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Naupliar Development of *Harpacticus nipponicus* Ito (Copepoda: Harpacticoida: Harpacticidae) Reared in the Laboratory

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ABSTRACT

The naupliar stages of *Harpacticus nipponicus* Ito reared in the laboratory are described and illustrated in detail. Ovigerous females of *H. nipponicus* were collected from the intertidal zone of Kuryongpo Yougil-gun Kyongangbuk-do, Korea. This species passed through 6 naupliar stages before metamorphosis to the first copepodid stage. At 20°C, the first copepodid stage was attained in 6-8 (mean 7) days after hatching. The nauplii of *Harpacticus* species can be distinguished from those of other Harpacticid genera by the maxillule, pereiopods, and caudal setae. The nauplii of *H. nipponicus* are similiar in morphology to those of *H. uniremis* but distinguished by the differences in naupliar size and appendage setation. A key to identify the naupliar stages of this species is briefly discussed.

Key words: Copepoda, Harpactiocoida, *Harpacticus nipponicus*, naupliar development.

INTRODUCTION

Harpacticus nipponicus Ito, 1976 has been reported in Japan from the west coast and along the coast of Hokkaido. It occurs also in the east coast of Korea, and lives on the alga, *Ulva pertusa* in the intertidal zone. Its copepodid stages were described by Ito (1976), but its naupliar stages were unknown.

In the genus *Harpacticus*, all the naupliar stags are known of *Harpacticus* sp. by Walker (1981), the early naupliar stages of *H. littoralis* by Griga (1960) and Castel (1976), and the first naupliar stages of *H. uniremis* by Dahms (1990) and Brian (1919).

The present study is the first description of the external morphology of all naupliar stages of *H.*

nipponicus. All the nauplii of *H. nipponicus* are compared with the previously described harpacticid nauplii, especially those of the *Harpacticus*.

MATERIALS AND METHODS

Nauplii of *Harpacticus nipponicus* used for this study were obtained by hatching eggs of ovigerous females collected from algae in intertidal zone of the Kuryongpo, Korea (36°N, 129°E), on August 1, 1993 and cultured in the laboratory for three generations.

Ovigerous females reared individually in Petri dish (55 mm diameter × 15 mm depth) containing filtered seawater of 33.3‰ until hatching. Newly hatched larvae were seperated into five groups of ten larvae per Petri dish (containing 25 ml filtered seawater) and kept at 20°C in a culture chamber with a light regime of 14:10 hr L:D. The diatom *Dunaliella tertiolecta* was provided as food, with daily changes of seawater.

Samples, dead larvae and all exuviae of each developmental stage were preserved in 5% neutral formalin. A Nikon FX II light microscope was used for examination at magnification of 400X-1000 X. Drawings were made with the aid of a camera lucida. The observations were based on ten specimens per stage. Measurements of larvae and tabular presentation of appendage setations followed those presented by Dahms (1987).

RESULTS

There are all six naupliar stages. Egg sac ovoid in outline (mean: 291 μm × 208 μm). Egg length 59 μm (52-75 μm), egg width 57 μm (49-65 μm). Nauplii are circular, almost as long as wide in early stages, but become somewhat elongate in later stages.

A red nauplius eye is located between the bases of the antennule (It loses colour and shape quite soon after embedding and thus is not figured). Maxillules appear from nauplius IV. Precursors of legs 1 and 2 appear first at the nauplius VI. Naupliar shield covers the hindbody only at nauplius I, in the later stages the caudal portion becomes prominent.

Nauplius I (Figs. 1A, 2A, 3A, 4A)

Body (Fig. 1A) 79 μm (78-80 μm) long, and 79 μm (78-80 μm) wide.

Labrum with a row of spinules laterally and distally.

Antennule (Fig. 2A) 3-segmented. First segment naked, second segment with 1 small seta, 1 long medial seta, and a tuft of spinules terminally. Third segment with 3 setae distally.

Antenna (Fig. 3A). Coxa with masticatory process with a small projection and spinules on distal edge. Basis with 1 spine-like process tipped with spinules and 1 simple seta.

Endopodite 2-segmented. First segment with 2 setae medially and a row of spinules. Second segment with a claw. Exopodite indistinctly 2-segmented. First segment with 1 small seta on midlength and 1 distal spinulose seta. Second segment with 1 small seta on midlength, 1 simple, and 1 spinulose setae distally.

