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Morphology and systematics of *Gelidiella tenuissima* (Gelidiales, Rhodophyta) from Gran Canaria (Canary Islands, Spain)

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Fertile male gametophytes of *Gelidiella tenuissima* are described for the first time, from cultured plants collected in Gran Canaria (Canary Islands, Spain). The morphology of male reproductive structures in this species is similar to other gametophytes referred to the Gelidiales, but different from the recently described male gametophyte of the type species of *Gelidiella, G. acerosa.* Morphological comparisons between these two species, using the male gametophyte and the tetrasporangial stichidia, and also molecular analyses, suggest that two different groups of species are included in the genus *Gelidiella.*

INTRODUCTION

The genus *Gelidiella* was proposed by Feldmann & Hamel (1934, p. 529) to include species previously included in the genera *Echinocaulon* and *Acrocarpus.* The main diagnostic character for this new genus was the absence of medullary hyphae (= rhizines), although the morphology of the stichidium was regarded as being important. Fan (1961) confirmed the placement of *Gelidiella* within the order Gelidiales and created a new family, the Gelidiellaceae, to include *Gelidiella* as the only genus. Fan (1961) also considered that the presence of tetrasporangia in stichidia characterized the genus *Gelidiella.* He noted that sexual reproduction was unknown in *Gelidiella* and, although not considered diagnostic by him, the seeming lack of a gametophytic stage has subsequently been considered a character of this genus by several authors. This has, in part, been because of the absence of substantiated records of female gametophytes and carposporophytes in species assigned to the genus. Cystocarps have been described from only two of the 21 species of *Gelidiella.* Sreenivasa Rao & Trivedi (1986) reported observing the cystocarp of an unnamed species of *Gelidiella* from India, but the description and illustrations are insufficient to judge the merit of this claim. This entity was subsequently invalidly named *G. diuens* Sreenivasa Rao & Trivedi (no Latin diagnosis provided: see Silva *et al.* 1996, p. 151). Guiry & Womersley (1992) also described and illustrated cystocarps in a supposed species of *Gelidiella, G. minima* Guiry & Womersley, from Australia. This latter species was subsequently transferred to *Pterocladiella* Santelices & Hommersand, based on cystocarp characters and presence of rhizines (Santelices & Hommersand 1997).

Despite a lack of observations of these life-history stages, there is some circumstantial evidence for sexual reproduction in the *Gelidiella* life history. A study of chromosomes in *G. acerosa* (Forsskål) Feldmann & Hamel indicated that meiosis occurs during the production of tetraspores (Sreenivasa Rao 1974). Likewise, in a study measuring nuclear DNA amounts in *G. acerosa,* Kapraun *et al.* (1994) found twice as much DNA in tetrasporophytes as in germlings grown from tetraspores. Recently, Santelices (1997) described spermatia from a putative male gametophyte of *G. acerosa.* These spermatangial sori are unique among the Gelidiales in that they protrude from the thallus surface as a result of proliferation of the surrounding inner cortical and external medullary cells (Santelices 1997). The position of male sori near the base of erect branches is also different from that reported for other species referred to the Gelidiales.

Feldmann & Hamel (1934) described two groups of *Gelidiella* species, based on the type of tetrasporangial stichidia present. The 'acerosa'-type has conical stichidia with unordered, compactly arranged tetraspores, whereas the 'pannosa'-type has flattened stichidia with tetrasporangia arranged in transverse rows. The present article reports the presence of fertile male gametophytes in *G. tenuissima* Feldmann & Hamel, which, as *G. pannosa* (Feldmann) Feldmann & Hamel, is the name-bringing, and presumably typical entity of the 'pannosa' group (*sensu* Feldmann & Hamel 1934) of *Gelidiella* species. A recent nomenclatural article (Santelices & Rico 2002) has proposed that the combination *G. tenuissima* Feldmann & Hamel is the valid name for this species and consequently, the group will be named the 'tenuissima' group from here on. Analyses of DNA sequence data are used to assess the relationships of *G. tenuissima* and *G. acerosa* within the Gelidiales.

