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Observations on the Structure and Life-cycle of the Digenetic Fish-trematode *Transversotrema patialense* (Soparkar)

by

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(With two plates and four text figures)

INTRODUCTION

Transversotrema patialense was first observed and described in North India (Soparkar, 1924) in its progenetic, protandrous, cercarial form, from the freshwater snail *Melanoides tuberculata*. It was named *Cercaria patialensis*. It was later recorded from South India (Anantaraman, 1948) in *M. tuberculata*, from the Belgian Congo (Brien, 1954) in *M. anomala*, and from Ceylon (Crusz, 1956) in *M. tuberculata*. Brien (1954) described in detail the redia and cercaria of this fluke.

Witenberg (1944) described for the first time the interesting adult trematode *Transversotrema haasi*, which presumably came from some Red Sea fish and was closely related to *Cercaria patialensis* and to another species, *C. koliensis* Olivier, 1947, from *Melanoides terebra* in the Solomon Islands. This suggested that the peculiar cercariae completed their life-cycles in fish (Crusz, 1956).

Velasquez (1958) described the metacercaria of a new species, *Transversotrema laruei*, from *Lates calcarifer*, a marine fish known to reach brackish waters and freshwaters in the Philippines. She recognized that all the related cercariae species belonged to the genus *Transversotrema*. Crusz & Sathananthan (1960) obtained metacercariae of *T. patialense* for the first time from the freshwater fish *Macropodus cupanus* in Ceylon. Almost at the same time came the interesting discovery (Velasquez, 1961) of the snail host, *Thiara riquettii*, of *T. laruei* and of mature, egg-producing metacercariae of this trematode beneath the scales and in the gill-cavity and muscle of several species of Philippine fish.

Our own work on *T. patialense* was meanwhile going on in field and laboratory. We have found the mature metacercariae in abundance under the scales of naturally infested *Macropodus cupanus* and *Ophicephalus punctatus*. Many of these metacercariae had large eggs in the uterus. The fish *M. cupanus*, *O. punctatus* and *Tilapia mossambica* have also been successfully infected in the laboratory with cercariae emitted from snails, and mature metacercariae, laden with eggs, have been recovered from under the scales. The life-cycle has also been 'closed' by infecting laboratory-bred, cercaria-free snails by exposing them to fish infested with mature metacercariae. Several details of structure of the mature metacercaria have also been more precisely observed and described.

MATERIAL AND METHODS

The freshwater channels of the Central Rice Breeding Station at Batalagoda, Ceylon, were chosen for extensive collections of snail and fish-hosts, since snails of the species *M. tuberculata* from these channels showed 40 per cent infestation with *C. patialensis* (Crusz and Sathananthan, 1960). While the snails were plentiful in all the channels and associated pools and in the paddy fields (rice fields) themselves, the fish were most easily caught in the broader areas of water near sluice No. 3 (Fig. 1, S 3). *Macropodus cupanus* (Cuv. et Val.) was confined to muddy pools which often became isolated during dry seasons and in which coconut leaves were generally rotting. Other species of fish, however, including *Ophicephalus punctatus* Bloch and *Tilapia mossambica* (Peters), were distributed widely. While *O. punctatus* was easily caught in the same pools, *T. mossambica* was collected in the slow-flowing channel adjoining them.

