

Redescription of the freshwater calanoid copepod *Neurodiaptomus formosus* with key to females of diaptomid species in Japan

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Received 8 March 2020; Accepted 26 March 2020 Responsible Editor: Shinji Shimode

doi: 10.3800/pbr.15.178

Abstract: *Neurodiaptomus formosus* (Kikuchi, 1928) is a calanoid copepod found in freshwater ponds in Japan. In the original description of this species, the illustration of the female habitus and the diagnostic description of the genital double somite were based on the copepodid V stage. Since there have been no studies describing the adult female of *N. formosus*, we redescribed fully both sexes of adults. The female genital double somite has a prominent projection at the right posterior corner, which is unique in this genus. Some differences between *N. formosus* and a previous description of the congener *N. tumidus* were found in the segmentation and setation of the cephalothoracic appendages. A key to females of 11 diaptomid species occurring in Japanese freshwaters is provided, because the previous keys so far were based mainly on male characters.

Key words: cephalothoracic appendage, freshwater copepod, *Neurodiaptomus formosus*, redescription

Introduction

Neurodiaptomus formosus (Kikuchi, 1928) is a freshwater calanoid copepod endemic to Japan (Ito 1965, Ito & Miura 1973, Makino et al. 2018). In the original description by Kikuchi (1928), the female habitus was illustrated based not on the adult but on the copepodid V stage, and the diagnostic description of the genital double somite was also not based on the adult. There has been no publication dealing with the genital double somite of the adult female of *N. formosus*, in spite of its importance for species identification.

Cephalothoracic appendages from the antenna to the maxilliped have been ignored in most taxonomic studies of diaptomid species. However, it may be not always true that these appendages are useless for species identification. Young (2002) studied the post-embryonic development of *Neurodiaptomus tumidus* Kiefer, 1937 from Taiwan, and

described all appendages of the adult in detail. We found some differences in the cephalothoracic appendages between our specimens of *N. formosus* and his description of *N. tumidus*; these differences are presented below. Makino et al. (2018) suggested the existence of cryptic species of *Acanthodiaptomus pacificus* (Burckhardt, 1913), which are different genetically but not distinguishable morphologically based on traditional diagnoses of the species. This implies that it is necessary for elucidation of the taxonomy of diaptomid species to examine not only the traditional diagnostic characters, such as body shape, antennule, and leg 5, but also other characters, including all the cephalothoracic appendages.

Eight genera and 11 valid species of the family Diaptomidae are known from freshwaters in Japan (Mizuno 2000). Although Mizuno (2000) presented a key to these species, his key cannot be used for the female species because the morphologies used are mostly of the males. There is no other identification key for female diaptomid species in Japanese waters at present, in spite of the common domi-

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nance of diaptomid copepods in freshwater zooplankton.

This paper fully describes *Neurodiaptomus formosus* from Japan, explains the differences in the cephalothoracic appendages between our specimens of *N. formosus* and the description of *N. tumidus* by Young et al. (2014), and provides an identification key to females of the diaptomid species in Japan. For non-taxonomists to use, morphologies used in this key are limited to those observable without dissection.

Materials and Methods

Specimens of *Neurodiaptomus formosus* were collected from Hiike (34.264°N, 132.684°E), a small pond at an altitude of about 800 m on Mt. Noro, Hiroshima Prefecture, western Japan, on 16 December 1996 and 8 March 2019. Sampling was done from a shore using a small conical plankton net with 0.1 mm mesh size. The samples were fixed and preserved in 1–3% buffered formalin in 1996 or 70% ethanol in 2019. Examination of specimens was done in 70% lactic acid or 50% glycerol under a microscope (Nikon E600) equipped with a drawing tube (Nikon Y-IDT) and a digital photo-microscopic camera system (Nikon DS-5M). Illustrations were prepared with the computer software package Adobe Illustrator® by tracing drawings and/or microscope photographs. Body length was measured with an ocular micrometer. Morphological terminology follows Boxshall & Halsey (2004), except for the following abbreviations: exp-1–exp-3 and enp-1–enp-3 for the first–third exopodal segments and the first–third endopodal segments, respectively.

Systematic Accounts

Family Diaptomidae Baird, 1850

Genus *Neurodiaptomus* Kiefer, 1937

Neurodiaptomus formosus (Kikuchi, 1928)

(Figs 1–3)

Synonym

Diaptomus formosus Kikuchi, 1928: p. 72, figs 33, 35–38.

