

Studies on the Cercaria of *Paragonimus mexicanus* in *Aroapyrgus alleei* from Colima, Mexico, with Special Reference to Its Morphology (Trematoda : Troglotrematidae)

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(Received for publication; September 27, 1984)

Key words: *Paragonimus mexicanus*, *Aroapyrgus alleei*, cercaria

Introduction

Even though several reports on the discovery of lung fluke cercariae were published in Central America, no report on its morphology has yet been published to date. Brenes *et al.* (1968, 1980) reported repeatedly the finding of cercaria of *Paragonimus mexicanus* in *Aroapyrgus costaricensis* from Costa Rica without any description on the cercarial morphology. Malek and Little (1971) also merely reported the finding of the cercaria of *Paragonimus caliensis* in *Aroapyrgus colombiensis* from Colombia, without any mention about the morphology of cercaria, because the main aim of that report was a proposition of a new name for the host snail with a detailed description of the snail only.

In Mexico, the first discovering report was made by one of the authors, Lamothe *et al.* (1983). According to them, one kind of hydrobiid snail was collected from near Madrid, the State of Colima, and discovered three out of several thousand snails being infected with

redia and cercaria of a species of *Paragonimus* in 1979. They identified the snail as *Aroapyrgus alleei*, and the cercaria as *Paragonimus mexicanus*. Nevertheless the paper was occupied mainly by the description and discussion on the taxonomical position of the host snail, so the description of the cercaria was left in the future.

In 1983, we had an opportunity to stay in the State of Colima, Mexico, as a commission of pathobiological studies on paragonimiasis in Mexico. Among five thousand snails collected from the same areas as reported by Lamothe *et al.* (1983), four cases of infected snails with redia and cercaria of *P. mexicanus* were found. The following is the description of redia and cercaria of *Paragonimus mexicanus*.

Materials and Methods

In the State of Colima, six species of fresh water snails were collected and examined for the cercarial infection. These were *Aroapyrgus alleei*, *Aroapyrgus* sp., *Trionia* sp., *Biomphalaria obstructa*?, *Physa osculans*? and *Helisoma* sp. As shown in Table 1, the cercaria of *Paragonimus mexicanus* was found only from *Aroapyrgus alleei* in Madrid, the infection rate being 0.08 %.

This snail belongs to Hydrobiidae, shells of 4 to 4.5 whorls measuring 1.48 to 3.13 mm in height, 1.11 to 1.68 mm in width, and aperture 0.83 to 1.3 mm in height and 0.57 to 0.97 mm in width. The locality of this snail was near Madrid, about 40 km far from Co-

This study was supported by a Grant-in-Aid for Overseas Scientific Survey from the Ministry of Education, Science and Culture (Subject No. 58041017).

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Table 1 Results of snail examination in the State of Colima, Mexico, during from July 26 to Aug. 5, 1983

Species of snail	Locality	No. of snails examined	No. of snails positive for	
			Cercaria of <i>Paragonimus</i>	Other cercariae
<i>Aroapyrgus alleei</i>	Madrid	4813	4 (0.08%)	10 *
<i>Aroapyrgus</i> sp.	Coquimatlan	192	0	2 †
<i>Trionia</i> sp.	Madrid	2631	0	3 ‡
<i>Biomphalaria obstructa</i> ?	Colima (Higueral)	166	0	0
<i>Physa osculans</i> ?	Colima (Higueral) and Madrid	46	0	0
<i>Helisoma</i> sp.	Colima (Higueral)	8	0	0

* Gymnocephalous cercaria...2, Xiphidiocercaria A...6, Xiphidiocercaria B...2.

† Gymnocephalous cercaria...2

‡ Gymnocephalous cercaria...1, Xiphidiocercaria C...2

lima City, and about 150 m above the sea level. The natural habitat of the snail where the present survey was carried on, is a pond filled with a natural underground spring and on the sides of concrete-lined canals leading from the pond. No submerged or emerging aquatic plants or weeds are found in these streams or the pond, but the submerged roots of *Piper hispidum* and *Ficus* sp. which are abundant along the banks, constitute a favorite habitat for the snail. Thousands of snails were attached to these roots.

