Plankton Benthos Res 17(4): 358–368, 2022

Plankton & Benthos Research

A new species of genus *Sapphirina* (Copepoda, Cyclopoida) from the Kuroshio Extension region in the western North Pacific Ocean

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Received 30 May 2021; Accepted 25 August 2022 Responsible Editor: Yuichiro Nishibe

doi: 10.3800/pbr.17.358

Abstract: A new species of sapphirinid copepod, *Sapphirina doliolettae* n. sp. was described from the Kuroshio Extension region in the western North Pacific Ocean. The new species is similar to *S. nigromaculata* and *S. scarlata* with respect to the following characteristics: 5-segmented antennule, one inner marginal process on caudal rami, and slender endopods with two apical spines on leg 4. However, it can be distinguished from these congeners by a combination of the following characteristics: the relative length of each segment of the antennule and antenna in both sexes, the width of the fourth pedigerous somite in females, the shape of the anterolateral corner of the genital somite in males and terminal process length of 3rd endopodal segment of leg 2 in males. The mitochondrial cytochrome oxidase subunit I sequences of *S. doliolettae* showed high interspecific variabilities from other *Sapphirina* species, including *S. nigromaculata* (20.2%) and *S. scarlata* (21.6–21.8%).

Key words: *Dolioletta*, predator, *Sapphirina doliolettae* n. sp., *Sapphirina nigromaculata*, surface layer, transition zone

Introduction

Copepods belonging to the genus *Sapphirina* reflect underwater light and shine owing to their subcutaneous integral multi-layer structure (Chae & Nishida 1994, 1999). Therefore, these organisms have attracted the attention of marine biologists for a long time, and most known species were described in the 19th century. Some species of this genus have been known to often associate with thaliaceans as parasitoids (Furuhashi 1966, Heron 1973, Harbison 1998, Takahashi et al. 2013, Gasca et al. 2015).

One of the authors (KT) observed the predatory behavior of a sapphirinid species on a *Dolioletta gegenbauri* (Uljanin, 1884) bloom that appeared in the Kuroshio Extension region and evaluated the effect of predation by this species on the growth of the *Dolioletta* population, as well as its contribution to the downward flux of carcasses of doliolids predated upon by the copepod species (Takahashi et al. 2013). This sapphirinid species was identified as *S. nigromaculata* Claus, 1863 on the basis of previous identification keys (eg Lehnhofer 1929, Mori 1937, Itoh 1997, Boxshall & Halsey 2004). However, our subsequent observations revealed that morphological characteristics of the species had remarkable differences from those reported in the descriptions of *S. nigromaculata* by Claus (1863)

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and Giesbrecht (1892), and these morphological differences were supported by genetic analyses. Thus, we describe this species as a new species.

Materials and Methods

Type specimens were collected on May 20, 2009 by a sediment trap, which was installed on May 19, 2009 at a depth of 50 m at a station (37.0002N, 149.0103E) in the Kuroshio Extension during the R/V "Wakataka-Maru" cruise of the Japan Fisheries Research and Education Agency and retrieved on May 20, 2009. The sediment trap was set up by filling the collection container with buffered 1% formalin/seawater solution. Fifteen adult females and 8 adult males were sorted and designated as type specimens.

Holotype, allotype, and paratype specimens were examined in a 70% glycerin/distilled water solution under a compound microscope according to the method described by Humes & Gooding (1964). Drawings were made using a camera lucida. Dissected appendages were mounted on glass slides by mount-quick aqueous solution (Daido Sangyo Co. Ltd.). Undissected specimens or dissected bodies were preserved in vials with 70% ethanol/distilled water solution or buffered 5% formalin/sea water solution. Type specimens were deposited at the National Museum of Nature and Science, Tokyo (NSMT).

Terminology for the description is according to Huys & Boxshall (1991). The anterior part of each urosomite is covered by the posterior part of the preceding urosomite, especially in the male sapphirinids; however, the anterior margin of each urosomite can be observed using transmitted light. Therefore, the length of each urosomite was measured from the anterior margin of the somite to the anterior margin of the succeeding somite in the dorsal view. The body length was measured from the tip of the prosome to the posterior end of the caudal ramus (excluding terminal seta). Prosome length was measured from the anterior margin of the cephalosome to the anterior margin of the fifth pedigerous somites and urosome length was measured from the anterior margin of the fifth pedigerous somites to the posterior end of the caudal ramus in dorsal view.

To obtain additional information on the morphological variation and differences among the related congeners and their geographical distribution, we sorted the present species and two related congeners (*Sapphirina nigromaculata* and *S. scarlata*) from plankton samples collected along the Pacific coast of Honshu, Japan, with the help of researchers at the Fisheries Research and Education Agency, Kanagawa prefectural fisheries technology center and the School of Marine Science and Technology, Tokai University. We measured body length for 31 females and 18 males of the present species (including the type specimen) and observed the discriminating characters in the antennule, antenna, 3rd and 4th pedigerous somites of females, genital somites of males and distal endopodal segments of leg 2 of males for 14 females and 12 males of the present species, 10 females and 5 males of *S. nigromaculata* and 4 females and 3 males of *S. scarlata*.

