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The Life Cycle of *Maritrema setoenensis* n. sp. (Trematoda : Microphallidae)

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In the fall of 1968, crabs, *Hemigrapsus sanguineus* (de Haan, 1835), *H. penicillatus* (de Haan, 1835) and *Macrophthalmus dilitatus* (de Haan, 1835), from the coastal and island intertidal zones of the Japan Inland Sea along the coast of Kagawa Prefecture were found to be parasitized with a microphallid metacercaria. This metacercaria, usually found between the lobules of the hepatopancreas, was examined and recognized to be a previously undescribed species belonging to the genus *Maritrema* Nicoll, 1907. The high incidence of crab infection in certain localities led to the discovery of the cercaria in the common snail, *Littorina brevicula* Philippi, 1844. Adult trematodes were found in the first half of the small intestine of the curlew, *Numenius madagascariensis* L.

Materials and Methods

Crabs, *Hemigrapsus sanguineus* (de Haan, 1835), *H. penicillatus* (de Haan, 1835) and *Macrophthalmus dilitatus* (de Haan, 1835) were collected from the intertidal zone of the Japan Inland Sea along the coast of Kagawa Prefecture between the towns of Tadotsu and Takuma. *H. sanguineus* and *H. penicillatus* were collected in large numbers from beneath small rocks where large rocks formed the major beach area. *M. dilitatus* was collected from a sandy beach area near the village of Mitachi. All crabs were collected at low tide by hand, and were successfully maintained in the laboratory in individual 20 cm diameter plastic pans of filtered diluted sea water (2 parts sea water to 1 part tap water). The crabs were fed pressed dry barley twice a week after which the pans were washed and the water changed.

Examination for metacercarial cysts in several organs was done with the aid of a low power dissecting microscopse. The crab tissue was prepared for examination by compression between two glass slides. Laboratory definitive hosts were exposed by feeding them metacercariae in infected tissue. These hosts were sacrificed and the digestive tract was removed, cut into 10 cm lengths, slit open and scraped into petri dishes containing 0.75 per cent saline. Trematodes were detected with the aid of a low power dissecting microscope and removed with a pipette.

Snails, *Littorina brevicula* Philippi, 1844, were collected in large numbers by hand from the rock surfaces in the same locations from which the two species of *Hemigrapsus* were taken. In the laboratory, those snails observed to be shedding cercariae were isolated for experimentation. The snails were maintained in the laboratory with filtered sea water in plastic containers. Snails were induced to shed by subjecting them to a slight elevation in temperature under an ordinary tungsten light bulb for several hours.

Crabs, from an area found to be uninfected, were exposed to cercariae by placing them together in small but deep glass containers filled with filtered sea water to a level of about 5 cm so that the crab was completely covered. In winter, the snails and crabs were preadapted to an incubator maintained at 30°C and connected to a time clock and

light which maintained summertime daylight lengths of 13 hours. Massive crab infections were obtained by placing the crabs in containers together with snails shedding cercariae for several hours or days.

For life cycle studies in the laboratory, only *H. sanguineus* was used. Crabs collected from the rocky south shore of the uninhabited Kikasa and Iwa Shima Islands located in the Inland Sea 2 km west of the village of Mitachi, were heavily infected, while those collected from the rocky shore adjacent to the village of Mitachi were never found to be infected during the study period. *H. sanguineus* from this uninfected area were used in exposure experiments.

Excystment of metacercariae in 0.75 per cent saline was accomplished with sharp needles; however, many worms excysted spontaneously when left to stand for 2 to 3 days at room temperature.

Both fixed and living material were used. Neutral red and nile-blue sulfate vital stains were used. Whole mounts were fixed in alcohol-formalin-acetic acid fixative and stained in Van Cleave's (1953) combination hematoxylin for mounting in Canada balsam. Specimens used for measurements were killed in hot water before being fixed to assure a standard state of body contraction. Drawings were made free hand and with the aid of a microprojector. All measurements in this paper are in millimeters unless otherwise specified.

Description of *Maritrema setoenensis* n. sp.

A. The Adult (Figures 1, 2 and 14)

Hosts: *Numenius madagascariensis* (L) Australian curlew [natural type host]; *Mus musculus albinus* (ICR strain) and *Rattus norvegicus albinus* (Wistar strain) [laboratory hosts].

Location: Proximal half of small intestine.

Frequency: Numerous in natural host.

Type Locality: Iwa Shima, a small rock island in the Japan Inland Sea 2 km West of Mitachi Village, Kagawa Prefecture, Japan.

Holotpye: U.S. Nat. Mus. Helminthol. Coll. No. *71756*.

