

# New Records of Creeping Ctenophores, Genus *Coeloplana* (Tentaculata: Platyctenida: Coeloplanidae), from Korea

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## ABSTRACT

Creeping ctenophores, *Coeloplana* species, were collected by SCUBA divers throughout the year (November 2006 to June 2010) from the branches and polyp masses of encrusting dendronephthys at a depth of 20-32 m off Munseom Island (Seogwipo-si, Jeju-do, Korea). A single individual of a newly recorded species in Korea, *Coeloplana bocki* Komai, 1920, was collected together with *C. anthostella* from the same location on 16 August 2009. A large number of individuals of each species were subsequently collected from the host *Dendronephthya* aff. *dendritica* on 20 June 2010. *C. bocki* can be distinguished from *C. anthostella* Song and Hwang, 2010 and *C. komaii* Utinomi, 1963 by its unique blue and orange colored stripes, and/or the branching and anastomosing milky-white stripes encircling the aboral sense organ towards the margin. The detailed morphology and molecular sequence information (nuclear 18S rDNA, internal transcribed spacer 1, and mitochondrial *cox1* gene sequences) for *C. bocki* is provided, and *C. bocki* and *C. anthostella* are compared.

**Keywords:** taxonomy, *Coeloplana bocki*, *Coeloplana anthostella*, 18S rDNA, ITS1, mtDNA *cox1*, Ctenophore, Korea

## INTRODUCTION

The family Coeloplanidae of comb jellies has a greatly compressed oral-aboral axis, and comprises the genera *Coeloplana* (24 species) and *Vallicula* (one species) (Mills, 1998). Reports of two platyctenid ctenophore species in Korean waters have been published, including the sessile *Lyrocteis imperatoris* (Song and Hwang, 2009) and a creeping form, *C. anthostella* (Song and Hwang, 2010). We report the identification of *C. bocki* associated with encrusting dendronephthys, which is a new record for Korea. In addition to a morphological comparison of *C. bocki* and *C. anthostella*, this study assessed the nucleotide sequences of nuclear and mitochondrial gene fragments (18S rDNA, internal transcribed spacer 1 [ITS1], and *cox1*) as molecular markers for the identification of these two Korean *Coeloplana* species.

## MATERIALS AND METHODS

### Taxonomic processes

Samples were collected by SCUBA from an approximately

20-32 m depth off Munseom Island (33° 13'32.45"N, 126° 34'06.84"E), offshore of Seogwipo-si, Jeju-do, Korea. A single individual of *C. bocki* was collected from the host, *Dendronephthya spinulosa* on 16 August 2009, together with *C. anthostella* from the same host at the same location. Several individuals of each of the two *Coeloplana* species were collected from *D. aff. dendritica* in this locality on 20 June 2010. The specimens were the creeping forms with undulating margins, and were attached firmly by the flat oral plane to branches and polyp masses of the rigida group of divaricate dendronephthys (Fig. 1A, B).

For species identification, the morphology was examined using a stereomicroscope (Stemi SV-6; Carl Zeiss, Jena, Germany) and optical microscope (Olympus BH2; Olympus, Tokyo, Japan), and measurements were made with an ocular micrometer before anesthetizing the animals with a MgCl<sub>2</sub> solution and fixing in 5% neutral formalin.

The morphology of *C. bocki* and *C. anthostella* is described in detail, with figures. The fragile specimens, which were attached to the branches and polyp masses of the host dendronephthys, were deposited at the Natural History Museum. Some were also deposited in the Korean Coral Resource

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**Table 1.** PCR primers used in this study

Gene	Primers	Sequence (5' → 3')	Primer source
18S	328 (forward)	TACCTGGTTGATCCTGCCAG	Nelles et al. (1984)
	329 (reverse)	TAATGATCCTTCCGCAGGT	Nelles et al. (1984)
ITS1	025 (forward)	TAACAAGGTTTCCGTAGGTG	White et al. (1990)
	026 (reverse)	AGCTRGCTGCGTTCTTCATCGA	White et al. (1990)
<i>cox1</i>	LCO1490 (forward)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCO2198 (reverse)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)

ITS, internal transcribed spacer.

Bank, Ewha Womans University (Seoul, Korea).

