Studies on the biology of *Cyathocephalus truncatus* (Pallas, 1781) (Cestoda: Spathebothridea) in its fish and crustacean hosts.

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ABSTRACT

During the examination of Salmo gairdneri, Salmo trutta and Thymallus thymallus from the Driffield section of the river Hull and its tributaries, Eastburn Beck and Driffield Beck, all fish were found to be infected with the cestode Cyathocephalus truncatus.

A prevalence of 2.2% was recorded for the procercoids of C. truncatus in the amphipod crustacean, Gammarus pulex in the same habitat. Other helminth parasites recorded in both fish and gammarid hosts include Echinorhynchus truttae, Echinorhynchus salmonis, Neoechinorhynchus rutili, Cystidicola farionis, Cucullanus truttae and Crepidostomum metoecus.

The life cycle of C. truncatus has been studied in the laboratory together with aspects of embryonic development, procercoid and adult morphology, establishment of infection and host-cestode interractions.

Hexacanth embryos of C. truncatus were found to develop optimally in eggs cultured at between 15°C and 20°C for about 25 days. Gammarus pulex became infected only by swallowing egg capsules containing hexacanth embryos fed to them on pieces of lettuce leaves. The young developing embryo grows over a period of about 10 weeks to the infective procercoid stage in the body cavity of the amphipod.

In fish, the tapeworm forms an attachment in the distal end of a pyloric caecum 3 days after infection and matures in 8-10 days with production of eggs. By the 15th day, the

attachment to host tissue has become so firm that it is impossible to separate the worm from it. The tapeworm's hold on the caecal wall is probably achieved by the sucking effect of the funnel-shaped scolex supplemented by the spike-like microtriches of the inner scolex surface.

The ultrastructure of the scolex and body wall of strobila of both the procercoid and adult tapeworm have been described. Hydrolytic enzymes such as alkaline phosphatase, acid phosphatase and non-specific esterase have been localised in both the procercoid and adult tapeworm.

Pathological effects of the tapeworm infection in fish are seen as swelling and proliferation of tissues of the caecal wall where the tapeworm forms an attachment. In long established infections erosion of the caecal wall, penetration by worms through the caecal wall into the body cavity and their attachment to the abdominal musculature of the fish host are common notable features.

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INTRODUCTION

Cyathocephalus truncatus is a tapeworm parasite most usually of salmonid fishes in some temperate freshwaters of the world. The life cycle involves one intermediate host always a crustacean. Amphipods are mainly known to serve as intermediate host but other crustaceans, possibly mysids (Amin, 1977) may also be utilized. Infections are usually associated with areas of shallow rivers and streams with slow running water and in most cases with water weeds.

Occurrence of the tapeworm in different species of primary and intermediate hosts in many parts of Europe and North America has been reported by several authors. There are now about 24 recorded primary host species and about 10 recorded intermediate host species.

Very little work has been done on the biology of the tapeworm - it includes investigations on distribution and ecology in Norway by Vik (1954, 1958), in Ladoga, U.S.S.R. by Bauer and Nikol'skaya (1957), and in Wales, U.K. by Awachie (1966a); on the effects of infection on fish in Sarajevo, Yugoslavia by Wisniewski (1932b)and Senk (1956), in Norway by Vik (1954, 1958) and in Italy by Catalini et al. (1978); on the site of occurrence in fish by Halvorsen and Macdonald (1972); and on stages of the life-cycle by Wisniewski (1932, a,b,c).

In most of the areas of previous studies such low levels of prevalence have been recorded that it has often not been possible to conduct useful experiments. In fact most of the previous accounts of C. truncatus consist of reports of single or occasional occurrences of relatively few tapeworms in already known hosts or in new hosts.

The life cycle of Cyathocephalus truncatus has not been fully investigated or experimentally verified. Although the general outline of development was first put forward by Wisniewski (1932c)there have been doubts concerning the true nature of the oncosphere infective to the amphipod and views have varied between the undifferentiated type described by Wisniewski (1932c)and the hexacanth type described by Gauthier (1923). Although the former description is at present accepted, as expressed by Freeman (1982), Gauthier's (1923) report has not been conclusively proved to be untrue of Cyathocephalus. Also Amin (1978) reported the maturation of C. truncatus procercoids in the intermediate host, Pontoporeia affinis, with production of eggs. This report thus created a new line of thought as to whether the tapeworm life-cycle could be completed using only the amphipod intermediate host in the absence of the fish primary host.

A peculiar and characteristic feature of C. truncatus infection is that the tapeworm is highly pathogenic and causes much tissue destruction at the site of attachment within the primary host. Its pathogenic effects have been

reported to be possibly lethal to the fish (Huitfeldt-Kaas, 1927; Wisniwski, 1932b; Vik, 1958) but much still remains to be elucidated about the method of attachment and the tapeworm's pathogenicity.

The area of present study (the river Hull and its tributaries at Driffield in Humberside, North East England) provided a supply of C. truncatus particularly in the primary host and to a lesser extent in the intermediate hosts thus making it possible to conduct studies on certain aspects of its biology that have not been previously thoroughly examined.

These include:

1. Monthly prevalence of infection in the intermediate host, Gammarus pulex.
2. Distribution of the tapeworm in the body of the primary hosts Salmo gairdneri, Salmo trutta and Thymallus thymallus.
3. Determination of the life cycle and the stages of development of the tapeworm in the intermediate and final hosts.
4. Structural and ultrastructural studies on the procercoid, the adult tapeworm, and the site of attachment of the adult tapeworm in its fish host.
5. Histochemical studies on some enzymes of the procercoid and adult tapeworms and the infected host tissues.
6. Observations on pathogenic effects of infection on the intermediate and final hosts.

Experimental infections were established in fish to enable the study of the stages of its life cycle and to investigate as far as possible the form of any pathogenic effects and the stage of infection at which they occurred.

CHAPTER 1

A REVIEW OF THE HISTORY, SYNONYMS, PRIMARY AND INTERMEDIATE HOSTS AND RECORDED OCCURRENCE OF *CYATHOCEPHALUS TRUNCATUS*

1.1 Outline history of Cyathocephalus truncatus

Cyathocephalus truncatus was first discovered and described by Pallas (1781) when he found it attached within the pyloric caeca in Esox lucius from Leningrad, U.S.S.R. He named it Taenia truncatus.

Zeder (1803) also recorded and described the tapeworm in E. lucius but he called it Echinorhynchus believing it to be an acanthocephalan with a withdrawn proboscis.

Rudophli (1810, 1819) described a similar tapeworm attached within the caeca of two fish, E. lucius and Perca fluviatilis and referred to it by the name Dubium Esocis lucii.

Diesing (1850) in his "Systema helminthum" placed the tapeworm in the category "Cephalocotylea subordine v. genere penitus dubia" and called it Cephalocotyleum Esocis lucii (Rudolphi).

Kessler (1868) first recorded the tapeworm in the trout, Salmo trutta and allocated it to a specific genus - Cyathocephalus - since the scolex appeared funnel-shaped. The characters he saw were different from those in already existing genera into which the tapeworm had been classified by previous authors. He named it Cyathocephalus truncatus (Pallas, 1781).

Grimm (1871) in a review discussed the anatomy and histology of the species and said he preferred to name it Monobothrium because of the shape of the scolex.

Olsson (1872) described as Acrobothrium typicum a new tapeworm species found in Lota vulgaris from Storsjøen in Sweden. Olsson (1893), referring to the same tapeworm he found in Thymallus vulgaris changed his mind after careful studies of its features and decided to call it Cyathocephalus truncatus (Kessler, 1868) with which it was characteristically similar. Using Olsson's work as a source of reference Linstow (1878) and Hofer (1904) in their separate reviews used the name Acrobothrium typicum (Olsson, 1872) for C. truncatus found in Lota vulgaris whereas for the same tapeworm found in Perca flaviatilis they used the name Cyathocephalus truncatus (Kessler, 1868)

Kraemer (1892) following Kessler's (1868) studies first gave a thorough description of the tapeworm accompanied by well-documented illustrations.

Riggenbach (1899) found a tapeworm in Solea vulgaris and named it Cyathocephalus catinatus. This tapeworm is referred to as a synonym of Cyathocephalus truncatus by Vik (1954). It is also referred to as a synonym of Bothrimonus (= Diplocotyle) (Cestoda: Spathebothridea) by Sandeman and Burt, (1972). It is thus difficult to say which of them is correct.

Lühe (1899, 1910) recommended the acceptance of the description by Kraemer (1892) and the nomenclature given by Kessler (1868) - Cyathocephalus truncatus (Pallas, 1781) - the name by which it is known today.

In whitefish, Coregonus clupeaformis from Lake Michigan in North America, Cooper (1918) found a species of Cyathocephalus which he named Cyathocephalus americanus. Nybelin (1922) found that

the apparent anatomical differences between the accounts of Kraemer (1892) and Cooper (1918) which according to Cooper justified the allocation of his specimens to a new species were uncertain and it was his (Nybelin's) opinion that the two species (termed by Cooper as European and American types) were identifical. Nybelin regarded Kraemer's (1892) description as poor in comparison with those of Cooper (1918), lacking in substance as well as being less detailed and accurate.

Nybelin (1922) also redescribed the anatomy of the tapeworm. Other reviews of the characters of the tapeworm have been published by Wardle (1932), Wardle and McLeod(1952), Vik (1954, 1958) and Amin (1977).

During considerations of the life cycle and intermediate hosts, Olsson (1893) assumed that the tapeworm was transmitted by Gammarus species which serve as the major food of pike in Storsjøen, Sweden. Wolf (1906) was the first to record the occurrence of the procercoid in Gammarus pulex but could not infect the amphipods with the ova-laden faeces of the infected fish, Salmo gairdneri and S. trutta.

Cooper (1918) indicated that species of the amphipod genus Pontoporeia, which form the major food of Coregonus clupeaformis, could possibly be the intermediate host of C. truncatus in Lake Michigan, North America. He was proved correct by Wardle (1932).

The occurence of C. truncatus procercoids in the amphipods Fontogammarus bosniacus and Rivulogammarus spinicandatus were also recorded in river Bosnia, Sarajevo, Yugoslavia by Schäferna (1922).

Gaulhier (1923) gave an account of the development of C. truncatus ova into hexacanth embryos but could not infect amphipods with them. Wisniewski (1932c)however disagreed with Gauthier's report and described a developed embryo lacking larval hooks and also recorded the stages of development of the tapeworm in the amphipods Fontogammarus bosniacus and Rivulogammarus spinicaudatus.

1.2 Synonyms of Cyathocephalus truncatus (Pallas, 1781)

Taenia truncatus	Pallas (1781)
Echinorhynchus sp.	Zeder (1803)
Dubium Esocis lucii	Rudolphi (1810, 1819)
Cephalocotylum Esocis lucii	Diesing (1850, 1851)
Monobothrium sp.	Grimm (1871)
Acrobothrium typicum	Olsson (1872)
Cyathocephalus americanus	Cooper (1918)
Cyathocephalus truncatus	Kessler (1868)

Only the species Cyathocephalus truncatus is known so far to exist. The single genus Cyathocephalus is classified under the family Cyathocephalidae which with two other families (Spathebothridae and Diplocotylidae) are placed in the order Spathebothridea of the class Cestoda.(Wardle and McLeod, 1952).

1.3 Primary host records

Primary hosts	Common Name	Relative frequency of occurrence as host
1. Salmo trutta Linnaeus, 1758	Brown trout	***
2. Salmo alpimus Linnaeus, 1758	Red char	***
3. Salmo irideus Gibbons 1855	Trout	**
4. Salmo gairdneri Richardson, 1836	Rainbow trout	***
5. Salmo obtrusirostrus Linnaeus, 1758	Trout	*
6. Salvelinus alpinus malma (Linnaeus, 1768)	Char	*
7. Coregonus albula (Linnaeus, 1768)	Houting	**
8. Coregonus clupeaformis Mitchill 1818	White fish	**
9. Coregonus laveratus (Linnaeus, 1758)	Houting	**
10. Thymallus thymallus (Linnaeus, 1758)	Grayling	**
11. Thymallus vulgaris (Nilsson 1855)	Grayling	**
12. Esox lucius (Linnaeus, 1758)	Pike	**
13. Tinca tinca (Linnaeus, 1758)	Tench	*
14. Leiciththys zenithicus (Valenciennes, 1822)	Whitefish	*
15. Luceoperca sandra (Linnaeus, 1758)	Zander (Pike perch)	*
16. Perca fluviatilis (Linnaeus, 1758)	Perch	*

17.	Lota lota Linnaeus, 1758)	Burbot	*
18.	Lota vulgaris Jenyns 1835	Burbot	*
19.	Anguilla anguilla (Linnaeus, 1758)	Eel	*
20.	Anguilla vulgaris Turton	Eel	*
21.	Cottus gobio Linnaeus, 1758	Miller's thumb/ bullhead	*
22.	Cottus cognatus Richardson 1836	Slimy sculpin	*
23.	Cotus asper Richardson 1836	Prickly sculpin	*
24.	Gasterosteus aculeatus Linnaeus 1758	Stickleback	*

*** Very common

** Frequent

* Rare

1.4 Intermediate host records

Intermediate hosts	Invertebrate crustacea order
1. Gammarus pulex Linnaeus,1758	Amphipoda
2. Gammarus lacustris G. O. Sars	"
3. Gammarus italicus Goedmakers and Pinkster,1977	"
4. Echinogammarus roco Karman, 1973	"
5. Echinogammarus tibaldi Pinkster and Stock, 1970	"
6. Fontogammarus bosniacus (Schäferna, 1922)	"
7. Rivulogammarus spinicaudatus (Schäferna 1922)	"
8. Pontoporeia affinis	"
9. Pallasea quadrispinosa	"
10. Mysis relica Loven 1934	Mysidacea

TABLE 1.—Records of the occurrence of C. truncatus in the final and intermediate hosts

	Author	Primary Host	Intermediate Host	Locality
	Pallas (1781) (as Taenia truncatus)	Esox lucius	Not recorded	Ieningrad, U.S.S.R
*	Batsch (1786) (as Taenia truncatus)	Esox lucius	Not recorded	Not indicated
	Zeder (1803) (as Echinorhynchus sp.)	Esox lucius	Not recorded	Germany
	Rudolphi (1810, 1819) (as Dubium Esocis lucii)	Esox lucius Perca fluviatilis	Not recorded	Germany
*	Kessler (1868)	Salmo trutta	Not recorded	Petersburg.
	Olsson (1872) (as Acrobothrium typicum)	Lota vulgaris	Not recorded	Storsjøen, Sweden
*	Braun (1884, 1892)	Esox lucius	Not recorded	Mecklenburg, Germany
	Zschokke (1884, 1903, 1933)	Coregonus albula, Lota lota and Salmo alpinus	Not recorded	Lake Leman Geneva, Switzerland.
	Kraemer (1891, 1892)	Lucioperca sandra	Not recorded	Sweden
	Olsson (1893)	Thymallus vulgaris	Not recorded	Jämtland, Sweden
	Linton (1898)	Coregonus clupeaformis	Not recorded	Lake Superior, North America.
*	Nufer (1905)	Salmo alpinus	Not recorded	Switzerland

*Quoted from Vik (1954)

Author	Primary Host	Intermediate Host	Locality
* Lühe (1899, 1900, 1910)	Salvelinus alpinus and trout	Not indicated	Not indicated
Wolf (1906)	Salmo gairdneri Salmo trutta	Gammarus pulex	South Germany
Huitfeldt-Kass (1913)	Salmo alpinus	Not recorded	Einasjøen, Sweden
Cooper (1918) (as Cyathocephalus americanus)	Coregonus clupeaformis	Not recorded	Lakes Huron and Michigan, North America
Jääskelainen (1921)	Trout	Not recorded	Finland
Schäferna (1922)	Not recorded	Rivulogammarus spinicaudatus and Fontogammarus bosniacus	River Bosnia, Sarajevo, Yugoslavia
Gauthier (1923)	Trout	Not recorded	Fishculture Laboratory l'Universite de Grenoble France
Huitfeldt-Kaas (1927)	Salmo alpinus	Not recorded	Lemonsjøen Vaga in Gudbrandsdalen Sweden
Joyeux et Baer (1936)	Salmo irideus	Not recorded	Paris, France
Baylis (1939)	Salmo trutta and Anguilla anguilla	Not recorded	River Test, Hants, U.K.
Guiart (1935)	Salvelinus alpinus	Not recorded	Spitsbergen, Germany
Janicki (1928)	Trout	Gammarus spp.	Yugoslavia

* Quoted from Vik (1954)

Author	Primary Host	Intermediate Host	Locality
Wardle (1932)	Coregonus clupeaformis Leicichthys zenithicus	Pontoporeia affinis	Lake Winnipeg, North America
	Salvelinus alpinus malma	Not recorded	Spray lakes Alberta, North America
Wisniewski (1932, 1933)	Salmo trutta and Salmo irideus	Rivulogammarus spinicaudatus and Fontogammarus bosniacus	River Bosnia Sarajevo, Yugoslavia
Zschokke (1933)	Salmo alpinus	Not recorded	River Rhine, Switzerland
Richard (1935)	Coregonus sp. & Trout	Not recorded	Spitsbergen, Germany
Bauer and Nikol'skaya (1957)	Coregonus laveratus	Pallaseq quadrispinosa	Lake Ladoga U.S.S.R
Šenk (1952, 1956)	Salmo trutta	R. spinicaudatus F. bosniacus	River Bosnia Sarajevo, Yugoslavia
Bangham and Adams (1954)	Cottus asper	Not recorded	British Columbia, North America
Vik. (1954)	Salmo trutta Salmo alpinus Anguilla vulgaris Gasterosteus aculeatus	Gammarus lacustris	Ånøya Water System Trøndelag, Norway
Bangham (1955)	Coregonus sp.	Not recorded	Lake Huron and Manitoba Island, North America

Author	Primary Host	Intermediate Host	Locality
Vik (1958)	Salmo trutta Salmo alpinus Lota sp. Coregonus sp.	Gammarus lacustris	Ångya Water System Trøndelag, Norway
DeGuisti and Budd (1959)	Coregonus sp.	Not recorded	South Bay Ontario, North America
Rukavina and Delić (1959)	Salmo trutta Salmo thymus obtusirostris Thymallus thymallus	Not recorded	Adriatic Conflux and Black Sea Conflux Central Europe
Alexander (1960)	Coregonus sp. & trout	Not recorded	Oregon State, North America
Malakhova (1961)	Lota lota	Not recorded	Lake Kouche, Karelia U.S.S.R.
Ćorić (1963)	Cottus gobio	Not recorded	River Bosnia Sarajevo, Yugoslavia
Dechtair and Loftus (1965)	Coregonus sp.	Not recorded	Lake Huron, North America
Awachie (1966)	Salmo trutta	Gammarus pulex	Afon Terrig, North Wales, U.K.
Aisa (1971)	Tinca tinca	Not recorded	Lake Trasimeno, Italy
Halvorsen and Macdonald (1972)	Salmo trutta	Not recorded	Norwegian Lakes, Norway
Rahim (1974)	Salmo trutta	Not recorded	River Alyn, Afon Glyn Afon Dyfrdwy Wales, U.K.

Author	Primary host	Intermediate Host	Locality
Leong (1975)	Coregonus clupeaformis	Not recorded	Cold Lake, Alberta, Canada, North America
Mudry and McCart (1976)	Salvelinus alpinus	Not recorded	Rivers and Lakes in Alaska and Canada North America
Amin (1977, 1978)	Not recorded	Pontoporeia affinis Mysis relica	Milwankee Harbour and Lake Michigan, North America
Paggi et al (1978)	Salmo trutta	Not recorded	River Tirino L'Aquila, Italy.
Orecchia et al (1978)	Salmo trutta	Echinogammarus roco Echinogammarus tibaldi Gammarus italicus	River Tirino L'Aquila, Italy.
Catalini et al (1978)	Salmo trutta	Not recorded	River Tirino L'Aquila, Italy
Henrickson and Nyman (1976)	Salvenlinus alpinus	Gammarus sp.	Lake Faatjaure Sweden
Petersson (1971)	Coregonus spp.	Not recorded	Sweden
Maren (1979)	Thymallus thymallus	Not recorded	Rhone river Lyon France
Reimchen (1982)	Gasterosteus aculeatus	Not recorded	Queen Charlotte Island British Columbia, Canada.

TABLE 1.2: British Records of Cyathocephalus truncatus

	Author	Primary and Intermediate Hosts	Location
1.	Baylis (1939)	Salmo trutta Anguilla anguilla	River Test, Hants, South England
2.	Robertson (1953) **	Salmo trutta	Loch Walton, Scotland
		Esox lucius	Loch Con, Loch Skiach and Loch Kinardochy, Scotland
3.	Awachie (1966)	Salmo trutta and Gammarus pulex (Intermediate host)	Afon Terrig, North Wales
4.	Powell (1966) *	Salmo trutta Anguilla anguilla	Lyn Padarn, Caerus, Wales
5.	Campbell (1974) **	Salmo trutta	Loch Leven, Morton Loch, Brother Loch, Loch Skiach, Scotland
		Salmo solar	Loch Kinardochy, Scotland
6.	Rahim (1974)*	Salmo trutta	River Alyn, Afon Dyfrdwy, Afon Glyn, Wales

NOTE: Records of Cyathocephalus truncatus in the United Kingdom are sparse, most are not full reports but as occasional finds during investigations into parasites of fish. They appear as indicated above: * Reports in PhD Theses
** Personal communication report in Kennedy (1974)

CHAPTER 2

ECOLOGICAL OBSERVATIONS ON *CYATHOCEPHALUS TRUNCATUS* IN THE FISH AND AMPHIPOD HOSTS.

2.1 Introduction

As noted in the previous chapter, only a few accounts have been given of occurrence of Cyathocephalus truncatus in Britain.

During the present investigation however C. truncatus was present in all trout caught from the Driffield end of the river Hull and its tributaries in Yorkshire, England. The area also harbours an abundant population of amphipods.

In the present study an account is given of the infection with Cyathocephalus truncatus and six other species of helminth parasites occuring in three species of fish (Salmo gairdneri, Salmo trutta and Thymallus thymallus) from the area of study. The intensity of infection of the tapeworm in different regions of the pyloric caeca is described.

An account is also given of the prevalence of infection with the tapeworm procercoids and the juvenile stages of six other species of helminth parasites in Gammarus pulex.

2.2 Literature review

All the published records show that Cyathocephalus truncatus infection is always associated with freshwater lakes, streams and rivers.

Senk (1952) reported that the infection rate of Salmo trutta with C. truncatus decreased along the river Bosnia, Sarajevo, Yugoslavia from its source to its mouth. The author indicated that infected Gammarus were mostly found in the tributaries Vaceria, Bukulas and Zejevina where the speed of

water flow was much reduced due to presence of water plants and where the amphipods were abundant. Vik (1954) in his investigations in the Ånøya water system in Trøndelag, Norway, believed that infection was governed by the level of abundance of intermediate host, Gammarus lacustris, which he found to occur mostly in slow running shallow water with water plants. Also Rukavina and Delić (1959) in their account of C. truncatus in salmon established that the occurrence of infection was mostly in the upper shallow streams of the rivers of the Black Sea conflux and the Adriatic conflux.

Most of the reports on the occurrence of C. truncatus are restricted to that in the fish primary hosts. The early reports although referring to light infections mostly concerned fish like the pike, perch, burbot and whitefish (Pallas, 1781; Zschokke, 1884; Olsson, 1872; Kraemer, 1892). Fishes of the family Salmonidae have been found to be the most frequently and most heavily infected (Huitfeldt-Kaas, 1913, 1927; Wisniewski, 1932a,b; Vik, 1954). There are however reports of occasional occurrence in certain other fish. Examples are the bullhead, eel and stickleback. While 4 of 37 Cottus cognatus examined by Amin (1977) were infected only 1 of the 344 Cottus asper was reported to have C. truncatus infection by Bangham and Adams (1954). Awachie (1966a) recorded no infection in 44 Cottus gobio whereas 25.8% of the 252 Salmo trutta were infected. Baylis (1939) stated that he had found C. truncatus in eels (Anguilla anguilla) while Vik (1954) made a similar discovery in the eel species Anguilla vulgaris but observed that the tapeworms

were never attached at any time in the intestine of the fish. Although Baylis did not give full details of how established the tapeworms were in the intestine of the fish he examined, Vik remarked that eels cannot possibly be a host for C. truncatus and that the worms he found had only newly escaped from the gammarids which the eel had eaten and would have been digested or expelled later on. Vik (1954) also reported the occurrence of a single tapeworm in a stickleback, Gasterosteus aculeatus which he regarded as unique since he was unable to infect G. aculeatus experimentally where he succeeded with Salmo trutta. In a recent report Reimchen (1982) also found the worm in the stickleback.

Kraemer (1892) stated that members of the species C. truncatus were rare and that probably only a few specimens live in a single host at the same time. The greatest number recorded in a single Salmo alpinus by Nufer (1905) was 35. Although distribution of the tapeworm is generally sparse and with low rate of occurrence, occasional heavy infections have been reported. Olsson (1893) reported that the tapeworm was common in Jämtland, Sweden, though he gave no detailed data of incidence. Huitfeldt-Kaas (1913) from Sweden, reported mass infection of Salmo alpinus and in 1927, he recorded a maximum infection of 200 tapeworms per fish. Even heavier infection was recorded by Wisniewski (1932b)who reported a 100% infection rate and a mean of 50 tapeworms per fish in the 60 Salmo trutta and Salmo irideus he examined from river Bosnia, Sarajevo, Yugoslavia. The most heavily infected fish harboured 400 tapeworms. Wisniewski inferred that the number of tapeworms occuring in

a fish was entirely a matter of chance -depending on the extent to which individual fish prey on infected shrimps. Vik (1954) recorded a maximum number of 250 tapeworms in a single infected fish in his 1943 collection and as many as 400 tapeworms in another single fish in the 1950 collection from the Ånøya Water System, Trøndelag, Norway. The percentage numbers of fish (Salmo trutta and Salmo alpinus) infected in the two years of collection were 22.5% and 16.7% for 1943 and 1950 respectively.

Only very few reports exist on the incidence of the larval stages in Gammarus. The levels are sometimes very low - only 0.07% prevalence in G. pulex recorded by Awachie (1966a) at Afon Terrig, Wales although a higher infection rate (7%) was noted in Fontogammarus bosniacus and Rivulogammarus spinicaudatus recorded by Wisniewski (1932b) from river Bosnia, Yugoslavia.

Although no regular monthly or seasonal sample data were taken, Vik (1954) and Wolf (1906) in their separate investigations believed that there was no variation in the intensity of infection at different periods of the year. Awachie (1966a) however observed that the maximum infection in Gammarus pulex occurred between January and February. Also the intensity of infection in fish increased at the same period of the year with a high prevalence usually tending to spread over the period October to April. Awachie thus concluded that there was an annual cycle and periodicity of occurrence of C. truncatus in the intermediate and definitive hosts at Afon Terrig, Wales.

Halvorsen and Macdonald (1972) reported that C. truncatus attached preferentially within the anterior pyloric caeca particularly in a small group of dorsally located caeca.

They also observed that in trout parasitized only by the digenean Crepidostomum metoecus, the anterior caeca were the preferred site of the fluke while in fish infected with both the cestode and fluke, C. metoecus was found mainly in the posterior caeca and small intestine with the tapeworm restricted to the anterior caeca.

2.3 Materials and Methods

2.3.1 Area of collection

The area investigated comprised part of the river Hull adjacent to Driffield Canal and the river's tributaries namely Driffield Beck, and Eastburn Beck.

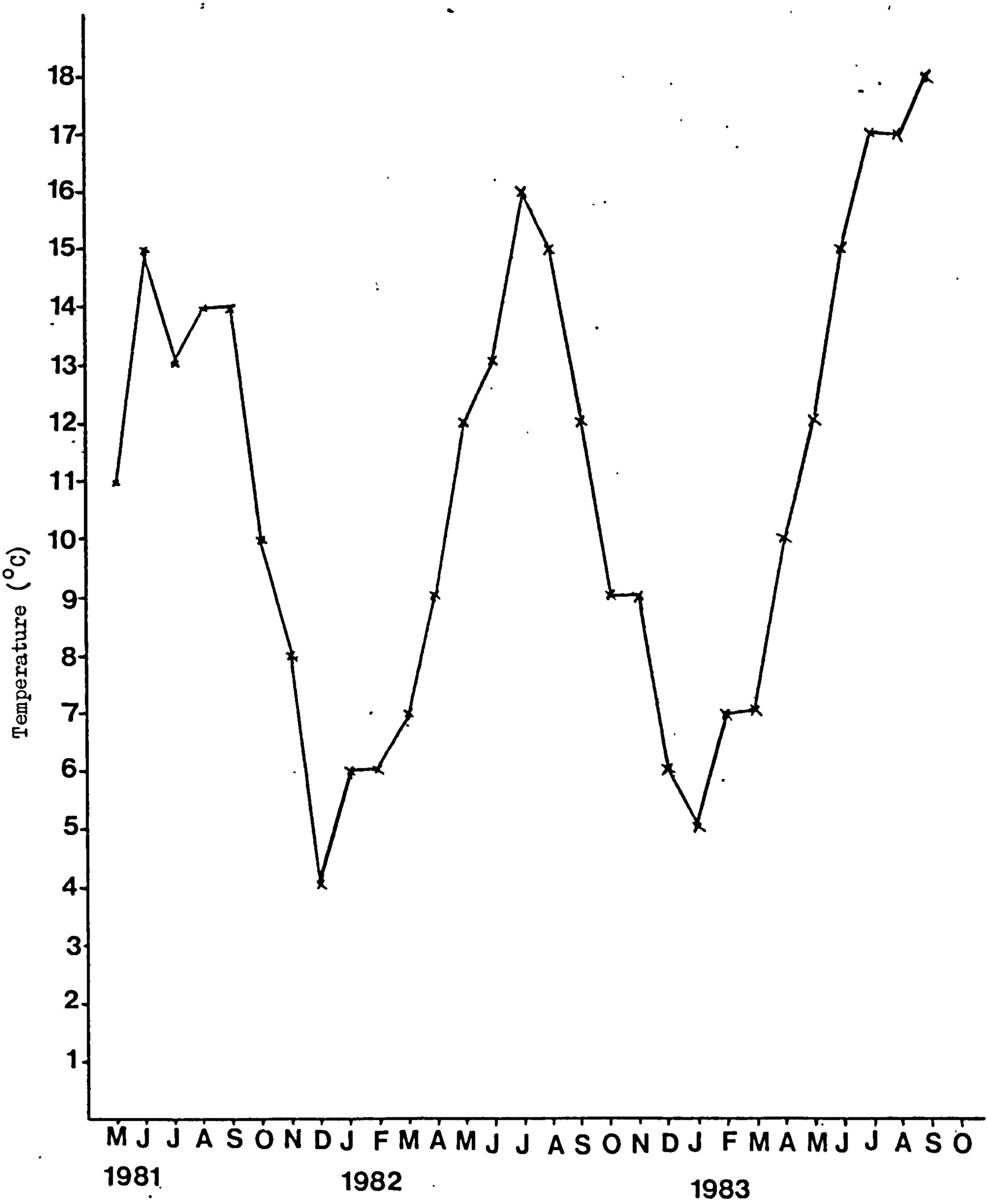
In the Ordnance Survey Sheet 107 (Landranger series of Great Britain) grid reference scale, the areas lie between TA 022 564 and TA 054 564. The water system is located in Driffield, North East of England and referred to as Driffield trout streams for convenience in the present account.

The water system is about a 10-mile stretch in the area of the Yorkshire Water Authority, East Division, but managed by the Angling Club of Driffield. Angling rights are restricted to members.

The water is shallow, usually about 1.3m deep but up to 2m deep in certain places. Its width varies from about 3m to 9m In areas where most specimens of fish or gammarids were collected, the water contains dense beds of vegetation, largely with plants of Ranunculus aquafalis and Fontinalis antipyrectica.

The presence of water plants allied with the slow speed of water flow afford a good environment for an abundant population

Fig: 2.1 Monthly water temperature at Driffield trout streams.


Temperature (°c)
18
17
16
15
14
13
12
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10
9
8
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1
M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O
1981
1982
1983
Months

of Gammarus pulex individuals of which apparently form the major food items of the fish and also serve as the intermediate host of the tapeworm Cyathocephalus truncatus and some other helminth parasites.

The water harbours fish including brown trout (Salmo trutta), rainbow trout (Salmo gairdneri), grayling (Thymallus thymallus), stickleback (Gasterosteus aculeatus), Miller's thumb or bullhead (Cottus gobio) and eel (Anguilla anguilla).

Temperature of water varied from about 4°C in colder months to about 18°C in warmer months (Fig 2.1)
There is a continuous flow of water in the stream throughout the year and it did not become ice-covered in winter months.

The water system does not appear to serve any navigational purpose but the vegetation is cut at intervals to facilitate fishing activities with unknown effects on the populations of the organisms living in it.

2.3.2. Collection and examination of specimens

Fish specimens (Salmo trutta, Salmo gairdneri and Thymallus thymallus) were netted. They were transported to the University Aquarium at Leeds in bins of aerated river water. Unfortunately the supply of fish was rather spasmodic and the numbers available for study generally low hence a comprehensive survey could not be conducted.

Some fish were examined for parasites within a few days of collection. Others were maintained in aquaria for long-term study and to provide a supply of parasite material for

experimental purposes and histological and histochemical studies. The viscera of fish which could not be inspected immediately were preserved in 4% formol saline. Faecal material of fish maintained in the laboratory was used as a source of tapeworm eggs in studies of the tapeworm's life cycle.

The external body surface, entire alimentary canal, gills, swimbladder, and the body cavity of the fish were examined for helminth parasites. The distributions of the tapeworm, C. truncatus and of other helminth parasites in different parts of the fish were noted.

Gammarus pulex were also collected from various accessible points of the river with the aid of hand nets. The amphipods were collected every fortnight and after each collection some specimens were examined immediately in the laboratory for the presence of the tapeworm and other helminth parasites. This was done initially in case infected shrimps might not survive if kept for long in laboratory aquaria as stated by Awachie (1965). It was however found that if gammarids were transported to the laboratory in open containers filled with damp water weeds, mortality was usually low; subsequently the survival of infected shrimps in the laboratory appeared to be similar to that of non-infected specimens.

On examination, the amphipods were teased apart with the aid of pins or needles and the intestine, hepatopancreas and body cavity were examined under a low power dissecting microscope for parasites. The stages of development of C. truncatus in the amphipods were categorised into 3 groups by Wisniewski (1932b). Based on their sizes and structure he described

the stages as early procercoid (fruhprocercoidstadien), middle procercoid (mittelprocercoidstadien) and mature procercoid (reifeprocercoidstadien). In the present study, it was thought appropriate to refer to them as procercoid stages I, II and III respectively.

2.3.3. Identification of parasites

For proper identification, the parasites found in fish and amphipod were first placed in 0.9% saline and examined live. Then whole mount preparations of them were made, stained with Borax carmine (Humason, 1962) or with hematoxylin using acetic acid as diluent and dehydrant - a technique devised by Chubb (1962).

2.4 Results

2.4.1 Incidence and Intensity of infection in fish.

200 specimens of fish from the 232 specimens obtained between May 1981 and December 1983 were examined. The remaining 32, all rainbow trout were maintained in aquaria and later used for other studies. The fish examined comprised 160 rainbow trout, 31 grayling and 9 brown trout. They ranged in size from 8cm to 36cm in total length and from 10g to 350g in fresh weight. Apart from the tapeworm, Cyathocephalus truncatus, other helminth parasites identified include

1. Echinorhynchus truttae Schrank, 1788 (Acanthocephala)
2. Echinorhynchus salmonis Muller, 1784 "
3. Neoechinorhynchus rutili (Muller, 1780) "
4. Cystidicola farionis Fischer, 1798 (Nematoda)
5. Crepidostomum metoecus Braun, 1900 (Digenea)
6. Cucullanus truttae (Fabricius, 1794) (Nematoda)

The summarised data on infection of fish with helminth parasites are given in Tables 2.1, 2.2 and 2.3.

All the fish examined were infected with Cyathocephalus truncatus and three other helminths: Echinorhynchus truttae, Echinorhynchus salmonis and Cystidicola farionis. Of the other species of helminth parasites found Crepidostomum metoecus occurred in 132 (82.5%) rainbow trout, 7 (77.8%) brown trout and 23 (74.2% graylings ; Neoechinorhynchus rutili in 128 (90.0%) rainbow trout, 4 (44.4%) brown trout and 16 (51.6%) grayling and Cucullanus truttae in 13 (8.1%) rainbow trout, 5 (55.6%) brown trout and 4 (12.9%) grayling.

TABLE 2.1: Occurrence of helminth parasites in Salmo gairdneri from Driffield trout streams

Date of Collection	No. of Fish Examined	Cyathocephalus truncatus		Echinorhynchus truttae		Echinorhynchus salmonis		Neoechinorhynchus rutili		Cystidicola farionis		Crepidostomum metoecus		Cucullanus truttae	
		No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms
20.5.81	13	13	528	13	751	13	269	10	121	13	498	13	158	-	-
3.7.81	5	5	251	5	263	5	254	2	17	5	163	-	-	-	-
22.7.81	22	22	1749	22	2606	22	691	15	377	22	1474	18	282	5	25
10.11.81	16	16	661	16	343	16	330	12	160	160	839	15	84	2	17
17.2.82	12	12	530	12	423	12	380	12	50	12	917	10	182	-	-
7.5.82	14	14	604	14	987	14	216	10	115	14	802	12	147	1	18
6.8.82	17	17	639	17	1075	17	236	17	51	17	1057	15	34	-	-
29.10.82	24	24	561	24	708	24	290	20	51	24	933	20	53	3	35
10.12.82	12	12	412	12	873	12	267	12	49	12	709	8	36	-	-
20.5.83	13	13	538	13	721	13	118	13	82	13	1025	12	64	-	-
16.12.83	12	12	395	12	855	12	315	5	63	12	877	9	21	2	13
Total	160	160	6868	160	9604	160	3366	128	1136	160	9294	132	1061	13	98
Percentage infection		100%		100%		100%		80%		100%		82.5%		8.1%	
Mean			42.9		60.0		21.0		7.1		58.1		6.6		0.6

TABLE 2.2: Occurrence of helminth parasites in Salmo trutta from Driffield trout stream

Date of Collection	No. of Fish Examined	Cyathocephalus truncatus		Echinorhynchus truttae		Echinorhynchus salmonis		Neoechinorhynchus rutili		Cystidicola farionis		Crepidostomum metoecus		Cucullanus truttae	
		No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms
20.5.82	2	2	13	2	92	2	17	-	-	2	70	1	7	1	14
3.7.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22.7.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.11.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17.2.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7.5.82	1	1	75	1	21	1	6	-	-	1	14	1	4	-	-
6.8.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29.10.82	2	2	51	2	54	2	7	1	3	2	76	2	26	2	47
10.12.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20.5.83	4	4	82	4	114	4	22	3	14	4	107	3	12	2	150
16.12.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	9	9	221	9	281	9	52	4	17	9	267	7	49	5	211
Percentage infection		100%		100%		100%		44.4%		100%		77.8%		55.6%	
Mean			24.6		31.6		5.8		1.9		29.7		5.4		23.4

TABLE 2.3: Occurrence of helminth parasites in Thymallus thymallus from Driffield trout streams

Date of Collection	No. of Fish Examined	Cyathocephalus truncatus		Echinorhynchus truttae		Echinorhynchus salmonis		Neoechinorhynchus rutili		Cystidicola farionis		Crepidostomum metoecus		Cucullanus truttae	
		No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms	No. of Fish Infected	No. of Worms
20.5.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.7.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22.7.81	8	8	358	8	365	8	139	6	75	8	323	8	93	1	3
10.11.81	4	4	183	4	72	4	7	-	-	4	202	4	26	-	-
17.2.82	4	4	161	4	51	4	22	2	9	4	273	3	25	2	17
7.5.82	6	6	271	6	254	6	44	2	12	6	312	2	13	-	-
6.8.82	8	8	304	8	405	8	67	5	43	8	447	5	50	1	7
29.10.82	1	1	71	1	28	1	5	1	2	1	45	1	5	-	-
10.12.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20.5.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16.12.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	31	31	1348	31	1175	31	284	16	141	31	1602	23	212	4	27
Percentage infection		100%		100%		100%		51.6%		100%		74.2%		12.9%	
Mean			43.5		37.5		9.2		4.5		51.7		6.8		0.9

None of the rainbow trout and grayling contained any species of Crepidostomum other than C. metoecus. Six specimens of Crepidostomum farionis were identified from 3 brown trout. Additionally 4 specimens of an unidentified trematode, possibly Allocreadium sp. was recorded from 4 brown trout.

Apart from Cystidicola farionis which inhabits the swim bladder all the other species listed above occupy various regions of the alimentary canal. Acanthocephalans were found in all the regions of the pyloric caeca and the small intestine whereas the trematode Crepidostomum metoecus and nematode Cucullanus truttae were located in the posterior pyloric caeca and proximal portion of small intestine. The distribution of Cyathocephalus truncatus in the body of the fish examined is given in Appendix 1 and described below.

(a) Cyathocephalus truncatus

The intensity of infection with C. truncatus ranged between 2 and 117 tapeworms per fish with a mean of 44 worms per fish in rainbow trout, 24 worms per fish in brown trout and 43 worms per fish in grayling. (Tables 2.1, 2.2 and 2.3).
The frequency distribution of the tapeworms in rainbow trout of different sizes is tabulated in Table 2.4 . Infection appears to be intense among the fish in the size groups 18.1 - 22 .0cm and 22.1 - 26.0cm which recorded a mean infection of 52 worms per fish and 68 worms per fish respectively. The small sized fish (10.0 - 14.0cm and 14.1 - 18.0 cm) recorded a mean infection of 14 worms per fish and 31 worms per fish respectively while the apparently large ones (26.1 - 30.0 cm, 30.1 - 34.0 cm and 34.1 - 38.0 cm)

recorded a mean infection of 33 worms per fish, 35 worms per fish and 22 worms per fish respectively.

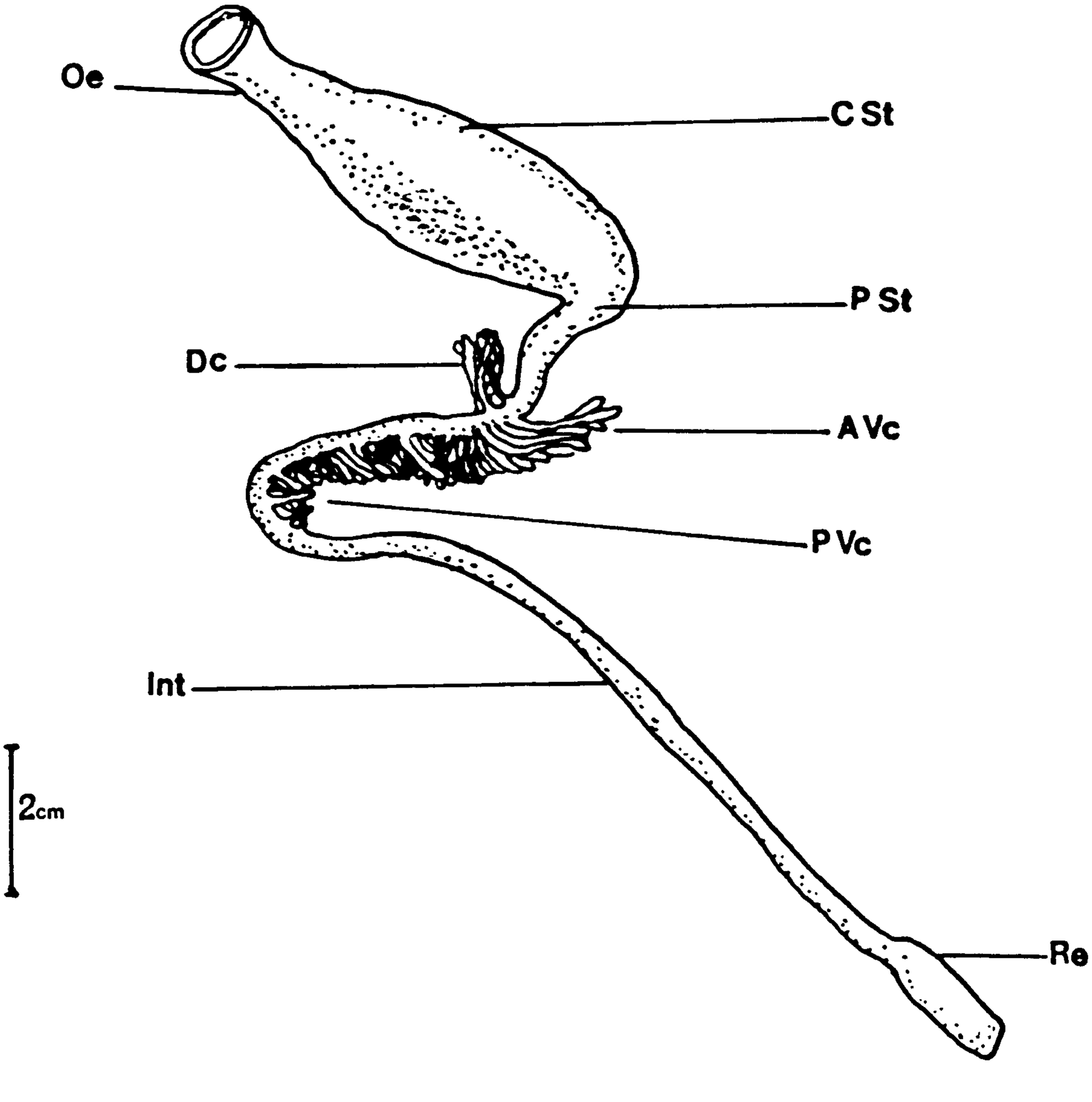
Most of the tapeworms were firmly attached to the distal ends of the pyloric caeca. In light infections usually only a single worm was present in each infected caecum but in heavy infections up to 6 individuals were recorded in separate caeca. In such cases one tapeworm occupied the distal portion of the caecum with the others attached to the mucosa along the length of the caecum.

The worms appear to establish mostly in the anterior dorsal caeca and the anterior ventral ceca but infection spreads to the posterior ventral caeca and the proximal region of the small intestine with the increase in number of tapeworms. The incidence of worms in the various regions of pyloric caeca of the rainbow trout examined is presented in Table 2.5. As the number of worms increases from the ranges 1-10 to 111-120, there is a lightly marked tendency for a decrease in percentage occurrence in the dorsal caeca from 65% to 36%; increase in the anterior ventral caeca from 32.6% to 43.7% and increase in the posterior ventral caeca from 2% to 10.8%. Also with increase in number of worms there is slight increase in percentage number of worms in the intestine from 0% to 2.6% and in the body cavity from 0% to 7.0%.

There is no clear line of demarcation between anterior ventral caeca and posterior ventral caeca other than the fact that the former are anterior-laterally directed and the latter posteriorly directed (see Fig. 2.2).

Fig: 2.2 The alimentary tract of rainbow trout (Salmo gairdneri)

A Vc	Anterior ventral caeca
C st	Cardiac stomach
Dc	Dorsal caeca
Int	Intestine
Oe	Oesophagus
Pst	Pyloric stomach
PVc	Posterior ventral caeca
Re	Rectum


Oe
C St
P St
Dc
A Vc
P Vc
Int
2cm
Re

In a small number of cases, some tapeworms were also found free in the body cavity or attached to the abdominal muscles. A few worms with deformed appearance and enclosed in a 'cyst' were also seen. A few of the latter worms were sometimes found dead among the worms that occurred outside the alimentary tract. Presence of such individuals in the body cavity indicate long standing infection during which time the worms have broken through the caecal wall.

Except for a few worms found free in the intestine and in the body cavity all other worms were attached. Most of the unattached worms found in the small intestine were dead and were evidently being eliminated from the alimentary canal. Some of the dead worms had no scolex perhaps indicating that the scolex may be so firmly attached to the host that it could no longer separate on its own even after the death of the worm. Others possessed a scolex carrying a lump of host tissue attached to the concavity of the funnel. These latter worms were usually found alive and had probably moved into the gut following the breakdown of host tissue at the distal end of the caeca to which they had been attached. It was not uncommon to find some caeca lacking their distal ends and others in which their distal ends had been transformed into a cyst-like structure encapsulating the tapeworm.

In the rainbow trout, all individuals of both sexes examined were infected with a mean number of 42 worms (Standard Deviation 28.1) per fish in the 95 males and a mean of 45 worms (S.D. 28.2) per fish in the 65 females (Table 2.6). Similarly, all the individuals of both sexes of the brown trout and grayling examined

Table 2.4 Frequency distribution of C. truncatus numbers in rainbow trout of different sizes.

Range of No. of tapeworms	NO. OF FISH IN THE SIZE RANGES						
	10 - 14cm	14.1 - 18cm	18.1 - 22cm	22.1 - 26cm	26.1 - 30cm	30.1 - 34cm	34.1 - 38cm
1 - 5	2	3	1				1
6 - 10	6		1			1	
11 - 15	11	6	1		1	1	
16 - 20	5	1	2		1		
21 - 25	1	2	1	1	1	2	
26 - 30		4	2		3		1
31 - 35	1	4	4	1	2		
36 - 40		2	3	1	1		1
41 - 45	1	2	3	2	1		
46 - 50		2	4	2			
51 - 55		2	8	1			
56 - 60		1	2	1			
61 - 65			4	6			
66 - 70		1	1	5			
71 - 75			3	2	1		
76 - 80		1	3	1			
81 - 85				2			
86 - 90			3	1			
91 - 95			1	3			
96 - 100				2			
101 - 105		1	1	1			
106 - 110				1			
111 - 115						1	
116 - 120			1				
No. of fish	27	32	49	33	11	5	3
No. of tapeworms	381	1007	2570	2257	361	175	67
Mean No. of worms per fish	14.1	31.5	52.4	68.4	32.8	35.0	22.3
Variance	60.8	447.0	587.8	454.1	258.4	198.7	316.3
St. D.	7.8	21.1	24.2	21.3	16.1	44.6	17.8

TABLE 2.5: Percentage occurrence of Cyathocephalus truncatus in the body of the rainbow trout from Driffield trout streams.

Range of No. of worms	PERCENTAGE OCCURRENCE (%) IN				
	Dorsal Caeca (10*)	Anterior ventral Caeca (50*)	Posterior Ventral Caeca (12*)	Intestine	Body Cavity
1- 10	65.2	32.6	2.2	-	-
11- 20	50.7	43.5	4.8	1.0	-
21- 30	48.8	45.1	5.8	1.0	0.3
31- 40	43.3	48.8	6.5	1.0	0.4
41- 50	45.2	46.4	6.6	1.3	0.5
51- 60	38.8	51.9	6.6	1.1	1.6
61- 70	34.2	54.2	8,7	1.2	1.7
71- 80	37.4	54.4	6.2	1.0	1.0
81- 90	35.1	53.8	7.8	1.2	2.1
91-100	35.5	55.0	8.1	0.4	1.0
101-110	35.6	53.6	7.7	1.4	1.7
111-120	35.9	43.7	10.8	2.6	7.0

* Approximate numbers of caeca in the region

Table 2.6: *Cyathocephalus truncatus* infection in male and female fish from Driffield trout streams

Fish species	MALES				FEMALES			
	No. (%) of infected fish	No. of worms	Mean No. of worms per fish	Standard Deviation	No. (%) of infected fish	No. of worms	Mean No. of worms per fish	Standard Deviation
Salmo gairdneri	95 (100)	3974	41.8	28.1	65 (100)	2894	44.5	28.2
Salmo trutta	5 (100)	110	22	30.1	4 (100)	111	27.8	20.2
Thymallus thymallus	17 (100)	804	47.3	28.1	14 (100)	544	38.9	29.5

were infected. A mean number of 22 worms (S.D. 30.1) per fish in males and 28 worms (S. D. 20.2) per fish in females was recorded for brown trout while a mean number of 47 worms (S.D. 28.1) per fish in males and 39 worms (S.D. 29.5) per fish in females was recorded for grayling.

(b) Other helminth parasites

Echinorhynchus truttae - The mean numbers in rainbow trout, brown trout and grayling were 60, 31 and 37 worms per fish respectively (Tables 2.1,2.2 and 2.3). The distribution of E. truttae in the alimentary tract of 40 of the rainbow trout examined is given in Table 2.7. This random number from the first group of the fish collected was chosen to demonstrate the occurrence of the worm in the intestinal tract. The prevalence of E. truttae in the pyloric caeca was low (10.4%) but high in the small intestine (89.6%). E. truttae occurred in very small numbers (in most cases fewer than 10 specimens) in the caeca as young orange coloured individuals but the great majority were located in the small intestine where they varied between brick-red coloured middle-sized individuals in the region of small intestine proximal to the pyloric caeca to dark coloured large and mature specimens near the rectum. They were easily distinguishable from other acanthocephalan species which are whitish or light grey in colour.

Table 2.7: Distribution of *Echinorhynchus truttae* in the alimentary canal of 40 rainbow trout examined between November 1981 and May 1982.

No.	Total Length (cm) of fish	Weight (g) of fish	Sex	Total Number of *E. truttae*	Number (%) in the caeca	Number (%) in the intestine
1	13.1	12.4	M	52	8(15.3)	44(84.6)
2	14.2	26.8	M	38	7(18.4)	31(81.5)
3	14.5	35.2	M	82	3(3.6)	79(96.3)
4	13.0	15.5	M	21	-	21(100)
5	28.0	208.7	M	85	4(4.7)	81(95.2)
6	20.7	84.4	F	57	2(3.5)	55(96.4)
7	18.3	54.0	F	85	5(5.8)	80(94.1)
8	14.6	28.2	M	102	3(2.9)	99(97.0)
9	22.5	115.9	F	42	6(14.2)	36(85.8)
10	22.2	128.2	F	87	1(1:1)	86(98.9)
11	17.0	58.1	M	25	-	25(100)
12	21.2	62.5	M	62	8(12.9)	54(87.0)
13	19.7	51.9	M	89	2(2.2)	87(97.7)
14	19.8	75.5	M	92	3(3.2)	89(96.7)
15	22.0	104.5	M	32	-	32(100)
16	20.0	86.4	F	77	6(7.7)	71(92.2)
17	18.5	65.2	M	35	8(22.8)	27(77.1)
18	17.0	46.5	M	83	11(13.2)	72(86.7)
19	34.0	246.2	M	132	17(12.8)	115(87.1)
20	22.5	126.8	M	42	7(16.6)	35(83.3)
21	22.2	132.0	M	97	14(14.4)	83(85.5)
22	21.5	119.8	F	110	10(9.0)	100(90.9)
23	23.0	120.0	M	39	2(5.1)	37(94.8)
24	20.8	92.0	F	103	13(12.6)	90(87.3)
25	22.1	116.7	M	47	6(12.7)	41(87.3)
26	22.4	145.0	F	33	4(12.1)	29(87.8)
27	21.6	94.5	M	113	14(12.3)	99(87.6)
28	20.0	104.0	F	108	2(1.8)	106(98.1)
29	18.7	78.5	M	23	9(39.1)	14(60.8)
30	22.0	112.5	M	43	12(27.9)	31(72.0)
31	21.3	96.8	M	26	5(19.2)	21(80.7)
32	18.2	70.8	F	26	10(38.4)	16(61.5)
33	17.2	67.0	F	34	3(8.8)	31(91.1)
34	12.8	20.0	M	5	1(20.0)	4(80.0
35	24.0	141.3	M	25	7(28.0)	18(72.0)
36	19.8	82.5	F	35	4(11.4)	31(88.5)
37	14.1	25.0	M	32	6(18.7)	26(81.2)
38	14.0	24.1	M	4	-	4(100)
39	22.2	112.2	F	75	13(17.4)	62(82.6)
40	11.3	12.2	F	14	4(28.5)	10(74.4)
Total	782	3399.8	-	2312	240(10.4)	2072 (89.6)
Mean	19.6	85.0	-	58	6	52

Echinorhynchus salmonis - The worm occurred in the mean numbers of 21, 5 and 9 worms per fish in the rainbow trout, brown trout and grayling respecitvely. Its distribution in the intestine of fish was similar to that of E. truttae except that E. salmonis was less prevalent.

Cystidicola farionis - The nematode recorded a mean number of 58 worms per fish in rainbow trout, 29 worms per fish in brown trout and 51 worms per fish in grayling.

Neoechinorhynchus rutili - The intensity of infection was low and the mean numbers recorded were 7, 2 and 5 worms per fish in the rainbow trout, brown trout and grayling respecitvely.

Cucullanus truttae - A mean number of 23 worms per fish was recorded in the brown trout whereas in the rainbow trout and grayling the mean number was less than 1.

The level of helminth infection observed in the fish was relatively high. The most heavily infected fish harboured 114 Cyathocephalus truncatus, 132 Echinorhynchus truttae, 47 Echinorhynchus salmonis, 30 Cystidicola farionis and 5 Crepidostomum metoecus. The maximum incidence number of each species of helminth parasites per fish excluding the tapeworm were 203 Echinorhynchus truttae, 37 E. salmonis, 36 Neoechinorhynchus rutili, 47 Cystidicola farionis, 34 Crepidostomum metoecus, 62 Cucullanus truttae.

The examination of a few specimens of other fish species present namely Cottus gobio, Gasterosteus aculeatus and Anguilla anguilla gave negative results except for the presence of small

numbers of Echinorhynchus truttae in some Cottus gobio.

2.4.2. Incidence and intensity of infection in Gammarus pulex.

Cyathocephalus truncatus, together with 5 other species of helminth parasite (Echinorhynchus truttae, Echinorhynchus salmonis, Neoechinorhynchus rutili, Cystidicola farionis and Crepidostomum metoecus) were found to utilize Gammarus pulex as an intermediate host. The juvenile stages of the above helminths as well as an unidentified cestode cysticercoid larva occurred in the examined gammarids.

Infection was usually high in the cold months of December to April and low in the warmer months of May to November. The prevalence of infection of G. pulex with the helminth juveniles reached a maximum level of 12.8% in January 1982 while the lowest level was 1.4% in May 1981 (Table 2.8 and Fig. 2.3) The juvenile stages of all species of the helminth parasites were recorded in the body cavity of Gammarus pulex lying free among the 2 pairs of hepatic caeca and the intestine except for the meta-cercariae of Crepidostomum metoecus which were found to be attached to the hepatic caeca or the external wall of the intestine.

Multiple infections of a single Gammarus pulex with 2 to 4 of the different species of helminth juveniles were common.

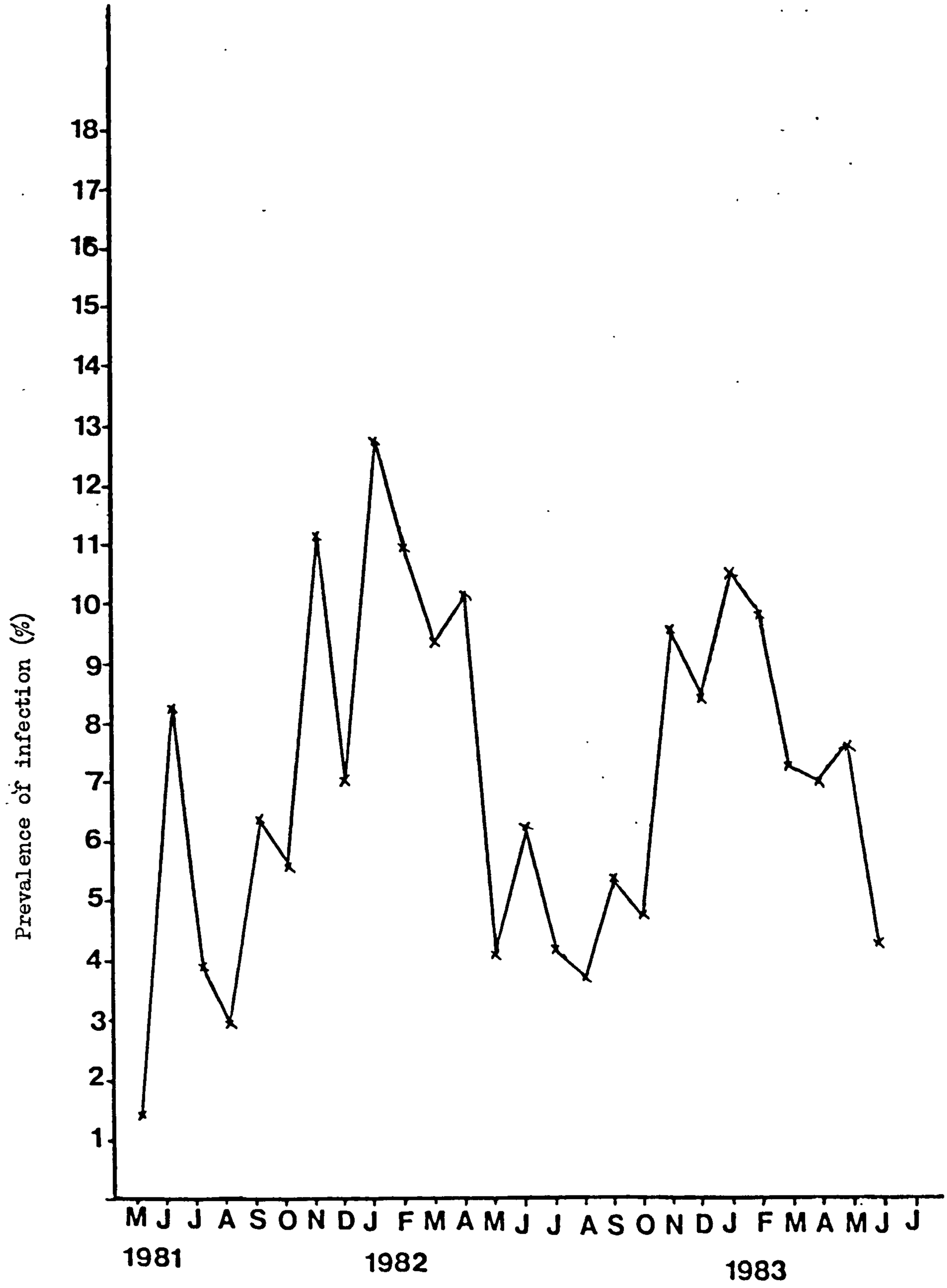
(a) Cyathocephalus truncatus procercoids

The prevalence of C. truncatus procercoids in Gammarus pulex was highest in January 1982 with 2.2.% of gammarids infected and lowest in May 1982 with 0.2%. No infection was recorded in gammarids

Table 2.8: Monthly prevalence of infection by helminth juvenile stages in Gammarus pulex from Driffield trout streams. 2,500 G. pulex examined per month.

Month	Total No. of Gammarus infected	Prevalence of infection (%)	Total No. of worms
May 1981	35	1.4	57
June	207	8.3	227
July	97	3.9	132
August	72	2.9	104
September	162	6.5	200
October	140	5.6	267
November	279	11.2	345
December	176	7.0	280
January 1982	321	12.8	393
February	276	11.0	338
March	234	9.4	281
April	255	10.2	288
May	102	4.1	189
June	157	6.3	253
July	106	4.2	159
August	93	3.7	124
September	135	5.4	207
October	121	4.8	272
November	240	9.6	317
December	211	8.4	229
January 1983	365	10.6	388
February	247	9.9	352
March	182	7.3	274
April	174	7.0	257
May	193	7.7	221
June	208	4.3	226

Fig: 2.3: Monthly prevalence of infections with the juveniles of at least one of the following helminths - *Cyathocephalus truncatus* *Echinorhynchus truttae*, *E. salmonis*, *Cystidicola farionis*, *Crepidostomum metoecus* and *Neoechinorhynchus rutili* in *Gammarus pulex* from Driffield trout streams.
2,500 *G. pulex* examined per month


Prevalence of infection (%)
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
M J J A S O N D J F M A M J J A S O N D J F M A M J J
1981
1982
1983
Months

in May 1981 (Table 2.9 and Fig. 2.4). As expressed above (page 28) the different stages of the procercoid of C. truncatus recovered from G. pulex in each month have been categorised into 3 groups - Stages I, II and III.

Stage I procercoid:- These are less than 2.5mm in length. Structurally they appear pear-shaped or slightly elongated and dark in colour. They lack the scolex and cercomer. This stage was rare and was found only in September 1981 with a prevalence of 0.1% and in September, October , November 1982 and March 1983 with a maximum prevalence of 0.2% in September 1982 (Fig. 2.4(a)). Only 12 such procercoids were recovered from gammarids between May 1981 and June 1983.

Stage II procercoid:- These are between 2mm and 5mm in length and each possess an anterior distinct tubular scolex and a posteriorly placed cercomer. The prevalence of this stage was at its highest, exceeding 0.5% during the period September 1981 to January 1982 inclusive and between October 1982 and February 1983 inclusive. At other periods of the year, the prevalence was low (Fig 2.4 (b)).

Stage III procercoid:- This stage includes specimens from 5mm to about 14mm in length similar in external morphology to the adults in fish except for the possession of a funnel-shaped scolex and the cercomer and is probably the only stage infective to trout. Specimens were found in amphipods throughout the year but most frequently in the colder months of January and February with a maximum prevalence of 1.2% in January 1982. Infected gammarids were least common during the warmer months of April, May and June (Fig. 2.4(c)).

Fig: 2.4 Monthly prevalence of stages I, II and III procercoids of Cyathocephalus truncatus in Gammarus pulex from Driffield trout streams between May 1981 and June 1983.
2500 G. pulex examined per month.

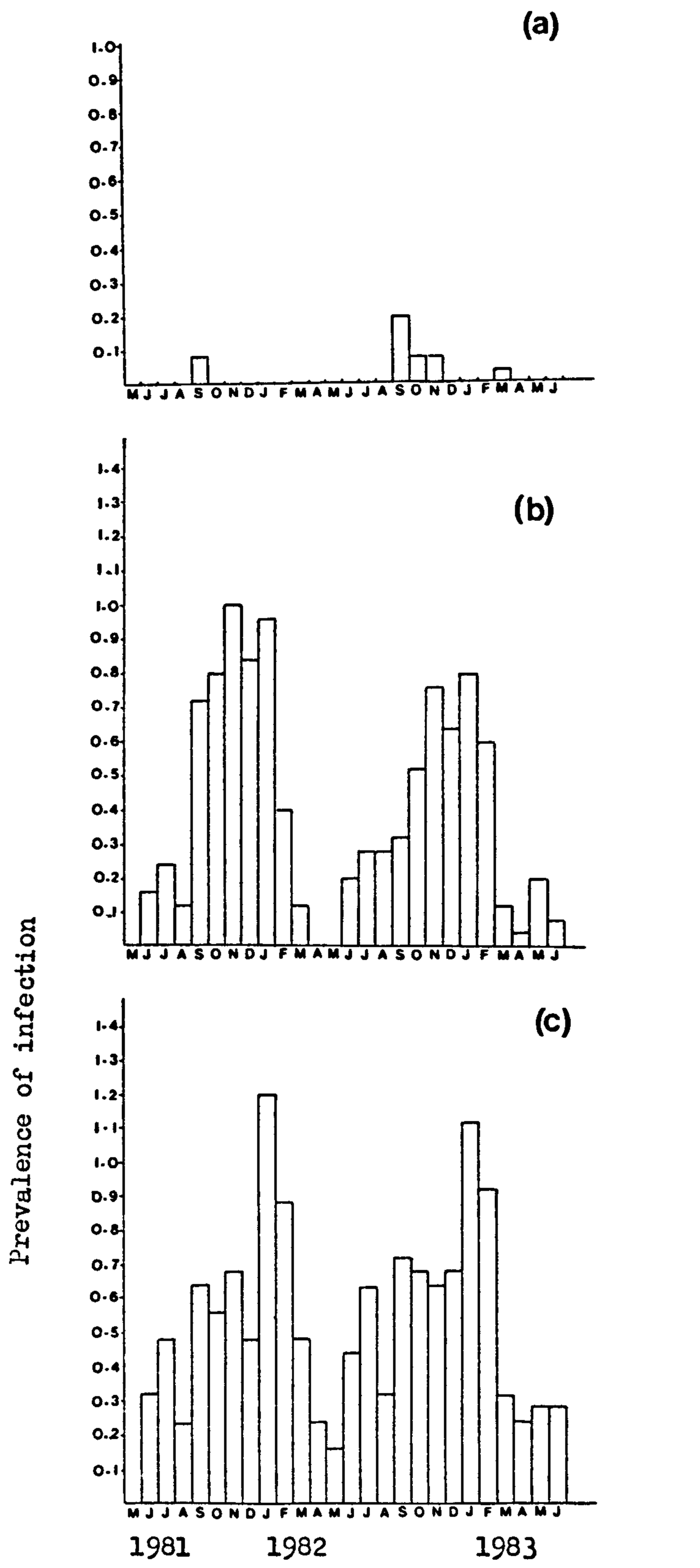
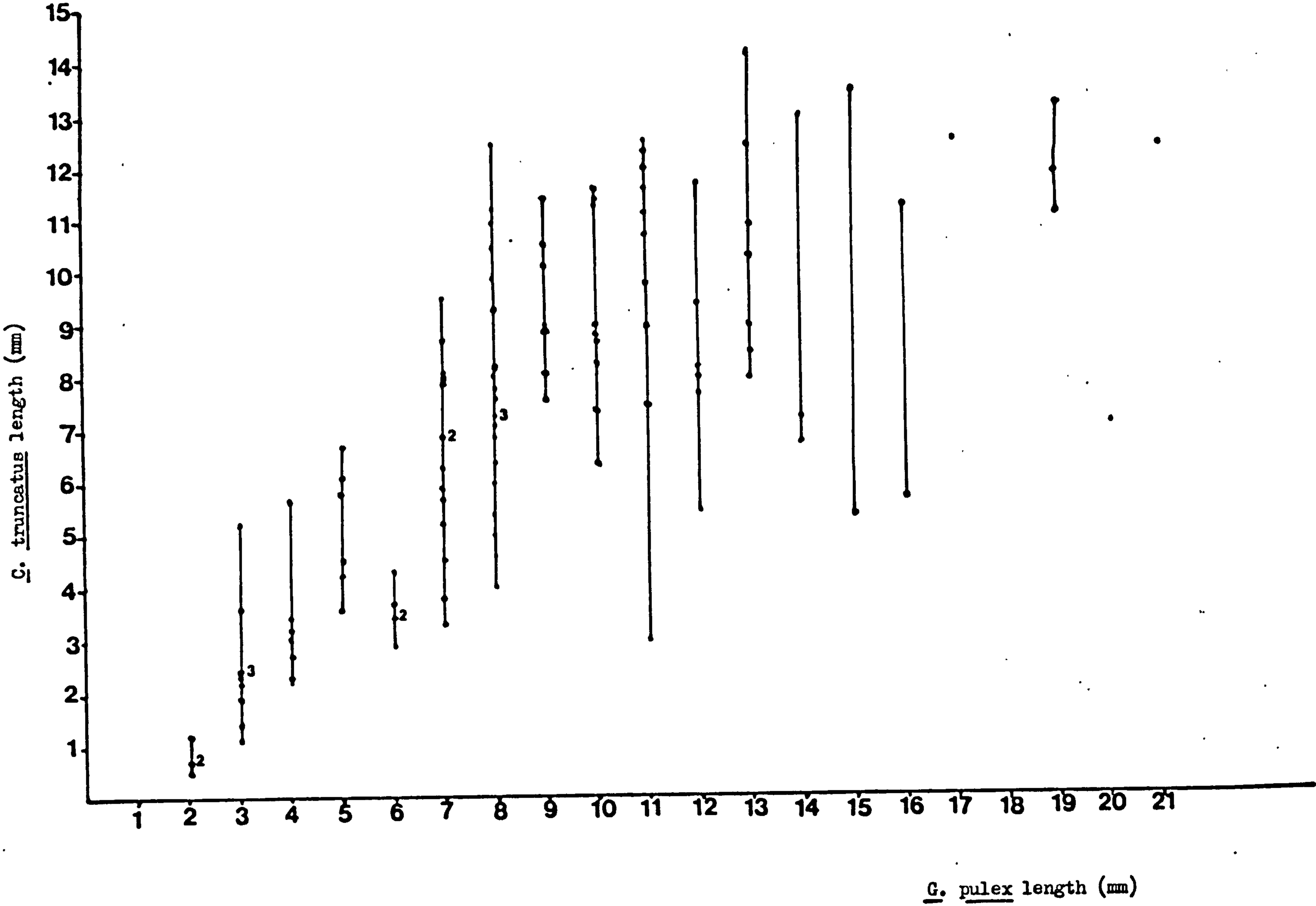

(a)
(b)
(c)
Prevalence of infection
M J J A S O N D J F M A M J J A S O N D J F M A M J
1981
1982
1983
Months

Fig: 2.5 The relationship between the length of C. truncatus procercoids and the host Gammarus pulex


C. truncatus length (mm)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
2
3
2
3
2
2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
G. pulex length (mm)

The Stage I procercoid was found mostly in small specimens of G. pulex approximately 3mm to 5mm in length; Stage II usually in gammarids 5mm to 7mm long and Stage III usually in gammarids measuring 5mm and above in length. The approximate distribution of the procercoid stages in different sizes of Gammarus pulex shown in Fig. 2.5 indicates that gammarids are only capable of being infected when young. It also indicates that there is little increase in length of the tapeworm procercoid as it reaches 12mm size.

Occasionally, infected gammarids harboured two tapeworms but most frequently only a single individual was present. Multiple infections of a single amphipod with the tapeworm and up to two of the other species of helminth juveniles were also frequently seen.

(b) Other helminth parasites

The prevalences of all helminth larvae in samples of 2,500 Gammarus pulex examined monthly between May 1981 and June 1983 are shown in Table 2.9.

Echinorhynchus truttae juveniles were more frequent in gammarids than any other helminth parasite, including C. truncatus. They showed their highest occurrence in the period January to June, inclusive with a maximum prevalence of 4.2% (104 gammarids infected) in March and April 1982. The two most conspicuous stages were the orange-coloured encysted cystacanths and the pink coloured excysted juvenile stage. The encysted cystacanths were most common between March and June (maximum prevalence of 2.6% [64 gammarids infected] in June 1981) while the encysted juveniles stage was most frequent between September and April, with a maximum prevalence of 2.7% (67 gammarids

infected) in February 1982 (Table 2.10). The number of E. truttae larvae per amphipod ranged between 1 and 4. Multiple infections of gammarids with this species and other helminth larvae were common.

Echinorhynchus salmonis juveniles occurred as white coloured encysted cystacanth and excysted juvenile stage in Gammarus pulex. Its prevalence was 1.6% (40 gammarids infected) at its peak in March 1982. The period of highest infection of amphipods with E. salmonis was between January and June. Although most infection consisted of single individuals a few gammarids harboured 2 specimens.

Neoechinorhynchus rutili juveniles found in gammarids were not encysted and were similar to the adults found in fish except that the proboscis was usually withdrawn. No cystacanth stage occurred. The prevalence was low and worms were most frequently found between October and March with the highest incidence of 1.2% (31 gammarids infected) in January 1982. Only single infection were recorded.

Cystidicola farionis larvae were most common between September and March with a peak prevalence of 3.2% (79 gammarids infected) in November 1981. Most infections consisted of single worms per gammarid but on a few cases, 2 or 3 worms were recorded.

Crepidostomum metoecus metacercaria were frequently seen in gammarids between October and April with a peak of 3.4% (84 gammarids infected) in January 1983. Number per gammarid ranged between 1 and 15.

