New records of *Gelidiella pannosa*, *Pterocladiella caerulescens* and *Pterocladiella caloglossoides* (Rhodophyta, Gelidiales) from Japan

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SUMMARY

Three gelidialean species, Gelidiella pannosa (Feldmann) Feldmann et Hamel, Pterocladiella caerulescens (Kützing) Santelices et Hommersand and Pterocladiella caloglossoides (Howe) Santelices, are newly reported from Japan, and their characteristic features are described. Monoecious plants of P. caerulescens produce spermatangial sori on: (i) fertile cystocarpic branchlets; (ii) special spermatangial branchlets on a cystocarpic axis; and (iii) branchlets of a special spermatangial axis. The latter two are newly reported in this species. Gelidiella pannosa has numerous unicellular independent points of attachment, whereas P. caerulescens and P. caloglossoides have the peg type of secondary rhizoidal anchorage. In the molecular phylogenetic study using small subunit ribosomal DNA sequences, G. pannosa is included in the Gelidiella clade with 100% bootstrap support in neighbor-joining (NJ) analysis and 99% in maximum parsimony (MP) analysis. Pterocladiella caerulescens and P. caloglossoides are included in the Pterocladiella clade with 99.7% bootstrap support in NJ analysis and 100% in MP analysis. Each type of secondary rhizoidal attachment is completely consistent with the respective genus clade, which suggests that this morphological characteristic reflects phylogeny within the order Gelidiales.

Key words: Gelidiales, *Gelidiella pannosa*, Japan, molecular phylogeny, morphology, *Pterocladiella caerulescens*, *Pterocladiella caloglossoides*, Rhodophyta, secondary rhizoidal attachment, SSU rDNA sequences.

INTRODUCTION

The Yaeyama Islands are situated in the south-westernmost, warmest part of Japan. Several studies of the marine algal flora of these islands have been reported (Yamada and Tanaka 1938; Segawa and Kamura 1960; Tanaka and Itono 1972; Akatsuka 1973; Ohba and Aruga 1982). Although a number of tropical species of *Gelidiella* and *Pterocladiella* (as *Pterocladia* spp.) have been reported in the Pacific Ocean (Dawson 1954; Santelices 1977; Hatta and Prud'homme van Reine 1991; Price and Scott 1992), only one species, *Gelidiella acerosa* (Forsskål) Feldmann et Hamel, has been recorded from the Yaeyama Islands, where numerous tropical algae are known (Yamada and Tanaka 1938; Segawa and Kamura 1960; Akatsuka 1973; Ohba and Aruga 1982). In the present paper, three tropical species, *Gelidiella pannosa* (Feldmann) Feldmann et Hamel, *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand and *Pterocladiella caloglossoides* (Howe) Santelices, are newly reported from the Yaeyama Islands. Their phylogenetic positions within the order Gelidiales are discussed with the aid of molecular phylogenetic study using nuclear-encoded small subunit ribosomal DNA (SSU rDNA) sequences and secondary rhizoidal attachments.

MATERIALS AND METHODS

Specimens examined were collected at localities shown in Table 1. The majority of specimens were fixed and preserved in 10% formalin/seawater and either dried or mounted in 30% Karo[™] corn syrup in distilled water on microscope slides as voucher specimens that are deposited in the Herbarium of the Graduate School of Science, Hokkaido University, Sapporo, Japan (SAP 071771–071778). Some plants were transported live to Hokkaido University for culture studies. Unialgal cultures were established from excised apical tips of branchlets and were grown in Tris-buffered medium (Van der Meer and Patwary 1991) at 20°C, 16:8 LD with the photon flux of 15–25 µmol m⁻² s⁻¹.

