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TWO NEW NOTOCOTYLID TREMATODES FROM BIRDS IN TASMANIA AND THEIR LIFE HISTORIES

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(with twelve tables and eight text-figures)

ABSTRACT

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Paramonostomum caeci n. sp., adults of which occur mainly in the intestinal caeca of *Anas superciliosa*, *Cygnus atratus* and *Poliocephalus poliocephalus*, is described. It is considered to closely resemble *P. maleri*schi from which it differs in being only one-third the size, having a spinose tegument and a cirrus covered with tubercles. *P. bursae* n.sp., adults of which have been found inhabiting the bursa of Fabricius of *Anas superciliosa* is described and considered to be most similar to *P. alveatum* and *P. parvum* but to differ from them in the distribution of its vitellaria, the position of its ovary relative to the testes and in having a greater number of uterine loops. The domestic duck, *Anas platyrhynchos*, was found to serve as an experimental host for both new species. A brackish water snail, *Coxiella badgerensis*, inhabiting Calverts Lagoon in southeast Tasmania serves as a natural intermediate host for *P. caeci* n.sp. and *P. bursae* n.sp. and also for two other undescribed notocotylids. Developmental stages (rediae, cercariae and metacercariae) of both new *Paramonostomum* species are described. The cercaria of *P. caeci* n. sp. belongs to the Imbricata group, and that of *P. bursae* n.sp. to the Yenchingensis group.

INTRODUCTION

Developmental stages of four species of notocotylid trematodes (subfamily Notocotylinae Kossack, 1911), have been found infecting the gastropod *Coxiella badgerensis* (Johnston) at Calverts Lagoon near Hobart, Tasmania (Smith 1981). The cercariae of these species leave the molluscan host and encyst free in the aquatic environment. Although cysts of each species were fed to laboratory ducklings, the adults of only two species became established. They have been identified as new species of the genus *Paramonostomum* and flukes considered conspecific with them have been found in black duck from the lagoon. Notocotylids identified with one of the species have also been recovered from hoary-headed grebe and black swan which frequent the lagoon.

There have been few reports of notocotylids in birds of the Australasian region: *Catatropis gallinulae* was recorded in the dusky moorhen in South Australia (Johnston 1928); an unidentified notocotylid was recorded in the mallard in Tasmania (Angel, pers. comm. in Munday & Green 1972); and *Uniserialis gippyensis*, *Catatropsis* sp. and *Notocotylus* sp. were recorded in various anatids in New Zealand (Rind 1974). In 1975, specimens of an unidentified notocotylid were found in the black swan at Lake Bookar, Queensland (Smith 1981, pp. 256-257).

Pre-adult stages of notocotylid species are distinguished principally by the form of the excretory ring of mature cercariae, and by the form and size of metacercarial cysts. Rothschild (1938) proposed that notocotylid cercariae be classified into three groups, according to the form of the excretory ring: Imbricata, Yenchingensis and Monostomi. The cercariae of two of the species at Calverts Lagoon belong to the Imbricata group, those of another belong to the Yenchingensis group and those of the fourth species belong to the Monostomi group.

Two Notocotylid Trematodes from Birds in Tasmania

Two of the notocotylid species are described in this paper. Both are named after their habitats in the definitive hosts: *Paramonostomum caeci* n.sp. which inhabits the intestinal caeca, and *P. bursae* n.sp. which inhabits the bursa of Fabricius.

MATERIALS AND METHODS

Individual *Coxiella badgerensis* from Calverts Lagoon were maintained at room temperature in filtered lagoon water and crystal dishes in the laboratory. Snails releasing notocotylid cercariae were either used to provide metacercarial cysts for experimental infection of ducklings or were dissected and examined for trematode developmental stages. Fully developed cercariae encysted on the host snail's shell or on the walls of the crystal dish.

Domestic ducklings, *Anas platyrhynchos* Linnaeus, purchased when one day old from Rutherglen Duck Farm, Rutherglen, Victoria, were maintained in the laboratory and when approximately one week old were fed gelatin capsules containing metacercarial cysts. The ducks were killed with chloroform after periods of up to five weeks. The periods of infection of these experimental hosts are referred to in this paper using an abbreviated style, i.e. 18 days and 23 hours post-infection is given as 18, 23 days P.I.

Two black ducks, *Anas superciliosa* Gmelin, two black swans, *Cygnus atratus* (Latham), and five hoary-headed grebes, *Poliocephalus poliocephalus* (Jardine and Selby), frequenting Calverts Lagoon were shot and taken to the laboratory where dissection was usually begun within four hours of death. When two birds were shot on the same day, one was stored at 5°C and dissected within 24 hours of death. The duck were shot on 27/4/78, the swan on 8/8/78 and the grebes on 31.7.78 (two), 14.8.78 (two) and 5.10.78 (one).

Observations of live trematodes on microscope slides were facilitated by slightly compressing the specimens in Hank's balanced salt solution under coverslips supported by a little vaseline. Specimens were examined under both ordinary and phase contrast light microscopes. Aqueous vital stain solutions 0.001% brilliant cresyl blue and 0.001% neutral red, were used to stain live cercariae.

Some specimens, slightly compressed under coverslip pressure, were relaxed by heating gently over a slide warmer and then fixed by drawing 70% ethanol under the coverslip with filter paper. Most specimens were fixed, without compression, in boiling 10% phosphate buffered formal saline. Generally, after fixation, worms were stained in alum carmine or Gower's carmine. 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 counterstained in Gower's carmine. Organ primordia, and the various stages of spermatogenesis in metacercariae and juvenile flukes, were detected in aceto-orcein squashes, or whole mounts. Permanent whole mounts of stained specimens were prepared by clearing in clove oil, and mounting in Canada balsam, or D.P.X., via standard procedures. Drawings were made with the aid of a camera lucida.

Unless otherwise stated all measurements are given in microns (mean first, followed by 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.

PARAMONOSTOMUM CAECI n.sp.

1. Life cycle (fig. 1)

Cercariae leave the primary intermediate host, *Coxiella badgerensis*, and encyst on its shell and operculum and on other submerged surfaces, such as the valves of ostracods, cuticle of amphipods and possibly plants. The hoary-headed grebe, black swan and black duck serve as definitive hosts.

