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The Morphological Development of *Macrobrachium rosenbergii* (de Man) Larvae

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INTRODUCTION

The propagation and culture of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) has extended to many countries with the establishment of hatchery techniques for the species. The morphology of the larval instars has been reported previously[1-3]. The prawn has been considered for about 20 years to metamorphose into postlarva after the completion of eleven zoeal stages; however during our work discrepancies were found with the earlier description[1].

The importance of correct identification of the larval stages of a crustacean of commercial value like *M. rosenbergii* resides in the possibility to improve hatchery production by means of optimizing the rearing conditions for each stage[4], or determining the nutritional requirements during development.

MATERIALS AND METHODS

Larvae were obtained from *M. rosenbergii* broodstock introduced from Taiwan, which is currently being cultured in our laboratory at constant temperature (28°C). Newly hatched-out zoeae were collected and transferred individually to 40 test tubes with a capacity of 20 cc and each of 20 beakers of the same volume. The containers were previously filled with oxygen-saturated brackish water at 28°C of temperature and 13.6 ppt of salinity. The test tubes were covered with glass marbles and the beakers with watch glasses to avoid evaporation of the water. The rearing was carried out in an incubator set at constant temperature and light. From the onset of the second zoea the larvae were fed with *Artemia salina* nauplii and the water was changed twice per day. Observations for molting and growth of the larvae were done 4 or 5 times per day. When molting was observed, the shed molt was fixed in buffered 10% formalin. The molts were stained with 0.5% Phloxine solution and mounted in glycerin for observation and sketching under the microscope. The lateral views of the larval instars were observed by using alive zoeae that were killed on a slide glass with 4% formalin solution and were immediately sketched. Body length measurements were performed on larvae fixed in formalin-glycerin to prevent bending of the abdomen. The larval stages are not described with all their appendages. Only the most important features for rapid recognition were selected.

RESULTS

First zoea (Fig. 1.1)

Body length: 1.90 mm; carapace length: 0.63 mm. The larva is transparent with non stalked eyes and a broad, spatula-shaped telson (Fig. 2.1). Rostrum horizontal with no rostral teeth (Fig. 6.1). The color (intensity) of the dorsal and orbital chromatophores can vary according to the nutritional conditions of the spawner. The scaphocerite presents segmentation in the distal region. The antennal flagellum is unisegmented (Fig. 3.1). The antennule is rudimentary (Fig. 4. 1). Oil droplets are visible in the cephalothorax (Fig. 1.1). Instar mean duration: 1.5 d.

Second zoea (Fig. 1.2)

Body length: 1.92 mm; carapace length: 0.68 mm. The eyes become stalked; supraorbital spines appear (Fig. 1.2). The telson is similar to that of the first zoea but the rudiments of the uropod exopodite are visible to each side (Fig. 2.2). Scaphocerite with four segments. The antennal flagellum is unisegmented (Fig. 3.2). Instar mean duration: 2.1 d.

Third zoea (Fig. 1.3)

Body length: 2.1 mm; carapace length: 0.65 mm. An epifrontal tooth appears in the carapace dorsum (Figs. 1.3, 6.3). Telson broad, spatular in shape. Uropods are formed, the exopodites separated. The endopodites appear under the telson, rudimentary, shorter than exopodite and without setae (Fig. 2.3). The antennal flagellum has three segments; scaphocerite without segmentation (Fig. 3.3). Instar mean duration: 2.0 d.

Fourth zoea (Fig. 1.4)

Body length: 2.3 mm; carapace length: 0.73 mm. A second tooth, the epigastric tooth appears in the carapace dorsum (Fig. 1.4). In some individuals the number of teeth is three. The telson is markedly broad in the distal margin, but with the spatular shape less pronounced than in the third zoea. Exopodite of uropods larger than endopodite. Endopodite is free, with setae only in the inner margin (Fig. 2.4). Antennal flagellum has three segments (Fig. 3.4). Instar mean duration: 1.2 d.

Fifth zoea (Fig. 1.5)

Body length: 2.50 mm; carapace length: 0.76 mm. Telson enlarged longitudinally, with the proximal margin (basis) narrower than the distal margin (tip of the telson). Exopodite and endopodite of uropods approximately of the same size, larger than those of the fourth zoea. The endopodite presents setae also in the external margin (Fig. 2.5). Antennal flagellum with three segments, the distal segment being somewhat enlarged (Fig. 3.5). Instar mean duration: 0.8 d.

Sixth zoea (Fig. 1.6)

Body length: 2.72 mm; carapace length: 0.85 mm. Telson elongated, rectangular in shape, with the proximal and distal margins of the same width (Fig. 2.6). Antennal flagellum with three segments (Fig. 3.6). The distal segment is enlarged, with or without rudiments of the articulation for the fourth segment. Antennular peduncle increased in size (Fig. 4.6) compared to that of the fifth zoea. Instar mean duration: 1.0 d.

Seventh zoea (Fig. 1.7)

Body length: 3.0 mm; carapace length: 0.96 mm. The distal margin (tip) of the telson is narrower than the proximal margin (basis); uropods more elogated (Fig. 2.7). The antennal

flagellum presents four segments (Fig. 3.7). Buds of pleopods are not present in the ventral region of the abdomen (Figs. 1.7, 5.7). Instar mean duration: 0.3 d.