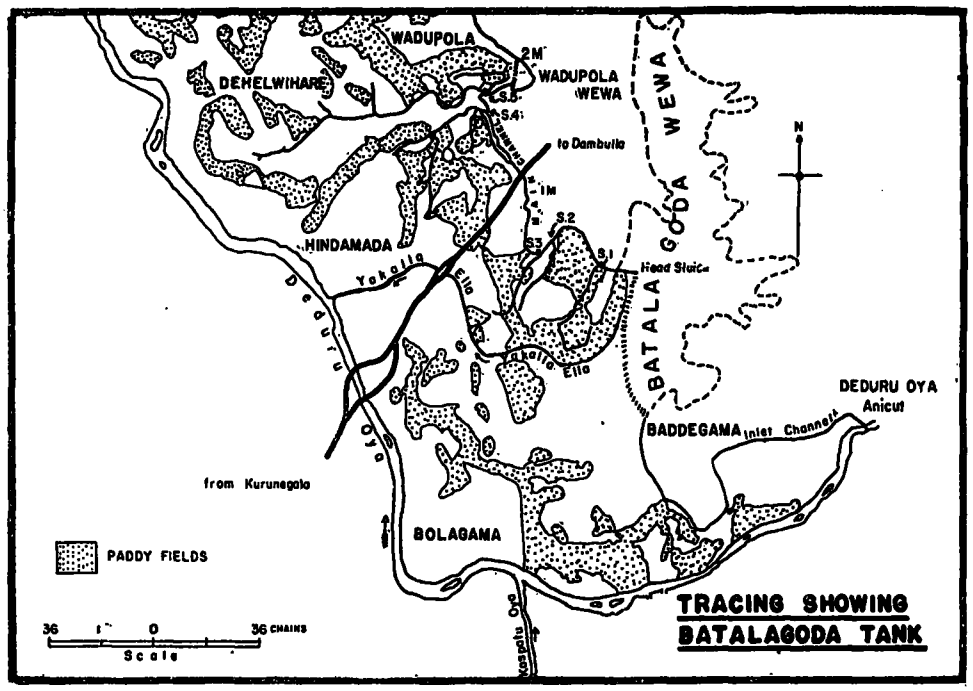

Fig. 1—Tracing showing Deduru Oya (river), Batalagoda tank (wewa or lake) and associated channels and paddy fields.

Snails and fish were taken directly to the laboratories of the Rice Breeding Station and examined without delay for various stages of *T. patialense*. So far only *O. punctatus* and *M. cupanus* have shown natural infestation with metacercariae of this species in this locality. The trematodes were observed and drawn in the living condition under the ordinary microscope or the phase-contrast microscope. Some were fixed in Bouin's fluid under coverglasses for subsequent staining and sectioning. Snails and fish were also taken to the University Zoological laboratories at Peradeniya and Colombo for more detailed study.

Bouin-fixed metacercariae were stained in borax-carmine, acetic acid alum carmine or Delafield's haematoxylin and eosin. Some were embedded in celloidin-paraffin (Peterfi's method) and serially sectioned, 4 to 8 microns thick, in all three planes. The sections were stained in borax carmine or Heidenhain's haematoxylin. Some metacercariae were fixed in 70 per cent alcohol and treated with catechol (Smyth's method). This stained the granules in the vitellaria, vitelline ducts, vitelline reservoir and eggs a very dark brown, showing the presence in them of polyphenol oxidases. Other metacercariae were fixed in Muller's fluid and counterstained in borax carmine, for demonstrating the polyphenols involved in egg-shell formation. The various stages of the trematode, particularly the mature, egg-producing metacercaria, were observed and drawn with the aid of a camera lucida or photographed through the microscope.

For experimental infestations, all of which were done in Peradeniya, snails were placed in Kilner jars containing tap water. When they began emitting cercariae, uninfested fish (*O. punctatus*, *M. cupanus* or *T. mossambica*) were introduced into the jars and kept in them for varying lengths of time. The fish were then isolated and examined after the lapse of certain definite periods. When they were found to be infested, the number of trematodes, site of infestation and stage of development of the parasites were noted. The trematodes were fixed and stained as before for drawing and photography.

The life-cycle was 'closed' by doing the converse experiment. Fish infested in the laboratory with egg-producing metacercariae were placed in a jar containing young, uninfested, laboratory-bred snails. Subsequent examination of these snails revealed heavy infestation with rediae and cercariae of the trematode. These were studied in the usual ways.

Transversotrema patialense (Soparkar, 1924)

DESCRIPTION OF MATURE METACERCARIA

The mature metacercaria (Plate I, Figs. 5, 6 and 7) is leaf-like in form and transversely elongate, with the anterior margin straight or slightly concave and the posterior margin convex and slightly indented medially. It measures 0.368 to 0·560 mm. long and 0·640 to 1·072 mm. wide. The ventral surface is slightly concave and carries short spines with their triangular free ends pointing backwards (Figs. 3 and 4). They are disposed in rows, the spines of one row alternating with those of the next. The dorsal surface is slightly convex and carries smaller spines similarly arranged. Two prominent, round ocelli, 0·016 to 0·024 mm. in diameter and 0·080 to 0·152 mm. apart, are situated dorsally in the anterior third of the body at the level of the posterior margin of the pharynx. The mouth is a median longitudinal slit, ventrally situated in the anterior third of the body. It opens directly into a globular pharynx, 0·056 to 0·096 mm. in diameter. The acetabulum is ventral, disc-shaped, pedunculate, lying just behind the level of the pharynx and covered ventrally with small spines (Fig. 4). It is 0·096 to 0·112 mm. in diameter. In the living specimen the margin of the acetabulum is seen to fold in and out in waves, so that it assumes a scalloped and floral appearance.

