

Naupliar Development among the Tisbidae (Copepoda: Harpacticidae) with a Phylogenetic Analysis and Naupliar Description of *Tisbe thailandensis* from Thailand

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Hans-U Dahms, Supawadee Chullasorn, Nikolaos V. Schizas, Pawana Kangtia, Wongpiya Anansatitporn, and Wan-Xi Yang (2009) Naupliar development among the Tisbidae (Copepoda: Harpacticidae) with a phylogenetic analysis and naupliar description of *Tisbe thailandensis* from Thailand. *Zoological Studies* 48(6): 780-796. Oviparous females of a recently described species *Tisbe thailandensis* were collected from the seaweed *Enteromorpha clathrata* on the eastern coast of Thailand and cultured in the laboratory. Six naupliar stages are described, and a key to the identification of the stages is provided. An unweighted analysis of the character matrix yielded a single most parsimonious tree with a length of 27, a consistency index of 0.778, a retention index of 0.786, and a rescaled consistency index of 0.611. There were 15 parsimoniously informative characters. The resulting cladogram, using nauplii from the harpacticoid genus *Tachidius* (Giesbrecht, 1881) for the outgroup comparison, supports the hypothesis that *T. gracilis* is the sister taxon of *T. cucumariae* (both of which belong to the *T. gracilis* group), and that *T. battagliai* is the sister taxon of *T. holothuriae* (both of which belong to the *T. holothuriae* group), with *Tisbe thailandensis* adjoining the *T. gracilis* group on an underived branch. Naupliar comparisons of copepodid morphology provided the greatest number of phylogenetically useful additional characters since copepodids essentially foreshadow the adult condition. Limits of the utility of naupliar characters for phylogenetic inference studies were apparent, as well as their potential and importance for future work in zoological systematics. It was shown that phylogenetic relationships of tisbids are difficult to evaluate on a morphological basis alone, because of the comparatively small morphological differences among them. However, more specific morphological characters can be found, provided studies are detailed enough and different stages of a sufficient number of species are known to allow meaningful comparisons.
<http://zoolstud.sinica.edu.tw/Journals/48.6/780.pdf>

Key words: Phylogenetic systematics, Naupliar characters, Ontogeny, Morphology, Tisbidae.

The harpacticoid copepod taxon, *Tisbe*, occurs worldwide especially in shallow marine waters and contains about 60 described species (Bodin 1997, Boxshall and Halsey 2004). Species of *Tisbe* are easy to collect, maintain, and rear in the laboratory, and they have short life cycles

compared to other Harpacticoida (Dahms and Qian 2004). For these reasons, *Tisbe* has served as a model taxon for a variety of morphological, ecological, and genetic studies (Dahms and Schminke 1995). There are few copepod genera that have been investigated as thoroughly as

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representatives of *Tisbe* (Dahms and Qian 2005). In particular, there are numerous life cycle studies (reviewed in Dahms et al. 2007). This taxon has become the focal point of several in depth studies, e.g., of its population ecology (Parise 1978), life history (Bergmans 1984), external and internal anatomy (Dahms et al. 1991), genetics (Dahms and Schminke 1995), and ecotoxicology (Bechmann 1999, Hutchinson et al. 1999).

Cross-breeding experiments showed that some *Tisbe* species actually contain a mixture of morphologically similar sibling species (e.g., Volkmann 1979). Molecular approaches other than those described by Dahms and Schminke (1995) are lacking for *Tisbe* as yet. Although ontogenetic stages evolve specific characters through the effects of natural selection, phylogenetic studies traditionally rely on adult characters (Dahms et al. 1991a). Only rarely are larval characters taken into consideration (Dahms 2000, Dahms et al. 2005, Ferrari and Dahms 2007, Ivanenko et al. 2008). The success of such attempts depends on the reliability and level of detail of the underlying descriptions. The taxon *Tisbe* is a good example illustrating how little of such information is available from the literature. Lopez (1980) found that nauplii differed more from each other than from their copepodid stages. This is not surprising since important details have previously been overlooked. In some cases, larvae do not even belong to the species claimed (Battaglia 1957, Barnard and Reish 1960, Clogston 1965). For this reason Dahms et al. (1991b) presented the first cladogram using non-conventional morphological characters based on naupliar morphology of the taxon *Tisbe*.

Naupliar development can be used in an updated study that is warranted because previous attempts were either incomplete or lacked sufficient detail. The aim of the present study was to provide a phylogenetic analysis on the basis of detailed morphological descriptions of all naupliar stages of a recently described species, *Tisbe thailandensis*, from Thailand. The results of this analysis and reliable character information from the literature are compared with existing hypotheses using adult characters to evaluate the respective conclusions. The separate treatment of adult and larval characters allows us to assess their respective contributions to the analysis of phylogenetic relationships among morphologically similar species as is the case in the taxon *Tisbe*.

MATERIALS AND METHODS

Collection of data

Ovigerous females of *T. thailandensis* (Chullasorn in press) were collected by S. Chullasorn and P. Kangtia from the seaweed, *Enteromorpha clathrata*, in Chonburi Province, on the eastern coast of Thailand on 23 Dec. 2006 and cultured in the laboratory. A single ovigerous female was subsequently selected from the cultures and transferred to a 250 ml beaker to provide the offspring studied here. This culture was kept at room temperature with an approximate light: dark cycle of 12:12 h. About 50% of the seawater was renewed each week, and it was obtained and prepared as described by Dahms et al. (2007). When the nauplii emerged, some of them were isolated in watch glasses, and the exuviae of subsequent molts were collected. *Isochrysis galbana*, *Tetraselmis* sp., and *Chaetoceros* sp. were used as mixed algal food. A few drops of a food suspension were added every few days. Small protozoan contaminants and algal growth were not prevented. No substratum was added since *Tisbe* sp. is an epistrate dweller, whereas harpacticoids on soft bottoms often require sediment substrates (Dahms and Qian 2005).

Preparation

Naupliar stages were fixed in 5% buffered formaldehyde and embedded in glycerol. Glycerol clarifies nonexuvial material within a few days, and this allows information on hidden posterior structures to be observed with phase contrast microscopy. Unfortunately, the natural color of the nauplii and the color and shape of the red naupliar eye are rapidly lost, and for this reason, the eye was not figured. Nauplii were mounted whole, and glass fibers were added to prevent them from being compressed and to facilitate rolling to allow inspection from all sides. 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) and Volkmann (1979) if not stated otherwise.

values were calculated for 1000 replicate searches using unweighted characters. The bootstrap technique is widely used to provide assessments of confidence for each clade of an observed tree, based on the proportion of bootstrap trees showing that same clade (Felsenstein 1985). It was shown that the bootstrap method is not systematically downwardly biased, and that it provides reasonable assessments of the error of an estimated tree (Efron et al. 1996). However, there are some caveats on the use of bootstrapping since the basic assumption of independence (1 observation evolves independently from all others) is violated. Additionally, since the data columns are not identically distributed (e.g., some data columns are more variable than others), the distribution of observations is not identically distributed.

RESULTS

Individuals of all 6 naupliar stages of *Tisbe* spp. are benthic, and crawl and swim crawl on surfaces. All developmental stages including nauplii can also freely swim in the water column. Nauplii are fat and unpigmented except for the red eye (Figs. 1-4).

Description of naupliar stages

Nauplius I (Fig. 2). Body form circular in outline. Body length 66.5 μm , body width 53.7 μm . Body slightly longer than wide and covered by a cephalic shield. Almost circular labrum furnished with hairy spinules along its lateral corner and distal margin. Sternal field unornamented. Hindbody bearing 1 seta (i.e., the initial furca). Three appendages present: A1, A2, and Md.

