Paralepidapedon g. n. (Trematoda: Lepocreadiidae), with Descriptions of Metacercariae of Paralepidapedon hoplognathi (Yamaguti, 1938) comb. n. and of Two Other Species from Sea Urchins

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ABSTRACT — Paralepidapedon g. n. (Trematoda: Lepocreadiidae) is erected to receive P. hoplognathi (Yamaguti, 1938) comb. n. (type species), P. antarcticum (Prudhoe et Bray, 1973) comb. n., P. dubium (Prudhoe et Bray, 1973) comb. n. and P. sebastisci (Yamaguti, 1938) comb. n. This genus is most closely related to Neolepidapedon Manter, 1954, but different from it in having the uroproct. Metacercariae of P. hoplognathi were found encysted in the sea urchins, Anthocidaris crassispina, Hemicentrotus pulcherrimus and Pseudocentrotus depressus, at Misaki, Kanagawa Prefecture, and developed to sexual maturity in the fish, Oplegnathus fasciatus, in feeding experiments. The metacercarial and adult worms are described. The development, structure and phylogenetic importance of the cirrus pouch in the genus are discussed. In addition, simultaneously obtained metacercariae of Proctoeces maculatus (Looss, 1901) Odhner, 1911 (Fellodistomidae), from A. crassispina, Diadema setosum and H. pulcherrimus, and of an unidentified trematode from A. crassispina are briefly described.

Metacercariae of a lepocreadiid trematode were found in sea urchins. They were fed to fish, from which juvenile and adult worms were recovered. This trematode was identified as *Neolepidapedon hoplognathi* (Yamaguti, 1938) Manter, 1954. Close examination of these metacercarial and adult flukes and Yamaguti's [1, 2] specimens of this species showed that the species represents a new lepocreadiid genus, which is proposed with it as the type species in this paper.

MATERIALS AND METHODS

Sea urchins, Anthocidaris crassispina, were collected from around the pier of the Misaki Marine Biological Station, University of Tokyo, Misaki, Kanagawa Prefecture, on several occasions from May 1980 to July 1983. They were crushed and examined organ by organ for parasites.

In July 1983, a small collection of *Diadema setosum*, *Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus* taken at the same locality was also examined.

In order to obtain adult flukes, two experiments were conducted to feed metacercariae isolated from A. crassispina to fish, Oplegnathus fasciatus, by means of a gastric tube. In the first experiment, about 30 metacercariae each were given to three fish. One of them was autopsied 1 day later and the rest were examined 3 days later. In the second experiment, about 110 metacercariae were fed to one fish, followed by about 130 metacercariae 2 days later, and it was autopsied 3 days after the last feeding. The fish used were commercially supplied and had been kept in an aquarium before use for a few weeks after capture in the sea.

Parasites were flattened, fixed in Schaudinn's solution, stained with Heidenhain's iron hematoxylin or alum carmine and mounted in Canada balsam. Infected organs of hosts were made into serial paraffin sections and stained with hema-

toxylin and eosin or with the PAS. Representatives of the specimens studied are deposited in the collection of the National Science Museum (Natural History), Tokyo. All measurements are given in millimeters.

Specimens including the holotypes and paratypes of species hitherto assigned to the genus *Neolepidapedon* Manter, 1954, were restudied, whenever possible, on loan from the Meguro Parasitological Museum, Tokyo; the U.S. National Museum Helminthological Collection, Beltsville, Maryland, U.S.A.; and the Harold W. Manter Laboratory, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A.

RESULTS

Results of surveys

Encysted metacercariae of the trematode (NSMT-Pl 2625-2636) were found mostly in the gonads and rarely in the muscles of Aristotle's lantern and ampullae of A. crassispina, H. pulcherrimus and Ps. depressus. The first was much more heavily infected than the two others. On July 10, 1980, 29 individuals (43 to 62 mm in testa diameter) of A. crassispina were examined, and 22 or 76% of them harbored 1 to 66 (mean, 14.9) parasites in the gonads. Besides these metacercariae, those of two other species of trematodes were obtained, and they will be dealt with later (see pp. 815-816).

