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OBSERVATIONS ON THE DEVELOPMENT OF TWO ECHINOSTOMES, ECHINOPARYPHIUM RECURVATUM AND ECHINOSTOMA REVOLUTUM, THE ANTAGONISTS OF HUMAN SCHISTOSOMES IN EGYPT

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Abstract. Detailed studies have been made on the complete life cycle of two species of echinostome trematodes, *Echinoparyphium recurvatum* and *Echinostoma revolutum*. In Egypt, the two species utilize for larval development the same snail intermediate hosts as do human schistosomes. The first intermediate host of *E. recurvatum* in Egypt is the snail *Bulinus truncatus;* the miracidia of the parasite penetrate the heart and aorta of the snail and develop there to sporocysts, while mother- and daughter rediae are located in the venous sinuses, and mainly in the hepatopancreas. At 22—24 °C, development in the intermediate hosts lasts from 29—35 days. The second intermediate hosts of *E. recurvatum* as determined experimentally are the snails *Bulinus truncatus*, *Biomphalaria alexandrina* and *Physa acuta*, the fishes *Gambusia affinis* and tadpoles of *Rana mascareniensis;* the cercariae encyst in the body of these intermediate hosts; metacercariae are located, generally, in the pericardiac sac and the kidneys, in fishes and tadpoles in the kidneys. The definitive hosts (experimental infection) are the domestic duck, chicken, mouse, rat, hamster and rabbit. The trematodes mature in the small intestine of these hosts within a period of 7—8 days. The development of the miracidium in the egg is greatly influenced by temperature: under optimal conditions, at 27 °C water temperature, miracidia hatch in as short a time as 5 days.

The first intermediate host of the trematode *Echinostoma revolutum* in Egypt is the snail *Biomphalaria alexandrina*. Sporocysts, mother- and daughter rediae develop in its inner organs; at 24 °C water temperature, development in the first intermediate host (to free-swimming cercariae) is completed in 25 days; at water temperatures of 16—17 °C, it lasts 72 days. The second intermediate hosts are the snails *Biomphalaria alexandrina*, *Bulinus truncatus*, *Physa acuta* and *Viviparus unicolor;* encysted metacercariae of this parasite were found also in the kindeys of frogs (*Rana esculenta*) with natural infection. Development of *E. revolutum* in the definitive host is completed in 7—12 days. Experimental infection disclosed these hosts: domestic duck, chicken, mouse, white rat, hamster and rabbit; immature trematodes were found in *Ardeola ibis*. The development of the miracidium in the egg was completed in 8—10 days at 28 °C water temperature, in 21 days at 17 °C. In addition to descriptions of the individual trematode stages, the taxonomic position of both species is discussed.

Our investigations, which took place in the vicinity of Cairo between the years 1971—1973, were concerned with establishing which organisms parasitize the snail hosts of *Schistosoma haematobium* and *Schistosoma mansoni* with a view to utilizing their pathogenic effect for the development of methods of biological control of human schistosomiasis in Egypt. Of the species found, the most suitable appeared to be echinostome trematodes, as previously noted by Ryšavý et al. (1973), and as indicated by observations on the competitive development of larval digenetic trematodes in snail intermediate hosts (Lie 1967, 1969; Heyneman et al. 1972, and others). For these reasons we studied in detail the life cycles of two of the most common members of the family Echinostomatidae in the locality under consideration, i.e., *Echinoparyphium recurvatum* (Linstow, 1873) and *Echinostoma revolutum* (Froelich, 1802). The results of these studies are presented in this paper.

METHODS

Eggs for studies on the life cycles of the trematodes *E. recurvatum* and *E. revolutum* were obtained either by teasing the body of adult trematodes, or by collecting eggs from definitive hosts with an experimental infection (white mice). Eggs were cultured in glass dishes (volume 500 ml) in tap water with continuous aeration and daily change of the water. Egg development was inspected daily with the microscope. We studied the miracidia both alive and fixed (in 2 % formalin). The same method was employed for studying the developmental stages of the trematodes extracted from the first intermediate host, and of the free cercariae.

Development in the first intermediate host was studied in laboratory-bred snails of the species *Biomphalaria alexandrina* (for *E. rèvolutum*) and *Bulinus truncatus* (for *E. recurvatum*). The snails measured from 4—7 mm, the infective dose was 20 miracidia per snail. Exposure to infection of the snails with an infective dose of miracidia (in 5 ml water) lasted 1.5 hr at +27 °C with additional light. The age of the miracidia used for snail infection did not surpass 2 hr. Hatching of the miracidia was activated in a flask (volume 1 liter) into which the egg culture was poured. Warm water (+32 °C) was added, and the flask was exposed to the sun for one hr. After this period the bulb of the flask was shaded and only the open narrow neck exposed to the sun for an additional 30 min. The miracidia concentrated in masses in the exposed neck of the flask and could be collected for snail infection. After experimental infection the snails were transferred into plastic vessels (volume 5l) with filtered Nile water and a sprinkle of chalk, and fed with lettuce *(ad libitum)*. The water in the vessels was changed each day.

The development in the first intermediate host was examined regularly at intervals of 2 days by the use of a compressorium, developmental stages of the trematodes being extracted, measured and drawn. The production of cercariae was followed daily by exposing snails to sunlight. For encystment of the cercariae we used either the snail species *B. truncatus* and *B. alexandrina*, or other Egyptian water snail species, sometimes also tadpoles and fishes. Metacercariae for study were released from the cyst either by the use of a mounted needle, or the cysts were digested with prepared fluids (Ždárská 1964).

Metacercariae were used for infection of various species of definitive hosts being fed to these hosts together with pieces of snail tissue. The results were confirmed by daily inspection of the faeces and finally by post-mortem examination for the presence of trematodes. The morphology of the adult trematodes was studied using live-, fixed and stained, and mounted material. In order to study the spines of adults, cercariae and metacercariae, the specimens were fixed and cleared with ammonium picrate. All drawing was by the aid of a camera lucida. Microphotographs were made of several phases.

RESULTS

A. DEVELOPMENT OF *ECHINOPARYPHIUM RECURVATUM* (LINSTOW, 1873)

Both field and experimental observations showed that *Bulinus truncatus* (Audouin) is the sole first intermediate host of *E. recurvatum* in Egypt. Development in the internal organs of this intermediate host is completed within 29—35 days (at 22—24 °C water temperatures). The second intermediate hosts as determined experimentally are the water snails *Bulinus truncatus* (Audouin), *Biomphalaria alexandrina* (Ehrenberg) and *Physa acuta* Draparnaud, the fishes *Gambusia affinis* (Baird et Girard), and tadpoles of *Rana mascareniensis* Dum. et Bibr. Ryšavý et al. (1972) found under field conditions in the vicinity of Cairo that metacercariae occurred also in the snails *Lanistes bolteniaunus* Chemnitz, *Cleopatra* sp., *Bithynia* sp., *Lymnaea taillaudi* Bourguignat, *Viviparus unicolor* Olivier. The definitive hosts, in the intestine of which the trematode matures within 7—8 days, can be either birds or mammals. Following artificial infection, adult trematodes were obtained from domestic duck (*Anas platyrhynchos f. dom.* L.)., chicken (*Gallus gallus f. dom.* L.), white mouse (*Mus musculus* L.), white rat (*Rattus norvegicus* [Erxleben]), golden hamster (*Mesocricetus auratus* [Waterhouse]), rabbit (*Oryctolagus cuniculus f. dom.* [L.]).

Egg: In *E. recurvatum* the eggs are widely ovoid to oval, with a thin yellowish brown shell. Measurements: 0.078—0.089 × 0.055—0.061.*) The wider pole of the egg forms an operculum. When laid, the eggs are uncleaved and contain an indifferently disposed light coloured germinal cell. The remaining inner space of the egg-shell is occupied by vitelline cells which are densely granulated.

As development proceeds, the egg cleaves, the embryo increases in size and is distinctly lighter in colour than the remaining vitelline cells. The granules of the vitelline cells disappear, they also are lighter in colour, their outlines are more distinct. At the period of miracidium formation they appear to have become partly merged into vacuolar formations surrounding the miracidium. Fully developed miracidia display considerable motility inside the egg and their apical papilla, the pigmented eye spots and flame cells can be seen through the egg shell. The emergent miracidium pushed the operculum aside by pressure and, apparently, with the aid of its excretions, and eventually emerges into the surrounding water.

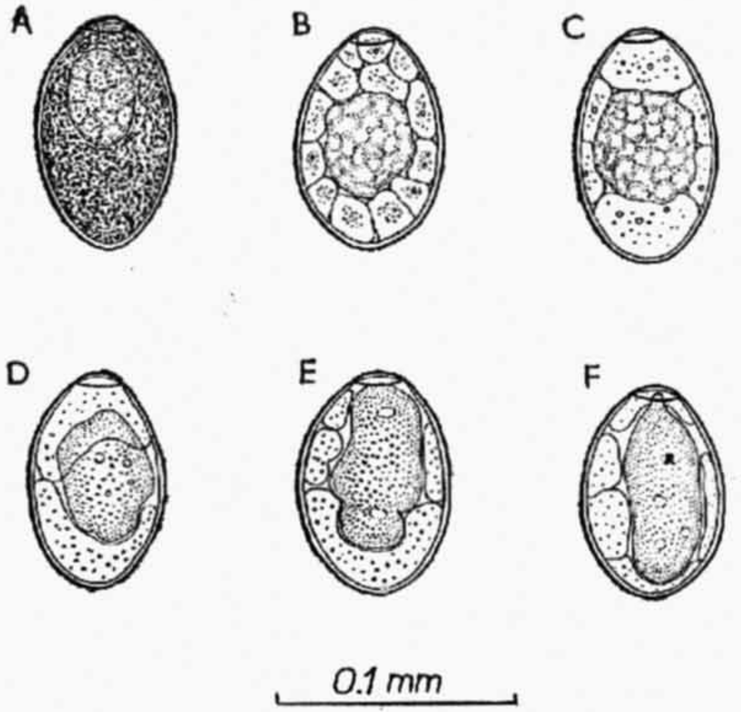

Fig. 1. *Echinoparyphium recurvatum* (Linstow, 1873)—development of the miracidium in the egg. A—on day 2; B—on day 3; C—on day 4; D, E—on day 6; F—on day 7.

