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Taxonomic revision of *Gracilaria* "*verrucosa*" from the Russian Far East based on morphological and molecular data

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Abstract

Gracilaria "*verrucosa*" is now regarded an invalid name, with *G. gracilis* being used in recent studies of the genus from the Russian coast of the East Sea (Sea of Japan). This has created nomenclatural confusion for members of the genus in this region. Here we revise the taxonomy of *G.* "*verrucosa*" from Peter the Great Bay (East Sea, Russia) based on morpho-anatomical characters and comparisons of nuclear small subunit rDNA, plastid *rbc*L and mitochondrial *cox*1 sequence data. No differences were found between sequences for attached and unattached forms of *G.* "*verrucosa*". Molecular data also indicate that *G.* "*verrucosa*" from Peter the Great Bay is conspecific with *Gracilaria vermiculophylla* from the northwestern Pacific, and the Russian population is closely related to Korean-Japanese populations from the East Sea rather than with populations on the Pacific coast of Japan.

Keywords: *Gracilaria gracilis*; *Gracilaria vermiculophylla*; *Gracilaria* "*verrucosa*"; Peter the Great Bay of Russia; taxonomic revision.

Introduction

The genus *Gracilaria* (Gracilariales, Rhodophyta) has a worldwide distribution and comprises more than 190 valid species (Guiry and Guiry 2008), many of which have high commercial value. Delimitation of gracilarioid taxa based on morphological characteristics is notoriously difficult because of feature variability (Oliveira et al. 2000, Gurgel and Fredericq 2004).

Three *Gracilaria* species are reported from the Russian Far East (Przhemenetskaya 1989, Perestenko 1994). Flat specimens have been referred to as *G. textorii* (Suringar) De Toni, whereas cylindrical specimens have been referred to as *G. austramaritima* Przhemenetskaya in Selivanova et al. (2008) and *G.* "*verrucosa*" (Hudson) Papenfuss. Steentoft et al. (1995) showed that *G. verrucosa* is a taxonomically invalid name and proposed a new combination, *G. gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham. Subsequent studies of the genus *Gracilaria* in the Russian Far East have used the name *G. gracilis* for cylindrical specimens (Skriptsova and Yakovleva 2002, Nabivailo et al. 2005).

The binomial *Gracilaria asiatica* Zhang *et* Xia was applied to Asian *G. verrucosa* in other studies (Yoshida et al. 1995), but *G. asiatica* has been reduced to a synonym of *G. vermiculophylla* (Ohmi) Papenfuss (Yoshida 1998). It is uncertain whether *G. gracilis* is the correct name for cylindrical *Gracilaria* specimens from the Russian Far East previously identified as *G.* "*verrucosa*".

In Peter the Great Bay [Russian coast of the East Sea (Sea of Japan)] *Gracilaria* "*verrucosa*" is present in both attached and unattached forms (Titlyanova et al. 1990). The attached form is distributed within the Russian Far East along the coast of the East Sea from Tatarsky Strait in the north to Peter the Great Bay in the south. It also occurs off the South Kuriles and Sakhalin Islands in the Okhotsk Sea. The unattached form is only found in the brackish-water lagoon system connected to Sivuchya Bay in the southern section of Peter the Great Bay. Furthermore, it is only found in the vegetative state. A physiological comparison of the two forms of *G.* "*verrucosa*" from the Russian coast of the East Sea showed large differences in photosynthetic rate, adaptation to light and salinity (Yakovleva 2007), suggesting that they are not genetically identical.

The goals of the present study were to evaluate the status of *Gracilaria* "*verrucosa*" from Peter the Great Bay and to determine whether the attached and unattached forms of *G.* "*verrucosa*" are conspecific. It should be stated that precise taxonomic identification of sterile cylindrical specimens is a difficult task, especially in light of morphological modifications due to the influence of particular biotic (e.g., herbivory) and abiotic (e.g., drifting habit, sand scour, nutrient limitation, current velocity) factors. Vegetative characteristics of algae are highly variable in these cases and molecular analyses are the most reliable approach for species identification.

Materials and methods

Morphological observations

Samples were collected in summer 2007 from four localities in Peter the Great Bay (East Sea). Specimens of the attached form were collected off Cape Rechnoy, Lasurnaya Bay and Sivuchya Bay, while specimens of the unattached form were collected from the brackish-water lagoon system connected to Sivuchya Bay (Table 1). Algal samples were sectioned with a freezing microtome and observed using a light microscope. They were subsequently dried and stored in plastic bags with silica gel.

Table 1 Russian samples of *Gracilaria* "*verrucosa*" included in morphological and molecular investigations.

Sample	Collection details	GenBank accession numbers		
		SSU	*rbc*L	*cox*1
CH1422	Unattached form; intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 1 May 2007	GQ292853	GQ292855	GQ292864
CH1431-1	Unattached form; intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 1 May 2007	–	GQ292856	GQ292865
CH1431-2	Unattached form; intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 1 May 2007	–	GQ292857	GQ292866
CH1471	Attached form; intertidal, Lazurnaya Bay (132°03′ E, 43°02′ N), Peter the Great Bay, 28 August 2007	–	GQ292858	GQ292867
CH1478	Attached form; intertidal, Cape Rechnoy (131°49′ E, 43°18′ N), Peter the Great Bay, 24 August 2007	–	GQ292859	GQ292868
CH1479	Unattached form; intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 12 June 2007	–	GQ292860	GQ292869
CH1480	Attached form; intertidal, Sivuchya Bay (130°46′ E, 41°27′ N), 12 June 2007	GQ292854	GQ292861	GQ292870
CH1482	Unattached form, intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 12 June 2007	–	GQ292862	GQ292871
CH1485	Unattached form, intertidal, lagoons of Khasan district (130°45′ E, 41°28′ N), 12 June 2007	–	GQ292863	GQ292872

Vouchers were pressed and exsiccatae deposited in the AV Zhirmunsky Institute of Marine Biology herbarium, Russian Academy of Science at Vladivostok.