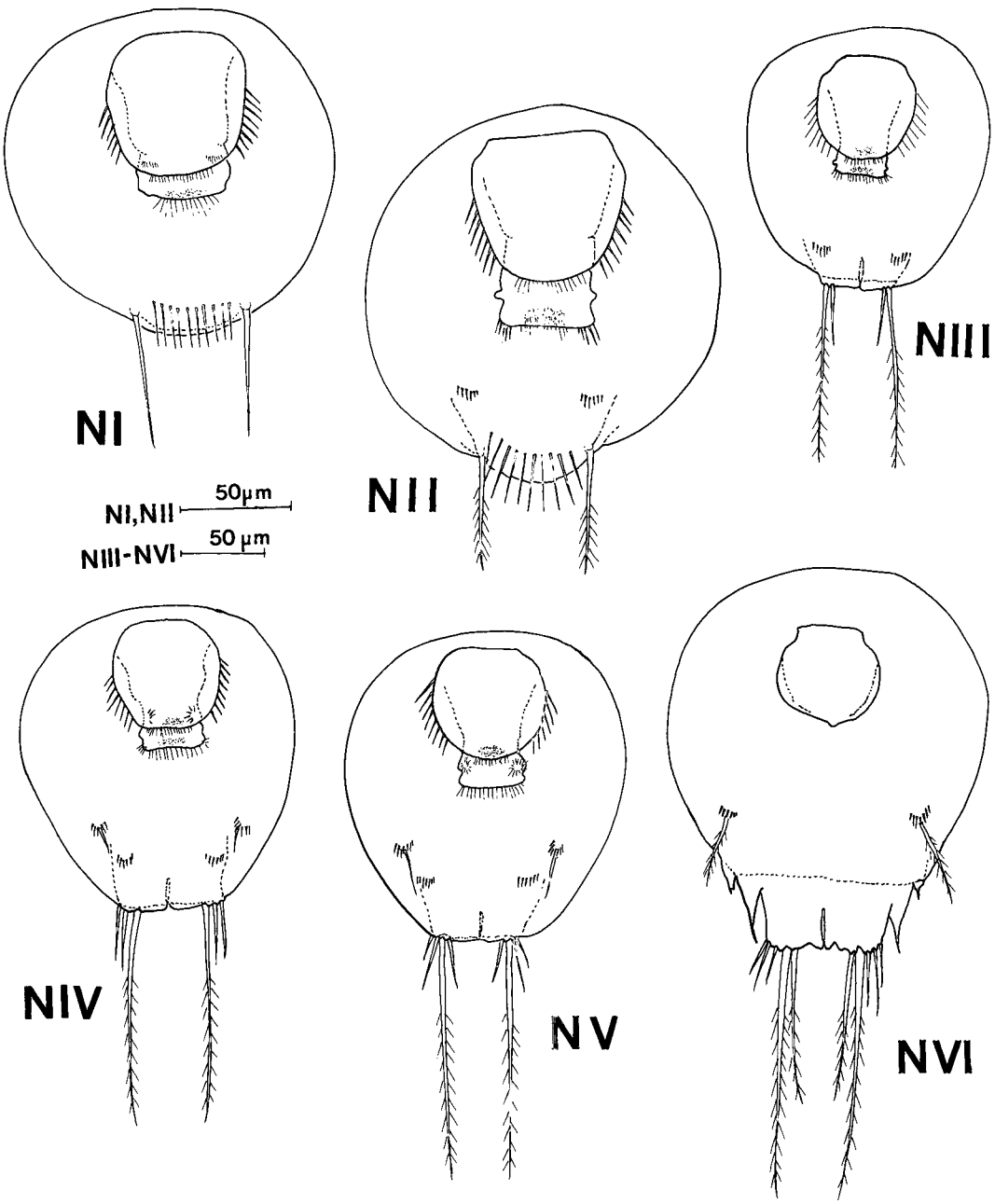

Fig. 1. *Harpacticus nipponicus.* Naupliar stages I to VI in ventral view.

Mandible (Fig. 4A). Basis with 1 spinulose spine and a row of spinules on inner side. Endopodite almost fused with basis, with 1 strong claw-like spine and 3 geniculate setae. Exopodite 1-segmented, with 1 simple seta proximally, 1 spinulose seta subtermially and 1 spinulose and simple setae on tip.

Caudal armature (Fig. 1A) with a pair of simple setae. Ventrocadudal crest of spinules present between caudal setae.