### Molecular techniques for DNA sequencing

After species identification by microscopy, the samples for molecular analysis were fixed in 100% ethanol before total genomic DNA extraction. The total genomic DNA was extracted using a DNA extraction kit (Qiagen Co., Valencia, CA, USA) according to the manufacturer's protocol. The target fragments from the three genes (18S, ITS1 and *cox1*) were amplified by PCR using universal primer sets (Table 1). The PCR reactions were carried out in a 50 µL reaction volume consisting of 10 units of *Taq* polymerase (Roche, Mannheim, Germany), a 2.5 mM mixture of dNTPs, 2.5 mM MgCl<sub>2</sub>, and 20 pmol of each primer. The amplification conditions involved an initial denaturation step at 94°C for 1 min followed by 35 cycles of denaturation at 94°C for 30 sec, primer annealing at 45-55°C (45°C for mtDNA *cox1*; 48°C for ITS1; 55°C for 30 sec for 18S), elongation at 72°C for 1-2 min, and extension at 72°C for 10 min. The amplified PCR products were subject to gel electrophoresis, and extracted using the TOPO gel purification reagents supplied with the TOPO XL cloning kit (Invitrogen Co., Carlsbad, CA, USA). After gel purification, each of the PCR products was ligated using a TOPO cloning kit, and transformed into competent *E. coli*. The cycle sequencing reactions for each of the PCR products were performed in both directions.

## SYSTEMATIC ACCOUNTS

Phylum Ctenophora Eschscholtz, 1829  
 Class Tentaculata Eschscholtz, 1825  
 Subclass Typhlocoela Ospovat, 1985  
 Order Platyctenida Bourne, 1900  
 Genus *Coeloplana* Kowalevsky, 1880

### *Coeloplana anthostella* Song and Hwang, 2010 (Fig. 1A-D)

*Coeloplana anthostella* Song and Hwang, 2010: 217.

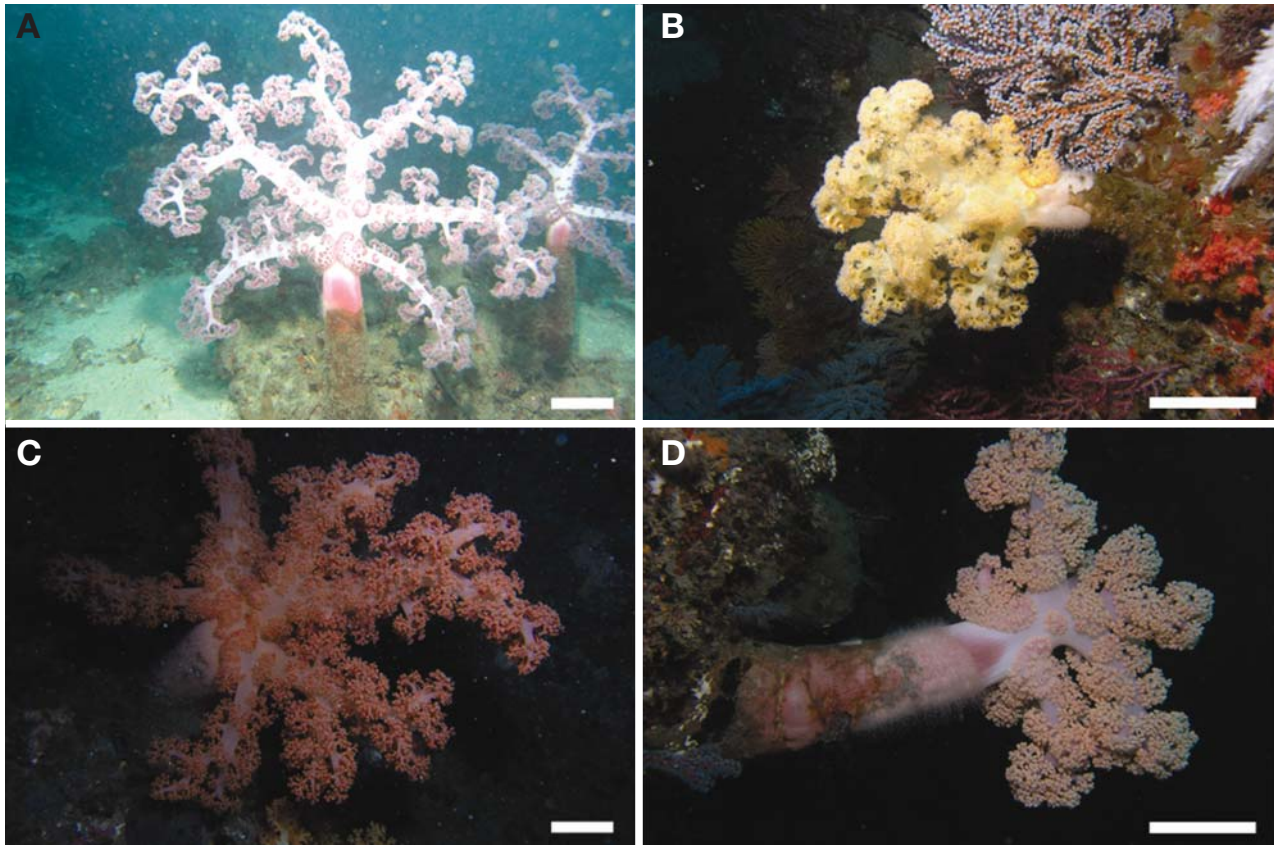
**Table 2.** Symbiotic relationship between one polyp mass of host dendronephthya species and the number of symbionts, *Coeloplana* spp.