Eighth zoea (Fig. 1.8)

Body length: 3.5 mm; carapace length: 1.2 mm. The tip of the telson narrows further (Fig. 2.8). The segment number of the antennal flagellum increases to five (Fig. 3.8). The main characteristic of this instar is the emergence of buds of the pleopods in the ventral region of the abdomen (Fig. 1.8). The number of buds vary with the individuals: either two, three of the five pairs of buds appear (Figs. 5.8a, 8b). Instar mean duration: 1.5 d.

Ninth zoea (Fig. 1.9)

Body length: 3.9 mm; carapace length: 1.35 mm. This stage can be distinguished from the previous instar in that the buds of the pleopods elongate slightly, sometimes curved downwards in the form of an "L" (Figs. 5.9a, 9b). If during the eighth zoea the complete set of pleopod buds was not present, they invariably develop when the larva molts to the ninth zoea. The tip of the telson is very narrow (Fig. 2.9) and the antennal flagellum is composed of six segments (Fig. 3.9). Instar mean duration: 0.4 d.

Tenth zoea (Fig. 1.10)

Body length: 3.93 mm; carapace length: 1.40 mm. The pleopods increase in size and become birramous (Fig. 5.10), but not articulated and without setae. Seven segments are present in the antennal flagellum (Fig. 3.10). From this stage on, the antennule is useful for identification. The inner antennular flagellum is unisegmented (Fig. 4.10). Instar mean duration: 2.0 d.

Eleventh zoea (Fig. 1.11)

Body length: 4.5 mm; carapace length: 1.55 mm. Setae can be observed in the tip of the exopodite of one or several of the pleopods (Fig. 5.11), except the fifth pair. Usually the setae are more conspicuous in the third pair. The antennal flagellum is composed of eight segments (Fig. 3.11). Inner antennular flagellum with two segments (Fig. 4.11). Instar mean duration: 1.6 d.

Twelfth zoea (Fig. 1.12)

Body length: 4.9 mm; carapace length: 1.76 mm. The articulations of the pleopodal exopodites and endopodites appear (Fig. 6.B.12). Setae develop in the exopodites of all pleopods. Antennal flagellum with nine segments: the inner antennular flagellum is bisegmented, as in the eleventh zoea (Fig. 4.12). Instar mean duration: 0.4 d.

Thirteenth zoea (Fig. 1.13)

Body length: 5.65 mm; carapace length: 2.13 mm. The number of setae in the pleopodal exopodites increases; the endopodites of the third and fourth pairs develop setae and appendix internae as well (Fig. 6.B.13). Nine or ten segments can be observed in the antennal flagellum (Fig. 1.13). The inner antennular flagellum shows three segments (Fig. 4.13). Instar mean duration: 2.0 d.

Fourteenth zoea (Fig. 1.14)

Body length: 5.90 mm; carapace length: 2.20 mm. Endopodites of the fifth pair of pleopods present setae, and their number in the exopodite increases. Appendix internae developed in all the pleopods, with the exception of the first pair (Fig. 6.B.14). The number of segments in the antennal flagellum becomes variable; this instar can present from nine to twelve segments (Fig. 1.14). Sometimes in the same individual the antenna from one side may have one segment more than its counterpart. However, the inner antennular segment number does not present this type

of variations. The inner flagellum lengthens, and four segments can be appreciated (Fig. 4.14). The rostrum tip is elongated (Fig. 6.A.14). Instar mean duration: 1.7 d.

Fifteenth zoea (Fig. 1.15)

Body length: 8.70 mm; carapace length: 2.46 mm. Antennal with flagellum eleven to fourteen segments (Fig. 1.15). Inner antennular flagellum invariably with five segments (Fig. 4.15). In the dorsal edge of the rostrum appear one to five teeth (Fig. 6.A.15). Instar mean duration: 0.4 d.

Sixteenth zoea (Fig. 1.16)

Body length: 8.96 mm; carapace length: 2.75 mm. Antennal flagellum with thirteen to fifteen segments (Fig. 1.16); inner antennular flagellum with six segments (Fig. 4.16). The number of teeth in the dorsal edge of the rostrum increases to six or more (Fig. 6.A.16). Larvae of this instar become very fast swimmers due to the advanced development of the pereiopods. They can also be identified with the naked eye because of the mode of swimming. Instar mean duration: 2.2 d.

Seventeenth zoea (Fig. 1.17)

Body length: 9.25 mm; carapace length: 2.9 mm. About 17 segments can be observed in the antennal flagellum (Fig. 1.17). The number of segments in the inner antennular flagellum increases to seven (Fig. 4.17). The number of rostral teeth in the dorsal edge augments, and in the ventral edge of the rostrum three or four teeth can be observed (Fig. 6.A.17). Contrary to the former instars characterized by a brownish carapace color from the onset of the fourth zoea on, the body of the seventeenth zoea regains a transparent coloration. This instar can further be identified with the naked eye by noting the presence of a conspicuous red and blue chromatophore situated longitudinally in the middle part of the ventral side of the abdomen. Instar mean duration: 2.0 d.

Postlarva (Fig. 1.18)

Body length: 7.26 mm; carapace length: 2.45 mm. The number of segments in the antennal flagellum increases markedly (more than two times the number of segments in the seventeenth zoea). Setae appear in the space between each of the rostral teeth (Fig. 6.A.18); the epigastric tooth disappears (Fig. 1.18). Inner antennular flagellum lengthens and presents eight to twelve segments (Fig. 4.18). Upon metamorphosis, the shape changes to that of an adult prawn (Fig. 1. 18); the body is transparent and the black eyespots are conspicuous. The behavior shifts to settlement in the bottom and swimming actively forwards by means of the pleopods. In about 24 hr the newly metamorphosed postlarva molts again.

