[Jpn. J. Parasitol., Vol. 36, No. 2, 53–62, April, 1987]

Life Cycle of *Philophthalmus gralli* (Mathis and Leger, 1910) (Trematoda: Philophthalmidae) in Azraq Oasis, Jordan

NAIM S. ISMAIL AND ISSAM M. ISSA

(Received for publication; March 19, 1986)

Abstract

This is the first report for the occurrence of *Philophthalmus gralli* in Jordan. It was found that this fluke utilizes the freshwater snail, *Melanoides tuberculata,* as an intermediate host and water geese, *Anser sp.*, as a definitive natural host. The overall infection rate of *M. tuberculata* snails with the *P. gralli* cercaria in Azraq Oasis was 1.0%. The mature cercariae swarmed out of the snail and encysted on solid subjects. The encysted metacercariae were excysted and inoculated around the eye orbit of one day old chicks. Mature *P. gralli* was recovered 26 days post-infection. Three groups of uninfected *M. tuberculata* were exposed to 3, 5 or 10 *P. gralli* miracidia per snail, respectively. It was found that the use of 5 miracidia per snail gave a high survival rate of snails (80%) and a high infection rate (92%).

Key words: *Philophthalmus,* Trematoda, Azraq Oasis, Jordan, Digenea

Introduction

Philophthalmus Looss, 1899 (Philophthalmidae Travassos, 1918) has been found in the eyes of many species of birds. The first species of this genus identified was the *P. palpebrarum* (Looss, 1899) recovered from the eyes of *Corvus cornix* in Egypt. To date 36 species of *Philophthalmus* have been reported in the literature. Based on the life cycles of various species of *Philophthalmus,* it is eveident that these flukes develop from distome gymnocephalous cercariae which utilize various species of freshwater and marine snails (Ching, 1961; West, 1961; Alicata, 1962; Murty, 1966; Rao and Rao, 1981). In China, Zhongzhang *et al.* (1980) and Xu (1981) reported that *P. gralli* utilizes the prosobranch freshwater snail *Melanoides tuberculata* as an intermediate host. Moreover, Zhongzhang *et al.* (1980) reported that *P. gralli* develop in *Thiara sp.* In Hawaii, *P. gralli* may develop in *Tarebia granifera* snails (Ching, 1961). Recently, Ismail and Saliba (1985) reported the occurrence of a gymnocphalous cercaria in *M. tuberculata* snails collected from Azraq Oasis. The present study was thus initiated to recover the adult fluke to which this cercaria belongs and to demonstrate its life cycle.

Department of Biological Sciences, Yarmouk University, Irbid, Jordan.

Materials and Methods

Collection and examination of *Melanoides tuberculata* snails:

Melanoides tuberculata snails were dominant along the edge and the bottom of the water pool at the Water Pumping Station of North Azraq. A total of 3,500 snails were collected during the period from June 1984 to July 1985. In the laboratory, the snails were transferred into an aquarium containing a thin layer of sediment and water which were brought from Azraq. The sediment was freezed and dried before use. Examination of snails for larval trematodes was made as described by Saliba *et al.* (1978). Observations on larval trematodes wre done as described by Ismail and Abdel-Hafez (1983).

Infected snails with the gymnocephalous cercaria were measured to the nearest mm, using a caliper. Moreover, the snails which did not shed cercariae were kept for two months and examined twice during that period for infection with larval trematodes. Snails which

were free from larval trematodes after that period were considered uninfected and used for further experiments. Snails were kept at ambient room temperature (21–29°C).

Encystment and excystment of cercariae and metacercariae:

The gymnocephalous cercariae usually encyst on solid subjects shortly after they emerge from the snail. Empty shells of snails were placed in the dish containing infected snail to enhance the encystment process. Then empty shells were placed in warm (40°C) normal saline solution to release metacercaria (Alicata and Ching, 1960; Howell and Bearup, 1967; Nollen, 1971). Excysted metacercariae were examined live, unstained or vitally stained, measured, and photographed. Metacercariae were also studied, fixed and stained with acetocarmine.

Experimental recovery of the adult fluke:

Eight, one-day-old chick, which were brought from an indoor commerical farm, were used in this experiment. Ten excysted metacercariae were inoculated around the orbits of both eyes of each chick using a Pasteur pipette as recommended by Alicata and Noda (1959) and Murty (1966). These chicks were examined 40 days post-infection. Flukes recovered were relaxed by cooling as recommended by Nollen (1971). This was done by placing the dishes containing the adult flukes in the refrigerator for one hour. The adult flukes were studied live and were then killed and fixed either by pipetting them into a hot AFA solution (Ethylalcohol-Formalin-Acetic Acid), or by placing them between two slides and fixing them in cold AFA. Fixed flukes were subsequently stained with acetocarmine. Measurements and drawings of stained worms were taken by the aid of a microprojector microscope.

