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Distribution pattern of a new record crustose red alga, *Lithophyllum neo-okamurae* (Corallinales, Rhodophyta), in Korea

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Contribution to Environmental Biology

- We present the inclusion of *Lithophyllum neo-okamurae* as a novel addition to the catalog of macroalgal flora in Korea.
- *Lithophyllum neo-okamurae* is distributed all the coastline in Korea while *Lithophyllum okamurae* is more commonly found in the southern part of Korea.

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Abstract: A crustose red alga, *Lithophyllum neo-okamurae* A.Kato, D.Basso, Caragnano, Rodondi, V.Peña & M.Baba, is reported a new record from Korea and its biogeographic distributions are extended in Korea. *Lithophyllum neo-okamurae* has been known as endemic species in Japan. Despite the resemblance between *L. neo-okamurae* and *L. okamurae*, our molecular and morphological analyses have revealed the simultaneous presence of both species in Korea. Although *L. neo-okamurae* and *L. okamurae* share morphological similarities, there are notable variations in gene sequences, with a range of 9.1–11.3% in *psb*A and 14.8–15.3% in *rbc*L. Specifically, while *L. neo-okamurae* is distributed throughout the entire coastline of Korea, *L. okamurae* is predominantly found in southern region. Furthermore, our analyses have revealed their detailed distributions in Korea.

Keywords: distribution, *Lithophyllum neo-okamurae*, *Lithophyllum okamurae*, phylogeny, taxonomy

1. INTRODUCTION

The crustose red coralline algal genus, *Lithophyllum*, initially described by Philippi. *Lithophyllum* comprises approximately 146 taxonomically accepted species worldwide (Guiry and Guiry 2024). Of them, seven species have been reported in Korea: *Lithophyllum canescens*, *L. corallinae*, *L. dispar*, *L. okamurae*, *L. shioense*, *L. tumidulum*, and *L. yessoense* (Lee and Kang 1986; Lee and Kang 2002; Lee 2008). Specifically, *Lithophyllum okamurae* is well recognized as a coralline red alga characterized by protuberances emerging from its encrusting thallus (Kato *et al.* 2022). It has been documented along the entire shoreline of Korea (Lee and Kang 2002).

Recently, Kato *et al.* (2022) described *Lithophyllum neo-okamurae*, using morpho-anatomical observations and DNA sequence analyses from herbarium specimens of *L. okamurae*, which included the type material, as well as newly collected samples from Japan. Despite the morphological similarities between the two species, *L. neo-okamurae* is distinguished by predominantly knobby protuberances, while *L. okamurae* displays a variety of protuberance forms, including knobby, tapering, and plate-like (foliose thalli) appearances. Notably, the size of the tetrasporangial conceptacle chambers further differentiates the two species, with *L. neo-okamurae* having larger chambers (248–380 μm) compared to the smaller chambers (167–341 μm) of *L. okamurae* (Kato *et al.* 2022). Kato *et al.* (2022) emphasize the necessity of molecular approaches for a definitive distinction between these species, given their shared morphological characteristics.

We collected 76 unidentified samples of crustose red algae from Korea. Through a combination of molecular and morphological analyses, we conducted a comprehensive identification, taxonomic classification, and biogeography of these specimens. In this study, we add a crustose red alga, *Lithophyllum neo-okamurae* in the list of Korean macroalgal flora and extend its biogeographic distribution to Korea.

2. MATERIALS AND METHODS

2.1. Taxon Sampling/Collections

Seventy-six samples of crustose red algae were collected from the intertidal zone in Korea from 2014 to 2023. Samples were either manually collected using a chisel or obtained from stone and shell substrata containing noticeable mixtures of crustose algae. They were air-dried until completely dehydrated and subsequently stored in zipper bags with silica gel for morphological and molecular analyses.