Host	<i>C. anthostella</i>	<i>C. bocki</i>
<i>Dendronephthya spinulosa</i> (n=19)	1-18 (mean, 7.5; n=143)	0-1 (mean, 0.05; n=1)
<i>Dendronephthya</i> aff. <i>decussatospinosa</i> (n=6)	3-14 (mean, 7.7; n=46)	0
<i>Dendronephthya</i> aff. <i>dendricata</i> (n=7)	1-13 (mean, 5.6; n=39)	1-20 (mean, 2.9; n=20)
<i>Dendronephthya</i> aff. <i>gracillima</i> (n=3)	2-11 (mean, 7.3; n=22)	0

**Material examined.** Many inds., Isl. Munseom, 26 Nov 2006, Hwang SJ; many inds., Isl. Munseom, 30 Nov 2006, Hwang SJ; many inds., Isl. Munseom, 2 Dec 2006, Hwang SJ; many inds., Isl. Munseom, 16 Aug 2009, Kim BI, 26-32 m deep by SCUBA; 2 inds., Isl. Munseom, 20 Jun 2010, Hwang SJ, 20-32 m deep by SCUBA.

**Molecular data.** The GenBank accession numbers: HQ43-5810 (18S rDNA), HQ435812 (ITS1), and HQ435811 (*cox1*).

**Remarks.** This species is distinguished from *C. bocki* (see Komai, 1920, 1922, 1942, 1955) and *C. komaii* (see Utinomi, 1963) by its smaller size and deep scarlet star-shaped markings, which are similar in color to the host dendronephthya. The creeping flattened ctenophores are oval-shaped, have an undulating margin, and are 0.88 × 0.53-3.50 × 2.88 (1.99 ± 0.87 × 1.38 ± 0.55, n=31) mm (tentacular axis × sagittal axis; length × width) in size. The polar plate is not visible on the aboral pole, but is raised above its periphery or lobed. A sense organ (0.3 × 0.4 mm in diameter) containing a statocyst (0.08 × 0.08 mm in diameter) can be observed in the center by optical microscopy. The statolith forms two masses, each of which has more than 20 granules located centrally in the cavity. All individuals collected since November 2006 were attached commensally to the branches and polyp masses of the encrusting alcyonaceans *D. spinulosa*, *D. aff. dendritica* and other dendronephthyas at 20-32 m depth off Munseom Island. Each polyp mass (size range=10-25 mm; mean=16.1



**Fig. 1.** Host dendronephthyas. A, *Dendronephthya spinulosa* with numerous individuals of *Coeloplana anthostella* and one ind. of *C. bocki*; B, *D. aff. dendritica* with few inds. of *C. anthostella* and numerous inds. of *C. bocki*; C, *D. aff. gracillima* with *C. anthostella*; D, *D. aff. decussatospinosa* with *C. anthostella*. Scale bars: A-D=10 cm.

mm; n=35) had 1-18 aggregated individuals (mean=7.1; n=250) (Table 2).

**Distribution.** Korea (Isl. Jeju-do).

<sup>1</sup>\**Coeloplana bocki* Komai, 1920 (Figs. 1A, B, 2, 3)

*Coeloplana bocki* Komai, 1920: 576; 1922: 1, Pls. 1-7; 1942: 214; 1955: 13; 1975: 305, fig. 12; Tokioka, 1958: 178, Pl. 89, fig. 4; 1968: 210; Nishimura and Noda, 1992: 173, Pl. 42, figs. 6, 7; Mills, 1998: 9.

