Korean Journal of Environmental Biology

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https://doi.org/10.11626/KJEB.2023.41.3.223

Korean J. Environ. Biol.
41(3) : 223-228 (2023)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

A new record of high temperature tolerance species, *Pyropia kitoi* com. nov. (Bangiaceae, Rhodophyta), from Korea

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Contribution to Environmental Biology

- We present the inclusion of *Pyropia kitoi* com. nov. as a novel addition to the catalog of macroalgal flora in Korea.
- *Pyropia kitoi* could be considered a valuable seaweed species due to its distinct characteristic of exhibiting high temperature tolerance, making it a promising candidate for marine cultivation.

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Received: 14 July 2023
Revised: 17 August 2023
Revision accepted: 28 August 2023

Abstract: *Neoporphyra kitoi* Ma. Abe, N. Kikuchi, Tamaki, Tom. Sato, Murase, Fujiyoshi & Mas. Kobayashi has been known as an endemic species in Japan. Its high temperature tolerance suggests that it could be advantageous for cultivation. In this study, we collected it from the Ulleungdo island, Korea and transferred it into *Pyropia* for a new combination, identified as *Pyropia kitoi* (Ma. Abe, N. Kikuchi, Tamaki, Tom. Sato, Murase, Fujiyoshi & Mas. Kobayashi) D.J. Kim, T.O. Cho & B.Y. Won comb. nov. based on morphological and molecular analyses. *Pyropia kitoi* is also reported as a new record species in the list of Korean macroalgal flora. Although we didn't observe the emergence of new blades from the rhizoidal cells, which is a key character for this species, our molecular analysis of *rbc*L revealed that our samples from Korea were congruent with "*Neoporphyra kitoi*" from Japan and were nested within the clade of *Pyropia*. The gene sequence divergence between the Korean and Japanese samples was 0–0.2%.

Keywords: morphology, *Neoporphyra kitoi*, phylogeny, *rbc*L, taxonomy

1. INTRODUCTION

The red algal order Bangiales Nägeli (Nägeli 1847) has traditionally been divided into two genera: *Porphyra* C.Agardh (Agardh 1824), which includes species with a foliose gametophyte, and *Bangia* Lyngbye (Lyngbye 1819), which encompasses species with a filamentous gametophyte morphology (Boedeker *et al.* 2008; Zheng and Li 2009). The foliose form of the order Bangiales has been composed of nine genera: *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Miuraea*, *Neothemis*, *Porphyra*, *Pyropia*, and *Wildemania* (Sanchez *et al.* 2014; Yang *et al.* 2017; Kikuchi *et al.* 2018). In 2020, Yang *et al.* redefined the genus *Pyropia* based on a molecular phylogenetic study of foliose Bangiales from China. They resurrected *Porphyrella* and proposed four additional genera: *Calidia*, *Neoporphyra*, *Neopyropia*, and *Uedaea*. However, recently, Zuccarello *et al.* (2022) synonymized all five of these newly proposed genera back into *Pyropia*. Currently there are nine genera with a foliose gametophyte: *Boreophyllum*, *Clymene*, *Fuscifolium*, *Lysithea*, *Neomiuraea* (formerly *Miuracea*), *Neothemis*, *Porphyra*, *Pyropia*, and *Wildemania*.

Pyropia ('Gim' in Korean) is an economically significant seaweed in Korea and has witnessed significant progress in maximizing production through advance-

ments in cultivation methods and selective breeding of high-quality varieties (Hwang and Park 2020; Hwang *et al.* 2020). Recently, seaweed cultivation is facing a direct threat from the increasing seawater temperatures caused by global warming (Kim *et al.* 2007; Hwang *et al.* 2020). It is important to find or develop high-temperature-tolerant species/strains of *Pyropia* (Choi *et al.* 2013; Xing *et al.* 2023).

"*Neoporphyra kitoi*" was initially described by Abe *et al.* in 2021 based on specimens collected from Isumi, Chiba, Japan. They noted that "*N. kitoi*" exhibited significant molecular differences in *rbc*L and SSU genes, as well as a distinct morphological characteristic of new blades emerging from rhizoidal cells even if foliose thalli "*N. kitoi*" were similar to *Pyropia pseudolinearis* in morphology. According to Niwa *et al.* (2022) "*N. kitoi*" show high potential as marine crop due to its tolerance to high temperature.

