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TWO STRIGEOID TREMATODES, *APATEMON (APATEMON) GRACILIS* (RUDOLPHI, 1819) AND *DIPLOSTOMUM (DOLICHORCHIS) GALAXIAE* n.sp., WHICH ENCYST IN THE FRESHWATER FISH *GALAXIAS AURATUS* JOHNSTON IN LAKE CRESCENT, TASMANIA.

by S.J. Smith and J.L. Hickman
The Friends' School, Hobart and Department of Zoology, University of Tasmania

(with nine tables, six text-figures and one plate)

ABSTRACT

SMITH, S.J. & HICKMAN, J.L., 1983 (31 viii): Two strigeoid trematodes, *Apatemon (Apatemon) gracilis* (Rudolphi, 1819) and *Diplostomum (Dolichorchis) galaxiae* n.sp., which encyst in the freshwater fish *Galaxias auratus* Johnston in Lake Crescent, Tasmania. *Pap. Proc. R. Soc. Tasm.*, 117: 21-39. https://doi.org/10.26749/rstpp.117.21 ISSN 0080-4703. The Friends' School, Hobart and Department of Zoology, University of Tasmania, Hobart, Tasmania.

The metacercaria and adult of *Apatemon (Apatemon) gracilis* (Rudolphi) and *Diplostomum (Dolichorchis) galaxiae* n.sp. are described and figured. Metacercarial cysts of these species occur in different parts of the endemic fish *Galaxias auratus: A. gracilis* in the body cavity, orbit and vitreous humour, and *D. galaxiae* n.sp. visible as black spots ('black-spot disease') in the musculature of the body and head. *A gracilis* which has not previously been recorded from Australia occurs in the black duck, *Anas superciliosa*, while *D. galaxiae* n.sp. infects the white-faced heron, *Ardea novaehollandiae*. The domestic duck, *Anas platyrhynchos*, is much more susceptible to experimental infection by *Apatemon gracilis* than by *D. galaxiae* n.sp. Both flukes inhabit the upper small intestine of their bird hosts. The reproductive system of *D. galaxiae* n.sp. is amphitypic. *D. galaxiae* n.sp. most closely resembles *D. heronei* Srivastava, 1954 and *D. ketupanensis* Vidyarthi, 1937; a key to species in the sub-genus *Dolichorchis* is given.

INTRODUCTION

Galaxias auratus Johnston, a small freshwater fish endemic to Lakes Sorell and Crescent in central Tasmania has been found to suffer from black-spot (or black-grub) disease (plate 1). This disease, caused by trematode cysts, has been reported from many species of freshwater fish of various taxonomic groups around the world (Singh 1956; Ganapati & Rao 1962; Hoffman 1967; and Berra & Au 1978), but has not previously been recorded in Australia. Investigation revealed that the black spots in *G. auratus* were cysts of *Diplostomum (Dolichorchis) galaxiae* n.sp., and that the fish also harboured cysts of *Apatemon (Apatemon) gracilis* (Rudolphi, 1819). *A gracilis* encysts in the body cavity, orbit and vitreous humour; and *D. galaxiae* n.sp. encysts in the musculature throughout the body and head.

In the laboratory gravid adults of both species were recovered from domestic ducklings, *Anas platyrhynchos* L., which had been fed with cysts from naturally infected *Galaxias auratus* from Lake Crescent. A black duck, *Anas superciliosa* Gmelin, killed at Calverts Lagoon southeast of Hobart, Tasmania, was found to be infected with adult flukes conforming to the description of *Apatemon gracilis*. Two white-faced herons, *Ardea novaehollandiae* Latham, killed while feeding on *Galaxias auratus* at Lake Crescent, were each found to harbour hundreds of flukes identical to adults of *Diplostomum galaxiae* n.sp. taken from experimentally infected ducklings.

Apatemon (Australapatemon) intermedius (Johnston, 1904) Dubois, 1937 is the only member of this genus previously recorded in Australia. In South Australia, it encysts in freshwater leeches, and matures in the black swan (Johnston & Angel 1951; Dubois & Pearson 1965). Six species of *Diplostomum* have previously been recorded in Australia: *D. (Adenodiplostomum) triangulare* (Johnston, 1904) Dubois & Pearson, 1967; *D. (Diplostomum) spathaceum murrayense* (Johnston & Cleland, 1938), adult described by Johnston & Angel (1941); *D. (Diplostomum) amygdalum* Dubois & Pearson, 1965; *D. (Dolichorchis) auriculosum*

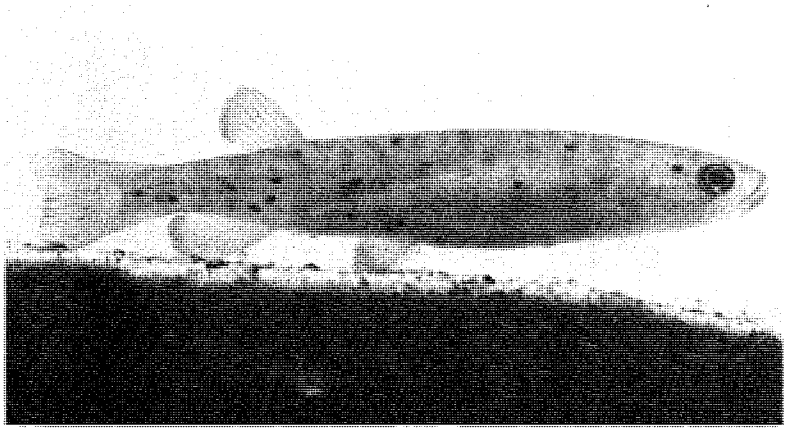

PLATE 1 - Golden galaxiid, *Galaxias auratus*, from Lake Crescent. Trematode cysts *(Diplostomum galaxiae* n.sp.*)* are visible as dense black spots in musculature of the body and head. Photo: J. Lim, x $^{3}/_{2}$.

Dubois & Pearson, 1967; *D. (Diplostomum) parvulum* Dubois & Angel, 1972; and *D. (Tylodelphys) podicipinum podicipinum* Kozicka & Niewiadomska, 1960, recorded in Australia by Dubois & Angel (1972). These *Diplostomum* species infest piscivorous birds of the families Alcedinidae, Anhingidae, Ardeidae and Laridae.

MATERIALS AND METHODS

Galaxias auratus were collected at Lake Crescent on 2/4/78 and 26/6/78, and brought back to the laboratory where they were maintained in a freshwater aquarium until required for autopsy. Cysts, on removal from the host, were placed in Hank's balanced salt solution (Hank's BSS). Domestic ducklings, raised under controlled conditions, were fed metacercarial cysts in gelatin capsules (Parke Davis and Co., Empty Gelatin Capsules No.4), and dissected after intervals from 1 hour to 30 days 19 hours in the case of those fed cysts of *Diplostomum galaxiae* n.sp. and after 18 hours to 30 days 19 hours for those fed cysts of *Apatemon gracilis*. An abbreviated method of referring to time, viz. 30,19 days for 30 days 19 hours is used throughout this paper. Two black ducks were shot at Calverts Lagoon on 27/4/78 and two white-faced herons were killed at Lake Crescent on 27/6/79.. The domestic ducklings were dissected immediately they were killed. The other birds were held at 5°C pending the autopsy which was undertaken within 24 hours. For the purpose of recording the location of trematodes in the put of birds the small intestine was divided into ten equal lengths, numbered (from the pylorus) S1 to S10. Trematodes were removed, rinsed in Hank's BSS, and fixed in boiling 10% phosphate buffered formol saline.