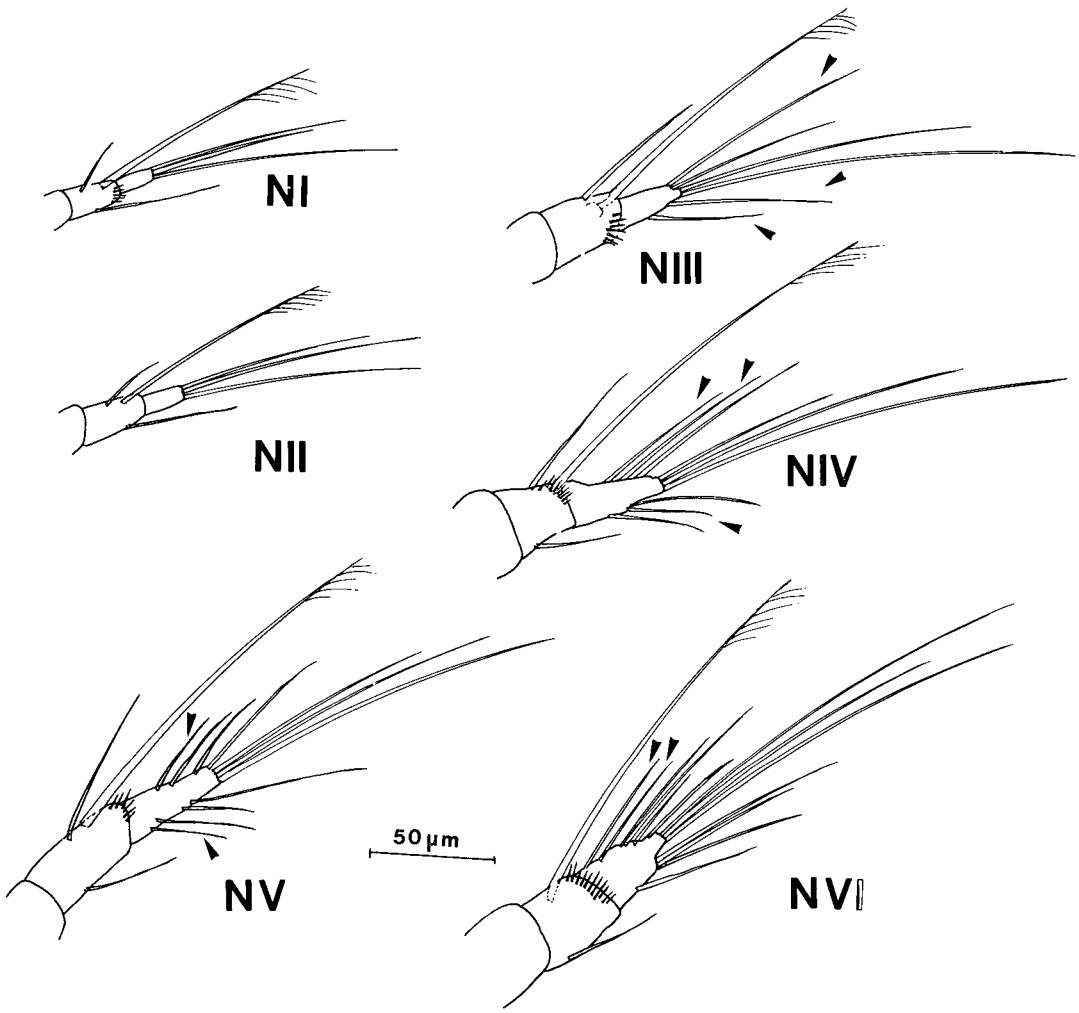

Fig. 2. *Harpacticus nipponicus.* Development of naupliar antennlues. Arrowheads indicate new structures as compared with the preceding stage

Nauplius II (Figs. 1B, 2B, 3B, 4B)

Body (Fig 1B) 96 μm (92-98 μm) long, 93 μm (90-96 μm) wide. Nauplius II differs from nauplius I as follows.

Antennule (Fig. 2B). Third segment with a row of spinules.

Antenna (Fig. 3B). Basis with 1 more small seta. Endopodite with 1 proximal seta on terminal claw.

Mandible (Fig. 4B). Exopodite with 1 more seta on outer distal edge.

Caudal armature (Fig. 1B). With pair of spinulose setae.

Nauplius III (Figs. 1C, 2C, 3C, 4C, 5C)

Body 108 μm (96-117 μm) long, 104 μm (98-111 μm) wide. Nauplius III differs from nauplius II as follows.

