

RESEARCH ARTICLE

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New record of three economic *Hypnea* species (Gigartinales) in Korea

Pil Joon Kang and Ki Wan Nam* 

Abstract

Three economic marine algae that have been used as food and carrageenan sources were collected from Korea during a survey of marine algal flora. They share the generic features of *Hypnea*, and three major clades supported by the sectional features were confirmed in a phylogenetic tree based on *rbcl* sequences. The first species, which belongs to a species group corresponding to the sect. *Spinuligerae*, nests in the same clade with *Hypnea yamadae* in a genetic distance of 0%. It is morphologically characterized by an entangled base, subcompressed or subterete to terete axes, somewhat percurrent main axis, irregularly alternately branching with wide angle, and rarely hooked spinous branchlets. The second one is also referred to the sect. *Spinuligerae* and formed the same clade as *Hypnea cenomyce*. The genetic distance between both sequences was calculated as 0.0–0.1%, which is considered to be intraspecific. This species is distinct by somewhat entangled thallus at the basal part, percurrent axis, short spine-like branchlets densely covering the axis, and medullary lenticular thickenings. The third alga, which forms a species group corresponding to the sect. *Pulvinatae*, nests in the same clade as *Hypnea nidulans* (no intraspecific divergence). It shows occasionally epiphytic habitat rather than epilithic habitat of low mat-forming growth and percurrent erect main axes with dense lateral branchlets. Based on these morphological and molecular data, the three Korean species are identified as *H. yamadae*, *H. cenomyce*, and *H. nidulans*. This is the first record of the *Hypnea* species in Korea.

Keywords: *Hypnea* species, Economic marine algae, *rbcl*, Molecular data, First record, Korea

Background

The marine red algal genus *Hypnea* J.V. Lamouroux, which has a wide geographical distribution around the world, is economically important as food and as one of the sources of carrageenan, particularly in the tropics (Mshigeni and Chapman 1994; Geraldino et al. 2010). This genus was established based on six species (Lamouroux 1813) but later lectotypified by *H. musciformis* (Wulfen) J.V. Lamouroux (Kyllin 1930; Papenfuss 1958).

Agardh (1852) proposed three sections within the genus *Hypnea* on the basis of thallus habit. The sect. *Pulvinatae* has creeping thalli of intricate cushion-like mat form. The sect. *Virgatae* is characterized by an erect, caespitose, and non-intricate thallus with main axis and dense lateral branchlets, while the sect. *Spinuligerae* in which the species show intricate and caespitose thalli with alternate branching and short spine-like branchlets. Recently, this infrageneric taxonomic scheme, with some exceptional

species, was strongly supported by molecular analyses (Geraldino et al. 2010).

Since Lamouroux (1813), many species have been reported in this genus (Agardh 1851, 1852; Tanaka 1941, 1960; Schneider and Searles 1976; Nauer et al. 2014, 2015; Geraldino et al. 2009, 2010; Jesus et al. 2013). Therefore, *Hypnea* is a large genus in which 60 species are currently accepted within the family Cystocloniaceae (Guiry and Guiry 2018). This genus is characterized by a terete to compressed, branched thalli with short lateral branchlets, protuberant globular cystocarps, and zonately divided tetrasporangia on short lateral branchlet (Lamouroux 1813; Mshigeni 1978; Womersley 1994). However, species delimitation is complicated by the high degree of morphological variation within individual species, which may be chiefly influenced by environmental factors occurring in specific habitats. Furthermore, the taxonomy based mainly on vegetative features, with a relatively simple and plastic morphology, has caused many difficulties in identifying the species within *Hypnea* (Nauer et al. 2014, 2016; the present study).

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In Korea, 13 species have been reported. During a survey of marine algal flora, three gigartinales species were collected from Korea. They share the generic morphological features of *Hypnea*. The *Hypnea* species are newly recorded in Korea based on the morphological and molecular analyses herein.

Methods

Specimens for this study were collected from Pohang and Sacheonjin located on the eastern coasts of Korea. Taxonomic data were obtained from fresh, liquid-preserved, and herbarium specimens. Liquid-preserved material was stored in a 10% solution of formalin/seawater. Blades dissected from the cleared materials were hand sectioned, transferred to a slide with distilled water, and mounted in pure glycerin. Measurements are given as width and length. For permanent slides, the glycerin was exchanged with 10–20% corn syrup.