In Table 1 the calculated median number of days required for the appearance of each instar is presented together with a concise explanation of the main characteristics for recognition of each stage.

DISCUSSION

The characteristics of the stages described above were corroborated upon observation of the moulted carapace corresponding to each instar. For some of the instars the calculated mean duration was of only several hours; we have observed that in effect, some larvae undergo the next molt in less than 24 hr. As the shed molt disintegrates very fast the repeated checking of the

Table 1. Characteristics for recognition of the zoeal larvae of *Macrobrachium rosenbergii* (de Man).

Zoea	Appearance day	Characteristics
1	0	Non-stalked eyes.
2	1.5	Stalked eyes.
3	3.6	Exopodite of uropod separates.
4	5.5	Endopodite of uropod with setae, smaller than exopodite. Tip of telson very broad.
5*	6.5	Telson enlarged, the tip broader than the basis. Antenna with three segments.
6	7.5	Telson rectangular in shape. Antennal flagellum with 3 segments.
7*	8.5	Tip of telson narrower than basis. Antennal flagellum with 5 segments.
8	8.8	Antennal flagellum with 5 segments. Buds of pleopods appear.
9*	10.3	Antennal flagellum with 6 segments. Buds of the pleopods elongate.
10	10.7	Antennal flagellum with 7 segments. Pleopods birramous, but not articulated. Inner antennular flagellum unisegmented.
11	12.7	Antennal flagellum with 8 segments. Setae in the tip of pleopod exopodites.
12*	14.3	Antennal flagellum with 9 segments. Inner antennular flagellum with 2 segments.
13	14.7	Antennal flagellum with 9-10 segments. Inner antennular flagellum with 3 segments.
14*	16.7	Antennal flagellum with 9-12 segments. Endopodites of fifth pair of pleopods with setae. Inner antennular flagellum with 4 segments.
15	18.4	Antennal flagellum with 11 -14 segments. Rostrum with 1-3 dorsal teeth. Inner antennular flagellum with 5 segments.
16*	18.9	Antennal flagellum with 13-15 segments. Rostrum with 6 or more dorsal teeth. Inner antennular flagellum with 6 segments.
17	21.1	Antennal flagellum with about 17 segments. Rostral teeth in the ventral edge. Inner antennular flagellum with 7 segments.
PL	23.1	The shape change to that of an adult prawn. Inner antennular flagellum with 8 segments.

Numbers with asterisk are instars not reported by Uno & Kwon[1]. The instar appearance time (days) is the calculated median.

culture containers ensured the recovery of the consecutive molts.

Although the body length of the larvae was measured on formalin-preserved specimens, we do not consider the size critical as a means of recognition of the different instars. When the larvae are mass-cultured, considerable variation in the body size occurs, as reported by Uno & Kwon[1]. It is interesting to note that the size of the larvae (i.e. metamorphosed postlarvae) can vary with such factors as rearing temperature and salinity, or the geographic origin of the broodstock (unpublished data).

We consider that earlier descriptions[1,2] are very complete from the taxonomical point of view; however the mass culture of the larvae used in the observations prevented these authors to determine the development with more detail. As an example, the newly metamorphosed postlarva reported by Uno & Kwon[1] is likely to be in reality the postlarva plus one molt, i.e. next instar.

In caridean prawns, several factors have been linked with variations in the number of larval molts[5,6]. Supernumerary molts usually result in repetitions of the previous instar, and in size increment. According to Williamson[7] the minimal number of molts should occur under optimal

conditions for survival. The rearing temperature and salinity used in this experiment are regarded as optimal, so no extra molts were observed in the larvae.

Macrobrachium rosenbergii distributes over a very wide area in the Indo-Pacific region. Different developmental rates have been reported[8] for the larvae from broodstocks of different geographic origins, so it would be interesting to clarify whether there are differences in the number of larval instars among the various geographic stocks of the species.

SUMMARY

Larvae of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) were reared individually at 28°C and 13.6 ppt of temperature and salinity conditions, to observe the development. Morphological observations were made on the shed carapaces after molting of each stage.

(1) The larvae metamorphose into postlarva after developing through 17 zoeal instars.

(2) Six new zoeal instars are introduced, in addition to the eleven instars reported elsewhere, and the main characteristics for identification are described.

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オニテナガエビ *Macrobrachium rosenbergii* (de Man) 幼生期の変態過程について

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オニテナガエビ *Macrobrachium rosenbergii* (de Man) の孵化幼生60尾を個体別に飼育し，各個体の成長にともなう脱皮について確認するとともに，その脱皮殻を用いて形態変化の観察を行った。その結果，本幼生は17期に分けられるゾエア期を経てポストラーバとなることが判った。これは，飼育個体群の観察から本種のゾエア期幼生を11期であるとした既往の報告と異なるものである。すなわち，脱皮の確認とその形態の特徴から，従来のゾエア第4～5期，第5～6期，第6～7期，第8～9期，第9～10期，第10～11期に，それぞれさらに1期を経ることが明らかになった。本論文は新たに認められたこれら6期を含めて，改めてゾエア第1～17およびポストラーバ各期の主要な形態的特徴について記載したものである。なお，本飼育実験は試験管またはビーカー中（何れも水容20cc）に1尾ずつを収容し，水温28℃・塩分濃度13.6 pptの条件下で，アルテミア幼生のみを餌料として行ったもので，今回の場合，ゾエア1期からポストラーバに至るまでに要した期間は約23日間であった。

EXPLANATION OF FIGURES

Fig. 1. *Macrobrachium rosenbergii* (de Man), ventral view. Numbers 1 to 17 indicate zoeal instar; 18, postlarva. Scale bars represent 1 mm.

