LARVAL TREMATODES FROM AUSTRALIAN FRESHWATER MOLLUSCS
PART VII

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Cercaria (Furcocercaria) trichofurcata n. sp.

Though several hundreds of the bivalve *Corbiculina angasi* (Prime) from the lower Murray River at Tailem Bend had been under observation prior to 7 February 1940, cercarial infection had not been detected. On that date, one specimen of the 243 collected was found to be giving off a large fork-tailed cercaria of a type quite new to us. It was seen subsequently in one of 840 on 26 February 1940, one of 289 on 8 March 1940, and in one of 70 on 1 May 1940. Even to the naked eye it appeared distinct from other furcocercariae observed by us. For a second or two the cercaria swims upwards rapidly, and then comes to rest with the body spherical and suspended by the furcae which form an angle of about 140° with each other, while the tail stem is vertical. In this resting state the spherical form of the body is more obvious than is usual with furcocercariae. From this position it sinks slowly until it is nearly at the bottom of the tube, when it swims upwards again. Examined under a cover-slip, the cercaria may draw up one of the furcae into a position more or less parallel with the main tail stem; both the furcae may become curved upwards and inwards so that the organism has somewhat the appearance of an anchor (fig. 5); or the body may be bent over to lie on the tail stem.

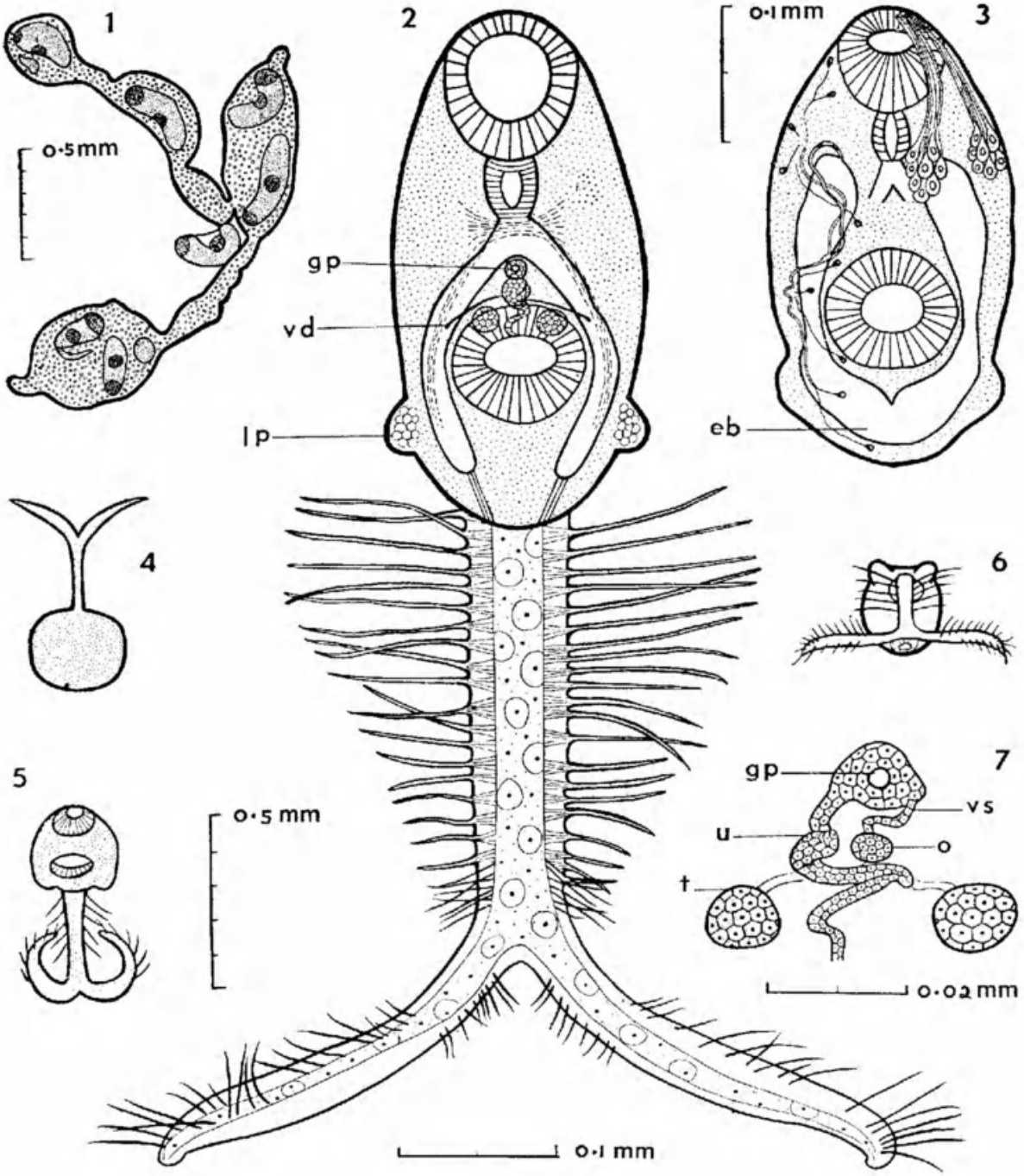
In formalinised material the body was slightly curved, but was easily flattened with the slight pressure of a cover-slip. The measurements of such specimens are: body, 276-384 μ long by 175-192 μ wide (average, 314 by 184 μ); tail stem, 267-301 μ by 63-71 μ (average, 284 by 67 μ); furcae, 234-284 μ by 33-42 μ (average, 250 by 38 μ); sucker ratio, oral:ventral = 6:7.

