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THREE NEW MICROPHALLID TREMATODES FROM TASMANIAN BIRDS

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(with seven text-figures)

ABSTRACT

The morphology of *Atrichophalophorus coxiellae* n.sp., *Maritrema calvertensis* n.sp., and *Microphallus tasmaniæ* n.sp. (Trematoda: Microphallidae) is described. The description of *A. coxiellae* is based on metacercariae found encysted in the hepatopancreas and gonads of the brackish water snail *Coxiella badgerensis* Johnston 1878. Adult, but sexually immature, trematodes taken from the small intestine of the dotterels *Charadrius cucullatus* Vieillot and *Charadrius ruficapillus* Temminck, which feed on *Coxiella badgerensis*, are considered identical with the metacercariae from the snail. The account of *Maritrema calvertensis* n.sp. is based on adult trematodes found in the lower intestine, caeca, and rectum of the duck, *Anas castanea* Eyton, and dotterels *Charadrius cucullatus* and *C. melanops* Vieillot. From a comparison of metacercariae taken from certain small cysts found in the amphipod *Austrochiltonia australis* Sayce 1901, and also in the alimentary tract of the birds, it is assumed that the amphipod is an intermediate host. The description of *Microphallus tasmaniæ* n.sp. is based on adult trematodes found in the intestine, caeca and rectum of *Anas castanea* and the dotterels *Charadrius cucullatus*, *C. melanops* and *C. ruficapillus*. Certain large cysts found in the amphipod *Austrochiltonia australis*, and also in the gut of the birds infected with *Microphallus tasmaniæ* n.sp., contained metacercariae almost identical with the adult. It is therefore assumed that the amphipod is an intermediate host of *M. tasmaniæ* n.sp. The definitive and intermediate hosts of the trematodes described were all taken at Calverts Lagoon, Tasmania.

INTRODUCTION

Yasin, (1967), in an account of the ecology of Calverts Lagoon, a permanent brackish water pond situated about 35 km south east of Hobart, Tasmania, reported the occurrence of larval digenetic trematodes in the snail *Coxiella badgerensis*. *C. badgerensis* is the only mollusc known to inhabit the lagoon, and therefore all the digenetic trematodes which complete their life cycles at the lagoon must pass through their early larval development in this snail.

An investigation of the trematodes of the fauna of the lagoon has revealed the presence of three new species of microphallids. The description of the three species forms the subject of this paper.

Only one microphallid trematode has previously been described from Tasmanian fauna, that being *Maritrema ornithorhynchi* Hickman 1955, which was found in the intestine of the platypus *Ornithorhynchus anatinus* Shaw. Many microphallid species have been recorded from fauna, mainly charadriiform and anatiform birds, on the Australian mainland, (Johnston 1916; Deblock and Pearson 1968, 1969).

MATERIALS AND METHODS

One *Anas castanea*, three *Charadrius cucullatus*, two *C. melanops*, and one *C. ruficapillus* inhabiting Calverts Lagoon were shot, taken to the laboratory and examined for adult trematodes. Hundreds of specimens of the snail, *Coxiella badgerensis*,

and the amphipod *Austrochiltonia australis* were collected from the lagoon and examined for trematode larval stages. Three types of metacercariae were found; one encysted in the snail and two encysted in the amphipod. The two types occurring in the amphipod were easily distinguished by a difference in size, one being twice the diameter of the other. To facilitate the examination of the metacercariae, the larvae were excysted. Excystment of those from the snail, and the larger of the two types from the amphipod was accomplished using a similar method to that described by Dixon (1966). After immersion in 0.3% pancreatin solution for 30 minutes the cysts were incubated in Hank's saline. Excystment of the smaller cysts from the amphipod was effected more successfully by immersion in sheep's bile, instead of the pancreatin solution. All media used for excystment were maintained at about 39°C. Metacercariae with thick cyst walls and accumulations of excretory granules in their excretory vesicles were found to excyst more readily than those with thin cyst walls and no excretory granules. Unsuccessful attempts were made to infect chickens and rats with the three types of metacercariae (Smith 1971). Metacercariae from the snail were found to excyst within chickens, however they were voided within a few hours.

Some live adult flukes removed from the intestines of wild birds, and some of the excysted metacercariae, were drawn and photographed prior to fixation. The vital stains neutral red and brilliant cresyl blue were sometimes used to aid the morphological studies. Observations of flame cells and excretory ducts were facilitated by mounting the live specimens in tap water, or Hank's saline, under a coverslip, and then allowing the slide to dry slowly.

