

***Gracilaria apiculata* and *G. flabelliformis*
(Gracilariaceae, Rhodophyta): restoring old names for
common tropical western Atlantic species, including the
recognition of three new subspecies, and a replacement
name for “*G. lacinulata*”¹**

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Abstract — The genus *Gracilaria* (Gracilariales, Rhodophyta) has long been one of the most taxonomically challenging groups of red algae as a result of the high number of species, small number of distinctive characters, and the high levels of morphological plasticity among vegetative and reproductive features. Recently, DNA sequence phylogenies based on the chloroplast-encoded *rbcL* gene have resolved many systematic questions in this genus. This study provides an *rbcL*-based phylogeny of the most derived lineage of *Gracilaria sensu stricto*, the assemblage containing the generitype, *G. bursa-pastoris*. In this clade, two poorly known and overlooked species of *Gracilaria*, *G. flabelliformis* and *G. apiculata*, were found to be very common members of the genus in the tropical western Atlantic. *Gracilaria apiculata* belongs to the *G. cervicornis* complex, a clade composed of highly branched species with terete to compressed main axes, while *G. flabelliformis* is a member of a clade composed only of Atlantic flat species. Exceedingly distinct phenotypes for each of these species were observed in our circumscription and are described as new subspecies, along with detailed morphological characterization for each of these taxa: *G. flabelliformis* subsp. *aionana* subsp. nov., *G. flabelliformis* subsp. *simplex* subsp. nov., and *G. apiculata* subsp. *candelabrififormis* subsp. nov., and *G. isabellana* nom. nov., a replacement name for *G. lacinulata* (Vahl) Howe which is a homonym of *G. lacinulata* (Kützinger) Piccone.

***Gracilaria* / Gracilariales / marine algae / morphology / phylogeny / *rbcL* / Rhodophyta / taxonomy / Western Atlantic**

Résumé — *Gracilaria flabelliformis* et *G. apiculata* (Gracilariaceae, Rhodophyta): restauration d'anciens noms pour les espèces communes de l'Atlantique tropical occidental, avec reconnaissance de trois nouvelles sous-espèces, et un nouveau nom pour “*G. lacinulata*”. Le genre *Gracilaria* (Gracilariales, Rhodophyta) est depuis longtemps un des groupes d'algues rouges dont la taxinomie est un perpétuel défi ; ceci est dû au grand nombre

1. It is our pleasure to dedicate this study to our colleague, friend and mentor, Prof. Isabella Aiona Abbott on the occasion of her 85th birthday.

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d'espèces, au petit nombre de caractères distinctifs et à une grande plasticité morphologique des caractéristiques reproductrices et végétatives. Récemment, des phylogénies basées sur l'analyse des séquences d'ADN du gène chloroplastique *rbcL* ont résolu de nombreuses questions systématiques dans le genre. La présente étude fournit une phylogénie construite à partir de l'analyse du gène *rbcL* de la lignée la plus dérivée de *Gracilaria sensu stricto*, cet ensemble contenant l'espèce type du genre, *G. bursa-pastoris*. Dans ce clade, deux espèces peu connues et passées inaperçues de *Gracilaria*: *G. flabelliformis* et *G. apiculata*, sont apparues comme deux taxons du genre, très communs dans l'Atlantique tropical occidental. *Gracilaria apiculata* appartient au complexe *G. cervicornis*, un clade composé d'espèces très ramifiées avec des axes principaux de section circulaire à aplatie. *G. flabelliformis*, en revanche, appartient à un clade composé seulement d'espèces atlantiques aplaties. Pour chacune de ces espèces, des phénotypes extrêmement distincts ont été observés dans notre circonscription des taxons; il sont décrits comme de nouvelles sous-espèces pour chacune desquelles une caractérisation détaillée est donnée: *G. flabelliformis* subsp. *aionana* subsp. nov., *G. flabelliformis* subsp. *simplex* subsp. nov., *G. apiculata* subsp. *candelabrififormis* subsp. nov., et *G. isabellana* nom. nov., pour remplacer *G. lacinulata* (Vahl) Howe, un homonyme de *G. lacinulata* (Kützinger) Piccone.

Algues marines / Atlantique occidental / Caraïbes / *Gracilaria* / Gracilariales / morphologie / phylogénie / *rbcL* / Rhodophyta / taxinomie /

INTRODUCTION

The genus *Gracilaria* Greville (1830: liv. 121) has long been one of the most taxonomically challenging groups of the red algae. While the name *Gracilaria* is derived from the Latin *gracilis*, meaning "thin, slender" (Brown, 1956: 376; Stearn, 2004: 421), taxonomic usage has also implied "simple", thus portraying a genus with a simplicity of vegetative organization, without ornaments, and the lack of morphological characters. The relatively small number of diagnostic features has long made the taxonomy of *Gracilaria* at the species level one of the most difficult, which has been further complicated by the high number of species described. In many cases species identification has often been considered nearly impossible, especially when only small, immature, sterile or atypical phenotypes are available (Taylor 1960). Foliose species of *Gracilaria* have also had taxonomic problems, such as members of the *G. mammillaris* complex in the Caribbean (Schneider, 1975; Ganesan, 1989; Gurgel *et al.*, 2004a), and *G. multipartita*-*G. foliifera* complex in the northeastern Atlantic (Guiry & Freamhainn, 1986).

