First record of non-indigenous colonial ascidian in the Korean coasts, confirmed by DNA barcoding

Taekjun Lee^{p1}, Michael Dadole Ubagan^{1, 2}, Seung Bin Park², Bal Dev Bhattrai¹, Sook Shin^{c1, 2}

¹Marine Biological Resource Institute, Sahmyook University, Seoul, Korea

²Department of Animal Biotechnology and Resource, Sahmyook University, Seoul, Korea

Corresponding author's e-mail: shins@syu.ac.kr

Introduction

- : Global invasive colonial ascidian, Botrylloides
- *Botrylloides* Milne Edwards, 1841 is a genus of popular colonial ascidians in the world and currently comprises 19 species (Shenkar et al., 2020).
- Among them, *B. violaceus* have been reported as a non-indigenous species (NIS) in western North America and Europe (Gittenberger and Moons, 2011; Simkanin et al., 2013; Viard et al., 2019).
- The identification of ascidians (especially colonial ascidians) based on morphological taxonomy is a difficult task owing to many limitations and this has led to misidentification of the species (Lambert 2009; Geller et al. 2010).
- The efficiency of DNA barcoding was confirmed for the identification of several species and resolved the limitations of identification through conventional taxonomy (Akram et al., 2017).

Materials & Methods

- Samples collecting
 - The colonial ascidians collected from artificial materials plate in 14 harbors of South Korea
 - > Incheon, Dangjin, Gunsan, Mokpo, Wando, Yeosu, Gwangyang, Tongyeong, Busan, Ulsan, Yangpo, Jukbyeon, Donghae and Sokcho
- DNA extraction and amplification
 - DNA extraction
 - o extracted from a single zooid in a colony
 - o following the DNeasy kit protocol (Qiagen, Hilden, Germany)
 - COX1 amplification
 - COX1 were amplified using primer pairs
 - ✓ LCO1490-HCO2198 (Folmer et al., 1994), dinF-Nux1R (Brunetti et al., 2017)
- Phylogenetic analysis
 - Calculation of pairwise distance (p-distance)
 - o Kimura 2-parameter model with 1000 bootstrapping replicates
 - Phylogenetic tree construction
 - Neighbor-joining method with 1000 bootstrapping replicates

: Morphological variations of *B. diegensis* in Korea

- Botryllus schlosseri and Botrylloides violaceus are common colonial ascidians in northwest Pacific region, but these species were known as non-indigenous species from North American Pacific coast (Cohen, 2011).
- Botrylloides violaceus in Korea has various color, but shown not two colored colony (Fig. 1).
- Botrylloides diegensis in Korea has two color types of colony: one colored (Fig. 1D-F) and two colored colony (Fig. 1A-C).
- Thus, one colored *B. diegenesis* is confused with *B. violaceus* in the field survey.



Figure 1. Morphological various features of *B. diegensis* and *B. violaceus* in Korea. A-C, two colored *B. diegensis*; D-F, one colored *B. diegensis*; G-I, B. violaceus.

Results : Distribution of *B. diegensis* in South Korea

- Installed artificial attachment monitoring plates (AMP) every 3 month from Nov. 2019.
- Our team surveyed 14 harbors in Korean coasts every 3 month and specimens collected at May 2020.
- We taken photographs all collected colonies on AMP in living condition.
- Among them, *Botrylloides diegenesis* is distributed in nine harbors along all coast line (Fig. 2).



Figure 2. Distribution and species composition of botryllids in South Korea. Bs, *Botryllus schlosseri* ; Bd, *Botrylloides diegensis*; Bv, *Botrylloides violaceus.* 5

: DNA Barcoding data of *B. diagensis* from Korea, based on *COX1*

• Haplotype and sequence count of *Botrylloides diagensis* in this study

Location	Region	H1	H2	H3	H4	H5	H6	H7
Incheon	Yellow Sea	2						
Gunsan	Yellow Sea				2	2		
Yeosu	Korea Strait				6			
Tongyeong	Korea Strait							2
Ulsan	East Sea				2		3	
Yangpo	East Sea		2	3			2	
Donghae	East Sea				2		2	
Sokcho	East Sea						2	

