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# *Cribrilina mutabilis* n. sp., an Eelgrass-Associated Bryozoan (Gymnolaemata: Cheilostomata) with Large Variation in Zooid Morphology Related to Life History

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We describe the cribrimorph cheilostome bryozoan *Cribrilina mutabilis* n. sp., which we detected as an epibiont on eelgrass (*Zostera marina*) at Akkeshi, Hokkaido, northern Japan. This species shows three distinct zooid types during summer: the R (rib), I (intermediate), and S (shield) types. Evidence indicates that zooids commit to development as a given type, rather than transform from one type to another with age. Differences in the frontal spinocyst among the types appear to be mediated by a simple developmental mechanism, acceleration or retardation in the production of lateral costal fusions as the costae elongate during ontogeny. Colonies of all three types were identical, or nearly so, in partial nucleotide sequences of the mitochondrial COI gene (555–631 bp), suggesting that they represent a single species. Zooid types varied temporally in overall frequency in the population: colonies contained nearly exclusively R-type zooids in mid-June; predominantly I-type, or both R- and I-type, zooids in mid-July; and I-type, S-type, or both I- and S-type zooids (interspersed or in discrete bands) in mid- to late August. Reproduction occurred throughout the season, but peaked in July, with only R- and I-type zooids reproducing. Reproductive zooids bear a vestigial compound (tripartite) oecium and brood internally; S-type zooids, first appearing in August, were non-reproductive, which suggests that they may serve as an overwintering stage. As this species is easily accessible, common, and simple in form, it is potentially useful as a model system for studying polyphenism at multiple levels (zooid, colony, and population) in the context of life-history adaptations.

**Key words:** COI, epibiont, haplotype, heterochrony, invasive species, Japan, ontogeny, plasticity, polyphenism, *Zostera marina*

## INTRODUCTION

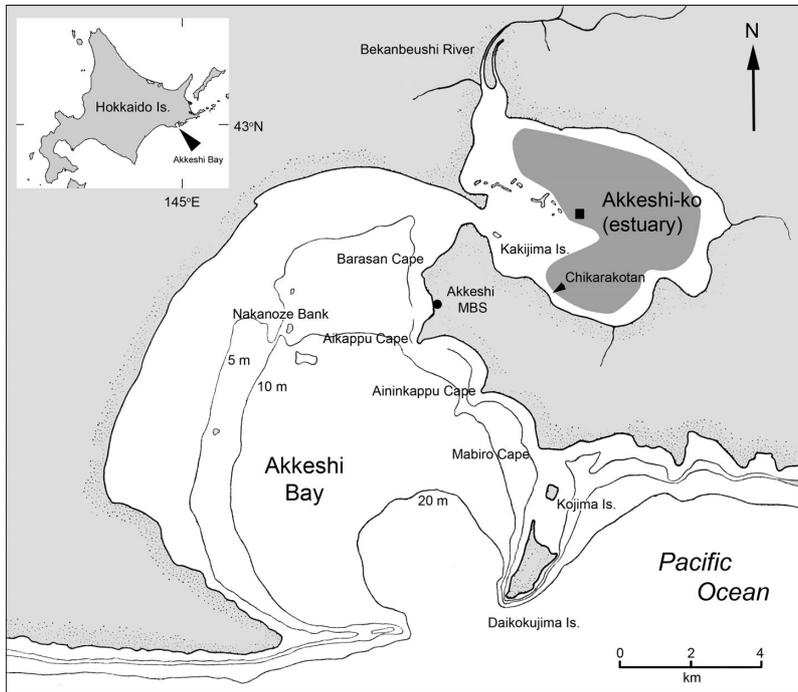
During a preliminary field survey in summer 2014 of eelgrass epibionts in Akkeshi-ko estuary, Hokkaido Island, northern Japan, we discovered a cribrimorph bryozoan that forms small, circular patches on eelgrass (*Zostera marina* L.). There were no published reports of this bryozoan from the Akkeshi vicinity despite considerable previous effort devoted to cheilostome bryozoans there (Mawatari and Mawatari, 1981; Mawatari, 1986; Ikezawa and Mawatari, 1993; Suwa and Mawatari, 1998; Grischenko et al., 2007), and its identity was unclear. Even more surprising was that the cribrimorph's zooid morphology early in summer differed markedly from that later in summer, with three alternative zooid types different enough that they appeared to represent different species. To confirm that these indeed represent the same species, we sequenced part of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) from colonies repre-

senting each of the three zooid types. Here we describe the cribrimorph as a new species in the genus *Cribrilina*, describe its temporal and astogenetic variation in zooid morphology, present the results of the COI analysis, and present preliminary ecological observations.

## MATERIALS AND METHODS

The study area was Akkeshi-ko (literally, 'Akkeshi Lake'), a sheltered estuary separated by a narrow neck from Akkeshi Bay, Japan (Fig. 1). The following summary of the environment of Akkeshi-ko is taken from Watanabe et al. (2005), Grischenko et al. (2007), and Yamada et al. (2007). Akkeshi-ko is 32 km<sup>2</sup> in area and generally 0.8–1.7 m in depth, although it reaches 10 m depth near the mouth. The Bekanbeushi River flows into the northwest corner of the estuary and accounts for 99% of the fluvial freshwater input, adding a volume equivalent to 5.8% of the volume of Akkeshi-ko daily. Salinity within Akkeshi-ko varies with proximity to the Bekanbeushi River outlet, season, and probably tidal stage; average monthly values from spring through fall range from 6.2 to 26.3 ppt in the northern part closest to the river outlet and from 24.5 to 27.6 ppt in the central and southern parts, which include our sampling sites. Akkeshi-ko typically freezes over from December to March. Mean monthly sea surface temperature recorded at the Akkeshi Marine Biological Station in Akkeshi Bay range from –0.3°C in February to

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**Fig. 1.** Map of Akkeshi Bay showing where specimens of *Cribrilina mutabilis* n. sp. were collected or observed, *in situ* in eelgrass bed (black square) or on drift eelgrass (Chikarakotan). Figure modified from Grischenko et al. (2007); eelgrass distribution (dark gray shading) in Akkeshi-ko from Yamada et al. (2007).

17.6°C in August; Akkeshi-ko probably reaches somewhat higher summer sea-surface temperatures than the outer bay. The maximum yearly range in tide level is 1.57 m; in Akkeshi-ko, extreme low tides expose scattered tidal flats. Patchy *Zostera marina* beds occupy the eastern two-thirds of Akkeshi-ko in sandy habitats (Fig. 1).

For the epibiont survey, non-quantitative collections of leaves of living eelgrass anchored *in situ* were made by snorkeling at low tide in central Akkeshi-ko (43°02.34'N, 144°53.11'E; black square in Fig. 1) on 18 June and 16 July 2014; at low tide, the floor of the eelgrass bed remained about 1 m deep. In addition, leaves of drift eelgrass were collected on the shore at Chikarakotan (43°01.40'N, 144°52.50'E) on 18 July, 10 August, and 21 August 2014. Finally, on 28 and 29 August 2014, samples of eelgrass leaves collected *in situ* at various sites throughout Akkeshi-ko were examined. For observation, leaf samples were taken back to the laboratory at the Akkeshi Marine Biological Station and rinsed gently with seawater to remove adhering sand and detritus. Leaves with bryozoan specimens were cut with scissors into smaller pieces, each bearing a single colony or a few colonies close together. Some colonies were observed alive with a Nikon SMA800 stereoscopic microscope and photographed with an attached Canon EOS Kiss X7 camera, after brushing with a fine brush, if necessary, to further remove detritus and epibionts. After observation, some specimens were preserved in 70% ethanol. In some cases, specimens were preserved in ethanol first and photographed later. Sample sizes (either observed or photographed) were ca. 30 colonies on 18 June; ca. 14 colonies on 18 July (mostly small, periancestrual colonies); three colonies on 10 August; and ca. 25 colonies on 21 August.

A 631-bp fragment of the COI gene was initially PCR-amplified from five specimens by using primers LCO1490 (Folmer et al., 1994) and BryoCOIH2161 (Mackie et al., 2006), and the amplicons were cloned to circumvent the problem of contaminating epibionts (Waeschbach et al., 2012) on the bryozoan colonies. However, no COI sequences were initially obtained from purely R-type colonies

(see below). To confirm that these were conspecific with the other types, two new primers (CribCOIF, AATGGTAGGAAGGGGGCTAAG; CribCOIR, CACCTCCTGCTGGTCAAAG) were designed to bind within the 631-bp region previously amplified, but not to the homologous sites in contaminant organisms previously identified by sequencing clones. With these new primers, a 555-bp fragment was amplified and directly sequenced (thus avoiding cloning) from each of three R-type colonies and an additional I-type colony.

