[Jap. J. Parasit., Vol. 29, No. 2, 69-76, 1980]

Two New Cercariae, *Cercaria brachycaeca* n. sp. and *Cercaria misakiana* n. sp., from Top Shells, *Batillus cornutus* and *Marmarostoma stenogyrum*, with Notes of Their Effects on the Hosts

SHIGERU SHIMURA* AND JIRO ITO†

(Received for publication; July 13, 1979)

Introduction

It has been known that the so-called "orange sickness" of top shells are caused by the infection with some larval trematodes. We studied two different species of larval trematodes obtained from top shells with this disease, with the result that they are to be described as new species of cercariae. A histological examination was also made to observe the effects of the parasites on the hosts.

Materials and Methods

About 1,000 top shells consisting of *Batillus cornutus* and *Marmarostoma stenogyrum* (Turbinidae) were collected from the rocky shores at Chikura, Kominato and Misaki, the Pacific coast of the Kanto district, Japan, from February 1974 to August 1978. Their shells were crushed, and smears made from the digestive gland and gonad of each of them were examined for larval trematodes under the binocular microscope. During this investigation were found two different species of cercariae.

* Department of Fisheries, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

† Department of Hygiene, Faculty of Education, Shizuoka University, Oya, Shizuoka City, 422 Japan

The following description is based on naturally emerged cercariae, both living and preserved in formalin, and on ones within their parthenitae. Neutral red was used as an intravitam stain. Measurements were made on twenty specimens each fixed in hot formalin, without excessive coverslip pressure.

For histological examination, the digestive gland, gonad, kidney and ctenidium of infected and uninfected snails were fixed in Bouin's solution or formalin, embedded in paraffin, and sectioned at 4–6 μm. Sections were stained with Ehrlich's haematoxylin and eosin, or Mallory's triple connective-tissue stain.

Results

Cercaria brachycaeca n. sp.

Presumptive adult form: unknown.
Snail host: *Batillus cornutus* (Turbinidae).
Date, locality and infection rate:
As a whole, 21 out of 1069, or 1.96%;
Feb. 8, 1974–Aug. 6, 1974, Chikura, 4 out of 86, or 4.7%;
Aug. 2, 1974–Aug. 8, 1974, Kominato, 2 out of 104, or 1.9%;
Apr. 2, 1974–Aug. 20, 1978, Misaki, 15 out of 879, or 1.7%.
Measurements:
body.....198(191–203) μm long × 83 (79–89) μm wide;

oral sucker..... 46(44–48) μm long × 52 (49–54) μm wide ;
stylet..... about 6 μm long ;
prepharynx..... about 4 μm long ;
pharynx..... 20(19–21) μm long × 18 (17–20) μm wide ;
acetabulum..... 51(48–57) μm long × 59 (56–62) μm wide.

Specific description : (Figs. 1–2)

Cercariaeum. Emerged larvae have no tail, although on the posterior end immature ones have a small knob-like process, which presumably represents the vestige of a tail. The body is oval in shape and very contractile in living specimens. The body surface is covered with minute, backward directed spines being more dense posteriorly than anteriorly. No sensory hairs could be observed. The oral sucker is well developed, globular in shape, and located in the anterior part of the body. A small, single-pointed, non-shouldered stylet is embedded in the oral sucker. The mouth opening is followed by a short prepharynx. The pharynx is globular. The oesophagus is short, bifurcating in front of the acetabulum into two short caeca reaching to the lateral fields of the equatorial level of the body. The caeca are thick-walled and contain ingesta easily stainable with neutral red. Eyespots are lacking. Six pairs of penetration gland cells are located in the middle part of the body. Their ducts on each side of the body twist into one bundle and open near the apical end of the stylet. The acetabulum is also well developed, larger than the oral sucker, and situated in the anterior part of the posterior third of the body. The genital primordium is not recognizable. A large excretory vesicle is triangulate or cordate in the posterior part of the body with a terminal pore. From each of its anterolateral corners arises a main collecting tube, which receives the anterior and the posterior branch, each forming two groups of two flame cells each ; thus the flame cell formula is 2 [(2+2)+(2+2)]=16.

