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Naupliar Development of *Neoergasilus japonicus* (Copepoda: Ergasilidae)*1

Shigehiko URAWA*2, Kiyokuni MUROGA*2, and Shogoro KASAHARA*2

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Neoergasilus japonicus is a parasitic copepod which has been reported from various freshwater fishes in Asia and Europe.

In this study, a morphology of the species in nauplius stages is given from the results of a rearing experiment.

According to the molts, the naupliar development of the species was divided into six stages like typical free-living copepods. They are capable of feeding throughout all the stages. The first maxilla appears in stage IV, and the rudimental first and second legs in stage VI. The second maxilla and the maxilliped are never found in any of the stages.

The result was compared with the descriptions of six other species of Ergasilidae in their developmental courses of nauplius stages, and some suspicion was cast on the results of the past workers.

Neoergasilus japonicus, a parasitic copepod belonging to the family Ergasilidae, has been recorded from various species of freshwater fishes in Eastern Asia and Europe.

The first record of the species was made by HARADA[1] from fishes in Formosa under the name of *Ergasilus japonicus*. In 1956, YIN[2] proposed a new genus, *Neoergasilus*, in the family Ergasilidae, and in the genus he placed *E. japonicus* and other two new species. Recently, *N. japonicus* has also been recorded in Czechoslovakia[3], Hungary[4], France[5] and also in Japan[6].

This parasitic copepod is widely distributed as above, however, the detailed study on the life cycle of the species has never been carried out. We have made a series of studies on the life cycle, the host range, and the distribution of the species in Japan.

In this paper, the naupliar development of *N. japonicus* was described on the basis of the results of a laboratory incubation, and the result was discussed from the comparative point of view with the development of other ergasilids.

Materials and Methods

Bluegills *Lepomis macrochirus* carrying *N. japonicus* on their fins were captured by angling in Chizuka pond in Fukuyama city, Hiroshima prefecture.

Egg sacs collected from the ovigerous females were placed in a petri-dish containing filtered freshwater. Soon after hatching, the nauplii were transferred to 1000 m*l* bottles containing the water from Chizuka pond and cultured by using a rolling incubator (Toyo, M1–5). This experiment was repeated at different temperatures ranging from 16°C to 30°C from July to October in both 1978 and 1979.

Detailed structures were observed on living specimens as well as specimens fixed with a solution of 5% formalin. Measurements of larvae were made on living specimens with an ocular micrometer, and drawings with the aid of a camera lucida.

Results

Out of 7 trials, the nauplii successfully developed to the copepodid stage I or II in 4 experiments. The success in the development seemed to be independent of the incubation temperatures tested. While, the duration required for the development was influenced by the temperature, and the relation between temperature and the development time will be reported in another paper.

According to the molts and the changes in the structural characteristics, the developmental course of the nauplius was divided into 6 stages.

Measurements of the body, the first and second

*1 Studies on *Neoergasilus japonicus* (Copepoda: Ergasilidae), a Parasite of Freshwater Fishes—I

*2 Faculty of Applied Biological Science, Hiroshima University, Fukuyama 720 (浦和茂彦・室賀清邦・笠原正五郎: 広島大学生物生産学部).

antenna, and the caudal armatures in each stage were given in Table 1.

Nauplius Stage I (Fig. 1, 7, 13, 19, 24)

Body (Fig. 1) oval, with small lob corresponding to rostrum on anterior end and hemispherical knob slightly flattened dorsoventrally on dorsal surface near caudal end. Median eye heavily pigmented red, placed just posterior to rostrum. Posterior end of body armed with pair of plumose setae (Fig. 24). Base of dorsal knob and caudal end pigmented reddish orange. Labium distinct. Narrow gullet running from mouth into stomach pigmented light brown. Intestine pigmented posteriorly light blue, running to anus near caudal setae. Newly hatched nauplii capable of active swimming, and beginnig to eat in this stage.

First antenna (Fig. 7) trimerous. Basal segment short, armed with no armature, middle one slightly longer than apical one, with unarmed seta in posterodistal corner. Apical segment armed with 1 long plumose and 1 short unarmed setae on distal margin.