Eudiaptomus formosus: Mizuno (1964), p. 94, pl. 33, fig. 2.

Neurodiaptomus formosus: Ito (1965), p. 471, fig. 479.1–3; Ito & Miura (1973), p. 437, fig. 20–50.7a–c; Mizuno (1984), p. 491, fig. 265.1–8; Mizuno (2000), p. 10, fig. 13 in p. 12.

Materials examined

Three adult females and 2 adult males in 1996; 2 adult females and 3 adult males in 2019.

Description

Female —**Body** (Fig. 1A, B): length 1.88, 1.92 and 1.99 mm in 1996 (n=3), and 1.92 mm in 2019 (n=1). Fourth and fifth pedigers incompletely fused. Posterior corners of prosome (Fig. 1A–D) greatly asymmetrical, with 2 pointed processes on each side (Fig. 1D, indicated by arrows); left

corner expanding dorsalward (Fig. 1C) with minute lateral process at tip without spinule; right corner producing lateralward with lateral process at tip without spinule, and posterior margin between 2 processes conspicuously concaved in dorsal view (Fig. 1D). **Genital double somite** (Fig. 1D, E, F): length 1.7–2.0 times width, longer than next 2 abdominal somites combined, without prominent lateral swelling at anterior part, with small pointed process at mid length of somite on each side; right process located more anteriorly than left one; left posterior corner with small projection; right posterior corner with prominent projection as in Fig. 1B, D, E; genital field as in Fig. 1F. **Caudal ramus** (Fig. 1A): length twice width; left ramus slightly longer than right one.

Antennule (Fig. 1G): 25-segmented, extending beyond caudal rami by 4–5 segments; setal formula, (1) I=2, (2) II–IV=4, (3) V=1, (4) VI=1, (5) VII=1, (6) VIII=1, (7) IX=1, (8) X=1+spine, (9) XI=2, (10) XII=1, (11) XIII=1, (12) XIV=1, (13) XV=1, (14) XVI=1, (15) XVII=1, (16) XVIII=1, (17) XIX=1, (18) XX=1, (19) XXI=1, (20) XXII=1, (21) XXIII=1, (22) XXIV=2, (23) XXV=2, (24) XXVI=2, (25) XXVII–XXVIII=4; aesthetasc absent. **Antenna** (Fig. 1H): coxa with 1 seta; basis with 2 setae; exopod 8-segmented with 2, 2, 1, 1, 1, 1, 1, 3 setae; endopod 2-segmented, with spinules on distolateral margin of each segment; enp-1 with 2 setae; enp-2 with 15 setae, 2 of them on medial margin, 6 on subdistal margin, and 6 long and 1 short setae on distal margin. **Mandible** (Fig. 1I): gnathobase with 8 teeth and short seta; basis with 4 setae; exopod 4-segmented with 1, 1, 1, 3 setae; endopod 2-segmented; enp-1 with 4 setae; enp-2 with 5 long and 3 short setae, and 2 groups of a few setules on lateral margin. **Maxillule** (Fig. 1J): praecoxal arthrite with 16 setae, 11 of them spiniform; coxa with 9 setae on epipodite and 4 setae on endite; basis with short seta on exite, 5 setae on proximal endite, and 4 setae on distal endite; exopod with 6 setae; endopod 2-segmented, with 4, 5 setae fused to segment. **Maxilla** (Fig. 2A): 2 praecoxal, 2 coxal, and 1 basal endites each with 2 long and 1 short setae; endopod 2-segmented; enp-1 with 2 setae; enp-2 with 3 long and 1 short setae. **Maxilliped** (Fig. 2B): syncoxa with 4 endites, first with 1 seta, second with row of fine setules proximally and 2 setae, third with 3 setae, fourth with 4 setae and row of fine setules distally; basis with row of fine setules on about half of medial margin and 3 setae distally; endopod 6-segmented, with 2, 3, 2, 2, 2, 4 setae.