It is only when relatively large collections of specimens are available that morphological patterns emerge and specimens can be grouped in different taxa. Still in some cases, due to their plasticity, the morphological boundaries of many species can overlap, and identifications often become tentative. However, as advocated by Bird (1995), molecular biology and more specifically DNA sequence technology, has significantly resolved many taxonomic questions in the Gracilariaceae. Gurgel *et al.* (2003a, b; 2004a, b) and Gurgel & Fredericq (2004) have shown that *rbcL*-based phylogenies are a powerful tool to recognize different species regardless of their morphological status.

Recently, a broad phylogenetic study based on *rbcL* DNA sequence analysis of 68 distinct species of Gracilariaceae showed the existence of at least six discrete evolutionary lineages in the genus *Gracilaria sensu stricto* (Gurgel & Fredericq, 2004). The present study focuses on the systematics of the most derived *rbcL* lineage, the *Gracilaria sensu stricto* lineage (= subgroup IX of the *Gracilaria*

sensu lato clade in Gurgel & Fredericq, 2004: Figs 1&2). This lineage contains the generitype, *G. compressa* (C. Agardh) Greville (1830: liv; a taxonomic synonym of *G. bursa-pastoris* (Gmelin) Silva 1952: 265; Steentoft, *et al.*, 1991; Silva, 1994), and is composed mainly of foliose species with spermatangial conceptacles of the 'textorii-type' (Yamamoto, 1978). Extensive collections based on new and herbarium-deposited specimens, including type material, were analyzed together with newly generated *rbcL*-based phylogenies.

Phylogenetically distinct species were identified based on morphology and on the type method by comparing recently collected specimens with type specimens and original descriptions. Our results recognized the existence of two distinct species and three new subspecies among the taxa analyzed within this lineage. We follow Prof. I. A. Abbott (1995: 186) who noted that "More important than describing new species will be re-describing old species whose names have been published for the more than 100 years but are as poorly known now as when their names were first published." Thus based on studies of type specimens, we conclude that our two "newly" recognized species of *Gracilaria sensu stricto* from the tropical Western Atlantic are poorly known and overlooked species, but have validly published names that have not been used in recent literature: *Gracilaria apiculata* P. et H. Crouan in Schramm et Mazé, and *G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel in Gurgel et Fredericq. The basionym of *G. lacinulata* (Kützinger) Piccone, i.e. *Sphaerococcus lacinulatus* Kützinger, is found to be a taxonomic synonym of *G. flabelliformis*; hence, the homonym, *G. lacinulata* (Vahl) Howe, is given a replacement name, *G. isabellana*.

MATERIAL AND METHODS

Molecular Analyses

Information regarding sample identification, site location, collector name(s), date of collection, and GenBank accession numbers for *rbcL* sequences produced in this study are all listed in the Appendix. Partial *rbcL* sequences were produced for thirty-three taxa representing fifteen species of *Gracilaria*. Methods of sample preparation, DNA extraction, automated DNA sequencing and sequence alignment follows those of Lin *et al.* (2001). The generated sequence data were compiled with Sequencher (Gene Codes Corp., Ann Arbor, MI), proofread with MacClade 4.0 (Maddison & Maddison, 2000), and aligned by hand in a nexus file. The *rbcL* alignment for phylogenetic analysis was composed of 33 sequences, 32 ingroup taxa and *G. chilensis* Bird, McLachlan et Oliveira (1986: 2928) as the outgroup. *Gracilaria chilensis* was selected due to its phylogenetic relationship to the ingroup (Gurgel & Fredericq, 2004) and comparative analysis of results obtained in several preliminary analysis of this very same dataset (data not shown).

Maximum Parsimony (MP) analysis was performed as implemented in PAUP* v.4.0 beta 10 (Swofford, 2003) under the Fitch criterion of equal weights for all substitutions (Fitch, 1971) and excluding the first 99 bp of the alignment for which too many missing data was present in most of the sequences. Maximum Parsimony (MP) trees were inferred based on an heuristic search of 5000 random sequence addition replicates, Tree-Bisecting Reconnection (TBR) algorithm, holding 25 trees at each step, with MULPARS, MULTREES options on. Consistency index (CI; Kluge & Farris, 1989) and retention index (RI; Farris, 1989) were calculated excluding uninformative characters. Support for nodes for MP analysis

was assessed by calculating bootstrap proportion values (BV) (Felsenstein, 1985) based on 2000 resamplings of heuristic searches, using the same parameters as implemented in their original heuristic search analysis (i.e. TBR, MULPARS and MULTREES options in effect) but the SIMPLE sequence addition options instead of RANDOM.

The Bayesian analysis was performed using the software MrBayes version 3.0 beta 4 (Huelsenbeck & Ronquist, 2001) without excluding the first 99 bp of the alignment. The optimal model of sequence evolution to fit the data alignments was estimated by hierarchical likelihood ratio test performed by Modeltest v.3.06 with $\alpha = 0.01$ (Posada & Crandall, 1998). The optimal model obtained was the TrN+ Γ +I and this model was used to set up the Markov Chain Monte Carlo (MCMC) search for Bayesian analysis (lset nst = 2 rates = invgamma). The starting parameter exact values (i.e. the estimated ti/tv, alpha shape parameter for the gamma distribution and exact proportion of invariable sites that maximize the likelihood values) were automatically set arbitrarily for substitution model parameters (= default options). This is because it normally makes little sense to override this behavior by forcing specific values onto prior parameters in Bayesian analysis. The Bayesian analysis was conducted by running six chains of the Markov Chain Monte Carlo (five hot, one cold), sampling one tree every 20 generations for 2,000,000 generations starting with a random tree. Reliability of the Bayesian consensus tree is given by the frequency at which each node appears among all saved trees after the "burn in" point, i.e. the moment when the maximum likelihood values from the trees sampled along the MCMC run reaches stationary. Inferences about the phylogeny were based on those trees sampled after the "burn-in" point and a 50% consensus tree (i.e. 50% majority rule consensus as implemented by PAUP*) was computed from the trees saved after this point. This frequency corresponds to the true probability of the clades, the posterior probability (PP) (Hall, 2001). Pairwise sequence divergences among intraspecific haplotypes were calculated based on *p* distances as implemented by PAUP*.

