



A new species of the genus *Porirualia* Huys & Mu, 2021 (Copepoda, Harpacticoida, Parastenheliidae) from the intertidal zone of Qingdao, China, with a key to species of the genus

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Abstract

A new species belonging to the genus *Porirualia* Huys & Mu, 2021 was identified and described here based on samples collected from No. 1 Bathing Beach, Qingdao, China. The new species differs from *Porirualia pyriformis* mainly in the following characteristics: P3 and P4 exp-3 with three inner setae (two inner setae in *P. pyriformis*), ratio of length to maximum width of female P5 3.83 (3.25 in *P. pyriformis*). The new species differs from *Porirualia megarostrum* by the characters including rostrum reaching to distal margin of third segment of antennule (reaching to distal margin of fifth segment of antennule in *P. megarostrum*), ratio of length to maximum width of female P5 3.83 (2.2 in *P. megarostrum*); male P2 and P3 two-segmented (three-segmented in *P. megarostrum*); male P5 bearing five elements (six in *P. megarostrum*). This is the first report of the genus *Porirualia* from the China Seas. The DNA barcode (COI) sequence of the new species was obtained and submitted to GenBank (PP761118), which is the first submission of COI sequence of the family Parastenheliidae to GenBank.

Key words: Crustacea, new taxa, taxonomy, morphology

Introduction

The harpacticoid copepod genus, *Porirualia* Huys & Mu, 2021, belonging to the family Parastenheliidae Lang, 1936, was proposed by Huys & Mu (2021) includes two species: *Porirualia megarostrum* (Wells, Hicks & Coull, 1982) distributed in New Zealand, and *P. pyriformis* (Song, Kim & Chang, 2003) distributed in Korea. So far, all *Porirualia* species are endemic and seems to be restricted to shallow marine habitats (Song *et al.* 2003; 2012; Huys & Mu 2021).

The taxonomic studies of marine parastenheliid harpacticoids are poorly carried in China. At present, only two species of Parastenheliidae Lang, 1936 from China seas were recorded: *Johnwellsia bipartite* Huys & Mu, 2021 collected from Dadeji Beach in Xiamen (Huys & Mu 2021), and *Parastenhelia spinosa* (Fischer, 1860) from Fujian (Lian *et al.* 2022). During a recent survey of the intertidal copepod fauna of No. 1 Bathing Beach in Qingdao, China, both sexes of a new species of the genus *Porirualia* were discovered. The new species, which is here described in detail, increases the species number of the genus *Porirualia* to three. This paper also provides an identification key to the species currently recognized in the genus *Porirualia*.

Materials and methods

Specimens were collected on 15 September 2021 from the gravelly sandy intertidal zone of No. 1 Bathing Beach (36°3'18"N, 120°20'20"E), Qingdao, Shandong, China. The specimens were extracted from benthic samples

using a 38 µm sieve and sorted using a light microscope (Nikon SMZ1270). The specimens were fixed in 100% alcohol, and refrigerated at -20 °C before DNA extraction. DNA was extracted from one individual prior to further morphological examination.

Non-destructive DNA extraction from each specimen followed Kim *et al.* (2020). Specimen was transferred to a 1.5 mL sterilized centrifuge tube containing 180 µL of ATL buffer and 20 µL of Proteinase K for non-destructive DNA extraction. The subsequent DNA extraction process was performed according to the protocol of QIAamp DNA Micro Kit (Qiagen, Hilden, Germany), except that the exoskeleton of each specimen was picked out and transferred into a new 1.5 mL sterilized centrifuge tube containing sterile distilled H₂O after the specimen was completely digested. Polymerase chain reaction (PCR) amplification was carried out in a reaction mix with 3 µL of the DNA, 12.5 µL of Premix Taq™ (Takara, Otsu, Shiga, Japan), 1 µL of each primer (10 mM) and 7.5 µL sterile distilled H₂O. The mitochondrial COI gene was amplified using the primer LCO1490/HCO2198 (Folmer *et al.* 1994). The PCR conditions were as follows: initial denaturation for 5 min at 94 °C, followed by 45 cycles of denaturation at 94 °C for 60 s, annealing at 48 °C for 90 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min. PCR products were purified using the Wizard™ SV Gel and PCR Clean-UP System (Promega, Madison, WI, United States) before sequencing. The purified PCR products were sequenced from both directions using the same forward and reverse primers for PCR amplification with ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, United States). Each sequence was BLASTed in the NCBI database to confirm it was not contaminated. Then, the DNA barcode sequence was submitted to GenBank. After DNA extraction, the exoskeleton of each specimen and other specimens preserved in 100% alcohol was dissected and observed under a stereomicroscope (Nikon SMZ1270).

Before dissection, the habitus was drawn, and the body length was measured. Specimens were dissected in lactic acid and mounted on slides, which were subsequently coated by coverslip and sealed with nail polish. The observations and drawings were taken by using a differential interference contrast microscope (Nikon Eclipse Ni), equipped with a drawing tube. Habitus were drawn at 400 magnification. Other figures were drawn at 1000 magnification, with an oil immersion lens.

The descriptive terminology follows that of Huys *et al.* (1996). Abbreviations used in the text and figures are: *aes*, aesthetasc; *A1*, antennule; *A2*, antenna; *P1–P6*, first to sixth thoracic legs; *exp(enp)-1(-2,-3)*, the proximal (middle, distal) segment of a ramus. Body length was measured from the anterior margin of the rostrum to the posterior margin of the caudal rami. The type materials are deposited in the Marine Biological Museum, Chinese Academy of Sciences, Qingdao, China (MBMCAS).

Systematics

Order Harpacticoida Sars, 1903

Family Parastenheliidae Lang, 1936

Genus *Porirualia* Huys & Mu, 2021

Type species. *Porirualia megarostrum* (Wells, Hicks & Coull, 1982).

Other species. *Porirualia pyriformis* (Song, Kim & Chang, 2003).

Porirualia ngankeae sp. nov.

