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Two New *Phyllopodopsyllus* Species (Harpacticoida, Tetragonicipitidae) from Korea †

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Abstract: Three species of the genus *Phyllopodopsyllus* T. Scott, 1906 (Copepoda: Harpacticoida: Tetragonicipitidae) were reported from the intertidal sand of Jeju Island and Busan in Korea, *Phyllopdopsyllus kitazimai* Karanovic, 2017, *Phyllopodopsyllus saywakimi* **sp. nov.** and *Phyllopodopsyllus similis* **sp. nov.** The latter two were described here. *Phyllopodopsyllus saywakimi* **sp. nov.** collected from only Udo, Jeju is similar to a Pacific species from Costa Rica, *Phyllopodopsyllus ancylus* Mielke, 1992. However, *P. saywakimi* **sp. nov.** differs from the Costa Rica species in the body size, the number of armatures on the antenna, the length ratio of endo- and exopod of the mandible, and the chaetotaxy of the fifth female pereiopod and the sixth male pereiopod. The second new species, *P. similis* **sp. nov.**, found in Busan and several localities of Jeju, significantly resembles the species complex related to *Phyllopodopsyllus aegypticus* Nicholls, 1944, which is widely distributed in the Indian, Pacific, and Atlantic oceans. *P. similis* **sp. nov.** can be distinguished from the *P. aegypticus* complex by the body size, the shape of the rostrum, the type of armatures, the length ratio of rami and armatures, and the chaetotaxy of the female P5. In addition to the species descriptions, partial fragments of small and large subunit ribosomal RNA genes, cytochrome b, and cytochrome c oxidase subunit I were provided. Using molecular markers, a phylogenetic analysis was also performed to estimate the relationships among the three Korean species *of Phyllopodopsyllus* for the first time.

Keywords: meiofauna; biodiversity; Crustacea; Copepoda; marine benthos

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1. Introduction

The genus *Phyllopdopsyllus* T. Scott, 1906 is one of the tetragonicipitid genera and contains more than half of all tetragonicipitid copepods [1]. Furthermore, it is one of the most diverse genera of marine harpacticoids [2]. *Phyllopdopsyllus* species typically inhabit shallow marine sediments, especially in the tropics [2], and have also been recorded from anchialine groundwaters [3]. All females of this genus share the large foliaceous fifth pereiopods that form a brood pouch with the urosome. Although this autapomorphic characteristic makes them easily distinguishable from other harpacticoids, the relationship among *Phyllopodopsyllus* species is still enigmatic. Lang [4] raised the genus *Paraphyllopodopsyllus* (Lang, 1944) to accommodate *Phyllopodopsyllus* species having no projection on the second segment of antennule, an inner seta on the first endopodal segment of the second pereiopod and no inner seta on the distal exopodal segment of the second and third pereiopods. Afterward, *Paraphyllopodopsyllus* was withdrawn by its author due to several subsequently described species that take an intermediate position between *Phyllopodopsyllus* and *Paraphyllopodopsyllus* [5]. Kunz [6] subdivided the genus into nine species-group with a review of this genus and several tables comparing characteristics such as the setation of the second to fourth pereiopods. Fiers [7] questioned the naturalness of his subdivision. Karanovic et al. [3] proposed the abandonment of Kunz's species group based on their new species that did not fit any group.

Currently, about 60 valid morpho- (sub)species of this genus have been described around the world [8]. In the Pacific ocean, 21 species of *Phyllopdopsyllus* have been reported: *P. aegypticus* Nicholls, 1944 (Hawaii, described as *P. gertrudi* [9] and Costa Rica, described as *P. gertrudi costaricensis*, [10]); *P. alatus* Fiers, 1986 (Papua New Guinea [11]); *P. angolensis* Kunz, 1984 (Galapagos Islands [12]); *P. busanensis* Karanovic, 2017 (Korea [8]); *P. borutzkyi* Lang, 1965 (California [5]); *P. carinatus* Mielke, 1992 (Costa Rica [10]); *P. furciger* Sars, 1907 (Galapagos Islands [12]); *P. galapagoensis* Mielke, 1989 (Galapagos Islands [12]); *P. geddesi* Kunz, 1984 (Hawaii [9]); *P. kitazimai* Karanovic, 2017 (Korea [8]); *P. kunzi* Mielke, 1989 (Galapagos Islands [12]); *P. longicaudatus* A. Scott, 1909 (Caroline Islands [13]); *P. longipalpatus hawaiiensis* Kunz, 1984 (Hawaii [9]); *P. mossmani chiloensis* Mielke, 1992 (Chile [14]); *P. paramossmani* Lang, 1934 (Stewart Island [15]); *P. parastigmosus* Gómez & Morales-Serna, 2015 (Mexico [16]); *P. petkovskii* Kunz, 1984 (Hawaii [9]); *P. punctatus* Kitazima, 1981 (Japan [17]); *P. setouchiensis* Kitazima, 1981 (Japan [17], Hawaii, described as *P. mielkei* [9], California, described as *P. mielkei californicus* [9], Costa Rica [10], and China [18]); *P. simplex* Kitazima, 1981 (Japan ([17]); *P. thiebaudi santacruzensis* Mielke, 1989 (Galapagos Islands [12] and Korea [19]).

In the present study, two new species of *Phyllopodopsyllus* from marine intertidal environments on the south coast of Korea were described. Detailed descriptions of the two new species were given, along with high-resolution images. A morphological comparison of them with their closely-related congeners was also executed. Additionally, we amplified partial sequences of two nucleic ribosomal RNA genes and two mitochondrial genes and inferred evolutionary relationships among three Korean species of *Phyllopodopsyllus*.

2. Material and Methods

2.1. Taxon Collecting and Examination

We collected meiofaunal assemblages from five localities in Korea (L1, 33°14′11.54″ N 126°21′35.56″ E; L2, 33°18′34.782″ N 126°49′53.328″ E; L3, 33°31′14.53″ N 126°57′29.64″ E; L4, 35°10′17.61″ N 129°11′52.68″ E; L5, 35°16′4.09″ N 129°14′39.46″ E) using the Karaman–Chappuis method (Figure 1). The collected material was transferred on a 38 μm mesh sieve and immediately sifted several times with fresh water until the filtered water looked clear. Subsequently, the residual material was washed with absolute ethanol to dehydrate and then preserved in the same ethanol concentration. The specimens of *Phyllopodopsyllus* were isolated under a stereo microscope, SZX-7 (Olympus, Tokyo, Japan), and stored at −20 °C with absolute ethanol.

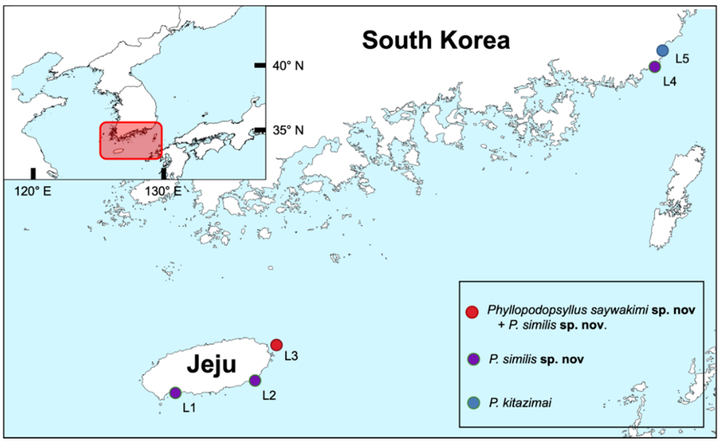

Figure 1. Map showing the localities of collecting specimens of *Phyllopodopsyllus saywakimi* **sp. nov.**, *P. similis* **sp. nov.**, and *P. kitazimai*. The red dot indicates the type locality of both new species; the purple and blue dots mark the locations where only *P. similis* **sp. nov.** and *P. kitazimai* were collected, respectively. This map was made under QGIS (3.22, downloaded from https://qgis.org/en/site/forusers/download.html) using map data of GADM (4.1, downloaded from https://gadm.org/data.html).

2.2. DNA Extraction

All specimens of *Phyllopodopsyllus* were used for total genomic DNA (gDNA) extraction. A non-destructive approach for subsequent morphological analyses was adopted to extract gDNA. Each specimen was soaked in ultra-pure water for 30 min to remove ethanol. Subsequently, gDNA was individually isolated from each whole specimen, using hot sodium hydroxide and Tris modified by [20] with a total volume of 110 µL, or DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The exoskeletons remaining after lysis were transferred separately in a 0.2 mL tube with 70% ethanol.

2.3. Taxon Collecting and Examination

We prepared each residual exoskeleton on a slide glass, identified them using a compound microscope, BX-51 (Olympus, Tokyo, Japan), and stored them separately in absolute ethanol at room temperature until later processing. We followed identification keys in [1,8,21] and original descriptions to identify specimens. Some specimens identified as two undescribed *Phyllopodopsyllus* species were dissected and mounted in lactophenol for more detailed observation. All dissections were made using a stereo microscope, SZX-7 (Olympus, Tokyo, Japan), or S APO (Leica microsystems, Wetzlar, Germany). Pencil drawings were made under a differential interference contrast microscope, DM2500 (Leica microsystems, Wetzlar, Germany)Using an iPad pro (Apple, Cupertino, CA, USA), line drawings were illustrated in Procreate (Savage Interactive, Hobart, Australia).

The type of material prepared in this study was deposited at the National Institute of Biological Resources (NIBR; Incheon, Republic of Korea) and the National Marine Biodiversity Institute of Korea (MABIK; Seochun, Republic of Korea). Detailed information regarding the type of material is listed in Table S1.

2.4. Image Acquisition

In both *Phyllopodopsyllus* species, several individuals of each sex were used for in-depth observation and image acquisition through a scanning electron microscope (SEM) and confocal laser scanning microscope (CLSM).

For SEM, the specimens were immersed in Hexamethyldisilazane (HMDS) [22,23] and placed in a drying oven until HMDS had evaporated completely. They were then attached to SEM stubs, coated with gold in an ion sputter coater, and observed using an SEM (EM-30, Coxem, Daejeon, Republic of Korea).

For CLSM, the individuals were transferred into distilled water to remove ethanol for about 30 min. Then, specimens were stained in a 1:1 mixture of Congo red and acid fuchsin overnight in a cabinet to avoid bleaching. Before staining, samples were soaked in distilled water until no stain leaked. To prepare slide glasses, self-adhesive reinforcement rings were used [24,25]. After adding a cover slip on prepared slide glasses, the samples were scanned using an Eclipse Ti CLSM (Nikon, Tokyo, Japan) with two lasers at different wavelengths (405 and 561 nm). CLSM image stacks were imported into Fiji [26] to make maximum-intensity z-projections. The z-stuck images were stitched for each individual using an affinity photo (Serif Ltd., West Bridgford, UK). The stitched CLSM images were used for body-length measurements using Fiji (Figures S1–S4).

2.5. Amplification and Phylogenetic Analysis

A partial fragment of two nucleic ribosomal RNA genes, 18S and 28S rDNA, and two mitochondrial genes, cytochrome b (cytb) and cytochrome c oxidase subunit I (cox1), were amplified by polymerase chain reaction (PCR). The primer pairs used in the amplification of each locus were the followings: 18A1 mod and 1800 mod for 18S rDNA [27]; 28S-F1a and 28S-R1a for 28S rDNA [28]; ucytb151 and ucytb270 [29] for cytb, and jgLCO1490 [30] and Cop-COI-2189R [31] for cox1. The gDNA from some *Phyllopodopsyllus* specimens were selected for amplification, and the others were stored at −80 °C for further study. PCR amplicons were sequenced for both strands using an ABI 3730XL DNA Analyzer

(Bionics, Korea) with the same primer sets used for the thermocycling. Additional four internal primers (F1, CF2, CR1, R2 [32]) were employed for 18S rDNA sequencing. All sequences obtained newly in this study were uploaded to GenBank (Accession numbers are in Table S1).

Both forward and reverse chromatograms were visualized, trimmed, edited, and assembled in Geneious Prime 2022.2.2 (https://www.geneious.com). The assembled partial sequences of two mitochondrial genes were used to calculate inter- and intraspecific divergences (uncorrected p-distance) using Mega11 [33] under the default parameter.

To estimate the evolutionary relationships among the three *Phyllopodopsyllus* species, both maximum likelihood (ML) and Bayesian inference (BI) were performed using two rDNA and cox1 sequences. The genetic information of three Korean *Phyllopodopsyllus*, three laophontids, a family belonging to taxon II in [34,35], two aegisthids, and two tetragonicipitids were used and listed in Table S1. Sequences of each gene were aligned using MAFFT (v7.490, [36]) with the E-INS-i algorithm in two nucleic rDNA and L-INS-i in cox1. Each alignment was trimmed with trimAl (v1.4, [37]) under gappyout mode and then trimmed alignments were concatenated into a single matrix in Geneious. The concatenated matrix was used to select the best-fit models for each partition in Modelfinder [38]. Subsequently, the phylogenetic tree was inferred with partitioned maximum likelihood in IQ-Tree (multicore version 1.6.12, [39]) with 3000 bootstrap replicates. We also examined the matrix used in BI using MrBayes (v3.2.7a, [40]). Akaike information criterion (AICc) was adopted for selecting the best-fit model in PartitionFinder2 (v2.1.1, [41]). Two independent analyses were performed for 10 million generations, with four Markov chains and with a sample frequency of 1000. The initial 25% of sampled trees were discarded as burn-in, and a majority-rule 50% consensus tree was generated. The post-burn-in trees were combined using a LogCombiner (v1.10.4, [42]), and then the combined tree was summarized on maximum-clade credibility (MCC) tree in a TreeAnnotator (v.1.10.4, [42]). The genetic information of three laophontids, two aegisthids, and two tetragonicipitids were downloaded from GenBank [43–46].

3. Results

3.1. Taxon Treatment

Class Copepoda, Milne-Edwards, 1840
Order Harpacticoida, Sars, 1903
Family Tetragonicipitidae, Lang, 1944
Genus *Phyllopodopsyllus*, T. Scott, 1906
Phyllopodopsyllus saywakimi, Kim & Lee, 2023 **sp. nov.**
Phyllopodopsyllus similis, Kim & Lee, 2023 **sp. nov.**

3.2. Phyllopodopsyllus saywakimi Kim & Lee, 2023 ***sp. nov.***

Figures 2–19.

Zoobank registration

urn:lsid:zoobank.org:act:36472E42-EBEE-4ACE-9E96-7E09D9D37AE1

Type locality

Intertidal sand of the rocky shore, Udo, Republic of Korea, 23 July 2020. (L3: 33°31′14.53″ N 126°57′29.64″ E)

Specimens examined

Holotype: 1 female (MABIKCR00252865)

Paratype: 9 females and 10 males (NIBRIV0000901821–NIBRIV0000901839). The voucher specimen information is given in detail in Table S1.

Etymology

The new species (saywakimi, Latin genitive, masculine) is named in honor of Prof. Saywa Kim, one of the first author's scientific mentors who contributed to the ecology of marine zooplankton from Korean waters.

Description of adult female (based on the holotype and paratype 1–9)

Body (Figures 2A, 3A–C, 4A–E, 5, 6A, 7A,B, 8, and 9A) nine-segmented, cylindrical, slightly constricted in middle, furnished with sensilla, cuticular pores, (micro)spinules, and/or (micro)setules (details in figures), and without dimples on all body surfaces except for cephalothorax (resulting from complete fusion of cephalosome and first pedigerous somite).

Prosome (Figures 2A, 3A,B,D, 4A–C, 6A, 7A,B, and 8A) four-segmented, slightly tapering distally and comprising cephalothorax and three free pedigerous somites. Cephalothorax somewhat longer than all succeeding prosomites combined and covered with vague punctures dorsally. Three free pedigerous somites nearly equal in length. Hyaline frills of prosomites smooth but frills of last prosomite finely serrated dorsally. Rostrum small, dimpled, weakly defined at base, semi-trapezoid in dorsal aspect, and with sensillum near each anterior corner. Eye not visible.

Urosome (Figures 2A, 3A,C, 4A,D,E, 5, 7A,B, 8B–D, and 9A,B) five-segmented, comprising fifth pedigerous somite, genital double-somite (resulting from fusion of second and third urosomites), two abdominal somites and anal somite armed with caudal rami, and slightly longer than prosome (including caudal rami). Fifth pedigerous somite shortest and wrinkled on ventral surface; hyaline frills of fifth pedigerous somite finely serrated and smooth laterally. Genital double-somite longest among urosomites, armed with sixth pereiopod (P6), and with vestigial original segmentation marked by pattern of surface ornamentation, ventral suture, and dorsal and lateral ridge, of which dorsal one faint; hyaline frills of genital double-somite finely fringed dorsally and smooth laterally and ventrally; terminal part of lateral frill ornamented with spinules regularly; genital field with single ovoid copulatory pore near ventral suture, short copulatory duct in distal part of genital somite, and two seminal receptacles. Antepenultimate somite slightly longer than first urosomite and with finely serrated fringes dorsally; hyaline frill of antepenultimate somite smooth ventrally and laterally; lateral frill ornamented with spinules regularly; ventral frill extended as lappet, furnished with hair-like elements, and ornamented with long spinules on terminal margin (details in Figures 5B and 9A). Penultimate somite about as long as preceding one; hyaline frill of penultimate somite finely serrated dorsally; lateral and ventral frills smooth and furnished with regular spinules terminally. Anal somite slightly narrower and longer than penultimate somite, cleft medially in posterior part; anal opening located in cleft; anal operculum rounded and densely ornamented with spinules terminally. Caudal rami cylindrical (outer margin longer than inner margin), about 3.3 times as long as greatest width, covered with microspinules throughout surface, ornamented with tube pore on outer distal margin, and armed with seven setae on each ramus, (three outer, one dorsal, and three terminal); setae I and II naked and set very close to each other dorsoventrally at outer margin medially; seta I tiny, thready, and about one fourth as long as seta II; seta III bipinnate, implanted near distal outer corner, and about as long as seta II; setae IV–VI bare and inserted inwardly; seta IV fused basally to seta V and about as long as caudal ramus; seta V modified into indefinable bulbous shape at base (see Figures 3C, 4E, 5C and 8B–D) and about 3.5 times as long as caudal ramus; seta VI fine and approximately half in length to seta IV; seta VII located at inner distal third and triarticulate.

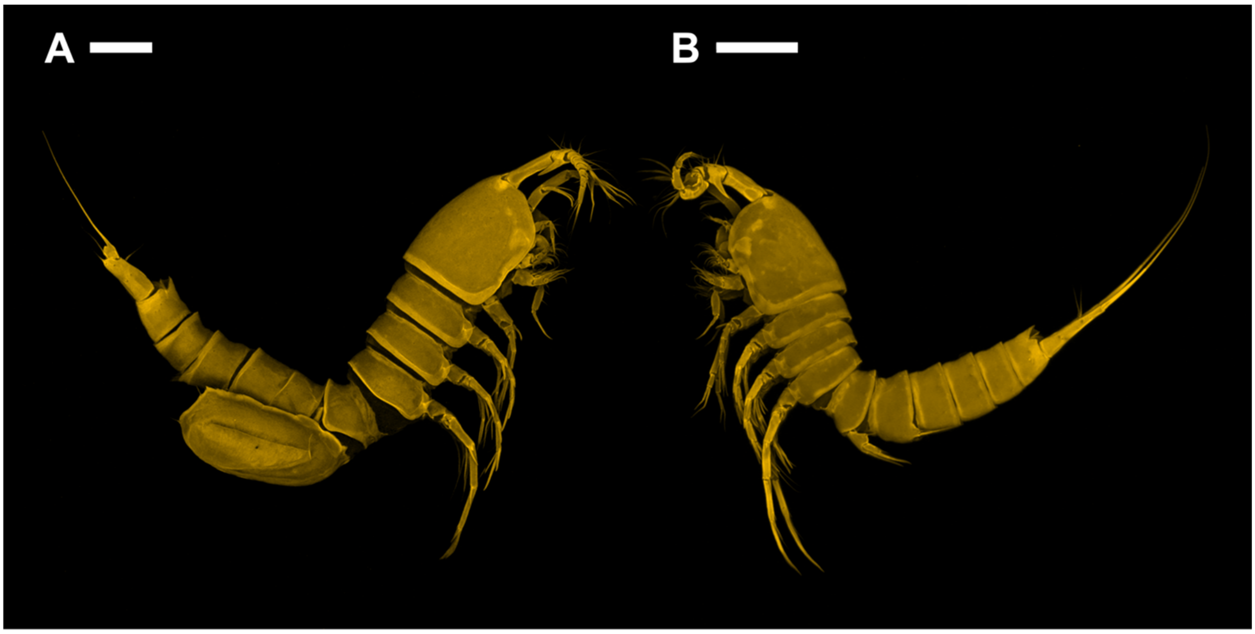

Figure 2. Confocal laser scanning microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** (**A**) female (paratype 1): habitus, lateral; (**B**) male (paratype 10): habitus, lateral. Scale bars: 100 μm.

Antennule (Figures 3D, 4F, 6B, and 10A) eight-segmented and covered with punctures; first segment elongated, longest, about four times longer than width, about 0.8 times as long as all succeeding segments combined, ornamented with row of small spinules on proximal inner margin and pore on dorsal surface proximally, and with pinnate seta on inner distal corner; second segment with robust pointy integumental projection on outer margin; fourth segment with pedestal armed with aesthetasc fused basally to long seta on inner distal corner; fifth segment shortest; eighth segment about as long as second one and with apical aesthetasc fused basally to two setae. Armature formula as follows: 1(1), 2(9), 3(8), 4(3+(ae+1)), 5(2), 6(4), 7(4), 8(5+(ae+2)); all setae naked and slender except for first two segments; all outer setae arising from penultimate and distal segments biarticulate.

Antenna (Figures 4F, 6C, and 10B) omposed of coxa, basis, endopod, and ex-opod. Coxa bare and about as long as greatest width. Basis unarmed, ornamented with longitudinal row of spinules along distal half of abexopodal margin, and about 2.2 times as long as width. Endopod two-segmented; first endopodal segment unarmed, about as long as basis, about 2.5 times as long as wide, and furnished with patch of spinules on proximal inner margin; second endopodal segment about 1.3 times longer than preceding segment, about 3.2 times as long as greatest width, decorated with patch of spinules on inner margin, row of strong spinules terminally, two rows of frills on middle and distal of outer margin, respectively, and armed with 11 armatures; four geniculate, two bare setae and one spine implanted terminally; two setae and two robust spines located in inner margin subapically; two lateral setae set very close to each other. Exopod one-segmented and with three pinnate setae (two apical and one lateral); outer apical seta fused basally to exopod.

Labrum (Figures 6D and 7C) well-developed, gradually tapering ventrally, covered with dimples on each lateral side, decorated with patch of long hairly setules on distal margin posteriorly; cutting edge furnished with setules and strong spinules on cutting edge, and with cuticular pore on outside of each cutting edge.

