Cryptonemia rotunda (Halymeniales) and *Schizymenia apoda* (Nemastomatales), two new records of red algae from Korea

Su Yeon Kim¹, Tae Ho Seo², Jeong Kwang Park¹, Ga Hun Boo¹, Kyeong Mi Kim¹ and Sung Min Boo^{1,*}

¹Department of Biology, Chungnam National University, Daejeon 305-764, Korea

²Department of Aqualife Science, Chonnam National University, Yeosu 550-749, Korea

Here we report the first finding of *Cryptonemia rotunda* and *Schizymenia apoda* in Korea based on plastid *rbcL* sequence analysis and morphological observations. *C. rotunda* occurred in the subtidal in Hongdo and Namyedo on the south coast. Thalli have short stipe with foliose, membranous blades with dentate margin, a length of 2-8 cm. *S. apoda* occurred in many locations along the coast of Korea. Thalli are foliose, simple or broadly lanceolate blades with irregular lobes and proliferations from the margins, sometimes undulate, soft fleshy, and slippery, up to 32 cm tall, and have cystocarps with ostioles on the blade. Plastid *rbcL* sequences reveal that each of these species is clearly separated from other species of their respective genera. *S. apoda* was well resolved within the genus. However, *C. rotunda* was distantly related to other members of the genus, and this result needs an urgent revision of the genus.

Key Words: *Cryptonemia rotunda*; molecular systematics; Rhodophyta; *Schizymenia apoda*

INTRODUCTION

Cryptonemia J. Agardh (Halymeniaceae) is a foliose red algal genus comprising of 43 species that are distributed from warm to cold temperate regions of the world (Guiry and Guiry 2011). The genus *Cryptonemia*, typified with *C. lomation* (Bertoloni) J. Agardh, is distinguished by the well-developed stipe and / or midrib, highly refractive medullary cells, and bushy ampullar filaments with up to four orders (Chiang 1970, Womersley 1994). In Korea, two species of *Cryptonemia* are reported; *C. lomation* [as *C. lactuca* (C. Agardh) J. Agardh, Lee 2008; see Guiry and Guiry 2011 for synonymy] and *C. tuniformis* (= *C. tunaeformis*) (Bertoloni) Zanardini (Kang 1966). Both species are rarely found in Korea (Kang 1966, Lee and Kang 2001, Lee 2008).

Schizymenia J. Agardh (Schizymeniaceae) consists of

10 species that are distributed in temperate and tropical waters, ranging from the subantarctic islands to Alaska (Gabriel et al. 2011, Guiry and Guiry 2011). It is distinguished by the presence of the gland cells resembling large, inverted tears, located terminally on cortical filaments (Dixon and Irvine 1977, Gabriel et al. 2011). Only *Schizymenia dubyi* (Chauvin ex Duby) J. Agardh is listed to date in the Korean inventory of marine algae (Kang 1966, Lee and Kang 2001, Lee 2008).

During floristic study of Korean marine algae, we found two unrecorded species; *Cryptonemia rotunda* (Okamura) Kawaguchi and *Schizymenia apoda* (J. Agardh) J. Agardh. In the present study, we analyzed *rbcL* sequences of both species together with previously recorded *S. dubyi* through extensive collections along the coasts of Korea,

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*Corresponding Author

E-mail: smbboo@cnu.ac.kr

Tel: +82-42-821-6555, Fax: +82-42-822-9690

observing details of morphology. Published sequences of species of *Cryptonemia* and *Schizymenia* (Kawaguchi et al. 2004, Gabriel et al. 2011, Huisman et al. 2011) allow us to directly compare our results with others from these two genera as a whole and with putative relatives. We found that both *Cryptonemia* and *Schizymenia* have the deep taxonomic problem and the lack of update in many areas of the world.

MATERIALS AND METHODS

Taxon sampling and morphology

Field collections of *Cryptonemia* and *Schizymenia* were made at many locations in Korea (Table 1). Materials were collected from intertidal zone or subtidal zone

using SCUBA diving. Tissues were sectioned with a freezing microtome (FX-80; Yamato Kohki Industrial Co. Ltd., Saitama, Japan). Photographs were taken with a DP-71 camera (Olympus, Tokyo, Japan) attached to a microscope (BX 51; Olympus). Voucher specimens are housed at the herbarium of Chungnam National University, Daejeon, Korea (CNUK).