The cercariae after emergence survived up to 3 days in sea water. They were usually inactive and attached the posterior end of the body to the substratum, although at times they wriggled by extending and contracting movement at the rate of about 10–20 times per minute.

Sporocyst.

Large, mature sporocysts are elongated saccular, 1.3–2.4 mm long and 0.19–0.38 mm wide, containing 20 to 40 cercariae in various stages of development. Their walls contain large masses of bright orange pigments. This made it possible to distinguish uninfected snails from infected ones with the naked eye ; the normal gonad was whitish yellow in male snails and dark green in female snails, but the infected gonad definitely orange in colour regardless of sex of snails.

Histological observations.

In the gonad of infected snails, reproductive cells were almost completely lacking and replaced by the sporocysts (Fig. 6). Although the digestive gland was generally uninfected, one snail collected at Chikura harboured sporocysts both in the digestive gland and in the gonad. The sporocysts in the gonad contained some mature cercariae, whereas only germ balls were observed in the sporocysts in the digestive gland (Figs. 6–7). Sometimes, unripe sporocysts containing only germ balls were found also in the ctenidium and the substantial part of the kidney (Fig. 8).

Mature cercariae, after leaving the sporocysts in the gonad, seemed to emerge through the renal coelom as reproductive cells do. The aberrant cercariae trapped in the renal tissue were found to cause distinct pathological changes ; they were walled off by several layers of epithelioid cells, and these layers were further partially covered by fibroblasts to form granuloma (Fig. 9).

Cercaria misakiana n. sp.

Presumptive adult form : Opecoelidae ?
Snail hosts : *Batillus cornutus* and *Marmarostoma stenogyrum* (Turbinidae).
Date, locality and infection rate :
As a whole, 12 out of 895, or 1.34 % ;

Aug. 15, 1974–Aug. 20, 1978, Misaki, 10 out of 879, or 1.1% in *B. cornutus*;
Aug. 10, 1974, Misaki, 1 out of 6, or 17% and
July 7, 1975, Misaki, 1 out of 10, or 10% in *M. stenogyrum*.

Measurements:

body.....186(175–198) μm long × 59 (56–63) μm wide;
oral sucker..... 37(34–38) μm long × 33 (30–34) μm wide;
stylet..... 12(11–13) μm long × 9(8–10) μm wide;
prepharynx..... 49(46–52) μm long;
pharynx..... 13(12–14) μm long × 16 (15–17) μm wide;
acetabulum..... 32(30–34) μm long × 32 (30–34) μm wide;
tail..... 25(22–28) μm long × 32 (29–35) μm wide.

Specific description: (Figs. 3–4)

Cotylocercous cercaria. The body is very contractile when alive, with about 15 pairs of sensory hairs set on papillae becoming thinner posteriorly. The oral sucker is well developed, subglobular in shape, located at the anterior end, and armed with a stylet cleft anteriorly with two points directed anterolaterally. The mouth leads into a long prepharynx, which is followed by a muscular pharynx. Other parts of the digestive system could not be worked out. A transverse nervous commissure is observed across the prepharynx. Eyespots are lacking. At the middle part of the body are located four pairs of penetration gland cells, but poorly defined, nor stainable with neutral red. Their ducts pass forward in two bundles each, which open separately at the anterior tip of the body. The acetabulum is well developed, protruded, smaller than the oral sucker, situated at the anterior part of the posterior third of the body, and provided with eight sensory hairs on its ventral surface. The genital primordium is not recognizable. The excretory vesicle is large and sac-like, occupying a great part of the postacetabular region of the body. It is lined with very large, apparently secretory cells, which project into the lumen. The vesicle opens with a thin-walled canal at the posterior extremity of the body proper. Each collecting tube, lined with seven cilia, runs forward from the anterolateral side of the vesicle to the level of the posterior margin of the pharynx, and there divides into the anterior and the posterior branch. The anterior branch has two flame cells. The posterior one subdivides further to produce four tubules, each of which ends in flame cell; thus the flame cell formula is $2[2+(2+2)]=12$. The tail is filled with eosinophilic granules and ten to twelve ducts open posteriorly.