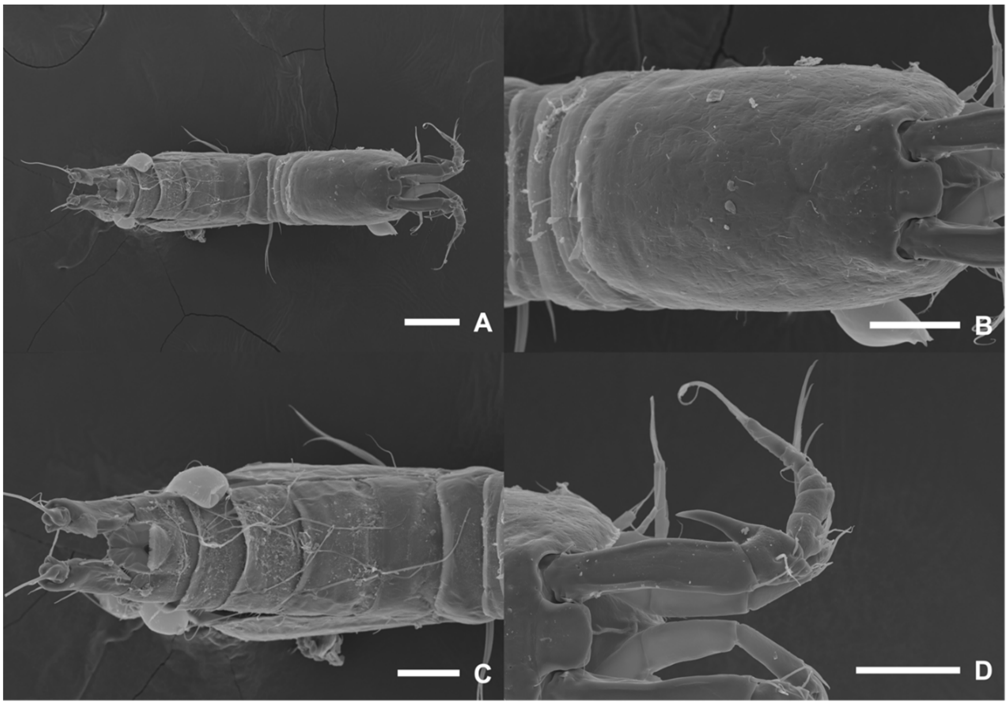

Figure 3. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** female (paratype 2) in dorsal view: (**A**) habitus; (**B**) prosome; (**C**) urosome; (**D**) antennule and rostrum. Scale bars: (**A**) 100 µm; (**B**–**D**) 50 µm.

Mandible (Figures 6C and 11A) composed of coxa, basis, endopod, and exopod. Coxa enlarged and with gnathobase bearing two large ventral teeth, pinnate dorsal seta and row of multicuspidate teeth. Basis gradually widening distally, subpentagonal, about as long as coxa, furnished with two rows of long spinules on anterior surface and slender setules along distal half of inner margin, and armed with two pinnate setae inserted near inner distal corner and one plumose seta originating from distal margin medially. Endopod one-segmented, about 0.8 times as long as basis, and armed with two lateral pinnate setae and seven apical bare setae; lateral setae implanted very close to each other in proximal third. Exopod one-segmented, slender, about 0.36 times as long as endopod, and with five setae; two lateral setae implanted in proximal quarter and midlength, respectively, and other three setae located terminally.

Paragnaths (Figure 6D) well-developed, composed of posterior and two lateral lobes; cutting edge of each lobe wrinkled; lateral robes ornamented with several rows of long and robust spinules on each inner side; posterior lobe decorated with some rows of spinules around wrinkles, medially.

Maxillule (Figures 6D,E and 11B) composed of praecoxa, coxa, basis, endopod and exopod. Praecoxa large, trapezoidal, ornamented with row of spinules on proximal third of inner margin of ventral surface, and armed with well-developed arthrite; praecoxal arthrite with two pinnate spines arising from posterior margin, of which proximal spine shorter, two apical setae arising from distal corner, of which longer one plumose, two bare setae implanted dorsally, and seven robust unipinnate spines around distal margin. Coxa about 0.3 times shorter than praecoxa, armed with cylindrical endite incorporated into coxa and epipodite represented by one plumose seta; coxal endite with five setae (one naked and four pinnate) and ornamented with minute spinules on posterior sur-face. Basis unornamented and with eight setae apically. Endopod hexagonal, about twice as long as wide, unornamented, and with four plumose setae, of which three setae located around terminal margin and other seta inserted in lateral margin. Exopod about 1.3 times as long as greatest width, ornamented with setules along ventral margin, and with three plumose setae located in terminal margin.

Maxilla (Figures 6E and 11C) composed of syncoxa, basis, and endopod. Syncoxa large and armed with four endites; endites with two, one, three, and three pinnate and/or plumose setae from proximal to distal, respectively; distal endite longest; proximal second endite shortest. Basis about 0.4 times as long as syncoxa, ornamented with row of spinules near terminal margin, and armed with cylindrical endite bearing one slender naked seta on distal margin, two robust apical spines and one bare seta arising from posterior surface; additional seta located in near boundary of basis and first endopodal segment (however not sure whether inserted in basis or following segment). Endopod two-segmented and much shorter than basis; first endopodal segment about 0.7 times as long as wide and with three setae of which two pinnate implanted around inner distal corner; other seta minute, thready, and located in anterior surface medially; second endopodal segment about equal to preceding segment in length and with four setae apically.

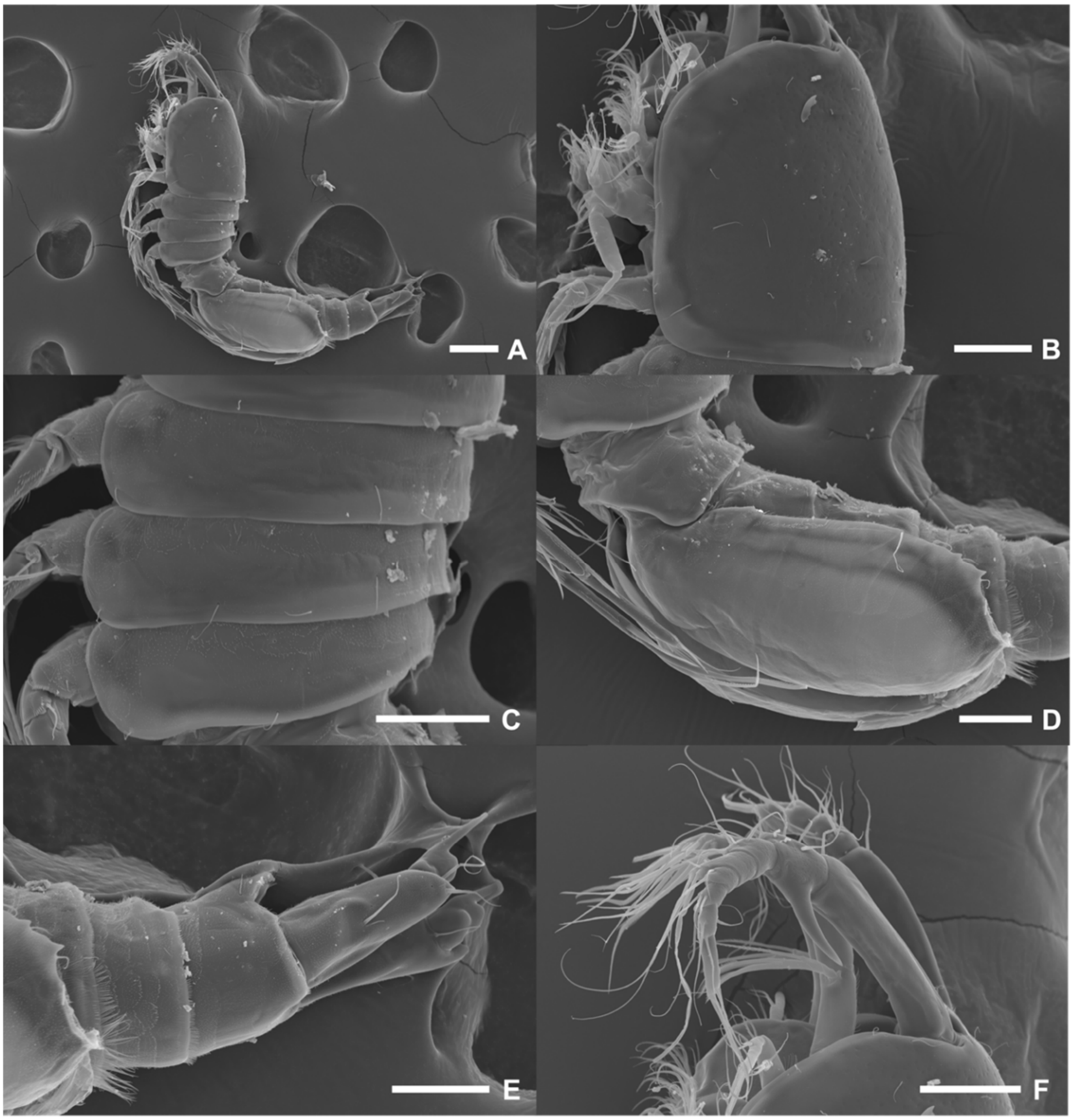

Figure 4. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** female (paratype 3) in lateral view: (**A**) habitus; (**B**) cephalothorax; (**C**) second to fourth prosomites; (**D**) first to third urosomites and P5; (**E**) fourth and fifth urosomites, and caudal rami; (**F**) antennule and antenna. Scale bars: (**A**) 100 µm; (**B**–**F**) 50 µm.

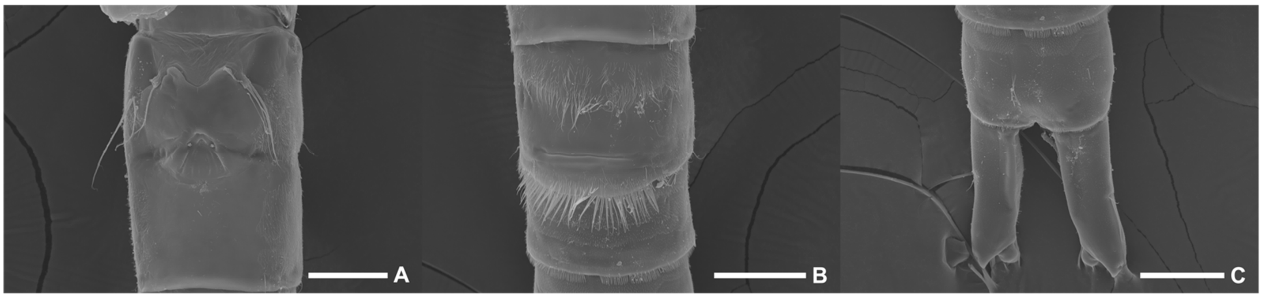

Figure 5. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** female (paratype 4) in ventral view: (**A**) genital double-somite; (**B**) third and fourth urosomites; (**C**) fifth urosomite and caudal rami. Scale bars: 50 μm.

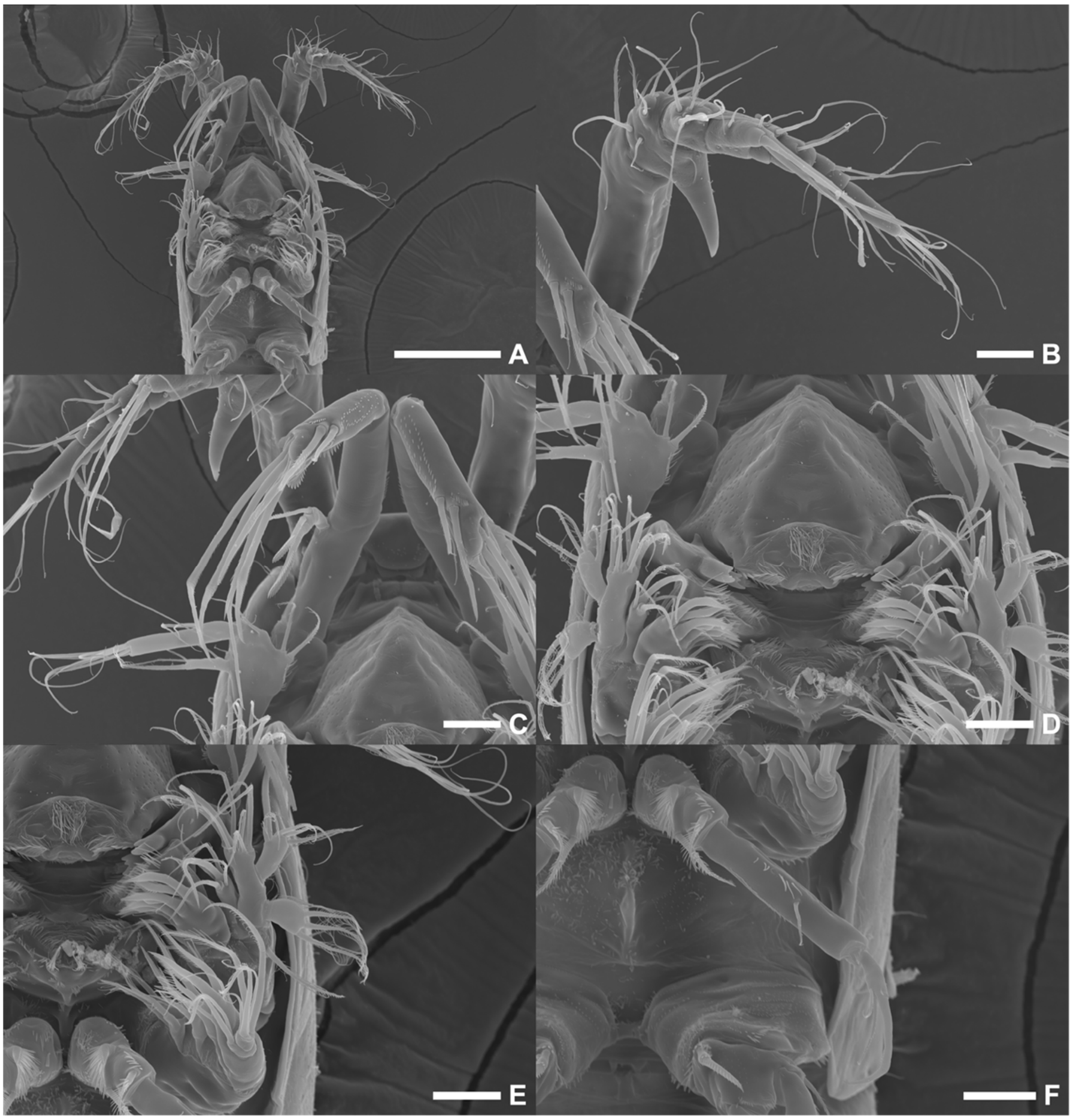

Figure 6. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** female (paratype 4) in ventral view: (**A**) cephalothorax; (**B**) antennule; (**C**) antenna and mandible (excluding coxa); (**D**) mouth parts around oral opening; (**E**) maxillule and maxilla; (**F**) maxilliped. Scale bars: (**A**) 100 μm; (**B**–**F**) 20 μm.

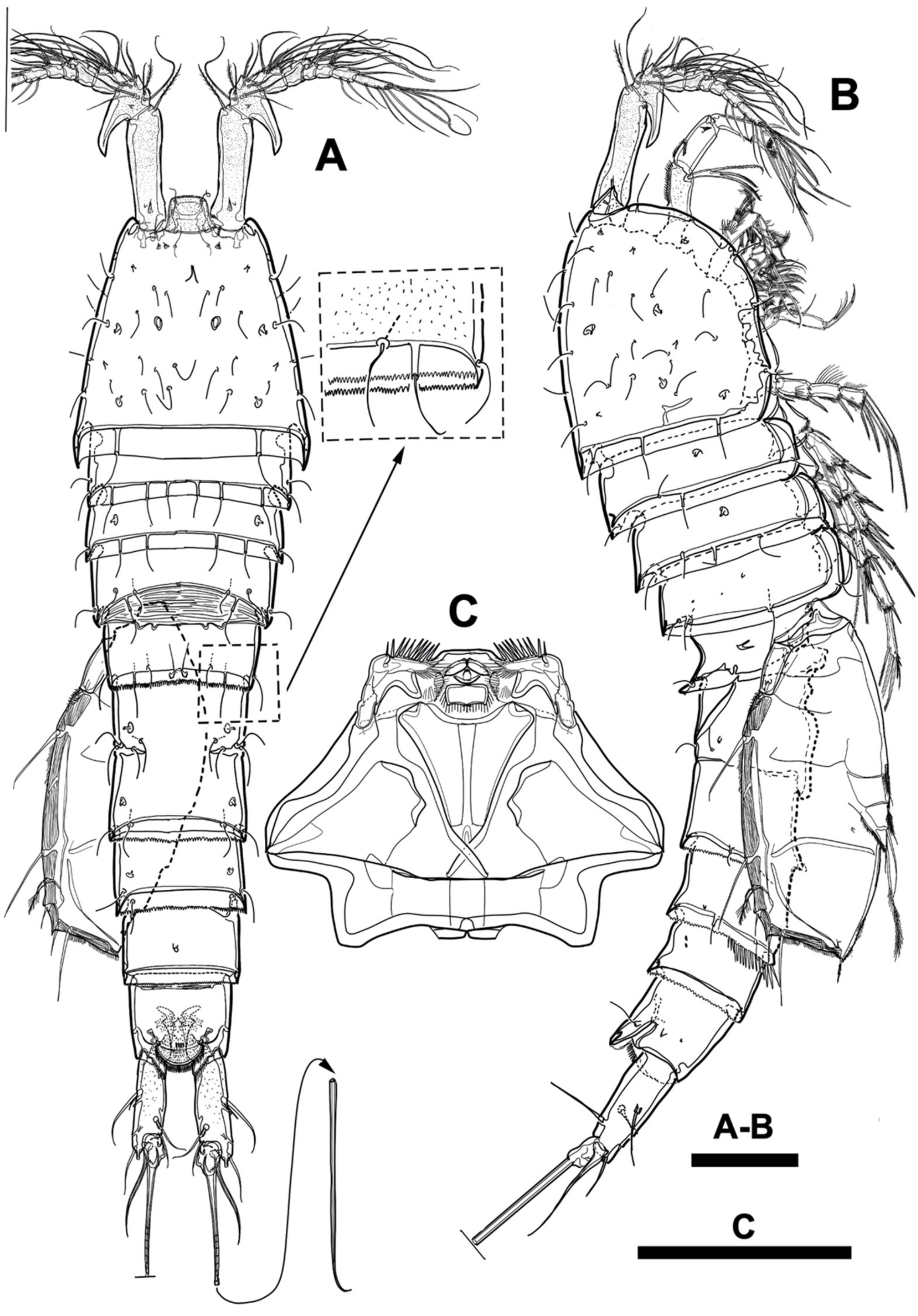

Figure 7. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) habitus, dorsal; (**B**) habitus, lateral; (**C**) rostrum, ventral. Scale bars: (**A**,**B**) 100 μm; (**C**) 50 μm.

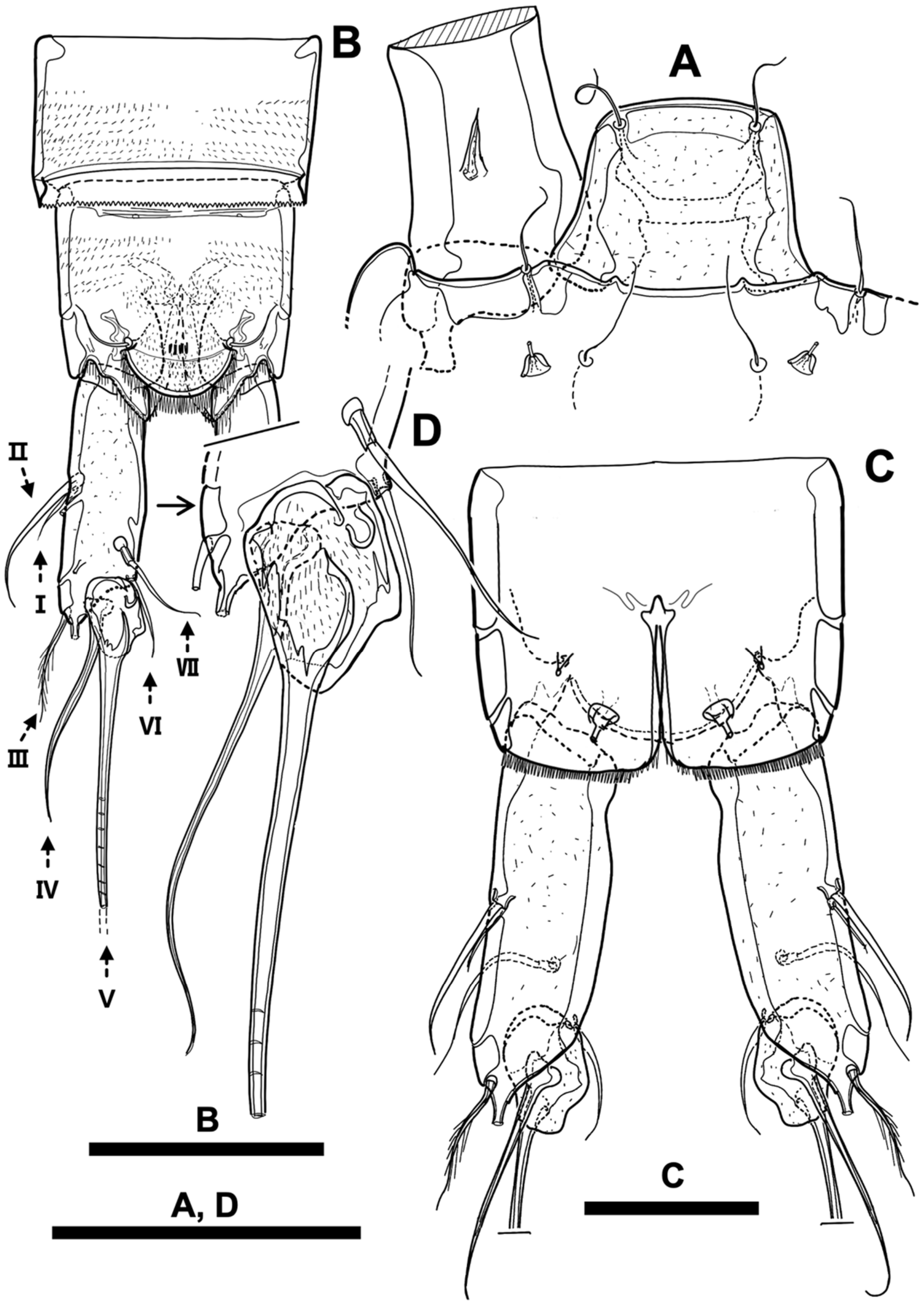

Figure 8. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) rostrum, dorsal; (**B**) fifth and caudal rami, dorsal; (**C**) fifth and caudal rami, ventral; (**D**) distal part of the caudal ramus, lateral. Scale bars: (**A**,**C**,**D**) 50 μm; (**B**) 100 μm.

Maxilliped (Figures 6F and 11D) subchelate and composed of syncoxa, basis, and endopod. Syncoxa almost 3.1 times as long as greatest width, constricted in middle of inner margin, ornamented with several rows of spinules, and with three inner setae; proximal seta, arising from distal third, robust and pinnate; other two bipinnate setae inserted in distal corner and near distal sixth of inner margin, respectively. Basis cylindrical, about equal to syncoxa in length, almost four times as long as maximum width, with pinnate seta located on inner surface medially, and ornamented with two inner rows of spinules; posterior spinules much shorter and denser than anterior row. Endopod one-segmented, almost 0.3 times longer and much narrower than preceding segment, about 3.3 times as long as wide, unornamented, and with three armatures (lateral seta arising from inner distal quarter and two apical armatures); apical spine robust, claw-like, and furnished with row of short spinules on inner end; apical seta pinnate and shorter than spine; lateral seta shortest.