Total genomic DNA was extracted from silica gel-preserved samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Before extraction, dried material was crushed with liquid nitrogen using a mortar and pestle. Extracted DNA was used for amplification of ribulose-1, 5-bisphosphate carboxylase large subunit (*rbcL*) regions. For *rbcL*, the gene was amplified in three overlapping parts with the primer pairs *FrbcL* start (5'-TGTGTTGTCGACATGCTCACTCTGTAGAAG-3') - R753 (5'-GCTCTTTCATACATACTTCC-3'), F492 (5'-CGTATGGATAAATTTGGTGG-3') - R1150 (5'-GCATTTGTCCGAGTGAATACC-3'), and F993 (5'-GGTACTGTTGTAGGTAATAGAAGG-3') - *RrbcS* (5'-TGTGTTGCGGCCGCCCTTGTGTTAGTCTCAC-3') (Freshwater and Rueness 1994). PCR amplifications were performed in a TaKaRa PCR Thermal Cycler Dice (TaKaRa Bio Inc., Otsu, Japan). PCR was performed with an initial denaturation step at 94 °C for 10 min, followed by 35 cycles of 30 s at 90 °C, 30 s at 50 °C, and 2 min at 72 °C, with a final 10-min extension cycle at 72 °C. The PCR products were moved to MacroGen Sequencing Service for sequencing (MacroGen, Seoul, Korea). The PCR primers were also used for sequencing.

Six *rbcL* sequences were generated. A total of 31 sequences from *Hypnea*, including *Cystoclonium purpureum* (Hudson) Batters as an outgroup, was aligned using 1356 base pairs (bp) of the *rbcL* gene using BioEdit (Hall 1999). Phylogenetic analyses were performed using neighbor-joining and maximum-likelihood methods using Mega 6 program. Bootstrap values were calculated with 1000 replications. *RbcL* sequences of other species were obtained from GenBank.

Results

Hypnea yamadae Tanaka 1960: 94

Type locality: Uji Islands, Kagoshima Prefecture, Japan (Yamagishi and Masuda 1997)

Korean name: Mit-eong-kin-ga-si-u-mu nom. nov. (신칭: 밀엥킨가시우무)

Specimens examined: NIBRAL0000146496, MGARBB000744, MGARBB000745, MGARBB000746 (Daebori: 07.viii.2014)

Habitat: Epilithic in upper to lower intertidal

Morphology: Thalli up to 10–15 cm high, epilithic, subcompressed or subterete to terete, somewhat entangled at base, without iridescence, light brown to dark brown in color, cartilaginous in texture (Fig. 1a); main axes more or less percurrent, issuing irregularly or alternately branches with wide angle and proliferations; branches bearing a few branchlets in alternate to spiral manner (Fig. 1b); branchlets short, spinous, slender, rarely hooked, without constriction near base, 5–20 mm long (Fig. 1d); apical cells distinct at the apices of axes (Fig. 1c); lenticular thickenings usually absent in the wall of medullary cells; cortex 2–3 cell layers thick; medullary cells round to elliptical in transverse section, linear to cylindrical shape in longitudinal section, with many pit connection between adjacent cells; tetrasporangia produced from cortical cell (Fig. 1f), restricted in ultimate branchlets (Fig. 1e), zonately divided, 25–35 × 55–70 μm. Sexual plants were not collected during the present study.

Hypnea cenomyce J. Agardh 1851: 452

Type locality: ad oras Novae Hollandiae (Silva et al. 1996)

Korean name: God-eun-ga-si-u-mu nom. nov. (신칭: 곱은가시우무)

Specimens examined: NIBRAL0000146479, MGARBB000747, MGARBB000748, MGARBB000749 (Sacheonjin: 03.vii.2014)

Habitat: Epilithic in upper to lower intertidal

Morphology: Thalli up to 10–15 cm high, epilithic, terete, somewhat entangled at basal part, without iridescence, dark red to brown in color, cartilaginous in texture (Fig. 2a); main axes often more or less percurrent, issuing irregular or alternate branches and proliferations; branches bearing abundant branchlets in alternate to spiral manner (Fig. 2b); branchlets short, spinous, usually with constriction near base, 5–15 mm long; apical cells distinct at the apices of axes (Fig. 2d); lenticular thickenings occasionally present in the medullary cell walls (Fig. 2c); cortex 2–3 cell layers thick; medullary cells round to elliptical in transverse section, linear to cylindrical shape in longitudinal section, with many pit connection between adjacent cells; tetrasporangia produced from cortical cell (Fig. 2f), restricted in ultimate branchlets (Fig. 2e), zonately divided, 10–20 × 20–40 μm. Sexual plants were not collected during the present study.

