

Naupliar Development of *Tigriopus japonicus* Mori, 1932 (Copepoda: Harpacticidae)

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Hans U. Dahms, Supawadee Chullasorn, Pawana Kangtia, Frank D. Ferrari, and Jiang-Shiou Hwang (2007) Naupliar development of *Tigriopus japonicus* Mori, 1932 (Copepoda: Harpacticidae). *Zoological Studies* 46(6): 746-759. Six naupliar stages of *Tigriopus japonicus* Mori, 1932 are described from the rocky coast off Keelung, northeastern Taiwan. A key for the identification of stages is provided. Naupliar morphology within the Harpacticidae differs among species and even more at the supraspecific level. The present study on nauplii of *Tigriopus japonicus* confirms the observation that representatives of the Tachidiidae and Harpacticidae (Copepoda-Harpacticoida) show a peculiar reduction of the oral structures from N V to N VI. The nauplii of the Harpacticidae are creeping larvae, and many are not able to swim; however, nauplii of *T. japonicus* are an exception to this rule. <http://zoolstud.sinica.edu.tw/Journals/46.6/746.pdf>

Key words: Naupliar development, Larval stages, Identification key, Evolution.

Like several other groups of Crustacea, harpacticoid copepods develop nauplius larvae as their early postembryonic stages (Bjørnberg 1986, Dahms 2000). Harpacticoids as do most free-living Copepoda, go through 6 naupliar and 6 copepodid stages (Ferrari and Dahms 2007). The naupliar phenotype is quite unlike the adults, and it is difficult to tell which nauplius belongs to which species, unless the development of isolated females is observed in the laboratory. Compared to other copepod taxa, naupliar development of harpacticoids has been far less studied (Dahms 1990, 2004a b). There are no fewer than 16 harpacticoid families for which nothing is known about the developmental instars. Naupliar instars are exposed to different selection pressures, and nauplii, therefore, have undergone remarkable adaptive radiation, leading to a diversity of structures, behavioral characteristics, and distribution patterns (Borutzky 1952). Due to their great abun-

dances and variety, nauplii may also play important ecological roles (Alekseev 2002, Dahms and Qian 2004). Life-history studies in the field and investigations of stage-specific phenomena in the laboratory are also hampered by a lack of descriptive information and missing keys for identification that are the basis for all work on stage-specific phenomena in the laboratory and heterogeneous assemblages in the field (Dahms 1993, Dahms et al. 2006). Much rearing and descriptive work has to precede any serious attempt to tackle ontogeny-related problems. This is particularly true for *Tigriopus* since this taxon has become the focal point of several in-depth studies, e.g., of its field ecology (Dethier 1980), life history (Koga 1970), internal anatomy (Itô 1973), chemical ecology (Kelly and Snell 1998), genetics of adaptation (Davenport et al. 1997, Burton et al. 1999), population genetics (Edmands and Burton 1998, Edmands 1999), and genomics in general

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(Machida et al. 2002, Jung et al. 2006).

Detailed and exact descriptions of postembryonic instars are also helpful for the elucidation of phylogenetic relationships in providing ontogenetic apomorphies for monophyletic groups that set the baseline for the reconstruction of phylogenies (Dahms et al. 2005). There are several reasons why naupliar characters have thus far been widely neglected in systematic and phylogenetic studies, e.g., difficulties in obtaining detailed information on naupliar characters (because nauplii provide fewer characters than later ontogenetic instars and adults), a lack of appropriate comparative data, and conflicting evidence when comparing adult and naupliar character states. In any case, it is more important to find new characters than merely to reinterpret those already known. Phylogenetically valuable apomorphies may be cryptic characters that are often camouflaged by superficial resemblances. Here, detailed resolution is a prerequisite for meaningful comparisons. Detailed studies, however, are more difficult the more-complex or smaller structures become. This is particularly true for minute nauplii, many of which do not exceed 50 μm in diameter at hatching.

Although there are 2 other studies dealing with the postembryonic development of *T. japonicus* (Itô 1970, Koga 1970) from Japanese populations, an updated study is warranted, since previous attempts are either incomplete or lack detail. The present study provides a thorough description of nauplii belonging to the harpacticoid species *Tigriopus japonicus* Mori, 1932. This will enable researchers to identify naupliar stages to the species level, which is important for various life history studies.

MATERIALS AND METHODS

Collection data

Adults of *T. japonicus* were collected by Mr. Kao Tzu from the coast of Badouzi Harbor, approximately 8 km east of Keelung, on the north-eastern coast of the main island of Taiwan in Oct. 2005. Ovigerous females which provided the developmental stages were collected in a beaker from a supralittoral splash-pool, that was subsequently decanted over a 50 μm -mesh screen. The residue containing only metazoans at all developmental stages of *T. japonicus* was rinsed into smaller bowls for transport to the laboratory. The developmental stages used in this study represent

the offspring of exclusively single-female cultures, unless indicated otherwise.

Cultivation was carried out in polystyrene Petri dishes. These were kept at 25°C in an incubator with a 12:12 h light: dark cycle. About 50% of the seawater was renewed each week, and it was obtained and prepared as described by Dahms (1990). Single ovigerous females were added to the Petri dishes. When nauplii emerged, some of them were isolated in watch glasses, and the exuviae of subsequent molts were collected. Various kinds of food were used: such as *Nitzschia* sp., Tetra-Min, or a mixture of these (see also Dahms et al. 2007). A few drops of a food suspension were added every few days which settled as a fine, even, semi-transparent coat over the entire bottom of the glass.

No attempt was made to exclude small protozoan contaminants or to prevent algal growth. No substratum had to be added in the case of the rock-surface dwelling *T. japonicus*, whereas harpacticoids from soft bottoms often require sediment substrates (Dahms and Qian 2005 2006).

Preparation

Stages were fixed in 5% buffered formaldehyde and embedded in glycerol. This clarifies nonexuvial material within a few weeks and provides information on hidden posterior structures when observed with phase-contrast microscopy. Unfortunately, the natural color of nauplii and the color and shape of the red nauplius eye were rapidly lost, and the eye was, thus, not figured. Nauplii were mounted whole, and broken glass-fibers were added to prevent them from being compressed and to facilitate rolling to allow inspection from all sides. Abnormalities were occasionally observed but were not illustrated. Body measurements are given from the frontal portion of the naupliar shield to the caudalmost protrusion of the hindbody (length), and the widest lateral tips of the naupliar shield (width); only specimens drawn were considered for length measurements. Otherwise, 2-5 specimens per stage were used for the investigation of stage-specific variability. Species identification was carried out with the aid of Lang (1948) if not stated otherwise.