Metacercariae were excysted *in vitro* using techniques similar to those used by Blair (1976) to excyst *Apatemon gracilis*, and by Mitchell *et al.* (1978) to excyst *Cotylurus erraticus*. Cysts were exposed to a sequence of solutions in test tubes held in an intermittently-shaking water-bath at 41 ± 1°C:

(i) 10 ml of 2% pepsin (B.D.H.) in Hank's BSS, adjusted to pH 2.0 with 0.1N HCl, for 10 minutes;
(ii) 10 ml of 0.2M sodium dithionite in Hank's BSS, for 15 minutes;
(iii) 10 ml of 0.5% pancreatin (Viokase) and 0.2% sodium taurocholate (B.D.H.) in Hank's BSS gassed to pH 7.4 with 5% CO_2 in air, for 60 minutes, or until excystment.

Between successive treatments cysts were transferred to crystal dishes under a dissecting microscope and quickly washed twice in Hank's BSS. Glassware and saline were kept at about 41°C.

In the following descriptions, unless otherwise stated, all measurements are given in microns (mean first, followed by size range in parenthesis). Drawings were made with the aid of a camera lucida.

Superfamily STRIGEOIDAE Railliet
Family STRIGEIDAE Railliet

Apatemon (Apatemon) gracilis (Rudolphi, 1819) Szidat, 1928

1. ADULT (fig. 1)

The dimensions of ovigerous adults from a naturally infected black duck and experimentally infected laboratory duklings, are presented in table 1.

Description

Body strongly flexed dorsally at junction of fore and hindbody. Cup-shaped forebody separated by deep constriction from larger, arcuate hindbody. Oral sucker round, mouth subterminal, pre-pharynx absent; oesophagus short, bifurcating immediately posterior to small round pharynx; caeca narrows (orange-brown contents conspicuous in live worms from black duck), extending to posterior of hindbody. Ventral sucker more or less round. Discrete ovoid gland at base of foliaceous holdfast organ. Paranephridial canals large, conspicuous throughout body; excretory pore terminal. Reproductive organs confined to hindbody. Testes lobed, contiguous, tandem, posterior to ovary. Seminal vesicle coils between posterior testis and genital cone, narrowing distally, joining terminal part of uterus to form hermaphrodite duct. Ovary oval, contiguous to anterior testis. Oviduct passes dorsally, from posterodorsal border of ovary, giving rise to Laurer's canal, which opens dorsal to anterior testis. Ciliated ootype overlies dorsal lobe of anterior testis. Vitellaria confined to hindbody, ventral and overlapping gonads. Vitelline reservoir dorsal, inter-testicular. Uterus loops anteroventrally from ootype, extending along ventral border of testes, joining seminal vesicle posterior to posterior testis. Hermaphrodite duct opens through protrusible genital cone, within subterminal-dorsal copulatory bursa. Uterus contains up to 14 eggs.

Hosts

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

Geographical location

Lake Crescent (fish intermediate host); Calverts Lagoon (black duck).

Date of collection

Fish 2/4/78, 26/6/78 (coll. R. White, R.B. Mawbey); black duck 27/4/78 (coll. R.B. Mawbey, S.J. Smith).

Habitat

Upper small intestine.

Material

Tasmanian Museum and Art Gallery: adults from black duck, Calverts Lagoon, K887; adults from laboratory duckling K888; excysted metacercariae K889.

Relationships

Adults recovered from a naturally infected black duck and experimentally infected laboratory ducklings fell within the range of previous descriptions of *Apatemon (Apatemon) gracilis* (Dubois 1951, Beverley-Burton 1961, Ricci & Carrescia 1961, and Vojtek 1964). This species is noted for its morphological variability and Dubois (1953) suggested that it be divided into ten sub-species. Beverley-Burton (1961), however, observed that specimens taken from the same host can be assigned to several of these sub-species, and hence considered it preferable to regard this cosmopolitan species with a wide host range, as polytypic, rather than as a collection of sub-species.

A. (Apatemon) gracilis and *A. (Australapatemon) minor* Yamaguti, 1933, have frequently been considered conspecific (Dubois 1968), and it is often impossible to determine which species is referred to when *A. gracilis* is reported, unless life-history studies have been

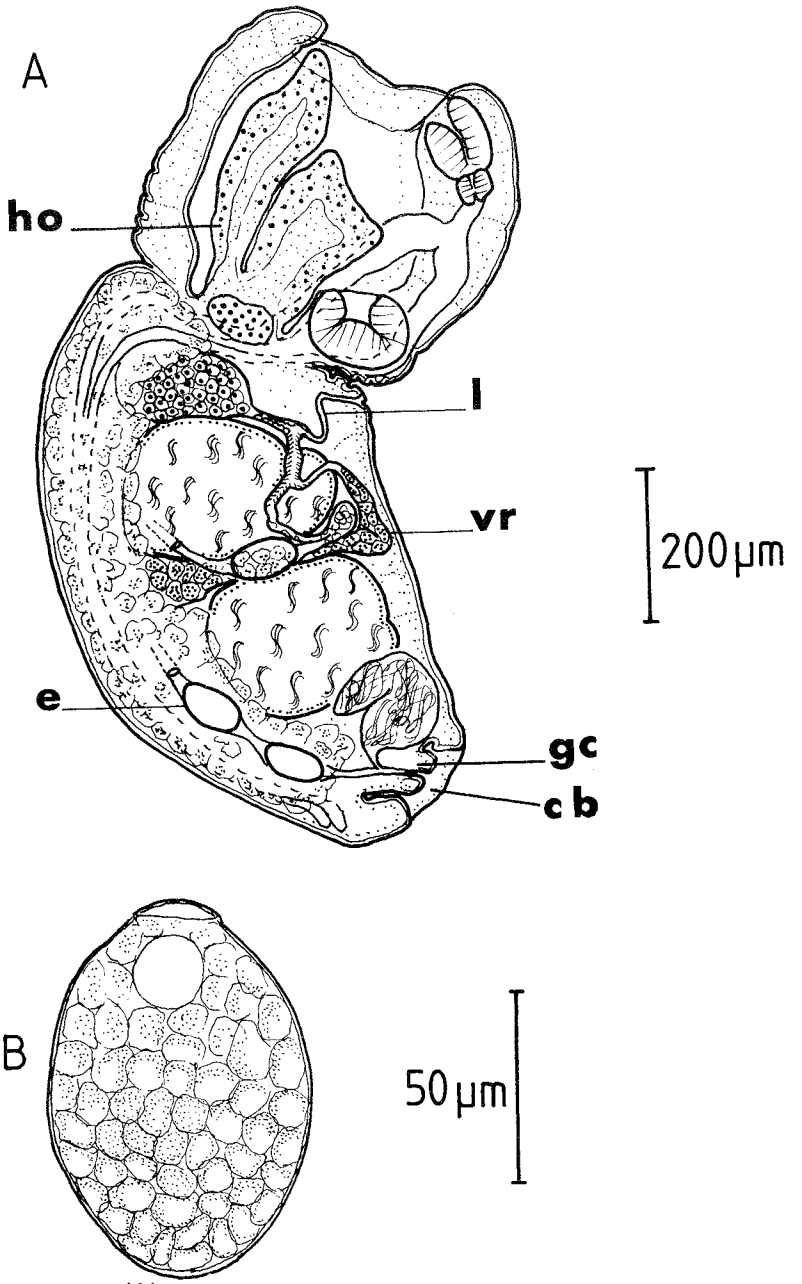

FIG. 1 - *Apatemon gracilis*. A: gravid adult after 4,21 days in experimentally infected duckling; B: egg deposited in duckling's intestine. (cb: copulatory bursa, e: egg, gc: genital cone, ho: holdfast organ, l: Laurer's canal, vr: vitelline reservoir).

made. The former species utilizes fish as intermediate hosts and the latter, leeches (Blair 1976). The present material evidently belongs to the former species.

