北海道忍路湾における浮遊性ハルパクチクス類(橈脚類),Microsetella norvegica Boeckの生活史について

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Biology of a Pelagic Harpacticoid Copepod, *Microsetella norvegica* BOECK in Oshoro Bay, Hokkaido[1)]

I. Life History

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Abstract

Concerning the field and laboratory cultured samples collected in Oshoro Bay, on the west coast of Hokkaido, during August, 1972, the life history of *Microsetella norvegica* BOECK was closely examined.

At a temperature of about 18°C, hatching of eggs took place within 3 days after ovigerous females were brought into culture vessels. Once hatched the young swam out from the egg sac successively, and this stage is called nauplius I. *M. norvegica* has 6 stages of nauplii and 6 of copepodids (the sixth being adult) as is the case of the most of harpacticoids. A remarkable characteristic is the formation of the rudimentary first maxilla and a strong median spine as the caudal armature at the nauplius stage I, but the latter disappears at the nauplius stage II.

Introduction

In Oshoro Bay on the west coast of Hokkaido, 13 species of harpacticoid copepods had been reported by ITŌ (1968, 1969, 1972) and MOTODA (1971). Among 5 pelagic species, *M. norvegica* is dominant in the bay during August; they occur approximately 80 % in number of individuals of all copepods. Although life histories of many non-pelagic species of harpacticoids were already studied by NICHOLLS (1935), FRASER (1936), SHAW (1938), JOHNSON and OLSON (1948), SMYLY (1957), BARNETT (1966), ITŌ (1970) and KOGA (1970), those of pelagic harpacticoids are only two species, *Euterpina acutifrons* and *Microsetella rosea* reported by BERNARD (1963) and BJÖRNBERG (1972) respectively.

M. norvegica is one of the most important copepod species in coastal waters and commonly distributed in the world oceans as reported by WILLIAMS (1906), BREEMEN (1908), SARS (1911), MORI (1937), LANG (1948), DAVIS (1949) and GONZALEZ and BOWMANN (1965). FISH (1955) mentioned that the generation time of this species in the boreal of the western Atlantic Ocean was about two months. Although FISH (1955) and OGILVIE (cited in LOVEGROVE, 1956) described on the morphological features of a partial body at some nauplius stages, an entire life history of the present species was unknown.

This study offers more complete knowledge of the life history of *M. norvegica* observed on field and laboratory cultured specimens obtained from Oshoro Bay during August, 1972.

Materials and Methods

The field samples by the surface tow were collected at least once every 3 days from 5

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to 26 August, 1972, between St. A (16 m in depth) and St. B (12 m in depth) in Oshoro Bay (Figure 1). The net used was 30 cm in mouth diameter and 100 cm in length made of pylen with 0.1 mm mesh openings. Additionally, 10 *l* of seawater were sampled by a Van Dorn water sampler and filtered through the Toyo Filter Paper No. 2 (pore size; *ca.* 0.8 μ) to obtain the nauplii, because some nauplius stages were unable to retain with the present net. These samples were immediately preserved in about 5 % of neutral formalin seawater.

To observe the hatching and the successive development of the larvae, the culture experiments were carried out in the Oshoro Marine Biological Station, Hokkaido University, from August 17 to 23. The 20 healthy females carrying egg sacs were sorted from the net samples collected at the surface near St. B on August 16 and transferred by individual into

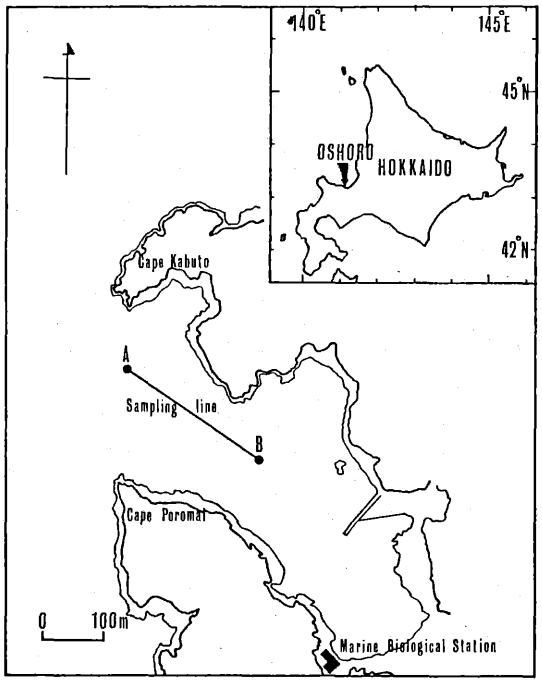

Fig. 1. Map showing sampling stations in Oshoro Bay.

test tubes filled with about 15 m*l* of filtered seawater. The seawater was filtered through a Whatman GF/C glass fiber filter at first and secondly through a 0.45 μ Millipore filter. Before culturing, an antibiotic, streptomycin (50 mg/*l*) was added to the filtered seawater and aerated. The materials were maintained under starvation. The test tubes were maintained at 18.6°C (±0.5°C) which was 2°−3°C lower than their natural habitat. During culture the seawater was exchanged with fresh filtered seawater once a day. The ovigerous females and the nauplii hatched were observed under a binocular microscope to identify their developmental stages. Then, a few individuals were fixed everyday with 5 % of neutral formalin seawater to follow the successive development of the nauplius stages. Development of nauplii from I to III stages was able to be observed and after the nauplius stage IV

unsuccessful because of starvation on the present culturing. Subsequently, development from the nauplius stage IV to the copepodite stage VI was observed on field samples.