Snails were crushed between two pieces of glasses and observed under a dissecting binocular microscope. The positive snail for the cercaria of *P. mexicanus* were isolated and washed with 0.4 % NaCl solution in a small special watch glass. It needs a careful attention for making a suitable microscopic specimen, because the cercaria was very few in number, usually less than 10 in one snail, and very weak and fragile for the pressure of cover glass. For observation of the living specimen, the margin of the cover glass was sealed with vaseline in order to avoid the evaporation of the medium, so that the material was kept in a good alive condition for a few hours. some internal organs such as the penetration glands were observed by the aid of vital staining with neutral red or Nile blue sulphate. Measurements were taken on 10

specimens fixed with 10 % hot formalin, because this fixing technic yields a comparative stable shape and size of the cercaria and parthenita. Semidiagrammatic drawings were made to scale from such measurements.

Description of cercaria and redia

Snail host: *Aroapyrgus alleei* Morrison, 1946

Date, locality and infection rate:

July 26, Madrid, 1 out of 660, or 0.15 %

July 28, Madrid, 1 out of 1513, or 0.07 %

Aug. 2, Madrid, 1 out of 1385, or 0.07 %

Aug. 5, Madrid, 1 out of 1255, or 0.08 %

Totally 4 out of 4813, or 0.08 %

Measurements of cercaria:

body...196 (158-245) × 76 (70-80) μm

oral sucker...48(45-50) × 41(40-45) μm

stylet...30 (27-32) × 7 (6-8) μm

acetabulum...31 (28-33) × 35 (30-38) μm

tail...19(15-25) × 18(15-20) μm

Morphology of cercaria:

The cercaria is a typical microcercous type. The body is ellipsoidal or cylindrical in shape, covered by a cuticle provided with many spines directed backward. The body surface is also beset with several sensory hairs on a minute papilliform base respectively. These hairs distribute more densely around the mouth opening. The ventral-posterior half of the body is occupied by a ellipsoidal peculiar stru-

cture which appears as if so-called "pseudo-sucker". It consists of a median wide groove from the acetabulum to the posterior end of the body, and the marginal striated hyaline membrane, measuring about 80-95 μm long and 60-70 μm wide. This pseudo-sucker-like structure is more prominent in the fixed specimen as shown in Fig. 1, 4, 7 and 8.

A well developed muscular oral sucker is situated at the anterior ventral side of the body. A prominent long, non-shouldered stylet with several longitudinal striations at its posterior part is embedded in the oral sucker at its dorsal side. Several radial muscles from the anterior body wall to the base of the stylet, make the stylet move incessantly. A pharynx is faintly recognized following the oral sucker with a very short prepharynx. The cecum is not observed except only a short esophagus. The nervous commissure is situated transversely across the pharynx. A ventral sucker, slightly smaller than the oral sucker, is located at a little behind the middle of the body. Seven pairs of penetration gland cells are observed between the pharynx and ventral sucker. The inner three pairs contain fine granules, and the outer four pairs contain coarse granules. Their ducts are grouped four bundles and open at both sides of the apical end of the stylet.

The posterior middle part of the body is occupied by an I-shaped excretory bladder lined with a layer of cuboid epithelia. From its antero-lateral corner the main collecting tube runs forwards in zigzag course, and divides into the anterior and posterior collecting tubes. The anterior tube runs forwards receiving 5 groups of three flame cells each. The posterior tube runs backwards receiving, as same as the anterior, 5 groups of three flame cells each. So the flame cell formula is constructed as $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$. But these 30 pairs of the flame cells are not equally developed. Usually the flame cells of the most anterior and posterior groups are well developed and larger than those in the middle part of the body, the latter being sometimes not yet developed completely.

At the ventral end of the body, a conical-shaped short tail is attached but detachable easily. It has a big power of contraction and extension reaching its length from 15 μm to 30 μm . The posterior end of the tail is provided with many solid and pointed spines giving a shaggy appearance.

Morphology of redia :

Only two matured daughter rediae were observed from two of four positive snails in the present investigation. They are bent cylindrical in shape, light yellow or yellowish-brown in color, measuring 440-460 μm long by 120-130 μm wide. They have neither collar nor locomotive appendix. The pharynx is about 40 μm in diameter, and is followed by about the same sized globular intestine. The intestine contains brownish food particles. Many sensory hairs are observed around the mouth opening of the redia. Each redia contains 3 or 1 matured cercariae, 7 or 5 immatured cercariae and 5 or 6 germ balls respectively.

Discussion

Since much attention was paid on the paragonimiasis in Central and South America, nearly twenty years have elapsed. During that time many reports on the morphology of adult and metacercaria of *Paragonimus* from Mexico, Colombia, Peru, Costa Rica, Ecuador, Guatemala and Panama have been published with special discussion on their taxonomic position. On the other hand, concerning with the cercarial morphology no report has ever been published up to date, even though there are several report on merely the discovering of cercariae of *Paragonimus*. Those reports are; Brenes *et al.* (1968, 1980) from Costa Rica, Malek *et al.* (1975) from Costa Rica, Malek and Little (1971) from Colombia, and Lamothé *et al.* (1983) from Mexico. Of course those reports are all very important and worthy on the determination of the first intermediate snail host of Latin American lung fluke, but they did not mention about the morphology of the cercaria, because of perhaps the scanty and fragile materials and the difficulty of the ob-

servation.

