

⟨Original article⟩

## Note on a Marine Algal Species, *Cryptonemia lomation* (Halymeniaceae) in Korea

Pil Joon Kang, Jae Woo An and Ki Wan Nam\*

Department of Marine Biology, Pukyong National University,  
Busan 48513, Republic of Korea

**Abstract** - During a survey of marine algal flora, a red algal species was collected from Giseong, Uljin located on the eastern coast of Korea. This species has the generic features of *Cryptonemia* belonging to Halymeniaceae, and is characterized by the presence of erect foliose thalli arising from a discoid holdfast, somewhat fan-shaped blade with an evanescent midrib at the base, narrow main axes with blade-like wings of slightly undulate margin, a perennial stalk, and entwined filamentous medulla with refractive stellate cells. In a phylogenetic tree based on *rbcL* sequence, the Korean alga nests in the same clade with *C. lomation* from France and *C. seminervis* from Spain. Genetic divergence among the sequences within the clade was not recognized thus suggesting that both the species are conspecific. The name *C. lomation* considered to be valid nomenclaturally is accepted for the entity. Based on the morphological and molecular analyses, the Korean alga is identified as *C. lomation*, originally described from Italy. This confirms the occurrence of *C. lomation* in Korea. The species appears to be distributed in the temperate region influenced more or less by the North Korea Cold Current.

**Keywords** : *Cryptonemia lomation*, red alga, Halymeniaceae, temperate region, Korea

### INTRODUCTION

*Cryptonemia* J. Agardh (1842), which involves 45 species, belongs to Halymeniaceae Bory, and is widely distributed from warm temperate to tropical waters (Guiry and Guiry 2018). This genus was established based on *C. lactuca* J. Agardh, which is currently regarded as a synonym of *C. lomation* (Bertoloni) J. Agardh. *Cryptonemia* shows morphological features of foliose or linear and cartilaginous erect thalli usually with small discoid holdfast, dichotomously branched or palmately lobed blade often with an evanescent midrib at the base, perennial stalk, multiaxial structure, entwined filamentous medulla with refractive stellate cells, bushy ampulla with conical outline and tetrasporangia usu-

ally restricted to proliferations (Chiang 1970; Irvine 1983; Womersley 1994; Nam and Kang 2013; D'Archino *et al.* 2014; Guiry and Guiry 2018). It is distinguished from other Korean genera, *Polyopes* (J. Agardh, *Kintokiocolax* T. Tanaka et Y. Nozawa, *Halymenia* C. Agardh and *Grateloupia* C. Agardh, within the Halymeniaceae, by a combination of the feature of thalli with or without midrib, presence of refractive cells in medulla and tetrasporangia usually produced in proliferations (Nam and Kang 2013). In particular, it is traditionally distinguished from the similar genus *Halymenia* by the feature of medullary filaments (D'Archino *et al.* 2014), but an exception was also reported (Womersley and Lewis 1994). The development type of auxiliary ampulla filament was also adopted for a distinction between both genera (Chiang 1970). However, this characteristic also appears not to be significant based on some other reports (Gargiulo *et al.* 1986; Guimarães and Fujii 1998; Kawaguchi *et al.* 2002, 2004).

\* Corresponding author: Ki Wan Nam, Tel. 051-629-5922,  
Fax. 051-629-5922, E-mail. [kwnam@pknu.ac.kr](mailto:kwnam@pknu.ac.kr)

These suggest that *Cryptonemia* is not easy to identify morphologically.

In Korea, since Kang (1966), three species of *Cryptonemia*, *C. tuniformis* (Bertoloni) Zanardini (Kang 1966), *C. lomation* (Lee *et al.* 2005 as *C. lactuca*) and *C. rotunda* (Okamura) Kawaguchi (Kim *et al.* 2012), had been listed (Kim *et al.* 2013). However, *C. rotunda* was recently transferred to *Galen* D'Archino et Zuccarello (D'Archino *et al.* 2014). It was reported that *C. lactuca* from Udo *sensu* Lee *et al.* (2005) is not *C. lomation* currently accepted, but is referred to newly described *C. asiatica* M.Y. Yang et M.S. Kim, based on a molecular analysis of specimens collected from the same locality (Yang and Kim 2014). Accordingly, two *Cryptonemia* species, *C. tuniformis* and *C. asiatica*, have been recognized in Korea.