2.2. Morphological analyses

Specimens of crustose red coralline algae were fully decalcified in 0.6 M nitric acid (HNO_3) until gas bubbles ceased emanating from the specimens. Decalcified samples were then gently rinsed with tap water. Sections of thallus were prepared using an embedding matrix (O.C.T.; CellPath, Ltd., Newtown, Wales, UK), sectioned using a freezing microtome (Shandon Cryotome FSE; Thermo Shandon, Ltd., UK), and stained with a 1 : 1 mixture of aqueous aniline blue and acetic acid. Photomicrographs were captured using an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan) and an Olympus DP71 camera. For observation with Scanning electron microscopy (SEM), the silica gel-dried samples were fractured using razor blade or chisel and small hammer. The fractured pieces were mounted on aluminum stubs using double-sided adhesive carbon tape (Nisshin EM Co., Ltd., Japan), and then coated with gold for 5 min using digital ion coater (SPT-20; COXEM Co., Ltd., Korea). The samples were observed with a COXEM EM-30 PLUS+ scanning electron microscope (Mini SEM; COXEM Co., Ltd., Korea).

The measurements were made using the ImageJ software (Schneider *et al.* 2012). The clarity of digitized images was improved using Adobe Photoshop software version 6.1 (Adobe Systems Inc., San Jose, CA, USA). Representative voucher specimens examined in this study were deposited in the herbarium of Chosun University (CUK) and the Marine Biodiversity Institute (MABIK), Korea.

2.3. Molecular analyses

Dried samples were ground with autoclaved sand for DNA extraction. Genomic DNA was manually extracted from silica-gel samples using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The *psb*A for red algae was amplified using primers psbAF1-psbA600R or psbAF1-psbAR2 primers (Yoon *et al.* 2002). The *rbc*L DNA amplifications for red algae were performed using primer sets F57-R753, F577-R1150, and F993-Rrbcst (Freshwater and Rueness 1994). Seventy-three *psb*A sequences and fourteen *rbc*L sequences of crustose red coralline algae were obtained in this study. The *psb*A and *rbc*L sequence that we generated, as well as those obtained from GenBank, were aligned using Geneious Prime® (v.2023.0.1 Biomatters Ltd.).

Phylogenetic analyses were performed using raxmlGUI1.5b2 (Silvestro and Michalak 2012). Maximum likelihood analyses were conducted using the General Time-Reversible + G (gamma distribution) + I (proportion invariant) 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 General Time-Reversible + G (gamma distribution) + I (proportion invariant) model evolutionary model for the first and third codon position and General Time-Reversible + G (gamma distribution) for second codon position in *psb*A and *rbc*L. Trees were sampled and printed every 1,000 generations, and summary trees were generated using a burn-in value of 25%. Interspecific pairwise distance was estimated using the p-distance model in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.* 2021).

3. RESULTS

3.1. Morphological observations

Class Florideophyceae Cronquist, 1960 진정홍조강
Order Corallinales P.C. Silva & H.W. Johansen, 1986 산호말목
Family Lithophyllaceae Athanasiadis, 2016 혹돌잎과
Genus *Lithophyllum* Philippi, 1837 혹돌잎속

Lithophyllum neo-okamurae **A.Kato, D.Basso, Caragnano, Rodondi, V.Peña & M.Baba**
신혹돌잎 (신칭) (Fig. 1)