: DNA barcode data comparison of *B. diegensis* from Korea with other *Botrylloides*

• Intraspecific p-distance between Korean and other *B.diegensis* from GenBank

	Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1	BD-H1_IC3-1																																						
2	BD-H1_IC3-2	0.00%																																					
3	BD-H2_YP5-1	0.72%	0.72%																																				
4	BD-H2_YP5-2	0.72%	0.72%	0.00%																																			
5	BD-H3_YP4-1	0.24%	0.24%	0.48%	0.48%																																		
6	BD-H3_YP4-2	0.24%	0.24%	0.48%	0.48%	0.00%																																	
7	BD-H3_YP8-2	0.24%	0.24%	0.48%	0.48%	0.00%	0.00%																																
8	BD-H4_DH1-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%																															
9	BD-H4_DH1-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%																														
10	BD-H4_GS12-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%																													
11	BD-H4_GS12-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%																												
12	BD-H4_US1-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%																											
13	BD-H4_US1-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%																										
14	BD-H4_YS2-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																									
15	BD-H4_YS2-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																								
16	BD-H4_YS7-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																							
17	BD-H4_YS7-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																						
18	BD-H4_YS8-1	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																					
19	BD-H4_YS8-2	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%																				
20	BD-H5_GS8-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%																			
21	BD-H5_GS8-2	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%																		
22	BD-H6_DH3-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%																	
23	BD-H6_DH3-2	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%																
24	BD-H6_SC1-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%															
25	BD-H6_SC1-2	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%														
26	BD-H6_US2-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%													
27	BD-H6_US2-2	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%												
28	BD-H6_US5-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%											
29	BD-H6_YP6-1	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%										
30	BD-H6_YP6-2	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%									
31	BD-H7_TY4-1	0.72%	0.72%	0.96%	0.96%	0.48%	0.48%	0.48%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%								
32	BD-H7_TY4-2	0.72%	0.72%	0.96%	0.96%	0.48%	0.48%	0.48%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	1.20%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.00%							
33	MK978806	0.72%	0.72%	0.00%	0.00%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.96%	0.96%						
34	MK978807	0.96%	0.96%	0.24%	0.24%	0.72%	0.72%	0.72%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.48%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	0.24%	1.20%	1.20%	0.24%					
35	MK978808	0.00%	0.00%	0.72%	0.72%	0.24%	0.24%	0.24%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.96%				
36	MK978809	0.24%	0.24%	0.96%	0.96%	0.48%	0.48%	0.48%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	1.21%	0.24%			
37	MK978810	0.24%	0.24%	0.96%	0.96%	0.48%	0.48%	0.48%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	1.21%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.48%	0.48%	0.96%	1.21%	0.24%	0.48%		
38	MK978811	0.48%	0.48%	0.72%	0.72%	0.24%	0.24%	0.24%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.96%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.72%	0.24%	0.24%	0.72%	0.96%	0.48%	0.72%	0.24%	

- within Korean specimens: Ave.=0.37%, Range=0.00-1.20%
- between Korean GenBank: Ave.=0.67%, Range=0.00-1.21%

: DNA barcode data comparison of *B. diegensis* from Korea with other *Botrylloides*

- Mean of Interspecific p-distance between *B. diegensis* and other *Botrylloides* from GenBank
 - ✓ B. diagensis other species: Ave.=22.07%
 - ✓ B. diagensis other Botrylloides: Ave.=19.61%
 - ✓ B.diagensis B.fuscus: Ave.=20.25%
 - ✓ B. diagensis B. giganteus: Ave.=19.48%
 - ✓ B. diagensis B. leachii: Ave.=18.16%
 - ✓ B. diagensis B. nigrum: Ave.=17.15%
 - ✓ B. diagensis B. perspicuus: Ave.=15.32%
 - ✓ B. diagensis B. simodensis: Ave.=16.74%
 - ✓ B. diagensis B. violaceus: Ave.=23.24%
 - ✓ B. diagensis Botrylloides sp.: Ave=20.31%
 - ✓ Botrylloides-Botryllus schlosseri: Ave.=21.07%
- Detailed all estimated values were presented next page

: Interspecific p-distance between *B. diegensis* and other *Botrylloides* from GenBank

