

STUDIES ON THE LIFE CYCLE OF *Haplorchis pumilio* (Looss, 1896) (Trematoda: Heterophyidae) IN VENEZUELA.

Estudios del Ciclo de vida de *Haplorchis pumilio* (Loos, 1896) (Trematoda: Heterophyidae) en Venezuela.

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ABSTRACT

The life cycle of *Haplorchis pumilio* was studied from redia to adult under natural and experimental conditions. The pre-cercarial stage (redia) was found in the digestive gland of *Melanoides tuberculata* (natural infection), collected from Aguasanta, Sucre State, Venezuela. Metacercariae were located in muscular tissues, under the scale of the caudal fin of *Rivulus harti* (natural infection). Metacercariae were supplied to ducks, chickens, rats and mice, and 3 days after infection, 28 adult *H. pumilio* were recovered from the small intestine of the ducks. *R. harti*, *Poecilia reticulata*, *Oreochromis mossambicus* and *Astyanax* spp. were exposed experimentally to cercariae emitted by *M. tuberculata*: only *R. harti* was found infected. The 30 days old metacercariae were experimentally supplied to ducks and chickens, and a total of 32 adult trematodes were found 3 days after inoculation of 20 metacercariae to each duck. *Butorides striatus* was found to be the natural final host of *H. pumilio*. Morphometric characteristics of adult *H. pumilio* from both natural and experimental hosts differ from adults described previously. These differences may be due to differences in host and geographical variations. *H. pumilio* in *R. harti* and *B. striatus* report new host records.

Key words: *Haplorchis pumilio*, life cycle, *Melanoides tuberculata*, fish hosts, new records, Venezuela.

RESUMEN

El ciclo de vida de *Haplorchis pumilio* se estudió del redia al adulto bajo condiciones naturales y experimentales. La etapa

pre-cercarial (redia) se encontró en la glándula digestiva de *Melanoides tuberculata* (infección natural), de la localidad de Aguasanta, estado Sucre, Venezuela. Las metacercarias se localizaron en los tejidos de la musculatura, debajo de las escamas de la aleta caudal de *Rivulus harti* (infección natural). Las metacercarias se suministraron a patos, pollos, ratas y ratones; y 3 días después de la infestación fueron encontrados en intestino delgado de los patos, 28 adultos de *H. pumilio*. Las cercarias producidas por *M. tuberculata* fueron usadas para intentar infestar experimentalmente a *R. harti*, *Poecilia reticulata*, *Oreochromis mossambicus* y *Astyanax* spp. y solamente *R. harti* fue infectado. Las metacercarias de 30 días de edad se usaron en ensayos experimentales en patos y pollos. Un total de 32 adultos de *H. pumilio* fueron encontrados en los patos, 3 días después de la infección con una inoculación de 40 metacercarias de *H. pumilio*. *Butorides striatus* resultó ser el hospedador definitivo natural de *H. pumilio*. Las características morfológicas de adultos de *H. pumilio* de los hospederos naturales y experimentales difieren de los descritos previamente. Estas diferencias pueden ser debidas a la diversidad de hospederos y a la distribución geográficas. *R. harti* y *B. striatus* son reportados como nuevos hospederos para *H. pumilio*.

Palabras clave: *Haplorchis pumilio*, ciclo de vida, *Melanoides tuberculata*, peces, nuevos reportes, Venezuela.

INTRODUCTION

Members of the family Heterophyidae (Odhner, 1914), especially those of the genus *Haplorchis* (Looss, 1899), are small parasites that inhabit the digestive systems of birds and mammals, including man and occasionally in reptiles. The infection of different heterophids is transmitted to man through

ingestion of raw or uncooked fish carrying metacercariae of *Haplorchis pumilio* (Looss, 1896), *H. taichui* (Nishigori, 1924) and *H. yokogawai* (Katsuka, 1932). *H. pumilio* is common parasite of many economically important freshwater fishes [17].

