## **Research Article**

Algae 2019, 34(3): 229-236 https://doi.org/10.4490/algae.2019.34.7.8

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# Genetic diversity and distribution of edible scytosiphonacean algae from Ulleungdo Island, Korea

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Despite the abundance of seaweeds from Ulleungdo Island, genetic diversity and distribution of edible brown algae from the island remain unstudied. We analyzed mitochondrial cox3 sequences from 86 specimens collected in the island and from the nearby Korean Peninsula. Our cox3 phylogeny for the first time confirmed the occurrence of fives species from Ulleungdo Island; Petalonia binghamiae, P. fascia, Planosiphon zosterifolius, and two cryptic species previously identified as Scytosiphon lomentaria. P. binghamiae was relatively homogeneous with three haplotypes. P. fascia comprised four haplotypes, which were grouped into two genetic lineages. S. lomentaria was heterogeneous with nine haplotypes and was divided into two cryptic species; one species clustered with taxa from cold waters while the other clustered with taxa from temperate and cold waters. Low genetic diversity in P. binghamiae while high genetic diversity in S. lomentaria from Ulleungdo Island are comparable to patterns observed from other species from the Korean peninsula. Ulleungdo Island, although small in size, is an ideal field laboratory to investigate genetic diversity and distributions of economic marine algae.

**Key Words:** haplotype; mitochondrial cox3; Petalonia; Planosiphon; Scytosiphon

#### INTRODUCTION

Scytosiphonacean brown algae have heteromorphic life histories with an alternation of macroscopic gametophytes and microscopic sporophytes (Wynne 1969, Kogame 1998, Boo 2010). Gametophytes are cylindrical to globose, and predominate usually during winter and spring. Sporophytes are crustose, and occur on rocks in summer and autumn. Sixteen genera of the family Scytosiphonaceae are accepted in AlgaeBase (Guiry and Guiry 2019), and many of the species classified to *Petalonia* and *Scytosiphon* are eaten by Koreans and Japanese.

The genus *Petalonia* is characterized by flattened thalli, sori without unicellular paraphyses, and rare to occasional phaeophycean hairs. The genus included only five species: *P. binghamiae* (J. Agardh) Vinogradova, *P. fascia* (O. F. Muller) Kuntze, *P. filiformis* (Batters) Kuntze, *P. tatewakii* Kogame & Kurihara, *P. tenuis* Matsumoto & Shimada, and *P. zosterifolia* (Reinke) Kuntze (Fletcher 1987, Kogame et al. 2011, Matsumoto et al. 2014). However, the last species was revised as *Planosiphon zosterifolius* (Reinke) McDevit & G. W. Saunders (McDevit and Saunders

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Received March 3, 2019, Accepted July 8, 2019

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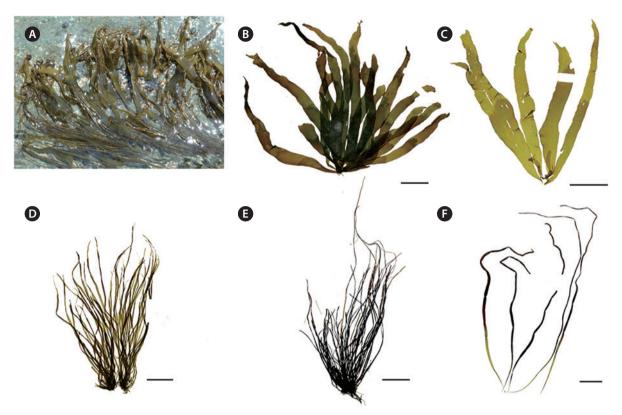


Fig. 1. Representative specimens of edible scytosiphonacean algae from Ulleungdo Island. (A) *Petalonia binghamiae* and *Scytosiphon lomentaria* plants growing on intertidal rocks at Naesujeon on Jun 19, 2018. (B) A specimen of *P. binghamiae* on Mar 12, 2018 at Cheonbu. (C) A specimen of *P. fascia* on Mar 13, 2018 at Naesujeon. (D) A specimen of *Planosiphon zosterifolius* on Mar 13, 2018 at Naesujeon. (E) A specimen of cryptic species 1 of *S. lomentaria* on Mar 13, 2018 at Hyeonpo. Scale bars represent: B-F, 2 cm. [Colour figure can be viewed at http://www.e-algae.org].