Fixation was accomplished using either T.C.A. modified Bouin's fixative, (T.C.A. replacing acetic acid), or 10% formal saline, after Dixon (1965). The trematodes were placed on a slide under slight coverslip pressure, relaxed in gentle heat, and then suddenly killed in hot fixative (Dawes 1946; Heard 1968). The flukes were stained whole in Horen's Trichrome (Horen 1957); Mallory's Heidenhain stain (Cason 1950); alum carmine; neutral red or toluidine blue. They were cleared in xylene, and mounted in either Canada Balsam, or D.P.X., via standard procedure.

In the following description all measurements were made on fixed material and are expressed in millimetres: the size range first, followed by the average in parenthesis.

Subfamily	MICROPHALLINAE	Ward 1901
Genus	ATRIOPHALLOPHORUS	Deblock and Rose 1964
	<i>Atriphallophorus coxiellae</i> n.sp.	

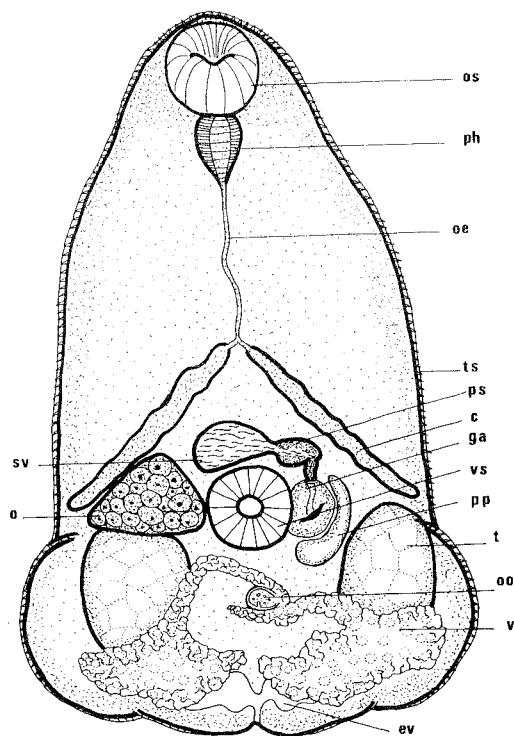
The following description is based on more than 50 well developed metacercariae, excysted experimentally, after removal from the snail *Coxiella badgerensis*. Motile spermatozoa were present in the seminal vesicles of some of the flukes.

Description (fig. 1)

Dimensions: Body length, 0.143-0.229 (0.195); maximum body width, 0.072-0.125 (0.090); oral sucker, 0.026-0.034 (0.029); ventral sucker, 0.026-0.031 (0.029); pharynx, 0.016-0.044 (0.020) X 0.015-0.020 (0.017); testes, 0.036-0.040 (0.039) maximum width; seminal vesicle, 0.029-0.036 (0.034) maximum width; ovary, 0.030-0.048 (0.035) maximum width; ootype, 0.018 x 0.013.

The body is pyriform to triangular; curved concave ventrally, particularly in the posterior region. The living fluke supports itself on the substratum by the oral sucker, and two posterior ambulatory buds. The outer tegument bears spines over the whole body, which are more prominent anteriorly. The spines are comb-like structures, triangular in lateral view. The round, subterminal, oral sucker is approximately equal in size to the ventral sucker; the latter is situated about two-thirds of the body length from the anterior end. The pharynx is spatulate; prepharynx is very short or absent, depending on the degree of contraction. The oesophagus varies in length as the fluke moves. The caeca may be slender or saccate; diverging widely and extending

0.050 mm



KEY TO SYMBOLS

c caecum; cs cirrus sac; e egg; ed excretory duct; eg excretory granules; ev excretory vesicle; fc flame cell; ga genital atrium; gp genital pore; o ovary; oe oesophagus; oo ootype; os oral sucker; ph pharynx; pp phallophore; pph prepharynx; ps pars prostatica; sv seminal vesicle; t testis; ts tegumental spines; v vitellaria; vs ventral sucker.

FIG. 1. - *Atriophallophorus coxiellae* n.sp., excysted metacercaria, showing reproductive and digestive systems, ventral view.

to the level of the ventral sucker.

