

Two new species of *Gelidium* (Rhodophyta, Gelidiales), *Gelidium tenuifolium* and *Gelidium koshikianum*, from Japan

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SUMMARY

Two new marine red algae, *Gelidium tenuifolium* sp. nov. and *Gelidium koshikianum* sp. nov. (Gelidiales, Gelidiaceae) are described from Japan. *Gelidium tenuifolium* with large-sized thalli (up to 30 cm tall) is distinguished from other species with such thalli by the production of wide, flattened and thin branches (up to 2 mm wide and 60–80 µm thick), the presence of an apical depression and simple determinate branches. *Gelidium koshikianum* with middle-sized thalli (5–8 cm tall) is distinguished from other species with such thalli by having wide axes (up to 2.5 mm wide) and short (2.0–3.2 mm), unbranched, second- and third-order branches issuing at short intervals (0.8–1.4 mm). In phylogenetic analyses of *rbcL* sequences, four *Gelidium* species that are chiefly distributed in Japan including *G. tenuifolium* were clustered together with 99% bootstrap value (Japanese *Gelidium*-complex clade). *Gelidium linooides* Kützinger came to the position of the sister group to *G. tenuifolium* with 99% bootstrap value. There were four substitutions (0.3% divergence) between *G. linooides* and *G. tenuifolium* sequences. *Gelidium koshikianum* and *Gelidium allanii* Chapman were clustered together with 100% bootstrap value and they came to the position of the sister group to the Japanese *Gelidium*-complex clade with 83% bootstrap value. There were six substitutions (0.4% divergence) between *G. koshikianum* and *G. allanii* sequences.

Key words: Gelidiales, *Gelidium koshikianum* sp. nov., *Gelidium tenuifolium* sp. nov., Japan, molecular phylogeny, morphology, *rbcL* gene, Rhodophyta, secondary rhizoidal attachment.

INTRODUCTION

The red algal genus *Gelidium* is the largest genus in the Gelidiales and includes approximately 100 species worldwide (Nelson *et al.* 1994). Although 14 species of *Gelidium* are recognized in Japanese waters (Yoshida 1998), other species have been reported, such as those of Segi (1955, 1957), but are not currently accepted,

and *Gelidium decumbensum* Okamura has recently been reduced to a synonym of *Pterocliadiella tenuis* (Okamura) Shimada, Horiguchi *et al.* Masuda (Shimada *et al.* 2000). There are still several species for which further investigations are necessary: for example, *Gelidium amamiense* Tanaka *et al.* Nozawa and *Gelidium isabelae* Taylor have not been reported since Tanaka's work (Tanaka 1965). Furthermore, some unrecorded or undescribed species may be present in Japanese waters (S. Shimada, unpubl. observation 1999) so that taxonomic studies on this genus in Japan are needed.

Recent molecular analyses have shown that species of the genus *Gelidium* are clustered into several monophyletic clades, each representing specific geographical areas (Freshwater *et al.* 1995; Shimada *et al.* 1999). Many Japanese *Gelidium* species are included in the Indo-Pacific/Caribbean *Gelidium*-complex; however, a few species such as *Gelidium pusillum* (Stackhouse) Le Jolis, *Gelidium divaricatum* Martens and *Gelidium vagum* Okamura are positioned outside the Indo-Pacific/Caribbean *Gelidium*-complex clade. These species are resolved in clades with other small Pacific species (*Gelidium coulteri* complex) or with other genera (*Capreolia* clade or *Acanthopeltis* clade), respectively (Shimada *et al.* 1999). It is therefore important to make clear the phylogenetic affinity of newly described species using molecular methods.

In the present paper, *Gelidium tenuifolium* and *Gelidium koshikianum* are described as new species from the Pacific coast of Japan. Their phylogenetic positions within the genus *Gelidium* are discussed based on analysis of *rbcL* sequences.

MATERIALS AND METHODS

Specimens of *Gelidium tenuifolium* were collected at Touji (25.ix.1996) and Shirahama (25.ix.1996, 28.iii.1998), Shimoda, Shizuoka Prefecture and Naminoura (29.ix.1997), Kushimoto, Wakayama Prefecture

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(Fig. 1). Specimens of *G. koshikianum* were collected at Nagahama (31.vii.1997), Shimo-Koshiki Island, Koshiki Islands, Kagoshima Prefecture and Yamata-tegami (2.viii.1997), Makurazaki, Kagoshima Prefecture (Fig. 1). The majority of materials were fixed and preserved in 10% formalin/seawater and some were dried as voucher herbarium specimens that are deposited in the Herbarium of the Graduate School of Science, Hokkaido University, Sapporo (SAP 070862–070877). Some plants were transported live to Hokkaido University for culture studies. Unialgal cultures were established from excised apical tips of branchlets of plants and grown in Provasoli enriched seawater medium (Provasoli 1968) at 15°C, 16 : 8 light : dark (LD) with the photon flux of 15–25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Sections were made by hand using a razor blade. Tissues of fixed materials were stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1 : 1 : 1 : 1) solution and mounted in 50% glycerol/seawater on microscope slides.