Antennule (Fig. 2C). Third segment with 3 more setae.

Antenna (Fig. 3C). Coxa with 1 innermost long seta toothed at tip and simple long seta. Basis with 1 more long seta. First segment of exopodite with 3 more setae.

Mandible (Fig. 4C). Endopodite with 4 geniculated setae with 3 rows of spinules on inner side. Exopodite with 1 more seta on inner margin and a row of spinules near the exopodite base.

Caudal armature (Fig. 5C). With a simple seta and a long spinulose seta on each side. A row of spinules disappearing between caudal setae.

Naplius IV (Figs. 1D, 2D, 3D, 4D, 5A, 5D)

Body (Fig. 1D) 128 μm (119-132 μm) long, 104 μm (100-107 μm) wide. Nauplius IV differs from nauplius III as follows.

Antennule (Fig. 2D). Third segment with 3 more setae.

Antenna (Fig. 3D). Second endopodite segment adding 1 tiny seta at base of claw.

Mandible (Fig. 4D). Basis with 2 spines. Endopodite with 1 more geniculate seta.

Maxillue (Fig. 5D). With a pair of simple seta and a row of spinules at base.

Maxilla (Fig. 5D). With some spinules.

Caudal armature (Fig. 5F). With 1 long and 2 shorter setae on each side.

Nauplius V (Figs. 1E, 2E, 3E, 4E, 5E)

Body (Fig. 1E) 146 μm (141-159 μm) long, 112 μm (108-114 μm) wide. Nauplius V differs from nauplius IV as follows.

Antennule (Fig. 2E). Third segment with 2 more setae.

Antenna (Fig. 3E). Endopodite with 1 geniculate seta and 2 small setae medially.

Maxillule (Fig. 5E). With 1 seta longer than in preceding stage.

Caudal armature (Fig. 5E). With 1 long spinulose seta, 2 medium-sized and 1 small seta on each side.

Nauplius VI (Figs. 1F, 2F, 3F, 4F, 5F)

Body (Fig. 1F) 161 μm (148-174 μm) long, 146 μm (137-153 μm) wide.

Nauplius VI differs from nauplius V as follows.

Antennule (Fig. 2F). Third segment with 2 more setae.

Antenna (Fig. 3F). Basis with 3 setae in place of masticatory process. Endopodite with 1 geniculate seta with a row of spinules medially and 1 tiny seta adding in 3 middle of endopodite spine.

Labrum (Fig. 5F). Spinules disappearing laterally and distrally.

Maxillule (Fig. 5F).

Maxilla (Fig. 5F). Setules disappear.

P1 (Fig. 5F). With 3 distal spines on lobe.

P2 (Fig. 5F). With 2 short setae on lobe.

