THE LIFE CYCLE OF *ORTHOCOELIUM SCOLIOCOELIUM* (FISCHOEDER, 1904) YAMAGUTI, 1971 (PARAMPHISTOMIDAE: ORTHOCOELIINAE) IN THE PHILIPPINES*

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ABSTRACT

The life cycle of *Orthocoelium scoliocoelium* (Fischoeder, 1904) Yamaguti, 1971 from adult to metacercaria is presented. Egg hatched to miracidium in 14-19 days at 26-31°C. The behaviour and morphology of the miracidium and the process of penetration into the snail host is discussed. *Gyraulus convexiusculus* was experimentally infected with miracidia of the species and successful development of the larval stages – sporocyst, redia and cercaria – occurred. No formation of daughter redia was observed. Sporocyst was observed to occur up to the 12th day post infection. Redia was observed as early as the 10th day and persisted even after mature cercariae were released. Mature cercariae were emitted by infected snail as early as the 32nd day post infection. Intramolluscan development was completed in 32-36 days. Larval stages were found concentrated in the region of the ovotestis, hepatopancreas and digestive glands of the snail host. Morphology and behaviour of these stages are described. Encystment of mature cercariae to metacercariae occurred within 15-30 minutes after emergence from infected snails and the process of encystment is described.

Introduction

A large number of paramphistomid species including *Orthocoelium scoliocoelium* have been reported to occur in ruminants in the Philippines (Eduardo, 1975; 1976; 1981, 1982b; 1983; 1984; 1985a & b; Eduardo and Manuel, 1975; 1976). However, the life cycle and intermediate hosts of these species in this country remain unknown.

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In the course of examining fresh water snails for larval trematodes, the snail *Gyraulus convexiusculus* collected in the province of Ilocos Norte was found naturally infected with amphistome cercariae. These cercariae encysted as metacercariae and when fed to a young goat yielded two species namely, *Orthocoelium scoliocoelium* and *Fischoederius elongatus.*

This study was conducted to follow-up in detail the development of both the free living and intramolluscan stages of *Orthocoelium scoliocoelium* using laboratory-reared *Gyraulus convexiusculus* as snail host.

Materials and Methods

Collection of eggs for hatching to miracidia

Adult *Orthocoelium scoliocoelium* were obtained from slaughtered cattle and water buffaloes and were kept alive either in Tyrode's or physiological salt solutions. Eggs were collected by straining live adults with the solution and were washed and concentrated following the sedimentation-concentration-technique using cone-shaped glass containers.

Concentrated eggs were placed in petri dishes with the hatching medium (aerated water) kept at room temperature under a flourescent lamp for a lighting period from 9:00 a.m. to 5:00 p.m. The medium was changed daily to prevent growth of fungi which may affect the development of the eggs.

Miracidia were concentrated in a container with aerated water ready for observation and infection of snails. Some miracidia were placed in Syracuse dishes with aerated water and observed for swimming behaviour and other movements. Longevity was also noted in the absence of a snail host. Representative specimens were fixed in hot water and processed for silver nitrate impregnation following the technique of Lynch as detailed and illustrated by Cable (1977). This will show the epidermal plates and reveal the surface pores and papillae.

Infection and examination of the snail host

Young *Gyraulus convexiusculus* reared and bred in the laboratory were exposed to the miracidia by two methods. The first involved exposing one snail to 10-15 miracidia. This was accomplished by placing a snail in a small vial or one snail per well of a microtiter plate with aerated water. The second involved exposing a group of snails (15-20) to a large number of miracidia (around 500-1,500) in petri dishes. In both cases, exposure time was overnight. Penetration of snails by miracidia was observed under the microscope.

Infected snails were examined live for the presence of developing stages. Representative specimens were dissected at the following period intervals to free any developing stage for detailed examination: 24 hours, 3rd day, 6th day, 8th day, 9th day, 10th day, 12th day post-infection and every other day until cercariae were released.

The various stages dissected out from infected snails were examined alive, some under slight coverslip pressure. Morphological characteristics were noted and measurements were made.

Freely emerged cercariae from infected snails were placed on glass slides with few drops of aerated water and observed for the process of encystment.

Collection of metacercariae

Snails in an advanced stage of infection were transferred to petri dishes lined with cellophane. The use of cellophane facilitates recovery of metacercariae which when attached to the cellophane could be harvested with ease. Petri dishes with infected snails were kept under flourescent light to stimulate release of cercariae.

Representative infective snails were processed for histopathological examination.

Results and Observations

Development of the free living stages

Description: The egg is oval in shape with the ends almost of the same width. It measures 142.1-151.9 by 88.2-137.1 microns. The anterior end is provided with an operculum while the posterior end has a slight thickening which sometimes could be mistaken for a spine. The embryos of freshly laid fertile eggs are usually in the four cell stage of division. The embryo measures 26.2-30.3 by 25-29 microns and is surrounded by numerous small yolk cells which can be distinguished from the former by their less dense appearance and presence of granules.

Development: Freshly voided eggs of *Orthocoelium scoliocoelium* are already in the early stage of segmentation. Most of the eggs are in the four-cell stage of development. On the 3rd day, the embryo appeared as one large round cell with smooth contour and measured 34-38.2 by 36.1-39.8 microns. From the 3rd to the 7th day, very little change was observed except for the irregular contour of the embryo as well as the increase of its size. The ovum on the 7th day measured 36.3-40.8 by 40.8-43.3 microns.

On the 9th day, the ovum started losing its spherical shape, becoming elongate with squarish corners. The yolk cells were observed to have decreased in number but had coalesced to form larger cells. The embryo rapidly increased in length with little increase in width on the 11th day. The anterior part also appeared broader at this period. The embryo observed on the 12th day had now the appearance of a miracidium because of its shape and the presence of several structures. It measured 128-132.1 microns in length and 49.2-53.6 microns in width. The anterior end of the embryo showed the formation of protrusion which indicated the start of the development of the apical papillae. The primitive gut could be vaguely outlined and the thickening of the four corners represented the developing epidermal plates. Yolk cells had further decreased in number.