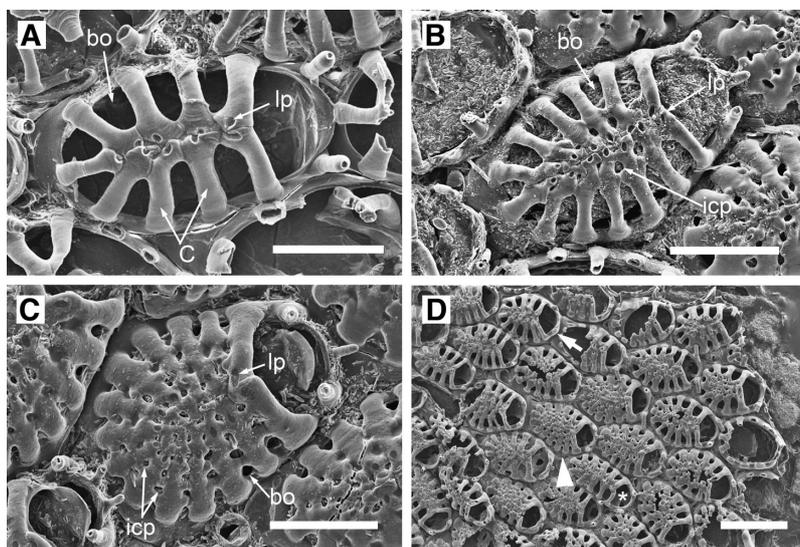
DNA was extracted from individual colonies preserved in 70% ethanol, by using a DNeasy Blood & Tissue Kit (Qiagen, Tokyo) following the manufacturer's standard protocol. In each case, half the colony was removed from the eelgrass with a sharp blade, and the other half remaining on the eelgrass was returned to ethanol as a voucher specimen. For both primer sets, PCR amplifications were conducted in 10.05- $\mu$ l volumes containing 1.0  $\mu$ l of 10 $\times$  TaKaRa *Ex Taq* buffer, 0.8  $\mu$ l of dNTP mixture (2.5 mM each dNTP), 0.5  $\mu$ l of each primer (10 pmol/ $\mu$ l), 0.05  $\mu$ l of TaKaRa *EX Taq* DNA polymerase (5 units/ $\mu$ l), and 1  $\mu$ l of DNA extract as the template source. PCR thermal cycling conditions were 95°C for 1 min; 35 cycles of 95°C for 30 sec, 50°C (LCO1490/BryoCOIH2161) or 58°C (CribCOIF/CribCOIR) for 1.5 min, and 72°C for 1 min; and 72°C for 7 min. Cloning (pGEM-T Easy Vector System, Promega), selection, and sequencing of amplicons (631 bp) from the LCO1490/BryoCOIH2161 primer set were performed as described in Dick et al. (2003). Nucleotide sequences of the 555-bp fragment were determined

by direct sequencing with the CribCOIF/CribCOIR primers. All sequences were determined by using a Big Dye Terminator Kit ver. 3.1 (Life Technologies) and an ABI 3730 DNA Analyzer. Sequences obtained in this study are available in the DNA Data Bank of Japan (DDBJ) under accession numbers LC061280–LC061293 (Table 1).

For scanning electron microscopy (SEM), sections of eelgrass leaf with attached bryozoan colonies, or in some cases detached colonies, were glued (after drying to remove ethanol, if necessary) with water-soluble glue onto aluminum SEM stubs. Some specimens were treated in a weak solution of household bleach (sodium hypochlorite, NaClO) to remove debris and most of the soft tissue, rinsed in tap water, dried, and glued onto stubs. Specimens on stubs were coated with Au in a Hitachi E1045 ion sputter coater and examined with a Hitachi S-3000N scanning electron microscope at 15 kV accelerating voltage. Images were stored electronically as bitmap files and later adjusted for size and clarity by using Adobe Photoshop CS6 ver. 13.0 software.

Zooidal characters were measured directly from SEM images by using ImageJ ver. 1.42q software (National Institutes of Health, USA; <http://rsb.info.nih.gov/ij/>). Measurements herein are presented in millimeters, with the range followed by the mean and standard deviation. Sample sizes are indicated as the number of zooids measured followed by the number of colonies involved; for example, (15, 1) indicates a character was measured on 15 zooids in a single colony.

The species described herein as *Cribrilina mutabilis* n. sp. is of the cribrimorph grade, to which a specialized and somewhat inconsistent terminology applies (Larwood, 1962; Bishop and Househam, 1987; Bock and Cook, 2001) (Fig. 2). Cribrimorphs have modified, often somewhat flattened, non-articulated marginal spines called 'costae' overarching the frontal membrane and usually fused together to a greater or lesser extent. Frequently each costa bears one or more small, membrane-covered openings called 'lumen pores' on the frontal surface. While the term 'pericyst' has been



**Fig. 2.** Categorization of zooid types in *Cribrilina mutabilis* n. sp. **(A)** R (rib) type; NSMT-Te 1041 (holotype, specimen MI-3). **(B)** I (intermediate) type; NSMT-Te 1043 (paratype, DNA voucher V-B4). **(C)** S (shield) type; NHMUK 2015.6.26.4 (paratype, DNA voucher V-C4). **(D)** Interspersed I-type (arrow) and S-type (arrowhead) zooids in single colony; zooid marked with asterisk has costae of I type proximally and R type distally; NHMUK 2015.6.26.3 (paratype, DNA voucher V-B3). Panels are SEM images of lightly bleached (A, D), moderately bleached (B), or dried, unbleached (C) specimens. Abbreviations: bo, basal opening; C, costae; lp, lumen pore; icp, intercostal pore. Scale bars: 200  $\mu$ m (A–C), 500  $\mu$ m (D).

used to refer generally to the frontal protective structure formed by overarching costae (e.g., Bock and Cook, 2001), we instead use the term ‘spinocyst’ (‘spinocystal frontal wall’) (Gordon, 1984) to describe this structure, and reserve the term ‘costal shield’ for a tight spinocyst composed of costae extensively fused medially and laterally. Costal shields usually have radiating columns of ‘intercostal pores’ between adjacent, laterally fused costae. We use the term ‘basal opening’ to refer to the most basal intercostal opening, bounded by adjacent costae and the opesia rim, and usually larger than the intercostal pores.

*Cribrilina mutabilis* shows variation in zooidal morphology that primarily involves varying degrees of lateral fusion between adjacent costae. Although we categorize three discrete zooidal types (Fig. 2A–C), they grade into one another, with some zooids of mixed type; in addition, more than one zooid type can occur in a single colony (Fig. 2D). We define the types as follows.

**R (rib) type** (Fig. 2A). The widely spaced costae meet and fuse only at and near the midline, leaving large basal openings, with no intercostal pores; the spinocyst appears like a vertebrate rib cage.

**I (intermediate) type** (Fig. 2B, D). The costae, narrower and more numerous than in the R type, are fused distally in the midline and laterally with adjacent costae at one or occasionally two points toward the distal end, leaving in the center of the spinocyst an elliptical shield of variable width bearing scattered intercostal pores. The basal openings remain large and about as wide as the costae. The most distal pair of costae is not thicker than the rest.

**S (shield) type** (Fig. 2C, D). The spinocyst comprises a costal shield, with adjacent costae fused intermittently along their length, leaving radial columns of intercostal pores. The basal openings are reduced, and narrower than the costae. The two costae forming the proximal official margin (‘apertural bar’; Larwood, 1962) are thicker than the rest, and each is separated from the adjacent costa by the basal opening and at least one intercostal pore.

To document changes in the frequency of zooid types and the proportion of reproductive zooids through at least part of the sum-

mer (2014), light micrographs of five colonies collected 18 June, two colonies collected 18 July, and five colonies collected 21 August were examined. For each colony, zooid type was determined for 20 zooids per colony, sampled in several columns of zooids running from near the ancestrula to the colony margin; for each colony, as many columns of zooids as necessary were examined to make up 20 zooids per colony. In each 20-zooid sample, the number of zooids bearing embryos was also counted.

The holotype and some paratype specimens from this study have been deposited in the National Museum of Nature and Science, Tsukuba, Japan, under catalog numbers prefixed with NSMT. Other paratypes have been deposited in the Natural History Museum, London, under numbers prefixed with NHMUK. The taxonomic classification is that of Gordon (2014). To conserve space, we do not include references for the authors of original descriptions of taxa other than bryozoans, nor for authors of bryozoan taxa at the family level and above.

## RESULTS

### Taxonomy

**Class GYMNOLEAMATA Allman, 1856**

**Order CHEILOSTOMATA Busk, 1852**

**Suborder NEOCHEILOSTOMINA d’Hondt, 1985**

**Superfamily CRIBRILINOIDEA Hincks, 1879**

**Family CRIBRILINIDAE Hincks, 1879**

**Genus *Cribrilina* Gray, 1848**

***Cribrilina mutabilis* n. sp.**

(Figs. 2–10)

? *Membraniporella aragoi*: Androsova (1971: 146); Kubanin (1984: 86, fig. 3.6A, map 18); Kubanin (1987: 121).

? *Membraniporella aragoni* [sic]: Kubanin (1975: 118).

? *Membraniporella* sp. aff. *aragoi*: Kubanin (1997: 123).

? *Membraniporella ex greg. M. aragoni* [sic]: Kubanin (1998: 304).

**Etymology.** The specific epithet is the Latin adjective *mutabilis* (changeable).

**Material examined.** All specimens are on (or were detached from) *Zostera marina* leaf fragments collected by M. Ito. Voucher specimens from which DNA was successfully sequenced are designated with ‘V’ followed by an alphanumeric combination. Some of the paratypes listed below are actually paratype lots, with several colonies on either the same or separate leaf fragments. Some lots contain a DNA voucher specimen, which is evident as the bisected colony, with half removed for DNA extraction.

