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An Integrative Approach for Identifying *Quinquelaophonte* (Harpacticoida, Laophontidae) Species from Korea with the Description of a New Species †

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Abstract: *Quinquelaophonte* is a genus of laophontid harpacticoid, including 13 valid species around the world. Many of them are known to possess inter- and/or intra-individual variations in their swimming legs. During a survey of the meiofaunal biodiversity of Jeju Island in Korea, specimens of *Quinquelaophonte* were collected from an intertidal zone off the west coast of Jeju Island. This study examined the morphological characteristics and amplified partial sequence of four genes (18S rRNA, 28S rRNA, COX1, and CYTB). As with other *Quinquelaophonte* species, specimens of this new species showed variability in the chaetotaxy of their swimming legs in both sexes. There was a setal arrangement pattern in females that could be considered a standard, whereas male individuals showed two sets of the chaetotaxy on the fourth swimming legs. The molecular data confirmed that individuals belonged to a single species, regardless of morphological variations. The result of the morphological comparison showed that the new *Quinquelaohponte* species shared some characteristics with congeneric species but included some specific morphological characters different from its congeners. The new species was named *Quinquelaophonte sominer* sp. nov. and was presented with detailed descriptions, illustrations, and confocal micrographs. Furthermore, phylogenetic analyses were conducted using newly obtained data plus the sequences of other copepods and two *Quinquelaophonte* species. The result suggested that *Q. sominer* sp. nov. and two congeners were classified as branched lineages. This discovery brings the total number of species to three in the Northwest Pacific region.

Keywords: meiofauna; biodiversity; variability; DNA barcoding; phylogeny

1. Introduction

The genus *Quinquelaophonte* Wells, Hicks & Coull, 1982 was erected to encompass the *quinquespinosa*-group of *Heterolaophonte* Lang, 1948. This taxon currently includes 13 species, excluding the questionable species *Laophonte brevicornis* Scott, 1894 [1]. *Laophonte brevicornis* was included in the *quinquespinosa*-group as *Heterolaophonte brevicornis* by Lang [2,3]. When Wells et al. [1] established the genus, they questioned the status and position of this species because of an enigmatic characteristic, the presence of an inner seta on the first exopodal segment of the fourth swimming leg, which is a feature not found in the family Laophontidae. Consequently, this species was classified as *incertae sedis*. Some *Quinquelaophonte* species have variable chaetotaxy in the second to fourth swimming legs, similar to other laophontids [4]. The variability appears between populations, within a population, and between left and right rami. *Quinquelaophonte* species inhabit various substrata, e.g., silty sand, intertidal mud, and gravel, and are widely distributed around the world, e.g., the Central Indo-Pacific [5], Western Indo-Pacific [6,7], Temperate Australasia [1,8,9], Temperate Northern Atlantic [10–12], Tropical Atlantic [13], Temperate South America [14,15], and Temperate Northern Pacific [16,17] realms. All *Quinquelaohponte* species were reported from

only the type locality or relatively close localities, with two exceptions, *Q. quinquespinosa* (Sewell, 1924) and *Q. capillata* (Wilson, 1932).

Quinquelaophonte quinquespinosa, the type species of the genus, was originally described from Chilka Lake in India by Sewell [18] as *Laophonte quinquespinosa*. Subsequently, this species was reported with few morphological differences from various localities [8,13,19–23]. Lee [16] suggested that *Q. quinquespinosa* is an example of a species complex and the isolated populations might be recognized as distinct species. Later, Gómez and Morales-Serna [24] reported *Q. quinquespinosa* from northwestern Mexico based on the examination of only two adult females.

The other widely distributed species, *Q. capillata*, was originally described as *Laophonte capillata* from Massachusetts, USA by Wilson [10]. Lang [3] claimed that *L. capillata* was a species complex on the basis of questions about the identity of the species and reassigned the female to a new species, *Paronychocamptus capillatus*, and the male to a new species of the genus *Heterolaophonte* Lang, 1948 as *H. noncapillata*. Subsequently, Coull [11] restored the specific name of *H. noncapillata* to *H. capillata*. He also found two female individuals of *H. capillata* in Wilson's vial and partially redescribed *H. capillata*. When the genus *Quinquelaophonte* was erected, *H. capillata* was translocated to the new genus [1]. Coull [12] then collected a population of *Q. capillata* from South Carolina, USA, which was identical to Wilson's type materials. Lastly, Gómez and Morales-Serna [24] reported *Q. capillata* from Sinaloa, Mexico, and noted that the chaetotaxy of Mexican females' swimming legs fully agreed with the observation of Coull [11]. By contrast, the armatures on the Mexican male's third and fourth swimming legs differed from the previous studies. However, they left the Mexican materials as *Q. capillata* due to the insufficient number of individuals observed. A more detailed chronology of the study of *Q. capillata* was summarized in Gómez and Morales-Serna [24].

Recent investigations on meiofaunal diversity in Jeju Island, Korea, led to the discovery of *Quinquelaophonte* specimens that showed the variable chaetotaxy of the swimming legs. In the present study, we used an integrative approach including both morphological and genetical characteristics to (I) confirm whether these organisms are conspecifics or members of a species complex; (II) propose a new *Quinquelaophonte* species with detailed descriptions and illustrations; (III) infer the phylogenetic relationships among the specimens obtained in this study and two congeners for which genetic information is available.

2. Materials and Methods

2.1. Sample Collection

A qualitative meiofauna assemblage was collected from an intertidal zone on the west coast of Jeju Island, Republic of Korea (33°13.61' N, 126°14.44' E; Figure 1). Silty sand was dug until the seawater table was reached. Subsequently, the sediment around the water level was transferred into a bucket and stirred with fresh water. The supernatant was immediately poured over a 38 μm sieve, and the material was sifted with fresh water until the filtered water appeared clear. This process was repeated several times. The fauna retained on the sieve was washed with absolute ethanol to dehydrate, followed by preservation.
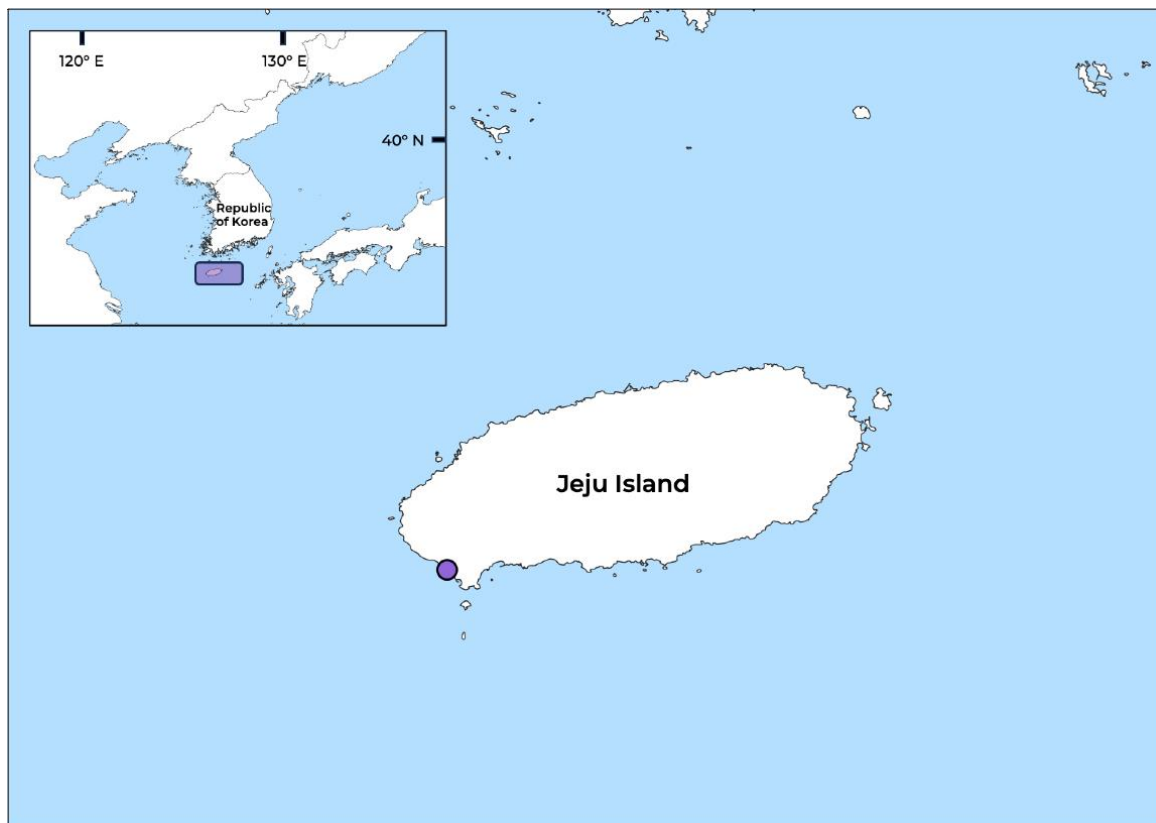
Figure 1. Map showing the type locality of *Quinquelaophonte sominer* sp. nov. The map was made under QGIS (3.32, downloaded from https://qgis.org/en/site/forusers/download.html accessed on 13 November 2023) using the map data of GADM (4.1, downloaded from https://gadm.org/data.html accessed on 13 November 2023).

