



New record of the red algae, *Halarachnion parvum* (Gigartinales) and *Champia lubrica* (Rhodymeniales), from Korea

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Abstract

We report the first finding of *Halarachnion parvum* and *Champia lubrica* from Korea based on morphology and the plastid *rbcL* sequence analyses. *H. parvum* occurs in the subtidal zone of Munseom, the southern part of Jeju. Thalli have short stipe, and elliptical to ovate fronds with marginal proliferations of up to 3 cm in height. *H. parvum* has zonately divided tetrasporangia and cystocarp immersed under the cortical layer. *Champia lubrica* appears in Namhae, Gyeongnam and Seopseom, Jeju. Thalli are erect, irregularly branched, terete, obtuse apex, up to 3–5 cm high, and have tetrahedrally divided tetrasporangia. Molecular analyses of the plastid *rbcL* gene reveal that two species are clearly separated from other species of their respective genera. *H. parvum* is sister with *Halarachnion latissimum* in 3.1–3.2% sequence divergence, and *C. lubrica* is closely related to the sample from Japan with 0.2% sequence divergence.

Key words: *Champia lubrica*, *Halarachnion parvum*, morphology, *rbcL*, Rhodophyta, taxonomy

INTRODUCTION

Red algae is one of the major group of marine algae, including about 7,000 species (Guiry and Guiry 2015), and predominates along the coastal and continental shelf areas of the ocean (Lüning 1990, Norris 2014). Meanwhile, the group of red algae has been well known as a difficult taxon to identify owing to their variable morphology and lack of conspicuous diagnostic characteristics (Saunders 2005). Cryptic species have often not been recognized as distinct species despite genetically different biological units (Saunders and Lehmkuhl 2005). Consequently, the approach of a molecular-assisted alpha taxonomy to identify species is increasing in use, which could help to confirm the species diversity of algal flora (Suzuki et al. 2013, Arakaki et al. 2014, Yang and Kim 2015).

Halarachnion Kützinger belongs to the family Furcellariaceae (Gigartinales), including five species that are

restricted to boreal regions in the north Atlantic and the north Pacific (Guiry and Guiry 2015). *Halarachnion*, typified with *H. ligulatum* (Woodward) Kützinger, is characterized by flattened frond, often marginally and superficially proliferous, tubular at the apex, and intercalary cells that are usually distended to one side (Knauss and Hommersand 1989). Only *Halarachnion latissimum* Okamura has been listed to date in the Korean algal inventory of marine algae (Oak et al. 2002).

Champia Desvaux, a genus of Champiaceae (Rhodymeniales), consists of 37 species that are distributed from subarctic to tropical regions of the world (Guiry and Guiry 2015). It is characterized by a hollow thallus in widely separated longitudinal filaments line, segments separated by monostromatic septa or diaphragms, multiaxial construction, and fully elevated cystocarp in having two-

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celled auxiliary cell branch from a supporting cell (Womersley 1996). In Korea, four species of *Champia* have been reported: *C. bifida* Okamura, *C. expansa* Yendo, *C. inkyua* Y.H. Koh, G.Y. Cho and M.S. Kim, and *C. recta* Noda (Koh et al. 2013).

There are about 590 species of red algae listed in the Korean algal inventory, less than the number (about 950) of Japanese red algal flora species (Masuda et al. 2006). In an effort to investigate the species diversity, several species in many groups of red algae have been added to the Korean marine algal inventory (Kim et al. 2012, Kang and Kim 2013, Koh et al. 2013, Jeong et al. 2013, Kim and Kim 2014, Lee and Kim 2014, Yang et al. 2015, Yang and Kim 2015). Our recent surveys along the Korean coasts revealed two unrecorded red algal species: *Halarachnion parvum* Yamada and *Champia lubrica* Mas. Suzuki & Yoshizaki. In order to report these two unknown species in Korea, we analyzed morphology and molecular analyses using *rbcL* gene.