Description: (Based on 20 hot water killed specimens from *Numenius madagascariensis*, average size in parentheses)

Microphallidae: *Maritrema*. Body elongate, with tapered anterior end, rounded posterior end sometimes with notch, 0.750 to 0.930 (0.812) long by 0.160 to 0.210 (0.192) wide. Integument of anterior 2/3 of body spined, surface glands present on fore body. Oral sucker subterminal rounded, 0.0500 to 0.0600 (0.0525) in diameter. Prepharynx 0.0550 to 0.1025 (0.0670) long. Pharynx rounded, 0.0250 to 0.0250 (0.0250) in diameter. Esophagus extending from pharynx to about mid body 0.1800 to 0.2300 (0.2005) long. Ceca two, rounded, lined with cells, extending posteriorly from cecal bifurcation at posterior end of esophagus; both ceca 0.1500 to 0.1850 (0.1660) long. Ventral sucker rounded, larger than oral sucker, 0.0750 to 0.0975 (0.0862) in diameter, located mesially at level of posterior tips of two ceca. Sucker ratio (based on average measurements) oral sucker/ventral sucker=1/1.64.

Genital pore immediately sinistral to ventral sucker. Testes, two, side by side, posterior to ventral sucker, edges smooth, rounded, partially obscured by numerous eggs in mature specimens, diameters: right testis 0.0500 to 0.0800 (0.0605); left testis 0.0475 to 0.0725 (0.0560). Vasa deferentia joining dorsally to ventral sucker, connecting with right margin of cirrus pouch. Cirrus pouch, 0.0800 to 0.1100 (0.1002) long by 0.0275 to 0.0400 (0.0327) wide, curved around and anterior and dorsal to ventral sucker. Cirrus pouch containing club-shaped seminal vesicle, tapered to from slender sperm duct surrounded by prostate gland cells with ducts entering near distil tip. Protrusible cirrus not observed in fixed or living specimens. Ovary between cirrus pouch and dextral testis, slightly overlapping ventral sucker, oval to triangular in outline, edges smooth, 0.0525 to 0.0925 (0.0655) long by 0.0400 to 0.0725 (0.0520) wide. Ovary connected to small

fertilization chamber which in turn is connected to uterus containing eggs filling posterior body. Uterus terminating in muscular metraterm which extends anteriorly toward posterior tip of sinistral cecum then loops posteriorly to enter genital pore dorsally. Vitellaria arranged in a complete ring in posterior half of body, arching anteriorly around testes and around posterior end of body. Uterine eggs numerous, operculate, 0.0225 to 0.0250 (0.0343) long by 0.0100 to 0.0125 (0.0105) wide. Excretory vesicle Y-shaped, main stem extending anteriorly from mesial excretory pore at posterior end of body, forking just anterior to posterior ring of vitellaria. Flame cell pattern 2[(2+2)+(2+2)]=16.

Comparisons: *Maritrema setoenensis* most closely resembles *M. linguilla* Jagerskiold, 1908, *M. afanassjewi* (Afanassjew, 1941) Belopolskaya, 1952, *M. elongata* Deblock, Capron and Biguet, 1961, *M. laricola* Ching, 1963, and *M. megametrios* Deblock and Rausch, 1968 but differs distinctly in various dimensions. *M. elongata* possesses a median ovary and like *M. laricola* and *M. megametrios* has a protrusible cirrus. In addition, these species and *M. linguilla* are clearly smaller in body dimensions, the largest body length measurement for *M. laricola*, *M. megametrios* and *M. linguilla* being considerably less than the smallest body length measurement for *M. setoenensis*. The body length dimensions of *M. afanessjewi* are comparable to *M. setoenensis*, but the body width, oral and ventral suckers, pharynx, testes and ovary dimensions are considerably larger, while the prepharynx and egg dimensions of *M. afanessjewi* are smaller.

The specific name, *setoenensis*, applied to this new species indicates the Japanese name of the Japan Inland Sea (Seto Nai Kai) where this microphallid was discovered.

Among the birds examined as suspected natural hosts were two gulls (one *Larus crassirostris* Vicillot and one *L. schistisagus* Stejineger), one loon (*Colymbus adamsii* G. R. Gray), one knot (*Caladris canutus* (L)) and two curlews (*Numenius madascariensis* (L)). Only the two curlews were found to harbor *M. setoenensis*, which were found in large numbers in the proximal half of the small intestine. The gut contents of the gulls and the loon showed no crab shell remains but only small fish, whereas the shore birds all contained crab remains in the gut. The species of these crab remains could not be determined.