For genetic analysis of the new species, zooplankton samples were collected from the Kuroshio-Oyashio transition region, where the paratypes were collected, in May 2018 (38.0016N, 147.2514E), aboard the R/V Wakataka-Maru. Zooplankton samples were preserved in 99% ethanol, and six females and one male were extracted. Samples were also collected from the East China Sea (25.990N, 126.015E) to add the genetic data of other *Sapphirina* species, including *S. nigromaculata*. Genomic DNA was extracted from each specimen using a DNeasy Blood & Tissue Kit (QIAGEN). Both mitochondrial cytochrome c oxidase subunit I (COI) and nuclear small subunit ribosomal RNA (18S) gene sequences were obtained. Primer pairs of L1384-COI (Machida et al. 2004) and HCO-2198Sapphirina (5′-WAMACYTCWGGRTGHCCAAAA AAYCA-3′; this study) were used for COI, and 18SE and 18SL (Questel et al. 2021) for 18S. PCR amplification was performed in 15 μL reaction mixtures containing 3.5 μL of distilled water, 1.5 μL of each primer (5 μM), 7.5 μL of KOD One PCR Master Mix (TOYOBO), and 1.0 μL of template DNA. PCR cycling was conducted as follows: 1 min denaturation at 94℃, followed by 35 cycles of 10 s denaturation at 98℃, 5 s annealing at 45℃ (COI) or 50℃ (18S), 5 s extension at 68℃, and a final extension at 68℃ for 1 min. After library preparations using ExoSap-IT (Applied Biosystems) and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), sequence data were obtained using a 3130 DNA Sequencer (Applied Biosystems). In addition to the primers used for PCR, primers from Machida & Knowlton (2015) were used for sequencing 18S. The obtained sequences were edited using the software Geneious (https://www.geneious.com). Sequence data from GenBank were added for available *Sapphirina* species, and all the sequences for each marker were aligned using MUSCLE (Edgar 2004). Intraspecific and interspecific sequence variabilities were calculated in MEGA (Kumar et al. 2016). All sequence data obtained in this study were registered in the NCBI GenBank, including sampling information such as location and date. The accession numbers for the sequences generated for this study are: *S. doliolettae* n. sp. (COI: LC711088–LC711094; 18S: LC711098–LC711101), *S. nigromaculata* (COI: LC711096; 18S: LC711103), *S. ovatolanceolata* (COI: LC711097; 18S: LC711104), *S. stellata* (COI: LC711095; 18S: LC711102).

Systematics

Family Sapphirinidae Thorell, 1859

Genus *Sapphirina* Thompson J. V., 1829

***Sapphirina doliolettae* Takahashi, Itoh and Hirai, new species**

(Figs. 1–3)

Sapphirina nigromaculata Farran (1926): 298, Pl. 9, Fig. 17.

Sapphirina nigromaculata Takahashi et al. (2013): in part.

Type locality

Western North Pacific Ocean, the Kuroshio Extension region (37.0002N, 149.0103E), at a depth of 50 m.

Material examined

The following material from five sites was used for the description of the present species.

(1) Western North Pacific Ocean, the Kuroshio Extension region, 37.0002N, 149.0103E: Stn. T1; R/V "Wakataka-Maru": collected on May 20, 2009 (Surface water temperature: 18.0°C) using a sediment trap suspended at a depth of 50 m.
   (a) Holotype: Adult female, appendages dissected and mounted on glass slides, dissected body in ethanol, NSMT-Cr30893.
   (b) Allotype: Adult male, appendages dissected and mounted on glass slides, body in ethanol, NSMT-Cr30894.
   (c) Paratypes: 14 adult females and 7 adult males, whole specimens in ethanol, NSMT-Cr30895 and NSMT-Cr30896, respectively.

(2) Kuroshio-Oyashio Transition region, 38.0016N, 147.2514E: Stn. A21; R/V "Wakataka-Maru": collected May 12, 2018 (Surface water temperature: 15.8°C) with a NORPAC net; 0–150 m: 4 females and 2 males in formalin, preserved by second author (HI).

(3) Sendai Bay, 37.2143N, 141.1800E: Stn. HRN100; R/V "Wakataka-Maru": collected July 3, 2020 (Surface water temperature: 16.2°C) with a BONGO net; 0–99 m.
   (a) Paratype: Adult female, dissected antennule, antenna, and body in a vial filled with ethanol, NSMT-Cr30897.
   (b) Six females and 6 males in formalin, preserved by HI.

(4) Suruga Bay, 35.0556N, 138.6833E: Stn. SR1; T/S "Hokuto": collected June 8, 2017 (Surface water temperature: 19.7°C) with a NORPAC net; 0–150 m: 2 females in formalin, preserved by HI.

(5) Tokyo Bay, 35.0716N, 139.7641E: Stn. 29; R/V "Sagami": collected June 2, 1994 (Surface water temperature: 20.6°C) with a NORPAC net; 0–605 m: 3 females and 2 males in formalin, preserved by HI.

Description

Female (holotype)-Body (Fig. 1A) length 2.74 mm, dorsoventrally flat and shaped like a rice scoop in dorsal view. Greatest width 1.16 mm at posterior 1/10 of cephalosome. Prosome length 1.5 times width and 1.6 times length of urosome. Width of the first to fourth pedigerous somites 94, 92, 69, and 67% of greatest width of cephalosome, respectively. Posterolateral corner of fourth pedigerous somite slightly protruded diagonally backwards. Head rounded in dorsal view, with one pair of lenses at frontal end, rostrum obscure (Fig. 1B). Length ratios of first to fifth urosomites and caudal ramus 12 : 20 : 11 : 13 : 15 : 29. Genital double somite (Fig. 1A, C) constricted laterally at anterior third, with genital apertures present just before this constriction, length 0.6 times width and 0.9 times smallest width at constriction. Anal somite short, length 0.5 times greatest width. Caudal ramus (Fig. 1D) leaf-shaped, 2.1 times longer than wide, with 2 lateral setae and additional minute seta on outer margin, 2 terminal setae near posterior end, dorsal seta near center, and 1 short process on inner distal margin.

Antennule (Fig. 1E) 5-segmented, length 0.17 times prosome, length ratios of first to fifth segments measured along anterior margin 15 : 44 : 18 : 10 : 13, formula for armature: 4, 19, 4+1 aesthete, 2+1 aesthete and 7+1 aesthete.

Antenna (Fig. 1F) 5 segmented, distal segment claw shaped; coxobasis with short seta on inner distal corner; first endopodal segment with short seta at mid-length of inner margin (11.1 times longer than seta); second endopodal segment with 3 setae near inner distal corner; third endopodal segment with 3 short setae and 2.23 times longer than terminal claw; terminal claw with 1 inner proximal small seta.