Seven specimens or specimen lots (23 colonies) from central Akkeshi-ko estuary, Hokkaido, Japan, on *Z. marina* growing *in situ*, 18 June 2014. Holotype NSMT-Te 1041, specimen MI-3 (R type), on SEM stub. Paratypes: NSMT-Te 1042, specimen MI-1 (R type), four colonies on SEM stub; NHMUK 2015.6.26.1, specimen MI-2 (R type), three colonies on SEM stub; NHMUK 2015.6.26.2, specimen MI-4 (R type), three colonies on SEM stub; NSMT-Te 1047, specimen V-R1 (R type), vial with colony in ethanol; NHMUK 2015.6.26.5, specimen V-R2 (R type), vial with five colonies in ethanol; NHMUK 2015.6.26.6, specimen V-R3 (R type),

vial with six colonies on *Z. marina* leaf in ethanol.

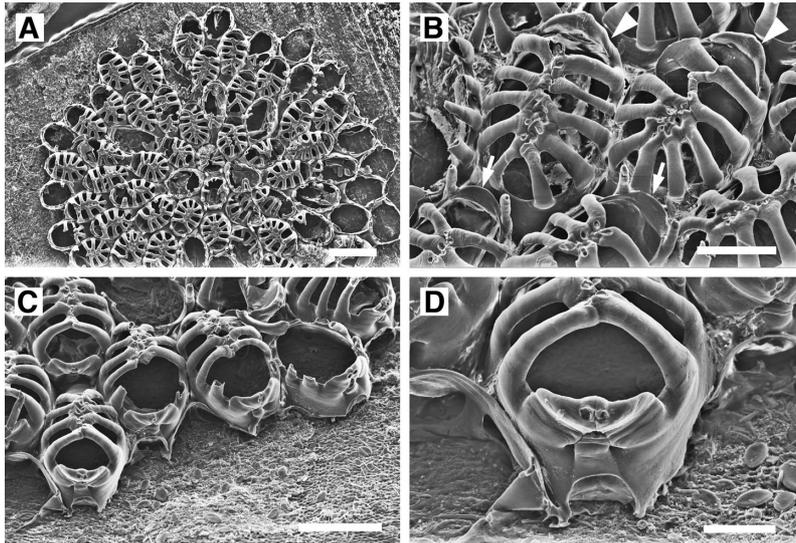
Nine paratype specimens or specimen lots (22 colonies) from drift *Z. marina* at Chikarakotan, Akkeshi-ko estuary, Hokkaido, Japan, coll. 21 August 2014 unless noted otherwise: NSMT-Te 1043, specimen V-B4 (mixed I and S types),

on SEM stub; NSMT-Te 1044, S-type ancestrula and specimen V-A5 (I type), on SEM stub; NSMT-Te 1045, V-C3 (mixed I and S types), on SEM stub; NHMUK 2015.6.26.3, specimen V-B3 (mixed I and S types), on SEM stub; NHMUK 2015.6.26.4, specimen V-C4 (S type), on SEM stub; NSMT-Te 1046, vial with three colonies in ethanol, coll. 10 Aug 2014; NHMUK 2015.6.26.7, specimen V-A3 (I type), vial with two colonies in ethanol; NSMT-Te 1048, vial with six colonies (mixed I and S types) in ethanol; NHMUK 2015.6.26.8, vial with five colonies (mixed I and S types) in ethanol.

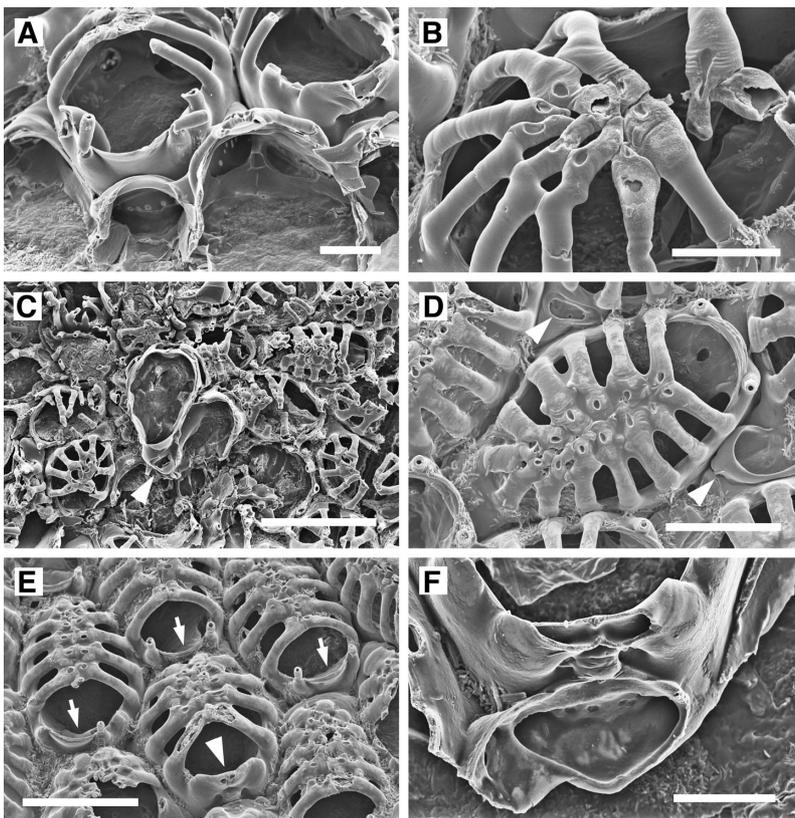
Other material examined: assorted specimens, photographed through stereoscopic microscope but not saved.

**Measurements.** R type: ZL, 0.46–0.58 ( $0.53 \pm 0.031$ ); ZW, 0.26–0.34 ( $0.30 \pm 0.019$ ) ( $n = 30, 2$ ). I type: ZL, 0.46–0.64 ( $0.54 \pm 0.038$ ); ZW, 0.29–0.43 ( $0.33 \pm 0.033$ ) ( $n = 30, 2$ ). S type: ZL, 0.51–0.69 ( $0.60 \pm 0.051$ ); ZW, 0.30–0.45 ( $0.38 \pm 0.039$ ) ( $n = 30, 3$ ).

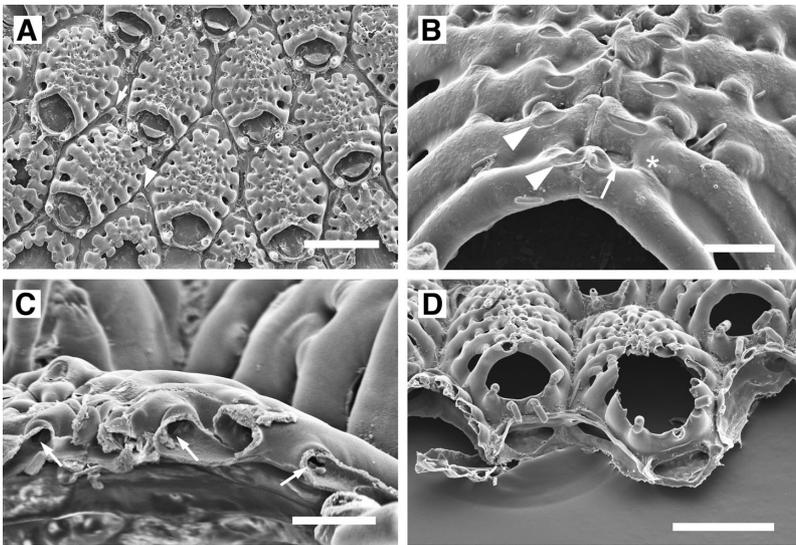
**Description.** Colony encrusting, usually unilaminar but sometimes with frontally budded zooids, forming sheet-like, roughly circular patches; mature colony typically around 5–7 mm across; whitish to light tan in color; embryos light yellowish to bright reddish orange. Zooids oval, distinct, separated by sharp groove; smooth gymnocyst in narrow zone proximally (e.g., Figs. 2A–C; 3B; 4D), scarcely evident laterally; with highly convex spinocyst composed of hollow, non-articulated costae; adjacent intracostal cavities continuous through lateral junctions (Fig. 5C). Opesia occupies entire frontal area inside raised mural rim (Fig. 2A, B), widest at or slightly proximal to middle; cryptocyst lacking, opesial margin smooth. Zooids lacking oecium have



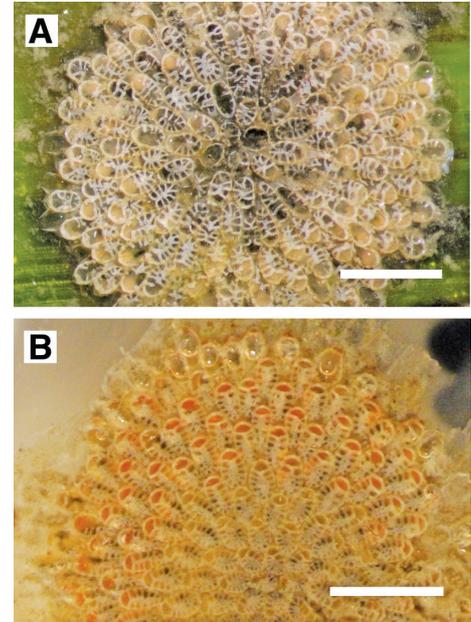
**Fig. 3.** *Cribrilina mutabilis* n. sp., R type, NSMT-Te 1041 (holotype, specimen MI-3). (A) Colony view. (B) Zooids with oecium (arrowheads) or distal lip (arrows), lacking oecium. (C) Oblique view of colony margin showing oecia and rib-like aspect of costae; note incipient or formed basal pore chambers. (D) Enlargement from panel (C) showing oecium. Panels are SEM images of a lightly bleached specimen. Scale bars: 500  $\mu\text{m}$  (A), 200  $\mu\text{m}$  (B), 300  $\mu\text{m}$  (C), 100  $\mu\text{m}$  (D).