Descriptive terminology

The following terms are defined according to their usage in the following text. The 1st to 6th naupliar stages are respectively abbreviated as N I

to VI. Nauplii of *T. japonicus*, as those of other harpacticoids, have at least 3 pairs of appendages: 1st and 2nd antennae and mandibles. The body is covered by a smooth nauplius shield; the hindbody protrudes from it in later stages. At the posterior end of the body is at least 1 caudal seta on each side of the anal area. The labrum originates as a lobular flap near the frontal margin of the body, between the bases of the 1st antennae, and extends posteriorly across the ventral surface of the body. The metasomal ventral body wall is a tongue-like structure arising at the base of the antennal protopod. The 1st antenna is uniramous. The 2nd antenna notably differs from that of the adult (and copepodids) in having a coxal masticatory process (= gnathobase). The 2nd antenna further consists of a coxa, basis, endopod, and exopod. The mandible is composed of the same elements except the precoxa. The endopod consists of an inner process and usually a lateral field of setae arising on the outer lateral margin. The postmandibular appendages (1st and 2nd maxilla, maxilliped, leg 1, and leg 2) may develop successively from N II onwards. The singular form is used for all appendages. Large outgrowths are called setae or spines. A typical seta is generally a flexible, finely attenuated element which is bare or has a double row of fine hair-like outgrowths. In the latter case it is called pinnate, or, if the fine spinules are more irregular, it is called spinulose. A typical spine is generally short, relatively inflexible, and usually bears a double row of tiny spinules. Very small, flexible elements originating from the endocuticle are referred to as setules. Aesthetascs (aesthetes sensu Gurney 1931) are presumed to be sensory elements of the 1st antennae with sclerotized bases and are more transparent than normal setae with blunt or rounded ends. The complement of setae, setules, spines, and aesthetascs are called elements of a particular structure and are referred to as the armature. In addition to setae, setules, and spines, the body segments or appendages present a variety of ornamenting cuticular projections. Spinules can be very fine hair-like cuticular extensions of setae and spines, the labrum, and ventral body wall, or, small, pointed, conical processes. Denticles are minute triangular outgrowths. The spinules and denticles are referred to as ornamentation.

DESCRIPTION

Individuals of all 6 naupliar stages are strictly

benthic, and crawl and swim-crawl on surfaces. All developmental stages including nauplii can also swim freely in the water column. Nauplii are about as wide as long and reddish and have a dark median eye. The cephalic shield covers only parts of the hindbody (Figs. 1, 2).

Nauplius I (Figs. 1, 3, 4, 6).

Body length 133 μm , body width 118 μm . Body slightly longer than wide and cephalic shield showing lobular bulges on both lateral sides. Hindbody bearing 1 seta (= the initial furca), arising from a protuberance on each caudal side and a row of short spinules in between. Almost rectangular labrum furnished with hairy spinules along its lateral corner and distal edge. Sternal field ornamented with 1 caudally curved row of slender spinules in between insertions of 2nd antennae.

First antenna 3-segmented. First segment armed with 1 small seta. Second segment bearing 2 small setae midlength and 1 large seta at distal margin of segment. Distal segment armed with 4 setae, 1 long and large outermost seta, 1 seta becoming an aesthetasc at later stages, and 2 small setae apically (Figs. 1, 3).

Second antenna bearing a ring-shaped unornamented coxa. Basis large and medially drawn out into a bifurcated masticatory process, becoming pincer-shaped distally (Figs. 1, 4) and pointing under labrum towards mouth. Basipodal endite bearing 2 setae on anterior surface; and 2 short rows of slender spinules, 1 in middle and 1 at inner corner. Cylindrical endopod 1-segmented, about 2.5 times as long as exp-1, with 2 subequal median inner setae midlength, 1 short terminal seta, and 1 large claw-shaped seta distally. Extended exopod 4-segmented. First segment furnished with 1 short row of spinules near distal margin and 1 spinulose seta at inner corner. Shorter 2nd segment bearing 1 long spinulose seta and 3rd segment armed with 1 small seta, 1 longer spinulose subterminal seta, and 2 subequal terminal setae (Fig. 4).

Mandible made of a short proximal portion (= initial coxa) with 1 smooth seta (Figs. 1, 6). Surface of large basipod furnished with a transverse row of slender spinules medially and at inner protrusion. Basopodal endite furnished with 5 small spinules midlength on an elongate claw-shaped spine and 3 long unfurnished setae. Exopod 3-segmented; 1st segment unarmed, 2nd segment with 1 terminal smooth seta; 3rd segment bearing 1 smaller spinulose inner seta, 1 outer

large spinulose seta, and 1 long attenuated spinulose seta distally (Fig. 6).

Nauplius II (Figs. 1, 3, 4, 6)

Body length 156 μm , body width 132 μm . N II

differs from N I in the following aspects.

Row of short spinules in between both caudal sides at hindbody not present (Fig. 1). First antenna with 1 additional median seta and 4 small spinules at anterior inner margin on its distal segment, and 1 former seta becoming aesthetasc