Of the stains used intravitam, orange G was the best. Neutral red, and the use of nile blue sulphate after neutral red, were also satisfactory. For permanent preparations, alum carmine gave the best results.

The tail stem is long, with furcae of approximately the same length as the main stem. The most noticeable feature of the cercaria is the presence of many long stout hairs or bristles on the tail, arranged on either side of the main stem. These are longest near the body of the animal (where the greatest length was 125 μ), and gradually diminish in size as they approach the junction of the furcae. Towards the distal end of the tail stem, on either side of the midline on the dorsal surface, is a collection of nine or ten finer, shorter hairs. On the furcae themselves, the setae are finer, shorter, and more hair-like, and are arranged differently; there are two rows which, instead of being placed laterally, take a somewhat oblique course on the dorsal and ventral surfaces respectively, the rows terminating near the tip of the corresponding furca. The two rows ending together give the appearance of a bunch of longer setae arising from the tip of the furca—the other setae being necessarily less obvious because of their dorsal and ventral positions. A third row, shorter in length, and composed of still smaller setae, commences on the inner side near the junction of the two furcae; it is not quite lateral, and the hairs are directed slightly backwards. In mounted specimens the edges of the tail stem are nearly always folded over, dorsally and ventrally respectively, so that the setae appear at first sight to arise near the midline. This may

indicate that, in swimming, the tail is twisted slightly. Longitudinal and transverse muscle fibres are present in the tail, and from the base of each "bristle" a small number of fibres radiate out to terminate around the main excretory canal (fig. 2). No caudal bodies were seen.

The body of the cercaria is beset with very small spines, regularly arranged. Near the posterior end of the body are two locomotor processes, situated dorsally



Figs. 1-7

Cercaria trichofurcata. Fig. 1, sporocyst with escaping cercaria; 2, 3, cercaria, anatomy; 4, cercaria, resting position; 5, 6, cercaria at rest, under coverslip; 7, genital system. Figs. 4, 5, 6, to same scale.

a, ventral sucker; act, anterior collecting tubule; b, brain; cg, cutaneous glands; eb, excretory bladder; ep, excretory pore; gp, genital pore; gr, genital rudiment; lp, locomotor process; o, ovary; pct, posterior collecting tubule; sdr, sex duct rudiment; t, testis; u, uterus; vd, vas deferens; vs, vesicula seminalis.

and projecting slightly. They have a definite cellular structure which stains deeply with neutral red, as well as with haematoxylin and other permanent stains. The surface is roughened.

