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Zoeal Stages of *Harrovia japonica* (Decapoda: Brachyura: Pilumnidae) with a Key to the Known Eumedoninid Zoeae from the Indo-Pacific

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Abstract: We obtained four zoeal stages of *Harrovia japonica* from laboratory-hatched material. They are described, illustrated in detail, and compared with those of other known species in the family Pilumnidae. The general zoeal morphology of *H. japonica* coincides well with those of other known species of the Eumedoninae (excluding *Echinoecus pentagonus*) and *Pilumnus minutus* of the Pilumninae. In order to facilitate the study of plankton-collected material, we provide a key for the identification of seven known zoeae of the Eumedoninae from the Indo-Pacific.

Key words: *Harrovia japonica*, *Pilumnus minutus*, Pilumnidae, Eumedoninae, zoeal morphology, key

INTRODUCTION

Classification of eumedonids has been confused whether the taxon should be designated as a distinct family (Stevcic et al., 1988; Chia and Ng, 2000; Martin and Davis, 2001) within the Xanthoidea or a subfamily (Lim and Ng, 1988) of the Pilumnidae Samouelle, 1819 (see Van Dover et al., 1986). Based on larval and adult characters, Ng and Clark (2000) has come to the conclusion that eumedonids should be a subfamily of the Pilumnidae. Currently the family Pilumnidae contains five subfamilies: Calmaniinae, Eumedoninae, Pilumninae, Rhizopinae, and Xenophthalmodinae (see Ng et al., 2008). However, larval descriptions are confined to two subfamilies, Eumedoninae and Pilumninae. In the Eumedoninae, larval stages of six species are known: *Echinoecus pentagonus* (A. Milne Edwards, 1879) by Van Dover et al. (1986), *Harrovia albolineata* Adams and White, 1848 by Chia et al. (1993), *Harrovia longipes* Lanchester, 1900 (as *H. albolineata*) by Lim and Ng (1988), *Permanotus purpureus* (Gordon, 1934) by Ng and Clark (2000), *Rhapdonotus pictus* A. Milne Edwards, 1879 by Chia and Ng (1995), and *Zebrida adamsii* White, 1847 by Mori et al. (1991). On the other hand, in the Pilumninae larval stages of 15 species are known: *Actumnus setifer* (De Haan, 1835) by Aikawa (1937), *Benthopanope eucratoides* (Stimpson, 1858) by Lim et al. (1986), *Benthopanope indica* (de Man, 1887) by Ko (1995), *Heteropanope glabra* Stimpson, 1858 by Greenwood and Fielder (1984a), *Heteropilumnus ciliatus* (Stimpson, 1858) by Ko and Yang (2003), *Parapilumnus trispinosus* Sakai, 1965 by Ko (1994a), *Pilumnopeus serratifrons* (Kinahan, 1856) by Greenwood and Fielder (1984b), *Pilumnopeus makiana* (Rathbun, 1931) by Lee (1993), *Pilumnopeus granulata* Balss, 1933 by Ko (1997), *Pilumnus dasypodus* Kingsley, 1879 by Bookhout and Costlow (1979), *Pilumnus vespertilio* (Fabricius, 1793) by Lim and Tan (1981), *Pilumnus hirtellus* (Linnaeus, 1761) by Salman (1982), *Pilumnus kempi* Deb, 1987 by Siddiqui and Tirmizi (1992), *Pilumnus minutus* De Haan, 1835 by Ko (1994b), and *Pilumnus longicornis* Hilgendorf, 1878 by Clark and Paula (2003).

The *Harrovia* Adams and White, 1849 is the largest genus in the Eumedoninae, and comprises seven described species (Ng et al., 2008). They are obligate symbiotic crabs on crinoids and restricted to the Indo-West Pacific region (Castro, 1989). Among them, *H. japonica* Balss, 1921 occurs from Japan, southern China and Korea (Chia and Ng, 1998; Lee and Ko, 2009), however, any larval description of it is not provided. The purpose of the present study is to describe all zoeal stages of this species, to compare their morphology with previously described zoeae of the subfamilies Eumedoninae and Pilumninae, and to provide a key for the identification of the known zoeae of the Eumedoninae from the Indo-Pacific region.

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MATERIALS AND METHODS

Ovigerous crab of *H. japonica* was collected by SCUBA diving from Geomun Island (34°02N, 127°18E), off the southern part of Korea on 3 August 2007. The zoeae hatched on 20 August. They were reared at constant water temperatures of 25°C and preserved in 10% neutral formalin. Zoeal specimens were dissected using Leitz zoom stereomicroscope and appendages were examined under a Leitz Laborlux S microscope. Appendages were mounted in polyvinyl lactophenol, and cover slips were sealed with clear nail varnish. Drawings were made with the aid of camera lucida. Setal counts and measurements were based on ten specimens for each zoeal stages. Description of zoeae is provided, based on the malacostracan somite plan, from anterior to posterior. Setal armature of appendages was described from proximal to distal segments and in order of endopod to exopod (Clark et al., 1998). The classification scheme follows Ng et al. (2008).