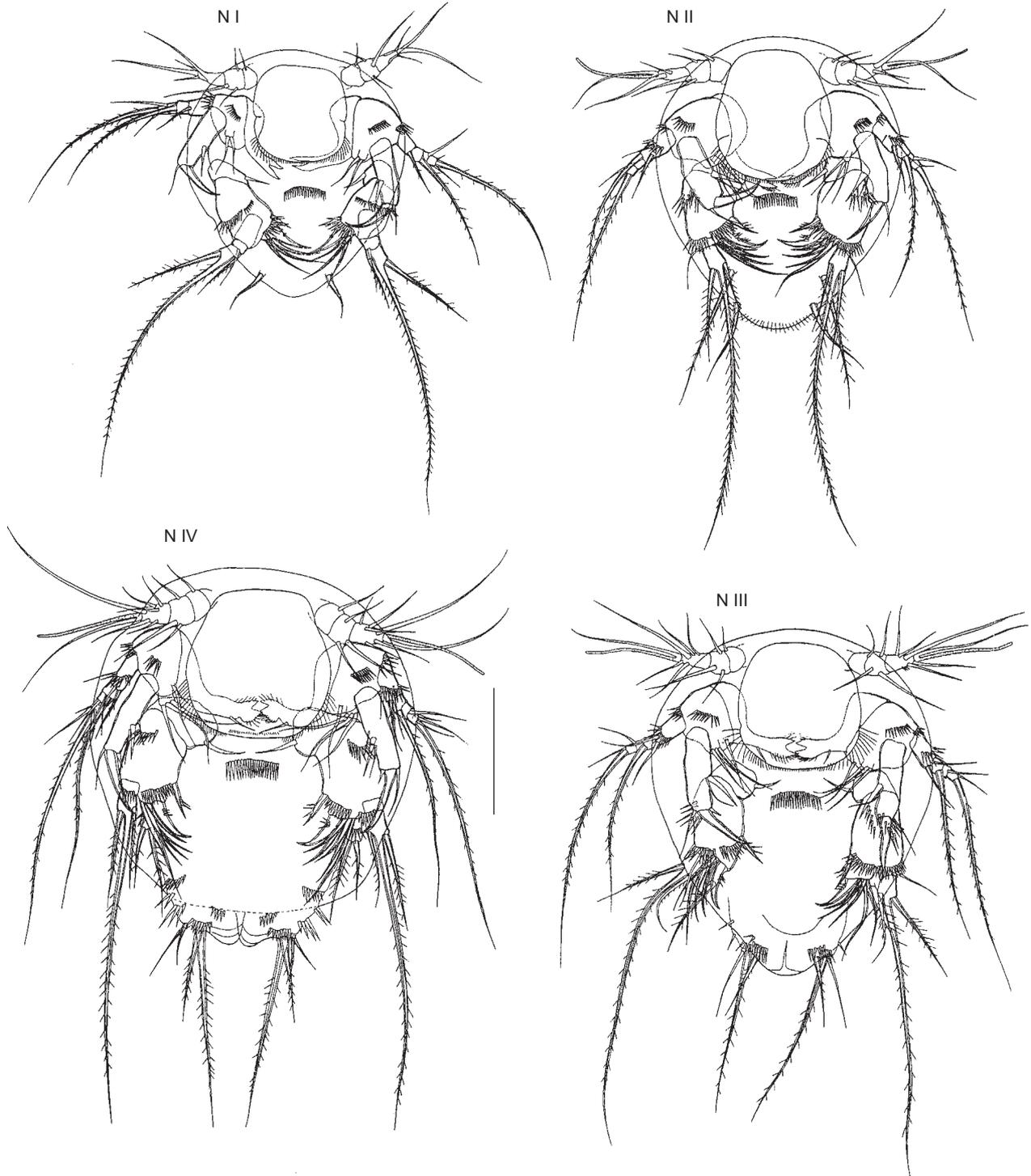


Fig. 1. *Tigriopus japonicus*. Naupliar stages I to IV in ventral view. Scale bar = 63 μm .

(Figs. 1, 3). Sternal field wall unornamented (Fig. 1). Bifurcated inner process of 2nd antenna armed with tiny spinules, and basipodal endite bearing 1 additional seta which is as long as entire gnathobase. Tubular endopod bearing 1 small spine at distal margin. Exopod very similar to that of N I (Figs. 1, 4). Mandibular basipod bearing a spinulose seta on its inner lateral corner and 1 additional seta on mandibular endopod. One additional short row of slender spinules at outer corner of basipod (Figs. 1, 6). First maxilla indicated as a spinulose seta.

Nauplius III (Figs. 1, 3, 4, 6)

Body length 188 μm , body width 153 μm . N III differs from N II in the following aspects.

First antenna with 2 additional lateral setae on anterior surface of its distal segment (Figs. 1, 3). Antennal gnathobase bearing 1 seta at midlength of masticatory process. Its basipod bearing 1 additional seta on anterior surface of gnathobase. Large claw-shaped seta of tubular endopod armed with very tiny spinules along inner side. Exopod very similar to that of N II (Figs. 1, 4). A protuberance appearing on anterior surface of mandibular basipod, becoming 1st maxilla in

later stages. Baseoendopod bearing 1 additional long seta (Figs. 1, 6). Hindbody with 2 additional inner subequal spinulose setae on either side (Fig. 1).

Nauplius IV (Figs. 1, 3, 4, 6)

Body length 205 μm , body width 175 μm . N IV differs from N III in the following aspects.

First antenna with 1 additional slender seta on anterior face of its distal segment (Figs. 1, 3). Cylindrical endopod of 2nd antenna bearing 1 additional slender spine at midlength close to former larger spine. Distal segment of exopod bearing 1 terminal slender seta (Figs. 1, 4). Spinulose seta of mandibular basipod bearing 2 additional small spinules on inner side and 2 small spinules on a large claw-shaped spine of basipod (Figs. 1, 6). Caudal sides furnished with groups of small spinules and folded in between. Hindbody with 1 additional outer smooth short seta on either side (Fig. 1).

Nauplius V (Figs. 2, 3, 5, 6)

Body length 249 μm , body width 204 μm . N V differs from N IV in the following aspects. First

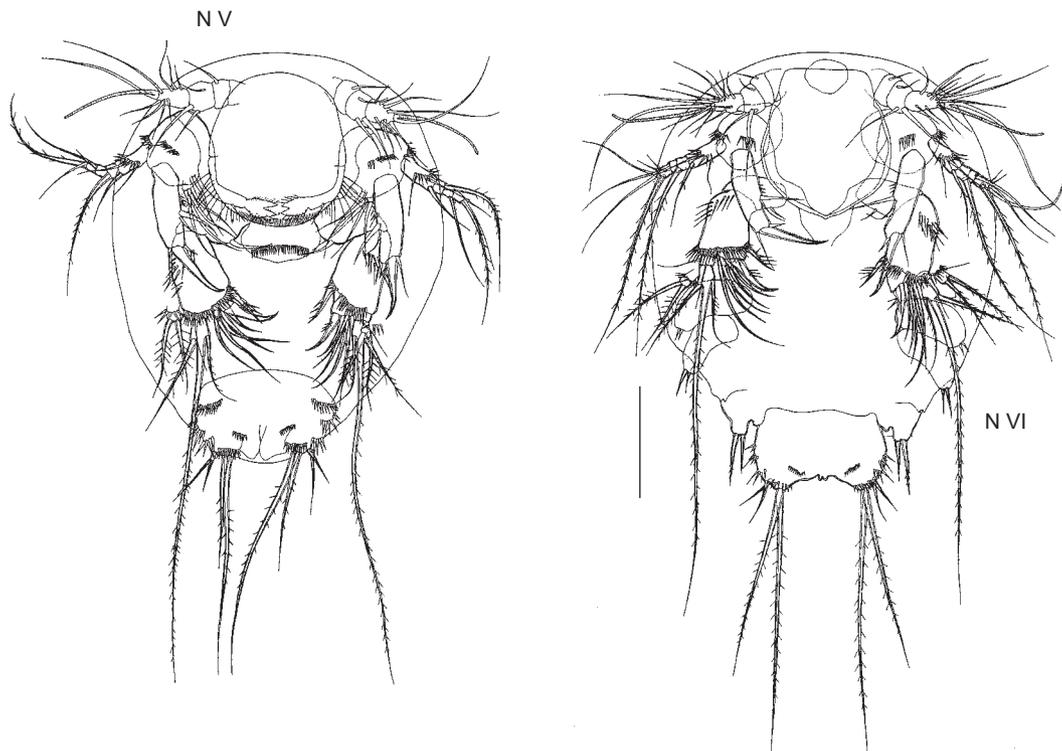


Fig. 2. *Tigriopus japonicus*. Naupliar stages V and VI in ventral view. Scale bar, 63 μm .