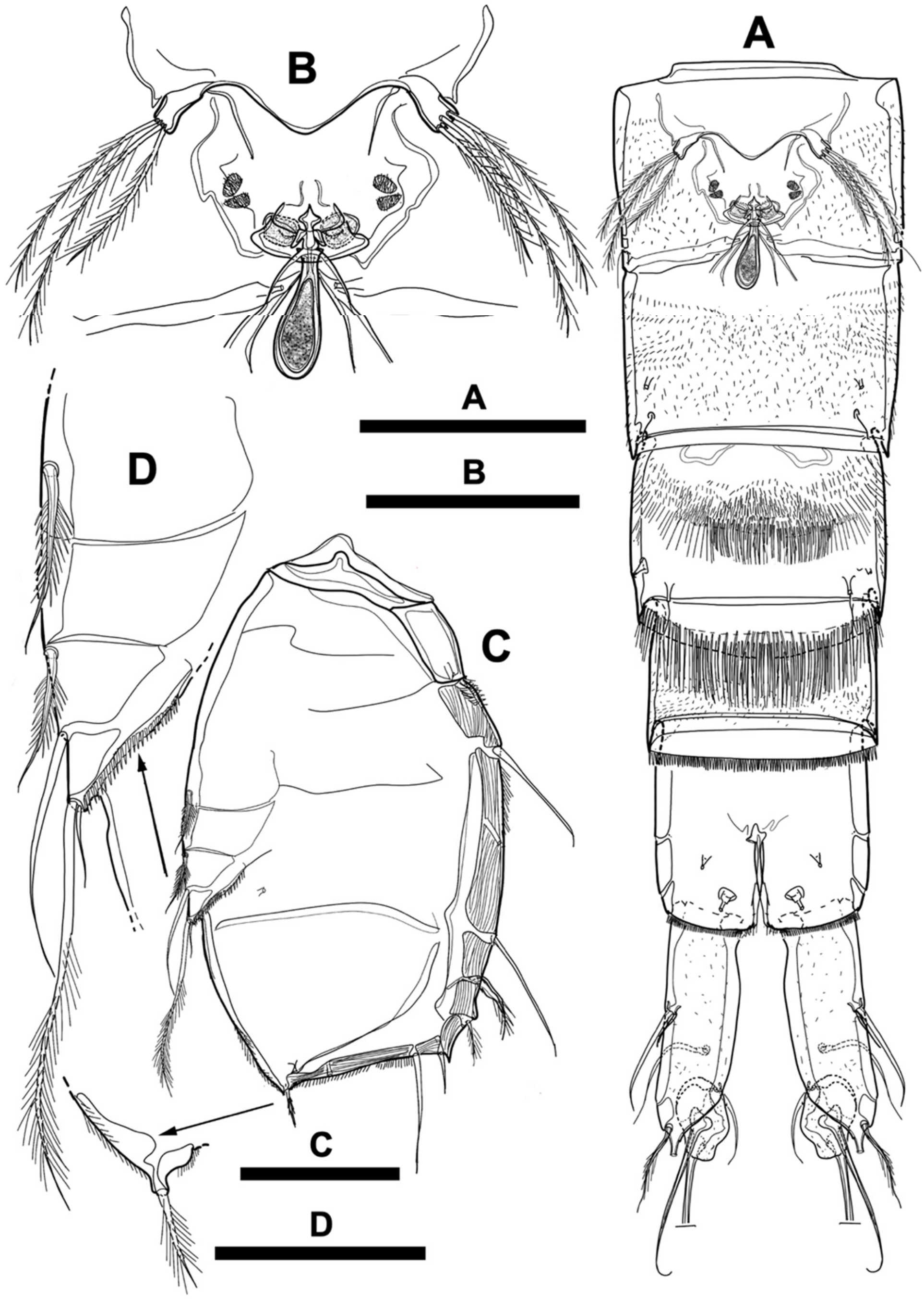

Figure 9. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) urosome excluding first urosomite, ventral; (**B**) genital field; (**C**) fifth pereiopod; (**D**) former baseoendopod of the fifth pereiopod. Scale bars: (**A**,**C**) 100 µm; (**B**,**D**) 50 µm.

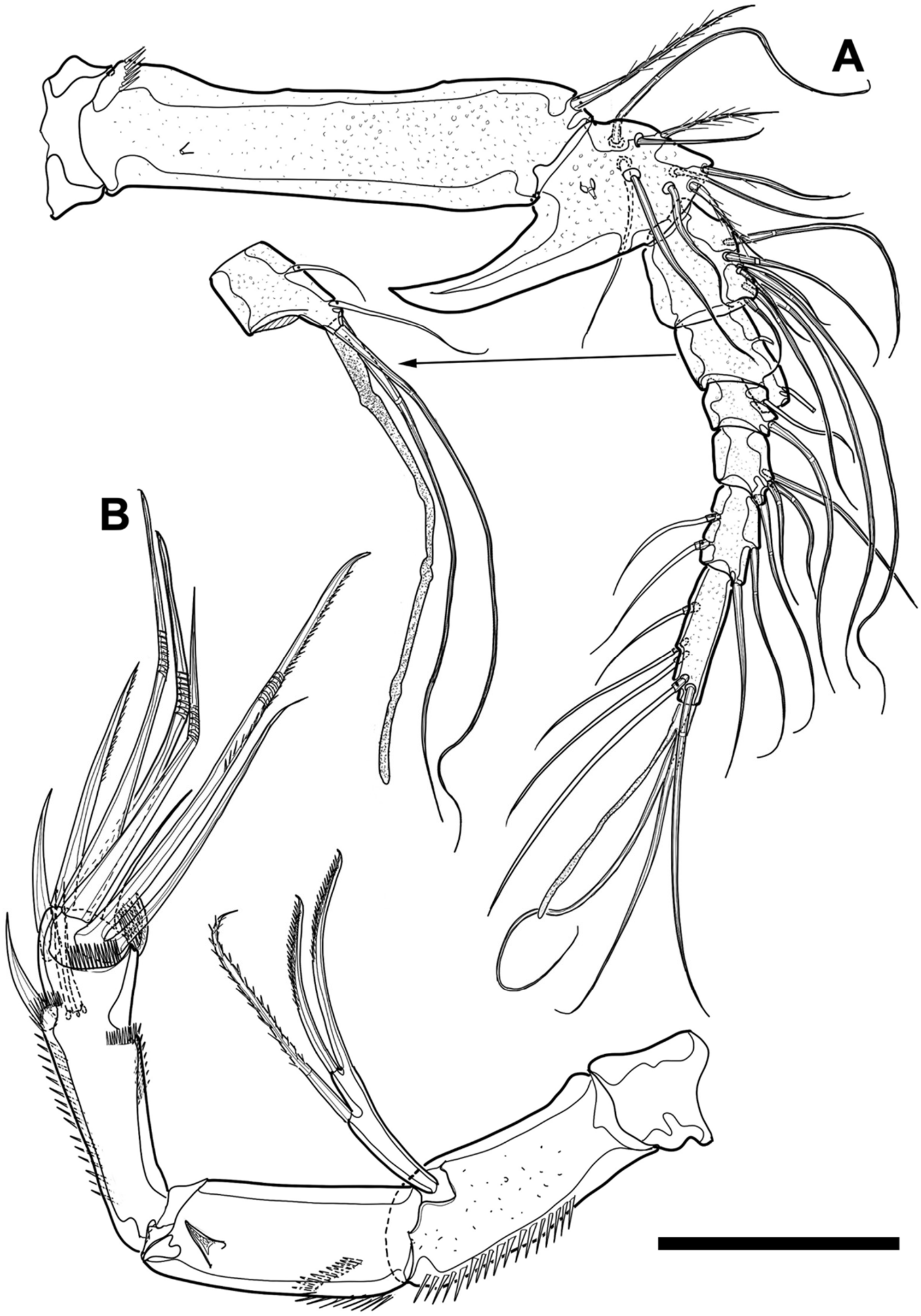

Figure 10. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) antennule, dorsal; (**B**) antenna, anterior. Scale bar: 50 µm.

First four pereiopods (P1–P4; Figures 12 and 13) biramous, composed of coxa, basis, two-segmented endopod, and three-segmented exopod. Protopod ornamented with numerous rows of various spinules on surface, but pattern of arrangement indefinable. Coxa rectangular and with small intercoxal sclerite connecting rami of each pair; cuticular pore located near outer distal corner, except for P4. Basis transformed into acute integumental structure on both anterior distal corners around endopod, apart from P1, and with cuticular pore on anterior surface near pedestal bearing outer basal seta. Exopod ornamented with rows of spinules or setules along inner and outer margins, but inner row absent on first segment; first two exopodal segments armed with bipinnate outer spine arising from distal corner; first two segments of P2–P4 decorated with frills on inner distal end and with cuticular extension transformed into thorn-like structure anteriorly, around base of outer spine, except for second segment of P4.

Figure 11. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) mandible, anterior; (**B**) maxillule, posterior; (**C**) maxilla, anterior; (**D**) maxilliped, anterior. Scale bar: 50 μm.

P1 (Figure 12A). Coxa about 0.9 times as long as greatest width and tapering distally. Basis shorter (about 0.6 times) and narrower (about 0.6 times) than coxa, and with two pedestals bearing inner and outer spine, respectively; inner pedestal ornamented with patch of long and short spinules; inner spine robust, bipinnate, and about as long as basis; outer spine shorter and finer than inner spine; distal margin bearing endopod furnished transversely with row of minute spinules. Endopod prehensile and longer than protopod (about 1.6 times) and exopod (about 1.5 times); first segment elongated, about seven times as long as wide, furnished with spinules along outer margin and setules from inner proximal quarter to inner distal quarter, armed with pinnate inner seta arising from distal quarter; inner seta about two-thirds of first segment in length; second segment much shorter (about 0.3 times) and slightly narrower than first segment, ornamented with row of spinules on outer margin, and armed with two geniculate setae arising from terminal margin; two apical setae much longer than distal endopodal segment and decorated terminally with row of minute spinules. Exopod not reaching distal end of first endopodal segment, without inner seta, and slightly getting shorter gradually (length ratio of three segments from proximal one to distal, 1:0.85:0.7); distal segment with two apical setae and two outer spines; apical setae geniculate and decorated with row of minute spinules terminally, inner of which longer than outer.

P2 (Figure 12B). Coxa about 1.4 times as broad as length. Basis ornamented with row of long setules on inner margin, shorter (about three quarters), and narrower (about 0.9 times) than coxa; basal spine bipinnate and almost as long as basis. Endopod reaching approximately midlength of distal exopodal segment, about as long as protopod, and shorter than exopod (about 0.6 times); first endopodal segment about twice as long as width, with pinnate seta inserted in inner distal third, and decorated with spinules along inner and outer margins; inner spinules longer and sparser than outer row; second segment much slenderer (about half) and longer (about 1.4 times) than preceding segment, ornamented sparsely with long spinules, and with three terminal setae of which middle seta bare and minute; other two setae pinnate, innermost of which longest and much longer than entire endopod. Exopod tapering distally; length ratio of three exopodal segments from first to third, 1:0.9:0.9; first segment widening distally and with inner robust seta; distal part of inner seta slightly curved and bipinnate terminally; second segment without inner seta; third segment with two outer spines, one apical spine, and one apical seta; apical spine longer than two outer spines; innermost seta slender and about as long as exopod.

P3 (Figure 13A). Coxa about 1.2 times as broad as length. Basis about 1.5 times as broad as length, shorter (about two-thirds), and narrower (about 0.9 times) than preceding segment; outer basal seta slender and much longer than basis. Endopod not reaching end of second exopodal segment, about 0.9 times as long as protopod, and shorter (about 0.4 times) than exopod; first endopodal segment about 1.5 times as long as greatest width, ornamented with spinules on inner and outer margins and with pinnate seta located in inner distal third; second segment slenderer (about 0.6 times) and longer (about twice) than preceding segment, furnished with spinules along inner and outer margins, and armed with three apical bipinnate armatures; innermost seta longest and much longer than entire endopod; middle seta shortest and thready; outmost armature spine-like. Exopod tapering distally; length ratio of three exopodal segments from proximal to distal, 1:1:1.1; first segment broadening distally and armed with robust seta arising from inner distal quarter; inner seta somewhat bent terminally and bipinnate; second segment without inner seta; third segment with four bipinnate armatures (two outer spines, one apical spine, and one innermost apical seta); apical spine much longer than outer spines; innermost seta about as long as entire exopod.

P4 (Figure 13B). Coxa about 0.9 times as long as width. Basis shorter (about 0.9 times) than coxa but almost equal in width; pedestal bearing outer seta smaller than two preceding pereiopods; basal seta slender and naked. Endopod about 0.6 times as long as protopod, much shorter (about 0.2 times) than entire exopod, and not reaching midlength of second exopodal segment; first endopodal segment minute, almost as long as wide, and armed with seta arising from inner margin; inner seta slightly longer than entire endopod; second endopodal segment more than five times as long as greatest width, decorated with minute spinules along inner, outer, and terminal margin, and armed with three bipinnate terminal armatures; outermost armature shortest and spine-like; innermost seta longest, longer than entire endopod, and exceeding end of second exopodal segment. Exopod elongate and tapering distally; length ratio of three exopodal segments from first to third, 1:1.3:1.4; first segment cylindrical and with strong pectinate seta originating from about inner distal third; second segment cylindrical and armed with thready inner seta implanted near distal end;

third segment with seven pinnate armatures (three inner setae, two terminal setae, and two outer spines); inner setae robust and long, middle of which longest; apical setae slender; outer spines minute.

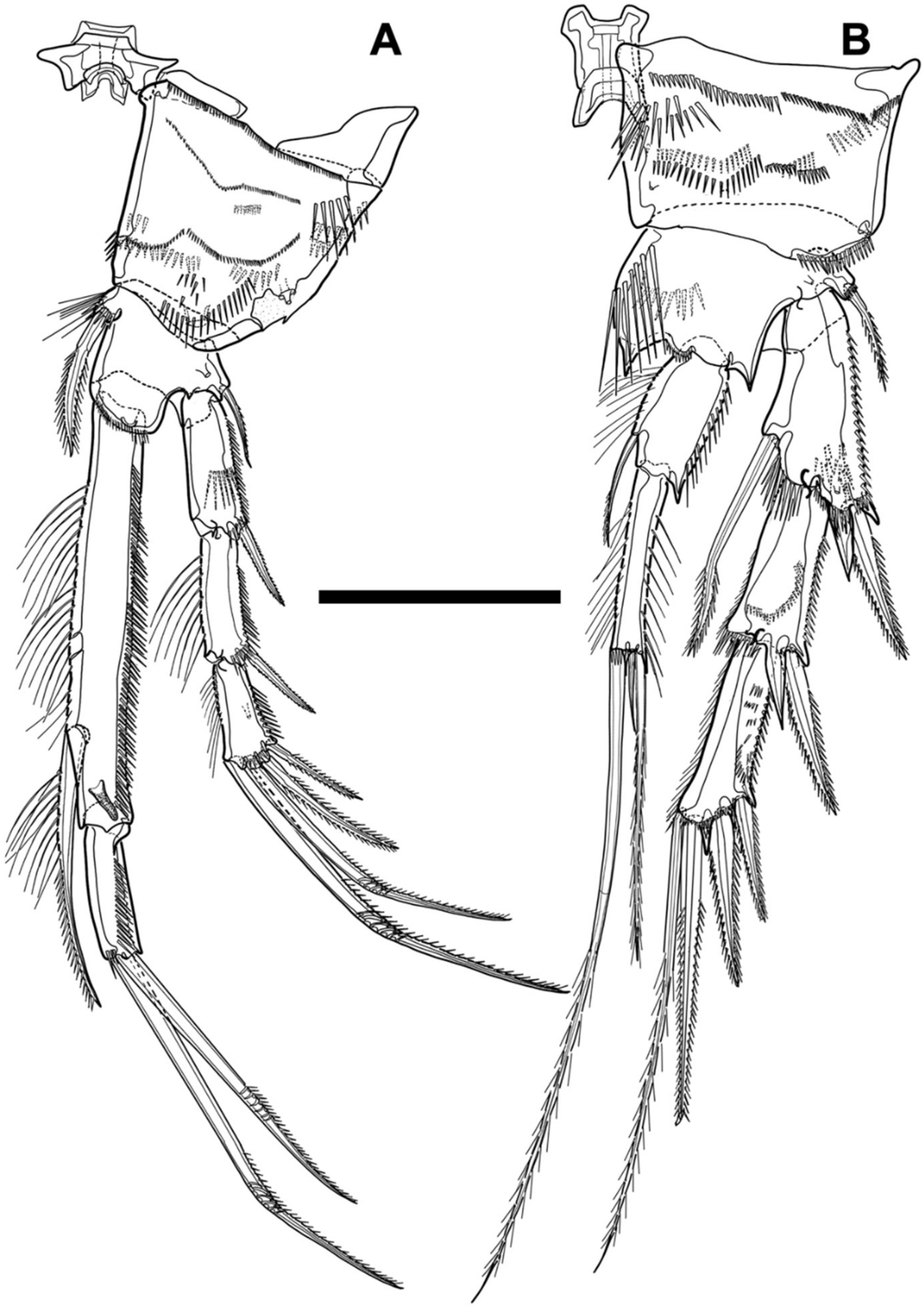

Figure 12. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) P1; (**B**) P2. Scale bar: 50 µm.

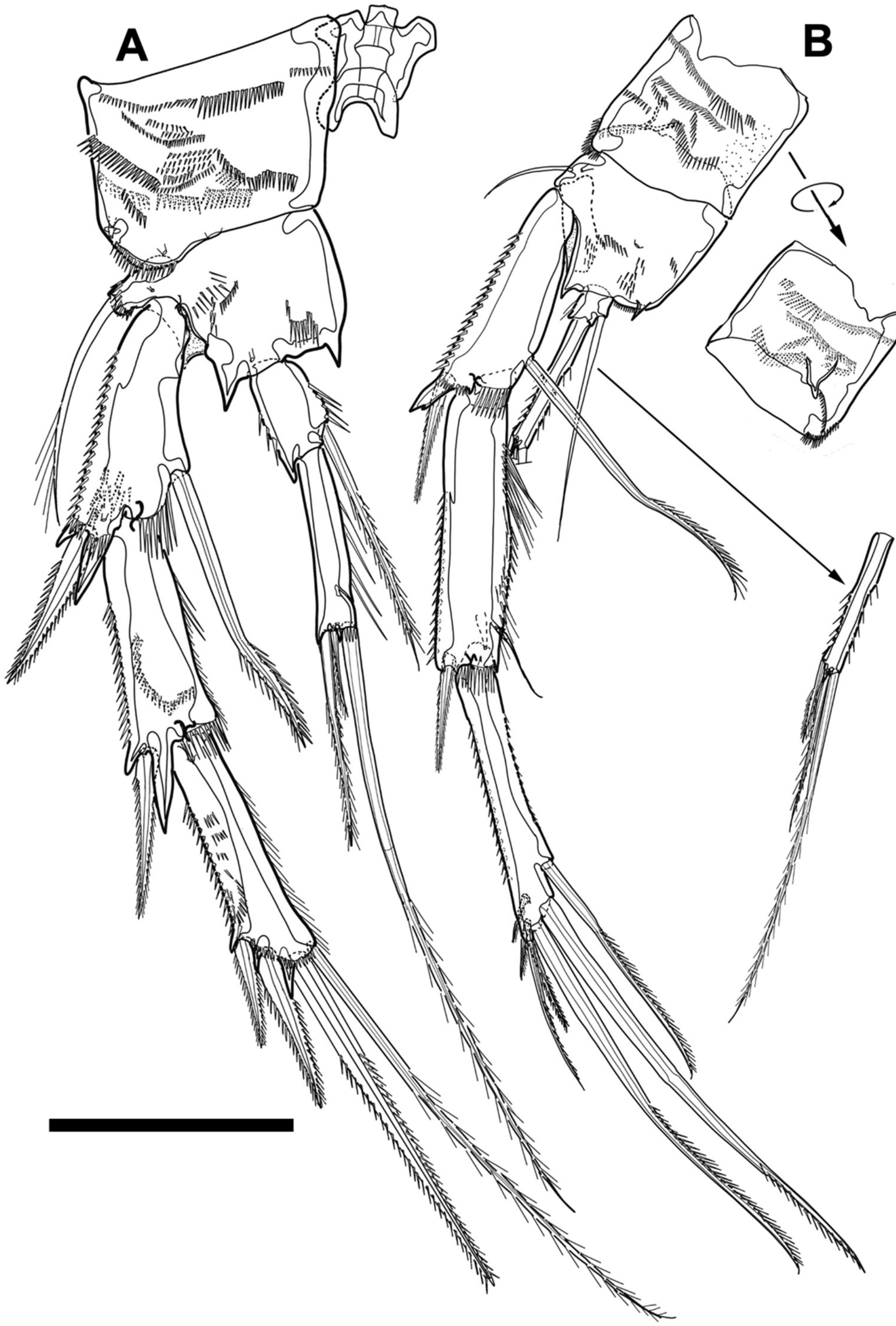

Figure 13. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** female (holotype): (**A**) P3; (**B**) P4. Scale bar: 50 μm.

The fifth pereiopod (P5, Figures 4D and 9C,D) enlarged, foliaceous, with confluence of baseoendopod and exopod, covered with microspinules and/or microsetules throughout surface, reaching end of third urosomite, formed into brood pouch, and armed with 12 setae (one basal, five baseoendopodal, and six exopodal); baseoendopodal setae located along inner edge; two proximalmost baseoendopodal setae short, bipinnate, and inserted on ventral surface; other three baseoendopodal setae located on small lobe; small baseoendopodal lobe ornamented with row of tiny spinules; two most distal baseoendopodal setae implanted very close to each other in end of lobe, of which proximal seta bipinnate, longest among baseoendopodal setae, and reaching distal end of P5; other distal seta hair-like and minute; basal seta bare and located in near proximal third of outer margin; three of exopodal setae located in distal margin; one of distal setae, arising from ventral corner, minute and bipinnate; central of distal setae much longer than other distal setae; other three exopodal setae located around distal third of dorsal edge; distalmost dorsal seta bipinnate and minute; central of dorsal setae small, bipinnate, and somewhat swollen at base; other dorsal seta about as long as central distal seta.

P6 (Figures 5A and 9A,B) P6 (Figures 5A and 9A,B) reduced and with three apical setae; outermost seta longest, reaching ventral suture of genital double-somite, and plumose.

Description of adult male (based on paratype 10–19)

Sexual dimorphism appeared in body ornamentation, segmentation of urosome, caudal rami, antennule, and P2–P6. The body ornamentations, such as hyaline frills, cuticular pores, sensilla, (micro)setules, (micro)spinules, and their arrangements are presented in Figures 2B, 14A–E, 15A–E, 16A–C, 17A and 18A.

Figure 14. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** male (paratype 11) in dorsal view: (**A**) habitus; (**B**) cephalothorax; (**C**) second prosomite to the second urosomite; (**D**) third to fifth urosomites; (**E**) ana somite and caudal rami; (**F**) antennule. Scale bars: 50 µm.

Urosome (Figures 2B, 14C–E, 15D,E, 16A–C, 17A and 18A,B) six-segmented and tapering gradually. First urosomite cylindrical and with smooth hyaline frill ventrally. Genital and first abdominal somite separated; genital somite armed with P6 and longest among urosomites; three free abdominal somites getting shorter posteriorly (length ratio of third to fifth urosomite in lateral aspect, 1:0.9:0.8) and with regularly spinulose hyaline frills ventrally. Caudal rami elongated, about 5.4 times as long as greatest width, with dimples on dorsolateral surface, covered with microspinules throughout surface, tapering from midlength to distal, and armed with seven setae (three lateral, three apical, and one dorsal); setae I and II, arising from proximal third, naked and inserted very close to each other dorsoventrally; seta I minute, hair-like, and less than one-tenth of seta II in length; seta III implanted in distal quarter and about as long as seta II; setae IV and VI slender and almost equal in length; seta V longest and robust; seta VII inserted in about distal third of dorsal surface and triarticulate.