The body length was measured from the tip of the head to the end of the anal segment.

Results

Hatching

An adult female carries an egg sac of about 0.12 mm long attached to the genopore (Figure 2-1 and -2). 6 to 10 eggs were obviously recognized in an egg sac and each was measured as 55-60 μ in diameter just before hatching. By 3 days before hatching, the egg

TABLE 1. SEGMENTATION AND SETATION OF *Microsetella norvegica* BOECK

Stage	No. of body segments	No. of segments in antennule	No. of setae in rudimentary 1st maxilla	No. of setae in rudimentary 2nd maxilla	Leg 1		Leg 2		No. of caudal setae
					Exopod	Endopod	Exopod	Endopod	
N. I	—	2	1	—	—	—	—	—	2
N. II	—	3	1	—	—	—	—	—	2
N. III	—	3	1	—	—	—	—	—	4
N. IV	2	3	2	—	—	—	—	—	6
N. V	3	3	2	—	Rudimentary		Rudimentary		6
N. VI	4	3	3	1	Rudimentary		Rudimentary		6

TABLE 2. SEGMENTATION OF *Microsetella norvegica* BOECK

Stage		No. of body segments			No. of segements in antennule	Leg 1	
		Cephalothorax	Abdomen	Total		Exopod	Endopod
C. I		3	2	5	3	1	1
C. II		4	2	6	4	2	2
C. III		5	2	7	4	2	2
C. IV		5	3	8	5	2	2
C. V		5	3	8	5	3	3
C. VI	F.	5	4	9	6	3	3
	M.	5	5	10	5	3	3

Stage		Leg 2		Leg 3		Leg 4		Leg 5	
		Exopod	Endopod	Exopod	Endopod	Exopod	Endopod	Exopod	Baso-endop.
C. I		1	1	Rudimentary		—	—	—	—
C. II		2	2	1	1	Rudimentary		—	—
C. III		2	2	2	2	1	1	Rudimentary	
C. IV		2	2	2	2	2	2	Rudimentary	
C. V		3	3	3	3	3	3	1	1
C. VI	F.	3	3	3	3	3	3	1	1
	M.	3	3	3	3	3	3	1	1

sac turned opaque. All the larvae of nauplius stage I emerged from the egg sac with each successive hatching and swam vigorously in the test tubes. This procedure is fundamentally similar to the hatching observed in other harpacticoids, *Tigriopus fulvus* (MARSHALL and ORR, 1954), *Tisbe furcata* (BATTAGLIA, 1970) and *Euterpina acutifrons* (HAQ, 1972).

Despite the careful examination, some morphological structures of the nauplii, particularly the setal number of the appendages and features of antennal gnathobases as a primitive jaw (FRASER, 1936), could not be exactly observed and clarified. These morphological structures had commonly been observed in other harpacticoid copepods (NICHOLLS, 1935; FRASER, 1936; JOHNSON and OLSON, 1948; BARNETT, 1966; KOGA, 1970).

Segmentations and setations of the nauplius and copepodite stages of *M. norvegica* are summarized in Tables 1 and 2.

Nauplius stages

Nauplius stage I (Figure 2-3)

Body length 0.084 mm, egg-shaped without segments, possessing a small red eye spot at the base of the antennules. Labrum halfcircular flap. Antennule two-segmented. Antenna strongly-built and one-segmented in the endopod and exopod. The mandible possesses a splender one-segmented exopod and the endopod with some short setae on the margin. Caudal region bears two strong and two flaccid setae on both sides and a single median spine acutely projected on the distal margin. The outermost caudal seta each on both sides is the rudimentary first maxilla.

Nauplius stage II (Figure 2-4)

Body length 0.096 mm, egg-shaped and without segments. Antennule three-segmented. Antenna one-segmented in the endopod and two-segmented in the exopod. The mandible possesses short spines on the distal margin of the endopod. The rudimentary first maxilla transfers from the posterior distal part to the inner part and more strongly-built than in the nauplius stage I. The obvious structural difference between the stage I and II is a disappearance of the median spine in the caudal region.

Nauplius stage III (Figure 2-5)

Body length 0.115 mm and without segments. Antennule three-segmented. Antenna one-segmented in the endopod and three-segmented in the exopod. Caudal region bears two strong and long setae, and two flaccid setae. Setation of the caudal region is similar to those of *Tigriopus fulvus* dscribed by FRASER (1936) and *Tisbe furcata* by JOHNSON and OLSON (1948).

Nauplius stage IV (Figure 2-6)

Body length 0.133 mm and has two segments in the ventral view. Antennule three-segmented. Antenna two-segmented in the endopod and four-segmented in the exopod. The rudimentary first maxilla one-segmented with one strong and one flaccid setae. Caudal armature consists of three pairs of terminal setae; two setae long with thick hair-like setae, and a short seta.