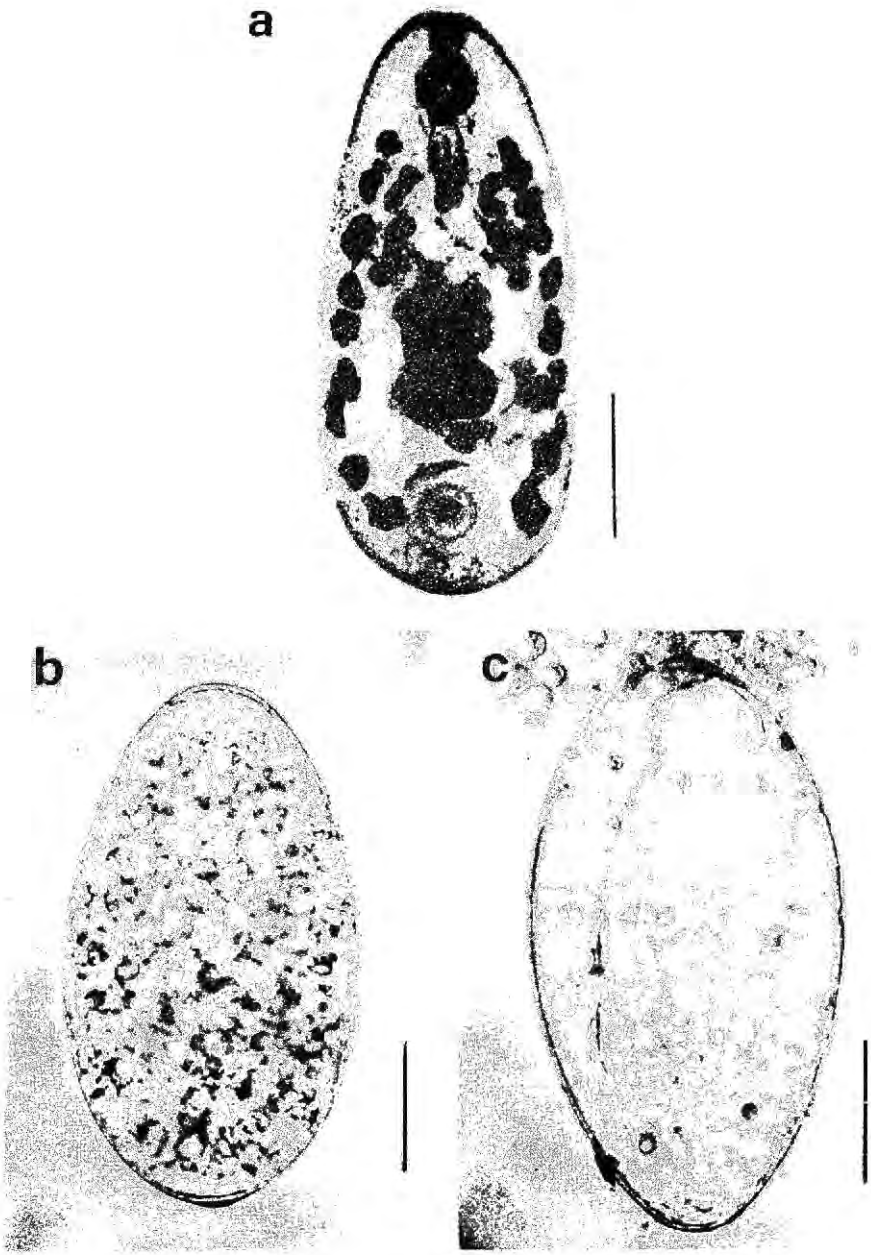

Fig. 1. a. Adult whole worm, ventral view (scale bar = 1 mm.); b. Freshly laid egg (scale bar = 35 microns); c. Egg at 12th day, note fully developed miracidium (scale bar = 35 microns).

On the 14th day, the embryo could now be called miracidium and it measured 130-136.3 microns in length and 45.8-55.7 microns in width. In longer miracidia, the body is slightly bent to be accommodated inside the egg shell. The primitive gut can be distinctly outlined during this period and the epidermal plates/ thickenings become more prominent. The miracidium is now covered with cilia and movement in the form of contractions was noted.

On the 16th day, almost all the eggs now contained well developed miracidia. These were slightly bent and displaced to one side by the remaining yolk cells which had coalesced to form one large cell. Movement was also observed to be more frequent at this period especially when exposed to light. The gut now showed a flask-shaped appearance and became more dense than the rest of the organs. Eggs hatched, producing free swimming miracidia on the 17th day.

The Miracidium

Hatching process: The fully formed miracidium inside the egg was observed to elongate and contract vigorously and the cilia beating rapidly. This activity usually lasted only for several minutes and the miracidium becomes quiescent for a short period. After a period of rest, the process was repeated. Eventually, the envelope containing the yolk material was ruptured because of the more vigorous movement of the cilia. The escape of the miracidium from the egg was usually completed within a short period of several seconds to four minutes. Hatching was observed to be stimulated by light as free swimming miracidia were seen 15-30 minutes after the flourescent lamp was turned on. It took 14-17 days for eggs to hatch to miracidia in warm summer months (28-31°C) and 17-19 days on cold months (26-28°C).

Swimming behaviour: Miracidium was observed to swim actively in a straight or linear direction, the body rotating in a clockwise direction. When it came in contact with an object which is not the snail host, it assumed a round form and flex the anterior end of the body to one side and swam around the obstruction. The longevity of the free swimming life in the absence of an intermediate host and at room temperature was about eight hours. Miracidium at the end of this period move in slow circling motion and finally sink to the bottom of the container.

Description of the miracidium: Unstained miracidium of *Orthocoelium scoliocoelium* appeared as pale brown, tear-shaped moving body covered with cilia. Silver nitrate-impregnated specimen took a golden brown hue. The epidermal plates are arranged in four tiers: from anterior to posterior, the first tier with six plates, the second with eight plates, the third with four plates and the fourth with two plates, giving a total of twenty epidermal plates.

