

No 19..76./..5.....

- NEW PROPOSAL
- CONTINUING PROJECT
- FINAL REPORT
- PROGRESS REPORT

# FISHING INDUSTRY RESEARCH TRUST ACCOUNT

**TITLE OF PROPOSAL/PROJECT:** \_\_\_\_\_

Establishment of a Centre for Research, Consultancy and Training on  
Parasites and Diseases Important to the Fishing Industry.

**ORGANISATION:** University of Queensland

**PERSON(S) RESPONSIBLE:** Dr. H.M.D. Hoyte

**FUNDS SOUGHT/GRANTED**

YEAR	SOUGHT	GRANTED
1976/77	_____	\$23,442
1977/78	_____	26,817
1978/79	_____	26,252
1979/80	_____	27,752
TOTAL, including \$3,157 supplementary grants.		\$107,420

**RELATED APPLICATIONS:** \_\_\_\_\_

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DISTRIBUTED 29.../...1.../1982

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*Judith Ho...*  
for Secretary  
Fishing Industry Research Council



SECRETARIAT NOTEFinal Report of FIRTA Project: Centre for Research  
Consultancy and Training on Parasites and Diseases  
Important to the Fishing Industry

The attached article (Annex A) by Dr R.J.G. Lester appeared in "Australian Fisheries" in October 1980. We are now advised following correspondence with Queensland University that the article is intended as the final report of the project. It should be noted that the report was also used to support in part the University's application for the current QX disease in oysters project.

The University has provided an addendum to the report (attached as Annex B) together with four articles published in scientific journals (attached as appendices 1 to 4). Copies of three further articles have been requested when available.

Secretariat  
January 1982

ATTACHMENTS

- ANNEX A: Marine Parasitology Research Aiding Industry  
- article by Dr R.J.G. Lester and final report
- ANNEX B: Addendum to Final Report
- APPENDIX 1: Descriptions of Two New Didymozoids from  
Australian Fishes
- APPENDIX 2: A New Perkinsus Species from the Abalone  
Haliotis Ruber
- APPENDIX 3: Host-Parasite Relations in Some Didymozoid  
Trematodes
- APPENDIX 4: Nematodes from Scallops and Turtles from  
Shark Bay, WA



# Marine parasitology research aiding industry

by R. J. G. Lester\*

WRITING an article on recent research in marine parasitology can present a delicate problem—in reading it some readers may actually be deterred from eating fish (although, of course, most of the other things we eat also have their share of parasites and diseases).

Leaving those readers aside, and adding that all the parasites and diseases mentioned below are harmless to man, I would like to explain how some of your hard-earned dollars, taken in various taxes and levies, have been spent on fish parasite research.

Over the last three years the Fishing Industry Research Trust Account (FIRTA) has provided a total of about \$80 800 to a unit at the University of Queensland for research, consultancy and training on parasites and diseases important to the fishing industry.

The unit consisted of a research fellow (the author), an able technician, a well-equipped laboratory in the Department of Parasitology (including three closed-circuit, controlled-temperature seawater aquarium systems) and additional facilities and expertise both within and outside the University as required.

During these three years, field work was carried out in Tasmania, Victoria, South Australia, Western Australia, Northern Territory and Queensland. Many fishermen generously provided facilities at sea, including Ralph Carter in the Northern Territory, Paul Conaty, George Mawhinney and Peter Spinner (Qld), Peter Glennan and Dirk Tober (Vic.), Mike Hardy (Tas.), and John

Kroezen and Mike Leech (SA).

Laboratory space and support were made available by Tasmanian Fisheries Development Authority, NT Fisheries, WA Marine Research Laboratories, SA Fisheries, and NorWest Whaling Co. Pty Ltd.

Their help, and that of the many fishermen, market managers and government officers who provided specimens or information is greatly appreciated.

The first problem tackled was a local one. Moreton Bay sand crabs (*Portunus pelagicus*) frequently carry the carbuncle-like parasite *Sacculina granifera*. It makes crabs, both male and female, sterile. Parasitized males frequently fail to reach the legal minimum size, and they take on the appearance of females, which are protected. Thus parasitized crabs were usually returned to the sea.

The loss to the industry was estimated at about \$60 000 a year. Experiments showed that it was not worth trying to save crabs by returning them to the water after killing the parasite.

As the meat was not affected we recommended the adoption of another strategy—that crab fishermen be allowed to keep and sell all parasitized crabs, whether males, females, or undersized. This was officially approved, and now the annual benefit to the crab fishermen far outweighs the original cost of the research.

The second project dealt with a disease in South Australian abalone, and required the combined input of specialists in parasitology, microbiology, and electron microscopy.

Up to a third of the black lip abalone (*Haliotis ruber*) from the Neptune Islands and some other areas off South Australia contain cavities in the meat, a few

millimetres in diameter, filled with soft yellowish material.

Naturally enough such abalone are not acceptable to the processing companies, and the disease results in a significant loss to the State's million-dollar abalone fishery. We sought to find its cause, and if possible to find means of discouraging it from spreading.

Within the cavities were colonies of some type of protozoan (a single-celled organism) that could not be identified directly. Therefore diseased tissue was incubated in an anaerobic medium under conditions similar to those that would be found in decomposing abalone tissue on the sea bed. Within two days the parasites swelled to five times their original size. Transferred to sea water, they subdivided and produced large numbers of a short-lived free-swimming stage, believed to spread the infection to other abalone. Abalone kept at 15°C recovered from the infection whereas those at 20°C succumbed.

The course of the development indicated that the parasite belonged to the genus *Perkinsus*. Its staining reactions and ultrastructure showed that it was a close relative of *P. marinus* (formerly known as *Dermocystidium marinum*), the cause of costly summer mortalities in oysters along the eastern coasts of North America. (A recent outbreak in Hawaii wiped out 90 per cent of the oysters in Pearl Harbour.) Attempts in the laboratory to infect Sydney rock oysters with the abalone parasite by a variety of means were unsuccessful.

So that all fisheries biologists working on molluscs in Australia will be aware of the parasite a full description of it has been

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submitted for publication in specialist scientific literature.

To prevent the unwitting spread of the infection by abalone divers it was recommended that infected abalone or parts thereof not be returned to the sea, but rather brought ashore and buried.

In an earlier article (*Australian Fisheries*, September 1978) I mentioned some ambitious overseas schemes designed to decrease the prevalence of a parasite in natural populations of fish. Many parasites have complex life cycles involving an obligatory series of different hosts. Increasing or decreasing the numbers of one host may have a dramatic effect on the numbers of parasites in another.

In Australia a parasite that appeared to lend itself to this approach was *Gymnorhynchus thyrstiae*, the cause of 'wormy 'couta' in Tasmania, Victoria and South Australia. The parasite affects the marketability of the fish to the degree that in South Australia, 'couta (*Leionura atun*, officially known as 'snoek'), because of the worms, are sold only as bait for cray pots.

The total value of the fishery in Australia is \$150 000 to \$300 000 annually. It would be much more valuable if the fish was free of the parasite.

The worm is a larva that matures in the gut of some type of shark. If we knew which shark, a small change in the management of the shark fishery could have a big effect on the prevalence of the parasite. I have since dissected over 70 sharks but to date the adult worm has not been found.

Because of the potential benefit to southern fishermen, let us hope that in the not too distant future someone will find the species and size of shark responsible for transmitting the infection.

A second project along similar lines had a quite different outcome. The tropical scallop *Amusium balloti* in Queensland and in Western Australia sometimes contains a brownish

blemish on the side of the muscle, which makes scallops unsuitable for export. This blemish is caused by a parasite.

An outbreak in scallops at Shark Bay, Western Australia, early last year, costing \$120 000 in lost markets during the year, prompted an examination of the offending parasite. It was tentatively identified as the larva of the nematode *Sulcascaaris sulcata* which is parasitic in turtles.

Two of us then visited Shark Bay to see what turtles were present and whether indeed they were carrying adult *S. sulcata*. We found that loggerhead turtles (*Caretta caretta*) were numerous in the Bay, and some of them had hundreds of adult *S. sulcata* in their stomachs.

Manipulating the population of these huge yet gentle animals, considered by the World Wildlife Fund to be in danger of extinction, is obviously out of the question.

Very little is known about loggerheads in terms of their growth rates, population sizes and migrations. Until more work is done it is difficult to see how the prevalence of the parasite could be reduced, though when more information about the turtles becomes available, with the results of work on the parasite currently underway at the University of Queensland, we may be able to see ways to alleviate the problem.

In the meantime two other approaches are being considered: the development of a stimulus to make the parasite leave affected muscles during processing; and the introduction of the nematode hyperparasite *Urosporidium spisuli*.

During the three years of the Unit's operation 78 inquiries regarding parasites or abnormalities in fish and shellfish have been handled by the consultancy service, mostly from the managers of fish processing plants and government fisheries officers.

Some could be answered immediately. Others required

microscopy and histological sections. One enquiry led to the discovery last year that the soft floury texture of some of the yellowtail kingfish (*Seriola grandis*) in Queensland when cooked was due to infection in the muscle by the rare and unusual protozoan *Unicapsula* sp.

To increase the value of kingfish in Queensland, where they are worth much less than in other States, we have merely to sort out and discard those fish that are parasitized before they get to the consumer. The parasite is not visible macroscopically but now we know the cause of the trouble it should be possible to work out a practicable procedure for sorting the fish.

Other problems raised, such as a disease in bream in oyster ponds in South Australia (a problem I think we have solved) and the cause of a muscle disease in barramundi, are the subjects of current research.

In addition to research, and the consultancy operation, a course on the parasites and parasitic diseases of marine animals was given each year to about 20 students in the Faculty of Science at Queensland University.

The one-semester course was designed to meet the needs of future fisheries biologists in Australia. No equivalent course is given elsewhere. At least one student has since joined CSIRO's Fisheries and Oceanography Division, and five students have gone on to specialize in marine parasitology at postgraduate level.

Marine parasitology is in its infancy in Australia. Without the support of FIRTA funds allocated by the Fishing Industry Research Committee it would have been many years before the work I have described would have been carried out.

Adequate funding being available, the unit at the University of Queensland will continue to provide the industry with information and expertise in this area.



ADDENDUM TO FINAL REPORT FOR 1977 - 1980

The above report was submitted in November 1980 as Appendix II to the application for funds to work on QX Disease and other problems associated with marine parasites. A copy of it is attached. Some additional comments to update it are given below, project by project.

1. Sacculina in sand crabs (Qld.)

There has been some buyer resistance to parasitized crabs, chiefly because of their small size. Nevertheless, crabbers are now keeping and selling them rather than throwing them back.

2. Perkinsus in abalone (S.A.)

Two abalone processors have requested more information about this work. A description of the disease and its cause have been published and reprints have been distributed throughout Australia.

3. Gymnorhynchus in barracouta (snoek) (Vic., Tas., S.A.)

This project was not completed. No further work is planned until catches of barracouta, which have been very poor for the past two years, improve.

4. Sulcascaris in scallops (W.A.)

The release of both published and unpublished results to the D.P.I. and to the main processing company involved helped the two parties to come to a satisfactory agreement. The problem has thus been overcome, at least for the moment.

The progress of the consultancy service and training program are as summarised in the report.

Not included in the final report were articles published in scientific journals. Four of these are attached. Three others have been accepted for publication but have not yet appeared.

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Capital items purchased in 1979

1.	Cooling unit	\$593	)	These are both being used in the work on QX Disease and associated projects funded by FIRTA for 1981/2.
	(+ installation	\$160	)	
			)	
2.	Fibreglass tank	\$130	)	

8.9.81

*J. G. Lester*



## DESCRIPTIONS OF TWO NEW DIDYMOZOIDS FROM AUSTRALIAN FISHES

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**ABSTRACT:** *Nematobothrium spinneri* sp. n. is described from cysts in the body musculature of *Acanthocybium solandri* (Cuvier) caught near Brisbane. It differs from other members of the genus in that the testes, ovary, and vitellaria show multiple branching, the vitellaria extend anteriorly as far as the testes, and the uterus descends on leaving the ootype. In addition, the posterior part of the worm is retractile.

*Neometadidymozoon helcis* sp. n. was recovered from cysts in the walls of the buccal cavity of *Platycephalus fuscus* Cuvier and Valenciennes caught near Brisbane. It differs from *N. polymorphis*, the only other member of the genus, in that it is smaller, constant in shape, its testes do not extend into the forebody, and the uterus forms 3 rather than 2 loops.

Wahoo, *Acanthocybium solandri* (Cuvier), caught off the Queensland coast frequently carry bluish cysts in the flesh. According to fishermen, about one-third of the wahoo are affected, and partly for that reason, the value of wahoo flesh on the local market is half that of other scombrid fish. The cysts are caused by a new didymozoid trematode species which is described below. This is followed by a description of a second new didymozoid from the buccal cavity of local flathead *Platycephalus fuscus* Cuvier and Valenciennes.

Didymozoids so far recorded in Australia are *Nematobothroides australiensis* Nikolaeva and Korotaeva 1970 from the gonad of *Scomber australasicus*, and *Nematobothrium filiforme* Yamaguti 1934 from the gills of the same fish (Korotaeva, 1974).

### MATERIALS AND METHODS

Twenty-one cysts from *A. solandri* were collected from fish caught off Point Lookout, Queensland, in February 1978. Five live worms were teased out, fixed in hot 10% formalin or flattened and fixed in cold formalin, and stained with hemalum.

Twenty infected flatheads *P. fuscus* were obtained from Moreton Bay, Queensland, in March 1978. Worms were treated as above or sectioned and stained with hematoxylin and eosin. Measurements are in mm unless otherwise indicated.

Received for publication 13 October 1978.

### *Nematobothrium spinneri* sp. n.

(Figs. 1-7)

#### Description

Based on two complete, though broken, specimens taken from one cyst, and parts of four others, three from one cyst and one from another; measurements from the holotype and, in parentheses, the paratype.

Body filiform, consisting of a dorsoventrally flattened anterior end, a cylindrical main portion, and a narrow retractable posterior part; length, excluding that part which is retracted in the two type specimens, 365 (355), maximum width 1.4 (1.5) near middle of worm. Acetabulum weakly developed 49 (59) × 65 (73) μm located 3.0 (3.8) from anterior end. Oral sucker spherical, terminal, 146 (160) μm in diameter, immediately followed by spherical pharynx 144 (161) μm in diameter. Oesophagus bifurcating 1.8 (1.8) from anterior end, poorly developed glandular cells. Caeca containing dark brown granular ingesta much of their length extend to posterior end of worm.

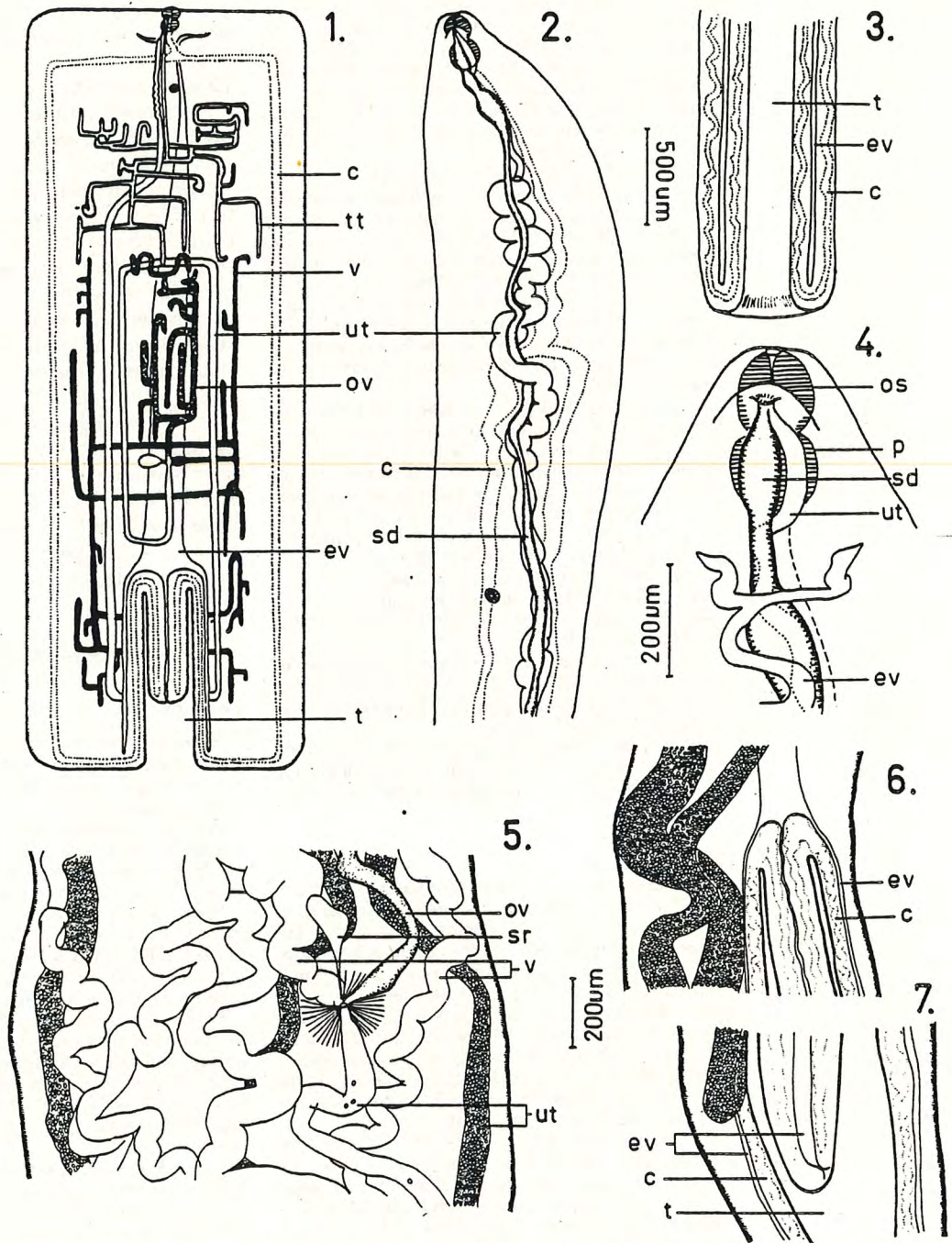
Testes tubular, branched, between levels 17 (23) and 120 (115) from anterior end, joining sperm duct about 44 (58) from anterior end. Sperm duct runs forward and opens at the genital pore on a ventral papilla at mid-level of the oral sucker.

Ovary tubular, branched, winding from the ootype to within 120 (115) of anterior end, overlapping testes by 3.8 (0.7). Ootype 202 (196) from anterior end; surrounded by very slender Mehli's gland cells about 120 μm in length. Seminal receptacle slightly elongate 300 × 80 μm, lying anterior to the ootype.

Uterus, containing few eggs in its initial part, winding posteriorly from ootype for 75 (33), turning

FIGURES 1-7. *Nematobothrium spinneri* sp. n. 1. Foreshortened diagrammatic representation of the anatomy of the holotype. Some of the links are tentative as the worm was broken in several places. 2. Ventral view of anterior end showing genital ducts, digestive tract, and acetabulum. 3. Posterior end of





worm with tail retracted showing intestinal caeca and part of cavity of excretory vesicle. 4. Ventral view of anterior end at higher magnification. 5. Female complex. 6 and 7. Proximal and distal ends of the retracted tail. Vitellaria and first descending loops of the intestinal caeca not shown (Same magnification as Fig. 5). Abbreviations: c, intestinal caecum; ev, excretory vesicle and cavities confluent with it; os, oral sucker; ov, ovary; p, pharynx; sd, sperm duct; sr, seminal receptacle; t, tube containing retracted tail; tt, testes; ut, uterus; v, vitellarium.



anteriorly as far as the vitellaria reach, descending again and reaching 143 (136) posterior to the ootype before turning anteriorly, narrowing abruptly about halfway between the intestinal bifurcation and the anterior end and then continuing to the genital pore. Eggs oval,  $15 \times 12 \mu\text{m}$  in the terminal uterus.

Vitellarium, tubular with multiple branches, extending from about the anterior bend of the uterus to near its posteriormost bend; a small vitelline reservoir evident or not.

Excretory vesicle, originating anteriorly in two arms which fuse  $455 (442) \mu\text{m}$  from the anterior end; terminating in a subterminal excretory pore at the end of the retractile part. In the holotype and paratype the end of the retractile part, the true end of the worm, is within the excretory vesicle about 20 mm from the apparent end. In a third specimen it extends 86 mm from the rest of the worm. Lying in the parenchyma and within the lumen of the gut caeca were unidentified structures thought to be hyperparasites.

One cyst contained the holotype and the paratype. A second contained only one worm (gravid), and a third three worms. Three other cysts contained an undetermined number of worms and the remaining 15 only eggs, pieces of dead worm, and host connective tissue. A formalin-fixed cyst containing worms was  $39 \times 23 \times 18$ . A cyst containing eggs and dead worm tissue was  $22 \times 10 \times 3$ .

*Type host:* *Acanthocybium solandri*.

*Site:* Encapsulated in body musculature.

*Type locality:* Point Lookout, Brisbane, Australia ( $27^{\circ}25'S$ ,  $153^{\circ}35'W$ ).

*Specimens deposited:* Holotype and paratype USNM Col. No. 75166-7 (nine slides). Pieces of other worms British Museum (Natural History) Col. No. 1979.3.20.5-6 (seven slides).

*Etymology:* Named in honor of Mr. Peter Spinner, commercial fisherman, who directed attention to the worm and provided many of the specimens.

#### Diagnosis

The occurrence of the worms in pairs in the muscles of a marine fish, and the general features of their anatomy place this species in the family Didymozoidae Poche 1907. As the worm is hermaphroditic and filariform, and as it has an acetabulum, seminal vesicle, and the ovary and testes lie anterior to the shell gland it belongs in the subfamily Nematobothriinae Ishii 1935 as defined by Yamaguti 1971. Within the subfamily it does not conveniently fit into any existing genus because the testes, ovary, and vitellarium are branched, and the posterior part of the worm is retractile.

The closest genus appears to be *Nematobothrium* van Beneden 1858, and it is into this genus that the worm is tentatively placed. It differs from all other members of the genus, in addition to the branching reproductive organs and the retractable posterior part mentioned above, in that the vitellarium extends anteriorly as far as the testes, and the uterus first descends on leaving the ootype.

#### Remarks

Evidently, the worm is able to retract its posterior part within the main body. In this respect, it resembles the ecsoma of hemiurids. However, as Dr. J. C. Pearson (University of Queensland) pointed out, the walls of a contracted ecsoma are folded, whereas in *N. spinneri* they appear to be uncompressed.

The mechanism producing the contraction is unclear. There are no large muscles that could withdraw the tail located within the excretory vesicle. Perhaps smaller muscles in the wall cause the section to turn inside out, thereby withdrawing it. A reduction in pressure within the excretory duct could have the same effect. The solution to this problem awaits study of additional material, as also does the question of the function of the tail, though presumably it is involved in some form of exchange or absorption as in the hemiurid ecsoma. That this type of organ should have developed in a didymozoid is interesting because of the probable close relationship of the didymozoids to the hemiurids (Cable, 1974).

#### *Neometadidymozoon helicis* sp. n.

(Figs. 8-12)

#### Description

Based on live material and five flattened specimens. Measurements from 10 specimens fixed in hot 5% formalin given as the mean followed by the range in parentheses.

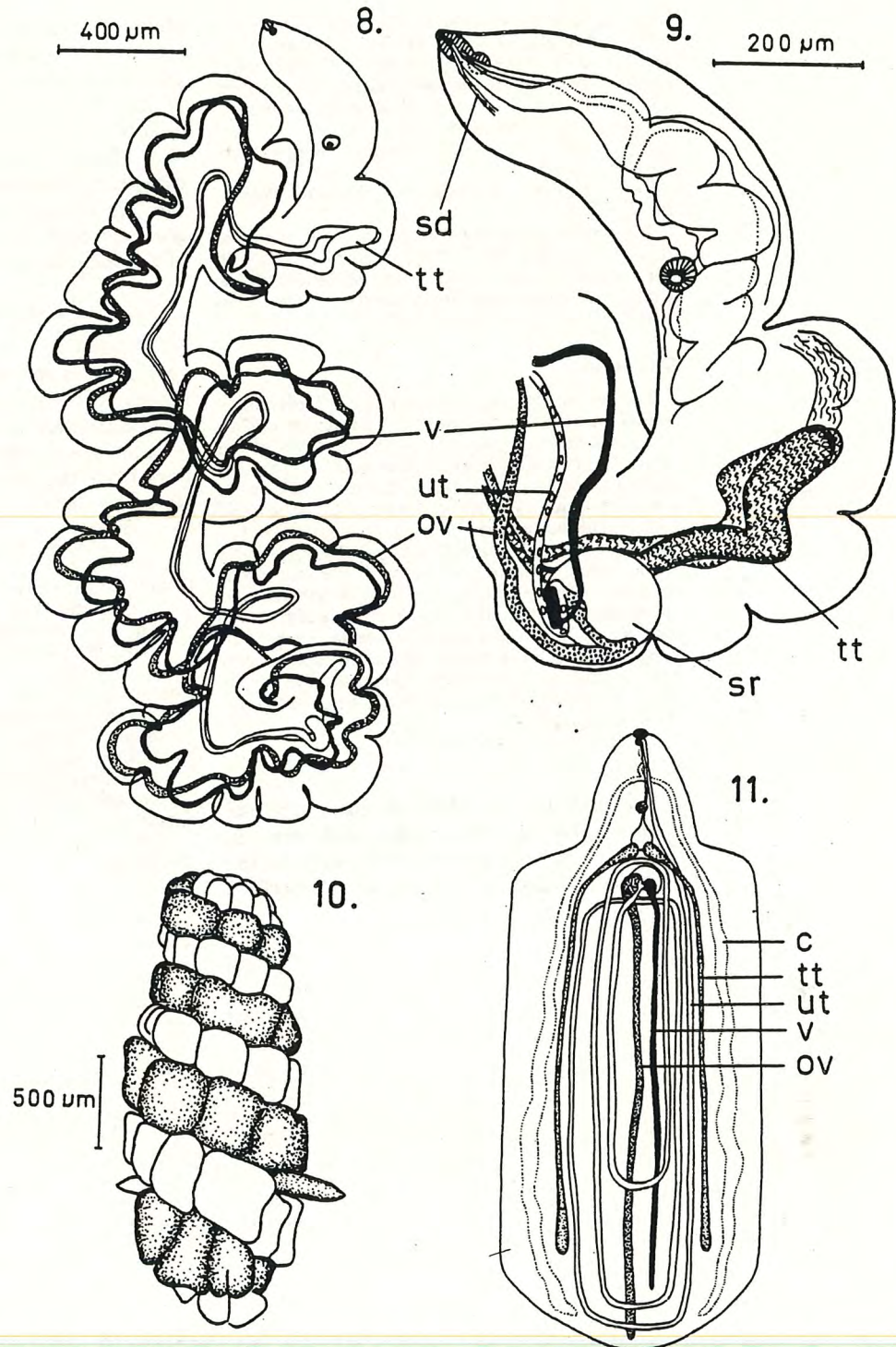
Worms invariably encysted in pairs, forming a double spiral. In life cysts usually yellowish in color, oval, visible in wall of buccal cavity, approximately  $3.5 \times 2.0$  mm in size.

Body clearly divided into 2 parts—a filiform forebody  $1.1 (1.0-1.7)$  long by  $0.19 (0.15-0.24)$  wide; hindbody  $0.5 (0.4-0.7)$  wide, a spiral  $3.3 (2.7-3.8)$  long,  $1.5 (1.2-2.0)$  wide, formed of 4 to 5 turns, outer wall thrown into folds.

Oral sucker terminal,  $50 (45-56)$  by  $30 (28-36) \mu\text{m}$  followed by a subspherical pharynx  $22 (17-24) \mu\text{m}$  in diameter. Oesophagus  $350 (290-450) \mu\text{m}$  long, ventral sucker  $48 (38-87) \mu\text{m}$  in diameter. Caeca thick walled to about level of the ootype, then thin walled almost to posterior extremity of worm.

Testes 2, ventral, elongate, extending almost full length of worm, average width about  $50 \mu\text{m}$ , wider at anterior and posterior ends. Sperm ducts join immediately anterior to testes and the common sperm duct accompanies uterus to the ventral genital pore at about midlevel of oral sucker. Ovary dorsal, full length of hindbody, average width about  $40 \mu\text{m}$ . A





FIGURES 8-11. *Neometadidymozoon helicis* sp. n. 8. Complete holotype showing reproductive structures. 9. Anterior end of holotype. 10. Typical shape of a pair of intertwined worms removed from capsule. 11. Diagram of the arrangement of the organs in *N. helicis*. Abbreviations as in Figs. 1-7.

large seminal receptacle overlaps the ootype. Vitellarium single, like ovary in size and extent. Uterus alternately descends and ascends 3 times before continuing to the genital pore. Eggs embryonated, 13 (11-14) by 6 (5-7)  $\mu\text{m}$  in final loop of uterus.

*Type host:* *Platycephalus fuscus*.

*Site:* Gill arches, walls of buccal cavity and underside of head.

*Type locality:* Moreton Bay, Brisbane, Australia.

*Specimens deposited:* Holotype and four paratypes USNM Col. No. 75168-9. Four paratypes British Museum (Natural History) Col. No. 1979.3.20.1-4. Five other specimens Queensland Museum, Brisbane, Australia Reg. No. G12103-7.

#### Diagnosis

The worms belong in the subfamily Didymozoinae (Ishii 1935) as defined by Yamaguti (1971). They are most closely related to members of the genus *Neometadidymozoon* Yamaguti 1971, though differing from his definition of the genus in that the hindbody is not cylindrical, the testes do not extend into the forebody, and the uterus forms three rather than two loops. In several additional respects the worm differs from *N. polymorphis*, the only other member of the genus. The forebody length and oral sucker diameter are about one-fifth those of *N. polymorphis*, and the pharynx and ventral sucker are much larger relative to the size of the oral sucker. Also, the shape is consistent, paired worms always forming a double spiral, and worms were not found in the body cavity.

#### Remarks

The probable life cycle of many didymozoids involves the death of the adult worm in the tissue and the release of the eggs on the death of the host. The first worm described

above, *N. spinneri*, evidently falls into this pattern as the majority of cysts examined contained eggs with only a few traces of the adult worms. This contrasts with observations on *N. helicis* as no dead worms or masses of eggs were found. Preliminary observations indicate that infections of *N. helicis* in Moreton Bay are annual, and that the adult parasites are shed from the walls of the buccal cavity of the flathead each spring.

#### ACKNOWLEDGMENTS

I thank Dr. J. C. Pearson for valuable discussions and criticism of an earlier draft of the manuscript. Mr. Con Boel provided able technical assistance, and Mr. V. R. M. Lester kindly translated papers in Russian. Financial support from the Australian Fishing Industry Research Council is gratefully acknowledged.

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## A New *Perkinsus* Species (Apicomplexa, Perkinsea) from the Abalone *Haliotis ruber*

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Received October 23, 1979

A new protozoan of the genus *Perkinsus* is described from the muscle and hemolymph of the blacklip abalone, *Haliotis ruber*, from South Australia. It occurs in the muscle of the adductor and mantle, and free and in brownish masses in the hemolymph. Cells cultured in thioglycollate medium produced a prezoosporangium which stained blue-black with iodine. The parasite differs from *Perkinsus marinus*, the only other member of the class, in having a much larger trophozoite, an eosinophilic vacuoplast when present, a short discharge tube, and appears to be uninfecive to oysters.