2017).

The genus *Scytosiphon* is distinguished by cylindrical to compressed hollow thalli, sori with unicellular paraphyses, and abundant phaeophycean hairs (Kogame 1998), and consists of seven species; *S. canaliculatus* (Setchell & Gardner) Kogame, *S. complanatus* (Rosenvinge) Doty, *S. crispus* Skottsberg, *S. dotyi* Wynne, *S. gracilis* Kogame, *S. lomentaria* (Lyngbye) Link, and *S. tenellus* Kogame (Wynne 1969, Kogame 1998).

In Korea, *Petalonia binghamiae*, *P. fascia* and *Planosiphon zosterifolius* are reported to occur, and two species for *Scytosiphon*, *S. gracilis* and *S. lomentaria*, commonly occur (Boo et al. 2011). Rhew and Boo (1991) studied the morphological variability of *P. binghamiae* on the east coast. However, there is no molecular studies on *P. binghamiae*. *S. lomentaria* predominates on intertidal rocks and tide pools in spring along all Korean coasts (Cho et al. 2002, Boo 2010, Boo and Ko 2012). Cho et al. (2007) reported that *S. lomentaria* from Korea contained two

cryptic species on the basis of plastid RuBisCO spacer and nuclear internal transcribed spacer (ITS) sequences.

Ulleungdo Island is a volcanic island (about 73 km<sup>2</sup>) in the East Sea, including 11 smaller isles, formed during the late Tertiary and early Pleistocene (Park and Park 1981). The island is roughly pentagonal in shape, lying about 137 km apart east from Imwon harbor, Gangwon Province. Previous studies focused on the flora of marine algae from Ulleungdo Island (Kang 1965, Lee and Boo 1981, Kim et al. 1996). However, there have been no genetic investigations on its economic marine algae. Here, we targeted the genera *Petalonia* and *Scytosiphon* which are common in winter and spring in Ulleungdo Island. Our aim was to investigate genetic diversity and haplotype structure of these edible scytosiphonacean genera. We analyzed mitochondrial cox3, a molecular marker that is commonly used in the taxonomy and biogeography of brown algae (e.g., Lee et al. 2009, 2013, 2014, Kogame et al. 2015a).

## **MATERIALS AND METHODS**

Specimens of *Petalonia* (Fig. 1A-C) were collected at four locations on Ulleungdo Islands from Mar 12-14, 2018, and additional specimens were collected at Naesujeon and Tonggumi on Jun 19, 2018. Specimens (Fig. 1A-F) were collected at three locations on Ulleungdo Island on the same dates. Plants were collected at intervals of 1 m. All collected specimens were kept in silica gel and epiphytes were removed under a dissecting microscope

in the laboratory prior DNA extraction. Additional specimens from Jejudo Island and Geojedo Island were available for comparative analyses. Voucher specimens were deposited in the herbarium of Chungnam National University, Daejeon, Korea. Information of specimens used in the present study is provided in Table 1.

A total of 86 individuals (71 from Ulleungdo and 15 from the Korean Peninsula) were analyzed (Table 1). DNA extraction, polymerase chain reaction amplification, and sequencing of the mitochondrial *cox*3 gene were per-

Table 1. Information of species, collection site and date, and cox3 sequence generated in the present study