After feeding metacercariae mixed with crab hepatopancreas to laboratory rats and mice, numerous mature worms were recovered from the proximal half of the small intestine 1, 2 and 3 days post-exposure. Only a few worms were recovered on days 5 and 6 (some of these from the distal half ot the small intestine), and none were found 7 days post-exposure. Four attempts to infect 2 to 5-day-old chicks in the laboratory yielded no worms from the intestine upon necropsy. A comparison of various dimensions of adult worms taken from the natural host, *Numenius madagascariensis*, and laboratory white mice is shown in Table 1. It is seen that in all the measurements, the mean sizes are very slightly larger for the worms from laboratory mice than from the natural host. The oral sucker/ventral sucker ratio based on means was 1/1.64 and 1/1.62 for the natural and mouse infections respectively.

B. The Metacercaria (Figures 8-13)

The metacercariae were enclosed in small rounded cysts 0.310 to 0.345 in diameter. The cysits were mainly located on and between the lobules of the hepatopancreas, but were occasionaly observed to be attached to the surface of the gonads, in other parts of the viscera or imbedded in the muscle ventral to the visceral cavity in heavy infections. In mature infections, there were three cyst wall layers (Fig. 13); the outer fibrous layer measured 0.0175 to 0.0275 while the tough middle hyaline layer measured 0.0075 to 0.0125 in thickness. The inner layer was a thin membrane 0.0010 to 0.0025 thick. Excysted metacercariae closely resembled the adults except for the absence of eggs, slightly enlarged testes and a more clearly visible

Table 1 *Maritrema setoenensis* range and mean (parentheses) body length and width, oral and ventral sucker diameter, and cirrus pouch length dimensions of adults from *Numenius madagascariensis* (natural host), 2-day infections from laboratory white mice and excysted metacercariae from *Hemigrapsus sanguineus*

	Adults from *N. madagascariensis*	Adults from mice	Excysted metacercariae
Body length	0.750–0.930 (0.812)	0.750–0.100 (0.859)	0.750–0.900 (0.815)
Body width	0.160–0.210 (0.192)	0.180–0.330 (0.234)	0.220–0.250 (0.231)
Oral sucker diameter	0.050–0.060 (0.0525)	0.050–0.070 (0.587)	0.0525–0.0625 (0.0552)
Ventral sucker diameter	0.075–0.0975 (0.0862)	0.075–0.1125 (0.0950)	0.075–0.100 (0.0865)
Cirrus pouch length	0.080–0.110 (0.1002)	0.0670–0.1250 (0.1049)	0.0850–0.1000 (0.0922)

* Measurements based on 20 hot-water-killed, whole mounted specimens per sample are in millimeters.

excretory bladder. The flame cell pattern was the same as for the adult. A comparison of some measurements of excysted metacercariae from *H. sanguineus* with adults from natural and laboratory hosts is shown in Table 1. The oral sucker/ventral sucker ratio based on means was 1/1.56 for the metacercariae.

Throughout the 20-month study period (November, 1968 to June, 1970), 78 per cent of the *H. sanguineus* examined from Kikasa island and 73 per cent from Iwa Shima island were found to be infected with *M. setoenensis* metacercariae. The crabs were lightly to moderately (1 to 50 cysts) infected at all seasons of the year; the incidence ranging from in 64 per cent January and February, 1969 to 92 per cent in July, 1969 and May, 1970. Crab infection probably takes place from the month of May on through the warm summer months, as young cysts were first seen in crab samples beginning about the middle of June in 1969 and 1970. Old thick-walled cysts are readily distinguished from young thin-walled cysts (Fig. 12), demonstrating that the crabs were not refractory to superinfection upon challenge to reinfection. *Hemigrapsus penicillatus* and *Macrophthalmus dilitatus* from infected areas were also occasionally found to be lightly infected.

It is of interest to note that although up to 92 per cent of the *H. sanguineus* samples collected from the rocky shores of uninhabited Iwa Shima and Kikasa islands were infected with *M. setoenensis*, only 2 km to the east, *H. sanguineus* samples collected in large numbers from the rocky shores near the village of Mitachi were never found to be infected. This observation was made use of for experimental studies as infected and uninfected *H. sanguineus* were easily obtained in good numbers at all times of the year from the field. Preceeding experimentation, at least 10 crabs from the sample were examined to determine the incidence of infection, if any.

In the laboratory, 20 *H. sanguineus*, and 5 each of *H. penicillatus*, *Macrophthalmus dilitatus*, *Helice tridens* and 3 species of *Sesarma* were exposed to cercariae from *Littorina brevicula*. Moderate to massive (10 to about 200 cysts) infections resulted in all *H. sanguineus* and *H. penicillatus*, light (1 to 5 cysts) infections in *M. dilitatus* (3 of 5 crabs) and all the other species of crabs were observed to be uninfected with *M. setoenensis* upon examination 6 to 9 weeks post exposure to cercariae. Exposure consisted of placing 5 crabs and 5 shedding snails together in a glass-covered plastic pan of filtered sea water under an electric lamp for 3 hr after which

the crabs and snails were left together without the lamp at room temperature for an additional 10 to 15 hr. Following exposure, the crabs were maintained for 6 to 9 weeks as previously described in individual containers. From one group of 5 *H. sanguineus* exposed together in the same pan, the density of infection upon examination, ranged from 10 to about 200 cysts per crab. This wide variation demonstrated that there are infection conditions which are unknown at this time.