KEY WORDS: *Haliotis ruber*; abalone, parasite of; *Perkinsus olseni*; protozoan parasite.

### INTRODUCTION

Blacklip abalone, *Haliotis ruber*, taken off South Australia sometimes have soft yellowish pustules in the flesh. These are of concern to commercial abalone divers because affected animals are not acceptable to the processing companies. High levels of infection were reported from the Neptune Islands and from near Port Lincoln. This study was initiated to determine the cause of the disease, first noticed in 1972, and to recommend methods for its control.

### MATERIAL AND METHODS

Ten live infected abalone, collected in June or August near Memory Cove, Port Lincoln, South Australia, were flown to Brisbane, maintained in recirculating sea water (30‰) at 15° or 20°C and fed dried marine algae. Parasites were examined in fresh smears and in sections fixed in Davidson's, Bouin's, and formalin fixatives. The material sectioned for electron microscopy (taken from the abalone which contained parasites with vacuoplasts), was fixed in 4% glutaraldehyde in cocodylate buffer, postfixed in osmium, and embedded in Araldite.

Cells from infected muscle and hemolymph were incubated in thioglycol-

late medium USP (Oxoid CM 173) containing 1 mg/ml Ampicillin (Austrapen, CSL) and incubated in the dark at 20°, 25°, and 28°C (Ray, 1966). After 3 to 13 days, cells were transferred to sea water and kept under the same conditions.

Local oysters, *Crassostrea commercialis*, were exposed to infection at 25°C in 30‰ sea water either by direct contact with the zoospores (three oysters) or by inoculation of trophozoites (five oysters). Others were exposed at 20°C in 20‰ sea water by inoculation of trophozoites only (three oysters). They were autopsied between 1 and 3 months after exposure. Part of each oyster was examined directly and the remainder cultured in thioglycollate for 3 days at 25°C before examination in dilute iodine.

Where possible, the terminology used to describe the various stages follows that normally used for the Apicomplexa. For stages not clearly homologous, botanical terms have been employed.

### RESULTS

Infected abalone had soft cream or brown necrotic foci 1 to 8 mm across on the surface or within the musculature of the foot and mantle. When near the surface, they

appeared as a hemispherical cream or brown swelling (Fig. 1). Lesions over 3 mm in diameter contained pus. Those adjacent to the shell caused the formation of a brown scar on the shell's internal surface.

Both live and dead parasites were found in fresh smears of the pustule contents (Figs. 2-5). Live trophozoites, each having a large vacuole and 50 to 100 smaller droplets, were commonly encountered in pustules of abalone kept at 20°C. Dividing cells, termed schizonts, were also frequently observed. In one abalone, the vacuole of some trophozoites contained a solid inclusion body of irregular shape, the vacuoplast (Fig. 2). Such an inclusion was not seen in the trophozoites from other animals.

Dead trophozoites, appearing as empty cells except for material clumped in the center, and dead schizonts which contained a few droplets and no evidence of a vacuole (Fig. 3), predominated in abalone kept at 15°C.

A very large type of cell was occasionally found in the debris-filled center of lesions over 3 mm in diameter. The cells were five to seven times larger than trophozoites, had prominent cell walls, and frequently contained a central group of large droplets. They stained blue-black with iodine and were considered to be developing prezoosporangia, though most of the ones examined appeared to be dead.

Sections of the pustules revealed a well-defined cellular organization. Adjacent

muscle was normal except for the presence of leukocytes. A loose wall of connective tissue fibers surrounded the infected area. Inside this were large numbers of leukocytes and scattered among them were clusters of parasites in trophozoite and schizont forms. Trophozoites that had been alive when fixed contained a vacuole that almost completely filled the cell, and a nucleus with a distinct nucleolus lying close to the prominent Periodic acid-Schiff (PAS) positive cell wall (Figs. 6, 13). In one series of sections, a few vacuoplasts were seen. They were weakly eosinophilic. Schizonts dividing unequally into 10 or more daughter cells were present (Fig. 7). Further toward the center of the lesion, the condition of the host cells deteriorated, and pyknotic nuclei and the remains of dead leukocytes were evident.

Using electron microscopy, the large vacuole of trophozoites was seen to contain uncondensed vacuoplast material (Fig. 15, arrow). Granules of similar material were present in smaller vacuoles in the cytoplasm. Also present in the cytoplasm were slightly opaque vacuoles with an electron lucent center (Fig. 15d). These were evidently the droplets visible in fresh smears. Similar cell components were present in cells that had undergone division but not yet separated (Fig. 16). The parasites observed were extracellular.

Hundreds of live trophozoites and schizonts were recovered from the hemolymph of 2 of the 10 infected abalone. The para-

FIG. 1. Dorsal view of abalone *Haliotis ruber* with shell removed, showing pustule (arrow) on surface of mantle. a, cut adductor muscle; t, tentacle. Bar = 10 mm.

FIGS. 2-7. At same magnification.

FIG. 2. Live trophozoite in fresh smear. Note vacuoplast (arrow). Bar = 10  $\mu$ m.

FIG. 3. Dead schizont in fresh smear.

FIG. 4, 5. Live trophozoites and schizont in fresh smear (phase contrast). Arrow, large vacuole.

FIG. 6. Trophozoites from PAS-stained section of muscle lesion. Note nuclei with prominent nucleoli (arrow), prominent cell walls, and absence of vacuoplasts.

FIG. 7. Schizont from H and E stained section.

FIGS. 8-11. At same magnification.

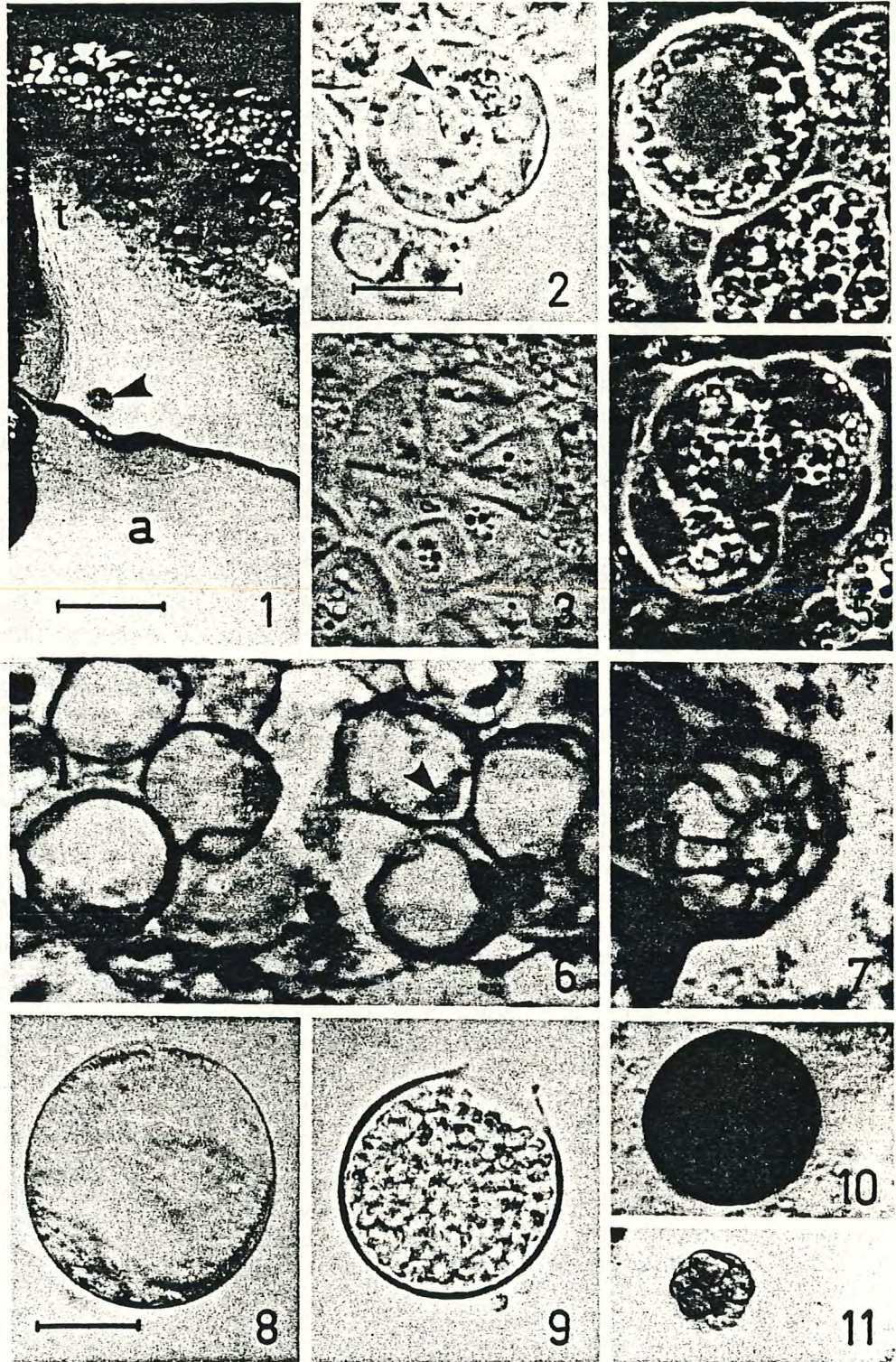
FIG. 8. Prezoosporangium from 14-day-old thioglycollate culture. Bar = 30  $\mu$ m.

FIG. 9. Mature zoosporangium after 2 days in thioglycollate and 5 days in sea water.

FIG. 10. Prezoosporangium treated with Lugol's iodine showing the densely staining wall.

FIG. 11. Schizont from the same preparation. The wall remained unstained.







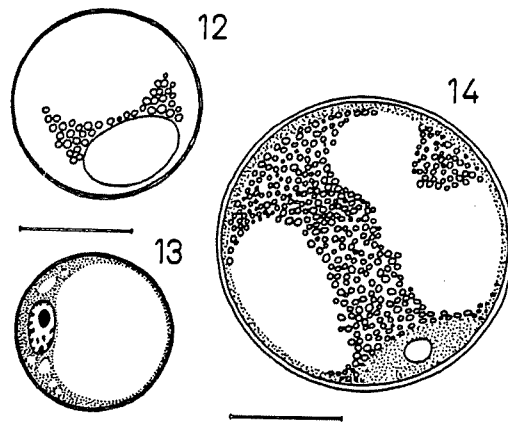


FIG. 12. Live trophozoite. A large vacuole is present together with a number of smaller droplets. Bar = 10  $\mu\text{m}$ .

FIG. 13. Fixed and stained trophozoite. Note nucleus with prominent nucleolus and vacuole almost completely filling cell. Magnification as in Fig. 12.

FIG. 14. Prezoosporangium from culture of haemolymph trophozoite (3 days in thioglycollate followed by 3 days in sea water at 20°C). Note droplets scattered over cell and peripheral mass of cytoplasm with inclusion. Bar = 30  $\mu\text{m}$ .

sites occurred in clusters either circulating freely or within aggregates of brown cells up to 1 mm long. The schizonts were dividing into two, four, or eight daughter cells of more or less equal size. No prezoosporangia were found in the hemolymph.

Trophozoites from both hemolymph and muscle were incubated in thioglycollate medium. Within 2 days they had swollen to more than five times their original size (Figs. 8, 14). The pustule wall ruptured and a cluster of enlarged cells, the prezoosporangia, were released onto the surface of the abalone. Within the cells, a mass of cytoplasm believed to contain the nucleus was located peripherally, and hundreds of fine droplets were scattered over the inside surface of the cell. When treated with Lugol's iodine, the cell wall, which varied in thickness from 1.7 to 4.2  $\mu\text{m}$ , stained blue-black and contracted, causing the cell

diameter to reduce to 0.6 of its original size (Fig. 10). The walls of trophozoites and schizonts remained unstained (Fig. 11).

Prezoosporangia stayed alive in the thioglycollate medium for at least 14 days. On being transferred to sea water, they remained clustered together on the bottom of the dish and underwent further development. By 4 days at 20°C the peripheral cytoplasmic mass had become more prominent. In some the vacuole had disappeared and the cytoplasm had contracted, now occupying the center of the cell. Shortly thereafter it began to divide (Fig. 9). The discharge tube, through which the zoospores were released, was poorly developed. Motile zoospores were visible by day 8 and empty sporangia were found on day 9. The development was not synchronized, though 90% of the cells had developed into sporangia and released zoospores by day 27. At higher temperatures development was more rapid, empty sporangia being found by day 3 at 28°C.

Protozoan parasites were not found in any of the experimentally exposed oysters.

Because the parasite is similar to but distinct from *Perkinsus marinus* (Mackin, Owen, and Collier, 1950) it is described as a new species below.

#### *Perkinsus olseni* n.sp

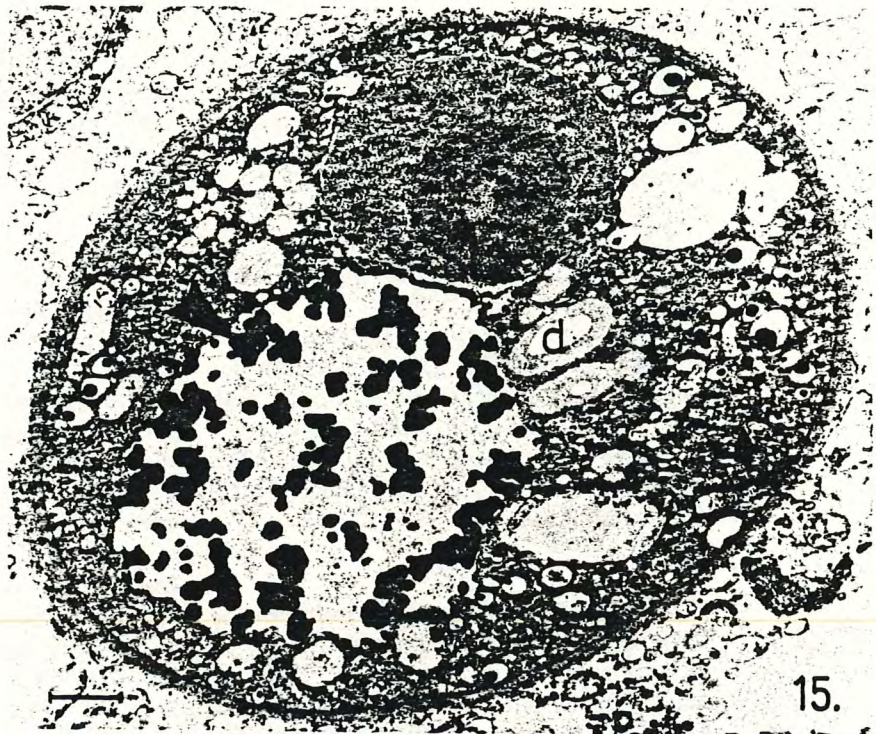
In fresh smears, spherical cells were 16.6  $\mu\text{m}$  (range of 10 measurements: 14–18  $\mu\text{m}$ ) in diameter with prominent walls. Live cells had a large vacuole up to 10  $\mu\text{m}$  in diameter and 50 to 100 droplets 1  $\mu\text{m}$  in diameter (Fig. 12). In fixed tissues, cells 13.8  $\mu\text{m}$  (13–16  $\mu\text{m}$ ) in diameter with peripheral nucleus and a large vacuole were found (Fig. 13). A vacuoplast when present was weakly eosinophilic. Dividing cells (19  $\mu\text{m}$  live, 15  $\mu\text{m}$  fixed and stained) divide into two, four, eight or more equal or subequal units.

Cells incubated in thioglycollate medium

FIG. 15. Electron micrograph of trophozoite from muscle lesion showing uncondensed vacuoplast material (arrow) and droplets (d). Bar = 1  $\mu\text{m}$ .

FIG. 16. Merozoites within schizont showing early development of vacuole, vacuoplast material, and prominent cell wall. Bar = 2  $\mu\text{m}$ .





15.



16.



develop into prezoosporangia  $74\ \mu\text{m}$  ( $56\text{--}94\ \mu\text{m}$ ) in diameter, the walls of which stain blue-black with Lugol's iodine. Cells returned to sea water divide to produce a large number of biflagellate zoospores. The parasite is not infective to oysters.

*Type host.* *Haliotis ruber*.

*Site.* Muscle and hemolymph.

*Type locality.* Near Port Lincoln, South Australia,  $35^{\circ}56'S$ ,  $135^{\circ}59'W$ .

*Histological sections deposited.* USNM Catalog. No. 30478, British Museum Accession No. 1980:11:4:1-3, South Australian Museum No. E. 1594.

*Etymology.* Named in honor of Mr. A. M. Olsen, formerly Chief Fisheries Officer, South Australia.

#### DISCUSSION

The ultrastructure of the zoospores needs to be examined before the parasite can be directly allocated to the class Perkinsea as defined by Levine (1978). Our preparations of these were unsuccessful. However, the stages observed bear many resemblances to those described for *P. marinus* by Perkins (1969) and Ray (1952). The trophozoite is spherical with an eccentric nucleus and large vacuole; the schizont divides into 2 to 10 or more subequal units; the trophozoite becomes greatly enlarged in anaerobic medium developing a wall that stains blue-black with iodine, and when returned to sea water develops flagellate zoospores; it is a parasite of a mollusk.

The parasite differs from *P. marinus*, the only species in the class, in several respects. The average size of the trophozoite cell is much larger,  $13\text{--}16\ \mu\text{m}$  in diameter in fixed tissues, compared to that of *P. marinus* which in fresh and stained preparations are  $3\text{--}10\ \mu\text{m}$  in diameter, generally  $5\text{--}7\ \mu\text{m}$  (Mackin et al., 1950). In sections the cell wall of *P. olseni* is more prominent. Its formation around merozoites still within a schizont indicates that the bulk of the wall is of parasite origin. In *P. marinus*, much of the wall is formed from membranes derived from the host (Perkins, 1969). In *P. olseni*

the vacuoplast, when present, is weakly eosinophilic whereas in *P. marinus* it stains darkly with hematoxylin. The discharge tube, prominent in *P. marinus*, is poorly developed in *P. olseni*.

Though *P. marinus* has been recorded only from the American oyster, *Crassostrea virginica*, similar organisms have been found in at least 12 other species of lamellibranchs (Andrews, 1954). This is the first report of a *Perkinsus* sp. from a gastropod.

The development and release of motile zoospores from the zoosporangia suggests that, as in *P. marinus*, a dispersal phase is produced in decaying host tissue (Perkins and Menzel, 1967). Thus, to help restrict the spread of the infection in South Australia, it is recommended that infected abalone or parts thereof found by abalone divers be disposed of on shore and not returned to the sea.

The course of the infection in the mollusk may involve circulation of the parasites in the hemolymph and the lodgment of them in the muscle. The pustule is evidently caused by the strong host reaction to the parasites once they have become localized. The preponderance of dead parasites in pustules of abalone kept at  $15^{\circ}\text{C}$ , and live parasites in abalone at  $20^{\circ}\text{C}$ , suggests that the balance between the host and the parasite is temperature dependent.

#### ACKNOWLEDGMENTS

We are greatly indebted to Mr. John Kroezen, commercial abalone diver, for generously providing the live material. Dr. D. E. Gardner, IMVS, Adelaide, Australia, and Dr. F. O. Perkins, Virginia Institute of Marine Science, Gloucester Point, Virginia, kindly sent slides of *P. olseni* n. sp. and *P. marinus*, respectively. Excellent technical help was provided by Miss S. Millyard, Mrs. M. Barrett, and Mr. C. Boel, University of Queensland. The project was supported financially by the Australian Fishing Industry Research Trust Account.

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## HOST-PARASITE RELATIONS IN SOME DIDYMOZOID TREMATODES

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**ABSTRACT:** Adult *Neometadidymozoon helicis* and *Nematobothrium spinneri* were found coiled in host connective tissue without any capsule wall of parasite origin. Eggs of *N. helicis* and *Didymozoon brevicolle* passed through the gut of a teleost without harm, suggesting predation as a possible mechanism for the release of eggs in these species. An alternative mechanism in *N. helicis* involved ulceration of the capsule wall and release of adults and eggs to the environment. The onset of ulceration was not dependent on temperature or on the sizes of the parasites, but may be associated with the host's spawning season. Larval didymozoids found in the intestinal wall of teleosts were ensheathed in a fine membrane of host connective tissue. When fed to other teleost species, one type from *Favonigobius exquisitus* burrowed into the gut wall and survived for at least a month in *Sillago analis*.

Information on the biology of didymozoids is scarce, possibly because the large pelagic scombrids in which most didymozoids occur are difficult to keep in captivity. The occurrence of several species of didymozoids in pelagic and benthic fishes off southeast Queensland permitted observations on three aspects of didymozoid biology—the structure of the didymozoid capsule, the mechanism for the release of eggs, and the location and infectivity of larval forms.

All hosts were from Moreton Bay except *Acanthocybium solandri*, which was caught off Point Lookout. One of the didymozoids discussed, *Nematobothrium spinneri*, is of commercial importance because its presence in muscle reduces the market value of *A. solandri*.

### OBSERVATIONS AND DISCUSSION

*Neometadidymozoon helicis* occurred in the dermis of the buccal cavity and gill arches of 90% of the flathead *Platycephalus fuscus* examined (Fig. 1). The worms appeared to be encapsulated by a discrete cyst, but sections stained for connective tissue showed little more than a parting of the dermal fibers to accommodate the parasites (Fig. 2). The much larger parasite, *Nematobothrium spinneri*, common in the musculature of *A. solandri*, was enclosed in a fibrous tissue wall comparable in thickness to that of an adjacent intermuscular septum (Figs. 4, 5). Connective tissue and blood capillaries interdigitated between the coils so that the whole of the sur-

face of the worm was in intimate contact with its host (Fig. 6). Though this may have facilitated the exchange of materials between the parasite and its host, the well-developed oral sucker and pharynx were evidently functional as dark deposits of partly digested host erythrocytes were in the caeca.

Neither of the species secreted a cyst wall. The only didymozoid so far found within a true cyst (Williams, 1959) is the highly modified *Köllikeria filicollis*. The capsule of *N. spinneri* resembled, in some respects, the nodule of *Onchocerca volvulus*, although far less connective tissue was present around the didymozoid.

Though all capsules of *H. helicis* examined contained live parasites, of 21 capsules of *N. spinneri* only six contained live worms. The other 15 were composed of masses of eggs within worm remains. Similarly, in a third didymozoid species examined, *Didymozoon brevicolle* from the stomach wall of *P. fuscus*, only two of 20 capsules contained live adults. Because the eggs remain in utero in the last two species, the uterine pore and terminal uterine segment, which are well developed, may exist for the acceptance of sperm rather than for the discharge of eggs.

As has been proposed for some other didymozoids (Noble, 1975), the egg masses of *N. spinneri* and *D. brevicolle* presumably are released to the environment only when the host is damaged or eaten by a predator. To test the capability of didymozoid eggs to pass through the gut of a teleost, gravid adults of *N. helicis* were fed to two whiting, *Sillago analis*. Eggs collected 24 hr later from the feces of both were broken open by coverslip

Received for publication 6 November 1979.

pressure in seawater and active miracidia emerged. In a second experiment, an egg mass of *D. brevicolle* from *P. fuscus* was fed by stomach tube to a second *P. fuscus*. Twenty-four hours later, feces containing eggs were recovered and all eggs found were intact. Eggs broken open released live miracidia, which were more active in physiological saline than in seawater. These experiments showed that the eggs of *N. helicis* and *D. brevicolle* may pass through the gut of teleosts without harm. The second experiment also indicates that the route of infection of the flathead by *D. brevicolle* is not by the ingestion of embryonated eggs.

Because all *N. helicis* examined were alive, it suggested that the parasites survived for the life of the fish. However, by keeping six infected flathead in the laboratory for several months, this was found not to be the case. The fish were kept at 25 C, the average summer bottom temperature of Moreton Bay, and fed frozen fish. They were examined periodically using ethyl-m-aminobenzoate (Sigma) as anesthetic. In the first 4 mo the number of capsules doubled (Fig. 10), presumably because larvae already present were moving into the gills, pairing up and maturing. The fish then became restless and tended to leap from the tanks or otherwise damage themselves. By 6 mo, most of the capsules in the two remaining fish had disappeared.

Monthly samples from commercially caught *P. fuscus* showed that there was a seasonal cycle in the occurrence of *N. helicis* in Moreton Bay (Fig. 11), most of the parasites disappearing between September and December. Of the 2,041 capsules examined at this

time, 123 (6%) were red as a result of capillaries hemorrhaging around the worms. Worms within hemorrhagic capsules appeared normal except that their caeca contained erythrocytes instead of clear fluid. One capsule was found open, with an occupant still alive and partly hanging free. In the same period, 75 depressions in the mucosa, believed to be the sites of former infection, were also recognized (Fig. 3). Evidently the capsule wall was breaking down and the worms were being released. The small number of hemorrhagic capsules observed suggested that this stage lasted for only a few days.

What triggered the wall breakdown was unclear. It was not capsule size, because capsules of all sizes were found hemorrhaging, and the mean monthly size of 4,500 capsules measured during the year did not significantly change. There was no significant correlation between host length and number of capsules ( $r = 0.012$ ). A rise in temperature was evidently not the cause because the laboratory-held fish also lost their worms. Leucocytes, while abundant in tissue adjacent to hemorrhaging capsules, did not surround or attach to the worms themselves, suggesting that an immune response was not directly responsible. Loss of parasites may be related to the sexual maturation of the host. Local *P. fuscus* start maturing in September and spawn between November and February (Dredge, 1976). Self et al. (1963) found that *Nematobothrium texomensis* developed only in host gonads that were maturing. They suggested that the development of the parasites was under the influence of host hormones.

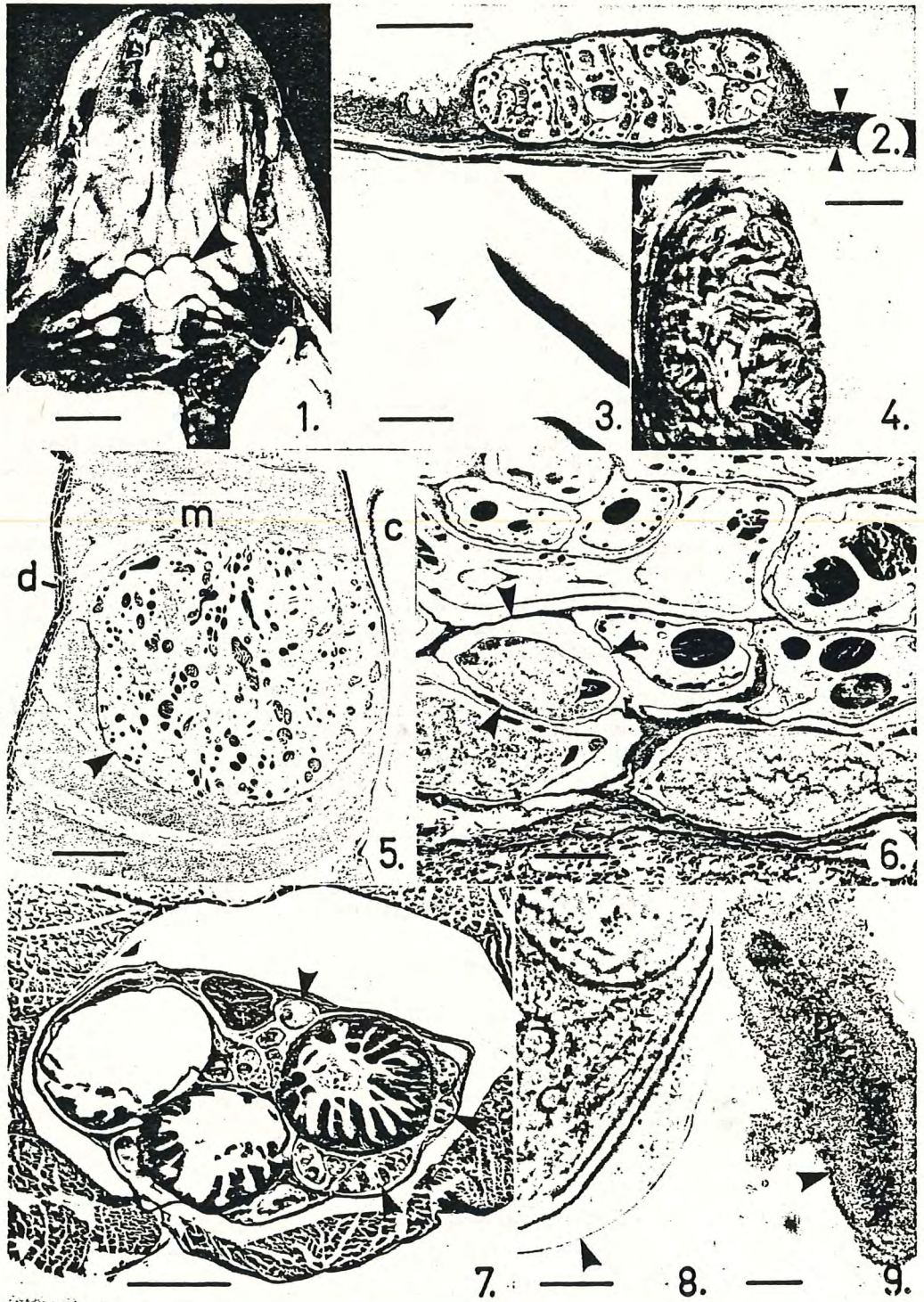
The loss of *N. helicis* through ulceration

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FIGURES 1-9. 1. Palate of *Platycephalus fuscus* infected with *Neometadidymozoon helicis*. The capsules (arrow), which are unusually large in this specimen, in life are bright yellow. Each contains two intertwined worms. Bar = 10 mm. 2. Section across *N. helicis* capsule showing parasites coiled up within the dermis. Dermis thickness indicated by arrows (Van Gieson stain; epidermis lost during processing). Bar = 1 mm. 3. Scar (arrow) in dorsal surface of lower jaw marking site of former *N. helicis* capsule. Bar = 5 mm. 4. Capsule of *Nematobothrium spinneri* in musculature of body wall of *Acanthocybium solandri*. Bar = 10 mm. 5. Section of capsule of *N. spinneri* (Van Gieson stain). Note the fine band of connective tissue surrounding the worm (arrow). c = body cavity; d = dermis; m = myotome. Bar = 3 mm. 6. Higher power showing the thin connective tissue wall and the connective tissue sheaths (arrows) enclosing the coils of the worm. Bar = 50  $\mu$ m. 7. Section of visceral mass of *Favonigobius exquisitus* containing larval didymozoids (arrows). Note that none are in the gut lumen. Bar = 400  $\mu$ m. 8. Posterior of larval didymozoid from viscera of *F. exquisitus* maintained for 32 days in the laboratory, showing thin connective tissue capsule (arrow). Bar = 20  $\mu$ m. 9. Dying larval didymozoid (p) from *P. fuscus* experimentally infected 2 days before. Note thick wall of host leucocytes (arrow). Bar = 100  $\mu$ m.