Holotype. SAP (the Herbarium of Faculty of Science, Hokkaido University, Sapporo, Japan) 115594, collected on 9 June 2013 by A. Kato and M. Baba.
Type locality. Misaki, Miura City, Kanagawa Prefecture, Japan.
Material examined. PH1151, Geobukbawi, Namyang-gil, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, Republic of Korea (37°27′35.5″N, 130°51′22.8″E), May 23,2023, T.O.Cho & D.J.Kim & G.W.Kim & S.H.Baek; TC10519, Yeonhwa-gil, Gijang-eup, Gijang-gun, Busan, Republic of Korea (35°12′56.46″N, 129°13′30.24″E), January 3, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & S.Y. & A.J.; TC10540, Yeonhwa-gil, Gijang-eup, Gijang-gun, Busan, Republic of Korea (35°12′56.46″N, 129°13′30.24″E), January 3, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & S.Y. & A.J.; TC10724, Cheoksa-gil, Gampo-eup, Gyeongju-si, Gyeongsangbuk-do, Republic of Korea (35°48′56.25″N, 129°30′46.51″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10740, Cheoksa-gil, Gampo-eup, Gyeongju-si, Gyeongsangbuk-do, Republic of Korea (35°48′56.25″N, 129°30′46.51″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10743, Homi-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°5′3.90″N, 129°33′7.40″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10746, Homi-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°5′3.90″N, 129°33′7.40″E), February 17, 2014, T.O. Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10747, Homi-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°5′3.90″N, 129°33′7.40″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10748, Homi-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°5′3.90″N, 129°33′7.40″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; MABIK AL00100607 (= TC10760), Haemaji-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°3′59.27″N, 129°34′13.38″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10762, Haemaji-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°3′59.27″N, 129°34′13.38″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10767, Haemaji-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°3′59.27″N, 129°34′13.38″E), February 17, 2014, T.O.Cho & S.Y. Jeong & D.B.M. & J.G.Lee; TC10769, Haemaji-ro, Homigot-myeon, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (36°3′59.27″N, 129°34′13.38″E), February 17, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10781, Seo Sang-ri, Seo-myeon, Namhae-gun, Gyeongsangnam-do, Republic of Korea (34°47′54.73″N, 127°50′10.95″E), February 18, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10804, Geumgap Beach, Geumgap-gil, Uisin-myeon, Jindo-gun, Jeollanam-do, Republic of Korea (34°23′39.84″N, 126°16′38.20″E), March 16, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10805, Geumgap Beach, Geumgap-gil, Uisin-myeon, Jindo-gun, Jeollanam-do, Republic of Korea (34°23′39.84″N, 126°16′38.20″E), March 16, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC10841, Geumgap Beach, Geumgap-gil, Uisin-myeon, Jindo-gun, Jeollanam-do, Republic of Korea (34°23′39.84″N, 126°16′38.20″E), March 16, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12047, Seobinbaeksa, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°30′8.98″N, 126°56′34.43″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12055, Seobinbaeksa, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°30′8.98″N, 126°56′34.43″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.