Figure 15. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** male (paratype 12) in lateral view: (**A**) habitus; (**B**) cephalothorax; (**C**) second to fourth prosomites; (**D**) first to third urosomites, P5, and P6; (**E**) fourth urosomite to anal somite and caudal rami; (**F**) antennule. Scale bars: (**A**) 100 µm; (**B**–**D**) 30 µm; (**E**,**F**) 20 µm.

Antennule (Figures 14F, 15F, 16D and 17A) seven-segmented, subchirocer, covered with dimples dorsally, and with geniculation between fourth and fifth segments; first segment longest, with cuticular pore on dorsal surface proximally, ornamented with row of spinules near dorsal pore, and armed with pinnate seta inserted in inner distal corner; second segment with integumental process as female; third segment small; fourth segment enlarged, shorter than all followings combined, and armed with aesthetasc fused basally to seta; sixth segment shortest; fourth to sixth segments modified into pincer-like structure with several cuticular ridges; seventh segment with short aesthetasc fused with two setae at base; most of setae arising from distal segment biarticulate. Armature formula as follow: 1(1), 2(11), 3(6), 4(13+(1+ae)), 5(1), 6(1), 7(9+(2+ae)).

It was difficult to determine whether: (a) the small lobe bearing two setae (marked by an empty arrow in Figure 14F) was a part of the third or the fourth segment; (b) the flame-like structures (marked by a hatched arrow in Figure 14F) on the fourth segment and the cuticular process (marked by a checkered arrow in Figure 14F) on the fifth segment were the transformed armatures or the integumental ridges. The above descriptions and formula were written, assuming they were a part of the fourth segment and the transformed armatures, respectively.

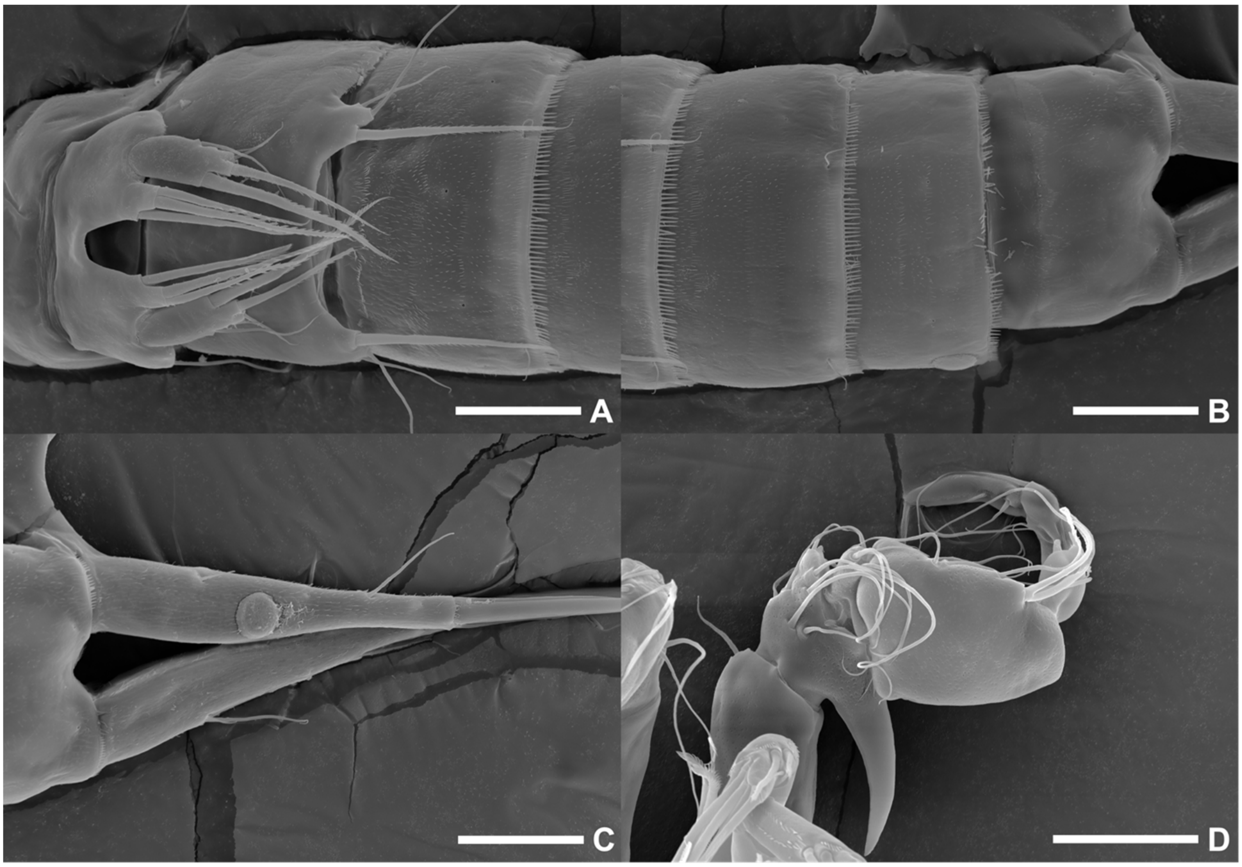

Figure 16. Scanning electron microscopy images of *Phyllopodopsyllus saywakimi* **sp. nov.** male (paratype 13) in ventral view: (**A**) second to third urosomites, P5, and P6; (**B**) fourth urosomite to anal somite; (**C**) caudal rami; (**D**) antennule. Scale bars: 30 μm.

P2 (Figure 19A) as in female excluding the following: Endopod reaching, at least, middle part of third exopodal segment; innermost seta of second endopodal segment shortest and hairy; middle seta of second endopodal segment longest.

P3 (Figure 19B) as in female excluding the following: Second endopodal segment longer (about 1.5 times) than preceding segment; outermost armature of second endopodal segment spine-like and shortest; other two setae inserted very close to each other.

P4 (Figure 19C) as in female excluding the following: Second endopodal segment about 4.4 times as long as greatest width, about 3.5 times longer than preceding segment, and armed with two pinnate spine-like armatures, of which outer armature much shorter. Distal segment of exopod with two inner setae, one apical seta, one apical spine, and two outer spines; two inner setae robust, pinnate, and much longer than other armatures.

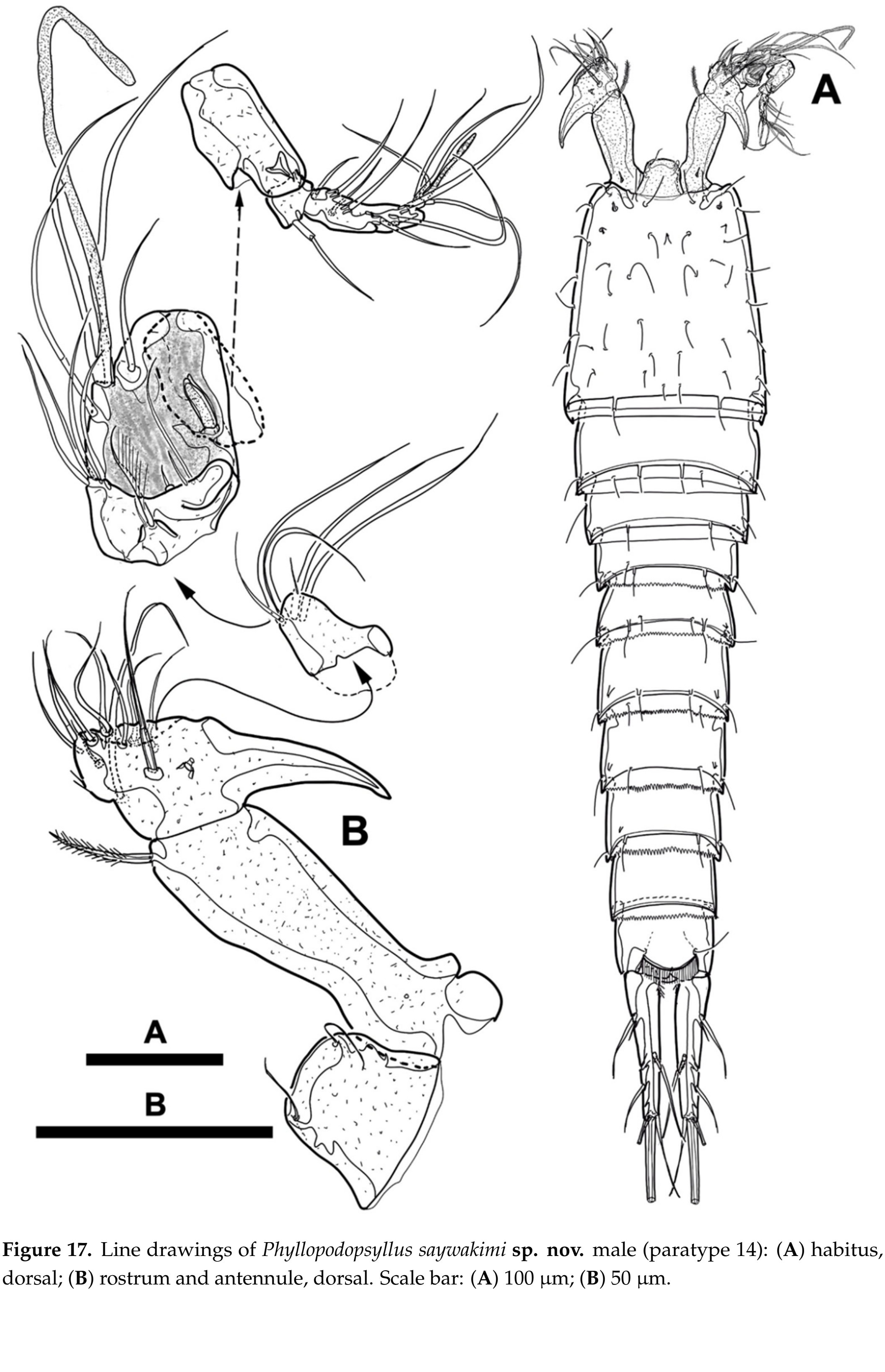

Figure 17. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** male (paratype 14): (**A**) habitus, dorsal; (**B**) rostrum and antennule, dorsal. Scale bar: (**A**) 100 µm; (**B**) 50 µm.

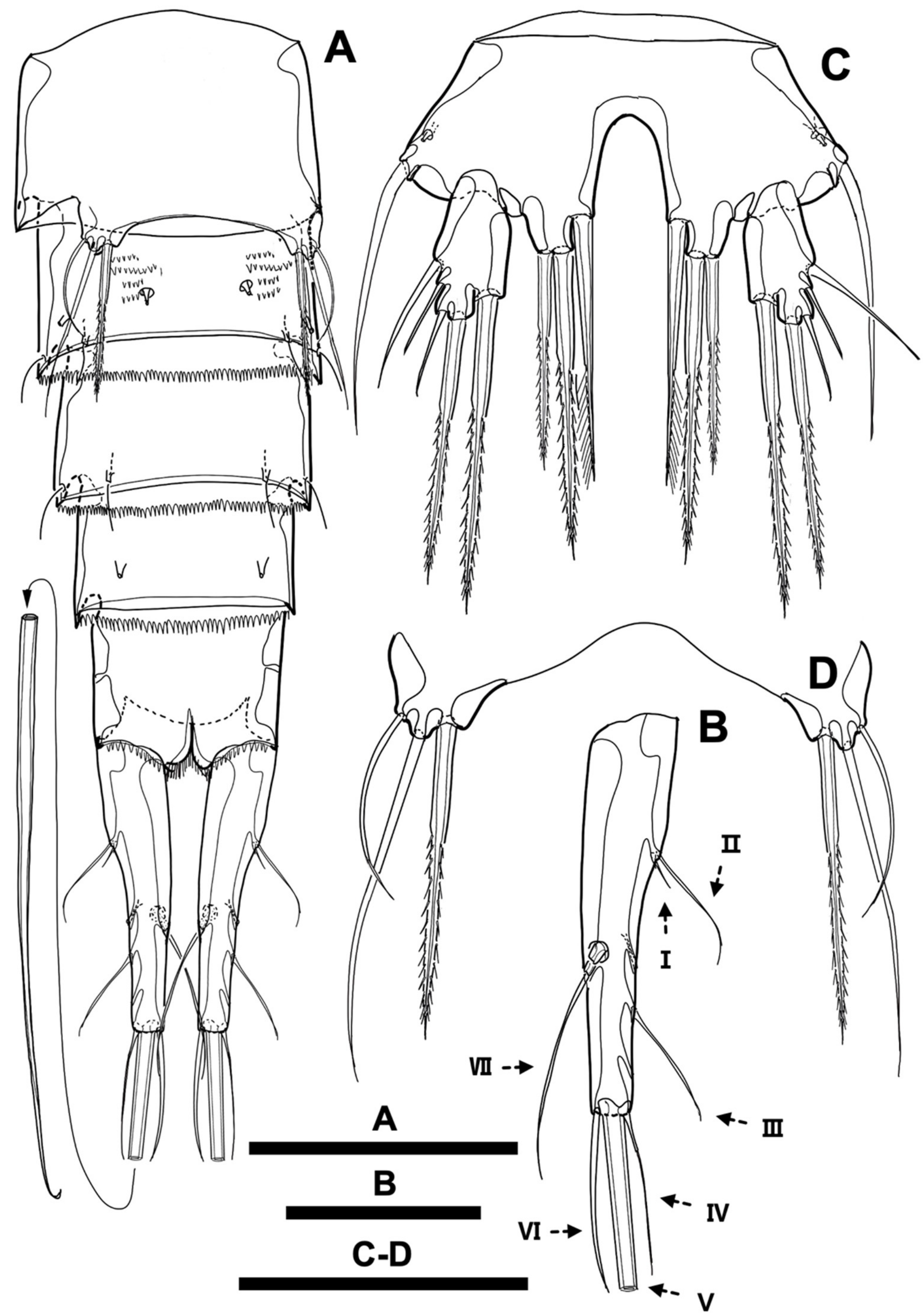

Figure 18. Line drawings of *Phyllopodopsyllus saywakimi* sp. nov. male (paratype 14): (**A**) urosome (excluding first urosomite); (**B**) caudal ramus; (**C**) P5; (**D**) P6. Scale bars: (**A**) 100 µm; (**B**–**D**) 50 µm.

P5 (Figure 18C) not foliaceous and composed of baseoendopod and exopod; both rami fused at central part completely. Baseoendopod large, with cuticular pore near base of basal seta, and armed with three pinnate setae on endopodal lobe; basal seta as long as P5 and slender; median seta longest among endopodal armatures; innermost seta bipinnate terminally. Exopod about twice as long as greatest width, shorter than baseoendopod, and armed with five setae; two innermost setae bipinnate; other setae slender and bare.

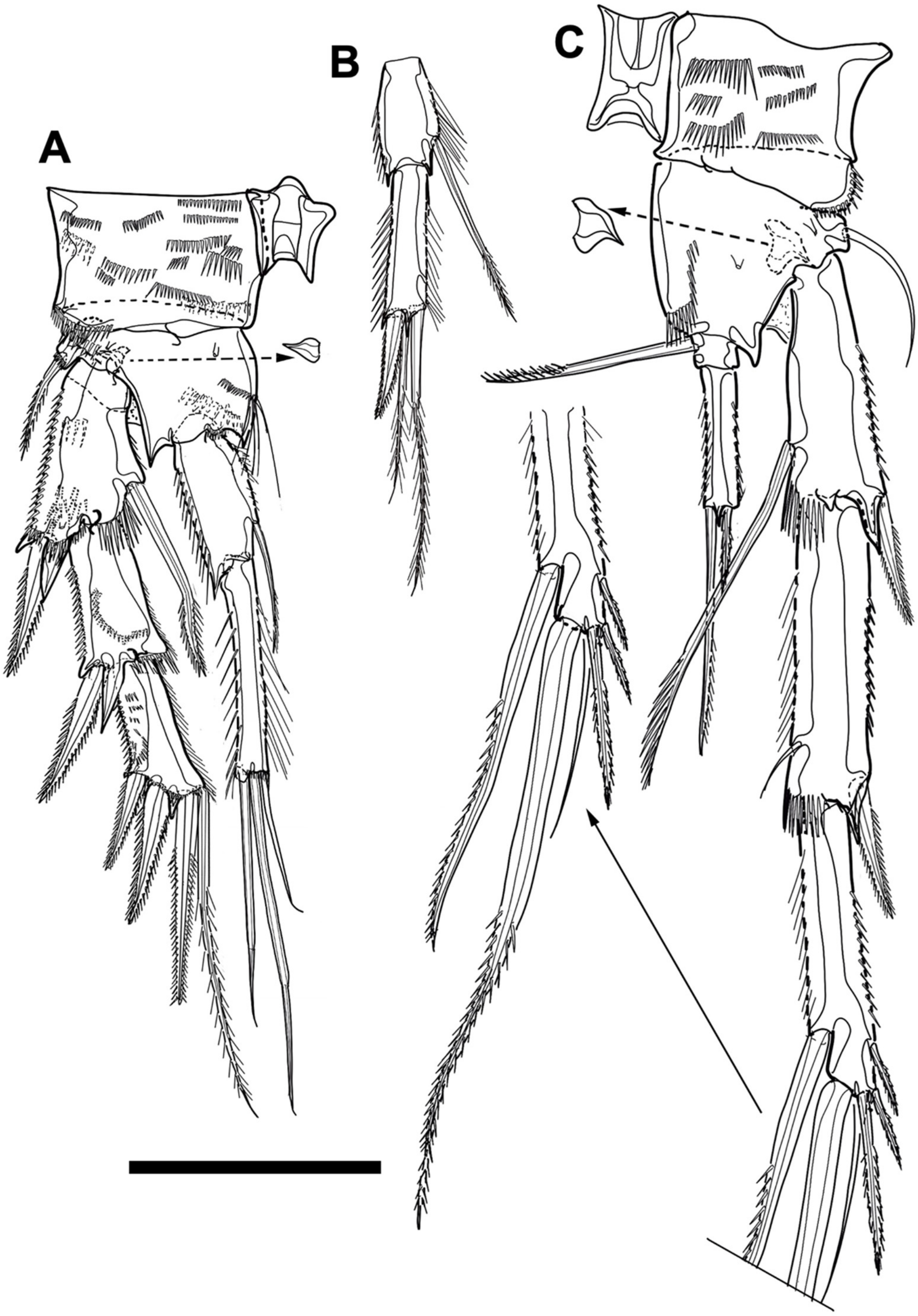

Figure 19. Line drawings of *Phyllopodopsyllus saywakimi* **sp. nov.** male (paratype 14): (**A**) P2; (**B**) endopod of P3; (**C**) P4. Scale bar: 50 µm.

P6 (Figure 18A,D) reduced into single plate, fused at base with second urosomite completely, and armed with three setae; outermost seta slender, shortest, and bare; meddle seta slender and naked; innermost seta longest, bipinnate, and robust.

Variability

The body length ranged from about 807.6–856.1 µm (mean = 829.3 µm, *n* = 3) in females (Figure S1) and about 554.6–640.8 µm (mean = 589.5 µm, *n* = 5) in males (Figure S2).

3.3. *Phyllopodopsyllus similis* Kim & Lee, 2023 *sp. nov.*

Figures 20–37 and S5–S12.

Zoobank registration

urn:lsid:zoobank.org:act:C0F18E60-A5B5-4672-BA24-0E85FF001B61

Type locality

Intertidal sand of the rocky shore, Udo, Republic of Korea, 23 July 2020. (L3: 33°31′14.53″ N 126°57′29.64″ E)

Other localities

The sandy habitat around the tidal pool near Daepyeong Pier in Jeju, Korea, 26 July 2020 (L1: 33°14′11.54″ N 126°21′35.56″ E); the intertidal sand near Jeju Ocean and Fisheries Research Institute in Jeju, Republic of Korea, 24 July 2020 (L2: 33°18′34.782″ N 126°49′53.328″ E); the intertidal sand of the rocky shore, Busan, Republic of Korea, 25 August 2022 (L4: 35°10′17.61″ N 129°11′52.68″ E).

Specimens examined

Holotype: 1 female (MABIKCR00252866)

Paratype: 20 females and 14 males (NIBRIV0000901840–NIBRIV0000901873). The voucher specimen information is given in detail in Table S1.

Etymology

The specific name *similis* is derived from the Latin adjective *similis*, meaning "similar". It alludes to the similarity between the new species and the widely distributed species, *Phyllopodopsyllus aegypticus* Nicholls, 1944.

Description of adult female (based on Holotype and Paratype 1–20)

Body (Figures 20A, 21A–D, 22A–E, 23, 24A, 25, 26A,B and S5–S7) nine-segmented, cylindrical, slightly constricted in middle, furnished with sensilla, cuticular pores, (micro)spinules, and/or (micro)setules (details in figures), and without dimples on all body surface except for anal somite.

Prosome (Figures 20A, 21A,B, 22A–C, 24A, 25, S5A,B,D, S6A–C, S8A, S9A, and S10A) four-segmented, slightly tapering distally, and composed of cephalothorax and three free pedigerous somites. Cephalothorax somewhat longer than all succeeding prosomites combined. Three free pedigerous somites nearly equal in length. Hyalin frills of prosomites smooth except for fourth somite; dorsal frills of last prosomite serrated but inconspicuous. Rostrum small, weakly defined at base, triangular in dorsal aspect, and with two sensilla near apex. Eye not visible.

Urosome (Figures 20A, 21A,C,D, 22A,C–E, 23, 25, 26A–C, S5A,C, S6A,D, S7, and S8E,F) five-segmented, about as long as prosome (including caudal rami), and composed of fifth pedigerous somite, genital double-somite, two abdominal somites, and anal somite armed with caudal rami. Fifth pedigerous somite shortest and wrinkled on ventral surface; hyaline frill of fifth pedigerous somite finely serrated dorsally and smooth laterally. Genital double-somite longest among urosomites, armed with P6, and with vestigial original segmentation marked by suture and pattern of surface ornamentation; hyaline frill of genital double-somite finely fringed dorsally but smooth laterally and ventrally; lateral frill ornamented with spinules regularly; genital field with single copulatory pore near ventral suture, short copulatory duct in distal part of genital somite, and two seminal receptacles. Antepenultimate somite slightly longer than first urosomite; hyaline frill of antepenultimate somite finely fringed dorsally; ventral part of frill smooth and ornamented with long spinules regularly on terminal margin. Penultimate somite about as long as first urosomite and finely fringed dorsally; ventral part of frill smooth and ornamented with regular spinules terminally. Anal somite about as long as antepenultimate somite, dimpled, cleft medially in posterior part; anal opening located in cleft; anal operculum dimpled, rounded, and ornamented densely with spinules on terminal margin. Caudal rami subconical, about 1.8 times as long as greatest width, dimpled laterally, swelled in proximal part of inner margin dorsally, furnished with one bunch of long setules near inner bulge, and armed with seven naked setae on each ramus, (three outer, one dorsal, and three terminal); setae I and II set very close to each other dorsoventrally at proximal third of outer margin; seta I tiny, thready, and about one-fourth as long as seta II; seta III inserted near outer distal quarter; seta V about as long as urosomite; setae IV and VI slender and equal in length; seta VII triarticulate and implanted in inner distal corner.