We collected 13 unidentified foliose red algae from the Korean coastline and recognized their taxonomic position based on both molecular and morphological analyses. Of them, one sample collected from Ulleungdo island has been added as *Pyropia kitoi* com. nov. to the marine algal inventory of Korea.

2. MATERIALS AND METHODS

2.1. Molecular analyses

Samples were collected from the Korean coastline. Genomic DNA was manually extracted from silica-gel samples using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany). The *rbc*L DNA amplifications were performed using primer sets F57-R753, F577-R1150, and F993-Rrbcst. PCR amplifications were carried out using a Veriti 96 well Thermal cycler (Applied Biosystem, Waltham, MA). The PCR products were then purified using a PCRquick-spin™ PCR product purification kit (iNtRON Biotechnology, Inc., Seongnam, Korea). The thirteen *rbc*L sequence obtained in this study had been deposited in GenBank. The *rbc*L sequence that we generated, as well as those obtained from GenBank, were aligned using ClustalW (Thompson *et al.* 1994).

Phylogenetic analyses were performed using raxml GUI1.5b2 (Silvestro and Michalak 2012). Maximum likelihood analyses were conducted using the GTR+G+I model, with 1,000 bootstrap replicates. Bayesian inference was performed using MrBayes 3.2.6 (Huelsenbeck and Ronguist 2001; Ronguist and Huelsenbeck 2003). Markov chain Monte Carlo runs were conducted for 2,000,000 generations, with one cold chain and three heated chains, using the GTR+Γ+I evolutionary model. Trees were sampled and printed every 1,000 generations and summary trees were generated using a burn-in value of 25%.

2.2. Morphological analyses

Samples were subsequently classified into voucher herbarium specimens, silica gel samples, and formalin samples. Formalin samples were preserved in a solution of 4−5% formalin mixed with seawater. Photomicrographs of the specimens were captured using an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) equipped with an Olympus DP71 camera. For long-term preservation, permanent slides were prepared using 70% karo syrup. Representative specimens examined in this study were deposited in the herbarium of Chosun University (CUK) and National Institute of Biological Resources (NIBR) in Korea.

3. RESULTS AND DISCUSSION

Phylogenetic analyses: The 1247 *rbc*L base pair (bp) portion of the 1467-bp *rbc*L gene (85% sequenced) was sequenced from samples from Korea. The phylogenetic trees were obtained from the alignment of the *rbc*L sequences newly generated and downloaded from Gen Bank (Fig. 1). *Smithora naiadum* (C. L. Anderson) Hollenb. and *Chlidophyllon kaspar* (W. A. Nelson et N. M. Adams) W. A. Nelson were included as outgroups. Phylogenetic analyses inferred from *rbc*L indicated that all thirteen samples from Korea were nested within *Pyropia* clade of the Bangiaceae, with 93% bootstrap support in the Maximum likelihood analysis and Bayesian posterior probabilities of 1 in the Bayesian tree. Our sequences cluster with one sequence belonging to *Py. kinositae*, one sequence belonging to *Py. Kuniedae*, one sequence belonging to *Py. pseudolinearis*, one sequence belonging to *Py. retorta*, five sequences belonging to *Py. seriata*, one sequence belonging to *Py. suborbiculata*, and two sequeces belonging to *Py. yezoensis* (Fig. 1). One collection from Ulleungdo island, Korea falls within the