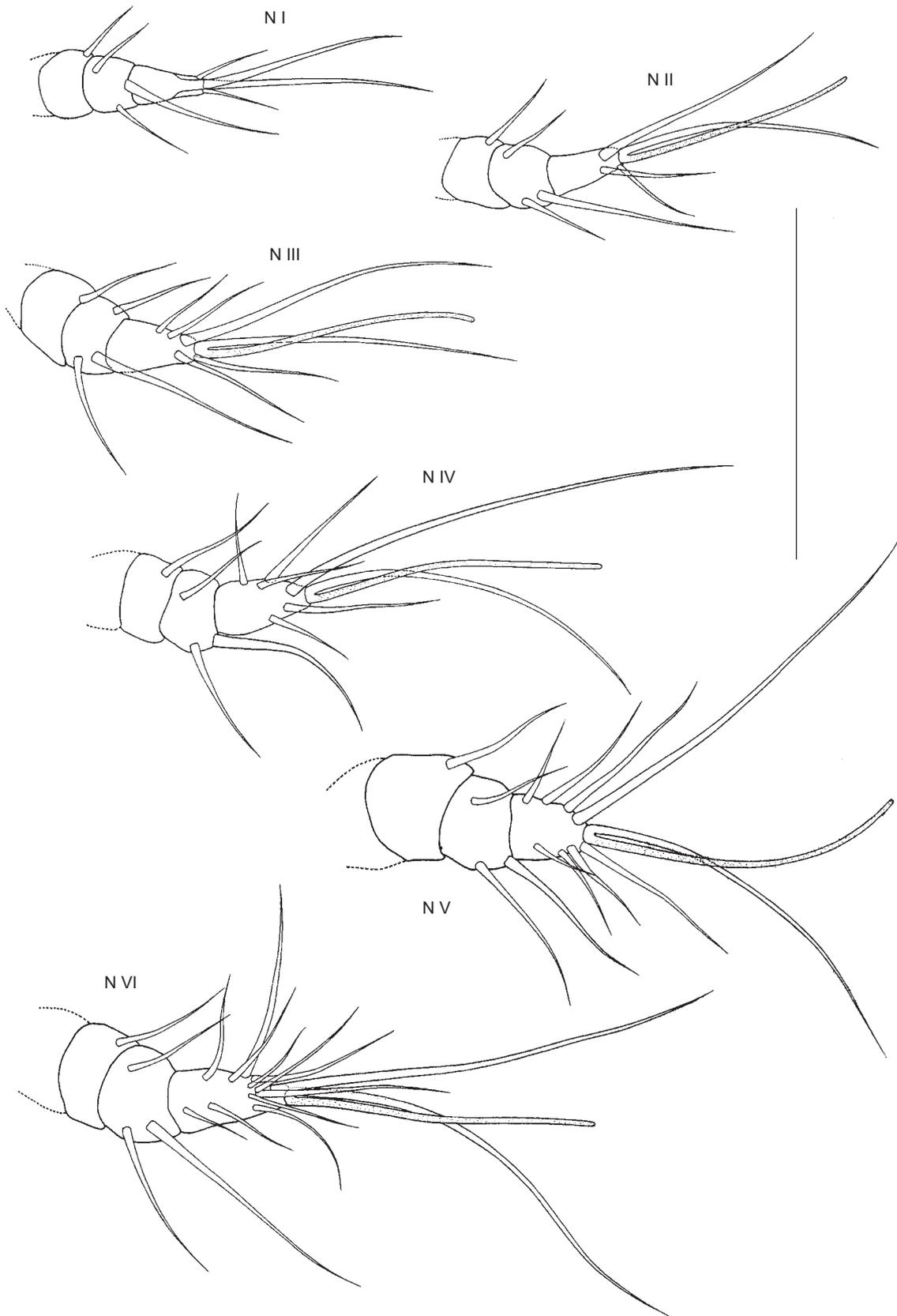


Fig. 3. *Tigriopus japonicus*. Development of the naupliar left 1st antenna of naupliar stages I to VI in anterior view. Scale bar = 63 μ m.

antenna with 2 additional small setae proximally on both sides of distal segment (Figs. 2, 3). Cylindrical endopod bearing 3 more small setae at midlength of its segment. Distal segment of exopod bearing 5 spinules at outer corner (Figs. 2, 5). Mandibular basipod with 4 spinules at inner corner (Figs. 2, 6). Hindbody showing 4 setae in total; 2 subequal spinulose setae and 2 subequal smooth

setae on each side, and bearing an abdominal bud. With 2 setae appearing next to mandibular exopod that becomes 1st maxilla of later stages (Fig. 2).

Nauplius VI (Figs. 2, 3, 5, 6)

Body length 267 μm , body width 212 μm .

