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THREE NEW SPECIES AND A NEW RECORD OF MICROPHALLID TREMATODES FROM TASMANIA, WITH OBSERVATIONS ON THEIR *IN VITRO* DEVELOPMENT.

by S.J. Smith
The Friends' School, Hobart

(with nine tables and six text-figures)

ABSTRACT

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Four species of microphallid trematodes occurring as metacercariae in the estuarine crab *Paragrapsus gaimardii* (M. Edw.) at Great Bay, Bruny Island, Tasmania, are described from specimens excysted and cultured to maturity *in vitro*. *Gynaecotyla hickmani* n.sp., and *G. macrocotylata* n.sp., occur in the green gland and body cavity, *Maritrema eroliae* Yamaguti, 1939 occurs in the body cavity and *Microphallis paragrapsi* n.sp. inhabits the nervous system of the crab.

INTRODUCTION

In 1968 Dr P.S. Lake, who at that time was a lecturer in the Department of Zoology, University of Tasmania, was engaged in neuro-endocrinological studies on the crab *Paragrapsus gaimardii* (M. Edw.) when he found in histological sections what he concluded were cysts of a parasite. He submitted the material to his colleague Dr J.L. Hickman who identified them as trematode cysts. On subsequent investigation *P. gaimardii* from Great Bay, Bruny Island, Tasmania, were found to be infected with metacercarial cysts of four microphallid species: *Gynaecotyla hickmani* n.sp., *G. macrocotylata* n.sp., *Maritrema eroliae* Yamaguti, 1939 and *Microphallus paragrapsi* n.sp.

The Tasmanian vertebrate and molluscan hosts of these trematodes have not yet been discovered. The following descriptions of the four species are based mainly on adults obtained *in vitro* by culturing excysted metacercariae to maturity.

MATERIALS AND METHODS

The estuarine crab *Paragrapsus gaimardii* was collected at low tide, from under rocks at Great Bay, Bruny Island, and maintained in running seawater in a marine aquarium for up to two weeks prior to dissection. Crabs were examined for trematodes in seawater, in plastic petri dishes. They were prepared for dissection by pithing, and then removal of limbs at the basi-ischium/merus joint, using strong scissors. The carapace was cut around the margins of the dorsal surface, and removed. Internal organs were first examined *in situ* under a dissecting microscope and then removed for further examination. The location of any trematodes present was recorded.

Metacercarial cysts were dissected free of host tissue, washed twice in isotonic saline and then transferred for excystment to test-tubes containing either 0.5% pancreatin or 0.5% pancreatin and 0.2% sodium taurocholate, dissolved in Hank's Balanced Salt Solution (Hank's BSS), gassed with CO_2 to pH 7.4 and pre-heated to 41 ± 1°C. The test-tubes were shaken for 30 seconds overy 15 minutes for a period of 60 minutes in an intermittently shaking water-bath kept at 41 ± 1°C. The trematodes were then rinsed free of the enzyme with Hank's BSS and maintained in Hank's BSS in the water-bath for a further 60 minutes. Excystment occurred in the enzyme solution and in the saline.

Observations on live excysted metacercariae were facilitated by slightly compressing specimens under coverslips supported by a little vaseline. For measurement, some excysted

metacercariae were fixed without flattening in boiling 10% phosphate buffered formol saline. Such worms were generally stained in alum carmine or Gower's carmine. Some excysted metacercariae and adults, flattened and fixed in 70% ethanol, were treated with Fast Red Salt B to stain phenolic egg-shell precursors in the vitellaria and vitelline ducts, and usually counter-stained in Gower's carmine. Permanent whole amounts of stained worms were prepared by clearing in clove oil, and mounting in Canada balsam, via standard procedures.

Some excysted metacercariae were cultured *in vitro*. Routine *in vitro* culture procedures were carried out under sterile conditions inside a laminar flow cabinet, using sterile culture media (Hank's BSS, Eagle's MEM and foetal calf serum), supplied by the Commonwealth Serum Laboratories. Serum was inactivated before use, by heating for one hour in a water-bath at 56°C. Excysted metacercariae were transferred to a sterile Universal vial at 41°C, and given four sterile washes of 10 minutes each in Hank's BSS before being transferred to the culture medium (Eagle's MEM, or Eagle's MEM plus 20% or 40% foetal calf serum) in a sterile Leighton tube. Cultures were maintained in an intermittently shaking (30 seconds every 13 minutes) water-bath at 41 ± 1°C. The pH of cultures was held at 7.4 by periodic gassing with 5% CO_2 in air.

Some metacercarial cysts of each microphallid species were placed in gelatine capsules and force fed to approximately one-week old uninfected ducklings, *Anas platyrhynchos* Linnaeus. The ducklings were killed with chloroform after periods of up to 5 weeks, dissected and examined for trematodes.

In the following descriptions, unless otherwise stated, all measurements are given in microns (the mean first, followed by the size range in parenthesis) and are based, except in the case of metacercarial cysts, on specimens fixed without compression. Cysts were measured live and uncompressed. Drawings were made with the aid of a camera lucida.

GYNAECOTYLA HICKMANI n.sp.

1. Adult

Dimensions of relatively mature, non-ovigerous, unflattened excysted metacercariae are shown in table 1. The adult is illustrated in fig. 1 and is described below from ovigerous adults obtained by *in vitro* culture of excysted metacercariae. Dimensions of unflattened ovigerous adults are not available.

Description

Body spatulate, dorsoventrally flattened, maximum width usually at level of gonads. Tegumental spines quincuncially arranged, extending to level of testes, diminishing in size posteriorly. Tegumental glands distributed over anterior body. Oral sucker round, mouth subterminal ventral. Prepharynx about one-quarter length of oesophagus. Pharynx oval, oesophagus bifurcates in anterior half of body. Caeca relatively short, terminating at or before mid-level of testes. Antiporal ventral sucker much larger, more muscular than weakly developed poral ventral sucker. Testes posterolateral, oval, symmetrical. Vesiculoprostatic pouch arcuate, between caeca, partly dorsal to antiporal ventral sucker. Large sausage-shaped seminal vesicle occupies proximal seven-eights; pars prostatica short, opening through ejaculatory orifice, between fleshy lobes of cornucotyle. Cornucotyle intermediate in size between ventral suckers, smaller than oral sucker. Lobes of cornucotyle side by side in same plane: outer lobe 40(38-42) × 14(11-15)μ, large, smooth; inner lobe smaller, 30(27-34) × 21(19-23)μ, surface studded with conical protuberances. Thin sclerotized layer, about 22 × 1μ, lines inner surface of outer lobe. Muscle fibres extend from posterior margin of vesiculoprostatic pouch to base of cornucotyle. Ovary round sinistral, between antiporal ventral sucker, sinistral testis, sinistral caecum. Oviduct short, passing posteromedially to ootype, posterior to ventral suckers. Uterus forms initial anterior loop, then looping posterior to testes. Metraterm enters genital atrium dorsally, opening adjacent to ejaculatory orifice. Vitelline glands clustered in distinct post-testicular groups. Vitelline ducts large, arising in centre of each group, passing anteromedially to small vitelline reservoir, ventral to ootype. Uterine eggs numerous, measuring 20(17-23) × 10(8-11)μ, (fixed, flattened). Flame-cell formula not determined. Excretory vesicle typical of genus, resembling Cross of Lorraine.