(Figs. 1–7)

Type material. *Holotype*: MBM189275, female dissected on three slides, China, the Yellow Sea, Qingdao, No. 1 Bathing Beach, 36°3'18"N, 120°20'20"E, intertidal zone, fine sand, 15 September 2021. *Allotype*: MBM189276, male dissected on three slides, collected with holotype. *Paratypes*: MBM189277, 1 female dissected on five slides, collected with holotype; MBM189278, 1 female dissected on three slides, collected with holotype; MBM189279, 1 female (DNA amplification successful) dissected on three slides, collected with holotype; MBM189280, 1 female dissected on three slides, collected with holotype; MBM189281, 1 male dissected on three slides; MBM189282, 1 male dissected on two slides, collected with holotype.

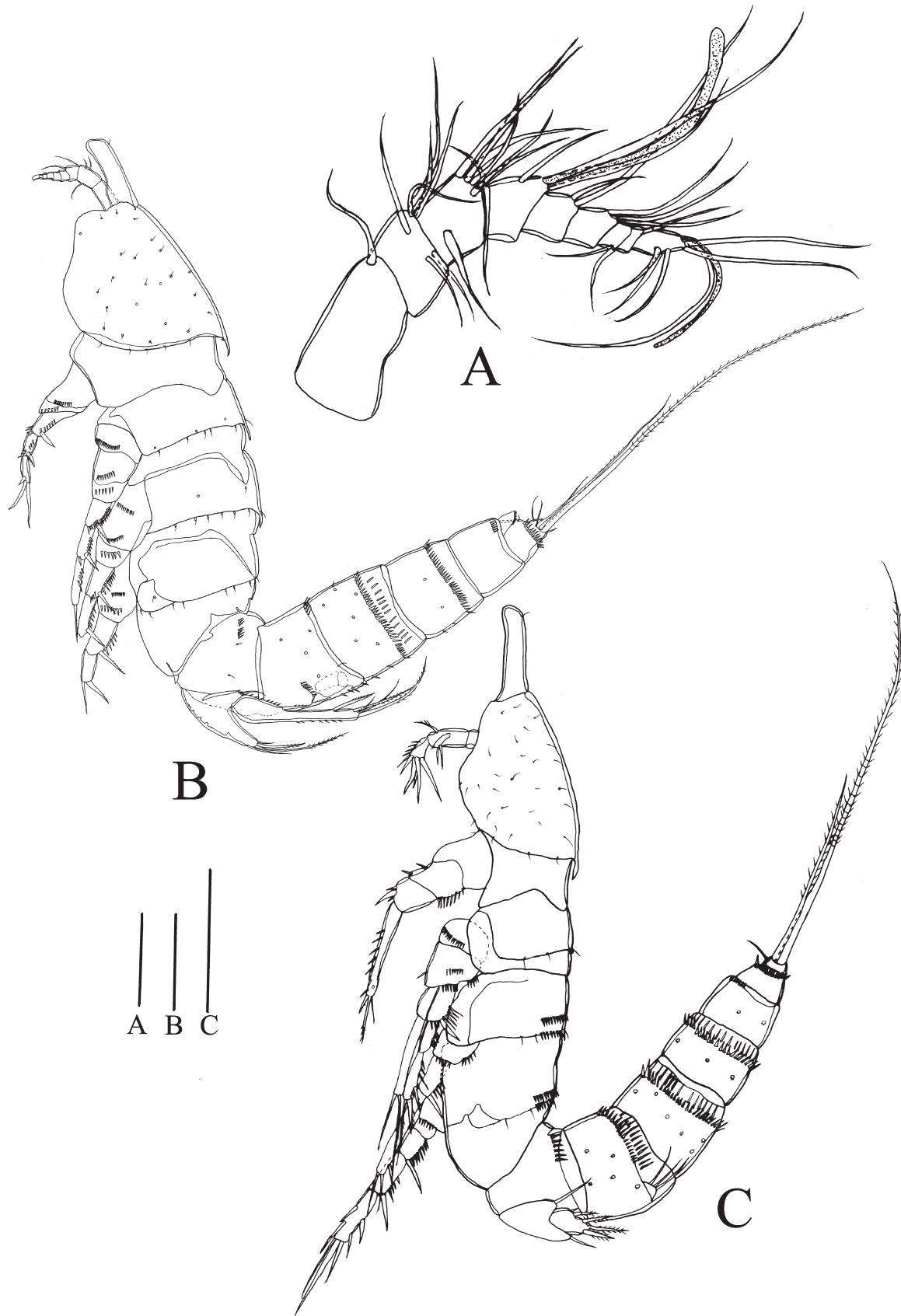


FIGURE 1. *Porirualia ngankeeae* sp. nov., female: A, antennule, dorsal; B, habitus, Lateral. male: C, habitus, lateral. Scale bars: A = 20 μ m, B–C = 100 μ m.