Methods for total DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of the *rbcl* gene were as described by Shimada *et al.* (1999). For this study, we determined six *rbcl* sequences: *Gelidium linoides* Kützinger collected at Shirahama, Shimoda, Shizuoka Prefecture (25.ix.1996;

AB030622); *Gelidium elegans* Kützinger collected at Touji, Shimoda, Shizuoka Prefecture (25.ix.1996; AB030623); *G. koshikianum* collected at Nagahama, Shimo-Koshiki Island, Koshiki Islands (31.vii.1997; AB030626); *Gelidium pacificum* Okamura collected at Enoshima, Kanagawa Prefecture (29.iii.1997; AB030627); *G. tenuifolium* collected at Touji, Shimoda, Shizuoka Prefecture (25.ix.1996) and at Shirahama, Shimoda, Shizuoka Prefecture (28.iii.1997; AB030628). The following 21 additional species were downloaded from GenBank and included in the alignment: *Gelidium pusillum* (Japanese material; AB017679), *Gelidium capense* (Gmelin) Silva (L22461), *Gelidium coulteri* Harvey (U00105), *Gelidium micropterum* Kützinger (U00446), *Suhria vittata* (Linnaeus) J. Agardh (U00112), *Gelidium sesquipedale* (Clemente) Thuret (L22071), *Gelidium pulchellum* (Turner) Kützinger (U01822), *Gelidium canariense* (Grunow) Seoane-Camba (L22460), *Gelidium latifolium* (Greville) Bornet et Thuret (U00112), *Gelidium attenuatum* (Turner) Thuret (U00110), *Gelidium floridanum* Taylor (U00106), *Gelidium purpurascens* Gardner (U00979), *Gelidium serrulatum* J. Agardh (U01042), *Gelidium americanum* (Taylor) Santelices (L22459), *Gelidium abbotiorum* Norris (U16829), *Gelidium pteridifolium* Norris, Hommersand et Fredericq (U16833), *Gelidium robustum* (Gardner) Hollenberg et Abbott (U01041), *Gelidium allanii* Chapman (L22458), *Gelidium elegans* from Chiba Prefecture (U16830), *Gelidium pacificum* from Chiba Prefecture (U16832) and *Ptilophora pinatifida* (J. Agardh) Norris (U16834). *Ptilophora pinnatifida* was used as an outgroup.

The distance matrix, maximum parsimony, and maximum likelihood methods were used to construct phylogenetic trees. For the distance matrix method, we used Kimura's (1980) two-parameter method to calculate the distance matrix and neighbor-joining (NJ) method (Saitou and Nei 1987) to construct the tree using the CLUSTAL W computer program (Thompson *et al.* 1994; Higgins *et al.* 1996). Maximum parsimony (MP) analysis was implemented with the PAUP program (version 3.1.1.; Swofford 1993) using heuristic search (simple addition). The maximum likelihood (ML) method was implemented with the fastDNAmI program (version 1.0.6c; Olsen *et al.* 1994) using the global search option. Bootstrap analyses based on 100 re-samplings of the data set (Felsenstein 1985) were calculated to evaluate the statistical reliability of the NJ and MP trees. The alignments are available from the corresponding author on request.

RESULTS

Gelidium tenuifolium sp. nov.

Axes erecti, 15–30 cm alti, cartilaginei, purpureorubri ad brunneoli-rubri, teres ad subteres, 480–720 μm

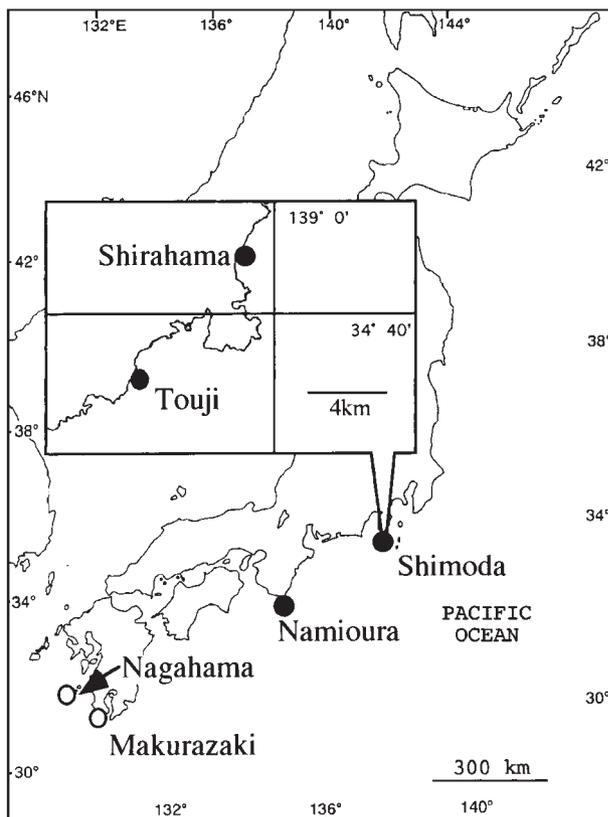


Fig. 1. Map showing collection localities of this study. (●) *Gelidium tenuifolium*; (○), *Gelidium koshikianum*.

diametro in parte basali, sursum compressescentes, attingentes 1.4–2.0 mm in latitudine et 280–340 μm in crassitudine in parte medio, oppositim vel alternatim-distichim ramosi 4-plo vel 5-plo; rami laterales numerosi breves, simplices, determinati sed aliqui indeterminati; rami indeterminati ordinis primae ad tertiae teres ad subteres, 400–560 μm diametro in parte proximali, sursum compressescentes ad complanatescentes, attingentes usque ad 2 mm in latitudine et 60–80 μm in crassitudine in parte distali; axes et rami indeterminati apicibus obtusis; cellulae apicales axium et ramorum indeterminatorum vulgo immersae in depressione apicali. Tetrasporangia irregulariter disposita ad apices ramorum determinatorum brevium, cruciatim vel decussatim divisa, 44–60 μm longa et 24–40 μm lata; cystocarpia facta in parte medio ramorum determinatorum brevium; spermatangia ignota.