No. Species Co	ode	1	2 3	4	5	6	7	B 9	10	11 1	2 13	14	15 16	17	18 19	20	21	22 23	24	25	26 27	28	29 30	31	32 3	33 34	35 3	6 37	38 3	89 40	41 42	43	44 4	5 46	47 48	49	50 51	52	53 54	55	56 57	58 59	60	61 62	63 64	4 65
1 B. diegensis BD-H1_N	IC3-1																																													
2 BD-H1_N	IC3-2	0.00%	77%																																											
4 BD-HZ Y	YP5-2	0.72%	0.72% 0	.00%																																										
5 BD-H3_Y	YP4-1	0.24%	0.24% 0	48% 0.4	8%																																									
6 BD-H3_Y	YP4-2	0.24%	0.24% 0	.48% 0.	8% 0.00	6																																								
7 BD-H3_Y	YP8-2	0.24%	0.24% 0	.48% 0.4	8% 0.00	6 0.00%																																								
8 BD-H4_D	DH1-1	0.96%	0.96% 0	.24% 0.3	4% 0.72	6 0.72%	0.72%																																							
9 BD-H4_D	DH1-2 G\$12-1	0.96%	0.96% 0	24% 0.	4% 0.72	6 0.72% 6 0.72%	0.72%	0.00%	a:																																					
10 BD-H4_C	G512-1 G512-2	0.96%	0.96% 0	.24% 0.	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%																																					
12 BD-H4_U	US1-1	0.96%	0.96% 0	24% 0.	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00%																																				
13 BD-H4_U	US1-2	0.96%	0.96% 0	.24% 0.3	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	.00%																																			
14 BD-H4_Y	Y52-1	0.96%	0.96% 0	.24% 0.:	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	.00% 0.00%																																			
15 BD-H4_Y	Y52-2	0.96%	0.96% 0	.24% 0.3	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	0.00% 0.00%	0.00%																																		
16 BD-H4_Y	YS7-1 YS7-2	0.96%	0.96% 0	24% 0.	4% 0.72	6 0.72% 6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	0.00% 0.00%	0.00%	0.00% 0.00%	*																																
19 BD-H4_Y	YS8-1	0.96%	0.96% 0	.24% 0.	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	.00% 0.00%	0.00%	0.00% 0.00%	× 0.00%																																
19 BD-H4_Y	Y58-2	0.96%	0.96% 0	24% 0.	4% 0.72	6 0.72%	0.72%	0.00% 0.00	% 0.00%	0.00% 0	.00% 0.00%	0.00%	0.00% 0.009	% 0.00%	0.00%																															
20 BD-H5_G	G58-1	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	.24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4%																														
21 BD-H5_G	G58-2	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	.24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%																														
22 BD-H6_D	DH3-1	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%	0.00%																													