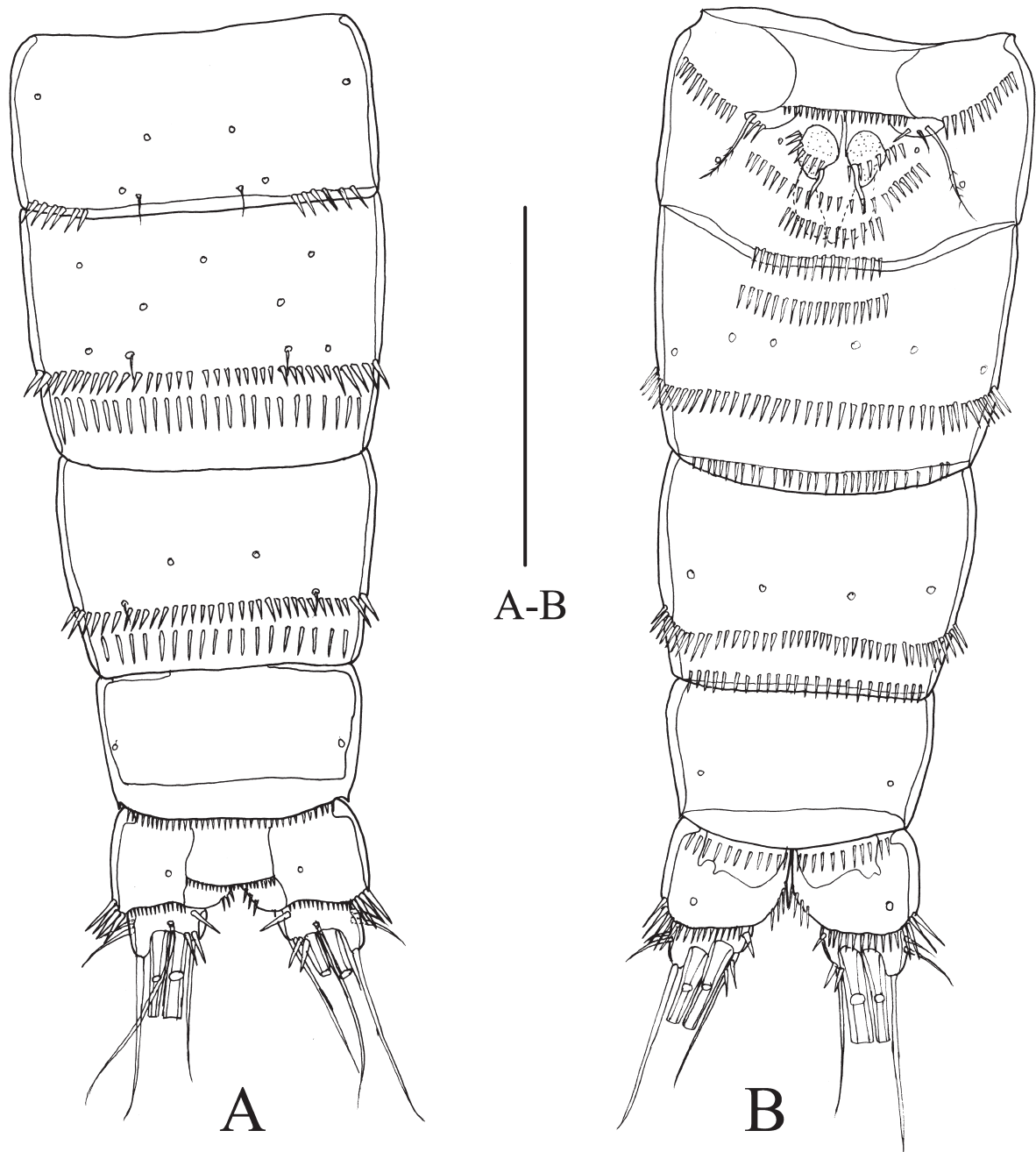


FIGURE 2. *Porirualia ngankeeae* sp. nov., female: A, urosome, dorsal; B, urosome, ventral. Scale bars = 100 μ m.

Other material examined. MBM189283, 3 females and 1 male, collected from type locality and preserved in 75 % ethanol.

Etymology. The species is named in honour of our very good and kind friend and colleague, the late Singapore carcinologist, Dr. Ngan Kee NG, for her great works in brachyuran crustacean taxonomy and systematics.

Description of female. Total body length, measured from anterior margin of rostrum to posterior margin of caudal rami, ranging from 542 to 587 μ m (mean = 563 μ m; $n = 5$).

Habitus (Figs. 1B, 2A–B). Fusiform, urosome usually curved to prosome dorsally. All prosomites with sensillae as illustrated. Somites with conspicuous pores as figured. Rostrum (Fig. 1A) defined at base, elongated and rounded apically, directed downward, with paired sensillae located near apex. Urosomites with obvious hyaline frills all around posterior margin. Genital somite ornamented with spinule rows on posterior margin dorsally; genital field with large copulatory pore and several spinule rows; genital apertures covered by vestigial P6 bearing three setae,

inner two short, outer one long and plumose. Anal operculum semicircular, bordered with fine spinules. Caudal ramus wider than long, with conspicuous spinular ornamentation; bearing seven setae, setae I and II naked, positioned ventrally near outer margin, seta III slender, longer than seta VI, originating from pedestal at outer distal corner, setae IV–V longest and bipinnate, seta VI slender and naked, issued at inner distal corner, seta VII triarticulated at base, arising from dorsal surface.

