

Article

A New Species of the Genus *Robertgurneya* Apostolov & Marinov, 1988 (Copepoda: Harpacticoida: Miraciidae) from a Sublittoral Zone of Jeju Island, Korea

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Abstract: A new species, *Robertgurneya jejuensis* sp. nov., was described from sandy sediment samples collected at a depth of 25 m on Mun Island, Jeju, in June 2018. The new species is morphologically similar to *Robertgurneya similis similis* (Scott A., 1896) and *Robertgurneya donghaensis* Bang, 2021; this is the second record of the genus *Robertgurneya* in South Korea. The morphological characteristics of the *similis* group within the genus *Robertgurneya*, to which the new species is ascribed, are summarized here. Furthermore, an identification key is provided based on the summary. Molecular identification of the collected specimens, based on the nuclear 18S ribosomal RNA (18S rRNA) and the mitochondrial cytochrome oxidase subunit I fragment, was obtained. Finally, a phylogenetic tree was constructed to present the position of *Robertgurneya* within the Miraciidae family based on 18S rRNA sequences which is relatively conserved. As a result, the relationship with sister genera morphologically similar to *Robertgurneya* was also molecularly confirmed.

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Keywords: taxonomy; Crustacea; meiofauna

1. Introduction

Miraciidae Dana, 1846, is a large family of benthic copepods, Harpacticoida [1]. It includes three subfamilies, 64 valid genera, and 506 species (WoRMS Editorial Board 2020), most of which were transferred from the former Diosaccidae family by Willen in 2002 [2]. In South Korea, 20 genera and 36 miraciid species identified at the species level have been reported to date, e.g., [3–8].

Sediment samples were collected from the sandy sediment in the subtidal zone at 25 m to investigate meiofauna. As a result, we collected a new species of *Robertgurneya* Apostolov & Marinov, 1988, on Mun Island, Jeju. The new species, *Robertgurneya jejuensis* sp. nov., is morphologically similar to the type species of *Robertgurneya similis similis* (Scott A., 1896) and the latest species, *Robertgurneya donghaensis* Bang, 2021. Morphological differences between these species and the new species were found in the number of setae in the mouth, the length ratio of A1 segment, the difference in type and relative length of setae, and the presence or absence of ornamentation on appendages. Currently, 19 valid species (and subspecies) exist worldwide in *Robertgurneya*; this is the second record of *Robertgurneya* in South Korea after the report of *R. donghaensis* [9].

The type species and diagnosis of genus *Robertgurneya* were established in 1988 [10] and the genus was formally named according to the International Commission on Zoological Nomenclature; however, this genus was first proposed by Lang in 1944 [11]. He divided the genus into two species groups and designated the type of each group as follows: *similis* group (type: *Stenhelia simulans* Norman & Scott T., 1905) and *spinulosus* group

(type: *Amphiascus spinulosus* Sars G.O., 1911). Lang (1948) [12] also commented on dividing these two groups into separate genera [13]. Gómez (2020) [14] supported this incomplete opinion by proposing the new genus *Robertgurneyella* Gómez, 2020, setting *Amphiascus spinulosus* Sars G.O., 1911 as the type species and suggested that the original *spinulosus* group was renamed the *rostrata* group.

This study summarizes the contents of comparing the morphological characteristics between the constituent species of the *similis* group to which the new species belongs. The identification key is presented based on this. In addition, the gene sequences obtained in this study are the first reported sequences in *Robertgurneyella*. Finally, a phylogenetic analysis was conducted to investigate the gene sequence-based phylogenetic relationship between *Robertgurneyella* and other genera in Miraciidae uploaded to NCBI.

2. Materials and Methods

2.1. Specimen Collection and Observation

Coarse sandy sediment samples from 25 m deep were collected from Mun Island, Jeju, South Korea (33°13'38.4" N 126°33'49.3" E) (Figure 1) by SCUBA diving in June 2018. Sandy sediments were collected by using acrylic corers and these were filtered through a 38 µm sieve and fixed with 99% ethanol.

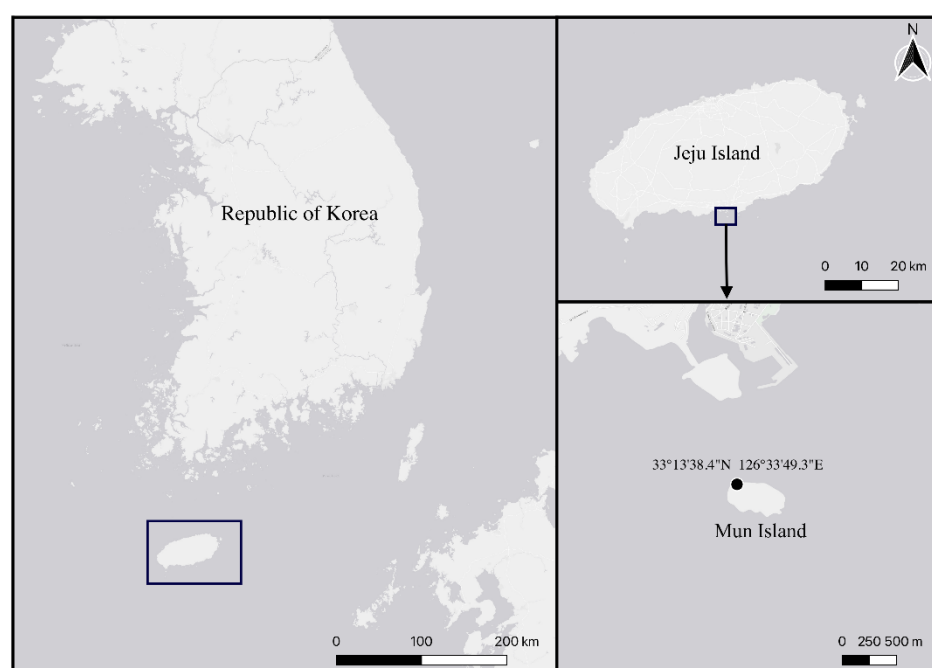


Figure 1. Map of sample locality.

Harpacticoids were identified following Huys et al. (1996) [15] and Wells (2007) [16] under a compound microscope at 400–1000X magnification. All drawings were prepared using a drawing tube on an Olympus BX51 differential interference contrast microscope (Olympus, Tokyo, Japan). Scanning electron micrographs were obtained using a Hitachi S-3400N scanning electron microscope (SEM) at Chung-Ang University. The descriptive terminology followed that of Huys et al. (1996) [15]. Abbreviations used in the text are as follows: A1, antennule; A2, antenna; benp, baseoendopod; ae, aesthetasc; exp, exopod; enp, endopod; P1–P6, first to sixth thoracopod; exp (enp)-1 (2, 3) denotes the proximal (middle, distal) segment of a three-segmented ramus. Specimens were deposited in the collection of the National Institute of Biological Resources (NIBR), voucher code: NIBRIV0000901874 - NIBRIV0000901877.

To compare the morphology more accurately with a domestically reported species (*Robertgurneya donghaensis* Bang, 2021), which is very similar in morphology to a new species, the paratypes stored in the NIBR were observed.