23 BD-H6_L	DH3-2	0.72%	1.72% 0	00% 0.	0% 0.48	6 0.48%	0.48%	0.24% 0.24 0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.24%	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	10%																											
25 BD-H6 S	SC1-2	0.72%	0.72% 0	.00% 0.	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.24%	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0% 0.00%																											
26 BD-H6_U	U52-1	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	.24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0% 0.00%	0.00%																										
27 BD-H6_U	U52-2	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	.24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0% 0.00%	0.00%	0.00%																									
28 BD-H6_U	US5-1	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0.00%	0.00%	0.00% 0.00	2%																								
29 BD-H6_Y	YP6-1	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.249	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0% 0.00%	0.00%	0.00% 0.00	0.00%																								
30 BD-H6_Y	YP6-2	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	20% 1.20%	0.24%	0.24% 0.24%	% 0.24% % 1.20%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0% 0.00%	0.00%	0.00% 0.00	0.00%	0.00%	96%																						
32 BD-H7 T	TY4-2	0.72%	0.72% 0	.96% 0.1	6% 0.48	6 0.48%	0.48%	1.20% 1.20	% 1.20%	1.20% 1	20% 1.20%	1.20%	1.20% 1.209	6 1.20%	1.20% 1.20	0% 0.96%	0.96%	0.96% 0.9	6% 0.96%	0.96%	0.96% 0.94	5% 0.96%	0.96% 0.	96% 0.00%																						
33 MK9788	306	0.72%	0.72% 0	.00% 0.1	0% 0.48	6 0.48%	0.48%	0.24% 0.24	% 0.24%	0.24% 0	24% 0.24%	0.24%	0.24% 0.24%	% 0.24%	0.24% 0.24	4% 0.00%	0.00%	0.00% 0.0	0.00%	0.00%	0.00% 0.00	0.00%	0.00% 0.	.00% 0.96%	0.96%																					
34 MK9788	807	0.96%	0.96% 0	.24% 0.3	4% 0.72	6 0.72%	0.72%	0.48% 0.48	% 0.48%	0.48% 0	.48% 0.48%	0.48%	0.48% 0.489	% 0.48%	0.48% 0.48	8% 0.24%	0.24%	0.24% 0.2	4% 0.24%	0.24%	0.24% 0.24	1% 0.24%	0.24% 0.	24% 1.20%	1.20%	0.24%																				
35 MK9788	808	0.00%	0.00% 0	.72% 0.1	2% 0.24	6 0.24%	0.24%	0.96% 0.96	% 0.96%	0.96% 0	.96% 0.96%	0.96%	0.96% 0.969	% 0.96%	0.96% 0.96	6% 0.72%	0.72%	0.72% 0.7	2% 0.72%	0.72%	0.72% 0.72	2% 0.72%	0.72% 0.	.72% 0.72%	0.72% 0	0.72% 0.965	К																			
36 MK9788	309	0.24%	0.24% 0	.96% 0.	6% 0.48	6 0.48%	0.48%	1.21% 1.21	% 1.21%	1.21% 1	21% 1.21%	1.21%	1.21% 1.219	% 1.21%	1.21% 1.21	1% 0.96%	0.96%	0.96% 0.9	6% 0.96%	0.96%	0.96% 0.98	5% 0.96%	0.96% 0.	96% 0.96%	0.96% 0	0.96% 1.215	% 0.24%																			
37 MK9788	811	0.48%	148% 0	72% 0	2% 0.48	6 0.48%	0.48%	1.21% 1.21	% 0.96%	0.96% 0	95% 0.95%	0.96%	0.96% 0.96%	% 0.96%	0.96% 0.96	5% 0.72%	0.96%	0.56% 0.5	2% 0.72%	0.98%	0.72% 0.7	2% 0.72%	0.72% 0	72% 0.48%	0.48%	0.50% 1.213	6 0.48% C	7.46% 1.72% 0.24%																		
