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A REVIEW OF NAUPLIAR DEVELOPMENT WITHIN THE HARPACTICIDAE, WITH NAUPLIAR DESCRIPTION OF ZAUS WONCHOELLEEI KANGTIA, DAHMS, SONG, MYOUNG, PARK & KHIM, 2014 (COPEPODA, HARPACTICOIDA)

BY

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ABSTRACT

All six naupliar stages of the harpacticoid copepod Zaus wonchoelleei Kangtia, Dahms, Song, Myoung, Park & Khim, 2014 are described. A key for the identification of the naupliar stages is provided. Stages can be distinguished by number of segments of the exopod of antenna 2, setation of the limbs including the bud of the caudal ramus, and presence and setation of the bud of maxilla 1. In phylogenetic reconstructions there are several characters which link two taxa of different harpacticoid groups, the Harpacticidae of Exanechentera and the Thalestridae of Podogennonta. The Harpacticidae and the free-living genera of the Thalestridae develop from a 3-segmented naupliar antennular precursor in Harpacticidae and a 1-segmented antennule in Thalestridae to a 6-segmented antennule at copepodid I. Both families also share a single, stout spine terminally on the inner process of the mandibular naupliar endopodite which is unique for harpacticoid nauplii. A peculiar medial bifid seta on the antennal basis at nauplius I and II is also unique. This seta is replaced by a medial seta of the coxa at nauplius III, which has the same structure as the aforementioned set which gets reduced.

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Detailed and exact descriptions of postembryonic instars are especially needed for the elucidation of phylogenetic relationships within the Copepoda (Dahms, 1990a, b). High resolution is a prerequisite for meaningful comparison, since phylogenetically valuable apomorphies may be cryptic characters which are often camouflaged by superficial resemblances (Dahms, 1992, 1994; Dahms & Fernando, 1992, 1993a, b, 1995a). But detailed studies are the more difficult the more complicated or smaller a structure is. This is especially true for minute nauplii, many of which do not exceed 50 μm in diameter at hatching. Current knowledge of phylogenetic relationships is mainly based on what can be deduced from adult morphology (Dahms, 1992). However, evidence from postembryonic stages should complement that gained from adult characters for a species exhibits different and significant characters at all phases of its ontogeny and these can be used as species-specific character patterns of evolutionary species (Dahms, 2000, 2004).
week. Seawater was obtained and prepared as described by Dahms (2000). When nauplii emerged, some of them were isolated in watch glasses and the exuviae of subsequent molts were collected. *Dunaliella salina* (Dunal) Teodoresco, 1905 was used as algal food. A few drops of food-suspension were added every few days.

Preparation.— Stages were fixed in 5% buffered formaldehyde and embedded in glycerine which clears the natural colour of nauplii, and colour and shape of the red nauplius eye is lost quite soon and the eye is, thus, not figured. Nauplii were mounted whole, broken cover-glass pieces being added to prevent them from being compressed and to facilitate rolling to allow inspection from all sides. Body lengths were measured from the anterior to the posterior end of the naupliar body: body width is given as the widest part of the nauplius. Drawings were made from single specimens; others (2-5) were checked for variability. The specimens have not been stained and that the naupliar appendages have not been dissected due to the small size of the nauplii.

Descriptive terminology.— The following terms are defined according to their usage in the following sections of this paper: The harpacticoid nauplius has at least three pairs of appendages: the antennules, antennae and mandibles. The body is completely covered by a usually smooth nauplius shield in the earlier stages, whereas the hind-body protrudes from it in later stages. At the posterior end of the body there is at least one caudal seta on both sides of the anal area. The labrum originates as a lobular flap near the frontal margin of the body, between the bases of the antennules, and extends posteriorly across the ventral surface of the body. The ventral body wall is a tongue-like structure arising at the base of the antennal protopodite. The antennule is uniramous. The antenna is noticeably different from that of the adult (and copepodids) in having a precoxa in some cases and a naupliar arthrite. The antenna further consists of a coxa, basis, endopodite and exopodite. The mandible is composed of the same elements. The endopodite consists of an inner process and usually an outer lateral field of setae arising on the outer lateral margin (except in Polyarthra where it is homologous to the 2nd segment and in Ectinosomatidae where it is vestigial). The postmandibular appendages (maxillule, maxilla, maxilliped, leg 1 and leg 2) may develop successively from nauplius II onwards. For all appendages the singular form is used. Large outgrowths are called setae or spines. A typical seta is generally a flexible, finely attenuate 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. The typical spine is generally short, relatively inflexible, and usually bears a double row of tiny spinules. Very small elements usually originating as extensions of the epicuticle are referred to as “setules” but only if they originate from the epicuticle of a seta.
Aesthetascs (aesthetes sensu Nishida & Ohtsuka, 1997) are presumed sensory elements of the antennule with sclerotized bases and are more transparent than normal setae with blunt or rounded ends. The complement of setae, setules, spines and aesthetascs is called element of a particular structure and is 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 could be very fine hair-like cuticular extensions of setae and spines, labrum and ventral body wall, or, small, pointed, conical processes. Denticles are minute triangular outgrowths. The spinules and denticles are considered as the ornamentation.