For growth and development of *M. setoenensis* in *H. sanguineus*, crabs were exposed to hundreds of cercariae for 1 hr in individual 5 cm diameter deep glass jars filled with enough filtered sea water to completely cover the crab. The crabs were then maintained in an incubator simulating summer time daylight conditions at 30°C for experimental studies. Crabs examined 1 to 2 hr post exposure demonstrated that infection took place when cercariae entered the gill chamber and penetrated the gill lamellae, since large numbers of cercariae without tails were observed in the gill lamellae and in the gill blood sinuses (Fig. 9). The development and growth of the metacercariae are summarized in Table 2. Growth began immediately in the gill blood sinuses where the metacercariae were observed to be constantly in squirming motion within a thin pliable cyst membrane (Fig. 10). By day-15, small thin-walled styleted metacercariae were observed floating in the hemocoel around the lobes of the hepatopancreas (Fig. 11). Subsequently as the metacercariae enlarged (Fig. 8), they became attached to the lobes of the hepatopancreas. By weeks 6 to 9, the metacercariae were surrounded by a three-layered cyst wall (Fig. 13) and were developed in size and form comparable to mature natural infections.

Metacercariae kept in 0.75 per cent saline at 40°C excysted in 12 to 24 hr. At room temperature excystment took up to 3 days. Although the worms appeared healthy and active and some lived as long as 3 days post-excystment at room temperature and at 40°C, no eggs were produced even though sperm were observed to be active and numerous in the seminal vesicle and the fertilization chamber.

C. The Ceacaria (Figures 3, 4 and 7)

The cercaria is a small "monostome" xiphidiocercaria with the characters of the Ubiquita group. Under light cover slip pressure, living specimens measured 0.1450 to 0.2050 long by 0.0325 to 0.0750 wide and were ovoid to elongate, and the tail with fine cuticular annulations was 0.0325 to 0.1150 long. Measurements of 10 hot water killed, fixed and stained specimens were as follows: Body 0.1450 to 0.1500 long by 0.0425 to 0.0475 wide. Tail 0.1000 to 0.1125 long by 0.0075 to 0.0085 wide at base. Oral sucker 0.0375 to 0.0400 in diameter. Stylet 0.0250

Table 2 Growth and development of *M. setoenensis* in *H. sanguineus*

Age, weeks	Location	Encysted metacercariae size range in millimeters	Development
1-2	Gill lamellae	0.1450-0.1950 by 0.1370-0.0550	Penetration gland cell ducts present, elongate to heart-shaped. Stylet present.
3-4	Hemocoel	0.1900-0.0255	Rounded cysts with one thin flexible wall. No penetration glands; stylet present; excretory bladder enlarged anlagen of gonads, acetabulum and ceca observed. Will not infect mouse.
4-6	On lobes of hepatopancreas	0.2450-0.3250	Cyst wall with 3 layers, outer layer thin. Stylet absent; adult organs observed but not well formed. Cuticular spines present. Will lightly infect mouse.
6-9	On lobes of hepatopancreas	0.3100-0.3450	Cyst wall with 3 layers, outer layer much thickened. All organs well formed. Will easily infect mouse.

long by 0.0025 wide (the same as in living specimens), base squared lightly colonnaded longitudinally, shaft cylindrical, then tapered to slightly ventrally directed point, asymmetrical in side view. Digestive system not observed. Four pairs of thin penetration gland cell ducts 0.0625 to 0.0750 long by 0.0020 to 0.0030 wide, opening anteriorly into the anterior portion of the oral sucker lateral to the stylet, connecting posteriorly to four pairs of penetration gland cells; the anterior pairs staining darker than the posterior pairs. Genital anlagen a small mass posterior to penetration glands. Excretory bladder heart-shaped. Flame cell pattern 2 [(1+1)+(1+1)].

Host: *Littorina brevicula* Philippi, 1844.

Type locality: Iwa Shima, a small rock island in the Japan Inland Sea 2 km west of the village of Mitachi, Kagawa Prefecture, Japan.

Frequency: Throughout the year, 3 to 8 per cent of snails collected.

Of the species of adult *Maritrema* compared to *M. setoenensis*, only the cercaria of *M. laricola* Ching, 1963 has been described. Although Ching's (1963) cercaria is comparable in form, it is in all respects smaller, except for tail length, than the cercaria of *M. setoenensis*. The cercaria of *M. laricola* was reported to have half the number of flame cells observed for *M. setoenensis*.

