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Morphological types of male copulatory organs of *Bicornucythere bisanensis* (Ostracoda, Crustacea) and the description of a new *Bicornucythere* species

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Abstract

This study is designed to evaluate the male copulatory organs and carapace size of the inner bay benthic ostracod *Bicornucythere bisanensis*. The male copulatory organs demonstrate noticeable intraspecific variation in the distal lobe, especially the length of the tip, which shows a gradual variation; whereas the thickness of the distal lobe is conservative within the species. The population from central Japan (Aburatsubo Cove) showed remarkable morphological variations, with four copulatory organ types, each type defined by a combination of four shapes of the right and left distal lobes (Shape R, r, L, and l). While the variety of carapace morphotypes have been previously reported, our findings suggest that one morphotype from western Japan (Misumi-cho) should be treated as a distinct species. Thus, we described it as *Bicornucythere misumiensis* **sp. nov.**

Key words: intraspecific variation, new species, taxonomy, mating frequency

Introduction

The genus *Bicornucythere* was established as a new genus by Schornikov & Shaitarov (1979), with *Bicornucythere bisanensis* (Okubo, 1975) as the type species, based on the presence of several small processes at the anterior margin and a pair of strong spines at the posterior margin of the carapace (Schornikov & Shaitarov 1979). Five extant species (*Bicornucythere adunca* (Brady, 1880), *Bicornucythere bisanensis* (Okubo, 1975), *Bicornucythere darwini* (Brady, 1868), *Bicornucythere euplectella* (Brady, 1869), and *Bicornucythere goujoni* (Brady, 1868) were included in the genus by Hanai *et al.* (1980). However, taxonomic re-examination of each species resulted in the re-allocation of four of these five species into other genera (*Alocopocythere goujoni* by Gou *et al.* 1983; *Lankacythere euplectella* by Whatley & Zhao 1987; *Neocytheretta adunca* by Whatley & Zhao 1988; *Venericythere darwini* by Mostafawi 1992). Consequently, the narrowly defined genus *Bicornucythere* includes just one species, *B. bisanensis*. The habitat of this species extends from the East China Sea to the seas around Japan (*e.g.*, East China Sea/Zhao & Wang 1988; Amur Bay/Zenina & Schornikov 2008; Aomori Bay/Ishizaki 1971; Yatsushiro Sea/Abe 1988). In Japan, *B. bisanensis* is found on muddy substrates of the inner bays of Honshu, Shikoku, and Kyushu Islands (Fig. 1 and Table 1).

Bicornucythere bisanensis was first identified in sediment samples collected from the Seto Inland Sea (probably near Loc. 13 in Fig. 1 and Table 1) during the voyage of H.M.S. Challenger from 1873 to 1876. It was originally identified as *Cythere hodgii* Brady, 1866, a species known from the Levant (eastern Mediterranean Coast) (Brady 1880). It was subsequently reclassified into the genus *Cythereis* based on a specimen from Aburatsubo Cove (Loc. 5 in Fig. 1), and designated *Cythereis hodgii* (by Kajiyama 1913). However, Okubo (1975) collected specimens from the Shibukawa Coast of the Seto Inland Sea (Loc. 13 in Fig. 1) and designated them as a new species, *Leguminocythereis bisanensis*, because of the unique carapace morphology, differentiating the species from *Cythere hodgii s.s.* found in the Mediterranean Sea (without an explanation about the unique carapace morphology). This species was then

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placed in the genus (subgenus) *Ruggieria* (*Keijella*) by Hanai *et al.* (1977), but without an explanation. However, currently, it is widely accepted that this species represents the type species of the genus *Bicornucythere* proposed by Schornikov & Shaitarov (1979).

TABLE 1. Reports of the species of *Bicornucythere bisanensis*.

			Carapace morphology			
Loc. No.	Site Name	Literature	Form A	Form M	Form P	Form G
1	Aomori Bay, Aomori Prefecture	Ishizaki 1971	○			
2	Sendai Bay, Miyagi Prefecture	Ikeya & Ito 1991	○			
3	Obitsu River Estuary, Chiba Prefecture	Nakao & Tsukagoshi 2002	○			
4	Tateyama Bay, Chiba Prefecture	Frydl 1982	△			
5	Aburatsubo Cove, Kanagawa Prefecture	Kajiyama 1913	◎			
6	Aburatsubo Cove, Kanagawa Prefecture	Okada 1981; 1982	○			
7	Aburatsubo Cove, Kanagawa Prefecture	Abe 1988	○			
8	Tsukumo Bay, Ishikawa Prefecture	Kamiya et al. 2001	○			
9	Ise and Mikawa Bay, Aichi Prefecture	Bodergat & Ikeya 1988	○			
10	Osaka Bay	Yasuhara & Irizuki 2001	○	○		
11	Kumihama Bay, Kyoto Prefecture	Masuma & Yamada 2014	○			
12	Harima Sea, Hyogo Prefecture	Irizuki et al. 2009	○	○		
13	Shibukawa, Okayama Prefecture	Okubo 1975	◎			
14	Lake Nakaumi and Shinji, Shimane Prefecture	Ishizaki 1969	○			
15	Lake Nakaumi and Shinji, Shimane Prefecture	Takayasu et al. 1990	○ (juvenile)			
16	Lake Nakaumi and Shinji, Shimane Prefecture	Tanaka et al. 1998	○			
17	Lake Nakaumi and Shinji, Shimane Prefecture	Irizuki et al. 2003	○			
18	Suo-nada Bay, Yamaguchi Prefecture	Irizuki et al. 2010	○			
19	Hiuchi-nada Bay, Ehime Prefecture	Yamane 1998	○			
20	Uranouchi Bay, Kochi Prefecture	Ishizaki 1968		○		
21	Uranouchi Bay, Kochi Prefecture	Irizuki et al. 2008		○		
22	Yatsushiro Sea, Kumamoto Prefecture	Abe 1988	○	○		
23	Kagoshima Bay, Kagoshima Prefecture	Bodergat et al. 2002	△			
X	Peter the Great Gulf, Russia	Schornikov & Shaitarov 1979			◎	
Y	Gamagyang Bay, Korea	Abe 1988; Abe & Choe 1988				○

◎, soft parts and valves; ○, valve only; △, report only.
The locality numbers correspond to those in Figure 1. Form M was assigned to *Bicornucythere misumiensis* **sp. nov.** in this study.

Bicornucythere bisanensis presents four intraspecific variations in its carapace morphology based on length/height ratios; these variations were originally described as Form A (Loc. 7 in Fig. 1 and Table 1), P (Loc. X in Fig. 1), G (Loc. Y in Fig. 1), and M (Loc. 22 in Fig. 1) (Abe 1988; Abe & Choe 1988). The specimens from Japan are

classified into one of two carapace types, namely, Form A and M. However, only Form A includes any description of the soft body parts, including the male copulatory organs (Kajiyama 1913; Okubo 1975; Schornikov & Shaitarov 1979); whereas, Form M has been reported without soft body part descriptions from Misumi-cho (Loc. 22 in Fig. 1) (Abe 1988) and Osaka Bay (Loc. 10 in Fig. 1) (Yasuhara & Irizuki 2001). In the present study, we compared the morphology of the male copulatory organs of various specimens of the genus *Bicornucythere*, which is widely distributed across the inner bays of Japan, to identify a new species and report patterns of morphological variation.

Our dissections of the soft body parts revealed that Form M is distinct from *Bicornucythere bisanensis*, and was thus referred to as *Bicornucythere misumiensis* **sp. nov.**