Labrum (Fig. 1G: on the basis of the paratype) deeply incised, with smooth ventral surface, covering mandibles. Maxillule, maxilla, and maxilliped visible in ventral view.

Mandible (Fig. 1H) shaped like a tapering blade, with 2 spinulose setae on proximal outer margin, dense spinules along proximal inner margin, 2 short spines on posterior surface, and several short spinules at distal end.

Maxillule (Fig. 1I) 1-segmented with two tufts of minute spinules distally, 1 spinulose seta and 3 naked setae.

Maxilla (Fig. 2A) 2-segmented; syncoxa with one tuft of spinules on posterior surface; basis with two spinulose setae on posterior surface and bifurcated; outer one of them shorter and naked and inner one furnished with spinulose terminal lash.

Maxilliped (Fig. 2B) 3-segmented; syncoxa with one tuft of minute spinules on outer distal corner; basis with 1 long spine and 1 short spine at mid-length of inner margin; endopod-shaped terminal claw, proximally with 1 lateral and 2 medial setae, and distally with several spinules along inner margin.

Legs 1–4 (Fig. 2C–F) with armature as follows (Roman

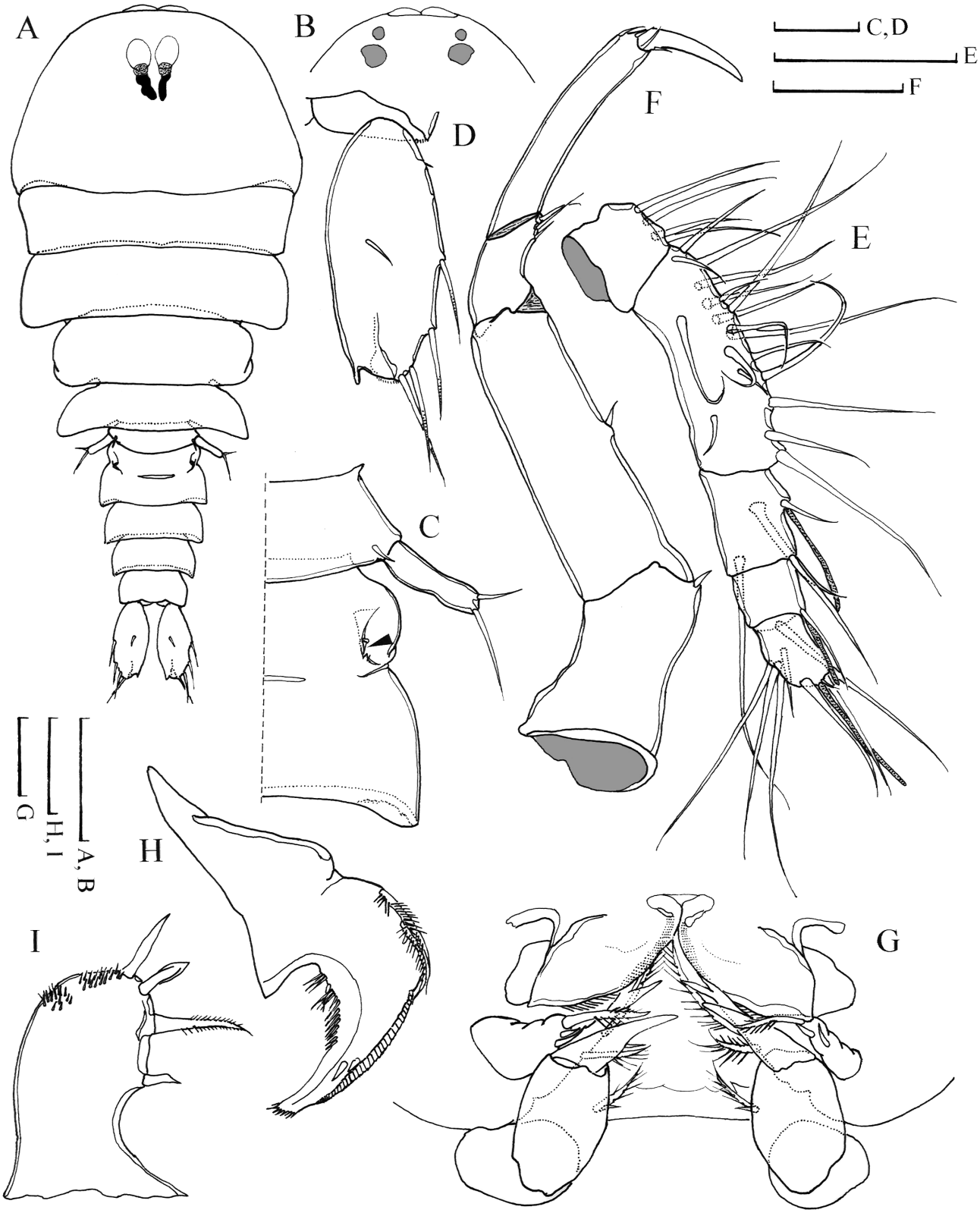

Fig. 1. *Sapphirina doliolettae* n. sp. Female (A–F, H–I, holotype; G, paratype): (A) whole animal, dorsal; (B) forehead, ventral; (C) fifth pedigerous somite and genital double somite, right side dorsal (arrowheads indicate 1 spine on sixth leg); (D) caudal rami, right dorsal; (E) antennule, right dorsal; (F) antenna, right posterior; (G) oral area, ventral; (H) mandible, right posterior; (I) maxillule, right posterior. Scale bars: A, B, 0.5 mm; C–F, 0.1 mm; G–I, 0.05 mm.

