

Studies on *Neoergasilus japonicus* (Copepoda: Ergasilidae), a Parasite of Freshwater Fishes – II Development in Copepodid Stage

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(Figs. 1–66, Table 1)

In the previous paper¹⁾, we have shown that *Neoergasilus japonicus* has six nauplius stages on the basis of the results of rearing experiments. In those experiments, however, the hatched nauplii developed successfully up to the copepodid stage II, yet failed to undergo further development.

It is generally known that ergasilids are present in plankton as nauplii, copepodids, males and nonovigerous females, and that only adult females are parasitic on fishes. In a pond of Hiroshima Prefecture, we have obtained many copepodids of *N. japonicus* by net tows. Here, we intend to describe the development in the copepodid stage of *N. japonicus* mainly from the results of examination of these net samples.

MATERIALS AND METHODS

Plankton samples were obtained by horizontal tows of a plankton-net (94 μ m mesh size) in Chizuka pond in Fukuyama City, Hiroshima Prefecture. Samplings were taken once or twice a month in 1977 and 1978, and the samples obtained were preserved in a solution of 5% formalin.

Under a dissecting microscope, the copepodids of the species were distinguished from other copepods by their prominent prehensile second antennae and brilliant-colored intestines.

Detailed observations were made on these net samples and on the samples obtained from the laboratory cultures, although the latter samples were restricted to the copepodid stage I and II as described above.

RESULTS

In the incubation experiments, 49 copepodids were obtained and they were divided into the copepodid stage I and II according to the molt and the difference in structural features.

Besides these, 953 copepodids of the species have been collected by 28 tows of net. Based on the detailed observations of these samples, the developmental course of the

Table 1. Morphological characteristics in the copepodid stages of *Neoergasilus japonicus*.

Stage	Sex	I		II		III		IV		V		VI	
						Female	Male	Female	Male	Female	Male	Female	Male
Number of sample		166	120	34	33	43	31	56	48	20	75		
Body length		352 (277-418)*	423 (365-474)	480 (401-556)	450 (384-522)	546 (469-623)	508 (469-589)	630 (528-760)	577 (460-639)	670 (573-795)	624 (549-716)		
Head	length	134 (115-160)	150 (120-170)	162 (145-185)	156 (140-185)	183 (160-205)	168 (160-193)	206 (180-259)	183 (163-203)	214 (185-260)	187 (160-210)		
	width	127 (105-158)	145 (120-173)	160 (134-203)	153 (133-188)	182 (158-215)	163 (144-200)	213 (180-263)	182 (155-213)	248 (225-283)	183 (163-230)		
Thorax	length	152 (100-193)	197 (153-245)	215 (173-268)	194 (150-238)	236 (180-280)	217 (175-255)	256 (190-325)	237 (175-283)	267 (200-365)	250 (193-305)		
	width	120 (99-148)	136 (105-165)	149 (133-184)	138 (120-178)	172 (150-200)	151 (135-193)	202 (180-250)	169 (145-200)	252 (210-295)	176 (155-215)		
Genital segment	No. of segment	4	5	5	5	5	5	5	5	5	5		
	length	lack	lack	23 (20-28)	22 (18- 28)	28 (20- 33)	28 (23- 33)	57 (48- 68)	37 (30- 43)	61 (53- 70)	45 (33- 53)		
Abdomen	width			41 (36- 45)	38 (33- 45)	48 (40- 55)	42 (39- 48)	61 (53- 70)	53 (45- 58)	75 (68- 88)	61 (55- 70)		
	length	46 (38- 55)	48 (38- 55)	45 (38- 58)	46 (38- 58)	58 (40- 73)	55 (48- 65)	63 (53- 75)	73 (63- 93)	74 (65- 85)	89 (73-108)		
Caudal rami	width	37 (30- 43)	37 (33- 41)	38 (34- 45)	37 (33- 43)	41 (35- 45)	35 (33- 40)	43 (38- 50)	40 (35- 45)	46 (40- 55)	41 (36- 48)		
	No. of segment	1	1	1	1	2	2	2	3	3	4		
Length of plumose setae	longer	20 (16- 25)	28 (23- 30)	34 (28- 40)	34 (25- 43)	41 (35- 48)	40 (36- 45)	49 (40- 58)	47 (40- 55)	53 (45- 60)	53 (45- 60)		
	shorter	16 (13- 18)	16 (14- 19)	16 (15- 18)	16 (15- 18)	17 (15- 20)	16 (15- 18)	18 (15- 20)	17 (14- 20)	16 (15- 20)	16 (13- 20)		
1st antenna	No. of setae	5	4	4	4	4	4	4	4	4	4		
	length	104 (85-125)	108 (88-130)	131 (118-150)	120 (105-133)	149 (125-170)	131 (118-155)	182 (150-230)	151 (128-170)	263 (250-280)	208 (188-233)		
2nd antenna	shorter	62 (50- 88)	78 (63- 95)	92 (88-108)	87 (80-108)	106 (88-130)	103 (90-118)	130 (110-145)	122 (100-138)	146 (135-165)	159 (140-188)		
	length	81 (70- 90)	91 (75-100)	104 (93-120)	100 (90-113)	120 (103-133)	112 (103-130)	138 (118-155)	128 (106-143)	145 (130-170)	137 (125-158)		
Maxilliped	No. of segment	4	4	4	4	4	4	5	5	6	6		
	length of apical claw	20 (19- 20)	26 (21- 28)	29 (28- 30)	29 (28- 29)	31 (30- 34)	27 (25- 28)	38 (33- 43)	31 (27- 35)	66 (60- 73)	35 (30- 38)		
Swimming legs**	length	lack	lack	lack	rudimentary	lack	rudimentary	lack	rudimentary	lack	4-segmented		
					20 (17- 25)		36 (28- 43)		67 (55- 83)		189 (168-226)		
1st leg	Exo	IV-4	I-0, III-5	I-0, III-5	I-0, III-5	I-0, III-5	I-0, III-5	I-0, III-6	I-0, III-6	I-0, I-1, II-5	I-0, I-1, II-5		
	Endo	II-5	0-1, II-4	0-1, II-5	0-1, II-5	0-1, II-5	0-1, II-5	0-1, II-5	0-1, II-5	0-1, 0-1, II-4	0-1, 0-1, II-4		
2nd leg	Exo	II-4	I-0, I-5	I-0, I-6	I-0, I-6	I-0, I-6	I-0, I-6	I-0, I-7	I-0, I-7	I-0, 0-1, I-6	I-0, 0-1, I-6		
	Endo	I-4	0-1, I-4	0-1, I-5	0-1, I-5	0-1, I-6	0-1, I-6	0-1, I-6	0-1, I-6	0-1, 0-2, I-4	0-1, 0-2, I-4		
3rd leg	Exo	rudimentary	II-4	I-0, I-5	I-0, I-5	I-0, 0(1)-6	I-0, 0(1)-6	I-0, 0-7	I-0, 0-7	I-0, 0-1, 0-6	I-0, 0-1, 0-6		
	Endo		I-4	0-1, I-4	0-1, I-4	0-1, I-5	0-1, I-5	0-1, I-6	0-1, I-6	0-1, 0-2, I-4	0-1, 0-2, I-4		
4th leg	Exo		rudimentary	0-5	0-3	0-5	0-3	0-4	0-3	0-4	0-3		
	Endo			0-2	0-2	0-2	0-2	0-2	0-2	0-2	0-2		
5th leg			rudimentary	rudimentary	0-3	rudimentary	0-3	rudimentary	0-3	rudimentary	0-3		

* mean and (range) μm

** arrangement of spines (indicated by Roman numerals) and setae (indicated by Arabic numerals).

copepodid stage of the species could be divided into six stages. Male and female are first distinguished from each other at the stage III, and both become mature at the stage VI.

Segmentations and measurements of the body and the appendages, and the armatures of swimming legs in each stage are shown in Table 1.

