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Two new records of *Laurencia decussata* and *L. pacifica* from Korea based on morphological structures and molecular data

Paola Romero-Orozco, Boo Yeon Won and Tae Oh Cho*

Department of Life Science, Chosun University, Gwangju 61452, Republic of Korea

Contribution to Environmental Biology

- We have included *Laurencia decussata* and *L. pacifica* in the inventory of macroalgal flora in Korea.
- *Laurencia decussata* has been reported in Australia and New Zealand, and *L. pacifica* in the USA and Mexico; this study expands their distribution to include Korea.

***Corresponding author**
Tae Oh Cho
Tel. 062-230-7161
E-mail. tocho@chosun.ac.kr

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Abstract: *Laurencia* is a red algal genus that was described by J.V. Lamouroux in 1813. The main characteristics of this genus have been known as the presence of four pericentral cells in an axial segment, secondary pit connections between adjacent epidermal cells, and the presence of *corps en cerise* in both epidermal and trichoblast cells. Additionally, the tetrasporangia are arranged in a parallel manner, and male branches feature terminal cup-shaped spermatangial pits. Currently, sixteen Korean *Laurencia* species have been reported based on their morphological characteristics. In this study, *Laurencia decussata* and *L. pacifica* have been added as new records to the Korean algal flora based on a combination of morphological observations and molecular analyses of *rbc*L sequences. *Laurencia decussata* has expanded from Australia and New Zealand to Korea, while the distribution of *L. pacifica* has expanded from USA and Mexico to Korea.

Keywords: *Laurencia pacifica*, *Laurencia decussata*, Phylogeny, *rbc*L, Taxonomy

1. INTRODUCTION

Laurencia is a red algal genus described by J.V. Lamouroux in 1813. It is composed of 133 species distributed in coastal areas and ranging from moderate to tropical climates (Guiry and Guiry 2023). This genus is characterized by following characteristics: 1) apical cell always sunk in apical pit of branchlet; 2) secondary pit connections present between adjacent epidermal cells; 3) four pericentral cells per segment with the first one positioned beneath a trichoblast; 4) *Corps en cerise* found in both epidermal and trichoblast cells; 5) presence of lenticular thickenings in wall of medullary cells in transverse section of branchlets; 6) a parallel arrangement of tetrasporangia; 7) spermatangial branches arising from suprabasal cells of trichoblasts in cup-shaped depressions (Saito 1964; Nam *et al.* 1994; Garbary and Harper 1998; Nam 2006, 2011; Fujii *et al.* 2012).

Laurencia is crucial to investigate the taxonomy and phylogeny of *Laurencia* species to better understand their ecological and evolutionary relationships and to identify valuable sources of bioactive compounds because its wide range of bioactive compounds with potential applications in pharmaceuticals and biotechnology (Harizani *et al.* 2016; Guiry and Guiry 2023). However, its species identification using morphological characters has been difficult due to the challenges of obtaining reproductive specimens and the limited observation of the anatomical features from herbari-

um specimens. Recently, molecular analysis plays an important role in the systematic reassessment of this genus (Rousseau *et al.* 2017). The genus *Laurencia* is composed of 133 species worldwide (Guiry and Guiry 2023). Of them, 16 species have been reported from Korea including two endemic species based on morphology (Lee and Kang 2001; Nam 2011): *L. brongniartii* J. Agardh, *L. chinensis* C.K. Tseng, *L. composita* Yamada, *L. dendroidea* J. Agardh, *L. glandulifera* (Kützing) Kützing, *L. hamata* Yamada, *L. intercalaris* K.W. Nam, *L. intricata* J.V. Lamouroux, *L. nidifica* J. Agardh, *L. nipponica* Yamada, *L. obtusa* (Hudson) J.V. Lamouroux, *L. okamurae* Yamada, *L. pinnata* Yamada, *L. succulenta* K.W. Nam, *L. tropica* Yamada, and *L. venusta* Yamada (Lee and Kang 2001; Nam 2011). However, these are not available to access molecular data for any of these species from Korea yet.

We collected the unidentified samples of *Laurencia*-like specimens from intertidal zones along the coast of Korea from 2009 to 2022. We observed their morphological characters and analyzed molecular data based on the plastid-encoded *rbc*L gene to investigate the phylogenetic relationships. In this study, we have added two *Laurencia* species, *L. decussata* and *L. pacifica*, to the Korean algal flora based on morphological and molecular data.

2. MATERIALS AND METHODS

2.1. Molecular analysis

Genomic DNA from a total 17 samples was extracted using either NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) or the DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA). The chloroplast-encoded *rbc*L was amplified using the following primer combinations: FrbcLstart-R753, F645-RrbcSstart, or F57-R753, F577-R1150, and F993-RrbcSstart (Freshwater and Rueness 1994; Lin *et al.* 2001). PCR amplification was conducted using a Veriti 96-well Thermal cycler (Applied Biosystem). The PCR amplification protocol consisted of an initial denaturation step at 94°C for 1 min, followed by 2 cycles of 1 min at 94°C, 1 min at 40°C, and then 40 cycles of 1 min at 94°C, 30 s at 42°C, and 1 min at 68°C, with a final extension step of 5 min at 72°C (Gavio and Fredericq 2002, modified).