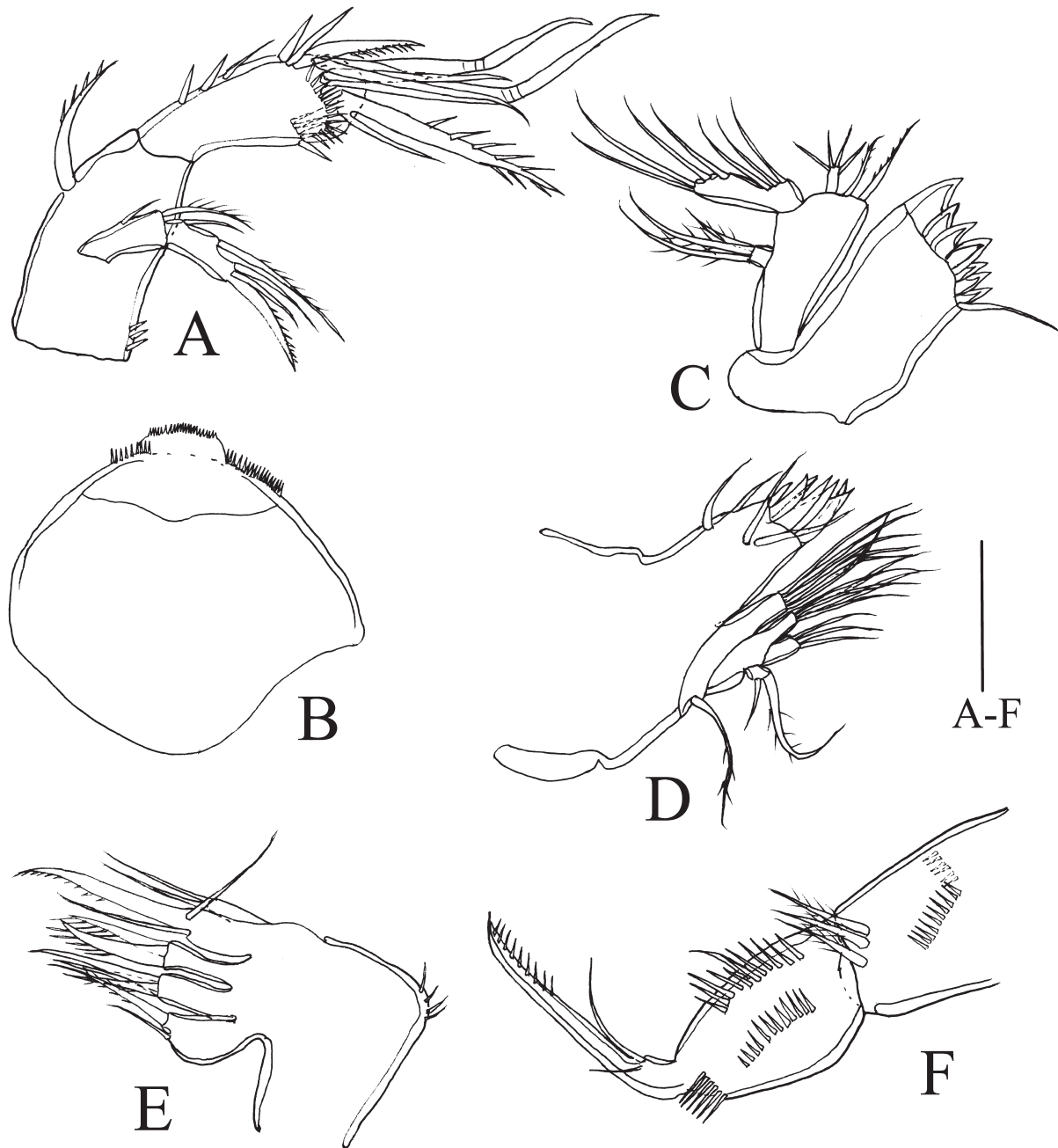


FIGURE 3. *Porirualia ngankeae* sp. nov., female: A, antenna; B, labrum; C, mandible; D, maxillule; E, maxilla; F, maxilliped. Scale bars = 20 μ m.

Antennule (Fig. 1A). Nine-segmented; segment 1 longest, surface of all segments smooth; with aesthetascs on fourth and distal segment. Armature formula: 1-[1], 2-[7], 3-[9], 4-[1 + (2 + aes)], 5-[2], 6-[3], 7-[2], 8-[1], 9-[3 + (3 + aes)].

Antenna (Fig. 3A). Allobasis with three spinules along outer margin; with one subdistal abexopodal seta. Exopod two-segmented; first segment with two setae, one subdistal and one distal; second segment with two lateral setae and three apical setae. Endopod one-segmented, with two transversal rows of spinules on anterior and posterior

surface respectively, three inner spinules along inner margin and some outer spinules subdistally; bearing nine elements: two small naked and one large unipinnate spines subdistally, two slender and naked setae distally, two geniculate apical setae, one spinose and one naked apical setae fused at base.

Labrum (Fig. 3B). Almost triangular, with many spinules around pointed apex.

Mandible (Fig. 3C). Gnathobase well developed, bearing several multicuspidate teeth along distal margin and one seta at dorsal corner. Basis elongated, carrying two plumose setae and bulbous seta distally. Endopod one-segmented, bearing seven setae as figured. Exopod much smaller than endopod, bearing three plumose setae.

Maxillule (Fig. 3D). Arthrite with two lateral setae, seven apical teeth, and two setae on anterior surface. Coxal endite with six setae and epipodite represented by one plumose seta. Basis with five apical setae. Endopod and exopod one-segmented, bearing three and two setae respectively.

Maxilla (Fig. 3E). Syncoxa ornamented with several setules along outer margin; with three endites, proximal endite bilobate with one naked setae on inner cusp and one plumose seta on outer cusp, middle endite with three setae, and outer endite with two pinnate setae. Allobasis with one strong claw bearing two naked setae. Endopod one-segmented, with two slender setae.