The first tier of plates are triangular in shape with apices meeting in the center while the bases are confluent and together form a circle. This layer as a whole appears like a dome on the anterior portion of the miracidium. Dark staining dots (believed to be papillae) were observed on the area where the apices met. Pigmented dots were found on the bases of the triangular plates, a pair on the middle and one

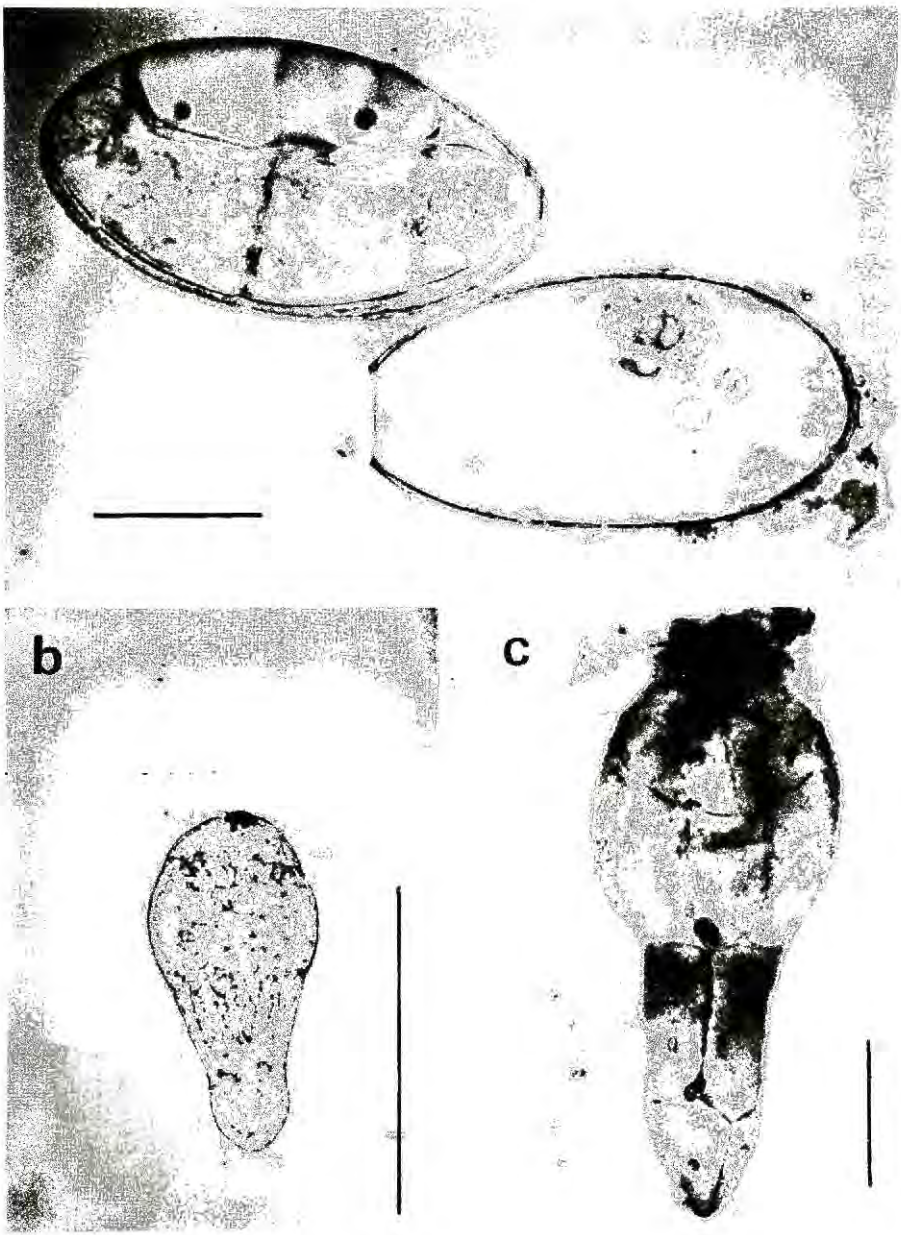

Fig. 2. a. Eggs, one with fully developed miracidium and the other empty from which the miracidium escaped (scale bar = 50 microns); b. Miracidium showing cilia covering body surface (scale bar = 60 microns); c. Miracidium impregnated with silver nitrate showing tier of plates (scale bar = 32 microns).

each on the lateral plates. The second tier of plates are rectangular in shape with a larger space interval between each plate. The lateral are larger than the middle plates. A single pigmented dot was also observed near the posterior limits of the right lateral pairs of plates. The third tier are also rectangular in shape. However, the corners appear to be more rounded. No pigmented dots were observed on or near these plates. The plates in the fourth tier are triangular but the apices are rounded.

The body of the miracidium is covered with cilia except at the apical or anterior region.

Penetration into the snail: In the presence of a suitable intermediate host, the miracidia were observed to swim around the snail instead of moving in a linear direction. Some tried to attack the exposed tissue of the snail but majority entered the pulmonic cavity through the pneumostome which is patent only in young snails. Once inside the cavity, they swam rapidly and attached themselves to the mantle area, though some tried to swim out. Those attached to the mantle area contracted and elongated and rotated in a boring fashion in order to penetrate deeper into the tissues.

If the snail is not a suitable intermediate host, the miracidia were observed to swim around the snail for some time then swim away and finally sink to the bottom as dead miracidia. This was observed when *Fischoederius cobboldi* miracidia were exposed to *Gyraulus convexiusculus*, a snail which is not a suitable host for this species.