Hypnea nidulans Setchell 1924: 161

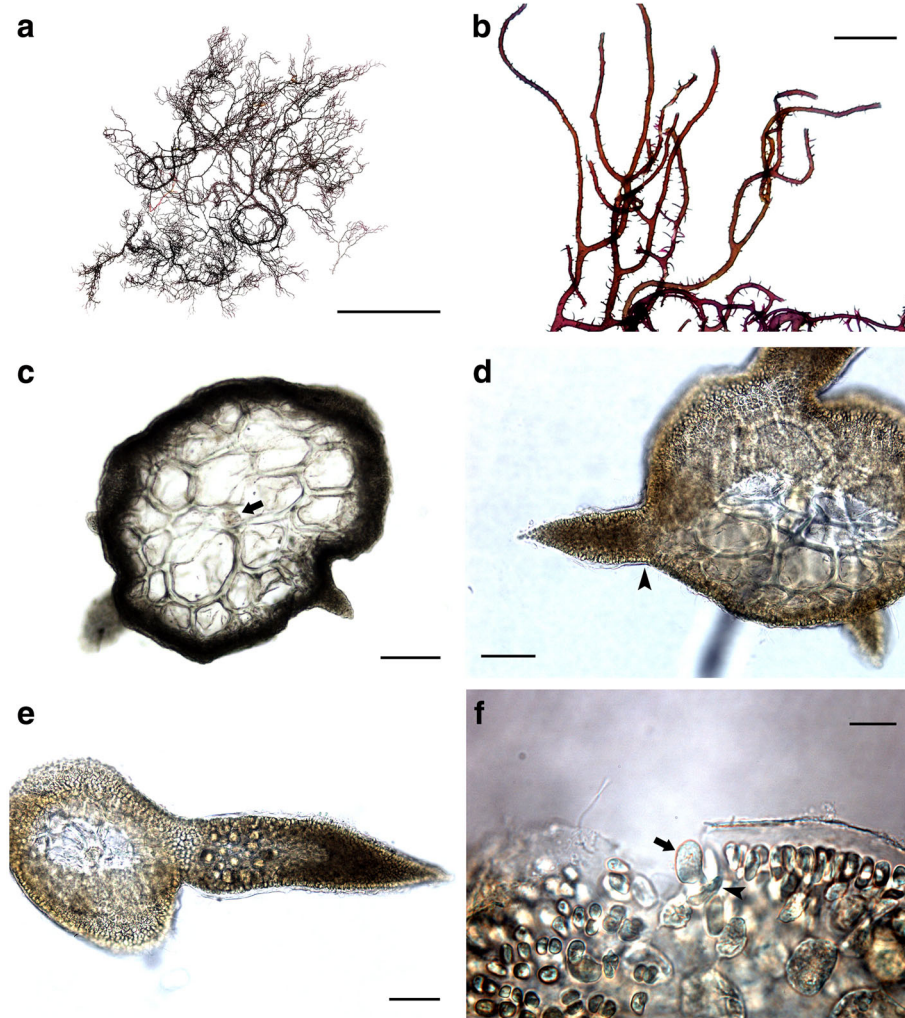


Fig. 1 *Hypnea yamadae* Tanaka. **a** Herbarium specimen entangled at the base. **b** Details of vegetative branches. **c** Subterete to subcompressed branch with the axial cell (arrow) in the transverse section. **d** Spinous branchlet without basal constriction (arrow). **e** Ultimate branchlet with tetrasporangia. **f** Tetrasporangial initial (arrow) produced from the cortical cell (arrowhead). Bars in **a** 3 cm, **b** 5 mm, **c** 150 μ m, **d** 80 μ m, **e** 80 μ m, **f** 20 μ m

Type locality: Tutuila Island, American Samoa (Silva et al. 1996; Setchell 1924)

Korean name: Gi-neun-ga-si-u-mu nom. nov. (신창: 기는가시우무)