As part of an extensive study on trematode fauna of fresh water molluscs from Sucre State, 633 molluscs, *Melanoides tuberculata* (Muller, 1774), a primary intermediate hosts of *Philophthalmus gralli* Mathis et Léger, 1910, and *Centrocestus formosanus* (Nishigori, 1924) Price, 1932 were collected from Aguasanta, Sucre State, Venezuela [2, 4], and also have been found to be intermediate host of a heterophid from the genus *Haplorchis* Looss, 1899. Of 633 molluscs examined, 13 were infected with cercariae from the Pleurolophocercous group, which turned out to be the cercaria of *Haplorchis pumilio*. Several specimens of *Rivulus hartii* (Boulenger, 1896), collected from the same locality were found to be infected naturally with metacercariae of this parasite. All developmental stages of this trematode were described from both natural and experimental hosts, using light microscopy. *R. hartii* and *Butorides striatus*, are reported as new hosts for this species. This study represents, the first record of *H. pumilio* from Venezuela.

MATERIALS AND METHODS

A total of 633 specimens of *Melanoides tuberculata* were collected and isolated in glass jars with water. Molluscs were examined at 12 hour intervals to observe the emission of cercariae, which occurred in 13 cases. The cercariae were identified as belonging to the *Pleurolophocercous* Sewell (1922) group on the basis of their morphological characteristics. Fresh water fishes; *R. hartii*, *Poecilia reticulata* (Peters, 1859) and species of *Astyanax* (Baird and Girard 1854), were caught by means of hand nets. All fish were examined thoroughly, and a few cysts of *H. pumilio* were detected under the fins of *R. hartii*. The naturally infected *R. hartii*, jointly with others of the same species, captured in Agua Blanca, Montes Municipality, Sucre State, and free of infections of any digenean were used for experimental infections to obtain mature parasites. The definitive experimental hosts, rats (*Rattus norvegicus*) and mice (*Mus musculus*) were provided by the Department of Biology, Universidad de Oriente, whilst one month old ducks (*Cairina moschata*) and chickens (*Gallus gallus domesticus*) were purchased from local poultry farms. All developmental stages of *H. pumilio* were studied live, or fixed in alcohol-formalin-acetic acid stained with Semichon's acetocarmin and mounted permanently in Canada balsam. All measurements are given in millimeters. The drawings were done with the help of a drawing tube. Keys of Sewell [14] and Yamaguti [19] were used to identify the trematodes.

RESULTS AND DISCUSSION

Redia (FIGS. 1, 2, 3, 4, 5): found in the digestive gland of infected mollusc, *Melanoides tuberculata*.

Description: Based on 30 live and some mounted specimens.

Body, 0.328-0.616 X 0.080-0.202, presents sensorial hair from birth in their anterior portion, and birth pore is located in the 1/3 portion anterior part of body. Neck and locomotory appendix absent. Pharynx, 0.040-0.060 X 0.024-0.045, well developed, followed by a short intestinal caeca in the shape of a sac. Two pairs of glandular cells with fine granular content are located in both postero-lateral sides of the pharynx. The body of redia is full with germinal masses and few cercariae of different developmental stages. No well developed cercariae were observed. The extreme posterior end of redia is covered by a group of glandular cells, probably adhesive cells. Protonephridial system was not observed.