Living cercariae were capable of markedly extending and contracting, particularly in the preacetabular region, and crept by alternate attachments of the oral sucker and acetabulum, and moved on about 15 mm per minute by about 70 movements. At times the larvae attached themselves to the substratum by the tail to wave or rotate their bodies.

Redia.

The redia is fusiform, 0.50–0.67 mm long, and more blunt at the anterior end, bearing setae and a birth pore at the head region. It contains several cercariae and some germ balls. The pharynx is very small and poorly defined.

Histological observations.

The digestive gland of *B. cornutus* consisted of tubular branches which opened into the stomach. These branches ramified and divided into a large number of tubules, each of which ended blindly. The rediae occurred in the intertubular spaces of the digestive gland, which led to the compression and displacement of the tubules (Fig. 10). The gonads of the infected snails were atrophied, however, reproductive cells were usually observed in them. In some cases, "overspilled" rediae were found to invade into the gonads (Fig. 11). When rediae grew fully ripe, the atrophied gonad was filled with the parasites and the germ cells of the infected snails disappeared from it. The kidney and ctenidium of the hosts were also affected by the mature rediae

(Fig. 12). In infected top shells, the whole surface of the visceral mass, mantle and ctenidium are slightly orange-coloured.

Discussion

According to Yamaguti (1975), tailless cercariae have been found sporadically in several families of trematodes : *Brachylaima virginianum*, *B. oesophagei*, *Postharmostomum gallinum*, *P. helicis* and *Panopistus pricei* in Brachylaimidae ; *Leucochloridium australiense*, *L. problematicum* and *Urotocus tholonetensis* in Leucochloridiidae ; *Asymphylodora tincae* and *Triganodistomum mutabile* in Monorchiidae ; and *Zoogonus rubellus*, *Z. laevis* and *Diphtherostomum brusinae* in Zoogonidae. Some other tailless cercariae such as *Cercariaeum reticulatum* (Stunkard, 1932), *Cercaria tenuans* (Cole, 1935) and *C. brachidontis* (Hopkins, 1954) were also reported. *Cercaria brachycaeca* is quite different from them in the shape of intestinal caeca, stylet, excretory vesicle, flame cell formula and several other morphological features.

During the course of this study, an adult trematode was obtained in the renal coelom of the top shell, *B. coruntus*. It was identical with *Proctoeces* sp. by Ichihara (1965) which was later described as a new species, *P. ichiharai*, by Shimura and Egusa (1979). Stunkard and Uzmann (1959) reported that an abbreviated life cycle of *P. maculatus* in *Mytilus edulis*. Consequently, a hypothesis that *C. brachycaeca* was the larval stage of *P. ichiharai* was suggested. However, any attempts to verify the hypothesis through infection experiment were unsuccessful (unpublished data). Moreover, a wide morphological difference between *C. brachycaeca* and the cercaria of *P. maculatus* was also recognized. Therefore, we conclude that *C. brachycaeca* is not the cercarial stage of *P. ichiharai*.

Concerning with *C. misakiana* and other cotylocercous cercariae, Stunkard (1932) described two cotylocercous cercariae, *Cercaria linearis* and *C. brachyura* from *Littorina littorea* and *Gibbula umbilicalis* respectively. *C. buccini* was reported from *Buccinum undatum* by Lebour (1911) and from *L. littorea* by Rees (1935). McCoy (1929) showed a cotylocercous cercaria, *Cercaria A* of Miller, to be the larval stage of *Hamacreadium mutabile*. McCoy (1930) further showed that *Cercaria B* of Miller was the larva of *H. gulella*. Cable (1963) described an opecoelid cercaria, *Cercaria caribbea LX*. Hunninen and Cable (1943) and Werding (1966) showed that *Podocotyle atomon* had a cotylocercous cercarial stage. Several other cotylocercous cercariae were reviewed by Yamaguti (1975).