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MATERIAL AND METHODS

Samples of tetrasporangial *G. tenuissima* were collected from intertidal rocks at La Garita, Gran Canaria, Canary Islands, in October 1993. Culture details are as in Guiry & Cunningham (1984) and Rico & Guiry (1997). Cultures obtained from germinated tetraspores were subjected to a range of photoperiods and temperatures, and examined periodically for the presence of fertile gametophytes.

Specimens from the following herbaria were examined: Herbario de Botánica, Universidad de La Laguna, Teneriffe, Spain (TFC), Herbario de la Cátedra de Botánica, Facultad de Farmacia de Barcelona, Spain (BCF), Laboratoire de Cryptogamie, Paris, France (PC), the Natural History Museum, London, UK (BM). Dried herbarium specimens were wetted with seawater. Rehydrated herbarium and freshly collected specimens were stained with Cotton Blue lactophenol and mounted in 60% Karo® for microscopical examination. Voucher specimens have been deposited in the Herbario de la Facultad de Biología, Oviedo, Spain (FCO).

Total genomic DNA was extracted from small pieces of the proposed lectotype specimen of *G. tenuissima* (Santelices & Rico 2002) and the Canary Islands culture isolate, following the protocol of Jeff Hughey (see Thomas & Freshwater 2001). Sequence data for the large subunit (LSU) of ribulose-1,5-bisphosphate carboxylase–oxygenase (*rbc*L) and the nuclear LSU RNA genes were generated as described by Freshwater & Rueness (1994) and Freshwater & Bailey (1998). These data have been deposited in GenBank under accession numbers AF309385, AF308799, AF320983 and AF320984. New sequence data were also generated for *P. capillacea* (S.G. Gmelin) Santelices & Hommersand from Italy (AF308797) and for an unidentified *Gelidiella* species from the Philippines (AF309386 and AF308798). Collection locations and GenBank accession numbers for the other taxa included in the sequence analyses are listed by Freshwater *et al.* (1995), Freshwater & Bailey (1998) and Thomas & Freshwater (2001).

Maximum parsimony (MP) and distance analyses of the sequence data sets were done using PAUP version 4.0b4a (Swofford 2000). Parsimony searches were performed with the branch-and-bound algorithm. Parsimony bootstrap analyses consisted of 1000 replications of either branch-and-bound searches (LSU) or heuristic searches using simple sequence addition, tree bisection–reconnection branch swapping and MULPARS (*rbc*L and combined). In distance analyses, neighbour-joining (NJ) trees (Saitou & Nei 1987) were constructed from Kimura 2-parameter distances (Kimura 1980). Distance bootstrap analyses consisted of 1000 replications of NJ tree construction with Kimura 2-parameter distances. Gaps were treated as missing in all analyses that included LSU data.

RESULTS

Morphology

Field-collected plants of *G. tenuissima* from Gran Canaria conformed well with the accepted concept of this species (Fig. 1). The plants formed entangled mats of 1–1.5 cm high within tufts of plants referred with some doubt to *Gelidium pusillum* (Stackhouse) Le Jolis, growing in crevices on intertidal rocks. All field-collected plants were either vegetative or tetrasporangial. No rhizines were observed in sections of these plants (Figs 2, 3), or in cultured isolates. In transverse section, the outer cortical cells appeared elongated tangentially and were 7–10 μm long and 3–4 μm in diameter (Fig. 3). Inwardly, more rounded, pigmented cortical cells and globose, unpigmented medullary cells (Figs 2, 3) were apparent. Rhizoids were unicellular, formed from outer cortical cells (Figs 2, 3) which produced protoplasmic filaments that developed within the rhizoid. The rhizoids were loosely arranged on the basal side and unaggregated throughout the prostrate axes (Figs 4, 7).