Recovery of eggs and miracidia:

Mature adult flukes were placed in autoclaved tap water to stimulate the release of eggs (Rao and Rao, 1981). Eggs were measured using an ocular micrometer on a compound microscope. Freshly hatched miracidia were studied live, unstained or vitally stained. The vital stain used was 0.5% neutal red. Measurements were made on killed miracidia with hot water (60°C). Miracidia were fixed in cold AFA and subsequently stained with acetocarmine.

Experimental infection of *Melanoides tuberculata* snails:

Three groups of uninfected *Melanoides tuberculata,* 30 snails each, were used in this experiment. Each snail was placed in a 4 ml vial with 1 ml filtered autoclaved tap water. Each snail of the first, second, and third groups was exposed to three, five, or ten miracidia, respectively. Snails were left in the vials with miracidia for one hour as recommended by Alicata (1962) and Rao and Rao (1981). Each vial was then shaken several times and then the snail removed. The remaining water in the vial was checked, using a dissecting microscope, and the miracidia which failed to penetrate the snail were counted. Each group of infected snails was kept in a separate aquarium at room temperature (21–29°C). Snails were examined 90, 105, 120 and 135 days post-infection. After that all snails were crushed to recover other larval stages of *philophthalmus.*

The natural definitive host:

A group of four water geese (*Anser sp.*) inhabiting the water pool of North Azraq Pumping Station were examined for eye flukes. Both eyes of each bird were washed and flushed with autoclaved normal saline solution (0.84%). Eye flukes were removed from the eyes using a Pasteur pipette. The flukes were studied as described earlier for flukes obtained experimentally.

Results

Natural infection of *Melanoides tuberculata* with *Philophthalmus gralli* cercaria:

Of the 3,500 *M. tuberculata* snails collected from Azraq between June 1984 and July 1985, 35 were infected with *P. gralli* cercaria (Table 1). The highest infection rate (1.58%) was during November 1984, while the lowest

(0.55%) was during February 1985. The overall infection rates among the various size classes of snails ranged from 0.03% in snails measuring 21–25 mm long to 0.69% for those measuring 11–15 mm long. The infection rate (0.20%) among the young snails (6–10 mm long) was significantly higher than the infection rate (0.09%) among the 16–20 mm long snails.

Experimental infection of *Melanoides tuberculata* with *Philophthalmus gralli*:

The survival rate of *M. tuberculata* snails four months after exposure to three *P. gralli* miracidia per snail was high (83%). However, the infection rate of this group was low (48%) (Table 2). Similarly, the survival rate of snails exposed to five miracidia per snail was high (80%), and the infection rate of this group was also high (92%). Although the highest infection rate (100%) was recorded for those exposed to 10 miracidia each, the survival rate of snails in this group was low (23%).

Stages of *Philophthalmus gralli* (Figs. 1 and 2):

Adult:

Flukes of *P. gralli* were found inhabiting the outer region of the nictitating membrane under the eye lids of chickens. They are elongated, flattened, and the tegument is smooth except for the remnant spines concentrated at the lateral region around the ventral sucker. The oral sucker is terminal and is slightly smaller than the ventral sucker. The ratio of transverse diameter of oral sucker to that of the ventral sucker is 1:1.1–1.3. The mouth is slightly subterminal. The alimentary canal includes a short prepharynx which leads into strongly muscular pharynx. Usually, the esophagus is slightly shorter than the pharynx and connects with the region of intestinal bifurcation at equidistant between the oral sucker and the ventral sucker. The intestinal caeca run posteriorly to the end of the body. The testes are tandem and located at the posterior third of the body. The anterior

Table 1 Infection rates of *Melanoides tuberculata* snails collected from Azraq with *Philophthalmus gralli* cercaria. Numbers in parenthesis are percentages

Date of collection	No. of snails examined	No. of snails infected at various size classed				Total
		6–10 (mm)	11–15 (mm)	16–20 (mm)	21–25 (mm)	
June 1984	521	0	4(0.80)	1(0.19)	0	5(0.96)
Oct. 1984	523	2(0.38)	4(0.76)	0	0	6(1.20)
Nov. 1984	573	2(0.34)	7(1.20)	0	0	9(1.58)
Feb. 1985	538	0	1(0.18)	1(0.18)	1(0.18)	3(0.55)
June 1985	592	1(0.17)	4(0.66)	0	0	5(0.85)
July 1985	753	2(0.27)	4(0.53)	1(0.13)	0	7(0.95)
Total	3500	7(0.20)	24(0.69)	3(0.09)	1(0.03)	35(1.00)