Molecular analyses

Collection information for specimens used in morphological and molecular investigations is provided in Table 1. Genomic DNA from silica-dried samples was extracted using a DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). After DNA extraction, the remaining silica-dried samples were deposited in the Korea Polar Research Institute (KOPRI) herbarium.

Nuclear small subunit (SSU) rDNA, plastid *rbc*L and mitochondrial *cox*1 were amplified from total genomic DNA using polymerase chain reaction (PCR) and the primer combinations of Saunders and Kraft (1994, 1996) for SSU rDNA, of Freshwater and Rueness (1994) for *rbc*L, and of Saunders (2005) for partial *cox*1. Agarose gel purification or direct purification on a High Pure™ PCR Product Purification Kit (Roche Diagnostics, Indianapolis, IN, USA) or LaboPass PCR Purification Kit (COSMO Genetech, Seoul, Korea) were used to clean PCR products before sequencing with a BigDye™ terminator cycle sequencing ready reaction kit (PE Applied Biosytems [ABI], Foster City, CA, USA). Sequence data were collected using an ABI PRISM 3730 DNA analyzer. Sequence data were edited using the SeqEd DNA sequence editor (ABI) software package and edited sequences were aligned using the SeqPup multiple alignment (Gilbert 1995) and MacClade 4 programs (Maddison and Maddison 2003).

The final alignments for SSU rDNA, *rbc*L and partial *cox*1 sequences consisted of 4, 27 and 36 taxa, respectively, including 2, 18 and 27 previously published SSU rDNA, *rbc*L and partial *cox*1 sequences for *Gracilaria* species as outgroups (Table 2). The 1793 (SSU), 1481 (*rbc*L) and 710 (*cox*1) aligned nucleotide positions were edited to remove the 5′ and 3′ PCR primer regions (Freshwater and Rueness 1994, Saunders and Kraft 1994, Saunders 2005), as well as ambiguously aligned regions, yielding 1720, 1386 and 427 base pairs, respectively, for phylogenetic inference.

Parsimony and distance analyses were completed in PAUP* 4.0b10 (Swofford 2002). Parsimony analyses (unweighted, gaps treated as missing data) were completed using a heuristic search strategy (100 random additions) with tree-bisection-reconnection (TBR) branch swapping. Distance analyses were completed with neighbor joining (Saito and Nei 1987) using the Kimura two-parameter model (Kimura 1980). To estimate the robustness of internal nodes, bootstrap resampling was carried out for the parsimony and distance analyses (1000 replicates with random additions set to 10 and 1000 replicates, respectively). In all analyses, unrooted trees were calculated and the ingroup taxa were subsequently rooted on *Gracilaria chilensis* (Bellorin et al. 2002, Gurgel and Fredericq 2004).

Minimum spanning networks were constructed by comparing both the most parsimonious trees generated in PAUP* 4.0b10 for 25 *rbc*L haplotypes and 23 partial *cox*1 haplotypes from the most closely related taxa to the samples of attached and unattached forms from Peter the Great Bay.

Results

Morphological observations

Mature *Gracilaria* "*verrucosa*" plants from Peter the Great Bay are brown, dark olive or dark red. Thalli are erect, cylindrical, up to 25–30 cm tall and attached to substrata by a discoid holdfast of 1–4 mm in diameter, with several thalli arising from a holdfast. The main axes are 1.5–2 mm in diameter, usually with 3–4 orders of lateral branches of various sizes at irregular intervals in an alternate to unilateral pattern (Figure 1). The branches are usually slightly constricted at their bases. The cortex has one to three layers of well-pigmented cells. Outer cortical cells are radially elongate, ovoid, and 4.5–7 μm in diameter. The subcortical cells are 12–25 μm×25–31 μm and

Table 2 *Gracilaria*: sources of additional SSU rDNA, *rbc*L and *cox*1 sequence data included in the multiple alignment.