39 B. fuscus L599254	45	19.79% 1	9.79% 20	43% 20.	3% 20.12	6 20.12%	20.12% 2	0.10% 20.10	% 20.10%	20.10% 20	10% 20.10%	20.10% 2	0.10% 20.109	% 20.10%	20.10% 20.10	0% 20.43%	20.43%	20.43% 20.4	3% 20.43%	20.43%	20.43% 20.43	8% 20.43%	20.43% 20.	43% 20.76%	20.76% 20	0.43% 20.765	6 19.79% 19	9.79% 20.12%	6 20.46%																	
40 B. giganteus LS99254	47	19.25% 1	9.25% 19	.61% 19.	1% 19.58	6 19.58%	19.58% 1	9.28% 19.28	% 19.28%	19.28% 19	.28% 19.28%	19.28% 1	9.28% 19.289	% 19.28%	19.28% 19.28	8% 19.61%	19.61%	19.61% 19.6	19.61%	19.61%	19.61% 19.63	1% 19.61%	19.61% 19.	61% 19.61%	19.61% 19	9.61% 19.95%	6 19.25% 19	9.58% 19.58%	6 19.91% 23	3.13%																
41 B. leachii MK9788	312	17.95% 1	.95% 18	24% 18.	4% 18.27	6 18.27%	18.27% 1	7.92% 17.92	% 17.92%	17.92% 17	.92% 17.92%	17.92% 1	7.92% 17.929	% 17.92%	17.92% 17.92	2% 18.24%	18.24%	18.24% 18.2	4% 18.24%	18.24%	18.24% 18.24	18.24%	18.24% 18	24% 18.62%	18.62% 11	8.24% 18.573	6 17.95% 18	3.27% 18.27%	6 18.59% 20	0.59% 17.68%																
42 MK9788	313	17.95% 1	.95% 18	.24% 18.	4% 18.27	6 18.27%	18.27% 1	7.92% 17.92	% 17.92%	17.92% 17	.92% 17.92%	17.92% 1	7.92% 17.929	% 17.92%	17.92% 17.92	2% 18.24%	18.24% 1	18.24% 18.2	4% 18.24%	18.24%	18.24% 18.24	18.24%	18.24% 18	24% 18.62%	18.62% 11	8.24% 18.573	6 17.95% 18	3.27% 18.27%	6 18.59% 20	0.25% 17.68%	0.24%															
43 MK9788	814	18.27% 1	3.27% 18 7.21% 17	57% 18. 60% 17.	7% 18.59	6 18.59% 6 17.62%	18.59% 1	8.24% 18.24	% 18.24%	18.24% 18	24% 18.24%	18.24% 1	8.24% 18.249	% 18.24% × 17.28%	18.24% 18.24	4% 18.57%	18.57%	18.57% 18.5	7% 18.57%	18.57%	18.57% 18.57	7% 18.57%	18.57% 18	57% 18.95%	18.95% 11	8.57% 18.899	6 18.27% 18 6 17.21% 17	3.59% 18.59%	6 18.92% 15 6 17.95% 16	9.91% 18.00%	0.48% 0.	24%														
45 MK9788	316	18.27% 1	3.27% 18	.57% 18.	7% 18.59	6 18.59%	18.59% 1	8.24% 18.24	% 18.24%	18.24% 18	24% 18.24%	18.24% 1	8.24% 18.249	% 18.24%	18.24% 18.24	4% 18.57%	18.57%	18.57% 18.5	7% 18.57%	18.57%	18.57% 18.57	7% 18.57%	18.57% 18	57% 18.95%	18.95% 11	8.57% 18.89	6 18.27% 18	8.59% 18.59%	6 18.92% 15	9.91% 17.36%	0.48% 0.	24% 0.48%	0.72%													
46 B. nigrum KU71178	82	15.99% 1	5.99% 16	.92% 16.	2% 16.31	6 16.31%	16.31% 1	5.60% 16.60	% 16.60%	16.60% 16	60% 16.60%	16.60% 1	6.60% 16.609	% 16.60%	16.60% 16.60	0% 16.92%	16.92%	16.92% 16.9	2% 16.92%	16.92%	16.92% 16.93	2% 16.92%	16.92% 16.	92% 16.92%	16.92% 16	6.92% 17.255	6 15.99% 16	5.31% 16.31%	6 16.63% 17	7.85% 20.86%	19.25% 19.	25% 19.58%	19.25% 18	1.92%												