Individuals of an unidentified species of cestode cysticercoid larva found in gammarids appear cyclophyllidean in type. Their

TABLE 2.9: Monthly prevalence of different species of helminth juvenile stages in Gammarus pulex from Driffield trout streams. 2500 G. pulex examined per month

Month	C. truncatus		E. truttae		E. salmonis		N. rutili		Cy. farionis		Metacercaria of Cr. metoecus		Unidentified cestode cysticercoid	
	No. of Gammarus infected	No. of Worms	No. of Gammarus infected	No. of Worms	No. of Gammarus infected	No. of Worms	No. of Gammarus infected	No. of worms	No. of Gammarus infected	No. of worms	No. of Gammarus infected	No. of Worms	No. of Gammarus infected	No. of worms
1981														
May	-	-	15	15	6	6	3	3	6	6	17	27	-	-
June	12	12	82	84	26	26	15	15	46	46	35	42	1	2
July	18	18	20	27	9	11	11	11	25	27	20	34	1	4
August	9	9	17	24	11	13	9	9	15	21	12	28	-	-
September	36	36	24	36	14	14	18	18	40	48	37	41	2	6
October	34	34	36	48	10	18	20	20	43	50	43	74	10	23
November	42	42	64	64	21	21	24	24	79	80	49	103	3	11
December	33	33	37	45	11	12	27	27	43	54	47	92	4	17
1982														
January	54	54	86	90	38	38	31	31	69	70	46	81	17	29
February	32	32	91	96	40	42	22	22	50	56	37	67	14	23
March	15	15	104	114	40	47	6	6	27	27	49	62	3	10
April	6	6	104	110	43	43	7	7	49	56	45	59	2	7
May	4	4	47	52	23	33	7	7	35	39	27	42	2	12
June	16	16	48	63	24	30	12	12	31	41	43	86	1	5
July	23	23	28	35	13	18	9	9	32	37	28	35	1	2
August	15	15	17	22	16	16	4	4	24	29	18	32	2	6
September	30	31	31	45	18	24	7	7	33	36	31	45	4	19
October	32	32	50	61	17	27	13	13	45	48	45	64	8	27
November	37	37	57	61	17	17	16	16	71	75	52	95	3	16
December	33	33	36	36	6	6	14	14	42	42	76	83	4	15
1983														
January	47	48	93	93	26	26	17	17	67	67	84	86	36	51
February	38	38	98	98	32	32	23	23	58	62	51	63	14	18
March	11	11	101	107	38	39	15	15	34	34	48	54	10	14
April	7	7	91	102	31	31	9	9	46	46	46	52	8	10
May	13	13	64	64	38	38	11	11	35	35	41	43	11	17
June	9	9	67	67	23	23	10	10	28	28	58	63	15	26

Table 2.10 Monthly occurrence of Echinorhynchus truttae juvenile stages in Gammarus pulex from Driffield trout streams. 2,500 G. pulex examined per month.

	Month	No. (prevalence) of infected gammarids	Total No. of Echinorhynchus truttae	ENCYSTED. CYSTACANTH		EXCYSTED JUVENILES	
				No. of Gammarus infected	No. of Worms	No. of Gammarus infected	No. of Worms
1	May 1981	15 (0.6)	15	11	11	4	4
2	June	82 (3.3)	84	64	66	18	18
3	July	20 (0.8)	27	14	21	6	6
4	August	17 (0.7)	24	9	16	8	8
5	September	24 (1.0)	36	10	19	14	17
6	October	36 (1.4)	48	16	25	20	23
7	November	64 (2.6)	64	21	21	43	43
8	December	37 (1.5)	45	12	14	25	31
9	January 1982	86 (3.4)	90	36	38	50	52
10	February	91 (3.6)	96	24	24	67	72
11	March	104 (4.2)	114	50	55	54	59
12	April	104 (4.2)	110	48	50	56	60
13	May	47 (1.9)	52	28	29	19	23
14	June	48 (1.9)	63	29	38	19	25
15	July	28 (1.1)	35	15	19	13	16
16	August	17 (0.7)	22	10	13	7	9

occurrence (Table 2.9) was highest in the cold months with a prevalence peak of 1.4% (36 gammarids infected) in January 1983. Numbers ranged between 1 and 10 per infected gammarid. These larvae were not infective when infected gammarids were fed to laboratory maintained brown trout.

2.5 Discussion

It is somewhat unusual for a parasite species to be found in every host individual examined but this has been the case with Cyathocephalus truncatus as well as with Echinorhynchus truttae, Echinorhynchus salmonis and Cystidicola farionis in rainbow trout, brown trout and grayling examined during the present investigation. A similar situation was also reported for C. truncatus by Wisniewski (1932b) from river Bosnia, Yugoslavia although he found a maximum of 400 worms per fish while in Driffield (for the present study) the maximum number of worms per fish was 117.

The ecology of the area investigated in the present study is similar to that of other environments in which C. truncatus occurrences have been studied (see Wolf 1906; Wisniewski 1932a, 1932b; Senk 1952; Vik 1954, 1958). The abundance of the intermediate host, Gammarus pulex and the fact that the amphipod appears to form the major food item of the fish in Driffield trout streams ensures the continuity of parasitic infections in the fishery. This is in spite of a relatively low level of prevalence of larval stages in gammarids. With a maximum tapeworm prevalence of 2.2% in Gammarus pulex, fish thus have to feed on large numbers of amphipods to become heavily infected - for example to reach

a total of 43 worms - the mean per fish recorded in rainbow trout - the fish would have to feed on about 2,250 gammarids, even if there were no losses of worms following the ingestion of procercoids by the fish.

Cyathocephalus truncatus appears to use amphipods as intermediate hosts almost exclusively. Although the report on occurrence of the procercoid in Mysis relica (see Amin, 1977) concerned only a single worm in a single infection it however showed that C. truncatus may also use other crustacean species as intermediate host. In the present investigation a critical study was not made experimentally to observe whether freshwater isopods or other species of crustacean could be hosts. This was due to the low rate of materials available and the fact that attention was given to other aspects of the tapeworms biology. A critical examination of other crustacean and planktonic organisms present in the area of collection is necessary to investigate the possible occurrence of C. truncatus procercoids in them.

C. truncatus however shows the ability to infect a wide range of fish species of various families. From available records the tapeworm occurs in fish of 11 Families belonging to 7 orders of the Class Pisces as shown below:

Order	Family	Example
Isospondyli	Salmonidae	Salmo trutta
	Coregonidae	Coregonus clupeaformis
	Thymallidae	Thymallus thymallus
Haplomi	Esocidae	Esox lucius
Ostariophysi	Cyprinidae	Tinca tinca
Anacanthini	Gradidae	Lota lota
Percomorphi	Pecidae	Perca fluviatilis
	Anarhichadidae	Luceoperca sandra
Scleroparei	Cottidae	Cottus gobio
	Gasterostidae	Gasterosteus aculeatus
Apodes	Anguillidae	Anguilla anguilla

The wide range of host specificity demonstrated by C. truncatus suggests that other fish species in the orders and families listed above but not yet known to harbour C. truncatus may possibly become infected when fed on amphipods harbouring the procercoid of the tapeworm.

The tapeworm has been found to establish remarkably well and in large numbers in host species of the order Isospondyli (families Salmonidae, Coregonidae and Thymallidae).

The heaviest infections recorded so far have been on species in the family Salmonidae - the maximum numbers recorded in single specimens of Salmo alpinus were 200 and 400 by Huitfeldt-Kaas(1927) and Wisniewski(1932a,b) respectively.

Additionally, fish species of the family Salmonidae have been found to be suitable for experimental infections (Vik, 1958)) and Chapter 3 of the present report) although other fish species have not been subjected to experimental infections.

Members of other genera for example Cottus and Anguilla appear to be less suitable as hosts for the tapeworm. In Cottus gobio only a few individuals have been recorded and in a particular case (Awachie, 1966a)were found to be uninfected whereas Salmo trutta occurring in the same water harboured the tapeworms. Vik (1954) reported that tapeworms recovered from Anguilla vulgaris were unattached and are evidently being voided with fish faeces. It is difficult to access the suitability of these two fish species as hosts of C. truncatus and it is possible that such information may be gained only by further experiments.

In the rainbow trout examined during the present study the tapeworms were most frequent in the middle sized fish (18:1cm to 26.0cm) than in smaller or larger individuals. This may be due to difference in food and feeding habits of the small and large sized fish from those of the middle sized fish. It may also be that they develop some form of resistance making them less susceptible to infection. Some large fish that are heavily infected may also die and are not represented in collections. Although there was no report of death to fish due to tapeworm infection, in the present study, Huitfeldt-Kaas (1927) and Wisniewski (1932a,b) reported that the tapeworm infection does result in fish mortality.

Observations on the establishment of the tapeworm sequentially in the dorsal caeca, the anterior ventral caeca then the posterior ventral caeca and the proximal portion of the small intestine of fish is similar to that reported in trout by Halvorsen and Macdonald (1972). While the reason for such an apparent preference for site of attachment is not fully known, these authors suggested that it could possibly be due to the fact that the bile duct opens through the region of the dorsal caeca into the intestine. Bile has been found to encourage the movement of cestode larvae (Smyth, 1969) and is important for their growth and development (Evans and Ryche, 1969; Smyth, 1969). Halvorsen and Macdonald(1972) also cited Williams and Halvorsen (1971) who reported that in cod, the cestode Abothrium gadi was found to attach preferentially in one of the two pyloric caeca nearest to the entrance of the bile duct. Although the effect of bile was not thoroughly investigated in the present study the fact that the tapeworms also establish successfully in the anterior ventral caeca posterior ventral caeca and in the proximal portion of the small intestine where there is no bile opening, suggests that the presence of bile may not be the only factor determining the attachment of C. truncatus in the pyloric caeca. It may be simply that the dorsal caeca and the anterior most ventral caeca are the regions where the tapeworms arrive first after being released from the disintegrating body of the G. pulex

intermediate host in the pyloric stomach. It is clear that the pyloric caeca constitute naturally suitable sites in which the tapeworms may obtain a firm attachment from which dislodgement is minimal.

The occurence of free, apparently moribund, tapeworms in the intestine still with a mass of host tissue lodged in the scolex possibly indicates that the tapeworm does not ever release its grip or change its site of attachment. Thus such worms are incapable of re-establishing and are voided along with the fish faeces.

The occurence of C. truncatus in the body cavity of the fish host noted by the present author was also reported by Vik (1954). He stated that worms found in this region are those that have penetrated through the caecal wall to which they were originally attached. A similar observation was made in the present study and additionally, damaged caeca which lack distal tips (point of attachment through the erosion of which some worms apparently move into the body cavity) were frequently seen. Some heavily infected fish with worms in the body cavity and abdominal musculature were examined histologically and the observations are presented below (page 189).

Worms lying free or attached in the body cavity of fish, except those found dead and usually enclosed in a cyst-like

capsule could possibly establish in larger fish preying upon already infected fish although this was not investigated. This may possibly be a means of dissemination of infection accounting for transmission of some C. truncatus to large trout or to other species such as pike. On the other hand such individual worms may not,as pointed out above,be able to reattach even if presented with the opportunity to do so.

The high rate of occurrence of other helminth parasites inhabiting the alimentary tract along with the tapeworm is noteworthy. Negative interraction is known to exist between two parasite species occurring simultaneously resulting in site displacement or exceptionally competitive exclusion. As reviewed by Kennedy (1983) interactive segregation resulting in site displacement of a cestode seems to be a more widespread feature of concurrent infections of cestodes and parasites of other groups especially acanthocephalans and nematodes rather than of concurrent infections involving cestodes only. Several authors including Chappell (1969) and Amin (1975) have reported on anterior displacement and partial segregation of tapeworm populations in concurrent infections of fish with tapeworms and acanthocephalans. In the present study there appears to be an overlap of site between the tapeworm and acanthocephalans although the tapeworm tend to group together in the pyloric caeca where the prevalence of thorny-headed worms are relatively low. The distribution of acanthocephalans - few and scattered in the caeca and proximal portion of small intestine but tending to

accumulate in the distal portion of the small intestine - appears in the present investigation as a normal attribute of the group rather than a reaction to the universal presence of Cyathocephalus truncatus. Individuals of Echinorhynchus truttae in single species infection are known to move gradually and take up a more posteriorly-placed sites in the small intestine with the development of maturity (Awachie, 1965).

Infections with the trematode Crepidostomum metoecus were located in the posterior ventral caeca and in the anterior portion of the small intestine. Since all the fish examined had C. truncatus infection with the tapeworm occupying in particular the anterior pyloric caeca, it is not known whether the trematode would prefer the anterior caeca in single species infections as reported by Halvorsen and Macdonald (1972).

The relative numbers of helminth parasites in fish recorded in the present investigation do not absolutely reflect the situation in the river in view of the small numbers of fish hosts. More rainbow trout were examined because they were more readily available by the Angling Club than brown trout while grayling were in any case less numerous.

Although the overall prevalence of infection with the procercoids of C. truncatus in Gammarus pulex was low throughout the year, there seems to be a seasonal pattern of occurrence of procercoids in the amphipods - high in the colder months and low in the warmer months. A similar trend in both fish and amphipod hosts was reported by Awachie (1966a).

Young procercoids of C. truncatus were found only in young specimens of Gammarus pulex with the infective procercoids of the tapeworm in older gammarids. It thus seems that amphipods are infected when they are young and both tapeworm and amphipod grow together until the tapeworm reaches the infective stage. Wisniewski (1932b) earlier suggested a similar trend adding that young gammarids appear more susceptible to infection while the older ones are somewhat resistant to infection.

Since the majority of infections with Stages I and II procercoids recorded between September and November occurred in small size specimens of amphipods, probably the young ones, it is most likely that the gammarids become infected mainly in the spring and summer months, harbouring the growing procercoids until the winter months when they become the Stage III procercoid. The latter stage is relatively more common during the winter months than at any other period of the year and thus coincides with the above supposition. The occurrence of Stage III procercoids in amphipods throughout the year is probably due to their persistence in ageing gammarids. Wolf (1906), Janicki (1928) and Wisniewski (1932b) stated that amphipods were capable of tolerating the infection and carrying it for over 1 year.

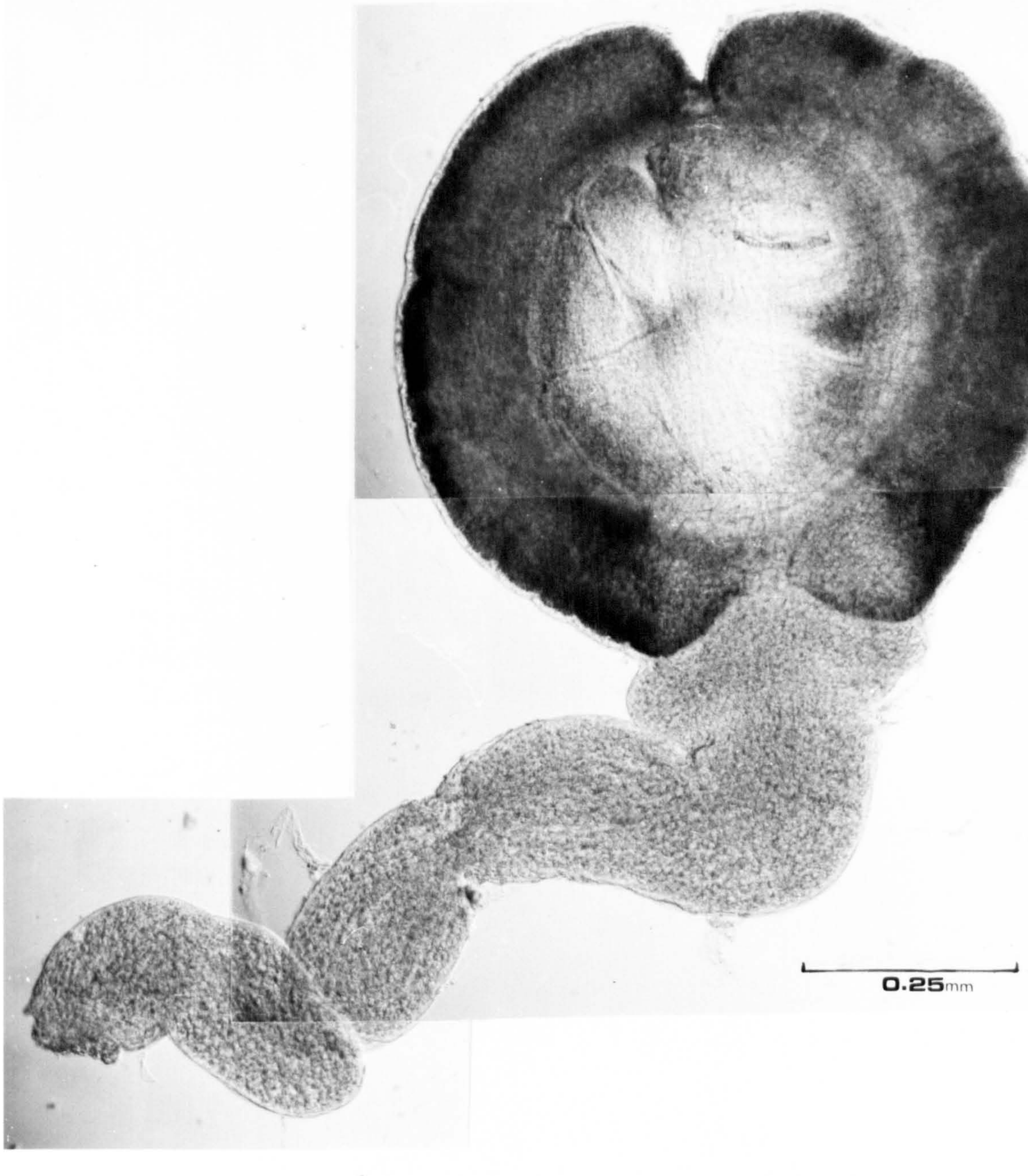
The comparative rarity of C. truncatus in amphipods but not in fish as might be expected cannot easily be explained. The possibility that interspecific competition with other helminth

parasites utilizing G. pulex as intermediate host may be responsible for the very low occurrence of the tapeworm in amphipods is ruled out by the observation that concurrent infection with the juveniles of other helminth parasites occur in nature. The low prevalence in G. pulex could even be due to possible death of some amphipods resulting from the infection.

There is evidently a similar seasonal pattern of occurrence of other helminth juvenile stages in Gammarus pulex to that of the tapeworm in the present study.

Fluctuations in the percentage infection of G. pulex with acanthocephalans appear seasonal in character similar to that reported by Awachie (1965)at Afon Terrig, Wales. However seasonal cycle could not be confirmed for the acanthocephalans since cystacanth and excysted larval stages were found to overlap in occurrence throughout the year. Also the occurrence of Crepidostomum metoecus metacercaria in G. pulex agree with the reports of Baylis (1931) and Awachie (1968).

Plate 2.1 Unidentified cestode cysticercoid larva in Gammarus pulex from Driffield trout streams. (Unstained) Scale - 0.25mm.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CHAPTER 3

THE LIFE CYCLE OF *CYATHOCEPHALUS TRUNCATUS*

3.1 Introduction

The life cycle of Cyathocephalus truncatus has been reported to involve certain species of fish as primary hosts and crustacea, usually amphipods, as intermediate hosts (see Chapter 1). The stages of development of the procercoid in gammarids, although originally described by Wisniewski (1932), have not yet been experimentally verified and established. Also the true structure of the infective embryo — whether it is a hexacanth embryo as described by Gauthier (1923) or an embryo lacking larval hooks as described by Wisniewski (1932)–still remains to be elucidated.

In the present study the processes of development of the egg structure of the oncosphere, mode of infection of the amphipod and stages of development of the embryo within the amphipod have been investigated experimentally in the laboratory, and the results discussed. Data on experimental infections in fish are also presented.

3.2 Literature review

As stated in Chapter 1, early reports on C. truncatus life-cycle dealt only with the incidence of the procercoid in the crustacean intermediate host. It was Gauthier (1923) who first tried to establish the life cycle under experimental condition. She described a hexacanth embryo which subsequently developed within the cultured egg capsule but did not succeed in infecting amphipods with it. No further details of experimental work were provided.

Wisniewski (1932) conducted a similar attempt and reported that it took about 35 days for the embryo in freshly laid eggs cultured in freshwater to develop to the infective stage. He further stated that eggs developed better when cultured in fish faeces. He described the developed embryo as lacking hooks and swimming organelles like cilia but appearing somewhat colourless or jelly-like within the egg capsule. Wisniewski stated that although operculated, the eggs did not hatch in water at any time to release the infective embryos. Although he did not succeed in infecting amphipods with the cultured eggs he believed that they required to be swallowed by the gammarids for infection to take place. He disagreed with Gauthier's (1923) description of the embryo as being a hexacanth and stated that Gauthier probably used a different species and not Cyathocephalus truncatus.

Wisniewski (1932b) described the developmental stages of the tapeworm he retrieved from the intermediate hosts Fontogammarus bosniacus and Rivulogammarus spinicaudatus. He also reported that within the gammarid host, the procercoid had developed clearly recognisable sexual organs and suggested that the worms may possibly attain maturiy in the intermediate host. He stated that gammarids usually produce a capsule around the worm and although the worms could free themselves from the capsules, the procercoids sometimes died within them.

No other report is available on attempts to infect amphipods with Cyathocephalus truncatus eggs. Sandeman and Burt (1972) dealing with the life cycle of Bothrimonus (= Diplocotyle) sturionis, which bears a close affinity to C. truncatus in the order Spathebothridea, described the operculated eggs and the infective non-ciliated hexacanth larvae. They stated that as in C. truncatus, eggs containing developed embryos did not hatch in water to release the oncospheres and that infection was certainly by ingestion of the eggs by gammarids. The authors were unsuccessful in attempts to infect gammarids but thought that this was due to the fact that amphipods probably crush the eggs with their powerful mandibles while feeding thereby destroying the oncospheres. Additionally they thought that gammarids appear somewhat resistant to infection.

Sandeman and Burt (1972) comparing the procercoid of Bothrimonus sturionis with that of Cyathocephalus truncatus described by Wisniewski (1932a,b) stated that B. sturionus procercoid attains sexual maturity within the gammarid host accompanied by the production of apparently normal eggs. They also reported on the presence of eggs in the uterus of a specimen of Cyathocephalus truncatus obtained from a gammarid by Mr. B. H. Hall of the University of Leicester, U.K., in the March of that year. The authors concluded that this progenetic development appeared to be characteristic among members of the order Spathebothridea.

The apparent maturity of C. truncatus procercoid in the intermediate host was again highlighted by Amin (1978) who recorded the production of eggs "which appeared viable" by gravid genital organs of the procercoid in the body cavity of the amphipod, Pontoporeia affinis. He raised the possibility that the fish host may not be necessary for the completion of C. truncatus lifecycle and that maturation in gammarids could also serve for the dissemination of infection.

Vik (1958) brought about experimental infection of fish by feeding them on infected Gammarus lacustris carrying Cyathocephalus truncatus procercoids. He reported that the worms matured and produced eggs 10 days after infection of fish and that worms could remain in the fish intestinal tract for up to 55 days. Apart from this report no experimental infection of fish has been published.

The affinities of C. truncatus and indeed of other tapeworms in the order Spathebothridea have not been conclusively ascertained. Wisniewski (1932) however stated that the tapeworm compares closely with Archigetes siebioldi (Cestoda Caryophyllaeidea) in its life-cycle adding that C. truncatus is a neotenic procercoid.

Species of Archigetes are known to become sexually mature in Oligochaetes (the original intermediate hosts). (Wisniewski, 1930; Calentine, 1964, 1965; Kennedy, 1965). This precocious development appear similar to that reported for C. truncatus procercoid by Wisniewski, (1932b) and Amin, (1978) as already stated in this review.

3.3 Materials and Methods

Eggs used for the study of the life cycle were obtained from two sources. Some were obtained from naturally infected fish - they were shed with fish faecal matter from which they could be separated by teasing them apart with the aid of needles under a dissecting microscope. Others were deposited by adult worms in 0.9% saline following the removal of the worm from the fish hosts.

The eggs were cultured in a petri-dish in about 0.5 cm depth of freshwater. The eggs were constantly checked and the freshwater replaced every other day. Experimental containers were left open to aid aeration. The above steps were very important as eggs cultured in deep water or in closed containers died soon and did not develop.

To investigate optimal conditions for development of embryo egg cultures were maintained at 0°C, 6°C, 12°C, 15°C, 20°C and 25°C. The success rates were expressed in percentages according to the proportion of eggs containing hexacanth embryos.

3.3.1 Infection of Gammarus pulex

Gammarus pulex for experimental use were collected from Loadpit Beck, Shipley Glen, Baildon near Leeds, an area where no helminth infections are known to occur. Initially, 3 groups of amphipods were used - those exceeding 4.9mm in length, those between 2mm and 4.9mm long and those less than 2mm long. In obtaining the latter size group, females carrying a brood were isolated in separate containers and left for a day or two until the young were released. Then they in turn were separated from the parent female. This group of young amphipods were used for subsequent experimental infections. Experiments were conducted in clear plastic containers measuring 17 cm long, 11cm wide and 5cm deep.

The method of infecting the amphipods was a slight modification of the technique of Hynes and Nicholas (1957). It involved placing eggs previously cultured to the infective stage either on vegetation collected along with Gammarus pulex or on pieces of fresh lettuce leaves so that when immersed in water the eggs were available to the feeding amphipods. The use of lettuce proved to be more successful in inducing the gammarids to feed and therefore was adopted in later experiments as the standard method.

Twenty experimental Gammarus pulex were introduced into each container half filled with freshwater. Pieces of leaves were immersed in the water and infective eggs were gently pipetted on them. An estimated number of about 1000 eggs were placed into the container. This was found to be ideal and

ensured that majority of the eggs remain available on the lettuce for the feeding G. pulex. The container was left undisturbed overnight or for 24 hours during which time the gammarids fed on the lettuce leaves and ingested some of the eggs. The amphipods were then removed and returned to labelled containers with fresh lettuce until required for examination.

3.3.2 Infection of Salmo trutta

Hatchery bred brown trout usually 10 cm to 14 cm in length collected from Washburn Valley trout farm, Otley and Kilnsey Park Hatchery Cottage at Kilnsey near Leeds, were used for experimental infection. Two methods were used for infecting the fish with the tapeworm procercoids.

1. The experimental fish was lightly anaesthetized with MS222 of concentration 1g per 15 litres of water. A procercoid of C. truncatus teased out of an infected Gammarus pulex was pipetted into the oesophagus and washed down into the stomach of the fish which was then allowed to recover in clean aerated water.

2. This method involved feeding fish with infected Gammarus pulex. Although the percentage of infected gammarids in the field was low it was relatively easy to detect and isolate infected individuals from groups examined in a dish. Infected amphipods were easily recognised since the cestode larvae can be seen through the body wall of intact gammarids.

Experimental fish were left to acclimatize in their tanks for about 2 days and were fed constantly with non-infected Gammarus pulex. Fish were maintained in numbers of 2 or 3 per tank since single fish kept in a tank showed little interest in feeding even when starved. When feeding Gammarus pulex to fish small numbers (20 to 30) of the amphipods were placed in the tank at frequent intervals. The fish tended to become very active and dashed around the tank looking for the amphipods, thus ensuring that all gammarids were rapidly consumed. Infected G. pulex were fed to the fish as follows:-

Eight tanks each containing 2 fish were used - 16 fish in all. In each tank 12 infected G. pulex were fed to the fish which were then fed everyday with uninfected gammarids until they were examined. One fish selected from those in tanks 1 to 5 was examined each day for 10 days while one fish from those in tanks 6 to 8 was examined at 5 day intervals starting from 15 days after infection. Faecal matter of fish was collected from all the tanks daily from the day fish were fed with infected G. pulex so as to know when tapeworm eggs first appeared in the fish faeces.

3.3.3 Microscopy and measurements

Whole mount preparations were made using Borax Carmine or Hematoxylin stains as stated above (page 28).

The eggs and the early procercoids in Gammarus pulex were examined unstained. Sizes of eggs and procercoids were measured under high power magnification with the light microscope.

3.3.4 Terminology

The terms used in the present account conform with those used by Stunkard (1983) and Wardle and McLeod (1952).

In Cyathocephalus truncatus, the linear sets of genital organs are not separated by partitions and therefore are not properly described as proglottides. For this reason it is thought appropriate in the present work to refer to each set of genital structures as genital areas.

The term "larva" which is used to describe the early stage of development of some worms has been avoided in the present study. This is because a larva by definition is very distinctly different from the sexually mature adults in form and require to metamorphose to become the adult. The helminth parasites found in Gammarus pulex in the present study were in some of their stages (especially the infective stages) similar morphologically to the adult stage in fish. They are therefore being referred to as juvenile stages in the present account.

As in previous reports, C. truncatus retrieved from Gammarus pulex has been referred to as procercoid in the present report.

3.4 Results

3.4.1 Egg production

Eggs egested with fish faeces occur in clusters embedded in a colourless mucus string within which they can be separated with the aid of needles and cultured in isolation from other faecal matter including undigested food items.

Adult tapeworms placed in 0.9% saline produce their first eggs within about 3 hours. The eggs cluster together around the female genital opening to which they retain attachment by means of a mucus-like string. Later eggs tend to push earlier egg clusters further away from the female genital opening. Eggs continue to be released for at least 7 hours and as time progresses, the rate of egg production decreases ceasing after about 14h. Eggs number between 12 and 50 (mean 30) per female genital opening all along the strobila (Table 3.1).

There were no observed differences between the number and structure of eggs produced in the anterior genital areas and those produced in the posterior ones. The first group of eggs laid were light brown in colour and very similar to eggs released with fish faeces but later ones did not possess any colouration possibly because any tanning process has not proceeded very far.

The total number of eggs laid per tapeworm depends on the total number of genital areas which range between 7 and 45 in *Cyathocephalus truncatus* (Amin, 1977).

Forty-three was the highest number of genital areas seen in an individual tapeworm during the present study.

Table 3.1; Numbers of eggs laid by individual C. truncatus in 0.9% saline.

Worm Number	No. of genital areas	Total No. of eggs laid	Mean no. of eggs per vagino-uterine opening
1	30	843	28
2	12	372	31
3	9	307	34
4	15	486	32
5	36	1193	33
6	17	427	25
7	21	632	30
8	24	736	30
9	27	813	30
10	33	1159	35
11	31	1003	32
12	22	724	33
13	18	583	32
14	29	988	34
15	38	1178	31
16	16	483	30
17	25	775	31
18	43	1216	28
19	23	739	32
20	36	1126	31
21	28	870	31
22	34	1023	30
23	37	1373	37
24	42	1346	32
25	13	402	31
Total	659	20797	751
Mean	26	832	30

3.4.2 The Egg

The egg capsule is ovoid,asymmetrically operculated at one pole and bearing a protrusion at the other (Fig 3.1A & Plate 3.1) The operculum is hard to see in freshly laid eggs. The capsules vary between 0.044mm and 0.052mm with a mean of 0.049mm in length and between 0.030mm and 0.040mm with a mean of 0.035mm in width (Table 3.2).

The embryo of the freshly laid egg is not differentiated and does not possess any colouration but is surrounded by yolk cells which are large and slightly dark in colour. The embryo is usually borne at the centre of the egg masked by the yolk cells or may be situated slightly to one side of the egg capsule always near the pole with the protuberance.

The egg capsule is surrounded by a thin and sticky film to which under natural conditions small faecal particles attach; additionally the film aids the adhesion of one egg to another causing eggs to appear in clusters as seen both in eggs produced by adult tapeworms in 0.9% saline and those shed with fish faeces. The egg capsules appear rigid and do not alter in size whether cultured or not; apparently there is no size difference between a freshly laid egg and that with a hexacanth embryo (Table 3.2).

3.4.3 Development of the embryo

Embryos developed in eggs which were cultured at temperatures of 12°C, 15°C and 20°C. Those cultured at 0°C, 6°C and 25°C did not develop; the contents of the capsule changed after 3 days of culture to a jelly-like mass - a state in which they remained throughout 40 days of observation as well as for a further period of 30 days when the temperature was later changed to one within the range 12°C to 20°C.

The phases of development of eggs cultured at 20°C is presented in Fig. 3.1. There was considerable variation in the speed of development of individual embryos; therefore the period stated here for each phase of development to occur is that required for approximately 50% of embryos to reach that phase.

Phase 1 is that of a freshly laid egg described above.

Phase 2 - The yolk cells are no longer distinguishable individually after 3 days but appear to have fused together. The embryo possesses a distinct central dark region termed the endodermal region by Wardle and McLeod (1952); the inner region is surrounded by a clear outer region termed the ectodermal region. Cells that probably constitute these inner and outer regions could not be identified individually at any time. The embryo is distinguishable from the yolk material within the egg capsule.

Phase 3 - this is seen about the twelfth day (Fig 3.1C) the endodermal region of the embryo begins to increase in size. It is at this stage that the operculum becomes very clearly visible.

Phase 4 - at about 15 days of culture, the embryo is seen to have increased in size reducing the apparent volume of the yolk material within the egg capsule. (Fig 3.1D) The inner region of the embryo becomes less dark in colour.

Phase 5 - After 19 to 20 days of culture the inner region of the embryo can barely be identified most of the space within the egg capsule becomes apparently occupied by the developing embryo which shows some weak movements.

Phase 6 - At 21 days, the embryo is still not distinctly marked out from remains of yolk but hooks can be seen in some eggs although partially masked by other material within the egg capsule.

Phase 7 - The hexacanth embryo is seen distinctly in some eggs at 22 days. The 6 hooks are very clearly marked out and additionally a pair of dark bodies is always present clear of the embryo and located at the edges of the capsule among the debris of yolk remains (Fig 3.1G and Plate 3.2).The embryo at this stage is the fully developed oncosphere infective to gammarids. It measures in length between 0.030mm and 0.036mm (mean 0.033mm) and in width between 0.019mm and 0.027mm (mean 0.023mm). (Table 3.2B).

An estimated 75% of the eggs appeared with hexacanth embryos 22 days after culture at 20°C but the great majority were developed by the 24th day.

Eggs cultured at 12°C and 15°C have similar phases of development as described for those at 20°C except for the

extended times occurring between one phase and another.

For eggs cultured at 12°C the durations were 4 days, 13 days, 18 days, 22 days, 27 days and 30 days for phases 2, 3, 4, 5, 6 and 7 to be seen respectively. A few hexacanth embryos were first seen in 30 days but an estimated 75% of eggs had hexacanth embryos at 35 days.

At 15°C, the durations for the different phases of development of embryos were 3 days, 12 days, 18 days 20 days, 23 days, and 25 days, for phases 2, 3, 4, 5, 6 and 7 to be seen respectively. The first few hexacanth embryos appear in 25 days but in 28 days a great majority of the eggs had hexacanth embryos.

The oncospheres did not hatch out of the egg capsules in water but remained intact and infective for a short period (5 to 12 days) after which they disintegrated within the egg capsules. Oncospheres of eggs shed with fish faeces usually remained infective for 12 days after maturity while those of eggs laid by adult tapeworms in 0.9% saline usually remained infective for only about 5 days after reaching maturity. Disintegration of oncospheres is brought about by the activity of microorganisms observed in the egg capsule apparently having moved in through inlets at the operculum slit. These inlets probably result from breakdown of the cement substance that keep the operculum closed and intact.

Eggs with infective oncospheres were made to hatch mechanically when put on a clean slide and slight pressure applied on a cover slip placed over the eggs. With the operculum forced open,

Fig 3.1 Appearance of the eggs of C. truncatus maintained at 20°C. (Not to scale)

A Phase 1

B " 2

C " 3

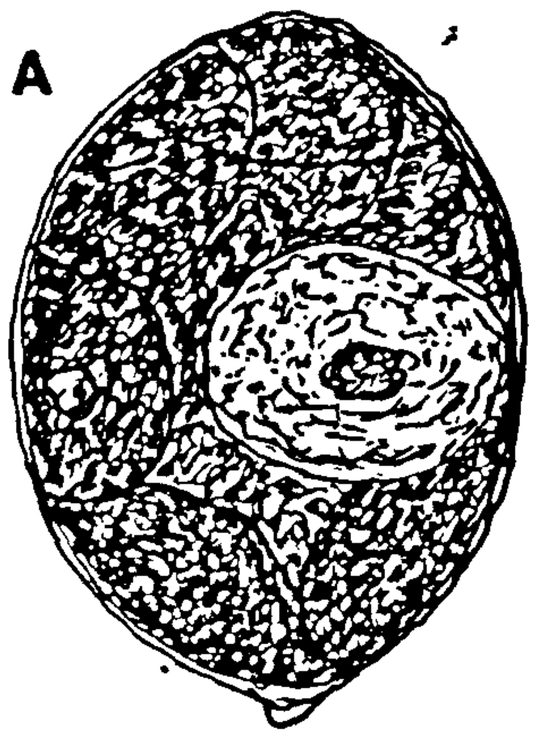
D " 4

E " 5

F " 6

G " 7

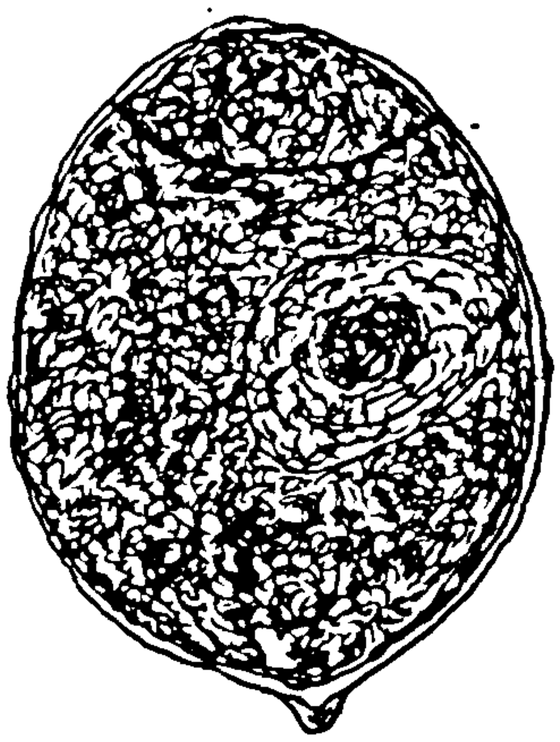
A



1 day old

Yolk cells clearly distinguishable. Operculum present but visible with difficulty.

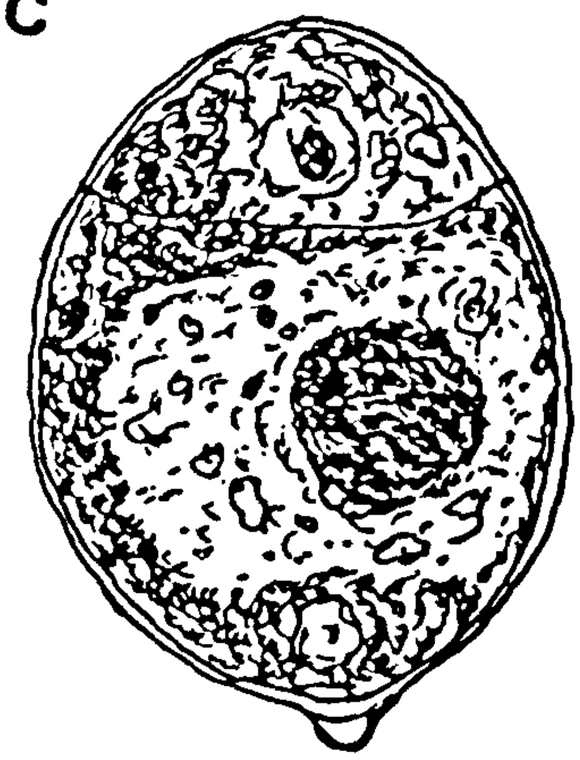
B



3 days old

Yolk cells no longer clearly distinguishable but appear to fuse together. Operculum not clearly visible.

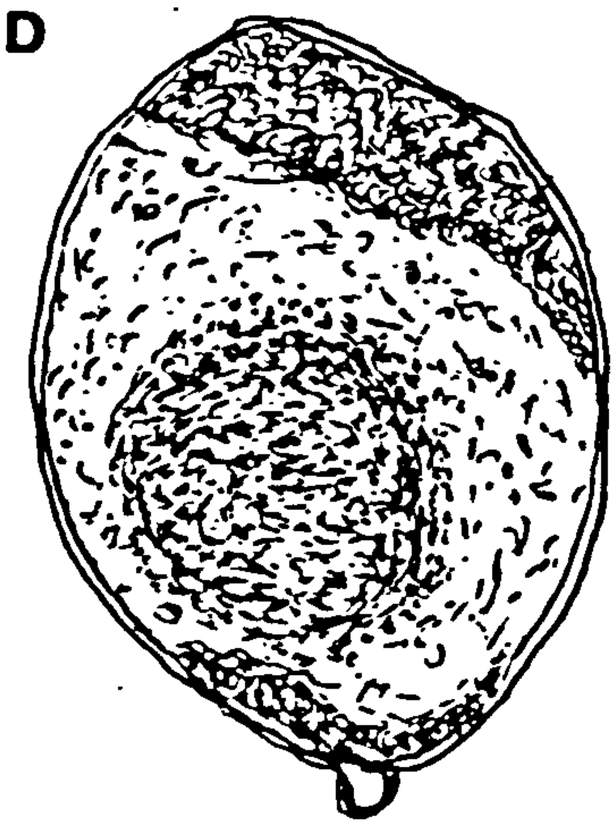
C



12 days old

Endodermal region of embryo become very distinct surrounded by a clearer ectodermal region. Yolk region still dense and occupying greater part of egg space. Operculum clearly visible.

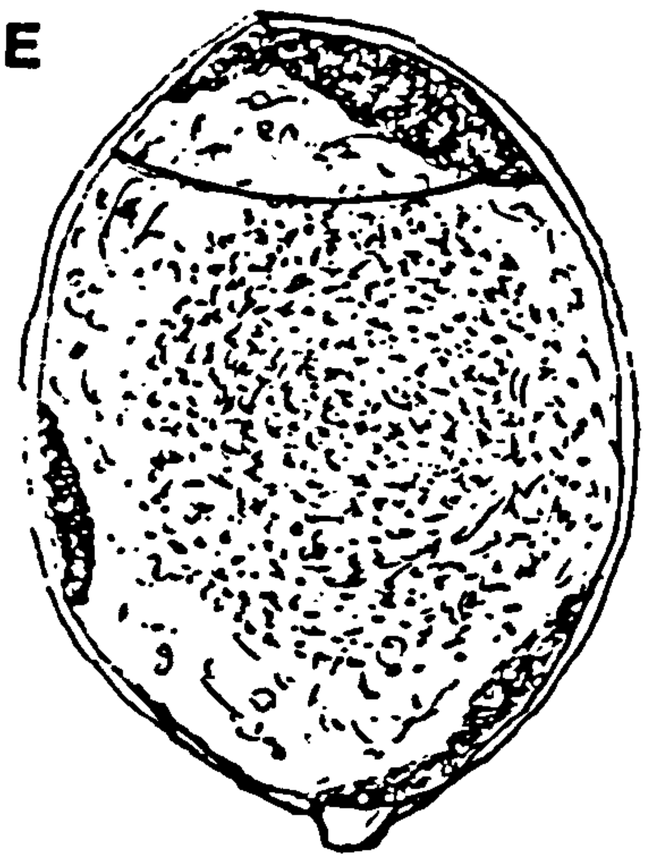
D



15 days old

The clear ectodermal region of embryo increases in size and expands reducing the size of the yolk. Also the dense endodermal region increase in size.

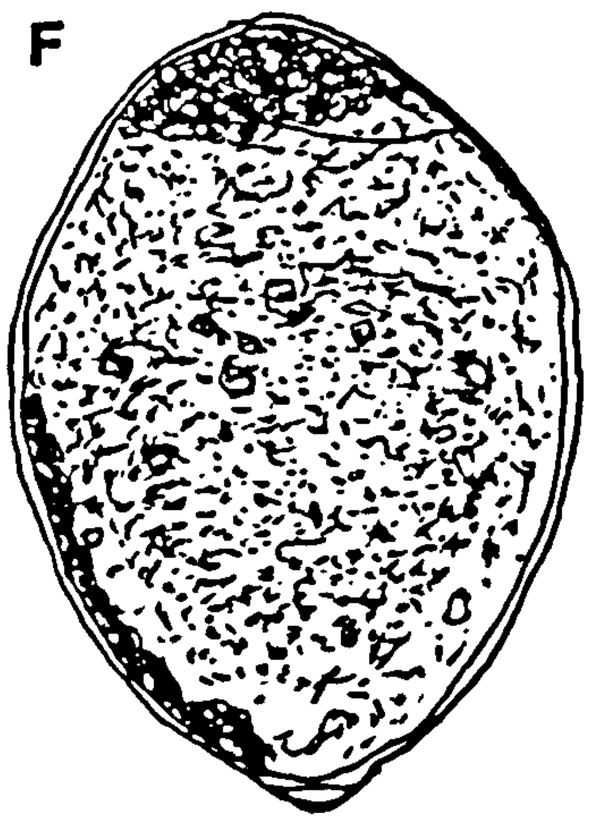
E



18 days old

Embryo expands in size and yolk only occupies small portions at both ends of the egg space. Endodermal region grows in size and become less dense in appearance.

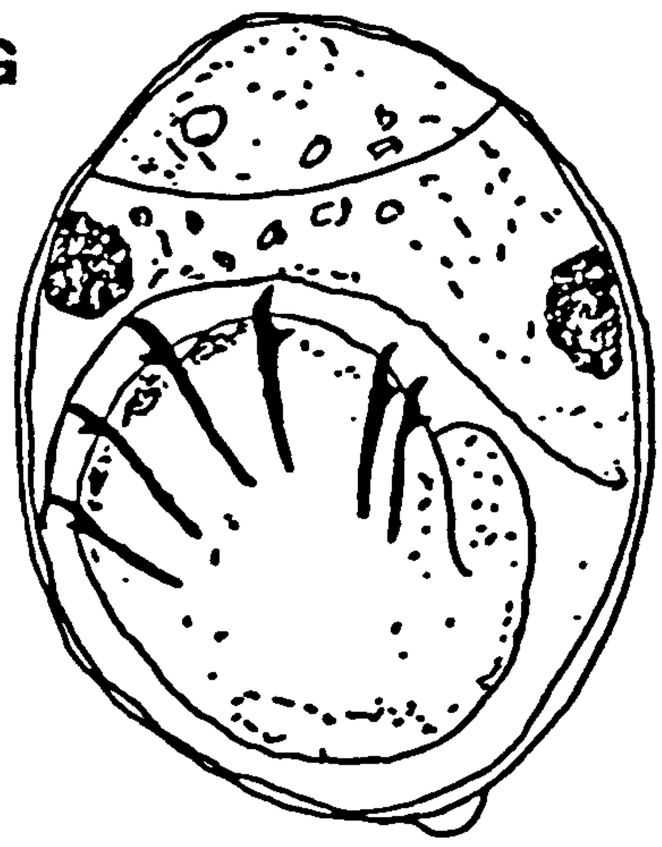
F



20 days old

The endodermal region can no longer be identified as a separate structure. Most of space within capsule apparently occupied by developing embryo showing weak movements.

G



22 days old

Fully developed embryo clearly separated from remains of yolk. Possesses 6 hooks which move weakly. A pair of dark bodies always present.

Table 3.2:Measurements of Cyathocephalus truncatus egg capsules and oncospheres. All measurements are in mm.

A. DEPOSITED EGGS BY INDIVIDUAL WORMS PLACED IN 0.9% SALINE

No.	Capsule Length	Capsule width
1	0.047	0.034
2	0.047	0.037
3	0.049	0.033
4	0.051	0.035
5	0.050	0.030
6	0.048	0.032
7	0.050	0.035
8	0.050	0.034
9	0.052	0.036
10	0.048	0.040
11	0.051	0.032
12	0.049	0.038
13	0.046	0.037
14	0.050	0.035
15	0.051	0.037
16	0.047	0.036
17	0.047	0.036
18	0.050	0.034
19	0.044	0.033
20	0.052	0.038
Mean	0.049	0.035

B. EGGS CONTAINING ONCOSPHERES AFTER CULTURE AT 20°C FOR 22 DAYS

No.	Capsule Length	Capsule width	Oncosphere Length	Oncosphere Width
1	0.045	0.036	0.031	0.026
2	0.051	0.040	0.036	0.026
3	0.045	0.034	0.030	0.022
4	0.047	0.032	0.030	0.020
5	0.050	0.035	0.031	0.025
6	0.048	0.038	0.035	0.027
7	0.049	0.031	0.035	0.025
8	0.052	0.033	0.036	0.024
9	0.050	0.033	0.035	0.024
10	0.046	0.040	0.032	0.020
11	0.044	0.038	0.030	0.020
12	0.048	0.035	0.031	0.019
Mean	0.048	0.035	0.033	0.023

C. SIZES OF ONCOSPHERES OF C. TRUNCATUS mechanically hatched FROM EGG CAPSULES

No.	Diameter
1	0.044
2	0.040
3	0.045
4	0.043
5	0.040
6	0.042
7	0.045
8	0.040
9	0.038
10	0.037
11	0.036
12	0.042
13	0.039
14	0.041
15	0.041
16	0.040
17	0.038
18	0.043
Mean	0.041

the true nature of the oncosphere was revealed (Plate 3.2C and 3.3.A). It has no swimming appendages like cilia and even when carefully forced out of the egg capsule, the oncosphere apparently is incapable of separate existence in water outside the capsule. The shape of each hook is similar to that described for other cestode oncospheres and consists of a straight, rodlike structure (the handle) and a shorted curved blade like portion. At the junction of the rod and the blade there is a slight englargement (the guard). The oncosphere hatched out of the egg is spherical in shape with a mean diameter of 0.041mm (Table 3.2C).

3.4.4 Infection of Gammarus pulex and development of procercoid

Amphipods examined 24h after feeding on infective eggs were found to contain only broken pieces of egg capsules in their intestine. Oncospheres freed from the egg capsules were not found in the intestine or in the body cavity; they were also not observed penetrating the intestinal wall.

However a young developing procercoid was first identified in G. pulex 5 days after it was exposed to infective eggs of C. truncatus. The young procercoid lies not entirely free within the body cavity but is enclosed within a transparent sheath attached either to the external wall of the alimentary canal or to one of the hepatopancreatic caeca The procercoids at this stage are pear-shaped (Plate 3.3B) and measure in length from 0.40mm to 0.50mm (mean 0.44mm) and in maximum width at the anterior end from 0.24mm to 0.38mm (mean 0.31mm).

The developing scolex appear as a slight depression at the anterior-most end while the posterior portion (the narrow end of the "pear") bears the remains of hooks which in most cases are masked and can barely be seen. (Plate 3.3.B).

In a series of trial infection experiments (See Table 3.3) a maximum of 25% Gammarus pulex were infected and the maximum number of larvae per amphipod was 5. This young procercoid stage was found in G. pulex, 5 to 10 days after their exposure to infective eggs. Young specimens of amphipods less than 2mm long appeared to be the most easily infected.

Table 3.4 is the record of infection in experimental Gammarus pulex fed with C. truncatus infective eggs and examined after 15 days, 25 days, 35 days, 50 days, 70 days and 84 days respectively. Measurements of the procercoids retried are given in Table 3.5.

The procercoid in Gammarus pulex examined 15 days after infection is elongated and slightly dark in colour (Plate 3.3C). The procercoids vary in length from 1.45mm to 1.52mm (mean 1.48mm); the width of the body vary from about 0.29mm to about 0.40mm (Table 3.5B). The developing scolex appear as an apical depression at the anterior end of the worm. The dark coloured appearance and fragile nature of the procercoid made it difficult to identify its anatomical details.

The procercoids retrieved from G. pulex 25 days after infection measured in length from 2.49mm to 2.54mm (mean 2.51mm).

The width of the body is about 0.38mm (Table 3.5C).

The body of the procercoid at this stage is being differentiated to form a strobila and formation of genital areas is seen to have commerced (Plate 3.4A). The scolex retains its appearance as a depression in the anterior region of the worm and the cercomer is beginning to develop at the posterior end.

The procercoid is still extremely fragile but gradually becoming less dark in colour. This stage is similar to the Stage I procercoid recovered from field-infected G. pulex as reported above (page 46).

At 35 days of infection, procercoids recovered from G. pulex measure in length between 3.85mm and 3.92mm (mean 3.88mm) as shown in Table 3.5D. The width of the strobila is about 0.50mm to 0.60mm. The procercoids show clearly the development of the genital areas which can be counted although the individual components are not yet distinguishable (Plate 3.4B). The posteriorly-placed cercomer is retained. Anteriorly the depression marking the scolex further deepens so that the scolex becomes almost tubular in shape. The worm at this stage is light in colour and to some extent details of its anatomy can be perceived including the osmoregulatory vessels in the strobila and the cercomer.

The procercoid obtained from a 50 days infected gammarid was 5.48mm in length (Table 3.5E). The positions of the developing sets of genitalia are well marked although the genital

organs themselves and their apertures remain difficult to identify individually (Plate 3.5). Compared to other experimental trials (stated in Table 3.4) where up to 20% infection of G. pulex was recorded, most of the gammarids in the 4 trial experiments meant for examination at 50 days of infection died prematurely and only 8 specimens remained alive for the whole period. The cause of such deaths is not known.

In the 70 days infected amphipods the procercoids recovered were similar in external morphology to that of the 50 day old procercoid described above except that they showed an increase in length which varied between 6.50mm and 6.55mm (6.52mm) (Plate 3.5B). They vary from about 0.60mm to 0.70mm in width of strobila. The genital apertures can be identified at this stage. The scolex with its tubular invagination is very clearly formed but it was observed that the edges were not of even thickness; the margins corresponding to the flat surfaces of the strobila are less thick than the lateral margins (Plate 3.6). Posteriorly the cercomer is also clearly formed.

The procercoids described in the 35 days -, 50 days - and 70 days - infected amphipods were similar to those categorised as Stage II procercoids in Chapter 2 (page 46).

The procercoids recovered from G. pulex examined 84 days after infection were similar to the Stage III procercoids described above (page 46). They varied in length between 7.31mm and 10.25mm and in most cases were longer than the amphipod host. In field-infected gammarids, the procercoid can

grow to about 14mm long. It always bears a funnel-shaped scolex and a cercomer (Plage 3.7). The genital plate bearing the genital openings (cirrus aperture and the vagino-uterine apterture) are conspicuously seen all along the strobila. A maximum of 15% Gammarus pulex were infected, all with single specimens (Table 3.4). Single infections of amphipods with C. truncatus procercoid were also frequently seen in gammarids collected in the field (Chapter 2).

The transparent sheath seen to envelop the young procercoid at 5 days of infection is also seen to envelop all other procercoid stages already described including the above Stage III procercoid. When dissected out of G. pulex the Stage III procercoid exhibits a vigorous movement accompanied by undulating contractions and relaxations of body wall muscles all along the strobila. During this time the transparent sheath becomes ruptured freeing the juvenile tapeworm (Plate 3.8).

Some field-infected amphipods with Stage III procercoids deliberately maintained in the laboratory were observed to harbour the procercoids for over 6 months with apparently no change in their condition. Despite the period spent in the amphipods, on examination the procercoids were never seen to mature to the egg-laying condition.

Table 3.3: Infections of Gammarus pulex with C. truncatus 7 days after exposure of the amphipods to infective eggs of the worm. 20 G. pulex were exposed to eggs in each trial.

Size of G. pulex (mm)	No. of Trials	No. of G. pulex surviving and examined	No. (%) of G. pulex infected	No. of worms	No. of worms per infected amphipod
≥ 5	1	20	-	-	-
	2	20	1 (5)	4	4
	3	18	-	-	-
	4	20	1 (5)	2	2
	5	20	-	-	-
2.1 to 4.9	1	19	-	-	-
	2	20	3 (15)	3	1; 1; 1
	3	17	3 (15)	5	1; 1; 3
	4	20	2 (10)	2	1; 1
	5	12	2 (10)	7	2; 5
≤ 2	1	18	3 (15)	3	1; 1; 1
	2	19	5 (25)	7	1; 1; 2; 2; 1
	3	20	2 (10)	6	1; 5
	4	16	3 (15)	8	2; 2; 4
	5	20	-	-	-
	6	20	-	-	-

≥ Greater than or equal to

≤ Less " " " "

TABLE 3.4: Infection of Gammarus pulex with Cyathocephalus truncatus at various periods following exposure of the amphipods to infective eggs of the worm. 20 G. pulex were exposed to eggs in each trial.

Period and No. of trial	No. of G. pulex surviving and examined	No. (%) of G. pulex infected	No. pf worms	No. of worms per infected amphipod
A. 15 DAYS AFTER INFECTION				
1.	8	1 (5)	1	1
2.	-	-	-	-
3.	16	2 (10)	7	4; 3
4.	13	2 (10)	9	6; 3
B. 25 DAYS AFTER INFECTION				
1.	12	3 (15)	8	2; 3; 3
2.	9	-	-	
3.	14	4 (20)	7	3; 1; 1; 2
4.	11	2 (10)	6	5; 1
C. 35 DAYS AFTER INFECTION				
1.	4	-	-	-
2.	15	3 (15)	9	3; 2; 4
3.	6	-	-	-
4.	10	4 (20)	5	1; 1; 1; 2
D. 50 DAYS AFTER INFECTION				
1.	2	-	-	-
2.	-	-	-	-
3.	6	1 (5)	1	1
4.	-	-	-	-

E. 70 DAYS AFTER INFECTION

1.	12	2 (10)	5	2; 3
2.	9	2 (10)	2	1; 1
3.	-	-	-	-
4.	7	-	-	-

F. 84 DAYS AFTER INFECTION

1.	2	1 (5)	1	1
2.	8	3 (15)	3	1; 1; 1
3.	-	-	-	-
4.	3	-	-	-
5.	-	-	-	-

Table 3.5: Measurements of Cyathocephalus truncatus procercoids from Gammarus pulex. All measurements are in mm.

A. 7 DAYS OLD PROCERCOIDS

Gammarus length		Serial No.	Length of worms	Max. anterior width
6	Multiple infection	1	0.45	0.38
		2	0.45	0.35
		3	0.40	0.31
		4	0.41	0.33
5	Double infection	5	0.48	0.28
		6	0.46	0.27
4		7	0.42	0.26
3		8	0.45	0.30
4		9	0.45	0.33
2	Multiple infection	10	0.40	0.24
		11	0.41	0.28
		12	0.50	0.36
		13	0.47	0.32
		14	0.46	0.30
2		15	0.43	0.30
		Mean	0.44	0.31

B. 15 DAYS OLD PROCERCOIDS

Gammarus length		Serial No.	Length of worms	Max. scolex width
2		1	1.48	0.30
3	Multiple infection	2	1.47	0.28
		3	1.47	0.31
		4	1.49	0.26
		5	1.46	0.28
4	Triple infection	6	1.48	0.26
		7	1.50	0.32
		8	1.47	0.30
3	Multiple infection	9	1.45	0.30
		10	1.51	0.31
		11	1.50	0.30
		12	1.50	0.30
		13	1.48	0.32
		14	1.52	0.31
		Mean	1.48	0.30

C. 25 DAYS OLD PROCERCOIDS

Gammarus length		Serial No.	Length of worm	Max. scolex width
4	Multiple infection	1	2.50	0.42
		2	2.52	0.40
		3	2.50	0.44
		4	2.50	0.40
		5	2.53	0.38
5		6	2.50	0.40
2	Double infection	7	2.51	0.35
		8	2.54	0.37
3	Triple infection	9	2.50	0.41
		10	2.51	0.41
		11	2.52	0.42
4	Triple infection	12	2.50	0.40
		13	2.49	0.40
		14	2.50	0.43
		Mean	2.51	0.40

D. 35 DAYS OLD PROCERCOIDS

Gammarus length		Serial No.	Length of worm	Max. scolex width
5	Double infection	1	3.90	0.43
		2	3.87	0.40
3	Multiple infection	3	3.84	0.42
		4	3.85	0.38
		5	3.90	0.39
		6	3.92	0.44
	Triple infection	7	3.88	0.42
		8	3.85	0.38
		9	3.90	0.40
		Mean	3.88	0.41

E. 50 DAYS OLD PROCERCOIDS

Gammarus length	Serial No.	Length of worm	Max. scolex width
	1	5.48	0.50

F. 70 DAYS OLD PROCERCOIDS

Gammarus length		Serial No.	Length of worm	Max. scolex width
3	Triple infection	1	6.52	0.52
		2	6.50	0.53
		3	6.50	0.50
6	Double infection	4	6.50	0.51
		5	6.53	0.53
5		6	6.55	0.49
5		7	6.51	0.50
		Mean	6.52	0.51

G. 84 DAYS OLD PROCERCOIDS

Gammarus length	Serial No.	Length of worm	Max. scolex width
6	1	10.25	0.64
4	2	9.18	0.60
5	3	10.00	0.62
5	4	7.31	0.60
	Mean	9.19	0.62

3.4.5 Infection and development of C. truncatus and Salmo trutta

Only 2 of the 10 Salmo trutta were infected in attempts made by pipetting tapeworm procercoids retrieved from G. pulex into the alimentary canal of the anaesthetised fish. In all other cases, the worms were regurgitated by the fish on recovery and when not regurgitated the worms were usually found dead in the intestine of fish 12 h after introduction. Such specimens were probably digested or expelled with the faecal matter.

Feeding infected Gammarus pulex to fish proved more successful in establishing infection with mature tapeworms. Of the 96 specimens introduced 67 (69.8%) became established and were recovered at autopsy. (Table 3.6). The worms evidently preferred the dorsal and anterior ventral caeca as sites of attachment - 52.2% incidence in the dorsal caeca, 46.3% in the anterior ventral caeca and 1.5% in the posterior ventral caeca. Observations made on the progressive development and attachment pattern of adult worms within the pyloric caeca of fish (shown in Table 3.6) are treated in detail below (see page 173).

Tapeworms were found already lodged in the pyloric caeca in fish examined 24 h after feeding with infected amphipods. These worms as well as those recovered from fish after 2 days of infection were found unattached in the pyloric caeca. Three days after infection the tapeworms were found to be attached to the mucosa at the distal end of the caeca but they could easily be dislodged on examination. Worms recovered from fish on the 7th day had formed a firm attachment with the mucosa. The worms in

15 days infected fish were found to be very strongly attached to the muscularis of the caeca having eroded the mucosa. This is discussed in detail below (page 175).

Evidence of maturity was first noted in tapeworms recovered 5 days after infection. The worms had gonads that appeared well developed but did not produce eggs in 0.9% saline. It was the tapeworms recovered after 8 days of infection that first produced eggs in 0.9% saline. Eggs were first noted in fish faeces 10 days after infection (Table 3.6). The cercomer was observed often to be shed following maturity although it was still intact in some egg-laying adults.

Adult tapeworms in fish vary in length usually between 5.0mm and 40mm and the number of genital areas vary accordingly. In the 25 worms measured (Table 3.7)the length varied between 5.56mm and 32.31mm (mean 19.07mm). The width of scolex is maximum at the tip (recording a mean of 0.59mm) and minimum at the base (recording a mean of 0.38mm). The scolex is about 0.59mm long; also the width of the strobila is about 1.07mm. The mean number of genital areas was 24.

Table 3.6 Infection of brown trout (Salmo trutta) with Cyathocephalus truncatus after feeding Gammarus pulex harbouring single procercoids. Two fish per tank. Twelve infected gammarids introduced to each tank.

Tank No.	No of worms recovered per fish	Occurrence in pyloric caeca: Dorsal caeca	Anterior ventral	Posterior ventral	Period of Infection (Days)	Remarks
1	5	3	2	-	1	Worms free in caecum. Cercomer present.
	2	1	1	-	2	Similar to day 1.
2	3	1	2	-	3	Worms attached to distal end of caeca but easily dislodged. Cercomer present.
	1	1	-	-	4	Similar to day 3.
3	3	2	1	-	5	Worms firmly attached to mucosa. No damage to caecum. Cercomer present.
	9	3	6	-	6	Similar to day 5.
4	2	2	-	-	7	Mucosa eroded at point of attachment. Very firm attachment. Cercomer present.
	6	4	2	-	8	Attachment similar to day 7. Eggs laid by worms in saline for the first time. Cercomer present.
5	5	2	3	-	9	Attachment similar to day 7. All worms produce eggs in saline. Cercome absent.
	4	3	1	-	10	Attachment similar to day 7. Eggs seen in fish faeces for the first time. All worms produce eggs in saline. Cercomer present only in 3 worms.

6	6	2	4	-	15	Worms attached to muscularis. Site of attachment swollen. Worms are all adults. Cercomer absent.
	5	3	2	-	20	Similar to day 15.
7	8	4	3	1	25	Similar to day 15; additionally swollen caeca get bigger.
	3	-	3	-	30	Points of attachment become hard and fuse with neighbouring caeca.
8	3	2	1	-	35	Tapeworm scolex embedded in tissue of attachment. Difficult to see scolex unless sectioned.
	2	2	-	-	40	Similar to day 35.
Total	67	35 (52.2%)	31 (46.3%)	1 (1.5%)		

Table 3.7 Measurements of 25 adult Cyathocephalus truncatus from laboratory infections of Salmo trutta. All measurements are in mm.

No.	Length of worm	No. pairs of genitalia	Scolex length	Scolex max. width	Scolex min. width
1	14.52	19	0.62	0.59	0.36
2	24.63	27	0.60	0.59	0.35
3	21.10	30	0.61	0.58	0.38
4	20.05	25	0.59	0.55	0.38
5	18.32	22	0.60	0.58	0.37
6	23.77	36	0.55	0.60	0.40
7	20.54	22	0.56	0.62	0.38
8	16.28	21	0.62	0.56	0.38
9	25.01	27	0.60	0.60	0.36
10	12.50	15	0.58	0.60	0.38
11	13.87	24	0.62	0.57	0.39
12	27.90	31	0.57	0.58	0.40
13	25.33	29	0.57	0.58	0.40
14	30.25	28	0.61	0.58	0.36
15	5.56	8	0.62	0.60	0.38
16	12.08	16	0.56	0.60	0.50
17	11.78	12	0.60	0.59	0.37
18	32.31	40	0.60	0.60	0.37
19	8.54	13	0.60	0.60	0.37
20	7.50	14	0.60	0.60	0.39
21	5.82	10	0.57	0.61	0.38
22	25.49	28	0.56	0.62	0.38
23	20.68	23	0.58	0.56	0.40
24	27.20	37	0.58	0.58	0.40
25	25.73	34	0.58	0.60	0.35
Mean	19.07	24	0.59	0.59	0.38

3.5 Discussion

The present observation on development and the occurrence of a hexacanth embryo in Cyathocephalus truncatus eggs agrees with the report of Gauthier (1923). The description of the hexcanth embryo is also similar to that of Gauthier (1923). Both reports above however contradict that of Wisniewski (1932b,c) who described an embryo that lacked larval hooks. Since Wisniewski did not state the temperature conditions under which he cultured the eggs and did not succeed in infecting amphipods with the mature embryo he described, it is difficult to say whether the embryo actually developed.

On the development of the embryo, the present study indicates that low temperatures of 6°C and below as well as high temperatures of 25°C and above are unsuitable for culture of the tapeworms eggs. It is important to emphasize that temperature and shallow well-aerated water are vital conditions for C. truncatus egg development; in the laboratory eggs develop optimally at between 15°C and 20°C in water about 1cm deep and which is replaced every other day. In the field, infections of amphipods are likely to be more frequent in the summer months when the higher temperature causes accelerated egg development so that infective stage III procercoids are common during the winter months. This would explain the apparent seasonal cycle of the procercoids in amphipods described in Chapter 2 (page 43).

Although eggs containing infective embryos did not hatch in the laboratory at any time it is not known whether they do so under natural conditions in the field. Observations show that

oncospheres lack cilia and the writer believes it is unlikely that they are normally liberated into water where they probably cannot survive. Moreover, since Gammarus pulex were only infected in the laboratory by feeding on eggs containing hexcanth embryos it is possible that this is the only way that amphipods can become infected.

The observation that younger specimens of Gammarus pulex appear more frequently infected than older ones under laboratory conditions shows that the former are probably more susceptible to infection and agreeswith observations on natural infections by Wisniewski (1932a,b). Subsequently it would appear that the chances of amphipods becoming infected is probably reduced by the masticatory effects of the amphipod mandibles that could crush the infective embryoswhen amphipods pick up the eggs while feeding. This was also suggested by Sandeman and Burt (1972) for infection of amphipods with the cestode Bothrimonus sturionis. Gammarus pulex may also have difficulty in tolerating multiple infections - whereas in the early procercoid stages up to 5 or 6 worms were recovered from a single gammarid (page 91) the number of procercoids per gammarid decreased to 1 as the worms grow to the Stage III procercoid (pages 91,92).

It seems probable that amphipods carrying multiple infections die before the procercoids grow to the infective stage. All the factors mentioned above are likely to be responsible for the low infection generally seen in G. pulex. Contrary to the reports of Sandeman and Burt (1972) and Amin (1978) no egg-laying

procercoid was ever recovered from Gammarus pulex in the present studies. Since the infective stage III procercoids recovered from G. pulex have conspicuous gonads and genital apertures it might be possible that these gonads mature and the procercoids become egg-laying within the amphipod.

In fish, the tapeworms emerge when the body of the amphipod hosts (preyed upon by the fish) start to disintegrate on being digested in the pyloric stomach and region of pyloric caeca rather than in the small intestine;hence the process of migrating to their site of final attachment is evidently rapid - individual worms are already found in the pyloric caeca 24h after being fed with infected gammarids. Establishment of the tapeworm in fish is also fast - becoming firmly attached by the 7th day of infection and initiating the processes of irritation, swelling, vascularisation and eroding of host tissues.

Observations that eggs were produced in fish faeces on the 10th day after infection agree with the report of Vik (1958) although from the present study the worms were actually found to mature and become egg-laying in 0.9% saline 8 days after infection. Thus the experimental infection demonstrated that tapeworm maturity is accelerated when procercoids enter the fish whereas similar procercoids in the body cavity of the amphipod host could remain for over 6 months in the laboratory without becoming mature. Maturity of C. truncatus in fish is accompanied by shedding of the cercomer and although it is sometimes retained by some egg-laying worms, its possession by the worm does not seem to play any functional part in the developmentof the tapeworm.

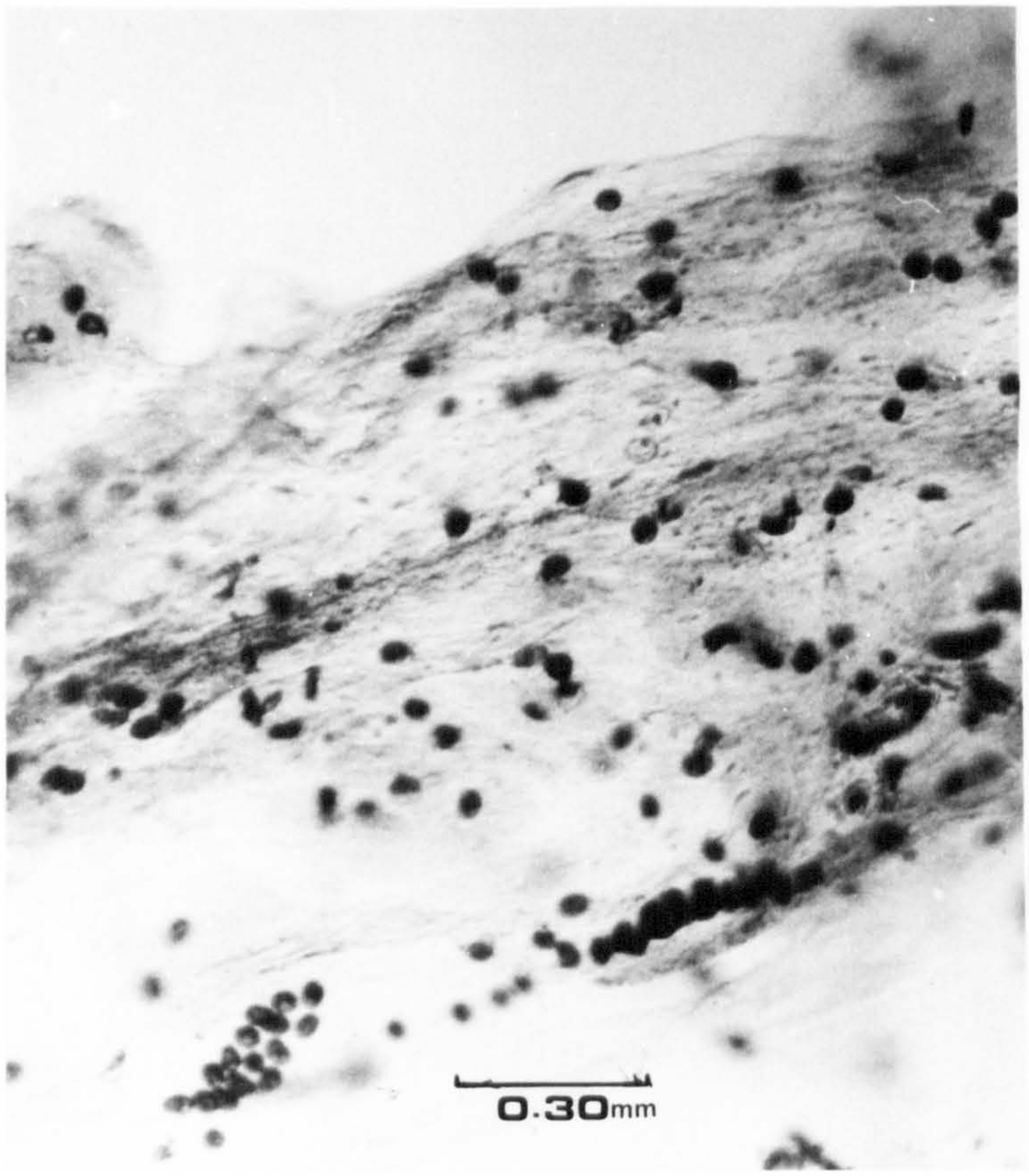
Because of the small number of infected amphipods available for experiments it was not possible to determine the actual longevity of tapeworm in both amphipod and fish hosts. In experimental infections, fish examined after 40 days still harboured tapeworms firmly established. Vik (1958) reported that tapeworms could remain for over 43 days and possibly up to 55 days in fish gut before being eliminated. In the present study, infected fish could not be left for a longer period than the one stated above because of their poor condition in the aquarium.