Nauplius stage V (Figure 3-1)

Body length 0.169 mm and has three segments. Posterior part of the third segment diverges right and left sides. The first, second and third segments have regular serrations on the posterior margins. Antennule three-segmented. Antenna two-segmented in the endopod and five-segmented in the exopod. Rudiments of the first and second pairs of swimming

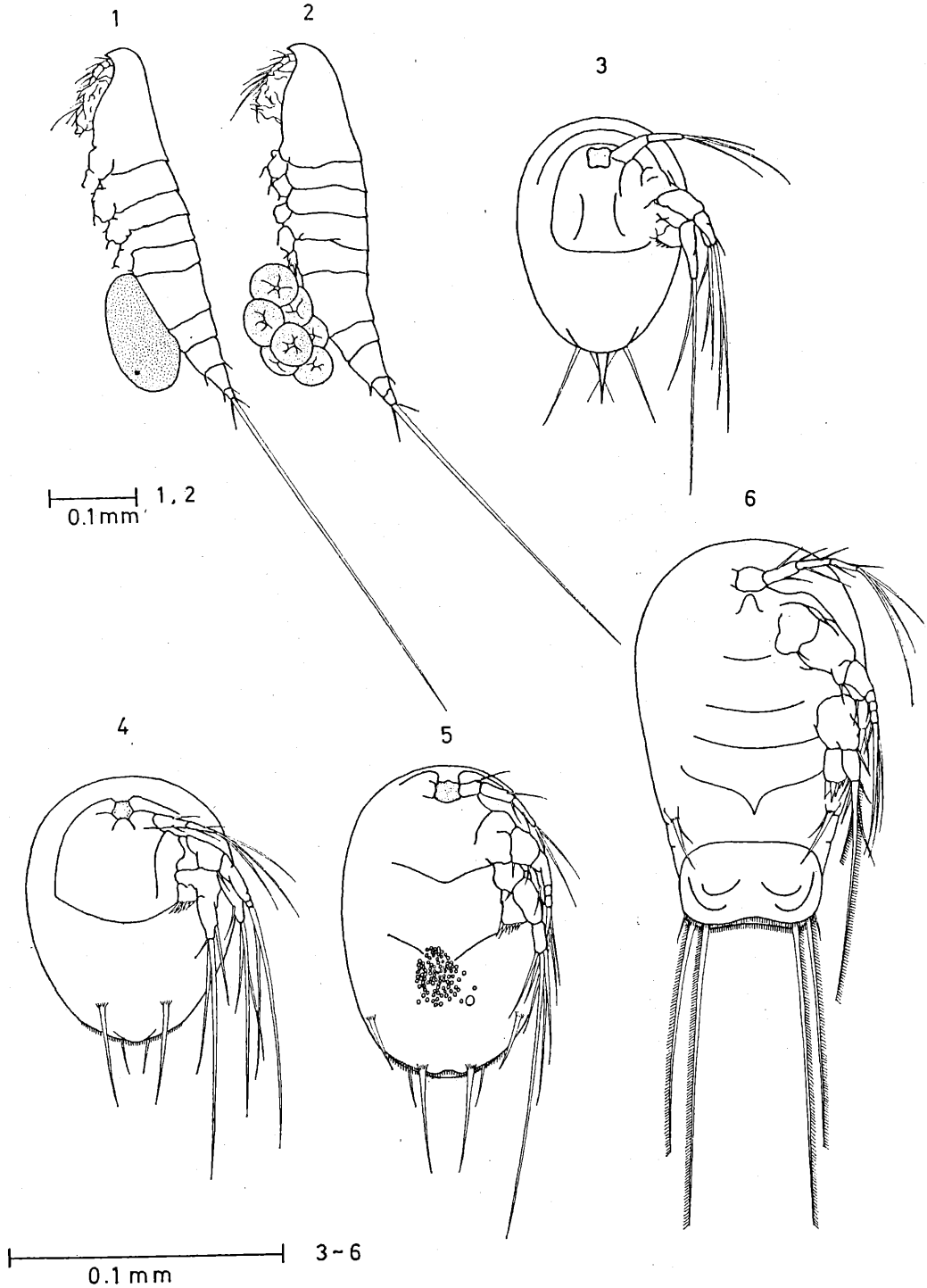

Fig. 2. 1. Female before segmentation of eggs occurs.
2. Female carrying eggs shortly before hatching.
3. Nauplius stage I, ventral view.
4. Nauplius stage II, ventral view.
5. Nauplius stage III, ventral view.
6. Nauplius stage IV, ventral view.

