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A new species of *Boholina* (Copepoda, Calanoida, Boholinidae) from Ganghwa Island in western Korea

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A new species of Boholina, B. ganghwaensis sp. nov. is described, based on specimens collected from burrows of the manicure crab, Cleistostoma dilatatum, in the tidal flat of Ganghwa Island in western Korea. The new species is closely similar to B. purgata and B. parapurgata by having a pointed process on the posterior angles of the second and third pedigerous somites and a similar rostrum in the female, but can be distinguished from its congeners by the following characters: in females by the genital double-somite with small hook-like process on each gonoporal plate, the setation of the distal endopodal segment of mandible, the basis and first endopodal segment of the maxillule incompletely separated, the inner distal spine/ outer terminal spine length ratio on P5; and in males by the distal spine present on the posterior surface of the basis of both P5 and the length/width ratio of the endopod of the right P5. This is the first Bololina species recorded from the north-west Pacific.

Keywords: taxonomy, Boholina, crustacean burrows, Ganghwa Island, north-west Pacific

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INTRODUCTION

The family Boholinidae Fosshagen & Iliffe, 1989 was established on the basis of morphological characteristics as follows: (1) well-developed mouthparts; (2) primitive threesegmented rami on P1-P4; (3) three-segmented exopod and slightly reduced two-segmented endopod in female P5; (4) male right antennule geniculate; and (5) highly complex grasping male P5 (Fosshagen & Iliffe, 1989). Its single genus, Boholina, was also described as having a three-segmented urosome in females and a four-segmented urosome in males. However, Boxshall & Jaume (2012) verified by examination of the types of B. crassicephala Fosshagen & Iliffe, 1989 that the anal somite of the urosome had been overlooked in both sexes and the species of the genus Boholina have four and five urosomites in the female and the male, respectively (Boxshall & Jaume, 2012). To date, boholinid copepods comprise four species within a single genus: B. crassicephala Fosshagen & Iliffe, 1989, B. purgata Fosshagen & Iliffe, 1989, B. parapurgata Boxshall & Jaume, 2012, and B. munaensis Boxshall & Jaume, 2012. Of these species, the first two were collected in the water column of an anchialine cave on the island of Bohol in the central Philippines (Fosshagen & Iliffe, 1989), and the last two were found in anchialine and brackish habitats on the coast of Muna Island, south-east of Sulawesi, Indonesia (Boxshall & Jaume, 2012).

We recently found a new *Boholina* species in burrows of the manicure crab, *Cleistostoma dilatatum* (De Haan, 1833), which predominantly occurs in muddy habitats along the north-west Pacific (Ono, 1965; Miura *et al.*, 2004). In calanoid copepods, such a symbiotic association with other organisms is very rare and only some members of the genus *Ridgewayia* Thompson & A. Scott, 1903 have been described in association with pearl oysters, cave corals, sea anemones, and cnidarians (Thompson & Scott, 1903; Humes & Smith, 1974; Fosshagen & Iliffe, 1991; Huys & Boxshall, 1991). This new species is described from the north-west Pacific and its morphological relationships with its congeners are discussed.

MATERIALS AND METHODS

Copepods were collected from an inter-tidal mudflat at Ganghwa Island in western Korea (37°36'19"N 126°30′56″E). Ganghwa Island, located in the mid-western part of the Korean Peninsula, has a large tidal range and tidal flats covering 105 km². In particular, the study area $(37^{\circ}36'19''N \ 126^{\circ}30'56''E)$ is open to the sea and harbours small tributary channels branching off from a main tidal channel. This area is affected by freshwater inflowing from the Han River through the major channel and tides are semidiurnal with a tidal range between 8 m at spring tide and 4 m at neap tide. There are many burrows constructed by a number of crabs: Cleistastoma dilatatum (De Haan, 1833) and Macrophthalmus (Mareotis) japonicus (De Haan, 1835) and polychaetes, Periserrula leucophryna Paik, 1977 and Nereis aibuhitensis (Grube, 1878) (Koo et al., 2005). The burrows extend to the small tidal channels. Copepods were captured using an improved flexible suction tube from burrows of the manicure crab, C. dilatatum, and washed using a hand-held net of 100 µm mesh size. Copepod specimens were preserved in 70% ethanol or 5% neutralized

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formalin in seawater. Before dissection and microscopic observation, specimens were immersed in lactic acid for about an hour. The dissection of specimens was carried out using the reverse slide method of Humes & Gooding (1964). The rostrum, genital double-somite and swimming legs were examined with a scanning electron microscope (Hitachi S-4700) to show some morphological details more clearly. The descriptive terminology proposed by Huys & Boxshall (1991) was adopted. Abbreviations used in text and figures are as follows: ae, aesthetasc; P1–P5, first to fifth swimming legs. The type specimens are deposited in the National Institute of Biological Resources (NIBR), Incheon, Korea.