Species, collection sites, date	Haplotype	GenBank accession No
Scytosiphon lomentaria (Lyngbye) Link		
Ulleungdo; Hyeonpo; Mar 12, 2018	S1	MN171303
Ulleungdo; Hyeonpo; Mar 14, 2018	S1	-
Ulleungdo; Naesujeon; Mar 13, 2018	S1 (4)	-
Ulleungdo; Naesujeon; Jun 19, 2018	S1 (2)	-
Ulleungdo; Hyeonpo; Mar 12, 2018	S2	MN171304
Ulleungdo; Hyeonpo; Mar 14, 2018	S2 (3)	-
Ulleungdo; Naesujeon; Mar 13, 2018	S2 (5)	-
Ulleungdo; Naesujeon; Mar 13, 2018	S3 (2)	MN171305
Ulleungdo; Naesujeon; Jun 19, 2018	S4	MN171306
Ulleungdo; Naesujeon; Mar 13, 2018	S4	-
Jejudo; Jocheon harbor; Mar 25, 2011	S4	-
Jejudo; Hado; Mar 25, 2011	S4	-
Ulleungdo; Naesujeon; Mar 13, 2018	S5	MN171307
Ulleungdo; Naesujeon; Mar 13, 2018	S6	MN171308
Ulleungdo; Naesujeon; Jun 19, 2018	S6 (2)	-
Busan; Gijang, Busan; Mar 27, 2009	S7	MN171309
Jejudo; Jocheon harbor; Mar 25, 2011	S8	MN171310
Jejudo; Hado; Mar 25, 2011	S8	-
Jejudo; Moonseom; May 16, 2012	S9	MN171311
Petalonia binghamiae (J. Agardh) K. L. Vinogradova		
Ulleungdo; Cheonbu; Mar 12, 2018	Pb1 (12)	MN171312
Ulleungdo; Naesujeon; Mar 13, 2018	Pb1 (8)	-
Ulleungdo; Hyeonpo; Mar 14, 2018	Pb1 (5)	-
Ulleungdo; Hyeonpo; Jun 19, 2018	Pb1 (3)	-
Ulleungdo; Tonggumi; Jun 19, 2018	Pb1 (2)	-
Guryongpo; Pohang; Apr 28, 2017	Pb1 (2)	-
Ulleungdo; Cheonbu; Mar 12, 2018	Pb2	MN171313
Ulleungdo; Tonggumi; Jun 19, 2018	Pb3	MN171314
Jejudo; Namwon; Mar 28, 2017	Pb4 (2)	MN171315
Jejudo; Haengwon; Mar 29, 2017	Pb5 (2)	MN171316
Petalonia fascia (O. F. Müller) Kuntze		
Ulleungdo; Cheonbu; Mar 12, 2018	Pf1	MN171317
Ulleungdo; Hyeonpo; Mar 12, 2018	Pf1	-
Ulleungdo; Hyeonpo; Mar 12, 2018	Pf2 (2)	MN171318
Ulleungdo; Hyeonpo; Mar 14, 2018	Pf2	-
Ulleungdo; Hyeonpo; Mar 12, 2018	Pf3	MN171319
Ulleungdo; Hyeonpo; Mar 14, 2018	Pf3	-
Ulleungdo; Naesujeon; Mar 13, 2018	Pf3 (2)	-
Gyeongju; Daebon; Apr 27, 2017	Pf3	<del>-</del>
Ulleungdo; Hyeonpo; Mar 14, 2018	Pf4	MN171320
Geojedo; Yeocha; Dec 5, 2017	Pf4 (2)	-
Planosiphon zosterifolius (Reinke) Kuntze		
Ulleungdo; Naesujeon; Mar 13, 2018	Pz1 (3)	MN171321
Ulleungdo; Hyeonpo; Mar 14, 2018	Pz1 (2)	
Ulleungdo; Naesujeon; Mar 13, 2018	Pz2	MN171322

Parenthesis indicates the number of identical haplotypes.