Species	Collection details	GenBank accession numbers		
		SSU rDNA	*rbc*L	*cox*1
Gracilaria chilensis Bird, McLachlan *et* Oliveira	Lachangua, De los Lagos, Chile	–	–	EF434915
Gracilaria gracilis (Stackhouse) Steentoft, Irvine *et* Farnham	Ile Verte, Roscoff, France	–	AY049399	–
G. gracilis	W. Angel Bay, Wales	–	AY049400	–
G. gracilis	Looe, England	–	–	DQ442908
G. gracilis	Roscoff, France	–	–	EF434919
G. gracilis	Sicily, Italy	–	–	EF434918
G. gracilis	Sidmouth 1, England	–	–	DQ191346
G. gracilis	Sidmouth 2, England	–	–	DQ191347
G. gracilis	Sidmouth 3, England	–	–	DQ191348
Gracilaria incurvata Okamura	Chiba, Japan	–	–	EF434920
Gracilaria parvispora Abbott	Jongdal, Jejudo, Korea	–	–	EF434921
G. parvispora	Hawaii, USA	–	–	EF434922
Gracilaria sp.	Batac, Philippines	–	–	EF434923
Gracilaria sp.	Mexico	AY468886	–	–
Gracilaria tenuistipitata var. *liui* Zhang *et* Xia	Orissa, India	–	–	EF434924
Gracilaria textorii (Suringar) De Toni	Kurohae Beach, Chiba, Japan	–	–	EF434925
Gracilaria vermiculophylla (Ohmi) Papenfuss	Japan	AY465828	–	–
G. vermiculophylla	Belon estuary, France	–	AY725172	–
G. vermiculophylla	Junchun 1, Donghae, Korea	–	EF434907	EF434926
G. vermiculophylla	Junchun 2, Donghae, Korea	–	EF434908	EF434927
G. vermiculophylla	Sangjokam 1, Gosung, Korea	–	DQ095819	EF434928
G. vermiculophylla	Sangjokam 2, Gosung, Korea	–	DQ095820	–
G. vermiculophylla	Daejung, Jejudo, Korea	–	EF434909	EF434929
G. vermiculophylla	Hado, Jejudo, Korea	–	DQ095815	EF434930
G. vermiculophylla	Haengwon, Jejudo, Korea	–	DQ095816	EF434931
G. vermiculophylla	Kimnyung 1, Jejudo, Korea	–	EF434910	EF434932
G. vermiculophylla	Kimnyung 2, Jejudo, Korea	–	EF434911	EF434933
G. vermiculophylla	Oidodong, Jejudo, Korea	–	DQ095818	EF434934
G. vermiculophylla	Sungsan, Jejudo, Korea	–	DQ095817	EF434935
G. vermiculophylla	Bangpo, Taean, Korea	–	DQ095821	EF434936
G. vermiculophylla	Inuwaka, Chiba, Japan	–	EF434912	EF434937
G. vermiculophylla	Shimoda, Shizuoka, Japan	–	DQ095822	EF434938
G. vermiculophylla	Konami, Oki Island, Japan	–	EF434913	EF434939

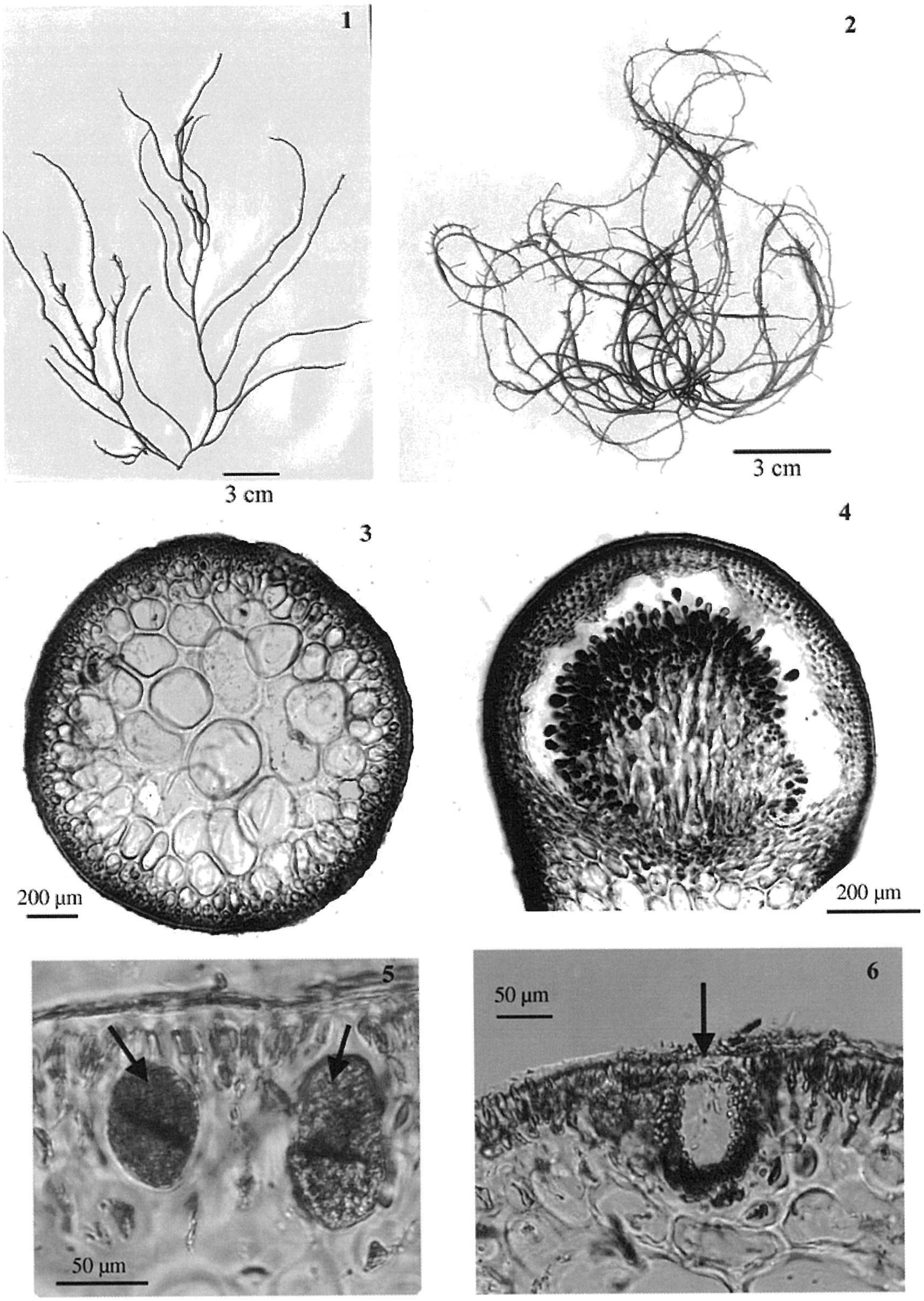
the medulla consists of large isodiametric cells 150–360 μm in diameter (Figure 3). Ruptured medullary cells occur on old branches, resulting in a partially hollow thallus.