C. misakiana is very similar to other cotylocercous cercariae, especially to *C. caribbea LX* and the cercaria of *P. atomon*, but differs from them in the shape of stylet, flame cell formula, development in rediae, and several other morphological features. The present species is referred tentatively to the family Opecoelidae on account of the morphological similarity, although cotylocercous cercariae in Opecoelidae generally develop in sporocysts and have a flame cell formula of $2[(2+2)+(2+2)]=16$.

The present observations showed some pathological changes in the gonad and digestive gland of the top shells infected with *C. brachycaeca* and *C. misakiana*. According to Wright (1966), the most common site of infection with larval trematodes is, in general, the interlobular haemocoel of the digestive gland, and in heavy infections an "overspill" of sporocysts or rediae may occur in almost any part of the body, particularly in the gonad. However, rarely is the gonad itself the primary site of infection as it is in the cystophorous cercariae. The present work indicates that the rediae of *C. misakiana* parasitize mainly the digestive gland, and that, on the other hand, the sporocysts of *C. brachycaeca* develop mainly in the gonad.

Several cases of encapsulation around parasites in molluscan hosts have been reported. Probert and Erasmus (1965) showed *Cercaria X* to be surrounded by host cells in *Lymnaea stagnalis*. Pauley and Becker (1968) reported that *Aspidogaster conchicola* parasitic in

Gonidea angulata became encapsulated in various tissues of the body, and that a marked haemocytic infiltration was induced into the surrounding tissues. Sparks (1962) observed metaplastic changes in the gut of the oyster, *Crassostrea gigas*, infected with the parasitic copepod, *Mytilicola orientalis*. He noted an apparent tendency of the underlying connective tissue towards fibrosis, suggesting a response of the host to protect the underlying tissue by encapsulation around the parasites. Cheng (1967) said that encapsulation appeared rather common as a defense mechanism in molluscs. The present investigation also indicated the distinct encapsulation around *C. brachycaeca* in the substantial part of the kidney.

Up to now the levels of infection of the top shells with the larval trematodes have been low and showed no sign to increase. However, considering that the larval trematodes do much damage to the gonad of the infected top shells, their potential enzootic and influence on seedling production of the top shells should be considered. Further studies may be required of their life cycles and ecological features.

Summary

Two new cercariae, *Cercaria brachycaeca* from *Batillus cornutus* and *Cercaria misakiana* from *B. cornutus* and *Marmarostoma stenogyrum* collected at the Pacific coast in the Kanto region of Japan are described.

C. brachycaeca is a tailless cercaria with a simple non-shouldered stylet, short intestinal caeca, a cordate excretory vesicle and a flame cell formula of $2[(2+2)+(2+2)]=16$. Sporocysts containing 20 to 40 mature cercariae were found in the gonad of the host snail, and the infected gonad was orange in colour. Some mature cercariae after leaving the sporocysts were found to be encapsulated in the host kidney.

C. misakiana is a cotylocercous cercaria with a double-pointed stylet and a flame cell formula of $2[2+(2+2)]=12$. Rediae containing several mature cercariae parasitize mainly in the digestive gland, and occasionally in the gonad, kidney and ctenidium of the infected top shell. The infected gonad was atrophied. The whole surface of the visceral mass, mantle and ctenidium are slightly orange-coloured.

Acknowledgements

We wish to thank Professor S. Egusa, Department of Fisheries, Faculty of Agriculture, University of Tokyo, for his suggestion of this original topic of research and for his kind advice throughout the work. Thanks are also due to Messrs. T. Sekimoto and M. Sekimoto, Misaki Marine Biological Station, and the staff of the Chiba Prefectural Fisheries Experimental Station for their aid in collecting the top shells, and Mr. N. Sueta for assistance in histological procedures.