2.2. DNA Extraction and Amplification

For DNA extraction and amplification, the two specimens, holotype and allotype, were transferred to ultrapure water for 1 h to remove the ethanol. The specimens were then prepared for non-destructive DNA extraction in worm lysis buffer [17]. Specimens were placed in tubes containing 25 µL lysis buffer and placed in a Takara thermocycler (Takara, Otsu, Shiga, Japan) with the following settings: 65 °C for 15 min, 95 °C for 20 min, and 15 °C for 2 min. The specimens were then maintained for morphological identification and described after genetic confirmation. Unpurified total genomic DNA was stored at -20 °C for long-term storage. Fragments from two genes, the nuclear 18S ribosomal RNA (18S rRNA) and mitochondrial cytochrome oxidase subunit I (mtCOI) genes, were amplified using a polymerase chain reaction (PCR) premix (BIONEER Co., Labopass, Korea), 2 µL of primers, 3 µL of genomic DNA as a template, and 5 µL of ultra-pure water.

The PCR primers that were used were 18S-F1, 18S-F3, 18S-R7, and 18S-R9 [18] for 18S ribosomal DNA. The amplification protocol consisted of an initial denaturation at 94 °C for 5 min, followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 47 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

For mtCOI, the LCO1490 and Cop-COI-2189R primer sets were used. The amplification protocol consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 2 min, extension at 72 °C for 3 min; and a final extension at 72 °C for 10 min [19].

Successful amplification was confirmed by electrophoresis on 1% agarose gel. The PCR products were sent to Macrogen (Seoul, Korea) for purification and DNA sequencing. DNA was sequenced on an ABI automatic capillary sequencer, using the same sets of primers as those used for amplification. All the obtained sequences were visualized using Finch TV version 1.4.0 (<https://digitalworldbiology.com/FinchTV>; Geospiza Inc., Denver, CO, USA, accessed on 13 August 2018). The quality of each sequence was evaluated, and low-resolution peaks were checked by comparing the forward and reverse strands. Basic Local Alignment Search Tool (BLAST) [20] searches revealed that the obtained sequences were of copepod origin and not contaminants.

2.3. Phylogenetic Analyses

Evolutionary analyses were performed using MEGA version 11 [21]. The sequences were aligned using the multiple sequence comparison by log-expectation (MUSCLE) method. Phylogenetic trees were constructed using neighbor-joining (NJ) and maximum likelihood (ML) methods. For the ML tree, the K2 + G + I model was selected according to the model test in MEGA version 11. Pairwise distance was computed in the same software, using the p-distance. The analysis of 18S rRNA involved 14 nucleotide sequences, including the sequences secured in this study. Among the sequences of the two individuals obtained in this study, the sequence with the longer length was included in the phylogenetic analysis. The sequences of other miraciid species and outgroups were obtained from NCBI (Table 1). Among the data uploaded to NCBI, it was determined that the sequences (350–450 bp) that were too short were not suitable for use in this study; therefore, only those that satisfy a length of 1600 bp or longer were used.

Table 1. GenBank numbers of 18S rRNA sequences used in phylogenetic analyses in this study.

Family	Genus	Species	Author, Year	NCBI No.	Authors
Miraciidae Dana, 1846	<i>Amphiascoides</i>	<i>atopus</i>	Lotufo and Flee-ger, 1995	KC815328	Gómez. et al., 2013, un-published data
	<i>Paramphiascella</i>	<i>fulvofasciata</i>	Rosenfield and Coull, 1974	EU380293	Huys. et al., 2009 [22]
	<i>Typhlamphiascus</i>	<i>typhlops</i>	(Sars G.O., 1906)	EU380292	Huys. et al., 2009 [22]
	<i>Sarsamphiascus</i>	<i>kawamurai</i>	(Ueda and Nagai, 2005)	MN541394	Yeom. and Lee., 2020 [23]
	<i>Sarsamphiascus</i>	<i>hawaiiensis</i>	Yeom and Lee, 2020	MN496456	Yeom. and Lee., 2020 [23]
	<i>Stenhelia</i>	sp.		EU380291	Huys. et al., 2009 [22]
	<i>Amonardia</i>	<i>coreana</i>	Song, Rho, and Kim W., 2007	KT030261	Baek. and Hwang., 2015, unpublished data
	<i>Diosaccus</i>	<i>ezoensis</i>	Itô, 1974	KR048740	Baek. and Hwang., 2015, unpublished data
	<i>Diosaccus</i>	<i>koreanus</i>	Lim, Bang, Moon, and Back, 2020	MT002900	Lim. et al., 2020 [24]
Dactylopusiidae Lang, 1936	<i>Miracia</i>	<i>efferata</i>	Dana, 1849	EU380294	Huys. et al., 2009 [22]
	<i>Robertgurneya</i>	<i>jejuensis</i> sp. nov.	Yeom and Lee, 2022	OP798781	This study
Dactylopusiidae Lang, 1936	<i>Dactylopusia</i>	<i>pauciarticulata</i>	Chang and Song, 1997	KR048735	Baek. and Hwang., 2015, unpublished data
	<i>Sewelliapusia</i>	<i>tropica</i>	(Sewell, 1940)	EU380299	Huys. et al., 2009 [22]
Thalestridae Sars G.O., 1905	<i>Parathalestris</i>	<i>verrucosa</i>	Itô, 1970	MT002906	Lim. et al., 2020 [24]

3. Results

3.1. Systematics

Class: Copepoda H. Milne-Edwards, 1840

Order: Harpacticoida Sars G.O., 1913

Family: Miraciidae Dana, 1846

Genus: *Robertgurneya* Apostolov & Marinov, 1988

***Robertgurneya jejuensis* sp. nov.**

(Figures 2–9)

ZooBank Registration LSID

Urn: lsid:zoobank.org:act:799CAA2D-009C-4A82-950E-44E4476DE931

Type locality: 33°13'38.4" N 126°33'49.3" E; Hangaechang, Mun Island, Seogwipo-si, Jeju Island, Korea; collected on 18 June 2018, by Wonchoel Lee and Raehyuk Jeong. Sedi-ment type: coarse sand; depth: 25 m; water temperature: 19 °C.

Material examined: Holotype; 1♀(NIBRIV0000901874) on 1 slide. Allotype; 1♂(NIBRIV0000901875) on 1 slide. Paratype; 2♀♀(NIBRIV0000901876) dissected on 20 slides, 2♀♀ and 2♂♂ on a SEM stub (NIBRIV0000901877).

Etymology: The species name was given as “*jejuensis*” because the species was dis-covered from Jeju Island, South Korea.

DNA-barcode: Sequences were submitted to GenBank. Genbank accession number: 18S rRNA (1755 base pairs); OP798781/mtCOI (531 base pairs); OP797675. The pairwise

distance based on mtCOI gene data of holotype and allotype specimens was 0.008 (standard error: 0.004), confirming that female and male individuals are of the same species.