Morphological analysis

Specimens studied, including vouchers, were fixed and liquid-preserved in buffered 5% Formalin/seawater, and/or some were pressed and air-dried on herbarium sheets and others were later herbarium-pressed from liquid-preserved specimens, and deposited in US Alg. Coll., LAF and ALCB (herbaria abbreviations follow Holmgren *et al.*, 1990). Specimens were photographed on a Zeiss Stemi 2000-C dissection scope (Carl Zeiss Inc., Thornwood, NY) attached to a Minolta 35mm camera (Minolta Corporation USA, Ramsey, NJ). Some were scanned into the computer either as 'wet' (liquid-preserved) specimens, or directly from a herbarium sheet using a Microtek ScanMaker III scanner (Microtek International, Hsinchu, Taiwan). Transverse sections for morphological studies were hand-made using stainless steel razor blades under a stereoscopic dissection microscope, and then stained in a 3% aqueous aniline blue solution for 10-15 minutes. The stained sections were fixed with 1 droplet of 3% acetic acid, rinsed with distilled water, and then mounted in a 50% Karo™ clear corn syrup/distilled water solution with phenol added as a preservative (Tsuda & Abbott, 1985). Photomicrographs were taken with a Polaroid DMC 1c digital camera (Polaroid, Inc., Cambridge, MA) attached to an Olympus BX50 microscope (Olympus, Melville, NY). Images were edited and assembled into plates using Photoshop v.6.0 (Adobe Systems Inc., San Jose, CA).

RESULTS

Molecular Data

Among the 33 partial *rbcL* sequences, 1105 bp were identical and 362 bp varied at least once. With the exclusion of the first 99 bp, there were 156 informative characters included in the MP analysis (including the outgroup sequence). Tree lengths of 100,000 randomly generated trees for this dataset had a skewed distribution ($g_1 = -0.634$, $p < 0.01$) indicating the presence of non-random structures and the presence of phylogenetic signal in the dataset (Hillis & Huelsenbeck, 1992; Hillis *et al.*, 1993).

Maximum parsimony analysis of the dataset resulted in 33 equally most parsimonious trees (MPT) of 378 steps, CI = 0.49 and RI = 0.62 (Fig. 1). All interspecific phylogenetic relationships among the 64 MPT were identical. The only topologies that differed among the 33 MPT occurred exclusively inside the haplotype-specific clades, i.e. the *G. apiculata*, *G. cervicornis*, *G. bursa-pastoris*, and *G. flabelliformis* intraspecific clades.

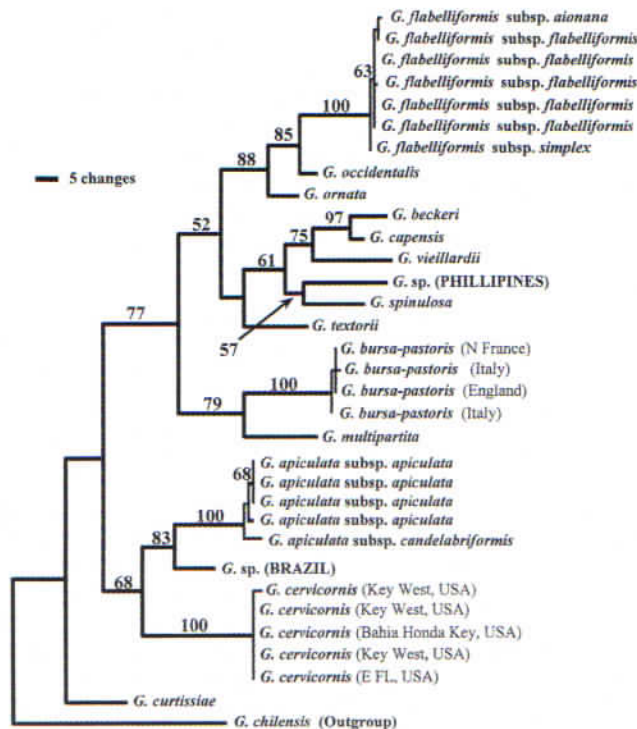


Fig. 1. One of 64 most parsimonious trees. Maximum parsimony phylogram of the most derived clade of the genus *Gracilaria sensu stricto* based on a *rbcL* DNA sequence analysis. Number above the branches correspond to bootstrap proportion values based on 2000 replicates; only values >50% are shown. Number of phylogenetically informative included characters = 156, tree length = 378 steps, CI = 0.49, RI = 0.62.