Copepodid stage I

Body (Fig. 1) composed of head, thorax of 4 segments, abdomen and 1 pair of caudal rami. On posterodorsal surface of head, vestigial dorsal knob. Caudal ramus (Fig. 8) provided with 2 long, plumose and 3 shorter, unarmed setae in tip.

First antenna (Fig. 2) 4-segmented, basal segment long, the others shorter. Setal formulae as follows (P, long plumose seta; p, shorter plumose seta; S, long unarmed seta; s, shorter unarmed seta; e, setule): segment 1—2S, 1s; 2—2P, 1s; 3—1P, 1s, 1e; 4—3P, 1p, 1S, 3s, 1e.

Second antenna (Fig. 3) 4-segmented. Basal segment robust with spiniform seta in distal corner, bearing vestigial exopod on distal margin. Second one cylindrical, with short spine on medial margin. Third one tapering slightly, with slender seta on postero-medial margin. Apical one forming terminal claw with no armature.

Labrum (Fig. 4, la) nearly orbicular, its midpart of distal margin heavily depressed.

Labium (Fig. 4, lb) U-shaped.

Mandible (Fig. 4, md) 2-segmented. Basal segment stout, with obsolete accessory on base. It also armed with palp fringed with short bristles. Apical segment curved forward, and its medial margin fringed with short, stiff setules.

First maxilla (Fig. 4, mx') in form of stout knob, with 4 structures: 2 unarmed setae on tip, 1 long unarmed seta on lateral margin and 1 short spiniform process on medial margin.

Second maxilla (Fig. 4, mx'') consisting of stout basal segment and short apical segment. Latter carrying thick tuft of spinule in tip and short seta in base.

First swimming leg (Fig. 5) biramous, with bimerous protopod. Basipod armed with plumose seta on lateral margin. Its distal margin between both rami slightly projected. Both rami 1-segmented.

Second leg (Fig. 6) closely resembling first leg, with few differences in armature. Distal margin of basipod not projected. Armature of rami of first and second legs shown in Table 1.

Third leg (Fig. 7) rudimental, rami both knob-like. Exopod armed with 2 short setae: inner one spiniform, outer one slender. Endopod tipped with 2 minute processes.

Copepodid stage II

General appearance (Fig. 9) resembling that of stage I, but thorax divided into 5 segments. Head not provided with dorsal knob. Caudal ramus (Fig. 17) equipped with 2 short unarmed and 2 long plumose setae. Inner plumose seta bifurcated near its base.

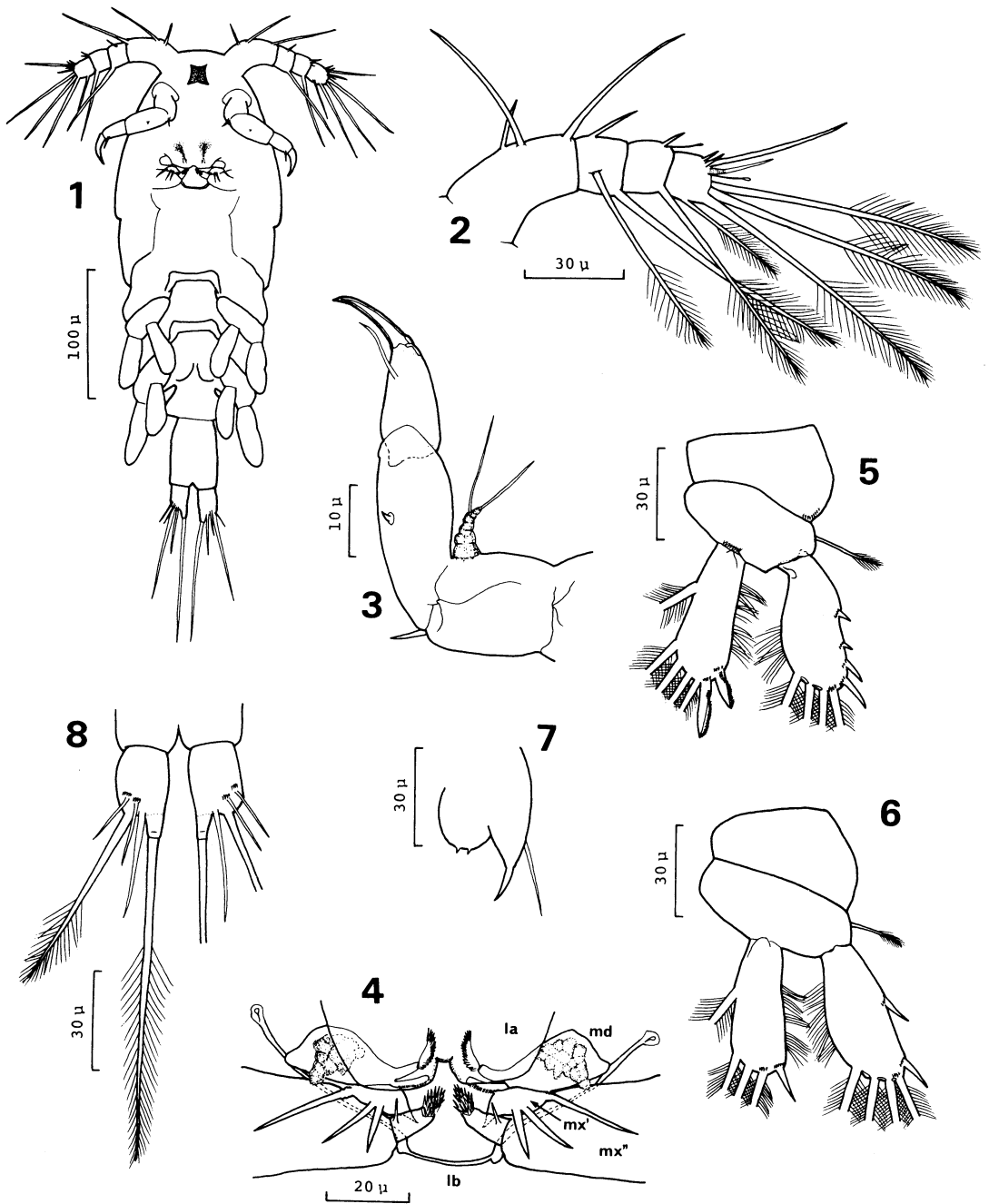


Fig. 1-8. Copepodid stage I of *Neoergasilus japonicus*.

1. Copepodid stage I, ventral; 2. First antenna, ventral; 3. Second antenna; 4. Mouth parts (la, labrum; lb, labium; md, mandible; mx', first maxilla; mx'', second maxilla); 5. First swimming leg; 6. Second leg; 7. Third leg; 8. Caudal rami, ventral.