First antenna 3 segmented (Fig. 3). First segment with 1 seta throughout naupliar phase. Second segment with 2 setae. Distal segment armed with 5 setae, one of which has a joint base with terminal seta transformed to an aesthetasc in N II.

Second antenna with coxa, basis, 4 segmented exopod, and 1 segmented endopod (Fig. 4). Coxa possessing setule row and naupliar arthrite articulating with a projection that terminates with a 2 tipped serrate fan. Basis with 2 setae on inner edge. First, 2nd and 3rd exopod segments with 1 terminal seta, 4th segment with 1 long and 1 shorter seta. Endopod with 2 small medial setae half-way along length, terminally with a claw and a small seta at base of claw.

Mandible with clearly separated coxa and basis, 4 segmented exopod, and 1 segmented endopod (Fig. 6). Coxa with 1 spine. Endopod of 1 article bearing a subterminal setule row and 2 strong terminal spines (innermost spine spinulated on labral side) and a dorsolateral hillock with 6 setae. First exopod segment bare, 2nd and 3rd exopod segments with 1 terminal seta each, 4th segment with 1 long plumose seta and 1 shorter seta.

Nauplius II (Figs. 1, 2). Body length 82.8 μm , body width 66.5 μm . Differing from N I as follows: body form ovoid (elongation beginning and is maximal in N VI).

First antenna, 2nd segment with 3 setae, 3rd segment with a setule row on inner side and seta next to terminal one with a joint socket transformed to an aesthetasc (Fig. 3).

Coxa of 2nd antenna bearing 1 newly developed seta at base of arthrite; exopod with 2 setae on 1st segment (Fig. 4).

Mandible basis with 2 spinulose setae; exopod with 1 seta on 1st segment (Fig. 5). First maxilla appearing as a seta posterior to mandible (Figs. 1, 2).

Nauplius III (Fig. 2). Body length 98.0 μm , body width 78.2 μm . N III differs from N II in the following aspects.

First antenna with 7 "setae" and 1 aesthetasc on 3rd segment (Fig. 3).

Basis of 2nd antenna bearing 3 setae; exopod with a tiny 3rd seta on its 1st segment and 2 long and 1 smaller setae on its 4th segment (Fig. 4).

Caudal area with 1 long and 1 shorter seta (Fig. 2).

Nauplius IV (Fig. 2). Body length 121.3 μm , body width 95.7 μm . N IV differs from N III in the following aspects.

First antenna with 8 setae and 1 aesthetasc on 3rd segment (Fig. 3).

Second antenna with a 3rd unfurnished seta between 2 spinulose ones on inner margin of basis (Fig. 4).

First maxilla appearing as a lobe with 4 setae (Fig. 2).

Caudal area with 4 setae, 2nd innermost one on each side longest (Figs. 1, 2).

Nauplius V (Fig. 2). Body length 133.0 μm , body width 102.7 μm . N V differs from N IV in the following aspects.

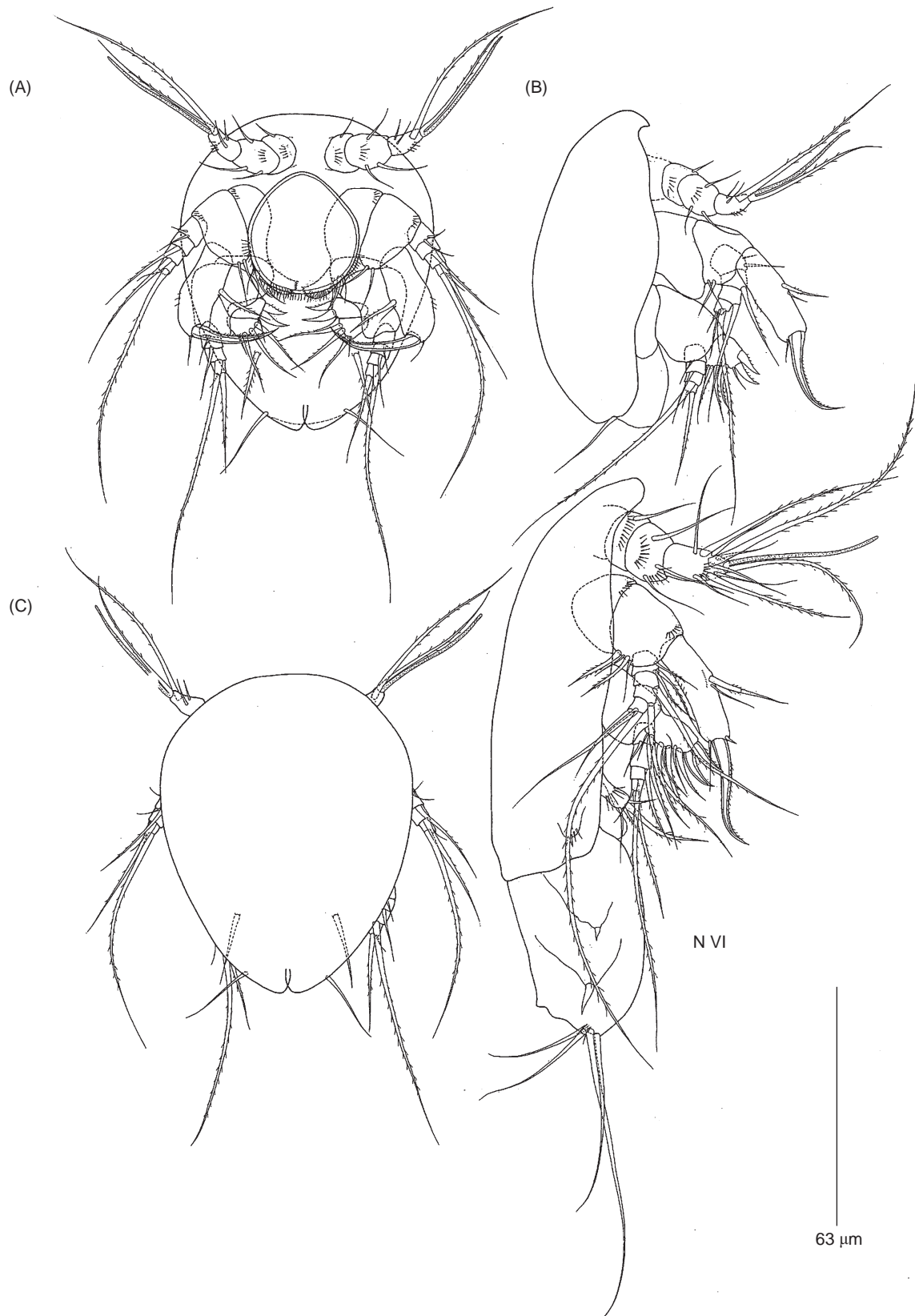


Fig. 1. *Tisbe* sp. naupliar stage II in ventral (A), lateral (B), and dorsal (C) views; stage VI in lateral view.