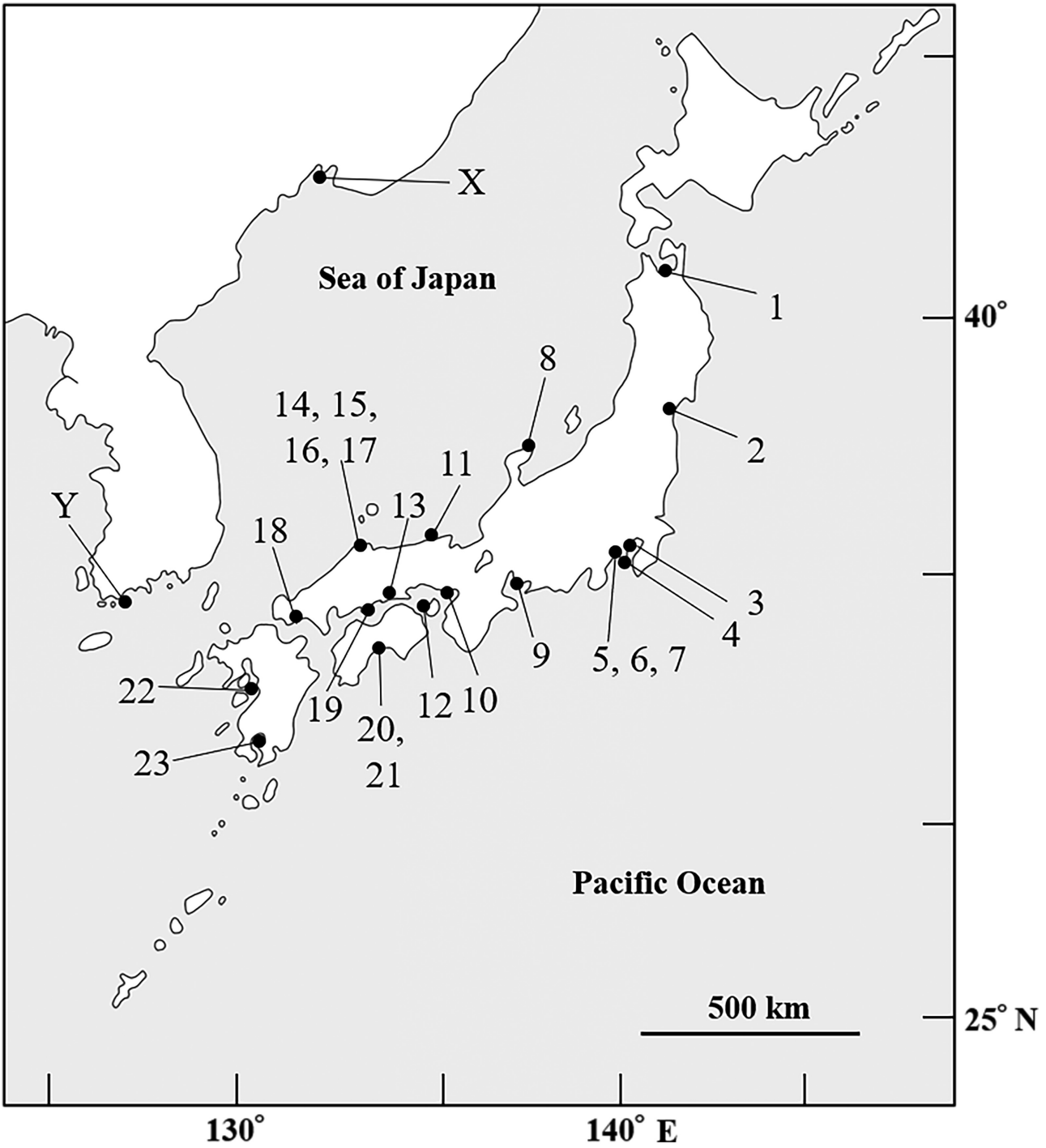

FIGURE 1. Map showing the locations of previous studies on *Bicornucythere bisanensis* from the seas around Japan. Locality numbers (1–23) and letters (X, Y) correspond to the list of reports in Table 1. The white map was taken from CraftMAP (2006).

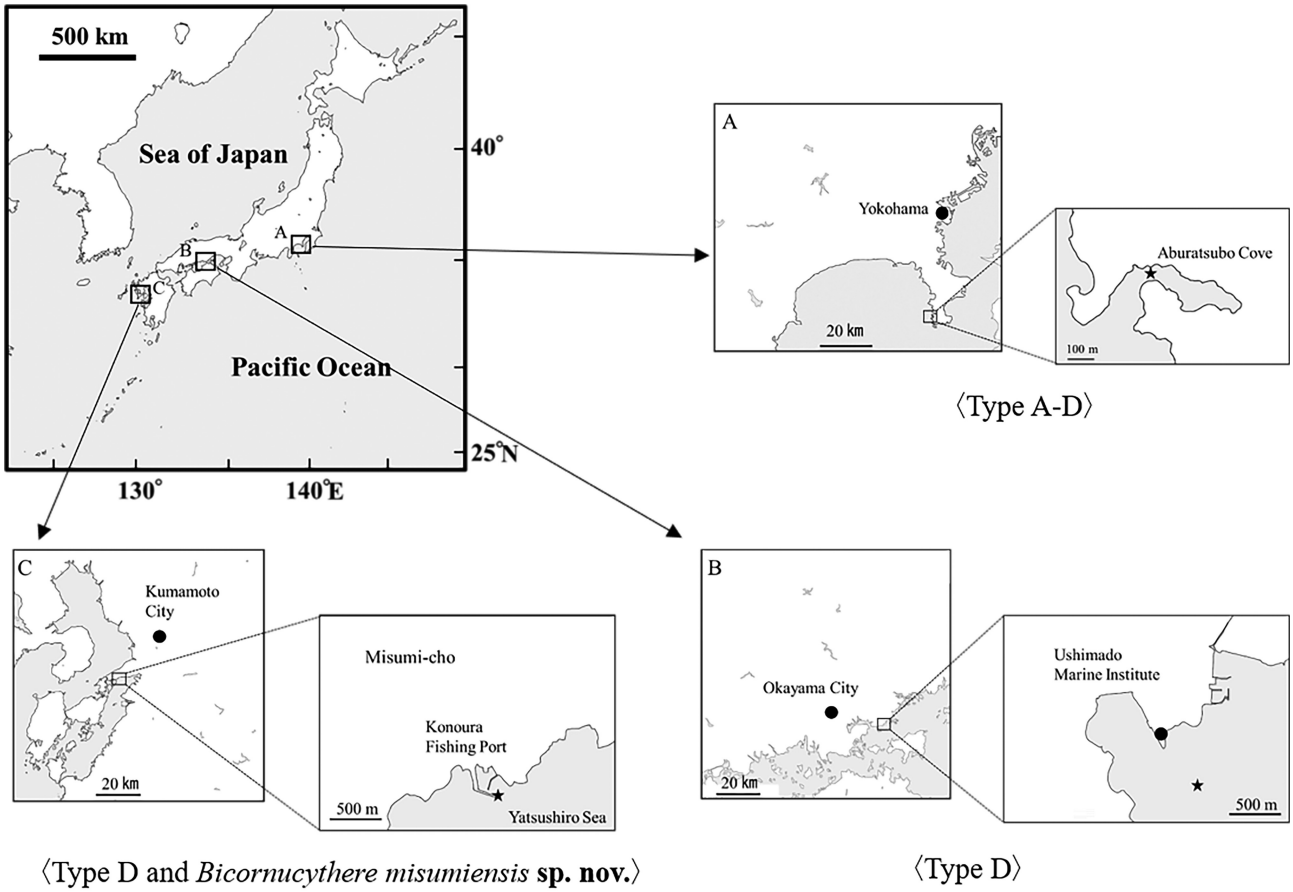

FIGURE 2. Map showing the locations evaluated in this study. A, Aburatsubo Cove. B, Ushimado. C, Misumi-cho. Misumi-cho is the type locality for *Bicornucythere misumiensis* **sp. nov.** and the description of each of these Types and/or species are linked to the type of male copulatory organ and their classification as either *B. bisanensis* or the new species in this study. Based on a map from CraftMAP (2006).

TABLE 2. Data describing the localities where the examined specimens were collected.

	Site	Latitude/Longitude	Date	Number of male specimens
Loc. A	Aburatsubo Cove, Kanagawa Prefecture	35°09'35.1 "N / 139°36'54.4 "E	15, March, 2018	103
Loc. B	Ushimado, Okayama Prefecture	34°36'22.5"N / 134°08'72"E	5, September, 2018	57
Loc. C	Konoura Fishing Port, Misumi-cho, Kumamoto Prefecture	32°37'4.06 "N / 130°32'1.08 "E	27, 28, November, 2019	10

Material and Methods

Locations, sampling, processing, and specimen evaluation

The results of the sampling in each site are summarised in Table 2. *Bicornucythere misumiensis* **sp. nov.** was collected from the type locality (Fig. 2C), close to the area where Form M of *Bicornucythere bisanensis* was first reported by Abe (1988). *B. bisanensis* was collected from Aburatsubo Cove (Fig. 2A) and Ushimado (Fig. 2B).

At the type locality of *Bicornucythere misumiensis* **sp. nov.**, a sampler net (opening: opening: 5 cm long × 6 cm wide, with a 0.05 mm mesh size net) was connected to a rope and thrown from a pier and pulled several meters along the mud bottom to collect sediment samples from the bay. Sediment samples were also collected from the

sandy-mud bottom of Aburatsubo Cove at a depth of 4–5 m using a dredge sampler (opening: 20 cm long × 40 cm wide, with 0.05 mm mesh size net) released from a boat. Sediment samples were also collected from the mud bottom of Ushimado Bay at a depth of 7–8 m using a large dredge sampler net (opening: 20 cm in length × 40 cm in width, with mesh size 0.05 mm net) released from a small boat. These sediments were washed using #16 (1 mm) and #200 mesh (0.074 mm), and the samples were then preserved in 70–80 % alcohol, before observation.

Only the male specimens used in our evaluations were enumerated for each location (Table 2). A total of 103 individuals from Aburatsubo Cove (Fig. 2A), 57 from Ushimado (Fig. 2B), and 10 from Misumi-cho were collected (Fig. 2C).