Total 95 sequences including 78 GenBank sequences were edited and aligned using Geneious Prime 2022.02, resulting a final alignment of 1,216 bp. Bayesian inference was performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using Metropolis coupled Markov chain Monte Carlo (MCMC). To evaluate posterior probabilities, we conducted two runs each with four chains (one cold chain and three heat), for 2,000,000 generations, sampling tree every 1,000 generations employing the GTR+Γ+I evolutionary model. A burn-in value of 25% was used to avoid suboptimal trees in the final consensus tree. Maximum likelihood analyses were performed using raxmlGUI 2.0 (Edler *et al.* 2021) with the GTR+G+I model, and 1,000 bootstrap replicates were used for statistical support. Interspecific pairwise distance was estimated using the p-distance model in MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura *et al.* 2021).

2.2. Morphological observations

Samples were collected from intertidal areas along the coastlines of Korea. Samples were photographed using a digital camera (Nikon D40; Nikon, Japan) and a stereomicroscope (SZX7; Olympus, Tokyo, Japan) for external morphology. Internal morphological observations were conducted using fresh material, rehydrated herbarium specimens, or samples preserved in a solution of 4% formalin and seawater. To examine the specimen's thallus, both cross and longitudinal sections were prepared using a stainless-steel razor blade and freezing microtome in 8−10 μm thickness (Shandon Cryotome FSE, Thermo Shandon, Ltd., Loughborough, UK). The sliced samples were transferred to a slide with distilled water and then stained with aniline blue. The stained sections were subsequently examined, and images were captured using an Olympus DP27 camera attached to an Olympus microscope (BX51TRF; Olympus, Tokyo, Japan). The measurements of the sections were made using the ImageJ software (Schneider *et al.* 2012).

3. RESULTS

3.1. Phylogenetic analyses

The alignment consisted of 1,216 base pairs to examine the relationship between the genus *Laurencia* and