RESULTS

Description of naupliar stages of *Zaus wonchoelleei*

The nauplii of *Zaus wonchoelleei* (figs. 1-7) have naupliar shields which are bare. Hind-body bears 1 seta on either side at nauplius I and nauplius II, 3 at nauplius III, 4 at nauplius IV and nauplius V, and 5 setae at nauplius VI. At nauplius V a protuberance with a pore at tip arises between uttermost seta and biarticulated dorsal one. A row of long subterminal spinules is present at caudal edge at nauplius I and nauplius II. Spinules are reduced in size but increase in number at nauplius II when anal area becomes indented. Spinules are scattered around the proximal part and subterminal part on caudal area. Anal-operculum is rounded and bare. Labrum is oval-shaped with spinule-row around lateral and caudal edge.

**Nauplius I**

Body slightly longer than wide with cephalic shield. Body length 112 μm, body width 100 μm. Antennule (fig. 4A) 3-segmented. Segment 1 unarmed, segment 2 with 2 small and 1 long medial seta distally with longer spinules distally which are extended caudally. Segment 3 with 1 subterminal seta and 2 long setae and an aesthetasc distally.

Antenna (fig. 5A) with coxa and basis, 4-segmented exopod and 1-segmented endopod. Coxa with row of denticles dorsally and 1 small seta and naupliar arthrite with 4 setae distally. Basis with setule row dorsally, with 1 thick seta bearing bifid tip and tiny setules on it, and with 2 bare setae. Exopod is shorter than endopod; segment 1 without ornamentation, segment 2 longest with 2 setae, and segments 3-4 with 1 and 2 setae, respectively. Endopod robust with 1 inner bare seta on middle, and with 1 stout claw bearing setule row on inner margin.

Mandible (fig. 7A) with coxa and basis. Coxa with 1 inner seta. Basis with 1 inner seta bearing setule row on inner margin, and 2 setule rows dorsally. Exopod 1-segmented, with 1 lateral seta proximally and 2 setae distally. Endopod
Fig. 1. *Zaus wonchoelleei* Kangtia, Dahms, Song, Myoung, Park & Khim, 2014, habitus. A, Nauplius I, ventral view; B-D, nauplius II, ventral, lateral and dorsal view, respectively.

1-segmented, with 3 outer setae and 1 stout spine bearing a setule row along inner margin.

**Nauplius II**

Differing from nauplius I in size and as follows: Body (fig. 1B-D) as long as wide and of circular form.

Antennule (fig. 4B), segment 2 with 1 long medial seta between 2 bare setae.

Antenna (fig. 5B), coxal naupliar arthrite well developed with denticles distally but without dorsal spinules. Basis with more setules dorsally, 1 thick seta modified
Fig. 2. *Zaus wonchoelleei* Kangtia, Dahms, Song, Myoung, Park & Khim, 2014, habitus. A, Nauplius III, ventral view; B, nauplius IV, ventral view.

bearing sharp tip. Exopod with 1 lateral seta added to segment 2. Endopod with 2 tiny setae added to inner margin and outer distal corner.

Mandible (fig. 7B). Basis with reduced dorsal setules. Exopod-2segmented, segment 1 unarmed, segment 2 with 1 bare seta proximally and 2 distal setae added. Endopod with 1 outer seta added.

Maxillule (fig. 1B, D) representated by 1 seta.

Nauplius III

Differing from nauplius II in size and as follows: Body (fig. 2A) length 120 μm, body width 112 μm.
Antennule (fig. 4C), segment 2 with setule row outerodistally, segment 3 with 8 setae added (now 11 + ae).

Antenna (fig. 5C), coxal naupliar arthrite well developed, and coxa with 1 seta bearing bifid tip (now 2 setae). Basis with 4 bare setae in total. Exopod segments 2
Fig. 4. *Zaus wonchoelleei* Kangtia, Dahms, Song, Myoung, Park & Khim, 2014. Antennule of nauplius I-VI.
Fig. 5. *Zaus wonchoelleei* Kangitia, Dahms, Song, Myoung, Park & Khim, 2014. Antenna of nauplius I-IV.
and 4 with 1 more bare seta, respectively (now 4 and 3). Endopod with 1 small seta added to inner margin.

Mandible (fig. 7C), endopod with setule row subdistally and 1 outer seta added (now 5).

Maxillule (fig. 2A) with 2 setae on small protuberance in total.