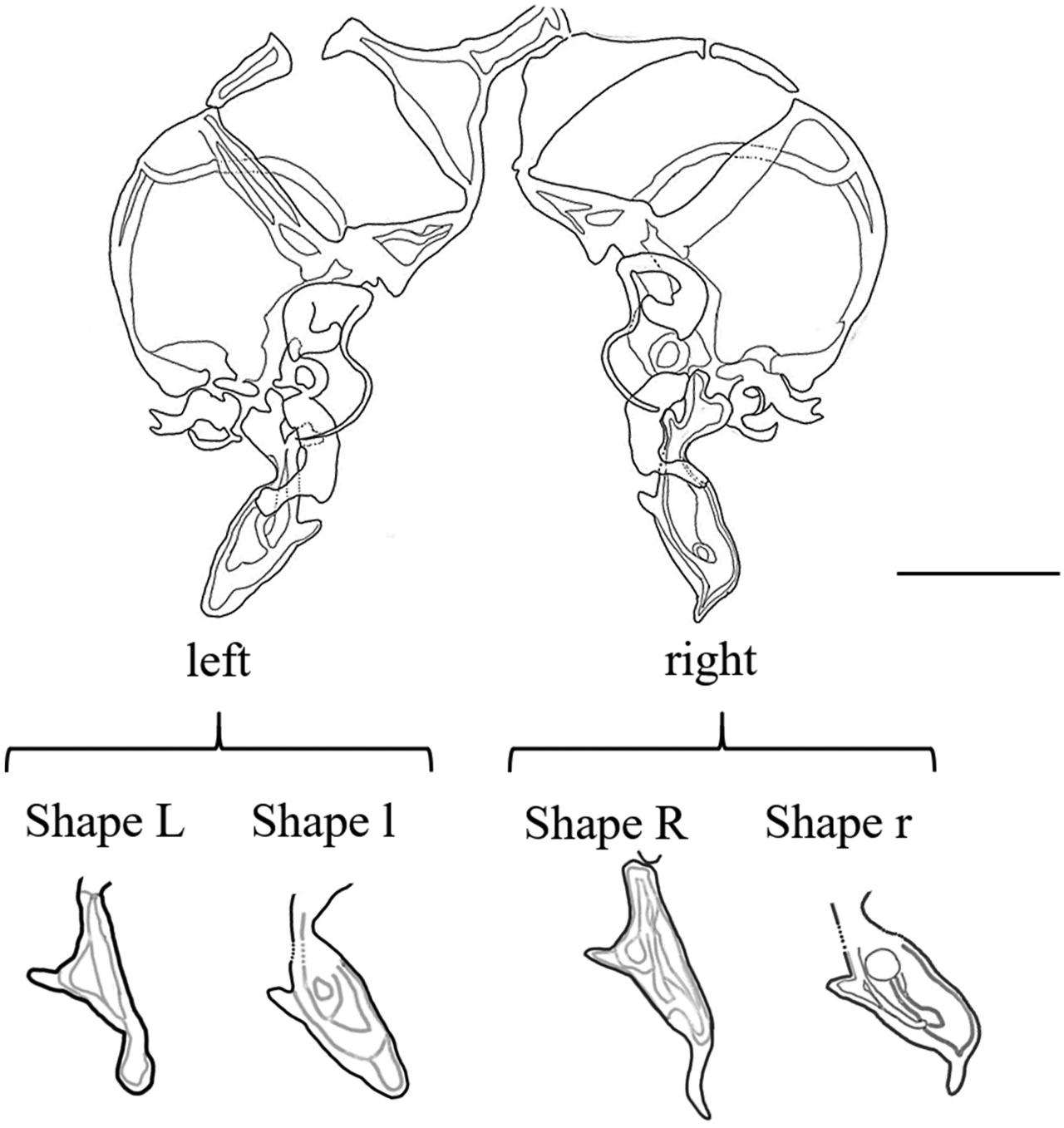

FIGURE 3. Typology of the distal lobes of male copulatory organs of *Bicornucythere bisanensis* from Aburatsubo Cove. Typology is based on the outline and shape of the tip and the right and left distal lobes, and were identified as R and r or L and l, respectively. Scale: 100 μm.

The soft parts were dissected and mounted in Neo-Shigaral medium on glass slides. The appendages and carapaces of the specimens were dissected under a stereomicroscope (SZ61, OLYMPUS) and the dissected specimens were then observed using a differential interference microscope (BX-50, OLYMPUS) with a camera lucida attached, and sketched. Several carapaces were observed using a scanning electron microscope (JSM-5600LV, JEOL) after freeze drying (JFD-310, JEOL) and coating with gold (JFC-1500, JEOL). The other specimens were air-dried and stored in two-hole slides.

Type material and all illustrated specimens have been deposited in the collection of the Shizuoka University Museum (836 Ohya, Suruga-ku, Shizuoka City, 422-8529 Japan) and identified by numbers with the prefix SUM-CO.

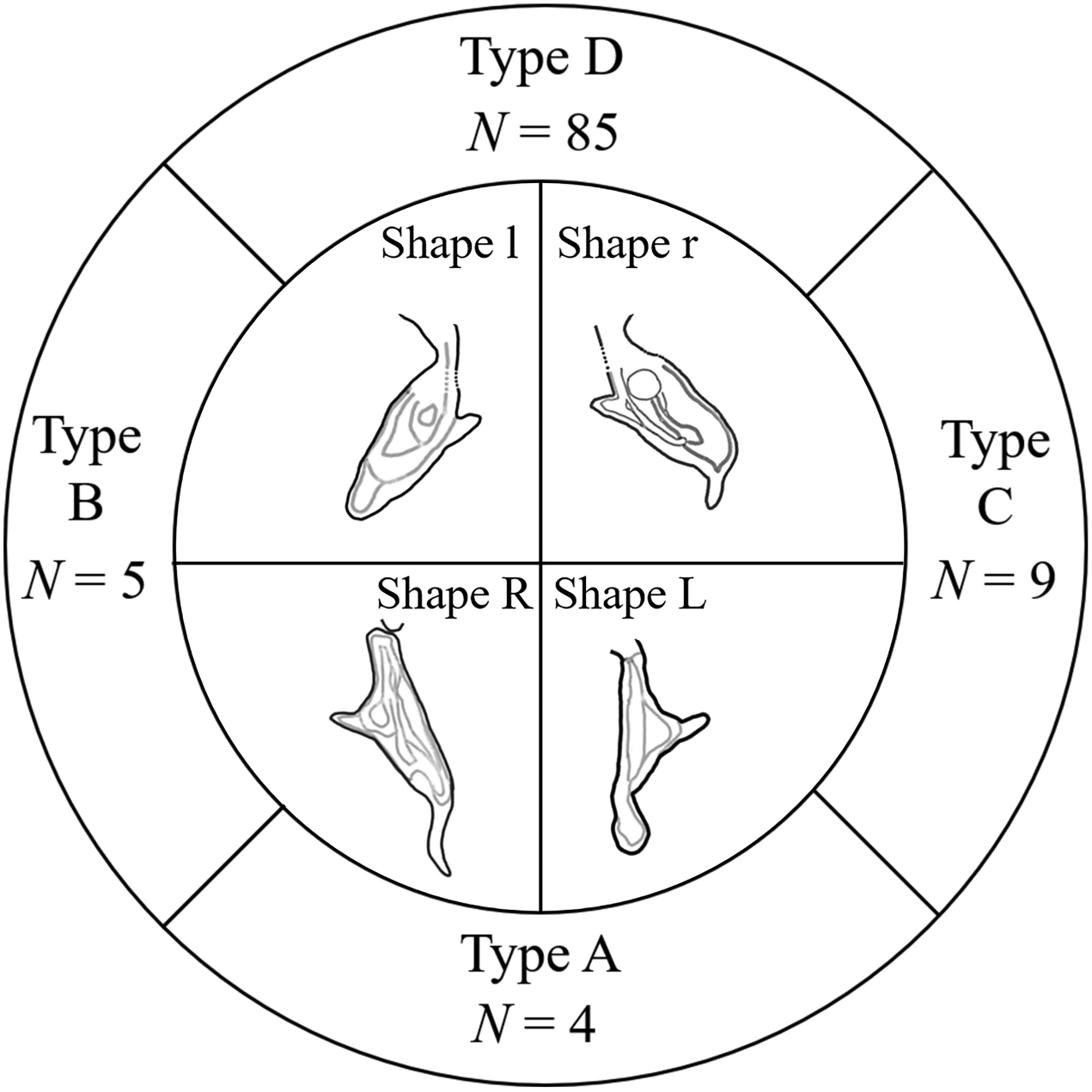

FIGURE 4. Diagram of the four Types of male copulatory organs identified in the Aburatsubo Cove samples. Each Type consists of a combination of right and left distal lobes that is, R, r, L, and l.

Evaluation of variations in the male copulatory organs and carapace morphology

As the distal lobes of the male copulatory organs of *Bicornucythere bisanensis* are highly asymmetrical, we examined both right and left hemipenes of the specimens from Aburatsubo Cove. We then used the outline of the distal lobe and the shape of the tip to identify four unique morphology types (Fig. 3) which could be described as follows:

Shape R: right hemipenis adopts a subtriangular shape which is tapered and curved at the tip

Shape r: right hemipenis adopts an elliptical shape with a tapered tip

Shape L: left hemipenis adopts a subtriangular shape with a thumb-shaped tip

Shape l: left hemipenis adopts a stocky shape with a rounded tip

All male copulatory organs were a combination of either Shape R and L, Shape R and l, Shape r and L, or Shape r and l, and they were designated as Type A, B, C, and D, respectively (Fig. 4).

Definitions of terms used in this study are as follows: Shape, soft part morphology based on the distal lobe, a part of the male copulatory organs, Type, soft part morphology of male copulatory organ based on the combination of distal lobe morphologies (*e.g*., Shape R and Shape L), Form, carapace morphology based on the length/height ratio.

The carapace morphologies are based on those identified by Abe (1988) and include: Form A with a carapace length of 0.78–0.85 mm and a height of 0.35–0.45 mm, and Form M with a carapace length of 0.70–0.76 mm and a height of 0.30–0.35 mm. Measurements were taken using a digital gauge (D-10S, PEACOCK) attached to a stereomicroscope (SMZ-10, Nikon), with both length and height evaluated using the left valve of the adult males. Four individuals randomly selected from each Type of male copulatory organ were then used for the carapace measurement of Aburatsubo Cove population and the Ushimado population, respectively, whereas 10 specimens, including *B*. *misumiensis*, were used to measure the Misumi-cho population (Tables 3, 4 and Fig. 5).

TABLE 3. Dimensions of the male left valves of *Bicornucythere bisanensis* from Aburatsubo Cove. Species types indicate the shape of the male copulatory organs.