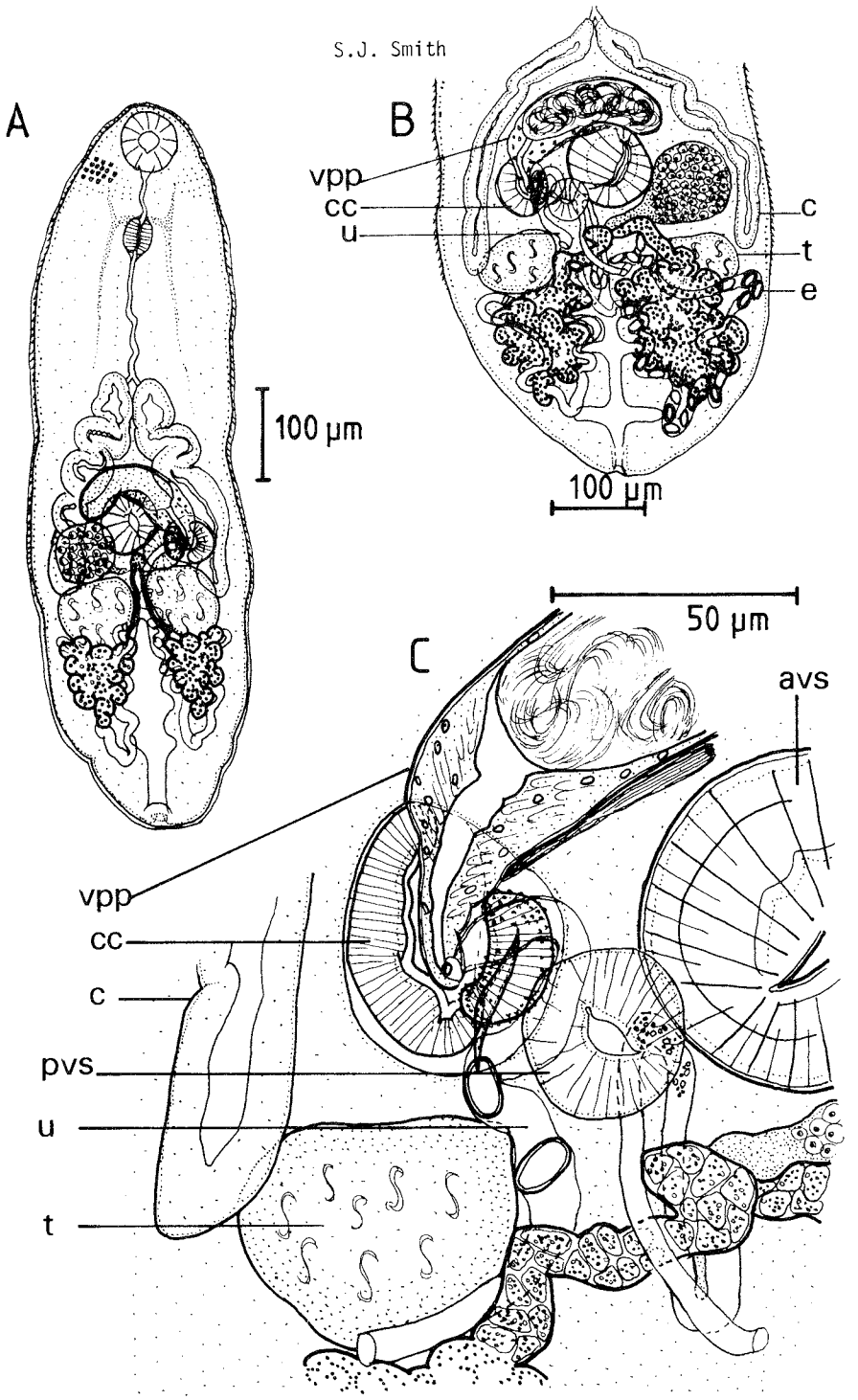

FIG. 1 - *Gynaecotyla hickmani* n.sp. A: holotype, mature excysted metacercariae after 12 hours at 41°C, dorsal view; B: slightly flattened gravid adult, ventral view; C: detail from B, of cornucotyle region. (avs: antiporal ventral sucker, c: caecum, cc: cornucotyle, e: egg, pvs: poral ventral sucker, t: testis, u: uterus, vpp: vesiculoprostatic pouch).

Crustacean intermediate host
Paragrapsus gaimardii (M. Edw.).

Geographical location
Great Bay, Bruny Island, Tasmania.

Site of encystment
Green gland, general body cavity.

Type material
Tasmanian Museum and Art Gallery - K895 holotype, mature excysted metacercaria (ringed); K896 paratypes, gravid adults (flattened); K897 and K898 paratypes, mature excysted metacercariae.

TABLE 1

EXCYSTED METACERCARIAE OF *GYNAECOTYLA HICKMANI* n.sp.
Dimensions of metacercariae excysted *in vitro:*
(a) after about 3 hours at 41°C, and (b) the holotype, after 12 hours at 41°C. All specimens relatively mature with active vitallaria, but no eggs.

	(a) n = 20	(b) n = 1
Body:length (BL)	735 (582-839)	703
width	220 (190-255)	217
Oral sucker:length (OS)	54 (48-61)	54
width	54 (49-61)	48
Prepharynx:length	43 (23-57)	38
Oesophagus:length	156 (118-190)	129
Pharynx:length	38 (34-42)	34
width	29 (25-32)	27
Left caecum:length	264 (220-304)	232
Right caecum:length	264 (224-304)	234
Antiporal ventral sucker:length (AVS)	69 (61-84)	68
width	65 (53-80)	65
Poral ventral sucker:length (PVS)	43 (38-48)	42
width	36 (32-40)	40
Cornucotyle:length (C)	44 (38-49)	42
width	46 (42-49)	-
Vesiculoprostatic pouch:length (VPL)	122 (106-148)	137
width	35 (30-40)	34
Ovary:length	60 (49-67)	72
width	53 (46-61)	63
Left testis:length	85 (63-99)	89
width	66 (53-84)	74
Right testis:length	81 (68-91)	91
width	63 (42-80)	68
VPL:BL ratio	0.17	0.19
Relative size (l+w) of organs	AVS > OS > C > PVS	

2. Metacercarial cyst (fig. 2)

The large round cyst occurs mainly in the green gland, but also in the body cavity of *Paragrapsus gaimardii*, either free or loosely bound by connective tissue. The cyst wall, about 28 µ thick, is composed of a wide translucent outer layer, often yellowish, and a narrow, clear inner layer. Dimensions of 20 cysts, their identity confirmed by *in vitro* excystment, are shown in table 2.

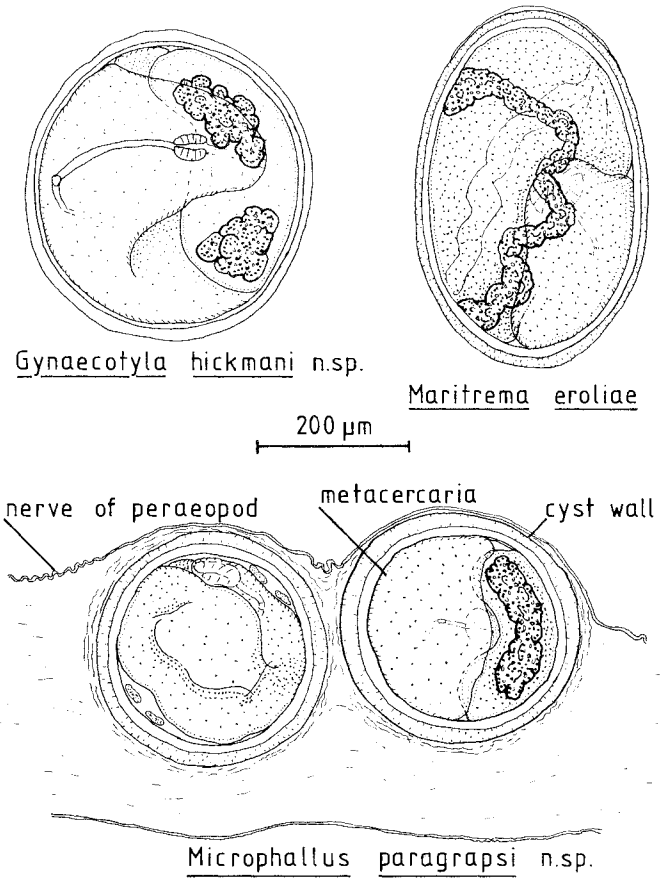

FIG. 2 - Trematode cysts infecting the crab *Paragrapsus gaimardii*. The cysts of *Gynaecotyla hickmani* n.sp. and *Maritrema eroliae* are shown free in the body cavity. Cysts of *Microphallus paragrapsi* n.sp. are embedded in a large nerve from the coxa of a peraeopod.

