Zoological Studies **51**(3): 372-382 (2012)

Zoological Studies

Naupliar Development of an Ancorabolid, *Paralaophontodes* sp. (Copepoda: Harpacticoida) Sheds Light on Harpacticoid Evolution

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(Accepted November 24, 2011)

Supawadee Chullasorn, Hans-U. Dahms, Nozomu Iwasaki, Pawana Kangtia, Frank D. Ferrari, Hyoung Joo Jeon, and Wan-Xi Yang (2012) Naupliar development of an ancorabolid, *Paralaophontodes* sp. (Copepoda: Harpacticoida) sheds light on harpacticoid evolution. *Zoological Studies* **51**(3): 372-382. Loss of the naupliar arthrite during the molt to naupliar stage (N) VI provides a developmental apomorphy for all Harpacticoida and sheds light on the evolution within this diverse copepod taxon. Naupliar development of *Paralaophontodes* sp. is unusual because the bud of swimming legs 1 and 2 does not bear setae at N VI and because the naupliar arthrite, present on the coxa of antenna 2 during the 1st 5 stages, fails to form during the molt to N VI; in addition, setal elements are lost from the basis and endopod of the mandible during this molt. This is only known from some harpacticoid copepods belonging to *Tisbe*; loss of setal elements on the mandible was also otherwise reported for species of the Tachidiidae and Harpacticidae. Five naupliar stages of the ancorabolid, *Paralaophontodes* sp., are described. A key to the identification of the stages is provided. Stages can be distinguished by the number of segments of the exopod of antenna 2, setation of the limbs including the bud of the caudal ramus, and the presence and setation of the bud of maxilla 1. This is the 1st description of nauplii of a species belonging to the oligoarthran family Ancorabolidae.
http://zoolstud.sinica.edu.tw/Journals/51.3/372.pdf

Key words: Naupliar characters, Evolution, Systematics, Phylogeny, Development, Ancorabolidae.

Larvae of copepods provide a rich source of information about their structure, genetics, behavior, systematics, evolution, and ecology (Dahms et al. 2007, Ferrari and Dahms 2007). This includes valuable information for reconstructing evolutionary histories and determining phylogenetic relationships (Barnett 1966, Dahms 1991 2004, Ferrari 1998, Chullasorn et al. 2009, Dahms et al. 2009, Ferrari et al. 2010). Development of copepod larvae can be divided into naupliar and copepodid phases (Dahms et al. 2005 2006). Copepod nauplii are much smaller relative to copepodids, and do not show clear external expression of somites, although the naupliar body increases in size from 1 stage to the next, and limb buds are often added in a way that predicts evolutionary trends of increasing somite numbers (Ferrari and Dahms 2007). The caudal rami of early nauplii have 3 well-developed, segmented appendages and seta-bearing buds. Buds of some limbs between the mandible and

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caudal ramus may be added during subsequent stages of naupliar development (Ivanenko et al. 2008). In contrast, during the copepodid phase, several somites are clearly separated by arthrodial membranes, limbs are segmented and functional with the exception of at most 1 limb bud, and the size of the copepodids significantly increases during their development (Ferrari and Dahms 2007). Yet, despite the organizational constraints on nauplii, relative to copepodids, nauplii may exhibit a significant diversification in morphology (Dahms 1990, Dahms and Qian 2004).

Naupliar development has not been reported for any species of the Ancorabolidae. *Paralaophontodes*, with only 4 species, is one of the least diverse among the 22 nominal genera belonging to this oligoarthran copepod family. The purposes of the present study were to discuss the unique and shared attributes of these interesting harpacticoid copepods in phylogenetic and evolutionary contexts, provide the 1st complete description of the naupliar stages of an unnamed species of *Paralaophontodes* of the family Ancorabolidae, which is similar to *P. echinata* (Fiers 1986 for a complete redescription), and provide a key for identifying its naupliar stages that can serve other disciplines, particularly systematics and ecology.