Metacercaria (Figs. 1 and 2)

Measurements based on 10 individuals. Cyst single-layered, globular to ellipsoidal, increasing in size to more than 0.9 in diameter with growth of

worm in it, about 0.02 in thickness. Body filiform, spinose, 1.14-4.80 long by 0.20-0.66 wide. Eye-spot pigment absent. Oral sucker funnelshaped, subterminal, 0.09-0.30 long by 0.07-0.32 wide. Prepharynx long, 0.24 - 0.60long. Pharynx 0.06-0.18 long by 0.05-0.14 wide. Esophagus very short, 0.02-0.12 long, one-fifth to -fourth of prepharynx length. Intestinal bifurcation near posterior one-third of forebody. Intestinal ceca a little undulating, containing reddish brown material, opening into excretory vesicle to form uroproct near posterior end of body. Ventral sucker 0.08-0.26 long by 0.09-0.25 wide, equatorial or slightly anterior to it; sucker width ratio 1: 0.81–1.22. Reproductive organs fairly well differentiated, similar to but less developed than those of sexually immature specimens found in the fish in the second experiment as will be described later. Testes postovarian, tandem, separated, less than 0.20 in diameter. Ovary 3- to 4-lobed, small, near middle of hindbody, less than 0.06 in diameter. Vitellaria not developed. Excretory vesicle tubular, reaching to ovary; main collecting canals extending laterally to near pharynx; flame cells numerous, formula not worked out; pore terminal.

Results of feeding experiments (Figs. 3 and 4)

In the first experiment, three juvenile worms (NSMT-Pl 2637) were found in the small intestine of the fish 1 day after feeding. Morphologically, they agreed well with the foregoing metacercaria. From the small intestine of one of the two fish examined 3 days after feeding, two gravid worm with several eggs in the uterus (NSMT-Pl 2638) were recovered. No worm was detected in the other fish. The morphology (Fig. 3) and measurements of these two adults were as follows.

Figs. 1-5. Paralepidapedon hoplognathi (Yamaguti, 1938) comb. n.

Fig. 1. Encysted metacercaria from Anthocidaris crassispina.

Fig. 2. Excysted metacercaria from A. crassispina, entire body (a) and posterior part of body (b), ventral view.

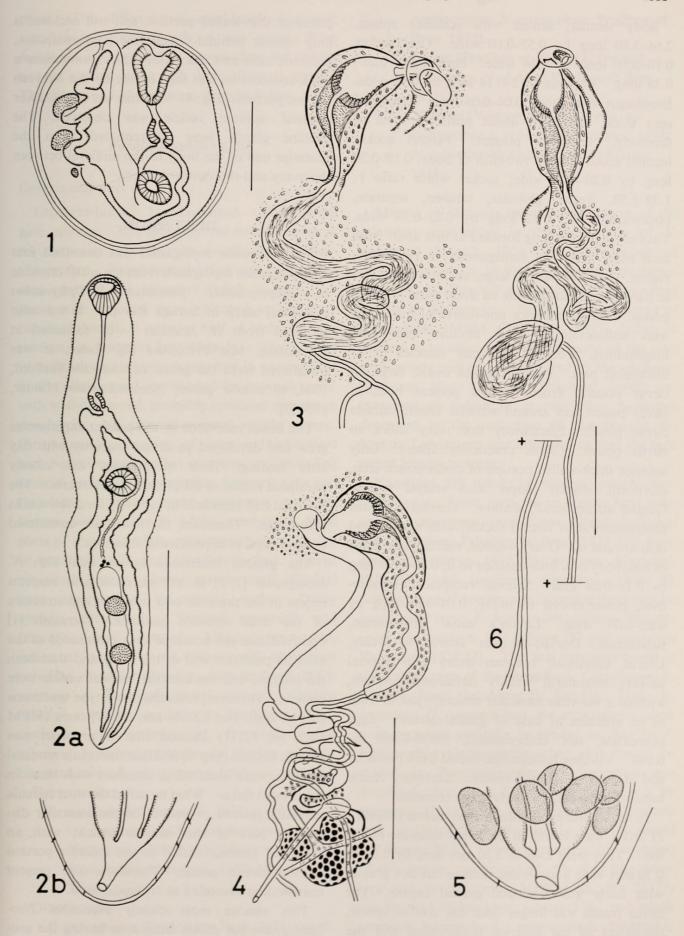
FIG. 3. Gravid worm recovered from *Oplegnathus fasciatus* 3 days after feeding of metacercariae, male terminal genitalia, ventral view.