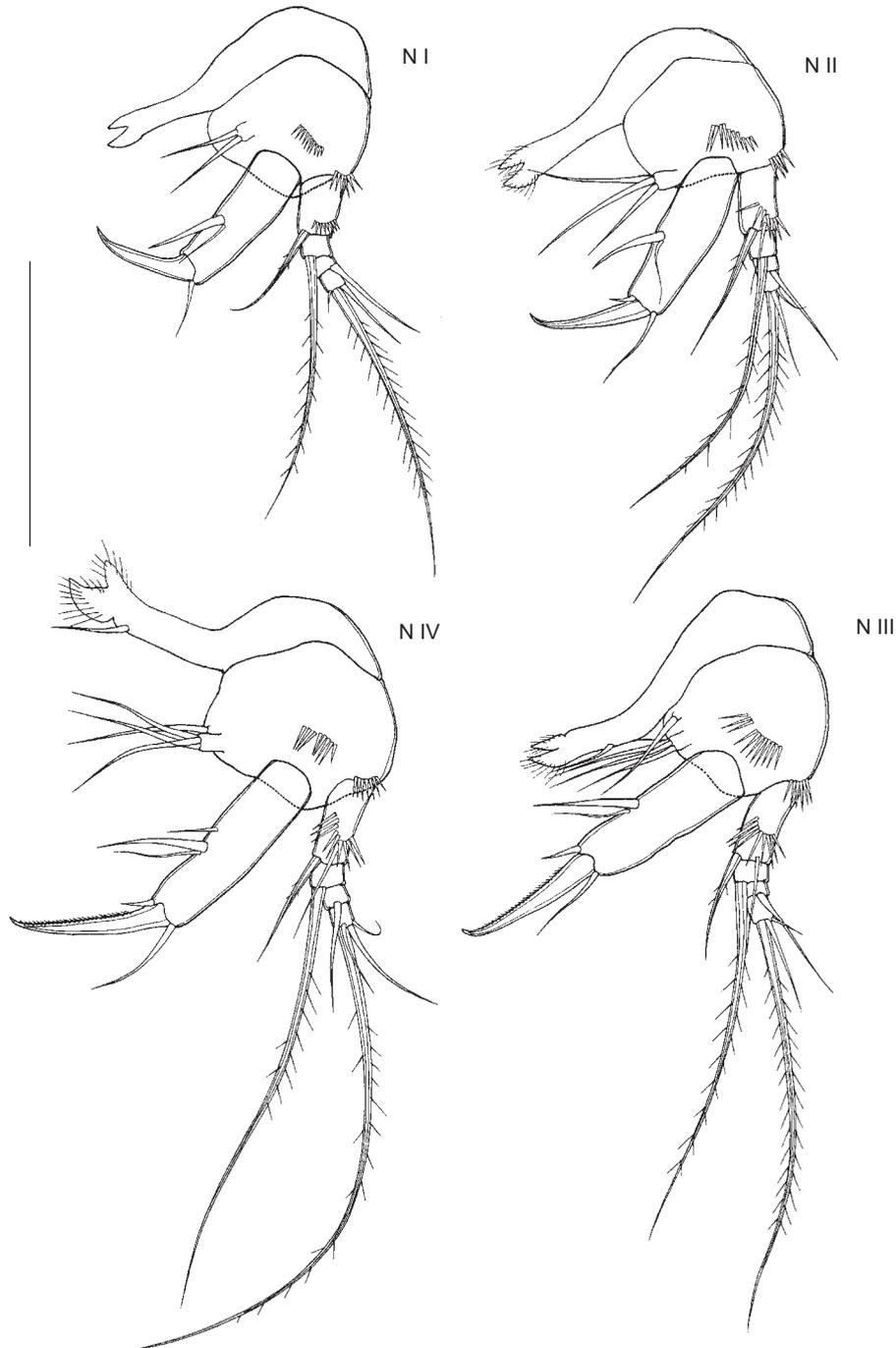


Fig. 4. *Tigriopus japonicus*. Development of the naupliar left 2nd antenna of naupliar stages I to IV in anterior view. Scale bar = 63 μm .

N VI differs from N V in the following aspects.

Distal segment of 1st antenna with 4 additional setae on anterior surface totaling 13 setae plus 1 aesthetasc (Figs. 4, 5). Second antenna with 2 additional small setae on distal segment of exopod (Fig. 6). Mandibular endopod expanded, now revealing its 2-segmented nature; basipodal projection enhanced; coxa still a lobe with 1 stylet-like seta (Fig. 6). Precursor of 1st maxilla developing a 3rd inner seta. Inner edge with enditic lobes; 1 seta on endopod, 1 seta on prospective exopod (Fig. 2). With 4 lobular precursors of postmandibular appendages developed on both lateral sides. Difficult to identify anterior 2 unarmed limb-buds. We assumed, however, that these represent 1st maxilla and maxilliped precursor. First 2 swimming legs indicated at 6th naupliar stage, as is also common in other copepods. We interpreted the lobe with 1 seta as corresponding to leg 1, and further caudally, the lobe with 2 setae as corresponding to leg 2 (Fig. 2), while 2nd maxilla not externally expressed.

Key for naupliar stages of *Tigriopus japonicus*

1. - Hindbody with 1 seta on each side 2
 - Hindbody with 3 setae on each side N III
 - Hindbody with 4 setae on each side; bud of 1st maxilla with 2 setae N IV
 - Hindbody with at least 4 setae on either side; 1st maxilla bud bearing 2 or more setae 3
2. - No indication of 1st maxilla N I
 - First maxilla appearing with 1 seta N II
3. - Hindbody with 4 setae on either side; distal segment of 1st antenna with 10 setae N V
 - Hindbody with 4 setae on either side; buds of 4 post-mandibular limbs present; inner part of oral appendages reduced N VI

DISCUSSION

As with apparently all Harpacticoida and most free-living Copepoda, nauplii of *T. japonicus* conform to the commonality of 6 naupliar instars (Ferrari and Dahms 2007). As in other harpacticoids, the nauplii are dorsoventrally flattened. The

