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On the Parasitic Organisms in a Krill, *Euphausia similis*, from Suruga Bay
IV. Metacercariae of the Digenetic Trematodes

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Introduction

Komaki (1970) and Shimazu (1971) have previously reported the progenetic metacerca of a digenetic trematode, *Pseudopecoelus japonicus* (Allocreadiidae), from a krill, *Euphausia similis*, from Suruga Bay. A more detailed survey was carried out on the infection of the *P. japonicus* metacercaria to *E. similis* collected in Sagami and Suruga bays during May to June, 1971. In the present survey, besides 136 metacercariae of *P. japonicus*, four other species of the trematode metacercariae together with the nematode, cestode, protozoan, and ellobiopsid parasites were also obtained from *E. similis*.

The present paper deals with the morphological descriptions and taxonomic problems of one metacercaria of *Syncoelium* sp., three of *Paronatrema* sp., and two of two different, unclassified species. The results concerning the remaining parasites obtained will appear elsewhere.

Materials and Methods

Euphausia similis Sars (Crustacea: Euphausiacea) examined was collected in Sagami and Suruga bays by the Research Vessel *Tansei Maru* of the Ocean Research Institute, Tokyo University, on her cruise KT-71-06 during May 25 to June 3, 1971. A total of 35,253 specimens of *E. similis* was examined for the parasites. In the laboratory, the formalin-preserved krills were individually dissected carefully with fine needles under a binocular dissecting microscope. At several stations, some fresh or frozen krills were examined in the vessel. Details of the sampling stations, methods of sampling, fixation, and examination will be given elsewhere.

The metacercariae were taken from the krills, and they were liberated from the cysts, if encysted. After being washed and macerated in the tap water for several days, they were carefully flattened under the pressure of a cover slip, stained with Delafield's hematoxylin, and then mounted in Canada balsam. After each of the whole mounts was studied, one of the *Paronatrema* metacercariae was dissolved off the slide glass and serially sectioned; the sections were restained with hematoxylin and eosin. Drawings were made with the aid of a microscope projector or a camera lucida. All measurements are in millimeter.

Description and Discussion

1. *Syncoelium* sp. metacercaria (Syncoeliidae)

Only one specimen was found free in the hemocoel of the gastric region of one of 255 krills from Station 22 (off Yaizu) in Suruga Bay.

Description: Metacercaria not encysted.

Body 3.23 long, cylindrical; forebody 2.10 long by 0.32 in maximum width, strongly arched ventrally; hindbody 1.13 long by 0.40 in maximum width, straight. Cuticle thick, smooth, with minute cuticular spines arranged in a ring on peduncle along near periphery of ventral sucker. Gland cells 0.017 to 0.032 in diameter, evenly dispersed in parenchyma under cuticle except in anterior- and posterior-most regions of body and in peduncle of ventral sucker. Stump of some organ very short, connected with posterior end of body proper. Oral sucker 0.26 in longitudinal diameter by 0.18 in depth, opening ventrally, surmounted with well-developed perioral lip. Prepharynx absent. Pharynx 0.14 long by 0.15 wide, globular. Esophagus very short. Intestines united posteriorly. Ventral sucker 0.35 in longitudinal diameter by 0.10 in depth, shallow, embedded slightly in peduncle; lateral rims thick-walled, notched irregularly; peduncle 0.41 long by 0.38 wide, cylindrical. Testes 13 in number, 0.038 to 0.094 by 0.133 to 0.168, ellipsoidal, situated in anterior half of hindbody. Seminal vesicle dorsal to metraterm. Prostatic cells not observed. Neither cirrus nor cirrus pouch present. Ovary five in number, 0.063 to 0.076 by 0.091 to 0.105, just posttesticular. Mehlis' gland 0.142 by 0.138, globular, ventral to posteriormost ovary, with thick-walled ootype measuring 0.040 in diameter in dorsal side. Uterus in hindbody not observed, but distally sinuous widely, muscular, lying forward from midlevel of forebody. Metraterm expanded to form a barrel measuring 0.11 long by 0.06 wide, with strong sphincters in both ends. Vitellaria about six follicles in number, postovarian. Hermaphroditic duct 0.32 long, muscular, expanded proximally to form a hermaphroditic pouch, passing medially through muscular structure, and then liberated and tapering distally in short genital atrium to form a genital papilla; muscular structure well-developed, composed chiefly of circular muscle fibers, swelling out like a cone measuring 0.07 high by 0.11 wide at base in a deep hollow located immediately behind ventral perioral lip. Excretory system not observed.

