A SURVEY OF HELMINTH PARASITES
IN SURFING WHELKS ON THE WESTERN CAPE COAST
OF SOUTH AFRICA

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CONTENTS

Page

ABSTRACT

Three species of sandy beach whelks of the genus *Bullia* were examined for helminth parasites.. Collection sites were at Ou Skip, Melkbos, Hout Bay, Noordhoek, Fish Hoek, Muizenberg and Mnandi on the Western Cape coast of South Africa.

Bullia digitalis was found to harbour: *Cercaria Z* (Family, Microphallidae); *Cercaria Y* (Superfamily, Allocreadiodea); *Cercaria X* (Family, Zoogonidae); *Metacercaria W* (Family, Azygiidae); *Metacercaria V* (Family, Ptychogonimidae),, and *Echinobothrium* sp. (Cestoda, Diphyllidea).

Bullia pura was found infected with: *Metacercaria W* , *Metacercaria V* , *Metacercaria U* (Family Ptychogonimidae), and *Echinobothrium* sp.

No helminths were found in a sample of 170 *B. rhodostoma*.

The likely intermediate host for *Cercaria Z* is the isopod *Eurydice longicornis*. That of *Cercaria Y* is the swimming crab *Ovalipes punctatus*.

Infection with *Cercaria Z*. abolishes sperm production in males. Infections with this parasite tend to occur in larger size whelks. The emergence of *Cercaria Z* from *Bullia* may be stimulated by injection of glucose.

Analysis of shell proportions in B. digitalis indicates no difference between infected and uninfected whelks. Shell proportions at different beaches are significantly different.

CHAPTER 1

1.1 INTRODUCTION

The genus *Bullia* (Gray) is a member of the gastropod family Nassariidae. The super family Buccinacea, of which the Nassariidae are part, are considered to be the most advanced super family of neogastropods (Brown 1982). The distribution of the Nassariidae is world-wide. Members of the subgroup containing the genera *Bullia* *Buccinanops* (d'Orbigny 1841) and *Dorsanum* are found predominantly in the southern hemisphere and tend to occur on soft substrates. With the exception of some members of the genus *Bullia* the subgroup shows a preference for sheltered beaches with little wave action (Brown 1982).

Published work on the subgroup containing *Bullia* has come from study sites on the south-west coast of India, South Africa and the Argentine coast of South America. In India, contributions to the knowledge of *Bullia melanoides* (Deshayes) have come from Crichton (1942a, b), Ansell and Trevallion (1969, 1970) and Ansell et al. (1972). In South Africa a great deal of publications have been produced covering all native members of the genus *Bullia*. Brown (1982) has produced the major source of work on *Bullia* native to South Africa. Penchasadeh (1971a, b, 1973) has published notable accounts of the biology of *Dorsanum* and *Buccinanops* from the Argentine coast.

The genus *Bullia* is concentrated around the southern land masses; its distribution, with few exceptions, may be described as Indo-Pacific. The full range of the genus has not been ascertained. Specimens have been collected as far afield as eastern India and West Africa (Brown, pers. comm.).

Approximately a dozen species of *Bullia* have been described from South Africa. The number is not precise due to the fluid nature of the systematics of some of the possibly spurious species, e.g. *Bullia lara* Bartsch, 1915 (Kilburn & Rippey 1982).

In the Western Cape there are three major intertidal species. *Bullia digitalis* (Dillwyn) is found alone on the west coast from Lüderitz to Cape Point. This is with the exception of a colony of *Bullia rhodostoma* (Gray) in Saldanha Bay (Brown 1977). *Bullia digitalis* is also found east of Cape Point but in company with *Bullia pura* (Melvill) and *Bullia rhodostoma*. *Bullia pura* is found sporadically along the coast, usually in much smaller numbers than either of the other species. *Bullia rhodostoma* may occur in greater or equal numbers than *Bullia digitalis* and becomes the dominant surfing *Bullia* as one moves away east from Cape Point. The above distributions are quoted from Branch and Branch (1981), Brown (1982), and Brown, pers. comm.

Notable subtidal species of *Bullia* include *Bullia laevissima* (Gmelin), an egg-shaped heavy-shelled species, and *Bullia annulata* (Lamarck) which has an angular, conical shell. Shells of both species are commonly washed up on Western Cape beaches. Living specimens may sometimes be caught at spring low tides. They may also be found on very sheltered beaches in shallow water.

All species of *Bullia* have a large expandable foot with which it is adept at burrowing in the substrate. The burrowing ability of the genus has given rise to the common name of "plough snail". A similar, though less vigorous action occurs during crawling across a waterlogged substrate. Surfing species have a proportionally larger and thinner foot than non-surfing species. In surfers, the specific gravity of the body is much less than in non-surfers. This is correlated to the increased surfing efficiency of the less dense *Bullia* (McGwynne 1980).

Bullia have no eyes and are functionally blind. *Bullia digitalis* shows a sensitivity to changes in light intensity, particularly from light to dark when an increase in activity occurs (Webb, unpubl.). The foot usually has two posterior cirri and may have anterior cirri also. The operculum is always small and entirely inadequate for closing the shell entrance. The whelk coincidentally shows a marked reluctance to retract into its shell even after mild provocation.

Bullia usually spends most of its time submerged or semi-submerged in the substrate and only emerges to feed on washed-up dead and decaying animal matter such as dead fish and stranded jellyfish.

Bullia detects the presence of food by using its inhalent siphon to sample water which is then passed over the osphradium, which has been shown by Brown (1961 , 1971b, 1982) to be a very sensitive detector of trimethylamine, a substance which is given off by decaying animal matter. The osphradium is sensitive to other compounds but the whelk's response is not always so sharply defined and is sometimes even negative.

The three species of *Bullia* dealt with in this study are the surfing species found on the western Cape beaches. *Bullia digitalis* ranges in size commonly from 6 mm to 45 mm; the shell is conical and may be classified into six common colour varieties (McGwynne 1980): cream with purple rays, cream with brown rays, purple, orange, cream and grey. The shell is relatively smooth. The operculum is triangular with only the base being devoid of several finger-like projections. *Bullia pura* is commonly found in the 10 mm to 35 mm size range. The shell may be coloured cream, brown, yellow or any combination. The shell shape is very similar to *B. digitalis* except for the shell of *B. pura* being sculpted with fine spiral grooves. *Bullia rhodostoma* has an ellipsoidal conical shell which is distinctive in shape from the other two species, this distinction is noticeable even from standing height. This facilitates collection. The shell is smooth and may be coloured cream or brown. The operculum is curved and smooth in outline. *Bullia rhodostoma* has the lowest specific gravity of the three species and tends to surf higher up the shore. More commonly, longshore segregation will be apparent, particularly between *Bullia rhodostoma* and *Bullia digitalis*. *Bullia pura* are commonly found with *Bullia digitalis* and show no tendency to segregation from *Bullia digitalis*.

On Eastern Cape beaches it has been estimated that the genera *Bullia* and *Donax* account for the vast majority of the biomass found on sandy beaches (McLachlan *et al*. 1980). In the Western Cape one can assume that broadly similar biomass figures apply. Assessment by repeated inspection at the collection sites in the Western Cape have tended to support this assumption.

A parasitological study of both *Donax* and *Bullia* would account for the majority of helminth parasites on Western Cape sandy beach ecosystems. The study of *Bullia* was undertaken as a step towards realizing this goal.

Bullia was chosen in preference to *Donax* because there is much more local knowledge of the distribution of populations of *Bullia* and collection of large numbers of *Bullia* is easier and more consistent than would be the case for *Donax*.

The present study aims to ascertain the types and levels of digenean trematode parasites in *Bullia* around the Western Cape. Morphometric studies will, it is hoped, shed light on sexual variation between whelks and parasite-induced aberrations of shell growth. It is hoped also that morphometric studies may be able to determine the significance of environmental factors such as wave action and water temperature on shell growth and proportions. Male whelk gamete production is examined to ascertain the effect of parasitism on secondary sexual features and on reproductive capacity. The shell colour varieties are examined to try to determine whether shell colour is governed by environmental or genetic factors.

1.2 THE STUDY AREA

Eight collection sites were chosen. Ou Skip, Melkbos, Hout Bay east and west, and Noordhoek are on the western coast of the Cape Peninsula. They have a water temperature which is 7 - 9°C lower than the collection sites at Fish Hoek, Muizenberg and Mnandi, all of which are in False Bay. The higher temperature in False Bay is primarily due to the limited depth and great area of the bay. It is warmed by the sun. The western side of the Peninsula is subject to cold upwelling during summer, driven by the south-easterly wind. The False Bay side may be periodically influenced by the warm Aghulas Current. The factors of ocean currents influencing the difference in temperature are considered to be of minor importance (Brown, pers. comm.).

The beaches may be graded from least to most exposure to waves in the following order (Brown 1973):

Hout Bay west
Fish Hoek
Hout Bay east
Muizenberg
Mnandi
Melkbos
Ou Skip
Noordhoek.

Beach slope closely follows exposure to wave action with the most exposed beaches having the greatest slope. A notable exception is Ou Skip which has a relatively gentle slope at most times of the year, the slope approximating to that of Fish Hoek beach. Sand-grain size changes drastically during the year and according to the wave action and weather Melkbos and, to a lesser extent Ou Skip and Hout Bay east, have pebbles from 1 - 4 cm as one approaches the low water mark. All other beaches tend to have sand only. Mnandi beach sand is consistently coarse throughout the year. At other beaches it is variable but never quite so coarse.

All collections were made at the upper limit of the surf zone at low tide.

Wind data were sought but found to be of little value. The mass of Table Mountain and other mountains on the Peninsula deflects and shields some of the beaches from the effects of wind from some angles. Centralized data from D.F. Malan Airport would be misleading in many cases.

See Figs 1,2 - 1,7 for more details of beach morphology. On each of the Figs 1,3 to 1,7 True North runs perpendicular to the scale axis.

TABLE 1.1 Showing latitude, longitude and map sheet index of collection site beaches

SITE	LATITUDE	LONGITUDE	SHEET INDEX
Ou Skip	33°41'20"S	18°26'10"E	3318 CB
Melkbos	33°42'30"S	18°26'40"E	3318 CB
Noordhoek	34°06'00"S	18°21'10"E	3418 AB/AD
Hout Bay E	34°02'30"S	18°21'35"E	3418 AB/AD
Hout Bay W	34°02'30"S	18°20'45"E	3418 AB/AD
Fish Hoek	34°08'25"S	18°26'00"E	3418 AB/AD
Mnandi	34°04'40"S	18°37'20"E	3418 BA
Muizenberg	34°06'20"S	18°29'20"E	3418 AB/AD

All maps are South Africa 1:50 000 Sheet

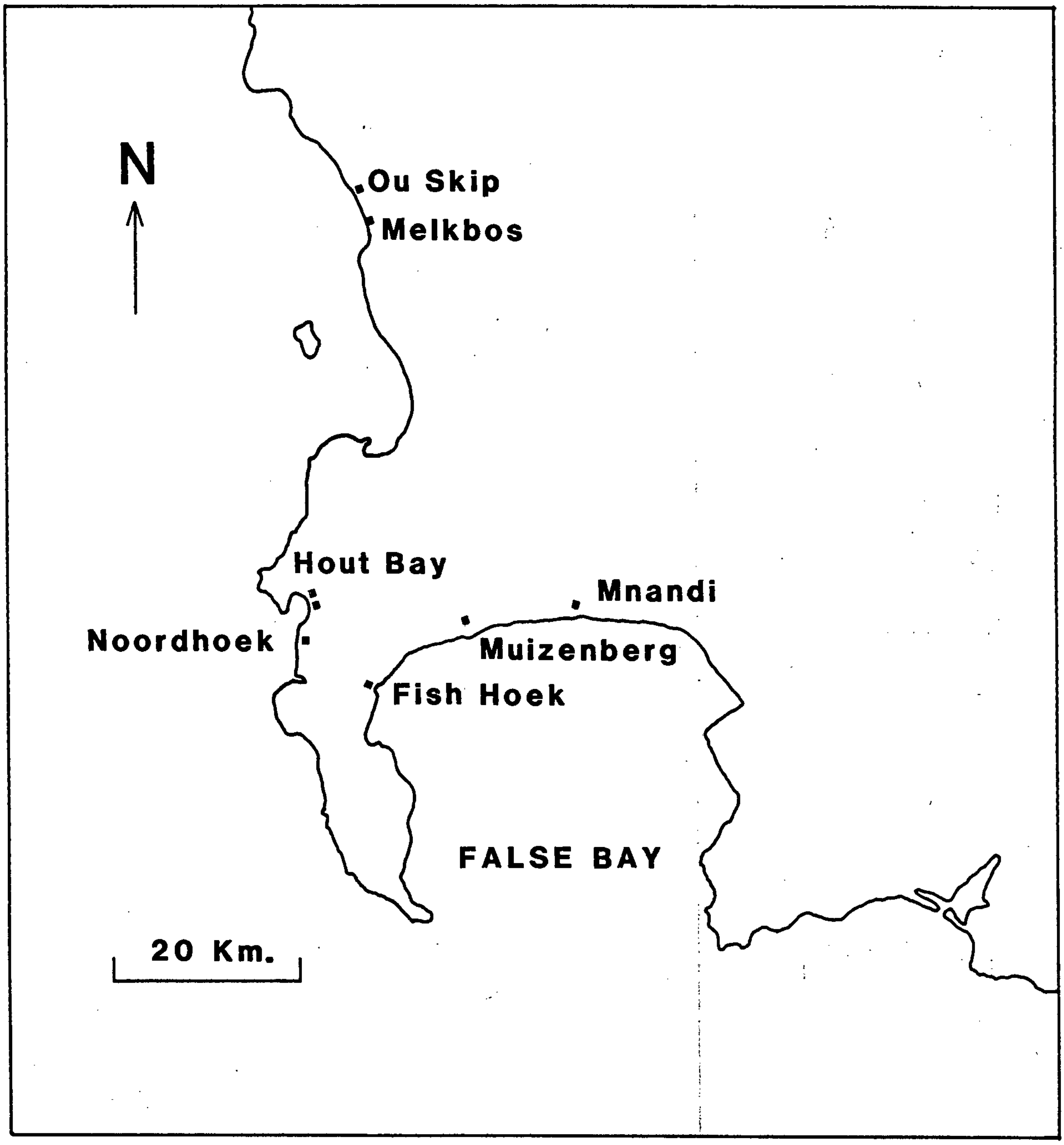


Fig. 1,1. Map of Cape Peninsula area showing collection sites.

Fig. 1,2. Collection sites at Melkbos and Ou Skip.

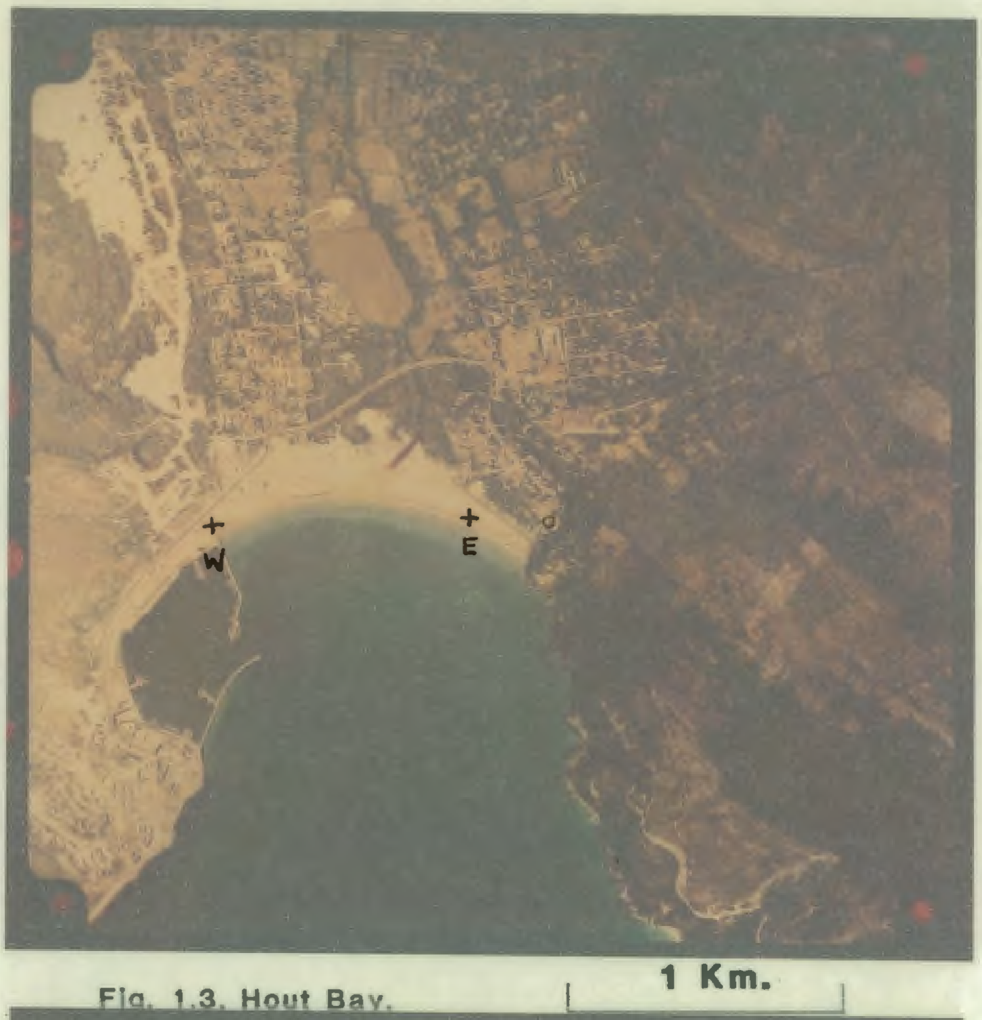


Fig. 1.3. Hout Bay.

Fig. 1,5. Fish Hoek.

Fig. 1,6. Muizenberg

Fig. 1,7. Mnandi.

CHAPTER 2

PARASITES OF BULLIA

2.1 INTRODUCTION

Major descriptive publications concerning the helminth parasites of Bullia relate only to the Indian species B. melanoides. Anantaraman (1963) gave a description and an estimation of prevalence of larval cestodes of Echinobothrium sp. (Beneden 1849) (Order Diphyllidea). Reimer (1975) described larval cestodes of Echinobothrium lateroporum (Subhapradha 1948), Polypocephalus sp. (Braun 1878) (Order Lecanicephalidea) and Christianella sp. (Guiant 1931) (Order Trypanorhyncha). In 1976 Reimer described a digenean trematode metacercaria Gonorchella sp. nov. (Family Halipegidae (Poche 1925)).

In the South African species Bullia digitalis, Brown (1971) has noted the presence of larval digenea in whelks from Western Cape beaches.

2.2 MATERIALS AND METHODS

Collections of Bullia took place at mid-rising tide from the upper edge of the surf zone. Fish bait was used in all collections after February 1984. A piece of skate or sunfish was dragged through the surf on a piece of string.

Thousands of Bullia would emerge from the sand and try to surf to the bait. Large numbers of Bullia could be collected with ease. Prior to February 1984, Bullia were collected without bait. This is a much longer process and it would sometimes take several hours to collect forty specimens. The whelks were kept in buckets of sea water in a temperature-controlled aquarium at about 15°C. The water was aerated and a steady through-flow of water was provided. Each bucket was provided with a layer of sand for the whelks to burrow into.

Whelks were examined as soon as possible after collection. In the laboratory the shell height, body whorl height and aperture height were measured (see Fig. 2.1) to the nearest 0,05 mm with vernier callipers. Shell colour was noted in the case of Bullia digitalis. The upper surface of the shell and extended foot was examined by transmitted and reflected light at high and low power with a dissection microscope. The whelk was induced to retract its foot. The shell was examined aperture uppermost, paying particular attention to the operculum. The water squirted from the mantle cavity was microscopically examined. Whelks were broken open with pliers and the exposed body was examined to determine its sex. This was achieved by noting an elongate penis in males and either a rudimentary short penis or a conspicuous orifice in females. In doubtful cases a gonad smear was examined at high power (1000x) to look for the presence of sperm or eggs. This sometimes helped to confirm or deny the indications from the secondary sexual characteristics. The size of the penis in males was noted. The internal organs of the whelk were examined for signs of parasitism.

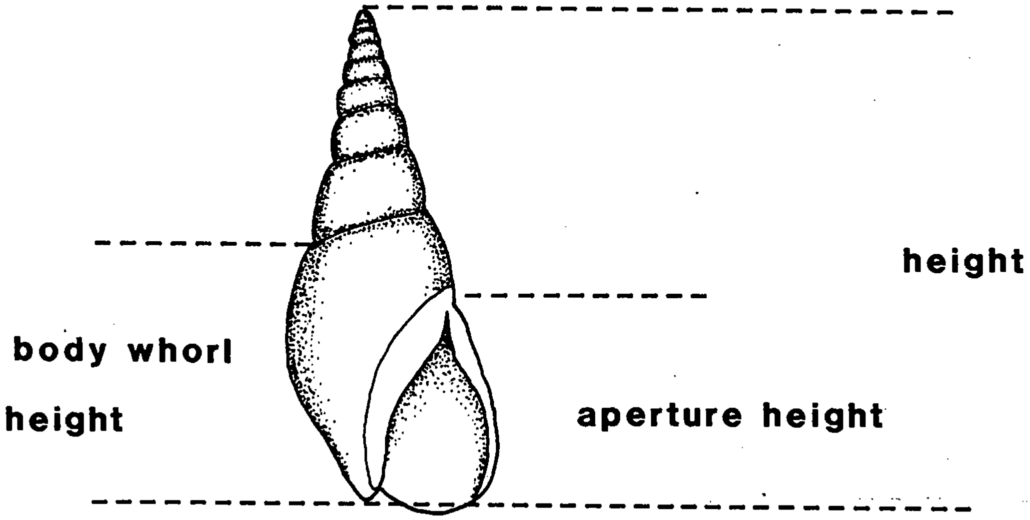


Fig. 2,1. Bullia shell measurements.

In some cases gonad colour was noted. The whelk was then progressively disrupted with fine forceps and examined under a dissecting microscope. Particular attention was paid to the foot musculature, alimentary tract and digestive gland complex. Each examination was completed by further agitation of the whelk in sea water and increasing the magnification of the microscope. Parasitism was determined by movement or by the presence of unusual structures in the whelk. Doubtful specimens were examined using a compound microscope.

For each type of parasite, attempts were made to induce them to leave the host spontaneously, so that mature emerged stages might be examined before cracking open the whelks. Storing whelks in petri dishes of sea water in different regimes of light, temperature and nutritional status was attempted. This will be related later.

Drawings were made of living parasites. It was felt that the living specimen is least liable to be affected by artefacts. Measurement of parasites was taken in living, heat-killed and 10% formalin in sea water killed specimens. Slides of living specimens were examined sixty to ninety minutes after initial examination because cercarial features sometimes become more clearly defined just before death.

The heat-killed specimens were killed by touching the slide on a 60W light bulb for a few seconds until the slide felt hot to touch. This was sufficient to kill the cercariae but not enough to damage them.

Cold formalin was used in preference to hot. Cold formalized specimens were found to be more consistent in shape. Hot formalin quite often fixed cercariae in contorted and unnatural shapes. Hot formalin in sea water is unpleasant and hazardous to use unless one has access to a fume cupboard.

Acetic orcein, bromocresol blue, nile blue sulphate, safranin, eosin, haematoxylin (Ehrlich's) and neutral red were used as intravital stains. In some cases parasites did not live long after administering some of these stains. Reactions to some stains were noted. Sections of parasitized host tissue were cut at 10 u and stained with Ehrlich's haematoxylin and eosin. All colour photographs of digestive gland tissue were of haematoxylin and eosin stained sections.

Photographs were taken using a Zeiss photomicroscope. Black and white pictures were taken on Ilford technical panchromatic film ASA 135/125 22 DIN. Colour photographs were taken using Fujicolor HR 100 colour film ASA 100, 21 DIN.

When attempting to identify parasites, it seemed that there were never enough illustrations in the literature for comparison.

Photomicrographs are limited in the recording of fine detail due to the small depth of focus that is available. In this study, a policy of one photograph to show one detail has been predominant. In a few cases, pairs of similar photographs have been included. Their inclusion is intended to show a slightly different aspect or to clarify a previous illustration.

Measurement of Parasites

Where possible, samples of ten measurements were taken of each feature. The results are tabulated giving descriptive statistics of the feature variability. These are the mean, standard deviation, standard error, maximum and minimum, all of which are expressed in micrometres (um). Coefficient of variance is given as a percentage. The symbol n indicates the sample size. In cases where n is considered too small, only significant descriptive statistics are tabulated. Features such as body and tail width were measured at three places, namely at 25%, 50% and 75% of the feature length when working from its anterior, backwards. In cases of measuring features with no specific anterior or posterior (such as some sporocysts) each specimen was measured starting arbitrarily at a point designated for this purpose. Cercarial stylet measurements indicate the maximum length, and the maximum width at the proximal end.

2.3 PRECISION OF MEASUREMENTS

Five specimens of Bullia digitalis taken from Melkbos were used. The size range of the specimens covers the range of the great majority of whelks encountered in this study. For each whelk the shell height, body whorl height and aperture height were measured. Measurements were taken from any of the whelks at random to ensure that the calliper is readjusted to the correct measurement and not merely refitted on the shell from the previous measurement. Twenty of each measurement were taken for the five shells. The results are shown below.

TABLE 2.1 Shell measurements repeated twenty times on five test specimens to ascertain precision. A: shell height; B: body whorl height; C: aperture height.

	MEAN mm	S.D. mm	S.E. mm	C.V. %	n
A.	5,61	0,08	0,019	1,5	20
	10,93	0,04	0,01	0,3	20
	18,23	0,06	0,01	0,3	20
	38,92	0,05	0,01	0,1	20
	55,36	0,11	0,02	0,2	20

- continued

Table 2.1 continued

	MEAN mm	S.D. mm	S.E. mm	C.V. %	n
B.	3,45	0,13	0,03	3,8	20
	5,98	0,09	0,02	1,5	20
	10,14	0,08	0,02	0,8	20
	22,82	0,31	0,07	1,4	20
	31,57	0,55	0,12	1,7	20
C.	2,44	0,13	0,03	5,3	20
	4,34	0,06	0,01	1,4	20
	7,23	0,13	0,03	1,8	20
	16,97	0,18	0,04	1,1	20
	26,13	0,27	0,06	1,0	20

In all cases the coefficient of variance is low, only in one instance is the C.V. higher than 5%. This only occurs in the extreme lower end of the measurement of aperture height.

2.4 LARVAL HELMINTH NOMENCLATURE

Nine species of larval digenea are described in this work. Following the example of *Cysticercus multiformis* (Hölldobler 1937) and *Sparganum proliferum* (Ijima 1905) the binomial appellation of larval stages referred to in this work shall consist of the larval stage as the generic name followed by the specific epithet. Thus terms such as *Cercaria Z* *Metacercaria V* and *Sporocyst B* will be encountered. It is hoped that by this usage the identity of the digenean larvae will be more clearly defined.

The larval stages from Z to U are found in *Bullia* and their descriptions are included in the main body of the thesis. Larval stages from T to R refer to species from the limpet *Siphonaria capensis* and the bivalve *Donax sordidus*. These species are described in the appendix.

An extensive survey of the literature failed to identify any of the larvae to species level. It is assumed that the specimens could be new species. Names taken from Greek are suggested below:

Cercaria Z	*Cercaria hastata* (adjective) Referring to the spear-like stylet.
Cercaria Y	*Cercaria bulliae* (Genitive) The cercaria of *Bullia*.

	(*Zoogonoides hapax*?)
Cercaria X	*Cercaria hapax* (adverb). Hapax means once. Found once in 3000 *Bullia*
Metacercaaria W	*Metacercaria erema* (adjective) The lonely metacercaria. It is found singly in the host.
Metacercaria V	*Metacercaria mnandiae* (Genitive) The metacercaria of Mnandi. Found only at Mnandi.
Metacercaria U	*Metacercaria chloromegala* (adjective) The green, large metacercaria.
Cercaria T	*Cercaria trinema* (noun in apposition) Trinema: three threads, referring to the filamentous tails.
Cercaria S	*Cercaria siphonariae* (Genitive) The cercaria of *Siphonaria*.
Cercaria R	*Cercaria notodonacis* (Genitive) The cercaria of southern *Donax*

CHAPTER 3

CERCARIA AND SPOROCYST TYPE Z

(FAMILY MICROPHALLIDAE TRAVASSOS, 1921)

HOST: BULLIA DIGITALIS

3.1 SPOROCYST

No mother sporocyst was located in any whelk. Infected whelks contained masses of daughter sporocysts in the digestive gland and gonad. In many cases sporocysts were found throughout the viscera.

Diagnosis

Circular cross-section but very variable in length. The sporocyst may be highly convoluted (see Figs 3.1.C, 3.3 and 3.4). The tegument is non-spinous and has no birth pore. All cercariae in any given sporocyst are always at the same stage of development. One end of the sporocyst usually has six to twelve deeply staining germ balls (stained with neutral red) (see Figs 3.1.A and 3.4). The germ balls are not found elsewhere in the sporocyst. The sporocyst wall is thin and lacking in musculature. No movement of the sporocyst was noted. Intravital staining with Erlich's haematoxylin and aceto-orcein rendered the sporocyst wall nuclei visible as dark staining spots (see Fig. 3.1.A).

Germinal masses were found outside of the mature sporocysts; these are

TABLE 3.1 Sporocyst Z measurements. A: living; B: heat-killed; C: Formalin-killed

	Mean	Standard Deviation	Standard Error	Coefficient of Variance	N	Maximum	Minimum
A							
Length	372,0	35,0	11,2	9,5	10	405	308
25%	123,0	28,0	9,0	23,0	10	162	73
Width 50%	114,0	24,0	7,6	21,1	10	146	81
75%	130,0	24,0	7,5	18,4	10	162	81
No. of cercariae	8,7	3,6	1,1	41,6	10	13	1
diameter of germ balls	19,0	3,2	1,0	16,6	10	22,5	15
B							
Length	360,0	62,0	19,6	17,2	10	446	243
25%	140,0	29,0	9,2	20,7	10	186	97
Width 50%	150,0	30,0	9,5	20,1	10	203	97
75%	141,0	29,0	9,3	20,9	10	203	97
No. of cercariae	7,5	2,8	0,9	37,3	10	13	4
C							
Length	365,0	55,0	17,4	15,2	10	467	316
25%	147,0	35,0	11,0	23,8	10	227	113
Width 50%	163,0	26,0	8,1	15,7	10	203	130
75%	150,0	30,0	9,4	19,9	10	203	113
No. of cercariae	8,4	2,6	0,8	31,4	10	12	5

probably immature sporocysts (see Fig. 3.1.B). It was not possible to determine the origin of the immature sporocysts. No details of the sporocyst excretory system could be ascertained at any stage.
In A, B and C the number of cercariae is, of course, unrelated to fixation or killing method.

3.2 CERCARIA

Diagnosis

Dorsoventrally flattened, tailed xiphidio cercaria. The broadest part of the body is about 45% from the front. The tegument is finely spinous. The length, and pitch between rows of spines is about 0,7 u. Bromocresol blue stains the spines when it is used intravitally. The oral sucker is modified and much reduced to a muscle system for control of the stylet. The oral sucker musculature is rendered visible by aceto orcein stain. The mouth is sub-terminal. No oral papillae can be seen with light microscopy. No prepharynx, pharynx or oesophagus is visible at any magnification. There is no ventral sucker. Densely staining clumps of cells at mid-body may be genital primordia.

The bladder is Y-shaped and occupies the rear 35% of the body. The contents of the bladder stain violet with nile blue sulphate. The stylet is bullet-shaped with a slightly domed base; it is held obliquely. There are two pairs of penetration glands. The posterior extent of the longer pair is made visible with nile blue sulphate.

TABLE 3.2 Measurements of Cercaria Z. A: Living; B: Heat-killed; C: Formalin-killed

	Mean	Standard Deviation	Standard Error	Coefficient of Variance	N	Maximum	Minimum
A							
Length extended*	196	31,0	9,8	15,8	10	250,0	125,0
contracted*	129	21,0	6,5	16,0	10	150,0	100,0
Width 25%	42	6,0	1,9	14,3	10	50,0	35,0
50%	48	8,0	2,5	16,2	10	60,0	35,0
75%	36	5,0	1,6	14,3	10	40,0	25,0
Tail length	71	19,0	5,9	26,3	10	110,0	50,0
Tail width 25%	14	2,4	0,8	16,6	10	17,5	10,0
50%	11	1,8	0,6	15,9	10	15,0	10,0
75%	8	1,1	0,3	13,2	10	10,0	7,5
B							
Length	139,0	6,7	2,1	4,8	10	150,0	130,0
Width 25%	49,0	3,4	1,1	7,0	10	55,0	45,0
50%	48,0	5,4	1,7	11,2	10	55,0	40,0
75%	30,0	1,8	0,6	6,1	10	35,0	27,5
Tail length	67,0	16,9	5,3	25,2	10	100,0	50,0
Tail width 25%	12,0	2,3	0,7	19,1	10	15,0	10,0
50%	10,3	1,9	0,6	18,6	10	14,5	7,5
75%	7,0	1,97	0,6	28,2	10	10,0	5,0
Stylet length	17,6	2,2	0,7	12,8	10	19,5	13,7
Stylet width	4,0	0,3	0,1	8,0	10	4,9	3,9
C							
Length	127,0	14,2	4,5	11,2	10	150,0	100,0
Width 25%	46,0	6,0	1,9	13,2	10	55,0	40,0
50%	50,0	5,5	1,7	11,1	10	60,0	40,0
75%	34,0	6,0	1,9	17,6	10	42,5	25,0
Tail length	92,0	24,4	7,7	26,7	10	115,0	40,0
Tail width 25%	12,2	2,4	0,8	19,6	10	15,0	10,0
50%	10,6	1,8	0,6	16,9	10	15,0	8,75
75%	7,4	1,5	0,4	20,3	10	10,0	5,0

* Measurements were taken at full extension and contraction

The openings of the penetration glands lie lateral and posterior to the point of the stylet in two pairs (see Fig 3.2.A and B).

Bromocresol blue stains the opening of the penetration glands a very conspicuous green. The rest of the glands are unstained. Four pairs of cystogenous glands are found lying ventral to the bladder, with ducts to the ventral surface. Nile blue sulphate is markedly toxic to the cercariae, causing rapid immobilisation; the emission of large quantities of penetration gland mucus is also stimulated. Neutral red is non-toxic. Intravital staining with safranin renders the cercaria green with orange borders. Aqueous eosin is slightly toxic.

There are four pairs of flame cells. No excretory ducts were visible. The arrangement of the flame cells (see Fig. 3.2.A) suggests that the flame cell formula is:

$$2\ (\ (2) + (2)\ )\ =\ 8 \text{ flame cells}$$

The excretory system appears to be mesostomate.

Movement

This cercaria is very active; moving when the tail is lashed laterally. The tail appears to have only slight powers of contraction and extension. No cercariae are able to swim or leave the substrate. Some progress can be made by the above activity. Leech-like movement has been noted from time to time. The cercaria is almost continuously moving and rest periods are very short, in the order of a few seconds. There is no response to light intensity. The above account applies only to extracted cercariae from broken whelks. Spontaneous emission of cercariae was almost non-existent. This will be dealt with in greater detail later.

3.3 EPIDEMIOLOGY OF TYPE Z IN BULLIA DIGITALIS

Overall prevalence at each site

The infection rate was calculated for the sample populations from each site. These values may be regarded as an estimate of prevalence of infection at each site. The results are given in Table 3.3

TABLE 3.3 Infection incidence of type Z in *B. digitalis* in samples from each collection site.

SITE	Sample Size	Infection Incidence %
Ou Skip	150	29,3
Melkbos	1378	45,6
Hout Bay W	151	7,3
Hout Bay E	150	2,0
Noordhoek	150	2,0
Fish Hoek	677	2,5
Mnandi	494	0,0

Seasonal prevalence at each site

The results given for Melkbos and Fish Hoek are derived from a series of collections. The results from each collection are presented in Table 3.4, in order to demonstrate any seasonal variation.

Analysis of infection incidence in male and female B. digitalis

The individual collections from Melkbos and Ou Skip all contained a large proportion of infected whelks. The collections are split into separate sexes and the size distribution and parasitism incidence shown graphically. The large proportion of infected individuals makes any infection/size relationship more obvious (see Figs 3.18 to 3.31).

TABLE 3.4 Seasonal variation of infection incidence at: A Melkbos, B Fish Hoek.

DATE	Sample n	Infection Rate %
A		
February 1984	49	85,7
March 1984	253	5,5
April 1984	108	25,0
May 1984	124	33,9
June 1984	108	18,5
July 1984	108	61,1
August 1984	104	65,4
September 1984	108	77,8
October 1984	108	80,6
November 1984	108	58,3
February 1985	50	58,0
B		
August 1983	150	1,3
March 1984	150	6,7
June 1984	162	0,6
November 1984	150	0,7
February 1985	65	4,6

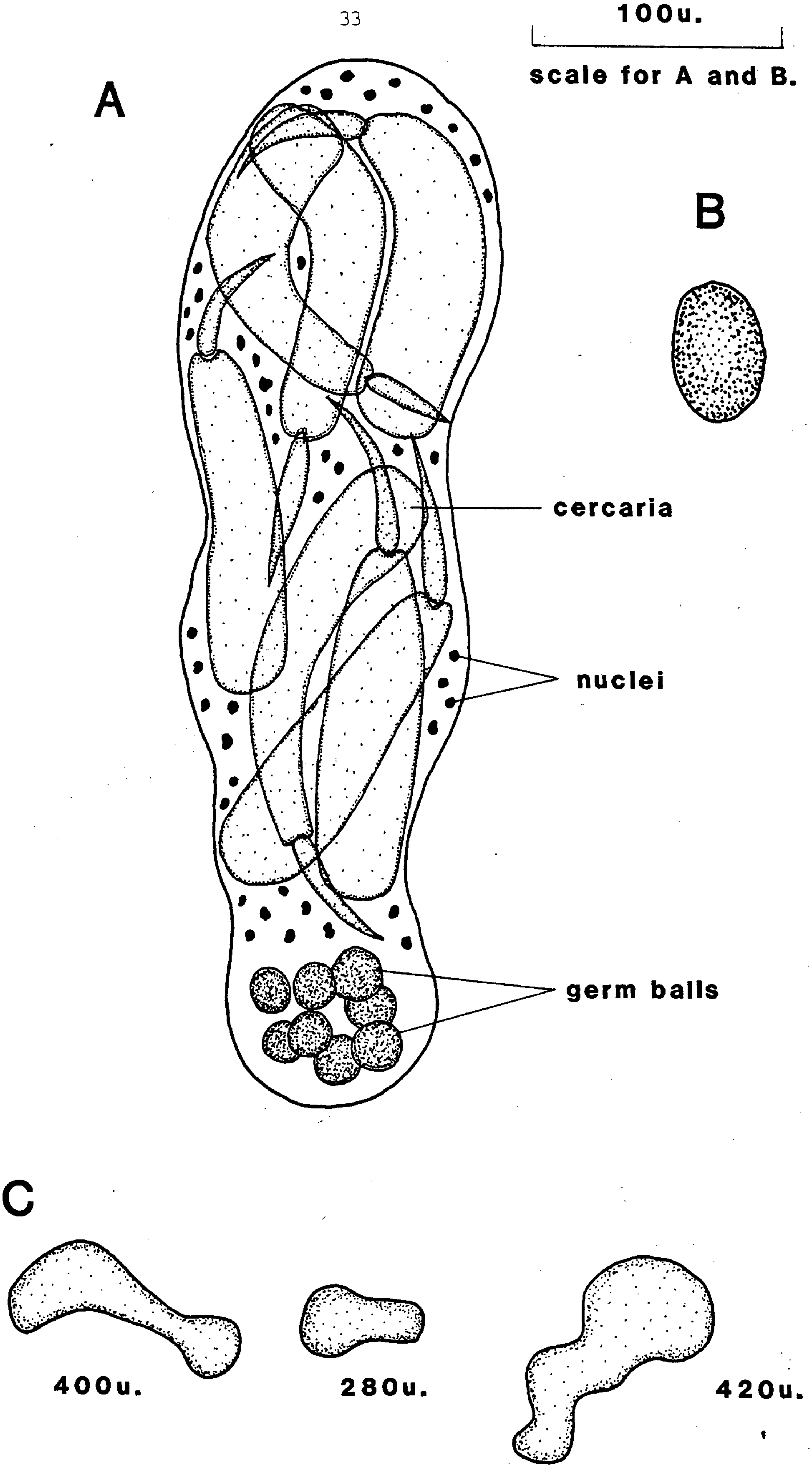


Fig. 3,1. Sporocyst Z A, mature sporocyst. B, immature sporocyst.

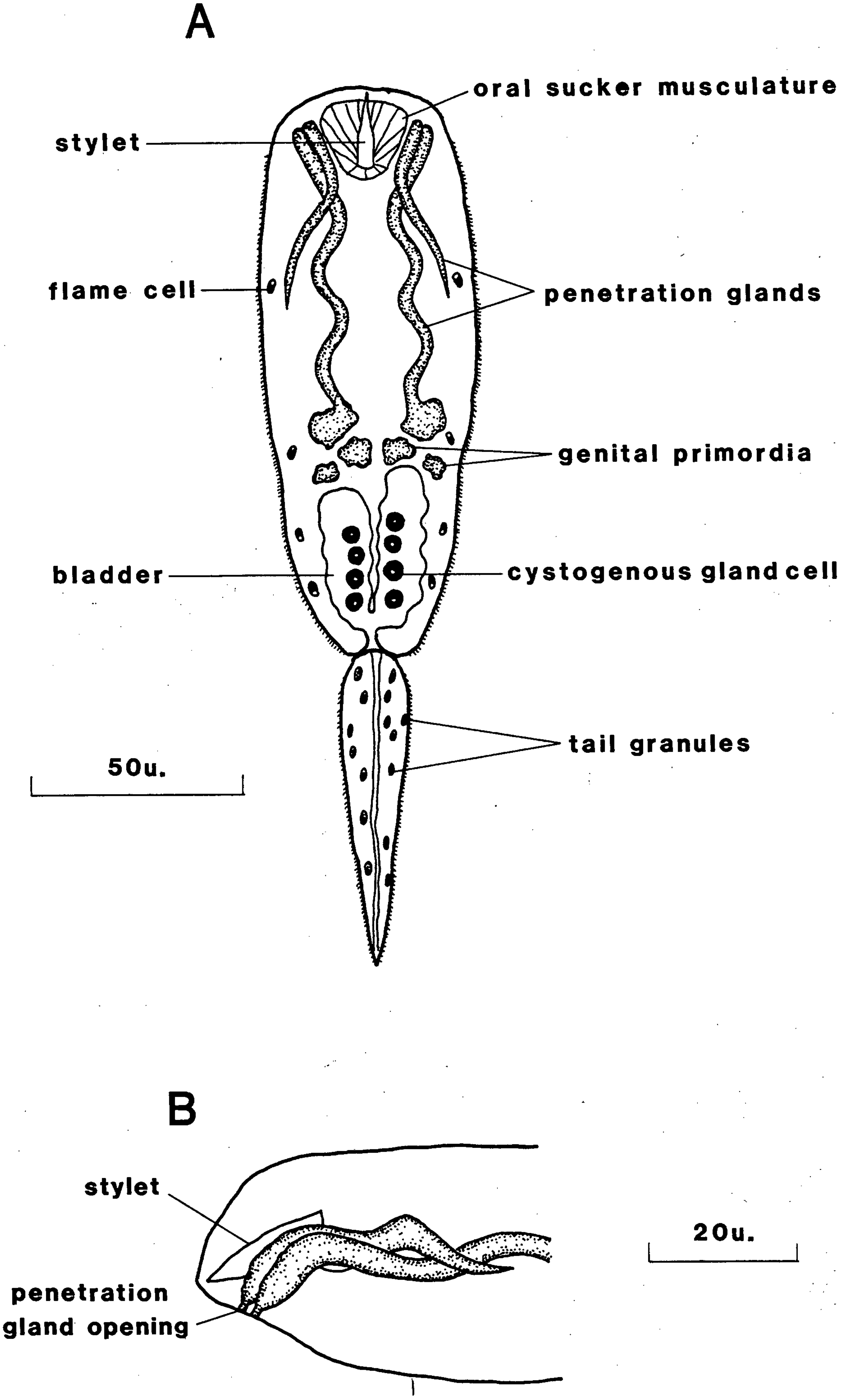
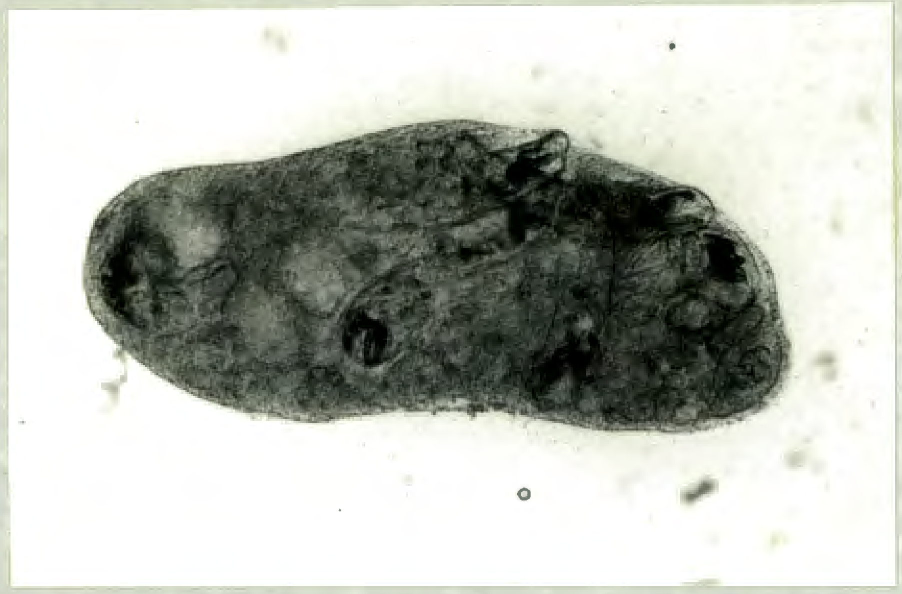


Fig. 3,2. <u>Cercaria Z</u> A, dorsal view. B, lateral view of anterior.



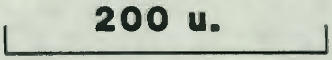


Fig 3,3. Sporocyst Z

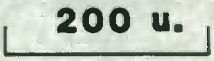
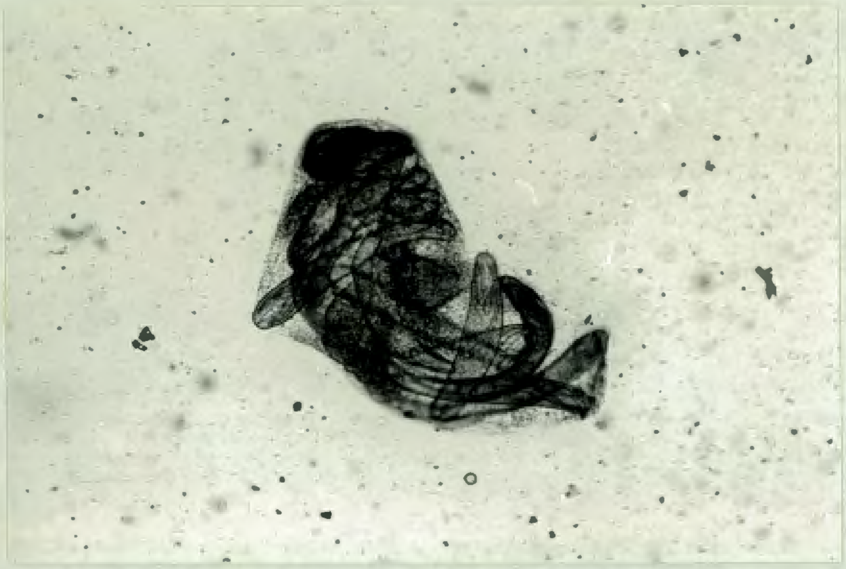


Fig 3,4. Sporocyst Z showing dark staining germ balls at the left end.



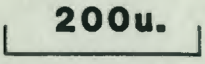


Fig. 3,5. Sporocyst Z disintegrating under assault from cercariae.

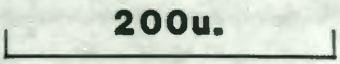


Fig. 3,6. Sporocyst Z and emerging cercariae.

Fig. 3,7. Cercaria Z after shedding its tail.

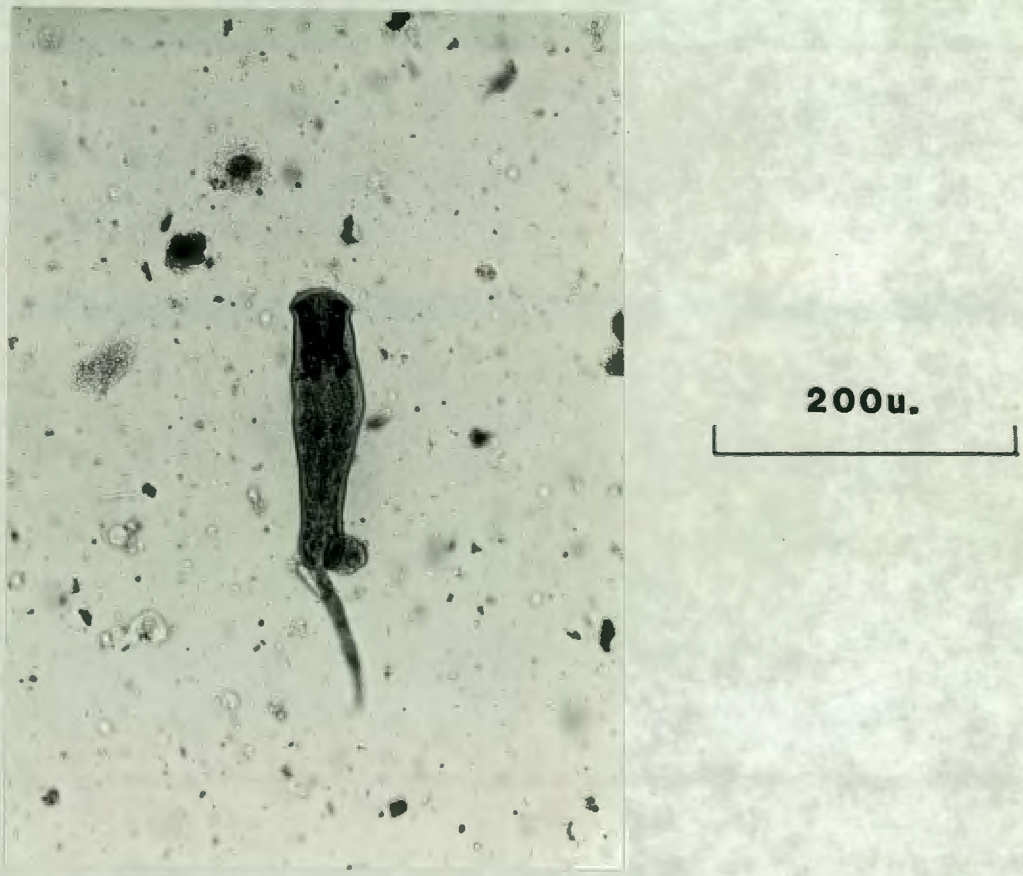
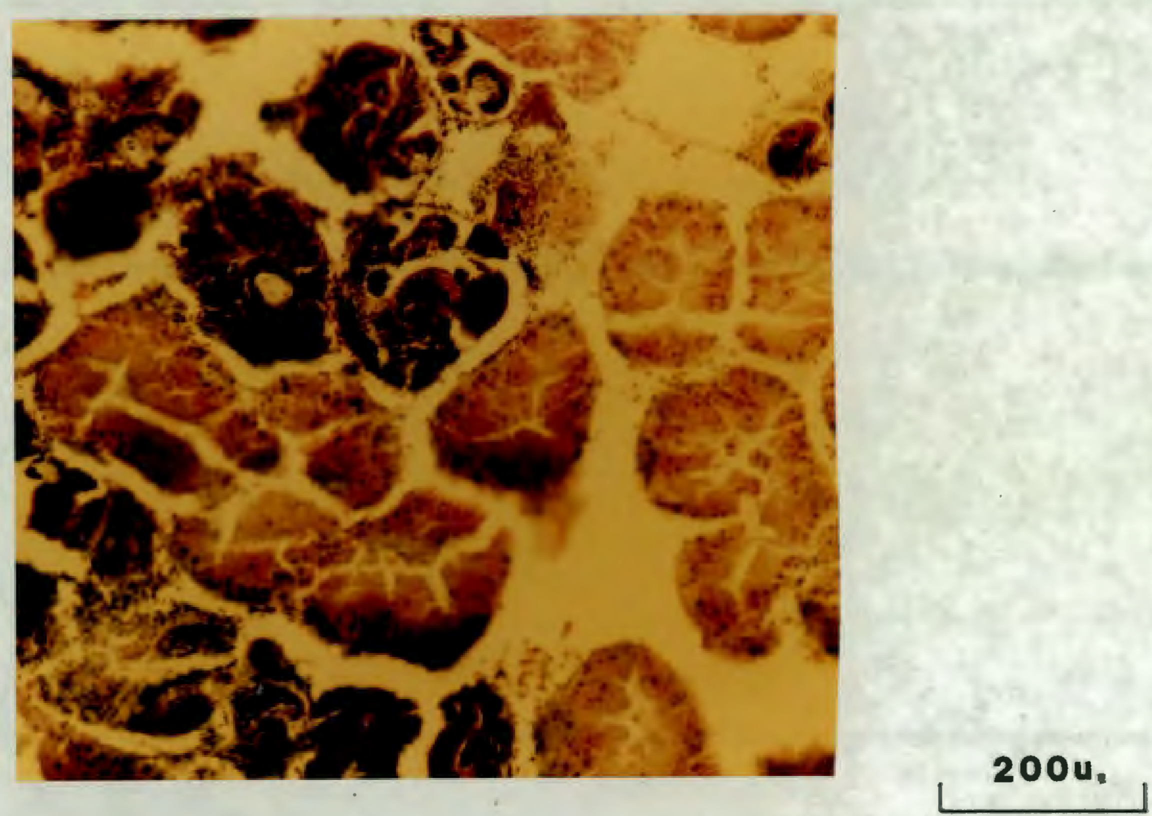


Fig. 3,9. Cercaria Z extended.

Fig. 3,10. Cercaria Z

Fig. 3,11. Cercaria Z showing rows of spines on the tail.



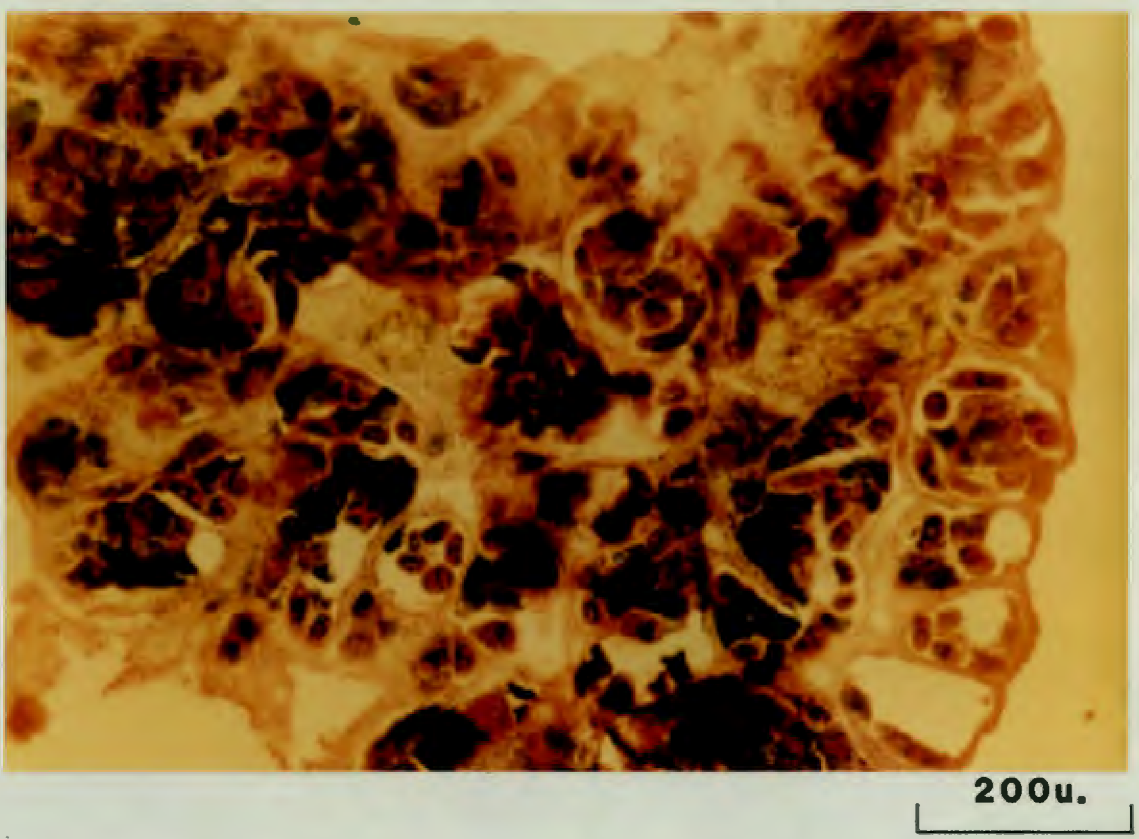


Fig. 3, 13. Digestive gland of B.digitalis infected with type Z.

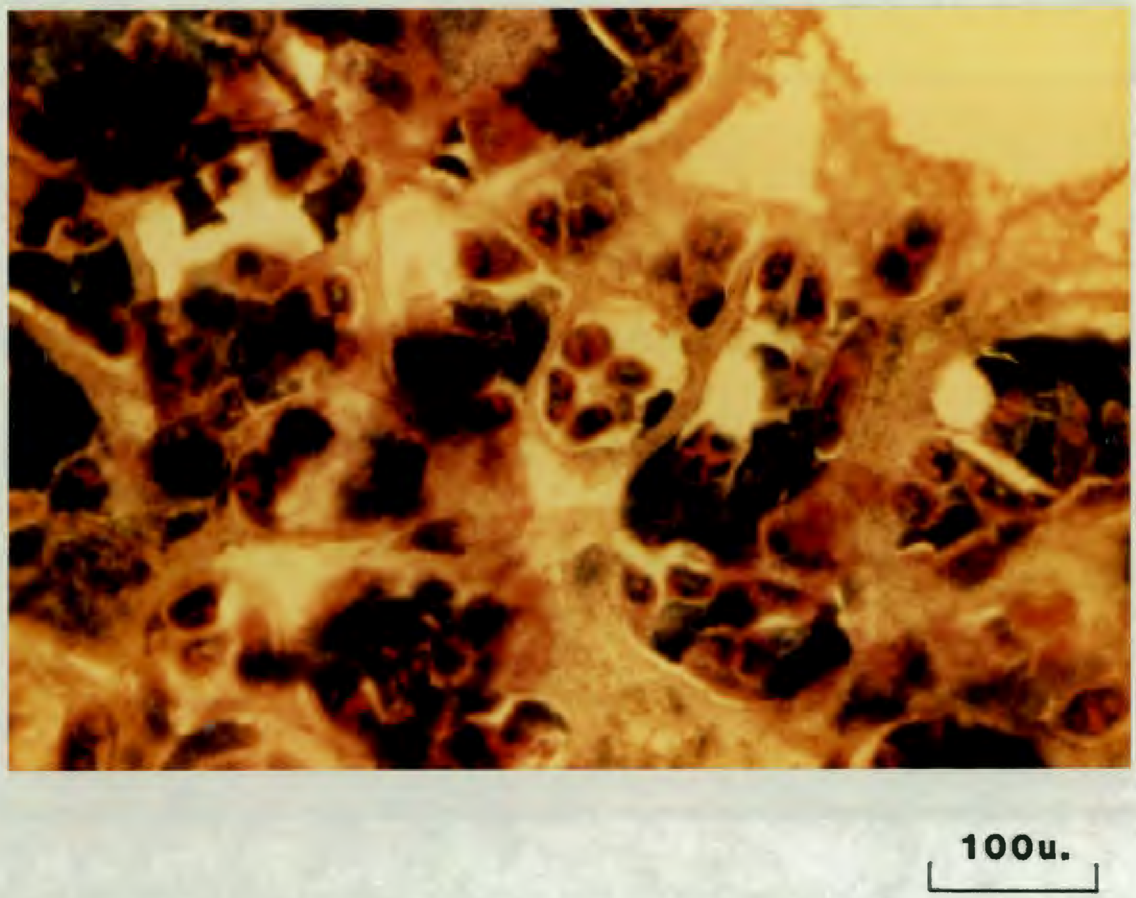


Fig. 3,14. Digestive gland of B. digitalis infected with type Z

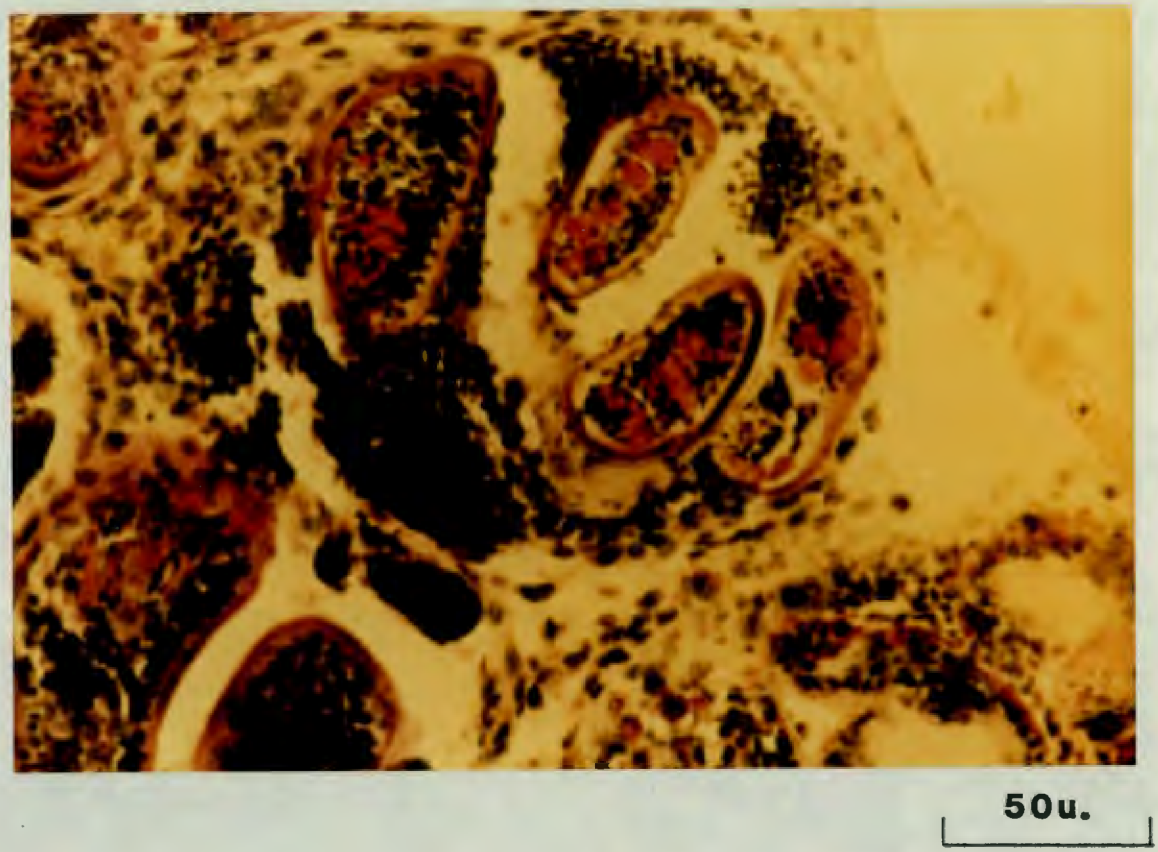


Fig. 3,15. Digestive gland of B. digitalis showing T/S. sporocyst and cercariae Z.

