Life History of *Pygidiopsis crassus* n. sp. (Trematoda, Digenea, Heterophyidae) in the Neotropical Region

Margarita Ostrowski de Núñez

Departamento de Ciências Biológicas, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Pabellon II, 1428 Buenos Aires, Argentina

The life cycle of Pygidiopsis crassus n. sp. was experimentally reproduced, starting from cercariae from naturally infected Littoridina parchappei collected from Lujan River and different ponds in Buenos Aires Province, Argentina. Metacercariae were found encysted in the body cavity of experimentally and naturally infected fishes Cnesterodon decemmaculatus and naturally infected Jenynsia lineata. *Adults were obtained experimentally in chicks and mice. The natural host is unknown. The new species is compared with Pygidiopsis macrostomum Travassos 1928, from* Rattus norvegicus *and from* Noctilio leporinus mastivus, *differing in body and egg sizes, in the size relation of oral and ventral sucker and the shape of excretory vesicle.*

Key words: *Pygidiopsis crassus* n. sp. - Digenea - Heterophyidae - life history - Neotropical region

The genus *Pygidiopsis* is composed by nine species parasitizing birds and mammals, which are very similar to each other in the adult stage, sometimes difficult to distinguish. Where life cycles are known (*P. summa* Onji and Nishio, 1916; *P. pindoramensis* Travassos, 1929; *P. genata* Looss, 1907, and *P. ardeae* Køie, 1990 in Yamaguti (1975), Ostrowski de Núñez (1976), Youssef et al. (1987), and Køie (1990b), respectively) morphology of larval stages and intermediate host species help to separate the species. In this paper the life cycle of a new species of *Pygidiopsis* is described, which differs in the cercaria and the adult from the other species in the genus.

MATERIALS AND METHODS

Naturally infected first intermediate host, *Littoridina parchappei* (D'Orbigny, 1935), were collected from Lujan River, near Mercedes City, from artificial ponds at the Zoological Garden and other ponds adjacent to the La Plata River, Buenos Aires Province. The second intermediate fish hosts, *Cnesterodon decemmaculatus* (Jenyns, 1842) Garman, 1895 and *Jenynsia lineata* (Jenyns, 1842) Günther, 1866 (Atheriniformes, Poecilidae, Jenynsidae) were collected only at the latter ponds. Emerging cercariae were collected by exposing snails to light in 5 ml of tap water. When emergence of cercariae ceased, snails were dissected to study intramolluscan stages. Laboratory reared *C. decemmaculatus* and *Gambusia affinis* were exposed to cercariae and metacercariae recovered on different days p.i. Seven white mice and six newly hatched, unfed chickens were force-fed mature metacercariae and autopsied one to four days p.i.

Cercarial emergence was analyzed every 2 hr during light periods (60 Watt electric light bulb) and at longer intervals during dark periods, changing the chronogram every 2-3 days, at room temperature (20-27°C). The samples were collected by an automatic apparatus modified after Disko (1978) and the cercariae counted removing them with a Pasteur pipette. All larval stages and adults were studied alive, with and without vital stains. Cysts were digested with trypsin at 37°C to free the metacercaria. Adults were fixed in 70% ethanol, stained with alcoholic hydrochloric carmine (Langeron 1949) and mounted in Canada balsam or cleared in lactophenol and mounted, unstained, in glycerine jelly. Measurements of heat killed and formalin fixed cercariae, whole mounts of metacercariae and adults, and of living metacercarial cysts are in µm, if not otherwise stated (minimum, maximum, followed by mean and standard deviation within parentheses).

RESULTS

Only four infected *L. parchappei* were found: one out of 500 (0.20%) from Lujan River (February 89); one out of 364 (0.27%) from the Zoological Garden (October 90), this specimen was also infected with cysts of a microphallid species; and two out of 2329 (0.08%) from seven samples between October 91-January 93, from a pond adjacent to the La Plata River. In the small Los Ranchos stream, which leads to the Lujan River, 4936 snails were collected in monthly samples