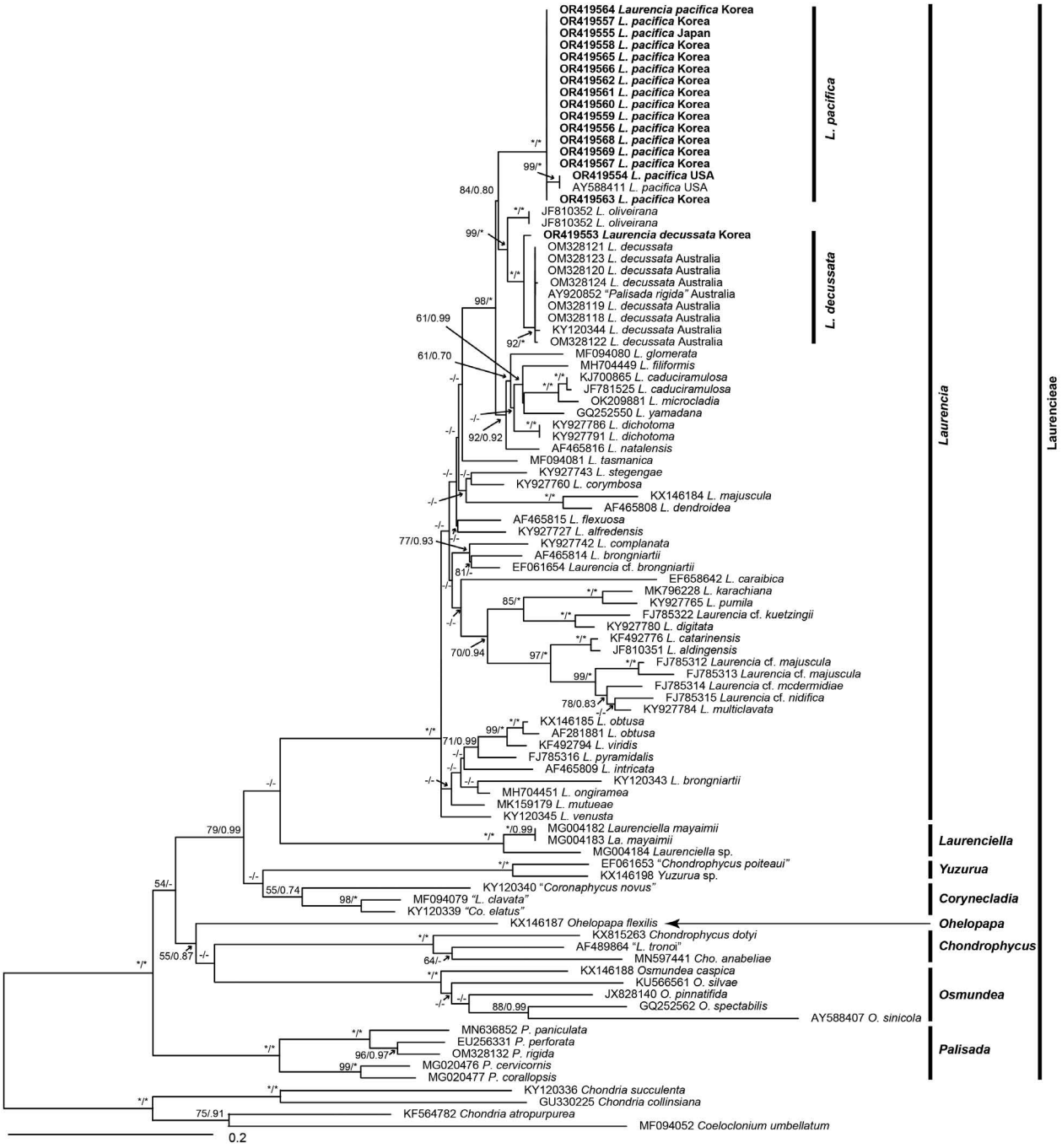

Fig. 1. Phylogenetic tree based on maximum likelihood and Bayesian analysis with *rbc*L sequences. The numerals assigned to the internal nodes represent bootstrap values obtained from 1,000 maximum likelihood bootstrap (BS) replicates and Bayesian posterior probability (BPP) analysis. Values positioned above the branches correspond to likelihood bootstrap values of ≥50% and BPP ≥0.75. Hyphens (-) denote values lower than 50% BS or 0.75 BPP, while asterisks (*) indicate values of 100 BS or 1.00 BPP.

related members in the tribe Laurencieae. The phylogenetic tree revealed that our *Laurencia*-like samples were nested in genus *Laurencia* and crusted in two well-supported clades (Fig. 1). The first clade consists of 16 generated sequences from Korea and grouped together with a GenBank sequence of *L. pacifica* near

the type locality. The second clade included the one remaining sequence from Korea and clustered with *L. decussata*. The gene sequence divergence within *L. pacifica* is from 0% to 0.6%, while *L. decussata* from 0% to 0.8%.

3.2. Morphological observations

Class Florideophyceae Cronquist, 1960 진정홍조강
Order Ceramiales Nägeli, 1847 비단풀목
Family Rhodomelaceae Horaninow, 1847
빨간검둥이과
Genus *Laurencia* J.V. Lamouroux, 1813 서실속

***Laurencia decussata* (A.B. Cribb) Metti 2022: 21 (Fig. 2)**

Holotype. BRI No. 3.1. AQ712542, collected on 11 Aug. 1948 from Miami, Queensland, Australia.
Type locality. Miami, Queensland, Australia (Cribb 1958)
Material examined. NIBRRD0000010616 (deposited in the National Institute of Biological Resources), PH464, Janggil-ri Complex Fishing Park, Janggil-ri, Guryongpo-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do, Korea (35°57′5.56″N, 129°32′52.40″E), January 8, 2022, T.O.Cho and B.Y.Won, at 1 m depth by hand.
Vegetative morphology. Thalli are red to dark purple, forming clumps of upright axes, percurrent growth habit, and 2–4 cm in height (Fig. 2A). The holdfast consists of densely intertwined stolons forming a complex network that gave rise to numerous upright thalli (Fig. 2B, C). The main axes are terete, columnar with a decussate opposite branching pattern, and 0.7–1.8 mm in diameter. The branching order is three or four (Fig. 2C). Ultimate branches are 1–3.5 mm in length. Branchlets on ultimate branch are closely pressed against the supporting axes appressed and 0.5–1.4 mm in length (Fig. 2C). The surface of thallus reveals the presence of secondary pit connections among the epidermal cells and the presence of one corps en cerise per cell with 7–16 μm in diameter (Fig. 2D). Thalli are composed of cortical and medullary structures (Fig. 2E). Four pericentral cells are produced from axial cell (Fig. 2F). The cortical cells are oblong shape lacking palisade arrangement and have undulate margin near apex and distinct coloration (Fig. 2G, H). They are not projected. The medullary cells are colorless, isodiametric, oblong, and have lenticular thickness (Fig. 2G, H). Apical pits of branches and branchlets contain short trichoblasts (Fig. 2I).
Habitat. Found at lower intertidal to subtidal (4 meters deep), on exposed and semi-exposed shores, always on rocky substrates.
World Distribution. Australia, New Zealand (Guiry and Guiry 2023), Korea (this study).

***Laurencia pacifica* Kylin 1941: 42 (Fig. 3)**

Holotype. Collected in June of 1922 by Kylin in La Jolla, California.
Type locality. La Jolla and Pacific Grove, California, USA (Kylin 1941).
Material examined. NIBRRD0000010617 (deposited in the National Institute of Biological Resources), TC854, Monterey Bay, south of the San Francisco Bay Area, California, United States (36°54′13.62″N 121°50′42.37″W), July 14, 2003, T.O.Cho and B.Y.Won, at 1 m depth by hand; TC4140, Hirakawa, Aomori Prefecture, Japan (31°55′13.13″N 130°13′13.59″E) April 19, 2008, T.O.Cho, at 1 m depth by hand; TC6664, Dangin-ri, Gunoe-myeon, Wando-gun, Jeollanam-do, Korea (34°19′43.41″N, 126°39′25.49″E), August 18, 2009, T.O.Cho, S.Y.Jeong, and J.K.Lee, at 1 m depth by hand; TC6669, Dangin-ri, Gunoe-myeon, Wando-gun, Jeollanam-do, Korea (34°19′43.41″N, 126°39′25.49″E), August 19, 2009, T.O.Cho, S.Y.Jeong, and J.K.Lee, at 1 m depth by hand; TC6734, Taprip-gil, Imhoe-myeon, Jindo-gun, Jeollanam-do, Korea (34°22′42.96″N, 126°14′37.71″E), August 20, 2009, T.O.Cho, S.Y.Jeong, and J.K.Lee, at 1 m depth by hand; TC7424, Sinchon-ri, Jocheon-eup, Jeju-si, Jeju-do, Korea (33°32′17.66″N, 126°37′1.69″E), June 28, 2011, T.O.Cho, S.Y.Jeong, Danilo, H.R.Lee and M.R.Yoo, at 1 m depth by hand; TC7458, Lighthouse, Yeonpyeong-ri, Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′48.26″N, 126°58′6.47″E), June 29, 2011, T.O.Cho, S.Y.Jeong, Danilo, H.R.Lee and M.R. Yoo, at 1 m depth by hand; TC9649, Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′48.26″N, 126°58′6.47″E) April 4, 2013, T.O.Cho, at 1 m depth by hand; TC9650, Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′48.26″N, 126°58′6.47″E) April 5, 2013, T.O.Cho, at 1 m depth by hand; TC9881, Chuja Port, Chuja-myeon, Jeju-si, Jeju-do, Korea (33°57′17.94″N, 126°17′30.5″E), June 24, 2013, T.O.Cho, at 1 m depth