**Material examined.** 1 ind., Isl. Munseom, 16 Aug 2009, Kim BI, 26-32 m deep by SCUBA; many inds., Isl. Munseom, 20 Jun 2010, Hwang SJ, 20-32 m deep by SCUBA, attached on polyp mass of *D. spinulosa* and *D. aff. dendrocata* (Table 2).

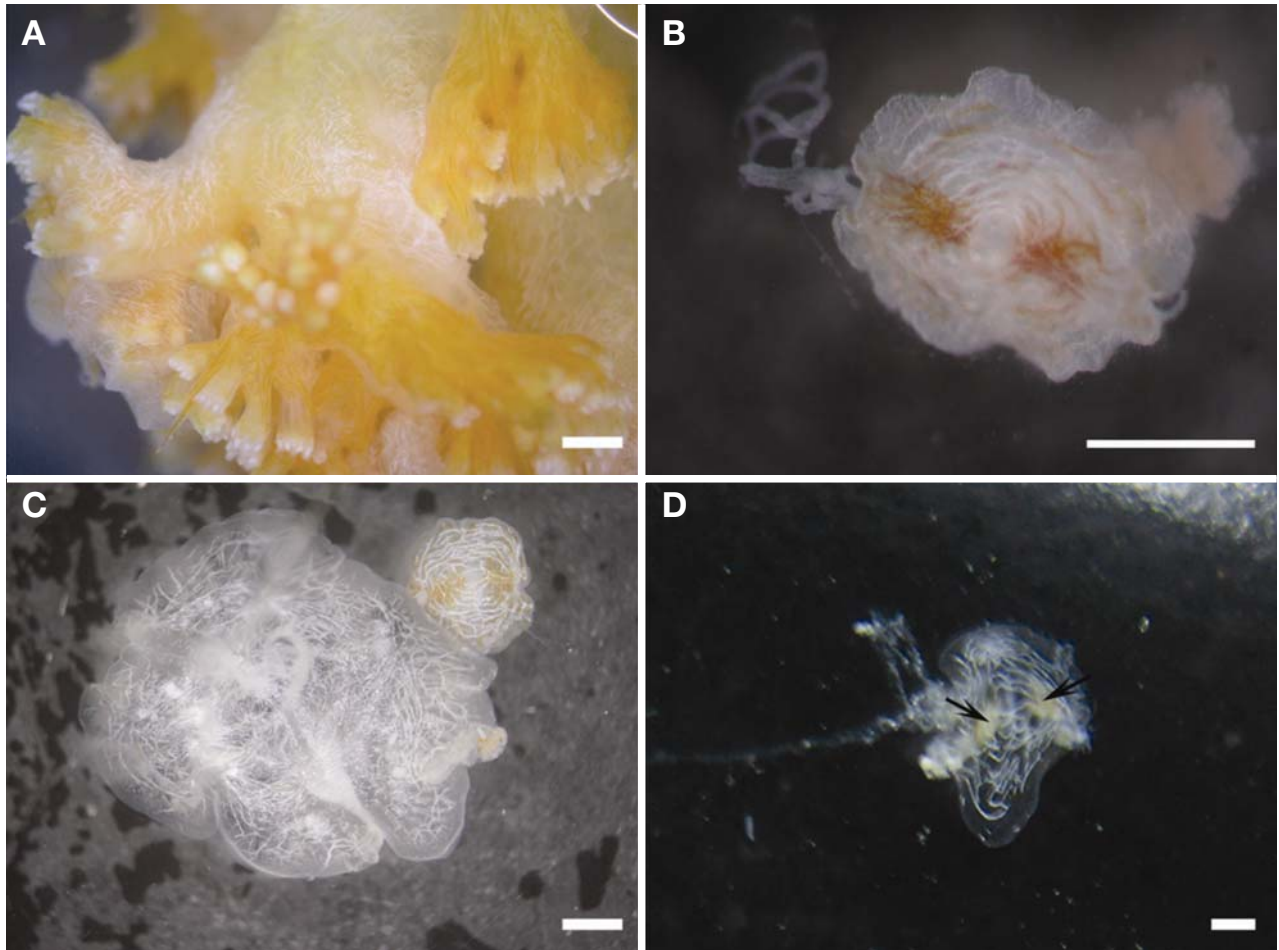
**Description.** Creeping flattened ctenophore represents somewhat hump-like elevation in its central parts of broad and thin basal expansion (Fig. 2B-D). Oval shape with undulating margin,  $2.2 \times 1.9-5.0 \times 4.4$  (n=7) mm (tentacular axis  $\times$  sagittal axis, length  $\times$  width) at individuals with orange stripes

(Fig. 2B), and  $3.7 \times 2.6-11.0 \times 5.7$  (n=4) mm at ones with milky white stripes (Fig. 2C, D).