TABLE 1

DIMENSIONS OF *APATEMON GRACILIS*.
(a) metacercariae, excysted *in vitro* after 2 hours at 41°C; (b), (c) and (d) adults recovered from experimentally infected ducklings after different periods of infection; and (e) adults from a naturally infected black duck.

	(a)	(b)	(c)	(d)	(e)
Infection period (days)	-	3,19	4,21	8,1	-
	n=4	n=5	n=6	n=5	n=3
Body length	590 (550-665)	957 (847-1028)	1335 (1225-1452)	1182 (1074-1300)	1542 (1315-1845)
Forebody:depth	264 (255-274)	355 (331-391)	397 (369-437)	360 (350-380)	510 (469-529)
length (FBL)	416 (393-469)	429 (348-499)	544 (499-590)	423 (393-469)	579 (469-741)
width	314 (300-327)	467 (433-502)	-	-	-
Hindbody:depth	144 (-)	312 (304-319)	402 (369-452)	354 (296-380)	479 (423-514)
length (HBL)	174 (166-197)	514 (454-559)	791 (650-907)	759 (680-832)	912 (786-1104)
width	129 (125-133)	346 (342-350)	-	-	-
Oral sucker:depth	72 (68-76)	87 (84-91)	103 (95-110)	85 (76-91)	86 (76-95)
length	78 (76-80)	94 (87-99)	103 (95-114)	95 (84-114)	108 (99-118)
width	80 (76-84)	93 (91-95)	-	-	-
Ventral sucker:depth	91 (87-95)	129 (114-141)	146 (122-167)	144 (129-156)	139 (114-156)
length	90 (84-99)	120 (106-133)	127 (122-137)	129 (122-133)	155 (137-167)
width	95 -	108 (99-118)	-	-	-
Pharynx:depth	34 -	52 -	64 (61-72)	64 (53-68)	34 -
length	34 -	60 (53-65)	57 (49-61)	62 (57-65)	46 -
width	32 (30-34)	44 (42-46)	-	-	-
Holdfast organ:depth	55 (49-61)	76 (53-99)	81 (61-95)	84 (76-95)	92 (84-106)
length	43 (34-46)	68 (65-72)	63 (46-76)	43 (34-53)	65 (53-72)
width	46 -	-	-	-	-
Ovary:depth	-	-	123 (103-144)	-	143 (122-175)
length			89 (84-95)	-	81 (72-125)
Anterior testis:depth	-	-	208 (175-266)	-	274 (217-346)
length	-	-	158 (114-220)	-	252 (213-304)
Posterior testis:depth	-	-	244 (213-281)	-	275 (171-388)
length	-	-	234 (220-255)	-	282 (163-418)
O.S. (l+w)/V.S. (l+w)	0.85	0.82	0.75	0.66	0.66
FBL/HBL	2.39	0.83	0.69	0.56	0.63
Eggs in uterus	-	+	+	+	+

Biology

Five out of seven ducklings fed cysts of *A. gracilis* from *Galaxias auratus* were infected, with from 2 to 37 flukes. The percentage of metacercariae recovered as adults varied from 8 to 93, and the maximum longevity recorded was 8,1 days. Adults were concentrated in S4 after 0.18 days, S2 after 3,19 days, S3 after 4,21 days, and S2 after 8,1 days. A black duck killed at Calverts Lagoon contained 27 adult *A. gracilis*, concentrated in S2. The distribution of adults in naturally and experimentally infected hosts is shown in figure 2.

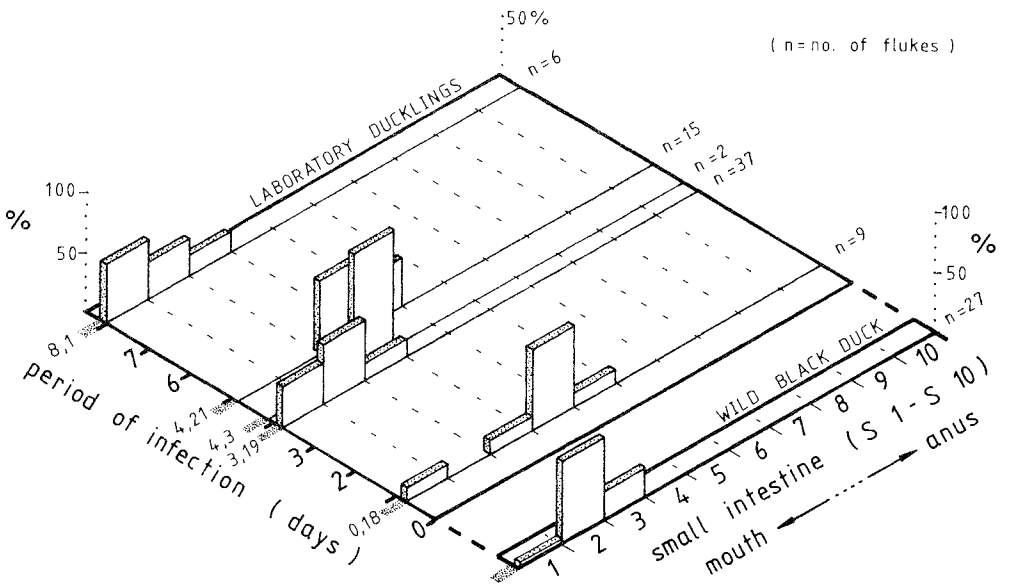

FIG. 2 - *Apatemon gracilis*. Distribution in the digestive tracts of a wild black duck, and laboratory ducklings. (n = number of flukes)

Excysted metacercariae are very immature and genital primordia barely discernible. After 0,18 days in a laboratory duckling little growth or development had occurred; however, growth proceeded rapidly over the next few days. As the reproductive organs developed, the hindbody grew from being a stumpy appendage to being larger than the forebody (fig. 3). After 3,19 days the FBL/HBL ratio had decreased from 2.39 for excysted metacercariae, to 0.83. At this stage the adults were mature and producing eggs. The hindbody continued to grow after egg production had commenced and after 8,1 days the FBL/HBL ratio was 0.66. The number of uterine eggs was not directly related to the age of ovigerous adults (table 2). A maximum of 14 eggs was found in flukes after 4,3 and 4,21 days; however, after 8,1 days the maximum number of eggs in any fluke was only five. The maximum number of eggs found in flukes infecting the black duck was seven.