Fig. 6. *Zaus wonchoelleei* Kangtia, Dahms, Song, Myoung, Park & Khim, 2014. Antenna of nauplius V-VI.
Fig. 7. *Zaus wonchoelleei* Kangtia, Dahms, Song, Myoung, Park & Khim, 2014. Mandible of nauplius I-VI.
Nauplius IV

Differing from nauplius III in size and as follows: Body (fig. 2B) length 136 $\mu$m, body width 129 $\mu$m.
Antennule (fig. 4D), segment 3 with 1 more seta (now 12 + ae).
Antenna (fig. 5D), exopod segment 1 with setules on surface, segment 2 with setules on surface. Endopod with 1 tiny seta added on inner margin.
Mandible (fig. 7D) remains unchanged.
Maxillule (fig. 2B) with 3 setae on small protuberance in total.

Nauplius V

Differing from nauplius IV in size and as follows: Body (fig. 3A) length 164 $\mu$m, body width 153 $\mu$m.
Antennule (fig. 4E) and antenna (fig. 6A) same as at nauplius IV.
Mandible (fig. 7E), with setule row dorsally on basis.
Maxillule remains unchanged and is not figured.

Nauplius VI

Differing from nauplius V in size and as follows: Body (fig. 3B) length 194 $\mu$m, body width 177 $\mu$m.
Antennule (fig. 4F) and mandible (fig. 7F) same as nauplius V.
Antenna (fig. 6B), coxal naupliar arthrite absent and with only 1 inner seta proximally. Basis with 3 inner setae totally. Endopod with setule row added on dorsal surface and 1 inner proximal seta absent (now 2 on inner margin).

Intraspecific variability

Variability was observed in the number of spinules, the length of setae and body size in general. Variability of ornamentation is not only restricted to different individuals but was also different for the two sides of the body.

**Key to Naupliar Stages of Zaus wonchoelleei**

1. Almost circular in shape; 1st maxilla not indicated .................. Nauplius I
   - 1st maxilla appears as 1 seta or a setose bud ........................... 2
2. 1st maxilla appears as 1 seta; not more than 2 caudal setae on each side ........ Nauplius II
   - More than 2 caudal setae on each side .................................... 3
3. Four (4) caudal setae on each side ........................................... Nauplius III
   - More than 4 caudal setae on each side .................................... 4
4. 1st maxilla present as lobe with 3 setae; no indication of leg 1 ............. Nauplius IV
   - Indication of leg 1 ................................................................. 5
5. Leg 1 indicated as an abdominal fold without setal armature .................. Nauplius V
   - Legs 1 and 2 indicated as abdominal lobes, each bearing setal armature .... Nauplius VI
As far as naupliar development is concerned in the Harpacticidae, *Tigriopus* is studied best by workers like Guiglia (1926), Fraser (1936) and Shaw (1938), studying *T. fulvus* (Fischer, 1860). Igarashi (1963), Ito (1970) and Koga (1970) studied the nauplii of *T. japonicus* Mori, 1938. Nauplii of the genus *Harpacticus* are known from a rough drawing of an early nauplius of *H. uniremis* Krøyer, 1842 by Brian (1919), from a single nauplius of *H. littoralis* Sars G. O., 1910 by Griga (1960), from the first 5 nauplii of the same species by Castel (1976) and from the detailed study by Walker (1981) on *Harpacticus* sp. The nauplius I of *Zaus spinatus* Goodsir, 1845 is shown by Lang (1948) and the first 5 nauplii of the same species are described by Clogston (1965).

The main characteristics of *Zaus wonchoelleei*, *Zaus* and the Harpacticidae are discussed below.

Reduction of feeding parts.— The reductions at nauplius VI in all the parts of the body being involved in feeding which are characteristic for the family are only reported by Itô (1970) for *T. japonicus* and by Walker (1981) on *Harpacticus* sp. All other authors do not mention any reductions in the text and figure and the nauplius VI is unchanged showing an unchanged masticatory apparatus.

Mandibular endopodite.— The mandible endopodite is elongate and bears an inner process armed with 1-2 terminal curved setae and usually an outer lateral bulge bearing 3-4 setae at nauplius I in Exanechentera and Podogennonta augmented to 5 or 6 in later stages. In Ectinosomatidae (as in Miracidae and Metidae) this bulge is represented by a small protuberance at nauplius I which is completely reduced in later stages. *Phyllognathopus viguieri* (Maupas, 1892) has an endopodite similar to that of the more advanced sections of Oligoarthra. Development of only 1 spiniform seta terminally on the inner process of endopodite is restricted to the following families: Harpacticidae, Thalestridae, Miracidae and Metidae. They belong to the thalestridimorph and metidimorph taxa of Podogennonta except Harpacticidae which belong to the Exanechentera.

