LIFE HISTORY OF THE TREMATODE, PETASIGER AUSTRALIS n. sp.

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[Read 11 September 1941]

Petasiger australis n. sp. is a minute echinostome occurring in the grebes, *Podiceps ruficollis novaehollandiae* Stephens and *P. poliocephalus* Jardine and Selby, in the swamps at Tailem Bend, South Australia. For assistance in collecting this material we are indebted to Messrs. G. and F. Jaensch and L. Ellis, of Tailem Bend. Our investigation has been assisted by the Commonwealth Research Grant to the University. Type material deposited in the South Australian Museum.

The largest parasite measures about ·7 mm. long, with its maximum breadth (·28 mm.) at the midacetabular level. Other dimensions are: width at sex pore, ·2 mm.; at level of ovary, ·26; across testicular zone, ·2-·25; at narrowest part of neck, ·12; across head collar, ·17. The anterior part of the body (except the head) is rather flattened and minutely spiny, with spinules arranged in closely-set rows. The posterior region is much thicker and devoid of spines. The reniform head bears 19 spines, 11 of them evenly spaced in a single unbroken series, and two pairs in each ventral angle, arranged more or less transversely to the long axis of the worm. One of each pair is slightly longer than the other, lengths ·054 and ·047 mm. respectively, while the remainder are about ·025 mm. long. The oral sucker is almost terminal and ·045 mm. broad by ·05 long. The acetabulum lies mainly in the hind-body, its anterior border being just in front of the midlength of the worm; it measures ·17 mm. broad by ·16 mm. long, the sucker ratio being about 1:3·7; sex pore near end of first third of body.

The prepharynx is about ·02 mm. long, the pharynx ·04 mm., the oesophagus about ·1 mm. and extending almost to the genital pore. The crura at first diverge widely to skirt the border of the cirrus sac, metraterm and acetabulum, terminating a short distance behind the level of the posterior testis.

The anterior testis is ·11 by ·07 mm. and obliquely placed on one side of the worm; the posterior testis is ·12 by ·08 mm., and the two glands are in contact along part of their inner surfaces. Both testes and ovary are in the posterior third of the body length. The large cirrus sac, ·13 mm. in length by ·11, occupies the region between the crura and the anterior border of the acetabulum on the same side of the body as the ovary and posterior testis. A considerable part of the sac is occupied by deeply-staining prostate cells, but a large seminal vesicle lies in its posterior portion.

The spherical ovary, ·06 mm. in diameter, lies dorsally between the posterior testis and the acetabulum. The shell gland is nearly median, beside and partly above the anterior testis. The uterus has two or three short convolutions. The metraterm lies on the opposite side of the acetabulum from the cirrus sac. The eggs are large (·085-·09 by ·05-·06 mm.) and few (one to seven, usually about four). Yolk glands obscure the intestinal crura and extend from just behind the level of the sex pore almost to the end of the worm, the opposite vitelline fields joining in a narrow zone behind the testes. They lie above and below the crura, as well as laterally from them. The transverse yolk duct is just in front of the posterior testis and above the anterior testis.

The smallest worm measured ·48 mm. long; the longest which had not yet become egg-bearing was ·57 mm. The smallest parasites containing each a single egg were ·59 and ·61 mm. long. Most specimens were ·66 mm. long.

Petasiger australis differs from *P. exaeretus* (Dietz, 1909; Davies, 1934) and *P. variospinosus* Odhner 1911 from *Phalacrocorax carbo* in the number of

collar spines (27 in each) and position of the testes. Several species, all with 19 collar spines, are known from grebes. The nearest known relative of *P. australis* is *P. nitidus* Linton 1928, redescribed by Beaver (1939), from *Colymbus auritus* from Massachusetts, but the two differ in the dimensions of the body, organs and collar spines and in the sucker ratio. Yamaguti (1933) described *P. lobatus* from *Podiceps ruficollis japonicus,* but that species differs from the Australian in the sucker ratio and especially in the form and arrangement of the testes. The account of *P. novemdecim* Lutz 1928 from a Venezuelan *Phalacrocorax* is not available to us. *P. pungens* (Linstow), from *Colymbus nigricans* in Central Europe, differs (judging from the summary account given by Lühe 1909) in general form, length of oesophageal region and the arrangement of the testes.