Erect axes 15–30 cm tall, cartilaginous, purplish to brownish-red, terete to subterete, 480–720 μm in diameter in the basal region, becoming compressed upward, reaching 1.4–2.0 mm in width and 280–340 μm in thickness in the mid-region, oppositely or alternate-distichously branched four or five times; many lateral branches short, undivided, determinate but a few indeterminate; indeterminate first- to third-order branches terete to subterete, 400–560 μm in diameter in the proximal region, becoming compressed to flattened upward, reaching up to 2 mm in width and 60–80 μm

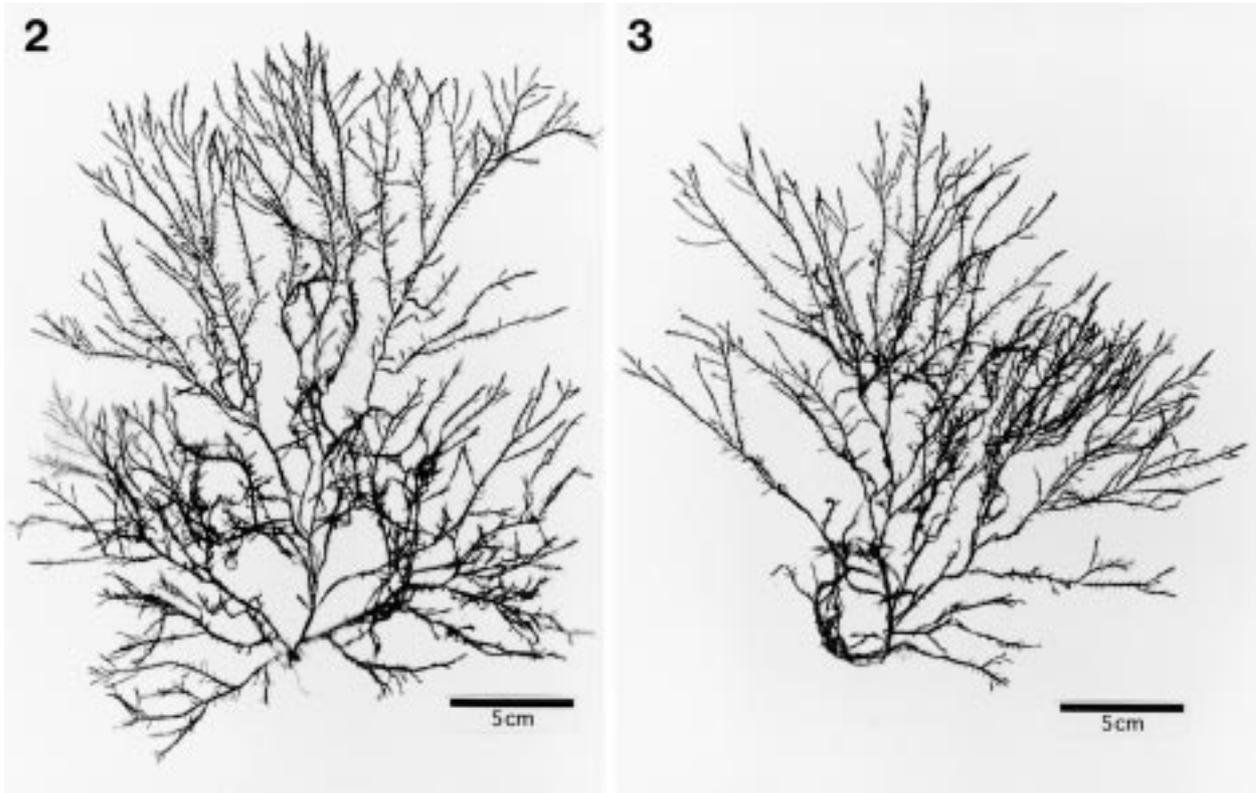
in thickness in the distal portion; axes and indeterminate branches having obtuse apices; apical cells of axes and indeterminate branches usually immersed in the apical depression. Tetrasporangia irregularly disposed at the apices of short determinate branches, cruciately or decussately divided, 44–60 μm long and 24–40 μm wide; cystocarps formed in the middle portion of short determinate branches; spermatangia unknown.

Holotype and type locality: A cystocarpic specimen (SAP 070868; Fig. 2), collected at Shirahama, Shizuoka Prefecture, on 28.iii.1998 by S. Shimada.

Etymology: The specific epithet refers to the thin upper portions of branches.

Japanese name: Usuba-tengusa.

Plants form tufts on bedrock in the lower intertidal to upper subtidal zones. They are 15–30 cm tall (Figs 2,3), cartilaginous, and purplish to brownish-red in color. Erect axes arise from creeping axes, which attach to the substratum by brush-like secondary rhizoidal attachments (Fig. 4) that are up to 680 μm in length and 10–14 μm in diameter. The creeping axes are subterete, up to 1.7 cm in length and 300–400 μm in diameter and are branched irregularly. Erect axes are terete to subterete, 480–720 μm in diameter in the basal region, becoming compressed upward, reaching 1.4–2.0 mm wide and 280–340 μm thick in the mid-region and becoming flattened distally (1.8 mm wide



Figs 2,3. Type specimens of *Gelidium tenuifolium*. 2. Holotype specimen collected at Shirahama, Shimoda, Shizuoka Prefecture (28.iii.1998; SAP 070868). 3. Isotype specimen collected at Shirahama, Shimoda, Shizuoka Prefecture (28.iii.1998; SAP 070869).