Development of the intramolluscan stages

The Sporocyst

Infected snails viewed live under the microscope 24 hours after infection showed sporocyst attached to the mantle and these were visible through the transparent shell. On dissection, some of the sporocyst appeared round while others were elongate. The elongate forms were miracidia which had just shed off their epidermal plates and cilia as indicated by the well distinct primitive gut. The round ones were later or more advanced forms in which the primitive gut had decreased in size. Both forms disintegrated half an hour after dissection. Sporocysts dissected out on the third day post infection showed no remarkable morphological changes, except that they were larger and they were observed to show movement of contraction at this period. Sporocysts recovered on the 5th day measured 0.18-0.29 by 0.22-0.26 mm and they now contained balls which were spherical in shape and measured 0.080-0.086 by 0.071-0.078 mm. The number of balls ranged from 5 to 7. Sporocysts on the 7th day increased in size measuring 0.21-0.39 by 0.25-0.30 mm. The redial balls had also increased in length, appearing more oval or elongate. Some of the redial balls showed the presence of pharynx. Dissection on the 9th day revealed not many changes, except in the increased size of the sporocysts and their redial contents. These forms were considered fully mature. They mea-

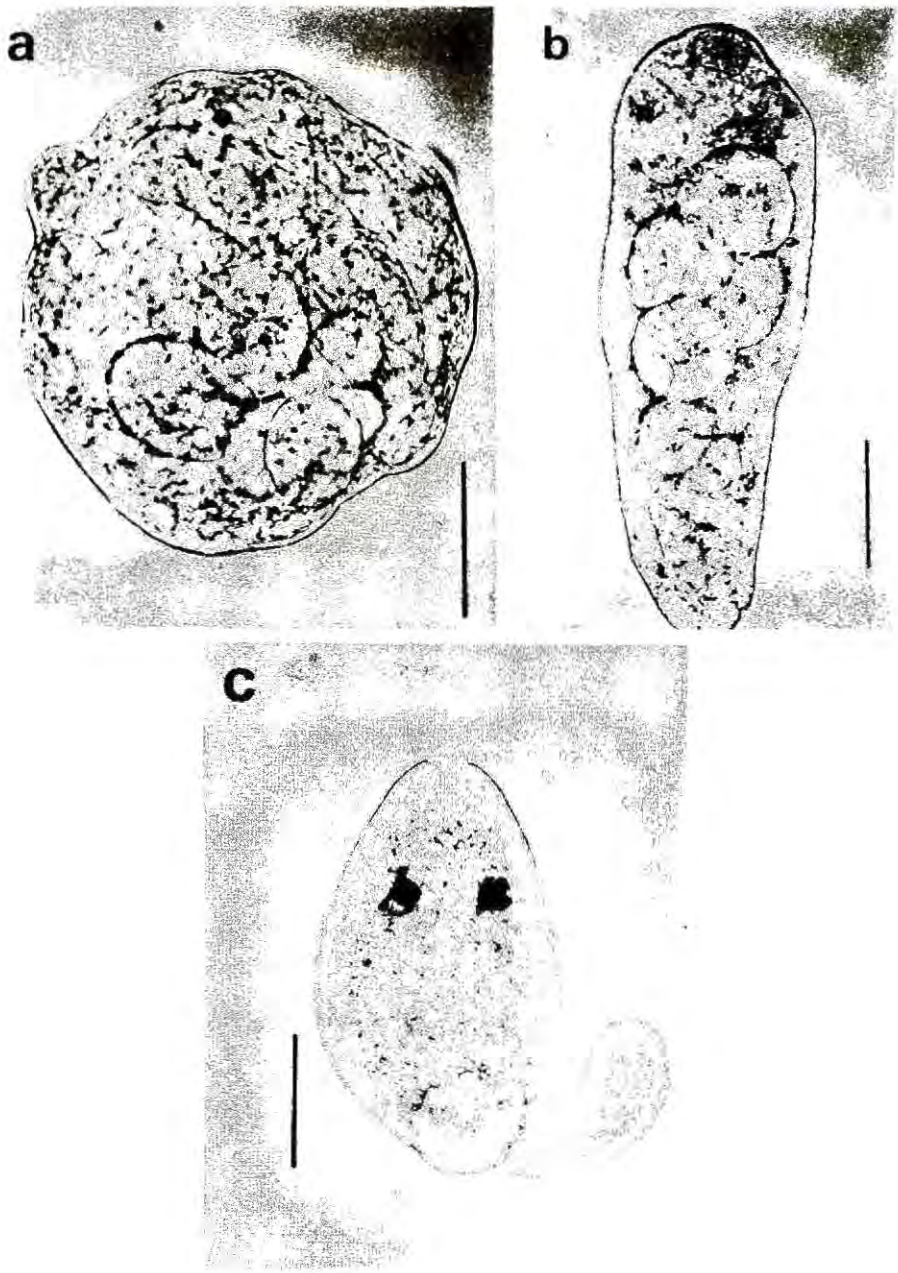

Fig. 3. a. Sporocyst, 9th day (scale bar = 92 microns); b. Redia, 16th day (scale bar = 84 microns); c. Immature cercaria, 27th day (scale bar = 100 microns).

sured 0.29-0.35 by 0.30-0.35 mm and they contained about 8 to 10 rediae. Dissected snails on the 10th showed free rediae together with mature sporocysts. It took therefore about 5 to 9 days for the development to mature sporocyst stage.

The Redia

Snails dissected from the 10th to the 12th day post infection showed free immature rediae with well developed pharynx. The rediae were filled with primordial germ cells with no evidence of cercarial balls. The rhabdocoele gut was only evident on the 12th day. Redia at this period measured 0.29-0.38 mm in length and 0.11-0.18 mm in greatest width. On the 14th day, rediae were found to contain small cercarial balls numbering 6 to 9. The largest ball lay closest to the pharynx while the smallest was farthest from it or near the posterior end. The rhabdocoele gut was visible at this stage. Redia at this period measured 0.39-0.45 mm in length and 0.10-0.18 mm in greatest width. Rediae on the 16th day now contained cercarial balls which were bigger and more numerous, each redia containing about 10 to 13 balls. Rediae at this period are considered mature as they contain cercariae about to be released. Snails dissected on the 18th days showed free immature cercariae with mature rediae. Rediae were observed to persist even after mature cercariae had been liberated from the snail. These rediae continued to increase in size measuring 0.48-0.56 mm in length and 0.16-0.20 mm in greatest width. It took about 5 to 7 days for the development to mature redia.

The Cercariae

The cercaria leaves the redia while still in a very immature condition. The intestine, oral sucker, acetabulum, tail rudiments and the excretory system, already laid down while cercaria is in redia, continue their development after emergence from redia.