2. EGG (fig. 1)

The broadly oval egg is operculate and densely packed with granular vitelline cells. Colourless when formed, the egg-shell is tanned golden as it passes through the uterus. A clear spherical body, about 19 µm diameter, underlies the operculum. The dimensions of live and fixed eggs in flukes from laboratory ducklings and fixed eggs in flukes from a wild black duck, are shown in table 3. There was little difference in the size of fixed eggs from different hosts, however, fixed eggs were smaller than live eggs.

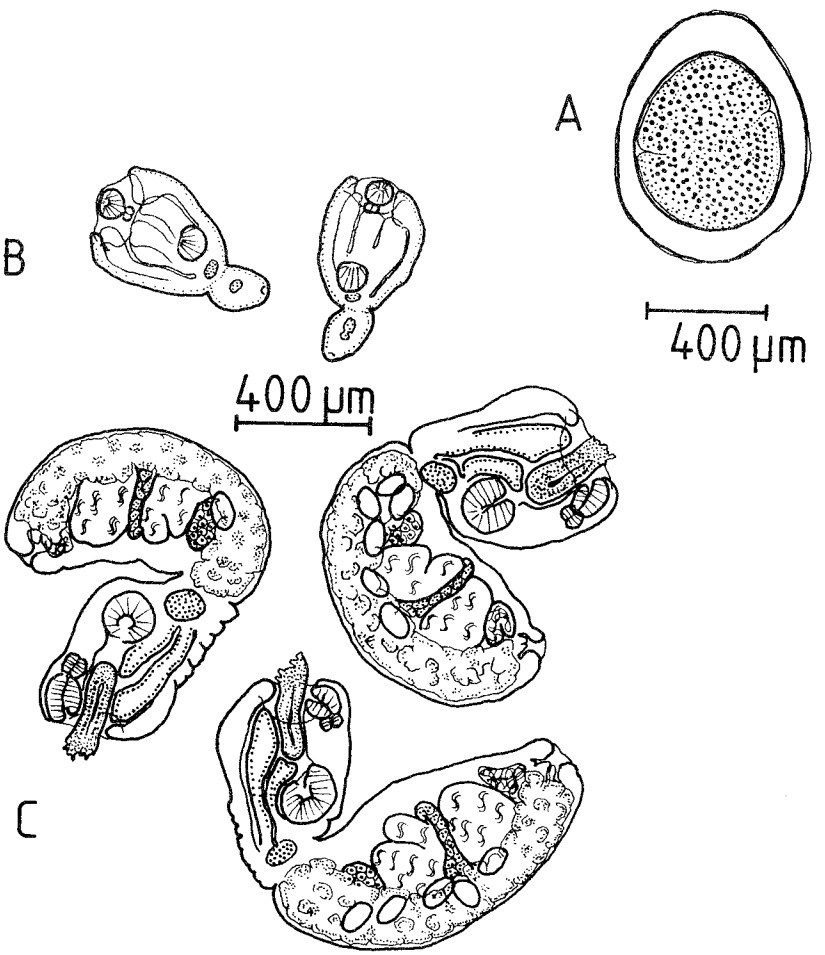

FIG. 3 - *Apatemon gracilis*. A: metacercarial cyst from *Galaxias auratus*; B: excysted metacercariae after 2 hours at 41°C; C: gravid adults after 8,1 days in laboratory duckling - forebody of each adult adhering to an intestinal villus from host (B and C drawn to same scale).

TABLE 2

THE NUMBER OF UTERINE EGGS IN ADULT *APATEMON GRACILIS*. (a) from experimentally infected ducklings, and (b) from wild black duck.

	Infection period (days)	Number of flukes	Number of uterine eggs	
			Mean	Range
(a)	3,19	30	0.5	(0-1)
	4,3	2	11.0	(8-14)
	4,21	11	3.5	(0-14)
	8,1	5	2.8	(1-5)
(b)	-	11	2.0	(0-7)

TABLE 3

DIMENSIONS OF EGGS OF *APATEMON GRACILIS*.

Host	Live/ fixed	Number of eggs	Length	Width
Duckling 1	live	6	103 (95-106)	71 (68-72)
Duckling 1	fixed	6	99 (95-103)	63 (61-68)
Duckling 2	fixed	9	99 (95-106)	61 (57-67)
Black duck	fixed	6	97 (87-103)	59 (53-68)

3. METACERCARIA

Metacercarial cyst (fig. 3)

The thick-walled, white, oval to pyriform cyst, most frequently occurs in the body cavity of *Galaxias auratus*, particularly in connective tissue adjacent to the intestine. It is also frequently associated with the eye, either in the vitreous humour, or just outside the eyeball, near the optic nerve. The cyst sometimes occurs between the peritoneum and muscle. Ten out of fourteen fish collected in June 1978 and June 1979, from the Clyde River where it enters Lake Crescent, were infected with *Apatemon gracilis*. The average number of cysts per infected fish was 5.5 (1-17) and the average size of the fish was 6.4 (4.8-9.9) cm. Eighty percent of these cysts were in or next to the body cavity, 18% were behind the eyeball next to the optic nerve and 2% were in the vitreous humour. The resilient cyst wall is translucent and homogenous, varying in thickness up to about 220 µm. A thin outer membrane, presumably of host origin, sometimes connects adjacent cysts. The densely packed contents of the cyst are opaque. Dimensions of ten cysts from the body cavity and two cysts from the vitreous humour of the eye, are shown in table 4. There is no marked difference in size or morphology of the cysts from different locations in the fish host.

TABLE 4

CYSTS OF *APATEMON GRACILIS* FROM DIFFERENT FISH HOSTS.
Dimensions of live metacercarial cysts, dissected from naturally infected *Galaxias auratus*; from the brook stickleback, *Eucalia inconstans*, in North Dakota, U.S.A. (Hoffman 1959); and from the rainbow trout *Salmo gairdneri*, in Scotland (Blair 1976).

Host fish	Number of cysts	External dimensions		Internal dimensions	
		Length	Width	Length	Width
G. auratus (body)	10	886(816-968)	718(650-756)	587(423-650)	493(348-552)
G. auratus (eye)	2	877(847-907)	665(650-680)	544(529-559)	529(484-575)
E. inconstans	-	1000	600	507	444
S. gairdneri	10	605(542-660)	396(356-426)	503(465-542)	293(279-310)

Excysted metacercaria (fig. 3)

The dimensions of some excysted metacercariae are presented in table 1. The forebody is cup-shaped and relatively larger than in the adult worm. The oral and ventral suckers, pharynx and holdfast organ are well-developed in the forebody, but genital primordia are barely discernible in the rudimentary hindbody. Body fluids and lipid droplets move through large paranephridial canals, throughout the body of live worms.

S.J. Smith and J.L. Hickman

Family DIPLOSTOMIDAE Poirier

DIPLOSTOMUM (DOLICHORCHIS) GALAXIAE n.sp.

1. ADULT (fig. 4)

The description of the adult of this new *Diplostomum* species is based on gravid flukes recovered from experimentally infected laboratory ducklings and naturally infected white-faced herons. The species is named after the endemic Tasmanian fish, *Galaxias auratus*, which serves as its second intermediate host. Dimensions of the holotype and other ovigerous and non-ovigerous adults, from naturally infected white-faced herons, are shown in tables 5 and 6.