Though any experiment of cercarial infection to the crab was not performed, the present cercaria was identified as *Paragonimus mexicanus* because of the following reasons. In the areas where the present survey was carried on, many crabs, *Pseudothelphusa dilatata*, were found side by side with the snail, *Aroapyrgus alleei*. This crab was already proved to be a second intermediate host of *Paragonimus mexicanus* by Lamothe *et al.* (1977), and also it was found by our present survey that 43 % of the crab was infected with the metacercaria of *Paragonimus*, all of which were proved to be *P. mexicanus* by an experimental infection to the cat. Moreover in the State of Colima, only one species of lung fluke, *P. mexicanus*, was reported from the natural definitive host, *Didelphis virginiana californica* by Lamothe *et al.* (1981).

The present cercarial description is the first one among Latin American lung fluke, so the comparative discussion with them, *i.e.* *P. peruvianus*, *P. caliensis*, *P. eadoriensis*, is impossible. As compared only with already known other species of lung fluke cercariae the general feature is almost same each other. But only one characteristic feature of the present cercaria was a "pseudo-sucker" like structure situated on the ventral-posterior half of the body. It consists of a median wide groove and the marginal striated hyaline membrane. As far as we are aware, no such structure was observed on other species of lung fluke cercariae. Further comparative studies among Latin American lung fluke cercariae will be necessary for the discussion on their taxonomic position in future.

Summary

The morphology of cercaria and redia of *Paragonimus mexicanus* was reported for the first time in Latin America in this paper. Materials were obtained from *Aroapyrgus alleei* in Colima, Mexico, the infection rate being 0.08 % only. Average size of the cercaria was, $196 \times 76 \mu\text{m}$ in body, $48 \times 41 \mu\text{m}$ in oral sucker, $30 \times 7 \mu\text{m}$ in stylet, $31 \times 35 \mu\text{m}$

in acetabulum and $19 \times 18 \mu\text{m}$ in tail. The flame cell formula was $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$. Though the general structure of this cercaria and redia resembled with those of other species of *Paragonimus*, the characteristic feature of the present one was a "pseudo-sucker" like structure on the ventral posterior half of the body, consisting of a median wide groove and the marginal membrane. Further comparative studies among Latin American *Paragonimus* on the morphology of cercariae will be necessary in future.

Acknowledgements

The authors wish to express their sincere thanks to the other members of the present research team, Prof. Dr. Moriyasu Tsuji, Prof. Dr. Somei Kojima, Dr. Yasumasa Tongu, Dr. Masashi Kobayashi, Mr. Raul Pineda-López and Mr. David Osorio for their kind help to collect materials and technical assistance in this study.

Thanks are due to the authorities concerned in Mexico and Japan for their close cooperation with this study.

The authors are also indebted to the Ministry of Education in Japan for the financial support by Grant-in-Aid for Scientific Research (Grant-in-Aid for Overseas Scientific Survey) in 1983, the subject no. 58041017.

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メキシコ、コリマ産の淡水貝 (*Aroapyrgus alleei*) に寄生するメキシコ肺吸虫 (*Paragonimus mexicanus*) のセルカリア、特にその形態に関する研究

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メキシコ肺吸虫のセルカリアはブレーネスら (1968, 1980), ラモテら (1983) によって淡水産の小巻貝 *Aroapyrgus* spp. から検出されたとの報告はあるが, その形態についてはいまだ報告されていない. 著者らは1983年にメキシコのコリマ地方において *Aroapyrgus alleei* 4,813個のうち4個からメキシコ肺吸虫のセルカリアを検出してその形態を観察することができた. 10%温フォルマリンによる固定標本の平均測定値は, 体 $196 \times 76 \mu\text{m}$, 口吸盤 $48 \times 41 \mu\text{m}$, 穿刺棘長 $30 \mu\text{m}$, 腹吸盤 $31 \times 35 \mu\text{m}$, 尾 $19 \times 18 \mu\text{m}$ で3倍体のウ肺吸虫セルカリアよりやや小型である. 焰細胞式は $2 [(3 + 3 + 3 + 3 + 3) + (3 + 3 + 3 + 3 + 3)] = 60$ であるが, 一般に体中央附近の焰

細胞の発育がおくれており, その数の少ないものもある. 他種肺吸虫セルカリアにみられない本セルカリアの特徴としては, 体後半腹面にある溝が拡大し, その周囲は線条のある透明な楕円形の膜で縁どられた“吸盤類似”の構造物のあることである.