Free immature cercariae still devoid of eyespots were observed on the 18th day post infection. The body measured 0.12-0.16 by 0.11-0.15 mm while the tail measured 0.06-0.09 mm in length. Eyespots were observed on cercariae examined on the 21st day. These were located on the anterior dorsal surface of the body. A small brain was also observed between the eyespots. Acetabulum and pharynx appeared well developed at this period. Changes observed from the 23rd to the 29th day included increase in size of the body and tail, spreading of eyespots into finger-like projections and increase in pigmentation of the body. Mature cercariae ready to be liberated were observed on the 31st day. They still had dendritic eyespots but the tail appeared longer than the body. Mature cercariae were observed emerging from the snail host on the 32nd day. Liberation was observed half an hour after exposure of snails to light. Development to mature cercaria took about 14 to 20 days.

Morphology of mature cercaria: The body of mature cercaria appeared oval to round, depending on the state of contraction. The tail was quite long. The body measured 0.39-0.44 by 0.31-0.35 mm and the tail measured 0.69-0.74 mm in

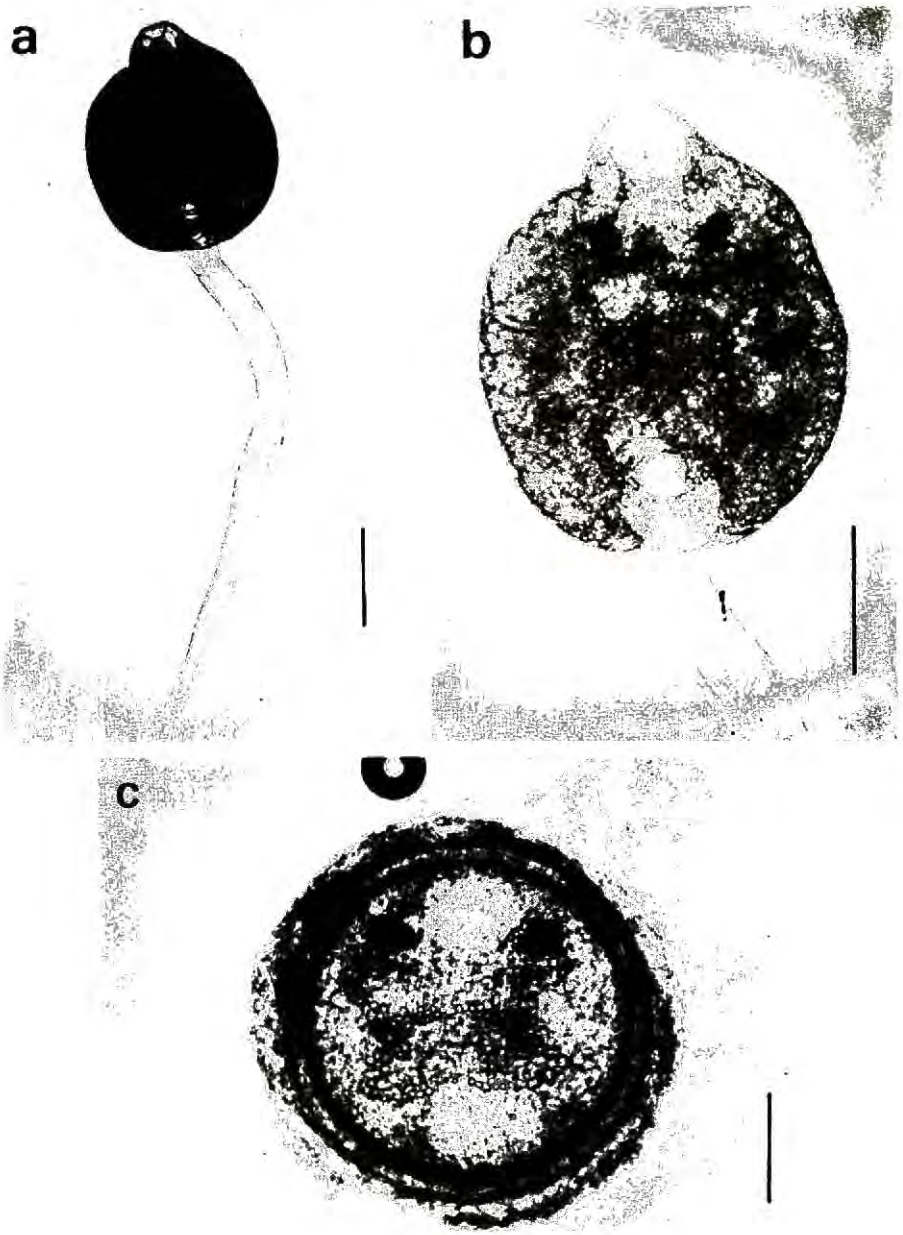

Fig. 4. a. Mature cercaria freely emerged from infected snail, 32nd day post infection (scale bar = 168 microns); b. Body of freely emerged cercaria (scale bar = 112 microns); c. Metacercaria (scale bar = 63 microns).