The rate of miracidial development inside the egg, and its hatching, is influenced by the temperature of the surrounding water. At +27 °C, egg development is very fast and motile miracidia appear in it in as short a time as 5 days. After a short exposure to the sun, miracidia hatched in 6 day-old eggs. At +20 °C, motile miracidia appeared in 16 day-old eggs. Hatching of the miracidia is considerably influenced by temperature and exposure to the sun. Hatching could be arrested by keeping the culture at a low temperature (+5—+8 °C). At these low temperatures a culture containing infective eggs could be kept for a period of 2 months as confirmed by experiments. But even under optimal conditions, hatching of the miracidia occurred consecutively, i.e., they did not all hatch at the same time. According to the temperature, hatching may last from 12 days to several weeks. We discovered in experiments that a miracidium does not

*) All measurements in mm.

hatch immediately after passage of the mature egg through the digestive tract of a snail intermediate host. On the other hand, eggs collected from snail faeces contained miracidia capable of both hatching and causing infection.

Miracidium: The miracidium changes considerably its bodily shape; a motile miracidium is generally club-shaped. The body is widest at the level of the pigmented eye spots. Body length 0.092—0.120, maximum width 0.049. A distinct, protractile apical „proboscis“ is situated on the anterior end. An apical gland is present in the space between the base of the apical "proboscis" and the eye spots opening at the peak of the pointed anterior end to the "proboscis". Eye spots submedian, close to one another, but distinctly separate, at 0.021 from anterior end of body. Close under eye spots (at 0.014 from anterior body end) we observed two fingerlike processes (sensory papillae) one at each side of the body. The surface of the miracidial body is densely covered with ciliae which appear to be uniform in length (0.011). The position of the two flame cells is slightly asymmetrical at each side of the body below its median line. Large germinal cells occupy the posterior half of the body. Facilitated by ciliary movements, the miracidia swim rapidly in the water (they are much faster than, e.g., miracidia of human schistosomes). Their phototaxis is positive, their geotaxis negative and this can be utilized for their concentration.

Sporocyst: it develops in the heart of the snail intermediate host *Bulinus truncatus*, where nutritive conditions are optimal. The sporocysts are tightly fixed to the tissues of the snail and, therefore, may escape attention when the snail is examined with the compressorium. On day 10 p.i., the sporocysts appear as sacular, almost immobile formations, length 0.240—0.384, maximum width 0.112—0.160. Body shape oval, sometimes pyriform. Sometimes, larger sporocysts harboured accretions of germinal cells and embryos of mother rediae. Simultaneously with these sporocysts we found also free, small mother rediae.

Mother redia: both redia generations are located in the venous sinuses, mainly in the hepatopancreas of the intermediate host, rarely in other organs. The first young mother rediae were found together with the sporocysts on day 10 p.i. The smallest mother redia measured 0.168 in length, 0.772 in width. Almost the entire anterior half of the redial body was occupied by a circular oesophagus (0.064 in diameter); the colourless, cyst-shaped gut (length 0.032) extended to approximately two third of the body length. We found neither a collar nor ventral locomotory processes. Rediae of the same age, but slightly more advanced in development, have a considerably elongate body (length 0.536, maximum width 0.128). Maximum body width is in the region of the oesophagus. Width of remaining body approximately 0.090. Diameter of circular oesophagus 0.069, length of cyst-shaped gut 0.080. The gut terminates at 0.216 from posterior body end. Close below we observed a marked collar encircling the redial body. At 0.112 from the posterior end of the body we found two ventral fingerlike locomotory processes (approximate length 0.040).

Mature mother rediae appeared in as late a time as 20 days p.i. The shape of the body is irregular, the cuticle smooth, of brownish colour. A remarkable feature is their large circular oesophagus (0.136 in diameter). The gut is short (0.120—0.130) of reddish-brown colour, densely occupied by granules. Overall length of redial body 0.728—0.998, maximum width 0.192—0.272. The locomotory ventral processes are relatively short. The body of the redia is occupied almost completely by embryos and young daughter rediae. The oesophagus of the latter, measured inside the mother rediae, is approximately 0.056 in diameter. These mother rediae react readily to osmotic pressure and easily rupture in the water particularly under the slightest pressure of the coverslip.

Daughter redia: the youngest daughter rediae appeared on day 20 p.i. together with the most advanced mother rediae. At this age the dauhgter rediae are translucent, their length is only 0.248—288, their width 0.088—0.112. The oesophagus measures 0.056—0.064 in diameter, the colourless, saclike gut extends to one half of the body and measures approximately 0.080. We observed neither ventral locomotory processes nor a collar. A daughter redia of the same age but slightly more advanced in development measured 0.704 in width, but the diameter of its oesophagus was the same as that of the youngest rediae (0.056); its gut, howerer, was considerably longer (0.280); it extended

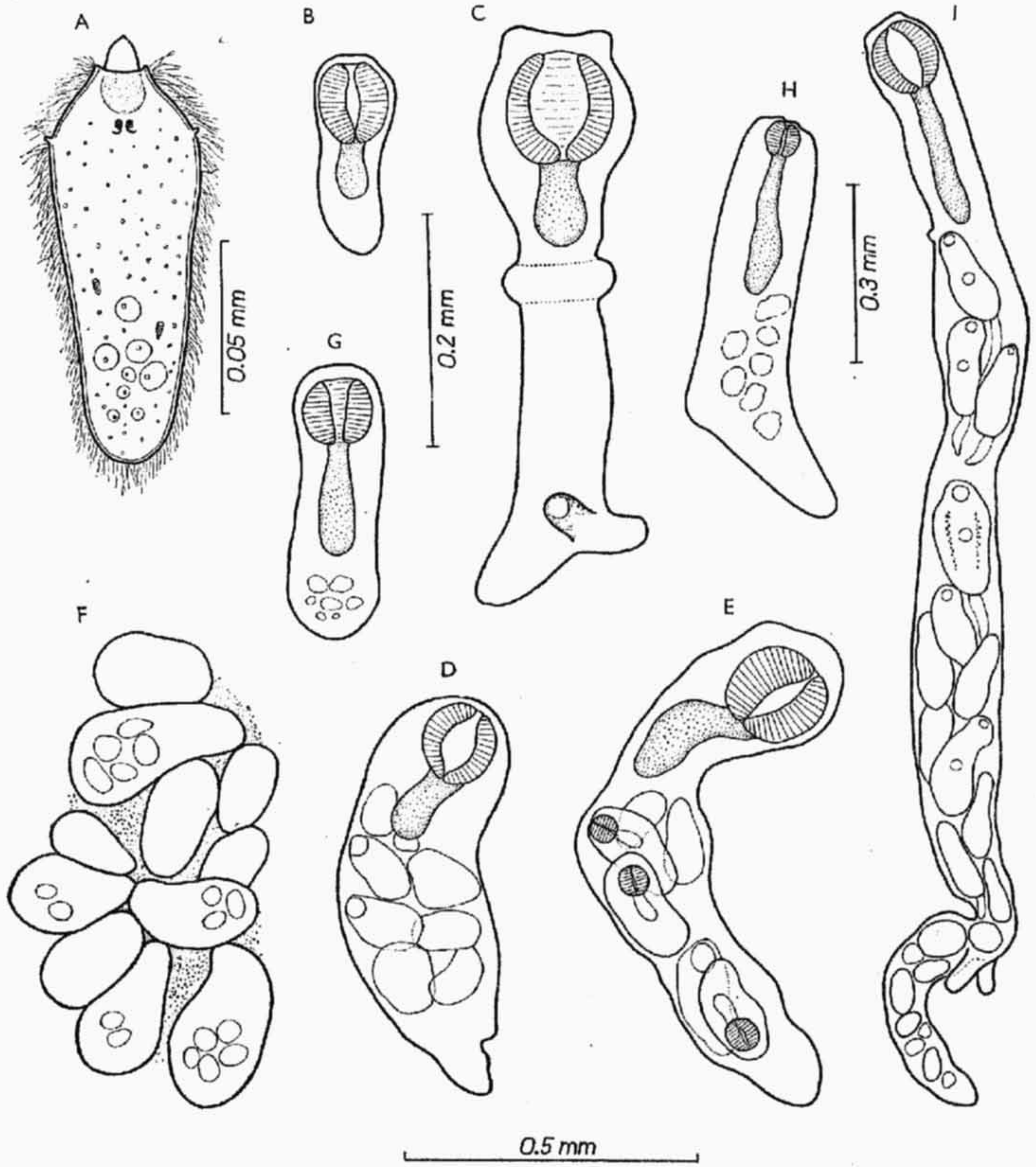

Fig. 2 *Echinoparyphium recurvatum* (Linstow, 1873). A—miracidium; B—E—mother rediae (B—smallest redia on day 10 p.i.; C—young redia on day 10 p.i.; D, E—more developed rediae on day 20 p.i.); F—sporocyst' cluster on the aorta of the snail; G—I—daughter rediae (G—smallest redia on day 20 p.i.; H—medium size redia on day 20 p.i.; I—mature redia on day 35 p.i.).

to above half of the body length. The ventral locomotory processes were, already, feebly outlined. Clusters of germinal cells could be distinguished in the redial body.