Metacercariae excyst at varying stages of maturity from those in which sex organs are not well developed, to those in which gametogenesis and vitellogenesis are advanced. Eggs are produced by more advanced specimens after one day in Eagle's MEM plus 20% foetal calf serum at 41°C.

TABLE 2

CYSTS OF *GYNAECOTYLA HICKMANI* n.sp.
Dimensions of live metacercarial cysts
dissected from naturally infected
Paragrapsus gaimardii (n=20).

External dimensions	length	416 (378-514)
	width	396 (348-499)
Internal dimensions	length	359 (334-384)
	width	342 (315-365)

3. Relationships

This new species of *Gynaecotyla* is named after Dr J.L. Hickman, in recognition of his initial investigations of the microphallids infecting the crab *P. gaimardii* at Great Bay. Using the key to species of the genus *Gynaecotyla*, proposed by Deblock (1974), *G. hickmani* n.sp. keys out with *G. brisbanensis* Deblock & Pearson, 1968a, and *G. bridgmani* Deblock, 1974. It is distinctly larger than both of those species, and although similar in shape and general anatomy, differs from them in several respects. The sclerotized pieces in the cornucotyle of *G. bridgmani*, arranged like a Y, are quite distinct. The form and ornamentation of the cornucotyle of *G. brisbanensis* are similar to those of *G. hickmani* n.sp. The arc of the vesiculoprostatic pouch of *G. brisbanensis* is not subtended by bundles of muscle fibres, and the diameter of the oral sucker is greater than or equal to the diameter of the antiporal ventral sucker; however, the vesiculoprostatic pouch of *G. hickmani* n.sp. is subtended by conspicuous muscle fibres, extending from the pouch to the cornucotyle, and the antiporal ventral sucker is much larger than the oral sucker.

GYNAECOTYLA MACROCOTYLATA n.sp.

1. Adult

A minority of *Gynaecotyla* metacercariae that excysted *in vitro* from large, round cysts taken from the body cavity of *Paragrapsus gaimardii*, were obviously different from *Gynaecotyla hickmani* n.sp., and were found to belong to another new species, *Gynaecotyla macrocotylata* n.sp.

Dimensions of relatively mature, non-ovigerous, unflattened excysted metacercariae of this species are presented in table 3. The adult, illustrated in figure 3, is described from ovigerous specimens cultured *in vitro*. Dimensions of the unflattened ovigerous holotype are included in table 3.

Description

Body elongate spatulate. Tegumental spines diminish in size posteriorly, extending to level of testes. Tegumental gland cells distributed in anterior of body. Oral sucker round, mouth subterminal ventral. Prepharynx about one-quarter length of oesophagus, pharynx oval; oesophagus bifurcates in anterior half of body. Caeca long, convoluted, terminating posterior to testes. Two ventral suckers well developed, antiporal ventral sucker slightly larger than poral ventral sucker. Testes posterolateral, oval, symmetrical. Vesiculoprostatic pouch arcuate, partly dorsal to ventral suckers, between caeca. Seminal vesicle occupies proximal two-thirds; pars prostatica opens through ejaculatory orifice, into genital atrium. Cornucotyle larger than oral and ventral suckers. External lobe largely ventral, enveloping smaller internal lobe, measures about 91 × 48 µ. Internal lobe measures about 47 × 42 µ. Surfaces of lobes smooth except for scattered small pointed projections. Three sclerotized pieces on, partly embedded in, anterior part of cornucotyle: according to scheme of Deblock (1974, p.323), no.2 elongate bent obliquely in middle, about 32 × 10 µ; no.5 rod shaped, about 20 × 7 µ; no.6 forked, about 17 × 8 µ. Two bundles of well developed muscles extend from piece no.6 to posterior wall of vesiculoprostatic pouch. Ovary round, sinistral, situated between sinistral testis and proximal end of vesiculoprostatic pouch, partly dorsal to antiporal ventral sucker. Oviduct passes posteromedially to median ootype. Uterus forms loops posterior to testes, then passes anteriad to genital atrium. Metraterm enters atrium dorsally, opening near ejaculatory orifice. Vitelline glands clustered in two distinct post-testicular bunches. Vitelline ducts pass anteriad, joining to form small round median vitelline reservoir. Eggs numerous, measure 17(16-19) × 12(11-13) µ (fixed, flattened). Flame-cell formula not determined. Excretory vesicle typical of genus.

Crustacean intermediate host

Paragrapsus gaimardii (M. Edw.).

Geographical location

Great Bay, Bruny Island, Tasmania.