ラテンアメリカの肺吸虫セルカリアの形態的報告は今回が始めてである. ラテンアメリカでは母虫のみによって, あるいはメタセルカリアを加味して肺吸虫の分類がなされ, 多少の混乱をきたしているが, 将来はセルカリアの形態をも加えて総合的に検討されることが必要であろう.

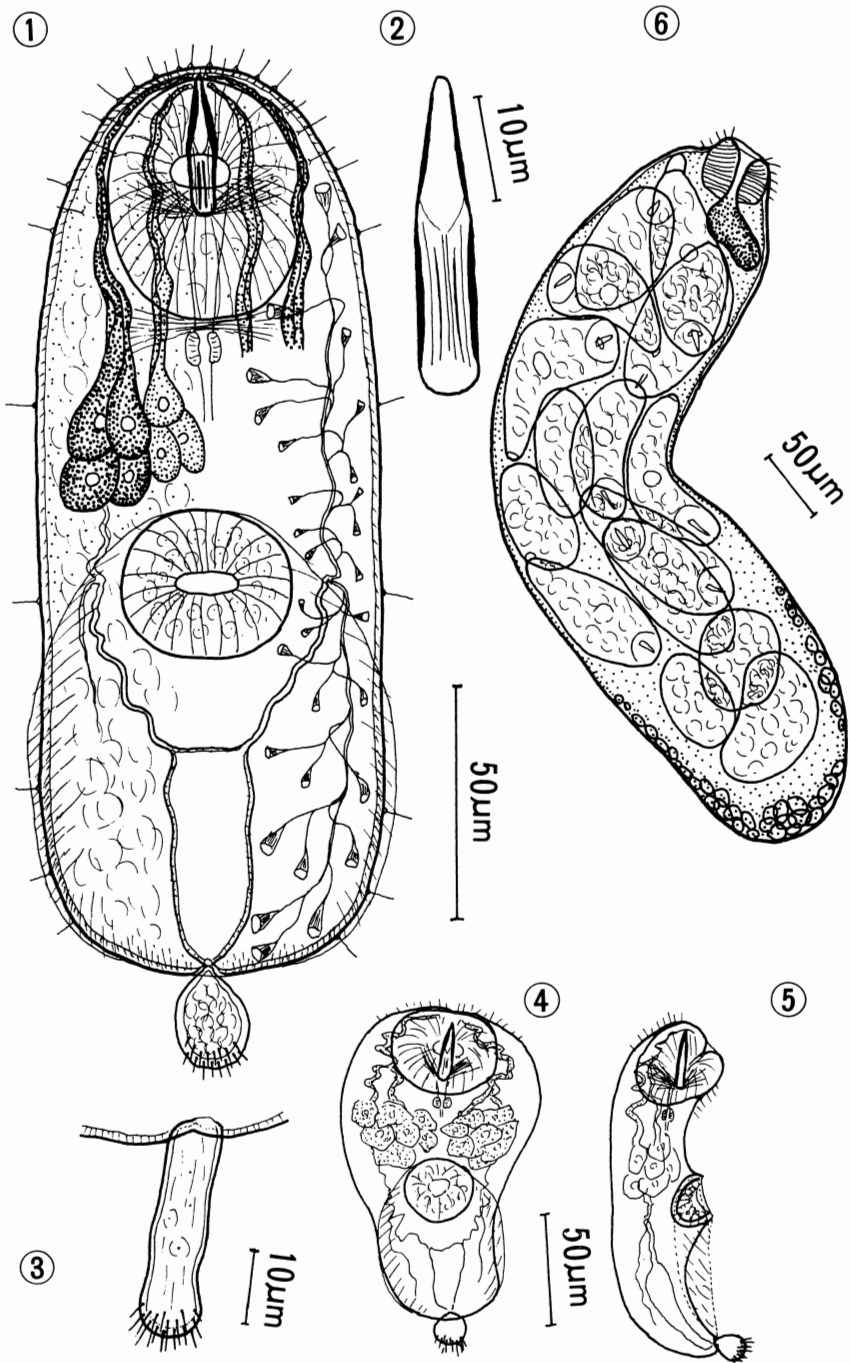


Fig. 1 Cercaria of *Paragonimus mexicanus*, semidiagrammatic drawing.
 Fig. 2 Magnification of the stylet.
 Fig. 3 The extended tail.
 Fig. 4 The cercaria of living posture.
 Fig. 5 The cercaria of lateral view.
 Fig. 6 The daughter redia of *P. mexicanus*, living material.