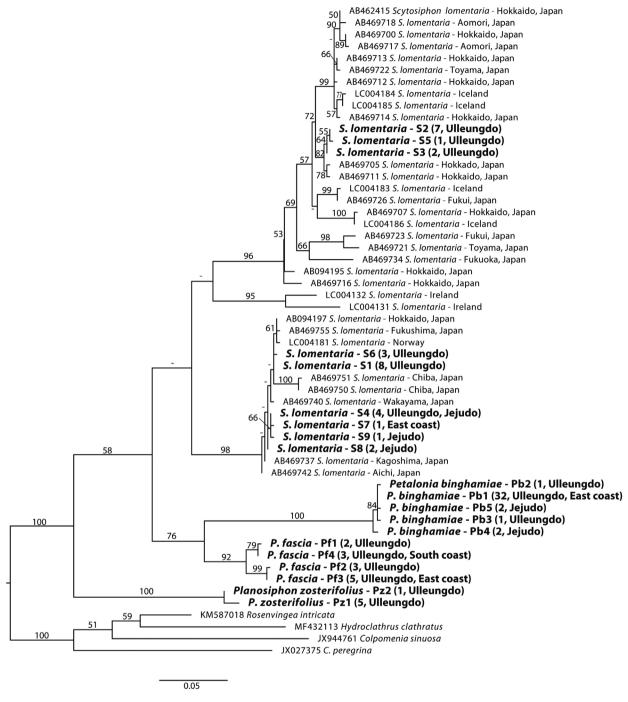
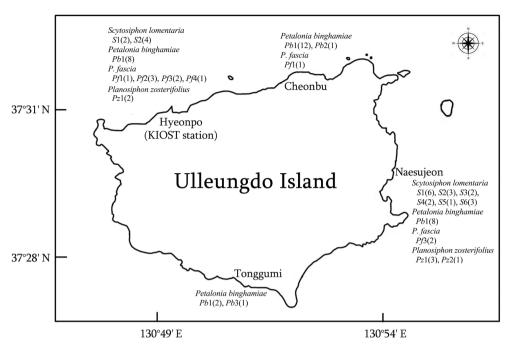


Fig. 2. Maximum likelihood phylogeny of edible scytosiphonacean algae from Ulleungdo Island using the cox3 sequences. The numbers above or below the nodes are RAxML bootstrap values. Only bootstrap values ≥50% are shown in the tree. Bold letter indicates sequence generated in the present study.

formed as described in Boo et al. (2011). The primers used for amplifying and sequencing were F49 and R20 (Boo et al. 2011). Unique haplotypes of *cox*3 generated in the present study are deposited in GenBank (Benson et al. 2018). Phylogenies of *cox*3 were inferred using maxi-

mum likelihood (ML). The ML analyses was performed using the Pthreads version of RAxML v8.0.X (Stamatakis 2014) set as follows: a rapid bootstrap analysis and search for the best-scoring ML tree in one single program run with 100 bootstrap replicates.



**Fig. 3.** Map of Ulleungdo Island showing collection sites and haplotype distributions of scytosiphonacean algae. Haplotypes are abbreviated as *Pb* for *Petalonia binghamiae*, *Pf* for *P. fascia*, *Pz* for *Planosiphon zosterifolius*, and *S* for *Scytosiphon lomentaria*. Parenthesis indicates the number of samples analyzed.

#### **RESULTS**

A total of 86 *cox*3 sequences from edible scytosiphonacean brown algae from Ulleungdo Island were obtained for a 480 bp alignment with 169 variable positions (35.2%) and 140 parsimoniously informative characters (29.2%). *Petalonia binghamiae* differed by up to 10.4% from *P. fascia. S. lomentaria* comprised two genetic lineages, both differed by up to 10.2%.

Mitochondrial *cox*3 phylogeny revealed that five species of scytosiphonacean edible algae occurred in Ulleungdo Island (Fig. 2); *P. binghamiae*, *P. fascia*, *Planosiphon zosterifolius* and two cryptic species previously recognized as *S. lomentaria*. *P. binghamiae* clustered with *P. fascia*. *S. lomentaria* was separated into two clades forming a sister relationship.