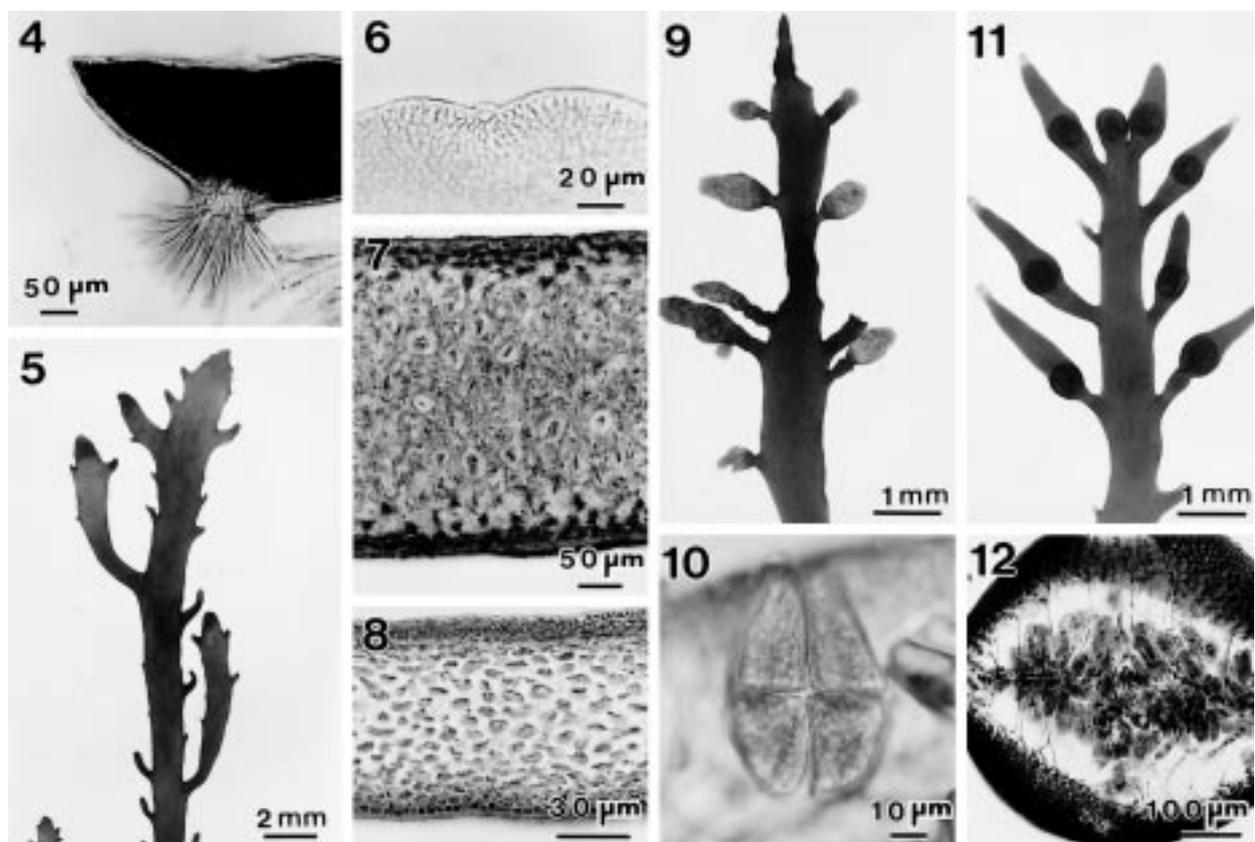
and 80–100 μm thick). They are oppositely or alternate-distichously branched four or five times. The vast majority of laterals become short simple determinate branches and a few of them grow into indeterminate branches (Fig. 5). Indeterminate first- to third-order branches are terete to subterete, 400–560 μm in diameter in the proximal region, becoming compressed to flattened upward, reaching up to 2 mm wide and 60–80 μm thick in the distal portion. Axes and indeterminate laterals have obtuse apices.

A dome-shaped apical cell is evident at the apices of axes and branches, as is typical of the Gelidiales. The majority of apical cells of axes and indeterminate branches are immersed in the apical depression (Fig. 6). Basal portions of axes (Fig. 7) consist of a medulla composed of a few large cells up to 80 μm in diameter and numerous rhizines (slender, thick-walled, internal, hypha-like filaments), and a cortex composed of three or four layers of smaller cells 4–8 μm in diameter. Distal portions of branches consist of a medulla composed of eight to 12 layers of cells up to 16 μm in diameter and a cortex composed of one or two layers of smaller cells 2–4 μm in diameter, and there are no rhizines (Fig. 8).

Tetrasporangia are irregularly disposed at the apices of short determinate branches (Fig. 9). Cruciatly or decussately divided sporangia are 44–60 μm long by 24–40 μm wide (Fig. 10). Cystocarps are formed in the middle portion of short determinate branches (Fig. 11), and are bilocular, having a distinct ostiole in each side. Carposporangia are 40–72 μm long by 16–40 μm wide (Fig. 12). Spermatangia were not found in our specimens.

Gelidium koshikianum sp. nov.