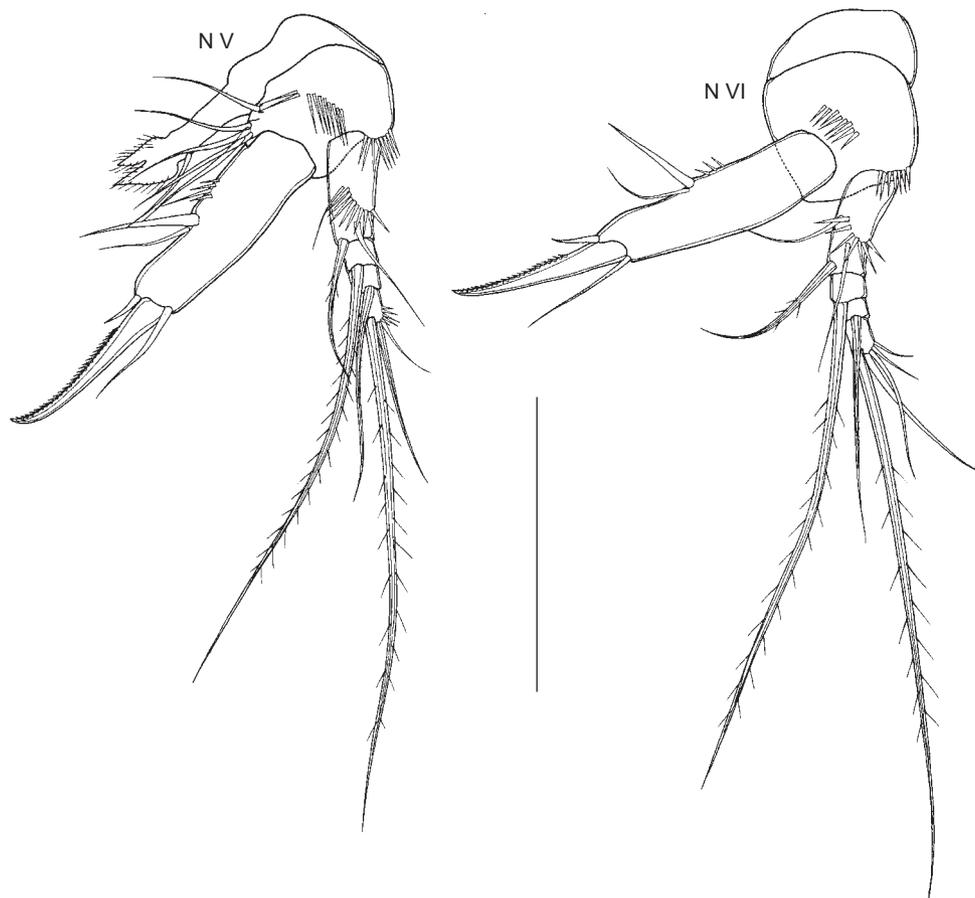


Fig. 5. *Tigriopus japonicus*. Development of the naupliar left 2nd antenna of naupliar stages V and VI in anterior view. Scale bar = 63 μm .

body shape is mostly circular in stage 1, then becomes elongated, and the hindbody emarginates at later stages. As in other copepods, the 1st stage nauplius (orthonauplius) of *T. japonicus* has 3 pairs of appendages: the uniramous 1st

antennae, the biramous 2nd antennae with trimerous protopods, and the biramous mandibles with bimerous protopods. As in other copepod nauplii, a medial red nauplius eye is situated dorsally between the bases of the 1st antennae. The hind-

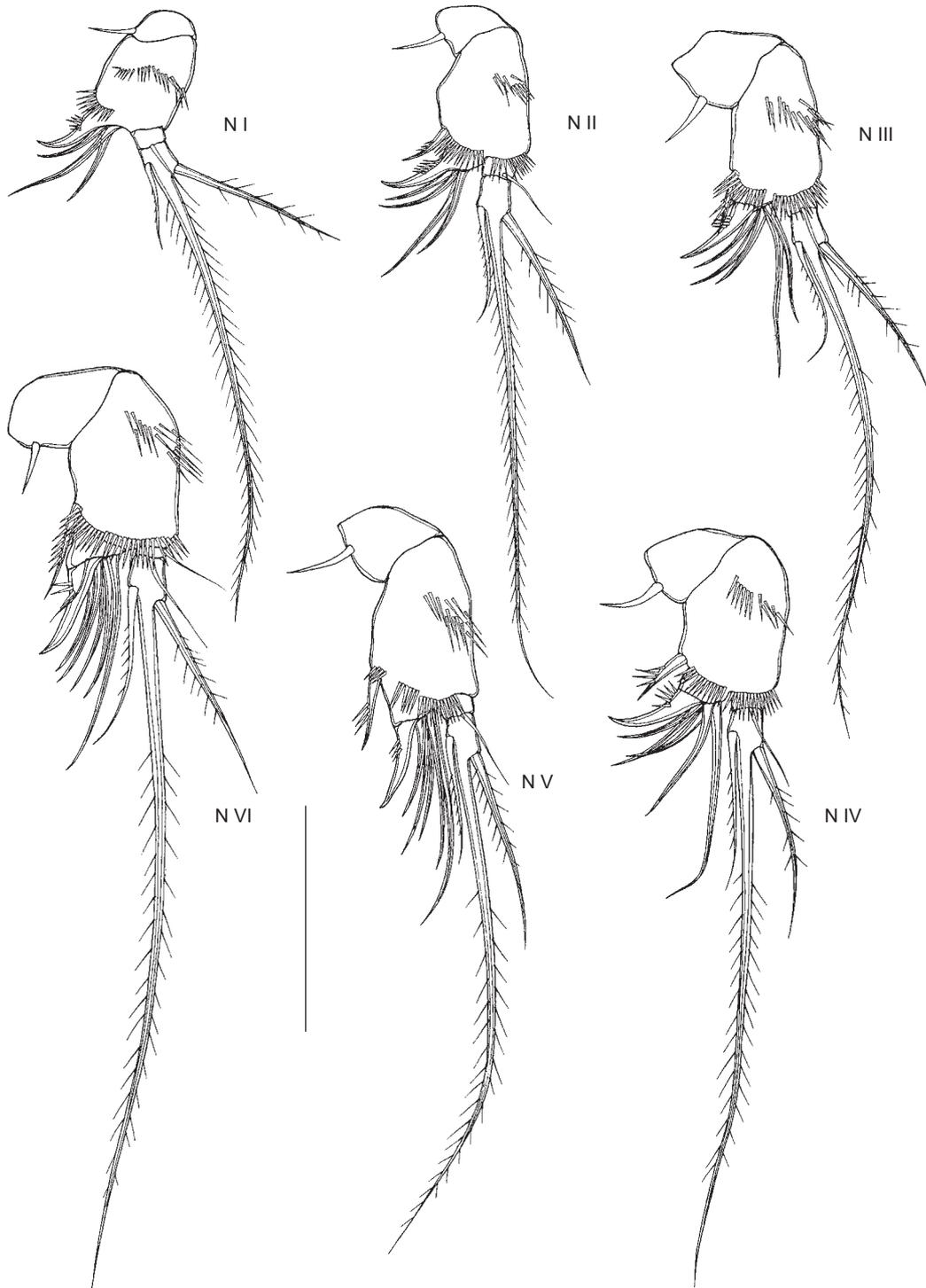


Fig. 6. *Tigriopus japonicus*. Development of the naupliar left mandible of naupliar stages I to VI in anterior view. Scale bar = 63 μ m.

body bears 1 seta on each side in N I and N II, whereas the seta number is remarkably variable in subsequent stages.

The 2nd nauplius stage (metanauplius) of *T. japonicus* develops the anlage of the 1st maxilla as a seta, usually arising from a protuberance. In the following stages, the number of anlagen appendages increases by 1 at each stage so that at N VI the anlage of leg 2 is present in most cases in *T. japonicus*. Other postmandibular appendages are hidden under the cuticle throughout the naupliar phase.

