

Experimental life cycle of *Philophthalmus gralli* (Trematoda: Philophthalmidae) in Venezuela

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Abstract: Descriptions of the miracidium, mother redia, daughter redia, granddaughter redia, cercaria, metacercaria and adult stages of *Philophthalmus gralli* Mathis and Leger, 1910 recovered from experimental infections are presented. The intermediate host, *Melanoides tuberculata*, was collected from freshwater rivulets in Aguasanta and Yaguaracal, Sucre State, Venezuela. Chicken were orally infected with cercariae and metacercariae, and metacercariae were introduced directly into the eyes by pipette. Both processes of infection produced adult worms. This is a new geographical record for *P. gralli*.

Key words: Trematoda, *Philophthalmus gralli*, chicken, life cycle, Venezuela, *Melanoides tuberculata*.

Looss (1899) created the genus *Philophthalmus* for *P. palpebrarum* from under the eyelids of birds from Egypt. Since then, over 36 species of this genus have been reported from Asia, Europe and America and adult *P. gralli* Mathis and Leger, 1910 were reported from the eyes of geese in the Azraq Oasis, Jordan by Ismael and Issa (1987). Ismael and Saliba (1985) isolated a gymnocephalous cercaria from *Melanoides tuberculata* snails in the same oasis, identified as *P. gralli* (Ismael and Issa 1987). This species has not been reported so far in Venezuela and this is the first natural occurrence of cercariae of *P. gralli* in a freshwater mollusc, *M. tuberculata*, from Aguasanta and Yaguaracal, Sucre State, Venezuela. In addition, Nasir and Diaz (1972) reported the occurrence of two more philophthalmid trematodes: *P. lacrymosus* and *Ophthalmotrema semipalmatus* in birds from Venezuela.

The present report describes all developmental stages of its life history acquired from experimental infection and compares the discrepancies and inconsistencies recorded in *P.*

gralli in other geographical regions.

MATERIALS AND METHODS

A total of 250 freshwater snails of *M. tuberculata* was collected from rivulets of Aguasanta and Yaguaracal, Sucre State, Venezuela during 1998 and 1999, and maintained in a laboratory in aquaria filled with water from the snails' natural habitat. Ten were infected with cercariae of *P. gralli*, which were maintained in separate glass bowls in dechlorinated water, and exposed to artificial lighting (tungsten tube light) to stimulate shedding of cercariae. Each container was examined every morning for emergent cercariae. Emerging cercariae encysted and preferably stayed at the bottom of the container.

All materials collected were deposited in the Laboratory of Parasitology, Department of

Biology, School of Science, Núcleo Sucre, Universidad de Oriente, Cumaná, Venezuela.

Experimental infection of the host snail:

Fifteen *M. tuberculata* snails cultured and grown in the laboratory were infected with miracidia of *P. gralli*. Miracidia were recovered from experimentally grown adult *P. gralli* (41 - 43 days old) in chicken. Snails were infected as follows: 3 uninfected snails were placed in a small glass dish with 10 cc of water and 10 miracidia were pipetted into each. These snails were later transferred to an aquarium, and 15 days post-infection, they were transferred to bowls and examined every morning for the emergence of cercariae. Two snails produced cercariae of *P. gralli* after 83 days of infection and (buried cysts at the bottom of the bowl.

RESULTS

(All descriptions are based on 25 live and mounted specimens.)

Experimental infection of chicken: A total of 103 adult *P. gralli* was recovered from the nictitant membrane of 11 experimentally infected chicken of 3 - 4 days old. These chickens were infected either orally or directly on the eyes using a Pasteur pipette. The results of the experimental infection are presented in

Table 1. Alicata and Ching (1960) and Ching (1961) recommended these methods of infection. Both cercariae and excysted metacercariae introduced in the chicken grew to sexually matured adult *P. gralli*. Two rats were fed orally 20 and 30 metacercariae and autopsied after 23 and 25 days, respectively. No parasites were found in the experimental hosts (Table 1). No conjunctivitis was observed in the eyes of infected chicken as mentioned by Alicata (1962).

Encystation (Figs. 12-13): Freshly emerged cercariae are not active swimmers. Swimming is slow and occurs by undulations of the body and tail in the form of an "S". They hang upside down by their tail tips when they are near the surface of the water during the period of swimming up and down. Possibly, the efflux of some sticky substance from the caudal end, aids cercariae in the act of suspending themselves from the surface film of the water. While hanging upside down, cercariae perform contractile movements constantly. Sooner or later, tail tips get detached, cercariae resume their undulatory and contractile movements, and reach the bottom of the container where they crawl with the help of their suckers. At times while crawling a cercaria

TABLE 1

Experimental infection of chicken and rats by cercariae and metacercariae of Philophthalmus gralli

Experimental host	Age of metacercariae (days)	Date of infection	Number of cysts fed to host	Location in host	Duration of infection (days)	No. of adults recovered
Chicken	2-3	4-5-98	24	Nicme	25	14
Chicken	2-3	4-5-98	31	"	25	3
Chicken	2-3	6-5-98	34	"	19	8
Chicken	2-3	12-5-98	53	"	17	1
Chicken	2-3	12-5-98	35	"	17	0
Chicken	Cercariae ¹	8-5-98	23	"	18	2
Chicken	2-3	24-11-98	32	"	22	10
Chicken	2-3	26-11-98	35	"	23	1
Chicken	2-3	26-11-98	39	"	23	6
Chicken	2-3	2-12-98	40	"	41	32
Chicken	2-3	2-12-98	40	"	43	26
Rat	2-3	26-11-98	20	Neg	23	0
Rat	2-3	26-11-98	30	Neg	25	0