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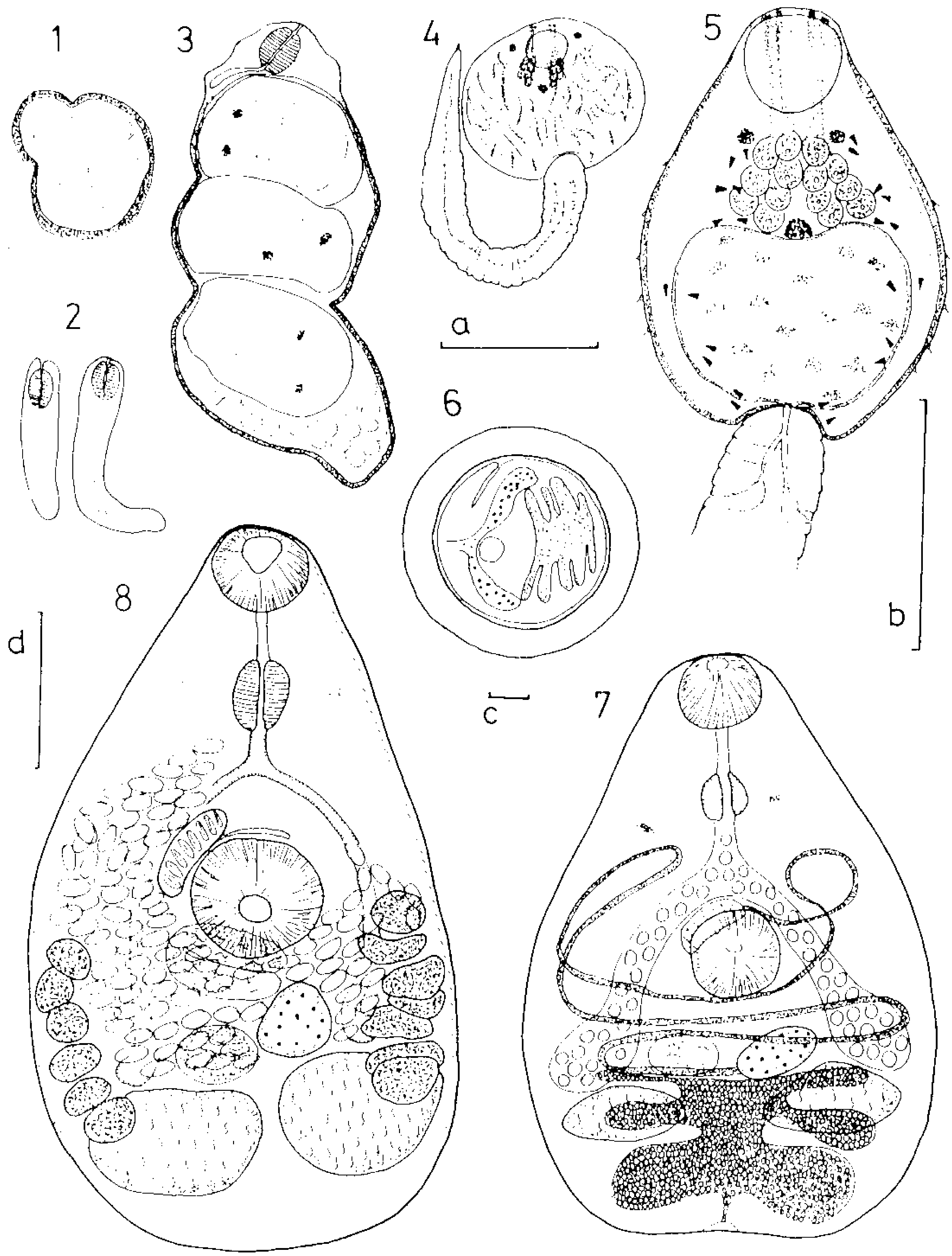
during 1988, but no infection with *P. crassus* n. sp. was observed.

The single sample of fishes collected in the ponds adjacent to the La Plata River was small. In *C. decemmaculatus*, 14 out of 37 (37.8%) were infected, with 1-4 cysts per infected fish. In *J. lineata* 8 out of 10 (80%) were infected, with 2-9 cysts per infected fish. The cichlid species of the



Pygidiopsis crassus n. sp. - Fig. 1: sporocyst. Fig. 2: immature rediae. Fig. 3: mature redia. Fig. 4: cercaria, showing distribution of pigment granules (Fig.1 - 4 scale a). Fig. 5: cercaria, body and proximal part of tail (scale b). Fig. 6: encysted metacercaria (scale c). Fig. 7: excysted metacercaria, dorsal view. Fig. 8: adult from a mouse, 2 days p.i., dorsal view (Fig.7, 8 scale d). All scale bars represent 100 μm.

same pond harbored no cysts (13 fishes dissected). In experimentally exposed *C. decemmaculatus* 80 out of 128 (62.5%) were infected with 1-54 cysts, 6 were negative, and 29 died without been dissected. Thirteen fishes were exposed individually with a counted number of cercariae (13-30) and 3.3% - 60% (mean 41.7%) metacercariae were recovered. Eight *G. affinis* exposed to cercariae were found not infected after dissection. The infection of the experimental hosts with the metacercarial cysts yielded 42 specimens from mice and 42 specimens from chickens one to four days p.i.

