A New Species of *Antennella* (Hydrozoa: Leptothecata: Halopterididae) from Korea

Hyo-Jin Yu, Seung-Joon Lee, Sung-Jin Hwang*

Department of Life Science, Woosuk University, Jincheon 27841, Korea

ABSTRACT

A taxonomic examination of hydroids collected from the subtidal zones of Ulleungdo and Dokdo located in the East Sea of Korea from 2017 to 2023 was conducted. Through this study, *Antennella aurantia* n. sp., a new species belonging to the family Halopterididae, was newly added to the hydrozoan fauna of Korea. This new species is clearly distinguished from similar species by the different morphology and size of the hydrothecae, lateral nematothecae, and gonothecae. By comparing the 16S rRNA sequence with 7 recorded species within the *Antennella*, the interspecific genetic distance to the new species was 8.2–28.9%, which is very high compared to the intraspecific variation of 0.0%, showing that it was clearly distinguished from other species. As a result of this study, a total of 4 *Antennella* species are reported in the hydrozoan fauna of Korea.

Keywords: unbranched halopteridids, Antennella aurantia n. sp., Ulleungdo Island, Dokdo Island, taxonomy

INTRODUCTION

Family Halopterididae Millard, 1962 currently has of 16 genera worldwide (Schuchert, 2023), and 6 species of 3 genera (*Antennella* Allman, 1877; *Halopteris* Allman, 1877; *Monostaechas* Allman, 1877) have been reported in Korea to date (Park, 2010). The genus *Antennella* was established with type species *Antennella gracilis* Allman, 1877 from the Carysfort Reef, FL, USA (Allman, 1877). *Antennella* is easily distinguished from other halopteridids by its unbranched colonial form and stems arising from creeping stolons (Schuchert, 1997). *Antennella* is widely distributed in the Mediterranean, Atlantic, Indian and Pacific Oceans, mainly in water depths of less than 100 m. However, species, such as *A. sinuosa*, are found in deeper water of less than 500 m (Hirohito, 1995; Schuchert, 1997; Ansín Agís et al., 2009).

To date, 24 valid species have been accepted in the genus *Antennella* (Schuchert, 2023). In Korea, 4 species, *A. africana* Broch, 1914, *A. integerrima* (Jäderholm, 1896), *A. secundaria* (Gmelin, 1791), and *A. siliquosa* (Hincks, 1877), were reported from all sea areas (Rho, 1967; Rho and Chang, 1972; Rho and Park, 1980; Park, 2010). In particular, *A. secundaria* is a cosmopolitan species, preferring temperate and warm seas and inhabiting all the waters of Korea (Schuchert, 1997;

Park, 2010). Among these species, *A. africana* has been synonymized as *A. quadriaurita* Ritchie, 1909 (Millard, 1977), and *A. integerrima* was transferred to the genus *Antennellopsis* Jäderholm, 1896 based on nematothecae fused to hydrothecae (van der Land, 2008). Consequently, 3 valid species from the genus *Antennella* are currently reported in Korea.

In this study, taxonomic and molecular examinations were performed for unbranched halopteridids collected from Ulleungdo and Dokdo located in the East Sea of Korea. One new species, *Antennella aurantia* n. sp., was added, and through this study, a total of 4 species in *Antennella* are reported in the hydrozoan fauna of Korea.

MATERIALS AND METHODS

Specimens were collected from the subtidal zone of Ulleungdo and Dokdo in the East Sea at depths of 10 m to 28 m by scuba diving from 2017 to 2023. Images of living colonies attached to rocks and mussel shells were taken with a digital camera (Tough TG-5; Olympus Digital Solution Corporation, Tokyo, Japan) to record their morphological (color and shape of colonies) and ecological features before fixation. After collection, specimens were anesthetized with menthol powder for

***To whom correspondence should be addressed** Tel: 82-43-531-2892, Fax: 82-43-531-2862 E-mail: buteo2@woosuk.ac.kr, buteo2@gmail.com

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less than 6 h and then fixed with 99% alcohol (v/v) for further morphological examination and molecular experiments.