(1 = Recently emitted cercariae; Nic. me = nictitating membrane of eye.)

attaches itself to the substratum by its tail tip, stands upright and performs swaying movements. During crawling, its forebody actively moves by contraction and expansion, apparently to select a suitable site for encystation. The cercarial body contracts as soon as attachment is secured, and then the body shortens and loses its characteristic shape. The body flattens and broadens out, particularly the forebody; the tail, too, contracts and lashes sideways. Subsequently, a cystogenous secretion oozes from the body and envelopes it. The cystogenous materials, due to their continuous exudation, spread from either side towards the anterior extremity of the cercarial body, gradually forming a cyst wall. While the process continues, posterior bodies of cercariae take the shape of a bottleneck and tails detach. The process of cyst formation is completed within a period of 40 s to 1 min. The anterior part of metacercariae in recently formed cysts is directed towards the bottom of the bottle but it changes its position within a short time. Cercariae tend to encyst on whichever object they attach to. They have even encysted on the tip of dissecting needles and pipettes.

Measurements of encysted metacercaria of *P. gralli*: Cyst 335-387 x 225 - 295 μm ; body 217 - 295 x 135 - 182 μm ; anterior part of cyst 26 - 53 x 86 - 99 μm ; oral sucker 48 - 64 x 51 - 67 μm ; prepharynx 5 - 21 x 5 - 5 μm ; pharynx 24 - 34 x 21 - 29 μm ; esophagus 18 - 26 x 13 - 16 μm and acetabulum 51 - 72 x 72 - 88 μm .

Miracidium (Fig. 1): The oval shaped miracidia measuring 94 - 156 μm long by 0.051 - 0.067 μm wide, totally ciliated except for the extreme anterior region which is occupied by apical papillae and separated by rows of epidermal plates. Cilia bundled together to form a tuft on each side of the apical papilla. Each side consists of 4 papillae with sensorial hairs. The apical gland with granulated materials opens to the apical papilla. Epidermal plates are arranged in 4 tiers. The 1st, 3rd and 4th rows consist of 6 plates and the 2nd of 8 plates. Eye spots located between the 1st and 2nd rows of epidermal plates, consist of one pair of dark brown pigmented bodies in the shape of an inverted "L". The nervous system

is composed of one large cephalic ganglion with numerous small nuclei. Penetration glands were not detected clearly, but two glandular ducts on each side of the apical gland were observed. Two large flame cells are located at midlevel on both sides of the body. A mother redia was encased in the miracidial body cavity.

Mother redia: Mother redia within a miracidium occupies about two-thirds of the body. Anterior parts of body possess muscular structures similar to a pharynx. A young mother redia in a miracidium measures 80 - 118 μm in length and 32 - 40 μm in width. The external surface lacks papillae and other structures. The posterior part possesses two ventro-lateral processes and a caudal appendix. There are 3 nucleated glandular cells which open to the anterior margin of the body of the redia and the right cell is larger than other two cells. The granular contents of these cells are fine. The redia possesses germinal cells and balls. The protonephridial system consists of symmetrical bilateral flame cells and their ramifications. Each half of the system consists of 2 flame cells which open to a small vessel on each side of body.

Daughter redia (Figs. 2-5): The elongated body measures 558 - 812 μm in length and 131 - 182 μm in width and its anterior margin is decorated with a row of sensory filaments.

The pharynx is muscular, measures 56 - 67 μm in length and 40 - 48 μm in width, followed by a short esophagus. The long intestinal caecum almost reaches the posterior part of the body of the redia, and contains brown-yellow granular materials. Birth pore is inconspicuous, and is located on one side of body just below the level of the pharynx. A pair of protrusive locomotory appendices are located at posterior part of body with a transverse pore, similar to ventral sucker. A prominent caudal appendix is present, measuring 80 - 215 μm long and 40 - 48 μm wide. Both the locomotory and caudal appendices are used for adhering to tissues of snail. The body cavity of rediae contains a maximum of 8 germ cells. Protonephridial system in some