Species type	Length (mm)			Height (mm)		
	Avg.	Range	*N*	Avg.	Range	*N*
Type A	0.82	0.78–0.85	4	0.38	0.36–0.38	4
Type B	0.81	0.78–0.83	4	0.37	0.35–0.37	4
Type C	0.81	0.79–0.82	4	0.37	0.37–0.38	4
Type D	0.80	0.78–0.80	4	0.36	0.35–0.38	4

TABLE 4. Dimensions of male left valves of the two *Bicornucythere* species from Ushimad and Misumi-cho. Species forms indicate the carapace Forms which were proposed by Abe (1988). Form M was identified as *B*. *misumiensis* **sp. nov.**

Species form	Length (mm)			Height (mm)			Locality
	Avg.	Range	*N*	Avg.	Range	*N*	
Form A	0.84	0.83–0.85	4	0.39	0.37–0.41	4	Ushimado
Form A	0.84	0.83–0.85	5	0.38	0.37–0.38	5	Misumi-cho
Form M	0.74	0.71–0.76	5	0.33	0.32–0.34	5	Misumi-cho

Results

Morphological variations of male copulatory organs in *Bicornucythere bisanensis* from the three locations

Variations in the degree of extension of the tip of the distal lobe in the right male copulatory organs of *Bicornucytere bisanensis* were observed in populations from both Aburatsubo Cove (Fig. 6A) and Ushimado (Fig. 6B). The specimens from Aburatsubo Cove could be classified undertwo shapes, namely, Shape r and Shape R (Figs 3, 4, and 6A).

In addition, we noted that the majority of *B. bisanensis* samples from:

Aburatsubo Cove (Figs 4 and 6A) could be classified as Type D (85 specimens), with the reminder as Type C (nine specimens), Type B (five specimens), and Type A (four specimens).

Type A: similar to the Kajiyama's (1913) description (Fig. 7A).

Type D: similar to Okubo's (1975) and Schornikov & Shaitarov's (1979) descriptions (Fig. 7B, C).

Type B: unique to this study.

Type C: unique to this study.

Ushimado (Figs 6B). All specimens were identified as Type D.

Misumi-cho. Five of 10 male specimens were identified as *B. bisanensis* of Type D. The other five specimens were identified as *B. misumiensis* **sp. nov.** (Figs 8–12). We attempted to quantify the results, but the seamless and indefinite variation hindered us from finding appropriate measurements (landmarks); therefore, we forwent to quantify them, and we only report variations of the distal lobes.

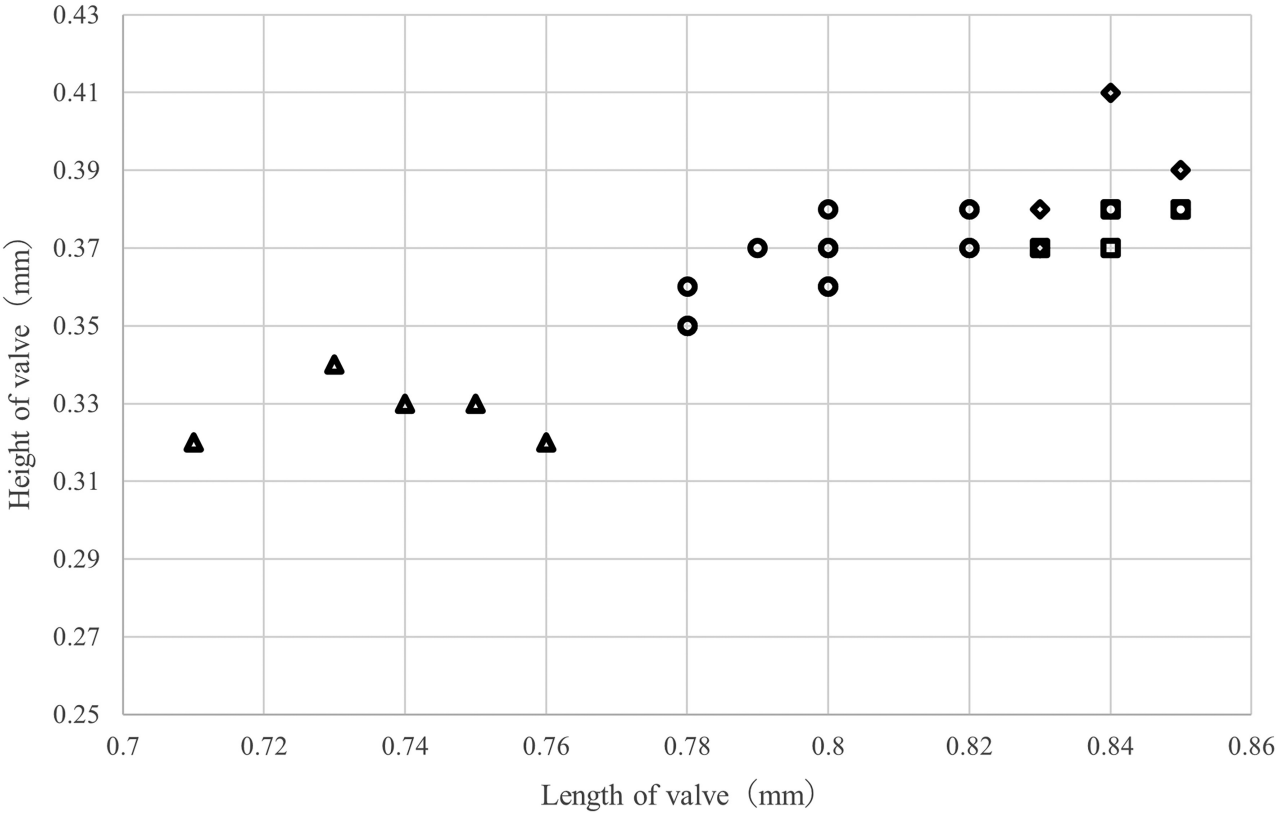

FIGURE 5. Size distribution of male carapace of *Bicornucythere* species. Circles = *Bicornucythere bisanensis* from Aburatsubo Cove; Rhombuses = *Bicornucythere bisanensis* from Ushimad; Squares = *Bicornucythere bisanensis* from Misumi-cho; Triangles = *B. misumiensis* **sp. nov.** from Misumi-cho.

Carapace evaluation

The carapace measurements (length and height) of specimens of all three locations are summarised in Tables 3 and 4 and Fig. 5.

Aburatsubo Cove (Table 3 and Fig. 5 with circles). All specimens were 0.78–0.85 mm in length and 0.35–0.45 mm in height, with no clear size differences among the examined specimens; they were identified as Form A.

Ushimado (Table 4 and Fig. 5 with rhombuses). All specimens were 0.78–0.85 mm in length and 0.35–0.45 mm in height, indicating that these specimens also belong to Form A.

Misumi-cho (Table 4 and Fig. 5 with squares and triangles). Ten specimens were evaluated. Five of the 10 specimens were 0.70–0.76 mm in length and 0.30–0.35 mm in height; they were identified as Form M, as previously described by Abe (1988). These findings combined with the copulatory organ differences enabled us to designate the species as *Bicornucytere misumiensis* **sp. nov.** The other five specimens were identified as Form A, of length 0.78–0.85 mm and heights of 0.35–0.45 mm.

