LIFE HISTORY OF THE TREMATODE, ECHINOCHASMUS PELECANI n. sp.

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[Read 11 May 1944]

Echinochasmus pelecani n. sp.

This small echinostome has been found in the small intestine of the pelican, *Pelecanus conspicillatus* Temm., at Tailem Bend, Murray River, on several occasions during the past six years, the number present being always small. The following measurements (in millimetres) have been taken from specimens which were egg-bearing, the average being based on ten worms in glycerine or methyl salicylate. Length 1·4-2·57 mm., average 1·92; maximum breadth ·29-·4 mm., average ·36, occurring in the vicinity of the acetabulum, though the width at the oral crown (excluding the oral spines) is in most cases almost equal to it. Oral sucker terminal, approximately circular, though sometimes the length is slightly greater, ·062-·075 mm. diameter. Acetabulum circular, ·24-·28 mm. diameter; distance of its anterior edge from head end of worm ·72-1·1 mm., the ratio of this distance to length of worm 1 : 2·2-2·9; acetabulum entirely in anterior half in larger specimens, more or less completely so in smaller worms, the post-acetabular length being relatively greatest in largest worms. The ratio of the breadths of the oral and ventral suckers is nearly 1 : 4 (1 : 3·7-4·0) in most specimens, but in the best-preserved material the oral sucker is ·087 mm. wide by ·070 long, and the acetabulum ·225 mm. in diameter, the ratio of breadths thus being approximately 1 : 2·6. The maximum breadth of such a worm was ·35 mm, in the vicinity of the acetabulum, while the oral crown measured ·31 mm. in width.

In most specimens the covering of body spines had disappeared, since the worms disintegrate rather rapidly. These triangular, scale-like spines are closely arranged, similarly to those figured for *E. donaldsoni* by Beaver (1941), and the series extends on the dorsal and ventral surfaces from the oral region at least as far as the level of the testicular region.

The collar spines are lost more or less completely soon after the death of the worms. The series is interrupted mid-dorsally where the interval between two spines is rather less than the diameter of the oral sucker. The majority of the spines are about ·075 mm. long by 16-17 μ, but the three situated on each ventral lobe are smaller and exhibit an alternate arrangement (fig. 10). The inmost is the smallest, ·045 mm. long by 12·5 μ; the next ·065-·07 mm. by 17 μ; and the next ·0575 by 15-16 μ. There are about 10 minute spinules on the anterior border of the oral sucker.

Prepharynx about as long as oral sucker; pharynx ·075-·103 mm. long, as long as or slightly longer than diameter of oral sucker, ·038-·07 mm. wide, usually ·055. Oesophagus long, widening posteriorly, bifurcating immediately in front of genital aperature. Crura extending to sides of excretory bladder. Lateral excretory siphons passing forwards laterally from caeca, oesophagus and pharynx, terminating each as a narrow canal close to prepharynx a short distance behind oral sucker.

Testes almost entirely in third quarter of worm; anterior ·11-·20 mm. long, ·15-·21 mm. broad, in contact with posterior testis; latter more elongate, usually rounded-triangular but occasionally almost elliptical, ·15-·275 mm. long, ·138-·20 mm. broad. Cirrus sac relatively large, lying largely in area bounded by crura and anterior border of acetabulum, but extending dorsally above latter to about its middle; ·175-·25 mm. long, ·112-·162 mm. broad; seminal vesicle consisting

of rounded anterior and posterior chambers; numerous prostate glands associated with most anterior part of cirrus sac; cirrus very short, simple.

Ovary more or less spherical, ·07-·08 mm. diameter, lying on right side of midline just in front of anterior testis; oviduct arising dorso-medianly, and curving downwards to enter Mehlis gland lying on left side of midline and continuing ventrally as the uterus; inner portion of latter sometimes considerably swollen with semen (receptaculum seminis uterinum of Yamaguti). Uterus thrown into a few convolutions closely crowded into region between anterior testis and acetabulum, then passing above latter to one side of, and somewhat ventral to, cirrus sac as the metraterm to terminate in the shallow genital atrium. Eggs 1-24 in uterus; ·075-·087 by ·050-·062 mm.; average of 20 eggs, ·081 x ·059. Vitellaria lateral, extending from anterior border of excretory vesicle to level of posterior border of acetabulum, fields more or less coalescing in post-testicular region; rarely with narrow irregular isthmuses crossing testicular zone. Transverse vitelline ducts lying immediately in front of anterior testis, one on more ventral level than the other; passing below corresponding crus to travel inwards and dorsally to enter the prominent yolk reservoir; latter approximately median, dorsal. Laurer's canal transverse, just in front of anterior testis.