Legs 1–4 (Fig. 2C–F): leg 1 with 2-segmented endopod; other legs with 3-segmented rami; setal formula as follows:

	coxa basis		exopod segment			endopod segment		
	1	2	1	2	3	1	2	3
leg 1	0-1	0-0	I-1;	0-1;	I,1,4	0-1;	1,2,3	
leg 2	0-1	0-0	I-1;	I-1;	I,1,5	0-1;	0-2;	2,2,3
leg 3	0-1	0-0	I-1;	I-1;	I,1,5	0-1;	0-2;	2,2,3
leg 4	0-1	0-0	I-1;	I-1;	I,1,5	0-1;	0-2;	2,2,3

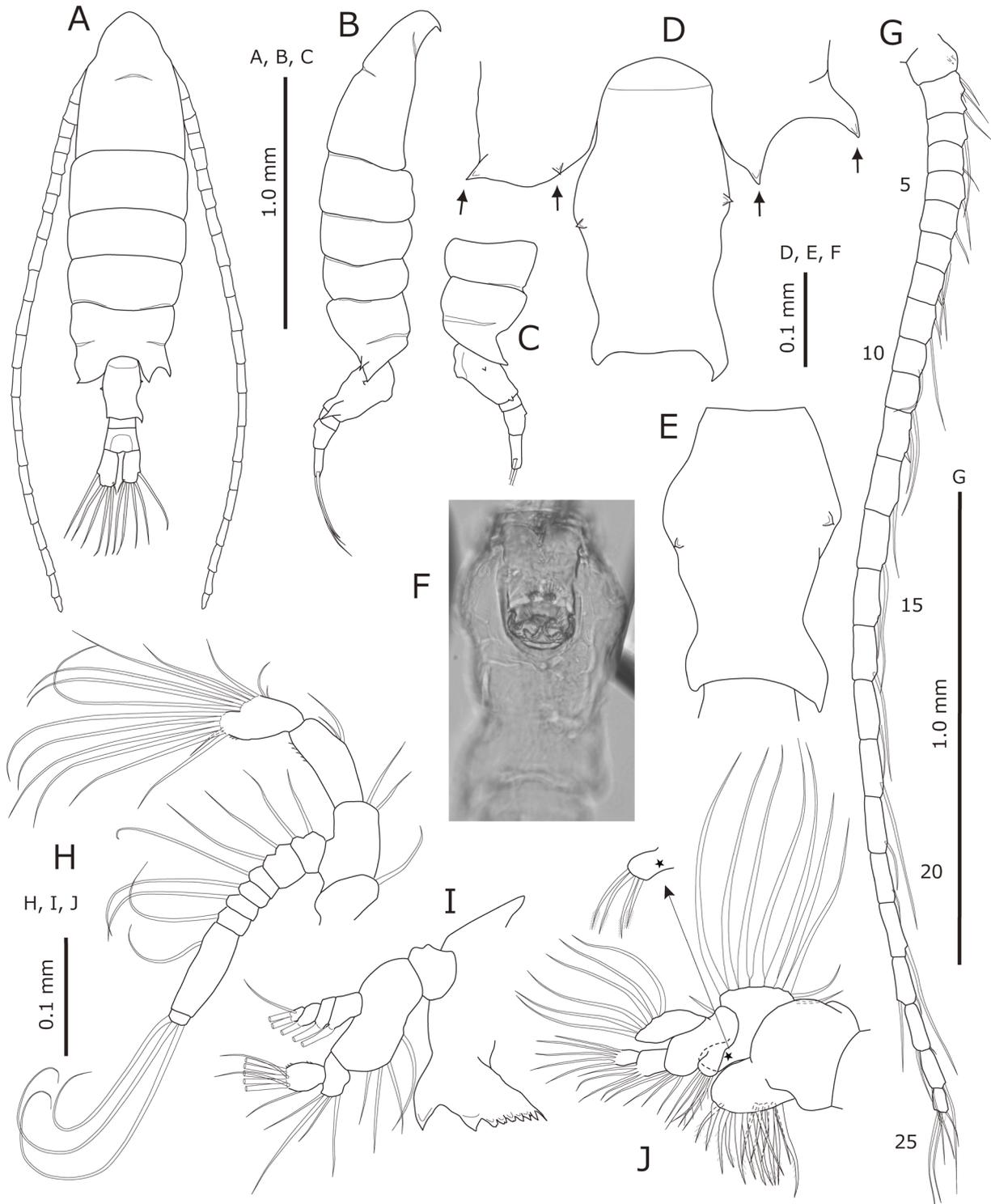


Fig. 1. *Neurodiaptomus formosus*, female (A–C, F, same specimen in 1996; D, G–J, same specimen in 2019; E, another specimen in 2019). A, habitus, dorsal; B, habitus, right lateral; C, posterior half of habitus, left lateral; D, posterior corners of prosome and genital double somite, dorsal; E, genital double somite, dorsal; F, photograph of genital field, ventral; G, antennule with segment numbers; H, antenna; I, mandible; J, maxillule (coxal endite is separately illustrated).