We have studied a large-tailed species of echinostome cercaria which agrees in general structure and reactions with that described by Beaver (1939) as being the larva of *Petasiger nitidus,* which, as already mentioned, is a 19-spined form from a North American grebe. Our cercaria obviously belongs to a species of *Petasiger,* closely related to *P. nitidus.* All known species possessing 19 spines have been taken from grebes, a group to which *Podiceps* spp. belong. We have not recognised *Petasiger* amongst the trematodes found in any other birds so far examined by us. The adult worms and the cercariae were found at the same time and in the same swamp. In view of these facts we have little doubt that they belong to the same species, though our attempt to infect a canary with metacercarial cysts in the hope of obtaining adult stages proved unsuccessful. Until actual proof has been established, we deem it advisable to describe the larval stages (redia, cercaria and metacercaria) under a distinctive name, *Cercaria gigantura* n. sp.

Cercaria gigantura n. sp.

Of 2,500 *Amerianna pyramidata* collected in February 1941 from the Murray swamps at Tailem Bend, 10 were found to be infected with a new type of echinostome cercaria. In the following March one of 424 of the snails was infected, in April none of 148, in May none of 64, and in June one of 27. Thus from April 1937 (when routine examination of the swamps was commenced) to June 1941, this cercaria has been found on only three occasions. We have found only 12 out of 3,163 *A. pyramidata* infected, *i.e.,* under ·4% during the summer and autumn of 1941. The distinctive feature of the cercaria is the relatively huge tail, which makes the swimming action clumsy. The tail does not appear to propel the body by the figure of eight movement characteristic of most echinostome cercariae, but lashes the water with a snake-like movement. The animal frequently hangs vertically with tail straight and body downwards, in which position it sinks gradually, though the resting period is not as a rule longer than six seconds. The cercariae emerge from the snail over-night (probably in the early morning) and up till midday, and have been observed to live for at least 30 hours. Although eye-spots are not discernible, the cercariae exhibit positive phototropism.

Probably because of the thickness of the tail, the cercaria does not perform sucker-to-sucker creeping movements under the compression of a coverslip. A pressure which will flatten the tail and keep it almost still, will allow a fair range of movement of the body, while sufficient pressure to enable the body to be studied in detail results in the loss of the tail. With only slight compression the animal oscillates about one axis, the tip of the tail describing almost 180°, while the body has a more limited range, probably a swimming movement limited to one plane.

Redia

The pale orange rediae are closely packed within the liver of the snail, where the individuals are clearly visible to the naked eye by reason of the black intestine. They vary in length from 0·8 to 1·4 mm., while the diameter is from 150 to 200 μ.

Each redia generally contains five or six cercariae. The birth pore was not seen, but a short distance behind the anterior end the collar shows as a definite protuberance. The foot processes are prominent, and behind them is a narrow terminal part, with somewhat the appearance of a tail. In this region there are generally a number of fairly large germ balls, and it seems probable that the nearly