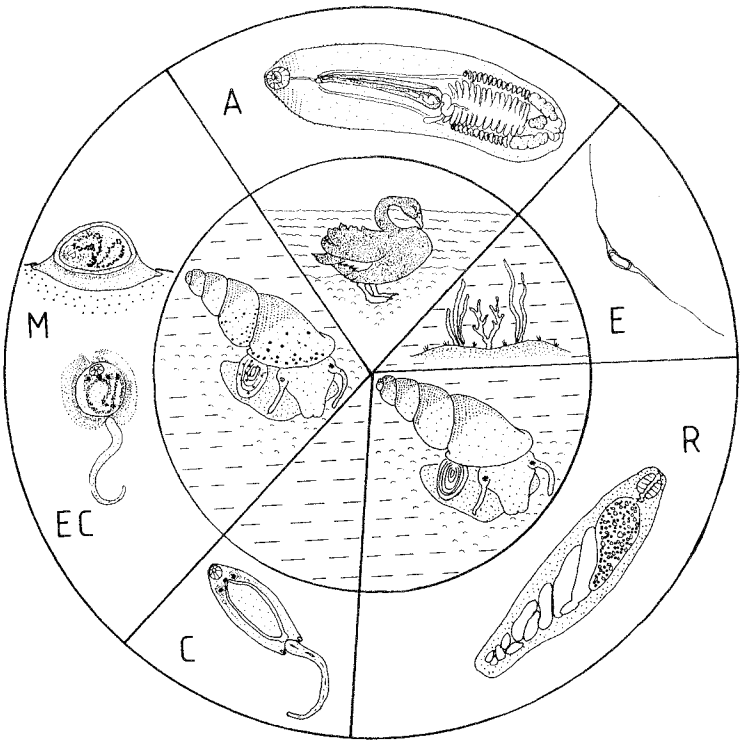

FIG. 1 - *Paramonostomum caeci* n.sp. Life-cycle diagram showing hosts (inner) circle) and parasite stages (outer circle). A: adult; E: egg; R: daughter redia; C: mature cercaria; EC: encysting cercaria; M: metacercarial cyst.

2. Adult (fig. 2)

Unfortunately, gravid specimens recovered from experimentally infected ducklings were all fixed under coverslip pressure. The dimensions of gravid and immature adults from these experimental hosts are shown in table 1. Dimensions of the holotype, and other gravid and immature adults from the hoary-headed grebe, fixed unflattened by standard methods, are shown in table 2.

Description

Dorsoventrally flattened, elongate-spatulate body, margins slightly curled ventrally. Tegumental spines in ventral surface, diminishing in size posteriorly; dorsal surface aspinous. Brown-black pigment dispersed in anterior one-third of body. No ventral glands or glandular ridges. Oral sucker oval, terminal; mouth terminal or subterminal ventral. Pharynx absent; oesophagus short; caeca simple, extending posteriorly between vitellaria and uterine loops, then between ovary and testes, terminating medial to posterior lobes of testes. Excretory ducts ramify throughout body, connecting with closed excretory ring. Excretory bladder large, lobate, opening through dorsal excretory pore. Testes posterolateral, elongate, deeply lobed. Common sperm duct convoluted, expanded serving as external seminal vesicle, entering base of cirrus pouch. Internal seminal vesicle bulbous, sinuous; pars prostatica well-developed, leading to ejaculatory duct within long, slender, coiled cirrus. Everted cirrus, about 171 × 19 µm, covered in small tubercles. Cirrus pouch elongate, clavate, extending from genital pore to near middle of body. Genital pore median, overlying or anterior to oesophageal bifurcation. Ovary lobate, intercaecal, at level of middle to anterior parts of testes. Oviduct short, leading anteriorly to ootype, surrounded by Mehlis' gland. Proximal uterus expanded, serving as seminal receptacle.

TABLE 1

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS OF ADULTS FROM EXPERIMENTALLY INFECTED DUCKLINGS. (a) 2,6 days P.I. (non-ovigerous); and (b) 13,0 days P.I. (ovigerous).

	(a) n = 10		(b)* n = 5	
Body: length	364	(310-416)	1194	(983-1467)
width	157	(144-166)	479	(423-575)
Oral sucker: length	44	(42-49)	81	(76-87)
width	52	(46-53)	89	(80-94)
Oesophagus: length	4	(4-8)	-	
Cirrus pouch: length	-		505	(460-551)
width	-		74	(72-76)
Ovary: length	23	(19-27)	109	(95-129)
width	19	(15-23)	49	(46-57)
Left testis: length	38	(34-42)	173	(163-182)
width	22	(19-23)	89	(87-91)
Right testis: length	40	(38-42)	182	(-)
width	25	(1-38)	87	(84-91)
Body length:body width ratio	2.32		2.49	
Distance from anterior of body to cirrus pouch base:body length ratio	-		0.52	(0.50-0.54)
Distance from posterior of body to anterior of vitellaria:body length ratio	-		0.35	(0.32-0.38)

* fixed under coverslip pressure

TABLE 2

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS OF ADULTS FROM A NATURALLY INFECTED HOARY-HEADED GREBE. (a) ovigerous specimens; (b) non-ovigerous specimens; and (c) dimensions of the holotype, a gravid specimen.

	(a) n = 10		(b) n = 10		(c) n = 1
Body: length	736	(597-907)	475	(333-590)	816
width	189	(151-219)	133	(83-197)	219
Oral sucker: length	53	(46-61)	47	(38-59)	51
width	59	(49-67)	47	(34-55)	57
Oesophagus: length	12	(0-46)	6	(4-8)	53
Cirrus pouch: length	331	(293-395)	224		361
width	33	(30-38)	19		32
Metraterm: length	287	(228-369)	-		312
width	16	(11-19)	-		14
Ovary: length	61	(53-87)	38	(27-49)	57
width	38	(27-42)	23	(19-27)	38
Left testis: length	108	(84-125)	93	(84-103)	110
width	45	(42-49)	40	(38-42)	49
Right testis: length	108	(87-122)	82	(68-95)	122
width	52	(42-65)	46	(42-49)	65
Body length:body width ratio	3.89		3.57		3.73
Distance from anterior of body to cirrus pouch base:body length ratio	0.55	(0.52-0.58)	-		0.56
Distance from posterior of body to anterior of vitellaria:body length ratio	0.37	(0.33-0.42)	-		0.39