Fully mature daughter rediae were found on day 35 p.i. Generally, their body was opaque, very elongate, 1.68—1.76 in length, 0.144—0.176 in maximum width. The oesophagus was circular, very muscular, 0.096—0.112 in diameter. The short, saclike gut (0.176—0.208) was of reddish brown to brown colour. The birth pore was situated generally on the papillar process at the dorsal side of the body, at 0.384—0.400 from the anterior body end. The collar close in front of the birth pore was mostly not very distinct. Two large fingerlike locomotory processes were situated at 0.320—0.480 from the posterior body end. The inside of the body, from the region of the birth pore to the posterior end, was occupied by embryos and cercariae at different stages of development. Simultaneously with these daugther rediae we found also free, fully developed cercariae. The emergence of cercariae from the intermediate host into the surrounding water, however, was observed on the following day, i.e. on day 36 p.i.

Cercaria: the body and tail of the cercaria are very contractile. Alive, the body without the tail measures approximately 0.416—0.600 in length, maximum 0.176 to 0.288. Both body and tail are covered with a cuticle with fine transverse striation which is finer on the tail. The anterior end of the body bears a collar with fine spines. This was less distinct in live than fixed specimens. The oral sucker is almost circular, and measures 0.043—0.058 by 0.049—0.064. A row of 4—8 pores of the penetration glands was clearly visible on the anterior margin of the oral sucker. The ventral sucker is slightly bigger (0.058—0.079 in diameter), almost circular and, upon side view, elevated above the body surface. The arrangement and number of collar spines are essentially those of the adult trematode (40—45); the first four spines are arranged in two rows, the dorsal spines are in alternate arrangement. The praepharynx is relatively short, the muscular pharynx is oval, length 0.018—0.030, width 0.015—0.024. The oesophagus is of considerable length, intestinal bifurcation starts close above the ventral sucker. The penetration glands situated in the oesophagus region were indistinct, their exact number could not be determined. The numerous, finely granulated cystogenic, unicellular glands form an almost continuous layer on the dorsal side of the cercaria. The excretory system is represented by two primary ducts of considerable width which extend along either side of the body and are filled with large, greatly refractile excretory granules. Each duct attenuates towards its anterior end and passes into a narrow duct, which forms a backward loop in the pharynx region and starts to branch. The primary lateral excretory ducts attenuate in the region of the ventral sucker, unite and pass through marked valves in a large excretory sac situated near the posterior end of the body. A thin median duct extends from this sac into the tail, bifurcates at 0.058 to 0.088 from the anterior end and its branches open at each side of the tail. The tail is very contractile, measuring 0.520—0.600 when extended. The surface of the tail is covered with epithelial cells with large nuclei. We did not observe caudal finlike folds. The tip of the tail is obtused.

Metacercaria: The second intermediate hosts of *E. recurvatum* are mainly various snail species, including the first intermediate host, less frequently fishes and amphibians. Cercariae may either emerge into the surrounding water and enter other or the same snails to encyst in them, or they may remain in their first intermediate host and encyst in its internal organs. If a cercaria cannot leave the daughter redia, it encysts directly in it. In snail intermediate hosts, cysts with metacercariae of *E. recurvatum* are located mainly in the pericardiac sac and in the kidneys, but may be present also in the remaining internal organs if the intensity of infection is high. In fishes and amphibians, however, they are always located in the kidneys.

Cysts obtained from *B. truncatus* with experimental infection were relatively small, but of regular spherical shape. The hyaline cyst wall was only 0.006 thick, the diameter of cysts was 0.135—0.144. Inside the cyst, the body of the metacercaria is doubled and the metacercaria changes frequently its position. Through the wall of the cyst it is possible to distinguish on the light-coloured body of the metacercaria the two primary excretory ducts filled with excretory granules, the suckers, the pharynx and the collar spines.

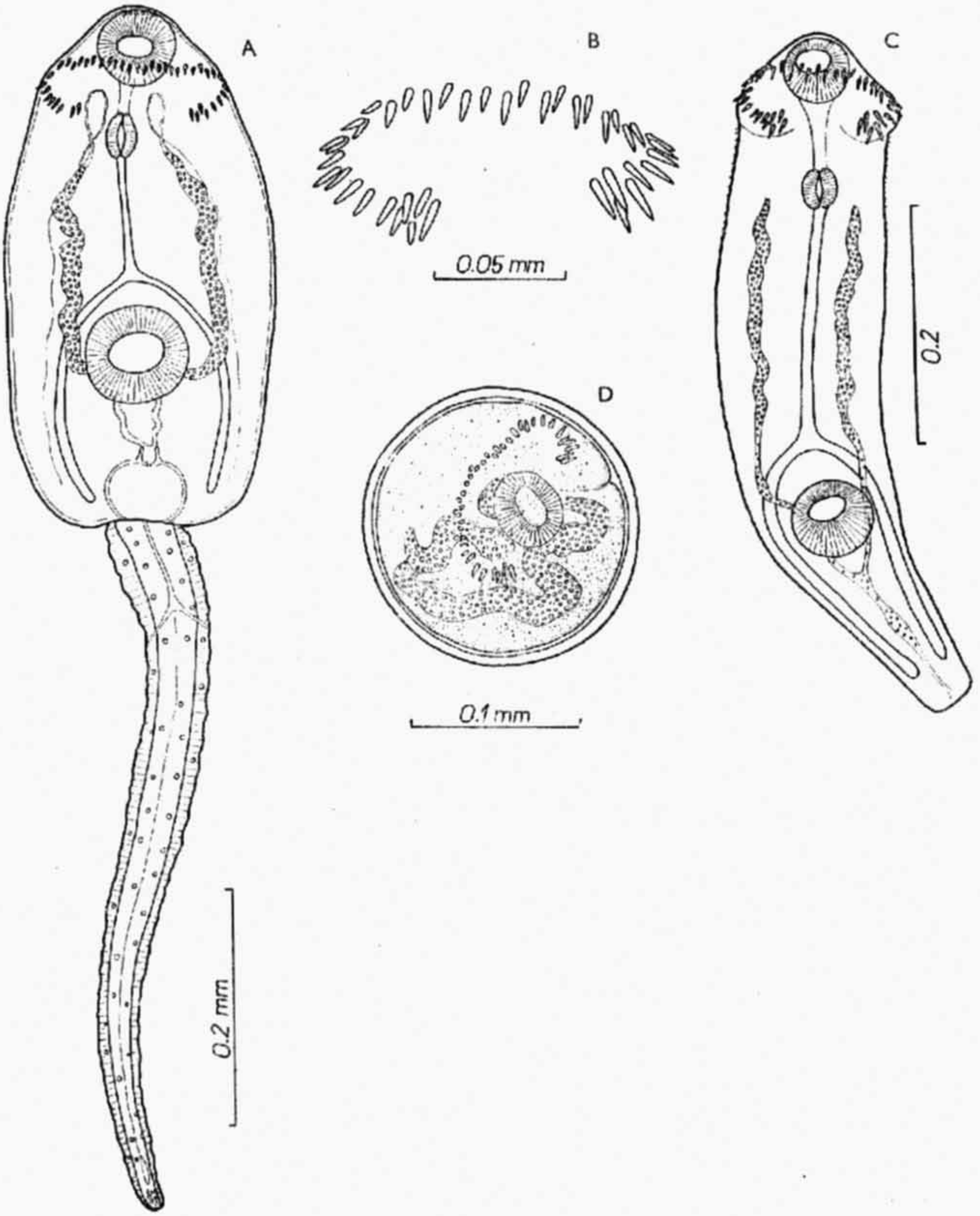

Fig. 3. *Echinoparyphium recurvatum* (Linstow, 1873). A—cercaria; B—arrangement of spines in the metacercaria; C—released metacercaria; D—metacercaria in the cyst.

The body of a released metacercaria is slender, elongate, with a maximum width in the collar region; it attenuates below the ventral sucker. Length 0.472—0.560, maximum width 0.128—0.160. The arrangement and number of spines on the distinct collar are analogous to those in the adult trematode. The oral sucker is circular, 0,040—0.081 by 0.064—0.081; the praepharynx considerably long, the greatly muscular pharynx oval, 0.023×0.026. The oesophagus very long; it bifurcates close above the ventral sucker in two caeca which extend almost to the end of the metacercarial body. The ventral sucker is very muscular arising above the surface of the body; it measures $0.064—0.072 \times 0.064$ to 0.080. The two primary excretory ducts extend along each side of the body from the pharynx downwards; they attenuate at the site of the intestinal bifurcation and unite