When shed into sea water, the cercariae swam with the posterior part of the body flexed ventrally and the tail lashing vigorously in S-shaped movements. From time to time, the swimming motion would stop, and the cercariae would creep along the bottom of the dish after which the swimming would be resumed. This type of activity aided the cercariae as they were drawn into the gill chamber of the crab for penetration of the gills. From 5 to 6 hrs post emergence from the snail, many of the cercariae dropped their tails but still remained alive on the bottom of the container. At room temperature, the cercariae remained alive up to 24 hours.

D. The Sporocyst (Figures 5 and 6)

There are probably two sporocyst generations of *M. setoenensis* in *Littorina brevicula*, but only the cercaria-producing one was observed in naturally infected snails in the laboratory. Crushed snails contained from 10 to about 60 round to elongata sporocysts ranging in size from 0.400 to 0.600 long by 0.175 to 0.225 wide under light cover glass pressure. Sporocysts were observed to contain from 10 to about 50 cercariae. The birth pore was terminal; the opposite end of the sporocyst contained rounded structures considered to be developing cercariae and germ balls. Attempts to infect snails in the laboratory by feeding them eggs from gravid specimens were unsuccessful.

Discussion

From Japan, the following species of *Maritrema* have been described: *M. eroliae* Yamaguti, 1939; *M. caridinae* Yamaguti and Nishimura, 1944; *M. macrovestibulum* Ogata, 1951; *M. urayasensis* Ogata, 1951; and *M. kitanensis* Shibue, 1953. Deblock and Pearson (1968) proposed that *M. magnicirrus* Belopolskaya, 1952, and *M. echinocirrata* Leonov, 1958 be placed in synonymy with *M. eroliae* Yamaguti, 1939. To this synonymy the author would suggest the addition of *M. urayasensis* Ogata, 1951, as specimens collected from *Macrophthalmus dilitatus* (de Haan, 1853) in this Inland Sea area demonstrated a complete as well as an incomplete posterior body ring of vitellarian follicles, casting doubt upon Ogata's (1951) basis for distinguishing this new species. A more detailed study of the morphology and life cycles of the *M. eroliae* synonyms is needed. Of the species of *Maritrema* from Japan, only the life cycle of *M. caridinae* has been described (Shibue, 1951). These species of *Maritrema* from Japan show no close similarities to *M. setoenensis*.

Size variations within the same species of trematodes is discussed by Rodhe (1966) and Neiland *et al.* (1970). Neiland *et al.* (1970), however, pointed out that the use of size variations appear to be meaningful criterion only in a proportional sense or in extreme cases for speciation of the genus *Nasitrema*

Ozaki, 1935 from porpoises. For microphallids, Baer (1943), Belopolskaya (1957), Stunkard (1960) and Bridgman (1969), have pointed out that growth takes place in the second intermediate host, which is usually a crustacean. Staged development of the growth of *M. setoenensis* metacercariae in the crab demonstrates the remarkable increase in body and organ size that takes place at this stage of the life cycle (Fig. 12). The reports of Hunter and Vernberg (1953), Ching (1963 a, b) and Bridgman (1969) are in agreement with this growth of the microphallid in the second intermediate host. It therefore follows that no significant growth takes place in adult microphallids during their short-term infections in the intestine of the definitive host either with time or between different hosts (Table 1. and Bridgman, 1969). The size of *M. setoenensis* adults, which is consistently and significantly different from the other morphologically comparable and closely related species of *Maritrema*, is therefore considered to be a valid criterion for distinguishing this new species.

The stylet and cephalic gland morphology of cercariae were considered to be of taxonomic significance by Cable (1956) and Cable *et al.* (1960). They maintained that definitive determination of the taxonomic position of some forms can not be done without a knowledge of the life cycle. Among the differences worthy of note between the known cercariae of *Maritrema* closely related to *M. setoenensis* are the dimensions of the stylet (*M. setoenensis*, 0.025 by 0.0025; *M. laricola* Ching, 1963, 0.013 to 0.019 by 0.001 to 0.003). Although the first and second intermediate hosts for *M. laricola* and *M. setoenensis* are members of the same genera (snails, *Littorina* and crabs, *Hemigrapsus*), it appears that as these snails and crabs evolved to become eastern and western Pacific Ocean species, this may have been paralleled by the development of closely related geographical species of *Maritrema*.