Table 2 Experimental infection of three groups of *Melanoides tuberculata* snails with *Philophthalmus gralli* miracidia

Groups	No. of miracidia used/snail	No. of snails exposed	No. of snails alive 4 months post-infection	% of survival	No. of snails infected	% of infection in survived snails
1	3	30	25	83	12	48
2	5	30	24	80	22	92
3	10	30	7	23	7	100

testis is slightly larger than the posterior one in some specimens. The vase efferentia leave the ventral surface of testes and unite anteriorly before reaching the cirrus sac forming the vas deference. The cirrus sac (0.84 mm long) is located lateral and posterior to ventral sucker. The seminal vesicle is found at the posterior border of the cirrus sac and leads into a short narrow tubular ejaculatory duct which opens in the genital pore. The genital pore is located ventral to the intestinal bifurcation. It is either at the median line of the intestinal bifurcation or slightly to the right or left sides of the intestinal bifurcation. The ovary is globular and located in front of the anterior testis. The oviduct extends posteriorly to giving off the Laurer's canal and receiving the duct form the vitelline reservoir to form the ootype. The Mehlis' gland, ootype and proximal portion of uterus are found posterior to the ovary. The uterus occupies the body from the testes to short of the ventral sucker. The vitellaria extend to 79–86% of the distance from the margin of the anterior testis to the ventral sucker. They are extracaecal, and basically tubular. However, the yolk material may be concentrated into 10–11 follicles within the tubes. The excretory bladder (0.18 mm wide) is T-shaped and opens to the outside by a terminal excretory pore. It receives two primary excretory canals. They run intracaecally up to the region of ventral sucker where they become extracaecally and run up to the level of the pharynx. These canals are surrouded by a large excretory vesicle which obscured the secondary canals and the arrangement of flame cells.

Flukes obtained experimentally were compared with those from the eyes of water geese in Azraq (Table 3). Both flukes obtained from naturally and experimentally infected birds were morphologically indistinguishable. However, flukes obtained experimentally were slightly longer (3.60 mm) and wider (1.10 mm) than those obtained from the water geese (3.35 mm x 0.99 mm). Moreover, the testes and the ovary of the former were slightly larger than the latter. The ventral and oral suckers of flukes obtained experimentally were slightly smaller than those in flukes of water geese. The pharynx, the length of the cirrus sac, and eggs were generally similar.

Eggs:

Two types of eggs were found in the uterus of mature *P. gralli* recovered experimentally from chickens. The mature eggs were found in the distal region of the uterus below the ventral sucker. They are broad and rounded at one end, but narrow at the other end. It encloses a fully mature mobile miracidium with eye spots. The miracidium may migrate out of the egg shell while it is still in the uterus. The eggs are not operculated and have thin shells. The immature eggs were found inhabiting the proximal region of the uterus. They contain undeveloped miracidia without eye spots, and measure 0.07–0.09 x 0.03–0.04 mm. The eggs of *P. gralli* obtained from the natural host were found similar to eggs of *P. gralli* obtained experimentally (Fig. 3). Eggs of both types of flukes range in length from 0.130–0.150 mm. However, 50% of the 100 eggs of *P. gralli* obtained experimentally measured 0.150 mm compared to 37% of the 30 eggs obtained from the flukes from the natural host.

Miracidium:

The freshly emerged miracidium is an active swimmer. It is ciliated and swims quickly in all directions. While swimming, it is oval in shape with the maximum width at the anterior end. It swims usually near the bottom. It may swim upward by rotating its body but as soon as it stops this rotation, it sinks to the bottom. It is positively phototactic.