Fig. 4. Juvenile worm recovered from O. fasciatus (3 days after feeding of metacercariae?), terminal genitalia and ovarian complex, dorsal view.

Fig. 5. Yamaguti's specimen (MPM Coll. No. 22111) from O. fasciatus, posterior part of body, ventral view.

Fig. 6. Yamaguti's specimen (MPM Coll. No. 22113) of *P. sebastisci* (Yamaguti, 1938) comb. n. from *Sebastes inermis*, male terminal genitalia, ventral view.

Scale bars: 1=0.3 mm; 2a=1 mm; 2b=0.1 mm; 3, 4, 6=0.2 mm; 5=0.1 mm.



Body slender, armed with scalelike spines, 2.64-3.80 long by 0.52-0.60 wide. Oral sucker 0.10-0.16 long by 0.14 wide. Prepharynx 0.22-0.38 long. Pharynx 0.12-0.14 long by 0.14 wide. Esophagus very short, 0.04-0.06 long. Intestinal ceca thick, bifurcated about posterior third of forebody. Uroproct present. Ventral sucker located about anteror two-fifth of body, 0.19-0.20 long by 0.20-0.21 wide; sucker width ratio 1: 1.39-1.59. Testes globular, tandem, separate, postovarian, 0.22-0.32 long by 0.22-0.28 wide. Vasa efferentia running forward at first apart from each other and then contiguously in preovarian region. Seminal vesicle large, sinuous, lying free in parenchyma but leaving its distal parts in cirrus pouch, in about anterior one-fourth of hindbody, with well-developed inner circular and outer longitudinal, probably sphincter muscles in its distalmost part. Pars prostatica ovoid, large, in cirrus pouch. Prostatic cells present both in cirrus pouch and around seminal vesicle outside cirrus pouch. Ejaculatory duct very short, in cirrus pouch. Cirrus practically absent. Only anterior thick-walled portion of cirrus pouch seen, claviform, slightly longer than ventral sucker. Genital atrium small, shallow. Genital pore just anterosinistral to ventral sucker, with small gland cells around it. Ovary 4-lobed, median, in middle of hindbody or a little anterior to it, 0.14-0.24 long by 0.19-0.24 wide. Seminal receptacle elongateoval, posterodorsal to ovary, 0.04-0.05 long by 0.02-0.04 wide. Laurer's canal postovarian, submedian. Ootype-complex lateral to ovary. Uterus intercecal, between ovary and ventral sucker; metraterm weakly developed, smooth, without a valvelike muscular structure just in front of its aperture at base of genital atrium. Eggs operculate, not embryonated, 0.056-0.060 by 0.040. Vitelline follicles distributed from posterior end of body to seminal vesicle. Excretory vesicle tubular, extending to ovary; pore terminal.

In the second experiment, seven worms (NSMT-Pl 2639) were found in the small intestine of the fish. They were 2.46 to 3.20 mm long by 0.50 to 0.70 mm wide and almost mature but not gravid, with fairly well-developed genital organs. The cirrus pouch was longer than the ventral sucker, composed of the anterior thick-walled and the

posterior thin-walled portion, and still enclosed a long tubular seminal vesicle, the pars prostatica, prostatic cells and ejaculatory duct; sometimes a weak constriction was seen on the border between the two portions (Fig. 4). Neither vas deferens nor external seminal vesicle was observed. The vitelline glands were differentiated from the posterior end of the body to the midlevel between the ovary and the ventral sucker.