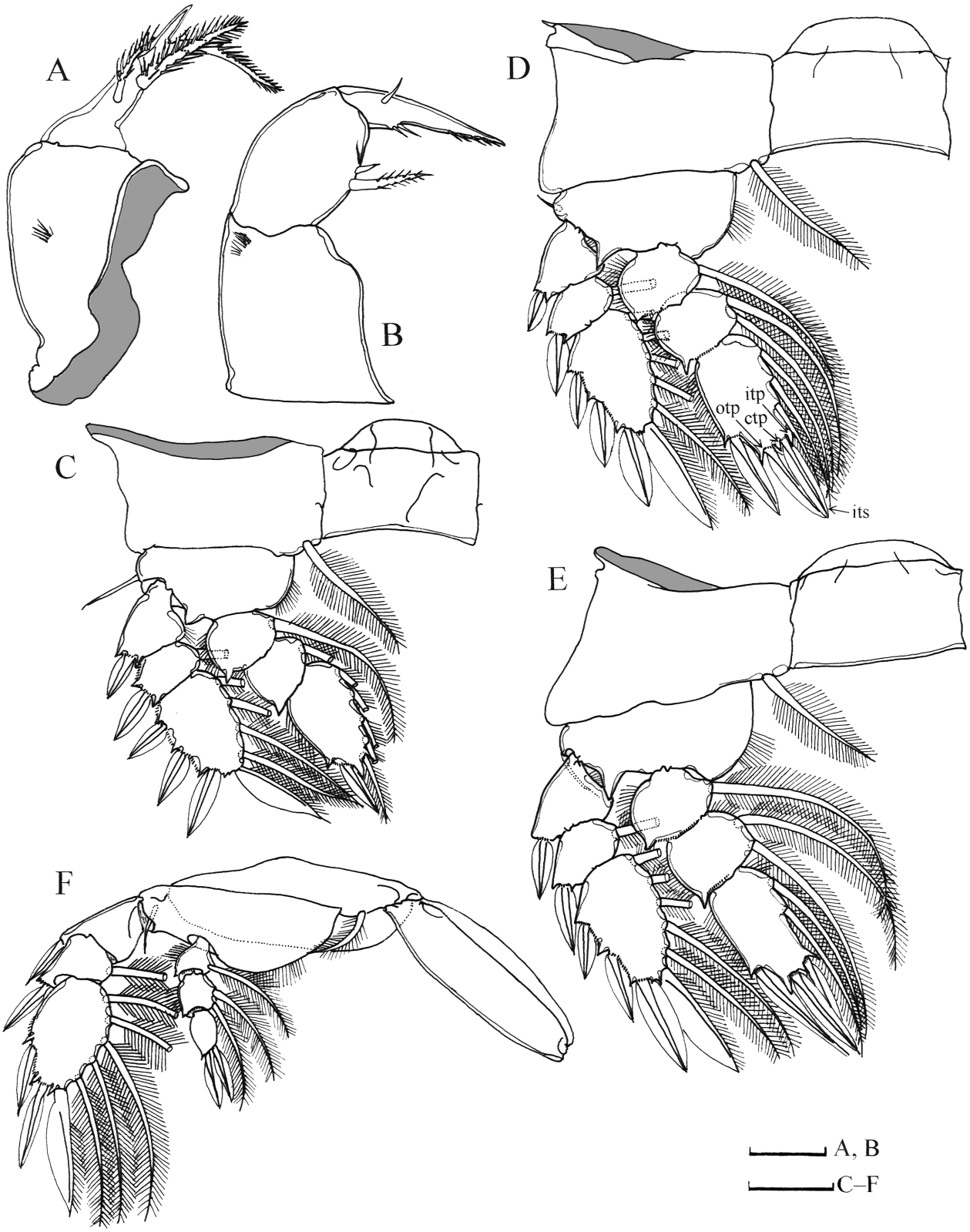

Fig. 2. *Sapphirina doliolettae* n. sp. Female (holotype): (A) maxilla, right posterior; (B) maxilliped, right posterior; (C) leg 1, right anterior; (D) leg 2, right anterior (otp, outer terminal process; ctp, central t. p.; itp, inner t. p.; its, inner terminal spine); (E) leg 3, right anterior; (F) leg 4, right anterior. Scale bars: A, B, 0.05 mm; C–F, 0.1 mm.