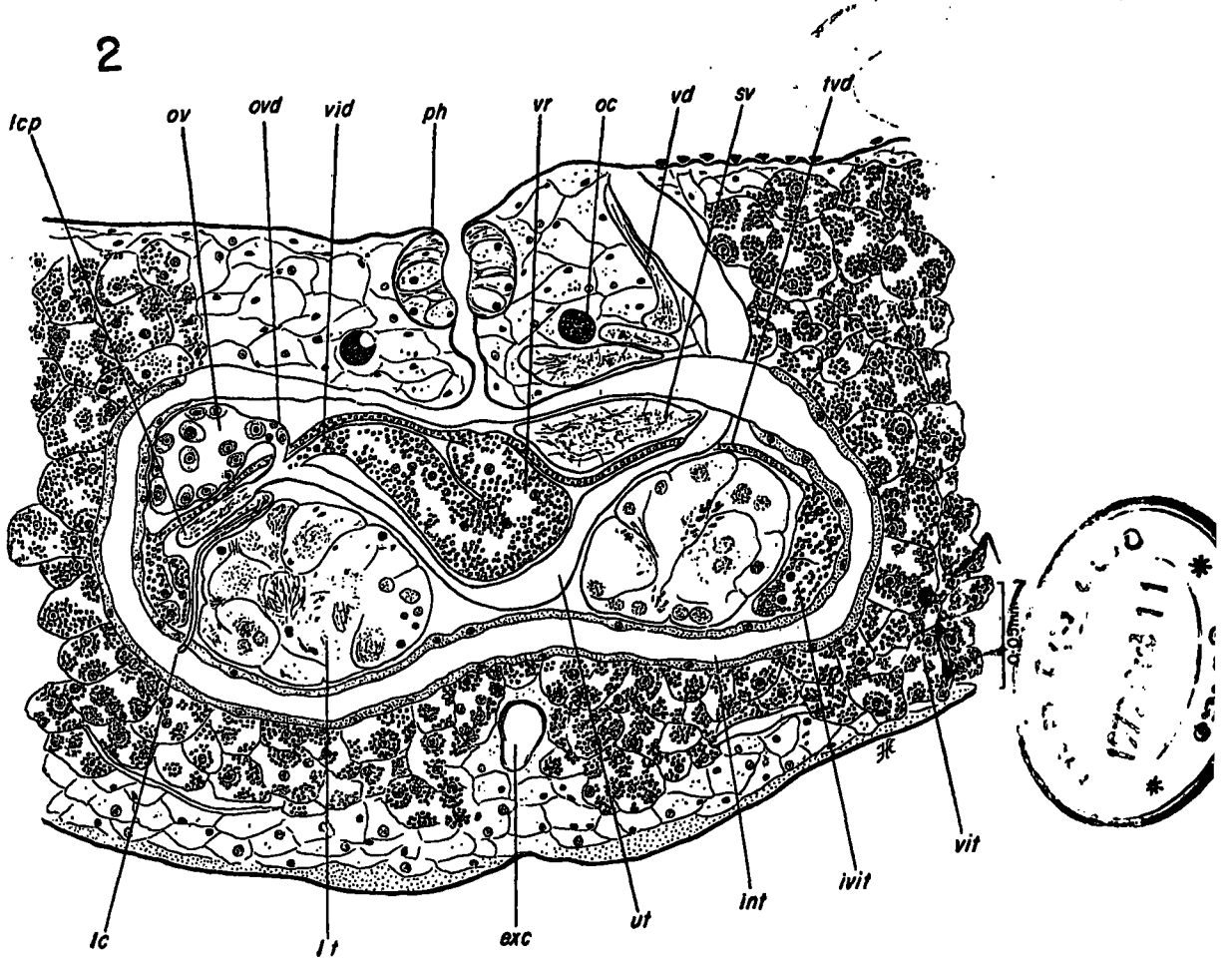

Fig. 2—Frontal (horizontal) section of metacercaria of *T. patialense*. (Partly reconstructed from serial sections, 6 microns thick, stained in Heidenhain's haematoxylin).

exc.—excretory vesicle; int.—intestine; ivit.—right intracaecal vitellaria; lc.—opening of Laurer's canal; lcp.—proximal part of Laurer's canal; lt.—left testis; oc.—ocellus; ov.—ovary; ovd.—oviduct; ph.—pharynx; sv.—seminal vesicle; tvd.—transverse vitelline duct; ut.—uterus; vd.—vas deferens; vid.—common vitelline duct; vit.—vitellaria; vr.—vitelline reservoir.

The pharynx leads into a narrow oesophagus which bifurcates just in front of the middle of the body. The two intestinal caeca run towards the sides of the body and together cover a distance of about one-third the body-width. They then turn and unite postero-medially to form a closed ring, whose posterior section is almost parallel to the posterior margin of the body. The intestinal wall often appears scalloped owing to contraction. The wall of the oesophagus and of the narrow, anterior, transverse portion of the intestinal ring is cuticular and refractile in appearance, while the rest of the intestinal wall looks glandular. This difference is well seen in sections (Figs. 2 and 4) and even in whole mounts of entire specimens stained in haematoxylin and eosin.