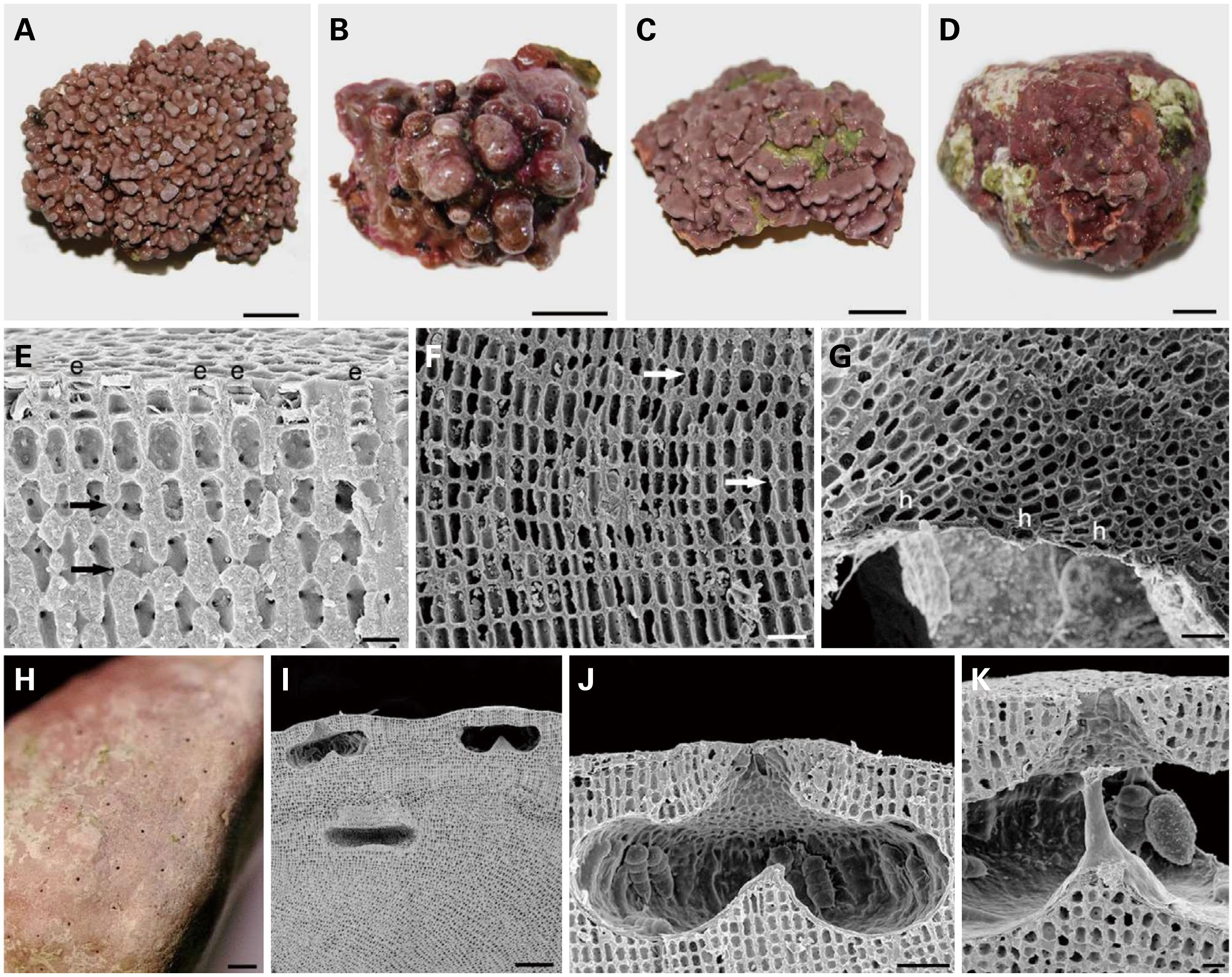

Fig. 1. Morphology of *Lithophyllum neo-okamurae*. A–D. Various external morphologies. E–F. Longitudinal section view of thallus showing epithallium cells (e) and perithallium cells with secondary pit connections (arrows) in inner thallus. G. Longitudinal section view showing unistratose hypothallium cells (h) in basal part. H. Surface view of tetrasporangial conceptacles with raised or sunken roofs. I. Longitudinal section view showing buried tetrasporangial conceptacle. J. Longitudinal section view of tetrasporangial conceptacle showing zonate tetrasporangia in the chamber. K. Longitudinal section view of tetrasporangial conceptacle showing a central columella. Scale bars represent: A–D = 1.0 cm, E, K = 10 μm, F–G = 25 μm, H = 200 μm, I = 100 μm, J = 50 μm.

G.Lee; TC12077, Biyangdo, Hallim-eup, Jeju-si, Jeju-do, Republic of Korea (33°24′21.9″N, 126°13′46.40″E), May 30, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12103, Udo Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°29′32.58″N, 126°57′29.96″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12104, Udo Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°29′32.58″N, 126°57′29.96″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12105, Udo Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°29′32.58″N, 126°57′29.96″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12106, Udo Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Republic of Korea (33°29′32.58″N, 126°57′29.96″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12118_1, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12119, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), May 29, 2014, T.O.Cho & S.Y.Jeong &