The long plumose natatory setae of the first and second maxillipeds were drawn truncated. A micrometer was used for measurements: CL (carapace length) was measured from the base of the rostral spine to the most posterior carapace margin and SL (spine to spine length) was from tip of the rostral spine to tip of the dorsal spine. The zoeae and the spent females were deposited in Silla University, Korea.

RESULTS

The larval stage consists of four zoeal and one megalopal stages. The minimum durations of the zoeal stages I to IV at 25°C were 3, 3, 3, and 4 days, respectively. Metamorphosis to the megalopa first occurred 13 days after the zoeae hatched from eggs. However, the preservation of the megalopae was not adequate, and consequently this phase could not properly be described and figured. The first zoeal stage is described and illustrated completely. For the second zoeal stage only the main differences from the first zoea are described.

First zoea (Fig. 1)

Size. CL 0.53±0.04 mm. SL 0.92±0.04 mm.

Carapace (Fig. 1A, E): Almost smooth, with weak dorsomedial protuberance; dorsal spine long, slightly curved, with minute tubercles; rostral spine short, straight; lateral spines short; 1 pair of posterodorsal setae present; each posteroventral margin with 7 denticles; eyes sessile.

Antennule (Fig. 1B): Exopod with 2 long, stout aesthetascs, 1 shorter, thinner aesthetasc and 2 small setae, all terminal.

Antenna (Fig. 1C): Protopod slightly longer than exopodal spinous process; both with 2 rows of spinules distally; exopod with 2 medial spines.

Mandibles (Fig. 1D): Asymmetrical; right molar with 6 unequal sized teeth and left molar with 2 teeth, confluent with incisor process.

Maxillule (Fig. 1F): Coxal endite with 7 setae; basal endite with 5 plumodenticulate setae; endopod 2-segmented, proximal segment with 1 seta, distal segment with 6 (2 subterminal+4 terminal) setae.

Maxilla (Fig. 1G): Coxal endite bilobed, with 6+4 setae; basal endite bilobed, with 5+4 setae; endopod bilobed, with 3+5 setae; exopod (scaphognathite) margin with 4 plumose setae plus distal stout process.

First maxilliped (Fig. 1H): Coxa without seta; basis with 10 setae arranged 2, 2, 3, 3; endopod 5-segmented with 3, 2, 1, 2, 5 (1 subterminal+4 terminal) setae, respectively; exopod 2-segmented, distal segment with 4 terminal natatory plumose setae.

Second maxilliped (Fig. 1I): Coxa without seta; basis with 4 setae arranged 1, 1, 1, 1; endopod 3-segmented, with 1, 1, 6 (3 subterminal+3 terminal) setae, respectively; exopod 2-segmented, distal segment with 4 terminal natatory plumose setae.

Third maxilliped: Absent.

Pereiopods: Absent.

Abdomen (Fig. 1J): Five somites; somites 1-5 with 1 pair of posterodorsal setae; somite 2 with anteriorly directed dorsolateral processes; somites 3-5 with posteriorly directed dorsolateral processes and posterolateral spines; somites 2-4 each with 2-3 dorsomedial denticles; somites 2-5 each with 7-8 denticles on posterodorsal margin; pleopods absent.

Telson (Fig. 1J): Posterior margin with 3 pairs of stout spinulate setae; each fork long, with 1 stout lateral spine, 1 minute lateral seta and 1 stout dorsomedial spine; dorsal surface of fork densely spinulate, tip smooth.

Second zoea (Fig. 2)

Size. CL 0.69±0.03 mm. SL 1.07±0.05 mm.

Carapace (Fig. 2A, E): Rostral spine with minute tubercles; dorsomedial protuberance prominent; region adjacent to base of dorsal spine tuberculated; dorsal spine heavily tuberculated; pairs of anterodorsal and posterodorsal setae present; each posteroventral margin with 2 setae; eyes stalked.

Antennule (Fig. 2B): Exopod with 3 long, stout aesthetascs, 2 shorter, thinner aesthetasc and 2 small setae.

Antenna (Fig. 2C): Endopod bud present.

Mandibles (Fig. 2D): Left molar with 4 teeth, confluent with incisor process.

Maxillule (Fig. 2F): Epipod seta present; basal endite with 8 plumodenticulate setae.