Second antenna (Fig. 13) biramous, with indistinctly bimerous protopod. Coxopod short, armed with large, strong, slightly unciform and tapering spine. Endopod unsegmented, short and provided with 2 long unarmed and 1 minute setae on distal margin. Exopod 5-segmented. Basal segment long, with long plumose seta in distal margin. Second segment short and with long plumose seta in distal corner. Segment 3 and 4 similar. Segment 5 small, with 2 apical setae plumosed.

Mandible (Fig. 19) biramous. Protopod provided with unarmed seta on medial margin. Endopod bimerous. Basal segment not clearly delimited from protopod, cylindrical, pointing anteriorly and armed with 2 long setae: inner one unarmed and outer with row of short cilia. Apical segment small, rhombic and armed with flattened lamina and 3 slender setae on distal margin. Exopod also bimerous, basal segment armed with long, plumose seta on posteromedial margin, and apical one with 2 long, plumose setae.

Nauplius Stage II (Fig. 2, 8, 14, 20, 25)

General appearance (Fig. 2) quite similar to that of stage I. Appendages also similar to those of stage I, except for those described below:

Labium pigmented reddish orange in adjacent part to mouth. Minute seta on endopod of second antenna reduced in length (Fig. 14). On mandible, long setule added midway on inner seta of basal

Table 1. Measurements on the nauplii of *Neoergasilus japonicus*

Nauplius stage	Body		First antenna length	Second antenna length*2	Caudal armatures*3				Number of sample
	Length	width			1	2	3	4	
I	92 (84–105)*1	55 (48– 60)	48 (45–54)	61 (59– 64)	29 (27–30)	—	—	—	235
II	109 (100–118)	65 (58– 74)	53 (49–58)	66 (63– 70)	29 (28–30)	—	—	—	105
III	123 (110–140)	72 (65– 80)	59 (53–65)	73 (69– 80)	30 (28–31)	18 (16–21)	—	—	150
IV	142 (128–155)	82 (75– 90)	64 (55–70)	85 (70– 90)	30 (28–33)	21 (18–25)	3 (2– 5)	—	136
V	155 (143–183)	91 (80–109)	68 (63–83)	86 (70–100)	29 (26–31)	23 (20–26)	7 (5–10)	4 (3– 5)	169
VI	180 (163–235)	103 (88–135)	75 (68–88)	92 (78–115)	30 (28–31)	23 (20–25)	13 (11–15)	11 (10–12)	123

*1 mean and (range) in μm.
*2 length from the base of coxopod to the external end of exopod.
*3 length of the setae of the posterior end of the body (1, 2, 3 or 4 as indicated in Fig. 29); a dash (—) indicates that the armature has not yet appeared at the stage.