DISCUSSION

Neolepidapedon hoplognathi was described first as Lepidapedon hoplognathi from the small intestine of Hoplognathus punctatus [=Oplegnathus punctatus] taken in Suruga Bay [1]. It was also recorded from H. fasciatus [=O. fasciatus] at Hamazima, Mie Prefecture [2]. Later, it was transferred from the genus Lepidapedon Stafford, 1904, to a new genus, Neolepidapedon Manter, 1954 [3].

The flukes recovered in the present experiments grew and developed to sexual maturity with day after feeding. Their morphology was closely paralleled to that of the present metacercaria. The fish used had been held in captivity for a few weeks before use. Therefore, the flukes are considered to have been of experimental infection.

The present trematode agrees well with N. hoplognathi [1, 2] in all morphological respects except in the presence of a uroproct and structure of the male terminal genitalia. Yamaguti [1] observed that the intestinal ceca terminated at the extreme posterior end of the body and that both the internal and the external seminal vesicle were present. However, restudies of the type specimens (MPM Coll. No. 22376) and his [2] ones (MPM Coll. No. 22111) showed that a uroproct was present in them (Fig. 5) and that their male terminal genitalia were identical in structure with those in the present fluke. What he called the oval vesicula seminalis interna proved to be the muscular distalmost part of the seminal vesicle, with an expanded lumen, located in the anterior portion of the cirrus pouch. Therefore, the present trematode is identified as N. hoplognathi.

This species most closely resembles Neolepidapedon but differs from it in having the uroproct. This difference is considered of generic significance. Consequently, a new genus, *Paralepidapedon*, is created to accomodate the species as type species, along with three others at present. The diagonosis of this genus is given below.

Paralepidapedon g. n.

Generic diagnosis

Lepocreadiidae: Lepidapedinae. Body elongate, spinose. Eye-spot pigment not seen even in metacercariae. Prepharynx usually long. Pharynx present. Esophagus short or not. Intestinal ceca opening into excretory vesicle to form uroproct with terminal pore. Ventral sucker comparatively small, in anterior half of body. Testes tandem, in posterior half of body. Greater part of seminal vesicle convoluted, free in parenchyma in the adult stage, surrounded by prostatic cells, with well-developed, probably sphincter muscles in distal end. No external seminal vesicle present. Cirrus pouch consisting of anterior thick-walled and posterior thin-walled portions and including seminal vesicle, pars prostatica, prostatic cells and ejaculatory duct in young specimens; only the former portion seen in adults, containing distal parts of seminal vesicle, pars prostatica, a number of prostatic cells and ejaculatory duct. Cirrus practically absent. Genital atrium small. Genital pore anterolateral to ventral sucker. Ovary median, pretesticular. Seminal receptacle and Laurer's canal present. Uterus preovarian, intercecal; metraterm not spinose, with or without valvelike muscular structure just in front of its aperture at base of genital atrium. Vitelline follicles extensive, confined to hindbody. Eggs operculate, not embryonated. Excretory vesicle tubular. Intestinal parasites of marine teleosts. Encysted metacrecariae known from sea urchins.

Type species:

P. hoplognathi (Yamaguti, 1938) comb. n. (synonyms: Lepidapedon hoplognathi Yamaguti, 1938; Neolepidapedon hoplognathi (Yamaguti, 1938) Manter, 1954).

Other species:

P. antarcticum (Prudhoe et Bray, 1973) comb.

- n. (synonym: *N. antarcticum* Prudhoe et Bray, 1973).
- P. dubium (Prudhoe et Bray, 1973) comb. n. (synonym: N. dubium Prudhoe et Bray, 1973).
- P. sebastisci (Yamaguti, 1938) comb. n. (synonyms: L. sebastisci Yamaguti, 1938; N. sebastisci (Yamaguti, 1938) Manter, 1954).