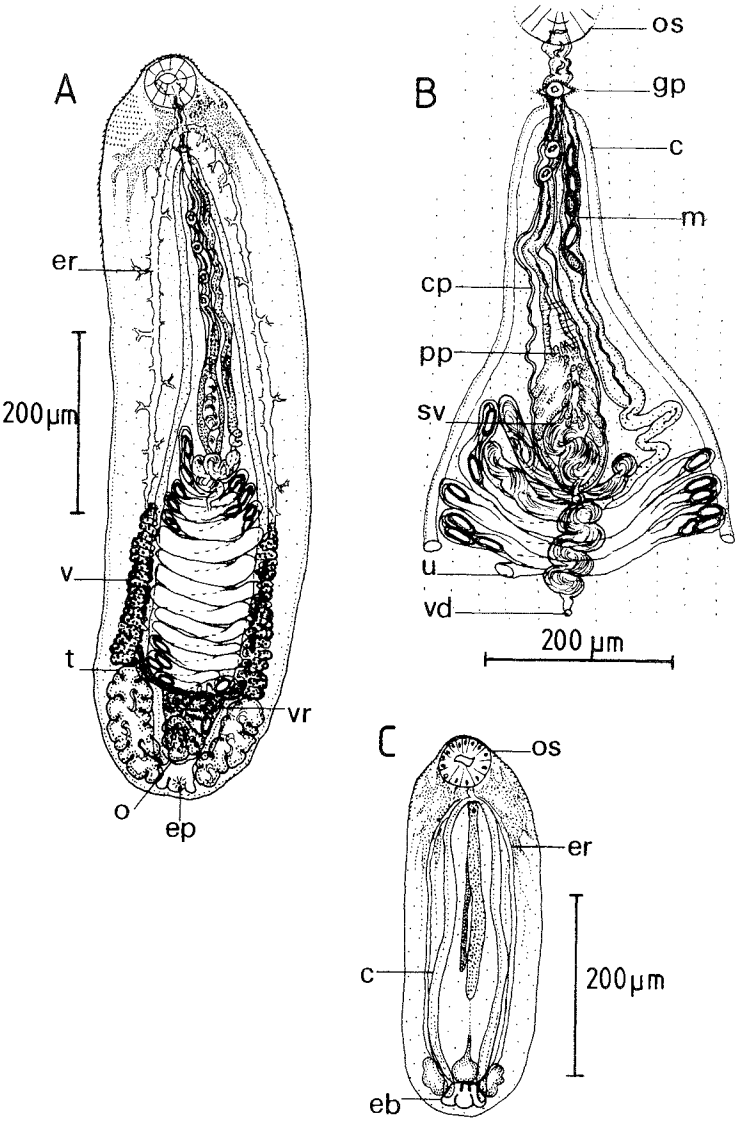

FIG. 2 - *Paramonostomum caeci* n.sp. A: gravid adult from hoary-headed grebe, ventral view; B: flattened gravid adult, after 13 days in laboratory duckling, detail of cirrus pouch region, ventral view; C: immature adult, after 2,6 days in laboratory duckling, dorsal view (c: caecum; cp: cirrus pouch; eb: excretory bladder; ep: excretory pore; er: excretory ring; gp: genital pore; m: metraterm; o: ovary; os: oral sucker; pp: pars prostatica; sd: sperm duct; sv: seminal vesicle; t: testis; u: uterus; v: vitellaria; vr: vitelline receptacle).

Uterus initially passing posteriorly, then forming 12-14 transverse loops between transverse vitelline ducts and cirrus pouch. Metraterm well-developed, opening with cirrus pouch at ventral genital pore. Ratio of length of metraterm to length of cirrus pouch = 0.9. Vitelline glands extracaecal, longitudinal, extending from testes to two-fifths body length from posterior end of body, never reaching middle of body. Vitelline ducts transverse, uniting between caeca, forming sinistral vitelline reservoir, which leads posteriorly to ootype, anteromedial to ovary. Hundreds of large eggs occupying uterus of gravid adult.

Vertebrate hosts

Anas platyrhynchos L. (experimental host); *A. superciliosa* Gmelin; *Cygnus atratus* (Latham); and *Poliocephalus poliocephalus* (Jardine and Selby).

Habitat

Mainly intestinal caeca, also lower small intestine and rectum.

Geographic location

Calverts Lagoon, southeast Tasmania.

Type material

Tasmanian Museum - K907, holotype (ringed), gravid adult; K907, paratypes (not ringed), gravid adults; K908, K909, K910 and K911, paratypes, adults.

Relationships

Paramonostomum caeci n.sp. clearly belongs to the *elongatum* group of Harwood (1939) and is most similar to the group of species: *P. elongatum*, *P. bucephalae* and *P. malerischi*, that Stunkard (1967) said were characterised by "linear, spatulate bodies, very long cirrus sacs that extend to the middle of the body, and short vitelline zones". From the position of the genital pore, overlying or anterior to the oesophageal bifurcation, and the relatively large eggs, *P. caeci* n.sp. appears closest to *P. malerischi*, a fluke inhabiting the caeca of the Emperor goose, *Philacte canagica*, in Alaska (Dunagan 1957). Apart from the major difference in size (*P. malerischi* is more than three times longer than the longest specimen of *P. caeci* n.sp.), the tegument of *P. malerischi* is aspinous and its cirrus in "unarmed", whereas the tegument of *P. caeci* n.sp. bears spines in the anterior ventral region, and its cirrus is covered with distinct tubercles.

Biology

Cysts varying in age from 11 to 42 days, were found to be infective to laboratory ducklings. Four out of nine ducklings became infected with from one to twenty-eight flukes. Up to 80% of metacercariae were recovered as adults, all of them living in the intestinal caeca. The adults are established in the caeca after 22 hours and can live there for at least 13 days.

This fluke naturally inhabits the caeca, and is sometimes also found in the lower small intestine and rectum of several bird species at Calverts Lagoon. One out of two black ducks harboured seven adults; two out of two black swans harboured 20 and 70 adults and five out of five hoary-headed grebes were heavily infected, each harbouring hundreds of adults.

The metacercaria undergoes considerable growth and development within the experimental bird host. After 2,6 days the genital primordia can be distinguished, and by 5,3 days eggs are being formed and occupy the proximal 11 loops of the uterus. After 13,0 days, the adult has grown considerably and eggs occupy 12 to 14 uterine loops. Adults removed from an experimentally infected duckling after 13,0 days, were maintained *in vitro* at 41°C for 2 days in 40% foetal calf serum in Eagles' M.E.M. Large numbers of eggs were shed into the culture medium.