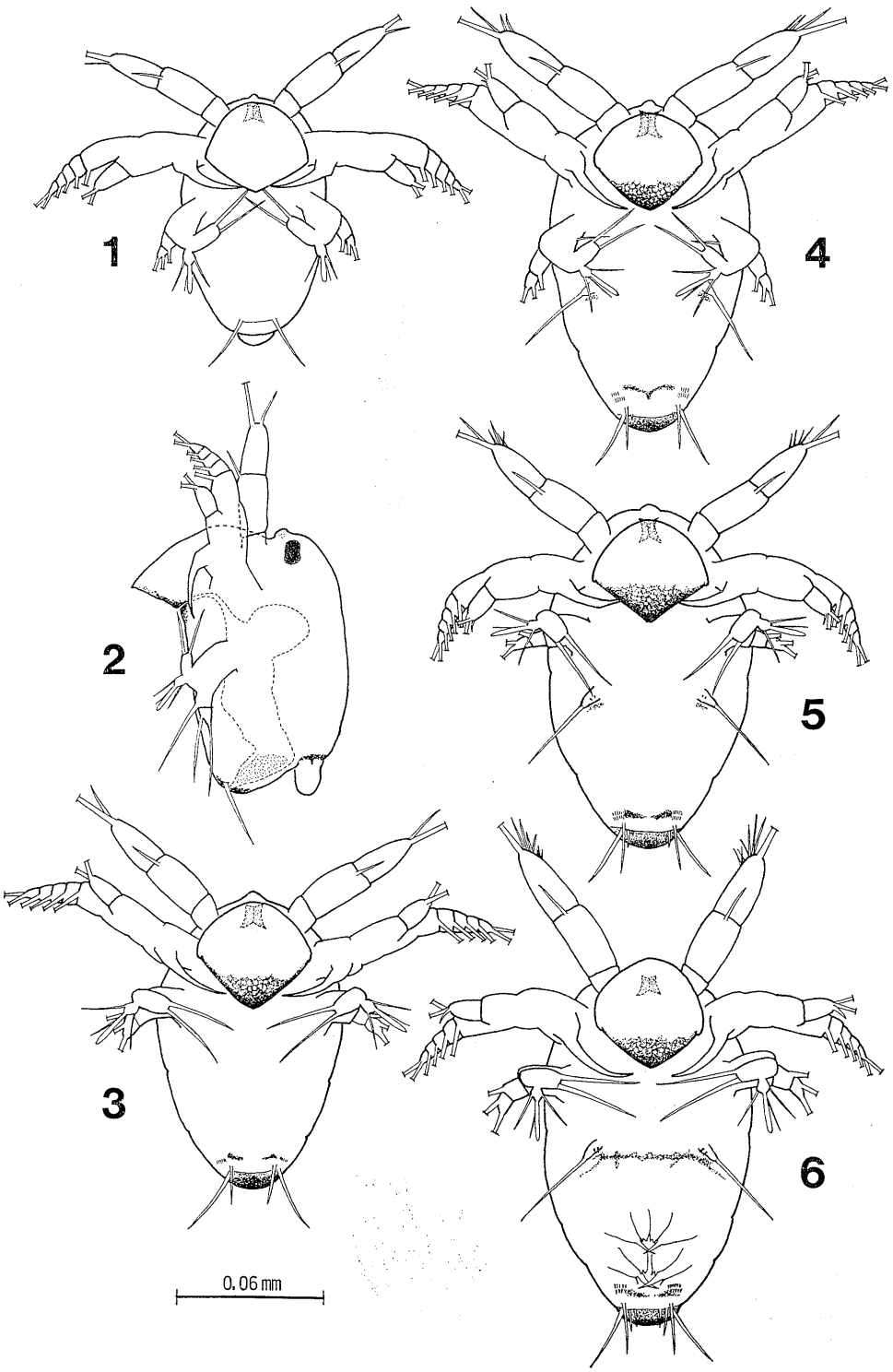

Fig. 1–6. Nauplius stages of *Neoergasilus japonicus*. 1. Nauplius stage I, ventral view; 2. Nauplius stage II, lateral view; 3. Nauplius stage III, ventral view; 4. Nauplius stage IV, ventral view; 5. Nauplius stage V, ventral view; 6. Nauplius stage VI, ventral view.