References

1) Cable, R. M. (1963): Marine cercariae from Curaçao and Jamaica. Z. Parasitenk., 23, 429–469.
2) Cheng, T. C. (1967): Marine molluscs as hosts for symbiosis with a review of known parasites of commercially important species. Advan. Mar. Biol., 5, 1–424.
3) Cole, H. A. (1935): On some larval trematode parasites of the mussel (*Mytilus edulis*) and the cockle (*Cardium edule*). Parasitol., 27, 276–280.
4) Hopkins, S. H. (1954): *Cercaria brachidontis* n. sp. from the hooked mussel in Louisiana. J. Parasit., 40, 29–31.
5) Hunninen, A. V. and Cable, R. M. (1943): The life history of *Podocotyle atomon* (Rudolphi) (Trematoda, Opecoelidae). Trans. Am. Microsc. Soc., 62, 57–68.
6) Ichihara, A. (1965): On a Trematoda (*Proctoeces* sp.) of the topshell, *Turbo* (*Batillus*) *cornutus* Solander, I. Geographical distribution in Japan and observations on the adult fluke. Jap. J. Parasit., 14, 426–436.
7) Lebour, M. V. (1911): A review of the British marine cercariae. Parasitol., 4, 416–456.
8) McCoy, O. R. (1929): The life-history of a marine trematode, *Hamacreadium mutabile* Linton, 1910. Parasitol., 21, 220–225.
9) McCoy, O. R. (1930): Experimental studies on two fish trematodes of the genus *Hamacreadium* (Family Allocreadiidae). J. Parasit.,

17, 1–13.
10) Pauley, G. B. and Becker, C. D. (1968): *Aspidogaster conchicola* in mollusks of the Columbia river system with comments on the host's pathological response. J. Parasit., 54, 917–920.
11) Probert, A. J. and Erasmus, D. A. (1965): The migration of *Cercaria X* Baylis (Strigeida) within the molluscan intermediate host *Lymnaea stagnalis*. Parasitol., 55, 77–91.
12) Rees, W. J. (1935): The anatomy of *Cercaria buccini* Lebour, 1911. Proc. Zool. Soc., Lond., 2, 309–312.
13) Shimura, S. and Egusa. S. (1979): A new digenetic trematode, *Proctoeces ichiharai* n. sp. (Fellodistomidae) from topshell *Batillus cornutus* (Gastropoda). Bull. Jap. Soc. Sci. Fish., 45, 1249–1253.
14) Sparks, A. K. (1962): Metaplasia of the gut of the oyster *Crassostrea gigas* (Thunberg) caused by infection with the copepod *Mytilicola orientalis* Mori. J. Insect Pathol., 4, 57–62.
15) Stunkared, H. W. (1932): Some larval trematodes from the coast in the region of Roscoff, Finistère. Parasitol., 24, 321–343.
16) Stunkard, H. W. and Uzmann, J. R. (1959): The life cycle of the digenetic trematode, *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 (syn. *P. subtenuis* (Linton, 1907) Hanson, 1950), and description of *Cercaria adranocerca* n. sp. Biol. Bull. Mar. Biol. Lab., Woods Hole, 116, 184–193.
17) Werding, B. (1969): Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der Strandschnecke *Littorina littorea*. Mar. Biol., 3, 306–333.
18) Wright, C. A. (1966): The pathogenesis of helminths in the Mollusca. Helminth. Abst., 35, 207–224.
19) Yamaguti, S. (1975): A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates. Keigaku Publishing Co. Tokyo, Japan. 590 pp with 219 plates.