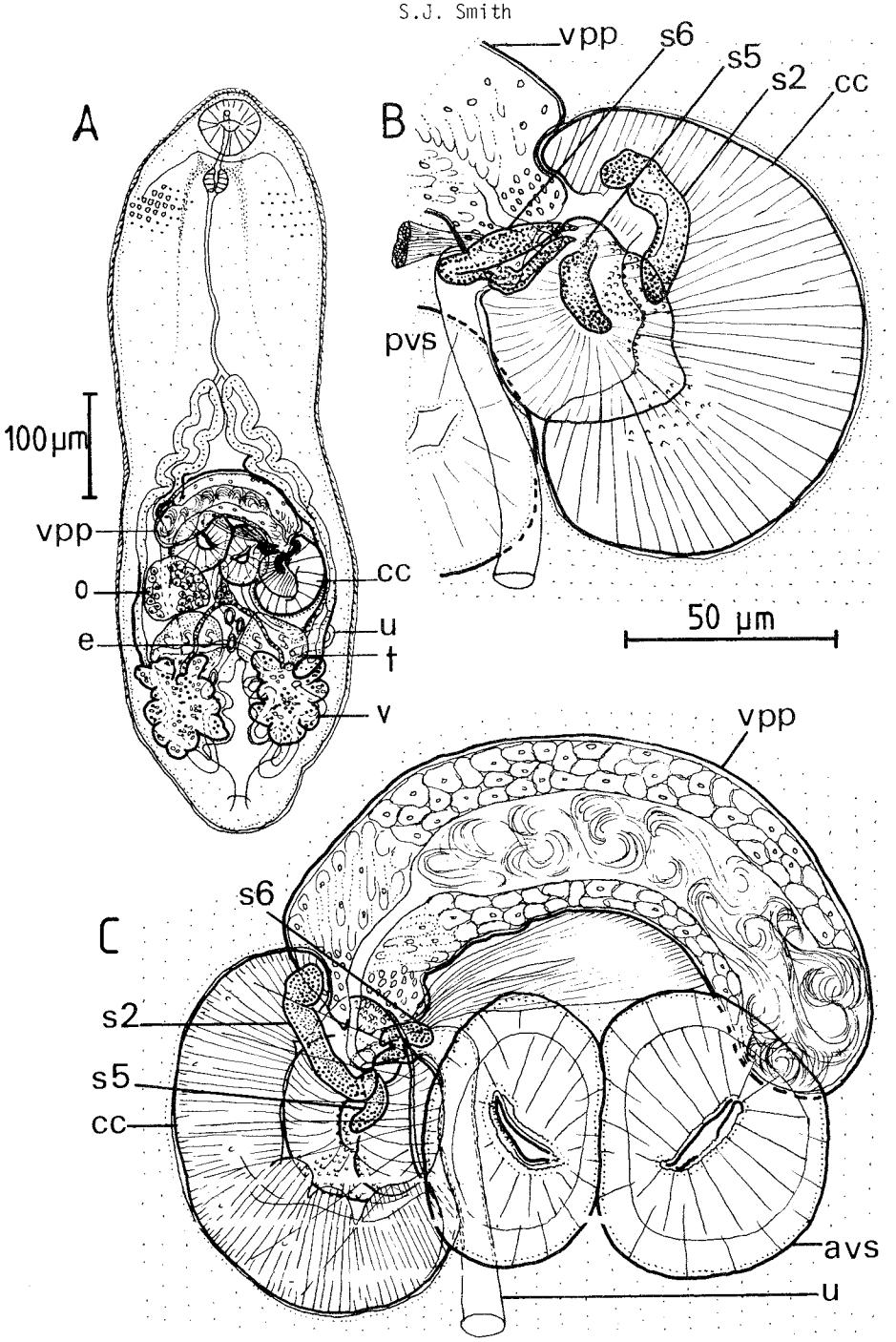

FIG. 3 - *Gynaecotyla macrocotylata* n.sp. A: holotype, gravid adult, dorsal view; B: flattened gravid adult, detail of cornucotyle, dorsal view; C: gravid adult, detail of vesiculoprostatic pouch and ventral suckers, ventral view. (avs: antiporal ventral sucker, cc: cornucotyle;, e: egg, o: ovary, pvs: poral ventral sucker, s2, s5, s6: sclerotized pieces in cornucotyle, t: testis, u: uterus, v: vitellaria, vpp: vesiculoprostatic pouch).

Site of encystment
Green gland, general body cavity.

Type material
Tasmanian Museum and Art Gallery - K899 holotype, gravid adult (ringed); K900 paratypes, gravid adults (flattened); K901 and K902 paratypes, mature excysted metacercariae.

TABLE 3

EXCYSTED METACERCARIAE OF *GYNAECOTYLA MACROCOTYLATA* n.sp.
Dimensions of; (a) mature, non-ovigerous specimens after about 3 hours *in vitro* at 41°C, and (b) the holotype, a gravid specimen, after about 12 hours *in vitro* at 41°C.

	(a) n = 12	(b) n = 1
Body:length (BL)	779 (665-937)	703
width	238 (224-258)	227
Oral sucker:length (OS)	55 (49-61)	48
width	56 (53-61)	53
Prepharynx length	44 (34-57)	38
Oesophagus length	201 (160-228)	160
Pharynx:length	40 (36-44)	36
width	30 (27-32)	27
Left caecum length	323 (289-372)	285
Right caecum length	329 (296-380)	308
Antiporal ventral sucker:length (AVS)	68 (61-74)	68
width	59 (53-68)	57
Poral ventral sucker:length (PVS)	63 (57-68)	61
width	46 (42-51)	48
Cornucotyle:length (C)	78 (65-95)	84
width	71 (68-72)	72
Vesiculoprostatic pouch:length (VPL)	166 (156-182)	160
width	45 (42-49)	46
Ovary:length (O)	65 (57-72)	70
width	57 (53-61)	61
Left testis:length	70 (65-80)	68
width	57 (51-65)	53
Right testis:length	66 (61-72)	65
width	58 (49-65)	53
VPL:BL ratio	0.21	0.23
Relative size (l+w) of organs	C > AVS > PVS ≃ OS	

2. Metacercarial cyst

The cysts are round and large, and occur mainly in the green gland, but also in the general body cavity of *Paragrapsus gaimardii*. To date they have not been distinguished from those of *G. hickmani* n.sp. In January 1980, about 50 large, round metacercarial cysts from the body cavity of *P. gaimardii* were measured, and then exposed to digestive enzymes *in vitro*, however all of the metacercariae that excysted were *G. hickmani* n.sp. In December 1979, similar, unmeasured cysts were exposed to digestive enzymes *in vitro*, and the ratio of the two *Gynaecotyla* species in 100 excysted metacercariae was 85 *G. hickmani* n.sp. to 15 *G. macrocotylata* n.sp.

The metacercaria of *G. macrocotylata* n.sp. excysts at varying stages of maturity. The more advanced specimens, with active vitellaria producing phenolic egg-shell precursors, become ovigerous after one day in Eagle's MEM plus 20% foetal calf serum at 41°C.

3. Relationships

The more distinct differences between *G. macrocotylata* n.sp. and *G. hickmani* n.sp. are that in the former the cornucotyle is larger than the oral and ventral suckers, the two ventral suckers are similar in muscular development, the poral ventral sucker is similar in size to the oral sucker, the cornucotyle contains three large sclerotized pieces, and the caeca are relatively long, terminating posterior to the testes; whereas in *G. hickmani* n.sp. the cornucotyle is smaller than the oral sucker and antiporal ventral sucker, the antiporal ventral sucker is more powerfully developed than the poral ventral sucker, the cornucotyle contains only a single thin sclerotized layer, and the caeca do not extend posterior to the testes.

According to the key to the species of *Gynaecotyla* presented by Deblock (1974), *G. macrocotylata* n.sp. most closely resembles the large form of *G. longiintestinata* Leonov, 1958 (syn. *G. gallica* Rebecq, 1961). In the latter species, however, the oral sucker is larger than the poral ventral sucker, and larger than or equal to the antiporal ventral sucker. The form and arrangement of sclerotized pieces in the cornucotyle, which are consistent within *Gynaecotyla* species, differ markedly between *G. macrocotylata* n.sp. and *G. longiintestinata*, e.g. in the latter species no.6 is not branched, and no.2 is absent, whereas in the former, no.6 is forked, and no.2 is well developed.

The name *G. macrocotylata* n.sp. is proposed for this new species as the cornucotyle is massive, and both ventral suckers are large and muscular.

MARITREMA EROLIAE YAMAGUTI, 1939

Syn. (according to Deblock, 1975): *M. urayensis* Ogata, 1951; *M. magnicirrus* Belopolskaia, 1952; *M. kitanensis* Shibue, 1953.

1. Adult

The adult, illustrated in figure 4, is described from ovigerous specimens cultured *in vitro*, and from one gravid fluke recovered from a laboratory duckling. Dimensions of mature excysted metacercariae, some of which have commenced egg production, are shown in table 4. Ovigerous and non-ovigerous specimens are similar in size.

Dimensions

Body elongate pyriform to triangular. Tegumental spines small, cover entire body, except at posterior extremity. Tegumental gland cells anterior to cirrus pouch. Oral sucker round. Prepharynx about half length of oesophagus; pharynx oval; oesophagal bifurcation about one-third body length from anterior end. Caeca diverge acutely, extend to midlevel of metraterm. Ventral sucker round, larger than oral sucker, located in posterior half of body. Oval testes symmetrical, situated posterolaterally. J-shaped cirrus pouch massive, about 2.5 µ thick. Flexure of pouch antero-dorsal to ventral sucker. Seminal vesicle large, clavate, in proximal half of dextral limb of cirrus pouch. Distal end of seminal vesicle tapers to short, narrow, folded seminal canal, which widens to voluminous, convoluted pars prostatica. Invaginated spiny cirrus leads from level of anterior seminal vesicle to sinistral genital atrium, proximal part of cirrus densely lined by simple sharp spines, increasing in size distally from 5 × 2 µ to 10 × 3 µ; distal part lined by large, flattened, thorn-like spines, variable in size, 12 (6-19) × 7 (5-10) µ. Evaginated cirrus, directed anteriorly, about 150 µ long, (fixed flattened), with small simple spines covering distal part, increasing in size to several rows of thorn-like spines near base of cirrus. Aspinous 'heel' at base of evaginated cirrus. Ovary triangular, multilobed (usually three main lobes), median, dorsal to ventral sucker, bounded anteriorly by cirrus pouch, posteriorly by metraterm. Oviduct leads posteriorly to median ootype, surrounded by well-developed Mehlis' gland. Uterus loops around each testis, mainly lying within vitelline ring; enters vast, muscular metraterm medially, posterior to ovary. Metraterm lining about 13 µ thick, traversed by radial fissures which widen towards lumen. Eggs numerous, oval 18 (16-19 × 9 (8-10) µ. Vitelline glands form post-ovarian ring, closed posteriorly, open near ootype. Vitelline ducts short, transverse, uniting medially to form short longitud-