47 KU71178	83	16.96% 1	5.96% 17	.90% 17.	0% 17.28	6 17.28%	17.28% 1	7.57% 17.57	% 17.57%	17.57% 17	.57% 17.57%	17.57% 1	7.57% 17.579	% 17.57%	17.57% 17.57	7% 17.90%	17.90%	17.90% 17.9	17.90%	17.90%	17.90% 17.90	17.90%	17.90% 17.	90% 17.90%	17.90% 1	7.90% 18.235	6 16.96% 17	7.28% 17.28%	6 17.61% 18	8.49% 20.86%	19.58% 19.	58% 19.91%	19.58% 15	.25% 0.72%												
48 B. perspicuus LS99255	51	14.69% 1	1.69% 15	.59% 15.	9% 15.00	6 15.00%	15.00% 1	5.28% 15.28	% 15.28%	15.28% 15	28% 15.28%	15.28% 1	5.28% 15.289	% 15.28%	15.28% 15.28	8% 15.59%	15.59%	15.59% 15.5	9% 15.59%	15.59%	15.59% 15.59	9% 15.59%	15.59% 15.	59% 15.28%	15.28% 1	5.59% 15.59%	6 14.69% 15	5.00% 15.00%	6 15.31% 18	8.22% 24.28%	21.20% 21.	20% 21.54%	20.52% 20	1.86% 17.09%	17.41%											
49 B. simodensis LS99254	46	15.88% 1	5.88% 16	.80% 16.	0% 16.19	6 16.19%	16.19% 1	7.12% 17.12	% 17.12%	17.12% 17	.12% 17.12%	17.12% 1	7.12% 17.129	% 17.12%	17.12% 17.12	2% 16.80%	16.80%	16.80% 16.8	16.80%	16.80%	16.80% 16.80	0% 16.80%	16.80% 16.	80% 16.80%	16.80% 16	6.80% 16.80%	6 15.88% 16	5.19% 16.19%	6 16.51% 19	9.19% 21.81%	23.65% 23.	55% 24.01%	22.93% 23	1.29% 17.68%	18.00% 9.09	1%										
50 B. VIOIDCEUS MK9788	300	22.82% Z	23	84% 233	4% 23.17 8% 23.21	6 23.17%	23.1/% 2	3.48% 23.48	% 23.48%	23.48% 23	48% 23.48%	23.48% 2	3.48% 23.48%	% 23.48%	23.48% 23.48	5% 23.84% 7% 73.88%	23.84%	23.84% 23.8 73.88% 73.8	14% 23.84%	23.84%	23.84% 23.84 23.88% 23.81	1% 23.84%	23.84% 23	84% 22.86% 88% 23.25%	22.86% 2:	3.84% 24.20	6 22.82% 23	2.82% 22.82%	6 23.17% 25 6 23.56% 25	5.79% 23.69%	23.35% 23.	55% 23.69%	23.37% 22	31% 22.71%	21.65% 23.5	5% 23.88%	0.72%									
52 MK9788	802	22.51% 2	2.51% 23	52% 23.	2% 22.86	6 22.86%	22.86% 2	3.17% 23.17	% 23.17%	23.17% 23	17% 23.17%	23.17% 2	3.17% 23.179	% 23.17%	23.17% 23.17	7% 23.52%	23.52%	23.52% 23.5	2% 23.52%	23.52%	23.52% 23.52	2% 23.52%	23.52% 23	52% 22.89%	22.89% 2	3.52% 23.885	6 22.51% 22	2.51% 22.86%	6 23.21% 24	4.73% 22.66%	22.31% 22.	31% 22.66%	23.02% 21	.96% 22.75%	21.69% 23.2	23.56%	0.96% 0.24	6								
53 MK9788	803	22.51% 2	2.51% 23	.52% 23.	2% 22.86	6 22.85%	22.86% 2	3.17% 23.17	% 23.17%	23.17% 23	17% 23.17%	23.17% 2	3.17% 23.179	% 23.17%	23.17% 23.17	7% 23.52%	23.52%	23.52% 23.5	2% 23.52%	23.52%	23.52% 23.52	2% 23.52%	23.52% 23	52% 22.89%	22.89% 23	3.52% 23.885	6 22.51% 22	2.51% 22.86%	6 23.21% 24	4.73% 22.66%	22.31% 22.	31% 22.66%	23.02% 21	.96% 22.04%	21.00% 23.2	23.56%	0.96% 0.24	6 0.48%								
54 MK9788	304	22.12% 2	2.12% 23	13% 23.	3% 22.47	6 22.47%	22.47% 2	2.78% 22.78	% 22.78%	22.78% 22	.78% 22.78%	22.78% 2	2.78% 22.789	% 22.78%	22.78% 22.78	8% 23.13%	23.13%	23.13% 23.1	3% 23.13%	23.13%	23.13% 23.13	3% 23.13%	23.13% 23	13% 22.16%	22.16% 23	3.13% 23.489	6 22.12% 23	2.12% 22.12%	6 22.47% 23	3.60% 23.69%	22.98% 22.	38% 23.33%	23.69% 22	.62% 21.73%	20.70% 22.4	% 22.47%	2.69% 2.43	6 2.68%	2.18%							