RESULTS

SYSTEMATICS

Subclass COPEPODA Milne-Edwards, 1830 Order CALANOIDA Sars, 1902 Family BOHOLINIDAE Fosshagen & Iliffe, 1989 Genus Boholina Fosshagen & Iliffe, 1989 Boholina ganghwaensis sp. nov. (Figures 1-5)

TYPE MATERIAL

Holotype: female (NIBRIV0000245276), allotype male (NIBRIV0000245277), six paratype females (NIBRIV0000245 278), and four paratype males (NIBRIV0000245279) preserved in 70% ethanol deposited in the NIBR. Dissected paratypes (2 QQ, 2 $Q^{3}Q^{3}$) are retained in the collection of the first author. All specimens from the type locality collected using a flexible suction tube from burrows of the manicure crab, *Cleistostoma dilatatum*, on 10 August 2010 by the first author (S.Y.M). The description below is based on the paratypes.

ADDITIONAL MATERIAL EXAMINED

One female and seven males preserved in 70% ethanol from the type locality from burrows of the manicure crab, *C. dila-tatum*, on 22 June 2011 by the first author (S.Y.M).

TYPE LOCALITY

Yellow Sea, Ganghwa Island $(37^\circ 36' 19'' N \ 126^\circ 30' 56'' E),$ western Korea.

ETYMOLOGY

The specific name of the new species refers to its type locality, Ganghwa Island, Korea.

FEMALE

Body (Figure 1A) robust, length 1.03-1.29 mm (mean 1.14 ± 0.13 mm, N = 5). Prosome five-segmented: cephalosome and first pedigerous somites completely separated; second and third pedigerous somites forming acute posteriorly directed points (arrows in Figure 1B); fourth and fifth pedigerous somites completely fused (Figure 1A, B), posterior corners of prosome slightly rounded (Figure 1D, E). Prosome-urosome ratio 2.28:1. Urosome four-segmented, comprising genital double-somite, two free abdominal somites and anal somite; length ratio of genital double-somite, first free abdominal somite, and anal

somite as 6.75:4.41:3.91:1. Genital double-somite (Figure 1D, E) symmetrical, widest midway, posterior border ornamented with smooth hyaline frill dorsally and strongly dentate hyaline frill ventrally, 1.06 times longer than wide; paired gonopores (Figures 1E, 5C, D) located ventro-medially with small hooklike process on each gonoporal plate; two pairs of sensillae present, one pair positioned adjacent to medial margin of gonopores (arrow in Figure 5D). First and second abdominal somites with transverse hyaline frill dorsally and ventrally. Anal somite shortest. Caudal rami (Figure 1D, E) with six setae, symmetrical, about 1.64 times longer than wide (62 imes38 µm), with pointed dorsal process midway of distal margin; caudal setae II to VII present (seta I lacking); seta II spiniform, seta III about half length of seta V, seta V longer than seta IV, both plumose; dorsal seta VII short, plumose. Rostrum (Figures 1C, 5A, B) in form of rounded plate terminated by small tube with distal pore (arrow in Figure 5B).

Antennules (Figure 1F) symmetrical, extending almost to middle area of fifth pedigerous somite; 24-segmented, with all articulations expressed, except between ancestral segments II–IV and XXVII–XXVIII, and partially expressed between ancestral segments X and XI; segmentation and setation pattern as follows (ancestral segment number-setae + aesthetasc): I-1 + ae, II–IV-6 + ae, V-2 + ae, VI-2, VII-2 + ae, VIII-2, IX-2-ae, X–XI-3 + 2ae, XII-1, XIII-1 + ae, XIV-1 + ae, XV-1 + ae, XVI-1 + ae, XVII-1 + ae, XVIII-1 + ae, XIV-1 + a, XXV-1 + ae + 1, XXVI-1 + 1, XXVII–XXVIII-5 + ae.