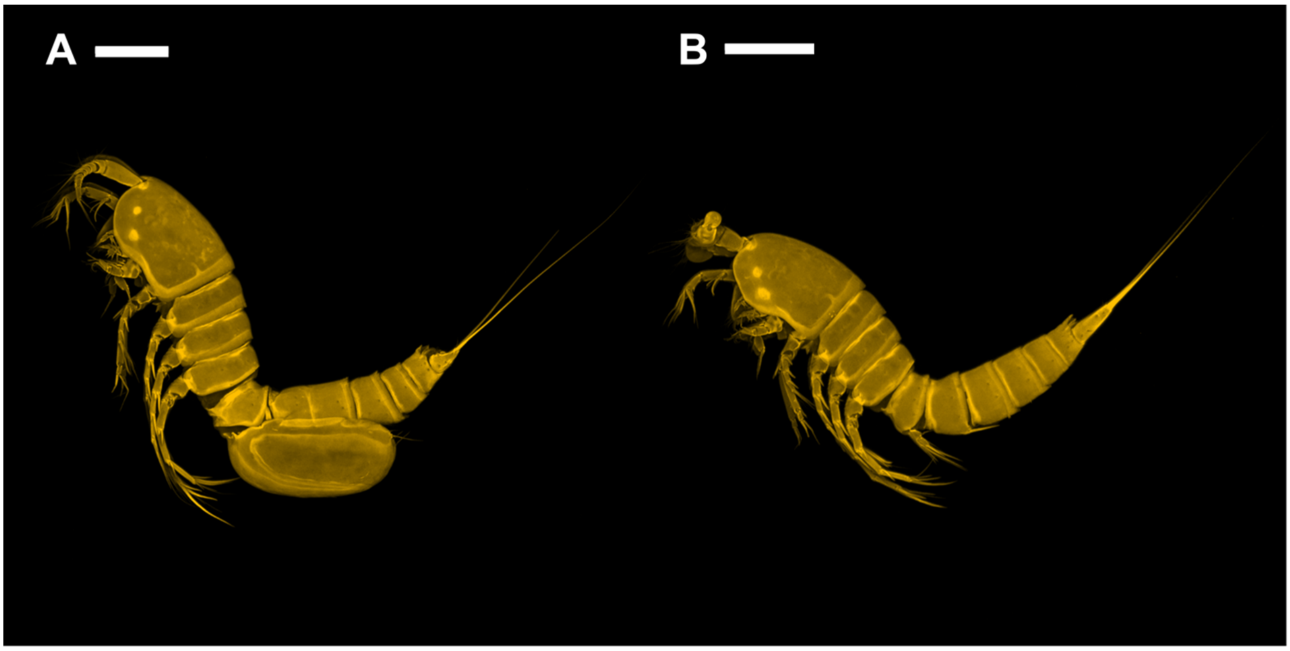

Figure 20. Confocal laser scanning microscopy images of *Phyllopodopsyllus similis* **sp. nov.** (**A**) female (paratype 1): habitus, lateral; (**B**) male (paratype 21): habitus, lateral. Scale bars: 100 µm.

Antennule (Figures 21E, 22F, 24B, 27A, S5D, S6B, S8B, S9B, S10B, and S11A) nine-segmented and covered with dimples; first segment elongated, longest, about three times longer than maximum width, about 0.6 times as long as all succeeding segments combined, ornamented with row of tiny spinules on proximal inner margin, and with pinnate seta on inner distal corner; second segment without protuberance and with cuticular pore on dorsal surface medially; fourth segment with pedestal armed with aesthetasc fused basally to long seta on inner distal corner; eighth segment shortest; ninth segment about 3.5 times as long as wide and with apical aesthetasc fused basally to two setae. Armature formula as follows: 1(1), 2(9), 3(8), 4(3+(ae+1)), 5(2), 6(4), 7(2), 8(2), 9(5+(ae+2)); all of setae naked and slender, except for first two segments; all outer setae arising from antepenultimate to distal segments biarticulate.

Antenna (Figures 22F, 24B, 27B, S8B, S9B, S10B, and S11A) composed of coxa, basis, endopod, and exopod. Coxa bare and about as long as greatest width. Basis unarmed, ornamented with longitudinal row of spinules along abexopodal margin, and about 2.5 times as long as wide. Endopod two-segmented; first endopodal segment bare, about as long as basis, about 2.7 times long as greatest width; second endopodal segment about as long as preceding segment, about 2.7 times as long as greatest width, decorated with patch of spinules on inner margin, row of strong spinules terminally, and two rows of frills on outer margin and armed with nine armatures (six apical and three lateral); one bare, one pinnate, and four geniculate setae located apically; three lateral armatures composed of two robust inner spines and one slender seta; one of inner spines located near distal corner and other spine inserted in distal third; lateral seta implanted between outer spines. Exopod one-segmented and with three pinnate setae (two apical and one lateral); outer apical seta completely fused basally to exopod.

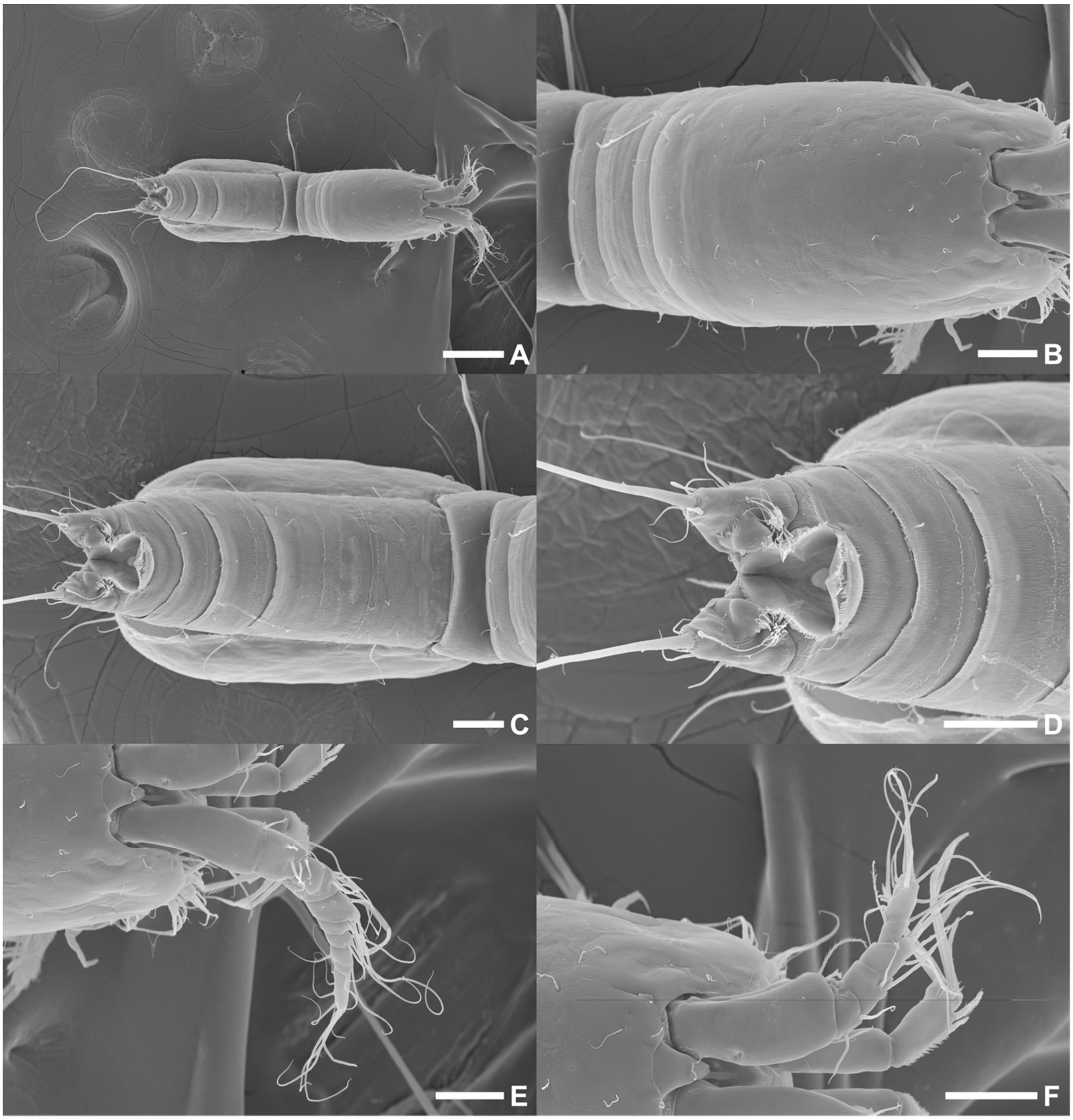

Figure 21. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 2) in dorsal view: (**A**) habitus; (**B**) prosome; (**C**) urosome; (**D**) anal somite and caudal rami; (**E**) antennule; (**F**) atypical antennule. Scale bars: (**A**) 100 µm; (**B**–**F**) 30 µm.

Labrum (Figures 24C, S8C, S9C, S10C, and S11B) well-developed, gradually tapering ventrally, decorated with patch of long setules along posterior margin distally, and covered with long and slender setules throughout cutting edge; anterior and lateral surfaces dimpled

Mandible (Figures 24C, 28A, S10C, and S11B) composed of coxa, basis, one-segmented endopod, and one-segmented exopod. Coxa enlarged and with gnathobase bearing pinnate dorsal seta and row of multicuspidate teeth. Basis gradually widening distally, pentagonal, shorter than coxa, furnished with longitudinal rows of long spinules on anterior surface, and armed with three pinnate setae originating from terminal margin. Endopod much shorter than basis and armed with two lateral setae and six apical bare setae. Exopod slightly narrower than endopod, about as long as endopod, and with five setae (three lateral and two apical); proximal-most of lateral setae plumose and long; middle seta naked, short, and thready; distal-most lateral seta naked; two apical setae plumose and long.

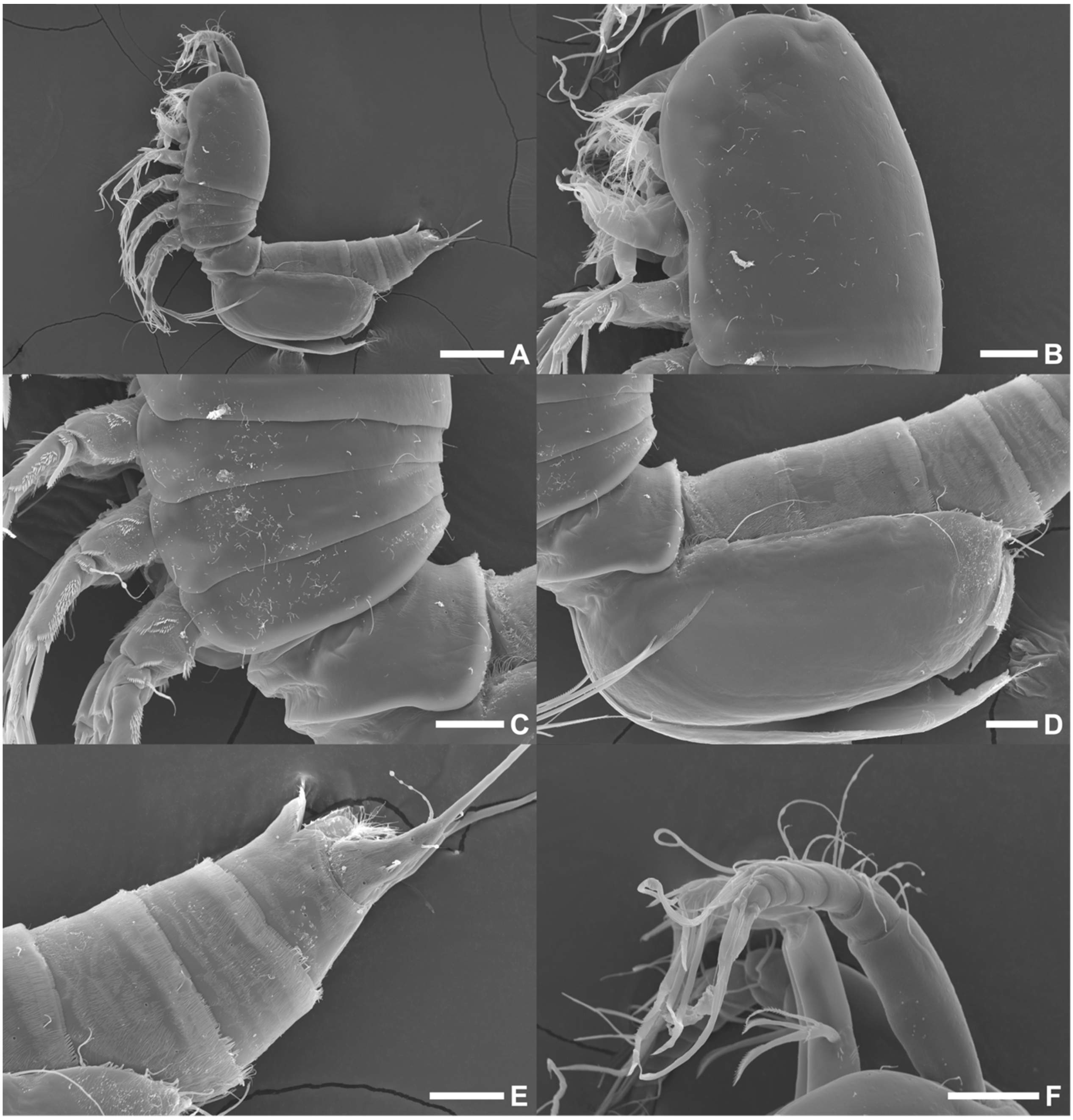

Figure 22. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 3) in lateral view: (**A**) habitus; (**B**) cephalothorax; (**C**) second prosomite to first urosomite; (**D**) P5 and genital double-somite; (**E**) third urosomite to caudal rami; (**F**) antennule and antenna. Scale bars: (**A**) 100 μm; (**B**–**F**) 30 μm.

Paragnaths (Figures 24C, S8C, S9C, and S10C) well-developed and composed of posterior and two lateral lobes; cutting edge of each lobe wrinkled; lateral lobes ornamented with several rows of long spinules on each inner side; posterior lobe decorated with fine setules around median wrinkles.

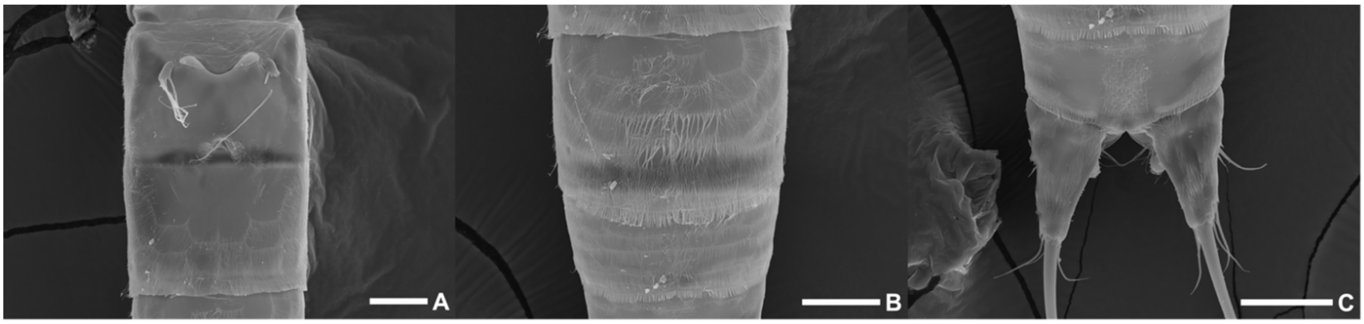

Figure 23. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 4) in ventral view: (**A**) genital double-somite; (**B**) third and fourth urosomites; (**C**) anal somite and caudal rami. Scale bars: 30 µm.

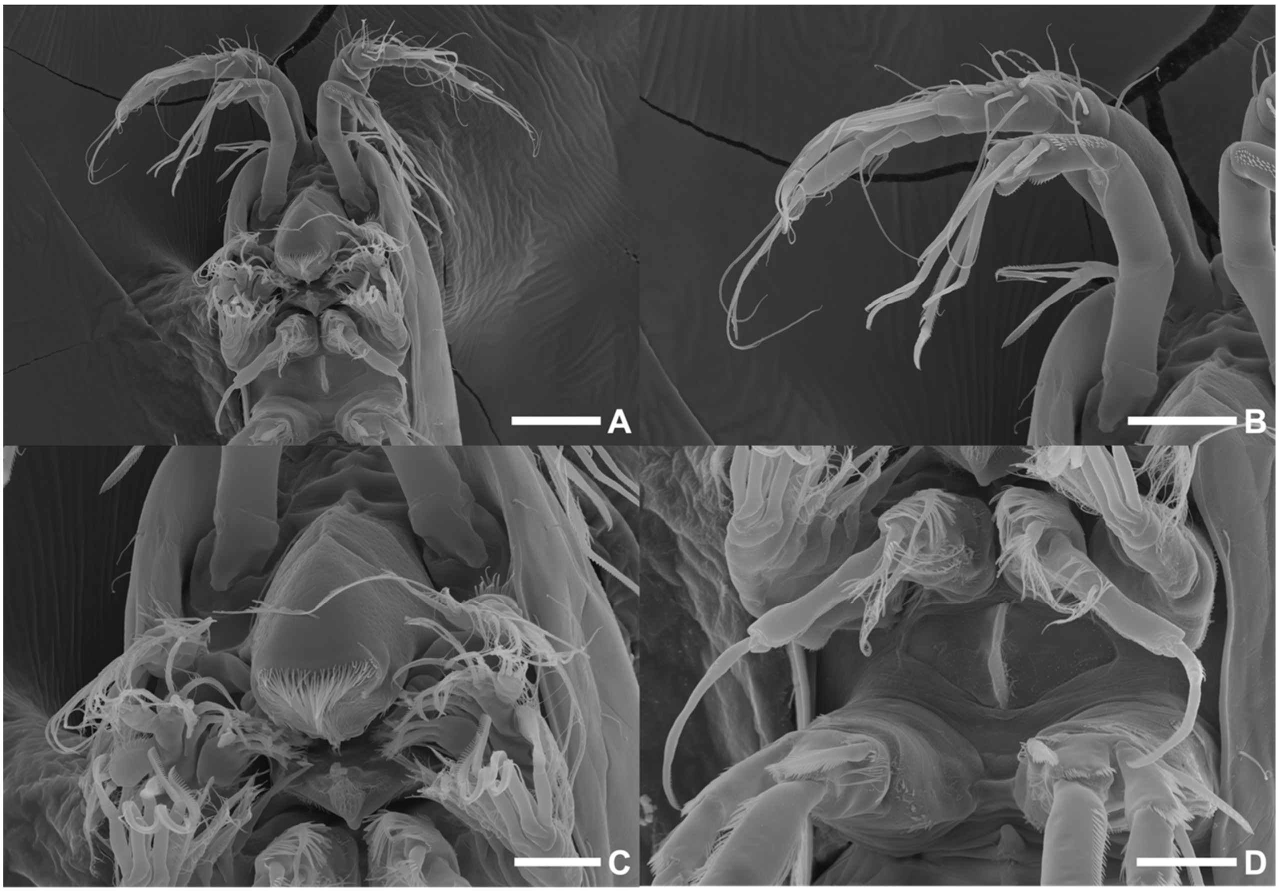

Figure 24. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 4) in ventral view: (**A**) cephalothorax; (**B**) antennule and antenna; (**C**) mouth parts around oral opening; (**D**) maxilliped. Scale bars: (**A**) 50 µm; (**B**) 30 µm; (**C**,**D**) 20 µm.

Maxillule (Figures 24C, 28B, S8C, S9C, S10C and S11B) composed of praecoxa, coxa, basis, endopod, and exopod. Praecoxa large, trapezoidal, ornamented with row of spinules on distal half of outer margin, and armed with well-developed arthrite; praecoxal arthrite armed with 13 elements and ornamented with row of spinules; two setae located in near posterior margin; one apical pinnate seta inserted in distal corner; two bare setae implanted in anterior surface; eight robust armatures arranged around distal margin. Coxa about half as long as praecoxa, with cylindrical endite incorporated into coxa, and armed with epipodite represented by one plumose seta; coxal endite with four setae. Basis unornamented and armed with six setae apically. Endopod about twice as long as greatest width, and with four plumose setae; three endopodal setae originating around terminal margin, and one seta inserted in inner margin subapically. Exopod broader than endopod, about as long as greatest width, ornamented with setules along inner margin, and with three plumose setae located apically.

Maxilla (Figures 24C, 28C, S8C, S9C and S11B) composed of syncoxa, basis, and endopod. Syncoxa large and armed with four endites; endites with two, one, three, and three pinnate and/or plumose setae from proximal to distal, respectively; proximal second endite shortest; other endites almost equal in length; Basis approximately half as long as syncoxa and armed with cylindrical endite bearing one slender plumose seta arising from posterior surface distally and two robust setae apically; Endopod two-segmented and much shorter than basis; first endopodal segment about 0.2 times as long as greatest width and armed with three setae (two robust pinnate setae and one slender bare seta); second endopodal segment about as long as preceding segment and armed with four setae apically (two slender naked setae and two pinnate robust setae).

Maxiliped (Figures 24D, 28D, S8D, S9D, S10D and S11B) subchelate and composed of syncoxa, basis, and endopod. Syncoxa about twice as long as greatest width, constricted in middle of inner margin, ornamented with several rows of spinules and setules, and with three inner setae; proximal-most seta, arising from distal third, robust and pinnate; central inner seta plumose; distal-most seta pinnate and located near distal corner. Basis cylindrical, almost as long as preceding segment, about 2.8 times as long as greatest width, armed with plumose seta inserted in inner margin medially, and ornamented with two rows of spinules along inner margin; posterior spinules much shorter and denser than anterior row. Endopod one-segmented, much narrower and shorter (about 0.3 times) than preceding segment, about 2.7 times as long as maximum width, unornamented, with claw-like spine originating from terminal margin; terminal spine robust and pinnate on inner end.

P1–P4 (Figures 29 and 30) biramous, composed of coxa, basis, two-segmented endopod, and three-segmented exopod. Protopod ornamented with several rows of spinules on surface but arrangement pattern indefinable. Coxa rectangular and with small intercoxal sclerite connecting rami of each pair; cuticular pore located near outer distal corner except for P4. Basis transformed into acute integumental structure on both anterior distal corners around endopod apart from P1 and with cuticular pore on anterior surface near pedestal bearing outer basal armature. Exopod ornamented with row of spinules or setules along outer margin of each segment; first two exopodal segments armed with bipinnate outer spine originating from distal corner; first two segments of P2–P4 decorated with frills on inner distal end and with cuticular extension transformed into thorn-like structure, anteriorly, near base of outer spine.

P1 (Figure 29A). Coxa about 0.6 times as long as greatest width and somewhat tapering distally. Basis shorter (about two-thirds) and narrower (about 0.6 times) than coxa, and with two bipinnate spines originating from inner and outer margins, respectively; base of inner spine ornamented with patch of long and short spinules; inner spine robust and longer than basis; distal margin bearing endopod furnished with row of spinules, transversely. Endopod longer than protopod (about 1.7 times); first segment elongated, about six times as long as greatest width, furnished with spinules and setules along outer and inner margins, respectively, and armed with pinnate inner seta arising from distal third; inner seta about half of first segment in length; second segment much shorter (about 0.2 times) and slightly narrower than preceding segment, ornamented with row of spinules on anterior surface, and with two geniculate setae apically; two apical setae much longer than distal endopodal segment, and pinnate terminally. Exopod reaching about midlength of first endopodal segment (about 0.7 times) and without inner seta; length ratio of three exopodal segments from proximal to distal, 1:0.8:0.8; distal segment with two apical setae and two outer spines; apical setae geniculate and pinnate terminally; inner apical seta longer than outer.