55 Botrylloides sp. MK9788	805	19.85% 1	9.85% 20	.55% 20.	5% 20.18	6 20.18%	20.18% 2	0.22% 20.22	% 20.22%	20.22% 20	22% 20.22%	20.22% 2	0.22% 20.229	% 20.22%	20.22% 20.22	2% 20.55%	20.55%	20.55% 20.5	5% 20.55%	20.55%	20.55% 20.55	5% 20.55%	20.55% 20.	55% 20.49%	20.49% 20	0.55% 20.225	6 19.85% 20	0.18% 19.85%	6 20.18% 22	2.86% 20.79%	21.41% 21.	41% 21.75%	21.41% 21	.75% 21.35%	21.00% 21.9	22.93%	24.60% 24.56	6 24.20%	24.20% 23.9	6%						
56 Botryllus schlosseri MK9788	517	20.52% 2	1.52% 20	15% 203	276 20.86	5 20.86%	20.86% 2/	0.45% 20.49	% 20.49%	20.49% 20	20.49%	20.49% 2	0.45% 20.499	n 20.49%	20.49% 20.45	20.82%	20.82%	20.82% 20.8	20.82%	20.82%	20.82% 20.8	20.82%	20.82% 20.	82% 21.51%	21.51% 20	0.15% 20.40	% 20.52% 20	20.86%	6 21.20% 22	2.16% 22.78%	23.13% 23.	13% 23.48%	22.78% 22	18.59%	19.58% 19.7	7% 19.41%	24.96% 25.37	5 25.74%	25.01% 23.9	6% 22.72%	0.72%					
58 MK9788	319	21.88% 2	1.88% 21	.85% 21.	5% 22.23	6 22.23%	22.23% 2	1.51% 21.51	% 21.51%	21.51% 21	51% 21.51%	21.51% 2	1.51% 21.519	% 21.51%	21.51% 21.51	1% 21.85%	21.85%	21.85% 21.8	15% 21.85%	21.85%	21.85% 21.85	5% 21.85%	21.85% 21	85% 22.89%	22.89% 2	1.85% 22.19	6 21.88% 22	2.23% 22.23%	6 22.58% 20	0.46% 22.44%	23.13% 23.	13% 23.48%	22.78% 22	1.78% 18.27%	19.25% 19.7	20.07%	25.33% 25.74	6 25.37%	25.37% 24.3	2% 24.12%	4.49% 3.7	196				
59 MK9788	320	20.18% 2	0.18% 20	.15% 20.	5% 20.52	6 20.52%	20.52% 1	9.82% 19.82	% 19.82%	19.82% 19	82% 19.82%	19.82% 1	9.82% 19.829	% 19.82%	19.82% 19.82	2% 20.15%	20.15%	20.15% 20.1	5% 20.15%	20.15%	20.15% 20.1	5% 20.15%	20.15% 20	15% 21.16%	21.16% 20	0.15% 20.499	6 20.18% 20	0.52% 20.52%	6 20.86% 20	0.46% 20.40%	21.75% 21.	75% 22.09%	21.41% 21	.41% 18.27%	19.25% 19.7	19.41%	25.33% 25.74	6 25.37%	25.37% 24.3	2% 22.03%	2.95% 2.2	2.45%				
60 MK9788	321	20.07% 2	0.07% 20	.38% 20.	8% 20.40	6 20.40%	20.40% 2	0.05% 20.05	% 20.05%	20.05% 20	.05% 20.05%	20.05% 2	0.05% 20.059	% 20.05%	20.05% 20.05	5% 20.38%	20.38%	20.38% 20.3	20.38%	20.38%	20.38% 20.31	3% 20.38%	20.38% 20	38% 20.76%	20.76% 20	0.38% 20.055	6 20.07% 20	20.40%	6 20.74% 15	9.41% 24.40%	22.51% 22.	51% 22.86%	22.16% 22	.16% 19.46%	19.79% 18.13	21.02%	26.93% 26.60	6 26.22%	26.22% 25.5	2% 21.07%	14.74% 14.4	3% 15.99% 14.3	.2%			
61 MK9788.	322	19.74% 1	9.74% 20	.05% 20.	5% 20.07	6 20.07%	20.07% 1	9.72% 19.72	% 19.72%	19.72% 19	.72% 19.72%	19.72% 1	9.72% 19.729	% 19.72%	19.72% 19.72	2% 20.05%	20.05%	20.05% 20.0	15% 20.05%	20.05%	20.05% 20.05	5% 20.05%	20.05% 20	.05% 20.43%	20.43% 20	0.05% 19.725	6 19.74% 19	9.74% 20.07%	6 20.40% 15	9.41% 24.05%	22.51% 22.	51% 22.86%	22.16% 22	.16% 18.49%	18.81% 18.1	20.35%	25.79% 25.47	6 25.10%	25.10% 24.4	1% 21.07%	14.74% 14.4	3% 15.99% 14.3	.2% 0.96%			