For identification, detailed morphological characteristics of colonies, stems, hydrothecae, nematothecae, and gonothecae were examined under a stereomicroscope (SteREO Discovery. V8; Carl Zeiss, Jena, Germany). All photographic images in detail were taken in detail using a CMOS sensor microscope digital cameras (KCS-2000SS; Korea Lab Tech, Seongnam, Korea). Measurements of the characteristics of each part were performed with an image analyzer (OptiView; Korea Lab Tech). After coating the air-dried specimens with gold, scanning electron microscopy (SEM) images of nematothecae and gonothecae were photographed with a Mini-SEM (SNE 3200M; SEC Co. Ltd., Suwon, Korea) at the Marine Bryozo-ans Resources Bank of Korea (MBRBK).

Molecular experiments were performed to compare genetic distances among Antennella species. Type specimens of the new species including the holotype and one A. secundaria specimen collected from Ulleungdo were used for analysis. After washing the specimens preserved in ethanol with distilled water, total DNA was extracted from each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer. The 16S rRNA sequences were partially amplified using FiMod primer pairs (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 performed on a T100 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) using TaKaRa Ex Taq (Takara Bio Inc., Kusatsu, Japan) for 50 µL reactions. The polymerase chain reaction conditions were an annealing temperature range of 40-55°C using thermal gradient functionas, and then were set to the follows: denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min; followed by a final extension step at 72°C for 10 min. Sequence editing and analysis were conducted using Geneious Prime software version 2023.1.1 (Biomatters, Auckland, New Zealand). Analysis of genetic distances and phylogeny was performed using a total of 20 16S rRNA sequences from 8 species of Antennella and 1 sequence from Dynamena crisioides as an outgroup. Molecular Evolutionary Genetics Analysis (MEGA) software version 11 was used to calculate intra- and interspecific genetic distances and construct a neighbor-joining (NJ) tree based on the Kimura 2-parameter (K2P) model (Tamura et al., 2021). Among 20 sequences used in the molecular analysis, 3 in A. aurantia n. sp. (OR944081, OR944082, OR944083) and 1 in A. secundaria (OR944084) were obtained through this study. The rest were obtained from the GenBank, and accession numOne holotype (MABIK CN00081413) and 1 paratype (MABIK CN00081412), were each deposited in the National Marine Biodiversity Institute of Korea, Seocheon and the remaining paratypes were stored separately in Cnidaria Bioresources Bank of Korea and Marine Biodiversity and Conservation Laboratory at Woosuk University, Jincheon (CBB18 CnHyE057, MBCL17CnHyE011, MBCL18CnHyE031).

SYSTEMATIC ACCOUNTS

Phylum Cnidaria Hatschek, 1888 Class Hydrozoa Owen, 1843 Subclass Hydroidolina Collins, 2000 Order Leptothecata Cornelius, 1992 Family Halopterididae Millard, 1962 Genus *Antennella* Allman, 1877

Key to species of the genus Antennella from Korea

1. Lateral nematothecae 2 pairs A. quadriaurite	a
- Lateral nematothecae 1 pair	2
2. Axillar nematothecae present A. secundaria	a
- Axillar nematothecae absent	3
3. Intersegment nematothecae 2 A. siliquose	a
- Intersegment nematotheca 1Antennella aurantia n. sp).

^{1*}Antennella aurantia n. sp. (Tables 1-3, Figs. 1-4)

ZooBank LSID: urn:lsid:zoobank.org:act:7B33F5ED-011A-4FA8-825D-78D5E30F144B.

Type locality. Korea: Gyeongsangbuk-do, Ulleung-gun, Ulleung-eup, Ssangjeongcho (37°33′26.54″N, 130°56′20.90″E), 24 m.