Methods for total DNA extraction, polymerase chain reaction (PCR) amplification and sequencing of the SSU rDNA gene were as described by Shimada *et al.* (1999). For this study, we determined four sequences: #326 *Gelidiella pannosa* collected at Nosoko, Ishigaki Island (AB031300); #321 *Pterocladiella caerulescens* collected at Sonai, Yonaguni Island (AB031301); #328 *Pterocladiella caloglossoides* collected at Oohama, Ishigaki Island (AB031302); and #83 *Pterocladiella*

*To whom correspondence should be addressed. Email: sshimada@sci.hokudai.ac.jp Communicating editor: K. W. Nam. Received 26 August 1999; accepted 17 February 2000. nana (Okamura) Shimada, Horiguchi et Masuda collected at Teuchi, Shimo-Koshiki Island, Koshiki Islands (31.vii.1997)(ABO313O3). An additional 13 sequences of other species (Table 2) were downloaded from GenBank and included in the alignment, and *Gracilaria tikvahiae* MacLachlan and *Chondrus crispus* Stackhouse were used as outgroups. SSU rDNA sequences were first aligned with the CLUSTAL w computer program (Thompson *et al.* 1994; Higgins *et al.* 1996) and then refined by eye. The alignments are available from the first author upon request.

The distance matrix and maximum parsimony 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 the neighbour-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. Bootstrap analyses based on 1000 resamplings of the data set (Felsenstein 1985) were calculated to evaluate statistical reliability.

RESULTS

Gelidiella pannosa (Feldmann) Feldmann et Hamel 1934: 534, figs 1, 2

Basionym: *Echinocaulon pannosum* Feldmann (1931).

Type locality: Biarritz, France.

Distribution: Tropical to temperate coasts of the world (Kraft and Abbott 1998).

Japanese name: Ito-shimatengusa (new name).

Plants grow gregariously on bedrock in the upper intertidal zone and are dark red. Individual plants consist of a creeping axis and numerous erect axes (Fig. 1), both of which are terete to subterete. Creeping axes are $80-135 \,\mu$ m in diameter, branched irregularly, and attach to the substratum by unicellular unaggregated rhizoids (Fig. 2) that are up to 200 μ m long by $10-25 \,\mu$ m wide. The majority of branches grow into erect axes and some become creeping branches that grow like the parental axis. Erect axes are up to 8 mm high and $65-115 \,\mu$ m wide. They are usually simple, but are sometimes irregularly branched.

A dome-shaped apical cell is evident at the apices of creeping and erect axes (Fig. 3), as is typical of the

Table 1.	List of newly	v found species in Jap	an, collection data,	habitats and SAP	numbers of voucher	herbarium specimens
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	Gelidiella pannosa	Pterocladiella caerulescens	Pterocladiella caloglossoides
Collection data	Agarizaki, Yonaguni Island (3.iii.1999) (24°27'30″N, 122°02'40″E) Oohama, Ishigaki Island (4.iii.1999) (24°20'10″N, 124°12'00″E) Nosoko, Ishigaki Island (6.iii.1999) (24°30'40″N, 124°152'00″E)	Sonai, Yonaguni Island (1.iii.1999) (24°27′55″N, 123°00′10″E) Irizaki, Yonaguni Island (2.iii.1999) (24°26′40″N, 122°54′00″E) Agarizaki, Yonaguni Island (3.iii.1999) (24°27′30″N, 122°02′40″E)	Oohama, Ishigaki Island (4.iii.1999) (24°20'10"N, 124°12'00"E)
		Oohama, Ishigaki Island (4.iii.1999) (24°20'10"N, 124°12'00"E)	
Habitats	Upper intertidal	Upper to middle intertidal	Middle intertidal
SAP number	71771–71773	71774–71777	71778

Table 2. List of species used in the molecular study and GenBank accession number

Species	Accession number
Acanthopeltis japonica Okamura	AB017664
Capreolia implexa Guiry et Womersley	U60344
Gelidiella acerosa (Forsskål) Feldmann et Hamel	U60342
Gelidiella ligulata Dawson	AB017669
Gelidiella pannosa (Feldmann) Feldmann et Hamel	AB031300
Gelidium americanum (Taylor) Santelices	U60347
Gelidium latifolium (Greville) Bornet et Thuret	U60350
Pterocladia lucida (Brown et Turner) J. Agardh	U60349
Pterocladiella caerulescens (Kützing) Santelices et Hommersand	AB031301
Pterocladiella caloglossoides (Howe) Santelices	AB031302
Pterocladiella capillacea (Gmelin) Santelices et Hommersand	AB017672
Pterocladiella melanoidea (Schousboe ex Bornet) Santelices et Hommersand	U60341
Pterocladiella nana (Okamura) Shimada, Horiguchi et Masuda	AB031303
Ptilophora pinnatifida (J. Agardh) Norris	U60345
Ptilophora subcostata (Okamura) Norris	U60348
Chondrus crispus Stackhouse	Z14140
Gracilaria tikvahiae MacLachlan	M33640