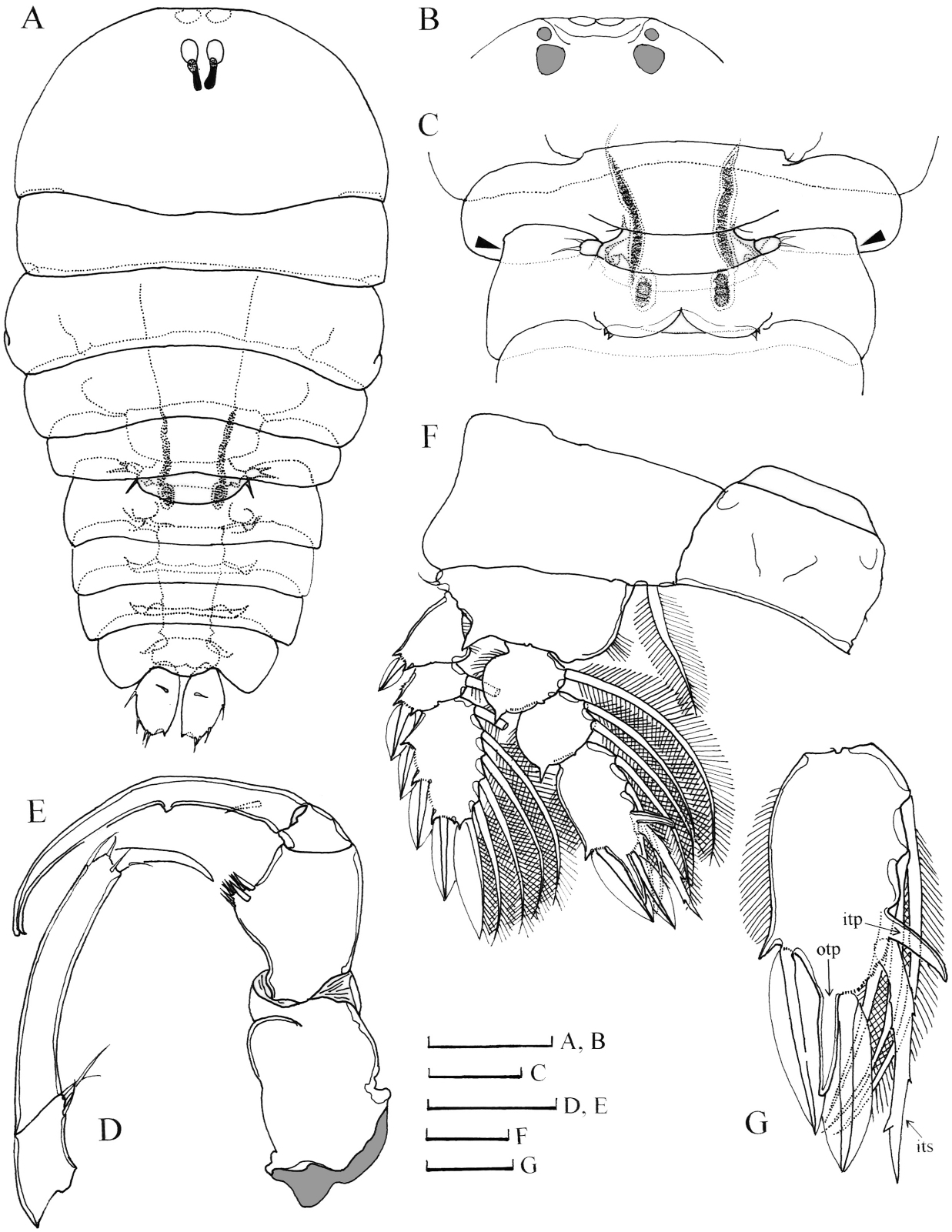

Fig. 3. *Sapphirina doliolettae* n. sp. Male (allotype): (A) whole animal, dorsal; (B) forehead, ventral; (C) fifth pedigerous somite and genital somite, ventral (arrowheads indicate concave anterolateral margins of genital somite); (D) second and third endopod and terminal claw of antenna, right posterior; (E) maxilliped, right anterior; (F) leg 2, right anterior; (G) third endopodal segment of leg 2, right anterior (otp, outer terminal process; itp, inner t. p.; its, inner terminal spine). Scale bars: A, B, 0.5 mm; C, 0.25 mm; D–F, 0.1 mm; G, 0.05 mm.

and Arabic numerals represent spines and setae, respectively):

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-0	I-0; I-1; III, I, 4	0-1; 0-1; I, 5
Leg 2	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; I, II, 3
Leg 3	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; I, II, 2
Leg 4	0-1	1-0	I-0; I-1; II, I, 5	0-1; 0-2; II

Outer spines of exopods and all spines of endopods with hyaline membrane on both sides and terminal spines of exopod with hyaline membrane on outer side and setules on inner side in all legs.

Leg 5 (Fig. 1C) comprising of small seta arising from fifth pedigerous somite and 1 free segment; segment length 3 times width, with 2 apical setae, inner seta of them 3 times longer than outer seta.

Leg 6 (Fig. 1C) represented by genital opercula with one small spine (arrowhead in Fig. 1C) and 2 processes.

Male (allotype)- Body (Fig. 3A) length 2.93 mm, dorsoventrally flat and shaped like a long egg in dorsal view. Greatest width 1.54 mm at posterior 1/3 of 2nd pedigerous somite. Prosome length 1.2 times width and 1.7 times length of urosome. Width of the first to fourth pedigerous somites 100, 102, 92, and 79% of greatest width of cephalosome, respectively. Distal portion of head flat and hides a pair of lenses in dorsal view (Fig. 3A). Rostrum absent (Fig. 3B). Length ratios of first to sixth urosomites and caudal ramus 8 : 16 : 17 : 14 : 9 : 10 : 26 (Fig. 3A). Genital somite (Fig. 3A, C) broad; length 0.17 times greatest width, anterolateral corner somewhat pointed and anterior part of lateral margin slightly concave (arrowheads in Fig. 3C). Anal somite (Fig. 3A) length 0.4 times greatest width, completely covered by the posterior part of fifth urosomite. Caudal ramus 1.9 times longer than wide.

Antennule, mandible, maxillule, and maxilla as in female. Antenna similar to that of female, medial seta on first endopodal segment longer than that of female (inner margin longer than 4.14 times length of seta), third endopodal segment 2.65 times longer than terminal claw (Fig. 3D).

Maxilliped (Fig. 3E) 4-segmented; syncoxa unarmed; basis with one tuft of spinules and 2 short setae on a process at midpoint of inner margin; first endopodal segment unarmed; terminal claw long and curved, much longer than basal two segments combined, with 1 short proximal seta and 1 tooth in middle of inner margin.

Legs 1, 3, and 4 as in female. Leg 2 (Fig. 3F) third endopodal segment (Fig. 3G) with outer and inner long terminal processes; central terminal process found in female (see also Fig. 2D) lacking in male; inner terminal spine slightly sinuated, with 2–3 teeth on each side.

Leg 5 (Fig. 3C) covered by fourth pedigerous somite in dorsal view; exopod short with 2 apical setae and 1.5 times longer than wide.

Leg 6 (Fig. 3C) represented by 2 short processes on posteroventral flap of genital somite.

Measurement data

Female: 2.09–2.84 mm (mean±standard deviation= 2.52±0.19 mm, n=31); male: 2.30–2.93 mm (2.67± 0.18 mm, n=18).

Etymology

The species is named after the genus of its prey/host *Dolioletta* (Takahashi et al. 2013). Both are often collected at the same time.