Material examined. Korea: Holotype (MABIK CN0008 1413), 1 colony, Gyeongsangbuk-do, Ulleung-gun, Ulleungeup, Ssangjeongcho (37°33'26.54"N, 130°56'20.90"E), 6 Nov 2017, Yang HJ, Jeong GJ, 24 m; Paratype (MBCL17CnHyE 011), 1 colony, Gyeongsangbuk-do, Ulleung-gun, Ulleungeup, Ssangjeongcho (37°33'26.54"N, 130°56'20.90"E), 6 Nov 2017, Yang HJ, Jeong GJ, 24 m; Paratypes, 2 colonies (MBCL 18CnHyE031, CBB18CnHyE057), Gyeongsangbuk-do, Ulleung-gun, Ulleung-eup, Dokdo-ri, Gajicho (37°14'39.99"N, 131°52'3.08"E), 21 Oct 2018, Hwang SJ, Kim BI, Park SH, 28 m; Paratype, 1 colony (MABIK CN00081412), Gyeong-sangbuk-do, Ulleung-gun, Ulleung-gun, Ulleung-eup, Jukdo straight wall, (37°31'54"N 130°56'10"E), 31 Aug 2023, Hwang SJ, Lee SJ, Yu HJ, Bae JK, 10–15 m. All specimens were collected by SCUBA diving.

ber of each sequence was given in the NJ tree (Fig. 4).

Korean name: ^{1*}주황깃히드라 (신칭)

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Fig. 1. Antennella aurantia n. sp., holotype, MABIK CN00081413: A, Living bright orange-colored colony attaching on barnacles; B, Faded specimen fixed in alcohol; C, Stems arising directly from creeping stolons; D, Male gonotheca arising just below hydrotheca; E, Main segment and intersegment in lateral view; F, Main segment and intersegment in frontal view. Scale bars: A, B=1 cm, C=5 mm, D-F=0.5 mm.

Description. Colonies composed of unbranched stems rising directly from ramified creeping stolons (Figs. 1, 2). Stems usually 6-13 mm in height, $84-108 \mu \text{m}$ in diameter, containing up to 19 hydrothecae. Stems somewhat curved at distal parts, divided into several (2–5) trasverse nodes at basal parts,

with one nematotheca above them, segmented alternately by oblique and transverse nodes at remaining parts. Transverse nodes sometimes indistinct, incomplete, absent (Fig. 3A). Main segments with a hydrotheca, three nematothecae: one median inferior, one pair of laterals, axillar nematothe-



Fig. 2. Antennella aurantia n. sp., paratype, MBCL17CnHyE011: A, Faded specimen fixed in alcohol; B, Stem in lateral view; paratype, MBCL18CnHyE031; C, Faded specimen fixed in alcohol; D, Stem with male gonotheca in lateral view; paratype, CBB18Cn-HyE057; E, Dyed specimen attached to seaweed; F, Stem with female gonothecae in lateral view; paratype, MABIK CN00081412; G, Faded specimen fixed in alcohol; H, Stem in lateral view. Scale bars: A, C, E, G=1 mm, B, D, F, H=0.5 mm.

ca absent, 372-478 µm in length (Figs. 1D, F, 2B, H). Intersegments with only one median nematotheca, 230-445 µm in length (Figs. 1E, 2F, 3A). Hydrothecae cylindrical, deep, almost straigt or slightly curved outward at rim, lowered laterally, at an angle of 30-54° to stem (Fig. 1E). Adcauline wall adnate to stem about half of total length, extends beyond transverse node, sometimes reaches basal part of intersegments nematotheca above. Nematothecae two chambered. Median inferior nematotheca usually dose not reach hydrotheca; adaxial wall of upper chamber emarginated widely and deeply, appears almost absent; looks like scoop (Fig. 3A, B). Lateral nematothecae on short apophyses; inner wall inrolled, with wide and deep emargination along median (Fig. 3C). Intersegments nematotheca similar to median inferior nematotheca, looks like scoop (Fig. 3B). Gonothecae rise singly next to median inferior nematotheca just below hydrothecae, and somewhat curved inward (Figs. 2D, F, 3A). Female and male gonothecae located on seperated stems (Figs. 1C, 2E). Female gonothecae larger than male ones. Female gonothecae narrow basally, broad and rounded distally, with somewhat flat capped opening at end, with two nematothace inside at base, and with two-segmented pedicel, 510-617 µm long, 437-486 µm