MATERIALS AND METHODS

Specimens were collected from intertidal or subtidal zone using Scuba diving (Table 1). Field-collected samples were first kept fresh in a cool box with seawater, and they were transported to the laboratory. Samples for DNA analyses were individually assigned identification numbers, and we detached a small piece from each to put in silica gel for desiccation. Voucher herbarium specimens were deposited in the Herbarium of Jeju National University (JNU). Sections were cut by hand with a razor blade or prepared by using a bench-top freezing microtome (MFS no. 222; Nippon Optical Works, Tokyo, Japan).

Sections were stained with 1% aniline blue acidified with 1% HCl, and mounted in 40% corn syrup solution. Photomicrographs were taken by BX 43 microscope (Olympus, Tokyo, Japan) with an EOS 600D digital camera (Canon, Tokyo, Japan). The images of pressed specimens were captured using a D 80 camera (Nikon, Tokyo, Japan). The digitized images of the specimens were imported into the Adobe Photoshop ver. 6.1 software (Adobe Systems Inc., San Jose, CA, USA) program and edited to produce the plates.

Seven specimens of *Halarachnion parvum* and two of *Champia lubrica* were used for molecular analyses. For the extraction of total DNA from the silica-gel dried specimens, we used the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol, and the extracted DNA was stored at -20°C . We used the following primer combinations: to amplify forward of *rbcL* gene, *rbcLF145-rbcLR898* (Kim et al. 2010) for *Halarachnion parvum* and *rbcLF7-R753* (Gavio and Fredericq 2002, Freshwater and Rueness 1994) for *Champia lubrica*. We used the primer to amplify reverse directions of the rear part of *rbcL* gene: *rbcLF762-rbcLR1442* (Kim et al. 2010) for *Halarachnion parvum* and *CrbclRF725-CrbclLR1407* (Koh et al. 2013) for *Champia lubrica*. For PCR amplifications, we used Swift MaxPro thermal cyclers (ESCO, Singapore) with an AccuPower PCR PreMix (Bioneer, Daejeon, Korea). PCR reactions for *rbcL* amplification were carried out with an initial denaturation at 96°C for 4 min, followed by 35 cycles of amplification (denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and an extension at 72°C for 2 min) with a final extension at 72°C for 7 min. PCR products were purified using the Accuprep PCR Purification Kit (Bioneer) according to the manufacturer's instructions. Sequencing of the forward

Table 1. List of specimens used in the molecular study and GenBank accession number

Taxa / Voucher	Collection data	Accession No.
<i>Champia lubrica</i> Mas. Suzuki & Yoshizaki		
CP120501	Namhae, Gyeonam, Korea; 20 May 2012	KT880066
CHCO110601	Seopseom, Jeju, Korea; 17 Jun 2011	KT880067
<i>Halarachnion parvum</i> Yamada		
140516-04	Munseom, Jeju, Korea; 16 May 2014	KT880071
140813-10	Munseom, Jeju, Korea; 13 Aug 2014	KT880069
140813-12	Munseom, Jeju, Korea; 13 Aug 2014	KT880068
140813-13	Munseom, Jeju, Korea; 13 Aug 2014	KT880070
150103-09	Munseom, Jeju, Korea; 03 Jan 2015	KT880072
150103-10	Munseom, Jeju, Korea; 03 Jan 2015	KT880073
150103-11	Munseom, Jeju, Korea; 03 Jan 2015	KT880074