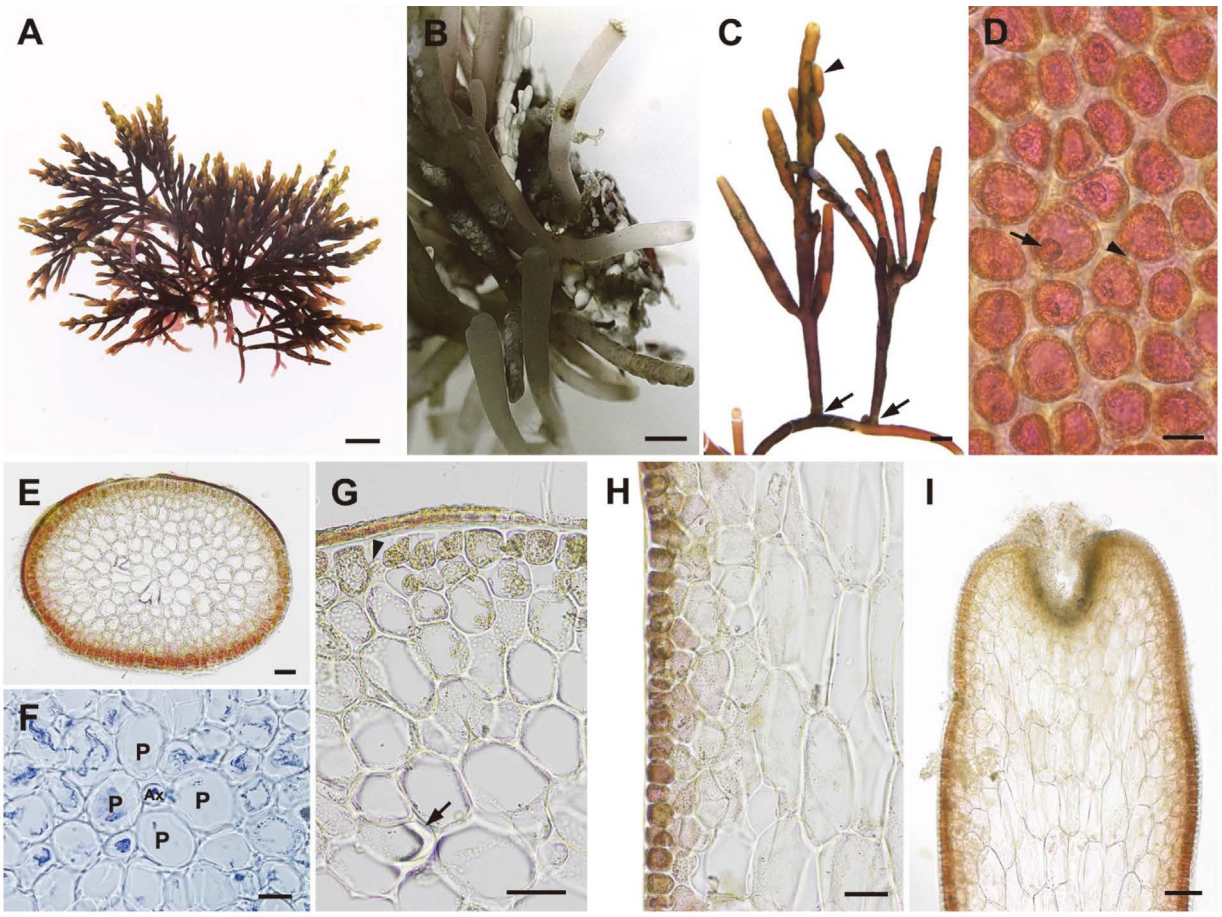

Fig. 2. *Laurencia decussata* from Korea. A. Habit of the sterile thallus. B. Stoloniferous holdfast. C. New thalli, showing stolons (arrows) and the last branchlets (arrowhead) pressed closely to the supporting axes. D. Surface view of the thallus, showing the *corps en cerise* (arrow) and secondary pit connection (arrowhead). E. Cross-section view of the middle thallus. F. Cross-section view, showing 4 pericentral cells (P) produced from an axial cell (Ax). G. Cross-section view, showing cortical cells with secondary pit connections (arrowhead) and lenticular thickening (arrow). H. Longitudinal section view. I. Longitudinal section view, showing short trichoblasts emerging from the apical pit. Scale bars: A = 0.5 cm, B, C = 1 mm, D = 20 μm, E, I = 100 μm, F–H = 50 μm.

