

Taxonomy and distribution of selected species of the agarophyte genus *Gelidium* (Gelidiales, Rhodophyta)

Ga Hun Boo · Kyeong Mi Kim · Wendy A. Nelson ·
Rafael Riosmena-Rodríguez · Kyung Ju Yoon · Sung Min Boo

Received: 3 May 2013 / Revised and accepted: 13 August 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Although the diverse uses of *Gelidium* as food and in the production of agar and paper pulp have increased research interest in this genus, the taxonomy and biogeography of several species of *Gelidium* remain largely unstudied. We conducted phylogenetic analyses of mitochondrial *cox1* and plastid *rbcL* sequences of selected species of *Gelidium*. The data revealed that *Gelidium allanii*, *Gelidium johnstonii*, and *Gelidium koshikianum*, species that share a similar morphology, formed a monophyletic clade with a wide distribution around the Pacific rim. Because *G. johnstonii* Setchell & Gardner has nomenclatural priority over *G. allanii* V.J. Chapman and *G. koshikianum* Shimada et al., we synonymize the latter two species with the former. Based on the extremely low genetic divergences (0.0–0.2 % for *rbcL* and 0.0–0.4 % for *cox1*) between Korean and Mexican specimens of *G. johnstonii* and its sister relationship with Asian species, we consider that *G. johnstonii* may have been recently dispersed by anthropogenic agents. The New Zealand endemic *Gelidium longipes* and *Gelidium crinale* from Australia were compared with both *rbcL* and *cox1*, and were found to be identical. Although the transfer of *G. longipes* to *G. crinale* is necessary, the Australasian group within *G. crinale* is separated from other populations of the species, and we therefore recognize it

as a subspecies. Biogeography of *Gelidium* on the basis of *rbcL* phylogeny of the 59 *Gelidium* species is briefly discussed.

Keywords Anthropogenic dispersal · Biogeography · *Gelidium crinale* subsp. *longipes* · *G. johnstonii* · Red algae · Systematics

Introduction

Members of the red algal family Gelidiaceae are distributed from intertidal to subtidal habitats and occur in most oceans, including tropical, warm, and cool temperate waters, although they are absent from polar waters. The family is commercially valuable and critically important to molecular biologists as a source of agarose gels. The taxonomy of this family has not been updated in some regions of the world. Biogeographic relationships in this family are interesting to study with several species distributed, for example, between oceans, between the northern and southern hemisphere, and on the western and eastern coastlines of the Pacific Ocean.

Gelidium is the most speciose genus in the family that currently includes 134 species (see Guiry and Guiry 2013). Previously published papers have greatly contributed to our understanding of diversity in the genus (Gardner 1927; Okamura 1934; Loomis 1949, 1960; Dixon 1958; Fan 1961a; Santelices 1977; Perrone et al. 2006). Recent molecular studies have provided further clarification of the boundaries of the genus (e.g., Tronchin et al. 2002; Boo et al. 2013). Molecular data have shown that an apparently widespread species may actually comprise one or many “cryptic” species, or alternately, what have been regarded as regionally isolated endemic species, may actually be a single widespread species. For example, *Gelidium crinale* (Hare ex Turner) Gaillon is confirmed to have a wide distribution over Atlantic and Pacific oceans, while *Gelidium pusillum* (Stackhouse) Le Jolis has actually a restricted distribution in the north Atlantic waters (Freshwater and

G. H. Boo · K. M. Kim · K. J. Yoon · S. M. Boo (✉)
Department of Biology, Chungnam National University,
Daejeon 305-764, South Korea
e-mail: smboo@cnu.ac.kr

W. A. Nelson
National Institute of Water and Atmospheric Research, Wellington
6241, and School of Biological Sciences, University of Auckland,
Auckland 1142, New Zealand

R. Riosmena-Rodríguez
Programa de Investigación en Botánica Marina, Departamento de
Biología Marina, Universidad Autónoma de Baja California Sur,
La Paz, B. C. S. CP 23080, Mexico

Rueness 1994; Millar and Freshwater 2005; Kim and Boo 2012). Thus, reports of *G. pusillum* outside of Europe and Atlantic North America should be treated with caution, and herbarium specimens identified as *G. pusillum* in East Asia, Australia, and North America should be reexamined.

Gelidium longipes J. Agardh is a small, mat-forming alga, described from specimens collected in 1874–1875 in the Bay of Islands, northeastern New Zealand, by the Swedish explorer Berggren (Agardh 1876; Nelson and Farr 2003). It has been considered to be a rare endemic species from northern New Zealand, not collected for 50 years before Nelson and Farr (2003) studied its ecology and morphology, based on material from the type locality. Although they found morphological similarity between *G. longipes* and *G. crinale*, Nelson and Farr (2003) kept both names until molecular sequence data could be compared. Another interesting group of species includes *Gelidium allanii* V.J. Chapman from New Zealand (Chapman 1969; Nelson et al. 1994) and *Gelidium koshikianum* Shimada, Horiguchi & Masuda from Japan. Shimada et al. (2000) found a close relationship in the *rbcL* sequences between these species when they described *G. koshikianum*. *Gelidium johnstonii* Setchell & Gardner from the Gulf of California, Mexico, which is similar to these former species (Setchell and Gardner 1924; Shimada et al. 2000), has not been studied using molecular markers.

In this paper, we analyzed *cox1* to test the conspecificity of *G. longipes* with *G. crinale*, and the identity of *G. johnstonii* and the conspecificity of *G. allanii* and *G. koshikianum*. For addressing these taxonomic issues, we used specimens collected in or near type localities or published sequences from the type localities, and observed type material. Then, we analyzed *rbcL* sequences including previously published sequences in GenBank to reconstruct a robust, inclusive molecular phylogeny of *Gelidium* and addressed taxonomic issues and current distribution patterns within the genus.