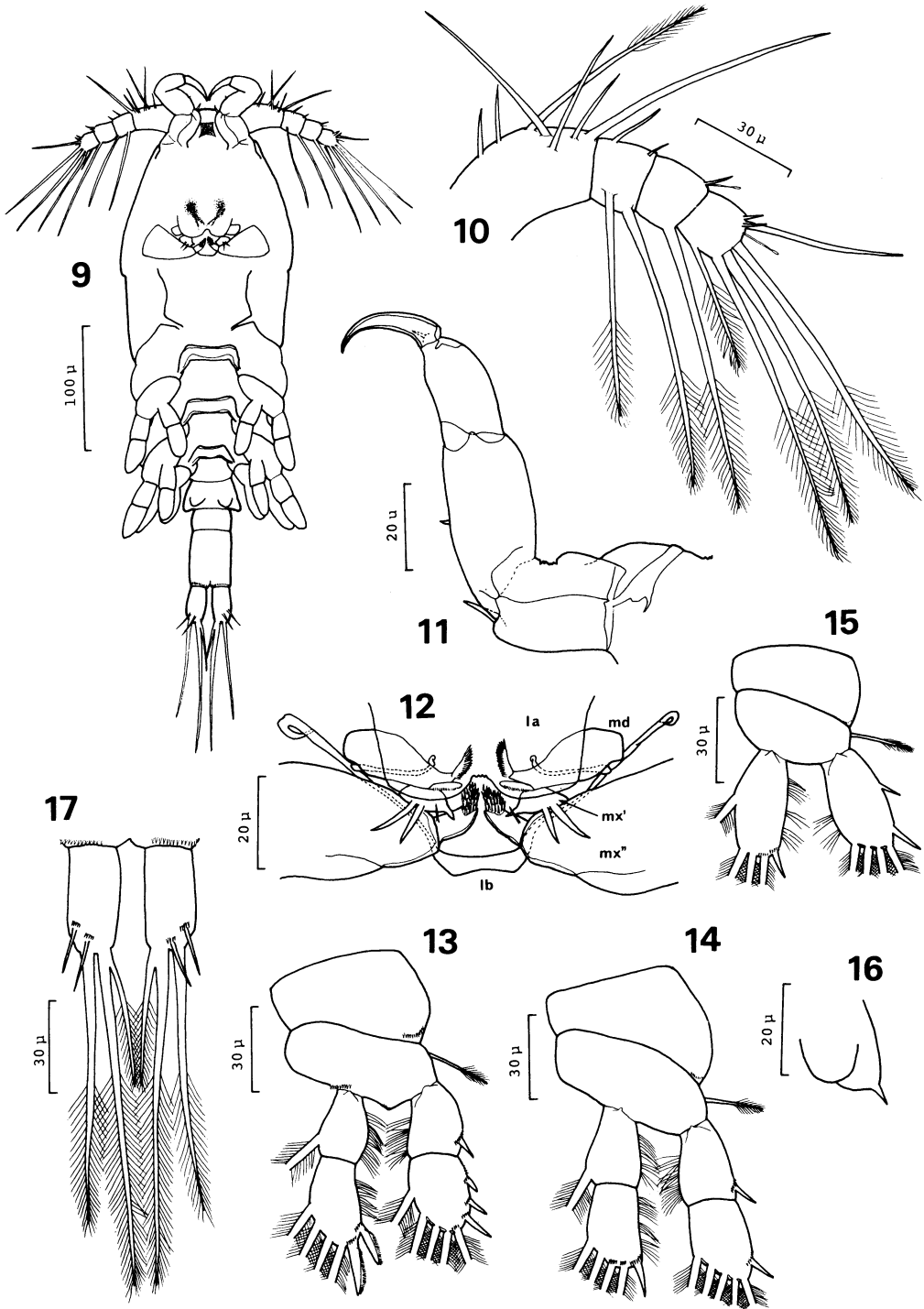


Fig. 9-17. Copepodid stage II of *Neoergasilus japonicus*.

9. Copepodid stage II, ventral; 10. First antenna, ventral; 11. Second antenna; 12. Mouth parts (la, labrum; lb, labium; md, mandible; mx', first maxilla; mx'', second maxilla); 13. First swimming leg; 14. Second leg; 15. Third leg; 16. Fourth leg; 17. Caudal rami, ventral.

First antenna (Fig. 10) retained its structure, but showing changes in armature. Setal formulae as follows: segment 1 – 1P, 3S, 3s; 2–2P, 2s; 3–1P, 1s, 1e; 4–3P, 1p, 1S, 2s, 1e.

Second antenna (Fig. 11) resembling that of the previous stage, but obsolete exopod of basal segment and slender seta of third segment not present. In addition, apical segment equipped with short seta near its base.

Mouth parts (Fig. 12) nearly reached their definitive conditions, obsolete accessory of mandible (md) and lateral seta of first maxilla (mx') disappeared.

Rami of first (Fig. 13) and second leg (Fig. 14) 2-segmented, and those of third leg (Fig. 15) 1-segmented. Fourth leg (Fig. 16) rudimental, knob-like and with spiniform seta on tip of exopod.

Copepodid stage III Female

General appearance (Fig. 18) resembling that of the previous stage, but genital segment present just posterior to fifth thoracic segment.

First antenna (Fig. 19) similar to that of the previous stage, except for armatures. Setal formulae as follows: segment 1–1P, 4S, 5s; 2–2P, 2s; 3–1P, 1s, 1e; 4–3P, 1p, 1S, 2s, 1e.

Second antenna (Fig. 20) resembling that of the previous stage, but slightly enlarged in size.

Rami of first to third legs (Fig. 21, 23, 24) 2-segmented, and those of fourth leg (Fig. 25) 1-segmented. Basal spine on second segment of first exopod slightly enlarged. Exopod of fourth leg with 3 plumose and 2 shorter, unarmed setae. Fifth leg rudimental and without armature.

Copepodid stage III Male

In general appearance, it resembles female very closely.

Appendages also similar to those of female, except for those described below.

Maxilliped (Fig. 27) present just behind second maxilla, rudimental, thumb-like, and with minute process.

First leg resembling that of female, but basal spine on second segment not enlarged (Fig. 22). Exopod of fourth leg (Fig. 26) provided with 3 plumose setae.

Copepodid stage IV Female

Body (Fig. 28) differed from that of the previous stage only in abdomen. Abdomen divided into 2 segments.

First antenna (Fig. 30) same as in the previous stage, but slightly longer.

Second antenna (Fig. 31) much similar to that of the previous stage, though somewhat larger.

First to fourth legs (Fig. 32, 34–36) resemble those of the previous stage, only with some differences in armature.

Fifth leg (Fig. 38) uniramous, with 1 lateral and 2 apical setae.

