

# New record of the family Porcellidiidae Boeck, 1865 (Harpacticoida, Copepoda) in Korea

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*Kushia zosterophila* Harris V.A. & Iwasaki, 1996 is newly collected and described from macroalgae in the intertidal region of Gijang-gun, along the southeastern coastal region of Korea. *Kushia zosterophila* can be distinguished from congeners by following morphological characteristics: the length of the first dorsal seta similar with the second dorsal seta of female P5, the length to width ratio of the female caudal ramus, and the presence of a conspicuous comb on the accessory lobe of the male antennule. Although there are some minor discrepancies, the main diagnostic characteristics of the specimen from the study area are well-matched with the original description. We herein provide detailed morphological descriptions and illustrations of this species. According to a survey of the location of the reported porcellidiid species in Korea, this specimen is the second record in Korean waters of the genus *Kushia*. A key to species of the family Porcellidiidae in Korea is provided. A partial sequence of the mitochondrial COI gene was obtained and provided as a DNA barcode for this species.

Keywords: distribution, Korean fauna, *Kushia*, mtCOI, taxonomy

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## INTRODUCTION

The family Porcellidiidae Boeck, 1865 has characteristic dorsoventrally flattened, shield-shaped morphology (Boxshall and Halsey, 2004), and 73 species in 16 genera have been reported (Walter and Boxshall, 2022). Most of the family Porcellidiidae can be found on macroalgae (Kim and Kim, 1997; Song *et al.*, 2012), and some of the species are associated with marine invertebrates such as hermit crabs (Kim and Kim, 1996; Kim, 2014). In NIBR (2022), 12 species in the family Porcellidiidae have been described in Korea so far. However, among these species, *Kioloaria brevicaudata* found in Hanrim, Jeju was established as a new species, *Kioloaria jejuensis* by Huys (2016). For this reason, the following 11 porcellidiid species have been reported in Korea until now: *Dilatatiocauda bipartita* (Kim I.H. & Kim H.S., 1997); *Kensakia acuta* (Kim I.H. & Kim H.S., 1997); *Kioloaria jejuensis* Huys, 2016; *Ki. paguri* (Ho, 1986); *Ki. similis* (Kim S.H. & Kim W., 1996); *Kushia gamoi* Harris V.A. & Iwasaki, 1996; *Porcellidium brevicavum* Kim I.H. & Kim H.S., 1997; *P. kiiroum* Harris V.A. & Iwasaki, 1996; *P. ofunatense* Harris V.A. & Iwasaki, 1996; *P. viride* (Philippi, 1840); *P. wan-*

*doensis* Kim I.H. & Kim H.S., 1997.

During a study of harpacticoid copepods in Korean coastal waters, we found one unrecorded species of the family Porcellidiidae. In this study, we describe the morphological characters and provide a key to species of the family Porcellidiidae reported in Korean waters.

## MATERIALS AND METHODS

### Sample collection and Identification

Samples were collected by washing and filtering (38 µm sieve) macroalgae in the intertidal region on the coast of Gijang-gun, Busan. Specimens were preserved in 99% ethanol. Specimens were dissected in lactic acid and mounted on slides with lactophenol. Preparations were sealed with transparent nail varnish. All drawings were prepared using a drawing tube attached to an Olympus BX53 differential interference contrast microscope. For scanning electron microscope (SEM) preparation, specimens were dehydrated in a series of graded ethanol solutions, then placed in hexamethyldisilazane (HMDS), dried, mounted on stubs, coated in gold, and observed

an EM-30 SEM (Coxem, Korea) at Hanyang University, Seoul, Korea. Descriptive terminology is adopted from Huys *et al.* (1996) and Harris & Iwasaki (1996b). Abbreviations used in the text are as follows: A1, antennule; A2, antenna; ae, aesthetasc; exp, exopod; enp, endopod; P1–P5, first to fifth thoracopod; exp (enp) - 1(2,3), proximal (middle, distal) segment of three-segment ramus; CR, caudal rami; numbered 1–4 setae of female P5, distal setae of female P5 from the most proximal to the most distal.

### DNA extraction and amplification

For genomic DNA extraction, the whole body of eight individuals was used without homogenization. Genomic DNA was extracted, individually, using DNeasy® Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. The mitochondrial cytochrome oxidase subunit I (mtCOI) gene was amplified using polymerase chain reaction (PCR) in 25 µL reaction volumes containing extracted DNA and a primer pair, jgLCO1490 (5'-TITCIACIAAYCAYAARGAYATTGG-3') and jgHCO 2198 (5'-TAIACYTCIGGRTGICCCRAARAAYCA-3') (Geller *et al.*, 2013). PCR conditions comprised initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min. This was followed by a final extension step at 72°C for 5 min. PCR products were evaluated by electrophoresing amplification products on 1% agarose gel and sequenced BTseq™ contiguous sequencing (Celemics, Korea).

### Systematics

Order Harpacticoida Sars, 1903  
 Family Porcellidiidae Boeck, 1865  
 Genus *Kushia* Harris V.A. & Iwasaki, 1996

#### *Kushia zosterophila* Harris V.A. & Iwasaki, 1996 (Figs. 1–6)

*Kushia zosterophila* Harris V.A. & Iwasaki, 1996b, P. 205–208, Figs. 3, 6A, B, 7C, D.

**Material examined.** 2 ♀♀ (MABIK CR00252831, CR00252832) and 1 ♂ (MABIK CR00252833) from the intertidal region (35°16'9.43"N, 129°14'36.70"E) of Gijang-gun, Busan on 7 May 2020. Eight individuals (5 ♀♀, 3 ♂♂) from same locality were used for length measurement. DNA-barcode (mtCOI) sequence and trace were submitted to GenBank (OP825029–OP825036, 658 bp).

**Description of female.** Four transverse bands of dark red-brown at the body segment (cephalosome, metasome, and urosome). Total body length 1,002–1,102 µm (mean ± SD = 1,049 ± 37 µm, n = 5) as measured from the anterior margin of cephalosome to the posterior margin of the cau-

dal rami. Dorsal pit present on prosome, urosome, P5 and CR (Fig. 6A). Body surface armed with some sensilla (Fig. 1A).