in width from frontal view (Figs. 2F, 3D). Male gonotheca oblong with round distal, with narrow opening without operculum at end, with one nematotheca inside at base, and with two-segmented pedicel, $391-444 \mu m \log 246-314 \mu m in$ width (Figs. 1D, 2D).

Comparison of detailed measurements between type specimens in Table 1.

Color. While alive, entire colonies are bright orange (Fig. 1A), but when preserved in alcohol, the colors fade and turn white (Fig. 1B). Sometimes, colonies attached to seaweed may be dyed by seaweed pigment when preserved in alcohol (Fig. 2E).

Ecology and habitat. Hydra colonies connected by creeping stolons attach to shells of barnacles and mussels, and the surface of seaweed leaves within 30 m of subtidal waters.

Distribution. Korea (Ulleungdo, Dokdo).

Etymology. The specific name, *aurantia*, is derived from the Latin word meaning orange-colored, referring to the vivid orange color of the living colonies.

DNA barcoding. An analysis of the inter- and intra-specific genetic distances based on the partial sequences of 16S rRNA of 8 *Antennella* species, including a new species, and 1 out-

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Fig. 3. Antennella aurantia n. sp., paratype, MBCL17CnHyE011: A, Stem with three main segments containing hydrothecae, median inferior and lateral nematothecae, and two intersegment with one nematotheca, female gonotheca rising just below middle hydro-theca; B, Scoop-shaped median inferior and intersegment nematothecae seen frontal view; Antennella aurantia n. sp., holotype, MABIK CN00081413; C, Lateral nematotheca with wide and deep emargination on inner side; D, Capped female gonothecae with nematotheca (arrows) inside base. ne, nematotheca; tn, transverse node. Scale bars: A, B, D=100 µm, C=50 µm.

group, was conducted. Intraspecific genetic distances were very low at 0.000, except for *A. secundaria* and species for which only one sequence was available in GenBank (*A. kiwiana*, *A. siliquosa*, *A. varians*) (Table 3). In contrast, the interspecific genetic distances among species were very high, ranging from 8.2% to 30.4%, showing the high resolution of DNA barcoding using 16S rRNA, similar to previous molecular phylogenetic studies (Moura et al., 2011; Zheng et al., 2014; Maronna et al., 2016). In particular, this new species appears to be more closely related to the morphologically similar species *A. ansini* and *A. kiwiana* than to other species (Fig. 4), but was clearly distinct from them, with average genetic distances of 8.2% and 19.7%, respectively. *A. secundaria*, which is a cosmopolitan species that has also been reported in Korea, showed relatively high intraspecific variation compared to other species, with intraspecific genetic variations of 0.7% to 13.9% (average 9.4%) and interspecific variations of 23.4% to 28.2%. In particular, the differences in genetic variations between the Korean samples and GenBank (overseas samples) were 9.5–13.9%, showing large differences among populations, suggesting the necessity of a taxonomic review of the species.

Remarks. Antennella is characterized by unbranched stems directly arising from the creeping stolons of the hydrorhiza. Hydrothecae are cup-shaped without marginal teeth and lids, and lack fused lateral nematothecae. Characteristics that dis-

Table 1. Comparison of detailed measurements of axillary and stem, hydrothecae, nematothecae and gonothecae in type specimens of *Antennella aurantia* n. sp.