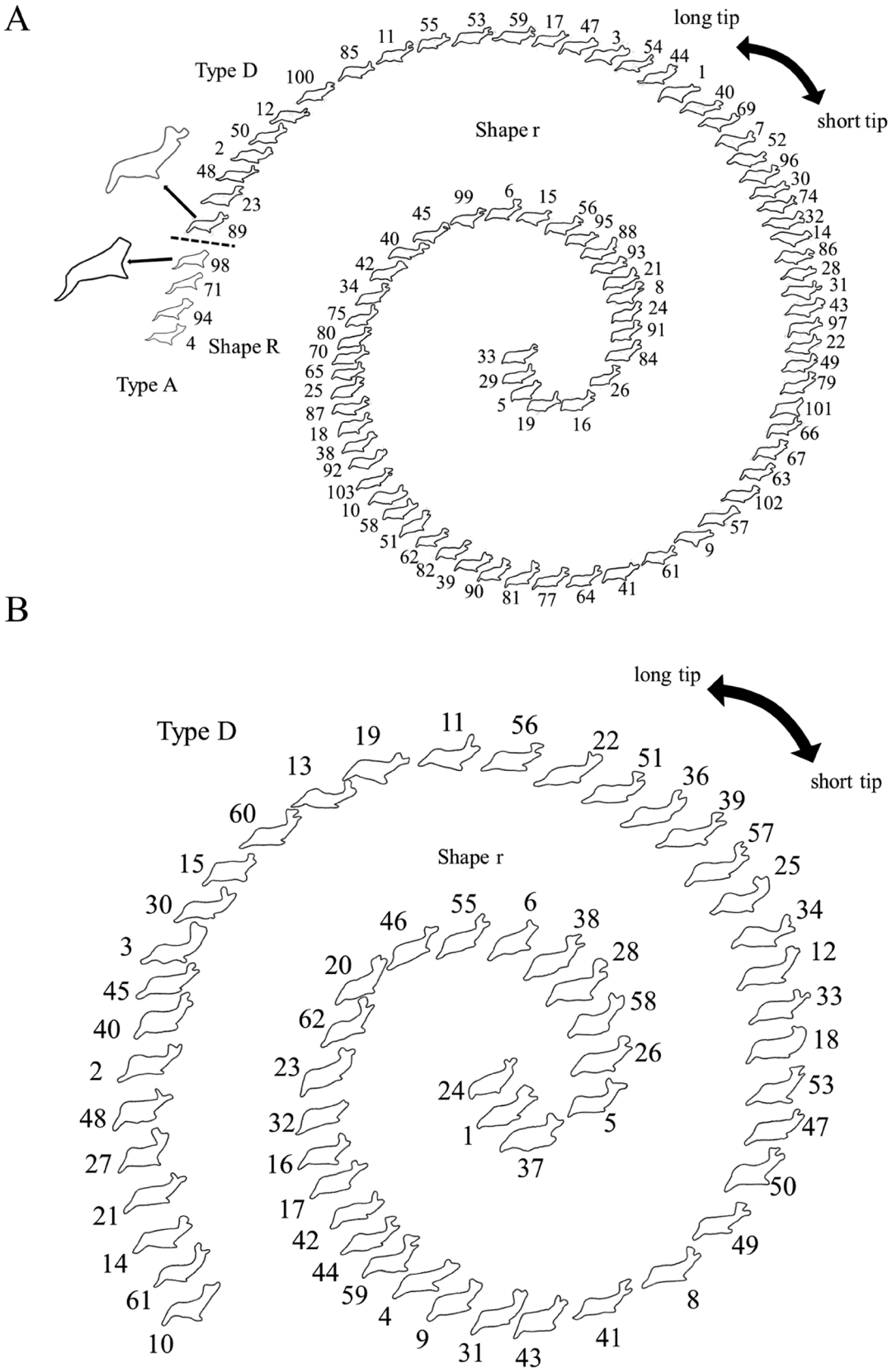

FIGURE 6. Morphological variation in the extension degree of the tip of Shape r in Type D and difference from Shape R in Type A. A, specimens from Aburatsubo Cove. B, specimens from Ushimado. The Aburatsubo Cove samples included some specimens from Shape R group, and they were distinguished from Shape r samples based on their elongated sub-triangular outline and tapering curved tip, which differed from the spindle-shaped outline and pointed tip observed in Shape r samples. Each number correlates with a specific specimen from each locality.

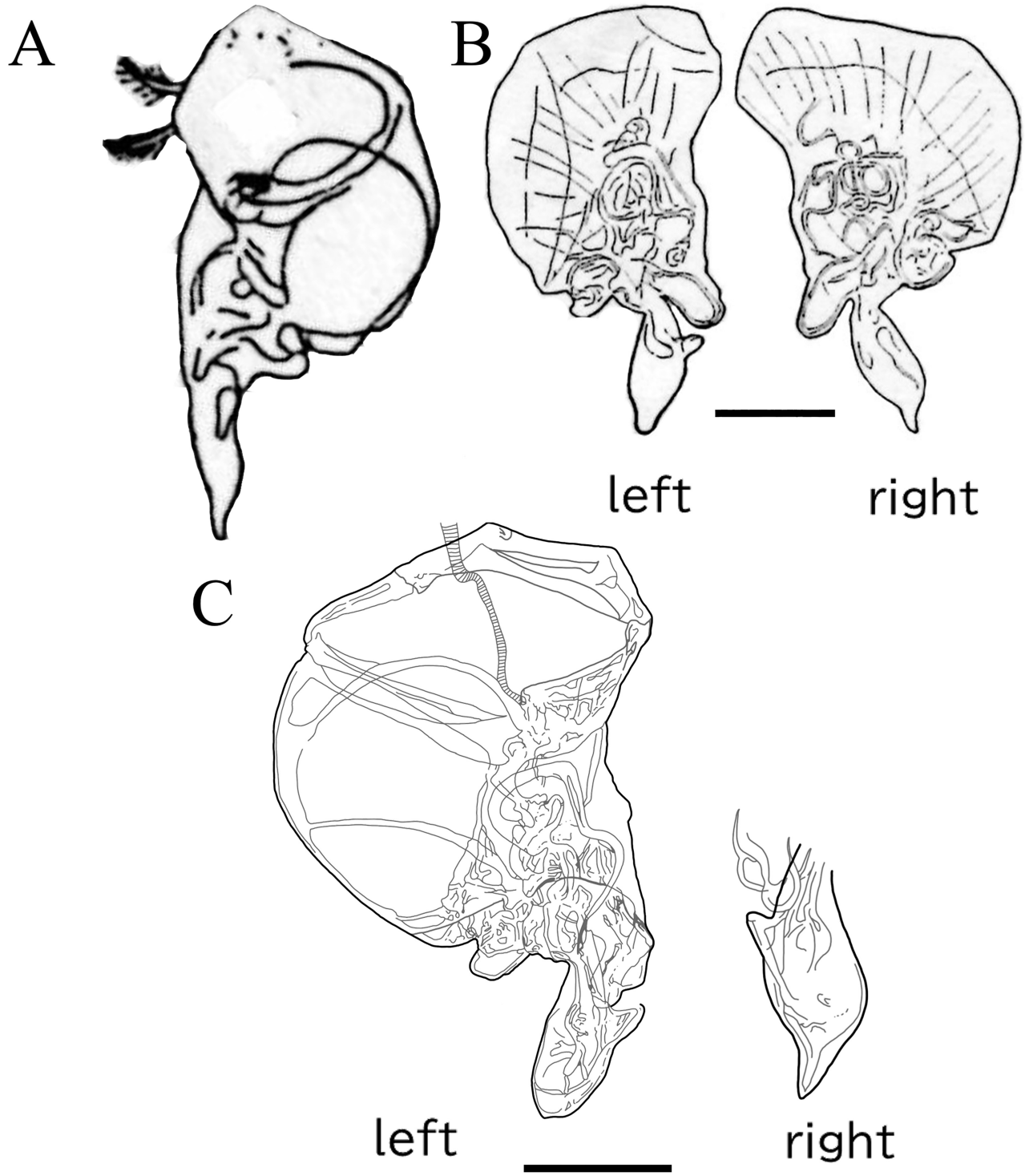

FIGURE 7. Male copulatory organs of *Bicornucythere bisanensis*. A, after Kajiyama (1913). B, after Okubo (1975). C, modified from Schornikov & Shaitarov (1979). Kajiyama 1913's description did not include a description of the position of the hemipenis. Permission was granted for Okubo (1975). For Schornikov & Shaitarov (1979), the figure has been replaced by a traced version due to difficulties in contacting the journal's editorial office. Scale: 100 μm.

Taxonomy

Order Podocopida Sars, 1866

Superfamily Cytheroidea Baird, 1850

Family Trachyleberididae Sylvester-Bradley, 1948

Genus *Bicornucythere* Schornikov & Shaitarov, 1979

Bicornucythere misumiensis sp. nov.

(Figs 8–12)

Type series. All specimens were collected from type locality on 28 November 2019. Holotype: adult male (SUM-CO-2501), right valve length 0.71 mm, height 0.32 mm, left valve length 0.76 mm, height 0.36 mm, appendages mounted on slide and valves preserved on SEM stub. Paratypes: 10 adult males (SUM-CO-2502 and 2503, 2505–2509, 2511, 2515 and 2516) and 5 adult females (SUM-CO-2504, 2510, 2512–2514). Depth about 5 m. Salinity ca. 30 ‰.

Etymology. After name of the type locality, Misumi-cho (western Japan).

Diagnosis. Carapace length is shorter in females than in males. Carapace reticulation slightly weak at anterior half in female. Second podomere of male right fifth limb divided into 3 parts (IIa, IIb, IIc). IIa and IIb completely fused, and only with remaining segment boundary. IIb and IIc with faint borderline remaining between podomeres. In male copulatory organ, right distal lobe with triangular outline and sharp linearly tip, left one with hump-shaped tip.

Description. *Carapace* (Figs 10, 11G–J, 12). Carapace lateral outline groundnut-like in male with greatest height at one-fourths from anterior margin, and sub-rectangular in female. Anterior margin obliquely rounded. Ventral margin concave at middle. In dorsal view, outline sunflower-seed like. In anterior view, outline sub-pentagonal, height of left valve slightly higher than right valve and left one overlapping right along dorsal margin. Five processes (Fig. 10I) along anterior ventral margin: some of them bifurcated at distal ends. One strong spine on posterior ventral margin of each valve. Surface fully covered by reticulation in male; vein-like ridges of reticulation with tendency of horizontal lines in posterior half. Reticulation weak at anterior half in female. Surface covered with more than 100 pore systems, classified as corolla-like pore with no seta (Fig. 11G, H), simple pore with broom-shape seta (Fig. 11I), and simple pore with simple seta (Fig. 11J). Four adductor muscle scars, but often a mutation to five is seen. Heart-shaped frontal scar located in front of adductor scars (Fig. 10J, black arrow). One elliptic mandibular scar in front of lowest adductor scar (Fig. 10J, gray arrow). Hingement amphidont-type; composed of 1 prominent fan-shaped tooth and 1 large socket present in anterior element, 1 prominent fan-shaped tooth in posterior element, and many sub-ovate sockets in median element in right valve (Fig. 10K). Complementary in left valve.

Antennula (Fig. 9A). Six articulated podomeres, length ratio among them from proximal to distal 36: 12: 10: 15: 23: 32. First podomere with numerous setulae along ventral margin. Second podomere with 1 very long annulated seta on posterior-distal corner, setulae along anterior and posterior margins. Third podomere with 1 medium to long seta on anterior-distal corner. Fourth podomere with 3 medium to long setae on anterior margin. Fifth podomere with 3 medium to long setae on distal end. Sixth podomere with 3 medium to long setae and 1 aesthetasc on distal end.

Antenna (Fig. 9B). Four articulated podomeres, length ratio among them from proximal to distal 9: 4: 9: 2. First podomere with long segmented exopodite in male and reduced segmented exopodite in female. Second podomere with 1 very long annulated seta on posterior-distal end. Third podomere with 2 long setae on ledge of anterior margin, 1 long and 1 short setae on posterior distal end, 1 long and 1 medium-length stout seta, and 1 short aesthetasc seta on ledge of posterior margin. Fourth podomere with 1 large claw-like seta, and 2 medium to long setae on distal end.