Prosome (Figs. 1A, 6A) four-segmented; comprising cephalosome and three free pedigerous somites. P1-bearing somite fused to cephalosome. Hyaline membrane present on lateral margin. Rostrum reversed triangular shaped, protrudes beyond the anterior margin of cephalosome.

Urosome (Figs. 1A, 2B) three-segmented, comprising the P5 somite, genital double somite and anal somite. Most of the anal somite is dorsally covered by genital double somite.

Caudal ramus (Figs. 1A, 2B, C, 6C) pentagonal; inner edge straight and longer than outer edge; distal edge bevelled at an outer corner; seta VII and I closed, inserted at proximal half; seta II small on bevelled edge; seta III annulate, inserted ventrally along bevelled edge; terminal seta IV, V and VI pinnate. Terminal fringe of fine setules present.

Antennule (Fig. 1B) six-segmented. Aesthetascs on segment 4 and 6. Armature formula: 1-[1], 2-[11], 3-[9], 4-[6 + ae], 5-[5], 6-[11 + ae].

Antenna (Fig. 2A) four-segmented, comprising with coxa, basis, and two-segmented endopod. Coxa short. Basis with spinules on the medial surface and a distal end. Exopod with three lateral and three distal setae. Enp-1 with spinules along of lateral margin; enp-2 with many spinules on the surface, four lateral setae, two annulate distal; three geniculate setae; one thick and denticulate setae.

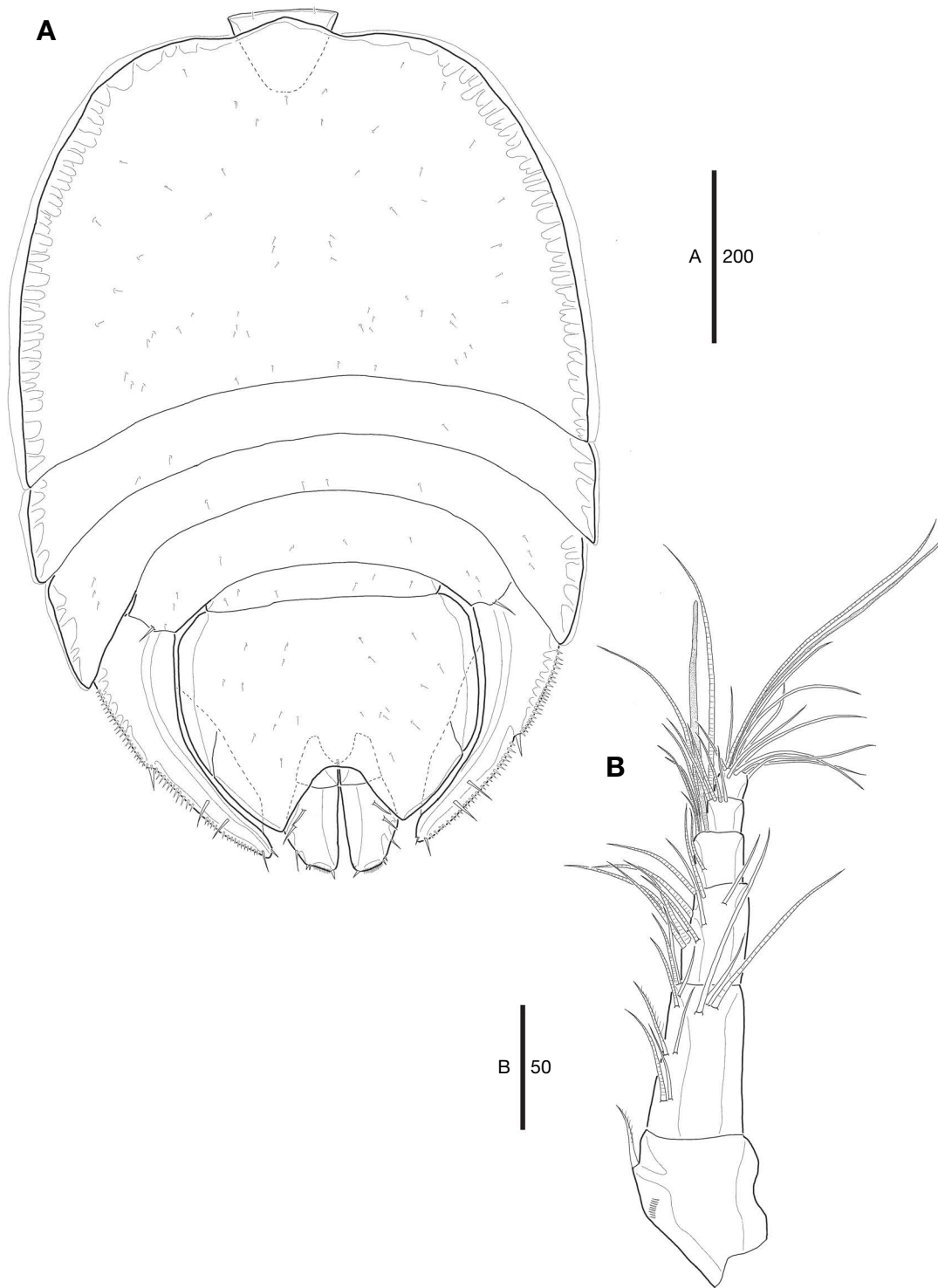
Mandible (Fig. 3A) with small coxa and gnathobase. Endopod with four pilous and thick setae on the anterior lobe; five pilous and thick setae, and one slender seta on the posterior lobe. Exopod one-segmented, with six pilous and thick setae, and three slender setae.

Maxillule (Fig. 3B) with nine plumose and two naked setae on arthrite. Coxa with five setae and spinules on coxal endite. Basis with two endites and four setae on each. Endopod with six plumose setae. Exopod with one plumose seta and spinules on distal margin.