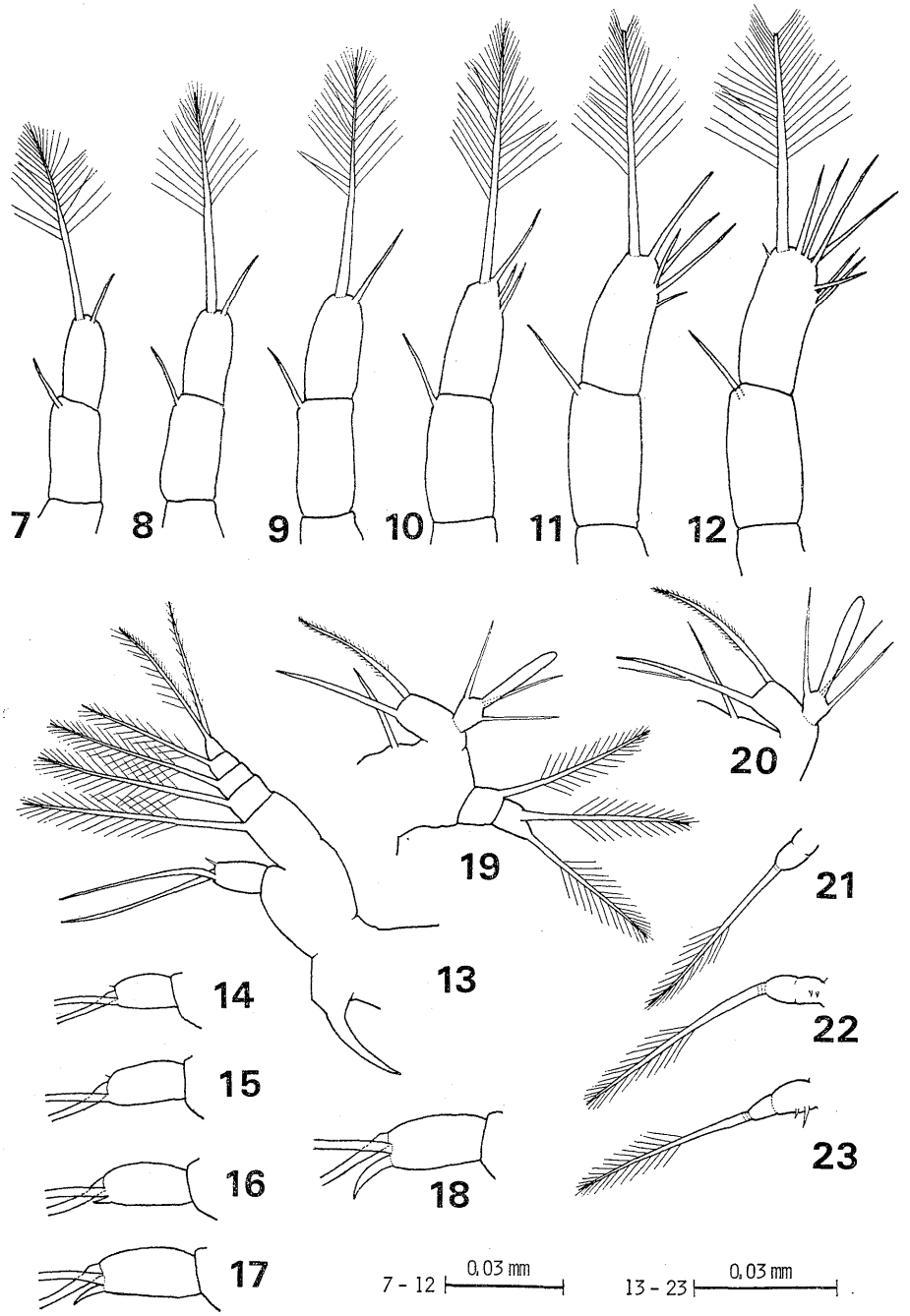

Fig. 7–23. Nauplius stages of *Neoergasilus japonicus*. 7–12. First antenna of nauplius stage I–VI, lateral view. 13. Second antenna of nauplius stage I, lateral view. 14–18. Endopod of second antenna of nauplius stage II–VI, lateral view. 19. Mandible of nauplius stage I, ventral view. 20. Endopod of mandible of nauplius stage II, ventral view. 21–23. First maxilla of nauplius stage IV–VI, ventral view.

segment of endopod (Fig. 20).

Nauplius Stage III (Fig. 3, 9, 15, 26)

On caudal end, one pair of caudal setae newly appeared in addition to plumose pair (Fig. 26). No changes apparent in first and second antenna and mandible.

Nauplius Stage IV (Fig. 4, 10, 16, 21, 27)

On caudal end, third pair of short spiniform setae appeared (Fig. 27). On tip of first antenna (Fig. 10), 2 new setae appeared. On second antenna, small spine added to distal margin of endopod (Fig. 16). Mandible as in preceding stage. First maxilla (Fig. 21) newly appeared on ventral surface just posterior to mandible, unsegmented process surmounted by long, plumose seta.

Nauplius Stage V (Fig. 5, 11, 17, 22, 28)

On caudal end, fourth pair of short spiniform setae added (Fig. 28). Two new setae appeared in tip of first antenna (Fig. 11). Spine on endopod of second antenna increased in length, and claw-like (Fig. 17). Mandible unchanged. On base of first maxilla (Fig. 22), 2 minute, spiniform setae appeared.