2.2. DNA Extraction

Total genomic DNA (gDNA) was extracted independently from 32 adult specimens of *Quinquelaophonte*. A non-destructive approach was employed to preserve the specimen for subsequent morphological analysis. Specimens were transferred into ultra-pure water separately to remove ethanol for up to 30 min. Subsequently, gDNA was isolated from each specimen using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The remaining exoskeletons were stored individually after lysis in a 0.2 mL tube.

2.3. Morphlogical Examination and Image Acquisition

All exoskeletons were individually prepared on temporary slides and examined under a differential interference contrast microscope (BX-51, Olympus, Tokyo, Japan). The individuals were identified according to the identification keys [4,25] and original descriptions. Even after lysis for DNA extraction, most individuals were covered with a layer of fine debris. It was difficult to discern the exact nature of the integumental ornaments on the body surface and their arrangement pattern.

Twenty-five individuals (10 females and 15 males) and three females were then used for image acquisition through a confocal laser scanning microscope (CLSM) and a scanning electron microscope (SEM), respectively. The method for each of the two micrographs was adopted from Kim et al. [26]. All CLSM images were used for body length measurements in Fiji v2.14.0 [27] (Figures S1 and S2). SEM images from three female individuals were provided as Supplementary Materials (Figures S3–S5).

Except for the four females that were excluded from the two types of micrographs, the remaining exoskeletons of each individual were dissected under a stereo microscope (S

APO, Leica microsystems, Germany). Dissections were mounted in permanent slides with lactophenol for more detailed observation. Pencil drawings of habitus and appendages were made under a compound microscope (DM2500, Leica Microsystems, Wetzlar, Germany). The line drawings were illustrated using Clip Studio Paint (Celsys, Tokyo, Japan).

A total of 32 type specimens, including 16 adult females and 16 adult males, that were prepared in this study were deposited at the National Institute of Biological Resources (Incheon, Republic of Korea). For detailed information on the type materials, see Table S1.

The descriptive terminology was adopted from Huys et al. [25]. Henceforth, abbreviations used in the text are as follows: ae, aesthetasc; P1–P6, first to sixth swimming legs; exp, exopod; enp, endopod; exp(enp)-1(-2, -3), first(second, third) exopodal(endopodal) segment.

2.4. Amplification and Phylogenetic Analysis

Partial fragments of two nucleic ribosomal RNAs (18S and 28S rRNA) and two mitochondrial genes, cytochrome c oxidase subunit I (COX1) and cytochrome b (CYTB) were amplified from all obtained gDNA templates. The pairs of primers for each amplification were as follows: 18A1 mod and 1800 mod for 18S rRNA gene [28]; 28S-F1a and 28S-R1a for 28S rRNA gene [29]; coxf and coxr2 for COX1 [30]; ucytb151F and ucytb270R for CYTB [31]. The gDNA templates were stored at $-80\ ^{\circ}$C for further study. PCR amplicons were sequenced for both strands on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the same primer sets used for the thermo-cycling at a commercial sequencing service provider, Bionics, Republic of Korea. For the sequencing of the 18S rRNA gene, additional internal primers (F1, CF2, R2, and CR1) [32] were used. All newly obtained sequences in this study were submitted to GenBank and were assigned accession numbers as follows: (I) 18S rRNA gene, OR656936–OR656938; (II) 28S rRNA gene, OR656939 and OR656940; (III) COX1, OR659900–OR659928; (IV) CYTB, OR667755–OR667778 (Table S1).

The chromatograms of both directions were visualized, edited, and assembled in Geneious Prime 2023.2.1 (https://www.geneious.com, accessed on 13 November 2023). The intra- and inter-specific uncorrected *p*-distances for two mitochondrial genes were calculated with two other *Quinquelaophonte* sequences (Table 1) using MEGA X [33] under the complete deletion parameter.

Table 1. GenBank accession numbers of the sequences used for genetic comparison and phylogenetic analyses in this study.

Species	Specimen ID	GenBank Accession Number				References
		18S	28S	COX1	CYTB	
Harpacticoida Sars G.O., 1903						
Quinquelaophonte sominer sp. nov.	sed81-06	OR656936	OR656939	OR659904	**OR667760** [1]	this study
	sed81-07	OR656937	-	OR659905	**OR667761**	this study
	sed81-08	OR656938	OR656940	OR659906	**OR667762**	this study
Q. aurantius Charry, Wells, Smith, Stringer & Tremblay, 2019	MA73574	MH444815	-	MH444814	-	[9]
Q. enormis Kim, Nam, & Lee, 2020	paratype09	-	-	**MT416598**	**MT422734**	[17]
	paratype10	-	-	**MT416599**	**MT422735**	[17]
	paratype11	-	-	**MT416600**	**MT422736**	[17]
	paratype12	-	-	**MT416601**	**MT422737**	[17]
	paratype13	MT410708	MT420735	MT416602	**MT422738**	[17]
	paratype16	-	-	**MT416603**	-	[17]
Paralaophonte congenera (Sars G.O., 1908)	LEGO-HAR027	KR048738	KR048877	KR049011	-	[34]
Pseudonychocamptus spinifer Lang, 1965	97	MF077714	MF077863	MF077898	-	Khodami et al. [2]
Lourinia armata (Claus, 1866)	LEGO-HAR030	KR048739	KR048877	KT030278	-	[34]
Phyllopodopsyllus similis Kim & Lee, 2023	sed86-01	OP923229	OP923708	OP897045	-	[26]
	sed86-02	OP923230	OP923709	OP897046	-	[26]
Calanoida Sars G.O., 1903						
Spinocalanus aspinosus Park, 1970	361	MF796503	MF796484	MF796470	-	[35]
Caudacalanus sp.	352	MF796505	MF796486	MF796472	-	[35]

[1] Sequences were used only to calculate uncorrected p-distances in bold. [2] a retracted study.

To infer the phylogenetic relationships among three *Quinquelaophonte* species (the new species, *Q. enormis*, and *Q. aurantius*), both maximum likelihood (ML) and Bayesian inference (BI) were conducted with two nucleic rRNA genes and COX1 sequences. We retrieved the corresponding sequences of four other laophontids (*Paralaophonte congenera* (Sars G.O., 1908), *Pseudonychocamptus spinifer* Lang, 1965, *Quinquelaophonte aurantius* Charry, Wells, Smith, Stringer & Tremblay, 2019, and *Q. enormis* Kim, Nam & Lee, 2020), two harpacticoids belonging to other families (*Lourinia armata* (Claus, 1868) and *Phyllopodopsyllus similis* Kim & Lee, 2023), and two calanoid copepods (*Caudacalanus* sp. and *Spinocalanus aspinosus* Park, 1970) from GenBank (Table 1). *Spinocalanus aspinosus* was set as the outgroup in both analyses. Sequences of each gene were aligned using MAFFT v7.490 [36] with the E-INS-i algorithm for two nucleic rRNA genes and L-INS-i for COX1. We trimmed sites, including missing data at both ends of each alignment, and then concatenated them into a single matrix in Geneious.

The ML estimation was conducted with IQ-TREE2 v2.2.2.7 [37]. The best partition scheme and best-fit model were selected using ModelFinder plus [38], considering the corrected Akaike information criterion (AICc) by merging partitions with edge-unlinked partition models. The branch support was assessed with 2000 replicates of the standard nonparametric bootstrap.

For BI, the best partition scheme and best-fit model selection were performed using PartitionFinder2 v2.1.1 [39] with branch lengths specified as unlinked under the AICc model selection and greed search algorithm. The characteristics of the concatenated sequences and the selected substitution models for each analysis are shown in Table 2.

Table 2. Substitution models for each partition and sequence characteristics used for phylogenetic analysis.

Fragment	For ML	For BI	Length (bp)	Constant (bp)	Parsimony-Informative (bp)
18S	TIM + F + R2	GTR + I + G	1735	1423	229
28S	GTR + F + G4	GTR + G	792	501	231
COX1	TIM + F + I + R3	GTR + I + G	500	259	210

Bayesian analysis was conducted with Mrbayes v3.2.7a [40]. We simultaneously executed two independent runs of four chains for 20 million generations with a sample frequency of 1000. The convergence of parameters was diagnosed using Tracer v1.7.1 [41]. We checked that the effective sample size of all parameters in the combined trace was greater than 200. Sampled trees were combined into a single file manually, and then a maximum clade credibility tree was constructed using Treeannotator v1.10.4 [42] with ignoring the first 12,000 of the sampled trees as burn-in.

3. Results

3.1. Taxonomical Account

Order Harpacticoida Sars G.O., 1903.
Family Laophontidae Scott T., 1904
Genus *Quinquelaophonte* Wells, Hicks & Coull, 1982
Type species *Quinquelaophonte quinquespinosa* (Sewell, 1924)

3.2. Quinquelaophonte sominer Kim & Lee, 2023 sp. nov.

Zoobank registration
https://zoobank.org/086C6A99-3907-429B-B048-103CA5FE9BB0 (accessed on 13 November 2023).