Maxilla (Fig. 3C) with syncoxa having two endites with five setae and two setae, respectively. Allobasis with three setae on the distal corner: one small slender, one thick and denticulate, and one spinulose seta. Endopod one-segmented, with two denticulate and two spinulose setae.

Maxilliped (Fig. 3D) with long hairs on the margin of syncoxa and basis. Syncoxa with one naked seta on the distal surface. Basis with the protruding and plumose process. Endopod two-segmented; enp-1 with one small seta and one thick seta; enp-2 with two thick setae.

P1 (Fig. 4A). Basis with one swollen and pilous seta. Endopod two-segmented; enp-1 large and triangular shape, with a seta on proximal inner margin; enp-2 with

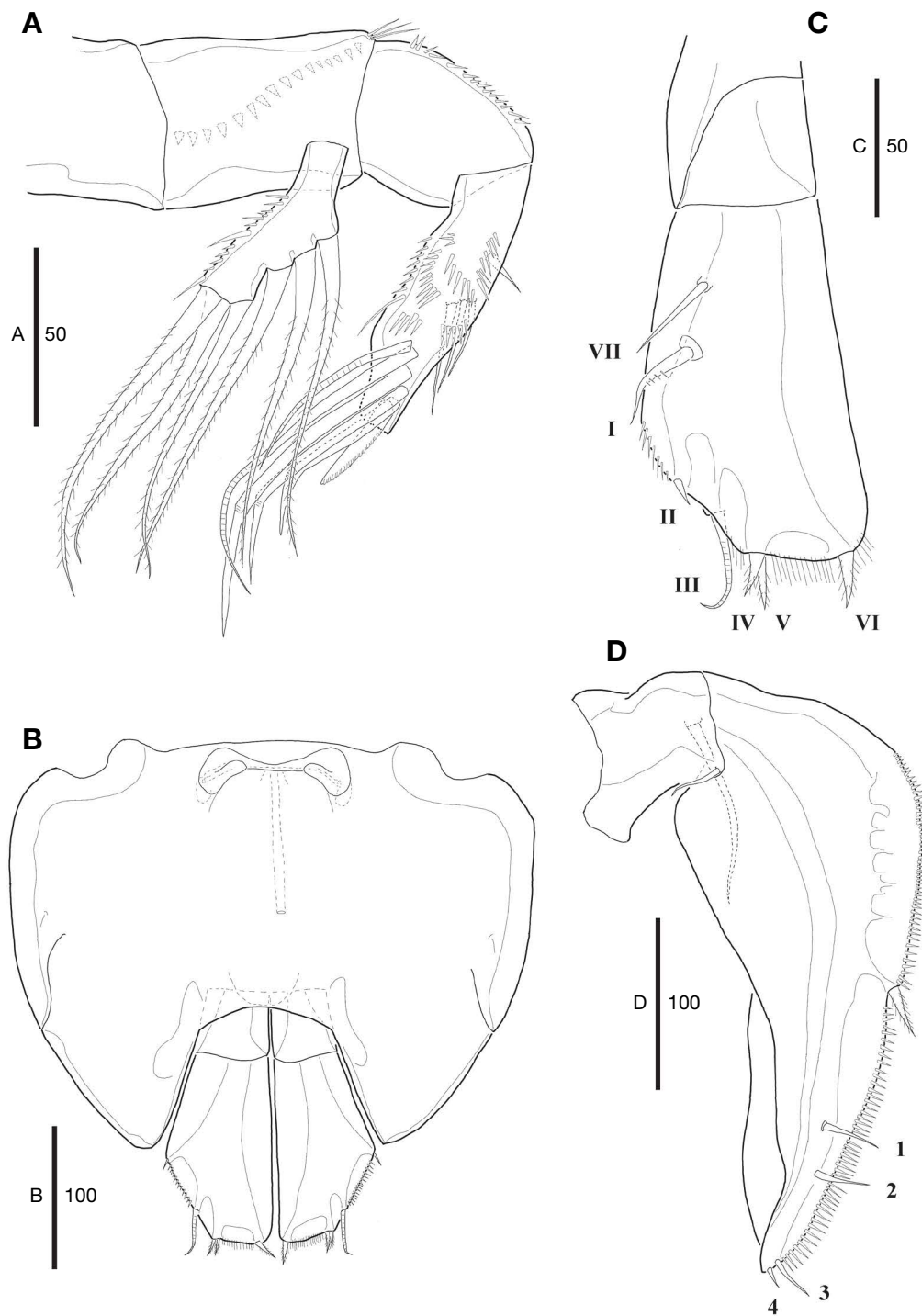


**Fig. 1.** *Kushia zosteraphila*. Female. A: Habitus, dorsal; B: Antennule. Scale bar in  $\mu\text{m}$ .

two thick claws distally. Exopod three-segmented; exp-1 with a swollen pilous outer seta; exp-2 with a swollen pilous outer seta; exp-3 with a plumose seta on the proximal inner margin, three swollen pilous setae on the outer

margin, one annulate and one swollen pilous seta on distal margin.