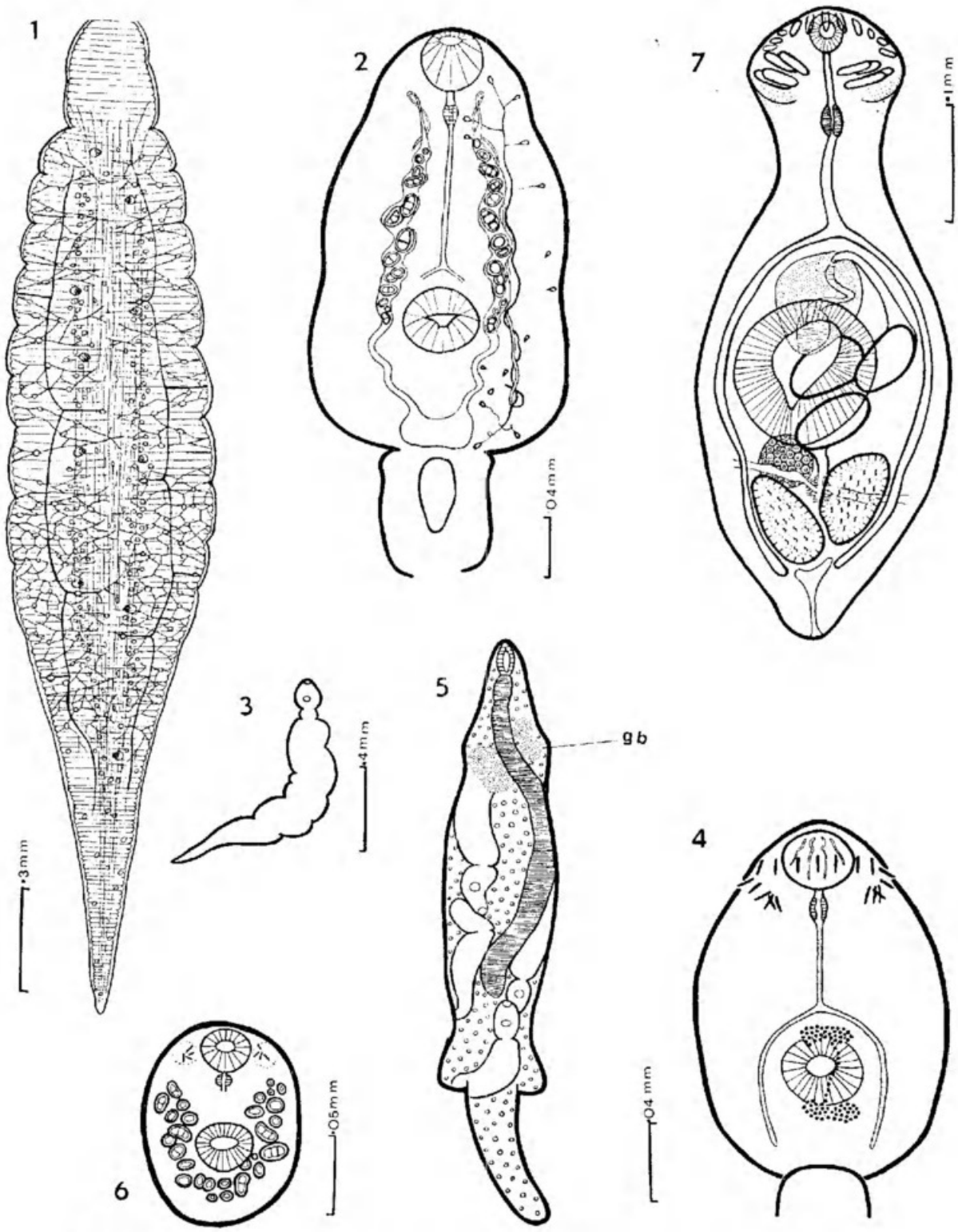

Fig. 1-6, *Cercaria gigantura:* 1, tail; 2, excretory system from living cercaria; 3, 4, formalinised cercariae (in fig. 4 arangement and size of spines probably not precisely accurate—errors due to difficulty of determination, compression of cercaria, etc.); 5, redia; 6, cyst. Fig. 7, *Pelasiger australis:* ventral view. Vitellaria not indicated. Fig. 1, 5, to same scale. g.b., germ balls.

mature cercariae do not remain in this region but migrate anteriorly, which would explain the smaller diameter of the part. The gut varies from half to seven-eighths (commonly three-quarters) the length of the redia.

Cercaria

For measurements, the cercariae were killed by the addition of an equal volume of boiling 10% formalin to the water containing them. This standard method resulted in the cercariae being fixed in different positions, from one of maximum contraction to one of great extension. In the former case the body measured 105 by 100 μ, while the most extended specimen was 267 by 70 μ. Actual range in ten specimens measured 105 to 267 μ long by 50 to 100 μ wide.