**Fig. 4.** *Cribrilina mutabilis* n. sp., I type. (A) Zooids at colony margin, showing basal pore chambers, each with row of pores. (B) Proximal end of spinocyst, showing costae with medial and also some lateral fusions (the latter delineating intercostal pores), and large basal openings. (C) Incompletely developed, frontally budded zooids (center); larger zooid has enlarged frontal pore chamber (arrowhead) on proximal gymnocyst. (D) Zooid with costae of I type proximally and R type distally; note frontal pore chambers (arrowheads) on proximal gymnocyst of some zooids. (E) Zooids in oblique frontodistal view, one having oecium (arrowhead), and others having oral spines and simple distal lip (arrows). (F) Enlargement of marginal zooid showing basal pore chamber and hollow spinous components associated with oecium. Panels are SEM images of lightly bleached DNA vouchers NSMT-Te 1044 (paratype, V-A5) (A–C) and NHMUK 2015.6.26.3 (paratype, V-B3) (D–F). Scale bars: 100  $\mu\text{m}$  (A, B, F), 500  $\mu\text{m}$  (C), 200  $\mu\text{m}$  (D), 300  $\mu\text{m}$  (E).



**Fig. 5.** *Cribrilina mutabilis* n. sp., S type. **(A)** Group of non-reproductive zooids, most of which have proximal frontal pore chamber (one marked with arrowhead); paired chambers present on partial zooid in bottom right corner; one lateral chamber evident (arrow). **(B)** Enlargement of costal shield, showing lumen pores (arrowheads), intercostal pores (one indicated by arrow), and lateral costal fusions (one indicated by asterisk). **(C)** Broken costal shield, showing internal cavities of adjacent costae form continuous connection through lateral fusions (arrows). **(D)** Colony margin, showing basal pore chambers, each with several simple pores; note basal articulation of distal spines, which droop after light bleaching. Panels are SEM images of NHMUK 2015.6.26.4 (paratype, unbleached, dried DNA voucher V-C4) (A, B) and NSMT-Te 1043 (paratype, moderately bleached DNA voucher V-B4) (C, D). Scale bars: 300  $\mu$ m (A, D), 50  $\mu$ m (B, C).

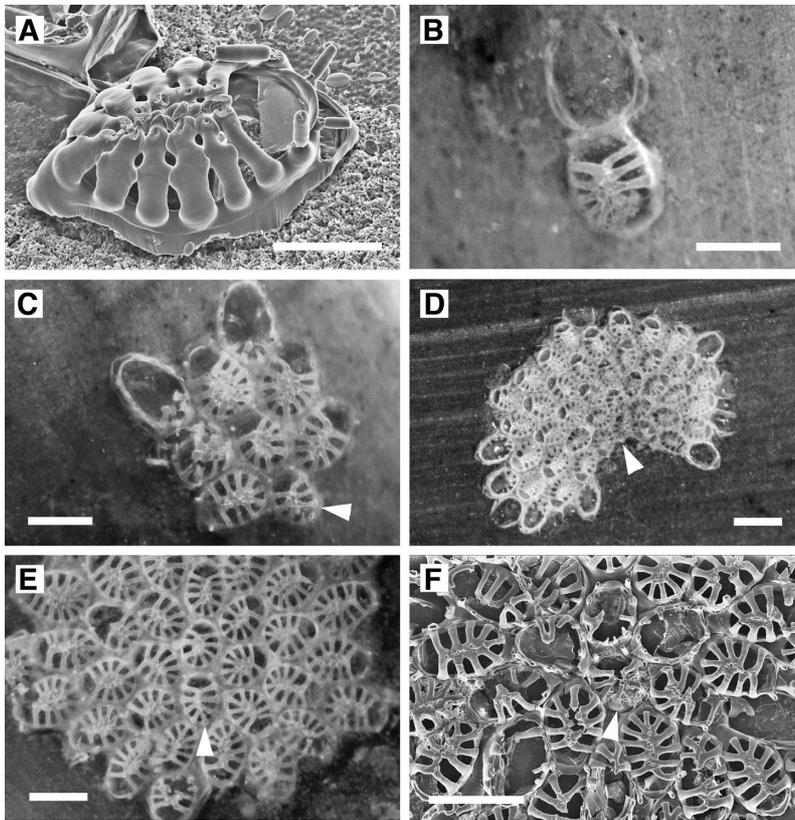


**Fig. 6.** *Cribrilina mutabilis* n. sp., intrazoooidal brooding. **(A)** Colony of predominantly R-type zooids, with a few I-type zooids interspersed, showing tan-colored embryos distally within zooids in peripheral zone; 18 June 2014. **(B)** I-type colony, showing bright orange-red embryos distally within zooids; 18 July 2014. Both panels are light micrographs of living colonies for which no vouchers remain. Scale bars: 2.0 mm.

sharp, raised lip distal to orifice (Figs. 2A–C; arrows in Figs. 3B, 4E), with pair of short, straight, vertically oriented, articulated distolateral oral spines, between which are 0–2 shorter, thinner articulated spines angled slightly distally (Figs. 2A–C; 5A, D). Zooids interconnect by row of small, single pores in transverse wall and each distolateral wall, each group of pores leading to large basal pore chamber (Figs. 4A, F; 5D). Basal wall not calcified, except for short distance around margin (Fig. 4A). Some zooids have one or two small, cup-shaped frontal pore chambers (Figs. 4D, 5A, arrowheads) on proximal gymnocyst, singly in midline or paired proximolaterally, with membranous cover, connecting to zooidal cavity by 3–5 small pores; similar frontally shifted pore chambers occasionally occur on lateral gymnocyst (Fig. 5A, arrow). Frontal pore chambers occur in all three zooid types, but most commonly in I and S types. Zooids brood internally, with single embryo occupying brood cavity immersed in distal part of cystid (Fig. 6). Reproductive zooids have vestigial compound (tripartite) oecium (Figs. 3C, D; 4E, F) around distal end, composed of pair of thick, tapering, non-articulated costae in place of distolateral oral spines, bent in medial direction and meeting in midline, each with irregular lumen pore; short, hollow, cylindrical or tapering median projection arises from roof of distal basal pore chamber (i.e., proximal-most gymnocyst of next-distal zooid) and meets lateral spinous components in midline (Figs. 3D, 4F). Frontal budding (Fig. 4C) occurs, but uncommonly; observed frontally budded zooids incompletely developed, lacking costae. Avicularia lacking. Ancestrula (Fig. 7) vari-

able; can be R, I, or S type (see Variation below); with pair of straight, articulated distolateral spines, often with one or two smaller, articulated spines between them. Ancestrula in mature colony typically surrounded by seven periancestrular zooids (Fig. 7E): one distal, two distolateral, two proximolateral, and two proximal. Ancestrula first buds distal zooid (Fig. 7B), then pair of distolateral zooids sequentially; one or two additional generations of distal and paired distolateral zooids follow, giving initial unidirectional growth of colony (Fig. 7C); only later are proximolateral and proximal periancestrular zooids produced, after colony has begun to surround ancestrula (Fig. 7D).

**Variation.** R type (Figs. 2A, 3, 6A, 7B, 7F) has eight to 12 costae (mode = 9;  $n = 32, 2$ ); costae wide at base; often narrower, and cylindrical or flattened, in mid-region; with distal end flattened and irregularly expanded or bent; costae complexly interlocking and fused in zooidal midline; each costa with oval lumen pore near or subterminal to distal end; lateral intercostal fusions and intercostal pores lacking; basal openings extend from opesia rim nearly to midline, width 1–2 times width of costae; distal pair of costae not conspicuously thicker than others. R-type colonies typically precocious, with most zooids starting about third generation from ancestrula reproductive, with vestigial oecium and bearing embryo in distal end of zooidal cavity. R-type ancestrula (Fig. 7B) with 8–10 costae fused in midline and pair of small, straight distolateral spines; some R-type ancestrulae tatform-like, having spines incompletely fused in midline (Fig. 7F).