The two testes lie, one on each side, within the postero-lateral borders of the intestinal ring. Each testis is well-developed and lobed. A seminal vesicle, formed by the union of the two vasa efferentia from the testes, lies near the right ocellus and is filled with spermatozoa (Fig. 2). Its coils lie immediately in front of and behind the intestinal caecum in

this region. It leads on the same side to the vas deferens which opens at the genital pore at the mid-anterior margin of the body.

The ovary lies anterior to the left testis, within the intestinal ring. It is a berry-like mass of female germ cells. A short oviduct leads from it to an ootype from where a prominent Laurer's canal runs to open at the dorsal surface (Fig. 2). The proximal part of this canal is a broad duct full of spermatozoa, whose active undulating movement in the living specimen makes the duct look like a large flame-cell. This proximal duct runs from the ootype, between ovary and testis, to a point near the left intracaecal vitellaria. It then takes a sharp turn ventrally and runs in the opposite direction under its dorsal portion to a point close against the mid-anterior margin of the left testis. Here it turns sharply again, towards the left, narrowing a great deal as it does so, and continues as a narrow canal along the margin of the testis to open at the dorsal surface opposite the lower left region of the intestinal ring (Figs. 2 and 3).

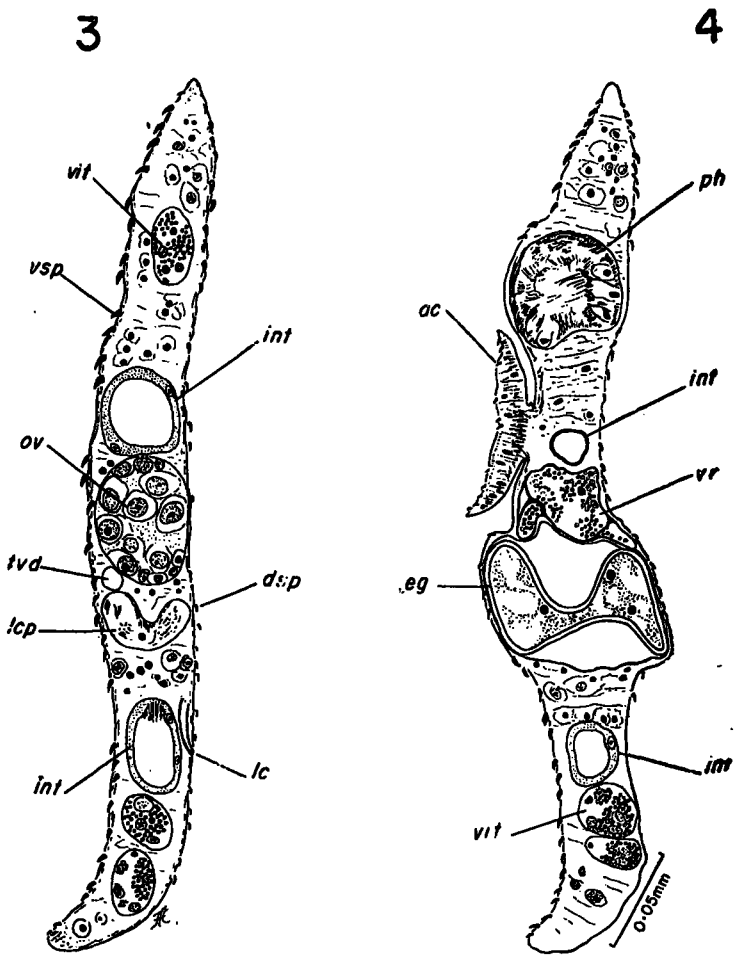

Fig. 3—Longitudinal (vertical) section of metacercaria, through ovary and opening of Laurer's canal. (Serial section, 6 microns thick, stained in Heidenhain's haematoxylin).

Fig. 4—Longitudinal (vertical) section of the same series, but nearly median in position.
ac.—acetabulum; dsp.—dorsal spine; eg.—egg in uterus; int.—intestine; lc.—opening of Laurer's canal, lcp.—proximal part of Laurer's canal; ov.—ovary; ph.—pharynx; tvd.—transverse vitelline duct; vit.—vitellaria; vr.—vitelline reservoir; vsp.—ventral spine.