Leg 5 (Fig. 2G, H): coxa with acute attenuation at disto-lateral corner on posterior surface; basis with seta at disto-lateral corner. Exopod 3-segmented; exp-1 length 4 times width, with 2 fine setules on lateral margin; exp-2 slightly

shorter than exp-1, curved at distal end, with short lateral spine proximal to exp-3 and teeth along distal half of medial margin; exp-3 small, with 1 long and 1 short spines. Endopod 1-segmented, slightly longer than half of exp-1,

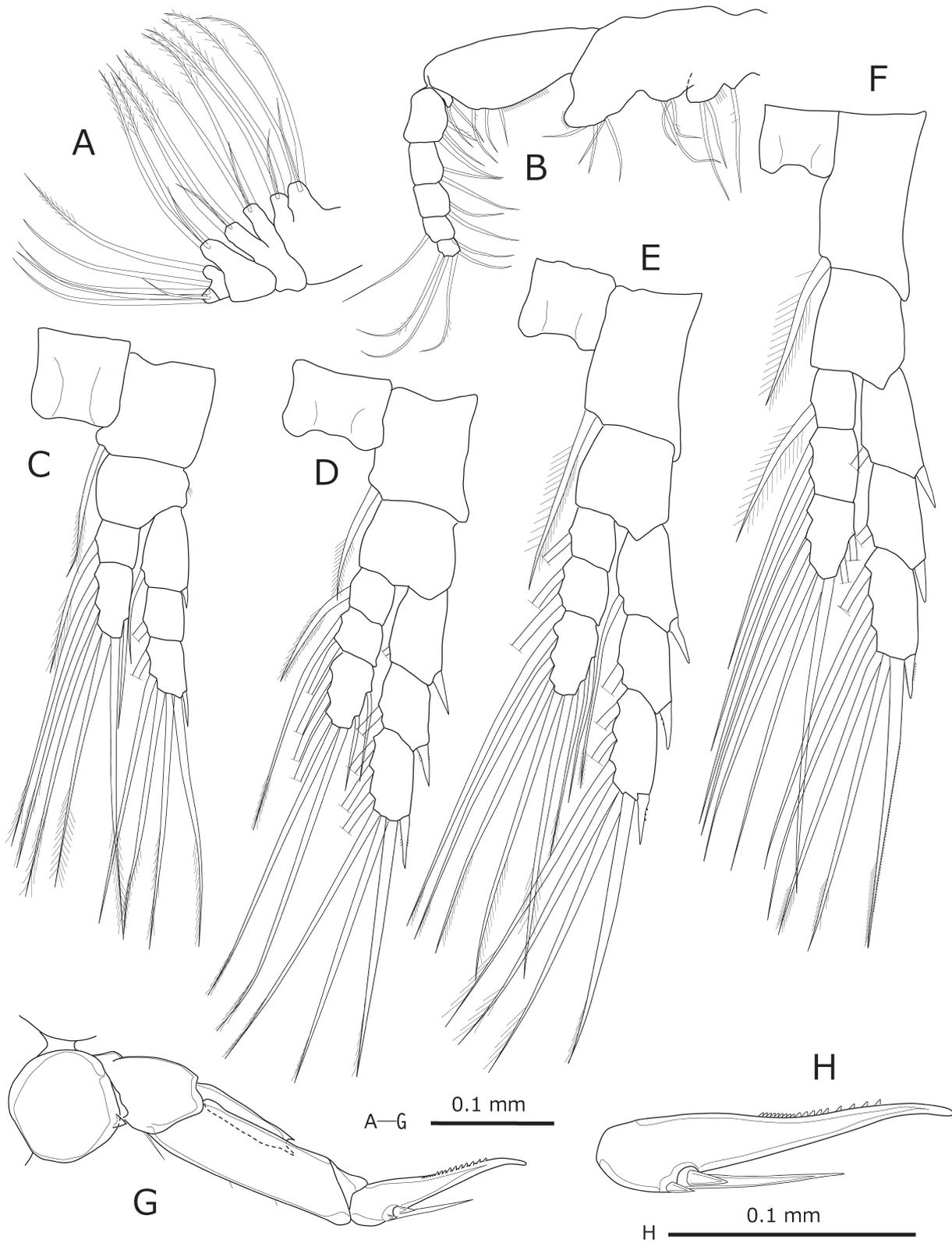


Fig. 2. *Neurodiaptomus formosus*, female (A, B, H, same specimen as Fig. 1D; C–G, same specimen as Fig. 1A). A, maxilla; B, maxilliped; C, leg 1, posterior; D, leg 2, posterior; E, leg 3, posterior; F, leg 4, posterior; G, leg 5, posterior; H, distal 2 segments of leg 5 exopod, posterior.