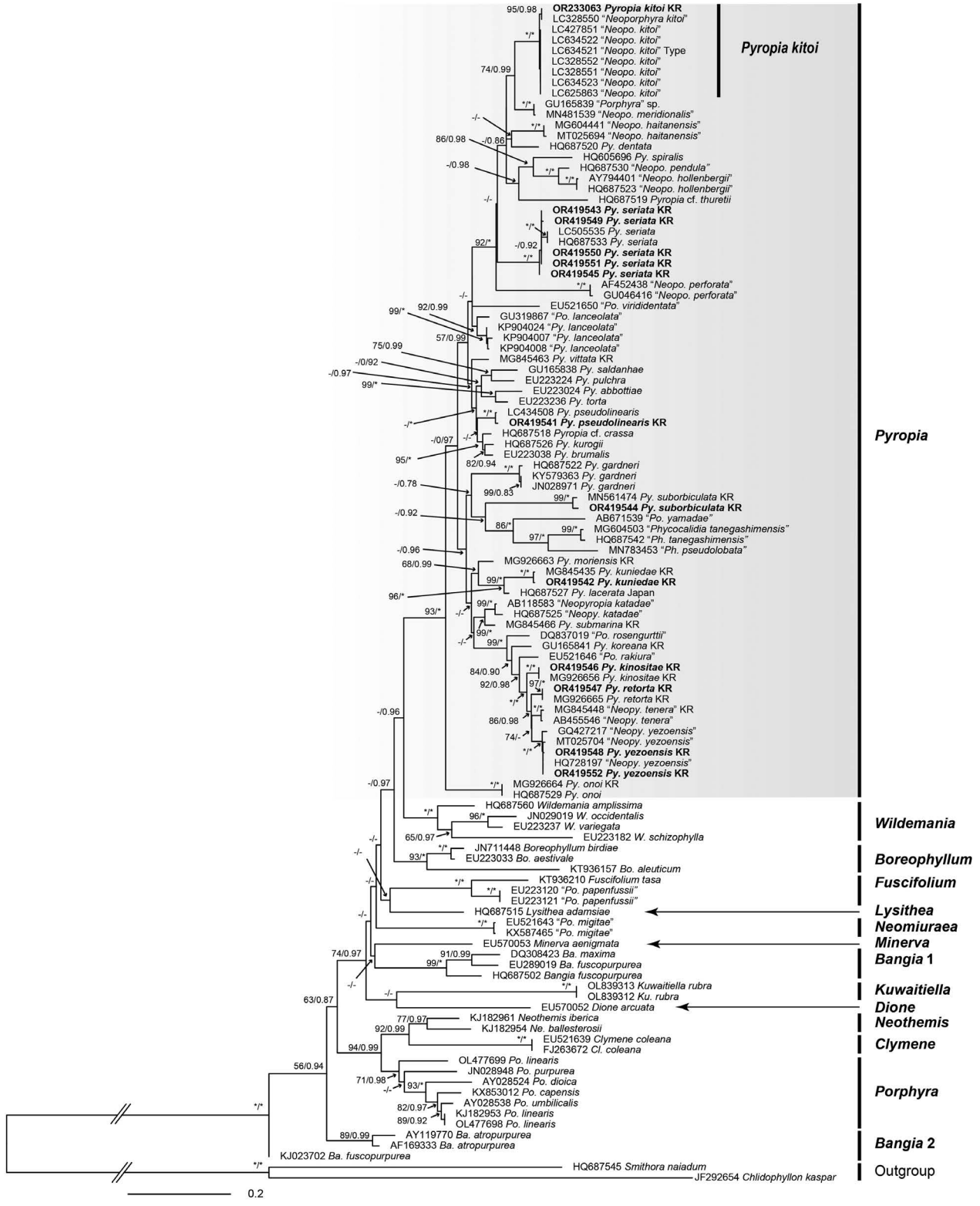

Fig. 1. Phylogenetic tree of Bangiaceae species based on ML and Bayesian analysis with *rbc*L gene sequences. Value above branches = Maximum likelihood bootstrap values in ≥50%/Bayesian posterior probabilities ≥0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (-). Values of BS 100 or BPP 1.00 are indicated by asterisks (*). Sequences generated in this study are highlighted in bold.