Rediae:

Three redial stages were found in the pericardial cavity and the digestive gland of the *M. tuberculata* snails. These include: the mother redia, the daughter redia, and the granddaughter redia. The mother redia measures 0.29 x 0.07 mm and has two lateral processes at the posterior end of the body. Its intestinal caecum is long and extends to the posterior end of the body. This redia contains a large number of germ cells. The daughter redia is larger in size

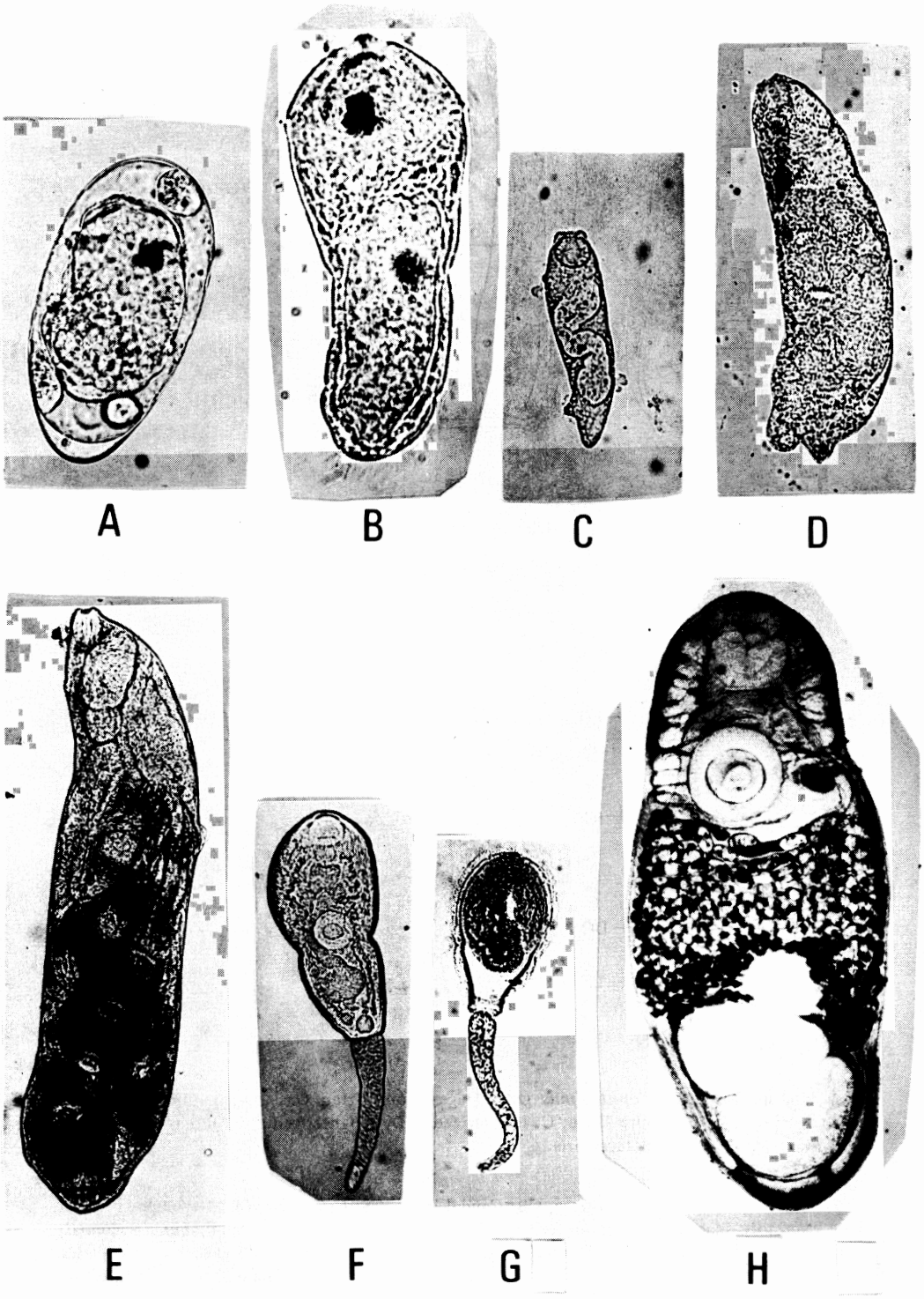

Fig. 1 Stages of *Philophthalmus gralli* from Azraq Oasis. A, egg (340X); B, miracidium (640X); C, mother redia (110X); D, daughter redia (110X); E, granddaughter redia (120X); F, cercaria (80X); G, encysted cercaria (80X); H, mature fluke (40X).