The oval testes are lateral, slightly diagonal, in the posteroacetabular region. The seminal vesicle, between the caecal arch and ventral sucker, is oval to reniform. Extending from right to left, it leads via a well-developed pars prostatica, and sinuous ejaculatory duct, to the male papilla, which is contained within a simple, round, sinistral genital atrium. A large semi-lunar, muscular chamber lies in juxtaposition to the left of the genital atrium. The dextral ovary is oval to triangular; located at the acetabular level, between the testes and caecum of the right side. A thick walled, oval, ootype is situated posteromedially, at the level of the testes. Vitellaria are in two symmetrical groups, posterior to, or overlapping the testes; vitelline ducts converge anteriorly, meeting next to the ootype. The receptaculum seminis, Laurer's canal and uterus were not seen.

The excretory system is shown in figure 2. Excretory ducts in the posterior region are obscured by reproductive structures, however the distribution of flame-cells is typical of the family (Stunkard 1958; James 1968); the flame-cell formula is $2((2+2)+(2+2)) = 16$. Dark brown granules fill the V-shaped excretory vesicle in older metacercariae.

The incidence of *A. coxiellae* n.sp. in *Coxiella badgerensis* varies seasonally. However, from 1035 snails examined between February and October 1970, 17.2% were infected with cysts of this microphallid species. The cyst diameter increases from

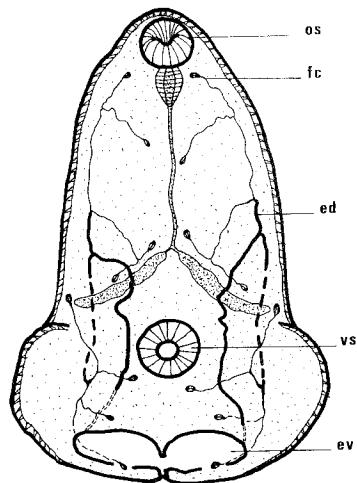


FIG. 2. - *Atriophallophorus coxiellae* n.sp., excysted metacercaria, showing excretory system, ventral view.

0.100
mm

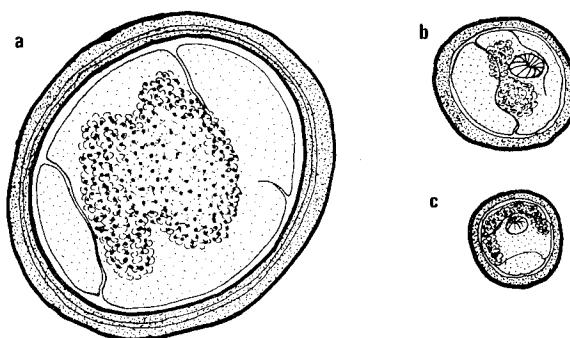


FIG. 3. - Cysts of (a) *Microphallus tasmaniensis* n.sp.; (b) *Maritrema calvertensis* n.sp.; and (c) *Atriophallophorus coxiellae* n.sp., drawn to scale.

Atriophallophorus Deblock and Rose 1964.

0.088 mm, newly formed, to 0.125 mm, in older cysts, (fig. 3). One of 3 specimens of the dotterel *Charadrius cucullatus* examined was infected with *A. coxiellae* n.sp. The only specimen of *Charadrius ruficapillus* examined was heavily infected with *A. coxiellae* n.sp. Specimens of *A. coxiellae* n.sp. obtained from the birds were identical in size and morphology to the excysted metacercariae from the snail - however they were not gravid, and details of the reproductive system were not clearly seen. Cysts, identical in size to those from the snail, and fragments of the snail were found in all of the infected birds.

Probable definitive hosts:

Charadrius cucullatus Vieillot, *C. ruficapillus* Temminck.

Habitat: small intestine and rectum.

Intermediate Host: *Coxiella badgerensis* Johnston.

Habitat: hepatopancreas and gonads.

Geographical Location: Calverts Lagoon (Tasmania).

Type Material: Tasmanian Museum - holotype K246; Paratypes K245, K247, K248.

DISCUSSION

No microphallids have previously been recorded from either of the two *Charadrius* species, *C. cucullatus* and *C. ruficapillus*.