Plate 3.1

A. Freshly laid eggs of C. truncatus embedded in mucus and eliminated from the fish host in faeces. Scale = 0.30 mm

B. Single freshly laid egg. Scale = 30µm.

A



B

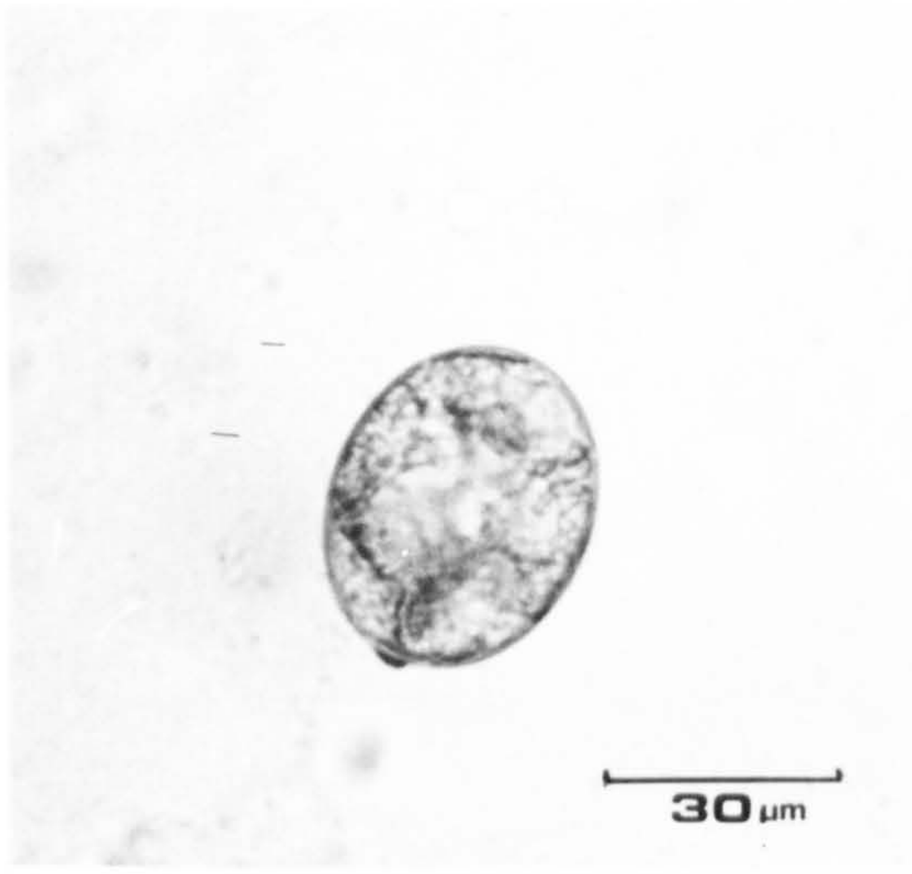
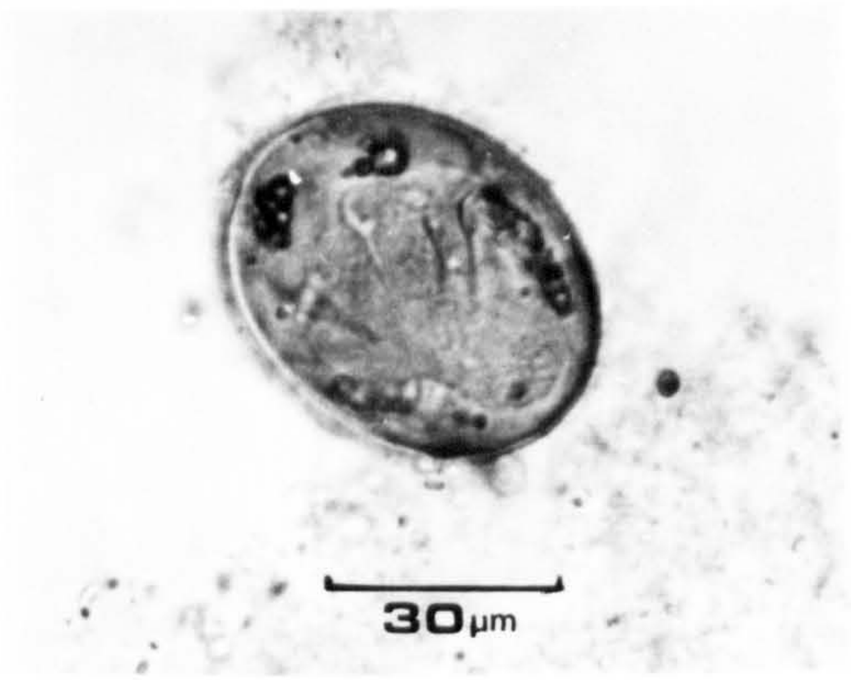


Plate 3.2

A. and B. Hexacanth embryos of C. truncatus within egg capsules. Scale = 30 µm

C. Eggs with hexacanth embryos hatched mechanically under the pressure of a cover slip. Scale = 20 µm.

A



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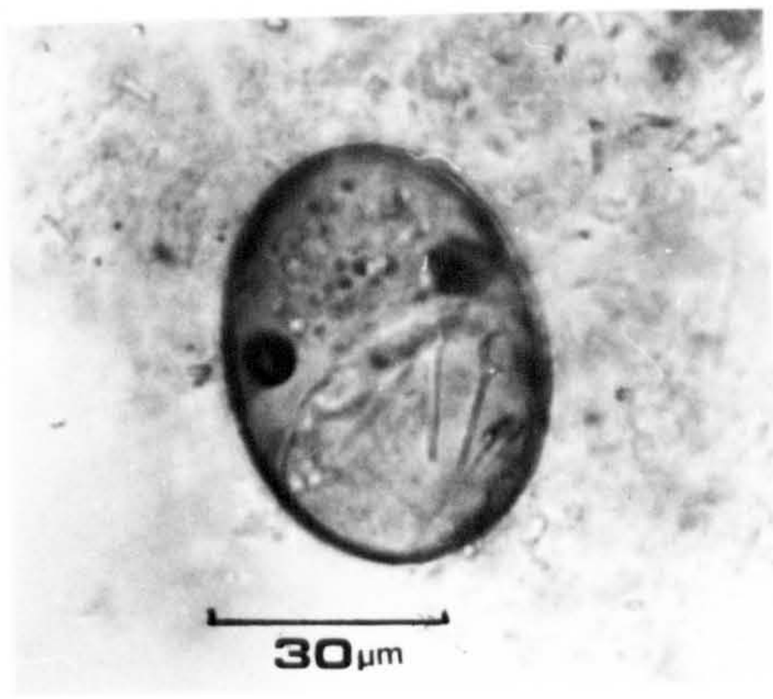


C

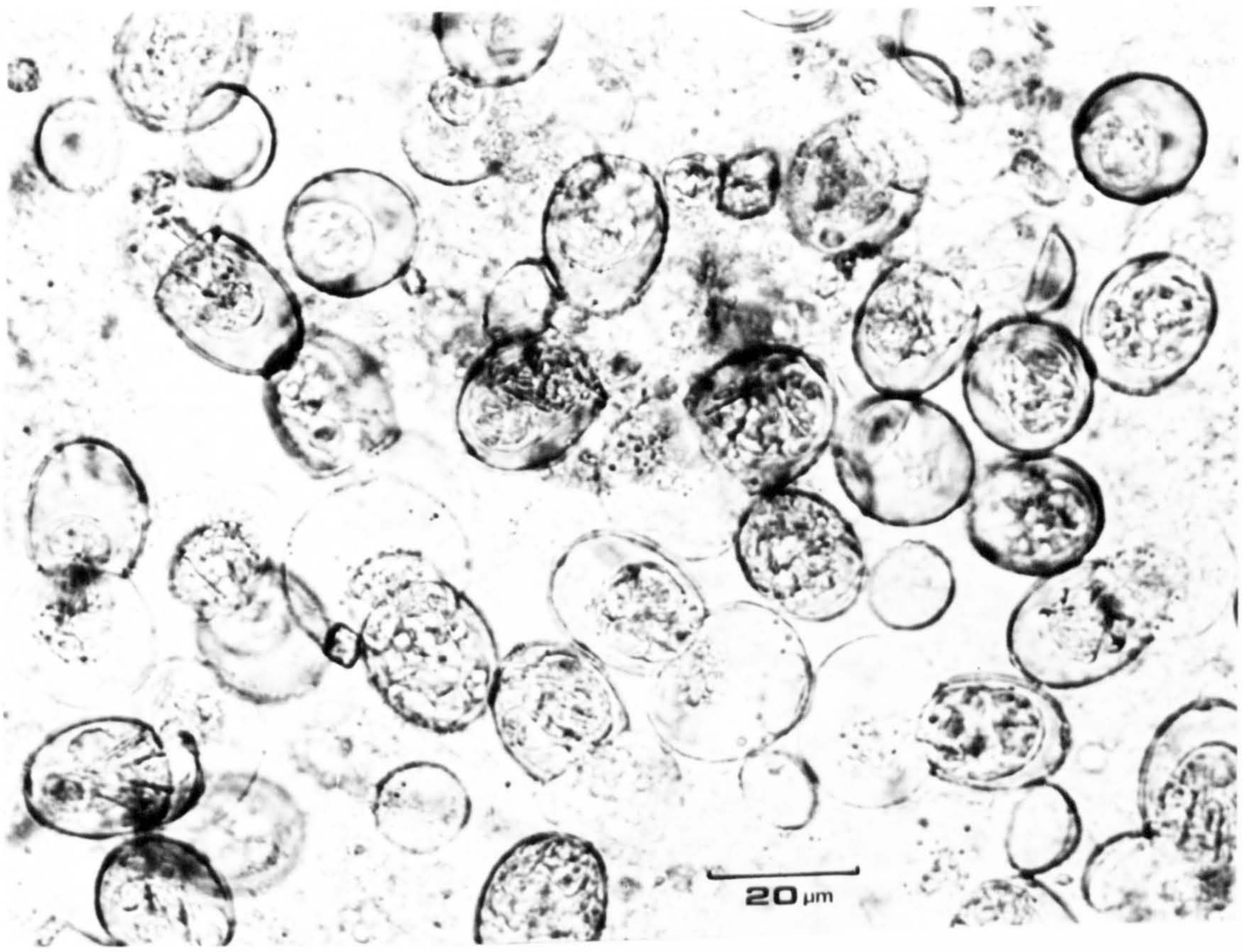


Plate 3.3

A. The released oncosphere of one of the mechanically hatched eggs of C. truncatus. Scale = 30 µm

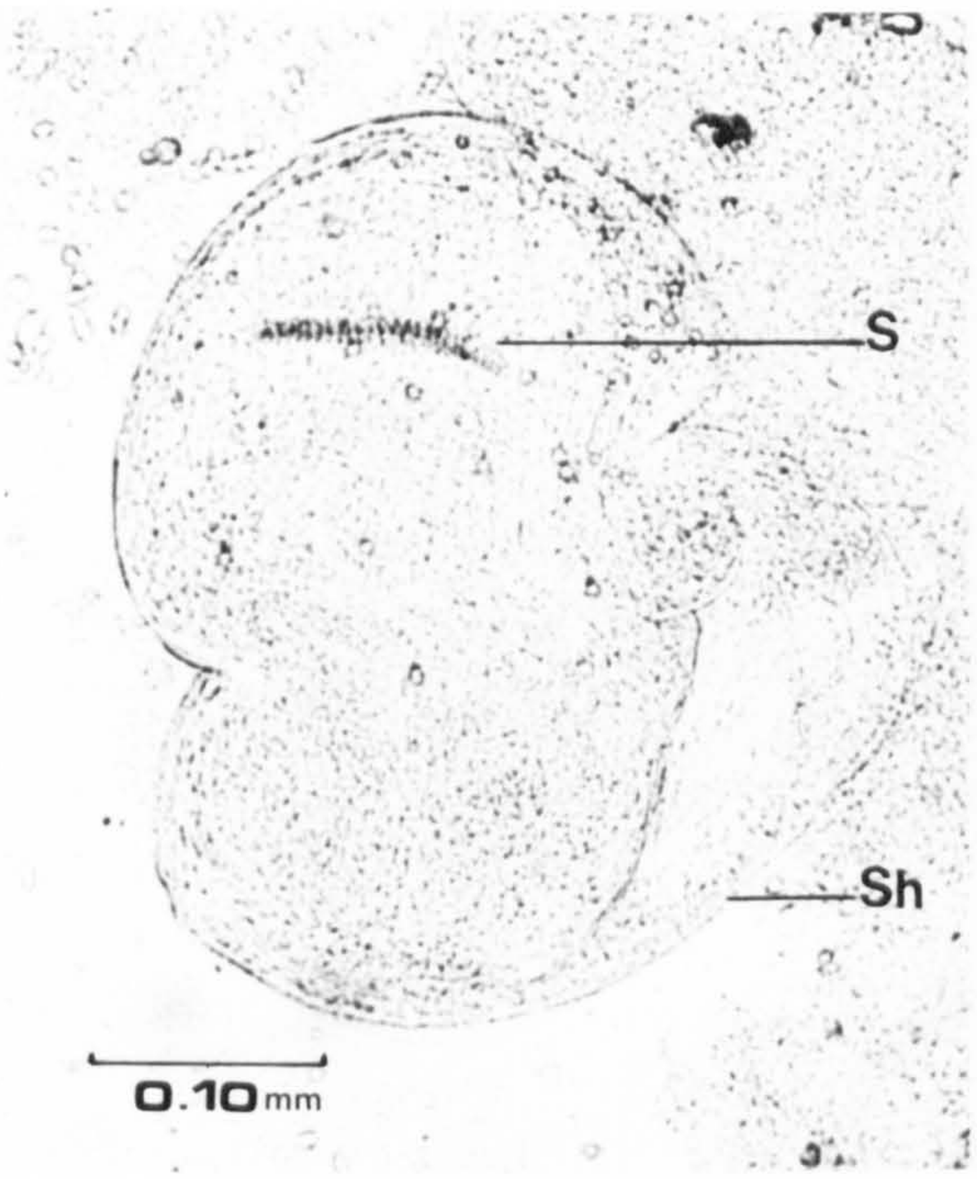
B. 7 days old procercoid of C. truncatus in body cavity of Gammarus pulex. (Unstained) Scale = 0.10mm

S ..... scolex
Sh ..... sheath

C. 15 days old procercoid of C. truncatus. (Unstained) Scale = 0.40mm

A

B



C

Plate 3.4

A. 25 days old procercoid of C. truncatus
Borax carmine . Scale = 0.70mm

B. 35 days old procercoid of C. truncatus
Borax carmine . Scale = 0.70mm

A

0.70mm

B

0.70mm

Plate 3.5

A. 50 days old procercoid of C. truncatus
Borax carmine Scale = 1.0mm

B. 70 days old procercoid of C. truncatus
Borax carmine Scale = 1.0mm

A

1.0mm

B

1.0mm

Plate 3.6

A. scolex of the Stage II procercoid of C. truncatus showing thickness of its lateral walls. Hematoxylin. Scale = 0.30mm

B. Diagrammatic representation transverse section of scolex of C. truncatus procercoids showing the relative thickness of the surficial and lateral walls. Scale = 0.30mm

(i) Scolex of Stage I procercoid
(ii) Scolex of Stage II procercoid

L ..... Lateral walls
S ..... Surficial walls.

A

B

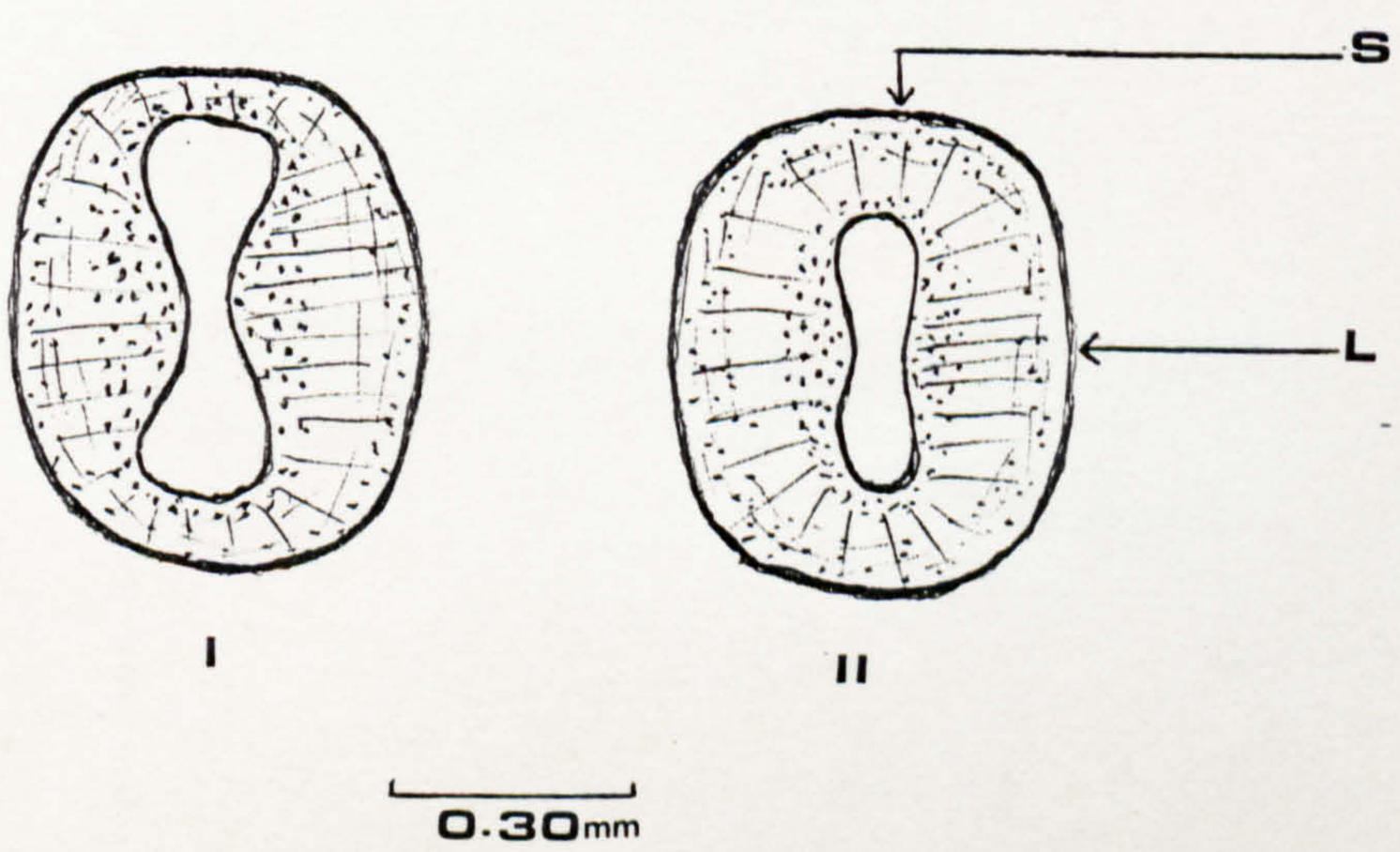


Plate 3.7 Stage III procercoid of C. truncatus
Hematoxylin • Scale = 1.0mm

C ..... Cercomer
U ..... Uteri
S ..... Scolex

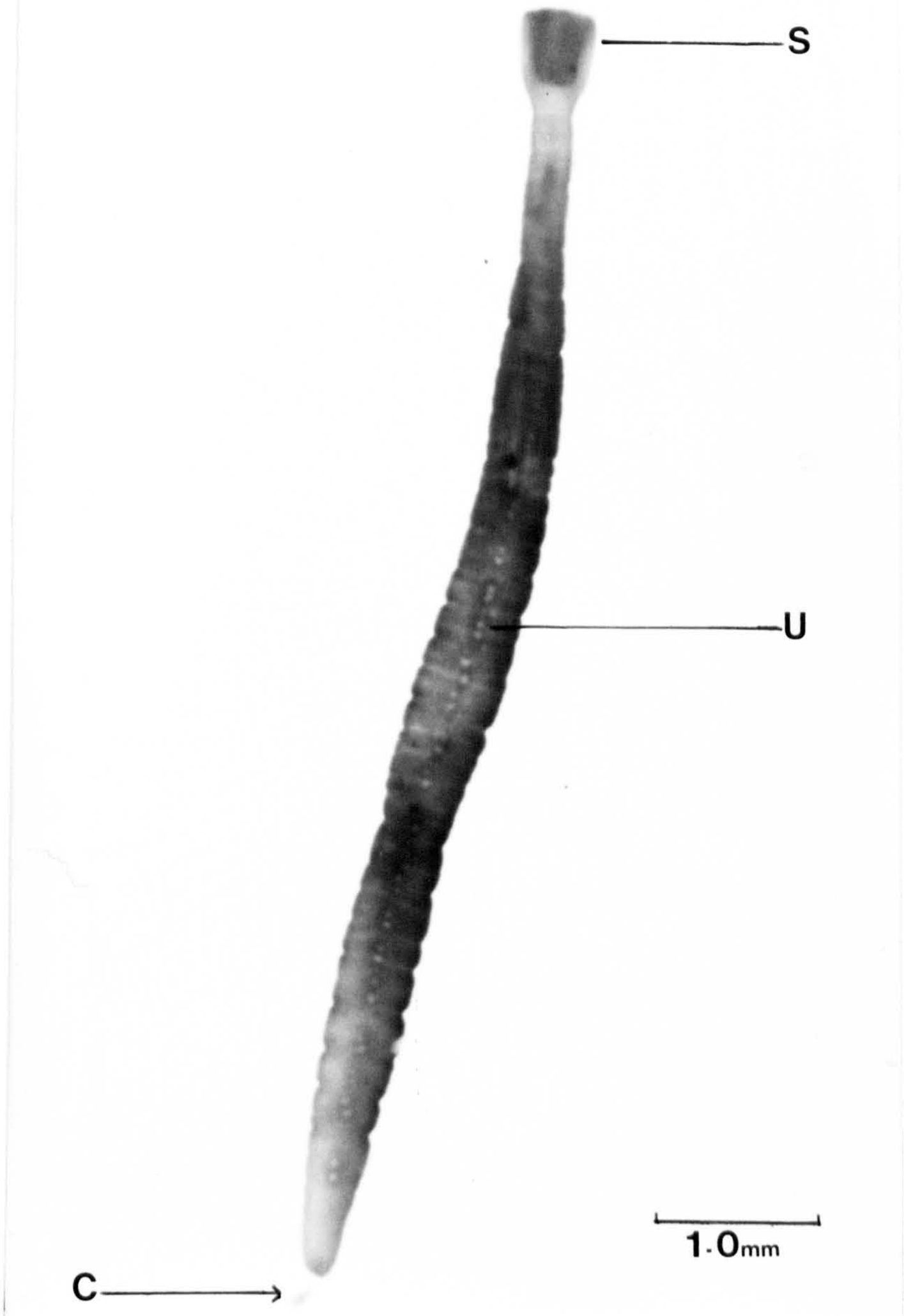

S
U
C
1·0mm

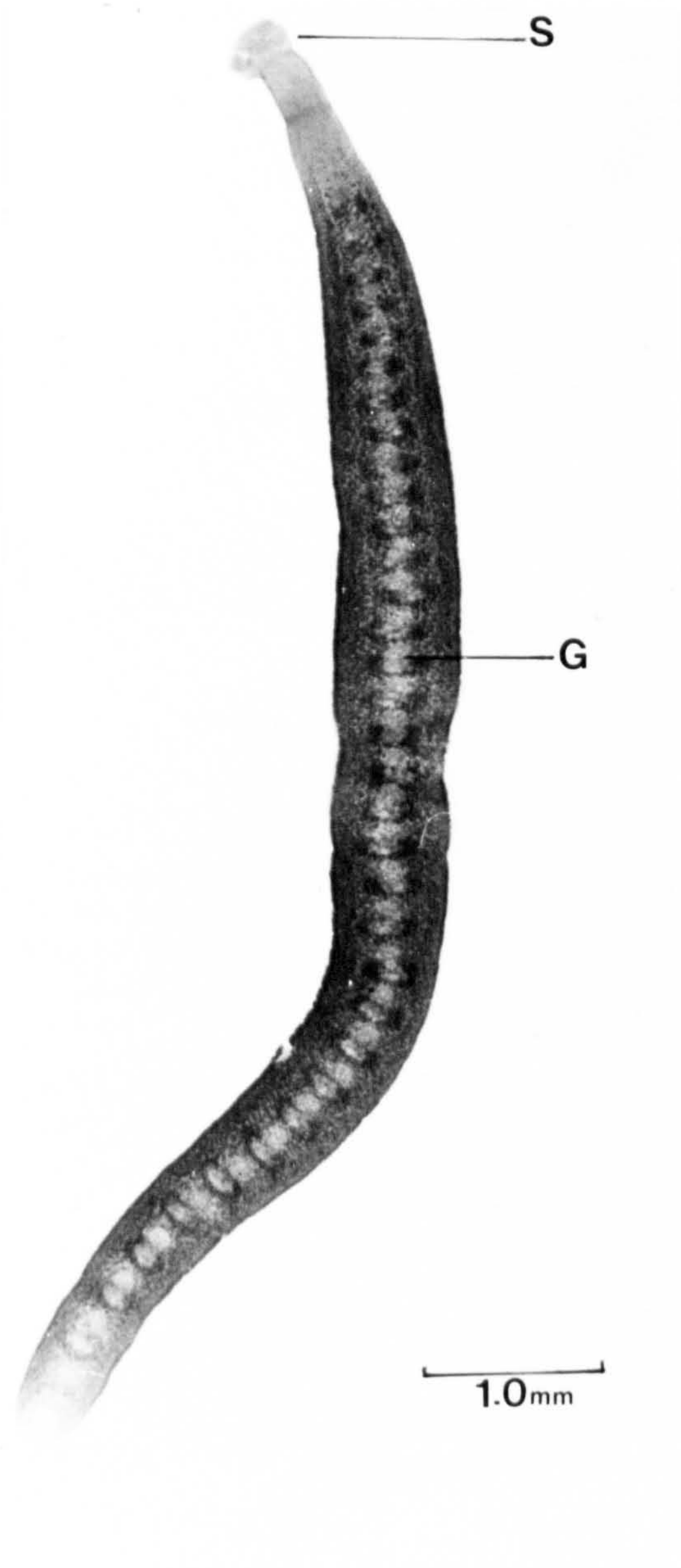
Plate 3.8 Procercoid of C. truncatus freshly removed from the gammarid host. The sheath arrowed is being shed. Scale = 1.0mm

A ..... Anterior and scolex end
B ..... Trunk (mid body)
C ..... Posterior end


A
B
C
1.0mm

Plate 3.9 Adult C. truncatus removed from pyloric caecum of S. trutta. Borax carmine . Scale = 1.0mm

G ..... Genital area
S ..... Scolex showing slight damage caused during removal from host.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CHAPTER 4

STUDIES ON THE PROCERCOID OF *CYATHOCEPHALUS TRUNCATUS* IN *GAMMARUS PULEX*

4.1 Introduction

Considerable work has been done on the structure and ultrastructure of metacestodes particularly of the cyclophyllidean cestodes, for example Hymenolepis spp. There have been no such reports on the procercoid of Cyathocephalus truncatus or of other related tapeworms in the order Spathebothridea.

Metacestodes of members of the order Pseudophyllidea which possibly bear a close affinity to the spathebothrids have been studied and reports published by several authors especially on species of Diphyllobothrium. Among others are the reports of Bråten (1968 a,b) in which he compared the procercoid, plerocercoid and adult of Diphyllobothrium latum and that of Gustafsson and Vaihela (1981) on the structure of the scolex and scolex glands in the plerocercoid and adult of Diphyllobothrium dendriticum.

In the present investigation, with the aid of light and electron microscopy, the structure and ultrastructure of the procercoid of C. truncatus have been studied with particular attention to the body wall and the scolex of the Stage III procercoid. Histochemical studies have been conducted on the procercoid and the scolex again studied in detail in an attempt to understand features that make it an efficient attachment organ when the procercoid reaches the adult stage in fish. The possible pathogenic effects of the procercoid on Gammarus pulex have also been considered.

4.2 Literature review

The structure of the metacestode presents a wide range of features - showing those inherited from the oncosphere and in particular the features that are developed by the metacestode which are utilizable in the adult stage. Features like scolex glands that develop in the procercoid enable it to penetrate host tisses and gain access to the body cavity or muscle for encystment. These glands are usually referred to as penetration glands and are known to contain proteinaceous substances and possibly enzymes in their secretions.

Gustafsson and Vaihela (1981) described two types of frontal glands in plerocercoids of Diphyllobothrium dendriticum Type I glands, they stated, disappeared while Type II remained as the plerocercoid developed to the adult worm. Although the precise chemical composition of the glands is not fully known the authors believed that they play an adhesive role -enabling the tapeworm to attach to host tissue. However Kwa (1972) did not find any glands in the scolex of the plerocercoid of Spirometra erinacei (Cestoda: Pseudophyllidea) but after ultrastructural observation described the presence of organelles which bear granules. He suggested that the granules might represent proteins transported to the outside and which probably aid in attachment to host tissue.

The body wall structure of metacestodes has been studied and is very well known. Ubelaker (1983) in his review, stated that all metacestodes have their external surfaces covered by a tegument inherited from the oncosphere. The tegument

consists of a distal cytoplasm connected to underlying cytons by thin cytoplasmic processes - the internuncial processes. The distal cytoplasm is distinguished by a brush-border of microtriches which are retained as the tapeworm develops to the adult stage. Charles (1970) identified microtriches in procercoids of Schistocephalus solidus and Ligula intestinalis as early as 2 to 4 days after penetration of oncospheres into the copepod haemocoel. Bråten (1968 a,b) compared the tegumentsof procercoid, plerocercoid and adult of Diphyllobothrium latum and revealed changes in the adult tapeworm including an increase in size and number of mitochondria, an increase in the length and number of microtriches and the disappearance of "lamellated bodies" present in the procercoid and plerocercoid.

Like adult cestodes, metacestodes depend on the host for nutritional requirements which are obtained via their body surfaces. Polysaccharides, fats and hydrolytic enzymes like alkaline phosphatase, acid phosphatase and non-specific esterase have been demonstrated by histochemical studies on some metacestodes. These enzymes wherever they occur also indicate intense metabolic activities. Takahasi (1959) reported on heavy glycogen deposits and positive reaction to tests for alkaline and acid phosphatases in the "cuticle" and"subcuticlar cells" of Diphyllobothrium mansoni. A similar report was repsented on the plerocercoid of Ligula intestinalis by Arme (1966).

Vik (1954), Awachie (1966a) and Amin (1978) have noted that the procercoid of C. truncatus can grow to a large size within the body cavity of the amphipod. Awachie (1966) emphasized

on the "rather large" procercoid in the haemocoel of Gammarus pulex but could not do any work on effects of infection on the gammarid because of the low rate of incidence of the tapeworm. He however quoted Beckman (1954) who reported that the larva of Cyathocephalus truncatus seemed to induce sterility in female amphipods which he stated lacked gonads. Apart from this report, there has been no other published reports on the possible pathogenic effects of the procercoid of C. truncatus on Gammarus pulex.

Among tapeworms of other families belonging to the order Spathebothridea, Scott and Bullock (1974) reported that females of the amphipods Psammonyx nobilis infected with the tapeworm Bothrimonus sturionis similarly lacked gonads whereas infected males appeared normal. Sandeman and Burt (1972) found that gammarids infected with Bothrimonus sturionis lost normal pigmentation to the extent that the outline of the larva could be distinguished through the body wall. Because of the size of the larva the authors believed that the parasite burden could weigh more than the crustacean host. Also, observations of Stack (1965) revealed that Gammarus zaddachi infected with Diplocotyle sp. became sluggish tending to rise in the water and sticking to the water surface, apparently tooweak to free themselves from the effects of surface tension. The above reports obviously demonstrate that spathebothrid larvae may be markedly pathogenic to the crustacean host. Further contributions on this topic are however required to enable a clear understanding of such host-parasite relationships.

4.3 Materials and methods

Infected Gammarus pulex were sorted out from field-collected amphipods and used in the studies. Only the Stage III procercoids were studied extensively since they were more readily available.

4.3.1 Light microscopy

Sections were at times obtained of both the amphipods and the procercoids intact in the body cavity. In other cases the procercoid was teased out of the amphipod and sectioned separately.

(a) Histology: Standard histological methods were used. Specimens were fixed in Bouin's fluid, washed and dehydrated in graded alcoholds (70%, 90% and 100%) and then cleared in xylene before being embedded in paraffin wax (m.p. 60°C). Sections 10µm thick were made and stained using hematoxylin/eosin and Masson's stains.

(b) Histochemical studies: Fresh living specimens of C. truncatus Stage III procercoids dissected out of G. pulex were washed briefly in 0.9% saline and fixed immediately in 10% buffered formalin at 4°C for 12h, washed and dehydrated rapidly in series of acetone of concentrations 70%, 90% and 100%. Specimens were then embedded in paraffin wax (m.p. 41°C). 10µm-thick sections were obtained, stained, washed in water and mounted directly in glycerol jelly.

(i) Periodic acid Schiff's (PAS) stain was used for demonstration of glycogen materials as stated by Humason (1962) and Pearse (1972).

(ii) Lipid was demonstrated by staining with oil-red-'O' (Humason, 1962).

(iii) Alcian blue stain was used for acid mucopoly-saccharide demonstration.

(c) Enzyme histochemistry: Sections were obtained as stated above for histochemical studies but different incubating media were used for demonstration of the enzymes thus:

(i) Alkaline phsophatse activity was demonstrated using the Naphthol - AS - BI phosphate method. (Appendix 2) The incubating medium containing the substrate (Naphthol AS - BI phosphate) and Red violet LB salt was maintained at pH 8.3. Sections were rapidly dewaxed in xylol and also hydrated rapidly in graded acetone in the order 100%, 90%, 70% and 50%, then washed in water before incubating at 37°C for 3h. Sections were then washed in water and mounted in glycerol jelly.

(ii) Acid phosphatase activity was demonstrated using the same method above for alkaline phosphatase but with the pH of the incubating medium maintained at 5.2 (see Pearse 1972; Hassall and Jennings, 1975). Components of the incubating medium is given as for alkaline phosphatase in Appendix 2.

(iii) Non-specific esterases was demonstrated by the Indigogenic method of Holt (1958) using an incubating medium containing O- acetyl- 5- bromoindoxyl as substrate (Appendix 3). The esterase was characterised into A-, B- and C- types as stated by Pearse (1972) and Hassall and Jennings (1975).

(iv) The distribution of lipase enzyme was investigated using an incubating medium containing Tween 60 (method of Gomori (1952) as given in Appendix 4).

Controls: Three control methods were used to ensure that the results obtained were accurate as reported.

1. Heat control Control sections were subjected to a temperature of 90°C before incubation. At such a temperature enzymes become denatured and on incubation gives no reaction thus enabling the elimination of any reaction that might be positive but is not enzymic.

2. Substrate elimination Control sections were incubated in a medium in which the substrate has been eliminated and substituted with 0.9% saline. This is also to avoid a false positive reaction that might be due to components of the incubating medium other than the substrate.

3. Tissues of the parasite host already known to be positive to the enzymes tested for also serve as controls.

4.3.2 Electron microscopy

A single specimen each of the stages I, II and III procercoids was studied by transmission electron microscopy.

The procercoids were teased out of the amphipods and fixed immediately for 2h at 18°C in 3% glutaraldehyde buffered to pH 7.2 with Millonigs buffer. Specimens were then cut into pieces of about 1mm thick, rinsed in buffer and dehydrated in graded acetone (70%, 90% and 100%); tissues were bathed in epoxyl propane before being embedded in araldite. (Appendix 5).

Semithin sections (0.5μm thick) were first cut stained with Toluidine blue and examined under the light microscope in order to find and identify the areas to be sectioned for ultra-structural studies. Then thin sections (60nm to 90nm thick silver and gold sections) were obtained, picked up on coated grids and double stained with 5% Uranyl acetate and 10% Reynold's lead citrate. Sections were then examined in an AEl EM 6B transmission electron microscope.

4.3.3 Observations on pathogenic effects

As well as conducting light microscope studies on histological sections of host tissues for possible damage of the tissues, visual observations were also made on the behaviour of the few Gammarus pulex harbouring large procercoids of Cyathocephalus truncatus. The infected gammarids were sorted out from the field-collected stock and observed together with a few non-infected ones.

Weights of infected G. pulex and of the procercoid were determined by teasing apart the infected amphipod releasing the tapeworm in a pre-weighed aluminium foil boat which was then put in a drying oven at 60°C overnight. By weighing separately the dried amphipod and the tapeworm with the aluminium foil boat, the dry weights of the G. pulex and of the tapeworm were determined. Similarly dry weights of non-infected G. pulex of the same length as the infected ones were determined for comparison.

4.4 Results and Observations

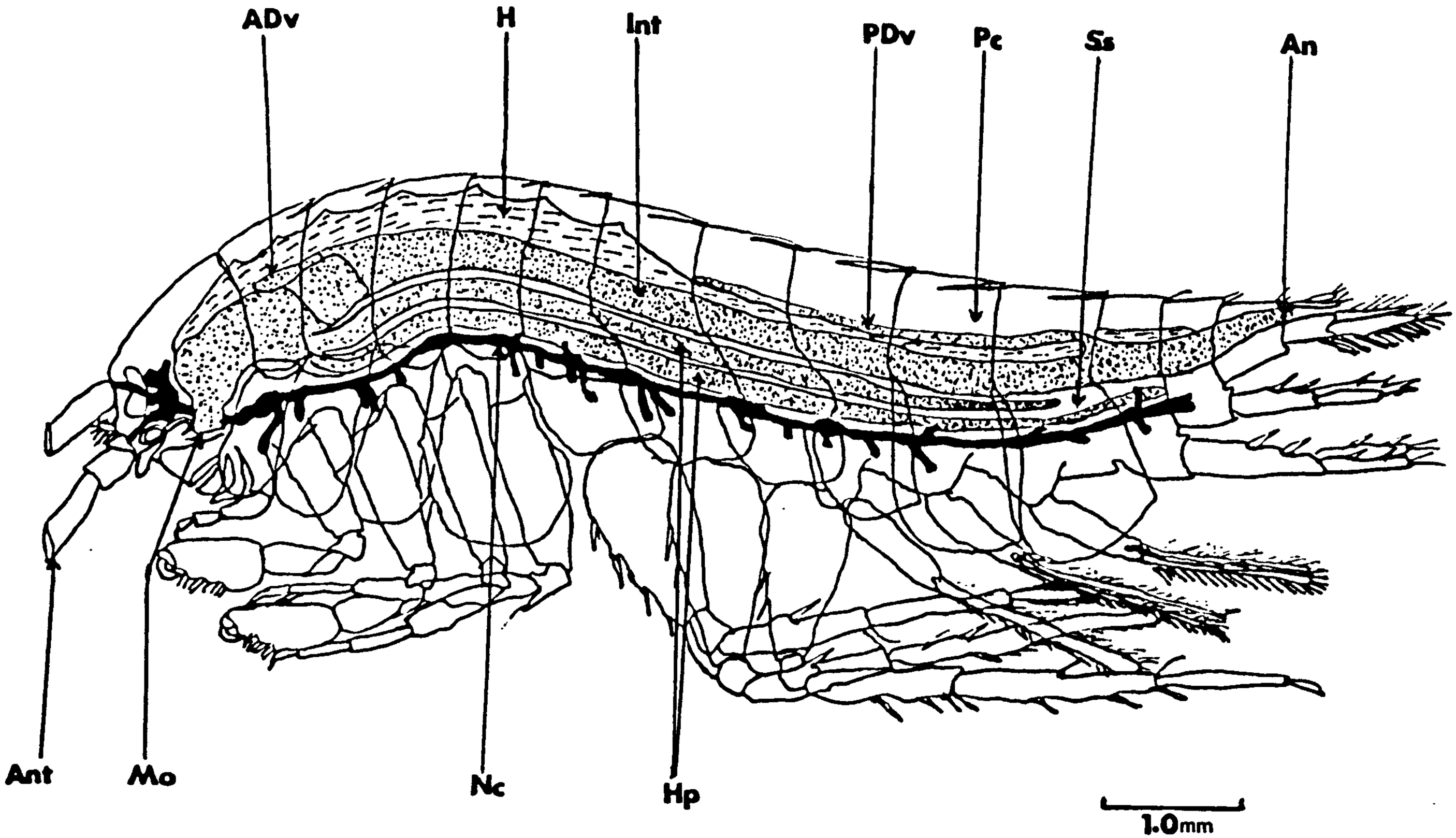
4.4.1 Structural studies

(a) Histological studies of C. truncatus procercoid and G. pulex host

The anatomy of Gammarus pulex has been well described by Cussans (1904), Lincoln (1979) and McLaughlin (1980). As shown diagramatically in Fig. 4.1 the alimentary system of G. pulex consists of a straight canal which branches antero-ventrally to form two pairs of backwardly directed and long diverticula (hepatic caeca or hepatopancreas). The members of one pair lie on either side of the intestine while those of the second pair are located ventral to the intestine as shown in a transverse section (Plate 4.2A). The intestinal canal also branches anterio-dorsally to form a single short anterior diverticulum. Posteriorly the intestinal canal again branches dorsally to form a pair of diverticula (posterior diverticula) that are long and forwardly directed. (Fig. 4.1)

Fig. 4.1 Anatomical representation of Gammarus pulex viewed from the left side.
(Musculature removed to show major organ systems)

ADv	..... Anterior diverticulum
An	..... Anus
Ant	..... Antenna (partly represented)
H	..... Heart
Hp	..... Hepatopancreas
Int	..... Intestine
Mo	..... Mouth
Nc	..... Nerve cord
PDv	..... Posterior diverticulum
Pc	..... Pericardial cavity
Ss	..... Sternal sinus


ADv
H
Int
PDv
Pc
Ss
An
Ant
Mo
Nc
Hp
1.0mm

The intestinal canal occupies a rather central position of the amphipod surrounded by its diverticula, connective tissues and fat bodies which occupy the greater part of the body cavity. The fat bodies although appearing free are actually held by fine fibres to the intestinal wall and the inner surface of the exoskeletal plates of the body wall. The single heart and pair of gonads are located dorsally to the intestinal canal.

(i) Site of occurrence of the procercoid in G. pulex :

Procercoids of Cyathocephalus truncatus were always found lying passively either among the two pairs of hepatic caeca ventral to the intestine or by the side of the intestine but were never seen to be located dorsal to it; the latter is the position occupied by the gammarid heart and gonads. Because of its large size the Stage III procercoid is usually folded over once or twice (Plate 4.1) within the ventral space thus displacing and pushing the hepatic caeca and intestine upwards to a doral position (Plate 4.2.B).

Although displaced, the intestinal wall and hepatopancreas of infected Gammarus pulex were similar in histological structure to those of non-infected gammarids. Similar observations were also made in amphipods infected with the juvenile stages of other helminths such as Echinorhynchus truttae. However infected amphipods usually have fewer fat bodies than non-infected individuals. In some cases, the fat bodies appear to be absent within the body cavity except for a few still stuck to the inner surface of the exoskeleton.

The apparent absence of structural damage to organs like the intestine and hepatopancreas in the body cavity of the amphipod caused by the procercoid is probably because the latter is entirely enclosed in a sheath seen in a transverse section as a thin superficial coat.

(ii) Structure of the procercoid: The external morphology of the Stage III procercoid has been described above (page 46). In its internal structure the procercoid is similar to the adult tapeworm except that some organs of the procercoid such as uteri, sperm cells and vitellaria of the reproductive systems are not fully developed; they are in most cases not clearly distinguishable.

In the scolex and strobila, the tegument, the medullary located band of longitudinal muscles, cortical transverse muscle fibres and subtegument longitudinal and circular muscles are clearly marked out. The testes are laterally borne in the medulla while the ovary is located at a central position in the modulla. Pairs of genital openings (cirrus apertures and vagino-uterine apertures) alternate irregularly on either flat side of the worm along the strobila. The cirrus and vagino-uterine apertures connect with the cirrus organ and vagino-uterine duct respectively which are both located in a subtegumental position.

The structures of these organs have been described in the adult Cyathocephalus truncatus by Cooper (1918), Wardle (1932) Wardle and McLeod (1952) and Vik (1954).

(b) Histochemical studies

The results of reactions shown by the procercoid of C. truncatus and organs of the body cavity of Gammarus pulex to certain histochemical procedure are presented in Tables 4.1 and 4.2.

(i) The procercoid: In the sheath, a strongly positive reaction was obtained for glycogen and acid mucopolysaccharide but only a weakly positive reaction for lipid (Plate 4.4). Strongly positive reactions were also demonstrated for glycogen, acid mucopolysaccharide and lipid in the tegument but in the reproductive system, apart from the ovary, reactions were weakly positive. Similar positive reactions were obtained in the scolex, musculature and excretory ducts.

(ii) Gammarus pulex: Strongly positive reactions were demonstrated in the lumen and mucosa of the intestine and hepatopancreas for glycogen, acid mucopolysaccharide and lipid. The gonads and fat bodies also gave positive reactions.

(c) Enzyme histochemistry

(i) The procercoid: The sheath gave a negative reaction to tests for alkaline and acid phosphatases, a weakly positive reaction for non-specific esterase and a weakly positive reaction for lipase. (Table 4.1 and Plates 4.5, 4.6). Positive reaction for the enzymes occurred in the distal cytoplasm. Tegumentary cytons, the musculature and parenchyma of the strobila showed only weakly positive or negative reactions to

tests for enzyme activity as shown in Table 4.1. In the scolex, though, there were extensive and strongly positive reactions particularly for phosphatases in the tegumentary cytons. (Plate 4.5)

(ii) Gammarus pulex: Acid and alkaline phosphatase reactions were found in the distal portion of the mucosa of the intestine and hepatopancreas, and in the gonads, Non-specific esterase activity was particulary prominent in the hepatopancreas and gonads (Plate 4.7). All these enzymic activities were either positive or weakly positive in the fat bodies.

(iii) Characterisation of the esterases:

A-, B- and C- type esterases are recognised by histochemists and according to Pearse (1972) these are convenient terms of reference for esterase enzyme systems that may include a variety of similar but not necessarily identical enzymes. Basically, A- type esterases are similar in reaction to protease - like enzymes, B-type esterases are similar to the cholinesterases type while C-type esterases resemble lipases.

In the present study, the effects of inhibitors and activators on the non-specific esterase in the procercoid of C. truncatus as well as in the Gammarus pulex host are given in Table 4.3. The esterase activity was not diminished by preincubation of sections in 10^{-5}M diethyl P- nitrophenyl phosphate (ie E-600) indicating the probable absence of cholinesterase and in general

B-type esterases in both the procercoid and G. pulex tissues; this was confirmed by the total inhibition observed with 10^{-2}M silver nitrate.

However sections of the procercoid and of tissues of Gammarus pulex were activated by 10^{-3}M Cysteine which is characteristic of A- esterases and by 10^{-4}M sodium p-chloromecuri-benzoate and 10^{-3}M lead nitrate which is characteristic of C-esterases. Thus it is concluded that the non-specific esterase reactions obtained at the sites named are produced by a mixture of A- and C-type esterases.

Table 4.1 Distribution of some enzymes and reactions to certain histochemical Stains in the procercoid of Cyathocephalus truncatus from Gammarus pulex.

Tissue or Organ tested	Alkaline phosphatase	Acid phosphatase	Non-specific esterase	Lipase	PAS stain	Alcian blue stain	Oil red 'O' stain
Scolex							
Sheath	-	-	*	*	***	***	*
Distal Cytoplasm	***	***	***	***	***	***	***
Teg. Cytons	***	***	***	***	***	***	***
Musculature	*	*	* ?	*	**	*	**
Parenchyma	*	*	*	*	*	*	*
Excretory ducts	**	*	-	*	**	**	**
Strobila							
Sheath	-	-	**	*	***	***	*
Distal Cytoplasm	***	***	***	***	***	***	***
Teg. Cytons	*	*?	*	*	***	***	***
Musculature	-	-	-	-	**	*?	*
Parenchyma	-	-	-	-	*	-	*
Excretory ducts	*?	-	-	-	*	*?	*
Vitellaria	-	-	-	-	-	-	-
Testes	-	-	-	-	-	-	-
Ovary	**	*	*	**	**	**	**
Uterus	**	*	*	**	*	*	*?
Cirrus	*	-	*?	*?	*	*	*
Vagino-uterine duct	*	-	*	*?	*	*	*

KEY

*** ..... Strongly positive
** ..... Positive
* ..... Weakly positive
*? ..... Reaction uncertain
- ..... Negative

Table 4.2 Distribution of some enzymes and reactions to certain histochemical stains in the alimentary tract and in body cavity of Gammarus pulex.

	Alkaline phosphatase	Acid phosphatase	Non-specific esterase	Lipase	PAS stain	Alcian blue stain	Oil red 'O' stain
Intestinal body wall	-	-	-	-	*	*	*
Intestinal lumen	**	**	*?	*?	***	***	**
Intestinal mucosa	**	**	*	*	***	***	***
Hepatopaucreas lumen	**	**	**	*	***	* **	***
Hepatopancreas mucosa	**	**	***	**	***	***	***
Hepatopancreas body wall	*?	*?	**	-	*	*?	*?
Fat bodies in body cavity	*	*	**	**	**	**	**
Gonads	**	**	***	*	**	**	**

KEY
*** ..... Strongly positive
** ..... Positive
* ..... Weakly positive
*? ..... Reaction uncertain
- ..... Negative

Table 4.3: Characterisations of esterases in the procercoid of C. truncatus and in tissues of Gammarus pulex using suitable activators and inhibitors.

	10^{-5}M E600	10^{-2}M $AgNo_3$	10^{-3}M Cysteine	10^{-4}M PCMB	10^{-3}M $Pb(No_3)_2$
(A) Procercoid larva					
Sheath	+	-	±	+	±
Distal Cytoplasm	+	-	+	+	+
Tegumentary Cytons	+	-	+	±	±
Musculature	+	-	+	-	±
Parenchyma	+	-	+	-	-
Excretory ducts	-	-	-	-	-
Vitellaria	-	-	-	-	-
Testes	-	-	-	-	-
Ovary	+	-	-	±	±
Uterus	+	-	+	+	+
Cirrus organ	-	-	-	-	-
Vagino-Uterine organ	-	-	-	-	-
(B) Gammarus pulex					
Small intestine body wall	-	-	-	-	-
Small intestine lumen	+	-	+	±	±
Small intestine mucosa	+	-	+	±	±
Hepatopancreas lumen	+	-	±	±	-
Hepatopancreas mucosa	+	-	+	+	+
Hepatopancreas body wall	+	-	+	±	±
Fat bodies in body cavity	+	-	+	-	±
Gonads	+	±	±	+	±

Note
$AgNo_3$ = Silver nitrate
PCMB = Sodium p-chloromecuribenzoate
$Pb(No_3)2$ = Lead nitrate
E-600 = diethyl p-nitrophenyl phosphate

Key
+ ..... No action
± ..... partial inhibition
- ..... Complete inhibition

4.4. 2. Ultrastructural studies

The structural plan of the procercoid of C. truncatus is similar to that previously described for metacestodes of other tapeworm species by several authors and included in the review of Ubelaker (1983). Each individual consists of a central core or medulla of parenchyma surrounded by a thin layer of distal cytoplasm the outer free surface of which is thrown into digitiform projections (the microtriches). Beneath the distal cytoplasm is the basal lamina underlain by layers of circular and longitudinal muscles. Cytoplasmic processes (internuncial processes) penetrate the basal lamina and pass between bundles of muscles at intervals connecting the distal cytoplasm with cell bodies of the basal epidermis (tegumentary cytons) located beneath the muscle layers in the region of the outer medullary parenchyma. The distal cytoplasm bears granules which appear to originate within the tegumentary cytons and are transported outwards via the internuncial processes.

Other structures such as those comprising excretory, nervous and reproductive systems are located in the medulla. These structures are however not highly elaborate in procercoids of C. truncatus and are not described in the present account. The present account deal mostly with the body wall structures of the procercoids and their variations in the Stages I, II and III forms.

Each of the procercoid stages possesses a sheath which entirely envelops the whole body as previously described (page 89). The sheath which is present continuously throughout the life of the procercoid is apparently produced by host reaction.

A description of the sheath as it appears in each procercoid stage is given below.

(a) Stage I procercoid

The Stage I procercoid of C. truncatus studied ultrastructurally measured 2.5mm long and 0.5mm in maximum width. A transverse section of the procercoid is represented diagrammatically in Fig. 4.2 and transmission electron micrographs of portions of it are shown in Plate 4.8.

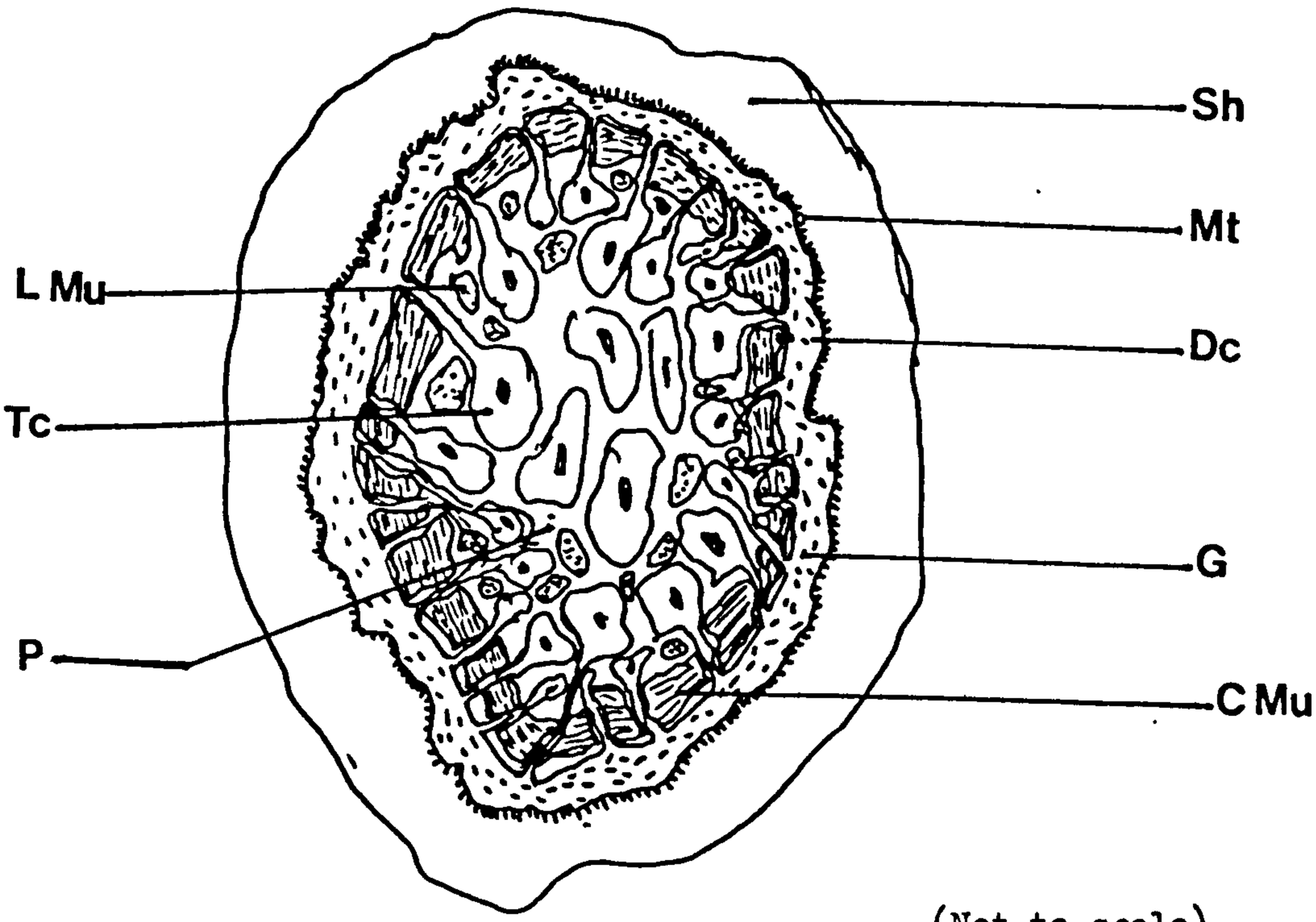
The sheath is about 6.5µm thick. The material constituting the sheath is difficult to describe ultrastructurally but appears to be tiny deposits of substance bonded together (Plate 4.8). Reactions of the sheath to certain histochemical tests are described on page 127.

The sheath does not only occur as an outer body covering but also its materials extend into the speaces between individual microthrix. The sheath thus appears to embed the microtriches.

The microtriches are few and small in size (Plage 4.8B) Each microthrix measures about 0.6µm long and consists of a stout basal part measuring 0.2µm long and an electron-dense apical cap which is about 0.4µm long. At the base plate, the microthrix is about 0.2µm thick.

The distal cytoplasm possesses an undulating outer surface (Fig. 4.2) and consequently it shows some variations in thickness (0.5µm to 2µm.) The granules within the distal cytoplasm are ovoid-shaped and rod-shaped.

Fig: 4.2 Diagrammatic representation of the transverse section of the Stage I procercoid of C. truncatus. Ultrastructure of the sheath and body wall are shown in Plate 4.8.



(Not to scale)

CMu ..... Circular muscle
Dc ..... Distal cytoplasm
L.Mu ..... Longitudinal muscle
Mt ..... Microtriches
Sh ..... Sheath
Tc ..... Tegumentary cyton
G ..... Granule in the distal cytoplasm.
P ..... Parenchyma

Bundles of circular and longitudinal muscles located in the outer medullary parenchyma are hot compacted together but have wide spaces of connective tissues between them. Each bundle of muscle fibres bear granules of glycogen and mitochondria.

Tegumentary cytons of the outer medullary parenchyma as well as cells of the inner medullary parenchyma are irregularly shaped and few in number. Most of the medulla is filled with the parenchyma which bears deposits of glycogen granules, tiny fibres, mitochondria and fat droplets.

(b) Stage II procercoid

The Stage II procercoid of C. truncatus studied ultrastructurally measured 4mm in length.

(i) The scolex:- Because of the tubular-shaped structure, the scolex possesses an outer surface and an inner surface (ie. in the scolex cavity). The scolex margin possesses the distal cytoplasm on both inner and outer surfaces with an inner medullary region between both surfaces as shown in Fig. 4.3.

In the Stage II procercoid of C. truncatus, both surfaces were not found to differ in structure and only the inner scolex surface is presented in Plate 4.9B.

The sheath at the scolex measures about 4µm in depth and appears in two bands - an upper denser band (about 0.6µm thick) lying beyond the zone of microtriches and less dense zone in the region of the microtriches (Plate 4.9B). The latter zone

bear vesicles whose origin is not clear but which appear to be released from the distal cytoplasm.

Microtriches are few and scattered and only one type is seen compared to two types found on the distal cytoplasm of the strobila as described below. The microtriches measure about 2.6µm in length.

The distal cytoplasm of the tegument is undulating in nature and varies in thickness between 0.5µm and 2µm. The bundles of muscle fibres occupy most of the outer medullary region. The only other structures occupying this region are the intermuncial processes and connective tissue. The tegumentary cytons that connect to the distal cytoplasm of the outer scolex surface and the inner scolex surface occur all over the medulla. The cytons have relatively small nuclei but large cytoplasmic space in which are borne numerous cytoplasmic inclusions. Prominent among these inclusions are granules and mitochondria.

(ii) <u>The strobila</u>: The sheath on the strobila is similar in structure to that on the scolex described above and measures about 6µm in depth.

The most distinguishing feature in the structure of the strobila body wall is the presence of two types of microtriches (Plate 4.9A). They are mixed together in occurrence and are of the same structural plan except that one type is longer than the other.

1. Type I: These are elongate and broad. Each measures about 5µm in length. The broad electron-dense apical cap is about 0.5µm long and 0.3µm wide at the base plate. The basal stalk connecting with the distal cytoplasm is about 4.5µm in length and 0.2µm across.

2. Type II: The shorter microtriches. Each of these is about half the length of type I and measure about 3µm long. Each microthrix also terminates in an electron-dense apical cap which measures about 0.3µm in length and about 0.1µm wide at the base plate.

Both types of microtriches have their apical tips posteriorly directed and angled to the basal stalk on which they are borne. Some of the type II microtriches, however, have their apical tips directed anteriorly or laterally as can be seen in Plate 4.9A.

The distal cytoplasm is about 5µm thick and is not an undulating layer as in the Stage I procercoid. The cytoplasm is rich in ovoid-shaped and rod-shaped granules. A felt-like layer of the basal lamina limits the distal cytoplasm with the medullary parenchyma. The muscle bands beneath the basal lamina are more compact than those of the Stage I procercoid and occupy a small area of the outer medulla. In the medulla are numerous tegumentary cytons and cell bodies which have not yet differentiated into any organ. Connective tissue surrounding these cells have been much reduced to tiny canals.

(c) Stage III procercoid

The Stage III procercoid of C. truncatus studied, measured 8mm in length.

(i) The scolex:

(1) Outer scolex surface:- The structure of the outer scolex surface is similar to that of the Stage II procercoid although there are a few differences. (Fig. 4.3).

As shown in Plate 4.10, the sheath is about 4µm in thickness and similar in structure to that of the scolex of Stage II procercoid.

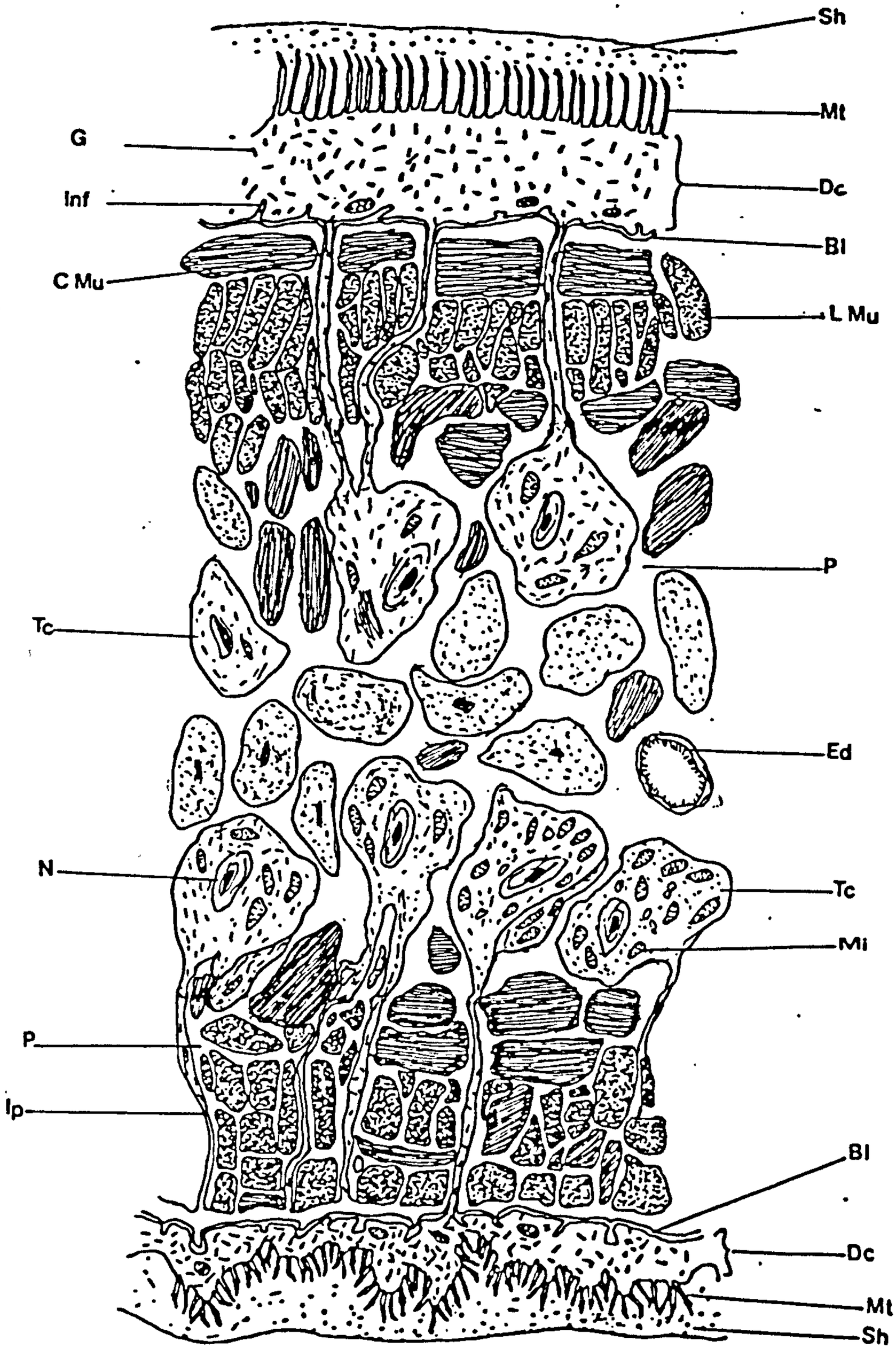
Only one type of microthriches is found in this region and each possesses an apical cap which is either slightly bent and posteriorly directed as those in the strobila or kept straight. The microtriches are rather short measuring 2µm and 2.5µm long and spike-like in form. They occur at different levels due to the undulating outer surface of the distal cytoplasm. The microtriches appear compact sometimes occurring as bunches on the upper elevation of the distal cytoplasm surface and at places two microtriches appear to branch from a single basal stem (Plate 4.10B). This feature was always observed among microtriches of the outer and inner scolex surfaces of the Stage III procercoid.

The distal cytoplasm possesses numerous granules and in addition there are vesicles and mitochondria. There are extensions of the felt-like layer of the basal lamina into the region of the distal cytoplasm. The connective tissue underlying the felt-like layer is about 0.3µm thick.

Fig 4.3: Diagramatic representation of transverse section ultrastructure of the scolex margin of Stages II and III procercoid of C. truncatus. Not to scale.

BL	.....	Basal lamina
CMu	.....	Circular muscle
Dc	.....	Distal cytoplasm
Ed	.....	Excretory duct
G	.....	Granule
Inf	.....	Extension of basal lamina in the region of distal cytoplasm
Ip	.....	Internuncial process
LMu	.....	Longitudinal muscle
Mt	.....	Microtriches
Mi	.....	Mitochondrion
N	.....	Nucleus
P	.....	Parenchyma
Sh	.....	Sheath
Tc	.....	Tegumentary cyton

OUTER SCOLEX SURFACE



INNER SCOLEX SURFACE

There is extensive musculature in the body wall of the outer region of the scolex. Beneath the basal lamina are closely packed bundles of longitudinal circular and oblique muscle fibres. Transverse fibres also exist in the region and across the medulla to connect with muscles of the body wall of the inner region of the scolex. (Plates 4.11 and 5.13A). The internuncial processes and connective tissues appear as tiny canals in between the bundles of muscle fibres.

Because of the extensive musculature, the tegumentary cytons that connect with the distal cytoplasm of both the outer and inner scolex surfaces occur all over the region of the medulla. These cytons possess relatively small nuclei and large cytoplasmic space in which are borne numerous electron-dense bodies, granules and mitchondria (Plate 4.12).
The cytons appear as secretory bodies and were positive to tests for phosphatase enzymes as reported above (page 128).

(2) Inner scolex surface: As shown in Plate 4.13, the sheath is similar to that described for the inner surface scolex of the Stage II procercoid.

The surface of the distal cytoplasm is highly undulating and irregular. In some places the distal cytoplasm is about 4µm thick while in others it is highly reduced to less than 1µm thick or is almost absent. The basal lamina pushes up at frequent intervals to form extensions in the region of the distal cytoplasm. In certain other places the extensions are deep.

Owing to the undulating surface of the distal cytoplasm the microtriches are borne at different levels. Some of them as shown at high magnification (Plate 4.13B) appear to be borne 2 to 3 on a common basal stem. The microtriches are spike-like and are essentially similar to those of the outer scolex surface described above.

The musculature below the distal cytoplasm is extensive and comprise of numerous bundles of circular, longitudinal and transverse fibres.

(ii) <u>The neck</u>: This region is prominent in the Stage III procercoid and less so in the Stage II procercoid.

The outer layer of the sheath (similar to that reported for the outer scolex surface) appears very dense and in form of a surface coat (Plate 4.14). This portion of the sheath measures about 1µm in thickness. Material of the sheath is also seen in the region of the microtriches.

Microtriches of the neck region is of one type (the type II) and measure about 2.5µm. The surface of the distal cytoplasm on which they are borne is slightly undulating. The distal cytoplasm measures about 4µm in thickness.

The most notable feature of the neck region is the musculature (Plate 4.14B). The muscles comprise of circular, longitudinal and oblique fibres similar to those of the scolex region. They occupy most part of the outer medullary parenchyma and are rich in deposits of glycogen granules and mitochondria. Tegumentary cytons in this region are few and are located in the region of the medulla.

(iii) The strobila: The surrounding sheath retains its structure and thickness similar to those of other regions already described.

As shown in Plate 4.15 the two types of microtriches retain their separate identities as in the Stage II procercoid and measure about 5µm long for type I and about 2.5µm long for type II.

Features of the distal cytoplasm and the musculature in the body wall of strobila of Stage III procercoid are similar to those described for the Stage II procercoid (page 137).

Cell bodies in the medullary parenchyma are compacted together and have not been differentiated into various recognisable organs. There are numerous glycogen granules, fat droplets, tiny fibres and mitochondria in the parenchyma. The excretory ducts and protonephridial organs are however distinctly distinguishable from the medullary cells. The excretory organ is similar to those of the adult tapeworm and of other cestodes as described by Lumsden and Hildreth (1983)

4.4.3 Effects of infection on G. pulex

The apparent lack of structural damage to tissues of infected Gammarus pulex has already been noted. Physiological effects resulting from the displacement of intestine and hepatopancreas of the amphipod by the tapeworm were not investigated.

Infected gammarids were observed to tolerate aquarium or laboratory environments and could live for as long a time as the non-infected amphipods. They also feed normally like the non-infected amphipods.

In movement, the infected G. pulex were usually sluggish, hanging on to leaves and stems of plants for most of the time. They usually stay resting on plants just beneath the water surface and rarely at the bottom of the container.

As well as possessing gonads which appear structurally normal (Plate 4.3) the infected amphipods were observed to be involved in copulation and female gammarids in addition were seen to carry their broods normally.

Table 4.4 shows the mean dry weights of G. pulex and of C. truncatus procercoids harboured by the gammarids. The dry weights of non-infected gammarids of similar length are also provided. From the result, the procercoid of C. truncatus is seen to account for a mean weight of 9% of the total dry weight of infected amphipods while that of the infected gammarid only accounts for the rest, 91%. In a comparison of the weights of gammarids of similar length, non-infected individuals were seen to weigh less than intact infected specimens plus tapeworm but weigh more than infected individuals excluding the weight of the worm. Consequently in terms of weight, the infected amphipod with the worm intact could be regarded as a good meal but with a price to pay for the fish.

Table 4.4: Dry weights in (g) of non-infected Gammarus pulex, those infected with procercoids of Cyathocephalus truncatus and of the procercoid harboured by the amphipod.

No.	Length G. pulex (mm)	Wt. uninfected G. pulex	Wt. infected G. pulex with tapeworm	Wt. infected G. pulex only	Length C. truncatus (mm)	Wt. C. truncatus
1	9	0.00915	0.00957	0.00877	11	0.00080
2	7	0.00683	0.00702	0.00646	8	0.00056
3	6	0.00625	0.00634	0.00600	6	0.00034
4	9	0.00891	0.00924	0.00848	11	0.00072
5	5	0.00306	0.00344	0.00273	9	0.00071
6	9	0.00913	0.00981	0.00874	15	0.00107
7	8	0.00727	0.00768	0.00684	12	0.00084
8	7	0.00719	0.00750	0.00699	8	0.00051
9	8	0.00782	0.00805	0.00757	8	0.00048
10	12	0.00934	0.00962	0.00899	10	0.00063
11	7	0.00662	0.00715	0.00642	9	0.00073
12	10	0.00897	0.00933	0.00879	8	0.00054
13	8	0.00721	0.00787	0.00706	12	0.00081
14	6	0.00620	0.00663	0.00597	9	0.00066
15	6	0.00619	0.00640	0.00603	5	0.00037
16	7	0.00705	0.00740	0.00658	10	0.00082
17	10	0.00933	0.00987	0.00893	13	0.00094
18	7	0.00680	0.00715	0.00653	9	0.00062
19	10	0.00906	0.00974	0.00871	15	0.00103
20	8	0.00712	0.00760	0.00684	10	0.00076
21	5	0.00587	0.00613	0.00552	8	0.00056
22	8	0.00723	0.00788	0.00703	12	0.00085
23	12	0.00958	0.01011	0.00905	17	0.00106
24	7	0.00664	0.00694	0.00647	8	0.00047
25	6	0.00608	0.00651	0.00587	9	0.00064
26	8	0.00712	0.00744	0.00691	8	0.00053
27	7	0.00691	0.00742	0.00664	11	0.00078
28	5	0.00578	0.00604	0.00561	7	0.00043
29	9	0.00816	0.00876	0.00782	13	0.00094
30	8	0.00694	0.00759	0.00676	10	0.00083
Mean	8	0.00733	0.00774	0.00704 (91%)	10	0.00070 (9%)

Discussion

The structure and ultrastructure of the procercoid of Cyathocephalus truncatus as observed in the present study show certain notable features.

One of these features is the sheath that envelops the tapeworm procercoid in the body cavity of the intermediate host. A similar transparent body was also observed to envelop the juveniles of other helminth parasites in Gammarus pulex notably the acanthocephalans. These were however not studied ultrastructurally in the present work.

The sheath is not a rigid body wall and it is possible that it undergoes a process of gradual wearing as well as continuous replacement from its source due to growth and metabolic activities of the tapeworm and the host. The origin of the sheath and its maintenance within the gammarid are not known. It is however not similar to the "capsule-like" cyst described by Wisniewski (1932b). He suggested that it was probably produced by 'hypodermal cells' of the amphipod and stated that although tapeworms could free themselves from the cysts they could also die within the cysts. This suggests that the cysts were hard surface coats. No such cyst was seen in this study in any of the procercoid stages of C. truncatus in Gammarus pulex.

The sheath seems to be protective in function - either for the host G. pulex against being physically harmed by the parasite or for the tapeworm against the reaction of host tissues to its presence. With positive reaction to tests for polysaccharides, lipids and glycogen and negative reaction to tests for enzyme

activities, the sheath might only be permeable to nutrient substances and does not seem to possess toxic substances harmful to the amphipod's body cavity organs with which the sheath get in contact. This is probably why the intestine and hepatopancreas were only seen to be displaced and also explains why infected amphipods are able to tolerate infection for months while still apparently physiologically active like non-infected individuals.

Another feature is the two types of microtriches in the strobila of C. truncatus procercoid. As can be distinguished from the Stage I procercoid which has only one type of microthrix, the origin of the two types in the Stages II and III procercoids is not known. It could also not be traced because of lack of regular specimens of the growing stages. Their origin can however be traced by ultrastructural study of the Stage I procercoid and all successive procercoids to it at the early stages of infection. The fate of the two types of microtriches when the procercoid arrives in the fish host is discussed below (page 185).

The scolex of the stage III procercoid presents features which give an insight to its suitability as an attachment organ. The elaborate and dense musculature in the neck and scolex presumably enable the funnel-shaped scolex to achieve a firm grip of the host tissue. Friction that could result from the sucker-like attachment is possibly eliminated by the sharp pointed microtriches which are pushed into the host tissue thereby holding the host tissue firmly with the scolex 'funnel'. The undulating nature of the inner scolex surface, the clustering and

the uneven nature of the microthrix layer could also ensure a firm grip to host tissue and counter forces that might dislodge it.

From observations on structural similarity between the inner and outer scolex surfaces of the Stage III procercoid it is also possible for attachment to be formed with the host tissue on the outer scolex surface. This could support observations in established adult tapeworms where the holdfast is sometimes seen to be totally embedded in the fish tissues to which it is attached. An ultrastructural observation of C. truncatus attachment is described below (page 182).

Actual glands were not seen in the scolex of C. truncatus procercoid but the tegumentary cytons have become modified into areas of high metabolic activity and appear glandular in that they are relatively larger in size than those of the strobila, give strongly positive reaction to tests for phosphatase enzymes and possess numerous granules and mitochondria in the large cytoplasmic space. These cytoplasmic bodies carried through the internuncial processes into the distal cytoplasm in the area of attachment probably contain proteinaceous substances which cause breakdown of tissues to which the worm is firmly attached when it gets into the fish host. Similar granules have been described to occur in penetration glands of Hymenolepis diminuta oncospheres by Lethbridge and Gijsbers (1974) who believe that the granules certainly play some roles which, although unknown, help the oncosphere to gain access into the body cavity of the intermediate host.

The possible functions of the gramules in the tegumentary cytons of the scolex in the Stage III procercoid is discussed with those of the adult tapeworm (in page 192).

The positive reactions demonstrated in the entire tegument and other organs of the procercoid to tests for phosphatase enzymes ~~and~~ and non-specific esterases is similar to the reports of Lee et al (1963) and Arme (1966), among other authors, of the presence of these enzymes in other tapeworms. This indicates that the mode of nutritional uptake in C. truncatus is possibly similar to that of other tapeworms. The absence of B-type esterase with the presence of A- and C- types in the procercoid of C. truncatus is also similar to the report of Arme (1966) on Ligula intestinalis. It seems also that in Gammarus pulex A- and C- types esterases as specifically characterised in the hepatopancreas could be constituent parts of the digestive secretions from the hepatic caeca into the anterior portion of the intestine.

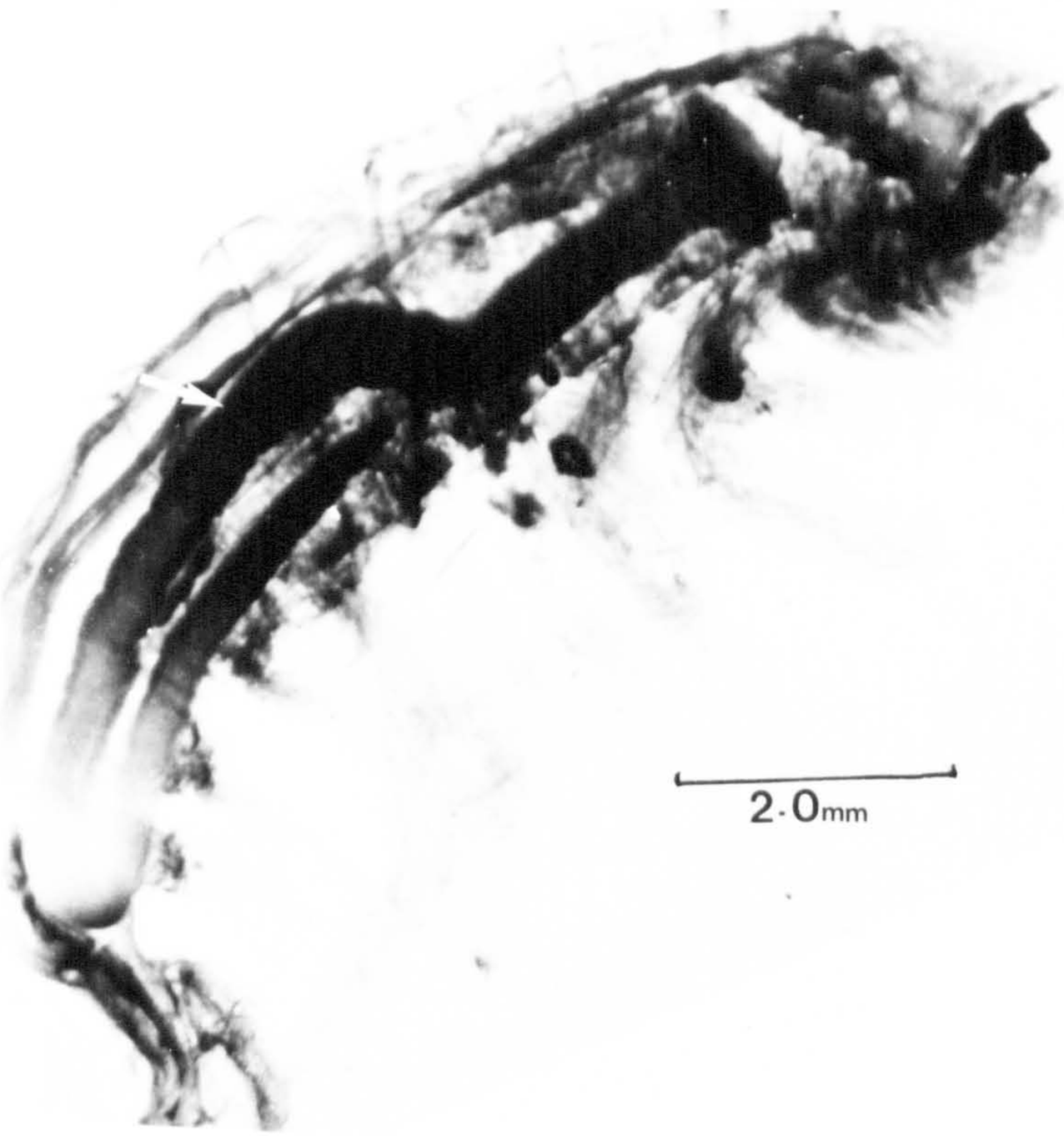
Pathogenic effects of the tapeworm on infected gammarids are evident and include the loss of fat bodies in the body cavity and the possible loss of weight. Since gammarids seem to tolerate the infection for sometime, most other pathogenic effects are likely to be physiological disturbances. The sluggishness of infected G. pulex suggests that the tapeworms certainly affect the amphipod physiologically and this behaviour probably makes them easy prey to fish.