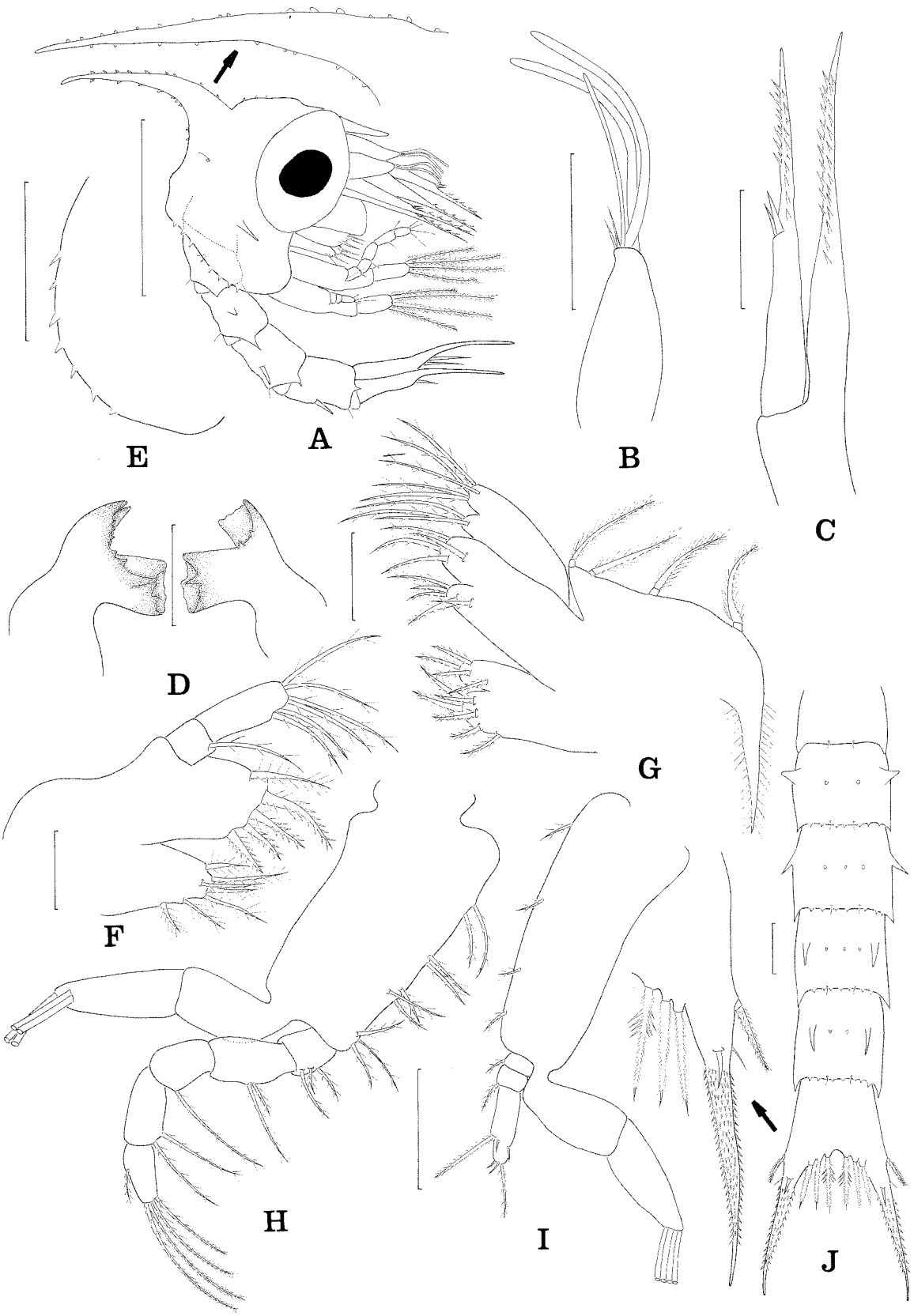

Fig. 1. *Harrovia japonica*, first zoeal stage. A, lateral view; B, antennule; C, antenna; D, mandibles; E, posterolateral margin of carapace; F, maxillule; G, maxilla; H, first maxilliped; I, second maxilliped; J, dorsal view of abdomen and telson. Scale bars = 0.5 mm (A), 0.1 mm (B-E, H-J), 0.05 mm (F, G).