terminally with subequal 2 acute attenuations.

Male — *Body* (Fig. 3A, B): length 1.63, 1.81 mm in 1996 (n=2) and 1.75, 1.78 and 1.82 mm in 2019 (n=3); fifth pedi-

ger (Fig. 3A, B, D) separated from fourth pediger, slightly asymmetrical, with 2 small spinules on each side (Fig. 3D, indicated by arrows). *Urosome* (Fig. 3C, D): genital somite

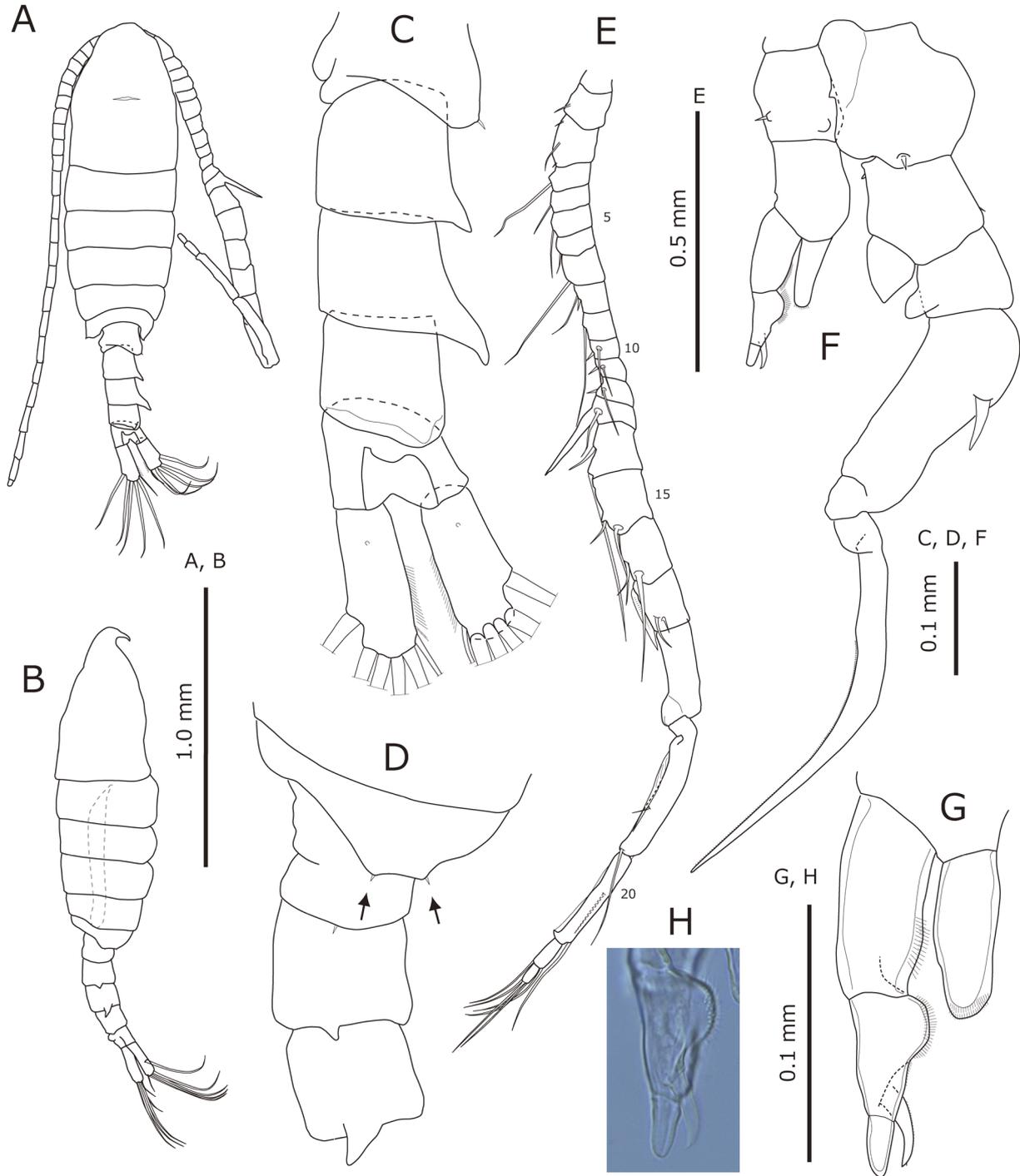


Fig. 3. *Neutrodiaptomus formosus*, male (A–E, same specimen in 1996; F–H, another specimen in 1996). A, habitus, dorsal; B, habitus, lateral; C, urosome, dorsal; D, fifth pediger and urosomites 1–3, right lateral; E, right antennule with segment numbers, ventral; F, leg 5, posterior; G, rami of left leg 5, posterior; H, photograph of distal 2 segments of left leg 5 exopod, posterior.

with spinule at right posterior margin; 1st and 2nd abdominal somites each with acute expansion at right posterior margins, expansion of second abdominal somite larger.

Left antennule (Fig. 3A): 25-segmented, reaching distal margin of caudal rami, setal formula as in female. *Right antennule* (Fig. 3E): 22-segmented; geniculation between segments 18 and 19; setal formula, (1) I=1, (2)

II–IV=3, (3) V=1, (4) VI=1, (5) VII=1, (6) VIII=1, (7) IX=1, (8) X=1+spine, (9) XI=1, (10) XII=1+spine, (11) XIII=1+spine, (12) XIV=1+spine, (13) XV=1, (14) XVI=2, (15) XVII=2, (16) XVIII=2, (17) XIX=2+large spine, (18) XX=0, (19) XXI–XXIII=1+large spine, (20) XXIV–XXV=2, (21) XXVI=1+1, (22) XXVII–XXVIII=4; segment 13 with long spiniform process; seg-

ment 20 with small hook at distal end and smooth hyaline membrane. *Left leg 5* (Fig. 3F–H): coxa posteriorly with small spine near lateral margin and small round process near distomedial corner; basis laterally with notch near distal corner; exopod 3-segmented; exp-1 length 2.5 times width, with dense fine setules on distomedial surface; exp-2 with dense fine setules on round proximomedial surface, curved thick spine on distal part of anterior surface; exp-3 finger-shaped, half length of exp-2; endopod as long as exp-1, with dense fine setules on round