Reproductive structures develop on all branches except near the apices. Mature cystocarps are subspherical and markedly basally constricted and up to 1.5–2 mm high. Inner gonimoblast cells are radially elongated, radiating from the cystocarp floor (Figure 4). The carposporangia are ovoid, 19–28 μm×39–50 μm, anticlinally elongated, and congregated in chains of 3–5 cells. Pericarps are 6–12 layers thick, consisting of ovoid cells up to 6–19 μm in diameter with secondary pit connections, not constituting rows. Tubular nutritive cells are only present on the floor of the cystocarp cavity. Cruciate-decussate, radially elongated tetrasporangia up to 28–42 μm×42–73 μm are produced in the outer cortex (Figure 5). Spermatangia (Figure 6) are produced in deep conceptacles of the *verrucosa* type (up to 50–84 μm deep and 42–56 μm wide).

Remarks: Two forms of *Gracilaria* “*verrucosa*” that are morphologically, physiologically and ecologically distinct can be recognized from the Russian coast of the East Sea. The attached variety forms extensive beds in the intertidal and upper sublittoral zones of shallow bays and estuaries of Peter the Great Bay. Specimens are attached to rocks or small pebbles, shells, or other substrata often covered with sand and mud. The vegetative anatomy and reproduction of the attached form are as described above. Unlike the attached form, thalli of the unattached form are free-floating and lay flat along the bottom, reaching 15–40 cm in length, and are irregularly branched to 3–5 (9) orders (Figure 2). The main axes of the thalli are ulterior; some axes arising from the initial fragment configure thalli into a globose shape. Reproductive structures have not been observed in the unattached form, which is believed to reproduce only by thallus fragmentation. This form lives on sandy or muddy bottoms in shallow brackish-water lagoons only in the southern part of Peter the Great Bay (Titlyanova et al. 1990).

DNA sequence data

Two SSU rDNA, nine *rbc*L, and nine partial *cox*1 sequences were newly generated in this study and deposited in GenBank (Table 1). No ambiguities were observed in the sequence data. The two SSU rDNA sequences generated for samples CH1422 (unattached form) and CH1480 (attached form) were identical (GenBank accession numbers GQ292853 and GQ292854). These sequences were also identical to those of *Gracilaria vermiculophylla* from Japan (AY465828) and Mexico (AF468886).



Figures 1–6 *Gracilaria vermiculophylla*: vegetative and reproductive structures of plants from Peter the Great Bay.
(1) Habit of the attached form. (2) Habit of the unattached form. (3) Cross-section of the old axis of a sterile plant. (4) Cross-section of a mature cystocarp. (5) Part of the cortex with tetrasporangia (arrows). (6) Fragment of a cross-section of the male gametophyte showing *verrucosa*-type spermatangial conceptacle (arrow).

The *rbc*L sequences for all nine samples from Russia (six unattached and three attached specimens) were also identical (GQ292855–GQ292863). These *rbc*L sequences differed by 0 to 4 point mutations from the sequence data of one French, three Japanese and 12 Korean *Gracilaria vermiculophylla* specimens in GenBank. Four different *rbc*L haplotypes were present among these 25 sequences (data not shown).

Table 3 *Gracilaria vermiculophylla*: sub-alignment for six haplotypes (A–F) of the partial *cox*1 sequence (664 bp) for specimens from Peter the Great Bay (Russia), Korea and Japan, showing positions in sequence and nucleotide changes in each haplotype (see Figure 7 for details).

Haplotype	*cox*1					
	308	439	469	550	556	565
A	**A**	T	**T**	G	T	A
B	G	**A**	C	G	T	A
C	G	T	C	G	T	A
D	G	T	C	G	**G**	A
E	G	T	C	**A**	T	A
F	G	T	C	G	T	**G**

Letters in bold indicate nucleotide changes in each haplotype.