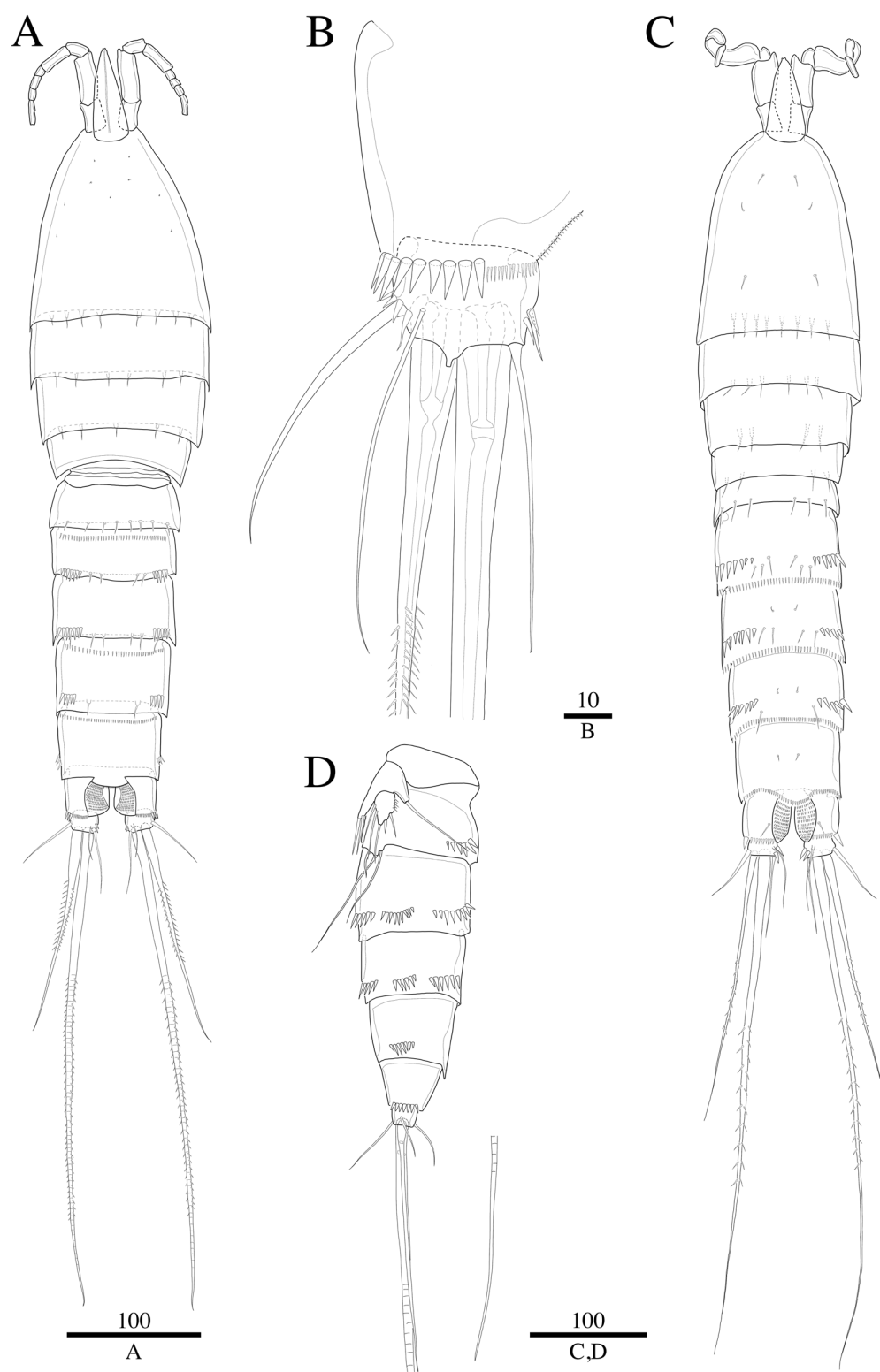


Figure 2. *Robertgurneya jejuensis* sp. nov. Female. (A) Habitus, dorsal. (B) Caudal ramus, ventral. Male. (C) Habitus, dorsal. (D) Urosome, lateral. Scale bars: 100 μ m (A, C, D); 10 μ m (B).

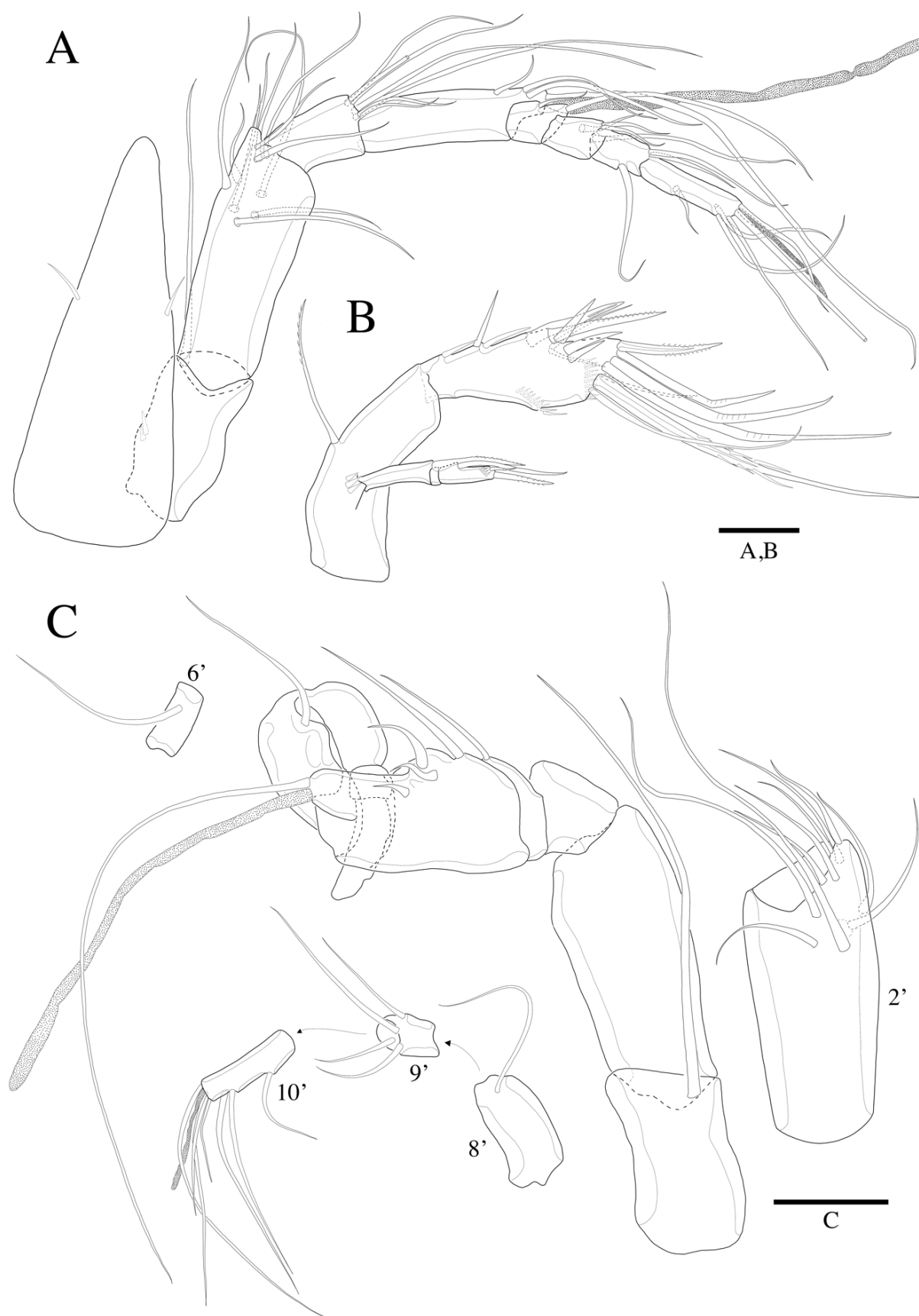


Figure 3. *Robertgurneya jejuensis* sp. nov. Female. (A) A1 and rostrum, dorsal. (B) A2. Male. (C) A1 and segments. Scale bars: 20 μ m.