Gelidiales. Thalli consist of a medulla composed of 3-5 layers of cells $6-12 \,\mu$ m in diameter and a cortex composed of one or two layers of slightly smaller cells $4-10 \,\mu$ m in diameter (Fig. 4). Rhizines (slender, thick-walled, hypha-like filaments) are absent throughout the thalli.

Tetrasporangia and sexual reproductive structures were not found in our specimens.

Pterocladiella caerulescens (Kützing) Santelices et Hommersand 1997: 118

Basionym: Gelidium caerulescens Kützing (1868).

Type locality: Vieillard, New Caledonia.

Distribution: Tropical regions in the Pacific (Santelices 1998).

Japanese name: Ao-obakusa (new name).

Plants form tufts on bedrock in the upper to middle intertidal zone, are up to 3.3 cm tall, and are gravish to blackish-green in colour. Individual thalli consist of a creeping axis and numerous erect axes (Fig. 5), the creeping axes attaching to the substratum by peg-like aggregates of rhizoids (Fig. 6) that are up to 360-480 µm in length and 95–145 µm in diameter. The creeping axes are subterete, 200-350 µm in diameter, and are sparingly branched. Erect axes are terete to subterete, 240-300 µm in diameter in the basal region, becoming compressed upward, reaching up to 1.3 mm wide and 150-175 µm thick in the middle region. They are oppositely or alternate-distichouly branched one to three times. First- to third-order branches are terete to subterete, up to 2.3 cm in length, 110-200 µm in diameter in the proximal region, becoming compressed to flattened upward, reaching up to 940 μ m wide and 130–160 μ m thick distally. Axes and first- to third-order branches have obtuse apices.

A dome-shaped apical cell is evident at the apices of axes and branches, the majority immersed in a very shallow, apical depression (Fig. 7). First-order branches consist of a medulla of four to seven layers of cells $10-15 \,\mu$ m in diameter and a cortex of three or four layers of slightly smaller cells $4-8 \,\mu$ m in diameter in the middle region (Fig. 8). Rhizines of first-order branches are abundant in the central medulla (Fig. 8).

Tetrasporangia are irregularly disposed in sori at the apices of axes and branches (Fig. 9). The cruciately or decussately divided sporangia are 24–36 μ m long by 44–64 μ m wide (Figs 10, 11).

Cystocarps are formed near the apices of branches (Fig. 12). Nutritive filaments grow centripetally and form a virtually solid cylinder around the central axis (Fig. 13). A cystocarp is attached to one side of the cystocarp floor or detachment of the floor in older cystocarps (Fig. 14), and produces chains of carposporangia from the remaining three sides (Fig. 14). Carposporangia are 20–30 μ m long by 14–22 μ m wide.

The majority of cystocarpic plants bear spermatangial sori on the cystocarpic branchlets. Some of them produce spermatangial sori on spermatangial branchlets (Fig. 15) that are independently formed from cystocarpic branchlets on the erect axes. A few cystocarpic plants bear a special erect axis that produces only spermatangial branchlets (Fig. 16). These three types of spermatangial sori are formed on different individuals and/or the same ones. Two spermatangia are cut off from an elongated spermatangial mother cell, and are $2-3 \,\mu$ m in diameter (Figs 17, 18).



Figs 1–4. *Gelidiella pannosa.* 1. Formalin/seawater-preserved specimen showing erect axes produced on a creeping axis. 2. Independent type of secondary rhizoidal attachments developing from a creeping axis (cultured plant grown at 20°C, 16:8 L:D for 1 month). 3. Dome-shaped apical cell at the apex of an erect axis. 4. Transverse section of an erect axis showing the absence of rhizines in the cortex and medulla.