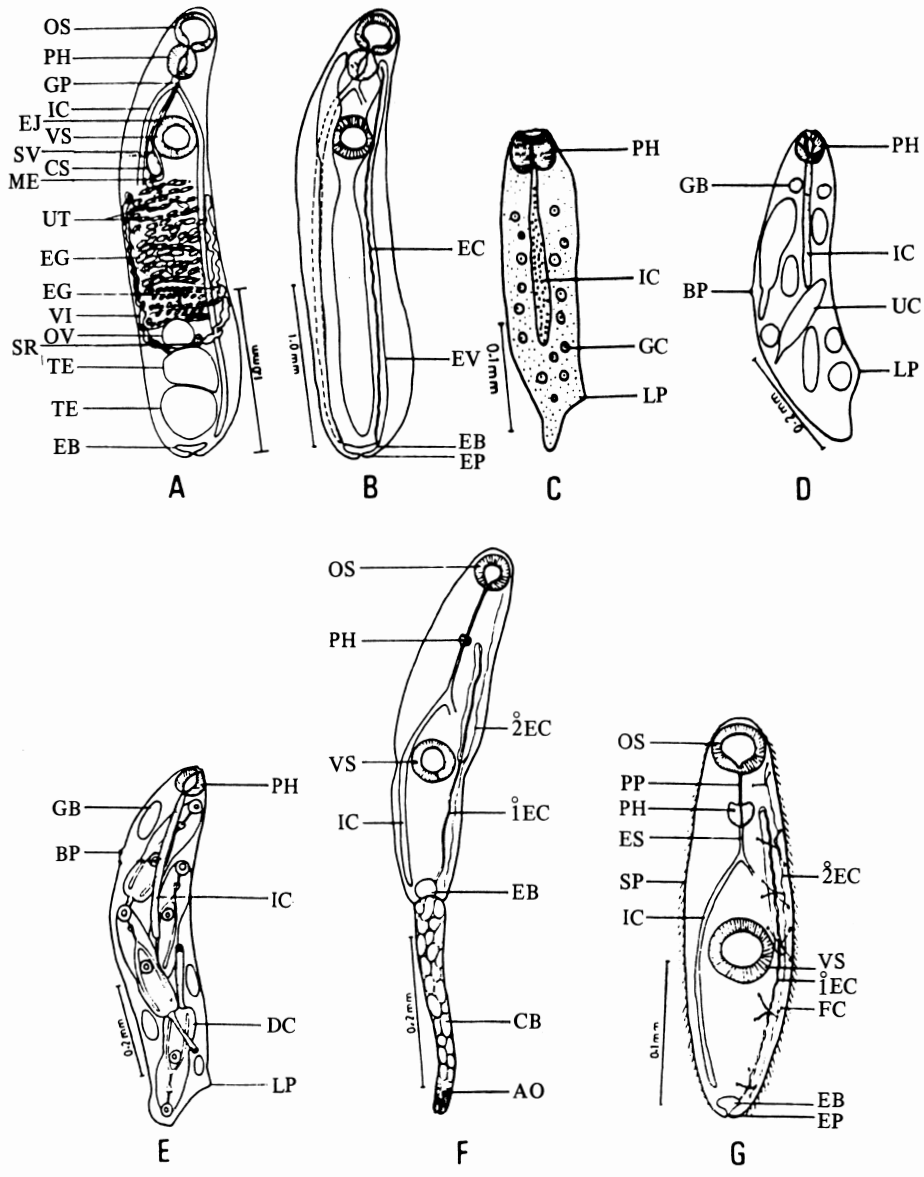

Fig. 2 Diagrams of *Philophthalmus gralli* stages from Azraq Oasis. A, mature fluke; B, excretory system of a mature fluke; C, mother redia; D, daughter redia; E, granddaughter redia; F, cercaria; G, excysted metacercaria.

Abbreviations

AO: Adhesive organ, BP: Birth pore, CB: Caudal body, CS: Cirrus sac, DC: Developed cercaria, EB: Excretory bladder, EC: Excretory canal, ^{1}EC: Primary excretory canal, ^{2}EC: Secondary excretory canal, EG: Egg, EJ: Ejaculatory duct, EP: Excretory pore, ES: Esophagus, EV: Excretory vesicle, FC: Flame cell, GB: Germ ball, GC: Germ cell, GP: Genital pore, IC: Intestinal caecum, LP: Lateral process, ME: Metraterm, OS: Oral sucker, OV: Ovary, PH: Pharynx, PP: Prepharynx, SP: Spine, SR: Seminal receptacle, SV: Seminal vesicle, TE: Testis, UC: Undeveloped cercaria, UT: Uterus, VI: Vitellaria, VS: Ventral sucker.