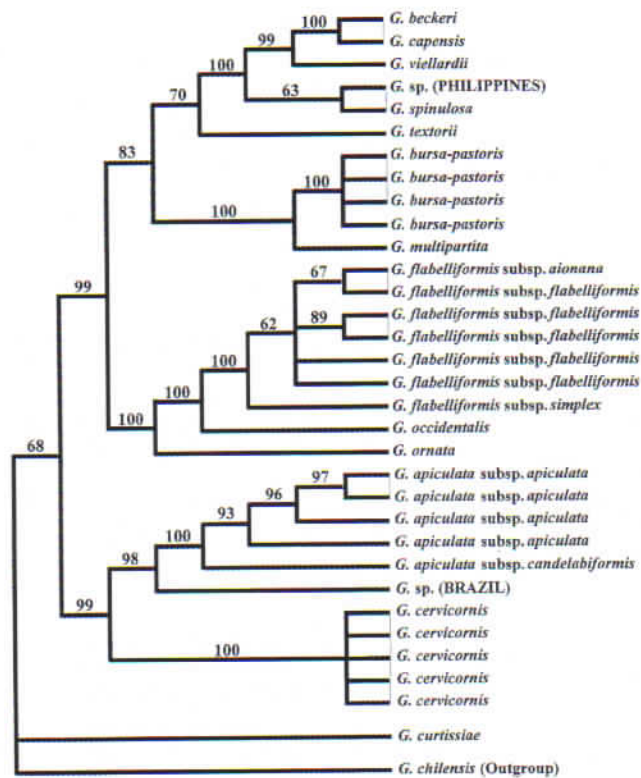


Fig. 2. Bayesian consensus tree (= 50% majority-rule consensus) of the most derived lineage in the genus *Gracilaria sensu stricto*. Consensus composed of 99,341+1 trees saved after the Metropolis-Coupled Markov Chain Monte Carlo run reached stationarity at generation 16,200. Burn-in equals to the first 659 discarded trees. Number of generations ran = 2.0×10^6 , sampling one tree every 20 generations. Number above the branches corresponds to posterior probabilities of the clades.

The Bayesian analysis reached stationarity at generation 13,200 and the first 659 trees were considered the burn-in point. The Bayesian tree resulted from the consensus of 99,341+1 trees saved after the burn-in point (Fig. 2). Four major clades can be recognized in the MP and the Bayesian results (Figs 1-2). Two are composed of Western Atlantic species, one is composed of eastern Atlantic species, and one is composed of Indo-Pacific species. The *G. cervicornis* clade includes two other morphologically similar but phylogenetically distinct species, *G. apiculata* and *Gracilaria* sp. from Brazil (= a specimen tentatively identified as "*G. aff. domingensis*" by Gurgel & Fredericq, 2004). In both phylogenetic methods *G. curtissiae* was left unresolved (Figs 1-2).

Four species were represented by multiple sequenced specimens: *G. apiculata* (5 sequences, 5 distinct haplotypes, with 0.14-0.9% pairwise sequence divergence), *G. bursa-pastoris* (4 sequences, 3 distinct haplotypes, with 0.07-0.44% pairwise sequence divergence), *G. cervicornis* (5 sequences, 2 distinct haplotypes,

with 0.21-0.23% pairwise sequence divergence), and *G. flabelliformis* (7 sequences, 7 distinct haplotypes, with 0.07-0.69% pairwise sequence divergence). While molecular biology and the use of phylogenies based on DNA sequence comparisons yielded a high degree of resolution in these systematic studies, the correct taxonomic circumscription for these species still strongly relies on the type method (e.g., Silva, 1952; Davis & Heywood, 1973).

Morphology and Taxonomy

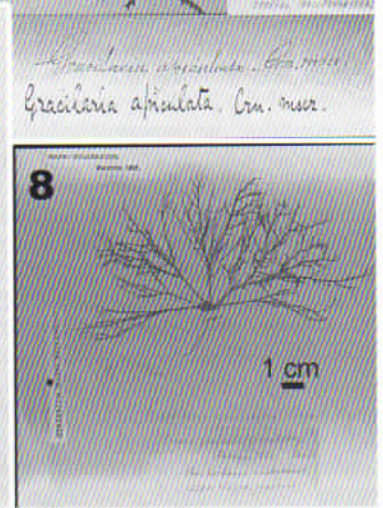
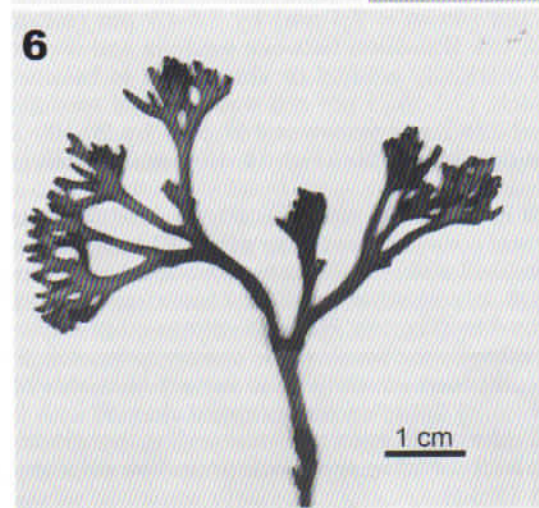
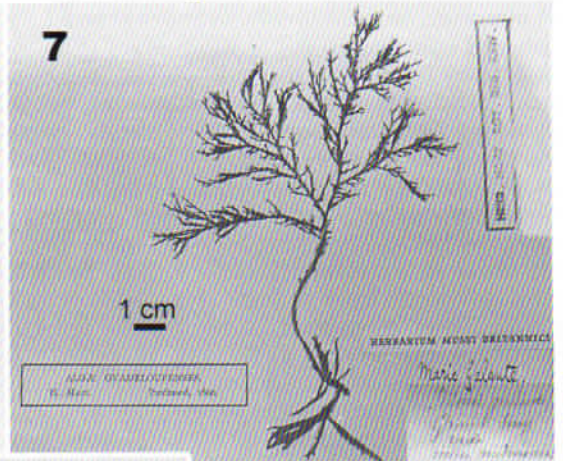
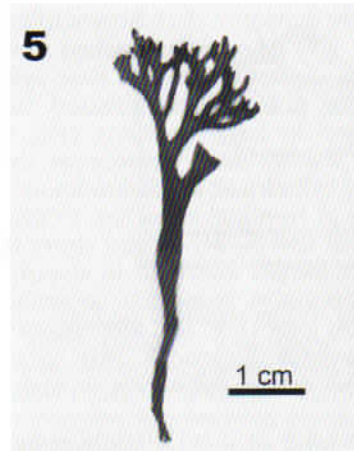
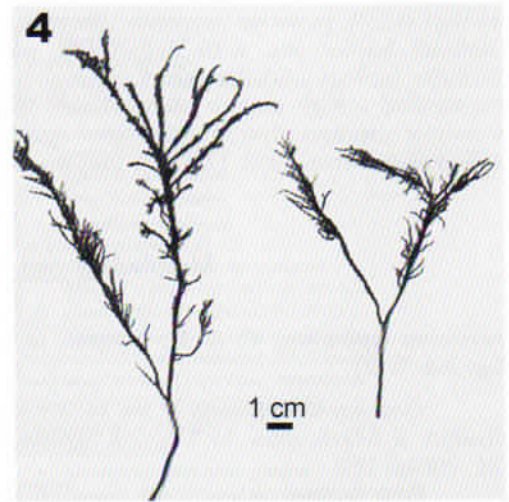
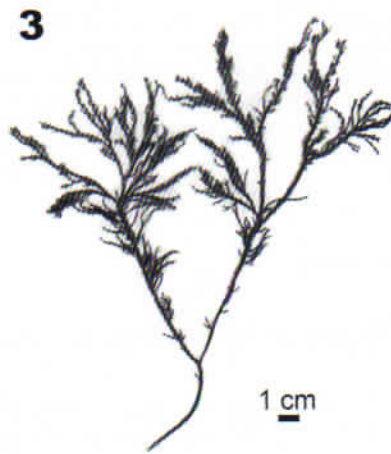
Gracilaria apiculata P. et H. Crouan in Schramm et Mazé subsp. *apiculata* (Figs 3-4, 7-17)