MATERIALS AND METHODS

Collection data

Nauplii of *Paralaophontodes* sp. were collected on 5 Jan. 2007 within sediments from an aquarium at Kerama Pearl, a company that cultures juvenile pearl oysters on Zamami I., Okinawa, Japan. Juvenile pearl oysters are collected locally, and maintained in aquaria with local seawater that has passed through a sand filter before entering the aquaria. A sample of sediment with all developmental stages of *Paralaophontodes* sp. was fixed in ca. 5% neutralized formalin immediately after capture, transferred to 70% ethanol, and subsequently placed in glycerol.

Preparation

Specimens in glycerol were placed on microscopic glass slides along with glass-fibers to prevent the whole mounts from being compressed by the cover glass and to facilitate rolling to allow inspection of structures from all sides. Mounted specimens were observed with phase-contrast microscopy. Measurements of the body were taken from the anterior edge of the cephalic shield to the posterior-most protrusion of the hind body (for the length), and across the greatest lateral distance of the cephalic shield (for the width). Only drawn specimens were considered for length measurements. In order to determine stage-specific variability, 2-5 specimens per stage were examined, and specimens that were drawn were chosen from those specimens that were measured.

Terms and identification

First to 6th naupliar stages (N) are abbreviated N I to N VI, respectively. Articulating elements of an appendage segment are setae, and variations in the size and shape of setae were described; modified chemosensory setae are called aesthetascs. The naupliar arthrite is a ventrally articulating element originating on the coxa of antenna 2, and is moved by a pair of intrinsic muscles (Ferrari et al. 2011). The species was identified with aids provided by Lang (1948 1965), Bodin (1977), Wells (1985), Huys et al. (1996), and Boxshall and Halsey (2004).

RESULTS

Naupliar stages are oval in shape and dorsoventrally flattened (Fig. 1); the cephalic shield is smooth throughout the naupliar phase.

Nauplius I (Fig. 2A)

Body slightly longer than wide, with cephalic shield. Body length 69.0 μm, width 58.0 μm. Subcircular labrum with spinules along lateral corner and posterior margin. Ventral field unornamented. First antenna 3-segmented (Fig. 4A). Proximal segment with 1 ventral seta. Middle segment with 2 thick, blunt setae ventrally and denticles dorsally. Distal segment ventrally with 1 thick, blunt seta; terminally with 2 setae and 1 aesthetasc terminally; with 1 small seta dorsally. Second antenna (Fig. 5A) with coxa, basis, 2-segmented exopod, and 1-segmented endopod. Coxa with row of denticles dorsally and naupliar arthrite ventrally with 1 seta near its base. Basis with denticles near distal edge. Exopod 2-segmented; proximal segment dorsally with 1 thick, blunt seta and 1 thin seta proximally;

terminal segment with 1 thick, blunt seta and 2 thin setae terminally. Endopod with 1 thin seta ventrally and with 1 long, thick, curved seta and 1 thin seta terminally. Mandible (Fig. 6A), protopod with unarmed coxa; basis with 1 thick, blunt seta ventrally and denticles distally. Exopod 4-segmented; proximal segment unarmed, antepenultimate segment dorsally with thick,

Fig. 1. *Paralaophontodes* sp. Naupliar stage III in ventral (A), lateral (B), and dorsal (C) views.

Fig. 2. *Paralaophontodes* sp. Naupliar stage (N) I (A), N III (B), N IV (C), and N V (D) in ventral view; the articulation plane of the 1st 3 pairs of appendages is indicated by the dashed line.

blunt seta, penultimate segment with 1 thick, long seta dorsally, terminal segment with 1 thick, long and with 1 thin, short setae terminally. Endopod 1-segmented with 2 thick setae terminally. Caudal ramus a bud with 1 seta.

Nauplius III (Fig. 2B)

Differs from N I as follows: body length 80.0 μm, body width 66.4 μm. First antenna distal segment with 1 short, thin seta dorsally and 1 long, thin seta ventrally (now 6 setae). Second antenna basis ventrally with 1 short, thin seta (now 4 setae); exopod 3-segmented, proximal segment with 2 thin setae, middle segment with 1 blunt, thick seta. First maxilla as a bud with 2 terminal setae and denticles.