Between the body and the tail proper is a relatively short neck, the diameter of which is less than that of the body. Most of the central part of the neck is occupied by an extension of the excretory bladder. The neck region is coloured by the same orange pigment as is present in the cystogenous cells of the body; it varies from 50 to 167 μ long by 17 to 54 μ wide. The rest of the tail, the measurements of which are necessarily only approximate because of the distorted positions in which it is fixed, varies from 434 to 584 μ long by 134 to 200 μ at the widest point. There is a definite apical region in the tail, which narrows suddenly, the tip so formed (which is very obvious in the resting position) ranging, in formalinised specimens, from 83 to 192 μ by 33 to 42 μ.

The tail has a transparent, glassy appearance, although at the margin, which is notched at intervals, the cuticle has a greenish tinge. The central third is occupied by longitudinal muscle fibres which appear to have their origin at the junction of the tail proper with the neck, and taper off towards the tip. Abundant myoblasts are associated with these fibres. A number of fine radial muscle fibres, arising from just beneath the cuticle, insert in the central longitudinal strand. In addition to the longitudinal and radial muscles, there is a somewhat complex system of fibres. Each unit of this system forms a reticulum which has its mid-point at one of the marginal notches and ends on either side about midway between notches, while centrally the muscle strands attach to the longitudinal fibres. About half-way down the tail this system forms a more or less continuous reticulum (fig. 1). Laterally to the main longitudinal muscles on either side is a very fine thread which continues throughout the length of the tail nearly to the tip, and is connected occasionally with the margin as well as with the central muscle strands.

The entire body is beset on both dorsal and ventral surfaces with parallel rows of spines. There are many cystogenous cells which are filled with long rod-like crystalline bodies of a bright orange colour. These cystogenous cells, which are arranged in a series of bands running longitudinally from the post-pharyngeal region to the posterior end of the body, are even more resistant to the passage of light than is ordinarily the case in echinostomes, and details of anatomy are consequently difficult to determine. No gland cells can be seen, even with intra-vitam staining, but what appear to be four very fine ducts are present in the region of the oral sucker. The collar region is more distinct than in other echinostome cercariae we have examined. The spines are arranged in a single row, uninterrupted dorsally; the total number is 19, of which four on each side form a corner group. There is no obvious differentiation in the size of the spines, the length of each being about 13 μ.

The acetabulum is slightly larger than the oral sucker, the diameter in formalinised specimens ranging from 28 μ across the transverse diameter by 21 μ lengthways to 38 by 30 μ for the acetabulum, and from 21 to 30 μ for the round oral sucker. The acetabulum is situated slightly behind the mid-line of the body, the ratio of length of the body in advance of the acetabulum to that part including the latter varying from 5:4 to 3:2.

The alimentary system is not obvious; there is a short prepharynx, and the oesophagus and intestinal crura are very narrow and filled with finely granular material. The crura extend almost to the posterior end of the body.

The excretory system conforms to the plan of the typical echinostome. The pre-acetabular part of the main excretory tubes is very prominent; each contains 12-14 large round or oval excretory bodies with doubly refractive margins. In some cases two or three granules, though maintaining their individuality, are enclosed in a common refractive envelope. In addition, there may be 4-6 smaller single granules. The anterior part of each main tube does not form a distinct triangle, as is usually the case in echinostomes, but each tube forms a small loop as it turns posteriorly. The posterior collecting tube is short and unites with the anterior tube at the side of the excretory bladder. Examination of the cercaria in equal parts of horse serum and water enabled us to see a number of flame cells which were invisible by other means. Even with this help it was not possible to demonstrate the exact connections of all the flame cells, but we are satisfied that they are arranged in groups of three, and we think that there are five or six groups on each side of the body, making a total of 30-36 flame cells. There is an extension of the bladder in the "neck" of the tail, but we could see no excretory tube in the tail itself. The excretory pore probably opens on the dorsal surface at the junction of the body with the tail.

