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Plerocercoids with blastocysts of the trypanorhynch cestode *Nybelinia surmenicola* found in the euphausiid crustacean *Euphausia pacifica*

Takeshi Shimazu

Nagano Prefectural College, 8–49–7 Miwa, Nagano 380–8525, Japan
E-mail: tshimazu@cb3.so-net.ne.jp

Plerocercoids enveloped in distinct blastocysts of *Nybelinia surmenicola* (Okada in Dollfus, 1929) Dollfus, 1929 (Cestoda: Trypanorhyncha: Tentacullariidae) were found in the hepatopancreas of *Euphausia pacifica* Hansen (Crustacea: Euphausiacea: Euphausiidae) from off the Pacific coast of Iwate Prefecture, Tohoku, Japan. The plerocercoids are described and illustrated. The euphausiid is considered the only true intermediate host for this cestode.

Key words: Plerocercoid, *Nybelinia surmenicola*, Trypanorhyncha, Cestoda, life cycle, *Euphausia pacifica*, Japan.

INTRODUCTION

The trypanorhynch cestode *Nybelinia surmenicola* (Okada in Dollfus, 1929a) Dollfus, 1929b (Tentacullariidae) was originally described as *Nybelinia* sp. by Dollfus (1929a) on the basis of a plerocercoid without a blastocyst found in the squid *Ommastrephes sloani pacificus* (Steenstrup) (=*Todarodes pacificus* Steenstrup) (Ommastrephidae) from Japanese waters. In a footnote, Dollfus (1929a: p. 338) mentioned that Y. K. Okada named this cestode *Tetrarhynchus surmenicola* n. sp. and later (1929b) established the combination *Nybelinia surmenicola* (Okada in Dollfus).

Plerocercoids of *N. surmenicola* without blastocysts were reported by Shimazu (1975a) from euphausiid crustaceans. Recently, a plerocercoid enclosed by a distinct blastocyst of this cestode has been found in a euphausiid. In this paper, the plerocercoid is described and the life cycle of the cestode is discussed briefly.

MATERIALS AND METHODS

Euphausiids *Euphausia pacifica* Hansen (Crustacea: Euphausiacea: Euphausiidae) were commercially fished off the Pacific coast of Iwate Prefecture, Tohoku, Japan. A total of about 172,000 nearly sexually mature adults of euphausiids were obtained from a fisherman at Otsuchi, Iwate Prefecture, during 18–27 March 1998. A majority of the euphausiids were dead but fresh when obtained. These were crushed in half-strength sea water and filtrated through a 5-mm mesh net. The filtrate was washed in half-strength sea water several times by the gravity sedimentation method. The last sediment was examined primarily for larvae of the nematode *Anisakis simplex* (Rudolphi, 1809) Baylis, 1920 under a binocular stereoscopic microscope. A minority of the euphausiids were obtained alive and examined alive under the same microscope.

A total of nine plerocercoids of *N. surmenicola* were found together with some helminth parasites of other species including *A. simplex*. Of the nine, seven were enclosed by blastocysts, but two did not have blastocysts. Three of the seven with blastocysts were pressed out of the blastocysts. These three, two still with blastocysts and two without blastocysts were fixed in 70% ethanol, stained with alum carmine and mounted in Canada balsam. The remaining two with blastocysts were fixed *in situ* in their respective hosts in cold 10% neutralized formalin, cut serially in 10-μm sections and stained with hematoxylin and eosin. Voucher specimens have been deposited in the National Science Museum, Tokyo (NSMT-Pl 4551). The helminth parasites of other species obtained will be reported elsewhere.

RESULTS

Of the nine plerocercoids of *N. surmenicola*, six with blastocysts (Figs. 1 and 3) were found in the live hosts, and one with a blastocyst and two without blastocysts (Fig. 4) were found in the sediment of the crushed hosts. In the sections of the two hosts, both plerocercoids were found in the hepatopancreas (Fig. 2, p, h). The lumen on the infected tubule (Fig. 2, t) of this organ was largely expanded due to the parasite in it. The morphology and measurements of the plerocercoids were briefly as follows.