3. Egg (figs 3A, 3B)

The operculate egg is without filaments when formed and is oblong to reniform in shape. Long polar filaments develop as it passes through the uterus, one filament forming at each end of the egg. The egg-shell is transparent, colourless and uniform in thickness, except where it thins at the operculum rim. Miracidial development was discernible within the egg after 3 days in lagoon water. No eggs had hatched after 6 days under normal light and temperature conditions. Ten uninfected snails, *Coxiella badgerensis*, were fed eggs but were found not to be infected when dissected 49 days later. Live eggs measure about 26 × 11 µm, and polar filaments extend up to 333 µm. Dimensions of fixed eggs (not including the polar filaments), are shown in table 3.

TABLE 3

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS OF UTERINE EGGS IN ADULT FLUKES. (a) from a naturally infected hoary-headed grebe; and (b) *from an experimentally infected duckling, 13,0 days P.I.

Host	Number of eggs	Length	Width
(a)	10	25 (23-29)	11 (8-14)
(b)	10	26 (24-27)	12 (11-12)

* flukes fixed under coverslip pressure

4. Redia (fig. 3C)

The rediae are more or less cylindrical to sausage-shaped, maximum width occurring near the middle and minimum width occurring at the posterior end. They range in size from very small individuals containing only germ balls, to the largest, containing germ balls and immature cercariae. The tegument is transparent and colourless, however the intestine appears brown-black, due to contained decomposing snail tissue. A terminal mouth opens into a large, barrel-shaped, muscular pharynx. A short sinuous oesophagus leads to the simple intestine, which is about one-half the body length in small rediae, but becomes relatively shorter as cercariae develop in the "brood chamber". Cercariae leave the redia through a birth pore situated at the mid-level of the pharynx and complete their growth and development free in the snail tissues.

The number of rediae found per infected snail, varied from 11 to 38. They occurred throughout the host's viscera, but were concentrated in the hepatopancreas. Dimensions of daughter rediae are shown in table 4.

TABLE 4

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS OF DAUGHTER REDIAE. Fifteen rediae selected at random

Body: length	597 (257-696)
width	160 (129-189)
Pharynx: length	66 (61-76)
width	62 (55-68)

5. Cercaria (fig. 4)

The body of this monostome cercaria is broadly oval to elongate, slightly concave ventrally. The tegument is speckled with numerous minute papillae, or spines. The tail is simple, usually longer than the body. Both the body and tail are very contractile. The tail, joined to the body by a slender peduncle, is attached terminally or slightly posterodorsally. The peduncle is flanked by two eversible, posterolateral locomotory appendages, each with an adhesive sucker or gland at its tip. A narrow excretory duct extends about three-quarters of the length of the tail. A round to oval oral sucker protrudes anteriorly. The mouth is subterminal ventral, surrounded by a ring of about 32 ciliated papillae. The oesophagus bifurcates posterior to the lateral eyespots, forming simple caeca which extend to the level of the excretory bladder. The parenchyma is packed, from the mid-level of the oral sucker to the excretory bladder, with unicellular cystogenous glands about 11 µm diameter, each filled with bacilliform rods about 2-3 µm long.

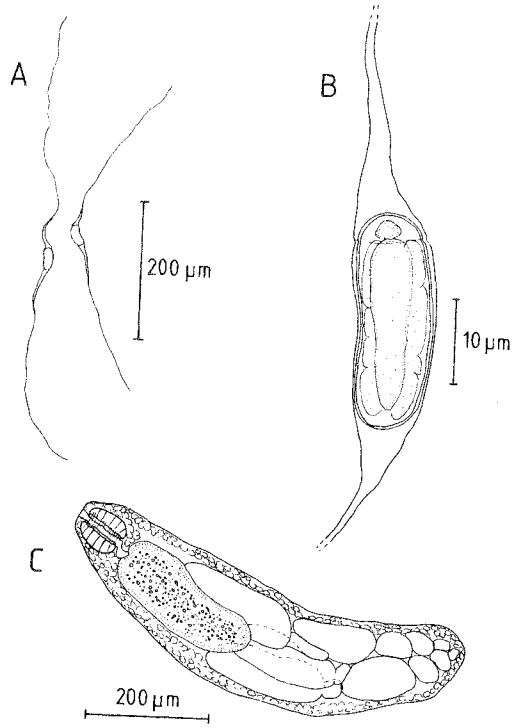

FIG. 3 - *Paramonostomum caeci* n.sp.
A: eggs deposited *in vitro* by fluke from experimentally infected duckling;
B: detail of similar egg after 3 days in lagoon water at room temperature;
C: daughter redia.

These glands make the body relatively opaque and obscure the anatomy. The excretory ring can only be seen when the body is flattened. It is of the Imbricata type, having an anterior loop that extends anteriad to the cerebral ganglion, overlapping, and sometimes extending anterior to the median eyespot. The ring is without diverticula and contains excretory granules, 5-8 µm diameter, along its length. There are about 4 granules across the posterior part of the ring, where it is about 20 µm wide, and from 1 to 3 granules across the anterior extremity of the ring, where it is only about 10 µm wide. The lateral eyespots are oval to round, composed of densely packed black pigment granules. The median eyespot is annular, composed of more dispersed brown pigment granules. This pigment is also distributed in dendritic strands around, and extending posteriad from, the lateral eyespots.

Under natural light and temperature conditions, mature cercariae emerged from the snail host between dawn and midday. Under controlled conditions, emergence occurred when the host snail was exposed to bright light, following a period of darkness. When snails were kept in constant darkness, no cercariae emerged. Free-swimming cercariae are positively phototactic, and in the laboratory swam near the water surface, encysting within an hour of emergence from the primary host. Although some cercariae encysted on the snail shell, most encysted on the side of the glass container. The cercariae swam an erratic course, the body leading and the tail describing the infinity symbol in the vertical plane.

Dimensions of the cercaria are shown in table 5.

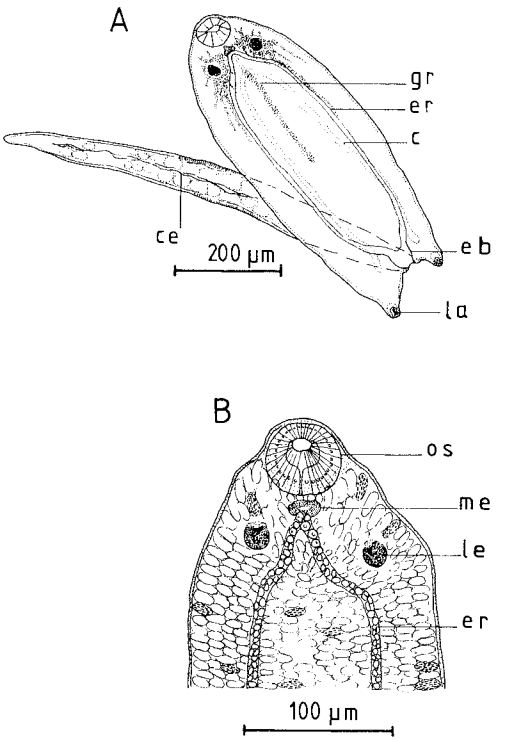

FIG. 4 - *Paramonostomum caeci* n.sp.
A: cercaria, dorsal view; B: flattened cercaria, detail of anterior region, ventral view (c: caecum; ce: caudal excretory duct; eb: excretory bladder; er: excretory ring; gr: genital rudiment; la: locomotory appendage; le: lateral eyespot; me: median eyespot; os: oral sucker).