Materials and methods

Collections and morphological observations

Representative specimens of *G. allanii*, *G. johnstonii*, and *G. longipes* were collected in the type locality or near the type locality in Mexico and New Zealand, and *G. koshikianum* was collected in the subtidal zone in Jeju, Korea (Table 1). Type specimens of these species were observed in UC at Berkeley or kindly borrowed from AK and SAP (Thiers 2013). Tissues were sectioned using a freezing microtome (FX-801, Yamato Kohki Industrial Co., Ltd., Japan) and stained with 1 % aqueous Aniline Blue. Photographs were taken with a DP-71 camera (Olympus, Japan) attached to a BX-51 microscope (Olympus). Voucher specimens were lodged at the herbarium of Chungnam National University (CNUK), Daejeon, Korea.

DNA extraction, sequencing, and phylogenetic analyses

Genomic DNA was extracted from ~5 mg of dried thallus ground in liquid nitrogen using a NucleoSpin Plant II Kit (Macherey–Nagel, Germany) according to the manufacturer's protocol. Two genes were sequenced: *cox1* and *rbcL*. Each gene was amplified by polymerase chain reaction (PCR) using the *TaKaRa Ex Taq* reagents (Takara Bio, Japan) in the following reaction mixture: 2.5 μ L 10 \times *Ex Taq* buffer (Mg²⁺ free), 2.5 μ L MgCl₂ (25 mM), 2 μ L dNTPs (2.5 mM each), 1 μ L of each forward and reverse primer (10 μ M), 0.15 μ L *TaKaRa Ex Taq*, and 3 μ L genomic DNA for a 25- μ L reaction. For mitochondrial *cox1*, the PCR protocol consisted of an initialization step of 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min (denaturation), 45 °C for 1 min (annealing), and 72 °C for 2 min (extension), and a final extension of 72 °C for 10 min. Amplification and sequencing of *cox1* was performed using the primer COXI43F and COXI1549R (Geraldino et al. 2006). For *rbcL*, the PCR protocol consisted of an initialization step of 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s (denaturation), 45 °C for 30 s (annealing), and 72 °C for 1 min (extension), and a final extension of 72 °C for 6 min. The primers used for amplifying and sequencing *rbcL* were F7, F645, R753, and RrbcS start (Freshwater and Rueness 1994; Lin et al. 2001; Gavio and Fredericq 2002). All sequences of the forward and reverse strands were determined for all taxa, and the electropherograms were edited using the program Chromas v.1.45 (Conor McCarthy, Griffith University, Australia, <http://www.technelysium.com.au/chromas.html>) and rechecked manually for consistency. Newly determined sequences were deposited in GenBank databases. We downloaded all previously published sequences of *cox1* and *rbcL* from major publications (Freshwater and Rueness 1994; Freshwater et al. 1995, 2010; Shimada et al. 1999, 2000; Tronchin et al. 2002; Millar and Freshwater 2005; Nelson et al. 2006; Tronchin and Freshwater 2007; Kim et al. 2011, 2012; Kim and Boo 2012) and GenBank. Nucleotide sequences were aligned using Se-Al v2.0a11 (Rambaut 2002).

Phylogenies of the *rbcL* and *cox1* data sets were reconstructed using maximum likelihood (ML). ML analyses were performed with RAxML v.7.2.8 (Stamatakis 2006) using the GTRGAMMA model. We used 300 independent tree inferences, applying options of automatically optimized SPR rearrangement and 25 distinct rate categories in the program to identify the best tree. Statistical support for each branch was obtained from 1,000 bootstrap replications with the same substitution model.

Bayesian analyses were performed with MrBayes v.3.2.1 (Ronquist et al. 2012) using the Metropolis-coupled Markov chain Monte Carlo with the GTR + Γ + I model. For each matrix, 20 million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The burn-in period was identified graphically by

Table 1 Specimens of *Gelidium* used for molecular analysis in the present study

Species	CNU code	Location	Date	<i>cox1/rbcL</i>
<i>G. allanii</i>	028416	McGregors Bay, Whangarei, New Zealand	June 23, 2007	KF381375/–
	NZ_WF	Doubtless Bay, North Island, New Zealand	November 23, 1992	KF381376/L22458 (Freshwater and Rueness 1994)
	LHI-2	Lord Howe Island, Australia	October 04, 2002	KF381377/AY350777 (Millar and Freshwater 2005)
<i>G. longipes</i>	ASD265	Tapeka Point, North Island, New Zealand	October 29, 2003	KF381378/KF381387
<i>G. koshikianum</i>	002155	Munseom, Jeju, Korea	May 16, 2011	KF384128/KF381386
		Nagahama, Koshiki Island, Japan	July 31, 1997	–/AB030626 (Shimada et al. 2000)
<i>G. johnstonii</i>	024849	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	KF381368/KF381379
	024864	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	KF381369/–
	AM379	Playa el Huerfanito, Baja California, Mexico	February 25, 2008	KF381370/–
	AM761	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	–/KF381380
	AM763	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	–/KF381381
	AM764	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	KF381371/KF381382
	AM765	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	–/KF381383
	AM768	Bahia de Kino, La Cruses, Sonora, Mexico	May 16, 2010	–/KF381384
	024922	La Choya, Puerto Penasco, Mexico	February 08, 2013	KF381372/–
	024969	San Carlos, Guaymas, Mexico	February 10, 2013	KF381373/KF381385
024974	San Carlos, Guaymas, Mexico	February 10, 2013	KF381374/–	

tracking the likelihoods at each generation to determine whether they reached a plateau. The 26,602 trees sampled at the stationary state were used to infer the Bayesian posterior probability.

Results

We aligned 46 *cox1* sequences (1,200 base pairs) from *Gelidium*, including *Pterocladia capillacea* (S.G. Gmelin) Santelices & Hommersand and *Gelidiella acerosa* (Forsskål) Feldmann & G. Hamel as outgroups. Variable sites were identified at 431 positions (35.9 %) and 364 positions (30.3 %) were parsimoniously informative. A total of nine *rbcL* sequences (1,266 bp) generated in the present study were aligned with 78 published sequences, including six outgroups (*Gelidium divaricatum* G. Martens, *Capreolia implexa* Guiry & Womersley, *Ptilophora scalaramosa* (Kraft) R.E. Norris, *Pterocladia lucida* (R. Brown ex Turner) J. Agardh, *P. capillacea*, *Gelidiella acerosa*). Among the 442 variable sites (34.9 %), 345 positions (27.3 %) were parsimoniously informative.