Nauplius Stage VI (Fig. 6, 12, 18, 23, 29)

General appearance (Fig. 6) different only slightly from that of preceding stage. Hindbody somewhat narrowed. Third and fourth caudal setae (Fig. 29) longer than in preceding stage. Ventral surface just behind first maxilla transversely pigmented reddish orange. Two short and one minute setae newly added in tip of first antenna (Fig. 12). Claw-like spine on endopod of second antenna (Fig. 18) longer than that of preceding stage. Setae on base of first maxilla (Fig. 23) also longer. Mandible unchanged. Rudiment of first and second pairs of swimming legs newly appeared in ventral surface of hindbody. Any signs of second maxilla and maxilliped not yet recognized.

The growth in body length is shown in Fig. 30.

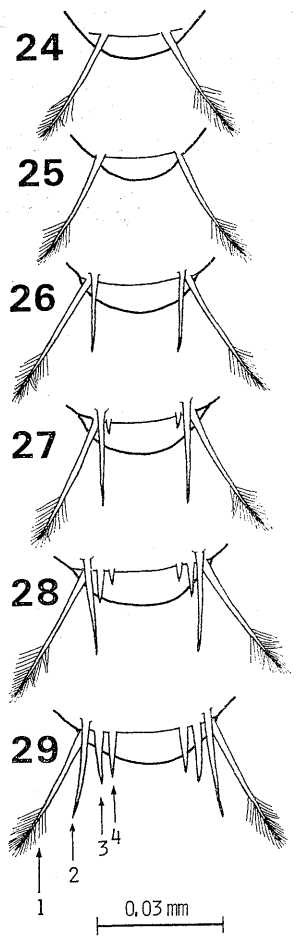

Fig. 24–29. Caudal armature of nauplius stage I–VI of *Neoergasilus japonicus*, ventral view.

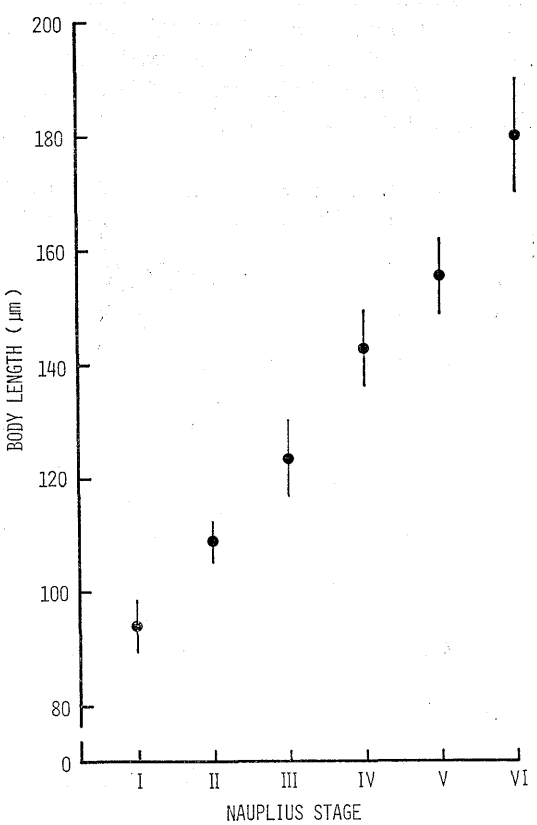

Fig. 30. Relationship between body length and nauplius stage of *Neoergasilus japonicus*. Closed circle indicates mean, and vertical bar indicates standard deviation. Number of sample observed is shown in Table 1.

As is evident from the figure, the body length has linearly increased, and the stage VI has become nearly twice as long as the stage I.

Discussion

In general, copepods have 6 nauplius stages, but in parasitic copepods, some of the nauplius stages tend to be abbreviated with the progress of adaptation in parasitism. It has been reported that there are 5 nauplius stages in some notodelphid copepods,[7] *Sarcotaces pacificus*,[8] *Colomatus pupa*[9] and *Ostrincola koe*,[10] 4 stages in *Lernaea cyprinacea*,[11] and 1 or 2 stages in some other parasitic copepods.