A. coxiellae n.sp. is very similar to the diminutive microphallid *A. minutus* (Price 1934) Deblock and Rose 1964 in morphology and life history. Both species have reduced life cycles, cysts being formed within the molluscan host, however *A. minutus* develops in the freshwater snails *Hydrobia minuta* and *Amnicola limosa*, and infect the duck *Nyroca affinis* (Stunkard 1958). *A. coxiellae* has a large clavate, muscular chamber adjacent to the genital atrium. This structure appears to be analogous to the phallophore, an hemispherical, muscular, periatrial envelope which is characteristic of

The new species, *A. coxiellae* n.sp., is named after the snail, *Coxiella badgerensis*, which serves as host for its larval stages.

Subfamily	MARITREMATINAE	Belopolskaia	1952
Genus	MARITREMA	Nicoll	1907
<i>Maritrema calvertensis</i> n.sp.			

The following description is based on about 50 ovigerous specimens obtained from the duck *Anas castanea* and dotterels *Charadrius cucullatus* and *C. melanops*. Apart from differences in size, no marked variations are apparent in specimens either from the one, or from all three hosts.

Description (fig. 4)

Dimensions: body length, 0.191-0.268 (0.215); maximum body width, 0.124-0.187 (0.144); oral sucker, 0.023-0.034 (0.029); ventral sucker, 0.027-0.031 (0.029); pharynx, 0.018-0.022 (0.020) long and wide; testes, 0.036-0.047 (0.040) maximum width; ovary, 0.025-0.030 (0.027) maximum width; eggs (0.018-0.023 (0.021) x 0.010-0.015 (0.012).

The body is ovoid to pyriform; maximum width occurring at the level of the anterior of the testes. The outer tegument is uniformly covered by minute, comb-shaped spines. The subterminal oral sucker, and medial ventral sucker are approximately equal in size. The prepharynx is short or absent depending on the state of contraction; pharynx is barrel-shaped. The short oesophagus bifurcates to form obtusely diverging caeca, which extend two-thirds of the way to the lateral body wall.

The ovoid testes are opposite, postero-lateral to the ventral sucker. A well-developed cirrus-sac extends from left to right, almost the body width, between the caecal arch and ventral sucker. Within the cirrus-sac is the clavate, seminal vesicle, tapering towards the pars prostatica. The distal end of the cirrus sac is hooked; ejaculatory duct curving postero-medially to the male papilla, and simple, sinistral, genital atrium.

The dextral, ovoid ovary, is situated between the right testes and the proximal end of the cirrus-sac. Vitellaria encircle the testiculo-uterine region in a dense, chain-like formation. Four to ten, non-operculate eggs are contained in the uterus at a time; the latter not passing forward of the ventral sucker.

The arms of the Y-shaped excretory vesicle extend forward to the anterior margin of the testes. The stem and arms may be dilated or contracted in living specimens. No flame cells were observed.

One specimen of *Anas castanea*, three specimens of *Charadrius cucullatus*, and one specimen of *C. melanops* were examined and all were infected with *M. calvertensis* n.sp. Apart from differences in size and sexual maturity, metacercariae excysted from the smaller type of trematode cyst present in the amphipod *Austrochiltonia australis*, (about 0.170 mm diameter), were identical to *Maritrema calvertensis* n.sp. (figs. 3, 5).

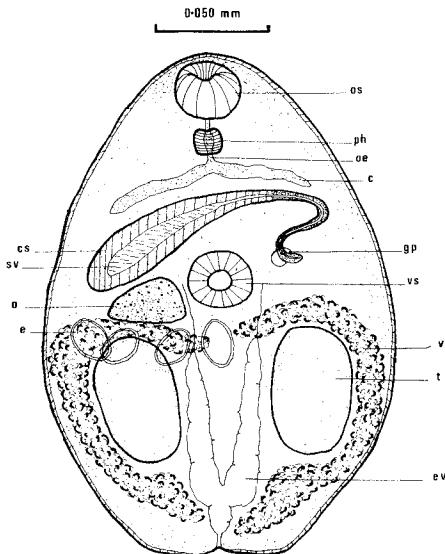


FIG. 4. - *Maritrema calvertensis* n.sp., adult, showing digestive and reproductive systems, and distribution of eggs, ventral view.

Similarly sized cysts, along with the remains of *A. australis*, were found in the alimentary tracts of birds infected with *M. calvertensis* n.sp. It is reasonable to assume that the metacercaria in the amphipod is a stage in the life-cycle of this trematode. The incidence of small cysts in *A. australis* is very high (about 90%) throughout the year.