We calculated *rbcL* pairwise divergence between sister species that formed a clade with high bootstrap support (Table 2). Usually, sister species varied from 1 to 2 %. However, *Gelidium linoides* Kützinger–*Gelidium tenuifolium* Shimada, Horiguchi & Masuda and *Gelidium isabellae* W.R. Taylor–*Gelidium microdenticum* W.R. Taylor had very low divergences (0.3 %, 4 bp; 0.6 %, 8 bp, respectively), and

Gelidium bernabei Millar & Freshwater–*Gelidium* sp. and *Gelidium pluma* Loomis–*Gelidium* sp. showed high pairwise divergences (4.0 %, 50 bp; 4.9 %, 62 bp, respectively).

The phylogenetic trees constructed by *cox1* and *rbcL* had similar topologies (Figs. 1 and 2). The monophyly of the genus *Gelidium* was strongly supported (80/1.0 for *cox1* and 99/1.0 for *rbcL*). *G. johnstonii*, *G. koshikianum*, and *G. allanii* formed a monophyletic group with maximum support, and pairwise divergences between these three species were 0.0 to 0.3 % in *rbcL* and 0.1 to 0.6 % in *cox1*. The monophyly of *G. longipes* and *G. crinale* was fully supported, and pairwise divergences between species were 0.1 to 1.3 % in *rbcL* and 0.2 to 1.9 % in *cox1*.

Morphologies of *G. allanii*, *G. johnstonii*, and *G. koshikianum* and anatomy of the latter species are shown in Fig. 3a–f. The tetrasporophyte of *G. johnstonii* is very similar to that of *G. koshikianum* (Fig. 3a, c). In *G. koshikianum*, refractive rhizoidal filament cells are congested in the medulla (Fig. 3d) and tetrasporangia arise from cortical cells and are divided cruciately (Fig. 3e, f).

Discussion

Taxonomic implications

One of the most striking observations in our study is the clade of *G. allanii*, *G. johnstonii*, and *G. koshikianum* resolved in both *cox1* and *rbcL* trees (Figs. 1 and 2). The clade of these

Table 2 List of species pairs with high statistical support (>90 %) in *rbcL* tree and their geographic distributions relative to each other

Clade	Species pair	Average pairwise divergence % (absolute difference, bp)	Distribution pattern
I	<i>G. bernabei</i> Australia– <i>Gelidium</i> sp. New Zealand	4.0 (50)	Sympatric
II	<i>G. asperum</i> Australia– <i>G. australe</i> Australia	2.1 (27)	Sympatric
VI	<i>G. eucorneum</i> Korea– <i>G. prostratum</i> Korea	1.0 (12)	Sympatric
VII	<i>G. micropterum</i> South Africa– <i>G. vittatum</i> Namibia	2.2 (27)	Sympatric
VII	<i>G. foliaceum</i> South Africa– <i>G. pristoides</i> South Africa	1.8 (23)	Sympatric
VIII	<i>G. attenuatum</i> Spain– <i>G. latifolium</i> France	2.1 (26)	Sympatric
IX	<i>G. elegans</i> Korea/Japan– <i>G. pacificum</i> Japan	1.0 (13)	Sympatric
IX	<i>G. linoides</i> Japan– <i>G. tenuifolium</i> Japan	0.3 (4)	Sympatric
VI	<i>G. chilense</i> Chile– <i>G. japonicum</i> Japan	2.0 (25)	Allopatric
V	<i>G. pluma</i> Hawaii– <i>Gelidium</i> sp. Oman	4.9 (62)	Allopatric
VII	<i>G. 'isabelae'</i> South Africa– <i>G. microdenticum</i> Costa Rica	0.6 (8)	Allopatric
VIII	<i>G. declerckii</i> Australia– <i>Gelidium</i> sp. Italy	1.4 (18)	Allopatric
IX	<i>G. americanum</i> USA(E)– <i>G. maggsiae</i> Spain	2.0 (25)	Allopatric
IX	<i>G. floridanum</i> Brazil– <i>G. sclerophyllum</i> Costa Rica	1.1 (14)	Allopatric