There is a pronounced acetabulum which is slightly larger than the oral sucker, and is beset with tiny papilla-like elevations.

Eye-spots and prepharynx are absent. The pronounced muscular pharynx is succeeded by a very short oesophagus. The intestinal crura extend well back towards the posterior end of the body. Muscle fibres pass from the lower end of each crus to the base of the body, on either side of the origin of the tail.

The gland cells seem to be arranged in two groups on each side, and lie at the hinder level of the pharynx, the inner group reaching the point of bifurcation of the intestinal crura, while the outer group does not extend so far. The inner group is perhaps composed of an anterior dorsal and a posterior ventral group. The cells themselves are small, and number eight, or probably more, to each group. They stain with neutral red used intravitam, but in formalinised specimens do not take up chlorazol black, showing that they have no glycogen. The ducts of the cells pass laterally to the anterior border of the oral sucker.

The excretory bladder is relatively very large, the hinder part occupying the greater portion of the posterior end of the body. It is bifurcated, and the two arms narrow as they pass laterally to the acetabulum, and then broaden out again towards their termination near the posterior level of the pharynx. On each side there are five flame cells in the posterior, and five in the anterior, half of the body. At the junction of the posterior and anterior collecting tubules is a small dilatation. Ciliary patches were seen only where each main collecting tube joined the bladder. The bladder continues into the tail as a wide channel occupying about half the diameter, and terminating close to the tip of each furca. In mounted specimens the portion of the bladder in the tail may appear only as a narrow tube. There are no flame cells in the tail.

Staining with orange G, as well as with nile blue sulphate following neutral red, showed a band of nervous tissue just behind the pharynx and a nerve cord extending from it down each side of the body near the corresponding crus almost to the posterior end.

The reproductive rudiments of *C. trichofurcata* have attained considerable differentiation. Near the anterior border of the acetabulum are two small rounded masses of cells lying at approximately the same level. These apparently are the testes. Anterior to the acetabulum, and median, is a thickened mass of cells, the future cirrus sac, which communicates with the ventral surface by an obvious genital pore. From this region a thick cord of cells, the uterus, twists posteriorly and becomes no longer recognisable just behind the testes. The ovary is represented by one or more small compact masses lying near the coils of the uterus immediately posterior to the cirrus sac. From each testis a faintly discernible cord of cells passes across ventrally to the uterus, and the anlagen of the common duct can be seen, anterior to the ovary, passing to the cirrus sac. On each side a very fine structure, presumably the rudiment of the vitelline duct, has its origin in the region of the intestinal crus, then crosses just in front of the corresponding testis, and becomes unrecognisable in the ovarian region.

The numerous, branching, dark grey sporocysts are scattered throughout the body of the mollusc, occurring in the gills, liver and reproductive gland. They vary a good deal in size, fig. 1 being taken from one of medium size. Because of opacity due to abundance of tiny globules, the contained cercariae can be seen only when pressure is put on the cover-slip. Even then the suckers were the only feature seen clearly.