Nauplius IV (Fig. 2C)

Differs from N III as follows: body length 91.0 μm, body width 73.6 μm. First antenna distal segment with 1 short, thin seta ventrally (now 7 setae). Second antenna exopod 4-segmented, penultimate segment added with 1 long, thin seta. Mandible endopod with 1 long, thin seta (now 5 setae).

Nauplius V (Fig. 3A)

Differs from NIV as follows: body length 93.4 μm, body width 70 μm. First antenna distal segment with 1 short, thin seta ventrally (now 8 setae). Caudal ramus as a bud with 3 setae.

Nauplius VI (Fig. 3B)

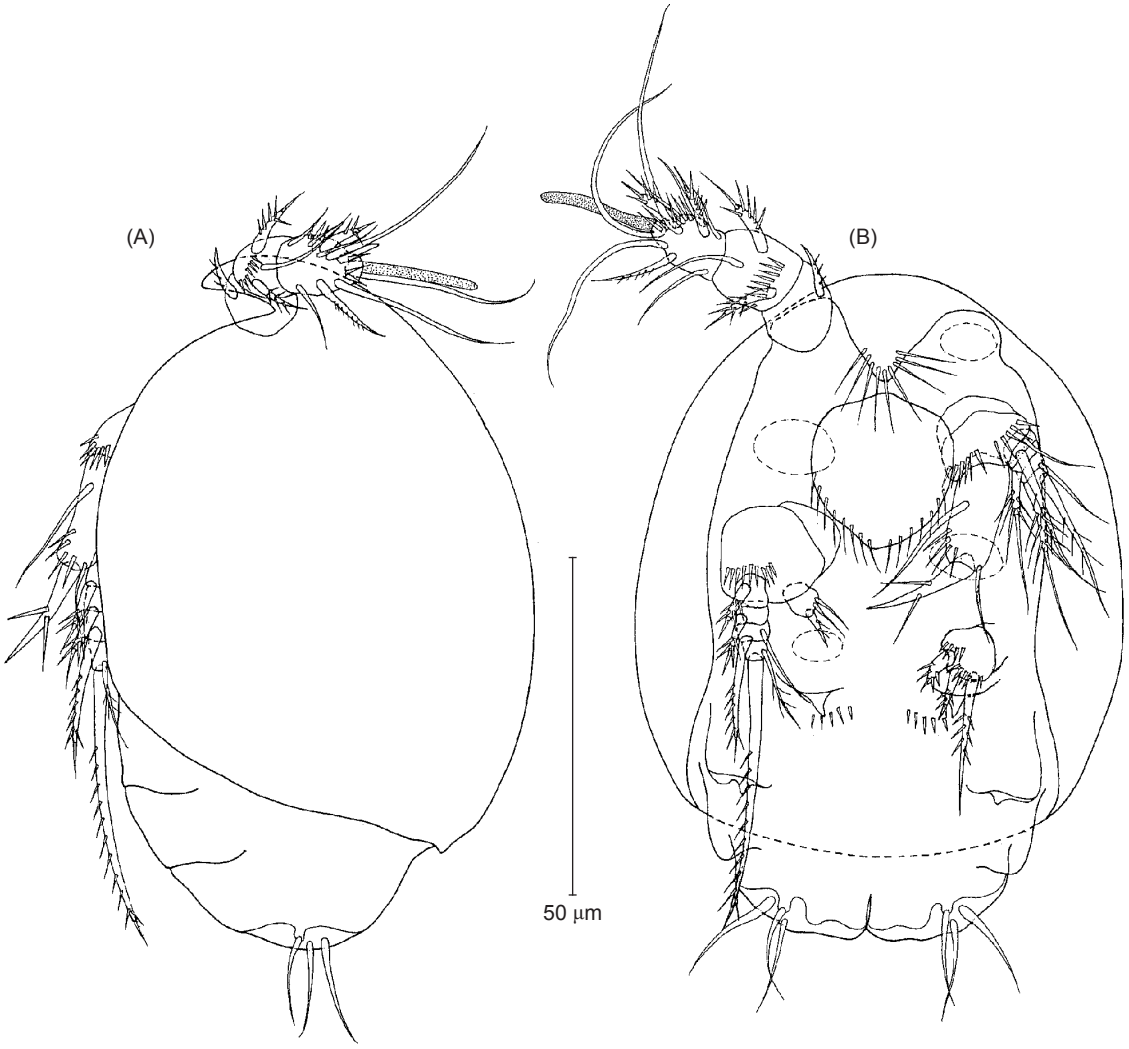

Fig. 3. *Paralaophontodes* sp. Naupliar stage (N) IV in lateral view (A), and N VI in ventral (B) view; the articulation plane of the 1st 3 pairs of appendages is indicated by the dashed line.

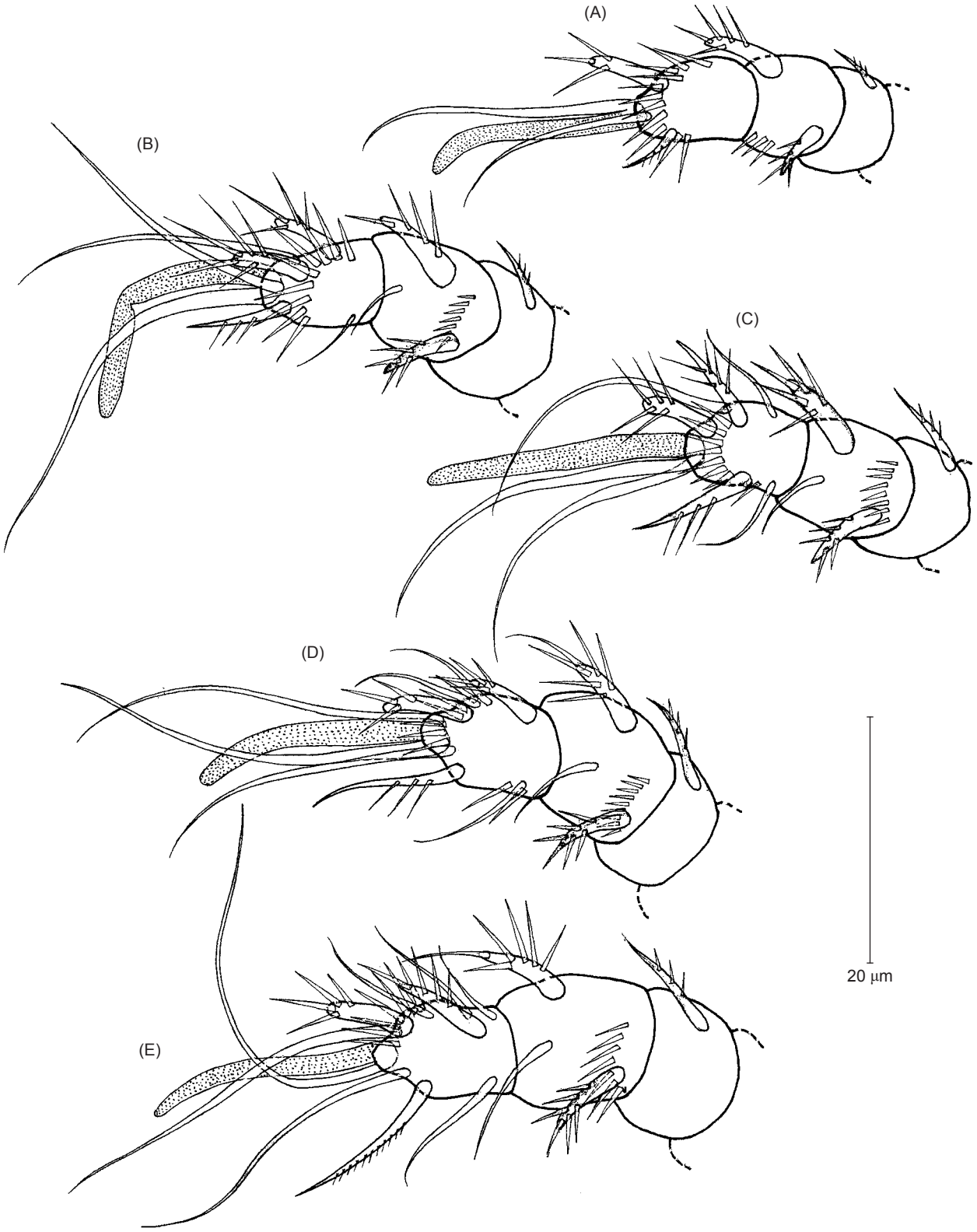

Fig. 4. *Paralaophontodes* sp. Development of the naupliar right 1st antenna of naupliar stages I, III, IV, V, and VI in anterior view.