TABLE 5

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS OF MATURE CERCARIAE.
Fifteen cercariae measured after emergence from *Coxiella badgerensis*.

Body:	length	534 (454-612)
	width	165 (166-197)
	depth	109 (91-144)
Tail:	length	655 (499-771)
	width	54 (45-68)
Oral sucker:	length	47 (42-53)
	width	49 (46-51)
	depth	48 (44-51)
Eyespot diameter		17 (15-19)

6. Metacercaria (fig. 5A)

When the cercaria stops swimming it creeps briefly, pauses, and then encystment commences. Cystogenous glands secrete bacilliform granules through the tegument, forming a flimsy outer cyst, which adheres to the substrate. The body contracts into a round shape and slow rhythmic contractions pass anteriorly along it. After a few minutes the body moves independently within the initial cyst wall. At this time the tail breaks free and swims away. The body rotates back and forth, as more cystogenous secretions are added internally to the cyst wall. The mature cyst is more or less hemispherical, with a thin flange around the base. Adherence to the substrate is by this peripheral flange, not by the basal cyst wall. The enclosed metacercaria appears light brown, with darker stripes, due to the conspicuous granular excretory ring.

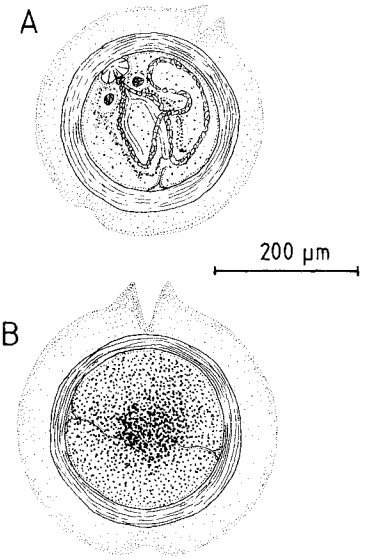

FIG. 5 - Notocotylid metacercarial cysts, plan view.
A: *Paramonostomum caeci* n.sp.
B: *Paramonostomum bursae* n.sp.

The cysts formed by cercariae from the same snail are similar in size, however there is variation between cysts formed by cercariae from different snails. Dimensions of metacercarial cysts are shown in table 6.

TABLE 6

PARAMONOSTOMUM CAECI n.sp. DIMENSIONS IN PLAN VIEW OF METACERCARIAL CYSTS.
Cysts formed by cercariae from two different snails.

	(a) n = 5	(b) n = 5
Interior: length	191 (186-194)	198 (194-201)
width	192 (190-194)	195 (190-201)
Exterior: length	253 (247-258)	252 (247-258)
width	255 (251-262)	248 (243-255)
Basal flange: length	306 (300-312)	327 (319-346)
width	310 (304-323)	304 (—)

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PARAMONOSTOMUM BURSAE n.sp.

1. Life-cycle

The life-cycle of *P. bursae* n.sp. is similar to that of *P. caeci* n.sp. (fig. 1). Cercariae, on leaving the primary intermediate host *Coxiella badgerensis*, encyst on the shell and operculum of the snail and on other submerged surfaces, such as plant stems. The black duck serves as the definitive host.

2. Adult (figs 6A and 7)

Dimensions of immature adults and mature adults with a few incomplete eggs, fixed by standard methods, from experimental hosts, are shown in table 7. Unfortunately gravid adults containing hundreds of fully formed eggs, from both experimental hosts and the black duck, were fixed under coverslip pressure. One such gravid adult from a laboratory duckling is designated as the holotype, and its dimensions are shown, with those of other gravid flukes, in table 8.

Description

Body oval to pyriform, flattened, leaf-like; margins sometimes folded ventrally. Tegument aspinous. Black-brown pigment grains distributed extensively in anterior region No ventral glands or glandular ridges. Oral suck round terminal; mouth terminal or subterminal ventral; pharynx absent; oesophagus short. Caeca simple convoluted, extending posteriorly between vitellaria and uterus, then between ovary and testes, terminating posterior to testes. Excretory ducts anastomose throughout body, from excretory ring. Excretory bladder lobate, opening through dorsal excretory pore. Testes lobate, posterolateral. Common sperm duct convoluted, expanded, serving as external seminal vesicle, entering proximal end of cirrus pouch. Internal seminal vesicle leading via well-developed pars prostatica to ejaculatory duct, within cirrus. Everted cirrus, elongate, about 300 × 35 µm, covered in large tubercles. Cirrus pouch clavate, extending posteriorly from genital pore to between one-third and two-fifths body length from anterior end of body. Genital pore median, immediately posterior to oesophageal bifurcation. Ovary lobate, sub-median dextral, inter-caecal; mid-level of ovary anterior to mid-level of testes; ovary usually extending anterior to testes, never posterior. Oviduct short, passing posterolateral to ootype, surrounded by median Mehlis' gland. Proximal uterus serving as seminal receptacle. Uterus convoluted, forming 12 to 15 transverse, intercaecal loops. Metraterm opening with cirrus pouch at ventral genital pore. Ratio of length of metraterm to cirrus pouch length from 0.6 to 0.8. Vitelline glands extra-caecal, longitudinal, extending from testes to middle of body. Vitelline ducts transverse, uniting to form longitudinal vitelline reservoir, which leads posteriorly to ootype. Hundreds of relatively large eggs occupying uterus of gravid adult.

Vertebrate hosts

Anas platyrhynchos L. (experimental host), *A. superciliosa* Gmelin.

Habitat

Bursa of Fabricius.

Geographical position

Calverts Lagoon, southeast Tasmania.

Type material

Tasmanian Museum - K912, holotype, gravid adult (flattened); K913, paratypes, gravid adults (flattened); K914 and K915, paratypes, immature adults.