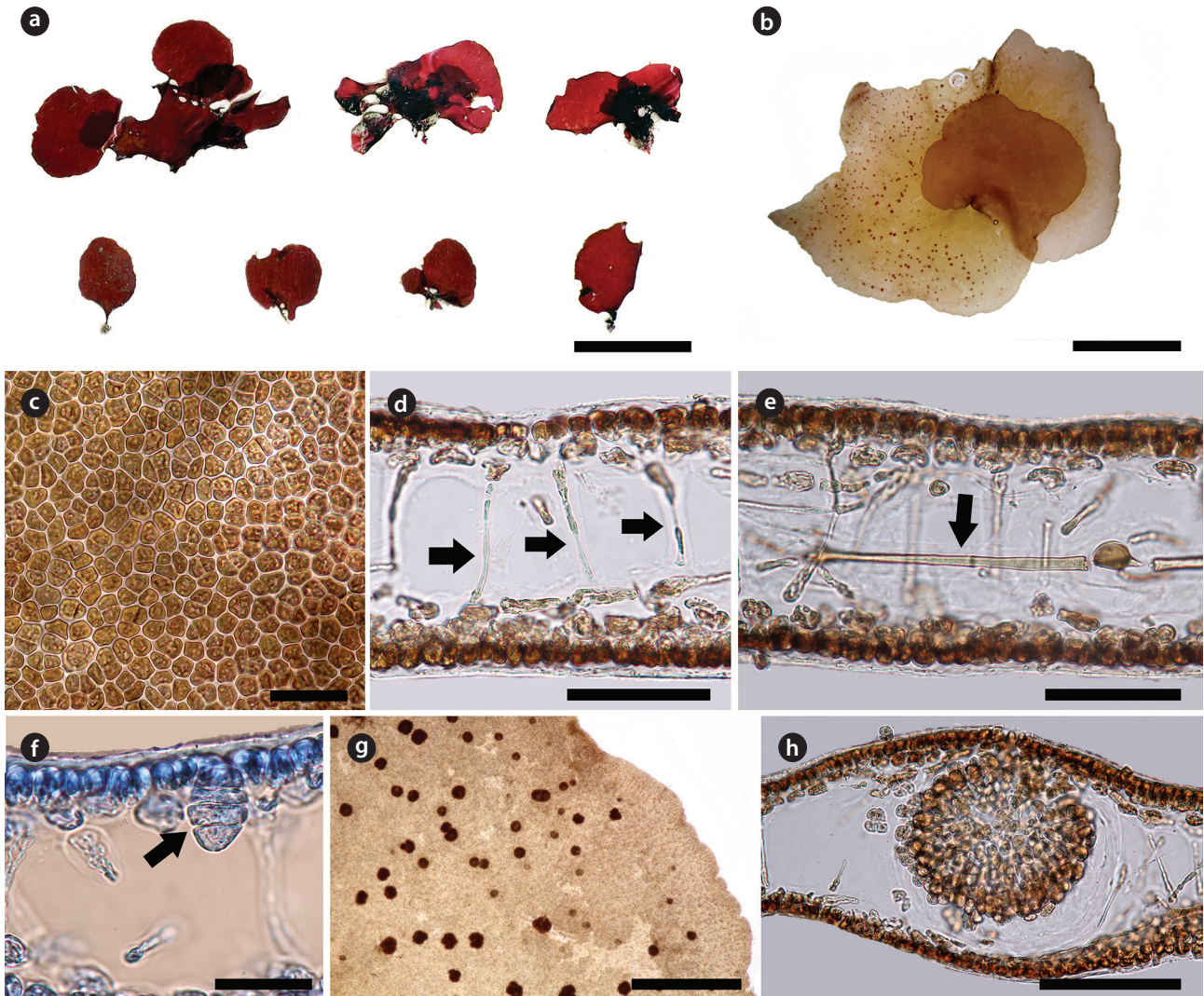


Fig. 1. *Halarachnion parvum* Yamada. (a) Morphology of thallus with short stipe and marginal proliferations, (b) living specimen of carposporophyte, (c) the surface of thallus showing subspherical to polygonal cells, (d) cross section of thallus showing the slender filaments, located to reach the opposite side of the blade (arrows), (e) cross section of thallus showing the medullary filament cell (arrow), (f) zonately divided tetrasporangia (arrow) in the cortical cells, (g) cystocarps scattered over the whole frond, (h) globular cystocarp immersed under the cortical layer. Scale bars: a, 2 cm; b, d, e, 50 μ m; c, f, 30 μ m; g, h, 100 μ m.

and reverse strands of purified PCR products were performed by Macrogen (Seoul, Korea). Both electropherogram outputs from each sample were edited with Chromas version 1.45 (Technelysium Pty. Ltd., Queensland, Australia). DNA sequences of *rbcL* gene were aligned using the multiple-sequence editing program BioEdit (Hall 1999) and then checked manually.