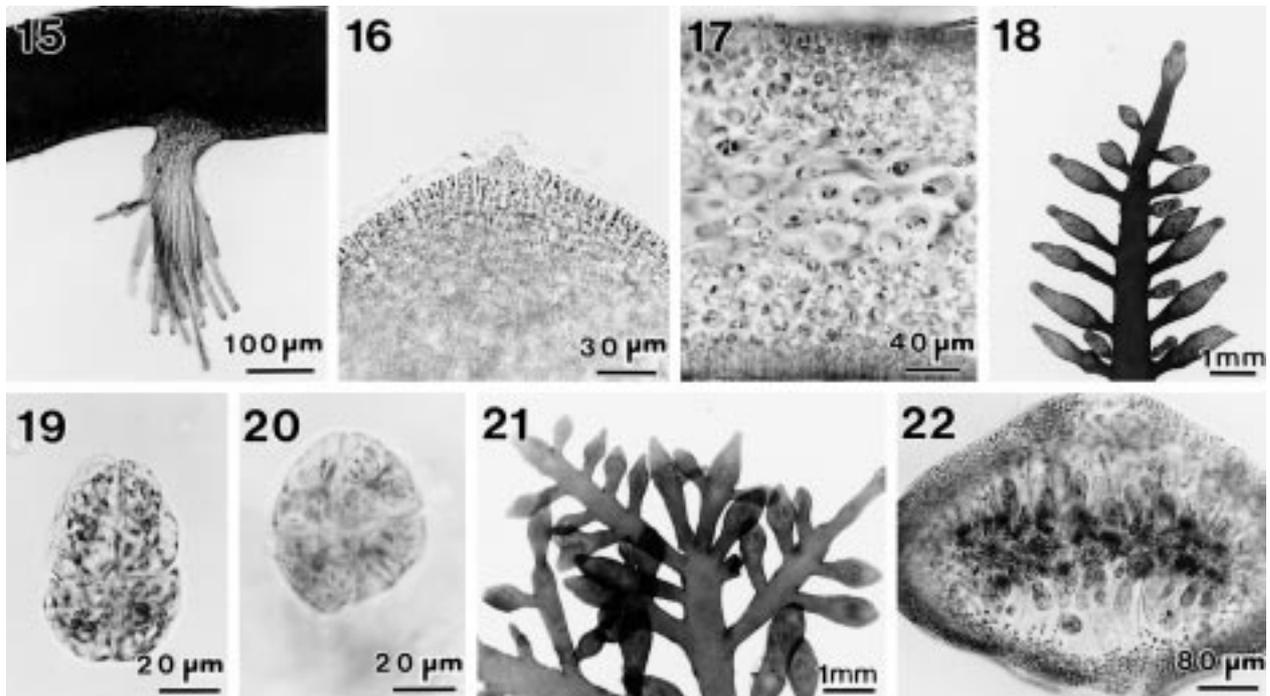
Axes erecti, 5–8 cm alti, cartilaginei, aurantiaci ad purpureorubri, teres ad subteres, 320–480 μm diametro in parte basali, sursum compressescentes, attingentes 2.5 mm in latitudine et 240–360 μm in crassitudine in parte medio, ferentes ramos laterales ordinum usque ad trium in modo opposito in intervallis 0.8–1.4 mm; rami indeterminati ordinis primae teres ad subteres, 400–640 μm diametro in parte proximali, sursum compressescentes, attingentes usque ad 1.6 mm in latitudine et 200–240 μm in crassitudine in parte medio. Tetrasporangia irregulariter disposita ad apices ramorum determinantum brevium, cruciatim vel decussatim



Figs 4–12. *Gelidium tenuifolium*. 4. Brush type of secondary rhizoidal attachments (cultured plant grown at 15°C, 16 : 8 LD for 1 month). 5. Upper portion of a plant, showing long, broad indeterminate branches and short simple determinate branchlets. 6. Dome-shaped apical cell in the apical depression. 7. Transverse section of a basal portion of the axis, showing a few large cells and numerous rhizines in the medulla. 8. Transverse section of the distal portion of a branch. 9. Tetrasporangial branches. 10. Cruciatly divided tetrasporangium. 11. Cystocarpic branches. 12. Transverse section of a bilocular cystocarp with a distinct ostiole in each side.



Figs 13,14. Type specimens of *Gelidium koshikianum*. 13. Holotype collected at Nagahama, Shimo-Koshiki Island, Koshiki Islands, Kagoshima Prefecture (2.viii.1997; SAP 070874). 14. Isotype collected at Nagahama, Shimo-Koshiki Island, Koshiki Islands, Kagoshima Prefecture (2.viii.1997; SAP 070875).



Figs 15–22. *Gelidium koshikianum*. 15. Brush type of secondary rhizoidal attachments (cultured plant grown at 15°C, 16 : 8 LD for 1 month). 16. Dome-shaped apical cell at the apex of a branch. 17. Transverse section of a first-order branch showing abundant rhizines in the inner cortex and rare in the central medulla. 18. Tetrasporangial branches. 19. Cruciately divided tetrasporangium. 20. Tetrasporangium showing an intermediate division between a cruciate and decussate manner. 21. Cystocarpic branches. 22. Transverse section of a bilocular cystocarp with a distinct ostiole in each side.

divisa, 48–64 μm longa et 20–44 μm lata; *cystocarpia facta in parte medio ramorum determinantum brevium*; *spermatangia ignota*.

Erect axes 5–8 cm tall, cartilaginous, orange to purplish-red, terete to subterete, 320–480 μm in diameter in the basal region, becoming compressed upward, reaching up to 2.5 mm in width and 240–360 μm in thickness in the mid-region, bearing lateral branches of up to three orders in an opposite manner at intervals of 0.8–1.4 mm; first-order indeterminate branches terete to subterete, 400–640 μm in diameter in the proximal region, becoming compressed upward, reaching up to 1.6 mm in width and 200–240 μm in thickness in the mid-region. Tetrasporangia irregularly disposed at the apices of short determinate branches, cruciately or decussately divided, 48–64 μm long and 20–44 μm wide; cystocarps formed in the middle portion of short

determinate branches; spermatangia unknown.