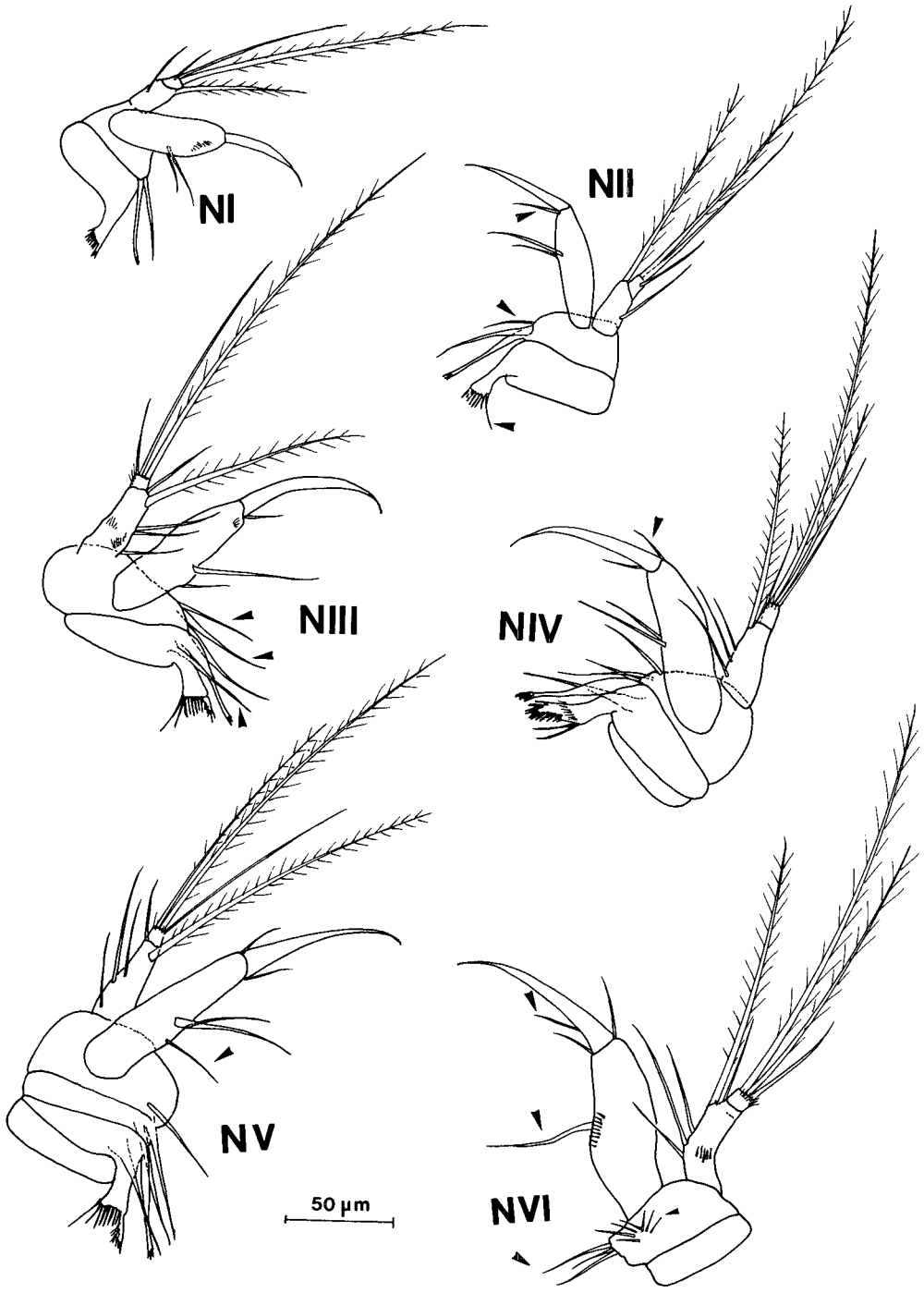

Fig. 3. *Harpacticus nipponicus.* Development of naupliar antennae. Arrowheads indicate new structures as compared with the preceding stage.

