped hood is withdrawn, the worm becomes truncated at the anterior end (Fig 8.1 & 8.2).

At the anterior extremity of the anterior sucker, the papillae are arranged in a semi-circle, similar to the anterior extremity of the sucker of metacercariae (Fig 7.4 & 8.2). These papillae represent two main types; pit papillae (p_2) and uniciliated papillae (p_3). The pit papillae are found in the middle of the truncated region. Three different types of pit papillae have been observed, which could not be clearly differentiated in the metacercarial stage. They are with single collar (p'_2), double collar (p''_2) and treble collar (p'''_2) (Fig 8.3). This anterior region is highly

ridged with interlocking tegumental folds and is devoid of spines (Fig 8.3). The interlocking of the tegumental folds are more complex in the adult, than in the metacercarial stage.

Inside the anterior sucker, two clear regions with characteristic variations are present. The anterior half of the inside roof of the sucker is armed with posteriorly directed spines, embedded between longitudinal ridges (Fig 8.4). The posterior half of the roof of the sucker, is devoid of spines and has parallel lateral foldings (Fig 8.5).

The spination along the rest of the body of the adult stage is similar to that of the metacercaria, armed with posteriorly directed spines. These spines are erect throughout the body, but progressively smaller in the posterior region (Fig 8.6 & 8.7).

There are more single ciliary papillae in the anterior region of the adult, especially posterior to the anterior sucker, than on the dorsal surface of the body. There are also long ciliary papillae (p_5), cilium measuring 8-10 µm long, on both sides, posterior to the sucker opening (Fig 8.6). These long ciliary papillae were not observed in the metacercarial and cercarial stages. There are uniciliary papillae with small cilium (p_3) around the excretory pore and genital pore (Fig 8.7).

Fig 8.1 Scanning electron micrograph of the ventro-lateral region of the adult of R. campanula showing the anterior sucker (a.s) and the truncated "hood" (arrowed). Note the posteriorly directed spines covering the whole body.

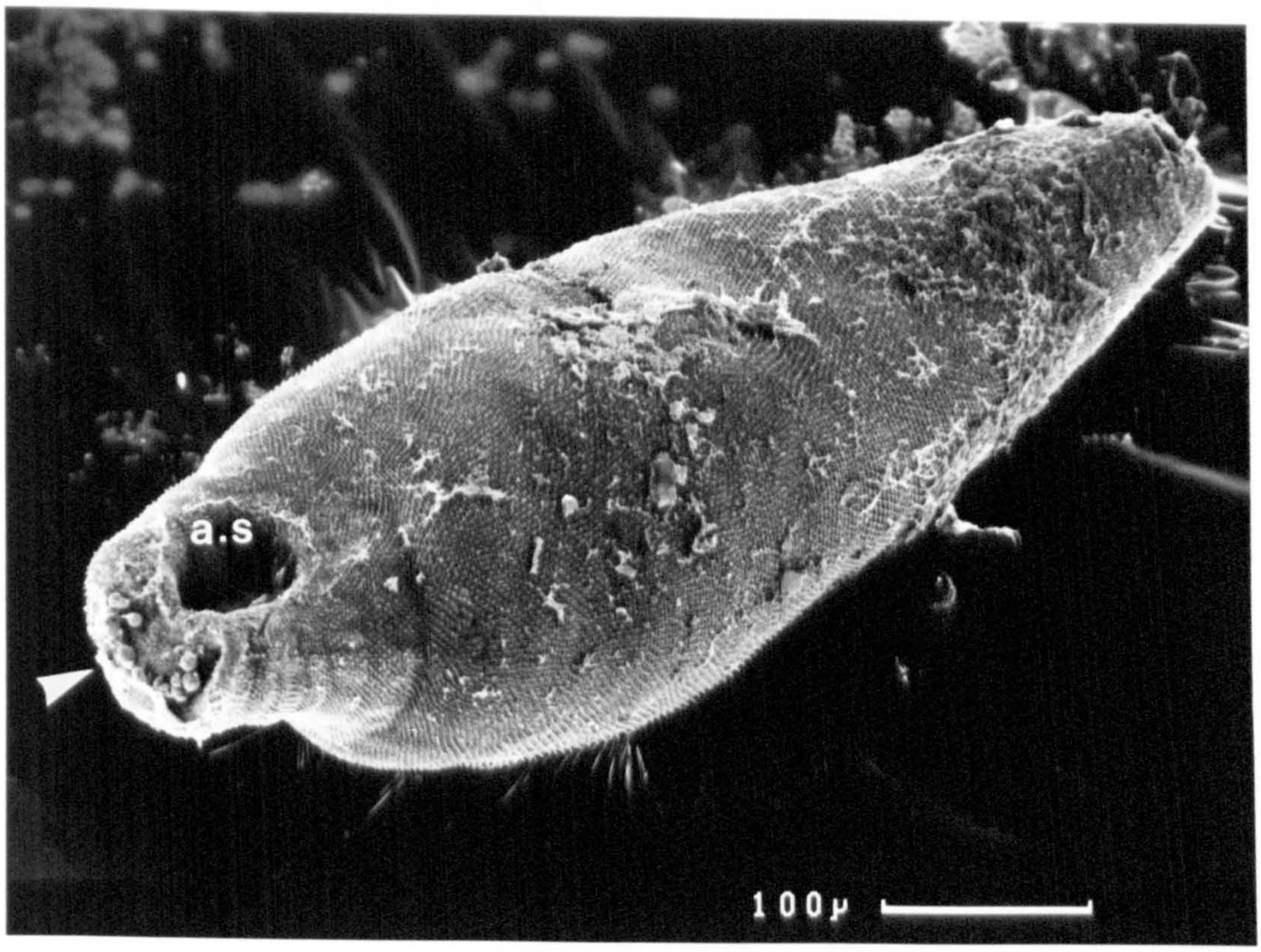
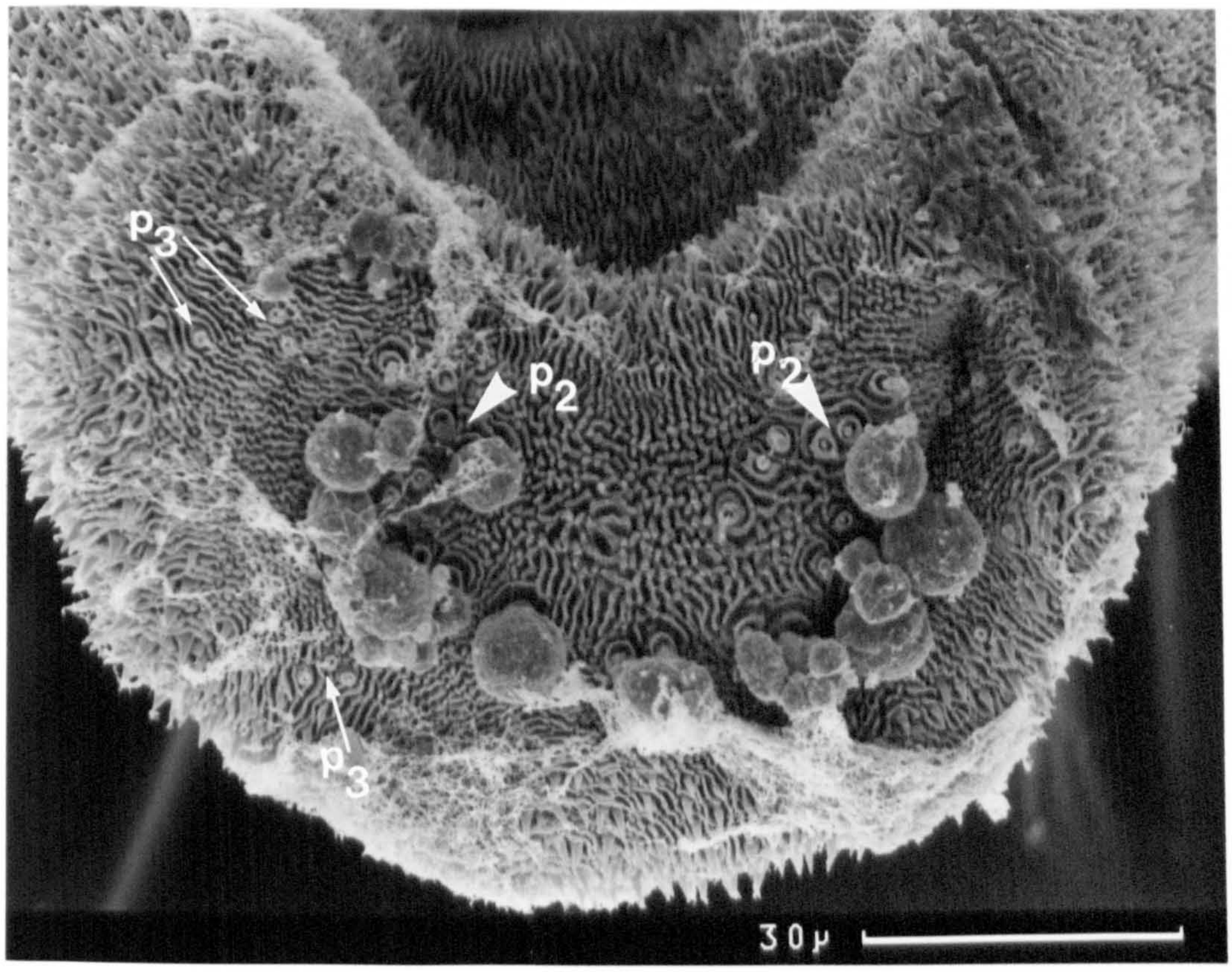

a.s
100μ

Fig 8.2 Scanning electron micrograph of the anterior extremity of the sucker showing pit papillae (p_2) arranged in a semi-circle. Note the interlocking tegumental ridges and absence of spines in this region; uniciliated papillae (p_3) are scattered outside the semi-circle of papillae.

Fig 8.3 Scanning electron micrograph of the anterior extremity of the sucker showing the double collar (p''_2) and treble collar (p'''_2) pit papillae.


p_3
p_2
p_2
p_3
30μ

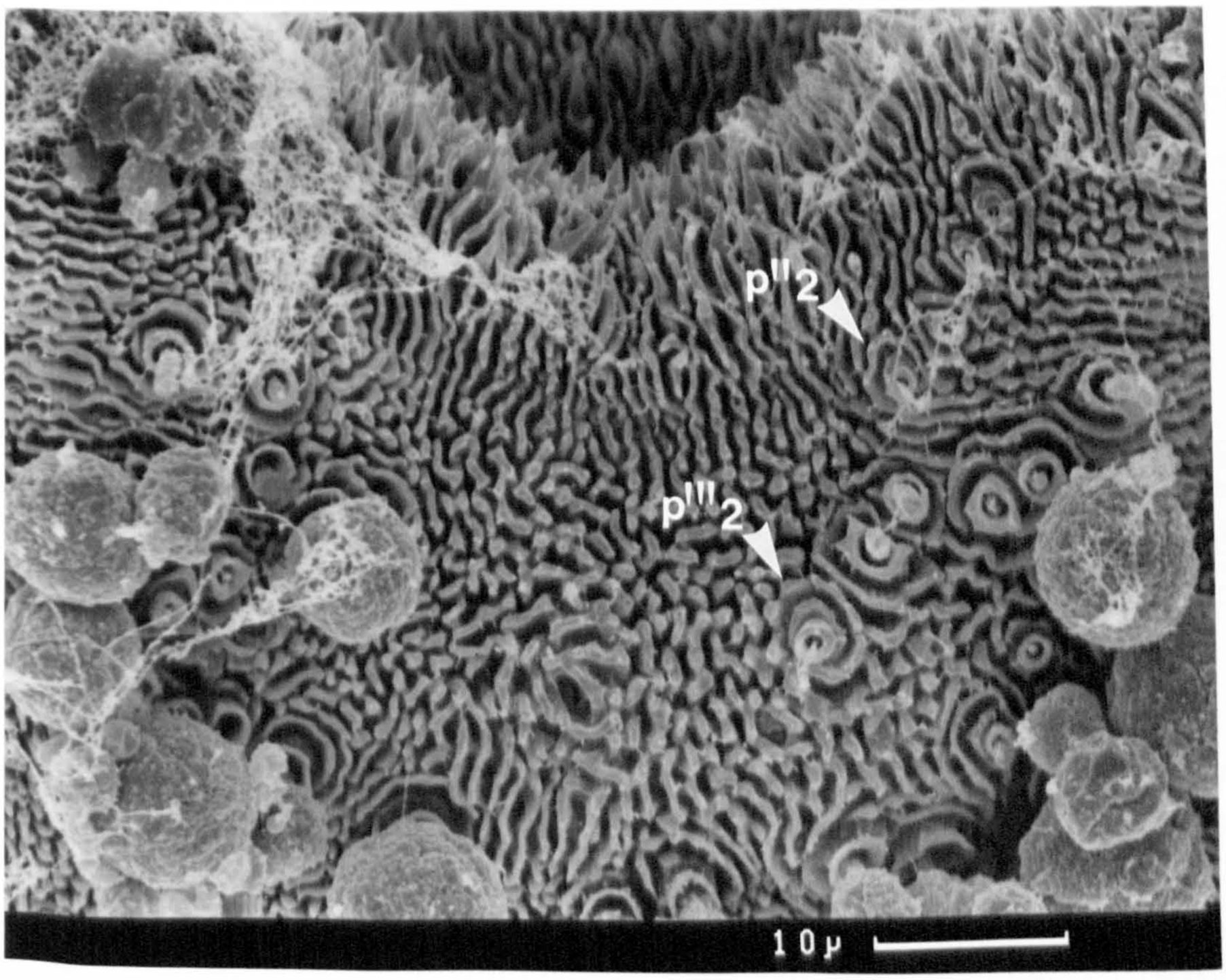
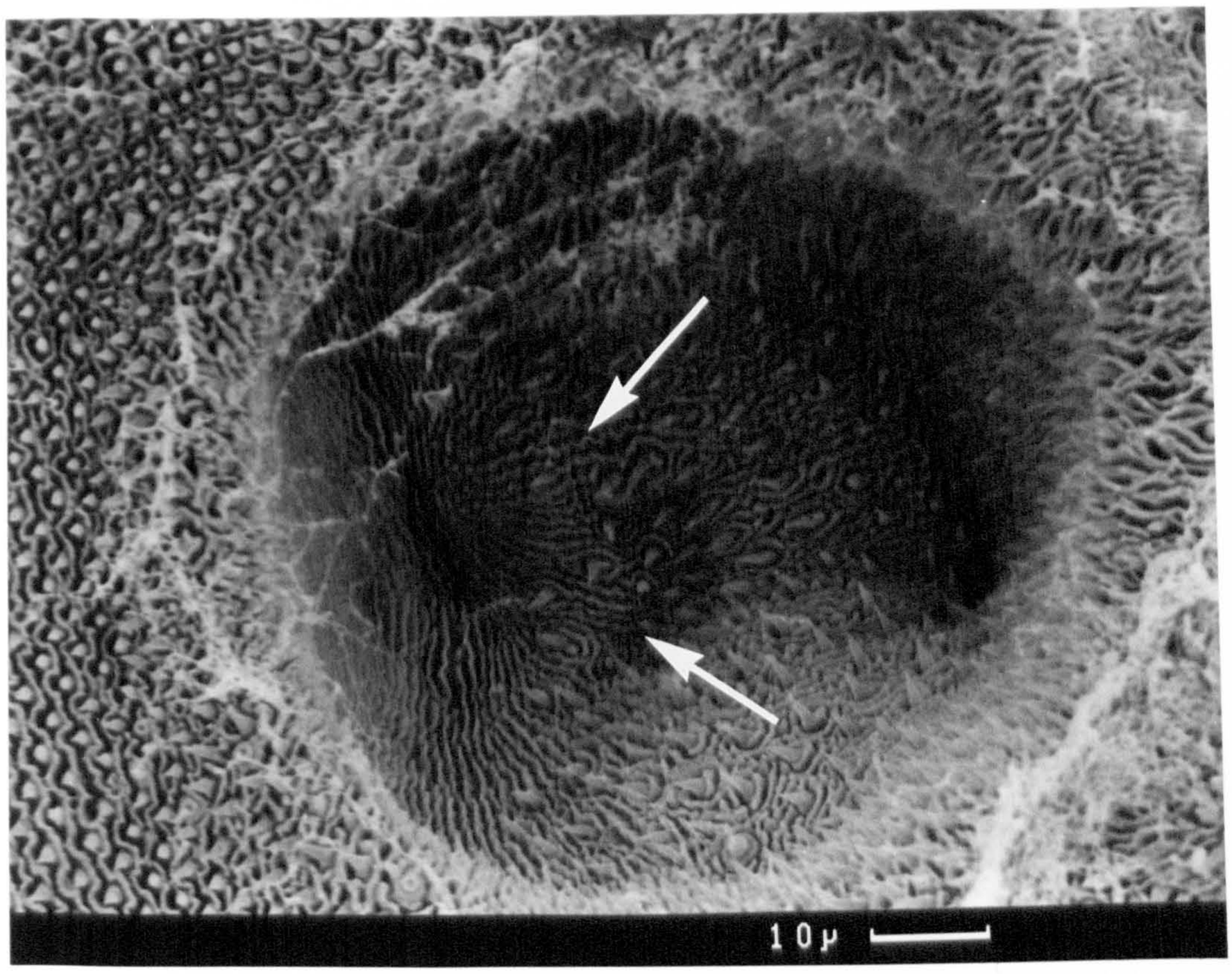

p''_2
p'''_2
10μ

Fig 8.4 Scanning electron micrograph of the innerside of the anterior sucker showing the posteriorly directed spines (sp) in the anterior half of the sucker and the longitudinal and inter-locking tegumental ridges.