Anterior portion of blastocyst bell-shaped, 3.10–3.20 mm long by 1.90–2.30 mm wide (Figs. 1 and 3). Anterior region of this portion like a capsule, consisting of an outer layer 28–80 μm thick and an inner layer 4 μm thick, with a small pore at about anterior tip; posterior region hanging like a collar from anterior region. Caudal portion of blastocyst conical, projecting through collar, 0.30–1.30 mm long, with excretory pore at posterior tip.

Body proper either straight or folded in cavity of anterior region of blastocyst, connected with posterior tip of posteriormost segment of strobila to center of bottom of cavity (Figs. 1 and 3). Scolex 3.00–4.20 mm long by 1.96–2.00 mm wide; velum 1.24–1.56 mm long (Fig. 4). Bothridia paired on dorsal and ventral sides, elongated, 2.20–2.70 mm long by 0.80 mm wide, bearing dense microtriches, with free outer and posterior margins. Tentacles four (abnormally three in one specimen), 1.70–2.20 mm long in completely invaginated state. "Ciliated pits" absent on bothridia. Armature homeoacanthous. Hooks solid, uncinate, numbering 16 per spiral row (Fig. 5); base length (Fig. 6, a) 30–36 μm at about mid-level of tentacle (20–22 μm long at basal part of tentacle), hook length (Fig. 6, b) 34–51 (18–21) μm, hook height (Fig. 6, c) 28–34 (16–20) μm. Tentacular sheaths undulating, not spiral; semicircular muscle with at

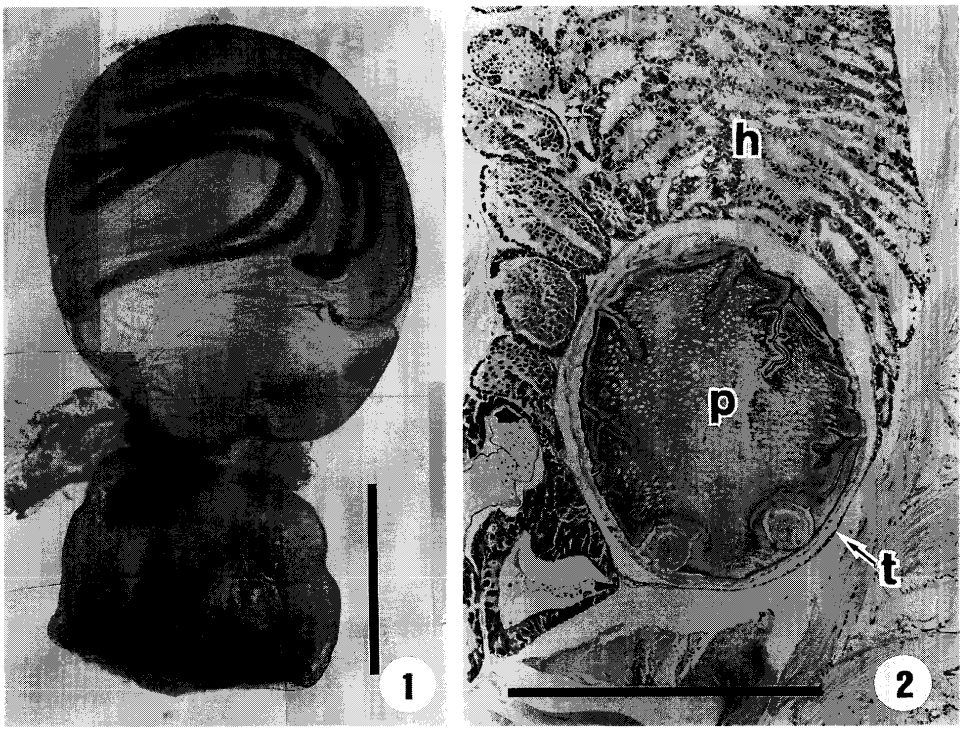


Fig. 1. Photomicrograph of a live plerocercoid of *Nybelinia surmenicola* found in *Euphausia pacifica*. Scale bar=1 mm.
Fig. 2. Photomicrograph of a section of *E. pacifica* infected with a plerocercoid of *N. surmenicola*. h: hepatopancreas; p: plerocercoid; t: tissues of infected tubule of hepatopancreas. Scale bar=1 mm.