Antenna (Figure 2A) biramous; coxa and basis separate, coxa with one and basis with two setae; endopod twosegmented, proximal segment with two setae, compound distal segment bilobed with eight and seven plumose setae subterminally and terminally, respectively, outer margin ornamented with small serrated process subdistally on medial margin; tiny spinule adjacent to serrated process; exopod ninesegmented, articulation between eighth and ninth segments not expressed, with setal formula of 1, 1, 1, 1, 1, 1, 1, 3.

Mandible (Figure 2B): well-developed coxal gnathobase, with ten short cuspidated or simple teeth plus small dorsal spinulose seta, ornamented with spinule rows on medioventral part; ventral-most teeth largest. Mandibular palp biramous; basis with four setae on inner margin. Exopod five-segmented, setal formula 1, 1, 1, 1, 2; endopod two-segmented, proximal with four setae, distal segment with 11 setae.

Maxillule (Figure 2C): praecoxa and coxa incompletely fused; praecoxal arthrite with ten marginal spines plus four stiff setae on posterior surface, rows of tiny spinules present on posterior surface of segment. Coxa with four setae on coxal endite, nine setae on epipodite, with rows of tiny spinules on outer surface; basis with four and five setae on basal endites 1 and 2, respectively, and one seta on basal exite; endopod apparently three-segmented, first and second segments partially separated, second and distal segments completely separated, with setal formula of 4, 4, 7; exopod completely fused to basis, with ten setae.

Maxilla (Figure 2D): praecoxa and coxa partially fused; praecoxa with five setae on proximal endite and three setae on distal endite; coxa with three setae on both endites; basis with four setae on basal endite; endopod four-segmented, setal formula 3, 2, 2, 3.

Maxilliped (Figure 2E): syncoxa robust, with setal formula 1, 2, 2, 3 and oblique rows of tiny spinules on posterior distal part; basis with three setae and row of setules on mediolateral



Fig. 1. Boholina ganghwaensis sp. nov. Female (paratype): (A) habitus, dorsal view; (B) habitus, left lateral view; (C) rostrum, ventral view; (D) urosome, dorsal view; (E) urosome, ventral view, hoop-like anal somite (arrowed); (F) antennule. Scale bars: A,B, 200 µm; C,D, 100 µm; E, 50 µm.

margin; endopod six-segmented, with setal formula 2, 4, 4, 3, 3 + 1, 4.

P1 (Figure 3A) biramous, with three-segmented rami: basis with finger-like, distally rounded process on anterior surface, and with row of spinules on posterior surface; second exopodal segment with outer rounded process with serrate distal part; first to third endopodal segments with pointed process on distolateral corners.

P2 (Figure 3B) biramous with three-segmented rami; basis with row of spinules on posterodistal edge; exopodal segments

with row of spinules on distal edge; first and second endopodal segments with row of spinules on distal edge, each with pointed process on distolateral corner.

P₃ (Figures 3C, 5E) and P₄ (Figure 3D) biramous, with threesegmented rami: basis with row of spinules; first and second endopodal segments with row of spinules on distal edge (arrow in Figure 5E), with pointed process on distolateral corner.

Armature formula of $P_1 - P_4$ are as shown in Table 1.

P5 (Figure 3E) biramous, with three-segmented exopod and two-segmented endopod, intercoxal sclerite smooth and



Fig. 2. Boholina ganghwaensis sp. nov. Female (paratype): (A) antenna; (B) mandible; (C) maxillule; (D) maxilla; (E) maxilliped. Scale bars: A-C, E, 50 µm; D, 20 µm.

unornamented; basis small, with acute process on posterior surface near base of exopod. Exopod much longer than endopod; tip of endopod reaching beyond level of proximal outer spine on third exopodal segment; distal endopodal segment 2.46 times longer ($66 \mu m$) than wide ($27 \mu m$); outer spines on exopod each with serrate frill bilaterally; terminal spine on exopod with serrate membrane externally and finely setulose frill intermedially, inner distal spine 1.03 times longer ($65 \mu m$) than outer terminal spine ($63 \mu m$), and shorter than distal exopodal segment ($71 \mu m$); first and second exopodal segments each with a pore on anterior surface at origin of outer spine; first exopodal segment with row of spinules on distal edge, third exopodal segment with rows of spinules on distal edge, and two pointed processes on distal edge; first endopodal segment with row of spinules on distal edge, second endopodal segment with pointed process on distal edge (see Table 1).