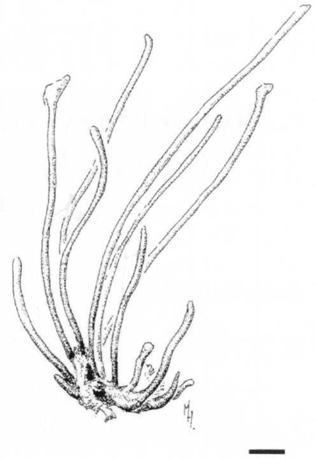


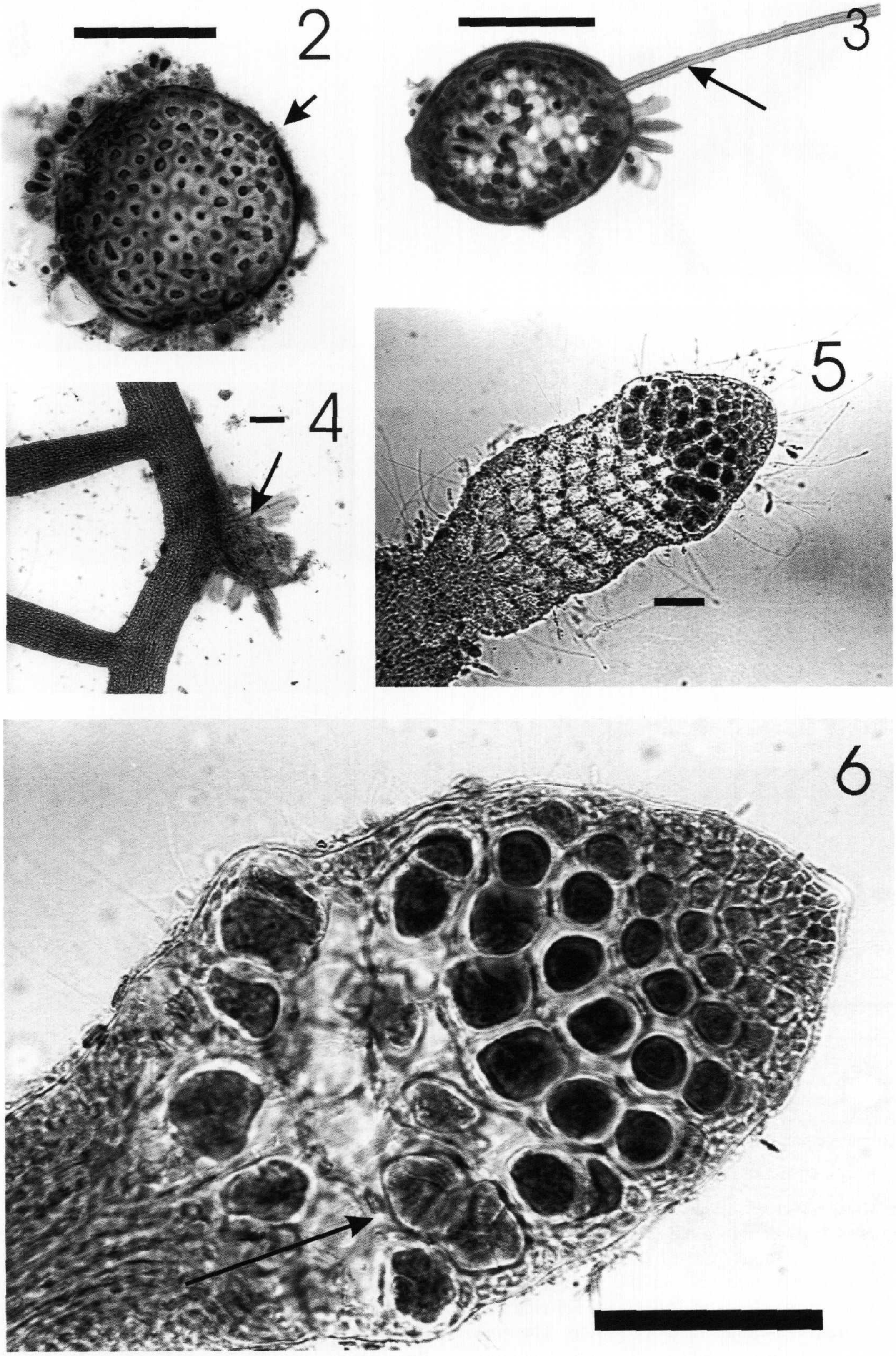
Fig. 1. Habit of *G. tenuissima* from Gran Canaria.

Tetrasporangia were borne on flattened stichidia at the apices of terminal branches; there was always one stichidium per branch. The tetrasporangia were arranged in regular rows of 3 + 3 (Fig. 5), with maturing tetrasporangia in the apical portion and discharged tetrasporangial cavities towards the base of the stichidium. The tetraspores appeared to be cleaved tetrahedrally (Fig. 6).