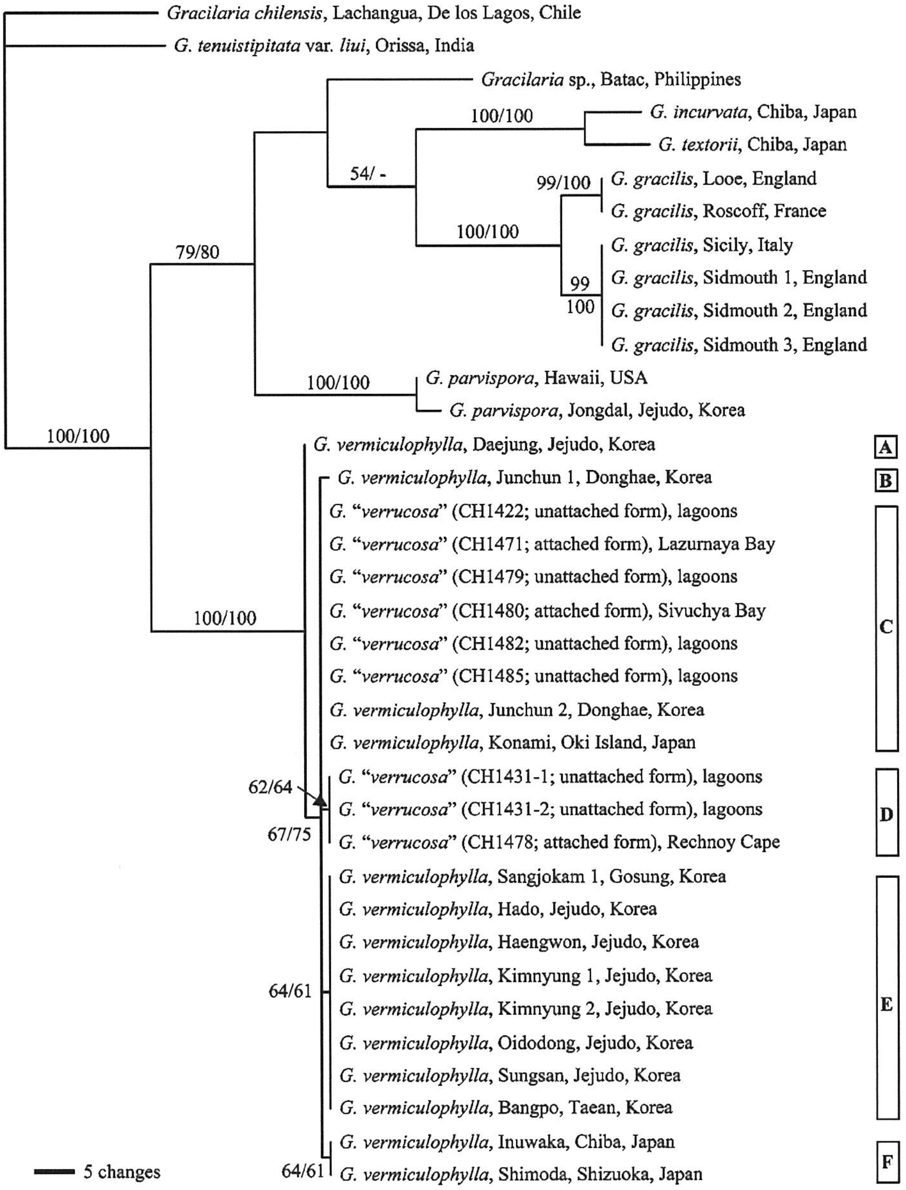


Figure 7 One of three equally parsimonious solutions for partial *cox*1 sequence data from *Gracilaria* spp. The numbers above the branches represent bootstrap values (≥50%) based on 1000 replicates for parsimony and distance analyses. Haplotypes of *cox*1 populations for *G. vermiculophylla* and *G. "verrucosa"* from Peter the Great Bay (Russian coast of the East Sea) are indicated.

Partial *cox*1 sequences for the nine Russian *Gracilaria "verrucosa"* samples differed from each other by 0–2 point mutations (GQ292864–GQ292872), and from the 11 Korean and three Japanese *G. vermiculophylla* sequences in GenBank by 2–3 point mutations. Six haplotypes were identified among these 23 sequences (Table 3).

Phylogeny of *Gracilaria "verrucosa"* from Peter the Great Bay and haplotype distribution

Maximum parsimony analysis produced a single most-parsimonious tree of 148 steps (consistency index 1.000, retention index 1.000) for partial *rbc*L sequence data for *Gracilaria "verrucosa"* from Peter the Great Bay and the outgroups (27 taxa with 1386 characters; data not shown). Four *rbc*L haplotypes were identified among the 25 sequences analyzed for *G. "verrucosa"* from Peter the Great Bay and *G. vermiculophylla* from GenBank. The Russian and some Korean samples from the East Sea and South Sea shared haplotype 2, and the remaining two haplotypes were found in the samples from the Pacific coast of Japan and a French sample, which represents an introduction from the northwestern Pacific region.

Maximum parsimony analysis of partial *cox*1 sequence data for 36 taxa (Table 2) resulted in three equally parsimonious solutions of 220 steps (consistency index 0.741, retention index 0.902) (Figure 7). Among the three equally parsimonious trees, there were no important differences within the *Gracilaria vermiculophylla* clade, which included *G. "verrucosa"* samples from Peter the Great Bay, a clade that was strongly supported in both parsimony and distance analyses (100% bootstrap support). The other included *Gracilaria* species from distinct clades that are not closely related to *G. vermiculophylla*. Six haplotypes (A–F) were identified in the 427 bp *cox*1 sub-alignments of 23 *G. vermiculophylla/Gracilaria "verrucosa"* sequences.

