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A New Record of Epizoic Hydroid, *Ectopleura radiata* (Hydrozoa: Anthoathecata: Tubulariidae), from Korea

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ABSTRACT

In 2022, during a survey of intertidal fauna around Jindo located in the Dadohaehaesang National Park in the South Sea, epizoic hydroids attaching onto seagrass (*Zostera* sp.) growing on soft sediments were collected. Through taxonomic examination, an unrecorded species, *Ectopleura radiata*, is newly added to the hydrozoan fauna of Korea. In addition, DNA barcoding for species from *Ectopleura* and *Tubularia* clarified the distinction among morphologically indistinguishable species without gonophores. Up to now, only one species, *E. crocea*, has been reported in Korea. Through this study, a total of two species belonging to genus *Ectopleura* have been reported in Korean waters so far.

Keywords: unrecorded hydroid, intertidal zone, seagrass, taxonomy, Korean fauna

INTRODUCTION

The genus *Ectopleura* L. Agassiz, 1862 belonging to family Tubulariidae was established with type species *Ectopleura dumortierii* (Van Beneden, 1844) originally described as *Tubularia dumortierii*. A total of seven genera [*Bouillonia* Petersen, 1990; *Ectopleura* L. Agassiz, 1862; *Hybocodon* L. Agassiz, 1860; *Lobataria* Watson, 2008; *Ralpharia* Watson, 1980; *Tubularia* Linnaeus, 1758; and *Zyzzyzus* Stechow, 1921] are now known in the family Tubulariidae. In Korea, only one species, *Tubularia mesembryanthemum* Allman, 1871, was first reported in 1941 in Incheon and then recorded in the South Sea and the East Sea (Kamita and Sato, 1941; Rho, 1969; Park, 2011). In 1970, Brinckmann-Voss synonymized *T. mesembryanthemum* as *Ectopleura crocea* (Agassiz, 1862) on the basis of no morphological differences between the two species.

Ectopleura can be distinguished from the genus *Tubularia* by its colonial or solitary forms, one whorl of oral tentacles, a hollow stem, and absence of longitudinal peripheral canals (Schuchert, 2010). *Ectopleura* hydroids usually form colonies with several polyps connected by stolonial hydrorhiza. Hydranth of *Ectopleura* has two whorls of tentacles, oral and aboral. Up to now, 34 valid species have been accepted in the genus *Ectopleura* (Schuchert, 2022). These species have been found all over the oceans. Of them, *E. dumortierii*, *Ectopleura larynx* (Ellis and Solander, 1786), and *E. crocea* are circum-global in (sub)tropical, in temperate and cold waters, and in temperate waters, respectively (Xu et al., 2007; Lin et al., 2010; Schuchert, 2010; Huang et al., 2015).

In this study, a taxonomic investigation was performed on hydroids collected in May 2022 from the intertidal zones of Jindo Island located in the Dadohaehaesang National Park in the South Sea. Taxonomic studies on hydroids inhabiting the waters of Jindo have been conducted by Park and Rho (1986), Rho and Park (1986), and Park (1995), reporting about 34 species. Through the present study, an unrecorded species, *Ectopleura radiata* (Uchida, 1937), is newly added to the hydrozoan fauna of Korea. In addition, DNA barcoding for species from *Ectopleura* and *Tubularia* was performed to clarify the distinction among morphologically indistinguishable species without gonophores.

MATERIALS AND METHODS

Specimens were collected from Jindo belonging to Dadohaehaesang National Park in the South Sea from intertidal zone

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in 2022. Living colonies attaching onto seagrasses were taken with a digital camera (Tough TG-5; Olympus Digital Solution Corporation, Tokyo, Japan) to record morphological and ecological features of colonies before fixation. After collection, they were transferred to a plastic container with seawater, and then anesthetized with finely ground menthol powder for 4–6 h. Anesthetized specimens were fixed with 99% alcohol (v/v) for further examination after removing menthol powder with running water.

For identification, detailed morphological characteristics of colony, hydrorihza, hydrocaulus, hydranth, and gonophore were examined under a stereomicroscope (SteREO Discovery. V8; Carl Zeiss, Jena, Germany). During the examination, all images in detail were captured using CMOS sensor microscope digital cameras (KCS-2000SS; Korea Lab Tech, Seongnam, Korea). All measurements were taken with an image analyzer (OptiView; Korea Lab Tech).