Figs 5–18. *Pterocladiella caerulescens.* 5. Formalin/seawater-preserved specimen. 6. Peg type of secondary rhizoidal attachments (cultured plant grown at 20°C, 16:8 L:D for 1 month). 7. Dome-shaped apical cell in the apical depression of a first-order branch. 8. Transverse section of the middle portion of a first-order branch. 9. Tetrasporangial branch. 10. Cruciately divided tetrasporangium. 11. Decussately divided tetrasporangium. 12. Cystocarpic branch. 13. Transverse section of a young female branchlet showing nutritive filaments which grow centripetally and form a virtually solid cylinder around the central axis. 14. Transverse section of a cystocarp with one ostiole in the upper side. 15. Cystocarpic (arrowheads) and spermatangial (double arrowhead) branchlets. 16. Spermatangial sori (arrowheads) occurring on the spermatangial erect axis. 17. Surface view of a spermatangial sorus. 18. Transverse section of a spermatangial sorus.

Pterocladiella caloglossoides (Howe) Santelices (1998): 244

Basionym: *Gelidium caloglossoides* Howe (1914). Type locality: San Lorenzo Island, Peru. Distribution: Tropical regions in the Pacific and

Indian ocean (Santelices 1998).

Japanese name: Hime-obakusa (new name).

Plants grow gregariously on bedrock in the middle intertidal zone and are purplish-red. Individual plants consist of a creeping axis and erect axes (Fig. 19), the former subterete to compressed, $110-160 \mu m$ in diameter and attached to the substratum by peg-like rhizoidal aggregations (Fig. 20) that are $135-455 \mu m$ long



Figs 19–24. *Pterocladiella caloglossoides.* 19. Formalin/seawater-preserved specimen. 20. Peg type of secondary rhizoidal attachments in a field-collected plant. 21. Dome-shaped apical cell at the apex of an erect axis. 22. Transverse section of the middle portion of an erect axis showing a single row of medullary cells. 23. Surface view of a tetrasporangial sorus showing transverse rows of tetrasporangia. 24. Surface view of a tetrasporangial sorus showing cruciately or decussately divided tetrasporangia.

by 65–90 μ m wide. Erect axes are up to 3 mm tall, terete to subterete, 60–240 μ m in diameter in the basal region, and become flattened upward, reaching up to 700 μ m wide and 65–115 μ m thick in the middle region. They are usually simple, but are sometimes irregularly branched.

A dome-shaped apical cell is evident at the apices of creeping axes and erect axes (Fig. 21). Erect axes consist of a single-layered medulla of cells 8–16 μ m in diameter and a cortex of two to four layers of slightly smaller cells 4–12 μ m in diameter (Fig. 22). Rhizines of first-order branches are rare in the central medulla (Fig. 22).

Tetrasporangia are regularly arranged in chevronlike rows (Figs 23), eight to 16 per row, are ovate in surface view and 16–32 μ m in diameter. They are divided cruciately or decussately (Fig. 24). Sexual reproductive structures were not found in our specimens.

Small subunit ribosomal DNA analysis

The phylogenetic trees obtained from NJ and MP analyses are shown in Figs 25 and 26 respectively. These two phylogenetic methods resulted in the same topology and levels of bootstrap support in the *Gelidiella* and *Pterocladiella* clades. *Gelidiella pannosa* was included in the *Gelidiella* clade (100% bootstrap support in NJ analysis and 99% in MP analysis) with *Gelidiella acerosa* and *Gelidiella ligulata*. *Pterocladiella caerul*- escens and *P. caloglossoides* were included in the *Pterocladiella* clade (99.7% bootstrap support in NJ analysis and 100% in MP analysis).