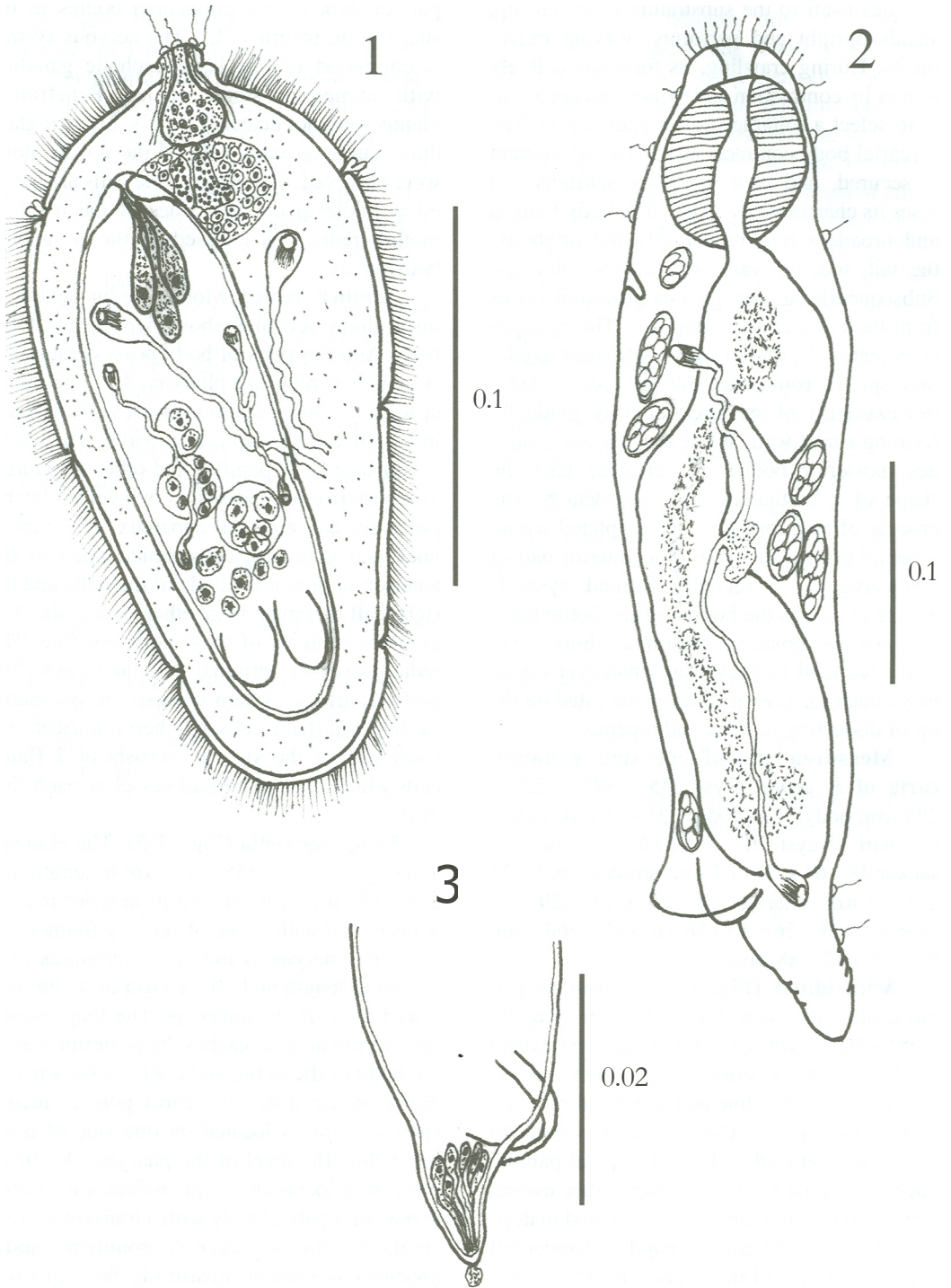


Fig. 1. Miracidium of *P. gralli*, including mother redia.
 Fig. 2. Daughter redia, showing pharynx and intestinal caecum.
 Fig. 3. Caudal appendix of a redia with adhesive glandular cell.

daughter rediae is similar to mother redia (2 flame cells) but in others varies between 4 - 8 flame cells in each redia.

Granddaughter redia (Figs. 6-7): The majority of these were found in abundance free in the digestive gland but some were found attached to tissues of snail. The structure of grand daughter rediae is somewhat similar to daughter rediae but much more developed. These appear subcylindrical with a blunt anterior end and a conical posterior end in live, and measures 548 - 1268 μm in length and 182 - 243 μm in breadth. The terminal mouth is surrounded by sensory filament and papillae. A birth pore is located anteriorly a little behind the pharynx. The pharynx is muscular and measures 51 - 64 X 45 - 56 μm ; the gut is long and extends beyond mid-body. The caudal process is well developed and the caudal appendix measures 18 - 269 X 13 - 88 μm . Besides germ cells, germ balls, 1 - 5 cercariae of different stages are found in these rediae. A protrusive excretory pore is situated on opposite to intestinal caecum and are observed only 5 flame cells.

Cercariae (Figs. 8-11): The body is elongated, the preacetabular region is wider than the postacetabular, beset with fine spines. It measures 456 - 598 μm in length and 101 - 162 μm in maximum width. The tegument is thick, beset with papillae with sensory filament. A tail terminal with small spine-like points measures 294 - 446 x 30 - 50 μm . Parenchymetic cells of different sizes and forms with nucleus are observed in the center of the tail. A short invagination observed at the end of the tail may function as an adhesive organ. It contains glandular cells with fine granular materials which secretes viscous substances, probably for adhesiveness of cercariae. Longitudinal and transverse muscles are observed in the tail of live cercariae, which may be responsible for undulation and expansion. An oral sucker sub-terminal measures 55 - 64 x 51 - 61 μm . The external border of the mouth is surrounded with papillae, with only 4 papillae on the inside border. The alimentary canal includes a prepharynx, measuring 10 - 61 x 5 - 10 μm and strong muscular pharynx, measuring 26 - 40 x

18 - 28 μm , followed by long esophagus, measuring 45 - 94 x 18 - 37 μm in mounted specimen. Esophageal bifurcation occurs near to the anterior third of the body and intestinal caeca extend posteriorly up to the hind end of the body. Acetabulum slightly posts equatorial and its internal and external borders are lined with papillae.

A mass of gland cells organized in two groups of 16 each are on either side of the esophagus. The ducts of these gland cells on each side run forward in a compact bundle and open independently at the anterior border of body. Genital primordium is composed of 2 masses of undifferentiated cells, localized immediately anterior and posterior to acetabulum. Nervous system consists of a transverse commissure, situated between oral sucker and pharynx, which give rise to 2 anterior and 2 posterior nerves. The short anterior nerves pass lateral to an oral sucker and longer posterior nerves pass posteriorly up to the end of the intestinal caecum.