Relationships

The broad, oval to pyriform body of *Paramonostomum bursae* n.sp. is characteristic of the *alveatum* group of the genus (Harwood 1939), however unlike many species of this shape, the vitellaria do not extend anteriad as far as the cirrus pouch, and do not extend

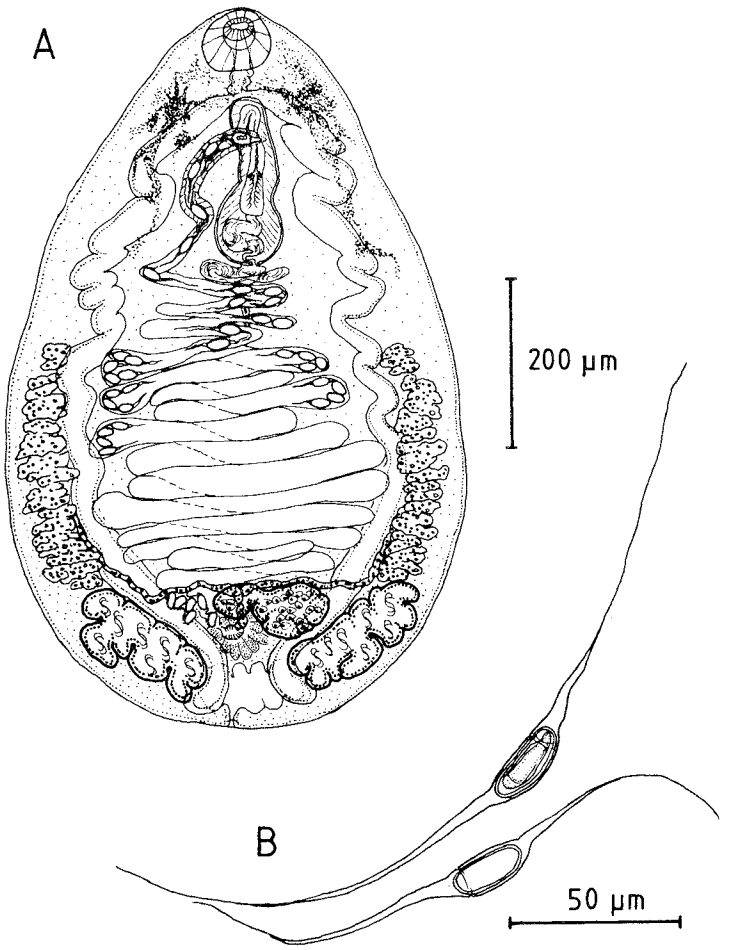

FIG. 6 - *Paramonostomum bursae* n.sp.
A: holotype, flattened gravid adult, from laboratory duckling 9 days after infection, dorsal view; B: eggs deposited *in vitro* by fluke taken from wild black duck.

into the anterior third of the body. In morphology and anatomy *P. bursae* n.sp. appears most similar to *P alveatum* and *P. parvum*. Like these species, it has a relatively short cirrus pouch, which extends only up to two-fifths body length from the anterior end of the body. It differs from both of these species, however, in the distribution of vitellaria. In *P. alveatum* and *P. parvum*, vitellaria extend into the anterior one-third of the body, according to Stunkard (1967). The position of the ovary also differs, being dextral and extending anterior to the testes in *P. bursae* n.sp.; and median, at the level of the posterior part of the testes in *P. alveatum* and *P. parvum*. The number of uterine loops of *P. bursae* n.sp. is 12-15, and that of *P. alveatum* and *P. parvum* is 10-12. *P. alveatum* and *P. parvum* inhabit the lumen of the intestine of ducks in North America (Stunkard & Dunihue 1931, Stunkard 1967) and *P. alveatum* also occurs in anatids in Europe (Yamaguti 1971). *P. bursae* n.sp. inhabits the bursa of Fabricius of ducks at Calverts Lagoon. The cercariae of *P. alveatum* and *P. parvum* are of the Monostomi type (Stunkard 1967), whereas the cercaria of *P. bursae* n.sp. is of the Yenchingensis type.

TABLE 7

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS OF ADULTS FROM EXPERIMENTALLY INFECTED DUCKLINGS. (a) 2,12 days P.I.; and (b) 5,3 days P.I. with newly formed eggs, lacking filaments, in the proximal uterus.

	(a) n = 15		(b) n = 4	
Body: length	443	(378-537)	764	(756-771)
width	181	(136-212)	314	(295-333)
Oral sucker: length	49	(46-53)	68	(67-68)
width	49	(46-51)	70	(68-72)
Oesophagus: length	58	(57-61)	49	(38-61)
Cirrus pouch: length	-		192	(171-213)
width	-		53	(49-57)
Metraterm: length	-		148	(144-152)
width	-		23	(-)
Ovary: length	-		60	(53-67)
width	-		72	(-)
Left testis: length	38	(-)	97	(87-103)
width	38	(34-42)	95	(87-103)
Right testis: length	47	(38-53)	108	(106-110)
width	38	(34-46)	105	(103-106)
Body length:body width ratio	2.45		2.43	

TABLE 8

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS OF GRAVID ADULTS.*
(a) from an experimentally infected duckling 9,0 days P.I.;
(b) from a naturally infected black duck; and (c) dimensions of the holotype, taken from (a).

	(a) n = 5		(b) n = 10		(c) n = 1
Body: length	650	(469-832)	1338	(953-1648)	832
width	416	(318-544)	764	(605-907)	544
Oral sucker: length	56	(49-65)	95	(76-106)	65
width	74	(72-80)	114	(87-129)	80
Cirrus pouch: length	151	(95-201)	267	(156-327)	201
width	62	(46-80)	103	(68-114)	80
Metraterm: length	152		169	(125-239)	152
width	27		37	(30-42)	27
Ovary: length	48	(38-61)	133	(76-182)	61
width	84	(76-95)	151	(122-198)	95
Left testis: length	127	(99-179)	208	(148-266)	171
width	60	(49-72)	164	(114-228)	68
Right testis: length	117	(80-171)	211	(148-239)	186
width	61	(49-76)	165	(129-209)	76
Body length:body width ratio	1.56		1.75		1.53
Distance from anterior of body to cirrus pouch base:body length ratio	0.38	(0.35-0.39)	0.35	(0.29-0.39)	0.36
Distance from posterior of body to anterior of vitellaria:body length ratio	0.53	(0.51-0.55)	0.55	(0.50-0.61)	0.52

* fixed under coverslip pressure

Biology

Cysts from 2-24 days old proved to be infective to laboratory ducklings. Up to about 60% of metacercariae were recovered as adults, and every adult inhabited the bursa of Fabricius. Five out of eight ducklings that were exposed to infection, harboured adults of *P. bursae* n.sp. From two to 24 adult worms were recovered from each infected bird. The maximum longevity of *P. bursae* n.sp. in the laboratory duckling was 9,0 days. One of two wild black ducks shot at Calverts Lagoon was infected with 16 adult *P. bursae* n.sp., all of which inhabited the bursa of Fabricius.