MALE

Body more slender than female (Figure 4A, B). Body 0.93 - 1.11 mm long (mean 1.07 ± 0.08 mm, N = 5). Cephalosome completely separated from first pedigerous somite; second



Fig. 3. Boholina ganghwaensis sp. nov. Female (paratype): (A) P1; (B) P2; (C) P3; (D) P4; (E) P5. Scale bars: A-E, 50 µm.

and third pedigerous somites with acute ventroposteriorly directed points (arrow in Figure 4B); fourth and fifth pedigerous somites completely fused; posterior corners of prosome rounded. Prosome-urosome ratio 1.81:1. Urosome five-segmented: first to fourth urosomal somites equal. Frontal margin of dorsal cephalic shield with transverse crest; ratio of first to fifth urosomal somites as 4.18:3.72:3.72:3.91:1. Caudal rami unequal, right slightly longer, about 1.94 times longer (74 μ m) than wide (38 μ m) with six setae, lacking dorsal seta I.

Antennules short, asymmetrical. Left antennule nongeniculate, 25-segmented and extending almost to posterior border of fifth pedigerous somite. Right antennule (Figure 4C) geniculate, 22-segmented, extending to middle of last pedigerous somite. Segmentation and setation patterns as follows: I-1 + ae; II-IV-6 + ae, V-2 + ae, VI-2, VII-2 + ae, VIII-2, IX-2 + ae, X-1 + ae, XI-1 + ae, XII-1, XIII-1 + ae, XIV-1 + ae, XV-1, XVI-2 + ae, XVII-1, XVIII-1, XIX-1, XX-1, XXI-XXIII-2 + ae, XXIV-XXV-4 + ae + process, XXVI-1 + 1, XXVII-XXVIII-5 + ae.

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

P5 strongly asymmetrical (Figures 4D-F, 5F-H); coxae and intercoxal sclerite fused to form common base. Left leg biramous: basis with seta and distal spine on posterior



Fig. 4. Boholina ganghwaensis sp. nov. Male (paratype): (A) habitus dorsal view, hoop-like anal somite arrowed; (B) habitus, left lateral view; (C) right antennule; (D) fifth legs, posterior view; (E) left P5 exopod, anterior view; (F) left P5 endopod, anterior view. Scale bars: A,B, 200 μm; C, 100 μm; D, 50 μm.

surface; exopod three-segmented, first segment with a long serrate outer spine (94 μ m); second segment modified, bearing strongly reflexed spine (101 μ m) on outer margin; third segment highly transformed bearing multiple short processes and one finger-like process; endopod forming an elongate lobe. Right leg biramous: basis with seta and distal spine on posterior surface; exopod unsegmented, with two long outer spines (proximal spine 68 μ m, distal spine 102 μ m), long terminal spine 49 μ m long, plus short stout spine on anterodistal margin; endopod as an elongate rounded lobe about 3.19 times longer than wide (102 × 32 μ m), armed with two unequal slender setae and tiny terminal pointed process, apical seta 59 μ m, subapical seta 39 μ m long.

REMARKS

Boholina ganghwaensis is most like *B. purgata* and *B. parapurgata*, but can be distinguished by the genital doublesomite, mandible, maxillule and rami of P5 in females, and antennule, mandible and fifth legs in males. A careful comparison is needed to distinguish *B. ganghwaensis* from *B. purgata* Fosshagen & Iliffe, 1989, because the differences between these two species are subtle. They may be distinguished by the following characteristics (see Table 2): (1) the body is larger in *B. ganghwaensis* than in *B. purgata* in both sexes; (2) a small hook-like process is present on the gonopore plate in the female (absent in *B. purgata*); (3) the two exopod segments of the mandible are separated in



Fig. 5. Scanning electron microscopy micrographs of *Boholina ganghwaensis* sp. nov. Female: (A, B) rostrum, ventral view; (C, D) genital aperture of genital double-somite, ventral view; (E) first and second segments on exopod of P3, ventral view. Male: (F) fifth legs, anterior view; (G) tip of exopod of right P5; (H) third exopodal segment of left P5. Arrows indicate the pore on rostrum, spinules, and process.

the female (not separated in *B. purgata*); (4) the distal endopodal segment of mandible has 11 setae (10 setae in *B. purgata*); (5) an apical spine is present on both basis of P5 in male (only present on the basis of right P5 basis in *B. purgata*); and (6) endopodal segment of right P5 about 3.2 times longer than wide (2.6 times longer than wide in *B. purgata*).