Uncorrected (p) pair-wise genetic distances among species were estimated using the software MEGA 4.0 (Tamura et al. 2007) to assess the genetic variations of *rbcL* sequences. Maximum likelihood (ML) phylogenetic analyses were performed with RAxML software (Stamata-

ki 2006) using the GTR + Γ + I model. To identify the best tree, we constructed 200 independent tree inferences using the *-#* option with default *-I* (automatically optimized Subtree Pruning-Regrafting rearrangement) and *-c* (25 distinct rate categories) software options. Statistical support for each branch was obtained from 1,000 bootstrap replications using the same substitution model and RAxML program setting. The outgroup, *Tichocarpus crinitus* (S.G. Gmelin) Ruprecht for *Halarachnion* and *Rhodymenia intricata* (Okamura) Okamura/*Lomentaria hakodatensis* Yendo for *Champia*, were chosen in accordance with previous studies (Koh et al. 2013, Arakaki et al. 2014).

RESULTS

Halarachnion parvum Yamada

Korean name: 애기곱단아 (신칭)

Type locality: Hayama, Sagami Prov., Japan (Yamada 1941).

Distribution: Japan (Yamada 1941) and Korea (this study).

Specimens examined: 140516-1-4, NIBRAL0000152588-89 (Munseom, Jeju; 16 May 2014); 140813-10-13 (Munseom, Jeju; 13 Aug 2014); NIBRAL0000152586-87, 150103-11-12 (Munseom, Jeju; 03 Jan 2015).

Description: Thalli are flat, blade-like, membranaceous, attached singly to the substratum by a small discoid holdfast, and extending from a short and narrow stipe (Fig. 1a and 1g). Thalli are erect, up to 3 cm tall, 2 cm broad, 80–100 μm thick, and simple or irregularly lobed, elliptical to ovate, and sometimes marginal proliferations arisen (Fig. 1a and 1b). The cortex is 1–2 cells thick, and cortical cells are subspherical to polygonal in surface with 7–8 μm in diameter (Fig. 1c and 1d). Subcortical cells are subspherical to stellate with 10–20 μm in diameter (Fig. 1d and 1e). Medulla are segmented, with few slender filaments which are loosely arranged and located to reach the opposite side of the blade (Fig. 1d). Some medullary cells are expanded to 80–90 μm wide in the central area of medulla (Fig. 1e).

Tetrasporangia are scattered over the frond and the shape is elliptic in cross section (Fig. 1f). They are zonately divided, 18 \times 20 μm broad and 24 \times 26 μm long (Fig. 1f). Carposporophytes are 0.7 cm long and 1.6 cm broad (Fig. 1b). Cystocarps are globular in shape, and 100–150 μm in diameter. They are scattered over the whole frond and immersed under the cortical layer (Fig. 1g and 1h). Spermatangia are not found.

Molecular analyses: Ten sequences of *Halarachnion* including seven new sequences of *H. parvum* generated in this study were aligned with 1,404 nucleotide base pairs. Seven sequences of *H. parvum* from Korea were differed from other species, and closely related with *Halarachnion latissimum* from Japan (KF709207) with 3.2–3.3% sequence variation, supported by 100% bootstrap value (Fig. 2). The clade of *Halarachnion* was monophyletic group by 99% bootstrap value, and interspecific divergences from those of 3.2% (*H. parvum* and *H. latissimum*) to 8.1% (*H. parvum* and *H. ligulatum*).

Champia lubrica Mas. Suzuki & Yoshizaki

Korean name: 미끌사슬풀 (신칭)

Type locality: Uranohama, Yamada, Shimohei County, Iwate Prefecture, Japan (Suzuki et al. 2013).

Distribution: Japan (Suzuki et al. 2013) and Korea (this study).

Specimens examined: NIBRAL0000152585 (CP120501; Namhae, Gyeongsangnam-do; 20 May 2012).