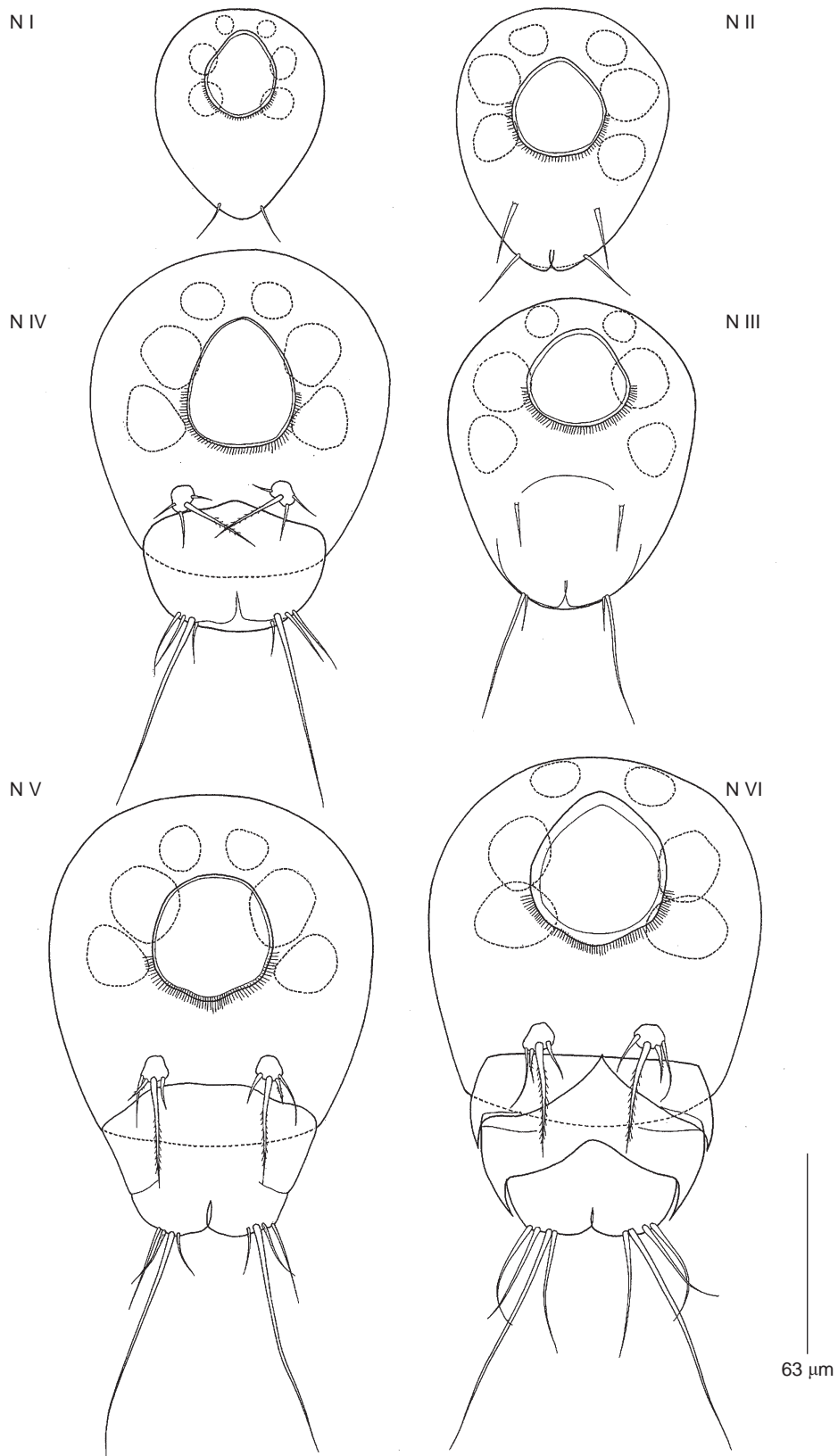


Fig. 2. *Tisbe* sp. naupliar stages I to VI in ventral view; the articulation plane of the 1st 3 pairs of appendages is indicated by a stroken line.

First antenna with 9 setae and 1 aesthetasc on terminal segment (Fig. 3).

Second antenna endopod with 2 small outer and inner setae proximal to terminal claw (Fig. 5).

Leg 1 indicated as an unfurnished lateral abdominal fold (Fig. 2).

Nauplius VI (Figs. 1, 2). Body length 151.7 μm , body width 103.8 μm . N VI differs from N V in the following aspects.

First antennal terminal segment with 10 setae and 1 aesthetasc (Fig. 3).

Legs 1 and 2 indicated as lateral abdominal lobes with 1 spiniform tip each (Fig. 2).

Key to naupliar stages of species belonging to *Tisbe*

1. Almost circular in shape; 1st maxilla not indicated..... N I
- Not so; 1st maxilla visible with at least 1 setae 2
2. One caudal seta on each side..... N II
- More than 1 caudal seta on each side..... 3
3. Two caudal setae..... N III
4. Four caudal setae on each side, 1st maxilla present as lobe with 4 setae, no indication of leg 1..... N IV
5. Leg 1 indicated as an abdominal fold; 5 caudal setae on each side N V
- Legs 1 and 2 indicated as abdominal lobes with acutiform tips N VI

Phylogenetic analysis

Naupliar characters are separated into structural, meristic, and stage dependent characters (i.e., the sequence of changes in the course of development). Stage dependent characters are rare in *Tisbe* nauplii. The only ones in the 7 *Tisbe* species investigated to date are described here.

The masticatory process of the antenna bears 2 setae in N II, after the addition of 1 proximal seta in N II of *T. thailandensis*, and 3 setae in N III of *T. gracilis*, *T. cucumariae*, and *T. bulbisetosa*. An outer small seta at the tip is not present in *T. furcata*, *T. battagliai*, and *T. holothuriae*; therefore, these 3 species have only 1 medial seta in N II and 2 setae in N III. This character accompanies the presence of a rounded fan of spinules at the masticatory tip in *T. gracilis*, *T. cucumariae*, and *T. bulbisetosa* and a more drawn-out acutiform tip in the remaining 3 species (this is the case in *T. battagliai* and *T. holothuriae* from N II to N V only, since the masticatory structures are completely reduced in N VI).

The 3rd small seta at the posterior base of

the endopodal claw of the antenna present in *T. gracilis*, *T. cucumariae*, and *T. furcata* fails to develop in the remaining 3 species in N V.

The anlage of the maxilla is already present in the N V stage as a row of 4 or 5 spinules in *T. gracilis*, *T. cucumariae*, and *T. furcata*, whereas it is not detectable in *T. holothuriae*, *T. battagliai*, or *T. bulbisetosa*.

Structural and meristic characters are best observed in the 6th naupliar stage because all characters acquired during the naupliar phase accumulate in this stage. No reductions occur during the naupliar phase except in the 2 species, *T. battagliai* and *T. holothuriae*. Both are characterized by a reduction of oral structures from N V to N VI. The same is otherwise known only from 2 families (Tachidiidae and Harpacticidae) of the Tachidiidimorpha (Dahms 1991).

Further differences between the 6th nauplius stage of *T. thailandensis* and those of the other species are described here.

In *T. battagliai* and *T. holothuriae*, the masticatory process of the antennal coxa is reduced to a spinulose seta; the proximal spinules on the antennal endopod are reduced in size and number, and the medial 2nd seta is tiny; and the 4th seta of the distal segment of the antennal exopod, which is common in the other species, is lacking in this pair of sibling species.

The outermost seta of the endopodal outer lateral field of the mandible is shorter in *T. gracilis* than in the other species.

In *T. holothuriae* and *T. battagliai*, the coxal seta of the mandible and the proximal seta of its basis are smaller than in the N V or N VI stage of the other species studied. The claw-like seta of the mandibular endopod is reduced in size (Fig. 5) in these 2 species.

Tisbe furcata bears longer spinules on the antennal coxa than those of all other species and only 2 or 3 spinules on its basis in contrast to 5-7 in the remaining species. In addition, the proximal seta of the 1st segment of its antennal exopod is bare, whereas it is spinulose in the other species.

The spinule crest on the 2nd segment of the mandibular endopod consists of longer spinules in *T. gracilis*, *T. cucumariae*, and *T. furcata* than those in the remaining species. This character is gradual and appears to be variable.