Pygidiopsis crassus n. sp.
(Fig. 8)

DESCRIPTION (measurements based on 16 egg bearing specimens from chickens, 2-3 days p.i., mounted *in toto* in Canada balsam; additional measurements in Table I) - Body pyriform, without ventral cavity, 294-454 (388.0; 45.5) long by 210-336 (280.3; 37.9) wide at ovarian level. Tegument with scale-like spines up to testicular level on dorsal side, up to end of body on ventral side. No oral spines present. Pigment granules from cercarial eyespots are scattered at pharyngeal level. Oral sucker subterminal, 42-57 (47.9; 4.1) long by 46-65 (53.2; 6.1) wide. Prepharynx 0-38 (12.8; 14.6) long. Pharynx 36-53 (42.4; 6.2) long by 29-40 (33.6; 3.2) wide, situated on mid way between oral sucker and esophagus bifurcation in well extended specimens. Esophagus 0-48 (25.8; 12.8) long; caecal bifurcation approximately on mid way between pharynx and ventral sucker. Caeca ending blindly at anterior border of testes. Acetabulum 48-63 (54.9; 4.9) long by 55-69 (61.9; 5.1) wide, at mid of body. Oral sucker to acetabulum width ratio: 1:1.0-1.54 (1:1.23, 0.13). Genital sac 19-40 (28.6; 8.9) long by 23-38 (31.6; 7.0) wide, containing a gonotyl with pockets, on left anterior border of acetabulum. The exact number of pockets could not be established, as the gonotyl never was seen everted. Genital pore immediately anterior to acetabulum. Testes opposite, oval, on posterior end of body; left testis 53-74 (58.8; 8.4) long by 67-95 (81.3; 9.6) wide, right testis 42-63 (55.0; 9.4) long by 74-95 (90.3; 8.4) wide; coiled seminal

TABLE I

Measurements of *Pygidiopsis crassus* n. sp. (specimens from mice, mounted in glycerine jelly) and of *P. macrostomum* after Travassos et al. (1969) and Odening (1969) (minimum, maximum, followed by mean and standard deviation in brackets; L: length, W: width)

		Mice (4 days p.i.) n = 14	Travassos et al. 1969 n = 1	Odening 1969[b] n = 10
Body	L	378-588 (451.8; 59.2)	840	543-822 (665; 90.1)
	W	294-386 (336.6; 25.1)	480	308-462 (383.2; 52.1)
Oral	L	48-63 (55.6; 4.2)	120 ø	76-114 (99.9; 14.2)
sucker	W	55-69 (59.3; 4.5)		79-121 (100.4; 13.6)
Preph.	L	0-50 (23.4; 14.8)		
Pharynx	L	42-46 (44.4; 1.6)	75	41-72 (60.4; 9.8)
	W	34-44 (37.5; 2.7)	56	35-69 (51.7; 10.5)
Esoph.	L	15-32 (20.5; 4.9)		21-62 (39.7; 10.3)
Acetab.	L	59-74 (65.0; 5.9)	97 ø	72-93 (82.1; 6.9)
	W	61-84 (71.6; 7.0)		72-93 (84.1; 7.6)
Ovary	L	38-50 (46.9; 6.9)	120[a]	55-90 (67.5; 10.9)
	W	63-84 (72.5; 6.5)	65[a]	52-131 (99.9; 24.2)
left	L	42-67 (55.9; 8.6)	175[a]	59-107 (78.7; 12.4)
testis	W	84-116 (100; 12.4)	100[a]	104-179 (135.4; 19.8)
right	L	42-69 (61.3; 7.0)	160[a]	
testis	W	84-130 (100.5; 13.4)	95[a]	
eggs	L	18.9-23.1 (20.4; 1.1)	24	18-19 (18)
	W	10.5-12.6 (11.6; 1.0)	13-16	9-11 (11)

a: measurements taken on the figure in Travassos et al. 1969.
b: calculated after measurements given of individual specimens.

vesicle posterior to acetabulum. Ovary in front of right testis, 42-48 (44.0; 3.0) long by 63-84 (75.3; 8.3) wide; seminal receptacle on its left side, overlapping sometimes posterior lateral part of ovary and anterior part of testis. Vitelline glands arranged in 6-10 transversely oval follicles, in part overlapping testes, between posterior border of acetabulum and mid level or posterior border of testes. Uterus extending in transverse, ascending and descending loops between testes and pharynx, overlapping caeca. Operculate eggs, 18.9-21 (20.0; 1.3) long by 10.5-12.6 (11.5; 1.3) wide. Excretory vesicle branched, excretory pore subterminal.