Differs from NV as follows: body length 104.5 μm, body width 72.7 μm. First antenna (Fig. 4F), distal segment with 1 short, thin seta ventrally (now 9 setae). Second antenna (Fig. 5F), naupliar arthrite on coxa and 3 ventral setae on basis failing to form. Mandible (Fig. 6D), thick, blunt seta on basis and 2 thick, blunt setae on endopod failing to form; endopod with 3 setae.

Remarks: All 6 naupliar stages of *Paralaophontodes* sp. have 3 pairs of segmented limbs: 1st and 2nd antennae and mandibles. The body is covered by a smooth cephalic shield, and the posterior region of the body is extended at later stages. The labrum originates as a lobe-like flap near the frontal margin of the body, between the bases of the 1st antennae, and extends posteriorly to cover the mouth and tips of the naupliar arthrite. The bud of maxilla 1 develops from N II onwards. The exopod of antenna 2 is the only ramus that adds segments during development, although

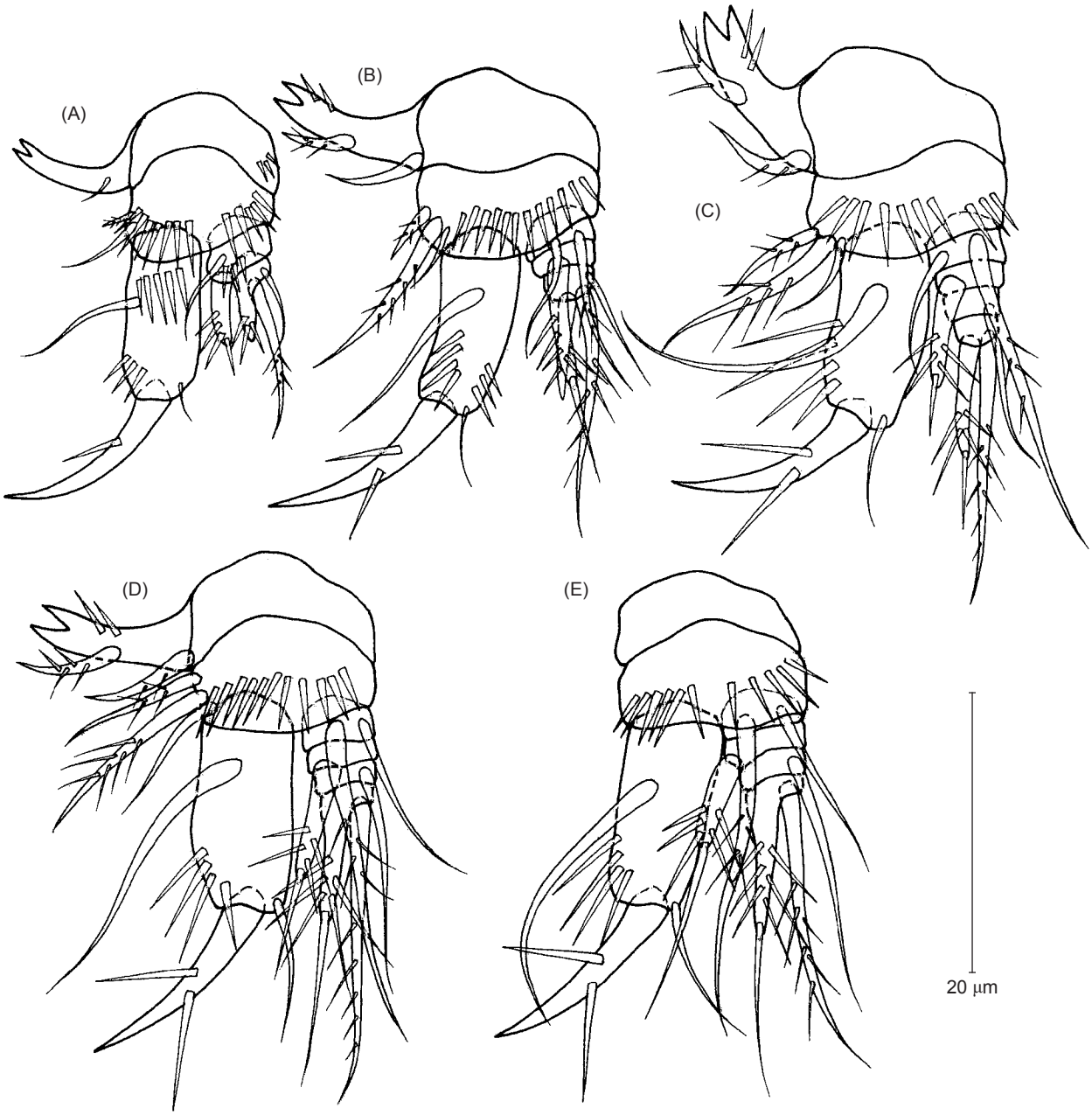

Fig. 5. *Paralaophontodes* sp. Development of the naupliar left 2nd antenna of naupliar stages I, III, IV, V, and VI in anterior view.