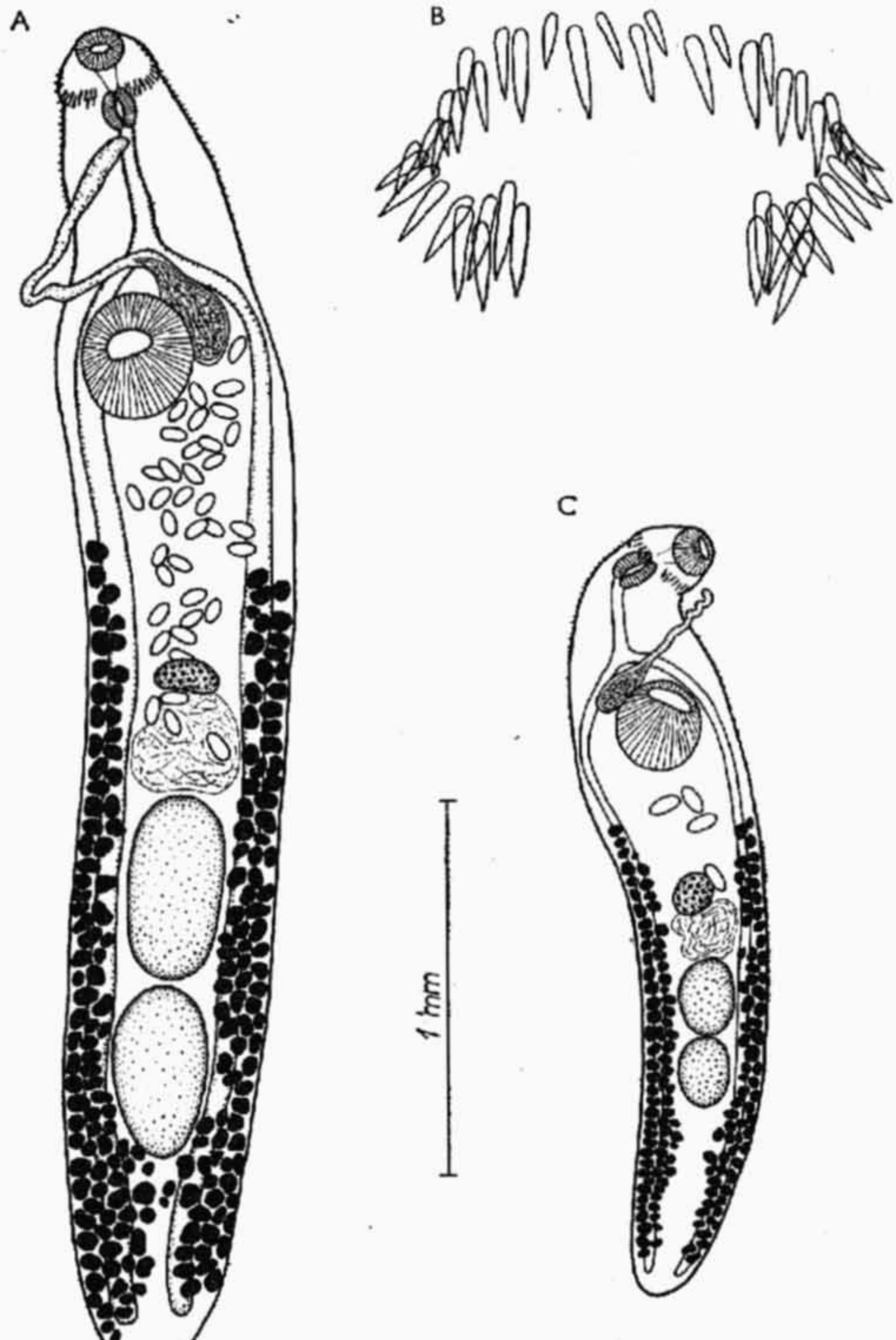

Fig. 4. *Echinoparyphium recurvatum* (Linstow, 1873) — adults from mice. A—largest specimen; B—arrangement of collar spines; C—smallest adult specimen.

in a relatively narrow common excretory sac below the ventral sucker, extending from there to the posterior end of the body. The primary excretory ducts are filled with large, highly refractile granules, which reduce both in number and size in the posterior part of the excretory system. On the anterior end of the body, the cuticle of the metacercaria is covered with transverse rows of small spines.

Adult: We observed in various bird and mammal hosts infected with encysted metacercariae from snails, that the metacercariae developed in the small intestine of the definitive host and attained the adult stage within 7—8 days.

The adult trematode is slender; its anterior end bears a distinct collar armed with a number of large spines. The length of the body of stained specimens from mice was 2.12—3.60, the maximum width 0.449—0.625. The cuticle on the anterior end of the body is covered with minute spines. The collar spines (40—45) measure 0.021—0.051 in length; the row of spines is not interrupted on the dorsal side. The oral sucker is subterminal, relatively small (0.111—0.123 × 0.120—0.135). The ventral sucker is situated close below the intestinal bifurcation, it is very muscular, almost circular, arising above the surface of the body in live specimens; its measurements are 0.249—0.330 × 0.240 to 0.315. The short praepharynx is followed by an oval or pyriform, greatly muscular pharynx (length 0.087—0.111, width 0.081—0.102). Oesophagus long (0.210—0.270); it bifurcates above the ventral sucker into two caeca which extend along each side of the body almost to its end. Two oval testes, tandem, almost equal in size (measurements of first testis 0.201—0.450 × 0.147—0.258) are situated in the space between the intestinal branches in the posterior part of the body. A cirrus sac of considerable size (length 0.198—0.300, width 0.090—0.150) containing a large seminal vesicle, is situated at the anterior margin of the ventral sucker. The cirrus is slender and very long (0.270 to 0.660). The ovary is small (0.099—0.174 × 0.114—0.117) irregularly shaped, situated close in front of the testes. Uterine loops occupy the space between the ventral sucker and the ovary. Eggs oval, with an operculum, measurements 0.078—0.089 × 0.055 to 0.061. The vitellaria are formed by large follicles; they extend from close below the ventral sucker along each side of the body to almost the posterior end of the trematode body. In older trematodes the vitelline follicles merge frequently in the space below the testes.

DISCUSSION

In Egypt, *Echinoparyphium recurvatum* was first reported by Sonsino (1892) who found 42 to 44-spined echinostome metacercarie in various snails; feeding these metacercariae to ducks and rabbits he obtained adult trematodes which he identified as *Echinostomum recurvatum* Linstow, 1873 (= *Echinoparyphium recurvatum*). It is evident that cercariae from *Biomphalaria alexandrina* identified as *Cercaria agilis* and considered to be conspecific with these metacercariae, must have belonged to a different species, apparently of the genus *Echinostoma*. Azim (1930) found cercariae with 43 to 45 collar spines produced in snails of the genus *Bulinus*. Metacercariae of this species have been recorded from various water snails. Feeding experiments with dogs and feral and white rats confirmed that these were larvae of *E. recurvatum*. In 1953, Kuntz studied in Egypt the development of the excretory system of *E. recurvatum* cercariae from *Bulinus truncatus*. El Gindy and Rushdi (1963) found these cercariae in the same intermediate host.

Conspecific cercariae from *Bulinus truncatus* collected in the environs of Cairo, were found by Ryšavý et al. (1973). Having regard mainly to the first intermediate host and to the number of collar spines, these authors assigned them provisionally to the species *Echinoparyphium bioccalerouxi* Dollfus, 1953, but considered, at the same time, their possible identity with *E. recurvatum*. Recent studies on the complete life cycle of this parasite under the conditions of Egypt indicate that there are no substantial differences between this trematode and the European species *E. recurvatum*. This applies to the morphology of the larvae and adults, to the range of definitive hosts, intermediate hosts, to the rate of development etc. Rašín (1933) suggested in his excellent paper that members of the genus *Lymnaea*, which are closely related to those of the genus *Bulinus*, are the first intermediate hosts of *E. recurvatum*, and that information on the development of this parasite in members of other families requires a revision. (Rašín obtained only negative results from experimental infection of various Planorbidae and other snails with this trematode.) In view of the fact that various waterbirds are, evidently, utilized by this trematode as natural definitive hosts, their frequent migratory flights from Europe to N. Africa and back may ensure the mixing of N. African and European populations of this parasite. Minute differences in the number of collar spines may be ascribed to interspecific variability recorded for echinostome trematodes by various authors (e.g., Azim 1930, Rašín 1933, Lie 1967, Lie and Nasemary 1973 etc.). For these reasons we consider the species *Echinoparyphium bioccalerouxi* Dollfus, 1953 to be in synonymy with *Echinoparyphium recurvatum* (Linstow, 1873); other synonyms of this species are *Echinostomum elegans* Loos, 1899 and *Echinostoma aegyptiaca* (Khalil et Abaza, 1924), as pointed out by Azim (1930) and Mathias (1927).

In view of the fact that larval stages of this parasite have a considerable pathogenic affect on their snail intermediate host (*Bulinus truncatus*), and clearly suppress larvae of *Schistosoma haematobium* in the case of a "double infection", there are good reasons to consider their utilization in the biological control of *S. haematobium* in Egypt.

B. DEVELOPMENT OF *ECHINOSTOMA REVOLUTUM* (FROELICH, 1802)

In Egypt, *E. revolutum* utilizes only one snail intermediate host species, i.e., *Biomphalaria alexandrina* (Ehrenberg). This fact has been confirmed both in experiments and in the field. The development of this parasite in the inner organs of the snail is greatly influenced by the temperature; at 24 °C water temperature, development in the intermediate host is completed in 25 days, at 21 °C in 37 days, at 16—17 °C in 71—72 days. The second intermediate hosts as determined experimentally are *Biomphalaria alexandrina* (Ehrenberg), *Bulinus truncatus* (Audouin), *Physa acuta* Draparnaud and *Viviparus unicolor* Olivier; encysted metacercariae were found also in the kidneys of frogs (*Rana esculenta* L.) with natural infection. Adult trematodes were obtained from artificially infected golden hamsters (*Mesocricetus auratus* [Waterhouse]), white rats (*Rattus norvegicus* [Erxleben]), white mice (*Mus musculus* L.), rabbits (*Oryctolagus cuniculus f. dom.* [L.]), domestic ducks (*Anas platyrhynchos f. dom. L.*), chickens (*Gallus gallus f. dom.* L.), and not fully mature trematodes also from herons (*Ardeola ibis* [L.]). In the definitive hosts the trematodes complete their development in 7—12 days; they are generally located in the small intestine; in several birds (chicken, heron) we found them in the large intestine and caecum, or in the cloaca, but these cases were rare. The trematodes survive in the intestine of the definitive host for a period of 1—2 months.

Egg: The eggs of *E. revolutum* are yellowish brown with an operculum at the narrower end, and covered with a thin shell. They are mostly ovoid, but both their shape and size shows considerable variation. Fresh eggs from mice with artificial infection measured from 0.118—0.134 × 0.069—0.072. Eggs were unembryonated when laid and we distinguished in them only the germinal cell which was distinctly lighter in colour than the surrounding, densely granulated, vitelline cells.