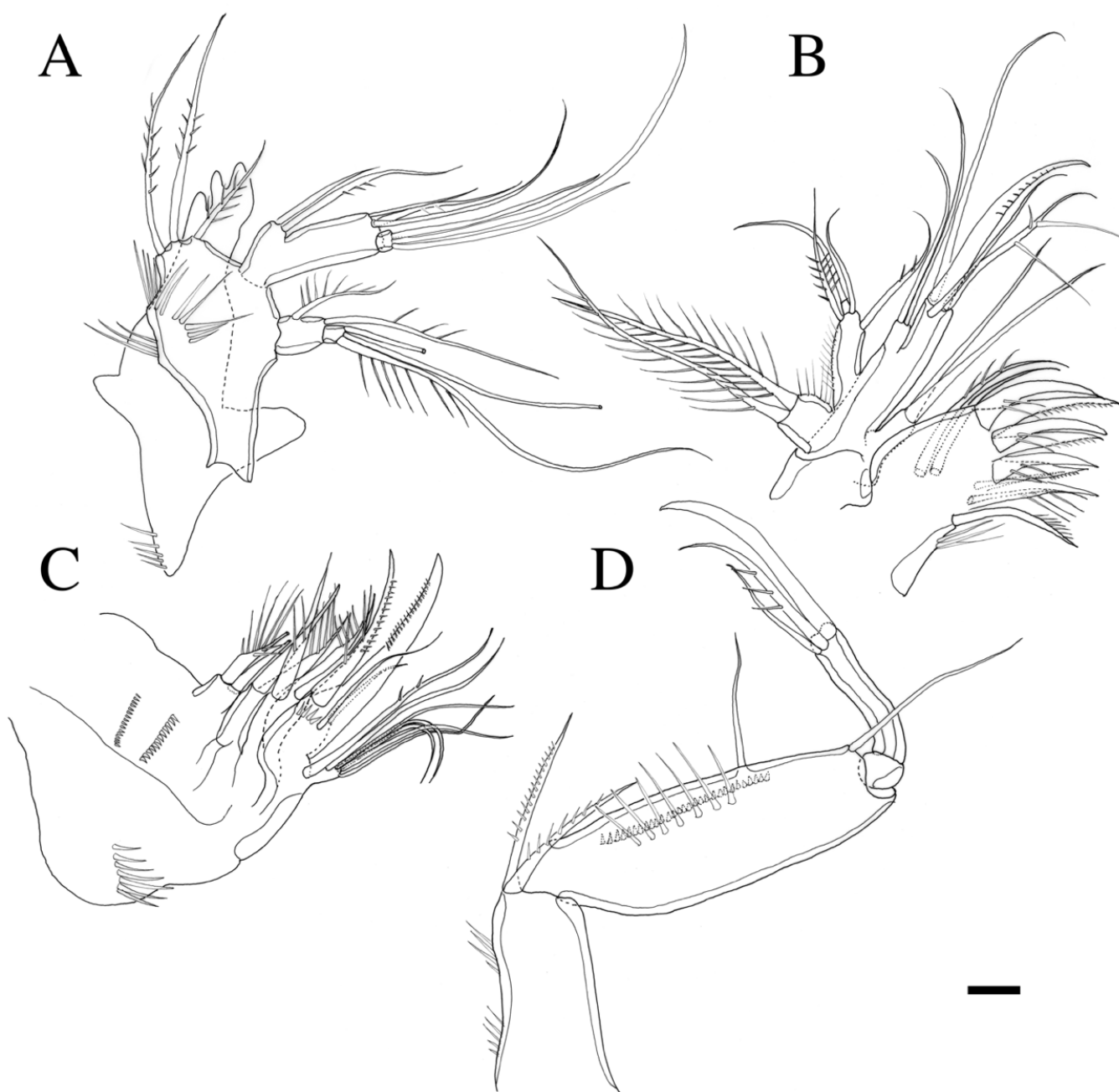


Figure 4. *Robertgurneya jejuensis* sp. nov. (A) Mandible. (B) Maxillule. (C) Maxilla. (D) Maxilliped. Scale bar: 10 μ m.

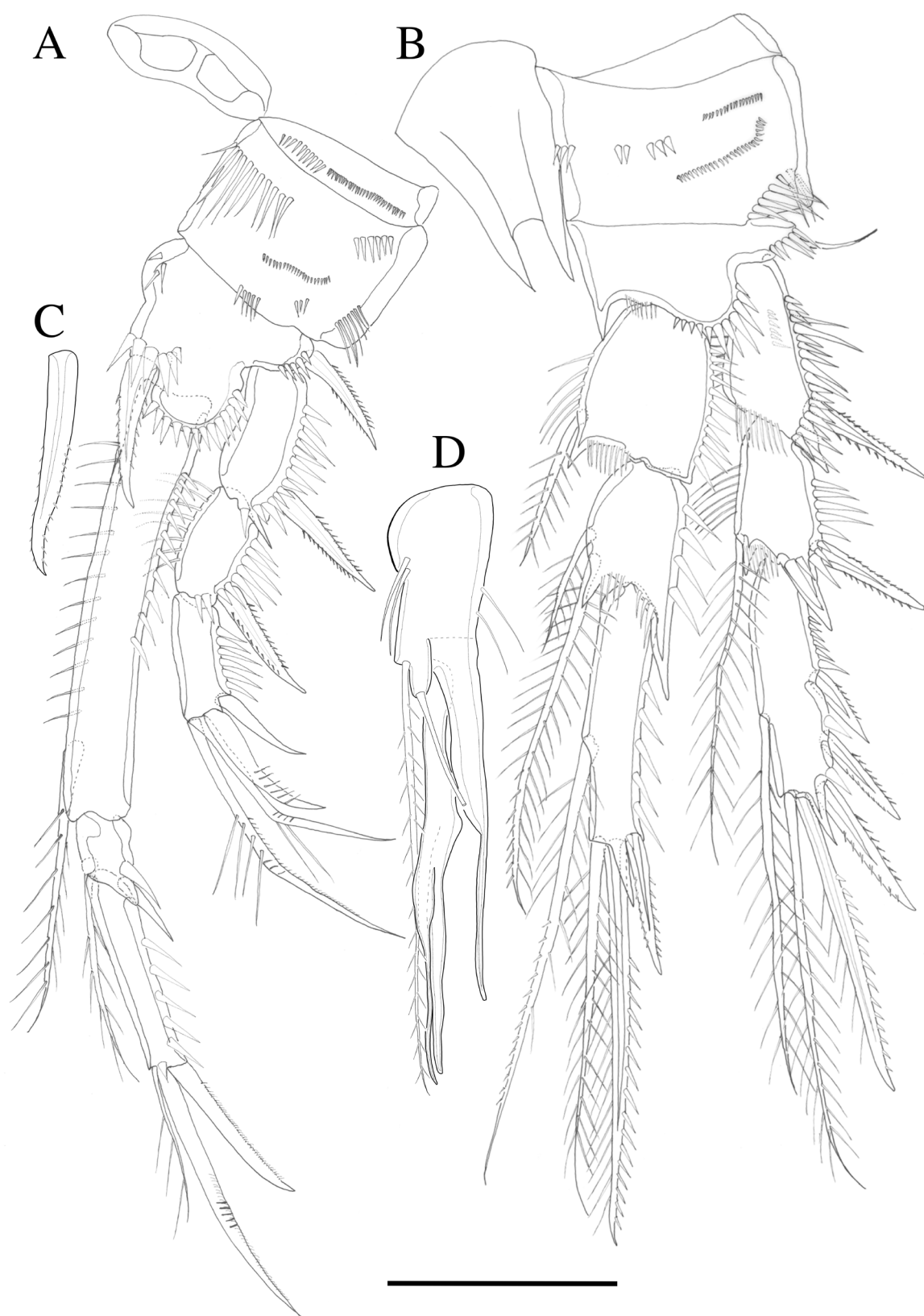


Figure 5. *Robertgurneya jejuensis* sp. nov. Female. (A) P1. (B) P2. Male. (C) Inner spine of P1 basis. (D) P2 enp-2. Scale bar: 50 μ m.