The inner seta of the maxillular inner lobe is shorter in *T. gracilis* and *T. cucumariae*, and there is only 1 spinule row in these species in contrast to 2 rows in *T. battagliai* and *T. holothuriae* and 3 in *T. bulbisetosa* and *T. furcata*.

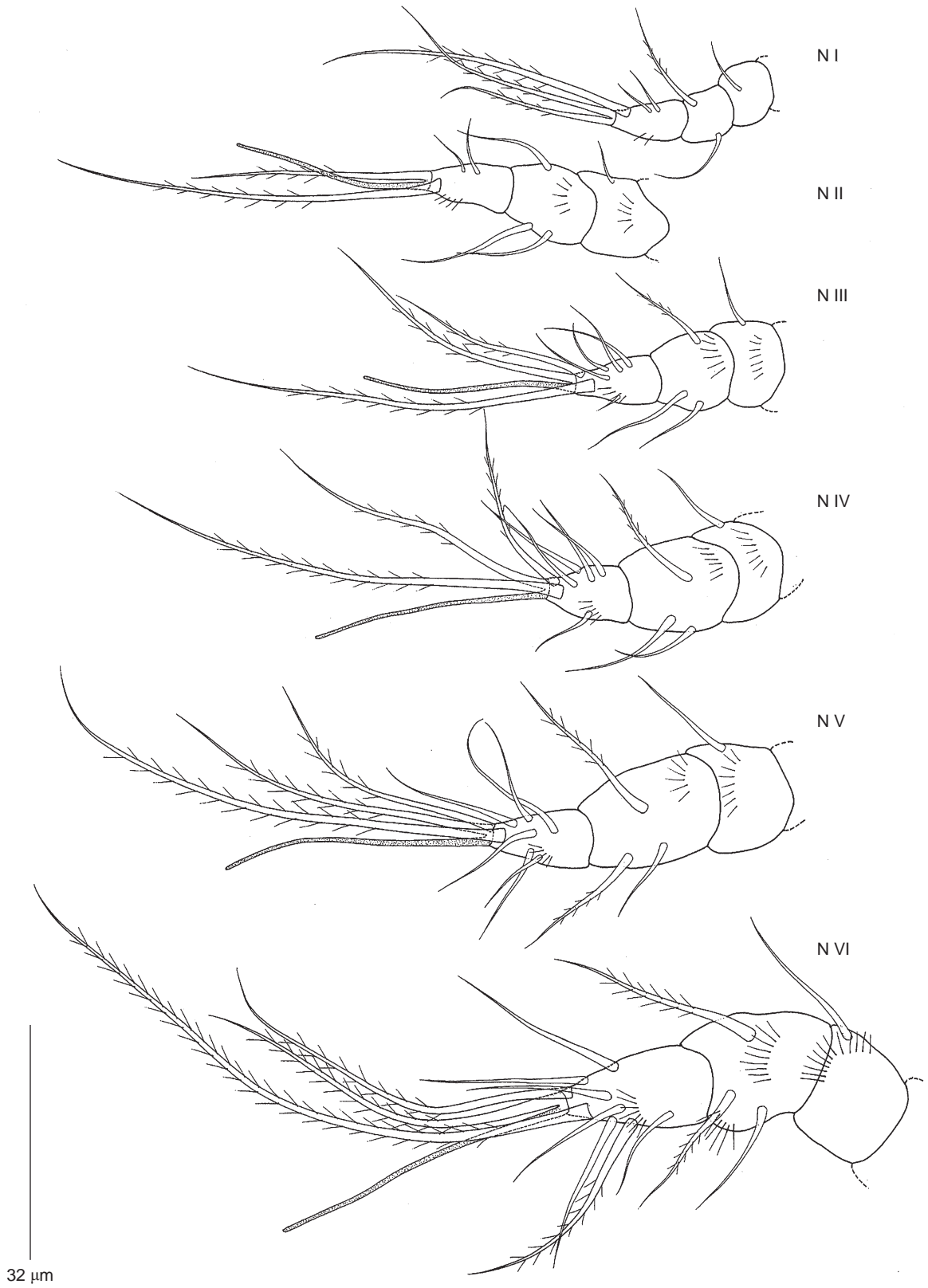


Fig. 3. *Tisbe* sp. development of the naupliar right 1st antenna of naupliar stages I to VI in anterior view.

The anlage of the maxilliped is represented by a spinule row in *T. gracilis* and *T. cucumariae*, by a blunt process ornamented with a spinule row in *T. bulbisetosa* and *T. furcata*, and by a process devoid of spinules in *T. battagliai* and *T. holothuriae*.

Lobes P1 and P2 are more flap like in *T. gracilis* and *T. cucumariae* with only minute emarginations in the 1st species. There are well developed denticuliform emarginations in *T. furcata*, *T. bulbisetosa*, *T. battagliai*, and *T. holothuriae*.

The spinules of the labral crest are more or less equal in length in *T. gracilis* and *T. cucumariae* but are longer medially in the remaining 4 species (this applies to the N V stage in *T. battagliai* and *T. holothuriae*).

The unweighted analysis of the character matrix (see character states section) yielded a single most parsimonious tree with a length of 27, a consistency index of 0.778, a retention index of 0.786, and a rescaled consistency index of 0.611. There were 15 parsimoniously informative characters. The resulting cladogram, using nauplii from the harpacticoid genus *Tachidius* (Giesbrecht, 1881) for the outgroup comparison, supports the hypothesis that *T. gracilis* is the sister taxon of *T. cucumariae* (both of which belong to the *T. gracilis* group), and that *T. battagliai* is the sister taxon of *T. holothuriae* (both of which belong to the *T. holothuriae* group) (Fig. 7). *Tisbe bulbisetosa* and *T. furcata* are more closely related to the pair, *T. battagliai* and *T. holothuriae*, than to the pair, *T. gracilis* and *T. cucumariae*; however, these relationships were only supported by low bootstrap values. The resulting cladogram shows a bifurcation with the *holothuriae* group, with *T. bulbisetosa* and *T. furcata* on 1 branch, and the *gracilis* group and *T. thailandensis* on the other branch. *Tisbe furcata* and *T. thailandensis* are respectively joined to one and the other branch with low bootstrap values.

DISCUSSION

Comparison of *T. thailandensis* nauplii described here with previous descriptions

There are more than 10 publications on the postembryonic development of *Tisbe*. However, several species of *Tisbe* have been erroneously identified (see Volkmann-Rocco 1971). *Tisbe cucumariae*, a representative of the *T. gracilis*

group, was studied by Battaglia (1957) and Lopez (1980). At least some of the material described by Barnard and Reish (1960) is not *T. gracilis* as stated by Volkmann (1979). Chua (1975) studied nauplii of *T. longisetosa*. Nauplii other than those of *T. furcata* were studied by Johnson and Olson (1948), Griga (1960), and Koga (1984). Vilela's (1969) study of the naupliar development of *Tisbe* sp. refers to *T. bulbisetosa* (pers. comm. with Dr. M. Bergmans). The study of the Battaglia and Talamini (1957) claiming to be of *T. gracilis* actually was of *T. cucumariae* (pers. comm. with Dr. M. Bergmans), and that by Barnard and Reish (1960) claiming to deal with *T. gracilis* has nothing to do with that species (Volkmann 1979). Clogston's unpublished observation that the antennal masticatory process is reduced in *T. gracilis* cannot be confirmed for that species. Such a reduction takes place in *T. battagliai* and *T. holothuriae*, which suggests that one of these species was investigated but certainly not *T. gracilis*. The latter species was described by Dahms and Bergmans (1988). The postembryonic development of *T. holothuriae* was described by Park (1976). Reduction of the antennal masticatory process in the 6th naupliar stage of that species is not mentioned in the text, but a coxal arm is indicated in the figures. The presence of such a pronounced coxal endite is doubtful, and other observations by Park (1976) are as well, such as the presence of 4 setae on the 2nd segment of the 1st antenna in N VI, only 2 setae on the antennal basis, no seta on the mandibular coxa, and only 4 caudal setae in N V and N VI.