Figure 25. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) habitus; dorsal; (**B**) habitus, lateral. Scale bar: 100 µm.

P2 (Figure 29B). Coxa almost as long as wide. Basis ornamented with row of long setules along inner margin, shorter (about half), and narrower (about 0.9 times) than coxa; basal spine bipinnate and longer than basis. Endopod not reaching end of second exopodal segment, about 0.7 times as long as protopod, and shorter than exopod (about 0.4 times); first endopodal segment unarmed, about 1.3 times as long as greatest width, and decorated with spinules along inner and outer margins; inner spinules longer and sparser than outer spinules; second segment narrower (about 0.7 times) than preceding segment but almost equal in length, ornamented with long spinules, and with three terminal armatures;

innermost seta longest; outermost armature spine-like and inserted deeper than other two setae. Exopod tapering distally; length ratio of three exopodal segments from first to third, 1:1:1.2; first segment widening distally and armed with inner bipinnate seta; second segment unarmed; third segment with five pinnate armatures (two outer spines, one apical spine, one apical seta, and one inner seta); apical spine much longer than outer spines.

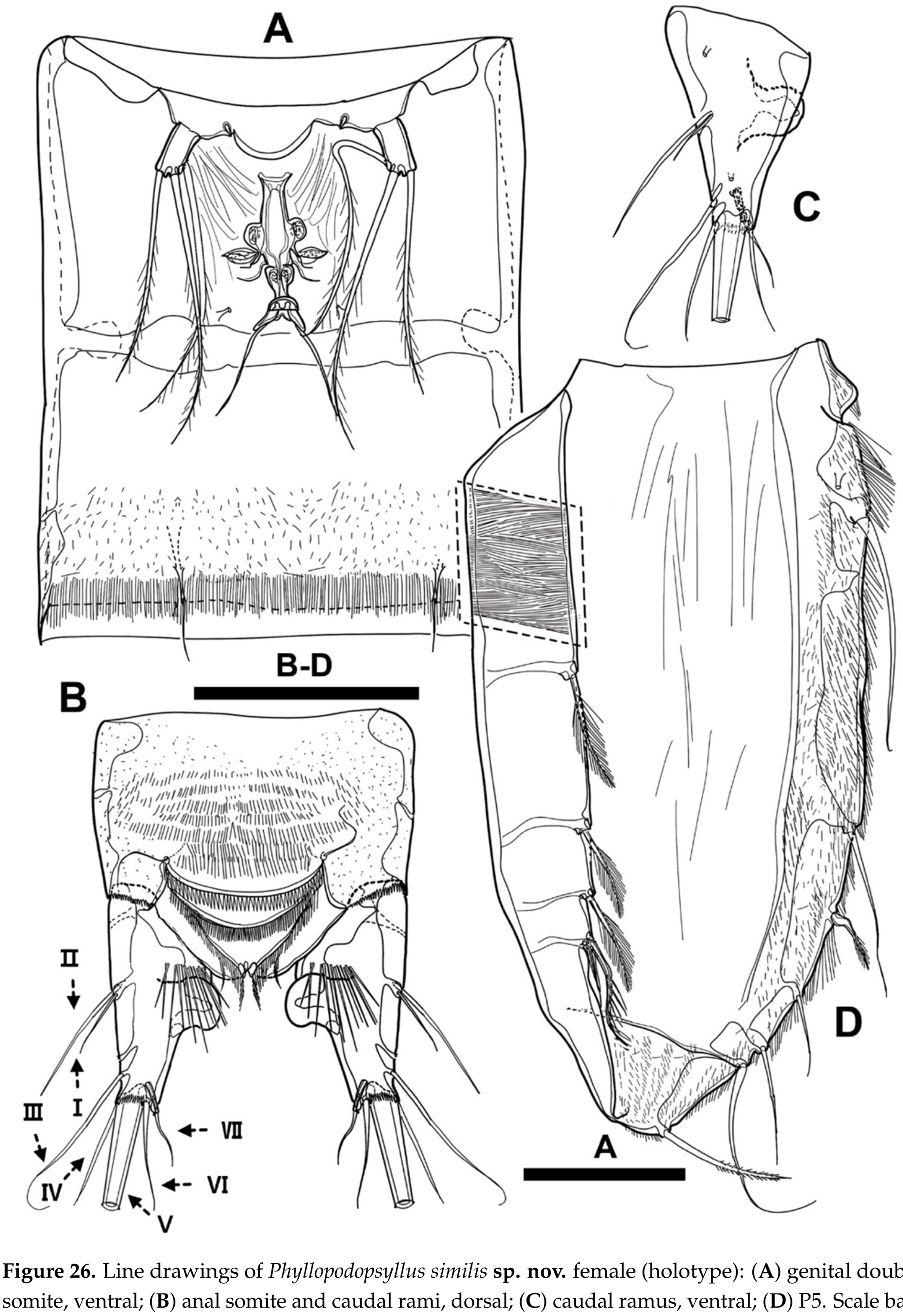

Figure 26. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) genital double-somite, ventral; (**B**) anal somite and caudal rami, dorsal; (**C**) caudal ramus, ventral; (**D**) P5. Scale bars: 50 μm.

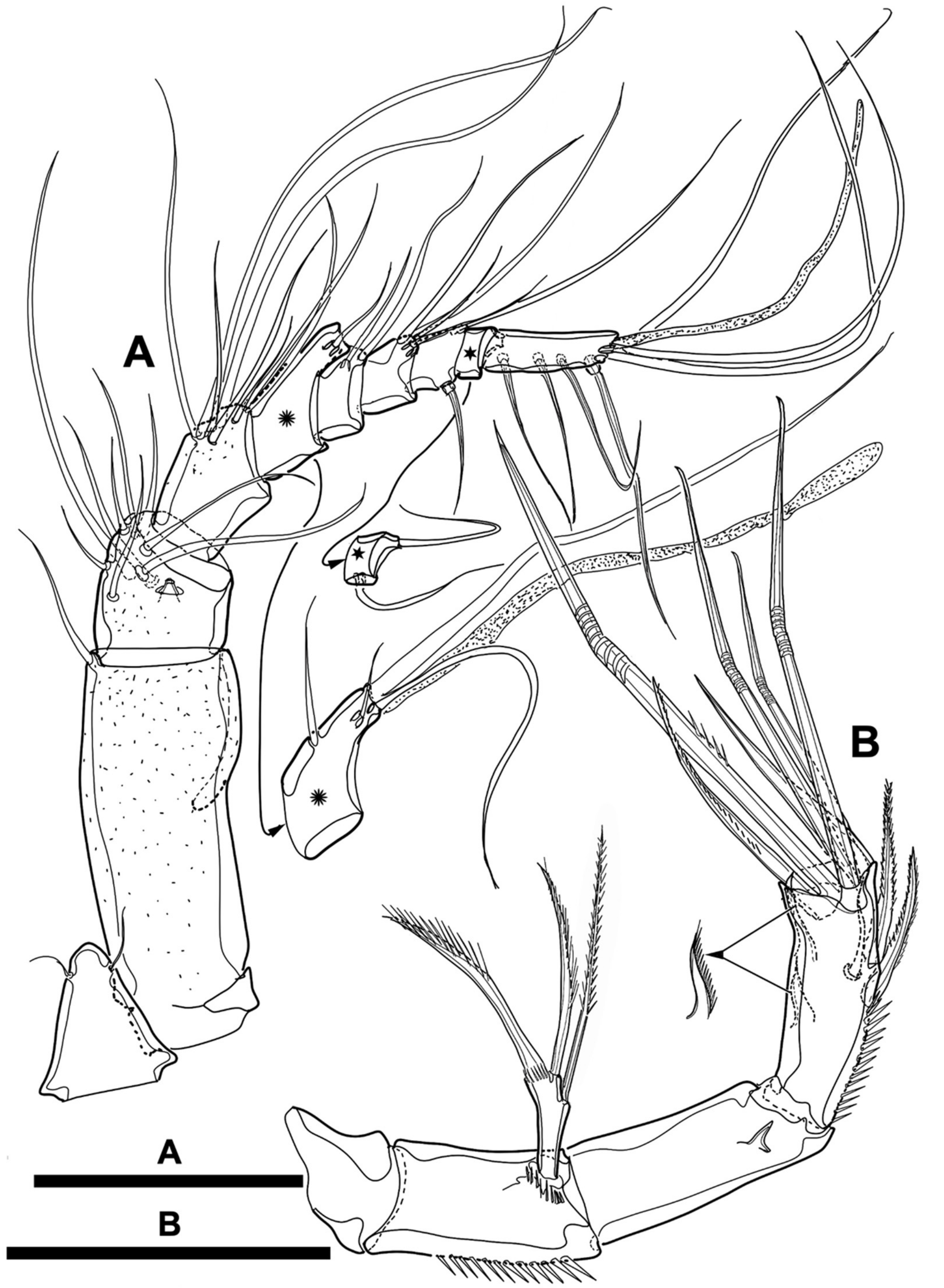

Figure 27. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) rostrum and antennule, dorsal; (**B**) antenna, anterior. Scale bars: 50 µm.

P3 (Figure 30A). Coxa almost as long as greatest width. Basis about half as long as wide, shorter (about 0.5 times) and narrower (about 0.9 times) than coxa; outer basal seta slender, naked, and much longer than basis. Endopod reaching midlength of second exopodal segment, about 0.7 times as long as protopod, and shorter than exopod (about 0.4 times); first endopodal segment unarmed, about 1.4 times as long as greatest width, ornamented with spinules on inner and outer margins; second segment slenderer (about 0.7 times) than first segment but almost equal in length and armed with three bipinnate armatures terminally; innermost seta longest and much longer than entire endopod; outmost armature spine-like and inserted deeper than other apical setae. Exopod tapering distally; length ratio of three exopodal segments from proximal to distal, 1:1:1.3; first segment broadening distally and armed with robust seta arising from inner distal corner; inner seta somewhat bent terminally and bipinnate; second segment unarmed; third segment with six pinnate armatures (two outer spines, two apical setae, and two inner setae).

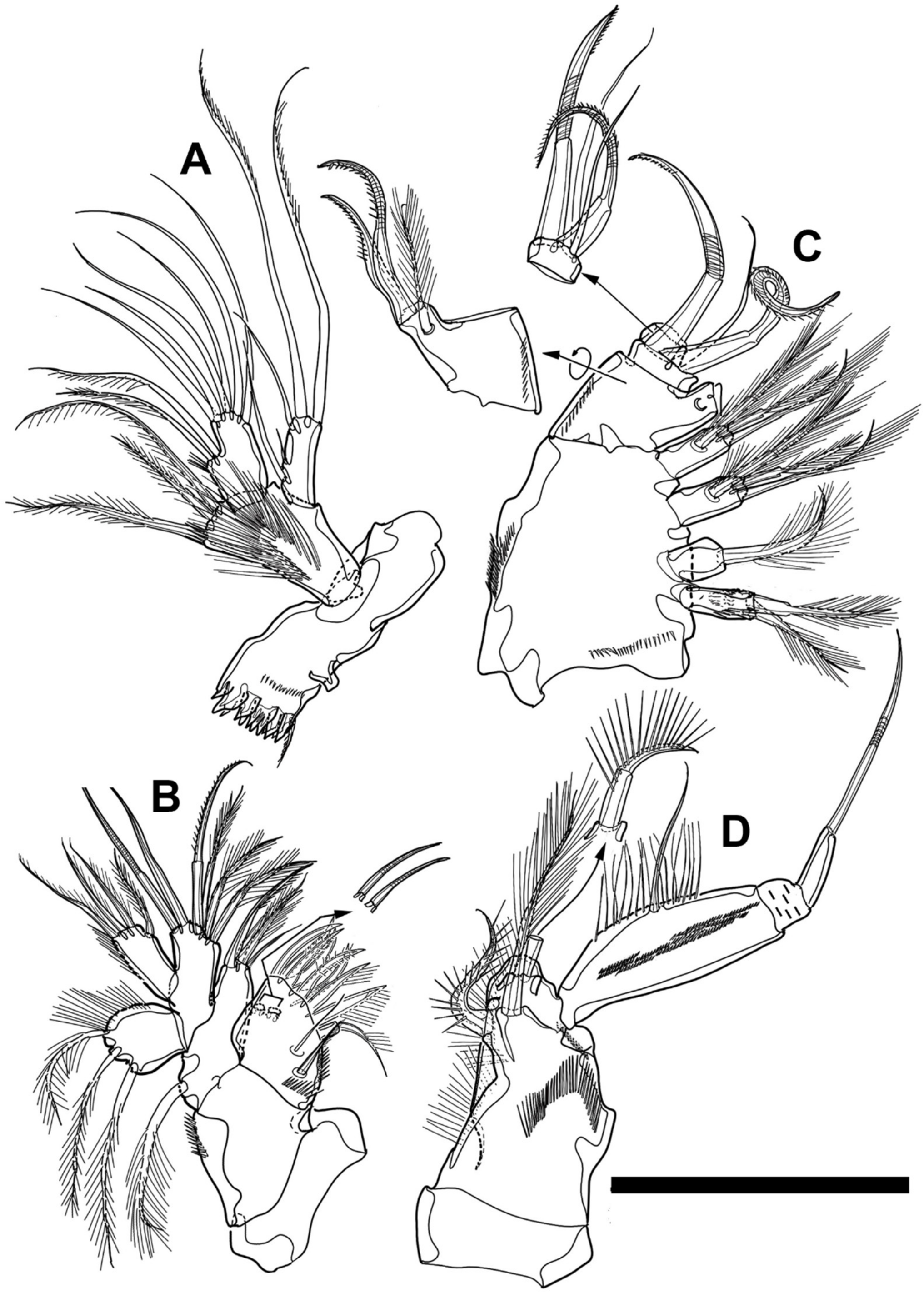

Figure 28. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) mandible; (**B**) maxillule; (**C**) maxilla; (**D**) maxilliped. Scale bar: 50 μm.

Figure 29. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) P1; (**B**) P2. Scale bars: 50 μm.

P4 (Figure 30B). Coxa about 1.1 times as broad as length. Basis shorter (about 0.8 times) than coxa but almost equal in maximum width; pedestal bearing outer basal seta smaller than ped-estals of two preceding pereiopods; basal seta slender and naked. Endopod about half as long as protopod, much shorter than exopod (about 0.2 times), and not reaching midlength of second exopodal segment; first endopodal segment minute, about 0.9 times as long as wide, and armed with inner pinnate seta; inner seta of first endopodal segment much longer than entire endopod; second endopodal segment about 2.4 times as long as greatest width and armed with three bipinnate terminal armatures; outermost spine-like and inserted deeper than other terminal setae; innermost seta longest, much longer than entire endopod, and exceeding end of second exopodal segment. Exopod elongated and tapering distally; length ratio of three exopodal segments from first to third segment, 1:1.4:1.4; first segment cylindrical and with pinnate seta originating from inner distal corner; second segment cylindrical and armed with long inner seta implanted near distal end; third segment with seven armatures (three inner setae, two terminal setae, and two outer spines); inner setae robust and pinnate terminally; middle one of inner setae longest; outer spines minute.

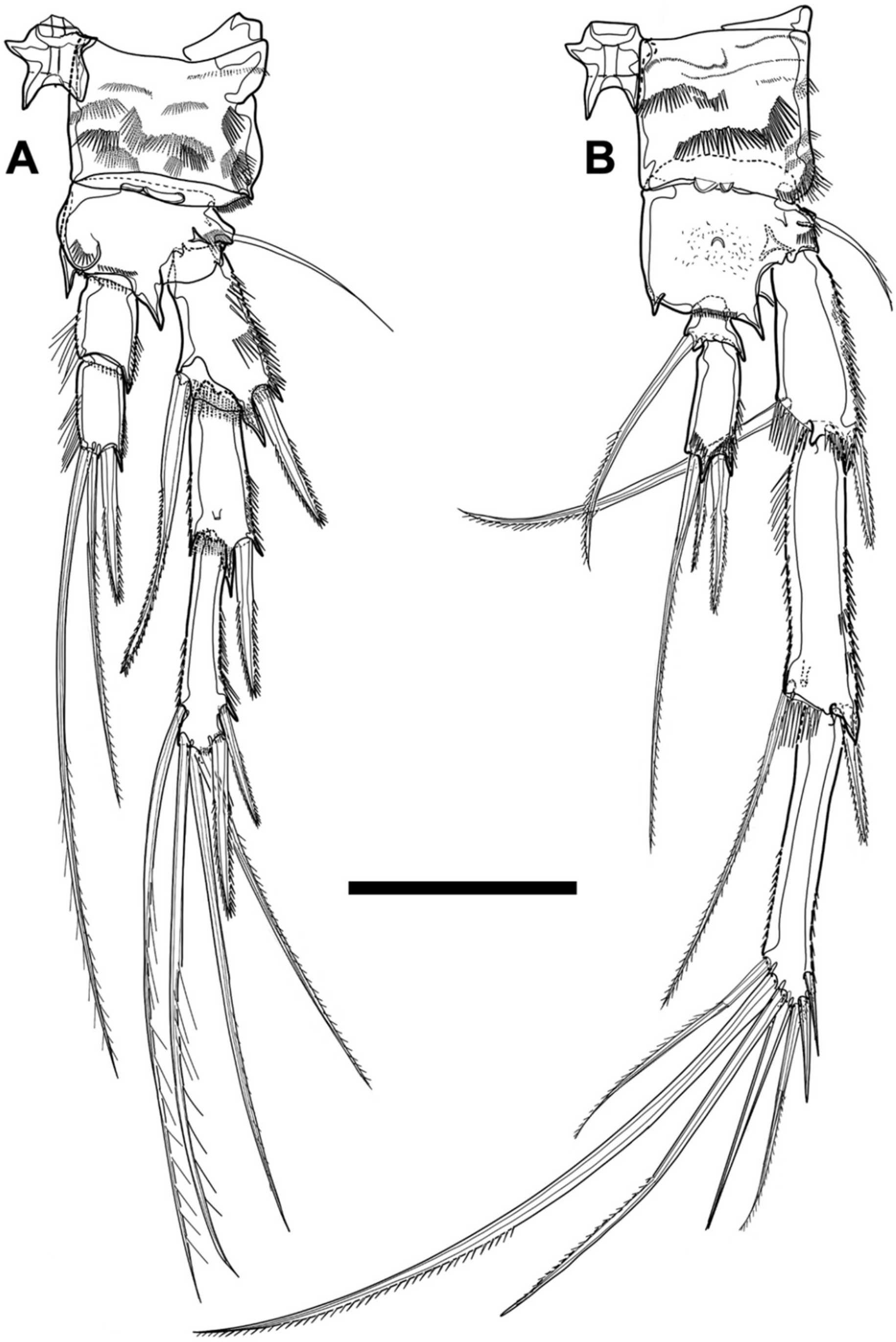

Figure 30. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** female (holotype): (**A**) P3; (**B**) P4. Scale bars: 50 μm.

P5 (Figures 22D and 26D) enlarged, foliaceous, formed into brood pouch, with confluence of baseoendopod and exopod, not reaching end of third urosomite, ornamented with microspinules and/or microsetules on posterior surface and dorsal margin, and armed with

12 setae (one basal, five baseoendopodal, six exopodal); baseoendopodal setae located along inner edge; two proximal-most baseoendopodal setae bipinnate; median seta plumose; two distal-most setae implanted very close to each other; distal-most baseoendopodal seta hair-like and minute; basal seta bare and inserted in proximal part of dorsal edge; exopodal setae located around from distal third of dorsal edge to distal corner; distal-most exopodal seta pinnate; second proximal seta pinnate, short, and some what swollen at base; other exopodal setae bare; second distal seta longest among exopodal setae and exceeding end of antepenultimate urosomite.

P6 (Figures 26A, S7A, and S8E) reduced, longer than maximum width, and armed with two apical setae and one inner seta; all setae plumose and almost equal in length.

Description of adult male (based on paratype 21–34)

Sexual dimorphism appeared in the body ornamentation, segmentation of urosome, caudal rami, antennule, and P2–P6. The body ornamentations, such as hyaline frills, cuticular pores, sensilla, (micro)setules, and (micro)spinules, and their arrangements, were presented in Figures 20B, 31A–C, 32A–E, 33A–C, 34, 35 and S12A.

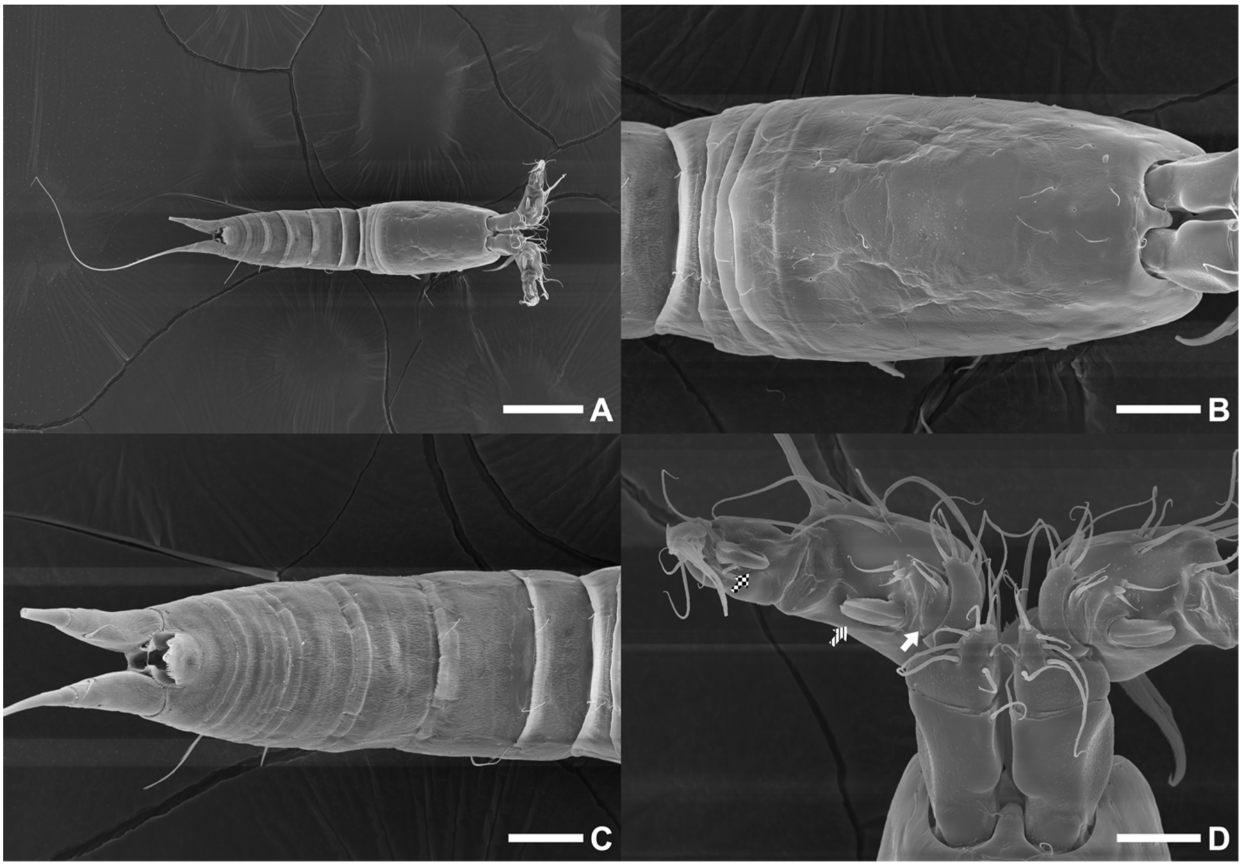

Figure 31. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 22) in dorsal view: (**A**) habitus; (**B**) prosome; (**C**) urosome; (**D**) antennule. Scale bars: (**A**) 100 µm; (**B**,**C**) 30 µm; (**D**) 20 µm.