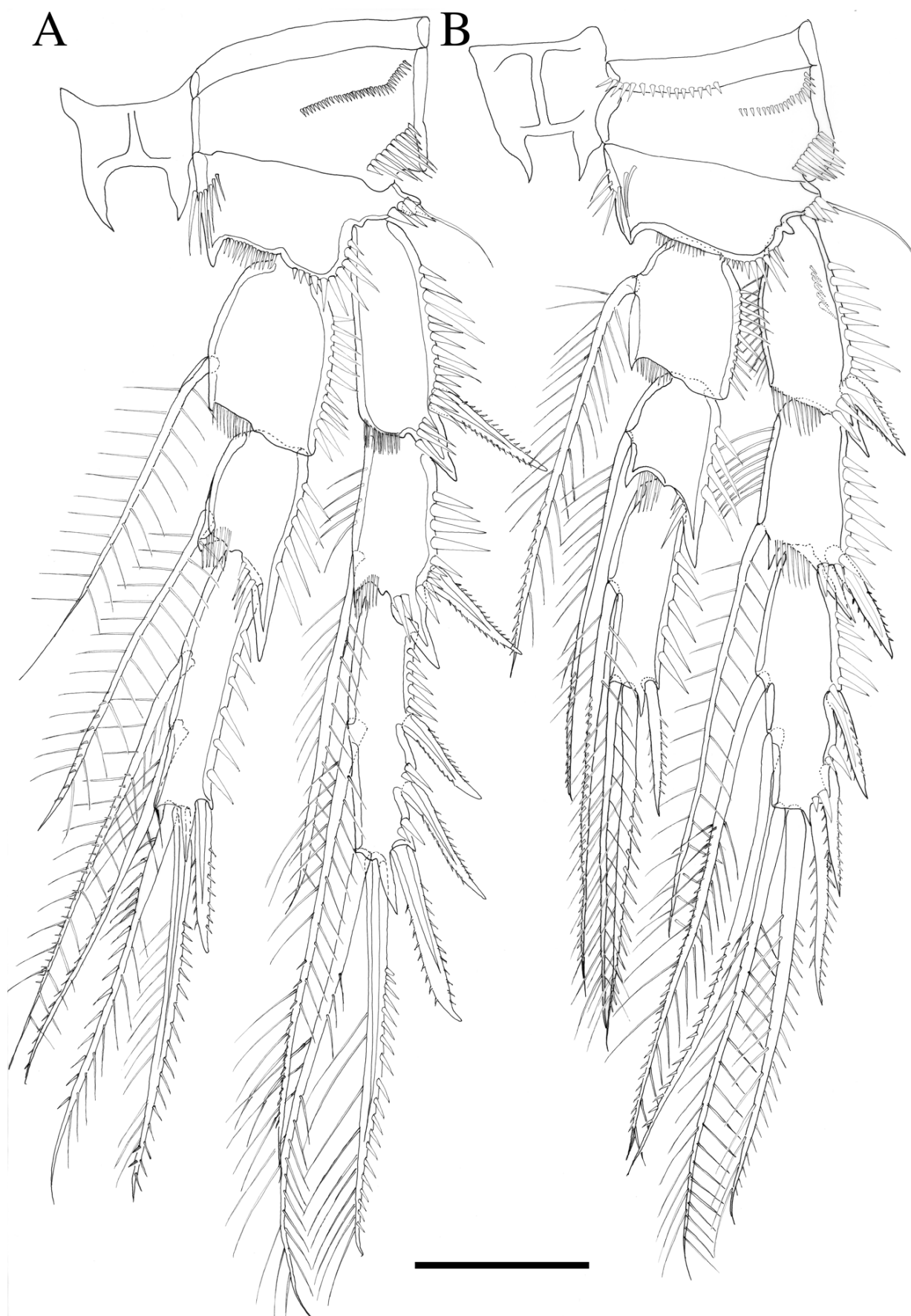


Figure 6. *Robertgurneya jejuensis* sp. nov. (A) P3. (B) P4. Scale bar: 50 μ m.

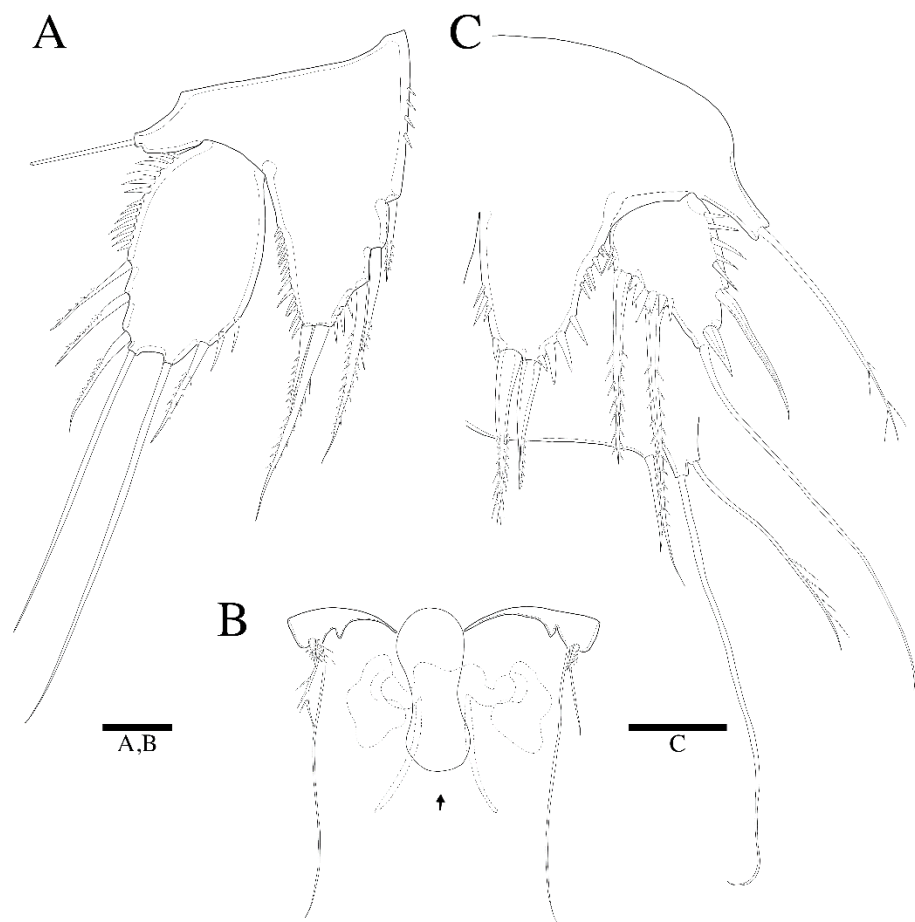


Figure 7. *Robertgurneya jejuensis* sp. nov. Female. (A) P5. (B) Genital field and P6 (arrow: epicopulatory bulb). Male. (C) P5 and P6. Scale bars: 20 µm.