With reference to the reports by Beckman (1954) and Stock and Bullock (1974) concerning the sterility of tapeworm-infected amphipods, while it is possible that the size of the tapeworm procercoid could preclude any form of development or proper physiological function, the infected gammarids examined in the present study were however found to have gonads and were reproductively viable. Further investigations, especially from large stock infection in the laboratory, are however necessary to enable proper understanding of the actual effects of infection.

Plate 4.1 A. Whole mount preparation of Gammarus pulex infected with C. truncatus procercoid (arrowed). Chubb's technique. Scale = 2.0mm

B. Longitudinal section of an infected G. pulex showing a C. truncatus procercoid lying in the haemocoel. Masson 3rees. Scale = 2.0mm.

A



B

Plate 4.2 Transverse sections at the mid-gut position of:

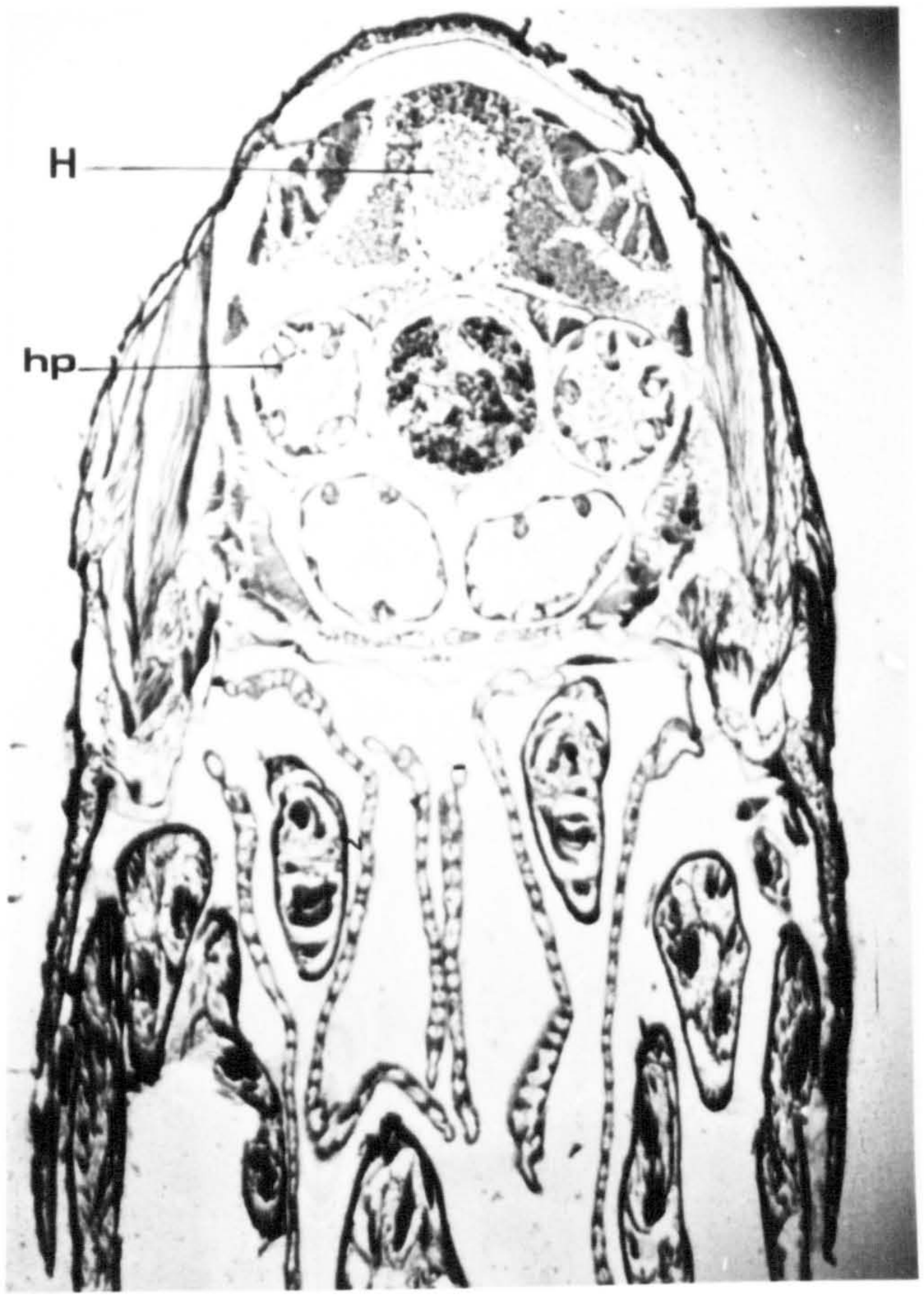
A. non-infected Gammarus pulex
Scale = 1.0mm

B. Gammarus pulex infected with C. truncatus
Scale = 1.0mm

Both with Masson green.

A	.....	Locomotory appendages
B	.....	Body cavity
H	.....	Heart
hp	.....	Hepatopancreas
I	.....	Intestinal canal
N	.....	Nerve cord
T	.....	C. truncatus procercoid

A



1·0mm

B

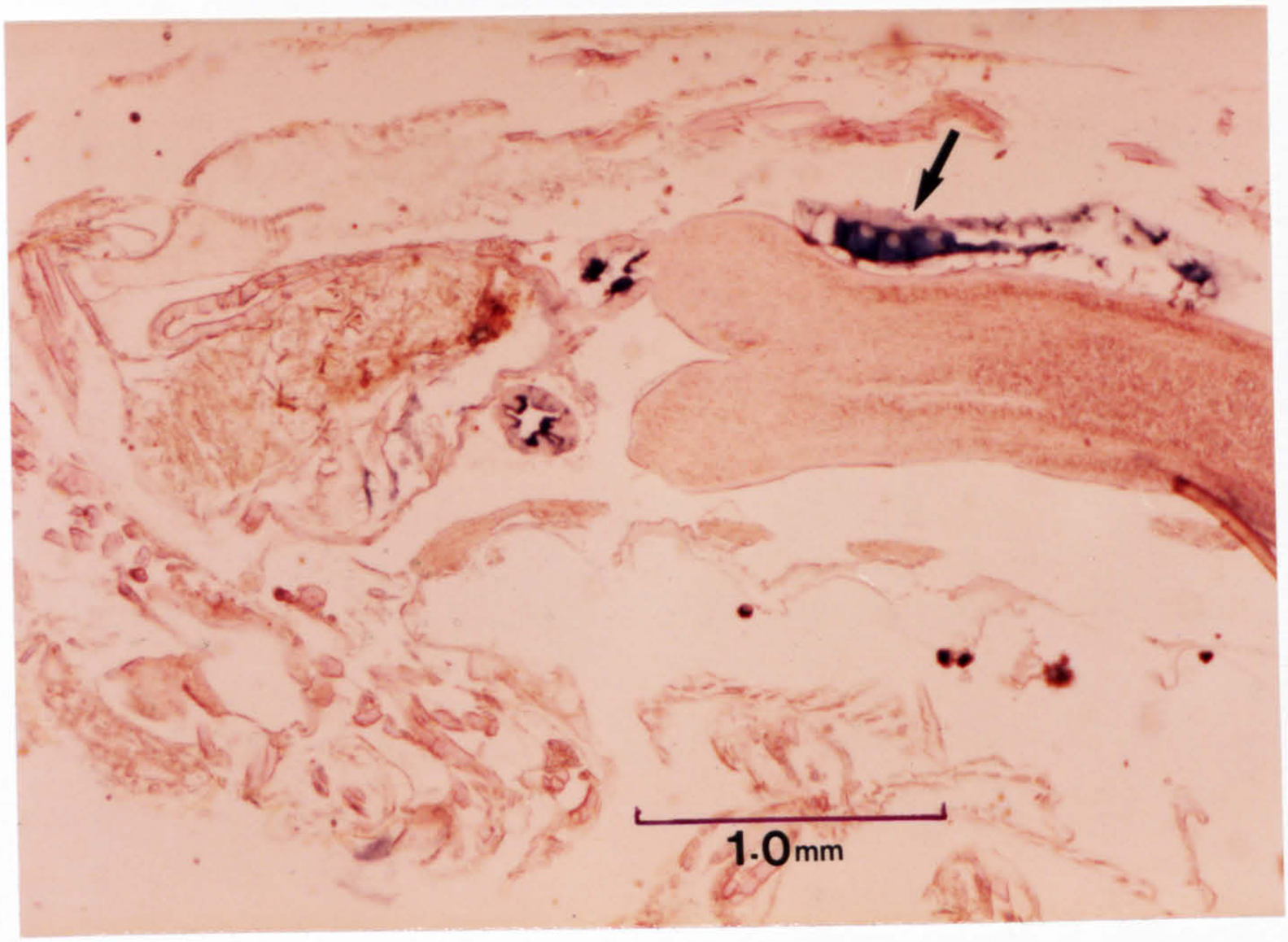
1.0mm

Plate 4.3 Longitudinal sections of infected *Gammarus pulex* showing the ovary (arrowed) in:

A. Infection with *Cyathocephalus truncatus*. Holt's indigogenic method for non-specific esterases. Scale = 1mm

B. Infection with *Echinorhynchus truttae*. Ehrlich's Hematoxylin and Eosin. Scale = 1mm

A



B

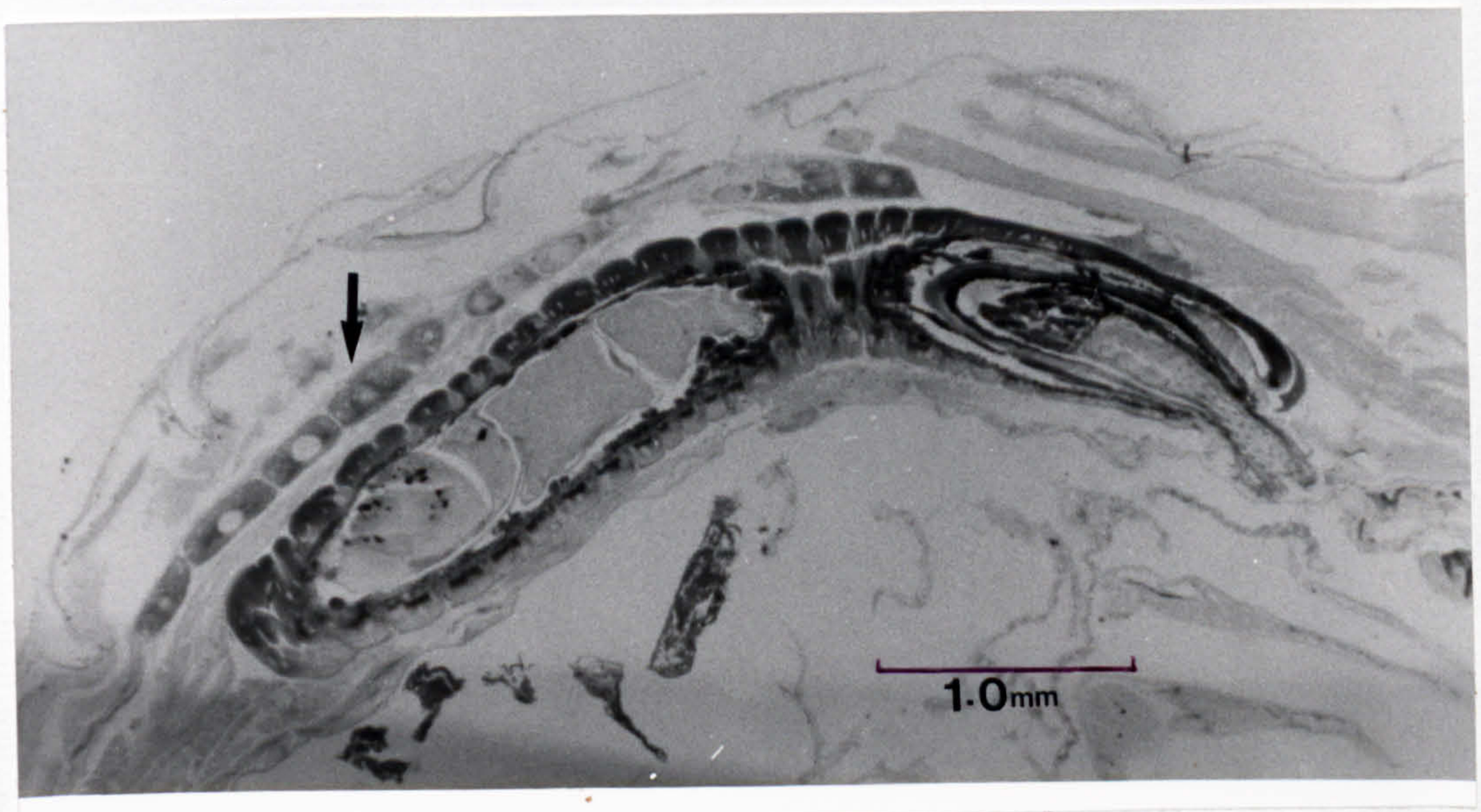
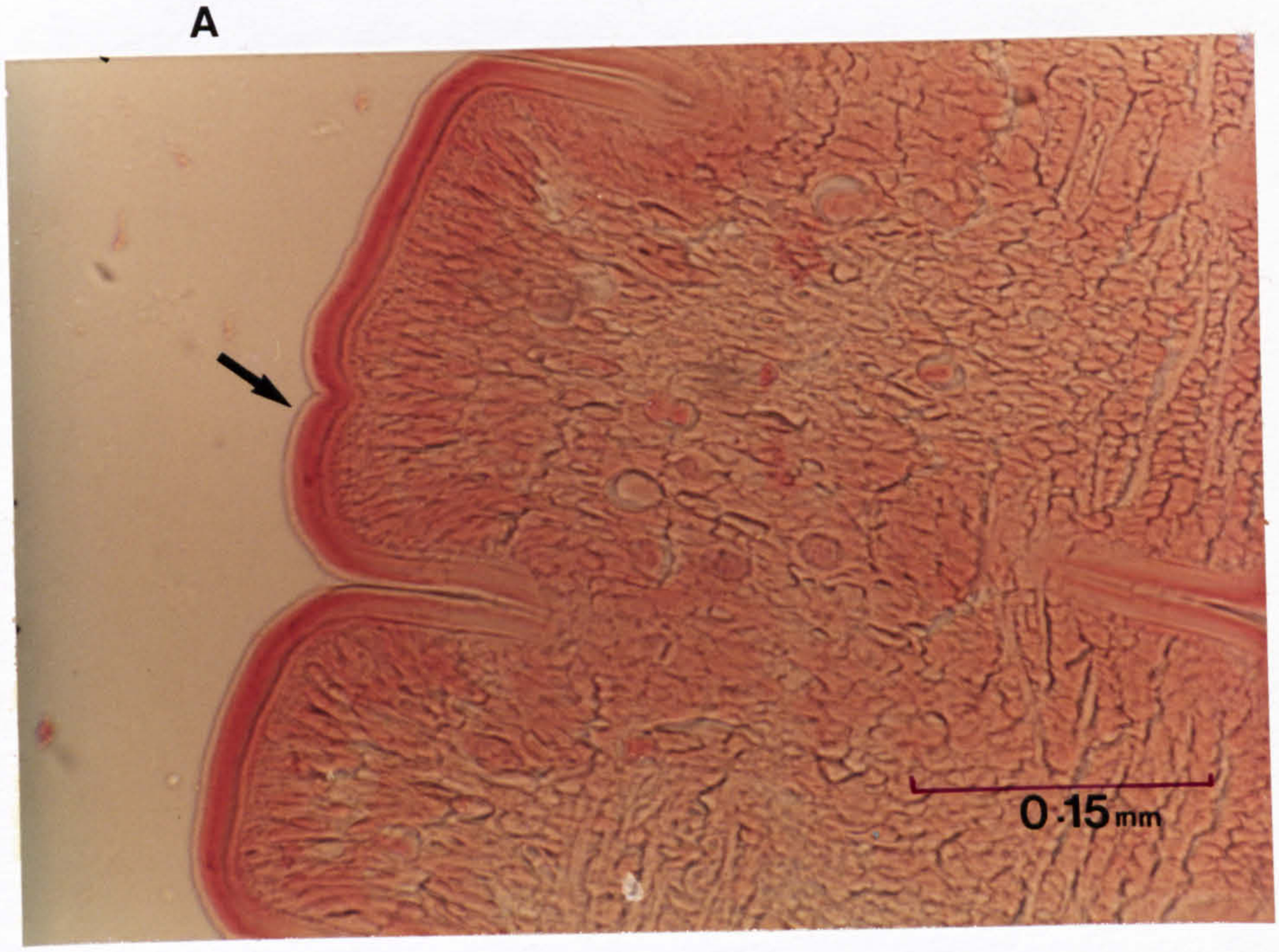


Plate 4.4

A. Demonstration of lipids in the tegument of the procercoid of C. truncatus in longitudinal section showing weakly positive reaction in the sheath (arrowed). Oil-red-'O' Scale = 0.15mm

B. Demonstration of acid mucopolysaccharide (seen blue in colour) in the sheath (arrowed). Alcian blue. Scale = 0.15mm.

A



B

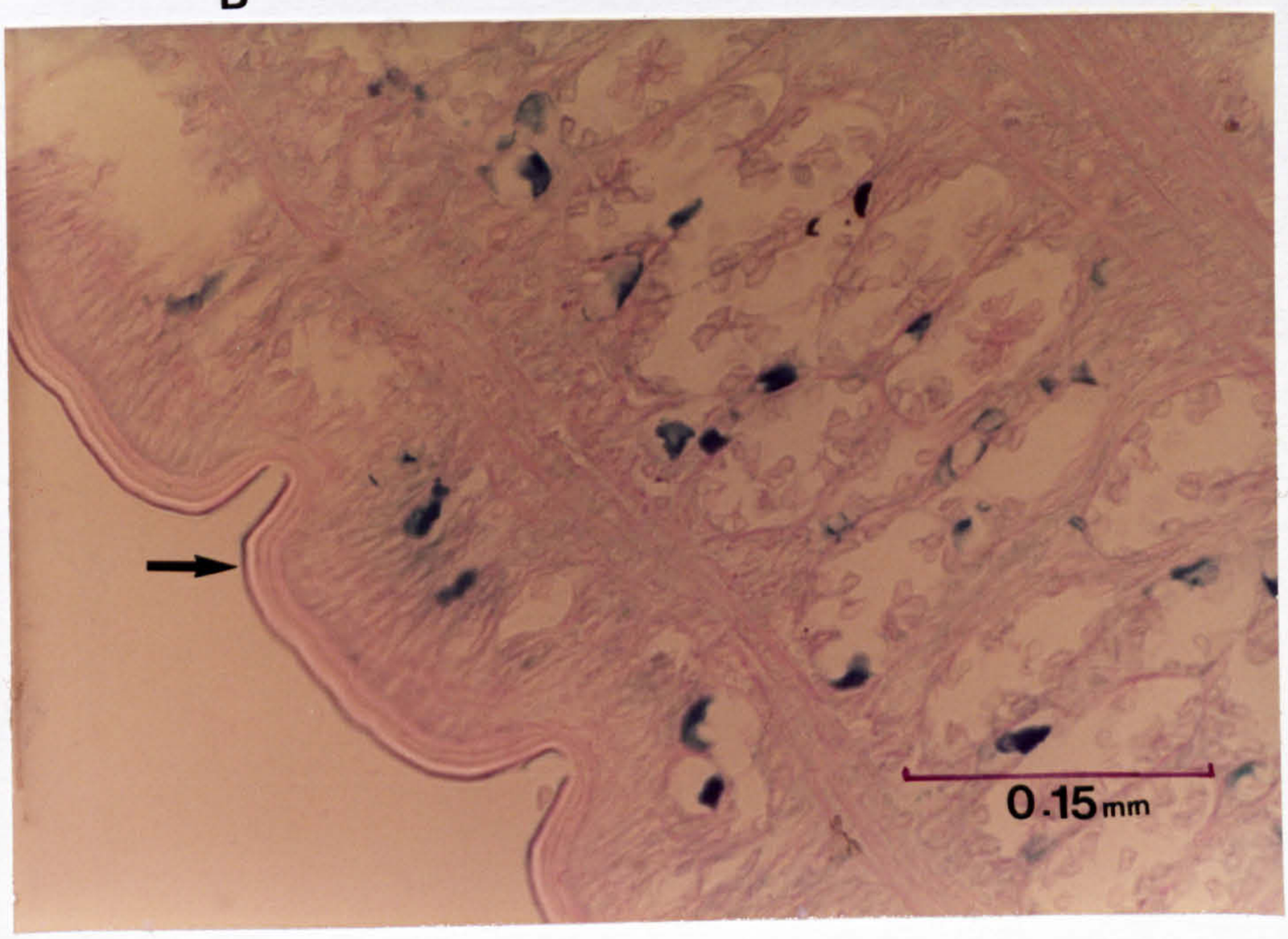
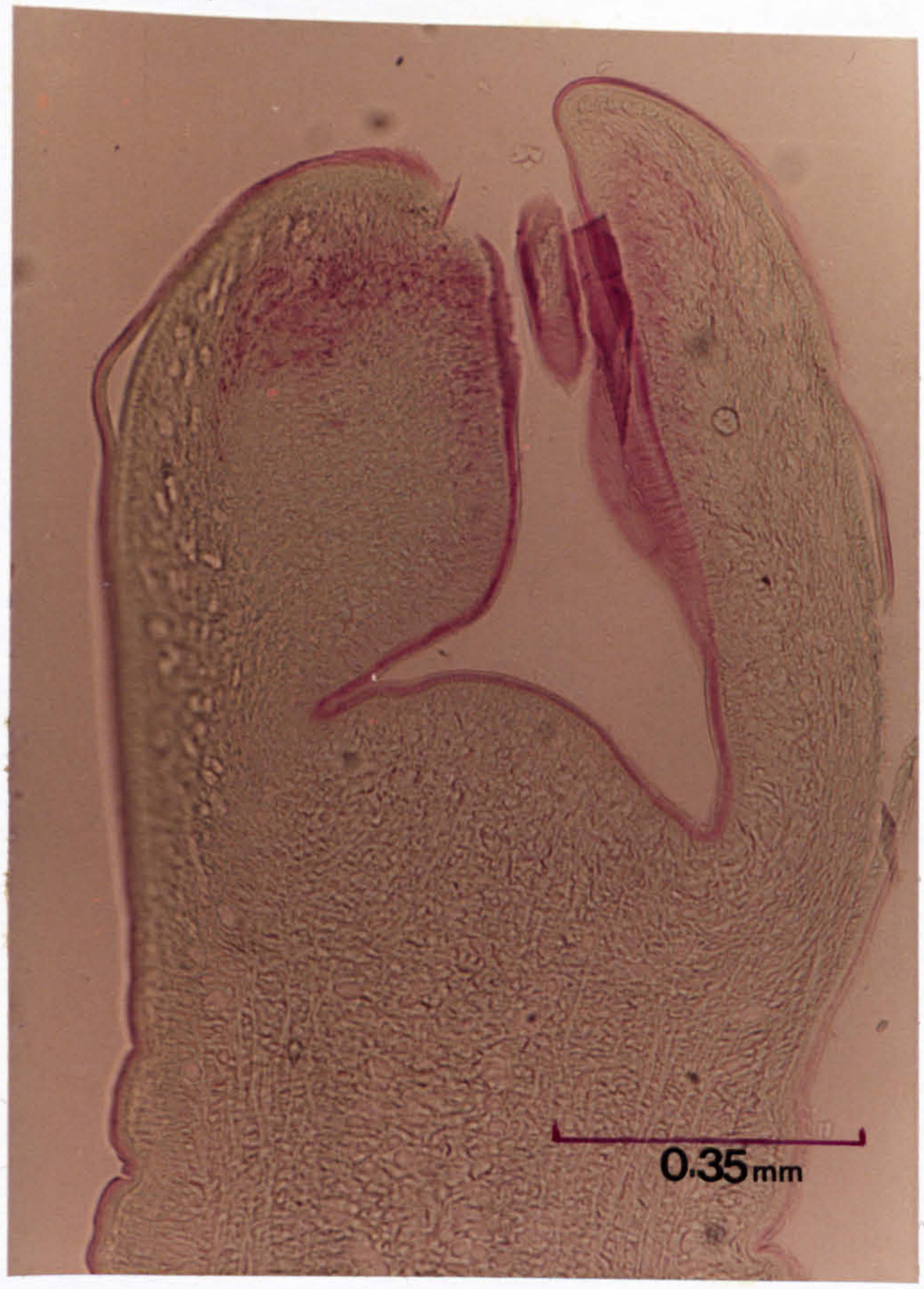


Plate 4.5 Demonstration of alkaline phosphatase acitivity (seen red in colour) in the procercoid of Cyathocephalus truncatus. Naphthol AS-BI phosphate method.

A. Longitudinal section of the tapeworms scolex region. Note extensive reaction in the tegumentary cytons. Scale = 0.35mm

B. Longitudinal section of body wall of the strobila. Scale = 0.15mm

A



B

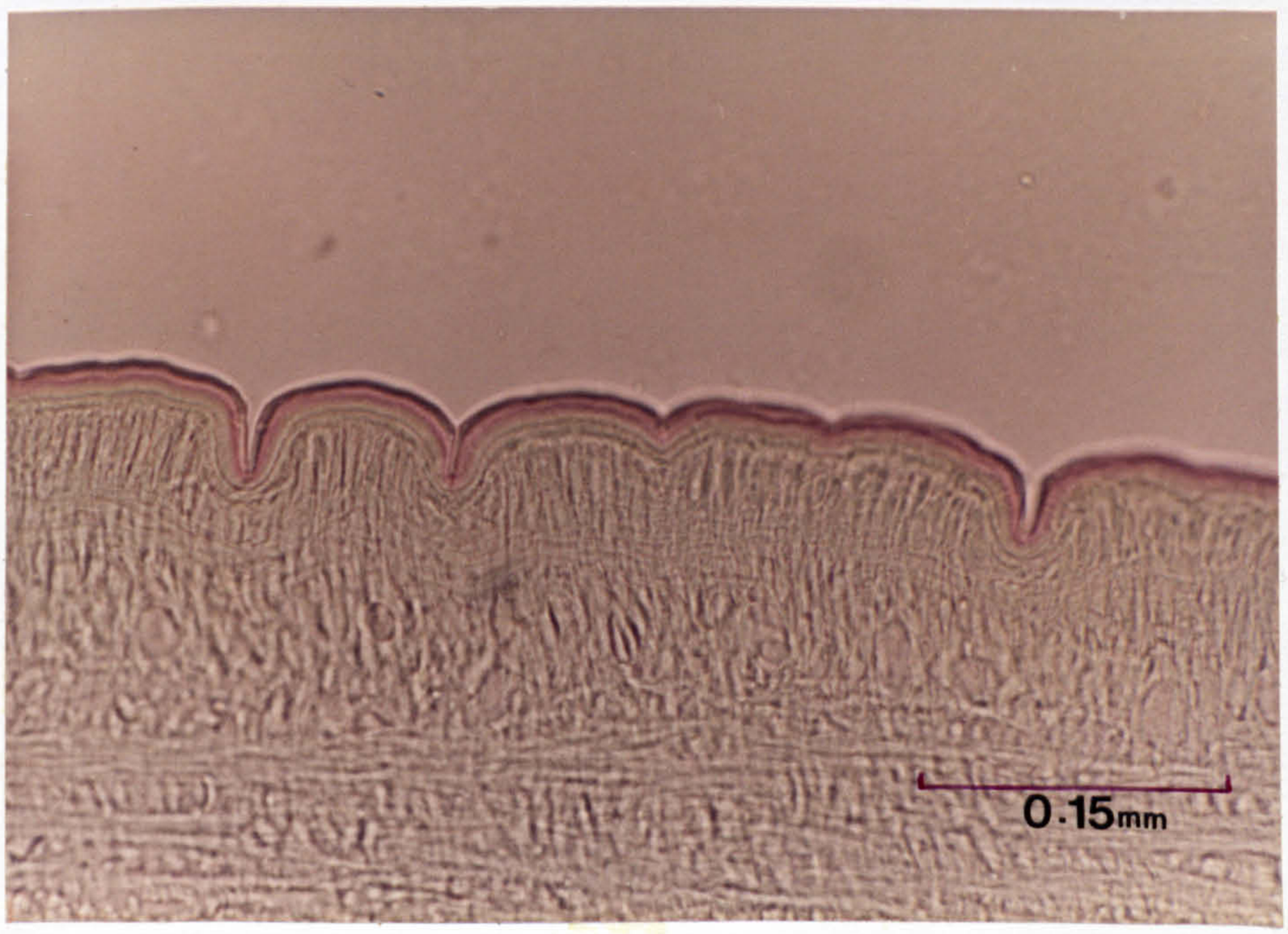


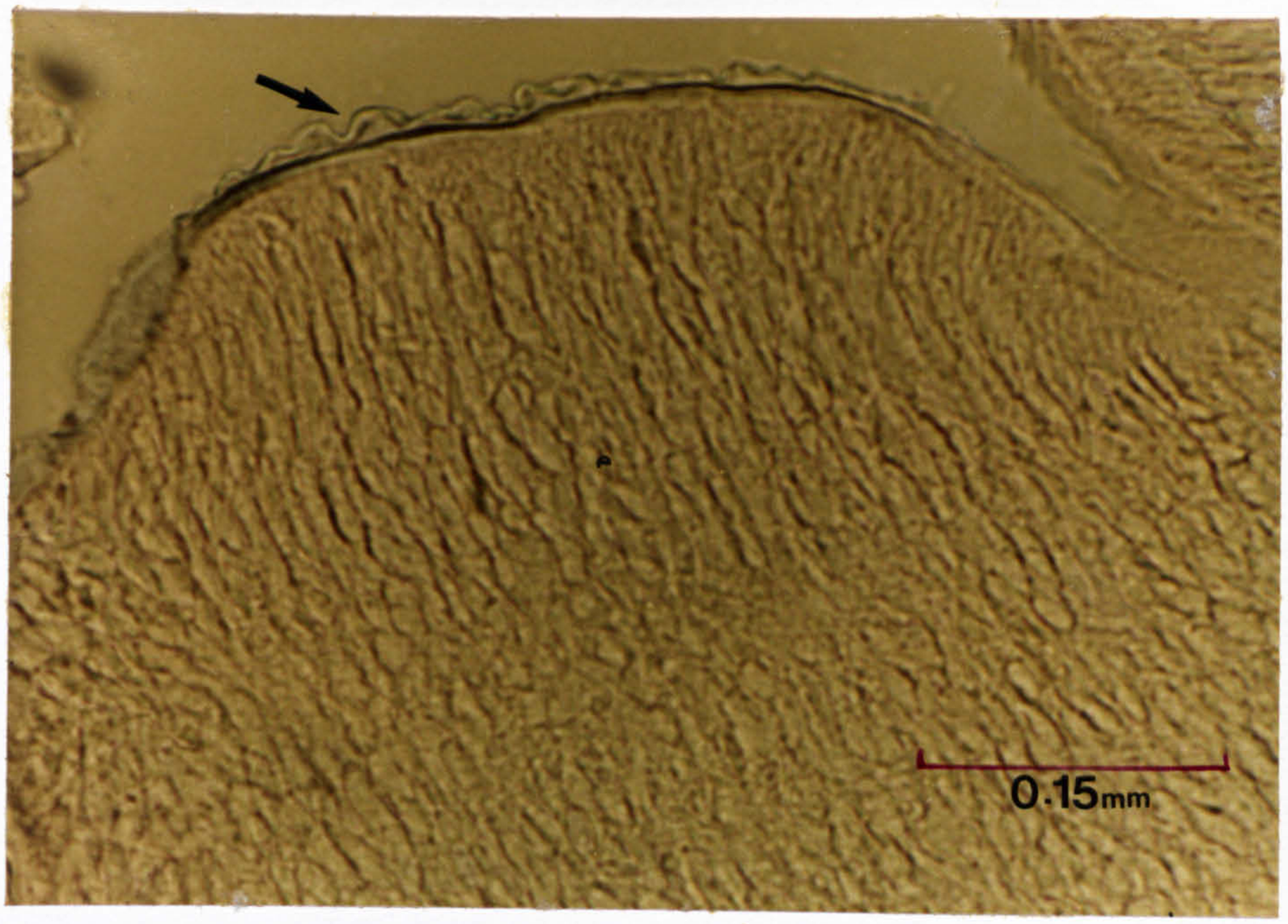
Plate 4.6 Demonstration of non-specific esterase activity (seen as deep blue colour) in the procercoid of C. truncatus. Holt's indigogenic method.

Longitudinal section of the scolex.
Note weakly positive reaction in the sheath (arrowed) and positive reaction in the distal cytoplasm. Scale = 0.15mm.

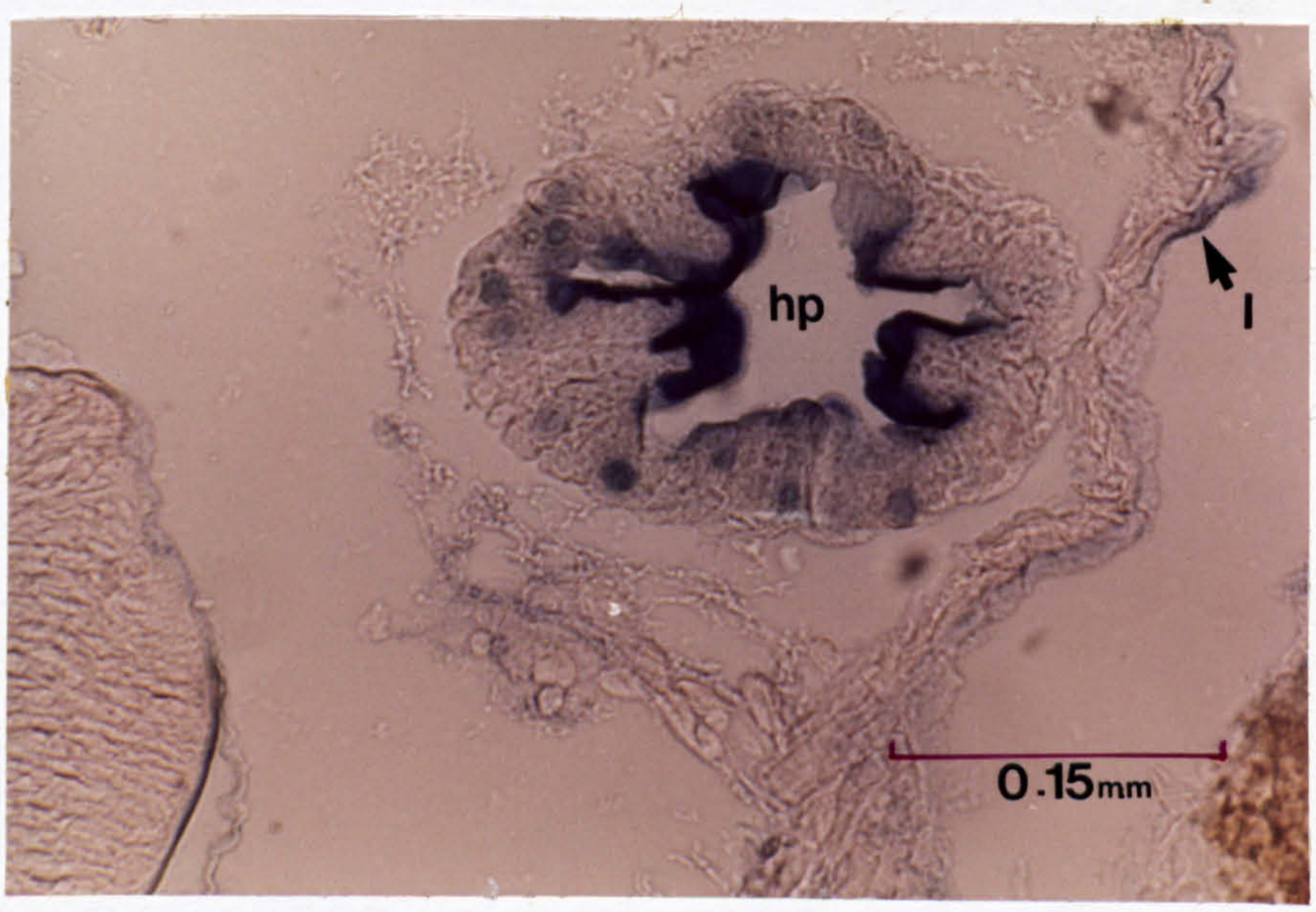
Plate 4.7 Demonstration of non-specific esterase activity in body cavity organs of Gammarus pulex. Holt's indigogenic method.

A. Hepatopancreas (hp) and intestinal wall (I). Scale = 0.15mm

B. Ovary. Scale = 0.15mm



A



B

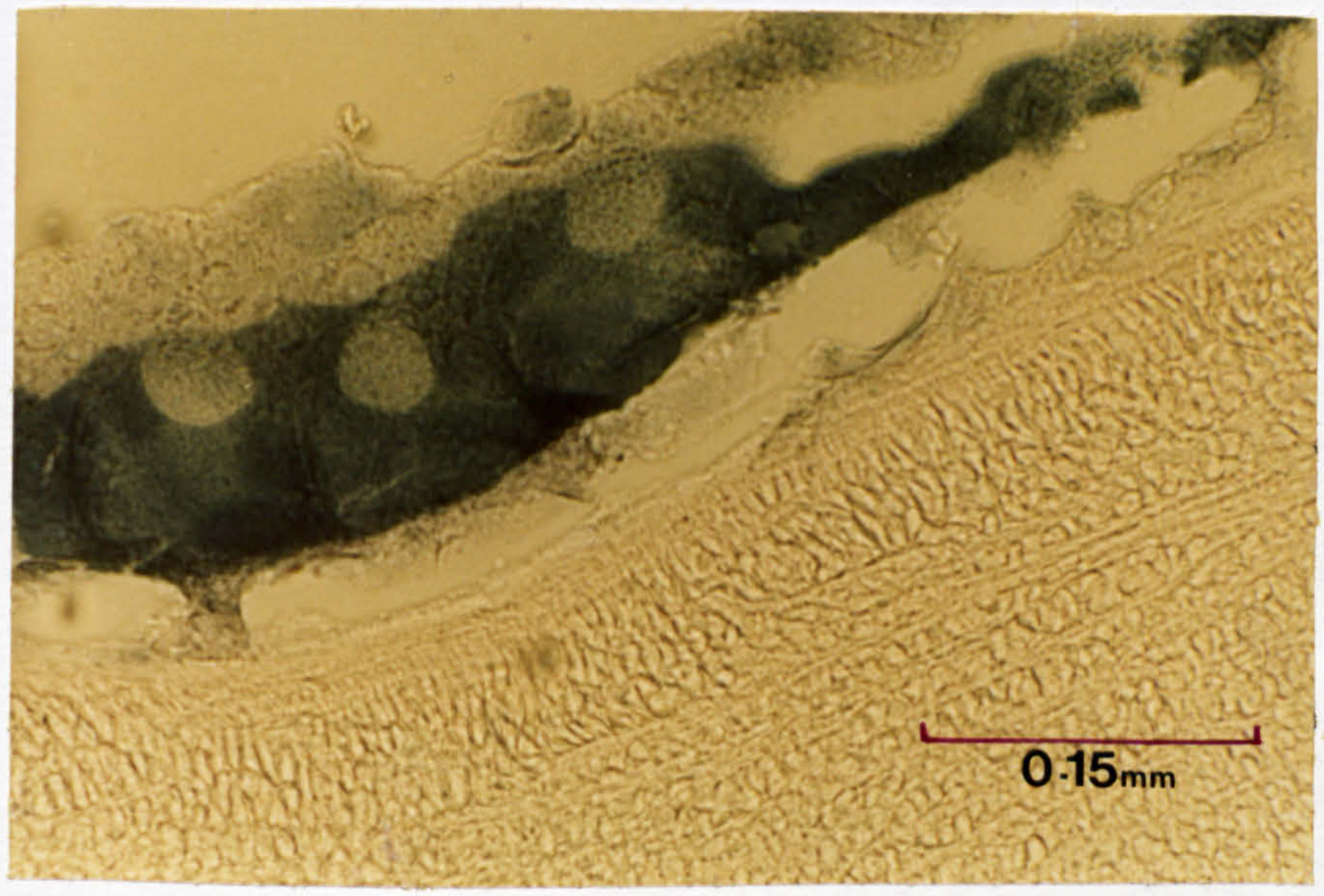


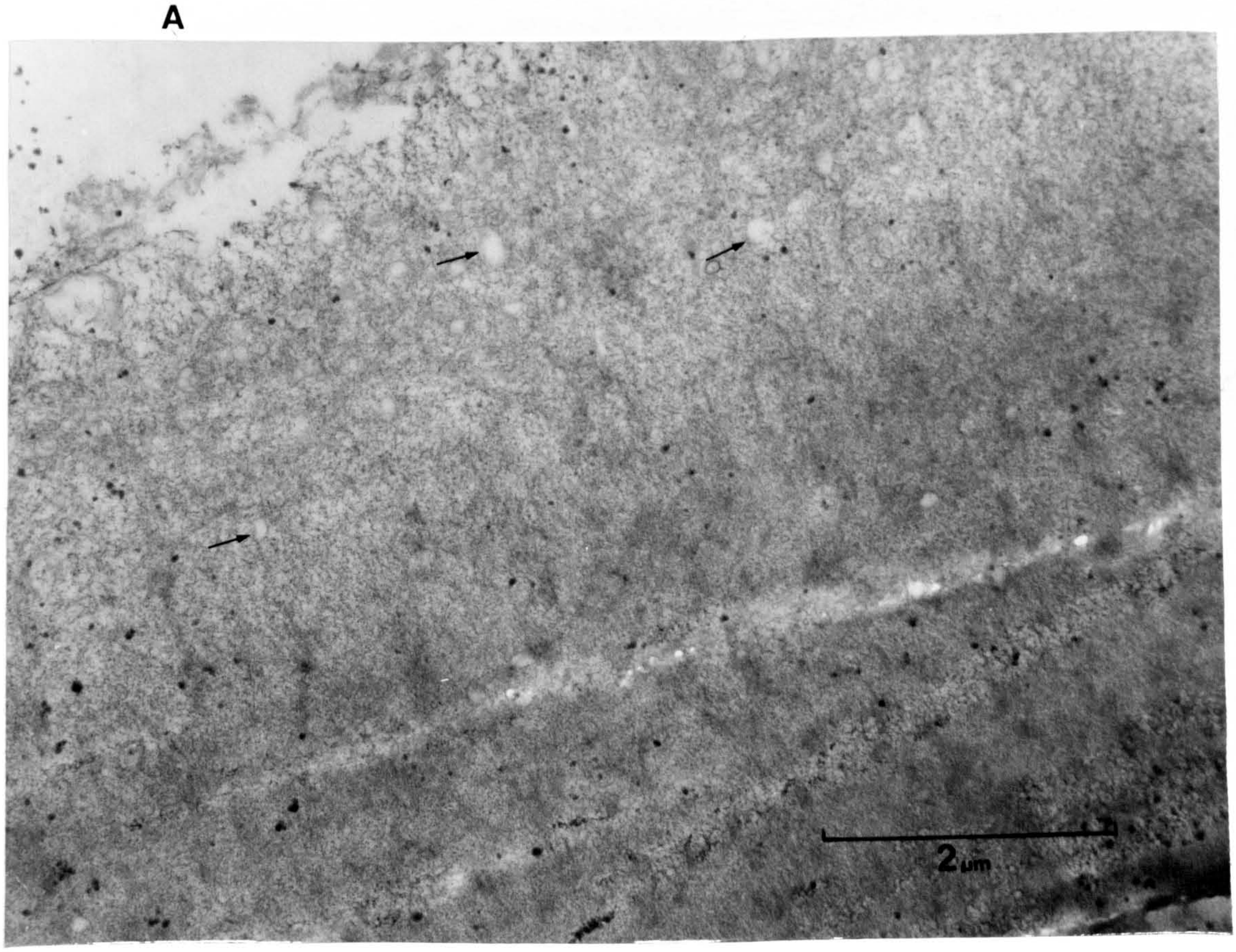
Plate 4.8 Transmission electron micrographs of a Stage I procercoid of C. truncatus.

A. A transverse section ultrastructure of the sheath. Note spaces (arrowed) within the material of the sheath.
Scale = 2µm.

B. A transverse section ultrastructure of the body wall. Scale = 1µm.

Dc ..... Distal cytoplasm
gl ..... Glycogen granules
Mt ..... Microtriches
Mu ..... Muscles
Sh ..... Sheath

A



B

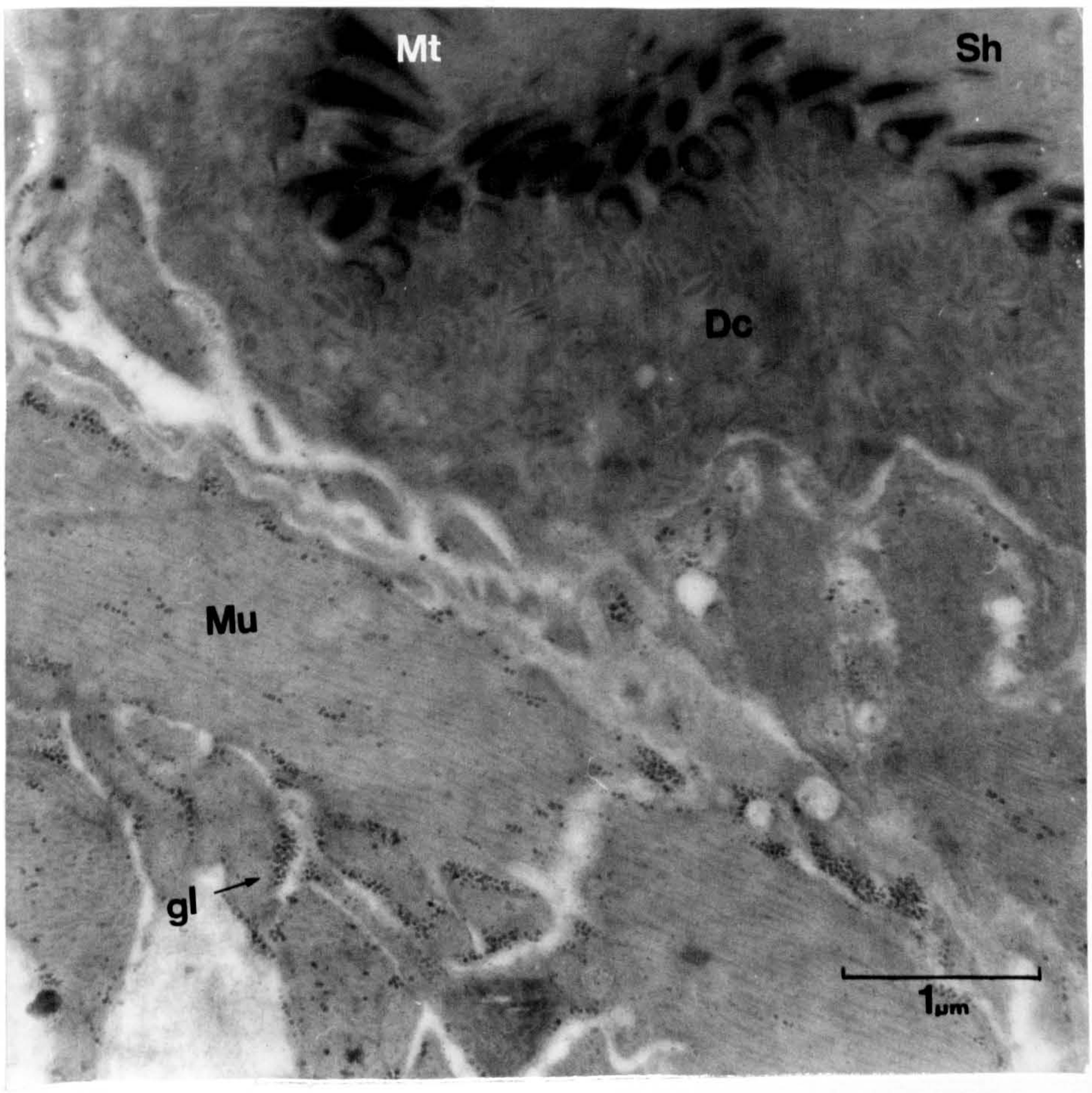


Plate 4.9 Transmission electron micrographs of the Stage II procercoid of C. truncatus in transverse section showing.

A. Two types of microtriches and part of the distal cytoplasm (Dc). Scale = 2µm.

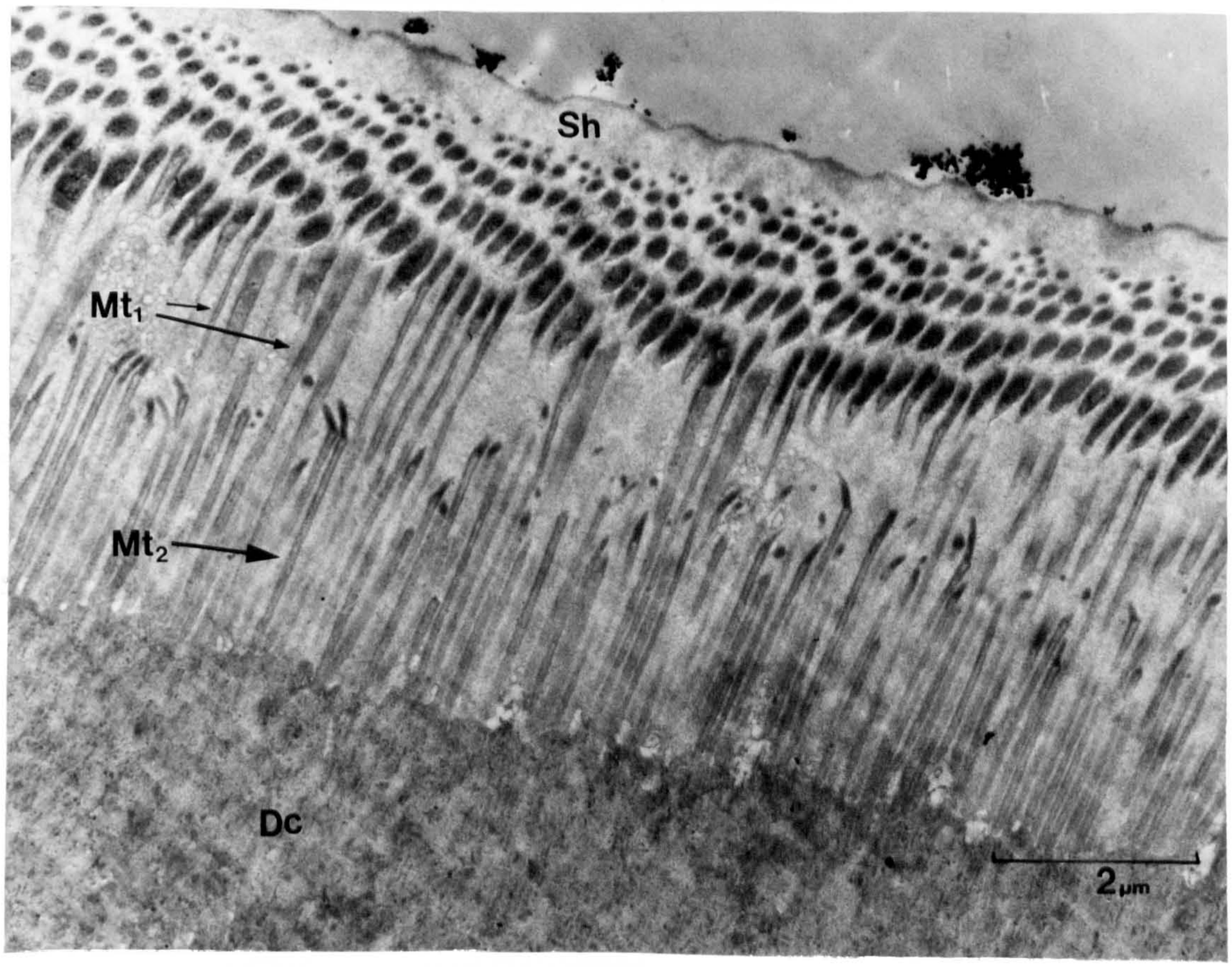
Mt_1 ..... Type I microtriches

Mt_2 ..... Type II microtriches

B. Body wall of the inner scolex surface Scale = 2µm

Sh ..... Sheath

A



B

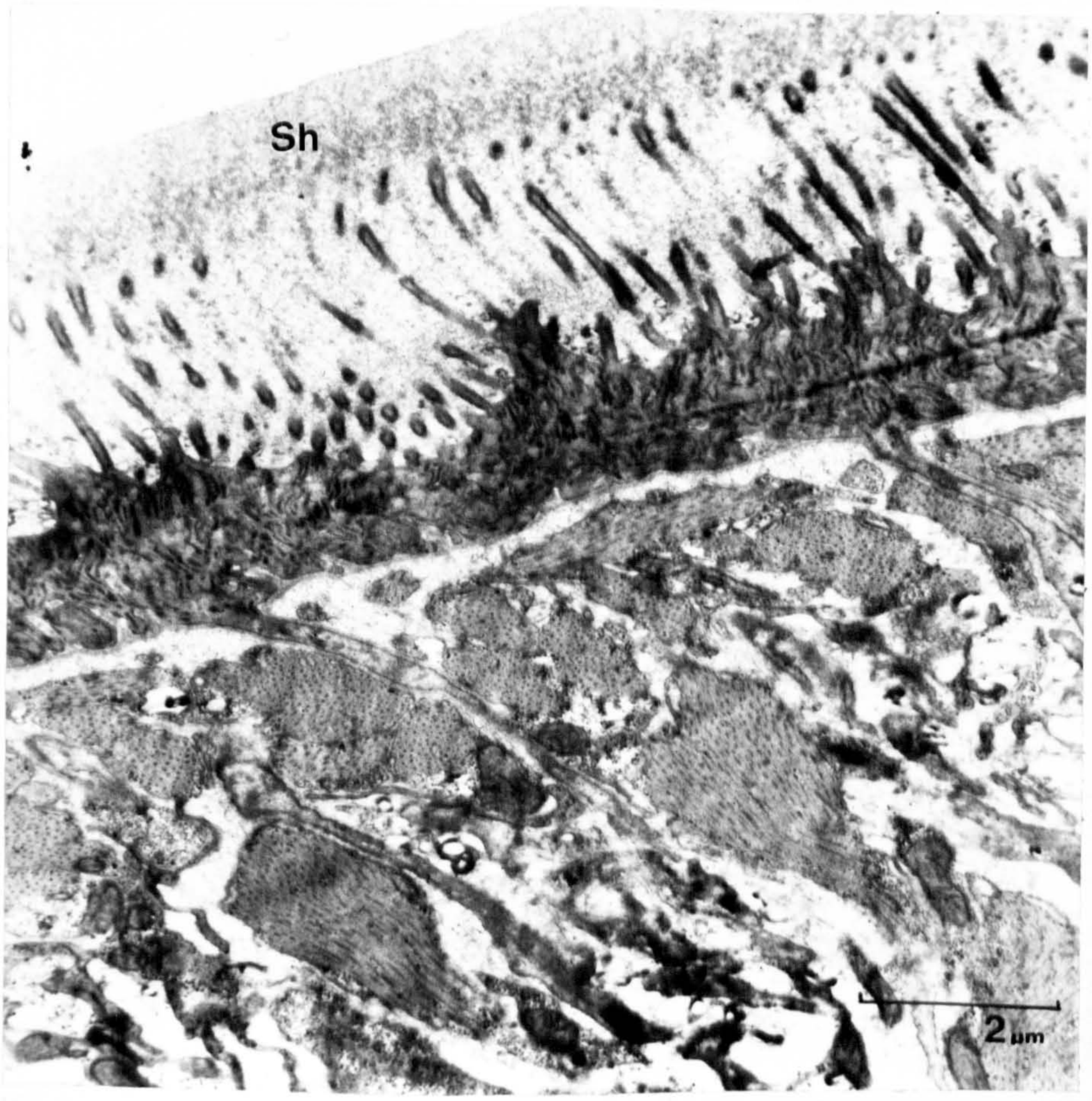


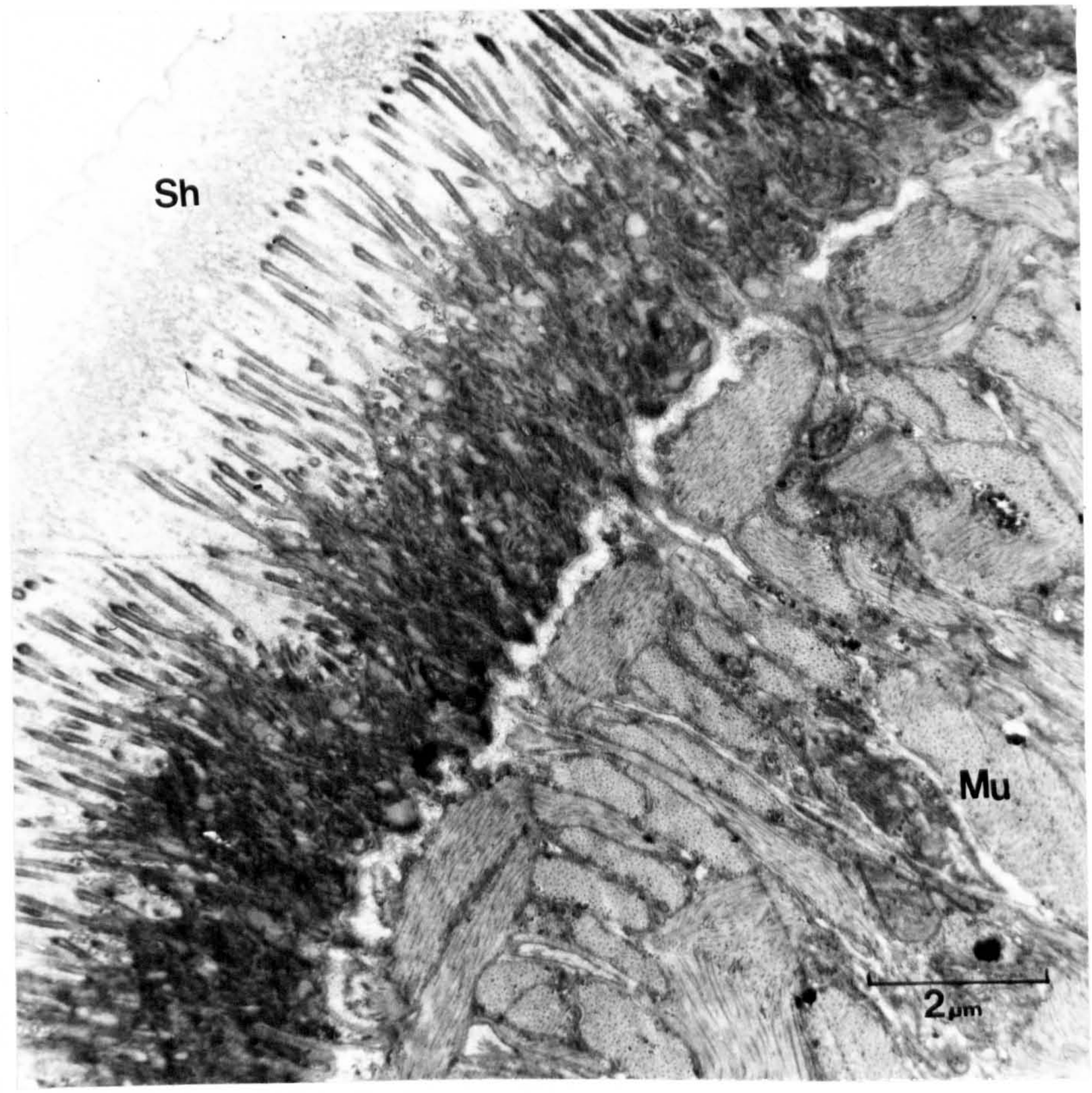
Plate 4.10

A. Transmission electron micrograph of outer scolex surface of the Stage III procercoid of C. truncatus in transverse section. Note the musculature (Mu) Scale = 2µm

Sh ..... Sheath

B. High power transmission electron micrograph of microtriches of the outer scolex surface. Note the bifurcations (arrowed)
Scale = 1µm

A



B

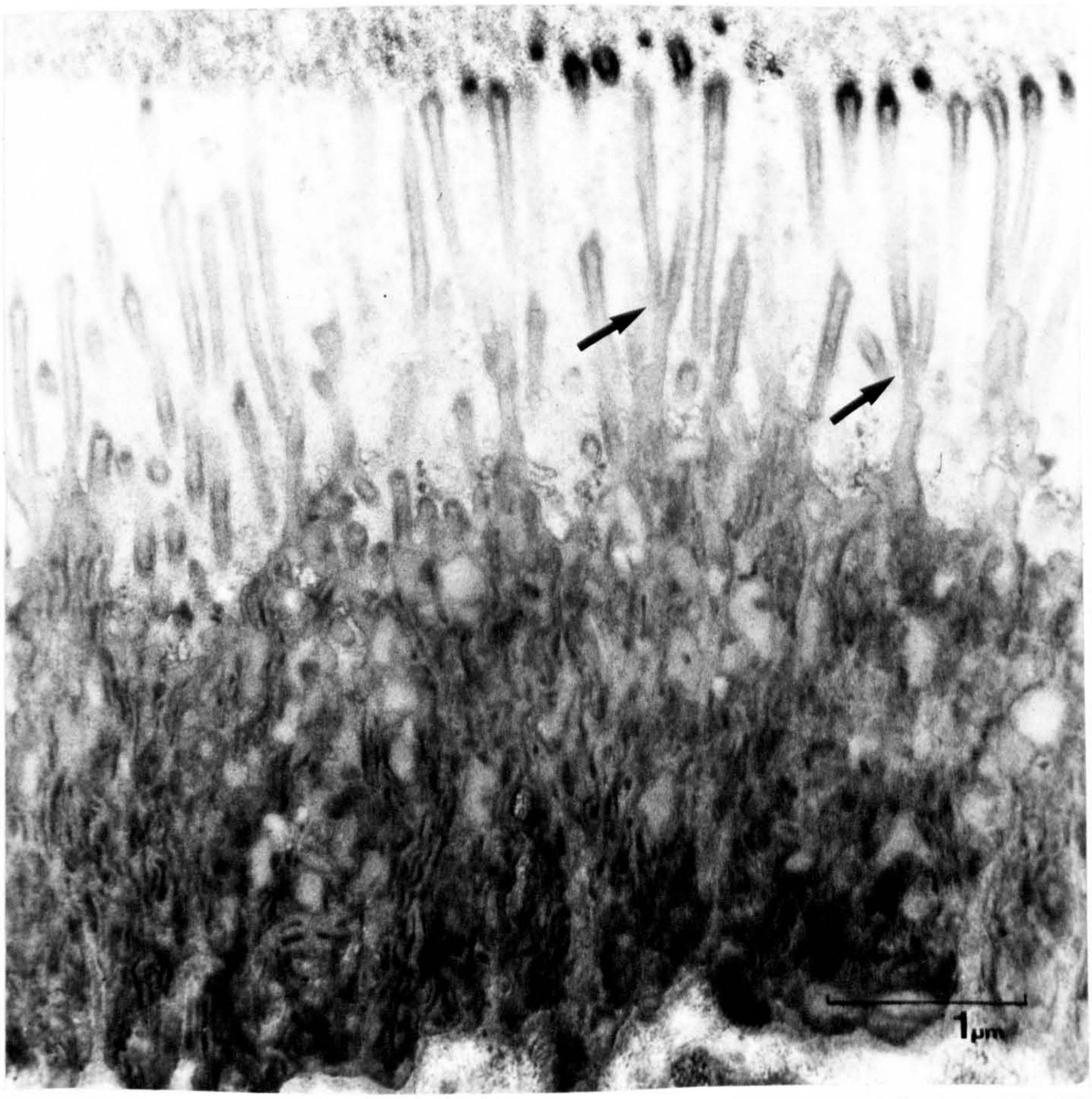


Plate 4.11

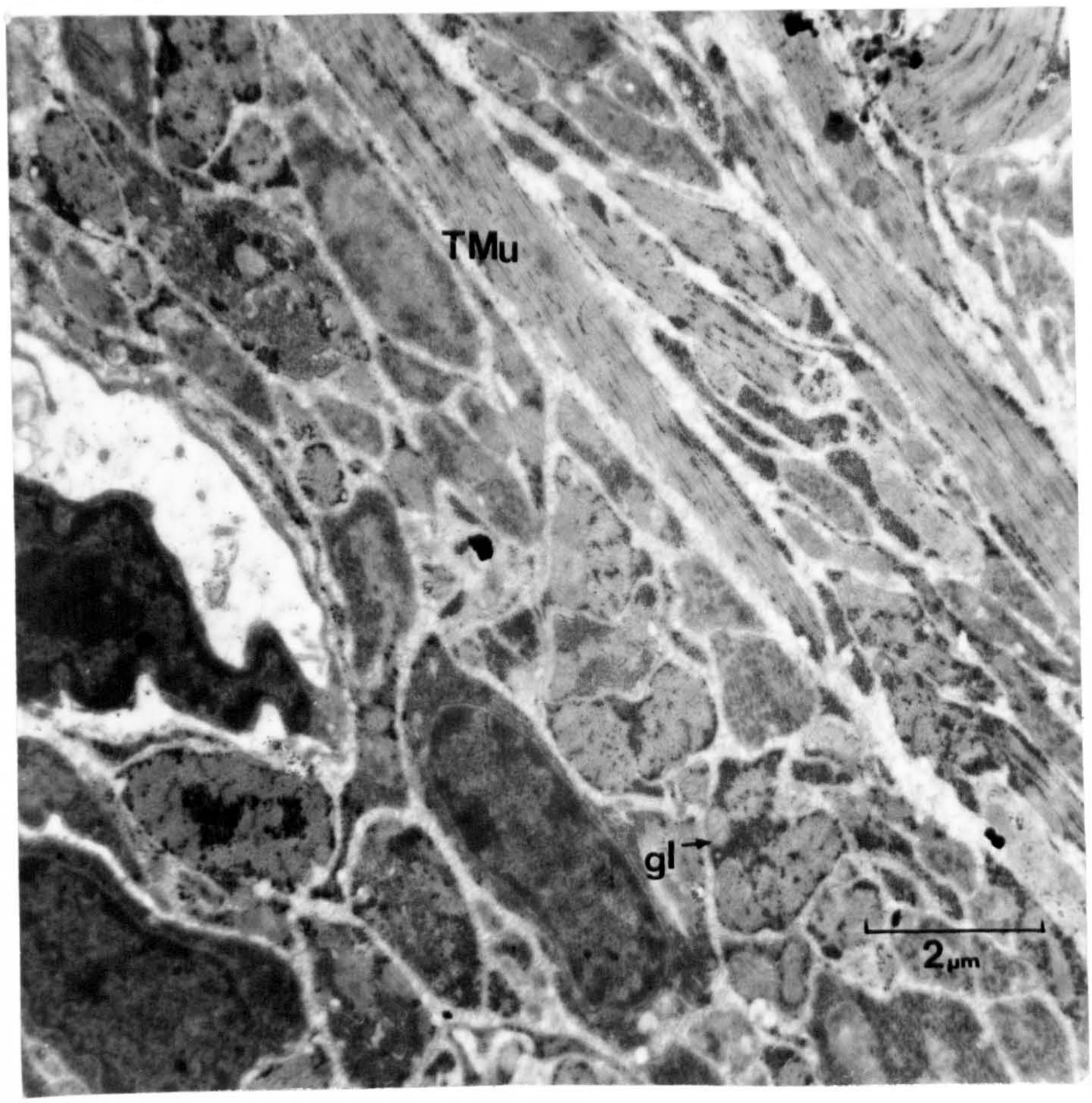
A. Transmission electron micrograph of outer medullary region beneath the outer scolex body wall of the Stage III procercoid of C. truncatus in transverse section showing the transverse muscles (Tmu). Scale = 2μm.

gl ..... Glycogen granules

B. Transverse section ultrastructure of medullary parenchyma of the scolex margin of Stage III procercoid. Transmission electron micrograph. Scale = 2μm

Ed ..... Excretory duct
P ..... Parenchyma
Fb ..... Fibres in the parenchyma

A



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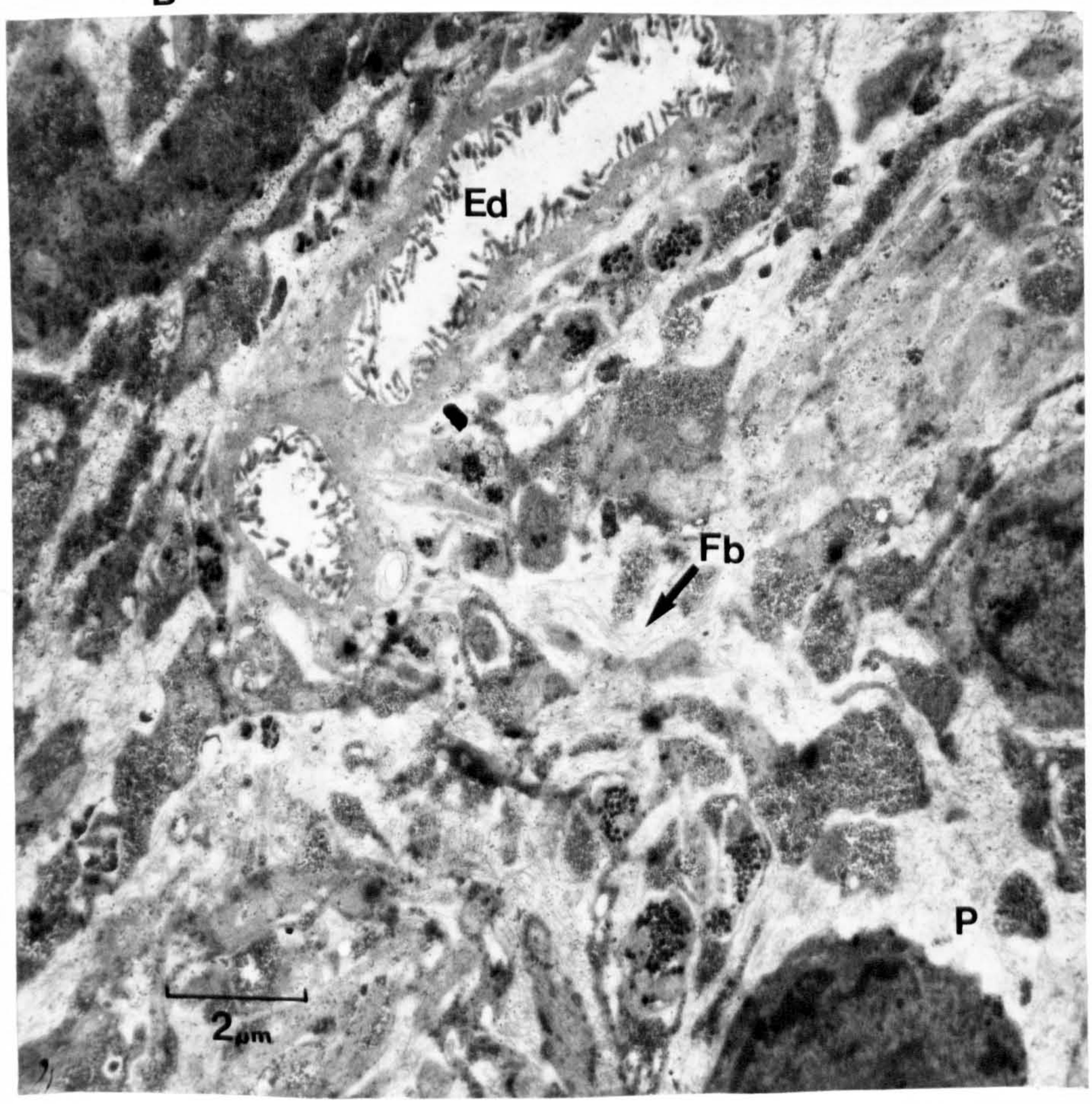
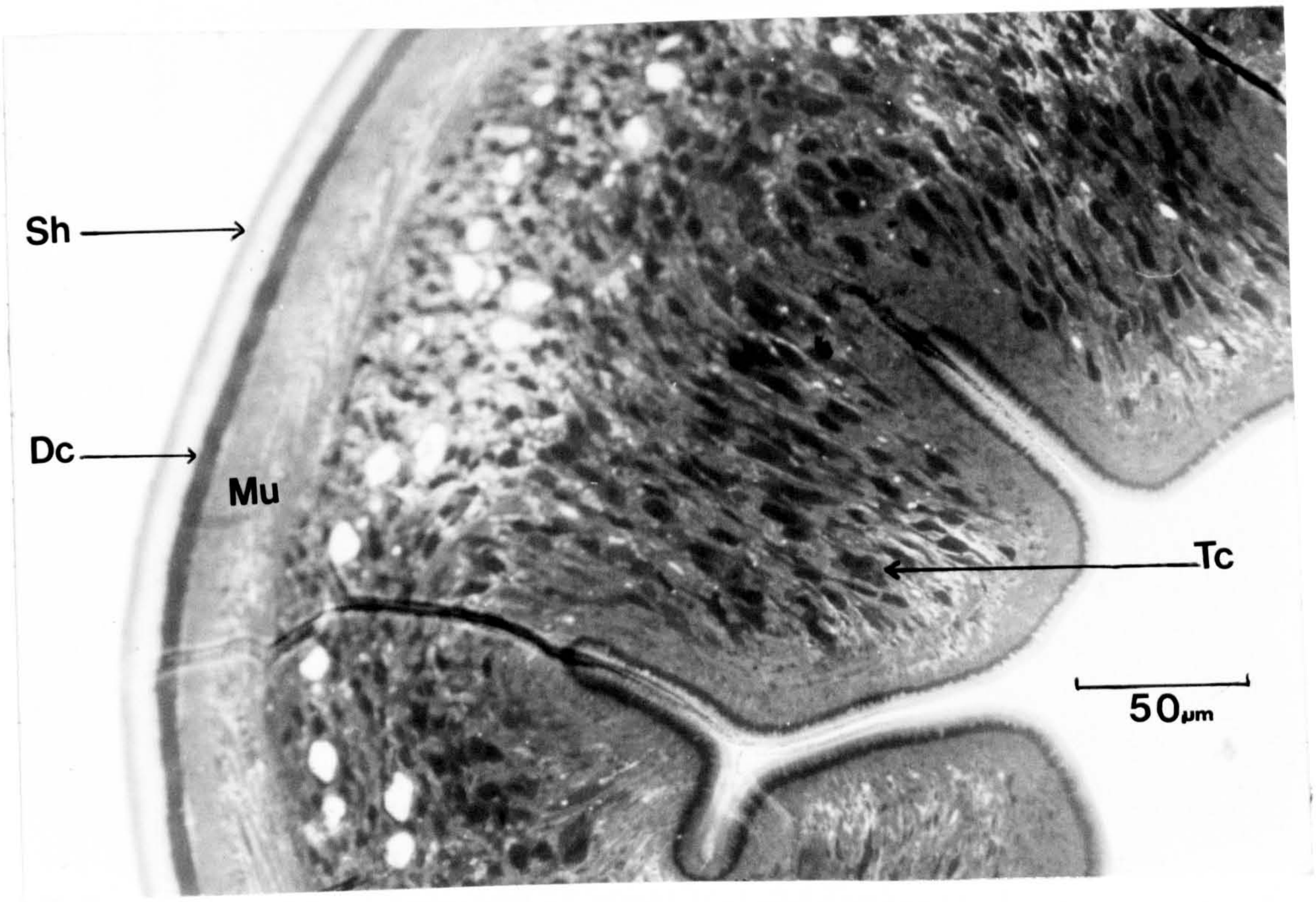


Plate 4.12

A. Semithin transverse section of the scolex margin of a Stage III procercoid of C. truncatus. Toluidine blue. Scale = 50µm.