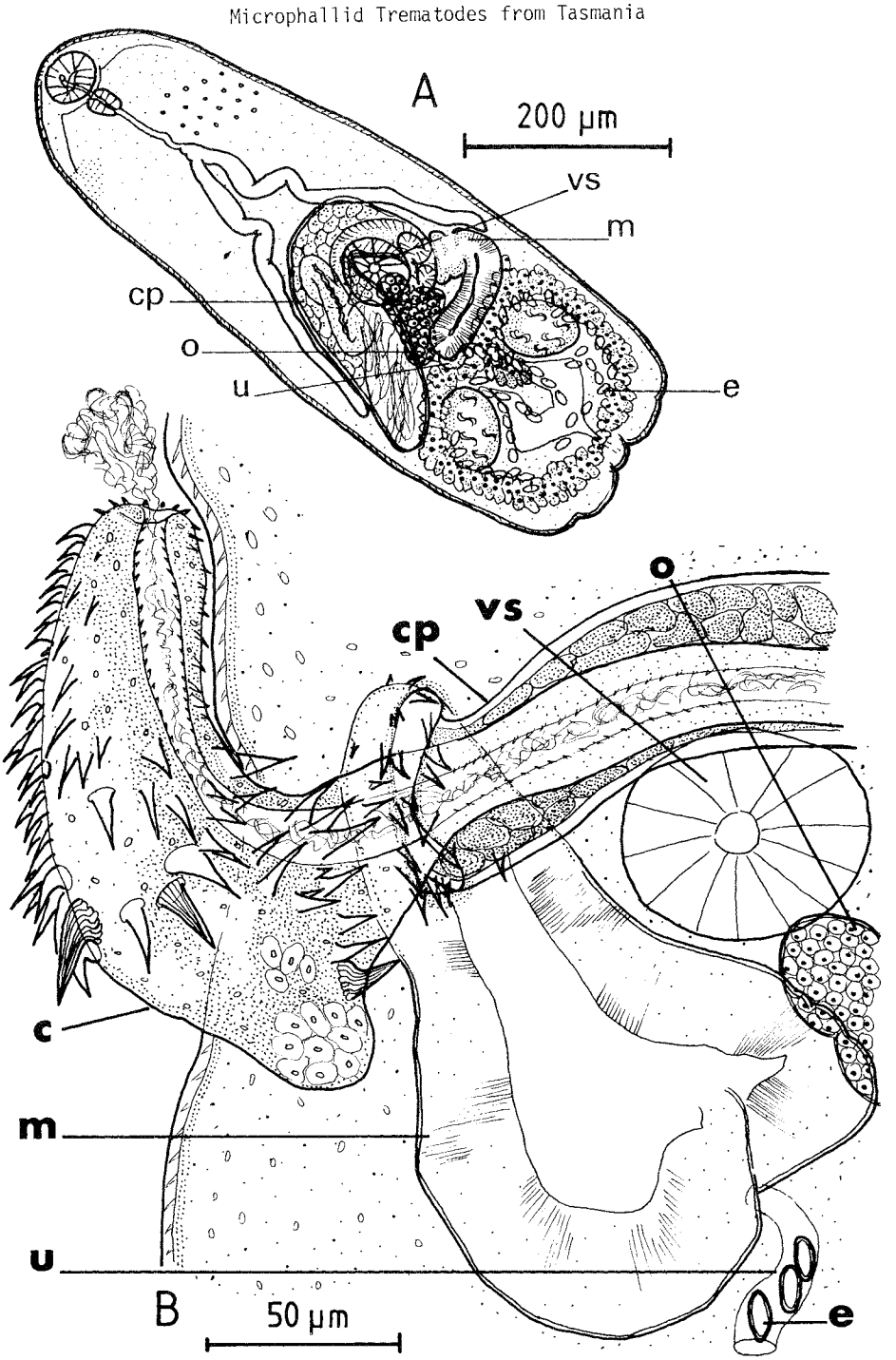

FIG. 4 - *Maritrema eroliae*. A: gravid adult, ventral view; B: flattened gravid adult, detail of everted cirrus, dorsal view. (c: cirrus, cp: cirrus pouch, e: egg, m: metraterm, o: ovary, vs: ventral sucker, u: uterus).

inal vitelline reservoir. Flame-cell formula not determined. Excretory vesicle Y-shaped, arms extending to anterior margins of testes.

Experimental definitive host
Anas platyrhynchos Linnaeus.

Crustacean intermediate host
Paragrapsus gaimardii (M. Edw.).

Geographical location
Great Bay, Bruny Island, Tasmania.

Site of encystment
Body cavity.

Material
Tasmanian Museum and Art Gallery - K892 gravid adult (flattened, cirrus everted); K893 gravid adults; K894 non-ovigerous, excysted metacercariae.

TABLE 4

EXCYSTED METACERCARIAE OF *MARITREMA EROLIAE*.
Dimensions of metacercariae excysted *in vitro*, after about 3 hours at 41°C. All metacercariae relatively mature, with active vitellaria (n = 15).

Body:length (BL)	683 (620-794)
width	258 (217-293)
Oral sucker:length (OS)	47 (40-53)
width	51 (46-57)
Prepharynx length	40 (30-53)
Oesophagus length	86 (65-133)
Pharynx:length	28 (23-30)
width	24 (21-27)
Left caecum length	292 (251-342)
Right caecum length	307 (274-357)
Ventral sucker:length (VS)	64 (61-70)
width	61 (57-67)
Cirrus pouch:length (CPL)	350 (323-380)
width	82 (68-91)
Seminal vesicle:length	141 (114-156)
width	59 (49-68)
Metraterm:length	117 (103-137)
width	62 (46-80)
Ovary:length	108 (87-125)
width	59 (46-76)
Left testis:length	71 (65-76)
width	52 (42-57)
Right testis:length	76 (65-87)
width	53 (46-61)
Roundness (BL/BW)	2.65
OS (l+w)/ VS (l+w)	0.78
CPL/BL	0.51

2. Metacercarial cyst (fig. 2)

The cyst is oval and quite large. Dimensions are presented in table 5. The cyst wall is composed of a clear uniform inner layer about 10 µ thick, overlain by a darker layer about 12 µ thick, traversed by fine radial striations or fissures. A thin outer membrane, about 1 µ wide, envelops the whole cyst. The cyst occurs free, or lightly bound by connective tissue, within the body cavity of *Paragrapsus gaimardii*.

Encysted metacercariae reach an advanced state of sexual development. Although they excyst at varying degrees af maturity, more advanced specimens have phenolic egg-shell precursors in the vitellaria, and commence egg production within a few hours at 41°C, in Hank's BSS and in Eagle's MEM.

TABLE 5

CYSTS OF *MARITREMA EROLIAE*.
Dimensions of live metacercarial cysts,
dissected from *Paragrapsus gaimardii*, (n=20).