Analysis of *rbcl* sequences

Forty-one specimens were available for extraction of DNA: 10 for *C. rotunda*, 7 for *S. apoda*, 24 for *S. dubyi* (Table 1). DNA extraction, polymerase chain reaction amplification, and sequencing follow Boo et al. (2010). Primer pairs for amplification and sequencing of *rbcl* were as follows: *rbcl*LF145-*rbcl*LR898 and *rbcl*LF762-*rbcl*LR1442 (Kim et al. 2010).

Table 1. Materials used in the present study

Taxa	Collection data	Voucher	GenBank accession No.
<i>Cryptonemia rotunda</i> (Okamura) Kawaguchi	Hongdo, Geojaedo, Korea; Jul 24, 2011 (2)	SH500	Same as JQ362446
	Namyedo, Geojaedo, Korea; Nov 3, 2011 (8)	SH341	JQ362446
<i>Schizymenia apoda</i> (J. Agardh) J. Agardh	Dolsando, Yesu, Korea; Mar 20, 2011 (2)	CNU1236	Same as JQ362442
	Hoidong, Jindo, Korea; Mar 1, 2010	S236	Same as JQ362442
	Hoidong, Jindo, Korea; Apr 5, 2008	G1101	JQ362441
	Ilkwang, Busan, Korea; Apr 4, 2011	CNU8698	JQ362442
	Namaehang, Yangyang, Korea; Feb 19, 2011	SH168	Same as JQ362442
	Seogeocho, Jindo, Korea; Apr 6, 2008	G849	Same as JQ362441
<i>Schizymenia dubyi</i> (Chauvin ex Duby) J. Agardh	Anin, Gangneung, Korea; Feb 18, 2011 (3)	SH171	Same as JQ362444
	Anin, Gangneung, Korea; Dec 21, 2011	SH361	Same as JQ362444
	Cheongjinri, Pohang, Korea; Feb 18, 2011	SH169	Same as JQ362443
	Gamchusa, Donghae, Korea; Mar 14, 2010	SH4	Same as JQ362444
	Gamchusa, Donghae, Korea; Dec 21, 2011	SH356	Same as JQ362444
	Gijang, Busan, Korea; Feb 28, 2010	SH1	Same as JQ362443
	Jeoncheon, Donghae, Korea; Apr 8, 2011 (2)	CNU1348	JQ362444
	Jeoncheon, Donghae, Korea; Mar 14, 2010 (2)	SH6	Same as JQ362444
	Namae Port, Yangyang, Korea; Feb 8, 2010	G7057	Same as JQ362444
	Sacheon, Gangneung, Korea; Apr 8, 2011 (2)	CNU1356	Same as JQ362444
	Sanbangsan, Jeju, Korea; Mar 13, 2009	G1868	Same as JQ362444
	Sagye, Jeju, Korea; Mar 14, 2009	G1847	JQ362445
	Sanbangsan, Jeju, Korea; Mar 13, 2009	G1870	Same as JQ362445
	Sodol, Gangneung, Korea; Apr 12, 2008	G802	Same as JQ362444
	Sodol, Gangneung, Korea; Mar 13, 2010	G7064	Same as JQ362444
	Suryeomri, Gyeongju, Korea; Apr 4, 2011	CNU8728	JQ362443
	Dalian, China; Nov 13, 2008	G1690	Same as JQ362443
	Tsuyazaki, Fukuoka, Japan; May 18, 2011	CNU9027	Same as JQ362444
	Tsuyazaki, Fukuoka, Japan; May 18, 2011	CNU9028	Same as JQ362445

The numeral in parenthesis indicates the number of specimens analyzed.

Fig. 1. Maximum likelihood (ML) tree of *Cryptonemia rotunda* inferred from *rbcL* sequences calculated using the GTR + Γ model. Numeral in parenthesis indicates the number of specimens with identical sequences. ML bootstrap values (≥ 50) are shown for each clade.

Phylogenetic analyses

Maximum likelihood (ML) phylogenetic analysis of *rbcL* was performed using the GTR + Γ model implemented in RAxML software (Stamatakis 2006). We used 200 independent tree inferences with the 'number of run' option, with default optimized subtree pruning and re-grafting rearrangement and 25 distinct rate categories to identify the best tree. Statistical support for each branch was obtained from 1,000 bootstrap replications using the same substitution model and RAxML program settings.