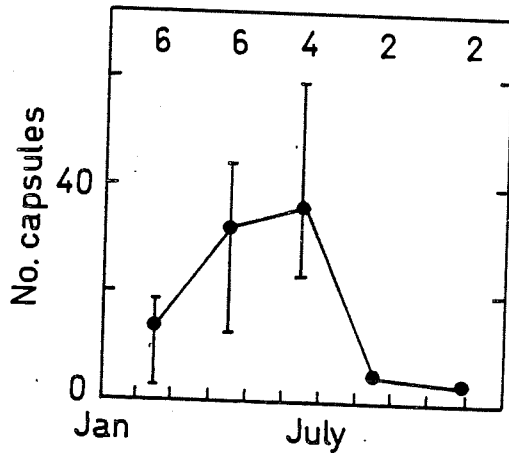


FIGURE 10. The mean numbers of *N. helicis* capsules present in two to six naturally infected *P. fuscus* kept in the laboratory at 25 C. Range and number of fish indicated.

supports the hypothesis of Timon-David (1937) who, after observing hemorrhage around *D. wedli*, suggested that the capsules reached a certain degree of maturity, then opened spontaneously and evacuated their contents to the exterior. Grabda (1947) reported occasionally finding blood suffusing inside the capsule of *N. sardae* "smothering the parasites," and Awachie (1972) observed tissue erosion associated with *Nematobothrium* infection. The release of eggs through ulceration may thus be widespread among the Didymozoidae.

Larval didymozoids were found in several species of fish (*Engraulis australis*, *Pranesus*

*ogilbyi*, *S. analis*, *S. maculata*, *Pelates quadrilineatus*, *Rhinogobius leftwichi*, *Favonigobius exquisitus*, and *Pseudogobius* sp.). In all cases, they occurred outside the gut lumen in the visceral mass (Fig. 7). They were enclosed within a sheath of connective tissue one cell thick (Fig. 8). To determine the host specificity of one of five larval types found (Fig. 12), approximately 40 larvae were fed to each of two decapods (*Portunus pelagicus*, *Grapsus* sp.) and 16 assorted teleosts (4 *Selenotoca multifasciata*, 3 *Neoarius australis*, 4 *Rhabdosargus sarba*, 3 *S. analis*, and 2 *P. fuscus*). Two to 8 days later, nothing was found in the crustaceans, but in the teleosts, parasites had penetrated into and in some cases through the gut wall. In the first three species only dead parasites were found. In *P. fuscus* there were a few live parasites, covered in host cells (Fig. 9), and many dead ones. In *S. analis*, parasites appeared unharmed and survived in a fourth fish for at least a month without eliciting any host response apart from the thin membrane of connective tissue, indicating that this fish could serve as a paratenic host. As maturation did not occur in any of the fish, the species to which the larvae belonged is unknown.

As suggested by Cable and Nahhas (1962) and Nikolaeva (1965), larvae probably are eaten by the definitive host while consuming prey. Presumably the larvae then burrow into the wall of the gut, as they were observed to in this work, and from there migrate to their normal site of maturation. Those like *D. brevicolle* developing to adults in the wall of the stomach thus are already in position. Those like *N. helicis* that are normally found

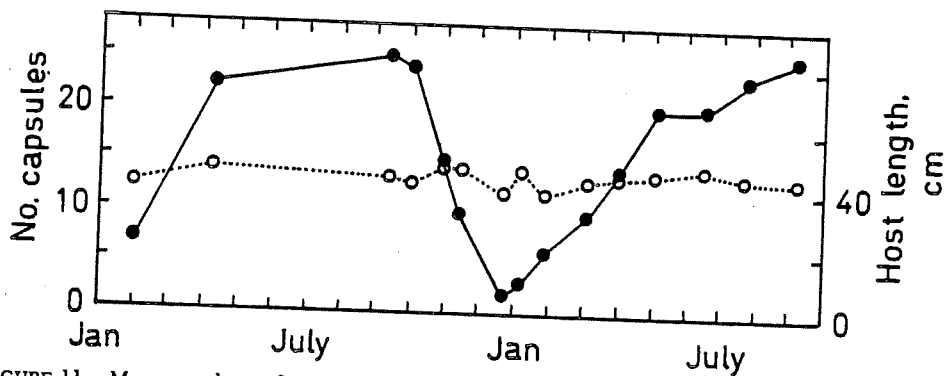


FIGURE 11. Mean numbers of *N. helicis* capsules (solid circles) in 583 *P. fuscus* 29 to 81 cm in length from Moreton Bay, Queensland. Open circles = mean host length. Each point from sample of at least 20 fish.



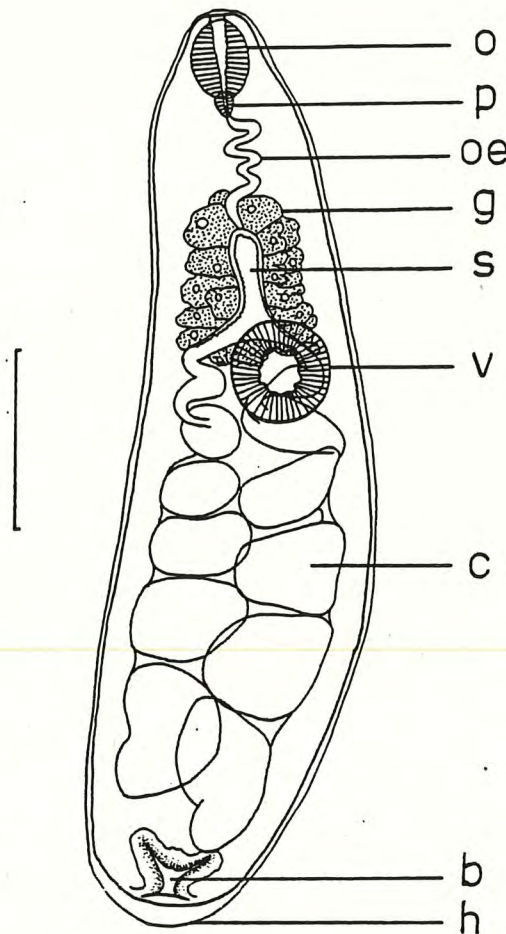


FIGURE 12. Larval didymozoid within fine sheath of host tissue from gut wall of *Favonigobius exquisitus*. Bar = 100  $\mu$ m. o = oral sucker; p = pharynx; oe = oesophagus; g = gland cell; s = stomach; v = ventral sucker; c = chamber of caecum; b = collapsed bladder; h = sheath of host tissue.

in the buccal region may have moved forward through the tissues of the oesophagus wall. Some of the species that develop in muscles may penetrate into them directly from the body cavity. Nodules of *N. spinneri* almost invariably occur in the muscle surrounding the viscera and because of this the "belly flap" usually is removed from local wahoo before marketing. Whatever route they take, preliminary observations suggest that the migration in *N. helicis* takes at least a week and may take several months, which is much slower than the speed at which some other fish

trematodes such as diplostomes reach their site for development (Betterton, 1974; Lester and Huizinga, 1977).

#### ACKNOWLEDGMENTS

I thank Drs. H. M. D. Hoyte and J. C. Pearson, University of Queensland, for help and advice, and Dr. D. F. Hoese, Australian Museum, for identifying the gobies. The article in Russian was kindly translated by Dr. K. M. Windle. Mr. C. Boel and Mrs. M. Barrett provided excellent technical assistance. The work was supported in part by the Australian Fishing Industry Research Trust Account.

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## Nematodes from Scallops and Turtles from Shark Bay, Western Australia

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### Abstract

Between 1971 and 1978 up to 64% of commercial-sized saucer scallops, *Amusium balloti*, in samples from the landed catch at Shark Bay were infected with the larval ascaridoid nematode *Sulcascaaris sulcata*. The presence of the nematodes and the brownish capsule in which they were found made some of the processed catch unsuitable for export. A small percentage of the scallops also contained a larval gnathostome, *Echinocephalus* sp.

Five of six loggerhead turtles, *Caretta caretta*, from the same area were found to contain adult *S. sulcata*. Also present in the turtles were the cucullanid *Cucullanus carettae*, the oxyuroid *Kathlania leptura* and the larval gnathostome *Echinocephalus* sp. Four of the turtles had been feeding chiefly on bivalves, one on crabs, and one on gastropods. Ulcers, probably associated with *S. sulcata* infection, occurred in the stomach, and small haemorrhages, probably associated with the feeding sites of *C. carettae*, occurred in the intestine.

Additional keywords: parasite, helminth, mollusc, reptile.

### Introduction

Many of the scallops taken commercially from Shark Bay, W.A., contain lesions caused by larval nematodes. As federal regulations prohibit the export of more than a small fraction of such affected scallops, the parasites reduce the value of the catch. Examination of a sample of the nematodes revealed that they were larval ascaridoids similar to those found by Cannon (1978) in Queensland scallops, which had been shown experimentally by Sprent (1977) to develop into *Sulcascaaris sulcata* (Rudolphi, 1819) in young loggerhead turtles, *Caretta caretta*.

Following the granting of a permit to the authors some adult turtles caught in prawn trawls in Shark Bay were made available for study. Normally turtles accidentally caught by commercial fishermen are returned to the sea. Information on all nematodes recovered from the turtles and scallops is given below, together with data on the prevalence of *S. sulcata* in the scallop.

### Materials and Methods

In 1971, 1977 and 1978, a sample of at least 50 scallops, *Amusium balloti*, was taken four to six times a year from commercial trawlers in Shark Bay. They were examined for nematode lesions by teasing apart the adductor muscle.

In July 1979, six *C. caretta* caught in the bay were killed and dissected within 48 h of capture. All were non-gravid females (oocyte diam. 4 mm). Nematodes were either fixed in glacial acetic acid and then transferred to 80% alcohol, or flown alive to Brisbane for life-cycle studies.



## Results

### Scallops

In 1971, 1977 and 1978, 63, 42 and 50%, respectively, of commercial-sized *A. balloti* sampled had brownish lesions 3–7 mm diameter across their adductor muscles as a result of infection by *S. sulcata* (Fig. 1). Each lesion contained one or more nematodes

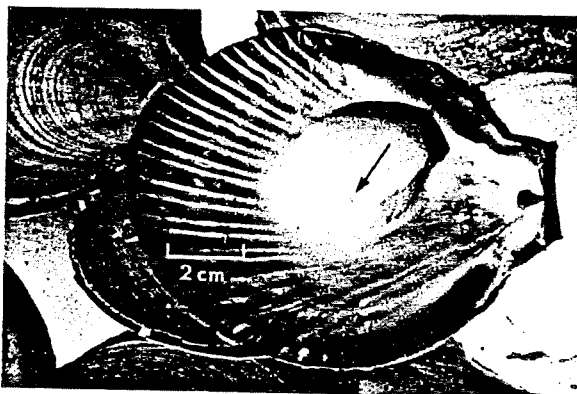


Fig. 1. *Amusium balloti* from Shark Bay showing brown lesion (arrow) as a result of *Sulcascaaris sulcata* infection in adductor muscle. Viscera of scallop removed.

30–50 mm long and more than one lesion was often present in a scallop. The number of nematodes per scallop increased with the size of the scallop. The mean numbers present in 916 scallops divided into six 10-mm size classes ranging from 56 to 115 mm shell height were 0, 0.1, 0.9, 0.44, 0.83, and 2.16. The coefficient of dispersion indicates that the distribution of the helminths among the scallops was clumped (mean 0.75, variance 1.71).

The only other nematodes found were two larval *Echinocephalus* sp., 11 and 18 mm in length, in a sample of 10 scallops dissected under the microscope.

Table 1. Nematodes recovered from six *C. caretta* from Shark Bay, Western Australia  
Number of parasites found is given in parentheses

Turtle	Host weight (kg)	Host length <sup>^</sup> (m)	Nematodes recovered from:	
			Stomach	Intestine
1	160	1.04	<i>Sulcascaaris sulcata</i> (70)	<i>S. sulcata</i> (1) <i>Cucullanus carettae</i> (23)
2	117	0.93	<i>S. sulcata</i> (35)	None
3	66	0.83	<i>S. sulcata</i> (9)	<i>C. carettae</i> (1)
4	125	0.98	None	<i>C. carettae</i> (34) <i>Echinocephalus</i> sp. (1)
5	111	0.96	<i>S. sulcata</i> (351) <i>Echinocephalus</i> sp. (1)	<i>S. sulcata</i> (3) <i>C. carettae</i> (68) <i>Echinocephalus</i> sp. (4)
6	—	0.93	<i>S. sulcata</i> (222)	<i>Kathlania leptura</i> (200) <i>C. carettae</i> (12) Immature oxyuroids (5000)

<sup>^</sup> Midline curved carapace length.

### Turtles

Five of the six loggerhead turtles contained *S. sulcata* (Table 1). All had ulcers which penetrated into the submucosa of the stomach wall (Fig. 2) and in four of the turtles most of the nematodes were attached within the ulcers.

Immature individuals of *Echinocephalus* sp., 16–19 mm in length, were found in the stomach of one turtle and in the intestine of two. The anterior one-third of the body was embedded in a whitish sleeve of host tissue in the mucosa. Around the attachment site the gut wall felt firmer than normal.

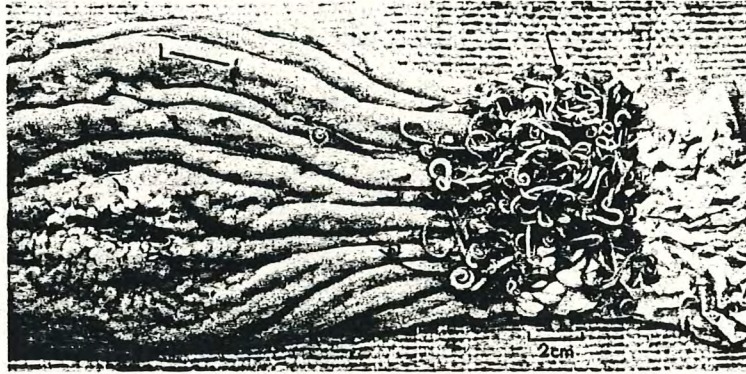


Fig. 2. Opened crop (to right) and stomach of *Caretta caretta* from Shark Bay. The nematodes (arrow) are all adults or fourth-stage larvae of *S. sulcata*. On the left is the stomach ulcer with its caseous white exudate. *L. Lophotaspis vallei*, an aspidogastreaean common in the stomachs.

A third nematode species, *Cucullanus carettae* Baylis, 1923, was restricted to the anterior 1–1.5 m of the intestine. Some of these parasites, attached by the anterior end with the body and tail projecting into the lumen, were completely ensheathed by host tissue. At the end of the sheath in the vicinity of the anus there was a dark mass, possibly of excreta, which rendered the worms more readily visible. Small haemorrhages marked the attachment sites.

One turtle contained about 200 individuals of the oxyroid *Kathlania leptura* (Rudolphi, 1819). These were swimming freely in the contents of the lower part of the intestine.

The six Shark Bay turtles were feeding on benthic invertebrates (Table 2). Four were feeding chiefly on bivalves, one chiefly on gastropods, and one chiefly on crabs. No remains of *Amusium balloti* were recognized.

### Discussion

Adult *S. sulcata* have been reported from *C. caretta* and the green turtle *Chelonia mydas*. Sprent (1977) identified the larval forms in *A. balloti* from Queensland by feeding them to laboratory-reared *C. caretta* and recovering adults. In Shark Bay both *A. balloti* and *C. caretta* are heavily infected with *S. sulcata*; however, the absence of *A. balloti* from the gut of the turtles suggests that a different mollusc may be the normal intermediate host. J. Penn (personal communication) has since recovered *S. sulcata* from two other species of scallop in Shark Bay: *Anachlamys leopardus* (2 infected out of 10 sampled) and *Chlamys asperrimus* (10 infected out of 10 sampled). In Queensland, *S. sulcata* is known from *A. balloti*, *Chlamys* sp., *Pinna menkei* and *Spondylus ducalis* (see Sprent 1977; Cannon 1978). In the United States, Lichtenfels *et al.* (1978) found *Sulcascaris* sp., presumably *S. sulcata*, in the bivalves *Spisula solidissima*, *Argopecten gibbus* and *A. irradians*, and the gastropods *Lunatia heros* and *Busycon canaliculata*.



The parasite thus appears to have a wide range of possible intermediate hosts among the Mollusca.

*Chelonia mydas* was thought to be a possible alternative definitive host as *S. sulcata* was originally described from this species. It is common in Shark Bay and a fishery for them ended in 1971. However, the parasite was not found in three medium-sized individuals washed up in the bay in September 1979 (curved carapace lengths 42, 44 and 51 cm; L. A. Smith and J. Penn, personal communications). Their stomachs

Table 2. Gut contents of *C. caretta* from Table 1

Turtle	Gut contents
1	Intestine filled with broken pinna shells ( <i>Atrina</i> sp.)
2	Mostly crabs ( <i>Eucrate</i> sp., with some pieces of portunid and dromid); also polychaete tubes and one polychaete ( <i>Chloea flava</i> )
3	Parts of intestine filled with broken bivalve shell ( <i>Venus laqueata</i> , <i>Megacardita incrassata</i> , <i>Fragum retusum</i> , <i>Tapes literatus</i> , <i>Paphia sulcosa</i> , <i>Callista planatella</i> , <i>Timoclea</i> sp., <i>Eucrassatella pulchra</i> , <i>Hytissa</i> sp., and <i>Circe sulcata</i> ); also several <i>Chloea flava</i> , pieces of <i>Pagurus</i> sp., stomatopod, ascidian ( <i>Phallusia depressiuscula</i> ), and a few gastropods ( <i>Strombus campbelli</i> and <i>Polinices albumen</i> )
4	Mostly <i>Callista planatella</i> and <i>Paphia sulcosa</i> ; pieces of <i>Phallusia depressiuscula</i> , and pagurid and leucosid crabs
5	Mostly <i>C. planatella</i> . Pieces of <i>Thalamita sima</i> (crab) and <i>Polinices albumen</i>
6	Intestine filled with <i>P. albumen</i> ; one piece of crab.

contained only vegetable matter. C. Limpus (personal communication) has found *Sulcascaris* sp. many times in *Caretta caretta* from Queensland but never in *Chelonia mydas*. Similarly, Sey (1977), during a parasitological survey of Egyptian turtles, found *S. sulcata* only in *Caretta caretta*. The above evidence, together with the different dietary preferences of the two turtles, *Chelonia mydas* being predominantly a herbivore, suggests that *Caretta caretta* is the more important host in the life cycle of the parasite.

The larval *Echinocephalus* sp. found in *A. balloti* appeared to be identical to those in *C. caretta*. Adult *Echinocephalus* species occur in the spiral valve of elasmobranchs (Ko 1975).

#### Acknowledgments

We thank Mr P. Rich, Mr S. Burke and other officers of the Nor'West Whaling Company for assistance in obtaining the turtles and in completing the dissections. The help of Dr D. A. Hancock and Mr B. Bartley, W.A. Marine Research Laboratories, is also greatly appreciated. Permission to take turtles was granted by Mr B. K. Bowen, W.A. Fisheries and Wildlife. Invertebrates were kindly identified by Ms S. Slack-Smith, W.A. Museum; Dr P. Mather and Mr P. Davie, Queensland Museum; Mrs H. Paxton, Macquarie University; and Mr J. Healy, Queensland University. Dr L. R. G. Cannon, Queensland Museum, confirmed the identity of the adult *S. sulcata*. Financial support from the Australian Fishing Industry Research Trust Account is gratefully acknowledged.

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Manuscript received 21 March 1980, accepted 27 May 1980





Figure 13.

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SWIM-BLADDER ECTASIA IN THE TREVALLY *CARANX GEORGIANUS*

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## ABSTRACT

All of 13 *C. georgianus* 470 to 540 mm in fork length caught off southeast Tasmania had cavities 2 to 20 mm across in the anterior musculature over the vertebral column. The cavities were lined with smooth muscle and connective tissue, and filled with gas. Cultures for bacteria were negative. No helminths were present, and histologically there was no sign of any protozoan infection or any inflammation. All the cavities on one side of a fish connected to a horizontal chamber up to 200 mm long that ran immediately dorsolateral to the vertebral column, and opened at the anterior end into the swim bladder via a small duct. A similar but not identical system of tubes was present on the other side of the fish. It is suggested that over a period of time pressure in the swim bladder had stretched the bladder wall and forced it to swell into the tissues. The cavities in the musculature reduced the marketability of the fish.

## INTRODUCTION

Silver trevally, *Caranx georgianus*, are caught by line and net fishermen in southern Australian waters, particularly in the summertime. A series of good catches in early 1981 focused attention on what were apparently holes in the musculature.



Though their existence in trevally has been known for many years, on this occasion processing companies refused to accept fish with these 'cancerous' abnormalities. The present study was therefore undertaken to determine their origin.

Possible causes that were considered were: bacteria, as *Aeromonas salmonicida* and *Renibacterium salmoninarum* in other fishes can cause breakdown of the musculature to produce discrete pus-filled cavities; protozoa, such as *Kodoo clupeiidae*; and parasitic helminths, as larval trypanorhynch tapeworms sometimes produce a bladder-like growth that may be found in the musculature.

#### MATERIALS AND METHODS

Thirteen fish, 470 to 540 mm in fork length, and one fish 180 mm long, caught off southeast Tasmania in February 1981, were dissected within 48 hours of being caught. Four other fish, 420 to 480 mm long and caught at the same time were used in making casts. Four fish 440 to 470 mm long, caught in the same area, were dissected fresh in May, 1978, and one formalin-fixed fish, 340 mm in length and caught in the Tamar River, northern Tasmania, in February, 1975, was also examined.

Attempts to culture bacteria were made on agar plates using the following media; Trypticase Soy Agar, Columbia Blood Agar, 0.1 Columbia Blood Agar, and Mueller-Hinton with cocarboxylase.<sup>1</sup> Inoculated media were incubated at 22° C for 5 days. Tissues were fixed in formalin and Bouin's fixatives, and stained with haematoxylin and eosin, Giemsa, and Verhoeff's elastin stain.

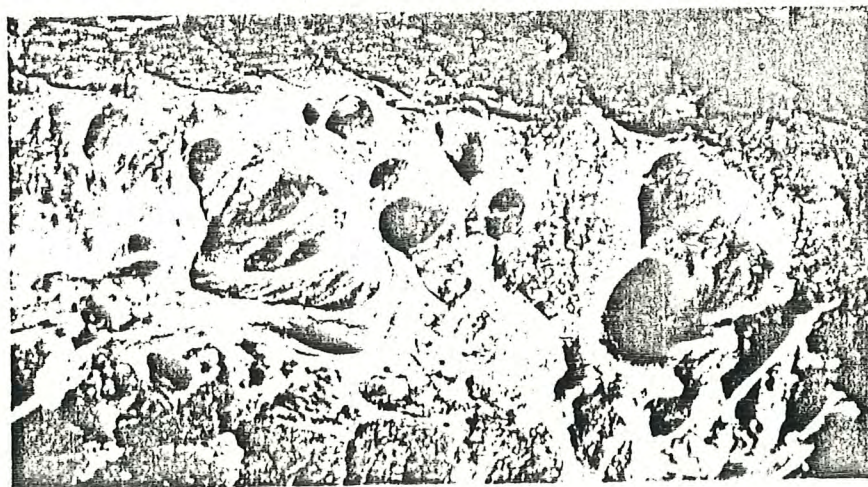
Fish to be injected with epoxy resin were held ventral side uppermost, the body cavity opened, an incision made in the swim bladder, and the resin introduced using a 20 ml syringe. In two of the fish, prior to injection, part of the musculature was removed to expose the ends of some of the canals, so that the resin could pass from the swim bladder through the canals to the outside, thus ensuring that the full length of the canal was filled. The resin used was Epirez 133 Hydrophobic Epoxy Binder 2 part mix. It was left to harden for 2 to 3 days after which the fish tissue was macerated within a solution of hot 1 molar sodium hydroxide and the casts freed.

Fish age was determined from the number of annuli on otoliths and scales.

#### RESULTS

All fish dissected over 330 mm had cavities in the musculature. The cavities ranged from 2 to 20 mm in diameter and were lined by a white pliable tissue (Figure 1). They enclosed no liquid or sign of necrotic tissue, and appeared to contain only gas. Cavities were grouped in the anterior part of the musculature dorsal to the vertebral column, and were more prevalent in some fish than others.

Figure 1. THE CAVITIES OF A FILLET OF *C. GEORGIANUS*.



In the 13 large fish dissected in 1981, the holes on one side were interconnected, and joined to a long horizontal chamber that lay immediately dorsolateral to the vertebral column (Figures 2 and 3). The length of this chamber varied between fish, and between the left and right sides of the same fish (Table 1). Finger-like projections from this chamber extended into the musculature, and it was these that appeared as holes in the fillets. The extrusions tended to run between the dorsal vertebral spines, though they did not cross the midline of the fish, and penetrated dorsally up to a muscular septum. The degree of development of the projections varied, some fish having a long horizontal chamber with only 2 projections, others having a short chamber with four or five well developed projections.

In all cases, however, at the anterior end of the chamber there was a small tube that ran ventrally around the vertebral column and opened into the swim bladder beneath. This connecting duct was between the 3rd and 4th vertebrae, or between the 4th and 5th vertebrae, sometimes being between different vertebrae on the two sides of the same fish (Figures 2 and 3).



Figure 2. THE LAYOUT OF THE EXTRUSIONS ON THE LEFT SIDE OF ONE FISH.

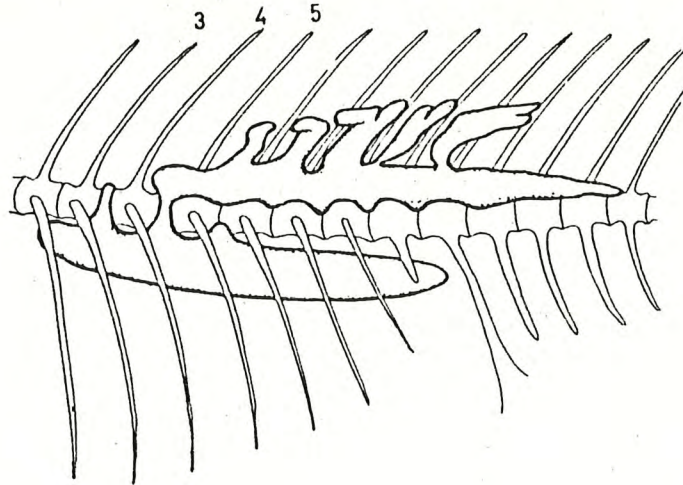
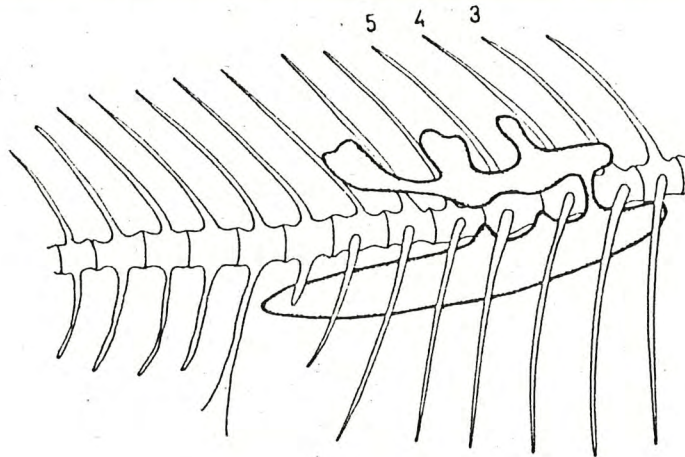


Figure 3. EXTRUSIONS ON THE RIGHT SIDE OF THE FISH SHOWN IN Fig. 2.



These connections and the shapes of the chambers were clearly demonstrated by the resin casts (Figure 4). Each swim bladder contained several dorsal pockets where it fitted between the ribs. Evidently one of these on each side of the fish had broken through into the tissue above. Two of the four casts showed a feature that had been overlooked in the dissections, a pair of horizontal chambers ventral to the vertebral column, running from the posterior of the swim bladder into the caudal musculature. These also had lateral projections though they were less well developed than those in the dorsal musculature.

Histologically, the cavities were lined with a thin mucosa and a mixed layer of smooth muscle and connective tissue. Behind these was an area of fat cells within which were thick sheets of collagen fibres. The fat cells were in contact with striated muscle fibres, some of which showed hyaline degeneration. There was no sign of inflammation, and no evidence of any bacterial, protozoal or helminth infection. The wall of the swim bladder proper also contained smooth muscle and connective tissue though these formed two separate layers, the tunica muscularis and the tunica serosa. Fat cells were absent.

No bacteria were cultured on the plates.

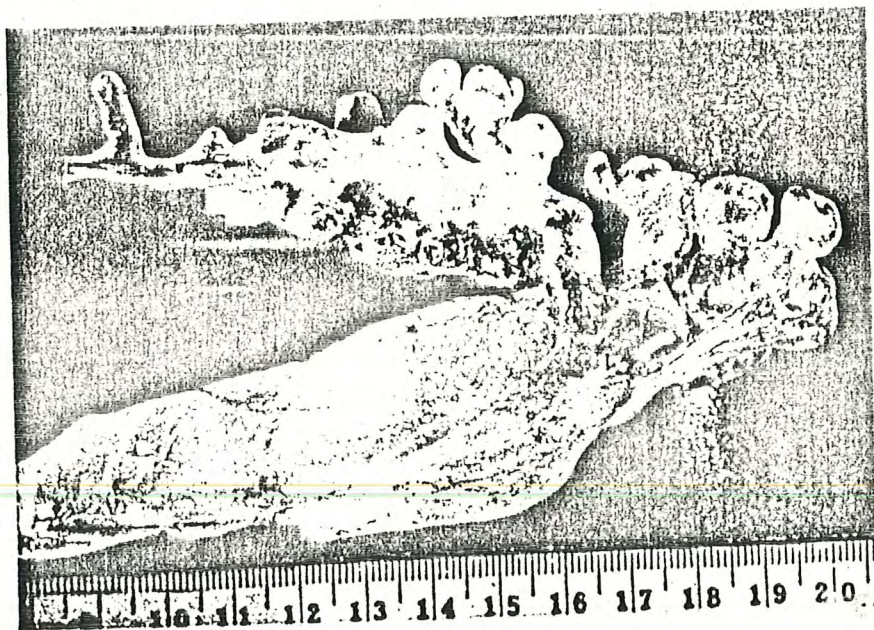
The 180 mm fish dissected was free of any swim bladder extrusions. Five of the fish 490 to 520 mm long were determined to be 7+ years old, whilst the 180 mm fish was 2+ years old.



Table 1. THE LENGTHS OF THE HORIZONTAL CHAMBERS IN TEN *C. GEORGIANUS*.

Fish length mm	left side mm	right side mm
470	130	75
480	45	55
490	85	110
490	190	40
500	200	75
500	42	40
520	160	80
520	120	130
540	160	78
550	110	80

Figure 4. A RESIN CAST FROM ONE FISH SHOWING THE SWIM BLADDER AND ITS EXTRUSIONS. ANTERIOR OF FISH TO RIGHT.