Fig. 2. *Macrobrachium rosenbergii* (de Man), telson. Numbers indicate the corresponding zoeal instar. Scale bars represent 0.2 mm.

Fig. 3. *Macrobrachium rosenbergii* (de Man), antenna. Numbers indicate the corresponding zoeal instar. Scale bars represent 0.2 mm.

Fig. 4. *Macrobrachium rosenbergii* (de Man), antennule. Numbers 1 to 17 indicate zoeal instar; 18, postlarva. Only the distal portion of the antennular peduncle is illustrated for the thirteen zoea to postlarva. Scale bars represent 0.2 mm.

Fig. 5. *Macrobrachium rosenbergii* (de Man). Morphological characteristics during the appearance of the pleopods from the sixth zoea to the eleventh zoea. Numbers indicate the respective zoeal instar; "a, b" represent variations for the same instar. Scale bar corresponds to 1 mm.

Fig. 6. *Macrobrachium rosenbergii* (de Man), A: rostrum. Numbers indicate the zoeal instar. From the first to the thirteenth zoea the scale bar represents 0.16 mm; from the fourteenth zoea to postlarva the scale bar represents 0.2 mm. B: pleopods. The pleopods of the twelfth to the sixteenth zoea are shown; in each group the rightmost pleopod is that of the first abdominal segment. The scale bar represents 0.2 mm.

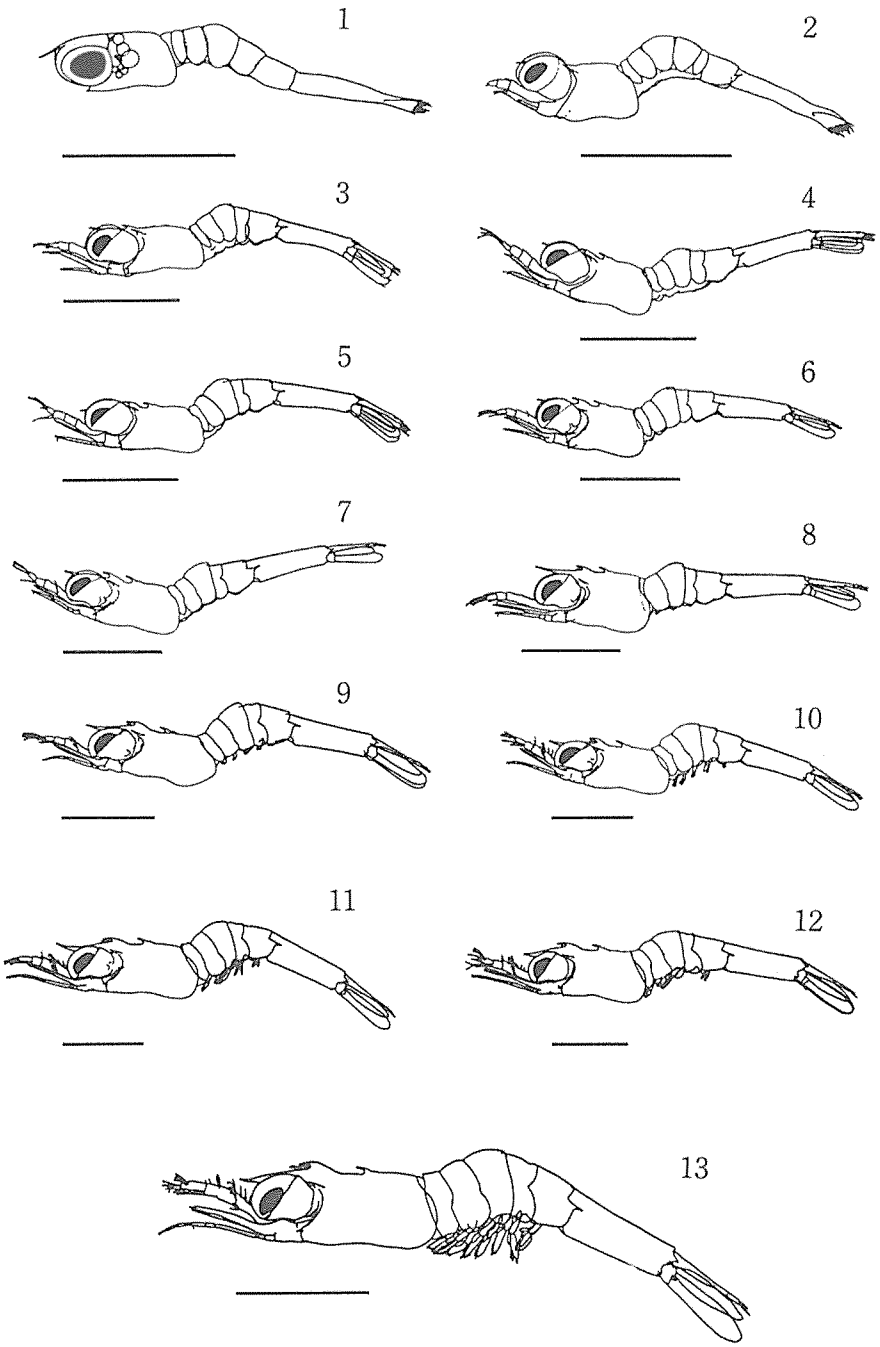
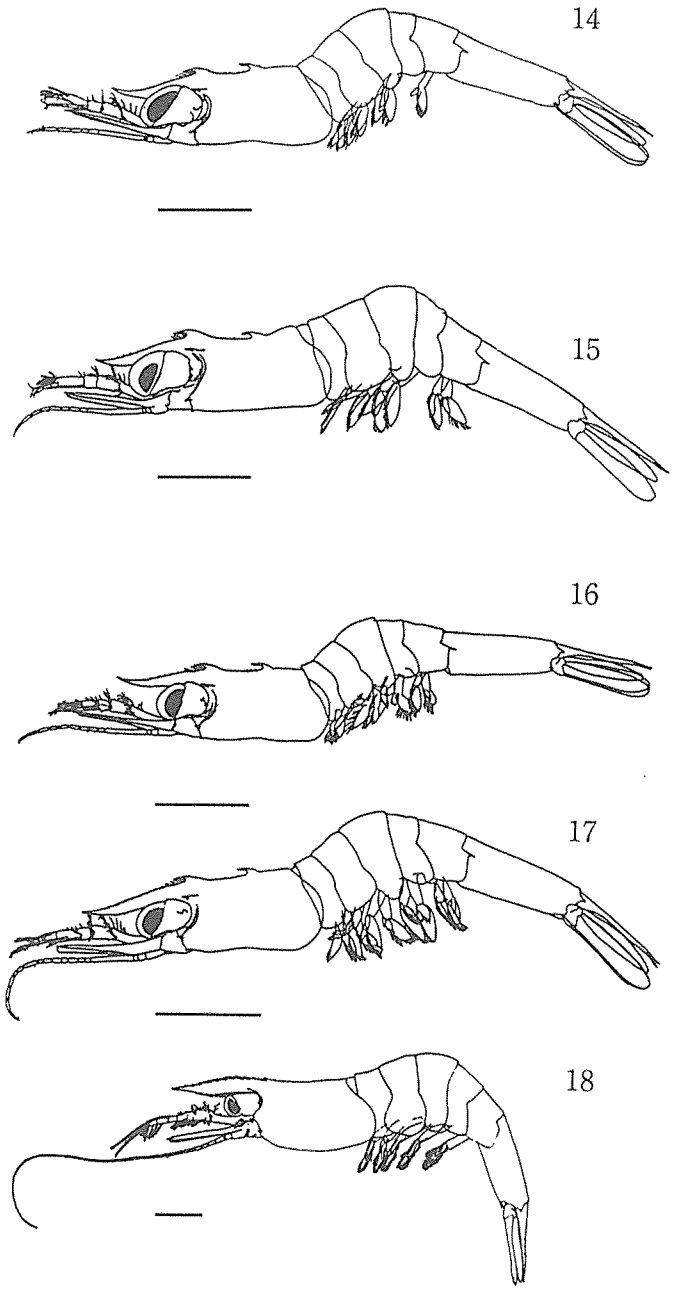