DISCUSSION

Four small-sized Gelidiella species have been reported from the western Pacific: Gelidiella adnata Dawson, Gelidiella bornetii (Weber-van Bosse) Feldmann et Hamel, Gelidiella myrioclada (Børgesen) Feldmann et Hamel and G. pannosa (Dawson 1954; Hatta and Prud'homme van Reine 1991; Kraft and Abbott 1998). Gelidiella bornetii can be distinguished from the other three species by its flattened creeping and erect axes (Kraft and Abbott 1998). Gelidiella myrioclada is characterized by alternately, oppositely or pectinately branched erect axes (Børgesen 1934). Gelidiella adnata is most similar to G. pannosa, but two marked differences separate these two species (Kraft and Abbott 1998). Gelidiella adnata has a dense rank of short unicellular unaggregated rhizoids that develop along the entire undersurface, and stichidia that are formed on special short erect axes, whereas G. pannosa has localized patches of unaggregated rhizoids and terminal stichidia on fertile branches. Although we have not observed tetrasporangial stichidia in our material, it has the G. pannosa type of attachment (Feldmann and Hamel 1934). We thus refer our material to this species.



Fig. 25. Phylogenetic tree inferred from small subunit sequences with the neighbour-joining (NJ) method based on Kimura's correction using the CLUSTAL w computer program. The numbers under the branches indicate bootstrap values (1000 replications) greater than 500. Scale bar = 1% divergence.



Fig. 26. Phylogenetic tree inferred from small subunit sequences with the maximum parsimony (MP) method using PAUP program. The numbers under the branches indicate bootstrap values (1000 replications) greater than 50%.

Pterocladiella caerulescens is one of the few monoecious species in the Gelidiales (Santelices and Flores 1995). Spermatangial sori, which are exclusively formed on cystocarpic branchlets, characterize this and five other gelidialean species: Gelidium howeii Acleto, Gelidium mcnabbiana (Dawson) Santelices, Gelidium pluma Loomis, Gelidium vagum Okamura and Onikusa japonica (Okamura) Akatsuka (Santelices and Flores 1995). Acanthopeltis japonica and Acanthopeltis hirsuta (Okamura) Shimada, Horiguchi et Masuda are also monoecious, but they have special spermatangial branchlets on the cystocarpic erect axes (Kaneko 1968; S. Shimada, T. Horiguchi and M. Masuda, unpubl. data). In this study, three types of spermatangial sori were found in Japanese populations of *P. caerulescens*: (i) on fertile cystocarpic branchlets; (ii) on special spermatangial branchlets of a cystocarpic axis; and (iii) on branchlets of a special spermatangial axis. The latter two are reported from *P. caerulescens* for the first time.

In the original description by Howe (1914), *Pterocladiella* (as *Pterocladia*) *caloglossoides* was characterized by the repent flattened thalli throughout, regularly arranged tetrasporangia, and the single layer of medullary cells. However, this species shows a wide range of morphological variations, with creeping axes being subterete to flattened and the length of erect axes ranging from 0.8 mm to 1.2 cm (Howe 1914; Santelices 1977; Norris 1987). Our Japanese material is similar to South African specimens that also have more terete creeping and longer erect axes (Norris 1987) than those originally described by Howe (1914). However, all descriptions of *P. caloglossoides* (Howe 1914; Santelices 1977; Norris 1987) emphasize the regular arrangement of tetrasporangia (chevron-like rows) as a defining feature of this species. Because the Japanese collections also possess such regularly arranged tetrasporangia, we refer them to *P. caloglossoides*.

Three types of secondary attachments are known in the Gelidiales: (i) unaggregated rhizoids [unicellular independent type termed by Shimada et al. (1999)]; (ii) rhizoids that are consolidated into cables [peg type termed by Shimada et al. (1999)]; and (iii) spreading, pseudoparenchymatous haptera [brush type termed by Shimada et al. (1999)]. Of the species examined in the present study, it is clear that Japanese Gelidiella pannosa has the first type and Pterocladiella caerulescens and P. caloglossoides have the second. Other species in the only distantly related Gelidiella and Pterocladiella clades (Fig. 25) have the same anchoring types as their Japanese counterparts (Shimada et al. 1999), suggesting that this morphological character may represent synapomorphies or sympleisiomorphies within particular genera of the Gelidiales.

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