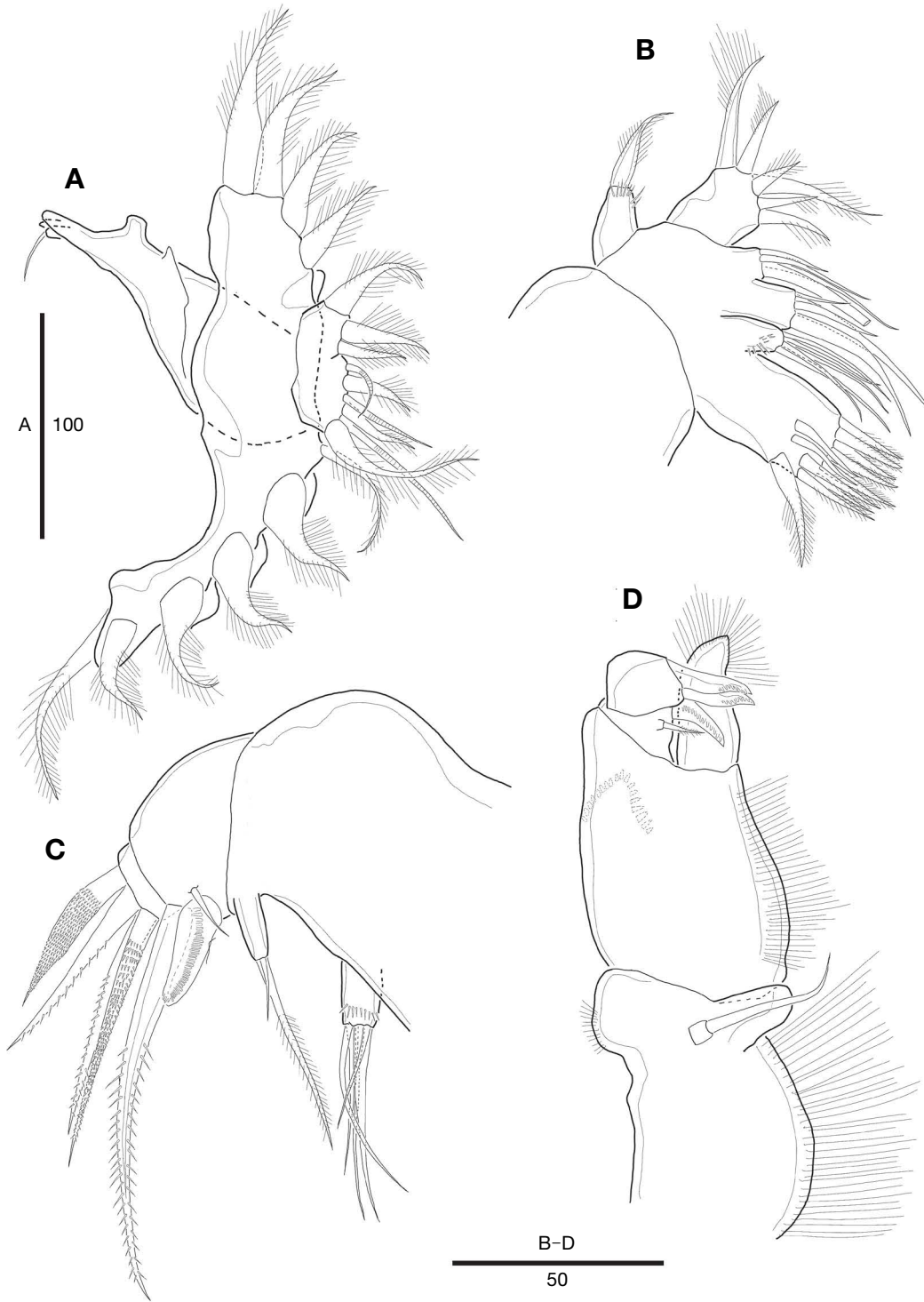
P2 (Fig. 4B). Basis shorter than wide, with slender outer seta and spinules on the outer lateral margin. Endopod



**Fig. 2.** *Kuschia zosterophila*. Female. A: Antenna; B: Urosome, ventral; C: Caudal rami; D: P5, numbered setae indicate distal setae from the most proximal to the distal. Scale bar in  $\mu\text{m}$ .

three-segmented and longer than exopod; enp-1 with hairs and a row of spinules along the outer lateral margin, with one plumose inner seta; enp-2 with hairs along the outer margin, spinules on middle anterior surface, with two

plumose inner setae; enp-3 with rows of spinules on the proximal and distal anterior surface, with one pinnate inner seta, two plumose distal setae, and one plumose outer seta. Exopod three-segmented and reaching to distal mar-