Type locality
Daejeong-eup, Seogwipo, Jeju Island, Republic of Korea (33°13.61′ N, 126°14.44′ E), intertidal zone, silty sand, sediment temperature: 27.7 °C.

Material examined

Holotype (NIBRIV0000909808) adult female, dissected and mounted onto eight slides. Paratypes 2–31 (NIBRIV0000909809–NIBRIV0000909839) fifteen adult females and sixteen adult males. All specimens were collected from the type locality on 30 April 2020 by Jaehyun Kim. For detailed information on type materials, see Table S1.

Etymology

The trivial name '*sominer*' is an anagram of '*enormis*'. We adopted the anagram to reflect the close resemblance of the new species to *Q. enormis* Kim, Nam & Lee, 2020.

Description of adult female

Body (Figures 2 and 3) length 743–804 μm (n = 10, mean = 772 μm; measured from the anterior tip of the rostrum to the posterior margin of caudal rami in lateral view); nine-segmented, gradually tapering posteriorly, slightly depressed, with sensilla and/or cuticular pores, covered with minute integumental ornaments throughout body surface. Tergites of second to fifth pedigers somewhat concave dorsally. Hyaline frills on all somites microserrate sparsely and rudimentary, except for cephalothorax (Figures S3 and S4).

Figure 2. *Quinquelaophonte sominer* sp. nov., confocal laser scanning microscope images, female (Paratype 12). (**Left**) habitus, dorsal view; (**Middle**) habitus, lateral view; (**Right**) habitus, ventral view. Scale bar = 100 μm.
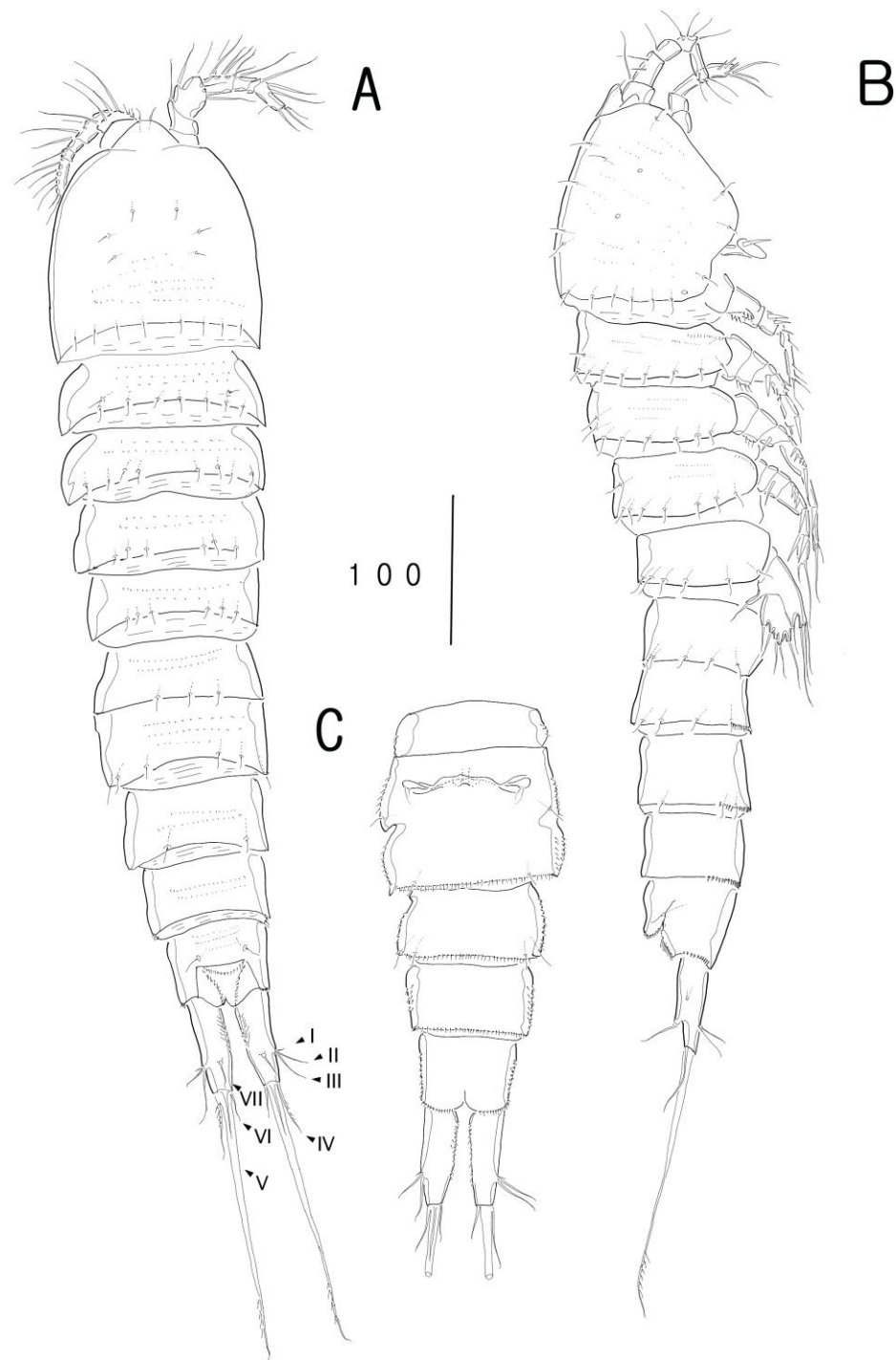
Figure 3. *Quinquelaophonte sominer* sp. nov., female (holotype). (**A**) habitus, dorsal view (I, antero-lateral accessory seta; II, dorsolateral seta; III, ventrolateral seta; IV, outer terminal seta; V, principal terminal seta; VI, terminal accessory seta; VII, dorsal set); (**B**) habitus, lateral view; (**C**) urosome, ventral view. Scale bar in μm.

Prosome (Figures 2 and 3A,B) four-segmented, comprising cephalothorax and three free pedigerous somites, wider than urosome. Cephalothorax slightly shorter than all succeeding prosomites combined. Lateral end of cephalic shield somewhat extended medially, bent toward ventral side. Free pedigerous somites nearly equal in length. Rostrum subtriangular, rounded at apex, concave medially, fused partially to cephalothorax, with two sensilla near apex. Eye not discernible.

Urosome (Figures 2 and 3B,C) five-segmented, somewhat longer than prosome, comprising fifth pedigerous somite, genital double-somite, two abdominal segments and anal somite armed with caudal rami. All urosomites, except genital double-somite, almost equal in length. Hyaline frills on urosomites with row of spinules ventrally, excluding first urosomite (Figures S3 and S4). Genital double-somite longest, with P6; original segmentation vestigial, marked by dorsal and lateral ridge, ventral suture, and pattern of surface ornamentation; genital field with single seminal receptacle. Anal operculum covered densely with microspinules dorsally, with row of minute setules terminally, flanked by sensillum on each side.

Caudal rami (Figures 2 and 3B,C) elongate, tapering posteriorly, about three times as long as maximum wide, with tube pore on outer distal corner ventrally; anterolateral accessory seta (I) tiny; dorsolateral seta (II) about four times as long as seta I; ventrolateral seta (III) long; outer terminal seta (IV) naked, about 2.5 times as long as terminal accessory seta (VI); principal terminal seta (V) with minute pinnules on distal half, about as long as penultimate urosomite, anal somite and caudal rami combined; seta VI short, bare, inserted in inner distal corner; dorsal seta (VII) triaticulate at base, located on distal third of ramus.

Antennule (Figure 4A) six-segmented, covered with minute integumental ornamentations; first segment with inner seta on anterior corner, with row of spinules near base of seta; second segment longest, about 1.3 times as long as wide, with small integumental protuberance on outer margin; third segment slightly shorter than preceding one, about 1.5 times as long as wide; fourth segment with apical aesthetasc fused basally to long seta arising from ventral pedestal; fifth segment shortest; sixth segment about as long as third one, with terminal aesthetasc fused basally to two setae. Armature formula as follows: 1(1), 2(8), 3(7), 4(1+(ae+1)), 5(1), 6(9+(ae+2)).

Antenna (Figure 4B) consisting of coxa, allobasis, endopod, and exopod. Coxa short, unarmed. Allobasis about 2.5 times as long as wide, with longitudinal row of spinules on proximal half of inner margin, with tiny abexopodal seta inserted at mid-length. Endopod one-segmented, broadening distally, about as long as allobasis, with bunch of spinules along inner margin, with two spines on distal part of inner margin, of which distal spine longer than proximal one, with row of spinules transversely along terminal margin, with two bare spines, three geniculate setae, and slender seta. Exopod arising from proximal third of allobasis, one-segmented, small, with three thready setae.

Mandible (Figure 4C) consisting of coxa, basis, endopod and exopod. Coxa enlarged, unarmed, and with well-developed gnathobase bearing distally multi-dentate cutting edge. Basis much smaller than preceding segment, with seta terminally. Endopod and exopod reduced beyond recognition; endopod represented by three setae; exopod represented by hairy seta.