Samples with specific haplotypes were: haplotype A, Korean samples from the South Sea; haplotype B, Korean samples from the East Sea; haplotype C, Russian-Korean-Japanese samples from the East Sea; haplotype D, Russian samples from the East Sea; haplotype E, Korean samples from the East Sea, South Sea and West Sea; and haplotype F, Japanese samples from the Pacific coast (Figures 7 and 8).

Discussion

The correct identification of red algae collected from around the globe and labeled as *Gracilaria verrucosa* has been the subject of numerous taxonomic studies. The history of the binomial *G. verrucosa* is complex and has already been reviewed exhaustively (e.g., Rice and Bird 1990, Irvine and Steentoft 1995). The type of *G. verrucosa* found in the British Isles is cylindrical with deep spermatangial conceptacles. Unfortunately, any cylindrical *Gracilaria* with deep spermatangial conceptacles has been indiscriminately assigned to *G. verrucosa*. Specimens fitting this description in the Russian Far East were originally called *G. verrucosa* (Schapova 1957), and after the work of Steentoft et al. (1995), the name *G. gracilis* was used for cylindrical *Gracilaria* from this region (Skriptsova and Yakovleva 2002, Nabivailo et al. 2005, Yakovleva 2007).

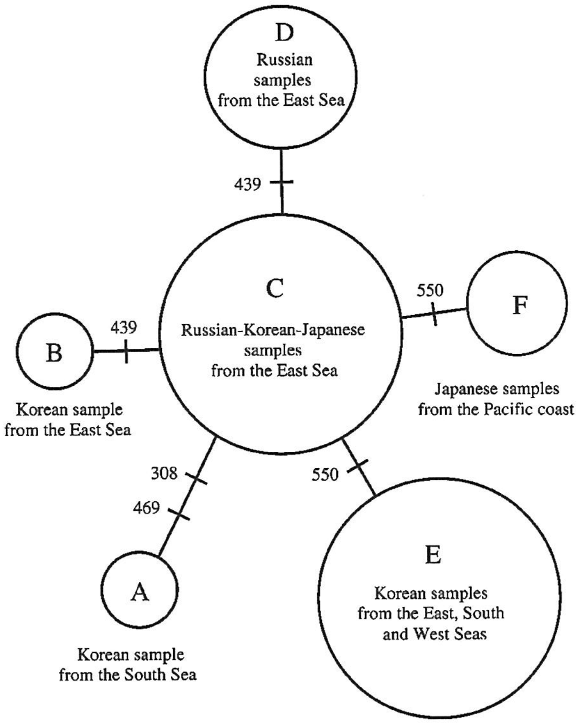


Figure 8 Parsimony network of six mitochondrial *cox*1 haplotypes.
Each circle represents one haplotype and its size is proportional to the frequency of that haplotype among all the samples. Numbers and characters on the cross line indicate the position and base of single-base-pair substitutions.

Molecular data, including nuclear SSU rDNA, plastid *rbc*L and mitochondrial *cox*1 sequence data, indicate that the dominant *Gracilaria* in Peter the Great Bay [East Sea (Sea of Japan)] is conspecific with *G. vermiculophylla* from the northwestern Pacific and clearly distinct from *G. gracilis*. Vegetative and reproductive morphology of Russian samples are in good accordance with the descriptions provided for *G. vermiculophylla* from Japan (Yamamoto 1978) and from the Pacific coast of North America (Bellorin et al. 2004). However, some morphological differences were found (Table 4). The diameter of major branches of the *Gracilaria* examined was smaller (1.5–2 mm) than that of *G. vermiculophylla* from the eastern Pacific coast (5 mm; Bellorin et al. 2004) and from Japan (2.5–3 mm; Yamamoto 1978). Furthermore, the depth of spermatangial conceptacles of the Russian samples (50–84 μm) was shallower than that reported for *G. vermiculopylla* from Japan, Korea, China and European waters (70–150 μm; Yamamoto 1978, Zhang and Xia 1985, Rueness 2005). Consistent conceptacle sizes were noted for European *G. gracilis* (50–60 μm; Steentoft et al. 1995) and the depth of spermatangial conceptacles has been considered to be one feature distinguishing between *G. gracilis* and *G. vermiculophylla* (Rueness 2005). Attached and unattached forms were distinguishable within the Russian *Gracilaria* examined by morpho-

Table 4 Morphological comparison of *Gracilaria gracilis*, *G. vermiculophylla* and two forms of *G.* "*verrucosa*" from the Russian coast of the East Sea (Sea of Japan).