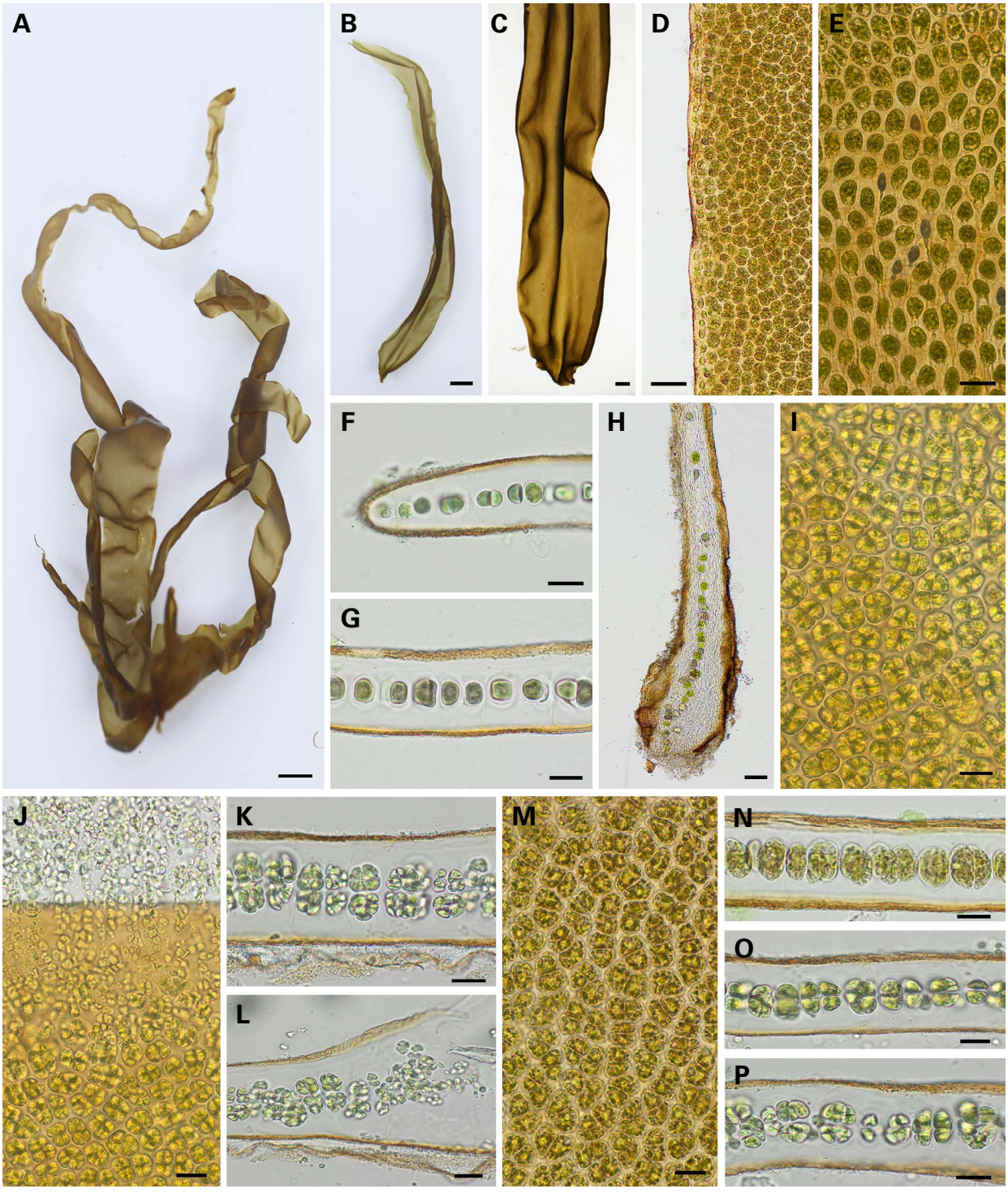

Fig. 2. *Pyropia kitoi* (CUK21236) from Ulleung-do, Gyeongsangbuk-do, Korea. A. Plant with multiple blades from palm-shaped base; B. Thallus with single blade; C. Rotund base in single blade; D. Marginal portion showing the entire margin; E. Basal cells projecting a rhizoidal filament in surface view. F and G. Cross-section views showing marginal (F) and central (G) portions of vegetative thalli; H. Longitudinal section view showing cells in the basal portion of vegetative thalli; I. Spermatangial portion in surface view; J. Surface view of spermatangial thallus releasing spermatangia; K and L. Longitudinal section views showing developmental series of spermatangia. M. Zygotosporangial portion in surface view; N–P. Cross-section views showing developmental series of Zygotosporangia; Scale bars: A, B = 0.5 cm; C = 0.1 cm; D, E, H = 50 μm; F, G, I–P = 20 μm.

same clade as "*Neoporphyra kitoi*", with only 3 base pair differences in *rbc*L squences (1247 bp). *Pyropia kitoi* com. nov was found to be closely related to *P. meridionalis* (Fig. 1).