External diameter	length	458 (423-484)
	width	304 (289-331)
Internal diameter	length	402 (370-423)
	width	254 (239-274)

3. Relationships

The anatomy of this microphallid, with very large cirrus pouch, spiny cirrus, vast metraterm and median ovary, is characteristic of a distinctive and homogeneous group of *Maritrema* species: *M. eroliae* Yamaguti, 1939, *M. echinocirrata* Leonov, 1958, *M. patulus* Coil, 1955, and *M. misenensis* (Palombi, 1940) Prevot, Bartoli and Deblock, 1976. The evaginated cirrus of the latter species is free of spines, except for a zone around the base, mainly proximal to a distinct "heel". *M. patulus* is morphologically identical to *M. eroliae*, and Prevot *et al.*, 1976, consider that when its life-cycle is discovered it may fall into synonymy with *M. eroliae*. *M. echinocirrata* differs from *M. eroliae* only in the absence of the large thorn-like spines at the base of the evaginated cirrus. The Tasmanian flukes fall within the range of variation described for *M. eroliae* by Deblock (1975).

The metacercariae of *M. eroliae* has previously been found encysting in various decapod crustaceans in Japan (Ogata 1951, Shibue 1953, and Bridgman *et al.* 1972). The oval cyst varies greatly in size and thickness, possibly in relation to the identity of the second intermediate host. The cysts found in Tasmanian crabs are intermediate in size between those infecting *Macrophthalmus dilatatus* , 520-690 × 360-450 µ (Ogata 1951; and Bridgman *et al.* 1972), and those infecting *Neocaridina denticulata*, 280-320 × 240-270 µ (Shibue 1953), and *Scopimer* spp., 310-360 × 210-240 µ (Ogata 1951). The adult of *M. eroliae* has been found only in birds inhabiting the eastern border of the Pacific Ocean (Deblock 1975); and in Australia, has been recorded in the Mongolian dotterel, *Charadrius mongolus* Pallas, in Queensland (Deblock & Pearson 1968b).

MICROPHALLUS PARAGRAPSI N.SP.

1. Adult

The adult, illustrated in fig. 5, is described below from ovigerous specimens cultured *in vitro* and non-ovigerous specimens taken from a laboratory duckling. Dimensions of unflattened, ovigerous specimens are not available, however gravid adults appear to be the same size as relatively mature excysted metacercariae (with phenolic egg-shell precursors in the vitellaria), dimensions of which are included in table 6b. Dimensions of very immature excysted metacercariae are shown in the same table. Non-ovigerous adults (some with phenolic egg-shell precursors), recovered from an experimentally infected duckling, were intermediate in size between immature and mature excysted metacercariae (table 7).

Description

Body varies from pyriform to elongate spatulate, frequently with distinct waist just anterior to seminal vesicle, causing dumb-bell appearance. Lateral body margins of worms cultured *in vitro* generally folded ventrally. Tegumental spines quincuncially

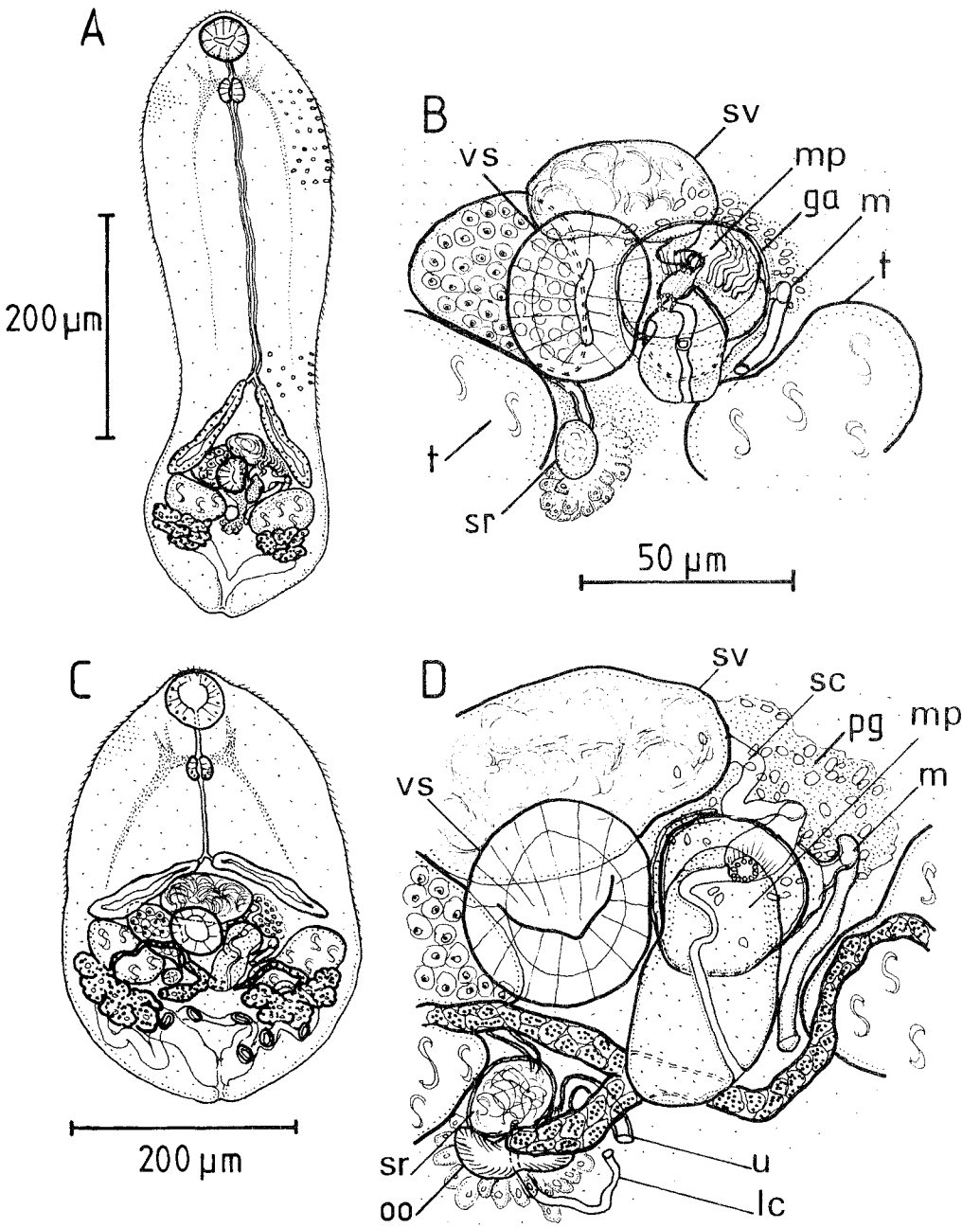

FIG. 5 - *Microphallus paragrapsi* n.sp. A: holotype, after 17 hours in laboratory duckling, ventral view; B: detail of ventral sucker region of holotype; C: flattened gravid adult, cultured *in vitro* at 41°C for 2 days, ventral view; D: detail of ventral sucker region of adult shown in C. (ga: genital atrium, lc: Laurer's canal, m: metraterm, mp: male papilla, oo: ootype, pg: prostate gland, sc: seminal canal, sr: seminal receptacle, sv: seminal vesicle, t: testis, u: uterus, vs: ventral sucker).

arranged, diminishing in size posteriorly, extending to waist level. Tegumental gland cells distributed over anterior body. Oral sucker transversely oval to round, mouth subterminal ventral. Prepharynx short, about 1/20th length of oesophagus. Oesophageal bifurcation in posterior half of body. Caeca relatively short, terminating at midlevel of ventral sucker. Ventral sucker round, smaller than oral sucker. Testes oval, equal, posterolateral. No cirrus pouch; seminal vesicle oval, partly dorsal to ventral sucker. Seminal canal short, leading posteriorly from seminal vesicle to expanded pars prostatica, about 6 μ diameter, at base of male papilla. Prostate gland cells located between seminal vesicle and male papilla, clustered around seminal canal; secreting through undetermined number of small ducts, into pars prostatica. Retracted male papilla coiled or folded within genital atrium, measures 34(30-38) × 29(27-30) μ. Everted male papilla, tubular, not lobed, directed posteromedially; length 66(53-76) μ, width at base 26(23-30) μ, width at tip 22(19-25) μ. Ejaculatory canal, about 4 μ diameter, mainly axial within male papilla, but opens eccentrically. Ovary oval, contiguous to dextral caecum and testis, dextral and partly dorsal to ventral sucker. Oviduct passes posteromedially to bulbous seminal receptacle, from which Laurer's duct leads to dorsal surface. Ootype located between testes, posterior to ventral sucker. Uterus forms post-testicular loops, overlapping each testis. Metraterm enters left side of genital atrium. Vitelline gland cells clustered in compact bunches posterior to each testis. Vitelline ducts pass anteromediad, joining to form longitudinal vitelline reservoir, posterior to ventral sucker. Oval eggs, numerous, 19(17-21) × 10(8-13) μ, (fixed, flattened). Flame-cell formula not determined. Excretory vesicle V-shaped, limbs extending to testes.