	G. gracilis[1]	*G. vermiculophylla*[2,3]	*G.* "*verrucosa*" attached form[4]	*G.* "*verrucosa*" unattached form[5]
Thallus	Erect to 60 (110) cm, cylindrical, branching irregular, branches of irregular length at irregular intervals, slightly basally constricted	Erect up to 100 cm, cylindrical, branching irregular, branches of irregular length at irregular intervals, usually lack basal constrictions or sometimes slightly basally constricted	Erect to 25–30 cm, cylindrical, branching irregular, branches of irregular length at irregular intervals, slightly basally constricted	Prostrate 15–40 cm (up to 100 cm) long, cylindrical, branching irregular, branches of irregular length at irregular intervals, occasionally slightly basally constricted
Major branches	1–2 (2.5) mm in diameter	Up to 5 mm in diameter	1.5–2 mm	0.5–1.5 mm
Color	Dark reddish-brown	Dark brown or reddish-brown	Brown, dark olive, dark red	Brown, dark olive
Number of branch orders	2–4	2–5	3–4	3–5 (9)
Cortex–medulla transition	No data	Abrupt	Abrupt	Abrupt
Cortex	2 cells thick	1–3 cells thick	1–4 cells thick	1–3 cells thick
Cortex cell shape	Ovoid	Ovoid	Ovoid	Ovoid
Cortex cell size	5–8 μm in diameter	9–16 μm long, 4.5–6 μm in diameter	4.5–7 μm in diameter	11–17 μm long, 4–9 μm in diameter
Medulla cell shape	Isodiametric or anticlinally elongated	Isodiametric or subspherical	Isodiametric	Isodiametric
Medulla cell size	260 (300)×240 μm in diameter	400–490×270–365 μm in diameter	150–360 μm in diameter	180–300 μm in diameter
Cystocarp	No data	Borne on all branches except apical parts	Borne on all branches except apical parts	Absent
Cystocarp size	No data	To 1.5 mm high	To 1.5–2 mm high	
Cystocarp shape	Superspherical, mammilate with marked basal constriction	Prominent superspherical, sometimes with basal constriction	Superspherical, mammilate with marked basal constriction	
Pericarp tissue	From thick-walled and pitted cells, do not constitute rows	6–10 layers thick, from stellate cells with abundant secondary pit connection, 5–7×10–17 μm in diameter anticlinally	From ovoid cells with secondary pit connection, 6–19 μm in diameter, do not constitute rows	
Traversing filaments	Common, connecting gonimoblast with pericarp and cystocarp floor	Common in young cystocarps only, absent in mature ones	Present only on floor of cystocarp cavity	
Carposporangia	Up to 5–6 cells, ovoid, 25–31×27–38 μm in diameter, anticlinally	Up to 5 cells, superspherical, 15–29 μm in diameter,	Up to 3–5 cells, ovoid, 19–28×39–50 μm in diameter, anticlinally	
Spermatangia	*verrucosa*-type conceptacles in cortex, 50 μm deep	*verrucosa*-type conceptacles in cortex, up to 90 μm deep and 60 μm wide	*verrucosa*-type conceptacles in cortex, 50–84 μm deep and 42–56 μm wide[4]	Absent
Tetrasporangia	Cruciately divided, 20–30 μm wide×30–40 μm long, in outer cortex, borne on young branches	Cruciately divided, up to 65 μm long, in outer cortex, borne on all branches	Cruciately divided, 28–42 μm wide, 42–73 μm long, in outer cortex, borne on all branches	Absent

[1] Steentoft et al. 1995; [2] Bellorin et al. 2004; [3] Yamamoto 1978; [4] data from this paper; [5] Perestenko 1994.

logical analysis of thallus shape, the number of branch orders and the presence or absence of reproductive and anchoring organs. Despite these morphological differences between the two forms of *G.* "*verrucosa*", molecular analyses showed that they are genetically identical. Based on these morphological and molecular data, we conclude that both forms of *G.* "*verrucosa*" in Peter the Great Bay on the Russian East Sea coast are indeed *G. vermiculophylla.*

At present, *Gracilaria gracilis* is known to be distributed across European waters (Ireland, Britain, Italy, Spain, Sweden, Turkey), Argentina, and Africa (Figure 9) (Guiry and Guiry 2008). In contrast, *G. vermicullophylla* is a western Pacific species distributed in Japan, China, Korea, and Vietnam (Tseng and Xia 1999); it has also been introduced to the western Atlantic (Thomsen et al. 2005, Freshwater et al. 2006), eastern Atlantic (Rueness 2005, Thomsen et al. 2007), and the eastern Pacific (Bellorin et al. 2004). The binominal *G. vermiculophylla* has been applied to all former *G. verrucosa* records in the northwestern Pacific (Yoshida et al. 1995, Yoshida 1998, Tseng and Xia 1999), except those from Russia. Our results change the reported distribution of *G. gracilis* and *G. vermiculophylla* by excluding the former and including the latter in the flora of the East Sea Russian coast. This was not unexpected because *G. gracilis* had previously been excluded from most other areas of the northwestern Pacific. Currently, *G. gracilis* is only reported in the Philippines as representative of the northwestern Pacific region (for a review see Tseng and Xia 1999). However, this report is likely based on misidentified specimens.

Our *rbc*L and partial *cox*1 sequence data for different *Gracilaria vermiculophylla* populations in the Asian Pacific region showed haplotypic diversity. Four *rbc*L and six *cox*1 (Figure 7) haplotypes were identified among the samples. The haplotype differences among populations from distant locations strongly suggest that these populations are structured geographically.