In the search for the secondary intermediate host of *C. trichofurcata,* negative results were obtained after subjecting the following animals to infection: tadpole, *Lymnodynastes* sp.; leech, *Glossiphonia* sp.; yabby, *Cherax destructor* Clark; freshwater amphipods, *Chiltonia subtenuis* (Sayce); mosquito larvae; chironomid larvae; larvae of the fly, *Eristalis tenax;* water bugs, *Agraptocorixa curynome* (Kirkaldy); gastropods, *Plotiopsis tatei* and *Ameria pyramidata;* lamellibranchs, *Hyridella australis* and *Corbiculina angasi; Tubifex* sp.; as well as the fish, *Gambusia affinis* and *Carassius auratus.* We observed cercariae being eaten by *Gambusia* and *Cherax.* After a number of *Corbiculina* and some *Hyridella* had been left over-night in a dish containing one of the former infected with *C. trichofurcata,* five of the *Corbiculina* and one *Hyridella* appeared to be giving off cercariae. That is to say, when these molluscs were isolated in tubes containing fresh water, several cercariae appeared in each tube. Since these particular molluscs were subsequently found (by dissection) to be uninfected, it appears that they must have been harbouring the cercariae, probably in the mantle cavities. This also affords additional evidence that *Hyridella* and *Corbiculina* do not act as secondary intermediate hosts.

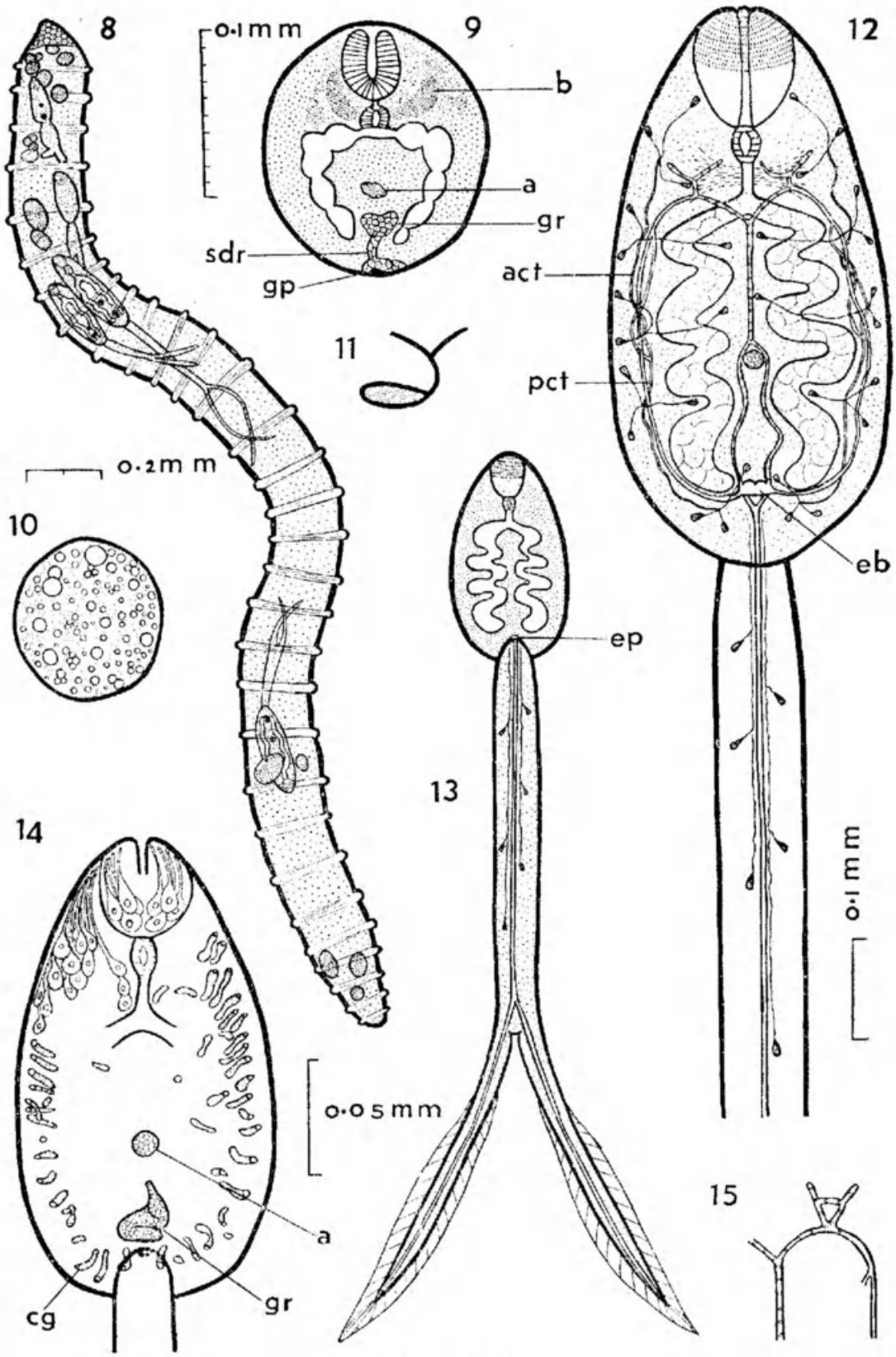
Cercaria trichofurcata does not belong to the Strigeoidea, as at present defined. It is not a Schistosome, because of the well-developed pharynx, and it is not a Strigeid because of the position of the genital pore anteriorly to the acetabulum. Miller (1926, 69) states that it is only the apharyngeal brevifurcata monostome group of furcocercariae in which flame cells are absent in the tail, but *C. trichofurcata,* which also has no flame cells in the tail, obviously does not belong to that group. A number of furcocercariae have been described as possessing "tactile hairs" or "scattered spines," or as having a "spiny tail," but none of them is suggestive of *C. trichofurcata* with its long, stout, densely placed tail spines. The possession of locomotor processes in this cercaria is also an outstanding character.