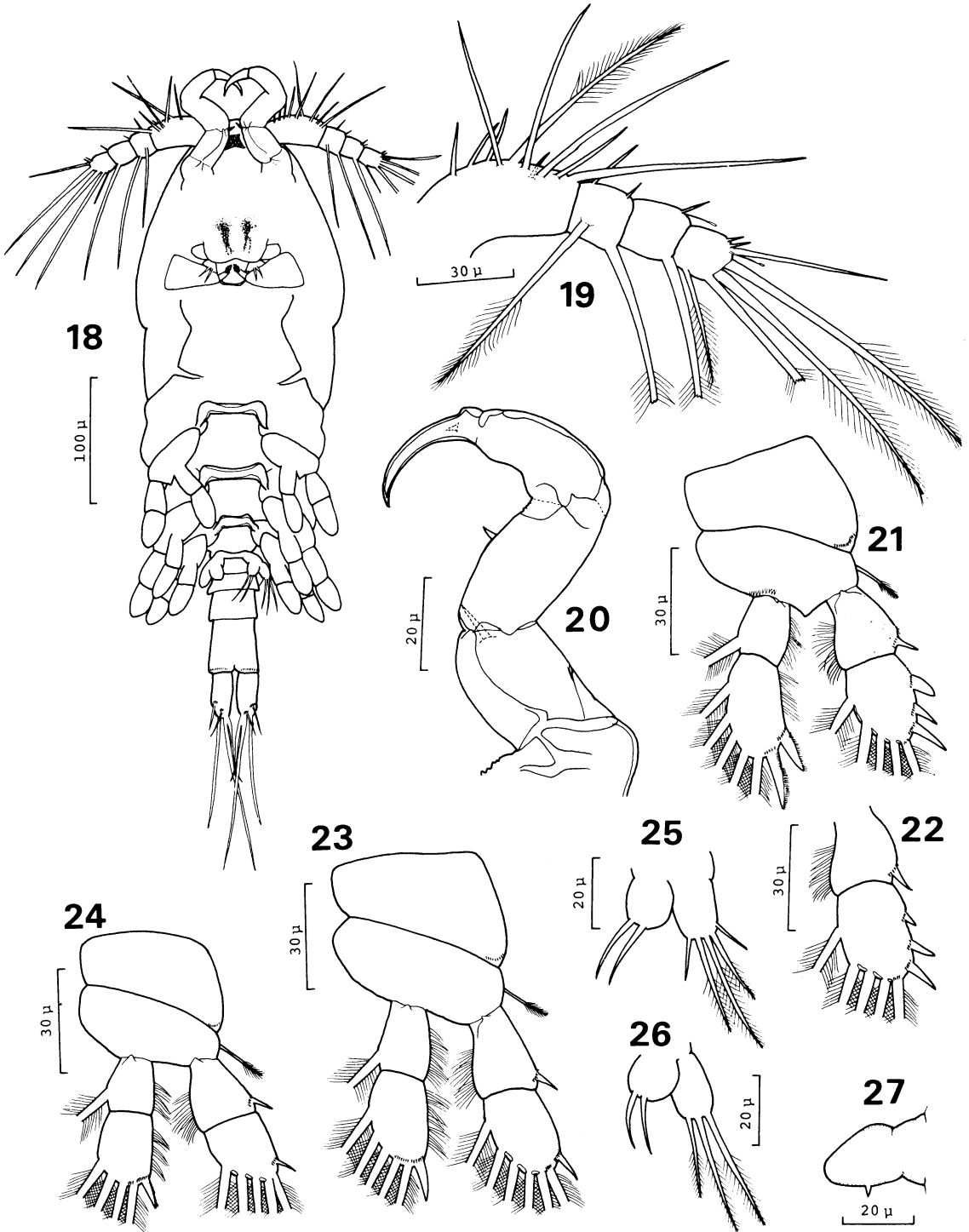


Fig. 18–27. Copepodid stage III of *Neoergasilus japonicus*.

18. Female, ventral; 19. Same, First antenna; 20. Same, Second antenna; 21. Same, First swimming leg; 22. Male, Exopod of First swimming leg; 23. Female, Second leg; 24. Same, Third leg; 25. Same, Fourth leg; 26. Male, Fourth leg; 27. Same, Maxilliped.

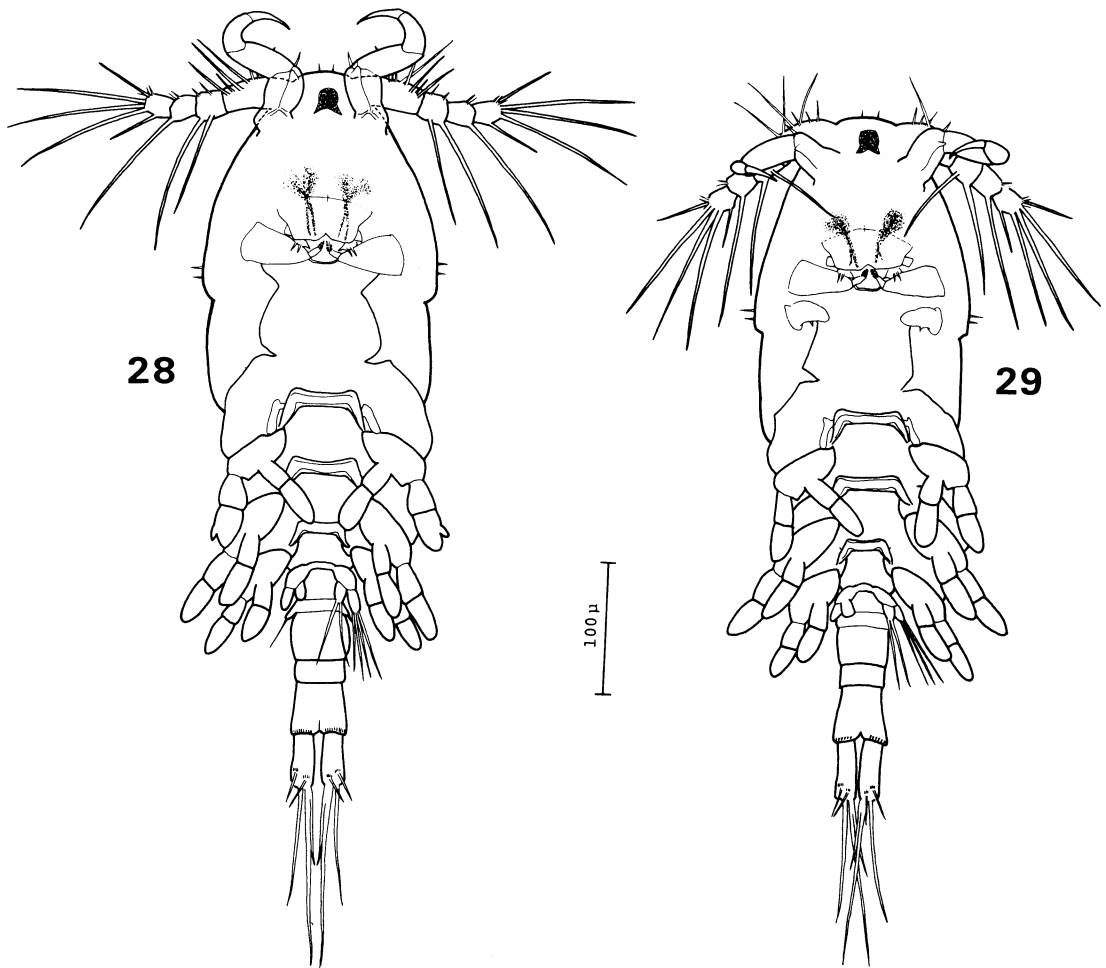


Fig. 28, 29. Copepodid stage IV of *Neoergasilus japonicus*, ventral.
28. Female; 29. Male.

Copepodid stage IV Male

General appearance (Fig. 29) resembles that of female.

Maxilliped (Fig. 39) somewhat enlarged in size, armed with short spiniform seta and minute process.

Spine on second segment of first exopod (Fig. 33) not enlarged. Fourth leg (Fig. 37) with 3 plumose setae on exopod as in the preceding stage. Fifth leg rudimental, with no armature.

Other appendages as in female.

Copepodid stage V Female

General appearance (Fig. 40) differed from that of the previous stage only in relatively developed genital segment.