Dc ..... Distal cytoplasm of outer scolex surface
Mu ..... Musculature
Sh ..... Sheath
Tc ..... Tegumentary cytons of the inner scolex body wall

B. High power transmission electron micrograph of the tegumentary cyton of Stage III procercoid showing numerous electron dense bodies (E) and packs of mitochondria (Mi) in the cytoplasm. The nucleus (not shown) is relatively small. Scale = 2µm.



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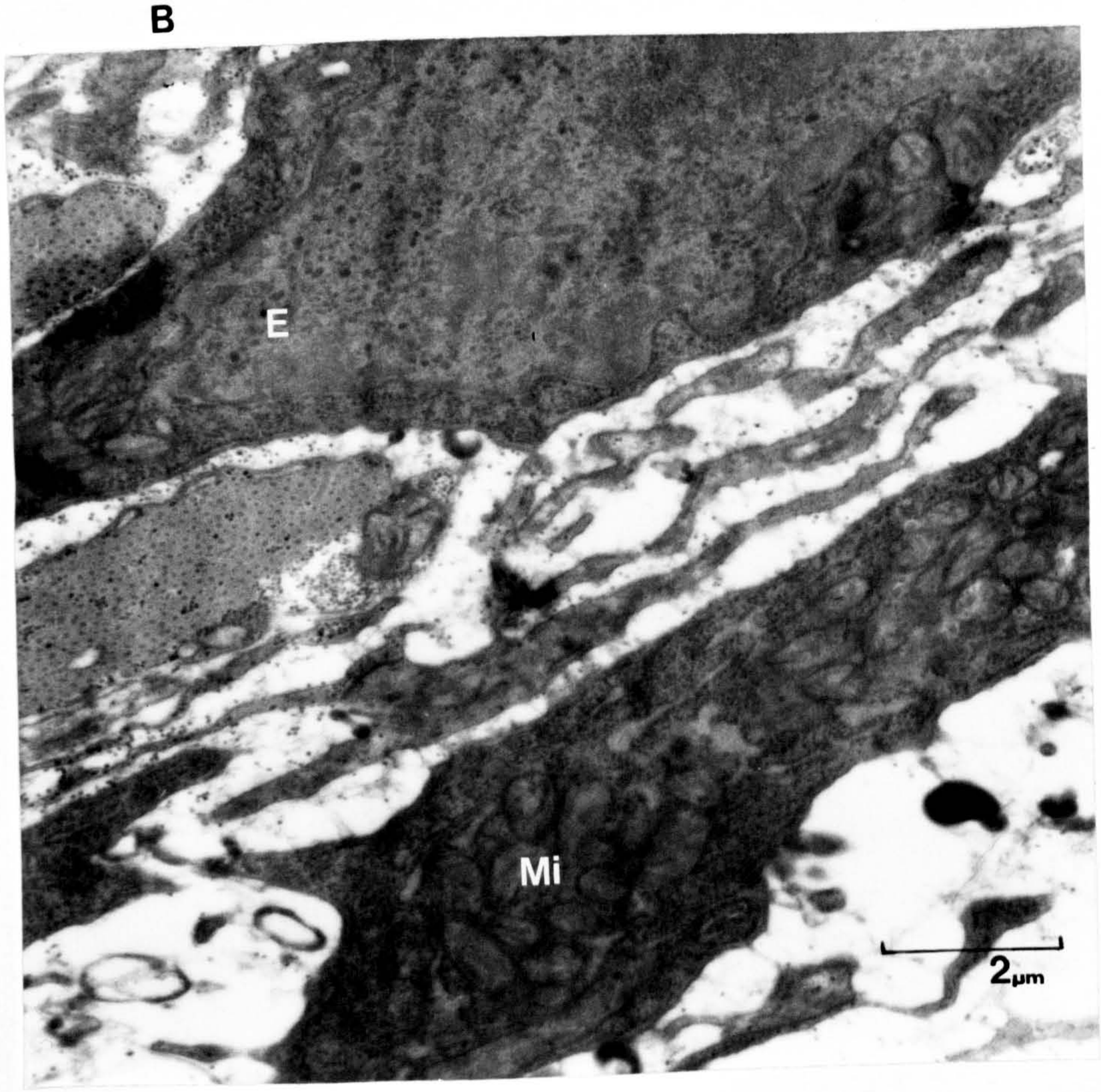


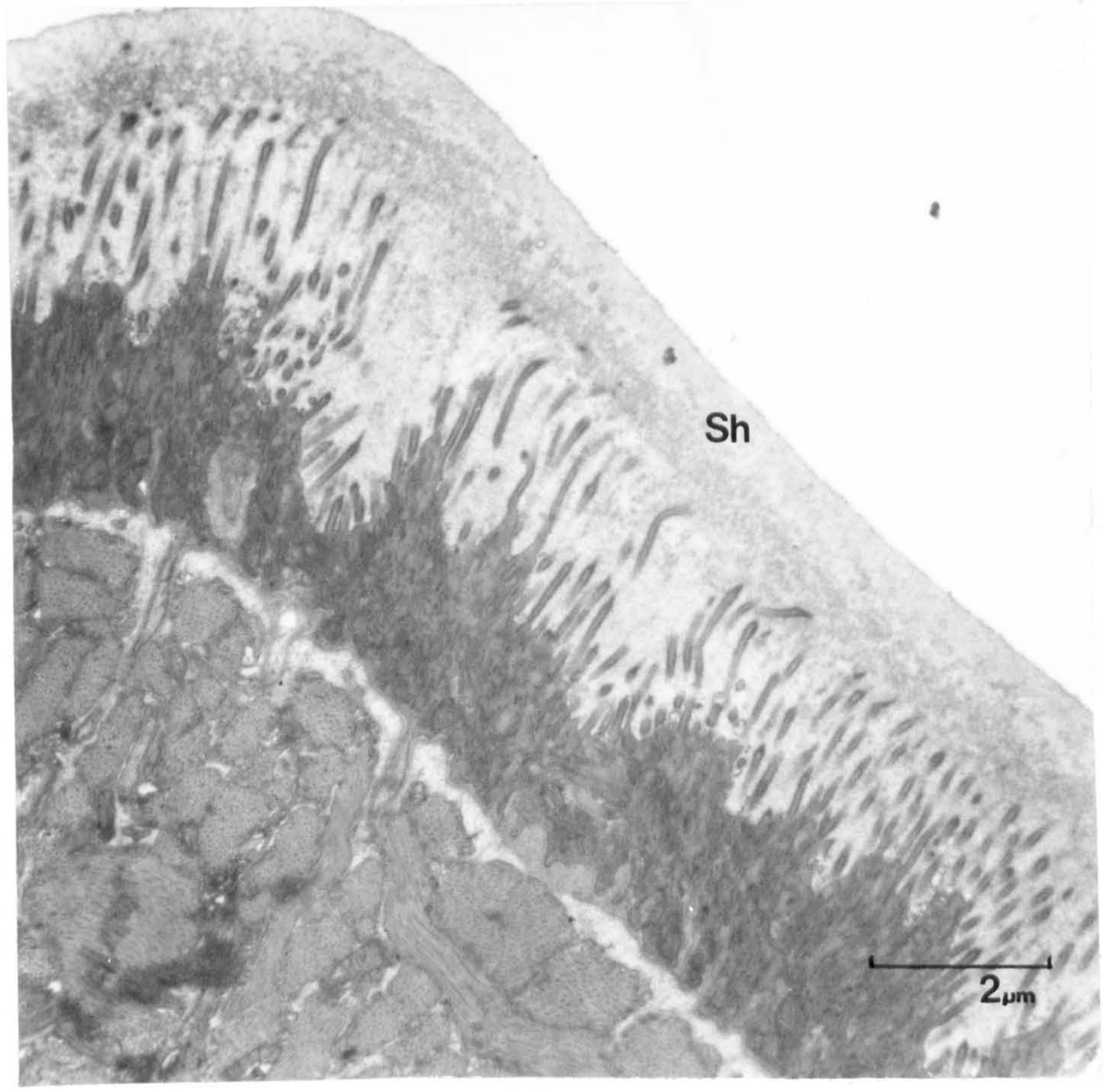
Plate 4.13

A. Transverse section ultrastructure of the inner scolex surface of the Stage III procercoid of *C. truncatus*. Note the undulating layer of the distal cytoplasm. Transmission electron micrograph. Scale = 2µm

Sh ..... Sheath

B. High power transmission electron micrograph of microtriches of the inner scolex surface (above) showing the structure of their electron dense apical cap (arrow 1) and a trifurcated microthrix (arrow 2). Scale = 1µm.

A



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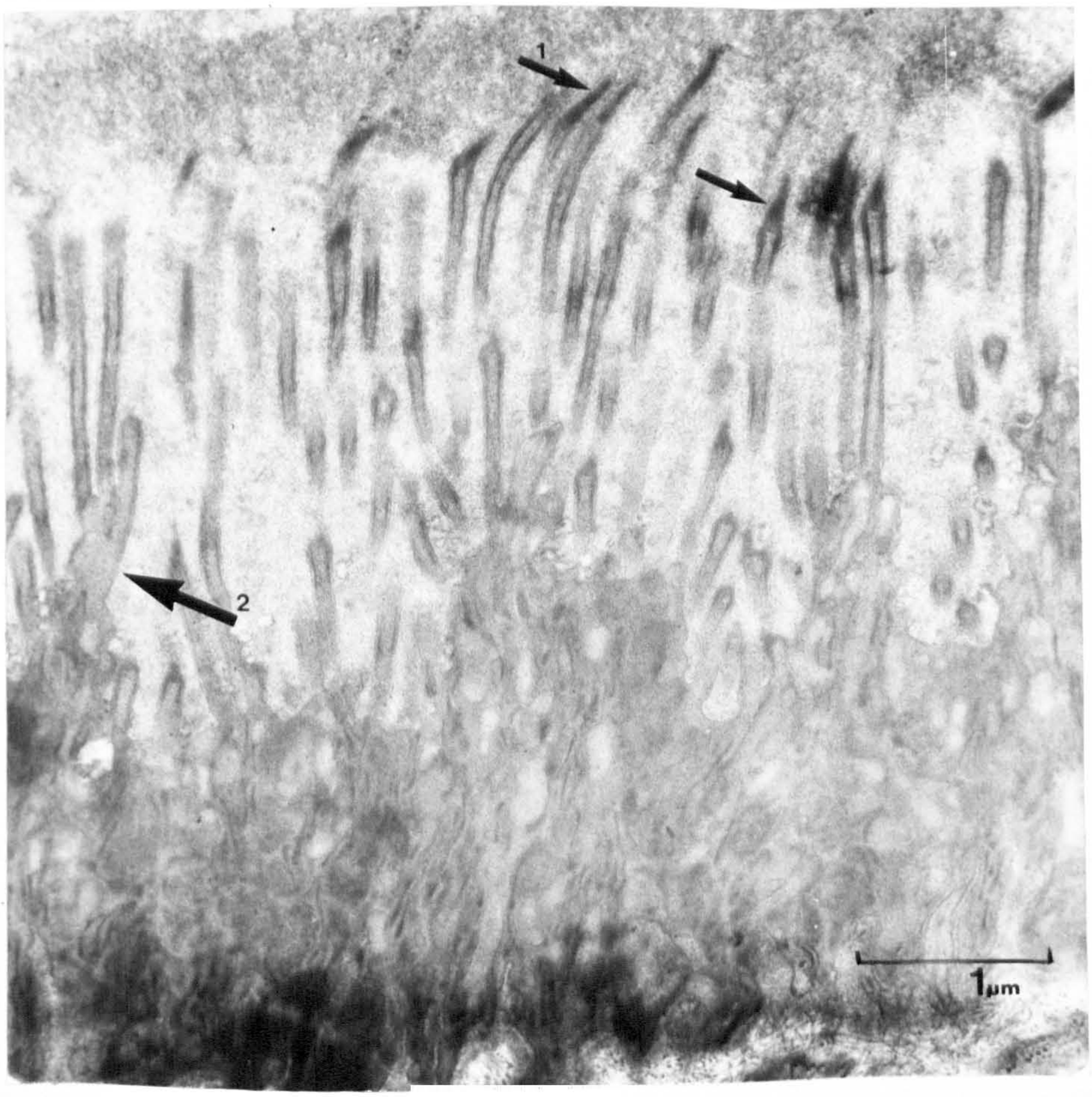


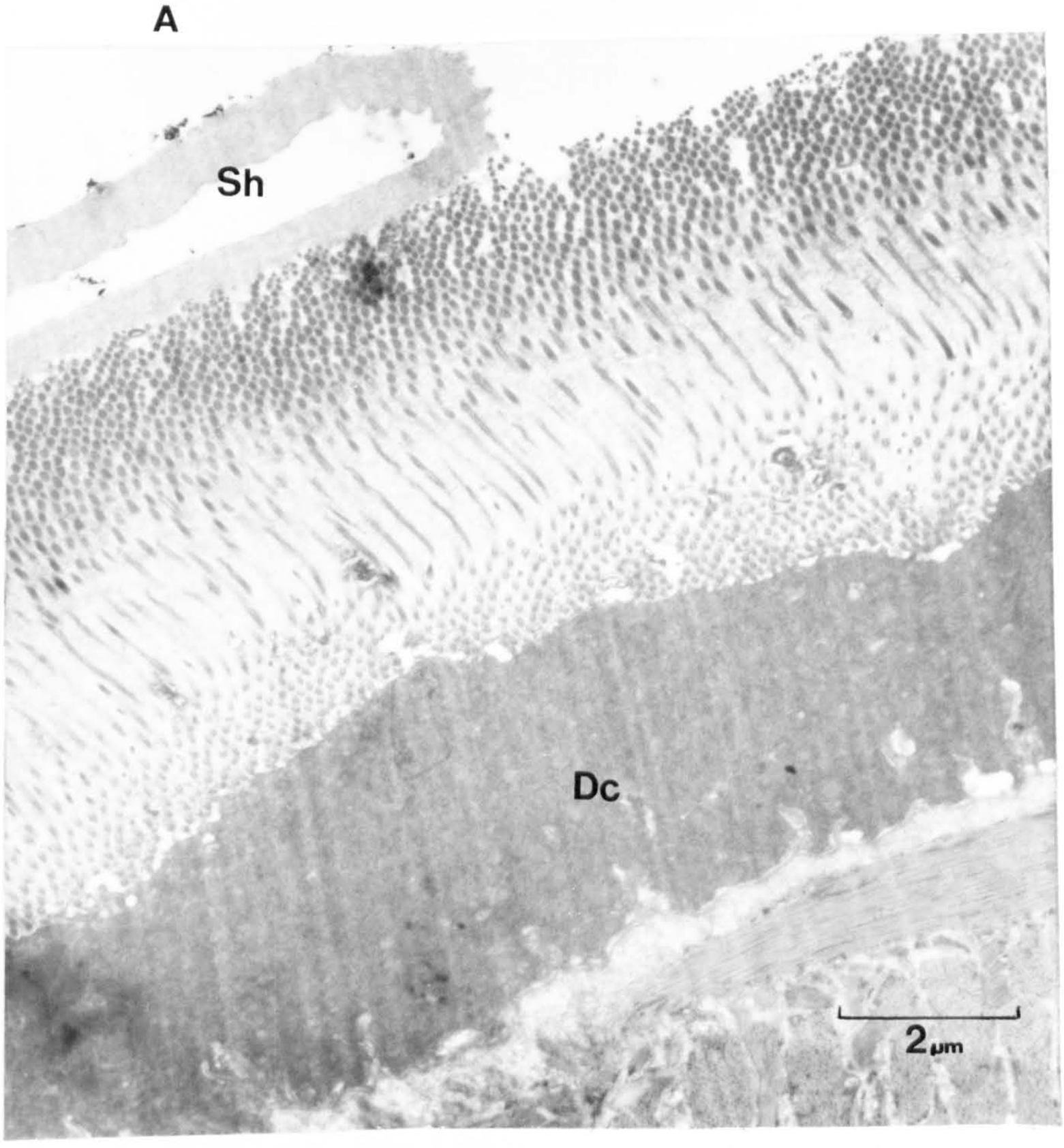
Plate 4.14 Transmission electron micrographs of the neck region of the Stage III procercoid of C. truncatus in transverse section.

A. The body wall. Scale = 2µm

Dc ..... Distal cytoplasm
Sh ..... Sheath

B. Region of the muscles beneath the distal cytoplasm. Note the densely packed nature of the bundles. Scale = 2µm.

A



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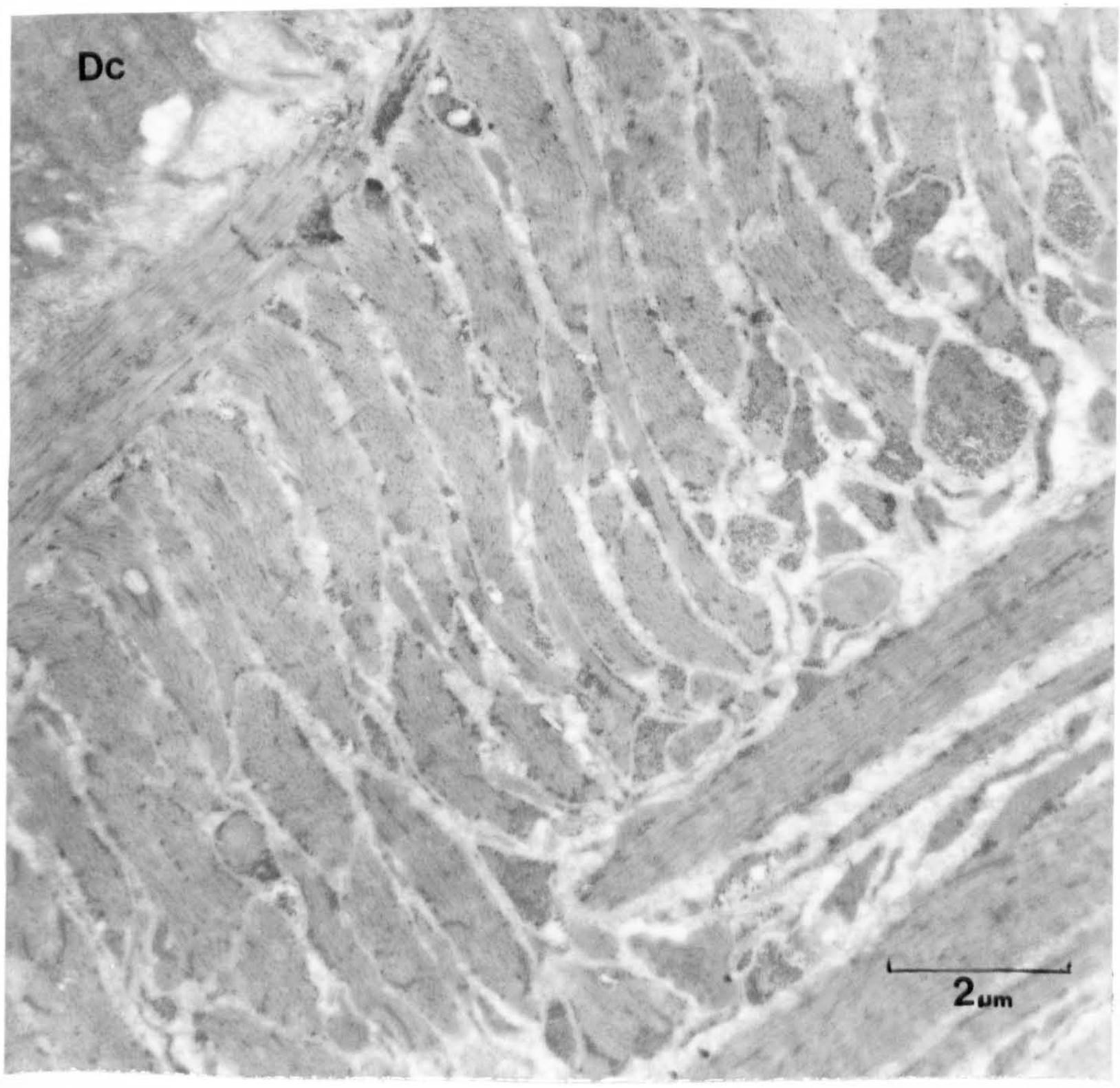


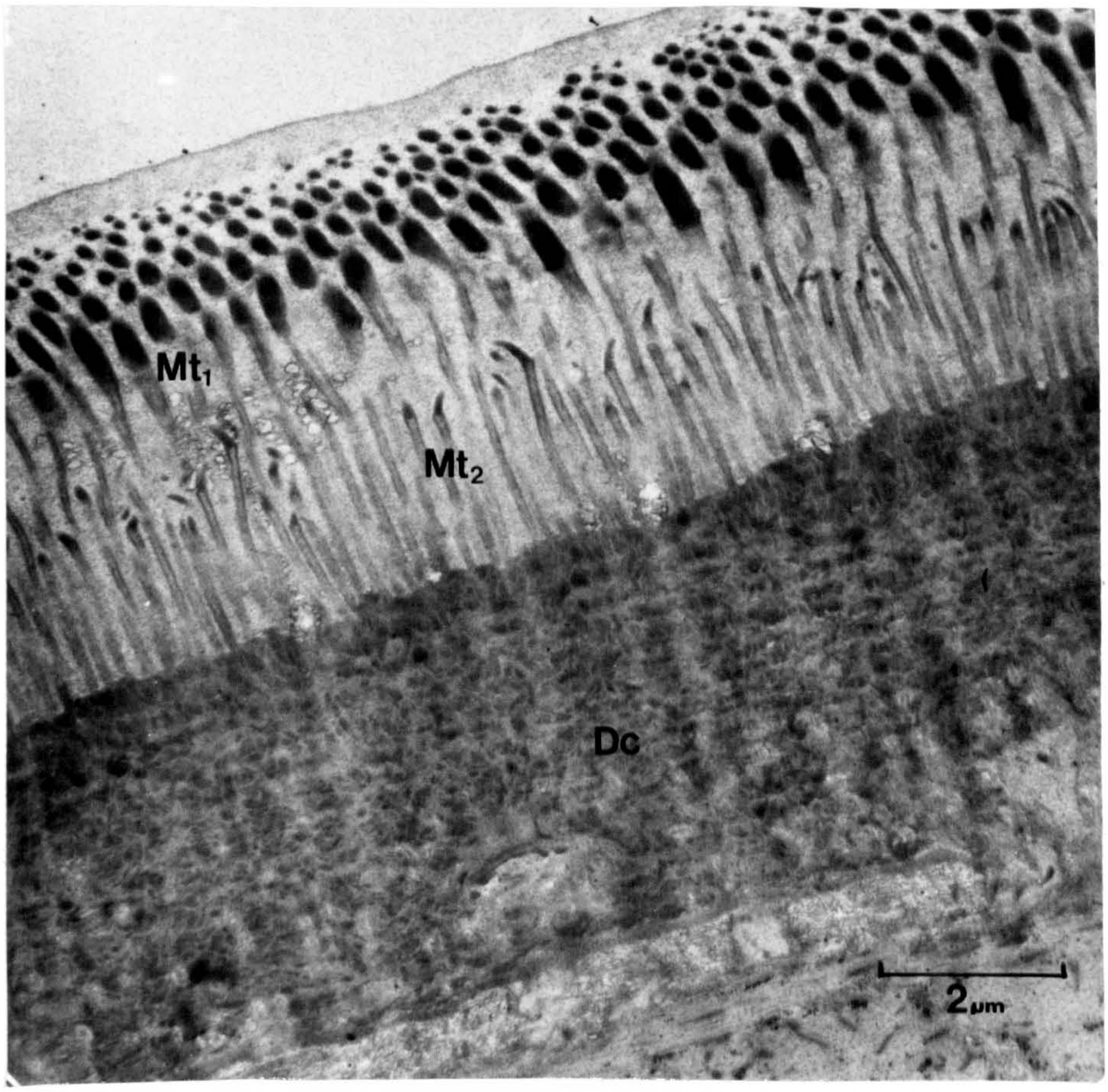
Plate 4.15 Transmission electron micrograph of strobila body wall of the Stage III procercoid of C. truncatus in transverse section

A. The distal cytoplasm and layer of microtriches. Scale = 2 µm.

Dc ..... Distal cytoplasm
Mt_1 ..... Type I microtriches
Mt_2 ..... Type II microtriches

B. Region of muscles beneath the distal cytoplasm. Note the deposits of glycogen granules (gl) Scale = 2µm.

A



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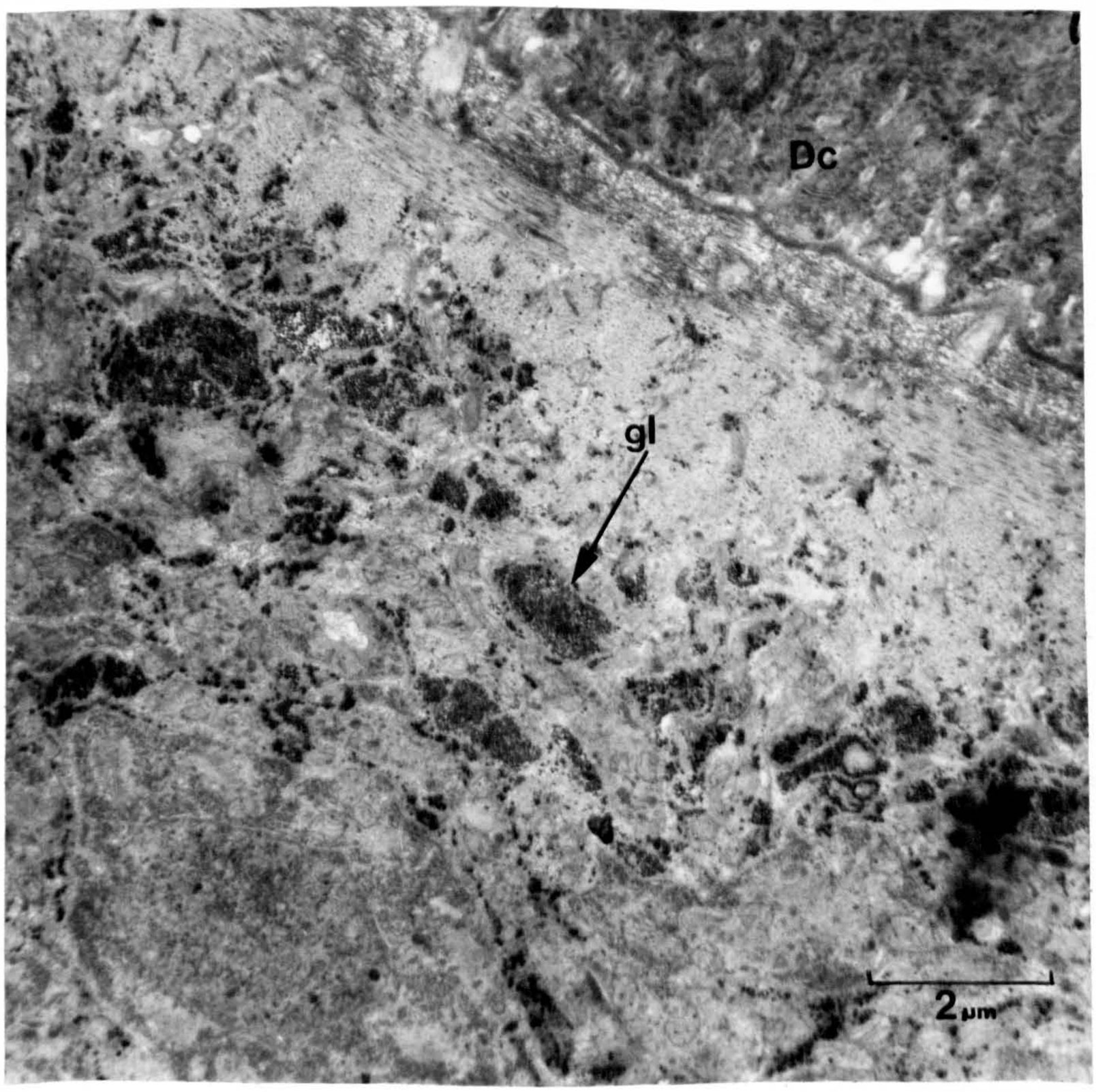


CHAPTER 5

STUDIES ON THE ADULT *CYATHOCEPHALUS TRUNCATUS* IN THE FISH HOST.

5.1 Introduction

Infections with Cyathocephalus truncatus in fish are known to result in severe pathological damage in the form of inflammation and loss of tissues by the host at the site of the tapeworm's attachment (Huitfeldt-Kaas, 1927; Wisniewski, 1932b; Vik 1958; Awachie 1966a; Catalini et al, 1978). However, very little is known concerning the mode of attachment which appears to give rise to most of the damage.

Since no previous report is available on the method of attachment of C. truncatus, it became necessary in the present work to study from experimentally infected fish the tapeworm's attachment and the progressive changes in host tissues as the worm became established. These have been studied structurally and ultrastructurally. Histochemical studies including reaction to certain histochemical stains and demonstration of some enzymes have been carried out on already established adult tapeworm and on host tissues at the area of infection. The level of tissue damage in field-infected fish has been observed and studied histologically.

5.2 Literature review

Some ~~adult~~ tapeworm infections in fish are known to be associated with pathogenic effects on the host. Examples include among others, Gasterosteus aculeatus infection with

Schistocephalus solidus by Vik (1954); Raja clavata infection with Prosobothrium armigerum by Euzet, (1959); Gadus species infection with Abothrium gadi by Williams, (1960); Scyliorhinus stellaris infection with Acanthobothrium coronatum by Rees and Williams (1965) and Gasterosteus aculeatus infection with Ligula intestinalis by Arme and Owen (1968).

Rees (1967) in a review on pathogenesis by adult cestodes noted that in some cases it is attributed to among other factors the intensity of infection, passive obstruction, migration to unusual sites and toxic actions of the tapeworms while in other cases it is attributed to irritative, inflammatory and traumatic actions resulting from tapeworms attachment. Pathogenesis may also be due to a combination of some of the factors listed above.

Rees (1967) remarked that in some adult pseudophyllidean infections as with Bothriocephalus scorpii the pathogenic effect is mainly inflammation of the host tissues and is usually localised - being restricted to the tissues immediately around the scolex and those engulfed by the bothria. In cases where the cestode is armed with hooks as with Acanthobothrium coronatum (Tetraphyllidea) serious damage resulting in total destruction of host tissue as well as haemorrhages may occur.

Cyathocephalus truncatus infection always appears to result in the inflammation of the host tissue to which it is attached.

Vik (1954, 1958) noted in experimental infections of Salmo trutta with C. truncatus that invaded pyloric caeca showed signs of

inflammation after 18 days with swelling after 28 days and the development of hard tissue after 43 days. He reported that after causing rupture of the wall of the pyloric caeca the worms were able to enter the abdominal cavity, thus explaining the discovery by earlier workers (Huitfeldt-Kaas 1913, 1927; Wisniewski, 1932b) of encysted worms in this location. The author concluded that even a light infection seemed to affect the host seriously and could possibly lead to death of the fish.

Still on effects of infection on host tissues, Awachie (1966a) reported that in long-standing infections the blind end of the infected caecum, where the tapeworm is attached, was transformed into a fibrous mass, part of which was sucked into the funnel-shaped scolex of the worm. He observed that such impaired caeca seem to be permanently incapacitated.

Senk (1956) dealt with aspects concerning reproduction in infected fish. He reported that C. truncatus infection affects the ovaries in such a way that the development of fertilized eggs and fry of infected fish was slow and with a high mortality rate compared to the non-infected fish. He added that even among the surviving fry of infected fish were a number which were of poor quality and much under weight compared to fry from non-infected fish.

Other reports, particularly those by earlier workers dealt with occasional heavy infections in fish. Huitfeldt-Kaas (1913) described epizootics in trout and red char as a result of severe infections with C. truncatus. The author was quoted by Vik (1954) as saying:

"The most severely infected fishes were very emaciated, besides which the flesh was decidedly decoloured and some inflamed swellings were observed on their appendices pyloricae. It was found that living fish specimens were anaemic."

Huitfeldt-Kaas reported a maximum number of 200 worms in the pyloric caeca of a single infected fish.

Wisniewski (1932a,b) also recorded mass infection from the river Bosnia, Yugoslavia reporting a maximum number of 400 tapeworms in a single infected fish. He stated that infected fish were retarded in growth and that inhabitants of the region around the sources of the river have complained of a decline in quantity and quality of fish. The author believed that it was due to the tapeworm infection.

From all the reports, it seems very clear that Cyathocephalus truncatus infections are always accompanied by serious pathogenic effects on the fish.

5.3 Materials and methods

Fish used for the study included both naturally infected individuals obtained from Driffield trout streams and those infected in the laboratory and monitored progressively as described in Chapter 3 (see page 98).

5.3.1 Light microscopy

(a) Histological studies:

Single infected caeca and other tissues harbouring tapeworms were removed by dissection from the fish body and processed for

histological studies using standard histological techniques as stated on page 119.

(b) Histochemistry:

Studies were conducted on already established adult tapeworms in fish. Techniques used for the determination of reaction shown by the tapeworm and fish tissues to certain histochemical stains were similar to those described for the procercoid in Chapter 4 (page 119).
Histochemical stains used were also PAS, Alcian blue and Oil-red-'O'.

For enzyme histochemistry similar techniques and incubating media used for the procercoid as described on page 120 were employed. Enzymes demonstrated were also alkaline phosphatase, acid phosphatase and non-specific esterase and lipases.

Characterisation of the non-specific esterases into A-, B- and C- type esterases was carried out as stated above (page 121).

Control methods are similar to those reported for the procercoid larva. (page 121).

5.3.2. Electron microscopy

(a) Transmission electron microscopy

A similar routine technique for preparation and examination of tissues for transmission electron microscopy described for the procercoid (page 122) was used.

(b) Scanning electron microscopy

For scanning electron microscopy, specimens were fixed in glutaraldehyde or buffered 10% formalin, dehydrated in a series of acetone or alcohols of concentrations 50%, 70% 90%. 100% in that order and critical dried in liquid carbon dioxide. The dried specimens were mounted on aluminium stubs precoated with a very thin layer of colloidal silver (Polaron Equipments Ltd). Specimens were gold-coated in polaron diode sputter coater and then examined in the Cam-Scan scanning electron microscope.

5.4 Results and Observations

5.4.1 Structural studies

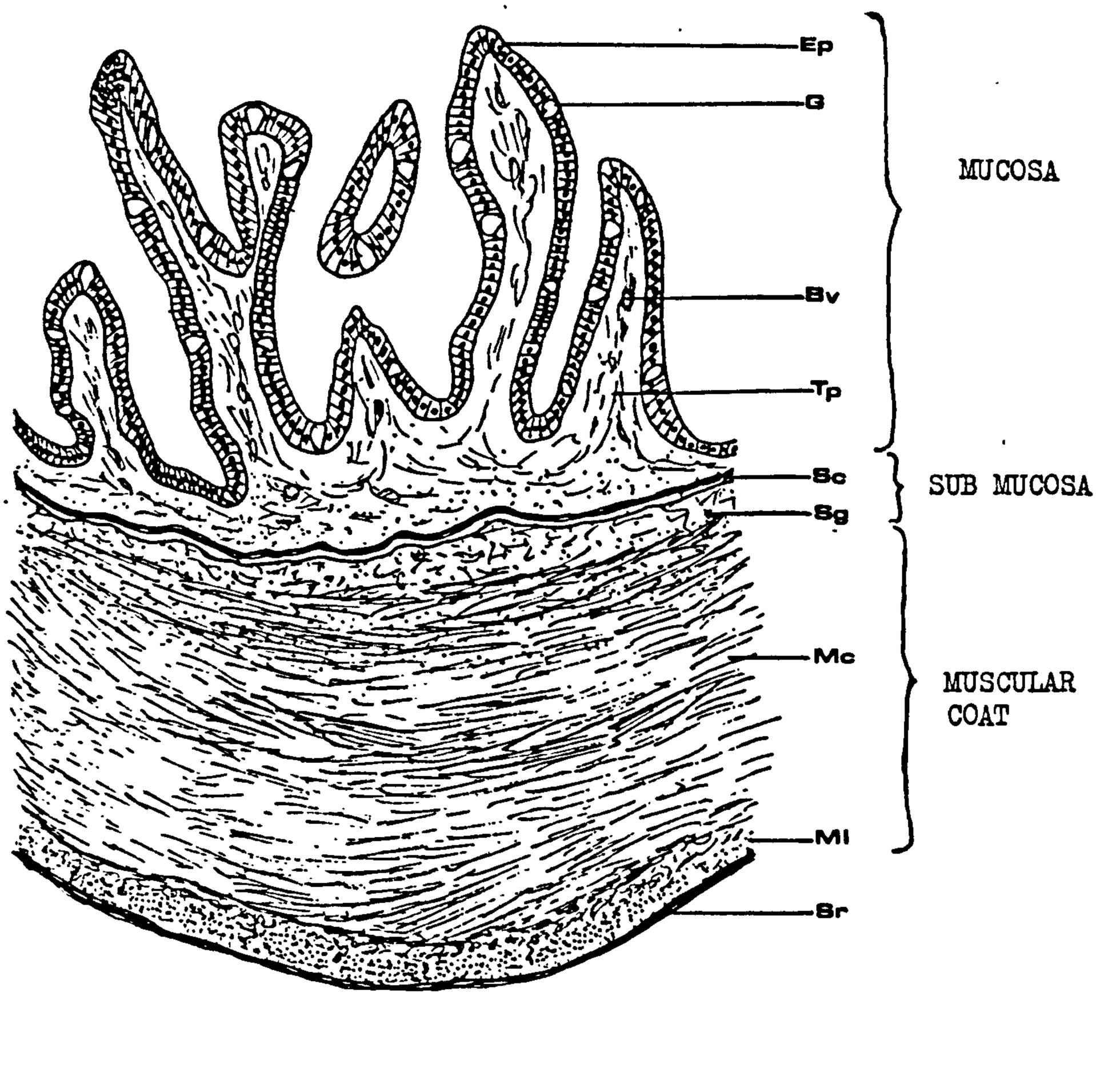
(a) Histology of uninfected caecum: The digestive tract of salmonids has been described by various authors including, among others, Greene (1912), Burnstock (1959), Ezeasor and Stockoe (1980, 1981). In rainbow and brown trout, histology of the pyloric caeca has been described by Weinreb and Bilstad (1955), Bullock (1963), Kimura (1973) and Ezeasor and Stockoe (1980, 1981). The structure of the pyloric caecum of uninfected trout in the present study is in agreement with that described by the earlier authors.

Fig. 5.1 is a diagramatic representation of the anatomy of a portion of typical caecal wall. It is composed of 4 layers: an inner mucosa, submucosa, muscular coat and an outer serosa.

The mucosa is thrown into folds and consists of the epithelium and a small amount of connective tissue. The folds (known as villi) project into the lumen of the caecum. The epithelial layer is made up of 2 basic cell types: the absorptive (columnar or

Fig: 5.1: Histological features of the wall of a pyloric caecum from Salmo gairdneri. Not to scale.

Bv	..... Blood vessel
Ep	..... Columnar epithelium
G	..... Goblet cell
Mc	..... Circular muscle
Ml	..... Longitudinal muscle
Sc	..... Stratum compactum
Sg	..... Stratum granulosum
Sr	..... Serosa
Tp	..... Tunica propria


Ep
G
Bv
Tp
Sc
Sg
Mc
Ml
Sr
MUCOSA
SUB MUCOSA
MUSCULAR COAT

or cylindrical) cell which dominates the layer and the goblet or mucus-secreting cell. Underlying the epithelium is a layer of loose connective tissue, the tunica propria (or lamina propria) which consists of granular cells, fibroblasts and blood vessels.

The submucosa comprises the stratum compactum and stratum granulosum. The former is a very prominent, thick and often undulating layer of non-cellular material. It gives a green stain with Masson's stain which shows it to be a homogeneous and compact mass evidently of collagenous material (Plate 5.1). Underlying the stratum compactum is the stratum granulosum which comprises granular cells, fibroblasts and fibres that merge with the inner region of the muscular layer. The stratum granulosum varies in thickness in different parts of the same section. It may be several cell layers thick or merely indicated by a few separated cells.

The muscular layer consists of a thick inner circular region separated from a thin outer longitudinal region by a layer of connective tissue of varying thickness.

The outer layer (the serosa) consists of a layer of connective tissue which is continuous with the mesentery and may contain adipose tissue and blood vessels.

(b) Tapeworm attachment and histopathology of infected tissues of caecum: In the non-infected caecum the ~~body~~ wall at the distal tip is naturally thicker than at the edges of the caecum. This blind end is the preferred site of attachment of the tapeworm Cyathocephalus truncatus.

(i) Three days old infection: The attachment formed by the tapeworm is restricted to the mucosa, mainly the villi (Plate 5.1). The columnar epithelium of the villi engulfed in the scolex loses its cellular structure and becomes a mass of non-fibrous, non-cellular material serving as the tissue of attachment for the tapeworm. The tissue gives a strong green colour reaction to Masson's stain similar to the stratum compactum of the submucosa. The scolex which retains the elongate funnel shape evident in the procercoid stage, appears to exert a sucking action on the caecal mucosa. At this stage the body wall at the distal tip (area of attachment) is of the same structure and thickness as that of the non-infected caeca.

(ii) Seven days old infection: The tapeworm's attachment has advanced into the region of the submucosa. The mucosa at the point of attachment has been lost and the scolex forms an attachment with the stratum compactum of the submucosa. The force exerted on the tissues of the muscular coat due to the sucking effect of the scolex is shown by the alignment of the tissues with the scolex. (Plate 5.2). The distal tip of the caecum has already started to swell and its muscular coat and serosa are considerably thicked than in other regions of the caecum. The increasing thickness results from the rapid production and proliferation of muscle fibres in the muscular coat - shown at light miscroscope level in Plate 5.2 and electron microscope level in Plate 5.17 and 5.18. Also present in the tissues held within the scolex and overlying it are fibres of collagenous

material which like the stratum compactum give a strong green colour reaction to Masson's stain (Plate 5.3). This collagenous material presumably produced from fibroblasts adds to an increase in thickness of the stratum compactum and is essentially the tissue to which the tapeworm is attached.

The villi of the mucosa in close proximity to the site of attachment develop more goblet cells compared to those of non-infected caeca. Blood vessels in the tunica propria in this region become slightly enlarged. However other tissues of the caecum outside the area of attachment appear histologically normal.

(iii) Fifteen days old infection: The tapeworms attachment has advanced further into the region of the muscular coat (Plate 5.2). The stratum compactum is retained in some places with the tissue to which worm is attached but in other places it is broken down. The collagenous material and the tissue of attachment are similar to that described for the 7 days old infection. The distal tip at which the tapeworm is attached is considerably swollen due to increased production of muscle fibres in the region of the muscular coat.

The tissues in the neighbourhood of the site of attachment are also slightly swollen. The epithelium of the mucosa in this region has evidently been modified into mucus-secreting cells. The blood vessels in the tunica propria and serosa are heavily swollen and the accumulation of blood in them can be seen around

the caecum tip when such caeca are fully excised from the fish gut. The spread of effects of infection is not seen in other regions of the caecum outside the distal tip (area of attachment).

(iv) Thirty days old infection: The tapeworm is firmly attached still in the region of the muscularis. The tissue to which the tapeworm is attached is similar to that described for the 7 days and 15 days old infections. The muscle fibres produced in the muscular coat as tissue reaction to the presence of the worm produce a thick layer spreading all around the scolex surface (Plate 5.4) forming a very heavily swollen bulb at the distal end of the caecum.

The situation in the neighbouring tissues is similar to that described for the 15 days old infection except that blood vessels in the tunica propria and serosa have become greatly enlarged.

(v) Forty days old infection: As shown in Table 3.6 of Chapter 3 (page 99) this was the last fish of the series to be examined. There was no difference in the histology of the host tissue at the region of attachment from observations in the 30 days old infection except that the tapeworm has almost destroyed the distal end of the caecum. The thick layer of muscular fibres produced from host reaction and the outer coat (serosa) at the distal tip have suffered breakages at a number of points.

In the uninfected sections of the caecum the mucosal epithelium was also considerably affected probably due to the presence of the tapeworm strobila. Some parts of the epithelium showed the development of additional goblet cells while other areas had been damaged with considerable loss of columnar cells.

(c) Histochemistry of tapeworm and tissues of the caecum:

Results of histochemical reactions to PAS, Alcian blue and Oil-red 'O' stains as well as reactions to tests for alkaline phosphatase, acid phosphatase, non-specific esterase and lipase are presented in Table 5.1.

(i) Tapeworm scolex and host tissue at point of attachment:

In both the inner and outer scolex surfaces, positive reactions for acid and alkaline phosphatases were demonstrated in the distal cytoplasm while it was weakly positive in the tegumentary cytons (Plate 5.6) No reaction was given by the host tissue to which the tapeworm is attached and in the collagenous tissue around the point of attachment. Non-specific esterase and lipase activities were weakly positive on the distal cytoplasm of the inner and outer scolex surfaces and negative in the infected tissues.

Tests for glycogen, mucopolysaccharides and lipid were positive in the tegument of the scolex. In the tissue of the caecum to which the worm is attached, reactions to these stains were positive (Plate 5.5) but in the infected

tissues around the site of attachment, reactions were only weakly positive or negative.

(ii) Tapeworm strobila and the uninfected parts of the pyloric caecum. Positive reactions to tests for enzyme activity were evident in the tapeworm strobila in the distal cytoplasm and in the tegumentary cytons; reactions for alkaline phosphatase (Plate 5.6) were more pronounced in the tegument than those of acid phosphatase (Plate 5.7) and lipase while that of non-specific esterase was weakly positive. Enzyme reactions in the reproductive organs were mainly confined to their ducts. With histochemical stains, strong positive reactions for glycogen, lipids and mucopolysaccharides were demonstrated in the distal cytoplasm and in the underlying cytons with a positive or weak positive reaction in the reproductive organs.

In the uninfected parts of the caeca, acid phosphatase, non-specific esterase and lipase activities were mainly positive in the columnar epithelium whereas alkaline phosphatase acitivity was present in the columnar epithelium and in tunica propria. Strong positive reactions were obtained in the distal mucosa of the intestine for glycogen, mucopolysaccharides and lipids.

(iii) Characterisation of the esterases:

As shown in Table 5.2 the esterase reaction was totally inhibited when treated with 10^{-2}M $AgNO_3$ suggesting the absence of B- type esterases or cholinesterases except at the distal cytoplasm of the inner scolex surface where there was

Table 5.1 Distribution of some enzymes and reaction to certain histochemical stains in adult C. truncatus and in the pyloric caeca of Salmo trutta.

	Alkaline phosphatase	Acid phosphatase	Non-specific esterase	Lipase	PAS stain	Alcian blue stain	Oil red 'O' stain
1. ADULT TAPEWORM							
Scolex							
Distal Cytoplasm	**	**	*	*	***	**	**
Teg. cytons	*	*	*	*	**	**	**
Musculature	-	-	-	-	*	-	*
Strobila							
Distal Cytoplasm	**	**	*	**	***	***	***
Teg. Cytons	**	*	*	*	**	*	**
Musculature	*	*?	-	*?	*	-	*
Parenchyma	*	-	-	*?	*	*?	*
Excretory ducts	-	-	-	*?	*	-	-
Vitellaria	*	*?	-	*	*	-	*
Testes	-	-	-	*?	*	*	*
Ovary	**	*	-	*?	*	*	*
Uterus	*	*	-	*?	*	*	*
Cirrus organ	*	*	*?	-	*	*?	*?
Vagino-uterine organ	**	*	-	*?	*	-	*?
Eggs	*	*	-	-	**	*?	*
2. PYLORIC CAECA OF FISH							
Point of worm attachment	-	-	-	-	**	**	**
Infected tissue	-	-	-	-	-	-	-
Lumen of caecum	**	**	*	**	**	**	**
Uninfected tissues mucosa	**	**	*	**	***	***	*
" submucosa	*	*	-	*	*	*	*?
" Muscularis	-	-	-	-	-	-	-
" Serosa	-	-	-	-	-	-	-

KEY: *** ..... Strongly positive ** ..... Positive
* ..... Weakly positive *? ..... Reaction uncertain - ..... Negative

Table 5.2: Characterisation of esterases in adult C. truncatus and in the pyloric caeca of brown trout using suitable activators and inhibitors.

	10^{-5}E600	10^{-2}MAgNo$_3$	10^{-3}MCysteine	10^{-4}MPCMB	10^{-3}Pb(No$_3$)$_2$
Tapeworm					
Scolex	+	-	±	+	±
Distal cytoplasm	+	-	±	±	+
Tegumentary cytons	+	-	±	±	±
Musculature	-	-	-	-	-
Parenchyma	-	-	-	-	-
Excretory ducts	-	-	-	-	-
Vitellaria	+	-	+	±	±
Testes	-	-	-	-	-
Ovary	-	-	-	-	-
Uterus	-	-	-	-	-
Cirrus organ	-	-	-	-	-
Vagino-uterine organ	-	-	-	-	-
Eggs	-	-	-	-	-
Pyloric caecum of fish					
Point of attachment	-	-	-	-	-
Infected tissues	-	-	-	-	-
Lumen of caecum	+	-	±	±	±
Mucosa of non-infected tissues	+	-	+	+	+
Submucosa "	-	-	-	-	-
Muscularis "	-	-	-	-	-
Serosa "	-	-	-	-	-

Note
E600 = diethyl p-nitrophenyl phosphate
AgNo$_3$ = Silver nitrate
PCMB = Sodium p-chloromecuribenzoate
Pb(No$_3$)$_2$ = Lead nitrate

Key
+ = No action
± = partial inhibition
- = complete inhibition

only partial inhibition. The esterases were thus predominantly A-and C-types as shown in the table and resemble, therefore, those of the procercoid (page 128).

5.4.2. Ultrastructural studies

I Scanning electron microscopy

For scanning electron miscroscope study, the tapeworm, C. truncatus, attached at the distal end within a caecum was removed from the caecum but with the attachment intact. The scolex of the tapeworm specimen with the host tissue attached is shown in plate 5.8A. After removal of the host tissue serving for attachment, by the use of fine dissecting needles the characteristic funnel-shape of the scolex is apparent (Plate 5.8B).

II Transmission electron microscopy

Ultrastructural studies were conducted on worms that were firmly established in the pyloric caeca. This was done so that the scolex and the attachment could be studied simultaneoulsy with the strobila.

(a) Seven days old infection

(i) The scolex: Ultrastructural studies was conducted on the scolex and the tissue of the caecum to which it is attached. The damage shown in different areas of the muscular coat lying outside the attachment material varies according to their proximity to the tapeworm - those areas close to the site

of attachment are more severely damaged than those further away in the caecal wall and close to the serosa. Three such areas in the muscular coat are identifiable and have been designated zones A, B and C according to their proximity to the tapeworm scolex (Fig. 5.2). The zones are more clearly marked out in the 15 days old infection than in the 7 days old infection due to the very firm attachment formed at the former. The characteristics of each zone are described below (page 186) for the 15 days old infection.

1. Inner scolex surface - The microtriches of the distal cytoplasm are embedded in the material tissue to which the worm is attached (Plate 5.9). The tissue is non-cellular and non-fibrous but a compact mass. Within the tissue are tiny vesicles (Plate 5.9B) presumably released by the tapeworm through the surface of attachment. The microtriches appear to have lost their apical caps as seen in the Stage III procercoid and instead possess at their tips electron-dense ring-like borders enclosing a matrix of homogenous material.

The distal cytoplasm is an undulating layer and vary between 2 µm and 6 µm in thickness. The most distinguishing feature in this region is the presence of large numbers of electron-dense bodies, mitochondria and vesicles (Plate 5.10) apparently passing from the tegumentary cytons through the internuncial processes. The electron-dense bodies appear to be located in the outer region of the distal cytoplasm, some even at the distal end of some microtriches (Plate 5:9B). The mitochondria

and vesicles on the other hand are usually located in the basal region of the distal cytoplasm (Plate 5.10). The electron-dense bodies occur in sizes ranging from about 0.1μm to about 1μm in width. They are also of different shapes including spherical, ovoid, discoid and rod-shaped. The discoidal bodies appear bilobed with both ends enlarged (Plate 5.10B). The electron-dense bodies in the distal cytoplasm of the inner scolex region differ in shape and size (being larger) from the granules found in the distal cytoplasm of the strobila.

The basal lamina is reduced to less than 1μm in thickness and bears numerous connections from the tegumentary cytons and the inner connective tissues. Numerous closely packed bundles of muscle fibres occupy an extensive area beneath the basal lamina (Plate 5.13B) and only several canals of connective tissue and internuncial processes occur between them. Large-sized mitochondria are sometimes found in the region associating with the bundles of muscle fibres. This is in addition to the several mitochondria in the internuncial processes, distal cytoplasm and the parenchyma.

The tegumentary cytons possess relatively small nuclei and large cytoplasmic spaces in which are borne numerous electron-dense bodies and mitochondria (Plate 5.12). Some of the bodies are more highly electron dense than others. How they are produced is not known but it is possible that they are formed during the procercoid stage. In the scolex of the Stage III procercoid (Plate 4.12) the tegumentary cytons of the inner

surface bear the electron-dense bodies as granules or particles and possibly these develop to the form described above for the adult stage. They may also be continuously produced in the adult stage as those formed initially are discharged into the distal cytoplasm.

2. Outer scolex surface - Tissues of the host are sometimes found to be associated with microtriches of the distal cytoplasm of the outer scolex surface as shown in Plate 5.4 although no attachment between host tissue and parasite microtriches has been demonstrated. These microtriches are short (measuring about 2µm long) and unlike those of the inner scolex surface they have dense apical caps at their tips.

The distal cytoplasm is heavily granulated mainly with rod- and discoid-shaped granules which appear smaller than the electron-dense bodies in the inner scolex tegument.

There is an extensive musculature in this region beneath the basal lamina. Like in the neck (Plate 5.14A) the muscle bands comprise an outer longitudinal underlying the basal lamina and an inner circular and oblique types. There are also transverse muscle bands occurring between the inner and outer scolex surfaces (Plate 5.13). Because of the extensive musculature beneath the distal cytoplasm in the inner and outer scolex surfaces the tegumentary cytons are located in the region of the medulla although they are mostly situated in the outer medullary position.

In the neck region, except for a few tegumentary cytons borne in the medulla beneath the tapering basal part of the

funnel-shaped scolex, the medulla consists mostly of muscle bands.

(ii) <u>The strobila</u>: The ultrastructure of the tapeworm body wall in a specimen recovered from fish 7 days after infection is presented in Plate 5.15. An interesting feature is the presence of only one type of microthri^ches, possibly type II in view of their size; they measure about 3 µm long. Numerous vesicles containing granular particles occur in the distal cytoplasm and appear to be released into the region of the microtriches. These vesicles are sometimes found within individual microtriches and appears to break out through the microthrix wall (Plate 5.15A). In some cases the vesicles appear to move along the length of the microthrix breaking out at the apical cap resulting in an accumulation of such vesicles and their contained particles in the region immediately distal to the microtriches. It is possible that some microtriches are being selectively eliminated and this may account for the loss of type I microtriches (observed in the Stage III procercoid) at the early stages of tapeworm infection in fish. The actual time of loss of the type I microtriches was not observed. Other parts of the body wall ultrastructure are essentially similar to that described for the stage III procercoid (page 143).

The tegumentary cytons of the strobila have numerous particles and granules in their cytoplasm, and it is presumably these particles and granules that are released through the body surface and microtriches of the distal cytoplasm.

(b) Fifteen days old infection

(i) The strobila: An electron micrograph of the longitudinal section of the strobila body wall shown in Plate 5.16 indicates the presence of one type of microthrix similar to that described for the 7 days infection but vesicles observed in the microtriches and distal cytoplasm at 7 days are apparently absent. All adult tapeworms recovered from fish after 15 days of infection show the features depicted in Plate 5.16. Other structures of the body wall are similar to those of other stages already described.

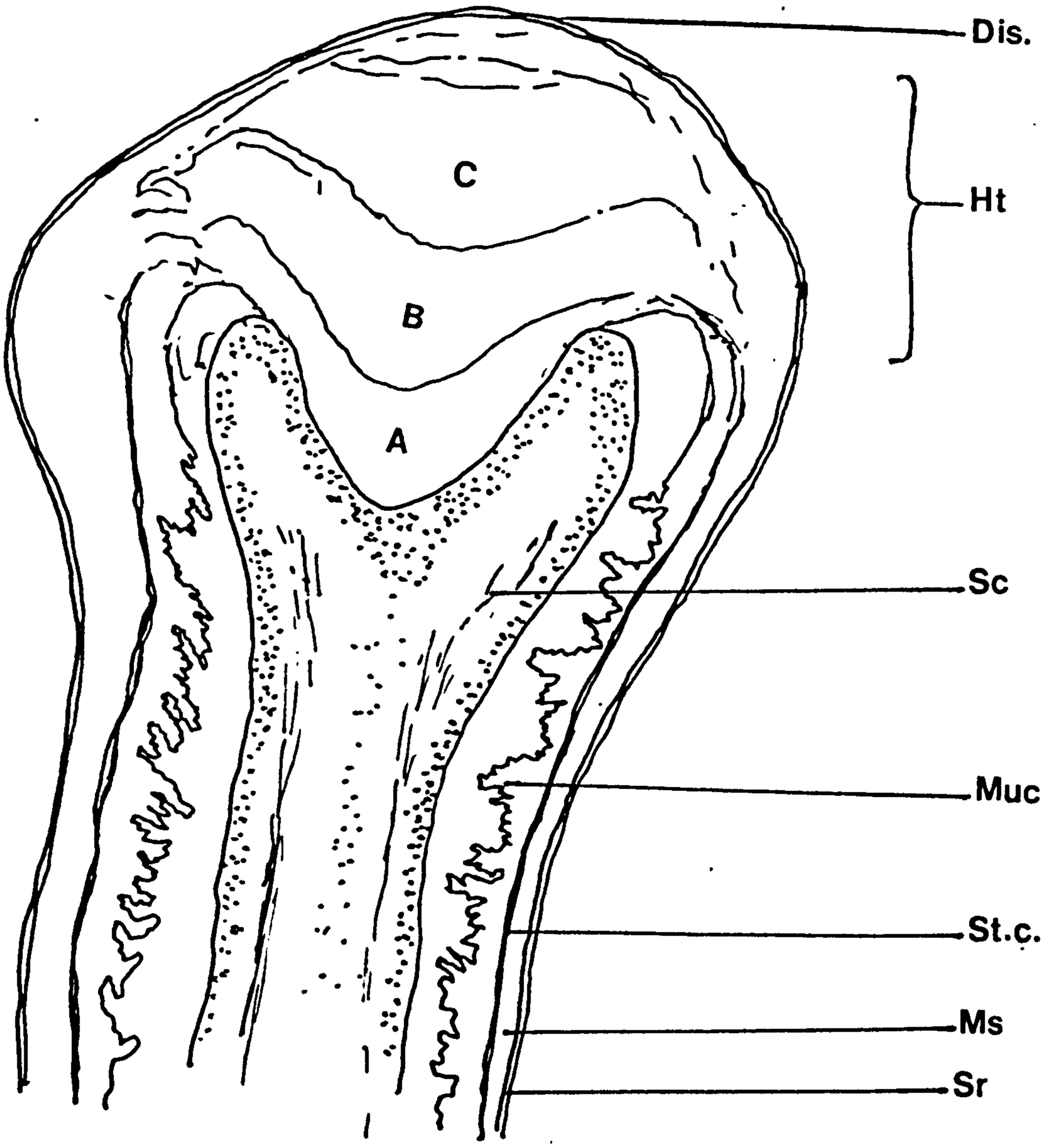
(ii) Host tissue at site of infection: The ultrastructure of the scolex and the tissues to which tapeworm is attached is similar to that of 7 days old infection. The degree of destruction seen in the host tissue above the attachment is directly related to the proximity of the tissues to the point of attachment.

Fig. 5.2 is an annotated diagram showing the location of zones A, B and C which have been identified at the distal ends of pyloric caeca containing C. truncatus.

1. Zone A - The tissue in which microtriches are embedded has no cellular structure as noted above (page 182). Overlying this tissue is the muscular coat (Plate 5.17) whose cells have apparently lost their cytoplasmic contents and are in a state of being broken down. The muscle fibres are irregularly arranged, loosely packed and appear to be embedded in a matrix-like substance evidently composed of degraded tissues.

Fig. 5.2 Diagramatic representation of a median L.S. of a pyloric caecum of Salmo trutta containing a mature Cyathocephalus truncatus. The limits of zones A, B and C (see text) are indicated (compare with Plates 5.17, 5.18 and 5.19). Not to scale.

Dis.	.....	Distal tip of caecum
Ht	.....	Swollen tissues of host
Muc	.....	Mucosa
Ms	.....	Muscular coat
Sc	.....	Scolex of tapeworm
St.c.	.....	Stratum compactum
Sr	.....	Serosa


Dis.
C
B
A
Ht
Sc
Muc
St.c.
Ms
Sr

2. Zone B - This region is the muscular coat sensu stricto. In a non-infected caecum, the muscular coat is seen to contain densely packed muscle fibres which are arranged in parallel.

In the infected caecum, as in Zone B (Plate 5.18), the bundles of muscle fibres are loosely packed. Large amounts of muscle fibres fill the greater part of the tissue The nature of these fibres can be clearly seen at high power (Plate 5.18B) and the electron-dense 'Z' lines of each fibre are clearly recognisable. In some cells in this region, the cytoplasmic content is greatly reduced with consequent distortion of the cell body. The nuclei, in most cases, appear destroyed and have a distorted appearance. Accumulations of lipid bodies in groups are also seen in this zone.

3. Zone C - Tissues of this zone in an infected caecum are more or less similar to those found at the muscular coat/ serosa of the non-infected caecum. In the infected caecum however (Plate 5.19) cells in the region appear to be losing their cytoplasmic contents followed by possible disintegration of the nuclei. There is much endoplasmic recticulum bearing numerous ribosomes. Also present are numerous muscle fibres loosely packed. Most of the fibres are smaller in size than those of Zone B and are probably newly formed fibres.

5.4.3. Musculature of the scolex and neck of C. truncatus

In the Stage III procercoid, the neck region is highly muscular with an outer layer of longitudinal muscles beneath

which are circular and oblique muscles. Within the medulla are numerous longitudinal muscles fibres which continue posteriorly and join to form two lateral longitudinal bands in the strobila.

Within the scolex, in addition to the outer longitudinal muslces and the underlying circular and oblique muscles, there are transverse muscle bands (Plate 5.13A) which connect with the inner and outer scolex surfaces.

The disposition of muscle layers in the scolex and neck of the adult C. truncatus is similar to that described above in the Stage III procercoid (see Plates 5.13B and 5.14). It is worth emphasizing here that the scolex and neck regions of C. truncatus are very muscular &these network of muscles function for attachment of the tapeworm to the caecal wall of the fish.

5.4.4. Pathogenic effects on tissues of infected fish.

The progressive damage of tissues at the distal tip of the caecum to which the worm is attached has already been described (page 173). In evidently long-standing infections caeca which contain one or more worms appear to fuse with adjacent non-infected caeca whose tissues become slightly swollen due to unusually large amounts of muscle fibres in them. In heavily infected fish, the entire region of the pyloric caeca becomes fused into a single mass (Plate 5.20) which may additionally fuse with the abdominal body wall immediately adjacent to it.

In some cases, tapeworms were seen to have penetrated through the caecal wall and were attached directly to the muscles

of the abdominal wall with the strobila still within the caecum. In other cases the whole tapeworm had moved through the perforated distal tip of the caecum and was found free in the body cavity (Plate 5.21). Worms could also be discharged into the intestinal tract with the detached distal end of the caecum still held in the scolex as described previously (Chapter 2, page 36).
Caeca with perforated tips are common in heavily infected fish.

At times tapeworms are even found immediately beneath the skin of fish (Plate 5.20) and it appears likely that individuals can penetrate through the entire body wall of the fish and become released to the outside with unpredictable results to the host.

Tapeworms were occasionally found to be completely enclosed in fibrous host tissues in the form of a cyst. Such tapeworms were always deformed in morphology and in most cases were found to be dead.

occurrence of more than single worm

Most of the serious damage to fish was caused by infection within a single caecum. A maximum number of six worms per caecum was recorded (page 34). The first individual to enter the caecum presumably attaches to the distal end while subsequent specimens attach to the side walls.

The scale of pathological damage in infected fish depends upon the intensity and duration of infection. During the present study the mean number of pyloric caeca for individual rainbow trout was about 65. In fish with small numbers of tapeworms obviously only a small number of caeca can be infected and in such cases

each infected caecum normally harbours only a single specimen. The cumulative level of damage to tissues of such fish is thus low and restricted to the caeca that are infected; but when the numbers of worms are large (say 117 worms - the highest recorded in this study) the majority of caeca are involved, some harbouring more than one tapeworm in each caecum. Consequently there is much damage to tissues of the fish.

The degree of damage suffered by each infected caecum becomes progressively more severe as the infection becomes more prolonged and especially when mutliple infections with other helminth parasites occur.

All the fish collected from Driffield in the course of the present study were infected and therefore it was not possible to make a comparion between infected and non-infected fish from the same water system. Most fish examined possessed distended abdomen, particularly when harbouring a heavy infection with C. truncatus. Such fish were often pale in colour, appearing very anaemic and showed blister-like pustules on the skin of the abdomen.

5.5 Discussion

In the present study, it has been seen that when the Stage III procercoid of C. truncatus enters into fish it first secures an attachment after which the onset of maturity is marked by the presence of eggs in the uteri.

It has also been seen that C. truncatus is similar to to other tapeworms in ultrastructure of the body wall of its strobila.

From observations made during the present study the mode of attachment is seen to be largely physical and possibly partly chemical. It is physical in the sense that the scolex acts as a muscular sucker. The attachment is probably achieved in the same way as with suckers of other tapeworms but the scolex of C. truncatus differs from others in its relatively large size, its funnel-shaped form and its markedly apical position. Its sucking effect is rendered very powerful by its dense musculature as well as that of the neck region and very firm by the manner by which host tissues taken into the funnel appear to become structurally fused with the inner surface of the funnel. In the latter case, the disposition of the microtriches and the irregular nature of the inner scolex surface could play the role of controlling friction. Furthermore, the microtriches have been found to be sunk into the host tissues; this probably causes the recorded inflammation and swelling at the distal tip of an infected caecum.

The attachment may also be chemical in that the scolex shows high metabolic activity in the distal cytoplasm of the inner surface. This is indicated by the presence of numerous mitochondria and the positive reaction to tests for enzyme activity at the point of attachment of the inner surface of the scolex to host tissue. Numerous electron-dense bodies and vesicles produced in the gland-like tegumentary cytons are possibly released into host tissue through the outer surfaces of the distal cytoplasm and microtriches. These electron-dense bodies and vesicles

probably contain certain proteinaceous substances that may weld the parasites scolex surface to the host tissue. The may also contain some chemical agents or enzymes which may contribute to the breakdown of host tissues. Similar electron-dense bodies have been noted ultrastructurally in the penetration glands of tapeworm metacestodes (Lethbridge and Gijsbers, 1974; Pence, 1970; Voge, 1973) and in the syncytial tegument of the anterior organ (an invagination at the central apical portion of the rostellum) in the scolex of adult Hymenolepis diminuta by Spacian and Lumsden (1980). The latter authors reported that the bodies were produced in the rostellar tegumentary cytons; in C. truncatus, the electron-dense bodies are similarly produced in the tegumentary cytons which has been described above as gland-like. The actual role of these bodies remain speculative as stated above and requires further investigation.

The mode of attachment of C. truncatus seems similar to that of Tetrabothrius affinis (Cyclophyllidea) described by Rees (1956). In both tapeworms, attachment involves engulfing of host tissue in the cavity or cavities of the scolex. However Rees reported that tissue destruction due to T. affinis attachment was restricted to those tissues engulfed in the four hemispherical suckers. The author further indicated that T. affinis needed to be attached for 'sometime' for hyperplasia to occur. From the present study C. truncatus attachment is seen to cause damage not only to tissue engulfed in the scolex cavity but also to neighbouring tissues. The attachment was found to have resulted in pronounced damage in the caecum wall of 7 days old infected fish.

Histochemical observations on infected pyloric caeca indicate that while normal nutritional and enzyme acitivity may continue over most of the internal surface of other regions of the caecum the distal portion where the tapeworm is attached may appear totally incapacitated. In the tegument of the tapeworm, hydrolytic enzymes like alkaline and acid phosphatases, non-specific esterases and lipases have been localised. This indicates that like other tapeworms, the body surface of C. truncatus is suited for nutritional uptake (see Lee, 1966; Lumsden, 1975; Lumsden and Hildreth, 1983).

The tendency of the tapeworm to penetrate the body wall of the caecum to which it is attached is very peculiar and difficult to understand. It is possible that the host tissues at the point of attachment are broken down due to the strong continual muscular action of the scolex. At the same time, the localisation of enzymes at the inner scolex surface could indicate that the tapeworm probably digests the host tissue to which it is attached and thereby penetrating the caecal wall. Also chemical agents may be contained in the electron-dense bodies and vesicles in the distal cytoplasm and microtriches of the inner scolex surface, as described ultrastructurally above, which result in dissolution of the caecal wall. Penetration of host tissue is a well known feature of procercoids of pseudophyllidean cestodes e.g. Diphyllobothrium latum Schistocephalus solidus and Ligula intestinalis. This penetration enables the procercoid to gain access into the body cavity for encystment

in the second intermediate host where it develops into the plerocercoid stage (see Vik, 1954; Smyth 1959 and Arme et al. 1983). In C. truncatus, the tendency to penetrate host tissue and get into the body cavity may represent a reminiscence of the behaviour of an ancestral procercoid boring through the gut wall of its intermediate host before becoming encysted in the body cavity or musculature. Wisniewski (1932b,c) regarded C. truncatus as possessing neotenic features and attaining maturity in fish at a stage when it should have been a plerocercoid.

However the result of the tendency by the tapeworm to migrate into the body cavity of the fish does not seem to be ultimately favourable to the fish or the tapeworm itself as presently observed. Whereas the tapeworm can become encapsulated in the host body cavity or even possibly shed outside through the body wall, the fish tissues and organs that associate with the tapeworm are destroyed.

The occurrence of the tapeworm in the body cavity observed in the present study confirms earlier reports by Vik (1954, 1958) of the ability of the tapeworm to destroy and penetrate host tissues at the point of attachment.

Even with a high prevalence and intensity of infection, specimens of C. truncatus tend to settle only in the pyloric caeca of fish and not in other regions of the alimentary canal; thus occurrence of more than one tapeworm in individual caeca may result. This may accentuate most of the pathogenic effects noted above and contribute to possible emaciation, retarded growth rate and loss of weight as reported by Huitfeldt-Kaas (1927) and Wisniewski (1932b).

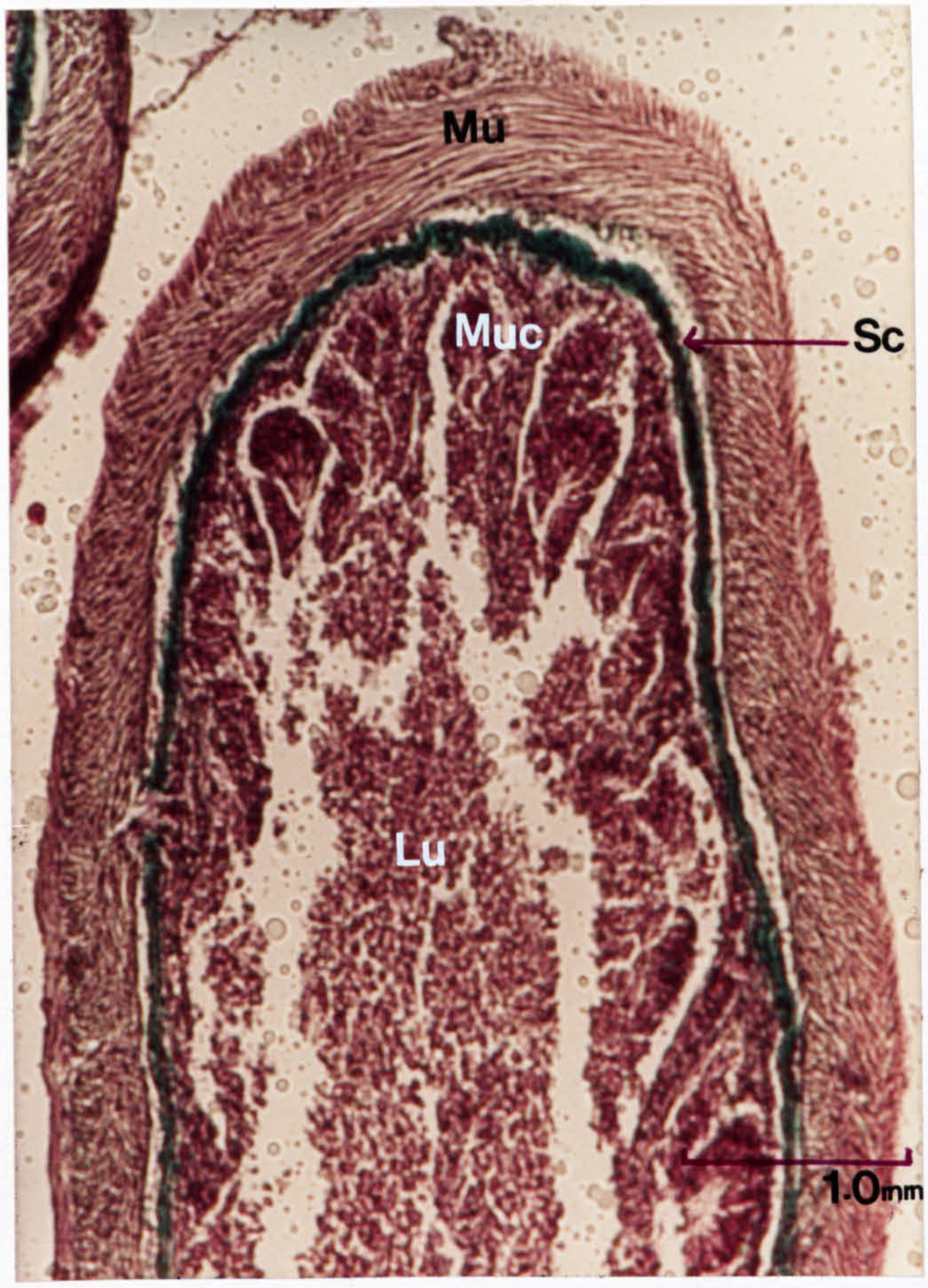
Plate 5.1

A. Longitudinal section of a non-infected caecum of Salmo trutta. Masson green Scale = 1mm.

Lu ..... Lumen of caecum
Mu ..... Muscullar coat
Muc ..... Mucosa
Sc ..... Stratum compactum

B. Longitudinal section of an infected caecum showing Cyathocephalus truncatus (T) attached to the distal portion in 3 days old infection. Masson green Scale = 2mm.