Segmentation of antennal and mandibular exopodite.— According to Walker (1981) in *Harpacticus* sp. is any segmentation lacking on both, the antennal and mandibular exopodite throughout the phase. This is in contrast to the present observation of a 3-segmented antennal and a 1-segmented mandibular exopodite in *H. uniremis* which is confirmed by Castel (1976) on *H. littoralis*. Clogston (1965) described the antennal exopodite of *Zaus spinatus* as being 2-segmented throughout the phase whereas it is 4-segmented in the present study. Clogston has missed nauplius II and describes the nauplius III as the nauplius II, nauplius IV as nauplius III and so forth until he misinterpreted the nauplius VI stage as nauplius V. Clogston (1965) shows an indication of the reduction process of the
oral appendages but in contrast to the present report it is said that there is no indication of the maxilla. The interpretation of 2 spinule rows on either side of the ventral hind-body of nauplius III of *Harpacticus* sp. (Walker, 1981) as rudiments of legs 1 and 2 is doubted here.

Appearance of postmandibular appendages.— As for the postmandibular appendages labeling the exuvial rudiments as they appear in the developmental sequence as maxillulae, maxillae etc. should not be considered a reliable method because a suppression of Anlagen can occur within this series (as the maxilla in Polyaarthra and *Zaus spinatus*). It has to be born in mind that chitinous structures at least within the Harpacticoida are observed to be modified only through molts. 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, buds of appendages) remain hidden there, not being expressed on the outside should be correlated with the exuvial Anlagen — if they are present—to verify their identity. This, however, may require histological studies in most of the cases.

Similarity between nauplii and copepodids.— As for the similarity between nauplii and copepodids, nauplii of *Zaus spinatus* bear a spinule fan on the antennal endopodite claw and on the seta of the mandibular endopodite process. Such a fan also exists on the copepodid endopodite leg 1 of this species.

Phylogenetic relationship between Harpacticidae and Thalestridae.— Phylogenetic reconstructions need to consider that there are several characters which link two taxa of different “subsections” (according to Lang, 1948): the Harpacticidae of Exanechentera with the Thalestridae of Podogenonta. To our knowledge this situation has so far passed unnoticed. However, this case is of conflicting evidence. All known genera of the Harpacticidae and the free-living (1) genera of the Thalestridae have a 6-segmented antennule at C I. This is a very peculiar character not known from any other harpacticoid species (Dahms, 1989). At C II, a 7-segmented antennule is acquired bearing the aesthetasc on segment 2 in both families Harpacticidae and Thalestridae. The 6-segmented antennule of C I develops from a 3-segmented naupliar precursor in the Harpacticidae but from a 1-segmented naupliar antennule in the Thalestridae. But, as shown by *Pseudotachidius* sp. and *Xouthous* (= *Idomene*) sp., a 3-segmented naupliar antennule is present in the Thalestridae as well (in case both these taxa are not to be removed from the Thalestridae for their otherwise derived characters).

The most specific naupliar character is the single, stout spine terminally on the inner process of the mandibular endopodite. This character is common to both,
Harpacticidae and Thalestridae (and for the Miracidae and Metidae belonging to the Thalestridimorpha) whereas it is not present in any other harpacticoid.

Furthermore, in both taxa (and in the Miracidae) a bifid seta is found medially on the coxa of nauplius III and later stages. The bifid seta on the basis of nauplius I and II is replaced by a simple seta of reduced length in nauplius III-VI. This then has the same structure as the aforementioned seta which itself is reduced in size and shape. Other common characters in species of the Harpacticidae and Thalestridae are as follows: the distal seta of the second antennular segment is long and bears long spinules distally (however, this is also indicated in the tisbids *Scutellidium hippolytes* (Krøyer, 1863) and in *Tisbe bulbirostra* Volkmann-Rocco, 1972) and the antennal masticatory process is heavily toothed. Another similarity is the presence of 2 setae with common base on the mandibular exopodite in some representatives at least (so in *Harpacticus uniremis* and *Zaus spinatus* for Harpacticidae and *Parathalestris harpactoides* (Claus, 1863) for Thalestridae). However, this character is also found in Tisbidae (see below).

As many of their representatives are phytal living forms, one could argue that both, Harpacticidae and Thalestridae have evolved these characters independently. However, as will be shown later, judged from larval evidence the Thalestridae are in good agreement with the taxa placed on the “thalestridimorph branch” by Lang (1948). Therefore, the Thalestridae should not be reallocated. A removal of the Harpacticidae from the “Tachidiidimorpha” would be more easily justified. The main argument against this is the peculiar reduction of the oral parts at nauplius VI uniting the Harpacticidae with the Tachidiidae. All that can be done for the moment is to emphasize the similarity of characters of both taxa demanding further investigation to clarify the relationships of Harpacticidae and Thalestridae.

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