Boholina ganghwaensis differs from *B. parapurgata* Boxshall & Jaume, 2012, because in the latter species (see

 Table 1. Armature formula of P1-P5 (female). Roman numerals indicate spines, Arabic numeral indicate setae.

Legs	Coxa	Basis	Exopod	Endopod		
P1	0-1	1-1	I-0; I-1; II,I,4	0-1; 0-1; 0,2(I + 1),3		
P2	0 - 1	0-0	I–1; I–1; II,I,5	0-1; 0-2; 2,2,4		
P3	0 - 1	1-0	I–1; I–1; III,I,5	0-1; 0-2; 2,2,4		
P4	0 - 1	1-0	I–1; I–1; III,I,5	0-1; 0-2; 2,2,3		
P5	0-1	1-0	I-0; I-1; III,I,3	0-1; 2,2,3		

Table 2): (1) the male body is larger; (2) a small hook-like process is present on the gonopore plate in the female (absent in *B. parapurgata*); (3) the setal formula 1, 1, 1, 1, 2 of the mandibular exopod of the female differs from the 0, 1, 1, 1, 2 of *B. parapurgata*; (4) the distal endopodal segment of mandible has 11 setae (vs 10 setae in *B. parapurgata*); (5) the terminal spine of the female right P5 is about 0.96 times longer than the inner distal spine (vs about 1.20 times longer in *B. parapurgata*); (6) the terminal spine of the female spine of the female P5 is shorter than the distal exopodal segment (vs longer in *B. parapurgata*); (7) an apical process is

present on the ancestral segment XXV of the male right antennule (vs absent in *B. parapurgata*); (8) an apical spine is present on both basis of the male P5 (vs present only on the right basis in *B. parapurgata*); and (9) the endopodal segment of male right P5 is about 3.2 times longer than wide (vs 3.6 times longer than wide in *B. parapurgata*).

DISCUSSION

The family Boholinidae Fosshagen & Iliffe, 1989 shows many similarities in its morphological characteristics with the families Ridgewayiidae M.S. Wilson, 1958 and Pseudocyclopidae Giesbrecht, 1893, but differs in the following characteristics: (1) the terminal spine on the distal edge of the third exopodal segment of the P4 is modified in the Boholinidae; and (2) there are no inner seta on the coxa of the female P5 in both Ridgewayiidae and Pseudocyclopidae (Fosshagen & Iliffe, 1989). Boholinid copepods now include four species (*B. crassicephala* Fosshagen & Iliffe, 1989, *B. munaensis* Boxshall & Jaume, 2012, *B. parapurgata*

Table 2. Comparison of morphological characteristics of the five species of genus *Boholina* Fosshagen & Iliffe, 1989. R, rostum; Gn, genital doublesomite; CR, caudal rami; R, rostrum; A1, antennule; A2, antenna; Mn, mandible; Mx1, maxillule; P5, fifth swimming leg; Re, exopod; Ri, endopod.