The rate of miracidial development inside the egg is influenced by the temperature of the surrounding water; it lasted from 8—21 days; hatching occurred several days later also depending on water temperature and light. The number of granules in the vitelline cells reduced gradually and their bodies became lighter and more distinct. At the time when the miracidium is clearly formed, the vitelline cells change into special vacuolar formations which occupy the space around the miracidium and, evidently, exert pressure to facilitate hatching. At a water temperature of +28 °C, motile miracidia were observed inside the egg on day 8, hatching was observed in the following 2—3 days. At a lower temperature (+17 °C) motile miracidia appeared on day 21. Hatching of the miracidia was not simultaneous; in dependence on the water temperature, hatching of miracidia from eggs of a culture of the same age lasted from several weeks to several months. Hatching can be arrested by transferring the culture to a temperature of +5 to +8 °C. At these temperatures, the miracidia retain their capability of hatching and infecting the snails for a period of at least two months as confirmed in our experiments.

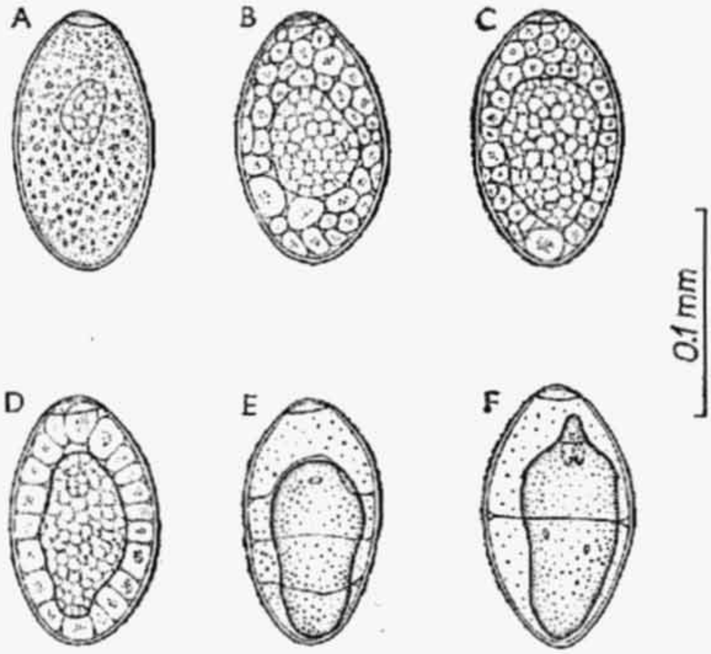

Fig. 5. *Echinostoma revolutum* (Froelich, 1802)—development of the miracidium in the egg. A—on day 2; B—on day 3; C—on day 4; D—on day 6; E—on day 7; F—on day 8.

M i r a c i d i u m : The shape of the body changes, but is generally elongate ovoid. The maximum width of the miracidial body was found generally at the level of the first third of total body length. The body length of live miracidia was 0.120—0.138, their maximum width 0.055—0.063. The anterior end of the body bears a large protractile apical papilla. The penetration gland (according to several authors "primitive gut") is voluminous, generally reaching the level of the pigmented eye spots, its length is approximately 0.025 mm. The eye spots, although close to one another, are distinctly separate. At approximately the level of the eye spots, or slightly above, we found a fingerlike process at each side of the body. The two flame cells are slightly asymmetrically placed near the centre of the body. Germinal cells are situated in the posterior half of the body. The body of the miracidium is densely covered with cilia of approximately the same length (0.018—0.020).

The swimming movements of the miracidia are quick; their phototaxis is positive, their geotaxis negative, which can be utilized for their concentration.

Sporocyst: The sporocyst develops in the heart and aorta of the snails, and is tightly fixed to the tissue. Young sporocysts obtained on day 10 p.i. were almost translucent, with an elongate body of irregular shape. The length was 0.38—0.46, their width approximately 0.16. More advanced sporocysts harboured in their body clusters of germinal cells, the largest of which measured 0.088—0.096 in diameter. Sporocysts obtained on day 16 p.i. were considerably bigger in size, measuring 0.736—0.880 in length, maximum width 0.208—0.296. They were whitish, of irregular shape, mostly pyriform, i.e., at one end clearly wider than at the opposite end. The body of these

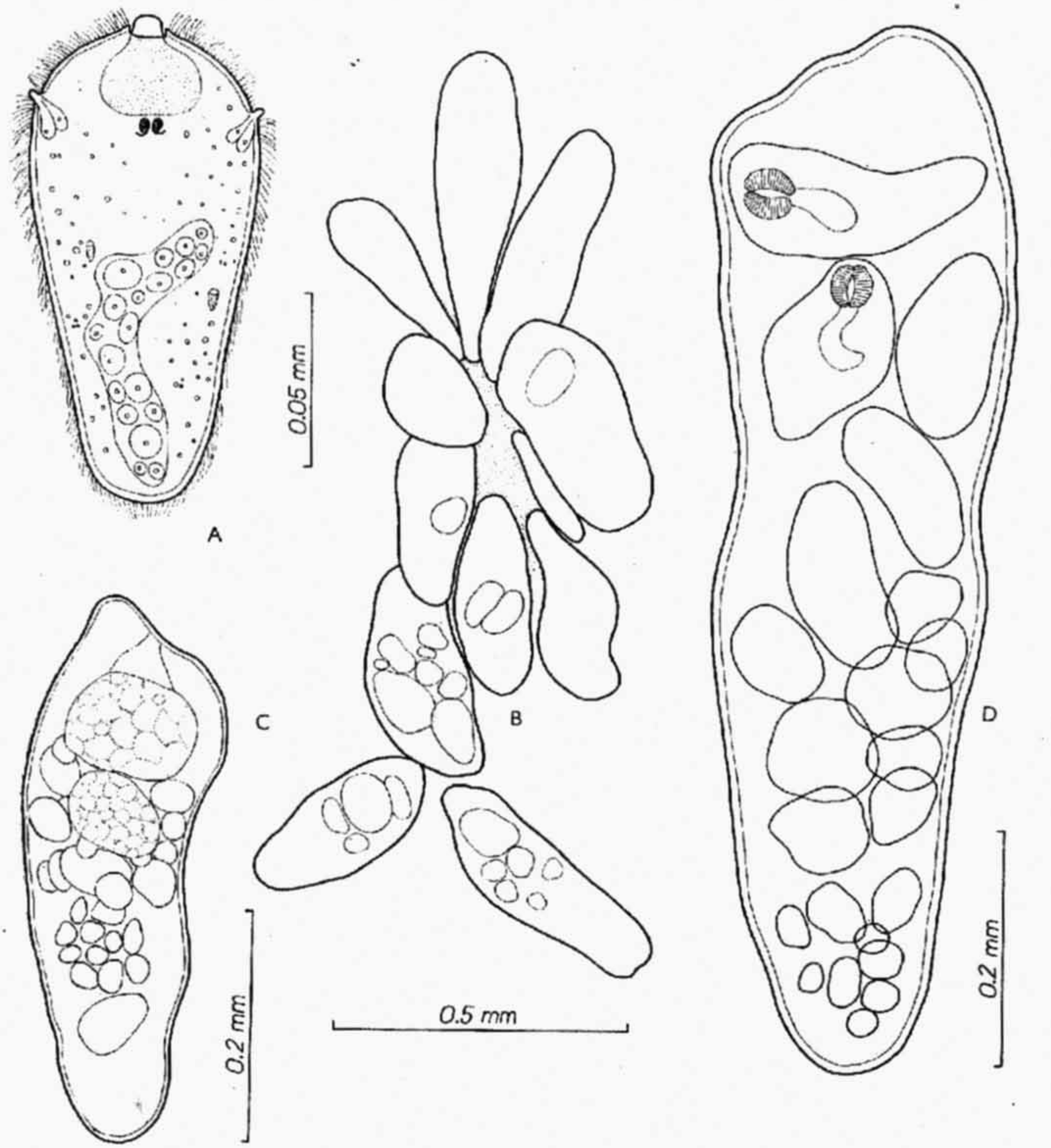

Fig. 6. *Echinostoma revolutum* (Froelich, 1802). A—miracidium; B—cluster of young sporocysts on the aorta of the first intermediate host (on day 10 p.i.); C—young sporocyst (on day 10 p.i.); D—older sporocyst (on day 16 p. i.).

sporocysts contained, in addition to the germinal clusters, young mother rediae with a distinct oesophagus and intestine. The sporocysts were almost motionless, their surface was smooth. The birth pore could not be seen.