A



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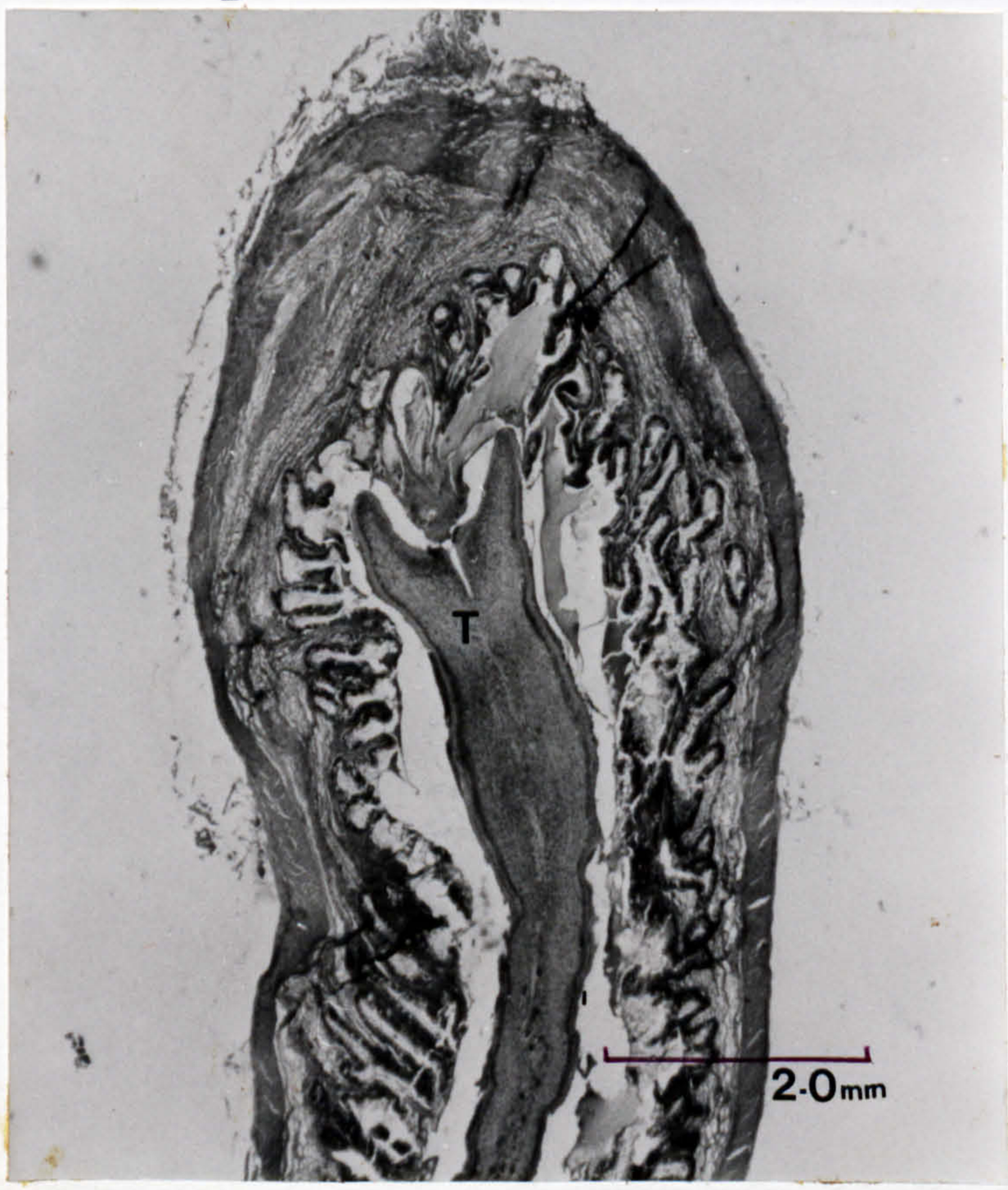


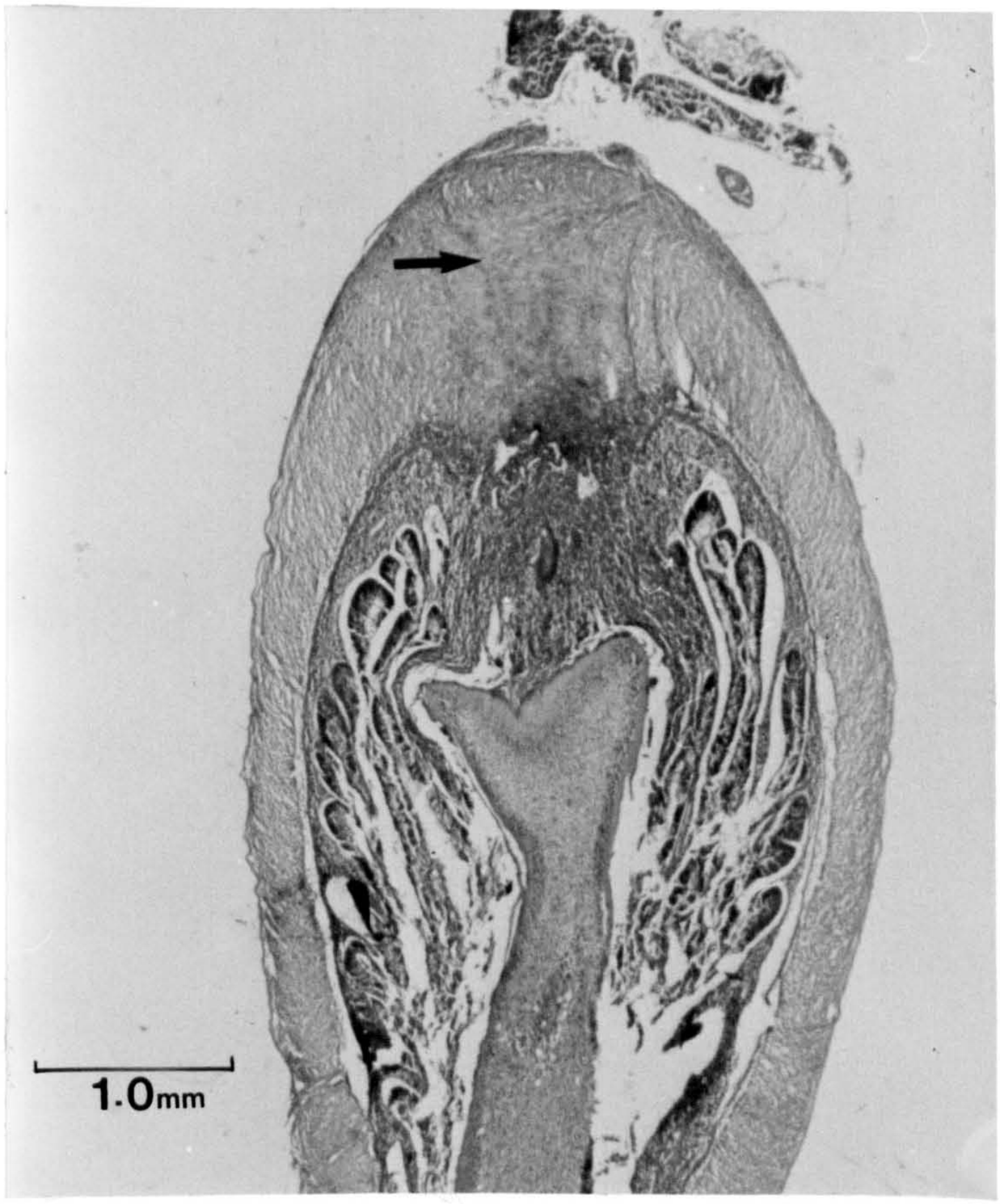
Plate 5.2 Longitudinal section of infected caeca of Salmo trutta showing attachment of C. truncatus at the distal end of pyloric caecum, in:

A. 7 days old infection. Masson green. Scale = 1mm

B. 15 days old infection. Masson green. Scale = 1mm.

Note the swollen nature of host tissue at area of attachment and the stress on host tissue (arrowed) resulting from tapeworms sucking effect.

A



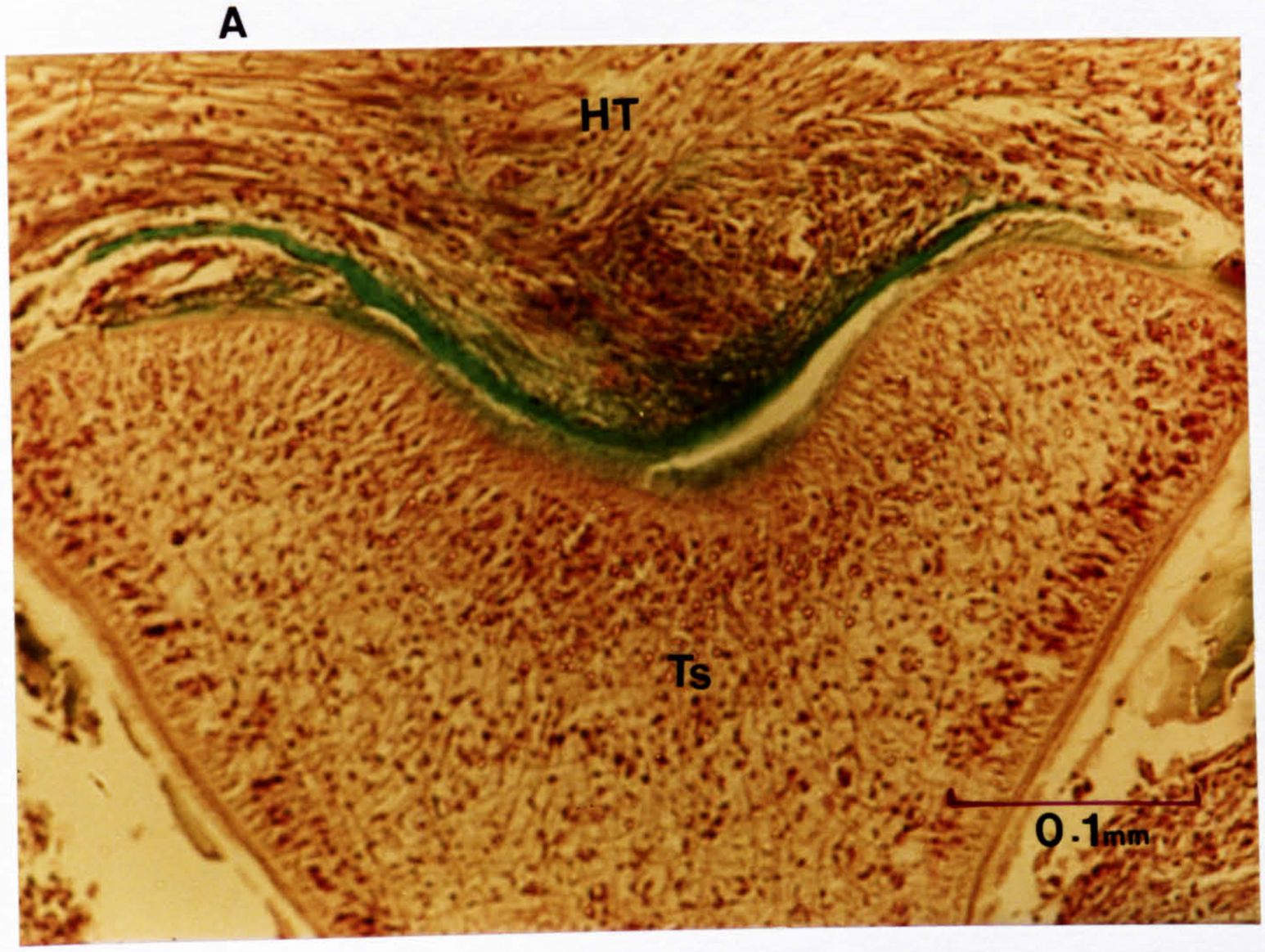
B

Plate 5.3 A. Longitudinal section of the scolex of Cyathocephalus truncatus and the host tissue at site of tapeworms attachment in a 7 days old infection showing the collagenous tissue (green in colour) serving for attachment. Masson green Scale = 0.1mm

HT ..... Host tissue
Ts ..... Tapeworm scolex

B. Longitudinal section of C. truncatus scolex and the host tissue at site of tapeworm's attachment in a 15 days old infection showing the occurrence of the collagenous material in the host tissue (HT) overlying the site of attachment as well as in tissue serving for attachment. Masson green Scale = 0.1mm

A



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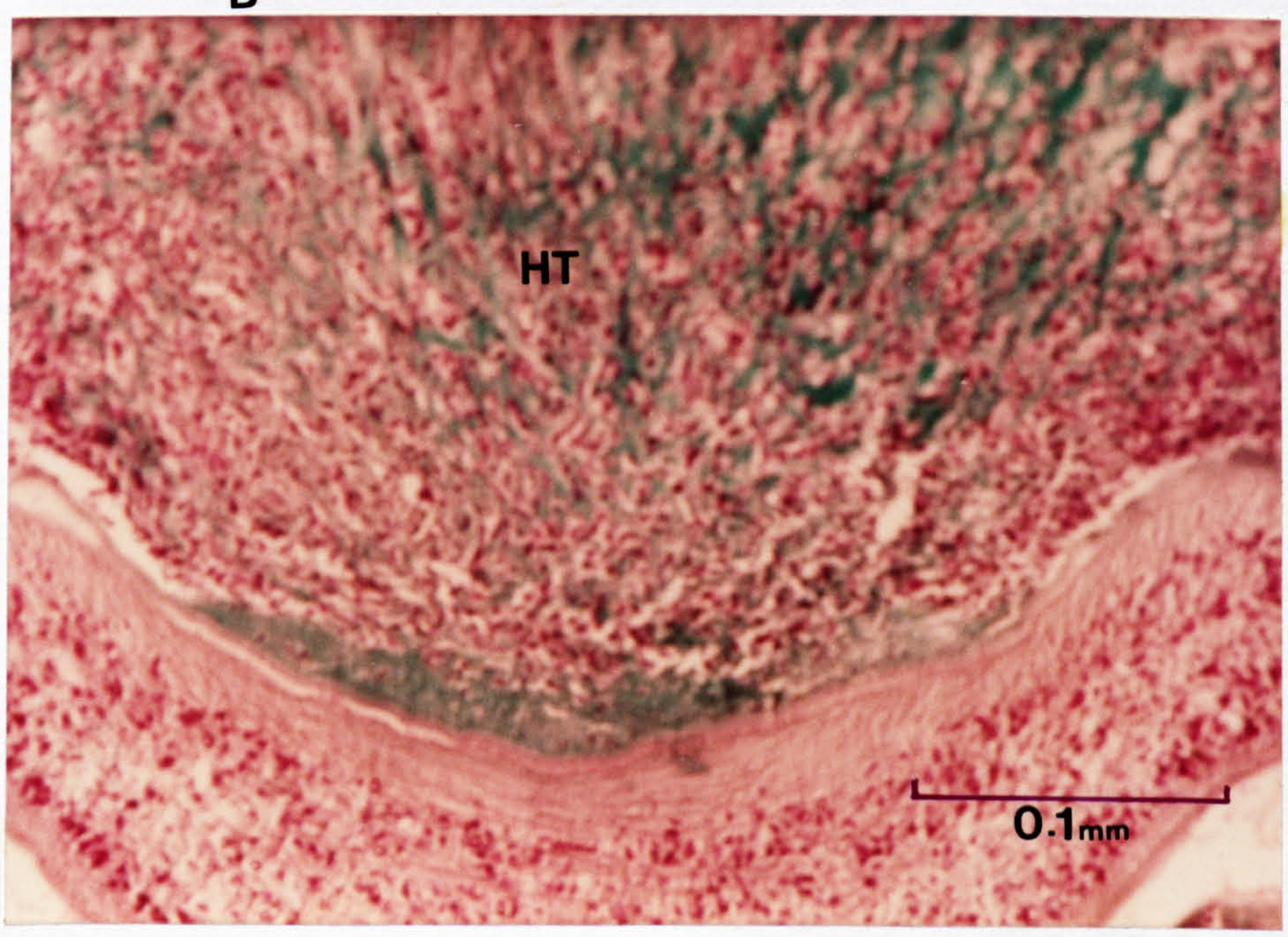


Plate 5.4 Longitudinal section of an infected caecum of Salmo trutta with Cyathocephalus truncatus attached at the distal portion in a 30 days old infection. Mallory. Scale = 1mm.

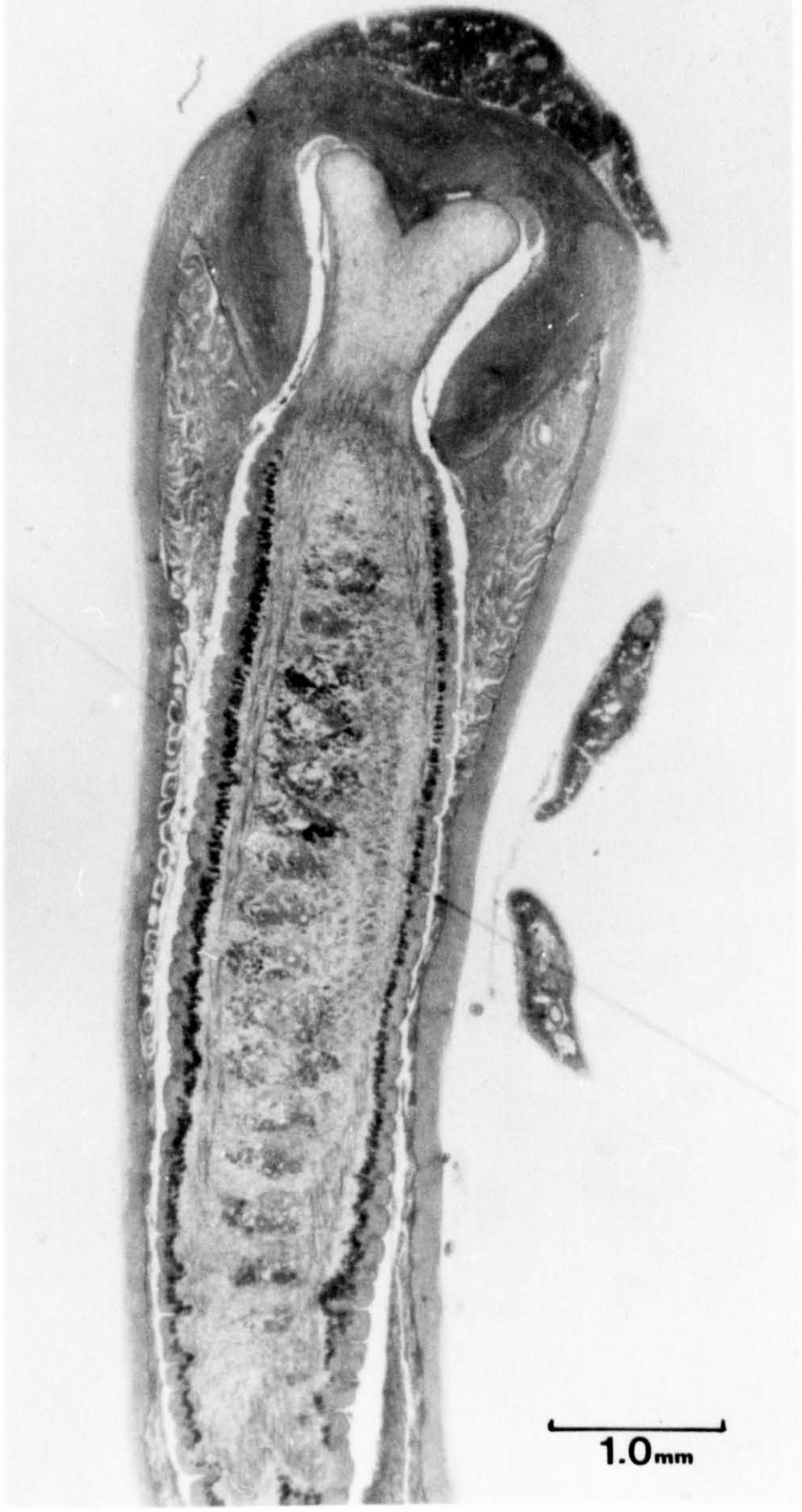

1.0mm

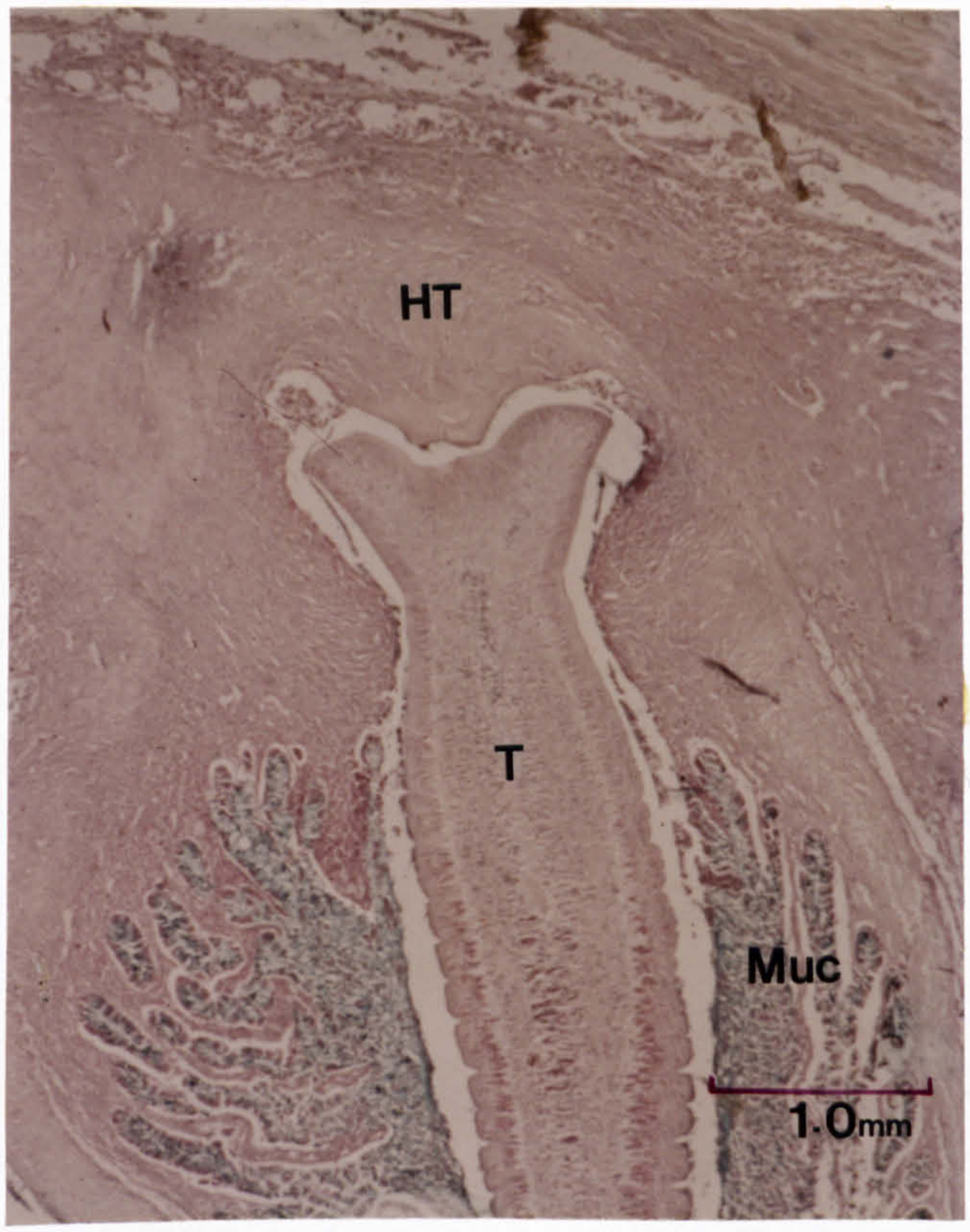
Plate 5.5 Longitudinal sections of the adult C. truncatus in the pyloric caecum of fish demonstrating.

A. Acid mucopolysaccharide (seen blue in colour). Alcian blue. Scale = 1mm

B. Glycogen (seen pink in colour). PAS/light green. Scale = 1mm

MUC ..... Mucosa
HT ..... Host tissue at site of tapeworm's attachment
T ..... Tapeworm

A



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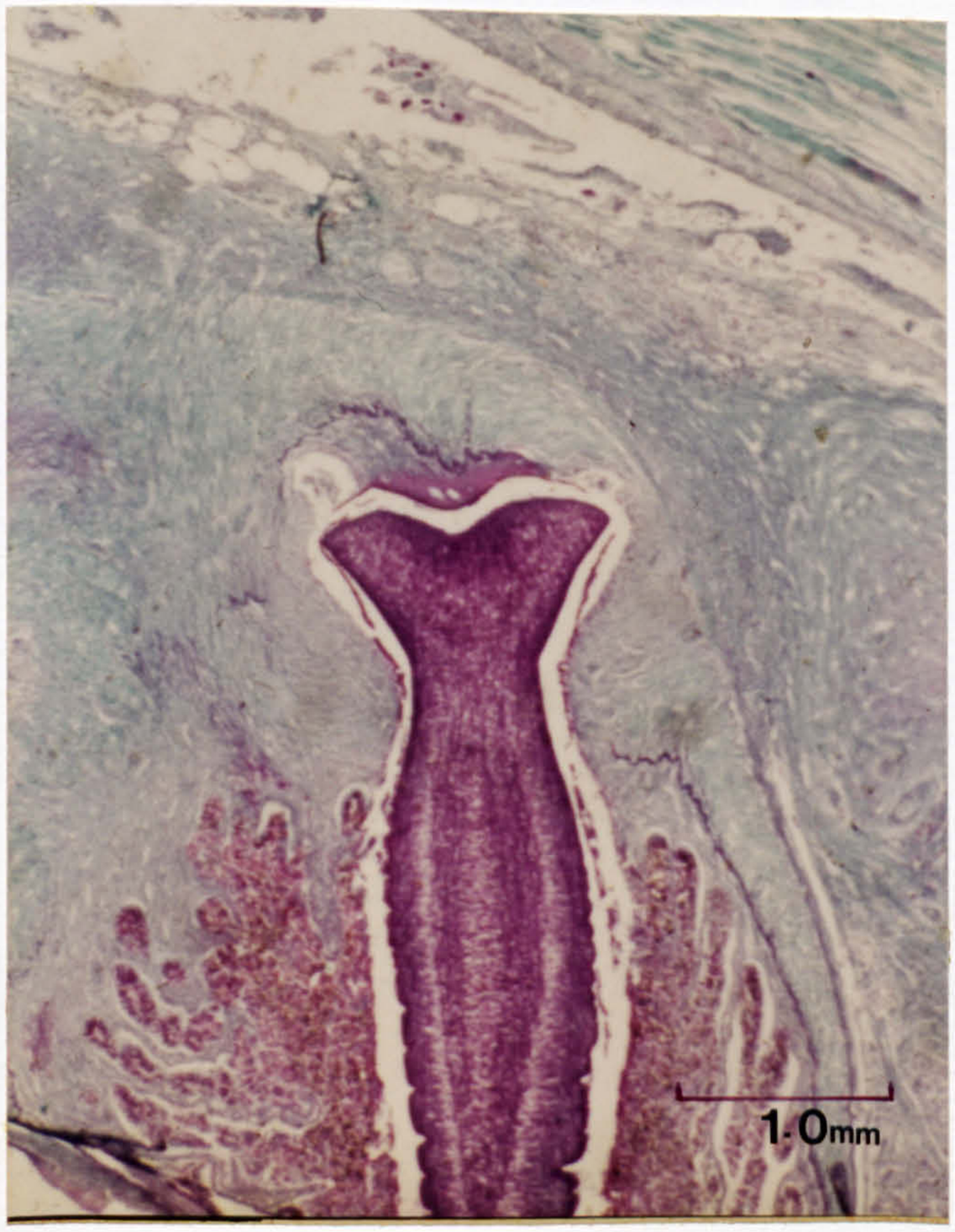


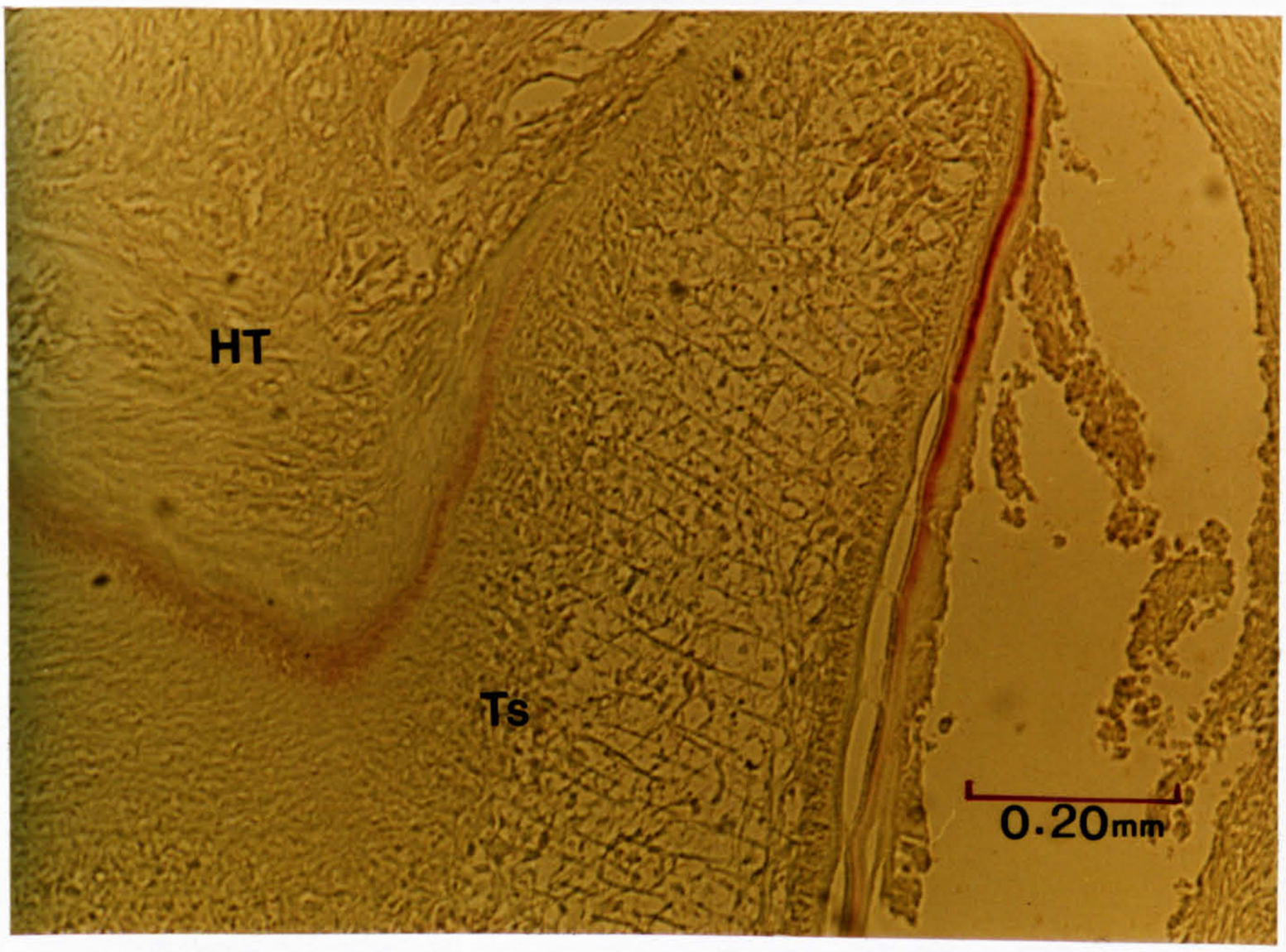
Plate 5.6 Demonstration of alkaline phosphatase activity (seen red in colour) in adult C. truncatus attached within the pyloric caecum. Naphthol AS-BI phosphate method pH.8.3

A. Longitudinal section of the tapeworms scolex (TS) showing the enzyme activity in outer scolex surface and inner scolex surface at point of attachment to host tissue (HT)

Scale = 0.2mm

B. Enzyme activities demonstrated in the tegument of the strobila body wall (S) and the mucosa (MUC) of pyloric caecum. Scale = 0.2mm

A



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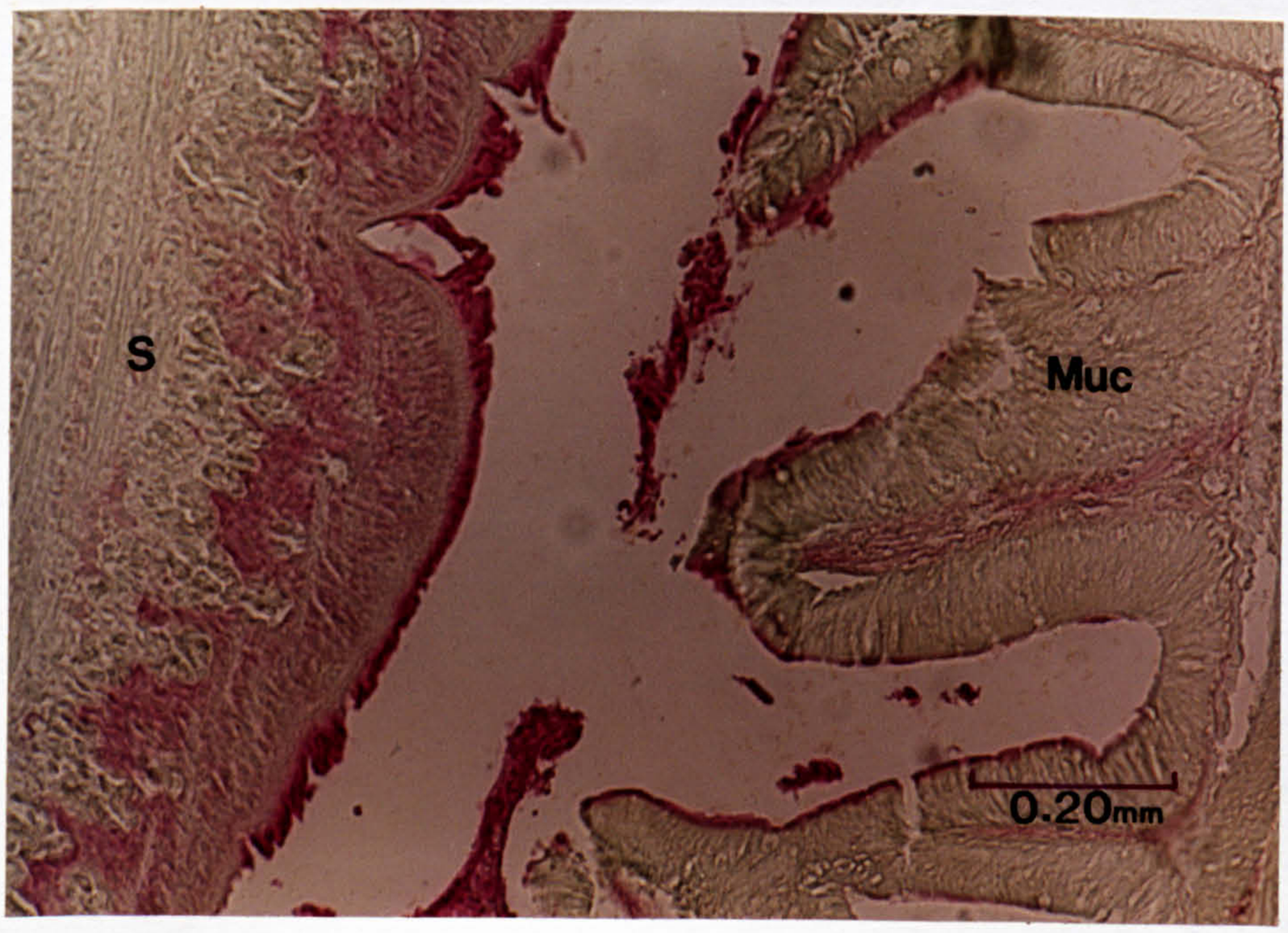


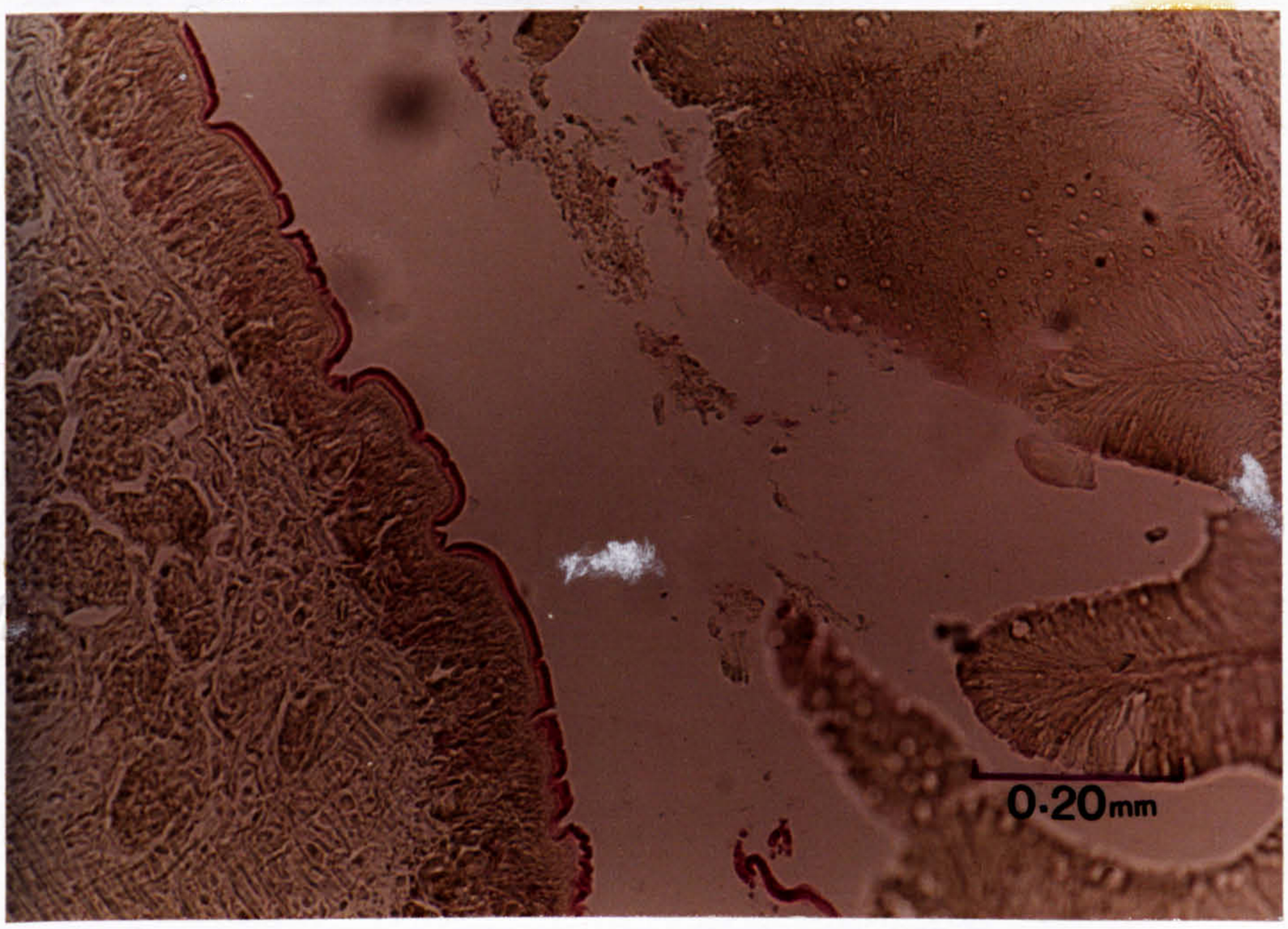
Plate 5.7

A. Demonstration of acid phosphatase activity (seen red in colour) in the distal cytoplasm of the strobila body wall of adult C. truncatus (in a longitudinal section). Activity in the mucosa of the pyloric caecum is sometimes negative or weakly positive. Scale = 0.2mm

B. Transverse section of the strobila showing acid phosphatase activity in the ovary ducts (arrowed). Scale = 0.2mm

Both prepared with Naphthol AS-BI phosphate method pH 5.3.

A



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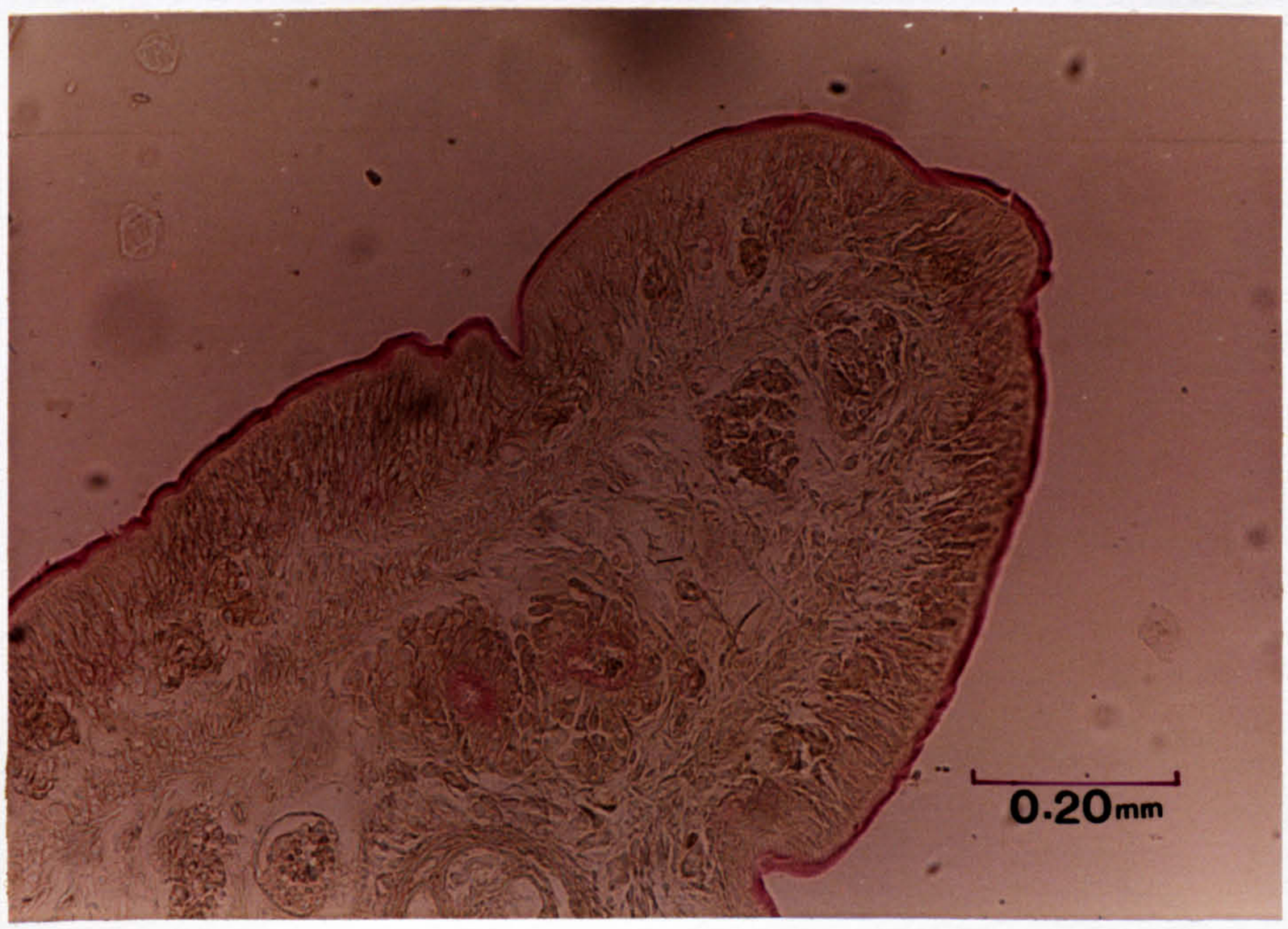
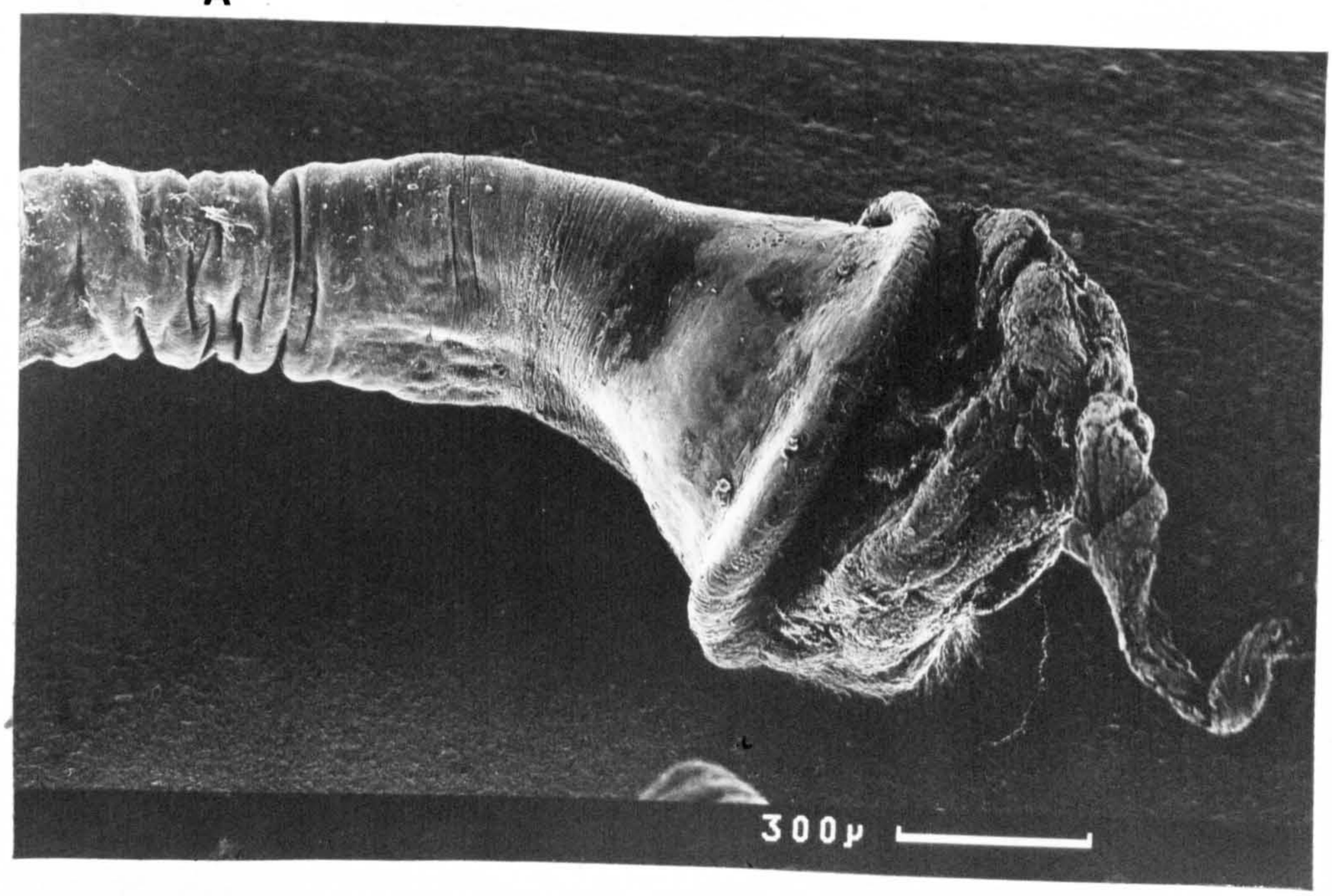


Plate 5.8 Scanning electron micrograph of the scolex and neck region of adult C. truncatus retrieved from fish caeca shown in:

A. With intact host tissue of attachment to the funnel shaped scolex. Scale 300µm

B. With host tissue of attachment pulled out of the scolex revealing its funnel-shaped structure. Scale = 300µm.

A



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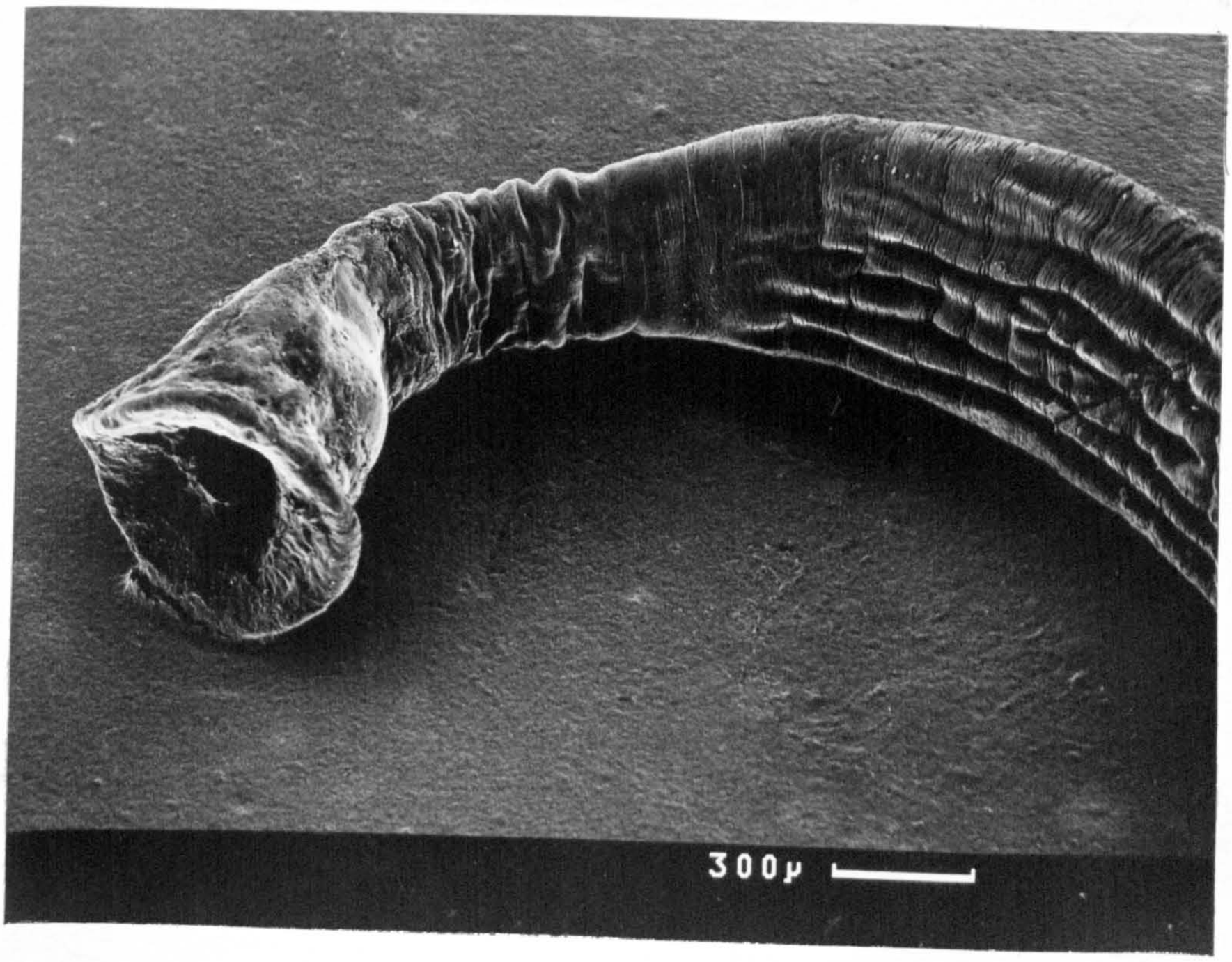


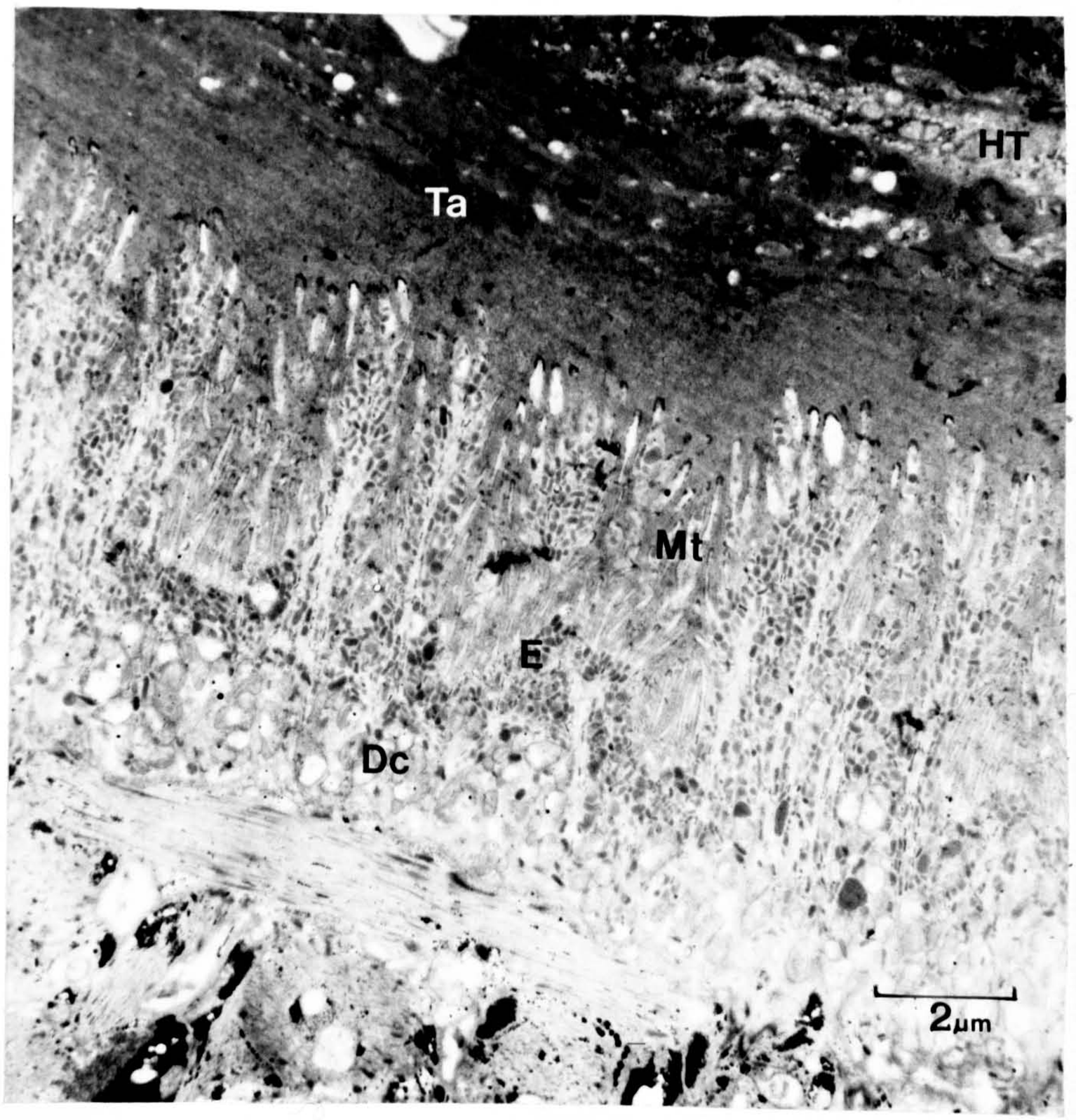
Plate 5.9 Transmission electron micrograph of the inner scolex surface of C. truncatus in longitudinal section and its attachment to host tissue.

A. A general view. Scale = 2µm.

Dc ..... Distal cytoplasm
E ..... Electron-dense bodies
HT ..... Host tissue
Mt ..... Microtriches
Ta ..... The non cellular tissue of attachment

B. The microtriches and the host tissue to which tapeworm is attached. Note shape of the apical cap of microtriches and the occurrence of numerous vesicles (arrowed) presumably produced by the tapeworm. Transmission electron micrograph. Scale = 1µm.

A

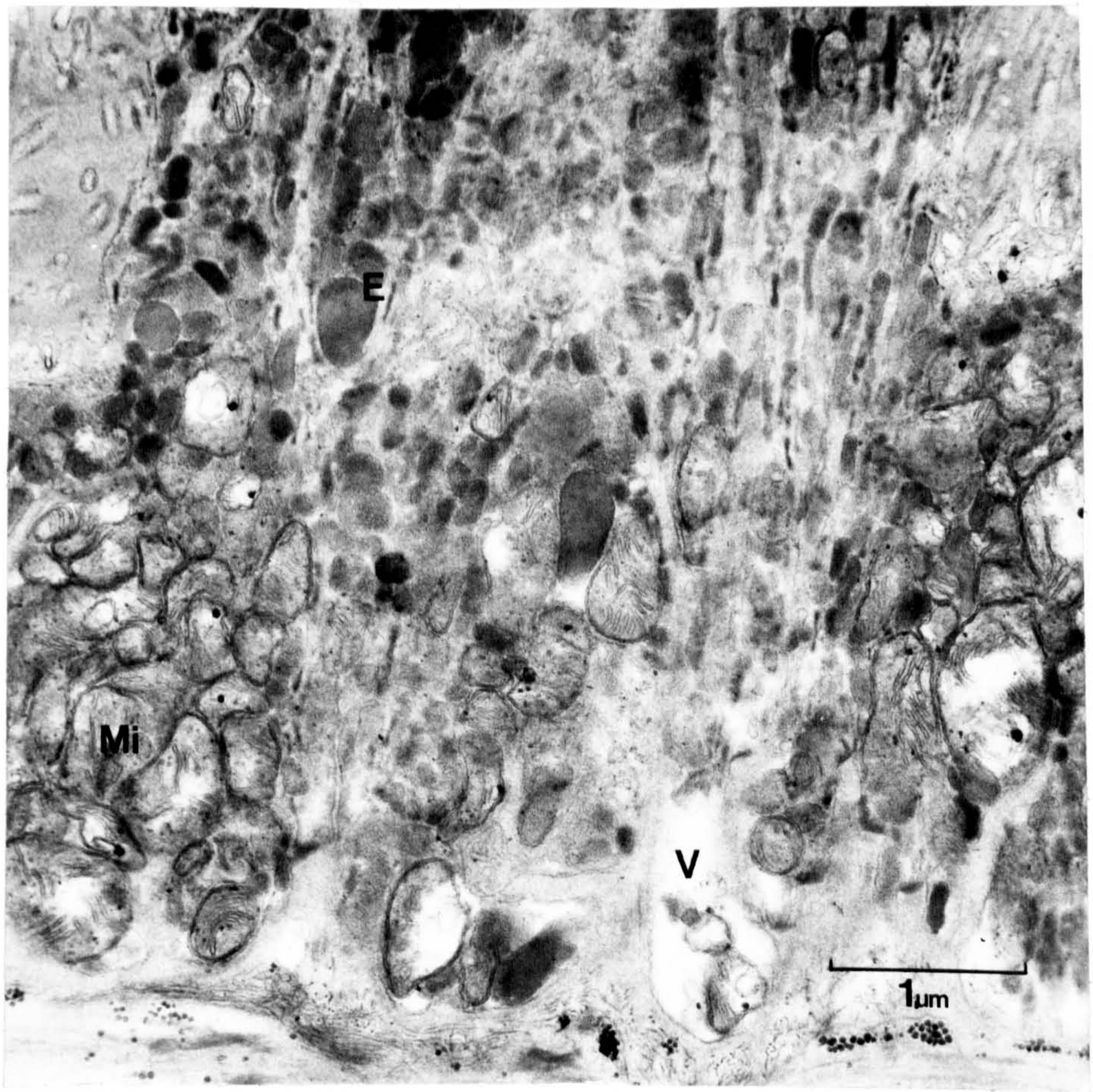


B

Plate 5.10 A.High power trans mission electron micrograph of the distal cytoplasm (shown in Plate 5.9) of the inner scolex surface showing the electron-dense bodies (E). mitochondria (Mi) and vesicles (V).
Scale = 1µm

B. High power transmission electron micrograph of the distal cytoplasm, basal lamina and muscules (Mu) of the inner scolex surface. Note large mitochondrion (Mi), glycogen granules (gl) and shape of electron-dense body (arrowed) in the internuncial process.
Scale = 2µm

A



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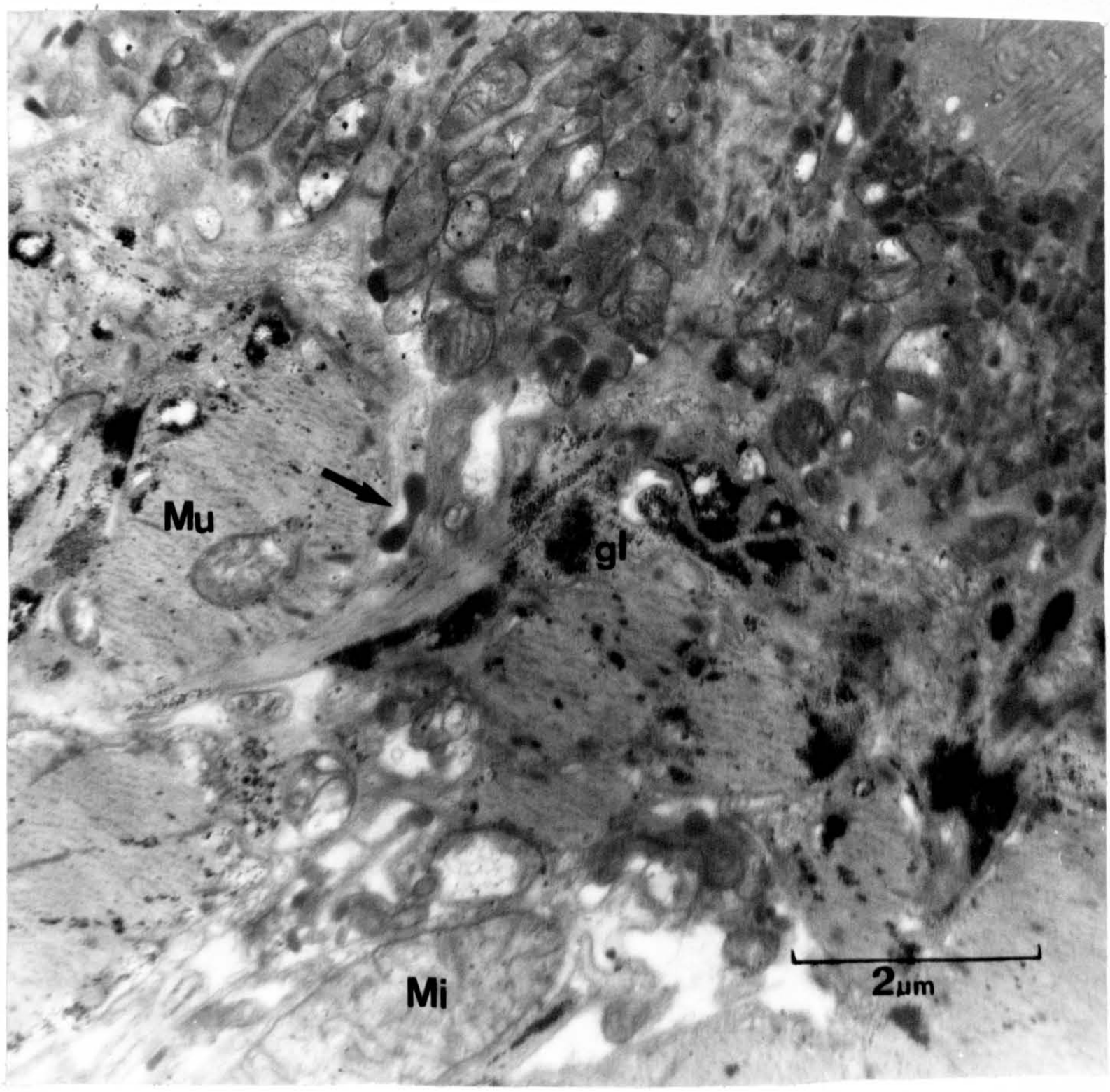
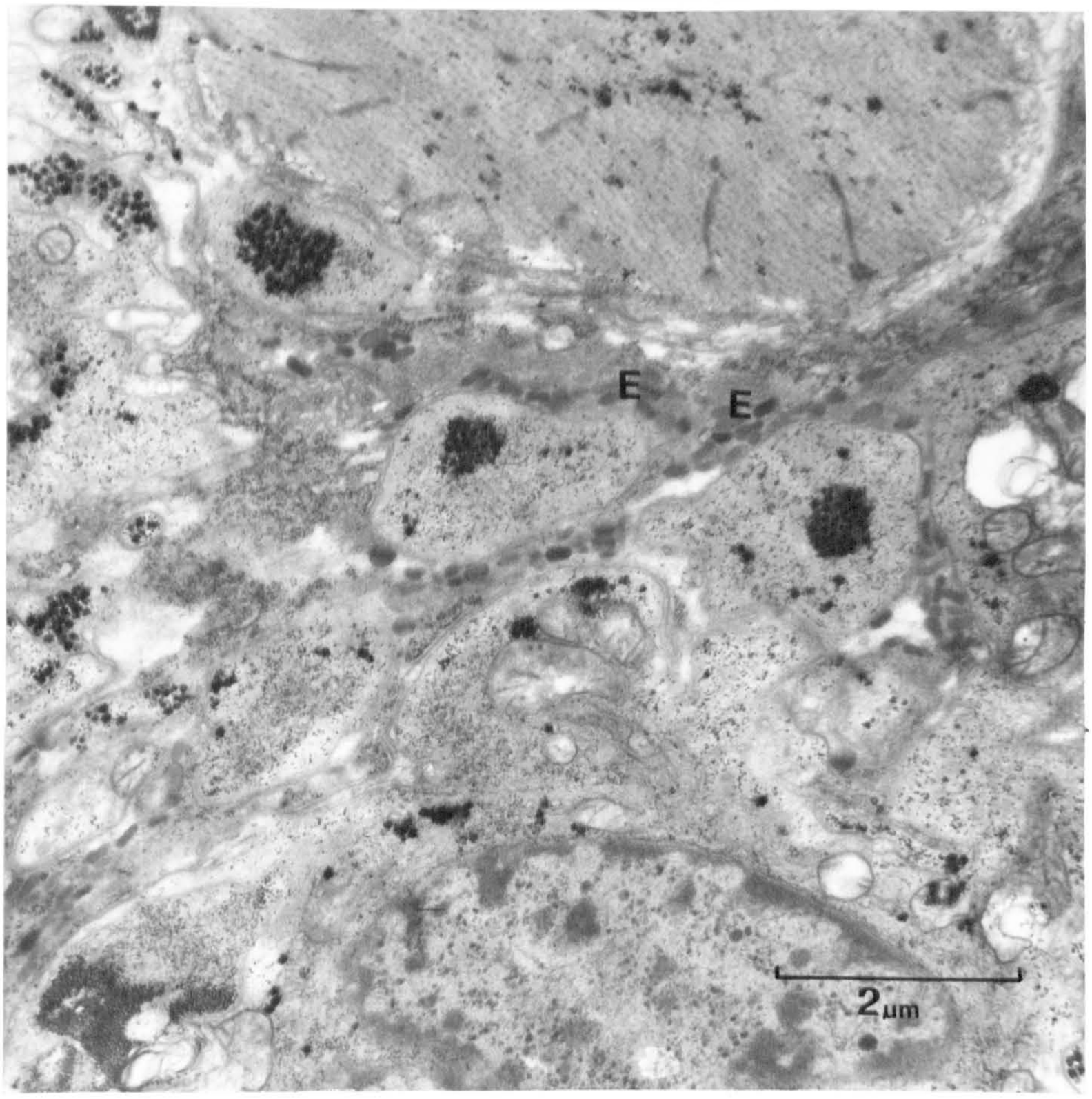


Plate 5.11 A. Transverse section ultrastructure of the outer medullary parenchyma beneath the muscle layers of the inner scolex surface showing the electron-dense bodies (E) in the internuncial process. Transmission electron micrograph. Scale = 2µm

B. The medullary parenchyma of the scolex margin. Transmission electron micrograph. Scale = 4µm

L ..... Lipid bodies

A



B

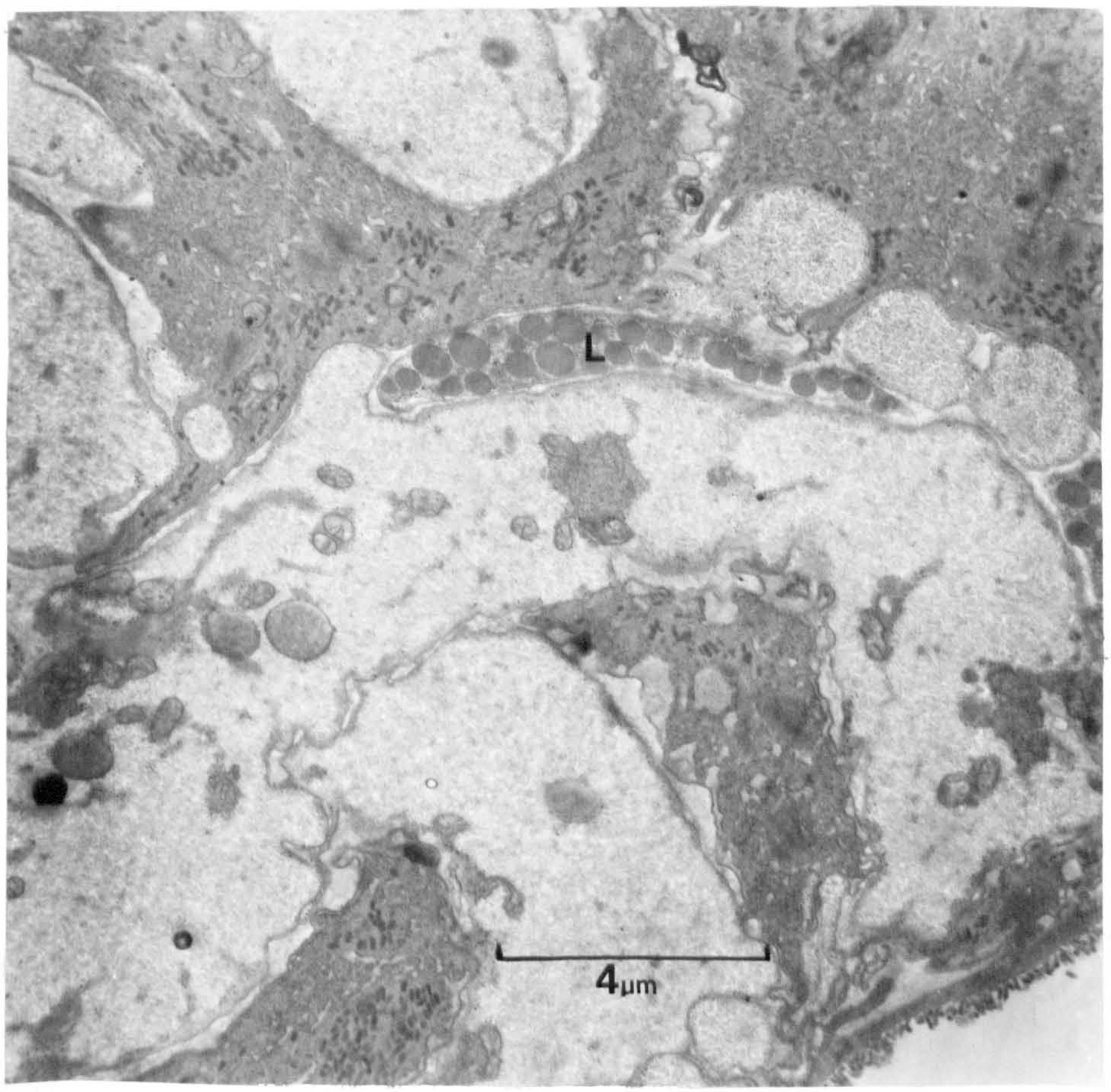


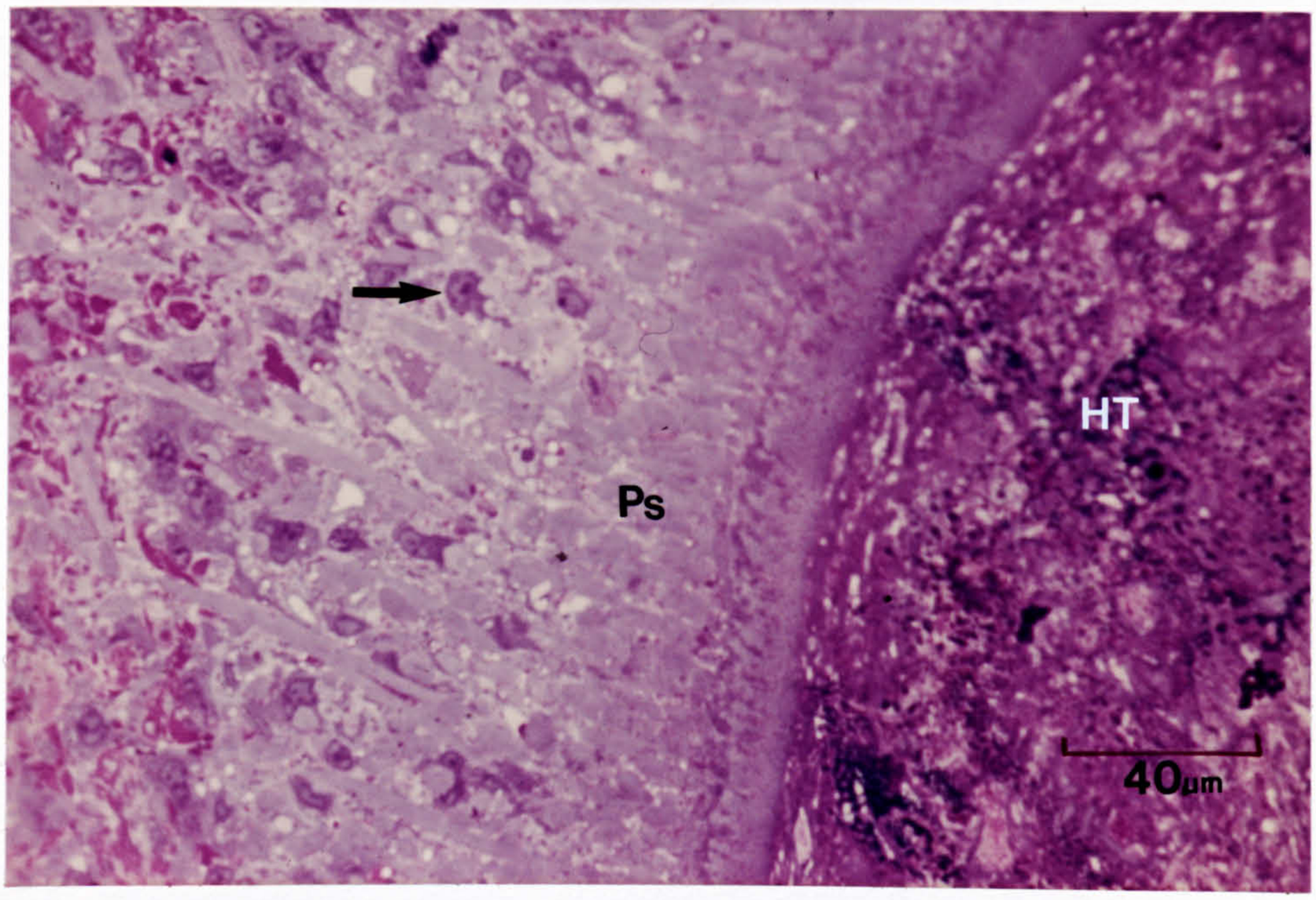
Plate 5.12 A. Semithin longitudinal section of the inner surface of scolex (PS) attached to the host tissue (HT) showing the tegumentary cytons (arrowed). Toluidine blue. Scale = 40µm

B. Transmission electron micrograph of the tegumentary cyton showing the numerous electron-dense bodies (E) and vesicles (V) in the cytoplasm.