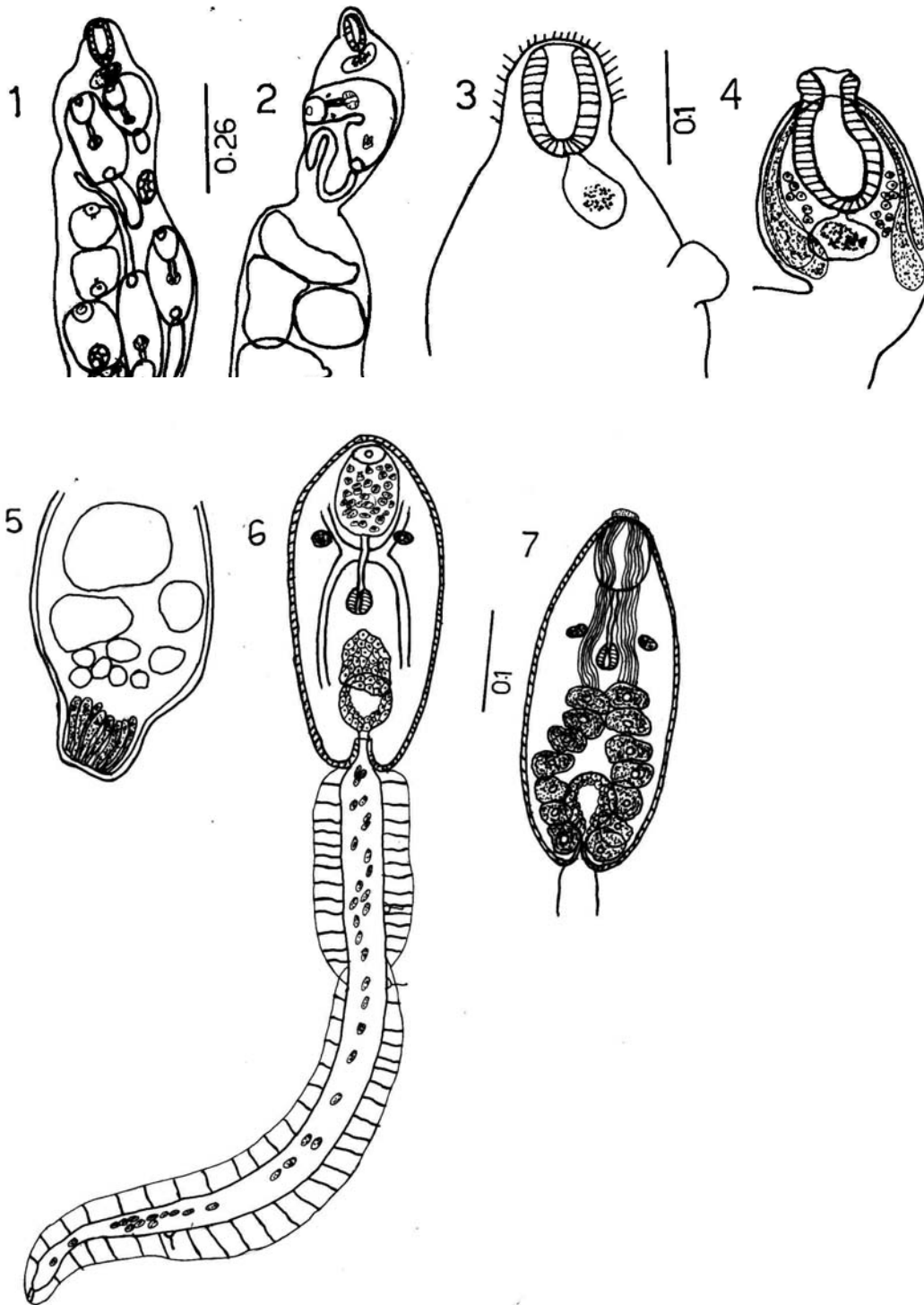
Cercaria (FIGS. 6, 7, 8, 9, 10, 11). Belongs to Pleurolophocercous group, in naturally infected mollusc, *M. tuberculata*. Cercariae emerge from the mollusc during the whole day, with vigorous swimming, alternating with short periods of rest, and have a life span of 72 hrs.

Description: Based on 30 live and some mounted specimens.

Body, 0.204-0.247 x 0.061-0.102, covered with spines, and two papillae with sensorial hair on one side. Tail, 0.429-0.873 x 0.015-0.030, long and thin, provided with undulated membrane dorsoventrally reaching to the end. Oral sucker subterminal, 0.043-0.059 x 0.029-0.048, surface is covered by structures of different shapes and sizes. A protrusible region was found with three rows of spines, carrying 8; 7; 6 spines in each row. Oral pore is covered with spines, pre-pharynx, 0.024-0.040 in length; followed by a muscular pharynx 0.010-0.016 x 0.013-0.016, Two ocular spots are located in each side above the pharynx; 3 ocular spots were observed in some cercaria; 2 on the left and one on the right side. Well developed acetabulum absent. A mass of rounded undifferentiated cells above the genital seems to be a rudimentary acetabulum. Seven pairs of penetration gland cells are located between the pharynx and the posterior part of body, with a thick granular content. The ducts extended towards the anterior region at the level of oral sucker and open anteriorly. Cystogenic gland cells with fine granular contents cover the whole body. Nervous system consists of two cerebral ganglion connected by a dorsal commissure. Anterior nerves pass laterally over the oral sucker and extend to the excretory bladder. The protonephridial system consists of an excretory bladder, 0.026-0.053 x 0.026-0.045; and main excretory ducts open to the excretory bladder anterolaterally, extending to the level of pharynx, where they bend and extending posteriorly. No other structures of the system could be observed due to intense dark body colour.