TABLE 5

OVIGEROUS ADULTS OF *DIPLOSTOMUM GALAXIAE* n.sp.
Dimensions of the holotype and other ovigerous adults,
of 'sinistral' and 'dextral' types, from the white-faced heron.

Sample size	'Sinistral' n = 11	'Dextral' n = 14	Holotype n = 1 ('sinistral')
Body length	957 (892-1058)	1002 (832-1074)	937
Forebody:depth	133 (121-166)	141 (106-±97)	-
length (FBL)	549 (484-665)	517 (393-635)	575
width	342 (302-401)	369 (287-438)	318
Hindbody:depth	269 (242-302)	266 (227-287)	-
length (HBL)	437 (348-499)	467 (378-499)	378
width	289 (272-302)	296 (204-333)	280
Oral sucker:depth	49 (46-43)	64 (53-76)	-
length	65 (57-76)	64 (57-68)	65
width	63 (61-67)	71 (59-84)	65
Ventral sucker:depth	-	53 -	-
length	63 (59-68)	62 (57-65)	53
width	73 (67-80)	70 (61-80)	70
Pharynx:length	49 (46-57)	48 (46-49)	46
width	36 (34-38)	34 -	38
Left lappet:length	87 (76-91)	81 (76-84)	91
width	46 (42-53)	36 (27-49)	53
Right lappet:length	87 (76-95)	81 (68-99)	76
width	49 (46-53)	40 (34-49)	53
Holdfast organ:depth	70 (68-72)	72 (68-76)	-
length	136 (110-160)	154 (133-179)	141
width	136 (114-148)	-	141
Ovary:depth	-	95 -	-
length	68 (61-76)	65 (42-84)	61
width	90 (80-95)	85 (65-106)	91
Anterior testis:depth	163 -	176 (141-194)	-
length	91 (80-114)	102 (78-144)	84
width	121 (87-141)	130 (84-198)	125
Posterior testis:			
left lobe depth	137 -	144 (122-167)	-
length	141 (114-198)	156 (110-213)	118
width	98 (76-114)	78 (61-95)	103
right lobe depth	123 (110-133)	143 (125-167)	-
length	158 (114-205)	164 (110-213)	133
width	96 (80-110)	97 (68-125)	87
O.S.(l+w)/V.S.(l+w)	0.94	1.02	1.06
FBL/HBL	1.26	1.11	1.52
Number of uterine eggs	1.90 (1-6)	3.14 (1-11)	2

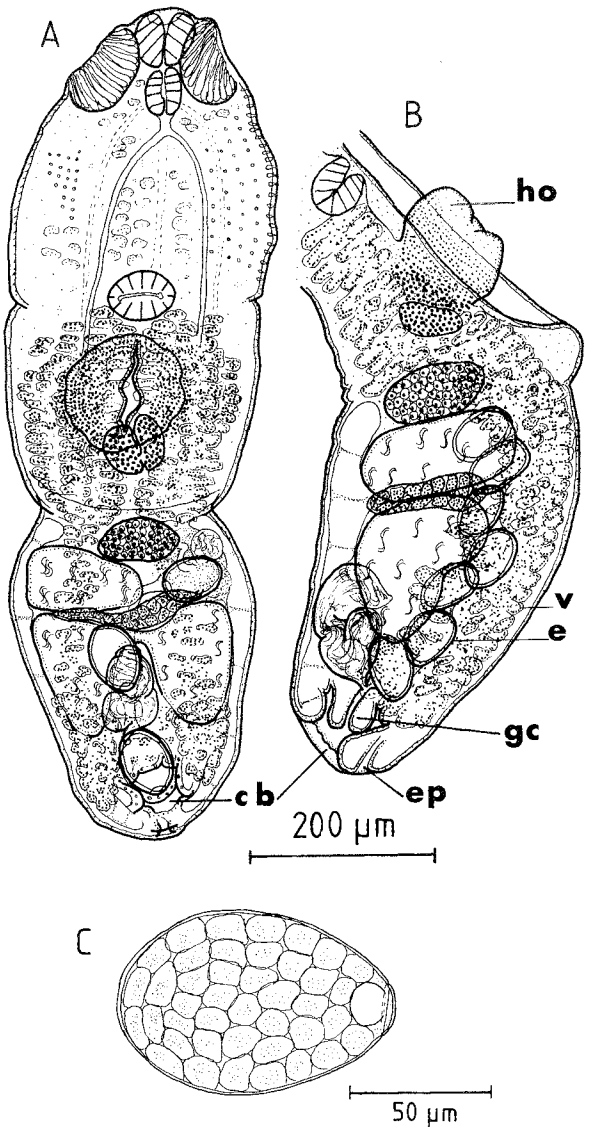

FIG. 4 - *Diplostomum galaxiae* n.sp.
A: gravid adult, sinistral anterior testis, from white-faced heron, dorsal view.
B: gravid adult, dextral anterior testis, from white-faced heron, lateral view of hindbody.
C: egg (cb: copulatory bursa, e: egg, ep: excretory pore, gc: genital cone, ho: holdfast organ, v: vitellaria).

Description

Forebody elongate, more or less rectangular, anterior body trilobate, posterior and posterolateral borders recurved ventrally. Hindbody conical, dorsally flexed, distinct from forebody. Tegumental spines on forebody diminishing in size posteriorly; hindbody aspinous. Tegumental gland cells distributed over anterior half of forebody. Oral sucker round, situated on prominent anterior protuberance bordered by well-developed crescentic lappets. Ventral sucker transversely oval; holdfast organ mobile, mushroom-shaped; both protrude into concavity of forebody. Paranephridial canals anastomose throughout body. Excretory pore terminal. Mouth subterminal ventral; prepharynx absent; caeca narrow inconspicuous, diverging immediately posterior to small oval pharynx, then extending into hindbody. Bipartite gland at base of large, holdfast organ. Anterior testis dextral or sinistral, round to wedge-shaped. Posterior testis bilobed, elongate lobes joined by narrow anterodorsal commissure. Seminal vesicle voluminous coiled, lying between lobes of posterior testis, narrowing posteriorly to form ejaculatory duct, which joins uterus. Ovary oval sub-median, situated anteriorly in hindbody. Oviduct extends posterolaterally to ootype. Ootype surrounded by Mehlis' gland, opposite posterior half of anterior testis. Laurer's canal opens dorsally, at level of anterior testis. Seminal receptacle lateral to ovary. Vitellaria occupy posterior half of forebody, extending anteriorly to posterior border of ventral sucker; distribution in hindbody mainly ventral, lateral. Vitelline reservoir intertesticular. Uterus loops antero-ventrally, then extends posteriorly, ventral to seminal vesicle, uniting with ejaculatory duct. Short hermaphordite duct traverses small protrusible genital cone, within subterminal dorsal copulatory bursa. Up to 11 eggs in uterus.

The reproductive system of this species is amphitypic. In one wild host, a white-faced heron, 58% of 92 mature adults (i.e. with vitellaria producing phenolic egg-shell precursors), had a sinistral anterior testis and the remaining 42% had a dextral anterior testis. The position of the testis is always clearly sinistral or dextral - there are no intermediates. No other distinct morphological differences could be found between the 'sinistral' and 'dextral' types (tables 5 and 6). However, only 30% of the 'sinistral' adults were ovigerous, compared with 54% of the 'dextral' adults; and there was a slight difference in the number of uterine eggs in each type: 1.9 (1-6) in 'sinistral' adults, and 3.1 (1-11) in 'dextral' adults. Fecundity of *D. galaxiae* n.sp. may be related to the position of the anterior testis.