Table 3 Comparison between *Philophthalmus gralli* flukes obtained experimentally and those obtained from a natural host. Measurements (in mm) were made on 10–15 live and 7–10 stained flukes

	Mature flukes from experimental host		Mature flukes from natural host	
	Live	Stained	Live	Stained
Length	*3.00–4.50 †(3.60±0.58)	2.68–2.88 (2.78±0.08)	2.75–3.75 (3.35±0.40)	2.45–2.75 (2.60±0.12)
Width	0.80–1.33 (1.10±0.18)	0.57–0.75 (0.66±0.08)	0.75–1.25 (0.99±0.15)	0.68–0.78 (0.73±0.04)
Ventral sucker (diam.)	0.35–0.48 (0.42±0.05)	0.27–0.36 (0.32±0.03)	0.40–0.50 (0.46±0.03)	0.26–0.34 (0.30±0.03)
Oral sucker	0.22–0.28×0.28–0.42 (0.25±0.02×0.35±0.06)	0.19–0.26×0.23–0.31 (0.23±0.03×0.26±0.03)	0.25–0.35×0.35–0.45 (0.30±0.04×0.39±0.04)	0.13–0.23×0.23–0.29 (0.20±0.02×0.27±0.02)
Anterior testis	0.35–0.50×0.45–0.70 (0.42±0.07×0.52±0.06)	0.25–0.31×0.27–0.35 (0.28±0.02×0.31±0.03)	0.30–0.38×0.45–0.58 (0.34±0.03×0.52±0.05)	0.20–0.26×0.25–0.35 (0.23±0.02×0.30±0.04)
Posterior testis	0.35–0.48×0.40–0.65 (0.42±0.05×0.52±0.10)	0.19–0.25×0.25–0.34 (0.22±0.02×0.29±0.03)	0.28–0.33×0.33–0.60 (0.29±0.02×0.46±0.10)	0.25–0.31×0.26–0.34 (0.28±0.03×0.30±0.03)
Ovary (diam.)	0.22–0.35 0.28±0.05	0.13–0.21 0.17±0.04	0.20–0.28 0.24±0.03	0.10–0.18 0.14±0.04
Ratio of transverse diam. of ovary to testes	1.00 : 1.95	1.00 : 1.80	1.00 : 2.00	1.00 : 2.10
Cirrus sac length	0.70–0.98 (0.84±0.10)	0.52–0.68 (0.60±0.06)	0.80–1.00 (0.89±0.08)	0.61–0.73 (0.67±0.05)

*Indicates the range.
†Number in parenthesis indicate the mean ± one standard deviation.

than the mother redia and it measures 0.52 × 0.14 mm. It has two lateral processes at the posterior end of the body. The intestinal caecum extends only to the middle of the body. The birth pore is present laterally near the middle of the body. The granddaughter redia is the largest form of rediae. It measures 0.78 × 0.16 mm. In general it resembles the daughter redia in structure, except that the birth pore is present at approximately the upper third of the body. It has two lateral processes and encloses several developed cercariae and several germ balls. The intestinal caecum extends to the middle of the body.

Cercaria:

The cercaria of *P. gralli* is relatively large. It measures 0.68–0.92 mm long. Its body is elongated with a slight constriction around the ventral sucker. It is densely packed with cystogenous cells. The tegument is about 3 μm thick and densely covered with fine spines. The tail is shorter than the body, and is filled with caudal bodies except near its posterior end. The adhesive organ is composed of two pairs

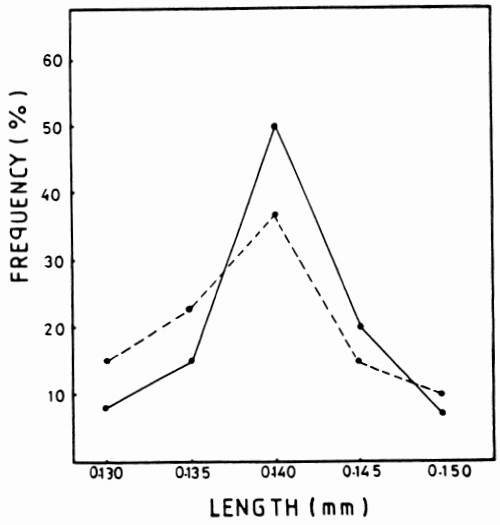

Fig. 3 Variation in length of 100 eggs of 26-day-old *Philophthalmus gralli* obtained experimentally from chickens (—), and of 30 eggs obtained from a natural host (- - -).

of glands of which the outer glands are larger than the inner ones. The gland ducts run posteriorly and open into a common pore at the posterior tip of the tail. The ventral sucker is

round (0.06 mm in diameter) in shape and is located near the middle of the body. It is slightly larger than the oral sucker, which measures 0.04 x 0.05 mm. The ratio of transverse diameter of the oral sucker to that of the ventral sucker is 1:1.2. The digestive system consists of a mouth that leads into a long prepharynx, which passes through a small muscular pharynx. The esophagus runs posteriorly to the region of the excretory bladder. The excretory system is well developed. The excretory bladder is globose and is located at the posterior part of the body. It receives two primary excretory ducts which extend anteriorly to the region of pharynx, where they recurve and unite with the main primary canals at the ventral sucker region. Then, two pairs of secondary tubules run forward and backward.