The genital anlage, which stains deeply with haematoxylin and other permanent stains, is represented by two masses of tissue, anterior and posterior respectively to the acetabulum and connected by a narrow string of cells.

Cyst and Metacercaria

The cercaria was found experimentally to encyst in the following Australian native fish, *Retropinna semoni, Philypnodon grandiceps, Nannoperca australis,* young *Tandanus tandanus;* and in the aquarium fish, *Phalloceros caudo-maculatus, Oryzias latipes, Carassius auratus* and *Gambusia affinis.* Of a large number of fish from the River Murray swamps which have been examined, none were found to be naturally infected with cysts of *Cercaria gigantura.* However, these cysts were found in *Nannoperca australis* from the River Finnis (collected in March 1941). In all cases the cysts were limited to the wall of the oesophagus and the pharyngeal region. Negative results followed attempts to infect tadpoles, *Lymnodynastes* spp.; the shrimp, *Paratya australiensis;* a triclad; and the molluscs, *Planorbis isingi, Limnaea lessoni* and *Corbiculina angasi.* A few of the host snails (*Amerianna pyramidata* and *A. pectorosa*) contained a number of cysts in the liver. Those snails which were examined in the summer contained no cysts, and we suggest that the occurrence just mentioned may not be a normal event, but was probably due to the lateness of the season, coupled with the fact that the snails had been isolated daily for some considerable time in small tubes, when the cercariae must encyst in the snail from which they had emerged, or perish.

The small, oval, rather flat cysts are remarkably uniform in size, being about 125 by 75 μ. They have thick walls through which the pale orange colour and the large dark excretory granules of the contained metacercaria show clearly.

In general features, the metacercaria shows no advance on the cercarial stage. Orange pigment is still present in the body, and cannot, therefore, be associated only with the cystogenous cells in the cercaria; it appears to be mainly in the hind end, and relatively scarce anterior to the acetabulum. The metacercaria could not be expressed from the cyst sufficiently intact to enable us to study it in the living state, and hence we have obtained no information regarding the excretory system. The collar spines are more definite than in the cercaria.

Cercaria gigantura resembles *C. Petasigeri-nitidi* Beaver 1939 very closely. If it should prove (as we anticipate) to be the cercaria of *Petasiger australis* it will be evident that here is a type of echinostome life-history in which the structure of the cercaria, apart from the collar spination, is an indication of the genus of the adult, the number of spines being a specific or group character.

Other characters in which the two cercariae are in general agreement are the following: length of life; resting and swimming action; positive phototropism; size; collar spination; relative sizes of oral sucker and acetabulum. They differ, however, in the following features: *C. Petasigeri-nitidi* colourless, *C. gigantura* orange-yellow; prepharynx long in former, short in latter; three pairs of gland cells present in former, not visible in latter; 20 to 25 excretory granules in *C. Petasigeri-nitidi,* 12 to 14 in *C. gigantura;* dorsal collar spines 5 to 6 μ in former, 13 μ in latter. They also differ in the shape of the bladder and in the flame cell formula. In *C. Petasigeri-nitidi* the longitudinal bands of the tail musculature are poorly developed and circulo-diagonal bands well developed, while in *C. gigantura* the longitudinal muscle is well developed, and there is no circulo-diagonal muscle; and there is no dorso-ventral flattening of the tail as in *C. Petasigeri-nitidi.*

The two rediae agree in most features; that of *C. gigantura* is probably smaller and the collar is not divided into four distinct folds. We confirm Beaver's observation that great masses of cercariae consistently were found free in the tissues, from which Beaver concluded that the cercariae apparently require a period of maturing in the snail's tissues, after emerging from the rediae.

For the cysts, Beaver states that, exclusive of host tissues, they vary round 85 by 68 μ; this makes the ratio of length to breadth 5:4, but his figure shows a ratio of 5:3, which corresponds with the ratio given by our measurements for *C. gigantura* (125 by 75 μ). As with *C. gigantura,* the cyst of *C. Petasigeri-nitidi* is slightly flattened.