**Fig. 7.** *Cribrilina mutabilis* n. sp., variation in ancestrula. **(A)** S-type ancestrula; NSMT-Te 1044 (paratype; settled adjacent to DNA voucher V-A5), 21 Aug. 2014. **(B)** R-type ancestrula with distally budded daughter zooid partly formed; 16–18 July 2014. **(C)** Early, unidirectional I-type colony produced by iterated budding of distal and distolateral zooids; 16–18 July 2014; arrowhead indicates ancestrula. **(D)** I-type ancestrula and colony, showing transition from unidirectional to circular colony, with pair of periancestrular zooids proximal to ancestrula (arrowhead) not yet formed; some peripheral zooids bear orange embryos; 21 August 2014. **(E)** R-type ancestrula (arrowhead) giving rise to I-type colony, showing typical arrangement of seven periancestrular zooids; 16–18 July 2014. **(F)** Tatiform-like ancestrula (arrowhead), with costae reaching but incompletely fused in midline; NSMT-Te 1041 (holotype, specimen MI-3), 18 June 2014. Panels are SEM images of lightly bleached specimens (A, F), or light micrographs (B–E) of freshly collected specimens in seawater, for which no vouchers remain. Scale bars: 100  $\mu\text{m}$  (A), 300  $\mu\text{m}$  (B, C, E), 500  $\mu\text{m}$  (D, F).

I type (Figs. 2B, 4, 6B, 7C–E, 8B-left, 8C-center, 9A) has 8–13 costae (mode = 11;  $n = 33, 2$ ); similar in form to R type, but with one or two subterminal lateral fusions delineating one or two corresponding intercostal pores between at least some adjacent costae (Figs. 4B, 9A). Median and lateral fusions create elliptical shield in center of spinocyst (Figs. 2B, D; 4B–E) with scattered intercostal pores; costae have one or occasionally two oval, elliptical, or drop-shaped lumen pores; basal openings shorter and narrower than in R type, as wide as or a little wider than costae; most-distal pair of costae not thicker than others. I-type colonies typically precocious, with most zooids starting about third generation from ancestrula bearing vestigial oecium and with embryo in distal end of zooidal cavity. I-type ancestrula (Fig. 7C) with around eight costae fused in midline, with lateral connections between some costae delineating intercostal pores; pair of small, straight, articulated oral spines, sometimes with one or two smaller spines between them.

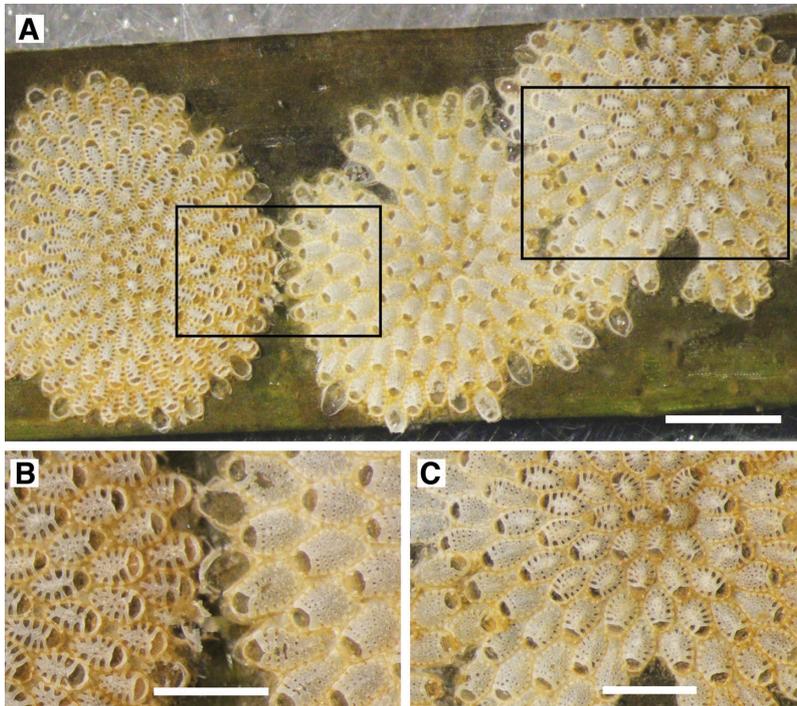
S type (Figs. 2C, D, 5, 7A, 8B-right, 8C-left, 9B) has 12–15 costae (mode = 14,  $n = 31, 3$ ), widest at base, tapering distally, with two or three lateral intercostal fusions between adjacent costae, delineating two or three corresponding intercostal pores. Intercostal pores arranged in radiating series. Most distal pair of costae usually thicker than others, with one or two intercostal pores between each distal costa and adjacent costa; basal openings small, much shorter than in other types and usually about half as wide as costae; oval lumen pores often subterminal. Zooids in colonies composed entirely of S form somewhat larger than in other types (see Measurements). S-type ancestrula (Fig. 7A) with around 11 costae, with one intercostal pore between pairs of adjacent costae in addition to basal opening; pair of articulated, straight oral spines, usually with one or two smaller articulated spines between them.

Two zooid types can occur in single colony (R and I types, or I and S types). Colony in Fig. 6A shows predominantly R-type zooids, with some I-type zooids present. Fig. 2D shows colony with I- and S-type zooids interspersed, while Fig. 8C shows colony with S-type ancestrula surrounded by central zone of I-type zooids, which gives way to peripheral zone of S-type zooids. Furthermore, single zooids can show mixed morphology, with one type of costae proximally and another distally; for example, single zooid can have I-type costae proximally but R type distally (Figs. 2D, asterisk; 4D).

**Remarks.** The first colonies of this species we observed, in mid-June, consisted almost exclusively of R-type zooids, with a few of I type. As the costae in the R type are fused near the midline but not laterally, this species initially seemed to belong in the genus *Klugerella* Moyano, 1991. However, a number of characters did not accord well with placement in

*Klugerella*, and our subsequent discovery of the S type suggested this species belongs in *Cribrilina*.

Characters diagnostic for *Klugerella* include usually bifurcate costae at least partly fused near the zooidal midline but not along their lateral margins; a hyperstomial ovicell with membranous ectooecium that after cleaning leaves a central zone of exposed endooecium, often as a triangular area at the proximal oocial margin; interzooidal connections alternatively described as “communication pores in lateral and distal rosette plates” (Moyano, 1991) or “uniporous mural pore chambers” (López de la Cuadra and García Gómez, 2000); avicularia present or absent, but vicarious if present; and a tatiform ancestrula (i.e., having a membranous frontal wall surrounded by marginal spines). We note, however, that comparative information is missing among the approximately 11 species (Bock, 2006) now assigned to *Klugerella*, as the nature of one or more key characters (oecium, ancestrula, or interzooidal communications) has not been



**Fig. 8.** *Cribrilina mutabilis* n. sp., variation in zooid type within and among colonies. **(A)** Three colonies on a *Zostera marina* leaf: left, I type; center, S type; right, mixed I and S types. **(B)** Enlargement of rectangle at left in panel (A), with I-type zooids to left and S-type zooids to right. **(C)** Enlargement of rectangle at right in panel (A); S-type ancestrula surrounded by central zone of I-type zooids, with peripheral zone of S-type zooids. Panels are from photomicrograph of living colonies collected 10 August 2014, preserved as paratype NSMT-Te 1046. Scale bars: 2.0 mm (A), 1.0 mm (B, C).

reported in some of these species. It is conceivable, for example, that species for which no ovicell has been reported (e.g., *K. musica* Gordon, 1993; *K. olaso* López de la Cuadra and García Gómez, 2000) have a vestigial oecium and internal brooding, as in *C. mutabilis*.

Characters diagnostic for *Cribrilina* (Levinsen, 1909; Hayward and Ryland, 1998) include a frontal shield formed from a series of flattened costae fused along the midline and at points along their lateral margins, with columns of intercostal pores between them; oecium hyperstomial or terminal, originating as an outfold formed by the distal zooid or as a kenozooid budded from the maternal zooid; ovicell ridged or smooth, with or without small pseudopores in the ectooecium (see Ostrovsky, 1998 for details); zooids interconnecting by large basal pore chambers, each having a few simple pores; and the ancestrula either tatiform, or smaller than but similar to subsequent autozooids, with a costal shield.

Several characters support placement of the Akkeshi seagrass-associated cribrimorph in *Cribrilina* rather than *Klugerella*, listed as follows. (1) The costae, even in the R type, which appears superficially like a *Klugerella* species, are not bifurcate. (2) The interzooidal connections are large basal pore chambers with a few simple pores, and are unlike those illustrated (Moyano, 1991) for either *Klugerella antarctica* (Kluge, 1914) (the type species) or *K. olaso* López de la Cuadra and García Gómez, 2000. (3) The ancestrula is variable (I, R, or S type) but usually not tati-

form, although in some R-type ancestrulae the costae are incompletely fused in the midline, resulting in a tatiform-like ancestrula; the S-type ancestrula looks very much like that in *Cribrilina annulata*. (4) The S type has an extensive costal shield with radiating columns of intercostal pores, which is the condition in *Cribrilina* rather than *Klugerella*. (5) *Cribrilina mutabilis* shares with at least two other *Cribrilina* species (*Cribrilina annulata* and *C. corbicula*) the presence of single or paired, cup-like frontal pore chambers located on the proximal gymnocyst of zooids, connecting to the parental zooid by several visible pores. These frontal pore chambers were previously reported as “frontal dietellae” (Powell, 1967) or “frontal septula” (Hayward and Ryland, 1998) in *Cribrilina annulata*, and are evident in SEM images of *C. corbicula* (M. Dick, unpublished data). The frontal pore chambers appear equivalent to basal pore chambers in which the chamber wall and pores have shifted to a more frontal position, with the opening directed frontally rather than peripherally. Although the occurrence of proximal frontal pore chambers is variable in *Cribrilina* (for example, the White Sea population of nominal *C. annulata* lacks them; A. Ostrovsky, pers. comm.) and thus they are not a diagnostic character for the genus, their occurrence in *C. mutabilis*, *C. annulata*, and *C. corbicula* may suggest a close relationship among these three species.