The uterus, which also leads from the ootype, passes obliquely above the anterior border of the left testis, descends to a position between the two testes and then passes obliquely above the anterior border of the right testis and ventral to the intestine (Fig. 2). It then proceeds anteriorly to the common genital opening. There is a receptaculum seminis uterinum. The vitellaria are follicular and heavily laden with yolk granules. They lie in a wide field outside the intestinal ring, but are wanting in a wide area anteriorly and in a small patch occupied by the excretory bladder posteriorly. They extend to just within the intestinal ring at its lateral lower ends. A vitelline duct starts off on each side from the intracaecal region of the vitellaria. The left vitelline duct runs towards the mid-line between ovary and left testis and ventral to the oviduct. The right duct runs along the anterior margin of the right testis, crosses the uterus ventrally and then proceeds towards the mid-line between seminal vesicle and uterus. Both ducts open just behind the intestinal bifurcation into an enormous vitelline reservoir lying close against the uterus. This reservoir veers to the right and bends sharply upon itself (in the manner of the early rudiment of a vertebrate heart) and proceeds to the left, along the anterior margin of the uterus, narrowing as it goes along (Fig. 2). From its left extremity leads a short, common vitelline duct to the ootype. When an egg forms in the uterus, the posterior portion of the vitelline reservoir gets pushed up against and spread out under and around the anterior portion (Fig. 4).

The eggs are large, ovoid and, according to their position in the uterus, yellow to dark brown in colour. They measure 0·104 to 0·176 mm. by 0·056 to 0·088 mm. One specimen had five eggs, lying one behind the other in the uterus, from its intertesticular region right up to the genital aperture (Plate I, Fig. 6). Some specimens had fewer eggs, while most had only one egg in the uterus (Plate I, Figs. 5 and 7).

EXPERIMENTAL INFESTATIONS

Experiment 1

Snails (*M. tuberculata*) collected at Batalagoda on 26th March 1962 were kept in tap-water in 10 one-pound Kilner jars, 10 snails to each jar. In four of the jars (A, B, C and D) they were seen to emit cercariae of *T. patialense* on the 4th, 6th, 9th and 12th April 1962, respectively, and they continued to do so for well over a month.

Three fish (*O. punctatus*) were collected on 26th March 1962 at Batalagoda. Two of them showed no signs of infestation, externally or internally. The third fish (an immature specimen, 51 mm. long) was placed successively in Kilner jars, A, B, C, and D, for a period of 24 hours in each jar. On 12th April 1962 it was removed to a seven-pound Kilner jar containing no snails. When examined seven days later, 16 large metacercariae of *T. patialense* were recovered from under the scales. One of them had a single, large egg, measuring 0·115 mm. by 0·072 mm.

Since the fish came from a locality where natural infestation with *T. patialense* is common, the result cannot be taken as conclusive proof of experimental infestation.

Experiment 2

Two fish (*O. punctatus*) were caught in a pond on the University campus at Peradeniya on 6th April 1962. This pond is noted for the absence of the snail *M. tuberculata*. One of the fish was thoroughly examined, externally and internally, for any signs of infestation with *T. patialense* and was found to be free of infestation. The other fish (an immature

specimen, 51 mm. long) was placed in Kilner jars B, C, and D on the 7th, 9th and 12th April 1962 respectively, for a period of 24 hours in each and finally removed to a seven-pound Kilner jar containing no snails. Twelve days later it was found to be teeming with metacercariae (over 100) under the scales (Plate II, Fig. 9). The heavy infestation made the fish extra slimy to the touch and its scales came off too easily when the body was scraped with a scalpel. The metacercariae were fixed in Bouin's fluid and stained in borax carmine.

Experiment 3

Two fish (*T. mossambica*), which were immature and 70 mm. long, were caught at the same pond in Peradeniya. One of them was exposed to infection (as in the previous experiments) in the four jars A, B, C and D, but on the 12th, 13th, 19th and 25th April 1962, and was examined on 4th May 1962. A similar heavy infestation was observed. Some of the metacercariae had well-developed eggs. They were fixed in Bouin's fluid and stained in borax carmine. One metacercaria showed a particularly large egg with distinct ovum and vitelline cells inside it (Plate I, Fig. 5 and Plate II, Fig. 10).

The other fish, which was not exposed to infection, was also examined externally and internally, but did not show any signs of infestation.

Experiment 4

One fish (*M. cupanus*) collected from Batalagoda on 26th March 1962 was kept isolated in the laboratory and away from snails until 4th May 1962. It did not show any external signs of infestation with *T. patialense*. It was then exposed to infection in Kilner jar D on 4th May 1962, by leaving it overnight in the jar. Fourteen days after it was isolated, 24 metacercariae were recovered from under the scales. These had eggs in the process of formation, but none fully formed. Smyth's catechol technique, used for the first time on five of the metacercariae, showed up the granules in the vitelline cells, wherever these cells were distributed (as seen in Plate I, Fig. 7, of Experiment 5). Six other specimens were treated with catechol and counterstained with borax carmine. A few were fixed in Bouin's fluid and stained in borax carmine.

Experiment 5

Fresh snails brought from Batalagoda on 10th May 1962 were kept in another set of one-pound Kilner jars, 10 snails to a jar. In three jars (E, F and G) the snails were emitting cercariae of *T. patialense*.