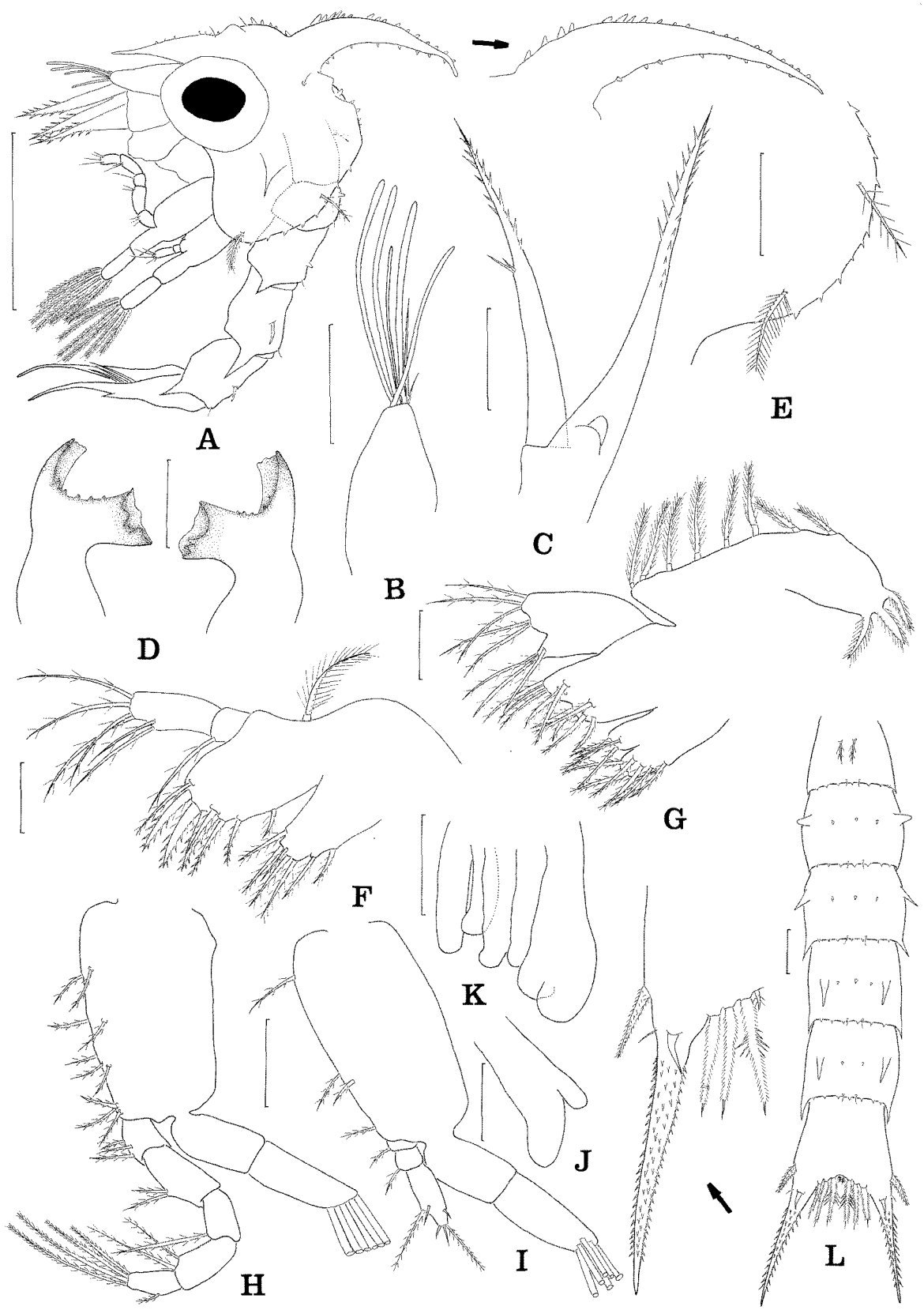

Fig. 2. *Harrovia japonica*, second zoeal stage. A, lateral view; B, antennule; C, antenna; D, mandibles; E, posterolateral margin of carapace; F, maxillule; G, maxilla; H, first maxilliped; I, second maxilliped; J, third maxilliped; K, pereiopods; L, dorsal view of abdomen and telson. Scale bars = 0.5 mm (A), 0.1 mm (B-E, H, I, K, L), 0.05 mm (F, G, J).

Maxilla (Fig. 2G): Basal endite bilobed, with 5+5 setae; exopod (scaphognathite) margin with 11 plumose setae.

First maxilliped (Fig. 2H): Exopod with 6 terminal natatory plumose setae.

Second maxilliped (Fig. 2I): Exopod with 6 terminal natatory plumose setae.

Third maxilliped (Fig. 2J): Present as bud.

Pereiopods (Fig. 2K): Present as buds; chela biramous.

Abdomen (Fig. 2L): Somite 1 with 2 dorsomedial setae; somites 1-5 each with 7-9 denticles on posterodorsal margin.

Telson (Fig. 2L): Posterior margin with 4 pairs of spinulate setae.