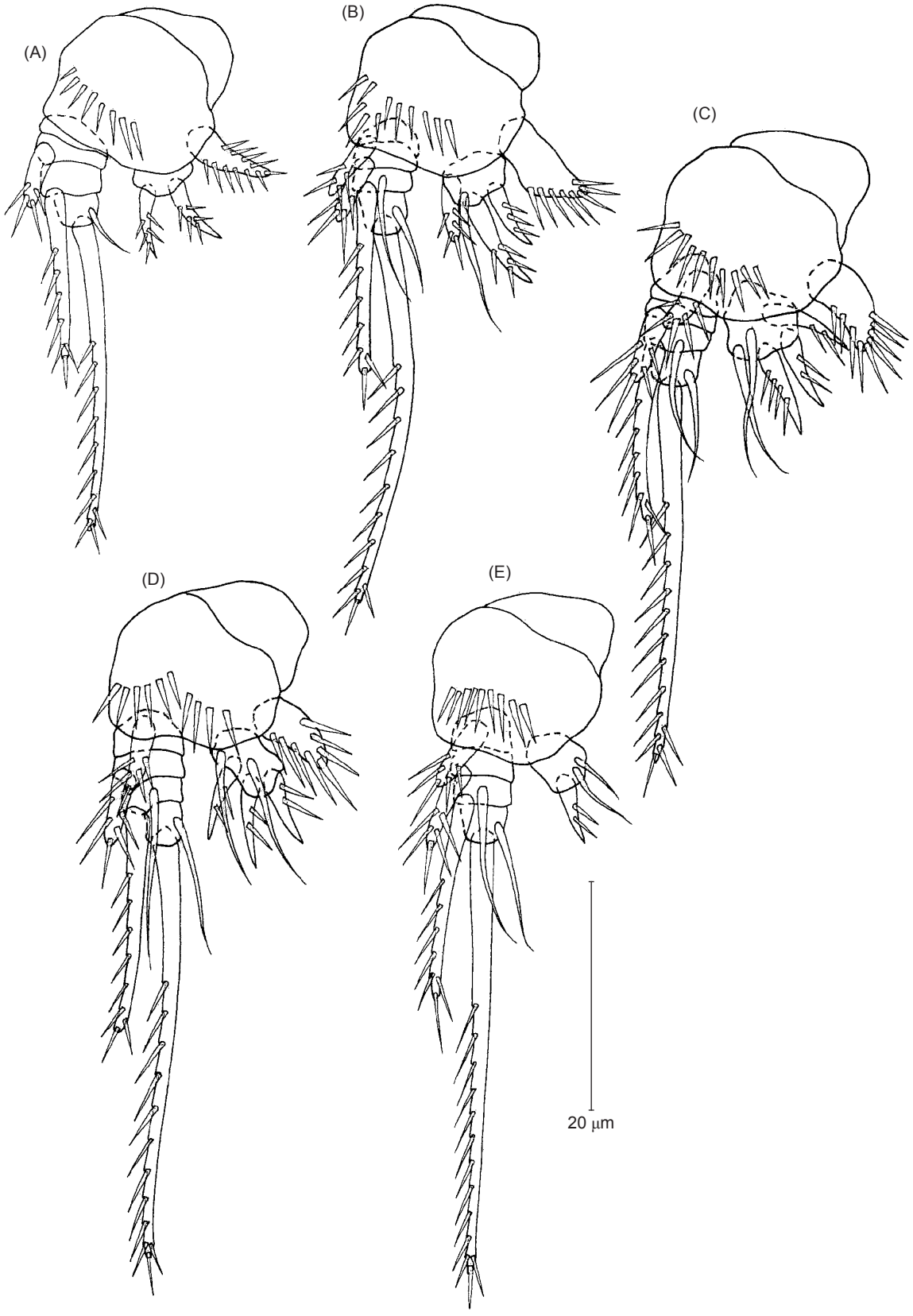

Fig. 6. *Paralaophontodes* sp. Development of the naupliar right mandible of naupliar stages I, III, IV, V, and VI in anterior view.

the number of setae on most limbs, including the bud of the 1st maxilla and caudal ramus, increase during naupliar development. Exceptions include the basis and endopod of the mandible, both of which lose setae during the molt to N VI. A naupliar arthrite, present on the coxa of antenna 2 from N I to N V, also fails to form during the molt to N VI, and setose buds of swimming legs 1 and 2 are not present at N VI. Such exceptions are unusual for copepods.

Key to naupliar stages of *Paralaophontodes* sp.

1. First maxilla absent; bud of caudal ramus with 1 seta; distal segment of 1st antenna with 3 setae ..... N I
- First maxilla present; bud of caudal ramus with more than 1 seta; exopod of 2nd antenna more than 2-segmented; distal segment of 1st antenna with more than 3 setae 2
2. First maxilla presumably a bud with 1 seta ..... N II
- First maxilla a large bud with 2 setae; exopod of 2nd antenna 2-segmented; distal segment of 1st antenna with more than 4 setae 3
3. Exopod of 2nd antenna 3-segmented; distal segment of 1st antenna with 6 setae ..... N III
- Exopod of 2nd antenna 4-segmented; distal segment of 1st antenna with more than 6 setae 4
4. Distal segment of 1st antenna with 7 setae ..... N IV
- Caudal ramus with 3 setae; distal segment of 1st antenna with more than 7 setae 5
5. Distal segment of 1st antenna with 8 setae ..... N V
- Naupliar arthrite absent; distal segment of 1st antenna with 9 setae ..... N VI

DISCUSSION