During a survey of marine algal flora, a red algal species was collected from Korea. It was identified based on morphological and molecular analyses, and a taxonomic note was included. This study confirms the occurrence of the species in Korea.

## MATERIALS AND METHODS

Specimens for this study were collected from Giseong, Uljin on the east coast 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 a silica-gel-preserved sample 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 the 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'-TGTGTTGTCGACATGTCTAACTCTGTA GAAG-3') - R753 (5'-GCTCTTTCATACATATCTTCC-3'), F492 (5'-CGTATGGATAAATTTGGTCG-3') - R1150 (5'-

GCATTTGTCCGCAGTGAATACC-3'), and F993 (5'-GGT ACTGTTGTAGGTA AATTAGAAGG-3') - *RrbcS* (5'-TGT GTTGC GGCCGCCCTTGTGTTAGTCTCAC-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 4 min, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, with a final 7-min extension at 72°C. The PCR products were moved to the MacroGen Sequencing Service for sequencing (MacroGen, Seoul, Korea). Sequences for the *rbcL* region were aligned using BioEdit (Hall 1999). Phylogenetic analyses were performed using neighbor joining and maximum-likelihood methods. Bootstrap values were calculated with 1,000 replications. *RbcL* sequences of other species were obtained from GenBank. *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva was used as an outgroup.

## RESULTS AND DISCUSSION

### *Cryptonemia lomation* (Bertoloni) J. Agardh 1851

Korean name: Sang-chu-ba-da-seon-in-jang (상추바다선인장).

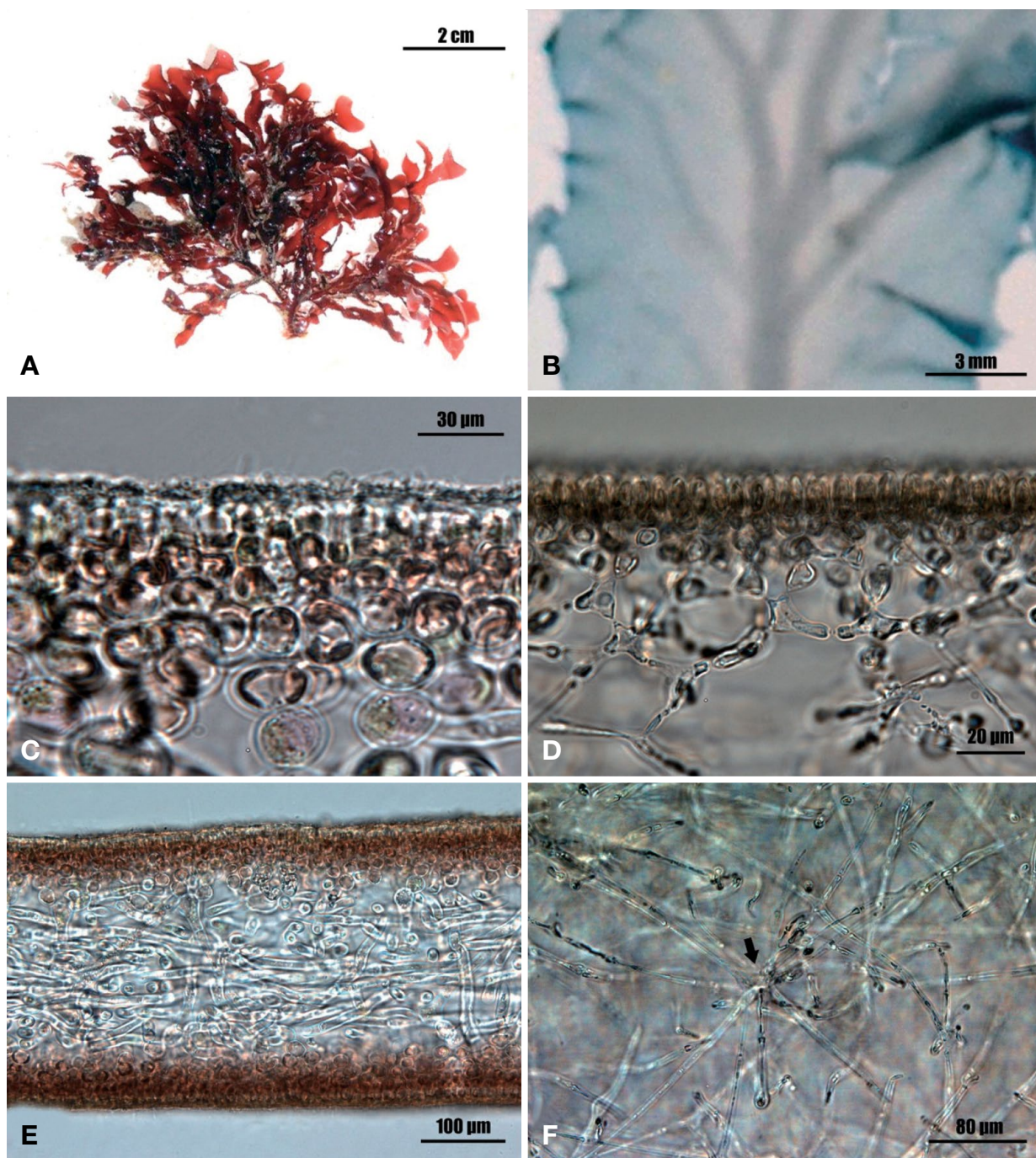
Type locality: La Spezia, Italy.