Maxillule (Figure 4D) consisting of praecoxa, coxa, basis, endopod, and exopod. Praecoxa with row of spinules on posterior surface, with strongly developed arthrite bearing naked seta and six spines of various types (e.g., dentate, cuspidate, and pinnate) apically; boundary between praecoxa and arthrite wrinkled. Coxa with cylindrical endite, with bunch of integumental elements on posterior surface; coxal endite with spinules on terminal margin anteriorly, with two terminal setae; posterior terminal seta pinnate, longer than anterior one. Basis with cylindrical endite; basal endite with several rows of spinules on anterior surface and ventral margin, with pinnate seta and two bare setae. Endopod fused to basis completely, with three naked setae. Exopod one-segmented, small, with two bare setae, of which inner seta much longer than outer seta.

Maxilla (Figure 4E) consisting of syncoxa, allobasis, and endopod. Syncoxa with several rows of spinules on proximal part of posterior surface, with row of long spinules along outer distal corner, with three endites; praecoxal endite small, with pinnate armature; proximal coxal endite with strong pectinate spine and two setae, of which posterior seta incorporated into endite; distal coxal endite with three setae, of which most posterior seta fused to endite basally. Allobasis transformed into strong pinnate curved claw, with row

of spinules on anterior surface, with three bare setae. Endopod reduced completely and represented by three naked setae, of which most posterior seta minute.
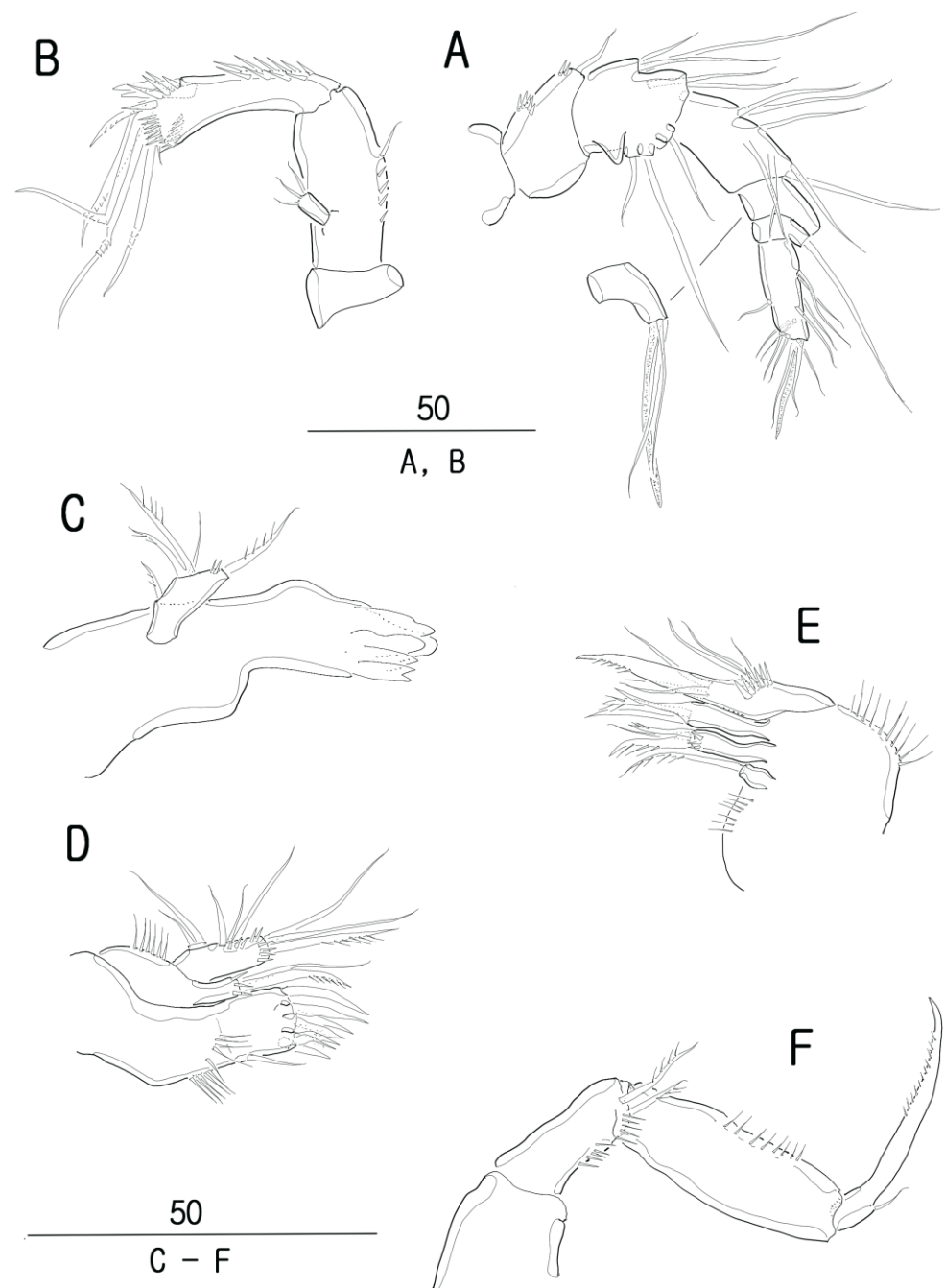

Figure 4. *Quinquelaophonte sominer* sp. nov., female (holotype). (**A**) Antennule; (**B**) Antenna; (**C**) Mandible; (**D**) Maxillule; (**E**) Maxilla; (**F**) Maxilliped. Scale bars in μm.

Maxilliped (Figure 4F) subchelate, consisting of syncoxa, basis and endopod. Syncoxa about twice as long as greatest wide, with several rows of spinules, with two setae on anterior surface terminally, of which inner seta pinnate and longer than outer one. Basis elongate, unarmed, about 1.5 times as long as preceding segment, about three times as long as greatest wide. Endopod one-segmented, reduced, with endopodal claw and accessory seta.

P1–P4 (Figures 5 and 6A,B) biramous, with three-segmented protopod (praecoxa, coxa, and basis), endopod, and exopod. Praecoxa well-developed, with anterior spinular surface ornamentation on outer margin terminally, except P1. Coxa broader than long, with

intercoxal sclerite broader than coxa (not illustrated in P1), with spinules around outer margin. Basis of P2–P4 about as long as coxa, broader than long, with outer armature arising from cylindrical extension, with anterior row of spinules on outer margin, with cuticular pore near anterior spinular ornamentation. Endopod two-segmented, shorter than exopod except P1, with setules on inner margin of each segment; enp-1 unarmed. Exopod three-segmented except P1, with several rows of spinules; exp-1 with outer spine, without inner seta.
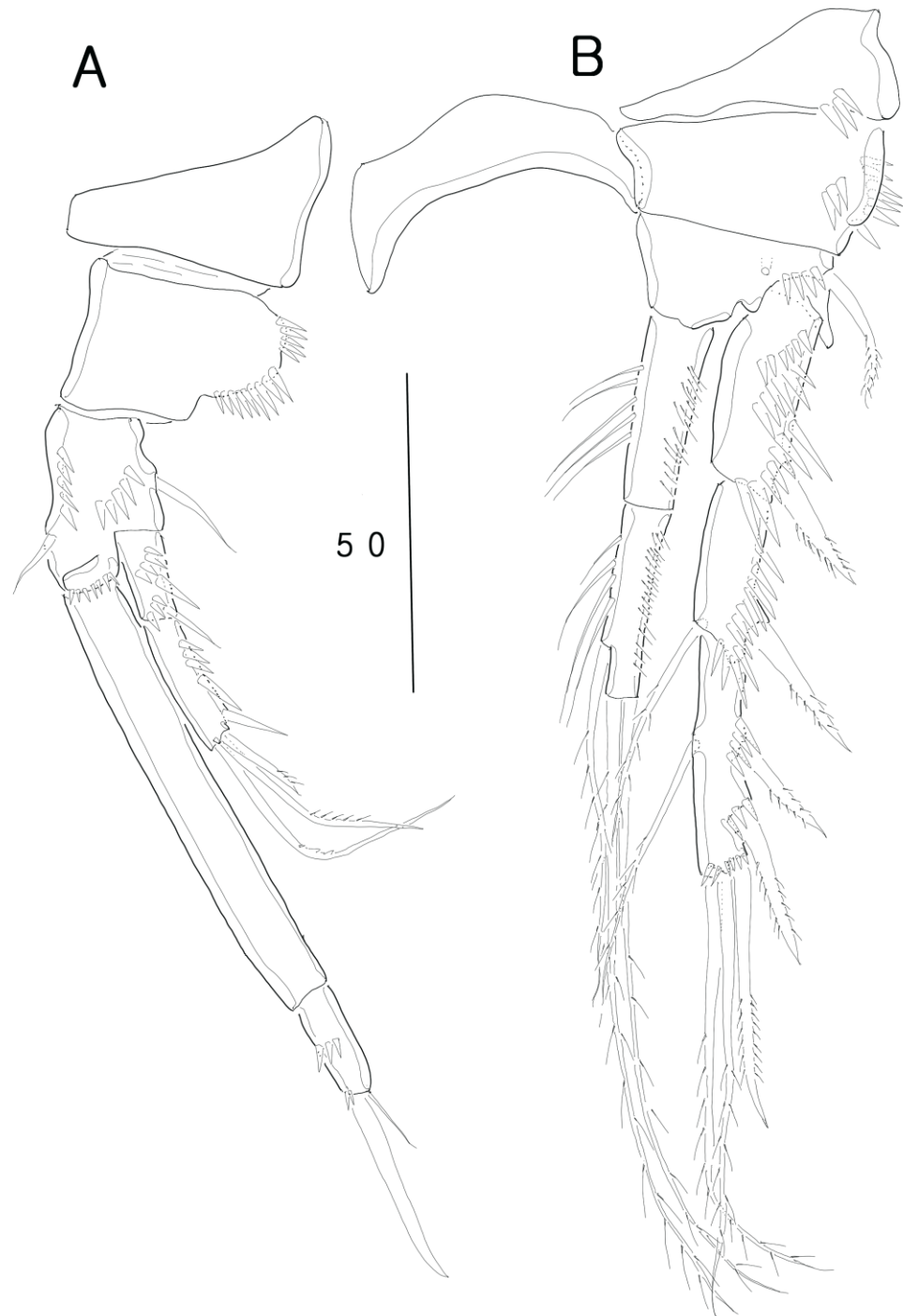