Discussion: In the genus *Syncoelium* Looss, 1899, seven known species from the marine fishes and one unidentified metacercaria from the copepod have been included. The present *Syncoelium* metacercaria from *E. similis* differs from *S. ragazzii* (Setti, 1897) Looss, 1899 in having a pedunculate ventral sucker and 13 testes. The metacercaria is similar to *S. filiferum* (Leuckart in Sars, 1885) Odhner, 1911 and *S. katuwo* Yamaguti, 1938 in general anatomy but different from them in having 13 testes. Manter (1954) stated that the small but distinct difference in the egg size seems to separate *S. filiferum* [= *S. thyrsitae* (Crowcroft, 1948) Manter, 1954] from *S. katuwo*. Lebedev (1968) concluded that either number of testes or egg size alone cannot be the decisive characteristic to separate species of the genus *Syncoelium*. As to the number of testes, this character-

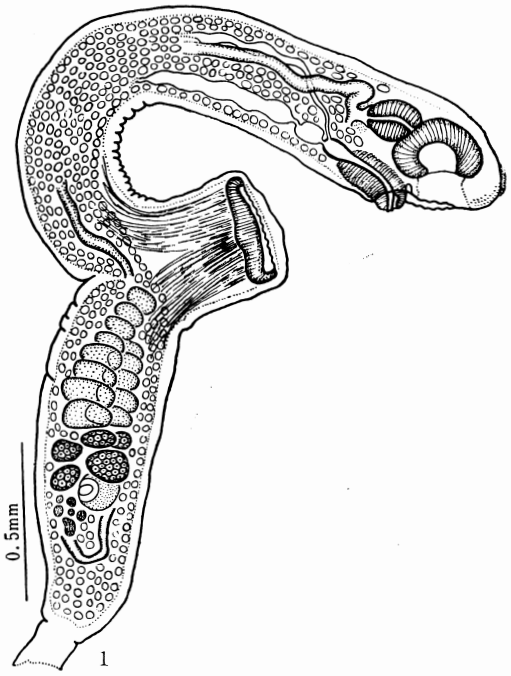

Fig. 1 *Syncoelium* sp. metacercaria; ventrolateral view.

istic is considerably variable according to the developmental stages of materials to be studied. This makes the identification of immature specimens quite difficult. Consequently, it is impossible to discuss further the relationships between the present metacercaria and those of known species, basing on a single immature specimen.

Sars (1885) and Leuckart (1889) briefly described and illustrated several immature specimens of *Distomum filiferum* Leuckart (in Sars, 1885) from the perivisceral cavity of krills, *Nematoscelis megalops* and *Thysanoessa gregaria*, collected during the *Challenger* Expedition in the South Atlantic Ocean. Later, Odhner (1911) transfered *D. filiferum* to the genus *Syncoelium* as *S. filiferum*, and he said of this species in a footnote that he had obtained an immature specimen free in the vertical net during the *Valdivia* Expedition in the South of the Cape. Oversteet (1970) lately reported an unidentified metacercaria of *Syncoelium* sp., which had attached externally to the thoracic spine of a copepod, *Candacia pachydactyla*, from the Atlantic Equatorial Current near the mouth of the Amazon River. Accordingly, the present paper is the fourth report for the larval *Syncoelium* found in the crustacean intermediate hosts.

The larvae from the krills from the South Atlantic had the peculiar byssus consisting of two strong filaments and a number of very adhesive fibers (Sars, 1885; Leuckart, 1889), but the metacercaria from the copepod did not possess such the byssus (Overstreet, 1970). In the present metacercaria, the short stump was connected with the posterior end of the body proper. This shows that the present *Syncoelium* species has such a certain organ as a tail at the metacercarial stage in the crustacean intermediate host. But the morphology of that organ remains unknown.