Specimens examined: NIBR0000051245 (Giseong, Uljin, Korea: 05.vi.2015), MGARBb000753–MGARBb000755 (Giseong, Uljin, Korea: 05.vi.2015).

Habitat: Epilithic or epiphytic on other algae near upper to lower intertidal.

Morphology: Thalli 3–5 cm high, erect, foliose, somewhat fan-shaped blade with evanescent midrib at the base, red to dark brown in color, cartilaginous in texture, attached to substratum by small discoid holdfast (Fig. 1A and B); main axes narrow, 2–3 mm wide, with blade-like wings of slightly undulate margin, 300–600 µm thick; cortex 6–10 cell layers, with 2–4 cell layers in the outer cortex; inner cortical cells ovoid to polygonal shape (Fig. 1C and D); medullary filaments loosely entwined, with refractive stellate cells (Fig. 1E and F). Tetrasporangial and sexual plants were not observed during the present study.

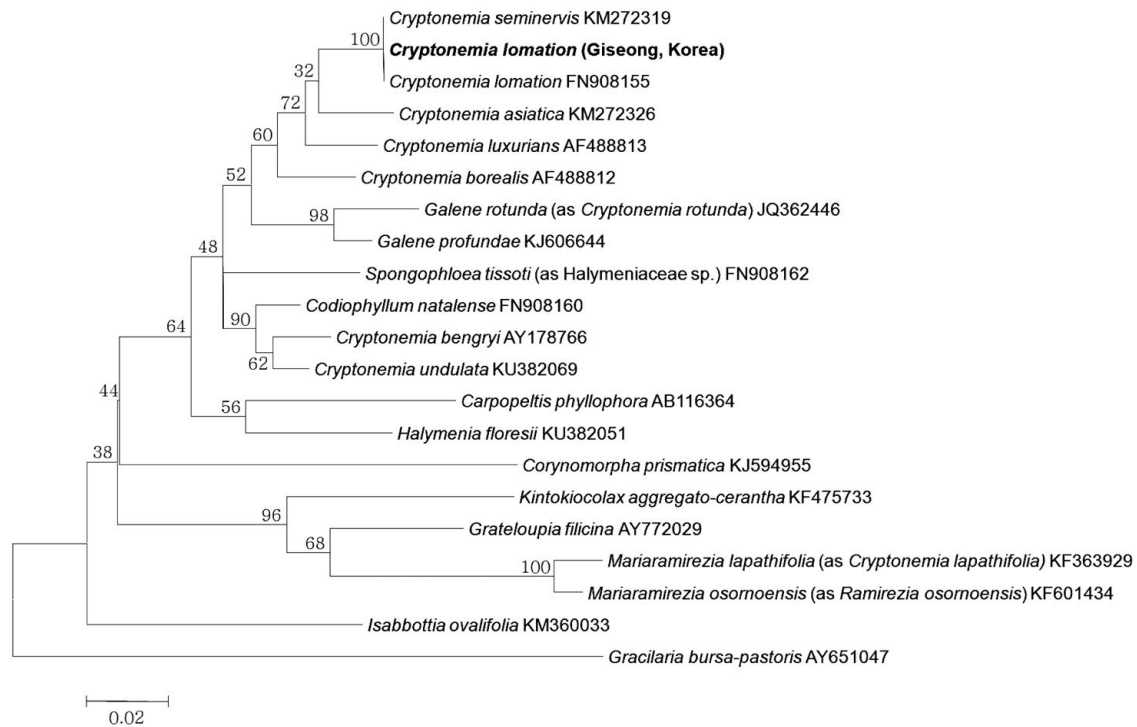
The Korean alga shares the generic features of *Cryptonemia*. *Cryptonemia lomation* is the generic type and was