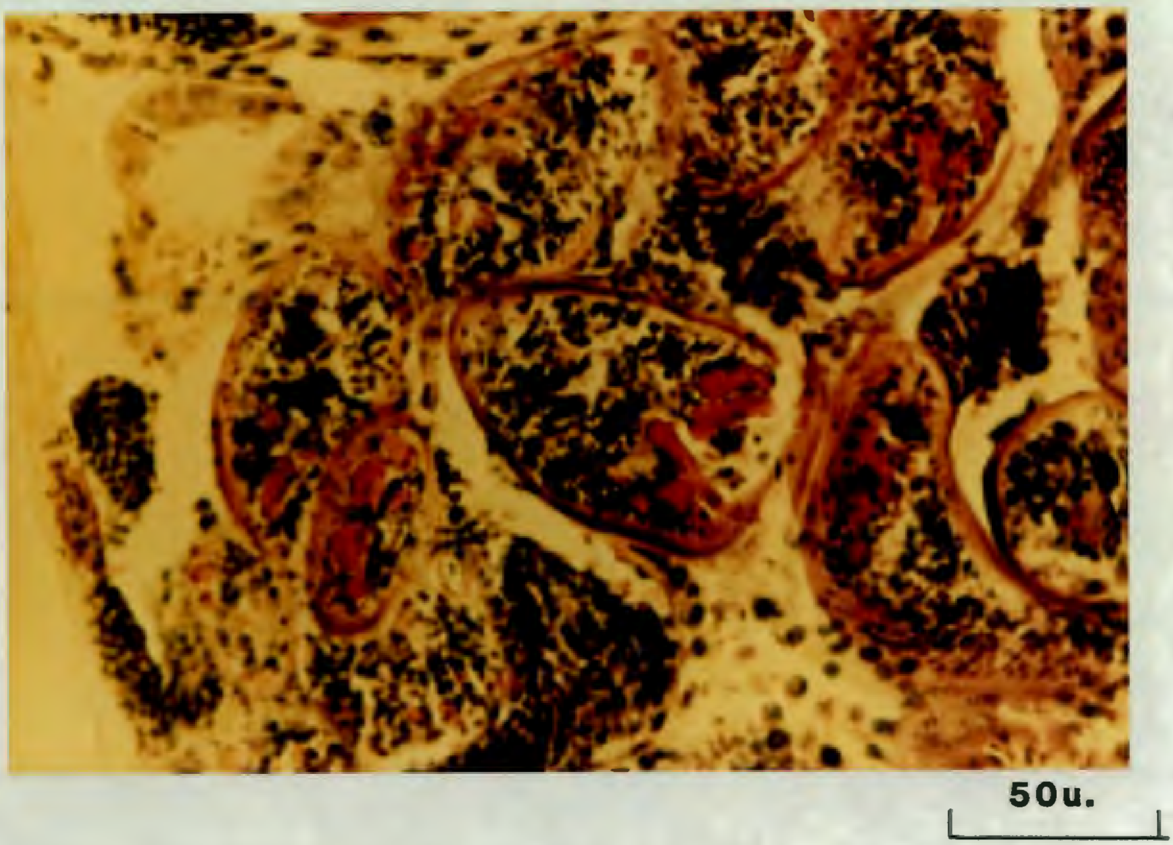


Fig. 3,16. Digestive gland of B.digitalis showing sections of cercariae

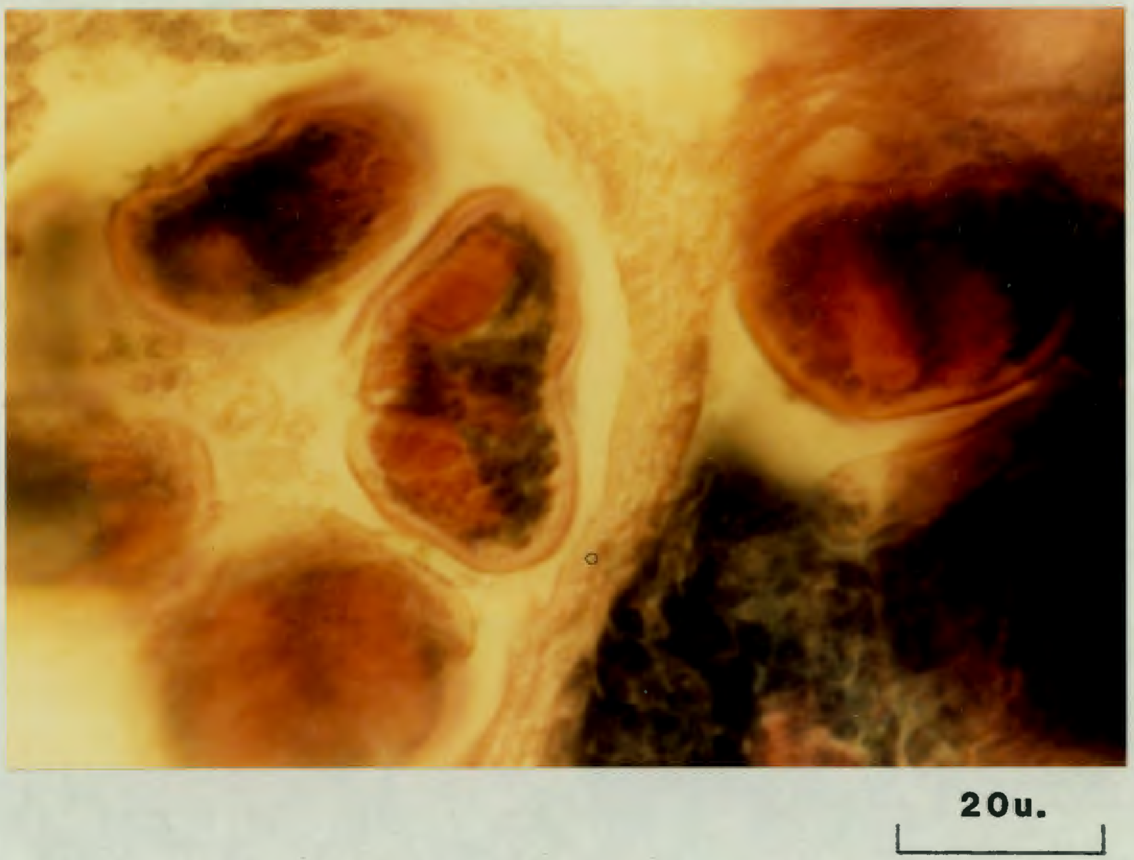


Fig. 3,17. Digestive gland of B. digitalis showing T/S. sporocysts and cercariae type Z.

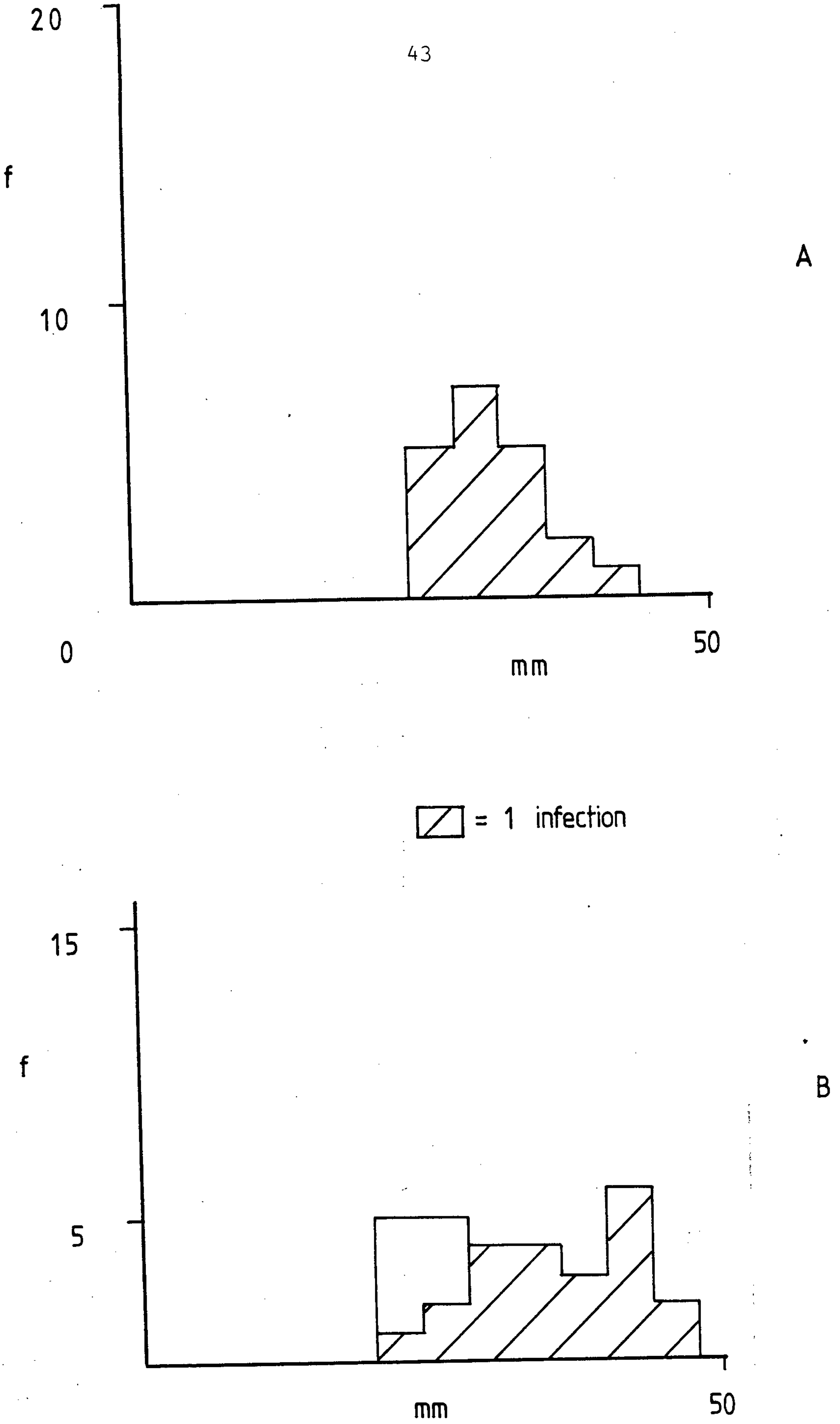
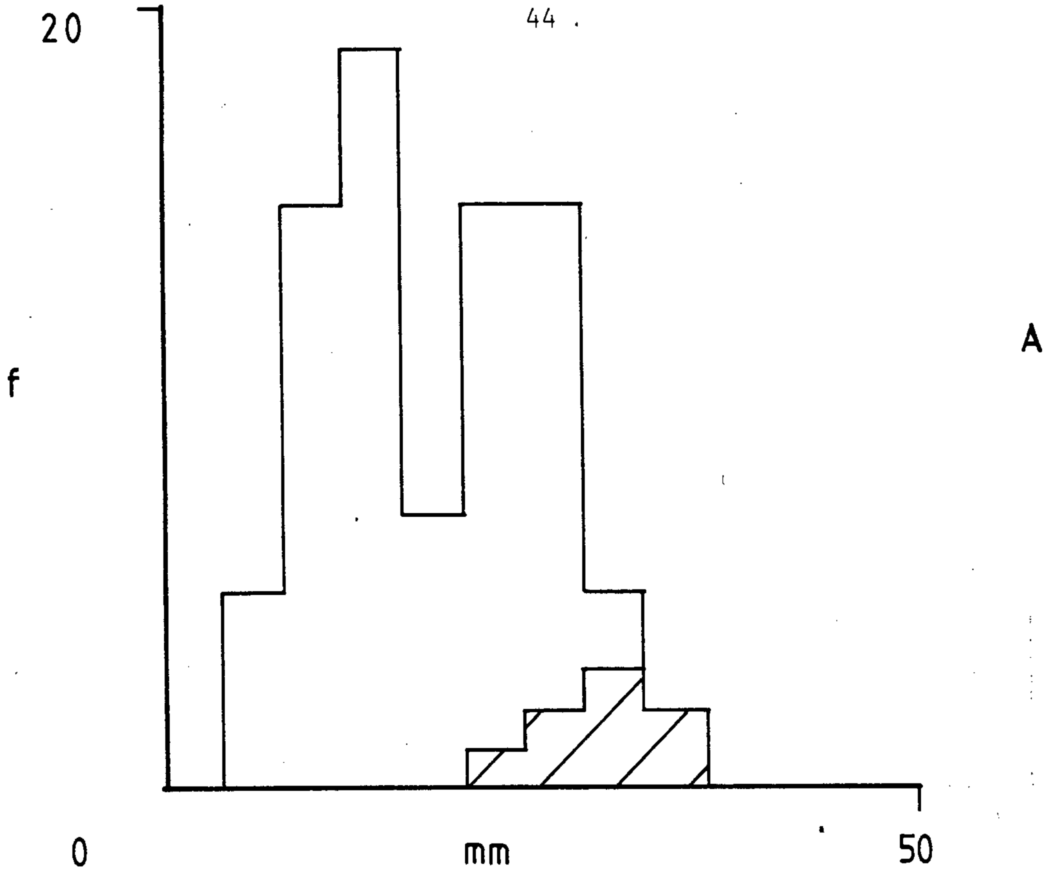


Fig. 3.18 Size and infection distribution in B. digitalis from Melkbos in February; A male, B female.



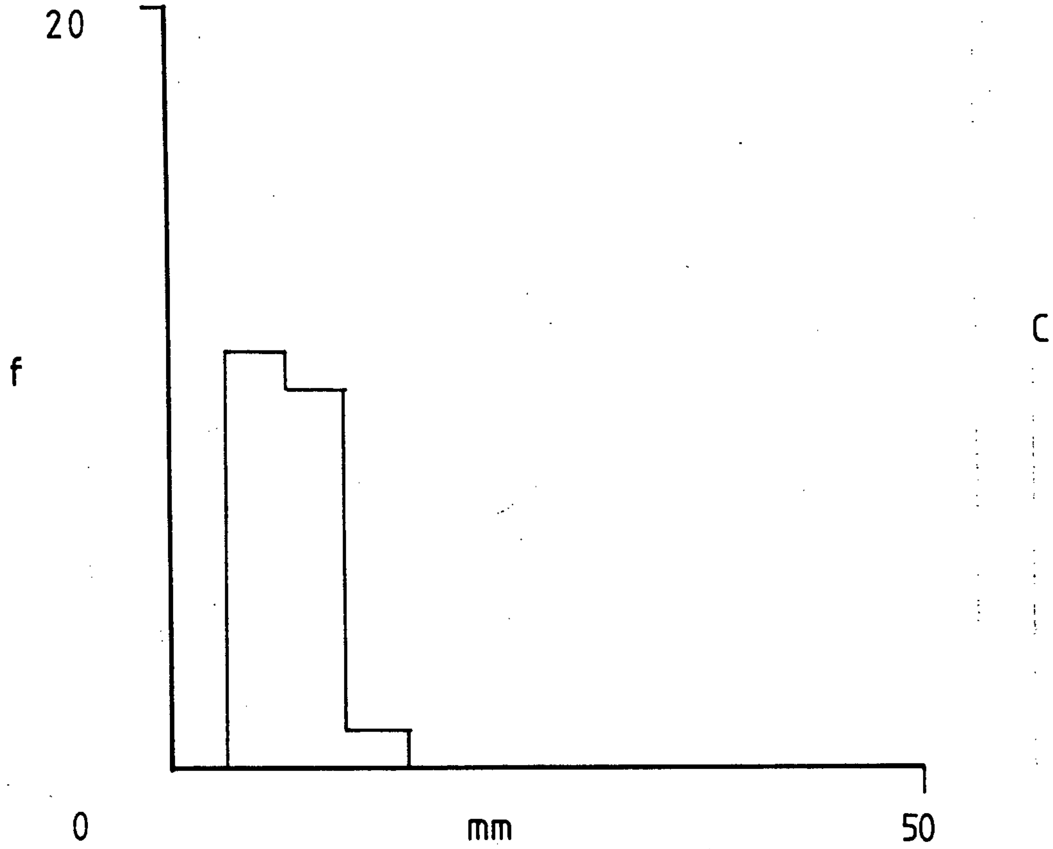


Fig. 3.19 Size and infection distribution in B. digitalis from Melkbos in March; A male, B female, C juveniles.

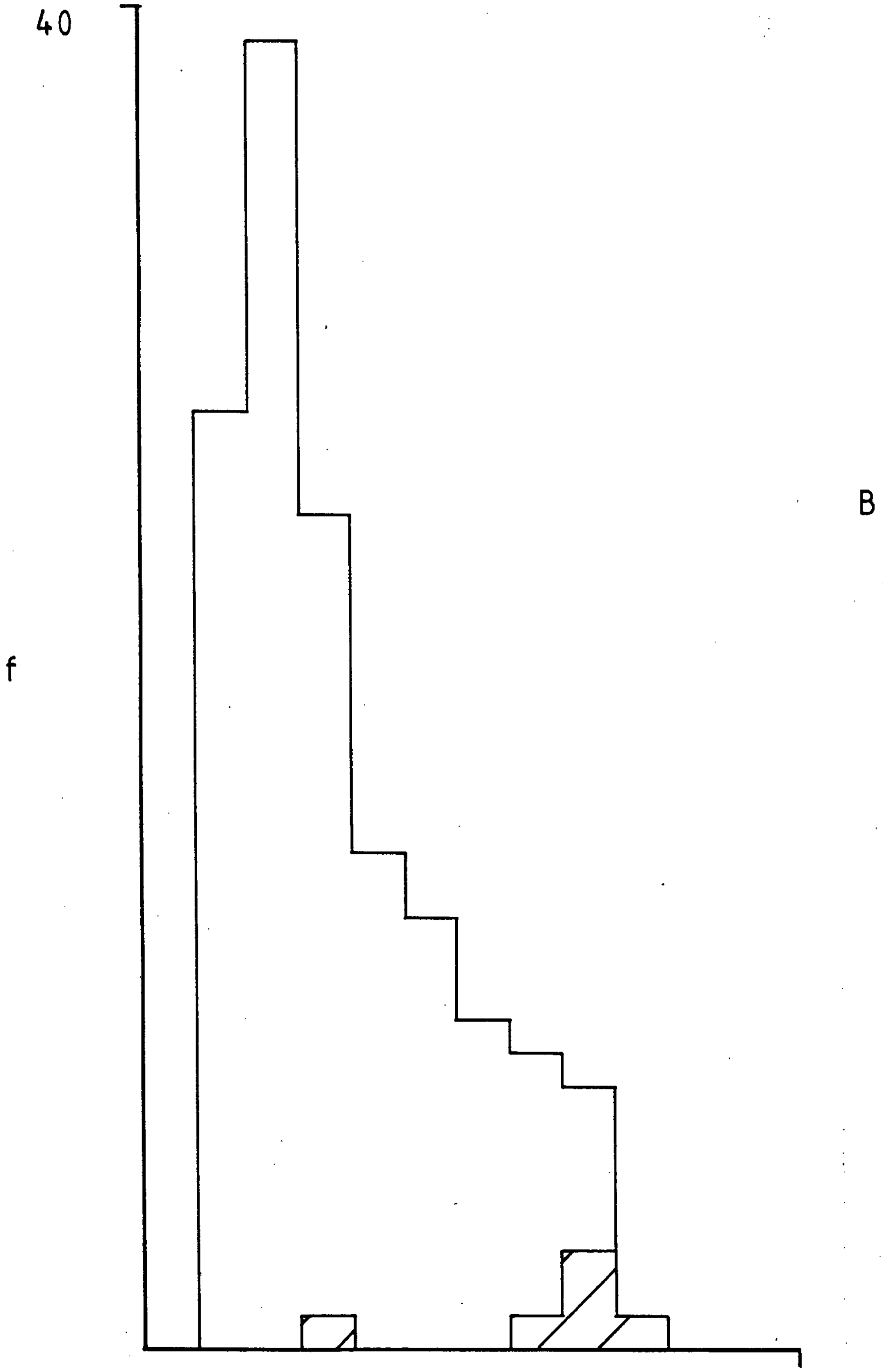


Fig. 3.19

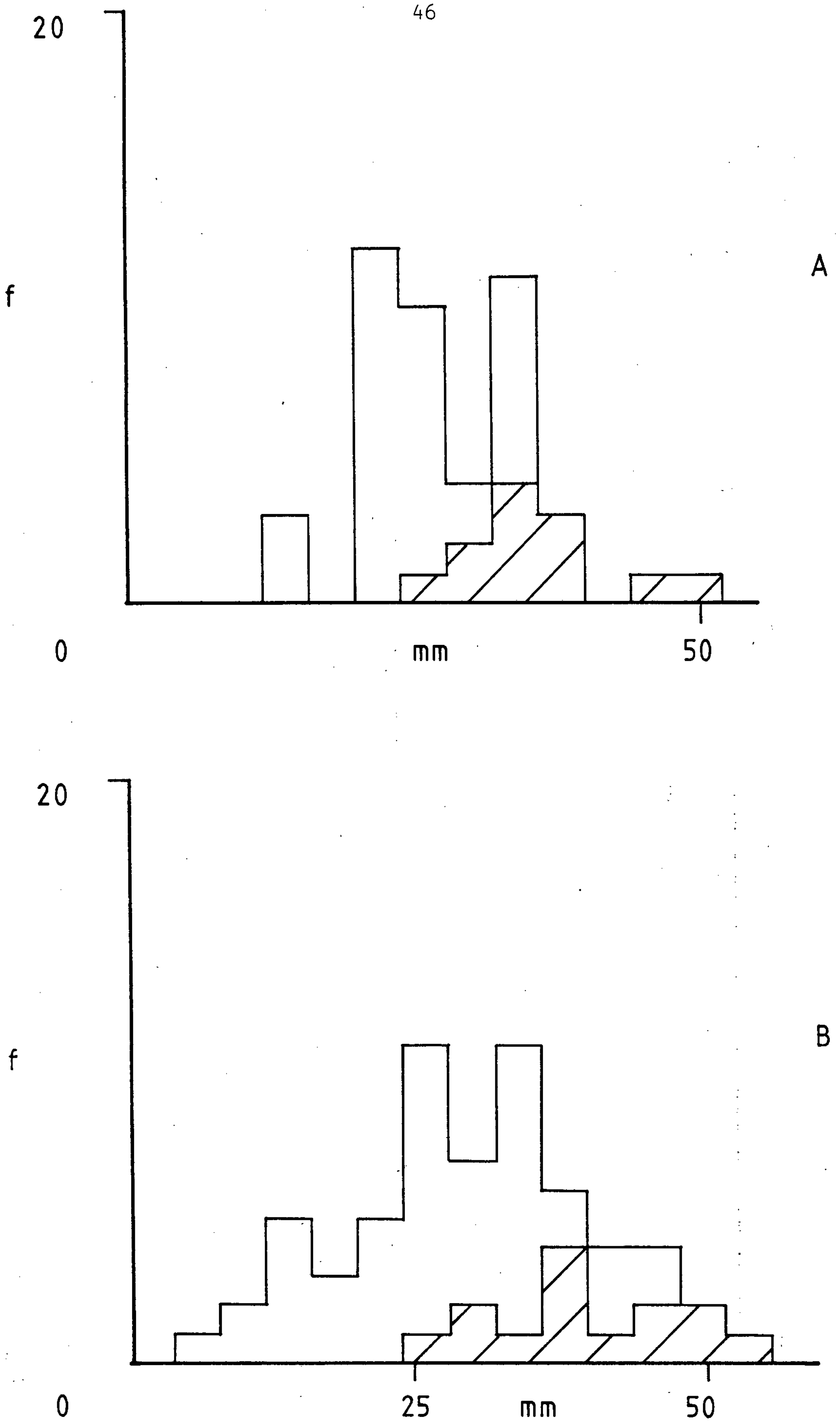


Fig. 3.20 Size and infection distribution in <u>B. digitalis</u> from Melkbos in April; A male, B female.

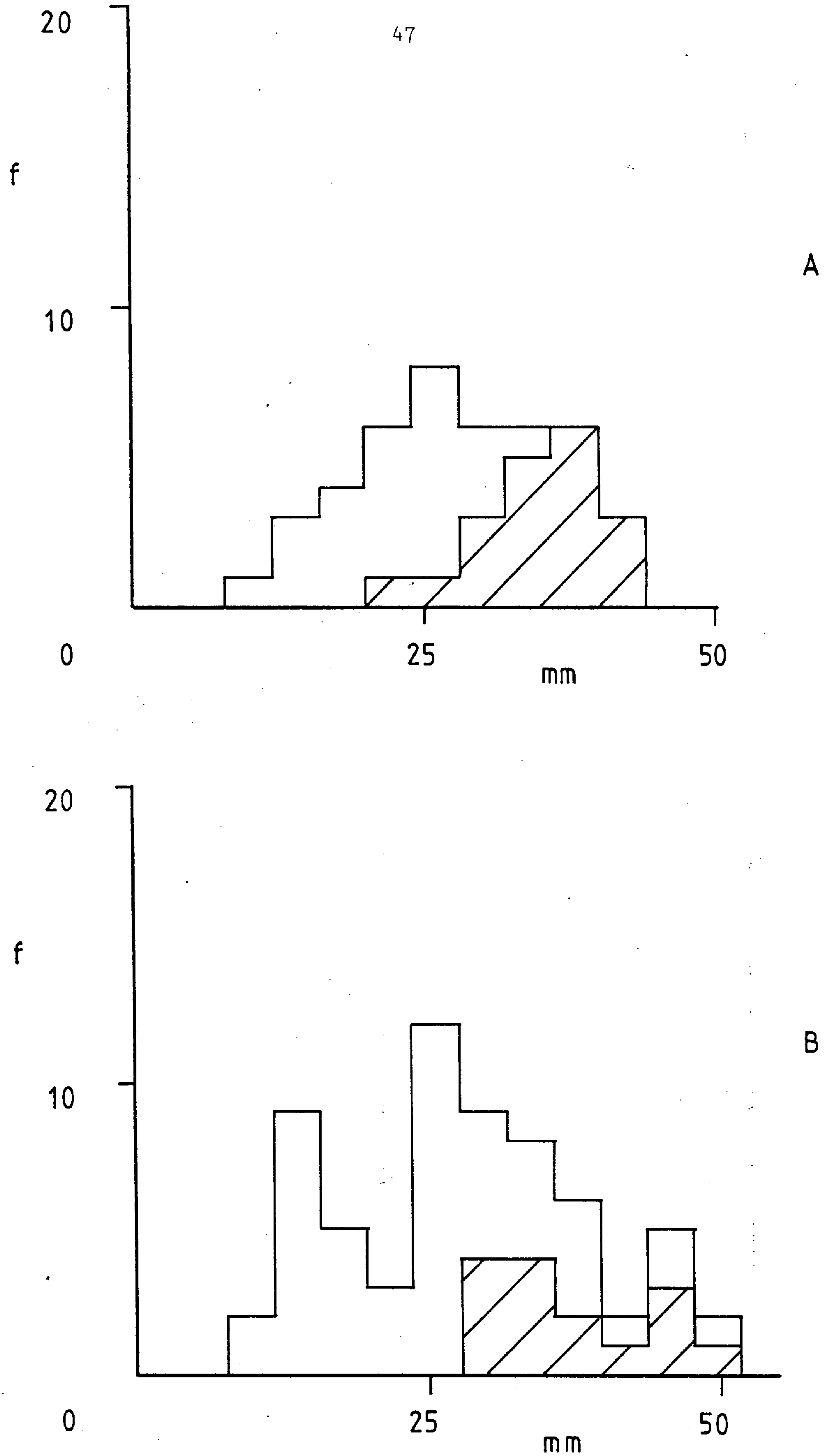


Fig. 3.21 Size and infection distribution in B. digitalis from Melkbos in May; A male, B female.

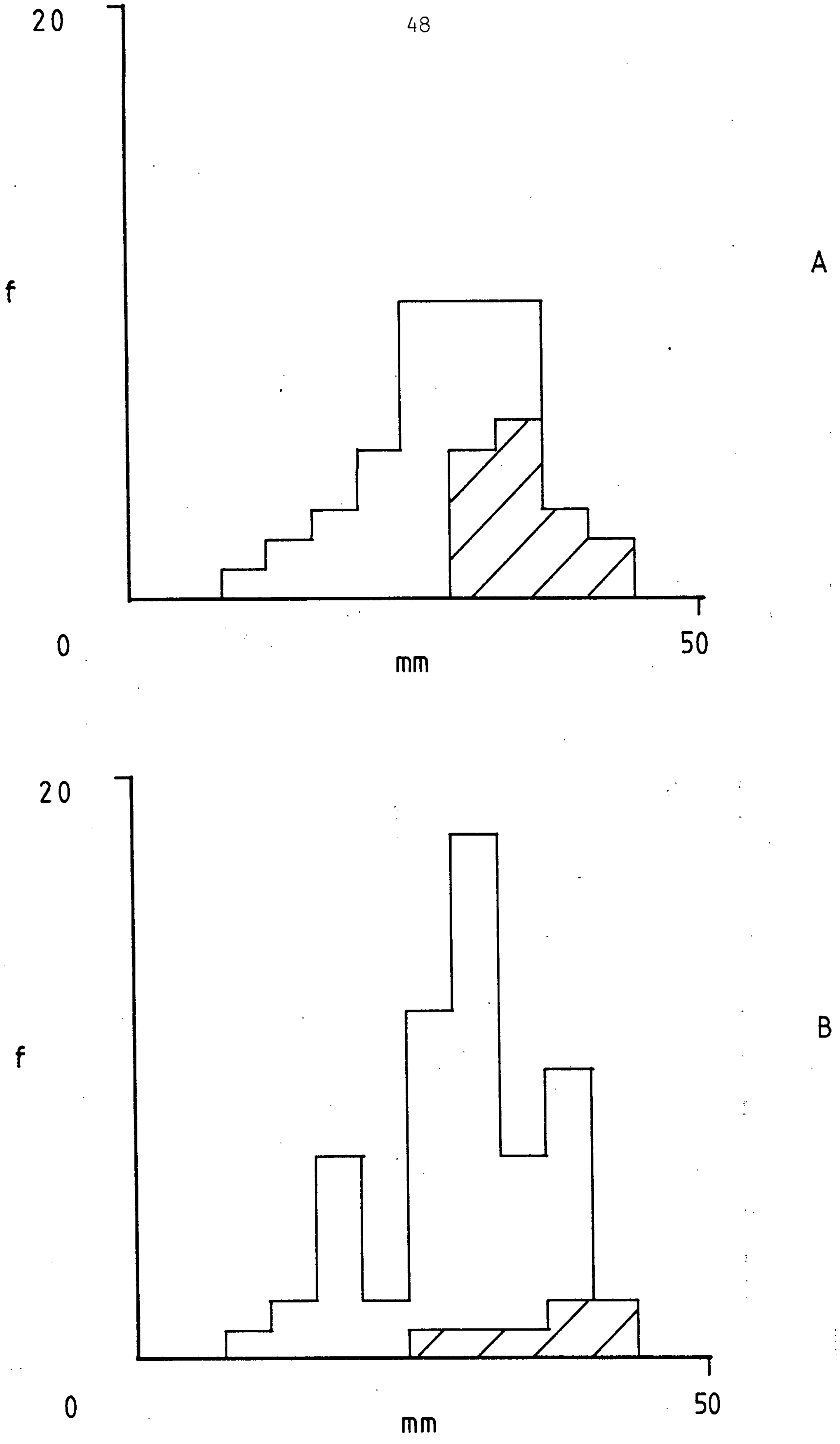


Fig. 3.22 Size and infection distribution in B. digitalis from Melkbos in June; A male, B female.

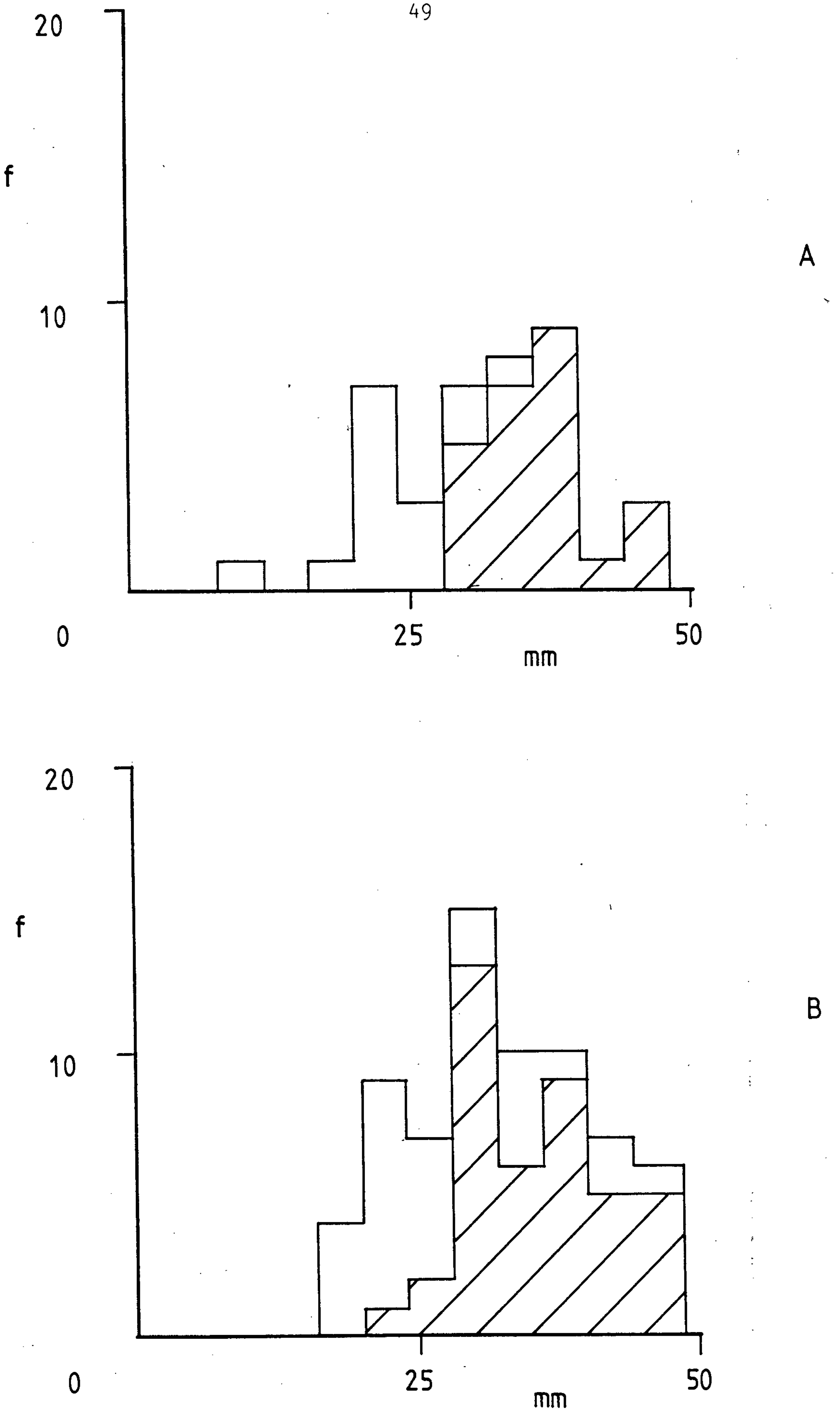
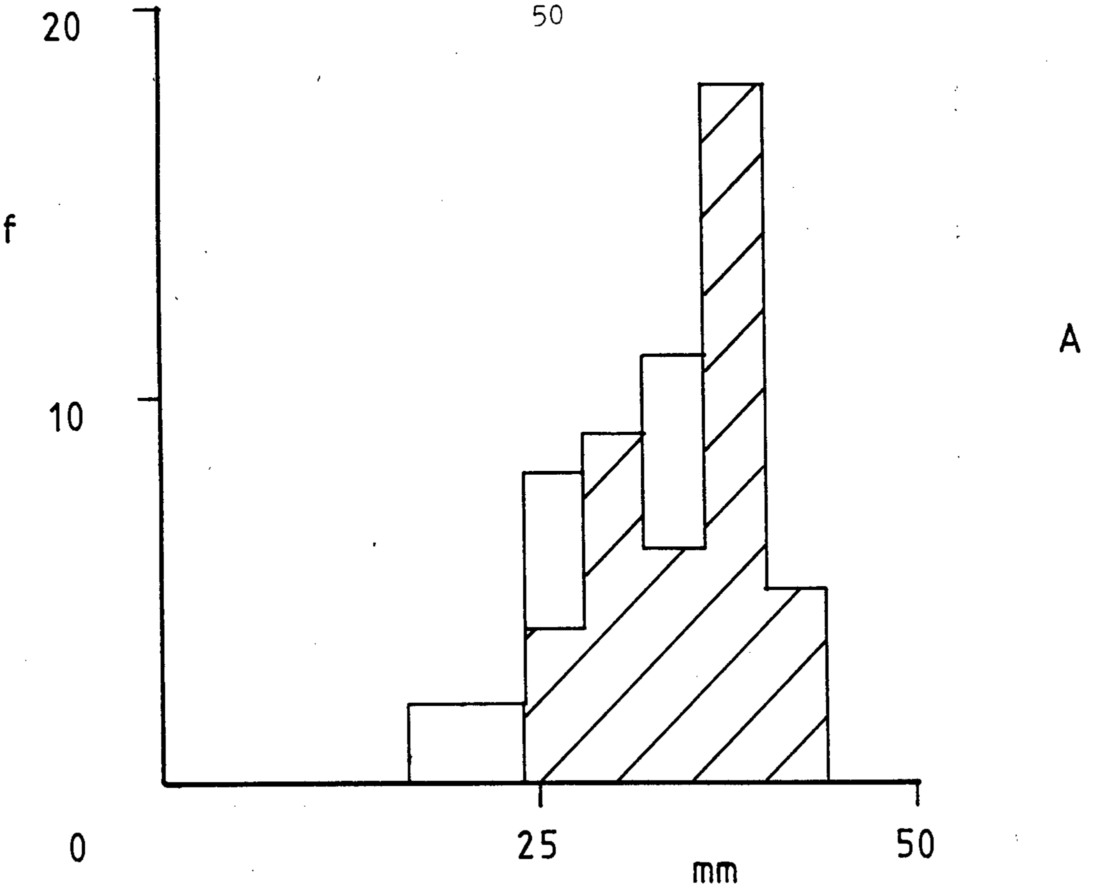


Fig. 3.23 Size and infection distribution in <u>B. digitalis</u> from Melkbos in July; A male, B female.



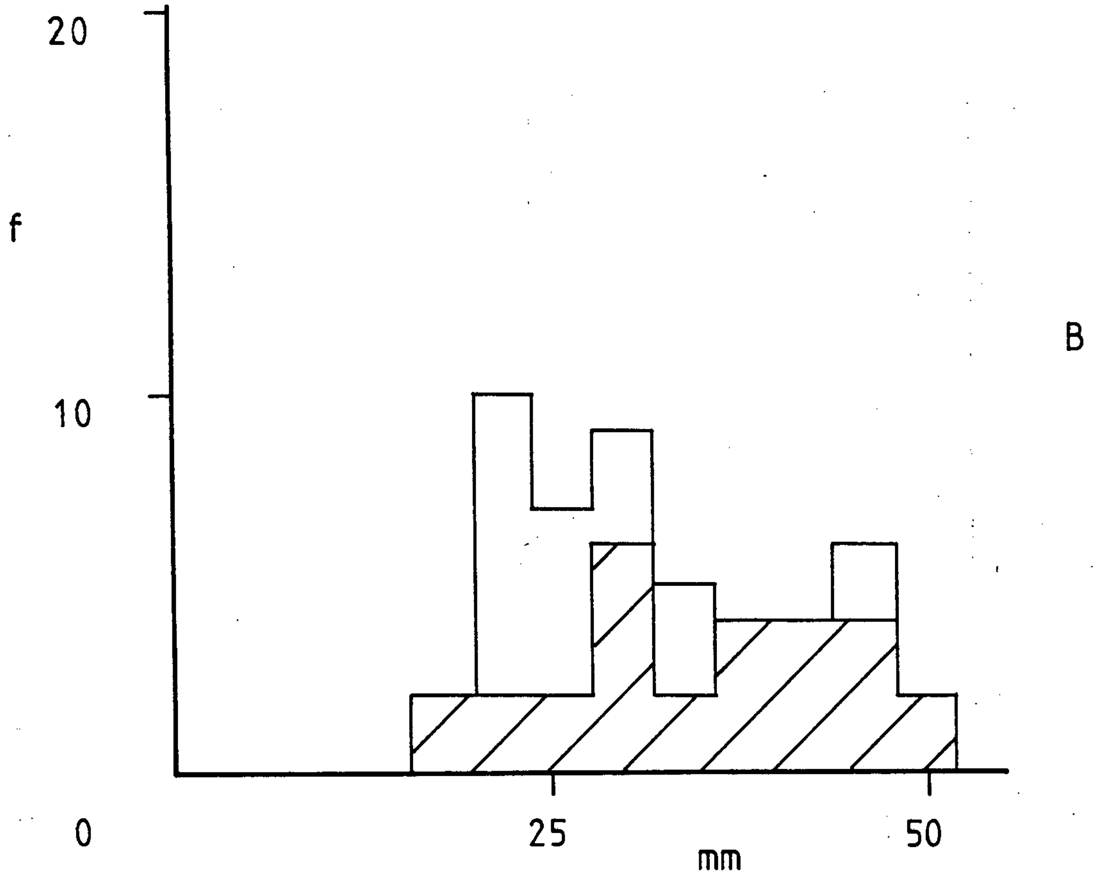


Fig. 3.24 Size and infection distribution in B. digitalis from Melkbos in August; A male, B female.

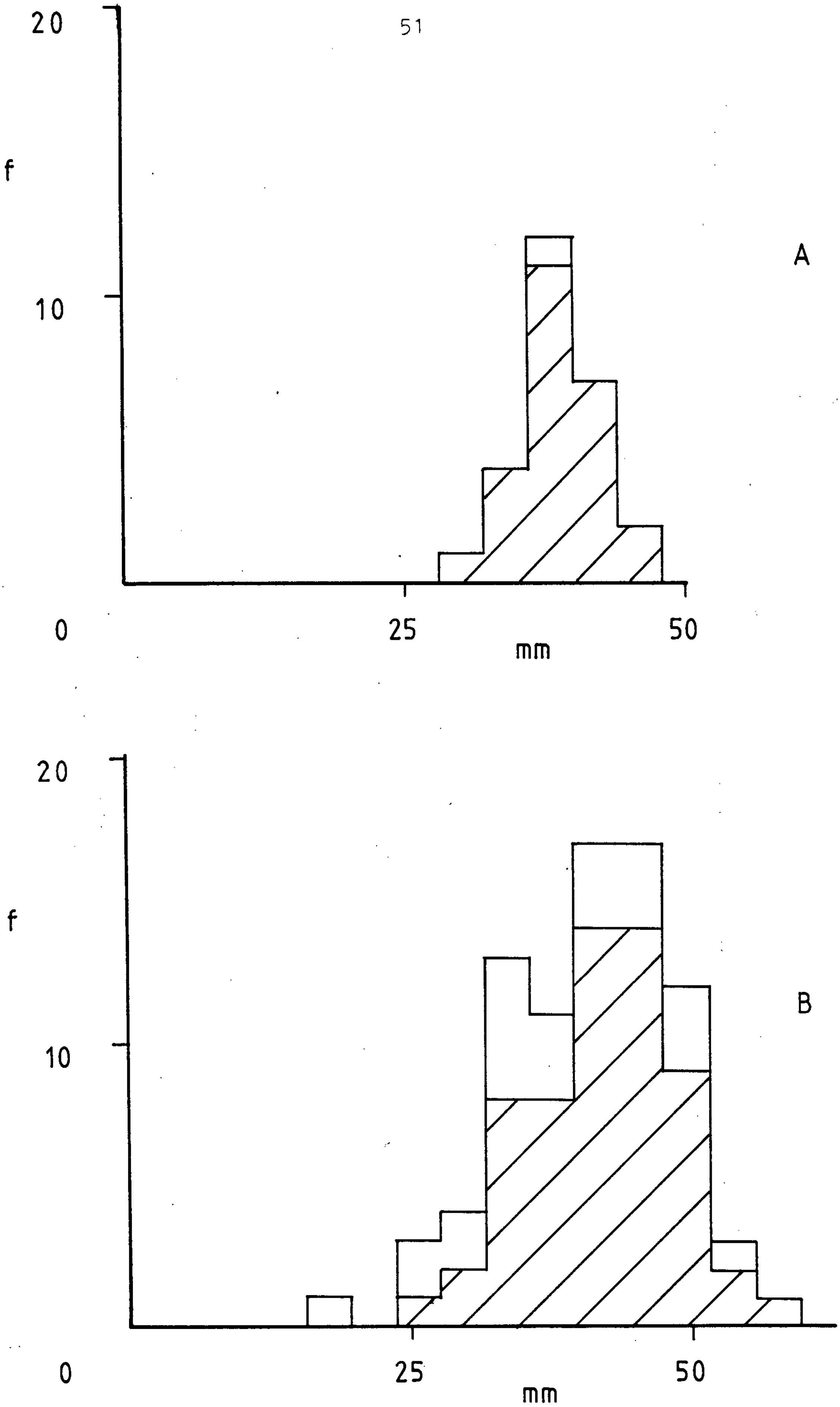


Fig. 3.25 Size and infection distribution in <u>B. digitalis</u> from Melkbos in September; A male, B female.

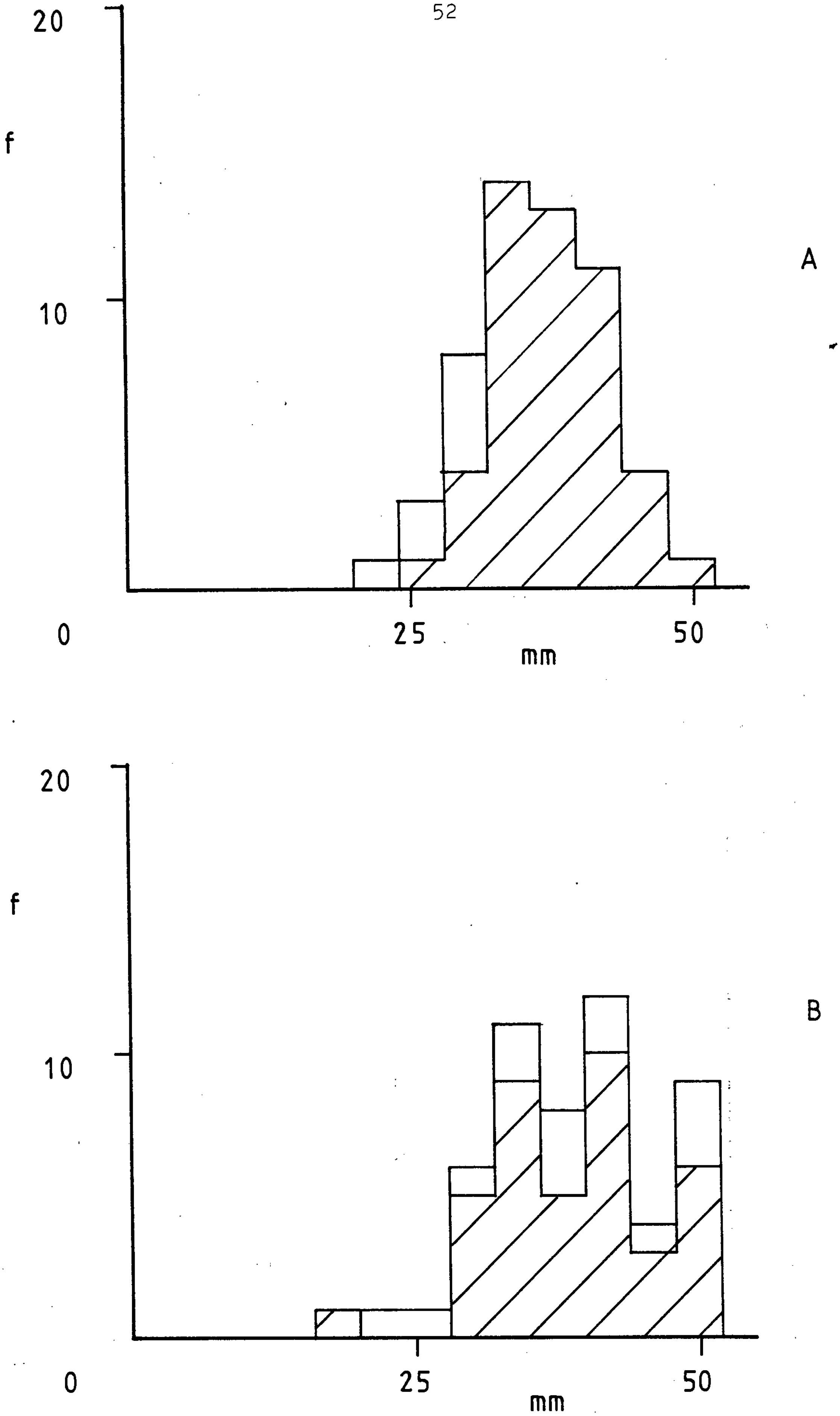
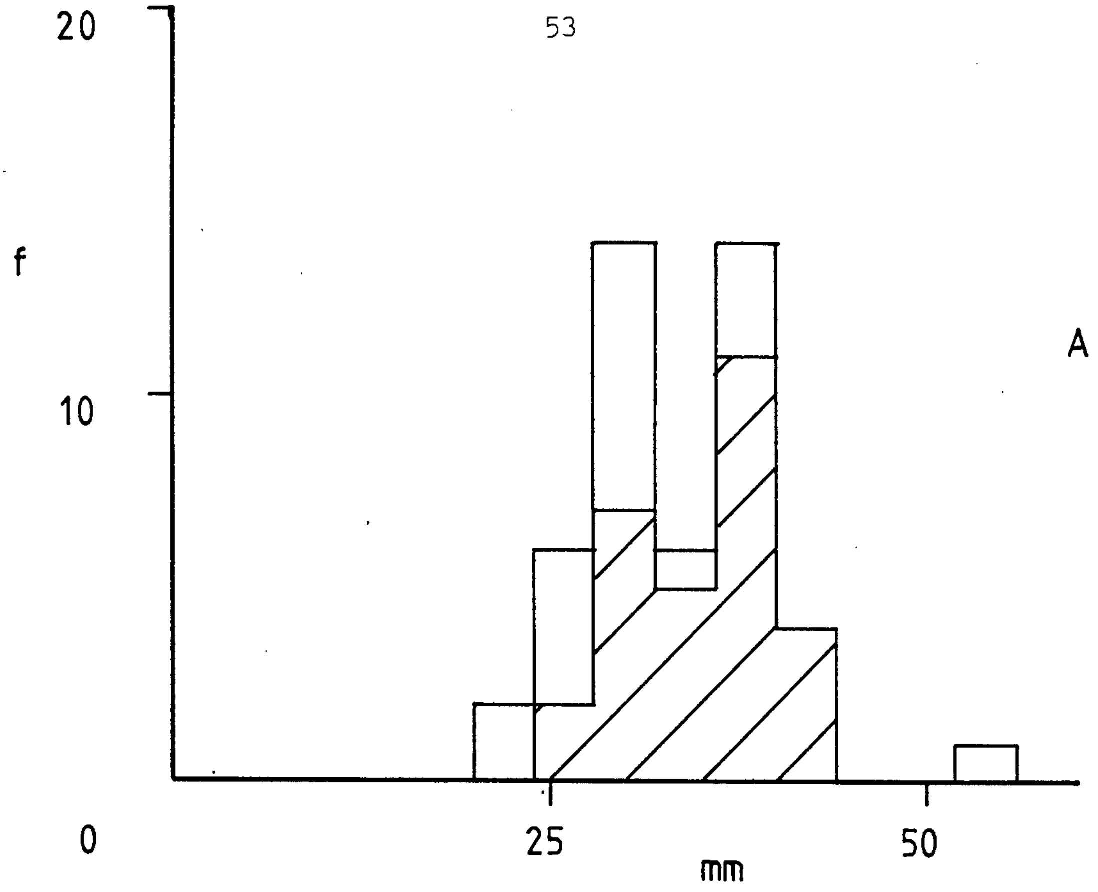


Fig. 3.26 Size and infection distribution in <u>B. digitalis</u> from Melkbos in October; A male, B female.



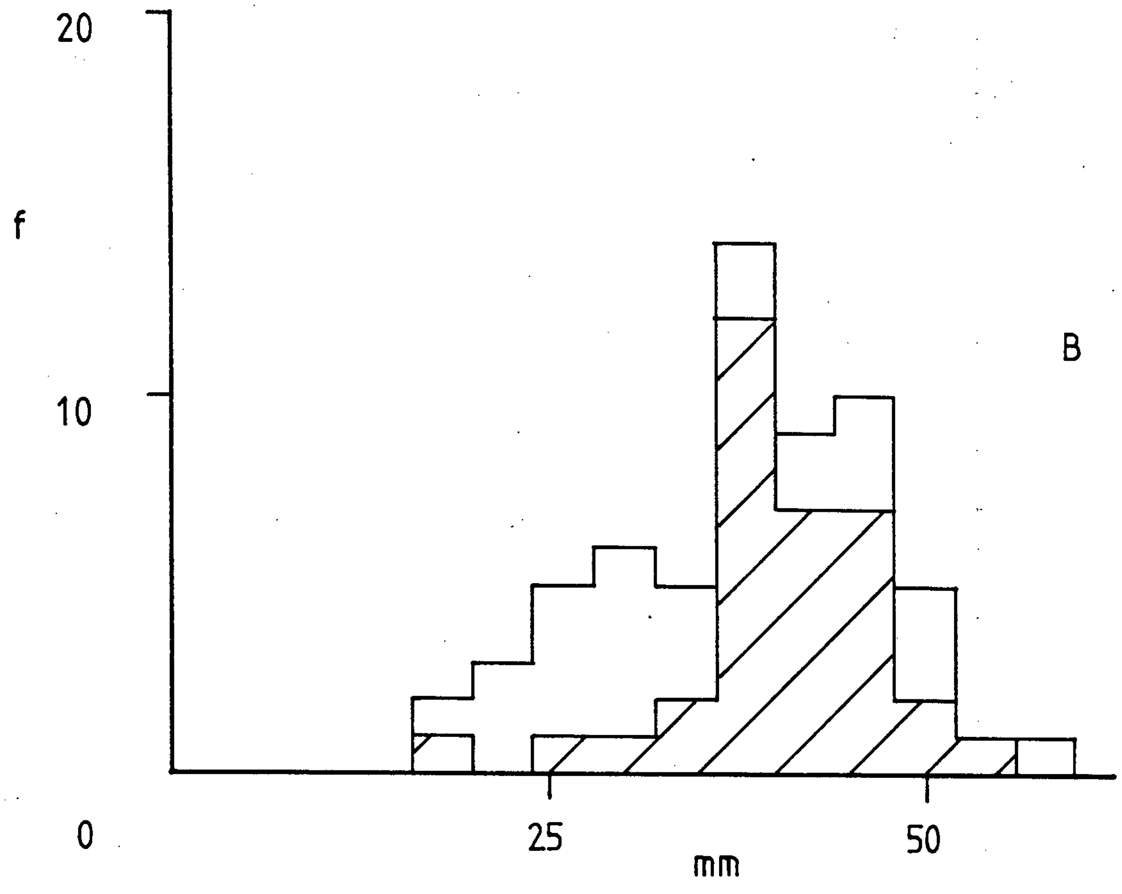
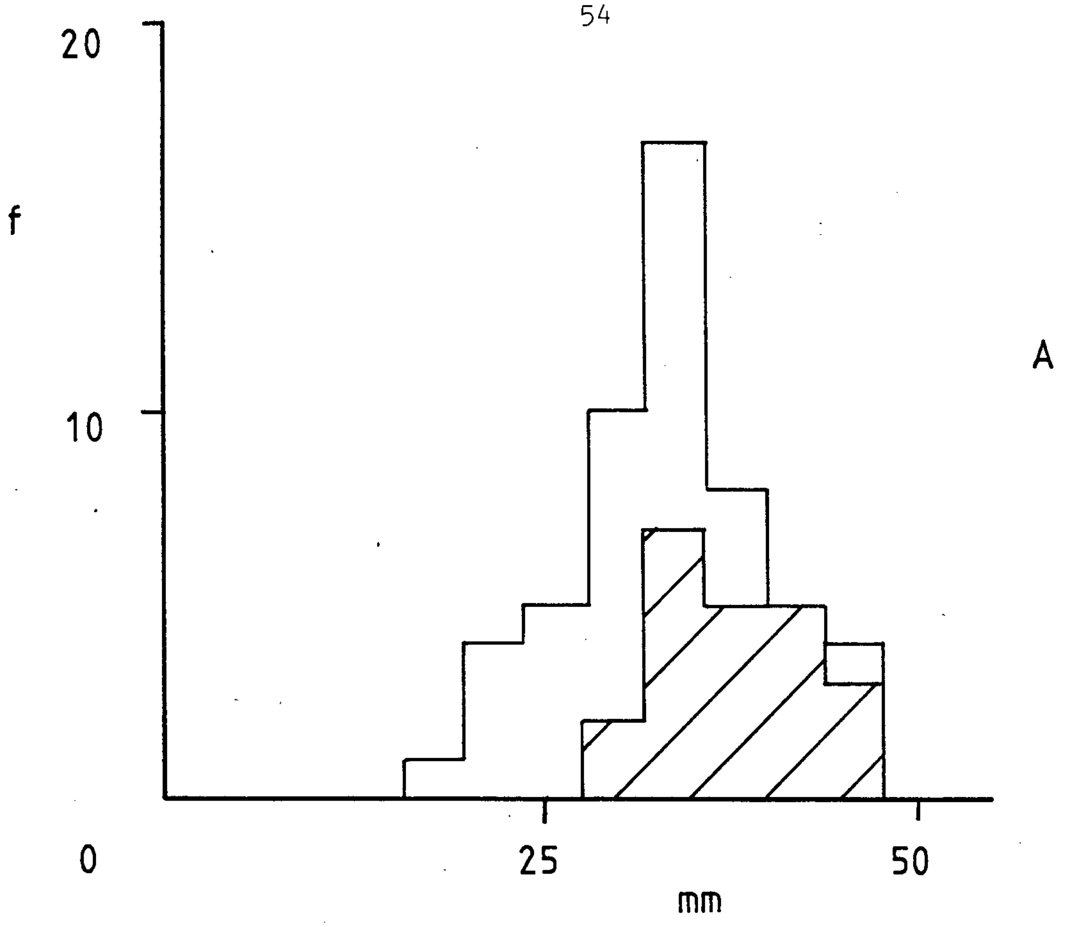


Fig. 3.27 Size and infection distribution in B. digitalis from Melkbos in November; A male, B female.



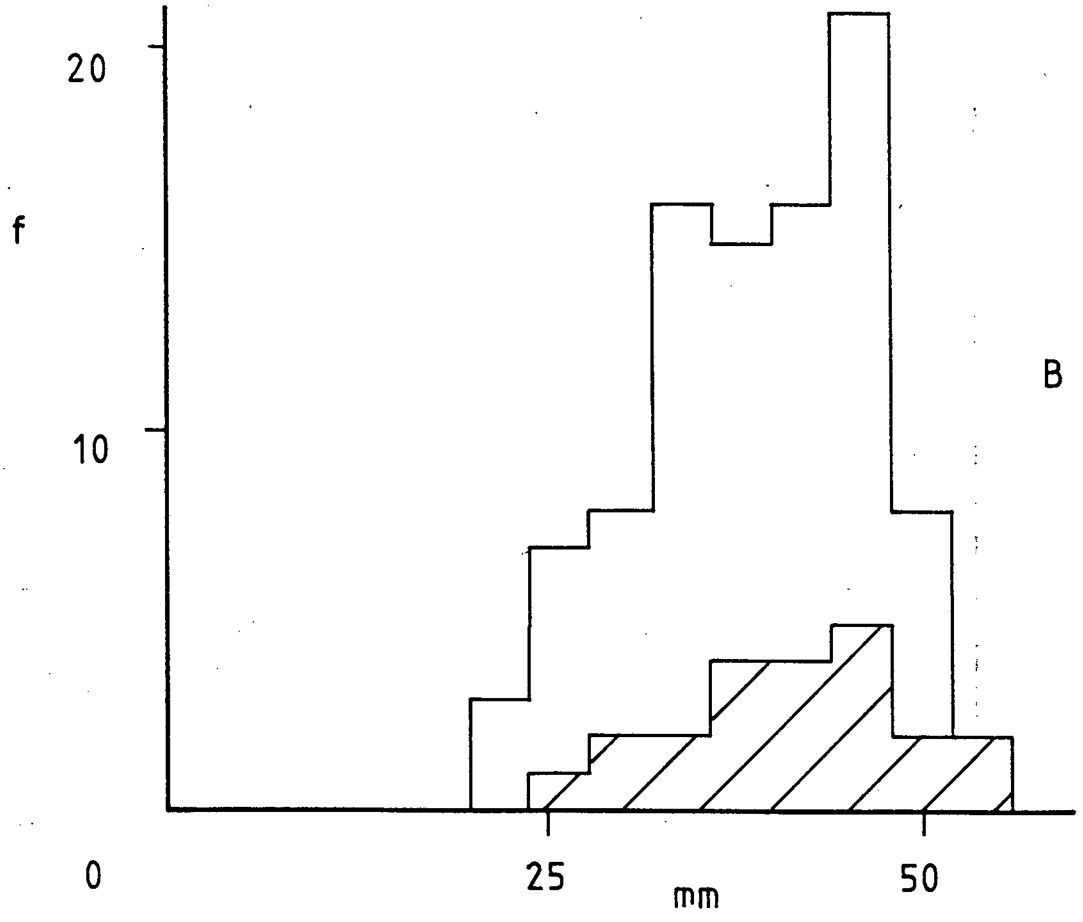


Fig. 3,28 Size and infection distribution in B. digitalis from Ou Skip in October: A male, B female.

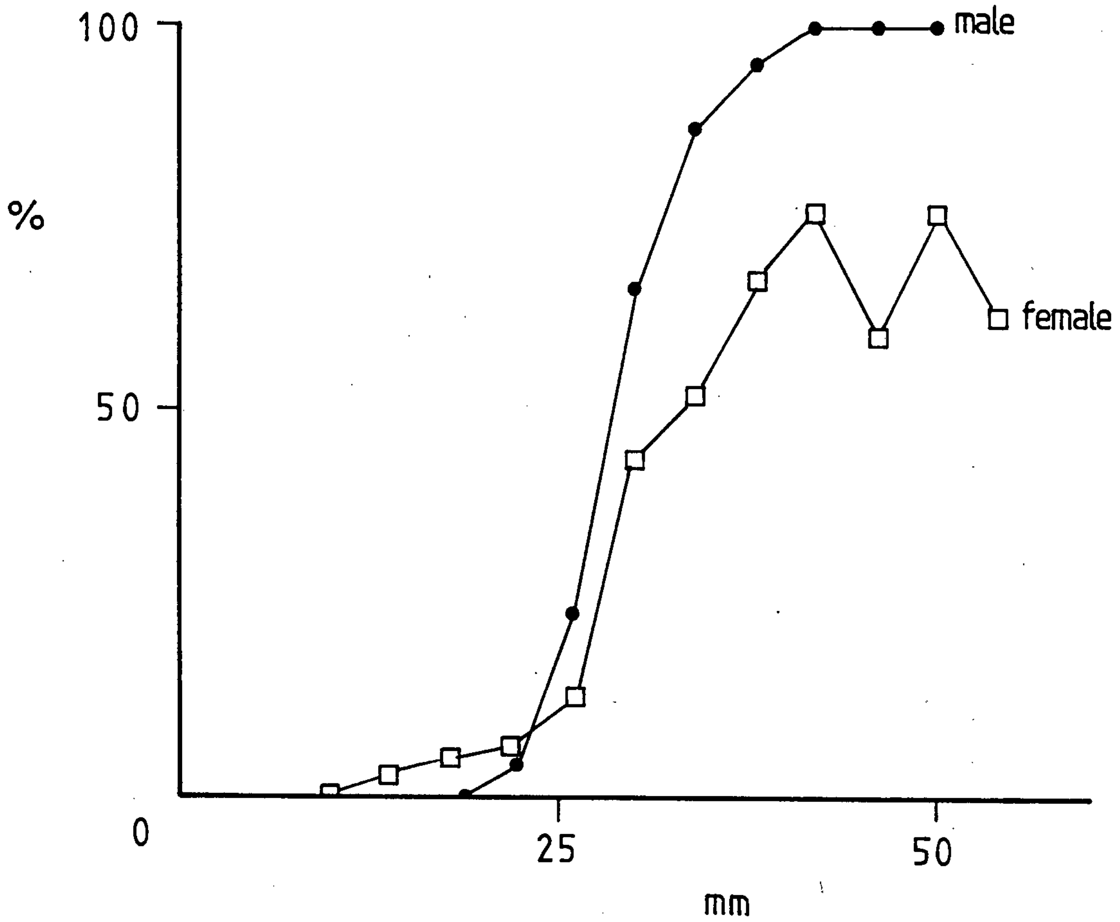


Fig. 3,29 Graph to show the relationship of infection incidence to shell height in B. digitalis at Melkbos.