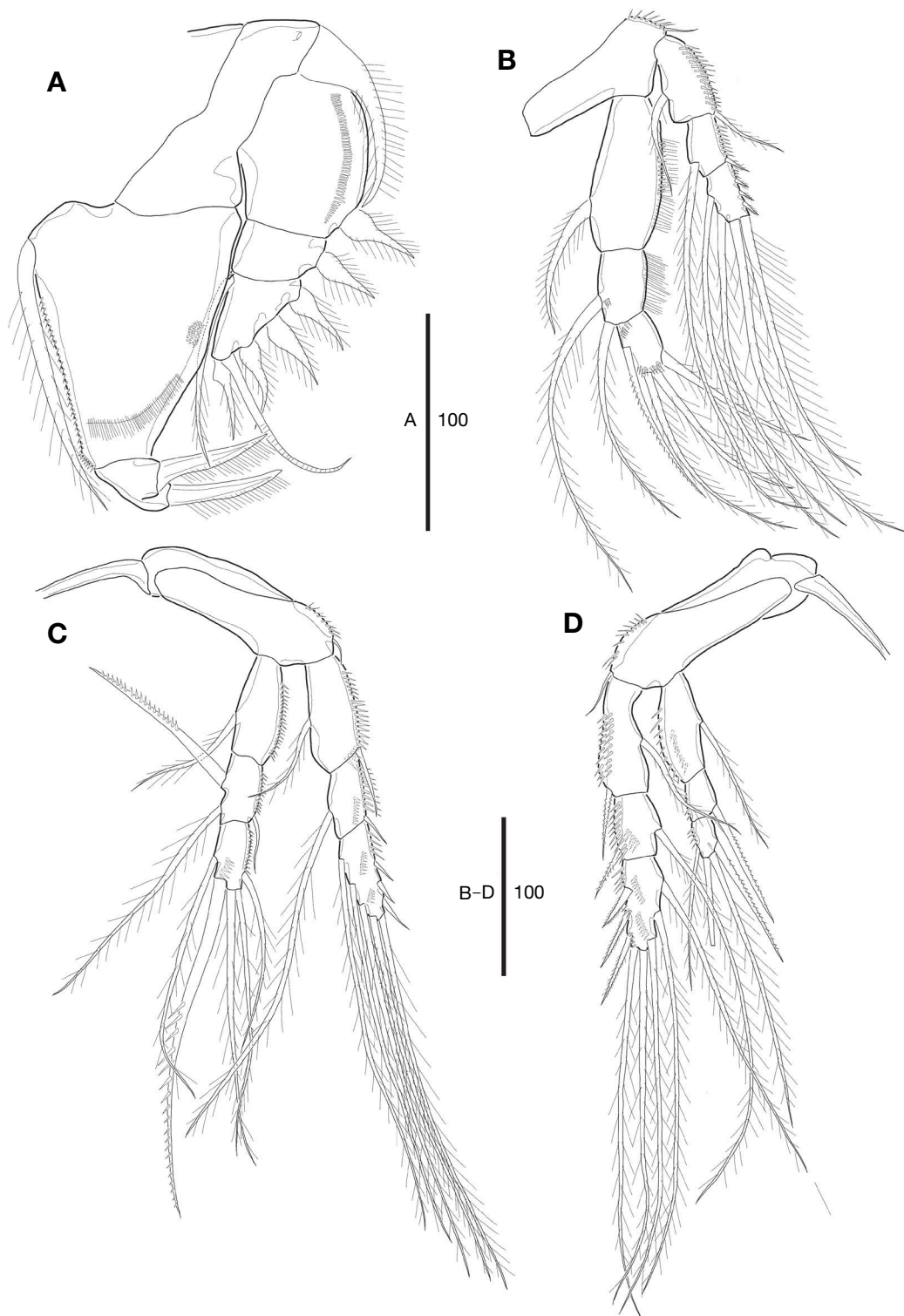


**Fig. 3.** *Kuschia zosteraphila*. Female. A: Mandible; B: Maxillule; C: Maxilla; D: Maxilliped. Scale bar in μm.

gin of enp-1; exp-1 with two rows of spinules along the outer lateral margin, with one plumose inner seta and one plumose outer seta; exp-2 with a row of spinules along the outer lateral margin, with one plumose inner seta and

one pinnate outer spine; exp-3 with a row of spinules on proximal lateral margin, with two plumose inner setae, two plumose distal setae, and three pinnate outer spines.

P3 (Fig. 4C). Intercoxal sclerite wide. Coxa broad. Basis

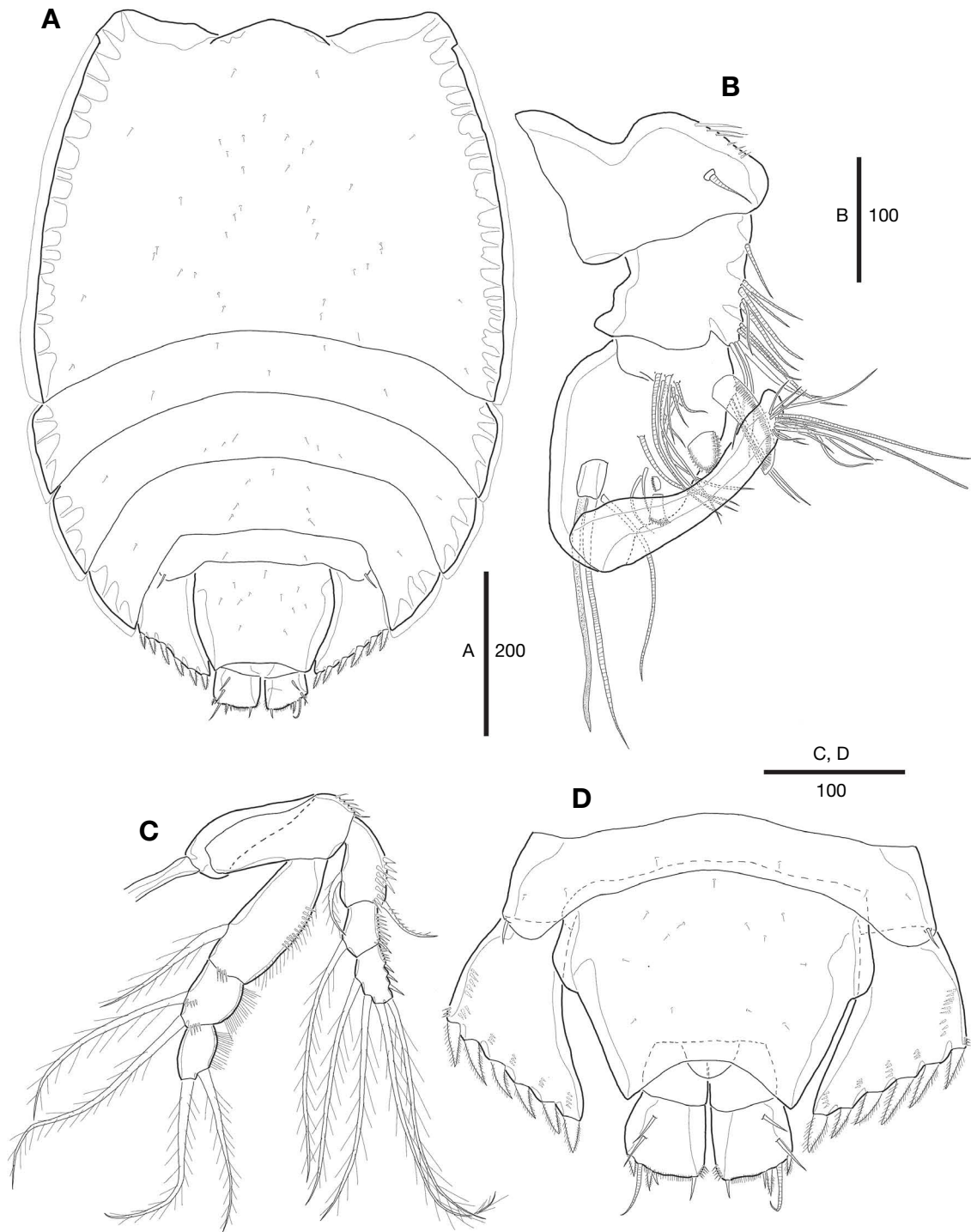


**Fig. 4.** *Kushia zosteraphila*. Female. A: P1; B: P2; C: P3; D: P4. Scale bar in  $\mu\text{m}$ .

shorter than wide and located on coxa, with slender outer seta and spinules on outer lateral margin. Endopod three-segmented and shorter than exopod, reaching almost

proximal half of exp-3; enp-1 with a row of spinules along the outer margin, with one plumose inner seta; enp-2 with a row of spinules along the outer margin, with one pin-