		<i>A. aurantia</i> n. sp.				
Characters	Holotype	Paratypes				
	MABIK CN00081413	MBCL17CnHyE011	MBCL18CnHyE031	CBB18CnHyE057	MABIK CN00081412	
Stem						
Height (mm)	9.8-12.8	6.4-7.2	3.03-7.81	7.7-10.2	9.1-10.1	
Main segment length	381.3-455.5	371.5-441.9	387.5-478.0	381.9-446.4	405.5-456.8	
Intersegment length	249.5-353.1	269.5-359.7	229.7-397.6	294.8-344.7	277.4-444.7	
No of hydrothecae per stem	13-19	8-12	7-10	12-16	13-17	
Diameter	91.0-108.5	85.9-95.6	84.4-89.8	85.6-101.8	99.5-108.2	
Hydrothecae						
Height	284.5-332.4	284.8-324.3	286.5-313.0	290.2-328.0	263.1-280.4	
Diameter	200.1-219.6	202.3-229.0	193.1-213.9	194.1-220.9	185.9-220.5	
Height : diameter	1.55:1	1.35:1	1.52:1	1.53:1	1.41:1	
Free adcauline wall length	159.3-175.4	162.1-170.1	157.0-170.9	144.1-172.0	134.0-145.1	
Abcauline wall length	247.8-304.0	273.9-304.6	268.6-302.5	253.6-280.8	232.7-259.9	
Angle (°) to stem	30.4-37.9	31.0-42.2	37.2-44.9	45.0-51.8	45.6-54.3	
Nematothecae						
Laterals length	49.9-60.8	54.9-59.9	50.6-63.7	48.2-56.8	60.7-70.2	
Gonotheca						
Female						
Length without pedicel	599.0-616.8	510.3-526.8	-	558.2-571.2	-	
Width	450.9-486.4	441.2-471.4	-	437.1-458.0	-	
Male						
Length without pedicel	436.5-443.8	-	391.4-412.5	-	-	
Width	307.9-314.3	-	245.7-268.2	-	-	
Color	Orange	Orange	Bright orange	Bright orange	Yellowish orange	

Size in µm.

tinguish species within Antennella include the morphological characteristics, number, and size of hydrothecae, nematothecae, gonothecae. Among 24 valid species, 5 species [Antennella ansini Peña Cantero and García Carrascosa, 2002, A. campanulaformis (Mulder & Trebilcock, 1909), A. kiwiana Schuchert, 1997, A. sinuosa Ansín Agís, Vervoort & Ramil, 2009, and A. tubulosa (Bale, 1894)] are similar to Antennella aurantia n. sp. in that they have 1 pair of lateral nematothecae, 1 median inferior nematotheca, and 1 intersegment nematotheca. However, Antennella aurantia n. sp. has characteristics that clearly distinguish it from the similar species.

First, *A. ansini* shows morphological differences from the new species in that the intersegments are longer than the main segments and the height and length of the hydrothecae are somewhat shorter (Peña Cantero and García Carrascosa, 2002). Second, *A. campanulaformis* differs from the new species in that the abcauline walls of the hydrothecae are shorter, and it has spanner-shaped lateral nematothecae and globular

female gonothecae (Schuchert, 1997). Third, *A. kiwiana* differs from the new species in that the lateral nematothecae are long and spanner-shaped, and the female gonothecae are approximately twice as long (Schuchert, 1997) (Table 2). Fourth, *A. sinuosa* is different in that the abcauline wall of the hydrotheca is sinuous, and the lateral nematothecae are very long and reach the hydrothecae (Ansín Agís et al., 2009). Lastly, *A. tubulosa* is distinguished from this new species by having an elongated tubular shape with a 2:1 ratio of hydrothecae height to diameter and spanner-shaped lateral nematothecae (Bale, 1894; Schuchert, 1997) (Table 2). In addition, genetic distances and NJ tree based on 16S rRNA sequences showed clear differences between *A. aurantia* and similar species. Thus, based on this information, the materials collected from Ulleungdo and Dokdo are described here as a new species.