Cytogenous cells are numerous throughout the body except in the region occupied by suckers and excretory bladder. The excretory bladder is single chambered and located at posterior end of body. The primary excretory duct arises from a short stem from the excretory vesicle. It runs forward up to the level of the pharynx where it recurves and continues posteriorly as secondary excretory ducts with three tufts of preacetabular cilia and one postacetabular, reaching the level of the excretory bladder where it forms a loop and gives rise to the tertiary excretory duct. Then, between the esophageal bifurcation and the ventral sucker, it bifurcates into an anterior and posterior collecting canal; of these two, the former runs forward up to the pharyngeal region, while the latter up to the caecal termination. Each is connected to the canaliculus of flame cells. The excretory pore is terminal. The flame cell formula is $2 [(3 + 3 + 3) + (2 + 2 + 2)] = 30$.

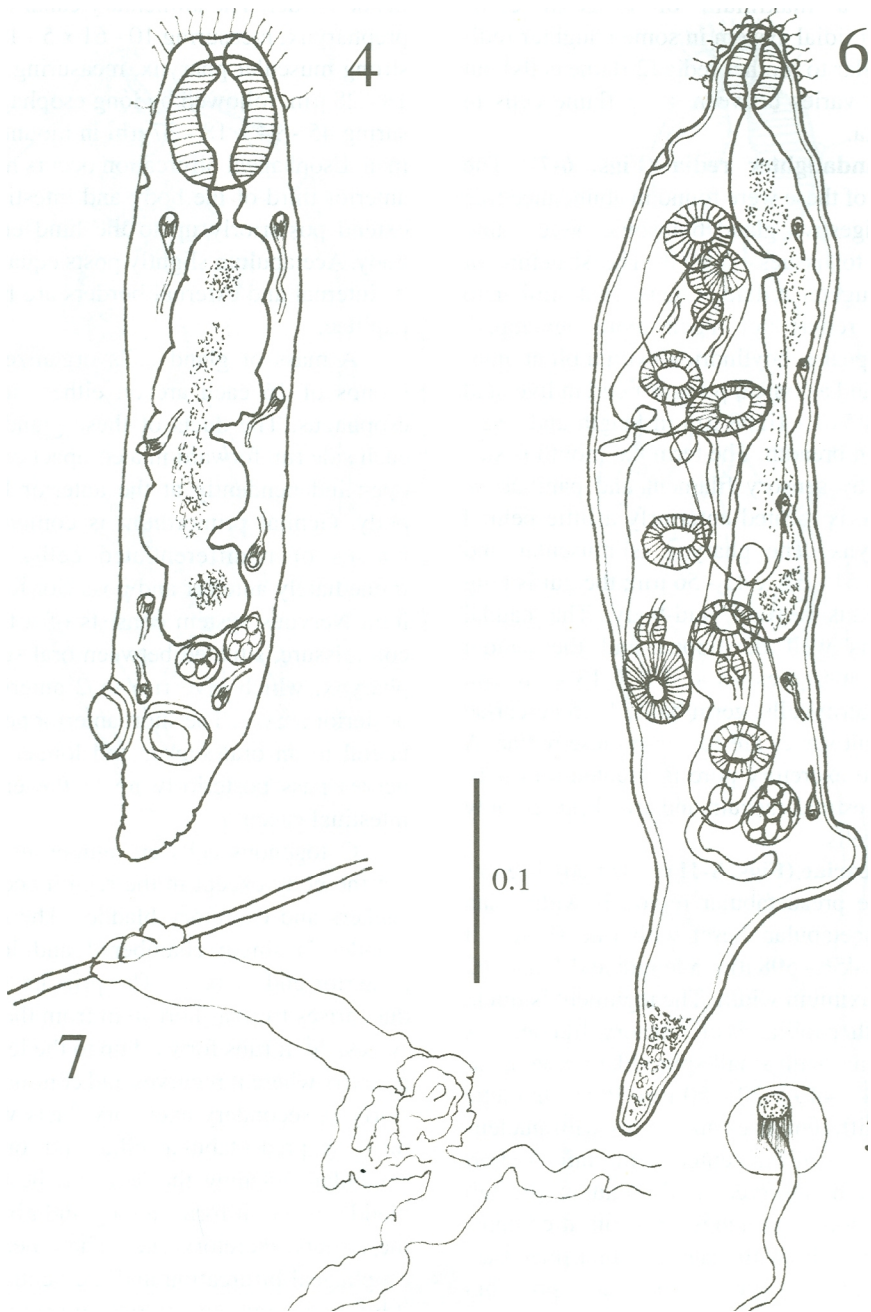


Fig. 4. Daughter redia with flame cells.

Fig. 5. Flame cells of daughter redia.

Fig. 6. Granddaughter redia containing cercariae of different stages of development.

Fig. 7. Excretory system of granddaughter redia with lobulated excretory pore.

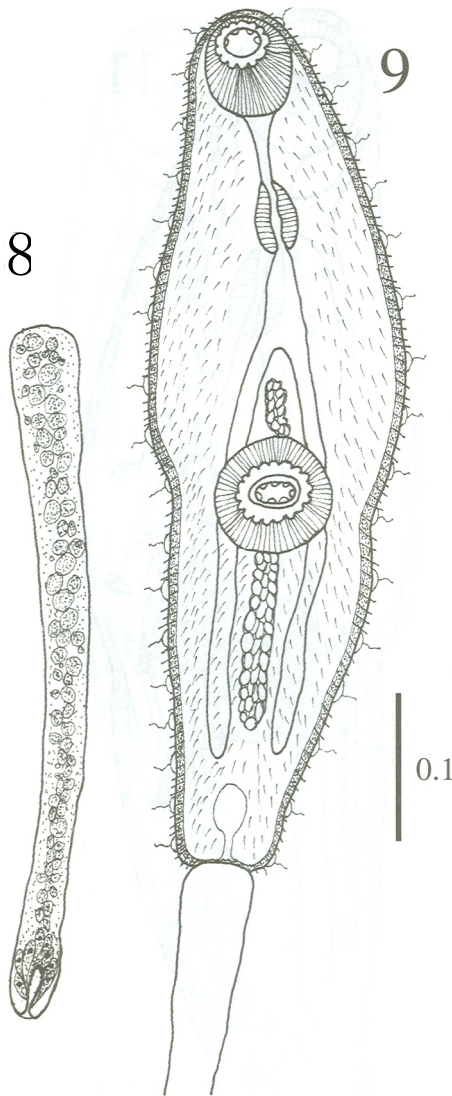


Fig. 8. Structure of tail of cercaria. Adhesive glandular cells at extreme posterior end.