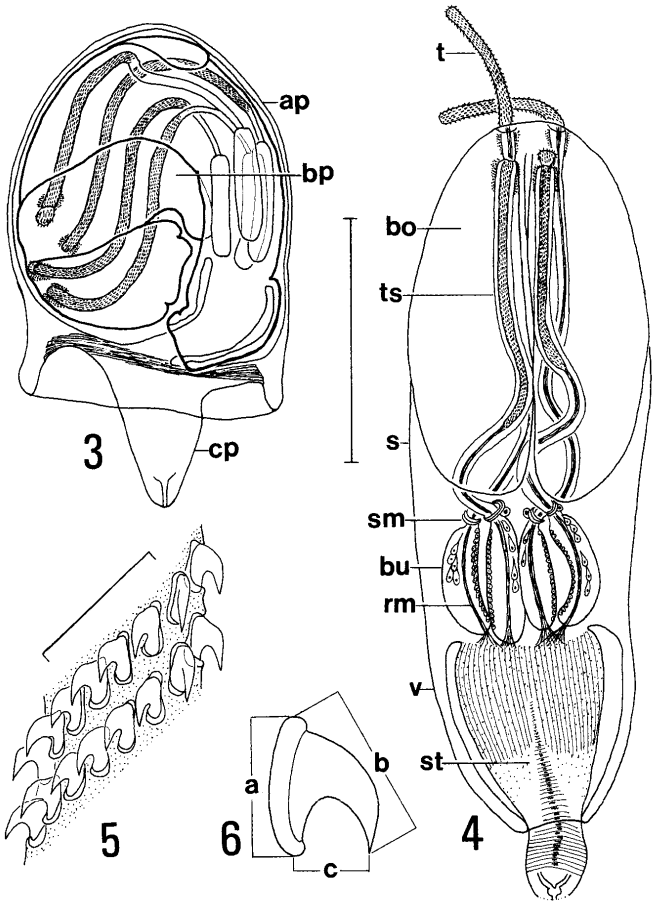
least one large gland cell present at basal part of each sheath; gland cells encircling aperture of each sheath. Bulbs banana-shaped, located posterior to bothridia, 0.50–0.86 mm long by 0.24–0.30 mm wide; numerous gland cells present in a band on one lateral side of whole length of each bulb. Retractor muscles reaching to posterior ends of respective bulbs. Strobila weakly developed, 0.94–2.10 mm long; posteriormost segment of strobila having no genital primordium, lacking tegument around excretory pore at posterior tip in plerocercoids without blastocysts (Fig. 4).

DISCUSSION

Shimazu (1975b) obtained numerous, developing worms of *N. surmenicola* from plerocercoids without blastocysts to gravid adults from a single, recently dead specimen of the shark *Lamna ditropis* Hubbs and Follett (Lamnidae) from the Bering Sea. The worms were found in the oral cavity, esophagus, stomach, intestine and even on the skin around the cloaca of the shark. Most of them may have migrated from the original site of infection, most presumably the intestine, to the other organs after the host's death. Plerocercoids without blastocysts of this cestode are common in the mantle, body cavity and viscera of the squid *T. pacificus* and in the musculature, body cavity and viscera of many species of teleosts in northern parts of the North Pacific Ocean (see Dollfus 1929a, Yamaguti 1934, Shimazu 1975b). Shimazu (1975a) described plerocercoids of the cestode from euphausiid crustaceans, as follows: (1) *E. pacifica* from off Sanriku (or the Pacific coast of Tohoku), (2) *Thysanoessa longipes* Brandt and an unidentified euphausiid from off the Pacific coast of the Kamchatka, and (3) *T. inermis* (Krøyer) from off the Pacific coast of the central Aleutians. None of them possessed a blastocyst.