D.B.M. & J.G.Lee; TC12121, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12126, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12249, Biyangdo, Hallim-eup, Jeju-si, Jeju-do, Republic of Korea (33°24′21.6″N, 126°13′46.7″E), May 30, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12250, Biyangdo, Hallim-eup, Jeju-si, Jeju-do, Republic of Korea (33°24′21.6″N, 126°13′46.7″E), May 30, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12256, Gapa Island, Daejeong-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°10′19.26″N, 126°15′58.24″E), May 29, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee; TC12488, Chuja Port, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′44.69″N, 126°17′47.12″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12489, Chuja Port, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′44.69″N, 126°17′47.12″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12500, Chuja Port, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′44.69″N, 126°17′47.12″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12507, Chujado, Chuja-ro, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′21.95″N, 126°19′51.3″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12508, Chujado, Chuja-ro, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′21.95″N, 126°19′51.3″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12509, Chujado, Chuja-ro, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′21.95″N, 126°19′51.3″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12514, Chujado, Chuja-ro, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°57′21.95″N, 126°19′51.3″E), June 27, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12534, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°59′21.3″N, 126°15′05.3″E), June 28, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12538, Chuja-myeon, Jeju-si, Jeju-do, Republic of Korea (33°59′21.3″N, 126°15′05.3″E), June 28, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12889, Daepo port, Daepohanghuimang-gil, Sokcho-si, Gangwon-do, Republic of Korea (38°10′23.50″N, 128°36′23.12″E), July 31, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12891, Daepo port, Daepohanghuimang-gil, Sokcho-si, Gangwon-do, Republic of Korea (38°10′23.50″N, 128°36′23.12″E), July 31, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12982, Jeongdo beach, Jeongdo 1-gil, Wando-eup, Wando-gun, Jeollanam-do, Republic of Korea (34°17′49.97″N, 126°42′17.39″E), September 26, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC12984, Cheonghaejinseo-ro, Gunoe-myeon, Wando-gun, Jeollanam-do, Republic of Korea (34°19′52.31″N, 126°39′8.81″E), September 26, 2014, T.O.Cho & S.Y.Jeong & D.B.M. & J.G.Lee & S.Y.Park; TC13094, Gyeongjeong Beach, Yeongdeokdaege-ro, Chuksan-myeon, Yeongdeok-gun, Gyeongsangbuk-do, Republic of Korea (36°28′54.77″N, 129°26′2.58″E), October 30, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC13095, Gyeongjeong Beach, Yeongdeokdaege-ro, Chuksan-myeon, Yeongdeok-gun, Gyeongsangbuk-do, Republic of Korea (36°28′54.77″N, 129°26′2.58″E), October 30, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC13139, Muchangpo Beach, Yeollinbada 1-gil, Ungcheon-eup, Boryeong-si, Chungcheongnam-do, Republic of Korea (36°14′49.86″N, 126°31′59.73″E), November 1, 2014, T.O.Cho & B.Y.Won; TC13220, Gapa Island, Gapa-ro, Daejeong-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°10′9.68″N, 126°16′45.52″E), November 6, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC13221, Gapa Island, Gapa-ro, Daejeong-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°10′9.68″N, 126°16′45.52″E), November 6, 2014, T.O.Cho & S.Y. Jeong & D.B.M.; TC13258, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), November 7, 2014, T.O.Cho & S.Y. Jeong & D.B.M.; TC13322, Guryongpo port, Homi-ro, Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (35°59′47.52″N, 129°33′59.87″E), February 13, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC13324, Guryongpo port, Homi-ro, Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (35°59′47.52″N, 129°33′59.87″E), December 13, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC13326, Guryongpo port, Homi-ro, Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Republic of Korea (35°59′47.52″N, 129°33′59.87″E), December 13, 2014, T.O.Cho & S.Y.Jeong & D.B.M.; TC14996, Seopjikoji-ro, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°25′26.3″N, 126°55′55.2″E), May 5, 2015,