As far as we know, six species of Ergasilidae have been studied on their naupliar developments. It was demonstrated that there were 3 nauplius stages (or 1 nauplius and 2 metanauplius stages) in the development of *Ergasilus centrarchidarum* by WILSON,[12] *E. briani* (syn. *E. minor*) by HALISCH,[13] *E. sieboldi* by ZMERZLAYA[14] and *Sinergasilus lieni* by MIRZOEVA.[15] GURNEY,[16] meanwhile, found 4 nauplius stages in the development of *Thersitina gasterostei* although he failed to keep them up to the copepodid stage. It was shown by YIN[2] that *Sinergasilus major* had 5 nauplius stages though he provided no information on the structure of the last three stages.

It has been confirmed that *N. japonicus* has 6 nauplius stages according to the molts and the changes in structures. Then, those copepods belonging to the same family, Ergasilidae, have different number of stages in their naupliar development.

Here, we made a comparison in nauplius stages between *N. japonicus* and other three species, *E. centrarchidarum, E. briani* and *E. sieboldi*, and the result is shown in Table 2.

It is evident from the table that the nauplius stage I of all the four species is much similar to each other.

On the naupliar development of *E. centrarchidarum*, WILSON mentioned that 3 molts occurred before transforming into the metanauplius stage I and 2 more molts occurred before the metanauplius stage II. So, if based on the number of molt, it can be said that *E. centrarchidarum* has 6 nauplius stages, and the metanauplius stage I and II of the species correspond to the nauplius stage IV and VI, respectively, of *N. japonicus*, though they differ from the latters in having second maxilla and maxil-

Table 2. Comparison in the naupliar characteristics among *Neoergasilus japonicus, Ergasilus centrarchidarum, E. briani* and *E. sieboldi*

Species	*Neoergasilus japonicus*		*Ergasilus centrarchidarum*		*Ergasilus briani*		*Ergasilus sieboldi*	
Author	Present authors		WILSON (1911)		HALISCH (1939)		ZMERZLAYA (1972)	
Number of setae on first antenna	NI–III*1	3	NI	3	NI	3	NI	3
	NIV	5	MI*2	5	MI	5	NII	4
	NV	7	MII	12	MII	6	NIII	6
	NVI	10						
Number of setae (S) and spine (H) on endopod of second antenna	NI–III	2S	NI	2S	NI	2S	NI	2S
	NIV–VI	2SlH	MI, II	4SlH	MI, II	3S	NII, III	2SlH
First maxilla	NI–III	absent	NI	absent	NI	absent	NI	absent
	NIV–VI	present	MI, II	present	MI, II	present	NII, III	present
Second maxilla	NI–VI	absent	NI	absent	NI, MI	absent	NI, II	absent
			MI, II	present	MII	?	NIII	present
Maxilliped	NI–VI	absent	NI	absent	NI, MI	absent	NI–III	absent
			MI, II	present	MII	present?		
First and second swimming legs	NI–V	absent	NI, MI	absent	NI, MI	absent	NI–III	absent
	NVI	present	MII	present	MII	present		
Number of caudal setae	NI, II	2	NI	2	NI	2	NI	2
	NIII	4	MI, II	6	MI, II	8	NII	4
	NIV	6					NIII	8
	NV, VI	8						

*1 N: Nauplius stage.
*2 M: Metanauplius stage.

liped, and in the armatures of some appendages.

In *E. briani*, the metanauplius stage I resembles the stage IV of *N. japonicus* except for the number of caudal setae. The metanauplius stage II is provided with 2 pairs of swimming legs like the stage VI of *N. japonicus*. Therefore, the metanauplius stage I and II can be interpreted to be comparable to the nauplius stage IV and VI of *N. japonicus* respectively.

The nauplius stage II and III of *E. sieboldi* can be assumed to correspond to the stage IV and V of *N. japonicus* because of the similarity of their appendages.

As reviewed above, some nauplius stages were reported to be abbreviated in some ergasilids. But we have some doubts about the results of the past workers. It is said that members of Ergasilidae are in the initial stages of the parasitic adaptations. If so, it seems reasonable that they keep the full developmental stages as demonstrated in the present species.

In any case, more work is needed to clear up the naupliar development of Ergasilidae.

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