A specimen which had not yet produced an egg but whose seminal vesicle was distended with sperms, possessed the following measurement in millimetres:— length 1·47 mm.; breadth of oral crown ·30, breadth at acetabulum ·286; oral sucker ·057 diameter; acetabulum ·20 by ·185; sucker ratio 1:2·6; front of acetabulum at ·72 mm. from head end, *i.e.*, at almost half body length. In three specimens which were each producing the first egg, their dimensions were 1·4 long, ·29 broad; 1·6, ·36; and 1·65, ·37 respectively, with the front of the acetabulum at 1:2·3-2·4 of body length distant from the anterior end.

Very young worms were taken on various occasions. The smallest worm (a well-preserved specimen, fig. 12) found in a pelican measured ·42 mm. long; ·162 mm. across the oral crown; ·125 mm. across the acetabular level; breadth of oral sucker ·045 mm., of acetabulum ·07, the ratio of widths thus being 1:1·6; the two testes and ovary were recognisable; and the oral crown showed the same relative sizes and positions of the oral spines as in the adult. In specimens ·7 (fig. 13) and ·8 mm. (fig. 14) long the corresponding measurements were:— ·187, ·20; ·125, ·187; ·375, ·40 (ratio 1:1·86, 1:2); ·05, ·06; and ·087, ·137 (ratio of widths of suckers 1:1·7, 1:2·3) respectively. Vitelline glands in an immature condition were abundant, but rather restricted in their distribution, in a specimen 0·99 mm. long; they were not seen in smaller worms.

We believe that our species is *E. mordax* (Looss) described (1899, 688) from an Egyptian pelican, *P. onocrotalus*, but the account of the parasite is brief and Looss' figure indicates a different arrangement of the ventral collar spines. We are not aware of any subsequent description of that species and consequently consider it wiser to describe our own as new, than to include it under *E. mordax*. We expect that re-examination of the latter will reveal an arrangement of the collar spines similar to that seen in the Australian parasite and will lead to the suppression of our specific name. The dimensions mentioned by Looss generally fall within the range stated by us; the general form is similar; the presence of 22 collar spines in both; the inmost ventral collar spine has a similar length; his figure shows that the ratio of the breadths of the oral sucker and acetabulum is about 1:2·6; the front end of the acetabulum is at about two-fifths of the body length; and the eggs have similar dimensions. The host in each case is a species of *Pelecanus*.

Echinostomum mordax was selected by Odhner (1910, 163) as the type of a new genus, *Heterechinostomum* (Echinochasminae), but Price (1931, 6)