Metacercariae:

The cercaria encysts within one hour of emergence from the snail. The cyst is pear-shaped and measures 0.30 x 0.20 mm. The encysted metacercaria emerges from the cyst immediately upon the addition of hot normal saline (40°C). The metacercaria needs at least five minutes after its encystment to respond to the addition of hot normal saline solution.

The newly excysted metacercaria is elongated. It measures 0.24–0.29 x 0.07–0.11 mm. The body is covered with numerous spines that are directed backward. As the excysted metacercaria migrates from the cyst it swims vertically, then sinks down to creep on the bottom. The ratio of the transverse diameter of the oral sucker to that of the ventral sucker is 1:1.15. The excretory system is as was described in the cercaria. It has the same arrangement of the primary and secondary canals. The flame cells formula is 2[(3+3+3) + (3+3)] = 36.

Discussion

This is the first report for the occurrence of *Philophthalmus gralli* in Jordan. It is evident now that *P. gralli* in Azraq Oasis, Jordan utilizes the *Melanoides tuberculata* snails as an intermediate host and the water geese as the definitive host. However, the occurrence of *P. gralli* in other waterfowls has to be investigated.

The total infection rate of 3500 snails of *M. tuberculata* with larval stages of *P. gralli*, collected from Azraq during June 1984 to July 1985, was generally low (1%). Higher rates (3.6%) were reported from *M. tuberculata* collected earlier from Azraq during May–September, 1981 (Ismail and Saliba, 1985). Moreover, Xu (1981) found that 34.0% of *M. tuberculata* in Southern China were infected with *P. gralli* cercaria. The low infection rate (1%) reported in this study may be due to the fact that waterfowls (geese) are not abundant in the study area.

It was possible to recognize three redial stages of *P. gralli* in the *M. tuberculata* snails, which included the mother, daughter, and granddaughter rediae. In general, the granddaughter redia resembled the daugher redia in structure. However, the birth pore is located at the upper third of the body in the former and near the middle of the body in the latter. Similarly, Alicata (1962) reported the presence of 3 redial stages of *P. gralli* in *Tarebia granifera mauiensis* and the differentiated between the daughter and granddaughter rediae on the basis of birth pore location and the lack of cuticular papillae on the surface of granddaughter redia.

Although, flukes obtained experimentally were morphologically indistinguishable from flukes obtained form the natural host, they were slightly longer and their testes larger than the latter flukes. The variations in the dimensions of the body and the reproductive organs of *P. gralli* due to age and type of hosts were also reported by Ching (1961) and Srivastava and Pande (1971).

Philophthalmus gralli has been reported from several localities in the world: China, Formosa and India (Srivastava and Pande, 1971; Zhongzhang *et al.*, 1980; Xu, 1981); United States (Nollen and Murray, 1978) and Hawaii (Ching, 1961; Alicata, 1962). The size

Table 4 Comparison among *Philophthalmus gralli* from different localities in the world

Type	Intermediate host	Natural definitive host	Egg size (mm)	Ratio of transverse diameter of ovary to testes	Extent of vitellaria	Reference
Chinese	*Melanoides tuberculata* *Thiara sp.*	Ducks Geese Cheickens	0.158×0.07	1:2.0	80–85%	Zu (1981); Zhongzhang *et al.* (1980); Ching (1961)
Indian	N.A.	*Anas acuta*	0.08×0.05	1:1.8	78–85%	Srivastava and Pande (1971)
American	*Tarebia granifera*	*Fulica americana*	N.A.	N.A.	79–89%	Nollen and Murray (1978)
Hawaian	*Tarebia granifera*	Chickens	0.14–0.157× 0.60–0.74	1:2.0	78–82%	Ching (1961); Alicata (1962)
Jordanian	*M. tuberculata*	Geese	0.14×0.06	1:2.1	78–87%	Present study

N.A.: Not available.

of eggs of *P. gralli* from Jordan is similar to those from China, Hawaii and America (Ching, 1961; Alicata, 1962; Nollen and Murray, 1978) (Table 4). The smaller size of eggs in the Indian *P. gralli* (Srivastava and Pande, 1971) may be due to the fact that the authors based their measurements on stained eggs which may have resulted in shrunken eggs. In general, the ratio of transverse diameter of the ovary to that of testes and the extent of vitellaria are similar among these flukes. Recently, Nollen *et al.* (1985) reported that the adult stages of the Texan and Hawaiian strains of *P. gralli* were indistinguishable based on sucker ratios, vitellaria, and egg sizes.