## DISCUSSION

The anomaly was evidently not the result of any bacterial or parasitic infection. Proliferation of the swim bladder as a result of a swim bladder tumour has been reported in three species of fish.<sup>2,4,5</sup> However, in these cases the tumours were solid masses of tissue quite unlike the gas filled spaces seen here. Finger-like projections are a normal feature of the swim bladder in some other families, particularly sciaenids.<sup>6</sup> However they have not been reported from carangids.

For several reasons, the trevally holes are thought to be abnormal. They are highly irregular in number and form in fish of the same size, and they develop differently on the two sides of individual fish. The fat cells surrounding the cavities suggest that muscles in the area have broken down, as adipose tissue is sometimes the product of muscle damage. Extrusions of the swim bladder have not been noticed in *C. georgianus* in New Zealand waters (B. Webb, personal communication).

The condition superficially resembles 'pneumothorax' of mammals. However, the gas had not been forced out of the swim bladder. Rather it was in the tissues still surrounded by an extension of the bladder wall. This integrity plus the lack of inflammation indicate that the condition had developed gradually, possibly as a result of a series of high pressures in the bladder. Increased pressure such as would result from a rapid change in depth would normally be compensated for by changes in bladder size, unless either a part of the wall was weakened in some way, or the bulk of the wall had lost some of its elasticity. This particular population of *C. georgianus* is prone to another abnormal development, osteomas on various pterygiophore and haemal spines.<sup>3</sup> The metabolic malfunction that results in the deposition of these masses may in some way be linked to the swim bladder extrusions described here.

Since completing this work the senior author has found swim bladder ectasia in an 88 cm snapper *Chrysophrys unicolor* from the Spencer Gulf, South Australia. The extrusions were demonstrated by x-ray photography and confirmed by dissection. Verbal reports indicate that 'holes' are not uncommon in the flesh of snapper in southern Australian waters.

That the same syndrome occurs in two unrelated species of fish raises the possibility that it is triggered by some environmental factor. A low pressure wave, for example, may cause the initial extrusion, which with time then progresses through the tissues as a result of the fish's normal changes in depth. Low pressure waves of suitable wavelengths are produced by some imploding devices used in seismic surveys and by cavitation of the propellers of large tugs and ocean-going vessels. However, if low pressure waves are the cause, one would expect swim bladder ectasia to be a world-wide phenomenon. As far as we can determine it is not, for there are no other reports of it in the literature. Until more information is available, we have to conclude that it is probably caused by a genetic abnormality in the fish populations concerned.

## ACKNOWLEDGEMENTS

We would like to thank Mr. R. Green of the Royal Victoria Museum, Launceston, for permitting dissection of the preserved trevally, Dr. K. Jones, Department of Fisheries, S.A. for the snapper, and Dr. B. Gannon, Flinders University, S.A., for advice on the casts. At the University of Queensland, Dr. W.R. Kelly, Dr. G.H.G. Davis, and Professor G.E.G. Sargent gave invaluable help and Mrs. M. Barrett provided able technical assistance. The project was supported financially by a grant to Dr. H.M.D. Hoyte, Department of Parasitology, University of Queensland, from the Australian Fishing Industry Research Trust Account.

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## TASMANIAN FISHERIES RESEARCH

No. 25, March 1983

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Published by the  
Tasmanian Fisheries Development Authority  
G.P.O. Box 619F  
Hobart, Tasmania

"ISSN" 0049-3015



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### TUMOUR-LIKE GROWTHS FROM SOUTHERN AUSTRALIAN MARINE FISH.

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#### Abstract

Three neoplasms and one growth of uncertain status from Australian marine fish are described. Osteomas were found on the pterygiophores of six *Caranx georgianus* and on the haemal spines of three. Two lipomas were identified, one from the body cavity of *Aldrichetta forsteri*, and one from the musculature of *Thunnus maccoyii*. A mass on the caudal peduncle of *Bigenier brownii* was believed to be the result of chronic inflammation rather than the development of neoplastic tissue.

#### INTRODUCTION

Two tumours have so far been reported from marine fish in Australian waters, a neurolemma and a lipoma. Both were found in *Platycephalus bassensis* during a survey for neoplasms in Port Phillip Bay, Victoria (Hard et al. 1979). In the northern hemisphere fish tumours have been linked to pollution. However, this is not invariably the case, and some fish populations are naturally predisposed to the formation of certain tumours. It is the purpose of this paper to record the existence of three other tumours from Australian marine fish, two of which seem to occur relatively frequently in unpolluted waters.

#### MATERIALS AND METHODS

Fish were caught by commercial fishermen. Trevally were examined fresh or preserved in formalin. The leatherjacket, mullet and tuna were received frozen. Unless otherwise stated all were caught in 1978.

Tissue samples were fixed in 10% neutral buffered formalin and, where necessary, decalcified in formic acid. Paraffin-embedded blocks were sectioned and stained with haematoxylin and eosin, and frozen sections of formalin-fixed lipomas were stained with Oil Red O.

Representative tissues from the four types described below have been deposited in the Registry of Tumours in Lower Animals, Smithsonian Institution, U.S.A. (accession numbers 2457, 2454, 2455 and 2456 respectively).

#### RESULTS

1. Osteoma in silver trevally *Caranx georgianus* (Figs. 1 & 2). Seven fish 34 to 57 cm in length were examined, all from Tasmania. Six were caught near Hobart, and one in the Tamar River near Launceston. All seven were affected. The distribution of the tumours within the fish is outlined in Table 1. They occurred on the proximal pterygiophore (an intervertebral spine) on the dorsal side between



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the second and third vertebrae, and on the haemal spines of the 15, 16, 17 or 18th vertebrae. They were absent from 30 other fish examined, 17 to 26 cm in length (29 from Hobart, 1 from Launceston).

The tumours were ovoid, up to 3.3 cm in largest diameter, smooth, white, bony in texture and more or less bilaterally symmetrical. They showed no tendency to invade surrounding tissue. In addition, in the seven largest fish the supraoccipital bone was expanded to form a smooth elongated bony mass. This was thought to be a normal developmental change similar to that occurring in the skull of the snapper *Chrysophrys auratus*.

Histological examination of two tumours showed that they consisted of a nodule of apparently normal bone enlarging by a process of orderly appositional ossification.

### 2. Caudal mass on leatherjacket *Bigener brownii* (Figs. 3 & 4).

The one affected fish examined, 28 cm long, was caught off Flinders Island, South Australia.

The growth was a sessile, slightly lobulated firm ovoid mass measuring 6 x 5 cm and attached to the left hand side of the caudal peduncle. It was irregularly blackened and ulcerated. The bulk of the tumour appeared to consist of bone. It was not attached to the caudal vertebrae.

Histologically it consisted of irregular trabeculae of mature bone overlain by thick hyperpigmented dermis. The bone was presumably derived from existing dermal bone. Cellular detail was obscured by postmortem degeneration from freezing and thawing.

The growth was evidently a chronic bony proliferation that had been traumatized and secondarily infected.

### 3. Lipoma from yellow eye mullet *Aldrichetta forsteri* (Figs. 5 & 6). One affected fish was examined, 28 cm in length, caught at Gippsland Lakes, Victoria.

This was a pale ovoid mass, 8.5 x 5 cm, firm to touch with a texture of solidified fat. On the surface it was smooth and showed no tendency to infiltrate adjacent tissue. It is not known whether it was free in the body cavity or originally attached as the fish had been opened prior to submission. Pieces of the tumour floated in formalin.

Histologically it was a well differentiated tumour filled with Oil Red O-positive material and containing a fine network of connective tissue.

### 4. Lipoma from bluefin tuna *Thunnus maccoyii* (Figs. 7 & 8). One affected fish examined, estimated length 102 cm, caught off southern Australia.

This tumour was pale, firm, and sharply demarcated from surrounding tissue. It was generally oval in shape, 21 x 16 cm, and had a series of lobules on its left dorsal side where it had grown around the dorsal vertebral spines. One particularly large lobule gave rise to speculation that it had started in the intermuscular



## INFORMATION ONLY

connective tissue on the left side of the fish and grown through the ventral rays into the muscle on the right. Pieces of the tumour floated in formalin.

Histologically, it contained a regular pattern of fibrous tissue, much denser than that in that in the mullet lipoma described above. This was interspersed with vacuoles in paraffin-embedded material. In frozen sections the vacuoles contained strongly Oil Red O-positive material.

### DISCUSSION

Osteomas have been reported from at least 18 different species of fish (Harshbarger, 1974, 1976, 1977; Mawdesley-Thomas, 1975). None have previously been reported from a Carangid. According to fishermen osteomas are common in *C. georgianus* off eastern Tasmania, and in fact the growths are generally believed to be a normal part of the fish. The limits of affected fish in Australian waters are not known though it is possible the osteomas are restricted to a single stock. Around New Zealand where the species is common less than 2% of the fish in the susceptible size range (over 35 cm) carry the tumours (Dr. G. James, Fisheries Research Division, Wellington, New Zealand).

The lesion in the leatherjacket appears to be similar to those reported by Hard et al. (1979) in *Meuschenia freycineti* (= *Navodon multiradiatus*), *Scobinichthys granulatus* and *Diodon* (= *Atopomycterus*) *nicthemerus* from Port Phillip Bay, Victoria. They considered these to be florid inflammatory excrescences and not neoplastic tissue. In *Bigener brownii* the growth covers an area on the caudal peduncle that would normally carry four small spines. The spines are probably used for defence and hence the lesion may be a result of a wound in this area.

Lipomas have been reported from at least 26 species of fish (Harshbarger, 1974, 1976, 1977, 1978; Mawdesley-Thomas, 1975) though only from isolated cases. This is the first record of a lipoma from a member of the Mugilidae. The lipoma from the bluefin tuna was an unusually large specimen of a type that occurs infrequently but regularly in tunas landed in South Australia. From the number found each year at the Safcol canning plant and the total weight of tuna processed there, it is estimated that about 1 in 10,000 fish carry a tumour large enough to be noticed by the processing staff (Mr. T. Griffin, personal communication). A bluefin tuna with a lipoma has been caught in the North Atlantic (Harshbarger, 1977).

The three neoplasms described appear to be benign. Few malignant fish tumours have been reported. Their apparent low incidence in wild populations may be due to the lowered survival chances of such affected fish.

### ACKNOWLEDGEMENTS

We thank Dr. T. Dix, Mr. P. Machin and Miss M. Fox of the Tasmanian Fisheries Development Authority, and Mr. R. Green of the Queen Victorian Museum, Launceston, for assistance in collecting specimens and data from *C. georgianus*. Other tumours were submitted by Mr. T. Castle, Elliston, South Australia, Dr. T. Walker, Melbourne and Dr. D. Bottrill, Adelaide, respectively. Financial support from the Fishing Industry Research Council is gratefully acknowledged.



## INFORMATION ONLY

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Table 1. Number of osteomas in five *Caranx georgianus*

Fish Length (L.C.F.)	Source	Pterygiophore	Haemal spines
57	Hobart	1	3 <sup>1</sup>
47		1	0
47		1	0
45		1	0
45		1	1
44		0	2 <sup>2</sup>
34	Launceston	1	- <sup>3</sup>

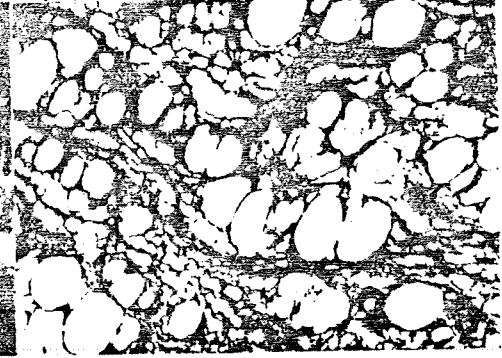
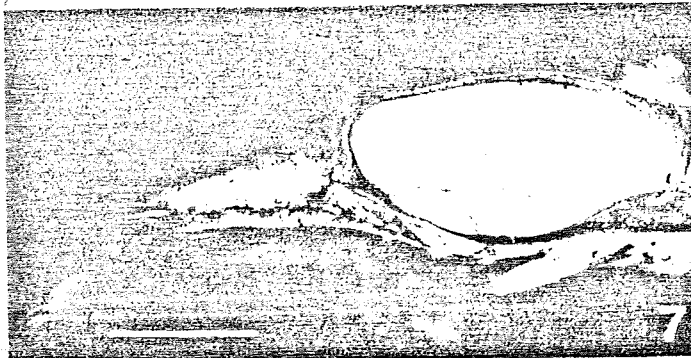
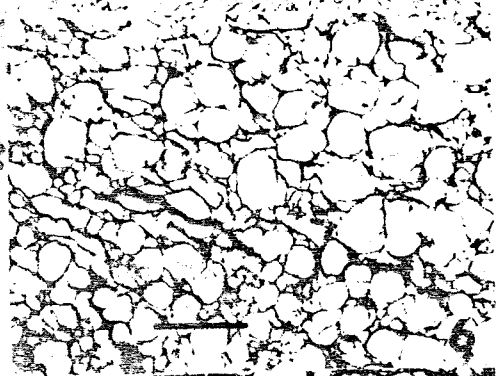
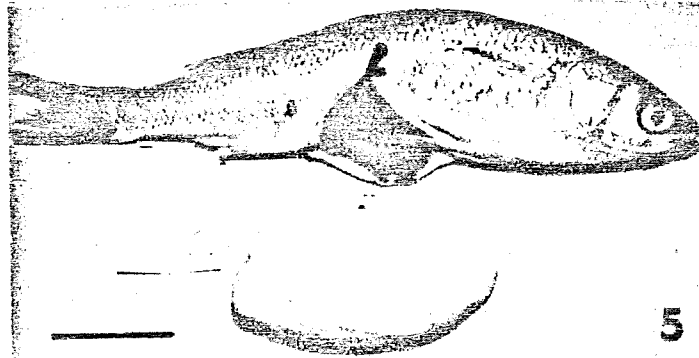
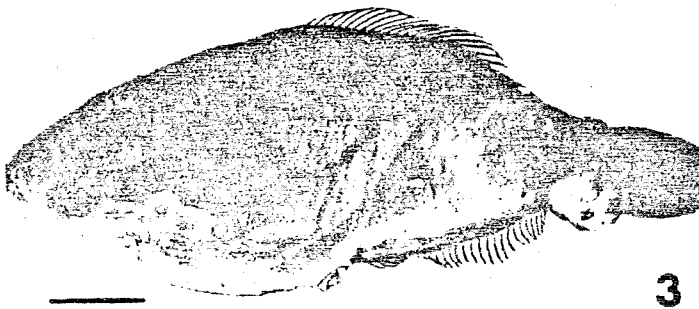
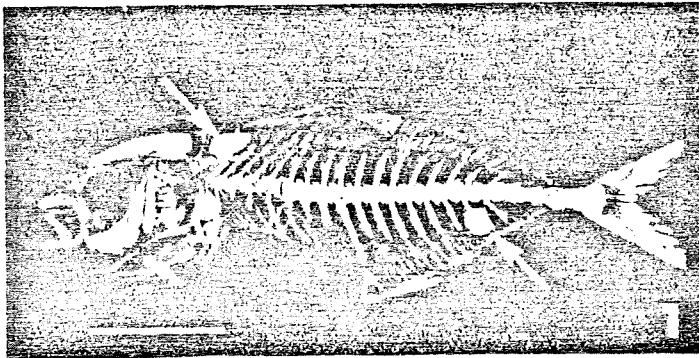
- <sup>1</sup> One on 18th ray much larger than those on 15th and 16th.
- <sup>2</sup> Both about the same size.
- <sup>3</sup> Not examined.



Figure captions

- Fig. 1. Two osteomas (arrows) attached to skeleton of *Caranx georgianus* from Hobart, Tasmania. Bar = 10 cm.
- Fig. 2. T.S. of osteoma (H. and E.). Note the orderly bone growth and the growth check line (arrow). External surface to left. Bar = 500  $\mu$ m.
- Fig. 3. Caudal mass attached to *Bigener brownii* from Elliston, South Australia. Bar = 5 cm.
- Fig. 4. Section of caudal mass (H. and E.). Note the bone and the abundant melanophores in the fibrous tissue (Same mag. at Fig.2).
- Fig. 5. Lipoma removed from body cavity of *Aldrichetta forsteri* from Gippsland Lakes, Victoria. Bar = 5 cm.
- Fig. 6. Section of lipoma (H. and E.). Note the fine network of connective tissue. Bar = 100  $\mu$ m.
- Fig. 7. Cut surface of lipoma in caudal musculature of *Thunnus maccoyii* caught off southern Australia. Bar = 10 cm.
- Fig. 8. Section of tuna lipoma (H. and E.). Note the much denser fibrous network compared to Fig. 6. (Same mag. as Fig. 6).

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## Some Digeneans (Platyhelminthes) Parasitic in the Loggerhead Turtle, *Caretta caretta* (L.), in Australia

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### Abstract

Digeneans parasitic in the loggerhead turtle *Caretta caretta* (L.) are reported for the first time from Australian waters. Six species, one of them new, are described or redescribed, figured, and their taxonomy discussed. They are: *Plesiochorus cymbiformis* (Rudolphi, 1819) Looss, 1901 (fam. Gorgoderidae) from the urinary bladder and cloaca; *Pachypsolus irroratus* (Rudolphi, 1819) Looss, 1902 (synonyms *P. lunatus* Looss, 1901; *P. ovalis* Linton, 1910; *P. tertius* Pratt, 1914; *P. brachus* Barker, 1922; *P. puertoricensis* Fischthal & Acholonu, 1976) (fam. Pachypsolidae) from the intestine; *Enodiotrema carettae*, sp. nov. (fam. Plagiorchiidae) from the liver; *Orchidasma amphiorchis* (Braun, 1899) Looss, 1900 (synonyms *O. indica* Simha, Rao & Chattopadhyaya, 1971; *O. vitelloconfluens* Rao, 1973) (fam. Telorchidae) from the intestine; *Cymatocarpus solearis* (Braun, 1899) Braun, 1901 (synonym *C. undulatus* (Looss, 1899) Looss, 1902) (fam. Brachycoeliidae) from the intestine; *Rhytidodes gelatinosus* (Rudolphi, 1819) Looss, 1901 (synonyms *R. secundus* Pratt, 1914; *R. indicus* Simha & Chattopadhyaya, 1969) (fam. Rhytidodidae) from the intestine.

### Introduction

Up to the present day there have been few records of digeneans parasitic in Australian sea turtles. T. H. Johnston (1912), S. J. Johnston (1913) and Prudhoe (1944) have reported seven species in the green turtle, *Chelonia mydas*, and the hawksbill, *Eretmochelys imbricata*. There are no previous records of flukes from the loggerhead turtle, *Caretta caretta*, in Australia. The present authors have had the opportunity to collect parasite material from Australian turtles, and this paper has been written to record some of the findings.

### Materials and Methods

Material was collected as and when the opportunity arose. Whenever live flukes were obtained, they were generally killed with hot (70°C) water, laid flat on moist filter paper and fixed in this position by the addition of a few drops of 10% formalin, or by a second piece of filter paper, soaked in formalin and placed on top of them. After initial fixation, flukes were stored in 10% formalin.

Whole mounts were stained with Gower's carmine and mounted in balsam. Serial sections, both transverse and sagittal, were cut at 8 µm and stained with haematoxylin and eosin.

Unless otherwise stated, the measurements given, either in tabular form or in the text of a description, were taken from ovigerous adult specimens seen in ventral view. They are expressed in millimetres as the range followed by (in parentheses) the mean and the number of observations upon which it was based.

Museums and other institutions from which specimens were borrowed, or in which specimens have been deposited, are indicated in the text by abbreviations. These are as follows:



BMNH	British Museum (Natural History), London
CIH	Commonwealth Institute of Health, University of Sydney
MNHN	Museum National d'Histoire Naturelle, Paris
NHR	Naturhistoriska Riksmuseet, Stockholm
QM	Queensland Museum, Brisbane
SAM	South Australian Museum, Adelaide
UNAM	Laboratorio de Helmintologia, Instituto de Biologia, Universidad Nacional Autonoma, Mexico
USNMHC	United States National Museum Helminthological Collection, Beltsville, Maryland
ZMB	Zoologisches Museum, Berlin

A full synonymy is not given for each species. Only descriptions and redescrptions and other major references are listed. A fuller synonymy for each species may be traced through the Index-Catalogue of Medical and Veterinary Zoology published by the United States Department of Agriculture. The family placing of each species follows Yamaguti (1971) and the excellent publication by Ernst and Ernst (1977).

### Family GORGODERIDAE

#### *Plesiochorus cymbiformis* (Rudolphi)

- Distoma cymbiforme* Rudolphi, 1819, pp. 96, 371 (urinary bladder, *Testudo mydas* = *Chelonia mydas*, Rimini, Adriatic Sea).
- Distomum cymbiforme* Rudolphi. Sonsino, 1893, pp. 183-5 (urinary bladder, *Chelonia caretta* = *Caretta caretta*, Pisa, Mediterranean Sea); Stossich, 1895, p. 38, pl. 4 fig. 1 (urinary bladder, *Thalassochelys caretta* = *Caretta caretta*, Trieste, Adriatic Sea); Stossich, 1897, p. 9 (*Thalassochelys corticata* = *Caretta caretta*, Corfu, Ionian Sea); Braun, 1899a, pp. 720-1.
- Spathidium cymbiforme* (Rudolphi). Looss, 1899, p. 605.
- Phyllodistomum cymbiforme* (Rudolphi). Braun, 1901, pp. 10-13, fig. 1.
- Plesiochorus cymbiformis* (Rudolphi). Looss, 1901a, pp. 205, 207, 209; Looss, 1901b, pp. 555-8 (*Thalassochelys corticata* = *Caretta caretta*, and *Chelonia mydas*, Egypt); Looss, 1902, pp. 469-85, figs 30-36 (urinary bladder, *Thalassochelys corticata* = *Caretta caretta*, and *Chelonia mydas*, Egypt); Pratt, 1914, pp. 420-3, figs 3, 13 (urinary bladder, rectum, *Caretta caretta*, Florida); Cary, 1930, pp. 325-6 (urinary bladder, *Caretta caretta*, Tortugas); Oguro, 1942, p. 164 (*Caretta olivacea* = *Lepidochelys olivacea*, Kurio, Yakusima, Japan); Caballero y C., 1954, pp. 32-4, figs 1, 2 (urinary bladder, *Chelonia mydas*, Gulf of Panama, Pacific Ocean); Chattopadhyaya, 1970, pp. 71-3, figs 5, 6 (liver of *Eretmochelys imbricata* and urinary bladder of *Chelonia mydas*, Gulf of Mannar, southern India); Fischthal and Acholonu, 1976, pp. 183-4 (small intestine, *Eretmochelys imbricata*, Puerto Rico, Caribbean Sea); Sey, 1977, p. 388 (*Caretta caretta*, Mediterranean coast of Egypt).
- Plesiochorus cymbiformis elongatus* Pigulevsky, 1953, p. 567, figs 151, 151a (urinary bladder, *Carettochelys insculpta* (*Thalassochelys corticata*), New Guinea).

**Hosts.** *Caretta caretta*, *Chelonia mydas*, *Lepidochelys olivacea*, *Eretmochelys imbricata*.

**Site in host.** Urinary bladder, rectum and cloaca, small intestine, liver (see *Discussion*).

**Geographical distribution.** Mediterranean Sea, Atlantic coast of Morocco, Gulf of Panama, Florida, Puerto Rico, India, Japan, Australia, ?New Guinea (see *Discussion*).

#### *Specimens Examined*

As whole mounts: 7, on two slides labelled '*Thalassochelys corticata* (Rondelet). Rabat 24.6.1925. R.Ph. Dollfus'. MNHN, Nos L54<sub>1</sub> L54<sub>2</sub>; 2, on one slide labelled '*Thalassochelys corticata* (Rondelet) dans le rectum mais venait evidement de la vessie. Rabat 25.6.1925. R.Ph. Dollfus Leg et determin.'. MNHN, No. L55<sub>1</sub>; 8, from urinary bladder, *C. caretta*, Shark Bay, W.A., 4.vii.1979, Blair and Lester; 4, from urinary bladder, *C. caretta*, Mon Repos, Bundaberg, Qld, summer 1975-76, Limpus.

In alcohol: 3  
Heron I., Qld, 1  
Bundaberg, Qld,  
W.A., 4.vii.1979  
As sections:  
Blair and Lester

*Specimens Depo*  
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In alcohol: 381 from urinary bladder and 12 from cloaca, subadult *C. caretta* in poor condition, Heron I., Qld. 15.v.1975. Limpus; 19, from urinary bladder, *C. caretta* (nesting female), Mon Repos, Bundaberg, Qld. 17.i.1979. Limpus; 62, from urinary bladder, *C. caretta* (2 host specimens), Shark Bay, W.A., 4.vii.1979. Blair and Lester; 2, same data. 5.vii.1979; 4, same data. 11.vii.1979.

As sections: 2, as transverse serial sections, urinary bladder, *C. caretta*, Shark Bay, W.A., 4.vii.1979. Blair and Lester.

#### Specimens Deposited

3, from urinary bladder, *C. caretta*, Shark Bay, W.A., 4.vii.1979, SAM Nos V3050-2; 2, same site and host, QM Nos GL 691-2.

#### Description

Body pyriform to spatulate (Fig. 1),  $2.97-7.21$  ( $5.47$ ,  $n = 12$ )  $\times$   $1.31-3.7$  ( $2.2$ ,  $n = 12$ ), often widening considerably posterior to ventral sucker and bluntly rounded at posterior end, strongly flattened in dorsoventral plane and often concave ventrally and convex dorsally. Tegument smooth, aspinous. Oral sucker sub-terminal, ventral,  $0.41-0.86$  ( $0.62$ ,  $n = 11$ )  $\times$   $0.42-0.85$  ( $0.64$ ,  $n = 12$ ), leading directly into a rounded pharynx which  $0.15-0.30$  ( $0.21$ ,  $n = 11$ )  $\times$   $0.18-0.37$  ( $0.25$ ,  $n = 11$ ); oesophagus very short; gut caeca thin-walled, following slightly sinuous course parallel to lateral margins of body and terminating close to posterior end. Gland cells, *c.* 12 on each side, lie just posterior to oral sucker. Their ducts pass anterad, but external openings were not seen. Ventral sucker larger than oral,  $0.5-1.26$  ( $0.95$ ,  $n = 12$ )  $\times$   $0.62-1.37$  ( $0.95$ ,  $n = 12$ ), in 2nd quarter of body in mature specimens.

Testes a pair,  $0.48-1.52$  ( $0.9$ ,  $n = 12$ )  $\times$   $0.43-1.15$  ( $0.7$ ,  $n = 12$ ), opposite or nearly so, in 3rd quarter of body, largely intercaecal and ventral to caeca, deeply dissected. A sperm duct arises from anterior region of each testis, passes anterad close to caecum then mediad close to anterior end of ventral sucker. Male terminal genitalia consist of convoluted, thin-walled seminal vesicle arising from junction of sperm ducts and lying around anterior edge of ventral sucker, then a thin-walled muscular prostatic chamber (Fig. 2) opening via a short muscular duct to genital pore in ventral midline just anterior to ventral sucker. Cirrus sac absent.

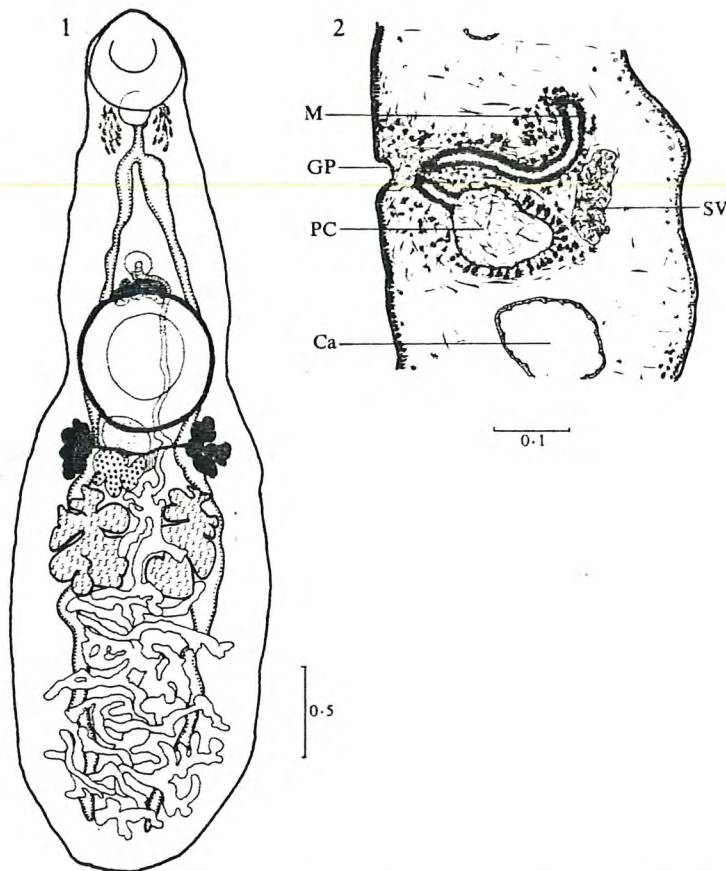
Ovary small,  $0.23-0.37$  ( $0.29$ ,  $n = 10$ )  $\times$   $0.21-0.37$  ( $0.29$ ,  $n = 10$ ), compactly lobed, submedian, to either side of midline, intercaecal, just posterior to ventral sucker. Seminal receptacle rounded, anterodorsal to ovary which it resembles in size. Mehlis' gland small, median to ovary and seminal receptacle, close to midline. Vitelline follicles a tight bunch on each side,  $0.19-0.42$  ( $0.32$ ,  $n = 12$ )  $\times$   $0.19-0.5$  ( $0.28$ ,  $n = 12$ ), extracaecal or ventral to caeca, just anterior to level of ovary. Oviduct leaves dorsal region of ovary, and passes mediad between ovary and seminal receptacle, receiving duct from latter before entering Mehlis' gland where it receives common vitelline duct from anteroventral side. Laurer's canal and vitelline reservoir absent. Uterus sinuous, initially passing posterad between testes, then looped in body posterior to testes, overreaching caeca laterally and sometimes posteriorly. Ascending limb of uterus lies along dorsal side of ventral sucker, its terminal portion a short muscular metraterm, surrounded by possibly glandular cells, lying just posterior or lateral to prostatic chamber (Fig. 2) and opening into genital pore. Eggs dissected from uterus measured  $0.030-0.047$  ( $0.038$ ,  $n = 20$ )  $\times$   $0.022-0.041$  ( $0.031$ ,  $n = 20$ ).

Excretory opening in dorsal midline a short distance from posterior end. Single, inconspicuous mid-dorsal excretory duct, apparently receiving no tributary ducts, could be traced forwards to between anterior edges of testes.



### Discussion

The published descriptions of *Plesiochorus cymbiformis* (see synonymy) are in general agreement with one another and with the observations of the present authors. Where discrepancies between descriptions occur, they often appear to represent no more than differing interpretations of similar structures, or have arisen because worms examined were processed in different ways. A few points are worth further discussion.



**Figs 1, 2.** *Plesiochorus cymbiformis*: 1, whole mount; 2, transverse section through body close to genital pore (GP). Ca, gut caecum; M, metraterm; PC, prostatic chamber; SV, seminal vesicle.