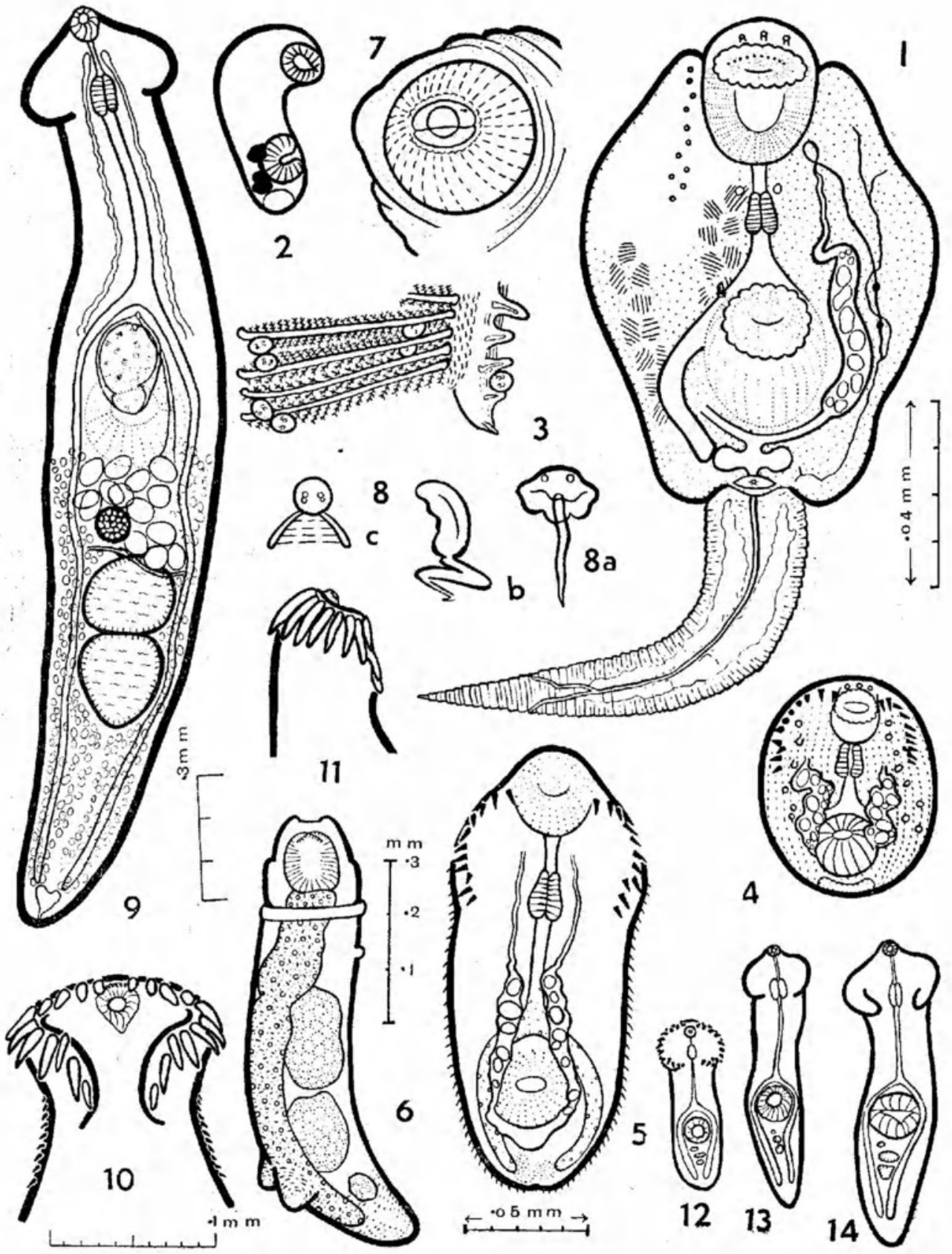

Fig. 1-10, *Echinochasmus pelecani*—1, cercaria; 2, lateral view of cercaria showing genital system; 3, cysts on gills of fish; 4, metacercaria in cyst; 5, metacercaria; 6, redia; 7, mouth of redia, end view, showing pharyngeal lips; 8, freehand sketches of cercaria: (a) in resting position, (b and c) when swimming; 9, adult, ventral view, collar crown and body spines omitted; 10, head region, ventral view; 11, head end, lateral view; 12-14, very young stages from pelican, spines omitted from 13, 14. Fig. 1, 4-6, 9-14, drawn with the aid of a camera lucida; fig. 4 and 5 to scale below fig. 5; 10 and 11 to scale below 10; 9, 12 to 14 to same scale.

suppressed the latter as a synonym of *Echinochasmus*, to which genus he transferred *H. mordax*.

Two Australian species of *Echinochasmus* have been described: *E. tenuicollis* S. J. Johnston 1919, from a cormorant in New South Wales; and *E. prosthovitellatus* Nicoll 1914 from a hawk in Queensland. The former species was transferred to *Paryphostomum* by Price (1931) and by Johnston and Angel (1942), and was subsequently shown by Johnston (1942) to be a synonym of *P. radiatum* (Rud.). *E. prosthovitellatus*, because of the forward distribution of its vitellaria, was transferred by Price (1931, 6) to *Episthmium*.

The presence of 22 spines on the oral collar links *E. mordax* and *E. pelecani* with a small number of other species of the genus. *E. schwartzi* Price (1931), from the muskrat and dog in U.S.A., seems to be the nearest species, but it differs in the sizes of the suckers, length of oesophagus, size of eggs, and arrangement of the oral spines on the ventral lobes. *E. milvi* Yamaguti 1939, from *Milvus migrans* in Japan, is a smaller species with a less extensive uterus containing very few eggs, with a shorter oesophagus, and with a different form of the testes; but it has a similar arrangement of the oral spines on the ventral lobes, and the eggs are of similar size. *E. dietzevi* Issaitchikov (1927), a 20-spined form, possesses three alternating angle spines on each side, as also do the 24-spined species. *E. bursicola* (according to Odhner 1910, pl. v, fig. 1) and *E. corvi* Bhalerao (1926). *E. bursicola* was placed under *Episthmium* by Lühe (1919), under *Echinochasmus* by Odhner (1910, 162), and restored to *Episthmium* by Price (1931).