Previous studies using nuclear markers have demonstrated that various geographical (e.g., distance, islands), hydrodynamic (current direction) and ecological barriers (e.g., differentiation by depth or temperature) can prevent gene flow in species and produce haplotypic differentiation (Billot et al. 2003, Uwai et al. 2006, 2007). However, the geographic isolation of haplotypes is usually not abrupt, and haplotypes with a higher frequency in one population can be found at a lower frequency in other geographically distinct populations. In general, samples collected in different seas have different haplotypes. However, sometimes, two haplotypes were found in close proximity (haplotypes C and D, lagoons of Khasan district of Peter the Great Bay; haplotypes B and C, Donghae; and haplotypes A and E, Jejudo Island; Figure 10). Current patterns and temperature gradients, or biological interactions, including competition and herbivory, might serve (or have served in the past) as barriers against dispersal or as impediments to recruitment among sites in close proximity. Previous studies using nuclear markers have revealed that a distance of only tens of meters to kilometers can prevent gene flow in species belonging to the order Laminariales (Billot et al. 2003). In general, Russian samples of *Gracilaria vermi-*

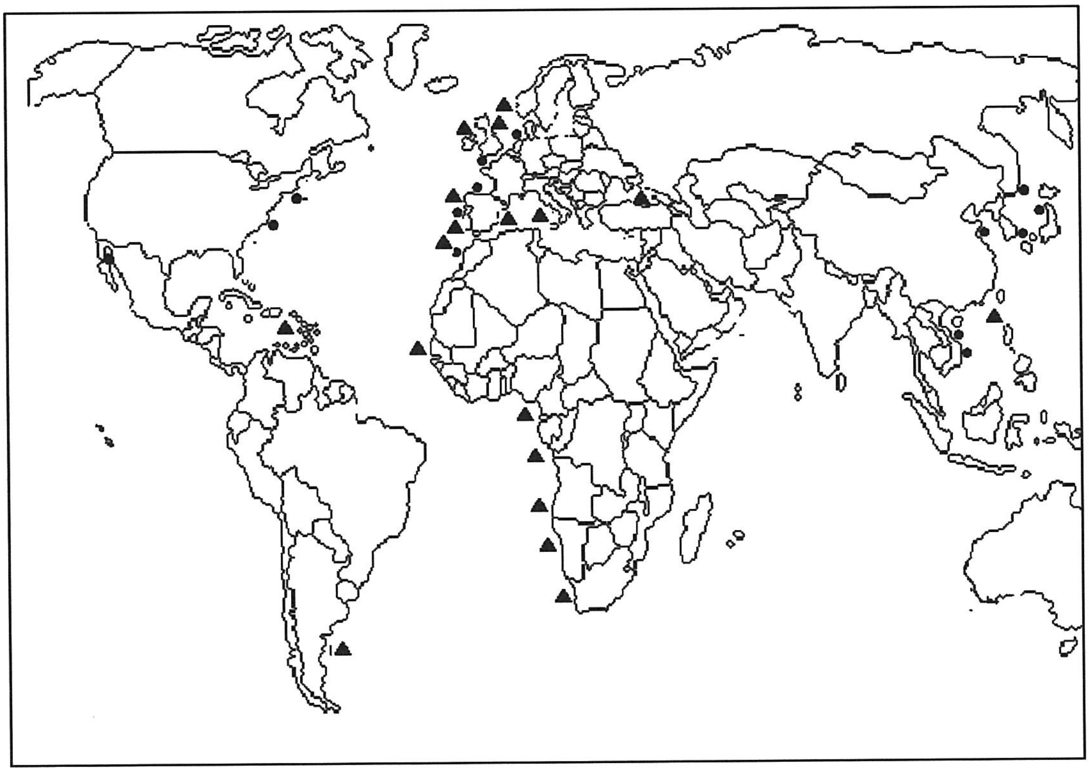


Figure 9 Corrected global distribution map for *Gracilaria gracilis* and *G. vermiculophylla* according to Guiry and Guiry (2008) and our own data.
Triangles correspond to locations with *G. gracilis* and circles to locations with *G. vermiculophylla*.

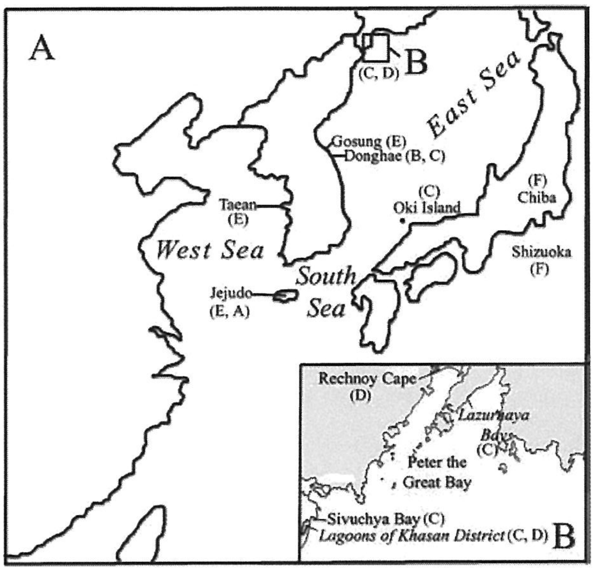


Figure 10 *Gracilaria vermiculophylla*: geographic distribution of haplotypes in the Asian Pacific.
Upper-case letters in brackets represent haplotype types (see Figures 8 and 9).

culophylla were closely related to Korean and Japanese populations from the East Sea (haplotype C) rather than to populations from the Pacific coast of Japan (haplotype F) and Jejudo Island (haplotype A) in the South Sea and the West Sea (haplotype E).

Our morphological and molecular data indicate that *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) is the correct name for cylindrical *Gracilaria* specimens from Peter the Great Bay that were previously identified as *G.* "*verrucosa*". In this region, *G. vermiculophylla* is represented by both attached and unattached forms that share the same *rbc*L and *cox*1 haplotypes and are closely related to other populations from the East Sea.

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