by hand; TC9882, Chuja Port, Chuja-myeon, Jeju-si, Jeju-do, Korea (33°57′17.94″N, 126°17′30.5″E), June 24, 2013, T.O.Cho, at 1 m depth by hand; TC9933, Yecho-ri, Chuja-do, Chuja-myeon, Jeju-do, Korea (33°57′13.57″N, 126°19′52.16″E) June 24, 2013, T.O. Cho, at 1 m depth by hand; TC12011, Lighthouse, Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′32.58″N, 126° 57′29.96″E) May 29, 2014. T.O.Cho, S.Y.Jeong, D.B.M., and J.G.Lee, at 1 m depth by hand; TC12222, Light-house, Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′ 32.58″N, 126°57′29.96″E) May 29, 2014. T.O.Cho, S.Y.Jeong, D.B.M., and J.G.Lee, at 1 m depth by hand; TC12607, Myeongsa Beach, Nambu-myeon, Geoje-si, Gyeongsangnam-do, Korea (34°43′36.45″N, 128°36′ 12.58″E) July 12, 2014, T.O.Cho, S.Y.Jeong, D.B.M., J.G.Lee, and S.Y.Park, at 1 m depth by hand; TC12862, Gyeongsangbuk-do, Uljin-gun, Uljin-eup, Yeonji-ri, Korea (37°0′8.17″N, 129°25′3.27″E), August, 1, 2018, T.O.Cho. S.Y.Jeong, D.B.M., J.G.Lee and S.Y.Park, at 1 m depth by hand; TC14784, Seopjikoji, Seong-san-eup, Seogwipo City, Jeju-si, Korea (33°25′26.0″N, 126°55′55.3″E), May 5, 2015, T.O.Cho, S.Y.Jeong, J.G.Lee and S.Y.Park, at 1 m depth by hand; TC14800, Seopjikoji, Seongsan-eup, Seogwipo City, Jeju-si, Korea (33°25′27.0″N, 126°55′55.4″E), May 5, 2015, T.O.Cho, S.Y.Jeong, J.G.Lee and S.Y.Park, at 1 m depth by hand; PH038, Gampo, Gyeongju-si, Gyeongsang-buk-do, Korea (35°48′27.5″N, 129°30′29.6″E), May 19,