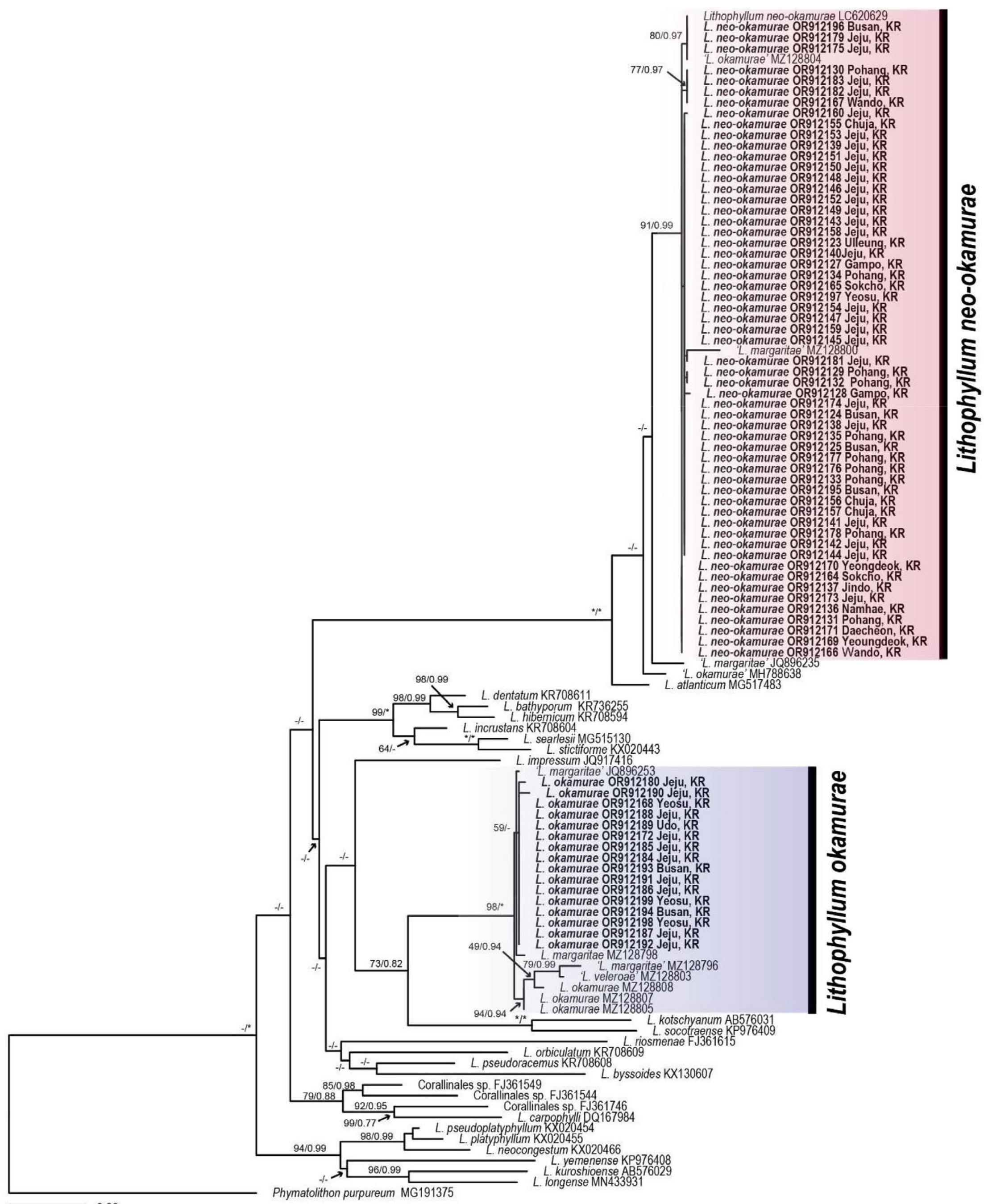

Fig. 2. Phylogenetic tree of *Lithophyllum* species based on Maximum likelihood (ML) and Bayesian analysis with *psb*A gene sequences. Value above branches = Maximum likelihood bootstrap values in % ≥50/Bayesian posterior probabilities ≥0.75. Values lower than BS (Bootstrap) 50 or BPP (Bayesian posterior probabilities) 0.75 are indicated by hyphens (–). Values of BPP 1.00 or BS 100 are indicated by asterisks (*). Sequences generated in this study are highlighted in bold.

T.O.Cho & S.Y.Jeong & J.G.Lee & S.Y.Park; TC16953, Seongsan, Seongsan-eup, Seogwipo-si, Jeju-do, Republic of Korea (33°27′42.60″N, 126°56′21.6″E), December 8, 2014, T.O.Cho & S.Y.Jeong; TC16959, Biyangdo, Hallim-eup, Jeju-si, Jeju-do, Republic of Korea (33°24′21.6″N, 126°13′46.7″E), March 18, 2016, T.O.Cho & S.Y.Jeong; TC16966, Biyangdo, Hallim-eup, Jeju-si, Jeju-do, Republic of Korea (33°24′21.6″N, 126°13′46.7″E), March 18, 2016, T.O.Cho & S.Y.Jeong; TC17742, Yeonhwa-gil, Gijang-eup, Gijang-gun, Busan, Republic of Korea (35°12′56.46″N, 129°13′30.24″E), January 12, 2017, T.O.Cho & B.Y.Won; TC17745, Yeonhwa-gil, Gijang-eup, Gijang-gun, Busan, Republic of Korea (35°12′56.46″N, 129°13′30.24″E), January 12, 2017, T.O.Cho & B.Y.Won; TC19118, Geomun-gil, Samsan-myeon, Yeosu-si, Jeollanam-do, Republic of Korea (34°1′44.44″N, 127°18′35.21″E), September 15, 2018, S.Y.Jeong & G.C.Choi.

Description. Thalli are encrusting, fruticose, lumpy, and warty and cover the hard substrate as they grow (Fig. 1A–D). The protuberances that grows from the encrusting thallus is up to 6–14 mm long, cylindrical or round hump shape. Apex of protuberance is blunt and enlarged. Color of living plants is light purple to scarlet. The cell walls are calcified. Epithallial cells are flattened, composed of 1–3 layers, 2–5 μm long and 6–8 μm in diameter (Fig. 1E). Cells of perithallial filaments are inconsistent rectangular, 13–20 μm long and 6–8 μm diameter (Fig. 1F). Secondary pit-connections are common (Fig. 1E–F). Dimerous construction with unistratose hypothallus is non-palisade, 4–6 μm long, 6–9 μm in diameter, and composed of approximately isodiametric or slightly elongate or wide cells (Fig. 1G). Cell fusions are absent. Trichocytes are not observed. Medullary regions in protuberances are coaxial.

