

## Studies on the Metacercaria of *Paragonimus peruvianus* (Trematoda : Troglotrematidae)\*

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### Introduction

Miyazaki, Ibáñez and Miranda (1969) described a new lung fluke, *Paragonimus peruvianus* in Cajamarca Province located in the northern part of Peru. In the same paper they also described *Paragonimus* metacercariae found in a crab, *Pseudothelphusa chilensis* collected in the same locality, which were considered to belong to *P. peruvianus* without getting adult worms by experiment. In September-October of 1970 the authors visited the above-mentioned locality, and investigated a lot of living *Paragonimus* metacercariae under the auspices of National University of Trujillo. Some larvae were fed to experimental cats both in Peru and in Japan, and they matured to adults of *P. peruvianus*. In this paper the authors wish to describe the newly identified metacercaria in detail.

### Materials and Methods

The crab, *Pseudothelphusa chilensis* was collected along Condebamba Valley on the

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Andes Mountains between Cajamarca City and Cajabamba City at the altitude of approximately 1,900 meters. The liver, gill, muscle and other organs of the crab were separately pressed between two glass plates, and were searched for *Paragonimus* metacercariae. Morphology of the larvae was carefully investigated, paying special attention to their cyst. Some larvae were photographed and then fixed with 10% formalin under slight coverglass pressure. They were brought to Japan, and stained with carmine and mounted in balsam for morphological study. Some larvae were experimentally fed to cats to get adult worms for identification. On the other hand, about 80 larvae were brought to Japan by the senior author in four small bottles containing physiological saline solution. Those which were still alive in Japan were immediately fed to two young cats to know their vitality.

### Results

Forty-nine crabs were examined, and 34 or 69% were positive for *Paragonimus* metacercariae, number of the larvae per crab varying from one to 73. Almost all of them were found in the liver of crabs. Eight crabs out of 49 mentioned above were most carefully examined by the senior author. The width of carapace ranged from 50 to 78 mm in five males and from 71 to 80 mm in three females. Six or 75% of the eight

crabs examined were proved to be infected with metacercariae, and the larvae counted respectively 2, 3, and 22 in three positive males and 18, 29, and 61 in three females, totaling 135 in all, of which only a single larva was found in the muscle and the remainder were all in the liver of crabs.

It was very peculiar and uncommon that all larvae were moving freely without encystment. In spite of particular attention, no cysts were recognized around the larval body. The larvae moved actively in the liver of crabs by using two powerful suckers. The body was large in size and provided with singly pointed cuticular spines all over. The ventral sucker was much larger than the oral sucker that was frequently armed with a short stylet. Two intestines appeared yellow in color owing to numerous yellowish granules in them. Occasionally, their color seemed blackish due to different granules occupying them (Fig. 8). It is most probable that the latter form is young larvae of the same species. The intestinal wall had many folds in life, but became less conspicuous after fixation (Figs. 5, 6). The excretory bladder was black in color and narrow in dorso-ventral view and wide in lateral view, extending to the bifurcation of intestines. The bladder had many folds, remarkably changing in shape by movement of the larva. Red granules were densely or scatteringly recognized mostly in the ventral side of larval body, and showed a beautiful appearance together with the black bladder and yellow intestines (Figs. 1-4).