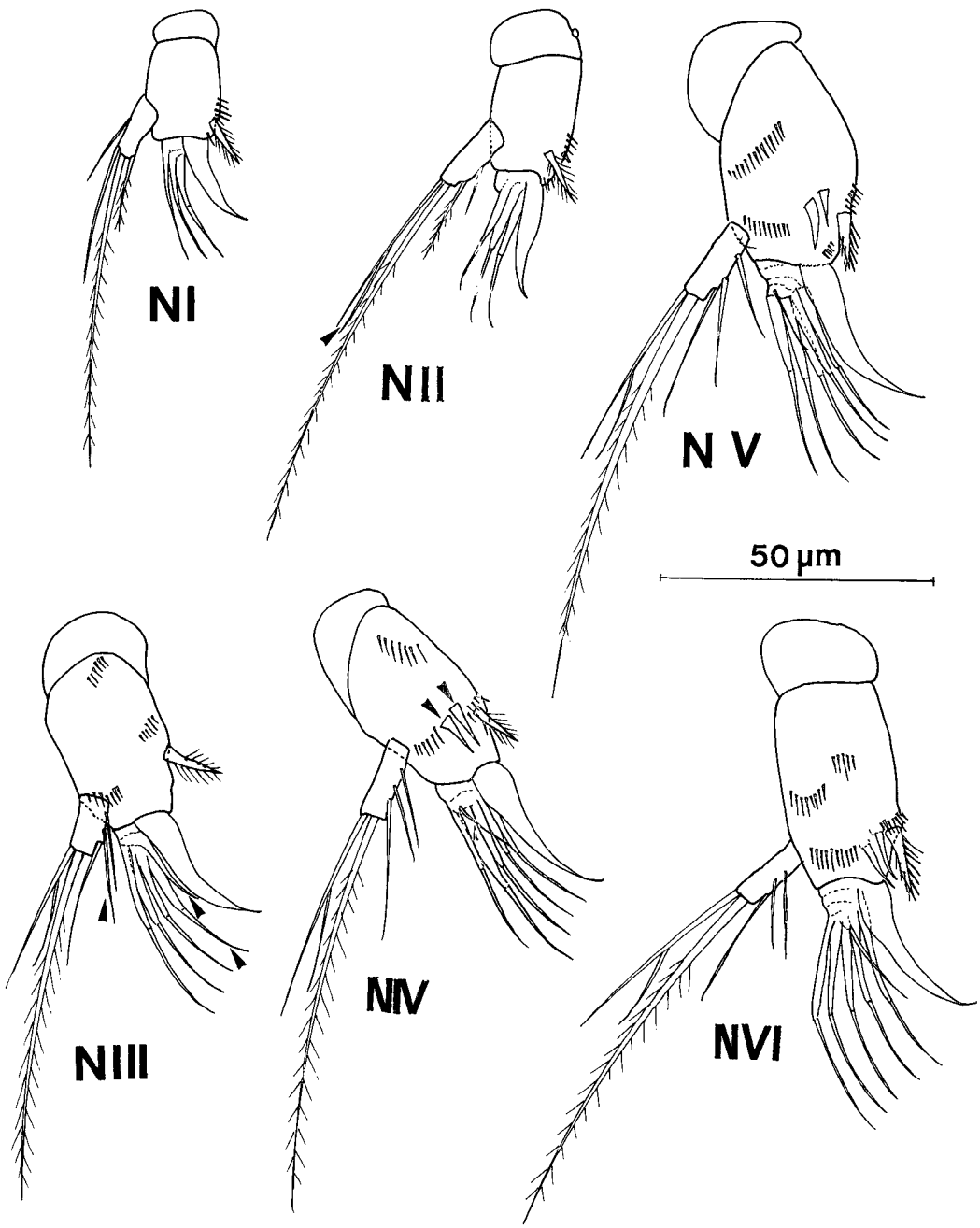

Fig. 4. *Harpacticus nipponicus.* Development of naupliar mandibles. Arrowheads indicate new structures as compared with the preceding stage.