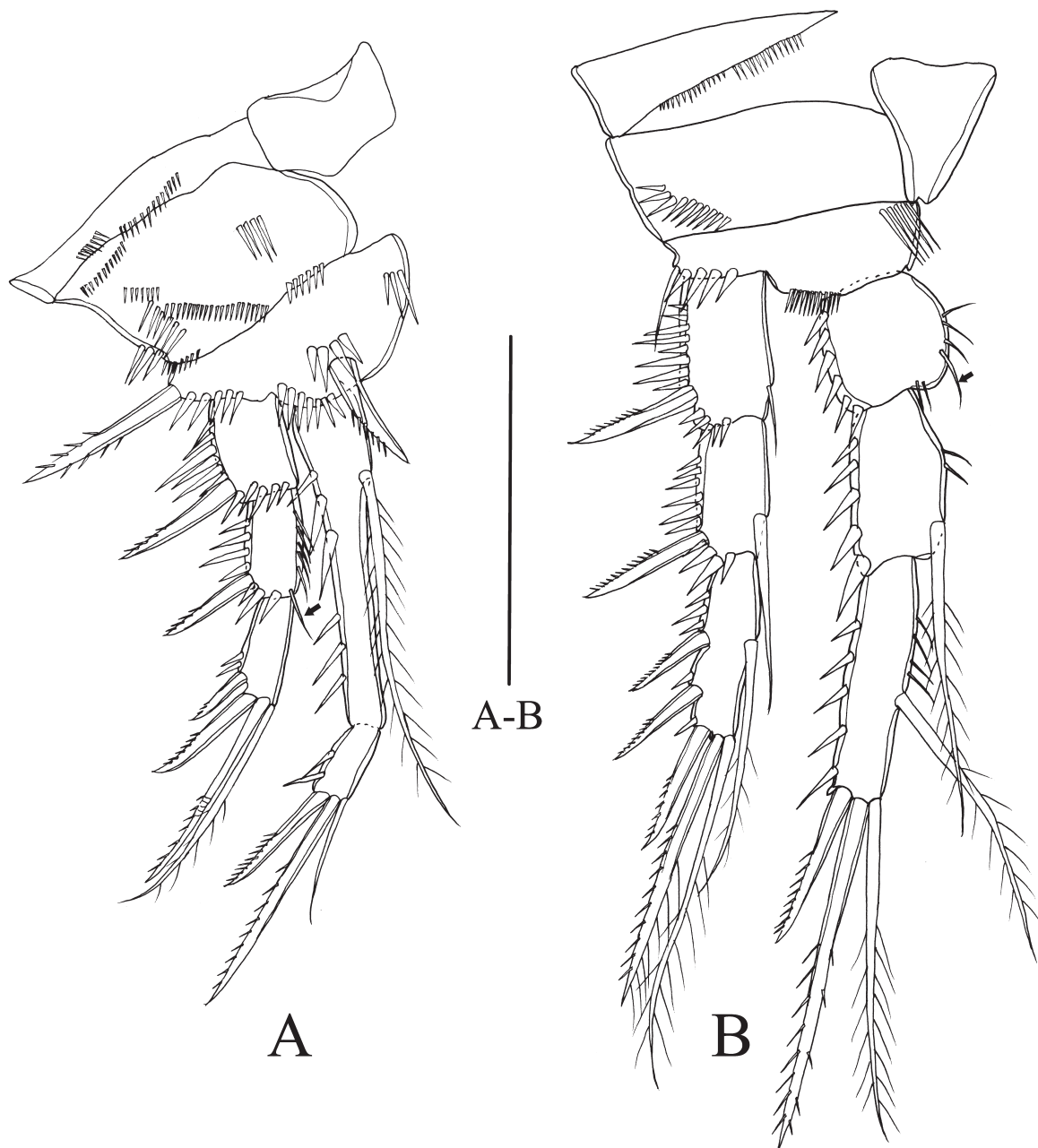


FIGURE 4. *Porirualia ngankeeae* sp. nov., female: A, P1, anterior; B, P2, anterior. Scale bar = 50 μ m.

Maxilliped (Fig. 3F). Syncoxa with two rows of spinules on surface and three setae subdistally. Basis with three spinule rows on palmar surface and one seta on medial margin. Endopod represented by one strong and unipinnate claw; bearing two accessory, naked setae.

P1 (Fig. 4A). Intercoxal sclerites smooth. Praecoxa narrow, with two rows of spinules subdistally. Coxa with several rows of spinules as figured. Basis almost trapezoidal, with one pinnate outer spine, one unipinnate inner spine, few spinules on surface, distal and inner margins. Exopod three-segmented, first segment with one spine and many spinules along outer margin; second segment slightly longer than first, with one tiny seta on inner distal corner (see arrow in Fig. 4A); distal segment with two outer spines, one geniculate seta and one plumose seta apically. Endopod two-segmented; proximal segment strikingly elongated, about 4.8 times as long as greatest width, overreaching apex of exopod, with one pinnate inner seta inserted proximally 1/4; distal segment slightly longer than wide, bearing one bare seta and two pinnate spines.

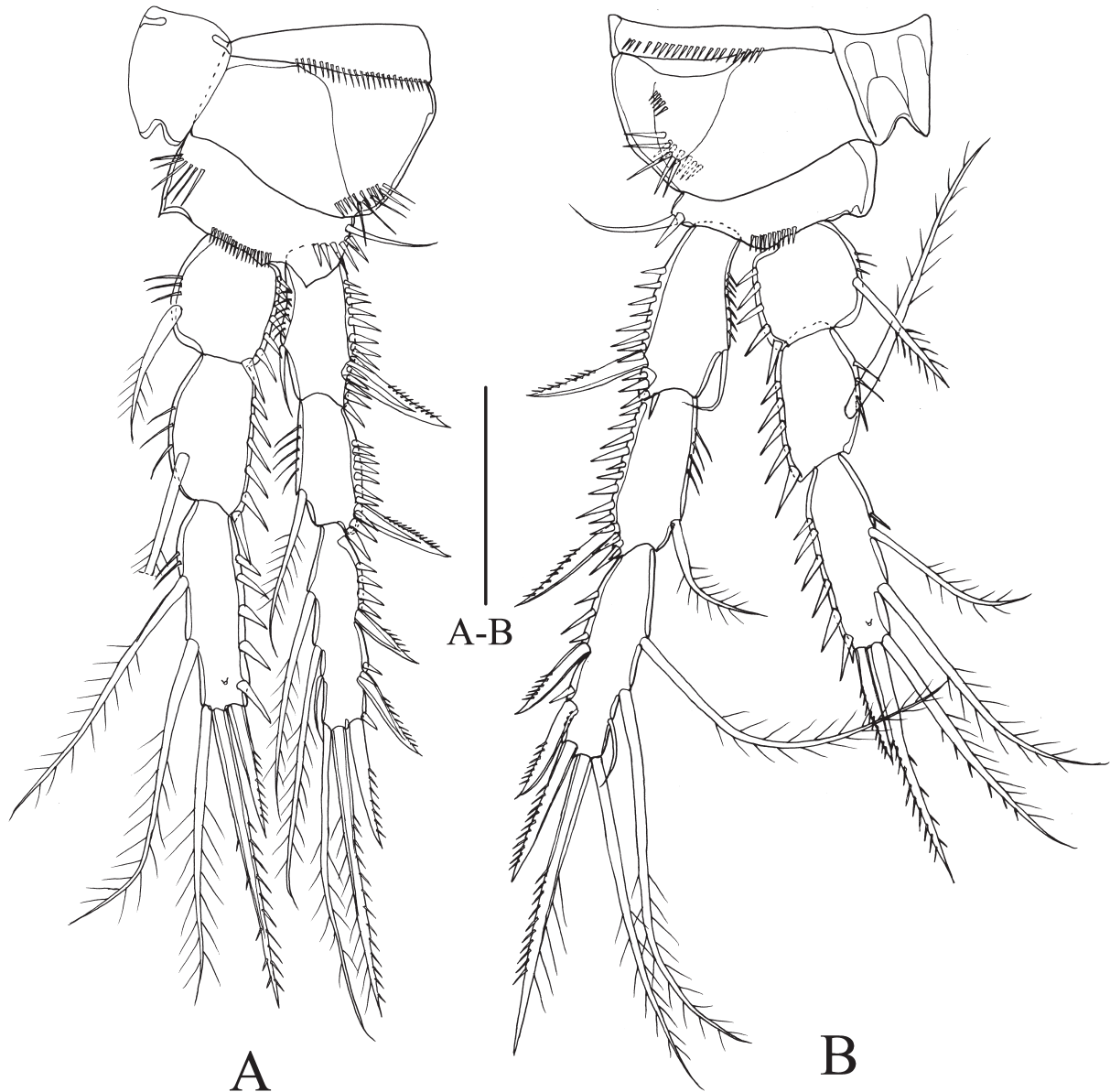


FIGURE 5. *Porirualia ngankeae* sp. nov., female: A, P3, anterior; B, P4, anterior. Scale bar = 50 μ m.