The present plerocercoids agree in morphology and measurements of the scolex and strobila with those of *N. surmenicola* as described by Dollfus (1929a, 1942), Yamaguti (1934) and Shimazu (1975a, b). The seven of the nine plerocercoids were enveloped in distinct blastocysts (Figs. 1 and 3). Each of them was connected with the posterior tip of the posteriormost segment of the strobila to the bottom of the cavity of the anterior region of the blastocyst (Figs. 1 and 3). The two others that were found in the sediment of the crushed hosts did not have blastocysts. The posteriormost segments of their strobilae lacked the tegument at their posterior ends (Fig. 4). This absence of the tegument suggests that the two plerocercoids were recently tore off from their blastocysts probably during the crushing procedure. It seems likely that all of Shimazu's (1975a) plerocercoids also lost their blastocysts when taken out of the highly pressed euphausiids during examination.

Knowledge is still limited about life cycles of trypanorhynchs. Final hosts for trypanorhynchs are exclusively elasmobranchs. Eggs, operculated or not, fully embryonate to contain oncospheres after having passed into sea water (Mudry and Dailey 1971). Kruse (1959) described a new species, *Parachristianella monomegacantha* (Eutetrarhynchidae), on the basis of plerocercoids with blastocysts found in a penaeid shrimp. Mudry and Dailey (1971) obtained adults of this species from a ray and experimentally demonstrated that (1) an unoperculated egg fully embryonated to contain a oncosphere without cilia in sea water; and (2) the oncosphere hatched from the egg after the egg was ingested by a copepod, in which it developed, through a "procercoid" without a cercomer, into a plerocercoid enclosed by a blastocyst. The plerocercoid of *P. monomegacantha* may directly infect the definitive host, and accordingly the life cycle of this species may involve only one intermediate host (Mudry and Dailey 1971). The life cycle of



Figs. 3–6. Plerocercoids of *Nybelinia surmenicola* found in *Euphausia pacifica*.

Fig. 3. Plerocercoid enveloped in a blastocyst. ap: anterior portion of blastocyst; bp: body proper; cp: caudal portion of blastocyst. Scale bar=2 mm.

Fig. 4 Plerocercoid without a blastocyst. bo: bothridium; bu: bulb; rm: retractor muscle; s: scolex; sm: semicircular muscle; st: strobila; t: tentacle; ts: tentacular sheath; v: velum. Scale bar=2 mm.

Fig. 5. Hooks at about mid-level of tentacle. Scale bar=0.1 mm.

Fig. 6. Tentacular hook. a: base length; b: hook length; c: hook height.

Prochristianella hispida (Linton, 1890) Campbell and Carvajal, 1975 (Eutetrarhynchidae), a parasite of dasyatid rays, is more complex. The plerocercoid with a blastocyst of this species is common in penaeid shrimps. In experimental infections, an unciliated oncosphere developed into early stages of a plerocercoid with a blastocyst in harpacticoid copepods but failed to infect a penaeid shrimp (Overstreet 1983). Whether shrimp acquires infections from eating infected copepods or eggs has not been established (Overstreet 1983). On the other hand, Sakanari and Moser (1985, 1989) experimentally completed three-host life cycles of *Lacistorhynchus tenuis* (van Beneden, 1858) Pintner, 1913 and *L. dollfusi* Beveridge and Sakanari, 1987 (Lacistorhynchidae), as follows: (1) a coracidium, after having hatched from an operculated egg in sea water, was ingested by a copepod (a first intermediate host), in which the oncosphere developed into a "procercoid" without a cercomer; (2) the procercoid was fed, along with the copepod, to a fish (a second intermediate host), in which it developed into a plerocercoid enclosed by a blastocyst; and (3) the plerocercoid was fed, along with the fish, to a shark (a final host), in which it attained sexual maturity.