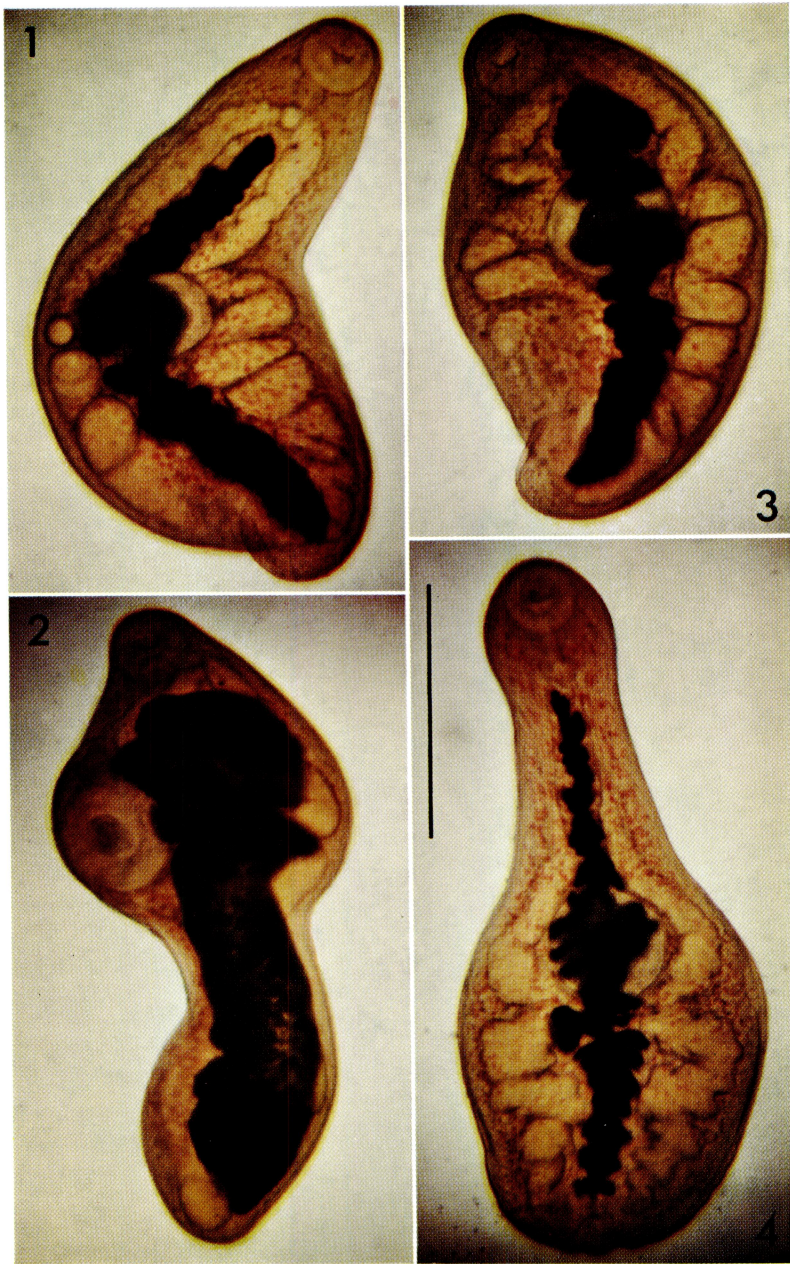
The following description was based on 30 larvae, which were stained with carmine and mounted in balsam. Measurements were in millimeters unless otherwise noted. Body 1.09 to 1.50 in length and 0.53 to 0.61 in width, averaging 1.30 by 0.56. Oral sucker 0.13 to 0.17 wide by 0.11 to 0.14 long, averaging 0.14 by 0.12. Ventral sucker, spherical and much larger than the former, 0.20 to 0.25 wide by 0.20 to 0.24 long (avg. 0.23 by 0.22). Small stylet in the oral sucker was clearly recognized in 19 specimens. Length of six stylets, which were fixed horizontally, measured 13

to 18 (avg. 16.5) micra. Intestines were reddish in color by the granules of various size and shape in them, which showed yellowish color in life. The contour of the excretory bladder was fairly recognized due to the cells of the bladder wall which were relatively big and densely arranged (Fig. 7). The male and female reproductive organs were situated, as clusters of cells, on both sides of the posterior half of excretory bladder and behind the ventral sucker, respectively. However, they are not so clear as those of the other species.