Since this is the 1st naupliar description of a representative of the family Ancorabolidae, a closer look at its hypothesized sister taxon, the Laophontidae, might be useful. The bulk of the literature dealing with laophontid postembryonic development refers to the genus *Laophonte*. Gurney (1931) described the N I of *L. brevirostris* and *L. cornuta*; Raibaut (1963) described 5 stages of *L. commensalis*; and Goswami (1977) described all naupliar stages of *L. setosa*. Barnett (1966) compared the N I of *Platychelipus littoralis* with that of *P. laophontoides*. With 1 exception, nauplii in all of those studies bear 1-segmented antennal and mandibular exopodites, whereas the mandibular exopodite of *Heterolaophonte minuta* of the present study is regarded as being 2-segmented. The exception is the antennal exopodite of *L. setosa* described by Goswami (1977) as being 3-segmented in all naupliar stages, which we consider doubtful. An interesting detail was given by Raibaut (1963) who described tiny spinules on the naupliar shield of *L. commensalis*. A spinulated naupliar shield is only shared with the cletodid, *Rhizothrix minuta*, described by Dahms (1990), whereas all other harpacticoid nauplii share a smooth shield. This corroborates with Lang's (1948) suggestion of a close phylogenetic relationship of the 2 families based on adult characters of the Laophontidae and Cletodidae. Larval characters support this hypothesis. Peculiar naupliar characters common to both families are as follows: an elongated labrum, several spinule rows on the antennule, a 1-segmented antennal exopodite, a 2-segmented mandibular exopodite and similar setation, the short terminal seta of the mandibular exopodite, and especially the above-mentioned ornamented naupliar shield shared by the Laophontidae and Cletodidae.