Taxonomy summary

Natural host: unknown

Experimental hosts: *Gallus domesticus, Mus musculus.*

Site of infection: intestine

Etymology: the specific name *crassus* from Latin, meaning "thick, heavy", refers to the relative big and thick specimens in comparison to the specimen of the other species of *Pygidiopsis* present in the area.

Specimens deposited: Holotype: No. 371/1, paratypes: No. 372/2 in the Helminthological Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires.

Remarks

A total of 84 adults were obtained from the upper intestine, near the stomach from chickens and mice. Adults recovered 24 hr p.i. are immature. At 2 days p.i., there is no significant difference in body size between adults from chicks and mice, but while in the latter egg production has just began with few, immature eggs, in the former there are nearly 100 eggs present. In two specimens mounted in glicerine gelly, one from a chicken and in one from a mouse, 2 days p.i., the acetabulum is slightly smaller than the oral sucker, being the difference 3.3 % and 3.6 % respectively. The adults recovered after 3 days p.i. from chicks and mice over 200 eggs in different stage of maturation are present in the uterus. In some specimens an arched fold in the wall of prepharynx at junction with pharynx, as described for *P. ardeae* by Køie (1990a) can be observed.

Larval forms

Sporocysts - Small, spherical, 90 m in diameter (Fig. 1) in the heart cavity of *L. parchappei*.

Rediae - Recently emerged rediae (Fig. 2) 95-168 long by 25-34 wide, pharynx 23-34 long by 17-21 wide. Small rediae migrated to the hepatopancreas. Rediae with mature cercaria (based on 15 formalin-fixed specimens; Fig. 3) 235-521 (376.4; 77.7) long by 84-126 (103.5; 12.7) wide, pharynx 34-40 (36.8; 2.1) long by 19-27 (21.8; 2.1) wide. Gut short, inconspicuous. No redia containing daughter rediae were found in the natural infections.

Cercaria - Measurements based on 30 heat killed and formalin fixed specimens (Figs 4, 5). Living cercaria with globose body, 164-178 (171.8; 3.9) long by 82-111 (94.5; 7.9) wide. Tegument covered with minute spines, anterior end more densely. Brownish pigment granules are distributed reticularly in the body and some are found in the tail. Papillae with short sensory hairs on lateral margin of body. Oral sucker spherical, 23-34 (29.8; 2.9) long by 25-32 (27.3; 2.3), protrusible. No acicular spines at anterior end present. One pair of pigmented eyespots posterior to oral sucker, 44-57 (53.7; 2.8) from anterior end. Seven pairs of penetration glands arranged in two groups of three and four gland cells, their ducts running forward together passing between eyespots and opening in sets of 3-4-4-3 pores at anterior extremity. Acetabulum not developed, on its site a cluster of small cells were observed. Big excretory bladder, which in living cercariae occupied more than the posterior half of body and is limited by scattered, irregularly shaped, epithelial cells. Excretory system with 24 flame cells, probably distributed as 2 (2+2+2) + (2+2+2) = 24. A short bifurcating excretory duct is present in anterior part of the tail.

Tail inserted terminally, 319-344 (327.5; 6.5) long by 42 (42.0; 0) wide, without fin folds, but with a tegumental crest on dorsal side. Conspicuous annulations in the tegument of whole length give to the borders a serrated appearance; the posterior fifth is smooth. A caudal spine is present at caudal extremity.

The cercariae swim near the water surface and live for approximately 48 hr.

Study of cercariae shedding pattern - Cercariae shedding patterns were studied during 17 consecutive days with the single snail collected in November 1992. During a photoperiod balanced L:D 12:12 hr (photophase 7:00-19:00) cercariae emergence occurred between 9:00 and 17:00, with marked peak at 9:00, declining to zero after 15:00. When light incidence is shifted from 7:00 to 9:00 or 11:00, the emergence peaked 2 hr later, at 11:00 and 13:00 respectively, but declined equally after 15:00 (Fig. 9). When the L:D cycle was reversed to photophase 7:00-19:00, the shedding pattern was analyzed every 30 minutes. Cercarial emergence began after 60 - 90 min, peaked after 120 min of light incidence and within 4 - 5 hr all cercariae had escaped (Fig. 10). During these photophases 83-168 (mean 115.8) cercariae escaped from the snail, while during the dark periods between them no cercariae emerged. During a constant dark period for three days only 69 cer-