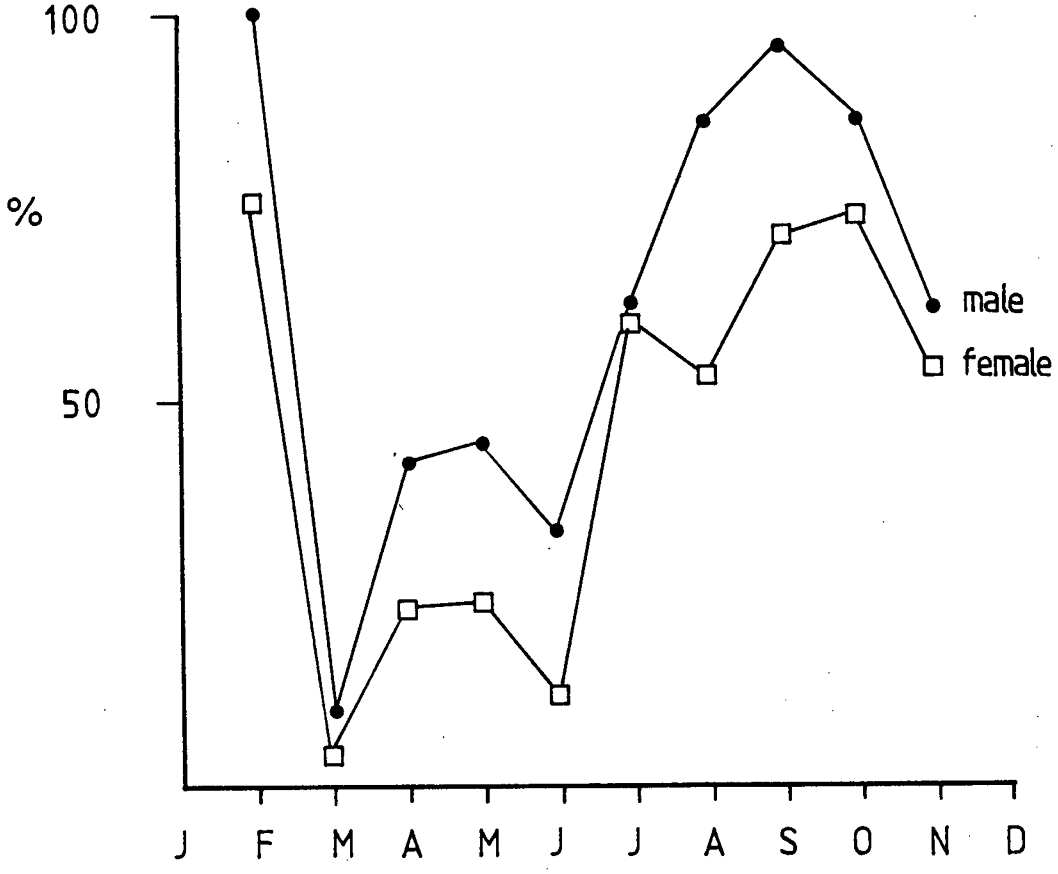


Fig. 3,30 Melkbos, seasonal infection incidence: all sizes.

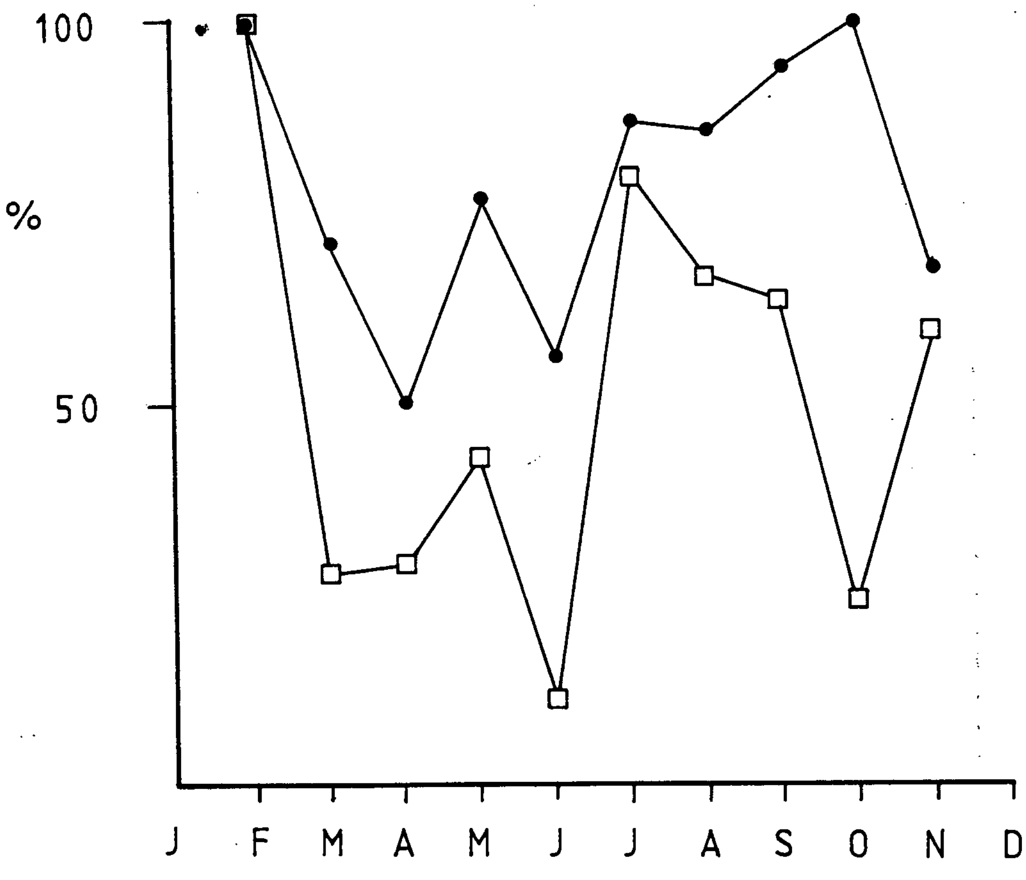


Fig. 3,31. Melkbos, seasonal infection incidence ,shell height: 28–40mm.

3.4 SECOND INTERMEDIATE HOST OF CERCARIA Z

Introduction

During collection of Bullia when using bait, it was noted that the isopod Eurydice longicornis (Stüder) was also attracted to the bait. Eurydice could be caught in large numbers by either shaking the bait in a bucket of water to dislodge them, or by sieving the sand at the highest point of the last wave where spume and detritus are deposited. A 1 mm sieve mesh size was used.

E. longicornis is an intertidal isopod which is found principally on beaches with moderate to heavy wave action. The range of E. longicornis is from Lüderitz in South West Africa, to the mouth of the Great Fish River (Day 1969; Day et al. 1970). E. longicornis feeds on decaying organic matter and small animals washed into the surf zone (Brown 1973).

Samples from Fish Hoek and Melkbos both showed a low level of infection with metacercarial cysts in the digestive gland. Commonly only one cyst would be found in any host isopod. Occasionally, however, two cysts were found in one host. The use of crustaceans as intermediate hosts of marine xiphidiocercariae is widespread. Any work on the biology of trematodes may confirm this.

Materials and Methods

Eurydice longicornis was collected from Fish Hoek and Melkbos using the bait and sieving methods. The isopods were kept in aerated static water buckets at 15°C; isopods would have been lost in any simple through-flow water system. The isopods could be kept in this manner for up to eight weeks. Interestingly, they also seem to thrive in water of low salinity. A large number were found in the laboratory sink 'U' bend after it had been blocked and unusable for over six weeks.

The isopods were examined as soon as possible after collection. Examination consisted of squeezing the isopods between microscope slides. In many cases the cysts could be seen and photographed through the intact isopod body. The isopods were then crushed and the cysts extracted using needles and a dissection microscope. The cysts were measured. Attempts were made to induce the metacercariae to excyst by using bird ringer, bird saline, distilled water and sea water at temperatures of 20°C, 35°C and 40°C. No excystment was obtained. The use of bird ringer and bird saline was suggested because the probable family to which the metacercaria belongs is the Microphallidae. This family has birds exclusively as the final host. The reason for the metacercaria being assigned to the Microphallidae will be elaborated later.

Attempts to extract the metacercaria by rupturing the cyst met with differing degrees of success. From the fragments that were extruded in a recognisable form a description of the metacercaria could be built up. A provisional description follows:

Metacercaria Z diagnosis:

Dorsoventrally flattened leaf-shaped metacercaria about 600 um long. The tegument is spinous overall. The oral sucker is terminal or slightly sub-terminal. The pre-pharynx is long, about the same length as the oral sucker. The pharynx is about half the diameter of the oral sucker and is spherical. The oesophagus is about twice the length of the pre-pharynx. The caecae are short and straight. They almost reach the lateral extremity of the body at the halfway point between front and rear.

The ventral sucker is small, about the same size as the pharynx. The cirrus arises behind the ventral sucker and passes it sinistraly; it opens to the exterior at about midway on the body on the left-hand side.

The testes are both larger than the ovary. They lie staggered on the left-hand side of the body. The ovary is about the size of the ventral sucker and lies dextrally in the posterior part of the body. The uterus is filled with yellowish eggs and occupies the extreme posterior of the worm. Due to the poor condition of the extruded samples, it was not possible to determine any of the features of the excretory system.

The above description is incomplete, but some of the features mentioned are typical of the Microphallidae, hence it can be said that this is probably a microphallid metacercaria.

Other evidence to tie Cercaria Z in Bullia digitalis to this Metacercaria is largely circumstantial but nevertheless persuasive.

The cysts occurred in *Eurydice longicornis* collected at Fish Hoek and Melkbos. *Bullia digitalis* infected with *Cercaria Z* also occur at both sites. At Mnandi, *Cercaria Z* is not present in *Bullia digitalis*. Interestingly *Eurydice longicornis* is also absent from Mnandi. *Eurydice longicornis* is replaced at Mnandi by the isopod *Exosphaeroma truncatitelson* (Barnard). There are few of these isopods on Mnandi. A sample of thirty eight *Exosphaeroma truncatitelson* were examined for cysts but none were found.

Partial success was achieved during laboratory infection of *Eurydice longicornis* with *Cercaria Z* extracted from crushed *Bullia digitalis*. Infected digestive glands from several whelks were added to a litre beaker of sea water containing several dozen *Eurydice longicornis*. After three days the infected whelk digestive gland showed no sign of living cercariae. Isopods were washed twice in sea water to dislodge any external cercariae that may have survived. Microscopic examination was used to confirm that there were no living cercariae adhering to the outside of the isopod. The isopods were then crushed and examined in the usual way. In ten consecutive examinations, living *Cercariae Z* were found in the isopod tissues. Each isopod was found to contain three to twenty cercariae. This proves that *Cercaria Z* can find its way into *Eurydice longicornis* and once inside, could survive longer than if it were free-living. The remainder of the *Eurydice longicornis* were kept to see if encystment would take place at a later stage. The heavy parasite burdens caused the deaths of all of the remaining isopods within seven days.

The encystment of Xiphidiocercariae is sometimes governed by the moulting cycle of the arthropod host (Erasmus 1972). In the case of *Eurydice longicornis*, moults occur at intervals of about one month (Brown 1973). Perhaps a repeat experiment with a lighter infection intensity would enable the isopods to survive to the next moult and thus encystment would be secured.

An experiment was carried out to investigate the upper lethal temperature of *Cercaria Z* (see Chapter 7.5).

The results indicate that the final host has a high body temperature and because of this is probably a bird rather than an ectotherm.

TABLE 3.3 Measurement of the diameter of metacercarial cyst type Z.

Mean u	S.D. u	S.E. u	C.V. %	n	Max u	Min u
331	52	24,2	15,6	8	403	277

3.4 EPIDEMIOLOGY OF TYPE Z IN *EURYDICE LONGICORNIS*

Eurydice from Melkbos and Fish Hoek were measured and the presence of metacercarial cysts noted. The results are presented as histograms. The hatched columns represent isopods containing cysts (see Fig 3.36 A and B).

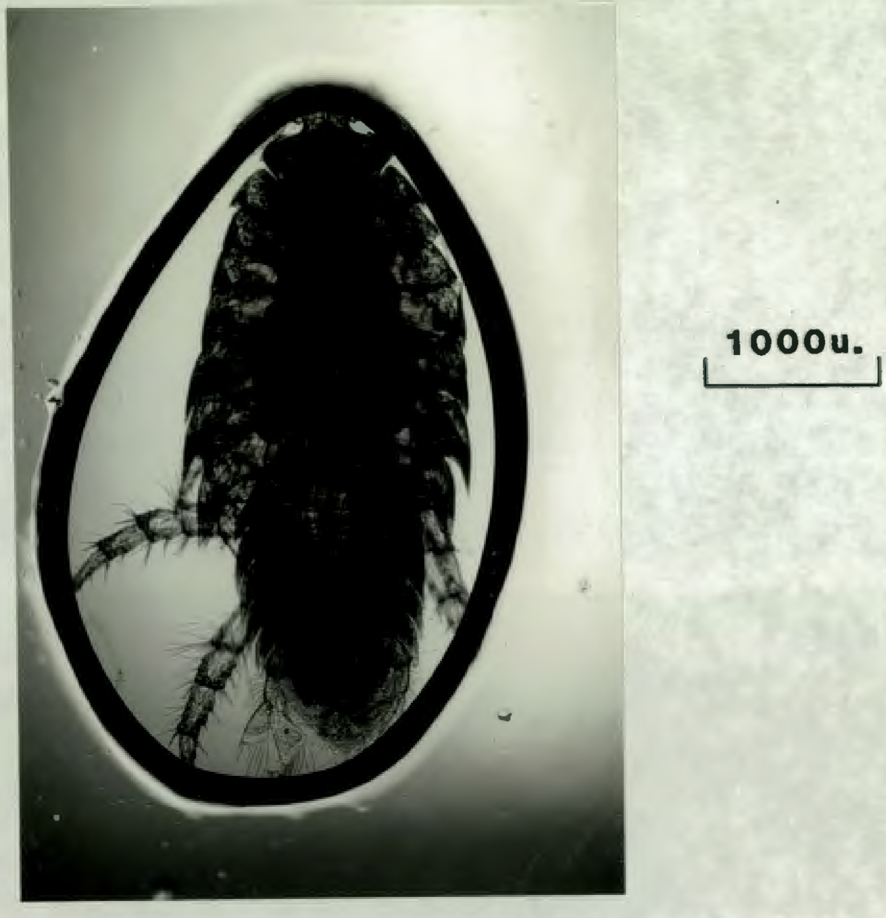


Fig. 3,32. E. longicornis with a metacercarial cyst in the digestive gland.

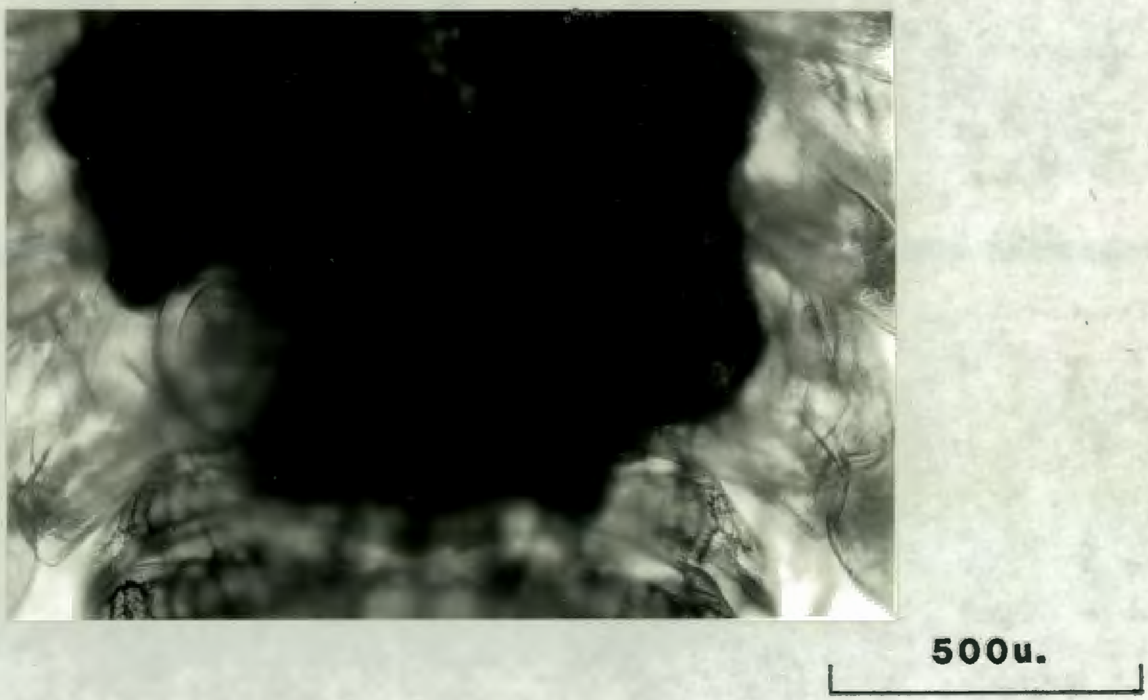


Fig. 3,33. Digestive gland of E. longicornis showing the metacercarial cyst on the left.

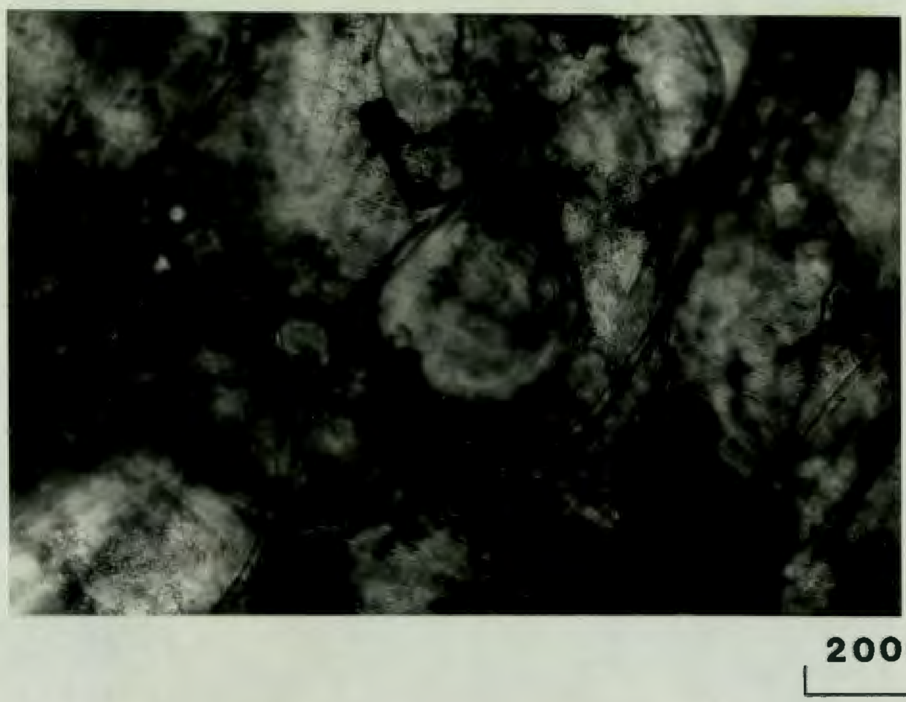


Fig. 3,34. Metacercarial cyst in E longicornis.

Fig. 3,35. Metacercarial cyst in E. longicornis.

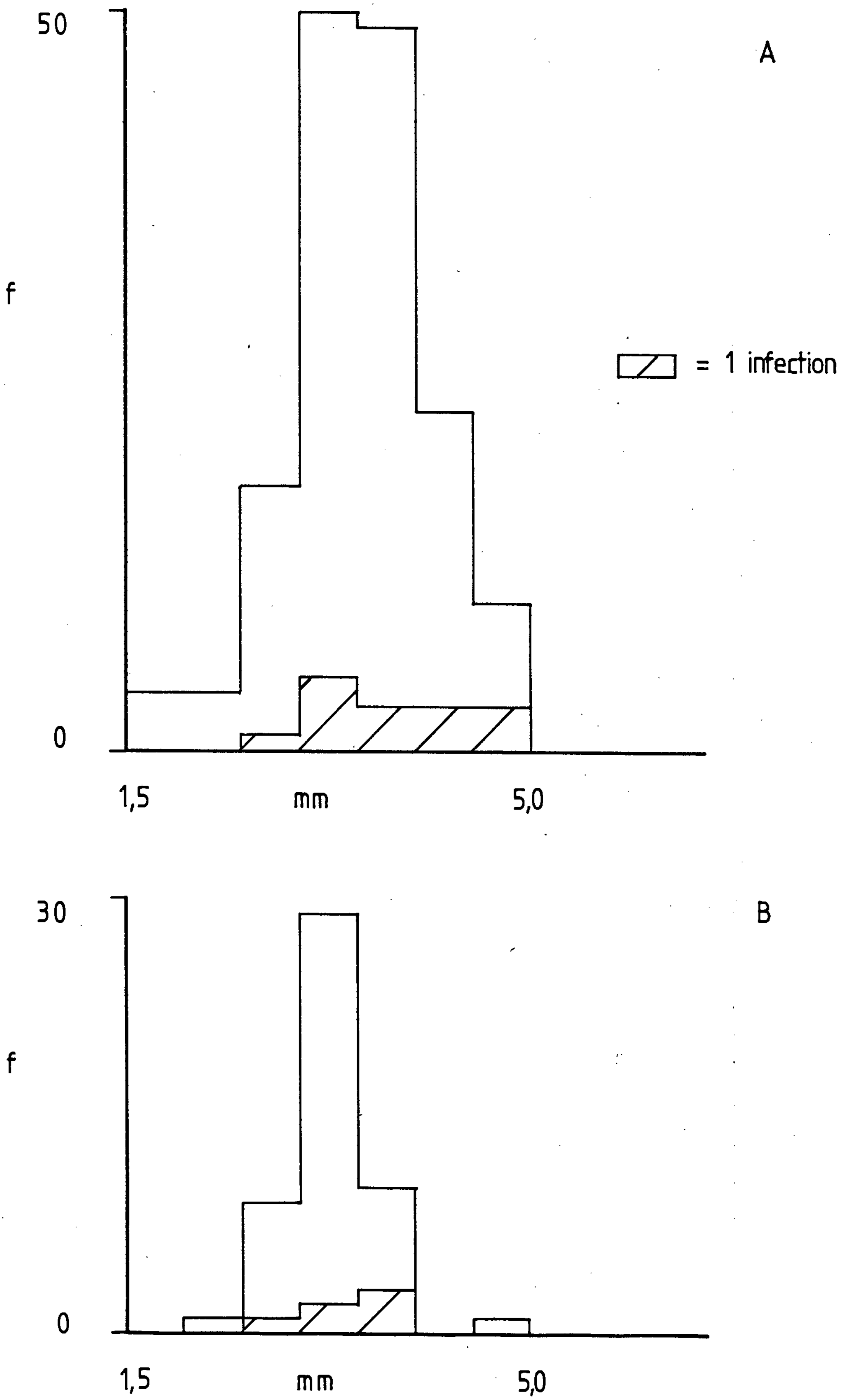


Fig. 3.36 Size and parasitism distribution in samples of E. longicornis from: A Fish Hoek; B Melkbos.

3.5 DISCUSSION

Cercaria Z resembles Cercaria lanceolata from Holliman (1961). The flame cell number in Cercaria Z is eight. There is no difference in number between extracted and emerged cercariae. In Cercaria lanceolata the flame cell formula is

$$2 \quad (2 + 2) + (2 + 2) \quad = 16$$

The microphallid Sogandaritrema progeneticus in Lotz and Corkum (1983) has a flame cell formula similar to Cercaria lanceolata. Microphallus pygmaeus has a flame cell formula of

$$2 \quad (1) + (1) \quad = 4$$ (James 1968).

The penetration glands of Cercaria Z are well developed. This contrasts with their absence in M. pygmaeus and only moderate development in S. progeneticus. According to Holliman (1961) four pairs of penetration glands are usual for a marine microphallid cercaria. Exceptions include: C. minima (Schell and Thomas 1955) and C. misensis (Palombi 1940), each with two pairs of glands; C. ubiquita (Lebour 1940) and C. ubiquitensis each with six pairs.

The presence of a stylet is common in microphallid cercariae. Its absence in M. pygmaeus may be related to deletion of the arthropod intermediate host.

The sporocyst of type Z is typical of other members of the family. The only anomalous feature being its convolution and bulbous form in some specimens. The disposition of germinal material in the sporocyst approximates to that of S. progeneticus.

The overall incidences of infection with type Z range from 45% at Melkbos to 2% at Noordhoek. These figures represent a useful crude estimate. Closer examination shows a marked difference in infection incidence for whelks of different sexes. This has been overlooked by Matthews et al. (1983). They chose to pool results from both sexes. Lysaght (1941) and Sousa (1983) have reported differential sex-related responses and resistance to infection.

In whelks collected from Melkbos, males showed a consistently higher incidence of infection than females. Both sexes showed a higher incidence in larger than in smaller whelks.

Evidence reinforcing the method of sex identification is found in the size distributions. Female whelks generally have a wider size range than males and tend to have more large members in each sample. If the sexing technique was defective one would expect no differences in distributions.

A number of factors may be at work in causing these differences. According to Moore (1937) immature females have a faster growth rate. This has also been reported by Underwood and McFadyen (1983) and Sousa (1983). A growth function was obtained for Bullia rhodostoma (McLachlan *et al*. 1979). This did not differentiate between the sexes. It is possible that the female grows at the same rate but lives longer. Both of these phenomena are complicated by the possibility of protandric hermaphroditism, as occurs in the neogastropod *Lora turricula* (Smith 1967).

The effect of parasitism on gastropod reproductive capacity has been extensively documented. Recent works include those by Sousa (1983), Baudoin (1975), Obreski (1975) and Kuris (1974).

If the premise that parasitism abolishes sexual difference is true then one would also expect growth difference to disappear. This would cause the height distributions to be identical. This is obviously not the case with type Z infections, even though whelk reproductive capacity is terminated in all infections. Sousa (1983) states that microphallid larval stages have no significant effect on growth in the snail *Cerithidea californica*.

It is possible that shell growth is sex-linked but not linked to the gonad or fecundity. (The gonad in *Bullia digitalis* is destroyed in type Z infections.) This would indicate a genetic sexual stability and not an animal that is prone to a change of sex.

In male *B. digitalis* infection occurs at a shell height of ≈ 23 mm. Sperm production in uninfected whelks starts at about the same size. This implies that infection occurs when the gonad is developed. The result would be to exploit the reproductive resources of the whelk without harming its vital functions.

Although the hepatopancreas is heavily involved in infections and appears quite sickly, infected whelks have survived starvation for over ten weeks. It appears that non-reproductive functions are not too badly disrupted.

The infection incidence in males follows a sigmoidal curve if plotted against shell height (see Fig. 3.30). Above about 42 mm all are infected. In females the curve is similar but does not reach 100% incidence. Some of the larger females are thus able to escape infection and contribute to reproduction. In males, sperm production is manifest from about 24 mm to about 36 mm, after which the infection incidence increases rapidly. Thus host and parasite accommodate each other's needs.

Seasonal infection incidence in both sexes follows the same pattern, the male showing a higher incidence. Incidences drop during March when recruitment takes place (see Fig. 3.31). To eliminate any fluctuation due to recruitment, whelks ranging form 28 mm to 40 mm are examined in Fig. 3.31 in the same way. Even in Fig. 3.31 there is still a low in March.

According to James (1968), the incidence of Microphallus pygmaeus in Littorina saxtilis tenebrosa shows two seasonal peaks: one in midsummer and a smaller peak in mid-winter. Until more is known about the development time and longevity of infection in Bullia one cannot speculate on the significance of these seasonal phenomena.

The result of a temperature tolerance experiment (in Chapter 7.4) reinforces the generalization that the final host of a microphallid is a bird. A possible candidate is the sanderling Calidris alba (Pallas 1764), due to its habit of eating the isopod Eurydice (Brown, pers. comm.)

CHAPTER 4

CERCARIA AND SPOROCYST TYPE Y

SUPERFAMILY ALLOCREADIODEA (Nicoll 1934)

HOST: BULLIA DIGITALIS

4.1 SPOROCYST

Daughter sporocyst stages only were found. The sporocysts form a mass next to the gut of the host. The mass appears yellowish in life. There appears to be less disruption of the digestive gland with Sporocyst Y than is the case with infections of Sporocyst Z In infections of Sporocyst Y the tissues surrounding the gut tissue appear to be more heavily affected.

The sporocysts are spherical or elipsoid. There is no birth pore. Sporocysts are quite opaque. Details of the contained cercariae are very difficult to distinguish unless the sporocyst is flattened under coverslip pressure.

Using light microscopy techniques, no tegumental structures could be distinguished. The use of intravital stains did not reveal the presence of contained germinal material. All of the cercariae in each sporocyst were at the same stage of development. The sporocyst did not exhibit any degree of mobility. No excretory structures were discernable.

4.2 CERCARIA

Diagnosis

Cercaria Y is a tailless pharyngeate distome Xiphidiocercaria. The tegument is covered with backward-facing spines, each about 2 u long. The transverse rows of spines are staggered. The distance between rows is about 4 u. The spines are triangular rather than needle-like. The stylet is very stout and conical, its base flares out slightly and is serrated around the circumference. There is a shoulder about half-way along the stylet, which then proceeds to a sharp point. The stylet is held horizontally and its base is embedded in the sucker musculature. On either side of the stylet point lie openings of the penetration glands. Just beyond the penetration gland openings at the extreme anterior is a row of four spines, each is about two or three times longer than a normal tegument spine (see Fig. 4.2.B).

The oral sucker is longer than broad and opens subterminally. There is a short pre-pharynx followed by an oval pharynx which is about half of the length of the oral sucker. The pharynx appears to have two sphincters at each end (see Fig. 4.2.C). The oesophagus runs back as far as the ventral sucker where it bifurcates to form two very short caecae.

The penetration glands arise just anterior to the ventral sucker and lead forwards in two bundles of ducts. There are four pairs of penetration glands. The ducts thicken posteriorly to the oral sucker, then each bundle bifurcates. The oval orifices of the penetration glands appear divided longitudinally. This division is more marked when nile blue sulphate is used to stain the glands. On each side the inner pair of glands stain blue far less heavily than the outer pair (see Fig. 4.2.A). Each penetration gland orifice appears darker anteriorly and lighter posteriorly.

Distributed throughout the body of the cercaria are about twenty five gland cells. These stain purple with nile blue sulphate.

The ventral sucker is broader than it is long. It is thick and muscular. The ventral sucker lining appears to consist of small polygonal "epithelial cells", each about 2 u in diameter. It is doubtful whether they are in fact epithelial cells as such, but they are a striking feature for recognition purposes. The ventral sucker is about twice the size of the oral sucker.

Posterior to the ventral sucker lies the bladder. The bladder is an irregular bag with no bifurcations. It is thick-walled and appears muscular (see Figs 4.15 and 4.16). The bladder contains a substance which is coagulated by fixing in formalin and sea water. It does not take up stain very well, but can be seen because of the way it shrinks and pulls away from the bladder wall (see Figs 4.19, 4.20, 4.21, 4.22) to see sections of cercarial bladders.

There are seven pairs of flame cells. Four pairs occupy positions in the body anterior to the ventral sucker. Three pairs lie posterior to the ventral sucker. It was not possible to work out the flame cell formula due to the extreme difficulty in finding the excretory ducts. The excretory pore is terminal. The bladder was seen to empty in about five to ten seconds.

Movement

Cercaria Y is always tailless and has no swimming ability. It is highly contractile and capable of very vigorous movement (see Figs 4.11 and 4.12) - they are separated by about five seconds). The ventral sucker is often seen being used as a holdfast. Response to nile blue sulphate is toxic, the cercaria usually dies within a few minutes.

Specimens which were subjected to heat for an insufficient period were able to produce metacercarial cysts, the measurements of which are given in Table 4.2.B.

4.3 EPIDEMIOLOGY OF TYPE Y IN B. DIGITALIS

Overall incidence at each site

The infection rate was calculated for the sample populations from each site. The results are given in Table 4.3.

TABLE 4.1 Measurements of Sporocyst Y. A: living, also included is an analysis of cercarial numbers in each sporocyst; B: heat-killed; C: formalin-killed.

	Mean u	S.D. u	S.E. u	C.V. %	n	Max. u	Min. u
A							
Length	511	77	24,2	14,9	10	607	364
Width	427	75	23,6	17,5	10	535	340
No. of cercariae	4,7	1,7	0,6	36,2	10	8	3
B							
Length	636	139	43,8	21,8	10	859	446
Width	500	113	35,7	22,5	10	689	365
C							
Length	457	56	17,8	12,3	10	567	389
Width	391	42	13,3	10,7	10	486	340

TABLE 4.2 Measurements of *Cercaria Y*. A: living; B: heat-killed; C: formalin-killed.

	Mean u	S.D. u	S.E. u	C.V. %	n	MAX. u	MIN. u
A							
length	689	106	33,5	15,4	10	859	446
width 25%	55	8,3	2,6	14,9	10	65	41
width 50%	56	7,7	2,5	14,2	10	73	49
width 75%	76	5,6	1,8	7,3	10	81	65
oral sucker length	66	9,9	3,1	14,9	10	78	49
width	52	6,3	2,0	12,2	10	59	49
pre-pharynx length	4,7	1,65	0,52	35,0	10	7,8	2,0
pharynx length	22	3,2	1,0	13,8	10	29	20
width	20	2,1	0,7	10,1	10	23	18
ventral sucker length	87	9,7	3,1	11,1	10	98	68
width	92	10,7	3,4	11,5	10	107	78
depth	69	9,4	3,0	13,6	10	78	59
stylet length	18,5	1,9	0,6	10,2	10	19,5	13,65
width	9,2	1,3	0,4	14,4	10	9,75	5,85
B							
length	41	39,0	12,3	9,4	10	486	356
width 25%	82	3,8	1,2	4,6	10	89	77
width 50%	86	5,6	1,8	6,5	10	97	81
width 75%	93	8,6	2,7	9,3	10	105	81
oral sucker length	69	7,6	2,4	11,0	10	78	59
width	61	6,6	2,1	10,7	10	68	49
pre-pharynx length	3,5	1,2	0,4	35,1	10	5,85	1,95
pharynx length	22,2	2,3	0,7	10,1	10	20	25
width	22,6	3,2	1,0	14,3	10	18	27
ventral sucker length	83	6,9	2,2	8,2	10	78	98
width	85	9,0	2,8	10,6	10	74	98
depth	72	12,0	3,8	16,5	10	49	88
stylet length	19,3	2,9	0,9	14,8	10	15,6	23,4
width	10,5	0,9	0,3	8,5	10	9,75	11,7
metacercarial cyst diameter	186	12	3,5	6,0	10	176	214
C							
length	259	53,3	16,9	20,6	10	348	210
width 25%	94	14,9	4,7	15,9	10	122	81
width 50%	11,4	15,5	4,9	13,7	10	130	81
width 75%	10,0	10,1	3,2	10,1	10	113	89

TABLE 4.3 Infection incidence of type Y in <u>B. digitalis</u> in samples from each collection site.

SITE	Sample Size	Infection incidence %
Fish Hoek	677	1,18
Mnandi	494	3,04
Hout Bay W	151	0,7
Hout Bay E	150	0,0
Noorhoek	150	0,0
Melkbos	1378	0,0
Ou Skip	150	0,0

Seasonal prevalence at each site

The results for Fish Hoek and Mnandi are broken down into individual collections (see Table 4.4).

TABLE 4.4 Seasonal variation of infection incidence with type Y in samples from: A Fish Hoek, B Mnandi.

DATE	SAMPLE n	INFECTION INCIDENCE %
A		
August 1983	150	0,0
March 1984	150	0,7
June 1984	162	1,2
November 1984	150	2,7
B		
May - July 1983	150	2,0
September 1984	152	5,9
May 1985	192	1,6

Due to the small numbers of parasitized whelks, no further analysis of the results would be profitable.

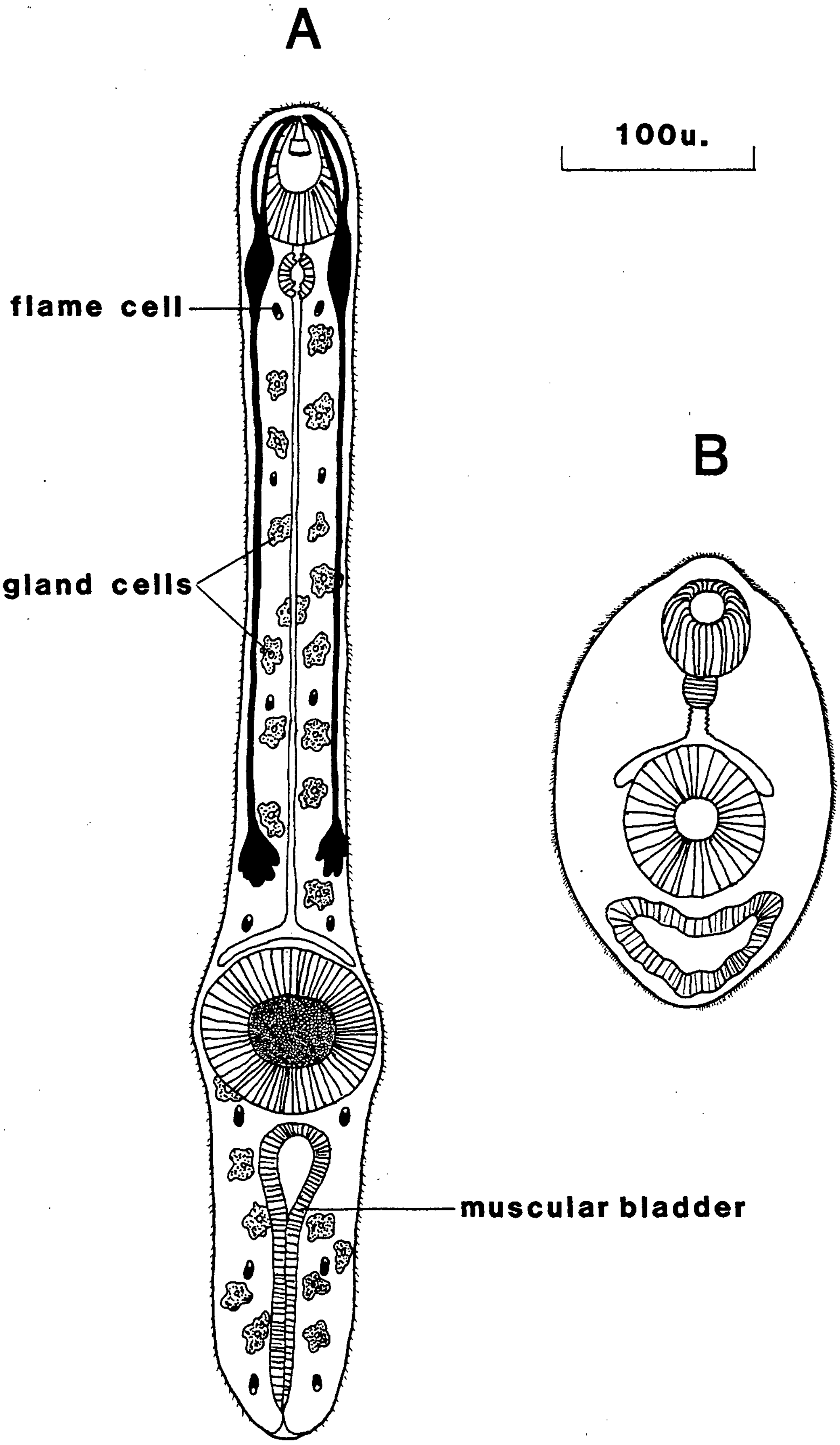


Fig. 4,1. *Cercaria Y* A, extended. B, contracted.

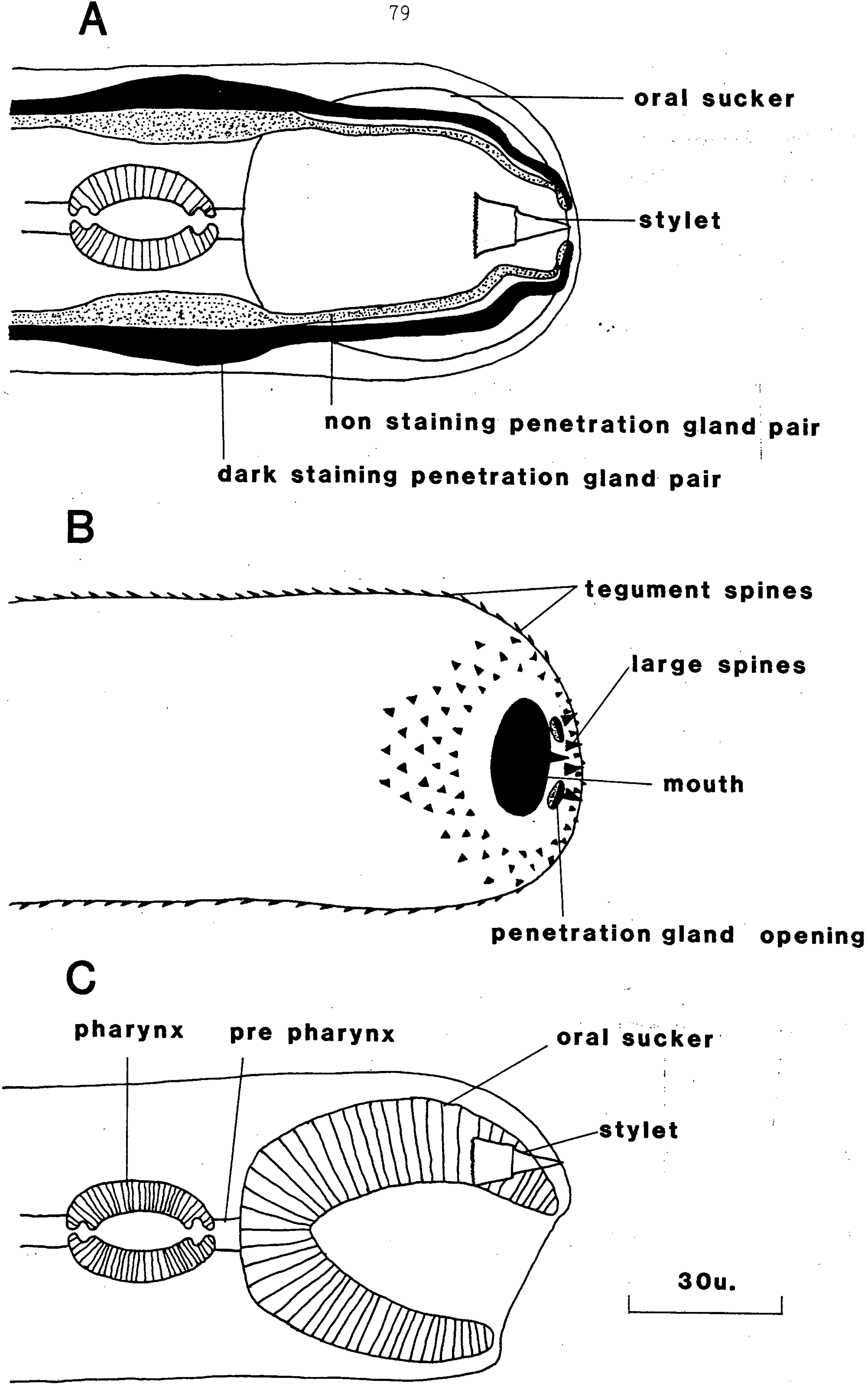


Fig. 4,2. Cercaria Y A, dorsal view of anterior internal features. B, ventral

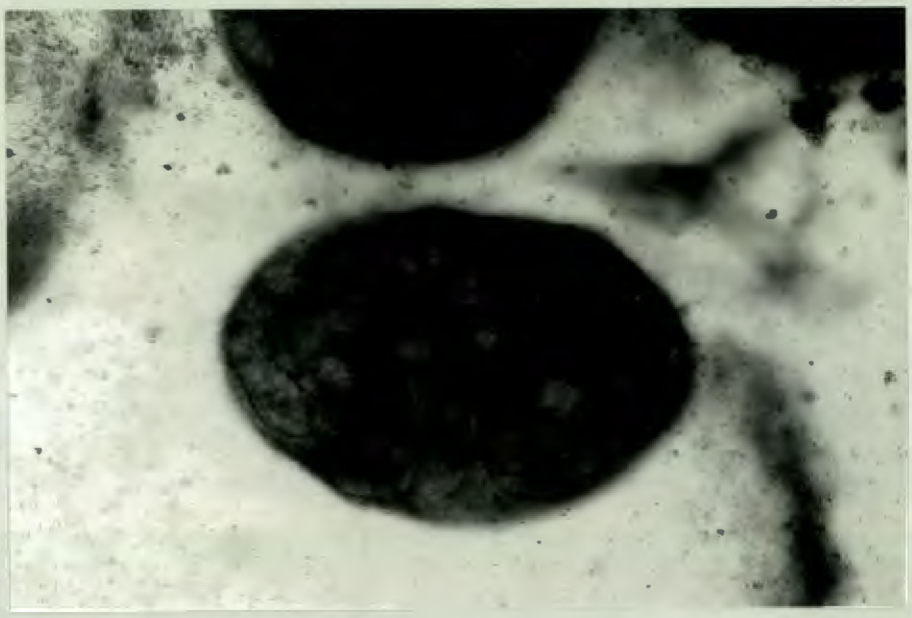


Fig. 4,3. <u>Sporocyst Y.</u>

Fig. 4,4. <u>Sporocyst Y</u> and escaping cercariae.

Fig 4,5. Sporocyst Y ruptured, releasing cercariae.

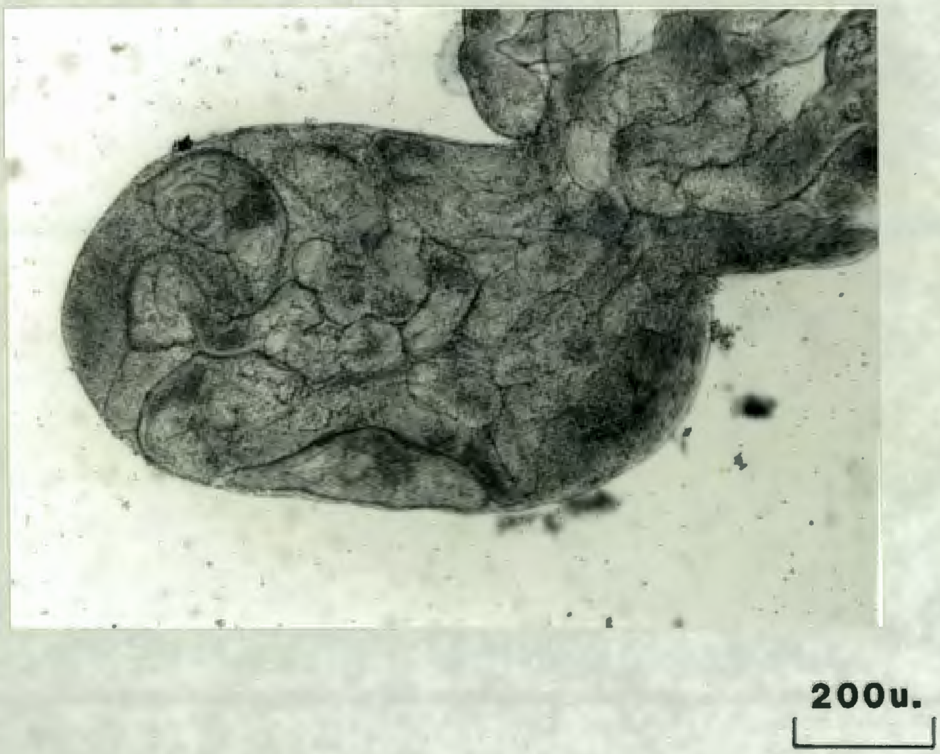


Fig. 4,6. Sporocyst Y ruptured, releasing cercariae.

Fig. 4,7. Sporocysts and cercariae type Y.

Fig. 4,8. Sporocyst and cercariae type Y.

Fig. 4,9. *Cercaria Y* escaping from sporocyst.

(Fig. 4.10. was taken ten seconds after fig. 4.9.)

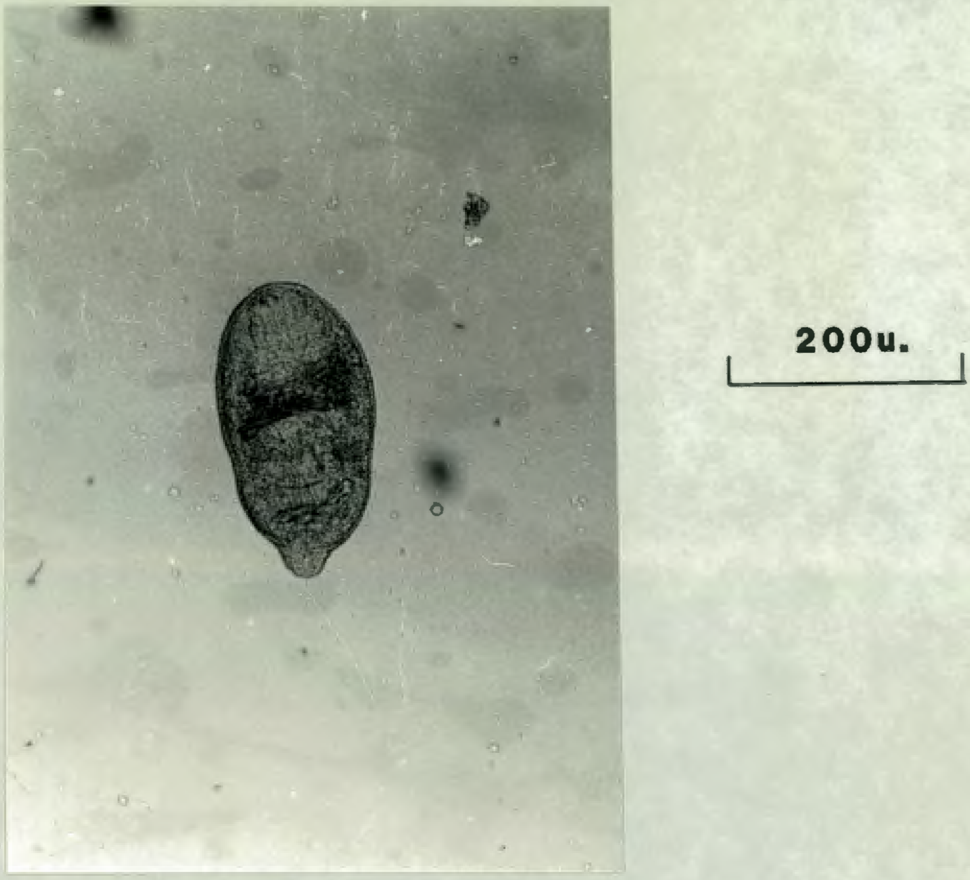


Fig. 4,11. *Cercaria Y* contracted.

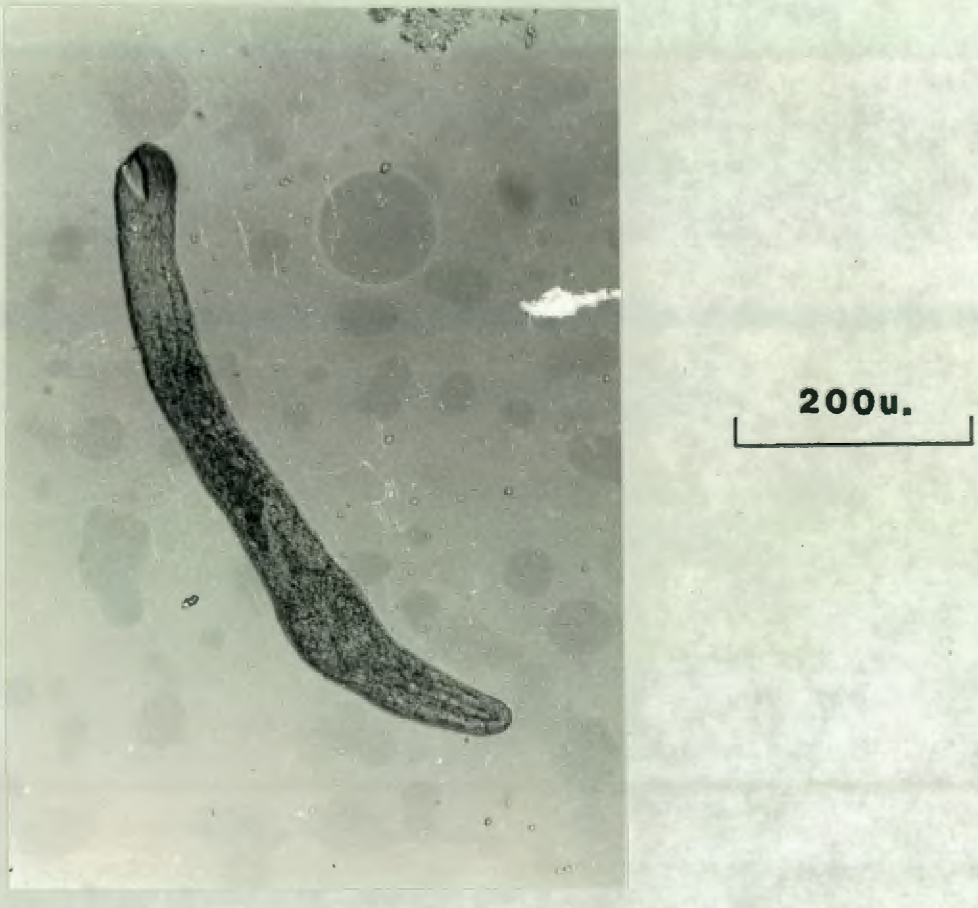


Fig. 4,13. Cercaria Y extended showing dorsal view of oral sucker.

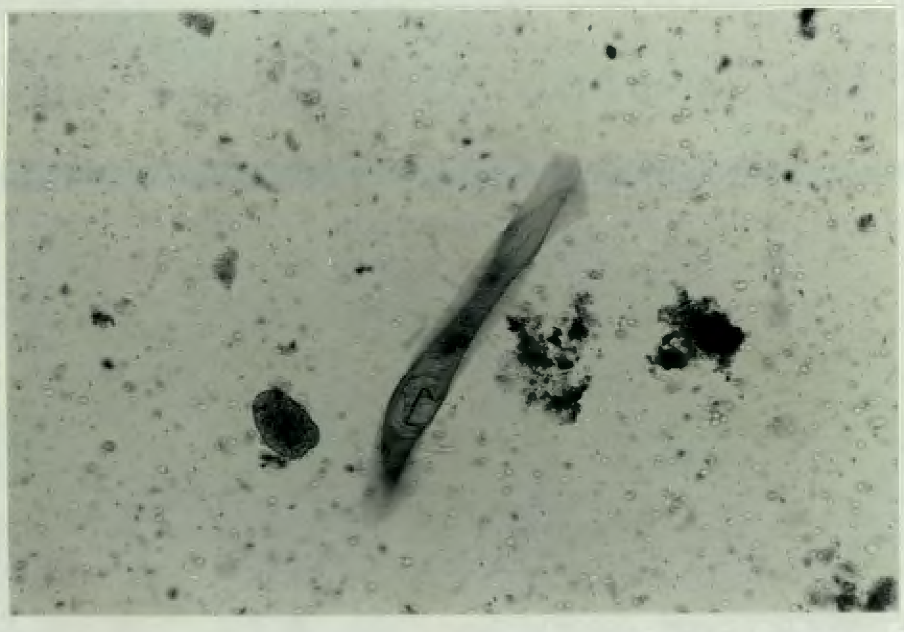


Fig. 4,14. Cercaria Y showing L/S ventral sucker.

Fig. 4,15. Cercaria Y showing full bladder.

Fig. 4,17. T/S. of gut and digestive gland of B. digitalis showing cercariae and sporocysts type Y.

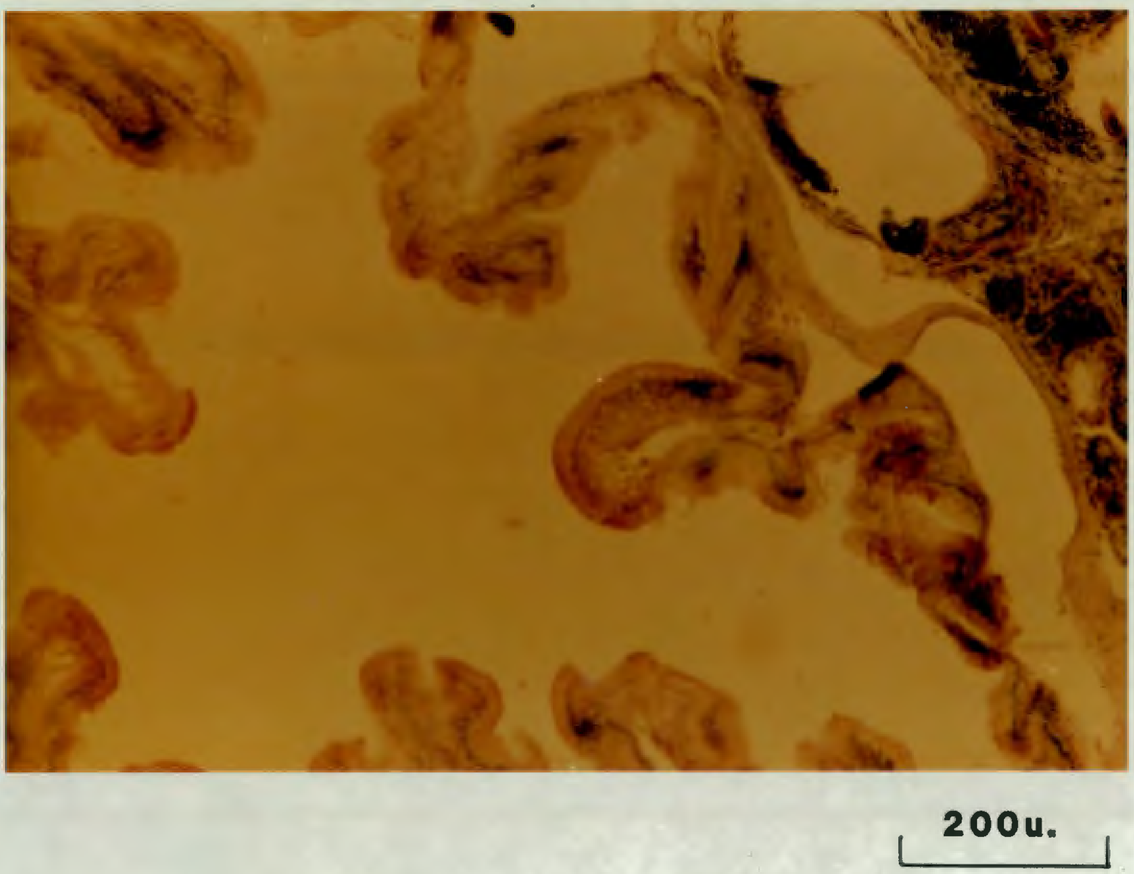


Fig. 4,18. Enlargement of fig.4,17. showing dark staining infected

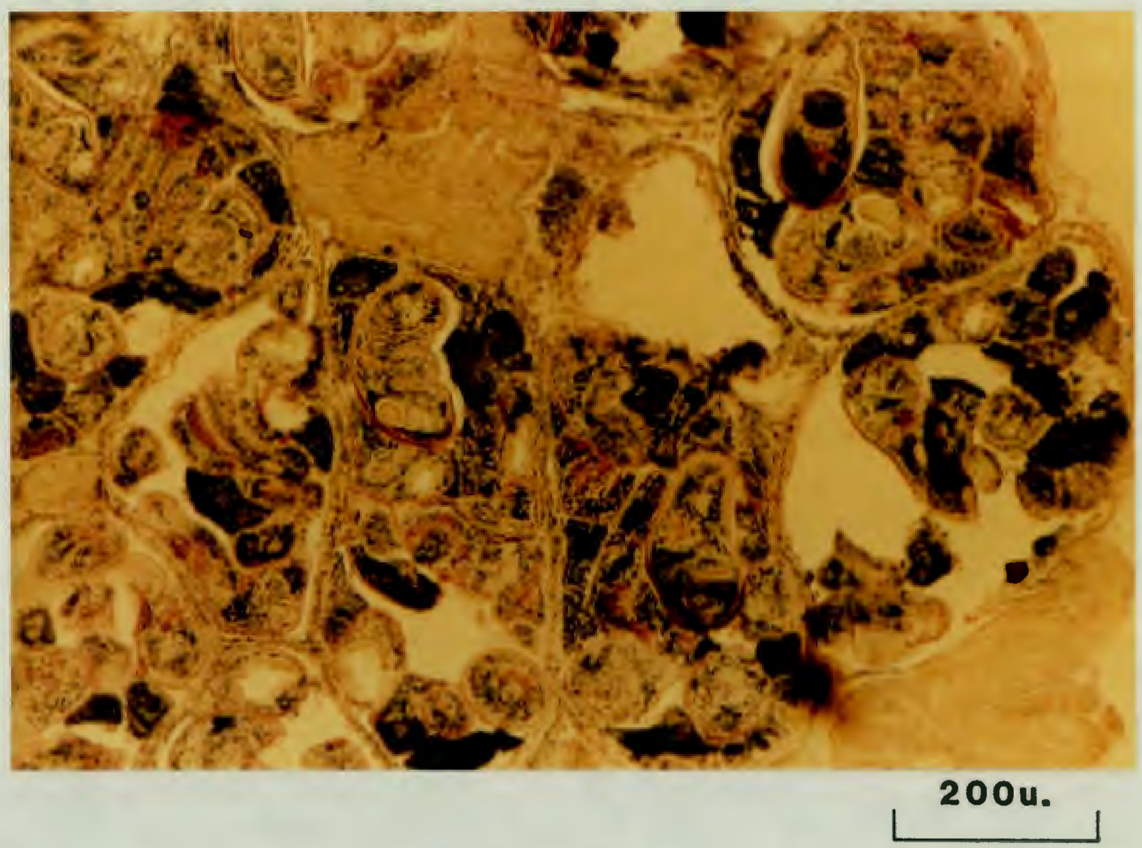


Fig. 4,19. Digestive gland of <u>B. digitalis</u> infected with cercariae and sporocysts <u>type Y</u>.