Fig. 9. Cercaria showing sensorial papillae, body spines and rudimentary genital primordium.

Excysted metacercaria (Fig. 14): The body is spinose and elongated and the preacetabular region is wider than the postacetabular region. It measures 153 - 190 μm in length and 36 - 47 μm in maximum width. The spines are more prominent and abundant in the post acetabular regions. There appears to be fine spines on the border of anterior portion of body. The suckers are more developed than in

the cercaria. Oral sucker measures 56 - 61 μm x 43 - 51 μm and ventral sucker, 40 - 72 μm x 56 - 72 μm .

The mouth leads through a long prepharynx measuring 29 - 45 x 5 - 8 μm and into a muscular pharynx measuring 29 - 34 x 21 - 24 μm . The esophagus is long, measuring 40 - 64 x 8 - 13 μm , and bifurcates near the ventral sucker. The intestinal caecum is long and narrow, extending backwards up to the excretory bladder. The rudimentary reproductive organs appear as two undifferentiated mass of cells on and below the ventral sucker. A mass of round gland cells with granular cytoplasm of unidentified number is located on either side of esophagus. About 20 - 22 gland cell pores with their respective ducts are observed on each side, close to the body margin. The nervous system is slightly better developed than in cercariae, but the posterior nerves are finer. The excretory bladder is increased in size but the arrangement of flame cells remained same as cercaria.

Description of adult (Figs. 15-17): The body is elongated, slightly attenuated at the anterior end and rounded posteriorly. Its length is 2.564 - 3.384 mm and its width is 0.512 - 1.205 mm. The tegument in the anterior part is covered with minute spines and, in some, it is difficult to observe. The oral sucker is subterminal, 237 - 297 μm long and 287 - 378 μm wide. The prepharynx is visible in the live worm. The pharynx is muscular, 227 - 323 μm long and 227 - 333 μm wide. The esophagus is 65 - 151 μm long and 80 - 141 μm wide, and bifurcates anteriorly to the acetabulum. The caecum is conspicuous, ending blindly in the posterior part of body at the level of excretory bladder. The acetabulum is pre-equatorial and very muscular, 388 - 544 μm long and 409 - 505 μm wide and the internal margin is surrounded by receptor sensorial papillae.

The testes are located one behind the other in the posterior part of the body, smooth or lobulated, measuring 272 - 378 x 424 - 530 μm long and 272 - 464 x 404 - 505 μm wide. The efferent ducts rise antero-laterally from each testes and unite to form a single duct