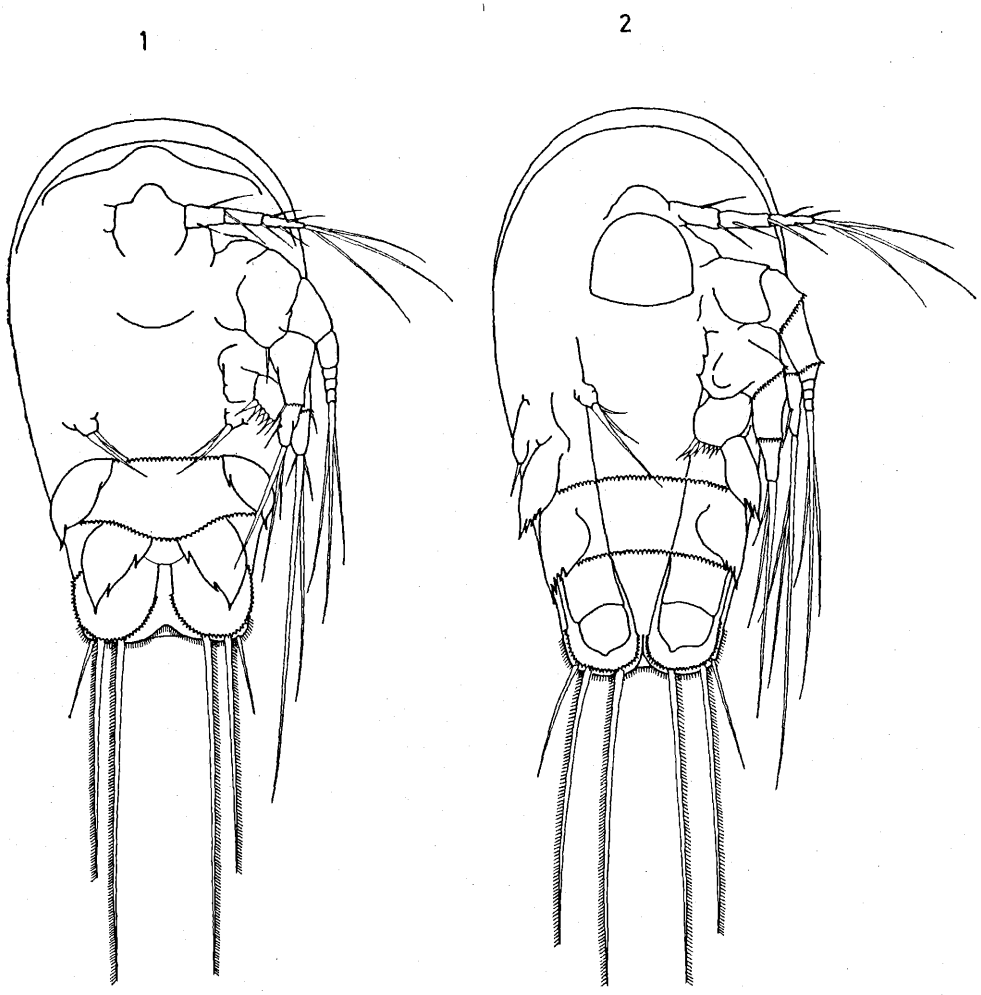

Fig. 3. 1. Nauplius stage V, ventral view.
2. Nauplius stage VI, ventral view.

legs appear on the posterior margins of first and second body segments. The outermost caudal seta appeared at the nauplius stage IV become now much longer than before.

Nauplius stage VI (Figure 3-2)

Body length 0.187 mm and has four segments. Antennule three-segmented. Antenna two-segmented in the endopod and five-segmented in the exopod. The rudimentary first maxilla has one strongly long seta and two short setae. The rudimentary second maxilla one-segmented with one short seta.

Copepodite stages

Copepodite stage I (Figure 4-1~4)

Body length 0.205 mm and has five segments, consisting of three cephalothoracic and two abdominal segments. The first, second and last segments bear a lot of flaccid setae on the posterior margins. The third and fourth segments bear serrations on the posterior margins. Three pairs of swimming legs appear in the first, second and third cephalothoracic segments; the first and second pairs biramous with biarticulate basipods, uniarticulate endopods and exopods, and the third pair flap-liked and rudimentary with several setae on the distal margins. Antennule three-segmented with several marginal setae; the apical segment with three long terminal setae. Each furcal ramus bears three terminal setae; the outer setae of them tiny, the innermost one the longest and about 1.3 times as long as the body length.

Copepodite stage II (Figure 4-5~10)

Body length 0.242 mm and has six segments, consisting of four cephalothoracic and two abdominal segments. Antennule four-segmented. Each cephalothoracic segment bears a pair of swimming legs. The first, second and third pairs biramous. The first and second pairs biarticulate in the endopods and exopods. The third pair possesses uniarticulate rami. The fourth pair rudimentary with several setae on the distal margins.

Copepodite stage III (Figure 4-11~14)

Body length 0.290 mm and has seven segments, consisting of five cephalothoracic and two abdominal segments. Antennule four-segmented. Each cephalothoracic segment bears a pair of swimming legs. The first three pairs biramous, each biarticulate in the both rami and the fourth pair biramous but uniarticulate, while the fifth pair flap-liked and rudimentary. The innermost furcal seta almost as long as the body length.

Copepodite stage IV (Figure 5-1~4)

Body length 0.314 mm and has eight segments, consisting of five cephalothoracic and three abdominal segments. Antennule five-segmented. The first four pairs of swimming legs biramous, and biarticulate in the both rami. The last pair rudimentary and the baso-endopod and exopod partly fused.