**Fig. 1.** *Cryptonemia lomation*. A. The habit of vegetative plant. B. Blade with evanescent midrib at the base. C. Cortex with 6–10 cell layers in the branch. D. Inner cortical cells with ovoid to polygonal shape. E. Entwined medullary filaments in transverse section of branch. F. Refractive stellate cells (arrow) in the medulla.

originally described from Italy as *Fucus lomation* Bertoloni (1818). According to Dixon (1961), *C. lactuca* (currently accepted as *C. lomation*) and *C. seminervis* which were collected from Herm, Channel Isles are inseparable from each

other. It was also suggested that *C. lactuca* is conspecific with *C. seminervis* (Irvine 1983). Athanasiadis (1987) noted that the type species appears to be closely related to the Atlantic *C. seminervis* (C. Ag.) J. Agardh, and that the two algae



**Fig. 2.** Phylogenetic tree of *Cryptonemia* species obtained from a maximum-likelihood method based on *rbcL* sequences. Bootstrap proportion values (1,000 replicates samples) are shown above the branches. Scale bar = 0.02 substitutions/site.

should be regarded as being conspecific based on a floristic account in the British Isles by the previous authors (Ardré 1970; Irvine and Farnham 1983). Guiry and Guiry (2018) also commented on the conspecificity of both species, even though they have treated them as two separate entities, like Silva *et al.* (1996). This is supported by molecular analysis in the present study (Fig. 2). The two entities nest in the same clade, and genetic distance between both sequences within the clade is not recognized. Therefore, *C. lomation*, which is considered to have nomenclatural priority (Guiry’s personal communication, see Guiry and Guiry 2018), is accepted taxonomically. *Cryptonemia luxurians* (C. Agardh) J. Agardh originally described from Brazil has been reduced to a synonym of *C. seminervis* (Guiry and Guiry 2018). However, this species appears to be genetically distinct from *C. seminervis* (Yang and Kim 2014; the present study).

*C. lomation* is characterized by the following features: erect foliose thalli arising from a discoid holdfast, fan-shaped or somewhat palmately lobed blade with an evanescent midrib at the base, a perennial stalk, and entwined filamentous medulla with refractive stellate cells (Irvine 1983, as *C. semi-*

*nervis*; Athanasiadis 1987). In particular, it appears to be distinguished from other species with a similar habit by the evanescent midrib at the basal blade and the stellate cells in medulla (Irvine 1983, as *C. seminervis*). These features are found in Korean specimens (Fig. 1B and F).

In a phylogenetic tree based on *rbcL* sequences, the Korean alga nests in the same clade with *C. lomation* (Fig. 2). In general, interspecific divergence in the Halymeniaceae is 2.6–11.6% (Sheng *et al.* 2012). In the present study, the value in *Cryptonemia* was calculated as 2.3–5.4%. The genetic distance between both sequences within the clade was 0.0%. Based on these morphological and molecular analyses, the alga that was collected from Giseong, Uljin on the east coast of Korea is identified as *C. lomation*, originally described from Italy. Yang and Kim (2014) reported that *C. lomation* from Udo, Jeju Island, is assignable to *C. asiatica*. *C. lomation* appear does not to be distributed in subtropical waters based on their report. This study confirms the occurrence of *C. lomation* in a temperate region influenced more or less by the North Korea Cold Current.

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