Specimens examined: NIBRAL0000146497, MGARBb000741, MGARBb000742, MGARBb000743 (Sachenjin: 20.vii.2017)

Habitat: Epilithic or occasionally epiphytic in upper to lower intertidal

Morphology: Thalli up to 5–10 cm high, interwoven, loosely entangled, terete to subterete, without iridescence, red to brown in color, cartilaginous in texture (Fig. 3a); main axes erect, often percurrent, issuing irregularly branches and proliferations; branches bearing numerous branchlets in alternate to spiral manner (Fig. 3b); branchlets short, linear to lanceolate, usually

with constriction near base, 2–10 mm long; apical cells distinct at the apices of axes; lenticular thickenings present in the wall of medullary cells (Fig. 3c, d); cortex 2–3 cell layers thick; medullary cells round to elliptical in transverse section, linear to cylindrical shape in longitudinal section, with many pit connection between adjacent cells; tetrasporangia produced from cortical cell (Fig. 3f), restricted in ultimate branchlets (Fig. 3e), zonately divided, 20–30 \times 45–60 μ m. Sexual plants were not collected during the present study.

Discussion

In a phylogenetic tree based on *rbcL* sequences, three major clades were confirmed (Fig. 4). These clades appear to be supported by the sectional features suggested by Agardh (1852).

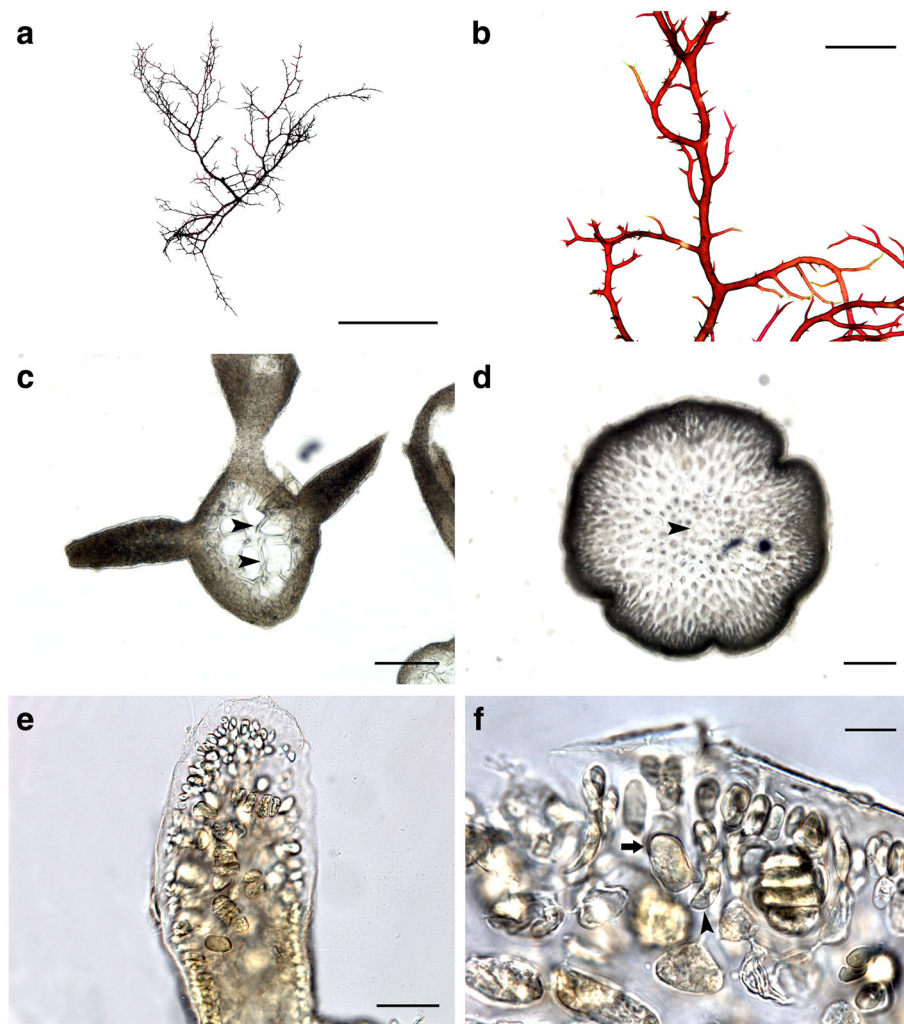


Fig. 2 *Hypnea cenomyce* J. Agardh. **a** Herbarium specimen. **b** Details of the tetrasporangial branches. **c** Lenticular thickenings (arrowheads) in the medullary cell walls. **d** Transverse section of the branch with axial cell (arrowhead). **e** Tetrasporangial branchlet. **f** Tetrasporangial initial (arrow) produced from the cortical cell (arrowhead). Bars in **a** 3 cm, **b** 5 mm, **c** 200 μ m, **d** 200 μ m, **e** 50 μ m, and **f** 15 μ m