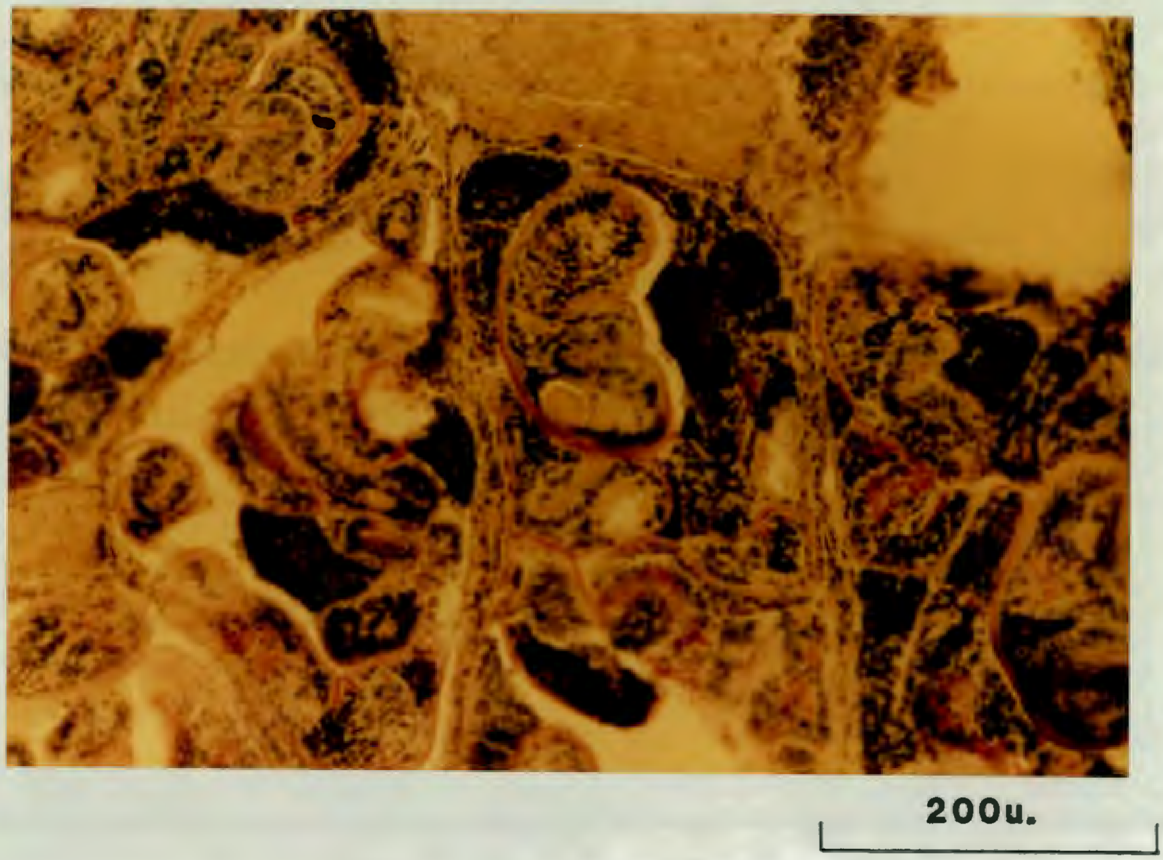


Fig. 4,20. Digestive gland of <u>B. digitalis</u> infected with cercariae and

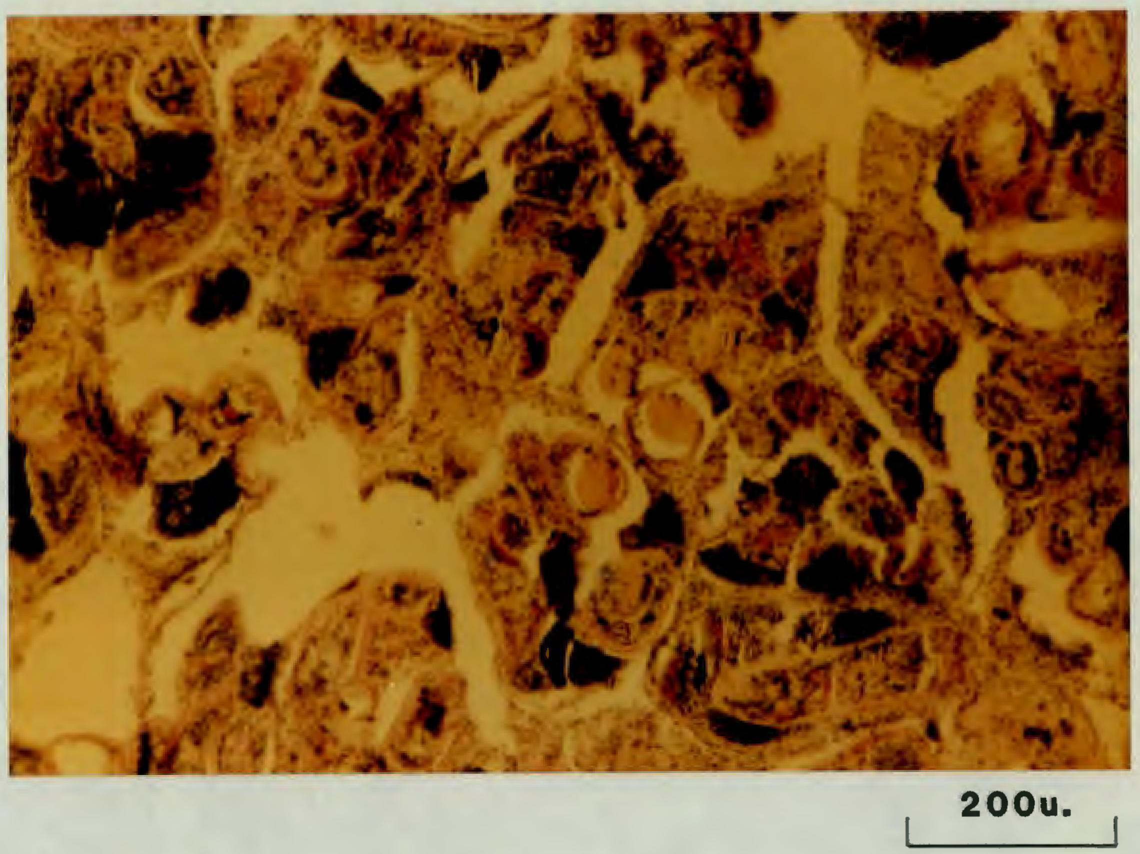


Fig. 4,21. Digestive gland of B. digitalis infected with cercaria and sporocysts type Y.

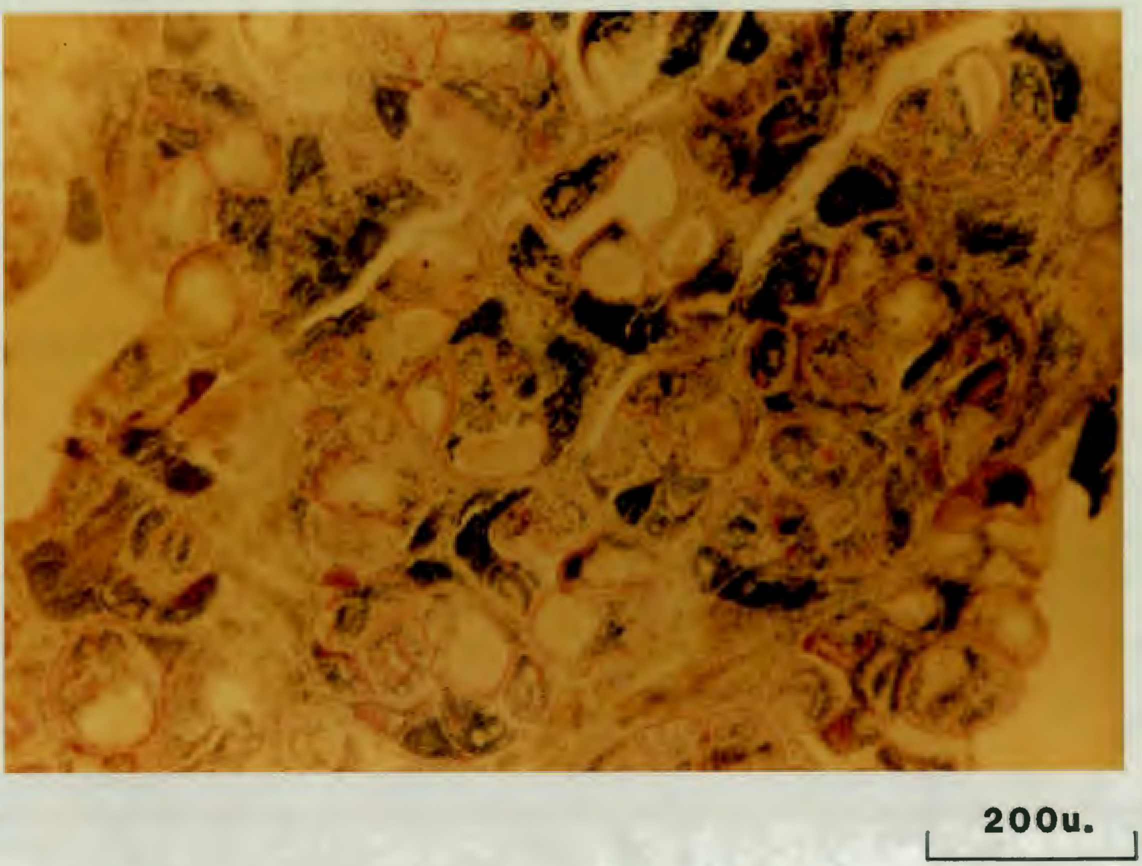


Fig. 4,22. Digestive gland of B. digitalis infected with cercariae and

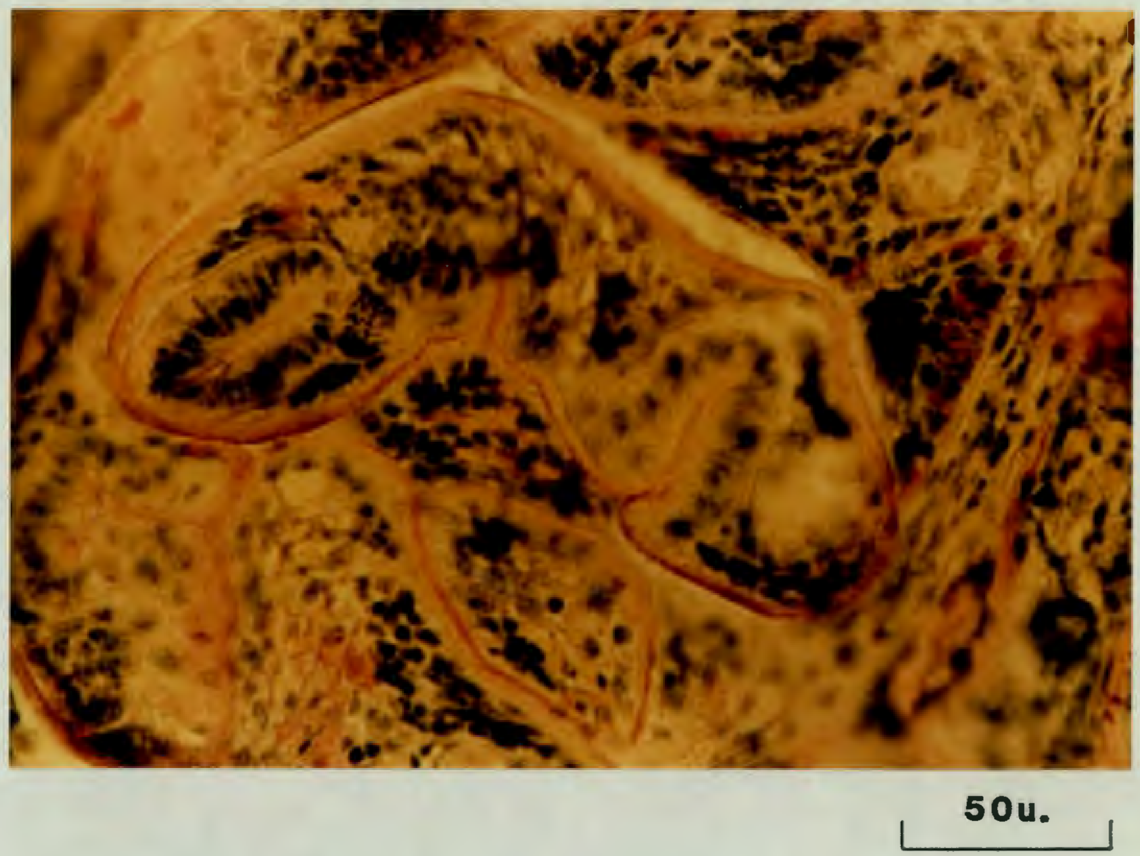


Fig. 4,23. L/S. of Cercaria Y.

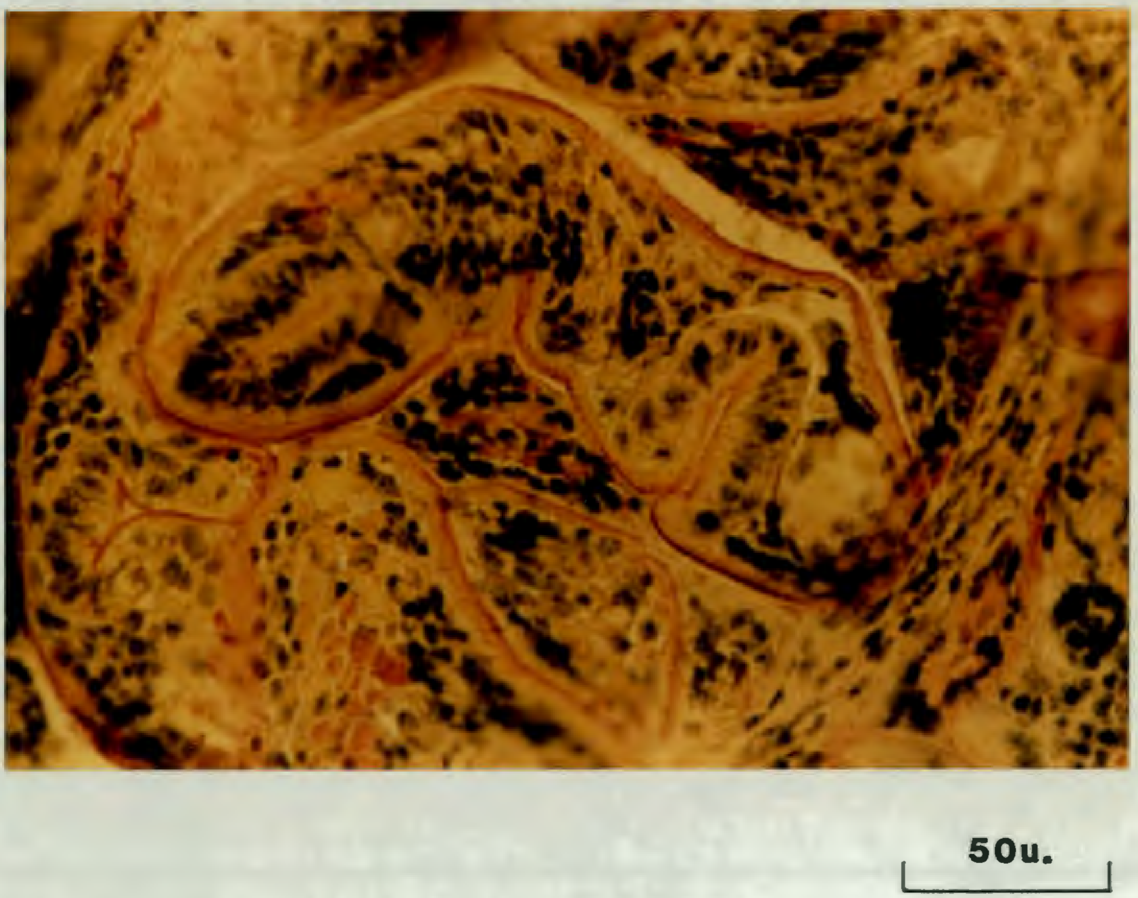


Fig. 4,24. L/S. of Cercaria Y. (Taken at different focus to fig .4,23.)

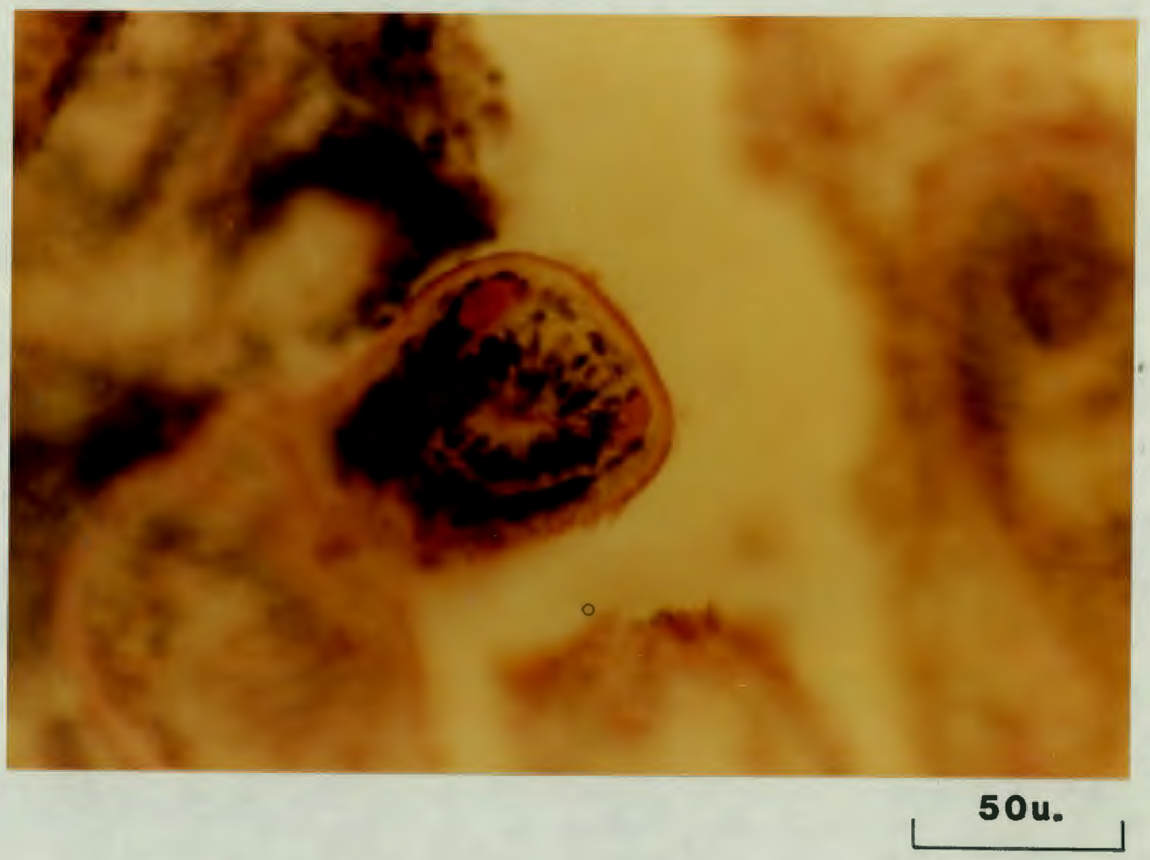


Fig. 4,25. T/S. of Cercaria Y showing oral sucker and penetration glands.

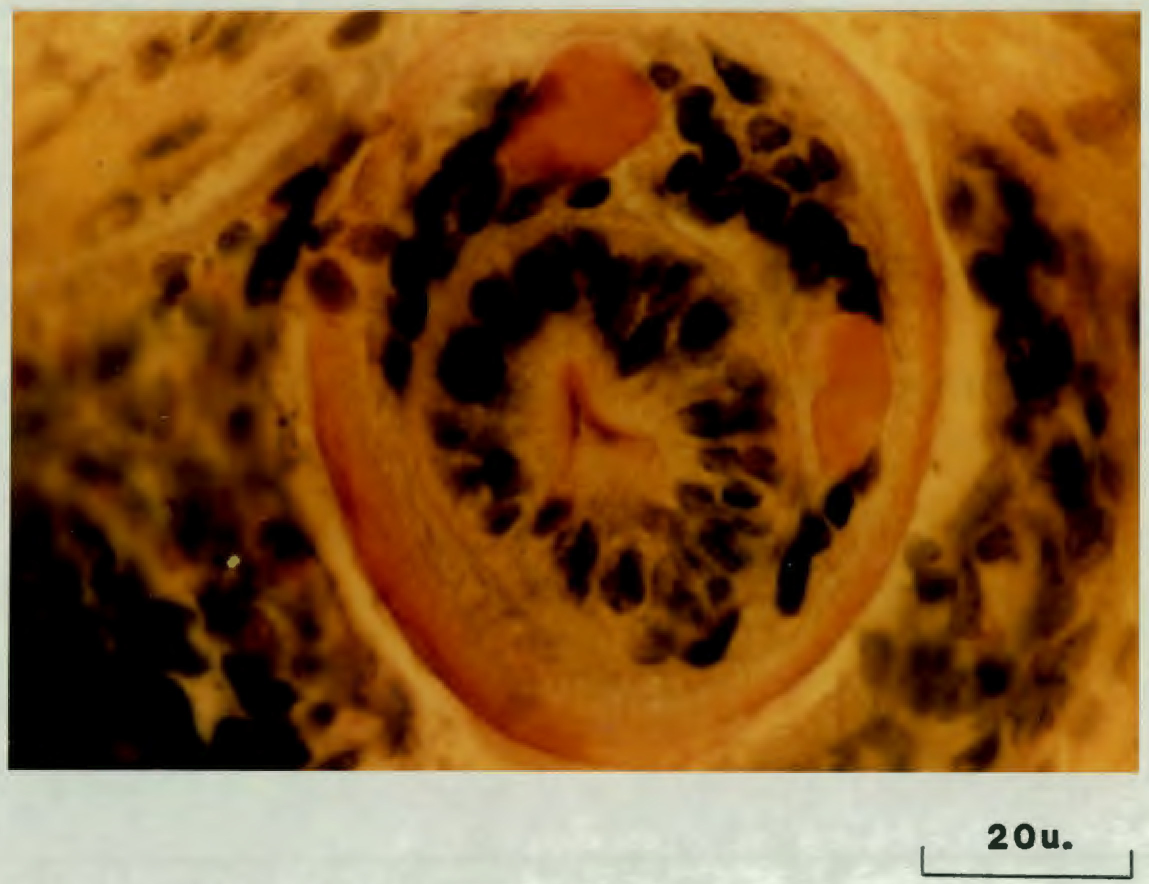


Fig. 4,26. T/S. of Cercaria Y showing oral sucker and penetration glands.

4.4 DISCUSSION

The superfamily Allocreadoidae is something of a taxonomic clearing house for dubious species. La Rue (1957) proposed a system in which the families Gorgoderidea : Gyliauchenidae, Troglotrematidae and Zoogonidae were provisionally assigned to the Allocreadoidea. It is probable that on further research, these families will be reassigned or have their taxonomic ranks revised.

Studies on the Allocreadoidea include those by Seitner (1951), Faust (1932), Holliman (1961), Ito (1964), Crowcroft (1951), Holton (1984). The descriptions of larval stages in the first four serve to underline the variability of the taxon.

Cercaria Y is never very common in Bullia but appears at a low level at Mnandi, Fish Hoek and Hout Bay. It is tailless and cannot swim. This suggests that the second intermediate host must come into close proximity. The apparent lack of cercarial emergence from Bullia supports this hypothesis (see Chapter 7.1).

Spontaneous encystment within Bullia was noted when infected digestive gland had been warmed. If this phenomenon was common it would imply that the second intermediate host had been eliminated. The presence of a stylet and well developed penetration glands, coupled with the abnormal temperature and rarity of encystment, refutes this. The ability of the cercariae to encyst indicates that they are fully mature. This is important as descriptions of the cercaria have been made from extracted specimens. The description could otherwise have been made from immature cercariae and be misleading.

The swimming crab ovalipes punctatus (De Haan) is a common predator of Bullia (McLachlan 1979; Du Preez 1981, 1984). The crab crushes the shell and tears the flesh of the whelk with its chelae. This would offer Cercaria Y the ideal opportunity to infect the crab.

Carcinus maenas, the green shore crab of the northern hemisphere, is known for its predation on intertidal gastropods. Elner and Raffaelli (1980) produced an account of Carcinus infecting itself with a microphallid digenean from Littorina spp.

In Chapter 7.4 the upper lethal temperature of Cercaria Y has been investigated. This temperature reflects the tolerance of the adult worm. In this case temperatures above 30°C were determined to be harmful; hence we can conclude that the final host is not warm-blooded. A crab-eating ray is suggested as the likely candidate. An examination of Rhinobatis spp. may be profitable.

CHAPTER 5

CERCARIA AND SPOROCYST TYPE X

(FAMILY ZOOGONIDAE ODHNER, 1911)

ZOOGONOIDES SP.?

HOST: *BULLIA DIGITALIS*

5.1 SPOROCYST

This simple sac-like sporocyst has a smooth tegument. The sporocyst wall is thin and is breached at one end by a birth pore. No flame cells or other excretory structures could be made out. In mature sporocysts, only two apparently mature cercariae were seen in the sporocyst. Other half formed cercariae and germ balls could be seen inside the mature sporocysts. Only daughter sporocysts were seen. Numerous immature sporocysts were seen, these were about half of the size of mature specimens and were almost entirely filled with germinal material.

In Table 5.1 the width was taken at intervals of 25%, 50% and 75% of the sporocyst body length starting at the end bearing the birth pore.

TABLE 5.1 Measurements of Sporocyst X A: living; B: formalin-killed

	Mean um	S.D. um	S.E. um	C.V. %	n	Max. um	Min. um
A							
Length	551	108	51,1	19,5	10	778	388
Width 25%	200	40	18,8	19,8	10	251	138
50%	227	23	11,0	10,2	10	267	194
75%	207	38	18,3	18,6	10	259	138
B							
Length	558	83	39,5	14,9	10	648	405
Width 25%	234	18	8,7	7,8	10	259	194
50%	272	45	21,3	16,6	10	364	226
75%	219	46	21,7	20,9	10	299	162

TABLE 5.2 Number of mature, recognisable cercariae in each sporocyst

Mean	S.D.	S.E.	C.V.	n	Max.	Min.
1,6	0,52	0,16	32,3	10	2	1

5.2 CERCARIA

Diagnosis

The tegument is heavily armed with spines all over its surface (see Figs 5.5, 5.8 and 5.9). The oral sucker is elipsoidal and opens subterminally. There is a simple conical stylet which has its flat base embedded in the sucker musculature. The stylet is held at 45° to the horizontal. The sucker has a series of oral papillae (see Fig. 5.3.A). Two papillae lie anterior and four lie posterior to the mouth. The pre-pharynx is very short and leads to a broad pharynx. The oesophagus passes posteriorly from the pharynx and becomes unobservable just anterior to the ventral sucker. The ventral sucker is about one third larger than the oral sucker and is oblate spheroid in shape. There are six flame cells, no excretory ducts could be distinguished. The bladder is thick-walled and muscular. It opens directly at the centre of the disc-like 'caudal sucker'. This structure is not a sucker in the sense of an oral or ventral sucker because it is a modification of the cercarial tail. Nevertheless it is often used as a holdfast and henceforth in this account it will be referred to as a caudal sucker. During body movements, the caudal sucker may disappear or even turn into a pointed termination of the body (see Fig. 5.3.C and D). The excretory pore is defended by eight large spines, each being from six to eight um long. The apices of the spines point to the opening of the pore. During microscopic examination no evidence of penetration glands was visible in any specimens examined. In Fig. 5.12, however, there are some dark staining patches (with neutral red) which could be evidence of penetration glands but this is not conclusive.

The spines and tegument take up neutral red stain very strongly. This stimulates production of stained mucus which can be seen in Fig. 5.11.

In preparations of infected *Bullia digitalis* digestive gland that are stained with Ehrlich's haematoxylin and eosin, it is interesting to note that some cercariae take up haematoxylin very strongly, while others barely stain. This phenomenon has been noticed even in a single sporocyst.

Movement

There is no swimming behaviour. Movement is worm-like with apparently aimless contraction and expansion. The oral and ventral suckers are not used in locomotion. The body is highly contractile and variable (see Figs 5.4 to 5.9). Neutral red stain elicits a toxic response.

5.3 EPIDEMIOLOGY OF TYPE X IN *B. DIGITALIS*

One whelk from a sample of 50 taken from Melkbos in February 1985 was found to be infected with type X. This gives an infection rate of 2% for the collection. overall 1378 whelks have been taken from Melkbos. This gives an infection rate of 0,07%.

TABLE 5.3 Measurements of *Cercaria X*. A: living; B: heat-killed; C: formalin-killed

	Mean u	S.D. u	S.E. u	C.V. %	n	MAX. u	MIN. u
A							
length	348	146	46,0	41,8	10	648	200
width 25%	106	46	14,5	43,3	10	202	40,5
width 50%	120	40	12,6	33,3	10	162	40,5
width 75%	112	39	12,3	34,6	10	162	56
oral sucker: length	60	8,8	2,8	14,7	10	75	50
width	53	12,1	3,8	22,8	10	75	35
pre-pharynx: length	1				1		
pharynx: length	25				2	25	25
width	27,5				2	30	25
ventral sucker:							
length	82	20,9	7,0	25,5	9	125	50
width	77	20,0	7,6	25,9	7	100	50
depth	71	25,3	12,6	35,5	5	100	50
caudal sucker width	72	7,4	2,8	10,3	7	81	64
B							
length	268	70	24,6	26,0	8	370	175
width 25%	101	22	7,4	21,9	9	146	72
width 50%	110	27	8,9	24,2	9	175	88
width 75%	89	12	4,1	13,7	9	98	64
depth	98				1		
oral sucker: length	60	8,5	2,7	14,2	10	78	49
width	59	13,9	4,4	23,6	10	78	29
pre-pharynx length	3,9				3	3,9	3,9
pharynx length	19,2	3,1	1,3	16,3	6	23,4	15,6
width	24	6,4	2,6	26,5	6	29,2	11,7
oesophagus: length	116				2	175	58,5
ventral sucker:							
length	76	11,8	3,9	15,6	9	97,5	58.5
width	80	10,8	3,6	13,5	9	101	68
caudal sucker: width	66	6,8	3,4	10,3	4	74	58,5
cuticle spines: length	2,4				2	2,4	2,4
pitch	2,1				3	2,4	1,6
stylet length	16,1	2,9	1,4	18,0	4	19,5	13,7
width	4,3	0,7	0,2	15,7	10	5,8	3,9

TABLE 5.3. (continued)

	Mean um	S.D. um	S.E. um	C.V. %	n	MAX. um	MIN. um
C							
length	340	47	14,9	13,8	10	409	273
width 25%	103	23	7,3	22,3	10	132	68
width 50%	123	21	6,6	17,0	10	156	88
width 75%	107	9	2,9	8,5	10	117	88
oral sucker: length	62,5	5,5	1,7	8,7	10	68	52,6
width	56,8	5,9	1,9	10,4	10	66,3	49
pre-pharynx: length	4,16	1,3	0,5	31,8	7	5,8	1,95
pharynx: length	18,6	3,9	1,3	21,0	9	23,4	11,7
width	19,9	2,5	0,9	12,8	9	23,4	15,6
oesophagus: length	78				2	87,8	68,2
ventral sucker:							
length	78,3	12,2	3,9	15,6	10	87,8	48,2
width	76,0	13,2	4,4	17,4	9	93,6	58
caudal sucker width	54	5,7	2,2	10,6	7	62,4	48,7
stylet length	18	3,0	1,5	16,7	4	19,5	13,65
width	4,1	0,7	0,2	16,6	8	5,85	3,9

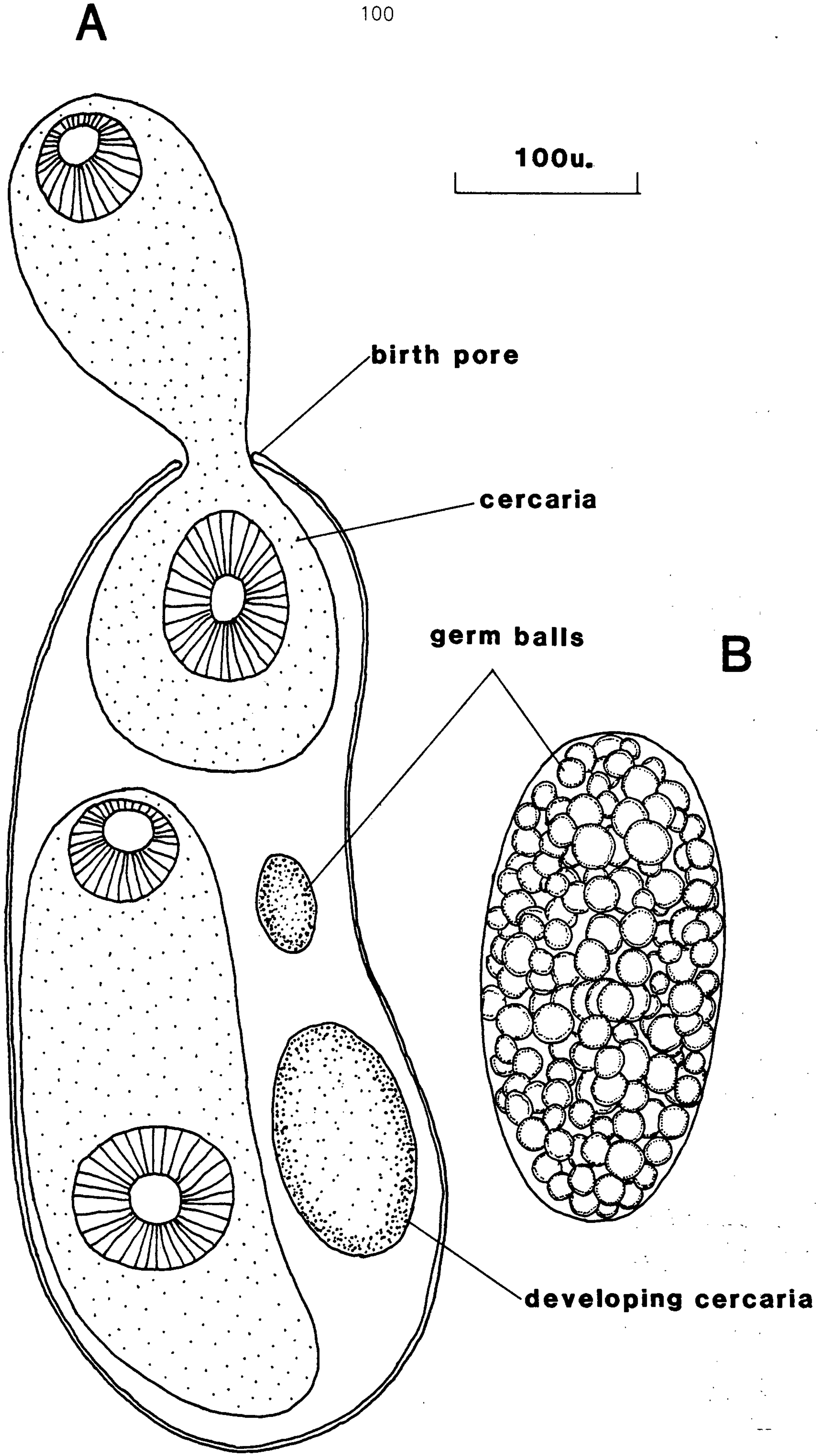


Fig. 5,1. Sporocyst X A, mature sporocyst. B, immature sporocyst.

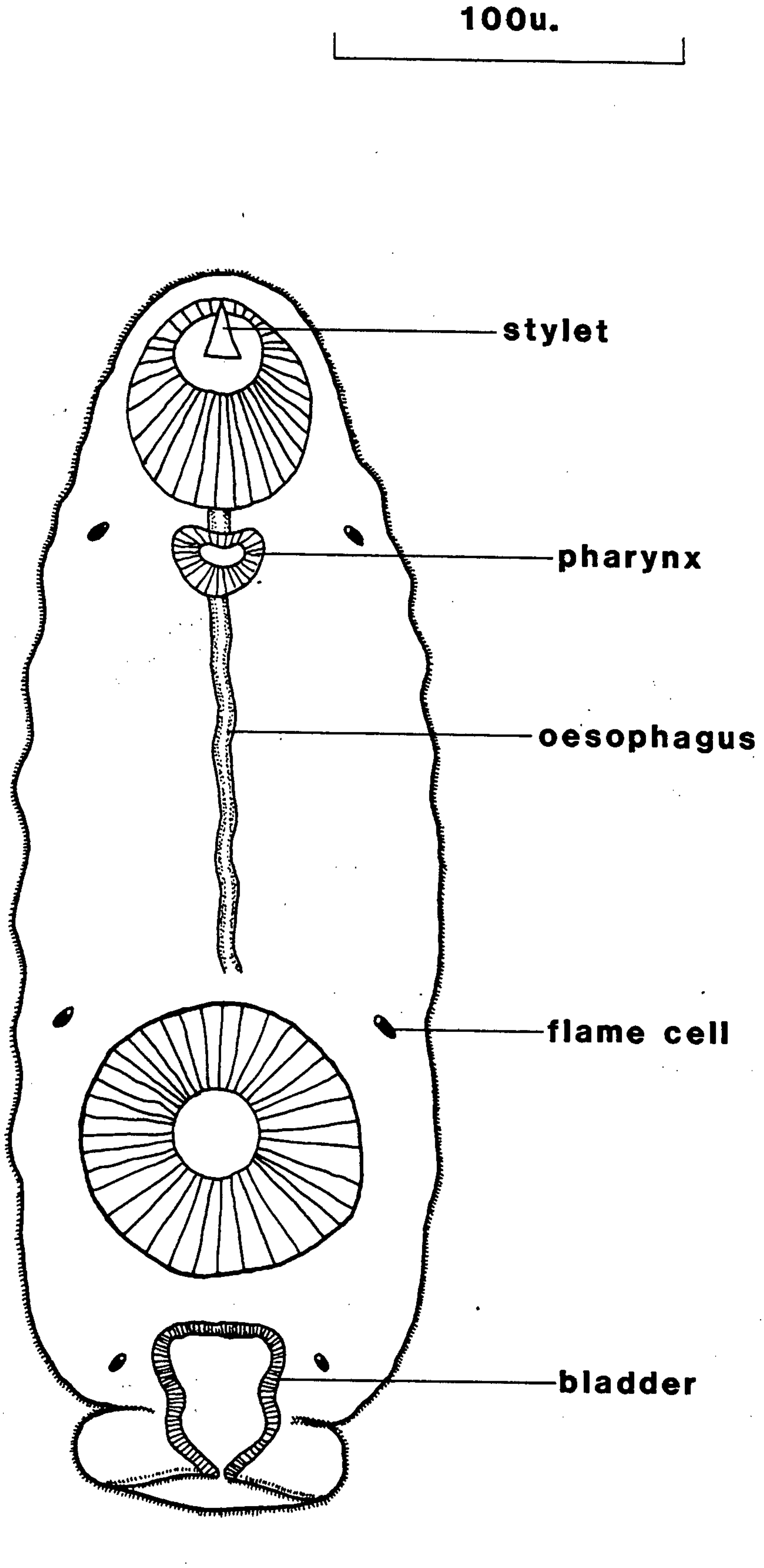


Fig. 5,2. Cercaria X ventral view showing internal structure.

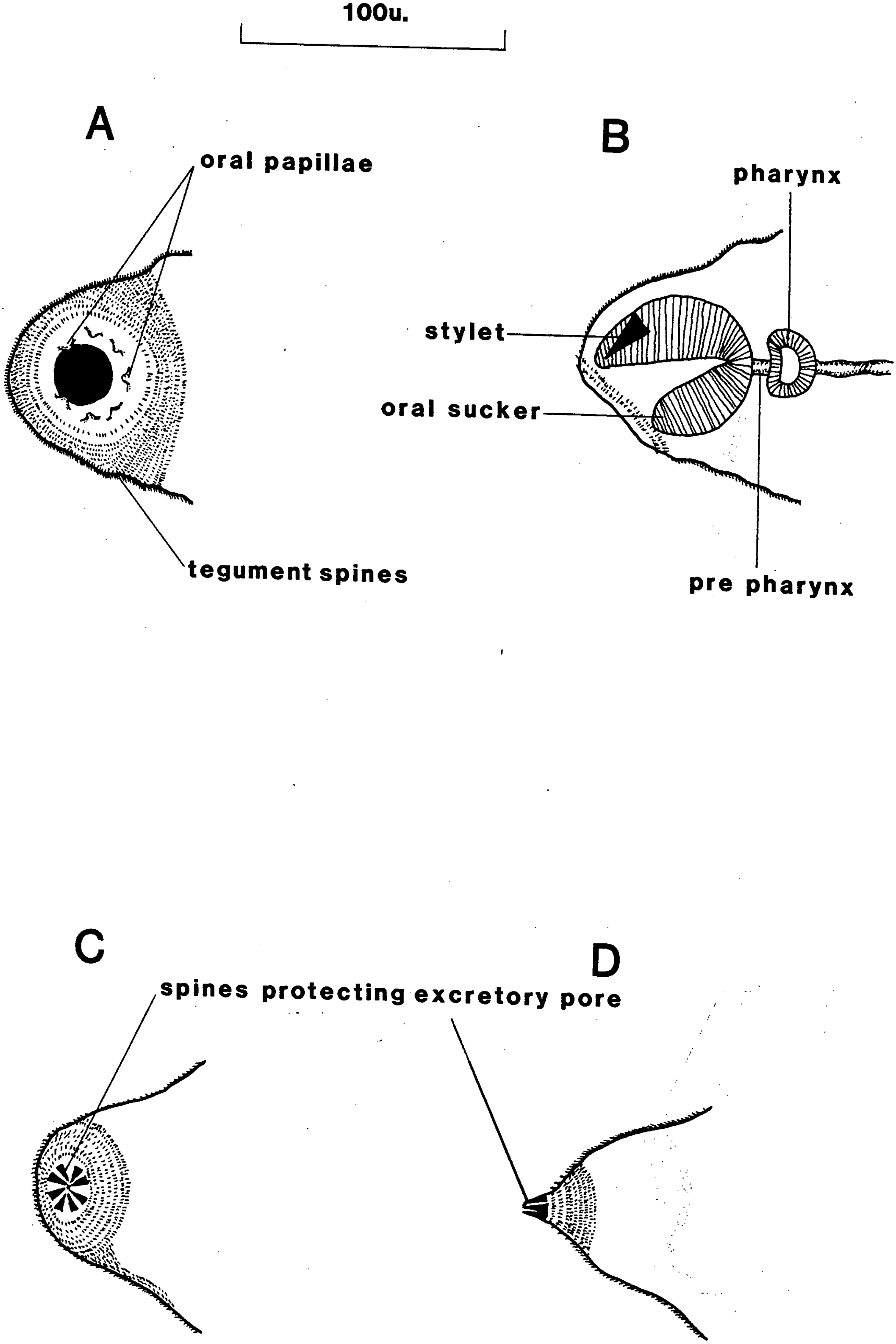


Fig. 5,3. Cercaria X A, external features of anterior. B, anterior in longitudinal section. C, caudal sucker evaginated. D, alternative appearance of

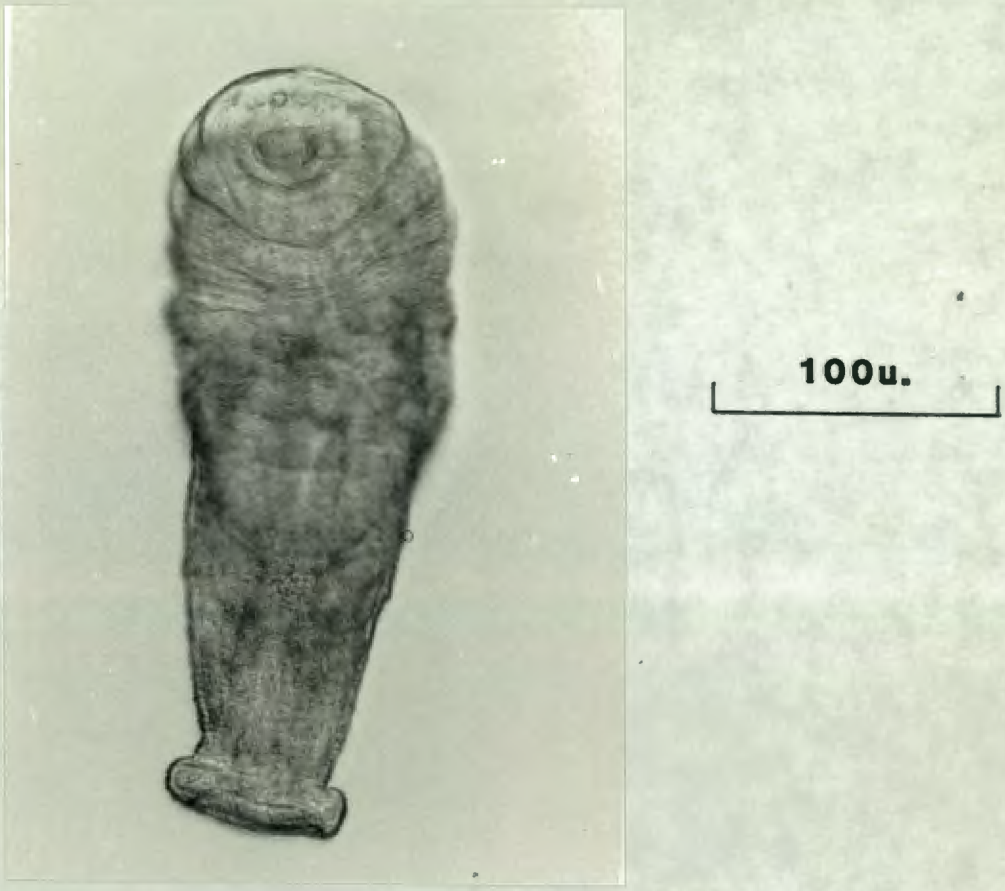


Fig. 5,4 Cercaria X

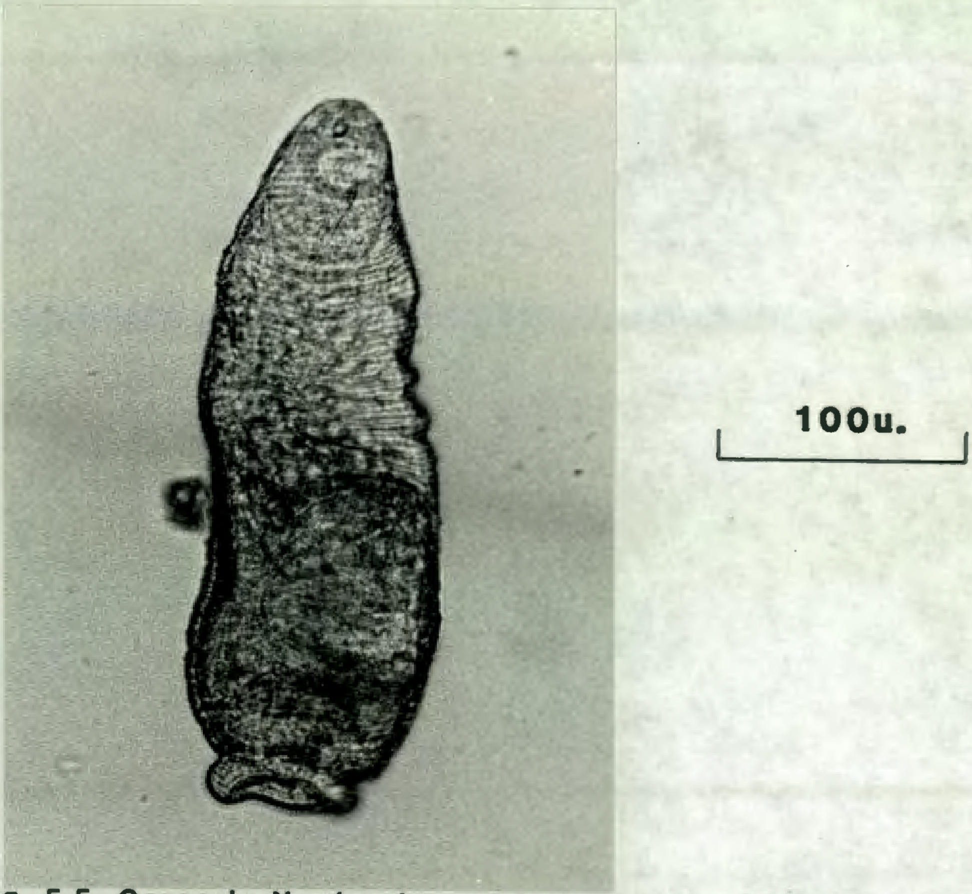


Fig. 5,5 Cercaria X showing striations caused by spines.

Fig.5,6. Cercaria X extended.

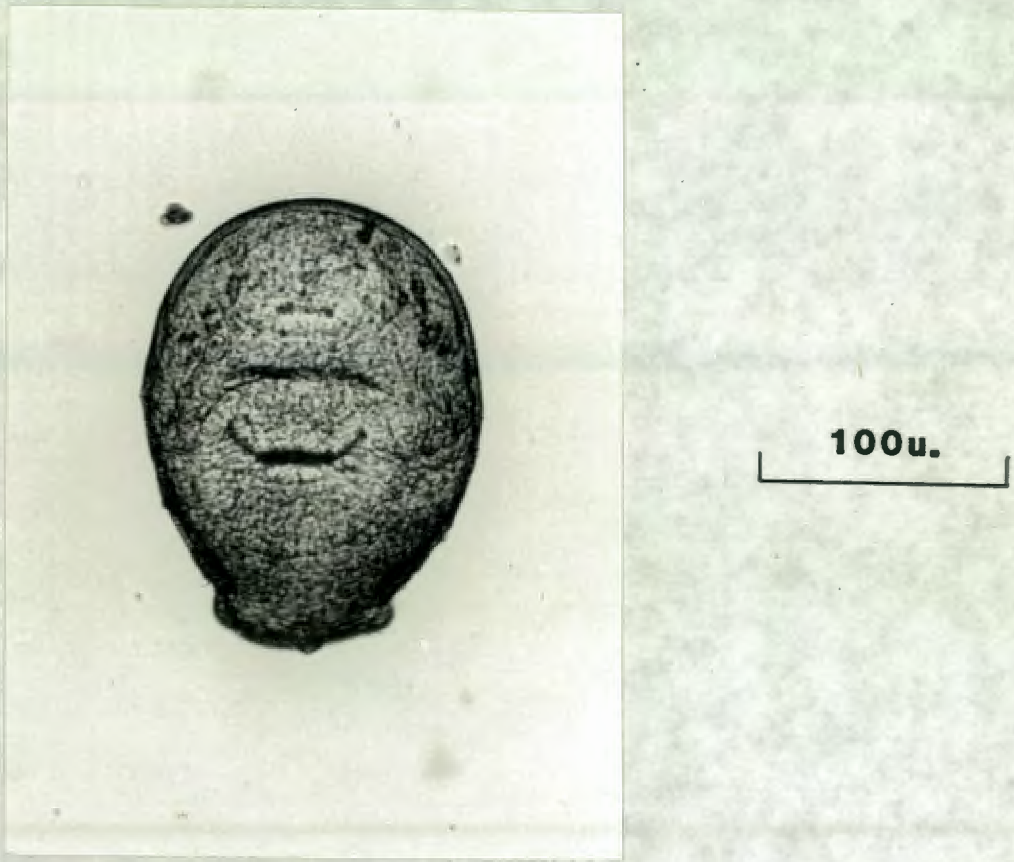


Fig.5,7. Cercaria X contracted.

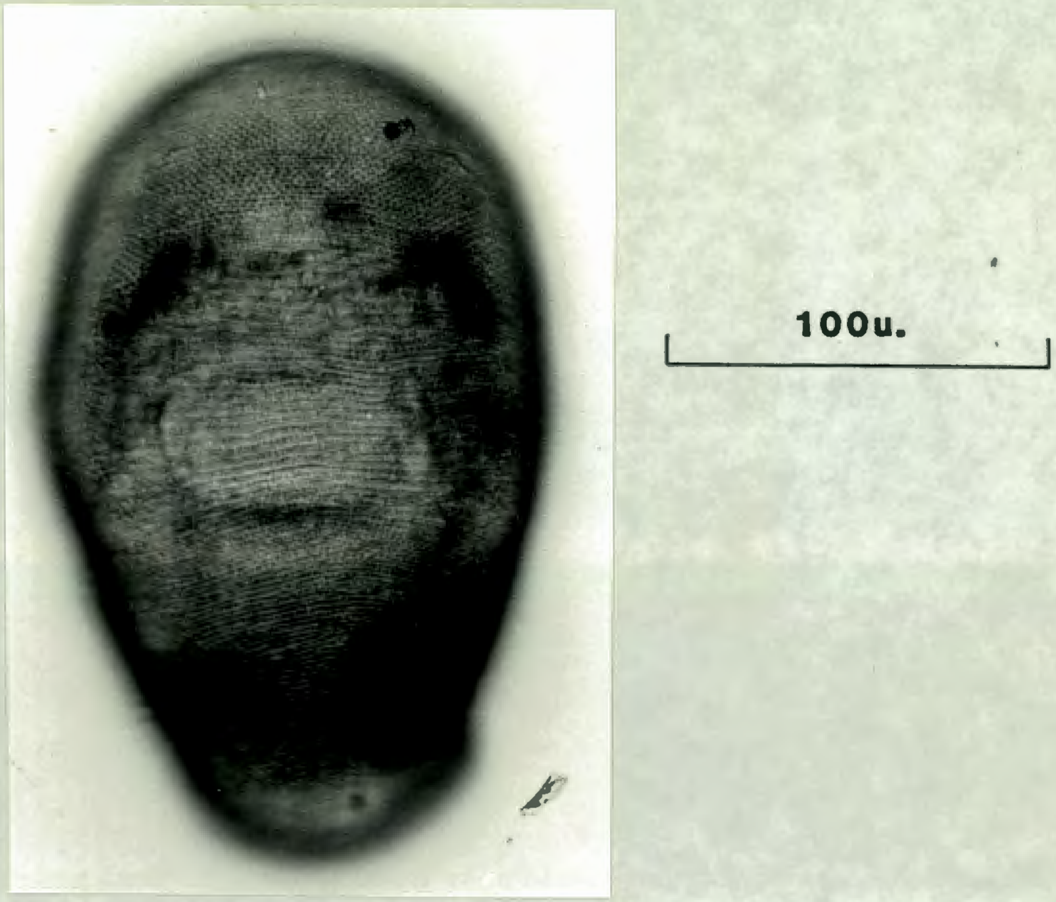


Fig. 5,8. Cercaria X showing tegument spines.

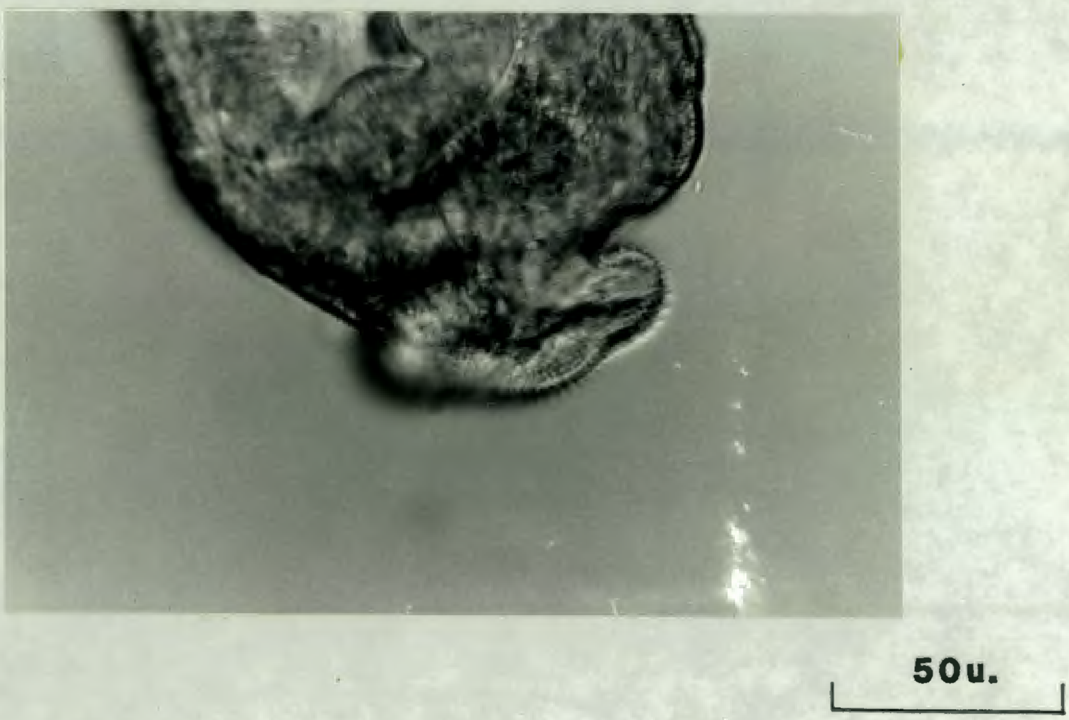


Fig. 5,9. Cercaria X

Fig. 5,10. Cercaria X showing L/S. of the oral sucker.

Fig. 5,11. Cercaria X releasing mucus from the tegument in response to neutral red stain.

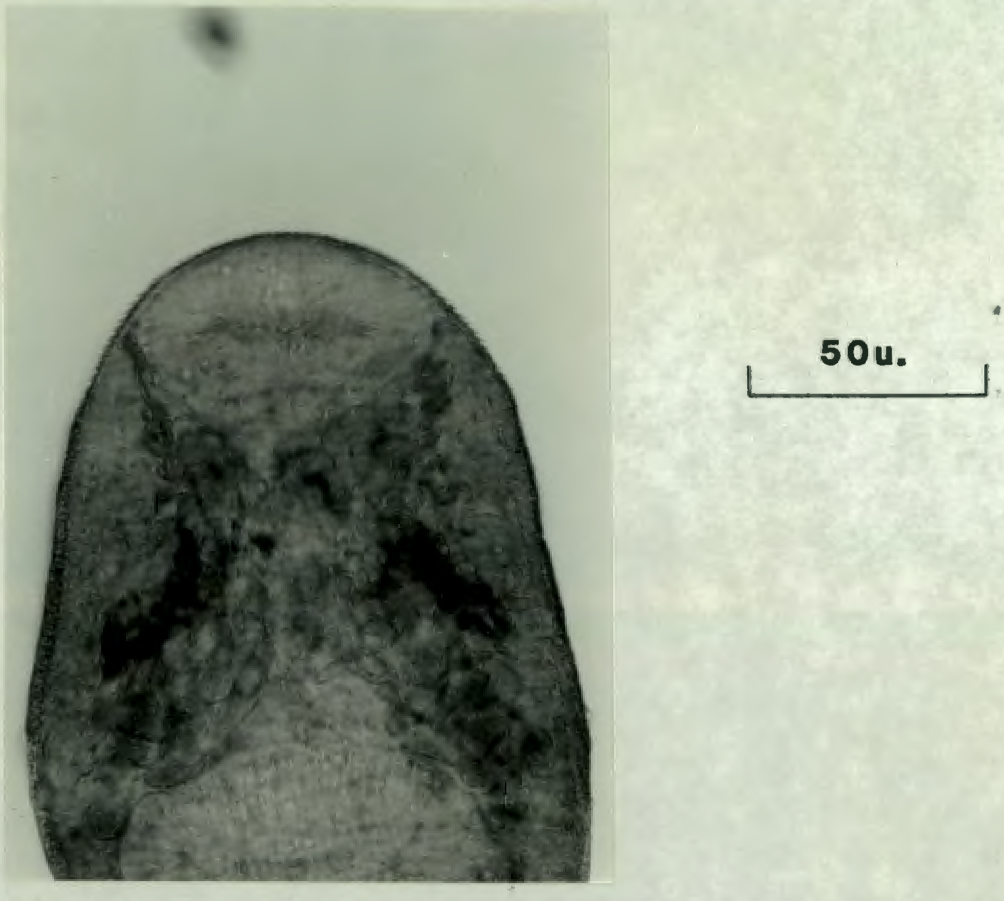


Fig 5,12. Cercaria X anterior.

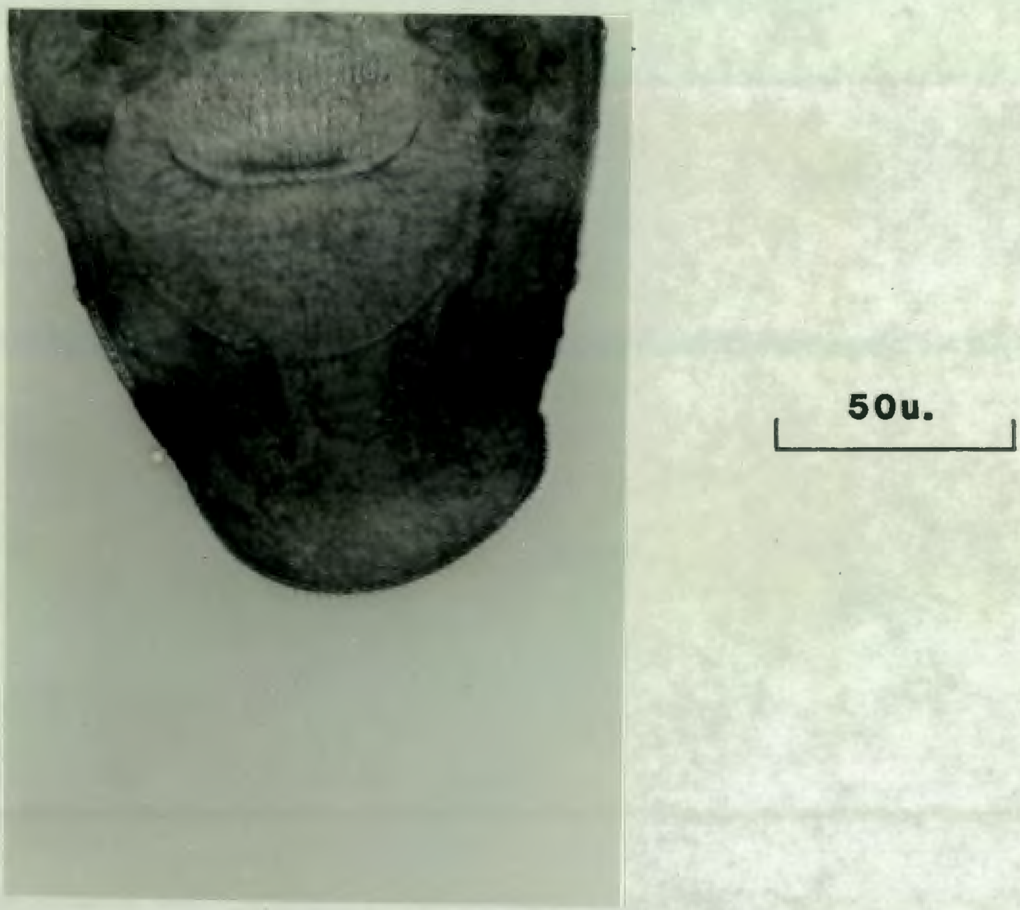


Fig. 5,14. Section through the gut and digestive gland of B. digitalis showing sporocysts and developing cercariae type X.