Third zoea (Fig. 3)

Size. CL 0.82±0.05 mm. SL 1.34±0.06 mm.

Carapace (Fig. 3A, E): With 1 stout spine between base of rostral spine and eye; dorsal spine heavily tuberculated, with 6 scattered setae; each posteroventral margin with 6 setae.

Antennule (Fig. 3B): Exopod with 1 shorter, thinner aesthetasc subterminally and 2 long, stout aesthetascs, 1 shorter, thinner aesthetasc plus 2 small setae terminally.

Antenna (Fig. 3C): Endopod shorter than half length of exopod.

Mandibles (Fig. 3D): Unchanged.

Maxillule (Fig. 3F): Coxal epipod seta present; basal endite with 9 plumodenticulate setae.

Maxilla (Fig. 3G): Basal endite bilobed, with 5+6 setae; exopod (scaphognathite) margin with 20 plumose setae.

First maxilliped (Fig. 3H): Distal segment of endopod with 6 (2 subterminal+4 terminal) setae; exopod with 8 terminal natatory plumose setae.

Second maxilliped (Fig. 3I): Exopod with 8 terminal natatory plumose setae.

Third maxilliped (Fig. 3J): Epipod present; endopod and exopod developing.

Abdomen (Fig. 3L): Six somites; somite 1 with 4 dorsomedial setae; somites 2-5 each with 3 dorsomedial denticles; somite 6 with 11-13 denticles on posterodorsal margin. Pleopods buds present.

Fourth zoea (Fig. 4)

Size. CL 1.08±0.05 mm. SL 1.76±0.05 mm.

Carapace (Fig. 4A, E): With tubercles; dorsal spine with 7 scattered setae; each posteroventral margin with 8 setae.

Antennule (Fig. 4B): Endopod bud present; exopod with total of 10 aesthetascs and 2 setae.

Antenna (Fig. 4C): Endopod about 2/3 times as long as exopod..

Mandibles (Fig. 4D): Endopod palp present.

Maxillule (Fig. 4F): Coxal and basal endites each with 10 and 11 plumodenticulate setae.

Maxilla (Fig. 4G): Basal endite bilobed, with 6+6 setae; exopod (scaphognathite) margin with 27 plumose setae.

First maxilliped (Fig. 4H): Exopod with 10 terminal natatory plumose setae.

Second maxilliped (Fig. 4I): Coxa with 1 seta; exopod with 10 terminal natatory plumose setae.

Third maxilliped (Fig. 4J): Segmental differentiation present.

Pereiopods (Fig. 4K): Some segmental differentiation into segments; chela prominent.

Abdomen (Fig. 4L): Somites 2-5 each with 4-5 dorsomedial denticles; somite 6 with 5-7 denticles on posterodorsal margin. Pleopods 1-4 with endopod buds.

Telson (Fig. 4L): With 2 dorsomedial setae.

DISCUSSION

The antenna of pilumnid zoeae is conservative, and it corresponds to that of Martin's (1984) xanthid group II, which is characterized by an antennal exopod acutely tipped, about equal in length to or slightly longer than the protopod, armed with small spinules distally and with a prominent outer seta about halfway along its length, and an antennal protopod is usually longer than rostrum. Recently Clark and Paula (2003) stated that having two medial spines on the antennal exopod is a common characteristic of this family, furthermore, the exopod is distally bilaterally spinulate as is the protopod. Among the known brachyuran zoeae having pilumnid antennal type, only zoeae of three species of *Halimede fragifera* (De Haan, 1835) (family Galenidae), *Parapilumnus trispinosus*, and *Pilumnus minutus* (see Terada, 1985; Ko, 1994a, b), and of six eumedoninid species (excluding *Echinoecus pentagonus*) have dorsolateral processes on the abdominal somites 2 to 5. In the former three species, dorsolateral processes on the abdominal somites 4 and 5 are vestigial or much smaller than lateral spines of telson, whereas in the latter eumedoninid zoeae, dorsolateral processes of the abdominal somites 4 and 5 are approximately equal in size to lateral spines of telson. Such feature could be considered as an important characteristic for distinguishing the eumedoninid zoeae (excluding *Echinoecus pentagonus*) from other brachyuran zoeae.