Overstreet (1970) briefly noted the presence of the minute spines in both suckers in the metacercaria from the copepod. In the present metacercaria, the minute cuticular spines were arranged in a ring on the peduncle along near the periphery of the ventral sucker. In the adult stage of the present metacercaria, both spines and thick-walled and notched lateral rims of the ventral sucker may be more suitable for the tight attachment to the certain organ such as the gill raker of the final host. In the branchial cavity of the host fish, there is the relatively strong flow of the respiratory water, particularly in the fast swimmers, for example, the bonito, flying fish, and salmon.

2. *Paronatrema* sp. metacercariae (Syncoeliidae)

Three specimens were found free in the hemocoel of the gastric region of *E. similis* collected along the innermost coastline of Suruga Bay, one from one of 1,118 female krills from Station 30–5 (off Kanbara), another from one of 2,937 females from Station 30–7 (off Kanbara), and a third from one of 1,857 females from Station 30–11 (off Yoshiwara). Because one of them was much damaged during the preparation, the following morphological description is based chiefly on the remaining two specimens. No satisfactory serial sections of the metacercaria were available for the examination.

Description: Metacercaria not encysted. Body 3.39 to 3.75 long, elongated, flattened dorso-ventrally; forebody 2.57 to 2.60 long by 0.60 to 0.75 wide; hindbody 0.82 to 1.15 long by 0.69 to 0.78 wide. Cuticle thick, smooth. Gland cells about 0.022 by 0.035, distributed evenly in parenchyma under cuticle except in lamellated perioral lip and in body wall lining ventral sucker. Parenchymatous cells minute. Eye-spots present or not, three in number, if present, represented by dark pigment masses; anterior two symmetrical, just lateral to intestines at level of anterior edge of ventral sucker, and the other one more posterior to anterior two in median field. Tail very large, forked distally; stem 0.35 to 0.55 long by 0.22 to 0.62 wide, small, narrow or globular; forks two, 0.84 to 1.80 long by 0.22 to 0.46 wide, symmetrical, club-shaped; cuticle smooth or slightly striated; muscle layers thin, consisting of outer layer of circular fibers and

inner one of longitudinal fibers, with no other specially developed muscles; cellular layer with minute nuclei present under muscle layers; parenchyma surrounded with cellular layer much looser in texture, containing numbers of ovoid cells; cells 0.021 to 0.027 by 0.029 to 0.042, fibrillar, stained with eosin, having a long winding stalk and two small cytoplasmic swellings having a large nucleus each. Oral sucker terminal, being of a funnel form, 0.54 in diameter by 0.18 in depth, lined with lamellated perioral lip like a scolloped collar measuring 0.67 in diameter, or being of a U-shaped form, 0.35 long by 0.36 wide, surmounted with perioral lip like a scolloped cuff; internal edge muscular, thick, scolloped at 0.033 to 0.035 intervals; suckerlike structures not observed. Ventral sucker 0.93 to 1.00 in diameter, large, lined dorsally with lamellated body wall; accessory suckers 40 or 42 in number, small, crescent-shaped, muscular, arranged at about 0.069 intervals in a ring around periphery of ventral sucker. Prepharynx absent. Pharynx 0.29 to 0.37 long by 0.22 to 0.27 wide, thick-walled, pyriform with narrowest diameter at junction with oral sucker. Esophagus very short, muscular, bifurcating into two muscular chambers extending directly to left and right for a very short distance and then joining intestines proper. Intestines sinuous in each lateral field, probably opening into excretory vesicle at same level near posterior end of body, not entering tail; no cecal pouches present. Testes tubular, 0.021 in diameter, irregularly segmented, arranged in many irregular rows spreading obliquely and overreaching intestines, within anterior two-thirds of hindbody. Seminal vesicle sinuous in median field of forebody. Pars prostatica 0.34 to 0.48 long by 0.08 wide, club-shaped, bending distally backward or not, lying just behind esophageal bifurcation. Neither cirrus nor cirrus pouch present. Prostatic cells free in parenchyma, lining externally anterior half or two-thirds of pars prostatica in dense layer. Ovary 0.099 by 0.073, globular or slightly indented, just post-testicular on median line. Ootype antero- or posterolateral to ovary, with Mehlis' gland closely around it. Laurer's canal opening dorsally. Uterus in distal part sinuous widely, winding dorsally to seminal vesicle. Metraterm muscular, largely winding dorsally to pars prostatica. Vitellaria tubular and irregularly flagmented, observed barely in lateral fields in posterior third of hindbody. Hermaphroditic duct simple, muscular, opening into succular genital atrium surrounded with a genital sucker measuring 0.039 by 0.063 to 0.068 which is located slightly to the right of the median line at the level of the midpharynx or base of the oral sucker. Excretory vesicle 0.127 long, short, T-shaped, lined internally with tall cylindrical cells, with short lateral branches extending dorsally to intestines; main excretory tubes running backward for a short distance and then forward in lateral fields to intestines; caudal excretory tube present; primary excretory pore(s) not observed.