The first species, which belongs to a species group corresponding to the sect. *Spinuligerae*, nests in the same clade with *H. yamadae* in a genetic distance of 0% (Fig. 4). It is also morphologically characterized by an entangled base of creeping branches, subcompressed or subterete to terete axes, somewhat percurrent main axis, irregular or alternate branching with wide angle, and rarely hooked spinous branchlets as *H. yamadae* reported previously by some authors (Tanaka 1960; Yamagishi and Masuda 1997). However, somewhat terete thalli and slender branchlets rarely hooked in the species differed from the original description of *H. yamadae* (Tanaka 1960) (Table 1). It was distinguished from its sister species, *H. rosea* Papenfuss (1947), by terete branch rather than subcompressed or subterete to terete one.

The second one is also referred to the sect. *Spinuligerae* and formed the same clade as *H. cenomyce* (Fig. 4). The genetic distance between both sequences within the clade was calculated as 0.0–0.1%. In general, the presence or absence of medullary lenticular thickenings is considered as one of the useful taxonomic characters in *Hypnea* (Tseng 1984; Chiang 1997; Xia and Wang 1997; Geraldino et al. 2010). It has thickenings in medullary cell walls like *Hypnea cenomyce* (Chiang 1997; Yoshida 1998) (Table 1) and is distinguished from other Korean *Hypnea* species, such as *H. boergesenii*, *H. cornuta*, *H. flexicaulis*, *H. japonica*, *H. saidana*, and *H. spinella*, by the presence of this feature. *H. flava* forming its sister clade is distinguished from *H. cenomyce* by epiphytic habitat and presence of anastomoses (Nauer et al. 2016, see table 2). In addition to the presence of lenticular thickenings in the medullary cell