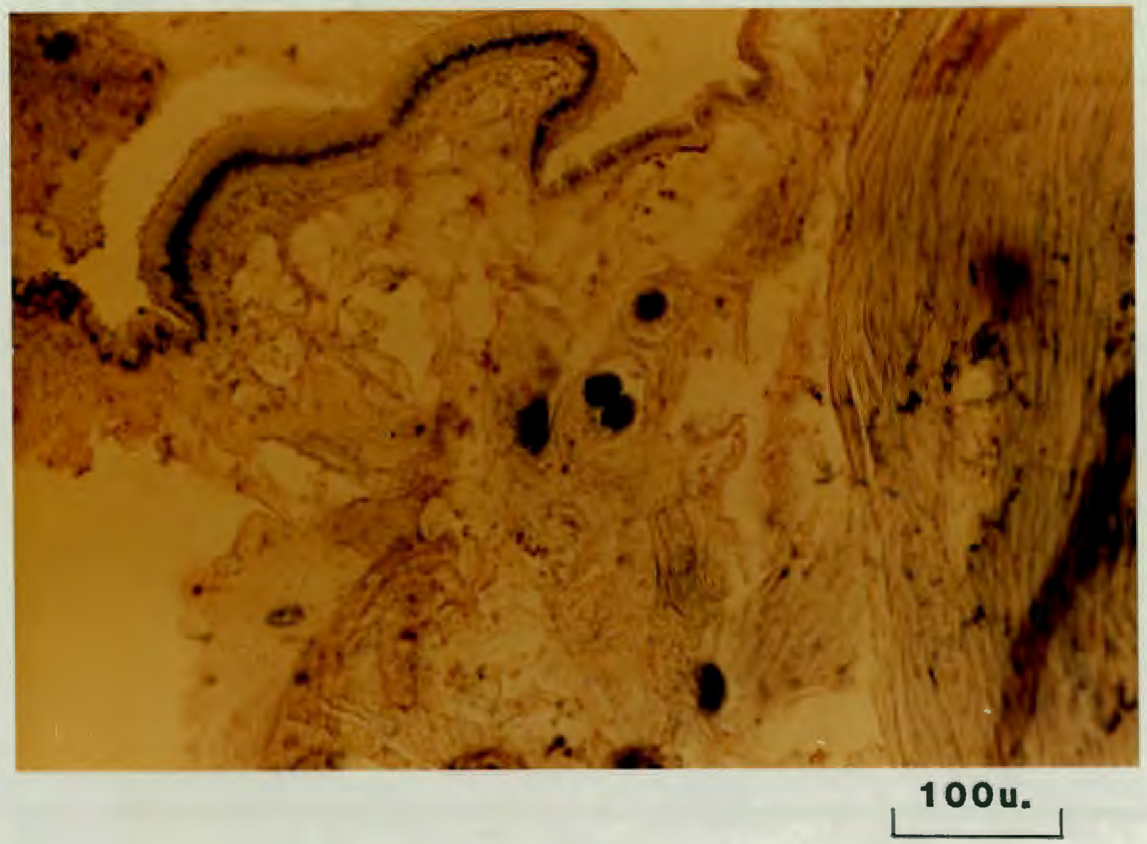


Fig. 5,15. Section through the gut and digestive gland of B. digitalis

5.4 DISCUSSION

The adults of the family Zoogonidae have featured in works by Bray (1985), Koie (1983), Holton (1983) and Fantham (1938). The account by Koie (1983) deals with Phyllodistomum tonaaatense (sic) in the teleost Coracinus multifasciatus from South Africa. Also from South Africa, Fantham (1938) described Lecithostaphylus spondyliosomae from the Hottentot fish Spondyliosoma blochii.

Larval stages of Zoogonoides viviparus have been described in Dawes (1946). Fretter and Graham (1962) also cite Buccinum undatum as the gastropod host.

Cercaria X bears a striking resemblance to Z. viviparus, except in the fine structure of the stylet. It is tentatively suggested that Cercaria X may belong to the same genus.

In common with other xiphidiocercariae it is most likely that Cercaria X penetrates and encysts in an arthropod second intermediate host. According to Stunkard (1938) Zoogonus spp. have been found in the gastropod Nassa obsoleta. The metacercariae have been found encysted in polychaets, especially Nereis virens. The final host are thought to be eels or toadfish. Stunkard (1941) found metacercariae of Zoogonus sp. in the sea urchin Psammechinus miliaris.

From Melkbos, the only site where Cercaria X was found, one whelk in over 1300 was infected. Possibly the infection is accidental in Bullia, the normal host living elsewhere. The presence of a zoogonid in the rocky shore, Hottentot fish, together with the elementary nature of this classification of Cercaria X makes such an hypothesis possible.

CHAPTER 6

METACERCARIA W

(FAMILY AZYGIIDAE ODHNER, 1911)

HOSTS: BULLIA DIGITALIS, BULLIA PURA

6.1 DIAGNOSIS

No parthenitae were found in any whelks. A dorsoventrally flattened tailless distome. The tegument is covered with fine transverse wrinkles. The crests of the wrinkles consist of rows of small nodules (see Fig. 6.2.A and B). The oral sucker opens sub-terminally. There is a bulge inside the sucker where the pre-pharynx opens into the sucker. The pre-pharynx is very short in comparison to the oesophagus. The pharynx is muscular and is about half of the diameter of the oral sucker. The pharynx is longer than broad. The oesophagus is about ten times longer than the pre-pharynx. The oesophageal junction with the caecae occurs anterior to the ventral sucker at about one-third of the body length from the front. The ventral sucker is about half as big again as the oral sucker diameter. The caecae terminate level with the anterior-most extent of the bladder. The bladder is relatively small and occupies the last twentieth of the body. The bladder is thick-walled and muscular. The excretory duct bifurcates at about three-quarters of the body length from the front. The two excretory ducts then proceed anterior, they recurve at the oral sucker and then run to the posterior extremity (see Fig. 6.1). The exceretory system is stenostomate. The ventral sucker has numerous brown nodules embedded in the muscle tissue. The nodules range in size from 4 um to 16 um.

The ovary is medial and posterior to the two testes. It is about half of the size of a testis. The testes lie laterally in the body, the left being staggered ahead of the right. The left vas efferens is shorter than the right. They meet to the left and dorsally to the ventral sucker. The vas deferens proceeds from here to the seminal vesicle. The cirrus lies in front of the ventral sucker and points obliquely to the left. The uterus is convoluted and extends from just posterior to the left testis to the margin of the ventral sucker on its right. It was impossible to ascertain the flame cell formula with the small number of specimens available (2). At least 24 flame cells were seen on each side, however. The positions of those seen are given on the left side of Fig. 6.1. The tegument is pigmented heavily at the anterior. The pigmentation becomes lighter towards the posterior, becoming light brown behind the ventral sucker.

6.2 MOVEMENT

The ventral sucker is used as a holdfast. The anterior is stretched and contracted vigorously in a leech-like manner. The posterior is not so mobile (see Figs 6.3, 6.4 and 6.5 for details of movement).

TABLE 6.1 Measurements of Metacercaria W living. Only one specimen was available for measurement.

		Units um
Length of body:	contracted	1078
	extended	2002
Oral sucker	length	202
	width	178
Ventral sucker	length	308
	width	283
Pharynx	length	89
	width	64
pre-pharynx	length	25
Ovary	diameter	49

6.3 INFECTION INCIDENCE

B. pura

One whelk in 150 was infected giving an incidence of 0,7%.

B. digitalis

Two whelks in 494 were infected giving an incidence of 0,4%.

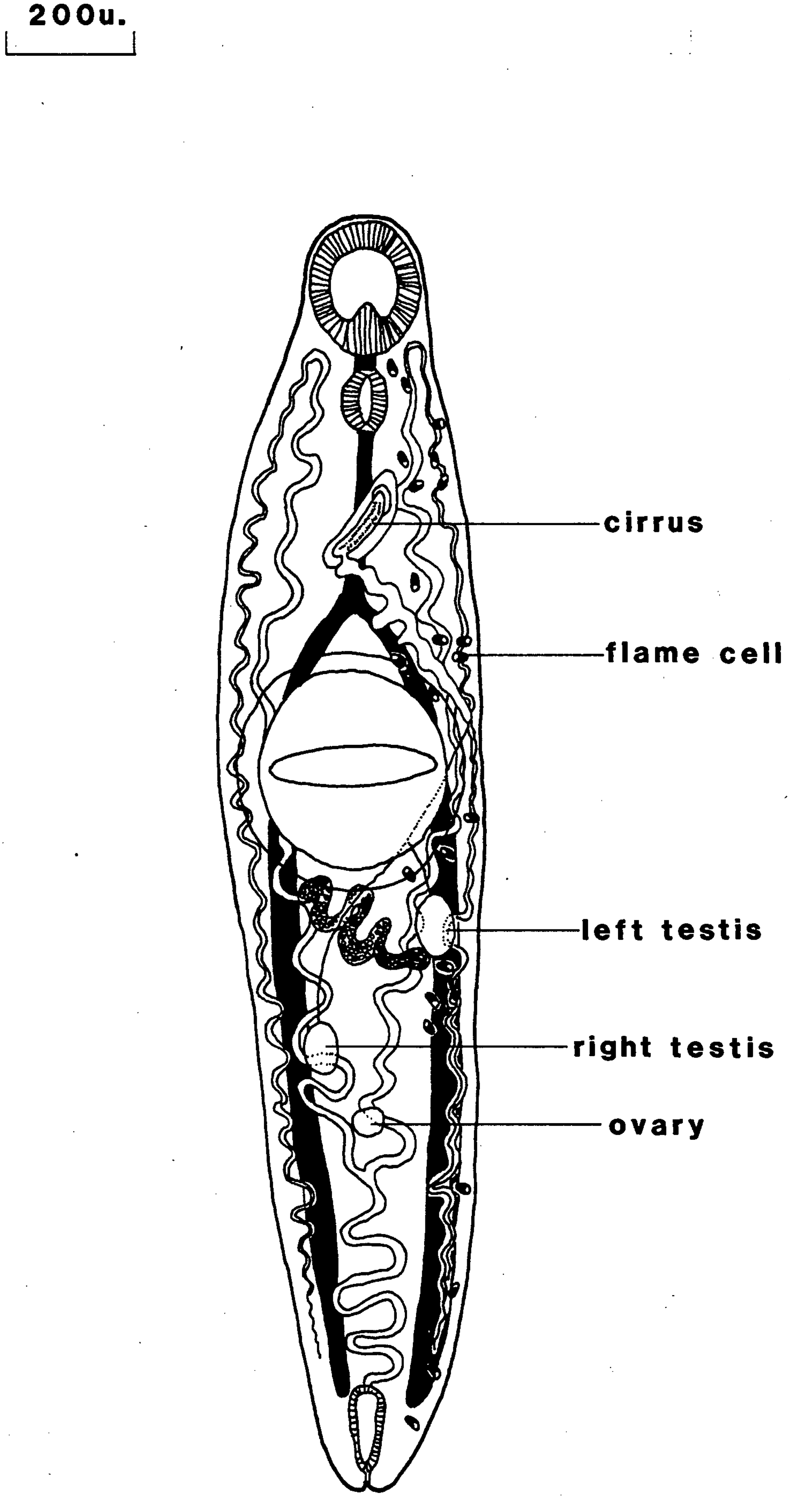


Fig. 6,1. Metacercaria W Ventral view showing internal details.

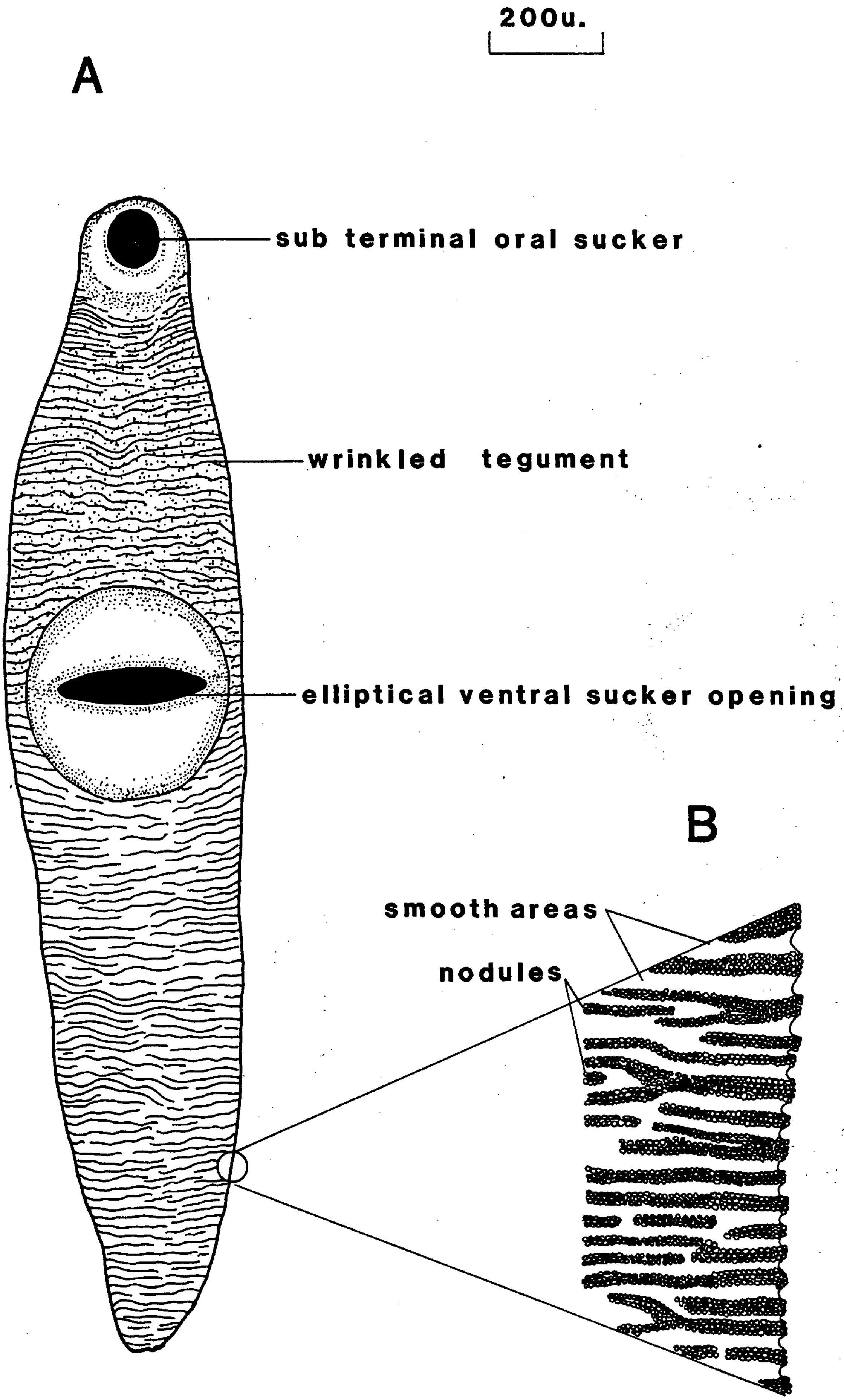


Fig. 6,2. Metacercaria W A, external features ventral view. B, part of tegument enlarged

Fig. 6,3. Metacercaria W.

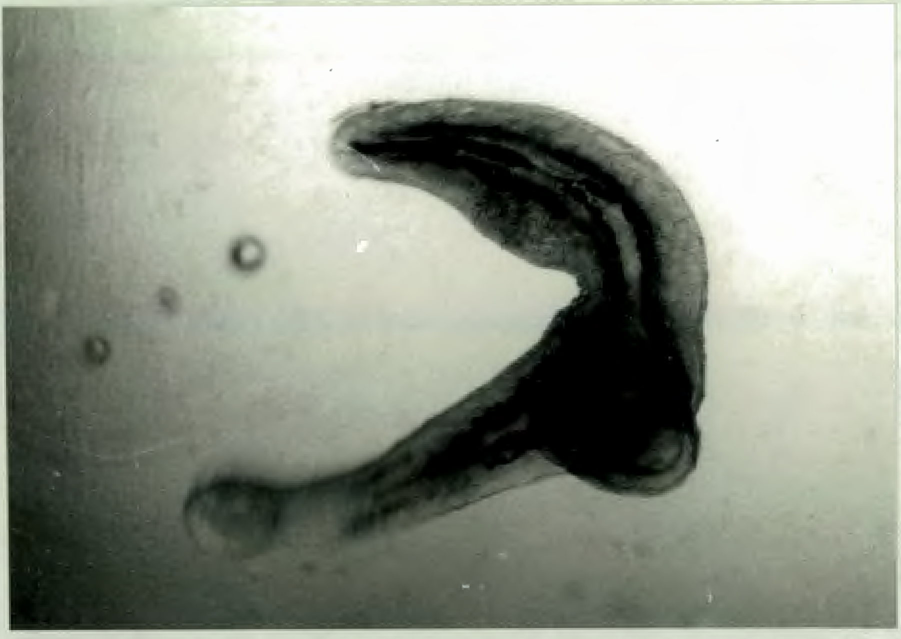


Fig. 6,4. Metacercaria W.

Fig. 6,5. Metacercaria W.

6.4 DISCUSSION

The family Azygiidae has two genera: *Otodistomum* and *Azygia*. Dawes (1946) states that the former is exclusive to the Selachii and the latter exclusive to the Teleostei.

Koie (1983) found a metacercaria of *Otodistomum* sp. in *Limanda limanda* (Osteichthyes : Pleuronectidae) from Danish waters. This apparently indicates less host specificity than had previously been thought.

The presence of *Metacercaria W* singly in *Bullia digitalis* combined with the absence of parthenitae suggests that *B. digitalis* is the second intermediate host.

One major difference between Mnandi and other collection sites is the very close proximity of an extensive rocky shore with the beach. Possibly it is a rocky-shore mollusc that is the first intermediate host. This would help explain the absence of metacercariae at the other sites.

CHAPTER 7

SOME ASPECTS OF THE PHYSIOLOGY OF LARVAL DIGENEA IN *BULLIA*

7.1 CERCARIAL EMERGENCE UNDER 'NATURAL' CONDITIONS

Introduction

Cercarial emergence from the molluscan host may be influenced by factors such as light, temperature, humidity, water turbulence, salinity, pH, season, movement of the host and host nutritional status (Rees 1947, Smyth 1966, Erasmus 1972). Some cercariae do not emerge at all but encyst within the body of the host as metacercarial cysts. The behaviour is specific and commonly invariable. From such activities one can throw light on the pattern of the life cycle, and speculate with more accuracy on the fate of the parasite.

Materials and Methods

Whelks were stored in petri dishes with lids. Each dish was about half full of sea water. The whelks were stored singly if the number was small (less than fifty) otherwise up to five whelks were placed in each dish. This may have introduced a difference in the environmental stress experienced between singly and multiply stored whelks, but the effect is probably not significant.

Whelks were always placed aperture down in the dish. The water level was adjusted to cover the aperture. Whelks stored aperture uppermost are unable to take in water for pedal expansion.

The whelks were then left in various light and temperature regimes for twenty four hours. Precise conditions will be elaborated for each experiment later. The whelks were then examined using the method set out in Chapter 2.2.

Results for Cercaria Z

Fifty nine infected *B. digitalis* were kept at 15°C for 24 hours in constant light. No emergence was detected.

Forty three infected *B. digitalis* were kept at 22°C for 24 hours in constant light. There was no emergence of living cercariae. Two whelks had released several dead cercariae each.

Two infected *B. digitalis* were kept at 22°C in the dark for 24 hours. No emergence was seen.

Three infected *B. digitalis* were kept at 15°C in the dark for 24 hours. No emergence was seen.

Results for Cercaria Y

One infected B. digitalis was kept at 15°C in the dark for 24 hours. No emergence was seen.

Six infected B. digitalis were kept at 22°C in light and dark (fifteen hours light, nine hours dark). No emergence.

Three infected B. digitalis were kept at 22°C in the dark for 24 hours. No emergence.

Results for Cercaria X

One Bullia digitalis was stored at 22°C (room temperature) in light and dark, at 10°C in the dark and 15°C in light. The whelk emitted cercariae continuously regardless of conditions at a rate of about twenty per hour.

Results for Metacercaria W

One infected Bullia digitalis was stored in light and dark for 24 hours with no emergence.

The Effect of Nutritional Status on Cercarial Emergence

One hundred Bullia digitalis from Melkbos were used. Subsequent examination of fifty whelks of this sample revealed an infection prevalence of 54% with Cercaria Z.

The whelks were placed in a tank of approximate dimensions sixty cm long, forty cm wide and forty cm high. The tank was arranged so that one end was about 10 cm higher than the other. Sand to a depth of about four cm followed by fifteen cm of water was put into the tank. The sand layer emerged from the water at the high end of the tank to simulate a beach slope. Two air stones were placed at the deep end of the tank and were regulated to produce vigorous streams of bubbles. This disturbed the water surface and caused ripples to impinge on the emerging sand to further enhance the beach simulation. Three samples each of the water column and the sand were examined for cercariae with no success. Fish bait was then added to the tank which caused emergence of the whelks from the sand. The whelks congregated around the bait and began feeding. Two infected whelks were removed from the tank with the bait they were feeding on. After about half an hour they were examined for cercarial emission with negative results.

The other whelks in the tank were left for twenty four hours with the bait. The following day three samples each were taken of the water and the sand. No cercariae were seen.

7.2 CERCARIAE IN HAEMOLYMPH OF BULLIA DIGITALIS AND THE STIMULATION OF CERCARIAL EMISSION BY INJECTION OF GLUCOSE

If cercariae are to migrate from the digestive gland to the point of exit from the snail then they must use a pathway through the host body. Migration through the haemolymph has been noted by Probert and Erasmus (1965).

Examination of haemolymph prior to injection

Bullia digitalis infected with type Z were examined for evidence of cercariae in the haemolymph. In all cases the whelks were previously examined externally to ascertain whether emission was occurring. The haemolymph was examined by two methods. The foot and head were removed with scissors and this part was then macerated in sea water. In other cases a fine hypodermic needle was used to remove 0,1 mℓ - 0,25 mℓ of haemolymph. The sample was examined at high power under a compound microscope.

Nine infected non-emitting whelks had the head and foot removed; on maceration one whelk produced one tail-less *Cercaria Z*.

Thirty five infected non-emitting whelks had haemolymph extracted from the pedal sinus. One whelk haemolymph sample contained one dead tail-less *Cercaria Z*.

Injection of glucose into infected B. digitalis

Two groups of *B. digitalis* infected with type Z were obtained. The control group had twelve infected whelks. The experimental group had eight infected whelks.

All of the whelks were injected, the controls with 0,2 mℓ of distilled water and the experimental animals with 0,2 mℓ of 2% glucose in water. The animals were placed in petri dishes at 15°C in constant light for 24 hours.

The experimental whelks were injected with a further 0,5 mℓ of 2% glucose and left in their petri dishes for a further 24 hours. They were then injected with 0,5 mℓ of 20% glucose and left for a further 24 hours. The results are in Table 7.1.

TABLE 7.1 The effect of glucose injection in B. digitalis on cercarial emergence.

		DAY 1	DAY 2	DAY 3
8 Controls	Treatment	Injected with 0,2 mℓ water	-	-
	Result	No emission	No emission	No emission
12 Experimental subjects	Treatment	Injected with 0,2 mℓ 2% glucose	Injected with 0,5 mℓ 2% glucose	Injected with 0,5 mℓ 20% glucose
	Result	No emission	Three whelks emitting	Five whelks emitting

The five experimental whelks were alive during emission of cercariae. The non-emitting whelks were dead.

The three emitting cercariae on day two had haemolymph samples taken. No cercariae were found.

7.3 UPPER LETHAL TEMPERATURE OF LARVAL TREMATODES FROM BULLIA DIGITALIS

Introduction

That the upper lethal temperature limit of larval trematodes gives an indication of the normal ambient temperature of the environment of the adult has been noted by Smyth (1966). Thus in looking for the potential final host of a trematode, the determination of temperature tolerance can distinguish between a homeothermic and a poikilothermic host.

Materials and Methods

Cercarial stages of parasites Z and Y were obtained from *B. digitalis*.

Two *B. digitalis* infected with type Z were obtained from Melkbos.

Two *B. digitalis* infected with type Z were obtained from Fish Hoek.

Two *B. digitalis* infected with type Y were obtained from Fish Hoek.

A large number of *B. digitalis* were examined from Fish Hoek to obtain the samples because the infection level is very low. The Fish Hoek samples were obtained first, followed by the Melkbos samples. In this way it was hoped that the waiting time between shell opening of *Bullia* and commencement of the experiment would be at a minimum for the majority of the samples.

The infected digestive gland of each of the whelks was divided into six and each sixth placed in an individual test tube with about 2 mℓ of sea water.

A series of three thermostatically controlled water baths was used to maintain the constant temperatures. The temperature of each bath was determined using one master thermometer and adjustments were made to the bath thermostats until the desired temperature was reached ± 0,5°C. In the first run the baths had a temperature of 25°C, 30°C and 35°C respectively.

The tubes were immersed for 90 minutes and then removed and left to cool for about ten minutes. The contents of each tube were then examined microscopically and the proportion of living to dead cercariae was noted. The criteria for death was complete lack of movement for longer than thirty seconds. The proportions were ascertained by counting ten cercariae at random in the field of view and noting movement in each. The process was repeated for temperatures of 40°C, 45°C and 50°C.

Cercarial samples were taken from each of the six whelks at the start of the experiment and also at the finish, four hours later, to ascertain the mortality rate of non-heat treated cercariae. This was to give a measure of control reference to the experiment. Before heat treatment all samples were stored at room temperature ≈ 20°C.

Results

TABLE 7.2 Cercarial mortality at increasing temperatures

	MORTALITY %			
Temp. °C	Cercaria Z Ou Skip	Cercaria Z Fish Hoek	Cercaria Y Fish Hoek	Remarks
25	0	0	0	No mortality
	0	0	0	
30	0	20	10	Cercariae Y becoming
	10	0	0	sluggish
35	0	20	0	All Cercariae Y contracted and
	0	0	0	immobile
40	10	0	100	Cercariae Y all dead
	10	30	100	Cercariae Z active
45	90	70	100	Only a few Cercariae
	90	100	100	Z active
50	100	100	100	All cercariae dead
	100	100	100	

TABLE 7.3 Showing mortality of cercariae with increasing time when stored at room temperature.

MORTALITY %					
2 HOURS AFTER OPENING SHELL			6 HOURS AFTER OPENING SHELL		
Z Ou Skip	Z Fish Hoek	Y Fish Hoek	Z Ou Skip	Z Fish Hoek	Y Fish Hoek
0	0	0	10	20	10
0	0	0	0	0	20

After 90 minutes at 35°C Cercaria Y was in all cases contracted and barely moving. At 40°C cercaria type Y were all quite dead but relaxed. In some cases the tegument was starting to separate from the cercarial body.

Cercaria Z remained very active up to and including 40°C. At 45°C most cercariae were dead but a small number were still feebly moving their tails. No temperature-dependent body contraction was seen in Cercaria Z.

7.4 DISCUSSION

Cercaria Z was never emitted alive under normal circumstances. On injection with relatively large doeses of glucose,cercarial emission could be stimulated. Conversely, feeding did not elicit initiation of emergence within a period of 24 hours. The factors are confusing as this implies that the cercariae stay in the whelk until it is opened by predation. *Eurydice* is hardly able to crack a whelk shell and besides, *Eurydice* has never been observed feeding on living *Bullia*. Due to the prolonged digestion time in *Bullia* (Brown, pers. comm.), it is possible that some days may elapse before the digestion products reach the haemolymph. At this time it is likely that *Bullia* is buried in the sand. *Eurydice* also spends much of its time buried and probably congregates around those whelks which have taken pieces of food with them. The burrowing of *Bullia* whilst holding food has been observed by McGwynne (1980). These conditions would be near-ideal for the worm-like movement of the cercaria to propel itself through the sand in relatively calm interstitial water and then penetrate *Eurydice*. The absence of spontaneous encystment even in cercariae that have been in a crushed *Bullia* for two days is supporting evidence.

Only two *Cercaria Z* were extracted in haemolymph samples. Both were tailless. Haemolymph extractions from emitting whelks after injection with glucose contained no cercariae. If the cercariae follow a similar path through *Bullia* as a strigeid cercaria does in *Lymnaea stagnalis* (Probert & Erasmus 1965), then the absence of cercariae in the cephalopedal sinus is not surprising. Probert and Erasmus found that most cercariae passed from the digestive gland to the visceral sinus, then to the mantle mainly via the rectal sinus. Otherwise they migrated via the sub-renal sinus through the kidney to the mantle. Any cercariae found in the cephalopedal sinus are probably 'lost'. The tailless cercariae extracted from this sinus in Bullia imply that this is an unproductive detour which lasts long enough for the cercariae to shed their tails and become moribund.

Given the limited data on cercarial temperature tolerance that the experiment in Chapter 7.3 has produced, it is obvious that Cercaria Z is more heat-tolerant than Cercaria Y These results are in agreement with the information available on these parasites and serve to support their identity.

That Cercaria Y appears to be reluctant to emerge from Bullia is not surprising if the second intermediate host is about to save it the effort.

Cercaria X was emitted from Bullia under any conditions. Possibly this high rate of cercarial production is linked to the second intermediate host being less common than Eurydice or Ovalipes. Any further speculation with so little evidence is probably unprofitable.

CHAPTER 8

METACERCARIA V

(FAMILY PTYCHOGONIMIDAE DOLLFUS, 1937)

HOSTS: BULLIA PURA AND BULLIA DIGITALIS

This metacercaria was found singly or in groups of two or three. Often Metacercaria V was found associated with Metacercaria U in the same whelk. It was found once only in Bullia digitalis. The worm is found adhering to the host's tissue after cracking open the whelk. Host tissue was found inside the ventral sucker; whether this occurred after the whelk had been disrupted by opening, or prior to this is not known. about half a dozen specimens were available to compile a description of this species.

8.1 DIAGNOSIS

No parthenitae were found in any host. The metacercaria is a large dorsoventrally flattened distome. The tegument is smooth and shows only slight transverse wrinkling during bodily contraction. The oral sucker opens sub-terminally. The opening between sucker and pharynx is always wide open. There is no pre-pharynx. The cerebral ganglia lie at the posterior extremity of the pharynx where they lie either side of the oesophagus. Several pairs (about five) of gland cells originate adjacent to the cerebral ganglia and lead forward to the periphery of the oral sucker opening. The gland cells stain deeply turquoise with nile blue sulphate. The oesophagus is very short, caecae appear almost immediately behind the pharynx. The caecae terminate close to the excretory pore at the posterior extremity. The ventral sucker is about twice the diameter of the oral sucker. There are four groups of gland cells symmetrically grouped around the ventral sucker (see Fig. 8.1.A and B). Two groups of gland cells lie anterior to the sucker and two groups lie posterior. The glands appear to empty at the opening of the sucker. The gland cells stain turquoise with nile blue sulphate. The sucker orifice is backward curving in many cases and is almost the width of the sucker. The cirrus lies immediately posterior to the ventral sucker and lies on the mid-line. The vas deferens is convoluted and divides to form a short left vas efferens and a longer right vas efferens. The testes lie slightly lateral to the centre line. The left testis lies anterior to the right. The ovary is anterior to the testes and is adjacent to the cirrus, on the right hand side of the body. The uterus is coiled and runs from the cirrus to the bladder where it recurves slightly. Full details of the female reproductive system were not visible. The bladder occupies the rear tenth of the body and is extensible. Running from the bladder the excretory duct bifurcates at the anterior level of the right testis. The ducts then proceed forwards to the mid-point between the ventral and oral sucker. At this point they terminate in a bulge; secondary excretory ducts enter the bulge laterally. Cilia lie in the lumen of the junction between primary and secondary ducts. The cilia beat in a similar manner to flame cells found elsewhere in the body. The secondary ducts run posteriorly and terminate very close to the excretory pore. There are four flame cells on each side of the body. The flame cells are of unusual structure; they are much larger than flame cells in other digenea so far examined. The cilia making up the flame cell are numerous and form a broad crown shape (see Fig. 8.1). The flame cell formula was not ascertained. The excretory system may be classed as stenostomate.

8.2 MOVEMENT

This trematode is very sluggish; very little attempt is made at locomotion. Leech-like movement involving the suckers was seen occasionally. There was no toxic reaction to neutral red or nile blue stains.

8.3 MEASUREMENTS

One complete set of measurements was taken from one specimen. It would have been advantageous to get more but other specimens were primarily used to obtain a description and drawing. often by the time that all of the morphological details had been noted the specimen was deformed or disintegrating. Incomplete sets of measurements are also included to give an idea of how variable these worms are.

TABLE 8.1 Metacercaria V. measurements

	um	um	um
Length	2464	810	2000
Width: 25%	370	324	
50%	616	405	
75%	462	243	
Ventral sucker length	462	228	300
width	493	228	300
Cirrus length	243		
Testes: length	284		
width	203		
Oral sucker length	238	162	200
width	242	122	

8.4 INFECTION INCIDENCE

B. pura

Two whelks out of 150 were infected giving an incidence of 1,3%.

B. digitalis

Two whelks out of 494 were infected giving an incidence of 0,4%.

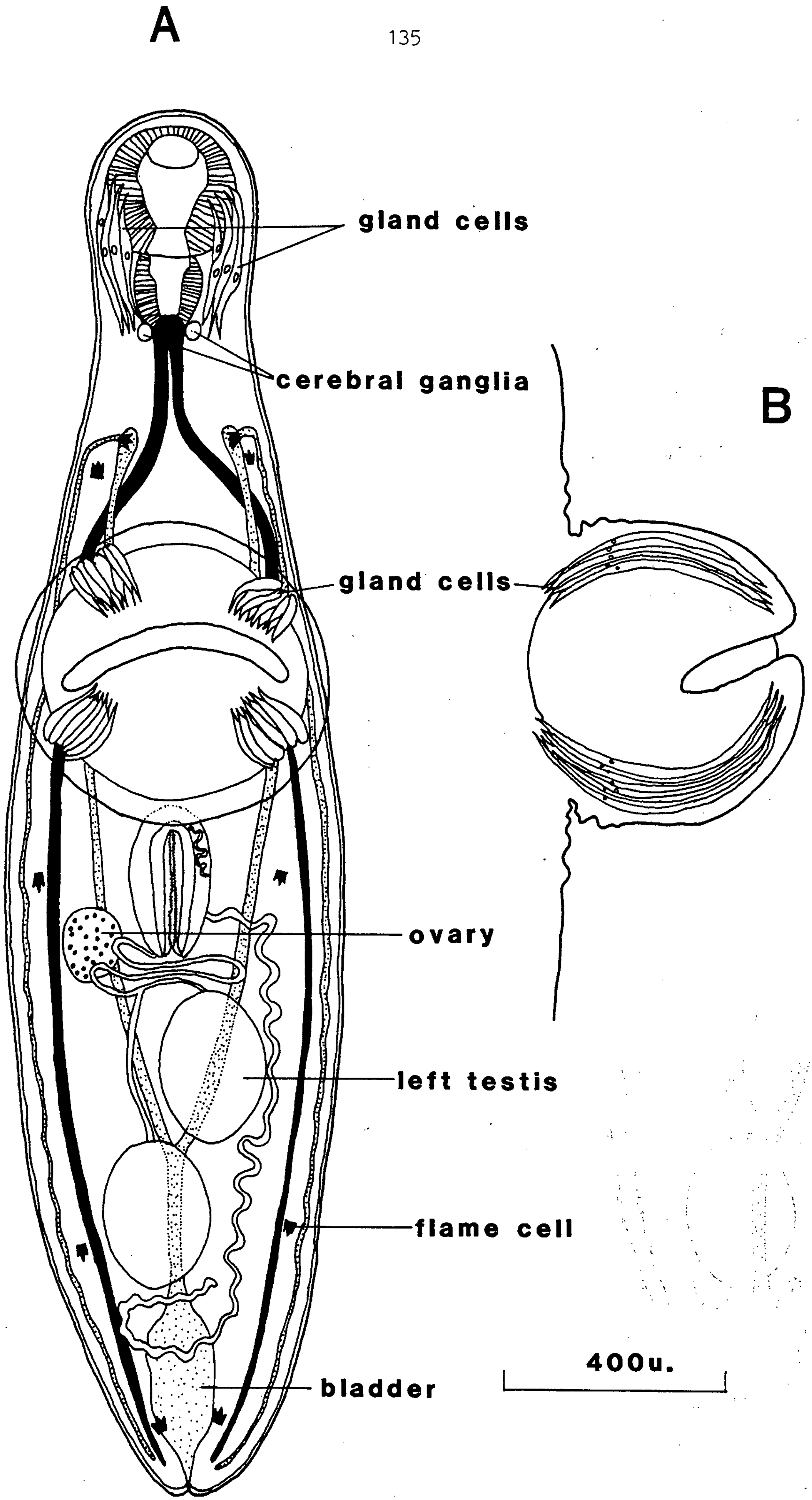


Fig. 8.1 Metacercaria V. A, ventral view of internal structure. B, lateral

Fig. 8,2. Metacercaria V

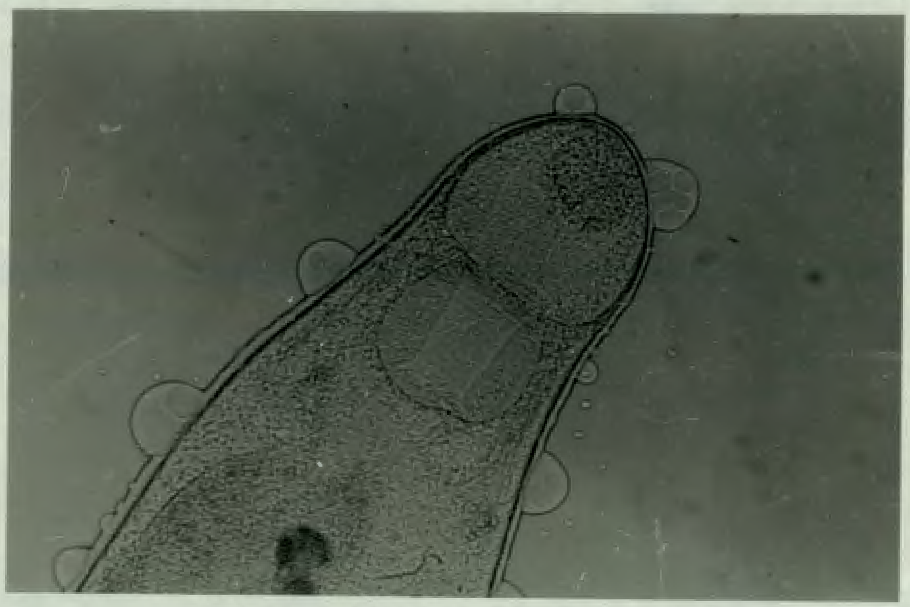


Fig 8,3. Metacercaria V anterior.

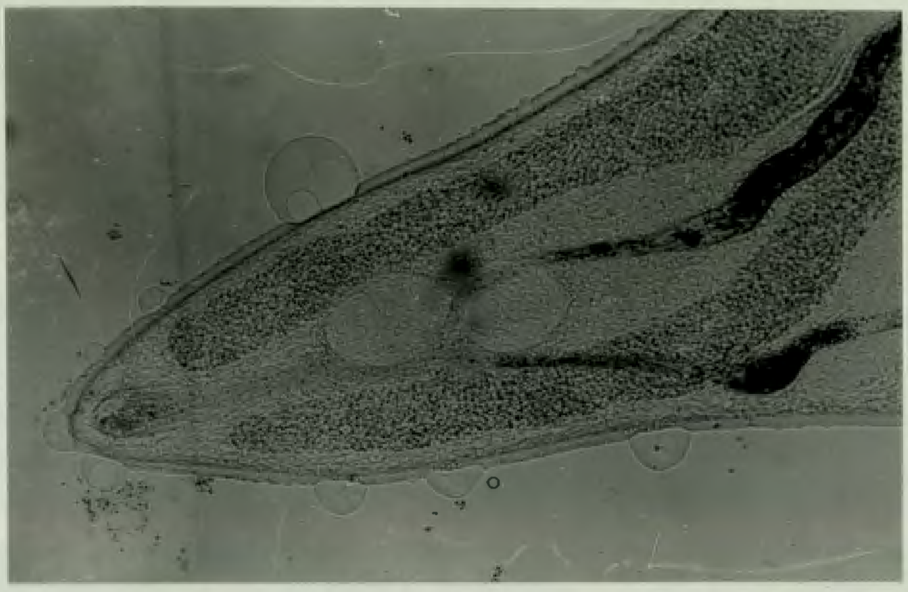


Fig. 8,4. Metacercaria V posterior showing testes.

8.5 DISCUSSION

The presence of metacercariae in Bullia has been noted by Reimer (1976). In addition to his account of G. indica in B. melanoides an alternative second intermediate gastropod host Sunnetta scripta (L.) is mentioned. Other gastropods mentioned were Thais rudolphi (Lamark) and Janthina globosa (Swainson), infected with Genolopa sp. (Family Monorchiidae) and Lecithocladium sp. (Family Dinuridae) respectively.

G. indica is a member of the family Halipegidae. Dawes (1946) cites the family as exclusive parasites of frogs as the final host. It is doubtful that frogs are the final host for G. indica. Holliman (1961) states that there are no marine representatives of the Halipegidae. Perhaps this anomaly can be explained by a case of accidental infection or, more likely a new habitat for the family has been discovered. If this is the case, more members are expected to appear in the marine environment.

The family Ptychogonimidae, of which both Metacercaria V and U are thought to be members, is exclusive to Selachian final hosts (Dawes 1946). A survey of Rhinobatis sp. from Mnandi would probably produce the adult stage. A possible reason for the exclusivity of metacercarial infections to Mnandi has already been suggested in Chapter 6.3.

CHAPTER 9

METACERCARIA U

(FAMILY PTYCHOGONIMIDAE DOLLFUS 1936)

HOST: BULLIA PURA

This large trematode was found in the mantle cavity of the host, where it was clinging to the gills and osphradium. It occurred singly or in pairs.

9.1 DIAGNOSIS

No parthenitae were observed. A large opaque, green coloured fusiform distome. The tegument is smooth generally but shows a tendency to wrinkle around the oral and ventral suckers and the excretory pore. The oral sucker opens terminally. The lining of the oral sucker consists of diamond-shaped areas, each about 6 um long. There is no pre-pharynx. The pharynx is elongated and may be as long as the oral sucker. The digestive caecae arise directly from the pharynx and run almost to the end of the body. The caecae are broad and lie halfway from the midline to the lateral margin of the body. The ventral sucker is about twice the diameter of the oral sucker and lies about one-third of the body length from the front. The cirrus lies directly behind the ventral sucker. The testes lie staggered behind the cirrus.

The left testis is anterior to the right. The ovary lies anterior to the testes and to the right of the cirrus. The ovary and testes are of comparable size; measurements show the ovary to be about 20% smaller. Other features of the reproductive system are hidden by the masses of green lobes or convolutions of a tubular organ. This gives the body a mottled appearance (see Fig. 9.2). The whole of the body of the worm is packed with countless small spheres, each with two long filaments. The filaments arise from the spheres at about the eleven and one o'clock positions (see Figs. 9.3.A and B). Before release the filaments are folded around the sphere. On exposure to sea water they spring apart. It was first thought that there may be eggs but they are rather too small. The excretory duct opens at the excretory pore. There is no bladder visible but the duct is probably extensible. The duct bifurcates adjacent to the testes. Each duct then curves out dorsally and laterally to lie above the caecae. At the anterior extent of the primary excretory ducts they curve back in, to lie medial to the caecae. The secondary duct arises just anterior to the ventral sucker at the termination of the primary duct. The secondary ducts terminate just beyond mid-way to the posterior. The excretory system is stenostomate. No flame cells could be seen due to the opaque nature of the body.

9.2 MEASUREMENTS OF METACERCARIA U

All measurements were taken of living material. Column 1 gives results taken from one specimen. Alternative measurements, where given, indicate variability between specimens. Generally the sample size is too small to give descriptive statistics.

TABLE 9.1 Measurements of Metacercaria U

	1 um	2 Max um	3 Min um	n
Length	3080	3400	1336	5
Width: 25%	486	801	405	3
50%	1093	1093	567	3
75%	1053	1053	324	3
Oral sucker length	202	202	162	3
width	283	283	243	2
Pharynx length	146	200	146	4
width	283	283	160	4
Ventral sucker length	365	500	259	5
width	348	364	259	4
Cirrus length	390	405	273	3
Testes: length (right)	202			
width (right)	202			
length (left)	186			
width (left)	202			
Ovary length	162			
width	146			

TABLE 9.2 Measurement of features of filamentous structures found in Metacercaria U.

	MEAN um	S.D. um	S.E. um	C.V. %	n	MAX.	MIN.
Sphere diameter	4,23	0,39	0,18	9,18	10	4,87	3,90
Filament span before release	10,4	1,66	0,79	15,95	10	12,67	8,77
Filament span when fully open	52,26	11,51	5,46	22,02	10	68,25	39,00

9.3 INFECTION INCIDENCE

Three infected B. pura were found in a sample of 150. This gives an incidence of 2%.

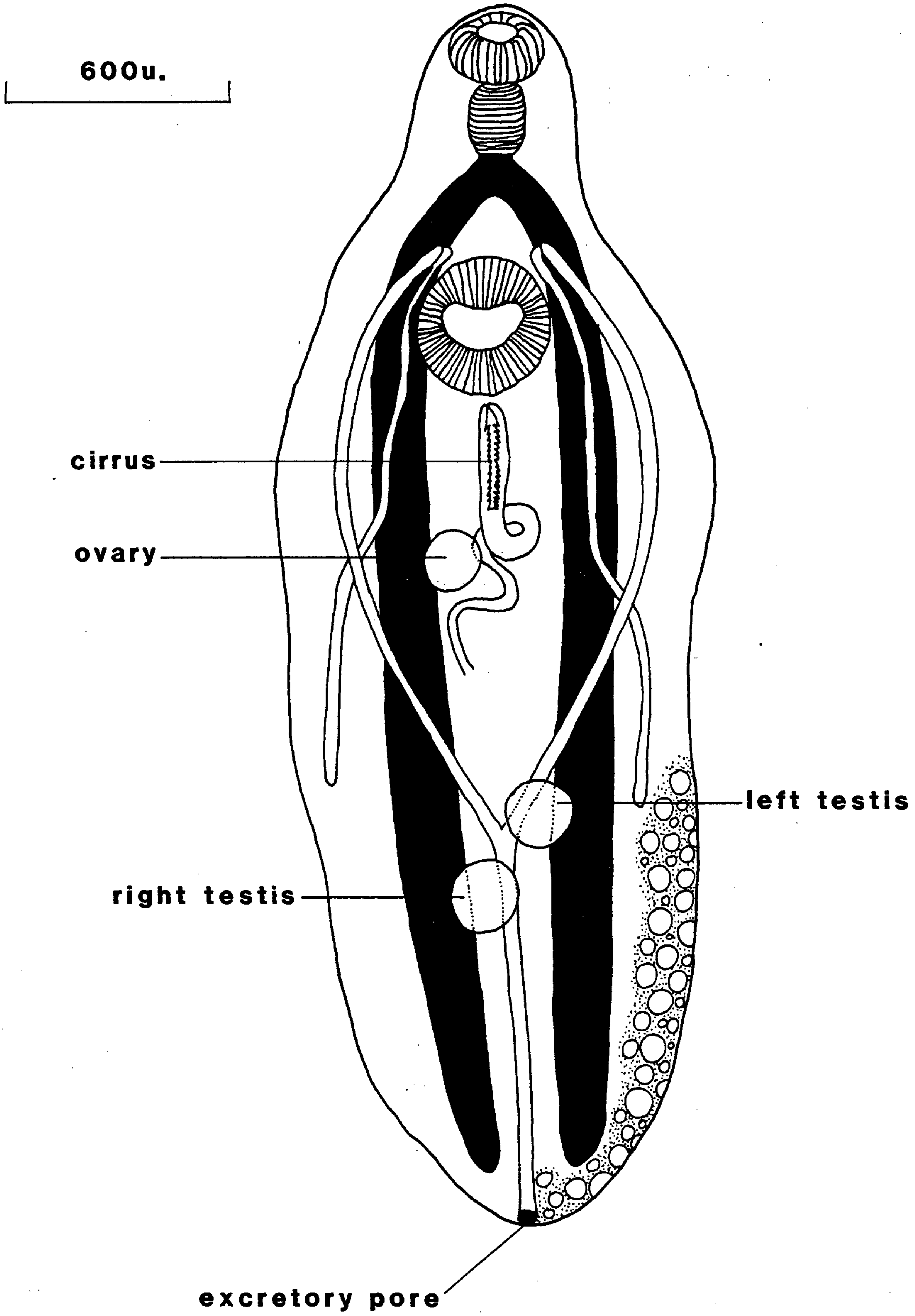


Fig. 9,1. Metacercaria U ventral view of internal structure.

Fig. 9,2. Metacercaria U

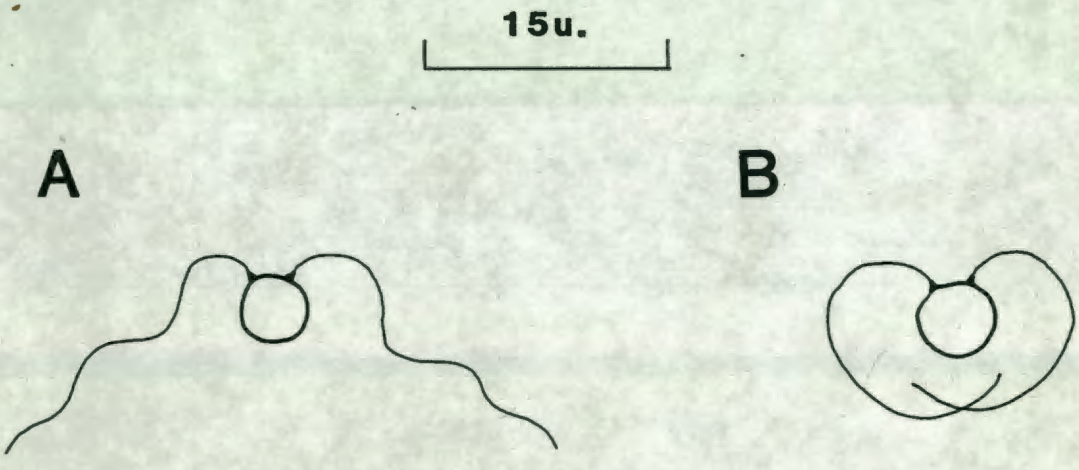


Fig. 9,3. Filamented bodies found in metacercaria U A,filaments extended.

B, filaments before extension.

9.4 DISCUSSION

The discussion in the last chapter dealt with many points concerning the family Ptychogonimidae. This metacercaria was only ever found in Bullia pura. On a few occasions this metacercaria was found in association with Metacercaria V A graduation in size was apparent with the smallest specimens in the whelk being Metacercaria V and the largest Metacercaria U Perhaps type U develops from type V and is not a separate species. The absence of Metacercaria U from B. digitalis throws doubt upon this hypothesis.

The large size and opaque nature of Metacercaria U suggests that it may be a progenetic adult. The phenomenon has been noted previously by Szidat (1956) in Genarchella genarchella when it completes development in the gastropod host Littoridina australis. Another example is the development of Asymphylodora progenetica described by Serkova and Bychowsky (1940). These two examples are cited from Erasmus (1972).

The presence of small filamented spheres is strange. They are much smaller than eggs but bear comparison to some trematode eggs in form. They were found consistently in all specimens which suggests that they may be symbionts.

CHAPTER 10

ECHINOBOTHRIUM sp.

(Van Beneden 1849)

ORDER DIPHYLLIDEA VAN BENEDEN ET CARUS 1863

HOST: BULLIA PURA AND BULLIA DIGITALIS

10.1 INTRODUCTION

Initially this cestode was overlooked as it lies quiescently in the host viscera and is often buried completely by tissue. The larval cysts are spherical or approximately so when in the host tissue. They elongate on being released from the host tissue (see Fig. 10.1.A and B.). At maximum extension the cyst appears vermiform.

10.2 DIAGNOSIS

This cysticercoid larval stage of Echinobothrium appears whitish with a red comb-like structure inside. This is the rostellum. The cysticercoid wall appears to have two layers: a thick muscular inner layer and a thin tegument. External to the cyst tegument the host has covered the parasite with a layer several cells thick of plate-like epithelial cells. The cyst contains one non-inverted larva. The cyst fluid is densely packed with oval plate-like calcareous bodies.

Each plate has bevelled edges and is dotted with small nodules on the flat surfaces (see Fig. 10.3.A.). The larva consists of a scolex with a crown of rostellar hooks and two bothria enclosing a neck, leading to an undeveloped strobilus. The rostellum is divided into two similar sections; each section has between 19 and 22 large hooks and from five to six small hooks on the medial side of the large hooks. The large hooks alternate in length and have a flattened mattock-like anchor at the proximal end. The small hooks have sharp points which join with the rest of the hook at a distinct thickening where they then maintain this thickness for about half of the total length of the hook (see Fig. 10.3.C.). The hook then either tapers to a point or broadens to a flat or curved proximal anchor. Each group of hooks is set on a curved muscular pad which bends around and almost meets the opposing pad (see Fig. 10.2). At the base of the scolex the two bothria arise and wrap around the neck in a leaf-like manner. The bothria are covered with small spines over the whole outer surface and edges. The bothria are capable of undulating in a wave-like motion. Flame cells may be seen in the bothria. Two excretory ducts arise from indistinct origins in the neck and run almost as far as the scolex before recurving and becoming indistinct at the same area as their origin (see Fig. 10.2). The strobilus is indistinct so no details could be ascertained. Crushing the cyst did not help to clarify any detail as usually the internal structure was disrupted.

TABLE 10.1 *Echinobothrium* sp. measurement of the cyst soon after extraction from the host tissue.

	MEAN um	S.D. um	S.E. um	C.V. %	n	MAX um	MIN um
Cyst length	954	170	78	17,7	9	1134	769,5
Cyst width	432	61	29	14,1	9	526,5	364,5

10.3 INCIDENCE OF INFECTION

B. pura

Sixty three whelks out of 150 were infected, giving an incidence of 42%.

B. digitalis

Two whelks out of 494 were infected, giving an incidence of 0,4%.

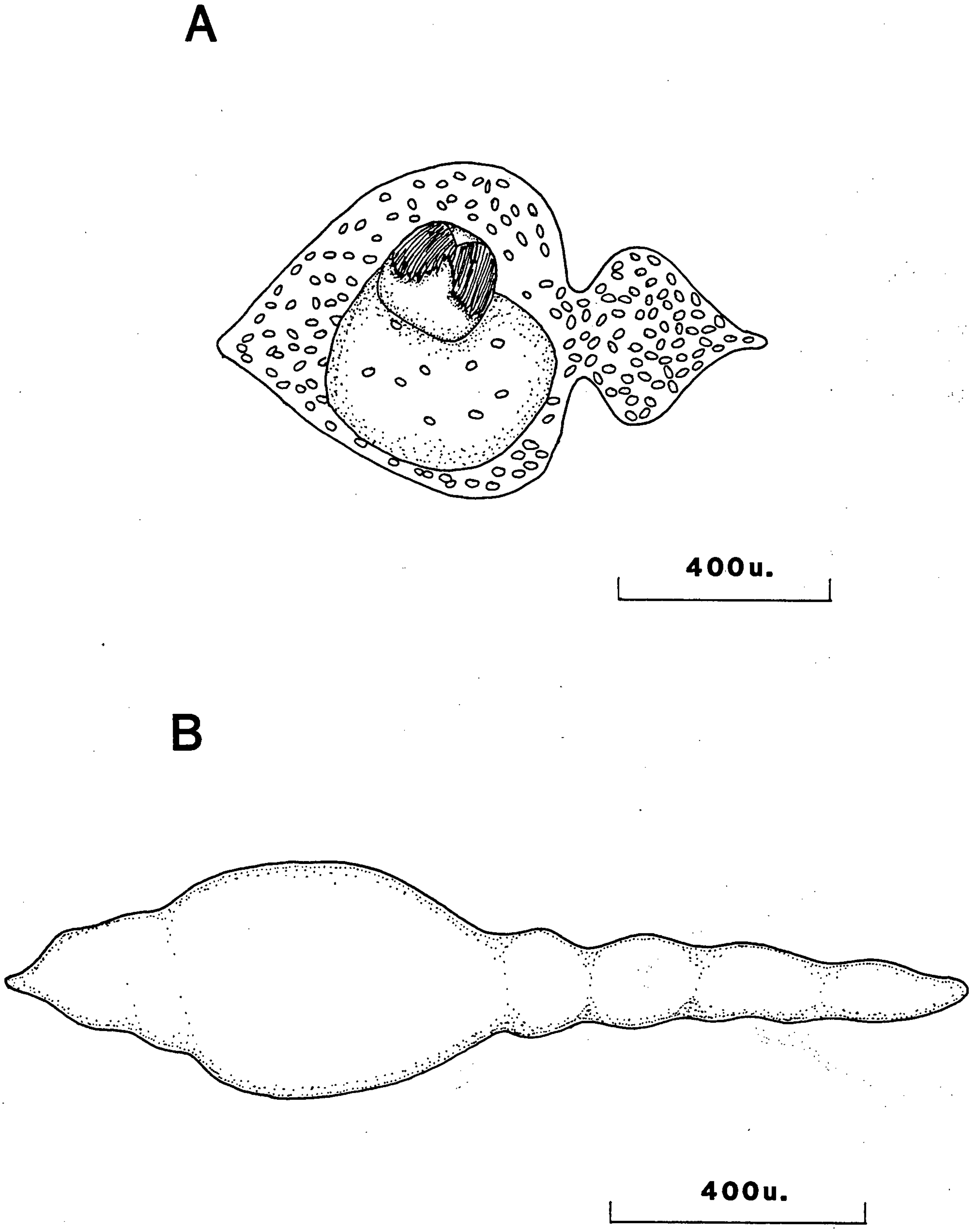
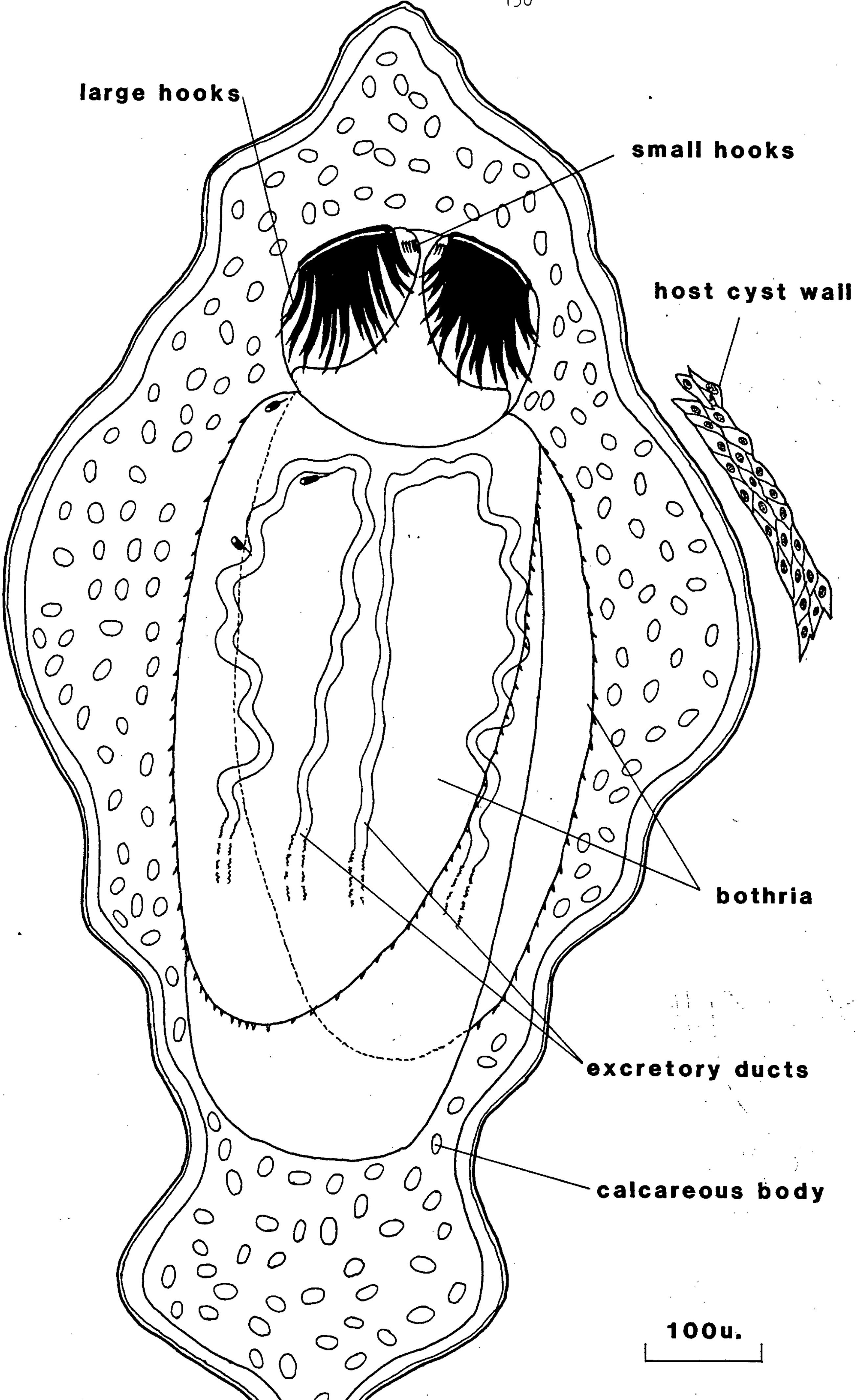


Fig. 10,1. *Echinobothrium sp.* A, freshly removed cyst. B, cyst one hour after removal.


large hooks
small hooks
host cyst wall
bothria
excretory ducts
calcareous body
100u.

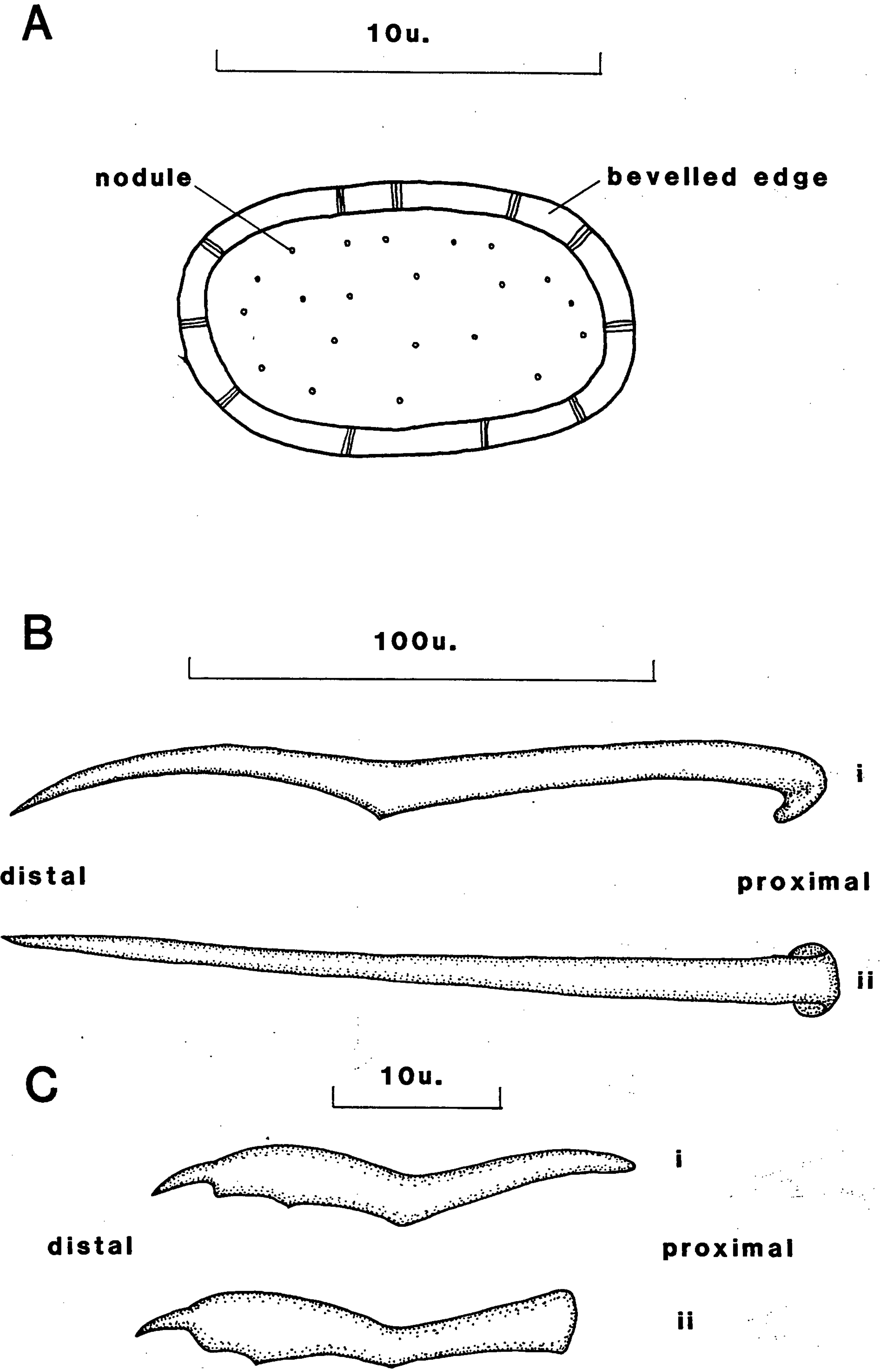


Fig 10,3. <u>Echinobothrium sp.</u> A, calcareous body. B, (i) large hook ,lateral view. (ii) large hook, top view. C, (i) small hook lateral view. (ii) small hook alternative form.