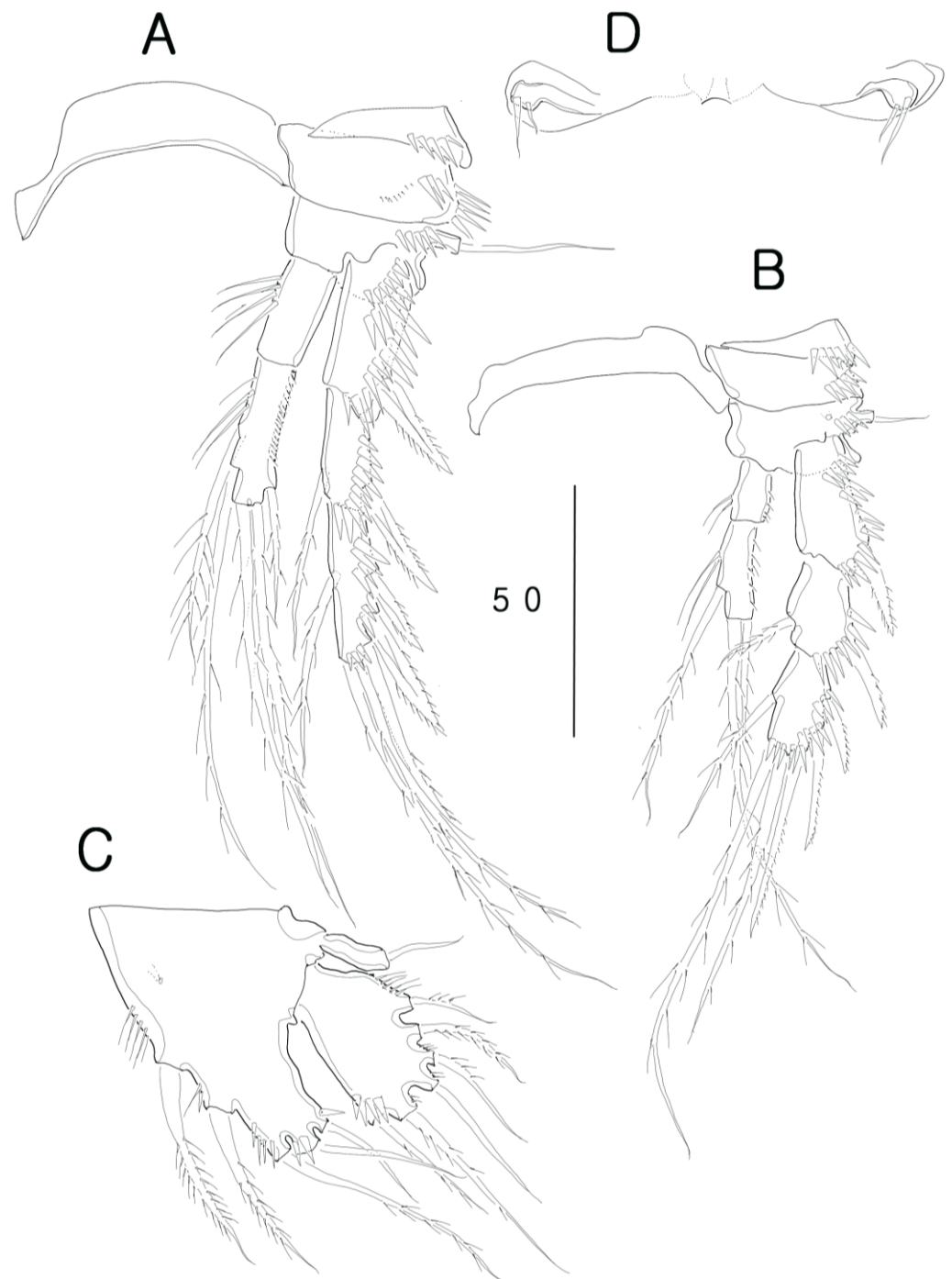**Figure 5.** *Quinquelaophonte sominer* sp. nov., female (holotype). (**A**) P1; (**B**) P2. Scale bar in μm.

Figure 6. *Quinquelaophonte sominer* sp. nov., female (holotype). (**A**) P3; (**B**) P4; (**C**) P5; (**D**) P6. Scale bar in μm.

P1 (Figure 5A) Coxa about 1.6 times broader than succeeding segment. Basis about 1.3 times as long as greatest width, slightly longer than coxa, with inner and outer elements; inner element located on distal quarter of outer anterior surface; outer element inserted in midlength of outer margin, arising from pedestal; integument of basis around boundary with endopod sclerotized, convex, with blunt frills terminally. Endopod prehensile, two-segmented; enp-1 elongate, about six times as long as wide; enp-2 about quarter of enp-1 in length, with spinules on terminal margin, with claw and thready seta apically. Exopod two-segmented, about 0.4 times as long as endopod, reaching about middle of enp-1; exp-2 about 1.3 times longer than exp-1, with three outer spines and two terminal geniculate

setae; inner terminal seta longer than outer one; most proximal spine inserted in midlength; distal two spines located near terminal corner.

P2 (Figure 5B) Endopod reaching about half of exp-3; enp-1 about three times as long as greatest wide, with tube pore on anterior end; enp-2 somewhat longer than preceding segment, about 4.5 times as long as maximum wide, with three long plumose setae (inner seta and two terminal setae). Exp-2 with outer bipinnate spine; exp-2 with inner pinnate seta inserted near distal corner; exp-3 with six armatures (three outer bipinnate spines, two apical plumose setae, and inner pinnate seta); inner one of two terminal setae slightly longer than outer; length ratio from exp-1 to exp-3, 1:0.8:1.

P3 (Figure 6A) Endopod reaching about proximal part of exp-3; enp-1, about twice as long as greatest wide; enp-2 about 1.3 times longer than enp-1, about 2.5 times as long as maximum wide, with five setae (two inner setae, two terminal setae, and outer seta); inner setae plumose, of which proximal one inserted in midlength; terminal setae plumose, longer than inner setae; outer seta bipinnate, shortest among endopodal armatures of P3. Exp-2 with inner pinnate seta inserted near distal corner, with outer bipinnate spine; exp-3 with six armatures (three outer bipinnate spines, two apical plumose setae, and inner pinnate seta); inner one of two terminal setae slightly longer than outer; length ratio from exp-1 to exp-3, 1:0.8:1.

P4 (Figure 6B) Endopod reaching at least midlength of exp-2; enp-1 slightly longer than wide; enp-2 about 1.5 times as long as enp-1, about 2.8 times as long as wide, with three plumose setae (inner seta and two terminal setae); inner setae inserted in distal third; outer terminal setae about half of inner terminal one in length, shortest among endopodal armatures of P4. Exp-2 with outer bipinnate spine, with inner pinnate seta inserted near distal corner; exp-3 with six armatures (three outer bipinnate spines, two apical plumose setae, and inner pinnate seta); inner one of two terminal setae slightly longer than outer; length ratio from exp-1 to exp-3, 1:0.8:1.

P5 (Figure 6C) comprising baseoendopod and exopod, with separated pair, with microspinules throughout surface but pattern of arrangement indefinable. Baseoendopod with basal seta arising from long articulated setophore, with setules and spinules on inner margin and terminal margin, with five setae (two inner and three terminal setae); inner setae bipinnate; innermost terminal seta longest; outermost seta shortest. Exopod exceeding end of besoendopod, not fused to basis, semi-ovoid, with spinules on inner distal corner, with minute setules along outer margin, with six setae, of which second innermost longest.