Comparison of detailed measurements and characteristics between *A. aurantia* n. sp. and similar species in Table 2.

						:
	A. ansını	A. campanulatormis	A. Kiwiana	A. sinuosa	A. tubulosa	<i>A. aurantıa</i> n. sp.
Characters	Peña Cantero & García Carrascosa, 2002 (Original description)	Schuchert, 1997 (Lectotype)	Schuchert, 1997 (Original description)	Ansín Agís et al., 2009 (Original description)	Schuchert, 1997 (Lectotype)	This study
Stem						
Height (mm)	I	8	15	17	8	3-13
Main segment lenght	310-320	330-450	310-390	610-700	320-420	372-478
Intersegment lenght	381-600	290-450	350-400	460-650	210-410	230-445
Hydrothecae per stem	I	18-22	15	I	12	7-19
Diameter	I	I	100-140	90-125	I	84-108
Hydrothecae						
Height	172-216	I	313-344	I	I	263-332
Diameter	192-208	210-280	170-200	270-290	160-170	186-229
Height : diameter	I	I	1.46:1		1.89:1	1.47:1
Free adcauline wall length	112-128	90-200	130-150	130-140	140-220	134-175
Abcauline wall length	164-216	150-250	270-290	450-480	220-350	233-305
Angle (°) to stem	I	I	30-40	I	50-60	30-54
Nematothecae						
Laterals lenght	48-60	I	60-70	150-160	I	48-70
Gonotheca						
Female						
Length	356-616	I	1,120-1,200	I	550	510-617
Width	360-448	600-700	I	I	,	437-486
Male						
Length	I		460-500	I	500	391-444
Width	160-258	300	I	I		246-314
Color	I	I	Transparent	I	Transparent or yellow	Orange
Type locality	Spain, Mediterranean sea	Australia, Indian ocean	New Zealand, Indian ocean	Australia, Indian ocean	Australia, Indian ocean	Korea, Pacific Ocean
Size in µm.						

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Table 3. Genetic distances within and between species of the genus Antennella based on partial 16S rRNA in 603	op le	ngt
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Species (number of sequences)	Within (mean±SD)	Among species of <i>Antennella</i> (minmax.)	Between <i>Antennella aurantia</i> n. sp. (mean±SD)
Antennella ansini (3)	0.000 ± 0.000	0.082-0.296	0.082±0.012
Antennella aurantia n. sp. (3)	0.000 ± 0.000	0.082-0.289	_
Antennella confusa (3)	0.000 ± 0.000	0.251-0.304	0.287 ± 0.025
Antennella kiwiana (1)	n/c	0.082-0.269	0.197 ± 0.020
Antennella secundaria (4)	0.094 ± 0.010	0.234-0.282	0.281 ± 0.023
Antennella siliquosa (1)	n/c	0.080-0.277	0.202 ± 0.023
Antennella similis (3)	0.000 ± 0.000	0.233-0.304	0.289 ± 0.026
Antennella varians (1)	n/c	0.209-0.293	0.237 ± 0.023

n/c, no calculated; SD, standard deivation.



Fig. 4. Neighbor-Joining (NJ) tree constructed from the 16S rRNA sequences of *Antennella* species including *Dynamena crisioides* as an outgroup. Numbers at the nodes indicate bootstrap values (1,000 replications). Newly sequenced in this study are in bold and GenBank accession numbers indicated next to scientific names.

ORCID

Hyo-Jin Yu: https://orcid.org/0000-0002-4143-1101 Seung-Joon Lee: https://orcid.org/0000-0003-4880-1547 Sung-Jin Hwang: https://orcid.org/0000-0002-1259-6775

CONFLICTS OF INTEREST

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

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