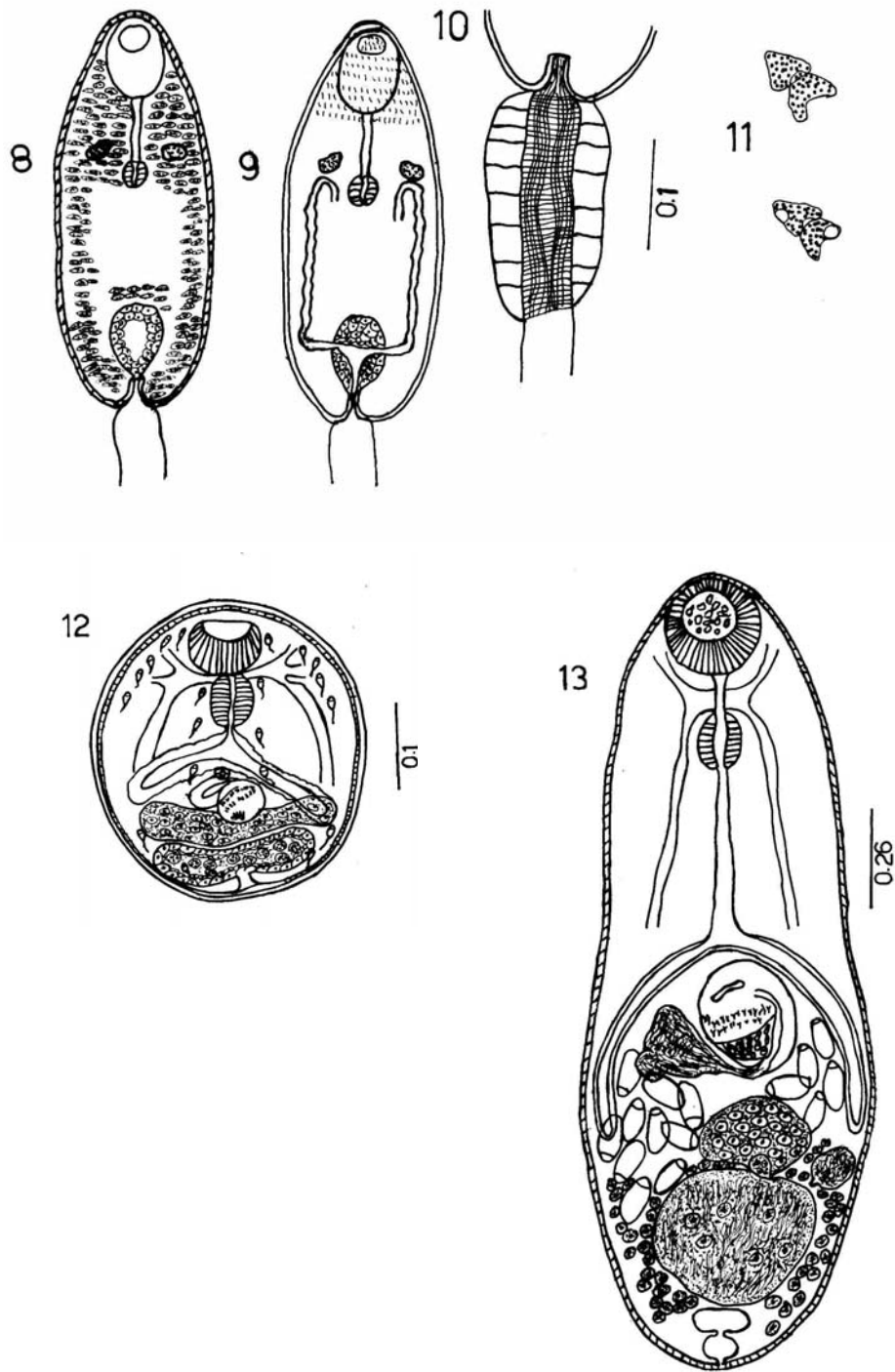
Metacercaria (FIG. 12): The cysts were extracted from muscular tissues of the base of caudal fins from the natural host *Rivulus hartii*.

