Morphological description of *Pentacheles laevis* postlarva collected from Suruga Bay, Japan (Crustacea, Decapoda, Polychelidae)

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Abstract.— A morphological description is given for the postlarval specimen of the polychelid lobster, *Pentacheles laevis*, collected from Suruga Bay, on the Pacific coast of Japan. The specimen has been previously identified using the DNA barcoding method. Diagnostic larval characters distinguishing the previously known postlarvae in three genera, *Pentacheles*, *Stereomastis*, and *Polycheles*, are also noted.

Key words: *Crustacea*, *Decapoda*, *Polychelidae*, *Pentacheles*, *Polycheles*, *Stereomastis*, *larval morphology*

■ Introduction

The deep-sea blind lobsters of the family Polychelidae are of interest to marine zoologists not only for their unique appearance, such as having four or five pairs of chelate walking legs and a caridean-like triangular telson, but also for their evolutionary lineage: the polychelids are sometimes referred to as “living fossils” due to their relationship to the extinct eryonoid families of the Mesozoic era. The family Polychelidae consists of 40 extant species in six genera (Galil, 2000; Ah Yong, 2009, 2012; Audo & Furrer, 2020). Among these, 12 species in five genera have been recorded in Japan and its surrounding waters (Okada *et al.*, 1966; Okutani, 1969; Galil, 2000; Kuramochi *et al.*, 2004; Yamamoto & Nagasawa, 2011; Komai & Tsuchida, 2014). Despite the progress made in adult taxonomy and palaeontology of other decapod species, little is known about polychelid life history, particularly its larval stages.

Recently, we collected polychelid postlarvae from the Pacific coast of Japan, and one of the specimens from Suruga Bay was identified as *Pentacheles laevis* using DNA barcoding analyses (Yanagimoto *et al.*, 2015, see Appendix).

The aim of the present paper is to describe the detailed morphology of *P. laevis* postlarva.

■ Materials and Methods

The polychelid postlarva was caught during research cruise of the R/V Hokuto (Tokai University) in Suruga Bay (34°56'N 138°38'E), on 14 May 2014, using a bottom larval net, at depths of 1304–1565 m. The observations and measurements of the postlarva were taken using an Olympus SZX10 stereomicroscope and a Nikon BX50 microscope equipped with a drawing tube. Carapace length (CL) was measured as the distance from the frontal margin to the posterior margin along the median line, and carapace width (CW) as the greatest distance across the carapace excluding the lateral spines (see Figs. 1A and 2B). The postlarva specimen used in the study was deposited at the Hokkaido University Museum under the accession No. ICHUM-6157.

■ Description of the postlarva of *Pentacheles laevis* Bate, 1878

Dimensions: CL = 17.2 mm, CW = 16.8 mm.

Color in life: Cephalothorax orange-red with

Fig. 1. Postlarva of *Pentacheles laevis*. A: whole body in dorsal view, A': frontal region of carapace (enlarged), B: pleon in dorsal view, C: pleon in lateral view, D: orbital region in ventral view, arrow indicating nerve cord, E: anterior pillar, F: posterior pillar. BR: branchial region, CL: carapace length, CW: carapace width, ap: anterior pillar, pp: posterior pillar.

Fig. 2. Postlarva of *Pentacheles laevis*. A: antennule, B: antenna, rp = renal process, C: outer (left) and inner (right) sides of the left mandible, D: maxillule, E: maxilla, F: maxilliped 1, G: maxilliped 2, H: maxilliped 3. ep: epipod, pg: paragnath, rp: renal process.

Fig. 3. Postlarva of *Pentacheles laevis*. A: pereopod 1 (first cheliped), B: pereopod 2, C: pereopod 3, D: pereopod 4, E: pereopod 5, F: pleopod 1, G: pleopod 2.

parts of the pleonites and telson transparent with reddish spots.

Carapace (Figs. 1A–D): Globular and pear-shaped in dorsal view, posterior margin slightly concave medially. CL slightly longer than CW. Posterolateral branchial region inflated. Spine arrangement on the middorsal line as “2r, 2, ap, 2, 2, pp, 2”, from the rostral spine to the posterior margin [ap: anterior pillar (Fig. 1E), pp: posterior pillar (Fig. 1F), r: rostral]. Four carinae with spines on the branchial region. Orbital sinus V-shaped with an acute spine on its inner side. Eyestalk with a frontal blunt projection in front. Terminal of nerve cord visible near the eyestalk base through the integument (Fig. 1D, arrow).

Antennule (Fig. 2A): Biramous. Peduncle tree-segmented, basal segment flattened with

one large and wide outer spine with outer margin bearing four spines, and one inner small spine. Exopod flagellum five-segmented, distal segment with three setae. Endopod flagellum multi-segmented with setae and numerous setules, approximately three times longer than outer flagellum.