distal margin. *Right leg 5* (Fig. 3F): coxa with small spine near posterodistal margin; basis laterally with small seta near distal corner and medially with small projection near base. Exopod 3-segmented; exp-1 with hyaline expansion medially; exp-2 longer than basis and exp-1 combined, with maximum width at near base; lateral spine of exp-2 about one-fifth segment length, inserted at about proximal one-third of segment; exp-3 (=terminal claw) as long as preceding 3 segments combined, curved at midlength, bearing minute teeth along distal four-fifths of medial margin. Endopod 1-segmented, triangle-shaped with broad base, naked.

Discussion

According to Walter & Boxshall (2018), the genus *Neurodiaptomus* consists of 16 species, including *N. okadai* (Horasawa, 1934), which has been synonymized with *N. pachypoditus* (Rylov, 1925) (Kikuchi 1940, Ito 1965, Mizuno 2000). Currently the genus consists of 15 valid species. In these 15 species, the female genital double somite of *N. formosus* is unique in having a prominent acute projection at the right posterior corner. Although the corresponding expansion was illustrated for *N. amurensis* (Rylov, 1918) by Rylov (1922), *N. lianshanensis* Sung et al., 1975 by Shen & Song (1979), *N. liaochengensis* Chen et al., 1991 by Chen & Guan (1991), and *N. sklyarovae* Markevich, 1985 by Markevich (1985), these are not acute and

are much weaker than in *N. formosus*. The anterior part of the genital double somite of *N. formosus* is not remarkably swollen. This is also a characteristic of this species, because the anterior asymmetrical swelling of the genital double somite is a diagnostic feature of the genus (Dussart & Defaye 2001). Most congeners have such swellings on the right or both sides of the genital double somite. Only *N. lianshanensis* has the similarly less-swollen genital double somite, but the somite is much shorter (as long as the next two somites combined, in the figure by Shen & Song 1979) than that of *N. formosus*, and it does not have the prominent projection at the right posterior corner.