Discussion: In the little-known genus *Paronatrema* Dollfus, 1937, only two species have been described from the marine elasmobranchs. Dollfus (1937) described *P. vaginicola* from the oviduct of a shark, *Squalus* sp. (?), from New Guinea. Manter (1940) reported *P. mantae* from the surface of the skin of a ray, *Manta birostris*, from Bahia Honda, Panama.

Dollfus (1966) recorded several metacercariae of *Metacercaria theomonodi* from the poorly preserved plankton samples collected from off Boavista in the Cape Verde Islands, presenting the photograph showing that the metacercaria was attached (externally?) to a krill, *Nyctiphanes couchii*. He supposed that *M. theomonodi* shows an affinity with the Accacoelioidea, particularly with *Accacladium serpentulus*, in having a well-developed copulatory apparatus (genital sucker). But he could not conclusively demonstrate the relationship between *M. theomonodi* and *A. serpentulus* because of the insufficient informations on the internal anatomy of the metacercaria. Manter and Pritchard, in a footnote in Dollfus' paper, said that *M. theomonodi*

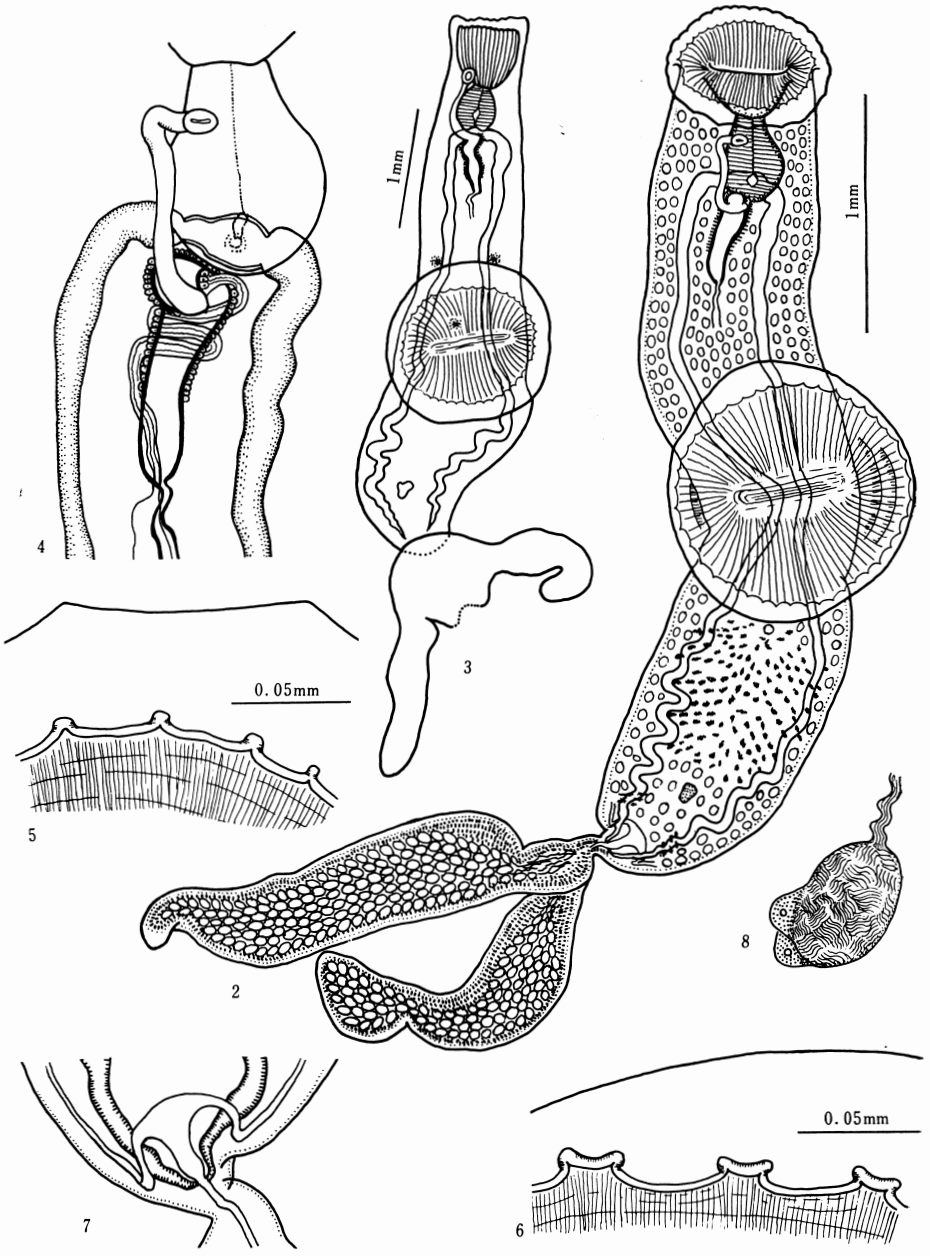

Fig. 2 *Paronatrema* sp. metacercaria (1) ; ventral view.
Fig. 3 *Paronatrema* sp. metacercaria (2) ; ventral view.
Fig. 4 *Paronatrema* sp. metacercaria (1) ; ventral view of terminal genitalia.
Fig. 5 *Paronatrema* sp. metacercaria (1) ; enlarged view of periphery of oral sucker.
Fig. 6 *Paronatrema* sp. metacercaria (1) ; enlarged view of accessory suckers of ventral suckers.
Fig. 7 *Paronatrema* sp. metacercaria (1) ; dorsal view of posterior end of body.
Fig. 8 *Paronatrema* sp. metacercaria (1) ; glandlike cell of tail.

relates to the genus *Paronatrema*. Overstreet (1970) lately concurred with this opinion.

In lacking the anterior ceca of intestines, *M. theomonodi* seems to belong to the genus *Paronatrema* rather than to the genus *Accacladium*. In *M. theomonodi*, an organ like "de poche conique" directed backward exists in the part of the esophagus, and the intestines do not penetrate into the posterior third of the hindbody. Such the organ has not been observed in *P. vaginicola*, in *P. mantae*, and in the present metacercariae from *E. similis*. And, in the latter three, the intestines extend into near the posterior end of the body. Accordingly, *M. theomonodi* differs from all of the known members of the genus *Paronatrema*, if the identification is correct.