Gracilaria apiculata P. et H. Crouan in Schramm et Mazé, 1865: 19; Schramm & Mazé, 1866: 44; Mazé & Schramm, 1878: 216; Wynne, 1998: 29; Gurgel et al., 2004a: 162.

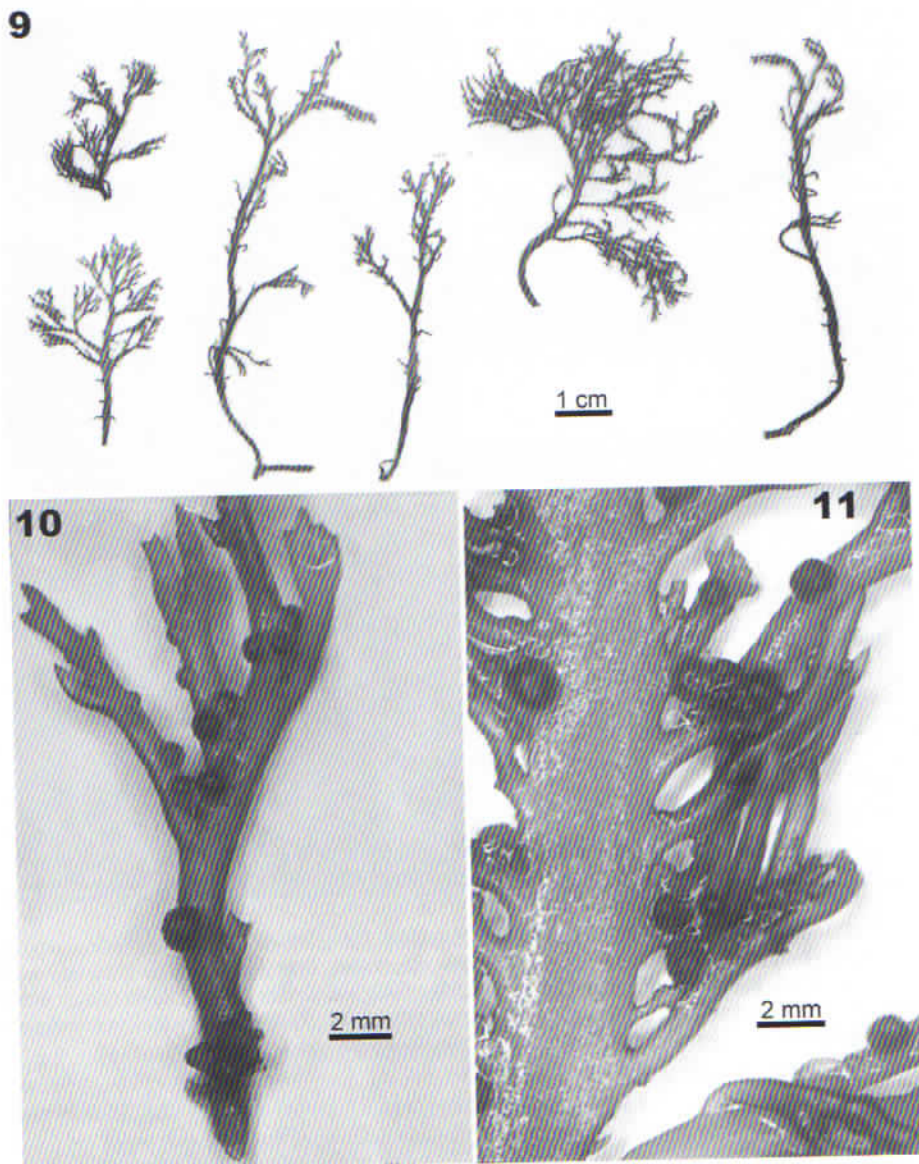
Description: Algae 5-25 cm tall, 0.5-0.6 mm thick; usually flattened, 2-4 mm wide, or occasionally cylindrical and 1.2 mm diameter; dark brown to purplish, not adhering to paper when dried (Figs 3-4, 7-9). Main axis attached to the substratum by a small discoid holdfast, 1-3 mm in diameter. Axes at base cylindrical, remaining distinct and prominent, becoming gradually compressed, then becoming flat and more slender, rarely branched in basal portions (Fig. 9). Branching up to three orders, mostly alternate, distally more proliferous than basally; branches not constricted at base, becoming cylindrical, subdichotomous to polychotomous or irregular branched near apices of terminal branches. In cylindrical specimens, branching becomes more abundant and evenly spaced above the first order of branching (Figs 3-4, 7-8). Branchlet initials alternate to opposite, spiny, not constricted at base (Figs 9-10), with uppermost branchlets sometimes oblong. Older branchlets longer, usually 0.8-4.0 cm long (Fig. 10), alternately or irregularly distributed along the margins, sometimes deciduous and secund (Fig. 9), occasionally abundant, branched up to two orders, sub-terete to slightly compressed with cylindrical bases.