Variations in shape and size were reported by most authors and discussed at some length. The largest specimen recorded was 12 mm (Looss 1902) and the smallest 2.5 mm (Caballero y C. 1954). Shapes varied from lanceolate to spoon-shaped. Such variations in shape should not come as a surprise, in view of the different treatments to which the worms were, apparently, subjected. The present authors have specimens tending towards either extreme. However, Pigulevsky (1953) has, apparently, used shape differences as a means of distinguishing subspecies within *P. cymbiformis*. He summarized, without comment or discussion, the description by Looss (1902) under the name *P. cymbiformis elongatus*. As an example of 'typical' *P. cymbiformis*, from which he excluded Looss' specimens, Pigulevsky figured one of the spoon-shaped

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specimens described by Stossich (1895). Pigulevsky listed the host of *P. cymbiformis elongatus* as (in translation) 'Turtle, *Carettochelys insculpta* (*Thalassochelys corticata*) from Family Carettochelyidae', and the locality as 'New Guinea Island'. His description is wholly based on that by Looss (1902), and the only literature reference given is 'Looss (1902) p. 469'. On this page, and those following, Looss lists the hosts of *P. cymbiformis* as *Thalassochelys corticata* and *Chelonia mydas*, but gives no locality. Elsewhere in Looss' writings, it is made clear that he collected material from the Mediterranean coast of Egypt (Looss 1901b). The current name of *Thalassochelys corticata* is *Caretta caretta* (family Cheloniidae), and not *Carettochelys insculpta* (family Carettochelyidae) as Pigulevsky implies. The latter species is a freshwater chelonian known only from New Guinea and northern Australia. It seems likely that Pigulevsky made a mistake in checking the current name for *Thalassochelys corticata*, and then assumed that the specimens must have come from New Guinea.

Chattopadhyaya (1970) has discussed variation within *P. cymbiformis*. She reported that the testes overlapped the caeca and that the vitellaria were extracaecal in some specimens, whereas in others these organs were intercaecal. Other workers generally report the testes to be ventral to the caeca and the vitelline glands to be extracaecal or ventral to the caeca. However, Caballero y C. (1954) described specimens in which the vitellaria were entirely intercaecal. The same author reported a Laurer's canal in his specimens. The male and female ducts are generally described as opening together into the genital pore. However, Chattopadhyaya (1970) implied that in her specimens the pores were distinctly separate. The seminal receptacle was described by Looss (1902) as sometimes approaching the ventral sucker in size. No other author, with the possible exception of Rudolphi (1819), has reported such a degree of development of this organ.

*Plesiochorus cymbiformis* has been reported most frequently from the urinary bladder, or from the cloaca or rectum, sites close to the opening of the bladder. However, a few specimens have been recorded from sites far from this region. Chattopadhyaya (1970) found a single specimen in the liver of *Eretmochelys imbricata*, and Fischthal and Acholonu (1976) found two specimens in the small intestine of the same host species.

There is no previous record of this species from Australian waters.

#### Family PACHYPSOLIDAE

##### *Pachypsolus irroratus* (Rudolphi)

- Distoma irroratum* Rudolphi, 1819, pp. 105, 393-4 (stomach, *Testudo midas* = *Chelonia mydas*, Rimini, Adriatic Sea).
- Distomum irroratum* Rudolphi. Braun, 1899a, pp. 717-18 (*Thalassochelys corticata* = *Caretta caretta*, Trieste, Adriatic Sea; unnamed turtle, Ralum, New Guinea); Braun, 1901, pp. 36-8, figs 27, 30, 32 (*Chelone mydas*, Red Sea; intestine, *Thalassochelys caretta* = *Caretta caretta*, Trieste, Adriatic Sea and Ralum, New Guinea).
- Pachypsolus lunatus* Looss, 1901b, p. 558 (stomach, *Thalassochelys corticata* = *Caretta caretta*, Trieste, Adriatic Sea).
- Pachypsolus irroratus* (Rudolphi). Looss, 1902, pp. 485-505, figs 37, 38, 169 (stomach, *Thalassochelys corticata* = *Caretta caretta*, Trieste, Adriatic Sea); Dollfus, 1937, pp. 507-14 (intestine, *Chelonia mydas*, coast of Mauritania, and *Thalassochelys corticata* = *Caretta caretta*, Rabat, Morocco); Euzet *et al.* 1972, pp. 158-65, figs 1, 2 (stomach, *Caretta caretta*, Banyuls-sur-Mer, Mediterranean coast of France).
- Pachypsolus ovalis* Linton, 1910, pp. 24-6, figs 7-14 (intestine, *Caretta caretta*, Tortugas, Florida); Caballero y C. *et al.*, 1955, pp. 153-7, figs 3, 4 (stomach, *Chelonia mydas*, Gulf of Panama);

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- Fischthal and Acholonu, 1976, p. 184. (stomach, *Eretmochelys imbricata*, Puerto Rico, Caribbean Sea).
- Pachypsolus tertius* Pratt, 1914, pp. 416–20, pl. 4 fig. 2, pl. 5 figs 8–11 (small intestine, *Caretta caretta*, off Florida coast, Gulf of Mexico).
- Pachypsolus brachus* Barker, 1922, pp. 215–22, figs 1–8, 12 (stomach, *Chelonia imbricata* = *Eretmochelys imbricata*, Bermuda); Caballero-Rodriguez, 1960, pp. 57–61, figs 18–20 (small intestine, *Chelone mydas*, Acapulco, Mexico).
- Pachypsolus puertoricensis* Fischthal and Acholonu, 1976, p. 181, figs 11, 12 (stomach, *Eretmochelys imbricata*, Puerto Rico, Caribbean Sea).

*Hosts.* *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*.

*Site in host.* Stomach, intestine.

*Geographical distribution.* Mediterranean Sea, Red Sea, Atlantic coast of north-west Africa, Florida, Puerto Rico, Pacific coasts of Panama and Mexico, New Guinea, Australia.

#### *Specimens Examined*

As whole mounts: 7, from stomach, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester; 2, from stomach wall, subadult *C. caretta* in poor condition, Heron I., Qld, 15.v.1975, Limpus; 7, from stomach, *Thalassochelys* (= *Caretta*), Trieste, 1908, NHR No. 263:3, from stomach, *Thalassochelys corticata* (= *C. caretta*), Trieste, Sept. 1901, Cori, NHR No. 578, Looss Coll. No. 1323 (see Looss 1902); 8, from intestines, *C. caretta*, Tortugas, Florida, 29.vi.1907, Linton, USNMHC No. 8443 (type and paratypes of *P. ovalis*, see Linton 1910); 1, from stomach, *Chelonia mydas*, Isla de Chepilla, Panama, UNAM Nos 212–25 (labelled *P. ovalis*, see Caballero y C. et al. 1955); 2, from stomach, *Eretmochelys imbricata*, Cabo Rojo, Puerto Rico, 13.v.1970, USNMHC No. 73341 (labelled *P. ovalis*, see Fischthal and Acholonu 1976); 1, same data, 25.v.1970, USNMHC No. 73320 (holotype of *P. puertoricensis*, see Fischthal and Acholonu 1976); 1, from small intestine, *Chelonia mydas*, Acapulco, Mexico, 18.i.1954, UNAM Nos 217–11 (labelled *P. brachus*, see Caballero-Rodriguez 1960); 2, from intestine, *Thalassochelys corticata* (= *C. caretta*), Rabat, Morocco, 24.vi.1925, Dollfus, MNHN, 2 slides Nos L59<sub>1</sub>, L59<sub>2</sub> (see Dollfus 1937); 1, from 'tube digestif', *C. caretta*, Sète, Hérault, France, 15.x.1951, MNHN, No. L60<sub>1</sub>; 7, from intestine, *Chelone mydas*, Mauritania (1st cruise of SS 'Boula-Matari'), 7.iv.1923, Monod, MNHN, nos L61<sub>1</sub>, L61<sub>2</sub>, L62 (last slide also holds 1 *Orchidasma amphiorchis*) (see Dollfus 1937).

As spirit specimens: 5, from stomach wall, subadult *C. caretta* in poor condition, Heron I., Qld, 15.v.1975, Limpus; 4, from stomach, *Chelonia mydas*, Rimini, Italy, Rudolphi, ZMB No. 1508 (syntypes of *P. irroratus*); 22, from *Thalassochelys corticata* (= *C. caretta*), Trieste and Berlin Aquarium, ZMB No. 2694 (see Braun 1899a); 1, from stomach, *Thalassochelys corticata* (= *C. caretta*), Trieste, USNMHC No. 50428, Cori (received from Looss, 1908; see Looss 1902); 6, from stomach, same data, Sept. 1901, NHR No. 578, Looss Coll. No. 1323 (see Looss 1902); 2, from stomach, *Thalassochelys* (= *Caretta*), Trieste, 1908?, NHR No. 263.

As sectioned material: 1, as transverse sections, stomach, *C. caretta*, Shark Bay, W.A., 4.vii.1979, Blair and Lester; 1, as sagittal sections, stomach wall, *C. caretta* (X 2083), Mon Repos, Qld, summer 1975–76, Limpus; 1, as transverse sections (2 slides from Looss Coll., Nos 1988<sup>a</sup>, 1988<sup>b</sup> in NHR); 2, as horizontal longitudinal sections, from intestines, *C. caretta*, Tortugas, Florida, 29.vi.1907, Linton, USNMHC No. 8443 (paratypes of *P. ovalis*; one slide includes portions of other specimens sectioned in different planes; see Linton 1910).

#### *Specimens Deposited*

2, from stomach, *C. caretta*, Shark Bay, W.A., 5.vii.1979, SAM Nos V3053–4; 2, same site and host, QM Nos GL693–4.

#### *Description* (Figs 3–17; Table 1)

Body elongate, subcylindrical, rounded at anterior end, broadest at or anterior to ventral sucker and tapering very gradually towards posterior end which is truncate or rounded (Fig. 15). Tegument without spines. Oral sucker large; oral opening subterminal, ventral; pharynx immediately posterodorsal to oral sucker; oesophagus very short or absent; caeca pass laterally initially with 2–3 short, anteriorly directed

Date  
5.vii.1979  
1908  
1901

a Type specimens of *P. ovalis*.  
b Labelled as *P. ovalis* by Fischthal and Acholonu (1976).



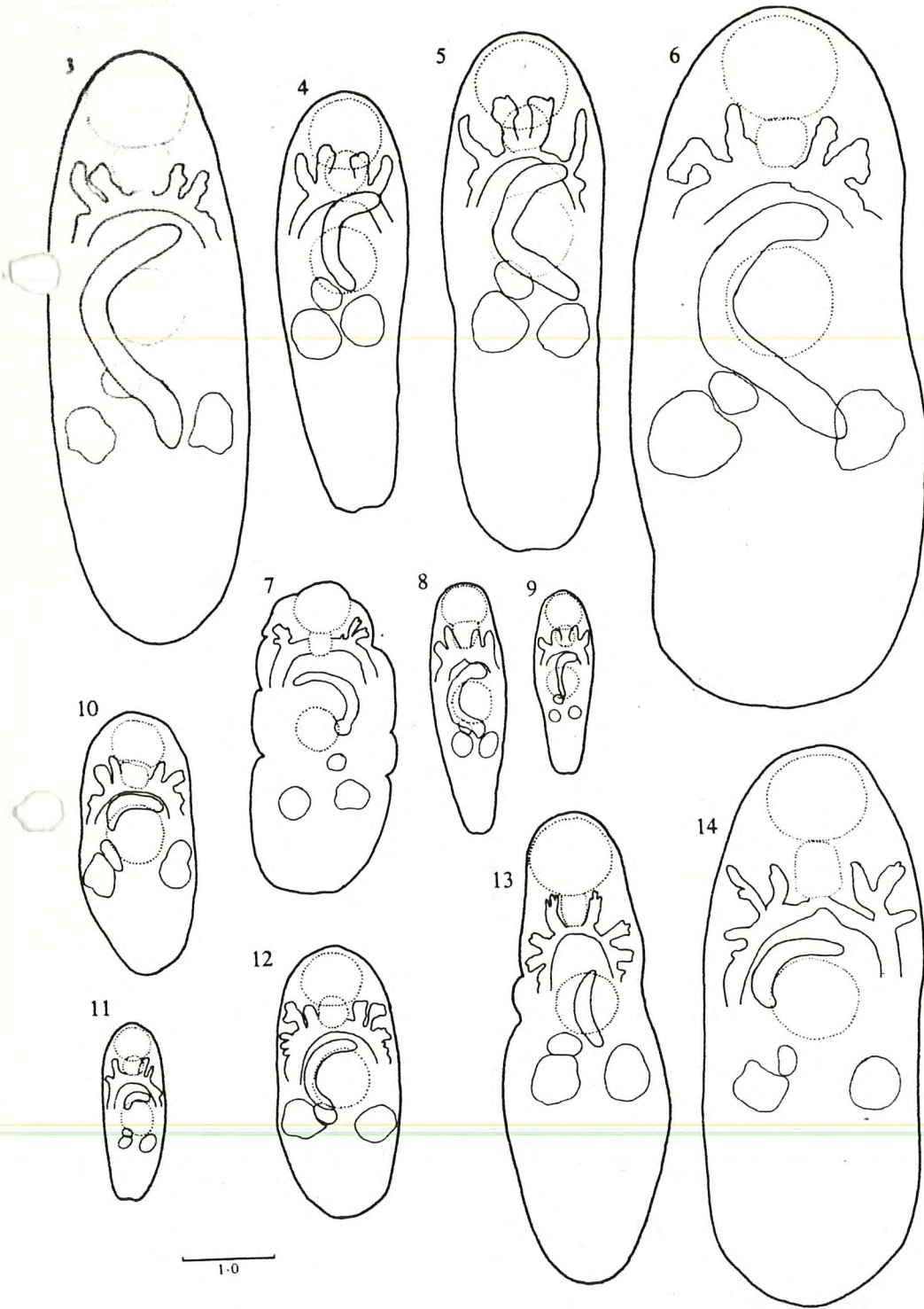
Table 1. Dimensions of *Pachypsolus irroratus* from hosts of three species  
All measurements in millimetres. Numbers of specimens measured are in parentheses

	Authors' coll.	NHR No. 263	NHR No. 578	MNHN Nos L61 <sub>1</sub> , L61 <sub>2</sub> , L62	USNMHC No. 8443 <sup>A</sup>	USNMHC No. 73341 <sup>B</sup>
Body, mean length, width	4.15, 1.26	3.27, 1.08	4.57, 1.55	4.29, 1.93	2.80, 1.14	—
Length, range	2.67-5.59(5)	2.57-4.54(6)	3.74-5.36(3)	3.23-6.97(4)	2.04-3.46(6)	3.37, 3.93(2)
Width, range	0.80-1.73(5)	0.87-1.52(6)	1.50-1.59(3)	1.40-3.30(4)	0.75-1.33(6)	1.10, 1.22(2)
Oral sucker, mean length, width	0.68, 0.73	0.52, 0.54	0.77, 0.75	0.74, 0.85	0.48, 0.53	—
Length, range	0.39-0.96(5)	0.42-0.66(6)	0.71-0.83(3)	0.59-1.05(4)	0.34-0.58(6)	0.40, 0.48(2)
Width, range	0.47-1.00(5)	0.42-0.74(6)	0.66-0.84(3)	0.65-1.39(4)	0.40-0.68(6)	0.40, 0.51(2)
Pharynx, mean length, width	0.38, 0.40	0.29, 0.34	0.44 ? (1)	0.45, 0.46	0.23, 0.27	—
Length, range	0.22-0.52(5)	0.28-0.30(5)	—	0.33-0.69(4)	0.19-0.26(6)	0.25, 0.26(2)
Width, range	0.28-0.48(5)	0.30-0.40(5)	—	0.30-0.72(4)	0.18-0.33(6)	0.25(1)
Ventral sucker	0.66, 0.65	0.50, 0.49	0.69, 0.70	0.77, 0.87	0.52, 0.52	—
Length, range	0.45-0.90(5)	0.41-0.65(6)	0.62-0.74(3)	0.61-1.19(4)	0.40-0.64(6)	0.36, 0.39(2)
Width, range	0.43-0.90(5)	0.41-0.64(6)	0.66-0.72(3)	0.67-1.40(4)	0.41-0.64(6)	0.35, 0.39(2)
Ovary, mean length, width	0.30, 0.28	0.23, 0.24	0.32, 0.29(1)	—	0.21, 0.22	—
Length, range	0.17-0.42(5)	0.16-0.31(5)	—	0.35, 0.37(2)	0.18-0.23(5)	0.17(1)
Width, range	0.15-0.42(5)	0.16-0.32(5)	—	0.30, 0.34(2)	0.16-0.28(5)	0.19, 0.21(2)
Testis, mean length, width	0.47, 0.39	0.36, 0.36	—	0.47, 0.43	0.39, 0.40	—
Length, range	0.25-0.68(5)	0.30-0.52(6)	0.51, 0.59(2)	0.43-0.50(3)	0.31-0.51(6)	0.38, 0.44(2)
Width, range	0.21-0.59(5)	0.24-0.47(6)	0.50, 0.51(2)	0.41-0.46(3)	0.25-0.45(6)	0.31, 0.49(2)
Egg length <i>in utero</i>	0.049-0.054	0.042-0.047	0.044-0.047	0.045-0.049	0.047-0.054	0.056-0.059
Host	<i>C. caretta</i>	<i>C. caretta</i>	<i>C. caretta</i>	<i>Chelonia mydas</i>	<i>C. caretta</i>	<i>Eretmochelys imbricata</i>
Locality	Shark Bay, W.A.	Trieste	Trieste	Mauritania	Florida	Puerto Rico
Date	5.vii.1979	1908	1901	—	—	1976

<sup>A</sup>Type specimens of *P. ovalis*.

<sup>B</sup>Labelled as *P. ovalis* by Fischthal and Acholonu (1976).





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*Discussion*

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diverticula on each side, then pass posterad, are simple, close to lateral margins of body and terminate close to posterior end of body. Ventral sucker smaller than or equal to oral, in anterior half of body.

Testes a pair, spherical or almost so, opposite or almost so, in middle third of body and posterior to ventral sucker. Sperm duct leaves dorsal face of each testis and passes to base of cirrus sac where each opens separately into internal seminal vesicle. Cirrus sac of variable size and shape, generally banana-shaped with its concave side directed towards the left, sometimes lying dorsal to ventral sucker and sometimes around side of ventral sucker, its posterior end being dorsal to ventral sucker, or extending as far posteriorly as posterior edges of testes on their dorsal side. Genital pore to left of midline, generally closer to ventral than to oral sucker. Male terminal genitalia consisting of coiled internal seminal vesicle within basal quarter of cirrus sac, then straight or slightly convoluted ejaculatory duct surrounded by prostatic cells and passing to genital opening. Terminal portion of ejaculatory duct acting as cirrus but lacking morphological specialization, may be protruded from genital pore or may extend a short distance into metraterm (Fig. 17). Eggs often occur throughout ejaculatory duct, sometimes more numerous than in metraterm.

Ovary spherical to ovoid, to right of midline, immediately anterodorsal to right testis and often partly dorsal to ventral sucker. Mehlis' gland posteromedial to ovary. Oviduct leaves left side of ovary and receives Laurer's canal and vitelline duct before entering ootype. Uterus leaves ventral side of Mehlis' gland, passes posterad and is extensively looped throughout entire body posterior to testes and overlapping caeca, ascending limb narrows dorsal to ventral sucker and becomes thick-walled metraterm. Laurer's canal opens in dorsal midline just posterior to ovarian mass, is looped in posterior region of Mehlis' gland then gives off small seminal receptacle before entering oviduct. Vitellaria lateral, extending towards dorsal midline in some specimens, in a field on each side starting between pharynx and ventral sucker and ending a short distance behind testes, arranged in a series of rosettes, probably 12-17 in number on each side and most clearly recognizable in large specimens (Fig. 16). Vitelline ducts, one from each rosette, unite into 2-3 main ducts which run towards the Mehlis' gland and finally unite into a single dorsal duct on each side. Vitelline reservoir ventral in Mehlis' gland, at junction of duct from each side.

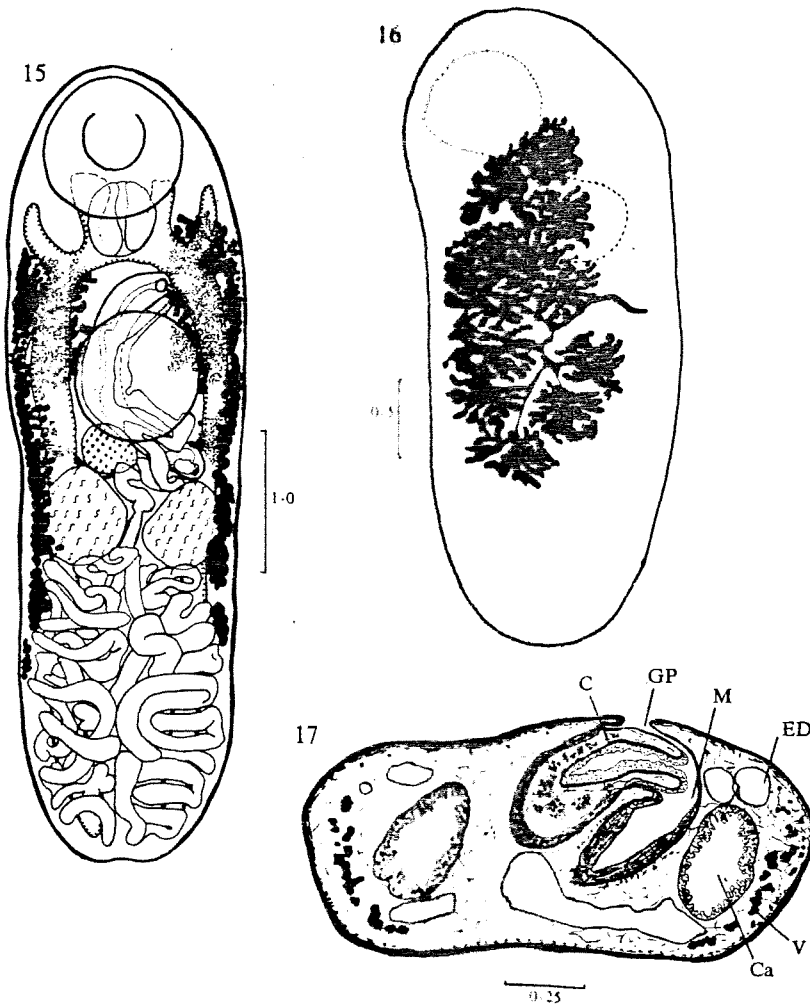
Principal excretory ducts a pair, united dorsal to oral sucker and forming single excretory stem at level of ovary. Anterior to ovary, paired ducts are united by several anastomoses and give off several diverticula, probably variable in number and location. Excretory stem with several short lateral diverticula. Excretory opening at posterior end.

#### Discussion

Seven specific names have been applied in this genus. These are: *P. irroratus* (Rudolphi, 1819) Looss, 1901; *P. lunatus* Looss, 1901; *P. ovalis* Linton, 1910; *P.*

**Figs 3-14.** *Pachypsolus irroratus*, outlines of specimens showing positions of suckers, testes, ovary, cirrus sac and anterior gut diverticula: 3, after Looss (1902); 4, 5, 8, 9, original, Australian material; 6, original, from slide L59<sub>2</sub>, MNHN; 7, after Braun (1901); 10-12, original, USNMHC No. 8443 (types of *P. ovalis*); 13, after Pratt (1914; *P. tertius*); 14, after Caballero-Rodriguez (1960; *P. brachus*). All to same scale, ovigerous specimens (except 9 and 11) in ventral view (possibly except 7), and from *C. caretta* (except 14, from *Chelonia mydas*).

*terius* Pratt, 1914; *P. brachus* Barker, 1922; *P. sclerops* Travassos, 1922; *P. puertoricensis* Fischthal & Acholonu, 1976. *Pachypsolus sclerops*, from crocodilians in South America, appears to stand distinct from the other members of the genus (Euzet *et al.* 1972). The remaining described forms closely resemble one another and



**Figs 15-17.** *Pachypsolus irroratus*: 15, whole mount; 16, dorsolateral view of a contracted specimen, to show distribution of vitellaria on one side; 17, transverse section through genital pore (GP) of specimen with cirrus (C) inserted into metraterm (M). Ca, gut caecum; Ed, excretory duct; V, vitellaria.

occur in the stomach and small intestine of marine turtles; the present authors consider them to represent a single species.

To set the scene for this discussion, it is appropriate to consider the history and variability of the type-species, *P. irroratus*. The first description of *Distoma irroratum* (= *P. irroratus*) was by Rudolphi (1819), who had 13 specimens from a sea turtle, identified as *Testudo midas*, from Rimini on the Adriatic Sea. Further original studies were not made until the end of the 19th century, when both Braun (1899a, 1901) and Looss (1899, 1901b, 1902) redescribed the species. The variability of form

exhibited by *P. i.* two authors. Braun (1899a) at Ralum and Dahl at Ralun at the posterior end of the body and supplemented the description by Braun at the Berlin Museum. The vitellaria were considered the variable by Braun to be described by Looss (1901) redescribed and answered the question. The vitellaria were not variations between

Looss (1901b) was dealing with specimens labelled as these three worms species, *Pachypsolus irroratum*. Looss stated that he was reluctant to further material for *P. irroratus*. He stated upon worms which changes in structure consider juvenile. In his discussion, Looss stated Braun (1899a) may not extend posteriorly in this view.

From the debate within *P. irroratum* in the number of suckers there were two suckers as a small lateral sucker variability in such equal size, where the ventral sucker than the ventral sucker observed: during the suckers tends to be larger sucker lies relatively the stellate or de-

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exhibited by *P. irroratus* led to an exchange of views in the literature between these two authors. Braun (1899a) gave a brief description of a single worm collected by Dahl at Ralum in New Guinea. In this worm, the vitellaria extended only to the posterior end of the testes. Braun considered his specimen to belong to *D. irroratum* and supplemented his description of it with a description of specimens from the Berlin Museum, including Rudolphi's types. In the latter specimens, he stated that the vitellaria sometimes extend to the posterior end of the body. Looss (1899) considered the variations in distribution of the vitellaria in the specimens described by Braun to be so great that two different species must have been involved. Braun (1901) redescribed *D. irroratum* from a slightly greater range of material. He answered the comment by Looss by stating that the differences in the distribution of vitellaria were not of specific value, as he had specimens exhibiting a range of variations between the extremes described in 1899.

Looss (1901b) accepted this statement, but marshalled further evidence that Braun was dealing with two different species. By this time, Looss had received three specimens labelled *Dist. irroratum* from Professor Cori in Trieste. He concluded that these three worms, along with part of Braun's material, should be placed in a new species, *Pachypsolus lunatus*. The remainder of Braun's material he left in *Dist. irroratum*. Looss considered the differences between the two species to be so great that he was reluctant even to place them in the same genus. However, after receiving further material from Trieste, he (1902) admitted that *P. lunatus* was a synonym of *P. irroratus*. He stated that his original description of *P. lunatus* (1901b) was based upon worms which he now recognized as immature. He had not appreciated the changes in structure that occur during growth in *P. irroratus*, and this had led him to consider juveniles and adults as separate species. Nevertheless, at the end of his discussion, Looss (1902) stated that the specimen from New Guinea described by Braun (1899a) must belong to another *Pachypsolus* species, because the vitellaria did not extend posterior to the hind end of the testes. The present authors do not share this view.

From the debate between Braun and Looss, a picture emerged of the variability within *P. irroratus*. Variations occur in the distribution of vitellaria (see above) and in the number of blind anterior diverticula of the caeca. Looss (1902) stated that there were two such diverticula on each side, with a third sometimes present, arising as a small lateral swelling of the caeca wall. He also commented on fixation-induced variability in sucker size. In one group of worms the oral and ventral suckers were of equal size, whereas in another group, treated differently, the oral sucker was larger than the ventral. Looss (1902) also summarized developmental changes that he observed: during growth, the suckers approach one another in size (the oral sucker tends to be larger in young worms), the hindbody elongates so that the ventral sucker lies relatively more anteriorly, the cirrus sac greatly increases in length, and the stellate or dendritic appearance of the vitellaria becomes less apparent.

Dollfus (1937) examined numerous specimens of *P. irroratus* from *Chelonia mydas* and *Caretta caretta* from north-west Africa. He could add nothing to the descriptions by Braun and Looss, but presented his observations on variability within the species. He found that the oral sucker could be larger or smaller than the ventral, and that the anterior extremity of the vitellaria did not extend anterior to the ventral sucker in some specimens, but did extend well anterior to it in others. In young worms, the cirrus sac lay dorsal to the ventral sucker but did not reach the posterior edge of the sucker. In mature specimens, the cirrus sac enlarges, comes to



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lie round the right side of the ventral sucker, and can extend posteriorly as far as the posterior edges of the testes (Fig. 3).

The Australian material examined by the present authors can add little new information. The body surface was devoid of the spines, scales or similar structures reported by some authors. In some immature and small mature worms, the cirrus sac had already reached its adult proportions, whereas in others it was still very small (Figs 8, 9). The number of vitelline rosettes was difficult to determine, but appeared to be 12–17 on each side when worms are examined in lateral view. This is a larger number than reported by other workers (with the exception of Fischthal and Acholonu 1976). However, most authors apparently did not examine their specimens in lateral view. Looss (1902) could see seven rosettes on each side under the dorsal surface of his specimens, but was aware of others under the lateral surface and extending ventrally. In Australian specimens, the stellate or dendritic appearance of the vitellaria is often more obvious in older specimens than in younger, contrary to the observations of Looss (1902).

Linton (1910) described *P. ovalis* (Figs 10–12) as a new species from the intestine of *Caretta caretta* from Florida. He considered that it differed from *P. irroratus* principally in possessing a shorter cirrus sac. The dimensions of Linton's types and paratypes from the U.S. National Museum Helminth Collection are shown in Table 1. It is clear that the specimens available to Linton were at the lower end of the size range of mature *P. irroratus*. Two of the specimens measured for inclusion in Table 1 were just beginning egg production, and a number of slightly smaller specimens (not measured) were immature. As Looss (1902) and Dollfus (1937) have pointed out, the cirrus sac of *P. irroratus* greatly increases in length during growth. It is clear (cf. Figs 4 and 12) that specimens of *P. ovalis* overlap those of *P. irroratus* in this respect. Therefore, *P. ovalis* Linton should be considered a junior synonym of *P. irroratus*.

A further description of *P. ovalis* by Caballero y C. *et al.* (1955) has provided no information to justify retention of the species. However, these authors drew attention to the fact, also observed by Linton (1910), that *P. ovalis* could have two or three anterior diverticula from the first part of the intestine on each side. This led them to synonymize *P. tertius* Pratt, 1914, with *P. ovalis*, a synonymy accepted by later authors. The specimens which Pratt (1914) examined were larger (the only specimen measured was 5 mm in length) than those seen by Linton (maximum 4 mm long). It is noteworthy that the cirrus sac in Pratt's material extended posterior to the ventral sucker, and 'in some cases to the anterior border of the testes' (Fig. 13). In this respect they were therefore identical with 'typical' *P. irroratus*.