Molecular identification was also performed to compare genetic distances among related species from *Ectopleura* and *Tubularia*. Total DNA was isolated from ethanol preserved whole individual using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Partial 16S rRNA sequences were amplified using primers (FiMod: 5′-TCG ACT GTT TAC CAA AAA CAT A-3′ and R2: 5′-ACG GAA TGA ACT CAA ATC ATG TAA G-3′) (Cunningham and Buss, 1993; Cartwright et al., 2008). Amplification was carried out on a MiniAmp thermal cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA) using TaKaRa Ex Taq (Takara Bio Inc., Kusatsu, Japan) for 50 μL reactions. PCR condition was fixed as follows: 5 min at 95°C, 35 cycles of 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C, followed by a final extension step at 72°C for 10 min. Analysis of genetic distances and phylogeny were performed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 11 (Tamura et al., 2021). Genetic distances were calculated based on the Kimura 2-parameter (K2P) model. Phylogenetic tree was constructed by the Neighbor-Joining (NJ) method using a total 21 16S rRNA sequences from five species of *Ectopleura* and *T. indivisia*. Among 21 sequences, two of *E. radiata* (GenBank accession No: OP879213) and *E. crocea* (OP872606) were obtained from this study. The rest were obtained from the GenBank. Accession numbers of sequences were given on the NJ tree.

Specimens were deposited in the National Marine Biodiversity Institute of Korea, Seocheon (MABIK CN00081226), and Cnidaria Bioresources Bank of Korea, Woosuk University, Jincheon (CBB22CnHyE002).

SYSTEMATIC ACCOUNTS

Phylum Cnidaria Hatschek, 1888
Class Hydrozoa Owen, 1843
Subclass Hydroidolina Collins, 2000
Order Anthoathecata Cornelius, 1992
Family Tubulariidae Goldfuss, 1818

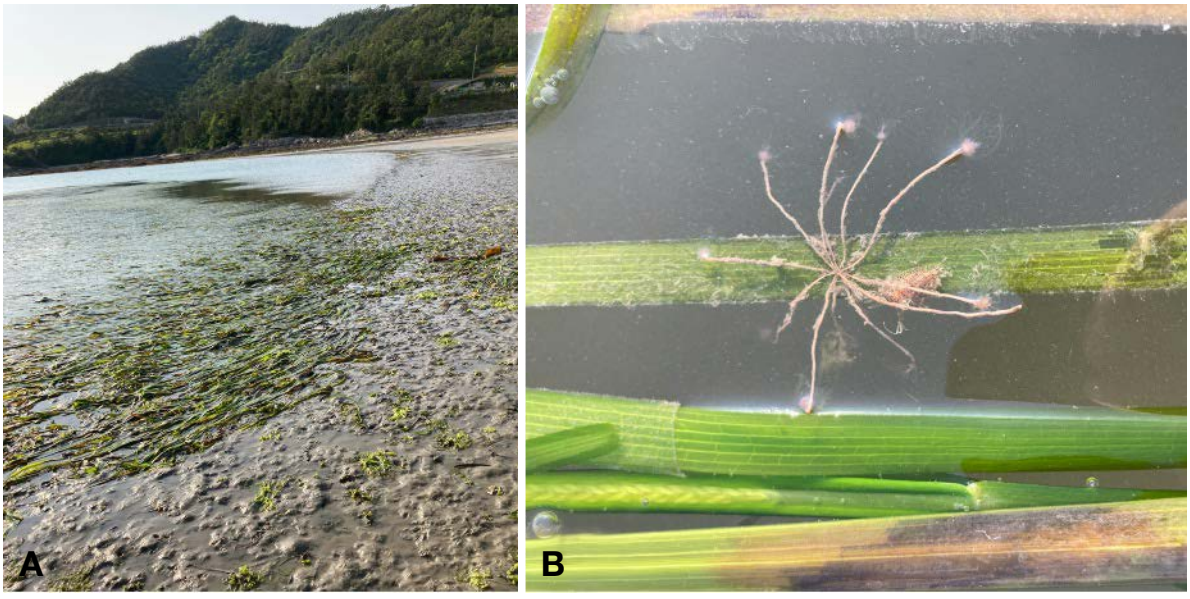

Fig. 1. Colonies of *Ectopleura radiata*. A, Seagrasses bed in intertidal zone of Geumgap Beach, Jindo; B, Radially branched living colony attached on *Zostera* sp.