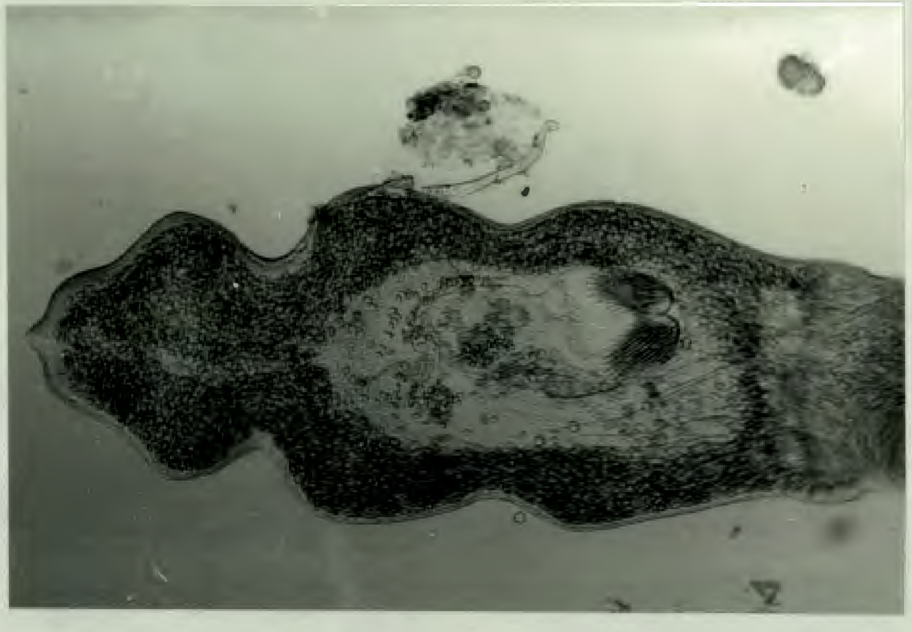


Fig. 10,4. Echinobothrium sp. cyst showing the rostellum.

Fig. 10,5. Echinobothrium sp. cyst compressed by cover slip pressure.

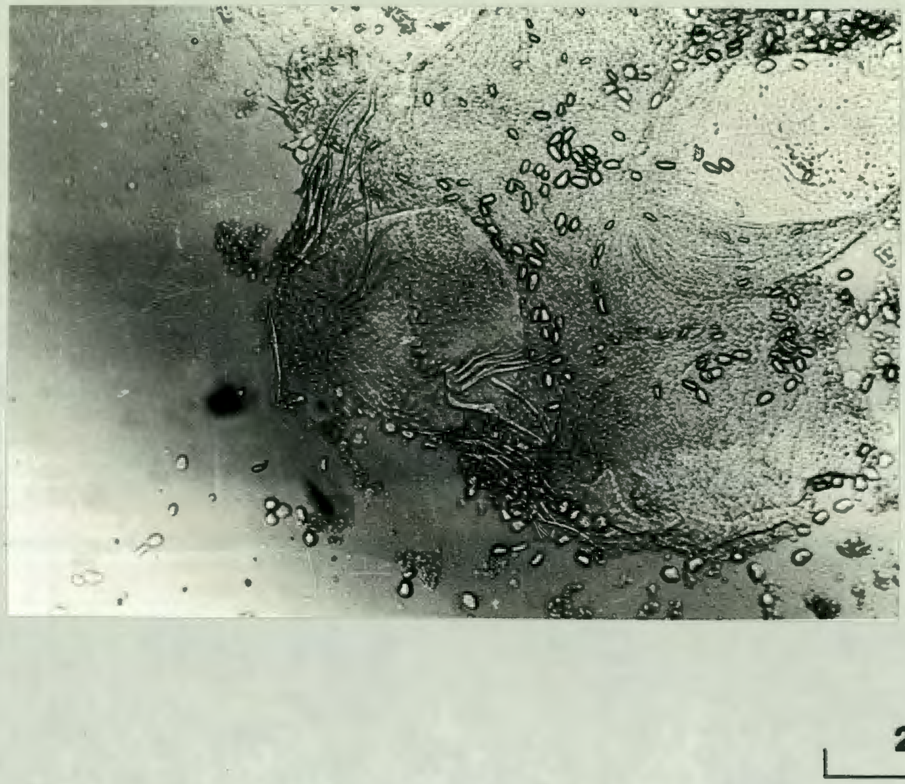


Fig. 10,6. Echinobothrium sp. cyst after squashing,showing rostellar hooks.

10.4 DISCUSSION

Bullia melanoides has been found to harbour larval stages of Echinobothrium sp. Anantaraman (1963) gave an account of Echinobothrium sp. which was also a parasite of Murex tropa. She reports finding the cestode in the gut of the crab Matuta victor and considers this an accidental infection. Possibly the crab is an alternative intermediate host in which the parasite may reside until the crab is eaten by the final host. Reimer (1975) found E. lateroporum in B. melanoides. Other cestode larvae mentioned by Riemer (1975) are referred to in Chapter 2.1.

Crude incidence of Echinobothrium sp. in B. pura is similar to the figure for B. melanoides given by Anantaraman (1963). The incidence of infection in B.digitalis is much lower.

Other works containing references to Echinobothrium sp. include: Chandra (1983), Hyman (1951), Wardle and McLeod (1952) and the detailed account of the anatomy of the adults of E. brachysoma (Pintner) and E. affine (Diesing) by Rees (1961).

Other intermediate hosts for members of this genus include: Nassa reticulata (Forbes & Hanley) for E. levicolle and E. musteli, Hippolyte varians (Leach) for E. benedeni (Ruszkowski); Solen vagina for Echinobothrium sp. and the crustaceans Gammarus locusta and Oedicerus longimanus for E. typus (Anantaraman 1963; Rees 1961).

CHAPTER 11

SEX-RELATED PHENOMENA

11.1 SPERM PRODUCTION IN BULLIA DIGITALIS

During disruptive examination of Bullia the presence or absence of sperm were noted. No differentiation was made between ripe and unripe reproductive status. If a whelk is in either state it is a good indication that it is fertile.

The results are presented on Fig. 11.1. The proportion of sperm producers to uninfected whelks is displayed. Infected whelks are invariably infertile and thus not part of the reproductive pool in the population.

11.2 PENIS CONDITION IN BULLIA DIGITALIS

Samples from the collection sites were examined to see if there is any relationship between infection, shell height and penis size. Four categories are recognised: medium/small penis; large penis; large penis and sperm production; small penis with a large stump. The results are laid out from Figs 11.2A to 11.2P. The size range of infected and uninfected whelks is indicated in each category.

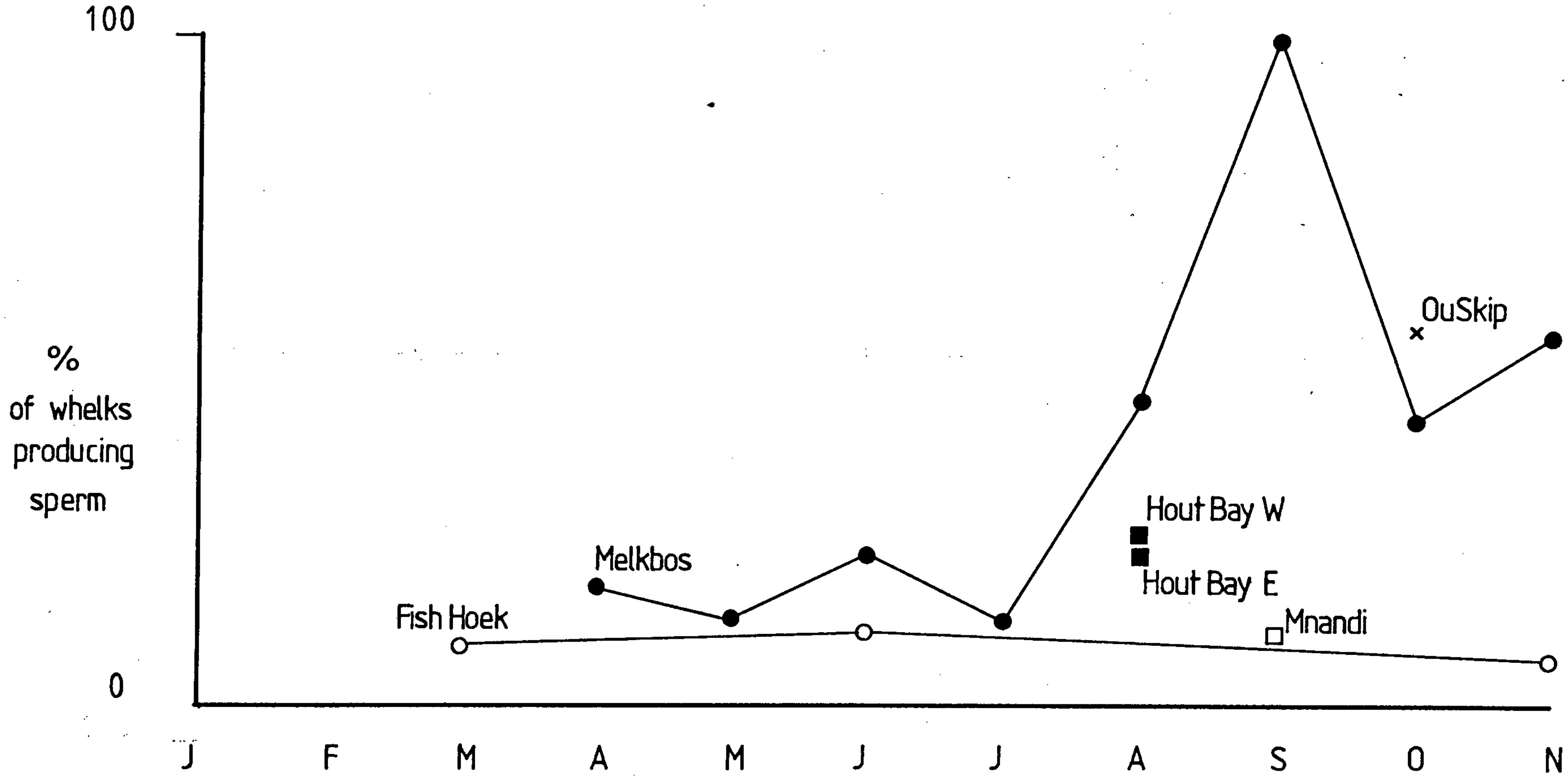


Fig. 11.1 Seasonal variation of sperm production in uninfected B. digitalis.

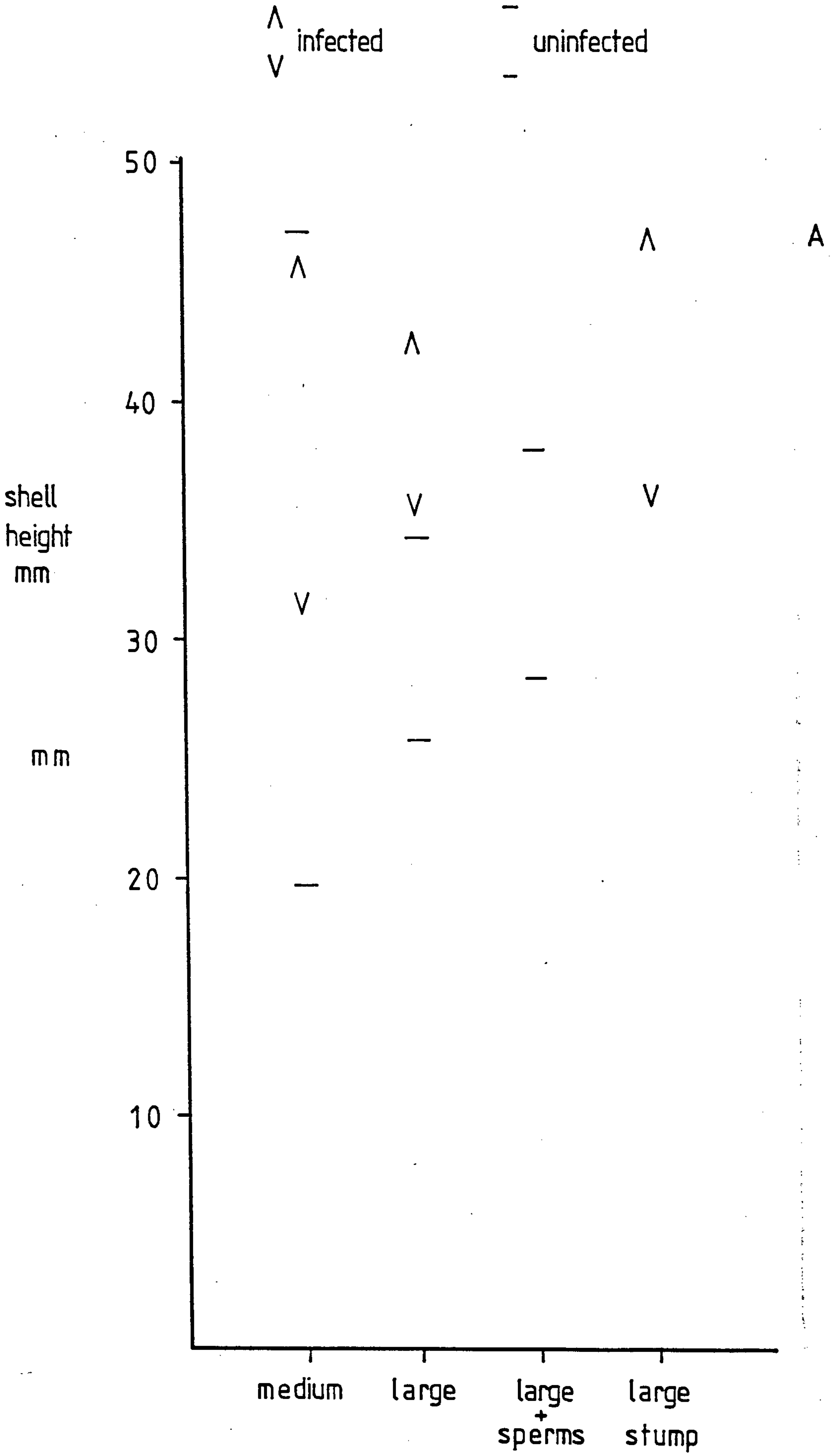
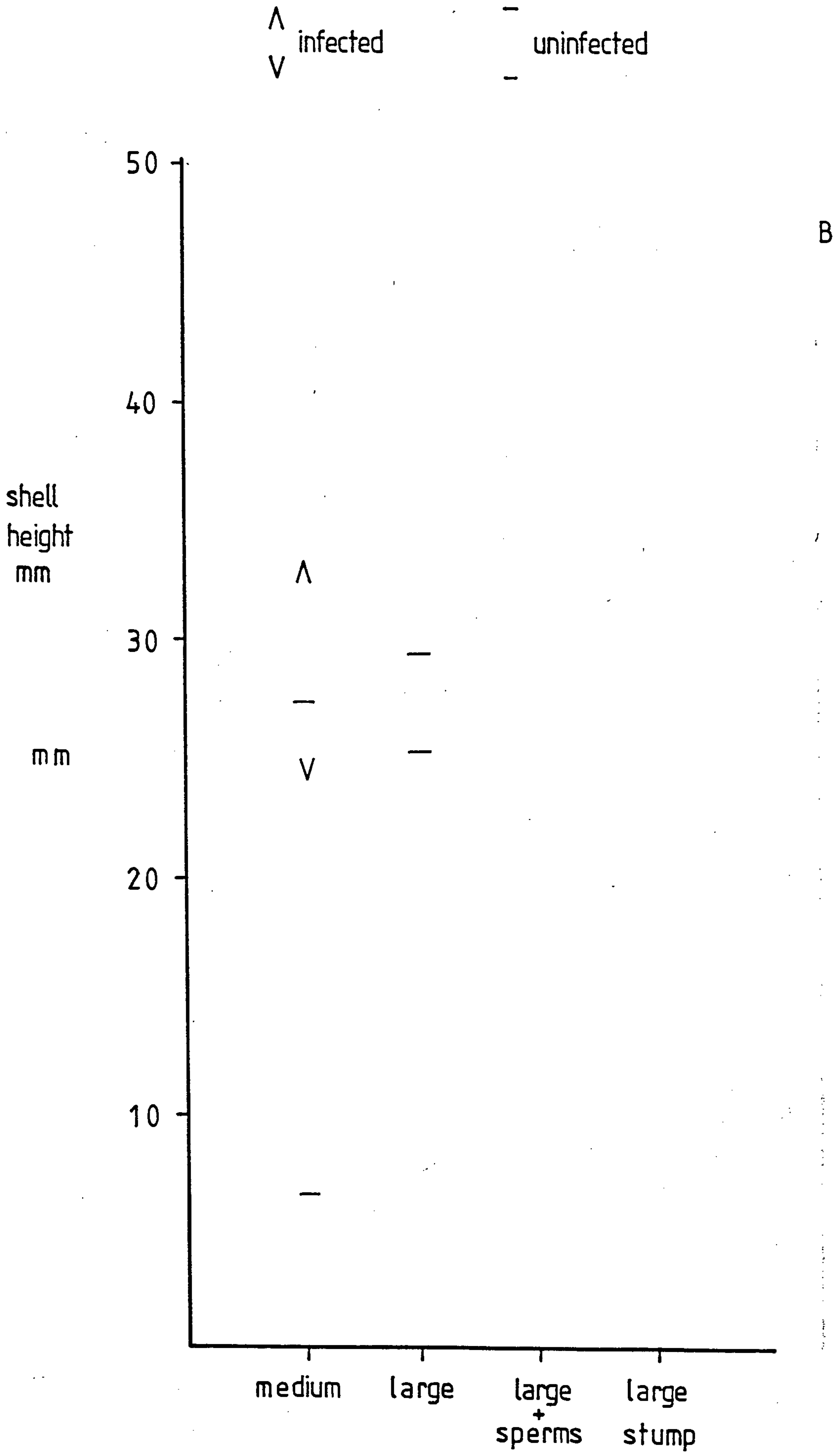


Fig. 11.2 The relationship of penis condition, shell height, sperm production and infections in male B. digitalis: A Ou Skip, October; B Melkbos, March; C Melkbos, April; D Melkbos, May; E Melkbos, June; F Melkbos, July; G Melkbos, August; H Melkbos, September; I Melkbos, October; J Melkbos, November; K Hout Bay west, August; L Hout Bay east, August;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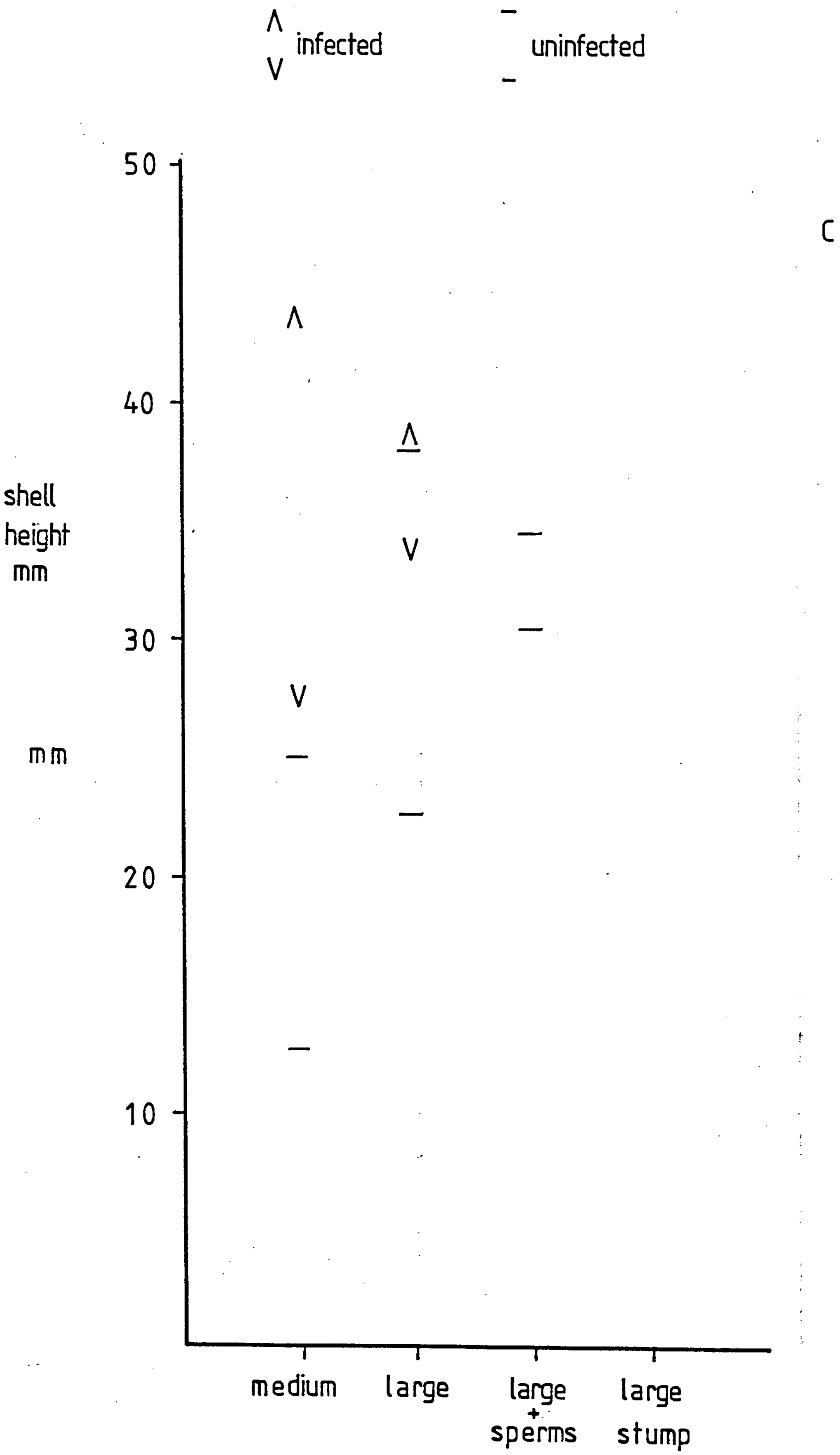
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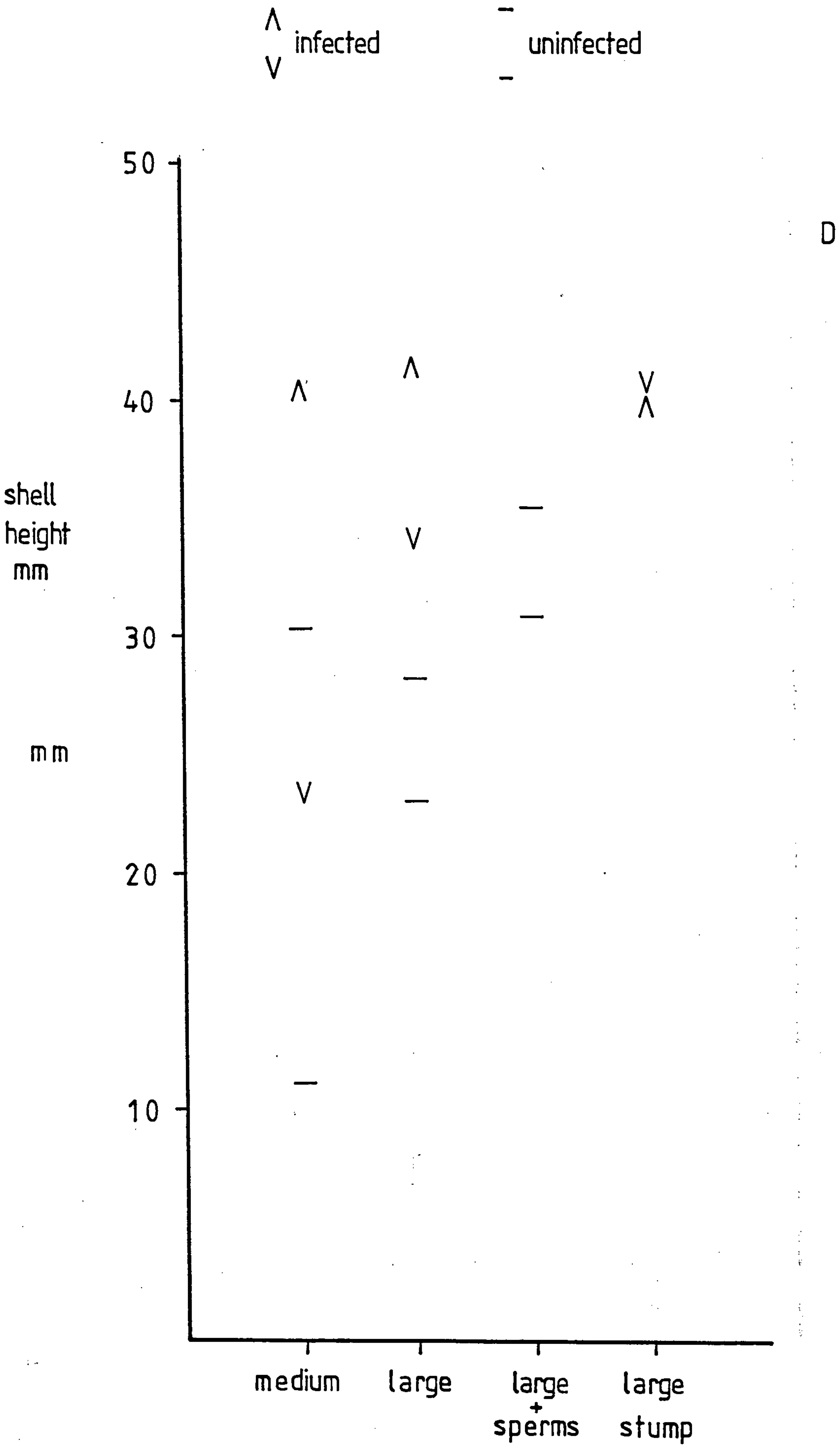
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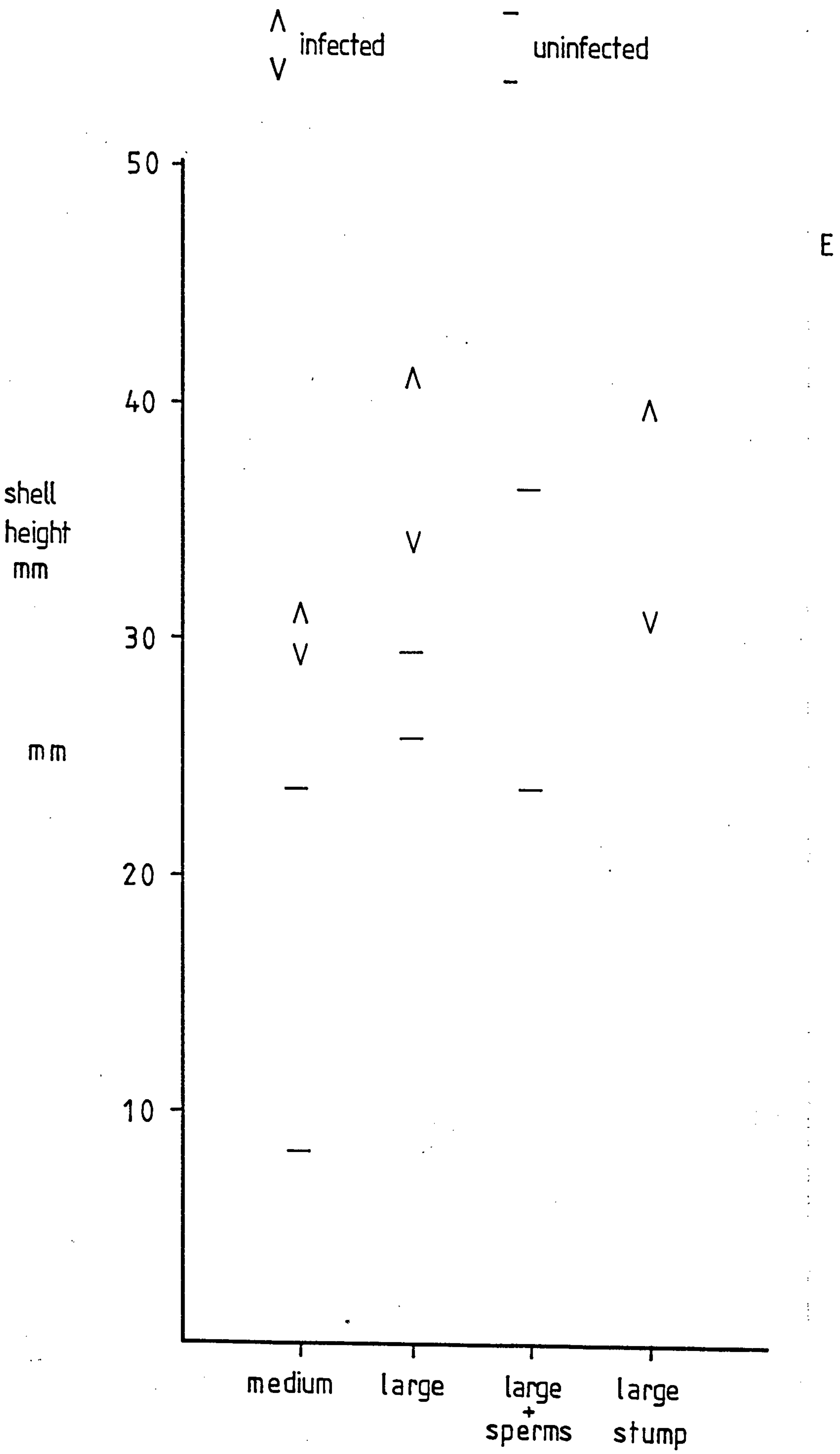
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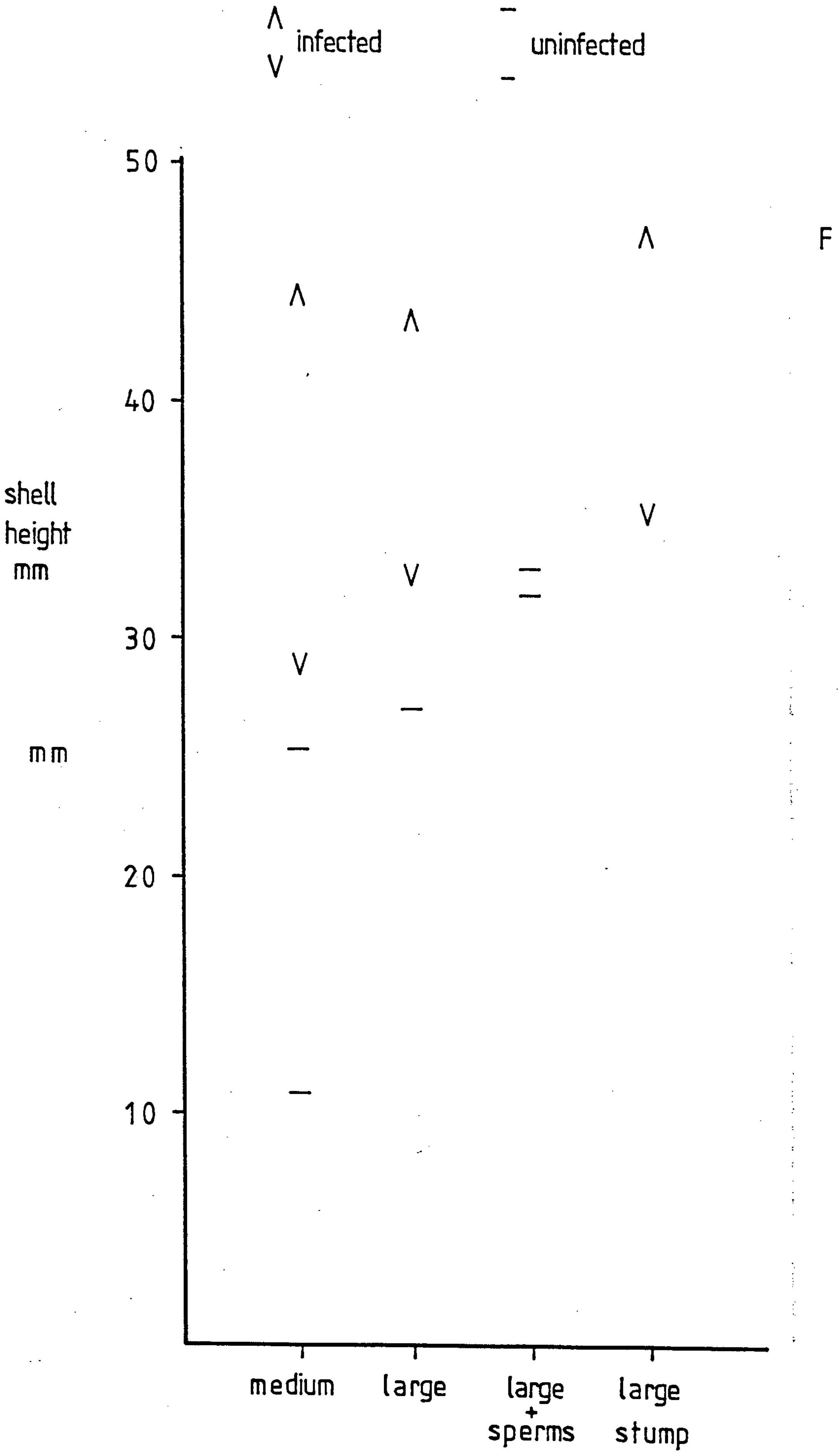
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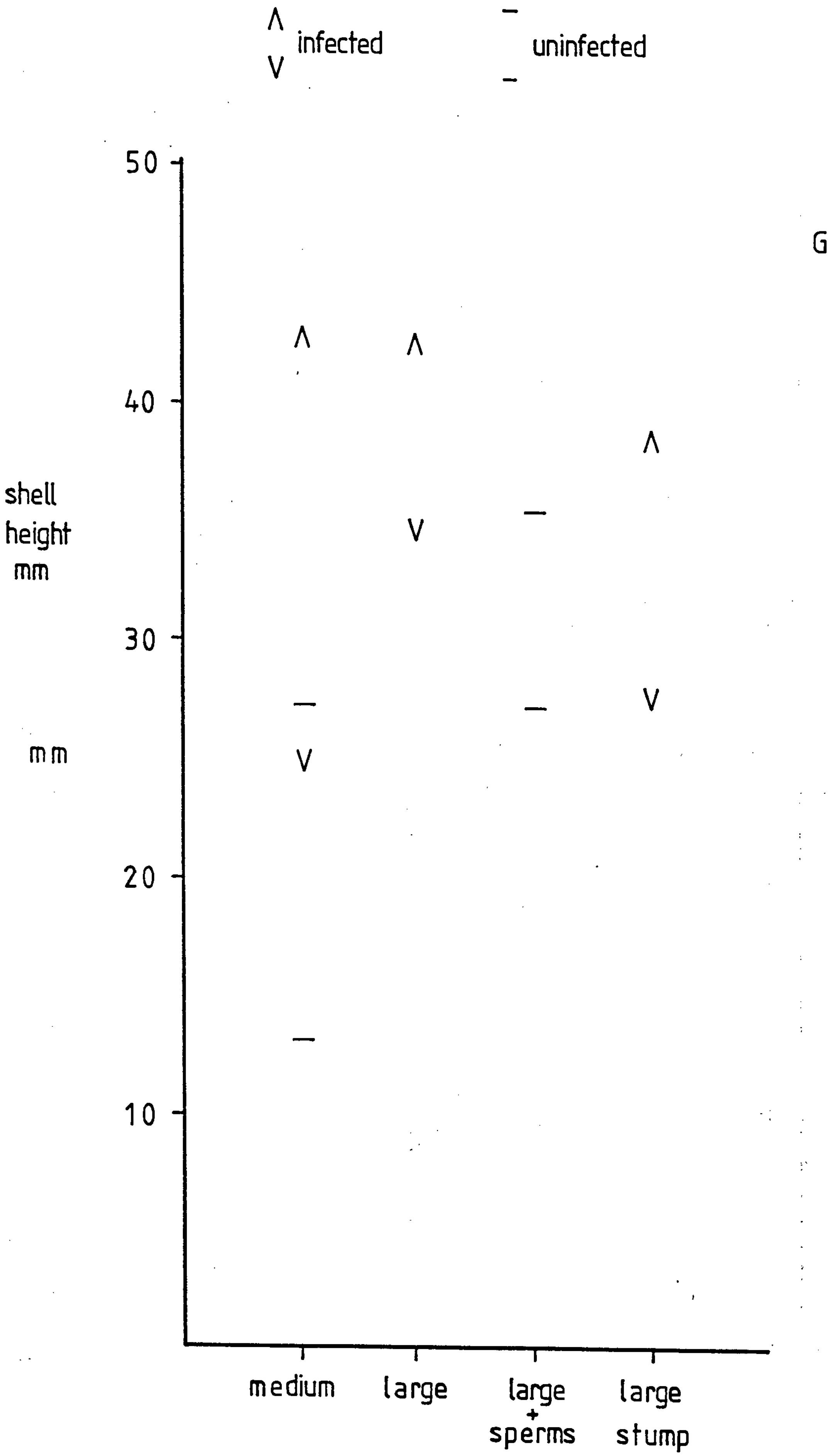
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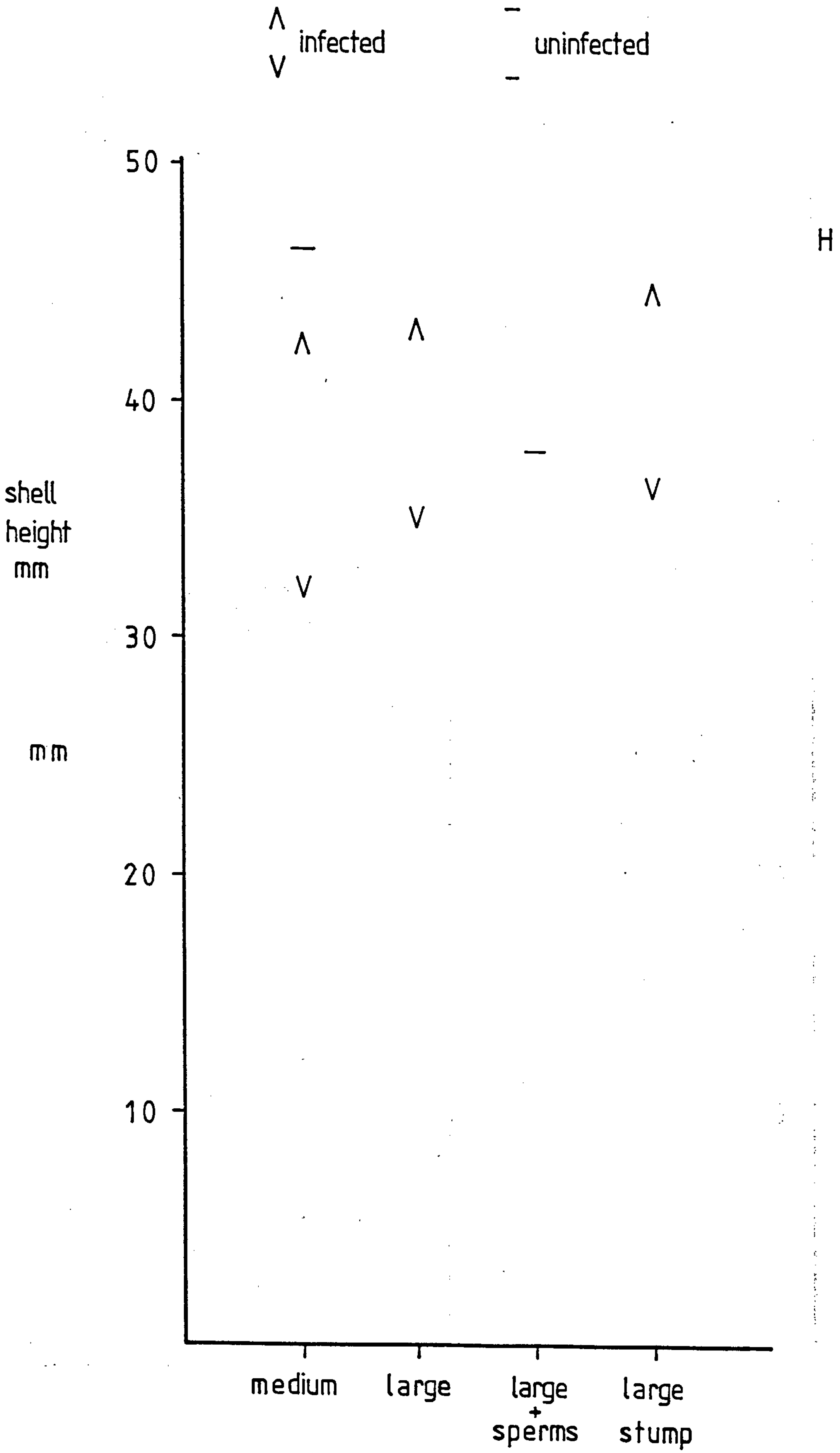
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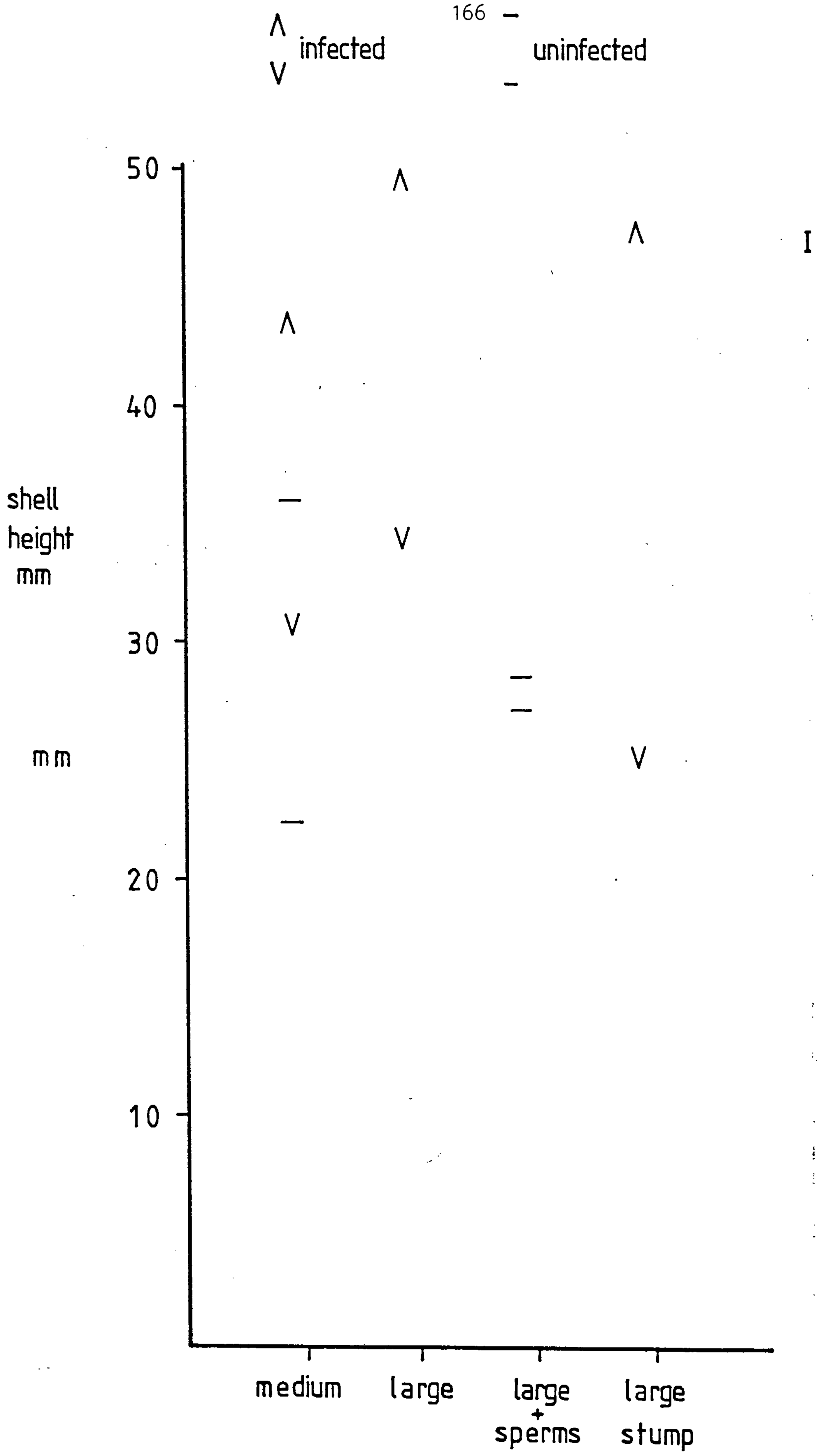

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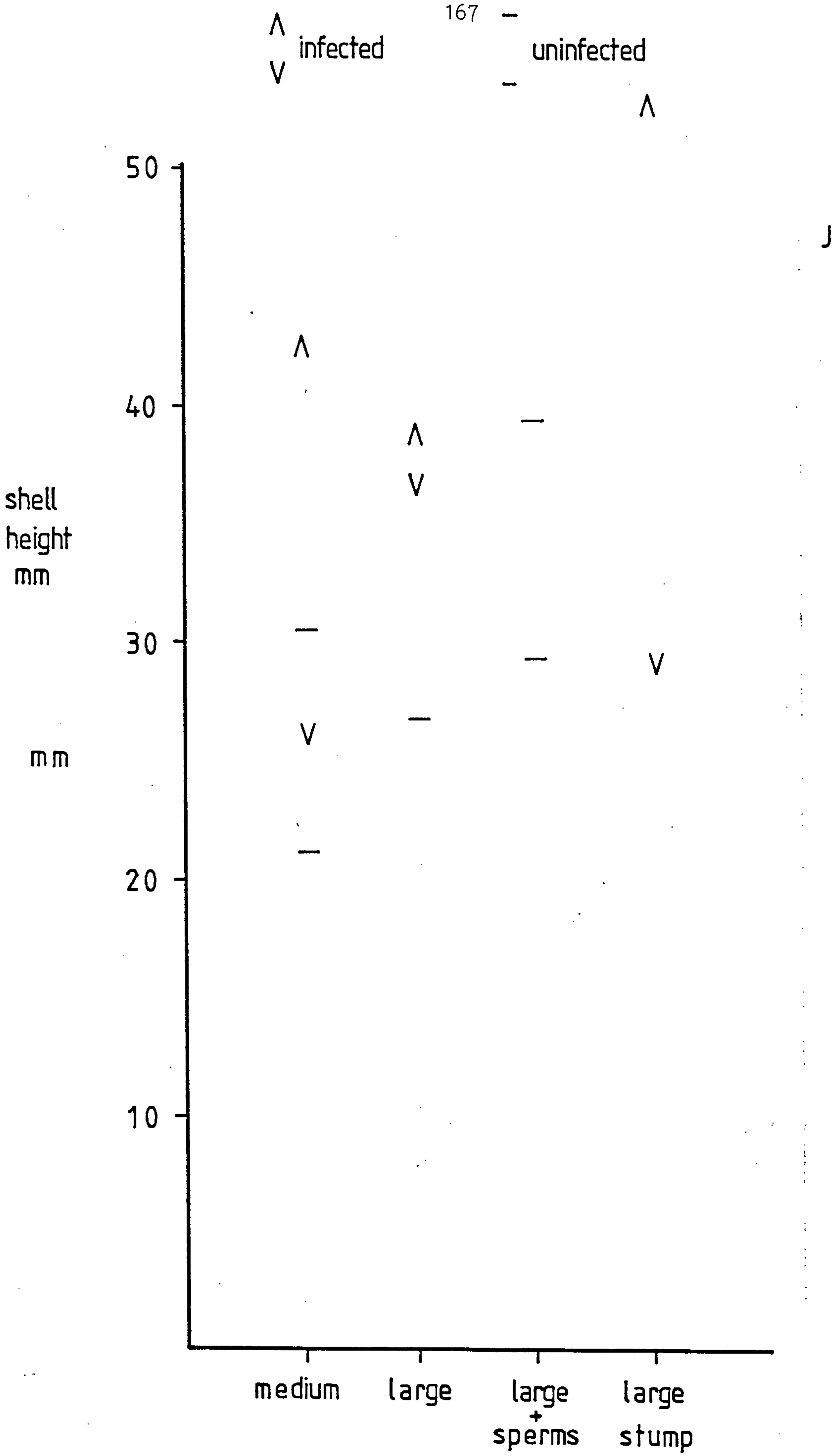
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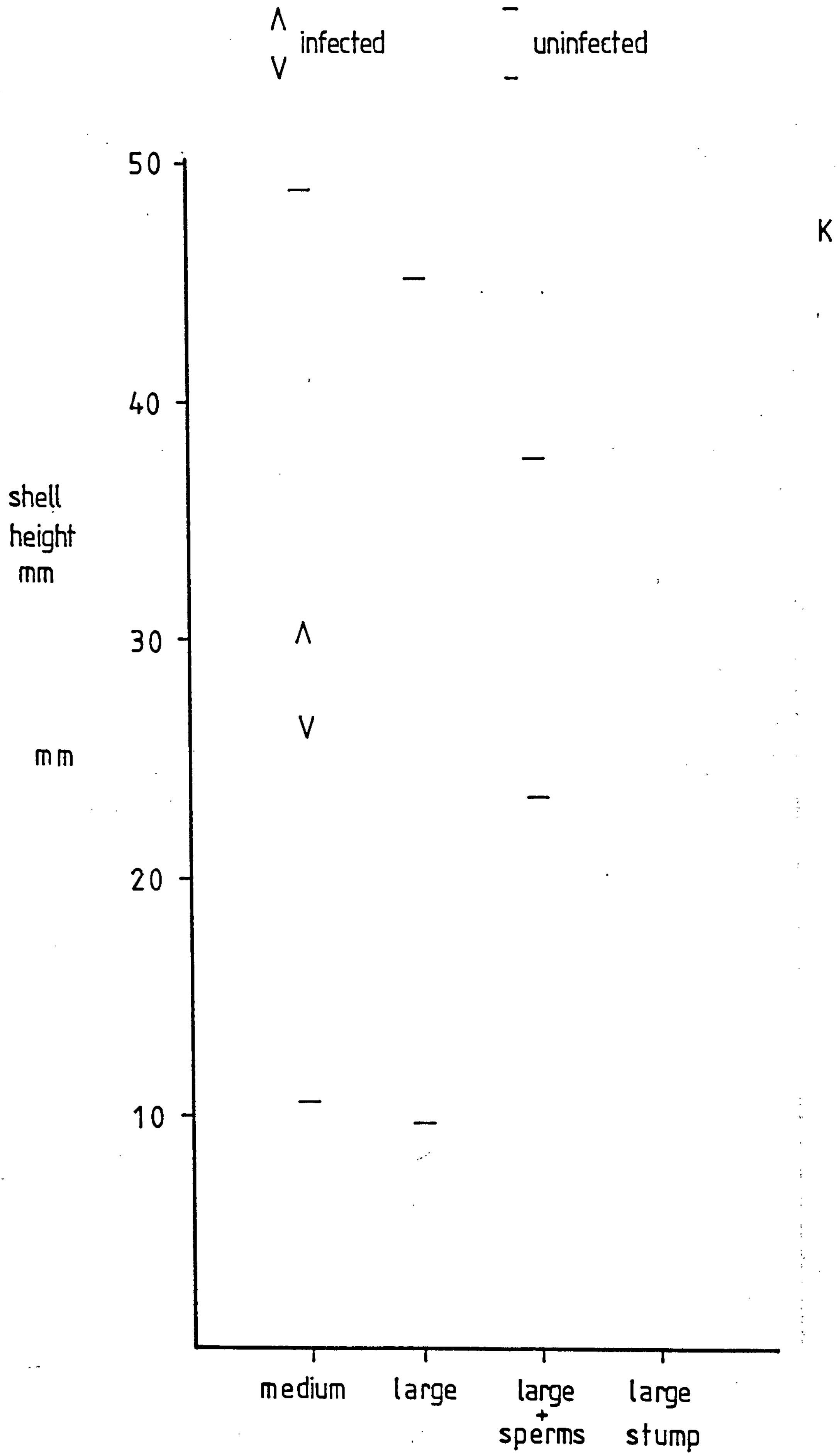
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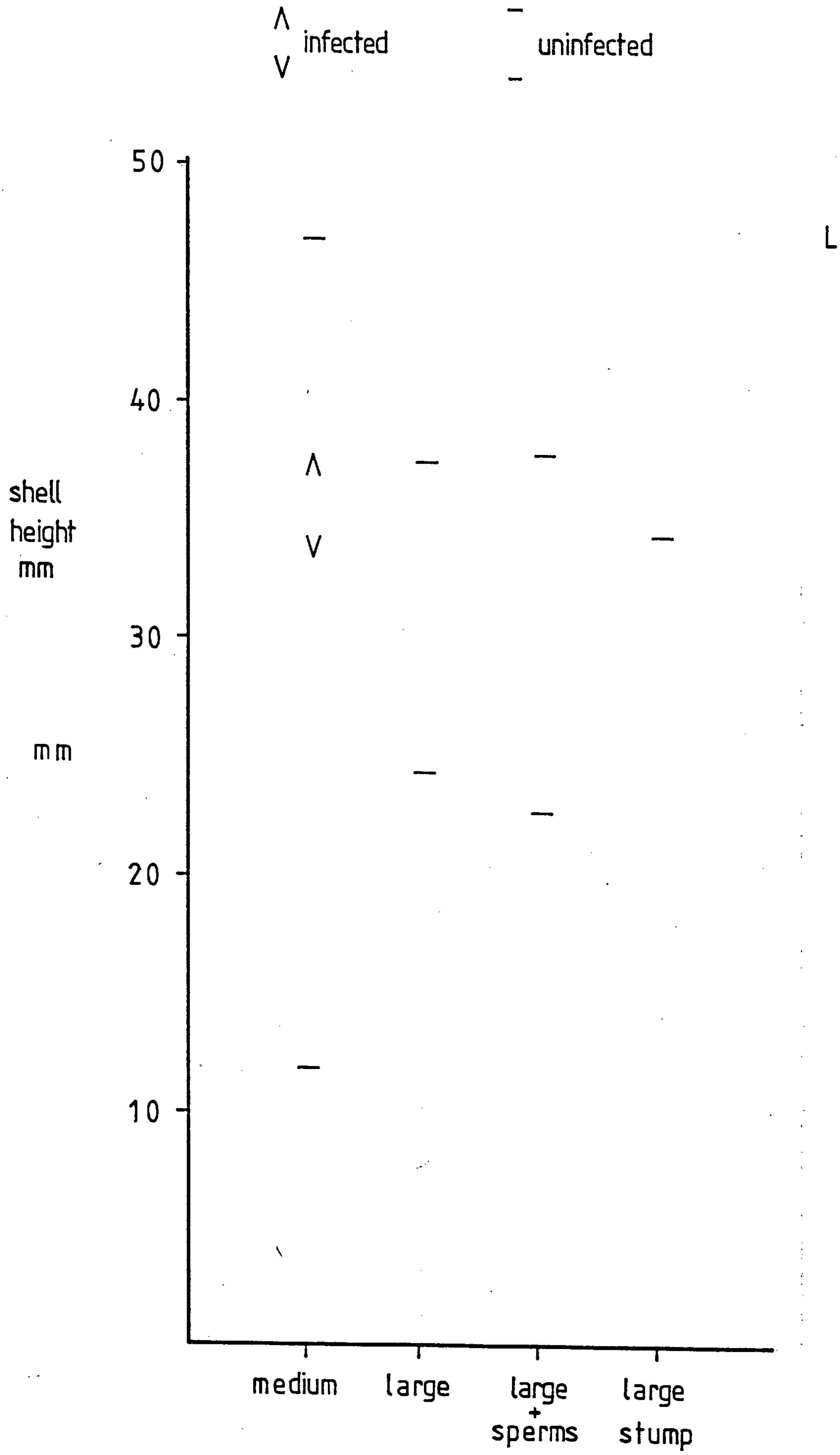
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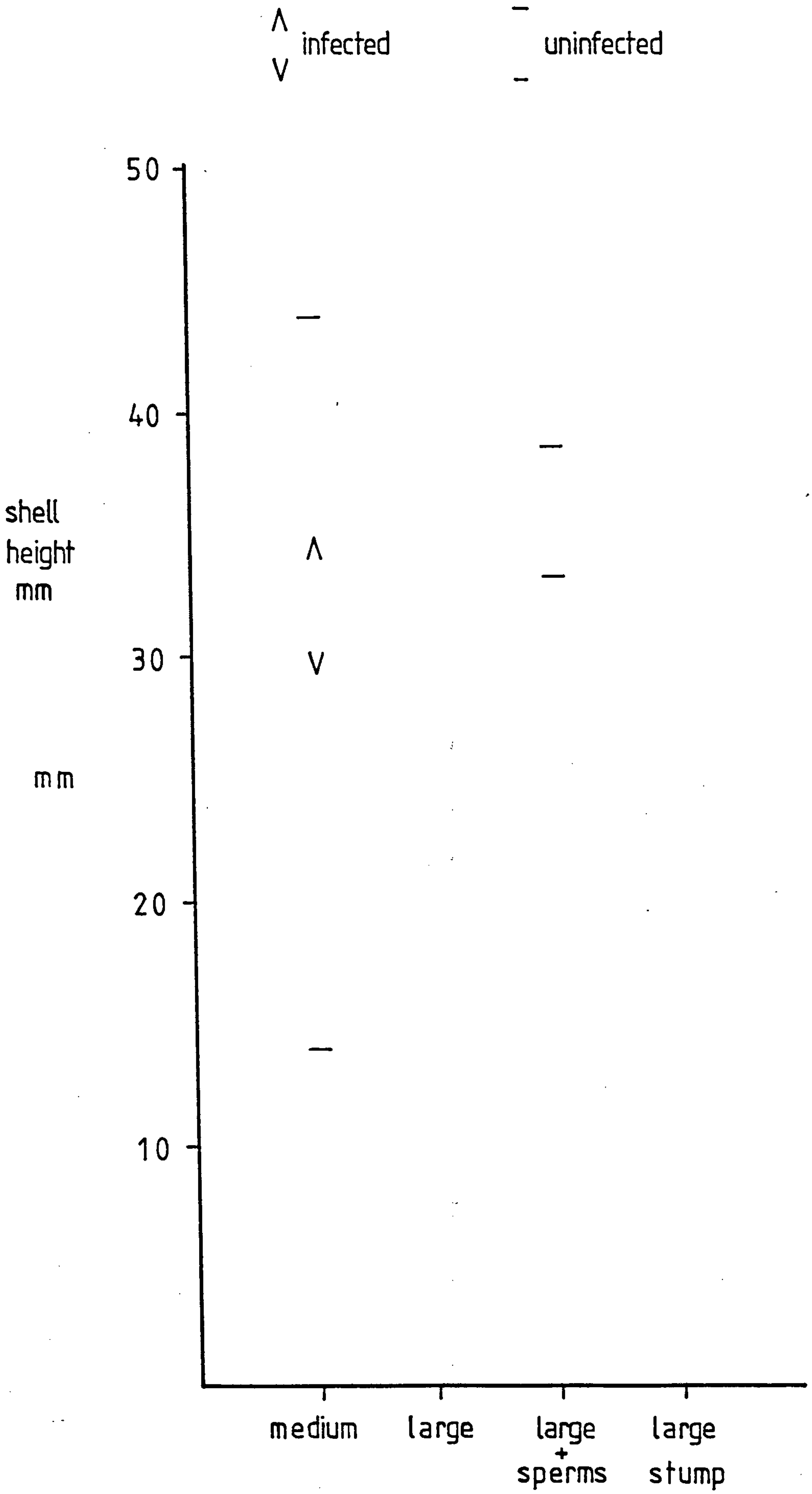
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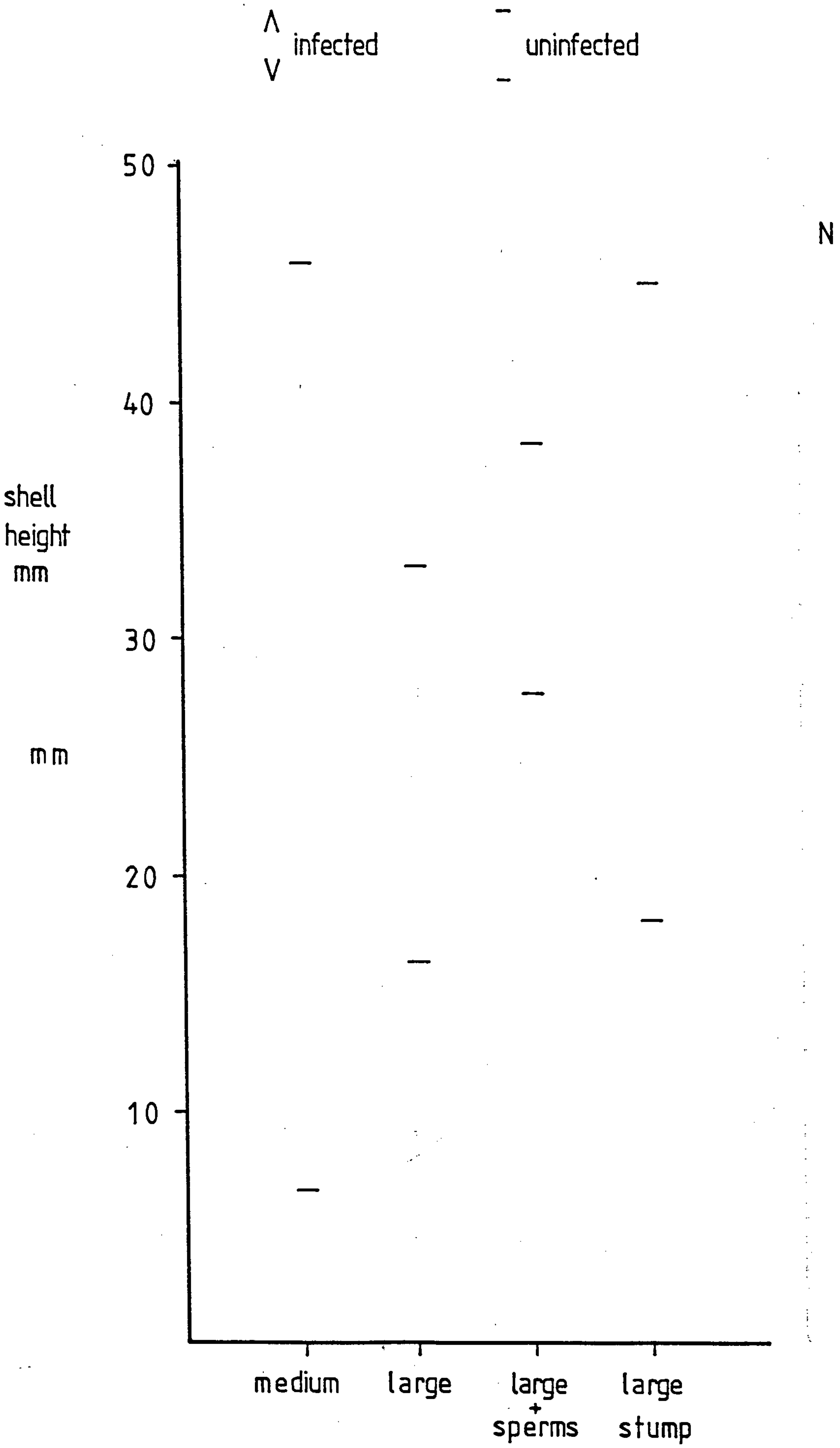
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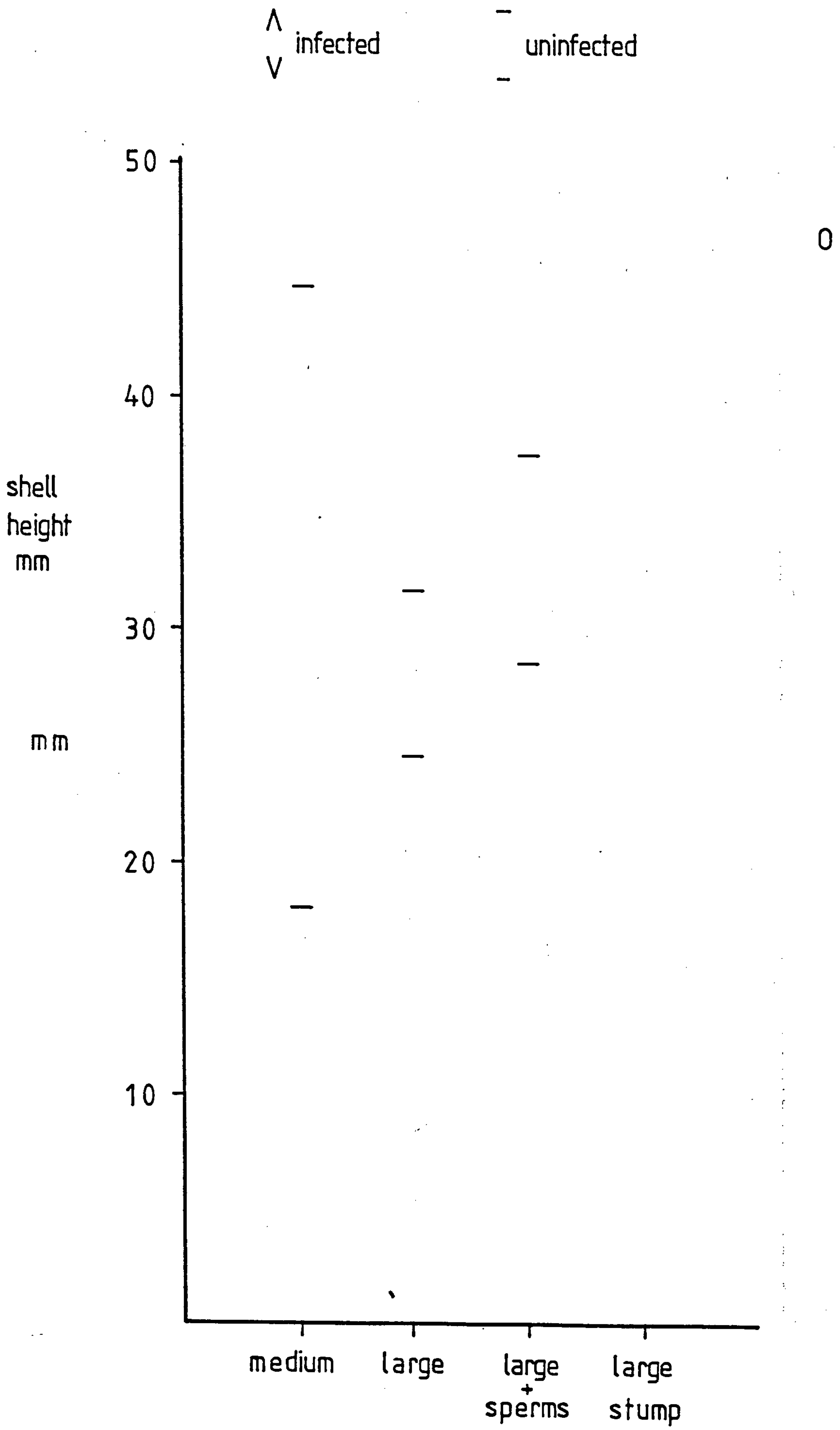
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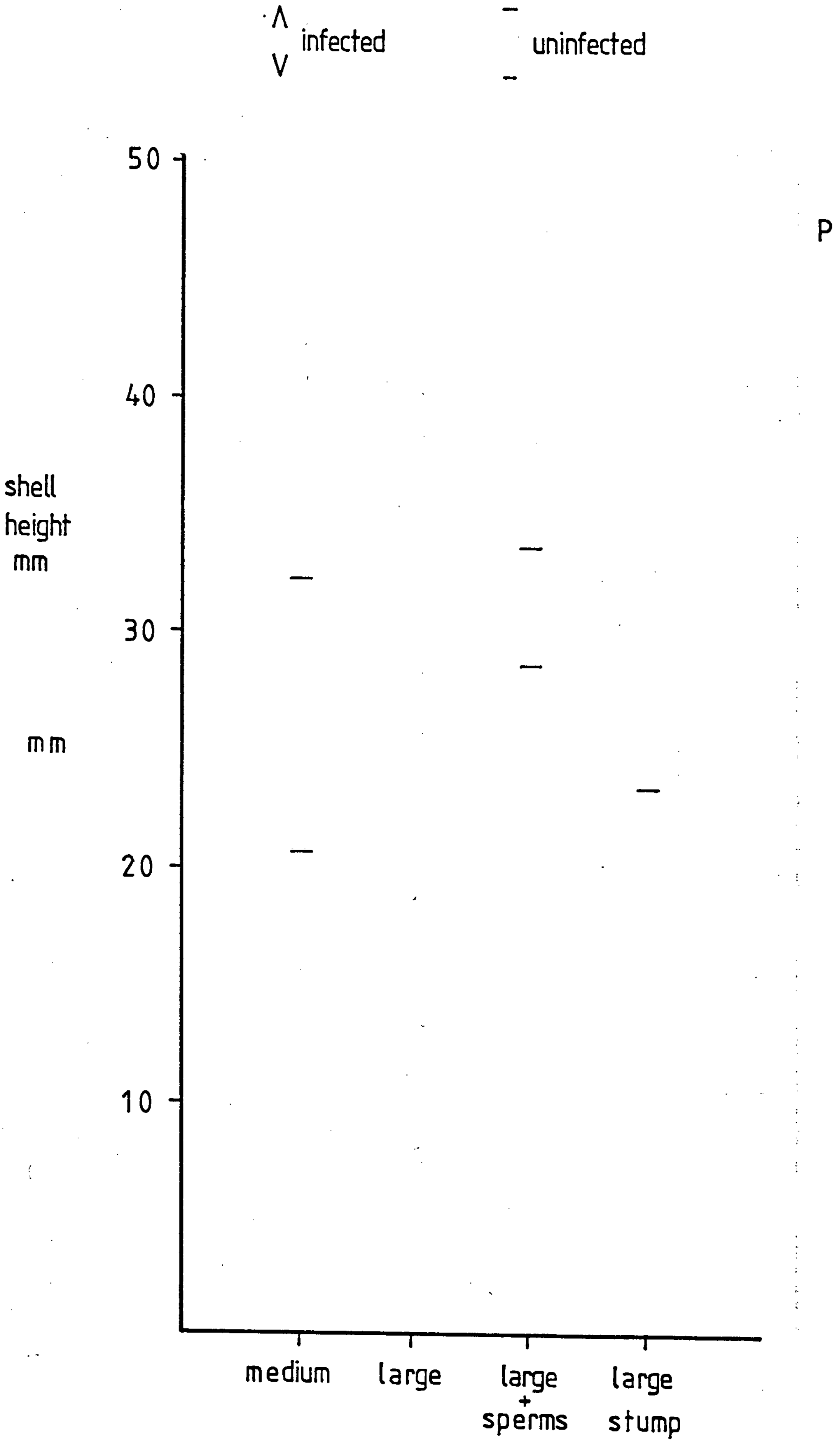
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large stump

11.3 SEX RATIO IN *B. DIGITALIS*

During examination for parasites, the sex of each whelk was determined. The results are presented as the sex ratio in uninfected whelks followed by the ratio in parasitized ones. All collections were made in 1984 unless otherwise stated.