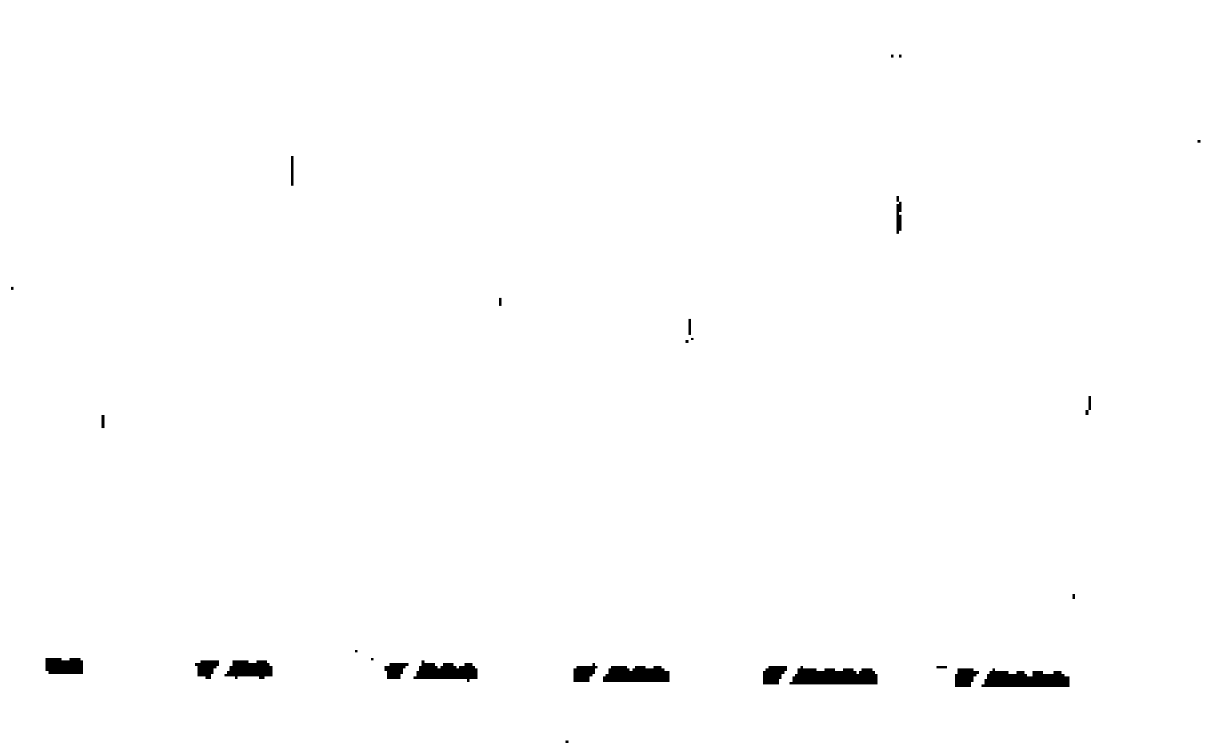


Fig. 9: emergence of cercariae, photoperiod L:D 12:12 hr, light incidence at 7:00, 9:00 and 11:00, measured every 2 hr. Temperature bars represent minimum and maximum °C/day.