Antenna (Fig. 2B): Biramous, coxal region with a long renal process (phymacerite), and basal region with a short anterolateral spine. Scaphocerite (exopod) forming spoon-shaped plate, with 29–31 marginal plumose setae. Endopod flagellum long, multi-segmented.

Mandible (Fig. 2C): No molar processes. Inner edge with 11 triangular teeth. Palp tree-segmented with numerous setae. Paragnath small, forming digitiform plate.

Maxillule (Fig. 2D): Biramous, basal endite

with two distal cuspidae and 20–22 marginal setae. Coxal endite with 18/20 setae. Endopod small, rudimentary lobe with six short setae distally.

Maxilla (Fig. 2E): Biramous. Coxal endite smaller than basal endite, with 19–21 setae. Basal endite with two stout spines and 20–22 setae. Endopod smaller than basal endite, unsegmented without setae. Scaphognathite large, elongate with numerous, marginal, plumose setae.

Maxilliped 1 (thoracopod 1) (Fig. 2F): Endopod with slender lobe. Exopodal lobe broad and elongate, anterior part bilobed.

Maxilliped 2 (thoracopod 2) (Fig. 2G): Endopod four-segmented, distal segment with three stout spines and two setae, and other segments with dense, long inner setae.

Maxilliped 3 (thoracopod 3) (Fig. 2H): Endopod five-segmented, with numerous long inner, and short outer setae. Coxal part with a long epipod fringed with 5–7 marginal setae.

Pereiopods (Fig. 3A–E): Pereiopod 1 chelate, largest, and more robust than the others. Inner margin of dactylus and propodus with minute cutting plates. Pereiopods 2–4 cheliform. Pereiopod 5 not chelate.

Pleon (Fig. 1B, C): Pleura of somite 1 with two lateral hook-like spines with rounded ends and one dorsal spine on the mid-posterior margin. Pleonite 2–5 with rounded triangular pleura with serrate margins; median carina bispinose, anterior spine larger than posterior one, and a pair of lateral spines.

Pleopods (Fig. 3F, G): Uniramous in pleonite 1. Biramous in pleonite 2–6; endopod with 20–23 plumose setae and an *appendix interna* bearing three cincinnuli on its distal end; exopod with 10–15 plumose setae.

Uropod (pleonite 6 pleopod): Endopod and exopod with numerous long plumose setae marginally.

Telson: Subtriangular with a bispinose mid-dorsal carina, lateral margin with 8–9 spines and numerous long setae.

Discussion

The developmental phase of the present specimen is classified as postlarval because it lacks a maxillipedal exopod with natatory setae and has functional pleopods. At present, however, a more accurate stage of the larval phase (i.e., the first postlarva (megalopa, decapodid) or subsequent postlarval stages) is not clear due to the lack of the morphological information on successive postlarval developments observed under the laboratory-rearing.

The comparison among selected morphological characters of different species of polychelid postlarvae, except for the so-called '*Eryoneicus* species', is shown in Table 1. Boyko (2006) examined the polychelid adult and larval specimens at the Yale Peabody Museum Collections, and morphologically identified the postlarvae up to the species level. He identified the larvae to *P. laevis* and *Stereomastis talismani* (as *Polycheles talismani*), although he gave no detailed morphological description of the larval specimens. In his paper, the postlarva of *P. laevis* was distinguished from that of *S. talismani* in four characters as follows: 1) two small rostral spines, 2) the antennal basal segment bear a single anterolateral spine, 3) inflated branchial regions of the carapace, and 4) a well-developed epipod on the maxilliped 3. However, the larval morphology of *Stereomastis* warrants re-examination as parentage has not been established by either larval rearing or DNA analyses. The basal antennal segment with double spines (Boyko, 2006), a diagnostic character of *Polycheles* and *Stereomastis* are not recognized by the other authors (Calman, 1925; Quintana & Retamal, 1984; Torres *et al.*, 2010). The middorsal spination observed in the present specimen differs from that of previously reported by having fewer spines, especially anterior to the anterior pillar. It may be possible that the spination decreases in successive stages of postlarval development. There is a dearth of morphological data on polychelid larvae

Table 1. Comparison of selected characters in polychelid postlarvae.

Genus Species		<i>Polycheles</i>		<i>Stereomastis</i>		<i>Pentacheles</i>	
		<i>Po. typhlops</i>	<i>S. talismani</i> [†]	<i>S. suhmi</i>	<i>S. pacifica</i>	<i>Pe. laevis</i>	<i>Pe. laevis</i>
Source of materials		P + L + D	P	P	P	P	P + D
References ^{††}		1)	2)	3)	4)	2)	Present study
Carapace	CL (mm)	8.5	21 ^{†††}	(26) ^{††††}	12	23 ^{†††}	17.2
	CL-CW lengths	CL > CW	CL > CW	CL > CW	CL > CW	CL ≥ CW	CL ≥ CW
	Branchial region	Not inflated	Not inflated	Not inflated	Not inflated	Inflated	Inflated
	Rostral spine	1	1	2	1	2	2
	Middorsal spination	1r, 1, 1, 1, 2, p, 2, 2, p, 2	ND	2r, 1, 1, 2, 2, p, 2, 2, 2, p, 2	1r, 2, 1, p, 2, 2, 1, 2	ND	2r, 2, p, 2, 2, p, 2
Eye stalk	Frontal spine	Acute end	ND	Blunt end	ND	ND	Blunt end
Maxilliped 3	Epipod	Reduced	ND	Reduced	Reduced	Developed	Developed

CL: carapace length, CW: carapace width, D: DNA analysis, L: laboratory-reared, ND: no data, P: plankton collection, p: pillar, r: rostral.