Description: Thalli are erect, up to 3–5 cm high, irregularly branched, and attached by a disc-like holdfast (Fig. 3a and 3b). They are terete, 1–1.5 mm in diameter, hollow, segmented by diaphragms, and slightly constricted at the nodal position (Fig. 3b–3d). Branching is alternate or opposite, and laterals are tapering to obtuse apex with the narrow base (Fig. 3a and 3b). Cortex is composed of single-layered large cell with randomly occurring small cells cut off obliquely from the large cell (Fig. 3c and 3e). The large cells are 50–120 μm long, whereas the small cells are 10–20 μm long (Fig. 3e). Longitudinal filaments are occurring peripherally throughout hollow axes and rarely in the center (Fig. 3c, 3d, and 3f). Gland cells are spherical with 10–20 μm diameter and toward the central cavity (Fig. 3d and 3f). Tetrasporangia are tetrahedrally divided, 40–60 μm in diameter, and transformed from cortical cells toward the inner hollow (Fig. 3g).

Molecular analyses: Twenty-six *rbcL* sequences of the genus *Champia* including two new sequences of *C. lubrica* were aligned with 1,241 nucleotide base pairs. Our *Champia* specimens collected from Namhae and Jeju were closely related to the sequence of *C. lubrica* from Japan (AB693118) supported by 100% bootstrap value (Fig. 4). The intraspecific divergence between Korean and Japanese specimens is 0.2%.

DISCUSSION

Halarachnion parvum has been known as an endemic species in the subtidal zone of Japan, and characterized by small habit about 1 cm in height (Yamada 1941). The molecular analyses using *rbcL* in the present study showed the monophyletic clade of *Halarachnion* with generitype *H. ligulatum* from Europe. Our sequences of *H. parvum* from Korea were separated from others in the genus *Halarachnion* and closely related with *H. latissimum* from Japan (Fig. 2). This result indicated that *H. parvum* is a distinct species and distributed in Korea and Japan. The morphology of specimens collected from Munseom, Jeju corresponds to the description of Yamada (1941). This is

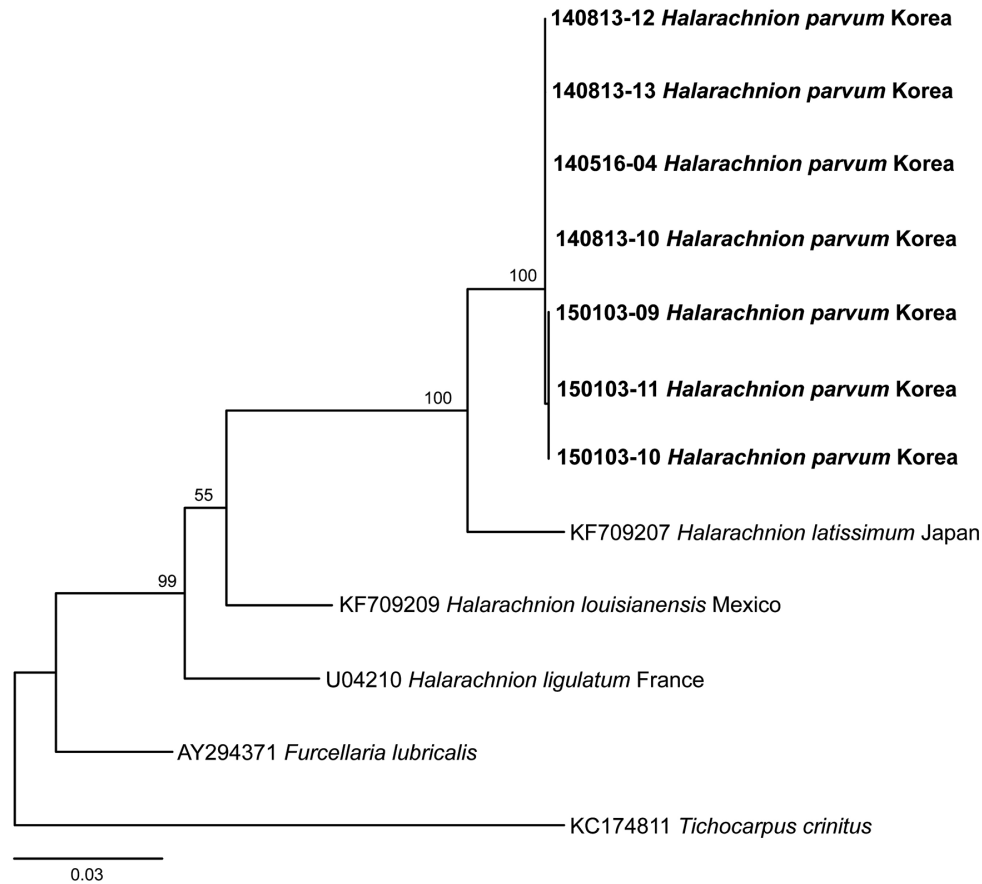


Fig. 2. Phylogenetic tree of the genus *Halarachnion* based on *rbcL* sequences inferred from maximum-likelihood analysis. The bootstrap value are shown above the branches. Scale bar, substitutions/site.