P6 (Figure 6D) reduced, represented by small segment, with two setae on each ramus; inner seta shorter than outer.

Description of adult male

The body ornamentation, the segmentation of the urosome antennule, and all swimming legs were sexually dimorphic.

Body (Figures 7 and 8A) length 671–761 μm (n = 15, mean = 725 μm; measured from the anterior tip of the rostrum to the posterior margin of caudal rami in lateral view; ten-segmented. Habitus largely as in female but urosome somewhat slender.

Urosome (Figures 7 and 8A) six-segmented, consisting of fifth and sixth pedigerous somites, three free abdominal somites, and anal somite armed with caudal rami. Anal somite and caudal rami as in female.

Antennule (Figure 8B) eight-segmented, subchirocer, with geniculation located between fifth and sixth segments. First segment with two spinular rows on dorsolateral margin and distal of inner side, respectively, with bare seta near inner row of spinules. Second segment about 1.5 times as long as greatest wide, with three setae on posterior margin dorsally and six setae located anteriorly; most proximal one of posterior setae shortest; middle proximal seta longest; two of anterior setae inserted near distal margin; four of anterior setae located dorsal and ventral surface, two on each side. Third segment with seven setae. Fourth segment shortest, with two setae. Fifth segment swollen, enlarged, with 13 elements, of which six on anterior margin and seven on ventral surface; two setae of ventral elements inserted medially, and other five (three setae and aesthetasc fused basally

to seta on pedestal) located distally. Sixth segment with three spinous processes on anterior surface. Seventh segment short, with naked seta on anterior corner terminally. Eighth segment with 11 armature elements; spiniform armature and seta located on anterior margin; six biarticulate setae inserted from proximal to midlength of outer margin; aethetasc fused to two naked setae at base, located on distal third. Armature formula: 1-[1], 2-[9], 3-[7], 4-[2], 5-[8 + 1 pectinate armature + 1 hook-like armature + 1 rod-like process + (ae + 1)], 6-[3 spinous processes], 7-[1], 8-[7 + 1 spiniform armature + (ae+2)].

Figure 7. Habitus of *Quinquelaophonte sominer* sp. nov., confocal laser scanning microscope images, male (paratype 5). (**Left**) habitus, dorsal view; (**Middle**) habitus, lateral view; (**Right**) habitus, ventral view. Scale bar = 100 μm.

Antenna as in female.
Mandible as in female.
Maxillule as in female.
Maxilla as in female.
Maxilliped as in female.
P1 as in female except for integument of basis around boundary with endopod; sclerotized integument without frills.
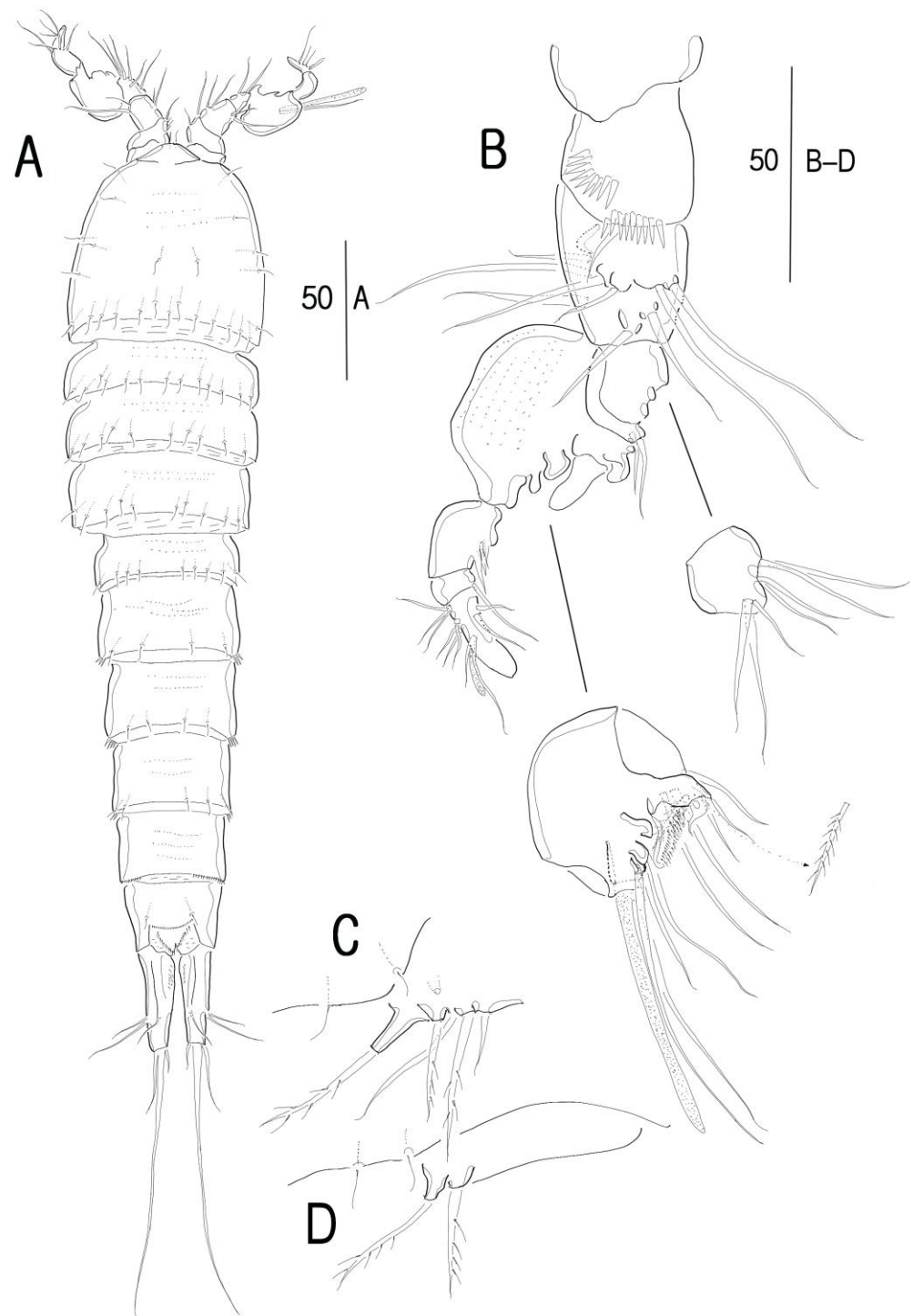
Figure 8. *Quinquelaophonte sominer* sp. nov., male (paratype 6). (**A**) habitus, dorsal view; (**B**) Antennule; (**C**) P5; (**D**) P6. Scale bar in μm.

P2–P4 (Figure 9) largely as in female. Exopod more robust than in female; inner seta of exp-2 inserted in distal quarter and much shorter than in female.
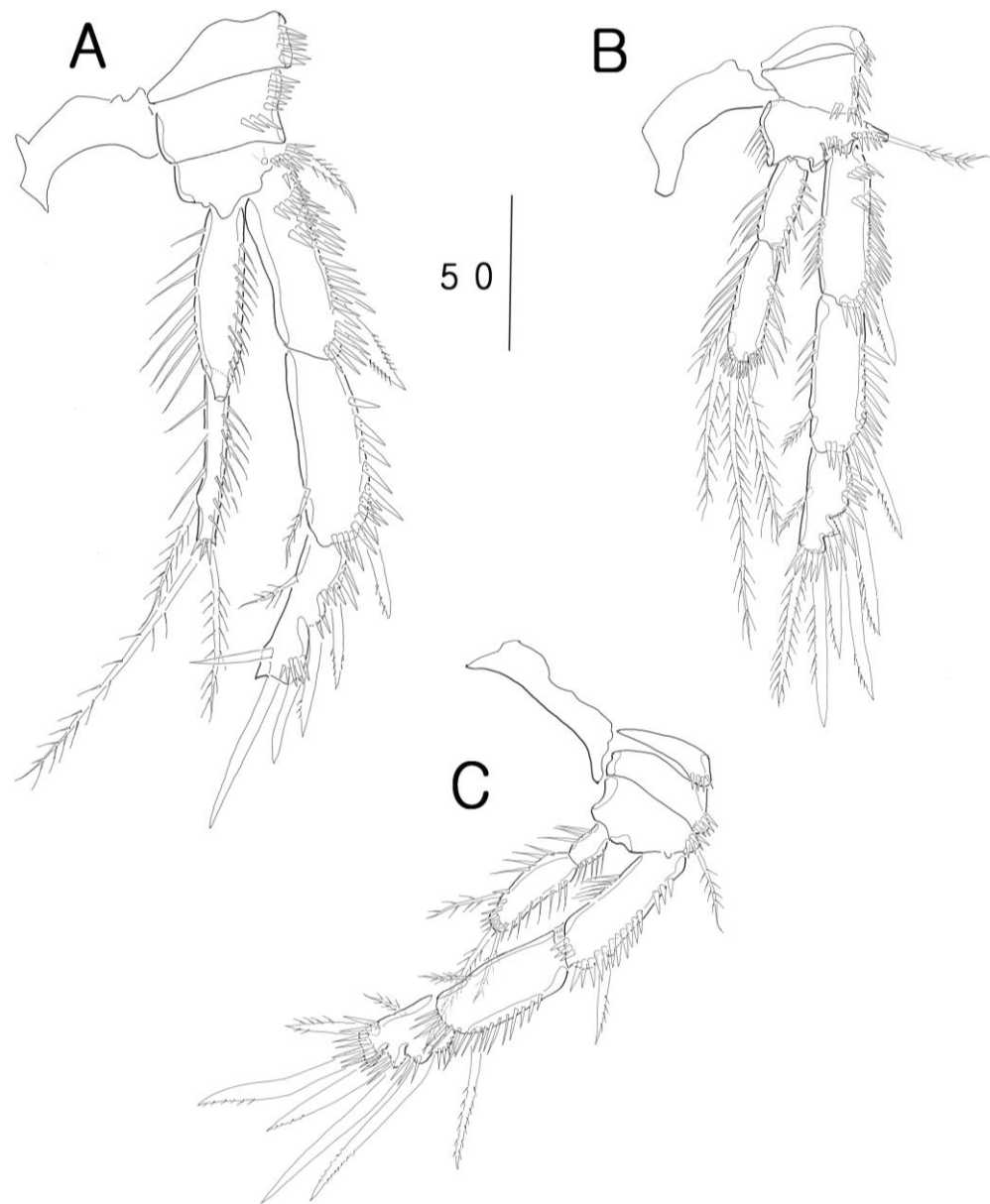
Figure 9. Line drawings of *Quinquelaophonte sominer* sp. nov., male (paratype 6). (**A**) P2; (**B**) P3; (**C**) P4. Scale bar in μm.