The present *Paronatrema* metacercariae can be distinguished from *P. vaginicola* by the seminal vesicle, genital sucker, and more numerous accessory suckers in the ventral sucker, and also from *P. mantae* in having the long tubular hermaphroditic duct, genital sucker, and more numerous accessory suckers in the ventral sucker, and in lacking the cecal pouches. The metacercariae resemble *M. theomonodi* in possessing a well-developed genital sucker but differ from the latter in having the tail and intestines which penetrate into near the posterior end of the body, and in lacking an uncertain organ as above mentioned. Consequently, the present metacercariae seems to be those of an undescribed species of the genus *Paronatrema*. It is not an appropriate manner to propose a new specific name for the present metacercariae basing on their cercarial form lacking eggs at present.

Both in *P. vaginicola* and in *P. mantae*, it has not been distinctly shown whether or not the intestines open into the excretory vesicle. In the present metacercariae, the intestines appeared to join to the excretory vesicle.

The metacercariae were found free in the krills. They remained a cercarial form, and one of them had three eye-spots. Their tails were very large, distally forked, and contained numbers of the conspicuous gland-like cells in the innermost parenchyma. In the morphology of the tail, the type of the present *Paronatrema* cercaria differs entirely from that of the so-called Furcocercous cercaria and also from that of the Diplocercous cercaria that was proposed by Dawes (1959) for *Cercaria owreae* (Hutton, 1954) obtained from the chaetognaths from the Florida Current. *C. owreae* possesses a pair of the peculiar appendages into which the intestines extend, one into each. Accordingly, the type of the *Paronatrema* cercaria may be new to science. The large tail probably assists floatation rather than swimming and serves to bring the cercaria from the vicinity of some botton-dwelling mollusk, in which the earlier stages of development occur, into the plankton zone.