Description: Based on 20 live specimens.



FIGURES 1-7. HAPLORCHIS PUMILIO (LOOSS, 1896) LOOSS, 1899.

1. REDIA/REDIA. 2. REDIA, SHOWING CERCARIAE IN SEVERAL STAGES OF DEVELOPMENT AND GERMINAL BALLS/REDIA MOSTRANDO CERCARIAS EN VARIOS ESTADOS DE DESARROLLO Y BOLAS GERMINALES.
3. ANTERIOR PART OF REDIA SHOWING PHARYNX AND INTESTINAL CAECUM/PARTE ANTERIOR DE LA REDIA MOSTRANDO LA FARINGE Y CIEGOS INTESTINAL. 4. ANTERIOR PART OF REDIA SHOWING GLANDULAR CELLS/PARTE ANTERIOR DE LA REDIA MOSTRANDO CELULAS GLANDULARES.
5. POSTERIOR PART OF REDIA SHOWING ADHESION GLANDULAR CELLS/PARTE POSTERIOR DE LA REDIA MOSTRANDO CELULAS GLANDULARES DE ADHESION.
6. CERCARIA SHOWING NERVOUS SYSTEM, EYE SPOTS AND RUDIMENTARY GENITAL ORGANS/CERCARIA MOSTRANDO SISTEMA NERVIOSO, MANCHAS OCULARES Y ORGANOS GENITALES RUDIMENTARIOS.
7. CERCARIA SHOWING PENETRATION GLAND CELLS/CERCARIA MOSTRANDO CELULAS GLANDULARES DE PENETRACION.



FIGURES 8-13. HAPLORCHIS PUMILIO (LOOSS, 1896) LOOSS, 1899.

8. CERCARIA SHOWING CYSTOGENIC GLAND CELLS/CERCARIA MOSTRANDO CELULAS GLANDULARES CISTOGENICAS.
 9. CERCARIA SHOWING PART OF PROTONEPHRIDIAL SYSTEM/CERCARIA MOSTRANDO PARTE DEL SISTEMA PROTONEFRIDIAL.
 10. CERCARIA, POSTERIOR PART SHOWING LONGITUDINAL AND CIRCULAR MUSCLES IN A SECTION OF THE TAIL/CERCARIA PARTE POSTERIOR MOSTRANDO MUSCULOS LONGITUDINALES Y CIRCULARES EN UNA SECCION DE COLA.
 11. EYE SPOTS OF THE CERCARIA/MANCHAS OCULARES DE LA CERCARIA. 12. ENCYSTED METACERCARIA/METACERCARIA ENQUISTADA. 13. ADULT/ADULTO.

The cyst is oval, with a fine and transparent wall, bearing metacercariae, body brown in color, 0.174-0.215 x 0.147-0.215, covered with spines. Small oral sucker, 0.018-0.053 x 0.045-0.064. Nervous system is clearly visible, dorsal commissure well developed, lateral nerve reaches anterior to oral sucker, and the posterior nerve is thicker. Digestive system developed. The mouth opens to mid oral sucker, followed by a short prepharynx, 0.008-0.021 in length, which opens to a muscular pharynx, 0.024-0.040 x 0.016-0.026, and ends in a short esophagus, 0.010-0.030 x 0.003-0.010, and arched intestinal caeca. Some metacercariae containing a light green disc or coin shaped inside the intestinal caeca. These structures are observed around the metacercaria inside the cyst. Penetration gland cell ducts are visible, but not their glands. Testes, 0.021-0.080 x 0.032-0.067, transversely elongated. Ventrogenital sac contains acetabulum, armed with several rows of spines, as of the adult. Genital pore located above the acetabulum. The rest of the reproductive system is still not well developed. Excretory bladder, 0.026-0.067 x 0.061-0.107, and 20 flame cells.