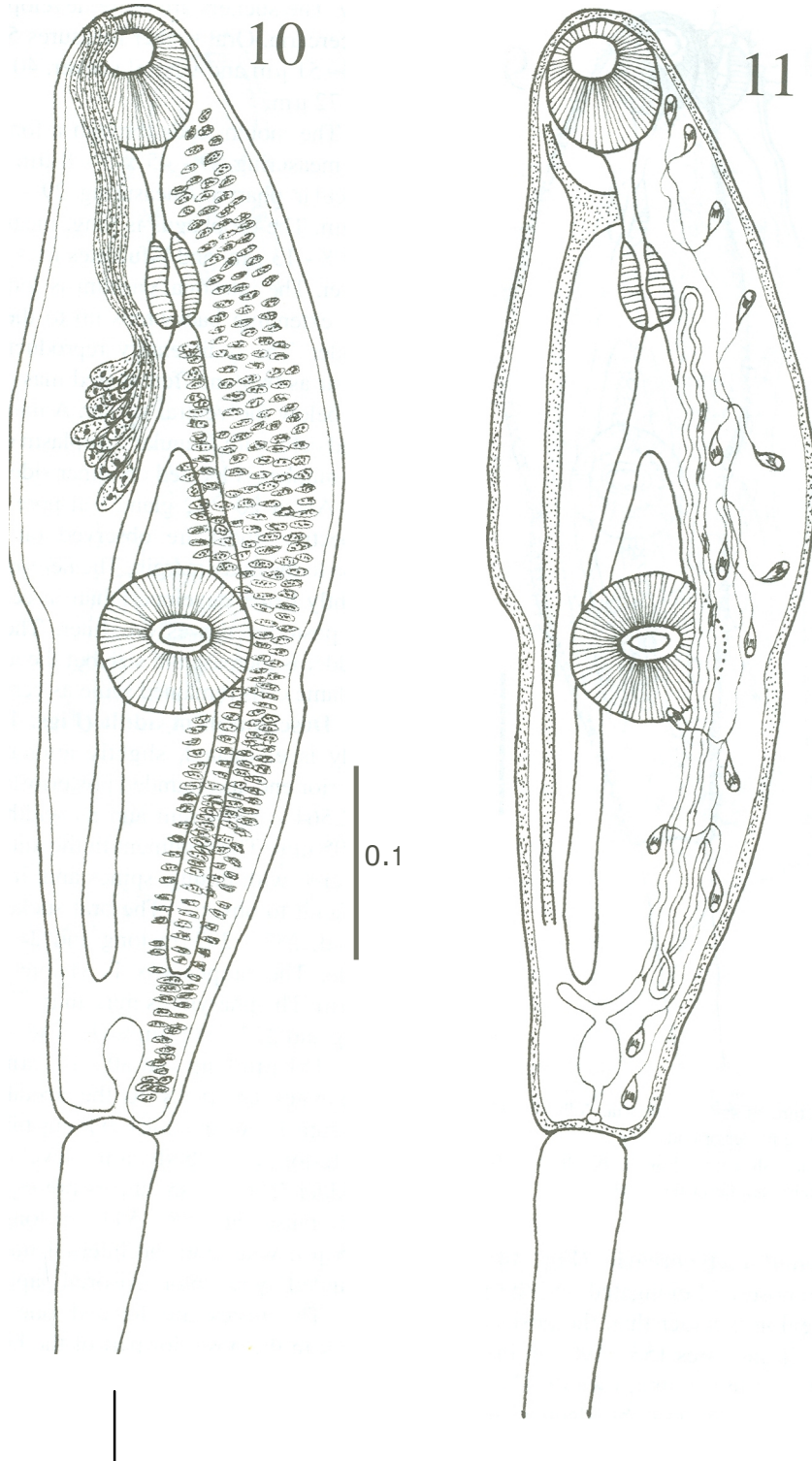


Fig. 10. Cercaria showing glandular penetration cells (left) and cystogenic glandular cells (right).
Fig. 11. Cercaria with protonrphridial system (right) and nervous system (left).

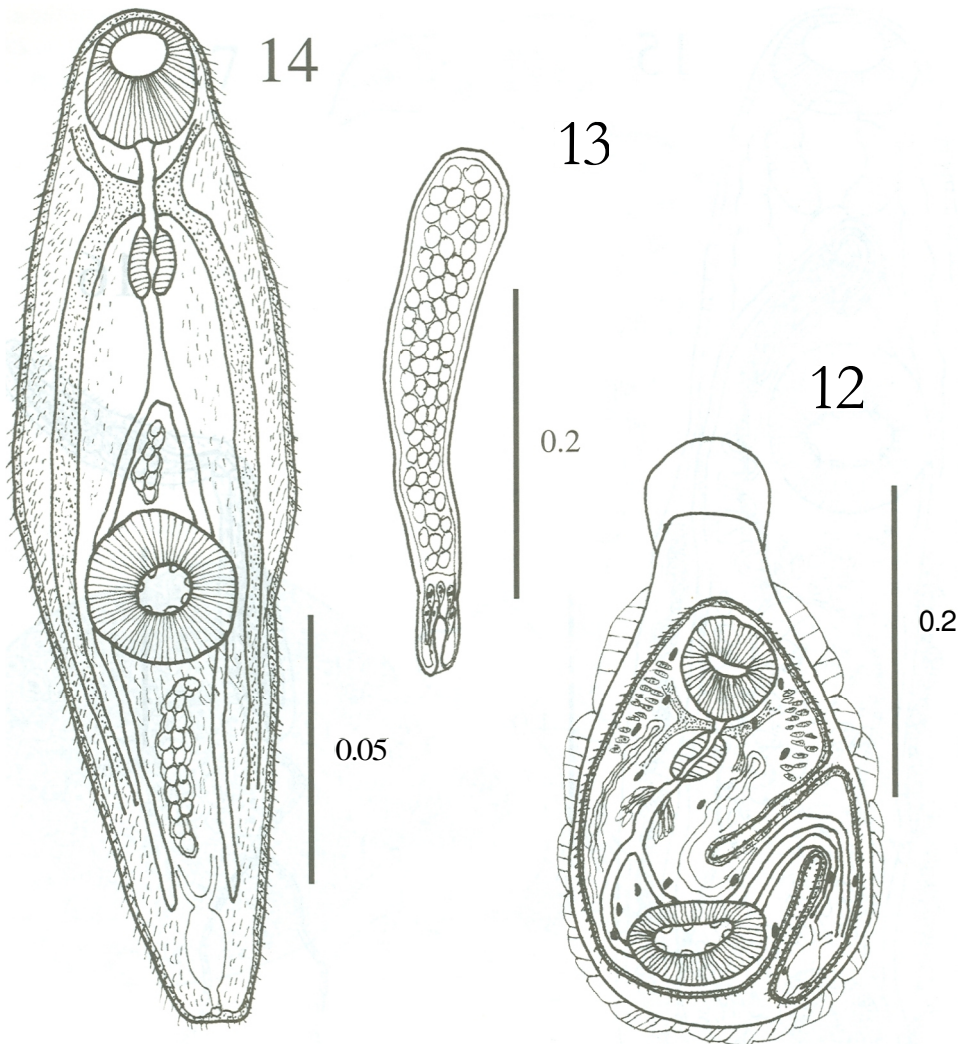


Fig. 12. Encysted metacercaria of *P. gralli*.
 Fig. 13. Vacuolated tail of encysted metacercaria.
 Fig. 14. Excysted metacercaria.

which opens to the cirrus pouch. The seminal vesicle may be elongate or globular. The seminal vesicle varies in its location depending on whether the cirrus extrudes from the cirrus pouch. The cirrus pouch is 717 - 868 μm long and 106 - 186 μm wide. The genital pore is close to esophageal bifurcation. The ovary is subglobular, 126 - 222 μm long and 151 - 252 μm wide, and is located in front of the anterior testes. The oviduct is muscular, rising from the dorsal posterior surface of ovary. It extends posteriorly to the Laurel canal. The

oviduct extends to join with the principal vitellarian duct which later forms an ootype surrounded by the gland cells of the Mehlis gland. The vitellaria is tubular, long and bilaterally symmetrical. The uterus is elongated, coiled and covers the space between the ventral sucker and the testes. The first part of uterus serves as a uterine seminal receptacle. The uterus is filled with eggs in different stages of development. The eggs measure 74 - 80 x 25 - 35 μm . The excretory bladder elongates transversally, with an excretory pore terminal which opens



Fig. 15. Adult *P. galli*.