Cortex composed of 1-2 cell layers; outer layer of small, slightly radially elongated, ellipsoid cells, 8.0-10.5 μm (mean = 9.5 μm) by 3.5-4.5 μm (mean = 4.0 μm); gland cells absent (Figs 14, 16). Transition between medulla and cortex is more or less gradual (*i.e.*, it is not markedly gradual or abrupt, but in between). Medulla of 6-12 cell layers, with thick, 12.5-16.25 μm cell walls; central medullary cells rounded to compressed, 125.7-174.7 μm (mean = 148 μm) by 70-100 μm (mean = 83 μm), decreasing in size towards cortex (Figs 14, 16); small intercalary medullary cells absent (Fig. 12).

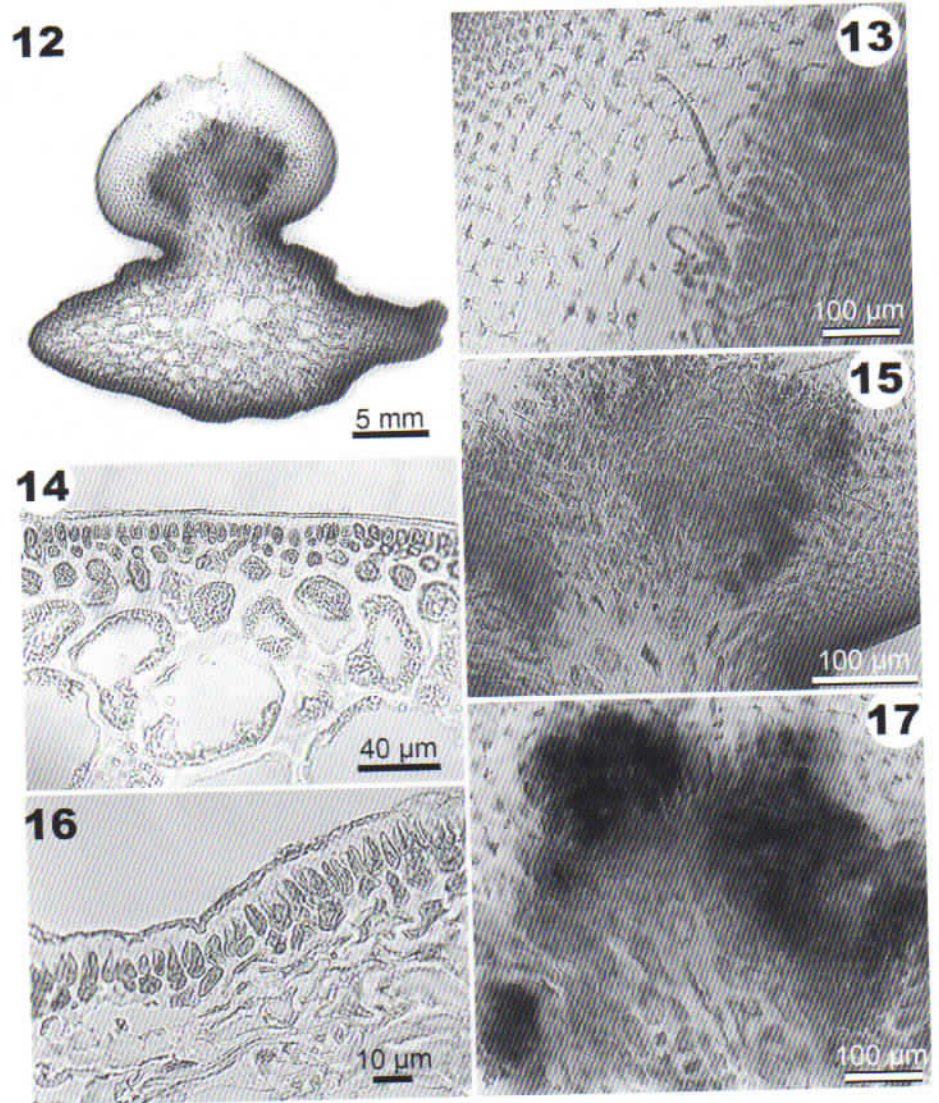
Mature cystocarps variable in shape and size (Figs 10-11), 1.0-1.6 mm tall, 1.5-2.0 mm wide, prominent, rostrate, constricted at base (Fig. 12), ampulliform; concentrated at upper part of thallus, isolated or in clusters, sometimes up to three partially fused but each retaining their respective ostioles; absent on main axes except at the very base of some branches (Fig. 11). Pericarp composed of 9-13 cell layers (Fig. 13); pericarp cells closest to gonimoblasts at the cystocarp base slightly radially elongated. Gonimoblasts composed of compact branched filaments of a few large slightly elongated cells, forming dense carposporangial clusters with terminal carposporangia (Fig. 17). Fusion cell enlarged, resembling neighboring expanded gonimoblast cells. Basal, lateral and upper tubular nutritive cells abundant in mature cystocarps (Figs 13, 15, 17).