Experimental Infections

Encysted *Haplorchis pumilio* metacercariae from the natural host, *R. harti* were supplied experimentally to ducks, chickens, rats and mice to study the complete life cycle (TABLE I). Fecal materials of these animals were examined daily for eggs of the trematode. The eggs were seen for the first time in the fecal material of ducks (Nº 1 and Nº 2), three days after infection. The ducks were necropsied and 29 gravid and 3 adult trematodes were collected from the small intestine. The remaining animals were examined for up to 8 and 9 days post infection, but no eggs were found.

The freshwater fishes, *Astyanax* spp., *R. harti* and *P. reticulata*, collected from Agua Blanca, Sucre State, where *H. pumilio* doesn't occur, and laboratory reared, *Oreochromis*

mosambicus, were placed with recently emitted cercariae of *H. pumilio* by *M. tuberculata* (TABLE II). Both experimental fishes and molluscs were maintained in aquariums. After 48 hrs, the caudal fin of one specimen of each species of fish, was observed live under the stereoscopic microscope to determine the presence of any metacercariae. The early formed encysted metacercariae, were only seen in the muscular tissue at the base of caudal fin under the scales of *R. harti*. These fishes were maintained in aquariums for up to 30 days to allow metacercarial development. The rest of the fish remained negative at 8; 12 and 15 days after ingestion.

A 3rd set of experiments was carried out with 2 chickens and 3 ducks using metacercariae obtained experimentally from *R. harti*, and birds were sacrificed 3 days post infection. No chicken were infected, but all three ducks were infected and 23 gravid and 3 adult worms were recovered from these hosts. All trematodes recovered from experimental hosts were identified as *H. pumilio* (Looss, 1896) Looss, 1899.

Butoridis striatus of Chirgua locality of Sucre State was found also as natural host of this trematode.

Adults (FIGS. 13,14,15,16,17). Recovered from small intestine of common duck, *Cairina moschata*.

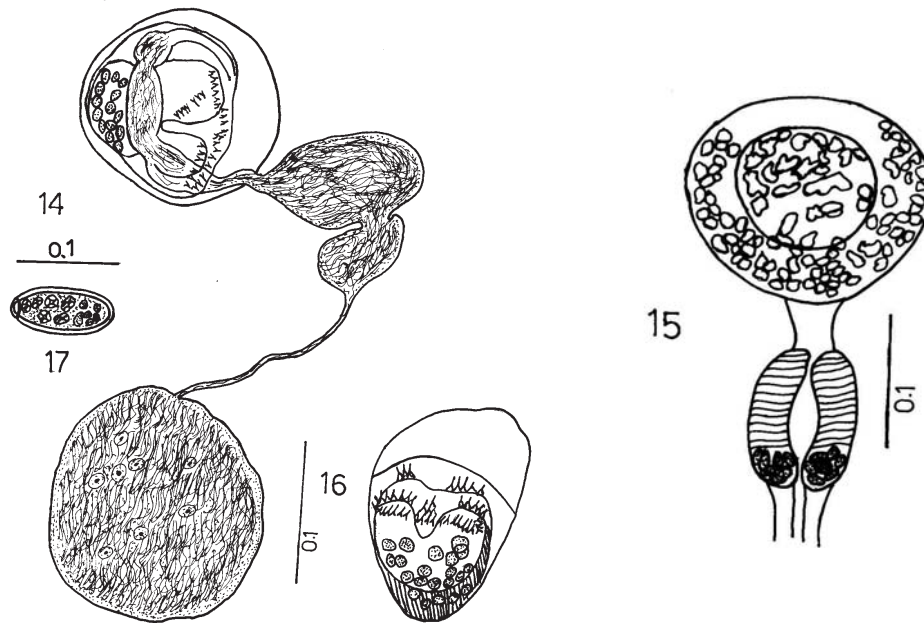
Description. Based on 20 mounted and live specimens. Body elongated, rounded at both ends, 0.365-0.517 x 0.121-0.192, covered entirely with spines. Oral sucker terminal, 0.040-0.053 x 0.048-0.067, Prepharynx, 0.010-0.026 x 0.005-0.010, long. Muscular pharynx, 0.026-0.040 x 0.018-0.029, well developed. Long oesophagus, 0.048-0.107 x 0.008-0.013, bifurcating in front of the ventro-genital complex in two intestinal caecae, reaching almost to the posterior part of the ovary. Complex ventrogenital, 0.054-0.075 x 0.040-0.067, located below the intestinal bifurcation, contains genital sac, acetabulum and the genital pore. Acetabulum small, 0.037-0.058 x 0.030-0.052, armed with a ring of 36-40 tiny hooks or spines. Two dorso-lateral lobes, one with 3-5 (right lobe) and the other with 4-5 (left lobe). Testes round, 0.056-0.083 x 0.059-0.102, located in the posterior part of the body. Deferent duct emerges from anterior border and extends anteriorly to enter into seminal vesicle. Seminal vesicle bipar-