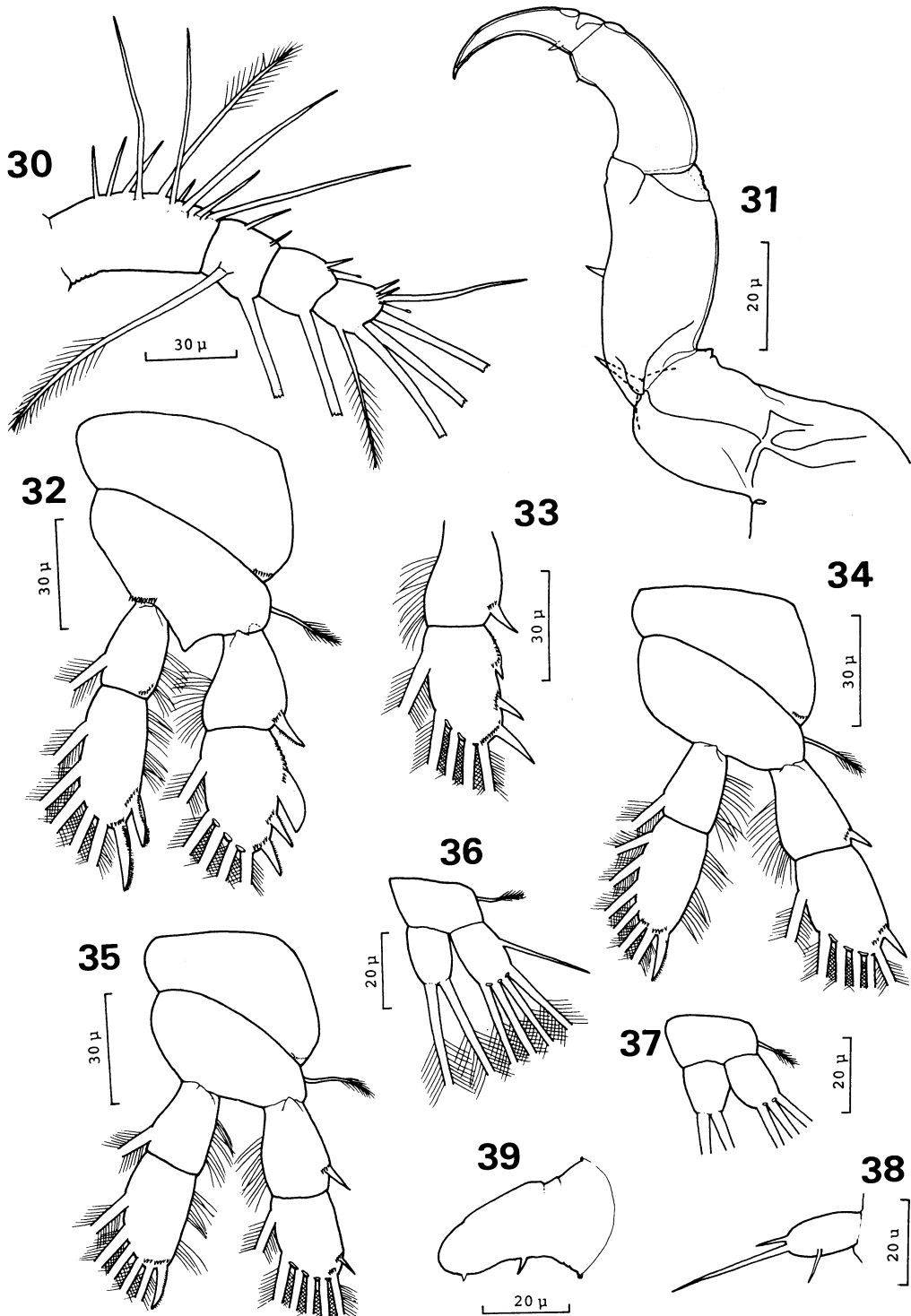


Fig. 30-39. Copepodid stage IV of *Neoergasilus japonicus*.

30. Female, First antenna, ventral; 31. Same, Second antenna; 32. Same, First swimming leg; 33. Male, Exopod of First leg; 34. Female, Second leg; 35. Same, Third leg; 36. Same, Fourth leg; 37. Male, Fourth leg; 38. Female, Fifth leg; 39. Male, Maxilliped.

First antenna (Fig. 42) divided into 5 segments, but border between basal and second segment indistinct. Setal formulae as follows: segment 1—1P, 1S, 7s; 2—4S, 2s; 3—2P, 2s; 4—1P, 1s, 1e; 5—3P, 1p, 1S, 2s, 1e.

Second antenna (Fig. 43) similar to that of the previous stage, but slightly increased in size.

Swimming legs resembling those of the previous stage, but with some differences in armature as shown in Table 1. Enlarged spine on second segment of exopod of first leg (Fig. 44) more developed up to about 1/2 of apical segment.

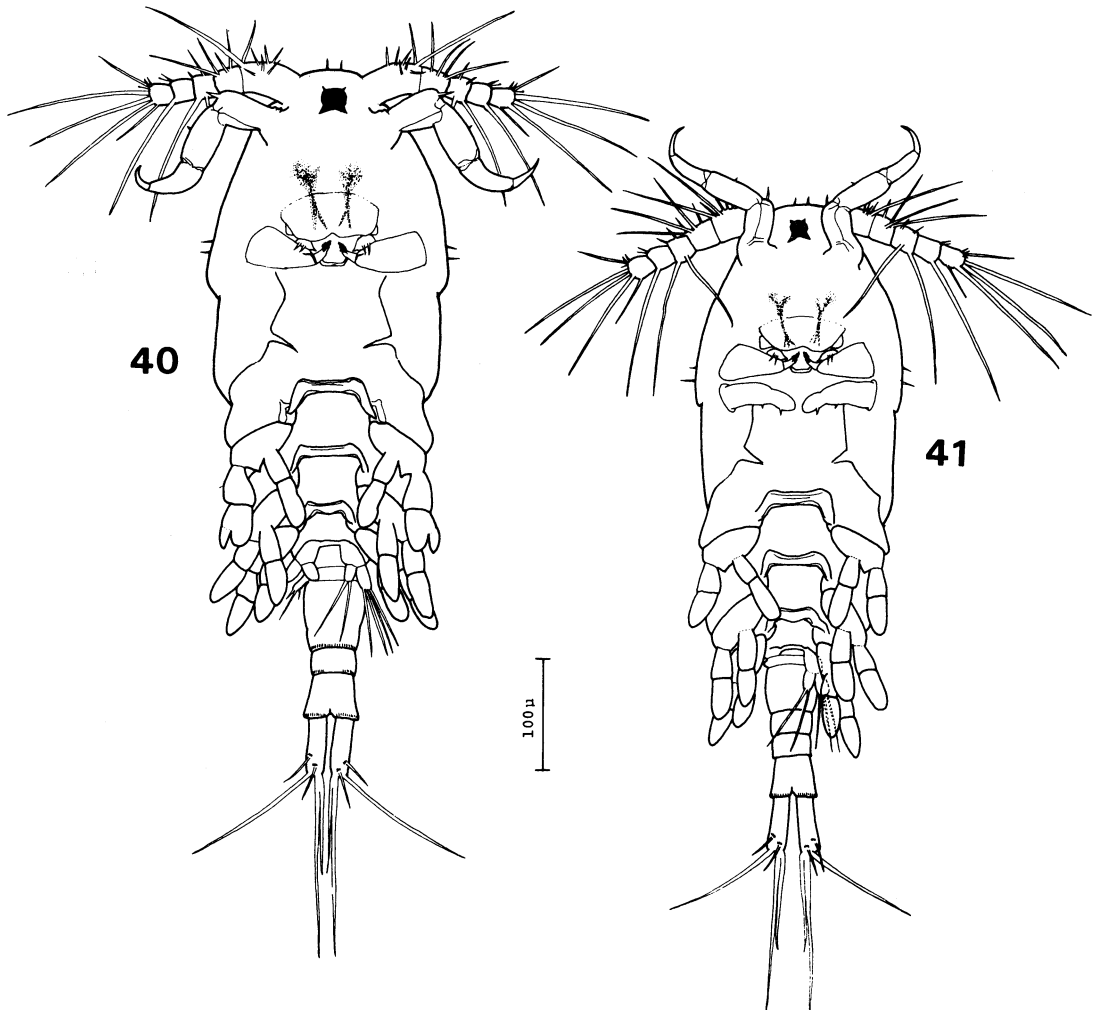


Fig. 40, 41. Copepodid stage V of *Neoergasilus japonicus*, ventral.
40. Female; 41. Male.