Figs 3-8. *Gracilaria apiculata*. 3-6: Phenotypic variation of the habit, population from Venezuela. 3-4: *G. apiculata* subsp. *apiculata*, #FG-20 (#US Alg. Coll.-208961). 5-6: *G. apiculata* subsp. *candelabriformis*, (5) Isotype (#LAF-7-14-99-02-01), (6) Holotype, #FG-34 (#US Alg. Coll.-208952). 7-8: Lectotype specimens of *G. apiculata* subsp. *apiculata* (BM!).



Figs 9-11. *Gracilaria apiculata* subsp. *apiculata* from México (#FG-149). 9: Phenotypic variation of the habit. 10: Cystocarp position on terminal cylindrical branches. 11: Cystocarp position on flattened main axis and marginal cylindrical branchlets.



Figs 12-17. *Gracilaria apiculata* subsp. *apiculata*: Transverse sections. **12**: Mature cystocarp. **13**: Pericarpic cells, and presence of tubular nutritive cells connecting gonimoblasts to upper pericarpic cells. **14**: A more or less gradual transition between the cortex and medulla (*i.e.*, it is not clearly gradual or abrupt) in main axis. **15**: Cystocarp base. **16**: Radially elongated cortical cells. **17**: Gonimoblast arrangement forming clusters of carposporangia.

Lectotype: BM!

Type locality: Grand-Bourg, Marie-Galante, Guadeloupe, French West Indies.

Distribution: México (southwestern Gulf of Mexico); Guadeloupe; Venezuela.

Habitat: Intertidal to shallow subtidal on rocky substratum; in Venezuela only found in wave exposed sites.

Specimens studied: *México* - Estado Tamaulipas: Laguna Madre, Punta Piedras, Ciudad Modero, Gulf of Mexico, coll. R. M. López-Bautista, #FG-149, 31 Jan 1992 (#LAF-01-31-92). *Venezuela* - Estado Falcón: La Vela de Coro, coll. C.F.D. Gurgel, J.E. Conde & C.S. Carmona, #FG-10 (cystocarpic), 13 Jul 1999, (#US Alg. Coll.-208960; #LAF-7-13-99-01-10); La Encrucijada, Peninsula Paraguaná, coll. C.F.D. Gurgel, J.E. Conde & C.S. Carmona, #FG-20, 13 Jul 1999 (#US Alg. Coll.-208959; #US Alg. Coll.-208961; #LAF-7-13-99-02-03); and Playa Barranquita, coll. C.F.D. Gurgel, J.E. Conde & C.S. Carmona, #FG-35, 14 Jul 1999 (#LAF-7-14-99-01-02).

Remarks: Male reproductive structures have not yet been described for *G. apiculata* and therefore cannot be used to help distinguish taxa at this time. *Gracilaria apiculata* subsp. *apiculata* from Venezuela (La Encrucijada, La Vela de Coro and Playa Barranquita, Estado Falcón) were 10-25 cm tall, with main axes 2-4 mm wide, whereas specimens from Ciudad Modero (Tamaulipas, Gulf of Mexico) were shorter, 4-8 cm long and more slender, with main axes 1.2 mm wide.

***Gracilaria apiculata* subsp. *candelabrifformis* Gurgel, Fredericq et J. Norris subsp. nov. (Figs 5-6)**

Latin diagnosis: *Algae breves lataeque, usque ad 1.5-4 cm longae, usque ad 4 mm latae, ramosae usque ad 6 (-8) ordines obscure rubrae ad flavidae; cartilagineae, crassulaceoideae, adhaerentes bene ad chartam ubi siccae. Hapteron parvum, discoideum, ferens stipitem usque ad 1 cm long. Compressescentem ad complanatescentem sursum subtentum ramos subdichotomos ad alternos. Ramificatio plano unico, non constricta basi, curvata, interdum palmate, densa apicaliter ubi thallus complanatus, subdichotomus usque ad polychotomus prope apicem. Cystocarpia supra ordinem ramificationis secundum quartumque. Tetra-sporophyti et thalli masculini non visi.*

Description: Algae short and broad, up to 5.5 cm long, up to 4 mm wide, branched up to 6 (-8) orders, dull red to yellowish; cartilaginous, crassulaceous; adhering well to paper when dried. A small, discoid, holdfast bears a cylindrical stalk, up to 1 cm long, becoming compressed to flattened upwards and subtending sub-dichotomous to alternate branches. Branching in one plane, not constricted at base, curved, sometimes palmate, concentrated at apex where thallus is flattened, subdichotomous to polychotomous closer to tips. Cystocarps present above the 2nd and 4th order of branching. Tetrasporophytes and spermatangial thalli not observed.