the second reported species of *Halarachnion* in the Korean inventory, together with *Halarachnion latissimum*. *H. latissimum* from Korea is different from *H. parvum* in having a thallus up to 20 cm high with no stipe (Oak et al. 2002). However, an examination between *H. parvum* and *H. latissimum* has been required because of morphological similarity and overlapping distributions (Arakaki et al. 2014). Recently, *H. louisianensis* from the Gulf of Mexico was newly described based on morphology and molecular analyses using *rbcL* gene (Arakaki et al. 2014). Although *H. louisianensis* from the original Gulf of Mexico is also similar to *H. parvum* in having a short stipe from the discoid holdfast, they are distinguished each other by the length of gametophyte thallus (up to 18 cm) and no proliferations in the thallus of *H. louisianensis* (Arakaki et al. 2014). The type species of *Halarachnion*, *H. ligulatum*, showed a heteromorphic life history in which tetrasporophyte formed discoid crust (Boillot 1965), whereas the shape of tetrasporophyte of *H. parvum* is fronds. This agrees well with the description that *H. parvum* has an isomorphic

life history (Yamada 1941). The discrepancies in the life history between *H. ligulatum* and *H. latissimum* led to question that these two species could be separated at the generic level (Oak et al. 2002), however, tetrasporophytes of *H. louisianensis* have not been confirmed (Arakaki et al. 2014). *H. pusillum* described from Tristan da Cunha has uncertain generic placement (Baardseth 1941), and it should be critically examined.

The second species added to the Korean algal inventory is *C. lubrica*, which has previously been recorded in Japan only (Suzuki et al. 2013). Our *rbcL* analyses showed that the specimens collected from Namhae and Seopseom are closely related to *C. lubrica* from Japan (AB693118) supported by 100% bootstrap value with 0.2% intraspecific sequence divergence (Fig. 4). *Champia lubrica* has been misidentified as *Champia parvula* (*C. Agardh*) Harvey from Japan, because *C. parvula* also has cylindrical thalli (Lee 1978, Lozada-Troche and Ballantine 2010, Koh et al. 2013). Although many features of *C. lubrica* are shared with *C. parvula*, Suzuki et al. (2013) suggested the new