Unfortunately, details of other postembryonic studies of *Tisbe* are also incomplete, incorrect, or contradictory. The postembryonic development of *T. cucumariae* was described by Battaglia (1957) and Lopez (1980). There are several differences between Lopez's study and our present results which cannot solely be attributed to geographical differences of the investigated populations. The 2nd antennal basis was found to bear 4 setae throughout the phase in *Tisbe* not 3, and its exopod has 3 setae not 2 on the 4th segment in N I and N II. The mandibular protopod bears 2 setae throughout the phase, and the exopod is already 3 segmented in the N I stage and not 1 segmented as stated by Lopez. There are 2 medial setae on the antennal endopod from N I onwards not only from N II onwards. The 3rd segment of the antenna bears only 1 seta not 2 as stated by Lopez (1980) for N VI of *T. cucumariae*. This applies also to other oligoarthran

harpacticoids if the 3rd segment is the penultimate one (Dahms 1990). The final number of caudal setae is reached in the N V stage not in N IV and is 5 per side, not 4.

In this study, the middle of the 3 terminal antennular setae in N I is described as an aesthetasc, although its morphology is only

indicated but not distinct at this stage. It only becomes distinct in N II. It should be emphasized that in contrast to most previous descriptions of harpacticoid development, the mandibular protopod bears 2 setae from N I, also in cases where the coxa and basis are not distinctly separated. In *Tisbe*, the proximal-most one is



Fig. 4. *Tisbe* sp. development of the naupliar left 2nd antenna of naupliar stages I to IV in anterior view.

interpreted as belonging to the coxa/ precoxa and the distal one to the basis (Dahms 1990).

The anlage of what several authors called the 2nd maxilla in some *Tisbe* nauplii in N V and always in N VI may also be the maxilliped for one if both are missing in N VI. For the sake of compatibility with previous studies, we assumed this to be indications of the maxillae.

The most remarkable naupliar differences

among *Tisbe* species are in the postmandibular appendages which are best developed in the N VI stage. The lateral P1 and P2 anlagen were usually overlooked by previous investigators. These morphological differences are believed to have been caused by the functionless state (at least concerning locomotion and feeding) of the limb buds which allows the expression of different genotypes without selective forces that would



Fig. 5. *Tisbe* sp. development of the naupliar left 2nd antenna of naupliar stages V and VI in anterior view.

act on functional structures. In early copepodids where the previous naupliar Anlagen (Mx1-P2) are adapted to presumably quite similar functional necessities, differences between species are less striking (unpubl. observ.), at least among the species of *Tisbe* studied herein.

A case in point is provided by the differences reported by Lopez (1980) between his own *Tisbe cucumariae* and “*T. gracilis*” as described by Battaglia (1957). In fact the latter paper also refers to *T. cucumariae* and not to *T. gracilis* (Volkman 1979), and the discrepancies may for the most part have been due to the incompleteness of the earlier description. *Tisbe gracilis* was originally described by T. Scott (1985). Since then several authors have mentioned this species, some with misidentifications (see Volkman 1979). The species, which seems to prefer low temperatures (Volkman 1979), is regularly found associated with living and dead mollusks and ascidians. Like other tisbids, it appears to be a scavenger (e.g., Lang 1948).

Atrophied feeding structures can be caused by a non-feeding mode of living as well as by lecithotrophy. 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). As many of their representatives are phytalliving forms, one could argue that some *Tisbe* species, such as the Harpacticidae and Thalestridae, have independently evolved these characters. Alternatively, the reduction in certain feeding structures may provide phylogenetically valuable characters.

In the *T. holothuriae* group (see Dahms et al. 1991) the labrum and ventral body wall have lost 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 in N V is replaced by 1 long and 3 tiny setae in N VI. Similar reductions occur in N VI of *Harpacticus uniremis* and all other representatives of the Tachidiidae and Harpacticidae investigated so far (Dahms 1990).

Variability

While the number of setae does not vary at all in nauplii of *Tisbe*, the number of spinules does vary. On the antennal basis in N VI, there are 2 or 3 spinules in *T. furcata* and 5-7 spinules in the remaining species. The inner proximal row of spinules of the antennal endopod in N V of *T. cucumariae* is replaced by 1 spinule. The regular ontogenetic replacement of spinule rows by single larger spinules or spines is demonstrated by the substitution of the spinule crest of the terminal part of the mandibular endopod in N I by an anterior seta in N II (in *Tisbe* as well as in other oligoarthran harpacticoids, see Dahms 1990).

The number of setae on the 3rd antennular segment shows the greatest difference of all naupliar features of *Tisbe* as it is highly variable. We attribute this to difficulties in distinguishing between the spinules of the crest and those setae. Nevertheless, in some specimens of *T. bulbisetosa* in N VI, the additional seta of this segment which appears in N VI is developed only as a tiny blunt-shaped process.

Phylogenetic implications of naupliar characters among species of *Tisbe*

The resulting cladogram of the present phylogenetic analysis shows a bifurcation with *T. battagliai*, *T. holothuriae*, *T. bulbisetosa*, and *T. furcata* on 1 branch, and *T. gracilis*, *T. cucumariae*, and *T. thailandensis* on the other branch (Fig. 7). It supports the close relationship of the pairs *T. holothuriae*/*T. battagliai* and *T. gracilis*/*T. cucumariae* with high bootstrap values. These species were respectively claimed to belong to the *T. holothuriae* group and *T. gracilis* group of *Tisbe* species by Volkman (1979). Related to the *holothuriae* group is *T. bulbisetosa*, followed by *T. furcata*. *Tisbe furcata* and *T. thailandensis* are adjoined to their respective branches with low bootstrap values.

Phylogenetic implications of naupliar characters among the Tisbidae

Developmental instars of other tisbid genera have been described by several authors. Although there are 25 genera belonging to the family Tisbidae, naupliar stages are known only from 4 genera: *Tisbe*, *Tisbintra*, *Scutellidium*, and *Drescheriella*. Ummerkutty (1960) studied the postembryonic development of *Tisbintra*

jonesi. Brian (1919) and Gurney (1933) described nauplii of *S. longicauda*. Clogston in his unpublished PhD thesis studied *S. arthuri*

and *S. idyoides*, and Branch (1974) described some stages of *S. patellarum*. The complete postembryonic development of *Drescheriella*