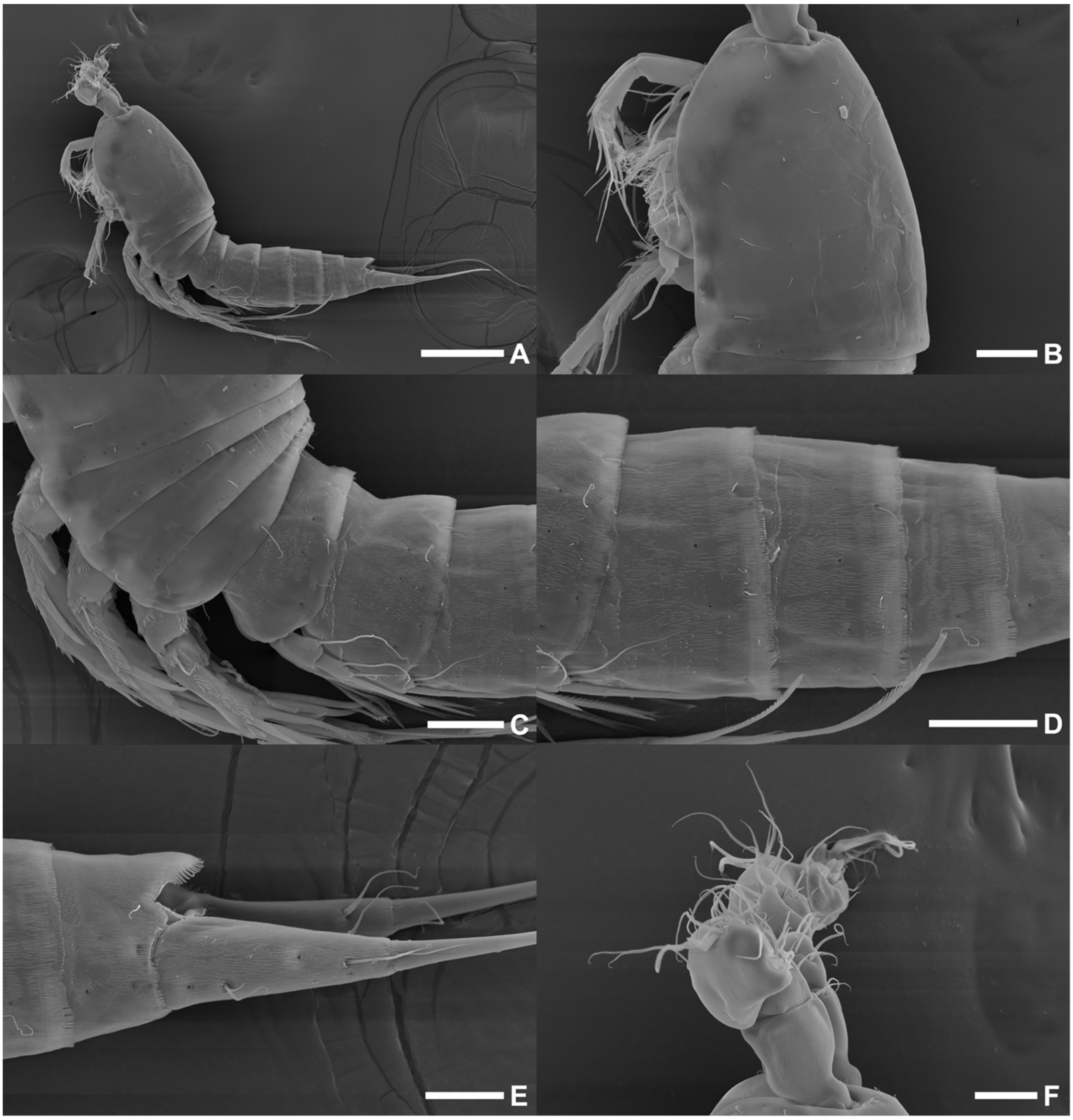

Figure 32. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 23) in lateral view: (**A**) habitus; (**B**) cephalothorax; (**C**) second prosomite to the second urosomite; (**D**) third to fifth urosomites; (**E**) anal somite and caudal rami; (**F**) antennule. Scale bars: (**A**) 100 µm; (**B**–**D**) 30 µm; (**E**,**F**) 20 µm.

Urosome (Figures 31C, 32C–E, 33A–C, 34, 35 and S12A) six-segmented and tapering gradually. First urosomite cylindrical and with smooth hyaline frill ventrally. Genital and first abdominal somite separated; genital somite armed with P6; three free abdominal somites getting shorter posteriorly (length ratio from third to fifth urosomite in lateral aspect, 1:0.9:0.7) and regularly ornamented with spinules along terminal margin. Caudal rami elongated, about 3.3 times as long as greatest width, with dimples on dorsal surface, covered with fine microsetules throughout surface, tapering distally, and armed with seven setae (one dorsal, three lateral, and three apical setae); setae I and II naked and inserted very close to each other dorsoventrally; seta I minute, hair-like, and about one-fourth of seta II in length; seta III about as long as seta VII and implanted in distal fifth; seta V longest and robust; setae IV and VI slender and almost equal in length; seta VII triarticulate and inserted in about distal third of inner margin.

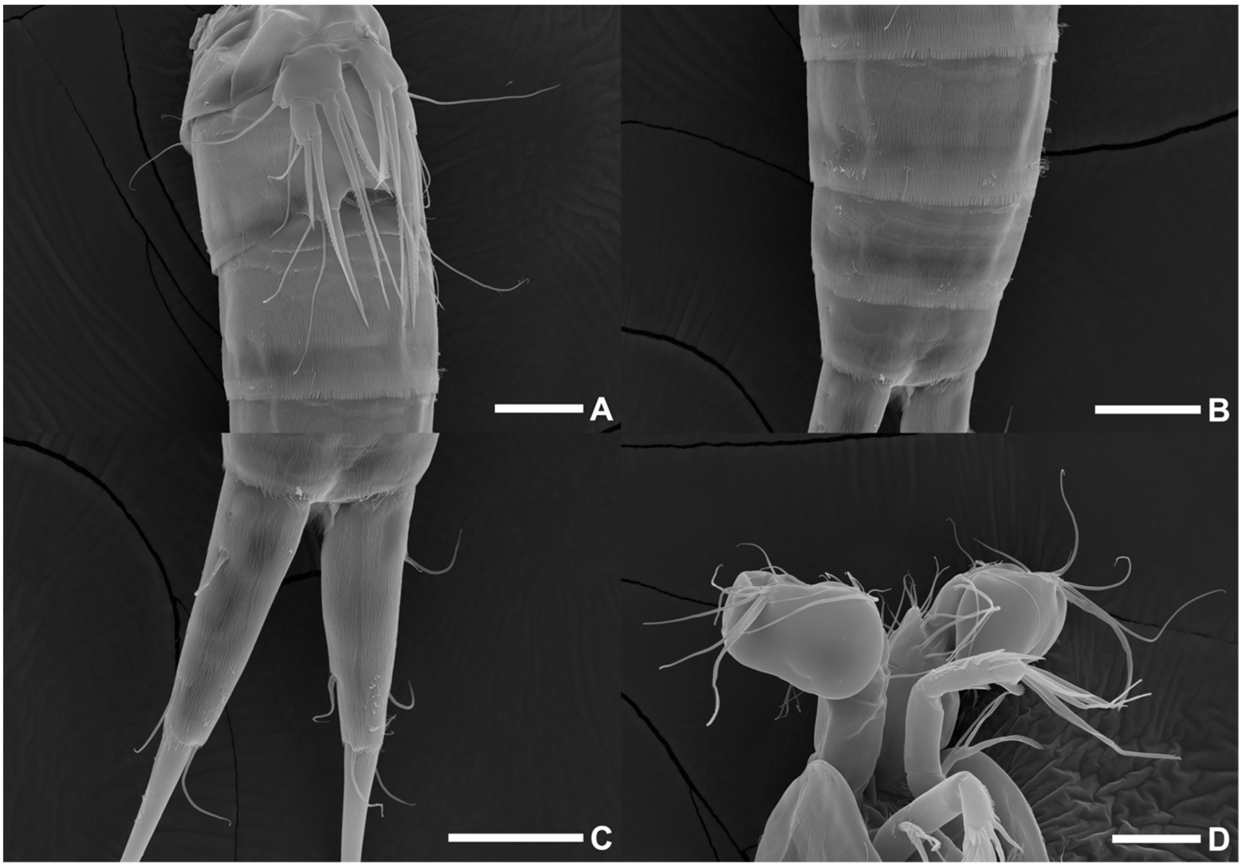

Figure 33. Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 24) in ventral view: (**A**) P5 and second to third urosomites; (**B**) fourth urosomite to anal somite; (**C**) caudal rami; (**D**) antennule and antenna. Scale bars: 30 µm.

Antennule (Figures 31D, 32F, 33D, 36A,B and S12B) seven-segmented, subchirocer, covered with dimples on first three segments, and with geniculation between fourth and fifth segments; first segment longest, dented in inner margin proximally, beveled in outer margin, ornamented with row of spinules on proximal concave surface, and armed with seta implanted in inner distal corner; second segment without cuticular projection and with cuticular pore near boundary with following segment dorsally; third segment shortest; fourth segment enlarged, shorter than all following segments combined, and armed with aesthetasc fused basally to seta; fifth segment unarmed; sixth segment about as long as maximum width; fourth to sixth segments modified into pincer-like structure with several cuticular ridges; seventh segment with short aesthetasc fused with two setae at base; most of setae on seventh segment biarticulate. Armature formula as follow: 1(1), 2(11), 3(7), 4(13+(1+ae)), 5(1), 6(1), 7(9+(2+ae)).

It was difficult to determine whether: (a) the small lobe bearing two setae (marked by an empty arrow in Figure 31D) was a part of the third or the fourth segment; (b) the coffee-bean-like projections on the fourth segment (marked by a hatched arrow in Figure 31D) and on the fifth segment (marked by a checkered arrow in Figure 31D) were the transformed armatures or the integumental ridges. The above descriptions and the formula were written assuming they were a part of the fourth segment and the transformed armatures, respectively.

P2 (Figure 36C) as in female excluding following: Endopod almost reaching end of second exopodal segfment; first endopodal segment about 1.5 times as long as greatest width; second endopodal segment shorter (about 0.8 times) than preceding segment; middle armature narrowed sharply from middle and longest; outermost spine fused with endopodal segment at base; other two setae inserted very close to each other; innermost seta shortest and hairly.

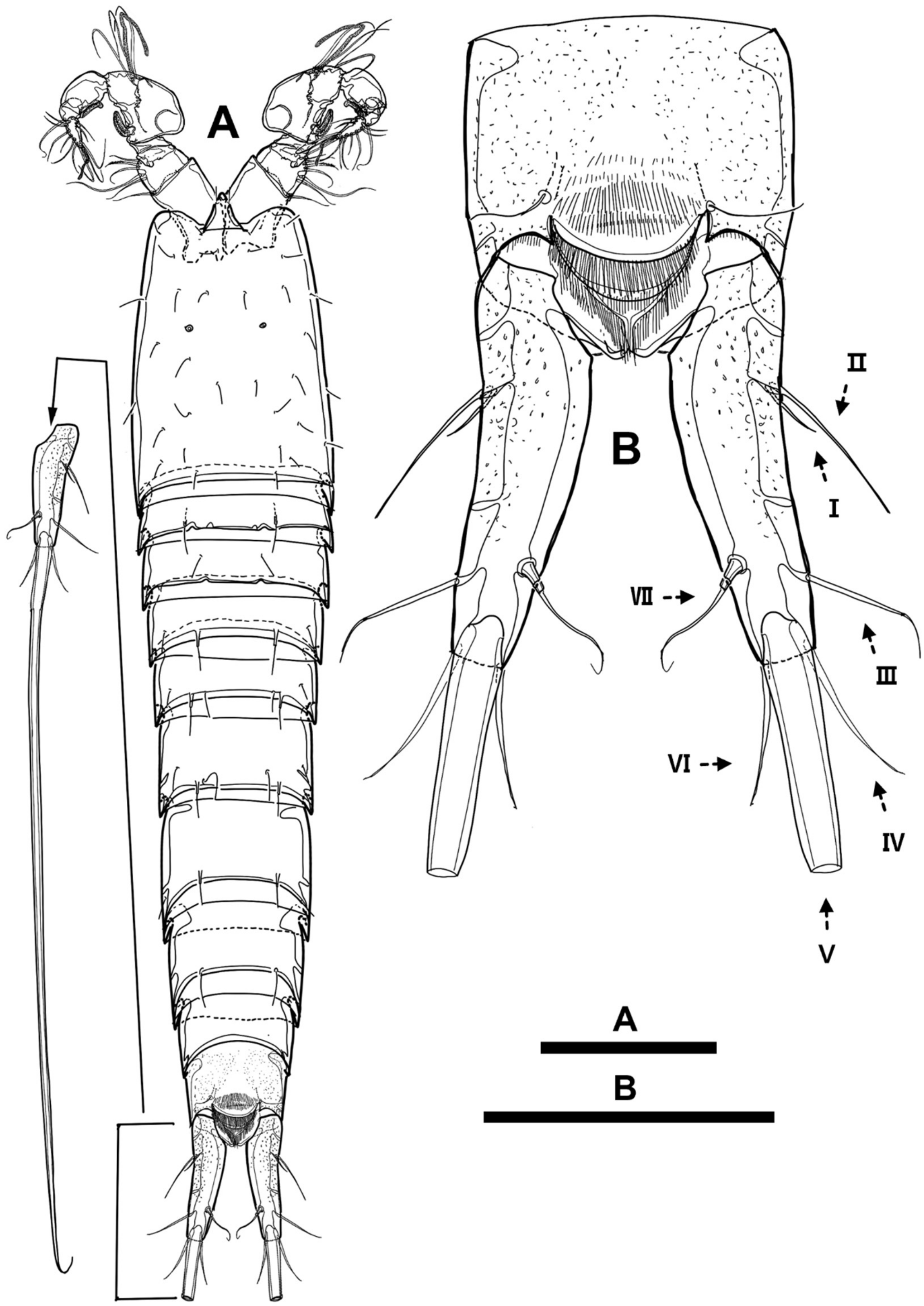

Figure 34. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 25): (**A**) habitus, dorsal; (**B**) anal somite, dorsal; Scale bars: (**A**) 100 µm; (**B**) 50 µm.

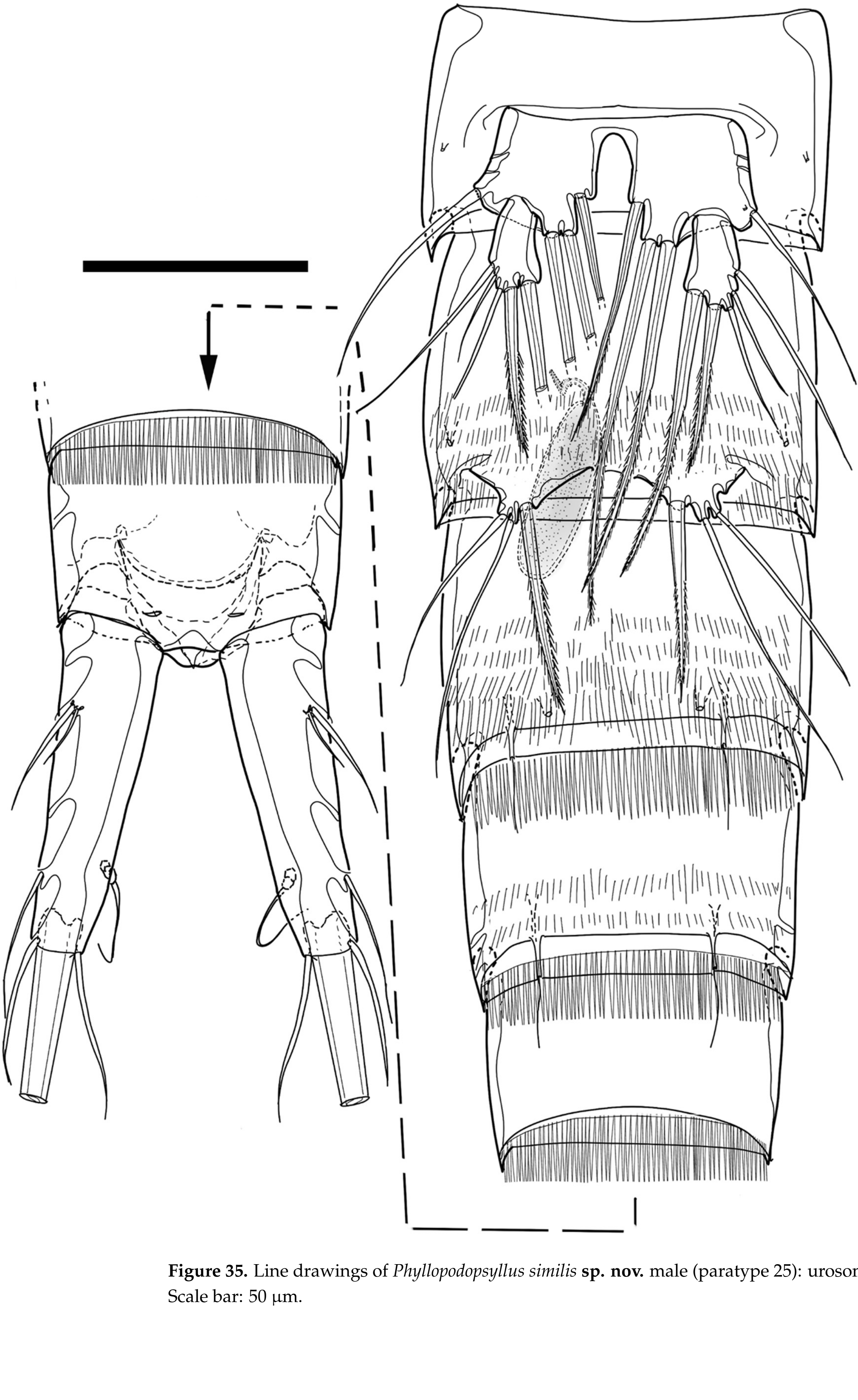

Figure 35. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 25): urosomite, ventral. Scale bar: 50 μm.

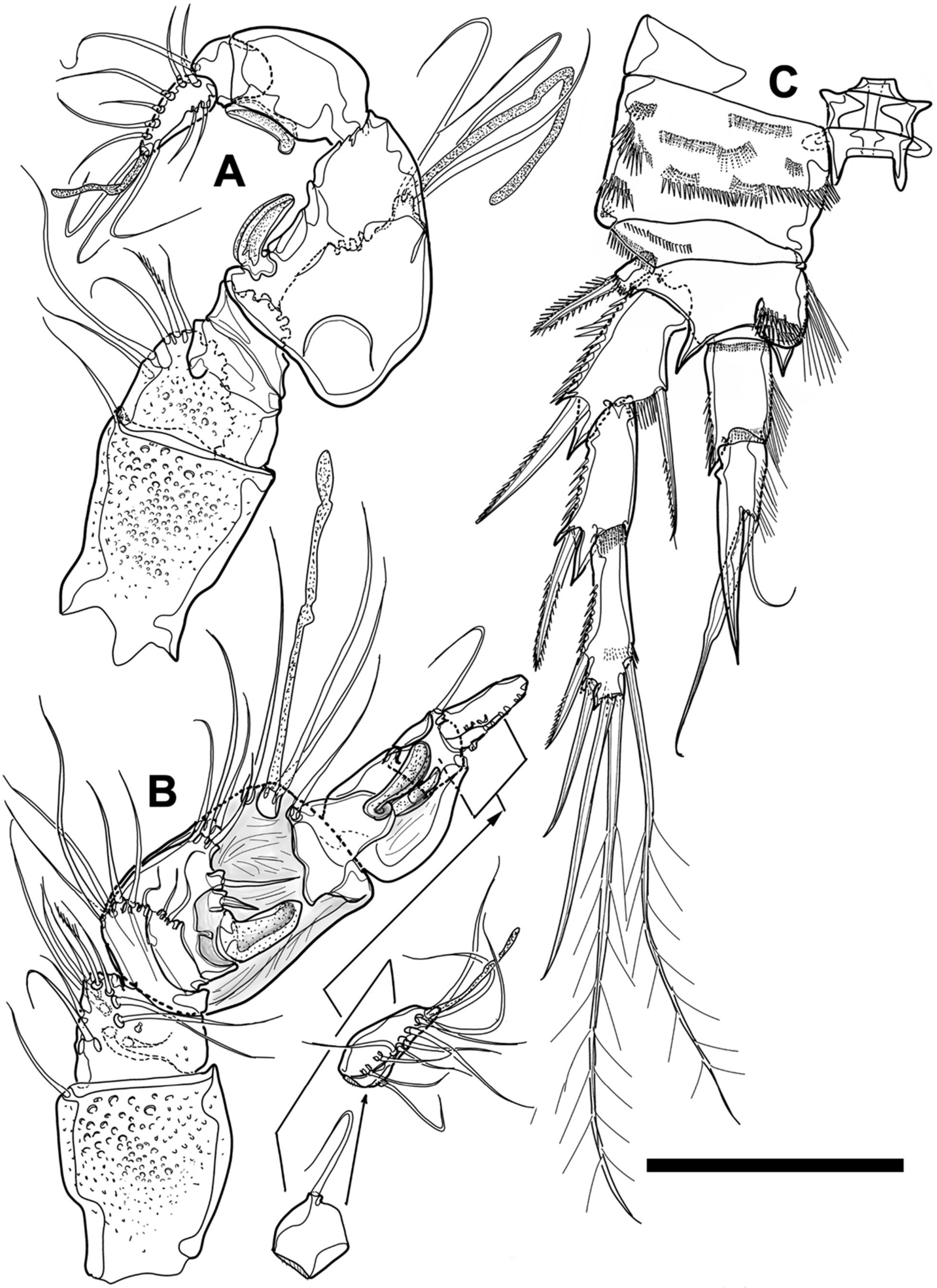

Figure 36. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 25): (**A**) antennule, lateral; (**B**) antennule, dorsal; (**C**) P2. Scale bar: 50 μm.