Considerable growth and development occurs within experimentally infected ducklings. After 1,23 days, the adult has reached the bursa of Fabricius. Its body is small and elongate and genital primordia are barely discernible. Black pigment is still concentrated in the regions of the cercarial eyespots. After 2,12 days, the body is broader and more pyriform, black pigment is dispersed in the anterior region, and the gonads and cirrus pouch have developed. The juvenile fluke nearly doubles in size over the next 3 days (fig. 7). At 5,3 days post-infection the reproductive system is fully developed, and egg production has commenced. At 9,0 days the uterus is filled with hundreds of eggs. Adults of *P. bursae* n.sp. are usually found deeply buried in the plicate lining of the bursa of Fabricius, their presence revealed by superficial white patches in the otherwise pink host tissue. When not buried, the dark anterior pigmentation makes the flukes conspicuous.

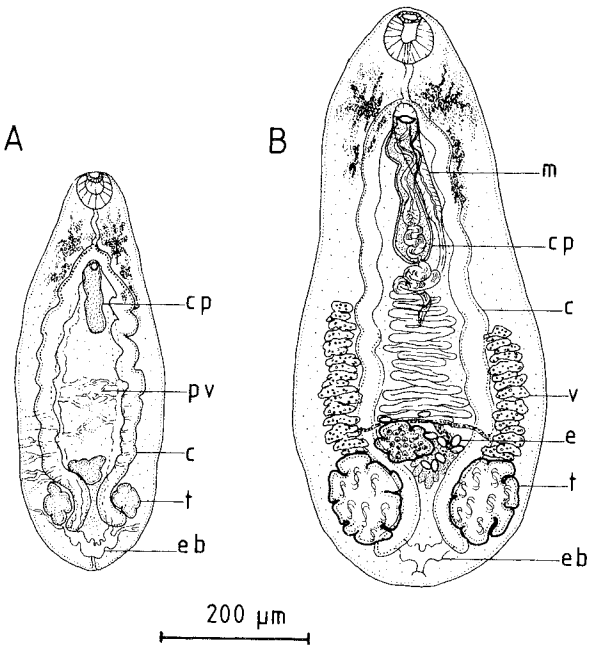

FIG. 7 - *Paramonostomum bursae* n.sp.
A: juvenile adult, after 2,12 days in laboratory duckling, ventral view;
B: mature adult with incompletely formed eggs in the proximal uterus, after 5,3 days in laboratory duckling, ventral view.
(c: caecum; cp: cirrus pouch; e: egg; eb: excretory bladder; m: metraterm; pv: paranephridial vessels; t: testis; v: vitellaria).

3. Eggs (fig. 6B)

The operculate egg, like that of *P. caeci* n.sp., is formed without polar filaments. Filaments form as the clear, colourless egg passes through the uterus. Dimensions of uterine eggs, not including the polar filaments, are shown in table 9. They are markedly smaller than those of *P. caeci* n.sp. Polar filaments are about 114 µm long.

TABLE 9

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS OF UTERINE EGGS IN FLUKES.*
(a) from an experimentally infected duckling, 9,0 days P.I.; and
(b) from a naturally infected black duck.

Host	Number of eggs	Length	Width
(a)	10	20 (19-21)	11 (8-11)
(b)	10	21 (18-23)	11 (10-12)

* flukes fixed under coverslip pressure

Miracidial development was evident in the egg after two days in lagoon water at room temperature, under normal light conditions. No eggs had hatched after 30 days under these conditions. Eggs incubated in lagoon water for two days were fed to ten laboratory-bred snails, *Coxiella badgerensis*. Five snails were dissected after 34 days, and five after 69 days; however, none were infected.

4. Redia (fig. 8A)

Rediae are cylindrical to sausage-shaped, maximum width occurring in the anterior one-third of the body. The terminal mouth opens into a relatively small pharynx and a short convoluted oesophagus leads to the simple intestine, which in larger specimens is filled with black-brown, granular, decomposing snail tissue. The gut becomes relatively shorter as the redia grows. Immature cercariae emerge through a birth pore lateral to the pharynx.

Rediae are found throughout the host viscera, mainly in the hepatopancreas. In one snail, 18 rediae were found, eight relatively mature specimens containing germ balls and developing cercariae, and ten immature specimens containing only germ balls. Dimensions of daughter rediae are shown in table 10.

TABLE 10

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS OF DAUGHTER REDIAE.
Ten daughter rediae selected at random.

Body: length	803 (544-953)
width	194 (151-257)
Pharynx: length	51 (38-61)
width	54 (38-61)

5. Cercaria (figs 8B and 8C)

Mature cercariae are similar in morphology and anatomy, but slightly larger than, those of *Paramonostomum caeci* n.sp. The tegument is speckled with minute papillae, or spines. Two lateral eyespots are composed of dense concentrations of black pigment grains. A third, median eyespot is composed of more dispersed brown pigment grains, which are extensively distributed in dendritic strands around the eyespots and extend posteriorly,

dorsal to the excretory ring. The tail is simple and attached terminally, between posterolateral, locomotory appendages. A caudal excretory duct extends through the middle of the tail, flanked by six pairs of large cells, which are stained intensely by neutral red. The round oral sucker protrudes slightly anteriorly; the mouth is terminal to subterminal ventral, encircled by a ring of prominent sensory papillae. The oesophagus and caeca are developed and the body densely packed with round cystogenous glands, containing bacilliform granules up to 5 µm long. The distinguishing characteristic of this cercaria is the excretory ring of the Yenchingensis type. It has a large single anterior diverticulum, that arises between the lateral eyespots and extends anterior to the median eyespot, sometimes as far as the oral sucker. The excretory ring is packed with granules which increase in diameter anteriorly from 5-11 µm. There are about four grains across the ring near the excretory bladder, and only one or two across the anterior part of the ring and diverticulum.