Manter [3] did not mention the nature of the intestinal ceca either in the description of *N. polyprioni* Manter, 1954, the type species of *Neolepidapedon*, or in the diagnosis of this genus. However, his Figure 7 shows the ceca terminating blindly in this species. Restudy of a paratype of the species (MPM Coll. No. 22385) failed to confirm it because the posterior parts of the ceca were poorly stained and obscured by the vitellaria. Professor Mary Hanson Pritchard kindly reexamined on behalf of us two other paratypes deposited in the collection of the Harold W. Manter Laboratory and informed us that the ceca ended blindly in them.

Yamaguti [4] listed 14 species under Neolepidapedon. Since then, seven species have been named in the genus: antarcticum, dubium, helicoleni and trematomi Prudhoe et Bray, 1973 [5]; histiopteri Korotaeva, 1975 [6]; belizense Fischthal, 1977 [7]; and israelense Fischthal, 1980 [8]. Among these 21 species, the following three besides hoplognathi (now P. hoplognathi) possess the uroproct: antarcticum [5], dubium [5] and sebastisci Yamaguti, 1938 [2]. The first two were found in the pyloric ceca of the same host, Coryphaenoides whitsoni, from the Antarctic Sea [5]. Their descriptions [5] suggest that the structure of their male terminal genitalia is similar to that in P. hoplognathi. Consequently, they also should be placed in Paralepidapedon. N. sebastisci was described first as Lepidapedon sebastisci by Yamaguti [1] from the small intestine and pyloric ceca of "Sebastiscus albofasciatus from Nagasaki and Suruga Bay." The type material (three slides) of this species was restudied. A slide (the holotype and a paratype; MPM Coll. No. 22377) was labelled "Opechona sebastisci n. sp.; small intestine; kasago [=S. marmoratus]; Nagasaki;

26/4 35." Another slide (four paratypes and a specimen of Plagioporus isaitschikowi identified by him; MPM Coll. No. 22112) was labelled "Opechona sebastisci; upper part of small intestine and pyloric ceca; ayamekasago [=S. albofasciatus]; Numazu; 13/4 35." A third slide (a paratype; MPM Coll. No. 22112) was labelled "Opechona sebastisci; upper part of small intestine; ayamekasago; Numazu; 13/4 35." The generic name Opechona was corrected to Lepidapedon on their labels by Shunya Kamegai on March 15, 1972. It seems likely that the type host is not S. albofasciatus but S. marmoratus and the type locality is Nagasaki. He [2] also recorded the species from "the intestine of Sebastiscus marmoratus Cuv. et Valenc. at Hamazima, Mie Prefecture." His slide (six specimens; MPM Coll. No. 22113) was labelled "Lepidapedon sebastisci; stomach and small intestine; mebaru [=Sebastes inermis]; Hamazima; 12/4 39." He may have made a mistake in description of the host name. Later, Manter [3] transferred the species from Lepidapedon to Neolepidapedon, presumably without knowing the presence of the uroproct in the species [2]. Reexamination of Yamaguti's [1, 2] specimens confirmed it and revealed that the male terminal genitalia in them (Fig. 6) was identical in anatomy with those in P. hoplognathi. The valvelike muscular structure in the genital atrium, which Yamaguti [1, 2] had not referred to, was observed. Therefore, the species also should be allocated to Paralepidapedon. P. hoplognathi is distinguishable from the three other species by having the prepharynx being much longer than the very short esophagus and by lacking the valvelike muscular structure in the genital atrium. The latter three are very like. P. sebastisci may be separated from the two others by a larger sucker width ratio, which was 1: 1.28 to 1.68 in nine better prepared ones of Yamaguti's specimens reexamined. P. dubium is said to differ from P. antarcticum in having the somewhat smaller size of body, oral sucker being distinctly smaller than the ventral sucker and definitely larger eggs [5].