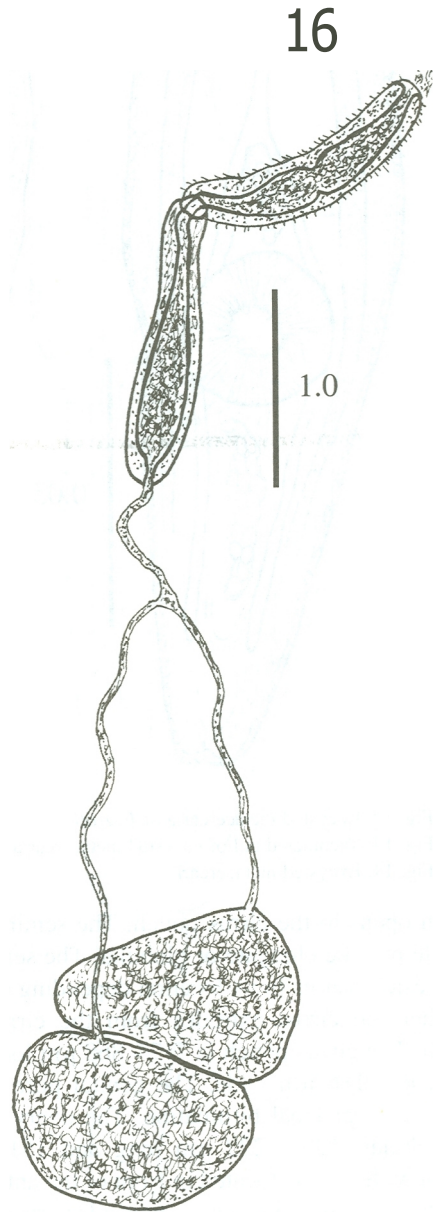


Fig. 16. Male reproductive system with everted cirrus.

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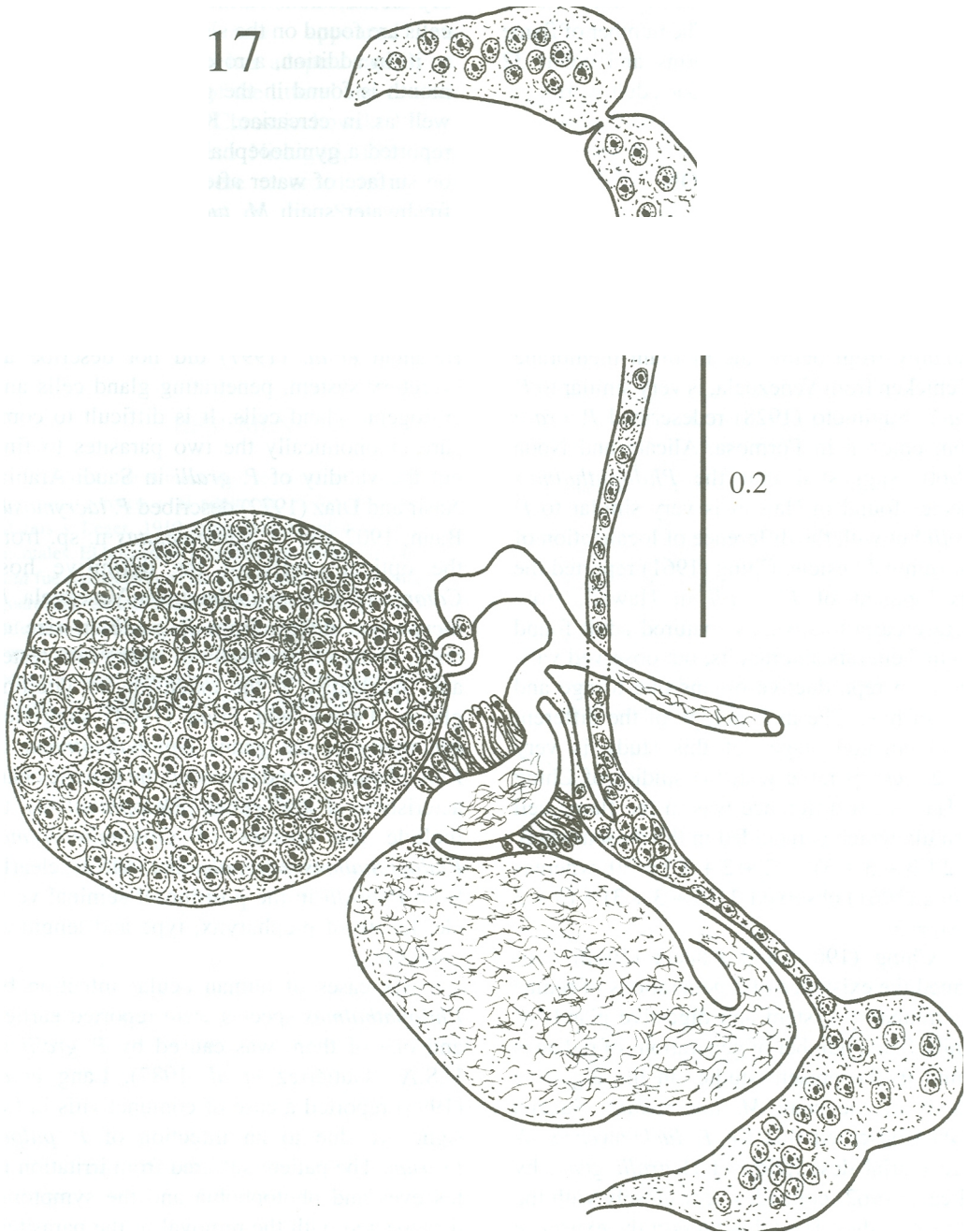


Fig. 17. Female reproductive system.

subterminally. The main excretory duct extends up to the level of pharynx. The number of flame cells increase in adult worms and count 32 flame cells on each side of one adult worm.