Fig 8.5 Scanning electron micrograph of the innerside of the anterior sucker showing the lateral parallel foldings (arrowed) and absence of spines in the posterior half of the sucker.

Scale bar = 10µm


10μ

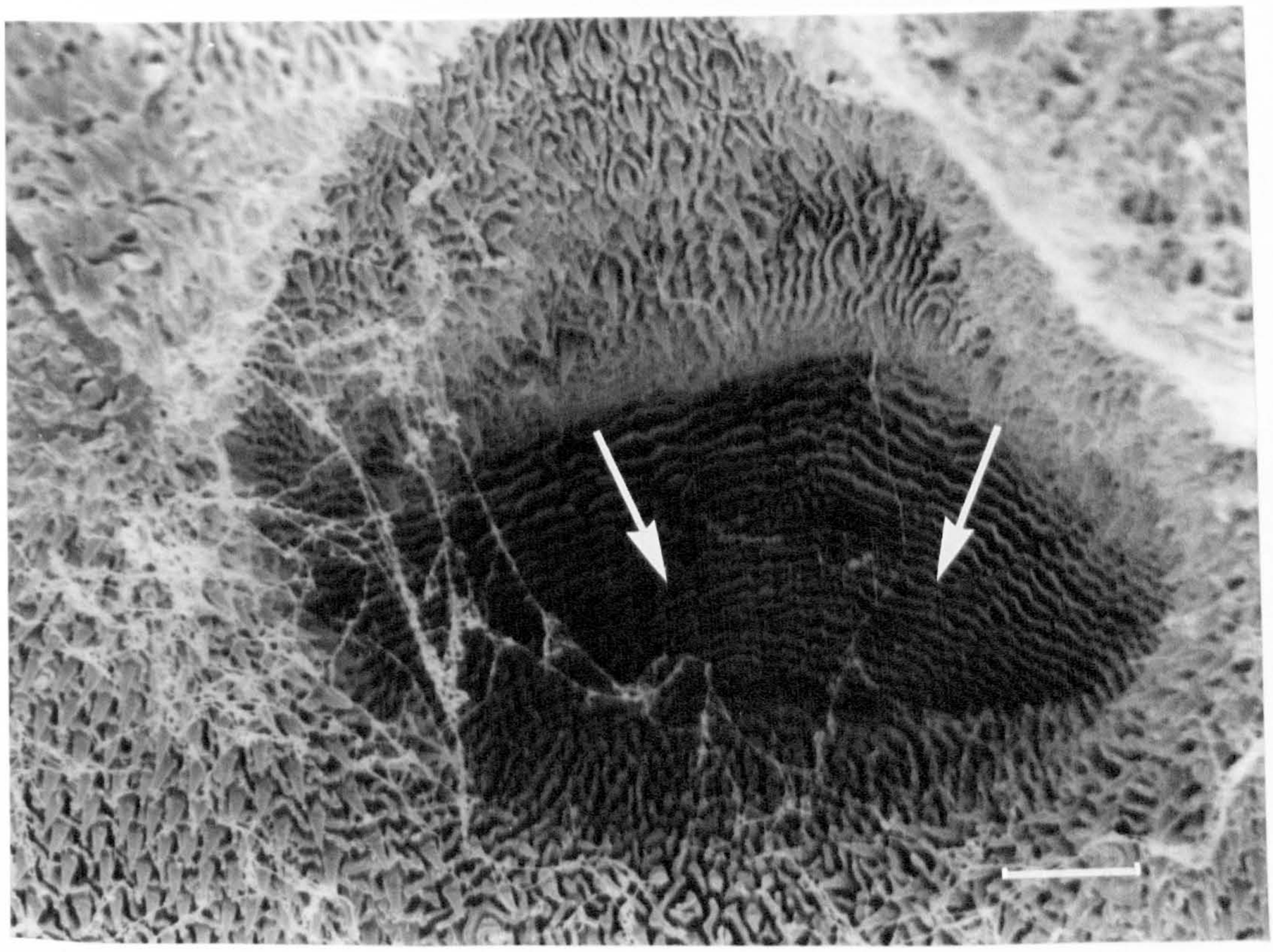
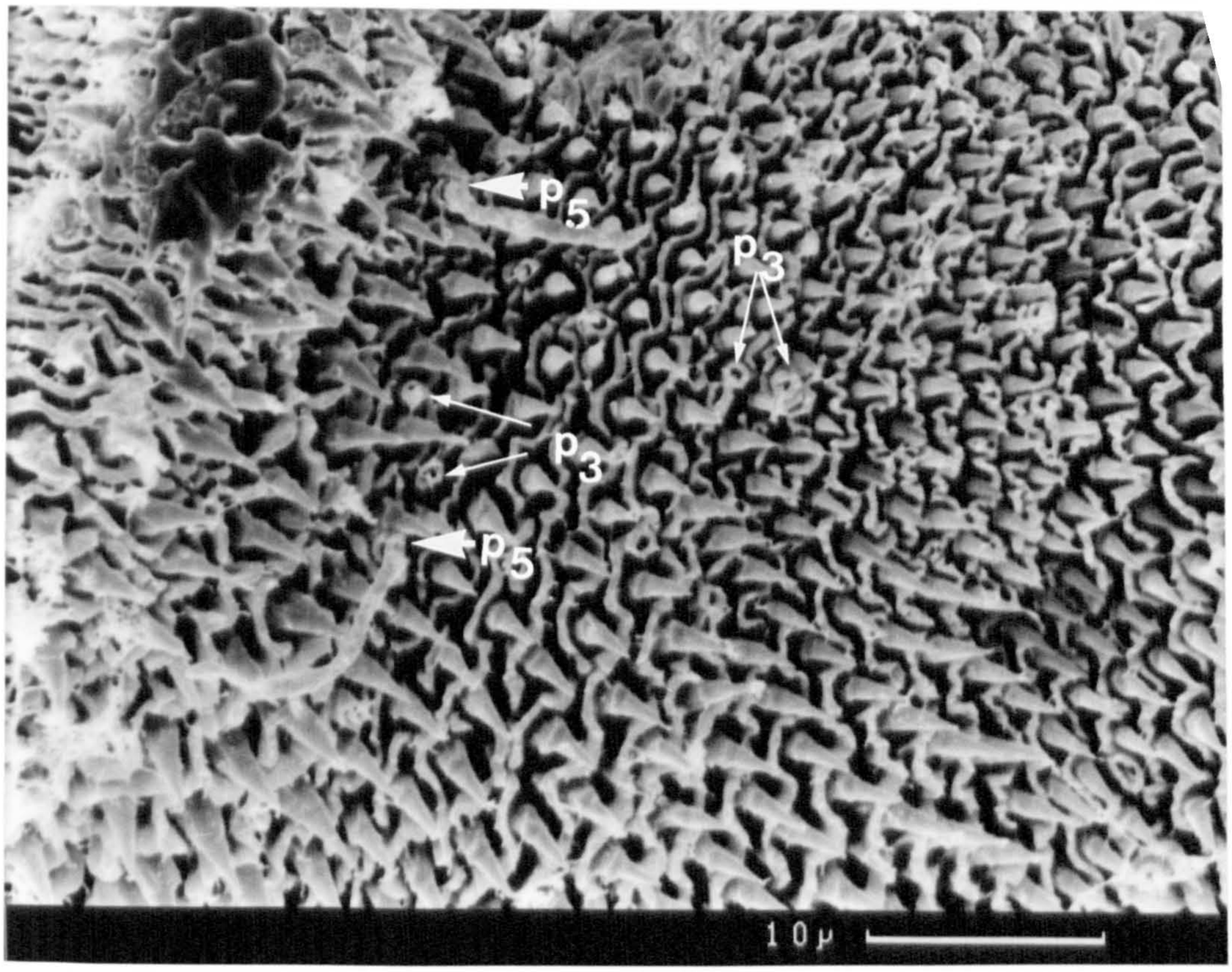
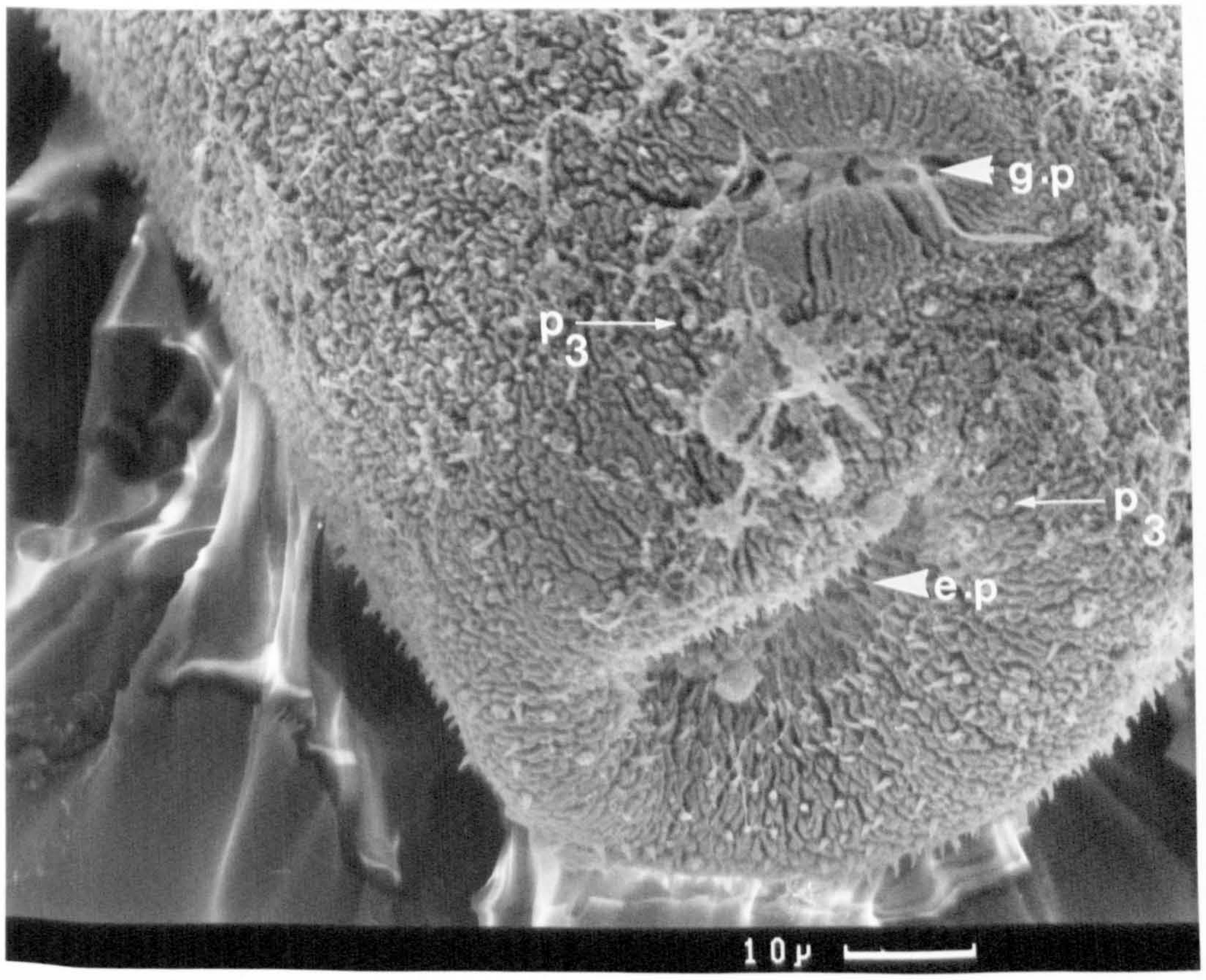


Fig 8.6 Scanning electron micrograph of the anterior ventral region behind the sucker of the adult of *R. campanula* showing the posteriorly directed spines (sp), short ciliary papillae (p_3) and long ciliary papillae (p_5).

Fig 8.7 Scanning electron micrograph of the posterior ventral region showing the genital pore (g.p) and excretory (e.p). Note the smaller scattered spines to that of the anterior region, and uniciliated papillae (p_3) with short cilium scattered on the region of the excretory (g.p) and genital pores (g.p)


p_5
p_3
p_3
p_5
10μ


g·p
p_3
p_3
e·p
10μ

8.2.2 Maturation of the adult

In two-weeks-old adults spermatogenesis and oogenesis had begun, but no spermatozoa were observed in the vesicula seminalis, cirrus sac or in the uterus. Following four weeks development, spermatozoa were seen in the vesicula seminalis and in the proximal part of the uterus. Vitellaria were fully developed and showed positive reactions to Fast red salt B (orange) (Fig 8.8) and Malachite green method (green) (Fig 8.9), demonstrating the presence of phenolic substances and protein. Oocytes were observed in the ovary and in the oviduct passing towards the ootype. Very few eggs were observed in the uterus leaving the ootype and none were observed further down the uterus. In six-weeks-old flukes, fully formed eggs were found along the uterus and no miracidial embryos were noted.

Spermatogenesis was followed in slightly squashed adults stained in 1% aceto-orcein. It was observed to follow a typical digenetic pattern (Fig 8.10). The primary spermatogonia are located within the wall of the testis and possess a relatively large spherical nucleus and very little cytoplasm. As the division approaches, the beginning of prophase is evidenced by the sixteen chromosomes becoming visible as elongated curved threads. Following a typical mitotic division each primary spermatogonia give rise to two secondary spermatogonia. These by further division

produce four spermatogonia and referred to as "tertiary spermatogonia" by Woodhead (1931) and Rees (1939). These are freed from the periphery of the testis into the lumen of the testis. These spermatogonia divide and produce eight cells and are connected together centrally. The general appearance of rosettes was not clearly observed as the cells were tightly grouped together.

These eight primary spermatocytes undergo meiotic division to produce sixteen secondary spermatocytes arranged in rosette formation. Each spermatocyte divides further into two spermatids, giving a total of thirty two; each spermatid develops into a mature spermatozoan. These rosette shape spermatocytes and spermatids are not very clear but the clusters of chromosomes are easily noticeable (Fig 8.8). Elongation of spermatozoa are very easily differentiated and form bundles of thirty two spermatids (Fig 8.10).

Live spermatozoa, observed in squashes of the vesicular seminalis, appeared as thread-like structures, and exhibiting rapid, undulatory movement along their entire length. A slightly thicker region is apparent in the anterior region, but no clear distinction can be made between head, mid and tail piece.