Ching (1963 a) reported infrequent immature *M. laricola* recovery from laboratory exposure to metacercariae of mice and chicks and moderate recovery from laboratory-hatched young gulls maintained on commercial cat food. After feeding metacercariae, *M. setoenensis* was not recovered from chicks, but by contrast, large numbers were recovered from laboratory mice and rats. Mature and immature worms were in all respects identical to those taken from the natural host. That the choice of definitive hosts is probably determined by the host food habits and the incidence and density of parasitism of the food is illustrated by the variation in natural definitive hosts presented by Deblock and Rausch (1969) where anseriform birds and brown rats were found to be naturally infected with *Maritrema megametrios*. Although the curlew, *Numenius madagascariensis*, was the only natural definitive host taken in this study, it is probable that a number of birds and possibly mammals could also serve for *M. setoenensis*.

The high incidence of infection in *Hemigrapsus sanguineus* from Iwa Shima and Kikasa Islands, whereas only 2 km away the same species of crabs were never found to be infected, can best be explained by the fact that the islands were uninhabited and were therefore frequently used as feeding and resting areas for a large variety of sea and shore birds throughout the year. The coastal shores of Kagawa Prefecture are heavily populated by humans and although birds came to the beaches, they were constantly moving about. *Littorina brevicula* were found in large numbers in both study areas, but snails shedding cercariae were found only in collections from the islands. The fact that snail collections throughout the year were found to be shedding *M. setoenensis* cercariae suggests that birds other than the migratory curlew are also parasitized, seeding the snails during the warmer seasons of the year.

Summary

The life cycle of *Maritrema setoenensis* n. sp. from the coast of Kagawa Prefecture in the Japan Inland Sea is described. Adult trematodes were found in the small intestine of *Numenius madagascariensis* L. Metacercariae were found between the lobes of the

hepatopancreas of *Hemigrapsus sanguineus* (de Haan, 1835). The common snail, *Littorina brevicula* Philippi, 1844, was found to shed cercariae. *Maritrema* from Japan are discussed and *M. setonensis* is compared with other similar and closely related species from North America and Europe.

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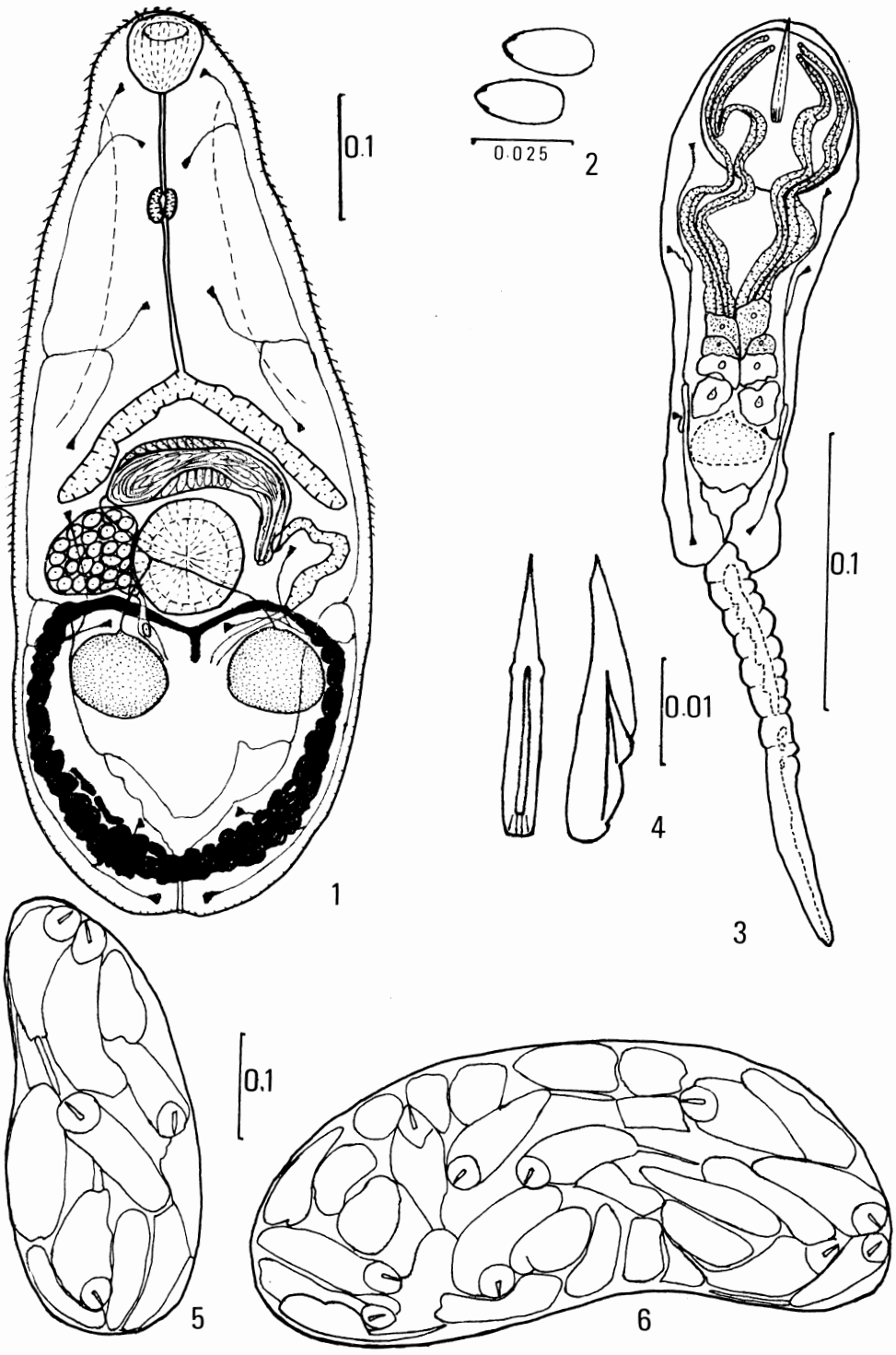
Literature Cited