Morphological comparison with congeneric species

According to WoRMS (Walter & Boxshall 2022), 22 species names are currently accepted within the genus *Sapphirina*. Among these, 20 species have comparable morphological information, with the exception of *S. indicator* Thompson J.V., 1829 and *S. granulosa* Giesbrecht, 1891 (Razouls et al. 2005–2022). Among these 20 species, the first and second endopodal segments of leg 4 of *S. angusta* Dana, 1849, *S. auronitens* Claus, 1863, *S. gastrica* Giesbrecht, 1891, *S. gemma* Dana, 1849, *S. iris* Dana, 1849, *S. lactens* Giesbrecht, 1892, *S. metallina* Dana, 1849, *S. ovatolanceolata* Dana, 1849, *S. pyrosomatis* Giesbrecht, 1892, *S. sali* Farran, 1929, *S. sinuicauda* Brady, 1883, and *S. vorax* Giesbrecht, 1891 are wide (more than 2/3 of the corresponding segments of the exopod; Giesbrecht 1892, Lehnhofer 1929). The remaining eight species and *S. doliolettae* n. sp. have narrow first and second endopodal segments (less than 1/2 of those of exopod). *Sapphirina doliolettae* n. sp. can be distinguished from *S. intestinata* Giesbrecht, 1891, *S. maculosa* Giesbrecht, 1892 and *S. stellata* Giesbrecht, 1891 by the presence of 2 terminal spines on the endopod of leg 4 (instead of 1 terminal spine), as well as the remaining five species. Among these, *S. darwinii* Haeckel, 1864 and *S. opalina* Dana, 1849, having a 3-segmented antennule, and *S. bicuspidata* Giesbrecht, 1891, having 2 inner processes on the caudal rami, are unique within the genus.

Sapphirina doliolettae n. sp. is similar to *S. nigromaculata* and *S. scarlata* with respect to the following characteristics: 5-segmented antennule, one inner marginal process on furcal caudal rami, and slender endopods with two apical spines on leg 4. The new species can be distinguished from these related species by the six characters shown in Table 1 and the shape of the anterolateral corner of the male genital segment shown in Fig. 4. Here, the criteria for significant differences between species was that the ranges of trait measurements did not overlap. As a result, a significant difference between this species and *S. nigromaculata* was found in the length ratio of the 3rd endopodal segment to the terminal claw of the antenna (female: 2.06–2.57 vs. 3.38–4.50, male: 2.56–2.87 vs. 3.19–4.40), the length ratio of the inner margin to medial seta of the first endopodal segment of the antenna in

Table 1. Comparison of data range for morphological characters among *Sapphirina doliolettae n. sp.* and two related congeners. ND, No data.

Species		*S. doliolettae* n. sp.			*S. nigromaculata*			*S. scarlata*		
Locality		Kuroshio Extension (type locality)	Sendai Bay	Tokyo Bay	Tokyo Bay Sagami Bay	Gulf of Naples	East China Sea/ off Kinkazan	Suruga Bay Tokyo Bay	Gulf of Naples	Korea Strait
Date		2009/5/20	2020/7/3	1994/6/2	1992/9–1994/9 (from June to September) 1993/10/15	ND	ND	1992/8/6 1994/7/4	ND	ND
Surface water temperature (℃)		18.0	16.2	20.6	18.6–24.4 ND	ND	ND	ND 22.7	ND	ND
Body length (mm)	Female	2.42–2.78 (*n*=5)	2.11–2.80 (n=6)	2.08–2.43 (n=3)	1.57–2.38 (*n*=10)	1.80–2.10	1.20–2.00	3.19–3.63 (*n*=4)	3.32	3.77
	Male	2.54–2.94 (*n*=5)	2.44–2.70 (n=5)	2.34–2.53 (n=2)	1.68–2.42 (*n*=5)	2.05–2.45	2.00–2.45	3.46–3.86 (*n*=3)	3.40–3.80	ND
Antennule: length (outer margin) ratio of the 2nd segment to distal three segments combined	Female	0.98–1.10 (*n*=5)	0.87–1.13 (n=6)	0.95–1.02 (n=3)	0.88–0.94 (*n*=10)	0.82	0.75	1.33–1.44 (*n*=4)	ND	1.42
	Male	0.90–1.08 (*n*=5)	0.92–1.04 (n=5)	0.96–0.98 (n=2)	0.80–0.91 (*n*=5)	ND	0.86	1.50–1.54 (*n*=2)	1.37	ND
Antenna: length (outer margin) ratio of the 3rd endopodal segment to the terminal claw	Female	2.06–2.38 (*n*=5)	2.27–2.57 (n=6)	2.06–2.37 (n=3)	3.41–4.50 (*n*=10)	3.38	ND	2.17–2.48 (*n*=3)	ND	2.22
	Male	2.61–2.87 (*n*=5)	2.56–2.84 (n=5)	2.73–2.76 (n=2)	3.58–4.40 (*n*=5)	3.19	4.18	2.86–2.88 (*n*=2)	2.69	ND
Antenna: length ratio of the inner margin to seta of 1st endopodal segment	Female	9.80–14.57 (*n*=5)	10.00–12.75 (n=6)	10.67–11.75 (n=3)	6.44–8.62 (n=9)	5.53	ND	12.04–14.00 (*n*=3)	ND	12.6
	Male	4.07–6.33 (*n*=5)	3.67–5.27 (n=5)	4.31–4.33 (n=2)	3.24–6.00 (n=4)	3.00	3.19	7.25–9.56 (*n*=2)	5.80	ND
Pedigerous somite: width ratio of the 4th to the 3rd somite	Female	0.91–0.96 (*n*=5)	0.87–0.99 (n=6)	0.89–0.95 (n=3)	0.78–0.86 (*n*=10)	0.81	0.75	0.76–0.78 (*n*=4)	0.80	0.79
The 3rd endopodal segment of Leg 2: length ratio of outer terminal process to inner terminal process	Male	1.19–1.36 (*n*=5)	1.11–1.40 (n=5)	1.20–1.26 (n=2)	0.77–1.04 (*n*=5)	1.07	1.08	1.50–1.69 (*n*=2)	1.27	ND
Reference		present study			present study	Giesbrecht (1892)	Mori (1937)	present study	Giesbrecht (1892)	Mori (1929)