Male reproductive structures were formed only in plants cultured at 20°C, irrespective of the photoperiod used (8 : 16 to 16 : 8 h light–dark). They were produced in ultimate unbranched branchlets, either terminally in erect axes or laterally from the main axis (Fig. 7). The spermatangial sori form colourless patches (Fig. 8) and, when fully mature, cover both surfaces of the fertile branchlet (Figs 7, 9). Spermatangial initials are formed from cortical cells and, when seen in surface view, can be seen to be arranged in tetrads (Fig. 8). A mucilaginous envelope covers the mature spermatangia (Fig. 9). Initials form spermatangia by transverse divisions (Figs 10, 11). The spermatangia are pyramidal in outline and about 3 or 4 μm high and have one or two refractile vacuoles (Figs 10, 11).

Molecular analysis

LSU and *rbc*L sequence data from 16 Gelidiales species were analysed separately and in combination, using parsimony and distance methods. Alignment of the LSU sequences required the inclusion of seven insertion–deletion mutations (indels) of 3 bp or less. The occurrence of one particular indel and the size of a second were diagnostic for *Gelidiella*. The alignment



Figs 2–6. Vegetative morphology of *G. tenuissima* from Gran Canaria. Scale bars = 50 μm, except Fig. 4 (100 μm).

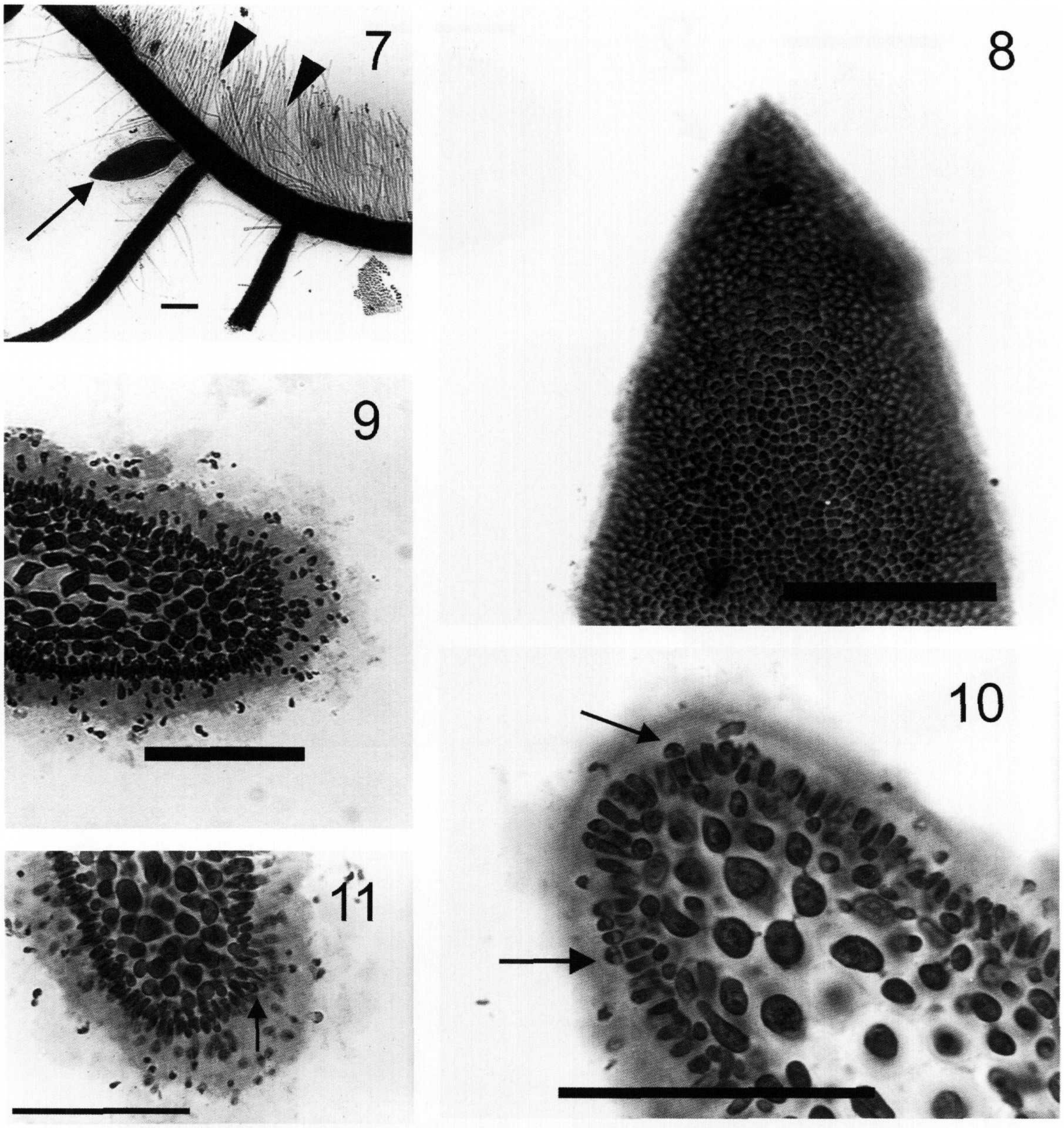
Fig. 2. Transverse section of erect axis. Note the pyriform cortical cell, which may be a rhizoidal initial (arrow).