The general anatomy of *C. trichofurcata* resembles that of *Tandanicola bancrofti* Johnston, 1927, from the swim-bladder of the freshwater catfish *Tandanus tandanus* Mitchell, in the relative sizes of the suckers, the form of the excretory bladder, the absence of a prepharynx, the general form of the alimentary system, the bilateral arrangement of the testes with the ovary median and in advance of the former, the position of the testes in relation to the anterior border of the acetabulum, and in the position of the genital pore anteriorly to the acetabulum. The resemblance is so close that we think it likely that *Tandanicola* is the adult stage of the cercaria.

The fact that all endeavours to trace the secondary intermediate host of the cercaria have been unsuccessful, combined with the relatively advanced development of the reproductive system in the cercaria, may suggest that the cyst stage is omitted in the life cycle, and that infection of the fish occurs by direct penetration of the cercaria, possibly through the gills or even by the alimentary canal.

Cercaria (Furcocercaria) tatei n. sp.

Cercaria tatei, a parasite of *Plotiopsis tatei* (Brazier), at Tailem Bend, Lower Murray River, was first discovered in April 1939, when one of 287 specimens of the gastropod was infected with it. This molluscan species was not examined again until last February, when two of 535 specimens were observed to be infected. Of these, one continued to give off cercariae for a little more than two months. On 1 May 1940, one of 132 specimens collected was parasitised by it. This snail exhibited double infection, *C. plotiopsis* Johnston and Simpson 1939, a Heterophyid, also being present.



Figs. 8-15

Cercaria tatei. Fig. 8, sporocyst; 9, metacercaria, stained and somewhat flattened; 10, cyst, partly under pressure; 11, cercaria in resting position; 12, cercaria, living, compressed; 13, cercaria formalinised with boiling 10% formalin (note difference in size from fig. 12, which is of living cercaria); 14, gland cells of cercaria; 15, anterior portion of excretory system of cercaria showing modification seen in two specimens. Fig. 8, 10, to same scale; fig. 12, 13, 15.

The cercaria swims tail first for a few seconds and then hangs suspended by the furcae with the body bent to form an angle with the tail stem, and the furcae with an angle of less than 90° between them. The resting stage is long (up to 27 seconds, though often only from three to eight seconds), and during this period the cercaria gradually sinks towards the bottom. It swims upwardly, and at times laterally also. There was no evidence of either positive or negative reaction to light. Specimens were observed to remain alive for at least 72 hours. Looss recorded that *C. vivax* Sonsino lived more than two days (Wesenberg-Lund, 1934, 159).