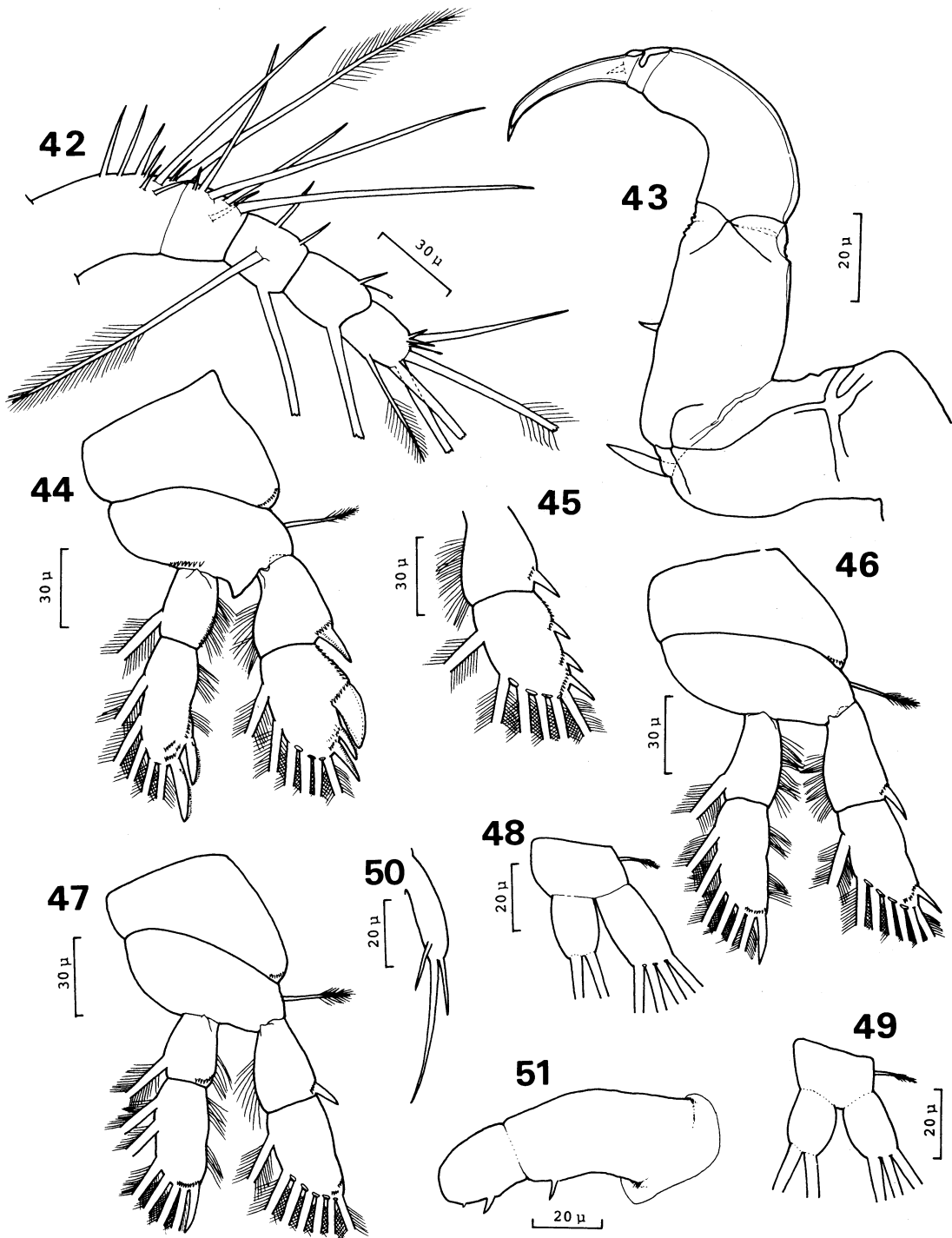


Fig. 42–51. Copepodid stage V of *Neoergasilus japonicus*.

42. Female, First antenna, ventral; 43. Same, Second antenna; 44. Same, First swimming leg; 45. Male, Exopod of First leg; 46. Female, Second leg; 47. Same, Third leg; 48. Same, Fourth leg; 49. Male, Fourth leg; 50. Female, Fifth leg; 51. Male, Maxilliped.

Copepodid stage V Male

General appearance (Fig. 41) similar to that of female, but abdomen divided into 3 segments.

First antenna resembling that of female, but second segment not armed with short seta near its base.

Maxilliped (Fig. 51) increased in length, indistinctly divided into 2 segments. Basal segment long, cylindrical and with spiniform seta on posterior margin. Apical one thumb-like, with spine and minute process.

Spine on second segment of first exopod (Fig. 45) not enlarged. Fourth (Fig. 49) and fifth leg as in the previous stage.

Other appendages similar to those of female.

Copepodid stage VI (Adult) Female

Body (Fig. 52) composed of head, thorax of 5 segments, genital segment, abdomen of 3 segments, and 1 pair of caudal rami. Genital segment barrel-shaped, with 1 pair of genital pores on dorsal surface. Caudal ramus similar to that of the previous stage, but inner plumose seta not bifurcated.

First antenna (Fig. 54) 6-segmented, setal formulae as follows: segment 1-2s; 2-1P, 1S, 5s; 3-3S, 3s; 4-2P, 2s; 5-1P, 1s, 1e; 6-3P, 1p, 1S, 2s, 1e.

Second antenna (Fig. 55) 4-segmented like previous stage, but remarkably enlarged, its apical claw nearly twice as long as that of the previous stage.

Mouth parts (Fig. 57) similar to those of the previous stage. Maxilliped absent.

Basipod of first leg (Fig. 59) protruded backward into triangular tooth between both rami. Both rami of first leg 3-segmented, second segment of exopod with thumb-like plate on its posterolateral corner. Second (Fig. 61) and third leg (Fig. 62) provided with 3-segmented rami, but in fourth leg (Fig. 63) both rami 1-segmented. Fifth leg (Fig. 65) uniramous, with 1 lateral and 2 apical setae.

Twenty adult females were captured by net tows, of which 17 individuals (85%) had spermatozoa in their seminal receptacles, and 4 individuals (20%) had incipient developing uterine processes. However, no specimens carried an egg sac.

Copepodid stage VI (Adult) Male

General appearance (Fig. 53) differed from that of female in genital segment and abdomen. Genital segment wedge-shaped, posterolateral margins slightly projected backward. Abdomen divided into 4 segments.

First antenna similar to that of female, but in third segment short seta near its base not present.

Second antenna (Fig. 56) much weaker than in female, and lateral surface of third segment covered with rows of spinules.

Mouth parts in same position similar to those of female. Maxilliped (Fig. 58) 4-segmented. Basal segment short, second one cylindrical, third one short with spine on