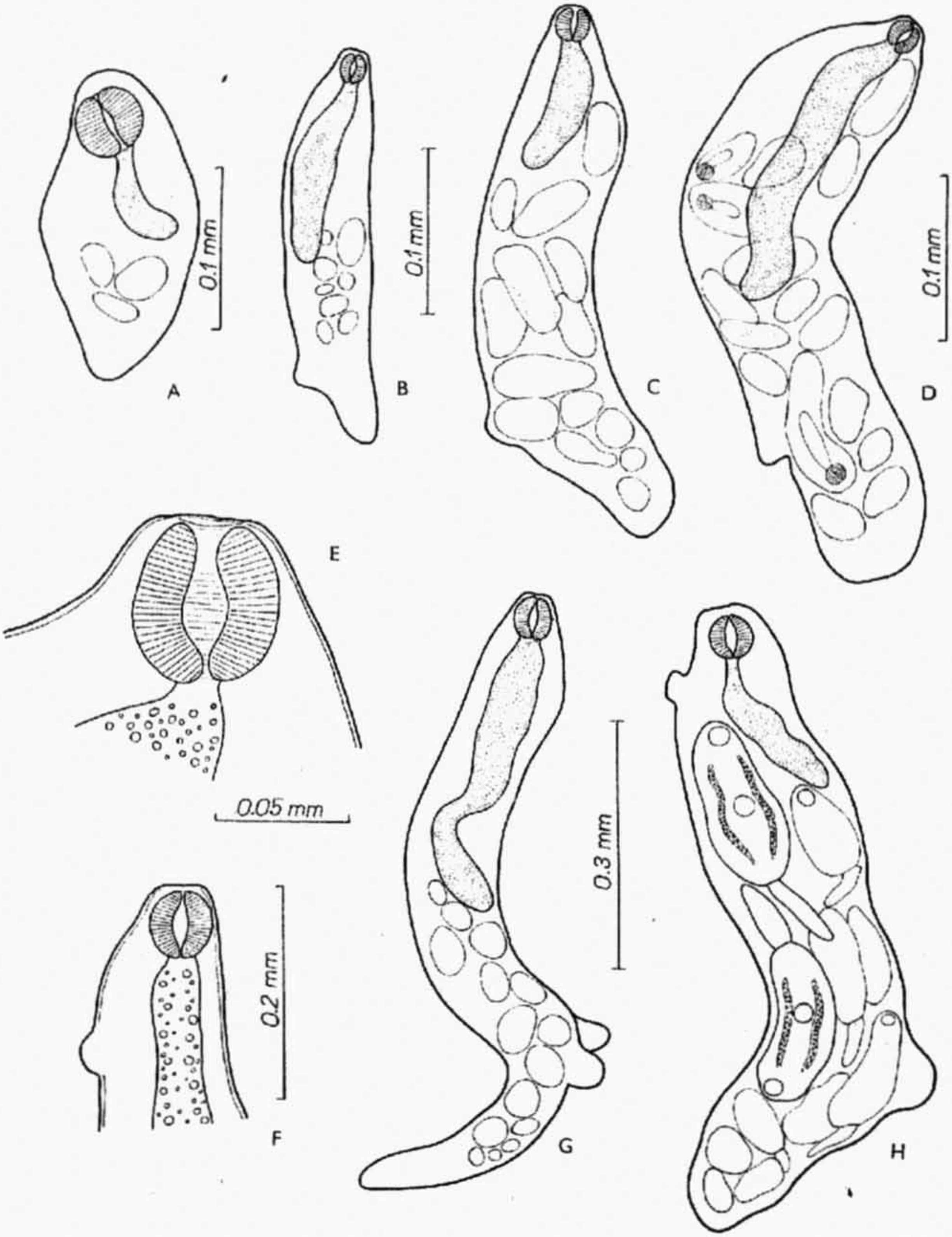

Fig. 7. *Echinostoma revolutum* (Froelich, 1802). A—E—mother rediae (A—on day 16 p.i.; B, C, D—on day 21 p.i.; E—anterior end of older mother redia on day 21 p.i.); F, G—younger daughter redia on day 37 p.i. (F—anterior part of body; G—general view); H—older daughter redia (on day 37 p.i.).

M o t h e r r e d i a e: The first mother rediae were observed on day 16 p.i. together with the appearance of mature sporocysts. Their body was whitish, their length 0.224 to 0.256, width 0.088—0.120. The circular oesophagus on the anterior end of the body (0.040—0.048) was followed by an as yet colourless, vacuolar intestine, length 0.040 to 0.056 reaching to at least half of the body length of the mother redia. The collar, birth pore and ventral locomotory processes were still indistinct in rediae of this age. The posterior part of the redial body contained indistinct clusters of germinal cells.

Fully developed mother rediae were found on day 21 p.i. Their length was 0.664 to 1.160, their width 0.144—0.224. The shape of the body varied, but generally the body was considerably elongate with a marked attenuation at the cephalic end. Pharynx circular, 0.040—0.056 in diameter, vacuolar intestine 0.312—0.440 long, its colour in less developed rediae an orange brown, coarsely granulated. On the anterior end of the body we observed a distinct collar followed dorsally by the birth pore. Approximately at the beginning of the last third of the body we found two small, ventral, locomotory processes. In addition to the clusters of germinal cells, the body of the more mature rediae contained already young daughter rediae of the second generation.

D a u g h t e r r e d i a : Free daughter rediae appeared first on day 21 p. i. At this time their length was only 0.088, their width 0.040. The circular pharynx measured approximately 0.016 in diameter. Large daughter rediae appeared in as late a time as 37 days p.i. They were relatively motile, whitish, with a marked orange brown intestine. The shape of their body was irregular, elongate, similar to that of the mother rediae, length 0.720—1.632, width 0.096—0.192. Diameter of circular oesophagus 0.032—0.048, length of vacuolar intestine 0.176—0.368. In less developed rediae the intestine reached half the body length, in older specimens approximately its first third. The cephalic collar was not very marked. The birth pore situated dorsally was approximately at 0.080—0.160 from the anterior end of the body; often, it was located on the papillar extension. The ventral locomotory processes were distinct, at 0.256—0.608 from the posterior end of the redial body. More advanced rediae (day 37 p.i.) harboured fully formed cercariae in addition to the germinal sacs.

C e r c a r i a : Free, young cercariae were first observed in *B. alexandrina* examined with the compressorium, on day 37 p.i., but their emergence occurred one day later after exposure of the intermediate host to the sun. The shape of the cercarial body was very changeable, mostly widest in its posterior third. Body length 0.350—0.420, maximum width 0.158—0.193. On the anterior end of the cercaria we observed a distinct collar armed with small, indistinct, sclerotized spines; their number (36—38) and location were analogous to those found in the adult trematode. In addition to the collar spines, the cuticle on the anterior half of the body was covered with very fine spines which reduced in size towards the posterior end. Oral sucker subterminal, almost circular, diameter 0.053—0.060. Circular ventral sucker situated slightly below mid-cercarial body, measurements 0.060—0.074. Below the oral sucker a short, thin-walled praepharynx leading into a greatly muscular, oval or pyriform pharynx (0.028—0.035 × 0.025). Oesophagus long (0.070—0.105), narrow; intestinal bifurcation starts close above the ventral sucker, the narrow caeca extend almost to the posterior end of the cercarial body. Penetration glands situated at oesophagus region, their exact number could not be determined. The outlets of these glands (8; exceptionally 4—6) are situated at the dorsal margin of the oral sucker. There are numerous unicellular cystogenic glands. Their granular contents obscure the view and, against the light, the colour of the cercarial body is dark. Tail 0.455—0.630 long, maximum width 0.046—0.077, with a sharply pointed tip. The epidermis which is thick on most parts of the tail surface, forms often dorsoventral and lateral, finlike folds; their number, shape and distribution show considerable variation. In live cercariae these finlike folds numbered from 2—5, on the tail of several specimens these folds were absent. In fixed cercariae we found always 5 fins (two dorsal, two ventral, one lateral). The excretory system consisted of a bipartite excretory bladder situated at the posterior end of the cercarial body which received the two primary collecting tubes at below the ventral sucker. These extend in

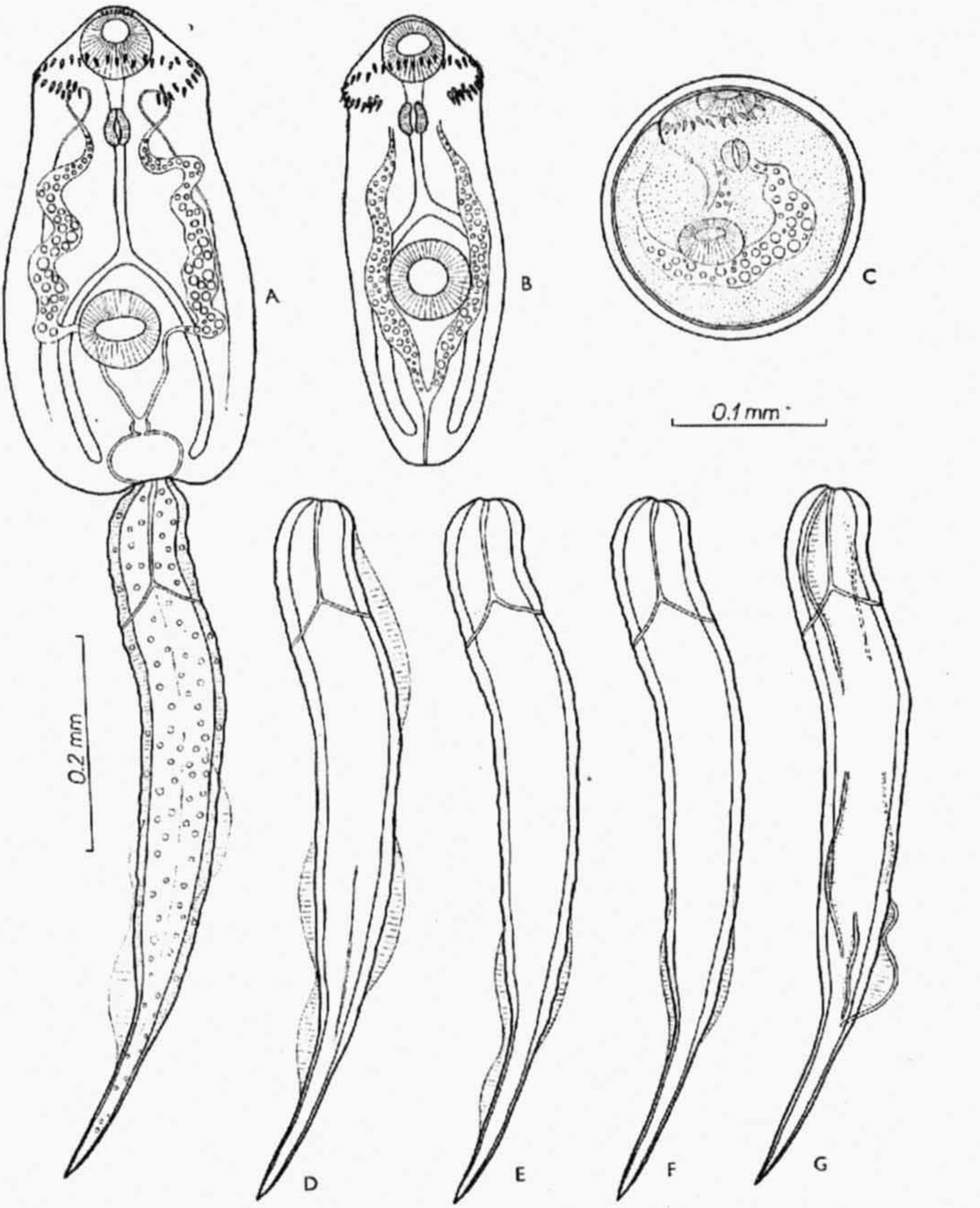

Fig. 8. *Echinostoma revolutum* (Froelich, 1802). A—cercaria; B—metacercaria released from the cyst; C—metacercaria inside the cyst; D, E, F—variability of the cercarial tail (observation *in vivo*); G—arrangement of the tail-fins in a cercaria fixed with formalin.

anterior direction along each side of the body; they are enlarged in the region between the ventral sucker and pharynx and filled with spherical highly refractile excretory granules. At the pharynx region, both primary tubes attenuate, form a loop and proceed in downwards direction. A thin median tube extending from the excretory bladder into the tail, bifurcates at 0.105—0.123 and opens in lateral excretory pores.