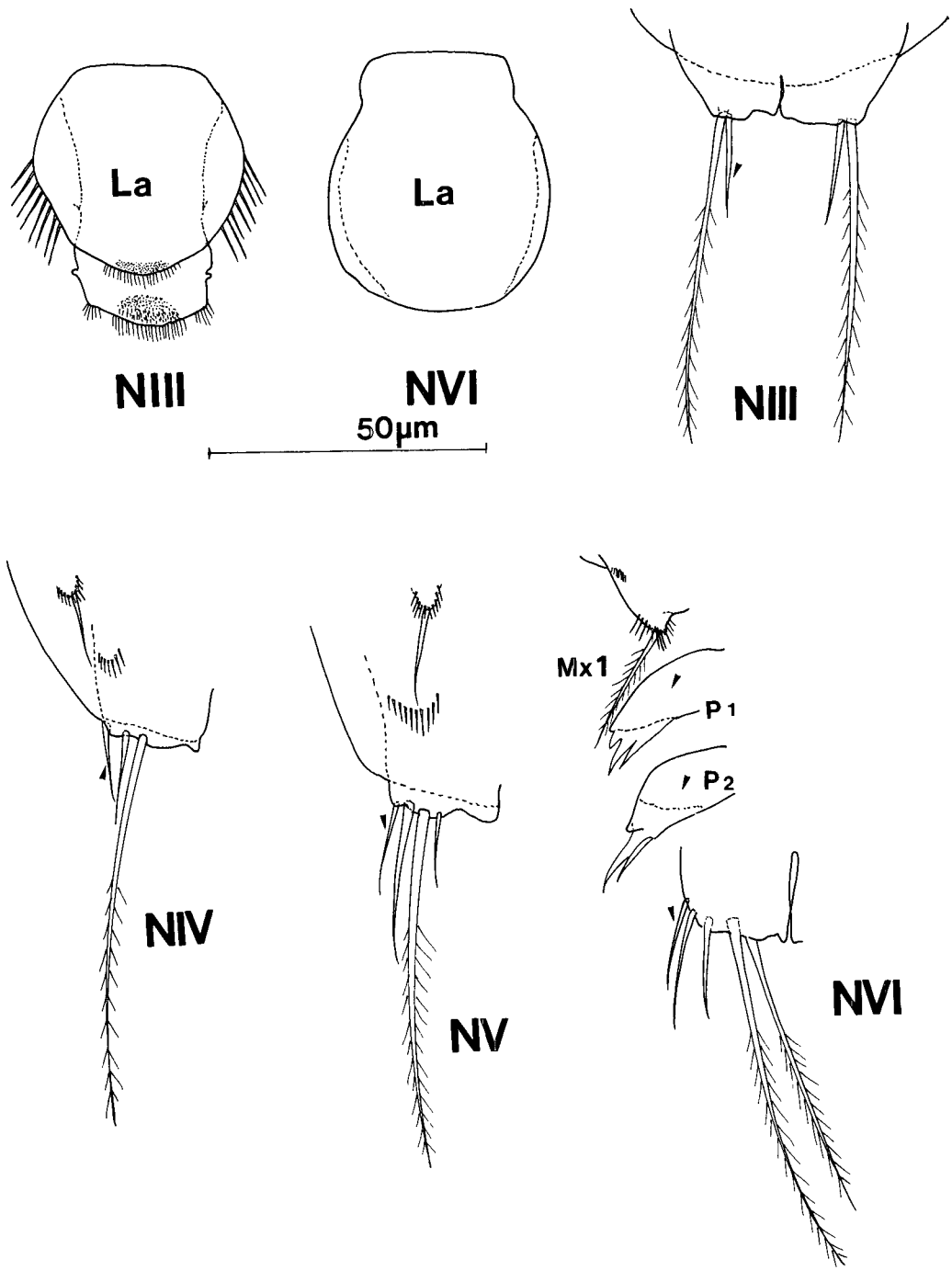

Fig. 5. *Harpacticus nipponicus.* Development of labrum, postmandiblar appendages and hindbody duri naupliar phase. Arrowheads indicate new structures as compared with the preceding stage (La = labrum, M × 1 maxillule, P1 – P2 = anlargen of thoracic locomotor appendages 1 and 2).

Caudal armature (Fig. 5F). With 2 long spinulose setae and 3 small setae on each side.

Key to the nauplius stages of *Harpacticus nipponicus*

1. Antennal basis with 2 setae ····· NI
— Antennal basis with 3 setae ····· 2
2. Caudal with 2 setae , mandibular endopodite with 3 geniculate setae ····· NII
— Caudal with 4-10 setae , mandibular endopodite with 5 geniculate setae ····· 3
3. Caudal with 4 setae, maxillule absent ····· NIII
— Caudal with 6-10 setae, maxillule present ····· 4

Table 1. Naupliar development of *Harpacticus nipponicus*

		NI	NII	NIII	NIV	NV	NVI
A1	seg.	3	3	3	3	3	3
	set.	0-3-3	0-3-3	0-3-6	0-3-9	0-3-11	0-3-13
A2	coxa	mast. proc.	mast. proc.	mast. proc.	mast. proc.	mast. proc	mast. proc
	basis	2s.	3s.	5s.	5s.	5s.	3s.
	enp						
	med.	2s.	2s.	2s.	2s.	3s.	1s.
	term.	1 claw	1 claw + 1s.	1 claw + 1s.	1 claw + 2s.	1 claw + 2s.	1 claw + 3s.
	exp						
	seg.	2	2	2	2	2	2
	set.	2-3	2-3	5-3	5-3	5-3	5-3
Md	basis	mast. sp.	mast. sp.	mast. sp.	mast. sp.	mast. sp.	mast. sp.
	end						
	proc.	1sp.	1sp.	1sp.	1sp.	1sp.	1sp.
	outer	3 genic.	3 genic.	5 genic. + 2h	5 genic. + 2h	5 genic. + 2h	
	field						
	exp.						
	seg.	1	1	1	1	1	1
	set.	5	5	6	6	6	6
Mx	1				1s.	1s.	1 spls.
Mx	2				setules	setules	
P	1						3 dentation
P	2						1 prot. + 2s
Labrum		hairs around margin until stage V					hairs lacking
Hindbody		2s.	2s.	4s.(2 spls.)	6s.(2 spls.)	8s.(2 spls.)	10s.(4 spls.)
Bodyform		circular	elongation	increase of			
		outline	begins	elongation			

A1. antennule; A2, antenna; Md, mandible; Mx1, maxillule; Mx2, maxilla; P1 and 2, anlargen of locomotor appendages 1 and 2; Seg., segment; Set., setation; Enp, endopodite; Exp., exopodite; Sp, spine; S, Seta; H, hair; Spls, spinules; Med, medium.