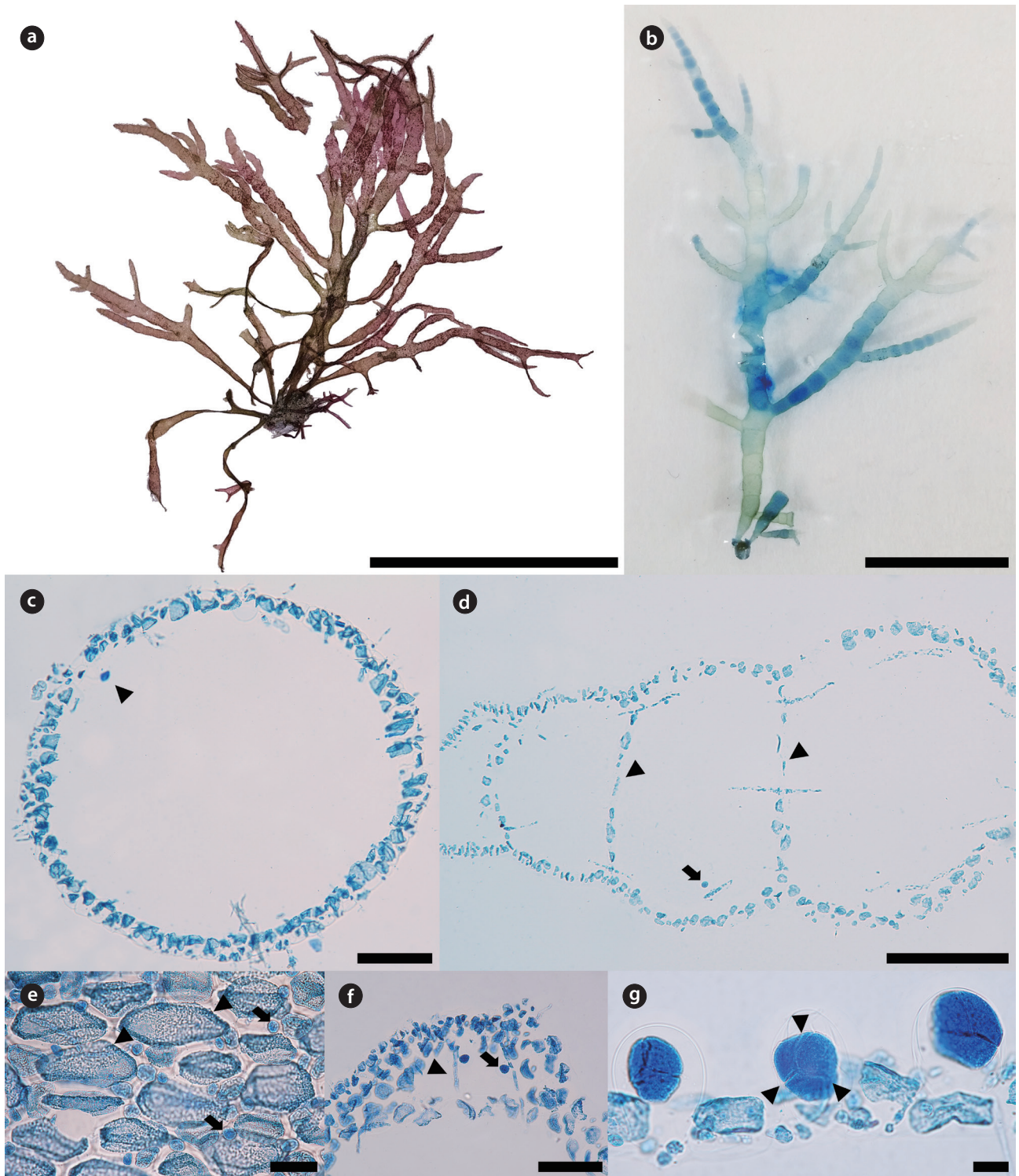


Fig. 3. *Champia lubrica* Mas. Suzuki & Yoshizaki. (a, b) Tetrasporophyte collected from Namhae, Korea, (c) transverse section of thallus showing longitudinal filament (arrowhead). (d) Longitudinal section of thallus showing single-layered diaphragms (arrowheads) and longitudinal filaments with gland cell (arrow), (e) surface view of thallus showing large (arrowheads) and small (arrows) cells, (f) longitudinal section of the apical portion showing longitudinal filaments (arrowhead) with gland cell (arrow), (g) tetraheadrally divided (arrowheads) tetrasporangia protruding from the surface layer into the central branch cavity. Scale bars: a, 2 cm; b, 1 cm; c, d, f, 200 μ m; e, 50 μ m; g, 20 μ m.