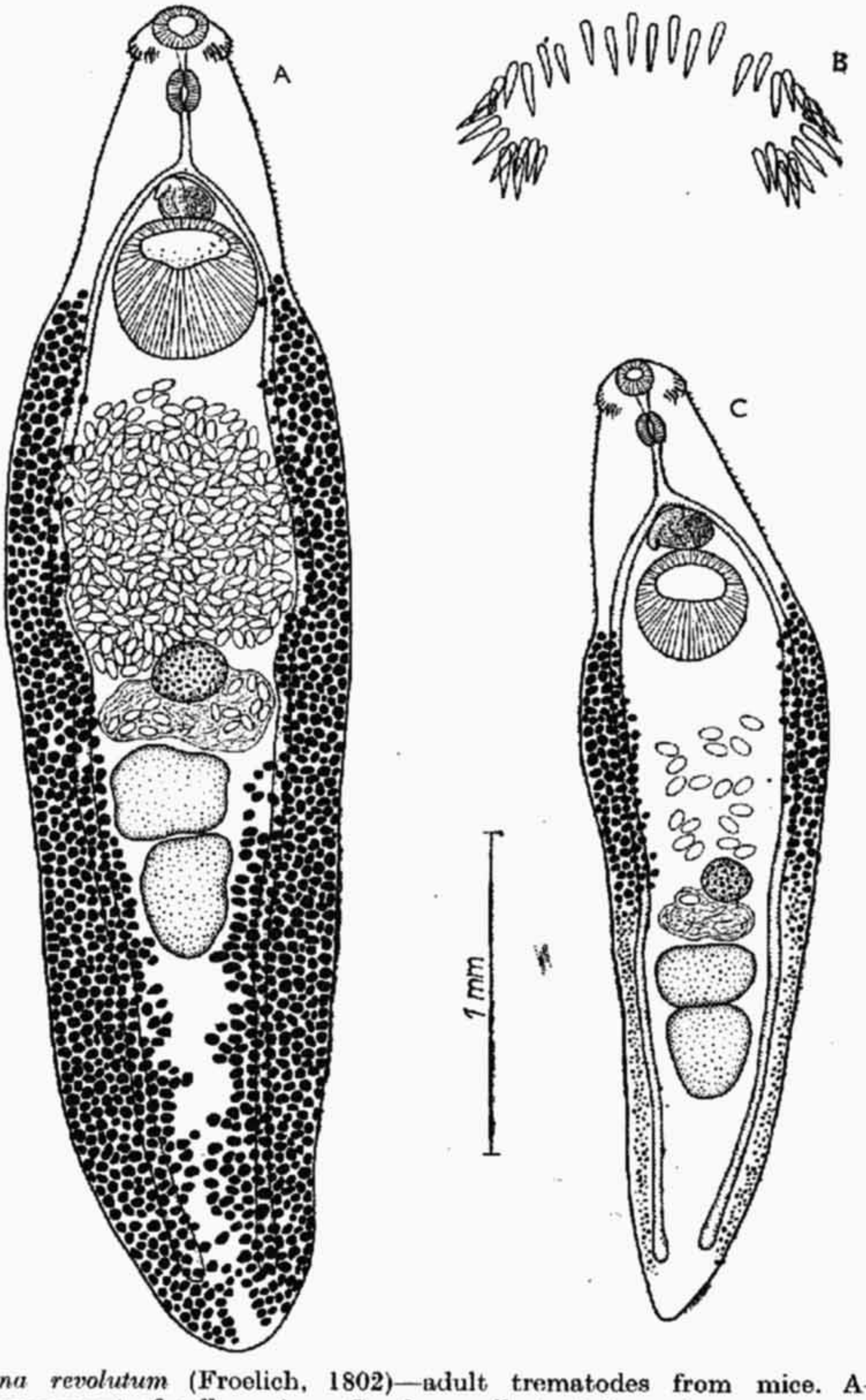

Fig. 9. *Echinostoma revolutum* (Froelich, 1802)—adult trematodes from mice. A—the largest specimen; B—arrangement of collar spines; C—the smallest adult specimen.

Metacercaria: Generally, cercariae emerging from the body of their first intermediate host into the surrounding water, enter the second intermediate host — various snail species, frog tadpoles and fishes. Several cercariae, however, stay in their first intermediate host and encyst there. The location of the cysts with the metacercariae in the second intermediate host — snails — is mostly in the pericardiac sac and kidneys; they were observed in other organs only if the intensity of infection was exceptionally high. In frogs they encyst in the kidneys.

Cysts obtained from *Biomphalaria alexandrina* with experimental infection were spherical, 0.158—0.161 in diameter. The cyst wall was thin (0.007), hyaline. The body of the metacercaria occupies the entire space in the cyst. Generally, the collar spines, the ventral and oral sucker, the oesophagus and excretory granules can be seen through the cyst wall.

The body of the metacercariae released from the cyst measured 0.361—0.385 in length, 0.123—0.151 in maximum width. The arrangement and number of collar spines were analogous to those in an adult trematode. In addition to collar spines, minute spines are present on the cuticle of the anterior part of the body; these reduce in size towards the posterior end of the body. Oral sucker subterminal, almost circular, size 0.049 to 0.060 × 0.049—0.063. Ventral sucker circular or moderately oval, 0.070 — 0.077 × 0.070, situated slightly below mid-body. Praepharynx short; pharynx oval, highly muscular 0.028 × 0.028—0.035, oesophagus (0.070 long) bifurcates close above the ventral sucker into intestinal caeca extending almost to the end of the body. Two excretory tubes of considerable width extend from the pharynx region along each side of the body; they are filled with spherical highly refractile excretory granules. Close under the ventral sucker, both primary excretory tubes unite to form a thin median tube which opens at the posterior end of the metacercarial body.

Adult: Adult trematodes were obtained from various animals with experimental infection, i.e., from mammals and birds. Metacercariae, released from the cysts after digestion in the digestive tract, fasten on the small intestine and mature within 7—12 days.

The body of fixed and stained adult trematodes from mice measured 2.92—6.32 in length, maximum width 1.13—1.77. The conically attenuated anterior portion bears a distinct collar armed with a row of large spines which continues uninterrupted on the dorsal side. A marked widening of the body occurs at the region of the ventral sucker; it attenuates again towards the posterior end. The cuticle covering approximately the anterior half of the body bears minute spines. The collar spines (36—38) measure 0.042—0.069 in length; they are arranged into a row which doubles at each ventral end, the dorsal spines alternate. Oral sucker subterminal, circular or oval, size 0.123 to 0.177 × 0.165—0.245. Ventral sucker large, 0.476—0.680 × 0.476—0.653, almost circular, greatly muscular, situated at approximately the end of the first fourth of body length. A short praepharynx is followed by a pyriform or oval, highly muscular pharynx, length 0.150—0.204, width 0.11—0.163. Oesophagus (0.240—0.258) bifurcates at a short distance above the ventral sucker and forms two intestinal caeca which extend along each side of the body almost to the posterior end. Testes almost equal in size, of irregular shape, generally irregularly oval, tandem, situated in the space between the caecal branches and the posterior half of the body. Size of anterior testis 0.231—0.449 × 0.625 to 0.544, posterior testis 0.381—0.558 × 0.126—0.435. Cirrus sac shortly ovoid (0.258 to 0.272 × 0.150—0.245) situated between the caecal bifurcation and ventral sucker, contains a seminal vesicle, pars prostatica and short cirrus. Ovary spherical or oval, 0.122—0.286 × 0.286—0.367, situated at a short distance above testes. Uterine coils occupy the space bordered laterally by the caeca, anteriorly by the ventral sucker, posteriorly by the ovary. Eggs yellowish brown, operculate, size 0.118—0.134 × 0.069 to 0.072. Vitellaria, formed by minute follicles, extend from approximately the level of the second half of the ventral sucker to the end of the body. In the anterior part of the body the follicles occupy the space between the caeca and the margin of the body forming two lateral bands which in older specimens coalesce in the posterior part of the body posterior to testes.

DISCUSSION

E. revolutum was first recorded from Egypt by Loos (1899) who found adults of this trematode in 3 bird species. Later, Jeyarasasingam et al. (1972) found 37-spined echinostome cercariae in *Biomphalaria alexandrina* from the vicinity of Cairo. They conducted experimental studies on the life cycle of this species with the snail *Biomphalaria glabrata* using it as first intermediate host, and obtained mature trematodes from experimental infection of various mammals and birds. They considered this trematode to be an independent species and named it *Echinostoma liei*. The species differed, alledgedly, from *E. revolutum* mainly in the different number and arrangement of finlike folds on the cercarial tail, and in several other morphological details of the cercaria (the absence of paraoesophageal cell outlets on the ventral surface of the oral sucker and the number of penetration gland outlets on the dorsal margin of the oral sucker), in the average smaller body measurements of the adult trematode, and in the first intermediate host.