As for the remaining 16 species in *Neolepidapedon* the exact nature of the intestinal ceca in them is not necessarily clear. The descriptions or figures indicate the blindly ending ceca in *cablei* Manter,

1954 [3]; dollfusi Durio et Manter, 1968 [9]; epinepheli Siddiqi et Cable, 1960 [10]; helicoleni [5]; histiopteri [6]; hypoplectri Nahhas et Cable, 1964 [11]; israelense [8]; macrum Overstreet, 1969 [12]; pugetense (Acena, 1947) Yamaguti, 1971 [13]; retrusum (Linton, 1940) Sogandares-Bernal et Hutton, 1960 [14]; and trematomi [5]. The descriptions and figures omit details of the ceca in belizense [7]; equilatum Siddiqi et Cable, 1960 [10]; medialunae Montgomery, 1957 [15]; mycteropercae and trachinoti Siddiqi et Cable, 1960 [10]. Restudy of four paratypes of cablei (HWML 0814), a paratype of dollfusi (HWML 0608), the holotype and a paratype (?) of epinepheli (USNM Helm. Coll. No. 39381), a paratype of hypoplectri (HWML 21631), and the holotypes of israelense, macrum, mycteropercae and trachinoti (USNM Helm. Coll. Nos. 75002, 71305, 39383 and 39380, respectively) ascertained that the ceca ended blindly Therefore, they are retained in in these species. Neolepidapedon. The species macrum is very like *mycteropercae* and appears to be synonymous with it. The holotypes of belizense, equilatum, medialunae and pugetense (USNM Helm. Coll. Nos. 74164, 39382, 38194 and 59587, respectively) and three paratypes of retrusum (USNM Helm. Coll. No. 8274) were reexamined, but it was impossible to trace the full extent of the ceca in them because the posterior parts of the ceca were hidden by the vitellaria. The species, helicoleni, histiopteri, pugetense, retrusum and trematomi, may also be preserved in the genus. The position of belizense, equilatum and medialunae remains indeterminate.

The present study demonstrates that the cirrus pouch in *P. hoplognathi* changes in structure as worms develop. During stages of development earlier than the adult stage, it was elongated, consisted of the anterior thick-walled and the posterior thin-walled portion, and included the seminal vesicle, pars prostatica, prostatic cells and ejaculatory duct. In the adult stage, on the other hand, only its anterior portion was seen containing the distal parts of the seminal vesicle, pars prostatica, a number of the prostatic cells and the ejaculatory duct; the greater part of the seminal vesicle and most of the prostatic cells were situated free in the parenchyma. It is suggested that, about

the time when worms attain sexual maturity, the posterior portion atrophies and eventually disappears, or bursts under too great pressure of the developing and enlarging seminal vesicle from the inside and wrinkles to become practically invisible, and that, as a result, the greater part of the seminal vesicle and most of the prostatic cells come to lie free in the parenchyma. It is as if this part of the seminal vesicle were a true external seminal vesicle. The same may be said of the other members of Paralepidapedon and of those of Neolepidapedon because the present reexamination of many of them showed that the cirrus pouch was thick and lacked a well-defined posterior end in them. The undeveloped cirrus pouch in the earlier stages in Paralepidapedon recalls Manter's [3] statement, "It [a membraneous sac surrounding the gland cells and the posterior portion of the seminal vesicle in Lepidapedon] is separated by a deep constriction or by a narrow isthmus from a more anterior, typical cirrus sac containing a seminal vesicle, prostatic cells, and cirrus. Its membrane is apparently continuous with the wall of the cirrus sac, and it is probably more correct to describe the cirrus sac as divided into two distinct portions." This cirrus pouch is identical with the undeveloped one in Paralepidapedon. As to the cirrus pouch, this type is regarded as primitive, and that in the adult stage of Paralepidapedon and Neolepidapedon secondary. These three genera are very like in all features except in the structure of the cirrus pouch and in the presence or absence of the uroproct. It is possible that Neolepidapedon was first descended from Lepidapedon and then Paralepidapedon originated from the former with acquisition of the uroproct. The family Lepocreadiidae (Odhner, 1905) Nicoll, 1935, comprises a large number of described genera [4]. It has long been said that an external seminal vesicle with or without the prostatic cells around it is present in some of them but absent in others. Evidently, however, reexamination of the structure and formation of the so-called external seminal vesicle in them is needed.