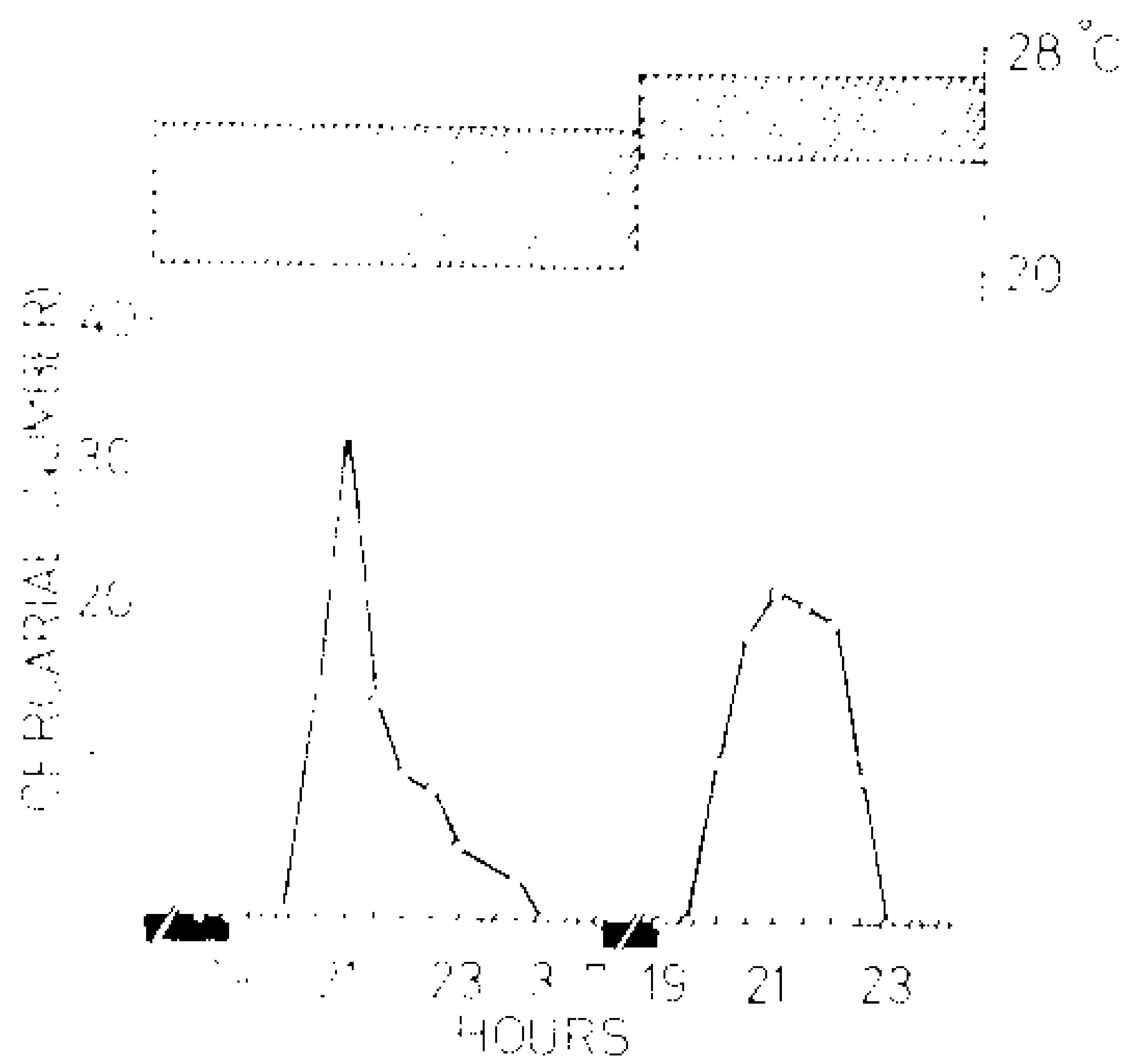


Fig. 10: emergence of cercariae, measured every 30 min during an inverted photoperiod. Temperature bars represent minimum and maximum °C/day.

cariae escaped. They accumulated in the snail, escaped in mass 2 hr after the next light incidence and the pattern normalized during the consecutive days (Fig. 11).

Metacercariae (Figs 6, 7) - In experimentally exposed fish, at summer temperature (approximately 20-30°C) small spherical pigmented cysts, 94 - 132 in diameter could be found in the body cavity after 48 hr p.i. On 12 days p.i. the cyst increased in size measuring 276 long by 286 wide; the digestive tract, the ventral sucker and the excretory bladder are developed. The cyst wall is thin. The cysts continuously increased in diameter until 38 days p.i., afterwards a slight reduction took place, and the "inner cyst", with the metacercaria in it, is smaller in size than the younger metacercariae, but the cyst wall continued to