DISCUSSION

Looss (1899) created the genus *Philophthalmus* to accommodate *P. palpebrarum*, a non-spinous trematode found in the eyes of a bird in Egypt. The trematode, obtained experimentally from below the nictitant membrane of chicken from Venezuela, is very similar to *P. gralli*. Sugimoto (1928) redescribed *P. gralli* from chicken in Formosa. Alicata and Noda (1960) suggested that the *Philophthalmus* species found in Hawaii is very similar to *P. gralli* but with the difference of localization of the seminal vesicle. Ching (1961) reported the development of *P. gralli* in Hawaii, from metacercaria to sexually matured adult found in chicken, rats and rabbits, but observed variations in reproductive organs due to age and type of host. The morphology of the different developmental stages of this study is very closely comparable with the studies of Ching in Hawaii. A difference was in the flame cell formula which is included in the present study as $2 [(3 + 3 + 3) + (2 + 2 + 2)] = 30$, whereas Ching (1961) observed $2 [(3 + 3 + 3) + (3 + 3 + 3)] = 36$.

Ching (1961) and Alicata (1962) confirmed the existence of 3 generations of rediae in circulatory system and digestive glands of snails. Same number of generations of rediae is found in the present studies in the digestive glands of the snail *M. tuberculata*. Saxena (1984) observed this for *P. lucknowensis*. A precercarial description of *P. gralli* given by Alicata (1962) coincides very closely with the present studies. He did not report the existence of 3 nucleated gland cells with fine granular contents in the mother redia, duct of which opens to the anterior body of rediae. The cavity in caudal appendix of both daughter and granddaughter rediae are filled with nucleated gland cells with fine granular contents, probably for adhesion. Various drops of these gland

cells are found on the slide and rediae attached to it. In addition, a rosette of papillae around mouth is found in the granddaughter rediae as well as in cercariae. Kalantan *et al.* (1997) reported a gymnocephalid cercariae encysting on surface of water after it emerged from the freshwater snail, *M. tuberculata*, which later developed experimentally to the adult stage and they identified it as *P. gralli*. According to them the cyst is pear-shaped, while the present study describes it as the shape of a bottleneck. Kalantan *et al.* (1997) did not describe an excretory system, penetrating gland cells and cystogenic gland cells. It is difficult to compare taxonomically the two parasites to find out the validity of *P. gralli* in Saudi Arabia. Nasir and Diaz (1972) described *P. lacrymosus* Baun, 1902 and *O. semipalmatus* n. sp. from the optical cavity of the definitive host *Catoptrophorus semipalmatus* in Venezuela. *P. lacrymosus* differs from *P. gralli* in size of body, pharynx, acetabulum, ovary and testes and, markedly, in the extension of vitelline glands. Penner and Fried (1963) suggested synonymy of the genus *Ophthalmotrema* to *Philophthalmus* and emended the generic characteristics of the genus *Philophthalmus* to include the species of *Ophthalmotrema*. *Philophthalmus semipalmatus* differs clearly from *P. gralli* in the position of seminal vesicle, length of prepharynx, type and length of vitellari a.

Six cases of human ocular infection by *Philophthalmus* species were reported earlier, and one of them was caused by *P. gralli* in U.S.A. (Gutierrez *et al.* 1987). Lang *et al.* (1993) reported a case of conjunctivitis in the right eye due to an infection of *P. palpebrarum*. The patient suffered from irritation in his eyes and photophobia and the symptoms disappeared with the removal of the parasites. These authors believe that human ocular infection by *Philophthalmus* is rare and caused by accidental zoonosis.

The introduction of thiarid snails into different habitats in Valencia, Venezuela resulted in the elimination of *Biomphalaria* sp., the

intermediate host of *Schistosoma* (Pointier *et al.* 1994). *M. tuberculata* appeared in Venezuela in 1972 and has adapted rapidly in all rivers and rivulets of the littoral and central region of the country (Chrosiecchowski 1973). *Centrocestus formosanus* Nishigori, 1924 and *Haplorchis pumilio* were recorded from this snail in two localities of this State (pers. comm.). *P. gralli* has been reported from human elsewhere and there may exist the possibility of human infection in Venezuela unless preventive measures are taken.

RESUMEN

Se describe el miracidio, redia madre, redia hija, redia nieta, cercaria y el adulto de *Philophthalmus gralli* Mathis y Leger, 1910, obtenidos de infecciones experimentales. El hospedero intermediario *Melanooides tuberculata* fue recolectado en las localidades de Aguasanta y Yaguaracual, estado Sucre, Venezuela. Varios pollos fueron infectados oralmente con cercarias y metacercarias y colocando metacercarias directamente en los ojos con una pipeta. En ambos procesos de infeccion, se obtuvo parasitos adultos. Se amplia la distribucion geografica de *P. gralli*.

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