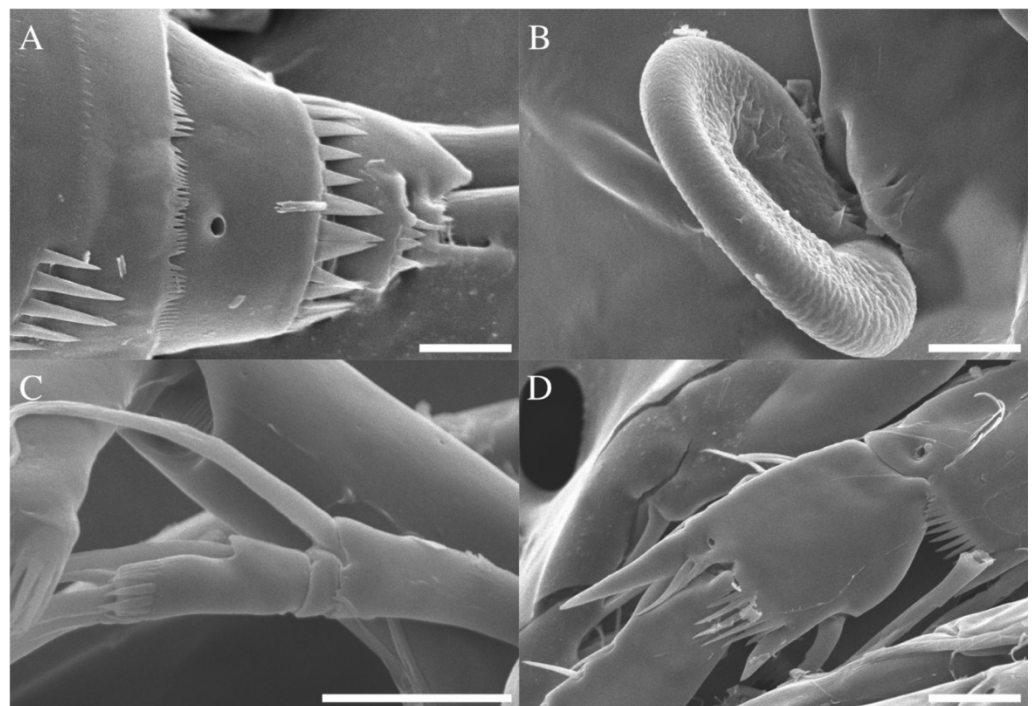


Figure 8. *Robertgurneya jejuensis* sp. nov. Female. SEM photographs. (A) Caudal ramus, lateral. (B) Epicopulatory bulb in genital field. (C) A2 exopod. (D) P2 segments with distal ornamentation. Scale bars: 10 µm.

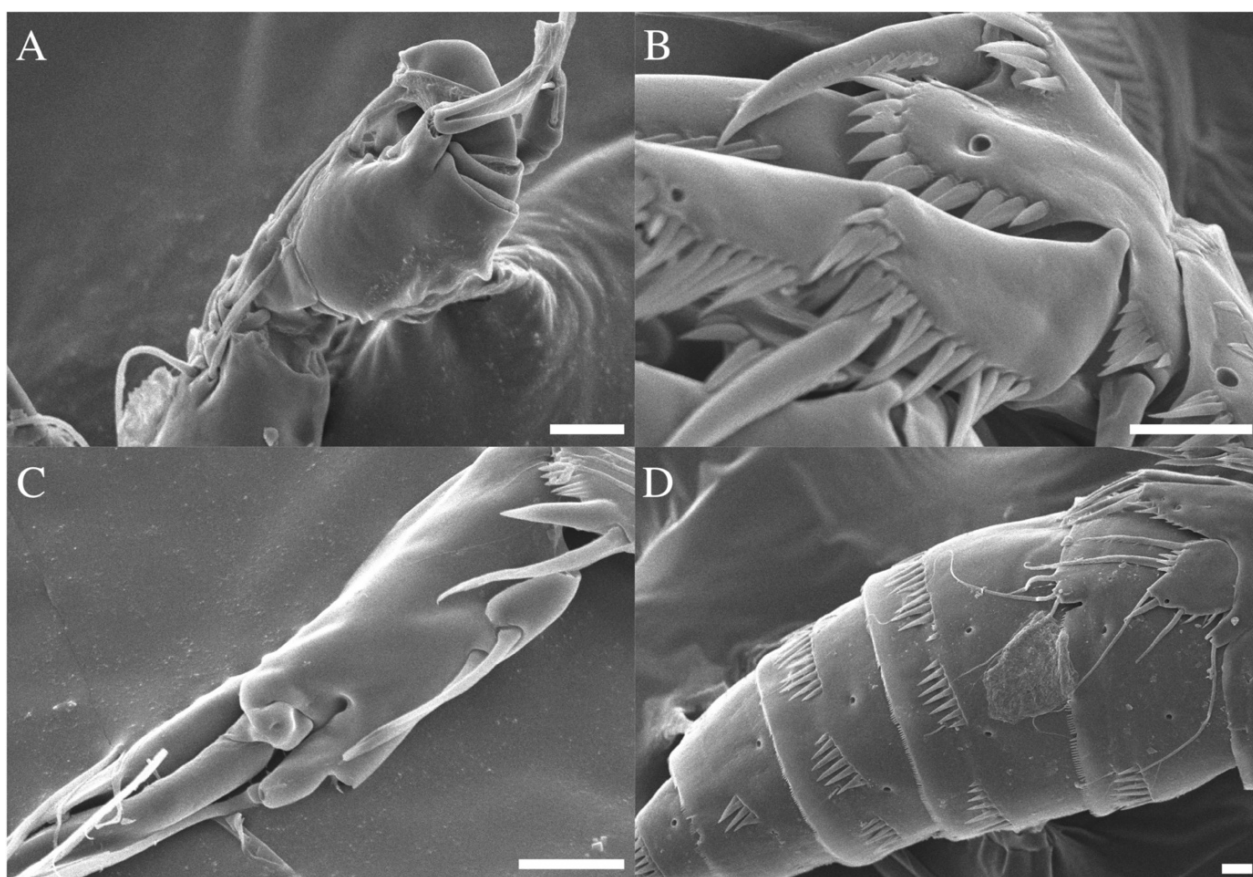


Figure 9. *Robertgurneya jejuensis* sp. nov. Male. SEM photographs. (A) A1. (B) P1 basis. (C) P2 enp-2. (D) Urosome, lateral. Scale bars: 10 μ m.

3.2. Description

3.2.1. Description of Female

Total body length 875 μ m (specimens of holotype and paratype, $n = 3$) (Figure 2A); body cylindrical, slightly tapering behind. Rostrum prominent, pointing upward, tapering distally, reaching mid-length of second antennular segment, defined at base as in Figures 2A and 3A; with two small sensilla. Cephalothorax bell-shaped.

Caudal ramus (Figures 2B and 8A). Approximately 0.7 times as long as greatest width, ventral margin armed with pore; each ramus armed with seven setae; caudal terminal setae pinnate, other setae all naked.

A1 (Figure 3A). Eight-segmented, slender; seg-2 about 1.7 times as long as seg-1; seg-4 2 times as long as seg-3. Armature formula (segment-[number of seta/setae]): 1-[1], 2-[11], 3-[7], 4-[4 + ae], 5-[2], 6-[4], 7-[3], 8-[4 + acrothek]. Setae all naked. Aesthetasc on seg-4 fused basally with adjacent seta and about 1.5 times as long as distal four segments combined. Apical acrothek consisting of aesthetasc and two bare setae.

A2 (Figure 3B). Endopod two-segmented, enp-2 with nine setae. Exopod three-segmented (Figure 8C), exp-1 with pinnate seta; exp-2 without seta and small; exp-3 with one seta laterally and two setae distally.

Mandible (Figure 4A). Gnathobase bearing chitinous projection on ventral surface; palp with three pinnate setae and setule rows; exopod two-segmented, exp-1 with two unipinnate setae laterally and distally, exp-2 with two bare setae and an unipinnate seta; endopod two-segmented, enp-1 with two proximal and three distal setae, enp-2 with three bare setae.