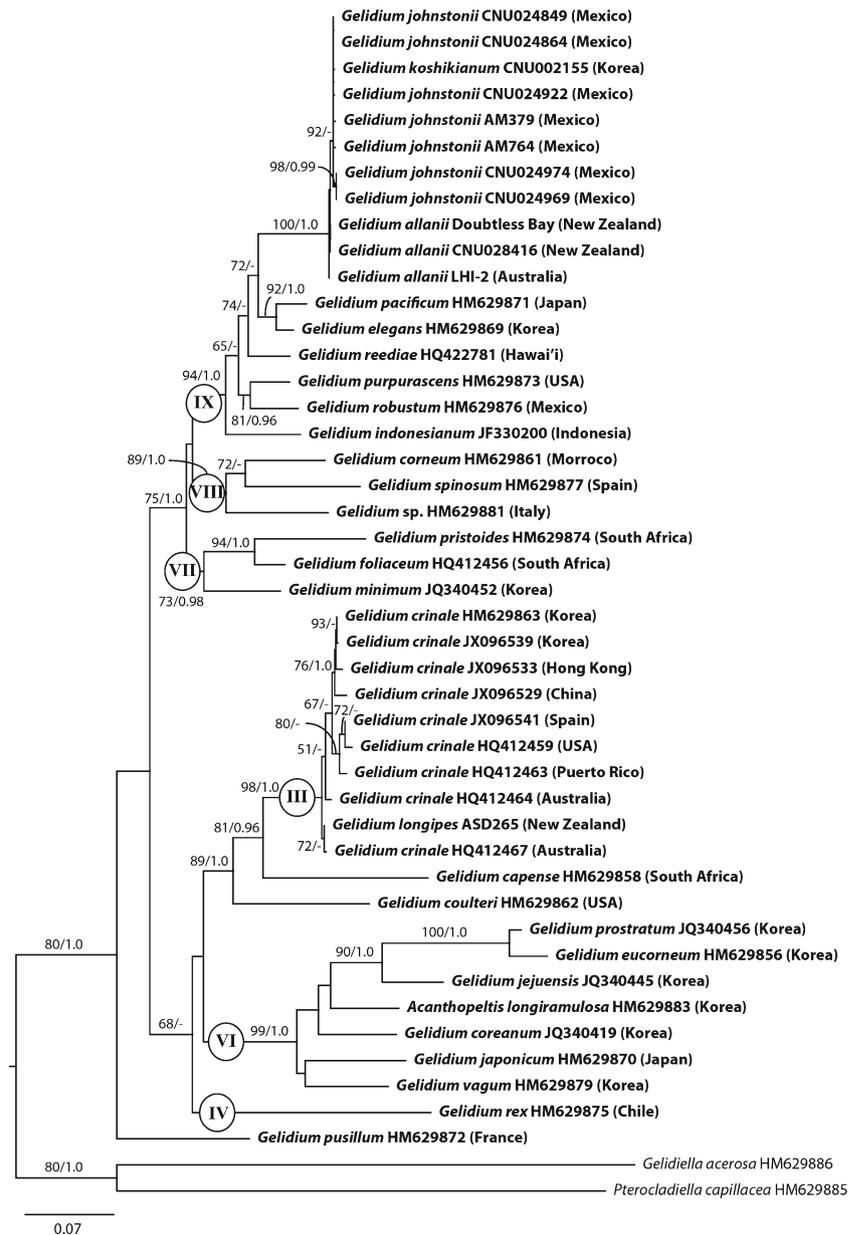
three species was clearly separated from morphologically similar species, *Gelidium abbottiorum* Norris (South Africa), *Gelidium americanum* (Taylor) Santelices, *Gelidium floridanum* Taylor (Pacific coast of America and Caribbean Sea), *Gelidium serrulatum* J. Agardh, and *Gelidium vagum* Okamura (Japan and Korea). In addition, the pairwise divergences among *G. allanii*, *G. johnstonii*, and *G. koshikianum* were very low (0.3 % in *rbcL* and 0.1 % to 0.6 % in *cox1*), compared to those between most of *Gelidium* species, strongly suggesting that these taxa are conspecific.

G. johnstonii was described by Setchell and Gardner (1924), based on specimens from San Francisquito Bay, Lower California, Mexico. It is characterized by decidedly flattened thalli, regularly pinnate branches, and more flattened and spatulate tetrasporangial ramuli. *G. johnstonii* is one of the most common species of *Gelidium* in Baja California (Dawson 1953). Although it was reported in Japan by Segi (1955) and also in Korea (Sohn and Kang 1978), subsequent publications exclude its occurrence in Japan and Korea (Lee 1988; Lee and Kim 1995; Yoshida 1998). However, our specimens collected in the subtidal region, Jeju Island, are identical with *G. johnstonii* from Baja Mexico in morphology and *cox1* and *rbcL* (Fig. 3). *G. koshikianum* from Japan has middle-sized thalli (5–8 cm) with wide axes (up to 2.5 mm) short (2.0–3.2 mm), unbranched, second- and third-order branches issuing at short intervals (0.8–1.4 mm) (Shimada et al. 2000). *G. allanii* from New Zealand has very prominent apical cells, narrower axes (up to 0.5 mm), longer second- and third-order branches, and tetrasporangia formed in elongate patches with sterile margins (Chapman 1969; Adams 1994; Nelson et al. 1994). Based on the above-mentioned *cox1* and *rbcL* phylogenies and their

pairwise divergences between these species and the lack of strong morphological differences, we propose that all three taxa actually belong to a single species. *G. johnstonii* (Setchell and Gardner 1924) has priority of publication, and thus following the International Code of Botanical Nomenclature, *G. allanii* (Chapman 1969) and *G. koshikianum* (Shimada et al. 2000) represent heterotypic synonyms of *G. johnstonii*. Another species requiring investigation is *Gelidium tsengii*, as it is similar to *G. johnstonii* in having flattened thalli with short opposite ramuli. Described by Fan (1961b), and based on specimens from White Sand Beach, Hong Kong, collected on May 9, 1933, molecular data from *G. tsengii* are needed to enable comparison with its congeners.

G. longipes, described from specimens in the Bay of Islands, northeastern New Zealand, is a small tufted alga that has elongate, terete axes that become flattened and branched at the tips (Chapman 1969; Nelson and Farr 2003). Adams (1994) noted that “*G. longipes*, described from New Zealand plants, includes at least some of the entities recorded as *G. crinale*, a European species”. Nelson and Farr (2003) also mentioned its similarity with *G. crinale* and necessity of molecular comparison, and Nelson et al. (2006) further found the close relationship of *G. longipes* with the *G. crinale*/*G. pusillum* complex in their *rbcL* tree. Kim and Boo (2012) concluded that that *G. crinale* is a cosmopolitan species with cylindrical axes while *G. pusillum*, restricted to European coasts, has flattened axes. Our *cox1* and *rbcL* trees clearly revealed that *G. longipes*, along with two Australian *G. crinale* sequences, was consistently placed in *G. crinale*, and that the *cox1* sequence of *G. longipes* from New Zealand is identical with that (HQ412466, Freshwater et al. 2010) of

Fig. 1 Maximum likelihood tree of the *cox1* gene data from Gelidiaceae. The numbers above or below the nodes are RAxML bootstrap values and Bayesian posterior probabilities. Only bootstrap values $\geq 50\%$ and ≥ 0.95 Bayesian posterior probabilities are shown on the tree. Roman numbers in circles denote clades as depicted in the text, but except I–III which were not available for *cox1* analysis



material collected from Old Gulch, Lord Howe Island, Australia. It is therefore concluded that *G. longipes* is an Australasian variant of *G. crinale*. However, because the *G. crinale* clade consisted of three geographically structured subgroups, Asian, Australian, and European/American groups, we propose that the Australasian clade of *G. crinale* should be recognized at the subspecies level at this time rather than as a forma or a separate species.