Four fish (*M. cupanus*) were exposed to infection in Kilner jar E on 14th May 1962 by leaving them in the jar overnight for 16 hours and then isolating them. These fish came from a collection of fish of this species which had been breeding for well over two years in the laboratory in Colombo, free from conditions that would have caused infestation with *T. patialense*. In fact, repeated examinations of these fish in Colombo failed to reveal any infestation with this trematode.

The results were as follows:—

(*a*) One fish (immature, 38 mm.) was examined 24 hours after isolation from jar E. Five small, lethargic metacercariae were recovered from under the scales. None of them had any vitellaria.

(*b*) Another fish (immature, 32 mm.) was examined eight days after isolation from jar E. Three metacercariae were obtained from under the scales. All had well-developed vitellaria and one had a fully formed egg half-way up the uterus. This specimen was fixed in 70 per cent alcohol and permanently mounted after treatment with catechol.

(*c*) The third fish (mature, 44 mm.) was examined nine days after isolation. Thirteen metacercariae were recovered from under the scales. Some had fully formed eggs, while others had eggs in the process of formation. All were large, actively moving specimens with well-developed vitellaria. Permanent preparations were made of specimens stained in either catechol (Plate I, Fig. 7) or borax carmine.

(*d*) The fourth and last fish (mature, 51 mm.) was examined 10 days after isolation from jar E. Sixteen egg-carrying metacercariae were recovered from the same site. Four of them were fixed in Muller's fluid.

All the metacercariae obtained from the four infested fish were those of *T. patialense*.

Experiment 6

Two laboratory-bred fish (*M. cupanus*) from Colombo were exposed to infection in Kilner jar E for five hours (from 11 a.m. to 4 p.m.) on 24th May 1962 and were isolated as in previous experiments. Six days later one fish was found to be missing from the jar. It had probably jumped out and decomposed on the floor. The other showed no signs of any infestation, externally or internally.

Experiment 7

One fish (*T.mossambica*, 63 mm.) was caught at the Peradeniya pond on 7th April 1962 and kept successively in jars E, F and G from 24th to 30th May 1962, for a period of 24 hours in each jar. It was then isolated and examined seven days after isolation. Fifty metacercariae were recovered from under the scales, which came off easily with plenty of mucus. Twenty of these metacercariae had one or two well-developed eggs each. One had five dark-brown eggs lying one behind the other in the uterus (Plate I, Fig. 6). The metacercariae were fixed in Bouin's fluid and stained in borax carmine. Of these, some were mounted in balsam, while others were embedded in celloidin-paraffin (Peterfi's method), serially sectioned 4 to 8 microns thick (T.S., L.S. and frontal sections) and stained in Heidenhain's haematoxylin.

The metacercaria with five eggs measured 0·528 mm. by 1·016 mm. Its eggs measured 0·088 to 0·144 mm. by 0·072 to 0·080 mm.

Experiment 8

Snails (*M. tuberculata*) collected at Batalagoda on 26th March 1962 bred freely in the laboratory at Peradeniya. Four newly hatched snails were isolated in a seven-pound Kilner jar and allowed to grow up away from the others. The infested fish *O. punctatus* (from Experiment 1) and the infested *M. cupanus* (from Experiment 4) were introduced into this jar on 19th April and 19th May 1962 respectively. On 24th May 1962 the snails were examined by crushing their shells and making smears of the internal contents. Three snails showed massive infestation with rediae and cercariae of *T. patialense* (Plate II, Fig. 8). There were also cercariae of the Pleurolophocerca group, very much like Cercaria Indica VII. Permanent preparations of these smears were made, after fixation in Bouin's fluid and staining in borax carmine.

Results and conclusions

1. Fish of the species *Ophicephalus punctatus*, *Macropodus cupanus* and *Tilapia mossambica* were susceptible to laboratory infection with the trematode *Transversotrema patialense* passed by infested snails of the species *Melanoides tuberculata*.

2. These fish were found to harbour metacercariae under their scales in anything from 1 to 22 days after exposure (lasting at least 16 hours) to infested snails emitting cercariae. Small metacercariae without vitellaria were recovered from fish a day after such exposure, while egg-laden metacercariae were obtained from fish at least 8 days after exposure.

3. Both the natural and the experimental infestations of fish so far studied showed metacercariae only under the scales and nowhere else on or in the body.

4. Uninfested snails exposed to fish infested with metacercariae carrying ripe eggs, themselves became infested with rediae and cercariae of this trematode, well within 35 days after exposure, thus experimentally 'closing' the life-cycle, although the miracidium was not observed.