Character	B. ganghwaensis sp. nov.	B. parapurgata	B. purgata	B. munaensis	B. crassicephala
	(This study)	Boxshall & Jaume, 2012	Fosshagen & Iliffe, 1989	Boxshall & Jaume, 2012	Fosshagen & Iliffe, 1989
Female					
Body length (mm)	1.03-1.29	0.93-1.11	0.73-0.79	0.70-0.77	0.75-0.85
Posterior angle of tergites of second and third pedigerous somites	Pointed	Pointed	Pointed	Rounded	Rounded
Shape of R	Narrow rounded	Narrow rounded	Narrow rounded	Transverse crest	Transverse crest
Gonopores on Gn	Either side of ventral midline	Either side of ventral midline	Either side of ventral midline	Ventrolaterally	Ventrolaterally
Small hook-like inner process on gonoporal plate	Yes	No	No	No	No
CR length:width ratio	1.6	1.5	?	1.5	?
A2 Re distal 2 segments	Completely separated	Completely separated	Not separated?	Completely separated	Not separated?
Mn distal Ri segment setae number	11	10	10	10	10
Setal formula on Re of Mn	1, 1, 1, 1, 2	0, 1, 1, 1, 2	1, 1, 1, 1, 2	0, 1, 1, 1, 2	1, 1, 1, 1, 2
Basis and first Ri segment of Mx1	Incompletely separated	Fused	Fused	Fused	Fused
Length/width ratio of distal Ri segment of P5	2.5	2.6	?	2.6	Ś
Length ratio of inner distal spine and outer terminal spine of P5	0.96	1.20	?	1.40	\$
Terminal spine longer than distal Re segment of P5 Male	No	Yes	No	No	Ś
Body length (mm)	0.87-0.93	0.66-0.71	0.64-0.73	0.68	0.70-0.77
Presence of process on right A1	Yes	No	Yes	No	Yes
Apical spine on basis of P5	Present on both legs	Present on right leg only P5	Present on right leg only P5	Absent	Absent
Length/width ratio of Ri of right P5	3.2	3.6	2.6	?	;
Ri of right P5 with large inner spiniform process	No	No	No	Yes	No
Ri of right P5 armature	2 slender spines	2 sigmoid spines	2 slender spines	Absent	2 slender spines
Number of spines on right Re of P5	3 strong spines + short spine vestige	3 strong spines + short spine vestige	3 strong spines + short spine vestige	4 strong spines	4 strong spines

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Boxshall & Jaume, 2012 and *B. purgata* Fosshagen & Iliffe, 1989) in a single genus. *Boholina ganghwaensis* is the fifth species, and shares the generic characteristics identified by Fosshagen & Iliffe (1989), which should be amended as follows: urosome comprising four somites in female and five somites in male, and female antennule 24-segmented, with segments X and XI incompletely separated. However, *B. ganghwaensis* differs from the rest of the members of the genus in the following characteristics (see Table 2): (1) hook-like inner process present on gonoporal plates of the genital doublesomite of female; (2) setal formula on the exopod of mandible; (3) length/width ratio of the distal endopodal segment of P5 in female; (4) length ratio of the inner and outer distal spine of P5 in female; and (5) shape of distal endopodal segment of left P5 in male.

Calanoid copepods have rarely been found in symbiotic associations, except with some bivalves, corals and sea anemones (Thompson & Scott, 1903; Humes & Smith, 1974; Forsshagen & Iliffe, 1991). Benthopelagic calanoids Exumella and Ridgewayia mainly occur in hyper- or epibenthic habitats, but have been occasionally reported in caves (Wilson, 1958; Fosshagen & Iliffe, 1998; Ohtsuka et al., 2000). Another genus, Placocalanus, is also regarded as an epibenthic species that may be able to burrow into sediments temporally (Fosshagen, 1970; Ohtsuka et al., 1996). Fosshagen & Iliffe (1989) first reported two Boholina species in marine anchialine cave habitats in the Philippines. Boxshall & Jaume (2012) also described two species of Boholina from anchialine and brackish habitats on the coastlines of Muna Island, south-east of Sulawesi, Indonesia. However, Boholina ganghwaensis was collected from the burrows of the manicure crab, *Cleistostoma dilatatum* in the inter-tidal mudflat of the Ganghwa Island, in western Korea. This is the first record of this genus living in an apparently symbiotic relationship with crabs, and cyclopoid copepods also inhabit these burrows. The burrow, being perpendicular to the surface of the mudflat, has only one entrance at the sediment surface and its depth is about 46 cm (Kim et al., 2011). Freshwater from the Han river intermittently inflows into the burrow at low tide, implying that B. ganghwaensis is adapted to brackish water conditions. This new observation suggests that the species of Boholina may be distributed extensively in the Indo-Pacific region. Research on the life history and geographical distribution of B. ganghwaensis in the inter tidal mudflats from the north-west Pacific is currently underway and will be dealt with in detail elsewhere.

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