4. Caudal with 6 setae, maxillule with 2 setae ..... NIV
— Maxillule with 1 seta, maxilla with spinules 5
5. Caudal with 8 setae, P_1 and P_2 absent ..... NV
— Caudal with 10 setae, P_1 with 3 spines, P_2 with 2 short setae ..... NVI

DISCUSSION

The known *Harpacticus* nauplii are so similar in morphology that it is very difficult to distinguish

Table 2. Comparision of nauplii 1 characters in four species of *Harpacticus*.

		H. nipponius (this study)	*H. uniremis* (damhs, 1990)	*H.* sp. (Walker, 1981)	*H. littoralis* (Castel, 1976)
A1	seg.	3	3	3	3
	set.	0-3-3	1-3-3	1-2-3	0-3-3
A2	coxa	mast. proc.	mast. proc.	mast. proc.	mast. proc.
	basis	2s.	4s.	3s.	3s.
	enp				
	med.	2s.	2s.	2s.(1spls).	2s.
	term.	1 claw	1 claw	1 claw	1 claw. + 1s.
	exp				
	seg.	2	3		3
	set.	2-3	3-1-3	4 spls.	2-0-2
Md	coxa			mast. sp.	
	basis	mast. sp.	1s. + setules		1 spls.
	enp				
	proc.	1 sp.	1 sp. + setules	1 sp.	1 sp.
	outer	3 genic.	4s.	3 genic. +	3s.
	field.			1 small s.	
	exp.				
	seg.	1	1		1
	set.	5s.	4s.	4s.	3s.
Mx	1				
Mx	2				
P	1				
P	2				
Labrum					
Hindbody		2s.	2s.	2s.	2s.

A1, antennule; A2, antenna; Md, mandible; Mx1, maxillule; Mx2, maxilla; P1 and 2, anlargen of locomotor appendages 1 and 2; Seg., segment; Set., setation; Enp, endopodite; Exp, exopodite; Sp, spine; S, Seta; H, hair; Spls, spinules; Med, medium.

them. The nauplii of *H. nipponicus* (Table 1) exhist extremely close morpological similarity with those of *H.* sp. described by Walker (1981). Except for in the second and third nauplius stages, the antennal basis is 2 setae in *H. nipponicus* whereas it is 3 setae in those of *H.* sp. Mandibular endopodite with 1 more geniculate seta in *H. nipponicus*. The maxillule clearly appears at nauplius IV stage as a pair of simple seta whereas it appears as small buds with hairs in nauplius II stage of *H.* sp.

The first nauplius stage descriptions of *Harpacticus* are available for *H. nipponicus* (present study), *H.* sp. (Walker, 1981), *H. uniremis* (Damhs, 1990), and *H. littoralis* (Castet, 1976) (Table 2). The antennal basis is 2 setae in *H. nipponicus* whereas it is 3 setae at least in those of *H.* sp., *H. uniremis*, and *H. littoralis*. The antennal exopodite with 2-segmented and 2, 3 setation in *H. nipponicus* whereas 3-segmented and 3, 1, 3 setation in the *H. uniremis* and 3 segmented 2, 0, 2 in *H. littoralis*. Mandibular exopodite with 5 setae in *H. nipponicus* and 4 setae in the *H.* sp. and *H. uniremis* whereas 3 setae in the *H. littoralis*.

It is important to discriminate the nauplii of *H. nipponicus* from those of *H. uniremis* species because they occur together in east sea of Korea. Although the larvae of these species, whose adults are also similar each other in most respect, descrimination between them may not be difficult if various minor features mentioned above are examined in detail.

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요각류, *Harpacticus nipponicus* Ito의 노우플리우스 유생 발생

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요 약

Harpacticus nipponicus Ito의 유생을 수온 20°C, 염분 농도 33.3‰, 광주기 14h light: 10h dark의 조건하에서 사육하여 각 유생 단계를 얻은 다음, 전체 유생기의 형태적인 특징을 상세히 기술 및 도시하였다. 이 종은 6개의 nauplius 유생기를 가지며, 부화후 6-8일 후에 copepodid 1기가 되었다. 이 종의 각 유생기는 제1작은턱다리, 가슴다리의 발달 시기, 그리고 caudal setae의 수 등의 특징에 따라 구별되었다. 또한, 이 종의 유생은 *H. uniremis* Kr yer와 *H.* sp. Walker의 유생과 외부적 형태가 비슷하나, 크기와 부속지의 자모식으로 쉽게 구별되었다. *Harpacticus nipponicus*의 유생을 속 내의 연구된 다른 종의 유생들과 비교, 토의하였다.