The present paper is the first record for the occurrence of the member of the genus *Paronatrema* in Japanese waters, and the second one for the parasitism of the larval form in the crustacean intermediate hosts.

3. Metacercaria A, unclassified

Only one encysted specimen was obtained from the gastric region of one of 1,857 krills from Station 30–11 (off Yoshiwara). The habitat in the host is unknown but at least not in the musculature.

Description : Cyst 0.49 by 0.42, ovoid ; cyst wall thick, elastic, transparent. Metacercaria lying double in cyst. Body 0.949 long by 0.416 wide, oval ; forebody 0.499 long. Cuticle smooth. Oral sucker 0.111 long by 0.119 wide, subterminal. Ventral sucker 0.147 in diameter, globular, located slightly anteriorly to midlevel of body, with small and round aperture. Prepharynx almost absent. Pharynx 0.058 long by 0.033 wide, rectangular. Esophagus narrow, short. Intestinal ceca reaching near posterior end of body. Testes two, 0.102 to 0.113 by 0.132 to 0.170, lying obliquely in middle of hindbody. External seminal vesicle probably present. Cirrus pouch curved, retort-shaped, lying on left of ventral sucker. Genital pore situated on left margin of body at level between

intestinal bifurcation and anterior edge of ventral sucker; cirrus and internal anatomy of cirrus pouch not observed in detail. Ovary 0.062 by 0.153, transversely elongated, anterior to testes. Ootype and Laurer's canal not observed. Metraterm running dorsally to cirrus pouch. Vitellaria not distinctly developed. Excretory vesicle 0.200 long by 0.107 wide, tubular, lined internally with large epithelial cells, reaching posterior edge of anterior testis; pore terminal.

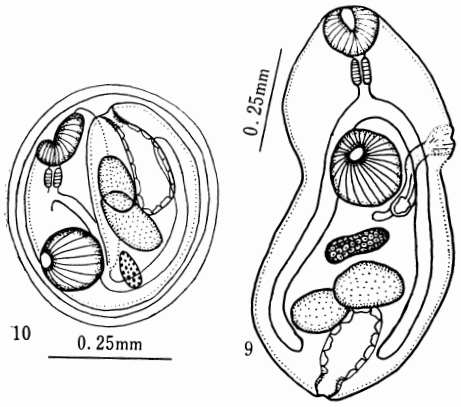

Fig. 9 Metacercaria A liberated from cyst; ventral view.
Fig. 10 Metacercaria A; cyst.

Discussion: This metacercaria appears to belong to the genus *Opisthogonoporus* (Lepocreadiidae) in having the marginal genital pore, external seminal vesicle, very short prepharynx, and relatively short tubular excretory vesicle, although the extents of its vitellaria and uterus were not observed. In the genus *Opisthogonoporus*, only one species *P. amadai* has been described by Yamaguti (1937) from the intestine of a marine fish, *Branchiostegus japonicus*, from the Sea of Japan and the Pacific. The present metacercaria is slightly different from *P. amadai* in possessing a smoth cuticle, a more anterior genital pore, a more transversely elongated ovary and testes, and a longer excretory vesicle.

4. Metacercaria B, unclassified

Only one encysted specimen was obtained from one of 645 female krills from Station 27-5 (in the center of Suruga Bay). The habitat in the host is unknown. Because the krills collected from this station had been frozen before fixation in formalin, they were considerably damaged, and, even worse, the metacercaria was completely destroyed during the preparation. Accordingly, the following morphological features can be merely noted here basing on the several freehand sketches and measurements made during the preparation.

The cyst was 0.45 by 0.42 and ellipsoidal. The cyst wall consisted of an inner hyaline layer and an outer brown one. The metacercaria was elongated. The oral sucker was 0.112 long by 0.105 wide and situated ventroterminally. The prepharynx was long. The muscular pharynx was globular. The narrow esophagus was short and bifurcated into two simple, relatively wide intestinal ceca which reached the posterior end of the body. The ventral sucker was located just behind the esophageal bifurcation at the anterior third of the body, and slightly larger than the oral sucker. Along the median line, some slender structure stained deeply with hematoxylin lied in the middle third of the hindbody. The excretory system was not observed.