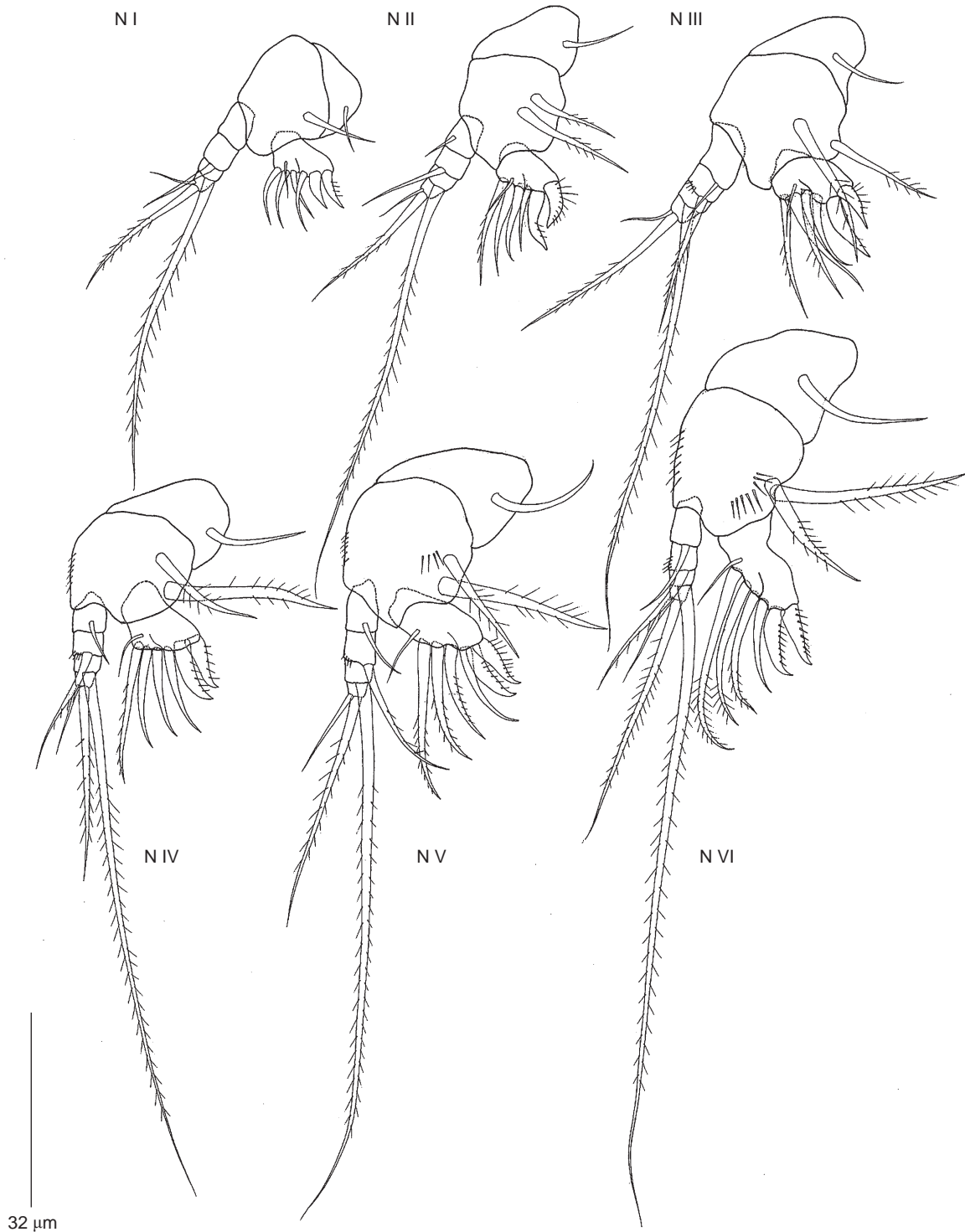


Fig. 6. *Tisbe* sp. development of the naupliar right mandible of naupliar stages I to VI in anterior view.

glacialis was investigated by Dahms and Dieckmann (1987). Dahms (1993a) compared the naupliar development of *Tisbe*, *Scutellidium*, and *Drescheriella*.

A sequence of reduction events in the segment number was observed in the mandibular exopod of the nauplii of 3 genera of the Tisbidae: *Scutellidium*, *Tisbe*, and *Drescheriella* (Dahms 1991 1993b). *Scutellidium hippolytes* bears 2 setae on the 1st segment of its 4 segmented mandibular exopod: the most proximal of these setae is absent from *T. cucumariae* and *D. glacialis*. *Scutellidium hippolytes* also bears 2 terminal setae on the small, knob like distal segment of its exopod; the border between the 4th segment of both of these setae is absent from the

3 segmented exopod of *T. cucumariae* and 5 other *Tisbe* species studied by Dahms et al. (1991b). In *D. glacialis* only 1 seta with an inflated base remains. In *T. cucumariae* the 2nd seta is present as a much smaller posterior seta in the middle of the proximal inflation at the base of the larger seta. In the apomorphic *D. glacialis* even this rudimentary seta has disappeared. The former 4th segment is only indicated as an inflated base of the outer seta in this species.

However, a close relationship of *Scutellidium* with *Tisbe* is widely accepted (Dahms 1993a). Lang's (1948) keys to genera of the Tisbidae does not allow a correct distinction of *Tisbe* and *Scutellidium*. According to Volkmann (1979), the members of 3 *Tisbe* groups (*Tisbe bermudensis* group, *T. angusta* group, and *T. tenella* groups) exhibit several morphological features and closely resemble *Scutellidium*. Among *Scutellidium*, *S. hippolytes* particularly resembles tisbids the most. There is additional evidence from copepodids to separate *S. hippolytes* from other species of *Scutellidium*. Clogston (unpubl.) described the proximal aesthetasc of *S. arthuri* as being situated on the 3rd segment and proximal proliferation of antennular segments taking place from CI to CII and from CII to CIII, whereas in *S. hippolytes*, as in other tisbids and Harpacticoida in general (Dahms 1989), segments come off distally from CI to CII, and the segment numbers of CII and CIII are the same.

Taking into account the special larval features of all species of *Scutellidium* known to him, but ignoring the larval features of *S. hippolytes*, Clogston (unpubl.) suggested removing the genus *Scutellidium* from the family Tisbidae and allocating it to the Porcellidiidae. There are several points against this proposal. The plesiomorphic state of *S. hippolytes* makes a clear distinction of a *Scutellidium* group impossible. Furthermore, there is no larval evidence for a sister group relationship between the Porcellidiidae and those species of *Scutellidium* bearing a larval sucker disc. The reduction of exopodal segments and the delayed development of the 1st maxilla may be adaptations to dorsoventral depression and holding onto surfaces by suction. They could, therefore, have evolved independently. Such reductions are shown by several unrelated forms, such as those species of the Diosaccidae with foreshortened nauplii and *Parathalestris harpacticoides* (Dahms 1990). The labrum of nauplii of the Porcellidiidae also does not resemble that of *Scutellidium* and is less structurally complex as can be seen from the

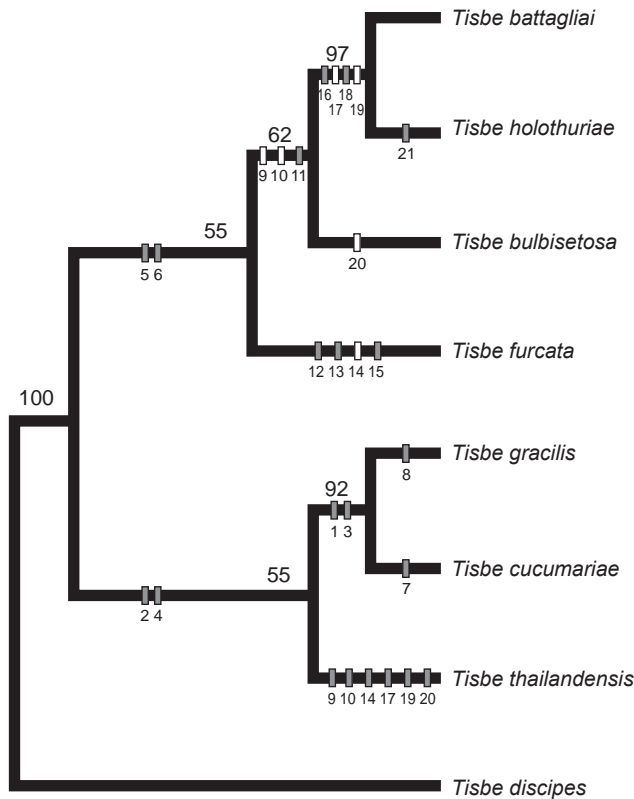


Fig. 7. Cladogram representing the most parsimonious tree (with a length of 27, a consistency index of 0.78, a retention index of 0.79, and an h index of 0.22) produced by an unweighted analysis of naupliar characters of *Tisbe*. Bootstrap values (1000 replicates) are shown for the resolved clades. Character numbers are shown below the nodes, and characters were optimized using an accelerated transformation. Homoplastic characters are indicated by open rectangles. The cladogram represents the most parsimonious tree produced by the unweighted analysis; bootstrap values are shown for the resolved clades.

drawings. If the genus *Scutellidium* were to have given rise to the Porcellidiidae, it is unreasonable that the complexity of this specialized structure would have been reduced (see Dahms 1993a).

