

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

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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
 - extracted from a single zooid in a colony
 - 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)
 - Kimura 2-parameter model with 1000 bootstrapping replicates
 - Phylogenetic tree construction
 - Neighbor-joining method with 1000 bootstrapping replicates

Results

: 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. diegensis* 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).

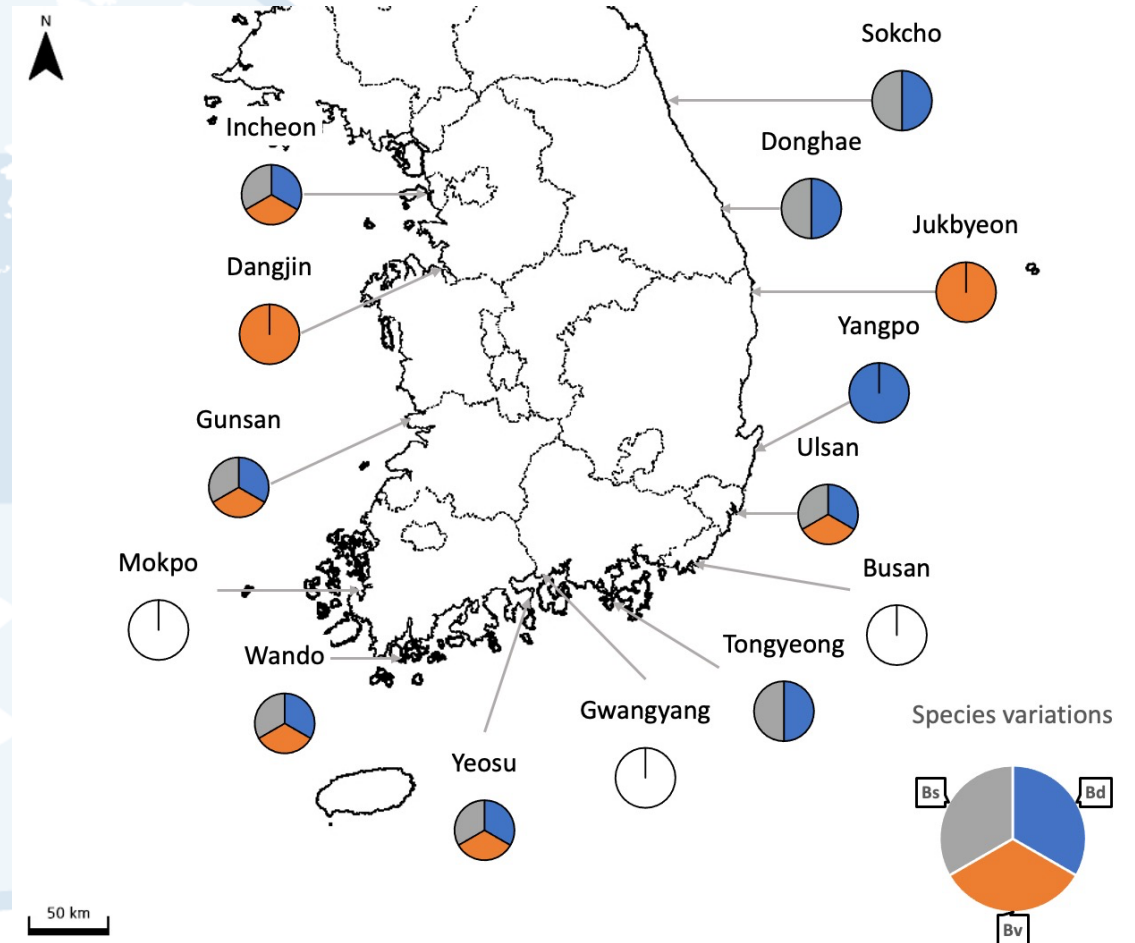


Figure 2. Distribution and species composition of botryllids in South Korea. Bs, *Botryllus schlosseri* ; Bd, *Botrylloides diegenensis*; Bv, *Botrylloides violaceus*.