We started our study on the life cycle of *E. revolutum* with cercariae from naturally infected *B. alexandrina* collected in the irrigation system near Cairo, i.e., from the same area and from the same snail species as indicated by Jeyarasasingam et al. (1972). In view of the fact that the morphology and measurements of all developmental stages were consistent with those in the description of *E. liei*, and that the parasite was very frequent in the area under consideration (Ryšavý et al. 1973, 1974), we had little doubt about the conspecificity of *E. revolutum* and *E. liei*. Our studies showed that the number and arrangement of the tail fins described by Jeyarasasingam et al. (1972) could be determined in fixed material only (Fig. 3 g), and even there the fins were frequently indistinct. In the live cercaria the number and arrangement of these "fins" appear to be highly variable (Figs. 3a, d, e, f). A comparison of this feature with European material seems almost impracticable, because too little attention has been given to the arrangement and number of fins in cercariae, and Beaver's (1937) data refer to North American trematodes. As shown in the paper by Lie and Basch (1967), and confirmed by us, the number of penetration gland outlets near the oral sucker is not necessarily a constant feature in cercariae of the genus *Echinostoma*. In view of the fact that the European *E. revolutum* also utilizes members of the family Planorbidae (Žďárská 1963, and others) as its first intermediate host, there is no reason to consider the Egyptian trematodes as an independent species and, therefore, they are, for us, *Echinostoma revolutum* (Froelich, 1802). Minute differences in body measurements of the adult trematodes may be explained as a result of the space available in the various definitive hosts. Differences in larval morphology may be due only to intraspecific variability which clearly is likely to be greater at the extreme limit of the area of distribution.

It has been indicated by both Heyneman et al. (1972) (experimental studies on the snail *B. glabrata*) and by our study conducted with natural intermediate host in Egypt (*B. alexandrina*) that the sporocysts, rediae and metacercariae of *E. revolutum* have a highly pathogenic effect on the snail intermediate hosts. In a double infection of the intermediate host with *Schistosoma mansoni*, larvae of *E. revolutum* suppress schistosome larvae. Therefore, it may be possible to utilize this species in the biological control of *S. mansoni* in Egypt and, perhaps, elsewhere.

НАБЛЮДЕНИЯ НАД ЦИКЛАМИ РАЗВИТИЯ ЭХИНОСТОМ ВИДОВ *ECHINOPARYPHIUM RECURVATUM* И *ECHINOSTOMA REVOLUTUM*, АНТАГОНИСТОВ ШИСТОСОМ ЧЕЛОВЕКА В ЕГИПЕТЕ

Ф. Моравец, В. Баруш, Б. Рышавы и Ф. Х. Юсиф

Резюме. Нами подробно изучались циклы развития двух видов трематод, *Echinoparyphium recurvatum* и *Echinostoma revolutum*. В Египете развитие личинок этих двух видов протекает в тех же самых промежуточных хозяевах — моллюсках как и развитие шистосом человека. Первым промежуточным хозяином вида *E. recurvatum* в Египете является моллюск *Bulinus truncatus;* мирацидии этого паразита проникают в сердце и аорту моллюска и превращаются в спороцисты, причем редии находятся в венозных синусах, преимущественно в гепатопанкреасе. При температуре 22—24 °C процесс развития в промежуточном хозяине происходит 29—35 дней. Вторым промежуточным хозяином вида *E. recurvatum* являются моллюски *Bulinus truncatus*, *Biomphalaria alexandrina* и *Physa acuta*, рыбы вида *Gambusia affinis* и головастики вида жаб *Rana mascareniensis;* церкарии инцистируются в теле этих промежуточных хозяев, метацеркарии находятся преимущественно в перикарде и почках, у рыб и головастиков в почках. Путем экспериментального заражения как окончательные хозяева были выявлены домашняя утка, курица, мышь, крыса, хомяк и кролик. Трематоды достигают половозрелости в тонкой кишке хозяев в течение 7—8 дней. Вылупление из яиц мирацидиев зависит от температуры: при оптимальной температуре воды 27 °C они вылупляются уже через 5 дней.

Первым промежуточным хозяином трематоды *E. revolutum* в Египете является моллюск *Biomphalaria alexandrina*. Спороцисты и редии развиваются в внутренних органах; при температуре воды 24 °C развитие церкарий по стадию в свободном состоянии происходит через 25 дней, при температуре воды 16—17 °C — через 72 дней. Вторым промежуточным хозяином являются моллюски видов *B. alexandrina*, *Bulinus truncatus*, *Physa acuta* и *Viviparus unicolor;* инцистированные метацеркарии находили также в почках естественно зараженных жаб (*Rana esculenta*). Развитие вида *E. revolutum* в окончательном хозяине происходит в течение 7—12 дней. Путем экспериментального заражения в качестве окончательных хозяев были выявлены: домашняя утка, курица, мышь, белая крыса, хомяк и кролик, причем неполовозрелые трематоды были обнаружены также в *Ardeola ibis*. Развитие мирацидия в яйце происходит в течение 8—10 дней при температуре воды 28 °C, в течение 21 дней при 17 °C. Кроме описаний отдельных стадий развития в статье приведены также таксономические данные по обоим видам трематод.

REFERENCES

AZIM M. A., On the identification and life history of *Echinostomum recurvatum* von Linstow, 1873. Ann. Trop. Med. Parasit. 24: 189—192, 1930.

BEAVER P. C., Experimental studies on *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. Illinois Biol. Monographs 15: 1—96, 1937.

DOLLFUS R. Ph., Sulla forma adulta di un Echinostomide (Trematoda, Digenea) ottenuta sperimentalmente nel ratto bianco di laboratorio. Atti Acad. naz. Lincei. Ren., Cl. Sci. fis., mat. et natur. 14: 658—665, 1953.

EL GINDY M. S., RUSHDY M. Z., Larval trematodes found in bulinid snails in Egypt. Proc. Zool. Soc. U.A.R. 1: 5—17, 1963.

HEYNEMAN D., LIM H. K., JEYARASASINGAM U., Antagonism of *Echinostoma liei* (Trematoda: Echinostomatidae) against the trematodes *Paryphostomum segregatum* and *Schistosoma mansoni*. Parasitology 65: 223—233, 1972.

JEYARASASINGAM U., HEYNEMAN D., LIM H. K., MANSOUR N., Life cycle of a new echinostome from Egypt, *Echinostoma liei* sp. nov. (Trematoda: Echinostomatidae). Parasitology 65: 203—222, 1972.

KHALIL M., ABAZA M. S., A new trematode parasite of the rat, *Echinostoma aegyptiaca*, nov. sp. Reports and Notes Publ. Health Labor., Cairo 6: 187—189, 1924.

KUNTZ R. E., Development of the cercaria of *Echinoparyphium recurvatum* (Linstow, 1873) Lühe, 1909, with emphasis on excretory system. Thapar Commemor. Vol., pp. 149—158, 1953.

LIE K. J., Antagonism of *Paryphostomum segregatum* rediae to *Schistosoma mansoni* sporocysts in the snail *Biomphalaria glabrata*. J. Parasit. 53: 969—976, 1967a.

—, Studies on Echinostomatidae (Trematoda) in Malaya. XV. The life history of *Echinostoma murinum* (Tubangui, 1931), Proc. Helm. Soc. Wash. 34: 139—143, 1967b.
—, BASCH P. F., The life history of *Echinostoma paraensei* sp. n. (Trematoda: Echinostomatidae). J. Parasit. 53: 1192—1199, 1967.
—, NASEMARY S., Studies on Echinostomatidae (Trematoda) in Malaysia. XVI. The life history of *Echinostoma ilocanum* (Garrison, 1908). Proc. Helm. Soc. Wash. 40: 59—65, 1973.
LOOS A., Weitere Beiträge zur Kenntnis der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. Zool. Jahrbuch, Abt. System. 12: 521—784, 1899.
MATHIAS P., Cyclo évolutif d'un trématode de la famille des Echinostomidae (*Echinoparyphium recurvatum* Linstow). Ann. Sc. nat. Zool. 10: 289—310, 1927.
RAŠÍN K., *Echinoparyphium recurvatum* (Linstow, 1873) a jeho vývoj. Biol. spisy VŠ zvěrolék., Brno 12 (fasc. 166—175): 1—104, 1933.
RYŠAVÝ B., Research of bilharziasis in Egypt. Report on the activities and results obtained by the Czechoslovak-Egyptian team in the period from April 1, 1971 till March 23, 1972. Prague-Cairo, pp. 1—48, 1972. (Unpublished).
—, ERGENS R., GROSCHAFT J., MORAVEC F., YOUSIF F., EL-HASSAN A. A., Preliminary report on the possibility of utilizing competition of the larval schistosomes and other larval trematodes in the intermediate hosts for the biological control of schistosomiasis. Folia parasitol. (Praha) 20: 293—296, 1973.
—, MORAVEC F., BARUŠ V., YOUSIF F., Some helminths of *Bulinus truncatus* and *Biomphalaria alexandrina* from the irrigation system near Cairo. Folia parasitol. (Praha) 21: 97—105, 1974.
SONSINO P., Studi sui parassiti di molluschi di acqua dolce nei dintorni di Cairo in Egitto. Festschr. zur 70. Leuckart's, Leipzig, pp. 134—146, 1892.
ŽĎÁRSKÁ Z., Larvální stadia motolic z vodních plžů na území ČSSR. Čs. parasitol. 10: 207—262, 1963.
—, Method for obtaining metacercariae from cysts *in vitro*. Čs. parasit. 11: 343—345, 1964.

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