Copepodite stage V (Figure 5-5~8)

Body length 0.326 mm and has eight segments as in the stage IV. Antennule five-segmented. The first four pairs triarticulate in the both rami. The baso-endopod of the fifth leg completely separated from the exopod. The baso-endopod bears three strong setae

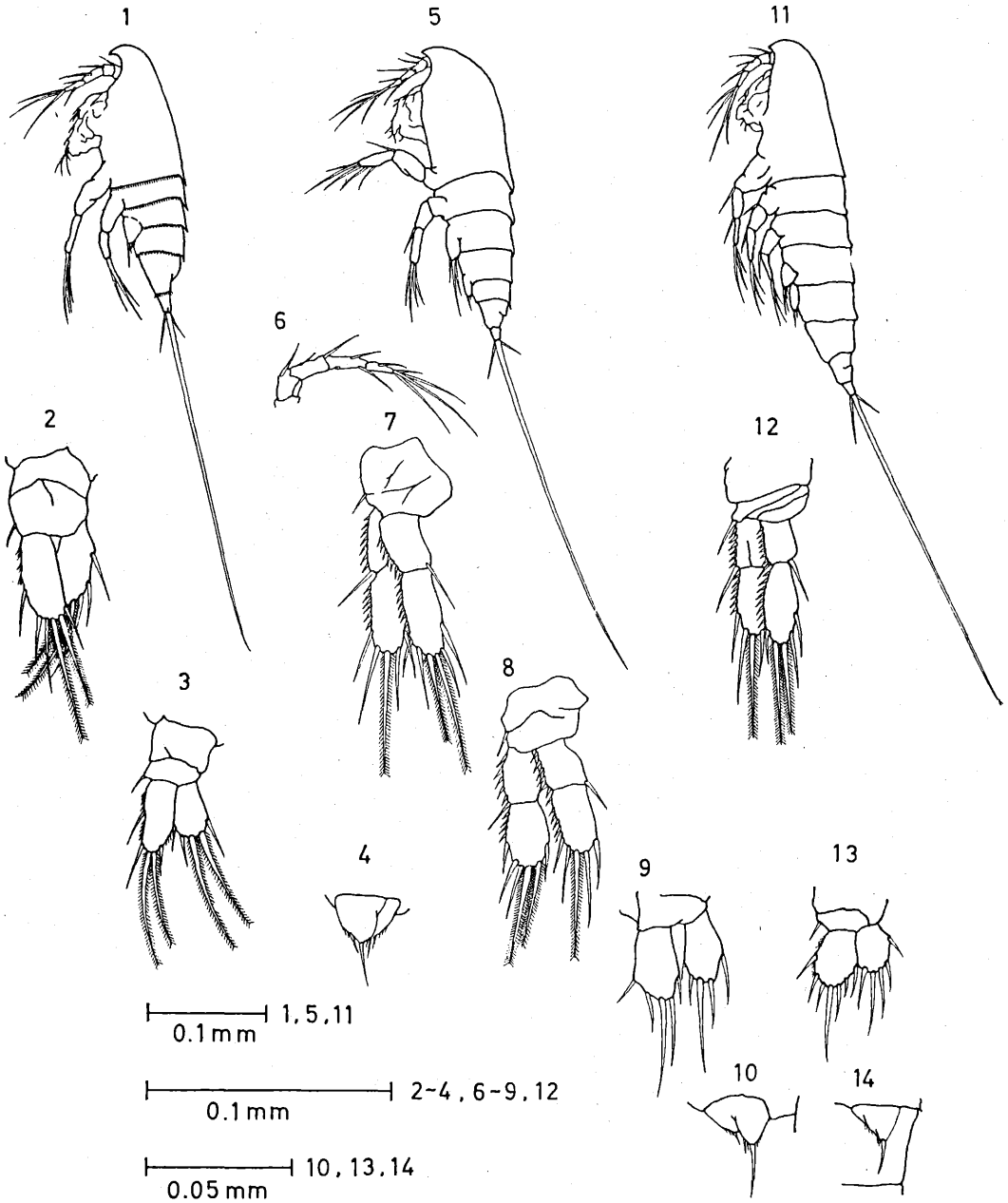

Fig. 4. 1. Copepodite stage I, lateral view.
2. Copepodite stage I, 1st swimming leg.
3. Copepodite stage I, 2nd swimming leg.
4. Copepodite stage I, 3rd swimming leg.
5. Copepodite stage II, lateral view.
6. Copepodite stage II, Antennule.
7. Copepodite stage II, 1st swimming leg.
8. Copepodite stage II, 2nd swimming leg.
9. Copepodite stage II, 3rd swimming leg.
10. Copepodite stage II, 4th swimming leg.
11. Copepodite stage III, lateral view.
12. Copepodite stage III, 1st swimming leg.
13. Copepodite stage III, 4th swimming leg.
14. Copepodite stage III, 5th swimming leg.