Two pinnate tentacles having colloblasts in their surface with one side branches (Figs. 2B, D, 3B) entirely retracted into each tentacle sheath at both ends of tentacular axis. Tip of tentacles globular ( $0.02 \times 0.02-0.04 \times 0.04$  mm in dia.), their side and main branches slender, 0.02-0.05 mm and 0.06-0.09 mm in dia. respectively. Tentacle sheath flask-shaped in aboral view, two tentacle bases separated each other at aboral pole and placed in transverse plane (Fig. 2D). Under stereomicroscope, ramification of gastrovascular system not clearly visible (Fig. 2C, D), but visible under light microscope (Fig. 3A). However, milky white stripes branched and anastomosing not related to each mesh consisting of thin reticulated gastrovascular canals (Fig. 3A).

On aboral pole, polar plate visible, with two lobe-like processes at its peripheral margin, and also raised above its periphery (Fig. 3C). Any other dorsal papillae undeveloped on its dorsal surface. At its center of small individual with

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**Fig. 2.** *Coeloplana bocki*. A, Polyp mass of *D. aff. dendritica* attaching numerous inds. of *C. bocki*; B, *C. bocki* with orange stripes; C, A small and a large inds. of *C. bocki* with milky white stripes; D, *C. bocki* showing two tentacle bases (arrows) and tentacles. Scale bars: A-D=1 mm.

orange stripes, sense organ (0.15 × 0.15 mm in dia.) with statocyst (0.04 × 0.04 mm in dia.) in its middle part clearly seen under light microscope (Fig. 3D). Statolith forming one mass of almost 40 granules in its central position of cavity. On oral side, one large mouth (0.6 × 0.7 mm in dia.) with many pharyngeal folds opened at center of ventral side.

In color, milky white, semi-transparent, with 7-10 blue and 4-6 orange colored branching and anastomosing stripes encircled aboral sense organ towards margin, and reddish orange colored radial stripes at both tentacle bases from one individual on August 16, 2009 (Fig. 2B, D). And also, milky white, semi-transparent, with 7-12 white branching and anastomosing stripes at small individuals and numerous stripes at large ones encircled aboral sense organ towards margin, and also orange colored radial stripes at both tentacle bases from lots of individuals on June 20, 2010 (Fig. 2B-D). However, all of blue and orange colored stripes disappeared in

formalin, except for yellowish tentacle bases.

**Habitat.** The single individual of this species together with many *C. anthostella* was found from *D. spinulosa* on August 16, 2009, and many individuals of the species together with *C. anthostella* were obtained from the host *D. aff. dendricata* on June 20, 2010 (Table 2). Of the number of symbionts, *Coeloplana* spp. was influenced by the another species attaching to the single host dendronephthya.