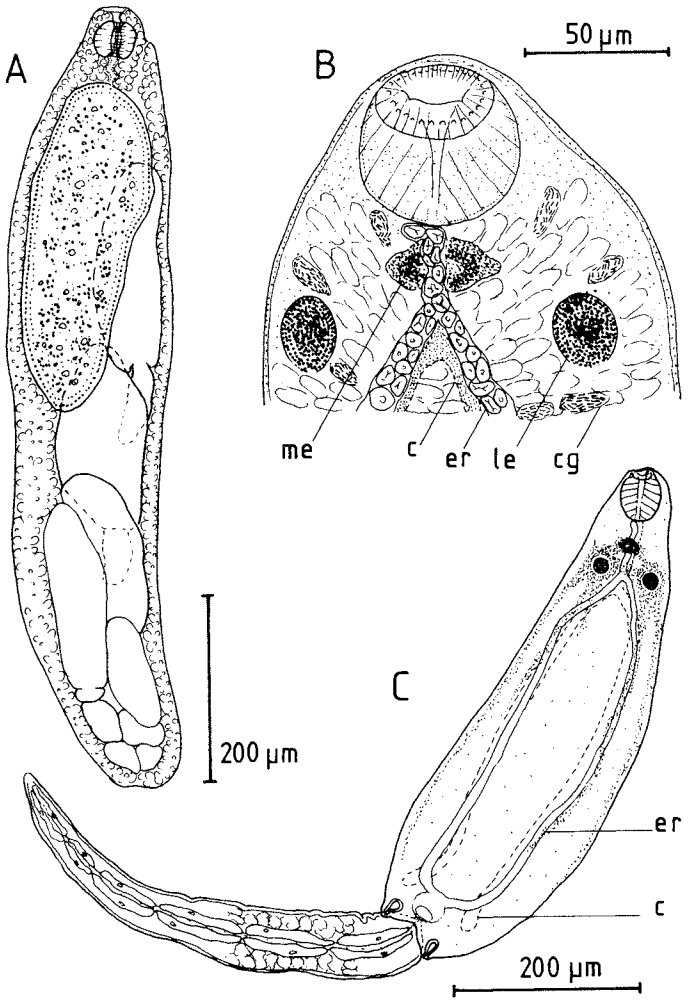

FIG. 8 - *Paramonostomum bursae* n.sp.
A: daughter redia;
B: flattened cercaria, detail of anterior region, ventral view;
C: cercaria, dorsal view. (c: caecum; cg: cystogenous gland; er: excretory ring; le: lateral eyespot; me: median eyespot).

In the laboratory, mature cercariae emerged from the snail host during mid-morning. After a variable period of swimming, from about 1-60 minutes, the positively phototactic cercariae encysted on the sides of the glass container, or on the host's shell and operculum. Dimensions of the cercaria are shown in table 11.

TABLE 11

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS OF MATURE CERCARIAE.
Fifteen cercariae measured after emergence from the snail host.

Body: length	534 (408-620)
width	180 (163-201)
depth	147 (106-179)
Tail: length	650 (559-786)
width	58 (49-65)
Oral sucker: length	51 (49-57)
width	46 (38-49)
depth	49 (—)
Eyespot diameter	24 (21-27)

6. Metacercaria (fig. 5B)

Encystment occurs in the same manner as described for *P. caeci* n.sp. The cyst is more or less hemispherical, with a thin basal flange. It is round, in plan view, with a markedly greater diameter than that of the cyst of *P. caeci* n.sp. The cyst contents of *P. bursae* n.sp. appear uniformly dark brown, and almost opaque, whereas those of *P. caeci* n.sp. appear striped, due to the translucent light brown parenchyma and opaque excretory ring. Dimensions of metacercarial cysts are shown in table 12.

TABLE 12

PARAMONOSTOMUM BURSAE n.sp. DIMENSIONS IN PLAN VIEW OF METACERCARIAL CYSTS.
Cysts formed by cercariae from two different snails.

	(a) n = 5	(b) n = 4
Interior: length	214 (209-220)	224 (220-228)
width	215 (209-224)	220 (213-228)
Exterior: length	—	280 (270-285)
width	—	282 (274-289)
Flange: length	345 (342-350)	365 (361-372)
width	350 (342-372)	367 (361-376)

DISCUSSION

Infection of one snail species by a number of notocotylid trematodes has been previously recorded. Rothschild (1941) reported a five-year study of the infection of *Peringia ulvae* Pennant in England by six notocotylid species. Three of the species belonged to the Yenchingensis group and three to the Monostomi group. Stunkard (1967) reported the discovery of five notocotylid species infecting *Hydrobia salsa* in the U.S.A. One of the species belonged to the Imbricata group, two to the Yenchingensis group and two to the Monostomi group.

Although the life-cycles of two of the notocotylids at Calverts Lagoon, *Paramonostomum caeci* n.sp. and *P. bursae* n.sp., have been elucidated, further studies are needed to identify another two notocotylid species that infect *Coxiella badgerensis* at the lagoon.

Rothschild (1941) found that two species of the Yenchingensis group developed into adults of the genus *Paramonostomum*, in the intestinal caeca of ducks. Stunkard (1967) found that the cercariae of *P. alveatum* and *P. parvum*, belong to the Monostomi group, and that the corresponding adults inhabit the lumen of the intestine of their bird host. Velasquez (1969) reported that the cercaria of *P. philippinensis* is of the Yenchingensis group, although his description and illustration indicate that it belongs to the Imbricata group. The adult of this species inhabits the intestinal caeca of the bird host. At Calverts Lagoon, the cercaria of *P. caeci* belongs to the Imbricata group, and the adult inhabits the intestinal caeca of ducks, whereas that of *P. bursae* n.sp. belong to the Yenchingensis group, and the adult inhabits the bursa of Fabricius of ducks. The cercariae of species currently classified in the genus *Paramonostomum*, thus belong to the Imbricata, Yenchingensis and Monostomi groups. Odening (1966) reported that the cercariae of five *Notocotylus* species belonged to the Monostomi group, whereas Stunkard (1960, 1966) found that the cercariae of two *Notocotylus* species belonged to the Yenchingensis group. A cercaria of the Yenchingensis group (Szidat & Szidat 1933) and one of the Monostomi group (Yamaguti 1938) are reported to develop into the same adult, *Notocotylus attenuatus*.

The apparent lack of correlation between cercarial types and notocotylid genera raises some doubts about the current classification within the family *Notocotylidae*. In his review of the genus *Paramonostomum*, Stunkard (1967) emphasized the importance of the discovery of life-cycles and the description of larval stages, to the taxonomy of the *Notocotylidae*. The present studies add support to that view.

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