Mandibula (Fig. 9C). Consisting of five podomeres. First podomere (coxa: C1) with masticatory part of 1 stout and several fine denticles. Basis with two short annulated setae on anterior margin and 5-branched exopodite. First podomere of endopodite with two long plumose setae on anterior distal corner. Second podomere with 1 long seta

and 1 plumose seta on anterior distal corner, 3 long and 2 medium to long setae on posterior dorsal corner and 1 medium-length plumose seta on posterior-proximal margin. Third podomere small and armed with 1 medium to long and 2 short setae on distal end.

Maxillula (Fig. 9D, 11F). Thin branchial plate (exopodite) bearing 14 plumose long setae and 1 short segmented seta. Basal podomere bearing 1 palp (endopodite), 3 masticatory endites and numerous setulae on anterior distal end. First podomere of endopodite with 2 medium to long setae and 1 medium-length seta on distal end. Second podomere of endopodite with 2 medium-length setae on distal end. Each endite with several short setae.

Right fifth limb (in male) (Fig. 9E). Consisting of 2 articulated podomeres, length ratio among them from proximal to distal 5: 7. First podomere with 1 annulated seta on middle of anterior margin, 2 annulated setae on anterior-distal end, numerous setulae along posterior margin and reduced exopodite on posterior-proximal margin. Second podomere divided into 3 parts (IIa, IIb, IIc). IIa and IIb completely fused, only with remaining segment boundary on posterior margin at one-third from posterior distal end (Fig. 9E1). Faint segment boundary between IIb and IIc (Fig. 9E2). IIa of second podomere with 2 fine setae on middle of anterior margin and 1 annulated seta on anterior distal end. IIc of second podomere bearing 1 stout terminal claw on distal end.

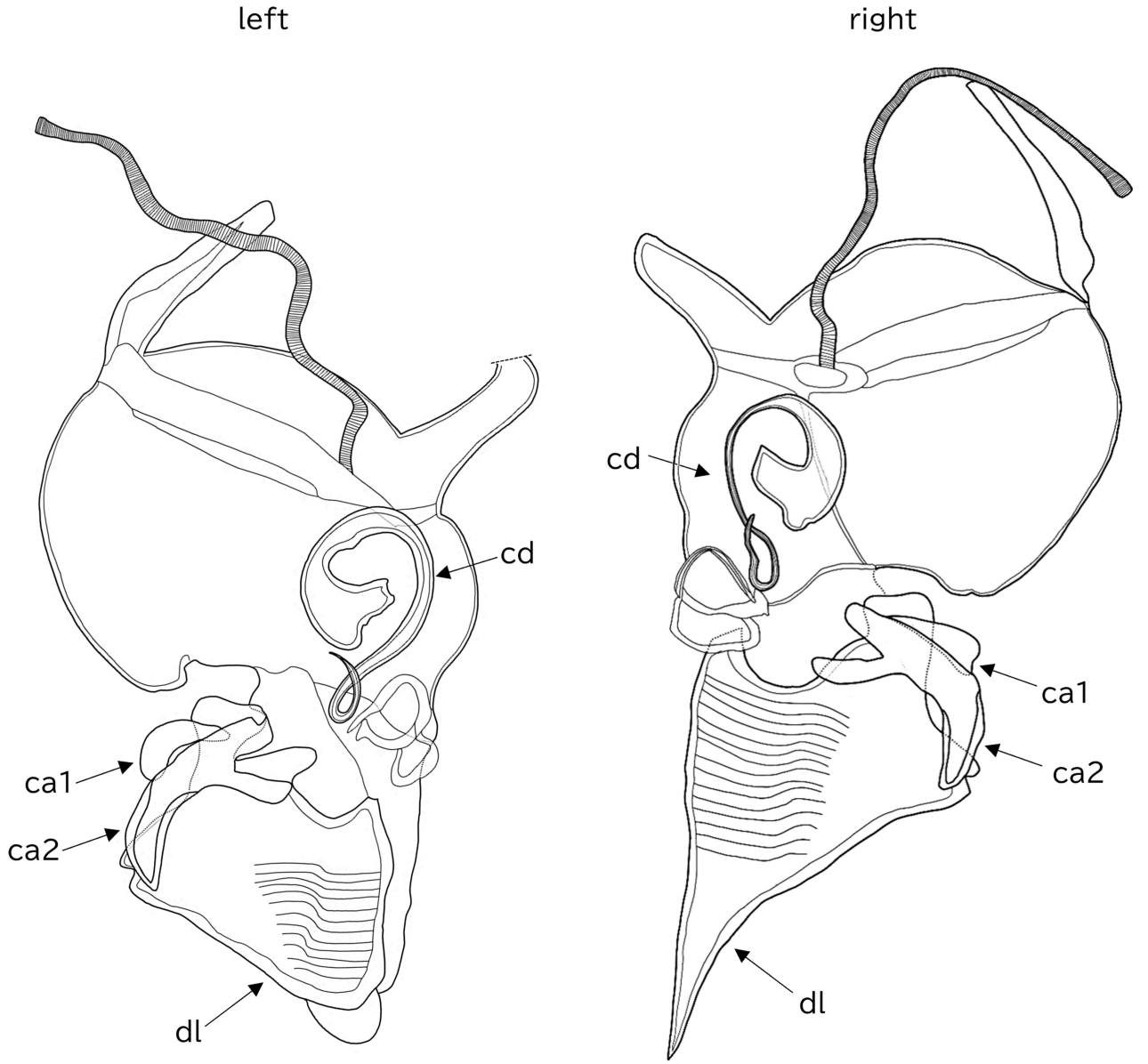

FIGURE 8. Male copulatory organs of *Bicornucythere misumiensis* **sp. nov.** (SUM-CO-2501, holotype). *ca*, clasping apparatus; *cd*, copulatory duct; *dl*, distal lobe. Scale: 100 μm.

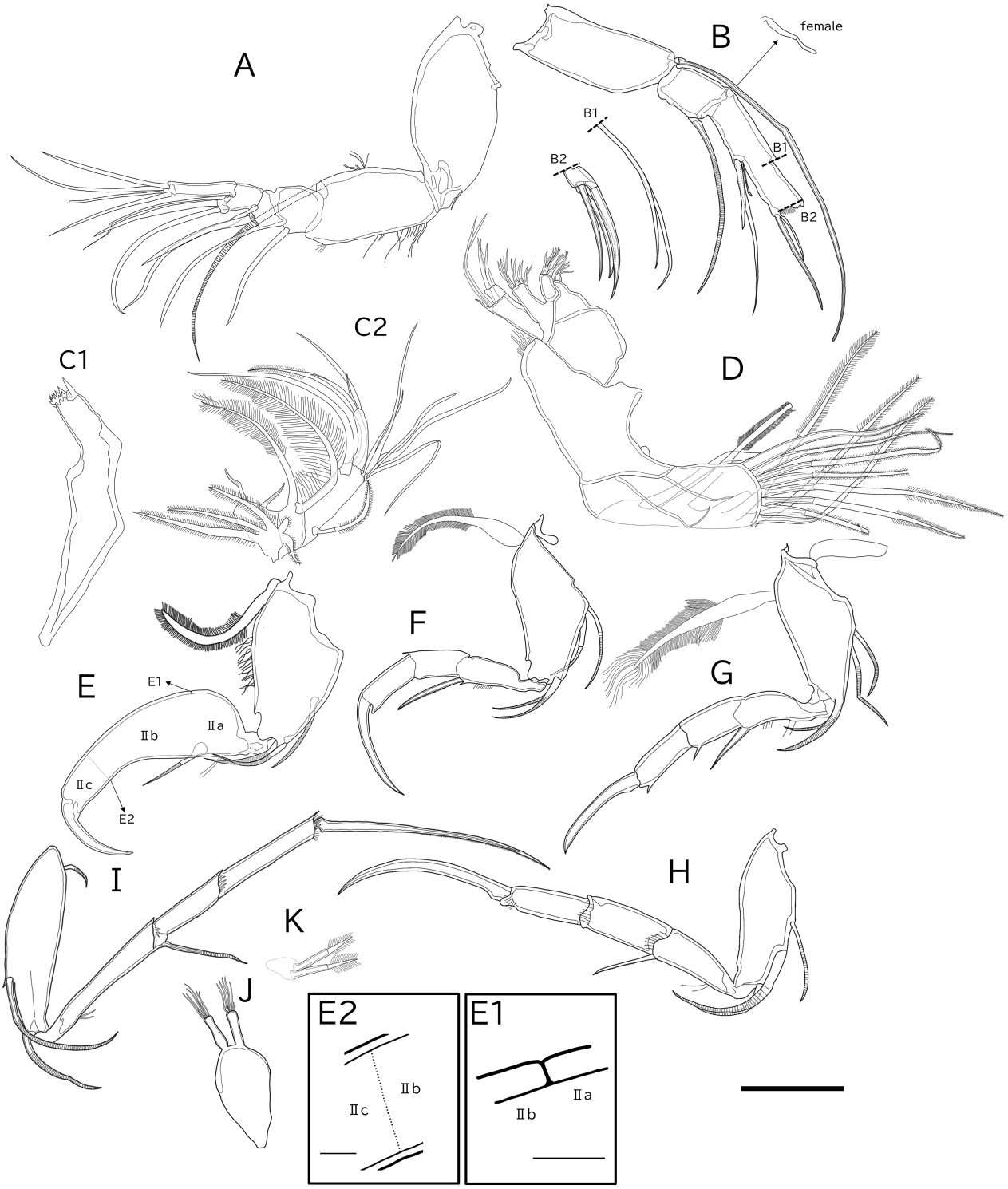

FIGURE 9. Appendages of *Bicornucythere misumiensis* **sp. nov.** A, antennule. B, antenna. C, mandibula. D, maxillula. E, male right 5th limb. E1, segment boundary in male right 5th limb. E2, faint segment boundary in male right 5th limb. F, female right 5th limb. G, left 5th limb. H, 6th limb. I, 7th limb. J, brush-shaped organ. K, left furca. A, C, J, K SUM-CO-2501, male holotype; B, E, G–I, L, M, SUM-CO-2502, male paratype; D, SUM-CO-2503, paratype; B, F, SUM-CO-2504, female paratype. Scale: 100 μm for A-K, 125 μm for E1, E2.