*P. binghamiae* contained three haplotypes (Pb1, Pb2 and Pb3); Pb1 was the most common, shared by 32 individuals from four locations on Ulleungdo Island (Fig. 3). It also occurred in Guryongpo on the east coast. Pb2 and Pb3 occurred at Cheonbu and Tonggumi, respectively. *P. fascia* contained four haplotypes (Pf1, Pf2, Pf3 and Pf4) from 10 specimens analyzed. All haplotypes occurred at Hyeonpo harbor. Pf3 occurred in Daebon on the southeast coast, and Pf4 occurred in Geojedo on the south coast. *Planosiphon zosterifolius* contained two haplo-

types (Pz1 and Pz2).

*S. lomentaria* comprised six haplotypes from Ulleungdo Island (Fig. 3), which were divided into two distinct genetic lineages. These two genetic lineages overlapped in three locations from Ulleungdo Island. Group I consisted of three haplotypes (S1, S4 and S6), and it clustered with haplotypes from south Korea as well as south and northern Japan. An identical haplotype (S4) occurred from Ulleungdo Island and Jejudo Island. Group II contained three haplotypes (S2, S3 and S5), and it clustered with haplotypes from Hokkaido, Japan. However, there were no identical haplotypes of the Group II between Ulleungdo and Korean Peninsula.

A distribution map of haplotypes of edible scytosiphonacean algae from Ulleungdo Island is given in Fig. 3.

## **DISCUSSION**

## Genetic diversity and distribution

The major findings of the present study are the low genetic diversity in *P. binghamiae*, showing only three haplotypes from 32 individuals, and the high genetic diversity in *S. lomentaria*, which contained six haplotypes from 23 individuals from Ulleungdo Island. The difference in

genetic diversity may reflect a difference in evolutionary history and contemporary dispersals experienced by each species.

The three *P. binghamiae* haplotypes from Ulleungdo Island comprise a common haplotype (Pb1 from 30 individuals) and two singletons (Pb2 from Cheonbu and Pb3 from Tonggumi). The common haplotype (Pb1) reveals the genetic connectivity of Ulleungdo Island with Gurvongpo on the southeast Korea. However, additional study will confirm whether singleton haplotypes are Ulleungdo-specific or shared from surrounding waters. The low genetic diversity in P. binghamiae from Ulleungdo Island is similar to the results of plastid rbcL and / or nuclear ITS from in previous studies (Cho et al. 2002, Kogame et al. 2011, Matsumoto et al. 2014). Petalonia fascia displayed a relatively high-level of genetic diversity, considering the occurrence of four cox3 haplotypes from 10 individuals from Ulleungdo Island. The presence of four haplotypes (Pf1, Pf2, Pf3 and Pf4) from Hyeonpo harbor may be the result of frequent fishery transportations by boat. P. fascia from Ulleungdo Island has genetic connectivity with the Korean Peninsula. The occurrence of two cox3 groups within *P. fascia* is supported by the ITS data by Cho et al. (2002). The two haplotypes were found for *Planosiphon* zosterifolius, indicating low genetic diversity, as shown in ITS sequences in the previous study (Cho et al. 2002).

S. lomentaria is heterogeneous, displaying six haplotypes from Ulleundo Island, segregated into two genetic lineages. These two genetic lineages are considered cryptic species because of their distant relationships in cox3 phylogeny but their morphological similarity. Group I, haplotypes S1, S4, S6, S7, S8 and S9, is considered an eurythermal species, occurring from Ulleungdo Island as well as south coast of Korea and southern Japan. Group II, including haplotypes S2, S3 and S5, is considered a cold-temperate species, occurring from Ulleungdo Island and middle to northern Japan. These two cryptic species overlapped at three locations in Ulleungdo Island where samples were collected. Ulleungdo Island was likely formed by volcanic eruptions between the late Pliocene (5.3-2.58 Ma) and early Pleistocene (2.58-1.8 Ma) (Park and Park 1981), and the time period is too short to make a 10.5% pairwise divergence between these cryptic species. We therefore hypothesize that contemporary coastal currents likely are driving forces for the arrival of two cryptic species in Ulleungdo Island. The eurythermal species reached Ulleungdo Island likely from the south coast of Korea along the East Korean Warm Current or nearby southern Japan, while the cold-temperate species arrived to Ulleungdo Island likely from North Korea or Far East Russia along the North Korean Cold Current. The presence of identical haplotypes of the eurythermal species between Ulleungdo Island and the Korean Peninsula indicates contemporary dispersals by warm water currents along the Korean strait to the East Sea. Additional studies are needed for dispersals of two cryptic species in Ulleungdo Island.