**Fig. 5.** *Kushia zosterophila*. Male: A: Habitus, dorsal; B: Antennule; C: P2; D: Urosome, dorsal. Scale bar in  $\mu\text{m}$ .

nate inner seta, one plumose inner seta, and one naked outer seta; enp-3 with rows of spinules along the outer margin and proximal surface, with one plumose inner seta and one pinnate inner seta, two plumose distal setae, and one plumose outer seta. Exopod three-segmented; exp-1

with a row of spinules along the outer margin, with one plumose inner seta and one naked outer seta; exp-2 with rows of spinules along the outer margin and posterior surface, with one plumose inner seta and one naked outer spine; exp-3 with rows of spinules on proximal lateral

margin and posterior surface, with three plumose inner setae, two plumose distal setae, and three naked outer spines.

P4 (Fig. 4D). Intercoxal sclerite wide. Coxa broad. Basis shorter than wide and located on coxa, with slender outer seta and spinules on the outer margin. Endopod three-segmented and shorter than exopod, reaching to distal margin of exp-2; enp-1 with two rows of spinules along the outer margin and posterior surface, with one plumose inner seta; enp-2 with a row of spinules along the outer margin, with one pinnate inner seta; enp-3 with a row of spinules along the outer margin, with one pinnate inner seta, two plumose distal setae, and one plumose outer seta. Exopod three-segmented; exp-1 with two rows of spinules along the outer margin, with one plumose inner seta and one pinnate outer spine; exp-2 with rows of spinules on lateral margin and posterior surface, with one plumose inner seta and one pinnate outer spine; exp-3 with rows of spinules on lateral margin and posterior surface, with three plumose inner setae, two plumose distal setae, and three pinnate outer spines.

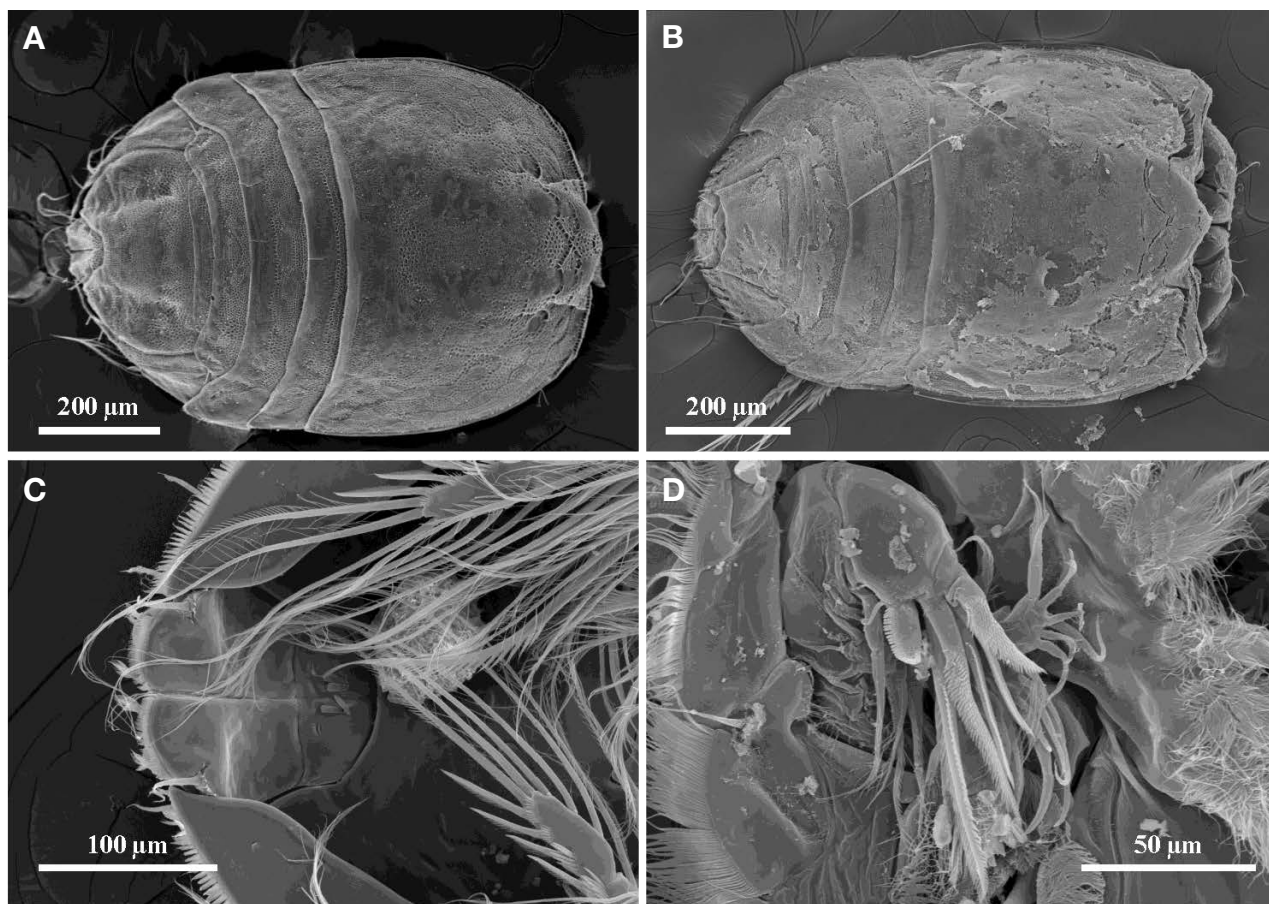
Armature formula for swimming legs:

	Exopod	Endopod
P1	0.0.123	1.2
P2	1.1.223	1.2.121
P3	1.1.323	1.2.221
P4	1.1.323	1.1.121

P5 (Fig. 2D). Baseoendopod with two distal setae each on the dorsal and ventral surface. Exopod forming keeled plate, tapering distally; a row of spinule along outer margin; exopod with five setae, one plumose seta on the medio-lateral margin, four setae on the distal half surface (distal seta 1 as long as seta 2 and 3; distal seta 4 shortest).