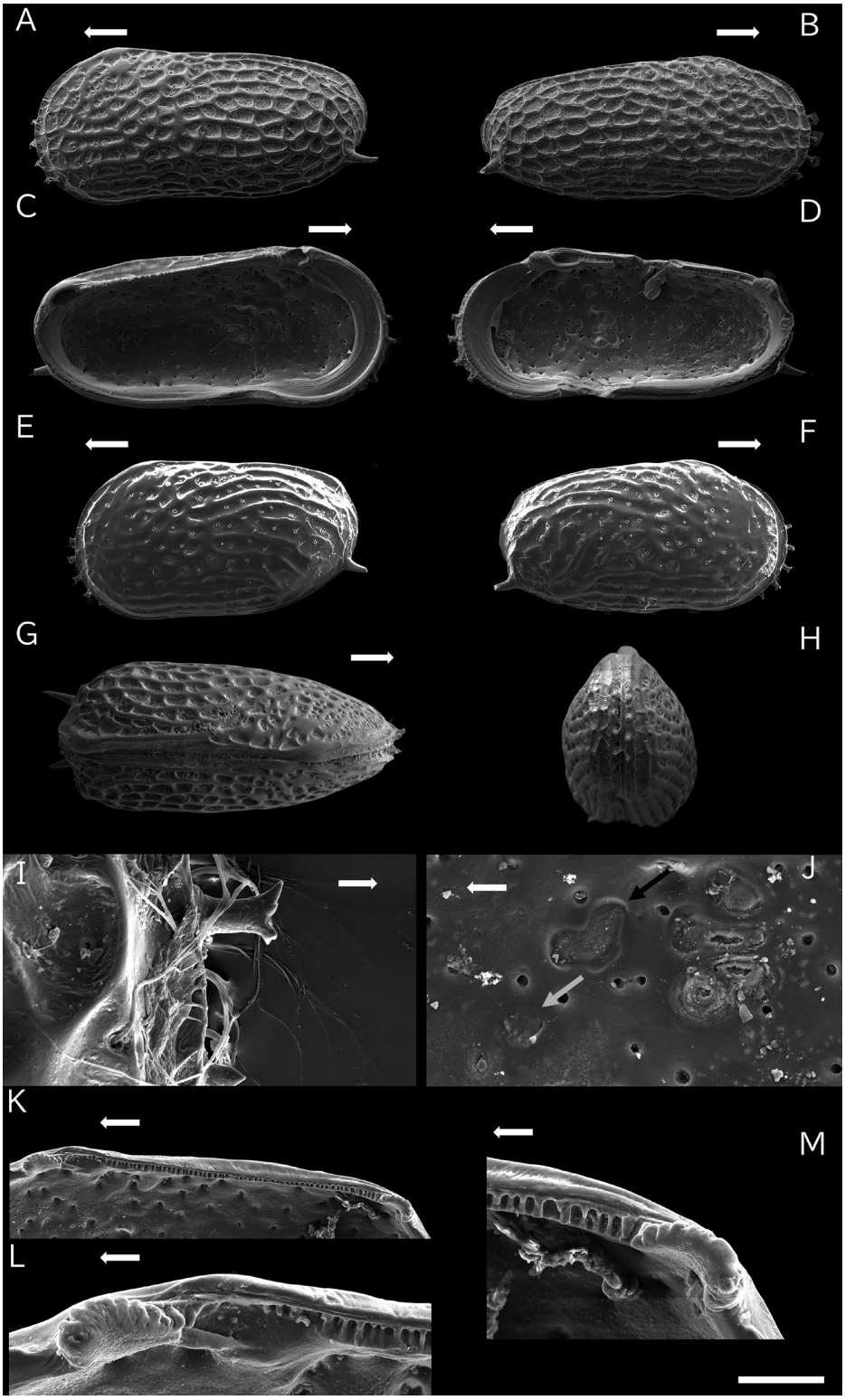

FIGURE 10. Carapace of *Bicornucythere misumiensis* **sp. nov.** A and B, male left and right valves in the external lateral view, respectively (SUM-CO-2501, holotype). C and D, male left and right valves in the internal lateral view, respectively (SUM-CO-2505, paratype). E and F, female left and right valves in the external lateral view, respectively (SUM-CO-2504, paratype). G, male dorsal view (SUM-CO-2506, paratype). H, male anterior view (SUM-CO-2507, paratype). I, process of left valve (SUM-CO-2508, paratype). J, muscle scars of right valve including adductor, frontal (black arrow), and mandibula scars (gray arrow) (SUM-CO-2509, paratype). K, hingement on male right valve (SUM-CO-2509, paratype). L and M, teeth and socket of hingement on male right valve (SUM-CO-2509, paratype). L, anterior element of right hingement. M, posterior element of right hingement. White arrows indicate anterior. Scale: 100 μm for A–H, K, 20 μm for I, J, L, M.

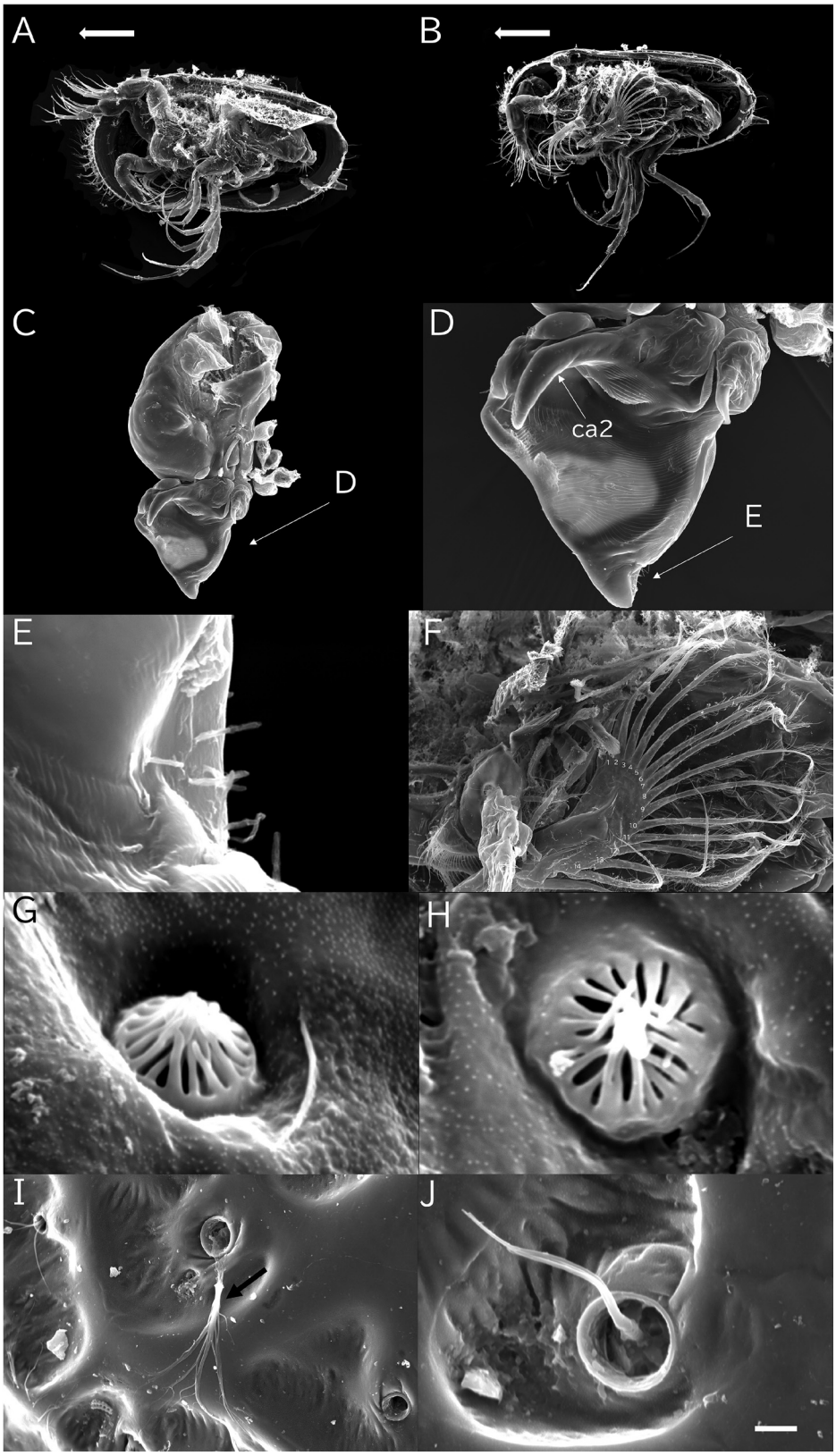

FIGURE 11. Exposed soft parts and pore systems of *Bicornucythere misumiensis* **sp. nov.** A and B, soft parts of female and male, respectively. C–E, male left copulatory organ (ventral view). C, whole image; D, extension of the distal lobe; E, tip of the distal lobe. F, maxillula of male. G–J, pore systems. G and H, corolla-like pore (reported by Okada, 1982 as a Ben-type pore). I, broom-shape seta (black arrow). J, simple seta. A, SUM-CO-2510, paratype; B–F, SUM-CO-2511, paratype; G–J, SUM-CO-2508, paratype. Arrows indicate anterior (A and B). Scale: 100 μm for A and B, 50 μm for C, 30 μm for F, 20 μm for D, 10 μm for I, 2 μm for E and J, 1 μm for G and H.