1) Baer, J. G. (1943) : Les trematodes parasites de la musarainge d'eau *Neomys fodiens* (Schreb). Bull. Soc. Neuchatel Sci. Nat., 68, 33-84.
2) Belopolskaya, M. M. (1957) : Die Fauna der Trematoden larven von *Gammarus locusta* L. aus der Ostsee. Tr. Lenigr. Obshch. Estestv., 73, 164-170, (in Russian ; German summary).
3) Bridgman, J. F. (1969) : Life cycles of *Carneophallus choanophallus* n. sp. and *C. basodactylophallus* n. sp. (Trematoda : Microphallidae), Tulane Stud. Zool. Bot., 15, 81-105.
4) Cable, R. M. (1956) : Marine cercariae of Puerto Rico. Sci. Survey of Puerto Rico and the Virgin Islands. N. Y. Acad. Sci., 16, 491-577.
5) Cable, R. M., Connor, R. S. and Balling, J. W. (1960) : Digenetic trematodes of Puerto Rican Shore Birds. Sci. Survey Puerto Rico and the Virgin Isl. N. Y. Acad. Sci., 17, 187-254.
6) Ching, Hilda Lei (1963 a) : The description and life cycle of *Maritrema laricola* sp. n. (Trematoda : Microphallidae). Canad. J. Zool., 41, 881-888.
7) Ching Hilda Lei (1963 b) : The life cycle and bionomics of *Levinseniella charadriformis* Young, 1949 (Trematoda : Microphallidae). Canad. J. Zool., 41, 889-899.
8) Deblock, S. and Pearson, J. C. (1968) : Contribution a l'etude des Microphallidae Travassos, 1920 (Trematoda) XV. De quelques especes d'Australie dont *Pseudolevinseniella ananteron* n. sp. Ann. Par. Hum. Comp. 43, 457-465.
9) Deblock, S. and Rausch, R. L. (1968) : Contribution a l'etude des microphallides Travassos, 1920 (Trematoda). XV. Description de *Maritrema megametrios* n. sp. parasite d'oiseaux de la cote ouest d'amerique du nord. Bull. Soc. Zool. France, 93, 317-323.
10) Hunter, W. S. and Vernberg, W. B. (1953) : Early stages in the life cycle of the trematode, *Gynaecotyla adunca* (Linton, 1905). Trans. Amer. Micro. Soc., 72, 163-170.