Maxillule (Figure 4B). Praecoxal arthrite bearing six elements distally with a pinnate seta, and three bare setae on surface; coxa with two setae; basis with six setae; endopod bearing four setae; exopod bearing two pinnate setae.

Maxilla (Figure 4C). Syncoxa with three endites; proximal and middle endites with two spines, respectively; distal endite with two pinnate spines and one naked seta; allobasis transformed to curved claw bearing pinnate spine and two thin bare setae on surface; endopod with seven setae.

Maxilliped (Figure 4D). Subchelate; syncoxa with two pinnate setae; basis with small spinules along inner margin and a row of fine spinules on surface, 3.5 times as long as broad, bearing two bare setae; endopod elongate, with a strong claw, a bare seta and an unipinnate seta.

P1 (Figure 5A). Basis inner margin with a bipinnate spine inner distally; exopod three-segmented; exp-1 inner margin naked, exp-1 and exp-2 with a bipinnate outer spine and ornamented with coarse spinules along outer margin, exp-2 inner margin with fine spinules without seta, exp-3 inner margin naked, exp-3 with two outer spines and a long geniculate seta and a relatively short, geniculate setae distally; endopod three-segmented; enp-1 longer than exopod, about 6.5 times longer than wide with one inner pinnate seta distally, inner and outer margin ornamented with fine spinules; enp-2 small, ornamented with coarse spinules along outer margin, with inner pinnate seta; enp-3 about three times longer than enp-2, outer margin ornamented with coarse spinules, bearing an unipinnate seta at inner distal corner, a spine and a geniculate seta apically.

P2 (Figure 5B). Rami three-segmented; basis with naked seta and inner margin with fine spinules; exp-1 without inner seta, hyaline frills on inner distal margin (Figure 8D), exp-2 with one inner pinnate seta at distal, both exp-1 and exp-2 with a bipinnate outer spine, inner margin ornamented and outer margin ornamented with coarse spinules, exp-3 with a plumose inner seta, a long plumose seta at inner terminal, a long seta with plumose inner side and pinnate outer side at outer terminal and three outer spines, proximal outer margin ornamented with coarse spinules; endopod about as long as exopod, enp-1 with a plumose inner seta, inner margin with setules; enp-2 with two inner setae; enp-3 with a plumose inner seta, two setae distally and a spine at outer distal corner; endopod segments outer margin ornamented with small spinules.

P3 (Figure 6A). Rami three-segmented; exp-1 without inner seta; exp-2 with a plumose inner seta at distal, both exp-1 and exp-2 hyaline frills on inner distal margin and outer margin ornamented with coarse spinules; exp-3 with a plumose inner seta, a long seta at inner terminal, a long seta with plumose inner side and pinnate outer side at outer terminal and three outer pinnate spines, proximal outer margin ornamented with coarse spinules; endopod shorter than exopod, both enp-1 and enp-2 with a plumose inner seta and hyaline frills on inner distal margin, enp-3 with two plumose inner setae, two setae distally, and a spine at outer distal corner, endopod segments outer margin ornamented with coarse spinules.

P4 (Figure 6B). Rami three-segmented; basis with bare outer seta; exp-1 posterior surface ornamented with coarse spinules, exp-1 without inner seta, both exp-1 and exp-2 inner margin ornamented with fine spinules, hyaline frills on inner distal margin and outer margin ornamented with coarse spinules; exp-3 with two long inner setae, a long seta at inner terminal, a long seta with plumose inner side and pinnate outer side at outer terminal and three outer spines, proximal outer margin ornamented with coarse spinules; endopod shorter than exopod, both enp-1 and enp-2 with one inner seta and hyaline frills on inner distal margin, enp-3 with one inner seta, two setae distally and a spine at outer distal corner, endopod segments outer margin ornamented with coarse spinules.

Armature formulae are shown in Table 2.

Table 2. Armature formulae of legs 1–4.

Setal Formulae	Exopod	Endopod
P1	0.0.022	1.1.111
P2	0.1.123	1.2.121
P3	0.1.123	1.1.221
P4	0.1.223	1.1.121

P5 (Figure 7A). Baseoendopod and exopod distinct, baseoendopod with naked outer basal seta; endopodal lobe bearing three inner spines and two bipinnate distal setae; exopod oval, elongated, 1.7 times longer than wide, with spinules along outer margins, two fine spinules on inner distal margin, bearing a bipinnate inner seta, two apical long bare setae and three unipinnate outer setae.

Genital area (as in Figure 7B). P6 with a long seta, a relatively short seta, and a short spiniform seta; kidney-shaped seminal receptacle. Genital double-somite with rows of spinules on ventrolateral surface with large epicopulatory bulb (arrowed in Figure 7B; Figure 8B).

3.2.2. Description of Male

General shape of body (Figure 2C) same as in the female. Urosome somites (Figures 2C,D and 9D) with several rows of spinule on dorsal, ventral, and lateral surface. Sexual dimorphism shown in antennule, inner projection on P1 basis, endopod of P2, P5, and P6.

A1 (Figures 3C and 9A). Subchirocer. Ten-segmented. The fifth segment swollen. Armature formula: 1-[1], 2-[10], 3-[8], 4-[1], 5-[4 + (1 + ae)], 6-[1], 7-[1], 8-[1], 9-[4], 10-[5 + acrothek]. Setae all naked.

P1 basis (Figures 5C and 9B) with strongly developed and slightly curved spine.

P2 endopod (Figures 5D and 9C). Two-segmented; enp-1 with inner pinnate seta; enp-2 modified, bearing two short naked inner setae and one long pinnate seta, terminal seta, and two outer distal spiniform projection.

P5 (Figures 7C and 9D). Baseoendopod and exopod distinct; endopodal lobe ornamented with spinules at outer margin, longer than exopod, armed with two pinnate spines. Exopod with five setae in total, including two pinnate inner setae covered with four setules on base of setae, long bare distal seta and two bare outer setae, inner margin and outer margin ornamented with spinules.

P6 (Figures 7C and 9D) represented by three setae, two inner naked and outer pinnate seta, on outer distal corner of genital operculum.

4. Discussion

Following the identification keys to harpacticoid species [12,25], this copepod is identifiable as *R. similis similis* (Scott A. 1896). However, the new species and *R. similis similis* were distinguished by a combination of the following morphological characteristics: (1) the length of the secondary segment of A1 (longer in the new species), (2) the type of inner setae on P2 enp-2 (plumose in the new species), (3) the inner margin of P4 exp-2 (with long setules on the inner margin in the new species), (4) the type and length of P5 setae (with longer distal setae on P5 exp and benp in the new species), and (5) the ornamentation and distance between P5 benp distal setae and inner setae (wider distance and setules in the new species).

The new species described here also has morphological features similar to those of the most recently reported Korean species, *R. donghaensis* Bang 2021 [9]. However, there were obvious differences in some features (Table 3). The morphological differences between the two species are noticeable in the number of setae in the mouth part, the length ratio of the A1 segments, the difference in setae length, and the presence or absence of ornamentations on the appendages and body surfaces. The male tends to be relatively less decorated in the new species.

Table 3. Morphological comparison of new species and *R. donghaensis*.