Right fifth limb (in female) (Fig. 9F). Four articulated podomeres, length ratio among them from proximal to distal 11: 6: 3: 4. First podomere with 2 annulated setae on anterior-proximal margin, 2 annulated setae on distal end and reduced exopodite on posterior-proximal margin. Second podomere with numerous setulae along anterior margin and 1 medium-length plumose seta on anterior distal end. Third podomere with acicular process on posterior distal corner. Fourth podomere with process on anterior distal corner and 1 terminal claw on distal end.

Left fifth limb (Fig. 9G). Four articulated podomeres, length ratio among them from proximal to distal 11: 6: 4: 5. First podomere with 2 annulated setae on middle of anterior margin, 2 annulated setae on anterior distal end and reduced exopodite on posterior-proximal margin. Second podomere with numerous setulae along anterior margin and 1 medium to long plumose seta on anterior distal end. Third podomere with acicular process on anterior distal corner. Fourth podomere with acicular process on anterior distal corner and 1 terminal claw on distal end.

Sixth limb (Fig. 9H). Four articulated podomeres, length ratio among them from proximal to distal 11: 7: 6: 5. First podomere with 1 annulated seta on anterior margin at one-thirds from proximal end, 1 stout annulated seta on anterior margin at two-thirds from proximal end and 1 annulated seta on distal end. Second podomere with 2 fine setae on middle of anterior margin, 1 medium to long plumose seta on anterior distal end and numerous setulae along distal margin. Third podomere with numerous setulae on distal end. Fourth podomere with numerous setulae and 1 terminal claw on distal end.

Seventh limb (Fig. 9I). Four articulated podomeres, length ratio among them from proximal to distal 12: 11: 5: 7. First podomere with 1 long annulated seta on anterior margin at three-fourths from proximal end, 1 medium to long annulated seta on distal end and 1 annulated short seta on posterior-proximal end. Second podomere with 2 fine setae on anterior margin at one-fourths from proximal end, 1 annulated medium to long seta on anterior distal end and numerous setulae along distal margin. Third podomere with numerous setulae on distal end. Fourth podomere with numerous setulae and 1 very long almost straight terminal claw with serrations on distal half.

Brush-shaped organ (in male) (Fig. 9J). Consisting of paired symmetrical lobes. Both brunches bearing about 6 long thin and 1 to 2 short thin setae.

Male copulatory organ (Fig. 8, 11C, D, E). Sub-elliptic capsule with long, annulated, arched structure. Distal lobe well-developed. Right distal lobe triangular outline with sharp linearly tip. Left distal lobe also triangular outline but hump-shaped tip. Coiled copulatory duct with thick base and arched. Two clasping apparatus (ca1, ca2) different in shape; proximal-most one (ca1) crescentic shaped, second one (ca2) two-forked shaped with beaked tip (Fig. 11D).

Furca (Fig. 9K). Consisting of paired symmetrical setulous setae.

General morphology of soft parts in Fig. 11A, B.

Dimensions. See Table 4.

Occurrence. Type locality is mud bottom of Konoura Fishing Port, Misumi-cho, Kumamoto Prefecture, Japan (32°37'4.06"N 130°32'1.08"E).

Remarks. This new species is different from *Bicornucythere bisanensis* (Okubo, 1975) with a smaller and more elongated carapace. The carapace surface reticulation is shallower in the frontal area than that in *B. bisanensis*. The well-developed asymmetrical distal lobe of the male copulatory organ is species-specific in the new species. The carapace of *B. concentrica* Yamaguchi *et al.*, 2015, which is a fossil species, shows a more ovate lateral outline, and the vein-like ridges show a concentric pattern.

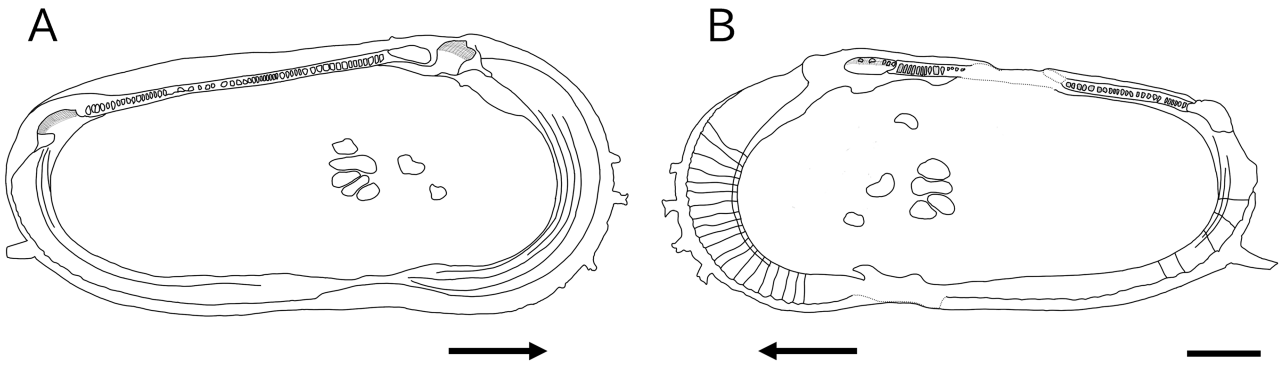

FIGURE 12. *Bicornucythere misumiensis* **sp. nov.** Internal lateral views of male carapace of left (A) and right (B) valves, respectively (SUM-CO-2505, paratype). Arrows indicate anterior. Scale: 100 μm.

Discussion

The four groups separated by carapace size (Forms A, M, P, and G) were described by Abe (1988) and Abe & Choe (1988). *Bicornucythere misumiensis* **sp. nov.** was found to belong to Form M based on carapace size. For the remaining three forms, Form P, which is the same species used by Schornikov & Shaitarov (1979), who proposed the genus *Bicornucythere*, demonstrated no clear differences in the male copulatory organs from Form A (Fig. 7B, C). Form G, first reported from Korea, was not included in these evaluations owing to a lack of clear descriptions of the copulatory organs. Therefore, it is necessary to collect new specimens of Form G and evaluate their male copulatory organs to confirm their taxonomic classification. Our finding also revealed extensive differences in the degree of extension in the distal lobe tip, which is a part of the male copulatory organ, in *B. bisanensis* specimens from both Aburatsubo Cove and Ushimado (Fig. 6). However, there was almost no variation in the specimens from Misumi-cho, although this may be due to the small number of specimens collected from this site (only five individuals).

We noted that the population from Aburatsubo Cove presented with all four Types of male copulatory organ. Individuals of Type D were the most abundant (85 individuals out of 103), followed by Type C (nine individuals), Type B (five individuals), and Type A (four individuals). These Types share common characteristics in their distal lobes, suggesting that they represent a continuum of morphologies likely produced via their interbreeding. There are several reports on dimorphism in the male copulatory organs of ostracods. Okubo (1979) and Higashi *et al.* (2011) reported its presence in *Xestoleberis setouchiensis* Okubo, 1979 and *Microloxoconcha dimorpha* Higashi *et al.,* 2011. However, as no dimorphism was observed in females of either species, the authors concluded that both species demonstrate intrasexual dimorphism. In contrast, to the best of our knowledge, this is the first report, at least in Ostracoda, of morphological variations of copulatory organs based on the combination of specific common characteristics. In addition, Types D and A do not share any characteristics that are "evenly distributed", and consequently we propose three hypotheses.

Firstly, Type A population is a subspecies-like population originating from Aburatsubo Cove that was geographically isolated from other populations around Japan, but when Type D population was introduced to Aburatsubo Cove, the population interbred with native Type A population.

Secondly, Type D population was inhabiting Aburatsubo Cove, and then, the Type A population emerged and formed a small cluster in a part of Aburatsubo Cove somewhere, with its microhabitat different from that of Type D. Types B and C have intermediate characters between Types A and D. These four morphotypes show the actual steps of morphological changes in male copulatory organ in this species, which is associated with speciation.

Thirdly, Aburatsubo Cove originally had several subpopulations with different life histories (*e.g.*, reproductive season) and the dominant population may have depended on different micro-environments (*e.g.*, micro-topography or sediment quality). In addition, it is known that male polymorphism is associated with differences in mating strategy and behaviour in several animal species (Gross 1996). Therefore, some similar differences may exist in each copulatory organ type among males in this species. The males of *Bicornucythere bisanensis* have asymmetrical fifth limbs and a well-developed right accessory limb (Okubo 1975; Schornikov & Shaitarov 1979), and Abe & Vannier (1991) reported that males use their well-developed right fifth limb to rotate females counter-clockwise before mating. There are also differences in the number of rotations among male individuals (Abe & Vannier 1991), suggesting that these behavioural differences may be due to differences in each Type, which may cause differences in mating frequency. Future studies should investigate the relationship among Types more multilaterally using genetic analysis and crossbreeding experiments, in addition to morphological observations.

The results of this study reveal that the male copulatory organs of *Bicornucythere bisanensis* show substantial morphological differences at both ends of the morphological range, and gradual morphological differences between the ends of the distal lobe. The length of the tip continuously varies within the same species. However, the thickness of the distal lobe is stable within each species, and clearly differs among species. Therefore, it can be inferred that it is not the length of the distal lobe tip but its thickness that is important as a taxonomic trait in this genus.

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