11) Neiland, K. A., Rice, D. W. and Holden, B. L. (1970) : Helminths of marine mammals, I. The genus *Nasitrema*, air sinus flukes of delphinid cetacea. J. Parasit., 56, 305-316.
12) Ogata, T. (1951) : Studies on the life histories of certain trematodes the intermediate hosts of which are brackish water crustaceans, with the discussion on the systematic position of the species. Jap. J. Parasit., 1, 17-35, (in Japanese).
13) Rodhe, Klaus. (1966) : On the trematode genera *Lutztrema* Travassos, 1941 and *Anchitrema* Looss, 1899 from Malayan Bats, with a discussion of allometric growth in helminths. Proc. Helminth. Soc. Wash., 33, 184-199.
14) Shibue, H. (1951) : The life history of cercaria Takahashi, a xiphidiocercaria found in *Oncomelania nosophora*. Jap. Med. Jour., 4 (5), 315-324.
15) Stunkard, H. W. (1960) : Problems of the generic and specific determination on digenetic trematodes with special reference to the genus *Microphallus* Ward, 1901. Lib. Hamenaje Dr. Eduardo Caballero y C. Mexico, D. F., pp. 299-309.
16) Van Cleave, H. J. (1953) : Acanthocephala of North American mammals III. Biol. Monogr., 23, 1-179.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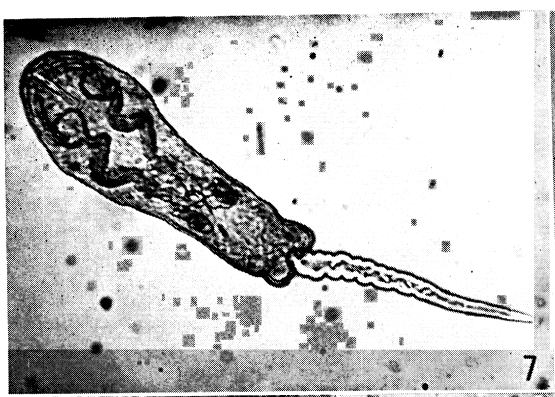
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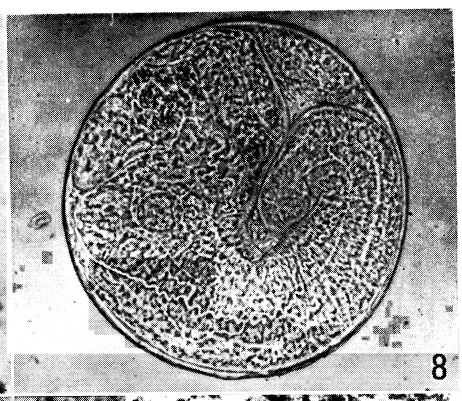
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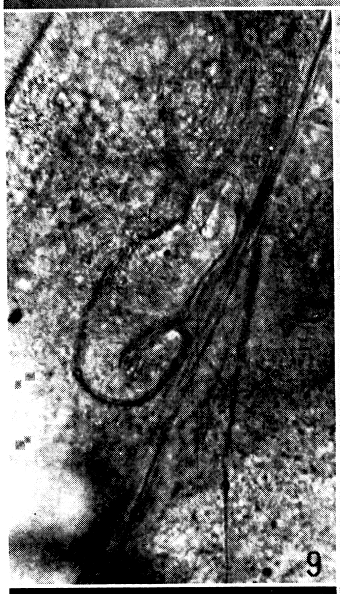
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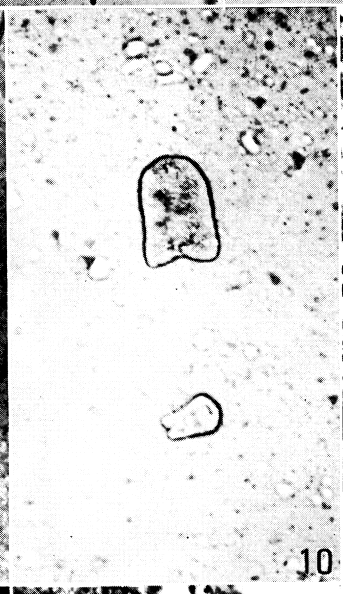
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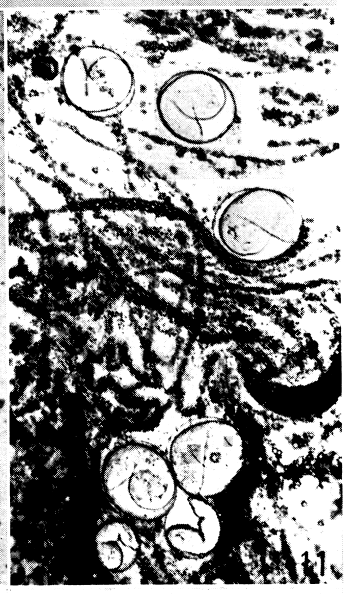
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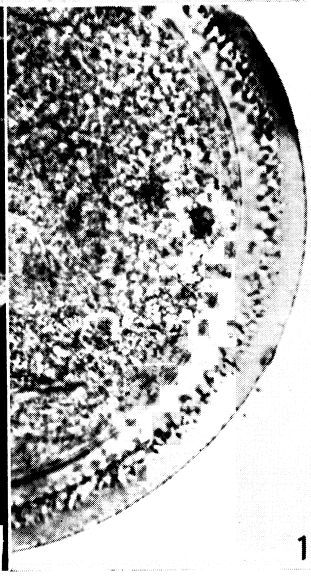
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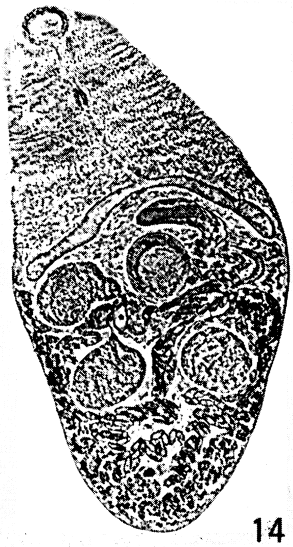
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