There are several larval characters distinguishing *S. hippolytes* from all other species of the genus where larvae are described. Nauplii of *S. hippolytes* lack any indication of a labrum serving as a sucking organ as shown by Dahms (1990), whereas this is known from *S.* (syn. *Psamathe*) *longicauda* (Brian 1919, Gurney 1933), *S. patellarum* (Branch 1974), *S. arthuri*, and *S. idyoides* (Clogston 1965). This organ enables the nauplii of all stages to withstand the surge and shock of wave actions and to cling to smooth surfaces of algal fronds without being swept away. The antennal exopod is 4 segmented in *S. hippolytes* as shown by Dahms (1990), whereas it is 3 segmented in *S.* (syn. *Psamathe*) *longicauda* (Gurney 1933), *S. patellarum* (Branch 1974), *S. arthuri*, and *S. idyoides* (Clogston, unpubl.). The mandibular exopod is 3 segmented in *S. hippolytes* but 2 segmented in *S. longicauda*, *S. arthuri*, and *S. idyoides* (the 4 segmented exopod of *S. patellarum* mentioned by Branch (1974) is doubtful as this statement is not backed up by drawings). The 1st maxilla appears in N II for *S. hippolytes* and *S. patellarum*, whereas it is reported no earlier than in N III for *S. arthuri* and *S. idyoides*. Thus, the naupliar characters of *S. hippolytes* resemble those of *Tisbe* more closely than those of the other species of *Scutellidium* which are more adapted to dwelling on smooth algal surfaces through development of a naupliar labral sucker.

In conclusion, *Scutellidium* appears to be the less apomorphic taxon for the plesiomorphic state of 8 characters, compared to an outgroup consisting of all oligoarthran nauplii and especially those of Exanechentera sensu Lang (1948). *Tisbe* is closely related to *Drescheriella*. According to Dahms (1993a), these 2 genera form a monophyletic group characterized by at least 8 synapomorphies. Dahms found only 1 apomorphy for *Tisbe*, namely the anlage of the 1st maxilla bearing 3 setae and 1 spinule row in N IV (compared to 4 setae and 2 spinule rows as the plesiomorphic state). This is a character in the reduced state which occurs in other oligoarthran nauplii as well. *Drescheriella* represents the apomorphic branch of the monophylum *Tisbe* and *Drescheriella*, because of its 4 secondarily derived modifications which are all characters in a reduced state (Dahms 1990).

The intergeneric comparison of naupliar

characters revealed that *S. hippolytes* has relatively more plesiomorphic characters (Dahms 1993a). *Tisbe* shares many specific larval characters with *Drescheriella*. Thus, the phylogenetic affinity of the latter 2 taxa should be closer than that between *Tisbe* and *Scutellidium*. *Drescheriella* exhibits relatively more apomorphic characters due to reductions. This was previously recognized by Dahms and Dieckmann (1987) who viewed *Drescheriella* as the apomorphic sister group of *Tisbe* on the basis of adult morphology and character differentiation of copepodid limb characters alone.

CONCLUSIONS

Specific naupliar similarities provide synapomorphies for taxa in several cases (e.g., the *T. holothuriae* group), whereas naupliar divergence casts doubt on the monophyletic status of certain taxa (e.g., *Scutellidium*) or suggests new phylogenetic affinities that were hitherto unrecognized (*T. gracilis* and *T. cucumariae*, and *Tisbe* and *Drescheriella*). Naupliar morphology provides the greatest number of phylogenetically useful additional characters, whereas copepodids essentially foreshadow the adult condition.

Limitations of the utility of naupliar characters for phylogenetic inference studies are apparent, as well as their potential and importance for future work in zoological systematics. The phylogenetic relationships of tsebids are difficult to evaluate on a morphological basis alone, because of the comparatively small morphological differences among them. However, more specific morphological characters can be found, provided studies are sufficiently detailed. Phylogenetic conclusions can only be drawn when the developmental stages of enough species are sufficiently well known to allow meaningful comparisons. Phylogenetic relationships should be studied with an integrative approach, where morphological characters of adults and larvae are used in concert with behavioral, ecological, biochemical, and molecular genetic characters (Dahms and Schminke 1995).

Life history studies in the field and investigations of stage specific phenomena in the laboratory are generally hampered by a lack of descriptive information and missing identification keys that are the basis of all work on stage specific phenomena in the laboratory and heterogeneous assemblages in the field (Dahms et al. 2006 2007).

Much rearing and descriptive work has to precede any serious attempt to tackle ontogeny related problems.