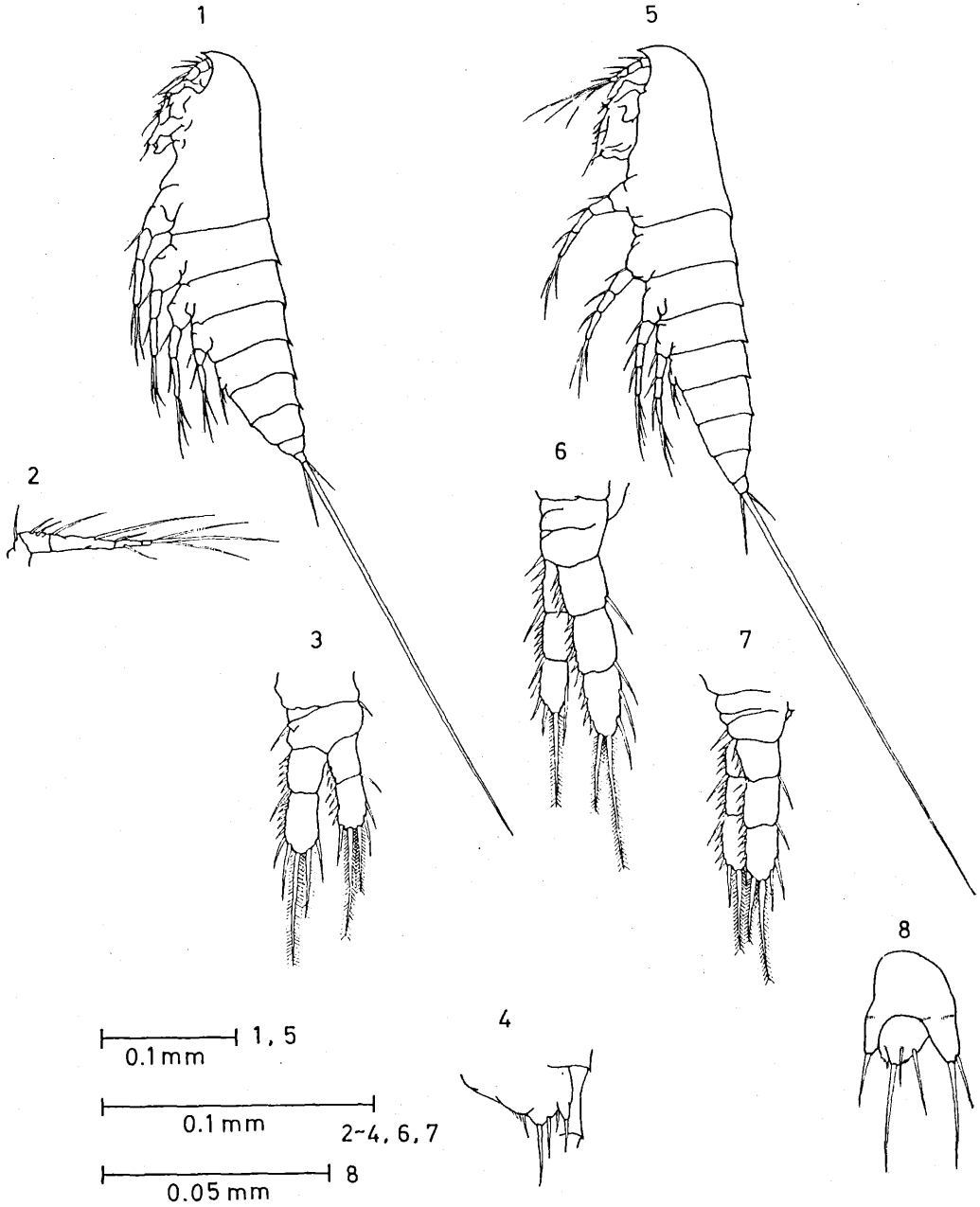

Fig. 5. 1. Copepodite stage IV, lateral view.
2. Copepodite stage IV, Antennule.
3. Copepodite stage IV, 4th swimming leg.
4. Copepodite stage IV, 5th swimming leg.
5. Copepodite stage V, lateral view.
6. Copepodite stage V, 1st swimmimg leg.
7. Copepodite stage V, 4th swimming leg.
8. Copepodite stage V, 5th swimming leg.