**Description of male.** Total body length 860–870  $\mu\text{m}$  (mean  $\pm$  SD =  $863 \pm 6 \mu\text{m}$ ,  $n = 3$ ) as measured from the anterior margin of cephalosome to the posterior margin of the caudal rami. Dorsal pit present on prosome, urosome, P5 and CR (Fig. 6B). Body surface armed with some sensilla (Fig. 5A).

Prosome (Figs. 5A, 6B) four-segmented; comprising cephalosome and three free pedigerous somites. P1-bearing somite fused to cephalosome. Hyaline membrane



**Fig. 6.** *Kushia zosterophila*. Scanning electron micrographs. A: Female, habitus; B: Male, habitus; C: Female, caudal rami, ventral view; D: Male, maxilla. Scale bar in  $\mu\text{m}$ .



present on lateral margin.

Urosome (Fig. 5D) three-segmented, comprising the P5 somite, genital somite and anal somite. Caudal ramus almost quadrate, without bevelled lateral edge. Seta III annulate and inserted on distal margin.

Sexual dimorphism in A1, P2, and P5.

Antennule (Fig. 5B) four-segmented. Aesthetasc on segment 3 and 4. 3rd segment with conspicuous comb on the accessory lobe, proximal coupling denticle with a double serrated edge. Armature formula: 1-[1], 2-[11], 3-[15 + ae], 4-[11 + ae]. Most of setae annulate.

P2 (Fig. 5C) exopod as in female. Endopod three-segmented and longer than exopod, with hairs along the outer margin of each segment; enp-1 with one plumose inner seta; enp-2 with two plumose inner setae; enp-3 with two plumose distal setae.

P5 (Fig. 5D) with six short and pinnate setae on the distal margin; rows of spinules on the ventral surface.

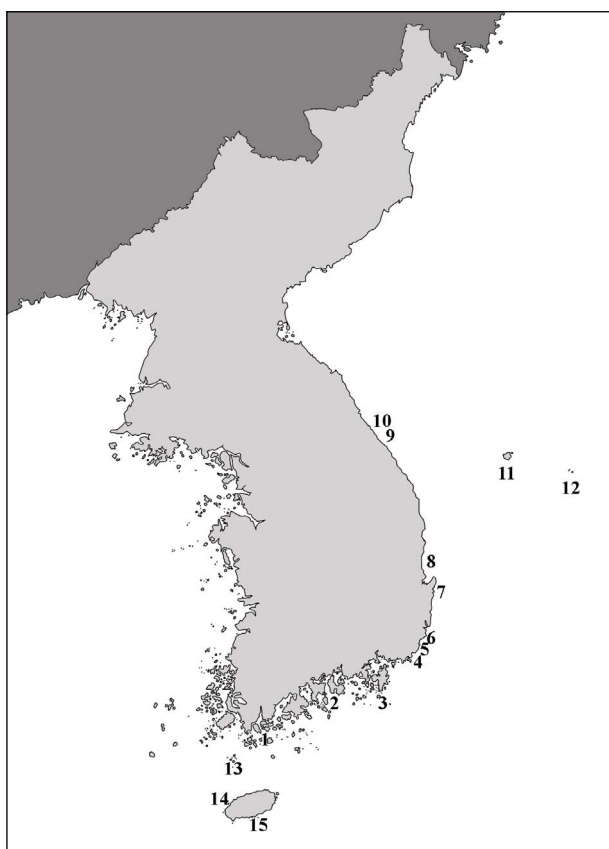
## DISCUSSION

Harris and Iwasaki (1996b) established the new genus, *Kushia*, including three new species (*Ku. gamoi*; *Ku. igaguria*, and *Ku. zosteraphila*) based on the following morphological characteristics: female P5, caudal ramus and male A1. These species were collected from macroalgae in the sublittoral region at Kadonohama Bay, Ofunato, Iwate Prefecture, Japan. After then, *Ku. spathoides* Harris V.A., 2014 has been published as a new species in Australia (Harris, 2014). As a result, the genus *Kushia* now contains four species. So far, only one species, *Ku. gamoi* has been reported in Korea, and this species has been collected at several localities: Dolsando, Gangneung, Gijang, Guryongpo, Maemuldo, and Wando (Kim and Kim, 1997).

The specimen described in this study belongs to the genus *Kushia* with the character set including the presence of a wide leaf-like ventral expansion of the female P5

**Table 1.** Distribution of species of the Family Porcellidiidae in Korea. Numbers in bracket indicate the numbers in Fig. 7.