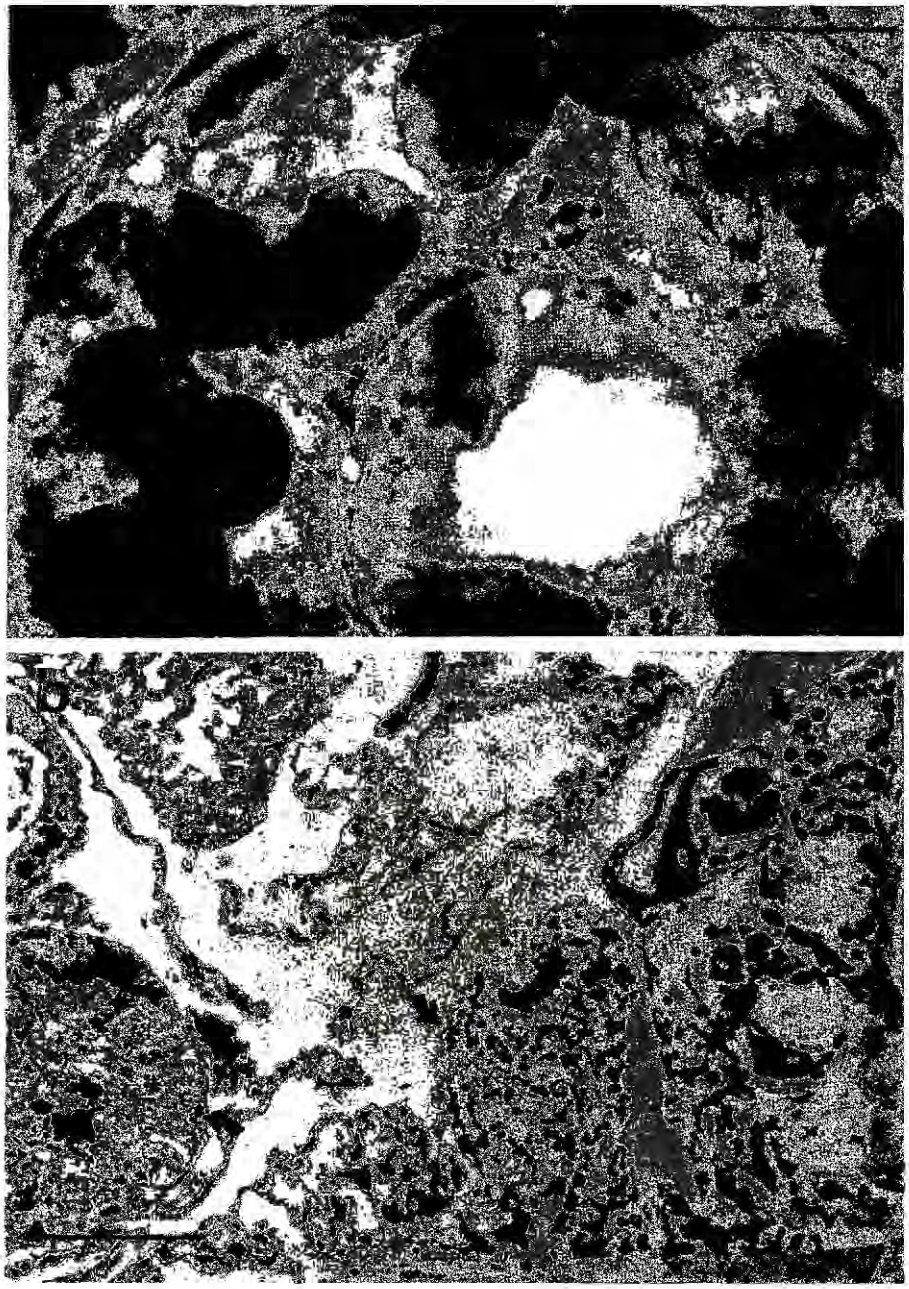

Fig. 5. a. Part of an infected snail showing cercariae visible through the transparent shell (scale bar = 270 microns); b. Section of an infected snail showing cercaria and other stages (arrows) (scale bar = 10 microns).

length. The body was pigmented all over except at the regions of the acetabulum and the oral end. The pigmentation made it very difficult to observe other internal features, including the flame cells. Cytogenous rods were distributed unevenly in the body. These were seen best when cercaria ruptured, thus releasing the rods. In mature cercariae, finger-like projections of the eyespots were lost, instead they become solid and compact. Eyespots are located at the level of the bifurcation of the caeca. The mouth is surrounded by a sucker which measured 0.061-0.065 by 0.053-0.056 mm. It leads to a short tubular oesophagus which bifurcates into two intestinal caeca which end blindly behind the middle of the body. The acetabulum is subterminal and located at the ventral side on the posterior end of the body. It measured 0.058-0.063 by 0.053-0.056 mm. The excretory bladder lies anterior to the acetabulum and has two main excretory tubules. These tubules are filled with excretory granules directed posteriorly towards the acetabulum. At the level of the intestinal caeca, a transverse vessel connects both tubules. The two tubules join to form one caudal excretory tube which runs along the middle of the tail and ends in an enlarged pouch.

Process of encystment: The process of encystment was very rapid. Freely emerged cercariae swim vigorously searching for a suitable encystment surface like vegetation, wall of petri dish and cellophane. The cercaria attached itself to the surface by means of the ventral sucker or acetabulum. It started to contract and elongate at intervals and appeared to undergo rapid squirming movements. The tail vibrated from side to side somewhat more slowly than in swimming. Cytogenous materials produced from the cytogenous granules oozed out rapidly over the surface of the body. The cyst wall formed rapidly and the tail was left attached to the outside of the cyst where it lashed violently and eventually was detached and may continue lashing about until it degenerated. The body of the cercaria inside the cyst twisted and turned about during the process as though molding the inner wall of the cyst. Encystment was completed in about 15 to 30 minutes. Finally, the cyst wall hardened, metacercaria coiled about in the cyst, become relatively motionless but periodically undergoing slight twitching and contractions in various regions of its body.

The Metacercaria

When viewed with the naked eye, the metacercaria appeared as a dark dot on the surface on which it was attached. It is dark brown in colour and darkens with age.

Metacercaria is round and measured 0.20-0.28 mm in diameter. The cyst wall is composed of three layers; the outermost is yellowish and measured 28-30 microns thick; the second layer measured 20.2-20.8 microns thick and the innermost layer is the thinnest and measured 8.1-8.6 microns thick. Slight movement of the cercaria within the cyst is observed whenever exposed to light. The black pigments of the eyespots and of the body persist throughout metacercarial life and those of the former are very distinct in mounted specimens.