P2 (Fig. 4B). Intercoxal sclerite unornamented, almost trapezoidal. Praecoxa triangular, with one row of spinules distally. Coxa almost rectangular, with one row of spinules on anterior surface close to outer margin. Basis with naked outer seta, few spinules on subdistal margin, row of long spinules close to proximal inner margin. Exopod three-segmented, all segments with few spinules along outer margin, one outer unipinnate spine and one inner seta,

respectively; second segment about equal to first; distal segment about 1.3 times as long as second. Endopod three-segmented; proximal segment short, with one weak seta (see arrow in Fig. 4B) on inner margin; second segment about 1.4 times as long as proximal, with one inner plumose seta; distal segment about 1.6 times as long as proximal, with one inner plumose seta, one plumose seta and one spine distally, one outer spine; all segments with few setules on inner margin, several spinules along outer margin.

P3 (Fig. 5A). Intercoxal sclerites smooth, with two distal blunt projections. Praecoxa narrow, with one row of spinules distally. Coxa with two rows of spinules on outer margin. Basis with naked outer seta, row of spinules on inner, subdistal and outer margins, respectively. Exopod three-segmented; first segment with few setules and one naked seta on inner margin, few large spinules and one unipinnate spine on outer margin; second segment similar as first, except inner seta plumose; distal segment about 1.5 times as long as second, with two plumose and one small inner setae, one plumose seta and one unipinnate spine apically, three unipinnate spines and several spinules along outer margin. Endopod three-segmented; proximal segment short, with one plumose seta and several setules on inner margin; second segment longer than first, with one long plumose seta and several setules on inner margin; distal segment about 1.6 times as long as second, with one pore on anterior middle surface close to distal margin, bearing two plumose inner setae, one plumose seta and one spinulose spine apically, one unipinnate outer spine; all segments with few spinules on outer margin.

P4 (Fig. 5B) Intercoxal sclerites smooth, with two distal blunt projections. Praecoxa narrow, with one row of spinules on anterior surface. Coxa slightly broader than long, with two rows of spinules on anterior surface and one row of spinules on posterior surface. Basis with one row of subdistal spinules and long, naked, outer seta. Exopod three-segmented; first segment with few small spinules and one naked seta on inner margin, few large spinules and one unipinnate spine on outer margin; second segment as long as first, with one plumose seta and few setules along inner margin, one unipinnate spine and few spinules on outer margin; distal segment about 1.4 times as long as second, with two plumose and one small inner setae, one plumose seta and one unipinnate spine apically, three unipinnate spines and several spinules along outer margin. Endopod three-segmented; proximal segment short, with one plumose seta and several setules on inner margin; second segment longer than first, with one long plumose seta and several setules on inner margin; distal segment about 1.6 times as long as second, with one pore on anterior middle surface close to distal margin, bearing two plumose inner setae, one plumose seta and one spinulose spine apically, one unipinnate outer spine; all segments with few spinules on outer margin.

P5 (Fig. 7A–B). Baseoendopods not fused medially. Baseoendopodal lobe extending to almost a proximal quarter of exopod, with five elements (two strong unipinnate spines, two pinnate setae, one slender and naked seta). Exopod elongated, about 3.83 times as long as greatest width, with two outer bipinnate, two subdistal (one bipinnate, one small and naked) and two distal setae (one bipinnate, one slender and naked).

Description of male. Total body length, measured from anterior margin of rostrum to posterior margin of caudal rami, ranging from 420 to 461 μm (mean = 446 μm ; $n = 3$).

Habitus (Figs. 1C, 6A–B). Smaller than female; generally as in female, except third and fourth prosomites bearing spinules along distal margin, second urosomite with continuous distal hyaline frills on dorsal surface and less spinules on ventral surface.

Antennule (Fig. 7F). Haplocer, 11-segmented, fifth segment slightly swollen; surface of all segments smooth; with aesthetasc on fifth and apical segments. Armature formula: 1-[1], 2-[3], 3-[5], 4-[4], 5-[3 + (1 + aes)], 6-[2], 7-[0], 8-[1], 9-[4], 10-[5], 11-[3 + aes].

Antenna, mandible, maxillule, maxilla, maxilliped, P1 and P4 as in female.

P2 (Fig. 7C). Exopod as in female. Endopod two-segmented, first segment with one minute inner seta; second segment with two inner setae (one naked and one plumose), one plumose seta and one bipinnate spine apically, one outer spine; all segments with few spinules along outer margin.

P3 (Fig. 7D). Exopod as in female. Endopod two-segmented, first segment with one inner seta; second segment with two inner setae, one plumose seta and one bipinnate spine apically, one outer spine; all segments with few spinules along outer margin.

P5 (Fig. 7E). Baseoendopodal lobe extending to almost proximal 3/4 of exopod, confluent on middle of its inner portion, with one spinulose seta and one dwarf seta. Exopod rectangular, bearing five elements (two apical bipinnate spines, one slender and bare seta, two bipinnate outer spines).

P6 (Fig. 6B). Bearing three setae, of which inner one shorter, middle one and outer one almost equal.