Holotype and type locality: A tetrasporangial specimen (SAP 070874; Fig. 12), collected at Nagahama, Shimo-Koshiki Island, Koshiki Islands, Kagoshima Prefecture, on 31.vii.1997 by S. Shimada.

Etymology: The specific epithet refers to the name of Koshiki Islands including the type locality where this species was first collected by the first author. Japanese name: Satsuma-tengusa.

Plants form tufts on bedrock in the middle to lower intertidal zones. They are 5–8 cm tall (Figs 13,14), cartilaginous, and orange to purplish-red in color. Erect axes arise from creeping axes, which attach to the substratum by brush-like secondary rhizoidal attachments (Fig. 15) that are up to 360 μm in length and 10–12 μm in diameter. The creeping axis is subterete,

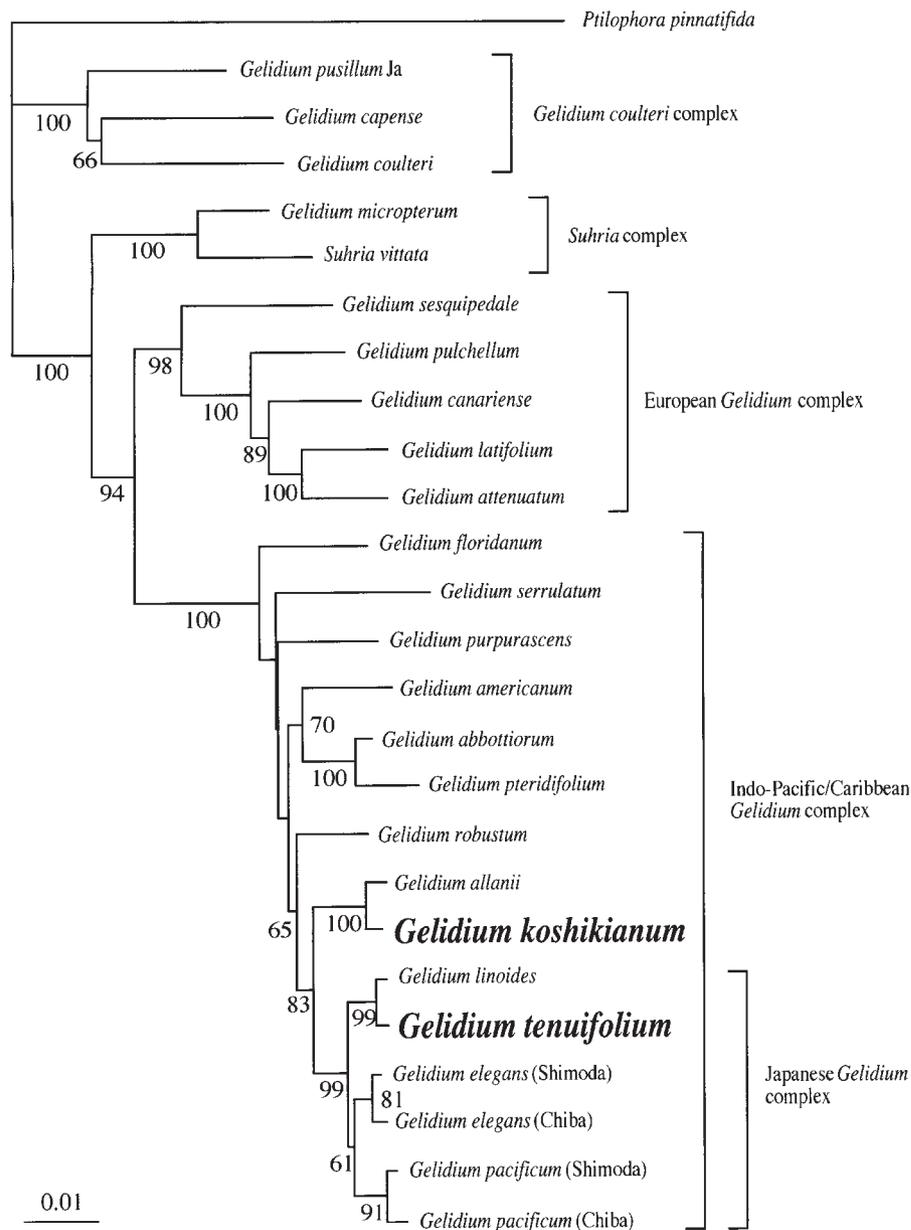


Fig. 23. Phylogenetic tree inferred from *rbcL* sequences with the neighbor-joining (NJ) method based on Kimura's correction using the CLUSTAL W computer program. The numbers under the branches indicate bootstrap values (100 replications) greater than 60. Scale bar corresponds to one change per 100 positions.

up to 400 µm in diameter and is branched irregularly. Erect axes are terete to subterete, 320–480 µm in diameter in the basal region, becoming compressed upward, reaching up to 2.5 mm wide and 240–360 µm thick in the mid-region. The erect axes produce lateral branches of up to three orders in an opposite manner at intervals of 0.8–1.4 mm. The vast majority of second- and third-order laterals remain short (2.0–3.2 mm) and unbranched. First-order indeterminate branches are terete to subterete, 400–640 µm in diameter in the proximal region, becoming compressed upward, reaching up to 1.6 mm wide and 200–240 µm thick in the mid-region. Apices of axes and branches are acute.