Fig. 1



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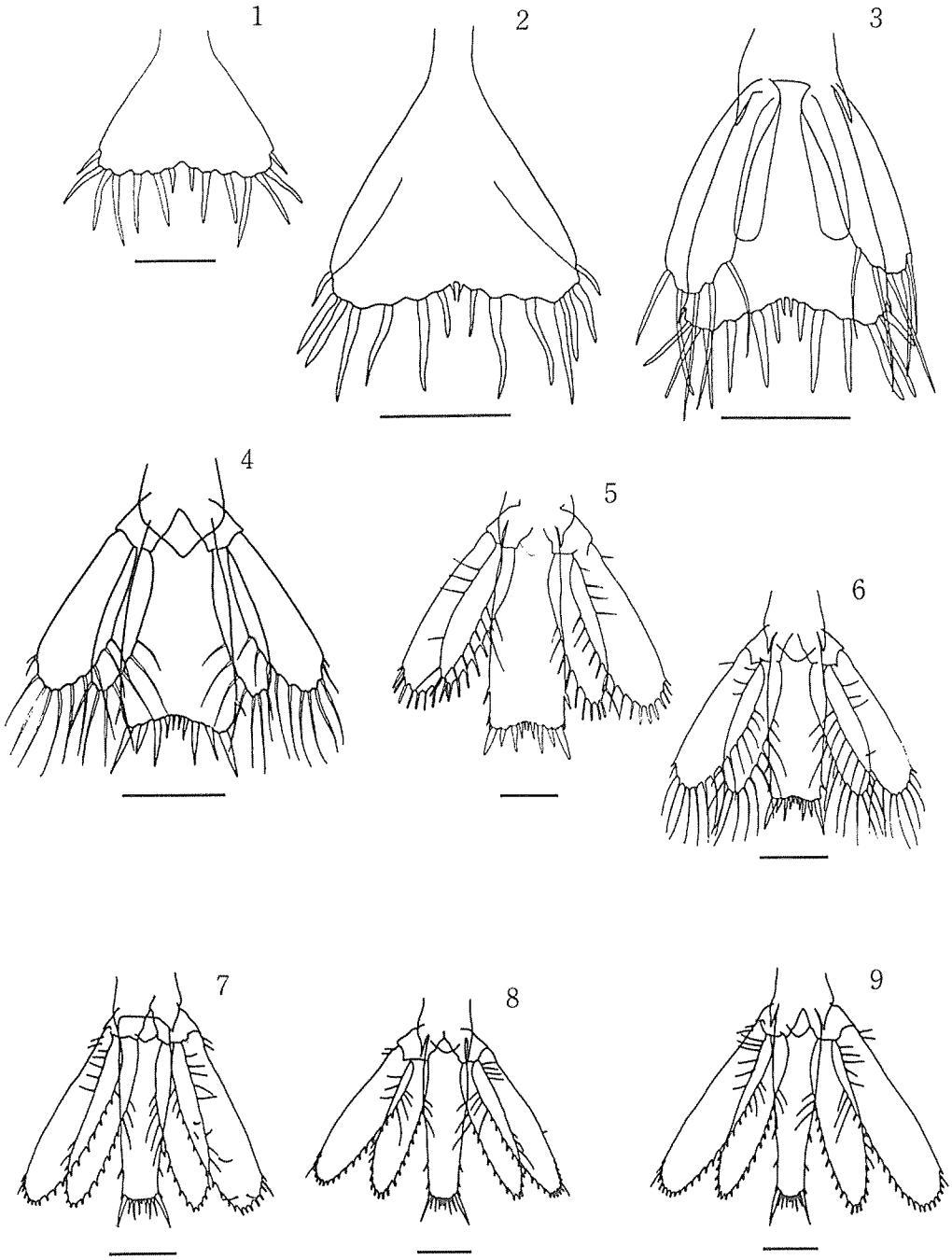
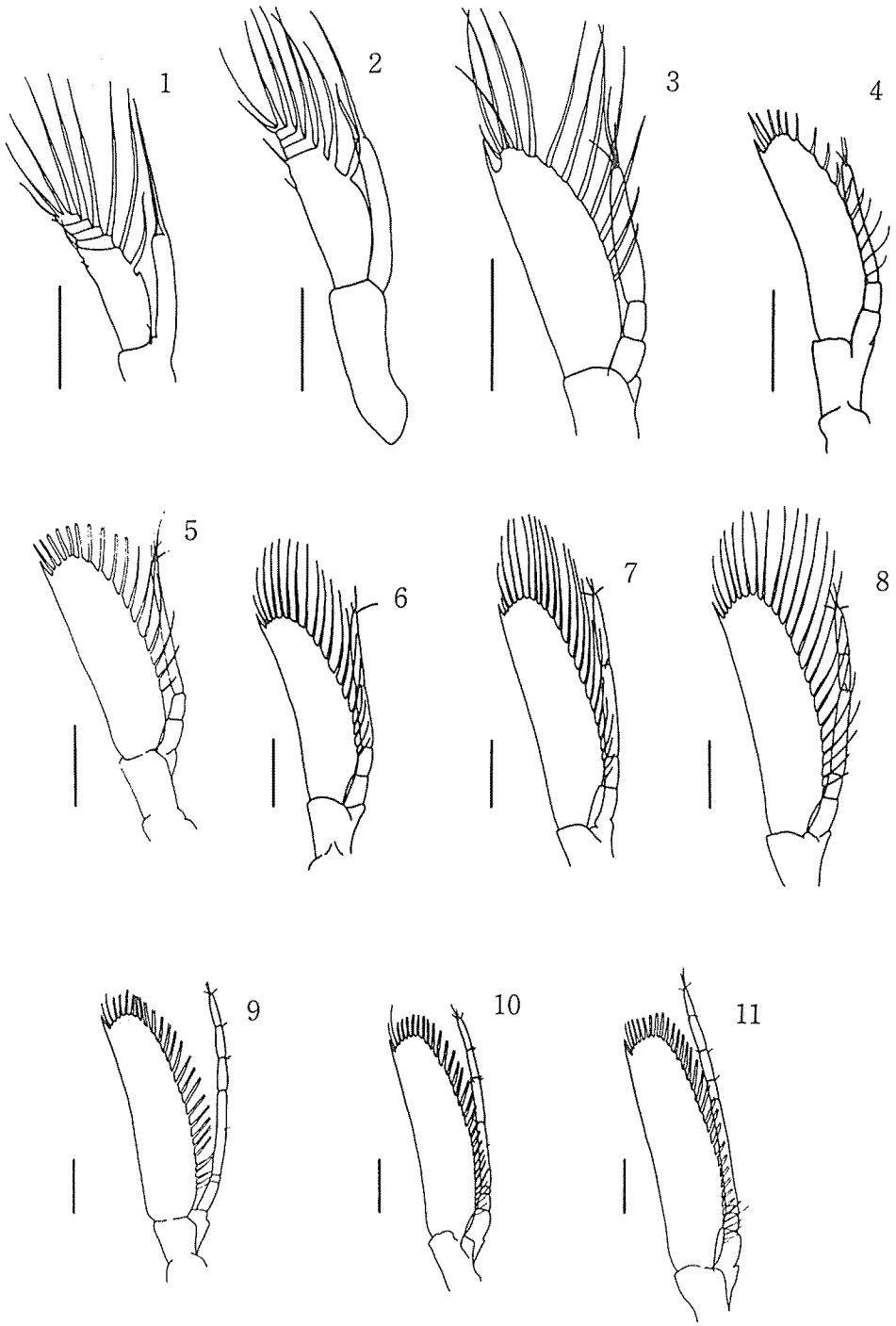