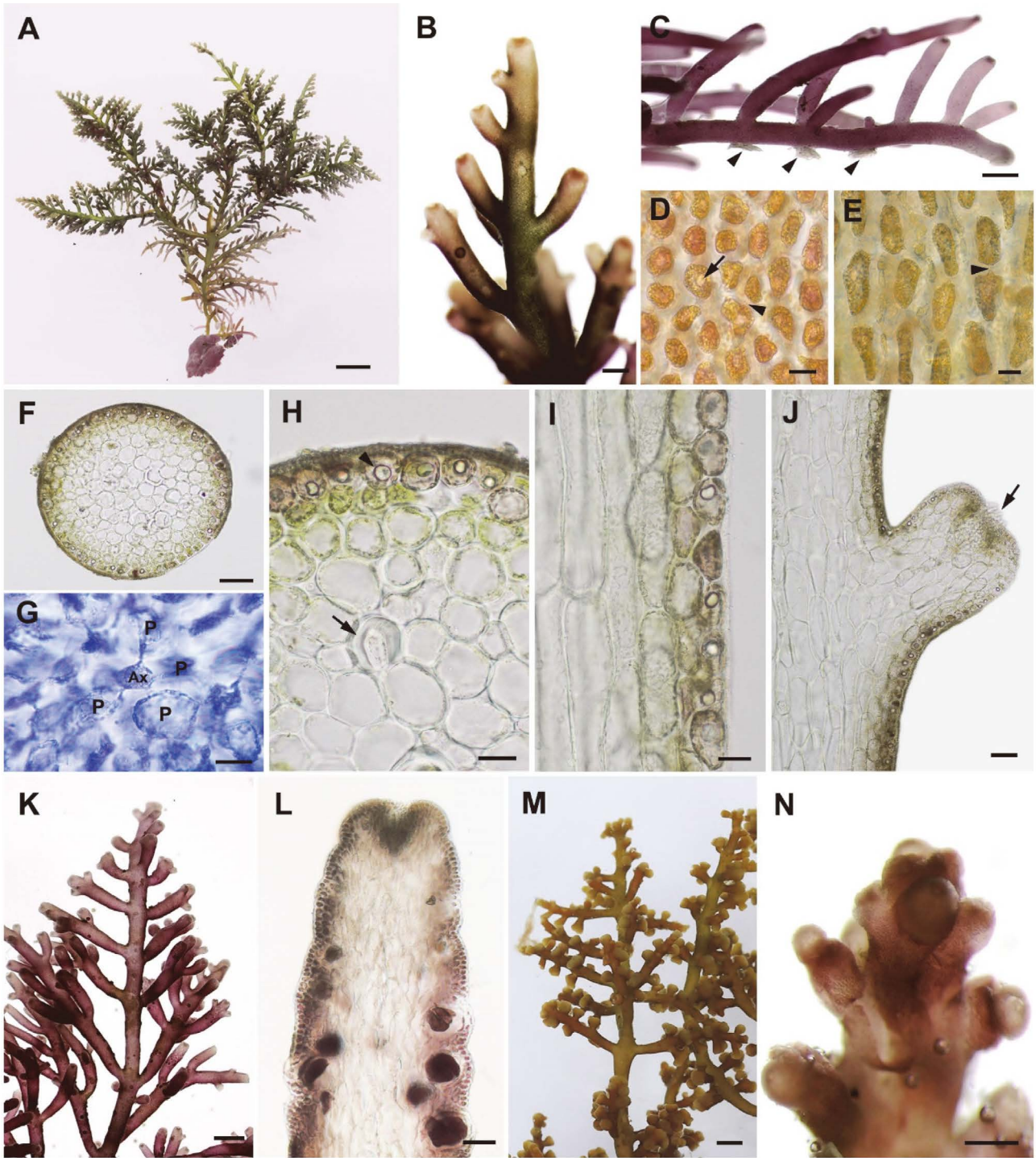

Fig. 3. *Laurencia pacifica* from Korea. A. Habit of the sterile thallus. B. Upper part of thallus with terete and irregular branchlets. C. New branches produced from stoloniferous holdfasts (arrowheads). D–E. Surface view of upper (D) and lower (E) thalli, showing the *corps en cerise* (arrow) and secondary pit connection (arrowhead), F. Cross-section view of the middle thallus. G. Cross-section view, showing 4 pericentral cells (P) produced from an axial cell (Ax). H. Cross-section view, showing the *corps en cerise* (arrowhead) and lenticular thickening (arrow). I. Longitudinal section view. J. Longitudinal section view, showing short trichoblasts (arrow) emerging from the apical pit. K. Tetrasporic thallus. L. Longitudinal section view of the tetrasporangial branchlet, showing the parallel arrangement of tetrasporangia. M. Carposporic thallus. N. Cystocarpic branchlet, showing rounded cystocarps. Scale bars: A = 0.5 cm, B, C, N = 0.5 mm, D, E = 20 μm, F, J = 50 μm, G–I = 25 μm, K, M = 1 mm, L = 100 μm.

2021, T.O.Cho and B.Y.Won, at 1 m depth by hand; PH287, 903-7 Jungnim-ri, Imhoe-myeon, Jindo-gun, Jeollanam-do, Korea (34°22′39.77″N, 126°14′36.33″E), October 3, 2021 T.O.Cho and B.Y.Won, at 1 m depth by hand; PH832, Gampo, Gyeongju-si, Gyeongsangbuk-do, Korea (35°48′27.5″N 129°30′28.6″E), July 2, 2022, T.O.Cho and B.Y.Won, at 1 m depth by hand.

Vegetative morphology. Thalli are brownish red to purple, upright, percurrent growth habit, and 2−11 cm in height (Fig. 3A). The main axes are terete, cylindrical with subopposite or subverticillate branching pattern, and 0.3−1.1 mm in thickness (Fig. 3B). The branching order is three or four. Ultimate branchlets are turbinate and 1−3 mm in length. The holdfast is discoid and attaches to substratum by numerous stoloniferous branches with small discs (Fig. 3C). One or more erect axes arise from basal creeping branches (Fig. 3C). The surface of thallus reveals the presence of secondary pit connections among the epidermal cells and the presence of one corps en cerise per cell with 7−16 μm in diameter (Fig. 3D, E). Thalli are composed of cortical and medullary structures (Fig. 3F). Four pericentral cells are produced from axial cell (Fig. 3G). The cortical cells are round shape lacking the formation of a palisade-like layer and display distinct coloration (Fig. 3H, I). They are not projected. The medullary cells are colorless and oblong and have annular and lenticular thickness (Fig. 3H, I). Apical pits of branches and branchlets contain short trichoblasts (Fig. 3J).

Reproductive structures. Tetrasporic thallus is 4.5−16.4 cm in height, 585−763 μm in diameter, and presenting a similar branching pattern and coloration to the vegetative thallus (Fig. 3K). In young tetrasporophyte, the branchlets are lanceolate, short, 0.3−0.9 mm in length, and arranged in a verticillate pattern. In mature tetrasporophyte, the branchlets are cylindrical, 0.6−1.9 mm in length (Fig. 3K). The tetrasporangia exhibit a parallel arrangement in a longitudinal section (Fig. 3L). In female gametophyte, thallus is 3 cm in height and presenting a similar branching pattern and coloration to the vegetative thallus (Fig. 3M). The cystocarps are located on upper branchlets, round shape without protuberant ostioles, and 863−923 μm in size. (Fig. 3N). Male gametophytes were not observed.

Habitat. Frequently encountered upon rocky substrates in the lower intertidal zone.

World Distribution. USA California and Mexico (Guiry and Guiry 2023), Japan and Korea (this study).