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REFERENCES

- Barnard JL, DJ Reish. 1960. Wood-browsing habits of the harpacticoid copepod *Tisbe gracilis* (T. Scott) in southern California. *Pac. Nat.* **22**: 9-12.
- Battaglia B. 1957. Ricerche sul ciclo biologico di *Tisbe gracilis* (T. Scott), (Copepoda, Harpacticoida), studiato in condizioni di laboratorio. *Archo Oceanogr. Limnol.* **11**: 29-46.
- Battaglia B, P Talamini. 1957. Osservazioni sullo sviluppo larvale di *Tisbe reticulata* Bocquet (Copepoda, Harpacticoida). *Archo Oceanogr. Limnol.* **11**: 63-68.
- Bechmann RK. 1999. Effect of the endocrine disrupter nonylphenol on the marine copepod *Tisbe battagliai*. *Sci. Total Environ.* **233**: 33-46.
- Bergmans M. 1984. Life history adaptation to demographic regime in laboratory-cultured *Tisbe furcata* (Copepoda, Harpacticoida). *Evolution* **38**: 292-299.
- Bodin P. 1997. Catalogue of the new marine harpacticoid copepods. 1997 ed. Studiedocumenten van het K.B.I.N. = Documents de Travail de l'I.R.Sc.N.B., 89. Brussels, Belgium: Koninklijk Belgisch Instituut voor Natuurwetenschappen, 304 pp.
- Boxshall GA, SH Halsey. 2004. An introduction to copepod diversity. 2 vols. London: The Ray Society, 966 pp.
- Branch GM. 1974. *Scutellidium patellarum* n. sp., a harpacticoid copepod associated with *Patella* spp. in South Africa, and a description of its larval development. *Crustaceana* **26**: 179-200.
- Brian A. 1919. Sviluppo larvale della *Psamathe longicauda* and *Harpacticus uniremis* Kröyer (Copepodi Harpacticoidi) (Descrizione della serie Copepodiforme). *Atti Soc. Ital. Sci. Nat.* **58**: 29-58.
- Chua TE. 1975. The developmental stages of *Tisbe longisetosa* Gurney, 1927 (Copepoda, Harpacticoida). *Crustaceana* **28**: 158-162.
- Clogston FL. 1965. Postembryonic development of species of harpacticoid copepods from the Pacific coast of the United States and an application of developmental patterns to their systematics. PhD dissertation, Univ. of Washington, Seattle, WA, 1-246.
- Dahms HU. 1987. Postembryonic development of *Drescheriella glacialis* Dahms and Dieckmann (Copepoda, Harpacticoida) reared in the laboratory. *Polar Biol.* **8**: 81-93.
- Dahms HU. 1989. First report of a lecithotrophic nauplius among Harpacticoida (Crustacea, Copepoda) from the eastern Weddell Sea. *Polar Biol.* **10**: 221-224.
- Dahms HU. 1990. Naupliar development of Harpacticoida (Crustacea, Copepoda) and its significance for phylogenetic systematics. *Mikrof. Mar.* **6**: 169-272.
- Dahms HU. 1991. Usefulness of postembryonic characters for phylogenetic reconstruction in Harpacticoida (Crustacea, Copepoda). *Bull. Plankt. Soc. Jpn. Spec. Vol.* **1991**: 87-104.
- Dahms HU. 1993a. Naupliar development of *Scutellidium hippolytes* (Copepoda, Harpacticoida) and a comparison of nauplii within Tisbidae. *Hydrobiologia* **250**: 1-14.
- Dahms HU. 1993b. Comparative copepodid development in Tisbidimorpha sensu Lang, 1948 (Copepoda, Harpacticoida) and its bearing on phylogenetic considerations. *Hydrobiologia* **250**: 15-37.
- Dahms HU. 2000. Phylogenetic implications of the crustacean nauplius. *Advances in copepod taxonomy. Hydrobiologia* **417**: 91-99.
- Dahms HU, M Bergmans. 1988. Postembryonic development of *Tisbe gracilis* (T. Scott) (Copepoda, Harpacticoida). *Zool. Scripta* **17**: 357-369.
- Dahms HU, S Chullasorn, P Kangtia, FD Ferrari, JS Hwang. 2007. Naupliar development of *Tigriopus japonicus* Mori, 1932 (Harpacticidae, Copepoda). *Zool. Stud.* **46**: 541-554.
- Dahms HU, GS Dieckmann. 1987. *Drescheriella glacialis* gen. nov., sp. nov. (Copepoda, Harpacticoida) from Antarctic sea ice. *Polar Biol.* **7**: 329-337.
- Dahms HU, JA Fornshell, BJ Fornshell. 2006. Key for the identification of crustacean nauplii. *Organisms Divers. Evol.* **6**: 47-56.
- Dahms HU, T Harder, PY Qian. 2007. Selective attraction and reproductive performance of a harpacticoid copepod in a response to biofilms. *J. Exp. Mar. Biol. Ecol.* **341**: 228-238.
- Dahms HU, S Lorenzen, HK Schminke. 1991a. Phylogenetic relationships within the taxon *Tisbe* (Copepoda, Harpacticoida) as evidenced by naupliar characters. *Z. zool. Syst. Evolutforsch.* **29**: 450-465.
- Dahms HU, PY Qian. 2004. Life histories of the Harpacticoida (Copepoda, Crustacea): a comparison with meiofauna and macrofauna. *J. Nat. Hist.* **38**: 1725-1734.
- Dahms HU, PY Qian. 2005. Exposure of biofilms to copepods affects the larval settlement of *Hydroides elegans* (Polychaeta). *Mar. Ecol.-Prog. Ser.* **297**: 203-214.
- Dahms HU, N Schizas, T Shirley. 2005. Naupliar evolutionary novelties of *Stenhelia peniculata* (Copepoda, Harpacticoida) from Alaska affirming taxa belonging to different categorical rank. *Invertebr. Zool.* **2**: 1-14.
- Dahms HU, HK Schminke. 1995. A multidisciplinary approach to the fine-systematics within *Tisbe* – an evaluation of morphological and molecular methods. *Hydrobiologia* **308**: 45-50.
- Dahms HU, HK Schminke, M Pottek. 1991b. A redescription of *Tisbe furcata* (Baird, 1837) (Copepoda, Harpacticoida) and its phylogenetic relationships within the Taxon *Tisbe*. *Z. zool. Syst. Evolutforsch.* **29**: 433-449.
- Efron B, E Halloran, S Holmes. 1996. Bootstrap confidence levels for phylogenetic Trees. *Proc. Natl. Acad. Sci. USA* **93**: 7085-7090.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
- Ferrari FD, HU Dahms. 2007. Postembryonic development of the Copepoda. *Crust. Monogr.* **8**: 1-232.
- Griga RE. 1960. Development of some Harpacticoida of the Black-Sea. *Trudy Sevastopol. Biol. Sta.* **13**: 68-77.
- Gurney R. 1933. Notes on some Copepoda from Plymouth. *J. Mar. Biol. Assoc. UK* **19**: 299-304.

- Hutchinson TH, NA Pounds, M Hampel, TD Williams. 1999. Life-cycle effects of 20-hydroxyecdysone and diethylstilbestrol on the marine copepod *Tisbe battagliai*. Environ. Toxicol. Chem. **18**: 2914-2920.
- Ivanenko VN, FD Ferrari, HU Dahms. 2008. Naupliar development of *Tegastes falcatus* (Copepoda, Harpacticoida). J. Crust. Biol. **28**: 270-280.
- Izawa K. 1987. Studies on the phylogenetic implications of ontogenetic features in the poecilostome nauplii (Copepoda: Cyclopoida). Publ. Seto Mar. Biol. Lab. **32**: 151-217.
- Johnson MW, JB Olson. 1948. The life history and biology of a marine harpacticoid copepod, *Tisbe furcata* (Baird). Biol. Bull. (Woods Hole) **95**: 320-332.
- Koga F. 1984. Morphology, ecology, classification and specialization of copepod nauplii. Bull. Nansei Reg. Fish. Res. Lab. **16**: 95-229.
- Lang K. 1948. Monographie der Harpacticiden I und II. Koenigstein, West Germany: Reprint Otto Koeltz Science Publ., pp. 1-1682.
- Lopez GW. 1980. Description of the larval stages of *Tisbe cucumariae* (Copepoda: Harpacticoida) and comparative development within the genus *Tisbe*. Mar. Biol. **57**: 61-71.
- Matthews JBL. 1964. On the biology of some bottom living copepods (Aetideidae and Phaennidae) from western Norway. Sarsia **16**: 1-46.
- Parise A. 1978. Population dynamics and food supply in a copepod of the genus *Tisbe*. Boll. Zool. **45**: 375-381.
- Park CW. 1976. The larval development of *Tisbe holothuriae* Humes (Copepoda, Harpacticoida) under the laboratory conditions. Publ. Inst. Mar. Sci. natn. Fish. Univ. Busan **9**: 33-48.
- Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sunderland, MA: Sinauer Associates.
- Ummerkutty ANP. 1960. Studies on Indian copepods 2. An account of the morphology and life history of a harpacticoid copepod, *Tisbintra jonesi*, sp. nov. from the Gulf of Mannar. J. Mar. Biol. Assoc. India **2**: 149-164.
- Vilela MH. 1969. The life cycle of *Tisbe* sp. (Copepoda, Harpacticoida) under laboratory conditions. Notas Estud. Inst. Biol. Mar. Lisb. **36**: 1-16.
- Volkman-Rocco B. 1971. Some critical remarks on the taxonomy of *Tisbe* (Copepoda, Harpacticoida). Crustaceana **21**: 127-132.
- Volkman B. 1979. A revision of the genus *Tisbe* (Copepoda, Harpacticoida). Part I. Archo Oceanogr. Limnol. **19(Supplement)**: 121-284.