**Molecular data.** The GenBank accession numbers: HQ43-5813 (18S rDNA), HQ435814 (ITS1), and HQ435815 (*cox1*).

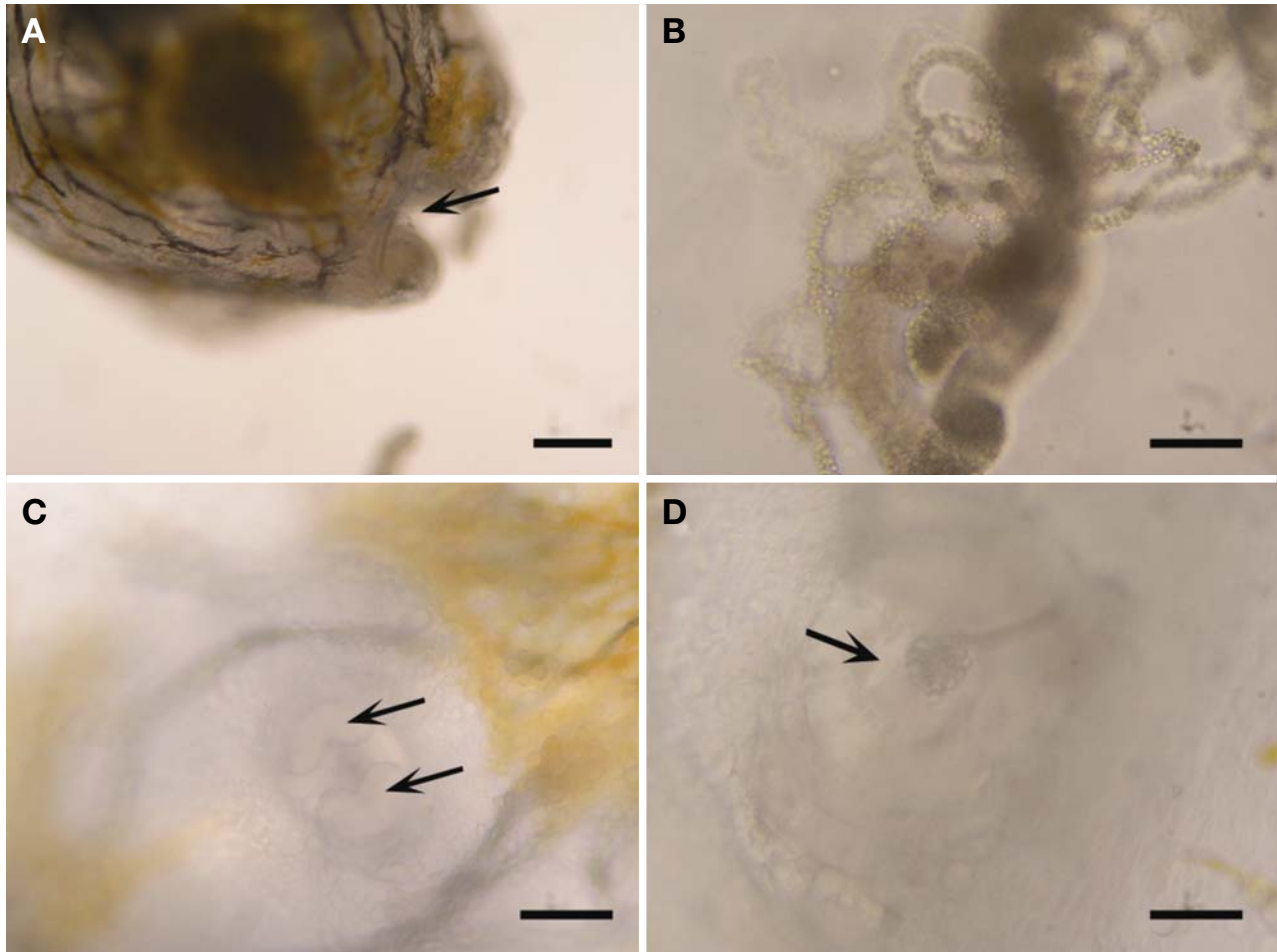
The nucleotide sequences of *C. bocki* differ from *C. anthostella* by 0.2%, 17.9%, and 11.7% for 18S, ITS1 (including inferred gaps), and *cox1*, respectively (Table 3). The high level of sequence divergence detected between the two *Coeloplana* species, particularly both from ITS1 and *cox1* indicates that they represent well-established distinct species.

**Remarks.** This species can be distinguished from *C. antho-*

**Table 3.** Nucleotide sequence differences between *Coeloplana anthostella* and *C. bocki* in three gene fragments

	18S		ITS-1		<i>cox1</i>	
	Length (bp)	Pairwise distance (%)	Length (bp)	Pairwise distance (%)	Length (bp)	Pairwise distance (%)
<i>C. anthostella</i>	1,768	0.2	271	17.9	664	11.7
<i>C. bocki</i>	1,768		271		664	

ITS, internal transcribed spacer.

**Fig. 3.** *Coeloplana bocki*. A, Opening of the tentacle sheath (arrow); B, Tentacle expelled from the tentacle sheath; C, Aboral sense organ showing polar plates (arrows); D, Sense organ (arrow) with a statocyst in the central part of aboral side. Scale bars: A=200  $\mu$ m, B, C=100  $\mu$ m, D=50  $\mu$ m.

*stella* (see Song and Hwang, 2010) and *C. komaii* (see Utinomi, 1963) by its unique blue and orange stripes, and/or branching and anastomosing milky-white stripes encircling the aboral sense organ towards the margin (Fig. 2B, D). These specimens were smaller than those in the original description (Komai, 1920, 1922), and have lobe-like processes at the peripheral margin of the polar plate.

**Distribution.** Korea (Isl. Jejudo), Japan (Sagami Bay, Tanabe, Misaki).

## ACKNOWLEDGEMENTS

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