SUMMARY

Progenetic, egg-laden metacercariae of the trematode *Transversotrema patialense* (Soparkar, 1924) (Digenea: Transversotrematidae) have been recovered from under the scales of the freshwater fish *Ophicephalus punctatus* Bloch and *Macropodus cupanus* (Cuv. et Val.) caught in Batalagoda, Ceylon. The fish *O. punctatus*, *M. cupanus* and *Tilapia mossambica* (Peters) have been infected in the laboratory with the cercariae of this trematode, emitted by snails of the species *Melanoides tuberculata* (Müller). Many of the metacercariae that developed under the scales of these fish had ripe eggs. Uninfested, laboratory-bred snails of the same species became infested with rediae and cercariae of *T. patialense*, after exposure to fish carrying the metacercariae. At no stage were the metacercariae found to be encysted. The mature metacercaria has been more precisely observed and described.

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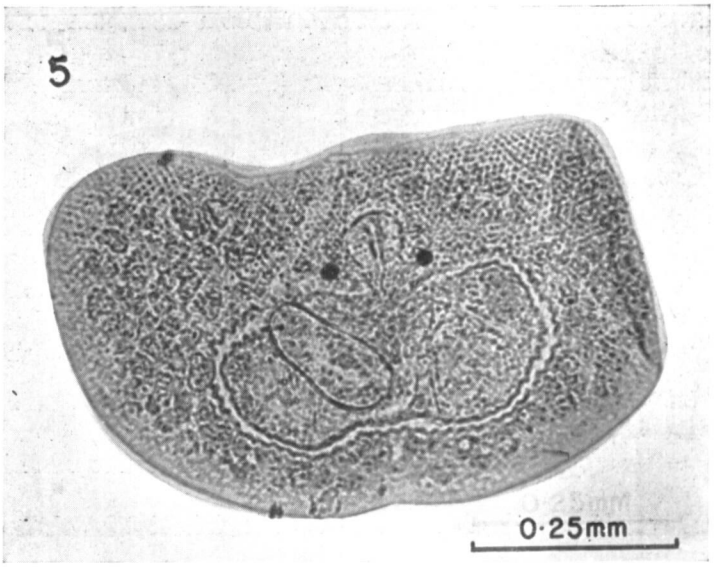
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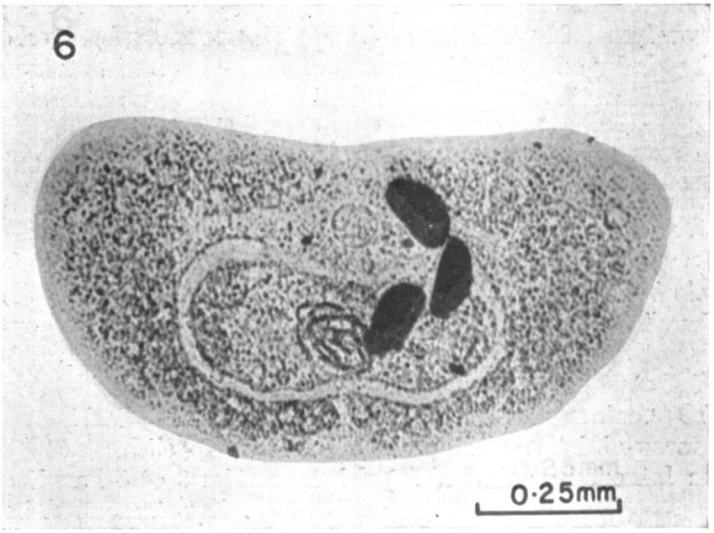
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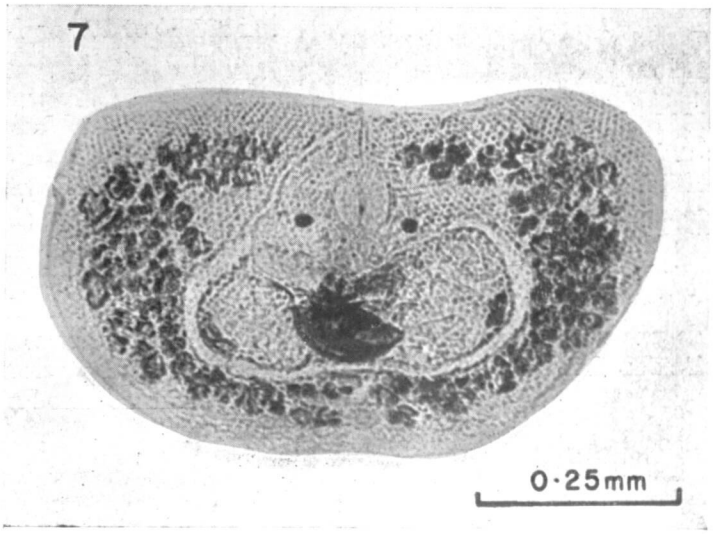