The 2nd antenna and mandible increase their armature and ornamentation, usually until the N V at the latest, when they reach their final naupliar appearance if no reduction occurs at the final molt. The 1st antenna and metanaupliar appendages, 1st and 2nd maxilla, maxilliped, leg 1, and leg 2 change their shape and increase their armature and ornamentation until the final naupliar stage.

As in most Harpacticoida, postmandibular appendages vary greatly in *T. japonicus* from the moment of their appearance as anlagen, in the total number of anlagen present at N VI, and in their shape and armature. At N VI, the anlagen of the 1st maxilla, 2nd maxilla, maxilliped, legs 1, and 2 may all be present, and they can then be identified according to their position from front to rear. However, if one or more of them is lacking as in the present description of *T. japonicus*, it is difficult to identify the remaining ones, for they are not easy to distinguish by their shape and armature. Even in nauplii with similar anlagen, their interpretation varies between authors. Labeling the exuvial rudiments as they appear in the developmental sequence as 1st and 2nd maxilla, etc., should not be considered a reliable method because suppression of the anlagen can occur within this series - as suppression of the 2nd maxilla in *T. japonicus* in the present study. Chitinous structures can usually be observed to be modified only through molts in the Arthropoda. Before each molt, the underlying tissue forms the anlagen of both modified and unmodified structures. It is not always easy to identify outer chitinous structures with their potential precursors formed under the cuticle, because the same tissue may form different chitinous structures. Sometimes anatomical changes under the cuticle (formation of segments and buds of appendages) remain hidden there, and are not expressed on the outside. Walker (1981) suggested that superficial subexuvial structures be correlated with the exuvial anlagen - if they are present - to verify their identity. This, however, may require

histological studies in most cases.

The armature and shape of the appendages on both sides of the body are symmetrical as in all non-calanoid copepod nauplii (Dahms et al. 2006). N VI shows all the characters acquired throughout that naupliar phase except ornamentation of the labrum, the ventral body wall, and those parts of the oral appendages which are involved in feeding. These are reduced in N V and N VI in *T. japonicus* as in all other harpacticid and some other harpacticoids (see below).

The nauplii of the Harpacticidae are creeping larvae and many are not able to swim; nauplii of *T. japonicus* are an exception to this rule.

Differences of *T. japonicus* nauplii described herein with previous descriptions

A morphological study, particularly from a very different island locality such as the island of Taiwan, of *T. japonicus* is warranted, despite there being 2 other studies dealing with the postembryonic development of *T. japonicus*. Previous work by Itô (1970) and Koga (1970) examined Japanese populations of this species. Whereas Koga's (1970) contribution lacks detail, particularly in the drawings, the study of Itô (1970) is useful, but it is missing naupliar stage 4. In particular, Koga (1970) documents the 1st maxilla from N III onwards, whereas it is expressed in Itô's and the present account at the N II stage. There is no mention of an aesthetasc developed on the 1st antenna in Koga's study, that both Itô and the present study found at later naupliar stages. There is, however, a strong gnathobase developed on the 2nd antenna in N I stage in Koga, that is described by Itô and in the present study as much more primordial and undifferentiated. There is no doubt, however, about its complete reduction in N VI (see discussion below); that was also shown by Itô, whereas Koga describes it as being as complete and strongly developed as in all preceding stages.

Larval peculiarities of the Harpacticidae

Postembryonic development of several genera within the Harpacticidae is known. *Tigriopus* was studied by Guiglia (1926), Fraser (1936), and Shaw (1938). Nauplii of *T. fulvus* were described by Igarashi (1963). Itô (1970) and Koga (1970) studied *T. japonicus*. The nauplii of *Harpacticus* are known from a rough drawing of an early nauplius of *H. uniremis* by Brian (1919), from a single nauplius of *H. littoralis* by Griga (1960), from the

1st 5 nauplii of the same species by Castel (1976), and from a detailed study by Walker (1981) of *Harpacticus* sp., by Dahms (1990b) of *Harpacticus uniremis*, and by Dahms and Schminke (1992) of *Harpacticus furcatus*. The N I of *Zaus spinatus* is shown by Lang (1948), and the 1st 5 nauplii of the same species are described by Clogston (1965). According to Walker (1981), segmentation is lacking on both the antennal and mandibular exopod in *Harpacticus* sp. throughout the phase. This is in contrast to the present observation of a 4-segmented antennal and a 2-segmented mandibular exopod in *T. japonicus*, the latter being confirmed by Castel (1976) from *H. littoralis*. Clogston (1965) described the antennal exopod of *Zaus spinatus* as being 2-segmented throughout the phase, whereas it is 4-segmented in the present study of *T. japonicus*. Clogston missed the N II of *Z. spinatus* and described N III as N II, N IV as N III, and so forth to the N VI stage as N V. In contrast to the present report on *T. japonicus*, he argued that there is no indication of the 1st maxilla in *Z. spinatus*.

The interpretation of 2 spinule rows on either side of the ventral hindbody in N III of *Harpacticus* sp. (Walker 1981) as rudiments of legs 1 and 2 is doubted here. The reductions in N VI of all parts of the body involved in feeding, which is characteristic of the family (Dahms 1990), are only reported by Itô (1970) for *T. japonicus* and by Walker (1981) for *Harpacticus* sp. None of the authors mentions any reductions in the text or figures, and N VI is shown with a masticatory apparatus, although this should be reduced as it is the case in several genera of Harpacticidae and Tachidiidae (Dahms 1990). The reductions are confirmed in the present study of *T. japonicus*.

In N VI of *Tachidius discipes*, the labrum and ventral body wall lose their ornamentation, and the labrum is reduced in size in N VI. The antennal endite is reduced to a spinous rudiment, the 4 setae of the basis are lost except for 1 tiny seta, and only one of the 3 medial setae of the antennal endopod is left. The seta of the mandibular coxa is replaced by a digitiform hump; the 3 setae of the basis are reduced; the anterior seta on the mandibular endopod is replaced in N V by 1 long and 3 tiny setae in N VI. Similar reductions take place in N VI of *Harpacticus uniremis* and all other representatives of the Tachidiidae and Harpacticidae investigated until now (Dahms 1990). These reductions may provide a naupliar apomorphy supporting the monophyletic status of a taxon comprising both the Tachidiidae and

Harpacticidae, which were already linked as the Tachidiidomorpha by Lang (1948). Among other Harpacticoida, such reductions are known only in the sibling-species *Tisbe holothuriae* and *T. battagliai* of the Tisbidae (Dahms et al. 1992). Such reductions are similar to structures convergently lost on the mandibles and 1st maxillae of many calanoid males belonging to different unrelated groups.