For the measurements, an average of ten formalinised specimens was taken: body length, 200 μ (range, 184-242 μ); breadth, 117 μ (range, 108-125 μ); length of tail stem, 401 μ (range, 384-417 μ); breadth of tail stem, 50 μ (range, 46-54 μ); length of furcae, 301 μ (range, 284-317 μ); breadth of furcae, 27 μ (range, 25-33 μ). A great disparity of size was noticeable between extended living and formalinised specimens (fig. 12, 13). On the body are rather widely separated rows of extremely minute spinules, giving the surface, especially in the region around the base of the tail, a punctate appearance.

The tail does not arise from the posterior border of the body, but at some distance from it on the dorsal surface, in this respect differing from Miller's classification of longifurcate larvae (Miller, 1925, 63). The long tail stem is about twice the length of the body, and the ratio of stem to furca is 4:3. The stem is simple, but the furcae each bear a fin-fold arising below the junction of the two furcae and continuous around the tip to a corresponding level on the outer side. Fine striations traverse the fin-fold obliquely and directed towards the tip. The furcae arise separately from the main stem.

There are numerous small pale green cells in the tail—about twelve across the diameter—some of them being apparently stalked These cells stain deeply with methylene blue (intravitam), and are probably myoblasts. Several of these cells, situated near the central canal, were seen to be swinging like pendulums, each from a narrow transparent stalk. Wesenberg-Lund (1934, 132) states that in *Cercaria* No. 4 of Petersen "the excretory tube has an irregular coating of parenchymatous cells," and that he "often saw this string, the excretory tube with its coating of cells, lifted up towards the anterior part of the tail by means of the oblique longitudinal muscles, and again lowered to the posterior part."

The musculature of the tail is complex. There is a series of comparatively massive oblique fibrils arising from the lateral borders and apparently terminating around the central canal of the tail. The transverse fibres are very fine, and the longitudinal series can be seen only in the central part of the tail, though these fibres are probably present throughout.

The anterior organ measures about 42 μ x 31 μ. The anterior half is beset with about 14 rows of small spines. The mouth opens terminally through the anterior organ into the pharynx immediately below the latter, there being no prepharynx. The narrow oesophagus soon bifurcates into broad intestinal crura which have a somewhat spiral course, forming, typically, four more or less regular bends, and extending almost to the posterior end of the body. The intestine stains vividly with neutral red. Its walls are formed of large epithelial cells, as described by Faust (1922, 257) for *C. leptoderma,* but in *C. latei* the outlines of the cells are distinct, and, in addition, the nuclei are large.

The ventral sucker is apparently represented by a small rounded parenchymatous mass of cells situated medially in the posterior half of the body.

The gland cells are not at all obvious, and can be seen only with careful study. Intravitam stains were used, but were not taken up by them. After treatment with neutral red, however, the cells became visible, although they were not coloured. The nuclei were not seen except in one or two of the cells of the most anterior group, and the shape of the cells could not be determined, since the margins were indefinite. In fact, the only indications of their presence were the finely granular nature of the protoplasm, and the ducts opening anteriorly. On either side of the midline and extending to the posterior border of the anterior organ is a group of four gland cells, the ducts of which pass laterally and terminate near the midline anteriorly. Behind the anterior organ is a mass of cells which appears to have no very regular arrangement. They extend down the sides of the pharynx and are then scattered across to the sides of the body, where they extend posteriorly as far as the level of the end of the oesophagus. They are too many, or too indefinite, to be counted. Intravitam staining also showed, distributed throughout the body, a number of cells in which there was a coarser granulation than in the gland cells. It was considered that they were probably not themselves gland cells.