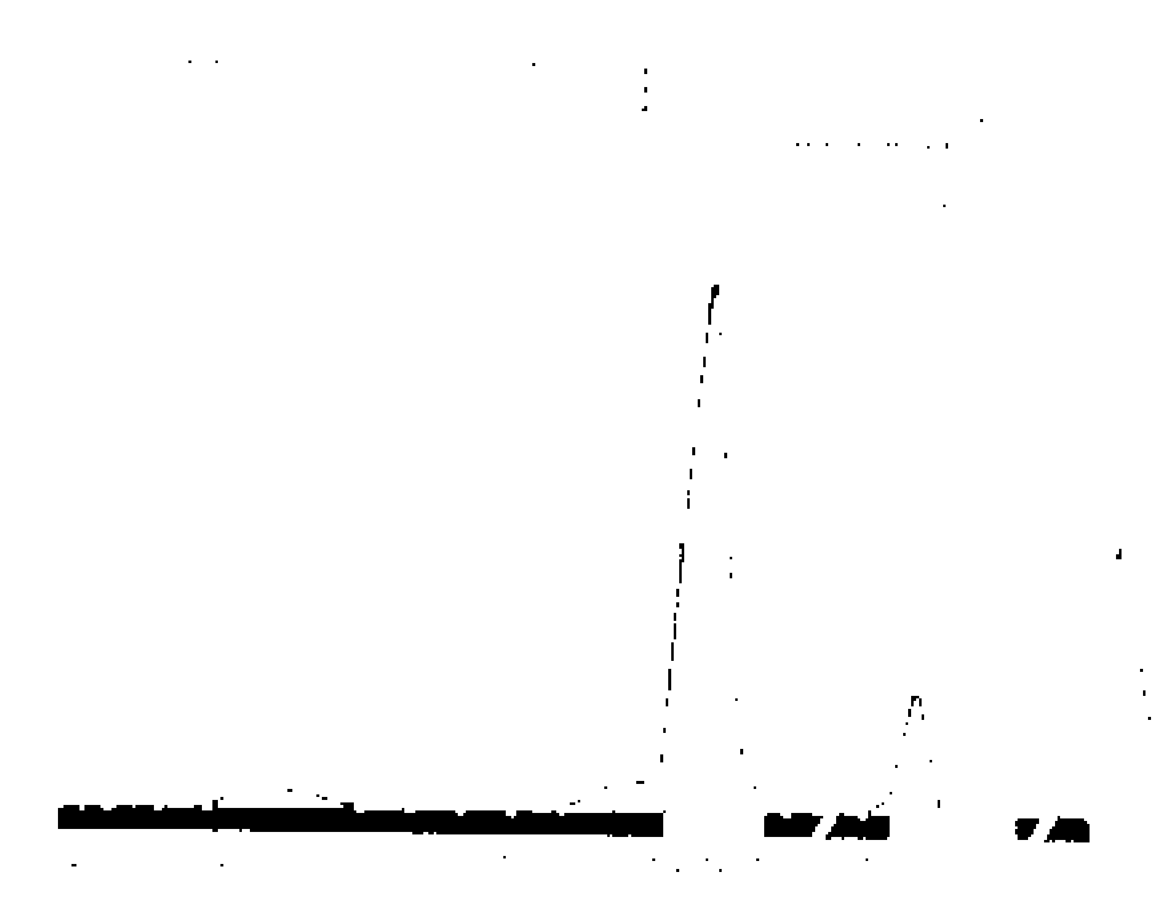


Fig. 11: emergence of cercariae, measured every 8 hr during constant darkness and every 2 hr after light incidence. Temperature bars represent minimum and maximum °C/day.

TABLE II

Growth of metacercaria

p.i.		Outer cyst Min.	Max.	Mean	Inner cyst Min.	Max.	Mean	Cyst wall Min.	Max.	Mean	n
48 hr	L	95	132	114					thin		5
	W	95	132	115							
8-12 days	L	235	353	276.4					thin		17
	W	235	344	286.2							
25 days	L	353	395	371.8	294	361	321.1	16.8	33.6	23.0	8
	W	344	395	362.4	294	336	314.9				
28 days	L	353	412	393.0	252	269	258.8	50	76	68.6	6
	W	353	412	392.0	252	260	256.0				
38 days	L	428	445	433.0	277	311	297.4	59	84	71.5	7
	W	403	437	423.6	260	302	274.7				
41-44 days	L	395	428	417.2	218	269	236.7	84	101	91.1	12
	W	395	428	416.5	227	260	236.6				

Min: minimum; Max: maximum; n: number of specimen measured; L: length, W: width grow thicker (Table II). The metacercaria is then fully developed, with a branched excretory bladder, platelets of 6 - 8.4 in diameter in the caeca, developed acetabulum, two testes, an ovary and an empty uterus. No spines are present on oral sucker, as in other *Pygidiopsis* species, but tegumental spines are distributed as in adults.

Cysts are easily distinguished from similar cysts of *Ascocotyle hadra* Ostrowski de Nuñez, 1992 by the thick, transparent, cyst wall, besides the morphological features of excysted metacercariae.

DISCUSSION

In the genus *Pygidiopsis* nine species had been described (*P. genata* Looss, 1907; *P. summa* Onji et Nishio, 1916; *P. plana* (Linton, 1928); *P. pindoramensis* Travassos, 1928; *P. macrostomum* Travassos, 1928; *P. maravillai* Refuerzo et Garcia, 1937; *P. phalacrocoracis* Yamaguti, 1939; *P. piclauromeli* Dollfus et Capron, 1958 and *P. ardeae* Køie, 1990). In all of them, except *P. macrostomum*, the vitellaria extend laterally between testes and ovary and the uterus between the testes and the ventral sucker. In *P. macrostomum* the vitellaria reached the posterior border of ventral sucker and the uterus extend up to the pharynx.