Barker (1922) described *P. brachus* from the stomach of *Eretmochelys imbricata* from Bermuda. In his discussion of the genus *Pachypsolus*, Barker placed great emphasis on the length of the cirrus sac: in his specimens, the sac was short and 'situated immediately anterior to the acetabulum, with its long axis nearly perpendicular to the frontal plane of the body'. He used this feature to separate *P. brachus* from other members of the genus. It is apparent from his description and figures that his specimens are small individuals which had contracted very strongly on fixation (see his fig. 2, which shows the relationship between the cirrus sac and the ventral sucker in a sagittal section). In relaxed specimens, it would seem likely that the cirrus sac and ventral sucker would come to lie relative to one another in the same way as is seen in *P. ovalis* (= *P. irroratus*). *P. brachus* is accordingly considered a synonym of *P. irroratus*.

Caballero-Figueroa and Rodriguez is Instituto de Biología (1955) (*P. irroratus*) was a sucker.

Fischthal and Acholonu (1976) described *Eretmochelys imbricata* *ovalis* and one from *P. ovalis* ventral sucker suckers. In other vitellaria are visible of specimens from *P. puertoricensis* therefore it is not the distribution are described from specimens (USNM) *P. puertoricensis*.

There is no p

Host. *Caretta caretta*  
Site in host. Y  
Geographical

#### Specimens Examined

As whole mounts  
Bay, W.A., 5.viii.1955  
In alcohol: 2,  
As sections: 2

#### Specimens Deposited

Holotype (which was 1 as transverse section GL695), in QM.

#### Description

Body elongate, parallel for most of its length. Spines present anteriorly and around a median ventral sucker, where tegumentary sucker subterminal pharynx 0.15–0.2 mm long (n = 4) long; caecum



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Caballero-Rodriguez (1960) has described and figured *P. brachus* from *Chelonia mydas* from Acapulco, Mexico (see Fig. 14). The specimen figured by Caballero-Rodriguez is specimen number 217-11 in the Laboratorio de Helminthologia, Instituto de Biología, Mexico. This specimen cannot be separated from *P. ovalis* (= *P. irroratus*) with respect to the size and relationships of the cirrus sac and ventral sucker.

Fischthal and Acholonu (1976) reported a number of trematodes from *Eretmochelys imbricata* from Puerto Rico. They identified four specimens as *P. ovalis* and one as a new species, *P. puertoricensis*. They separated their new species from *P. ovalis* by the distribution of the vitelline rosettes (confluent dorsal to the ventral sucker in *P. puertoricensis*, not so in *P. ovalis*) and by the smaller size of the suckers. In one of Linton's specimens of *P. ovalis* (USNMHC No. 8443) the vitellaria are virtually confluent dorsal to the ventral sucker, whereas in the majority of specimens they are not. As Fischthal and Acholonu had only a single specimen of *P. puertoricensis*, the range of variation in their species was not established, and therefore it is not possible to separate *P. puertoricensis* and *P. ovalis* on the basis of the distribution of vitellaria. The suckers of *P. puertoricensis* are rather smaller than are described for *P. ovalis*. However, they closely resemble in size the suckers of specimens (USNMHC No. 73341) identified by Fischthal and Acholonu as *P. ovalis*. *P. puertoricensis* should be recognized as a synonym of *P. irroratus*.

There is no previous record of *Pachysoles irroratus* from Australia.

#### Family PLAGIORCHIIDAE

##### *Enodiotrema carettae*, sp. nov.

*Host.* *Caretta caretta*.

*Site in host.* Liver.

*Geographical distribution.* Australia.

##### *Specimens Examined*

As whole mounts: 4, from liver washings and from gall bladder, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester.

In alcohol: 2, same data.

As sections: 2 as transverse serial sections, 1 as sagittal serial sections, same data.

##### *Specimens Deposited*

Holotype (whole mount, No. V3055) and two paratypes (1 whole mount, V3056, 1 as transverse serial sections V3057), in SAM. One paratype (whole mount, No. GL695), in QM.

##### *Description*

Body elongate, 5.48-8.12 (7.0,  $n = 4$ )  $\times$  0.54-1.08 (0.89,  $n = 4$ ), with margins parallel for most of length, rounded at each end (Fig. 18), dorsoventrally compressed. Spines present, very fine, densest towards anterior end, entirely absent only in and around a median or submedian ventral furrow immediately posterior to ventral sucker, where tegument thicker and underlaid by possible glandular cells. Oral sucker subterminal, ventral, 0.26-0.32 (0.30,  $n = 4$ )  $\times$  0.26-0.34 (0.31,  $n = 4$ ); pharynx 0.15-0.16  $\times$  0.09-0.16 (0.13,  $n = 4$ ); oesophagus 0.14-0.28 (0.21,  $n = 4$ ) long; caeca simple, lateral in body, almost straight, terminating c.  $\frac{1}{6}$  from



posterior end. Ventral sucker 0.24-0.31 (0.29,  $n = 4$ )  $\times$  0.24-0.30 (0.29,  $n = 4$ ), situated  $c. \frac{1}{3}$  from anterior end.

Testes a pair, 0.21-0.43 (0.37,  $n = 4$ )  $\times$  0.18-0.41 (0.34,  $n = 4$ ), spherical, oblique, situated in 2nd quarter of body. Sperm duct from anterior end of each testis runs anterad to very short common duct at base of cirrus sac. Cirrus sac 0.66-0.90 (0.79,  $n = 4$ )  $\times$  0.30-0.54 (0.46,  $n = 4$ ), comma-shaped in ventral view with concave side directed towards the left (Fig. 19), extending from ovary to  $c. 1$  sucker diameter anterior to ventral sucker. Genital pore to left of midline, surrounded externally by glandular cells in region of genital opening. Male terminal genitalia consist of convoluted internal seminal vesicle within basal half of cirrus sac, narrow prostatic duct and short spined cirrus. Prostatic cells numerous, occupying all available space within cirrus sac. Diverticulum arising from genital sinus, dorsal to cirrus within cirrus sac, spined internally and larger than cirrus, receives end of metraterm immediately before opening at genital pore and may contain eggs distally.

Ovary spherical, 0.1-0.29 (0.22,  $n = 4$ ) in diameter, usually submedian to right side of midline, sometimes median, immediately anterior to testes. Oviduct arising at posterior end of ovary and passing into Mehlis' gland posteromedial to ovary, receiving Laurer's canal and the common vitelline duct before passing into ootype. Uterus leaves ventral side of Mehlis' gland and initially passes posterad, looped throughout body to just posterior to ends of caeca, may overlap caeca, especially ventrally, terminal portion ventral to gonads and becomes metraterm just anterior to ovary; eggs *in utero* 0.035-0.042  $\times$  0.022-0.027 ( $n = 10$ ). Laurer's canal opens in dorsal midline towards posterior end of first testis, runs anterad and gives off conspicuous seminal receptacle immediately posterior to ovary before passing into oviduct.

Vitelline follicles lobed in mature specimens, 11-12 on right side of body, 15-17 on left, lateral to caeca in a field on each side from level of posterior edge of ventral sucker to halfway along region of uterine loops. Vitelline reservoir ventral in Mehlis' gland, formed by fusion of vitelline duct from each side.

Excretory ducts a pair anteriorly, each arising by fusion of several smaller ducts in region of oesophagus, apparently fusing to form single duct between testes and opening through subterminal sphincter at posterior end.

#### Discussion

Looss (1902) gave detailed descriptions of four species (*megachondrus*, *instar*, *reductum* and *acariaeum*) in the genus *Enodiotrema*. All came from the intestines of *Caretta caretta* and *Chelonia mydas* from Egypt. Yamaguti (1958) placed *E. acariaeum* in the genus *Paralepoderma*, presumably because in this species the vitellaria are in the acetabular region and the testes are opposite in the contracted specimen figured by Looss (in other *Enodiotrema* species, the testes are diagonal in extended specimens but may lie opposite one another in contracted specimens: cp. figs 47 and 49 of Looss 1902). However, the terminal genitalia of *E. acariaeum* have the same structure as in other members of the genus. It seems preferable to retain *E. acariaeum* in *Enodiotrema*.

Chattopadhyaya (1970) described *E. microvitellatus* from the intestine of *Eretmochelys imbricata* from India. In the same paper, she erected a new genus and species, *Neoparalepoderma chitinoides*, for a worm from the intestine of *Chelonia mydas*. This worm may belong to *Enodiotrema*. It was described as possessing a

spined cirrus (see below beside the acetabulum described by Looss (although not conspi

18



Figs 18, 19. *Enodiotrema* (omitted).

Figs 20, 21. *Orchidastrea* (omitted).  
C, cirrus; D, spinous diverticulum; M, metraterm; S, seminal vesicle; V, vitelline duct.

Gupta and Mehlis (1968) described *Enodiotrema schikhobalovae*, from the intestine of *Chelonia mydas*. As far as can be seen from the description, it is a member of the genus *Enodiotrema*. The diverticulum. Looss (1902) described it as consisting of a



C. J. Limpus

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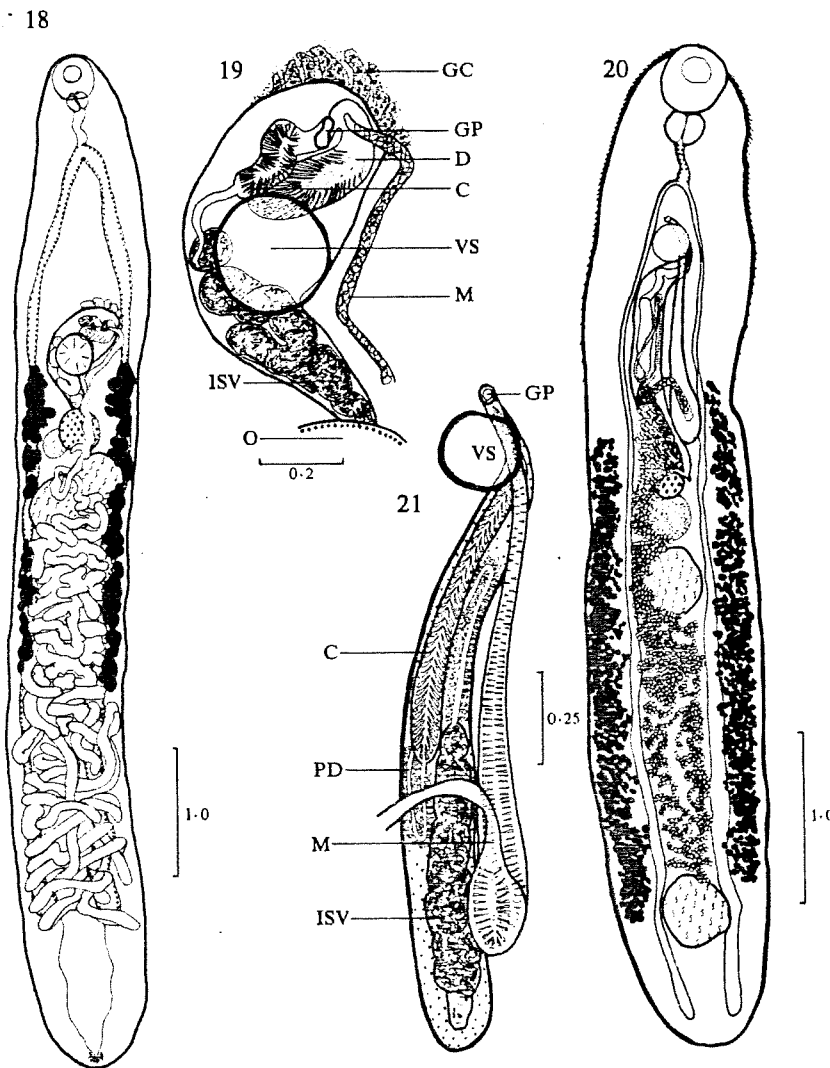
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spined cirrus (see below) and an 'oval chitinous structure below the genital pore and beside the acetabulum'. The latter structure could well represent the ventral furrow described by Looss (1902) in this position in some *Enodiotrema* species, and present, although not conspicuous, in *E. carettae*.



Figs 18, 19. *Endiotrema carettae*: 18, whole mount; 19, ventral view of terminal genitalia (prostatic cells omitted).

Figs 20, 21. *Orchidasma amphiorchis*: 20, whole mount; 21, ventral view of terminal genitalia.

C, cirrus; D, spinous diverticulum from genital sinus; GC, gland cells; GP, genital pore; ISV, internal seminal vesicle; M, metraterm; O, ovary; PD, prostatic duct; VS, ventral sucker.

Gupta and Mehrotra (1976) described a second species from India. This was *E. schikhobalovae*, from the oesophagus and stomach of *Eretmochelys imbricata*.

As far as can be determined from the literature, *E. carettae* differs from all other members of the genus in having spines within the cirrus as well as in the diverticulum. Looss (1902) described the male terminal genitalia in his four species as consisting of an internal seminal vesicle followed by a prostatic region, then an

unarmed ejaculatory duct giving off a large spinous diverticulum close to the genital pore (we have examined some of Looss' material, and can confirm this). In *E. carettae* the same arrangement is found, but the ejaculatory duct (here considered a cirrus), as well as the diverticulum, bear spines within their lumina. All descriptions or redescrptions of *Enodiotrema* species since those by Looss (1902) have tended to gloss over the structure of the male ducts. Several (Caballero y C. 1954; Caballero-Rodriguez 1960; Euzet and Combes 1962; Chattopadhyaya 1970; Gupta and Mehrotra 1976) speak of a spined cirrus, but make no mention of a diverticulum from the distal end of the cirrus. It seems likely that these authors have made the same mistake as Looss (1899, corrected 1902) in supposing the diverticulum to be the cirrus. Figures by Euzet and Combes (1962) and Gupta and Mehrotra (1976) show structures which may represent a diverticulum, although this is not mentioned in their text. No descriptions mention glandular cells surrounding the distal end of the cirrus sac, as is seen in *E. carettae*.

*Enodiotrema carettae* has more vitelline follicles than other members of the genus (with the possible exception of *E. schikhobalovae*) and these extend along a greater length of the body than in any other species, from the posterior edge of the ventral sucker to well posterior to the testes. Only in *E. instar* do the vitellaria approach this distribution.

All previously described species of *Enodiotrema* were taken from the intestines of their hosts. *E. carettae* occurs in the liver and gall bladder.

## Family TELORCHIIDAE

### *Orchidasma amphiorchis* (Braun)

- Distomum amphiorchis* Braun, 1899a, p. 719 (intestine, *Thalassochelys corticata* = *Caretta caretta*, Trieste, Adriatic Sea; *Chelone mydas*, Red Sea; *Podocnemis expansa*); Braun, 1899b, p. 629 (*Thalassochelys caretta* = *Caretta caretta*, Naples, Mediterranean Sea).
- Anadasmus amphiorchis* (Braun). Looss, 1899, pp. 568-9.
- Orchidasma amphiorchis* (Braun). Looss, 1900, p. 602; Braun, 1901, pp. 20-2, figs 7-11; Looss, 1901b, p. 560 (*Thalassochelys corticata* = *Caretta caretta*, Mediterranean coast of Egypt); Looss, 1902, pp. 463-8, figs 25-29, 39 (small intestine, *Thalassochelys corticata* = *Caretta caretta*, and hind intestine, *Chelone mydas*, Mediterranean coast of Egypt; *Thalassochelys* = *Caretta caretta*, Adriatic Sea); Linton, 1910, pp. 28-9, figs 23-28 (stomach, *Caretta caretta*, Dry Tortugas, Florida); Baylis, 1928, p. 332 (*Thalassochelys caretta* = *Caretta caretta*, Lancashire, England); Manter, 1931, p. 387 (*Caretta caretta* and *Coryphaena hippurus* (Pisces), Dry Tortugas, Florida); Yamaguti, 1934, pp. 649-50 (stomach and small intestine, *Caretta olivacea* = *Lepidochelys olivacea*, Pacific Ocean); Luhman, 1935, p. 275 (*Caretta caretta*, Florida); Dollfus, 1937, pp. 504-6 (intestine, *Chelone mydas*, Mauritania); Teixeira de Freitas and Lent, 1938, pp. 80-1, pl. 1 figs 1-3, pl. 2 figs 1-3, pl. 3 figs 1, 2 (stomach and small intestine, *Chelone mydas*, Rio de Janeiro, Brazil); Oguro, 1942, p. 164 (*Caretta olivacea* = *Lepidochelys olivacea* and *Chelonia japonica* = *C. mydas*, Japan); Pearse, 1949, p. 36 (intestine, *Caretta caretta*, North Carolina); Caballero y C. and Zerecero y D., 1950, pp. 123-9, drawing 2 and fig. 1 (small intestine, *Eretmochelys imbricata*, Gulf of Tehuantepec, Pacific coast of Mexico); Caballero y C. *et al.*, 1955, pp. 187-90, figs 24, 25 (small intestine, *Chelone mydas*, Gulf of Panama, Panama); Caballero y C., 1962, pp. 47-51, fig. 1 (small intestine, *Chelone mydas*, Soto la Marina, Gulf of Mexico, Mexico); Boero and Led, 1974, pp. 16-17, fig. 5 (small intestine, *Thalassochelys caretta* = *Caretta caretta*, Argentina); Fischthal and Acholonu, 1976, p. 184 (stomach, *Eretmochelys imbricata*, Puerto Rico, Caribbean Sea); Sey, 1977, p. 388 (*Caretta caretta*, Alexandria, Egypt).
- Orchidasma indica* Simha, Rao & Chattopadhyaya, 1971, p. 22 (intestine, *Eretmochelys squamosa* [sic] = *E. imbricata*, India).
- Orchidasma vitelloconfluens* Rao, 1973, pp. 181-4, fig. 1 (intestine, *Chelonia mydas*, south India).

*Hosts. Caretta olivacea, Podocnemis expansa.*  
*Site in host. Geographical distribution.*  
Florida, North Carolina, Panama, Puerto Rico.

*Specimens Examined.*  
As whole mounts: No. 1231; 2, same. 29.vi.1907, Linton, No. 51750; 40 (on *Podocnemis*, No. 36981 (see Peckham & Peckham, USNMHC No. 7341). 1923, Monod, MNHN, Shark Bay, W.A., 4. 1938, Mon Repos, Bundaberg, Queensland. As spirit specimens: No. 1231, Linton, 1907. As sectioned material: No. 1231, Linton, 1907. Intestine, *C. carettae*.

*Specimens Deposited.*  
3, from upper small intestine, QM Nos GL 1231-1233.

### Description

Body elongate, margins parallel, anteriorly compressed. Spines progressively smaller towards posterior end of body. Oral sucker opening subterminally. Testes ( $n = 7$ ), immediately posterior to oral sucker; glandular cells; cirrus terminate at posterior end, weak, 0.16-0.20. Cirrus anterior  $\frac{1}{4}$  of body length. Testes a pair (0.33,  $n = 7$ ) × 0.25-0.43 (0.33-0.43), with ovarian loops. Sac without enteric diverticulum or slightly undulate. Cirrus and passing to posterior (Fig. 21). Terminal half of cirrus sac before entering internal seminal sac throughout its length.



*Hosts.* *Caretta caretta*, *Eretmochelys imbricata*, *Chelonia mydas*, *Lepidochelys olivacea*, *Podocnemis expansa*.

*Site in host.* Stomach and intestine (especially small intestine).

*Geographical distribution.* Mediterranean Sea, Red Sea, Mauritania, England, Florida, North Carolina, Mexico (Gulf of Mexico and Pacific coast), Pacific coast of Panama, Puerto Rico, Brazil, India, Japan, Australia.

#### *Specimens Examined*

As whole mounts: 2, from *Eretmochelys imbricata*, Torres Strait, north Queensland, Breinl, CIH No. 1231; 2, same data, BMNH No. 1950.12.6.73-4; 3, from intestine, *C. caretta*, Tortugas, Florida, 29.vi.1907, Linton, USNMHC No. 8445 (see Linton 1910); 1, from *C. caretta*, ? Manter, USNMHC No. 51750; 40 (on 2 slides), from *C. caretta*, Beaufort, N. Carolina, 19.vi.1946, Pearse, USNMHC No. 36981 (see Pearse 1949); 3, from stomach, *Eretmochelys imbricata*, Puerto Rico, 13.v.1970, USNMHC No. 73460 (see Fischthal and Acholonu 1976); 4, from intestine, *Chelonia mydas*, Mauritania, 1923, Monod, MNHN Nos L51, L52, L62 (see Dollfus 1937); 13, from upper small intestine, *C. caretta*, Shark Bay, W.A., 4.vii.1979, Blair and Lester; 5, from upper small intestine, *C. caretta* (nesting female), Mon Repos, Bundaberg, Qld, 17.i.1979, Limpus.

As spirit specimens: 2, from upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester.

As sectioned material: 1, as transverse serial sections, 1 as sagittal serial sections, from upper small intestine, *C. caretta*, Shark Bay, W.A., 4.vii.1979, Blair and Lester.

#### *Specimens Deposited*

3, from upper small intestine, *C. caretta*, Shark Bay, W.A., 4.vii.1979, SAM Nos V3058-60; 2, same data, QM Nos GL696-7.

#### *Description*

Body elongate, 4.87-6.95 (5.93,  $n = 7$ )  $\times$  0.66-1.12 (0.89,  $n = 7$ ), with margins parallel for much of length (Fig. 20), rounded at each end, dorsoventrally compressed. Spines on tegument numerous on anterior half of body, becoming progressively smaller and sparser posteriorly, very sparse or absent on posterior  $\frac{1}{3}$  of body. Oral sucker small, 0.3-0.39 (0.35,  $n = 7$ )  $\times$  0.31-0.42 (0.37,  $n = 7$ ); oral opening subterminal, ventral; pharynx 0.14-0.2 (0.17,  $n = 7$ )  $\times$  0.18-0.30 (0.25,  $n = 7$ ), immediately posterior to oral sucker; oesophagus very short, surrounded by glandular cells; caeca simple, rather narrow, following more or less direct course to terminate at posterior end of body where they may be slightly dilated. Ventral sucker weak, 0.16-0.22 (0.19,  $n = 7$ )  $\times$  0.16-0.22 (0.19,  $n = 7$ ), smaller than oral, in anterior  $\frac{1}{4}$  of body.

Testes a pair, nearly equal in size, spherical to ovoid, tandem, one 0.26-0.4 (0.33,  $n = 7$ )  $\times$  0.27-0.37 (0.32,  $n = 7$ ), located at beginning of 2nd half of body immediately posterior to ovarian complex, the other 0.32-0.48 (0.38,  $n = 7$ )  $\times$  0.25-0.43 (0.33,  $n = 7$ ), close to posterior end of body immediately posterior to ovarian loops. Sperm duct from each testis appears to pass directly to base of cirrus sac without entering a common duct. Cirrus sac 1.2-2.1 (1.58,  $n = 7$ ) long, straight or slightly undulating, median or just submedian, arising dorsally at level of ovary and passing to genital pore in median line immediately anterior to ventral sucker (Fig. 21). Terminal genitalia consist of large, simple, internal seminal vesicle in basal half of cirrus sac, then prostatic duct looped once alongside and posterior to cirrus before entering base of cirrus which located slightly posterior to anterior end of internal seminal vesicle; cirrus straight or looped within cirrus sac, densely spined throughout its length, opening at genital pore.

Ovary 0.13–0.17 (0.15,  $n = 7$ ) × 0.11–0.18 (0.15,  $n = 7$ ), spherical to ovoid, approximately halfway along body, median or almost so, posteroventral to base of cirrus sac. Mehlis' gland anterodorsal to ovary. Oviduct receives Laurer's canal then vitelline duct as it passes to ootype. Uterus looped between caeca from base of cirrus sac as far posteriorly as 2nd testis. Metraterm 0.91–1.51 (1.25;  $n = 7$ ), long, initially a narrow muscular tube arising close to level of base of cirrus sac, then passing posterad into dilated region, bearing numerous spines internally, then turns anterad to lie along left-hand side of cirrus sac, then ventral to cirrus sac just before reaching genital pore; spinous region of metraterm tapers towards genital pore and spines, which, in mature specimens, are not as large as those in cirrus, decrease in size anteriorly and do not extend quite to genital pore. Eggs numerous, thick-shelled, 0.042–0.047 (0.045,  $n = 7$ ) × 0.035–0.04 (0.037,  $n = 7$ ). Laurer's canal convoluted, opening to one side of dorsal midline, enters Mehlis' gland posteriorly where it gives off large seminal receptacle which extends well posterior to Mehlis' gland. Vitellaria consisting of numerous small follicles external to caeca, in lateral field on each side from level of base of cirrus to slightly anterior to 2nd testis, or may extend almost to end of 2nd testis; collecting duct from each side enters vitelline reservoir in posteroventral region of Mehlis' gland.

Excretory stem dorsal, median, running from just posterior to ovary to dorsal median subterminal excretory pore; other details not worked out.

#### Discussion

From published descriptions, it is clear that *O. amphiorchis* is quite variable with respect to some of its morphological features. Looss (1902) and Caballero y C. (1962) state that the metraterm is always longer than the cirrus sac, whereas Yamaguti (1934) observed that, depending on the specimen, either structure could be the longer. In Australian material the cirrus sac is usually, but not invariably, longer than the metraterm.

The distribution of vitelline glands is quite variable. They normally lie in lateral fields from the level of the posterior half of the cirrus sac to slightly anterior to the second testis. In some Australian specimens the vitellaria extend to the posterior end of the second testis. Fischthal and Acholonu (1976) had three specimens of *O. amphiorchis*: in one, the vitellaria extended to the posterior end of the body, and in the other two they extended about midway between the second testis and the end of the body. Most authors speak of breaks in the vitelline fields, so that up to nine groups of follicles may be apparent. However, most (e.g. Looss 1902; Caballero y C. and Zerecero 1950) also point out that this is very variable, and some specimens display unbroken lateral fields. None of the Australian specimens showed clear breaks in the lateral fields.

Simha *et al.* (1971), in an abstract, reported a new species of *Orchidasma* from the intestine of *Eretmochelys imbricata* in India. They did not figure or name the worm, and the only morphological data given were that 'the species is characterised by the presence of a spined cirrus and the vitellaria extending from the middle of the second quarter of the body to the caudal end'. Neither of these features can distinguish their species from *Orchidasma amphiorchis*, in which the cirrus is spined, and the vitellaria commonly start in the second quarter of the body and, as reported by Fischthal and Acholonu (1976), may also reach the posterior end of the body.

Rao (1973) applied the name *Orchidasma indica* to the species reported by Simha *et al.* (1971). In the same paper, he proposed another species, *O. vitelloconfluens*, in

which the cirrus dorsal midline, cirrus sac and lies ventral to example of the terminal g for the vitelline reported that dorsally for a not seem to r

Braun (1899) *Amphistoma g* is unlikely that occur in a fresh the specimen *Podocnemis ex*

There is no

*Distomum sole*  
*Cymatocarpus*  
*Cymatocarpus*  
= *Caretta*  
(intestine, C  
Florida); D  
Persian Gul  
(*Caretta oli*  
Caballero y  
Caballero-R  
y C., 1959).

*Hosts. Caret*  
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of Mexico, Jap

*Specimens Examined*  
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*C. caretta*, Shark B

*Specimens Deposited*  
2, from small int  
different host, QM



which the cirrus sac crosses the metraterm and the vitellaria are confluent in the dorsal midline for a short region at the level of the ovary. It is no surprise that the cirrus sac and metraterm should cross in some specimens, as the metraterm normally lies ventral to the cirrus sac distally. Linton's (1910) fig. 24 shows an excellent example of this. Pressure applied to a specimen during flattening could well lead to the terminal genitalia appearing as in fig. 1 of Rao (1973). In *O. amphiorchis* it is rare for the vitellaria to extend between the caeca; however, Caballero y C. (1962) reported that this sometimes happens. In *O. vitelloconfluens* the vitellaria meet dorsally for a very short distance only. The characters discussed by Rao (1973) do not seem to merit the erection of a new species.

Braun (1899a) found a young specimen of *Orchidasma amphiorchis* in a tube of *Amphistoma grande* from the Brazilian freshwater chelonian *Podocnemis expansa*. It is unlikely that a parasite otherwise known only from marine turtles should also occur in a freshwater species. However, it is impossible to be certain whether or not the specimen seen by Braun had been mislabelled. Dollfus (1937) implies that *Podocnemis expansa* is an accidental host for *Orchidasma amphiorchis*.

There is no previous record of *Orchidasma amphiorchis* from Australia.

#### Family BRACHYCOELIIDAE

##### *Cymatocarpus solearis* (Braun)

*Distomum soleare* Braun, 1899b, pp. 629-630.

*Cymatocarpus solearis* (Braun, 1899b) Braun, 1901, pp. 22-3, fig. 8 (*Chelone mydas*).

*Cymatocarpus undulatus* Looss, 1899, pp. 711-12, figs 32-34 (small intestine, *Thalassochelys corticata* = *Caretta caretta*, Abukir, Mediterranean coast of Egypt); Linton, 1910, pp. 26-8, figs 15-22 (intestine, *Caretta caretta*, Dry Tortugas, Florida); Pratt, 1914, p. 426 (duodenum, *Caretta caretta*, Florida); Dollfus, 1927, pp. 1352-5, fig. 1 (metacercariae encysted in muscles of *Pagurus tinctor*, Persian Gulf); Luhman, 1935, p. 274 (*Caretta caretta*, Tortugas, Florida); Oguro, 1942, p. 164 (*Caretta olivacea* = *Lepidochelys olivacea*, Kurio, Yakusima and Naha, Okinawazima, Japan); Caballero y C., 1959, pp. 159-66, figs 1, 2 (small intestine, *Chelone mydas*, Acapulco, Mexico); Caballero-Rodriguez, 1960, pp. 62-6, figs 21-22 (redescription of specimen reported by Caballero y C., 1959).

*Hosts.* *Caretta caretta*, *Lepidochelys olivacea*, *Chelonia mydas*.

*Site in host.* Intestine, especially small intestine.

*Geographical distribution.* Mediterranean Sea, Persian Gulf, Florida, Pacific coast of Mexico, Japan, Australia.

##### *Specimens Examined*

As whole mounts: 4, from intestine, *C. caretta*, Tortugas, Florida, 1.vii.1906, Linton, 1 slide, USNMHC No. 8444 (see Linton 1910); 1, from cyst, *C. caretta*, 2.vi.1912, Pratt, USNMHC No. 36502; 1, from upper small intestine, *C. caretta*, Shark Bay, W.A., 4.vii.1979, Blair and Lester; 8, from small intestine, same data but 2 hosts, 5.vii.1979; 1, from first part intestine, *C. caretta* (nesting female), Mon Repos, Bundaberg, Qld, 17.i.1979, Limpus.

As spirit specimens: 75, tube labelled 'Cymatocarpus sp. Hafskoldpadda in intest., Tortugas, Pratt leg', NHR No. 101; 17, from upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester; 34, from first part intestine, *C. caretta* nesting female, Mon Repos, Bundaberg, Qld, 17.i.1979, Limpus.