TABLE I
EXPERIMENTAL INFECTION OF DIFFERENT ANIMALS WITH ENCYSTED METACERCARIAE OF HAPLORCHIS PUMILIO FROM RIVULUS HARTI (INFECCIÓN EXPERIMENTAL DE DIFERENTES ANIMALES CON METACERCARIAE ENQUISTADO DE *H. PUMILIO* DE *R. HARTI*). 20 CYSTS WERE SUPPLIED TO EACH EXPERIMENTAL HOST. (20 QUISTES ADMINISTRADOS A CADA HUESPED EXPERIMENTAL).

Animal	Examined after (days)	Results
Duck 1	3	15 gravids +1 adult
Duck 2	3	14gravids + 2 adults
Rat 1	8	Negative
Rat 2	8	Negative
Mice 1	9	Negative
Mice 2	9	Negative
Chicken 1	8	Negative
Chicken 2	8	Negative

TABLE II
EXPERIMENTAL INFECTION OF FRESHWATER FISHES FROM AGUA BLANCA WITH CERCARIAE of HAPLORCHIS PUMILIO RECOVERED FROM NATURALLY INFECTED MELANOIDES TUBERCULATA (INFECCIÓN EXPERIMENTAL DE PECES DE AGUA BLANCA CON CERCARIAE RECUPERADO DE *M.TUBERCULATA*).

Fish species	Nº of fish	Autopsied after (days)	Nº of fish infected
<i>O. mosambicus</i>	4	12	0
<i>P. reticulata</i>	7	8	0
<i>R. harti</i>	10	30	10
<i>Astyanax</i> spp.	4	15	0



FIGURES 14-17. HAPLORCHIS PUMILIO (LOOSS, 1896) LOOSS, 1899.

14. MALE REPRODUCTIVE SYSTEM/SISTEMA REPRODUCTOR MASCULINO. 15. ORAL SUCKER AND PHARYNX SHOWING STRUCTURES ON THE SURFACE/VENTOSA ORAL Y FARINGE MOSTRANDO ESTRUCTURAS EN SU SUPERFICIE. 16. ACETABULUM SHOWING HOOKS ON ITS DORSAL SURFACE/ACETABULUM MOSTRANDO ESPINAS Y GANCHOS EN SU SUPERFICIE. 17. EGG, NON EMBRYONATED/HUEVO NO EMBRIONADO.

tide; anterior part larger, and posterior part slimming to form a prostatic duct that penetrates to the ventro-genital sac. Ovary, 0.026-0.061 x 0.032-0.067, pretesticular. Seminal receptacle, 0.020-0.032 x 0.019-0.030, round, variable in size, located at the right side of the ovary and above the testes. Vitelline gland cells formed by small follicles distributed mainly in the post-ovarian region. Lateral vitelline ducts unite below the ovary to form a small vitelline reservoir, located between the ovary and testes. Uterine knobs occupy the space between ventro-genital complex and testes. Eggs large, 0.026-0.034 x 0.013-0.018, not very numerous, non embryonated. Excretory bladder formed by two chambers, communicating with short neck. Excretory pore terminal.