MONTH	UNINFECTED			INFECTED		
	n Males	n Females	F/M	n Males	n Females	F/M
Feb	0	7	-	20	22	1,1
March	75	142	1,89	8	6	0,75
April	33	48	1,45	12	14	1,17
May	24	48	2,0	19	15	0,79
June	31	55	1,77	15	7	0,47
July	15	27	1,8	25	35	1,4
Aug	13	23	1,77	42	26	0,62
Sept	1	23	23,0	25	59	2,36
Oct	7	14	2,0	48	39	0,81
Nov	18	28	1,56	29	33	1,14
Fish Hoek						
March	114	24	0,21			
June	119	30	0,25			
Aug '83	120	28	0,23 = F/intersexes			
Nov	117	28	0,24			

MONTH	UNINFECTED n Males	 n Females	 F/M	INFECTED n Males	 n Females	 F/M
Ou Skip						
Oct	32	74	2,31	22	22	1,0
Mnandi						
Sept	57	80	1,40			
Hout Bay W						
Aug	131	8	0,06			
Hout Bay E						
Aug	141	6	0,04			
Noordhoek						
July '83	60	87	1,45			
Sex ratio in <u>B. pura</u>						
	49	95	1,94			
Sex ratio in <u>B. rhodostoma</u>						
	72	78	1,08			

11.4 DISCUSSION

Seasonal sperm production in B. digitalis shows considerable differences at different sites. Comparison of the proportion of males producing sperm at Melkbos with those at Fish Hoek suggests that Melkbos males have an unusually high proportion of sperm producers. Single collections at Mnandi and both sites in Hout Bay approximate to the proportions found at Fish Hoek. In a collection from Ou Skip the proportion of male sperm producers approximates to that at Melkbos. Among the factors that may account for this are temperature, genetics and parasitism. The temperature at Hout Bay is similar to that at Melkbos and Ou Skip but the sperm production figures are widely different. One sample of 300 whelks from Hout Bay is not a lot of evidence but it may be significant in dismissing temperature as a factor. A genetic difference is possible but would it make that much difference? Assuming that these factors are not operative, how could parasite levels affect sperm production? It seems paradoxical that castrating parasites are enhancing sperm production in non-infected whelks. if sterile females are still sexually receptive or at least passive, then reproductive males would waste time and energy attempting to fertilize non-existent eggs. This would lead to more copulations being needed for each successful spawning. Sperm production could be increased by increasing the effort of individual whelks or by increasing the proportion of sperm producers. If the latter occurred then this would satisfy the hypothesis.

The peak of sperm production at Melkbos coincides with copulation in

B. pura and *B. rhodostoma* on Eastern Cape beaches (McGwynne 1980). The peak is somewhat early compared to *B. digitalis* from the Eastern Cape.

It was noted that males did not all possess a penis of the same size. A similar phenomenon was noted in *B. rhodostoma* on Eastern Cape beaches (McGwynne 1980). McGwynne also noticed that some females possessed a small penis. Similar findings have been noted by Blaber (1970) in *Nucella lapillus*.

Initially it was thought that parasitism in *B. digitalis* was a contributing factor influencing penis size. Sperm producers were never infected concurrently. All had a large penis. Many non-producers had a large penis and were considerably larger than the size range of the producers. Many large whelks had a small penis. This phenomenon is masked by, but independent of, infection. This is demonstrated by the occurrence of uninfected whelks at the upper range of the non-producing categories, where they often exceed the size of any sperm producer.

Inspection of Figs 11.2 A-P shows that the lower indices of the range for whelks move up as one goes from under-developed to sperm-producing whelks. This probably means that sexual maturity is size-dependent and that juvenile whelks are progressing to reproductive size.

McLachlan *et al*. (1979) determined that sexual maturity was reached in

B. rhodostoma at about 15 mm shell height. In B. digitalis sperm production does not begin until about 24 mm. Further reference to shell height and growth may be found in Chapter 12.

Parasitized whelks are found always towards the top of the range in each category. Much has been written about parasitic gigantism (Rothschild 1936, 1941; Lysaght 1941, and Sousa 1983). Sousa summed up the phenomenon of parasite-influenced growth by stating that parasitic gigantism will occur in short-lived semelparous species because they have a relatively greater expenditure on reproduction each year. This is to make up for their shorter life. Long-lived or iteroparous specimens will probably show a reduction in growth if any change. This is due to the relatively much smaller expenditure on reproduction each year. Sousa found that microphallid infections have little effect on growth.

Bullia is an iteroparous snail and can be heavily infected with microphallid trematodes. If Sousa's statement is true, then larger whelks tend to have a higher incidence because size is a function of time and therefore larger whelks have had longer exposure, and thus a greater chance of infection. The range of infected whelks is remarkably uniform regardless of their penis condition. A considerable proportion of whelks having a large penis are infected. One would not expect a large penis to persist if such an infection stimulates sex reversal. It is suggested that infection abolishes reproductive capacity but has no effect on secondary sexual characteristics, positive or otherwise.

Whelks collected from Melkbos sometimes had a large stump and a small withered penis. All of these were infected. It seems easy to alot cause and effect until one sees the results for Fish Hoek and Mnandi (Fig. 11.2. L, N, P.). Here none of the whelks with a whithered penis is infected. The phenomenon of penis shedding or re-absorption has been noted in Nassarius obsoletus, Littorina littorea, L. saxatilis and the crustacean Balanus balanoides (Grahame 1969). Hoagland (1978) gave an account of penis regression in which Crepidula sp. is noted to have its organ reduced to a stump, due to sex lability.

It is possible that penis shedding is as common in B. digitalis as it is in L. littorea, but that it is generally hidden by the rapid regeneration time as in L. littorea. Assuming that the rate of shedding is the same infected or uninfected, infected whelks, having a non-functioning gonad, may take much longer to regenerate a penis, if at all. This would account for the preponderance of infected whelks with penis stumps.

Whelks so far unaccounted for are the large uninfected, underdeveloped, sperm non-producers. These could be either a second class of facultative males or transient intersexes. Protandry is a recurring theme in the gastropods. Perhaps this mechanism is working here. Bullia has features in its mode of life that might be taken as indicators of protandry. Ghislen (1969) stated that brooding of young is a common occurrence in protandrous species. (Larger animals are better able to protect their young.) Hoagland (1978) states that sexual lability is linked to gregariousness. That the female does not select a mate according to size or age is a factor predisposing to sexual lability. Bullia is well known for brooding (Brown 1982; Ansell & trevallion 1970; Crichton 1942b). The surfing Bullia are gregarious and in close contact, particularly when feeding. Generally, female gastropods are not mate selective by size (Fetter & Graham 1962), if anything the size difference assists adhesion during copulation. The concept of male dimorphism is not confined to lower taxa. Gadgil (1972) reported dimorphism in male red deer.

CHAPTER 12

ANALYSIS OF BULLIA SHELL PROPORTIONS

12.1 COMPARISON OF SHELL PROPORTIONS IN BOTH SEXES OF BULLIA

The shell height to body whorl height ratio in male and female whelks was compared. *Bullia digitalis*, *B. pura* and *B. rhodostoma* were examined. Any significant difference between the sexes would imply a difference in the growth process. Students t-test was used to compare ratios.

Results for *B. pura* from Mnandi:

Height/Body whorl ratio

	Male	Female
Mean	1,6764	1,7031
Variance	0,0073	0,0069
	n = 49	n = 94

$H_o : U_1 = U_2$ Both means are the same

$H_1 : U_1 > U_2$

$\alpha = 0,05$

Critical region ts > t (0,05 : 141) = 1,658

ts = 1,793

H_o is rejected.

There is a difference between male and female B. pura height/body whorl ratios.

The male ratio is significantly greater at $\alpha = 0{,}05$.

Results for B. rhodostoma from Fish Hoek:

	Height/Body whorl ratio	
	Male	Female
Mean	1,6436	1,6415
Variance	0,0043	0,0038
	n = 72	n = 78

$H_o : U_1 = U_2$

$H_1 : U_1 > U_2$

$\alpha = 0{,}05$

Critical region ts $>$ (0,05 : 194) $=$ 1,658

ts $=$ 0,203

H_o is accepted.

There is no difference between male and female height/body whorl ratios, significant to $\alpha = 0{,}05$.

Result for B. digitalis from Mnandi

	Height/Body whorl ratio	
	Male	Female
Mean	1,803	1,801
Variance	0,0072	0,0062
	n = 47	n = 99

$H_o : U_1 = U_2$

$H_1 : U_1 > U_2$

$= 0,05$

Critical region $ts > (0,05 : 145) = 1,658$

$ts = 0,140$

H_o is accepted.

There is no difference between male and female height/body whorl ratios, significant to $\alpha = 0,05$.

12.2 HEIGHT/BODY WHORL RATIOS AT DIFFERENT BEACHES

Samples of 60 B. digitalis were taken from the following categories:

Ou Skip	Female
Melkbos	Female, infected
Melkbos	Female
Melkbos	Male, infected
Melkbos	Male
Hout Bay W	Male
Hout Bay E	Male
Noordhoek	Female
Noordhoek	Male
Fish Hoek	Female
Fish Hoek	Male
Mnandi	Female
Mnandi	Male

A one-way ANOVA test was performed

H_o : all means are equal

H_1 : not all means are equal

$\alpha = 0,05$

Critical region > (F 0,05, 12, 767) = 1,78

Calculated from data: F = 9,91

H_o is rejected.

To find which means are equal the Newman Keuls range test is used.

12.3 HEIGHT/APERTURE RATIOS ON DIFFERENT BEACHES

The same samples of B. digitalis from 12.2 were used in this determination. A one-way ANOVA test was performed.

H_o : all means are equal

H_1 : not all means are equal

= 0,05

Critical region > (F 0,05, 12, 767). = 1,78

Calculated from data: F = 15,93.

H_o is rejected.

Newmans Keuls test was applied to find out which means were equal.

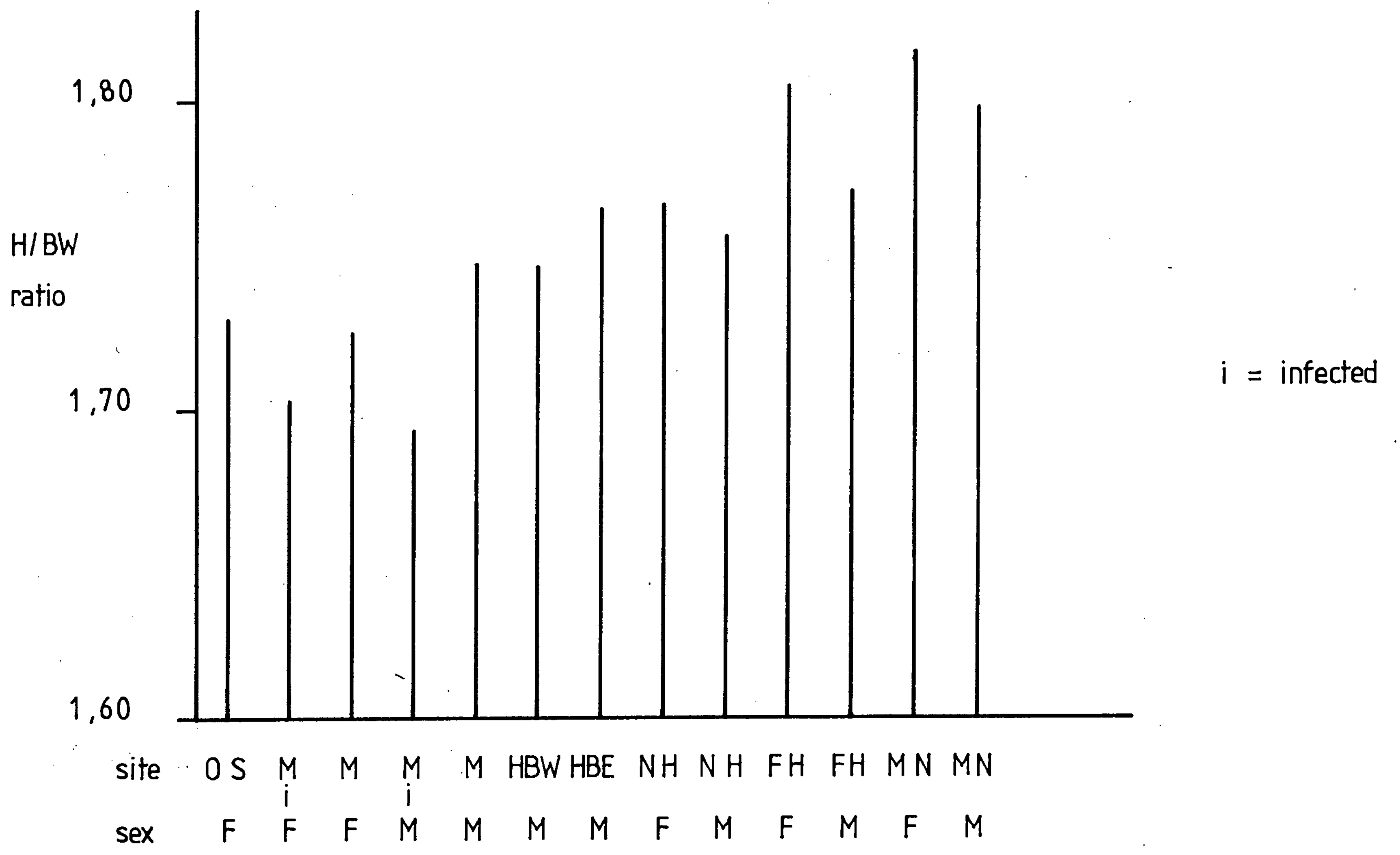

Fig. 12.1 Variation of shell height to body whorl height ratio in <u>B. digitalis</u> from different sites.

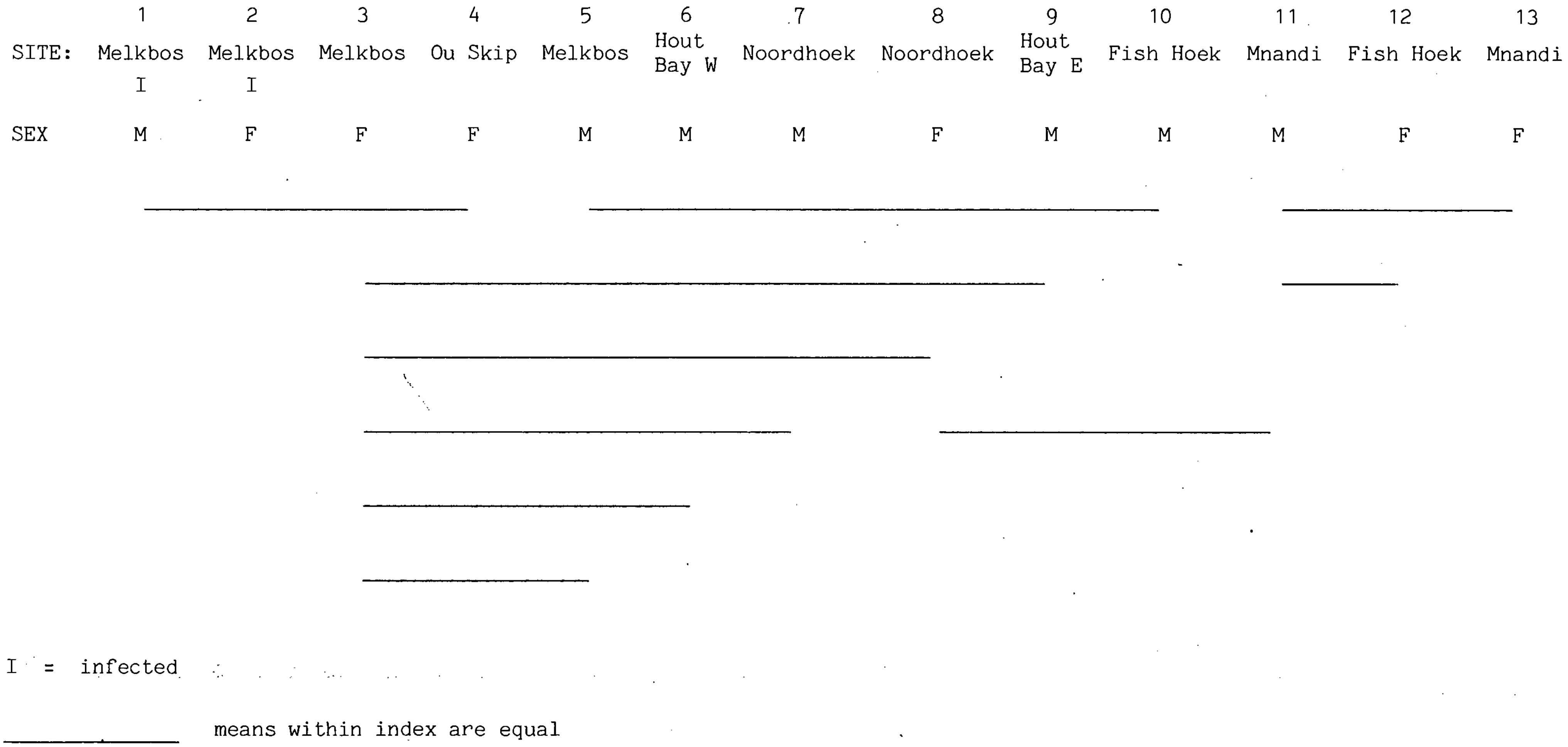

Fig. 12.2 Results of Newmans Keuls range test to differentiate height/body whorl ratios.

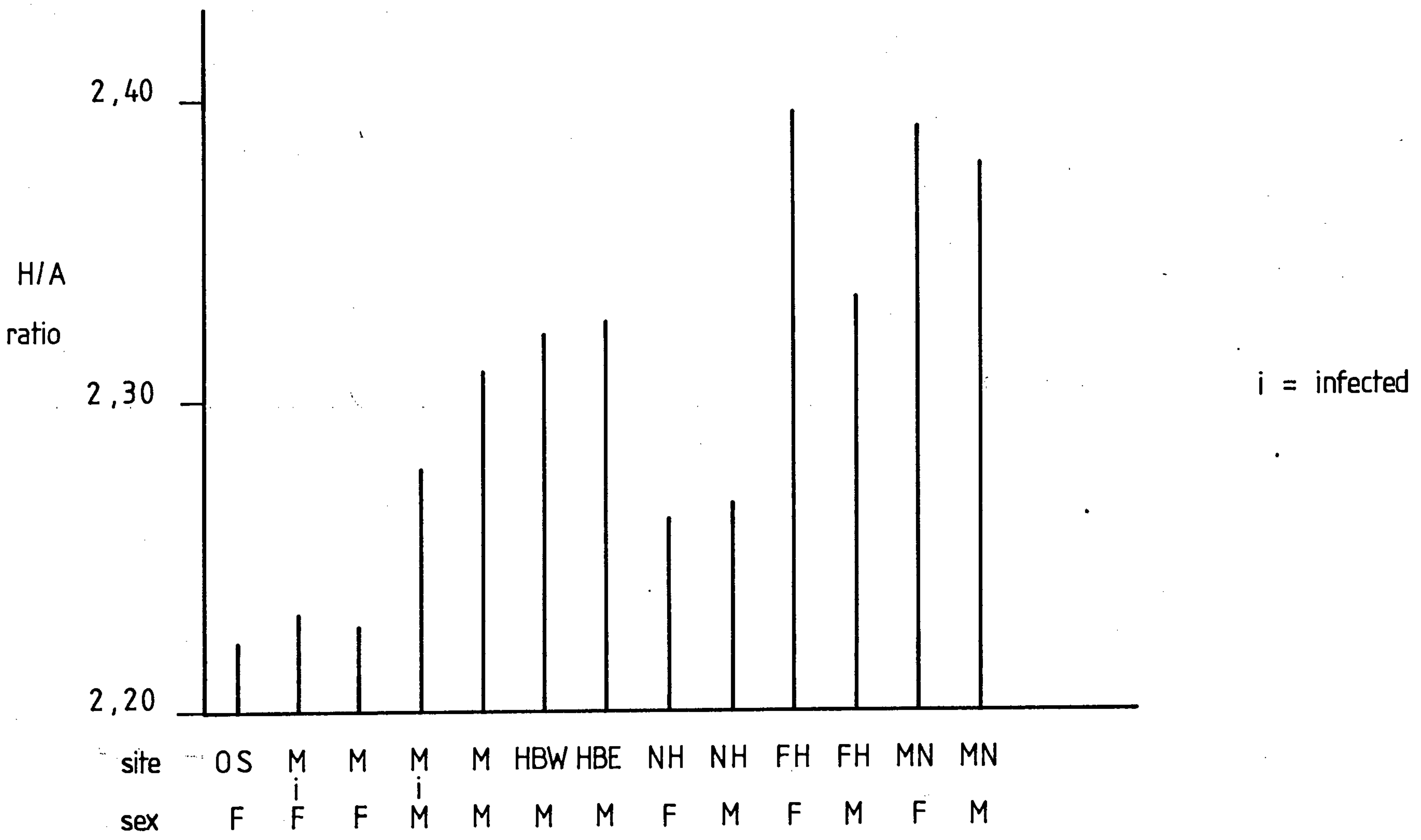

Fig. 12,3 Variation of shell height to aperture height ratio in B. digitalis from different sites.

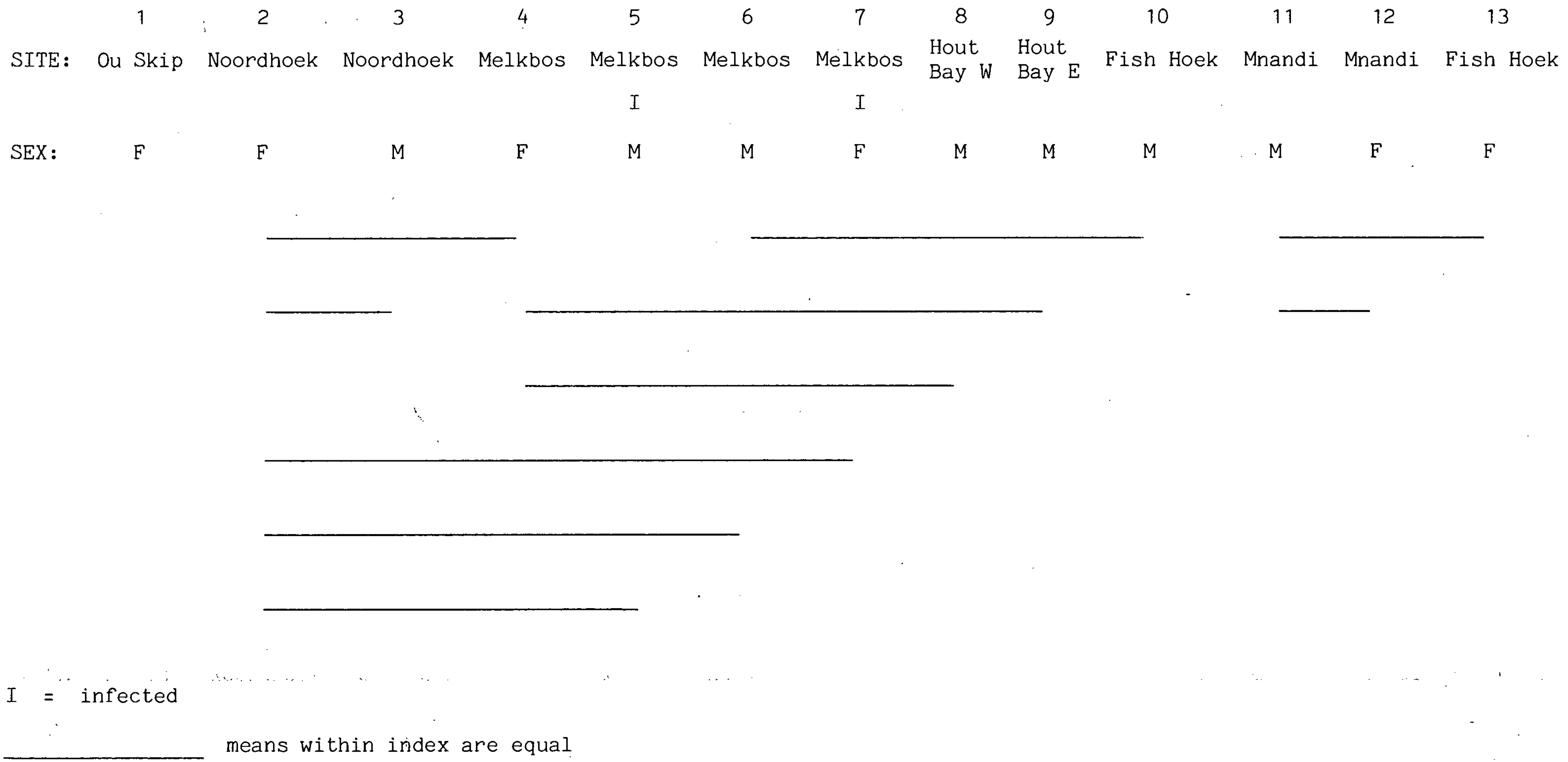

Fig. 12.4 Results of Newman Keuls range test to differentiate height/aperture ratios

TABLE 12.1 Size distribution of uninfected B. digitalis from Melkbos. A males; B females

	SHELL HEIGHT CATEGORIES mm													
	4	8	12	16	20	24	28	32	36	40	44	48	52	56
A														
March April May	5	16	25	11	33	33	15	19	9	3	1	1	0	
June July August	0	2	2	6	14	21	26	29	30	8	3	0		
September October November	0	0	0	0	3	9	23	24	39	22	6	1	1	0
B														
March April May	29	43	39	23	21	33	25	27	13	6	9	4	1	0
June July August	0	1	2	13	21	26	43	22	24	13	12	2	2	0
September October November	0	0	0	4	4	9	16	29	33	38	31	26	4	2

TABLE 12.2 Size distribution of uninfected <u>B.digitalis</u> from Fish Hoek

	SHELL HEIGHT CATEGORIES mm													
	4	8	12	16	20	24	28	32	36	40	44	48	52	56
March	0	3	6	28	41	25	7	9	9	10	1	0		
June	1	10	26	10	17	19	20	11	11	16	15	1	0	
August	0	6	22	71	29	9	0	1	4	7	0			
November	0	0	2	4	26	33	27	19	11	19	4	0		

12.4 GROWTH

Melkbos

Pooled data from the collections of B. digitalis made at Melkbos are tabulated. The three categories are: March, April and May; June, July and August; September, October and November. Each of the three are divided by sex (Table 12.1).

Fish Hoek

Four collections are tabulated. March, June and November are from 1984. August is from 1983. The data is not divided by sex (Table 12.2).

12.5 DISCUSSION

Differences in proportions of Bullia rhodostoma shells have been reported by Brown (1971). Male shells were thought to be slimmer than females. McLachlan (1979) reported no difference in shell proportions between the sexes of B. rhodostoma.

Environmental influences have been cited as major determinants of shell proportions. Crothers (1973, 1979a, 1979b, 1981, 1983, 1984, 1985). Kitching et al. (1966) and Cambridge and Kitching (1982) have been concerned with Nucella sp. High wave action was determined to be responsible for selecting against slim shells due to the smaller proportional foot area and thus power of adhesion. Predation by crabs was deemed a factor in selecting against squat shells due to their greater vulnerability. Thus the proportions of the two morphs could tell much about the level of exposure of any beach. The results by Crothers, (1984) and Crothers and Cowell (1979) are less convincing than their other works. Generally, though, his findings have a sound basis. Reimchen (1982), Newkirk and Doyle (1975), Smith (1981) and to a lesser extent, Struhraker (1968) deal with morphometric differences in Littorina sp. Wave action is generally thought to be a major influence, much as in Nucella.

In the comparisons of males with females in the species B. digitalis, B. rhodostoma and B. pura, only B. pura was found to have a significant, $\alpha = 0,05$, difference in shell proportions. Females were apparently narrower than males. Males are not markedly smaller than females. Thus it is unlikely that the ratio is size-dependent.

If the difference is biologically significant it would rule out the possibility of protandry in the species and casts doubt on the phenomenon in the genus. It has been shown by Da Silva-Tatley (1985) that B. pura is genetically the most different of the three whelks. This, coupled with their relative rarity (Hoagland 1977) and unambiguous sexuality, tends to reinforce the hypothesis of absence of protandry in this species. B. pura thus is different to the other two species and might not be representative of them.

The relationship of shell height to body whorl height was investigated.

Analysis graphically and statistically using ANOVA and Newmans Keuls test (Zar 1974) showed a trend from Ou Skip to Mnandi. The whelks became proportionally narrower as Mnandi was approached. Wave action is not thought to be a dominant factor. Noordhoek has the highest wave action yet is in the middle of the distribution. Temperature may play a part but no significant difference was detected between immediately adjacent beaches on the Atlantic side and False Bay. One would expect a greater separation between Noordhoek and Fish Hoek if temperature was a major factor. The two collections at Hout Bay were from an area of low wave action and an exposed area. There is no significant difference in shell proportions between the two sites. The mean of the shell ratios is opposite to expected if one is applying the principles of Crothers findings. Perhaps predation by crabs and wave action are not antagonistic in selection for shell proportion in Bullia. Short fat whelks would have less adhesive power but what is there to fasten to on a sandy beach? One might expect a stout individual to have a proportionally larger foot with which it could surf more efficiently to escape predation. Obviously a lot more must be known before any solid inferences can be made. If predation, wave action and temperature are not major influences then perhaps the difference is genetic. The trend of increasing shell change with distance suggests this. Genetic difference has been detected between B. digitalis from Ou Skip and Muizenberg by Brown and Da Silva (1979) in terms of oxygen consumption at low temperatures.

Shell height and aperture height ratios were examined. There appears to be a slight relationship between shell proportion and wave action with the stouter shells being found at Melkbos, Ou Skip and Noordhoek. The lack of significant difference between either end of Hout Bay tends to detract from these results. As in the examination of shell height to body whorl height ratio there is a trend of narrow whelks becoming dominant as one moves towards Mnandi. The same factors are probably responsible for the change in height to aperture height with distance.

A scatter diagram of shell height versus height to body whorl height ratio was constructed. The result showed points all over the graph with no trend appearing. There does not appear to be a change of ratio with height.

Growth in *Bullia* has been examined by Crichton, 1942b in *B. vittata* from India, by Brown (1971) in *B. rhodostoma*, and by McLachlan *et al*. (1979) in *B. rhodostoma*. The growth rate of the Indian species appears to be much higher than that of *B. rhodostoma*. *B. digitalis*, coming from a colder habitat may be expected to have a lower growth rate. The data of shell height for collections of *B. digitalis* are arranged in Table to show the size distributions. The numbers of whelks in the collections from Melkbos were deemed too low and the numbers of collections from Fish Hoek inadequate to apply von Bertalanffy growth equations. A rough estimate using the lower size limit whelk at each collection was made. Assuming that recruitment is a discreet and single event each year, then the smallest whelk in each collection must come from that recruitment. Whilst acknowledging that this is fraught with potential inaccuracies a growth rate of about 9 mm in six months was obtained. So in August/September one would expect the smallest whelks to be about 13 mm long.

McLachlan et al. (1979) determined that sexual maturity in B. rhodostoma occurs at about 15 mm shell height or two years. Sperm production in B. digitalis commonly occurs at about 23 mm. If the growth rate for B. digitalis is correct then it appears that it might get to sexual maturity at a comparable time to B. rhodostoma due to its higher growth rate at this stage. Due to the dubious nature of the growth rate then any further inferences should be treated with even greater caution.

CHAPTER 13

SHELL COLOUR IN *BULLIA DIGITALIS*

13.1 MATERIALS AND METHODS

Whelks were examined during parasitological work. Initially the colour categories were the same as those used by McGwynne (1980) (see Chapter 1.1). It was noticed that purple and brown may commonly occur on the same shell; differentiation between the two colours is difficult in transitional forms. The other colour varieties could occur also in any combination on one shell. These factors, coupled with the inconclusive results of McGwynne's work prompted a new classification:

Stripes, shells with spiral bands of colour
Colours, shells of any non-banded colour
Cream, unpigmented shells.

13.2 RESULTS

TABLE 13.1 Shell colour in B. digitalis from Melkbos collected in June 1983 (infected with type Z).

	Female		Male	
	n	%	n	%
Stripes	19	38,8	12	32,4
Colours	20	40,8	21	56,7
Cream	10	20,4	4	10,1

TABLE 13.2 Shell colour in B. digitalis from Melkbos collected in June 1983

	Female		Male	
	n	%	n	%
Stripes	20	40	4	28,6
Colours	21	42	8	57,1
Cream	9	18	2	14,2

TABLE 13.3 Shell colour in B. digitalis from Melkbos collected in October 1984 (infected with type Z).

	Female		Male	
	n	%	n	%
Stripes	11	28,2	14	29,2
Colours	27	69,2	33	68,8
Cream	1	2,6	1	2,1

TABLE 13.4 Shell colour in B. digitalis from Melkbos collected in March 1984.

	Female		Male	
	n	%	n	%
Stripes	32	22,5	15	20,0
Colours	108	76,1	58	77,33
Cream	2	1,4	2	2,67

TABLE 13.5 Shell colour in B. digitalis from Ou Skip collected in October 1984 (including infected whelks).

	Female		Male	
	n	%	n	%
Stripes	56	58,3	24	44,4
Colours	36	37,5	27	50,0
Cream	4	4,2	3	5,6

TABLE 13.6 Shell colour in B. digitalis from Hout Bay East, collected in August 1984.

	Male	
	n	%
Stripes	87	61,7
Colours	54	38,3
Cream	0	0

TABLE 13.7 Shell colour in B. digitalis from Hout Bay West, collected in August 1984.

	Male	
	n	%
Stripes	65	49,6
Colours	59	45,0
Cream	7	5,3

TABLE 13.8 Shell colour in B. digitalis from Noordhoek collected in July 1983.

	Female		Male	
	n	%	n	%
Stripes	52	59,8	35	58,3
Colours	33	37,9	20	33,3
Cream	2	2,3	5	8,3

TABLE 13.9 Shell colour in *B. digitalis* from Fish Hoek collected in August 1983.

	Female		Male	
	n	%	n	%
Stripes	13	46,4	83	69,2
Colours	9	32,1	30	25,0
Cream	6	21,4	7	5,9

TABLE 13.10 Shell colour in *B. digitalis* from Fish Hoek collected in March 1984.

	Female		Male	
	n	%	n	%
Stripes	12	50,0	38	55,9
Colours	10	41,7	19	27,9
Cream	2	8,3	11	16,2

TABLE 13.11 Shell colour in *B. digitalis* from Fish Hoek collected in June 1984.

	Female		Male	
	n	%	n	%
Stripes	18	60,0	60	68,2
Colours	7	23,3	17	19,3
Cream	5	16,7	11	12,5

TABLE 13.12 Shell colour in B. digitalis from Fish Hoek collected in November 1984.

	Female		Male	
	n	%	n	%
Stripes	9	32,1	62	52,9
Colours	14	50,0	44	37,6
Cream	5	17,9	11	9,4

TABLE 13.13 Shell colour in B. digitalis from Mnandi collected in September 1984.

	Female		Male	
	n	%	n	%
Stripes	59	73,8	37	67,3
Colours	15	18,8	9	16,4
Cream	6	7,5	9	16,4

13.3 DISCUSSION

Mollusc shell colour variation has been investigated by: Newkirk (1980) in Mytilus edulis the blue mussel; Hoagland (1977) in Crepidula the slipper limpet; Smith (1971) in Donax rugosus; Hyman (1967) and Moore (1936). In the first three cases polymorphism is attributed to genetic factors. In the last two accounts, nutritional factors have been cited. Hoagland (1977) stated that predation upon locally conspicuous specimens resulted in selection of populations with different colour distributions. The differences were according to the hue of the substrate.

The colour polymorphism in Bullia digitalis has been examined in whelks from Eastern Cape beaches (McGwynne 1980). The distribution of discreet colour forms and the categorisation of shells into cryptic and conspicuous forms was analysed with inconclusive results.

In samples taken from the Western Cape beaches, cream unpigmented shells were in the minority. Striped and coloured shells showed differing proportions according to the collection site. At Ou Skip and Melkbos there was rough parity or smaller numbers of stripes. At Hout Bay east, Noordhoek, Fish and Mnandi, stripes were dominant. At Hout Bay west, there was parity between stripes and colours.

Comparison of infected and non-infected whelks from the same collection showed no major differences. All collections were divided by sex, no marked sexual dimorphism was noted.

McGwynne (1980) suggested reclassifying shells as conspicuous or cryptic. Then the proportions of each were analysed to see if proportions changed with whelk population densities as proposed by Smith (1970).

Such reclassifying of Western Cape shells would probably have very little relation to shell varieties, especially in whelks from the Atlantic side. These shells usually are pitted with abrasion and symbiotic sponges. Sometimes they are green with algae. The shell condition of whelks from Mnandi is quite good. Further work at this site is clearly indicated.

CHAPTER 14

SUGGESTIONS FOR FURTHER WORK

Each of the foregoing chapters has ended with a discussion of the content of that chapter. These discussions have not only aimed at putting the work on Bullia parasites, and certain other information, into perspective, but have in particular demonstrated how little is still known about the whelk and its parasites.

It has thus been decided, rather than attempt a list of conclusions in this final section, to outline a few suggestions for further work.

1. The study areas are in general insufficiently known. Two sites - one on the west and one on the east coast of the Peninsula - could be chosen and monthly investigations carried out so as to build up an intimate knowledge of them. In this regard, publications such as that of Bally (1981) would provide invaluable assistance.

2. The parasites recorded in this thesis - all for the first time - have been only barely described. Karyotyping, following the techniques of Britt (1947) and Ciordia (1950), would clearly be useful, particularly in tying up life cycles when laboratory animals are reluctant hosts.

3. Histochemical methods, as outlined by Jennings and LeFore (1972) and Rees (1967), might be used to shed light in particular on glandular secretions, and give greater insight into the emergence and penetration of cercariae.

4. The physical features of the parasites could be more precisely charted by the use of techniques developed by Cardell (1962); Rees (1966), Dixon and Mercer (1965) and Cardell and Philpott (1960). In addition to the above accounts of light and electron microscopy, the techniques developed by Short and Cartrett (1973) and Short and Kuntz (1976) are a useful aid in locating argentophilic papillae on cercarial bodies.

5. The effects of parasitism on the hosts' tissues would shed light on any seasonal or chronic pathology. James (1965) is a useful source work on this subject.

6. The phenomenon of sex lability has been strongly hinted at. A concerted effort to find the cross-over point would go a long way toward modelling the phenomenon.

7. Is _Bullia pura_ really sexually dimorphic in shell shape? If so, and if the other intertidal _Bullia_ are not, then perhaps another look at its classification would be in order.

8. Shell colour polymorphism would be best studied at Mnandi beach. The shells are clean and colours definite. These are just a few of the main questions. It would have been useful to have had such a list at the outset of this work.

Bullia is remarkably easy to work with and is very forgiving and hardy. It is waiting on the shore for the next project.

CHAPTER 15

SUMMARY

1. At least six species of digenean trematode larvae occur in *Bullia*. *B. digitalis* is host to cercariae and sporocysts of the taxa Microphallidae, Allocreadiodea, Zoogonidae and metacercariae of the families Azygiidae and Ptychogonimidae. *B. pura* is host to metacercariae of the families Azygiidae and Ptychogonimidae.

2. The microphallid infection in *B. digitalis* abolishes gamete production and tends to occur in larger size whelks.

3. Larval stages of the cestode *Echinobothrium* sp. were found commonly (40%) in *B. pura* and rarely (less than 1%) in *B. digitalis*.

4. Injection of glucose into the haemolymph of *B. digitalis* infected with larval microphallids elicits cercarial emergence.

5. Penis size in *B. digitalis* is independent of infection with microphallid larvae.

6. Male *B. digitalis* are more prone to infection with microphallids than are females.

7. There is some evidence that sex change occurs in some male *B. digitalis*.

8. The variation of shell proportions in *B. digitalis* appears to be largely governed by genetics. Other factors, particularly wave action and predation by crabs, are probably subsidiary factors.

9. Statistical analysis of shell proportions in male and female *B. pura* have revealed a sexual dimorphism ($\alpha < 0,05$).

10. *Bullia digitalis* becomes sexually mature at a larger size than does *B. rhodostoma*.

11. Elementary analysis of shell colour of *B. digitalis* has revealed no obvious patterns of distribution, except that there is no major difference in shell pattern between infected and uninfected whelks.

CHAPTER 16

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APPENDIX 1

A SYMBIONT FOUND ON THE CEPHALIC TENTACLE OF BULLIA DIGITALIS

Bullia digitalis from Melkbos, Hout Bay west, Fish Hoek and Mnandi were found to harbour rotifers on their tentacles, penis and margin of the foot. The incidence is 0,2%, 0,7%, 0,4% and 0,7% respectively. The whelk sample sizes were: Melkbos 876, Hout Bay 151, Fish Hoek 462, and Mnandi 152.

No pathological damage was evident in the whelks. The exploitation of the whelk as a host for the rotifers is probably due to there being nowhere else suitable within the sandy beach surf zone. The rotifer has conflicting requirements. It needs to be exposed to currents of fresh water and at the same time it needs to be protected from the abrasive action of water-borne sand. No rotifers were found on the whelk shell. This is not surprising as the shells are subject to extreme abrasion during surfing.

It would be interesting to see if rotifers also live in the siphons and mantle cavities of the white mussel Donax. This would appear to be a similar niche.

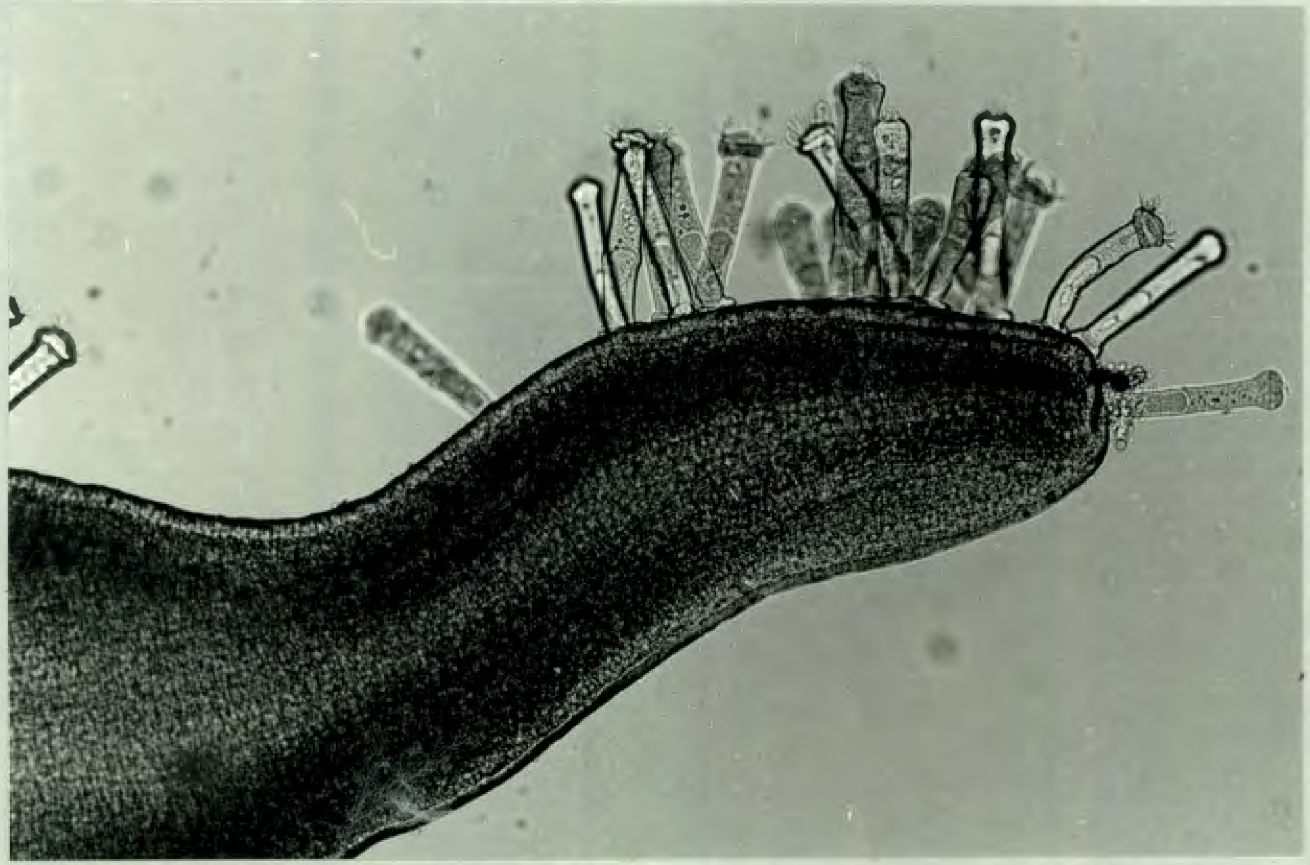

Fig. A,1. Tentacle of *B. digitalis* with symbionts.

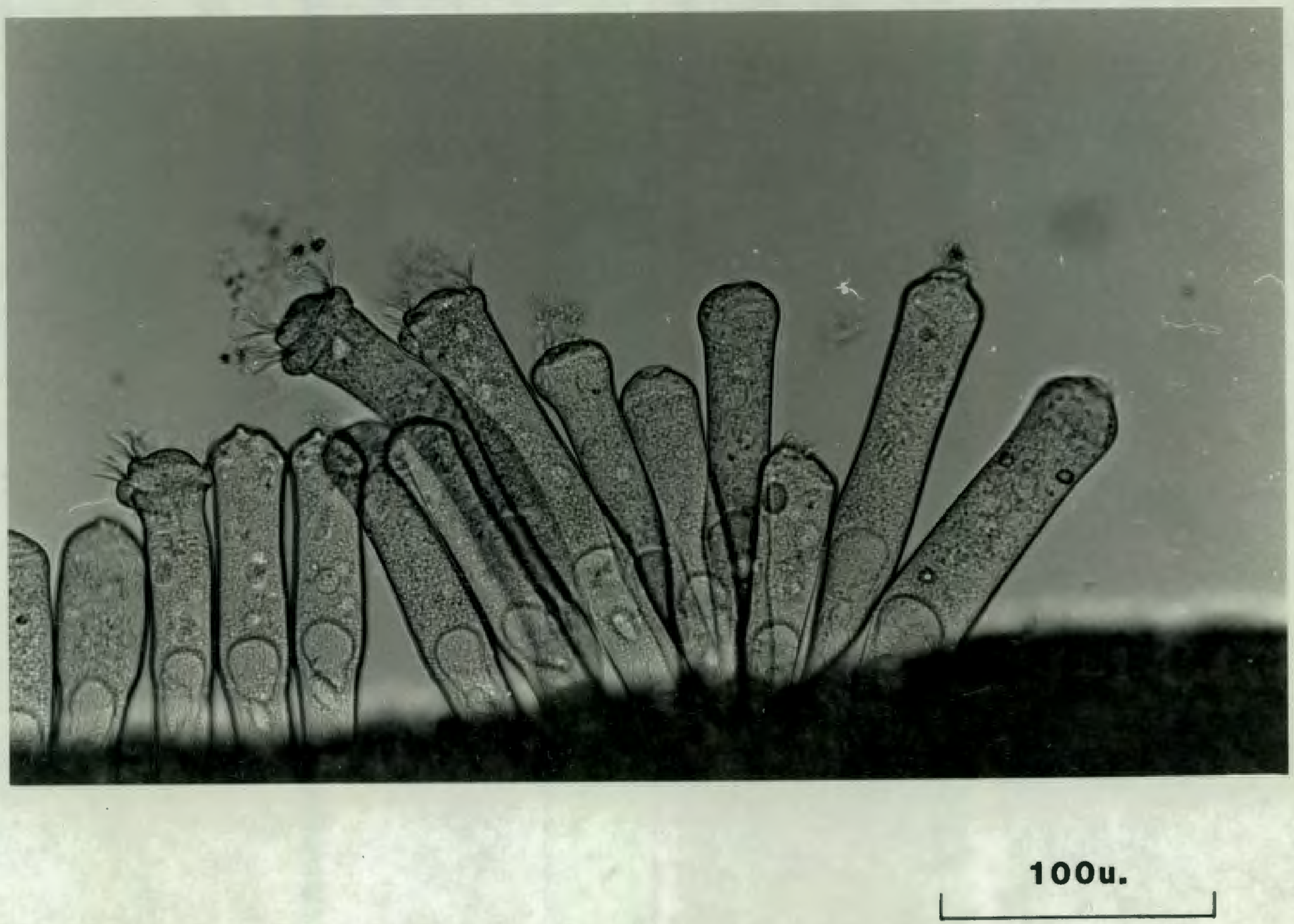

Fig. A,3. Tentacle of B. digitalis with symbionts.

APPENDIX 2

SPOROCYST AND CERCARIA TYPE T

HOST: SIPHONARIA CAPENSIS QUOY AND GAIMARD 1833

Sporocyst

The sporocyst is much-branched and filamentous. The filaments are approximately 140 um wide. The sporocyst contains hundreds of cercariae.

Cercaria

Naturally emitted cercariae have three long filamentous tails, each about 270 um long by 2 um wide. Cercariae extracted from crushed limpets do not exhibit these tails, otherwise the structures and measurements of naturally emerged and dissected out cercariae are identical. The tegument on the body is transversely wrinkled. The three tails meet at a large basal structure which has a loose and folded tegument. There are no spines or hard structures on any part of the cercarial tegument. This is as far as can be ascertained by light microscopy. The anterior sucker opens sub-terminally (see Figs. 5.A and B). The sucker is thick-walled and muscular. The excretory system consists of two pairs of flame cells connected to the excretory tubules in a stenostomate configuration. Two excretory ducts join and immediately become convoluted as they pass posteriorly to a diamond-shaped, thin-walled bladder. The bladder appears to be an extension of the duct and has corrugated walls. The excretory pore occurs at the termination of the body which lies inside the tail basal structure. It has not been ascertained how the excretory products escape from the tail. No further cercarial details could be distinguished.

Development

Various protocercarial structures were found in the host after dissection. There appeared to be stages in a development process which is elucidated below. The cercariae start as germballs which bud once to form a germball with a smaller sphere attached to the posterior end. The smaller germball changes shape and becomes more irregular (see Fig. A6,E). Two knob-ended filaments are seen growing from the ventral part of the smaller germball which is nearest the body germball. The filament grows posteriorly and turns through 270° in a diameter of about 15 um. The distal part of the filament is now pointing anteriorly. This distal part starts to elongate either at that point or from a point in the tail. The filaments have transverse banding at regular intervals. These bands grow apart as the filament increases in length. This indicates that the filaments grow over their whole length rather than at a proximal growing point. the filaments terminate in a knob-like lump about 10 um in diameter. The body germball elongates to become a long elipsoid. The above observations only account for the development of two filaments. The origin of the third filament is not known.

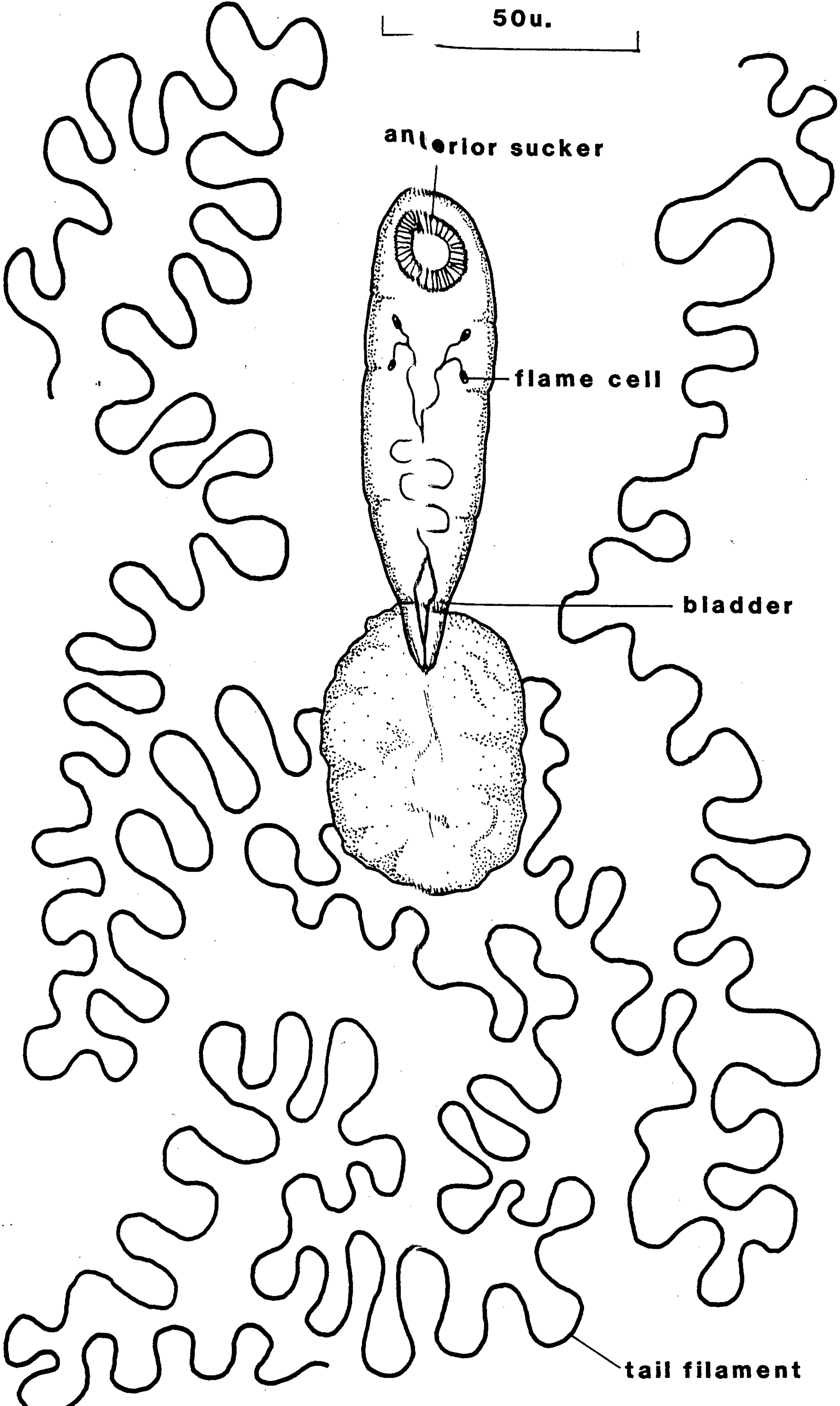

Fig. A.4. Cercaria T. dorsal view

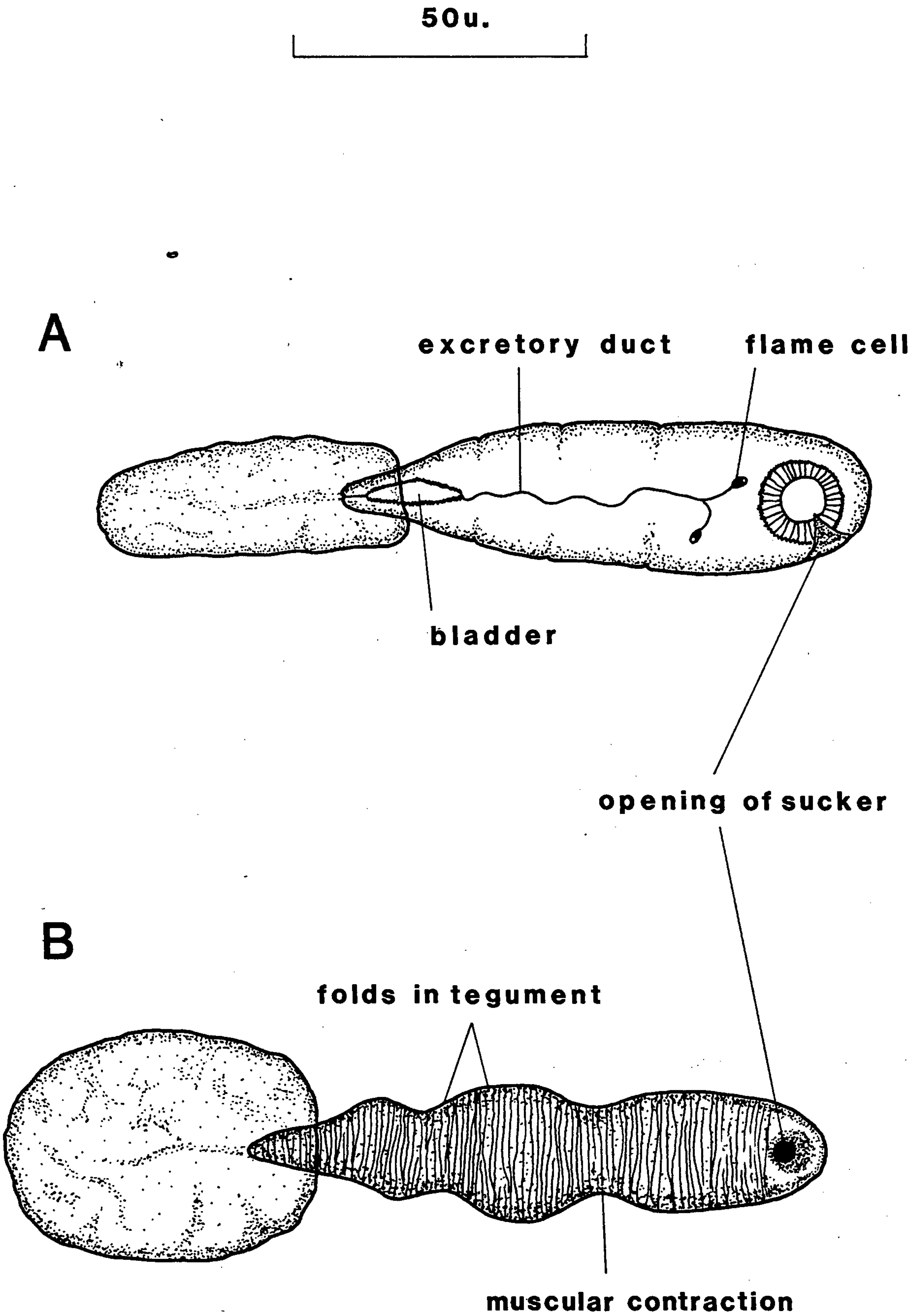

Fig. A,5. Cercaria T A, lateral view. B, ventral view. (Tail filaments deleted from A&B.)