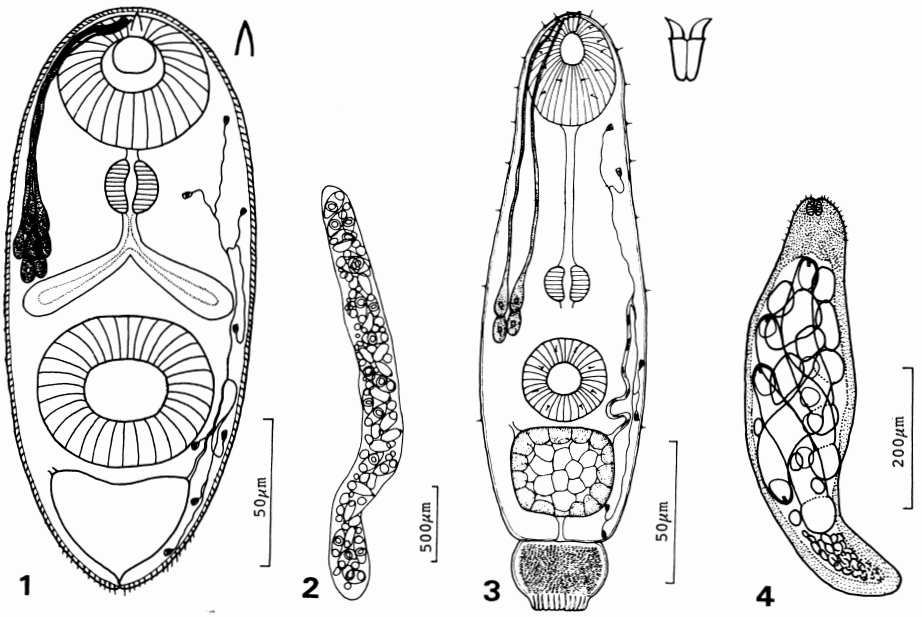
サザエ (*Batillus cornutus*) とコシダカサザエ (*Marmarostoma stenogyrum*) に寄生するセルカリアの2新種, *Cercaria brachycaeca* n. sp. と *Cercaria misakiana* n. sp. の形態と宿主への影響

志村　茂
(東京大学農学部水産学科)
伊藤　二郎
(静岡大学教育学部保健学教室)

神奈川県三崎産と千葉県千倉と小湊産のサザエ (*Batillus cornutus*) から無尾セルカリアを検出して新種 *Cercaria brachycaeca* n. sp. として，さらに，三崎産のサザエとコシダカサザエ (*Marmarostoma stenogyrum*) から短尾セルカリアを検出して新種 *Cercaria misakiana* n. sp. として命名記載した．

C. brachycaeca は口吸盤に単純な穿刺棘を有し，短い腸をもつ．侵入腺細胞は7対，排泄嚢は心臓形で，焔細胞式は 2[(2+2)+(2+2)]=16 である．単性虫はスポロシストで，20～40個体のセルカリアを含み，サザエの生殖腺に寄生する．本種の寄生を受けた生殖腺は，スポロシストの体壁中の色素のために，橙色を呈する．また，宿主の腎臓組織内にセルカリアが迷入して肉芽腫を形成する例が観察された．

C. misakiana は口吸盤に2尖頭の穿刺棘をもつ．侵入腺細胞は4対で，焔細胞式は 2[2+(2+2)]=12 である．単性虫はレディアで，セルカリアを数個体含み，中腸腺に寄生する．本種の寄生を受けたサザエの軟体部は全体に橙色を帯び，生殖腺の萎縮が認められた．