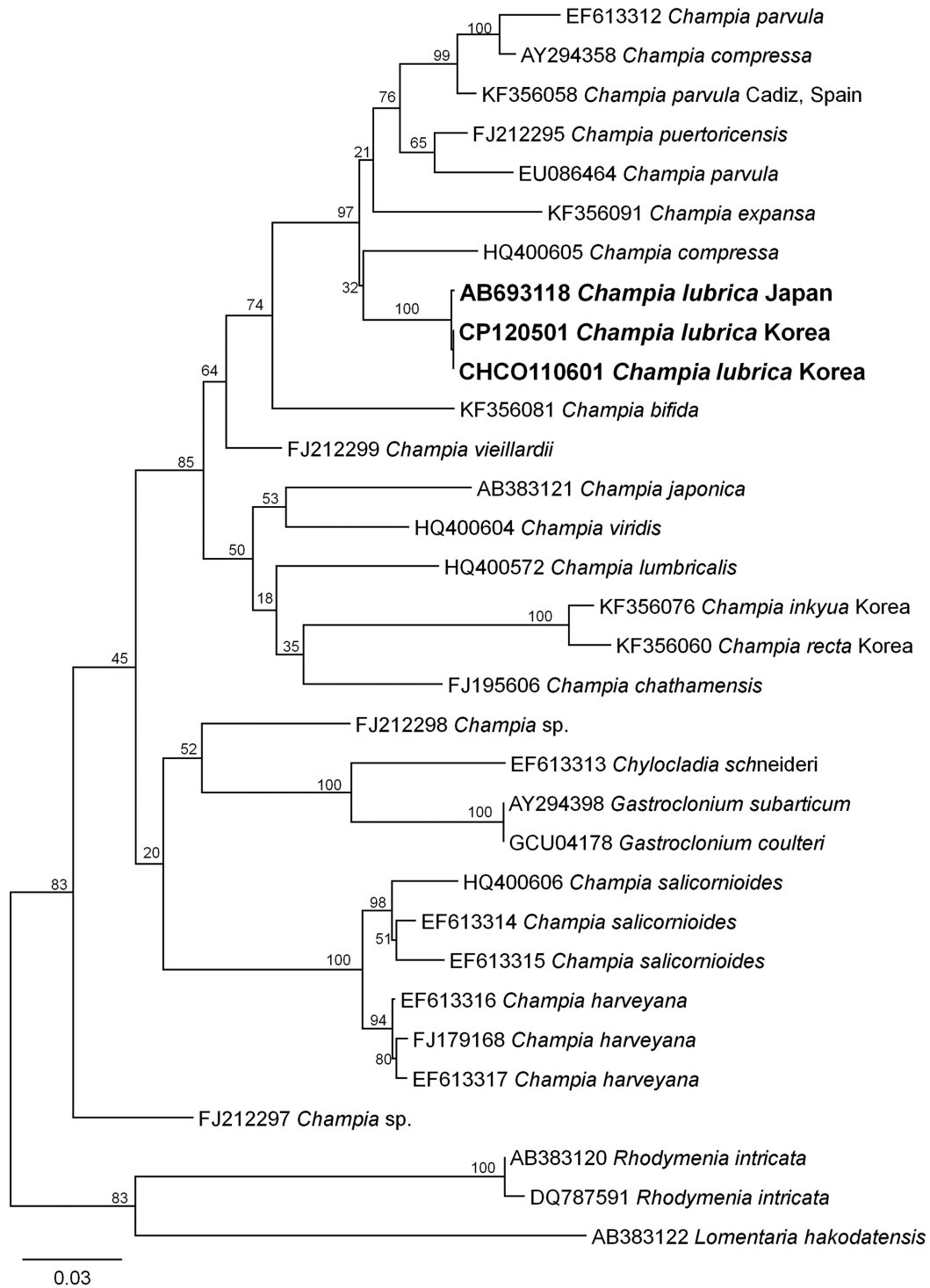


Fig. 4. Phylogenetic tree of the genus *Champia* based on *rbcL* sequences inferred from maximum-likelihood analysis. The bootstrap value are shown above the branches. Scale bar, substitutions/site.

species, *C. lubrica*, based on *rbcL* analyses and subtidal habitat. The specimens of *C. lubrica* collected from Korea are growing on bedrock at 3–5 m depth of subtidal zone. They showed morphological similarity with the original description of *C. lubrica* (Suzuki et al. 2013). The results of molecular analyses and morphological observations prove that the Korean and Japanese species are same species. Therefore, we present *C. lubrica* as a new record of Champiacean species from Korea.

Two new records in this study have not been recognized as distinct species from Korea because of small size for *H. parvum* and lack of distinguishable characteristics for *C. lubrica*. Morphological observations combined with molecular analyses allow us to identify the cryptic species (Koh et al. 2013, Lee and Kim 2014, Kim and Kim 2014, Yang and Kim 2015). Our results indicated that careful observations will be required to identify cryptic species, and molecular result could be resolve the taxonomic problems for species having wide morphological variation.

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