females (9.80–14.5 vs. 5.53–8.62), the width ratio of the fourth pedigerous somite to the third pedigerous somite of females (0.87–0.99 vs. 0.75–0.86) and the length ratio of the outer terminal process to inner terminal process of the third endopodal segment of leg 2 of males (1.11–1.40 vs. 0.77–1.08). Significant differences between this species and *S. scarlata* were found in the body length (female: 2.08–2.80 mm vs. 3.19–3.77 mm, male: 2.34–2.94 mm vs. 3.40–3.86 mm), the length ratio of second segments to the terminal three segments of the antenna (female: 0.87–1.13 vs. 1.33–1.44, male: 0.90–1.08 vs. 1.37–1.54) and the width ratio of the fourth pedigerous somite to the third pedigerous somite of females (0.87–0.99 vs. 0.76–0.80). The high width ratio of the fourth pedigerous somite in females of the new species is due to the posterolateral corner being protruded diagonally backwards (Fig. 1A) and is unique within the three species. In addition, the anterolateral corner of the genital somite of males is somewhat pointed and the anterior part of its lateral margin is slightly concave in *S. doliolettae* n. sp. (Fig. 4), whereas the anterolateral corner of the corresponding somite is rounded in the two congeners.

These characters concerning body segments are not found in most previous descriptions of congeners (e.g., Al-Yamani & Prusova 2003, Chen & Zhang 1974,Giesbrecht, 1892, Mori 1929, 1937, Itoh 1997), whereas the width ratio of the 4th to the 3rd pedigerous somite is 0.96 (corresponding to value of the new species) in the female specimen described as *S. nigromaculata* by Farran (1926) from Biscay Bay. He mentioned that the shape of the pedigerous somites of the specimen differs from that described by Giesbrecht (1892). It is highly possible that the specimen is this new species. Razouls et al. (2005–2022) listed *Sapphirina inaequalis* Dana, 1852 and *S. lomae* Esterly, 1905 as synonyms of *S. nigromaculata*. The female of *S. inaequalis* is similar to the new species in having a wide fourth pedigerous somite. However, females in the original description had an acute posterolateral corner on the fourth pediger-

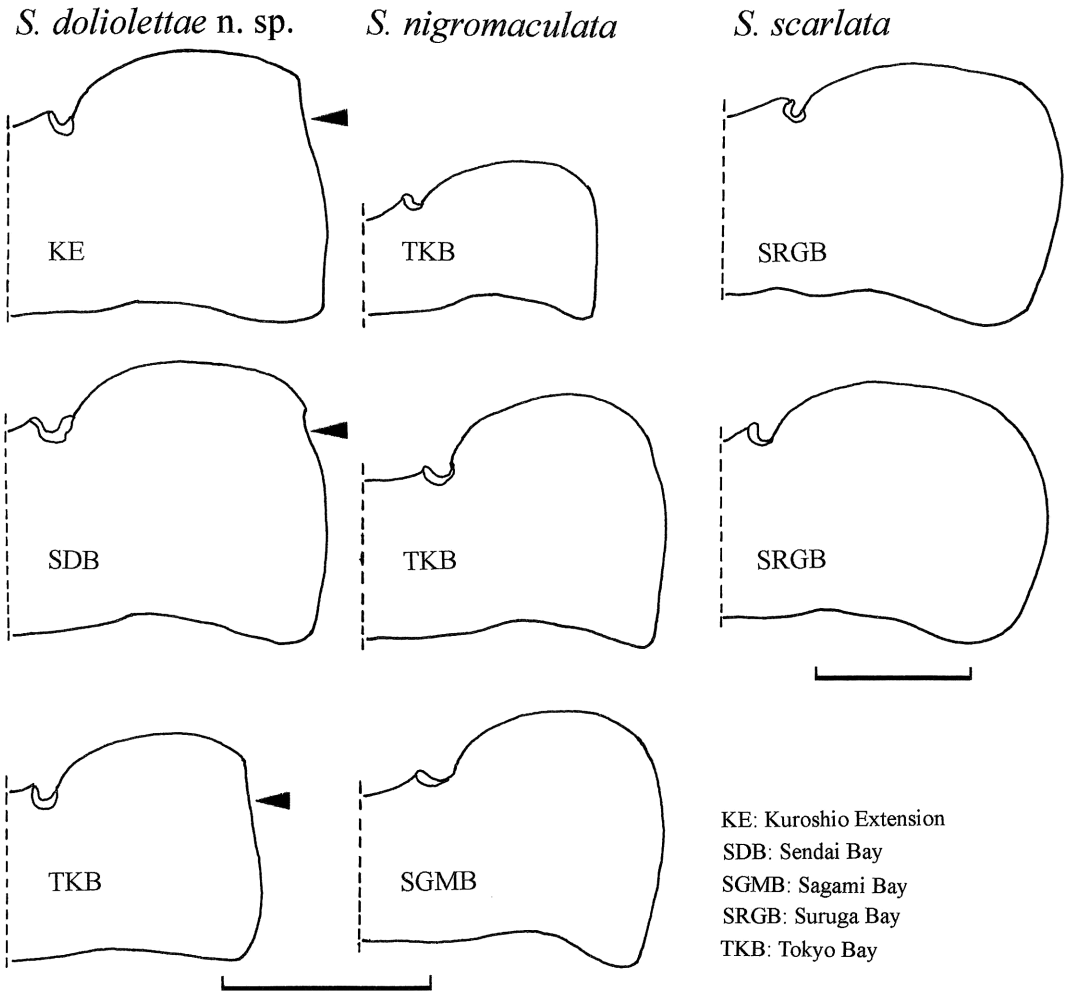

Fig. 4. Comparison of genital somite (right half) of male between *S. doliolettae* n. sp. and two related congeners. Uppercase alphabetic symbols indicate sampling sites. Scale bars: 0.25 mm. Arrowheads indicate concave anterolateral margin of genital somite.