Masses of sperms are stored in the vesicula seminalis (Fig 8.9), and sperm were also found in the portion of the uterus following the ootype. This may be the place where fertilization occurs. In few specimens, sperm were found in the uterus away from the ootype. Eggs

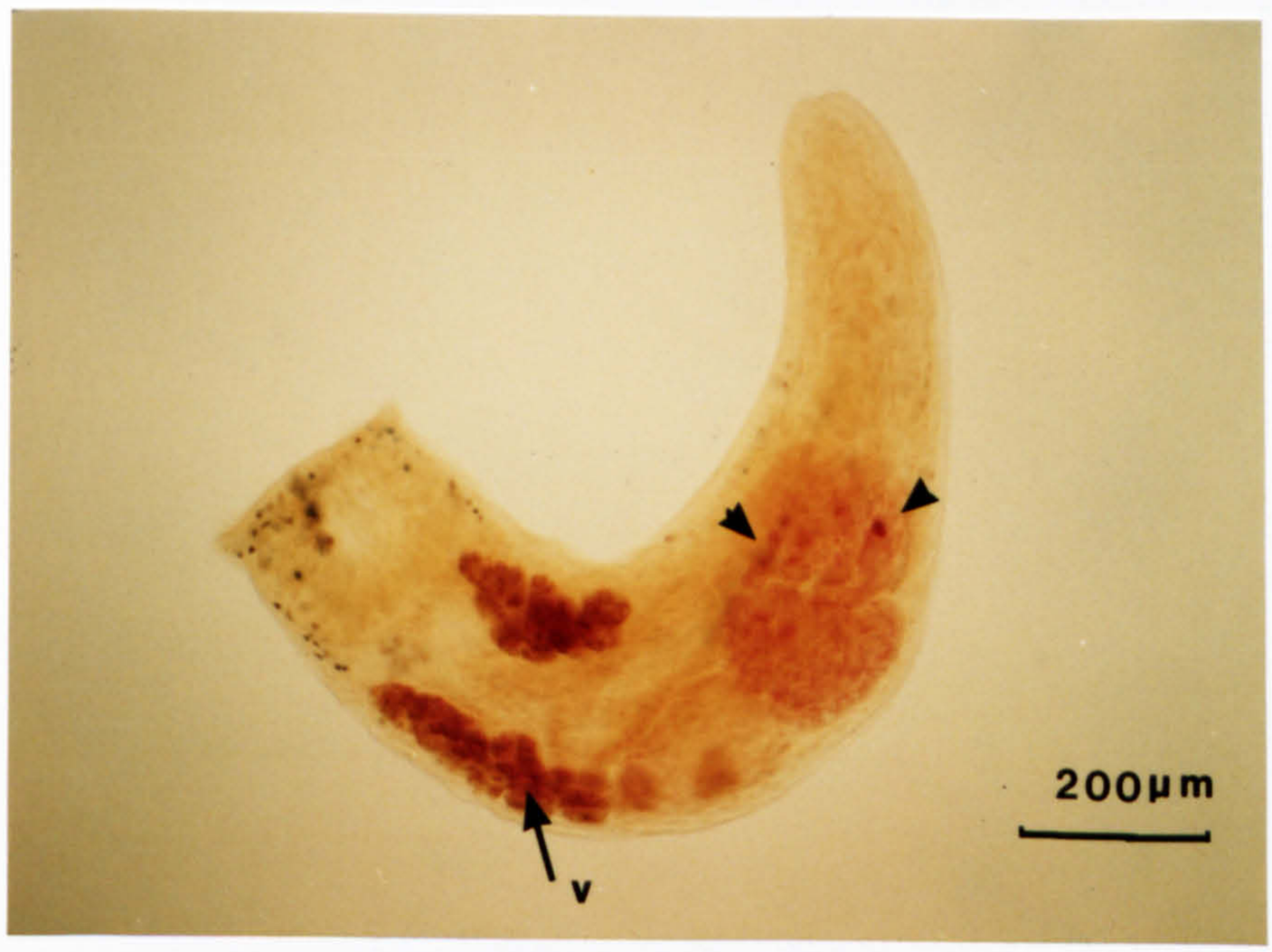
are stored in the uterus and these are probably, passed one at a time through the short duct called "metraterm" (which connects the uterus to the genital atrium), into the genital atrium.

The development of the eggs were followed in sections and whole mounts of six weeks old adult worms, stained in haematoxylin & eosin and also in 1% Fast red salt B. The cells lining the ovary in the internal surface have a spherical nucleus with a well developed nucleolus and a little cytoplasm. The oogonia divide by ordinary mitotic division to give rise to the oocytes.

The oocytes separate from the wall of the ovary and grow in size. Fully grown oocytes are found towards the entrance of the oviduct and in the centre of the ovary. These oocytes are found closely packed in the ovary and likely to liberate individually into the oviduct. These oocytes pass the entrance of Lauren's canal and vitelline duct into the ootype which is surrounded by Mehlis' gland. The formation of the egg capsule and fertilization were not observed closely but the whole eggs were observed surrounded by a mass of sperms during its passage through the downward loop of the uterus. The eggs in the upper loops of the uterus and near the cirrus sac, contained nucleus and few cells but no developed miracidium was observed. The fish were kept in the aquarium at temperatures between 9° C-11° C and probably the development of the miracidium is delayed due to the low temperature.

Fig 8.8 Four weeks old adult of *R. campanula* showing positive reaction in the vitelline cells (v) for phenolic compounds. Note the clusters of developing spermatids in the testis (arrowed). Stained with 1% Fast Red salt B and counter stained in 0.5% Neutral red.

Fig 8.9 Four weeks old adult of *R. campanula* showing positive reaction in the vitelline cells (v) to basic proteins. Note the clusters of sperms in the vesicula seminalis (arrowed). Stained with 0.5% malachite green and counter stained in 0.5% Neutral red.


v
200µm

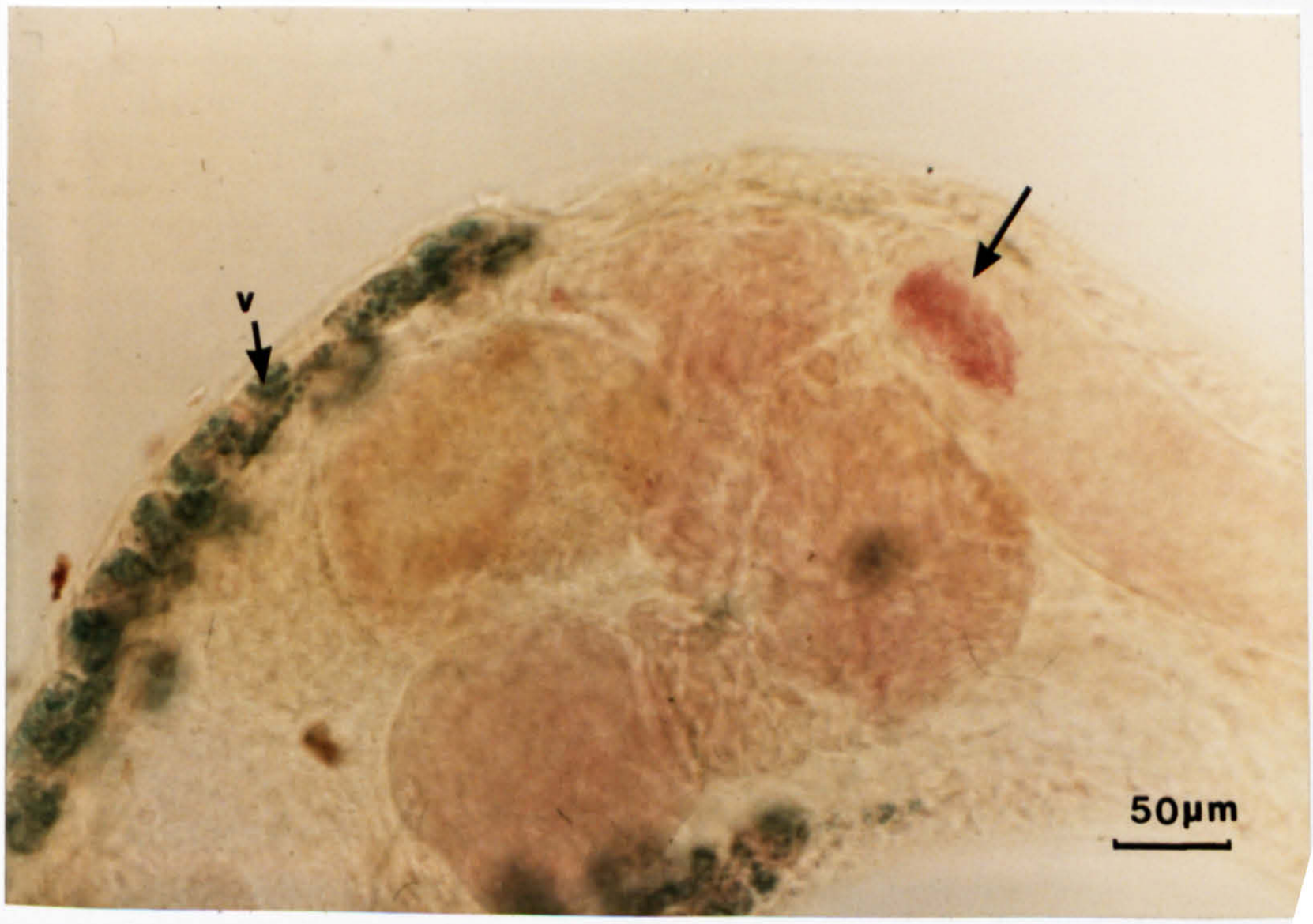

v
50µm

Fig 8.10

Spermatogenesis

Primordial germ cell

↓

Primary Spermatogonia (one cell stage)

↓ 1st mitotic division

Secondary Spermatogonoia (two cell stage)

↓ 2nd mitotic division

Tertiary Spermatogonia (four stage)

↓ 3rd mitotic division

Primary Spermatocytes (eight cell stage)

↓ 1st meiotic division

Secondary Spermatocytes (sixteen cells)

↓ 2nd meiotic division

32 Spermatids

(matures into functional spermatozoa)

8.3 Discussion

The scanning electron microscopical studies of the external surface of the adult and metacercarial and cercarial stages of *R. campanula* have clearly shown important structural variations in relation to the mode of life.

The anterior pit papillae in the adult stage are clearly defined compared to that of the cercarial and metacercarial stages. Therefore, most probably the papillae observed in the metacercariae in the similar sites to that of the adults are also pit papillae and may be in the process of development. These papillae most probably become functionally significant only in the adult. The nature and structure of the glands associated with these papillae are not studied and thus the true functions of these secretions can not be explained.

These pit type papillae have been reported in newly excysted metacercariae of Japanese lung flukes (Higo & Ishii, 1987), metacercariae of *C. sinensis* (Fijino et al, 1979) and *F. hepatica* (Bennett, 1976). The clearly developed single, double and treble tegumental collars surrounding the pit of the papillae in *R. campanula* adult and to a certain extent in the metacercariae have not been clearly defined in the above reported species. Higo & Ishii (1987) mentioned that these pit papillae function as tango-receptors and chemo-receptors for the migration and ingestion in the

gut of the host. As the anterior sucker in *R. campanula* is not associated with feeding as the mouth is mid-venrally placed in the body , these secretions are not likely to involve in digestion processess. These pit papillae observed in the adult of *R. campanula* are different from the pit papillae observed on the tail-stem of cercariae. These papillae on the tail-stem are more likely to be secretory organs as mentioned by Edwards et al (1977).

Study of the five species of the newly excysted Japanese lung flukes led Higo & Ishii (1987) to conclude that the arrangement of the pit and domed papillae around the suckers would contribute to distinguish the species. The arrangement of the pit papillae in bucephalids may eventually lead to distinguish species, if more studies are done on other species of bucephalids.

The development of the anterior sucker is highly advanced in relation to that of the metacercariae, in having the inner sucker walls with spines and lateral foldings to secure the attachment to the walls of the posterior intestine and rectum. As the metacercariae are enclosed in a cyst, there is no necessity for secure attachment.

The reproductive system of *R. campanula* is basically similar to that of *B. longicornutus* (Howell, 1966) and *Prosorhynchus crucibulum* (Matthews, 1973) in the way in which oviduct continues into the ootype, receiving Laurer's canal and the median vitelline duct; ootype receiving Mehlis' gland; opening of the uterus

into the genital atrium and the vas deferens entering the male pouch and dilates to form a vesicula seminalis. The arrangement and position of vitelline glands, position and size of ovary and testes, and the coiling of the uterus vary between species which are normally used to characterise species in bucephalids.

In *Prosorhynchus crucibulum*, histochemically separable two sections of Mehlis' glands were observed; those of the proximal glands containg neutral mucopoly-saccharide secretions and the distal glands containg lipid secretions (matthews, 1974). No detailed study was carried out in *R. campanula* to study the secretions of Mehlis' glands but in other Digenea two types of secretory cells have been described in these glands. It is probable that similar secretions may be present in *R. campanula*. Burton (1967) suggested that their secretions activate the sperm, lubricate the capsule-filled uterus and act as an emulsifying agent enabling the shell globules in the vitelline cells to coalesce and form the capsule. Later Erasmus (1972) and Smyth and Halton (1983) commented that the role of Mehlis' gland is still uncertain, and interpretations is hindered by its indefinite histochemical charecteristics.

The vitelline cells of *R. campanula* contains a protein which is positive to Malachite green, similar to *P. crucibulum* (matthews, 1973), and phenolic compounds which are positive to fast red salt B. Both believed to contribute to the egg capsule formation .

The developmental sequence of spermatogenesis in *R. campanula* is similar to those reported for other Digenea (Rees, 1939; Bell & Hopkins, 1956). Spermatogenesis commences with a rosette of eight spermatocytes, formed by mitotic divisions of spermatogonial cells, which give rise to sixteen secondary spermatocytes and thirty two spermatids by meiosis. The maturity of spermatids by spermiogenesis which have been studied electron microscopically in *Bucephaloides gracilescens*, have not been studied in *R. campanula*. The spermatozoa produced are liberated into the intercellular spaces of the testis.

Brief studies on *R. campanula* showed that the maturing spermatozoa are found only in the adult in the final host and no development is observed in the metacercarial stage. In *B. gracilescens* maturing spermatozoa are observed in all stages of post-cercarial development, from immature metacercarial to mature adults; no significant differences in ultrastructure of the testes are apparent, except for the size and numbers of cells present (Erwin & Halton, 1983). Also they noted high density of spermatozoa in the seminal vesicles of immature adults which is very clearly not observed until after two weeks of development of *R. campanula* in the rectum of final host. Further, they observed in *B. gracilescens* a continuous production of spermatozoa in the adult without seasonal variations. In the present studies temperature effect on the development of adult of

R. campanula in the final host is clearly noted, as lower the temperature, the longer the worms took to produce spermatozoa.

The conical penis observed in *R. campanula* is typical of a gasterostome character, lying permanently in the genital atrium. The ductus ejaculatorius opens dorsally near the base of the penis and opposite the uterus. The penis becomes spatula-shaped, the dorsal surface being drawn inwards forming a median groove . Jones (1943) suggested in *P. acleatus* that its shape was an adaptation to prevent self-fertilization but later Matthews (1968) observing the similar structure in *R. johnstoni* noted self-fertilization. It appears that asymmetry relate to a high degree of versatility of function to this structure.

Studies on B. gracilescens indicate that whilst the male system is highly progenetic, the female system is most immature and in mature metacercariae examined is less well developed (Erwin & Halton, 1983). Similarly in *R. campanula* female maturity is observed only in the final host. Consequently, Erwin & Halton (1983) indicated that the male system is able to discharge viable spermatozoa immediately . on excystment, ensuring cross-fertilization with ova from a more mature adult partner. In *R. campanula*, there is no clear indication whether cross-fertilization is taking place.