Beaver mentioned four other described species of large-tailed echinostome cercariae, all of which closely resemble *C. Petasigeri-nitidi* . We have examined the accounts of these cercariae—*C. magnacauda* O'Roke 1917,[1] *C. caudadena* Faust 1921, *C. cita* Miller 1925 (not described till 1929) and *C. oscillatoria* Brown 1931—and find that *C. gigantura* differs from each of them. Since Beaver has listed the features separating them from *C. Petasigeri-nitidi,* in many of which they differ also from *C. gigantura,* we do not propose to distinguish between them specifically. *C. cita* appears to resemble *C. gigantura* most closely, but the description of the former does not include either the number of collar spines or the flame cell formula, and Miller did not find the cyst. In addition to this, *C. cita* is described as having gland cells from the acetabulum to the pharynx, and this in itself we regard as sufficient to separate it from *C. gigantura,* in which it was not possible for us to identify any gland cells.

Szidat (1937) described *C. gigantocerca,* which he regarded as belonging to the Psilostomidae, near *Sphaeridiotrema;* the body of this cercaria, as figured, was very similar to that of an echinostome cercaria, but lacked collar and spines. The tail was huge, and though the figure is lacking in microscopic detail the relative sizes of the tail and body in *C. gigantura* and *C. gigantocerca* Szidat appear to be comparable, while the absolute sizes are very similar. The close resemblance of these two forms is further evidence of the relationship between the Echinostomidae and the Psilostomidae.

In describing *C. oscillatoria,* Brown stated that he found cysts in the tissues of the cercarial host snail, and that they appeared to be the encysted stage of

[1] O'Roke described *C. magnacauda* as a megalurous cercaria, but Miller who examined a slide of *C. magnacauda* (in 1929) lent him by O'Roke, identified it as an echinostome.

C. oscillatoria. Beaver (1939) thought that Brown probably observed another species of echinostome—evidently on the grounds that he (Beaver) did not expect this type of cercaria to encyst in a snail. However, we have indicated that *C. gigantura* will encyst in the snail host. The main point of difference is that Brown's cyst was spherical, while the cysts of *C. gigantura* and *C. Petasigeri-nitidi* are of the same oval, partly flattened, type. However, Brown stated that the compound nature of the calcareous concretions in the main collecting tubules was suggestive of *C. oscillatoria,* and we are inclined to agree that the cyst was the encysted form of *C. oscillatoria.*

The occurrence of these cercariae, which have been definitely identified as echinostome larvae, obviously calls for some modification of the accepted classification scheme for echinostome cercariae. Brumpt (1936), following Luhë's classification (Luhë 1909), which Brumpt says has been adopted by most authors, lists echinostome cercariae under Leptocercariae, with tail narrower than the body. In cercariae of the *magnacauda* group the tail is greater in length, breadth and thickness than the body, and these cercariae must be regarded as an aberrant type which develops into a typical echinostome cyst and adult. Sewell (1922) proposed three sub-groups for the echinostome cercariae, but this separation appears to us to be no longer satisfactory in view of the large number of echinostome cercariae which have been described since then.

In an effort to obtain the adult of *C. gigantura* experimentally, a canary and two pigeons were fed with cysts of the cercaria over a number of days, but on dissection of the birds some fortnight later no trematodes were found.

Summary

1 *Petasiger australis* n. sp. is described (from hosts *Podiceps ruficollis novaehollandiae* and *P. poliocephalus*) from South Australia.

2 A large-tailed echinostome cercaria, *C. gigantura,* is recorded (from *Amerianna pyramidata*) from the same locality, and is considered to be the larval form of *Petasiger australis.*

3 The redia, cercaria, cyst and metacercaria of *C. gigantura* are described, and their intermediate hosts recorded.

4 *C. gigantura* is compared with other known large-tailed echinostome cercariae.

5 The relationship of the Psilostomidae with the Echinostomidae is further shown by comparison of *C. gigantocerca* Szidat and *C. gigantura.*

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