As discussed earlier, the outline of the life cycle of *N. surmenicola* in northern parts of the North Pacific Ocean has been elucidated. The uterine egg includes the egg cell in the one-cell stage and a few yolk cells in a thin, unoperculated shell (Shimazu 1975b). Neither the oncosphere nor the procercoid is known. It may be possible that *E. pacifica*, *T. inermis* and *T. longipes* (second intermediate hosts) acquire infections from eating copepods (first intermediate hosts) harboring procercoids. However, this seems unlikely to take place. Euphausiids of the genera *Euphausia* and *Thysanoessa* feed on detritus, phytoplankton and zooplankton by filter feeding. *E. pacifica*, *T. inermis* and *T. longipes* do eat nauplii and adults of copepods by raptorial feeding. They pierce the integuments of copepods by the mouthparts, suck the "juices" leaving their empty husks, and further break up the sucked material in the stomach. Besides, their stomach contents also include the antennules, mandibles, swimming legs and urosomes of copepods (Mauchline and Fisher 1969, Y. Endo pers. comm.). They may not eat copepods whole. Accordingly, it seems impossible that the euphausiids can ingest procercoids, which have grown to some extent in the copepods, without injuring them seriously. Instead, it is probable that, as in the case of *P. monomegacantha* (see above), (1) the egg containing a fully-formed oncosphere without cilia is swallowed by these euphausiids by filter feeding; and (2) the oncosphere hatches from the egg in the alimentary canal and migrates into the hepatopancreas, where it fully develops into a plerocercoid enveloped in a blastocyst. If so, it is conceivable that the euphausiids serve as the only true intermediate host for *N. surmenicola*. The squid and teleosts may be infected with plerocercoids from eating euphausiids harboring fully-developed plerocercoids with blastocysts or from eating squids or teleosts already harboring plerocercoids without blastocysts or from both. They are considered ecologically essential as paratenic hosts to transmit plerocercoids to the final host *L. ditropis* (Shimazu 1975b).

The present study shows that *N. surmenicola* forms a plerocercoid with a distinct blastocyst in the intermediate host. Previously, Dollfus (1960) reported a plerocercoid with a spherical blastocyst of *N. africana* Dollfus, 1960 from the inner surface of the pharynx of a fish. The plerocercoid may have recently been liberated from a crustacean intermediate host ingested by the fish. Palm (1997) proposed a new classification of the order Trypanorhyncha Diesing, 1863. In this classification, the presence or absence of the blastocyst in the plerocercoid was used as one of the characters at the family level. The family Tentaculariidae Poche, 1926, *sensu* Palm, 1997 including the genus *Nybelinia* Poche, 1926 was placed in the group without the blastocyst. In addition, the presence or absence of the prebulbar organs was regarded as one of the characters at the suborder level. The family Tentaculariidae was assigned to the suborder Tentacularioidea Poche, 1926 without the prebulbar organs. However, the semicircular muscles at the basal parts of the respective tentacular sheaths that were observed in the present plerocercoids (Fig. 4) and those from the squid *T. pacificus* (unpubl. data) appear to be similar to the enigmatical prebulbar organs illustrated by Campbell

and Beveridge (1994). Therefore, the classification of the order Trypanorhyncha seems to require much more study.

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ツノナシオキアミ *Euphausia pacifica* から検出された，四吻条虫 *Nybelinia surmenicola* の被嚢性プレロセルコイド

嶋津　武

長野県短期大学　〒380–8525　長野市三輪8–49–7

1998年3月に，岩手県大槌港に水揚げされたツノナシオキアミ *Euphausia pacifica* Hansen (Crustacea: Euphausiacea: Euphausiidae)の肝膵臓から四吻条虫 *Nybelinia surmenicola* (Okada in Dollfus, 1929) Dollfus, 1929 (Cestoda: Trypanorhyncha: Tentacullariidae)のプレロセルコイドを検出し，記載した．このプレロセルコイドは被嚢していた．オキアミはこの条虫にとって唯一の真性中間宿主と考えられた．

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