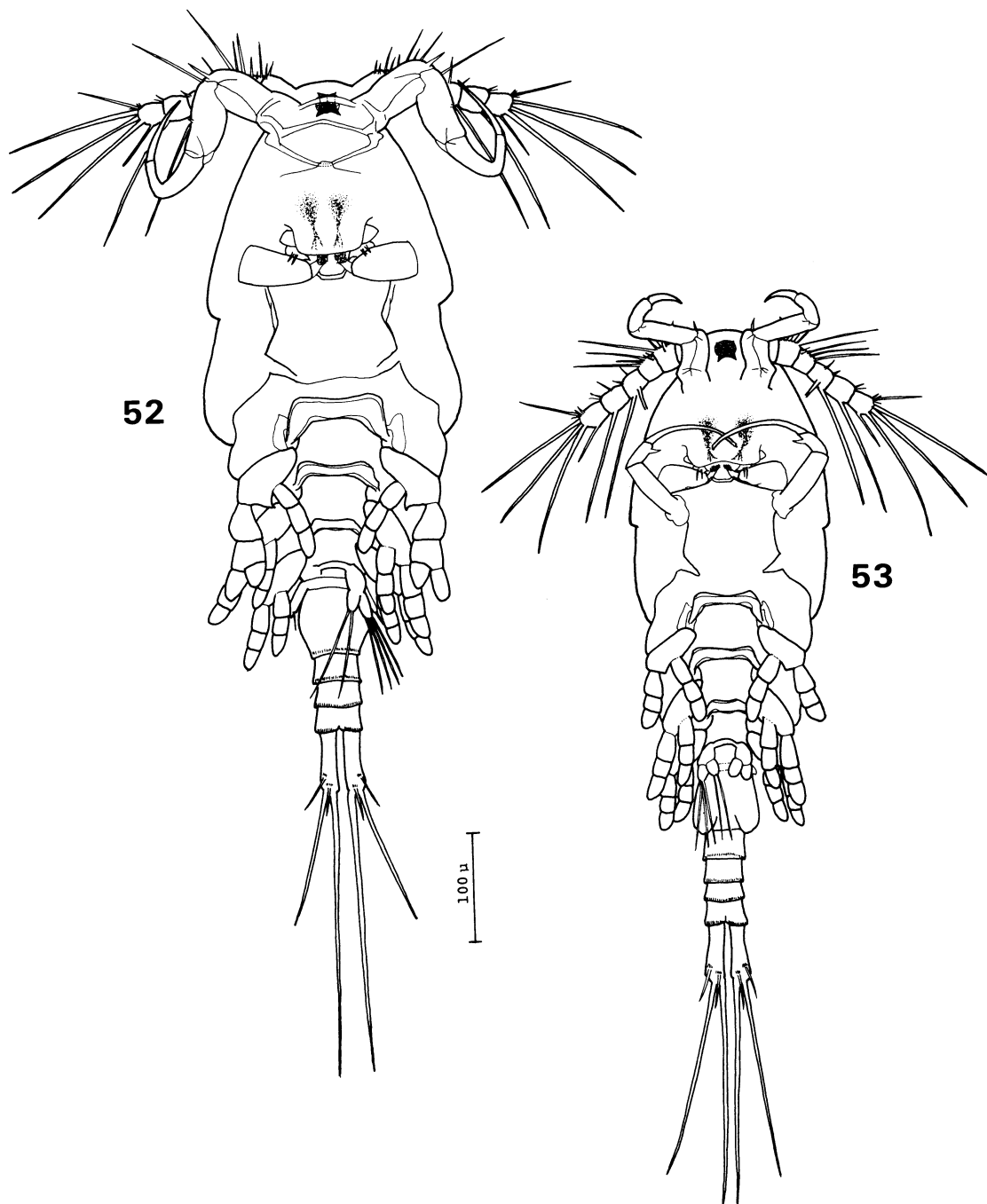


Fig. 52, 53. Copepodid stage VI (Adult) of *Neoergasilus japonicus*, ventral.
52. Female; 53. Male.

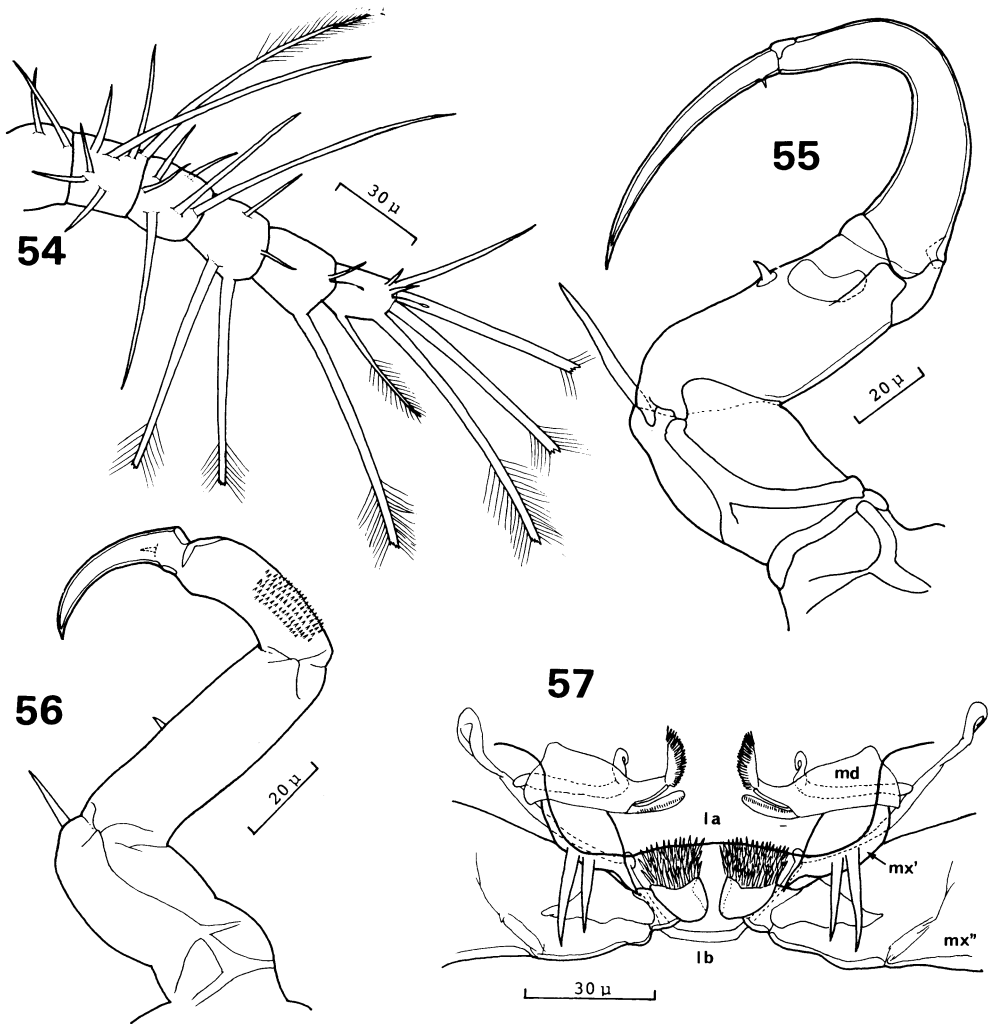


Fig. 54–57. Copepodid stage VI (Adult) of *Neoergasilus japonicus*.

54. Female, First antenna, ventral; 55. Same, Second antenna; 56. Male, Second antenna; 57. Female, Mouth parts (la, labrum; lb, labium; md, mandible; mx', first maxilla; mx'', second maxilla).

medial margin, and apical one long, slender and slightly curved inward.

Swimming legs like those of female, except for some differences in first, fourth and fifth legs. Second segment of first exopod (Fig. 60) provided with small spine corresponding to thumb-like plate in female. Exopod of fourth leg (Fig. 64) armed with 3 plumose setae. HARADA²⁾ mentioned that fifth leg is similar to that of female, and YIN³⁾ mentioned that sixth leg is represented by a small seta in posterior corner of genital segment. In the present specimens, however, fifth leg is rudimental and provided with no armature, and there is no sixth leg (Fig. 64).