*Cribrilina mutabilis* at Akkeshi might be conspecific with material reported as *Membraniporella aragoi* (Audouin, 1826) in Russian literature on the northeastern Asian coast. Androsova (1971) reported a large number of young colonies of *M. aragoi* in summer in closed areas of Posyet Bay, from 0.4 m depth on algae (*Sargassum miyabei*) and the seagrasses *Zostera asiatica* and *Zostera marina*. Kubanin (1975) reported *M. aragoi* [sic] from laminarian algae and mollusc shells in Vostok Bay, the Rimskiy-Korsakov Island group, and Posyet Bay (all in Peter the Great Bay in the vicinity of Vladivostok), noting it occurred at 0.5 m depth and was one of the most abundant bryozoan species, also frequently occurring in fouling communities on wharfs and fishing boats, and on experimental acrylic-plastic plates. In that report, he described it as having a spinocyst formed of five or six pairs of costae; noted the occurrence of “orange ova” in zooids of colonies collected in August 1971; and noted that settlement in 1971 occurred in September. Kubanin (1984) reported *M. aragoi* from Aniva Gulf (Sea of Okhotsk, southern end of Sakhalin Island), the Sovetskaya Gavan vicinity (most northern part of the Sea of Japan), and Peter the Great Bay (including Posyet Bay); he provided no description, but illustrated a single zooid that closely resembles an R-type zooid of *C. mutabilis*. Kubanin (1987) listed *M. aragoi* intertidally from Furugelm Island, Peter the Great Bay.

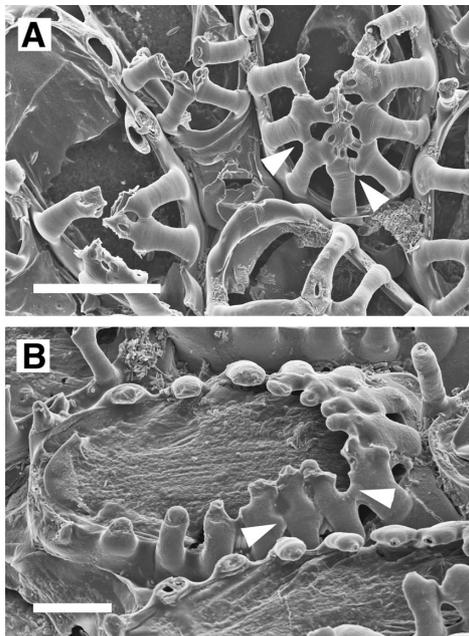
In later regional faunal lists, Kubanin expressed doubt over the identity of nominal *M. aragoi* along the northeastern Asian coast, listing it as “*Membraniporella* sp. aff. *aragoi*”

(Kubanin, 1997: 123) and “*Membraniporella ex greg. M. aragoni*” [sic] (Kubanin, 1998: 304). His material is certainly not *M. aragoi* Audouin, which according to a revision by López de la Cuadra and García Gómez (2000) has bifurcate costae and a conspicuous hyperstomial ovicell. However, unlike *C. mutabilis* at Akkeshi, which we detected only on eelgrass, the species reported by Androsova and Kubanin occurred on a variety of substrates, and there was no mention of alternative zooid types. Until more information is available on the potential utilization by *C. mutabilis* at Akkeshi of substrates other than eelgrass, and the morphology and natural history of populations along the northeastern Asian coast, it remains unclear whether a single species is involved.

It is important to emphasize that the three zooid types in *C. mutabilis* do not appear to be successive ontogenetic stages in single zooids, with R type developing to I type and thence to S type; once the spinocyst has developed, its morphology appears to be fixed. Evidence supporting this conclusion is as follows. (1) If R-type zooids developed into I type, and I-type zooids into S type, cases should be common in which newly forming lateral projections between adjacent, fully formed costae (i.e., those that have reached the zooidal midline) are growing toward one another across the basal opening but have not yet met and fused. We saw no instances in SEM images of these incipient, late-developing, lateral fusions. The costae rapidly calcify proximal to the growing tip as they form (e.g., Fig. 9B) and thus are able to form lateral projections only at or near the distal

end (where they are not yet calcified) as they grow. (2) In circular, encrusting bryozoan colonies, zooids are ontogenetically youngest near the colony periphery and oldest at the colony center. If zooids progressed ontogenetically from R to I type and thence to S type, one would expect R-type zooids to consistently occur at the periphery and S-type zooids in the center, with I-type zooids in between, and with a gradual transition among types. Observed astogenetic patterns, however, clearly do not conform to this expectation. The colony in Fig. 8C, for example, has a central zone of older, I-type zooids and a peripheral zone of younger, S-type zooids; if I-type zooids were gradually transforming to S-type zooids with age, the zones would be reversed and not as clearly demarcated. The colony in Fig. 2C has I-type and S-type zooids interspersed, with some I-type zooids younger and others older than intervening S-type zooids; furthermore, as mentioned above, no incipient (incomplete) lateral connections are evident, as would be expected if costae were transforming from I type to S type. (3) Ooecia were common in R- and I-type zooids, but were not observed in S-type zooids. This is difficult to explain if the zooid types represent an ontogenetic progression, in which case I-type zooids bearing ooecia should convert into S-type zooids bearing ooecia; changes associated with the aging of the spinocyst would be expected to occur independently of the presence or absence of an ooecium. (4) The zooid types differ in the range and especially the modal number of costae. Costae develop as the cystid develops and are not added later after the spinocyst is complete. If zooids were progressing from one type to another with age, there should be no differences among the types in the number of costae. Finally, we note that no other species in *Cribrilina* show R- or I-type zooids. In other *Cribrilina* species, zooids initially develop as S type, with lateral connections arising between adjacent costae in a basal-to-distal progression as the costae grow (as in Fig. 9B for S-type *C. mutabilis*)—once the S-type spinocyst has formed, it undergoes little further change with age, and older zooids at the beginning of the zone of astogenetic repetition typically look much like younger zooids near the colony periphery; see, for example, fig. 1 in Powell (1967).

From these observations, we concluded that zooids develop as one type or another, although zooids showing costae characteristic of more than one type in a single spinocyst are not uncommon. The main morphological difference among the types has to do with the number of lateral fusions between adjacent costae. This appears to be a developmentally simple change effected by retardation or acceleration of production of the matching lateral projections on adjacent costae that result in points of lateral fusion (Fig. 5B, C)—ontogenetic heterochrony *sensu* Lidgard et al. (2012)—although the degree of proliferation of lateral fusions could itself be a downstream effect of variation in the number and width of costae, which affect costal spacing. In R-type zooids, with the fewest costae and widest basal openings among the three types, no fusion occurs until at or near the midline, where the costae become splayed or irregular (e.g., Fig. 2A). The other types have more costae and narrower basal openings. In I-type zooids, the costae grow for some distance without lateral projections, but produce one or two toward the distal end, leading to a partial median



**Fig. 9.** *Cribrilina mutabilis* n. sp., ontogenetic heterochrony in production of lateral costal projections. **(A)** I-type zooid (top right), with lateral costal fusions (arrowheads) delayed for about two-thirds length of costae; NSMT-Te 1044 (paratype, lightly bleached DNA voucher V-A5). **(B)** Early stage of spinocyst development in S-type zooid, showing accelerated production of lateral fusions at bases of costae; NHMUK 2015.6.26.4 (paratype, unbleached DNA voucher V-C4). Both panels are SEM images. Scale bars: 200  $\mu$ m (A), 100  $\mu$ m (B).

shield with a few intercostal pores (Fig. 9A). In S-type zooids, the production of lateral projections is accelerated, beginning at the bases of the costae (Fig. 9B), leading to small basal openings and columns of intercostal pores.

**Occurrence.** Although bryozoan researchers in Japan had apparently not detected *C. mutabilis*, we have anecdotal information that seagrass researchers have been aware of its existence in northern Japan for some time, under the name *Membraniporella aragoi*. Ms Naoko Kouchi (pers. comm.) noted what was “probably this species” in seagrass beds in Otsuchi Bay, northeastern Honshu Island, around the year 2000 and subsequently. Similarly, Prof. Hiroshi Mukai (pers. comm.) stated that he has been aware of the occurrence of this species for “probably more than a decade” in Akkeshi-ko.