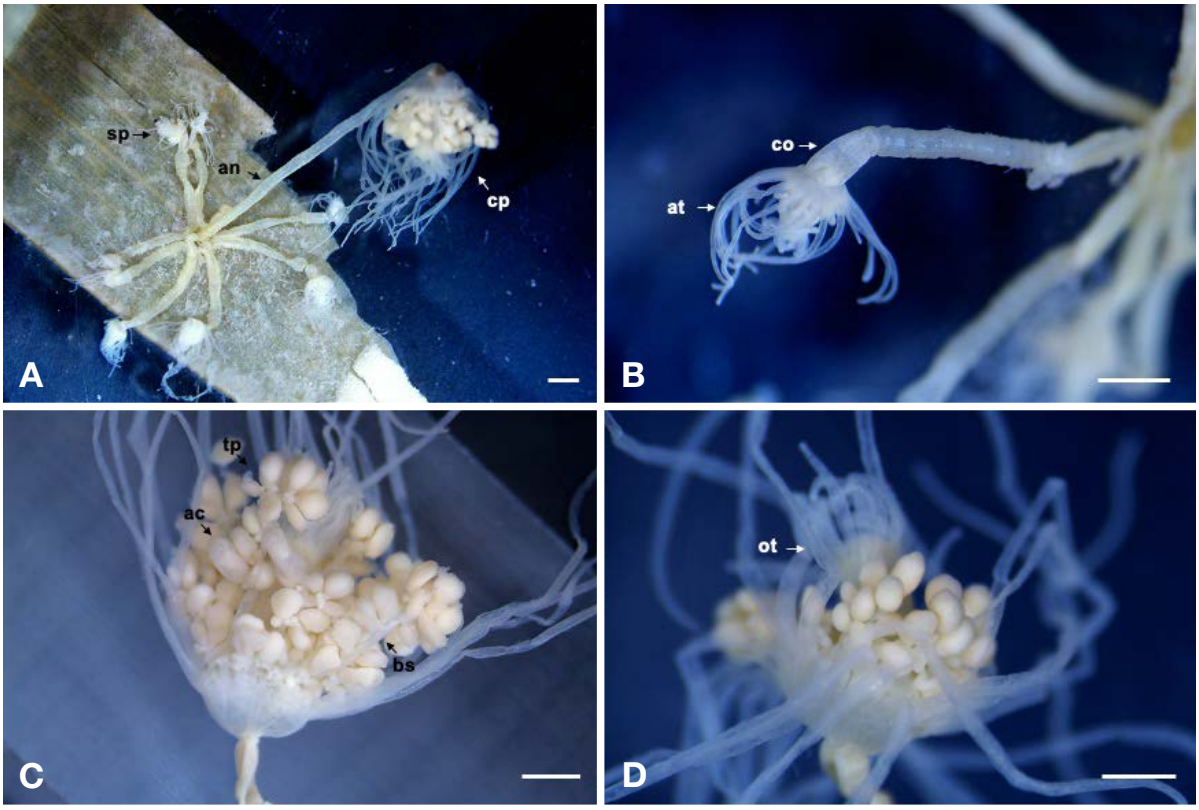

Fig. 2. Stereo microscope images of *Ectopleura radiata*. A, Preserved whole colony consisting of central and side polyps; B, Young polyp with annulated hydrocaulus and collar; C, Vasiform hydranth with blastostyles bearing matured female gonophores; D, Hydranth with blastostyles bearing matured male gonophores between two whorls of tentacles. ac, actinula; an, annulation; at, aboral tentacle; bs, blastostyle stalk; co, collar; cp, center polyp; ot, oral tentacle; sp, side polyp; tp, tentacle-like processes. Scale bars: A, C, D = 1 mm, B = 0.5 mm.

[1*]**Genus *Ectopleura* L. Agassiz, 1862**

Acharadria Wright, 1863: 378
Parypha Agassiz, 1860: 46.
Pinauay Marques and Migotto, 2001: 479–480.
Thamnocnidia Agassiz, 1860: 46.
Vorticlava Alder, 1856: 353.