P2 (Figure 9A) Intercoxal sclerite as broad as coxa. Endopod reaching about end of exp-2; enp-1 about four times as long as wide, with tube pore (more conspicuous than in female); enp-2 nearly as long as enp-1, about 6.5 times as long as greatest wide; all armatures on enp-2 generally shorter compared to female. Exp-3 with six armatures as in female, but with two apical spines, of which inner one much shorter than outer; inner seta of exp-3 much shorter than in female. Length ratio of from exp-1 to exp-3, 1:0.9:0.8.

P3 (Figure 9B) largely as in female. Endopod reaching about midlength of exp-2; enp-1 about 2.4 times as long as greatest width; enp-2 about three times as long as greatest width; all armature elements on enp-2 generally shorter compared to female; outer armature transformed into spine-like process. Exp-3 with six armatures as in female, but with two apical pinnate spines; inner terminal spine somewhat shorter than outer; inner seta of exp-3 much shorter than in female. Length ratio from exp-1 to exp-3, 1:0.8:0.6.

P4 (Figure 9C) largely as in female. Endopod not reaching midlength of exp-2; enp-1 about 1.5 times as long as greatest width; enp-2 about 2.3 times longer than enp-2, about three times as long as maximum width; all armatures of enp-2 generally shorter compared

to female. exp-3 with six armatures as in female, but with two apical spines; inner apical spine much shorter than outer; inner seta of exp-3 much shorter than in female. Length ratio from exp-1 to exp-3, 1:0.8:0.5.

P5 (Figure 8C) rudimentary, fused to distal edge of somite, with four setae, with elongate setophore bearing basal seta; innermost seta and second outermost seta thready; innermost seta shortest; second innermost seta longest.

P6 (Figure 8D) asymmetrical (with sinistral and dextral configurations), represented by small plate, with two setae on outer corner; outer seta much longer than inner one.

Variability

Intra-specific variation was present in the chaetotaxy of the P2–P4 and male P6. Ten of the 16 female individuals examined had two inner setae on the P3 enp-2, while four had only one. On the P4 enp-2, 13 females had an inner seta, and two had none. Male's swimming legs also exhibited setal variation, which differs slightly from that of females. Of the 16 males we examined, eight had a single inner seta on the P3 enp-2, whereas six had two inner setae. The P4 enp-2 lacked inner seta in nine of the 16 male individuals (one had a broken right ramus), but an inner seta was present in six individuals. Both sexes also displayed intra-individual variation in the P2–P4. Out of 16 males, ten had an articulated plate of P6 on the right side, while six had it on the left. For the detailed armature formula of the P2–P4, and the position of the articulated plate of the male P6 for each individual, see Table S2.

Morphological abnormality was observed in the P4 exopod of a single female individual (paratype 9; not illustrated). The left terminal segment of the abnormal exopod had one inner seta, three-terminal armatures, and one outer spine. Compared to the normal condition, the number of armatures was one less (two fewer outer spines and one more terminal seta). Additionally, the innermost terminal seta was slightly swollen at the base.

3.3. Molecular Data and Phylogenetic Analysis

We obtained at most 3820 bp for each specimen, including 2639 bp nuclear (18S rDNA:1758 bp; 28S rDNA: 881 bp) and 1181 bp mitochondrial (COX1: 820 bp; CYTB: 361 bp) sequences. The GenBank accession numbers of all sequences obtained in this study are provided in Table S1. The intra-specific distance of *Quinquelaophonte sominer* sp. nov. was 0.002 for COX and 0.001 for CYTB. The uncorrected distances based on the COX1 sequences between the new species and two congeners (*Q. enormis*/*Q. aurantius*) were 0.226 between *Q. sominer* and *Q. enormis* and 0.207 between *Q. sominer* and *Q. aurantius*. The mean *p*-distance for the CYTB gene fragment between *Q. sominer* and *Q. enormis* was 0.310.

The phylogenetic trees (Figure 10) from the ML and BI based on the combined dataset are identical in topology. All of the nodes in both trees were well supported. A minimum bootstrap support value in the ML tree was 77 for the relationship between *Q. enormis* and *Q. aurantius*, and maximal supports (posterior probability = 1.00) were found for all clades in the BI tree. The new species was grouped with a clade of two other *Quinquelaophonte* species.
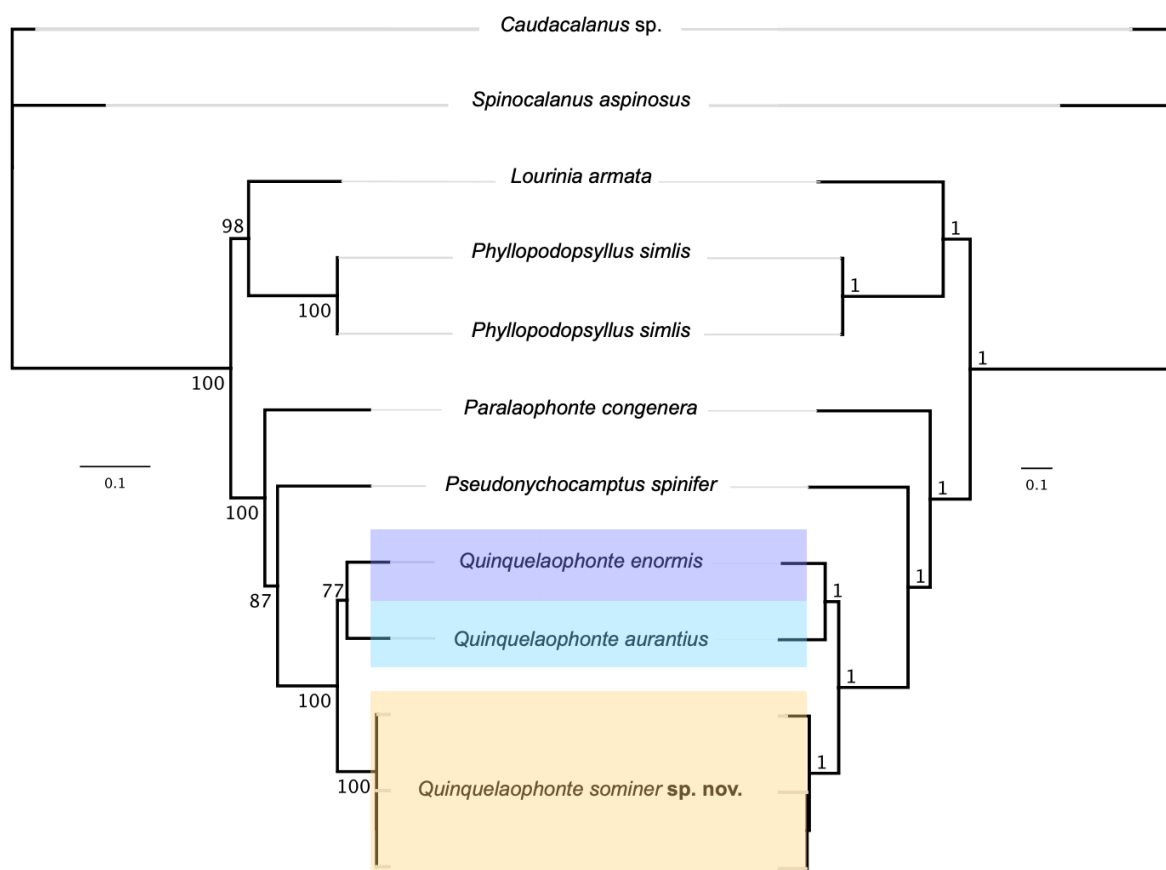
Figure 10. Two phylogenetic trees based on the concatenated matrix. (**Left**) maximum likelihood tree; (**Right**) maximum clade credibility tree of Bayesian inference. Scale bars = substitutions per site.