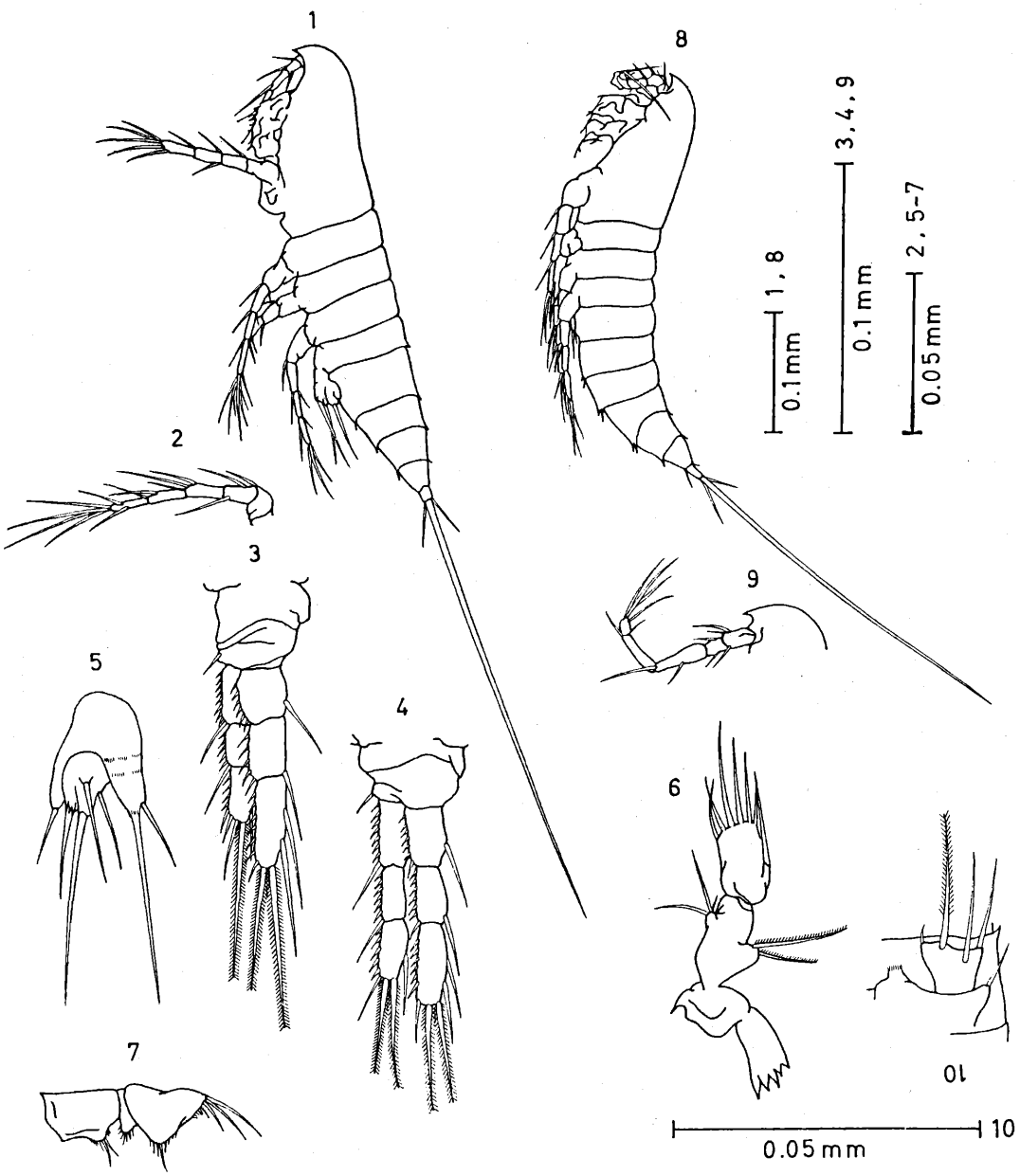

Fig. 6. 1. Copepodite stage VI, female, lateral view.
2. Copepodite stage VI, female, Antennule.
3. Copepodite stage VI, female, 1st swimming leg.
4. Copepodite stage VI, female, 4th swimming leg.
5. Copepodite stage VI, female, 5th swimming leg.
6. Copepodite stage VI, female, Mandible.
7. Copepodite stage VI, female, 2nd maxilla.
8. Copepodite stage VI, male, lateral view.
9. Copepodite stage VI, male, Antennule.
10. Copepodite stage VI, male, 5th swimming leg.

on the distal margins and the exopod with several flaccid setae and two strong setae on the distal margin.

Copepodite stage VI (Figure 6-1~7 and -8~10)

Female: Body length 0.365 mm and has nine segments, consisting of five cephalothoracic and four abdominal segments. Antennule six-segmented. The first four pairs triarticulate in the both rami. The fifth pair biramous but uniarticulate. The baso-endopod possesses one seta on the outer distal margin and two setae on the inner distal margin; one of them about 2 times as long as the other. Exopod of the same leg possesses four setae; apical one of them about 2 times as long as the other.

Male: Body length 0.338 mm and has ten segments, consisting of five cephalothoracic and five abdominal segments. The first four pairs triarticulate in the both rami and the fifth pair biramous but uniarticulate as the female. The fifth leg possesses one seta at the outer edge of the baso-endopod, three strong setae and one very short seta on the distal margin of the exopod. Antennule five-segmented and modified into a robust claw-like structure; the first to the third segments geniculate.

Discussion

Microsetella norvegica has 6 stages of nauplii and 6 of copepodids (the sixth being adult) as in other harpacticoids, excluding *Tigriopus fulvus* (FRASER, 1936) and *Macrosetella gracilis* (TOKIOKA and BIERI, 1966) in which only 5 stages of nauplii have been recognized.

In *Microsetella rosea* (BJÖRNBERG, 1972), the female was first observed at the copepodite stage IV on the basis of the fifth pair of legs much more developed than that of the male in the copepodite stage V and VI. Moreover, in most calanoid, cyclopoid and harpacticoid copepods the sexual dimorphism has commonly been observed as early as copepodite stage IV. In this study, all the specimens of copepodite stages IV and V were possibly females of *M. norvegica*, because the setal numbers on the distal margin of the fifth pair of legs are equal to those of stage IV or more numerous at stage V than those of males in the copepodite stage VI (Figs. 5-4, 8 and 6-10). Therefore, exact differences according to sexes at early developmental stages could not be observed in the present specimens.