Hosts

Anas platyrhynchos L. (experimental); *Ardea novaehollandiae* Latham.

Geographical location

Lake Crescent.

Date of collection

Fish intermediate host 2/4/78, 26/6/78 (coll. R. White, R.B. Mawbey); white-faced herons 27/6/79 (coll. R.B. Mawbey, S.J. Smith).

Habitat

Upper small intestine.

Type material

Tasmanian Museum and Art Gallery: holotype K884, ringed (gravid adult); paratypes K885 (adults) and K886 (excysted metacercariae).

Relationships

This species has the characteristics of the subgenus *Dolichorchis* Dubois, 1961, i.e. asymmetrical anterior testis, elongated lobes of the posterior testis joined by a narrow dorsal commissure and the presence of a genital cone. Seven species of *Diplostomum* have previously been assigned to the subgenus *Dolichorchis*, however only one of these, *Diplostomum auriculosum*, has been recorded in Australia. All seven species reach maturity in piscivorous birds. No intramolluscan developmental stages have yet been recorded. The metacercarial stage has only been described for two of the species: *Diplostomum heronei* Srivastava, 1954 (syn. *D. ketupanensis* sensu Ganapati and Rao, 1962 nec Vidyarthi, 1937, according to Williams, 1967), and *D. tregenna* Nazmi, 1932. The metacercaria of *D. heronei* encysts in the musculature of freshwater fish in India; however, according to Khalil (1963),

the metacercaria of *D. tregenna* remains unencysted in the brain of *Clarias*, a fish of the River Nile. The only other previous record of the metacercaria of a *Diplostomum* species encysting in fish is the metacercaria, *Diplostomulum pigmentata* Singh, 1956, which encysts in the muscles of freshwater cyprinoid fish in the Allahabad region of India, where *D. heronei* and *D. ketupanensis* Vidyarthi, 1937, have been recorded.

Of the seven species previously assigned to the subgenus *Dolichorchis*, *Diplostomum galaxiae* n.sp. most closely resembles *D. heronei* and *D. ketupanensis* in general morphology, distribution of vitellaria, shape and size of anterior testis and relative size of lappets. In all three species, vitellaria are extensively distributed in the posterior part of the forebody, extending anteriorly as far as the ventral sucker, whereas in *D. marahoueense* Baer, 1957, and *D. auriculosum* Dubois and Pearson, 1967, vitellaria are restricted in the forebody, to a narrow zone around the holdfast organ, and do not extend anteriorly as far as the ventral sucker. In *D. buteii* Vidyarthi, 1937, and *D. tregenna*, vitellaria are distributed anteriorly well beyond the ventral sucker nearly to the pharynx. Dubois (1968), considers that *D. duboisi* Anantaraman and Balasubramaniam, 1953, is a synonym of *D. buteii* and the present authors agree.

Diplostomum ketupanensis Vidyarthi, 1937 is very similar in morphology to *D. heronei*, however, the body and organs of the former are about two to three times larger than those of the latter, and whereas uterine eggs are absent in the former, four or more eggs may occur in the uterus of the latter. Williams (1967), considered that *D. ketupanensis* sensu Ganapati and Rao, 1962, but not *D. ketupanensis* Vidyarthi, 1937, is a synonym of *D. heronei*, and the present authors concur. *D. galaxiae* n.sp. is significantly smaller than *D. ketupanensis* Vidyarthi, 1937, has a more elongate forebody, and contains up to 11 uterine eggs. The position of the anterior testis is variable in the former and sinistral in the latter.

D. heronei and *D. galaxiae* n.sp. are similar in size, however the former has a flat, round forebody, whereas that of *D. galaxiae* n.sp. is elongate and very concave. The anterior testis of *D. heronei* is dextral and the posterior testis is relatively longer than that of *D. galaxiae* n.sp. The excysted metacercariae of *D. heronei* is markedly smaller and has a relatively rounder forebody and larger hindbody than that of *D. galaxiae* n.sp. The discovery of the intramolluscan developmental stages of these species would elucidate their relationship, however it is presently considered that differences in morphology of adults and metacercariae and the different intermediate and definitive hosts, warrant their specific separation.

A key to the species of the subgenus *Dolichorchis* is given below.

1a. Anterior testis L-shaped; extends width of hindbody.
Intestinal parasite of the Indian pariah kite, in S.E. India; and *Buteo rufinus rufinus*, in N. India. ... *D. buteii* Vidyarthi, 1937
Syn.: *Bolbophorus orientalis* Vidyarthi, 1938
D. duboisi Anantaraman and Balasubramaniam, 1953

1b. Anterior testis not L-shaped; lateral in anterior hindbody ... 2

2a. Vitellaria not distributed anteriorly beyond ventral sucker ... 3

2b. Vitellaria distributed anteriorly beyond ventral sucker.
Diplostomulum encysted in brain of freshwater fish in River Nile.
Adult intestinal parasite of Egyptian kite, Cairo, Egypt
..... *D. tregenna* Nazmi, 1932

3a. Vitellaria restricted to region of holdfast organ; anterior distribution distribution not reaching ventral sucker ... 4

3b. Vitellaria distributed widely in posterior forebody; anterior distribution reaching ventral sucker ... 5

4a. Ventral sucker larger than oral sucker. Lappets projecting anterior to oral sucker.
Intestinal parasite of darter, in Queensland, Australia
..... *D. auriculosum* Dubois and Pearson, 1967

4b. Oral sucker larger than ventral sucker. Lappets not projecting anterior to oral sucker.

Parasite of upper small intestine of fishing owl, Ivory Coast
..... *D. marahoueense* Baer, 1957

5a. Small flukes, body length less than 1.5 mm ... 6

5b. Large flukes, body length more than 1.5 mm.
Intestinal parasite of northern brown fishing owl, in N India
..... *D. ketupanensis* Vidyarthi, 1937

6a. Forebody flat, round; anterior testis dextral.
Metacercaria encysts in muscles of freshwater fish, Andhra Pradesh.
Adult intestinal parasite of grey pond heron in N. and E. India
..... *D. heronei* Srivastava, 1954
Syn.: *D. ketupanensis* sensu Ganapati and Rao, 1962

6b. Forebody elongate, ± rectangular, concave; anterior testis variable (reproductive system amphitypic).
Metacercaria encysts in muscles of freshwater fish, Lake Crescent.
Adult parasite in upper small intestine of white-faced heron, Tasmania, Australia ..... *D. galaxiae* n.sp.

TABLE 6

NON-OVIGEROUS ADULTS OF *DIPLOSTOMUM GALAXIAE* n.sp.

Dimensions of mature (i.e. vitellaria producing phenolic egg-shell precursors), non-ovigerous adults of 'sinistral' and 'dextral' types, from the white-faced heron.