In the family Pilumnidae, larval descriptions are presently available for 15 species of the Pilumninae and for seven species of the Eumedoninae. So, we make a comparison between species of two subfamilies based on the zoeal characteristics. According to Table 1, the eumedoninid zoeae are more similar to the zoeae of the genus *Pilumnus*, than to other pilumninid genera in having short rostral carapace spine, the lateral carapace spine, and short dorsal carapace spine curved distally. Especially, we find the zoea

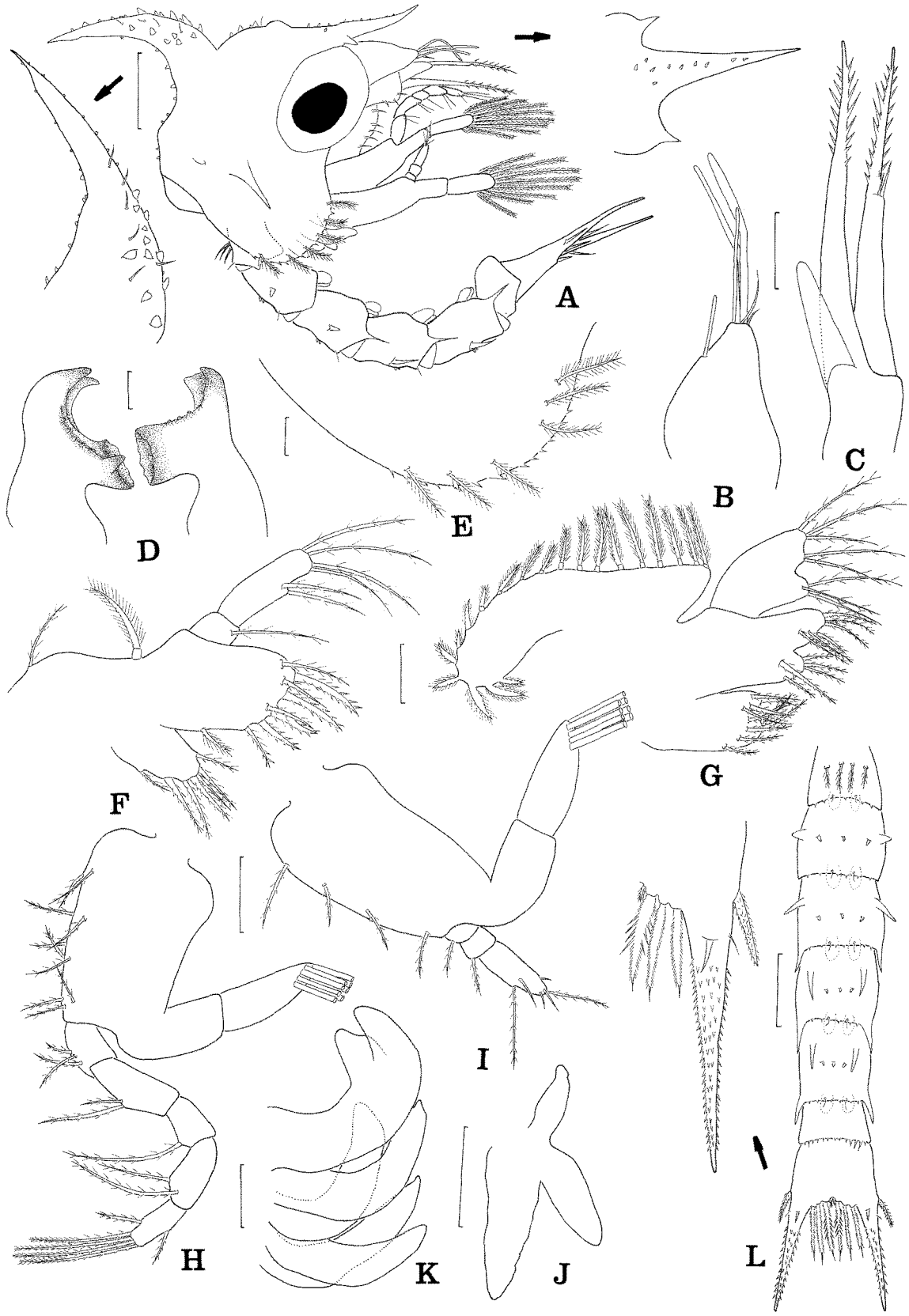

Fig. 3. *Harrovia japonica*, third zoeal stage. A, lateral view; B, antennule; C, antenna; D, mandibles; E, posterolateral margin of carapace; F, maxillule; G, maxilla; H, first maxilliped; I, second maxilliped; J, third maxilliped; K, pereiopods; L, dorsal view of abdomen and telson. Scale bars = 0.25 mm (A, L), 0.1 mm (B, C, H-K), 0.05 mm (D-G).