In the *R. campanula*, when the eggs are passed through the uterus leading to the ootype (referred as

receptaculum uterinum), mass of sperms are found surrounding the eggs but no case of polyspermy was noted.

In the uterus, the eggs contained few cells of different sizes and no clear differentiation of structure was noted. As mentioned by Erasmus (1972) further development of miracidium may require a developmental period in the external environment as in *F. hepatica* and *D. spathaceum* before miracidial formation is completed. In bucephalids miracidial stages have been reported only in *B. elegans* (Woodhead, 1929), *R. papillosum* (Woodhead, 1930) and *R. septpapillata* (Kniskern, 1952b), all North American fresh water species. Therefore, more work should be done in *R. campanula* and in other bucephalids to study the development of the miracidial stage.

GENERAL DISCUSSION

General discussion

The life-cycle of *Rhipidocotyle campanula* (Dujardin,1845) is typical of a gasterostome trematode, involving three hosts of which the primary host is the mussel, secondary host is a small fish and the final host a carnivorous fish. The natural primary host of *R. campanula* is *Anodonta anatina* (duck mussel), the secondary host is *Phoxinus phoxinus* (minnow) and the experimental final hosts were *Perca fluviatilis* (perch) and *Salmo trutta* (trout). The natural final hosts in the River Aire, Keighley could not be investigated. There are reports of pike and perch (Chappell ,1967 and Shillcock, 1972) as final hosts in British waters, but it was not possible to obtain a licence to fish for these. Even though adult worms in the experiments, developed in trout, the percentage of developing worms were very low compared to perch. This is the first record of trout being susceptible to infection in Britain and also in Europe.

The life-cycle study of *R. campanula* shows there is still no way of determining the generic status of a cercaria other than by infection experiments. However there may be possibilities of identifying the generic status of a cercaria by its swimming and resting phases and method of penetration and entering into the secondary host, in addition to the suggested possibilities of studying the flame cell distribution (Hopkins 1954, 1956;

LaRue 1957; Stunkard 1974d, 1975) and chaetotaxy (Richard 1968, 1971; Bayssade-Defour, 1979). The counting of flame cells is very tedious, and long and repeated observations may be required to complete the pattern for any one species. Similarly the conventional silver impregnation method, used to study the distribution of papillae, stains the gland duct openings and may mask the actual distribution of the papillae. A combination of these possibilities may eventually solve the problem of formulating a suitable key to the identification of species at cercarial level. To obtain a suitable key for the identification of bucephalid cercariae, more detailed studies on morphology, biology and behaviour are needed.

The generic identity of the bucephalid metacercariae is difficult to determine in specimens where the reproductive structures are not fully developed as in the adult, and its specific identity must be obtained from experimental infections. The metacercaria of *R. campanula* shows similarities to that of *Bucephalus longicornutus* (Howell, 1966) and *B. elegans* (Woodhead, 1930): they all lack the tentacles characteristic of the genus *Bucephalus*, and have partly developed reproductive structures. The absence of tentacles from the anterior extremity of *B. elegans* and *B. longicornutus*, given an appearance similar to the characteristic "hood" of *Rhipidocotyle*. Care must therefore be taken in assigning the metacercaria to species level on comparative morphology of metacercaria and adult. The scanning

electron micrographs of metacercariae and adult of *R. campanula* (Fig 7.3 & 8.1) confirm this view by showing remarkable differences in the anterior sucker.

The behavioural experiments revealed that the maximum survival time is 95h in 0.15% saline at temperatures 15° C and 20° C. This shows the ample opportunity of the cercariae to infect the secondary host the minnows, which occupy the area of the mussel beds mainly during spawning and otherwise are dispersed in deeper waters and away from the mussel beds. At the same time frequent resting phases save energy and prolong the active life of the cercaria, thus increasing the chances of infecting the secondary host. Moreover, because of their neutral responses to both light and gravity, cercariae were distributed throughout the full depth of water (8cm in the experiment). This would increase the chances of cercariae infecting minnows which occupy the mid and bottom waters of the river.

The infection of minnows with cysts is observed in almost all fish above 2cm in length, with an increase in number in older fish. This is probably coincidental, as the minnows may be using the mussel beds as spawing grounds, since these beds are only a few yards downstream from the area where the regular samples of minnows were taken. Observations from Baturo (1977) revealed that only 4.8% of *Rutilus rutilus* (roach) and 6.3% of *Scardinius erythrophthalmus* (rudd) were infected with the metacercarial cysts. Similarly Wierzbicka (1977) reported very

low incidence of infection of *Abramis brama*, *A. ballerus* and *Blicca bjoercna* with the metacercarial cysts of *R. campanula*.

The only natural intermediate hosts to harbour the metacercarial cysts of *R. campanula* in the River Aire at Keighley are minnows (*Phoxinus phoxinus*). There are, however, records of twelve other species of fish harbouring the parasite in Europe; four of these species are carnivorous and known as final hosts (pike, perch, pike perch and ruffe), but these are most likely to be accidental hosts. The observations from the experimental studies revealed a host specificity to minnows; cercariae which entered the trout fry (*Salmo trutta*), died within 2 days of entry. Apart from minnows no other cyprinid fishes could be obtained during the present study in order to analyse the specificity to this family; other published records show a definite tendency towards specificity to cyprinid fishes.

Kozicka (1959) describes cysts along the fins, muscles (usually at the base of the tail), in the eyes and brain, and Chernogorenko (1983) and Ivantsiv & Chernogorenko (1984) reports cysts in the fins and tail (of intense concentration) apart from gills and subcutaneous tissue in the pharyngeal region. These infections may be accidental, or may be the cysts of *B. polymorphus* since these sites are generally those where encystment has been observed (Baturo,1977). The present observations clearly reveal that cercariae of *R.*

campanula enter passively through the respiratory and food currents, and encyst in the subcutaneous fatty tissue beneath the lining of the pharynx and in some cases in the gill arches. The cercariae of *B. polymorphus* actively penetrate and encyst along the fins and at the bases of the fins (Baturo, 1977). Kozicka (1959), Wierzbicka (1977) and Baturo (1977) report of finding the metacercariae of these two species together in the same fish. Chernogorenko (1983) and Ivantsiv & Chernogorenko (1984) found these two species in the same species of fish but gave no clear indication whether simultaneously both these were present within individual hosts. Also Kozicka (1959) reported that when the tentacles which are characteristic of *B. polymorphus* are withdrawn, its appearance is similar to that of *R. campanula*. It seems most probable that Kozicka (1959) and Chernogorenko (1983) and Ivantsiv & Chernogorenko (1984) have confused the identification of these two species, which have proved very difficult to separate when they infect together. Since only one species, *R. campanula*, is found in the River Aire at Keighley, a comparative study of the two species could not be made to look more deeply into the identification problems.

Comparison of measurements of cercariae and metacercariae of *R. campanula* from River Aire, Keighley (cercaria,194-266µm x 94-141µm & metacercaria, 680-930µm x 155-178µm) with that of Polish species (Baturo, 1977) (cercaria, 340-580µm x 111-160µm & metacercaria,

33?-799µm x 140-261µm) and Russian species (Ivantsiv & Chernogorenko, 1984) (cercaria, 430-650µm x 120-200µm & metacercaria, 1185-1207µm x 316-372µm) show remarkable variations in size. This could clearly explain that the differences are not due to variations in technique, but most likely to geographical variations, and may represent different strains of the same species.

The characteristic seven lips in the anterior region, of the cercaria of *R. campanula* totally disappear, and an anterior sucker is formed in the metacercaria, becoming well developed in the adult. The rudimentary sucker seen in the cercaria of *R. campanula* is reported only in cercariae of *B. haimeanus* (Matthews, 1973) among bucephalid cercariae. The scanning electron micrographs of cercaria (Fig 6.4), metacercaria (Fig 7.3) and adult (Fig 8.1) show that the rudimentary anterior sucker of the cercaria not seen in metacercaria and adult, is likely to develop into the true sucker. Even though this progressive development has not been observed, this view is further supported by the observations of papillae which are seen on both sides of the rudimentary sucker in the cercaria (Fig 6.4). These are also seen in the anterior extremity of the sucker of metacercaria (Fig 7.4) and adult (Fig 8.2). This is the first comparative study by SEM of cercaria, metacercaria and adult of *R.campanula* or of any bucephalids.

SUMMARY

Summary

(1) A brief review of the literature and a survey of published records for the family Bucephalidae have been made, and problems of systematics and taxonomy discussed.

(2) Tabular summaries are given for the previous life-cycle studies of bucephalids, for synonyms and the total number of species in each of the 24 valid genera belonging to the six subfamilies of bucephalids, and for the previous reports on the becephalid cercariae.

(3) The studies on the previous bucephalid cercariae revealed that many descriptions are inadequate for comparative purposes and also that the extent of variation in the morphology between the cercaria, metacercaria and adult of a given species is unknown. It is impossible to predict adult characters and taxonomic status from larvae. More studies on the excretory patterns and chaetotaxy along with behavioural and detailed morphology may help to create a new system of classification of cercariae of bucephalids.

(4) Life-cycle studies have been completed for only six fresh- water and twelve marine species of bucephalids. Until more information on life-cycles is available, taxonomic allocations of species can not be definitive.

(5) A systematic review of *Rhipidocotyle campanula* is discussed along with the problems of differentiating the two freshwater bucephalids, *Bucephalus polymorphus* and *Rhipidocotyle campanula* in Europe.

(6) The experimental life-cycle studies confirm the identity of the parasite studied as *Rhipidocotyle campanula*, and that this species is the only one which occurs in the River Aire at Keighley.

(7) *Anodonta anatina* is the primary host of *R. campanula* and the only species identified in the area sampled in the river. The secondary host is *Phoxinus phoxinus* (minnow). Due to difficulties in obtaining a licence for fishing larger carnivorous fish, the final host in the river could not be confirmed.

(8) Only 8% of the mussels were infected with the sporocysts, and no mussels less than 3 years old were infected. The incidence of infection increases with age.

(9) The liberation of cercariae varies considerably in individual mussels depending on their state of infection, and is intermittent rather than cyclic or continuous. Both the activity of the mussel and higher temperatures (15°C to 18°C) influence emission of cercariae.

(10) The postulations regarding the release of cercariae from the sporocyts in bucephalids are confirmed by the observations of birthpores and broken sporocyst tubules near brood chambers which contain mature cercariae.

(11) For descriptive purposes the cercarial movements have been divided into active swimming, dropping, creeping and resting phases. The behaviour of the furcae of the swimming phase and the resting phase (furcae folded over the body) may be used as a generic identity of the cercaria.

(12) Prolonged survival time (>95h in 0.15% saline at 15°C & 20°C) with intermittent resting phases and no definite responses to light and gravity are adaptive features of cercariae to increase their chances of entry into the secondary host, minnows, which occupy the area of mussel beds during spawning.

(13) Cercariae enter passively through the respiratory and food currents; they encyst in the subcutaneous fatty tissue beneath the lining of the pharynx, and in some cases in the gill arches of the minnow. *R. campanula* is the only bucephalid known to enter passively into the secondary host. Some specificity to the host is also indicated.

(14) Entry and encystment is very rapid and a membraneous cyst forms within 4h of entry of cercariae. In 6 weeks the metacercaria attains its full size but at this time the reproductive system is not as advanced as in some other bucephalids.

(15) In the samples surveyed almost all the minnows above 2 cm in length are infected with the cysts. The number of cysts per fish increases with the age of the fish. An unequal distribution of cysts within each length group

of fish, and a greater variation in the bigger fish are noticeable.

(16) Gravid specimens of *R. campanula* were recovered from the rectum of perch, 5 weeks after feeding between 9°C-11°C, and 3 weeks after feeding between 14°C-15°C. Influence of temperature on the maturation of worms is evident.

(17) The chromosome number is 16.

(18) Attempts to obtain miracidia were not successful.

(19) The flame cell formula of the cercaria is 2[(5+5+5+5) + (5+5+5)] = 70, and is the same for the metacercaria. Previously the flame cell formulae were known for 25 spp. of bucephalid cercariae.

(20) The epidermis at different regions of the body of the cercaria reveals striking differences which explain the significant role of the epidermis in the free-living stage of the cercaria.

(21) The epidermis of the body of the cercaria has four types of tegumental bodies and the tail only one type. The suggested functions of these bodies are protection and secretion of the cyst wall in the metacercarial stage, but their functional significance is largely undetermined.

(22) There is no glycocalyx on the surface of the free-living cercaria but the discharge of the secretory bodies in places is noticeable. An acid mucopolysaccharide coat is present in the developed cercaria in the molluscan host.

(23) The variation in size and amount in the distribution of mitochondria along the body and tail of the cercaria is noticeable and this clearly corresponds to the energy requirements and related functions of different regions of the tegument.

(24) Significant variations in the external features of the anterior region of the cercaria, metacercaria and adult are noticeable in SEM:

(a) loss of the anterior lips of the cercaria and formation of anterior sucker in metacercaria and adult.

(b) rudimentary anterior sucker appears to become the true anterior sucker in the metacercaria and adult. This is evident from the papillae on either side of the rudimentary sucker moving to the anterior extremity of the true sucker.

(c) presence of long ciliary and double & treble collar pit papillae in the adult, which are not found in the cercaria.

(25) The excretory system & alimentary system are basically the same in cercaria, metacercaria and adult. The nervous system of the cercaria and metacercaria are the same except the nerves are more prominent in the metacercaria. The distribution and type of papillae differ in cercaria, metacercaria and adult.

(26) Calcareous corpuscles are present in the excretory bladder and tubules of the cercaria and not in the metacercaria and adult. The role of these corpuscles is not clear.

(27) Histochemical tests of cercaria revealed the presence of acid phosphatase in the pharynx, intestine, excretory bladder, anterior gland and secretory vesicles along the tail; non-specific esterase in the pharynx, intestine and nervous system and acetyl cholinesterase along the nervous system of cercaria and metacercaria. As the tests were done on wholemount preparations, the precise locations of these enzymes are not known. The possible functions are discussed.

REFERENCES

REFERENCES

ADEROUNMU, E. A. 1966. A comparative account of the parasite fauna of brown trout *Salmo trutta* L. from a lake and a hatchery. *Parasit.*, **56**: 10p.

AMATO, J. F. R. 1982. Digenetic trematodes of percoid fishes of Florianopolis, Southern Brasil, Bucephalidae. *Revista Brasileira de Biologia*, **42(4)**: 667-680.

ANDERSON, R. M & WHITFIELD, P. J. 1975. Survival characteristics of the free-living cercarial population of the ectoparasitic digenean, *Transversostrema patialense* (Soparkar,1924). *Parasit.*, **70**: 295-310.

ANDREV, B. 1949. Sobre la presencia de dos cercarias en el ovario de almeja (*Tapes aureus* Gmelin) en la Bahia de Santander. *Bol. Inst. Esp. Oceanogr.*, **22**: 1-7. (English summary, p.7)

ANGEL, L. M. 1961. Larval trematodes from Australian fresh-water molluscs. Part XV. *Trans. Roy. Soc. S. Aust.*, **84**: 63-70.

ASANJI, M. F & WILLIAMS, M. O. 1973. The structure and histochemistry of trematode metacercarial cysts. *J. Helminth.*, **47(4)**: 353-368.

BADCOCK, J. 1875. Some remarks on *Bucephalus polymorphus*, together with translations from papers of von Baer, Lacaze - Duthiers and Alf. Giard, on *B.polymorphus*

and *haimeanus* by Henry J. Slack. *Mon. Micros. Jour.*, 13: 141-146.

BAER, K. E. von. 1827. Beitrage zur kenntnis der niedern Thiere. *Nova. Acta. Acad. Nat. Curios.*, 13(2): 532-562. (English translation obtained)

BAKKE, T. A. 1978. *Urogonimus macrostomum* (Rudolphi, 1883) (Digenea) : its taxonomy and morphology as revealed by light and Scanning electron microscopy. *Can. J. Zool.*, 56: 2280-2291.