4. DISCUSSION

The genus *Laurencia* described by J.V. Lamouroux, includes intertidal and upper subtidal species found globally (Guiry and Guiry 2023). Although *Laurencia* has distinct taxonomic group within the family Rhodomelaceae, the identification of *Laurencia* species is challenging due to substantial intraspecific morphological variation (Cassano *et al.* 2012; Machín-Sánchez *et al.* 2012; Metti *et al.* 2013). In this study, we have added *Laurencia decussata* and *Laurencia pacifica* as new records in the list of Korean algal flora based on molecular and morphological analyses.

Laurencia decussata was originally described as *L. heteroclada* f. *decussata* from Australia by A.B. Cribb in 1958. Metti (2022) was reevaluated as an independent species, named *L. decussata*, based on morphological and molecular analyses. Its distribution has been known from Australia to New Zealand (Guiry and Guiry 2023). In our phylogenetic analyses of *rbc*L, *L. decussata* from Pohang, Korea was clustered with sequences from Australia and exhibited 0% to 0.8% of genetic divergence (Fig. 1). Furthermore, our Korean collection of *L. decussata* has the same morphological characteristics to the designated type material: intertwined stolon holdfasts, 3 to 4 ordered branching patterns, cylindrical branches with closely pressed branchlets, and oblong epidermal cells with undulating margins (Cribb 1958; Metti 2022; Guiry and Guiry 2023).

Laurencia pacifica was proposed by Kylin in 1941 based on collection from California (Harvey 1852). Its distribution has been known from California to Mexico (Guiry and Guiry 2023). In phylogenetic analyses of *rbc*L, the genetic divergence of *L. pacifica* was identical between our sequence from the sample of California (near the type locality) and GenBank sequence. However, there is a genetic divergence of 0% to 0.4% between samples from Korea and California. Our Korean collection of *L. pacifica* has the same morphological characteristics to the type material: cortical cells with a singular *corps en cerise* structure per cell, discoid holdfast with stolon, tetrasporangia forming dense clusters, round-shaped cystocarps without protuberant ostioles (Harvey 1852; Kylin 1941; Abbott and Hollenberg 1992).

Laurencia decussata and *L. pacifica* are similar with several *Laurencia* species reported in Korea (Table 1).

Table 1. Comparison of the morphological characteristics of *Laurencia* species with morphological characteristics from Korean specimens.

Features	*Laurencia decussata*	*L. pacifica*	*L. composita*	*L. glandulifera*	*L. intercalaris*	*L. nidifica*	*L. nipponica*	*L. obtusa*	*L. okamurae*	*L. tropica*
Type locality	Australia	USA	Japan	Italy	Korea	Hawaiian Islands	Japan	Southern England	Japan	Saipan, Marian Island
Thallus color	Light orange-red to dark purple	Deep reddish-purple	Greenish or dark purple	Bright orange to deep red	Brown or yellowish brown	Pale green with pink tips.	Brown or purplish red	Purplish pink, deep brown, or pale green	Purplish green, pale green, brown	Dark brown to brownish red
Thallus height	2–7 cm	6–30 cm	6–14 cm	up to 7 cm	up to 10 cm	up to 10 cm	30–40 cm	6–10 cm	up to 20 cm	up to 10 cm
Texture	Cartilaginous, not rigid	Cartilaginous, not rigid	Soft	Soft	fleshy to subcartilaginous, soft	cartilaginous, not very rigid	subcartilaginous, not so firm	flesh and soft	fleshy to cartilaginous, not rigid	flesh and soft
Thallus attachment	Composed of a dense mass of stolons	Discoid holdfast with postrate stolon-like branches	Small Discoid holdfast with stolon-like branches	Discoid holdfast with postrate stolon-like branches	Fibrous accessory branches	Stolon-like branches	Numerous stoloniferous branches	Discoid holdfast	Discoid holdfast with stolon-like branches	Discoid holdfast
Branching pattern of ultimate branches	pressed against the supporting branch	turbinate	spiral	sub-verticillate	regularly alternate	spiral	turbinate	sub-opposite, or sub-verticillate	alternate	radial
Lenticular thickening	Rarely found	Presence to Rarely found	Rarely found	Absence	Presence	Presence	Presence to Rarely found	Absence	Presence	Absence
Projection of the epidermal cell	Slightly-none	Slightly-none	Absent	Slightly	Slightly-none	Absent	Slightly-none	Slightly-none	Absent	Slightly-none
Spermatangial pit width	–	–	320–1,020 μm	–	380–580 μm	700–800 μm	860–1,500 μm	700–900 μm	400–900 μm	300–450 μm
Spermatangia nucleous position and intercalary formation	Apical	–	Apical	–	Apical, present	Apical	Central, absent	Apical, absent	Apical, absent	Apical
Cystocarps	Ovoid, circular with non-protuberant ostiole	Ovoid with non-protuberant ostiole	Ovoid with non-protuberant ostiole	Ovoid with protuberant ostiole	–	Ovoid with protuberant ostiole	Ovoid with non-protuberant ostiole	Ovoid with non-protuberant ostiole	Ovoid with protuberant ostiole	Ovoid with non-protuberant ostiole
Cystocarp diameter	–	1,000–1,200 μm	550–740 μm	500–700 μm	–	600–700 μm	600–1,000 μm	800–830 μm	472–820 μm	700–800 μm
Tetrasporangia branching pattern	Compound, numerous	Verticillate clusters	Straightforward from unexpected branches.	Sub-verticillate arranged	–	–	–	–	–	–
References	Cribb (1958), Metti (2022), This study	Abbott and Hollenberg (1992), This study	Oliveira-Filho (1969), Masuda *et al.* (1996), Nam (2011)	Cecere *et al.* (1996), Rindi *et al.* (1996), Furnari *et al.* (2016)	Nam (1994), Nam (2011)	McDermid (1988), Wynne *et al.* (2005), Nam (2011)	Nam *et al.* (1991), Abe *et al.* (1997), Nam (2011)	Nam (1994), Nam (2011)	Saito (1967), Masuda *et al.* (1996), Nam (2011)	Nam (2011)