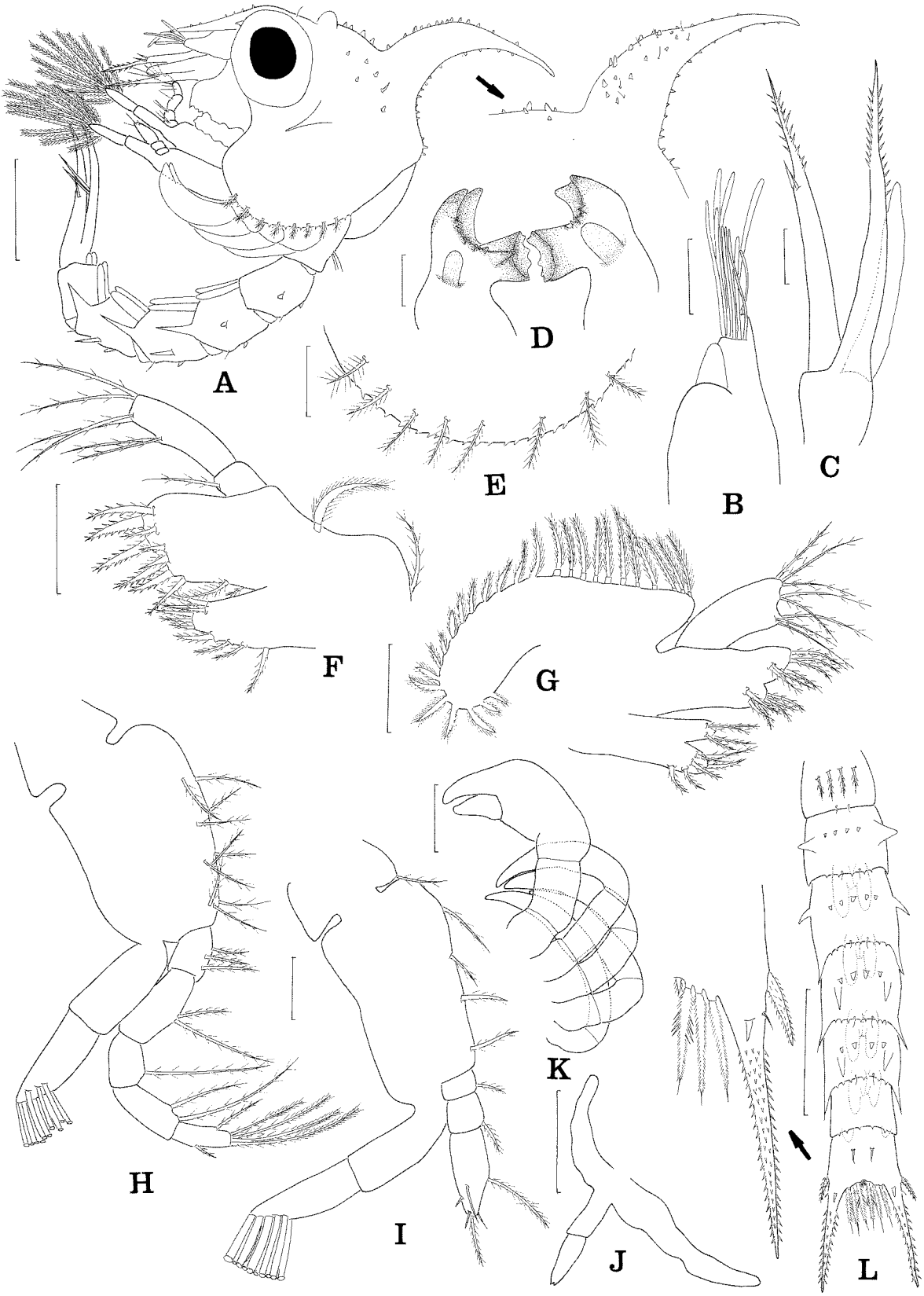

Fig. 4. *Harrovia japonica*, fourth zoeal stage. A, lateral view; B, antennule; C, antenna; D, mandibles; E, posterolateral margin of carapace; F, maxillule; G, maxilla; H, first maxilliped; I, second maxilliped; J, third maxilliped; K, pereiopods; L, dorsal view of abdomen and telson. Scale bars = 0.5 mm (A, L), 0.25 mm (K, J), 0.1 mm (B-I).