***Pyropia kitoi* (Ma.Abe, N.Kikuchi, Tamaki, Tom. Sato, Murase, Fujiyoshi & Mas.Kobayashi) D.J. Kim, T.O. Cho & B.Y. Won com. nov.**
(길쭉돌김) (Fig. 2)

Basionym: *Neoporphyra kitoi* Ma. Abe, N. Kikuchi, Tamaki, Tom. Sato, Murase, Fujiyoshi & Mas. Kobayashi 2021.

Holotype. SAP (the Herbarium of Faculty of Science, Hokkaido University, Sapporo, Japan) 115 574, collected on 11 March 2019 by N. Kikuchi from Iwafune (35° 12′36″N, 140°23′32″E), Isumi, Chiba, Japan.
Material examined. CUK21236 (Herbarium of Chosun University) & NIBRRD0000010448 (National Institute of Biological Resources), Cheonbu-ri, Buk-myeon, Ulleung-gun, Gyeongsangbuk-do, Korea (37°32′ 40.7″N, 130°54′31.5″E), April 09, 2023, T.O. Cho & B.Y. Won, at 1m depth by hand.
Habitat. Thalli grow on rocks or on *Scytosiphon lomentaria* in intertidal zone.
GenBank accession number. OR233063 (*rbc*L).
Morphological observation. Thalli are dark red purple or reddish brown (Fig. 2A, B). Thalli are membranous, monostromatic, linear and narrow elliptic, 10−15 cm long, 1.0−1.5 cm broad, with cuneate, obtuse or rotund base, and with entire margin (Fig. 2A−E). Vegetative cells are oblong or irregular tetragonal to polygonal with rounded angles (Fig. 2F, G). Basal cells capitate with projected rhizoidal filaments (Fig. 2E). Rhizoidal filaments are arranged in both directions in sectional view (Fig. 2H). Thalli mixed monoecious. Spermatangia are formed in parallel with zygotosporangial patches along margins (Fig. 2I, J). They are small (Fig. 2K, L). Zygotosporangia are large (Fig. 2M−P) and prototrichogyne are conspicuous and acute to obtuse (Fig. 2N).
Remarks. *Pyropia*, the most species-rich genus in Bangiales, comprises approximately 76 taxonomically accepted species worldwide (Guiry and Guiry 2023). While Yang *et al.* (2020) divided *Pyropia* into several genera within Bangiaceae, Zuccarello *et al.* (2022) synonymized these genera including *Neoporphyra* back into *Pyropia*. Zuccarello *et al.* in 2022 mentioned that it is essential to ensure that phylogenetic analyses integrate the most current reconstruction techniques, model selection methodologies, and a variety of support measures when proposing new genera without alternative morphological diagnostic features (Simon 2022; Zuccarello *et al.* 2022). Our phylogenetic tree, based on Maximum Likelihood analysis, also indicated weak support for the clades proposed as genera by Yang *et al.* (2020) (Fig. 1). In our study, *Pyropia kitoi* is proposed as a new combination with *N. kitoi* Ma. Abe, N. Kikuchi, Tamaki, Tom. Sato, Murase, Fujiyoshi & Mas. Kobayashi 2021 based on the detailed molecular and morphology analyses. We also report *P. kitoi* as a new record in the list of Korean macroalgal flora. *Pyropia kitoi* may be one of beneficial seaweed species to have a characteristic of having potential for marine crop with high temperature tolerance (Niwa *et al.* 2022).

CRediT authorship contribution statement

DJ Kim: Formal analysis, Writing-Original draft. P Romero-Orozco: Resources. GW Kim: Resources. SH Baek: Resources. TO Cho: Funding acquisition, Supervision. BY Won: Funding acquisition, Visualization.

Declaration of Competing Interest

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1I1A2059577), by the Ministry of Ocean and Fisheries (Marine Biotics Project, 20210469), and by the National Marine Biodiversity Institute of Korea (the management of Marine Fishery Bio-resources Center 2023) to Tae Oh Cho. This research was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1I1A1A01051909) to Boo Yeon Won.

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