Table 2. Intraspecific and interspecific sequence variabilities (%) of COI (below) and 18S (above) gene sequences in *Sapphirina* species. Intraspecific variabilities, indicated with grey hatching, are listed for COI (left) and 18S (right). In addition to sequences obtained in this study (see Materials and Methods), sequences data were obtained from the GenBank for *S. angusta* (COI: GU171328, KT345967–KT345969), *S. auronitens* (COI: KU049704–KU049708), *S. darwinii* (COI: MW125205, MW125190, MW125193; 18S: GU969173), *S. metallina* (COI: HM045344, KF985240, KT429933, KP861444, KU144690, KU144691, KU200948, MW125202, MW125206, MW125209), *S. opalina* (COI: JX503000, JX503001, KU158879–KU158883), *S. scarlata* (COI: HM045348, KT351342–KT351344; 18S: GU969208), *S. stellata* (COI: KT354294), and *S. vorax* (COI: KX454156). ND, no data.

		1	2	3	4	5	6	7	8	9	10	11
1	*S. doliolettae* n. sp.	0–0.2/0	ND	ND	3.2	ND	2.2	ND	4.9	2.5	1.6	ND
2	*S. angusta*	22.5	0/ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	*S. auronitens*	20.2	20.7	0/ND	ND	ND	ND	ND	ND	ND	ND	ND
4	*S. darwinii*	24.1–24.5	23.2–23.4	23.4–23.6	0–0.2/ND	ND	4.0	ND	3.2	2.5	1.8	ND
5	*S. metallina*	22.1–23.2	23.8–24.8	24.8–25.4	27.5–28.8	0–7.7/ND	ND	ND	ND	ND	ND	ND
6	*S. nigromaculata*	20.2	24.5	22.3	21.1–21.3	25.4–26.3	ND/ND	ND	5.9	3.8	3.1	ND
7	*S. opalina*	22.3–23.4	23.8–24.6	21.3–22.3	22.7–23.6	24.3–26.4	22.9–24.1	0–4.6/ND	ND	ND	ND	ND
8	*S. ovatolanceolata*	24.1	23.2	23.6	24.8–25.0	25.0–26.1	25.0	23.6–23.8	ND/ND	4.6	4.0	ND
9	*S. scarlata*	21.6–21.8	23.8	23.2	24.8–25.0	22.5–23.0	23.4	22.5–23.9	26.1	0/ND	0.8	ND
10	*S. stellata*	22.0–22.2	25.5–25.7	24.3–24.5	24.5–24.8	24.5–25.2	24.1–24.3	22.1–23.2	25.9–26.1	21.6–21.8	0.2/ND	ND
11	*S. vorax*	24.6–24.8	23.4	24.1	25.0–25.2	25.0–25.5	22.9	24.1–24.5	24.5	23.4	27.7–27.9	ND/ND

ous somite (rounded in the new species) and the slender cephalosome was 0.78 times longer than its width (0.63 in the new species). The female of Brady (1883) has a 6-segmented antennule (5- in the new species) and a relatively long medial seta on the inner margin of the first endopodal segment of the antenna (length ratio to inner margin: 2.4). *S. lomae* was described on the basis of broken male specimens and differs from the new species in the higher length ratio (3.1) of the third endopodal segment to the terminal claw of the antenna and the long outer terminal spine being significantly longer than the inner terminal spine on the third endopodal segment of leg 2 (Esterly 1905).

Genetic variability

The COI sequence of *S. doliolettae* n. sp. (560 bp) showed high interspecific genetic variabilities of 20.2–24.8% from the other 10 *Sapphirina* species studied (Table 2). The smallest interspecific variation (20.2%) from the new species was observed in *S. auronitens* and *S. nigromaculata*, followed by *S. scarlata* (21.6–21.8%). The intraspecific genetic variability of COI was low (0–0.2%) within the new species. Sequence variabilities in 18S (1,716 bp) between *S. doliolettae* n. sp. and five other *Sapphirina* species, including *S. nigromaculata*, were 1.0–3.1%, and no clear intraspecific variabilities in 18S (0%) were observed for *S. doliolettae* n. sp.

Ecological Remarks

As listed in the 'Material examined,' the new species has been found in the Kuroshio Extension region, the Kuroshio-Oyashio Transition region, Sendai Bay, Tokyo Bay and Suruga Bay from spring to early summer (surface water temperature: 15.8–20.6℃). The specimens of *S. nigromaculata* and *S. scarlata* were collected mainly from summer to autumn (surface water temperature: 18.6–24.4℃ and more than 22.7℃, respectively). In comparison with these related congeners living in the tropical and subtropical waters (Razouls et al. 2005–2022), the new species may prefer temperate water and relatively low water temperatures.

Takahashi et al. (2013) confirmed that *Sapphirina doliolettae* n. sp. (as *S. nigromaculata*) is an active predator of *Dolioletta gegenbauri* at the type locality for the new species by observations on board and by a video plankton recorder (VPR). The mesozoolankton community at the site was dominated by *D. gegenbauri*. Doliolids belonging to genus *Dolioletta* were also collected from all the other sites in the present study (data not shown). These observations indicate that the occurrence of the new species is strongly correlated with that of the doliolids, as pointed out by Takahashi et al. (2015a). The new species show a circadian rhythm in male colouration pattern between iridescent and transparent phases (Takahashi et al. 2015b).

Acknowledgements

We are grateful to Dr. Y. Okazaki of the Fisheries Research and Education Agency, Mr. Y. Yamada of Kanagawa Prefectural Fisheries Technology Center (presently Kanagawa Prefectural Eastern Region Fishing Ports Management Office) and Dr. H. Matsuura of the School of Marine Science and Technology, Tokai University, who provided the plankton samples and the environmental data. The specimens obtained from the research and assessment program for fisheries resources, the Fisheries Agency of Japan, were used for genetic comparison with related species. We also thank three anonymous reviewers for their critical comments and Dr. S. Nishida of the Atmosphere and Ocean Research Institution, The University of Tokyo, and Dr. C. Walter of the Smithsonian National Museum of Natural History for information on the type specimen. This study was partially supported by grants from the Japan Society for the Promotion of Science (KAKENHI; grant numbers16H04950 and 20H03059).

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