Results

: 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	

Results

: 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. diegensis* – other species: Ave.=22.07%
 - ✓ *B. diegensis* – other *Botrylloides*: Ave.=19.61%
 - ✓ *B. diegensis* – *B. fuscus*: Ave.=20.25%
 - ✓ *B. diegensis* – *B. giganteus*: Ave.=19.48%
 - ✓ *B. diegensis* – *B. leachii*: Ave.=18.16%
 - ✓ *B. diegensis* – *B. nigrum*: Ave.=17.15%
 - ✓ *B. diegensis* – *B. perspicuus*: Ave.=15.32%
 - ✓ *B. diegensis* – *B. simodensis*: Ave.=16.74%
 - ✓ *B. diegensis* – *B. violaceus*: Ave.=23.24%
 - ✓ *B. diegensis* – *Botrylloides* sp.: Ave=20.31%
 - ✓ *Botrylloides-Botryllus schlosseri*: Ave.=21.07%
- Detailed all estimated values were presented next page

Results

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

- Phylogenetic tree of *B. diegensis* and other *Botrylloides*

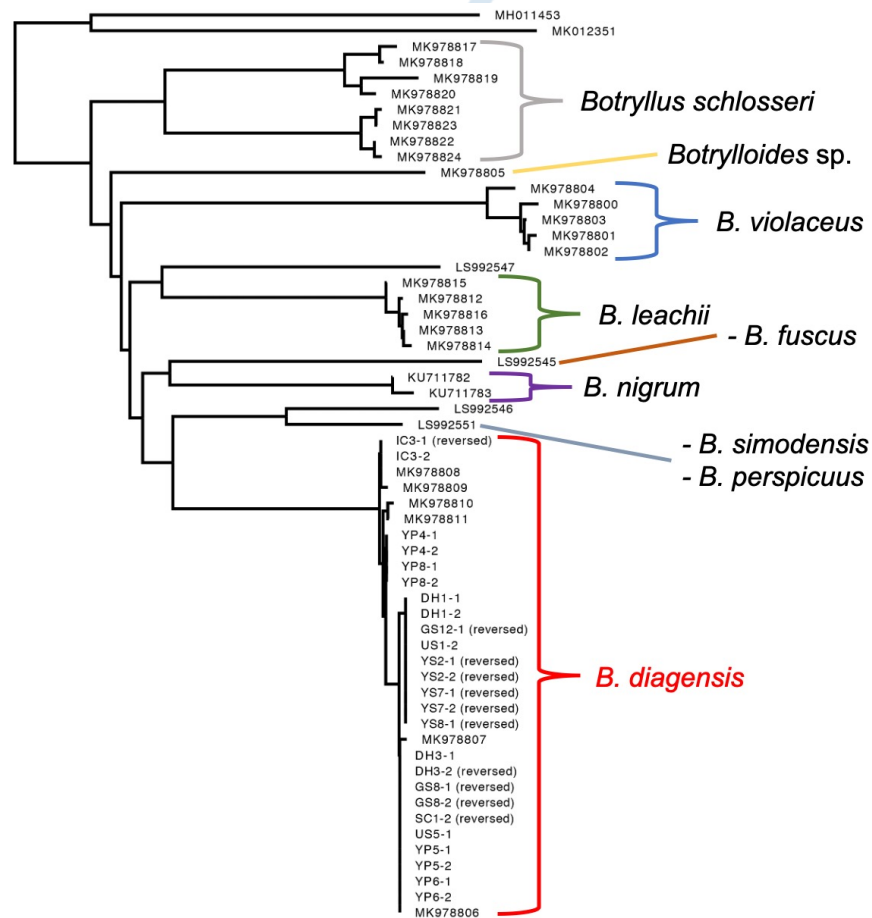


Fig. 3. Phylogenetic tree constructed using the neighbor-joining method based on the Kimura 2-parameter model, with 1000 bootstrap replicates

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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