Fig. 3. Transverse section of prostrate axis. A unicellular rhizoid is clearly visible, with a cortical cell forming a long cytoplasmic intrusion into rhizoid (arrow).

Fig. 4. Fragment of prostrate axis, showing unicellular rhizoids (arrow).

Fig. 5. Tetrasporangial stichidium. Note tetrasporangia forming on the apical side and empty tetrasporangial cavities on the basal side.

Fig. 6. Detail of tetrasporangial stichidium, showing tetrahedral cleavage of tetraspores (arrow).



Figs 7–11. Morphology of the male gametophyte of *G. tenuissima* from Gran Canaria. Scale bar = 50 μm, except Fig. 7 (100 μm).
Fig. 7. Spermatangial branchlet (arrow). Note the unicellular rhizoids on the basal side (arrowheads).
Fig. 8. Surface view of spermatangial branchlet, showing arrangement of spermatangial initials in tetrads.
Fig. 9. Transverse section of mature spermatangial branchlet, showing mucilaginous envelope.
Fig. 10. Transverse section of mature spermatangial branchlet. Note the transverse division of the spermatangial initials (arrows).
Fig. 11. Transverse section of spermatangial branchlet with forming spermatia (arrow).

included 1159 sites, of which 121 were parsimony informative. MP analysis of LSU data resulted in a single tree of 252 steps with a consistency index (CI) of 0.684. The LSU NJ tree differed from the MP tree only in the arrangement of *Capreolia* Guiry & Womersley and *Ptilophora* Kützing, and required only a single additional mutational step. The *rbc*L data set contained 1400 sites, of which 385 were parsimony informative. MP analyses of these sequences resulted in a single tree of 1207 steps with a CI of 0.498. As in the LSU analyses, the *rbc*L NJ tree differed from the parsimony tree only in the arrangement of the *Capreolia* and *Ptilophora* lineages and required two additional mutational steps.

The results of the separate LSU and *rbc*L analyses showed no conflict between well-supported relationships and so the data sets were combined for further analyses. The combined data set included 2559 sites, of which 506 were parsimony informative. MP analysis resulted in a single tree of 1469 steps with a CI of 0.524 (Fig. 12). The NJ tree resulting from the distance analysis was identical to the parsimony tree. In this tree, the four lineages distinguished by different nutritive strategies for the developing carposporophyte (Bailey & Freshwater 1997) are strongly supported as monophyletic groups (bootstrap proportion = 100%). The lectotype of *G. tenuissima* is within the monophyletic clade of *Gelidiella* spe-