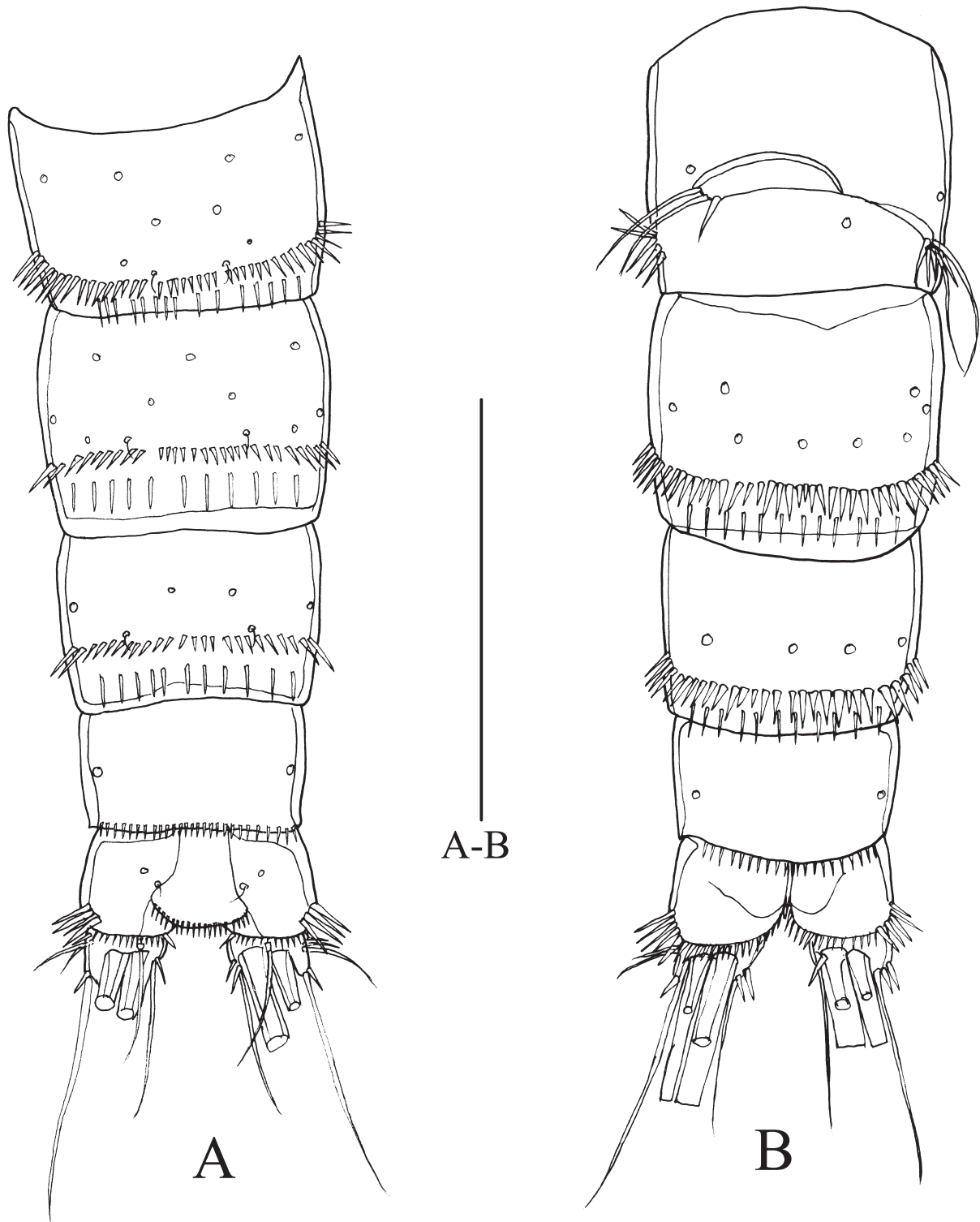


FIGURE 6. *Porirualia ngankeeae* sp. nov., male: A, urosome, dorsal; B, urosome, ventral. Scale bars = 100 μ m.

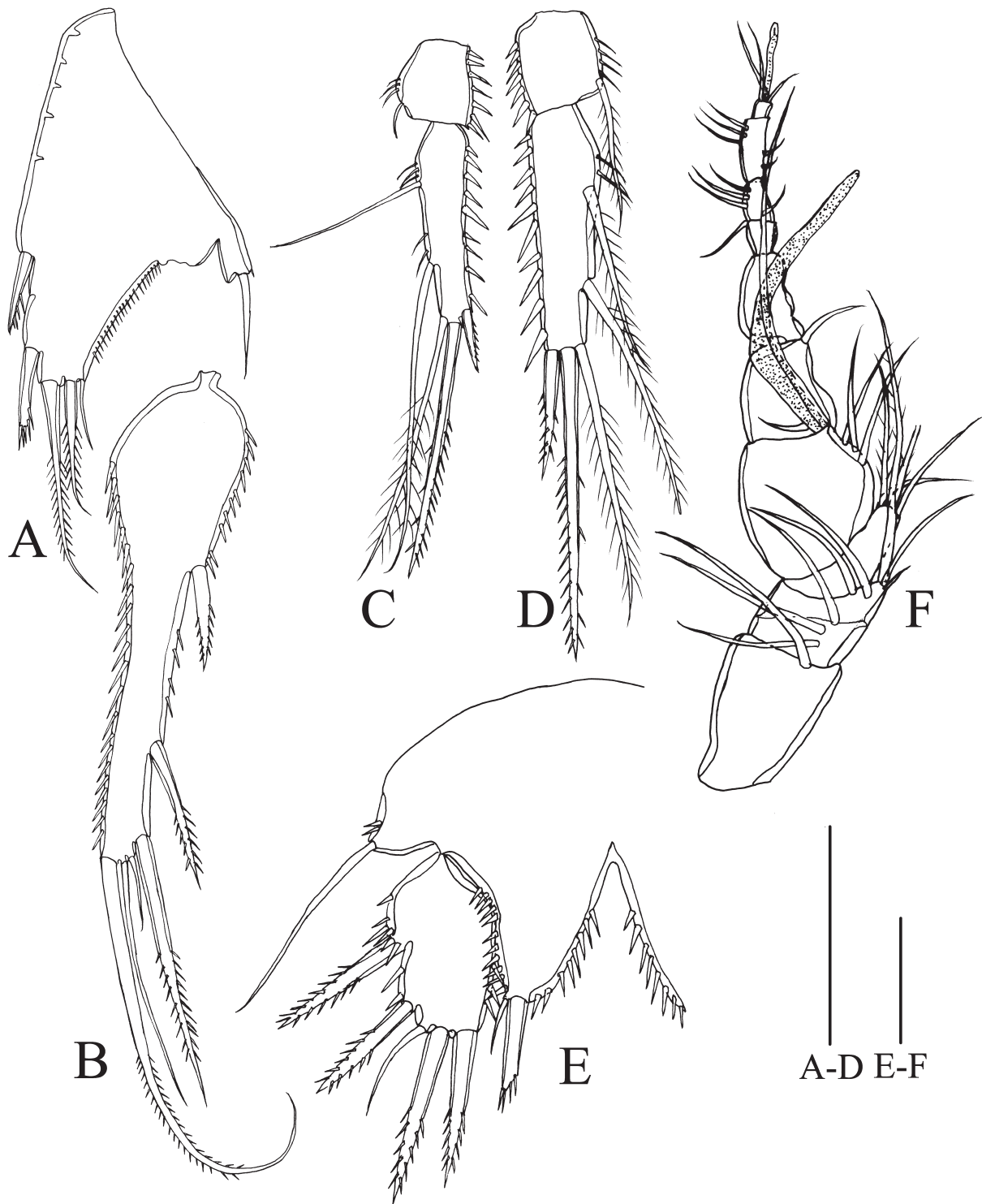


FIGURE 7. *Porirualia ngankeeae* **sp. nov.**, female: A, P5 baseoendopod, anterior; B, P5 exopod, anterior. male: C, P2 endopod, anterior; D, P3 endopod, anterior; E, P5, anterior; F, antennule, dorsal. Scale bars: A–D = 50 µm, E–F = 20 µm.