BAKKE, T. A. 1982. The morphology and taxonomy of *Leucochloridium variae* McIntosh (Digenea: Leucochloridiidae) from the Nearctic as revealed by light and scanning electron microscopy. *Zool. Scr.*, 11: 87-100.

BASCH, P. F. & NATALKIA, BASCH. 1982. *Schistoma mansoni*: Scanning electron microscopy of Schistosomula, adults and eggs grown in vitro. *Parasit.*, 85: 333-338.

BATURO, B. 1977. *Bucephalus polymorphus* Baer, 1827 and *Rhipidocotyle illense* (Ziegler,1883) (Trematoda, Bucephalidae) : morphology and biology of developmental stages. *Acta Parasitologica Polonica*, 24: 203-219.

BATURO, B. 1979. *Bucephalus* Baer, 1827, and *B. polymorphus* Baer, 1827 (Trematoda): proposed use of the plenary powers to conserve these names in accordance with general use. *Bull. Zool. Nomencl.*, 36(1): 30-36.

BATURO, B. 1980. Pathological changes in cyprinid fry infected by *Bucephalus polymorphus* Baer,1827 and *Rhipidocotyle illense* (Ziegler, 1883) metacercariae (Trematoda, Bucephalidae). *Acta Parasitologica Polonica*, **27**: 241-246.

BAYSSADE-DEFOUR, C. 1979. L'appareil sensoriel des cercaires et la systematique des Trematodes digenetiques. *Mem. Mus. natn. Hist. nat., Paris, Seris A, Zool.*, **113**: 1-81. (English translation obtained)

BELL, E. J & HOPKINS, C. A. 1956. The development of *Diplostomum phoxini* (Strigeida, Trematoda). *Annals Trop. .med. and Parasit.*, **50**: 275-282.

BELL, E. J & SMYTH, J. D. 1958. Cytological and histochemical criteria for evaluating development of Trematodes and Pseudophyllidean cestodes invivo and invitro. *Parasit.*,**48**: 137-148.

BELTON, M. C. & HARRIS, J. P. 1967. Fine structure of the cuticle of the cercaria of *Acanthatrium oregonense* (Macy). *J. Parasit.*, **53**: 715-724.

BENNETT, C. E. 1975. Surface features, sensory structures and movement of the newly excysted juvenile *Fasciola hepatica* L. *J. Parasit.*, **61**: 886-891.

BENNETT, C. E. 1977. *Fasciola hepatica*: development of excretory and parenchymal systems during migration in the mouse. *Expt. Parasit.*, **41**: 43-53.

BENNETT, C. E & THREADGOLD, L. T. 1973. Electron microscope studies of *Fasciola hepatica*. xiii. Fine structure of newly excysted juveniles. *Expt. Parasit.*, 34: 85-99.

BEVELANDER, G. 1933. The behaviour of the cercariae of *Bucephalus elegans* with special reference to the effect of light and temperature. *Phy. Zool.*, 6: 289-520.

BIBBY, M. C. 1972. Population biology of the helminth parasites of *Phoxinus phoxinus* (L), the minnow, in a Cardiganshire Lake. *J. Fish Bio.*, 4: 289-300.

BIBBY, M. C. & REES, G. 1971. The ultrastructure of the epidermis and associated structures in the meta-cercaria, cercaria and sporocyst of *Diplostomum phoxini* (Faust, 1918). *Zeit. fur Parasitenk.*, 37: 169-186.

BILQEES, F. M. 1976a. Two trematodes of the genus *Prosorhynchus* Odhner, 1905 (Bucephalidae) including a new species *P. erumenis* from the fish *Psettodes erumei* (Bl. and Schn.) of the Karachi Coast. *Norw. J. Zool.*, 24: 345-348.

BILQEES, F. M. 1976b. A comment on the relationship of *Prosorhynchus thapari* Manter, 1953 (Trematoda) from *Plectorhynchus cinctus* (T.S) off the Karachi coast, with a note on it's surface ultra-structure. *Proceedings of the Pakistan Academy of Science*, 13(1): 29-33.

BILQEES, F. M. 1978. Surface ultrastructure of three trematodes (Bucephalidae) from the fishes of Karachi Coast. *In abstracts of the Asian Congress of Parasitology, Bombay, India*. 243.

BLAIR, D. 1976. Observations on the life - cycle of the strigeoid trematode *Apatemon* (*Apatemon*) *gracilis* (Rudolphi, 1819) Szidat, 1928. *J. Helminth.*, 50(2): 125-132.

BRAND, T, VON; WEINBACH, E. C & CLAGGETT, C. E. 1965. Incorporation of phosphate into the soft tissues and calcareous corpuscles of larval *Taenia taeniaeformis*. *Comp. Biochem. Physiol.*, 14: 11-20.

BRETON, G. 1970. An epizootic of larvae of *Bucephalus mytili* Cole, affecting natural and cultivated populations of *Mytilis edulis* L. in the region of Coutances (Manche). *C. r. hebd. Seanc. Acad. Sci., Paris, Ser. D*, 271(12): 1049 - 1052.

BRUCKNER, D. A & VOGE, M. 1974. The nervous system of larval *Schistosoma mansoni* as revealed by acetyl-cholinesterase staining. *J. Parasit.*, 60: 437-446.

BURSTONE, M. S. 1958. Histochemical demonstration of acid phosphatase with naphthol AS-phosphates. *J. Nat. Cancer Ins.*, 21: 523-539.

BUSTA, J. 1985. Scanning electronmicroscopy of the cercaria, metacercaria and adult of *Plagiorchis elegans* (Rudolphi,1802) (Trematoda:Plagiorchidae). *Folia Parasitologica*, 32: 317-321.

BYKHOVSKAYA - PAVLOVSKAYA, I. E. et al. 1964. *Key to parasites of freshwater fish in the U.S.S.R.* Israel Program for Scientific Translations, Jerusalem.

CABLE, R. M. 1956. Scientific Survey of Puerto Rico and Virgin Islands. Marine cercariae of Puerto Rico. *New York Acad. Sci., Part 4*, **16**: 490 - 577.

CAMPBELL, A. D. 1974. The parasites of fish in Loch Leven. *Proc. R. Soc. Edinb.*, B, **74**: 347 - 364.

CARDELL, R. R. 1962. Observations on the ultrastructure of the bodyof the cercaria of *Himasthla quissetensis* (Miller & Northup 1926). *Trans. Amer. Microsc. Soc.* **81**: 124-131.

CARDELL, R. R & PHILPOTT, D. E. C. 1960. The ultrastructure of the tail of the cercaria of *Himasthla quissetensis* (Miller & Northup, 1926). *Trans. Amer. microsc. Soc.*, **79**: 442-450.

CHAPMAN, H. D. 1973. The functional organization and fine structure of the tail musculature of the cercariae of *Cryptocotyle lingua* and *Himasthla secunda*. *Parasit.*, **66**: 487-497.

CHAPMAN, H. D & WILSON, R. A. 1973. The propulsion of the cercaria of *Himasthla secunda* and *Cryptocotyle lingua*. *Parasit.*, **67**: 1-15.

CHAPPELL, L. H. 1967. Ecological and experimental studies on the parasites of freshwater fishes in Northern England. Ph. D. Thesis. (Leeds).

CHAPPELL, L. H. AND OWEN, R. W. 1969. A reference list of parasite species recorded in freshwater fish from Great Britain and Ireland. *J. Nat. Hist.*, 3: 197-216

CHENG, T. C 1963. The effects of *Echinoparyphium* larvae on the structure of the glycogen deposition in the hepatopancreas of *Helisoma trivolvis* and glycogenesis in the parasite larvae. *Malacologia*, 1: 291-303.

CHENG, T. C. 1964. Studies on phosphatase system in hepatopancreatic cells of the molluscan host of *Echinoparyphium* sp. and in the redia and cercaria of this trematode. *Parasit.*, 54: 73-79.

CHENG, T. C. and SNYDER, R. W. Jr. 1962. Studies on the host-parasite relationships between larval trematodes and their hosts. 1. A review. 11. The utilisation of the hosts glycogen by the intra-molluscan larvae of *Glypthelmins pennsylvaniensis* Cheng, and associated phenomenon.*Trans. Amer. Microsc Soc.*, 81: 209-228.

CHENG, T. C & BURTON, R. W. 1966. Relationships between *Bucephalus* sp. and *Crassostrea virginica*: a histochemical study of some carbohydrates and carbohydrate complexes occuring in the host and and parasite. *Parasit.*,56: 111-122.

CHERNOGORENKO, M. I. 1980. Periodicity, diurnal rhythm and ecological factors influencing the rate of issuance of cercariae from molluscan hosts. *Gidrobiol. ZH.* 18(3): 64-72. (In English)

CHERNOGORENKO, M. I. 1983. [Trematode larvae in molluscs in the Dneper river and its water reservoirs,

fauna, biology, features of it's formation.] "*Naukova Dumka*" 212pp. (In Russian, English translation obtained).

CHERNOGORENKO, M. I. and IVANTSIV, V. V. 1980. Life-cycle of the trematode *Rhipidocotyle illense*. In *Viprosy Parazitologii Vodnykh Bespozvonochnykh* by Zhivotnykh p.105-106. (In Russian, English translation obtained)

CHOUBISA, S. L. 1986. Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy. *Proc. Indian Acad. Sci (Anim. Sci).*, **95(5)**: 623-628.

CHOWDHURY, A. B; DASGUPTA, B. & RAY, H. N. 1962. On the nature and the structure of the calcareous corpuscles in *Taenia saginata*. *Parasit.*, **52**: 153-157.

CHU, H. J. 1950. *Bucephalopsis kweiyangensis* n.sp. from the giant salamander, *Megalobatrachus japonicus* Temm. in Kweichow, China. *J. Parasit.*, **36**: 120-122.

CHUBB, J. C. 1965. Report on the parasites of freshwater fishes of Lancashire and Cheshire. *Lancs. Chesh. Fauna Commn.*, No.50.

CHUBB, J. C. 1970. The parasite fauna of British freah-water fish. *Symp. Br. Soc. Parasit.*, **8**: 119-144.

CHUBB, J. C. 1975. A review of seasonal occurrence of maturation of adult helminths in freshwater fish in the British Isles. *Parasit.*, **71(2)**: iii-iv.

(Proceedings of the British Society for Parasitology, Univ. of East Anglia, Norwich, 1975).

CHUBRIK, G. K. 1952. [The life-cycle of *Prosorhynchus squamatus* Odhner, 1905.] *Dokl. Akad. Nauk.*, S. S. R **83(2)**: 327-329. (In Russian, English translation obtained).

CHUBRIK, G. K. 1966. Fauna and ecology of larval trematodes in molluscs of Barents and White Seas. Life-cycle of parasitic worms of North Seas. *Tr. Akad. Nauk.*, S.S.R **10(4)**: 78-158. (In Russian, English translation obtained).

CHUN, A. K. 1974. Histopathology and site of infection of oyster by *Bucephalus* sp. on the Southern coast of Korea. *Publ. Mar. Lab. Busan Fish. Coll.*, 7: 77-85. (In Korean, English abstract consulted in Hel. Abs. 1976(45A), No. 1758)

CIORDIA, H. 1956. Cytological studies of the germ cell cycle of the trematode family Bucephalidae. *Trans. Amer. Microsc. Soc.* **75(1)**: 103-116.

CLAPAREDE, E. 1863. Beobachtung uber Anatomie und Entwicklung wirbelloser Thiere, an den Kuste der ,kc Normandie angestellt. Leipzig. (Quoted by Badcock, 1875).

COIL, W. H. 1958. Alkaline phosphatase in the trematode excretory system. *Proc. Helminth. Soc. Wash.*, **25**: 137-138.

COLE, H. A. 1935. On some larval trematode parasites of the mussel (*Mytilus edulis*) and the cockle (*Cardium edule*). *Parasit.*, **27**: 276-280.

CONDE-DEL PINO, E; PEREZ-VILAR, M; CINTRON-RIVERA, A. A & SENERIZ, R. 1966. Studies in *Schistosoma mansoni*. 1. Malic and lactic dehydrogenase of adult worms and cercariae. *Expt. Parasit.*, **18**: 320-326.

CONDEL-DEL PINO, E; ANNEXY-MARTINEZ, A. M; PEREZ-VILAR, M & CINTRON-RIVERA, A. A. 1968. Studies in *Schistosoma mansoni*. 11. Iso-enzyme patterns for alkaline phosphatase, isocitric dehydrogenase, glutamic oxalacetic transaminase and glucose 6-phosphate dehydrogenase adult worms and cercariae. *Expt. Parasit.*, **22**: 228-294.

CORT, W. W. 1917. Homologies of the excretory system of the forked-tailed cercariae. *J. Parasit.*, **4**: 49-57.

CROFTON, H. D. AND FRASER, P.G. 1955. The mode of infection of the Hake, *Merluccius merluccius* (L) by the trematode *Bucephalopsis gracilescens* (Rud.) *Proc. Zool. Soc. Lond.*, **124**: 105-109.

CROWCROFT, P. W. 1947. The anatomy of two new digenetic trematodes from Tasmanian food fishes. *Proc. of the Linn. Soc. N. S. Wales*, **71(3/4)**: 108-118.

CULLING, C. F. A. 1974. *Hand book of Histopathological and Histochemical techniques*. 3rd Edn., Butterworth, London. xiv - 712pp.

DAWES, B. 1946. *The trematodes with special reference to British and other European fishes*. Camb. Univ. Press. 644 pp.

DAYAL, J. 1948. Trematode parasites of Indian fishes. Part 1. New trematodes of the family Bucephalidae Poche, 1907. *Indian J. Helminth.*, **1(1)**: 47-62.

DICKERMANN, E. E. 1954. *Paurorhynchus hiodontis*, a new genus and species of Trematoda (Bucephalidae: paurorhynchinae new sub fam.) from the mooneye fish, *Hiodon tergisus*. *J. Parasit.*, **40(3)**: 311-315.

DIESING, K. M. 1858. Revision der Myzhelminthen. Abtheilung Trematoden. *Sitzungsb. Akad. Wissensch.*, Wien, Math-Naturw, **32**: 307-390. (English translation obtained).

DIXON, K. E. 1965. The structure and histochemistry of the cystwall of the metacercariae of *Fasciola hepatica*. *Parasit.*, **55**: 215-226.

DIXON, K. E. 1966. A morphological and histological study of the cystogenic cells of the cercaria *Fasciola hepatica* L. *Parasit.*, **56**: 287-297.

DIXON, K. E. & MERCER, E. H. 1965. The fine structure of the nervous system of the cercaria of the liver fluke, *Fasciola hepatica* L. *J. Parasit.*, **51**: 967-976.

DOLLFUS, R. P. 1929. Helmintha 1. Trematoda et Acanthocephala. *Faune colonies Francaises*, Paris, **3(2)**: 73-114. (English translation obtained)

DOLLFUS, R. P. 1968. Les trematodes de l'histoire naturelle des helminthes de Felix Dujardin (1845). *Mem. Mus. natn. Hist. nat., Paris, Serie A, Zool.*, **54(3)**: 119-196. (English translation obtained)

DUJARDIN, F. 1845. *Histoire naturelle des helminthes ou vers intestinaux*. Paris, 654 pp. (English translation obtained).

DURIO, W. O. & MANTER, H. W. 1968. Some digenetic trematodes of marine fishes of New Caledonia. Part 1 Bucephalidae, Monorchiidae and some smaller families. *Proc. Helminth. Soc. Wash.*, **35(2)**: 143-153.

DUSANIC, D. G. 1959. Histochemical observations of alkaline phosphatase in *Schistosoma mansoni*. *J. Infect. Dis.*, **105**: 1-8.

DUTT, S. C & SRIVASTAVA, H. D. 1962. Biological studies on *Orientobilharzia dattai* (Dutt & Srivastava,1952) Dutt and Srivastava, 1955 - a blood fluke of ruminents. *Indian J. Vet. Sci.*, **32**: 216-228.

ECKMANN, F. 1932. Beitrage zur kenntnis der Trematoden-familie Bucephalidae. *Z. Parasitenk.*, **5**: 94-111. (English abstract consulted in Hel. Abs. 1932-1933, (1), 319d).