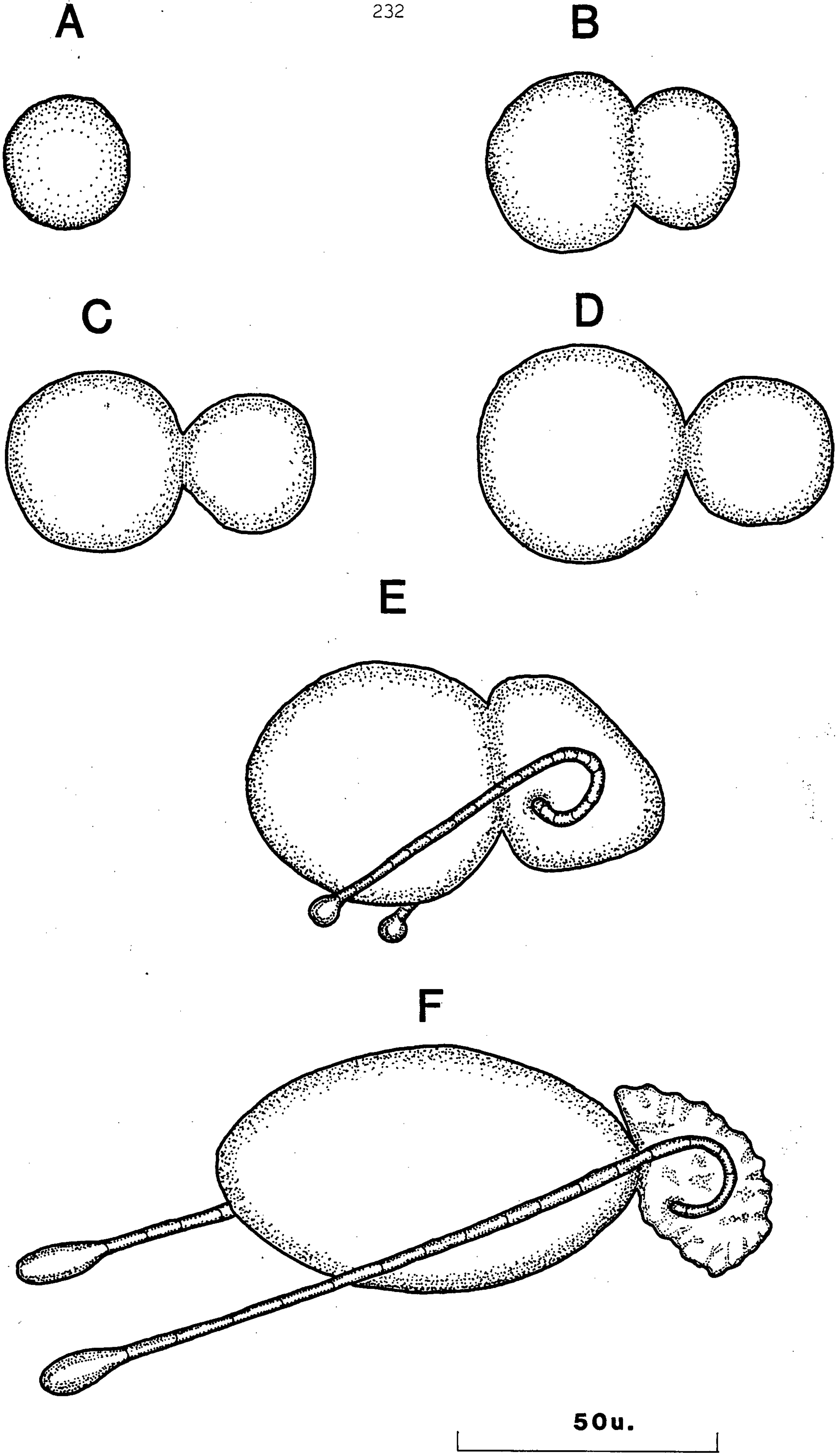
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Fig. A,7. <u>Cercaria T</u> lateral view showing entrance to anterior sucker.

Fig. A,8. <u>Cercaria T</u> ventral view.

Fig. A,9. Cercaria T showing anterior sucker and three tail filaments.

Fig. A,10. Cercaria T showing the junction of the tail and filaments.

Fig. A,11. Cercaria T showing the tail filaments.

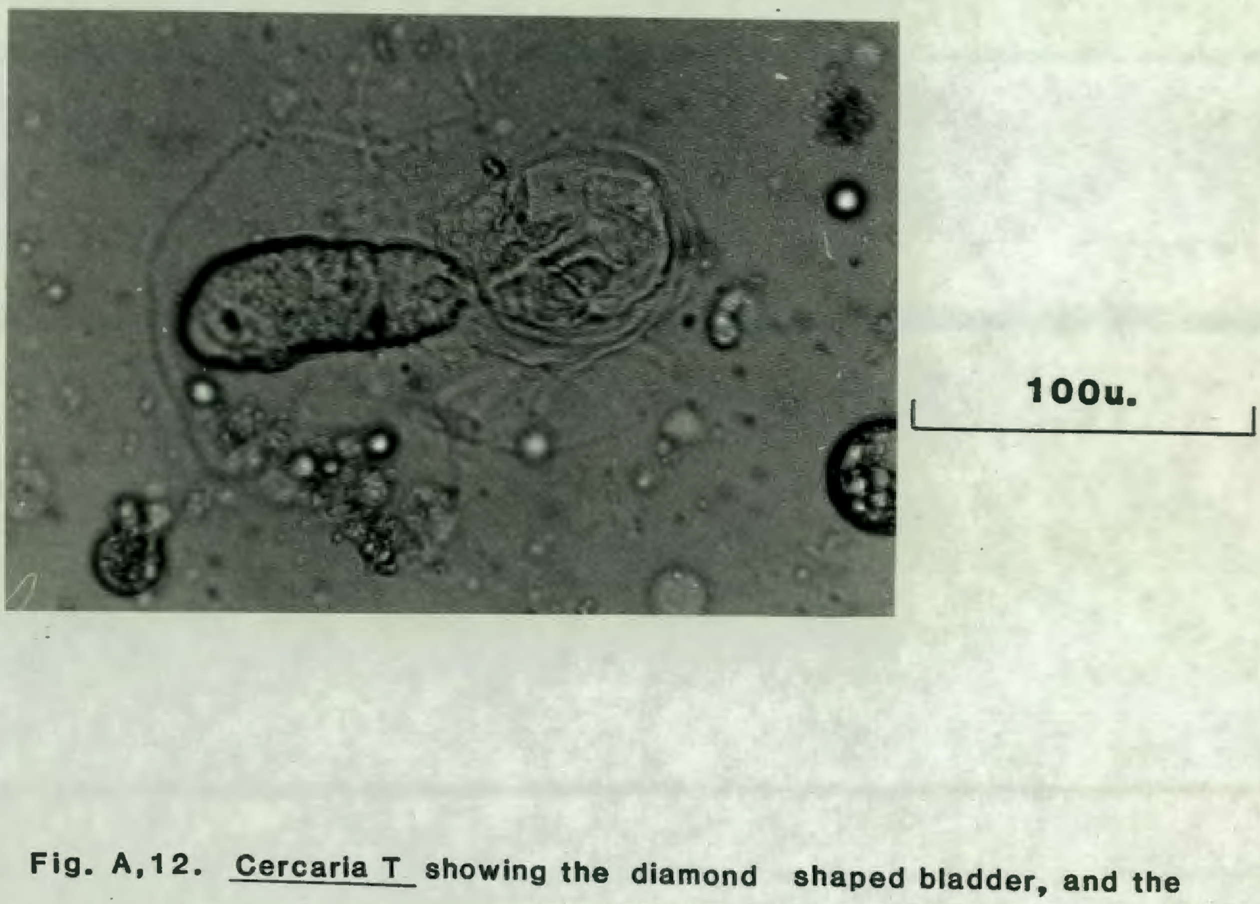


Fig. A,12. Cercaria T showing the diamond shaped bladder, and the

APPENDIX 2

DISCUSSION

This cercaria has a combination of features which are found in two widely separated taxa. The development of the cercaria as described earlier, follows closely that of the cystophorus cercaria Halipegus occidualis, a hemiurid investigated by Hussey (1941). The major difference in Cercaria T. is that Halipegus only has one knob-ended delivery tube. Cercaria T. has two during development. These apparently are joined by a third filament and become greatly elongated on maturation. Naturally emerged specimens of Cercaria T. were never observed to withdraw into the tail cyst as Halipegus commonly does. Members of the suborder Hemiurata develop in rediae. Cercaria T. develops in long-branched sporocysts similar to those of gasterostomes. The flame cell configuration is mesostomate in common with gasterostomes. The oral sucker has no trace of a gut leading from it. No gasterostomate mouth was found either. The digestive system in Cercaria T. remains a mystery. There was no ventral sucker. Multiple-tailed hemiurid cercariae such as Cercaria introverta (Ito 1964) have been reported but none with such long filaments. Perhaps the method of karyotyping suggested by Britt (1947) would go a long way to sorting out this problem. Meanwhile this cercaria remains anomalous.

Movement

The carcarial body is capable of waves of peristaltic contraction (see Fig. A,5 B). The body can bend considerably (see Fig. A,7). All movement is very slow. The tail structures appear entirely passive.

TABLE A1 Cercaria T measurements of living specimens

	MEAN um	S.D. um	S.E. um	C.V. %	n	MAX. um	MIN. um
Body length	84,8	8,4	2,7	9,9	10	98	68
width 25%	24,8	3,5	1,1	13,9	10	31	21
50%	22,5	3,3	1,0	14,6	10	27	18
75%	22,9	2,8	0,9	12,1	10	29	20
Oral sucker length	14,3	2,0	0,8	13,7	6	18	12
width	14,7	1,6	0,7	11,1	6	16	12
Tail base length	55,5	3,8	1,2	6,9	10	62	51
width 25%	39,2	9,6	3,0	25,2	10	57	19
50%	39,2	10,1	3,2	25,8	10	51	19
75%	32,5	8,7	2,8	26,9	10	39	12

EPIDEMIOLOGY

Seventy five Siphonaria were examined. Prior to dissection they were placed in petri dishes with sea water over night at about 23°C. Four Siphonaria emitted Cercaria T. On dissection 13 limpets were found to be infected. This gives an infection rate of 17,3% (see Fig. A26 for details of the frequency distribution of infections and shell lengths).

APPENDIX 3

FAMILY MICROPHALLIDAE

SPOROCYST AND CERCARIA TYPE S

HOST: SIPHONARIA CAPENSIS

Sporocyst

The sporocysts are found in masses in the digestive gland. They are unbranched tubular structures, but they may be contorted and swollen in sections (see Figs A14 and A15). There is usually one end which is swollen and club-shaped. The sporocyst is non-motile and appears to have poor sub-tegumentary musculature. There is no birth pore. Each sporocyst contains around 30 cercariae. Each cercaria being at the same stage of development. The sporocyst tegument was devoid of any spines or other distinguishing structures. No part of the sporocyst excretory system was visible.

TABLE A2 Sporocyst S measurements of living specimens

	MEAN um	S.D. um	S.E. um	C.V. %	n	MAX. um	MIN. um
Length	863	193	61,0	22,3	10	1215	648
Width 25%	191	39	12,3	20,5	10	243	145
50%	190	28	9,0	14,9	10	243	162
75%	178	38	12,1	21,5	10	243	121

Cercaria S.

Diagnosis:

Monostome tailed xiphidiocercaria. Dorsoventrally flattened with a concavity of the ventral surface. The body plan is eliptical at the front and squared off at the back. The tegument is faintly spinous. The tail appears more spinous than the body. The spines are too small to measure using light microscopy and are under 1 um in length. The rows of spines on the tail are about 4 um apart. The oral sucker opens subterminally and is feebly developed. No further part of the digestive tract is evident. The stylet is held at an angle of 45° and is embedded in the sucker musculature. The stylet is a slim cone with a flat base. The angle of the cone increases in the terminal 2 - 3 um to form a robust point. A pair of penetration glands open on either side of the stylet point. They have their cell bodies just anterior to the bladder. Each pair has a duct extension which zigzags forward. This convolution is in both the vertical and horizontal planes (see Figs A13, A and B). As the gland ducts approach the stylet they bifurcate and proceed to the penetration gland openings individually. There are four ducts traversing the oral sucker. There is a pattern of penetration gland openings, two either side of the stylet. The medial openings are anterior to the lateral openings (see Figs A13, A & B). The more dorsal gland ducts run to the medial openings. The ventral ducts run to the lateral openings. The gland cells stain with neutral red and nile blue sulphate intravitally. After sectioning, the glands stain red with eosin. The bladder is fairly thin-walled. The bladder walls appear finely wrinkled. The bladder is Y-shaped and has an excretory pore at the body tail junction. There is a gap between the tail and the invagination of the body. It is assumed that the bladder contents escape from this gap by pores at the junction. The tail is single and tapering. The body to tail length ratio is variable around 1 to 0,7 in the living specimen. The tail shows periodic transverse constrictions. There are four pairs of flame cells in total. No excretory ducts could be seen. The flame cell formula may be:

2 ((2) + (2)) = 8

As in Cercaria Z. the excretory system is mesostomate.

Movement:

The cercaria is capable of swimming, the body is flexed to form a concave dorsal surface. The tail is bent posteriorly and the cercaria is propelled front end first by lashing the tail from side to side (see Fig. A13, C). Swimming may proceed for one minute followed by a pause of 2 minutes or more. The body is capable of worm-like bending and stretching movements. The tail is fairly contractile and capable of considerable movement.

Cercaria S.

A total of 75 Siphonaria were examined. Before dissection they were kept in petri dishes at about 23°C overnight. No cercarial emission was seen. Two limpets were found to be infected on dissection. This gives an infection rate of 2,7%.

TABLE A3 Cercaria S. measurements. A: living; B: heat-killed; C: formalin-killed.

	MEAN um	S.D. um	S.E. um	C.V. %	n	MAX. um	MIN. um
A							
Length	100,5	5,0	1,6	5,0	10	110	95
Width 25%	36,5	4,1	1,3	11,3	10	40	30
50%	44,7	4,4	1,4	10,0	10	50	35
75%	35,5	4,4	1,4	12,3	10	40	30
Stylet: length	10,8	1,0	0,3	9,4	10	11,7	8.8
width	1,95	0,1	0,03	4,7	10	2,1	1,8
Tail: length	69,0	20,9	6,6	30,3	10	110	40
width 25%	8,5	4,1	1,3	48,4	10	15	5
50%	7,1	3,7	1,2	52,9	10	15	3,5
75%	4,4	2,3	0,7	52,5	10	10	2,5
B							
Length	104,5	10,4	3,3	10,0	10	125	90
Width 25%	40,1	3,5	1,1	8,8	10	45	35
50%	49,7	6,7	2,1	13,6	10	65	45
75%	36,3	4,0	1,3	11,0	10	40	30
Stylet: length	12,1	1,6	0,7	13,3	5	13,6	9,75
width	2,3	0,5	0,2	22,3	5	2,9	1,95
Tail: length	105,0	10,0	3,2	9,5	10	120	90
width 25%	10,5	2,6	0,8	24,6	10	15	7,5
50%	8,5	2,1	0,7	24,8	10	10,0	5,0
75%	5,9	1,8	0,6	31,2	10	7,5	2,5
C							
Length	96,0	14,3	4,5	14,9	10	110	70
Width 25%	36,5	7,1	2,2	19,4	10	50	25
50%	42,5	6,9	2,2	16,2	10	55	35
75%	30,0	4,1	1,3	13,6	10	40	25
Stylet: length	11,4	1,1	0,35	9,7	10	13,6	9,75
width	1,96	0,24	0,07	12,0	10	2,34	1,56
Tail: length	62,0	18,9	6,0	35,0	10	110	50
width 25%	9,8	0,8	0,25	8,1	10	10	7,5
50%	9,5	1,6	0,50	16,6	10	10	5,0
75%	7,8	1,8	0,58	23,8	10	10	5,0

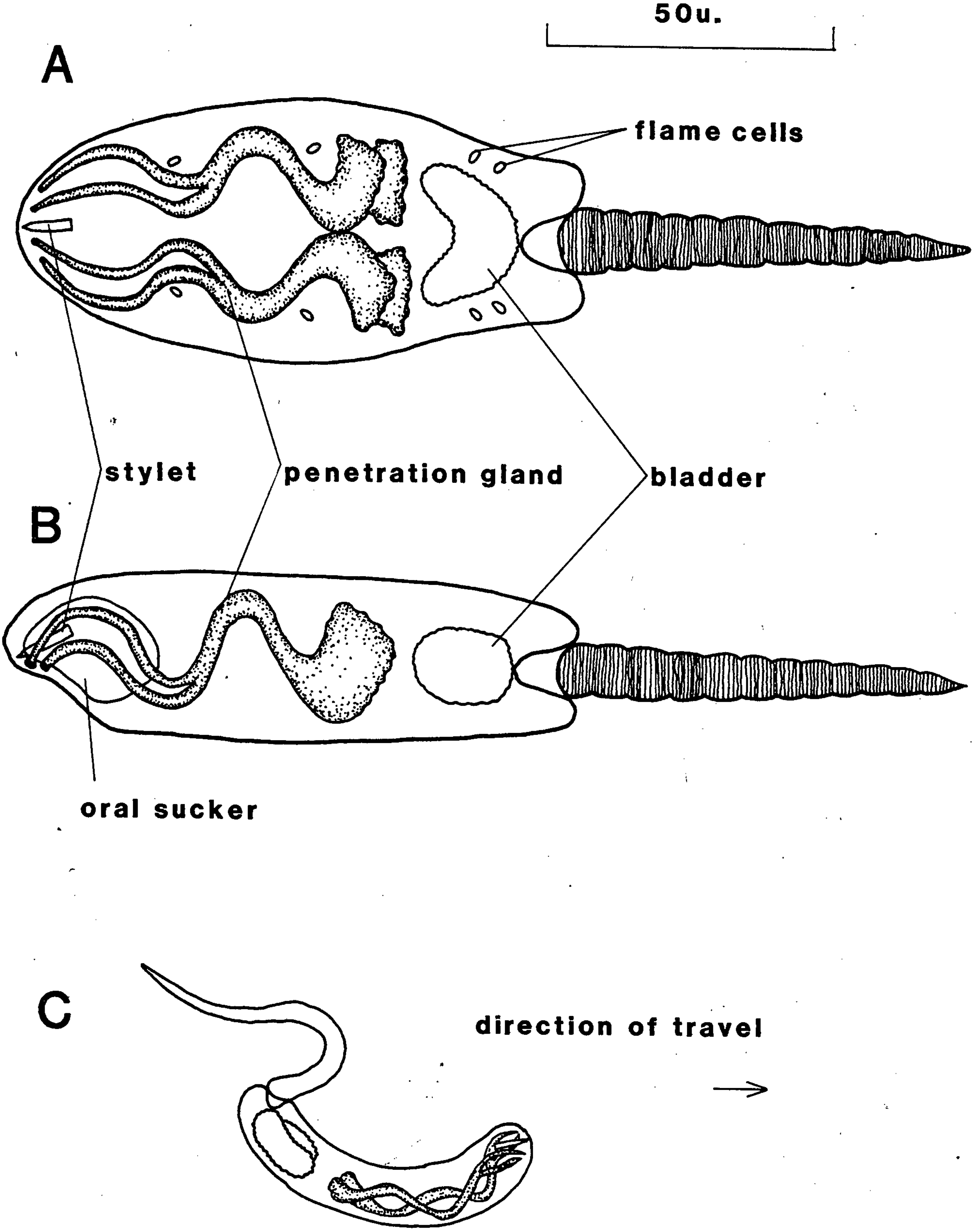


Fig. A,13. Cercaria S A,dorsal view. B, lateral view. C, position during swimming.

Fig. A,14. Sporocyst S

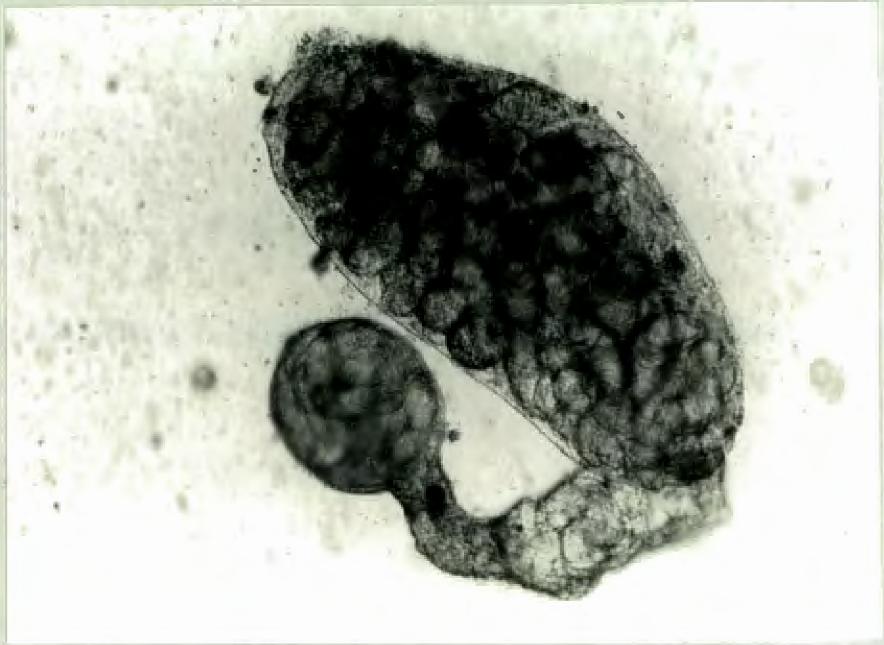


Fig. A,15. Sporocyst S

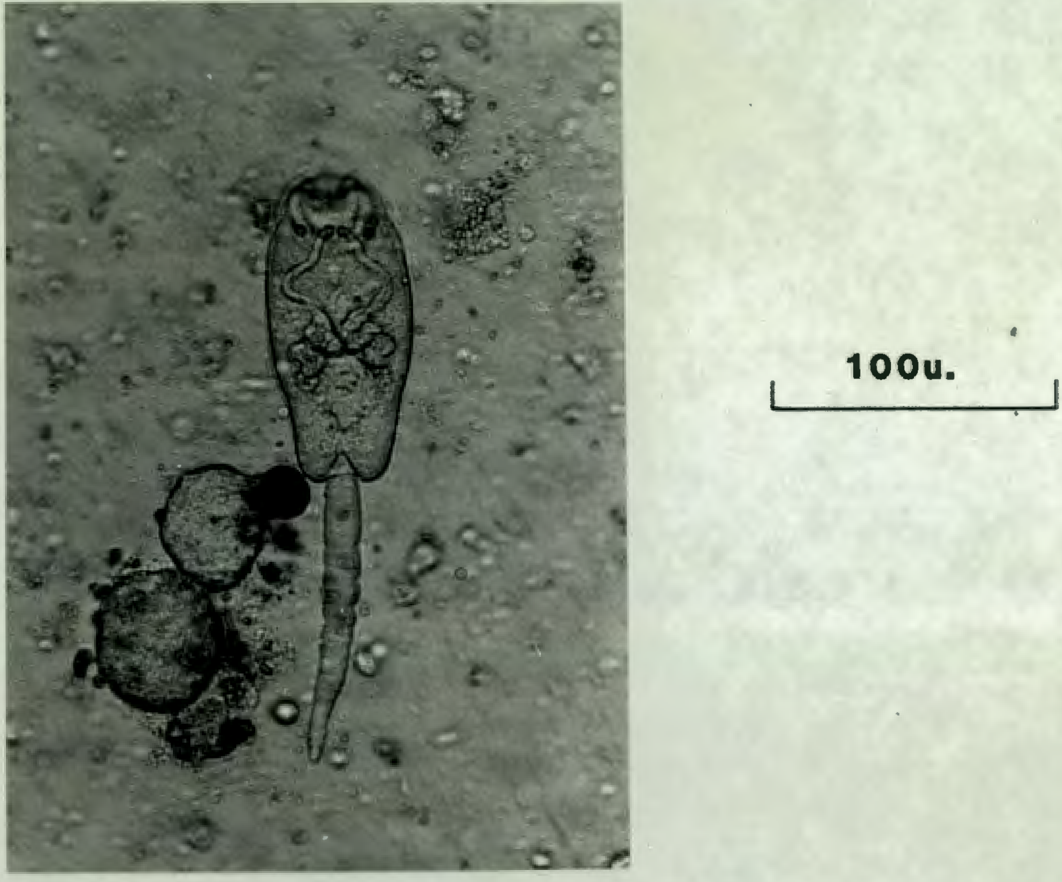
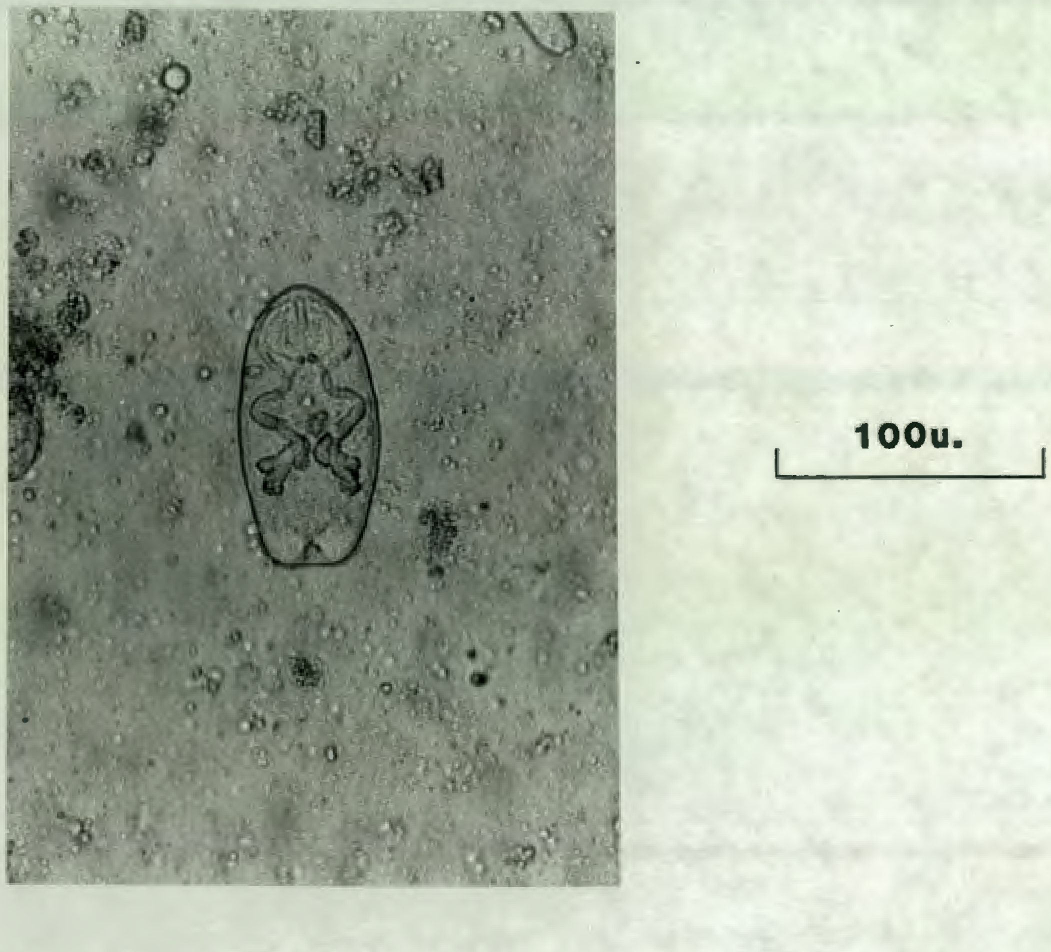


Fig. A,16. Cercaria S



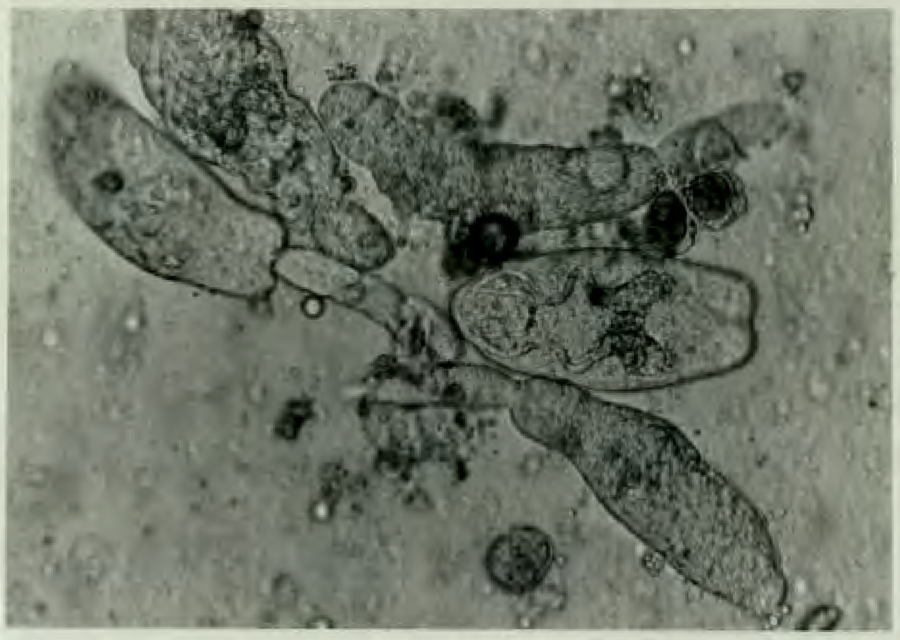


Fig. A,18. Cercaria S immature and mature specimens.

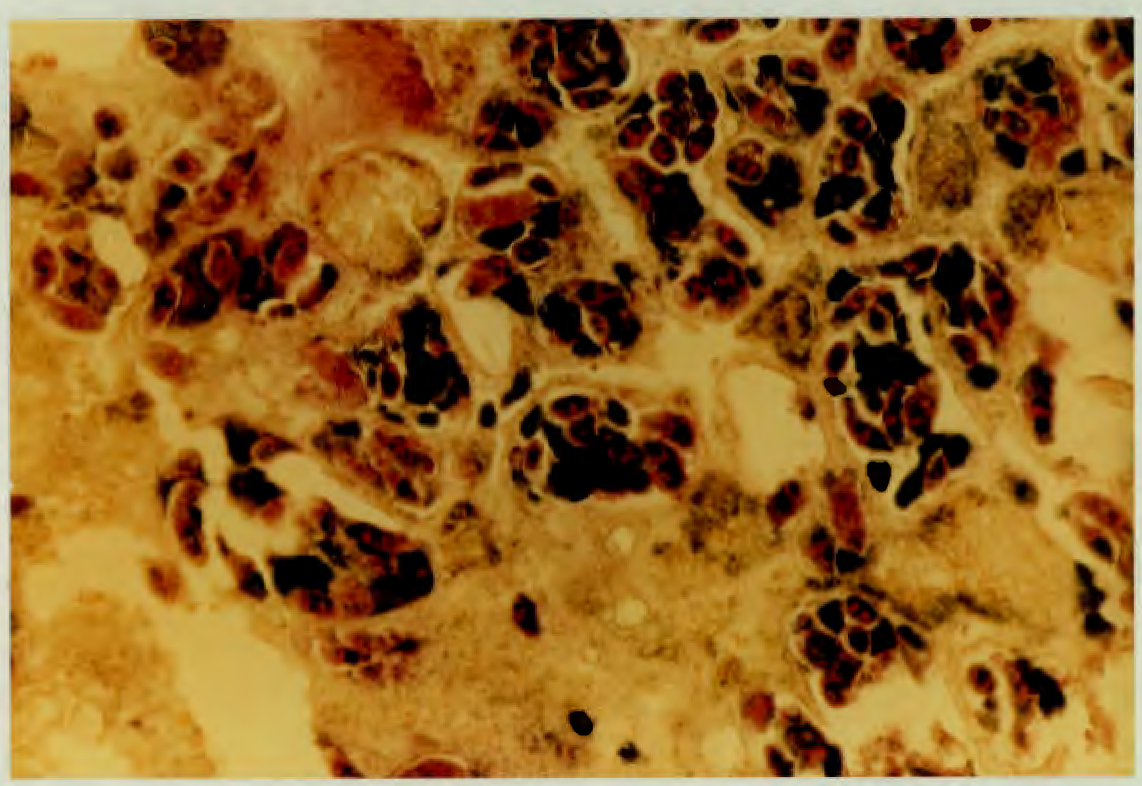


Fig. A.19. Digestive gland of S. capensis infected with type S.

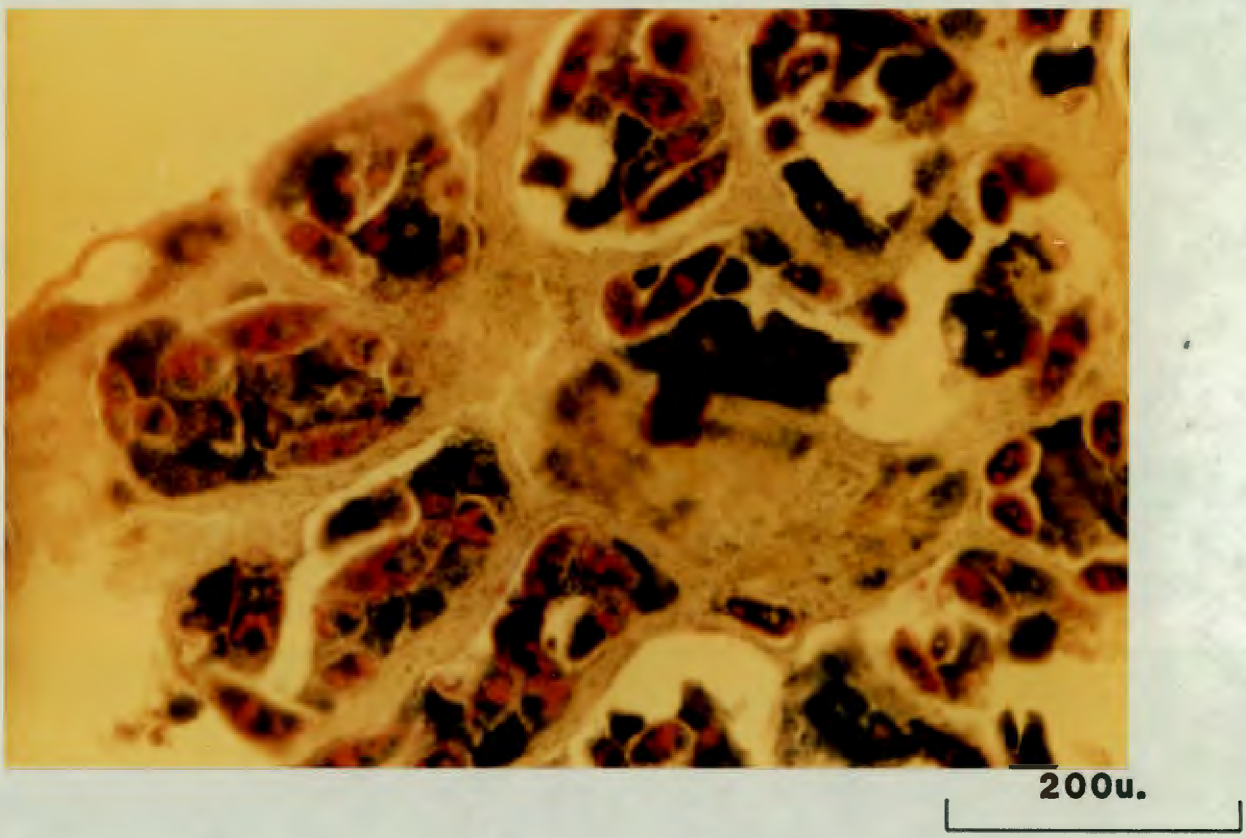


Fig. A,20. Digestive gland of S. capensis infected with type S.

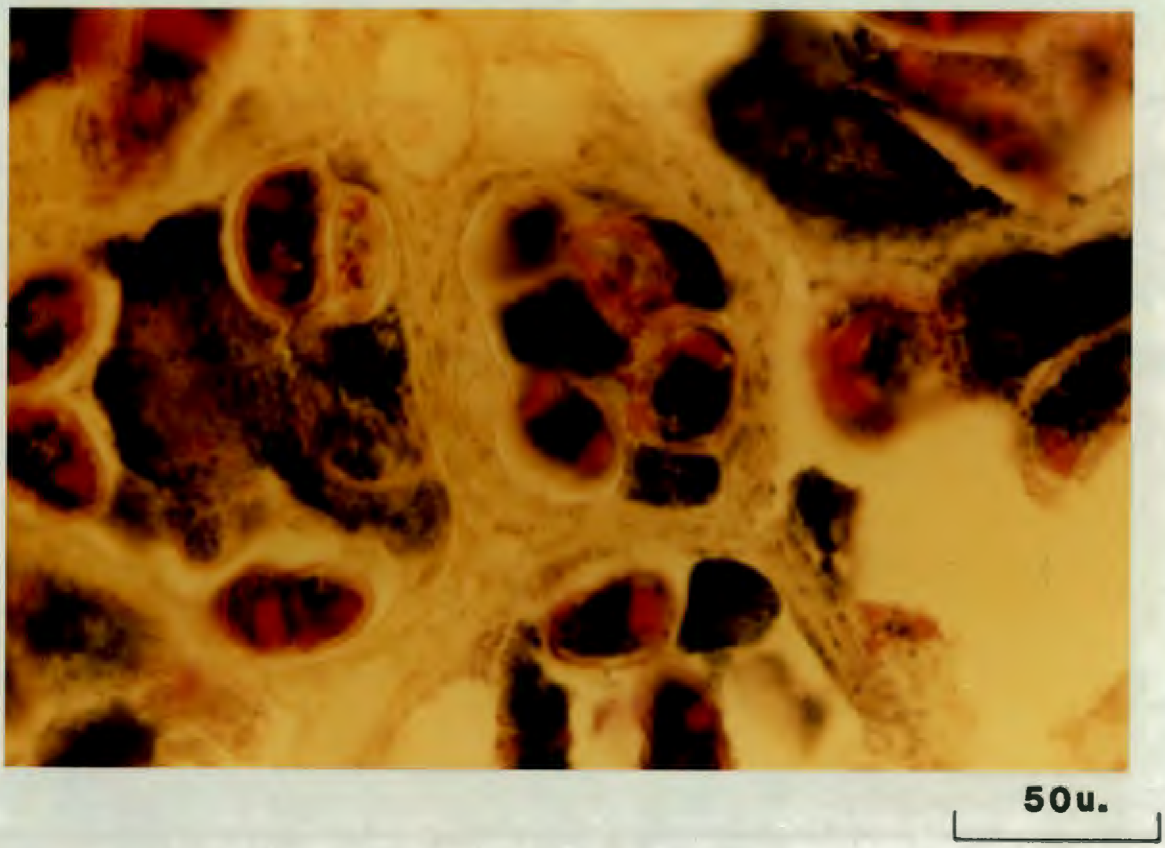


Fig. A,21. Digestive gland of S. capensis showing cercariae and

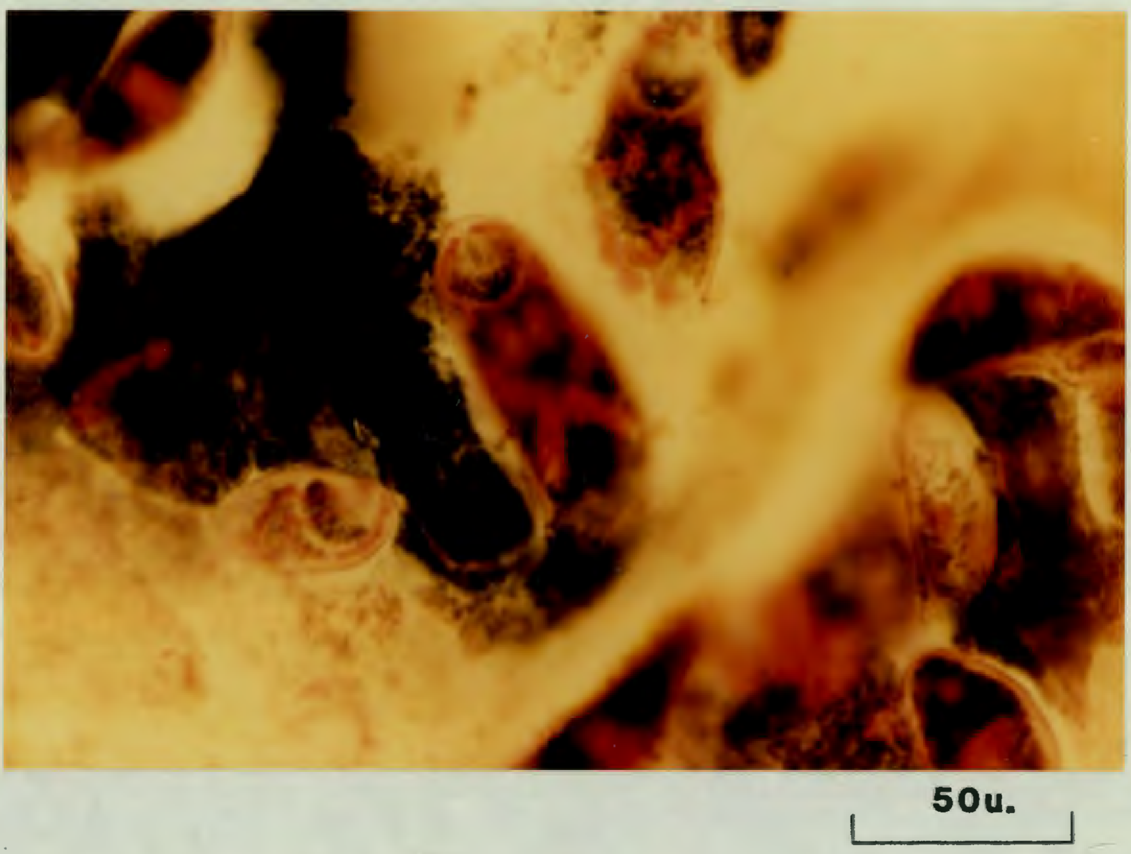


Fig. A,22. Digestive gland of _S. capensis_ showing _Cercaria S._

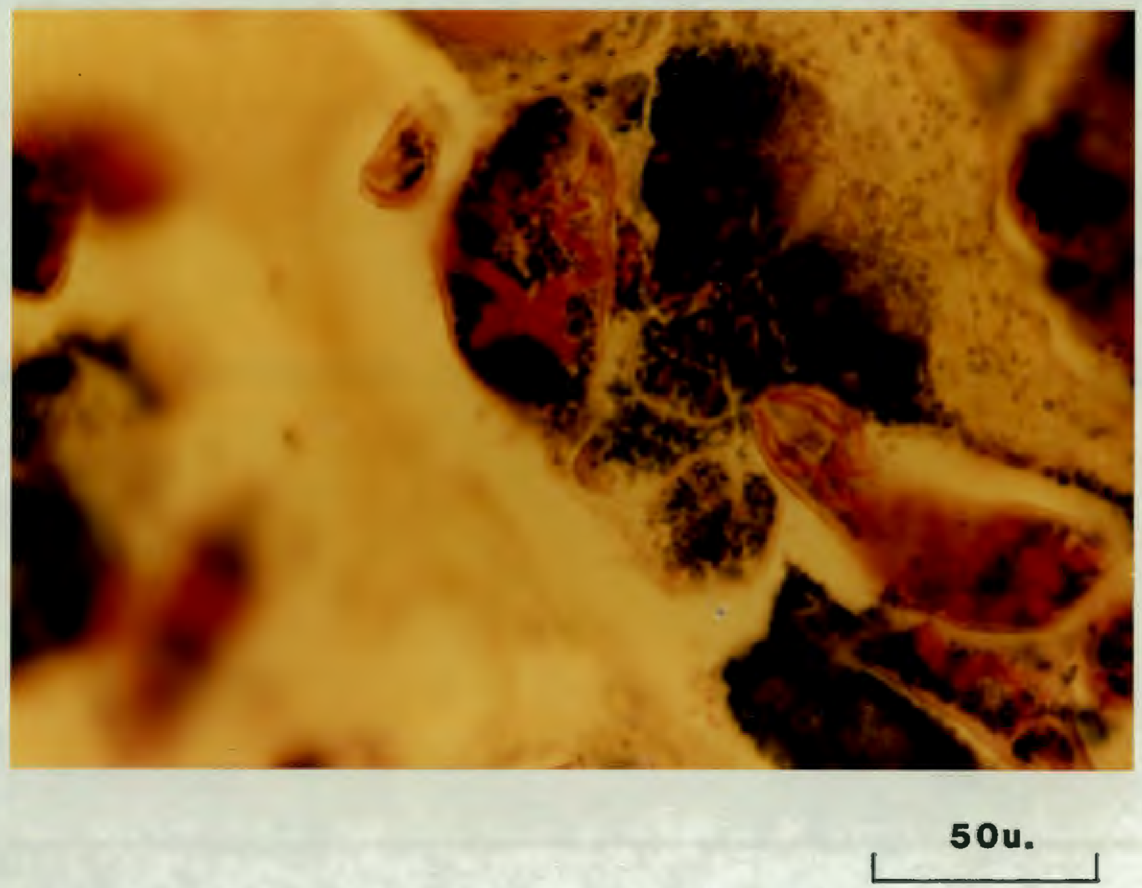


Fig. A,23. Digestive gland of _S. capensis_ showing _Cercaria S._

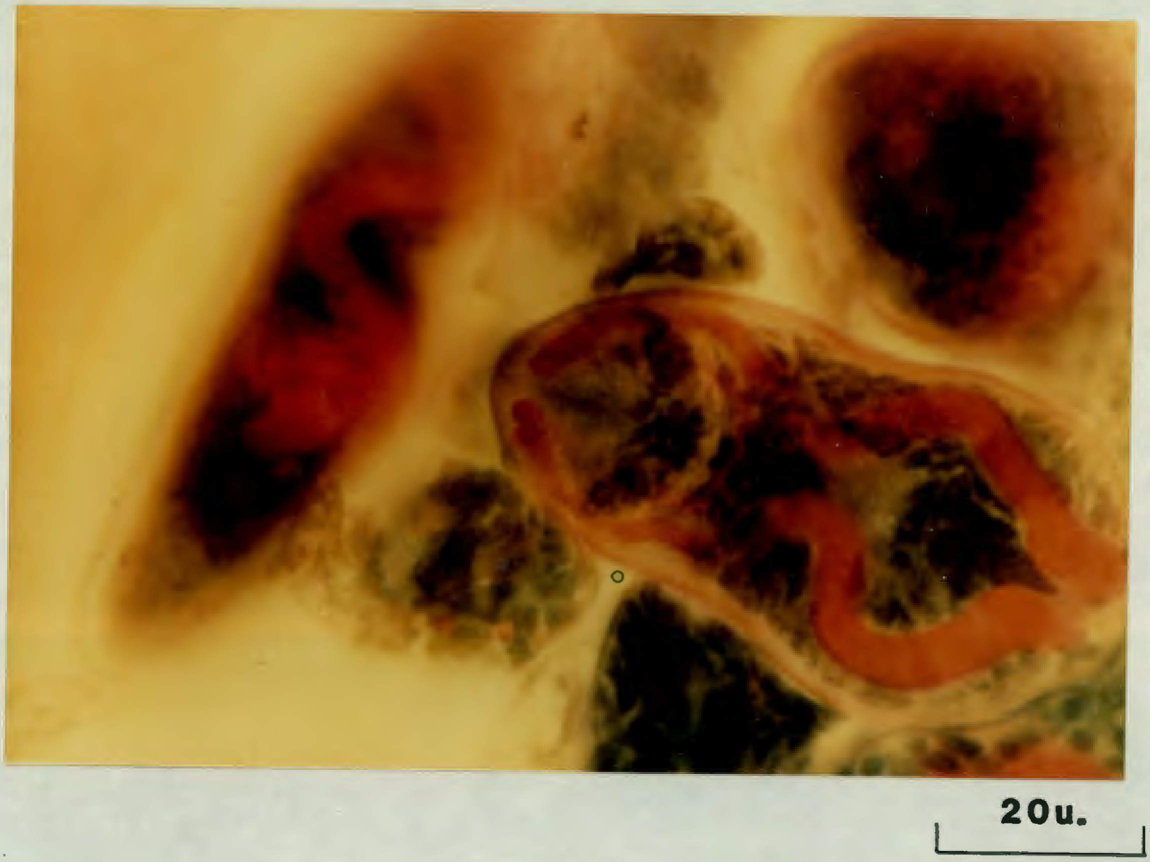
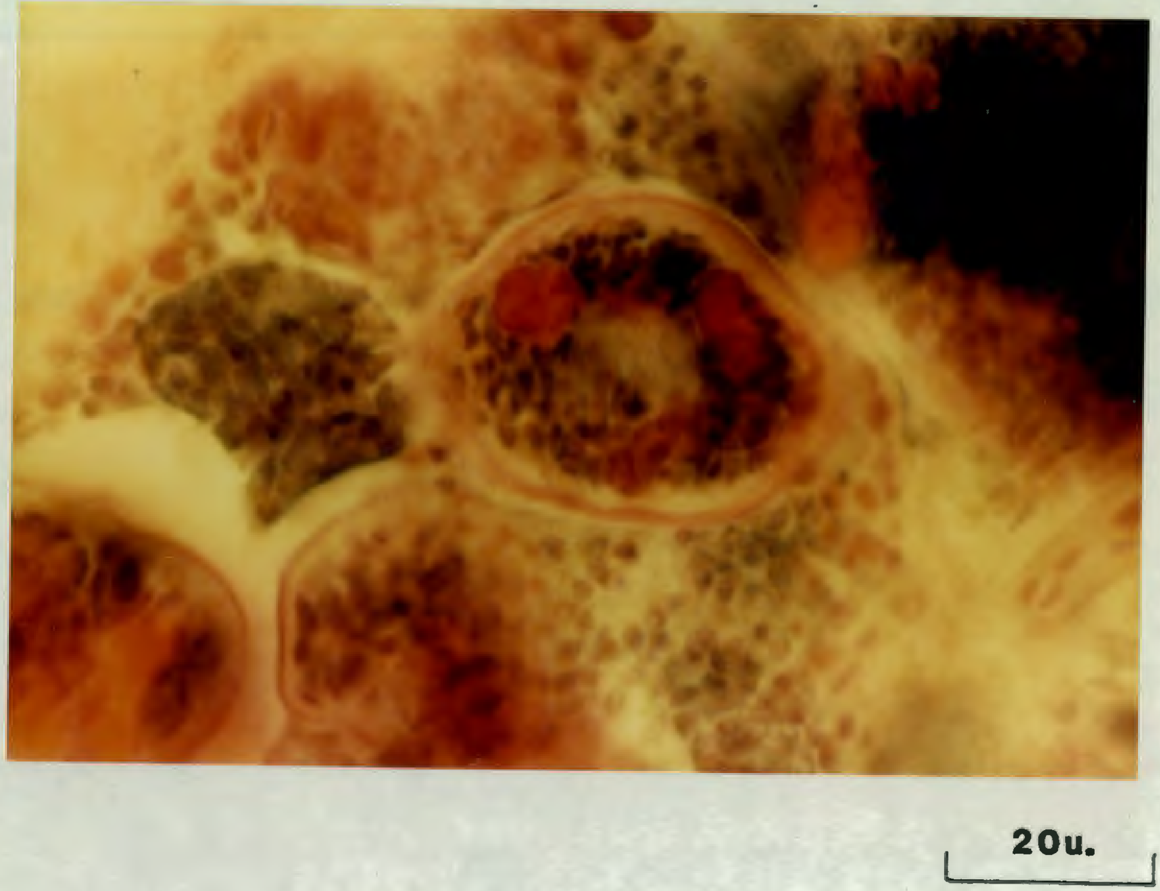


Fig. A,24. Cercaria S in the digestive gland of S. capensis, note stylet musculature and penetration gland openings.



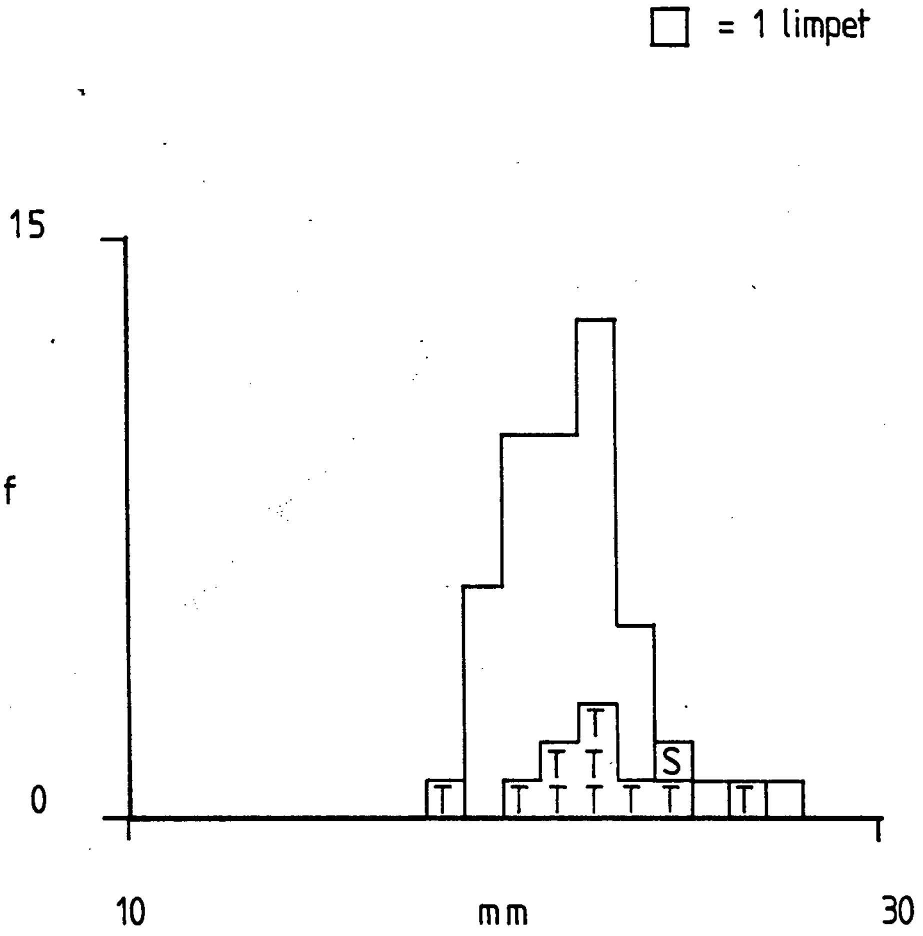


Fig. A26 Shell length and infection distribution in Siphonaria capensis.

APPENDIX 3

DISCUSSION

This cercaria is very similar to Cercaria Z. from B. digitalis. Its measurements, shape and swimming ability differentiate it from Cercaria Z.

The same number of flame cells, similar organs of penetration and bladder shape suggest that they are in the same genus. Examination of adult specimens would of course clarify this.

For further general discussion concerning the family Microphallidae see Chapter 3.5.

APPENDIX 4

CERCARIA R

HOST: DONAX SORDIDUS

A small number of Donax sordidus were collected from Muizenberg beach. On examination one was found to be infected with a single specimen of Cercaria R.

Diagnosis

Dorsoventrally flattened distome. Length of the body varied between 180 um contracted to 220 um when fully extended. The tegument is spinous anteriorly, very few spines being found on the posterior half of the body. The oral sucker opens slightly sub-terminally. There is a pair of penetration glands on either side of the oral sucker. The gland cell bodies lie posterior to the sucker and a thin duct runs forward to the sucker opening. There is no pre-pharynx. The pharynx is longer than broad and is about as long as the oral sucker diameter. The oesophagus is short and leads to the caecae which terminate at about half the body length. The ventral sucker lies centrally about two thirds of the way from the anterior and protrudes beyond the ventral surface. The ventral sucker is about two thirds of the size of the oral sucker. The excretory system does not appear to have a bladder. At the junction of the excretory ducts there are six diverticulae, two lie posterior to the junction, one on either side of the resultant single duct. Two diverticulae are anterior to the junction and lie laterally to each duct. The final two are also anterior to the junction but lie medially to the ducts and adjacent to the posterior edge of the ventral sucker (see Fig. A27). The excretory vessels proceed forward and terminate next to the oral sucker. Lateral to the ventral sucker on either side are a group of five cells; these are probably cystogenous gland cells. On extension, an undulating structure lying between the oral and ventral suckers can be seen. This is probably the genital primordia.

Movement

The cercaria is capable of considerable contraction and expansion as Fig. A27 shows. Such movement causes considerable deformation of the internal structures.

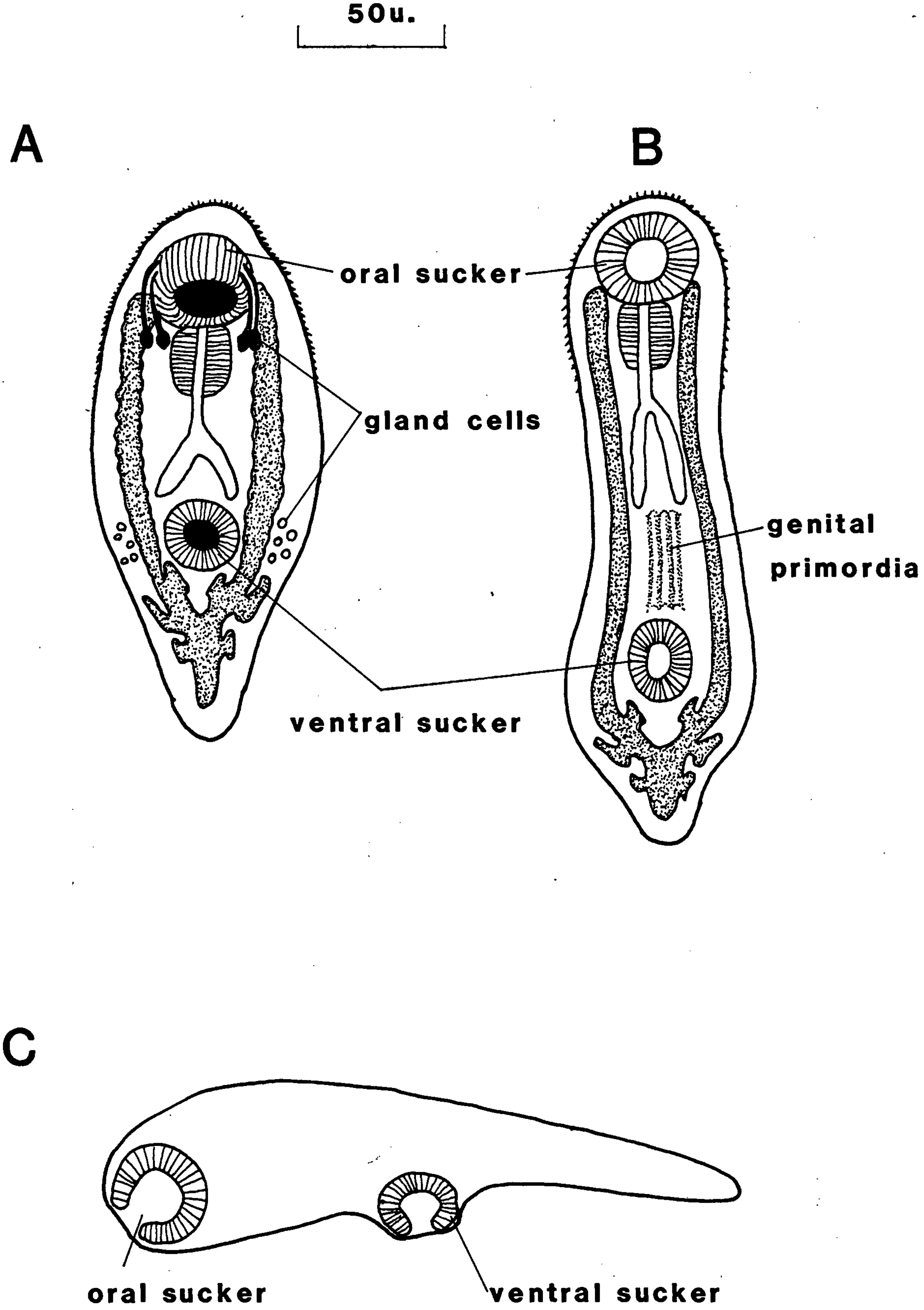


Fig. A,27. Cercaria R. A, ventral view (contracted). B, ventral view (extended). C, lateral view showing sucker positions.

APPENDIX REFERENCES

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ITO, J. 1964. A monograph of cercariae in Japan and adjacent territories. Progress in Medical Parasitology in Japan 1, 396-550.