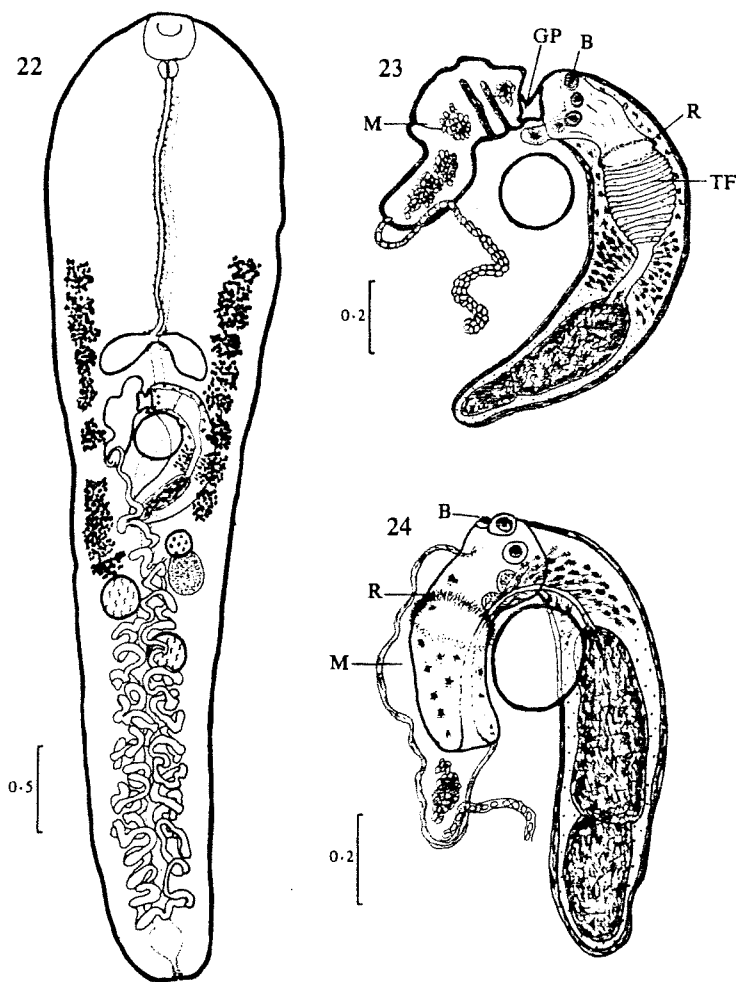
As sectioned material: 2 as transverse serial sections, 1 as sagittal serial sections, upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester.

##### *Specimens Deposited*

2, from small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, SAM Nos V3061-2; 2, same data but different host, QM Nos GL698-9.

### Description

Body elongate, 2.55–5.69 (3.80,  $n = 10$ ) × 0.87–1.45 (1.11,  $n = 10$ ), spatulate in relaxed specimens, broadest a short distance behind oral sucker, rounded at anterior end and tapering gradually towards bluntly rounded posterior end (Fig. 22),



Figs 22–24. *Cymatocarpus undulatus*. 22, whole mount; 23, ventral view of terminal genitalia, showing structures within cirrus sac; 24, same, but with cirrus everted through genital pore. B, spinous bursa; GP, genital pore; M, metraterm; R, ring of spines in cirrus; TF, transverse folds in cirrus wall.

dorsoventrally compressed. Tegument thick, bearing numerous tiny spines over entire surface. Oral sucker small, 0.18–0.26 (0.22,  $n = 10$ ) × 0.20–0.31 (0.24,  $n = 10$ ); oral opening subterminal ventral; pharynx small, 0.07–0.13 (0.1,  $n = 10$ ) × 0.08–0.14 (0.11,  $n = 10$ ); oesophagus 0.36–1.51 (0.90,  $n = 10$ ) long, narrow, muscular, surrounded by glandular cells especially numerous towards posterior end; caeca short, broad, pyriform, arising a short distance anterior to genital opening and passing posterolaterally to terminate anterior to ventral sucker. Ventral sucker small, 0.18–0.26 (0.21,  $n = 10$ ) × 0.18–0.28 (0.22,  $n = 10$ ), in 2nd quarter of body.

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### Discussion

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Testes a pair, each 0.17–0.25 (0.20,  $n = 10$ )  $\times$  0.19–0.28 (0.23,  $n = 10$ ), oblique, in 3rd quarter of body, separated by folds of uterus, spherical or almost so. A sperm duct leaves anterior end of each testis and passes separately to enter base of cirrus sac. Cirrus sac large, curved, arising in midline at level of ovary, then curved round left side of ventral sucker to open at genital pore, median or just to right of midline, immediately anterior to ventral sucker. Internal seminal vesicle usually bipartite, within basal  $\frac{1}{3}$  of cirrus sac, then short prostatic duct opening into cirrus which occupies  $\frac{1}{3}$ – $\frac{1}{2}$  of cirrus sac; cirrus muscular, sometimes shortened by numerous transverse folds along its proximal half (Fig. 23); lumen lined with a ring of spines about halfway along its length and bearing 5–7 spinous bursae near distal end, most typically arranged with 2 larger bursae dorsally and 3–4 smaller ones ventrally (Figs 23, 24). Bursae evaginated in everted cirrus (Fig. 24).

Ovary spherical to ovoid, 0.11–0.18 (0.13,  $n = 10$ )  $\times$  0.14–0.19 (0.16,  $n = 10$ ), on left side of midline at beginning of 2nd half of body, anterior to testes. Mehlis' gland posteromedial to ovary. Oviduct arises on medial face of ovary, passes into Mehlis' gland and receives Laurer's canal and vitelline duct. Uterus arises posteroventrally, sometimes initially containing sperm, then passes posterad between testes to form descending limb looped on left side of midline and reaching almost to posterior end, then ascending limb on right side of midline and entering muscular sac-like metraterm just posterior to ventral sucker; metraterm surrounded by glandular cells visible in sections, passes around right side of ventral sucker to genital pore. Eggs *in utero* 0.023  $\times$  0.012 ( $n = 10$ ). Laurer's canal opens in dorsal midline posterior to ovary, passes into Mehlis' gland and gives off duct to large seminal receptacle posterior to ovary. Vitellaria small, forming a series of clusters (sometimes indistinct), ventral, in lateral field commencing slightly anterior to caecal junction on each side, 9 clusters in line extending to just anterior to ovary on left side and 11–12 clusters extending to just anterior to first testis on right side but sometimes with gaps or other variations on this side. Vitelline duct from each side enters vitelline reservoir in ventral region of Mehlis' gland.

Excretory stem conspicuous in sections, cylindrical except where compressed by other organs, median, arising posterior to oral sucker and passing to distinct sphincter at terminal excretory pore. Tributary ducts or branches not seen.

#### Discussion

In a paper published on 25 November 1899, Braun (1899b) described *Distomum soleare* from specimens in the collection of the Vienna Museum. The host was *Chelonia mydas*, but no other collection data were given. Later Braun (1901) placed *D. soleare* in the genus *Cymatocarpus* Looss and compared it with *Cymatocarpus undulatus* described by Looss in a paper published on 28 December 1899 (information on dates in Index-Catalogue of Medical and Veterinary Zoology). Braun (1901) and Looss (1901b, 1902) considered the two species to be distinct from one another largely on the basis of the size of the body, internal organs and eggs. Braun did not observe the spinous bursae in the cirrus of his specimens, and stated that he could not see the metraterm (although something resembling this latter structure is figured by him (Braun 1901) and possibly mistaken for part of the male genitalia). However, as he had only a few small and badly preserved specimens, Braun could have missed seeing or correctly interpreting these structures. Neither Braun nor Looss observed a ring of spines in the cirrus of their specimens.

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There seems little doubt that *C. undulatus* is synonymous with *C. solearis*. The apparent differences between the species in their terminal genitalia are probably due in part to differing interpretations by Braun and Looss, and such differences are also to be found in descriptions of *C. undulatus* by later authors. Dollfus (1927), describing a well advanced metacercaria in a crab, Caballero y C. (1959) and Caballero-Rodriguez (1960) made no mention either of the spinous bursae or of the ring of spines in the cirrus. Only Linton (1910) reported both these features. Looss and Braun considered their species to differ largely on the basis of size (*C. undulatus* 5 mm long, contracting to 3.5 mm on preservation; *C. solearis* about 2 mm long). Specimens reported by Linton (1910) and Australian specimens examined by the present authors bridge the gap in size between these. We agree with Caballero y C. (1959) that difference in size is not a good character for separating these species.

Looss (1899) stated that the excretory bladder was Y-shaped, dividing into two branches immediately posterior to the ventral sucker. All subsequent authors have observed the bladder to be a simple tubular structure running anterad as far as the pharynx.

There is no previous record of *C. solearis* from Australia.

#### Family RHYTIDODIDAE

##### *Rhytidodes gelatinosus* (Rudolphi)

- Distoma gelatinosum* Rudolphi, 1819, pp. 102, 386-7 (intestine, *Testudo mydas* = *Chelonia mydas*, Rimini, Adriatic Sea).
- Distomum gelatinosum* Rudolphi. Sonsino, 1890, p. 106 (*Chelonia* (*Thalassochelys*) *caretta* = *Caretta caretta*); Sonsino, 1893, pp. 183-4 (intestine, *Chelonia caretta* = *Caretta caretta*); Stossich, 1895, p. 37, figs 2, 3 (intestine, *Thalassochelys caretta* = *Caretta caretta*, Trieste, Adriatic Sea); Stossich, 1898, p. 43 (intestine, *Thalassochelys caretta* = *Caretta caretta*, Trieste); Braun, 1899a, pp. 716-17 (*Chelonia mydas*, *Thalassochelys corticata* = *Caretta caretta*, and unidentified turtle species, Trieste, Adriatic Sea, and New Guinea); Looss, 1899, pp. 570, 579, 580; Braun, 1901, pp. 29-34, figs 6, 12, 19.
- Rhytidodes gelatinosus* (Rudolphi) Looss, 1901b, pp. 563-5 (small intestine, *Thalassochelys corticata* = *Caretta caretta*, Egypt, Trieste); Looss, 1902, pp. 445-57, figs 19-24; Luhman, 1935, p. 274 (*Caretta caretta*, Tortugas, Florida); Caballero y C., 1954, pp. 46-9, figs 13, 14 (intestine, *Chelonia mydas*, Gulf of Panama, Pacific Ocean); Perez-Vigueras, 1955, pp. 63-5, fig. 18 (intestine, *Eretmochelys imbricata*, Cuba); Caballero-Rodriguez, 1960, pp. 17-22, figs 1-4 (small intestine, *Chelonia mydas*, Acapulco, Mexico); Euzet and Combes, 1962, pp. 16-18, figs 1, 3 (intestine, *Thalassochelys caretta* = *Caretta caretta*, Sète, Mediterranean coast of France); Simha and Chattopadhyaya, 1969, pp. 96-7, figs 1-3 (intestine, *Eretmochelys imbricata*, Camp Mandapam, Gulf of Mannar, southern India); Euzet *et al.*, 1972, pp. 157-8 (duodenum, *Caretta caretta*, Banyuls-sur-Mer, Mediterranean coast of France); Bilqees, 1974, pp. 300-2, fig. 3 (intestine, *Chelonia mydas*, Karachi, Pakistan); Fischthal and Acholonu, 1976, p. 184 (small intestine, *Eretmochelys imbricata*, Puerto Rico, Caribbean Sea); Sey, 1977, p. 388 (*Caretta caretta*, Mediterranean coast of Egypt).
- Rhytidodes secundus* Pratt, 1914, pp. 423-6, pl. 4, fig. 4 (duodenum, *Caretta caretta*, Loggerhead Key, Florida).
- Rhytidodes indicus* Simha and Chattopadhyaya, 1969, pp. 97-9, figs 4-7 (intestine, *Eretmochelys squamosa* = *E. imbricata*, Rameswaran, India).

*Hosts.* *Caretta caretta*, *Chelonia mydas*, *Eretmochelys imbricata*.

*Site in host.* Intestine (? small intestine).

*Geographical distribution.* Mediterranean Sea, Atlantic coast of Morocco, Red Sea, Florida, Puerto Rico, Cuba, Mexico, Gulf of Panama, India, Pakistan, New Guinea, Australia.

##### *Specimens Examined*

As whole mounts (see Bilqees 1974, Sète (Hérault) *Thalassochelys caretta*, 1, same label as Caballero y C. 1959, Bundaberg, Queensland, 5.vii.1979, Blair).

In alcohol: *Chelonia mydas*, 1, 5.vii.1979, Blair.

As sections: *Chelonia mydas*, 1, 5.vii.1979, Blair.

##### *Specimens Deposited*

1, from small intestine, GL700-1.

##### *Description*

Body elongate, wrinkled, especially anteriorly compressed on expansion on margins (Figs 13, 14). Long, not surmounting caeca simple, margins for most in anterior  $\frac{1}{5}$ .

Testes a pair, each occupying a quarter of body width and one duct very short, passing ventral sucker back on itself, surrounded by conspicuous genital sucker. Body immediately to

Ovary spherical, Mehlis' gland arises posteriorly, it receives vitelline anterad ventral terminal portion, canal opens mid-ventrally but sperm son filamentous, rare caecum on each ventral sucker especially at level vitelline filamen



*Specimens Examined*

As whole mounts: 1, from intestine, *Chelonia mydas*, Karachi, Pakistan, BMNH No. 1973-10.12.4 (see Bilquees 1974); 1, labelled '*Rhytidodes gelatinosus* (Rudolphi, 1819), tube digestif, *Caretta caretta* (L.) Sète (Hérault) 15.10.1951', MNHN No. L63<sub>1</sub>; 1, labelled '*Rhytidodes gelatinosus* (Rud. 1819) Looss, 1901. *Thalassochelys corticata* (Rondelet). Rabat 25.6.1925. R.Ph. Dollfus leg. et déterminé', MNHN No. L64<sub>1</sub>; 1, same label data but 24.vi.1925, MNHN No. L64<sub>2</sub>; 1, from small intestine, *C. caretta*, Mon Repos, Bundaberg, Qld. 19.xii.1976, Limpus; 6, from upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester.

In alcohol: 3, from intestine, *Chelonia mydas*, Rimini, Rudolphi, ZMB No. 1491 (syntypes); 3, from *Chelonia mydas*, Red Sea, Hemprich and Ehrenberg, ZMB No. 3883.

As sections: 1 as transverse serial sections, from upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, Blair and Lester; 1 as sagittal serial sections, same data.

*Specimens Deposited*

1, from small intestine, *C. caretta*, Mon Repos, Bundaberg, Qld. 19.xii.1976, SAM No. V3064; 1, from upper small intestine, *C. caretta*, Shark Bay, W.A., 5.vii.1979, SAM No. V3063; 2, same data, QM No. GL700-1.

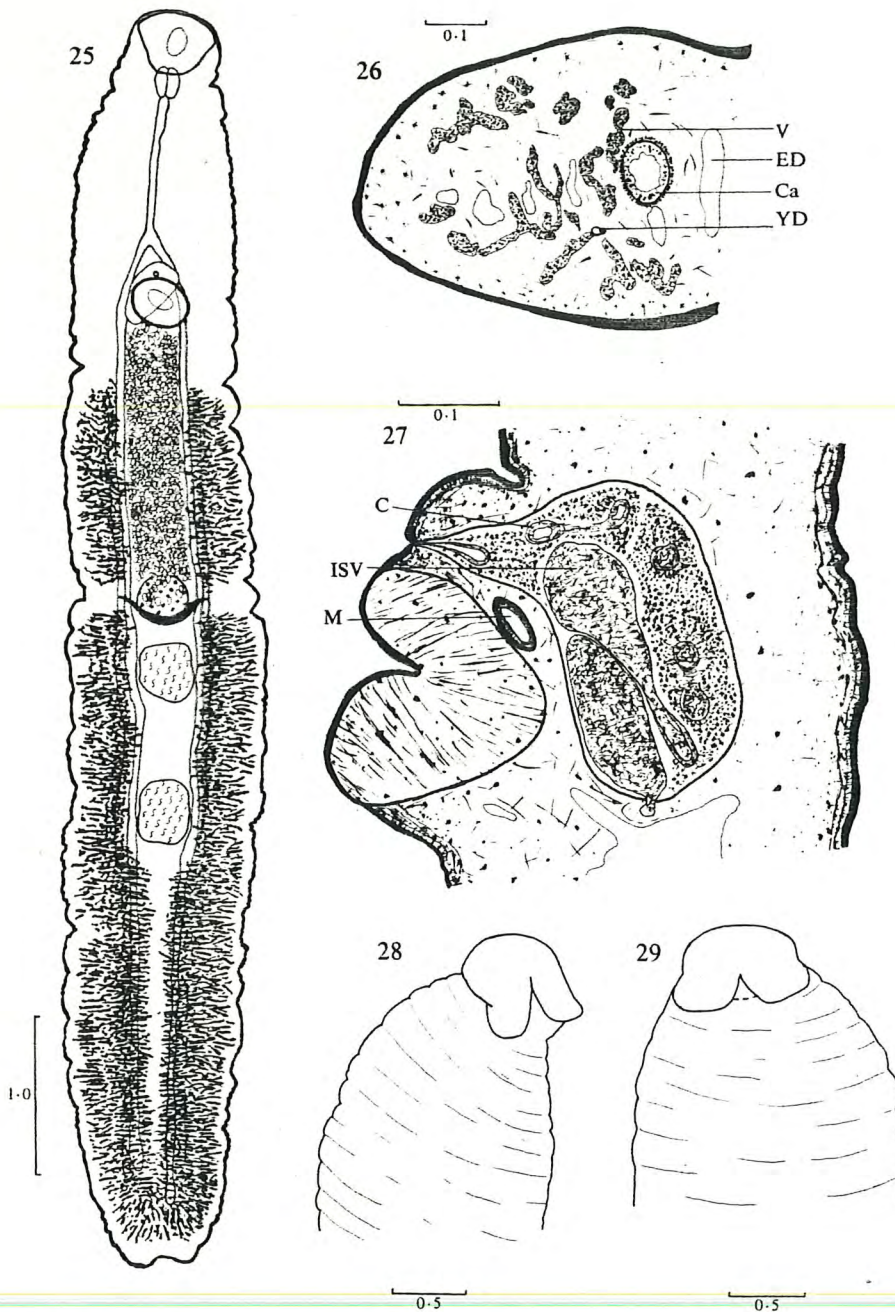
*Description*

Body elongate, margins parallel for most of length and sometimes crenate or wrinkled, especially in contracted specimens, bluntly rounded at each end, dorsoventrally compressed (Fig. 25). Tegument aspinous. Oral sucker small with ventrolateral expansion on each side and transverse dorsal ridge extending onto dorsolateral margins (Figs 28, 29); oral opening subterminal, ventral; pharynx small; oesophagus long, not surrounded by gland cells, bifurcating slightly anterior to ventral sucker; caeca simple, parallel, approximately midway between midline of body and its margins for most of length, terminating close to posterior end. Ventral sucker small, in anterior  $\frac{1}{5}$  of body.

Testes a pair, rounded or ovoid and similar in size, tandem in middle third or 3rd quarter of body, between caeca. A sperm duct arises from anterior end of each testis, and one duct passes anterad medial or ventromedial to each caecum; common duct very short, passing into base of cirrus sac which is located dorsal and anterior to ventral sucker. Cirrus sac short, pyriform, containing internal seminal vesicle curved back on itself in posteroventral region of sac, then coiled muscular cirrus (Fig. 27) surrounded throughout its length by prostatic cells and opening through inconspicuous genital pore in or near ventral midline immediately anterior to ventral sucker. Body surface raised in a small region anterior to ventral sucker and immediately to left of genital pore.

Ovary spherical, median or almost so, in 2nd quarter of body anterior to testes. Mehlis' gland ill-defined, posteroventral to ovary and slightly to one side. Oviduct arises posterodorsally, receives Laurer's canal then passes into Mehlis' gland where it receives vitelline duct before entering ootype. Uterus arises ventrally, passes anterad ventral to ovary, then looped between caeca to level of ventral sucker; terminal portion a short muscular metraterm passing into genital opening. Laurer's canal opens mid-dorsally just posterior to level of ovary. Seminal receptacle absent but sperm sometimes present in first few loops of uterus. Vitellaria dendritic or filamentous, radiating from a narrow muscular longitudinal duct ventrolateral to caecum on each side (Fig. 26), in a field extending from a short distance posterior to ventral sucker to posterior end of body (short breaks in vitelline field may occur, especially at level of ovary), lateral to caeca except posterior to 2nd testis where vitelline filaments may overlap caeca and extend to midline dorsally and ventrally.





Figs 25–29. *Rhytidodes gelatinosus*: 25, whole mount; 26, transverse section through region of vitelline follicles; 27, sagittal section through cirrus sac; 28, ventrolateral view of anterior end of rather contracted specimen, showing appearance of oral sucker (mouth not shown); 29, same, in anteroventral view, with tip flexed ventrally to give an almost apical view of sucker. C, cirrus; Ca, gut caecum; ED, excretory duct; ISV, internal seminal vesicle; M, metraterm; V, vitellaria; YD, yolk duct.

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#### Discussion

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Common collecting duct from each side enters ventral region of Mehlis' gland where they become greatly dilated to form transverse crescentic vitelline reservoir.

Excretory system not fully worked out. Apparently 4 vessels emerge from plexus of ducts in pharyngeal region and pass posterad, 2 pairs submedian, medial to caeca for most of length, and 2 pairs closer to lateral margins, lateral to caeca; lateral ducts bear numerous diverticula and occasionally anastomose with submedian ducts which unite posterior to testes to form central excretory stem which opens through terminal excretory pore.

#### Discussion

Rudolphi (1819) described *Distoma gelatinosum* from *Chelonia mydas* from Rimini on the Adriatic Sea. Dujardin (1845) and Diesing (1850) were the next authors to mention the species, but apparently they both confused Rudolphi's species with some other form (Braun 1901). Dujardin (1845) repeated the original description, and supplemented it with observations on fragments of worms from the Vienna Museum. Diesing (1850), working in Vienna, described under the name of *Distomum gelatinosum* worms from the Amazonian chelonian *Podocnemis expansa*, collected by Natterer. Braun (1901) could find no specimens of *D. gelatinosum* in the Vienna collection and concluded that both Dujardin and Diesing had made errors in identification. Possibly unaware of Braun's views, Viana (1924) and Travassos *et al.* (1969) have included *Rhytidodes gelatinosus* in their lists of trematodes from Brazil.

Pratt (1914) distinguished his new species, *R. secundus*, from *R. gelatinosus* apparently solely on the basis of body size. Looss (1902) had given the size range of the species as 13–28 mm, describing the largest specimens as being stretched and compressed. Pratt's description was based on 27 specimens all between 4 and 9 mm long, the largest of which were mature. Later authors generally regarded *R. secundus* as a synonym of *R. gelatinosus*, arguing that size alone was not a good basis for the separation of species (Caballero-Rodriguez 1960; Bilqees 1974). The present authors have specimens in two ranges of size (see Table 2), a single relaxed and extended specimen 17.13 mm long, and six rather contracted specimens ranging from 5.87 to 7.9 mm long.

Simha and Chattopadhyaya (1969) proposed the name *Rhytidodes indicus* for three specimens from *Eretmochelys imbricata* from India. Diagnostic features given for this species were as follows: (1) The presence of a long muscular cirrus (small in the type-species). The figure of *R. indicus* shows an apparently compressed whole mount with the cirrus protruding. However, Looss (1902) observed that the ejaculatory duct was everted in his compressed specimens, and in the compressed specimen figured by Bilqees (1974) the ejaculatory duct is everted. The degree of protrusion of the ejaculatory duct or cirrus (which presumably Simha and Chattopadhyaya took as an indication of cirrus length) in compressed material cannot be accepted as a specific character. (2) The presence of a sucker-like structure posterior to the posterior testis. Concerning this, Simha and Chattopadhyaya (1969) said 'The exact nature and function of this structure could not be assessed'. From their description and figure, it is scarcely possible to make any useful comment. (3) The presence of a ventrolateral and a dorsolateral ridge on each side of the oral sucker. In *R. gelatinosus* the oral sucker bears a ventrolateral expansion on each side and a transverse dorsal ridge (Figs 28, 29), the latter extending onto the dorsolateral regions in such a way as to appear as a dorsolateral lip in flattened whole mounts. Only in unmounted specimens can the true nature of the dorsolateral ridge be seen.

A number of authors have commented on the distribution of vitellaria in *R. gelatinosus*. There is usually, but not invariably, a break in the vitelline field at the level of the ovary. Other breaks sometimes occur at the level of either of the testes. Caballero y C. (1954) speaks of the vitellaria being arranged in four or five lateral groups. This is clearly a variable feature in *R. gelatinosus* and may depend in part on the degree of contraction of individual specimens.

Table 2. Dimensions of *Rhytidodes gelatinosus* from *C. caretta*  
All measurements in millimetres

	Extended specimen	Contracted specimens		N
		Mean	Range	
Body, length × width	17.13 × 1.61	6.80 × 1.35	5.85–7.90 × 1.19–1.54	6, 6
Oral sucker,				
length × width	0.46 × 0.74	0.38 × 0.58	0.35–0.41 × 0.50–0.71	5, 6
Pharynx, length × width	0.22 × 0.24	0.22 × 0.16	0.18–0.24 × 0.15–0.17	4, 5
Oesophagus, length	2.4	0.78	0.58–0.90	5
Ventral sucker,				
length × width	0.38 × 0.42	0.29 × 0.34	0.23–0.32 × 0.30–0.37	6, 6
Cirrus sac, length				
× width	0.53 × 0.42	0.35 × 0.31	0.30–0.37 × 0.24–0.40	6, 6
Ovary, length × width	0.19 × 0.33	0.15 × 0.22	0.14–0.18 × 0.18–0.26	6, 6
First testis, length				
× width	0.74 × 0.33	0.34 × 0.39	0.29–0.40 × 0.33–0.42	6, 6
Second testis, length				
× width	0.89 × 0.38	0.40 × 0.33	0.28–0.44 × 0.28–0.36	6, 6
Egg, length × width	0.067 × 0.035		0.063–0.066 × 0.035–0.040	
Locality	Mon Repos, Qld		Shark Bay, W.A.	
Date	19.xii.1976		5.vii.1979	

Looss (1902) reported that the genital pore opens on a small bulge just in front of the ventral sucker. Pratt (1914) could see no such structure. Other authors appear to have made no mention of this feature. In the Australian material, there is a bulge in front of the ventral sucker, but this lies mostly to the left of the genital pore.

This is the first record of *R. gelatinosus* from Australia. The species was included in Johnston's (1912) 'Census of Australian Reptilian Entozoa' (see also Ernst and Ernst 1977), but the specimens referred to had been collected in New Guinea by Dahl (see Braun 1901).

#### Acknowledgments

We wish to thank all those who helped this study by supplying or lending specimens or assisting in other ways. Special thanks must go to Dr R. J. G. Lester and Mr B. Bartley. Employees of the Nor'West Whaling Company supplied invaluable aid during field work in Western Australia. Travel money for this field work was provided by the Fishing Industry Research Trust Account. To the following we are indebted for the loan of parasite specimens: Dr J-L. Albaret, Dr A. Andersson, Dr J. R. Lichtenfels and Mr J. Walker. This work was carried out during the tenure of a University of Queensland Post-Doctoral Fellowship awarded to the senior author. The second author collected specimens as part of the Queensland Turtle Research Project of the Queensland National Parks and Wildlife Service, funded in part by the Australian National Parks and Wildlife Service.

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 Euzet, L., and *Fr.* 87, 15–22.  
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1976/5

## Further Observations on a Bucephalid Trematode Infection in Scallops (*Pecten alba*) in Port Phillip Bay, Victoria

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### Abstract

Of 1250 scallops sampled from four areas over a 16-month period, 387 (31%) were infected by the trematode *Bucephalus* sp.

Parasitized gonads reached their maximum weight in January, 3 months after non-parasitized gonads had reached their maximum and 2 months after the normal spawning period. In the same month, cercariae first appeared within the sporocysts. They were present for the next 5 months during which time the weight of the gonad and muscle decreased. The muscle weight returned to and exceeded the weight of controls in September-October, a time when cercariae were not being produced and when normal unparasitized scallops were spawning.

The characteristic red colour of parasitized gonads occurred at all times of the year. It was not related to the stage of maturity of the parasite.

Additional keywords: parasite, mollusc.

### Introduction

Bucephalid trematodes, which parasitize many commercially important bivalves, frequently destroy the host's reproductive tissue (for review see Howell 1967). Little else is known about their gross effects though Menzel and Hopkins (1955) noticed that *Crassostrea virginica* infected with *Bucephalus cuculus* grew faster than uninfected oysters. They added that in the field bucephalid-infected oysters did not become watery during the spawning season, but 'remained fat and retained an excellent flavour all summer'.

This paper reports incidental observations on a bucephalid infection, gathered during a study on the biology of the scallop *Pecten alba*. The presence of the parasite, well known to Bass Strait fishermen because it turns the gonad bright red, was first reported by Sanders (1966). The specific identity of the parasite, which normally sterilizes the host, is still unknown. Sections of parasitized tissue have been lodged in the South Australian Museum, Adelaide (Accession No. A.H.C. S499-502, ST503-506).

### Materials and Methods

Samples of 15-25 scallops mostly 75-100 mm in length were taken monthly from January 1965 to April 1966 from two to four places in Port Phillip Bay, Victoria: off Altona, Brighton, Portarlington, and Point Cook. Length was recorded to the nearest millimetre. The tissues were separated into muscle, gonad, or waste, and weighed to the nearest gram. Representative gonads were photographed in colour. Tissue fixed in Davidson's fixative was sectioned, stained with haematoxylin and eosin, and examined for the presence of cercariae.

The relationship between tissue weight  $W$  and shell length  $l$  was determined for the unparasitized scallops in each sample using the equation

$$\log(W) = \log(a) + b \log(l).$$

The slopes for the samples were compared, and significant differences were found between samples at Brighton for waste weight, and between samples at Portarlinton for muscle and gonad weights. However, in the former case there appeared to be no systematic differences and in the latter case the increase in the slopes was not great. The additional reduction in the error mean square that resulted from assuming different slopes for each sample was small, so that it was considered justified to use the same slope for all samples of the same tissue, with a considerable gain in simplicity of analysis. Using the pooled slopes, a theoretical tissue weight ( $w$ ) at a standard length of 85 cm was calculated for each sample. Seasonal variations in the adjusted weights were assumed to follow the form:

$$\begin{aligned} \log(w) &= A + B \cos[2\pi(T - T_0)/12] \\ &= A + C \cos(2\pi T/12) + D \sin(2\pi T/12). \end{aligned}$$

where  $T$  is the month of the year,  $A$  is a constant non-seasonal term,  $B$  is the amplitude of the variation and  $T_0$  is the month of highest weight. The data from the four different areas were combined. The values of  $A$ ,  $B$  and  $T_0$  were estimated by finding the regression of  $\log(w)$  against  $\cos(2\pi T/12)$  and  $\sin(2\pi T/12)$ . The data from the parasitized scallops were analysed in the same way.

Hypotheses on the equality of parameters were tested by the following method: the sum of squares of deviations and error mean square were calculated assuming the parameters to be different. The sum of squares of deviations which resulted when the hypothesis was assumed to hold was calculated and the increase in the sum of squares of deviations was divided by the additional degrees of freedom. This mean square was compared to the error mean square by means of  $F$ -tables to give a significance probability, small values of which caused rejection of the hypothesis.