However, the nauplius of *Heterolaophonte minuta* of the Laophontidae was shown with a wide, blade-like pinnate seta on the mandibular basis by Dahms (1990). Otherwise, this character is present only in the Canthocamptidae. This would support the proposed affinity of the Cletodidimorpha and Ameiridimorpha if one assumes its secondary reduction among some representatives of the remaining taxa belonging to both groups.

Most nauplii do not feed in the 1st naupliar stage, even if later stages are planktotrophic. The antennal gnathobase is, therefore, little differentiated in the 1st stage (Dahms 1990). A naupliar arthrite might not be present in the 1st stage of many harpacticoid nauplii that do not feed in the 1st stage, even if later stages presumably feed and are planktotrophic (Dahms 1990). This delayed formation of the naupliar arthrite is also found among calanoids like *Calanus finmarchicus* (Ferrari and Dahms 2007). Copepods in which a naupliar arthrite fails to form may also pass through fewer naupliar stages than copepods that pass through 6 naupliar stages (Matthews 1964, Izawa 1987, Dahms 1989). In addition, nauplii of calanoid copepods without a naupliar arthrite also may lack a labrum, a mouth opening, and an open gut, e.g., *Euchaeta norvegica* (Nicholls 1934) of the Euchaetidae, and species of *Aetideus* and *Chiridius* in the family Aetideidae (Matthews 1964).

Atrophied feeding structures such as observed here in the N VI stage of *Paralaophonte* sp. can be caused by cessation of feeding due to a developmental transition from the naupliar to the copepodid phase of development. Lecithotrophic nauplii that do not feed, as they live on yolk supplyd by their eggs, occur in different taxa

of the Crustacea, such as in the Penaeidea, Euphausiacea, Copepoda, and Branchiopoda. Lecithotrophy often, but not necessarily, leads to an abbreviation of naupliar stages (Matthews 1964, Izawa 1987, Dahms 1989). Free-living copepod groups such as the bathypelagic Misophrioida also have non-feeding nauplii (Ferrari and Dahms 2007). Also, the calanoid *Euchaeta norvegica* has nauplii that lack a labrum, mouth opening, and gut tube (Nicholls 1934). Similar nauplii occur with similar reductions due to lecithotrophy in *Aetideus* and *Chiridius* of the calanoid family Aetideidae (Matthews 1964). As many of their representatives are phytal-living forms such as the Harpacticidae, Thalestridae, and some *Tisbe* species, it is possible that those representatives have independently evolved such characters. In the *T. holothuriae*-group, as in *T. battagliai* and *T. holothuriae*, the masticatory process of the antennal coxa is reduced to a spinulose seta; the proximal spinules on the endopod of the antenna are reduced in number and size; the medial 2nd seta is tiny; and the 4th seta of the distal segment of the antennal exopod is missing among this pair of sibling species (Dahms et al. 1991).

In *Paralaophontodes* sp. the labrum is smaller at N VI, and the ventral body labrum has lost its ornamentation. The naupliar arthrite is reduced to a simple 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 remains. The seta of the mandibular coxa is replaced by a hump-like structure along the ventral margin; the basal 3 setae are reduced in length; and 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 to date (Dahms 1990).

Acknowledgments. The work of S. Chullasorn is supported by Ramkhamhaeng University (Bangkok). The work of H.-U. Dahms is supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-A001-0057). The work of W.X. Yang is supported by the National Nature Science Foundation of China (Grant No. 31072198 and 30671606).

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