Species	Type locality	Habitat	Korean localities	References
<i>Dilatatiocauda bipartita</i> (Kim I.H. & Kim H.S., 1997)	Maemuldo, Korea	Macroalgae	Maemuldo (3)	Kim and Kim, 1997
<i>Kensakia acuta</i> (Kim I.H. & Kim H.S., 1997)	Maemuldo, Korea	Macroalgae	Gangneung (9), Maemuldo (3), Munseom (15)	Kim and Kim, 1997 Lee et al., 2012
<i>Kioloaria jejuensis</i> Huys, 2016	Hanrim, Korea	Hermit Crab	Hanrim (14), Maemuldo (3), Munseom (15)	Kim and Kim, 1996 Lee et al., 2012
<i>Kioloaria paguri</i> (Ho, 1986)	Tassha bay, Japan	Hermit Crab	Weolpo (8)	Kim, 2014
<i>Kioloaria similis</i> (Kim S.H. & Kim W., 1996)	Ulleungdo, Korea	Hermit Crab	Chujado (13), Dokdo (12), Haeundae (4), Munseom (15), Ulleungdo (11)	Kim and Kim, 1996
<i>Kushia gamoi</i> Harris V.A. & Iwasaki, 1996	Kadonohama bay, Japan	Macroalgae	Dolsando (2), Gangneung (9), Gijang (5), Guryongpo (7), Maemuldo (3), Wando (1)	Kim and Kim, 1997
<i>Kushia zosteraphila</i> Harris V.A. & Iwasaki, 1996	Kadonohama bay, Japan	Macroalgae	Gijang (5)	Present study
<i>Porcellidium brevicavum</i> Kim I.H. & Kim H.S., 1997	Maemuldo, Korea	Macroalgae	Maemuldo (3)	Kim and Kim, 1997
<i>Porcellidium kiiroum</i> Harris V.A. & Iwasaki, 1996	Kadonohama bay, Japan	Macroalgae	Weolpo (8)	Kim, 2014
<i>Porcellidium ofunatense</i> Harris V.A. & Iwasaki, 1996	Kadonohama bay, Japan	Macroalgae	Dolsando (2), Gangneung (9), Gijang (5), Guryongpo (7), Namae (10), Wando (1)	Kim and Kim, 1997 Lee et al., 2012
<i>Porcellidium viride</i> (Philippi, 1840)	Sorrento, Italy	Macroalgae	Gori (6)	Yoo and Lee, 1995
<i>Porcellidium wandoensis</i> Kim I.H. & Kim H.S., 1997	Wando, Korea	Macroalgae	Dolsando (2), Gijang (5), Guryongpo (7), Wando (1)	Kim and Kim, 1997



**Fig. 7.** Reported localities of Porcellidiid species in Korea. 1. Wando; 2. Dolsando; 3. Maemuldo; 4. Haeundae; 5. Gijang; 6. Gori; 7. Guryongpo; 8. Weolpo; 9. Gangneung; 10. Namae; 11. Ulleungdo; 12. Dokdo; 13. Chujado; 14. Hanrim; 15. Munseom.

that lies under the lateral edge of the urosome, caudal seta III inserted ventrally along the bevelled edge of caudal rami, and the presence of a conspicuous comb on the accessory lobe of male antennule. Also, this specimen matches to *Ku. zosteraphila* based on the combination of the following character sets: (1) distinct color band across body, (2) first dorsal seta of female P5 as long as second dorsal seta, (3) length to width ratio of the female caudal ramus ( $< 2$ ), and (4) double serrated edge of proximal coupling denticle on male A1. However, the Korean specimens have some minor differences from the original description as follows: 1) mean body length (female 1,049  $\mu\text{m}$ ; male 863  $\mu\text{m}$ ) compared from the 930  $\mu\text{m}$  (female) and 760  $\mu\text{m}$  (male) in Harris and Iwasaki (1996b); 2) length of seta on the lateral margin of female P5 is similar with first dorsal seta, but it is shorter than first dorsal seta in Harris and Iwasaki (1996b); and 3) armature formula of male antennule is different with original description. The above characters between the original description and the present study are regarded as minor discrepancies within the range of observational errors, or individual variations.

So far, species of the family Porcellidiidae occurring in Korea, have been mainly reported on the southern and eastern coasts of Korea (Fig. 7 and Table 1), and most of them have been published as new species. The unrecorded species, *Ku. zosteraphila*, identified in this study was found from Gijang-gun, and several species (*Ku. gamoi*, *Porcellidium ofunatense*, and *P. wandoensis*) have been reported in this region (Lee *et al.*, 2012). The appearance of multiple species in the same region means that more species can be discovered if further study is conducted, and a DNA barcode would be a useful tool.

Molecular analysis with DNA barcoding is an efficient method to compare and identify species, especially those having difficult morphological characteristics (Hebert *et al.*, 2003; Karanovic *et al.*, 2018; Lee *et al.*, 2019). We obtained a 658-bp partial sequence of mtCOI, but molecular comparisons could not proceed because there were not enough sequences from congeners to date for comparison. If molecular results are accumulated for various species that occurred in Korea in the future, it would be useful to study the diversity of the family Porcellidiidae in Korea.

#### Key to species of the family Porcellidiidae Boeck, 1865 in Korea

1. Presence of seta IV on female caudal rami ..... 2
  - Absence of seta IV on female caudal rami ..... *Kensakia acuta*
2. Female caudal rami rhomboidal or trapezoidal shaped ..... 3
  - Female caudal rami not rhomboidal shaped (rectangular or pentagonal) ..... 4
3. P3 and P4 endopod-3 with four and three setae ..... *Kioloaria paguri*
  - P3 and P4 endopod-3 with five and four setae ..... *Kioloaria jejuensis*
4. Female caudal rami pentagonal shaped ..... 5
  - Female caudal rami square or rectangular shaped ..... 6
5. Length of female P5 distal seta 1 is similar with seta 2 ..... *Kushia zosteraphila*
  - Length of female P5 distal seta 1 is shorter than seta 2 ..... *Kushia gamoi*
6. Female P5 extend to posterior margin of caudal rami ..... *Kioloaria similis*
  - Female P5 not extend to posterior margin of caudal rami ..... 7
7. Seta I of female caudal rami locates on distal half surface of caudal rami ..... *Porcellidium brevicavum*
  - Seta I of female caudal rami locates on middle surface of caudal rami ..... 8
8. Basis of maxilliped without fimbriate edge ..... *Dilatatiocauda bipartita*

- Basis of maxilliped with fimbriate edge ..... 9
- 9. Male A1 with 'peg' like process on proximal surface of 3rd segment ..... *Porcellidium viride*
- Male A1 without process on 3rd segment ..... 10
- 10. Lateral edge of female genital double somite with a distinct cleft ..... *Porcellidium kiiroum*
- Lateral edge of female genital double somite with a notch ..... 11
- 11. First denticle on 4-segment of Male A1 pointed and comb-like shape ..... *Porcellidium ofunatense*
- Denticles on 4-segment of Male A1 bulbous shape ..... *Porcellidium wandoensis*

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