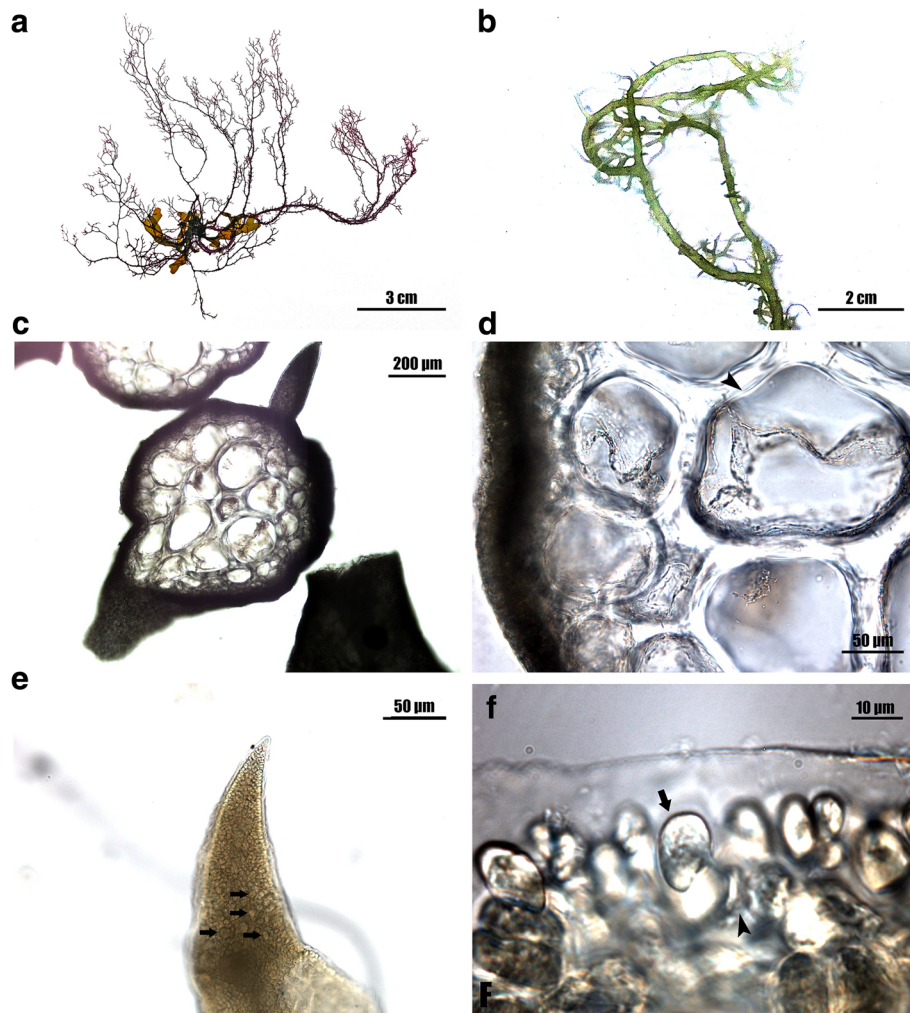


Fig. 3 *Hypnea nidulans* Setchell. **a** Herbarium specimen. **b** Details of vegetative branches. **c** Transverse section of the branch. **d** Lenticular thickening (arrowhead). **e** Branchlet with tetrasporangia (arrows). **f** Tetrasporangia initial (arrow) produced from the cortical cell (arrowhead). Bars in **a** 3 cm, **b** 2 cm, **c** 200 μm , **d** 50 μm , **e** 50 μm , and **f** 10 μm

walls, this Korean alga is characterized by somewhat entangled thallus at the basal part, percurrent axis, and short spine-like branchlets densely covering axis.

The third alga, which forms a species group corresponding to the sect. *Pulvinatae*, nests in the same clade as *H. nidulans* (no intraspecific divergence) (Fig. 4). According to Tanaka (1941), *H. nidulans* is loosely entangled, and tetrasporangial sori are saddle-shaped, while *H. pannosa* has densely entangled plants and tetrasporangial sori usually growing on one side of the branchlets, then gradually completely surrounding the branchlets. Dawson (1954) distinguished *H. pannosa* from *H. nidulans* by the small size and compact tufts of the former species, but he reduced *H. nidulans* to the synonymy of *H. pannosa* based on an examination of the type specimens of both species (Dawson 1961). Later, however, Silva et al. (1996) retained

it as a separate species. This is currently accepted (Guiry and Guiry 2018) and is also supported by the present study. *H. nidulans* appears to be distinguished from those species forming a sister group, such as *H. japonica*, *H. caespitosa*, *H. pannosa*, and *H. viridis*, by occasionally epiphytic habitat rather than epilithic habitat of low mat-forming growth, as based on the present observation. Percurrent erect main axes, with dense lateral branchlets, also distinguish *H. nidulans* from those species. This is a feature of the sect. *Virgatae* rather than the sect. *Pulvinatae*.

Conclusions

In general, the value of interspecific divergence in the Gigartinales varies from 2.8 to 16.5% (Hommersand et al. 1994; Kato et al. 2009). The interspecific divergence range within the genus *Hypnea* was calculated as 0.6–7.6% in