ECKMANN, F. 1934. Rectifications de nomenclature. *Ann. de Parasit. humaine et comp.*, **12(3)**: 256. (English abstract consulted in Hel. Abs. 1934-1935, (3), 56d)

EDWARDS, H. H; NOLLEN, P. M & NADAKAVUKAREN M. J.1977. Scanning and transmission electron microscopy of oral sucker papillae of *Philophthalmus megalurus*. *Int. J. Parasit.*, **7**: 429-437.

ERASMUS, D. A. 1958. Studies on the morphology, biology and development of a strigeid cercaria (*Cercaria X*, Baylis 1930). *Parasit.*, **48**: 312-335.

ERASMUS, D. A. 1967a. Ultrastructural observations on the reserve bladder system of *Cyathocotyle bushiensis* Kahn,1962 (Trematoda: Strigeoidea) with special reference to lipid excretion. *J. Parasit.*, **53**: 525-536.

ERASMUS, D. A. 1967b. The host-parasite interface of *Cyathocotyle bushiensis* Khan, 1962 (Trematoda : Strigeoidea). 11. Electron microscope studies of the tegument. *J. Parasit..*, **53**: 703-714.

ERASMUS, D. A. 1972. *The biology of trematodes*. Edward Arnold (Publishers) Ltd. pp.viii +312

ERCOLANI, G. 1881. Dell' adattamento delle specie all' ambiente nuove recerche sulla storia genetica dei Trematodi. 1. *Mem. R. Accad. Bologna, 4th ser.*, **2**: 237-334. (Reference from Howell, 1966).

ERCOLANI, G. 1882. Memoria. 11. *Mem. R. Accad. Bologna, 4th ser.*, **3**: 43-111. (Reference from Howell, 1966).

ERWIN, B. E. & HALTON, D. W. 1983. Fine structural observations on spermatogenesis in a progenetic trematode *Bucephaloides gracilescens*. *Inter. J. Parasit.*, **13(5)**: 413-426.

FARLEY, J. 1962. The effect of temperature and pH on the longevity of *Schistosomatium douthitti* miracidia. *Canadian J. Zool.*, **40**: 615-620.

FAUST, E. C. 1919. The excretory sysrem in Digenea. 2 Observations on the excretory system in distome cercariae. *Biol. Bull.*, **36**: 322-339.

FAUST, E. C. 1924. Notes on the larval flukes from China. *Amer. Jour. Hygiene*, **4**: 241-301.

FAUST, E. C. 1926. Further observations on South African larval tremmatodes. *Parasit.*, **18(1)**: 101-126.

FAUST, E. C. 1932. The excretory system as a method of classification of digenetic trematodes. *Quart. Rev. Biol.*, **7**: 458-468.

FRIPP, P. J. 1967. Histochemical localization of esterase activity in Schistomes. *Expt. Parasit.*, **21**: 380-390

FUJINO, T; ISHII, J & CHOI, D.W. 1979. Surface ultrastructure of the tegument of *Clonorchis sinensis* newly excysted juveniles and adult worms. *J. Parasit.*, **65**: 579-590.

GIARD, A. 1874. Sur l'encystment du *Bucephalus haimeanus* *C. R. Acad. Sci., Paris*, **79**: 485-487. (English Translation obtained)

GOMORI, G. 1952. *Microscopic Histochemistry*. Univ. of Chicago press, Chicago. 273pp.

GRABDA-KAZUBSKA, B & MOCZON, T. 1981. Nervous system and chaetotaxy in the cercaria of *Haplometra cylindracea* (Zeder, 1860) (Digenea, Plagiorchiidae). *Z. Parasitenk*, **65**: 53-61

GUPTA, V. & AHMAD, J. 1976. Digenetic trematodes of marine fishes. On some new and known digenetic trematodes of the family Bucephalidae poche,1907 from marine fishes of Pori, Orissa, India. *Anales del Instituto de Biologia, Univ. Ersidad National Autonoma de Mexico, Serie Zoologia*, **47(2)**: 9-18.

GUPTA, S. P. & TIWARI, M. 1983a. Trematode parasites of marine fishes. *Ind. J. of Helminth.*, **35(2)**: 93-111.

GUPTA, S. P. & TANDON, V. L. 1983b. On some digenetic trematodes from marine fishes of Pori, Orissa. *Ind. J. of Helminth.*, **35(2)**: 112-136. (1983,publ 1985)

HALTON, D. W. 1967a. Observations on the nutrition of digenetic trematodes. *Parasit.*, **57**: 639-660.

HALTON, D. W. 1967b. Studies on phosphatase activity in trematodes. *J. Parasit.*, **53**: 46-54.

HALTON, D. W. 1979. The morphology and development of a Gasterostome Trematode, *Bucephaloides gracilescens*. *Parasit.*, **79(3)**: V (Proceedings of the British Society for Parasitology, April 1979, Univ. of Keele).

HALTON, D. W & MORRIS, G. P. 1969. Occurrence of cholinesterase and ciliated sensory structures in a fish gill fluke, *Diclidophora merlangi* (Trematoda : Monogenea). *Zeit. fur Parasitenk.*, **33**: 21-30.

HALTON, D. W. & JOHNSTON, B. R. 1982. Functional morphology of the metacercarial cyst of *Bucephaloides gracilescens* (Trematoda:Bucephalidae). *Parasit.*, **85(1)**: 45-52.

HALTON, D. W. & MCCRAE, J. M. 1985. Development of the tegument and alimentary tract in a digenetic trematode, *Fellodistomum fellis*. *Parasit.*, **90**: 193-204.

HAMAJIMA, F; FUJINO, T; YAMAGUTI, K; FUKUDA, K. 1982. Mitochondria in the body wall of life-cycle stages of lung flukes of the genus *Paragonimus* and mitochondrial cytochrome components of the adult worm. *Comp. Biochem. Physiol. A*, **71**: 149-156.

HANNA, R. E. B. 1980. *Fasciola hepatica* : glycocalyx replacement in the juvenile as a possible mechanism for protection against host immunity. *Expt. Parasit.*, 50: 103-114.

HASWELL, W. A. 1903. On two remarkable sporocysts occurring in *Mytilus latus* on the coast of New Zealand. *Proc. Linn. Soc. N. S. Wales*, 27(4): 497-515.

HIGGINS, J. C. 1977. Nutrient uptake by the metacercarial stage of *B.haimeanus*. *Parasit.*,75(2): XX-XXl.

HIGGINS, J. C. 1980. Formation of cyst wall and related changes in the structure of the tegument of *Bucephalus haimeanus* (Lacaze- Duthiers,1854) during it's metamorphosis from the cercarial to the metacercarial stage. *Parasit.*, 81: 47-59.

HIGGINS, J. C., WRIGHT, D. E. & MATTHEWS, R. A. 1977. The ultrastructure and histochemistry of the cyst wall of *Bucephalus haimeanus* (Lacaze-Duthiers, 1854). *Parasit.*, 75(2): 207-214.

HIGO, H & ISHII, Y. 1987. Comparative studies on surface ultrastructure of newly encysted metacercariae of Japanese lung flukes. *Parasit. Res.*, 73: 541-549.

HOCKLEY, J. DAVID. 1968. Scanning electron microscopy of *Schistosoma mansoni* cercaria. *J. of Parasit.*, 54(6): 1241-1243.

HOCKLEY, J. DAVID. 1972. *Schistosoma mansoni*: the development of the cercarial tegument. *Parasit.*, 64: 245-252.

HOFFMAN, G. L. 1967. *Parasites of North American freshwater fishes*. Univ. Calif. Press, Berkeley and Los Angeles. 486p.

HOLLIMAN, R. B. 1961. Larval trematodes from the Apalachee Bay area, Florida, with a check list of known marine cercariae arranged in a key to their super-families. *Tulane Stud. Zool.*, 9(1): 1-74.

HOLT, S. J & WITHERS, R. G. J. 1952. Cytochemical localisation of esterase using indoxyl derivatives. *Nature*, 170: 1012-1014.

HOPKINS, S. H. 1954. The American species of Trematode confused with *Bucephalus* (*Bucephalopsis*) *haimeanus*. *Parasit.*, 44: 353-370.

HOPKINS, S. H. 1956. Two new trematodes from Louisiana, and the excretory system of Bucephalidae. *Trans. Amer. Microsc. Soc.*, 75: 129-135.

HOPKINS, S. H. 1958. Trematode parasites of *Donax variabilis* at Mustang Island, Texas. *Univ. Texas Pub. Inst. Mar. Sci.*, 5: 301-311.

HOWELL, M. 1966. A contribution to the life - history of *B. longicornutus* (Manter, 1954). *Zool. Publ. Victoria Univ. Wellington.*, 40: 1-42.

HOWELLS, R. E; RAMATHO-PINTO, F. J; GAZZINELLI, G; OLIVEIRA, C. C. de; FIGUEREDO, E. A & PELLAGRINO, J: 1974 *Schistosoma mansoni*: The mechanism of cercarial tail loss and its significance to host penetration. *Expt Parasit.*, 36: 373-385.

HUET, L. 1888. Note sur le *Bucephalus haimeanus*. *Bull. Soc. Linn. Normandie*, 2: 145-149. (English translation obtained)

HUET, L. 1893. Nouvelle note sur le *Bucephalus haimeanus*. *Bull. Soc. Linn. Normandie*, 7: 40-41. (English translation obtained)

HUMASON, H. T. 1972. *Animal Tissue Techniques*. 3rd Edn. W. H. Freeman & Co. vii - 641pp.

HUNT, J. S. 1953. A method of preparation of whole mounts of miracidia and cercariae. *J. Parasit.*, 39: suppl,24.

HUSSEY, K. 1943. Further studies on the comparative embryological development of the excretory system in digenetic trematodes. *Trans. Amer. Micros. Soc.*, 62(3): 271-279.

International code of Zoological Nomenclature, on the status of subgeneric names . *In Proceedings of the Biological Society of Washington*. 1926, 39: 75-104.

ISSAITSCHIKOW, I. M. 1928. [Zur kenntniss der parasitischeen Wurmer einiger Gruppen von Wirbeltieren der russischn Arktis]. *Trudy. Morsk. Nauch. Inst. Moskva.*, 3(2): 5-79. (In Russian, English translation obtained).

IVAVTSIV, V. V. & CHERNOGORENKO, M. I. 1984. [The life-cycle of *R. illense* (Trematoda, Bucephalidae)]. *Vestnik Zoologii*, 2: 66-69. (In Russian, English translation obtained).

JAMES, B. L. 1965. The effects of parasitism by larval Digenea on the digestive gland of the intertidal prosobranch, *Littorina saxatilis* (Olivi) subsp. *tenebrosa* (Montagu). Parasit., 55: 93-115

JAMES, B. L; BOWERS, E. A; RICHARDS, J. G. 1966. The ultrastructure of the sporocyst of *Cercaria bucephalopsis haimeana* - Duthiers, 1854 (Digenea: Bucephalidae) from the edible cockle, *Cardium edule* *Parasit.*, **56**: 753-762.

JAMES, B. L & BOWERS, E. A. 1967a. The effects of parasitism by the daughter sporocyst of *Cercaria bucephalopsis haimeanus* Lacaze-Duthiers, 1854, on the digestive tract of the cockle, *Cardium edule* L. *Parasit.*, **57**: 67-77.

JAMES, B. L. & BOWERS, E. A. 1967b. Histochemical observations on the occurrence of carbohydrates lipids and enzymes in the daughter sporoycst of *Cercaria bucephalopsis haimeana* Lacaze-Duthiers, 1854 (Digenea : Bucephalidae). *Parasit.*, **57**: 79-86.

JAMES, B. L & BOWERS, E. A. 1967c. Reproduction in the daughter sporocyst of *Cercaria bucephalopsis haimeana* (Lucaze-Duthiers, 1854) (Bucephalidae) and *Cercaria dichotoma* Labour, 1911 (non Muller) (Gymnophallidae). *Parasit.*, **57**: 607-625.

JENNINGS, J. B. & LEFLORE, W. B. 1972. The histochemical demonstration of certain aspects of cercarial morphology. *Trans. Amer. Micros. Soc.*, **91(1)**: 56-62.

JOHNSTONE, J. 1904. Internal parasites and diseased conditions of fishes. *Rep. Lancs. Sea Fish. Lab.*, **12**: 98-120.

JOHNSTON, B. R. & HALTON, D. W. 1981a. Excystation in vitro of *Bucephaloides gracilescens* metacercaria (Trematoda:Bucephalidae). *Zeit. fur Parasitenk.*, **65(1)**: 71-78.

JOHNSTON, B. R. & HALTON, D. W. 1981b. Occurrence of *Bucephaloides gracilescens* metacercariae in three species of gadoid fish. *J. Fish Biol.*, **18(6)**: 685-691.

JOHRI,L.N. AND SMYTH,J.D. 1956. A histochemical approach to the study of Helminth morphology. *Parasit.*, **46**: 107-116.

JONES, D. O. 1943. The anatomy of three digenetic trematodes, *Skrjabiniella aculeatus* (Odhner), *Lecithochirium rufoviride* (Rud.) and *Sterrhurus fusiformis* (Luhe) from *Conger conger* (Linn.). *Parasit.*, **35**: 40-51

JOSEPH, M. M. 1978. Observations on the larval trematode *Bucephalus* sp. parasitic in the oyster *Crassostrea madrasensis*. *J. Invert. Path.*, **32(3)**: 381-383.

KELLOG, J. L. 1915. Ciliary mechanism of lamellibranchs with description of anatomy. *J. Morph.* **26**: 625-701.

KELLY, H. M. 1899. A statistical study of the parasites of the Unionidae. *Bull. Ill. State Lab. nat. Hist.*, **5**: 399-418.

Kinkelin P. de, Tuffery, G., Leynaud, G and Arrignon, J. 1968

Etude epizootiologique de la bucephalose larvaire (Bucephalus polymorphus) dans le peuplement piscicole du Bassin de la Seine.

Rech. Vekr., 1 : 77-98

KENNEDY, C. R. 1966. The helminth parasites of some Irish freshwater fish. *Ir. Nat. J.*, **15**: 196-199.

KENNEDY, C. R. 1974. A checklist of British and Irish freshwater fish parasites with notes on their distribution. *J. Fish Biol.*, **6**: 613-644.

KENNEDY, M. J. 1979. The responses of miracidia and cercariae of *Bunodera mediovitellata* (Trematoda : Allocreadiidae) to light and to gravity. *Can. J. Zool.*, **57**: 603-609.

KNISKERN, V. B. 1950. *Rhipidocotyle septpapillata* Krull, 1934 (Trematoda); The cercaria and notes on the life - history. *J. Parasit.*, **36**: 155-156.

KNISKERN, V. B. 1952a. Studies on the trematode family Bucephalidae, Poche, 1907. Part 1. A Systematic review of the family Bucephalidae. *Trans. Amer. Microsc. Soc.*, **71**: 253-266.

KNISKERN, V. B. 1952b. Studies on the trematode family Bucephalidae, Poche, 1907. Part 11. The life-history of *Rhipidocotyle septpapillata* Krull 1934. *Trans. Amer. Microsc. Soc.* **71(3)**: 317-340.

KOHN, A. 1962. [Sobre um nova genero de trematodeo bucephaliforme parasito de peixe de agua doce]. *Revista Brasileira de Biologia*, **22(4)**: 351-355. (In Portugese, English abstract consulted in Hel. Abs. 1964, 33A, No. 1522)

KOHN, A. 1967. [Sobre um novo genero de Prosorhynchinae Nicoll, 1914 e noros dados sobie *Prosorhynchus bulbosus* Kohn, 1961 e *Rhipidocotyle quadriculatum*

Kohn, 1961 (Trematoda, Bucephalidae)] *Mems. Inst. Oswaldo Cruz*, **65(1)**: 107-114. (In Portugese, English abstract consulted in Hel. Abs. 1970, 39A, No. 345)

KOHN, A. 1970a. [*Chabaudtrema rarus* gen. n., sp. n. trematodes bucephaliforme parasito de peixe]. *Atlas Soc. Biol. Rio de J.*, **13(3/4)**: 147-148. (In Portugese English abstract consulted in Hel. Abs. 1971, 40A, No. 397).