TABLE 6

EXCYSTED METACERCARIAE OF *MICROPHALLUS PARAGRAPSI* N.SP.
Dimensions of metacercariae excysted *in vitro*, after about 3 hours at 41°C:
(a) smaller, less mature specimens; (b) larger, more mature specimens,
some with active vitellaria.

	(a) n = 20	(b) n = 20
Body:length (BL)	355 (287-469)	579 (484-665)
width (BW)	156 (137-117)	184 (156-209)
Oral sucker:length (OS)	38 (34-40)	40 (34-46)
width	40 (36-42)	44 (42-49)
Prepharynx length	4 (4-49)	12 (4-27)
Oesophagus length	115 (87-114)	275 (220-319)
Pharynx: length	22 (17-23)	25 (19-27)
width	18 (17-19)	21 (19-25)
Left caecum length	88 (76-99)	128 (110-156)
Right caecum length	87 (72-99)	126 (103-137)
Ventral sucker:length (VS)	35 (30-38)	40 (34-46)
width	31 (23-36)	40 (34-44)
Seminal vesicle:length	-	39 (34-42)
width	-	27 (23-36)
Ovary:length	31 (23-34)	41 (38-46)
width	25 (17-30)	34 (30-38)
Left testis:length	45 (34-53)	59 (57-61)
width	38 (29-46)	51 (38-57)
Right testis:length	50 (34-61)	61 (53-68)
width	35 (27-42)	46 (42-51)
BL/BW	2.28	3.15
OS (1+w)/VS (1+w)	1.18	1.05

Experimental definitive host
Anas platyrhynchos Linnaeus

Crustacean intermediate host
Paragrapsus gaimardii (M.Edw.)

Geographical location
Great Bay, Bruny Island, Tasmania

Site of encystment
Nerves of legs and claws

Type material
Tasmanian Museum and Art Gallery - K903 holotype, non-ovigerous adult (ringed); K903 paratypes, non-ovigerous adults (not ringed); K904 paratypes, ovigerous adults (ringed, flattened); K905 and K906 paratypes, excysted metacercariae.

TABLE 7

NON-OVIGEROUS ADULTS OF *MICROPHALLUS PARAGRAPSI* N.SP.
Dimensions of the holotype (a), and other non-ovigerous adults (b), from an experimentally infected duckling 17 hours after infection.

	(a) n=1	(b) n=9
Body:length (BL)	544	460 (325-544)
width (BW)	171	159 (137-179)
Oral sucker:length (OS)	42	38 (34-40)
width	44	41 (36-44)
Prepharynx length	11	14 (8-23)
Oesophagus length	243	199 (110-258)
Pharynx:length	27	22 (19-25)
width	19	19 (17-23)
Left caecum length	114	95 (72-110)
Right caecum length	114	98 (80-110)
Ventral sucker:length (VS)	40	39 (34-42)
width	34	34 (30-38)
Ovary:length	38	40 (36-46)
width	34	34 (27-38)
Left testis:length	57	55 (46-65)
width	38	40 (30-46)
Right testis:length	55	54 (46-61)
width	46	46 (38-49)
BL/BW	3.18	2.89
OS (l+w)/VS (l+w)	1.16	1.08

2. Metacercarial cyst (fig. 2)

The cyst is round, colourless, and quite thick-walled. Its dimensions are shown in table 8. The uniformly thick cyst wall, about 36(27-44) µ wide, is composed of two layers: a clear, homogeneous inner layer, 14(10-19) µ, and a darker, outer layer with fine radial striae, 22(17-27) µ. The cyst occurs within the large nerves innervating the legs and claw muscles of the crab host, particularly near the coxae. No behavioural changes related to this infection have been noticed in the crab host.

Great variation was recorded in the time required by metacercariae for excystment at 41°C. The time was directly related to the size and maturity of excysted metacercariae: smaller, less mature metacercariae, possibly with incompletely formed cyst walls, excysted sooner than larger more mature specimens (table 9). Advanced metacercariae have phenolic

egg-shell precursors in the vitellaria, and produce eggs *in vitro* after 2 days at 41°C, in Eagle's MEM plus 20% foetal calf serum.

TABLE 8

CYSTS OF *MICROPHALLUS PARAGRAPSI* N.SP.
Dimensions of live metacercarial cysts, dissected from *Paragrapsus gaimardii*, (n=20).

External diameter	length	319 (300-353)
	width	310 (285-331)
Internal diameter	length	246 (228-266)
	width	239 (224-251)

TABLE 9

SIZE VARIATION IN EXCYSTED METACERCARIAE OF *MICROPHALLUS PARAGRAPSI* N.SP.
Size of metacercariae excysted *in vitro* at 41°C: (a) after 1½ hours; and (b) from 1½ to 12 hours.

	(a) n=20	(b) n=20
length	351 (249-507)	479 (333-665)
width	154 (125-175)	185 (144-213)

3. Relationships

The species of *Microphallus* encysting in the nerves of the crab *Paragrapsus gaimardii* in Tasmania is considered to be new, and is named after its second intermediate host. Only one other microphallid species is known to encyst in the nerves of the limbs of its crustacean host: *Microphallus pachygrapsi* Deblock & Prevot, 1968, encysts in the crab *Pachygrapsus marmoratus* on the Mediterranean coast of France. *Microphallus paragrapsi* n.sp. and *M. pachygrapsi* adults are similar in size, but differ in the following respects: the former has OS:VS greater than 1, and male papilla length greater than VS diameter; the latter has OS:VS less than 1, and male papilla length less than VS diameter. Metacercarial cysts of *M. paragrapsi* n.sp. are round whereas those of *M. pachygrapsi* are oval. According to the key to species of *Microphallus* presented by Deblock (1971), the species most similar to *M. paragrapsi* n.sp. is *M. minutus*, a tiny fluke discovered in water rats captured on the banks of the Murray River at Tailem Bend, South Australia (Johnston 1948) and rediscovered and redescribed by Deblock (Pearson (1969), in the same host, captured near Brisbane, Queensland. The adults of *M. paragrapsi* n.sp. and *M. minutus* are very similar, however the average size of gravid *M. minutus* adults, 290 × 160 µ (Deblock & Pearson 1969), is slightly less than the average size of the more immature excysted metacercariae of *M. paragrapsi* n.sp., 355 × 156 µ, and markedly less than the average size of mature excysted metacercariae of *M. paragrapsi* n.sp., 579 × 184 µ. The male papilla of *M. minutus*. 53 × 27 µ, is less elongate than that of *M. paragrapsi* n.sp., 66 × 26 µ. In relation to body size, the oral and ventral suckers of *M. minutus* are much larger than those of *M. paragrapsi* n.sp. On the basis of these differences, and in the absence of further life-history information, *M. paragrapsi* n.sp. and *M. minutus* are considered to be distinct, but closely related species. Four other species of *Microphallus* has been recorded in Australia (Deblock & Pearson 1969), however, all are readily distinguished from *M. paragrapsi* n.sp. by details of the reproductive system: *M. minutus* has a large, robust male papilla of the "papillorobustus" type; *M. papillornatus* has an ornamented male papilla; *M. vaginosus* has an unusual, large metraterm, with thick folded walls; and *Microphallus* sp. has a voluminous ovoid papilla, about the same width as the ventral sucker.