62 MK9788	323	19.74% 1	9.74% 20	.05% 20.	5% 20.07	6 20.07%	20.07% 1	9.72% 19.72	% 19.72%	19.72% 19	.72% 19.72%	19.72% 1	9.72% 19.729	% 19.72%	19.72% 19.72	2% 20.05%	20.05%	20.05% 20.0	15% 20.05%	20.05%	20.05% 20.05	5% 20.05%	20.05% 20.	05% 20.43%	20.43% 20	0.05% 19.725	6 19.74% 19	9.74% 20.07%	6 20.40% 15	9.74% 24.76%	22.16% 22.	16% 22.51%	21.81% 22	.51% 19.79%	20.12% 18.4	21.35%	27.31% 26.98	6 26.60%	26.60% 25.5	0% 20.74%	15.05% 14.7	16.31% 14.4	3% 0.24%	1.21%		
64 S. clava MK9788	524	20.07% 20	1.07% 20	.58% 20. 73% 30	8% 20.40 3% 30.79	5 20.40% 5 30.79%	20.40% 2/	0.05% 20.05 0.73% 30.73	% 20.05% % 30.73%	20.05% 20	20.05% 20.05%	20.05% 2	0.05% 20.05%	n 20.05%	20.05% 20.05	5% 20.38%	20.38%	20.38% 20.3	8% 20.38%	20.38%	20.38% 20.31	5% 20.38%	20.38% 20.	38% 20.76%	20.76% 20	0.58% 20.055	n 20.07% 20 6 30.39% 30	20.40% 20.40%	6 20.74% 15 6 30.79% 34	9.74% 24.40% 4.33% 31.27%	22.16% 22.	16% 22.51% 25% 28.63%	22.51% 21	.81% 18.81%	19.14% 18.4	20.68%	25.42% 25.10	6 24.73% 6 32.30%	24.73% 24.0	5% 21.41% 3% 30.28%	15.05% 14.7	15.31% 14.4 1% 27.67% 27.6	5% 1.21%	U.24% 1.45%	29.01%	
65 S. plicata MH0114	453	28.67% 2	3.67% 27	.58% 27.	8% 28.29	6 28.29%	28.29% 2	7.58% 27.58	% 27.58%	27.58% 27	.58% 27.58%	27.58% 2	7.58% 27.589	% 27.58%	27.58% 27.58	8% 27.58%	27.58%	27.58% 27.5	8% 27.58%	27.58%	27.58% 27.51	3% 27.58%	27.58% 27.	58% 28.25%	28.25% 23	7.58% 27.95	6 28.67% 29	3.06% 28.67%	6 28.29% 33	3.03% 28.42%	30.33% 30.	33% 30.72%	29.95% 25	.95% 32.32%	32.72% 28.2	% 31.21%	36.22% 36.55	6 36.16%	36.16% 37.0	9% 28.96%	29.65% 29.6	5% 29.65% 28.5	30% 28.05%	9.56% 28.42%	29.95% 28.	1.25%

: DNA barcode data comparison of *B. diegensis* from Korea with other *Botrylloides*

• Phylogenetic tree of *B. diagensis* and other *Botrylloides*



Fig. 3. Phylogenetic tree constructed using the neighbor-joining method based on the Kimura 2-parameter model, with 1000 bootstrap replicates

Conclusion

- Two color types of botrylloids in Korea is *B. diegensis* based on DNA barcoding analysis. This study present the DNA barcoding data of *B. diegensis* and *B. violaceus* in Korea, respectively.
- This study is the first report that *B. diegensis* is distributed in northwest Pacific region.
- *Botrylloides diegensis* is widely distributed in Korea and it means that marine environment of near Korea is appropriate for inhabit of *B. diegensis*.
- Accordingly, *B. diegensis* is seems to be widely distributed in northwest Pacific region, including coasts of Japan and China.

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