RESULTS

Cryptonemia rotunda (Okamura) Kawaguchi

Molecular analyses. Thirty-three *rbcL* sequences consisting of 23 taxa of Halymeniaceae including ten new sequences of *C. rotunda* analyzed in the present study were aligned with 1,129 bp. All *Cryptonemia* specimens collected from Hongdo and Namyedo belong to a single species consistent with the GenBank sequence of *C. rotunda* from Japan (Fig. 1).

Plastid *rbcL* tree revealed that the genus *Cryptonemia* was not monophyletic (Fig. 1). *C. rotunda* was not monophyletic with the other members of the genus. The pairwise divergence between *C. rotunda* and *Halymenia floridana* was 5.2%, while *C. rotunda* was in distance of 5.6-6.3% from other members of the genus.

Fig. 2. *Cryptonemia rotunda* (Okamura) Kawaguchi. (A) Morphology of thallus with stipe and small protuberances in margin (upper right). (B) Angular cells on the surface of thallus. (C) Cross section of blade showing refractive cells (arrows). (D) Cross section of stipe showing growth rings (arrow). (E) Cross section of female plant having cystocarps (arrowheads). (F) Magnification of Fig. 2E showing mature cystocarp (arrowhead). Scale bars represent: A, 1 cm; inset in A, 200 μm; B, 10 μm; C, 50 μm; D, 200 μm; E, 100 μm; F, 20 μm.

Fig. 3. Maximum likelihood (ML) tree of *Schizymenia apoda* inferred from *rbcL* sequences calculated using the GTR + Γ model. Numeral in parenthesis indicates the number of specimens with identical sequences. ML bootstrap values (≥ 50) are shown for each clade.

Korean name. 둥근바다선인장 (신칭) (Fig. 2A-F)

Description. Thalli are medium to dark red, membranous, circular or ellipsoid, rarely irregularly shaped, up to 8 cm in length and 4 cm in width, expanding from a stipe (Fig. 2A). Blades are thickened along the margin and beset with numerous, small protuberances, looking toothed (Fig. 2A), up to 200 μm thick (margins 300-370 μm thick). Cells at the surface of blade are angular (Fig. 2B). Tissue consists of cortex and medulla (Fig. 2C). Cortices are compact with 2-5 layers of cortical cells and 20-30 μm thick. Medulla are 120-180 μm thick, of entwined refractive filaments 1-4 μm in diameter (Fig. 2C). Holdfasts are discoid, 1-3 mm across, bearing stiff stipe which have several growth rings in cross section (Fig. 2D).

Female reproductive structures are scattered around

upper side of the blade. Cystocarps (Fig. 2E & F) are subglobose, 80-100 μm across, and swollen on the surface of blades with small ostiole. Both male plants and tetrasporangial plants were not found.

Thalli grow on rocks at a depth of 14-18 m in the subtidal zones. Thalli are larger in July than in November.

Schizymenia apoda (J. Agardh) J. Agardh

Molecular analyses. A total of 48 *rbcL* sequences of Schizymeniaceae including 31 new sequences were aligned with 503 bp. Seven specimens of Korean *S. apoda* clustered with those from China, the Azores, and Namibia (Fig. 3). Twenty-four specimens of *S. dubyi* from Korea clustered with those from China, Japan, Argentina,

Fig. 4. *Schizymenia apoda* (J. Agardh) J. Agardh. (A) Morphology of thallus showing undulated margins. (B) Small angular cells on the surface of blade. (C) Transverse section of blade showing hyphae cells (arrows) in medulla. (D) Cystocarpic thallus showing ostioles on the surface of blade. (E) Cystocarps (arrowheads) on both side of blade. (F) Enlarged view of Fig. 4E. Scale bars represent: A, 5 cm; B, 20 µm; C-E, 100 µm; F, 50 µm.

France and Chile.