4. Discussion

4.1. Taxonomic Remarks

Quinquelaophonte sominer sp. nov. is most similar to species that are characterized by long caudal rami (length/breadth ≥ 3), the number of setae on the exopod of the antenna (three setae), a minute accessory seta on the P1 enp-2, and the number of armature elements on the P3 exp-3 (less than seven). These morphological characteristics of the new species are shared with *Q. aestuarii* [15], *Q. aurantius* [9], *Q. capillata* [10–12,24], *Q. enormis* [17], *Q. longifurcata* [43], *Q. parasigmoides* [6,7], and *Q. varians* [14].

The new species can be distinguished from *Q. aestuarii* by the number of setae on the mandibular palp and the gnathobase. *Quinquelaophonte aestuarii* possesses seven setae on the palp and two on the gnathobase (versus five setae on the palp and setae lacking on the gnathobase in *Q. sominer*).

The northern Atlantic populations of *Q. capillata* [11,12] can be differentiated as follows: the outer element on the second endopodal segment of the male P3 is a seta in *Q. capillata*, but an acute and short spine-like process in the new species; the setae on the second endopodal segment of the male P4 are almost equal in length in *Q. capillata*. However outer terminal seta is about half of the inner terminal one in the new species. *Quinquelaophonte capillata* recorded from north-western Mexico [24] also differs from the new species by the relative length of the setae on the male P4 as the Atlantic populations. Additionally, the new species is distinct from the Mexican *Q. capillata* as follows: the new species possesses five setae on the mandibular palp, whereas four setae in the Mexican *Q. capillata*; the new species has two setae on the syncoxa of maxilliped, but only single seta in the Mexican *Q. capillata*.

The number of setae on the syncoxa of the maxilliped is also different from *Q. varians*, which has a single seta on the segment (vs. two setae in *Q. sominer*). Moreover, these two

species also show differences in the chaetotaxy of the male P3–P4. An inner seta is present on the distal endopodal segment of both P3 and P4 in the male of *Q. varians*. However, this set of the setal arrangement is not observed in the new species, except for intra-individual variations.

Three other Pacific species, *Q. enormis*, *Q. aurantius*, and *Q. longifurcata*, are closely related to the new species. On the other hand, these species share, except for their variation within a single individual, the lack of an inner seta on the third exopodal segment of the male P4, which contrasts them from the new species. In addition, *Q. longifurcata* differs from the others as follows: an outer armature is present on the distal segment of P4 endopod in both sexes of *Q. longifurcata* (vs. absent in *Q. aurantius*, *Q. enormis*, and *Q. sominer*); the syncoxa of maxilliped bears a seta on the terminal margin (vs. two setae). *Quinquelaophonte enormis* also exhibits a suit of characteristics that allow it to be readily distinguished from *Q. aurantius*, *Q. longifurcata*, and the new species: the characteristics are devoid of an inner seta on the terminal exopodal segment of both P3 and P4 in females, possessing no inner seta on the second exopodal segment of P4 in both sexes with one exception in a single female specimen.

A western Indian species, *Q. parasigmoides*, is distinct from the new species due to the setal arrangement of the P3 endopod. *Quinquelaophonte parasigmoides* has an inner seta on the P3 enp-1 in both sexes and six armatures on the P3 enp-2 in females. In contrast, the new species has no inner seta and, at most, five setae, respectively.

4.2. Difficulties of Morphological Analysis for Quinquelaophonte Species

Including the new species, the aforementioned *Quinquelaophonte* species were superficially very similar in body structure. The habitus showed subtle differences, such as body length and the length/width ratios of caudal rami. This difficulty can make it hard to recognize diagnostic characteristics from each specimen under a stereo microscope. At the same time, inter- and/or intra-individual variation occurs in the chaetotaxy on the swimming legs of some *Quinquelaophonte* species, e.g., *Q. aurantius*, *Q. candelabrum*, *Q. longifurcata* [1,9,43]. The new species exhibits two sets of the setal arrangement patterns on the endopod of the P3 and P4 in males ('221, 120' and '121, 020'; Table S2). The arrangement of armatures on the swimming legs is the most commonly used morphological characteristic for identifying harpacticoid species [3,4,25,43]. However, the chaetotaxy could become inapplicable for identifying *Quinquelaoophonte* species if additional species with complex morphological variability are discovered.

In some species of the genus *Quinquelaophonte*, including the new species, body surfaces are covered with a layer of fine debris or particles [1,9]. It can also be challenging to examine specimens on a temporary mount, so dissecting the cephalic and thoracic appendages of more individuals than necessary for exact species identification may be unavoidable.

4.3. Molecular Data and Phylogeny

The two mitochondrial genes, COX1 and CYTB, of the new species presented low intra-specific variability, regardless of the various patterns of the pereiopodal chaetotaxy. It demonstrated that the specimens studied in this study belonged to a single species, not a species complex. Additionally, the COX1 sequences of the new species and two congeners (*Q. aurantius* and *Q. enormis*) differed by ~137 bp and ~150 bp out of 654 bp, respectively, and in the CYTB sequences, 113 bp out of 361 bp were different between the new species and *Q. enormis*. The results of these analyses, in addition to the preceding morphological comparisons, strongly indicate that *Q. sominer* was not conspecific with either of the two species, and each of these species had a long independent evolutionary history.

To assess the phylogenetic relationship among the new species and two other Pacific *Quinquelaophonte* species whose genetic information had been previously reported, we conducted phylogenetic analyses based on the concatenated matrix of mitochondrial (COX1) and two nuclear (18S rRNA and 28S rRNA) genes utilizing the ML and the BI.

The new species was unambiguously embedded within the family Laophontidae in both phylogenetic trees (Figure 9). Our phylogenetic analyses inferred that *Q. sominer* was sister to two congeners with strong support (bootstrap support value: = 100; posterior probability = 1.00; Figure 9). This is reflected in their setal arrangement on the endopod of the male P4. However, due to the limited available information, it is premature to suggest a phylogeographic history and evolutionary relationships among the examined species. However, we noticed that *Q. enormis* is geographically closer to the new species (Republic of Korea [17]) than *Q. aurantius*, which was reported from the opposite hemisphere (New Zealand [9]). In Korean fauna, in addition to these two species, there is one more species, *Q. koreana*, which is intuitively distinguishable (e.g., short caudal rami, two setae on the exopod of the antenna, two inner setae on the P3 exp-3 in both sexes, four armatures on the distal endopal segment of the P4 in both sexes, and the shape of the female P5) and was reported from the coast of the Yellow sea [16]. This seemed to imply that there could be much more hidden diversity in this genus yet to be discovered.

5. Conclusions

In this study, we collected laophontid harpacticoids belonging to the genus *Quinquelaophonte* from Jeju Island in Republic of Korea. These organisms were superficially indistinguishable from morphologically similar congeneric species in habitus and exhibited complex variability in their pereiopods, with two distinct types of chaetotaxy in males. We confirmed that these *Quinquelaophonte* individuals were conspecific and distinct from congeners using an integrative approach, including morphological and genetic data. The discovery of the new species is the third in the Northwest Pacific, following *Q. koreana* (Taean, Republic of Korea [16]) and *Q. enormis* (Busan, Republic of Korea [17]). Moreover, we inferred the phylogenetic relationships among the new species and two congeners (*Q. aurantius* and *Q. enormis*). The analyses showed that the new species had a sister relationship with two Pacific congeners.

The genus *Quinquelaophonte* is facing multiple challenges, such as a lack of genetic information, widely distributed species, undiscovered diversity, and complex morphological variability. To resolve this situation, future taxonomic descriptions should be based on a thorough morphological analysis along with additional available sources, e.g., molecular data, biogeography, behavior, and ecology [44–46]. Our study documented the chaetotaxy of the swimming legs of all individuals and the variation in the position of articulated lappets of the male P6 through careful examinations. We hope that this study might serve as a first step to stimulate future intensive taxonomic work on the genus *Quinquelaophonte*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15121168/s1, Figure S1: Measurement of the body length of female individuals of *Quinquelaophonte sominer* sp. nov., CLSM images; Figure S2: Measurement of the body length of male individuals of *Quinquelaophonte sominer* sp. nov., CLSM images; Figure S3: *Quinquelaophonte sominer* sp. nov., SEM images, female (Paratype 15); Figure S4: *Quinquelaophonte sominer* sp. nov., SEM images, female (Paratype 4); Figure S5: *Quinquelaophonte sominer* sp. nov., SEM images, female (Paratype 14); Table S1: Information of type specimens of *Quinquelaophonte sominer* sp. nov. and the Genbank accession number of 18S, 28S, COX1, and CYTB; Table S2: Armature formula of the segment on P2–P4 displaying variability in the chaetotaxy and the position of the articulated male P6 plate in each individual of *Quinquelaophonte sominer* sp. nov.

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