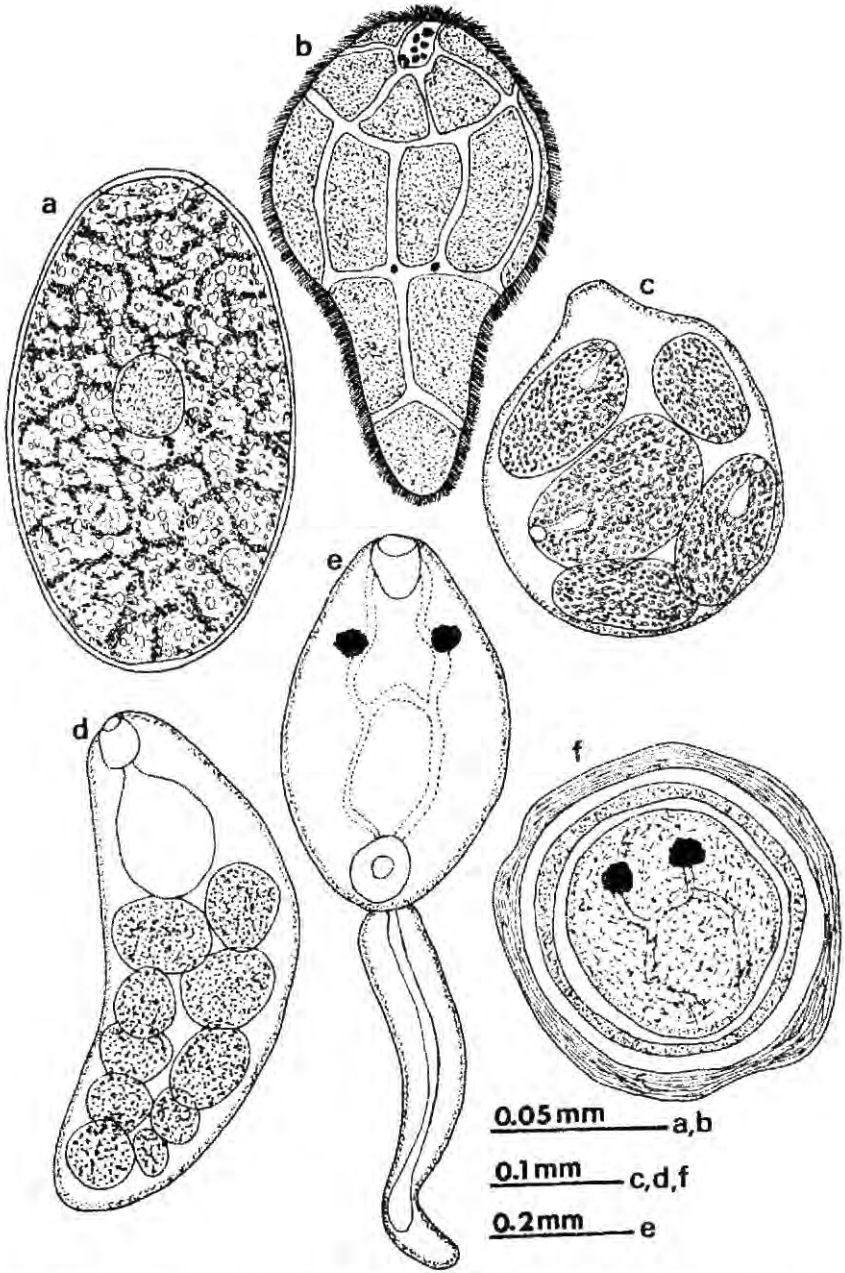

Fig 6. Schematic representation of the pre-adult stages: a. Egg; b. Miracidum; c. Sporocyst; d. Redia; e. Cercaria; f. Metacercaria.

Discussion

It is evident from the result of this study that *Gyraulus convexiusculus* is a suitable snail host of *Orthocoelium scoliocoelium* as indicated by the development of the various stages up to the infective form under Philippine conditions. In other countries, other snail species serve as intermediate host for the species. These are *Anisus natalensis* in Kenya (Dinnik, 1954) and *Bulimus pulchellus* in India (Jain and Srivastava, 1969).

The morphology of the egg of *O. scoliocoelium* is similar to that of other species of Paramphistomoidea whose eggs are already described like *Orthocoelium streptocoelium* (Durie, 1953); *Paramphistomum cervi* (Odening *et al.*, 1978; 1979); *Fischoederius elongatus* (Mukherjee, 1966); *Calicophoron microbothrium* (Lengy, 1960) and *Calicophoron daubneyi* (Sey, 1972). It is apparent that eggs among the paramphistomids appear similar; hence, specific identification based on this character alone would not be reliable. At 26-31°C, the eggs of *O. scoliocoelium* would require about 14-19 days to hatch to miracidum. This observation is comparable with those obtained by authors with other species of paramphistomids. Durie (1953) and Sey (1972) observed that at 27°C, it took 16 and 10-11 days for the eggs of *O. streptocoelium* and *Calicophoron daubneyi* respectively to hatch to miracidium. At 28°C, *Calicophoron microbothrium* needs 17 days to develop to miracidium (Lengy, 1960). Durie (1953), however, has shown that development and hatching to miracidium of *Paramphistomum ichikawai* was more rapid than *O. streptocoelium* maintained under similar conditions. The embryo of *P. ichikawai* at the end of the six-day period corresponds closely in size and structural development with that of *O. streptocoelium* after 11-12 days. Eggs hatch after 12 days for the former and 16 days for the latter species.

As observed in the present work, the miracidium of *O. scoliocoelium* enters the snail through the mantle cavity and remains in this tissue in the early sporocyst stage before it penetrates deeper into the tissues and then to the various organs of the snail. Upon penetration, the epidermal plates and cilia are shed off. The same pattern of penetration was also observed for *C. microbothrium, O. streptocoelium* and *P. ichikawai* (Lengy, 1960 and Durie, 1953). Other species have been shown to differ from this in that there was no loss of structure during penetration as in *Cotylophoron cotylophorum* (Bennett, 1938) and there was penetration of the exposed part of the snail as in *Cotylophoron indicum* (Mukherjee, 1960). It is interesting to note here that while some workers had difficulty in demonstrating early sporocyst in their respective life cycle studies, sporocyst in the present work was recovered as early as 24 hours after snail infection and persisted until the 12th day. This early form was found in the mantle of the snail. Dinnik and Dinnik (1954) and Jain (1978) were able to demonstrate sporocyst only on the 11th day for *C. microbothrium* and 5th day for *Gigantocotyle bathycotyle* respectively, that is, only after the sporocyst had grown to a larger and recognizable size. It is possible that early sporocyst in these species are already in the deeper tissues