Discussion

Huys & Mu (2021) erected the genus *Porirualia* in accordance with Mielke (1990)'s and Gee (2006)'s views, which suggested removing *Parastenhelia megarostrum* Wells, Hicks & Coull, 1982 and/or *Pa. pyriformis* from the genus *Parastenhelia* because they lack the characteristic sexual dimorphism on the male P3 endopod that defines this genus. Based on species *Porirualia megarostrum* (Wells, Hicks & Coull, 1982) and *P. pyriformis* (Song, Kim & Chang,

2003), Huys & Mu (2021) gave apomorphic characters of the genus *Porirualia*, including large rostrum reaching to at least halfway the fourth antennular segment in the female, P1 enp-1 elongated, with inner seta extending beyond distal margin of segment; female P5 with series of transverse striae along inner margin of endopodal lobe. Except these apomorphic characters, the genus *Porirualia* also has the characters as mandible exopod one-segmented, with three setae; maxillipedal basis with one seta. The new species accords with the diagnosis of the genus *Porirualia* given by Huys & Mu (2021), except the rostrum of the present new species just reaching the end of the third antennular segment in the female. So we assigned the new species in the genus *Porirualia*.

TABLE 1. Armature formulae of P1–P4

| | Exopod | Endopod |
|----|---------|-----------------------|
| P1 | 0.1.022 | 1.120 |
| P2 | 1.1.123 | 1.1.121 (male 1. 221) |
| P3 | 1.1.323 | 1.1.221 (male 1.221) |
| P4 | 1.1.323 | 1.1.221 |

TABLE 2. Morphological characters of the *Porirualia* species (amended from Song *et al.* 2003 and Huys & Mu 2021)

| Morphological character | <i>Porirualia megarostrum</i> (Wells, Hicks & Coull, 1982) | <i>Porirualia pyriformis</i> (Song, Kim & Chang, 2003) | <i>Porirualia ngankeae</i> sp. nov. |
|---|--|--|---|
| Rostrum size | reaching to distal margin of segment 5 of antennule | reaching to margin of segment 2 of antennule | reaching to distal margin of segment 3 of antennule |
| Antennule | segment 1 longest | segment 2 longest | segment 1 longest |
| Antennary | exp-2 with four setae/spines | exp-2 with five elements | exp-2 with five elements |
| Armature of mandibular basis | with one spine and three setae | with one spine and two setae | with three setae |
| Origin of P1 enp-1 inner seta | proximal 1/3 | proximal 1/4 | proximal 1/4 |
| Length:width ratio of P1 enp-2 | 2.2 | 1.3 | 2.1 |
| Armature of female P2 exp/enp | 0(1), 1, 123/0(1), 1, 121 | 1, 1, 123/1, 1, 121 | 1, 1, 123/1, 1, 121 |
| Armature of P3–P4 exp | 0(1), 1, 323 | 1, 1, 223 | 1, 1, 323 |
| P5 female L: maximum W ratio | 2.2 | 3.25 | 3.83 |
| Segment of male P2 Enp (setae armature) | 3(1,1,4) | 2(1, 5) | 2(1, 5) |
| Segment of male P3 Enp (setae armature) | 3(1,1,4) | 2(1, 5) | 2(1, 5) |
| Number of elements on male P5 exp | 6 | 5 | 5 |
| Operculum | fine hairs | spinulose | spinulose |
| References | Wells <i>et al.</i> 1982 | Song <i>et al.</i> 2003 | present study |

In Table 2, all currently valid species of *Porirualia* are listed, together with some of their most prominent morphological characters. All morphological characters were collected from publications, except for the new species described here. *Porirualia ngankeeae* **sp. nov.** is most related to *P. pyriformis* in sharing the following characters combination: A2 exp-2 with five elements; female P5 exopod elongated; male P3 and P4 endopod two-segmented; male P5 with five setae; operculum spinulose. *Porirualia ngankeeae* **sp. nov.** is distinguished from *P. pyriformis* mainly on ornamentation type of habitus, where *P. ngankeeae* **sp. nov.** has more hyaline frills in genital field and less spinules ventrally (Fig 2A, 2B), rostrum reaching to distal margin of third segment of antennule (Fig. 1B), P3 and P4 exp-3 with three inner setae (Fig. 5A, 5B) and exopod of female P5 elongated, about 3.83 times as long as greatest width (Fig. 7B). *Porirualia ngankeeae* **sp. nov.** is distinguished from *P. megarostrum* by the characters including rostrum reaching to distal margin of third segment of antennule (*vs* reaching to distal margin of fifth segment of antennule in *P. megarostrum*), ratio of length/width of female P5 exopod 3.83 (*vs* 2.2 in *P. megarostrum*), male P2 and P3 two-segmented (*vs* three-segmented in *P. megarostrum*), male P5 bearing five elements (*vs* six in *P. megarostrum*).

Based on morphological characters of publications, a key to the species of the genus *Porirualia* were given.

Key to species of the genus *Porirualia* Huys & Mu, 2021

1. P3 and P4 exp-3 with two inner setae *Porirualia pyriformis* (Song, Kim & Chang, 2003)
- P3 and P4 exp-3 with three inner setae 2
2. P5 female exopod elongated, ratio of length to maximum width 3.83. *Porirualia ngankeeae* **sp. nov.**
- P5 female exopod not elongated, ratio of length to maximum width 2.2
 *Porirualia megarostrum* (Wells, Hicks & Coull, 1982)

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