Scale = 2µm

N ..... Nucleus

A



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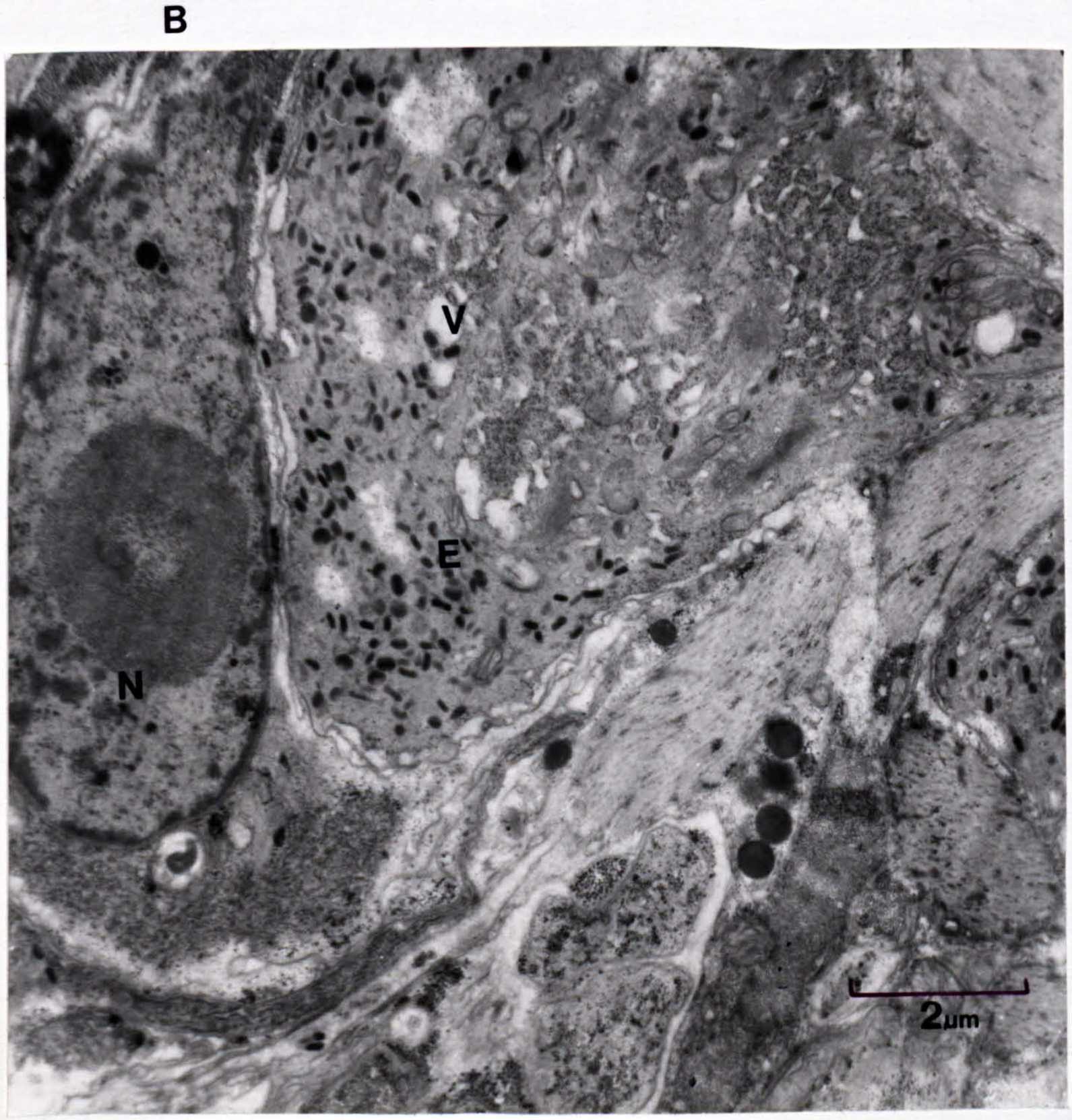


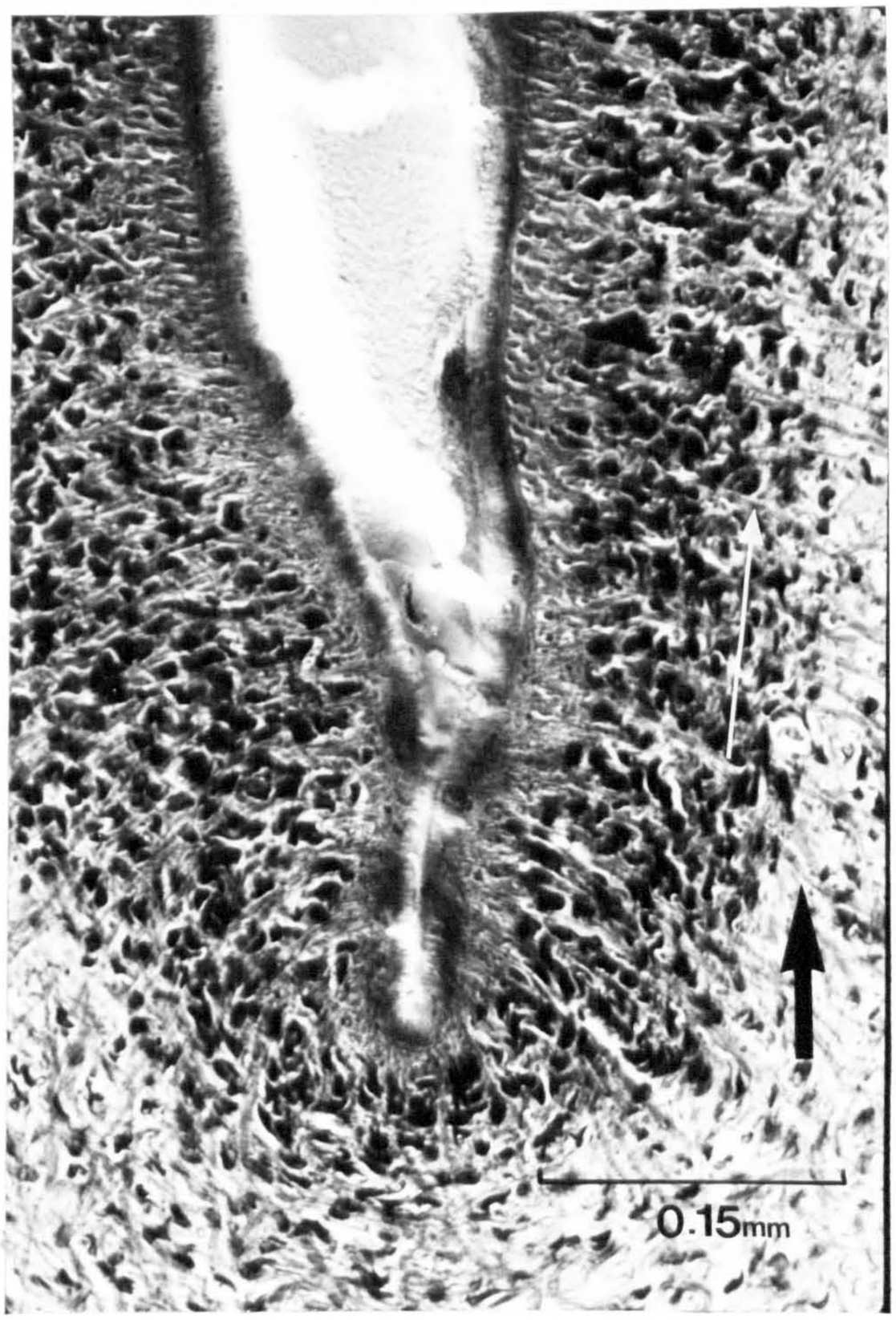
Plate 5.13

A. Longitudinal section of inner scolex surfaces of the stage III procercoid of C. truncatus showing transverse muscle fibres (arrowed) that connect inner scolex and outer scolex surfaces. Masson green Scale = 0.15mm

B. Low power transverse section transmission electron micrograph of the inner scolex surface of attachment of the adult tapeworm showing the musculature beneath the distal cytoplasm (Dc) Scale = 2µm

CMu ..... Circular muscle
LMu ..... Longitudinal muscles
TMu ..... Transverse muscles

A



B

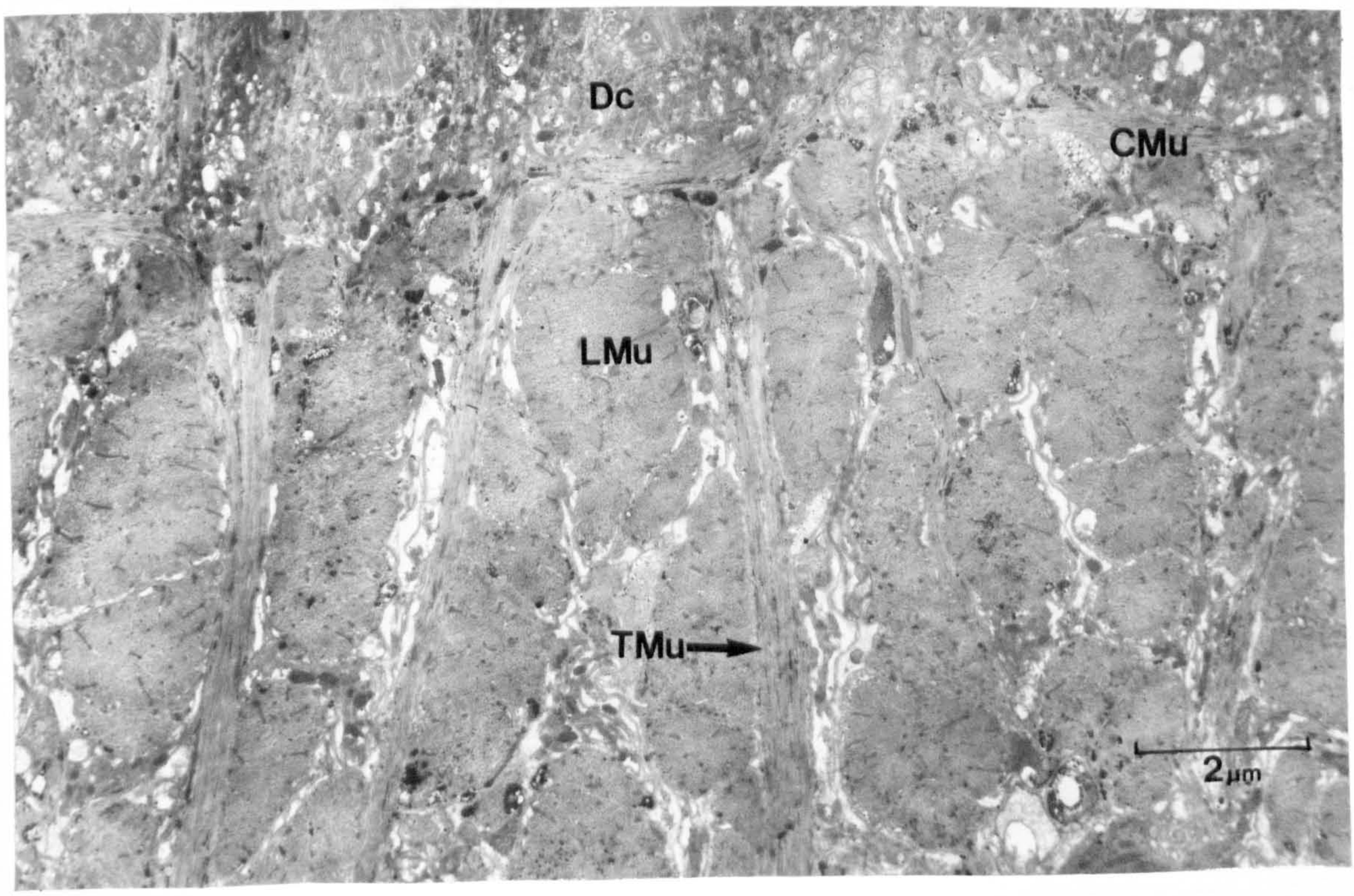
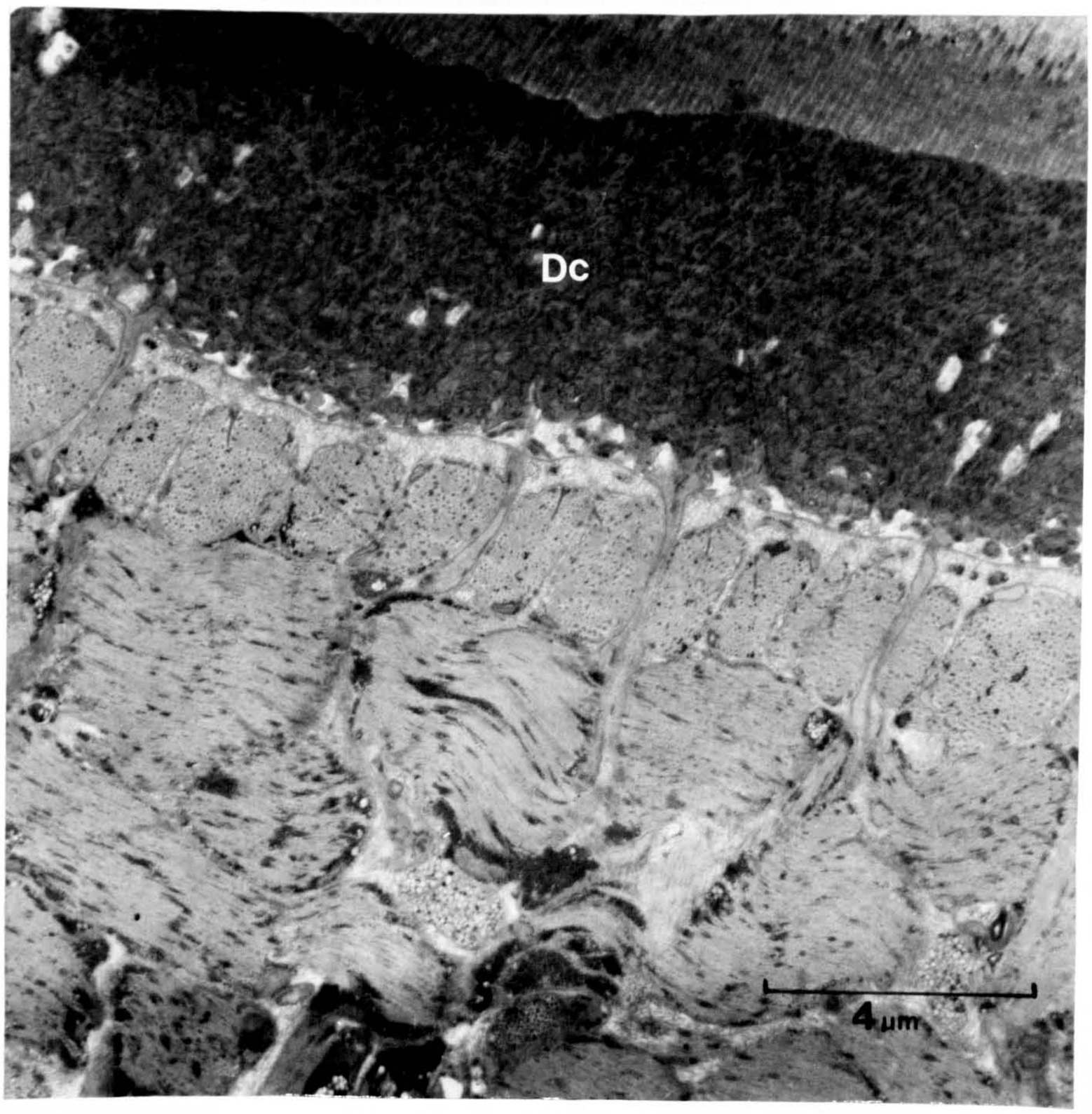


Plate 5.14 A. Transverse section ultrastructure of body wall at the neck region of adult Cyathocephalus truncatus showing the extensive musculature beneath the distal cytoplasm (Dc). Transmission electron micrograph. Scale = 4µm

B. Outer scolex surface transverse section ultrastructure of an attached adult Cyathocephalus truncatus. Transmission electron micrograph. Scale = 2µm

Hc ..... Host cell of the neighbouring tissues at area of attachment

A



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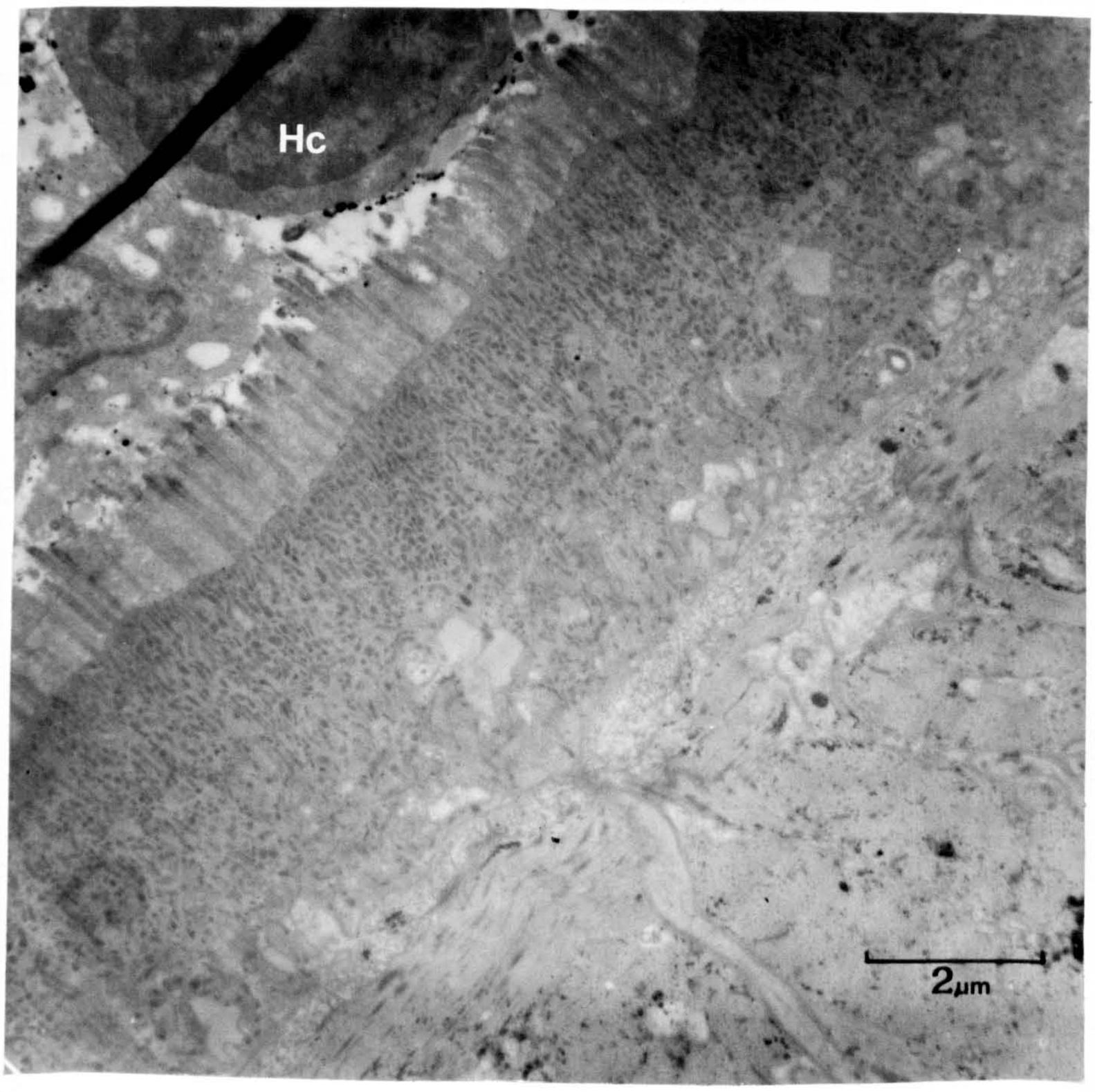
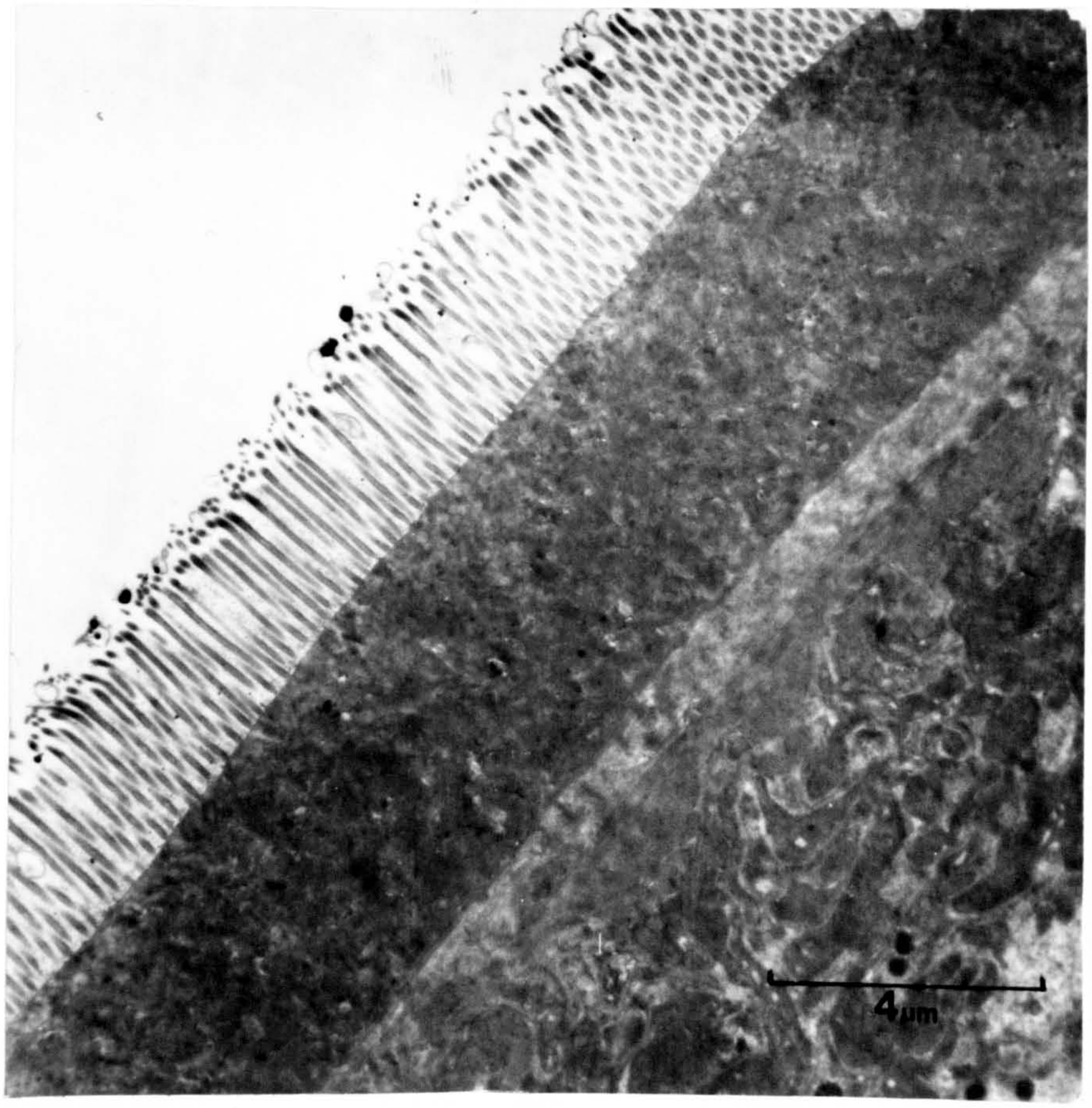


Plate 5.15 A. Ultrastructural transverse section of C. truncatus strobila showing the body wall in the 7 days old infection in S. trutta. Scale = 4 µm

B. High power transmission electron micrograph of the layer of microtriches of the tapeworm of 7 days old infection (above) showing vesicles containing granular particles (arrowed) within individual microthrix. Scale = 1µm

A



B

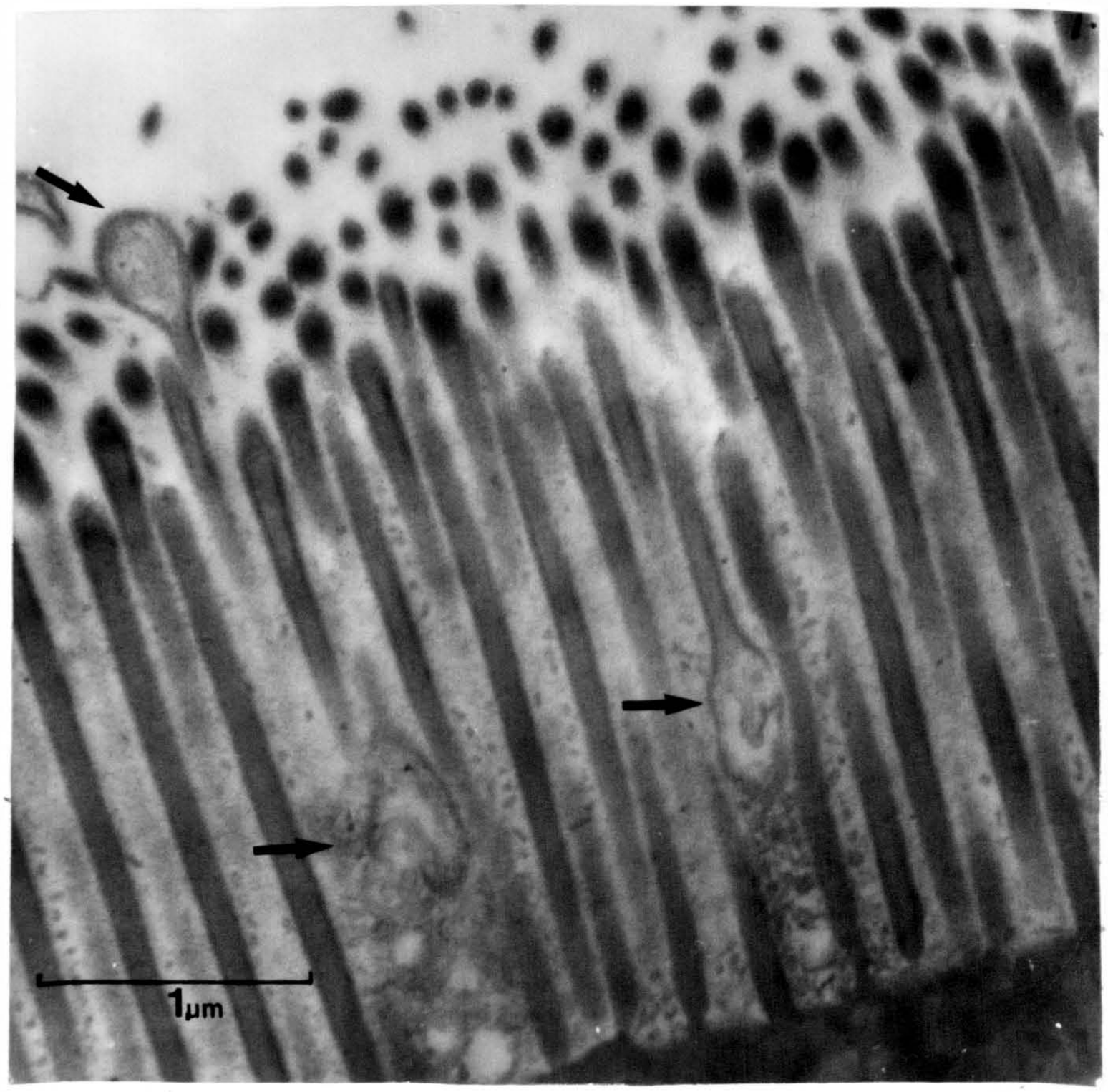


Plate 5.16 Transmission electron micrograph of a longitudinal section of the strobila of C. truncatus in the 15 days old infection of Salmo trutta

Dc ..... Distal cytoplasm
Mt ..... Microtriches

Scale = 2µm

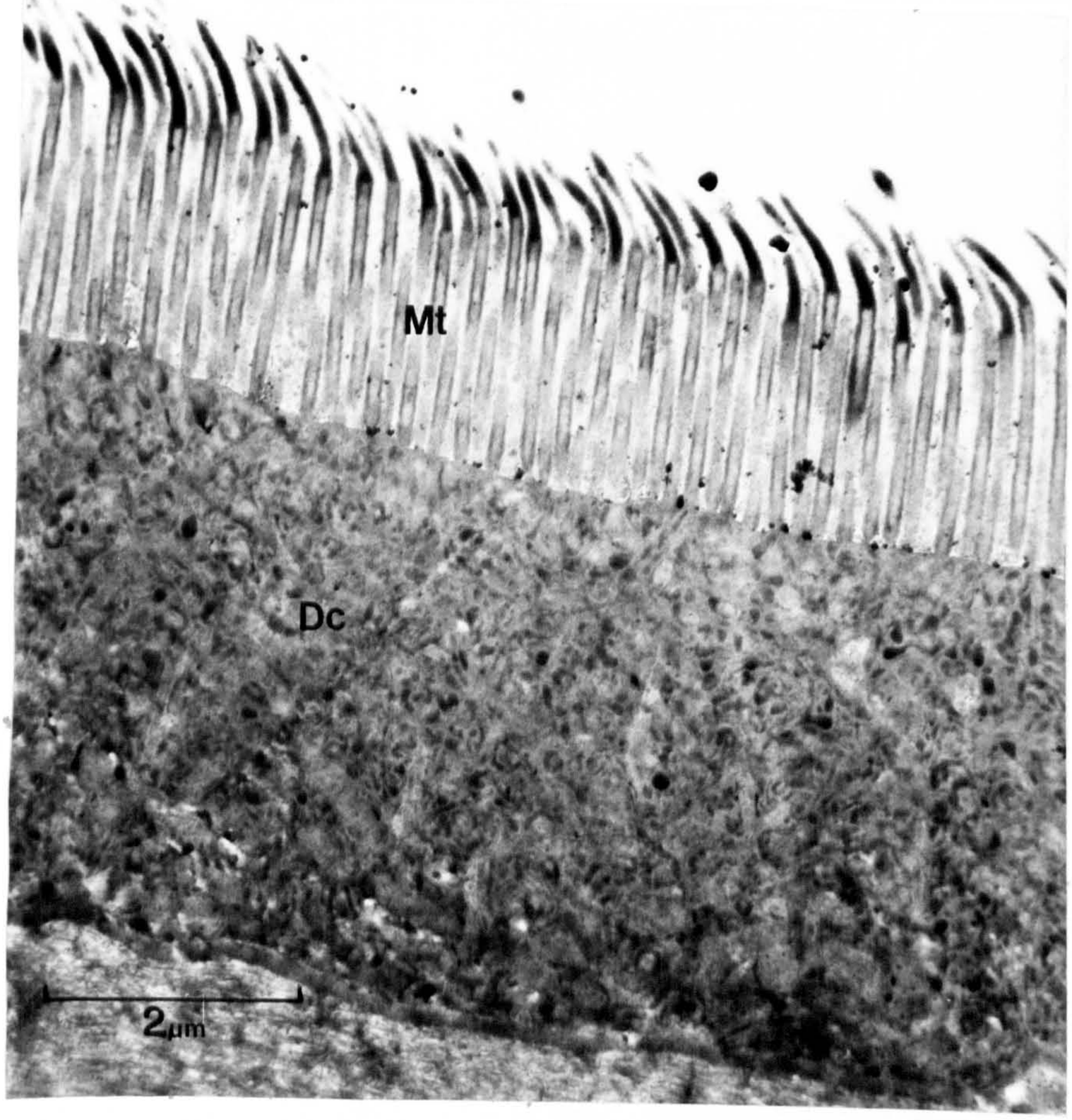

Mt
Dc
2μm

Plate 5.17 Longitudinal section ultrastructure of zone 'A' of the infected host tissue showing:

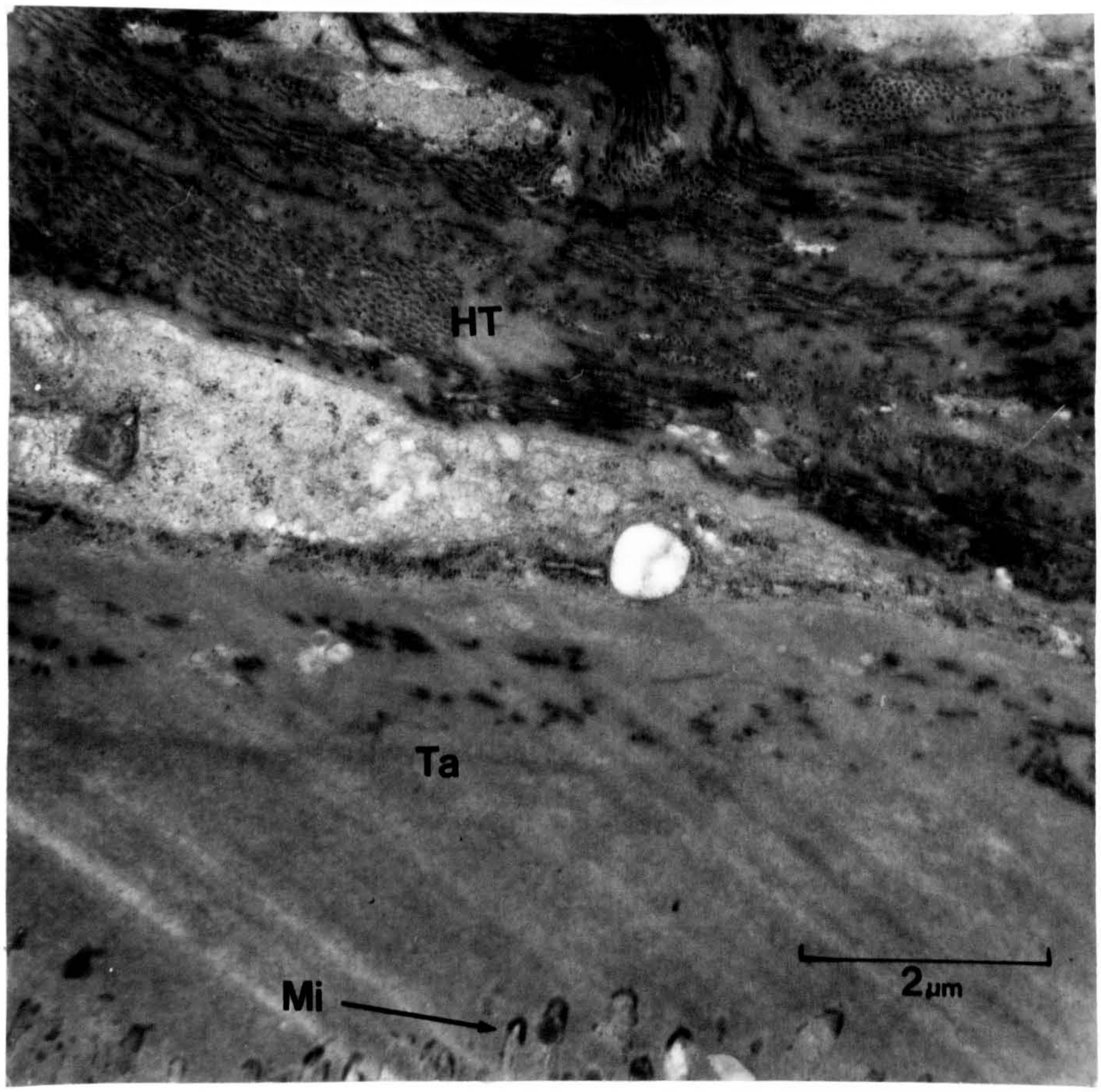
A. The host tissue (Ta) that serves for tapeworm attachment and endings of the microtriches (Mi) of distal cytoplasm. Transmission electron micrograph. Scale = 2µm

HT ..... Host tissue

B. Region of the muscular coat of host tissue overlying the tissue of attachment. Transmission electron micrograph. Scale = 2µm

RER ..... Endoplasmic recticulum with ribosomes
Muf ..... Muscle fibres
N ..... Nucleus

A



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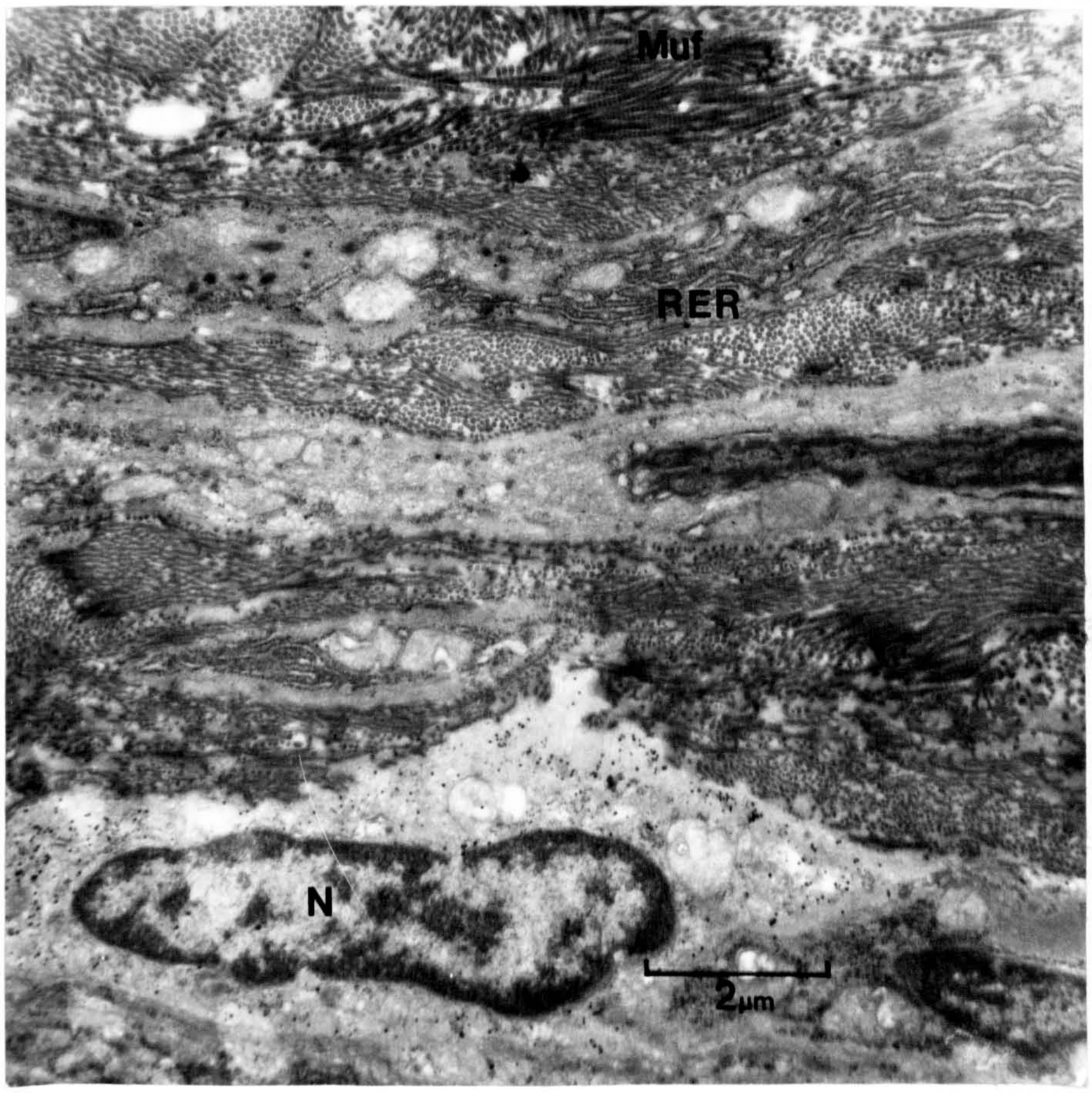


Plate 5.18 Longitudinal section ultrastructure of zone B of host tissue at site of tapeworm's attachment

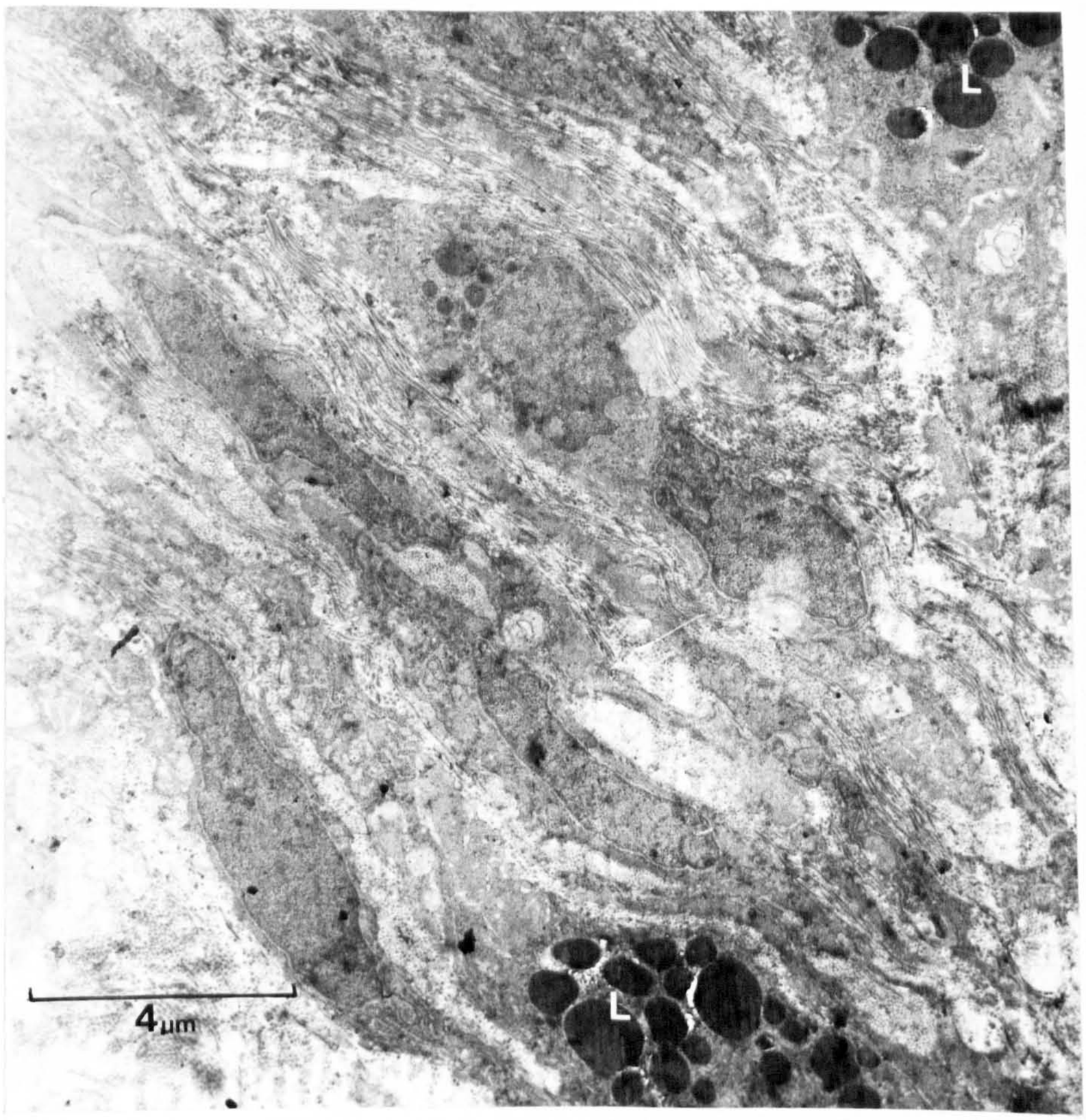
A. General view of the zone. Transmission electron micrograph. Scale = 4 µm

L ..... Lipid bodies

B. High power transmission electron micrograph of the zone. Scale = 2 µm

Muf .....Muscle fibres

A



B

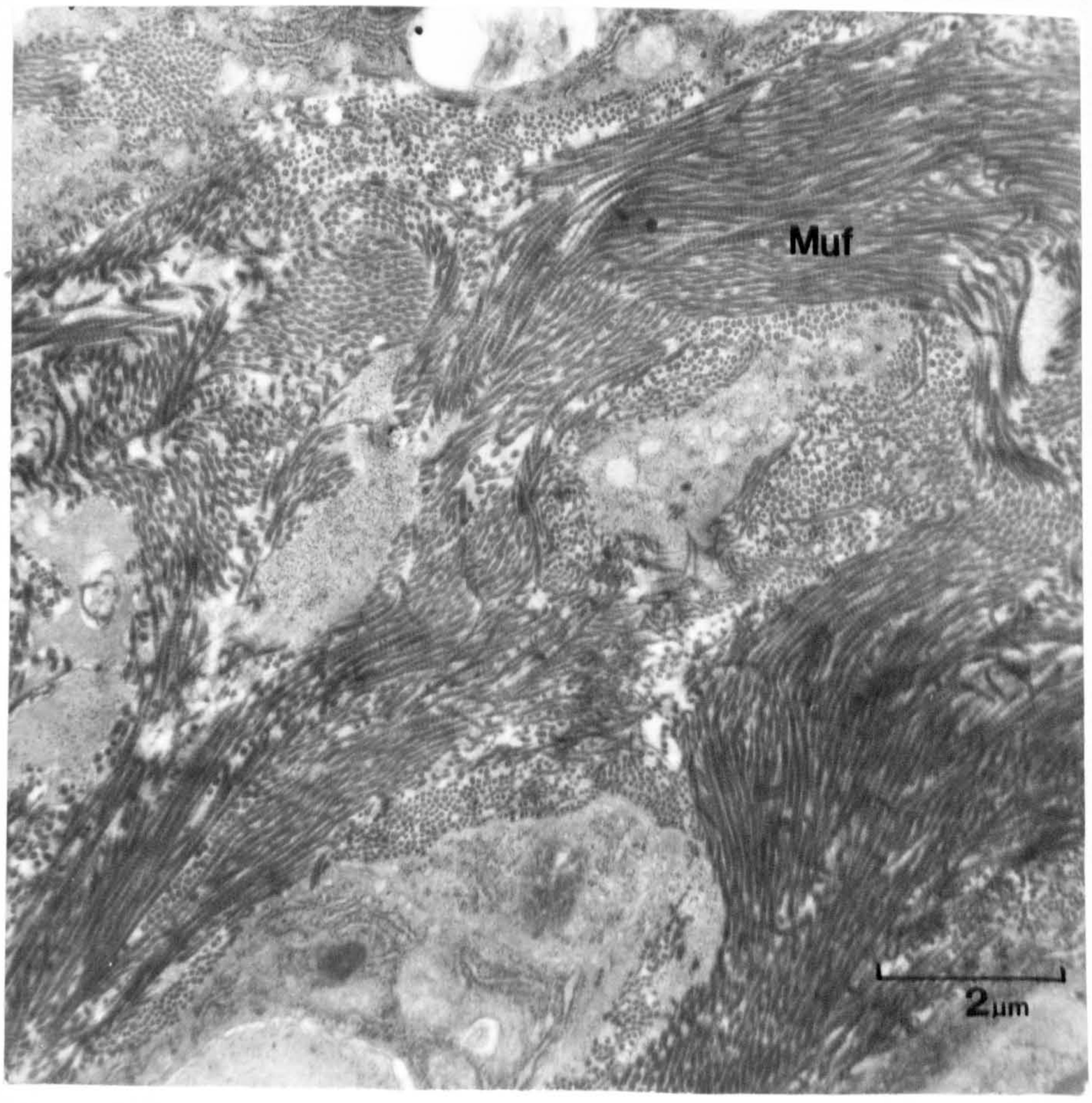


Plate 5.19 Longitudinal section ultrastructure of zone C of host tissue at site of tapeworm's attachment. Transmission electron micrograph. Scale = 4 µm

ER ..... Endoplasmic recticulum
Muf ..... Muscle fibres
N ..... Nucleus

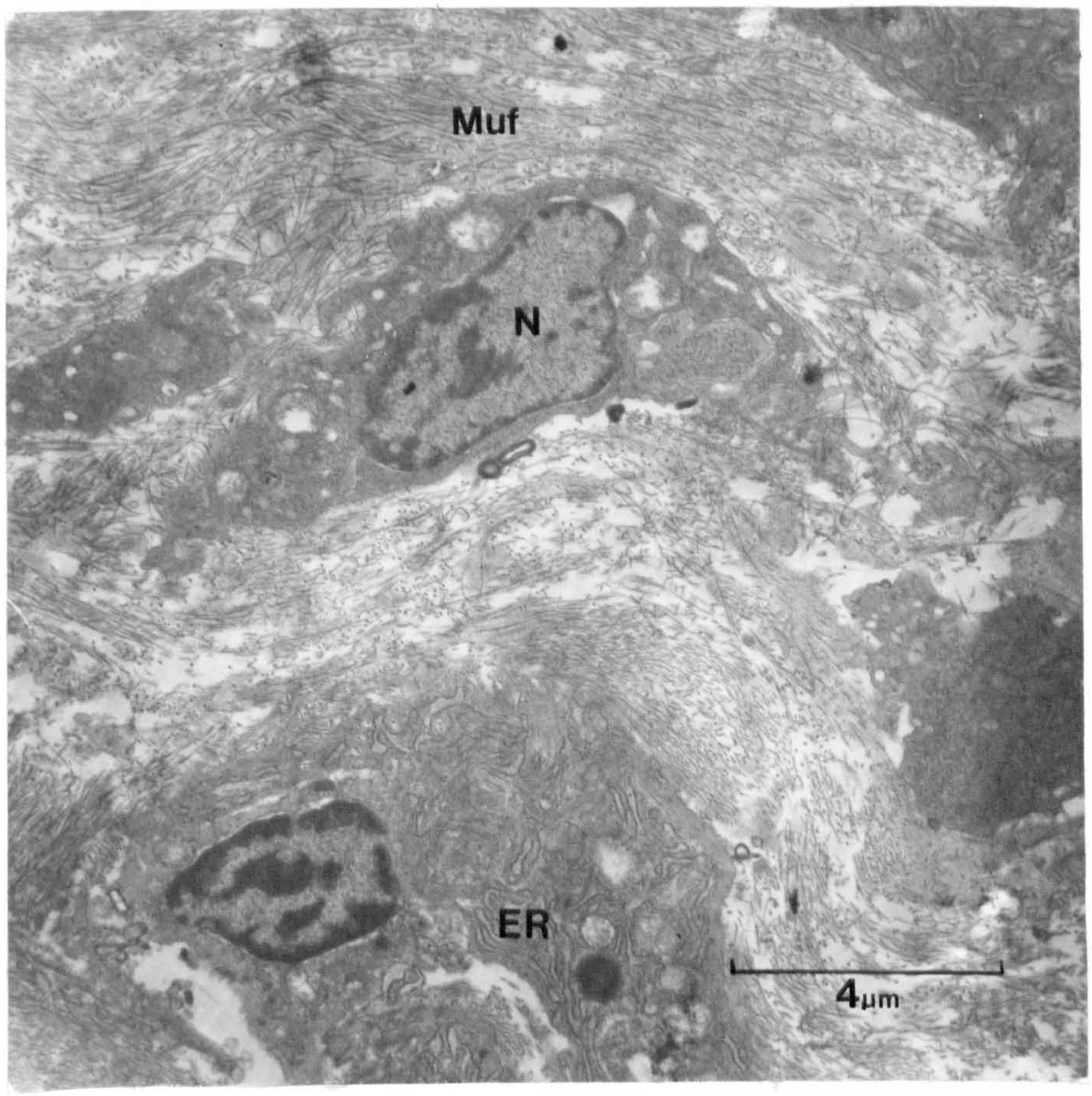

Muf
N
ER
4µm

Plate 5.20 Tissue damage by Cyathocephalus truncatus in Salmo trutta

A. Transverse section of pyloric caeca and abdominal body wall of S. trutta showing tissue erosion at the distal tip of an infected caecum with the tapeworm approaching the body cavity. Two other worms in cross section (arrowed) are already located in the abdominal musculature beneath the skin. Masson green. Scale = 3mm

B. Transverse section through the region of pyloric caeca of infected fish. Note the severe damage. Masson green. Scale = 3mm

AMu ..... Abdominal musculature
Bc ..... Body cavity of fish
Sk ..... Skin of fish
T ..... Tapeworm, C. truncatus

A

B

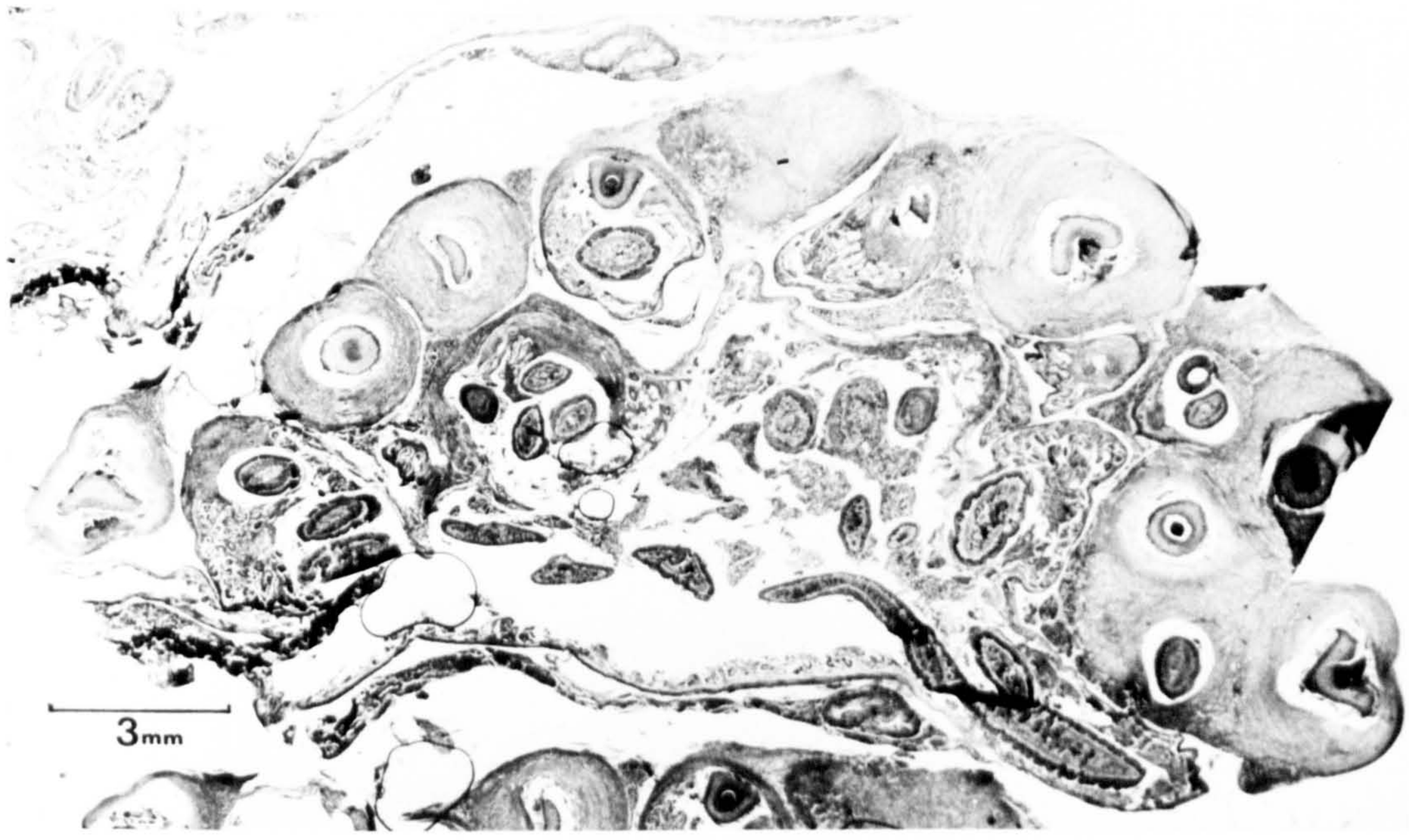


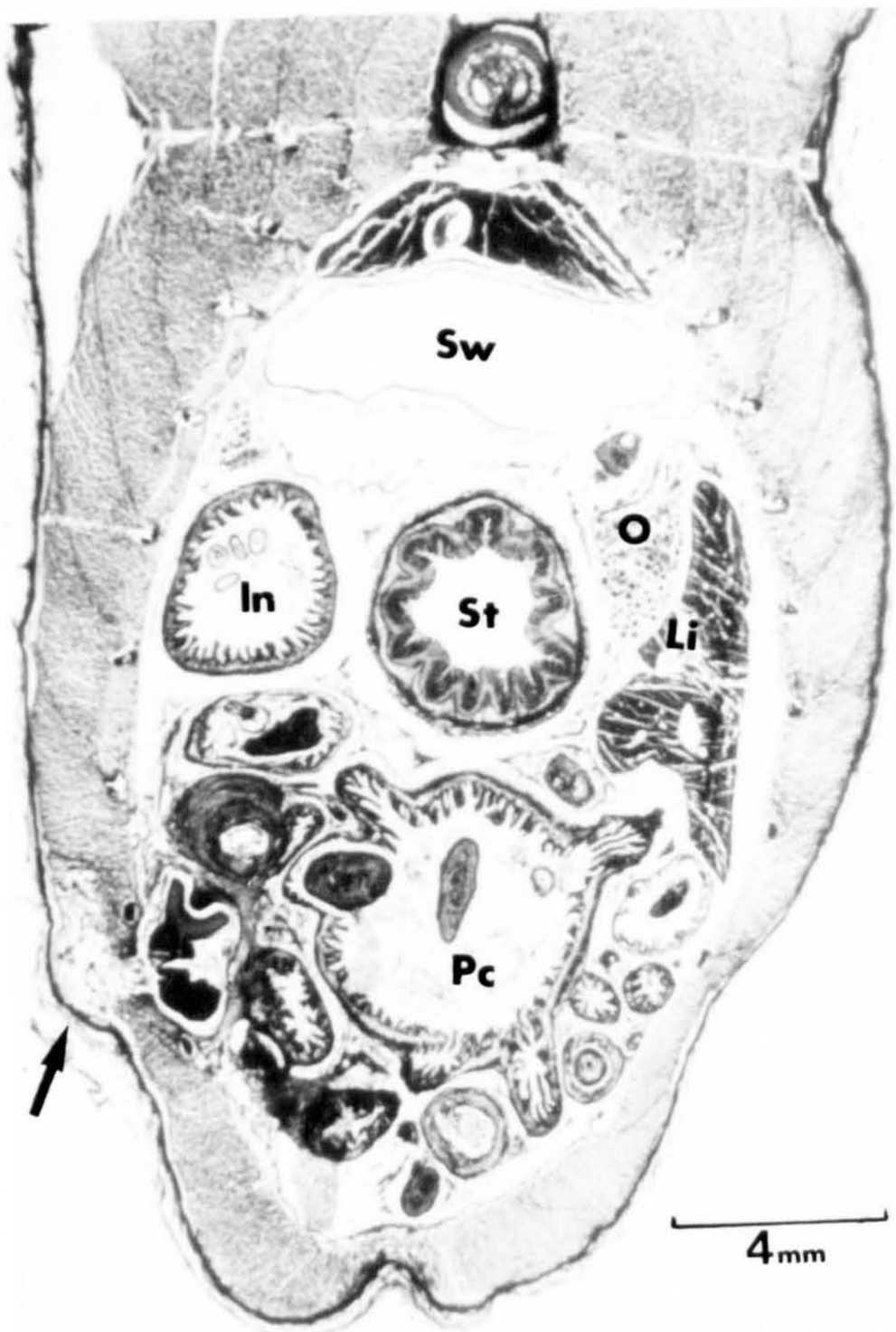
Plate 5.21 Transverse section of Salmo trutta at the trunk showing in

A. Occurrence of Cyathocephalus truncatus in the body cavity of fish. Note the damage done to the abdominal musculature (arrowed).
Masson green Scale = 4mm

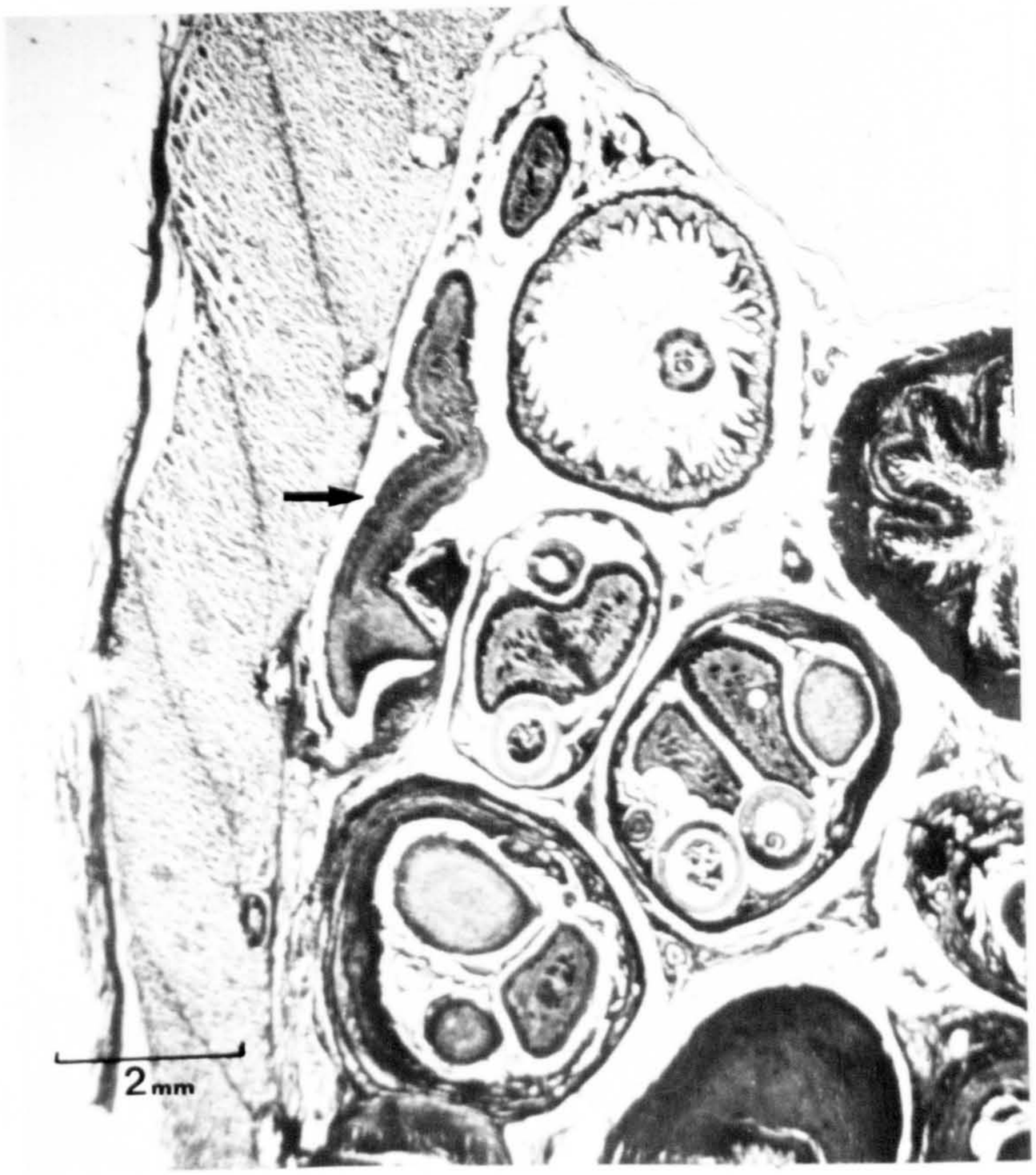
In .....Small intestine
Li .....Liver
O .....Ovary
Pc .....Region of Pyloric caeca
St .....Stomach
Sw .....Swimbladder

B. Occurrence of the tapeworm in the body cavity of fish. Masson green Scale = 2mm

A



B



GENERAL DISCUSSION

The present investigation has clarified a number of important issues in the life cycle of Cyathocephalus truncatus. The presence of a hexacanth embryo has been demonstrated and the stages of development in Gammarus pulex have been described from naturally-infected amphipods and from experimental infections established in the laboratory. In both instances as well as with natural infections of gammarids with acanthocephalans and digeneans the infection rate of amphipods is very low.

C. truncatus infections recorded by Wisniewski (1932b), Vik (1954, 1958) and Awachie (1966a) were similarly marked by low prevalence in the amphipod intermediate host and a high prevalence in the fish primary host. The general low infection rate of gammarids with the tapeworm and the other helminth parasites could be due to a number of factors.

As with C. truncatus infection, one of such factors may be that a number of the infective embryos are crushed by the mandibles of the gammarids during ingestion resulting in a diminution in their number. This has been discussed in Chapter 3 (page 102). During attempts to produce experimental infections of G. pulex it was observed that the presence of more than a single worm per gammarid probably results in the death of the host (page 91). Some amphipods in the field may have died as a result of the large size to which the contained procercoid of C. truncatus may grow or of the presence of a number of

procercoids within the body cavity.

The occurrence of a large population of G. pulex in the area of present study could also tend to obscure the actual prevalence of the tapeworm procercoid. Hynes (1955) pointed out that Gammarus pulex breeds twice in the year - first between April and June, maturing in the winter months of December and January and secondly in September and November, over-wintering as juveniles and maturing about March. It is possible that infections with C. truncatus only establish during the summer breeding season when the temperature is suitable for egg development (as suggested above on page 101). Thus the number of infected amphipods could appear low when new individuals are added to the population in winter months, effectively diluting the number carrying procercoids.

Although the tapeworm appears to have a seasonal pattern of occurrence in the intermediate host a study of their seasonal occurrence in fish is necessary to clarify their actual pattern of prevalence in the hosts from Driffield trout streams.

In the early stages of development of C. truncatus procercoid, the terminal invagination that develops to form the scolex is similar to that of the procercoid of some pseudophyllidean species such as Diphyllobothrium latum. But whereas the scolex in D. latum develops to form a pair of bothria after gaining access into the second intermediate host, that of C. truncatus does not vary but develops further into a terminal funnel-shaped scolex. The uneven thickness of the scolex margin

observed in the procercoid during its early stages of development (page 88) might be reminiscent of the probable positions of the bothria in the ancestral form and have become fused as in present day C. truncatus. However C. truncatus differs considerably from the pseudophyllideans in that two hosts (crustacea and fish) are required for completion of the life cycle. In the pseudophyllideans as in Ligula intestinalis the life cycle is completed in three hosts: the first intermediate host is a ccpepod in which the procercoid develops; the second intermediate host is a fish in which the plerocercoid occurs and the definitive host is a fish-eating bird. The dorminant phase of Ligula intestinalis life-cycle, according to Arme et al (1983), is the plerocercoid stage in fish. Cyathocephalus truncatus probably originally showed a similar pattern of life cycle and later evolved to become an adult in the fish. Moreover, C. truncatus may attain maturity in the crustacean host as indicated by Amin (1978) who reported egg production by C. truncatus procercoid in Pontoporeia affinis. With Amin's report, C. truncatus can be compared with the genus Archigetes whose procercoids are known to attain maturity in fresh water annelids (intermediate host) as well as in the fish definitive host (Wisniewski, 1930, Calentine, 1964; Kennedy, 1983).

However, egg- production by the procercoid in the gammarid host has not been observed in the present study. The state of the reproductive system observed in the present study certainly shows that egg production may be possible but the inability of

the amphipods to tolerate the increasing size of the procercoid may constitute a limiting factor for them serving as definitive hosts.

Wisniewski (1932b,c) was of the opinion that C. truncatus must have originally passed through a complete life-cycle of three hosts with a true strobila probably formed within the final host which he thought to be an animal now extinct. He also interpreted the precocious development of genital organs and excretory ducts in the procercoid as a tendency to neoteny thus shortening the developmental cycle.

Since several fish species are reported to serve as hosts for Cyathocephalus truncatus the level of host specificity may be characterised as low. As remarked above (page 55). it is possible that additional fish species may be found to act as final hosts.

In the present study, the pathogenicity of the worm in the fish host appears to persist in the use of the funnel-shaped scolex for further attachment following the breakdown of the caecal tissues to which it was initially attached thus eventually penetrating the caecum wall. The structure and shape of the scolex of C. truncatus shows it to be a muscular sucking organ rather than a penetrating one like the scolex, of pseudophyllidean procercoids e.g. Diphyllobothrium species.Of particular note, adult specimens of another pseudophyllidean cestode, Penetrocephalus ganapatti, as reported by Rao (1954, 1960) and Subhapradha (1954) have a remarkable feature of penetrating the intestinal wall of the host, Saurida tumbil, with the scolex to reach the liver while the strobila lies inside the intestine of the host. The scolex and neck regions that lie outside the intestine are enclosed in a thick tough

sheath of host tissue resulting from host-parasite interaction. The mode of penetration of this tapeworm is however not known but the tapeworm's penetrating ability and the damage it causes at the site of infection in the host appear similar to what has been observed in C. truncatus infection. Moreover the life cycle of P. ganapatii has not been worked out and may produce additional informations to enable an effective comparison of the tapeworm with Cyathocephalus truncatus.

It is not known for certain whether the electron-dense bodies and vesicles produced in the gland-like tegumentary cytons of the scolex and released through the inner scolex surface into the host tissue contain chemical agents which cause lysis of the tissues but this seems very likely.

The overall pathological effects seen in infected fish in the present study are similar to those reported by previous authors (Wisniewski, 1932b; Vik 1954, 1958; Awachie, 1966). The damage to tissues of the host in the course of infection is severe. Although no dead infected fish have been reported from the area of the present study, the fish examined were certainly in poor condition. However, death of fish due to C. truncatus infection has been reported by Huitfeldt-Kaas (1927) and Wisniewski (1932b).

SUMMARY

1. All the specimens of Salmo gairdneri (160), Salmo trutta (9) and Thymallus thymallus (31) examined from the Driffield section of the river Hull and its tributaries, the Driffield Beck and the Eastburn Beck, were infected with Cyathocephalus truncatus and other helminth parasites including Echinorhynchus truttae, Echinorhynchus salmonis and Cystidicola farionis. Other helminth parasites recorded include Neoechinorhynchus rutili, Cucullanus truttae and Crepidostomum metoecus.

2. Juvenile stages of various helminth parasites were recorded in the amphipod crustacea, Gammarus pulex, collected from the same water and include (with their maximum prevalence), the procercoids of Cyathocephalus truncatus (2.2%), the juveniles of Echinorhynchus truttae (4.2%), Echinorhynchus salmonis (1.6%), Neochinorhynchus rutili (1.2%), Cystidicola farionis (3.2%), the metacercaria of Crepidostomum metoecus (3.4%) and an unidentified cysticercoid of a cestode (1.4%).

3. The life cycle of C. truncatus involves Gammarus pulex as the intermediate host and the fish as the primary host. Hexacanth embryos were found to develop in 22 days when eggs of the tapeworm were cultured at 20°C; 25 days at 15°C and 35 days at 12°C but at 6°C and below and 25°C and above no development occurred.

4. Experimental infections of Gammarus pulex with C. truncatus were obtained only after the ingestion of egg capsules containing oncospheres which were fed to the amphipods on pieces of lettuce leaves. The procercoid develops in the body cavity of the amphipod over a period of about 10 weeks to attain the infective stage. Within the gammarid, the procercoid is entirely enveloped in a sheath which is lost when the worm enters the fish host. Ultrastructural study showed two types of microtriches on the body surface of the procercoid.

5. Specimens of Salmo trutta were infected by feeding them with specimens of Gammarus pulex harbouring the infective procercoids of Cyathocephalus truncatus. The tapeworm matures in 8 to 10 days accompanied by production of eggs. The adult tapeworm possesses on the body surface only one type of microtriches and is similar in body wall ultrastructure to other tapeworms.

6. The tapeworm forms an attachment at the distal tip position within the pyloric caecum of fish 3 days after infection. The attachment becomes very firm by the 15th day and is accompanied by proliferation and swelling of host tissue overlying the site of attachment. The tissue of attachment is seen structurally to consist of collagenous material. Ultrastructurally, microtriches of the scolex surface at point of attachment are seen to be embedded in the attachment tissue which is also seen as non-cellular and non-fibrous.

7. The mode of attachment of C. truncatus is mainly by muscular sucking action of the funnel-shaped scolex. It is not known whether the electron-dense bodies and vesicles produced in the gland-like tegumentary cytons of the scolex and possibly discharged into the host tissue through the scolex surface of attachment perform any chemical role in the tapeworms mode of attachment.

8. The procercoid of C. truncatus attains a rather large size in the body cavity of G. pulex and although no damage was seen structurally in organs such as hepathopancreas and gonads of the amphipod, movements of infected amphipods were observed to be sluggish.

9. In fish, Cyathocephalus truncatus is seen to be capable of penetrating the caecal wall to which it is attached and to enter the body cavity.

BIBLIOGRAPHY

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

AISA, E. (1971): Infection by Cyathocephalus truncatus (Pallas) (Cestoda: Cyathocephalidae) in a population of Tinca tinca L. from Lake Trasimeno. Atti della soieta Italiana delle Scienze Verterinarie, 25, 470-471

ALEXANDER, C. G. (1960): A survey of parasites of Oregon trout. Report to Oregon State Game Commission. pp 1-34

AL-HUSSAINI, A. H. (1946): The anatomy and histology of the alimentary tract of the bottom-feeder Mulloides auriflamma (Forsk.). Journal of Morphology, 78, 121-153

ALLISON, V. F., UBELAKER, J. E. and COOPER, N.B.(1972): The fine structure of the cysticercoid of Hymenolepis diminuta. II. The inner wall of the capsule. Z. parasitKde, 39, 137-147

AMIN, O.M. (1975). Host and seasonal associations of Acanthocephalus parksidei Amin, 1974 (Acanthocephala: Echinorhynchidae in Wisconsin fishes. J. Parasit, 61, 318-329

AMIN, O.M. (1977): Helminth parasites of some southwestern Lake Michigan fishes. Proc. helminth. Soc. Wash. 44, 210-217

AMIN, O.M. (1978): On the crustacean hosts of larval acanthocephalan and cestode parasites in southwestern Lake Michigan. J. Parasit 64, 842-845

ANDERSEN, K. (1975): Ultrastructural studies on Diphyllbothrium ditremum and D. dendriticum (Cestoda: Pseudophyllidea) with emphasis on the scolex tegument and the tegument in the area around the genital atrium. Z. Parasitkde, 46, 253-264

ARME, C. (1966): Histochemical and biochemical studies on some enzymes of Ligula intestinalis (Cestoda: Pseudophyllidea). J. Parasit. 52, 63-68.

ARME, C. (1968): Effects of the plerocercoid larva of a pseudophyllidean cestode, Ligula intestinalis on the pituitary gland and gonads of its host. Biological Bulletin 134, 15-25

ARME, C. and OWEN, R. W. (1967): Infections of the three-spined stickleback. Gasterosteus aculeatus L., with the plerocercoid larvae of Schistocephalus solidus (Muller, 1776), with special reference to pathological effects. Parasitology, 57, 301-314

ARME, C. and OWEN, R.W. (1968): Occurrences and pathology of Ligula intestinalis infections in British fishes. J. Parasit. 54, 272-280.

ARME, C. and OWEN, R. W. (1970): Observations on a tissue response within the body cavity of fish infected with the plerocercoid larvae of Ligula intestinalis (L). (Cestoda: Pseudophyllidea). J. Fish Biol. 2, 35-37

ARME, C. and READ, C. P. (1970): A surface enzyme in Hymenolepis dimnuta (Cestoda) J. Parasit., 56, 514-516

ARME, C., BRIDGES, J. F. and HOOLE, D. (1983): Pathology of cestode infections in the vertebrate host. In "Biology of the Eucestoda Vol. 2" (C. Arme and P. W. Pappas eds.) pp 499-538 Academic Press, London.

AWACHIE, J.B.E. (1963): The ecology of intestinal helminth parasites of the fish of Afon Terrig, North Wales. PhD. Thesis, University of Liverpool.

AWACHIE, J.B.E. (1965): The ecology of Echinorhynchus truttae Schrank 1788 (Acanthocephala) in a trout stream in North Wales. Parasitology, 55, 757-762

AWACHIE, J.B.E. (1966a): Observations on Cyathocephalus truncatus Pallas, 1781 (Cestoda: Spathebothriidea) in its intermediate and definitive hosts in a trout stream, North Wales. J. Helminth. 40, 1-10.

AWACHIE, J.B.E. (1966b): The development and life history of Echinorhynchus truttae Schrank, 1788 (Acanthocephala). J. Helminth. 40, 11-32.

AWACHIE, J.B.E. (1968): On the bionomics of Crepidostomum metoecus (Braun 1900) and Crepidostomum farionis (Muller 1784) (Trematoda: Allocreadiidae), Parasitology, 58, 307-324

AWACHIE, J.B.E. and CHUBB, J. C. (1964): Observations on the occurrence and distribution of Crepidostomum metoecus (Braun, 1900) in the British Isles. Parasitology, 54, 13P

BANGHAM, R. V. (1955): Studies on fish parasites of Lake Huron and Manitoalin Island. Am. Midl. Nat. 53, 184-194

BANGHAM, R. V. and ADAMS J. R. (1954): A survey of the parasites of freshwater fishes from the warmland of British Columbia. J. Fish. Res. Bd. of Canada, 11(b), 673-708

BARON, P. J. (1971): On the histology, histochemistry and ulstrastructure of the cysticercoid of Raillietina cesticillus (Molin, 1858) Fuhrmann, 1920 (Cestoda: Cyclophllidea). Parasitology, 62, 233-245

BATSCH, A. J. G. C. (1786): Naturgeschichte der Bandwurmgattung iiberhaupt und ihrer Arten insbesondere, nach den neweren Beobachtungen in einem systematischen Auszuge 298 pp 6.pl. Halle

BAUER, O.N. (1957): Diseases of carp in fish ponds in Leningrad, Velikie and Novogorod Oblasts) Izvestiya Vsesoyuznogo Nauchno Issledoratell'skogo Instituta Ozernogo i Rechnogo Rybnogo Khozyaistra 42, 67-88 (In Russian: translation Isreal Program for Scientific Translations Cat, No. 105.)