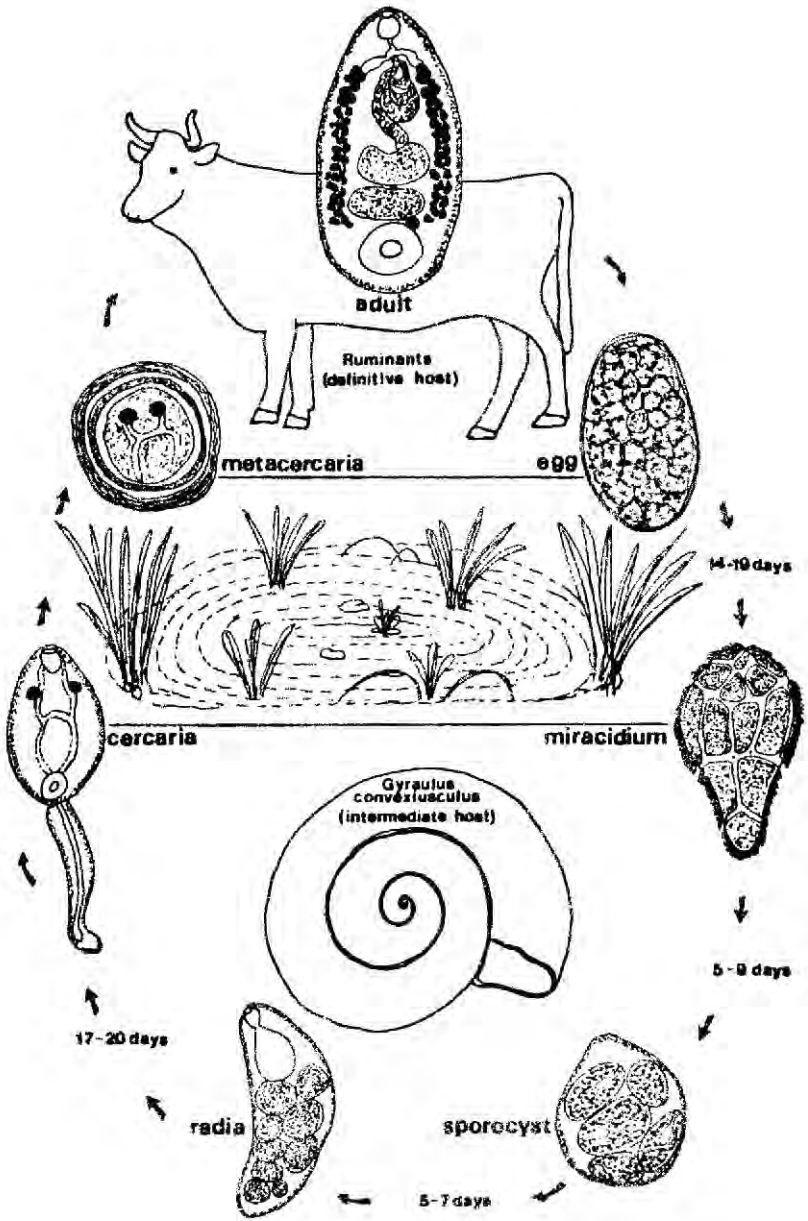

Fig. 7. The life cycle of *Orthocoelium scoliocoelium* in the Philippines (Schematic representation).

making it difficult for recovery after dissection except in careful and very systematic teasing of tissues.

The redia of *O. scoliocoelium* follows the same pattern of development as described for amphistome species whose life cycles have been worked out. It differs, however, in the majority of these species in the absence of daughter redia formation. In no occasion was this particular stage observed and all mature redia examined contained either cercarial balls or immature cercaria. Three species of paramphistomid so far have been found not to produce daughter redia and these are *C. cotylophorum, G. explanatum* and *G. bathycotyle* (Bennett, 1939; Singh, 1958 and Jain, 1978, respectively). Durie (1953) claimed, but without certainty and direct evidence, that daughter redia formation is induced by changes in temperature. However, Singh (1958) has shown that snail infected with *G. bathycotyle* and examined both in the summer and winter months produced not a single redia with daughter redia.

The cercaria of *O. scoliocoelium*, as in other species of amphistomes, leaves the redia in the immature stage and further development occurs in the tissues of the snail. However, unlike the cercaria of *G. bathycotyle* and *C. daubneyi* which are already provided with eyespots while still inside the redia (Jain, 1978; Dinnik, 1962), that of *O. scoliocoelium* is without eyespots. The eyespots develop rapidly and only after the cercaria leaves the redia. Similarly, this development has been observed for *C. microbothrium, O. streptocoelium, P. ichikawai, C. indicum* and *G. bathycotyle* (Dinnik and Dinnik, 1954; Lengy, 1960; Durie, 1953; Mukherjee, 1968 and Singh, 1958).

The distribution of the larval stages of *O. scoliocoelium* in *Gyraulus convexiusculus* as already pointed earlier conforms to that of *Paramphistomum cervi* in *Planorbis planorbis.* In this species, rediae and cercariae were noted to be heavily concentrated in the ovotestis, hepatopancreas and digestive glands. It is probable that affinity for these areas is due to the high amounts of nutritive materials present in these regions which have a rich blood supply necessary for further development of the larval stages.

This study has shown that *Gyraulus convexiusculus* is a suitable intermediate host for *Orthocoelium scoliocoelium.* The intramolluscan stages conform with the general pattern earlier established for the paramphistomid group namely, sporocyst, redia, cercaria but no formation of daughter redia. Under Philippine conditions, intramolluscan development is completed in 32-36 days; hence, infective stage (metacercariae) is already available at the end of this period. *G. convexiusculus* is a very small snail and could easily be missed in cursory examination of areas for molluscan surveys. In such case, examination must be done systematically in order not to overlook it or similar snails. Careful search of literature showed no report on the distribution of this snail in the Philippines. Future studies should therefore be directed along this line.

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