Diagnosis. Colonial or solitary form, hydranth vasiform with two whorls of oral and aboral tentacles, stem (hydrocaulus) hollow and with thin periderm and longitudinal ridges, without longitudinal peripheral canals, gonophores arising on blastostyles.

[2*]***Ectopleura radiata* (Uchida, 1937) (Table 1, Figs. 1–3)**

Tubularia radiata Uchida, 1937: 20–21, figs. 1, 2; Yamada, 1959: 16; Hirohito, 1988: 18.

Material examined. Korea: 9 colonies, Jeollanam-do, Jindo-gun, Uisin-myeon, Geumgap-gil, Geumgap Beach (34°23′40″ N, 126°16′37″E), 16 May 2022, Hwang SJ, Seo SY, intertidal zone.

Description. Colony consist of 3–13 polyps. Polyps connected to each other by radiating hydrorhiza, oldest (usually longest) polyp located at center, new ones emerged from radially formed stolons from old one (Fig. 1B). Stem (hydrocaulus) unbranched, erect but slightly bent just below hydranth in old polyp, covered with periderm, annulated along a whole length, annulation especially prominent in young polyp, fully grown up to 45 mm in height, 0.30–0.51 mm in width, narrow towards base (Fig. 2A). Distal portion of stem making collar with longitudinal stripes on surface, 1.04 mm long (Fig. 2B). Hydranth vasiform with long tubular hypostome, with two whorls of tentacles in oral and aboral regions, approximately 5 mm and 1 mm high in old and young polyps, respectively (Fig. 2B–D). Oral tentacles filiform, short, tapered distally, about 18 in number, 1.19–1.86 mm long, 0.12–0.18 mm wide in large

Korean name: [1*]빈관히드라속 (신칭), [2*]방사관히드라 (신칭)