*Experimental infection to cats:* In Peru, four cats were fed with active larvae to get adult worms, and were sacrificed after 45, 60, 75, and 105 days of infection, respectively. The worms obtained were all mature, having a lot of eggs in their uterus. On the other hand, 18 larvae were still moving when they arrived at Fukuoka, Japan, which had been collected from the crabs in Peru from six to ten days before. They were fed to two young cats by nines on the same day. Unfortunately, however, both kittens died 47 and 49 days after infection. A single worm was found in the pleural cavity of the first kitten, and four worms obtained from two worm-cysts of the right lung of the second kitten. One couple from the larger worm-cyst were both mature, but the other couple from the smaller cyst and the worm found in the first kitten were nearly mature, but without eggs in their uterus. By morphological features of them these worms were all identified as *P. peruvianus*.

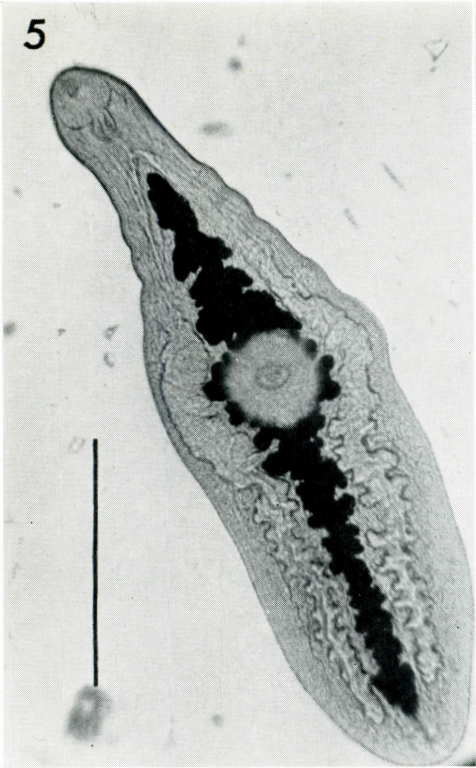
### Discussion

It was surprising that the metacercaria of *P. peruvianus* was entirely nonencysted, because all known species of *Paragonimus* metacercariae are enveloped in one or two kinds of cysts in their crustacean hosts. Accordingly, in the present study particular attention was paid to find any kind of cyst around the larval body, but all efforts were in vain. On the other hand, Brenes, Zeledón and Rojas (1968) briefly communicated that a kind of *Paragonimus* metacercaria (uniden-



#### Explanation of Figures

Figs. 1-4: Living metacercariae of *P. peruvianus* under the same magnification, natural color. Scale: 0.5 mm. 1, 3, 4: Dorsal or ventral view. Note yellow intestines and red granules. 2: Ventrolateral view. Note red granules in ventral side. (Photo by Ichiro Miyazaki)



tified) which was found in two species of Costa Rican river crabs, *Pseudohelphusa tristani* and *P. magna*, appeared crawling free on the liver of the crabs at the moment of examining them. Prior to this communication, Sogandares and Smalley (1965) reported *Paragonimus* metacercariae from preserved specimens of *Pseudohelphusa tristani* collected in Costa Rica. But, they did not describe any cyst around the larval body. The larvae reported in these two papers are most probably identical, and in case they are really nonencysted, the larvae may possibly be very close to those of *P. peruvianus*. Judging from the figure by Sogandares and Smalley (1965), on the other hand, the Costa Rican *Paragonimus* reported above-mentioned two papers seems to be different from *P. peruvianus*, because the ventral sucker of the larva is apparently larger in the former than the latter as compared with respective oral sucker. In order to solve these questions, more detailed investigation of the Costa Rican *Paragonimus* is quite necessary.

Generally speaking, the metacercaria enveloped in a thin and fragile cyst, such as that of *Paragonimus iloktsuenensis* Chen, 1940, excysts very easily by the slightest pressure and it appears as if it was originally nonencysted. Therefore, special attention must be paid to this point at the time of examination. At any rate it is very interesting to know that the metacercaria of *P. peruvianus* was quite free from encystation in the crab host. But it may be reasonable to suppose that the larva is encysted in early stage of intracrustacean development and after maturity it becomes free. This supposition must be verified in the future by experimental infection of the crab host with cercariae of

the present fluke. Yellowish color of the intestine is also characteristic of larval *P. peruvianus* in life, which can be used to some extent for distinguishing it from other known species.