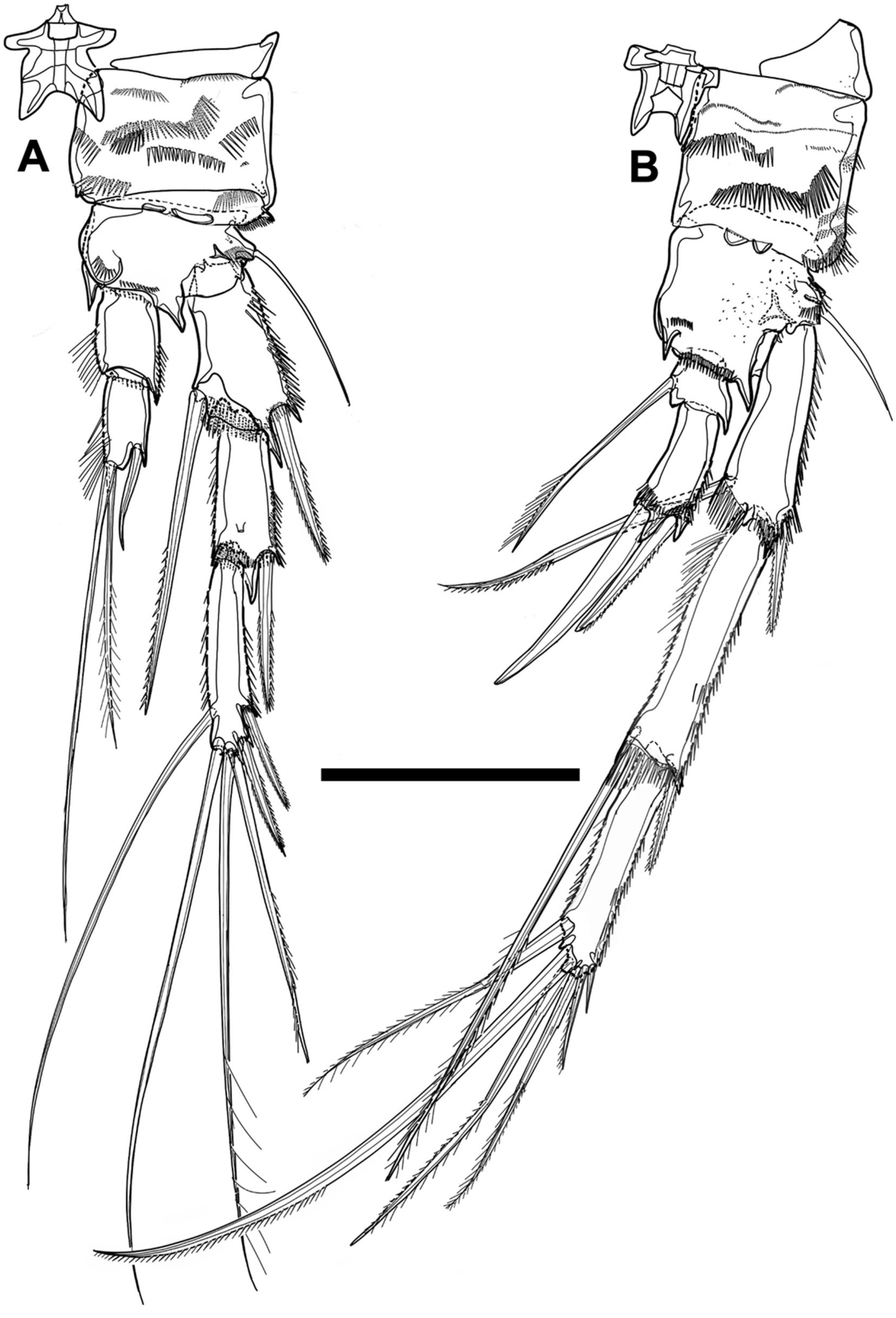

Figure 37. Line drawings of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 25): (**A**) P3; (**B**) P4. Scale bar: 50 µm.

P3 (Figure 37A) as in female excluding following: Outer armature of second endopodal segment somewhat bent terminally, and much deeper embedded than other two setae; other two setae inserted very close to each other

P4 (Figure 37B). as in female excluding following: Second endopodal segment about twice as long as wide and armed with two stout spines apically; inner spine longer than outer spine; third exopodal segment with two inner setae, two terminal setae, and two outer spines.

P5 (Figures 33A, 35 and S12A) not foliaceous and composed of baseoendopod and exopod; both rami completely fused at central part. Baseoendopod large, with cuticular pore near base of basal seta, and armed with three pinnate setae on endopodal lobe; basal seta bare, slender, and much longer than P5; two apical setae equal in length; distal half of innermost seta bipinnate. Exopod about 1.6 times as long as greatest width, shorter than baseoendopod, and armed with five setae; two innermost setae robust and bipinnate, of which inner seta longest; other three setae slender.

P6 (Figure 33A, 35 and S12A) reduced into single plate, completely fused at base with second urosomite, and armed with three armatures; innermost spine bipinnate and robust; middle seta longest, bare, and slender; outermost seta naked and about as long as innermost spine.

Variability

The body length ranged from about 561.5–685.9 µm (mean = 608.4 µm, n = 8) in females (Figure S3) and about 461.6–520.6 µm (mean = 485.7 µm, n = 5) in males (Figure S4). Another morphological variation appears in a single female individual (paratype 2). The left antennule of this individual shows the abnormalities, such as segmentation, chaetotaxy, and the shape of each segment (Figure 21F).

3.4. DNA Sequences and Phylogenetic Analysis

From 52 individuals (20 of *Phyllopodopsyllus saywakimi* **sp. nov.**, 28 of *P. similis* sp. nov., and four of *P. kitazimai*), partial fragments (361 bp) of cytb were obtained, representing six haplotypes in *P. saywakimi* **sp. nov.** and three haplotypes in *P. similis* **sp. nov.** There is a 31.1% genetic divergence between the two new species. *Phyllopodopsyllus kitazimai* has a mean distance of 31.6% and 31.9% between *P. saywakimi* **sp. nov.** and *P. similis* **sp. nov.**, respectively. *Phyllopodopsyllus saywakmi* **sp. nov.** and *P. kitazimai* each bear a 0.4% and 0.6% mean p-distance, whereas *P. similis* **sp. nov.** has a mean distance of 0.1% divergence (Table S2).

The sequences of cox1 from 40 individuals (20 of *P. saywakimi* **sp. nov.**, 16 of *P. similis* **sp. nov.**, and four of *P. kitazimai*) were composed of 655 bp with six haplotypes in *P. saywakimi* **sp. nov.** and five haplotypes in *P. similis* **sp. nov.**. The interspecific genetic divergence of the two new species is a 21.6% mean p-distance. *Phyllopodopsyllus kitazimai* has a mean p-distance of 22.6% and 20.6%, each with *P. saywakimi* **sp. nov.** and *P. similis* **sp. nov**. Within each new species, there is only 0.1% sequence divergence (Table S3).

Two phylogenetic trees inferred from ML and BI approaches show that the three species of *Phyllopodopsyllus* were grouped with two unidentified tetragonicipids (Figure 38). Strong statistical values supported all nodes in both trees; A clade including *Phyllopodopsyllus saywakimi* **sp. nov.** and *P. similis* **sp. nov.** has the minimum bootstrap value 87 in the ML tree (Figure 38A). In the MCC tree, the posterior probability is one at all nodes (Figure 38B). Three *Phyllopodopsyllus* species formed a single clade.

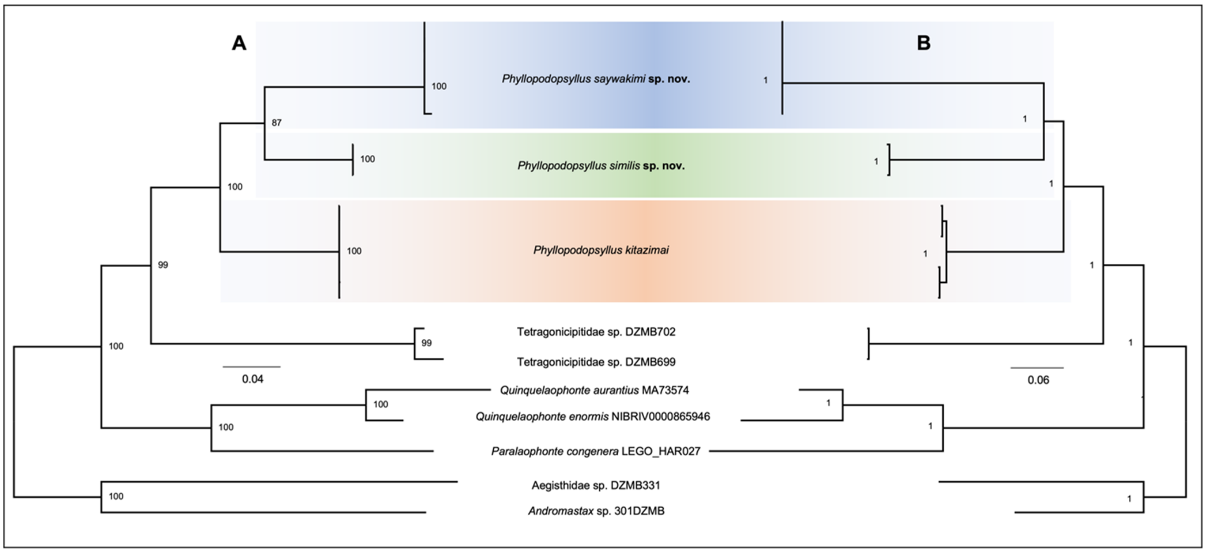

Figure 38. The two rooted trees of three Korean *Phyllopodopsyllus* species and some harpacticoids based on 18S rDNA, 28S rDNA, and cox1: (**A**) Maximum likelihood tree; (**B**) MCC tree. Numbers on each node are bootstrap values in (**A**) and posterior probabilities in (**B**). Scale bars: substitutions per site.

4. Discussion

Phyllopdopsyllus is readily distinguishable from other tetragonicipitid genera, according to the identification keys in [1,7], and by the combination of the following characteristics: cephalothorax without unguiform process on posterior lateral corner; antennule eight- or nine-segmented; first segment of antennule up to three times as long as second segment and without projection; fourth segment of antennule with aesthetasc; P1 endopod two-segmented; female P5 large and foliaceous, forming a brood pouch. Two new species described above are placed in *Phyllopodopsyllus* on account of showing these characteristics, with one exception: the first segment of *Phyllopdopsyllus saywakimi* **sp. nov.** is more than four times as long as the second segment. In addition to the preceding features, the two species are similar in several morphological characteristics, such as the presence of the inner seta on the second exopodal segment of the P4 in both sexes, the number of armatures on the distal segment of P4 in both sexes, and the number of setae on female P5.

The two new species also clearly differ in a significant number of morphological features, such as body length (*Phyllopodopsyllus saywakimi* **sp. nov.** averaged about 829.3 µm in females and 589.5 µm in males, while *Phyllopodopsyllus similis* **sp. nov.** averaged about 608.4 µm in females and 485.7 µm in males), the shape of the rostrum in the dorsal aspect (semi-trapezoid in *P. saywakimi* **sp. nov.**, while triangular in *P. similis* **sp. nov.**), the shape of female caudal rami (cylindrical in *P. saywakimi* **sp. nov.**, while subconical in *P. similis* **sp. nov.**), the principal terminal seta of the female caudal rami (bulbous at base in *P. saywakimi* **sp. nov.**, while not modified in *P. similis* **sp. nov.**), the segmentation and the shape of the antennule (eight-segmented and with pointy projection on second segment in *P. saywakimi* **sp. nov.**, while nine-segmented and without integumental process in *P. similis* **sp. nov.**), the chaetotaxy on the second endopodal segment of the antenna (11 armatures in *P. saywakimi* **sp. nov.**, while nine in *P. similis* **sp. nov.**), the shape and chaetotaxy of the mandible (endopod with nine setae and about 0.8 times as long as basis in *P. saywakimi* **sp. nov.**, while with eight setae and much shorter than basis in *P. similis* **sp. nov.**), the chaetotaxy of the maxillule (with five setae on coxa and eight on basis in *P. saywakimi* **sp. nov.**, while with four on coxa and six on basis in *P. similis* **sp. nov.**), the chaetotaxy of the maxilliped (one spine and two setae on endopod in *P. saywakimi* **sp. nov.**, while only one spine on endopod in *P. similis* **sp. nov.**), the setation of the P2–P3 endopod in both sexes (with inner seta on first segment in *P. saywakimi* **sp. nov.**, while without inner seta in *P. similis* **sp. nov.**), and the setation of the P2–P3 exopod in both sexes (without inner seta on distal segment in *P. saywakimi* **sp. nov.**, while with one and two inner setae on distal segment of P2 and P3, respectively, in *P. similis* **sp. nov.**). Besides the above differences between these two species, there are other differences, for instance, in the number and the arrangement pattern of cuticular ornaments (pores, sensilla, (micro)setules, and (micro)spinules), the length/width ratio of segments, the type and the location of the armatures. These numerous morphological dissimilarities suggest that the two new species could be distantly related within the genus. Additionally, the high divergences of *cytb* (31.1%) and *cox1* (21.6%) were in concordance with our comparison (Tables S2 and S3).

Phyllopodopsyllus saywakimi **sp. nov.** has a superficial resemblance to *P. ancylus* Mielke, 1992 described from the Pacific beach in Costa Rica. These two species differ from their congeners in the presence of an acute projection on the second segment of the antennule, the setation and the segmentation of P2–P4, the shape of female caudal rami, and the bulbous base of the principal terminal seta. Nevertheless, *P. saywakimi* **sp. nov.** can be distinguished from *P. ancylus* by the following morphological differences (Table 1): body length (*P. saywakimi* **sp. nov.** averaged about 829.3 μm in females and 589.5 μm in males, while *P. ancylus* ranged from 0.44–0.49 mm in females and 0.43–0.45 mm in males); chaetotaxy of the antenna (second endopodal segment with 11 armatures in *P. saywakimi* **sp. nov.**, while with ten armatures in *P. ancylus*); the length ratio of the mandibular endopod and exopod (endopod about three times longer than exopod in *P. saywakimi* **sp. nov.**, while endopod less than twice as long as exopod in *P. ancylus*, see Figure 18D in [10]); chaetotaxy of the female P5 (armed with five setae on inner edge in *P. saywakimi* **sp. nov.**, while with four setae in *P. ancylus)*; length of armature on male P2 (inner seta on first endopodal segment about as long as first endopodal segment and reaching midlength of second endopodal segment in *P. saywakimi* **sp. nov.**, while inner seta on first endopodal segment is shorter than first endopodal segment and barely exceeding end of first endopodal segment in *P. ancylus*, see Figure 21B in [10]); chaetotaxy of male P6 (in *P. saywakimi* **sp. nov.**, male P6 armed with three armatures, while armed with two armatures in *P. ancylus*); location of seta on the caudal rami of male (posterolateral seta inserted in distal fifth in *P. saywakimi* **sp. nov.**, while posterolateral seta located medially in *P. ancylus*, see Figure 21G in [10]). *Phyllopodopsyllus saywakimi* **sp. nov.** and *P. ancylus* are the only two species in the genus with a unique combination of characteristics (the presence of acute projection on the second antennular segment and the setation of P2–P4). The fact shows that the two species are very closely related. However, the numerous morphological differences indicate that *P. saywakimi* **sp. nov.** is a separate species.

Table 1. Morphological comparison of *Phyllopodopsyllus saywakimi* **sp. nov.** and *P. ancylus*.

	Characteristics	***P. saywakimi* sp. nov.**	***P. ancylus***
female	body length	0.81–0.86 mm	0.44–0.49 mm
	second endopodal segment of antenna	with 11 armatures	10 armatures
	length ratio of endo- and exopod of mandible	endopod = 3x exopod	endopod < 2x exopod
	number of setae on the inner margin of P5	5	4
male	body length	0.56–0.69 mm	0.43–0.45 mm
	length of inner seta on first endopodal segment of P2	=first endopodal segment	<first endopodal segment
	number of setae on P6	3	2
	seta III location on caudal rami	distal fifth	midlength

As it is one of the harpacticoid genera containing numerous species, *Phyllopodopsyllus* is rich in synonyms [3]. One such example, *Phyllopodopsyllus aegypticus* Nicholls, 1944, was first reported from Ghardaqa in the Red Sea without examination of the male individual [47]. According to Nicholls' description and illustration, *P. aegypticus* has a body length of 0.67 mm, no seta originating from epipodite on the coxa of maxillule, four setae on the inner edge of female P5, and six armatures on the distal exopodal segment of the P4. *Phyllopodopsyllus aegypticus* was subsequently reported by Wells and Rao [48], including descriptions and illustrations of the male from the South Andaman and Nicobar Islands. They demonstrated that their specimens agreed with Nicholls' description except for two characteristics. They pointed out a difference in the setation, where the setal formula of the distal segment of the P4 exopod of Nicholls' specimen was 321, while their specimens showed 322. Another exception was the female body length which is 810 µm. Subsequently, Kunz [9] described Hawaiian specimens as a new species, *Phyllopodopsyllus gertrudi* Kunz, 1984, based on the number of armatures on the distal exopodal segment of P3 (five armatures in *P. gertrudi*, while six in *P. aegypticus)*. The new subspecies, *P. gertrudi costaricensis*, was found on the Caribbean and Pacific coasts [10]. This subspecies differed from *P. gertrudi* in the setation of the distal exopodal segment of P3, the setation of the distal exopodal segment of male P4, and the length ratio of apical armatures on the distal endopodal segment of female P2–P3. After that, Karanovic et al. [3] synonymized these two Pacific (sub)species with *P. aegypticus*. Lastly, Björnberg & Kihara [49] reported *P. aegypticus* from the Atlantic beach of Brazil; however, although they had adult individuals of both sexes, only naupliar stages were described and illustrated.

Phyllopodopsyllus similis **sp. nov.** significantly resembles *P. aegypticus* in the segmentation of the antennule, the absence of a unguiform projection on the second segment of antennule, the setation of the P2–P4, the caudal rami shape of female, and the length ratio of the first endopodal segment of P1 and exopod. Although *P. aegypticus* has been reported with intraspecific variation over a wide distribution area as mentioned above, *P. similis* **sp. nov.** is distinguishable from each population in the following combination: (1) comparison with the Egyptian population [47], seta originating from epipodite on the coxa of maxillule (present in *P. similis* **sp. nov.**, while absent in *P. aegypticus*); setae on the inner edge of female P5 (five setae in *P. similis* **sp. nov.**, while four setae in *P. aegypticus*); (2) comparison with the South Andaman and Nicobar Islands' population [48], body length (in *P. similis* **sp. nov.**, about 561.5–685.9 µm in female and about 461.6–520.6 µm in male, while, in *P. aegyptus*, 810 µm in female and 540–550 µm in male); the length/width ratio of the male caudal rami (more than three times as long as width in *P. similis* **sp. nov.**, while about twice as long as width in *P. aegypticus*); the type of armatures on the male P6 (two long setae and one innermost spine in *P. similis* **sp. nov.**, but two long setae and one outermost spine in *P. aegypticus*); (3) comparison with Hawaiian population [9], the shape of the rostrum (triangular in *P. similis* **sp. nov.**, while rounded in *P. aegypticus*); length of the exopod and the endopod of mandible (similar in *P. similis* **sp. nov.**, while endopod much longer than exopod in *P. aegypticus*); seta originating from epipodite on the coxa of maxillule (present in *P. similis* **sp. nov.**, while absent in *P. aegypticus*); chaetotaxy of female P3 (third exopodal segment armed with six armatures in *P. similis* **sp. nov.**, while armed with five armatures in *P. aegypticus*); length of setae on the second endopodal segment of female P2–P3 (three armature of different lengths in *P. similis* **sp. nov.**, while two innermost setae of equal length in *P. aegypticus*); length of innermost seta on the distal endopodal segment of female P4 (innermost seta reaching midlength of distal exopodal segment in *P. similis* **sp. nov.**, while not exceeding end of second exopodal segment in *P. aegypticus*); length of two innermost seta on the distal endopodal segment of male P3 (different in *P. similis* **sp. nov.**, while almost equal in *P. aegypticus*); length of setae on female P5 (second distal seta among three apical exopodal setae about as long as basal seta in *P. similis* **sp. nov.**, while much shorter than basal seta in *P. aegypticus)*; Kunz [9] described and illustrated the exopod of the mandible and the inner edge of female P5 having three and four setae, respectively. These characteristics differ from Nicholl's illustration [47] and our specimens. In Nicholls' illustration [47], the two distal lateral setae on the mandibular exopod were presented as minute and thready, and in our specimen, the median lateral seta was so. The two distal-most setae on the inner edge of female P5 are located very close to each other, and one of the setae is very minute in Nicholls' illustration [47] and our specimens. Since the minute setae can be easily overlooked, we assumed that the Hawaiian *P. aegypticus* has more than three exopodal setae of the mandible and five baseoendopodal setae on female P5; (4) comparison with Costa Rican populations [10], body length (in female, 0.54–0.60 mm and in male, 0.41–0.45 mm in the Atlantic population, and a single individual showing body length of 0.42 mm in the Pacific coast); length of seta on the second endopodal segment of female P3 (middle seta exceeding end of exopod in *P. similis* **sp. nov.**, while not reaching midlength of distal exopodal segment in *P. aegypticus*); length of seta on the second endopodal segment of female P4 (innermost seta reaching midlength of third exopodal segment in *P. similis* **sp. nov.**, while reaching end of second segment in *P. aegypticus*); chaetotaxy of male P4 (third exopodal segment armed with six armatures in *P. similis* **sp. nov.**, while armed with five armatures in *P. aegypticus*). These distinctions suggest that *P. similis* **sp. nov.** is a different species from the *P. aegypticus* complex.

The presence or absence of a unguiform projection on the second segment of the antennule is a characteristic that has frequently been employed in attempts to subdivide the genus *Phyllopodopsyllus* along with the chaetotaxy of the second and the fourth pereiopods [4,6]. Two of the three *Phyllopodopsyllus* species collected in this study, *P. similis* **sp. nov.** and *P. kitazimai,* share the absence of the acute process on the second segment of the antennule and the absence of the inner seta on the first endopodal segment of the second and the third pereiopods. However, the phylogenetic trees reconstructed in this study show that the two new species are more closely related than *P. kitazimai*. Furthermore, *P. saywakimi* **sp. nov.** and *P. kitazimai* share some morphological features, such as the segmentation of the antennule, the presence of additional setae other than the claw on the endopod of the maxilliped (two additional setae in *P. saywakimi* **sp. nov.**; one seta in *P. kitazimai*), and the absence of the inner setae on the distal exopodal segment of the second and the third pereiopods. Although it only includes three of about 60 species of *Phyllopodopsyllus,* the characteristics that have been used so far, especially the shape of the antennule, might be unsuitable for the subdivision of this genus. The two new species have some characteristics that distinguish them from *P. kitazimai*, for instance, the number of setae on the distal endopodal segment of the second to the fourth pereiopods in females (three setae in two new species, but two setae in *P. kitazimai*) and the number of setae on the inner edge on the fifth pereiopod in females (two new species have five setae, but only three setae in *P. kitazimai*).

In addition to utilizing molecular information, we tried to analyze the phylogeny of *Phyllopodopsyllus* species using their morphological characteristics. However, we cannot understand their relationships because considerable inter- and intraspecific variations exist in this genus, and many descriptions and illustrations need to be revised to resolve this situation. Although we suggested a phylogenetic relationship among three *Phyllopodopsyllus* species using newly obtained molecular makers, there is almost no genetic information derived from tetragonicipitid copepods in the database, such as NCBI and Barcode of Life Data System. For constructing the phylogeny of *Phyllopodopsyllus* species within family Tetragonicipitidae, additional molecular evidence must be accumulated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15010097/s1, Table S1: Information of type specimens and sequences from GenBank; Table S2: Uncorrected pairwise p-distance for cytb among individuals of three *Phyllopodopsyllus* species; Table S3: Uncorrected pairwise p-distance for cox1 among individuals of three *Phyllopodopsyllus* species; Figure S1: Measurements of body length of *Phyllopodopsyllus saywakimi* **sp. nov.** females; Figure S2: Measurements of body length of *Phyllopodopsyllus saywakimi* **sp. nov.** males; Figure S3: Measurements of body length of *Phyllopodopsyllus similis* **sp. nov.** females; Figure S4: Measurements of body length of *Phyllopodopsyllus similis* **sp. nov.** males; Figure S5: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 12); Figure S6: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 13); Figure S7: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 14); Figure S8: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 15); Figure S9: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 16); Figure S10: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 14); Figure S11: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** female (paratype 17); Figure S12: Scanning electron microscopy images of *Phyllopodopsyllus similis* **sp. nov.** male (paratype 30).

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