Pygidiopsis crassus n. sp. is similar in general body shape and distribution of genital organs to *P. macrostomum* as described by Travassos (1928; after Travassos et al. 1969), upon a single specimen from *Rattus norvegicus* (Erxleben, 1777) in Brazil, and to those described by Odening (1969) from *Noctilio leporinus mastivus* Ribeiro, 1914 in Cuba.

Pygidiopsis crassus n.sp. differs from *P. macrostomum* by its 40-75% smaller body, smaller eggs and in the oral sucker to acetabulum width ratio. While in *P.crassus* n. sp. the oral sucker is 4-15% smaller than the acetabulum, in *P. macrostomum* this condition is reversed, being the oral sucker 20 % larger than the acetabulum, as well in the specimen of Travassos as in those of Odening. Odening (1969) made some observations on living material and described an I-shaped excretory vesicle and 24 flame cells arranged in a formula 2[(2+2+2)+(2+2+2)]=24. It is difficult to compare the present material with the single specimen of Travassos, as no variation could be considered, e.g. in the relation of body and sucker sizes. All other species of *Pygidiopsis* differ from each other in few details as body and/or sucker sizes, characteristics of prepharynx and the structures of gonotyl, the latter are not easy to observe. Since the condition of the oral sucker to acetabulum width ratio is 1:0.80 in the single specimen of Travassos and 1:0.83 in the specimens of Odening, it seems that this is a stable character. Due to this difference in the present material, adding the different body and egg size and the shape of excretory vesicle, *P. crassus* is considered a new species.

The heart cavity of the first intermediate host is the site of infection of the sporocyst of several species of *Philophthalmus*, *Echinostoma* and *Echinopharyphium*, and for *Hypoderaeum dingeri* and *Euparyphium murinum* as reviewed by Yamaguti (1975). Little is known about the sporocyst generation in Opisthorchioidea. Martin (1950) described sporocysts of *Parastictodora hancocki* Martin, 1950 as simple, saccular, sausage shaped structures up to 3 mm in length to 1 mm in width, with yellow to brownish pigment. They contain hundreds of rediae of varying size, and are localized in the digestive gland of the snail. Martin (1958) described the sporocysts of *Centrocestus formosanus* (Nishigori, 1924) as slender, elongate, up to 1 cm in length, localized in the mantle tissue near the gill of the snail. Besides these, the sporocysts of *Stellacantchasmus falcatus* Onji and Nishio, 1924, *Opisthorchis felineus* (Rivolta 1884), *O. viverini* (Poirrier, 1886) and *Clonorchis sinensis* (Cobbold, 1875) are described (cited after Yamaguti, 1975). Maillard (1976) described small, spherical sporocysts of 15-20 m in diameter in *Acanthostomum imbutiforme* (Molin, 1855) and *Timoniella praeterita* (Looss, 1901) (Acanthostomidae), localized at the level of the stomach of the snails. In all the cited species, except *S.falcatus*, only one generation of redia is described. In *Acanthostomum brauni* Mañé Garzon and Gil, 1961 small sporocysts could be found in the heart cavity of the snail host (Ostrowski de Núñez, unpublished data), similarly as in *P. crassus* n. sp. In these two species one generation of redia seems to be present.

The cercaria of *P.crassus* n. sp. is similar to other cercariae described within the genus *Pygidiopsis* in having seven pairs of penetration glands, but differs from that of *P. pindoramensis* which has hooklets on oral sucker, a flame cell formula 2(2+2)+(2+2)=16, smaller body and no annulations on the tail (Ostrowski de Núñez 1976), and from *P.ardeae*, which in addition to the previous characteristics, has an inconspicous pharynx and acetabulum (Køie 1990b) *P. genata* and *P. summa* differed in having well developed fin folds (Youssef et al. 1987, Yamaguti 1975). The cercaria of *P.crassus* n. sp. is similar to that of *Ascocotyle hadra* Ostrowski de Núñez, 1992, in having the same flame cell formula, but differs in its smaller body dimensions, smaller excretory vesicle with a different epithelial layer and less pigment granules.

It is noticeable, that species in the same genus, as *Pygidiopsis*, show different cercariae: *P. genata* has a cercaria similar to Acanthostomidae and Opisthorchidae, *P. ardeae* and *P. pindoramensis* to *Phagicola* (Heterophyidae), and *P. crassus* n. sp. to *Ascocotyle mcintoshi* Price, 1936 and to the recently described cercaria of *A. hadra*.

Pygidiopsis crassus n. sp. may not be very specific for the definitive host, as concluded from the fact that chicks and mice could be experimentally infected. Piscivorous birds, as egrets, herons, ibis, etc. or perhaps rats, frequent in the area, are suspected to be the natural hosts.

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