In the male, the broad-based triangular endopod of right leg 5 of *Neurodiaptomus formosus* is unique in the genus. This endopod in the other species is mostly rod-shaped with a pointed or spiniform end. A triangular endopod is seen only in *N. ostroumovi* (Stepanova, 1981) (Stepanova 1981), but its base is much narrower (a half length of the height) than that of *N. formosus*.

Differences in the segmentation and setation of the cephalothoracic appendages between *Neurodiaptomus formosus* in the present study and the congener *N. tumidus* described by Young (2002) are as follows (corresponding characters of *N. tumidus* in brackets): (1) endopod of the antenna 2-segmented, with 17 setae [4-segmented, with 18 setae]; (2) enp-2 of the mandible with 12 setae [10 setae]; (3) coxal endite of the maxillule with 4 setae [5 setae]; (4) 5 endites of the maxilla each with 3 setae [4, 3, 4, 3, 3 setae, respectively]; (6) the first syncoxal endite, enp-2 and enp-3 of the maxilliped with 1, 3, and 2 setae, respectively [2, 4, and 1 setae]; (7) the male right antennule 23-segmented and the segments 10–12 each with a spine [22-segmented and the segments 10, 11, 16 each with a spine]. Although some of these differences may be due to overlooking or missing of setae or spines, further studies are necessary to examine if these are interspecific variations within the genus.

Key to females of 11 diaptomid species occurring in Japan

1. Last prosomal somite with horn-like dorsal attenuation; right posterior corner of prosome producing lateralward with pointed tip 2
- Last prosomal somite without pointed dorsal attenuation; right posterior corner of prosome not producing lateralward 3
2. Antennule extending beyond caudal rami by 2 segments; process on distolateral corner of leg 5 coxa extending to exopod *Sinodiaptomus sarsi* (Rylov, 1923)
- Antennule extending beyond caudal rami by 6 segments; process on distolateral corner of leg 5 coxa not reaching endopod *Sinodiaptomus valkanovi* Kiefer, 1938
3. Genital double somite with prominent pointed projection at right posterolateral corner *Neurodiaptomus formosus*
- Genital double somite without prominent pointed projection at right posterolateral corner 4
4. Urosome comprised of 2 somites (genital double somite and anal somite) *Tropodiaptomus oryzanus* Kiefer, 1937
- Urosome comprised of 3 somites 5
5. Genital double somite nearly symmetrical without prominent lateral swelling 6
- Genital double somite apparently asymmetrical or with prominent lateral swelling on anterior part 8
6. Last prosomal somite with round posterior corners; caudal rami length 2 times width 6
- *Heliodiaptomus nipponicus* (Kokubo, 1912)
- Last prosomal somite with acute posterior corners; caudal rami length 1.5 times or less width 7

7. Prosome length 3 times or more width; anterior part of genital double somite wider than posterior part *Eodiaptomus japonicus* (Burckhardt, 1913)
- Prosome length 2.5 times or less width; anterior part of genital double somite as wide as posterior part *Acanthodiaptomus pacificus* (Burckhardt, 1913)
8. Right posterior corner of prosome without pointed projection; genital double somite without prominent swelling on anterior part *Heliodiaptomus kikuchii* Kiefer, 1932
- Right posterior corner of prosome with pointed projection or genital double somite with prominent lateral swelling on anterior part 9
9. Genital double somite length 1.5 times maximum width; 2 spines on leg 5 enp-3 short and equal in length *Nordodiaptomus alaskaensis* Wilson M.S., 1951
- Genital double somite as long as maximum width; one spine on leg 5 enp-3 much longer than the other spine 10
10. Tip of right expansion of genital double somite directed toward posterior; leg 5 exp-1 length 3 times width *Neurodiaptomus pachypoditus* (Rylov, 1925)
- Tip of right expansion of genital double somite directed laterally; leg 5 exp-1 length 1.5 times width *Neodiaptomus schmackeri* (Pope & Richard, 1892)

Acknowledgements

We are grateful to Dr. Chad Walter of Smithsonian Institution and Dr. Wataru Makino of Tohoku University for kindly providing us the references necessary for this study. This study was partially supported by a grant-in-aid from the Japanese Society of the Promotion of Science (KAKEN No. 19H03032, awarded to SO).

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