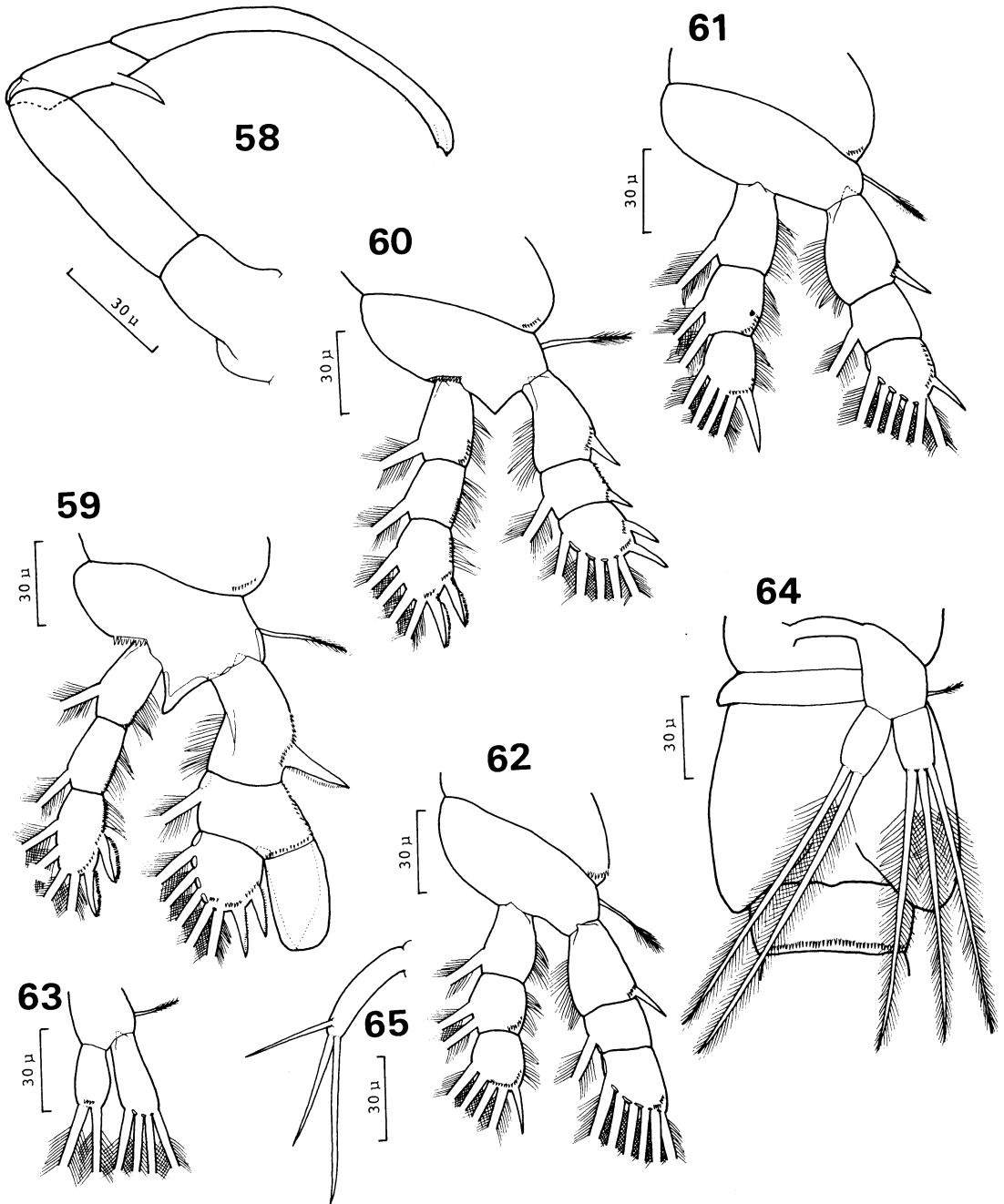


Fig. 58–65. Copepodid stage VI (Adult) of *Neergasilus japonicus*.

58. Male, Maxilliped; 59. Female, First swimming leg; 60. Male, First leg; 61. Female, Second leg; 62. Same, Third leg; 63. Same, Fourth leg; 64. Male, Fourth leg, fifth thoracic segment and genital segment; 65. Female, Fifth leg.

The growth in body length of each stage is shown in Fig. 66. It is clear from the figure that the body length has increased linearly, and that females are slightly larger than males.

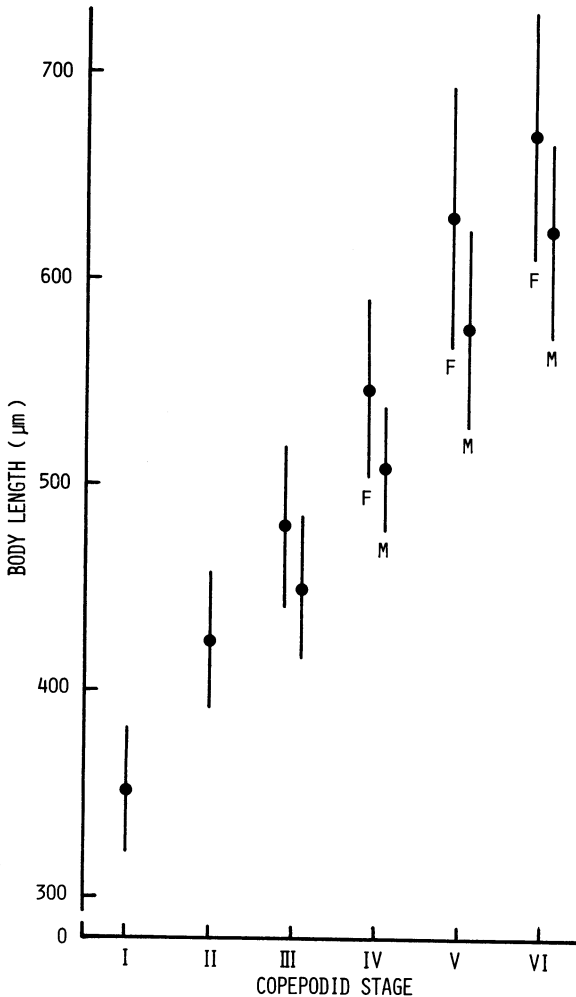


Fig. 66. Relationship between body length and copepodid stage of *Neoergasilus japonicus*. Closed circle indicates mean, and vertical bar indicates standard deviation. Number of samples is shown in Table 1. F, Female; M, Male.

DISCUSSION

Among the members of Ergasilidae, only five species have been studied on their developments in the copepodid stage.

HALISCH⁴⁾ studied the life cycle of *Ergasilus briani* (*E. minor*) and described four copepodid stages including the adult one. Meanwhile, it was reported that there were six copepodid stages in *Thersitina gasterostei* by GURNEY⁵⁾, *Sinergasilus major* by YIN³⁾, and *S. lienii* by MIRZOEVA⁶⁾. In addition, ZMERZLAYA⁷⁾ has successfully observed the life cycle of *E. sieboldi* by a rearing experiment with cages immersed in a lake, and confirmed that the species has also six copepodid stages.

As described in the result, it has been made evident that *N. japonicus* has six copepodid stages like the other four species referred above. So, only *E. briani* differs from the other five species in the number of copepodid stages, however we suspect that some copepodid stages of that species had been overlooked by HALISCH.

On careful comparison of the five species except for *E. briani*, it has become evident that the basic structure in each copepodid stage is quite similar one another, except for some points. For example, in copepodid stage III, the male of *N. japonicus* is provided with the rudimental maxilliped which makes it possible to be distinguished from the female. As for *T. gasterostei*, *S. lienii* and *E. Sieboldi*, however, maxilliped appears in copepodid stage IV.

In *N. japonicus*, the protrusion of the first basipod between both rami, which is one of the important features of the genus *Neoergasilus*, can be recognized in copepodid stage I. In addition, another characteristic feature of the genus, the elongation of the spine on the second segment of the first exopod of the female has been recognized in copepodid stage III.

Though the copulation has not been observed, it can be assumed that it occurs in copepodid stage VI (adult). Because only adult females have been occupied with spermatophores in their seminal receptacles, and male's maxilliped has never been completed before copepodid stage VI.

Among ergasilids, one species, *E. chautauquaensis*, is known to spend its full life in free-living mode⁸⁾⁻¹⁰⁾. In the case of *N. japonicus*, some free-living adult females were observed and they were provided with developed uterine processes, but none carried an egg sac. Therefore, it can be considered that *N. japonicus* can not complete its life cycle without parasitism.

Summarized these observations, the development of *N. japonicus* can be explained as follows. The hatched larvae pass through 6 nauplius and 5 copepodid stages and are transformed into the last stage, i.e. adult stage. The sexual maturation of the copepod occurs in the last stage. After the copulation, females enter into parasitic life by finding hosts. Mature males are still free-living or die.

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淡水魚に寄生する橈脚類 *Neoergasilus japonicus*

(Ergasilidae) に関する研究 — II

コペポディッド期の発育

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前報に引き続き、寄生性橈脚類 *Neoergasilus japonicus* のコペポディッド期の発育形態を記載した。ノープリウスの場合は飼育実験により得られた各期の虫体に基づいて記載したが、飼育実験ではコペポディッドⅡ期までの虫体しか得られなかったため、本報では主として福山市の千塚池におけるネット採集により得られたコペポディッド期標本に基づき記載を行なった。

その結果、本種のコペポディッド期は6期から成ることが明らかとなった。*Neoergasilus* 属の特徴である第1遊泳脚底節における歯状突起の発達には既にコペポディッドⅠ期に認められ、同じく第1遊泳脚外肢第2節の発達した棘(遊泳板)は第Ⅲ期に認められるようになる。

雌雄の分化は第Ⅲ期に始まり、この期より雄は顎脚を備えるが、雌では終始顎脚は認められない。そして第Ⅵ期に至り、共に成熟し、交尾をする。その後雌は宿主をみつけて寄生し、産卵するが、雄は自由生活に終わるものと思われる。