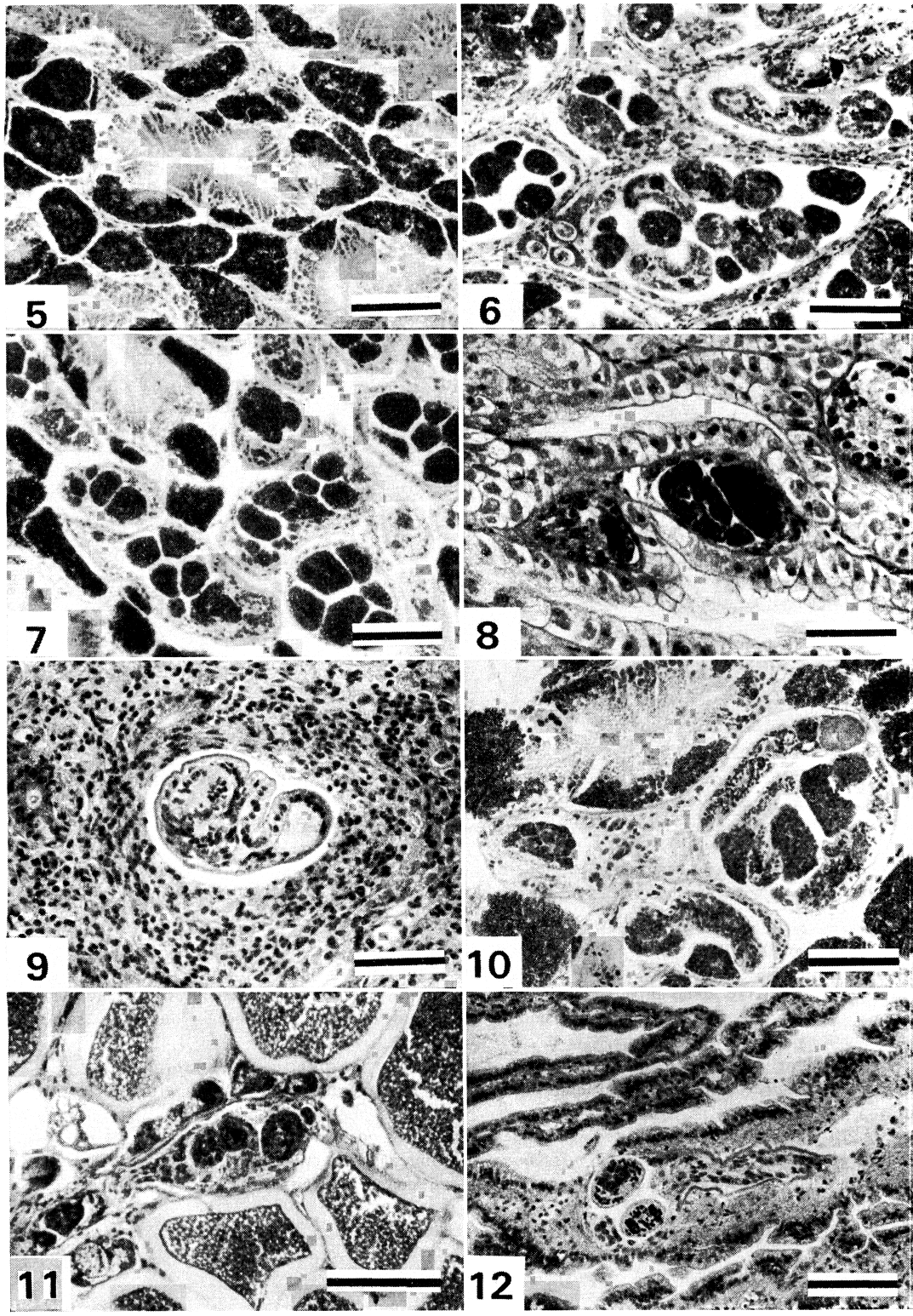


Explanation of Figures

Fig. 1 *Cercaria brachycaeca* n. sp., cercaria, ventral view.
Fig. 2 *C. brachycaeca*, ripe sporocyst with mature and developing cercariae.
Fig. 3 *Cercaria misakiana* n. sp., cercaria, ventral view.
Fig. 4 *C. misakiana*, ripe redia with mature cercariae and germ balls.

Photomicrographs of transverse sections of uninfected (Fig. 5) and infected (Figs. 6–12) top shells stained with haematoxylin and eosin. Line bars: 5–7, 10–12, 100 μm; 8 and 9, 50 μm.

Fig. 5 Uninfected healthy digestive gland.
Fig. 6 Ovary infected with *C. brachycaeca*, showing sporocysts containing mature and developing cercariae and germ balls. Ova of the host are observed.
Fig. 7 Digestive gland infected with *C. brachycaeca*. Sporocysts in the digestive gland contain only germ balls and are smaller and less developed than those in the ovary (same specimen in Fig. 6).
Fig. 8 Unripe sporocysts of *C. brachycaeca* invading in the intertubular space of kidney.
Fig. 9 *C. brachycaeca* trapped and encapsulated by several layers of epithelioid cells of kidney.
Fig. 10 Digestive gland infected with ripe rediae of *C. misakiana*.
Fig. 11 Ovary infected with *C. misakiana*, showing a early stage of infection with small active rediae. The rediae invaded along connective tissue. Eggs of the host are present.
Fig. 12 Ctenidium infected with "overspilled" ripe rediae of *C. misakiana*.


5
6
7
8
9
10
11
12