Definitive hosts: *Anas castanea* Eyton, *Charadrius cucullatus* Vieillot, and *C. melanops* Vieillot.

Habitat: Lower intestine, caeca and rectum.

Probable Intermediate Hosts: *Coxiella badgerensis* Johnston and *Austrochiltonia australis* Sayce.

Habitat: Hepatopancreas and haemocoel respectively.

Geographical Location: Calverts Lagoon (Tasmania).

Type Material: Tasmanian Museum - holotype K250 (ringed and arrowed); paratypes K250 (ringed but no arrow). K249 (ringed on slide), K251 (ringed on slide), K252 (metacercaria).

Discussion

M. calvertensis n.sp. is similar to *M. oocysta* (Lebour) (syn. *M. humile* Nicoll and *M. imiae* Leonov) and *M. sobolevi* Kurotsckin, which may be synonyms of the same species (Deblock and Combes 1965). *M. calvertensis* differs from these species in having a smaller body, and a much shorter prepharynx, which is equal in length to the oesophagus. It also has a markedly different life cycle. *M. oocysta* encysts in the molluscan host, and lives in charadriiform birds in Western Europe, and lariform birds in Eastern Europe (Reimer 1963; Deblock and Combes 1965); *M. sobolevi* encysts in an amphipod, and lives in seals in the Caspian sea (Deblock and Combes 1965); and *M. calvertensis* n.sp. develops in the brackish water snail *Coxiella badgerensis*, encysts in the amphipod *Austrochiltonia australis*, and reaches sexual maturity in non-migratory Tasmanian dotterels and ducks.

The new species is named after the lagoon in which the definitive and intermediate hosts were found.

Subfamily	MICROPHALLINAE	Ward 1901
Genus	MICROPHALLUS	Ward 1901
Subgenus	MICROPHALLUS	Deblock and Pearson 1969
	<i>Microphallus tasmaniae</i> n.sp.	

The following description is based on more than 50 ovigerous specimens obtained from the duck *Anas castanea*, and dotterels *Charadrius cucullatus*, *C. melanops* and *C. ruficapillus*. Apart from differences in size, no marked variations are apparent in specimens either from the one, or from all four hosts.

Description (fig. 6)

Dimensions: body length, 0.491-1.810 (0.880); maximum body width, 0.220-0.364 (0.307); oral sucker, 0.055-0.085 (0.073); ventral sucker, 0.048-0.070 (0.062); pharynx, 0.020-0.052 (0.042) x 0.023-0.034 (0.029); testes, 0.072-0.098 (0.078) maximum width; genital atrium, 0.030-0.039 (0.036); ovary, 0.040-0.078 (0.070) maximum width; eggs, 0.021-0.025 (0.023) x 0.012-0.019 (0.016).

The body is elongate pyriform; maximum width occurring at the level of the testes. The anterior two-thirds is covered with transverse rows of spines, which diminish in size posteriorly. The ventral sucker is usually slightly smaller than the oral sucker. The prepharynx is long; barrel-shaped pharynx situated mediad; oesophagus varying in length as the fluke moves. Two saccular caeca diverge acutely, extending to the level of the anterior border of the ventral sucker.

The testes are ovoid, opposite, situated posterolaterally to the ventral sucker. The transverse, oval to reniform seminal vesicle, between the caecal arch and ventral sucker, is connected by a well-developed pars prostatica, and ejaculatory duct to the male papilla in a simple, round, sinistral, genital atrium. The ovary is oval to triangular, between the testes and caecum of the right side. The vitellaria consist of numerous follicles, grouped posterior to, or overlapping, the testes. The uterus, entirely post-acetabular, passes backwards forming a series of loops over the right testis, crossing and looping over the left testis, before entering the sinistral, genital atrium. The oval, non-operculate eggs, numbering 35 to 100 within the uterus, are not embryonated on deposition.

No flame cells were observed.

One specimen of *Anas castanea*, three specimens of *Charadrius cucullatus*, one specimen of *C. melanops*, and one specimen of *C. ruficapillus* were infected with *Microphallus tasmaniæ* n.sp. Metacercariae excysted from the larger type of trematode cyst (about 0.437 mm in diameter) present in the amphipod *Austrochiltonia australis*, were found to be almost identical with *Microphallus tasmaniæ* n.sp. differing only slightly in size and sexual maturity (figs. 3, 7). Similarly sized cysts, along with the remains of the amphipod, were present in the digestive tracts of the birds infected with *M. tasmaniæ* n.sp. It is assumed therefore that the metacercariae of these cysts represent a larval stage of *M. tasmaniæ* n.sp. The incidence of these large cysts in *A. australis* is about 90%, throughout the year.