Table 1. Comparison of the first zoeal charactersistics for the two pilumnid subfamilies Pilumninae and Eumedoninae

Species	Rostral carapace spine	Lateral carapace spine	Dorsal carapace spine	Endopod of maxilla	Endopod of maxilliped 2	Dorsolateral process of abdominal somites	Lateral armature of telson fork	Sources
PILUMNINAE								
Heteropanope grabra	long	present	straight, long	8 setae	1,1,6 setation	2, 3	1 seta	Greenwood and Fielder, 1984a
Heteropilumnus ciliatus	long	present	straight, long	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Ko and Yang, 2003
Pilumnopeus makiana	intermediate	present	straight, long	7 setae	1,1,5 setation	2, 3	1 spine, 1 seta	Lee, 1993
Pilumnopeus serratifrons	vestigial	present	straight, long	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Greenwood and Fielder, 1984b
Pilumnopeus granulata	vestigial	present	straight, short	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Ko, 1997
Actumnus setifer	intermediate	present	curved, short	8 setae	1,1,5 setation	2, 3	1 spine, 1 seta	Aikawa, 1937
Pilumnus dasypodus	short	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine	Bookhout and Costlow, 1979
Pilumnus hirtellus	intermediate	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Salman, 1982
Pilumnus vespertilio	vestigial	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine. 1 seta	Lim and Tan, 1981
Pilumnus kempi	vestigial	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine. 1 seta	Siddiqui and Tirmizi, 1992
Pilumnus longicornis	intermediate	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Clark and Paula, 2003
Pilumnus minutus	short	present	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine, 1 seta	Ko, 1994b
Parapilumnus trispinosus	short	absent	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine, 1 seta	Ko, 1994a
Benthopanope indica	vestigial	absent	curved, short	8 setae	1,1,6 setation	2	1 spine, 1 seta	Ko, 1995
Benthopanope eucratoides	short	absent	curved, short	8 setae	1,1,6 setation	2	1 spine, 1 seta	Lim et al., 1986
EUMEDONINAE								
Echinoecus pentagonus	short	present	curved, short	8 setae	1,1,6 setation	2, 3	1 spine, 1 seta	Van Dover et al., 1986
Zebrida adamsii	short	present, extremely short	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine, 1 seta	Mori et al., 1991
Permanotus purpureus	short	present	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine, 1 seta	Ng and Clark, 2000
Harrovia japonica	short	present	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine, 1 seta	Present study
Harrovia longipes	short	present	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine	Lim and Ng, 1988
Harrovia albolineata	short	present	curved, short	8 setae	1,1,5 setation	2, 3, 4, 5	1 spine	Chia et al., 1993
Rhabdonotus pictus	short	present	curved, short	8 setae	1,1,6 setation	2, 3, 4, 5	1 spine	Chia and Ng, 1995

of *H. japonica* strongly resembles that of *Pilumnus minutus* by having abdominal somites 2 to 5 with dorsolateral processes and the telson fork with a spine and a seta laterally.

Although zoeal descriptions are limited to seven species in the Eumedoninae, the general zoeal morphology of the subfamily can be summarized as follows: (1) surface of carapace is either tuberculated [*Rhapdonotus* and *Harrovia*] or smooth [*Echinoecus*, *Permanotus*, and *Zebrida*]; (2) rostral carapace spine is short, lateral carapace spine is present; dorsal carapace spine is short, curved distally; (3) antennal exopod is subequal to protopod, both spinous processes are armed with spinules distally, exopod is with 2 medial spines; (4) endopodal setation of maxillule is 1, 2+4; (5) endopodal setation of maxilla is 3+2+3 [8]; (6) basipodal and endopodal setations of first maxilliped are 2, 2, 3, 3 and 3, 2, 1, 2, 5; (7) basipodal and endopodal setations of second maxilliped are 1, 1, 1, 1 and 1, 1, 6 [1, 1, 5 in *Harrovia albolineata*]; (8) dorsolateral processes are on abdominal somites 2-5 [abdominal somites 2, 3 in *Echinoecus pentagonus*]; and (9) telson fork has either 1 spine and 1 seta or 1 spine laterally.

The following provisional key is provided to aid in the identification of seven zoeae of the Eumedoninae. The characteristics employed are usually consistent during the zoeal development.

A key to the zoeae of seven known eumedoninid species from the Indo-Pacific

1. Dorsolateral processes on abdominal somites 2, 3 ..... *Echinoecus pentagonus*
- Dorsolateral processes on abdominal somites 2-5 2
2. Endopod setation of maxilliped 2 with 1, 1, 6 3
- Endopod setation of maxilliped 2 with 1, 1, 5 ..... *Harrovia albolineata*
3. Lateral armature of telson fork with 1 spine 4
- Lateral armature of telson fork with 1 spine and 1 seta 5
4. Dorsal carapace spine with heavy tubercles ..... *Rhabdonotus pictus*
- Dorsal carapace spine smooth ..... *Harrovia longipes*
5. Lateral carapace spine short 6
- Lateral carapace spine extremely short ..... *Zebrida adamsii*
6. Dorsal carapace spine with tubercles ..... *Harrovia japonica*
- Dorsal carapace spine smooth ..... *Permanotus purpureus*

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