Tetrasporangial conceptacles are uniporate with roof, raised above surrounding thallus surface or sunken below thallus surface (Fig. 1H). Buried conceptacles are observed (Fig. 1I). Conceptacle chambers are 258–333 μm in diameter and 75–125 μm high. Pore canals are triangular, tapering towards surface, and 53–67 μm long (Fig. 1J). A central columella is present (Fig. 1K). A calcified hump (four to five cell layers) below central columella is present. Tetrasporangia are zonately divided, 27–65 μm long and 12–42 μm in diameter, and peripherally arranged in conceptacle chamber (Fig. 1J).

Habitat. Growing on bedrock, small stones, or free living in the intertidal and upper subtidal zone.

Distribution. Japan, Korea.

3.2. Phylogenetic analyses

The 866 bp of *psb*A and 686 bases pair (bp) of *rbc*L were sequenced from samples collected in Korea. The phylogenetic trees were constructed by aligning the newly generated *psb*A and *rbc*L sequences with those downloaded from GenBank (Figs. 2, 3). *Phymatolithon purpureum* (P.Crouan & H.Crouan) Woelkerling & L.M.Irvine was included as outgroup. Phylogenetic analyses inferred from *psb*A and *rbc*L indicated that all seventy-six samples of crustose red algae from Korea were nested within *Lithophyllum* clade of the Lithophyllaceae (Figs. 2, 3) and that our *Lithophyllum*-like samples were crusted in two well-supported clades, *L. okamurae* and *L. neo-okamurae*. The genetic divergences between *L. neo-okamurae* and *L. okamurae* are 9.7–11.7% in *psb*A (866 bp) and 14.2–15.4% in *rbc*L (681 bp) respectively. The intraspecific genetic variations between specimens from Korea and the holotype of *Lithophyllum neo-okamurae* are 0–0.3% in the *psb*A region (822 bp) and 0.1–0.5% in the *rbc*L region (681 bp).

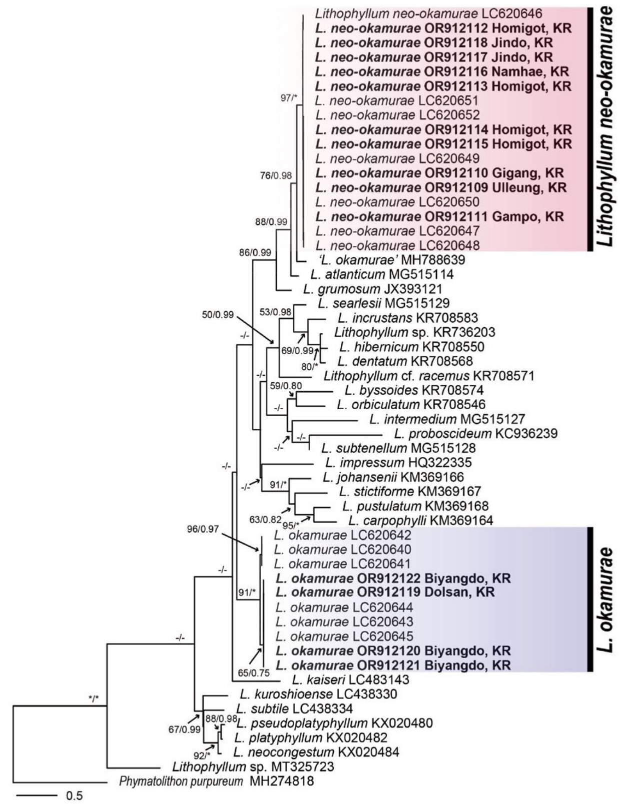

Fig. 3. Phylogenetic tree of *Lithophyllum* species based on ML and Bayesian analysis with *rbc*L gene sequences. Value above branches = Maximum likelihood bootstrap values in % ≥50/Bayesian posterior probabilities ≥0.75. Values lower than BS 50 or BPP 0.75 are indicated by hyphens (–). Values of BPP 1.00 or BS 100 are indicated by asterisks (*). Sequences generated in this study are highlighted in bold.