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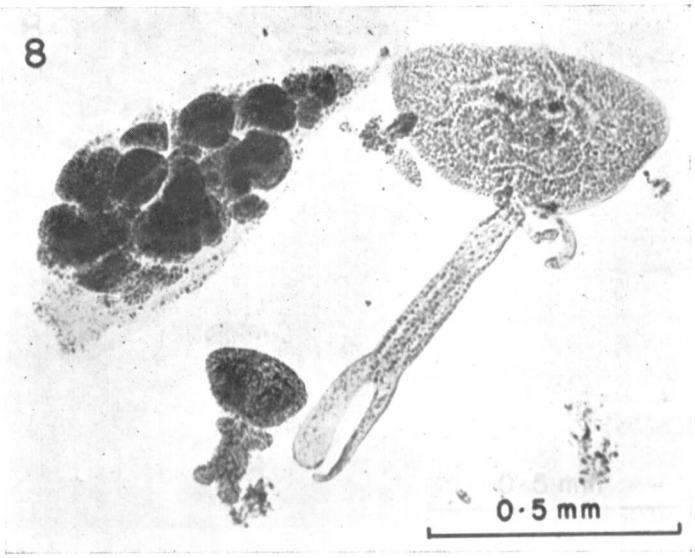
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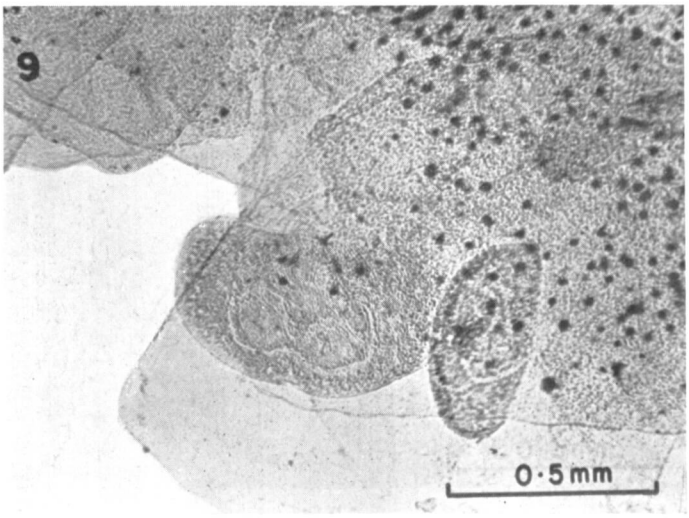
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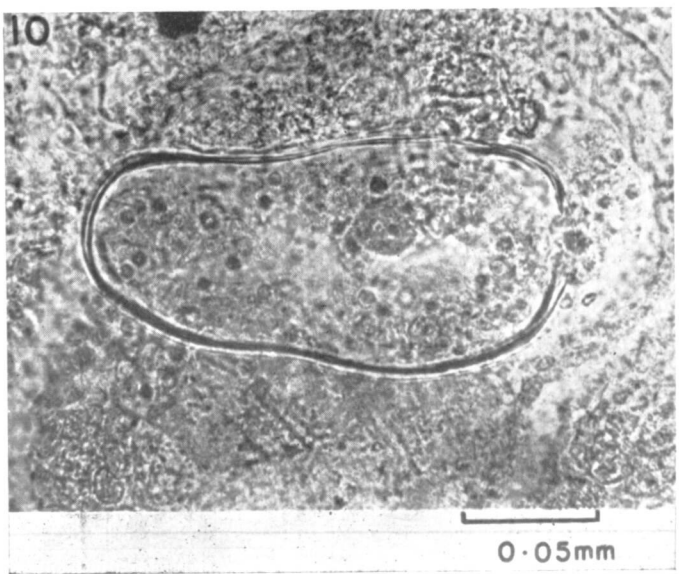
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EXPLANATION OF PLATES

(Photomicrographs by George Webster)

PLATE I

Fig. 5—Metacercaria of *T. patialense* with one large egg, from experimentally infested fish *T. mossambica*. Ventral view. (Bouin's fluid, borax carmine).

Fig. 6—Metacercaria with five eggs, from experimentally infested *T. mossambica*. Dorsal view. (Bouin's fluid, borax carmine).

Fig. 7—Metacercaria with one egg, from experimentally infested *M. cupanus*. Ventral view. (70 per cent alcohol, Smyth's catechol stain for polyphenol oxidases of vitelline cells).

PLATE II

Fig. 8—Redia and cercariae of *T. patialense* from experimentally infested snail *M. tuberculata*. (Bouin's fluid, borax carmine).

Fig. 9—Metacercaria of *T. patialense* beneath scales of experimentally infested fish *O. punctatus*. (Bouin's fluid, borax carmine).

Fig. 10—Egg of metacercaria in Fig. 5, under high power, showing distinct ovum and vitelline cells.

(MS. received 1. 11. 63).