Definitive Hosts: *Anas castanea* Eyton, *Charadrius cucullatus* Vieillot, *C. melanops* Vieillot, and *C. ruficapillus* Temminck.

Habitat: Mainly caecum; also intestine and rectum.

Probable Intermediate Hosts: *Coxiella badgerensis* Johnston, *Austrochiltonia australis* Sayce.

Habitat: Hepatopancreas and haemocoel respectively.

Geographical Location: Calverts Lagoon, (Tasmania).

Type Material: Tasmanian Museum - holotype K254 (ringed); paratypes K254 (not ringed), K253, K255, K256, K257 (metacercaria), K258 (metacercariae).

Discussion

Five species of the genus *Microphallus* have previously been described from Australian fauna (Deblock and Pearson 1969). Three of these species were found infecting

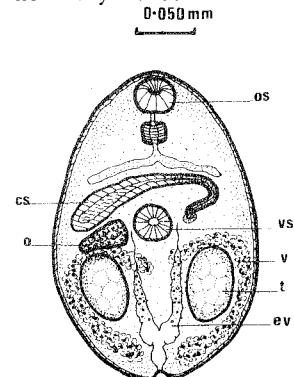


FIG. 5.- *Maritrema calvertensis* n.sp., excysted metacercaria, ventral view.

0.200 mm

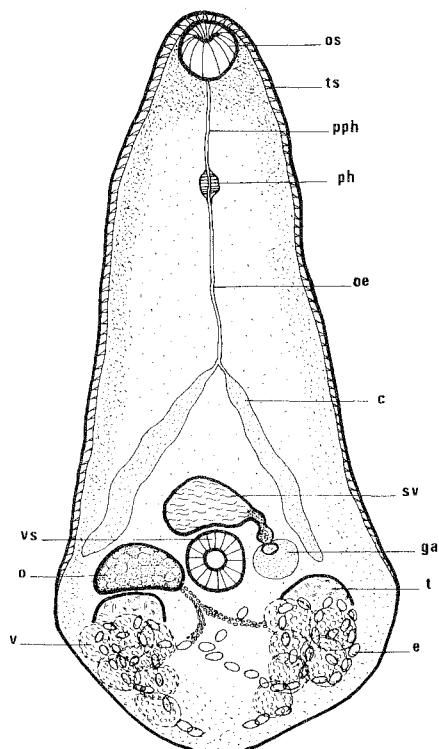


FIG. 6.- *Microphallus tasmaniæ* n.sp., adult, showing general organization, and distribution of eggs, ventral view.

the dotterels, *Charadrius dominicus* Mull and *C. mongolus* Pallas, in Queensland.

M. tasmaniæ n.sp. is similar to *M. papillorobustus* (Rankin 1940) (syn. *M. chabaudi*, *M. magnipapillata*) Deblock and Pearson 1969; and

in both species an amphipod serves as an intermediate host (Belopolskaia 1952). *M. papillorobustus* encysts in *Gammarus locusta*, and infects charadriiform and lari-form birds of the old and new worlds; and *M. tasmaniæ* n.sp. encysts in *Astrochiltonia australis*, and infects non-migratory Tasmanian dotterels and ducks. *M. tasmaniæ* n.sp. differs from *M. papillorobustus* in having a smaller, less conspicuous male papilla, longer body, and larger ventral sucker.

The name *Microphallus tasmaniæ* n.sp. is proposed because this is the first species of *Microphallus* to be described from Tasmania.

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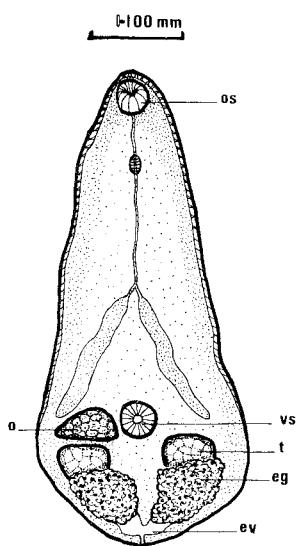


FIG. 7.- *Microphallus tasmaniæ* n.sp., excysted metacercaria, ventral view.

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