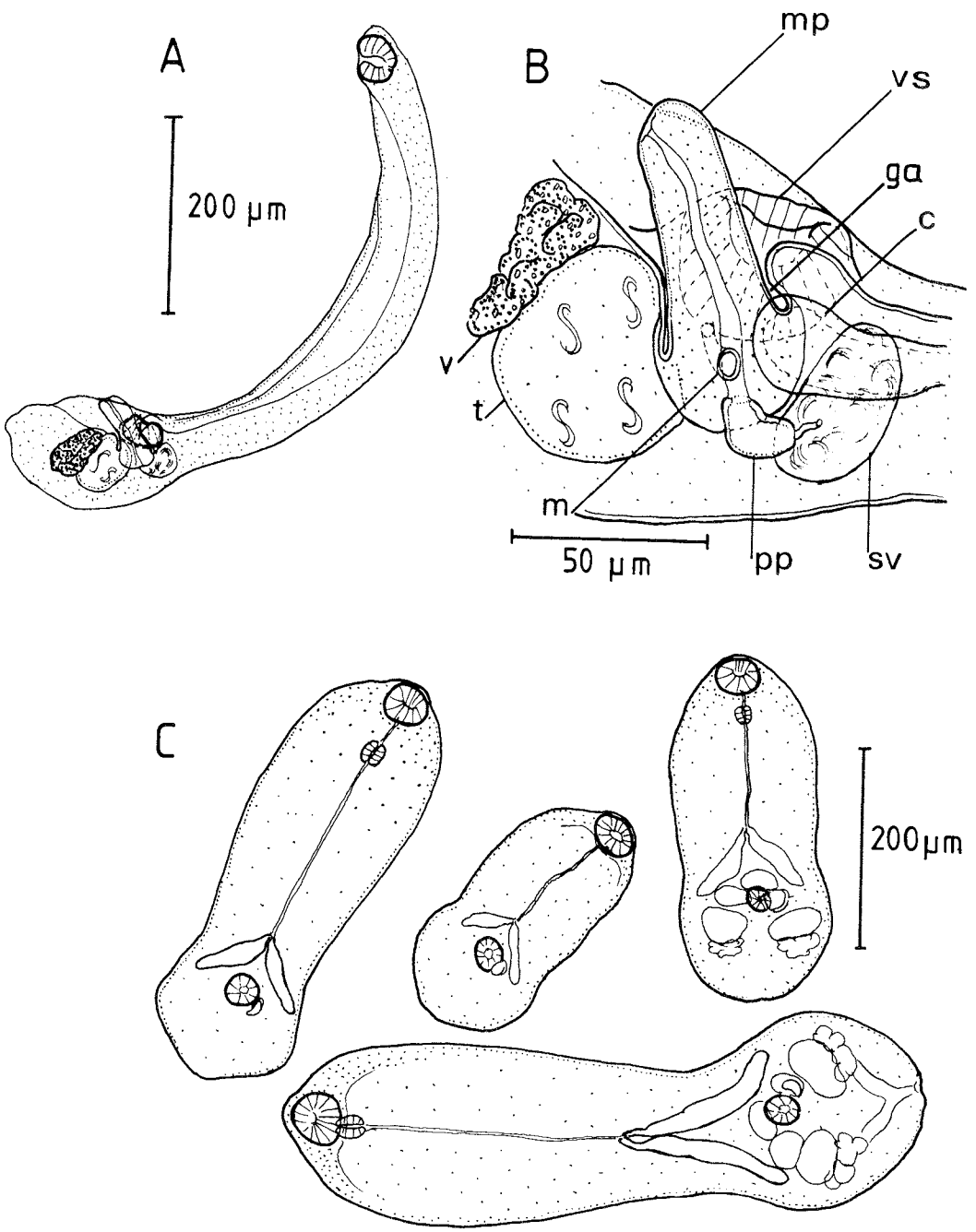

FIG. 6 - *Microphallus paragrapsi* n.sp. A: excysted metacercaria, after 12 hours at 41°C, lateral view; B: detail of everted male papilla of metacercaria shown in A; C: variation in form and size of excysted metacercariae, after 12 hours at 41°C. (c: caecum, ga: genital atrium, m: metraterm, mp: male papilla, pp: pars prostatica, sv: seminal vesicle, t: testis, v: vitellaria, vs: ventral sucker).

DISCUSSION

The incidence of infection of *Paragrapsus gaimardii* with microphallid trematodes has not been determined, however the impression gained from dissection of many crabs from Great Bay, at irregular intervals over several years, is that almost all adult crabs, of both sexes, are infected by *Gynaecotyla* spp., *Maritrema eroliae*, and *Microphallus paragrapsi* n.sp., throughout the year. Two large male crabs dissected in July 1977 were infected by an average of 197(118-275) metacercarial cysts of *Gynaecotyla* spp., 195(90-300) of *M. paragrapsi* n.sp., and 14(2-26) of *Maritrema eroliae*. These crabs also contained an average of 19(15-23) large, unencysted immature metacercariae of *Gynaecotyla* spp. These specimens had an oral sucker, a large excretory bladder, and some individuals also had ventral suckers and rudimentary digestive and reproductive systems. Delayed encystment is known in the life-cycles of a number of *Gynaecotyla* species, and in *G. adunca* and *G. longiintestinata*, a small percentage of metacercariae mature and produce eggs, without encysting (Deblock 1977). The cysts of the two *Gynaecotyla* species infecting *P. gaimardii* have not been distinguished, however, the ratio of the two species in 100 metacercariae, excysted *in vitro* from cysts dissected in December 1979, was about 6 *G. hickmani* n.sp. to 1 *G. macrocotylata* n.sp. The molluscan hosts of these four microphallid species are as yet unknown. The Mongolian dotterel, which harbours *Maritrema eroliae* in Queensland, is an infrequent visitor to Tasmanian shores, and might have introduced this trematode to the fauna of Great Bay. An estuarine fish, the leatherjacket, caught in the Derwent River, was found by Last (1975) to harbour a single microphallid in its intestine, which appears to be an immature adult of *Maritrema eroliae*. As experimental evidence indicates that a temperature of about 40°C is required for oviproduction in *M. eroliae*, eggs are unlikely to be produced in this host. Attempts to experimentally infect eight domestic ducklings with hundreds of cysts of these four Tasmanian microphallids were not very successful. No ducklings were infected by the two *Gynaecotyla* species; three ducklings, dissected 17, 18 and 26 hours after being fed with cysts, were infected with 16, 1 and 1 immature adults of *Microphallus paragrapsi* n.sp. respectively; and one duckling dissected after 17 hours was infected by one gravid adult of *Maritrema eroliae* that contained 52 uterine eggs.

The discovery and identification of four microphallid species concurrently infecting a single species of crab in Tasmania presents many challenging problems. The site of encystment of *Microphallus paragrapsi* n.sp. is unusual, and presumably of selective value to the parasite. This trematode may increase its chances of transmission by making its intermediate host more vulnerable to predation. An investigation of the effect of *M. paragrapsi* n.sp. cysts on the function of the nerves and the behaviour of *Paragrapsus gaimardii*, may help to explain why two microphallids, *M. paragrapsi* n.sp. in Tasmania and *M. pachygrapsi* in France, invade the nerves of their crustacean hosts. The life history of none of the four microphallids infecting *P. gaimardii* is known. Elucidation of their life-cycles at Great Bay will facilitate discovery of their life-cycles at other locations in Australia, and the life-cycle of *Maritrema eroliae* in Japan.

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