Haplorchis pumilio was originally described from two species of birds, *Pelecanus onocrotalus* and *Milvus migrans* in Egypt [6]. Later, it was reported from China, Japan, Palestine, The Philippines, Thailand, Laos, Australia, Malaysia, Kenya and Mexico, infecting a diverse array of animals, such as: birds, reptiles and mammals, including man. Nasir and Díaz [8] described a cercaria belonging to the *Pleurolophocercous* group, from the freshwater mollusc, *Stenophysa venezuelensis*, from Laguna de los Patos, Sucre state, Venezuela, which was similar to *H.pumilio* but differed in the number and ar-

range of the penetration gland cells. Scholz *et al.* [13] reported for the first time, the occurrence of *H.pumilio* cercaria emitted by *M.tuberculata* in Mexico. Scholz *et al.* [12] did not describe the cercaria in detail but referred to the similarity with the morphology described by Martin [7] and Shen [15]. There are 26 flame cells in FIG 3F of this study, which does not coincide with the 18 flame cells reported by Khalifa *et al.* [5], 36 of adult by Sommerville [16] and 20 in the metacercaria of the present study; and did not refer to the precercarial stages. Chen [1] redescribed *H.pumilio* from intestines of dogs, cats, rabbits, rats, mice, birds and humans in China. Chen [1] considered that the descriptions of certain important structures of this parasite, published by previous authors [3, 9, 18] were inadequate. Many authors took the size of eggs and the position of ovary as important taxonomic characters, while Chen [1] found them very variable even within the same species. Chen [1] emphasized the importance of elucidating detail structures of the armature of the acetabulum which seems to provide more stable characters for this genus. On the basis of detailed studies of the armature of the acetabulum, he considered *H.taihukui* Nishigori (1924) and *H.pumilio* as similar species, but, *H.taichui* Nishigori (1924) as a valid species, an opinion shared by Pearson [10], Khalifa *et al.* [5], Pearson and Ow-

yang [11] and the present authors. The present species of *H. pumilio* is very similar to the one redescribed by Chen [1] for the smaller size of the body and seminal receptacle, and the larger size of the seminal vesicle. The number of hooks observed in the acetabulum varied from 35 to 37 and 38 to 40 in natural and experimental hosts in this study. Chen [1], however, did not mention the two small dorso-lateral lobes with spines, or the central group of long spines present in the acetabulum described in this study. Pearson [10] redescribed *H. pumilio* from the intestine of the water rat, *Hydromys chrysogaster* in Australia. There are no marked differences between the Australian *H. pumilio* and the one found in the present study, except for slight variations in the number of spines in the dorso-lateral lobes and the central group of spines. The only notable difference is the presence of a single deferent vessel from this study, compared with two from the specimen described by Pearson [10]. Pearson and Ow-Yang [11] reported a *H. pumilio* from the small intestine of *Haliastur indus* in Malaysia and included a detailed description of the position and number of acetabular spines, spines from the lateral lobes and the central group, which are very similar to those described in the present study. Sommerville [16] described the life cycle of *H. pumilio* in Africa, infecting different species of fresh water fish with cercariae emitted by naturally infected *M. tuberculata*. The cysts were found in muscles, gills and caudal fins of fish hosts which were fed to pigeons, chickens and ducks. Adults were recovered only from pigeons, but no parasites were found in chickens and ducks. This author did not provide any description or illustration of the different stages of this trematode. Khalifa et al. [5] described the life cycle of *H. pumilio* in Egypt, which is closely similar to the one described here, with slight variations in the morphology of different larval stages. The Prepharynx and pharynx of cercariae found in the present study are well developed, and there are also differences in the number and arrangement of the penetration spines. The description of adults is also very close to the one described in this study, with slight differences in body size, testes, position of the acetabulum spines and egg development. Thus, there are slight differences in some morphological details of *H. pumilio* described by different authors from different regions of the globe which could be attributed to geographical and interspecific, rather than taxonomic variation.

CONCLUSIONS

The life cycle of *Haplorchis pumilio* was studied from reidia to adult both under natural and experimental conditions. All developmental stages were described and illustrated.

Of five freshwater fishes used as experimental hosts, only *Rivulus harti* could be infected with cercariae emitted by *Melanoides tuberculata*. Metacercariae of *H. pumilio* were experimentally fed to ducks, chicken, rats and mice to define the possible definitive host; adult trematodes were recovered only from the ducks.

Butorides striatus and *R. harti* were found to be new natural host of *H. pumilio*. The present finding is the first record of *H. pumilio* from Venezuela.

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