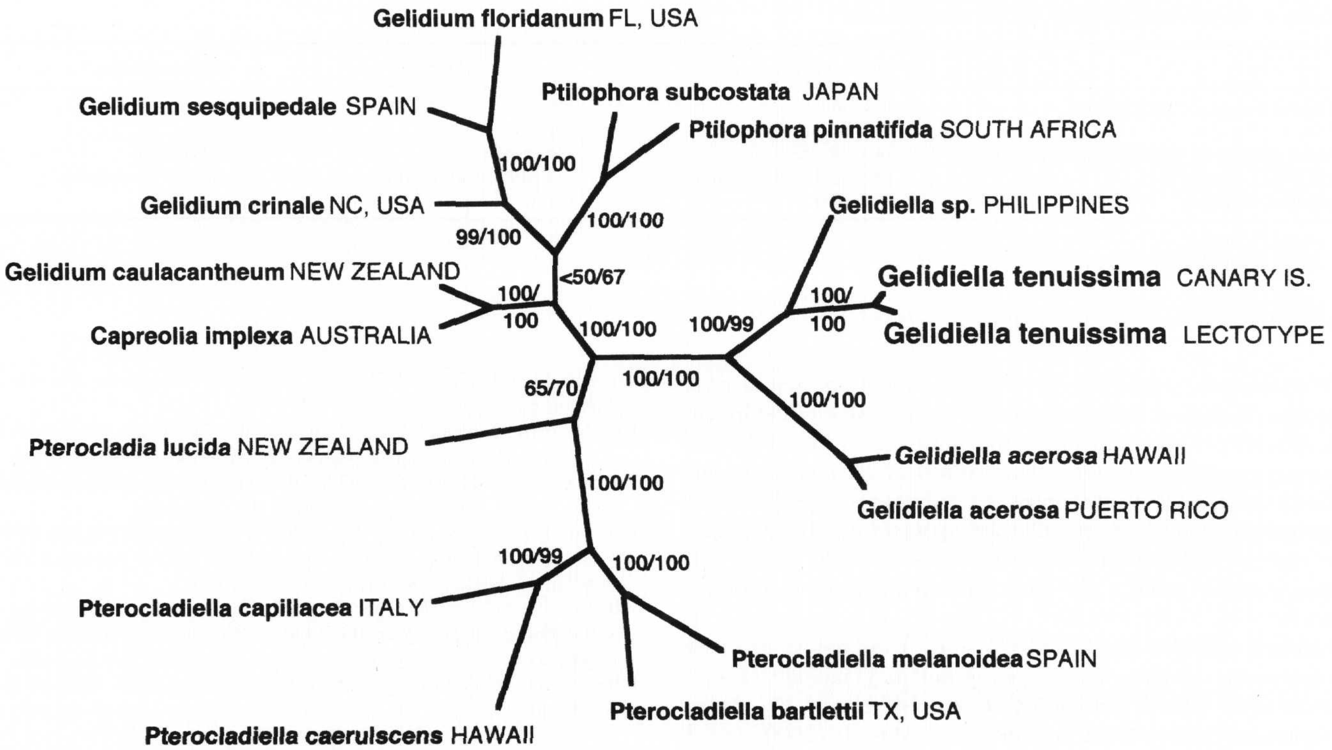


Fig. 12. Single minimal tree (L = 1469, CI = 0.524) resulting from maximum parsimony (MP) analysis of the combined *rbc*L + large subunit data set. This topology is identical to that obtained by neighbour-joining (NJ) analysis of Kimura 2-parameter distances. MP and NJ bootstrap values (1000 replications each) are shown for each branch, in that order.

cies that terminate one of the four major lineages and is sister to the Canary Islands *G. tenuissima* collection; it differs from it at only one LSU site and 1.75% of the *rbc*L sites compared. Other taxa in the *Gelidiella* clade include samples of *G. acerosa* from the Pacific and Caribbean and an unidentified *Gelidiella* species from the Philippines (Fig. 12). The assignment of this specimen to *Gelidiella* was confirmed in culture: cultured specimens developed tetrasporangial stichidia that had a '*tenuissima*-type' of tetrasporangial arrangement and produced unordered unicellular rhizoidal attachments; no rhizines were observed in sections of these plants.