In the third week of June 2015, Dr. Andrew Ostrovsky (pers. comm.) found *Cribrilina mutabilis* to occur abundantly at depths of 1–3 m in the vicinity of the Sven Lovén Center for Marine Sciences at Kristineberg, on the western (North Sea) coast of Sweden (58°N latitude). That population appears to differ from the Akkeshi population both in substrate utilization and the phenology of the zooid types. At Kristineberg, the species occurred at least on *Zostera*, *Laminaria*, and *Fucus* substrates; hard substrata were not examined. Colonies of R type, I type, or mixed R and I type were most common, but S-type zooids and colonies also occurred, with some colonies containing all three zooid types. In contrast, at Akkeshi colonies in the third week in June were predominantly R type.

It is unclear whether *C. mutabilis* is native in northern Japan, but the recent discovery at the Kristineberg Center (established in 1877) in Sweden, and the very low genetic diversity detected for COI in the Akkeshi population (see the next section), suggest that *C. mutabilis* may have expanded in range in recent decades to northern Japan and the northwestern Atlantic, possibly as an anthropogenically dispersed invasive species. Identity of *C. mutabilis* with ‘*Membraniporella aragoi*’ from the western and northern Sea of Japan, Sakhalin Island, and the southern Sea of Okhotsk remains unclear because I- and S-type zooids have not been reported in those populations. However, if they are conspecific with *C. mutabilis*, they may represent the source population prior to expansion of the range, as records extend back to Androsova (1971) and Kubanin (1975) ca. 45 years ago. Genetic data from ‘*M. aragoi*’ / *C. mutabilis* populations in coastal eastern Asia and Sweden will help resolve these issues.

### COI analysis

We sequenced part of the COI gene for nine colonies with zooid morphologies predominantly or entirely of one or another of the three zooid types (Table 1). Although the DNA sequences of five of the colonies were 631 bp long (see Materials and Methods), the comparable sequence region for all nine colonies was 555 bp long. A BLAST

**Table 1.** COI haplotype, zooid type, and other information for DNA voucher specimens of *C. mutabilis* collected at Akkeshi in 2014. Zooid type: R, rib; I, intermediate; S, shield. Parentheses indicate a few zooids of one form present in a colony predominantly of another form. DDBJ, DNA Data Bank of Japan; NSMT, National Museum of Nature and Science, Tsukuba; NHMUK, Natural History Museum, London. All specimens listed are paratypes.

DNA voucher	Haplotype	Zooid type	Collection date	Length (bp)	DDBJ numbers	Museum catalog number
V-R2	H1	R	18 June	555	LC061292	NHMUK 2015.6.26.5
V-R3	H1	R	18 June	555	LC061293	NHMUK 2015.6.26.6
V-A3	H1	I	21 Aug.	555	LC061285	NHMUK 2015.6.26.7
V-A5	H1	I	21 Aug.	631 555	LC061280 LC061286	NSMT-Te 1044
V-B3	H1	I (S)	21 Aug.	631 555	LC061281 LC061288	NHMUK 2015.6.26.3
V-B4	H1	S (I)	21 Aug.	631 555	LC061282 LC061287	NSMT-Te 1043
V-C4	H1	S	21 Aug.	631 555	LC061284 LC061290	NHMUK 2015.6.26.4
V-R1	H2	R	18 June	555	LC061291	NSMT-Te 1047
V-C3	H2	I (S)	21 Aug.	631 555	LC061283 LC061289	NSMT-Te 1045

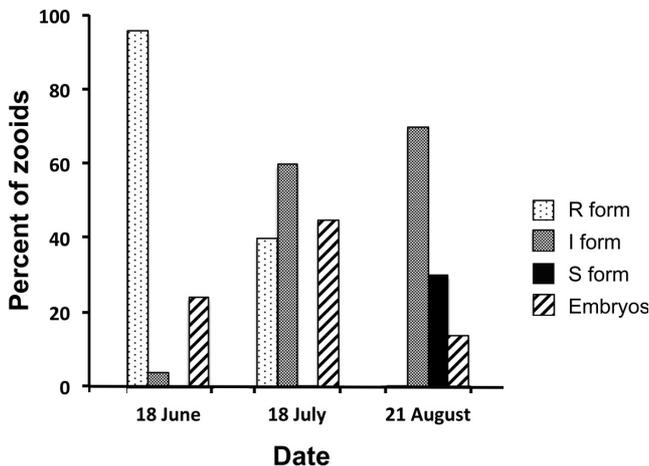
search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the GenBank nucleotide database identified the sequence most similar (81% identical) to the 631-bp *C. mutabilis* COI sequence to be a 615-bp region of COI from the ascophoran bryozoan *Calloporina angustipora* (accession number JF950411.1), indicating that the COI fragment we amplified was from *C. mutabilis* rather than from an associated epibiont or other contaminant.

For the 555-bp region, we detected two haplotypes (designated H1 and H2) differing by only two synonymous substitutions in codon third positions; the divergence between the haplotypes was thus 0.36%. Seven colonies (78%) were haplotype H1, representing all three zooid types (R, I, S, or predominantly I or S; Table 1). Two colonies (22%) were haplotype H2, with one R-type colony and the other predominantly I type. These results indicate that colonies having the alternative zooid types represent a single species, with high variation in zooid morphology despite low genetic variation for COI.

### Life history and ecology

In 2014, we first observed *C. mutabilis* colonies on 18 June, attached to *Z. marina* leaves anchored in sediment in the central part of Akkeshi-ko (black square in Fig. 1). Colonies (Figs. 3A, 6A) were circular, roughly 4–6 mm in diameter, and nearly entirely (96%) composed of R-type zooids, with only a few I-type zooids among them (Fig. 10); both zooid types and 24% of all zooids examined contained embryos, which were light tan in color. We noted no new recruits in the form of single ancestrulae or young, unidirectional colonies.

At the same locality on 16 July, the overall proportion (Fig. 10) of R-type zooids in colonies sampled had decreased to 40%, and that of I-type zooids had increased to 60% (Fig. 6B); both zooid types and nearly half (45%) of all zooids contained embryos, which were bright reddish orange in color. On this date, we noted circular, reproductive



**Fig. 10.** Temporal variation (2014) in the frequency of zooid types and in embryo production in *C. mutabilis* n. sp. Sample sizes (total zooids, number of colonies): 100, 5 (18 June); 40, 2 (18 July); 100, 5 (21 August).

colonies up to about 7 mm in diameter, as well as single ancestrulae (Fig. 7B) and small, unidirectional colonies (Fig. 7C) representing recent recruits. Specimens collected among drift *Z. marina* at Chikarakotan on 18 July mirrored the condition among specimens collected *in situ* on 16 July.

On 10 and 21 August, we collected only from drift *Z. marina* floating close to shore at Chikarakotan, where we found circular colonies up to about 7 mm in diameter and new recruits in the form of single ancestrulae (Fig. 7A) and young, unidirectional colonies. We observed no R-type zooids; zooid frequencies in our sample were 70% I-type and 30% S-type, with the latter appearing for the first time. Colonies in August varied greatly in composition; they consisted entirely of I-type zooids (Fig. 8A, left colony), entirely of S-type zooids (Fig. 8A, center colony), or of a central zone of I-type zooids giving rise to a peripheral zone of S-type zooids (Fig. 8C). Zooids bearing embryos were lower in frequency (14% of all zooids) than in June or July, and were all I type, occurring in both exclusively I-type colonies and mixed I- and S-type colonies; we observed no S-type zooids to bear oocysts or contain embryos. In spot checks of *Z. marina* around the entirety of Akkeshi-ko on 28 and 29 August, we found *C. mutabilis* remaining on *Z. marina* still anchored in the seagrass bed only in one small area between the central area sampled in June and July (black square in Fig. 1) and the shore at Chikarakotan.

We made two additional field observations. Throughout the summer season (mid-June through August), most colonies of *C. mutabilis* we collected were on green, living leaves of *Z. marina*; the exception was in the drift collections at Chikarakotan, where some living colonies were attached to recently uprooted leaves, but others remained on brownish (dying or dead) leaves. In addition, while we primarily examined epibionts on *Zostera marina*, we did not detect *C. mutabilis* in a cursory examination of other seagrasses (*Z. asiatica* Miki, *Z. japonica* Ascherson and Graebner, and *Phyllospadix iwataensis* Makino) occurring in Akkeshi-ko and Akkeshi Bay.

## DISCUSSION

We present very preliminary information on the life history and ecology of *C. mutabilis*. We first detected this species in June 2014 in a general survey of calcareous epibionts on *Zostera marina*. At that time, we observed only colonies with a high proportion of R-type zooids and did not recognize the few I-type zooids as different; after SEM examination, we tentatively identified this species as belonging in the genus *Klugerella* Moyano, 1991. We examined a small amount of additional material in July 2014, but considered the somewhat different I-type zooids to represent ontogenetic maturation from R-type zooids. Thus it was not until we observed colonies of I type, S type, or both (but no R type) in mid-August that we realized the Akkeshi-ko population was displaying different zooid types in varying frequencies as the summer season progressed. Due to small samples of complete colonies for zooid counts, Fig. 10 only roughly reflects the overall frequencies of zooid types and reproductive zooids in the population in 2014, and it does not address at all the frequencies in the population of I-type, S-type, and mixed I- and S-type colonies in August. However, it accurately reflects the salient features of temporal variation in zooid morphology (predominantly R type in June; predominantly I type in July and August, with the S type appearing only in August) and reproduction (reproductive at least from mid-June through mid-August, with oocysts and embryos observed in R- and I-type, but not S-type, zooids). All aspects of the annual life history need to be investigated through thrice-monthly sampling from early spring through late fall, with occasional sampling in winter.