This is the first paper to report the metacercaria of *P. hoplognathi* from the sea urchins. At Misaki, metacercariae of a trematode were recorded from the ovary of about 25% of *Mespilia*

globulus taken in 1930 to 1931 [16]. Since this record is without any morphological description or figure, it is quite impossible now to identify this trematode. No individuals of this sea urchin could be collected around the Station in the present study.

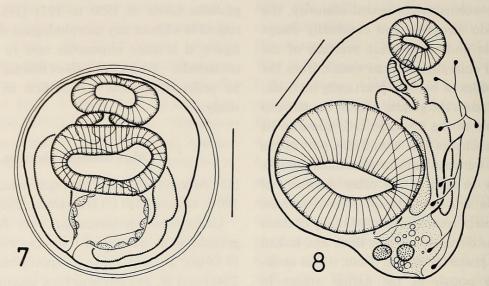
Other helminth parasites

(1) Metacercariae of Proctoeces maculatus (Looss, 1901) Odhner, 1911 (Trematoda: Fellodistomidae)

Unencysted metacercariae were found in the gonads of *A. crassispina* (two specimens, NSMT–Pl 2640), *D. setosum* (two specimens, NSMT–Pl 2641) and *H. pulcherrimus* (one specimen, NSMT–Pl 2642). The incidence of infection of each of these sea urchins was not recorded. The morphology and measurements of the five metacercariae were: body immature, 1.80–4.20 long by 0.44–1.00 wide; oral sucker 0.26–0.56 long by 0.26–0.54 wide; pharynx 0.16–0.32 long by 0.12–0.34 wide; ventral sucker 0.36–0.76 long by 0.40–0.86 wide; sucker width ratio 1: 1.40–1.81; testes 0.10–0.44 in diameter; cirrus pouch 0.28–1.00 long; ovary rounded or trilobed, 0.06–0.30 in diameter.

(2) Metacercariae of an unidentified trematode (Figs. 7 and 8)

Encysted metacercariae were found in the gonads of A. crassipsina (NSMT-Pl 2643-2645). The incidence and intensity of infection were not recorded. The morphology (Figs. 7 and 8) and measurements (based on five specimens) were: cyst globular, single-layered, 0.16-0.25 by 0.18-0.25 in size, 0.004–0.008 in thickness; body delicate, oval, smooth, 0.28-0.34 long by 0.12-0.16 wide; eye-spot pigment absent; oral sucker subterminal, 0.05–0.07 long by 0.06–0.08 wide; prepharynx 0.02 long; pharynx 0.03-0.05 long by 0.03-0.04 wide; esophagus 0.02-0.04 long; intestinal ceca bifurcated about halfway between two suckers, ending blindly at some distance from posterior end of body; ventral sucker large, a little postequatorial, 0.13-0.17 long by 0.13–0.20 wide; sucker width ratio 1: 1.97-2.45; testes symmetrical, small, located near posterior ends of ceca; other gential organs (ovarian complex, uterus and terminal genitalia) located median between excretory vesicle and



Figs. 7-8. Metacercariae of an unidentified trematode from *Anthocidaris crassispina*.

Fig. 7. Encysted metacercaria.

Fig. 8. Excysted metacercaria, flame cells and their canals shown only on left side of body, ventral view.

Scale bars: 7, 8=0.1 mm.

intestinal bifurcation; vitellaria not developed; excretory vesicle succular, filled with concretions of various sizes, in posterior two-thirds of hindbody; flame cell formula 2[(2+2)+(2+2)]=16; pore terminal. The identification of this trematode is impossible at present for lack of the adult stage.

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