Korean name. 자루갈래잎(신칭) (Fig. 4A-F)

Description. Thalli are bright to dark red, soft fleshy, circular or ellipsoid with undulated margins, up to 32 cm in length and 15 cm in width (Fig. 4A). Cells at the surface of blade are irregularly elongate (Fig. 4B). Tissues are compactly composed, and 300-330 μ m thick (Fig. 4C). Cortices are 5-8 layers of cortical cells and 40-60 μ m thick. Medulla are composed of thin filaments that are densely interwoven with one another, and 200-240 μ m thick.

Female reproductive structures are scattered over entire blade, and easily distinguished by visible ostioles (Fig. 4D). Cystocarps (Fig. 4E & F) are subglobose, 40-80 μ m across. Spermatangia and tetrasporangia are not found.

Thalli grow on the rock with coralline algae at intertidal zones. They were collected in February, March, and April from 2008 to 2011.

DISCUSSION

Plastid *rbcL* sequences of ten specimens of *C. rotunda* collected from the subtidal in Hongdo and Namyedo islets on the southeastern coast of Korea were identical with that (AB061375) of a Japanese specimen. Thalli have substantial stipe and circular, membranous blade, and highly refractive cells in medulla. Morphology and anatomy of the Korean specimens correspond well to the description of Okamura (1936, as *Halymenia rotunda* Okamura) and Kawaguchi (1993). This is the third species in the Korean *Cryptonemia* inventory, together with *C. lomation* and *C. tuniformis*. *C. lomation* and *C. tuniformis* from Korea are different from *C. rotunda* in having midribs (Kang 1966, Lee 2008). *C. lomation* occurs only in Udo, Jeju Island (Lee 2008), while *C. tuniformis* has not been reported after Kang (1966, 1968) who described it based on a single fragment cast up ashore in Jijindo, now included to Yeochon city, on south coast. Molecular analysis of *C. lomation* and *C. tuniformis* is necessary to confirm their genetic identities because both species are mainly distributed in Europe (see Guiry and Guiry 2011). The topology of our *rbcL* tree is similar to that of previous studies on the family Halymeniaceae (Kawaguchi et al. 2004, Huisman et al. 2011). However, our tree reveals that *Cryptonemia* is not monophyletic and *C. rotunda* is distantly related to other species of *Cryptonemia*. More sampling is necessary for the urgent revision of the genus *Cryptonemia*.

The second species added to the algal inventory of Korea is *S. apoda*, which has, to date, been recorded in South Africa, Europe, and China (Gabriel et al. 2011). Thalli of

Korean *S. apoda* are similar to those from the Azores (Gabriel et al. 2011). Thalli are foliose, simple or broadly lanceolate blades with irregular lobes and proliferations from the margins, sometimes undulate, soft fleshy, and slippery, up to 32 cm tall. Cystocarps on the blade surface have ostioles. In the present study, we found *S. apoda* in the same (e.g., Namaehang in Yangyang) or nearby locations where *S. dubyi* was recorded in Korea (see Table 1). *S. dubyi* is very common from Sokcho to Guryongpo on the east coast, and Busan to Heuksando on the south coast (Kang 1966), and Jeju Island (Kang 1966, Lee 2008). The mixed occurrences of *S. apoda* with *S. dubyi* in Korea imply that *S. apoda* thalli have likely been misidentified as morphological variants of *S. dubyi*. Our results support the suggestion of Gabriel et al. (2011) that at least several of the Japanese *S. dubyi* records (e.g., Yoshida 1998) are likely to be *S. apoda*.

All specimens of Korean *S. apoda* always formed a clade with those from the Azores, China, and Namibia in *rbcL* phylogeny (Fig. 3). However, genetic diversity within *S. apoda* is likely higher than *S. dubyi* (up to 0.6% for *S. apoda*, 0.4% for *S. dubyi*). All specimens of *S. dubyi* in Korea formed a clade with those from Argentina, Chile, China, France and Japan. *S. apoda* and *S. dubyi* formed a sister clade.

Together with the previous study by Gabriel et al. (2011), our result demonstrates that both *S. apoda* and *S. dubyi* have disjunct global distributions: China, Korea, the Azores, Namibia for *S. apoda* and Argentina, Chile, China, France, Japan, and Korea for *S. dubyi*. It is interesting to study whether their disjunct distributions can be explained by either natural dispersal or anthropogenic transport, using more taxon sampling and more variable molecular markers such as mitochondrial *cox1*.

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