Acknowledgements

This research was supported by a grant from Yarmouk University Research Council. The authors wish to thank Dr. S. K. Abdel-Hafez, Yarmouk University, for his valuable suggestions and comments. The help of Prof. I. Vassilev, Bulgarian Academy of Sciences, in the identification of the adult fluke is appreciated. The authors wish also to thank Mr. S. M. Bdair for his help in the field and laboratory work.

References

1) Alicata, J. E. (1962): Life cycle and developmental stages of *Philophthalmus gralli* in the intermediate and final hosts. J. Parasitol., 48, 47–54.
2) Alicata, J. E. and Ching, H. L. (1960): On the infection of birds and mammals with the cercaria and metacercaria of the eye fluke, *Philophthalmus.* J. Parasitol., 46, 16.
3) Alicata, J. E. and Noda, K. (1959): The life history of a species of *Philophthalmus,* an eye fluke of birds in Hawaiian Islands. J. Parasitol., 45, 22.
4) Ching, H. L. (1961): The developmental variation of *Philophthalmus gralli* (Mathis and Leger, 1910) with a comparison of species of *Philophthalmus* (Looss, 1899). Proc. Helminth Soc. Wash., 28, 130–138.
5) Howell, M. J. and Bearup, A. J. (1967): The life history of two bird trematodes of the family philophthalmidae. Proc. Linn. Soc. N.S.W., 92, 182–194.
6) Ismail, N. S. and Abdel-Hafez, S. K. (1983): Larval stages of digenetic trematodes of *Melanopsis praemorsa* (L. 1758, Buccinum) (Thiaridae) snails from Yarmouk River, Jordan. Z. Parasitenkd., 69, 613–626.
7) Ismail, N. S. and Saliba, E. K. (1985): Studies on larval stages of digenetic trematodes of *Melanoides tuberculata* (Muller) snails from Azraq Oasis, Jordan. Riv. Parasitlogia, 46, 263–

271.

8) Murty, A. S. (1966): Experimental demonstration of the life cycle of *Philophthalmus sp.* (Tremadoda: Philophthalmidae). Curr. Sci., 36, 366–367.
9) Nollen, P. M. (1971): Studies on growth and infection of *Philophthalmus megalurus* (Cort, 1914) (Trematoda) in chick. J. Parasitol., 57, 261–266.
10) Nollen, P. M. and Murray, H. D. (1978): *Philophthalmus gralli*: infection, growth characteristics, and treatment of an Oriental eye fluke of birds introduced into the Continental United States. J. Parasitol., 64, 178–180.
11) Nollen, P. M., Leslie, J. F., Cain, G. D. and MacNab, R. K. (1985): A comparison of Texan and Hawaiian strains of the avian eyefluke, *Philophthalmus gralli,* with a cautionary note on the importation of exotic animals. J. Parasit., 71(5), 618–624.
12) Rao, B. P. and Rao, B. V. (1981): Developmental stages of *Philophthalmus anatinus* (Sugimoto, 1928) in the snail. Indian Vet. J., 58, 525.
13) Saliba, E. K., Lutfy, R. G. and Ismail, N. S. (1978): Fascioliasis in Azraq Oasis, Jordan. II. Infection of *Lymnaea auricularia* snails with *Fasciola gigantica* (Cobb, 1885) cercariae. Acta Parasit. Pol., 25, 341–345.
14) Srivastava, S. C. and Pande, B. P. (1971): The eye-fluke *Philophthalmus gralli* (Mathis and Leger, 1910) in two anatid hosts with remarks on the genus. Indian J. Anim. Sci., 41, 369–404.
15) West, A. F. (1961): Studies on the biology of *Philophthalmus gralli* (Mathis and Leger, 1910) (Trematoda: Digenea). Am. Midl. Nat., 66, 363–383.
16) Xu, P. R. (1981): On the epidemiology of *Philophthalmus* of domestic fowls in Guangdong province. J. South China Agricultural College, 2, 19–27.
17) Zhongzhang, T., Zingquan, C., Xiumin, L., Yulin, W., Tucheng, H. and Chongti, T. (1980): *Philophthalmus* of domestic fowls in Fugian, China. Acta Zool. Sin., 26, 232–242.