Atrophied feeding structures can be caused by a non-feeding mode of living or by lecithotrophy. The reduction of certain feeding structures may mask phylogenetically valuable characters (see Bjørnberg 1986). Lecithotrophy often, but not necessarily, leads to an abbreviation of naupliar stages (see Matthews 1964, Izawa 1987). Structural reductions are most pronounced in the feeding parts of the 2nd antenna and mandible. Most nauplii do not feed in the 1st stage, even if later stages are planktotrophic. The antennal gnathobase is, therefore, little differentiated in the 1st stage (Dahms 1990). In some unrelated harpacticoids, nauplii become non-feeding in the 6th stage. They show peculiar reductions of the oral structures in N V and N VI, such as the loss of the labrum and ventral body wall ornamentation, as well as the antennal and mandibular protopod armature. This holds for all nauplii of Tachidiidae and Harpacticidae so far studied and for the *Tisbe holothuriae* group (Dahms et al. 1992).

Allometry and heterochrony

The antennal coxal process acquires 1 proximal seta in N II and 1 medial seta in N III in several Harpacticoida-Oligoarthra. This seta is a strong geniculating arm in many cases, probably assisting in the feeding process. Exclusively in the Harpacticidae, Diosaccidae, and Thalestridae, this seta replaces a seta of the basis which is similarly shaped in N I and N II but is reduced to a smaller seta in N III when the medial seta of the coxa develops. The proposed functional shift from a seta of the basis to the medial coxal seta in N III is illustrated for a representative of the Harpacticidae (as for *T. japonicus* see figure 4) and Thalestridae. This functional shift takes place at particular molt stages providing a sound synapomorphy of the 4 taxa, which are not considered to be related.

Phylogenetic considerations with regards to naupliar characters of *Tigriopus japonicus*

As for the Harpacticidae, the nauplii of

Tigriopus brevicornis are characterized by the lack of a masticatory seta on the 2nd antennal basis (in N I and N II), or on the 2nd antennal coxa (in N III) (Dahms 1990). The same holds for *T. japonicus*. The presence of this particular seta is regarded as being apomorphic, since this character is only found in the remaining harpacticid genera studied (and in the Thalestridae and Diosaccidae, see above) - its lack is thought to be plesiomorphic for *Tigriopus*.

The masticatory process of the 2nd antenna is strongly bifurcated in *T. japonicus*. In *Zaus spinatus* it is shuffle-shaped until N V but reduced to 2 tiny setae in N VI (Dahms 1990). In N II, a proximal seta and in N III, a strong, toothed medial seta are developed. The basis bears 4 setae throughout the naupliar phase, among which the innermost is long and toothed at its tip and serves as a masticatory aid. In N III, the shuffle-like seta is reduced to a seta of common length and shape, whereas its function is taken over by a newly developed medial seta of the masticatory process, which also has a similar shape and is toothed terminally. The same phenomenon of replacement is observed in N III of *Harpacticus uniremis* and *H. obscurus*, whereas no such strong seta occurs in *T. brevicornis*, where the medial seta of the antennal coxa also develops in N III as small and plain.

There are also some peculiar naupliar features linking the Tachidiidae with the Harpacticidae, both of which belong to the "Tachidiidimorpha" (sensu Lang 1948):

- a strongly toothed antennal masticatory process (lacking in the Maxillipedasphalea and Tisbidimorpha);
- a particular terminal process of the mandibular endopod (the inner of the 2 setae is spiniform and originates subterminally in the Tachidiidae, while only 1 spine is present in the Harpacticidae); and
- a reduction of the parts of the body involved in feeding in N V and N VI.

There are several characters which link the 2 taxa of different "subsections" (according to Lang 1948): the Harpacticidae of the "Exanechentera" with the Thalestridae of the "Podogennonta". This case is an example of conflicting evidence as the interpretation is not easy: all known genera of the Harpacticidae and the free-living genera of the Thalestridae have a 6-segmented 1st antenna in C I. This is a very peculiar character not known from any other harpacticoid species (Dahms 1989). In C II, a 7-segmented antenna is acquired, bearing the aesthetasc on segment 2. The 6-seg-

mented antenna of C I develops from a 3-segmented naupliar precursor in the Harpacticidae, but from a 1-segmented naupliar antenna in the Thalestridae. However, as shown by *Idomene* sp. and *Pseudotachidius* sp., a 3-segmented naupliar 1st antenna is present in the Thalestridae as well (in case these taxa are not removed from the Thalestridae for their otherwise derived characters).

The most specific naupliar character is a single, stout spine terminally on the inner process of the mandibular endopod. This character is common in both the Harpacticidae and Thalestridae (and common in the Miracidae and Metidae, which belong to the Thalestridimorpha), whereas it is not present in any other harpacticoid. Furthermore, in both taxa (and in the Diosaccidae), a peculiarly bifid seta of the antennal basis is present in N I and N II, probably assisting in the feeding process. This seta is functionally replaced by a medial seta of the coxa in N I, which then has the same structure as the aforementioned seta, which itself is reduced in size and shape. Other common characters of the Harpacticidae and Thalestridae are as follows: the distal seta of the 2nd segment of the 1st antenna is long and bears long spinules distally (however, this is also indicated in *Scutellidium hippolytes* and *Tisbe bulbisetosa*), and the antennal masticatory process is heavily toothed. Another similarity is the presence of 2 setae with a common base on the mandibular exopod in some representatives at least (as in *Harpacticus uniremis* and *Zaus spinatus* of the Harpacticidae and *Parathalestris harpactoides* of the Thalestridae). However, this character is also found in the Tisbidae (see below). As many of their representatives are phytal-living forms, one could argue that both the Harpacticidae and Thalestridae have evolved these characters independently.

In our opinion, the singularity and particularity of some of the described characters do not allow such an interpretation. However, as will be shown later, judging from larval evidence, the Thalestridae is in good agreement with the taxa placed on the "thalestridimorph branch" by Lang (1948). Therefore, the Thalestridae should not be shifted. A removal of the Harpacticidae from the "Tachidiidimorpha" could be more reasonably justified. The main argument against this is the peculiar reduction in the oral parts in N VI uniting the Harpacticidae with the Tachidiidae (both families comprise the Tachidiidimorpha sensu Lang 1948). All that can be done at the moment is to empha-

size the similarity of characters of both taxa which demands further investigation to clarify the relationships of the Harpacticoidae and Thalestridae.

This study has shown that developmental stages can provide valuable insights into relationships at various systematic levels. However, many more detailed descriptions of life cycles are required before conclusive evidence can be drawn from developmental characters for hypotheses about phylogenetic relationships within the Harpacticoida.

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