In mounted specimens it is noticed that the contour of excretory bladder is relatively clear and the ventral sucker is much larger than the oral sucker. These features are somewhat useful for identification of the present metacercaria, together with the biggest body size among known species of *Paragonimus*.

The vitality of larval *P. peruvianus* is considered to be very high, judging from the facts that the larvae were alive in saline solution for six to ten days without any cysts protecting larval body, and that they could easily infect cats in naked condition. As mentioned previously, the crabs in the endemic area of Cajamarca Province are highly infected with this vigorous metacercaria, and are frequently eaten uncooked by inhabitants. Accordingly, it is a matter of course that cases of paragonimiasis are not infrequently occurring among inhabitants in that area. Incidentally, no metacercariae of *P. westermanni* (Kerbert, 1878) and other known species of the genus were found in the crabs in the endemic area stated above.

Finally, the authors wish to emend the description of the present metacercaria in their previous paper (1969). On page 127 they described, "Living metacercaria is oval in shape and enveloped with one cyst. Since the cyst wall is thin and fragile, the larva excysts very easily". This erroneous description was based on private communications from one of the authors in Peru that he sometimes recognized very thin membrane-

#### Explanation of Figures

Figs. 5-8: Metacercariae of *P. peruvianus*.

- 5: Crawling larva. Note remarkable folds on the wall of intestines and excretory bladder. Scale: 0.5 mm.
- 6: Same larva fixed under pressure. Magnification same as figure 5.
- 7: Mounted larva, stained with carmine. Contour of excretory bladder clear. Scale: 0.2 mm.
- 8: Living larva under pressure. Presumably young form of the same species. Note blackish intestines. Magnification same as figure 5. (Photo by Ichiro Miyazaki)

like substance close to the larval body, and on the preconception of the senior author that *Paragonimus metacercaria* is enveloped in the cyst, without investigating fresh materials of the present worm.

### Summary

The crab, *Pseudothelphusa chilensis*, which is frequently eaten by inhabitants uncooked, was examined for *Paragonimus metacercariae* in Cajamarca Province, Peru. Thirty-four or 69% of 49 crabs were infected with larval *Paragonimus peruvianus* Miyazaki, Ibáñez et Miranda, 1969 and the number of the larvae varied from one to 73 per crab. However, not a single larva of *P. westermani* and other known species was found in these crabs. It was quite uncommon and characteristic that the larvae were not enveloped in any cyst. They showed high vitality, moving in saline solution for six to ten days after removal from the crab and infecting cats easily without protective cyst. Eggs from patients completely agreed in morphology with those from experimental cats. The previous description of the present metacercaria in 1969 was emended concerning the encystation of the larva.

### Acknowledgement

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## ペルー肺吸虫のメタセルカリア

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著者らは1970年の9~10月、ペルー北部、カハマルカ州の流行地で、住民に生食されているチリーサワガニをしらべ、高率に肺吸虫の幼虫をみつけた。その一部をネコに与えて成虫をえた結果、ペルー肺吸虫であることをたしかめた。そして、ネコからの虫卵と患者のものと同じ特長を示したことや、上記のカニに他種肺吸虫の幼虫が全くみつからなかつたことなどから、上記流行地の病原虫として、ペルー肺吸虫を重視した。この幼虫は、

これまでの種類にみられぬような大型であるばかりでなく、全然フクロをかぶっていない点、きわめて特異であつた。生活力が非常につよく、フクロなしで、カニの体外でも、かなり長く生存することができ、かつ、容易にネコに感染した。色もゆたかで美しいので、その容姿をカラー写真で示した。(この大要は1971年4月、日本寄生虫学会総会で報告した)。