DISCUSSION

DNA sequence analysis is a well-established method for exploring relationships within the red algal order Gelidiales (e.g. Freshwater *et al.* 1995; Shimada *et al.* 1999, 2000). In analyses of *rbc*L and LSU sequence data, species of *Gelidiella* formed a well-resolved branch within the Gelidiales (Fig. 12). This monophyletic *Gelidiella* clade contained the generitype, *G. acerosa*, the lectotype of *G. tenuissima*, field-collected plants of *G. tenuissima* from the Canaries, and an unidentified *Gelidiella* species from the Philippines. Guiry & Womersley (1992), while discussing the generic reallocation of species of *Gelidium* J.V. Lamouroux and *Pterocladia* J. Agardh, suggested that the presence or absence of rhizines and the morphology of the cystocarp were critical in separating species into groups, and proposed four potential genera: *Pterocladia sensu stricto,* which was confirmed by cystocarp morphology described by Santelices & Hommersand (1997); *Gelidium,* as presently defined by the type species *G. sesquipedale* (Clemente) Bornet; *Gelidiella,* with unknown cystocarp morphology and unordered tetrasporangia; and an unnamed genus to allocate species not included in the previous three. Santelices & Hommersand (1997) defined *Pterocladiella* on the basis of cystocarp morphology and included in it several species that demonstrated conflicting generic character states. Amongst the species transferred to *Pterocladiella* was the entity that was initially used to propose this reassessment, viz. *G. minima,* which has rhizines (Guiry & Womersley 1992, p. 176). Another species initially described as *G. calcicola* Maggs & Guiry (Maggs & Guiry 1988) was transferred to *Gelidium* by Norris (1992), who suggested that the presence or absence of rhizines should remain a decisive factor in generic delimitation. Thus, rhizines should be present in all species of the genera *Gelidium, Pterocladia* and *Pterocladiella.* Melo (1992) confirmed that the type species of *Gelidiella, G. acerosa,* lacked rhizines, and our observations show that *G. tenuissima* also lacks them, thus establishing this character as valid for separating the *Gelidium–Pterocladia–Pterocladiella* complex from *Gelidiella.*

The discovery of male plants of *G. acerosa* (Santelices 1997) and those reported here for *G. tenuissima* confirm that the absence of a sexual phase cannot be used as a diagnostic character for *Gelidiella.* It is probably only a matter of time before reproductive female gametophytes are discovered, and then the relationship of the ontogeny and morphology of the female reproductive system and cystocarp in *Gelidiella* and other Gelidiales can be resolved.

The morphology of the spermatangial sori in *G. acerosa,* however, differs considerably from those described here for

Table 1. Comparison between species of *Gelidiella*.

	G. acerosa[1]	*G. adnata*[2]	*G. tenuissima*[3]
Tetrasporangial arrangement	Irregular	Regular (3 + 3)	Regular (3 + 3)
Apical division	Decussate	Lateral	Lateral
Arrangement of spermatangial patches	In elevated sori	Unknown	*Gelidium*-type
Rhizoidal attachment	Multicellular filaments[4]	Unordered unicellular	Unordered unicellular
Internal structure	Irregular	Central row	Irregular

[1] Melo (1992).
[2] Santelices (2002).
[3] This study.
[4] Norris (1992).

G. tenuissima, supporting the view that there are two distinct groups of species within the genus, as proposed by Feldmann & Hamel (1934), who noted the presence of two different tetrasporangial types. Our observations of *G. tenuissima* indicate that *Gelidiella* comprises two subgroups: one has raised spermatangial sori, an irregular arrangement of tetrasporangia in conical stichidia and decussate division of the apical cell (the 'acerosa' group); the other has '*Gelidium*-type' spermatangial sori, a chevron-like arrangement of tetrasporangia in flattened stichidia and lateral division of the apical cell (the 'tenuissima' group). This latter group may include several small-sized species like *Gelidiella tenuissima* and *G. adnata* (Santelices 2002) (Table 1). Shimada & Masuda (2000) added another diagnostic character for *G. tenuissima* (as *G. pannosa*) from Japan, viz. the presence of unordered rhizoidal attachments, as opposed to peg-like rhizoidal attachments or multicellular attachments such as those found in *G. acerosa* (Norris 1992). The sequence analyses presented here also show two strongly supported lineages within *Gelidiella*, but sequence data from other *Gelidiella* species are needed to verify this. The separation of the genus into two distinct entities may be warranted, just as has been done, on the basis of cystocarp ontogeny and morphology, with *Pterocladiella* and *Pterocladia* (Santelices & Hommersand 1997). Accordingly, proposal of four genera by Guiry & Womersley (1992) may be conservative and at least one further genus may need to be added to *Gelidiella, Pterocladia, Pterocladiella* and *Gelidium*. Although this idea is attractive, additional sequence data and further observations, especially of cystocarp morphology, are needed to confirm it.

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