Based on the above results, we propose taxonomic revision as follows:

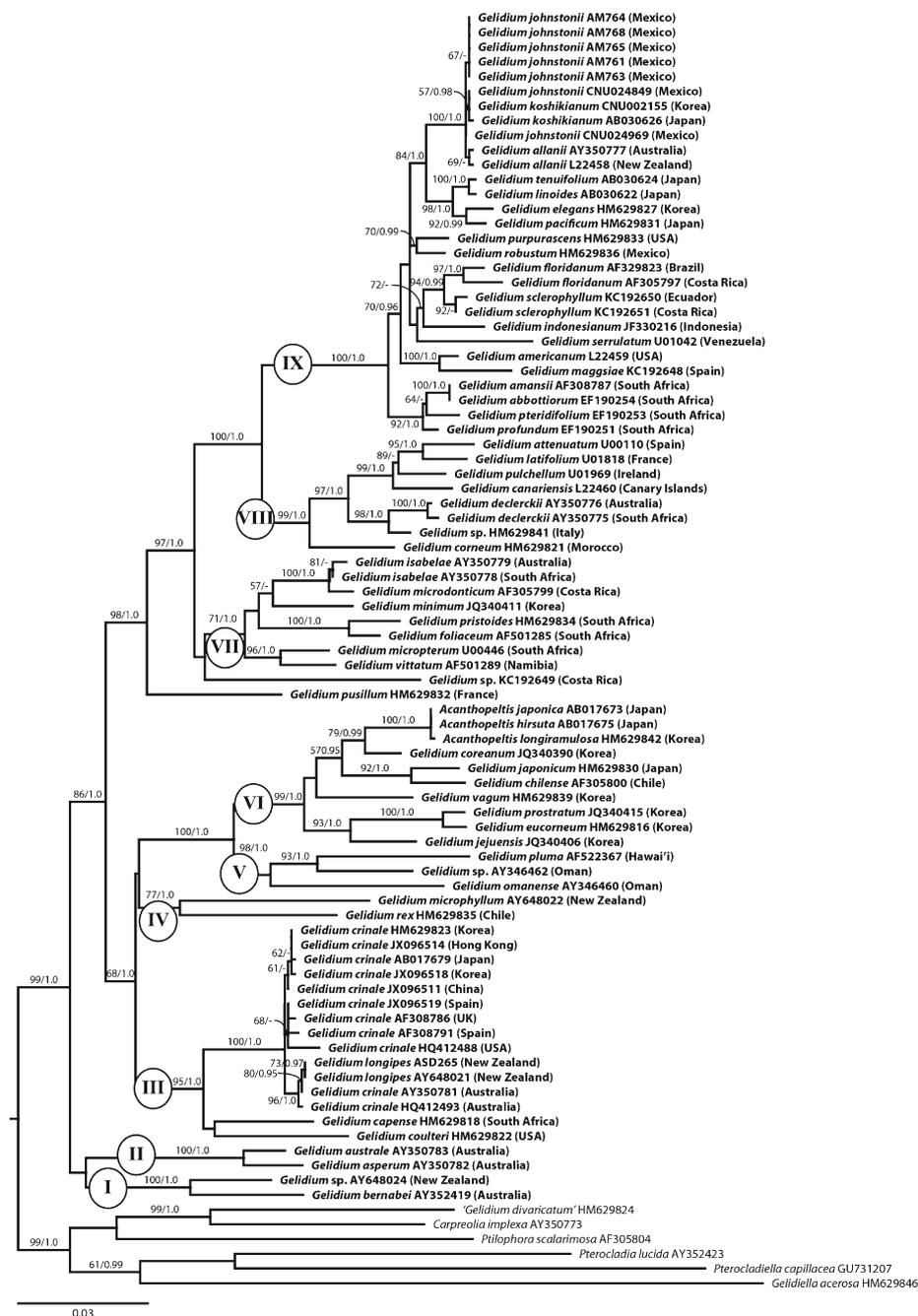
Gelidium johnstonii Setchell & Gardner 1924 (in Calif. Acad. Sci 12: 742, pls 72–3). Holotype: No. 1343 (image of paratype in Fig. 3a), Herb. Calif. Acad. Sci., collected by Ivan M. Johnston (no. 27), at San Francisquito Bay, Lower California, Mexico.

Synonyms: *Gelidium allanii* V.J. Chapman 1969 (Mar alg. New Zealand 3: 98, pl. 33, A.). Holotype: AK280542 (image in Fig. 3b); Waitata Rock, Russell, New Zealand, 20.xi.1937, V.W. Lindauer.

Gelidium koshikianum S. Shimada, T. Horiguchi & M. Masuda 2000 (Phycol Res. 48:37–46, Figs 13–22). Holotype: SAP 070874 (image in Fig. 3c), Nagahama, Shimo-Koshiki Island, Koshiki Islands, Kagoshima Prefecture, 31.vii.1997, S. Shimada.

Gelidium crinale subsp. *longipes* (J. Agardh) W.A. Nelson & G.H. Boo subsp. nov. (“*G. longipes* J. Agardh” in Sp. alg. 3: 547, 1876). Holotype: the Bay of Islands, northeastern New Zealand (Berggren) (L.).