Around the lateral borders of the body are a number of cutaneous glands, of comparatively uniform diameter throughout. These lie just below the ventral surface, and take a slightly curved course before they terminate on the latter by a narrow opening. There are 50 or 60 of these around the margin of the body, and a few throughout the ventral surface. These cutaneous glands can sometimes be seen in living specimens, but show up most clearly in those stained with chlorazol black, the glands appearing dark grey. Structures similar to these were recorded by Lutz (1933, 366-7) for *Dicranocercaria utriculata*. Wesenberg-Lund (1934, 132; pl. xxix, fig. 2) recorded for *Cercaria* No. 4 of Petersen "a series of 12-15 bright, clear bodies with a dark point along the borders of the body," and stated that he was "quite ignorant of their function."

In describing the encystment of *C. vivax* Sonsino, Azim (1933, 433) mentioned "cystogenic glands, previously described as cutaneous glands by Looss." We have not had access to this paper of Looss', but it seems probable that the structures mentioned by these four authors are similar to those which we have called cutaneous glands in *C. tatei*, and that these are, in reality, cystogenous glands.

The small body of the excretory bladder lies immediately anterior to the origin of the tail, the pore opening dorsally in this position. The comparatively narrow inner arms of the bladder pass upwards in the intercrural region. Just above the ventral sucker they unite into a single tube which passes forwards to a point immediately posterior to the origin of the intestinal caeca, where it bifurcates. Each tube so formed takes a wide swing laterally, and passes back along the outside of the crus to open into the bladder again. Where each tube lies above the corresponding crus, it gives off, anteriorly, an extremely short branch which soon divides into two short widely separated blindly ending arms. The intercrural parts of the excretory system, together with these arms, contain small refracting granules which are absent from the extracrural arms of the bladder. The main collecting tube joins the lateral arm at the level of the first intestinal bend, and passes back to the level of the mid-intestinal length where it bifurcates into an anterior and a posterior collecting tubule. There are 15 flame cells on each side of the body, nine to each anterior, and six to each posterior tubule. These are arranged in groups of three. A branch of the bladder extends into the tail, terminating near the tip of each furca. An island of Cort is present. Two collecting tubes in the tail extend far back into the main stem, and each

receives the ducts of three flame cells. The connection of these tubes with the main system was not definitely determined, but it is thought that they connect with the posterior collecting tubes of the body, and, if this is the case, the flame cell formula would be 2 (9 + 9) rather than 2 (9 + 6 + 3).

Sporocysts occur in the mantle cavity of the mollusc. They are long and narrow, and at regular intervals there are pronounced muscle bands which give rise to projections on the surface, so that the structure has the general appearance of a tapeworm. Between the muscle bands the wall contains a number of finer circular muscular fibres and very minute fat globules. Each sporocyst contains cercariae as well as germ balls which may be oval or round, exhibiting early segmenting and later stages. All of these move freely in the sporocyst as it undergoes muscular contraction and expansion. At one end (? anterior) of the sporocyst is a pointed cap of cells, the nuclei of which are a prominent feature in stained preparations.

The genital rudiment lies just above the origin of the tail and near the posterior border of the body. It is more ventral anteriorly, and then curves posteriorly and dorsally to terminate near the excretory pore.