A dome-shaped apical cell is evident at the apices of creeping axes and branches (Fig. 16), as is typical of the Gelidiales. Basal portions of axes consist of a medulla composed of a few large cells 40–60 µm in diameter and numerous rhizines, and a cortex composed of three or four layers of smaller cells 2–4 µm in diameter. First-order branches consist of a medulla composed of 10–14 layers of cells up to 30 µm in diameter and a cortex composed of one or two layers of smaller cells 2–4 µm in diameter in the mid-region (Fig. 17). Rhizines of first-order branches are abundant in the inner cortex, but rare in the central medulla.

Tetrasporangia are irregularly disposed at the apices

of short determinate branches (Fig. 18). Tetrasporangia are divided in a cruciate manner (Fig. 19) or a decussate manner, and some intermediate conditions between these two are found (Fig. 20). Mature tetrasporangia are 48–64 µm long by 20–44 µm wide (Figs 19,20). Cystocarps are formed in the middle portion of short determinate branches (Fig. 21), and are bilocular, having a distinct ostiole in each side. Carposporangia are 36–64 µm long by 14–30 µm wide (Fig. 22). Spermatangia were not found in our specimens.

rbcl analysis

Sequences from the two collections of *G. tenuifolium* (Touji and Shirama) were found to be completely identical. All three phylogenetic methods (NJ, MP and ML) resulted in the same overall topology and levels of bootstrap support. The positions and inferred relationships of *G. tenuifolium* and *G. koshikianum* were identical in three analyses and only the NJ result is shown here (Fig. 23). *Gelidium tenuifolium* was included in the Japanese *Gelidium*-complex clade (99% bootstrap value) that contains three other Japanese *Gelidium* species. *Gelidium linooides* came to the position of the sister group to *G. tenuifolium* (99% bootstrap value). Four substitutions (0.3% divergence) were found

Table 1. A comparison of six closely related species of *Gelidium*

| | <i>G. tenuifolium</i> | <i>G. linooides</i> ^a | <i>G. elegans</i> ^a | <i>G. pacificum</i> ^a | <i>G. koshikianum</i> | <i>G. allanii</i> ^b |
|---------------------------|------------------------------|----------------------------------|--------------------------------|----------------------------------|------------------------------|---------------------------------|
| Thallus length | 15–30 cm | 25–30 cm | 10–30 cm | 15–35 cm | 5–8 cm | Up to 13 cm |
| Thallus color | Purplish to brownish-red | Purplish to brownish-red | Dark red | Dark red | Orange to purplish-red | Purplish to dark red |
| Upper portion of branches | Wide, flattened and thin | Linear, flattened and thin | Subterete to compressed | Subterete to compressed | Subterete to compressed | Subterete to compressed |
| Apex of branches | Obtuse | Acute | Acute | Acute | Acute | Acute |
| Location of apical cell | Depression | Apex | Apex | Apex | Apex | Apex |
| Branchlets | Determinate | Determinate | Indeterminate | Indeterminate | Determinate | Indeterminate |
| Midrib of axes | Absent | Absent | Absent | Slight | Absent | Absent |
| Width of axes | 1.4–2.0 mm | Up to 2.0 mm | Up to 2.0 mm | Up to 2.5 mm | Up to 2.5 mm | Up to 0.5 mm |
| Width of branches | Up to 2.0 mm | 0.2–0.5 mm | Up to 1.5 mm | Up to 2.0 mm | Up to 1.6 mm | Unknown |
| Tetrasporangia | Short determinate branchlets | Short determinate branchlets | Indeterminate branchlets | Assembled branchlets | Short determinate branchlets | Branchlets with sterile margins |

^aData from Okamura (1934). Okamura did not distinguish axes and branches so the present authors measured the width of axes and branches from specimens used by Okamura (1934).

^bData from Chapman (1969) and Nelson *et al.* (1994)

between *Gelidium linoides* and *G. tenuifolium*. *Gelidium koshikianum* was clustered with *G. allanii* (100% bootstrap value) in a clade that is sister to the Japanese *Gelidium*-complex clade (83% bootstrap value). Six substitutions (0.4% divergence) were found between *G. koshikianum* and *G. allanii*. Intraspecific divergence between Shimoda and Chiba populations of *G. elegans* was 0.2% divergence (three substitutions), while in *G. pacificum* 0.4% divergence (six substitutions) was found.

DISCUSSION

Gelidium tenuifolium

Gelidium tenuifolium is one of the largest species in this genus. Some species with large thalli are known in the Pacific ocean: *Gelidium linoides*, *G. pacificum*, *G. elegans* (Japan), *G. latiusculum* Okamura (Taiwan), *G. nudifrons* Gardner, *G. purpurascens* Gardner, *G. robustum* (Gardner) Hollenberg et Abbott (California), *G. asperum* (C. Agardh) Greville, and *G. australe* J. Agardh (Australia) (Okamura 1934, 1935; Abbott and Hollenberg 1976; Womersley 1994). Primarily, *G. tenuifolium* is characterized by the nature of the upper portion of the branches, which are wide, flattened and thin, and possess apical depressions that are formed at obtuse apices. Secondarily, it bears short, pinnate, simple determinate branches. Although most branches have apical depressions that are formed at obtuse apices, a few branches possess acute apices. Rodríguez and Collado-Vides (1996) have demonstrated that apex morphology is changeable depending on the developmental stage of the thallus, and each species has its own specific apical morphology. The presence of both depressed and acute apices in this species thus represents the presence of branches in different developmental stages. It is important to note that most branches of this species possess apical depressions. Therefore, despite the fact that some parts of a plant possess acute apices, we regard apical depressions as one of the diagnostic characters for *G. tenuifolium* because closely related species (*G. linoides*, *G. elegans* and *G. pacificum*) are known to have acute apices only.