Sample size	'Sinistral' n=17	'Dextral' n=14
Body length	889 (771-1058)	983 (847-1210)
Forebody:depth	106 (91-121)	132 (121-151)
length (FBL)	496 (423-635)	581 (469-711)
width	351 (318-408)	363 (302-408)
Hindbody:depth	234 (227-257)	259 (242-272)
length (HBL)	396 (333-484)	429 (348-544)
width	292 (272-333)	292 (272-318)
Oral sucker:depth	60 (49-76)	66 (57-70)
length	63 (49-68)	65 (49-72)
width	70 (61-76)	67 (61-70)
Ventral sucker:depth	61 -	70 (65-76)
length	62 (53-68)	63 (57-72)
width	76 (68-84)	72 (61-84)
Pharynx:length	51 (42-57)	53 -
width	38 (30-46)	35 (32-38)
Left lappet:length	81 (72-99)	84 (72-91)
width	40 (30-53)	39 (34-46)
Right lappet:length	80 (68-95)	81 (76-87)
width	41 (30-57)	39 (30-46)
Holdfast organ:depth	75 (68-84)	81 (68-91)
length	125 (114-133)	138 (114-167)
width	137 (129-148)	130 (114-156)
Ovary:depth	76 -	-
length	76 (68-91)	66 (65-68)
width	89 (76-106)	82 (80-84)
Anterior testis:depth	148 -	103 (91-114)
length	100 (80-114)	109 (95-118)
width	117 (95-171)	108 (103-114)
Posterior testis:		
left lobe depth	122 -	115 (95-137)
length	162 (148-190)	163 (137-190)
width	106 (95-118)	103 -
right lobe depth	109 (95-114)	108 (99-114)
length	164 (129-190)	126 (103-152)
width	101 (87-118)	99 -
O.S.(l+w)/V.S.(l+w)	0.96	0.98
FBL/HBL	1.25	1.35

Biology

Three out of six ducklings fed cysts of *D. galaxiae* n.sp. from *Galaxias auratus* became infected. Each infected bird, however, harboured only one fluke. The average infectivity rate of metacercariae in these ducklings was about 1 in 100. Although ducklings are not very susceptible hosts for *D. galaxiae* n.sp., two flukes did grow to maturity. Excysted metacercariae are about half adult size, and genital primordia are discernible in the relatively short, stumpy hindbody. As growth occurs, the reproductive system develops and the hindbody increases in size relative to the forebody. One small, immature fluke was found in S7, 10 hours after infection of a duckling (fig. 5). After 4,3 days, one specimen was living in S1, and had grown to adult size and form. It was sexually mature, with vitellaria producing phenolic egg-shell precursors. Sperm filled the seminal vesicle, but no uterine eggs were present. After 12,4 days, one specimen was living in S1, and contained one uterine egg.

The distribution of adults in two white-faced herons killed while feeding on galaxiids at Lake Crescent, is shown in figure 5. They occurred from S1 to S6, but were concentrated in S2 and S3.

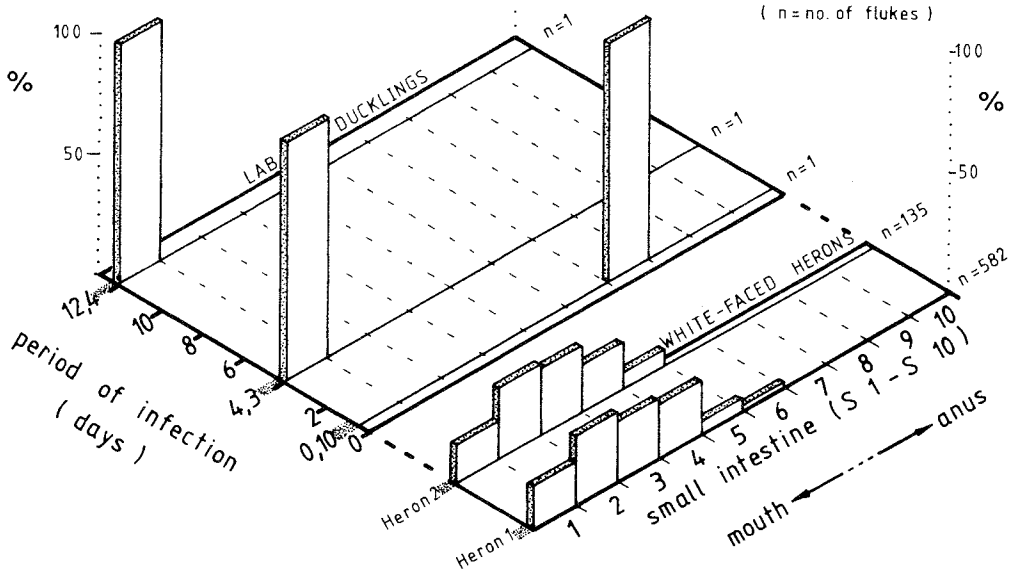

FIG. 5 - *Diplostomum galaxiae* n.sp. Distribution in digestive tracts of laboratory ducklings, and wild, white-faced herons.

2. EGG (fig. 4)

The broadly oval egg has a small round operculum and is densely packed with granular vitelline cells. The egg-shell is relatively thin, clear when formed, but becomes tanned yellow. A clear sphere about 24 µm diameter, underlies the operculum. The dimensions of live eggs taken from the lumen of the upper small intestine of infected herons, and dimensions of fixed uterine eggs of 'sinistral' and 'dextral' adults from the same hosts are shown in table 7.

TABLE 7

DIMENSIONS OF EGGS OF *DIPLOSTOMUM GALAXIAE* n.sp.
Dimensions of live and fixed eggs produced by adults in naturally infected white-faced herons.

Type	Live/ fixed	Number of eggs	Length	Width
Mixed	live	15	95 (89-101)	65 (63-67)
'Sinistral'	fixed	10	98 (91-103)	59 (53-65)
'Dextral'	fixed	12	97 (87-99)	60 (53-65)

3. METACERCARIA

Metacercarial cyst (fig. 6)

The entire black cyst is composed of a thin, but resilient, transparent inner layer of parasite origin, and a thick opaque cellular outer layer of host origin. The 'inner cyst', which separates readily from the 'outer cyst', is oval. It encloses a curled metacercaria, partially surrounded by densely packed coarse lipid droplets. The outermost layer of the 'outer cyst', or host reaction coat, consists of black pigmented melanocytes, which make even deep-seated cysts conspicuous in the translucent body of *Galaxias auratus*. Cysts are distributed widely in the musculature of the body and head, causing 'black spot' or 'black grub' disease. The dimensions of the cyst are shown in table 8.

TABLE 8

CYSTS OF *DIPLOSTOMUM GALAXIAE* n.sp.
Dimensions of live metacercarial cysts, dissected from naturally infected *Galaxias auratus*.

	Number	Length	Width
External dimensions 'outer cyst'	10	1125 (1043-1210)	866 (771-937)
External dimensions 'inner cyst'	10	629 (575-643)	354 (333-386)

All of fourteen fish collected in June 1978 and June 1979, from the Clyde River where it enters Lake Crescent, were infected with *Diplostomum galaxiae* n.sp. The average number of cysts per fish was 42.7 (10-104), and the average size of the fish was 6.4 (4.8-9.9) cm.