## **Taxonomic implications**

This study confirms the presence of five edible scytosiphonacean species from Ulleungdo Island; *Petalonia binghamiae*, *P. fascia*, *Planosiphon zosterifolius*, and two cryptic species previously recognized as *S. lomentaria*. *P. binghamiae* (type locality, vicinity of Santa Barbara, California, USA) has been documented elsewhere (Vinogradova 1973, Brophy and Murray 1989, Rhew and Boo 1991). Our mitochondrial *cox*3 dataset demonstrates its monophyly, as in studies of *psa*A, *rbc*L and nrDNA ITS sequences (Cho et al. 2006, Kogame et al. 2011, Matsumoto et al. 2014).

*P. fascia* (type locality, near Kristiansand, Norway), a species that is similar in morphology to *P. binghamiae*, has a complex taxonomic history. It occurs from winter to spring along the entire Korean coast, including Ulleungdo Island (Boo 2010). *Planosiphon zosterifolius* is confirmed for the first time to occur from Ulleungdo Island, therefore extending the range of *Planosiphon zosterifolius* to Ulleungdo Island. Its presence from Guryongpo to Ayajin on the east coast of Korea was confirmed using nrDNA ITS by Cho et al. (2002). *Planosiphon zosterifolius* is common during early spring on the east coast of Korea (Cho et al. 2002) and Japan (Kogame et al. 2011).

S. lomentaria currently represents five cryptic species; two from Atlantic and three from Pacific (Cho et al. 2007, Kogame et al. 2015a, 2015b, Hoshino et al. 2018). Our cox3 phylogeny confirms the occurrence of two cryptic species from Ulleungdo Island, as reported in Korea based on nrDNA ITS and plastid rbcL sequences (Cho et al. 2007). The taxonomy of S. lomentaria thus remains incomplete, and a thorough systematic proposal is necessary for this species complex. The first step to stabilizing the taxonomy of S. lomentaria is to perform high-throughput sequencing on the type material deposited in the Lyngbye herbarium of Copenhagen (Womersley 1987). Recent molecular analysis from archival type specimens of marine algae have shown that intact DNA is retrievable and yields common plastid and mitochondrial markers (Hughey et al. 2014, Boo et al. 2016). However, if the type is not available for analyzing mitogenome, sequencing topotype material (type locality, Quivig, Faroe Island) is an additional option (Kogame et al. 2015*b*).

In conclusion, this is the first attempt to investigate the genetic diversity of edible scytosiphonacean algae from Ulleungdo Island and the occurrence of Ulleungdo-specific haplotypes. Ulleungdo Island reveals a low genetic diversity in P. binghamiae, but a high genetic diversity in S. lomentaria, showing patterns similar to those species in the Korean Peninsula. The contrasting patterns in these two species may result from its position in the midst of the East Sea. Ulleungdo Island, being small in size but harboring diverse haplotypes, is an ideal field laboratory to investigate the effects of ocean warming in shaping genetic diversity and the distributions of intertidal edible brown algae and other economic marine algae. Our study provides baseline data for future culture studies of edible scytosiphonacean algae from Ulleungdo Island.

#### **ACKNOWLEDGEMENTS**

This study was supported by a 2018 research grant from Ulleungdo-Dokdo Ocean Science Station, Korean Institute of Ocean Science and Technology (PE99713). We thank Eun Chan Yang for phylogenetic analysis and two reviewers for their critical reviews.

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