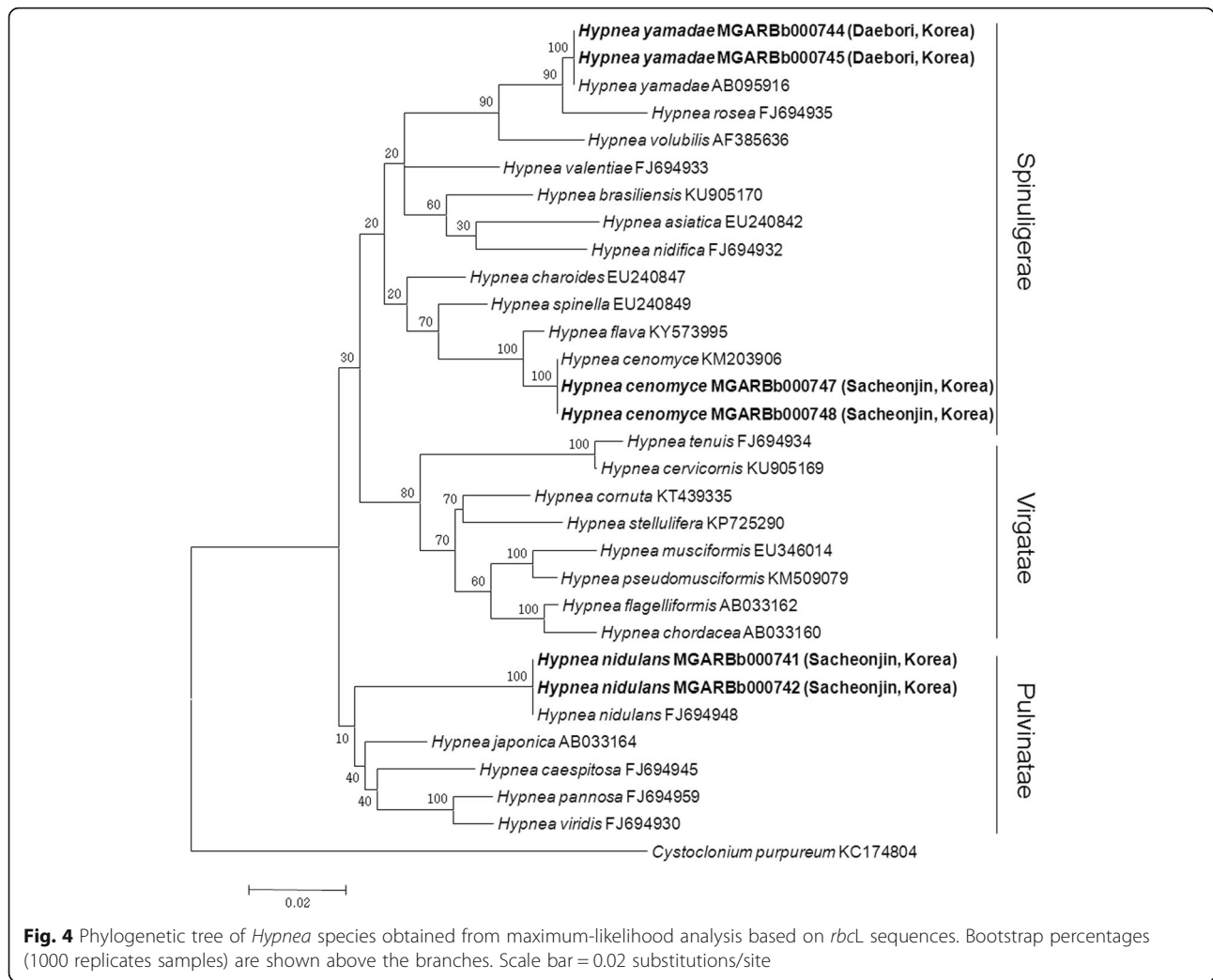


Table 1 Comparison of morphological features found in the three *Hypnea* species

Characters	Species		
	<i>H. yamadae</i>	<i>H. cenomyce</i>	<i>H. nidulans</i>
Habitat	Epilithic in intertidal to subtidal	Epilithic in intertidal	Epilithic or occasionally epiphytic in intertidal
Thalli	Terete to compressed	Terete	Terete to subterete
Size (cm)	9–37	4–15	2–10
Branching type	Irregular, alternate or dichotomous	Alternate or irregular	Alternate to spiral, irregular
Branchlets	A few	Several (or abundant)	Numerous
Basal constriction of branchlets	Absent	Present	Present
Hooked branchlets	Absent or rarely present	Absent	Absent
Lenticular thickening	Absent	Often or occasionally present	Present
Size of tetrasporangia (µm)	–	50 × 25	12 × 58
References	Tanaka 1960; Yamagishi and Masuda 1997; the present study	Chiang 1997; the present study	Chiang 1997; Yamagishi and Masuda 1997; the present study

the present study. This indicates that the genetic distance of 0.0–0.1% between both sequences within each clade formed by the three Korean species is intraspecific within the genus. Based on these morphological and molecular analyses, these Korean *Hypnea* species are identified as *H. yamadae*, *H. cenomyce*, and *H. nidulans*. This is the first record of these *Hypnea* species in Korea.

Abbreviation

rbcl: Ribulose-1, 5-bisphosphate carboxylase large subunit

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Availability of data and materials

All datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PJK conducted the research, analyzed the materials, and prepared the draft manuscript. KWN designed and directed the study and finalized the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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