We speculate that larvae produced by an overwintering population (possibly of S-type colonies) settled in a concentrated burst in late May or early June and developed into colonies of predominantly R type that rapidly grew and became reproductive—as evidenced by our observation on 18 June of colonies of rather uniform size, with few or no new recruits and with most colonies containing embryos. The thin, widely spaced costae in R-type zooids may function in allowing energy to be diverted from carbonate production toward rapid growth and reproduction.

We observed no purely R-type colonies on 16–18 July; colonies were of I-type, or of mixed R- and I-type zooids, with the latter type predominant; new recruits (evidenced by recently metamorphosed ancestrulae and small, periances-trular colonies) were common. If (as argued above) R-type zooids do not transform into I-type zooids within colonies, the replacement of predominantly R-type with predominantly I-type colonies in the population from June to July is difficult to explain unless R-type colonies disintegrate and disappear from the population after reproduction—and such short-lived colonies would be highly unusual. While the increase in the proportion of I-type zooids and the relatively high frequency of new recruits in July might suggest that the species is multivoltine (i.e., that embryos produced by R-type colonies in June settled from June to mid-July as a second annual generation of predominantly I-type colonies), this possibility needs to be carefully checked through increased sampling frequency and tracking of individual colonies. Reproduction appeared to peak in July, when the frequency of zooids with embryos was highest (Fig. 10). The function of the I-type

spinocyst is unclear; we speculate that it offers increased protection against specific predators appearing in mid-summer. Yamada et al. (2007) listed 20 crustacean and five gastropod species of epiphytic fauna firmly attached to seagrass substrates in Akkeshi-ko, and another 27 species in these groups in the water column surrounding the seagrass beds, and this largely crustacean seagrass-associated fauna potentially includes predators on *C. mutabilis*.

On 10 and 21 August, all our samples came from drift eelgrass leaves, on which we observed I-type, S-type (appearing for the first time), and mixed I- and S-type colonies, as well as new recruits (Fig. 7A). Reproduction was reduced; only 14% of all zooids (and only I-type zooids) contained embryos. We observed no reproductive S-type zooids, suggesting that this may be an overwintering type that has diverted energy from reproduction toward producing a complete costal shield, which should be stronger and more resistant to disintegration under stormy winter conditions than the other types. In any case, whatever their form, we speculate that overwintering colonies become reproductive from late May to early June the following year to produce the burst of recruitment leading to R-type colonies such as we observed in mid-June.

It is unknown where or in what form *C. mutabilis* overwinters; our collections on 10 and 21 August were exclusively from drift seagrass, a habitat hardly conducive for overwintering, although we did observe *C. mutabilis* on *Z. marina* leaves *in situ* in a very local area in Akkeshi-ko on 28–29 August. A sampling-intensive study of cheilostomes at 10 rocky intertidal sites in Akkeshi Bay and Akkeshi-ko (Grischenko et al., 2007) did not detect *C. mutabilis*. On the chance that Grischenko et al. (2007) had misidentified S-type *C. mutabilis* as *Cribrilina annulata*, we reexamined specimens collected in that study in early June 2004 on hard substrates and red algal fronds at two sites in Akkeshi-ko, but these proved to have been correctly identified as *C. annulata*.

*Cribrilina mutabilis* is unusual for several reasons. First, the highly reduced, tripartite oecium appears to be a novel type among cheilostomes. This oecium is superficially similar to the oecium in *C. annulata*, in which the external aspect shows a pair of medially fused oecial spines comprising the proximal margin, with a distal cap of calcified ectoecium bearing pseudopores extending between them (e.g., Ostrovsky, 1998, fig. 9; Dick et al., 2005, fig. 7B). The oecium in *C. mutabilis* likewise has a pair of spinous elements and at first glance appears to differ only in having the intervening cap (oecium proper) reduced to a cylindrical median projection. However, the two oecia are fundamentally different. The oecial fold (evident externally as the distal cap) in *C. annulata* is kenozooidal (type 2 oecium; Ostrovsky 1998, 2013), budded distally from and with a direct pore connection to the maternal zooid. The central projection in the reduced oecium of *C. mutabilis* is not kenozooidal, but instead is a hollow projection from the most proximal part of the gymnocyst of the next-distal zooid, with no pore connections to the maternal zooid. Whereas kenozooidal oecia are rare, formation of the oecium as an outfold of the next-distal zooid (type 1 oecium; Ostrovsky, 1998, 2013) is the common condition in cheilostomes (Ostrovsky et al., 2007, 2009a). However, the oecial out-

fold in *C. mutabilis* is unique in its extreme reduction, and homology of the oecial costae to the oecial spines in *C. annulata* is unclear.

In conjunction with the vestigial oecium, *C. mutabilis* is an internal brooder, a condition widespread in cheilostomes, with internally brooding species known or suspected in roughly one-quarter of neocheilostome families (Ostrovsky et al., 2009b). However, anatomical details of the internal brood sac in *C. mutabilis*—whether it arises from the vestibule or the distal zooidal wall, for example, and its relationship to the reduced oecium—remain to be elucidated through histological sectioning. We noted differences in embryo color at difference times of the season (Fig. 6), though the causes of these differences are unknown. Ostrovsky et al. (2009b) similarly noted variation in embryo color within and among colonies in another internally brooding species, *Discoporella marcusorum* (Cupuladriidae), and discussed possible causes of this variation.

Another unusual aspect of *C. mutabilis* is that while cheilostome autozooids typically exhibit a more-or-less uniform progression of changes in morphology in successive zooid generations in the zone of astogenetic change early in the growth of a colony, and can undergo gradual ontogenetic changes as individual zooids age and become more heavily calcified, few species show discrete, extreme variants in autozooid morphology during their life history. The difference in the spinocyst between R-type and S-type zooids in *C. mutabilis* is of a magnitude that one would normally expect between different genera in the family Cribrilinidae. A similar example is the cribrilinid *Corbulipora tubulifera* (Hincks, 1881), which shows three different zooid types, with superfamily-level differences among them (Bock and Cook, 1994, 2001).

Finally, *C. mutabilis* is unusual in that, at Akkeshi, it appears to be an obligate eelgrass epibiont. While 189 bryozoan species have been reported from Recent seagrass habitats worldwide (Di Martino and Taylor, 2014), with most species documented on other substrates as well as on the leaves and rhizomes of the seagrasses themselves, very few obligate seagrass-epibiotic bryozoans are known. The best-studied example is the bryozoan *Electra posidoniae* Gautier, 1954, an obligate epibiont on the endemic Mediterranean seagrass *Posidonia oceanica* (L.) (e.g., Lepoint et al., 2014). Another example, which is largely but not exclusively epibiotic on *Zostera* in the Mediterranean and Black Seas, is *Tendra zostericola* Nordmann, 1839 (Gontar, 2014). Finally, though less specifically obligate, the cheilostome *Microporella trigonellata* Suwa and Mawatari, 1998, in northern Japan had previously been reported only from the seagrasses *Phyllospadix iwatensis* (Suwa and Mawatari, 1998) and *Zostera caulescens* Miki (Kouchi et al., 2006); in 2014, we found it also to co-occur with *C. mutabilis* on *Z. marina* in Akkeshi-ko. Substrate specificity may vary among regions, however, as Grischenko and Zvyagintsev (2012) reported *M. trigonellata* from a wharf fouling community in Amur Bay within Peter the Great Bay, northwestern Sea of Japan.

*Zostera marina* is a perennial seagrass widely distributed in littoral habitats at temperate to subarctic latitudes in the Northern Hemisphere. In northern Hokkaido, shoot recruitment by rhizome branching and seed germination occurs from October to April, flowering from late April to

June or July, and fruiting from June or July to August or September; there is considerable annual variation in standing biomass and shoot density (generally highest from May to July, lowest from October to December) (Nakaoka and Aioi, 2001). Whether or how annual variation in zooid types in the life history of *Cribrilina mutabilis* is an adaptation to the dynamic life history of the substrate eelgrass are open questions. In the classification of Fusco and Minelli (2010), the zooidal plasticity in *C. mutabilis* is within- or across-generation polyphenism (i.e., occurrence of different phenotypes that are not the result of genetic differences; Mayr, 1963). The expression of alternative phenotypes might be environmentally triggered, but not directly environmentally induced (as when a change in temperature, for example, effects morphological changes by directly affecting physiological processes such as reaction kinetics or membrane permeability)—although this is problematic, since zooids of two types can be interspersed in a single colony (Fig. 2D). Because *C. mutabilis* is fairly common, easily accessible, forms flat, circular colonies in which embryos are easily observed, and displays phenotypic plasticity at several levels (among costae within zooids, among zooids within colonies, and among colonies within the population), it is possibly a useful model system for studying how extreme plasticity arises, is maintained, and is environmentally cued as an adaptive feature of life histories.

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