[†] as *Polycheles talismani*. ^{††} 1) Torres *et al.* (2010), 2) Boyko (2006), 3) Calman (1925), 4) Quintana & Retamal (1984).

^{†††} measured from the figures. ^{††††} total length.

such that a discussion on development of mid-dorsal spination is premature. The other four genera i.e., *Cardus*, *Homeryon*, *Stereomastis*, and *Willemoesia* virtually unknown. The comparison of polychelid postlarvae (Table 1) is only preliminary at present. Nonetheless, we propose an additional character in distinguishing postlarvae in the previously known three genera: i.e., the eyestalk spine either having a long blunt or a short acute end. Thus, postlarvae of *Pentacheles* differs from those of *Polycheles* and *Stereomastis* in having inflated branchial carapace region and well-developed epipod on maxilliped 3, while postlarvae of *Polycheles* differs from those of *Pentacheles* and *Stereomastis* in having an acute-ended spine on eyestalk.

Since Bate's (1882) description, under the name of '*Eryonoeicus*,' which was believed to be a pelagic decapod genus, until Sund (1915) revealed their parentage, and the increased interest of planktologists due to its large body size and unique appearance (Martin, 2014), a few larval forms of the species have been described (e.g. Bernard, 1953). These larval descriptions were done independently from adult classification creating the "*Eryonoeicus* problem." As a result, this rather generic name was suppressed in 1964 by the International

Commission on Zoological Nomenclature (ICZN, 1964; Boyko, 2006). To date, parentages in the polychelid larvae have been confirmed in only one species, *Polycheles typhlops* by laboratory-rearing and DNA analyses (Guerao & Abelló, 1996; Torres, *et al.*, 2014). Williamson (1983) reported on the hatching of *P. laevis* (as *Polycheles granulatus*) prezoaea, but he gave no morphological description and rearing data. The present larval description is the second one of which parentage is confirmed in polychelid larvae.

In addition to the extant polychelids, it is noteworthy about recent progress in larval studies on extinct decapod crustaceans. Discoveries of fossil polychelid larvae from the Mesozoic era show different larval forms and developmental patterns of spination from those of the extant species (Haug *et al.*, 2015; Eiler & Haug, 2016). Larval morphological study of polychelids is therefore indispensable for understanding of the history of larval evolution in decapod crustaceans.

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Appendix—Summary of DNA barcoding methods in our previous work

Muscle samples from the postlarval pleon to be used in PCR amplification of the COI and ITS1 gene were extracted using Quick-Gene-810¹⁾. The amplification was done using the universal primers LCO1490 and HCO2198. The PCR reactions were carried out in a volume of 10 μ L containing 20–50 ng template DNA, 1 μ L of 10 $\times$ PCR buffer, 0.8 μ L of dNTP (2.5 mM), 1 μ L of each primer (10 μ M), 0.05 μ L of 5 U/ μ L Ex Taq Polymerase Hot Start Version²⁾ in a Model 9700 Thermal Cycler³⁾. The amplified products were electrophoresed on a 1.5% agarose gel, and stained with EtBr. The remnants of the primers and dNTP were removed by treatment of the amplicons with ExoSAP-IT⁴⁾ after which they were subjected to direct sequencing using the PCR primers. DNA for cycle sequencing reactions were carried out using a BigDye Terminator Ver3.1 kit³⁾, and sequencing was conducted on an ABI3730XL Automatic Sequencer³⁾ with forward and reverse primers. The primers used for sequencing were the same as those for PCR amplification. The ITS1 region was amplified with primers MD-1 and 5.8SH. PCR products with ITS sequences were cloned into electro-competent *E. coli* using a TOPO TA cloning kit⁵⁾. Eight white colonies were randomly selected, then amplified by PCR were conducted using primers of M13 and its reverse primers. The resultant sequences were edited and aligned using ATGC software⁶⁾. The determination of Kimura's 2 parameter (K2P) genetic distance, and construction of the phylogenetic tree were performed using MEGA 5.05 software.

The nucleotide sequences data of the postlarva were entered into a DNA Database under accession numbers of LC022125 for the ITS1 region and LC022130 for the COI.

1) Wako, 2) TAKARA, 3) Applied Biosystems, 4) GE Healthcare, 5) Invitrogen, 6) GENETYX.