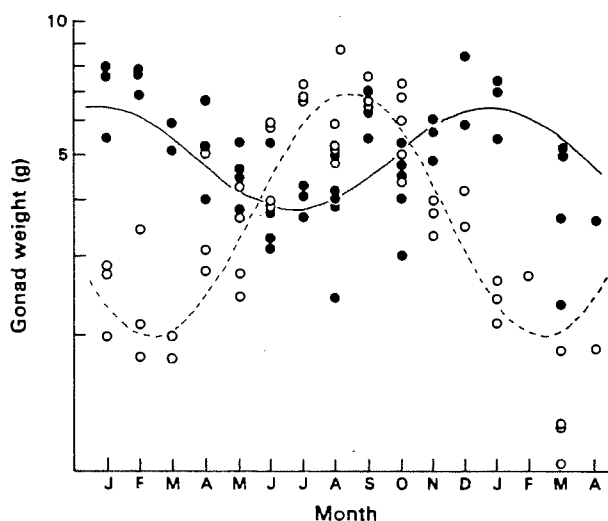


Fig. 1. Mean gonad weight per sample (adjusted for a standard 85-mm scallop) plotted against month. ● Scallops with bucephalid infection. ○ Unparasitized scallops.

## Results

As expected, the gonad weights of uninfected scallops showed marked seasonal variation (Fig. 1, ○), the peak in August coinciding with the onset of the spawning season. The weights of parasitized scallops (Fig. 1, ●) also showed seasonal variation but peaked in January. The muscle weights of uninfected scallops (Fig. 2, ○) showed little seasonal change. However, the muscles of parasitized scallops (Fig. 2, ●) underwent a marked fluctuation and were clearly heaviest in September–October. The weights of the remaining tissue, the waste, of both normal and parasitized scallops were very variable. There were slight seasonal changes and both appeared marginally heavier in September–October compared with other times of the year.



Statistical ( $F$ ) tests on the amplitudes and phases of the fitted curves showed no significant differences between the four locations in the bay; however, scallops from Brighton and Point Cook were generally heavier (for the same length) than scallops from Portarlington and Altona.

The histological sections showed that the parasitized gonads contained sporocysts throughout the year. The proportions of parasitized scallops that contained cercariae, the next stage in the life cycle of the parasite, are given in the following tabulation:

Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M
No. examined per month	3	7	5	6	13	8	12	14	8	10	5	3	6	2	5
Proportion with cercariae	1.0	1.0	1.0	1.0	0.8	0.9	0.8	0.7	0.4	0	0	0	1.0	1.0	0.8

Cercariae were readily seen within sporocysts collected in most seasons but were absent in October, November and December. The tabulation includes data from very light infections in March, May, June, July and October in which little gonad was destroyed. Sporocysts in these infections contained no cercariae and were believed to be relatively new. The tabulation also includes two cases in the August sample where the cercariae were apparently dying.

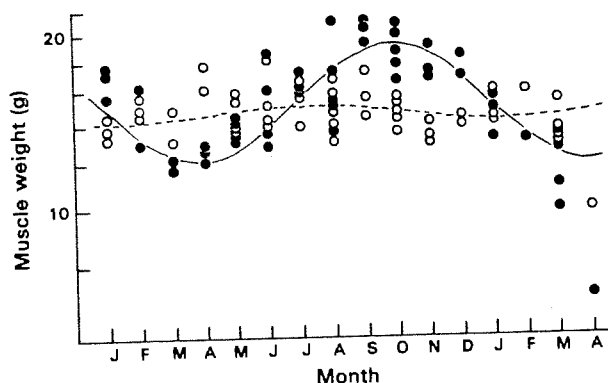


Fig. 2. Mean muscle weight per sample (adjusted for a standard 85-mm scallop) plotted against month. ● Parasitized scallops. ○ Unparasitized scallops.

About one-third of the parasitized gonads were red. Others were orange or brownish pink. Red gonads occurred in all months of the year including October, November and December. The colour was unrelated to the state of maturity of the parasite.

### Discussion

The lack of cercariae and the presence of dying cercariae suggested that there was little cercarial production between August and December. During this time the parasitized gonad greatly increased in weight, from a minimum in July to a maximum in January. This was evidently due to an increase in the biomass of the parasite as in histological sections the parasite was seen to occupy the bulk of the organ. The energy for this increase may have come from the supplies that would normally have been used to develop and maintain the host gonads through the spawning season (September–November).

During the spawning season the muscles of parasitized scallops were heavier than normal. An abnormal increase in size has been reported in many molluscs with trematode infections (see Cheng 1967). However, in the scallop the trend did not

continue and the muscle weight began to drop in January prior to the production of cercariae. Over the following 6 months while cercariae were being produced, the average weight of the muscle fell below that of unparasitized scallops, perhaps reflecting a strain on the energy resources of the host.

The red colour of parasitized gonads, as it was not related to the state of maturity of the parasite, may be a reflection of the age of the infection.

#### Acknowledgments

Mr David Powell and Pamela Davies, both of the Arthur Rylah Institute of Environmental Research, Heidelberg, Vic., are gratefully acknowledged for undertaking data analyses. The junior author thanks the Australian Fishing Industry Research Committee for financial support.

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Manuscript received 6 August 1980, accepted 27 February 1981

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## Description of and observations on *Grillotia branchi* n.sp., a larval trypanorhynch from the branchial arches of the Spanish mackerel *Scomberomorus commersoni*

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### Summary

A new species of trypanorhynch is described from blastocysts found within the gill arches of *Scomberomorus commersoni* caught off eastern Australia. It differs from other members of the genus in having 9 to 10 hooks per principle row, scattered intercalary hooks that merge with the band of small hooks, and a well-developed basal armature. Developing stages were found both within and outside branchial blood vessels. Thousands of fully developed blastocysts were within the gill arches. Around and between them was fibrous tissue, haemorrhage, and evidence of chronic inflammation.

### Introduction

During a survey for marine parasites, fishermen drew attention to larval tapeworms in the narrow-barred Spanish mackerel *Scomberomorus commersoni* (Lacépède). The most obvious worms, occurring in the body-cavity, were a species of *Pterobothrium*. Further examination of the fish revealed large numbers of a second much smaller species within the gill arches. These, members of the genus *Grillotia*, were of interest for several reasons. They represented a previously undescribed species, they appeared adversely to affect their host, and, as they were not distributed uniformly over the host's range, they were potentially valuable as a 'biological tag'. About 20 species of *Grillotia* have been

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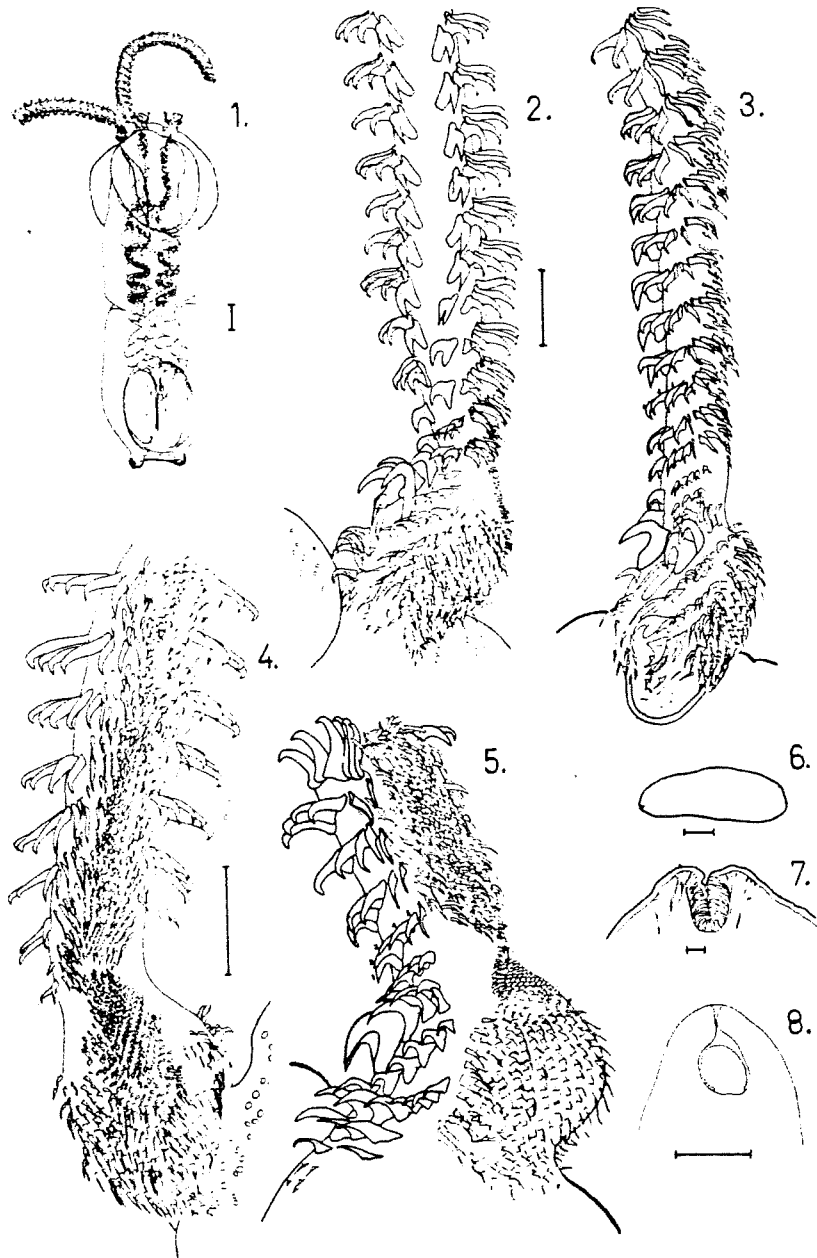
described. None has previously been reported from *Scomberomorus*.

The description given below is based upon the metacestode as adults were not available for study. However, the scolex was well developed in the larva and it is the features of this organ that are used primarily for separating members of the genus. The description and diagnosis are followed by observations on the biology of the larva and on the interaction between it and its host.

### Material and methods

Parasites were collected from 28 *Scomberomorus commersoni* caught off eastern Australia, and five off Pulau Ketam, Malaysia. As only the head of the fish was available for dissection in some cases, all fish sizes are given in terms of head length (distance from tip of jaw to posterior edge of operculum;  $x$  mm). These can be converted to fork length ( $y$  mm) using the formula  $y = 450 + 2.8x$  which was calculated from measurements of 25 fish.

The branchial arches from one side of each fish were removed, measured, and the number of metacestodes present estimated by counting the cysts in 10 mm of each gill arch. Representative scolices were excysted by dissection, relaxed in tap water for 30 min and fixed in 10% formalin. Whole mounts were stained with haematoxylin. Histological sections of gill arches were stained with haematoxylin and eosin (H & E), Prussian blue and Van Gieson. Drawings were done using a camera lucida. Measurements are in micrometers unless otherwise stated. The mean is followed in parentheses by the



Figs. 1 to 5. Fully developed metacestode of *Grillotia branchi* n.sp.

Fig. 1. Scolex. Bar = 100  $\mu$ m.

Fig. 2. Internal surface of tentacle (twisted at level of metabasal armature to show part of bothridial surface). Bar = 50  $\mu$ m.

Fig. 3. Bothridial surface. Same scale as preceding figure.

Fig. 4. External surface. Bar = 50  $\mu$ m.

Fig. 5. Antibothridial surface. Same scale as preceding figure.

Figs. 6 to 8. Developing metacestodes of *G. branchi*.

Fig. 6. Most immature form found. Bar = 500  $\mu$ m.

Fig. 7. Anterior end of most immature form. Bar = 10  $\mu$ m.

Fig. 8. Anterior end of a later stage showing invagination and area of development of future scolex. Bar = 500  $\mu$ m.



U.S.N.M. Nos. 76386-7. Three paratypes. B.M. (N.H.) Accession Nos. 1981.3.8.1-3. Three paratypes, Queensland Museum, Brisbane, Australia, Col. Nos, GL4-6.

#### Other observations

Metacestodes were found in various stages of development. What appeared to be the youngest stage was a cylindrical whitish worm about 2.5 mm in length with a muscular sucker at the anterior end (Figs. 6, 7). This was found within one of the blood vessels. The next stage, found both within and around blood vessels, was an elongate worm up to 10 mm in length with signs of a developing scolex at the anterior end (Figs. 8, 9). Bothridia formed before tentacle bulbs and tentacles. By the time the scolex was fully developed a thin capsule had formed around the worm (Fig. 9). With time, the worm evidently decreased in size and, in so doing, caused the capsule wall to crinkle, so that eventually the worm was reduced to a small opaque mass lying in clear gelatinous material within a tough horny wrinkled capsule.

Black pigment, possibly melanin, was laid down around cysts and this made them visible through the translucent walls of the intact gill arch (Fig. 10). Histological sections showed that the cysts were packed together filling most of the cavity within the arch (Fig. 11). Fibrous tissue was present around the cysts especially in the ventral part of the cavity, and there were areas of extensive cellular infiltration suggesting inflammation. Masses of brown material that stained positive with the Prussian blue test were evidently haemoglobin breakdown products from earlier sites of haemorrhage. The bone of the gill arch adjacent to cysts was eroded.

The parasites were abundant in the fish. In 23 fish from Point Lookout, mean head length 190 (range 140 to 250) mm, there were estimated to be an average of 1800 (80 to 5900) parasites per fish (Fig. 12). In 5 fish from Cairns, mean head length 180 (150 to 190) mm there were 1250 (390 to 2400) per fish, and in 5 fish from Pulau Ketam, Malaysia, (head length 160 (150 to 210) mm) there were 65 (26

to 150) parasites per fish. In all samples there was a weak but significant positive correlation between parasite abundance and fish size (Fig. 12).

#### Discussion

The developing stages of *G. branchi* resemble those of *Lacistorhynchus tenuis* described by Dollfus (1942) and Mudry & Dailey (1971), and *Gilquinia squali* by Mackenzie (1975). According to Dollfus (1942), as the scolex of *L. tenuis* develops there is a decrease in cyst size resulting in the formation of ridges in the walls of the pyriform blastocyst. This corresponds to the features observed in this study

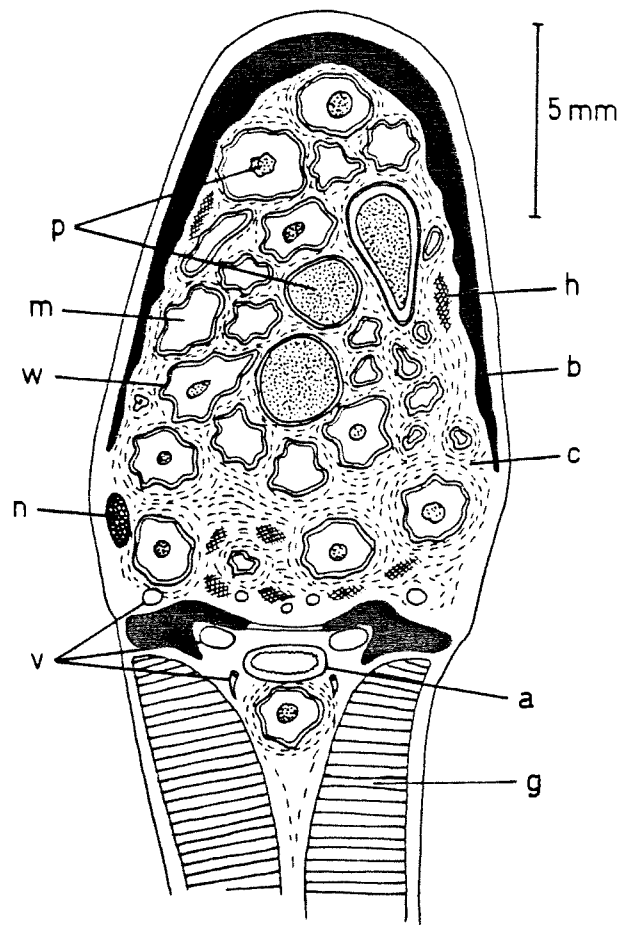


Fig. 11. Diagram of section of gill arch showing metacestodes (p), gelatinous matrix (m), blastocyst walls (w), and areas of haemorrhage (h). Other symbols: a, afferent artery; b, bone; c, connective tissue; g, gills; n, nerve; v, blood vessels.

# The taxonomy of the family Paramphistomidae Fiscoeder, 1901 with special reference to the morphology of species occurring in ruminants.

## I. General considerations\*

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### Summary

The various published schemes of classification of the family Paramphistomidae Fiscoeder, 1901 are reviewed. The morphological and histological characters employed in the classification of paramphistomid trematodes of ruminants are discussed and assessed. The value of histological details (Näsmark's system) and of tegumental papillae as specific characters is recognized. Different types (some new) of acetabulum, pharynx and terminal genitalium (newly defined) in median sagittal section are defined and illustrated and a key to their separation is provided. Various types of lining and muscular development of the oesophagus are illustrated and scanning electron photomicrographs of the different types of tegumental papillae observed on the anterior region of some species are provided.

### Introduction

#### *The family Paramphistomidae*

The family Paramphistomidae was established by Fiscoeder (1901, 1902, 1903) for two new subfamilies, Paramphistominae and Cladorchiinae. Stiles & Goldberger (1910) erected the superfamily

\* Part of a thesis approved by the University of London for the award of the Ph.D. degree.

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Paramphistomoidea and assigned to it the Paramphistomidae Fiscoeder, 1901 and two new families, the Gastrodiscidae and Gastrothylacidae. They restricted the Paramphistomidae to the subfamilies Paramphistominae Fiscoeder, 1901, Cladorchiinae Fiscoeder, 1901 and Diplodiscinae Cohn, 1904. Since then there have been additions to and changes in the structure of the family. The major systems of classification proposed since 1910 are those of Stunkard (1917, 1925), Mapestone (1923), Fuhrmann (1928), Fukui (1929), Travassos (1934), Näsmark (1937), Southwell & Kirshner (1937), Szidat (1939), Skrjabin (1949), Yamaguti (1958, 1971) and Baer & Joyeux (1961).

Opinions on the scope and subdivision of the family vary greatly because authors differ widely about which characters are of family, subfamily or generic value. Furthermore, some systems were based only on analysis of the literature and not on extensive and critical examination of specimens. No single system yet published is entirely satisfactory but that proposed by Yamaguti (1971) is the most comprehensive and is largely followed in this work. The ten genera and their synonyms in this study are therefore arranged as follows:

Paramphistomoidea	
Paramphistomidae	
Paramphistominae	<i>Paramphistomum</i> (Syn.: <i>Liorchis</i> , <i>Srivastavaia</i> )
	<i>Calicophoron</i> (Syn.: <i>Bothriophoron</i> )
	<i>Gigantocotyle</i>



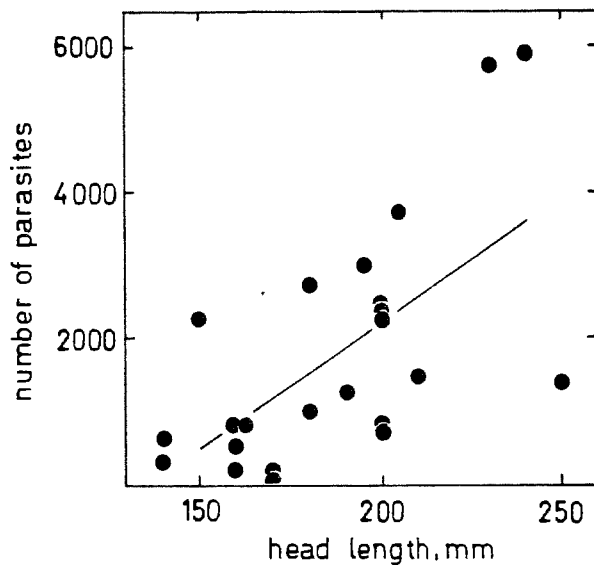


Fig. 12. Fish head length v. estimated total number of parasites per fish, for 23 fish from Point Lookout. (Correlation coefficient = 0.64.)

to pick up infection. Adult *S. commersoni* are and is evidently the way in which the long, white, cylindrical body is transformed to the wrinkled cyst.

The occurrence of larval cestodes resembling procercoids within the gill arches and the positive correlation between the number of parasites and fish size indicate that adult fish were continuing specimens of *L. tenuis* and *G. perelica* (paratypes) predatory fishes feeding almost solely on medium sized teleosts (Hu, 1973). If one of these is the source of infection, *G. branchi* may have a life-cycle similar to that described by Nakajima & Egusa (1969) for *Callitetrarhynchus gracilis*.

The large number of metacestodes packed within the gill arches may impair the performance of the fish. The eroded bone revealed in histological sections would certainly weaken the gill arch but the effect on the vascular system may be of greater significance. Immature metacestodes appear to break from blood vessels, presumably the afferent artery and its branches, causing damage to the vessel walls. Most of the parasites eventually come to lie within a cavity in the gill arch. Their bulk plus that of the inflammatory tissue that they elicit during their migration may restrict expansion of the efferent arteries and cause lymph ducts to collapse.

This could result in oedema in the gill lamellae and affect respiratory exchange.

#### Acknowledgements

Fish were kindly supplied by Mr. P. Spinner and Mr. L. Nash, commercial fishermen and Mr. G. MacPherson, Queensland Fisheries Service. Dr. M. Dailey, California State College, and Dr. J.R. Lichtenfels, U.S.N.M., generously loaned us specimens of *L. tenuis* and *G. perelica* (paratypes) respectively. Dr. J.C. Pearson and Dr. H.M.D. Hoyte, University of Queensland, read an earlier draft of the manuscript. For financial support we thank the Australian Fishing Industry Research Committee and, for an overseas scholarship to the senior author, the Universiti Pertanian Malaysia.

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Accepted for publication 10th February, 1981.

4 *Grillotia branchi* n.sp. on *Scomberomorus commersoni*

*Type host:* *Scomberomorus commersoni*

*Site:* Encapsulated within the gill arches. Also found adjacent to the ventral aorta.

*Type locality:* Point Lookout, Brisbane, Australia

(27° 25' S, 153° 25' W). Also found in *S. commersoni* from Cairns, Australia, and Pulau Ketam, Malaysia.

*Specimens deposited:* Holotype and three paratypes.

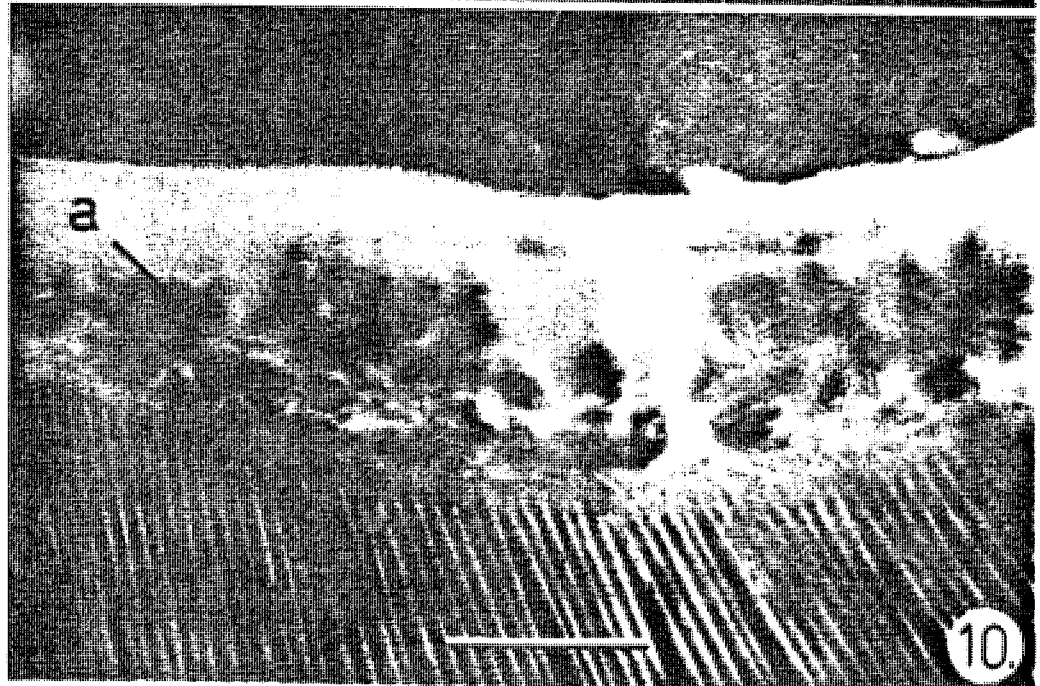
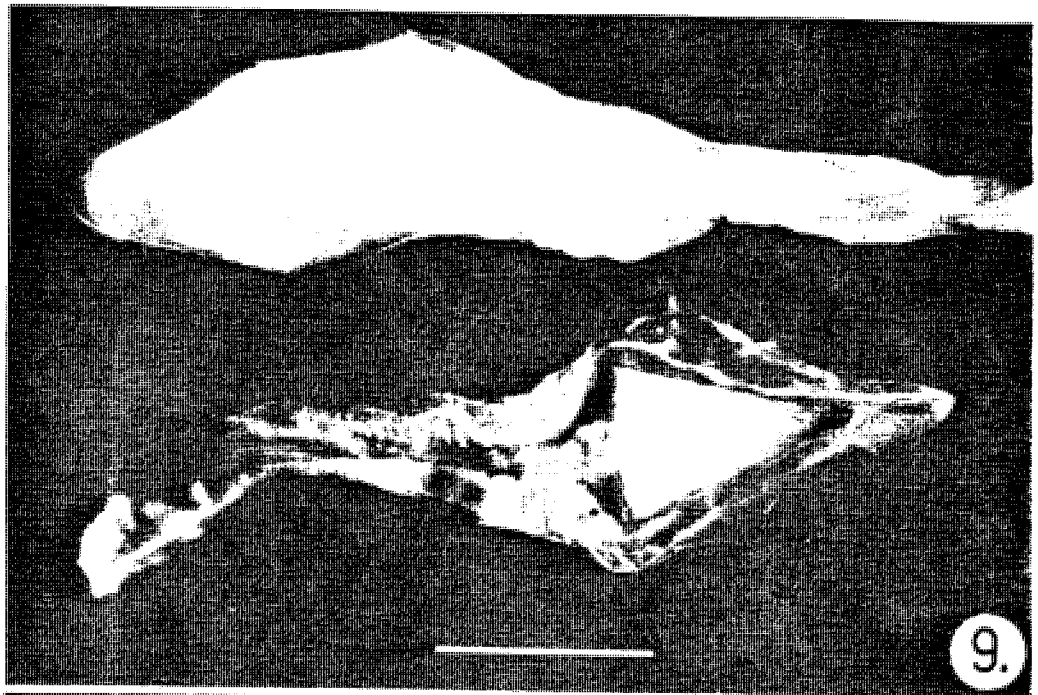


Fig. 9. Blastocysts of *G. branchi*. The lower one is believed to be the older of the two. Bar = 2 mm.

Fig. 10. Gill arch of *S. commersoni* infected with *G. branchi*. Note the dark areas (a) marking the positions of the cysts. Bar = 10 mm.



range. The terminology used to describe the worms is similar to that used by Dollfus (1942).

## Results

*Grillotia branchi* n.sp. (Figs. 1 to 5)

*Description* (Based on 10 mounted scolices from fully developed blastocysts):

Scolex length 1510 (1120 to 2000); two bothridia 495 (340 to 530) by 330 (100 to 510), each weakly notched posteriorly and with thickened lateral and ventral margins. Pars vaginalis 975 (740 to 1350) by 330 (285 to 450); pars bulbosa 340 (285 to 450) by 410 (310 to 600); pars post bulbosa 52 (42 to 68) by 182 (150 to 230) with a posterior involution covered with spines about 4 long. Tentacle bulbs 330 (285 to 370) by 105 (85 to 120); retractor muscle attached one third of the way down. Tentacles 1400 (1200 to 1500) by 46 (40 to 55). Tentacle sheaths coiled.

Metabasal armature poeciloacanthus; basal armature present. In metabasal armature, each half turn has 9 to 10 hooks: hooks 1 (1') on inner side largest, base 24 (18 to 27), length 26 (23 to 31), height 15 (14 to 16) (Fig. 2). Hooks 2 (2') to 9 (9') are more slender, less recurved, and gradually decrease in length from 24 to 14 (Fig. 3, counting from left to right). Between the 6th and 9th hook are a group of 5 to 6 intercalary hooks, 7 long. These merge with the band of small hooks, each 6 to 11 long, which runs up the middle of the external face (Fig. 4). The band is about 5 hooks wide. Basal armature: on internal surface 6 rows of hooks, the first 3 weakly recurved, the second 3 more strongly recurved (Fig. 5). Largest hook base 34, length 37, height 24. Number of hooks in each half turn 3 to 7. On external surface, proximal part bears longitudinal rows of spines 6 to 11 in length. Above these is a field of small hooks each 2 long, covering an area 46 by 31. On either side are bare areas devoid of hooks or spines. The basal armature is on a swollen part of the tentacle 110 (81 to 116) long by 68 (52 to 75) wide.

Scolices occurred within a tough horny capsule 3 mm wide and up to 12 mm long.

## Diagnosis

The presence of the band of small hooks, two bothridia, and a short acraspedote scolex puts the helminth into the genus *Grillotia*. Of the 16 *Grillotia* species where the tentacle armature is known, only 6 have a clearly defined region at the base of the tentacles where there are modified hooks. These are *G. smarigora* (Wagener, 1854), *G. pseudoerinae* Dollfus, 1969, *G. microthrix* Dollfus, 1969, *G. angeli* Dollfus, 1969, *G. bothridiopunctata* Dollfus, 1969 and *G. perelica* (Shuler, 1938). *G. branchi* differs in many respects from the first five species but is most easily distinguished from them by the number of hooks per principal row. *G. branchi* has 9 to 10 and these species all have 4.

*G. branchi* most closely resembles *G. perelica*, a species with 10 or 11 hooks per principle row. It can, however, be readily separated from it by the intercalary hooks. *G. perelica* has 7 to 8 intercalary hooks, quite distinct from the band of small hooks, and 5 to 6 of them are arranged in a straight line, whereas those of *G. branchi* merge with the band of small hooks and are evenly distributed. In addition, the first 6 rows of hooks on the internal surface contain 6 to 11 hooks (versus 3 to 7 for *G. branchi*), and the largest hooks in these rows are about the same size as hooks 1/(1') on the tentacle proper. In *G. branchi* the largest basal hooks are 1.5 times the size of any on the tentacle proper. The area of spines at the base of the external surface, prominent in *G. branchi*, is greatly reduced in *G. perelica*, and the retractor muscle is attached at the bottom of the bulb, not one third of the way down as in *G. branchi*.

Dollfus (1969) recognized two subgenera within *Grillotia*: *Paragrillotia* and *Grillotia*. *G. branchi* falls within the former as the intercalary hooks are not clearly separate from the band of small hooks and the bothridia can appear non-patelliform. However, it is easily separated from the two members of this subgenus, *G. simmonsii* Dollfus, 1969 and *G. rowei* Campbell, 1977, by the presence of the special basal armature.

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