Rudiments of the first maxillae are already formed in nauplius stage I of *M. norvegica* as well as *Longipedia coronata*, *L. scotti*, *L. minor* (Longipediidae) (NICHOLLS, 1935), *Microsetella rosea* (Ectinosomidae) (BJÖRNBERG, 1972), though those of *Tigriopus fulvus* (FRASER, 1936), *T. japonicus* (Harpacticidae) (ITŌ, 1970) and *Tisbe furcata* (Tisbidae) (JOHNSON and OLSON, 1948) are formed later at nauplius stage II. JOHNSON and OLSON (1948) mentioned that the formation of the first maxillae of harpacticoids is characteristically observed in early nauplius stages as well as cyclopoids. On the other hand, rudimentary first maxillae of calanoids are formed after nauplius stages III or IV. In this respect, harpacticoids are much closer to cyclopoids than calanoids.

Moreover, the nauplius of stage I shows a morphological characteristic taking together a acutely long median spine on the caudal region as in the case of the genus *Longspedia*

(NICHOLLS, 1935) and *M. norvegica* illustrated by OGILVIE (cited in LOVEGROVE, 1956). Although BJÖRNBERG (1972) made no mention of the formation of the median spine in the life history of *M. rosea*, it is found that only nauplius of stage I has a projected and spine-like distal margin of the caudal region, as is the case of *Longipedia* and *M. norvegica*, according to his illustration. LANG (1948) mentioned that *Longipedia* and Canuellidae occupy the most primitive group in harpacticoids because their nauplii are most characteristically simple in appearance among more than 30 families of harpacticoids. Recently, BJÖRNBERG (1972) observed morphological features of young developmental stages such as nauplius stages to discuss the phylogenic positions of copepod families. As a result, BJÖRNBERG (1972) suggested that the Ectinosomidae including *Microsetella* and Tachidiidae are derived from the primitive group (Canuellidae and Longipediidae) and occupy closer systematic position to that group. The present observation revealed that the morphology of nauplius stage I of *M. norvegica* is very similar to those of *Longipedia* (NICHOLLS, 1935) and *M. rosea* (BJÖRNBERG, 1972), *i.e.*, nauplii of three species having rudimentary first maxillae and a median spine on caudal region (Fig. 2-3). Therefore, as KOGA (1973) pointed out, the author also emphasizes that the caudal armature of the nauplius is the appropriate material to follow the evolutional changes at least in the harpacticoids.

It is generally recognized that the first pair of legs of copepods shows very specialized features according to their habitats, *e.g.*, swimming, creeping on and attaching to the substrata of their habitats. In harpacticoids, non-pelagic species, *Longipedia coronata*, *Tisbe furcata*, *Tisbe pori*, *Tigriopus japonicus*, *Tigriopus fulvus* and *Harpacticus uniremis* have their first pair of legs adapting morphologically to their habits such as benthic or ephytic habits. On the other hand, pelagic harpacticoids, *Euterpina acutifrons*, *Macrosetella gracilis*, *Microsetella rosea*, *M. norvegica* and *Aegithus mucronatus* have their legs adapted for swimming as those of calanoids. Apart from the first pair of legs, some nauplii among non-pelagic species have their strong spines much more developed than those of *M. norvegica* and *M. rosea* (BJÖRNBERG, 1972) on the endopods of both antennae and mandibles and they are also specially modified into strongly-built claws looking like prehensile hooks to adapt themselves to benthic or epiphytic lives (NICHOLLS, 1935; FRASER, 1936; JOHNSON and OLSON, 1948; ITO, 1970; KOGA, 1970). Among the pelagic harpacticoids, *Macrosetella gracilis* spends for nauplius and early copepodite stages attaching to the filaments of clumps of planktonic blue green algae *Trichodesmium* by means of specially adapted hooks of the mandibles and antennae (CALEF and GRICE, 1966; TOKIOKA and BIERI, 1966; BJÖRNBERG, 1972) but for late copepodite stages as free living pelagic lives (WILSON, 1932). However, *Microsetella norvegica* and *M. rosea* have no such well-developed and specialized structures throughout their entire life histories. Therefore, in the present study, *M. norvegica* as well as *M. rosea* is recognized to be truly pelagic throughout its life by the morphological observations from nauplius stage I to adult. This as well as eurythermal and euryhaline habits possibly make *M. norvegica* distribute widely from coast to open seas in the world oceans.

Summary

1) In *Microsetella norvegica* Boeck, there are 6 stages of nauplii and 6 of copepodids (the sixth being adult) as in other harpacticoids. The sex differentiation could not be exactly observed at the copepodite stage IV.

2) Rudiments of the first maxillae and a median spine acutely projected on the caudal region are clearly observed at the nauplius stage I the same as those of *Longipedia* and *Microsetella rosea*. The spine of *M. norvegica* disappears after nauplius stage II.

3) No specially adapted appendages to benthic or epiphytic habits as reported in other many non-pelagic harpacticoids are observed throughout the entire life history of *M. norvegica*. Therefore, this species should be regarded as holoplankton or spending pelagic lives at any developmental stages.

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