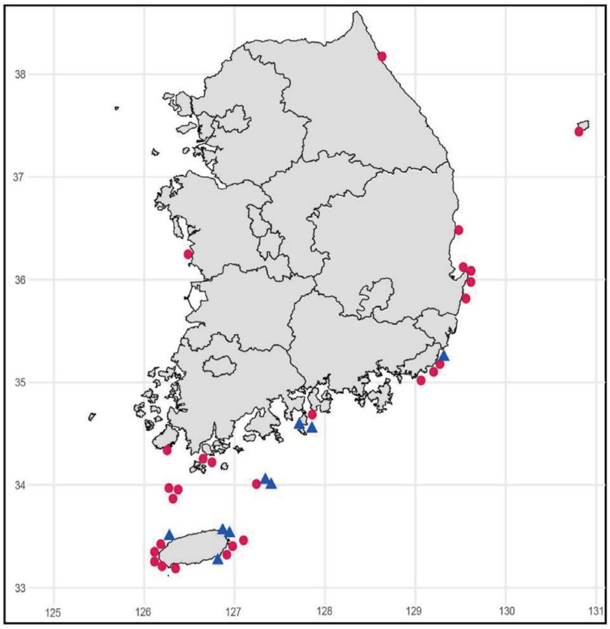

Fig. 4. Geographic distributions of *Lithophyllum okamurae* (▲) and *L. neo-okamurae* (●) in Korea.

4. DISCUSSION

Lithophyllum are known for forming rhodoliths and are commonly found in marine environments, including coral reefs, rocky shores and rhodolith beds (Riosmena-Rodríguez *et al.* 1999; Harvey and Woelkerling 2007). In this study, we add *L. neo-okamurae* with the presence in the catalog of Korean marine flora based on morphological and molecular analyses. Despite the resemblance between *L. neo-okamurae* and *L. okamurae*, *L. neo-okamurae* has been morphologically distinguished by knobby protuberances and larger tetrasporangial conceptacle chambers (Kato *et al.* 2022). However, in our study, the various appearances including long, knobby, tapering, and plate-like protuberances are observed in both of *L. neo-okamurae* and *L. okamurae* collected from Korea. In spite of the larger size of the tetrasporangial conceptacle chamber in *L. neo-okamurae*, there are morphological similarities and size overlap among tetrasporangial conceptacle chambers. Our morphological analysis suggests that the shape of the protuberance and the size of the tetrasporangial conceptacle chamber might not serve as reliable distinguishing characteristics for these species. Emphasizing the necessity of molecular approaches for a definitive distinction between these species, Kato *et al.* (2022) highlighted the shared morphological characteristics that make differentiation challenging. Our molecular analyses based on *psb*A and *rbc*L reveal that there are two distinct clades for *L. neo-okamurae* and *L. okamurae* from Korea with high support values (Figs. 2, 3). Additionally, the gene sequences divergence between the two species is 9.1–11.3% in *psb*A and 14.8–15.3% in *rbc*L respectively. *Lithophyllum okamurae* is recognized as a significant constituent of rhodolith beds within the northwestern Pacific Ocean (Kato *et al.* 2017). It has been well reported as crustose red coralline algae with the presence along the entire coastline of Korea (Lee and Kang 2002). However, our study presents different understanding of its distribution pattern in Korea. *Lithophyllum neo-okamurae* exhibits a distribution that spans the entirety of the Korean coastline, while *L. okamurae* is distributed in the southern region of Korea (Fig. 4). The findings of our study show that it is necessary to collect samples from additional regions of the world where *L. okamurae* has been documented and carry out taxonomic studies to elucidate the distribution of both species.

In conclusion, our study significantly contributes to the marine algal inventory of Korea by documenting the presence of one previously unreported crustose algae: *Lithophyllum neo-okamurae* (Corallinales, Rhodophyta). Our findings will contribute to a more comprehensive understanding of marine biodiversity, distribution pattern, and ecological dynamics of these crustose red algae.

CRediT authorship contribution statement

GW Kim: Formal analysis, Writing-Original draft, Resources. **SY Jeong:** Formal analysis, Writing-Original draft, Resources. **BY Won:** Funding acquisition, Visualization. **TO Cho:** Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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