Fig. 2 Maximum likelihood tree of the *rbcL* gene data from Gelidiaceae. The numbers above or below the nodes are RAxML bootstrap values and Bayesian posterior probabilities. Only bootstrap values $\geq 50\%$ and ≥ 0.95 Bayesian posterior probabilities are shown on the tree. Roman numbers in circles denote clades as depicted in the text



Recent introduction

Our *cox 1* and *rbcL* trees revealed the close relationship of *G. johnstonii* with the clade of Asian species, *Gelidium elegans* Kützinger, *Gelidium pacificum* Okamura, *G. tenuifolium*, and *G. linoides*. This relationship suggests that all these five species might have diverged in Asian waters and then *G. johnstonii* may be an Asian element rather than a northeastern Pacific species. The very low pairwise divergence of specimens of *G. johnstonii* from Korea/Japan and Baja California, Mexico indicates that its distribution in Mexico is likely to be

the result of recent anthropogenic agents. We found that some thalli of *G. johnstonii* grew on shells in San Carlos, Guaymas, Mexico. The same is true for *G. vagum*, introduced to California, USA and Netherlands (Hughey et al. 1996; Renfrew et al. 1989; Mineur et al. 2012), and which has often been found on shells. Growing on shells may be one of dispersal vectors for this species. In this context, introduction of *G. johnstonii* to Mexico may be linked to oyster mariculture in California and Washington, USA, as in case of other red algal invaders (e.g., Miller et al. 2011, Gracia-Rodriguez et al. 2013). If our scenario is correct, *G. johnstonii* becomes

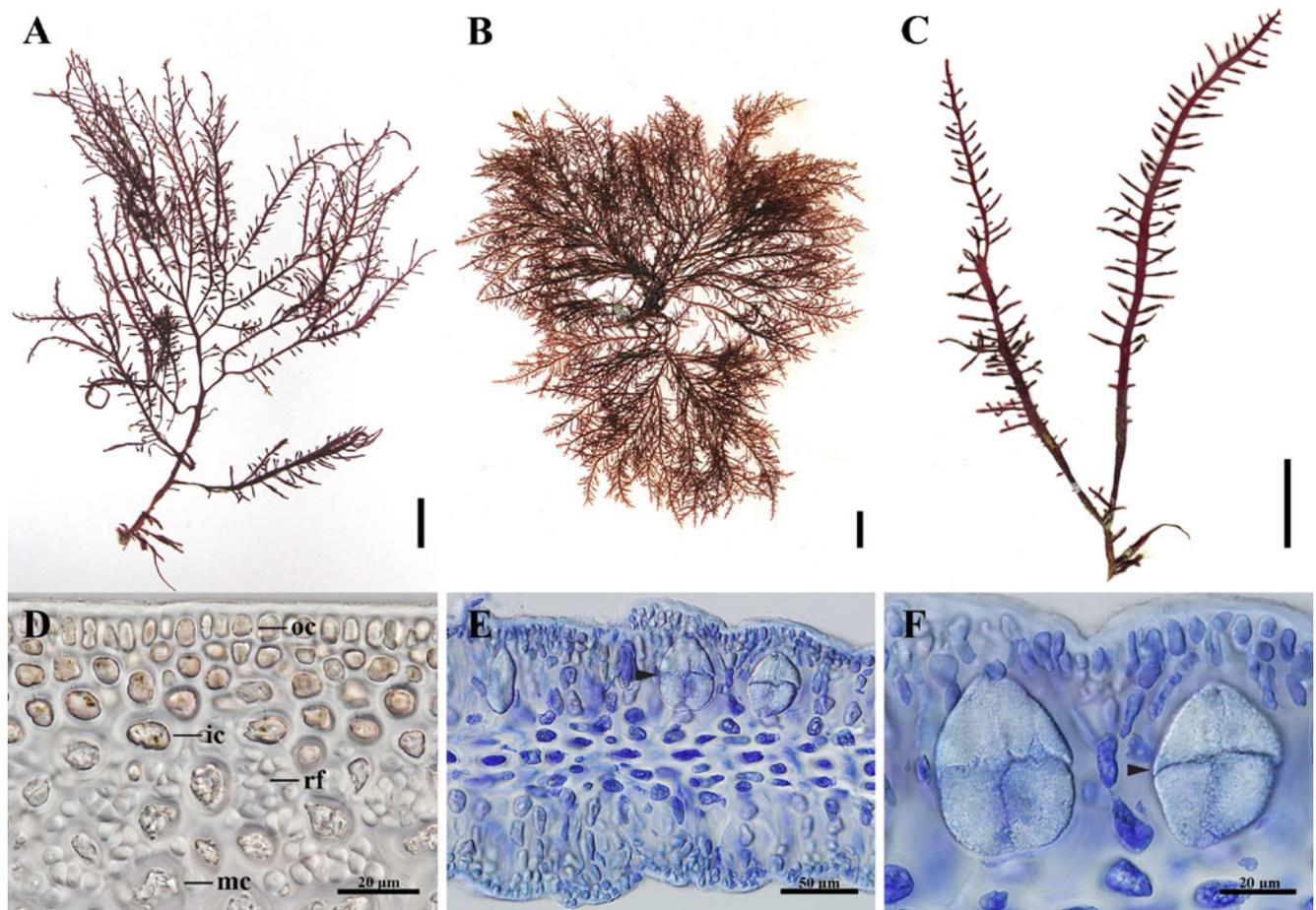


Fig. 3 **a** *Gelidium johnstonii*, paratype specimen, UC484390; San Marcos Island, Gulf of California, Mexico; June 19, 1921 (scale=1 cm). **b** *G. allanii*, holotype specimen, AK280542; Waitata Rocks, Russell, New Zealand; November 20, 1937 (scale=1 cm). **c–f**, *G. koshikianum*, CNU018554; **c** tetrasporophyte from Munseom, Jeju, Korea; May 16,

2011 (scale=1 cm). **d** Transverse section of axis, showing outermost cortical cells (*oc*), inner cortical cells (*ic*), rhizoidal filaments (*rf*), and medullary cells (*mc*). **e** Transverse section of a tetrasporangial branch with tetrasporangia (*arrowhead*). **f** Enlargement of a part in **e**, showing cruciately divided tetrasporangia (*arrowhead*)

the second invading species in the genus after *G. vagum*. There have been no reports of introduced macroalgae associated with the introduction of oysters for marine farms in New Zealand. Although Freshwater et al. (1995) suggested that *G. johnstonii* (as *G. allanii*) may be an exotic Japanese species that has spread to New Zealand based on morphological similarity and its sister relationships with Japanese *Gelidium* species, it is difficult to reconcile this hypothesis with the distribution of *G. allanii* populations in the intertidal zone of remote areas of northern New Zealand.