Gelidium tenuifolium can be distinguished from other large species as follows: *G. linoides* differs from *G. tenuifolium* in having linear branches (200–500 µm in width, Table 1) and acute apices of branches (the location of each apical cell being the acute apex) (Okamura 1934; Table 1); *G. pacificum* and *G. latiusculum* have slightly bulged midribs (Okamura 1934, 1935; Table 1); *G. elegans* differs from *G. tenuifolium* in having a darker reddish color, subterete to slightly compressed branches, acute apices and elongating and branching branchlets (Okamura 1934; Table 1); *G. nudifrons* is compressed to flattened throughout

(Abbott and Hollenberg 1976); *G. robustum* possesses thicker (up to 1.7 mm) axes (Abbott and Hollenberg 1976); *G. asperum* and *G. australe* possess subterete to slightly compressed branches (Nelson *et al.* 1994; Womersley 1994).

As Freshwater *et al.* (1995) have suggested, certain genera, species and populations of the order Gelidiales can be assembled in groups based on geographical distribution. In this study, four *Gelidium* species, including *G. tenuifolium*, chiefly distributed in Japan are clustered together with 99% bootstrap value (Japanese *Gelidium*-complex clade). *Gelidium linoides* was shown to be most closely related to *G. tenuifolium* with only four substitutions being found between the two species. In the Gelidiales, intraspecific sequence divergence has been shown to have a range of 0.0–1.8%, while the value for interspecific divergence is 0.0–11.5% (Freshwater and Rueness 1994; Bailey and Freshwater 1997; Shimada *et al.* 1999, 2000). Thus, the sequence divergence found between *G. tenuifolium* and *G. linoides* falls into either intra- or interspecific variations of other taxa. As Bird *et al.* (1992) pointed out, the taxonomic significance of molecular sequence divergence must be evaluated on a case-by-case basis, and it is important to interpret these values with the combination of other features such as morphology and geographical distribution when one decides whether these values indicate species difference or merely an intraspecific difference. In this case, the geographical distributions of *G. tenuifolium* and *G. linoides* overlap and they often occur sympatrically: for example, in Shirahama, Shimoda, Shizuoka Prefecture, where the morphology of each species can be clearly distinguished in the field as mentioned above (Table 1), and no intermediate forms have been observed. These facts strongly indicate that these two algae should be referred to as separate species rather than as intraspecific taxa.

Gelidium koshikianum

Gelidium koshikianum is a middle-sized species in this genus. In the Indo-Pacific regions and the Caribbean Sea, a number of middle-sized species have been reported. These include *Gelidium tenue* Okamura, *G. vagum*, *G. yamadae* Fan (Japan), *G. planiusculum* Okamura, *G. kintaroi* (Okamura) Yamada (Taiwan), *G. americanum* (Taylor) Santelices, *G. serrulatum* J. Agardh, *G. johnstonii* Setchell et Gardner, *G. floridanum* Taylor (Pacific coast of America and Caribbean Sea), *G. abbottiorum* Norris (South Africa), and *G. allanii* Chapman (New Zealand) (Setchell and Gardner 1924; Gardner 1927; Okamura 1934, 1935; Taylor 1943, 1960; Segi 1955; Chapman 1969; Abbott and Hollenberg 1976; Stewart and Norris 1981; Norris 1990; Rodríguez 1991).

Gelidium koshikianum can be distinguished from other middle-sized species as follows: *G. tenue* possesses slightly broader lateral branches (up to 2 mm)

and lacks short second- and third-order determinate branches (Okamura 1934); *G. vagum* has twisted axes and branches (Okamura 1934); *G. yamadae* has densely arranged branches (Okamura 1935, as *G. densum* Okamura; Fan 1951); *G. planiusculum* has numerous, linear branches (Okamura 1935); *G. kintaroi* has clavate branches (Okamura 1934, as *G. clavatum* Okamura; Segi 1955); *G. americanum* has obtuse apices of branches and lacks short second- and third-order determinate branches (Taylor 1943; Santelices 1976; Rodriguez 1991); *G. serrulatum* has serrate branchlets (Taylor 1960; Rodriguez 1991); *G. johnstonii* has glossy color and flattened branchlets (Setchell and Gardner 1924; Segi 1955; Stewart and Norris 1981); *G. floridanum* has very notable congestion of reproductive branchlets (Taylor 1943; Rodriguez 1991); *G. abbottiorum* has longer branching intervals (3–4 mm) (Norris 1990); and *G. allanii* 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; Adam 1994; Nelson *et al.* 1994; Table 1).

Gelidium allanii was shown to be most closely related to *G. koshikianum* in analyses of *rbcl* sequences, and only six substitutions were found between the two taxa. *Gelidium allanii* was originally described from a single locality of New Zealand (Chapman 1969). To date, it has been found in six localities and is thought to be endemic to northern New Zealand with a highly localized distribution (Nelson *et al.* 1994). The geographical distributions of both species, therefore, are widely separated from each other. Although our molecular data indicate that these two entities are closely related, the vegetative and reproductive morphology of *G. koshikianum* and *G. allanii* can be clearly distinguished as mentioned above (Table 1). Taking these clear differences in morphology into consideration, we conclude that these entities should be treated as independent species.

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