Negative results were obtained when experimental infections with the cercariae were attempted using the molluscs *Ameria pyramidata* and *pectorosa*, *Planorbis isingi*, *Plotiopsis tatei*, *Corbiculina angasi*, the tadpole, *Limnodynastes* sp., the leech, *Glossiphonia* sp., and the yabby, *Cherax destructor*. However, the cercariae were found to encyst in the muscles and body cavity of the fish *Gambusia affinis*. These cysts conformed to the descriptions given by other workers for related cercariae (Azim, 1933, for *C. vivax* Sonsino; Szidat, 1933, for *C. monostomi viviparae*) in that the enclosed metacercaria was an apparently structureless mass containing numerous small fatty globules with some larger ones (fig. 10). The fish had been subjected to infection for nearly six weeks (from March to May), and the cysts were consequently at different stages. Of these, the smallest were from 250 to 280 μ in diameter; the cyst wall was quite thin, and the cercaria occupied almost the whole of the cyst. The next group ranged from 300 to 330 μ, and in these the cyst wall was thicker, and the metacercaria occupied only about half the cyst. In what was apparently the most mature group the cyst wall was thick and the metacercaria very dark. Similar pigmentation was noted by Azim (1933, 433) who stated that in the metacercaria of *C. vivax* Sonsino black pigment began to be deposited about ten days after encystment, and that this continued until "a deep black figure" was formed inside the cyst. These mature cysts of *C. tatei* ranged from 384 to 418 μ. Some of the metacercariae were released from these cysts, but it was impossible to distinguish any structure in them until after staining, which showed that there was some differentiation. Another *Gambusia* subjected to infection for about 25 days yielded over 50 unpigmented cysts about 267 x 284 μ, the apparently structureless metacercaria occupying the whole or part of the cyst. Careful examination revealed the presence of an anterior organ, pharynx, and intestinal crura, and at least one flame cell was seen. When these metacercariae were stained a large depression on one surface was revealed. At the posterior end of this depression was a rounded structure with thick muscular edges; this is probably the developing tribocytic organ. A ventral sucker and genital rudiments were present but did not exhibit any advance on their state of development as seen in the cercaria. The oesophagus was rather long and narrow, and the crura wide.

A *Gambusia affinis* containing a number of cysts of *C. tatei* was fed to a rat on 23 May 1940. The faeces of the latter were examined several times, with negative results. It was killed on 19 June, but no trematodes were found.

The specific name is given as a tribute to Professor Ralph Tate.

Of the cercariae described by Sewell (1922), Wesenberg-Lund (1934), Szidat (1933), Tubangui (1928), Lutz (1933), Faust (1922, 1926, 1930), and others, as being related to *C. vivax*, *C. leptoderma* Faust (1922) is the only one possessing the same number of flame cells as *C. tatei*, but in other respects the two forms show marked differences. In *C. leptoderma* each group of three flame cells is described as having its own collecting tube, so that there are six pairs of secondary tubules, while in *C. tatei* the groups of three open (as far as we were able to observe) into the anterior or posterior collecting tubules as the case may be. The "secondary tubules" open into the "main collecting tubules" midway along the course of the latter in *C. leptoderma*, whereas in *C. tatei* the junction is more anterior. There is no X-shaped extension of the "main collecting tubules" anteriorly in *C. leptoderma*. The latter is brevifurcate, has gland cells differentiated into two kinds, has differently-shaped intestinal caeca, and is devoid of a ventral sucker, while the presence of a fin-fold is not mentioned. Its sporocysts occur in the liver; of *C. tatei* in the mantle cavity of the host.

C. tatei appears to be closely related to *C. vivax* (Looss, 1896), but unfortunately the number of flame cells in the body is not recorded for the latter. The two forms agree in the presence of a ventral sucker and of three pairs of flame cells in the tail. The cercariae are found in closely related gastropods (*C. vivax* in *Cleopatra bulimoides* Jick and *Melanopsis praemorsa* Linn., and *C. tatei* in *Plotiopsis tatei*), and the metacercariae occur in fish (*Gambusia affinis*).

Azim (1933, 433) has shown that *C. vivax* is the larval form of *Prohemistomum spinulosum* (= *P. vivax*) and other related cercariae have also been shown to belong to the Cyathocotylidae. The metacercaria of *C. tatei* appears to us to be closely related to the genera *Cyathocotyle* and *Cyathocotyloides*. We expect that the adult of *C. tatei* will be found in a fish-eating bird that frequents the River Murray region.

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