We have not succeeded in our attempts to infect with eggs of *E. pelecani*, *Ameria* spp., *Limnaea lessoni*, *Plotiopsis* and *Corbiculina angasi*, the chief molluscan species occurring in the Murray and its swamps at Tailem Bend. However, we have encountered very commonly in *Plotiopsis tatei* (Melaniidae) as a natural infection, a cercaria which, though gymnocephalous, enters fish and gives rise to an echinostome metacercaria belonging to *Echinochasmus*. This larva possesses the same number of collar spines, and these have a similar arrangement to that present in *E. pelecani*. Such similarity has not been observed in any other adult and larva known to us. *Plotiopsis* is restricted in the region to the banks of the Murray, occupying a zone about three to six feet in depth, pelicans utilising the adjacent bank as a resting place. We had not used the mollusc for our subsequent attemps at infection because we had not yet succeeded in rearing the species in our aquaria. For the reasons given above we consider that the redia, cercaria and metacercaria to be described are the larval stages of *E. pelecani*.

Cercaria Stage

This cercaria is the commonest of those observed by us to be emitted from *Plotiopsis tatei* (Melaniidae) at Tailem Bend and Swan Reach, and was found in 514 out of 7,123 examined, the percentage of infected individuals being 7·2. The highest infection rate was observed on 12 December 1937, when 227 out of 519 individuals collected harboured the parasite, the percentage of infection being nearly 44. The cercaria has been met with fairly regularly since then during the period November to May of each year. It has not been looked for during the remaining months.

The swimming movements of this small active cercaria resemble those of an echinostome. The resting position is typical (fig. 2), the organisms hanging as a fine cloud in the water in that part of the tube which is of optimum light intensity. The cercariae are given off in great numbers usually before 8 o'clock in the morning for the first few days in the laboratory, but on succeeding days few cercariae emerge. Snails kept in captivity over the winter have not been observed to give

off cercariae in the following December, although small rediae were found in the liver.

In ten specimens killed in boiling 10% formalin the body length varied from 114 to 179 μ (average 129 μ), and the breadth from 68 to 87 μ (average 79 μ). The anterior sucker varied in length from 30 to 38 μ, and in breadth from 27 to 30 μ; and the posterior sucker measured 27 to 30 μ long by 27 to 30 μ broad. The sucker ratio is 17 : 16. The distance of the posterior sucker from the anterior end varied from 61 to 152 μ (average 84 μ). The short annulate tail measured 91 to 178 μ long (average 122 μ). Both suckers have a frilled edge. The anterior sucker is retractile and can be withdrawn for some distance into the body of the cercaria, which then acts as a hood. Three refractive structures having the appearance of ducts are present on the dorsal part of the sucker, and ventral to them are 10 minute spinules. The body cells stain heavily with neutral red. Cystogenous cells are arranged in four longitudinal groups and fill the central parts of the body. They are pale yellow and are filled with rod-shaped granules. In nearly every specimen two refractive spots were seen on either side of the pharynx. These were the nuclei of the most anterior cystogenous cells of the two median rows. The body has a fine granular appearance, due to minute spines on the surface. Collar spines are not apparent, but in a few specimens refractive dots (apparently the immature collar spines) are seen arranged vertically as in fig. 1.

Following the mouth is a prepharynx. The pharynx is pear-shaped and is consistently on its side when the cercaria contracts. The oesophagus is long and the intestine is large and refractive and reaches to the bladder. The first part of the intestine is particularly hard to see.

The excretory system resembles that of an echinostome. The bladder is bilobed and a small duct leads posteriorly to a wide prominent opening at the junction of tail and body. The two main excretory tubes meet before entering the bladder by a median duct. About nine excretory granules, some of them compound, are present. Before these excretory tubes reach the anterior sucker, they form a loop and descend to the level of the middle of the ventral sucker. Two ciliated patches are present in these parts (fig. 1). The tube then divides into two; the ascending ramus, travelling anteriorly, gives off a secondary branch near the level of the pharynx; and the posterior ramus divides into two in the region of the bladder. Flame cells were not seen, though consistently looked for, and immature cercariae were examined. The excretory system in the tail is seldom seen. It consists of a median tube dividing into two in the distal part of the tail. This was confirmed by the study of immature cercariae.

The reproductive apparatus is represented by two masses of cells medially placed, dorsal to the posterior sucker. They are connected by a strand of cells (fig. 2) . Their position indicates that the anterior is the anlagen of the cirrus sac and associated structures, and that the posterior mass will differentiate into the gonads.

Redia Stage

Rediae (fig. 6) are present in all stages of development in the tissues of the snail. The liver is not usually discoloured but contains rediae and cercariae, which are often present in far greater numbers elsewhere in the body. These parts are coloured orange, and in heavily infected snails are compact masses of cercariae and rediae. The former are present in such numbers that they must, after birth, remain in the tissues of the snail several days before being emitted.