Fig. 2



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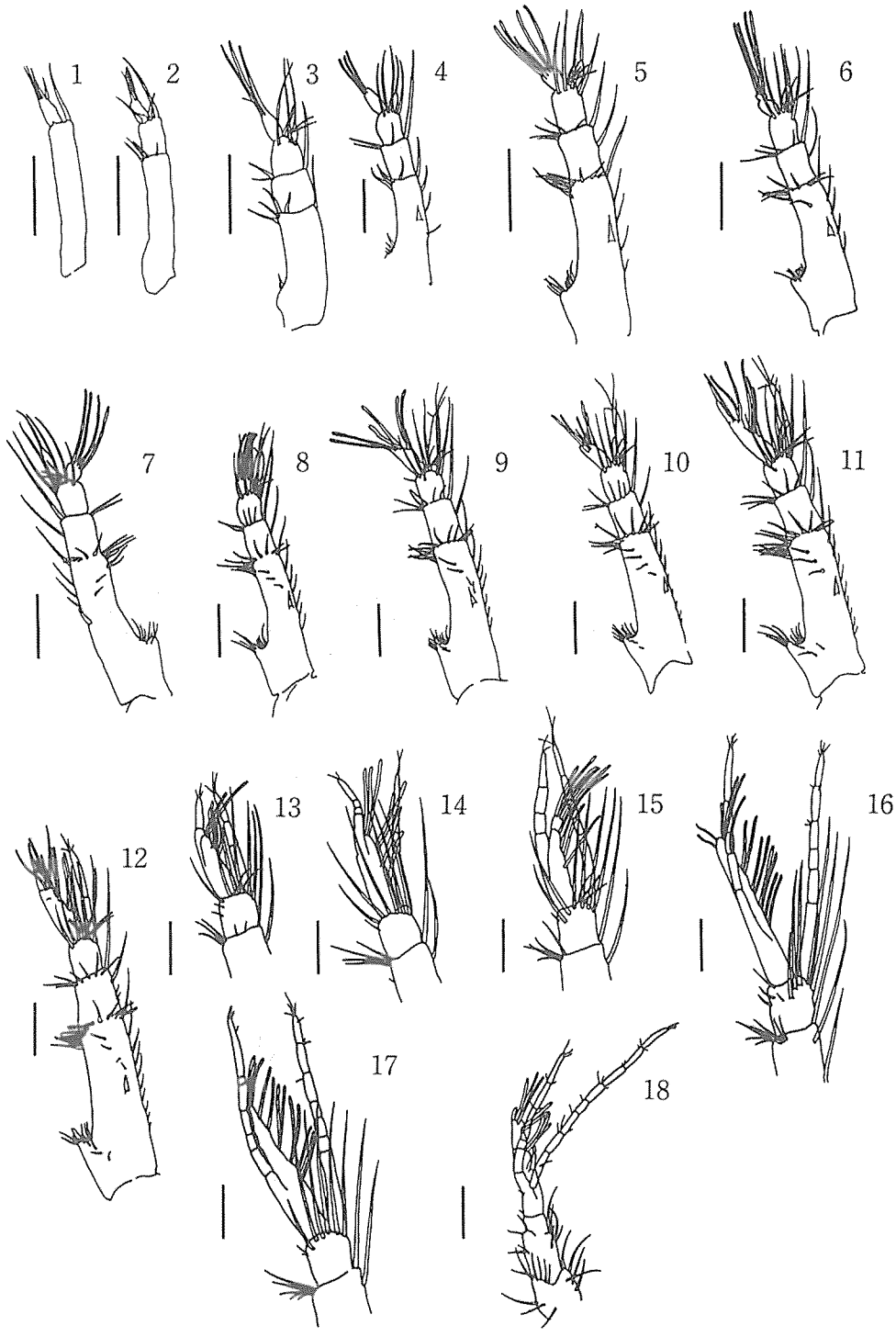

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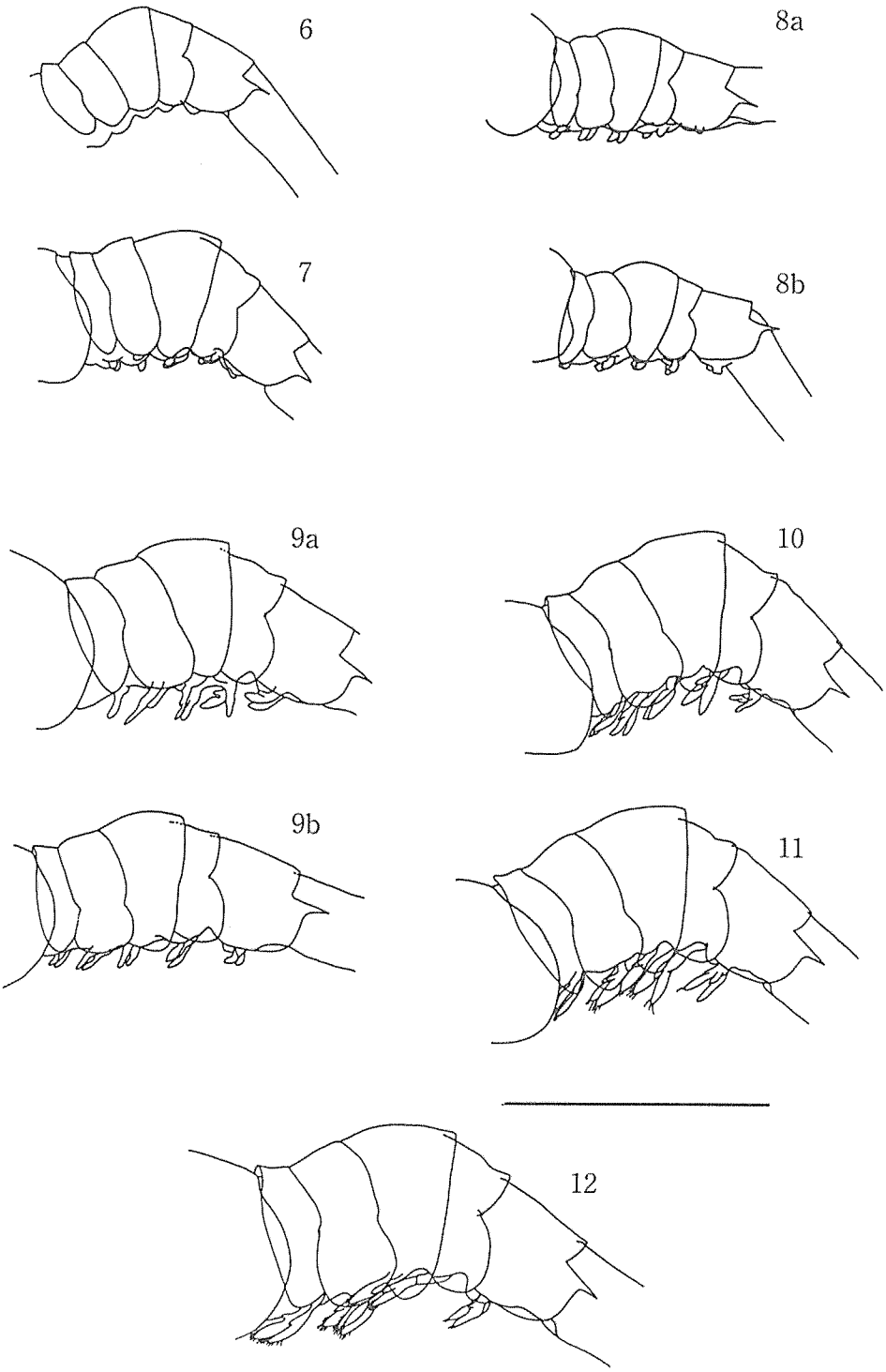