BAUER, O.N. and NIKOL'SKAYA, N.P. (1957): Dynamics of the parasitofauna of the whitefish Coregonus lavaretus from Lake Ladoga and its epizootic importance) Izvestiya Gosudarstvenn-ogo Nauchno-Issledovatel'skogo Instituta Ozernogo i Rechnogo Khozyaistra 42, 227-242 (In Russian: translation Israel Program for Scientific Translations Cat. No. 105)

BAUER, O.N. MUSSELIUS, V. A. and STRELKOV, Yu. A. (1969) ("Diseases of Pond Fish" pp. 1-335. Izdatel'stvo "Kolos", Muscow. (In Russian: translation Israel Program for Scientific Translations Cat. No. 601189)

BAYLIS, H. A. (1931): Gammarus pulex as an intermediate host for trout parasites. Ann. Mag. Nat. Hist., 7, 431-435.

BAYLIS, H. A. (1939): Further records of parasitic worms from British vertebrates. Ann. Mag. Nat. Hist. Ser. 11(4), 473-498

BECKMAN, M. Yu. (1954): "Biologiya Gammarus lacustris Sars pribaikal'skikhozer." Trudy Baikal'sko Limnobogicheskoi Stantsii 14.

BLAKE, I. H. (1930): Studies on comparative histology of the digestive tube of certain teleost fishes. I. A. predaceous fish, the sea bass. Centropristes striatus. Journal of Morphology 50, 39-70

BRATEN, T. (1968a): An electron microscope study of the tegument and associated structures of the procercoid of Diphyllobothrium latum (L). Z. Parasitkde, 30, 95-103

BRATEN, T. (1968b): The fine structure of the tegument of Diphllobothrium latum (L). A comparison of the plerocercoid and adult stages. Z. Parasitkde, 30, 104-112.

BRAUN, M. (1892) Verzeichniss von Eingeweidewurmern aus Mecklenburg Arch. Ver. Fr. Natg. Mecklenburg. 45, 97-117 Gustrow

BRINK, P. (1945): Ett bidrag till Kann edomen om rodingens biologi. K. Fysiogr. Sallsk. Lund Forh. 15, 170-184

BULLOCK, W. L. (1949): Histochmeical studies on Acanthocephala. I. The distribution of lipases and phosphatases. Journal of Morphology, 84, 185-199

BULLOCK, W. L. (1963): Intestinal histology of some salmonid fishes with particular reference to the histopathology of acanthocephalan infections. Journal of Morphology., 112, 23-24

BURNSTOCK, G. (1959): The morphology of the gut of the brown trout (Salmo trutta) Q. Jl. microsc. Sci. 100, 183-198.

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

BURT, M.D.B. and SANDEMAN, I. M. (1969): Biology of Bothrimonus (=Diplocotyle) (Pseudophyllidea: Cestoda). Part 1. History description, synonym and systematics. Journal of Fish. Res. Bd. of Canada, 26, 975-996

CALENTINE, R. L. (1964): The life cycle of Archigetes iowensis (Cestoda: Caryophyllidea). J. Parasit. 50, 454-458

CALENTINE, R. L. (1965): Larval development of four Caryophyllid cestodes. Proc. Iowa. Acad. Sci., 72, 418-242

CATALINI, N., ORECCHIA, P., PAGGI, L. and TODARO, P. (1978): Parassitofauna di Salmo trutta L. del Fiume Tiriono. Nota.111, Osservazioni istologiche sui parassiti reperti a Livello intestinale. Parassitologia, 20, 169-170

CHAPPELL, L. H. (1969): Competitive exclusion between two intestinal parasites of the three-spined stickleback, Gasterosteus aculeatus L. J. Parasit. 55, 775-778

CHAPPELL, L. H. and OWEN, R. W. (1969): A reference list of parasite species recorded in freshwater fish from Great Britain and Ireland. Journal of Natural History, 3, 179-216

CHAPPELL, L. H. (1980): The biology of the external surfaces of helminth parasites. Proce. R. Soc. Edinb., 79B, 145-171

CHARLES, G. H. (1970): The ultrastructure of the developing pseudophyllid tegument (epidermis) with reference to the larval stages of Schistocephalus solidus and Ligula intestinalis 2nd. Intl. Congress parasit. Washington, D. C. Resume No. 929. J. Parasit. 57, sect. II Part 1

CHARLES, G. H. and ORR, T.S. C. (1968): Comparative fine structure of outer tegument of Ligula intestinalis and Schistocephalus solidus. Exp. Parasit., 22, 137-149

CHUBB, J. C. (1962): Acetic acid as a diluent and dehydrant in the preparation of whole stained helminths Stain Technology 37, 179-184

CHUBB,J. C. (1967): A review of seasonal occurrence and maturation of tapeworms in British freshwater fish. Parasitology, 57, 13-14P.

CHUBB, J. C. (1980): Seasonal occurrence of helminths in freshwater fishes. Part III. Larval Cestoda and Nematodd. In: "Advances in Parasitology" (W.H.R. Lumsden, R. Muller and J. R. Baker, eds) Vol. 18, pp 1-120. Academic Press, London.

CHUBB, J. C. (1982): Seasonal occurrence of helminths in freshwater fishes, Part IV: Adult Cestoda, Nematoda and Acanthocephala. In: "Advances in Parasitology" (W.H.R. Lumsden, R. Muller and J. R. Baker, eds). Vol. 20, pp 1-292. Academic Press, London.

COLLIN, W. K. (1970): Electron microscopy of post-embryonic stages of the tapeworm Hymenolepis citelli. J. Parasit. 56, 1159-1170

COOPER, A. R. (1918): North American pseudophyllidean cestodes from fishes. Illinois. Biol. Monograph. Vol IV No. 4.

COOPER, N. F., ALLISON V, F, and UBELAKE, J. E. (1975) The fine structure of the cysticercoid of Hymenolepis diminuta III. The scolex. Z. Parasitkde, 46, 229-239.

ĆORIĆ, B. (1963): Cyathocephalus truncatus kod pesa (Cottus gobio) Veterinaria Sarajevo, 12 (1), 117-118.

CURRY, E. (1939): The histology of the digestive tube of the carp, Cyprinus carpio communis. Journal of Morphology, 65, 53-78.

CUSSANS, M. (1904). Gammarus. Liverpool Manchester Biological Commission Memmoir 12, 1-47.

DECHTIAR, A. O. and LOFTUS, K. H. (1965): Two new hosts for Cyathocephalus truncatus (Pallas 1781) Cestoda: Cyathocephallidae) in Lake Huron. Canadian Journal of Zoology, 43, 407-408.

DEGUISTI, D. L. and BUDD, J. (1959): A three-year survey of the infection rate of Echinorhynchus coregoni and Cyathocephalus truncatus in their intermediate host Pontoporeia affinis from South Bay Mouth, Ontario. Journal of Parasitology, 45, (4) Sect. 2. 25.

DIESING, C. M. (1850): Systema helminthum 1. 769pp. Wien.

DIESING, C. M. (1851): Systema helminthum 2. 588 pp. Wien.

DIKE, S. C. and READ, C. P. (1971): Tegumentary phosphohydrolases of Hymenolepis diminuta J. Parasit. 57, 81-87

ESCH, G. W. (1983): The population and community Ecology of Cestodes. In "Biology of the Eucestoda Vol 1" (C. Arme and P. W. Pappas, eds) pp 81-137. Academic Press, London.

ERASMUS, D. and OHMAN,J.C. (1965): Electron microscope studies of the gland cells and host-parasite interface of the adhesive organ of Cyathocotyle bushiensis Khan, 1962 Journal of Parasitology 51, 761-769.

EVANS, W. S. and de RYCHE, P. H. (1969): The in vitro cultivation of Hymenolepis. Parasitology 59, 20p

EZEASOR, D. N. and STOKOE, W. M. (1980): Scanning electron microscopic study on the gu mucosa of the rainbow trout (Salmo gairdneri) J. Fish Biol. 17, 619-634

EZEASOR, D. N. and STOKOE W. M. (1981): Light and electron microscopic studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, Salmo gairdneri, Rich. Journal of Fish Biology, 18, 527-544

EUZET, L. (1951): Echinobothrium mathiasi n.sp. (Cestode Diphyllidea) parasite d'une Raie: (Leiobatis aquila, L.). Bull. Soc. Zool. Fr. 76, No. 3., 182-188

EUZET, L. (1959): Recherches sur les cestodes tetraphyllides des selaciens des cotes de Frances. Montpelier; Causse, Graille et Castelneau, These. 262 pp.

FREEMAN, R. S. (1982): Do any Anonchotaenia, Cyathocephalus, Echeneibothrium or Tetragonocephalum (= Tylocephalum) (Eucestoda) have hookless oncospheres or coracidia ? J. Parasit., 68, 737-743.

FREEMAN, R. S. (1983): Pathology of the Invertebrate Host - Metacestode Relationship, In: "Biology of the Eucestoda" Vol. 2. (C. Arme and P. W. Pappas, eds.) pp. 441-497. Academic Press. London.

GABRION, C. (1975): Etude experimentale du development larvaire d'Anomotaenia constricta (Molin, 1858) Cohn, 1900 chez un coleoptere Pimelia sulcata Geoffr. Z. Parasitkde, 47, 249-262

GABRION, C. (1977): Etude experimentale du developpement larvaire d'Hymenolepis stylosa (Rudolphi, 1809) Railliet, 1899 (Cestode, Cyclphyllide). Annls. Parasit. Hum. Comp. 52, 117-130

GABRION, C. and GABRION, J. (1976): Etude ultrastructurale de la larve de Anomotaenia constricta (Cestoda, Cyclophyllidea) Z. Parasitkde, 49, 161-177

GABRION, C. and VERDIER, J. M. (1978): Etude ultrastructurale du cysticercoide de Hymenolepis stylosa (Cestoda, Cyclphyllidea) Annls. Parasit. Hum. Comp., 53, 131-146.

GAUTHIER, M. (1923): Development de l'oeut et embryon due Cyathocephale parasite de la Truite. Compte Reudus, 177 (2), 913-916. Paris.

GOMORI, G. (1946): A new histochemical test for glycogen and mucin. Am. J. Chin. Path. Tech. Sect. 10, 177-179

GOMORI, G. (1952): Microscopic histochemistry. Principles and practise. Chap. 8. University of Chicago, Chicago.

GREENE, C. W. (1911): The absorption of fats by the alimentary tract, with special reference to the function of the pyloric caeca in the King Salmon, Oncorhynchus tschawytscha. Trans Amer. Fish. Soc. (1911), pp. 261-270

GREENE, C. W. (1912): Anatomy and histology of the Alimentary tract of the King Salmon Bulletin of the U.S. Bur. Fish., 32, 75-101

GRIMM, O. (1871): Zur Anatomie der Binnenwurmer. Zeits wiss Zool. 21, 499-504. Leipzig.

GUIART, J. (1935): Cestodes parasites provenant des campagnes scientifiques de S.A.S. le Prince Albert ler de Monaco. Res. Camp. Sci. Monaco 91, 1-100. 5pl.

GUSTAFSSON, M.K.S. and VAIHELA, B. (1981): Two types of frontal glands in Diphyllobothrium dendriticum (Cestoda: Pseudophyllidea) and their fate during maturation of the worm. Z. Parasitkde, 66, 145-154.

HALVORSEN, O. and MACDONALD, S. (1972): Studies of the Helminth Fauna of Norway XXVI: The distribution of Cyathocephalus truncatus (Pallas) in the intestine of brown trout (Salmo trutta L.) Norw. J. Zool. 20, 265-272

HASSALL, M. and JENNINGS, J. B. (1975): Adaptive features of gut structure and digestive physiology in the terrestrial isopod Philosica muscorum (Scopoli) 1763. Biol. Bull. 149, 348-364

HAYHOE, F. G. L. QUAGLINO, D. and DOLL, R. (1964): The Cytology and Cytochemistry of acute leukaemias. Spec. Rep. Ser. Med. Res. Coun. No. 314

HENRICSON, J. and NYMAN, L. (1976): The ecological and genetical segregation of two sympatric species of dwarfed char (Salvelinus alpinus (L) species compelx). Institute of Freshwater Research Drottningholm. Report No. 55.

HOCKLEY, D. J. (1973): Ultrastructure of the tegument of Schistosoma In "Advances in Parasitology" (B. Dawes, ed). 11, pp 233-305. Academic Press, London.

HOFER, B. (1904): Handbuch der Fischkrankheiten. 359pp. 18 Taf. 222 Fig. Munchen

HOLMES, P. F. (1960): The brown trout of Malham Tarn, Yorkshire. Sam. Trout. Mag. 159, 127-145

HOLMES, J. C. (1961): Effects of concurrent infections on Hymenolepis diminuta (Cestoda) and Moniliformis dubius (Acanthocephala). I. General effects and comparison to crowding Journal of Parasitology 47, 209-216

HOOLE, D. and ARME, C. (1982): Ultrastructural studies on the cellular response of roach, Rutilus rutilus L. to the plerocercoid larva of the pseudophyllidean cestode, Ligula intestinalis. Journal of Fish Diseases, 5, 131-144

HOPKINS C. A., LAW, L. M. and THREADGOLD, L. T. (1978): Schistocephalus solidus: pinocytosis by the plerocercoid tegument. Expl. Parasit. 44, 161-172.

HUITFELDT-KASS, H. (1913): Fiskeribiologiske Undersokelser i Vande i Trondhjemsamterne. Kgl. Norske Vid. Selsk. Skr. Nr. 14. 75pp.

HUITFELDT-KASS, H. (1927): Cyathocephalus truncatus - Pallas als ursache von Fisch-Epizootien. Nytt. Mag. Naturv., 65, 145-151

HUMASON, G. L. (1962): Animal Tissue Techniques. W. H. Freeman and Co, Publishers, London.

HUTTON, J. A. (1923): The parasites of Salmon. Salm. Trout. Mag. (Dec.) 1-11.

HYNES, H.B.N. (1955): The reproductive cycle of some British Freshwater Gammaridae. Journal of Animal Ecology 24, 352-387

HYNES, H.B.N. and NICHOLAS, W. L. (1957): The development of Polymorphus minutus (Goeze 1782) (Acanthocephala) in the intermediate host. Ann. trop. Med. Parasit., 51, 380-391.

HYNES, H.B.N. and NICHOLAS W.L. (1958): The resistance of Gammarus spp. to infection by Polymorphus minutus (Goeze, 1782) (Acanthocephala). Ann. trop. Med. Parasit., 52, 376-383

INGHAM, L. and ARME, C. (1973): Intestinal helminths in Rainbow trout, Salmo gairdneri (Richardson): absence of effects on nutrient absorption and fish growth. J. Fish. Biol. 5, 309-313

JÄÄSKELAINEN, V. (1921): Uber die Nahrung und die Parasiten der Fische in Ladogasee. Ann. Acad. Scient. Fennicae. Ser. A. Tom 14 No. 3., 55pp. Helsinki.

JANICKI, C. (1928): Die Lebensgesch, von Amphilina fol. Wag. Parasiten des Wolgasterlets, nach Beobacht. u. Exper. Arb. d. Wolga-Stat. Bd.X Nr. 3. Saratow.

JANICKI, C. (1930): Uber die jungsten Zustande v. Amphilina foliacea in d. Fischleibeshohle u.s.w. Zool. Anz. 90

JANICKI, C. and ROSEN, F. (1917): "The Life cycle of Diphyllobothrium latus L." Translated from French In: Tropical Medicine and Parasitology Vol II (1978) pp. 659 - 673

JENNINGS, J. B. (1957): Studies on Feeding, digestion and food storage in free-living flatworms. (Platyhelminthes: Turbellaria) Biological Bulletin, 112, 63-80.

JENNINGS, J. B. (1962): A histochemical study of digestion and digestive enzymes in the rhynchocoelan Lineus ruber (O.F Muller). Biological Bulletin, 122, 63-72

JOYEAUX, C. H. and BAER, J. G. (1936): Cestodes. Faune de France. 30. Paris.

JOYEAUX, C. H. and BAER, J. G. (1939): Recherches Biologiques sur quelques cestode Pseudophyllidea. Volumen jubilare pro Prof. Sadao Yoshida. 11, 203-210. Japan.

JOYEAUX C. H. and BAER J. G. (1961): Classe des cestodes. In: "Traite de Zoologie" (P.P. Grasse, ed). Vol IV pp. 347-560. Masson, Paris.

KANE, M. B. (1966): Parasites of Irish fishes. Sciet. proc. R. Dubl. Soc. Bl, 205-220

KENNEDY, C. R. (1965): The life-history of Archigetes limnodrili (Yamaguti) (Cestoda: Caryophyllidea) and its development in the invertebrate host. Parasitology. 55, 427-437

KENNEDY, C. R. (1974): A checklist of British and Irish fresh-water fish parasites with notes on their distribution. Journal of Fish Biology. 6, 613-644.

KENNEDY, C.R. (1983): General Ecology. In: "Biology of the Eucestoda Vol 1". (C. Arme and P. W. Pappas, eds). pp. 27-80. Academic Press. London.

KESSLER, K. T. (1868): Bietrage zur zoologischen Kenntniss des Onegasee's und dessen Umgebung. Beil.z.d.Arb. d.l. russ. naturf. Vers. Petersburg. 183 pp. 8pl. St. Petersburg

KILEJIAN, A., SCHINAZI, L. A. and SCHWABE, C.W. (1961): Host-parasite relationship in echinococcosis. V. Histochemical observation on Echinococcus granulosus. Journal of Parasitology, 47, 181-188.

KIMURA, N. (1973): Fine structure of the epithelial cells in the pyloric caecum of the rainbow trout, Salmo gairdneri. Jap. J. Ichthyol., 20, 13-24.

KOTIKOVA, E. A. and KUPERMAN, B. I. (1978): (New data on the structure of the nervous system in Pseudophyllidea) Biologiya Morya, Vladivostock. 6, 41-46 (In Russian) Lab. of Evolutionary Morphology, Zool. Inst., Acad. of Sci. of the USSR, Leningrad, U.S.S.R.

KRAEMER, A. (1891): Vorlaufige Mittheilung uber Cyathocephalus truncatus (Pallas) Kessler. Zool. Anz. 14, 451-453. Leipzig

KRAEMER, A. (1892): Beitrage Zur Anatomie und Histologie der Cestoden der Susswasserfische Zeitz. Wiss. Zool. 53, 646-722. Leipzig.

KRASNOSCEKOV, G. P. (1978): Ul'trastruktura cerkomera cisticerkoida Trichocephaloides megalocephala. Parazitologiya, 12, 206-209.

KUHLOW, F. (1953): Bau und Differentialdiagnose heimischer Diphyllobothrium - Plerocercoide. Z. Tropenmed. Parasit. 4, 186-202

KWA, B. H. (1972): Studies on the Sparganum of Spirometra erinacei. Int. J. Parasit., 2, 23-43

LARKIN, P. A. (1948): Pontoporeia and Mysis in Athabasca, Great Bear and Great Slave lakes. Bulletin of the Fisheries Research Bd. of Canada., 78, 1-33.

LEE, D. L. (1966): The structure and composition of helminth cuticle. In: "Advances in Parasitology Vol IV". (B. Dawes ed.) 4, pp 187-254. Academic Press, London.

LEE, D. L., ROTHMAN, A. H. and SENTURIA, J. B. (1963): Esterases in Hymenolepis and Hydatigera. Expl. Parasitology, 14, 285-295

LEE, R. L. G. (1977): The serpentine fish and their parasites. London Naturalist, 56, 57-70

LEFEVERE, S. (1952): Sur la phosphatase alkaline chez les cestodes. Bulletin de l'institut Royale des Sciences Naturelles de Belgique. 28, 1-4

LEONG, R. T. (1975): Metazoan parasites of fishes of Cold Lake, Alberta: a community analysis. PhD Thesis, University of Alberta.

LETHBRIDGE, R. C. (1971): The hatching of Hymenolepis diminuta eggs and penetration of hexacanth in Tenebrio molitor. beetles Parasitology, 62, 445-456.

LETHBRIDGE R.C. and GIJSBERS, M. F. (1974: Penetration gland secretion by hexcanths of Hymenolepis diminuta. Parasitology 68, 303-311.

LINCOLN, R. J. (1979): British Marine Amphipoda: Gammaridea. British Museum (Natural History) 658pp. London.

LINSTOW, O. (1878) Compendium der Helminthologie. 382pp. Hanover.

LINSTOW, O. (1889): Compendium der Helminthologie. Nachtrag. Die Litterature der Jahre 1878-1889. 151pp. Hanover.

LINTON, E. (1898): Notes on cestode parasites of Fishes. Proc. U.S. Nat. Mus., 20, 423-456

LLEWELLYN, J. and OWEN, I.O. (1960): The attachment of the monogenea Discocotyle sagittata Leikart to the gills of Salmo trutta. Parasitology, 50, 51-59.

LUHE, M. (1899): Zur Anatomie und systematik der Bothriocephalidien. Verh. Deutsch. Zool. Ges. 9: 30-55. Leipzig.

LUHE,M. (1900): Uber Bothrimonus Duv. und verwandte Bothriocephaliden. Zool. Anz. 23: 8-14 Leipzig.

LUHE, M. (1910) Parasitische Plattwurmer 2. Cestodes in: Die suswasserfaung Deutschlands. Heft. 18.151pp. Jena.

LUMSDEN, R. D. (1975): Surface ultrastructure and cytochemistry of parasitici helminths. Experimental Parasitology, 37, 267-339

LUMSDEN, R. D. and SPECIAN, R. (1980): The morphology, histology and fine structure of the adult stage of the cyclophyllidean tapeworm Hymenolepis diminuta. In: "Biology of the Tapeworm Hymenolepis diminuta" (H. P. Arai, ed. pp. 157-280. Academic Press London.

LUMSDEN, R. D. and HILDRETH, M. B. (1983): The fine structure of Adult Tapeworms. In: "Biology of the Eucestoda Vol.1" (C. Arme and P. W. Pappas, eds.) pp. 177-233. Academic Press, London.

LYONS, K. M. (1978): The biology of Helminth Parasites. The Institute of Biology's Studies in Biology No. 102 Edward Arnold Publs., London.

MAITLAND, P. S. (1972): Key to British freshwater fishes. Scient. Publs. Freshwat. Biol. Ass. No. 27.

MALAKHOVA, R. P. (1961): Seasonal changes in the parasitofauna of certain freshwater fishes from Karelian lakes (Lake Konche). Trudy Karelskogo Filiala. Akademiya Nauk SSSR 30, 55-78.

MAREN, M. J. van (1979): The amphipod Gammarus fossarum Koch (Crustacea) as intermediate host for some helminth parasites with notes on their occurrence in the final host. Bijdragen Tot de Dierkunde, 48 (2), 97-110.

McLAREN, D. J. (1980): Schistosoma mansoni: The parasite surface in relation to host immunity. Research studies press, John Wiley and Sons Ltd., Chichester.

McLAUGHLIN, P. A. (1980): Comparative morphology of recent crustacea. W. H. Freeman and Co. San Francisco.

McMANUS, J.F.A. (1946): Histochemical demonstration of mucin after periodii acid. Nature, London., 158, 202.

McVICAR, A. H. (1972): The ultrasturcture of the parasite - host interface of three tetraphyllidean tapeworms of the elasmobranch Raja naevus. Parasitology 65, 77-88

MEEK, G. A. (1970): Practical Electron Microscopy for Biologists. Wiley-Interscience (a division of John Wiley and Sons Ltd). London.

MONNE, L. (1959): On the external cuticles of various helminths and their role in the host-parasite relationship. A histochemical study. Arkiv. for Zoologi., 12, 343-358

MOORE, J. (1984): Parasites that change the behaviour of their host. Scientific America Vol. 250, No. 5, 82-89

MORSETH, D. J. (1966): The fine structure of the tegument of adult Echinococcus granulosus, Taenia hydatigera and Taenia pisiformis. Journal of parasitology, 52, 1074 - 1088

MUDRY, D. R. and McCART, P. J. (1976): Metazoan parasites of Arctic char (Salvelinus alpinus) from the north slope of Canada and Alaska. Journal of the Fisheries Res. Bd. of Canada., 33, 271-275

NILSSON, L. M. (1977): Incubation time, growth and mortality of the amphipod, Gammarus pulex under laboratory conditions. Oikos, 29, 93-98

NUFER (1905): Die Fische des Vierwalds ta ttersees und ihre Parasiten. 232pp. 4 Taf. Luzern.

NYBELIN, O. (1922): Anatomisch-Systematische studien uber Pseudophyllideen. Goteborgs K. Vet. Och. Vitterh. Samh. Handl Fjarde Foljden. 26 (1), 1-228. 11 Fig.

OAKS, J. (1970): The functional morphology of integuments of parasites and free living platyhelminthes. PhD Thesis, Tulane University.

OHMAN-JAMES, C. (1973): Cytology and Cytochemistry of the scolex gland cells in Diphyllobothrium ditremum. Z. Parasitkde, 46, 77-86.

OLSSON, P. (1872): Om sarcotaces och Acrobothrium tva nya parasitslagten fran fiskar. Stockholm Vet. Akad. Ofvers No. 9 37-44.

OLSSON, P. (1893): Bidrag till skandinaviens Helminthfauna 2. Stockholm Vet. Akad. Handl. 25 (12), 3-41

ORECCHIA, P., PAGGI, L., MANILLA, G. and ROSSI, G. (1978): Parassitofauna di Salmo trutta L. Del fiume Tirino. Nota IV. Ricerca degli ospiti intermedi di Cyathocephalus truncatus (Pallas, 1781) E. Dentitruncus truttae Sinzar, 1955. Parassitologia. Vol. 20 pp. 175-181.

PAGGI, L., ORECCHIA, P., DEL-MARRO, M., IORI, A. and MANILLA, G. (1978): Parassitofauna di Salmo trutta L. Del fiume Tirino. Nota III: Le interazioni parassitaospite delle specie elmintiche reperite Parassitologia Vol. 20, 161-168

PALLAS, P. C. (1781): Bemerkungen uber die Bandwurmer in Menschen und Thieren. Newe nord. Bietrage Phys. geogr. Erd-Volkerbeschr. Natg. Okon 1, 39-112. 2 Taf. St. Petersburg and Leipzig.

PEARSE A. G. E. (1972): Histochemistry: Theoretical and Applied Vols I and II, 1518 pp. Churchill Livingstone (Longman Grp. Ltd) Edinburgh and London.

PEARSE, A. S. (1924): The parasites of Lake fishes. Trans Wisc. Acad. Sci. Arts and Lett., 21, 161-195.

PENCE, D. B., (1970): Electron microscope and histochemical studies on the egg of Hymenolepis diminuta. J. Parasit., 56, 84-97.

PENNOIT-DE COOMAN, E. (1940): Recherches sur le metabolisme des cestodes. Anales de la Societe Royale Zoologique de Belgique, 71, 36-76

PESSON, B. and LEGER, N. (1978): Hymenolepis nana var. fraterna (Cestoda: Hymenolepididae) chez Leucophaca maderae (Dictyoptera: Blattidae): l'expression due conflit' hote-parasite apres inhibition experimentale de la reaction hemocytaire. Annls. Parasit. Hum. Comp., 53, 147-154

PETERSSON, A. (1971): The effect of lake regulation on populations of cestodan parasites of Swedish Whitefish, Coregonus. Oikos 22 (1) 74-83

POWELLS, A. M. (1966): A primary investigation of the biology and parasite fauna of the Charr. (Salvelinus alpinus perisii) PhD. Thesis, University of Liverpool.

RAHIM, M. A. (1974): Biological investigations in the regulated, unregulated and the polluted streams of the Dee watershed. PhD Thesis, University of Liverpool.

RAO, K. H. (1954): A new bothriocephalid parasite (Cestoda) from the gut of the fish Saurida tumbil (Bloch). Current Sciences, 23, 333-334.

RAO, K. H. (1960): Studies on Penetrocephalus ganapatti, a new genus (Cestoda: Pseudophyllidea) from the marine teleost Saurida tumbil (Bloch). Parasitology, 50, 155-163

RAWSON, D. (1952): The occurrence of parasitic worms in British freshwater fishes. Ann. Mag. Nat. Hist. Ser. 12. (5), 877-888.

RAWSON, D. (1957): The anatomy of Eubothrium crassum (Bloch) from the pyloric caeca and small intestine of Salmo trutta L. J. Helminth, 31, 103-120

READ, C. P. (1951): The crowding effects in tapeworm infections. J. Parasit., 37, 174-178

READ, C. (1966): Nutrition of intestinal helminths. In: "Biology of Parasites" (E. Soulsby, ed) pp. 101-126. Academic Press, London.

REES, G. (1956): The scolex of Tetrabothrius affinis (Lonnberg) a cestode from Balaenoptera musculus L. the blue whale. Parasitology, 46, 425-442.

REES, G. (1958): A comparison of the structure of the scolex of Bothriocephalus scorpii (Muller 1776) and Clestobothrium crassiceps (Rud. 1819) and the mode of attachment of the scolex to the intestine of the host. Parasitology, 48, 468-492.

REES, G. (1961): Studies on the functional morphology of the scolex and of the genitalia of Echinobothrium brachysoma Pintner and E. afine Diesing from Raja clavata L. Parasitology, 51, 193-226.

REES, G. (1967): Pathogenesis of Adult Cestodes. Helminthological Abstracts Vol. 36, Part 1, 1-23.

RICHARD, M. J. (1935): Resultats des campagnes Scientifiques, 41, 55-56. 2 fig.

REES, G. and WILLIAMS, H. H. (1965): The functional morphology of the scolex and the genitalia of Acanthobothrium coronatum (Rud.) (Cestoda: Tetraphyllidea). Parasitology, 55 (4), 617-651.

REIMCHEM, T. E. (1982): Incidence and Intesity of Cyathocephalus truncatus and Schistocephalus solidus infection in Gasterosteus aculeatus. Canadian Journal of Zoology, 60 (5), 1091-1095

RICHARDS, K. S. and ARME, C. (1981): The ultrastructure of the scolex-neck syncytium, neck cells and frontal gland cells of Caryophyllaeus laticeps (Caryophyllidea: Cestoda). Parasitology, 83, 477-487.

RIGGENBACH,E. (1899): Cyathocephalus catinatus n.sp. Zool. Jahrb. 12, 154-160. Jena.

ROBERTS, R. J. (1978): Fish Pathology. Bailliere Tindal, London 318pp.

ROGERS, W. P. (1947)L Histological distribution of alkaline phosphatases in helminth parasites. Nature 159, 374-375

ROTHMAN, A. H. (1960) Ultramicroscopic evidences of absorptive function in cestodes. Journal of Parasitology 46 (suppl.) 10

ROTHAMN, A. H. (1963): Electron microscope studies of tapeworms: The surface structures of Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891. Trans. Am. micros. Soc., 82, 22-30

RUDOLPHI, C. A. (1793): Observationes circa vermes intestinalis. Pars 1, Diss praes. Quistorp. Fasc. 2 praes. C. E. Weigal. Cryphiswaldiae.

RUDOLPHI C. A. (1808): Entozoorum sive vermium intestinalium historica naturalis. Vol. 1, 57pp. 6pl. Amstelaedami.

RUDOLPHI, C.A. (1809): Ibid. Vol. 2(1), 457 pp. pl.7-12.

RUDOLPHI, C. A. (1810): Entozoorum sive vermium intestinalium historia naturalis. Vol. 2.2.389pp.

RUDOLPHI, C. A. (1819) Entozoorum synopsis cui accendunt mantissa duplex et indices locupletissimi. 821 pp. 3pl. Berolini

RUKAVINA, J. and DELIĆ, S. (1959): Cyathocephalus truncatus Kod Riba U Nekim Vodama Bosne 1 Hercegorine. Veterinaria Sarajevo 8, 547-553

RUMPUS, A. E. (1973): The ecology of the parasites of Gammarus pulex of the River Avon, Hampshire. PhD Thesis, University of Exter.

SANDEMAN, I. M. and BURT, M. D. B. (1972): Biology of Bothrimonus (= Diplocotyle) Pseudophyllidea: Cestoda): ecology, life cycle and evolution; a review and synthesis. J. Fish Res. Bd. Canada 29, 1381-1385

SCHÄFERNA, K. (1922): Amphipoda balcanica. Vestnik Kral. Ceske Spol. Nauk. tr. 2.

SCHARDEIN, J. L. and WAITZ, J. A. (1965): Histochemical studies of esterase in the cuticle and nerve cords of cour cyclophyllidean cestodes. Journal of Parasitology, 51, 356-363

SCHMIDT, G. D. (1970): The tapeworms. How to know. Pictured key nature series. 8° Dubuque Iowa.

SCHWABE, C. W. (1955): Helminth parasites and neoplasia. Am. J. vet. Res. 16, 485-491

SCOTT, K. J. and BULLOCK, W. L. (1974): Paammonyx nobilis (Amphipoda: Lysianassidae), a new host for Bothrimonus sturionis (Cestoda: Pseudophyllidae (sic)) Proc. helminth. Soc. Wash., 41, 256-257.

SENK, O. (1952): Cyathocephalus truncatus Pallas, rasirenost u izvorskom dijelu rijeke Bosne. Veterinaria Sarajevo 1, Part 8/10, 740-751.

SENK, O. (1956): Cyathocephalus truncatus, Pallas - Uticaj Na Rasplodne Elemente portocnih Pastmki (Salmo trutta fario) Veterinaria Sarajevo 5 (4), 607-615.

SEXTON, E. W. (1928): On the rearing and breeding of Gammarus in laboratory conditions. Journal of the Marine Biological Association, U. K. 15, 33-35

SINDERMANN, C. J. (1970): Principal diseases of Marine Fish and Shell fish. Academic Press, London and New York.

SLUSARSKI, W. (1958): Helminth fauna of fishes (Salmonidae) of the lakes in Polish part of the High Tatra. Kliad. Parazyt. 4, 651-653

SMIT, G. L., HATTINGH, J. and BURGER, A. D. (1979): Haematological assessment of the effects of the anaesthetic MS222 in natural and neutralized form in three freshwater fish species. J. Fish Biol. 15, 633-665

SMYTH, J. D. (1959): Maturation of larval pseudophyllidean cestods and strigeid trematodes under axenic conditions: the significance of nutritional levels in platyhelminth development. Annals of New York Academy of Sciences 77, 102-125

SMYTH, J. D. (1969): The physiology of Cestodes. University review in biology II 8° Edinburgh.

SMYTH, J. D. (1976): Introduction to Animal Parasitology. 2nd edn. Hodder and Stoughton, London.

SMYTH, J. D. and HASLEWOOD, G.A.D. (1963): The biochemistry of bile as a factor in determining host specificity in intestinal parasites with particular reference to Echinococcus granulosus. Ann. New York Acad. Sci. 113, 234-260

SMYTH, J. D. and HALTON, D. W. (1983): The physiology of trematodes. 2nd Edition. Cambridge University Press, Cambridge.

SPECIAN, R. D. and LUMSDEN, R. D. (1980): The microanatomy and fine structure of the rostellum of Hymenolepis diminuta. Z. Parasitkde, 63, 71-88.

STACK, G.T. C. (1965): Diplocotyle (Eucestoda) a parasite of Gammarus zaddachi in the estuary of the Yorkshire Esk, Britain. Parasitology, 55, 415-420

STIREWALT, M. A. (1966): Skin penetration mechanisms of helminths. In Biology of Prasites (E.J.L. Soulsby, ed) pp 41-58. Academic Press, London and New York.

STUNKARK, H. W. (1983): Evolution and Systematics. In "Biology of the Eucestoda Vol. 1" (C. Arme and P. W. Pappas, eds) pp. 1-25

SUBHAPRADHA, C. K. (1954): Two new bothriocephalids from the marine fish Saurida tumbil. (Bloch). Proc. Indian Acad. Sci. 41 (1)B, 20-30.

SWEETING, R. A. (1977): Studies on Ligula intestinalis: some aspects of the pathology in the second intermediate host. Journal of Fish Biology 10, 43-50

TAKAHASI, T. (1959): Studies on Diphyllobothrium mansoni 2. Histochemical studies on plerocercoid. Jap. J. Parasit. 8, 669-676

THOMAS, J. D. (1922): The food and growth of brown trout (Salmo trutta L.) and its feeding relationships with the Salmon Parr (Salmo solar L.) and the Eel (Anguilla anguilla L.) in the river Teify, West Wales. Journal of Animal Ecology, 31, 175-205

THOMAS, J. D. (1958): Studies on Crepidostomum metoecus (Braun) and C. farionis (Muller) parasitic in Salmo trutta L. in Britain Parasitology, 48, 336-352

THOMAS, J. D. (1964): Studies on population of helminth parasites in brown trout (Salmo trutta L.) Journal of Animal Ecology, 33, 83-95

THREADGOLD, L. T. (1962): An electron microscope study of the tegument and associated structures of Dipylidium caninum. Quarterly Journal of Microscopical Sciences, 103, 135-140

THREADGOLD, L. T. and HOPKINS, C. A. (1981): Schistocephalus solidus and Ligula intestinalis pinocytosis by the tegument. Expl. Parasit., 51, 444-456

UBELAKER, J. E. (1980): Structure and ultrastructure of the larvae and metacestodes of Hymenolepis diminuta. In: "Biology of the tapeworm Hymenolepis diminuta" (H. P. Arai ed.) pp 59-156. Academic Press, London.

UBELAKER, J.E. (1983): Metacestodes: Morphology and Development. In. "Biology of the Eucestoda Vol. 1" (C. Arme and P. W. Pappas, eds) pp. 137-176. Academic Press, London and New York.

UBELAKER, J. E. (1983): The Morphology, Development and Evolution of Tapeworm Larvae. In "Biology of the Eucestoda Vol.1" (C. Arme and P. W. Pappas, eds) pp. 235-296. Academic Press, London and New York.

UBELAKER, J. E., COOPER, N. B. and ALLISON, V.F. (1970): The fine structure of the cysticercoid of Hymenolepis diminuta. 1. The outer wall of the capsule. Z. Parasitkde, 35, 258-270

VIK, R. (1954): Investigations on the pseudophyllidean cestodes of fishes, birds and mammals in the Ånoya Water system in Trøndelag. Part I. Cyathocephalus truncatus and Schistocephalus solidus Nytt. Mag. Zool. 2, 5-51.

VIK, R. (1958): Studies of the Helminth Fauna of Norway. II. Distribution and life cycle of Cyathocephalus truncatus (Pallas, 1781) (Cestoda). Nytt. Mag. Zool., 6, 97-110.

VOGE, M. (1973): The post embryonic developmental stages of cestodes. In: "Advances in Parasitology" (B. Dawes ed.) 11, pp. 707-730. Academic Press, London and New York.

VOGE, M. and HEYMEMAN, D. (1957): Development of Hymenolepis nana and Hymenolepis diminuta (Cestoda: Hymenolepididae) in the intermediate host Tribolium confusum. University of California Zool. Publications 59, 549-580.

WAITZ, J. A. and SCHARDEIN, J. L. (1964): Histochemical studies of four cyclphyllidean cestodes. J. parasit., 50, 271-277

WARDLE, R. A. (1932): The cestoda of Canadian fishes. II. The Hudson Bay drainage system. Contrib. Canad. Biol. Fish No. 7. 379-403.

WARDLE, R.A. and McLEOD, J. A. (1952): The zoology of tapeworms. The University of Minnesota Press, Minneapolis. 780pp.

WARDLE , R. A., McLEOD, J. A. and RADINOVSKY, S. (1974): Advances in the Zoology of tapeworms 1950-1970, 272pp. University of Minnesota Press, Minneapolis.

WARE, D. M. (1971): Predation by rainbow trout: the effect of experience. J. Fish Res. Bd. Canada 28: 1847-1852

WEAKLEY, B. S. (1981): A Beginner's Handbook in Biological Transmission Electron Microscopy. Churchill Livingstone Publications, Edinburgh, London and New York.

WEINREB, E. L. and BILSTAD, N. M. (1955): Histology of the digestive tract and adjacent structures of rainbow trout Salmo gairdneri irideus. Copeia, 3, 194-204

WELTON, J. S. (1979): Life history and production of the amphipod Gammarus pulex in a Dorset chalk stream. Freshwater Biology, 9, 263-275

WELTON, J. S. and CLARKE, R. T. (1980): Laboratory studies on the reproduction and growth of the amphipods, Gammarus pulex Journal of Animal Ecology, 49 (2) 581-592

WHEELER, A. (1969): The Fishes of the British Isles and North-West Europe. 1st Ed. Macmillan London, Melbourne, Toronto.

WILLIAMS, H. H. (1960): Some observations on Parabothrium gadi-pollachii (Rudolphi, 1810) and Abothrium gadi van Beneden 1870 (Cestoda: Pseudophyllidea) includuing an account of their mode of attachment and of variation in the two species. Parasitology, 50, 303-322.

WILLIAMS, H. H. and HALVORSEN, O. (1971): The incidence and degree of infection of Gadus morhua L. 1758 with Abothrium gadi Beneden 1871 (Cestoda: Pseudophyllidea). Norw. J. Zool. 19, 193-199

WISNIEWSKI, L. W. (1930): Das Genus Archigetes R. Leuck. Eine studie zur Anatomie Histogenese Systematik und Biologie. Memories de l'Academie Polonaise des Sciences et des Lettres. Classe des Sciences Mathematique et Naturelles Serie B.2, 1-160

WISNIEWSKI, L. W. (1932a): Zur postembryonalen Entwicklung von Cyathocephalus truncatus Pallas. Zoologischer Anzeiger 98, 213-218

WISNIEWSKI, L. W. (1932b): Cyathocephalus truncatus Pallas - I. Rozwojpostembrjonalny i biologja - Cyathocephalus truncatus Pallas - 1. Die Postembryonalentwicklung und Biologie. Bull int. Acad. pol. Sci. Cl. Math Nat. B3, 237-252.

WISNIEWSKI, L.W. (1932e): Cyathocephalus truncatus Pallas - II. Ognolna morfologia - Cyathocephalus truncatus Pallas. II Allgemeine Morphologie. Bull. int. Acad. pol. Sci. Classe. Math Nat. B3, 311-327

WOLF, E. (1906): Beitrage zur Entwicklungsgeschichte von Cyathocephalus truncatus - Pallas. Zool. Anz. 30, 37-45

WOOTEN, R. (1973): The metazoan parasite fauna of fish from Hanningfield Reservoir, Essex, in relation to features of the habitat and host populations. J. Zool. Lond., 171, 323-331.

YAMAGUTI, S. (1959): Systema Helminthum Vol. II - The cestodes of vertebrates. Interscience Publishers Ltd. London and New York.

YAMANE, Y. (1968): On the fine structure of Diphyllobothrium erinacei with special reference to the tegument. Yonago Acta med. 12, 169-181.

ZEDER, J. G. H. (1803): Anleitung zur Naturgeschichte der Eingeweidewurmer, 448pp. 4pl. Bamberg.

ZSCHOKKE, F. (1884): Recherches sur l'organisation et la distribution zoologique des vers parasites des poissons d'eau douce. Archives de Biologie, 5, 153-241

ZSCHOKKE, F. (1891): Die Parasitenfauna von Trutta Salar. Zentralblatt fur Bakteriologie Parasitenkunde Infektionskrankheiten und Hygiene Abteilung 1, Originale 10, 694-699, 738-745, 792-801 and 829-838.

ZSCHOKKE, F. (1896): Zur Faunistik der parasitischen Wurmer von Susswasserfischen, Central. f. Bakt. un Parasit. 19, 772-784

ZSCHOKKE, F. (1903): Marine Schmarotzer in Susswasserfischen. Verh. Naturf. Ges. Basel. 16, 118-157.

ZSCHOKKE, F. (1933): Die Parasitenfauna der Gattung Coregonus. Revue Suisse Zool. 40: 32, 559-634

APPENDIX

Appendix I: Incidence anddistribution of Cyathocephalus truncatus in the fish collected from Driffield trout streams.

(A) Rainbow trout (Salmo gairdneri)

No.	Total Length (cm)	Weight (g)	Sex	Total No. of Tapeworms	Incidence & Distribution of Cyathocephalus truncatus: Dorsal caeca No. of Caeca	Dorsal caeca No. of worms	Dorsal caeca %	Anterior Ventral caeca No. of Caeca	Anterior Ventral caeca No. of worms	Anterior Ventral caeca %	Posterior Ventral caeca No. of Caeca	Posterior Ventral caeca No. of worms	Posterior Ventral caeca %	Intestine No.	Intestine %	Body cavity No.	Body cavity %	No. of C.truncatus: Attached No.	Attached %	Free No.	Free %
1	13.1	12.5	M	14	10	4	28.5	35	3	21.4	10	3	21.4	2	14.2	2	14.2	12	85.7	2	14.3
2	14.2	26.8	M	13	8	5	38.4	42	6	46.1	10	2	15.3	-	-	-	-	13	100	-	-
3	14.5	35.2	M	51	9	30	58.8	45	14	27.4	8	1	1.9	4	7.8	2	3.9	45	88.2	6	11.8
4	13.0	15.5	M	19	10	10	52.6	40	9	47.3	8	-	-	-	-	-	-	19	100	6	-
5	28.0	208.7	M	73	8	38	52.0	45	27	36.9	10	8	10.9	-	-	-	-	73	100	-	-
6	20.7	84.4	F	62	10	21	33.8	38	25	40.3	10	7	11.2	4	6.4	5	8.0	56	90.3	6	9.7
7	18.3	54.0	F	34	10	15	44.1	35	14	41.1	12	3	8.8	-	-	2	5.8	34	100	-	-
8	14.6	28.2	M	105	8	39	37.1	42	52	49.5	8	10	9.5	1	0.9	3	2.8	103	98.0	1	2.0
9	22.5	115.9	F	33	10	16	48.4	35	12	36.3	6	4	12.1	1	3.0	-	-	33	100	1	-
10	22.2	128.2	F	94	8	22	23.4	37	65	69.1	5	6	6.3	-	-	1	1.0	93	98.9	1	1.1
11	17.0	58.1	M	32	10	10	31.2	38	17	53.1	7	5	15.6	-	-	-	-	32	100	-	-
12	21.2	62.5	M	57	10	19	51.3	36	30	52.0	8	8	14.0	-	-	-	-	57	100	-	-
13	19.7	51.9	M	61	10	22	36.0	40	31	50.8	8	8	13.1	-	-	-	-	61	100	-	-
14	19.8	75.5	M	76	9	30	39.4	35	37	48.6	5	8	10.5	-	-	1	1.3	76	100	-	-
15	22.0	104.5	M	35	10	20	57.1	30	11	31.4	6	4	11.4	-	-	-	-	35	100	-	-
16	20.0	86.4	F	52	10	15	28.8	37	30	57.6	9	6	11.5	1	1.9	-	-	52	100	-	-
17	18.5	65.2	M	26	10	9	34.6	32	12	46.1	8	5	19.2	-	-	-	-	26	100	-	-
18	17.0	46.5	M	67	10	25	37.3	37	32	47.7	7	7	10.4	2	2.9	1	1.4	63	94.0	4	6.0
19	34.0	246.2	M	114	8	40	35.0	43	58	50.8	9	10	8.7	2	1.7	4	3.5	114	96.4	4	3.6
20	22.5	126.8	M	106	8	36	33.9	39	62	58.4	6	7	6.6	1	0.9	-	-	106	100	-	-
21	22.2	132.0	M	66	7	19	28.7	35	41	62.1	6	6	9.0	-	-	-	-	66	100	-	-
22	21.5	119.8	F	102	8	31	30.3	34	53	51.9	7	10	9.8	4	3.9	4	3.9	102	100	-	-
23	23.0	120.5	M	42	10	13	30.9	42	20	47.6	8	8	19.0	1	2.3	-	-	42	100	-	-
24	20.8	92.0	F	67	8	18	26.8	42	43	64.1	5	6	8.9	-	-	-	-	67	100	-	-
25	22.1	116.7	M	75	10	23	30.6	36	46	61.3	7	6	8.0	-	-	-	-	75	100	-	-

26	22.4	145.0	F	87	10	25	28.7	40	53	60.9	7	6	8.0	3	3.4	-	-	87	100	-	-
27	21.6	94.5	M	91	10	31	34.0	38	51	56.0	6	6	8.7	1	1.0	-	-	91	100	-	-
28	20.0	104.0	F	117	6	43	36.7	37	43	36.7	5	15	12.8	3	2.5	13	11.1	116	99.1	1	0.9
29	18.7	78.5	M	17	8	11	64.7	41	5	29.4	8	1	5.8	-	-	-	-	17	100	-	-
30	22.0	112.5	M	19	8	6	31.5	35	10	52.6	5	3	15.7	-	-	-	-	19	100	-	-
31	21.3	96.8	M	40	8	18	45.0	33	15	37.5	6	6	15.0	1	2.5	-	-	39	97.5	1	2.5
32	18.2	70.8	F	33	8	12	36.3	30	17	51.5	6	4	12.1	-	-	-	-	33	100	-	-
33	17.2	67.0	F	25	9	13	52.0	32	10	40.0	7	2	8.0	-	-	-	-	25	100	-	-
34	12.8	20.0	M	13	10	7	53.8	30	4	30.7	6	-	-	1	7.6	1	7.6	11	84.6	2	15.4
35	24.0	141.3	M	47	9	18	38.2	34	26	55.3	6	3	6.3	-	-	-	-	47	100	-	-
36	19.8	82.5	F	52	8	19	36.5	34	30	38.4	8	3	5.7	-	-	-	-	52	100	-	-
37	14.1	25.0	M	26	8	14	53.8	42	11	42.3	7	1	3.8	-	-	-	-	26	100	-	-
38	14.0	24.1	M	7	8	4	57.1	42	2	28.5	8	-	-	1	14.2	-	-	7	100	-	-
39	22.5	112.2	F	68	8	24	35.2	40	34	50.0	10	5	7.3	2	2.9	3	4.4	66	97.1	2	2.9
40	18.5	66.1	F	57	10	22	38.5	42	24	43.8	8	4	7.0	1	1.7	5	8.7	56	98.3	1	1.7
41	17.8	63.5	M	11	8	6	54.5	40	4	36.3	8	-	-	-	-	1	9.0	11	100	-	-
42	15.6	43.3	F	19	8	9	47.3	38	8	42.1	6	2	10.5	-	-	-	-	19	100	-	-
43	16.0	30.3	M	12	8	6	50.0	40	4	33.3	8	1	8.3	1	8.3	-	-	12	100	-	-
44	14.3	35.1	M	4	8	3	75.0	36	1	25.0	8	-	-	-	-	-	-	4	100	-	-
45	19.5	71.6	F	5	10	3	60.0	43	2	40.0	8	-	-	-	-	-	-	5	100	-	-
46	17.1	90.2	M	12	8	7	58.3	40	4	33.3	7	1	8.3	-	-	-	-	12	100	-	-
47	18.3	70.0	M	8	10	4	50.0	45	4	50.0	8	-	-	-	-	-	-	8	100	-	-
48	23.0	120.3	F	22	10	6	27.2	44	14	63.6	9	1	4.5	-	-	1	4.5	22	100	-	-
49	20.0	100.3	F	43	10	13	30.2	43	21	48.8	7	6	13.9	2	4.6	1	2.3	41	95.4	2	4.6
50	12.9	23.7	M	9	8	3	33.3	40	5	55.5	5	-	-	-	-	1	11.1	9	100	-	-

51	16.2	50.4	M	27	8	9	33.3	40	17	62.9	6	1	3.7	-	-	-	-	27	100	-	-
52	18.7	76.1	M	21	10	6	28.5	42	12	57.1	8	2	9.5	1	4.7	-	-	20	95.3	1	4.7
53	30.0	125.7	F	31	10	13	41.9	45	17	54.8	9	-	-	-	-	1	3.2	30	96.8	1	3.2
54	20.4	109.6	M	14	10	8	57.1	43	5	35.7	8	-	-	-	-	1	7.1	14	100	-	-
55	15.2	26.4	F	2	8	2	100	43	-	-	7	-	-	-	-	-	-	2	100	-	-
56	14.4	22.0	M	5	8	4	80.0	40	1	20.0	5	-	-	-	-	-	-	5	100	-	-
57	25.4	75.8	F	63	9	18	28.5	43	40	63.4	9	3	4.7	2	3.1	-	-	61	96.9	2	3.1
58	18.0	56.2	M	57	8	19	33.3	43	31	54.3	8	4	7.0	1	1.7	2	3.5	56	98.3	1	1.7
59	20.4	90.8	M	86	8	25	29.0	40	47	54.6	8	10	11.6	1	1.1	3	3.4	82	95.4	4	4.6
60	34.2	318.4	F	38	10	17	44.7	42	20	52.6	8	1	2.6	-	-	-	-	38	100	-	-
61	21.0	68.3	M	46	8	18	39.1	44	23	50.0	7	5	10.8	-	-	-	-	46	100	-	-
62	23.5	85.4	M	62	8	21	33.8	42	37	59.6	10	4	6.4	-	-	-	-	62	100	-	-
63	20.0	75.0	F	53	9	15	28.3	43	37	69.8	8	1	1.8	-	-	-	-	53	100	-	-
64	18.2	62.8	F	71	8	21	29.5	42	46	64.7	7	3	4.2	-	-	1	1.4	70	98.6	1	1.4
65	23.8	88.3	F	98	7	24	24.4	35	58	59.1	8	12	12.2	1	1.0	3	3.0	94	96.0	4	4.0
66	22.0	69.5	M	32	10	14	43.7	43	14	43.7	10	3	9.3	-	-	1	3.1	32	100	-	-
67	21.6	72.0	M	78	9	15	19.2	42	46	58.9	10	13	16.6	1	1.2	3	3.8	76	97.5	2	2.5
68	18.5	60.6	F	37	8	14	37.8	40	20	54.0	7	3	8.1	-	-	-	-	37	100	-	-
69	20.0	67.3	M	28	10	14	50.0	45	12	42.8	10	2	7.1	-	-	-	-	28	100	-	-
70	29.0	206.7	M	15	10	9	60.0	45	5	33.3	10	-	-	-	-	1	6.6	14	100	-	-
71	24.8	95.2	M	41	9	16	39.0	42	20	48.7	8	5	12.1	-	-	-	-	41	100	-	-
72	22.4	73.5	M	52	9	18	34.6	38	31	59.6	8	3	5.7	-	-	-	-	52	100	-	-
73	20.4	99.2	F	87	9	21	24.1	36	52	59.7	8	12	13.7	-	-	2	2.2	86	98.9	-	-
74	25.5	165.3	M	68	8	20	29.4	40	40	58.8	8	8	11.7	-	-	-	-	68	100	-	-
75	23.3	154.4	M	62	8	18	29.0	38	34	54.8	8	8	12.9	-	-	2	3.2	61	98.4	1	1.6

76	13.4	15.29	F	32	8	12	37.5	40	10	31.3	8	7	21.9	3	9.4	-	-	30	93.8	2	6.2
77	14.0	18.5	M	41	8	11	26.8	40	16	39.0	8	10	24.4	3	7.3	1	2.4	40	97.6	1	2.4
78	23.6	20.3	M	67	10	23	34.3	42	30	44.8	10	10	14.9	2	3.0	2	3.0	66	98.5	1	1.5
79	21.3	19.5	M	51	8	20	39,2	40	30	58.8	8	1	2.0	-	-	-	-	51	100	-	-
80	12.8	13.4	M	11	8	6	54.5	40	5	45.5	8	0	-	-	-	-	-	11	100	-	-
81	22.5	26.4	F	63	10	31	49.2	42	28	44.4	10	1	1.6	-	-	3	4.8	63	100	-	-
82	13.4	12.7	M	4	8	3	75.0	40	1	25.0	9	-	-	-	-	-	-	4	100	-	-
83	10.7	13.6	F	21	8	13	61.9	40	8	38.1	8	-	-	-	-	-	-	21	100	-	-
84	14.4	17.8	F	37	8	11	29.7	40	26	70.3	7	-	-	-	-	-	-	37	100	-	-
85	21.7	24.8	F	52	8	12	23.1	40	28	53.8	8	8	15.4	-	-	4	7.7	51	98.1	1	1.9
86	12.6	25.1	F	17	8	9	52.9	40	7	41.2	8	1	5.8	-	-	-	-	17	100	-	-
87	12.3	35.2	M	12	8	3	25.0	40	9	75.0	7	-	-	-	-	-	-	12	100	-	-
88	12.8	22.0	F	15	8	5	33.3	40	10	66.7	6	-	-	-	-	-	-	15	100	-	-
89	34.2	113.6	F	3	10	3	100.0	43	-	-	9	-	-	-	-	-	-	3	100	-	-
90	24.0	32.8	M	92	8	32	34.8	43	52	56.6	10	8	8.7	-	-	-	-	92	100	-	-
91	23.6	25.7	M	58	10	35	60.3	42	21	36.2	8	2	3.4	-	-	-	-	58	100	-	-
92	20.4	30.6	M	45	8	13	28.9	41	27	60.0	8	3	6.7	2	4.4	-	-	45	100	-	-
93	23.1	43.0	M	80	10	24	30.0	42	46	57.5	9	6	7.5	1	1.3	3	3.8	80	100	-	-
94	21.8	26.7	F	74	8	29	39.2	40	38	51.4	10	4	5.4	1	1.4	2	2.7	73	98.6	1	1.4
95	13.0	25.1	M	14	8	9	64.3	40	5	35.7	8	-	-	-	-	-	-	14	100	-	-
96	11.2	28.4	F	9	8	9	100.0	38	-	-	6	-	-	-	-	-	-	9	100	-	-
97	25.2	32.5	F	103	10	42	40.8	40	56	54.4	10	5	4.9	-	-	-	-	103	100	-	-
98	18.3	40.8	M	41	8	28	58.3	40	13	31.7	8	-	-	-	-	-	-	41	100	-	-
99	19.7	42.3	M	65	8	31	47.7	40	30	46.2	10	4	6.2	-	-	-	-	64	100	-	-
100	11.6	36.5	M	12	8	10	83.3	38	2	16.7	8	-	-	-	-	-	-	12	100	-	-

101	10.3	40.6	M	5	0	3	60.0	36	2	40.0	8	-	-	-	-	-	-	5	100	-	-
102	12.0	38.3	M	18	8	7	38.9	40	11	61.1	8	-	-	-	-	-	-	18	100	-	-
103	16.3	52.2	M	33	8	16	48.5	40	15	45.5	8	2	6.1	-	-	-	-	33	100	-	-
104	15.8	57.0	F	29	8	14	48.3	40	11	37.9	8	2	6.9	1	3.4	1	3.4	29	100	-	-
105	17.1	50.4	M	43	8	19	44.2	40	22	51.2	8	1	2.3	1	2.3	-	-	43	100	-	-
106	12.3	30.8	F	16	6	10	62.5	37	6	37.5	8	-	-	-	-	-	-	16	100	-	-
107	11.8	23.0	M	10	8	5	50.0	38	5	50.0	8	-	-	-	-	-	-	10	100	-	-
108	20.2	82.5	F	46	10	24	52.2	40	22	47.8	10	-	-	-	-	-	-	46	100	-	-
109	14.6	35.2	M	24	8	13	54.2	40	10	41.7	8	1	4.2	-	-	-	-	24	100	-	-
110	11.2	32.6	M	13	8	7	53.8	40	4	30.8	8	2	15.2	-	-	-	-	13	100	-	-
111	25.4	73.4	F	73	12	37	50.7	42	36	49.3	10	-	-	-	-	-	-	73	100	-	-
112	10.5	30.8	F	16	0	5	31.3	40	9	56.3	8	1	6.3	1	6.3	-	-	15	93.7	1	6.3
113	12.3	24.5	F	13	8	6	46.2	40	7	53.8	9	-	-	-	-	-	-	13	100	-	-
114	20.5	94.7	F	36	10	10	27.8	41	23	63.9	9	2	5.6	-	-	1	2.8	36	100	-	-
115	10.3	38.4	M	8	8	7	87.5	40	1	12.5	7	-	-	-	-	-	-	8	100	-	-
116	11.5	42.3	F	12	6	9	75.0	36	3	25.0	6	-	-	-	-	-	-	12	100	-	-
117	18.6	36.4	M	73	8	31	42.5		40	54.8	8	2	2.7	-	-	-	-	73	100	-	-
118	34.3	120.6	F	26	10	14	53.8	42	12	46.2	10	-	-	-	-	-	-	26	100	-	-
119	17.2	32.3	F	76	8	33	43.4	40	42	55.2	8	-	-	1	1.3	-	-	75	98.7	1	1.3
120	28.4	103.7	M	25	9	15	60.0	40	9	36.0	10	1	4.0	-	-	-	-	25	100	-	-
121	16.9	25.3	M	50	8	27	54.0	40	19	38.0	8	3	6.0	-	-	1	2.0	50	100	-	-
122	12.1	40.8	M	6	8	6	100.0	38	-	-	8	-	-	-	-	-	-	6	100	-	-
123	29.3	98.5	M	28	10	13	46.4	42	15	53.6	11	-	-	-	-	-	-	28	100	-	-
124	19.4	31.4	M	86	8	34	39.5	40	41	47.7	8	6	7.0	1	1.2	4	4.7	84	97.7	2	2.3
125	17.1	40.0	M	53	8	22	41.5	40	31	58.5	8	-	-	-	-	-	-	53	100	-	-

126	18.5	30.7	M	77	8	28	36.4	40	45	58.4	8	2	2.6	-	-	2	2.6	77	100	-	-
127	16.2	25.3	F	46	8	31	67.4	40	15	32.6	8	-	-	-	-	-	-	46	100	-	-
128	15.4	26.9	F	41	8	17	41.5	40	20	48.8	8	3	7.3	1	2.4	-	-	41	100	-	-
129	14.5	28.0	F	12	6	7	58.3	40	5	41.7	8	-	-	-	-	-	-	12	100	-	-
130	23.3	21.0	M	98	11	41	41.8	41	53	54.1	9	4	4.1	-	-	-	-	98	100	-	-
131	13.0	33.4	M	14	6	9	64.3	40	5	35.7	8	-	-	-	-	-	-	14	100	-	-
132	20.1	40.3	M	51	10	21	41.2	40	27	52.9	8	2	3.9	-	-	1	2.0	51	100	-	-
133	30.3	88.5	/	21	10	13	51.9	43	7	33.3	12	-	-	1	4.8	-	-	21	100	-	-
134	32.6	118.2	M	17	12	1	14.3	41	6	85.7	10	-	-	-	-	-	-	7	100	-	-
135	24.5	35.3	M	83	10	37	44.6	42	44	53.0	9	2	2.4	-	-	-	-	83	100	-	-
136	28.3	97.1	M	35	10	19	54.3	39	16	45.7	10	-	-	-	-	-	-	35	100	-	-
137	27.4	83.8	F	39	10	17	43.6	42	21	53.8	10	1	2.6	-	-	-	-	39	100	-	-
138	27.7	92.0	M	30	10	14	46.7	41	12	40.0	10	4	13.3	-	-	-	-	30	100	-	-
139	25.3	41.6	F	68	10	18	26.5	41	45	66.2	9	3	4.4	1	1.5	1	1.5	66	97.1	2	2.9
140	30.0	163.5	F	14	8	11	78.6	44	3	21.4	11	-	-	-	-	-	-	14	100	-	-
141	23.4	36.4	F	62	9	22	35.5	39	31	50.0	9	9	14.5	-	-	-	-	62	100	-	-
142	31.8	147.0	F	12	10	4	33.3	42	7	58.3	12	-	-	1	8.3	-	-	12	100	-	-
143	17.5	56.8	M	37	7	15	40.5	40	22	59.5	8	-	-	-	-	-	-	37	100	-	-
144	15.4	50.5	F	15	8	5	33.3	40	7	46.7	8	3	20.0	-	-	-	-	15	100	-	-
145	18.9	54.3	F	46	8	24	52.2	40	21	45.7	8	1	2.2	-	-	-	-	46	100	-	-
146	31.6	47.6	M	21	9	10	47.6	44	11	52.4	8	-	-	-	-	-	-	21	100	-	-
147	27.8	77.4	F	27	10	16	59.3	43	10	37.0	9	-	-	1	3.7	-	-	27	100	-	-
148	22.5	23.0	M	84	8	38	45.2	40	39	46.4	8	4	4.8	1	1.2	2	2.4	83	98.8	1	1.2
149	17.3	64.7	M	31	8	23	74.2	40	8	25.8	8	-	-	-	-	-	-	31	100	-	-
150	28.0	160.5	M	44	10	25	56.8	40	19	43.2	7	-	-	-	-	-	-	44	100	-	-

151	20.6	85.7	F	65	8	21	32.3	40	36	55.4	8	6	9.2	-	-	2	3.1	65	100	-	-
152	14.3	18.1	F	28	8	16	57.1	40	12	42.9	8	-	-	-	-	-	-	28	100	-	-
153	17.1	28.4	F	34	8	12	35.3	40	22	64.7	8	-	-	-	-	-	-	34	100	-	-
154	21.7	42.5	F	51	8	31	60.8	40	13	25.5	8	7	13.7	-	-	-	-	51	100	-	-
155	26.0	47.2	M	36	8	17	47.2	42	19	52.8	8	-	-	-	-	-	-	36	100	-	-
156	23.4	54.2	F	92	8	51	55.4	41	32	34.8	8	8	8.7	-	-	1	1.1	92	100	-	-
157	25.6	72.5	M	65	10	24	36.9	42	40	61.5	10	1	1.5	-	-	-	-	65	100	-	-
158	19.3	48.3	M	54	8	13	24.1	40	38	70.4	8	3	5.5	-	-	-	-	54	100	-	-
159	18.5	50.5	M	50	8	25	50.0	40	25	50.0	8	-	-	-	-	-	-	50	100	-	-
160	26.0	70.7	M	47	9	21	44.7	40	23	48.9	9	2	4.3	-	-	1	2.1	47	100	-	-

Incidence and distribution of Cyathocephalus truncatus in the fish collected from Driffield trout streams.

(B) Brown trout (Salmo trutta)

No.	Total Length (cm)	Weight (g)	Sex	Total No. of Tapeworms	Incidence & Distribution of Cyathocephalus truncatus														No. of C. truncatus			
					Dorsal caeca			Anterior Ventral caeca			Posterior Ventral caeca			Intestine		Body cavity		Attached		Free	
					No. of caeca	No. of worms	%	No. of caeca	No. of Worms	%	No. of Caeca	No. of worms	%	No.	%	No.	%	No.	%	No.	%
1	15.5	125.6	M	3	10	3	100.0	40	-	-	8	-	-	-	-	-	-	3	100	-	-
2	18.8	117.8	F	10	8	7	70.0	38	3	30.0	6	-	-	-	-	-	-	10	100	-	-
3	17.6	115.6	M	16	6	8	50.0	38	7	43.8	6	1	6.2	-	-	-	-	16	100	-	-
4	19.1	84.3	F	35	6	13	37.1	40	22	62.9	10	-	-	-	-	-	-	35	100	-	-
5	28.3	105.7	M	4	10	4	100.0	42	-	-	8	-	-	-	-	-	-	4	100	-	-
6	18.0	65.5	M	12	6	10	83.3	38	1	8.3	8	-	-	1	8.3	-	-	12	100	-	-
7	20.5	124.0	M	75	6	38	50.7	40	32	42.7	8	2	2.7	2	2.7	1	1.2	74	98.7	1	1.3
8	22.7	96.3	F	53	8	13	24.5	40	26	49.0	8	4	7.5	3	5.7	7	13.2	51	96.2	2	3.8
9	30.2	132.4	F	13	10	7	53.8	42	6	46.2	10	-	-	-	-	-	-	13	100	-	-

Incidence and distribution of Cyathocephalus truncatus in the fish collected from Driffield trout streams.

(C) Grayling (Thymallus thymallus)

No.	Total Length (cm)	Weight (g)	Sex	Total No. of Tapeworms	Incidence & Distribution of Cyathocephalus truncatus: Dorsal caeca No. of Caeca	Dorsal caeca No. of worms	%	Anterior Ventral caeca No. of Caeca	Anterior Ventral caeca No. of worms	%	Posterior Ventral caeca No. of Caeca	Posterior Ventral caeca No. of Worms	%	Intestine No.	Intestine %	Body cavity No.	Body cavity %	No. of C. truncatus: Attached No.	Attached %	Free No.	Free %
1	18.3	62.5	M	46	6	27	58.7	37	19	41.3	6	-	-	-	-	-	-	46	100	-	-
2	21.7	50.2	M	91	6	40	44.0	38	45	49.5	9	4	4.3	1	1.1	1	1.1	90	98.9	1	1.1
3	24.5	47.0	F	52	8	23	44.2	40	27	51.9	10	2	3.8	-	-	-	-	52	100	-	-
4	28.3	58.3	F	24	8	11	45.8	42	13	54.2	10	-	-	-	-	-	-	24	100	-	-
5	17.6	42.6	F	30	6	16	53.3	38	11	36.7	6	2	6.7	1	3.3	-	-	29	96.7	1	3.3
6	17.3	45.1	M	24	6	13	54.2	38	11	45.8	6	-	-	-	-	-	-	24	100	-	-
7	20.1	52.5	F	71	6	30	42.3	38	36	50.7	9	4	5.6	-	-	1	1.4	71	100	-	-
8	25.0	87.2	F	58	8	28	48.3	41	29	50.0	8	1	1.7	-	-	-	-	58	100	-	-
9	30.2	120.8	M	13	10	7	53.8	42	6	46.2	10	-	-	-	-	-	-	13	100	-	-
10	28.5	76.4	M	106	7	38	35.8	39	51	48.1	7	13	12.3	1	1.0	3	2.8	105	99.0	1	1.0
11	22.0	57.7	M	73	8	33	45.2	39	31	42.5	8	6	8.2	-	-	3	4.1	73	100	-	-
12	17.8	84.3	F	16	8	10	62.5	39	6	37.5	6	-	-	-	-	-	-	16	100	-	-
13	16.5	70.5	M	23	6	13	56.5	40	10	43.5	6	-	-	-	-	-	-	23	100	-	-
14	18.4	46.2	M	48	8	23	47.9	39	25	52.1	6	-	-	-	-	-	-	48	100	-	-
15	25.1	82.9	M	55	10	27	49.1	42	28	50.9	8	-	-	-	-	-	-	55	100	-	-
16	10.7	28.7	M	12	6	9	75.0	38	3	25.0	6	-	-	-	-	-	-	12	100	-	-
17	12.3	34.2	F	18	6	6	33.3	38	12	66.7	6	-	-	-	-	-	-	18	100	-	-
18	13.0	40.5	F	15	6	10	66.7	38	5	33.3	6	-	-	-	-	-	-	15	100	-	-
19	19.4	37.2	M	43	7	21	48.8	40	22	51.2	8	-	-	-	-	-	-	43	100	-	-
20	20.7	41.3	M	87	7	34	39.1	41	43	49.4	10	5	5.7	1	1.1	4	4.6	87	100	-	-
21	19.2	40.3	F	53	8	19	35.8	40	23	43.4	6	8	15.1	2	3.8	1	1.9	51	96.2	2	3.8
22	21.5	36.5	F	112	8	40	35.7	40	52	46.4	10	13	11.6	1	0.9	6	5.4	109	97.3	3	2.7
23	28.6	130.6	F	7	10	7	100.0	43	-	-	10	-	-	-	-	-	-	7	100	-	-
24	17.2	47.0	M	38	8	18	47.4	38	18	47.4	6	2	5.2	-	-	-	-	38	100	-	-
25	19.6	40.2	M	45	7	23	51.1	38	22	48.9	6	-	-	-	-	-	-	45	100	-	-
26	20.8	103.8	F	40	10	16	40.0	41	24	60.0	8	-	-	-	-	-	-	40	100	-	-
27	11.5	38.0	F	5	8	4	80.0	37	1	20.0	6	-	-	-	-	-	-	5	100	-	-
28	19.2	37.1	F	43	8	18	41.9	40	22	51.2	8	3	6.9	-	-	-	-	43	100	-	-
29	20.0	92.4	M	56	8	19	33.9	38	31	55.4	10	4	7.1	2	3.6	-	-	54	96.4	2	3.6
30	27.4	120.2	M	25	10	14	56.0	39	11	44.0	10	-	-	-	-	-	-	25	100	-	-
31	14.3	41.7	M	19	7	9	47.4	38	10	52.6	6	-	-	-	-	-	-	19	100	-	-

APPENDIX 2

(A) ACID PHOSPHATASE (after Burstone, 1958)

Standard Naphthol as phosphate method

Paraffin embedded sections (max. m.p. 41°C. Blocks kept at 4°C) Clear in Xylol and hydrate rapidly in series of acetone (100%. 90%, 70%, 50%)

Incubating medium

	4mg	Naphthol AS-Bl phosphate
		dissolved in 0.25 ml dimethyl formamide
*Add	25ml	0.2M Tris maleate buffer (pH 5.2)
Add	35mg	Red violet L.B. salt
		2 drops of 10% maganese chloride.

Incubate at 37°C for about 1 hour

Wash in distilled water and mount in Glycerol Jelly

(B) * For Alkaline Phosphatase

Change for 25ml ... 0.2M Tris maleate buffer (pH.8.3)

APPENDIX 3

NON-SPECIFIC ESTERASES (Holt, 1958)

(Also ref. Pearse, (1972); Humason (1962)

Indigogenic method

Paraffin embedded sections (wax m.p. 41°C; Blocks kept at 4°C) Clear in Xylol and hydrate sections quickly in series of acetone (100%. 90%, 70%, 50%).

Incubating medium

0 - acetyl - 5 bromoindoxyl	1.3mg
Ethanol	1.0ml

Allow to dissolve and add

0.1m Tris (hydroxylmethyl) aminonethane /HCl buffer (pH 6-8.5)	2.0ml
0.05m - Potassium ferricyanide	1.0ml
0.05m - Potassium ferrocyanide	1.0ml
0.1m - Calcium chloride	1.0ml

Add water to make up to 10ml of solution

Incubate (10 secs to 15 hrs) at 27 - 30°C

Incubation medium always to be prepared fresh.

APPENDIX 4

LIPASES (Gomori, 1952)

STOCK SOLUTION

A. Substrate:

Tween 60	5.0 gm
Distilled water	100.0 ml

B. Tris buffer, pH. 7.2-7.4:

(i) Buffer stock solution

Maleic acid	29.0 gm
Tris (hydroxylmethyl) aminomethane	30.3 gm
Distilled water	500.0 ml
Charcoal	2.0 gm

Shake, let stand 10 minutes and filter.

(ii) Buffer working solution

Stock solution	40.0 ml
4% NaOH	20.0 ml

dilute to a total of 100 ml

(Preserve substrate and buffer with 0.25% chloretone or crystal of thymol)

C. Calcium chloride:

Calcium chloride	10.0 gm
Distilled water	100.0 ml

WORKING SOLUTION

Buffer working solution	5.0 ml
10% Calcium chloride	2.0 ml
Substrate	2.0 ml
Distilled water	40.0 ml

Procedure:

1. Deparraffinize and transfer slides to absolute acetone.
2. Hydrate rapidly to water.
3. Incubate for 12-48 hours.
4. Wash in distilled water.
5. Treat with 1% Lead nitrate (1gm/100ml. water) for 15 minutes (calcium stearate transformed to lead stearate).
6. Wash in several changes of distilled water.
7. Treat with dilute ammonium sulfide (1:5): few minutes (brown sulfide produced).
8. Rinse in tap water.
9. Counterstain lightly with hematoxylin and eosin if desired.
10. Dehydrate and mount in glycerol jelly.

APPENDIX 5

Specimen preparation for Electron microscopy

Tissues usually about 1cm thick.

Glutaraldehyde (fixative)	½ - 3hrs
Buffer rinse	15 min
Osminm textroxide	1 hr
Buffer rinse	15 min
50% alcohol	20 min
70% alcohol	20 min
95% alcohol	20 min
Absolute alcohol 1	20 min
Absolute alcohol 2	20 min
Epoxyl propane 1	10 min
E.P. 2	10 min
E.P. Epon (1:1)	Overnight
Embed in fresh Epon (or Araldite)	
(or Araldite/Epon)	
Polymerise 60°C	24 hrs.