Biogeography of major *Gelidium* clades

The *rbcL* phylogeny of *Gelidium* revealed biogeographically interesting patterns. The monophyly of *Gelidium* including 59 species (47 % of total 126 species, see Guiry and Guiry 2013) was well supported. All nine groups of species, except *G. pusillum* and *Gelidium* sp. from Costa Rica, belong to two categories: either geographically restricted or with a more

cosmopolitan distribution. Groups I (*Gelidium bernabei* Millar & Freshwater and *Gelidium* sp.) and II (*Gelidium asperum* (C. Agardh) Greville and *Gelidium australe* J. Agardh) were consistently placed outside the clade of remaining species of *Gelidium*. Because all these four taxa occur in Australia and/or New Zealand (Womersley 1994; Millar and Freshwater 2005), we suggest the Australasian origin of *Gelidium*. This hypothesis has also been proposed by Hommersand (2007) who postulated that the Gelidiales may have arisen in the southern hemisphere, pointing largely to *P. lucida* and *Pterocladia rectangularis* (Lucas) Womersley & Guiry. The separation of Asian (Group VI) from Atlantic species in Group VIII suggests the hypothesis of vicariance. However, inclusion of Chilean *Gelidium chilense* (Montagne) Santelices & Montalva within the Asian clade appears anomalous and should be reconsidered after additional sampling. The most complex pattern occurs in Group IX, composed of many subgroups from the Pacific, Southeast Africa, Australasia, and the Caribbean Sea. This

group implies genetic connectivity within Indo-Pacific waters and between Pacific and Caribbean waters. The number of sympatric species pairs is similar to that of allopatric species pairs. However, more taxon sampling will enable us to examine which distribution pattern is more common or if both patterns occur in similar proportions in *Gelidium* (Table 2). More extensive taxon sampling and more markers will help to illuminate evolution within *Gelidium* and the current distribution patterns shown by members of this genus.

Acknowledgments We thank Ewen Cameron from Auckland Museum for providing images of types and Kazuhiro Kogame from Hokkaido University for providing isotype specimen on loan, Wilson Freshwater for two DNAs, and Paul C. Silva for his hospitality in observation of paratype specimens in Berkeley. This work was supported by Basic Science Grant (2012-0704), Korean Research Foundation, and MarineBio21 program grants from the Ministry of Maritime Affairs & Fisheries to SMB, MBIE Core Funding to NIWA (COBR1301 - WN), and by CONACYT SEP for field work to RRR.