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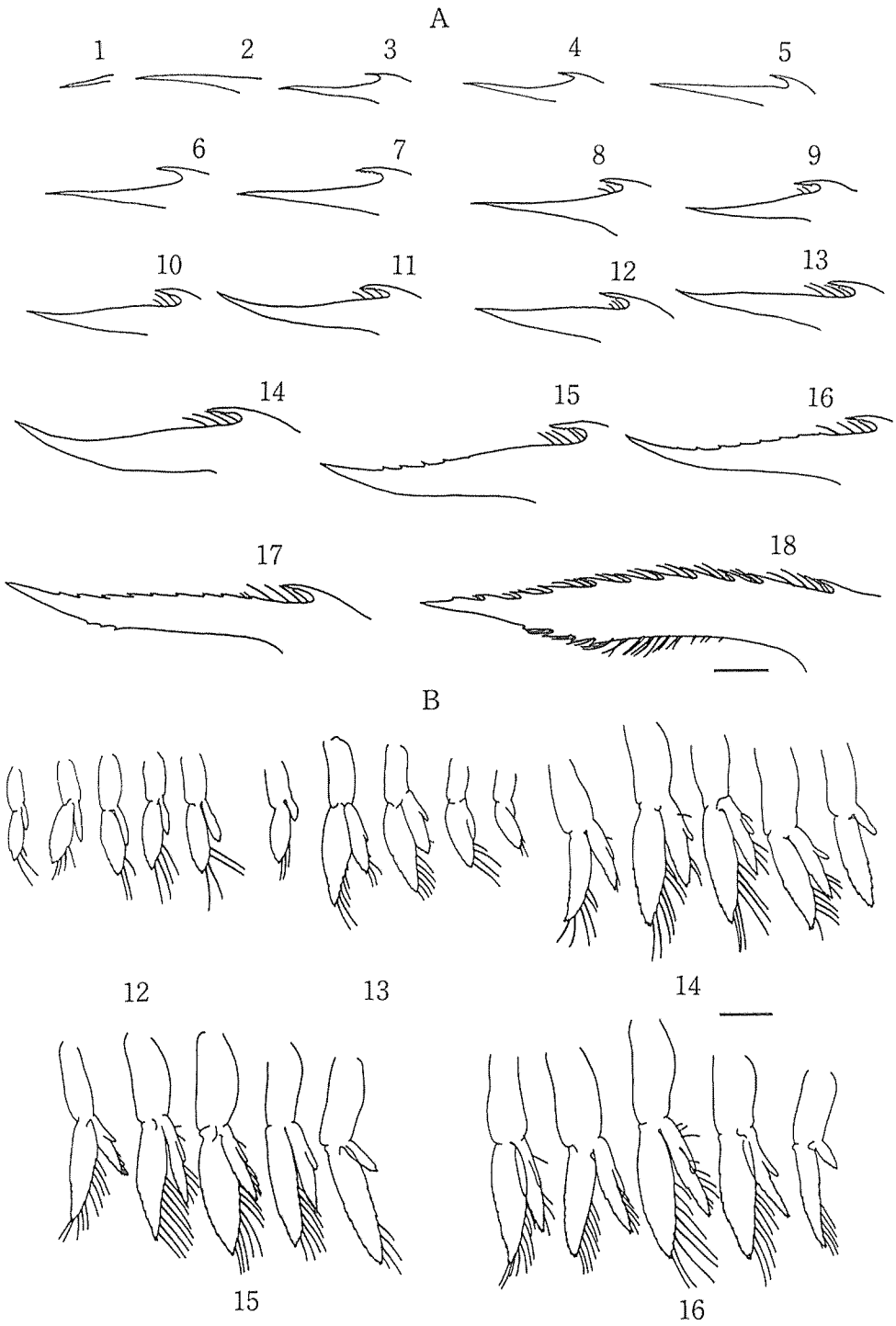

Fig. 6