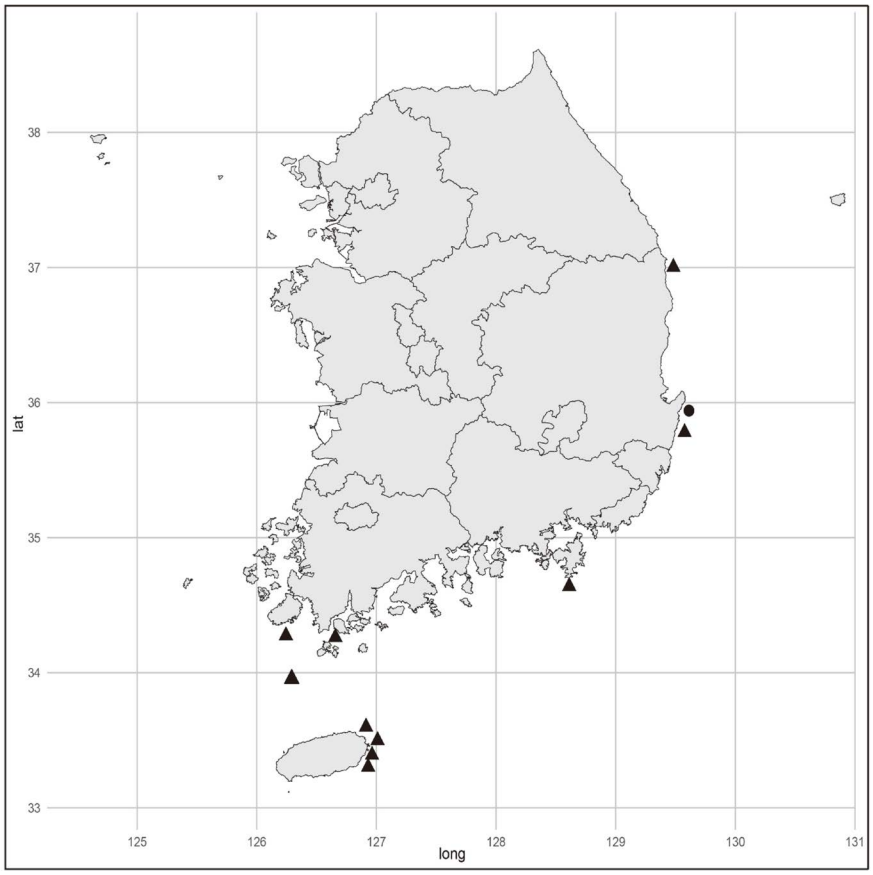

Fig. 4. Geographic distribution of *Laurencia decussata* (●) and *L. pacifica* (▲) in Korea.

However, *Laurencia decussata* is distinguished from *L. composita*, *L. glandulifera*, *L. obtusa* and *L. tropica* by having dense mass of stolons (Cecere *et al.* 1996; Nam 2011). It also differs from *L. intercalaris*, *L. nipponica*, *L. okamurae*, and *L. nidifica* by unique decussate opposite branching pattern, pressed branchlets against the supporting axes (Cribb 1958; McDermid 1988; Metti 2022). *Laurencia pacifica* differs from *L. intercalaris*, *L. nidifica*, *L. nipponica*, and *L. okamurae* by discoid holdfast with numerous stolon branches (McDermid 1988; Nam *et al.* 1991; Nam 1994, 2011). *Laurencia pacifica* is also distinguished from *L. composita*, *L. obtusa*, *L. glandulifera* and *L. tropica* by the presence of annular and lenticular thickness (Cecere *et al.* 1996; Abe *et al.* 1998; Nam 2011). Additionally, it differs from *L. glandulifera* and *L. nidifica* by ovoid cystocarp without a protuberant ostiole (Abbott and Hollenberg 1992; Cecere *et al.* 1996; Nam 2011).

Based on molecular and morphological analyses, we confirm that our samples are recognized as new record species from Korea, *Laurencia decussata* and *L. pacifica*. *Laurencia decussata* is distributed in eastern coast of Korea, while *L. pacifica* along the southern and eastern coasts of Korea (Fig. 4). In addition, this study shows that distribution of *L. decussata* has expanded from Australia and New Zealand to Korea and *L. pacifica* has distributed from USA and Mexico to Korea. These findings highlight the broader geographic range of these species and contribute to our understanding of their global distribution.

CRediT authorship contribution statement

P Romero-Orozco: Data analyses, Writing - Original draft. **BY Won:** Funding acquisition, Visualization, Writing - Review & editing. **TO Cho:** Funding acquisition, Supervision, Writing - Review & editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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