References

- Adams NM (1994) Seaweeds of New Zealand. An illustrated guide. Canterbury Univ Press, Christchurch, 360 pp
- Agardh JG (1876) Species genera et ordines algarum, seu descriptions succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumen tertium: de Florideis curae posterioris. Part I. CWK Gleerup, Lipsiae, 724 pp
- Boo GH, Park JG, Gerung G, Boo SM (2013) Transfer of the red alga *Gelidium zollingeri* Sonder (Gelidiales) to *Yonagunia* (Halimiales) based on morphological and molecular evidence. *Phycologia* 52:279–287
- Chapman VJ (1969) The marine algae of New Zealand. Part III. Rhodophyceae, Issue 1. Bangiophycidae and Florideophycidae (Nemalionales, Bonnemaisoniales, Gelidiales). Cramer, Lehre, 113 pp
- Dawson EY (1953) Marine red algae of Pacific Mexico. Part I. Bangiales to Corallinaceae subf. Corallinoidae. *Allan Hancock Pac Exp* 17:1–239
- Dixon PS (1958) The structure and development of the thallus in the British species of *Gelidium* and *Pterocladia*. *Ann Bot* 22:353–368
- Fan KC (1961a) Morphological studies of the Gelidiales. *Univ Calif Publ Bot* 32:315–368
- Fan KC (1961b) Two new species of *Gelidium* from China. *Bot Mar* 2: 247–249
- Freshwater DW, Ruess J (1994) Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based on *rbcL* nucleotide sequence analysis. *Phycologia* 33:187–194
- Freshwater DW, Fredericq S, Hommersand MH (1995) A molecular phylogeny of the Gelidiales (Rhodophyta) based on analysis of plastid *rbcL* nucleotide sequences. *J Phycol* 31:616–632
- Freshwater DW, Tudor K, O'Shaughnessy K, Wysor B (2010) DNA barcoding in the red algal order Gelidiales: comparison of COI with *rbcL* and verification of the “barcoding gap”. *Cryptogamie Algol* 31: 435–449
- Gardner NL (1927) New species of *Gelidium* on the Pacific coast of North America. *Univ Calif Publ Bot* 13:273–318
- Gavio B, Fredericq S (2002) *Grateloupia turuturu* (Halymeniaceae, Rhodophyta) is the correct name of the non-native species in the Atlantic known as *Grateloupia doryphora*. *Eur J Phycol* 37:349–359
- Geraldino P, Yang EC, Boo SM (2006) Morphology and molecular phylogeny of *Hypnea flexicaulis* (Gigartinales, Rhodophyta) from Korea. *Algae* 21:417–423
- Gracia-Rodriguez LD, Riosmena-Rodriguez R, Kim SY, Lopez-Meyer M, Ordunas-Rojas J, Boo SM (2013) Recent introduction of *Gracilaria parvispora* (Gracilariales, Rhodophyta) in Baja California, Mexico. *Bot Mar* 56:143–150
- Guiry MD, Guiry GM (2013) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: <http://www.algaebase.org>. Accessed 5 March 2013
- Hommersand MH (2007) Global biogeography and relationships of the Australasian marine macroalgae. In: McCarthy PM (ed) *Algae of Australia: introduction*. Australian Biological Resources Study and CSIRO Publishing, Canberra, pp 511–542
- Hughey JR, Kieltson CK, Silva PC, Moe RL, Decew TC (1996) Noteworthy collections: California. *Madrono* 43:432–436
- Kim KM, Boo SM (2012) Relationships and distribution of *Gelidium crinale* and *G. pusillum* (Gelidiales, Rhodophyta) based on *cox1* and *rbcL* sequences. *Algae* 27:83–97
- Kim KM, Hwang IK, Park JK, Boo SM (2011) A new agarophyte species, *Gelidium eucorneum* sp. nov. (Gelidiales, Rhodophyta), based on molecular and morphological data. *J Phycol* 47:904–910
- Kim KM, Hwang IK, Yoon HS, Boo SM (2012) Four novel *Gelidium* species (Gelidiales, Rhodophyta) discovered in Korea: *G. coreanum*, *G. jejuensis*, *G. minimum* and *G. prostratum*. *Phycologia* 51:461–474
- Lee YP (1988) Taxonomic studies on the Gelidiales (Rhodophyta) in Cheju Island I. Some members of *Gelidium*. *Korean J Plant Tax* 18: 95–113
- Lee HB, Kim JI (1995) Notes on Gelidiales species from Korea. In: Abbott IA (ed) *Taxonomy of economic seaweeds*. Vol. 5. California Sea Grant College System, La Jolla, pp 161–174
- Lin SM, Fredericq S, Hommersand MH (2001) Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbcL* sequences, including the Phycodryoideae subfam. nov. *J Phycol* 37:881–899
- Loomis NH (1949) New species of *Gelidium* and *Pterocladia* with notes on the structure of the thalli in these genera. *Allan Hancock Occas Pap* 6:1–29
- Loomis NH (1960) New species of *Gelidium* and *Pterocladia* from the Pacific coast of the United States and the Hawaiian Islands. *Allan Hancock Occas Pap* 24:1–35
- Millar AJK, Freshwater DW (2005) Morphology and molecular phylogeny of the marine algal order Gelidiales (Rhodophyta) from New South Wales, including Lord Howe and Norfolk islands. *Aust Syst Bot* 18:215–263
- Miller KA, Aguilar-Rosas LE, Pedroche FF (2011) A review of non-native seaweeds from California, USA and Baja California, Mexico. *Hidrobiológica* 21:365–379
- Mineur F, Le Roux A, Stegenga H, Verlaque M, Maggs CA (2012) Four new exotic red seaweeds on European shores. *Biol Invas* 14:1635–1641
- Nelson WA, Farr TJ (2003) Field and morphological observations of *Gelidium longipes* (Gelidiales, Rhodophyta), a rare endemic red alga from northern New Zealand. *NZ J Bot* 18:707–713
- Nelson WA, Knight GA, Falshaw R, Furneaux RH, Falshaw A, Lynds SM (1994) Characterisation of the enigmatic, endemic red alga *Gelidium allanii* (Gelidiales) from northern New Zealand—morphology, distribution, agar chemistry. *J Appl Phycol* 6:497–507
- Nelson WA, Farr TJ, Broom JES (2006) Phylogenetic diversity of New Zealand Gelidiales as revealed by *rbcL* sequence data. *J Appl Phycol* 18:653–661
- Okamura K (1934) On *Gelidium* and *Pterocladia* of Japan. *Jourm Imp Fish Inst (Tokyo)* 29:47–67
- Perrone C, Felicini GP, Bottalico A (2006) The prostrate system of the Gelidiales: diagnostic and taxonomic importance. *Bot Mar* 49:23–33

- Rambaut A (2002) SeAl, v. 2.0. Institute of Evolutionary Biology. Ashworth Laboratories, Edinburgh
- Renfrew DE, Gabrielson PW, Scagel RF (1989) The marine algae of British Columbia, northern Washington, and southeast Alaska: division Rhodophyta (red algae), class Rhodophyceae, order Gelidiales. *Can J Bot* 67:3295–3314
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542
- Santelices B (1977) A taxonomic review of Hawaiian Gelidiales (Rhodophyta). *Pac Sci* 31:61–84
- Segi T (1955) The species of the genus *Gelidium* from Japan and its vicinity (I). *Rep Fac Fish Mie Pref Univ* 2:124–136
- Setchell WA, Gardner NL (1924) Expedition of the California Academy of Sciences to the Gulf of California in 1921. *Proc Calif Acad Sci* 12:695–949
- Shimada S, Horiguchi T, Masuda M (1999) Phylogenetic affinities of genera *Acanthopeltis* and *Yatabella* (Gelidiales, Rhodophyta) inferred from molecular analyses. *Phycologia* 38:528–540
- Shimada S, Horiguchi T, Masuda M (2000) Two new species of *Gelidium* (Gelidiales, Rhodophyta), *Gelidium tenuifolium* and *Gelidium koshikianum*, from Japan. *Phycol Res* 48:37–46
- Sohn CH, Kang JW (1978) The classification of family Gelidiaceae (Rhodophyta) in Korea. *Publ Inst Mar Sci Nat Fish Univ Busan* 11:29–40
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690
- Thiers B (2013) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>. Accessed 13 March 2013
- Tronchin EM, Freshwater DW (2007) Four Gelidiales (Rhodophyta) new to southern Africa, *Aphanta pachyrrhiza* gen. et sp. nov., *Gelidium profundum* sp. nov., *Pterocliadiella caerulea* and *P. psammophila* sp. nov. *Phycologia* 46:325–348
- Tronchin EM, Freshwater DW, Bolton JJ, Anderson RJ (2002) A reassessment and reclassification of species in the genera *Onikusa* Akatsuka and *Suhria* J. Agardh ex Endlicher (Gelidiales, Rhodophyta) based on molecular and morphological data. *Bot Mar* 45:548–555
- Womersley HBS (1994) The marine benthic flora of Southern Australia—Part IIIA—Bangiophyceae and Florideophyceae (Acrochaetales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales sensu lato). Australian Biological Resources Study, Canberra, 508 pp
- Yoshida T (1998) Marine algae of Japan. Uchida Rokakuho, Tokyo, p 1222