KOHN, A. 1970b. [*Pararhipidocotyle jeffersoni* gen.n., sp.n., trematodeo bucephaliforme parasito de dourado]. *Atlas Soc. Biol. Rio de J.*, **13(5/6)**: 181-183. (In Portugese, English abstract consulted in Hel. Abs. 1971, 40A, No. 1839).

KOHN, A. 1971. [Contribuicao a sistematica dos trematodes] *Atlas Soc. Biol. Rio de J.*, **14(3/4)**: 65-66. (In Portugese, English translation obtained)

KOIE, M. 1971. On the histochemistry and ultrastructure of the tegument and associated structures of the cercaria of *Zoogonoides viviparus* in the first intermediate host. *Ophelia*, **9**: 165-206.

KOTIKOVA, E. A; JOFFE, B. I; RUNOVSKAYA, I. V. 1984 . [Nervous system of *Prosorhynchus squamatus* (Trematoda:Bucephalidae)]. Parasitologiya, 18(5): 408-412. (In Russian; English summary, p:412).

KOUBEK, P. 1977. [Occurrence of the trematode *Aspidogaster conchicola* Baer,1827 and cercariae of *Bucephalus polymorphus* Baer, 1827 in our mussels. (Czechoslovakia)]. *Helminthologicky Sbornik V*, 18

(9,Biologie 62): 47-53. (In Czechoslovakian, English abstract consulted in Hel. Abs. 1980, 40A, No. 96).

KOVAL, V. P. 1949. [A new species of *Bucephalus* in Dnieper fishes]. *Dokladi Akademi Nauk*, S. S. S. R. **68(1)**: 205-208. (In Russian, English translation obtained).

KOZICKA, J. 1959. Parasites of fishes of Druzno Lake. *Acta Parasit. Polonica*, **7**: 1-72.

KRULL, W. H. 1934. Studies on the life-history of a trematode *Rhipidocotyle septpapillata* n.sp. *Trans. Amer. Micros. Soc.*, **53(4)**: 408-415.

KUNTZ, R. E. 1950. Embryonic development of the excretory system in fork-tailed cercariae of the schistosomes and in a blunt-tailed brachylaemid cercaria. *Trans. Amer. microac. Soc.*, **69**: 1-20.

LACAZE - DUTHIERS, H. DE. 1854. Memoire sur le Bucephala Haime (*Bucephalus haimeanus*) Helminthe parasite des Huitres et des bucardes. *Annls. Sci. nat., Paris, Zool.*, **4(1)**: 294-302. (English translation obtained)

LAIRD, M. 1961. Microecological factors and oyster epizootics. *Canad. J. Zool.*, **39**: 449-485.

LARUE, G. R. 1957. The classification of digenetic Trematoda: A review and a new system. *Exp. Parasit.*, **6(3)**: 306-344.

LEBOUR, M. V. 1907. Fish trematodes of the Northumberland coast. *Northumberland Sea Fish Report*, 23-67.

LEBOUR, M. V. 1911. A review of British marine cercariae. *Parasit.*, **4**: 416-456.

LEE, D. L. 1966. The structure and composition of the helminth cuticle. *Advances in Parasit.*, 4: 187-254.

LEFLORE, W. B. 1978. *Plagiorchis elegans*: Histochemical localization of dehydrogenases in the cercarial stage. *Expt. Parasit.*, 46: 83-91.

LEFLORE, W. B. 1979. Histochemical observations on hydrolytic enzymes in cercariae of *Plagiorchis elegans* with notes on morphology of Nervous system. *Trans Amer. Micros. Soc.*, 98(2): 225-232.

LEFLORE, W. B; BASS, H. S & SMITH, B. F. 1980. The occurrence of some histochemically demonstrable dehydrogenases in cercariae of *Cloacitrema michiganensis* (Trematoda: Philophthalmidae). *Comp. Biochem. Physiol.*, 66B: 593-596.

LEVINSEN, G. M. R. 1881. [Bidrag til kundskab ons Gronlands Trematod fauna] *Overs. K. Danske. Vidensk. Selsk. Fork.*, 1: 52-84. (English abstract consulted in Zool. Record, vol.XVlll, Vermes, 4)

LO, S. J; HALL, J. E; ALLENDER, P. A & KLAINER, S. A 1975 Scanning electron microscopy of an opecoelid cercaria and its encystment and encapsulation in an insect host. *J. Parasit.*, 61: 413-417.

LONG, S & LEE, W. C. 1964. Worm parasites from Taihu fishes. Digenetic trematodes Vl. Bucephalidae. *Acta. Zool. Sin.*, 16(4): 567-580. (In Chinese with English summary)

LUHE, M. 1909. *Prasitische Plattwurmer. 1. Trematodes.* Die Suswasser fauna Deutschlands. (Ed. A. Brauer), H. 17, 217pp. (English translation obtained)

LUSHCHIVA, V. G. 1985. [Development cycle of the trematode *Bucephalus marinum* in Black sea fish]. *Ekologiya Morya Kiev*, **20**: 48-50. (In Russian with English summary)

MAC - CALLUM, G. A. 1917. Some new forms of parasitic worms. *Zoopathologica. Sci. Contrib. New York Zool. Soc.*, **1(2)**: 46-75.

MAILLARD, C. & SAAD-FARES, A. 1981. (*Bucephalus baeri*- n.sp., a trematode parasite of the teleost *Dicentrarchus labrax*. Description and life-cycle). *Zeit. fur Parasitenk.*, **66(1)**: 31-40. (In French)

MANTER, H. W. 1953. Two new species of Paurorhynchinae (Trematoda: Gasterostomata) from the Fiji Islands. *In Thapar Commemoration Vol.*, 193-200. (Edited by J. Dayal & K. S. Singh)

MANTER, H. W. 1961. Studies on digenetic trematodes of Hawaiian fishes : Family Bucephalidae. *J. Parasit.*, **47(3)**: 479-482.

MARGOLIS, L.& ARTHUR, J. R. 1979. Synopsis of the parasites of fishes of Canada. *Bull. Fish. Res. Board of Canada*. **No.199**: 269pp.

MARTIN, W. E & BILS, R. F. 1964. Trematode excretory concretions: formation and fine structure. *J. Parasit.*, **50**: 337-344.

MATRICON-GONDRAN, M. 1971. [Origine et differenciation du tegument D'un trematode digenetique : etude ultra-structurale chez *Cercaria pectinata* (larav de *Bacciger bacciger*, Fellodistomatides)]. *Z. Zellforsch*, **120**: 488-524.

MATTHEWS, R. A. 1968. Studies on the helminth parasites of some marine (teleost) fishes. Ph.D Thesis. University college of Wales, Aberystwyth.

MATTHEWS, R. A. 1972. The life-history of *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905 and a comparison of it's cercaria with that of *Prosorhynchus squamatus* Odhner, 1905. *Parasit.*, **66**: 133-164.

MATTHEWS, R. A. 1973. The life-cycle of *Bucephalus haimeanus* Lacaze-Duthiers, 1854 from *Cardium edule* L *Parasit.*, **67**: 341-350.

MATTHEWS, R. A. 1974. The life-cycle of *Bucephaloides gracilescens* (Rudolphi,1819) Hopkins,1954 (Digenea: Gasterostomata). *Parasit.*, **68**: 1-12.

MC -CRADY, J. 1874. Observations on the food and reproductive organs of *Ostrea virginica*, with some account of *Bucephalus cuculus*. *Proc. Bostan Soc. Nat. Hist.*, **16**: 170-192.

MELLORS, P. J. & OWEN, R. W. 1980. Some observations on the biology of a gasterostome digenetic fluke *Rhipidocotyle campanula* of fish in the River Aire, Yorkshire. (Proceedings for the British Society for Parasitoly Leeds, England). *Parasit.*, **81(2)**: XLV111.

MELLORS, P. J. 1985. Investigations on the biology of a Gasterostome Trematode from fish in the River Aire. M. Phil Thesis (Leeds).

MERCER, E. H. & DIXON, K. E. 1967. The fine structure of cystogenic gland cells of the cercaria of *Fasciola hepatica* L. *Z. Zellforsch.*, **77**: 331-344.

Milland, C and Saad-Fares, A. 1981

[Bucephalus baeri n.sp., a trematode parasite of the teleost Dicentrarchus labrax. Description & life-cycle]

Zeitschrift fur Parasitenkunde, 66(1): 31-40

MILLER, H. M. 1925. A preliminary report on the larval trematodes infesting certain molluscs from Dry Tortugas. *Carnegie Inst. Year book, Washington*, 24: 222-238.

MITCHELL, C. W. 1974. Ultrastructure of the metacercarial cyst of *Posthodiplostomum minimum* (MacCallum, 1921). *J. Parasit.*, 60: 67-74.

MITCHELL, C. W & CRAG, R. E. 1976. *Phosthodiplostomum minimum*: examination of cyst wall and metacercariae containing calcareous concretions with scanning electron microscopy and X-ray microanalysis. *Expt. Parasit.*, 40: 309-313.

MORRIS, G. 1971. The fine structure of the tegument and associated structures of the cercaria of *Schistosoma mansoni*. *Zeit. fur Parasitenk*, 36: 15-31

MUSS, BENT J. & DAHLSTROM, P. 1978. *Freshwater fishes of Britain and Europe*. Pub. Collins Clear-Type Press, U.K.

NAGATY, H. F. 1937. Trematodes of fishes from the Red Sea. Part 1. Studies on the family Bucephalidae Poche,1907. *Pub. Egypt. Univ. Fac. Med.*, 12: 1-172.

NAHHAS, F. M. & CABLE, R. M. 1964. Digenetic and aspidogastrid trematodes from marine fishes of Curaçao and Jamaica. *Tulane Studies Zool.*, 11: 167-228.

NICOLL, W. 1914. Trematode parasites of fishes from the English Channel. *J. Marine Biol. Assoc., Plymouth*, 10: 466-505.

NICOLL, W. 1924. A reference list of the freshwater parasites of British freshwater fishes. *Parasit.*, **16**: 127-144.

NIEWIADOMSKA, K & MOCZON, T. 1982. The nervous system of *Diplostomum pseudospathaceum* Niewiadomska (Digenea, Diplostomatidae). 1. Nervous system and chaetotaxy in cercaria. *Zeit. fur Parasitenk.*, 68: 295-304.

NIEWIADOMSKA, K & MOCZON, T. 1984. The nervous system of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Trematoda, Diplostomatidae). 11. Structure and development of the nervous system in metacercaria. *Z. parasitenk.*, **70**: 537-648.

NIEWIADOMSKA, K & MOCZON, T. 1987. The nervous system of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Trematoda, Diplostomatidae). 111. Structure of the nervous system in the adult stage. *Parasit. Res.*, 73: 46-49.

NOLLEN, P.M. 1983. Patterns of sexual reproductions among parasitic Platyhelminthes. *Parasit.*,**86**: 99-120.

NUTTMAN, C. T. 1971. The fine structure of ciliated nerve endings in the cercaria of *Schistosoma mansoni*. *J. Parasit.*, **57**: 855-859.

ODHNER,T. 1905. Die Trematoden des arktischen Gebietes. *Fauna Arctica*, **4(2)**: 291-372. (In German, Eng. translation obtained).

OGBA, M. G. 1982. Scanning electronmicroscopy of the tegumental surface of adult and developing *Schistosoma margrebowiei* Le Roux, 1933. *Int. J. Parasit.*, **12**: 191-198.

ORR, T. S. C. 1967. Parasites of freshwater fish in the Glasgow area. *Glasg. Nat.*, **18**: 503-504.

OVERSTREET, R. M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. *Tulane Studies Zool.*, **15**: 119-176.

OZAKI, Y. 1924. Gasterostomatous trematodes and three new genera of them. *Zool. Mag.*, **36**: 173-210.

OZAKI, H. 1960. On two new gasterostome cercariae. *Hiroshima Daigaku Igakubu Gyosekishu*, **10**: 77-81. (In Japanese, English translation obtained)

OZAKI, Y AND ISHIBASHI, C. 1934. Notes on the cercaria of the Pearl Oyster. *Proc. Jap. Acad. Tokyo.*, **10**: 439-441.

PAGE, R. M; NADAKAVUKAREN, J. M; HUIZINGA, W. H. 1979. *Ribeiroia marini* : Surface ultrastructure of Redia, Cercaria and Adult. *Int. J. Parasit.*, **10**: 5-12.

PAGENSTECHER, H. A. 1857. Trematodenlarven und Trematoden Helminthologischer Beitrag. (Heidelberg, 56pp). (From, Ziegler, 1883)

PALOMBI, A. 1934. [Gli stadi larvali dei Trematodi dei Gulfo di Napoli. 1. Contributo ollo studio della morfologia e sistematica delle cercarie marine]. *Publ. Staz. Zool. Napali*, **14(1)**: 51-94.

PARISELLE, A & MATRICON-GONDRAN, M. 1985. A new type ciliated sensory receptor in the cercariae of *Nicolla gallica* (Trematoda). *Zeit. fur Parasitenk.*, **71**: 353-364.

PEARSE, A. G. E. 1972. *Histochemistry: theoretical and applied*. 3rd edn. Churchill Livingstone, Edinburgh.

PELSENEER, P. 1906. Trematodes parasites de mollusques marins. *Bull. Sci. France et Belgique*, 40: 161-186.

PIGULEUSKY, S. 1932. Fisch parasiten des dnjeprbassins. *Annu. Mws. Zool. Acda. Leningrad*, 32: 425-450. (In Russian, German summary, pp. 451-452)

PIKE, ALAN W. & ERSMUS, D. A. 1967. The formation, structure and histochemistry of the metacercarial cyst of 3 spp. of digenetic trematodes. *Parasit.*,57: 683-694.

POCHE, E. 1907. Einige Bemerkungen zur Nomenclature der Trematoden. *Zool. Anz.*, 31(1): 124-126. (In German)

POJMANSKA, T. 1985. An analysis of seasonality of incidence and maturation of some fish parasites, with regard to thermal factor. lV. *B. polymorphus* Baer,1827. *Acta Parasit. Polonica*, 30(1/11): 25-34.

PORTER, C. W & HALL, J. E. 1970. Histochemistry of a cotylocercous cercaria. 11. Hydrolytic and oxidative enzymes in *Plagiorchis lepomis*. *Expt. Parasit.*, 27: 378-387.

PROBERT, A. J. 1966. Studies on phosphatase system in hepatopancreatic cells of the molluscan host of *Echinoparyphium* sp. and in the redia and cercaria of this trematode. *Parasit.*, 54: 73-79.

RAI, P. 1979. On the hitherto known bucephalid flukes of some of the freshwater fishes with remarks on the pathogenic significance of their metacercariae. *Vet. Res. Bull.*, 2(1): 20-27.

RAWSON, D. 1952. The occurrence of parasitic worms in British freshwater fishes. *Ann. Mag. Nat. Hist*, Ser. 12, 5: 877-888.

REES, G. 1934. *Cercaria patellae* Lebour, 1911, and its effect on the digestive gland and gonads of *Patella vulgata*. *Proc. Zool. Soc. Lond.*, **1**: 45-53.

REES, G. 1939. Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part 1 Anatomy of the genitalia and gametogenesis in the adult. *Parasit.*, **31**: 417-433.

REES, G. 1967. The histochemistry of cystogenous gland cells and cyst wall of *Parorchis acanthus* Nicoll and some details of the morphology and fine structure of the cercaria. *Parasit.*, **57**: 87-110

REES, G. 1971a. The ultrastructure of the epidermis of the redia and cercaria of *Parorchis acanthus*, Nicoll A study of scanning and transmission electron microscopy. *Parasit.*,**62**: 479-488.

REES, G. 1971b. Locomation of the cercaria of *Parorchis acanthus* Nicoll, and the ultrastructure of the tail. *Parasit.*, **62**: 489-503.