Excysted metacercaria (fig. 6)

The size and development of excysted metacercariae varies greatly (table 9). The forebody is relatively large and elongate and the hindbody, a rudimentary stump. Gonadal primordia are visible in advanced metacercariae, and 'dextral' and 'sinistral' types are often discernible. When 39 metacercariae, dissected from fish collected in June 1979, were excysted, 51% were mature enough to distinguish the position of the anterior testis; 55% of these were 'dextral' and 45% 'sinistral'. In advanced metacercariae, the oral sucker, pharynx and caeca are formed, lappets and holdfast organs are well-developed and the bilobed gland at the base of the holdfast organ is conspicuous. The forebody tegument is spinous, spines diminishing in size posteriorly, and the hindbody tegument is aspinous. Large paranephridial canals, through which body fluids and small lipid droplets move, anastomose throughout the body. The genital pore is slightly dorsal to the terminal excretory pore.

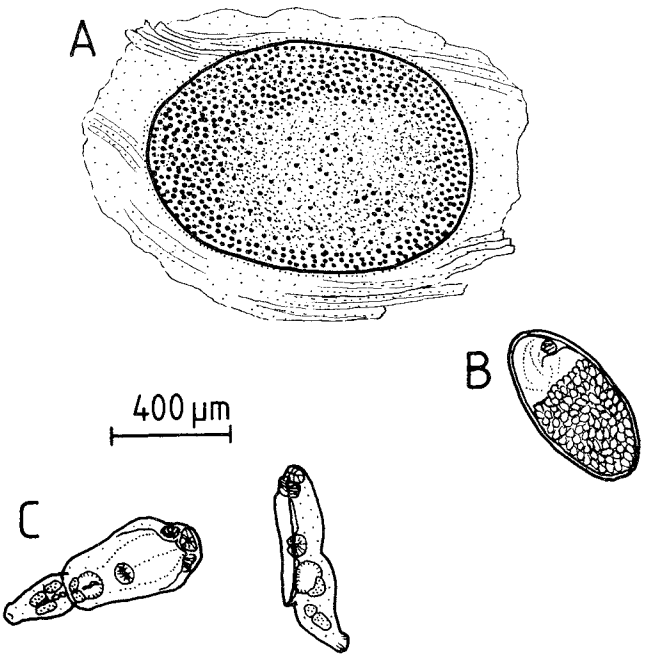

FIG. 6 - *Diplostomum galaxiae* n.sp.
A: black metacercarial cyst embedded in muscle of fish host.
B: oval 'inner cyst' removed from thick cellular 'outer cyst'.
C: excysted metacercariae after 3 hours at 41°C. (A,B and C are drawn to same scale.)

DISCUSSION

Neither of the two strigeoid species encysting in *Galaxias auratus* at Lake Crescent, has previously been recorded in Australia. *Apatemon (Apatemon) gracilis* and *Diplostomum (Dolichorchis) galaxiae* n.sp. encyst in different tissues of the fish host and infect different definitive hosts. Domestic ducklings are much more susceptible to experimental infection by *A. gracilis* than by *D. galaxiae* n.sp. - not surprisingly considering that anatids are the main definitive hosts for *A. gracilis* around the world, whereas there are no previous records of a species in the subgenus *Dolichorchis* infecting an anatid. A black duck, *Anas superciliosa*, shot at Calverts Lagoon 100 km from Lake Crescent, was found to harbour *A. gracilis*, but not *D. galaxiae* n.sp., and two white-faced herons, shot at Lake Crescent, harboured only *D. galaxiae* n.sp., despite the fact that about 70% of the fish being eaten by the herons contained cysts of *A. gracilis*.

The cercariae and metacercariae of *A. gracilis* are known to occur in Iceland and Scotland (Blair 1976), Wales (Crocombe 1959), central Europe (Vojtek 1964), Japan (Yamaguti 1933), and North America (Hoffman 1959, Lester 1974). The genera *Ancylastrum* and *Lymnaea* serve as molluscan hosts and the fish intermediate hosts include members of the families Cobitidae, Cottidae, Eleotridae, Gasterosteidae, Gobiidae and Salmonidae. Cysts from these fish intermediate hosts are very similar in morphology, but vary in size. The sizes of cysts from fish in Tasmania, the U.S.A. and Scotland, were shown in table 4. The external cyst dimensions vary much more between host species than do the internal cyst dimensions. Cyst wall thickness is apparently related to the identity of the fish host.

TABLE 9

EXCYSTED METACERCARIAE OF *DIPLOSTOMUM GALAXIAE* n.sp.
Dimensions of metacercariae excysted *in vitro* after 3 hours at 41°C: (a) too immature to distinguish anterior testis, (b) 'sinistral' type, (c) 'dextral' type.

	(a) n=11	(b) n=7	(c) n=13
Body length	401 (355-461)	691 (582-816)	665 (575-756)
Forebody:length	319 (287-363)	513 (438-605)	479 (393-559)
width	176 (156-228)	213 (194-232)	218 (194-247)
Hindbody:length	82 (68-98)	183 (144-227)	186 (151-204)
width	68 (57-72)	123 (106-137)	123 (114-148)
Oral sucker:length	46 (40-49)	54 (51-57)	55 (46-61)
width	45 (42-46)	49 (46-53)	52 (42-65)
Ventral sucker:length	40 (38-46)	42 (38-46)	45 (38-51)
width	44 (42-48)	47 (42-51)	52 (42-63)
Pharynx:length	29 (25-34)	33 (30-40)	36 (30-42)
width	21 (19-23)	25 (23-29)	25 (23-29)
Left lappet:length	49 (46-49)	70 (61-72)	70 (61-72)
width	31 (27-34)	38 (34-42)	41 (36-42)
Right lappet:length	46 (42-49)	69 (57-76)	71 (61-80)
width	30 (27-34)	38 (30-44)	43 (34-61)
Holdfast organ:length	69 (68-72)	104 (99-114)	107 (91-114)
width	74 (53-76)	81 (76-87)	81 (72-91)
Ovary:length	-	22 (19-23)	27 -
width	-	24 (23-25)	23 -
Anterior testis:length	-	38 (34-46)	39 (34-46)
width	-	30 (23-38)	34 (27-42)
Posterior testis:			
left lobe length	-	41 (34-49)	47 (38-53)
width	-	27 (23-30)	29 (23-34)
right lobe length	-	42 (34-49)	43 (34-49)
width	-	28 (27-30)	31 (27-38)
O.S.(l+w)/V.S.(l+w)	1.08	1.16	1.10
FBL/HBL	3.89	2.80	2.58

The molluscan hosts of the strigeoid species at Lake Crescent are not known, however *Potomopyrgus* spp. (Timms 1978), *Physastra gibbosa* and *Rivisessor gunni* inhabit the lake. At Calverts Lagoon in southeast Tasmania trematode developmental stages conforming to those of *A. gracilis* (Blair 1976) were found in *Coxiella badgerensis* on one occasion (Smith 1981).

The discovery of *A. gracilis* and *D. galaxiae* n.sp. in Tasmania may be cause for concern for local trout fishing and trout farming interests. The specificity of *D. galaxiae* n.sp. at the secondary intermediate host level is unknown, however *A. gracilis* infects rainbow and brown trout in Scotland. These fish are widespread and important in Tasmania. In Scotland, experimentally infected trout of both species were found to have cysts of *A. gracilis* concentrated around the head with a small percentage occurring in the eye (Blair 1976). Further studies are needed to determine the extent of these parasites in Tasmania and whether their intermediate host ranges extend to introduced fish species.

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