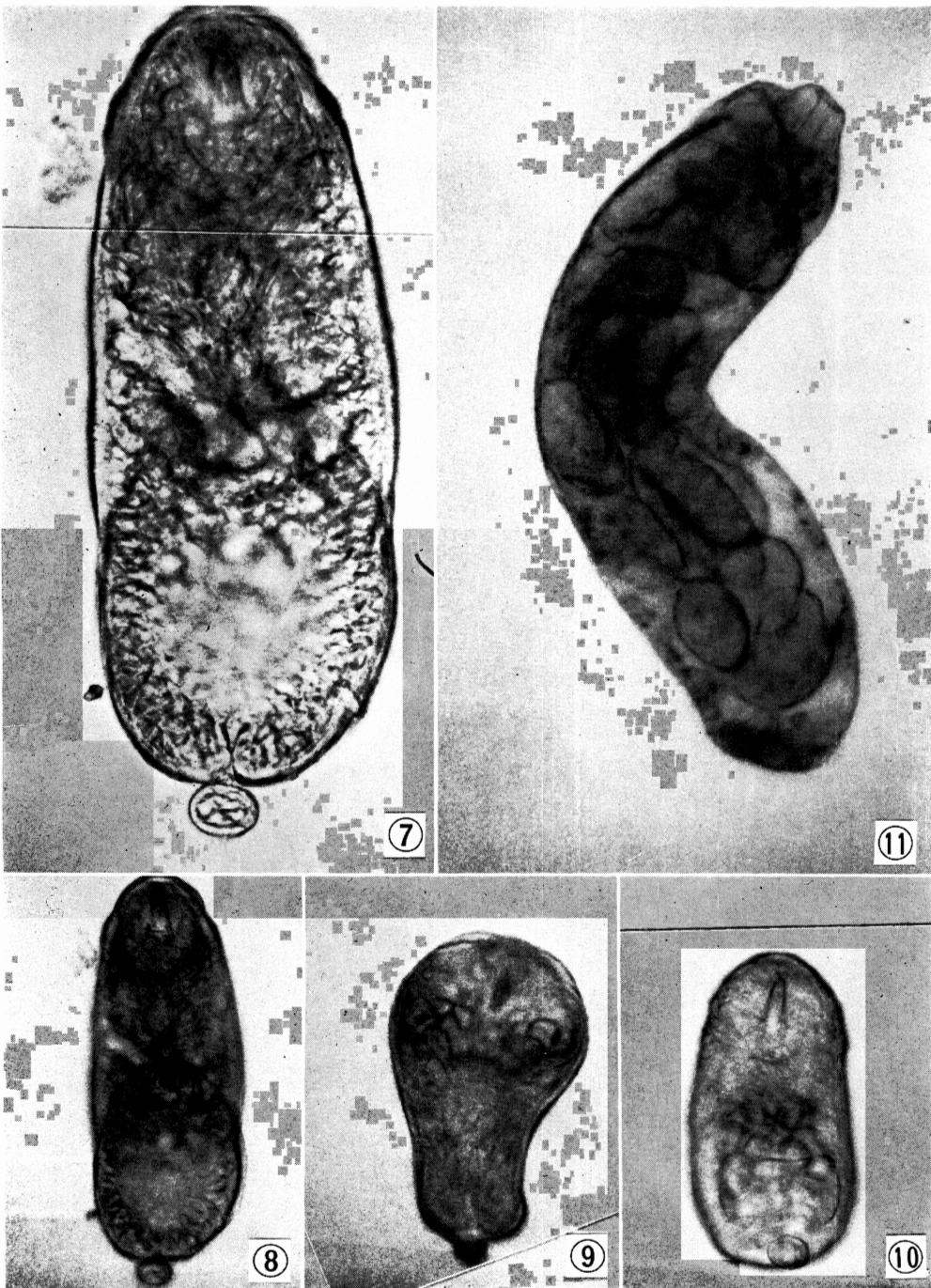


Fig. 7 Cercaria of *Paragonimus mexicanus*, fixed with 10% hot formalin.
 Fig. 8 Ditto.
 Fig. 9 The cercaria of living posture, dorsal view.
 Fig. 10 Ditto, ventral view.
 Fig. 11 The daughter redia of *P. mexicanus*, living material.