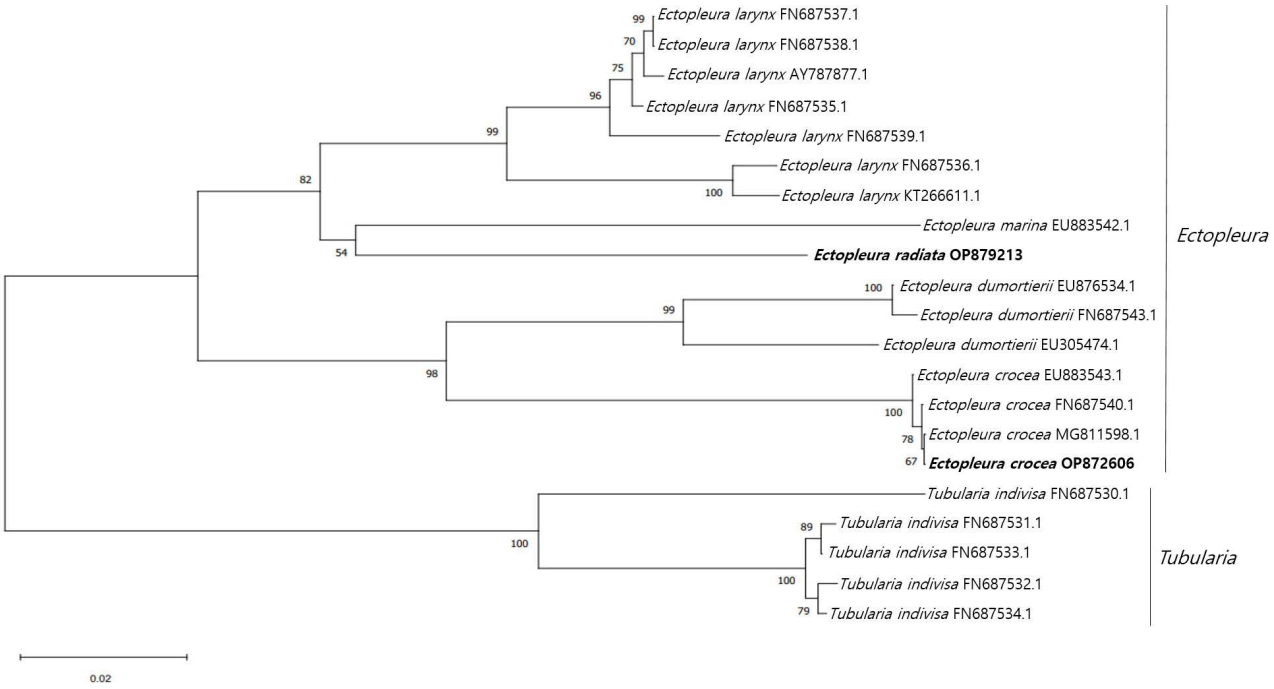

Fig. 3. Neighbor-Joining (NJ) tree constructed from the 16S rRNA sequences of *Ectopleura radiata* and related species. Numbers at the nodes indicate bootstrap values (1,000 replications). Newly sequenced in this study are in bold.

Table 1. Comparison of interspecific and intraspecific genetic distances of *Ectopleura radiata* and related species

Species	Within (mean±SD)	Between species of *Ectopleura* (Min.–Max.)	Between *T. indivisa* (mean±SD)
Ectopleura crocea	0.000±0.000	0.107–0.150	0.204±0.020
Ectopleura dumortierii	0.034±0.006	0.107–0.173	0.217±0.020
Ectopleura larynx	0.030±0.005	0.105–0.150	0.181±0.018
Ectopleura marina	n/c	0.118–0.173	0.217±0.021
Ectopleura radiata	n/c	0.105–0.146	0.202±0.020
Tubularia indivisa	0.035±0.005	0.181–0.217	n/c

n/c, no calculated.

polyps. Aboral tentacles filiform, long, slender, slightly tapered at tip, 28–30 in number, 6.10–12.50 mm long, 0.14–0.27 mm wide, distance between adjacent tentacles 22.03–27.26 μm at base in large polyps. Blastostyles between two tentacles whorls, arising just above aboral tentacles, with clusters of gonophores, raceme in form, 9–10 in number, stalk 2.69–2.93 mm long and 0.18–0.22 mm wide (Fig. 2C, D). Female gonophore oval shape, with short pedicel and tentacle-like processes at opening, developing actinulae observed in fully matured ones, 607.47–701.29×486.24–587.38 μm in diameter (Fig. 2C). Fully matured male gonophore elongated oval shape, connected to stalk with short pedicels, 455.73–580.94×325.86–360.29 μm in diameter (Fig. 2D).

Color. When alive, stems are light beige, hydranths (excluding tentacles) and upper stems are pink, and tentacles are transparent. All colors become opaque in alcohol.

Ecology and habitat. Colonies lives on the surface of seagrasses (*Zostera* sp.) in the intertidal zone composed of sand and mudflats (Fig. 1).

Distribution. Pacific Ocean: Korea (Jindo), Japan (Lake Akkeshi).

Remarks. This species was first described by Uchida (1937) in the Lake Akkeshi of Hokkaido, Japan. It has not been reported elsewhere since then. The Lake Akkeshi has been designated a Ramsar site covered with seagrasses bed (*Zostera marina*) (Yoon et al., 2011). It is famous for aquacul-

ture of oyster and clam. The habitat of our sample collection site, the Geumgap Beach, is very similar to the type locality. Our materials consisted of nine colonies. Each colony had the longest (oldest) and centered polyp with short and young side polyps. Old polyps contained well developed blastostyles with matured gonophores. Young side polyps also had developing blastostyles with immature gonophores. Although the number of polyps forming colony was rather small, these materials agreed with the original description, in particular the radiating hydrorhiza form, adherence to seagrasses, and shape of gonophore undoubtedly identify the species. According to Uchida's description, this species apparently resembles in *Tubularia indivisa* Linnaeus, 1758 in the appearance of hydranth, but differs in the shape of gonophores. Differences between similar species, including *T. indivisia*, were also evident in partial 16S rRNA sequences (Fig. 3). *Ectopleura radiata* was clearly distinct from the four species of *Ectopleura* and *T. indivisia* with interspecific distances of 10.5−14.6% and 20.2%, respectively. *T. indivisia* was outside the *Ectopleura* clade in the NJ tree, showing genetic distances of 18.1−21.7%. Detailed genetic distances within and between species are shown in Table 1.

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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