	<i>R. jejuensis</i> sp. nov.	<i>R. donghaensis</i>
Body length	875 (n = 3)	795 (n = 5)
Maxillule basis setae	6	5
Maxilla allobasis and enp setae	9	7
A1 1st/2nd seg length	0.7	0.4
P1 enp-1 inner seta length	~middle of enp-3 (long)	~ enp-2 (short)

	P2 exp-2 inner margin ornamentation	present	absent
	P3 enp and exp length	enp < exp	enp ≥ exp
	P5 benp distal setae length	double	same
	P5 benp ornamentation between seta II and III	relatively wide spacing with ornamentation	without
	P5 exp shape (l/b ratio)	oval (2)	swollen distal margin (1.7)
	P5 exp outer setae type	pinnate	naked
	P6 mid seta (A), the longest seta (B) length	3A = B	2A = B
	CR ornamentation	inner margin	inner distal margin
	CR l/b ratio	0.7	1
	CR lateral setae type	naked	pinnate
Male	Ornamentation on urosome segments surface	Setules on ventral, lateral, and dorsal surface	Setules on ventral surface
	Ornamentation on A1 seg-1	absent	present
	A1 seg-2 seta type	all naked	1 pinnate
	A1 seg-5 distal surface	without element	2 pinnate elements
	P2 enp-2 two inner setae type	naked	plumose
	P2 enp-2 outer margin ornamentation	absent	present
	P6 setae type	3 naked	1 pinnate and 2 naked

Gómez (2020) suggested a key to *Robertgurneya* species [14]. After this, as two new species of the *similis* group were additionally reported, the updated identification key in this study includes 11 species of the *similis* group. This group is a set of species within the genus that have in common that P2 enp-2 has two inner setae. Table S1 compares several morphological features of female congeners in the *similis* group and new species. Because these species are generally similar in morphological characteristics, minor features were selected for the comparison. Based on the contents of Table S1, the following identification key was organized.

Key to the Species of the *Robertgurneya similis* group:

1. P1 enp-2 without inner seta *R. remanei*
P1 enp-2 with inner seta 2
2. P2 enp-1 without inner seta; P3 enp-3 with one inner seta; female P5 setae benp: exp = 4:5 *R. brevipes*
P2 enp-1 with inner seta; P3 enp-3 with two inner setae; female P5 setae benp: exp = 5:6 3
3. P5 exp l/b = 1.5 4
P5 exp l/b > 1.5 7
4. P1 enp-1/exp = 1; P1 enp-1 inner seta reaching 2/3 of enp-3 *R. hopkinsi*
P1 enp-1 > exp; P1 enp-1 inner seta reaching the middle of enp-3 5
5. P5 exp distal margin swollen between setae II and III in both sexes *R. smithi*
P5 exp distal margin normal, oval-shaped 6
6. P1 basis inner spine of male reaching tip of P1 exp *R. falklandiensis*
P1 basis inner spine of male reaching P1 exp-2 *R. diversa*
7. Male P5 exp with six setae *R. simulans*
Male P5 exp with five setae 8
8. Female P5 exp l/b = 2; P5 benp without ornamentation between seta II and III 9
Female P5 exp l/b < 2; P5 benp with ornamentation between seta II and III *R. jejuensis* sp. nov.
9. P1 exp/enp-1 = 2/3; female P5 exp distal margin swollen *R. donghaensis*
P1 exp/enp-1 = 4/5; female P5 exp distal margin normal, oval-shaped 10
10. A1 first/second segment length ratio = 0.38; female P5 exp outer setae short and bulbous; P5 benp distal setae length ratio I/II = 1/4 *R. similis bulbamphiascoides*

A1 first/second segment length ratio = 1; female P5 exp outer setae slender and pinnate; P5 benign distal setae length ratio I/II = 1 *R. similis similis*

When identification was based on another identification key [26], this species was morphologically closely identified to *Amphiascoides subdebilis* (Willey, 1935). The morphological characteristics of the species that differed from this species are P2 exp-3 without inner seta, P5 exp with five setae, and A1 with stubby segments.

In this study, a phylogenetic study was conducted on genera within Miraciidae based on the nuclear 18S rRNA fragment sequences, which is highly conserved. To make the most of the miraciid sequences from the NCBI database, the analysis was performed using only the 18S rRNA, which had the most diverse genera uploaded. When comparing the new species with other miraciids using 18S rRNA sequences, it appeared similar to *Amphiascoides* on the phylogenetic tree (Figure 10). Indeed, many *Amphiascoides* species have undergone genus changes. Eight species were transferred to *Robertgurneya* [13], and nine were transferred to *Paramphiascella* [12]. Therefore, it can be inferred that these three genera are closely related, both morphologically and molecularly. Meanwhile, Gómez et al. (2021) conducted a phylogenetic analysis based on morphological features and confirmed that *Robertgurneya*, *Amphiascoides*, and *Paramphiascella* formed the closest clade in the phylogenetic tree [27]. Therefore, the phylogenetic tree in this study provides genetic support for the results of Gómez et al. (2021) based on morphological characteristics. It is, therefore, necessary to conduct a morphological and molecularly integrated review of these three genera in the future. The mtCOI sequence (NCBI no. OP797675) obtained in this study will be useful as basic data for future research.

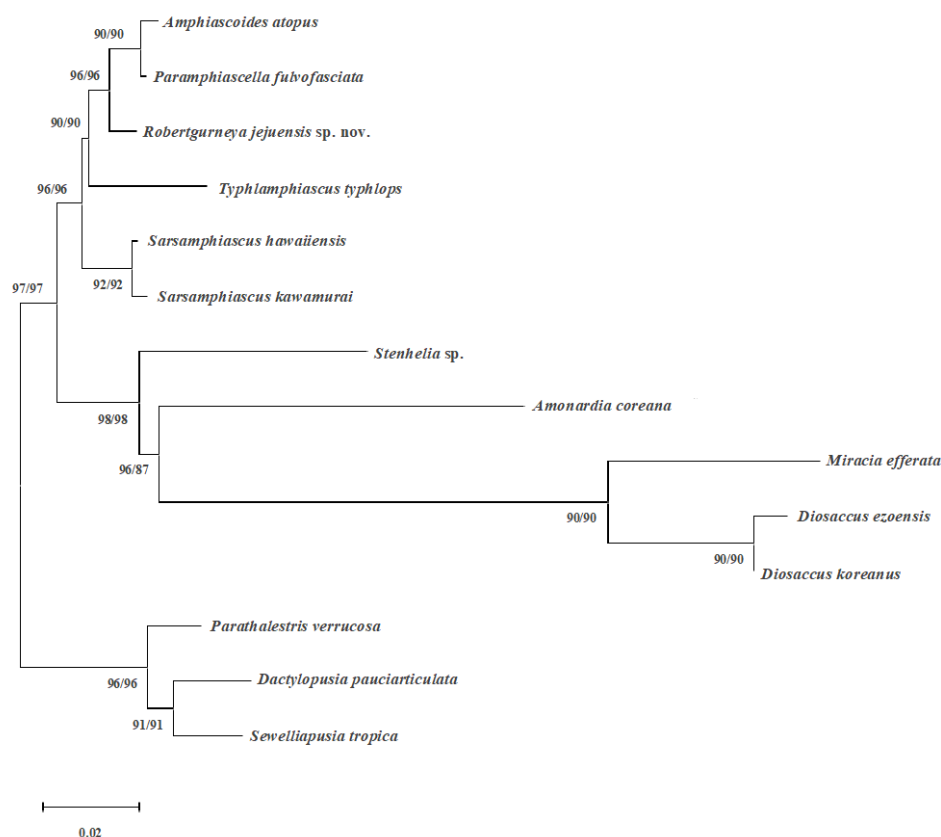


Figure 10. Maximum likelihood phylogenetic tree including nine genera of Miraciidae and outgroups based on 18S rRNA sequences. Tree shows bootstrap values (ML/NJ, %) together in one tree because the topologies of the two trees created in this study were the same.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14121127/s1>, Table S1: Morphological comparison of new species and congeners in *similis* group.

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