The slender structure lying along the median line of the hindboy is probably the anlagen of the genital glands. This metacercaria is separated from those of *P. japonicus* and the Metacercaria A by the longer prepharynx, wider intestinal ceca, and genital glands recognized as the slender anlagen.

Summary

From a total of 35,253 specimens of *Euphausia similis* Sars (Crustacea: Euphausiacea) collected from Sagami and Suruga bays during May 25 to June3, 1971, five species of the digenetic trematode metacercariae were obtained. The present paper dealt with one metacercaria of *Syncoelium* sp. (Syncoeliidae), three of *Paronatrema* sp. (Syncoeliidae), one of Metaceraria A, and one of Metacercaria B. One hundred and

thirty-six metacercariae of *Pseudopecoelus japonicus* (Allocreadiidae) will be reported elsewhere.

The unencysted *Syncoelium* metacercaria could not be identified specifically because of only a single immature specimen. The posterior tip of the specimen indicated that an organ such as a tail exists at the metacercarial stage of the present *Syncoelium* species.

The unencysted *Paronatrema* metacercariae remained a cercarial form possessing the tail and three eye-spots. The tail consisted of a short stem and two long, club-shaped forks, and it contained numbers of the conspicuous glandlike cells in the innermost parenchyma. Because of this unique tail, the type of the *Paronatrema* cercaria seemed to be new to science. The metacercariae were considered to be those of an undescribed species.

The encysted Metacercaria A appeared to be that of a species belonging to the genus *Opisthogonoporus* (Lepocreadiidae) in having the marginal genital pore, external seminal vesicle, short prepharynx, and short tubular excretory vesicle.

The encysted Metacercaria B could be only recorded because of its complete destruction during the preparation.

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駿河湾産オキアミ *Euphausia similis* に見られた寄生生物について
IV. 吸虫類のメタセルカリアについて

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東京大学海洋研究所所属調査船淡青丸の KT-71-06 次航海（1971年5月25日～6月3日）において，相模湾と駿河湾とから採集されたオキアミ *Euphausia similis* Sars (Crustacea : Euphausiacea) の35,253個体について寄生虫を調べた．この調査において，5種類の吸虫のメタセルカリアとともに，線虫，条虫，原生動物，それに ellobiopsid の寄生虫をえた．この報文では，そのうち，4種類の吸虫のメタセルカリア，すなわち *Syncoelium* 属 (Syncoeliidae) のもの1個体，*Paronatrema* 属 (Syncoeliidae) のもの3個体，そして未同定種2種のもの2個体（仮に Metacercaria AとB）を記載し，各々の分類について考察した．残りの吸虫の1種，*Pseudopecoelus japonicus* (Allocreadiidae) のメタセルカリア136個体と，吸虫以外の寄生虫は各々別々に詳しく報告する予定である．

Syncoelium 属のメタセルカリアはオキアミの胃域の血体腔内に遊離状態でみつかり，被嚢していなかつた．未成熟な標本が一つであるために，種の同定はできない．メタセルカリアは体後端にある種の器官の痕跡を付けており，この痕跡は，メタセルカリア期においても，この種では尾の様な付属器官をもつことを示している．

Paronatrema 属のメタセルカリアもまたオキアミの胃域の血体腔内に遊離状態でみつかり，被嚢していなく，そして尾と眼点などを具えており，いまだにセルカリアの形態を保つていた．分類上，メタセルカリアは未記載種のものと考えられる．尾は短い尾幹と長い棍棒状岐部からなる岐尾型で，尾の中央部の柔組織には特殊な腺様細胞を満していた．この特異な尾の形態によつて，この *Paronatrema* 属のセルカリアの型は新記載のものとされる．

Metacercaria AとBとは被嚢しており，その寄生部位は明らかでない．分類上，Aは *Opisthogonoporus* 属 (Lepocreadiidae) のある種のものと考えられる．Bは標本作製中に著しく破損したために，ここでは，Bがオキアミに寄生していたことを記録することにとどめる．