REES, G. 1974. The ultrastructure of the body wall and associated structures of the cercaria of *Cryptocotyle lingua* (Creplin) (Digenea : Heterophyidae) from *Littorina littorea* (L). *Zeits. fur Parasitenk.*, **44**: 239-265.

REES, G. 1975. The arrangement and ultrastructure of the musculature, nerves and epidermis, in the tail of the cercaria of *Cryptocotyle lingua* (Creplin) from *Littorina littorea* (L). *Proc. R. Soc. London*, B. **190**: 165-186.

REES, G & DAY, M. F. 1976. The origin and development of the epidermis and associated structures in the cercaria of *Cryptocotyle lingua* (Creplin) (Digenea : Heterophyidae) from *Littorina littorea* (L). *Proc. R. Soc. London*, B. **192**: 299-321.

REES, G. 1977. The development of the tail and the excretory system in the cercaria of *Cryptocotyle lingua* (Creplin) (Digenea : Heterophyidae) from *Littorina littorea* (L). *Proc. R. Soc. London*, B. **195**: 425-452.

REGER, J. F. 1976. Studies on the fine structure of cercarial tail muscle of *Schistosoma* sp. (Trematoda) *J. Parasit.*, **70(6)**: 999-1000.

RICHARD, J. 1968. La chetotaxie des cercaires. *Valeur Systematique C. R. Acad. Sci (D), Paris*, **266**: 371-374. (English translation obtained)

RICHARD, J. 1971. La chetotaxie des cercaires. Valeur Systematique et phyletique. Mem. Mus. Nat. Hist. Nat., Ser. A, Zool., 67, 1-179. (Eng. summary, pp:174-176)

ROBSON, R. T.&ERASMUS, D. A. 1970. The ultrastructure, based on sterescan obervation, of the oral sucker of the cercaria of *Schistosoma mansoni* with special reference to penetration. *Zeit. fur Parasitenk.*, **35**: 76-86.

ROBINSON, R. D. & HALTON, D. W. 1983. Functional morphology of the tegument of *Corrigia vitta* (Trematoda : Dicrocoeliidae). *Zeit. fur Parasitenk.* **69**: 319-333.

ROUGHLEY, T. C. 1933. Life history of the Australian oyster (*Ostrea commercialis*). *Proc. Linn. Soc. N.S.W* **58**: 279-333.

RUDOLPHI, C. A. 1819. Entozoorum synopsis cui accedunt mantissa duplex et indicus locupletissimi. x - 811pp. Berolini. (Reference obtained from Ziegler, 1883)

SAMUEL, D. 1978. A digenetic trematode infection in the edible oyster *Crassostrea madrasensis* (Preston). *Ind. J. of Fish.*, **23**): 153-159.

SAMUELSON, JOHN, C; QUINN, JOHN, J. & CAUFIELD, JOHN, P. 1984. Video microscopy of swimming and secreting cercariae of *Schistosoma mansoni*. *J. Parasit.*, **70(6)**: 996-999.

SANDERS, M. J. 1966. Parasitic castration of the scallop *Pecten alba* (Tate) by a Bucephalid Trematode. *Nature*, **212**: 307-308.

SHILLOCOCK, D. J. 1972. Ecological studies on the platyhelminth fauna of freshwater fish from a lake and a river in Essex with special reference to the roach. Ph.D Thesis. (University of London).

SHOTTER, R. A. 1972. Notes on helminth parasites of the Whiting *Odontogadus merlangus* (L) from the Northern Irish sea. *J. fish Biol.*, **4**: 117-136.

SIDDIQI, A. H & LUTZ, P. L. 1966. Osmotic and ionic regulation in *Fasciola hepatica* (Trematoda: Digenea). *Expt. Parasit.*, **19**: 348-357.

Sannia, A and James, B.L (1977)

The Digenea in marine molluscs from Eyjafjordur, North Iceland,

Opelia, 16(1): 97-109.

SIDDIQI, A. H; ISLAM, M. W & NIZAMI, W. A. 1975. Osmotic and ionic behaviour of some digenetic trematodes. *Comp. Biochem. Physiol.*, **51A**: 929-935.

SIEBOLD, C. T. VON. 1848. Lehrbuch der vergleichenden Anatomie der Wirbellosen Thiere. xiv - 679pp. Berlin (Translation by Waldo I. Burnett, M. D. (1854) - Anatomy of the Invertebrate by Siebold. Vol. 1. pp. XlV-470).

SINGH, R. N. & RAI, S. L. 1965. "Studies on a bucephalid cercaria, *Cercaria katangii*, sp.nov. (Trematoda: Bucephalidae)". *Ind. J. Helminth.*, **17(2)**: 104 117.

SINITZIN, D. F. 1909. Studies uber die Phylogenie der Trematoden. 11. *Bucephalus* v. Baer und *Cercaria ocellata* De La Vall. *Zeit. Wiss. Zool.*, **94(2)**: 299-325.

SINITZIN, D. F. 1911. La generation parthenogenetique des trematodes sa descendance dans les mollusques dela Mer Noire. *Mem. Acad. Sci. St. Petersb.*, **30(8)**: 1-127.

SMYTH, J. D.1956. Studies on tapeworm physiology 1X. A histochemical study of egg-shell formation in *Schistocephalus solidus* (Pseudophyllidean). *Expt. Parasit.*, **5**: 519-540.

SMYTH. J. D & HALTON, D. W. 1983. *The Physiology of Trematodes*. Cambridge University Press. 2nd Edn.

SOBHON, P; ANUPUNPISIT, V; YUAN, H. C; UPATHAM, E. S; SAITONGDEE, P. 1988. *Schistosoma japonicum* (Chinese): Changes of the tegument surface in cercariae, schistosomula and juvenile. *J. Parasit.*, **78**: 1093-1104.

SRIVASTAVA, C. B. 1938. Studies on the Gasterostomatous parasites of Indian food fishes. *Ind. J. Anim. Husb. & Vet. Sci.* **8**: 317-340.

SRIVASTAVA, C. B. 1963. "On three new sp. of the genus *Bucephalus* Baer, 1827 (Trematoda: Bucephalidae Poche, 1907), with remarks on the systematic position of *B. indicus* Srivastava, 1938. *Ind. J. Helmin.*, **15(1)**: 36-44

SRIVASTAVA, C. B. and CHAUHAN, B. S. 1972. A review of Indian gasterostomes. (Trematoda). *Rec. Zool. Survey India*, **67**: 1-13.

STEWART, C. 1875. "Notes on *Bucephalus polymorphus*". *Month. Micros. Jour.*, **vol.XIV**: 1-2.

STILES, W. C. & HASSALL, A. 1908. Index Catalogue of Medical and Veterinary Zoology. Trematode and trematode diseases. *Hyg. Lab. U. S. Pub. Health, Marine Hosp. Service, Bull.*, **37**: 1-401. Washington.

STIREWALT, M. A. 1963. Chemical biology of secretions of larval helminths. *Annals of the New York Acad. Sci.*, **113**: 36-53.

STIREWALT, M. A & WALTERS, M. 1964. Histochemical assay of glands of cercariae of *Schistosoma mansoni*. *J. Parasit.*, **50**: suppl. No.94, p.44.

STUNKARD, H. W. 1974a. Identity and specificity in Bucephalid trematodes. *3rd International Congress of Parasit., Munich*. **Proc.,1,(373)**.

STUNKARD, H. W. 1974b. The life-cycle of the gasterostome trematodes, *Rhipidocotyle transversale* Chandler,1935

and *Rhipidocotyle lintoni* Hopkins, 1954. *Biol. Bull.* **147(2)**: 500-501.

STUNKARD, H. W. 1974c. *Rhipidocotyle heptathelata* n.sp., a bucephalid trematode from *Thynnus thunnina* taken in the Red Sea. *Trans. Amer. Micros. Soc.*, **93(2)**: 260-261.

STUNKARD, H. W. 1974d. The trematode family Bucephalidae-Problems of morphology, development and systematics. Description of *Rudolphinus* gen.Nov. *Trans. of the New York Acad. of Sci.*, **36(2)**: 143-170.

STUNKARD, H. W. 1975. The excretory system and systematics of the Gasterostome Trematodes. *Dr. B. S. Chauhan Comm. vol.*, 1-11.

STUNKARD, H. W. 1976a. *R. campanula* (Dujardin, 1845) Dollfus, 1968. *J. Parasit.*, **62(5)**: 817.

STUNKARD, H. W. 1976b. The life-cycles, intermediate hosts, and larval stages of *Rhipidocotyle transversale* Chandler, 1935 and *Rhipidocotyle lintoni* Hopkins, 1954: Life- cycles and systematics of Bucephalid trematodes. *Biol. Bull.*, **150**: 294-317.

TANG, C. and TANG, Z. 1976. [Studies on nine species of gasterostomes from Fujian, with observations on the life-cycles of two species.] *Acta Zoologica Sinica*, **22(3)**: 263-278. (In Chinese, Eng. summary, pp. 272-274)

TENNENT, D. H. 1905. Feeding experiments for determining the life-history of an oyster parasite. *Biol. Bull.*, **8**: 233-235.

TENNENT, D. H. 1906. A study of the life-history of *Bucephalus haimeanus*; a parasite of oyster. *Quart. Jour. MIcros. Sci.*, **49**: 635-690.

TENNENT, D. H. 1909. An account of experiments for determining the complete life-history of *Gasterostomum gracilescens*. *Science*, **29**: 432-433.

THREADGOLD, L. T. 1968. The tegument and associated structures of *Haplometra cylindracea*. *Parasit.*, **56**: 1-7.

TUFFERY, G. 1978. (Research on bucephalopsis due to *B. polymorphus*). *Bulletin de l'Acade'mie Veterinaire de France*, **51(2)**: 143-145.

ULICNY, J. 1878. Helminthologische Beitrage. Arch. Nat., **44**: 211-218. (From Ziegler, 1883)

ULMER, M. J. AND RHODE, K. 1981. Morphology and taxonomy of parasitic helminths. *Rev. of Adv. in Parasit.*, 153-168. Warsaw.

VAULLEGEARD, A. 1894. Note sur le presence du *Bucephalus haimeanus* (Lacaze-Duthiers) dans le *Tapes decussatus* (Linne) et dans le *Tapes pullestra* (Montague). *Bull. Soc. Linn. Normandie*, **ser.7,4**: 23-29. (In French, Eng. translation obtained).

VEJNAR, F. 1956. [Prisperek k helminthofaune nasich okounovitych ryb]. Sbornik Vysoke Skoly Zemedetske a Lesnicke Fakulty v Brne. Rada B. Spisy Fakulty Veterinarni, **4(3)**: 161-176. (In Czechoslavakian, German & Russian summaries, p. 174)

VELASQUEZ, C. C. 1959. "Studies on the family Bucephalidae Poche, 1909, (Trematoda) from Philippine food fishes." *J. Parasit.*, **45**: 135-146.

VERMA, S. C. 1936a. Studies on the family Bucephalidae (Gasterostomata) Part 1. Description of new forms from Indian fresh water fishes. *Proc. Nat. Acad. Sci.*, India. **6**: 66-89.

VERMA, S. C. 1936b. Studies on the family Bucephalidae (Gasterosomata) Part 11. Description of two new forms from Indian marine fishes. *Proc. Nat. Acad. Sci.*, India. **6**: 252-260.

VONGPAYABAL, P; SOBHON, E. S. & UPATHAM etal. 1982. Scanning electron microscpic study of the tegumental surface of adult *Schitosoma mekongi*. *Parasit.*, **85**: 325-332.

WAGENER, G. R. 1952. Enthelmintica No 3. Ueber eine distomen Gattung Gasterostomata von Siebold. *Arch. Anat. Phys. Wissensch.* Berlin, 557-567. (In German, English translation obtained)

WAGENER, G. R. 1858. Enthemінthica 1V. uber *Distoma campanula* (*Gasterostomum fimbriatum* Siebold) Duj. und *Monostomum bipartitum* Wedl. *Arch. Naturges*, **24**: 250-256. (In German, Eng. translation obtained)

WAGNER, ALVIN. 1961. Papillae on three spp. of Schistosome cercariae. *J. of Parasit.*, **47**: 614-618.

WALLET, M & LAMBERT, A. 1984. [Characterization of the cercaria of *Bucephalus polymorphus* Baer, 1827 (Trematode: Bucephalidae): chaetotaxy and excretory

system]. *Ann. Parasit. Hum. Comp.*, **59(6)**: 583-588. (In French).

WALLET, M.; THERON, A. & LAMBERT, A. 1985. (Shedding pattern of *Bucephalus polymorphus* Baer,1827, cercariae (Trematoda: Bucephalidae) in relation to the activity of it's snail host *Dreissena polymorpha*). *Ann. Parasit. Hum. Comp.* **60(6)**: 675-684. (In French).

WEDL, C. 1858. Anatomische Beobachtungen Uber Trematoden. *Distoma campanula* (Dujardin) Sitzungsber. *Saechs Akad. Wiss. Leipzig Math- Naturwiss*, **26**: 241-278. (In German, Eng. translation obtained).

WESENBERG-LUND, C. 1934. Contributions on the development of the Trematoda, Digenea. Part 11. The biology of freshwater cercariae in Danish freshwater. *Mem. Acad. Roy. Sci. Lett.*, Denemark, *Sect.sci., 9, ser,* **5**: 1-223. (Abs. from Hel. Abs. (1934-1935), 3: 363)

WHEELER, A. 1969. *The fishes of the British Isles and North West Europe*. W & J. Mackay & Co Ltd. Chatham. pp. 1-613

WIERZBIKKA, J. 1977. Trematodes of *Abramis brama*, *A. ballerus* and *Blicca bjoercna* from the Dabie lake, Poland. *Acta Parasit. Polonica*, **25**: 1-16.

WILSON, R. A. 1967. The protonephridial system in the miracidium of the liver fluke, *Fasciola hepatica* L. *Comp. Biochem. Physiol.*, **20**: 337-342.

WILSON, R. A & WEBSTER, L. A. 1974. Protonephridia. *Biol. Rev.*, **49**: 127-160.

WOODHEAD, A. E. 1927. Concerning the encystment of *Bucephalus* cercariae. *Science*, **65**: 232.

WOODHEAD, A. E. 1929. Life-history studies on the trematode family Bucephalidae. *Trans. Amer. Micros. Soc.*, **48(3)**: 256-275.

WOODHEAD, A. E. 1930. Life-history studies on the trematode family Bucephalidae. No 11. *Trans. Amer. Micros. Soc.*, 49(1): 1-17.

WOODHEAD, A. E. 1931a. The germ cell cycle in the trematode family Bucephalidae. *Trans. Amer. Micros. Soc.*, **50(3)**: 169-187.

WOODHEAD, A. E. 1931b. The redia of the gasterostomes. *Science*, **74**: 463.

WOODHEAD, A. E. 1936. A study of the gasterostome cercariae of the Huron river. *Trans. Amer. Micros. Soc.*, **55(4)**: 465-676.

WUNDER, W. 1924. Die Schwimmbewegung von *Bucephalus polymorphus* v Baer. *Z. Morph. Physiol.*, **1**: 289.

YAMAGUTI, S. 1938. Studies on the helminth fauna of Japan. Part 21. *Trematodes of fishes*. **IV**. Kyoto, 139pp.

YAMAGUTI, S. 1958. *Systema Helminthum*. **Vol. 1.** The digenetic trematodes of vertebrates. Inter Science Pub., Part 1 & 11. 1575pp.

YAMAGUTI, S. 1971. *Synopsis of digenetic trematodes of vertebrates*. **Part 1 & 11.** Keigaku Publishing Co., Tokyo.

YAMAGUTI, S. 1975. *A synoptical review of life-histories of digenetic trematodes of vertebrates with special reference to the morphology of their larval forms.* Keigaku Pub. Co. Tokyo. pp. 591.

Ziegler, H. E. 1883. Bucephalus und Gasterostomum. *Zeit. Wiss. Zool.* **39**: 537-571. (In German, Eng. translation obtained).