One well developed redia measured about 1·68 mm. and contained numerous developing cercariae and germ balls. The mouth leads into a small vestibule just in front of a well-developed pharynx. The mouth of the pharynx (fig. 7) has

two semi-circular lips with thickened (probably chitinised) edges which form strong biting jaws. The intestine is large and extends beyond the two foot processes. The collar and birth pore are usually readily seen. The walls contain orange pigment. Some snails giving off this cercaria during the summer were kept through the winter and retested in the following December; although no cercariae had been given off in the aquarium during the latter period, a number of small rediae were found in the liver.

Metacercaria Stage

The cercaria has been found experimentally to encyst in the laboratory in the fish, *Oryzias latipes* and *Gambusia affinis*. Tadpoles (*Crinia* sp.) and the snails, *Limnaea lessoni* and *Ameria* spp. were tried, but with negative result. The metacercariae (fig. 3) are found only on the gills of the fish, where they may be present in great numbers. They are oval and fairly uniform in size. Of ten specimens the average measurements were 88 μ by 68 μ. In the laboratory all the cysts were dead on the third day after the death of the fish. The cyst is thin-walled and easily broken and the expressed metacercariae die almost immediately, thus making examination difficult. The addition of horse serum to the water did not increase their longevity.

Free metacercariae measure about 0·18 mm. long, with a maximum width (across the oral crown) of ·075 mm. Small spines in transverse and longitudinal rows completely cover the body, giving a fine, but distinctly hairy, appearance to the metacercaria. Ventrally the collar spines become smaller and the first and third spines from the ventral end are markedly smaller than any of the others. The third spine, set at an angle to the others, is the most anterior in position. No separate group of corner spines was seen. Body spines, slightly smaller than the rest, are present between the mid-dorsal gap of the collar spines and continue up to the anterior sucker. The ten oral spinules noticed in the cercaria are still present, though not readily seen. The globules (usually eight or nine on each side) present in the metacercaria are probably the ducts of small gland (cystogenous) cells. They stain the same shade as the three oral ducts.

We fed to a pigeon and to a rat numerous small fish which had been infected in the laboratory, an estimated total of about 200 cysts being fed in each case, but no adult stages were recovered. Yamaguti (1933) described various stages in the life cycle of some Japanese species (*E. elongatus*, *E. rugosus* and *E. rediodupli-catus*), having obtained his adults by feeding to rats, mice or dogs, infected molluscs or tadpoles in which the metacercaria stage occurred. He also figured the miracidium of *E. rugosus* (1933, 113). Cuirea (1931, 292) reported that cysts of *E. liliputanus* occurred on the gills of Roumanian marine fish and obtained adults by feeding the latter to dogs. Kurisu (1931) found that, from a cercaria from *Melania*, adult stages of *E. grandis* could be obtained from experimental rats and dogs. Beaver (1941) published an excellent account of the life history of *E. donaldsoni* from a grebe.

Our cercaria may be compared with that of *Echinochasmus donaldsoni*, as described by Beaver (1941). The behaviour and general characteristics are similar. The latter cercaria is distinctly smaller and the spination present on the ventral sucker and ventral lip of the anterior sucker were not noticed in our specimens. The body spines and collar spines have been seen, though with difficulty, in our form. Slight differences in the excretory system are apparent. The number of excretory granules is less and their position more restricted in our cercaria, and the bladder seen in the proximal part of the tail is not present in our form, unless the excretory sac, consistently present below the dorsal excretory pore, be it. The difference between the two is more marked in the metacercaria, where the collar spines number 22 in our form but only 20 in *E. donaldsoni*.

Rediae are similar in both forms, though in our species they are considerably larger, and the lips of the pharynx are reinforced.

Cercaria indica XLI (Sewell 1922) is probably the cercaria of an *Echinochasmus*. Its habits and general appearance, including the three oral ducts, are similar to those of our cercaria. Obvious differences are the much greater size of the Indian form, the presence of diverticula at the base of the caudal excretory canal, and the presence in the redia of an intestine which does not reach the level of the foot processes.

Type specimens of the various stages have been deposited in the South Australian Museum. We desire to acknowledge generous assistance rendered by Messrs. G. G., F., and Bryce Jaensch and L. Ellis, of Tailem Bend, in regard to collecting host material. The work was carried out with the aid of the Commonwealth Research Grant to the University of Adelaide.

Summary

1. The anatomy of *Echinochasmus pelecani* n. sp. from *Pelecanus conspicillatus* is described.

2. Cercariae and rediae from *Plotiopsis tatei* are regarded as its larval stages, the metacercaria developing experimentally in freshwater fish (*Oryzias latipes, Gambusia affinis*).

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