Epithet: The subspecies name, *candelabrifformis*, is from "*candelabrum*" (L.) (Brown 1956: 182) and refers to the shape of the thallus which resembles a candelabrum (a long stalk distally bearing branches pointing upwards in one plane bearing several branchlets terminally).

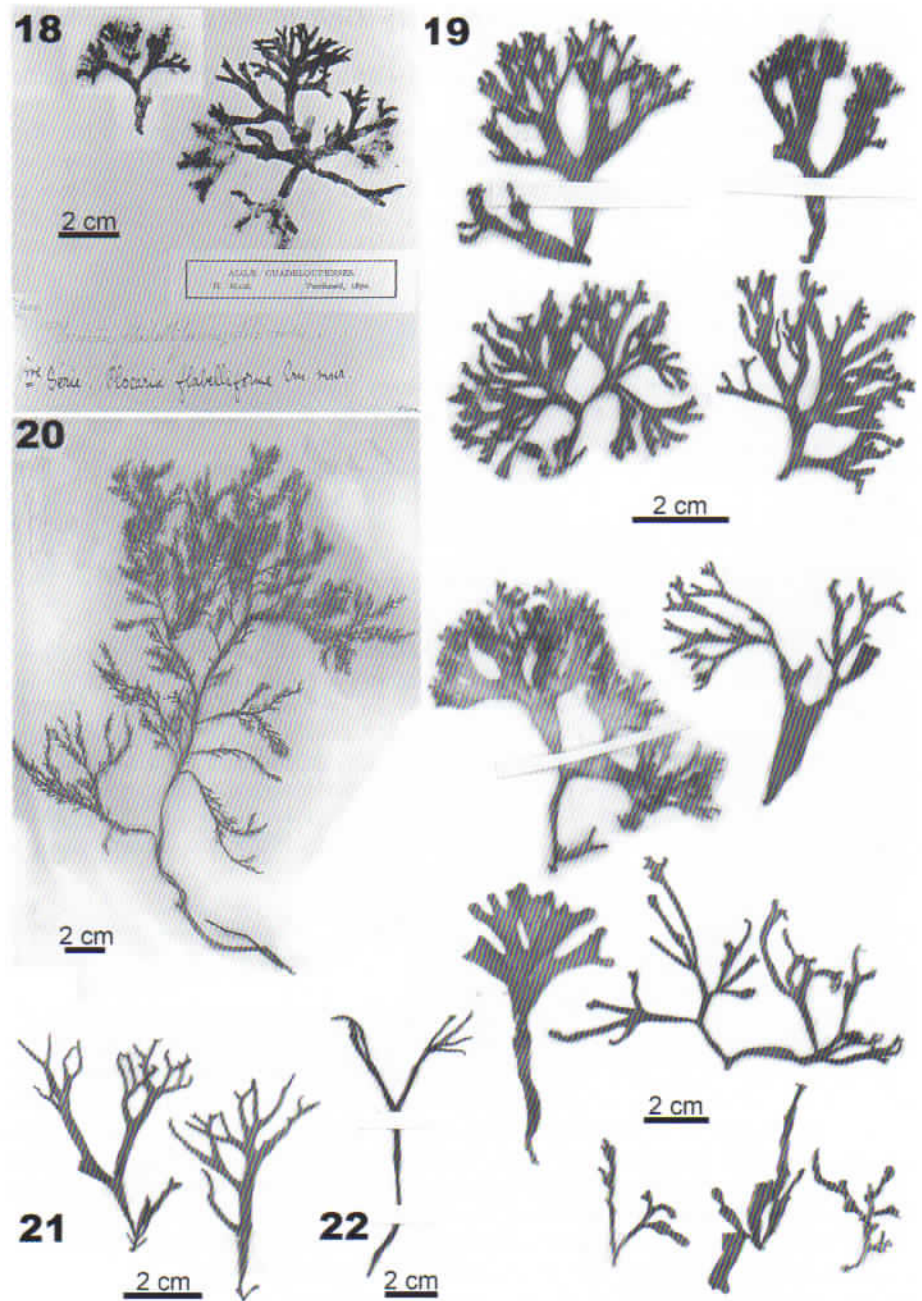
Holotype: #US Alg. Coll.-208952: coll. C.F.D. Gurgel, J.E. Conde and C.S. Carmona, #FG-34 (cystocarpic), 14 Jul 1999; GenBank *rbcL* sequence = AY049341.

Isotypes: #LAF-7-14-99-02-01 (5 cystocarpic specimens).

Type Locality: Playa Barranquita, Estado Falcón, Venezuela.

Distribution: Venezuela.

Remarks: *Gracilaria apiculata* subsp. *candelabrifformis* can be easily distinguished from *G. apiculata* subsp. *apiculata* by its more stout and flattened thallus, and denser branching concentrated at the distal region of the thallus (Figs 5-6).



Figs 18-22. *Gracilaria flabelliformis* subsp. *flabelliformis*. **18**: Lectotype specimens (BM!). **19**: Range of phenotypic variation; habit of typical specimen matching the lectotype (upper six specimens, #FG-67). **20**: Holotype of *G. flabelliformis* subsp. *aionana* (#US Alg. Coll.-208953). **21**: Paratypes of *G. flabelliformis* subsp. *simplex* from México (#US Alg. Coll.-208955). **22**: Holotype of *G. flabelliformis* subsp. *simplex* from Venezuela (#US Alg. Coll.-208954).

***Gracilaria flabelliformis* (P. et H. Crouan in Mazé et Schramm) Fredericq et Gurgel in Gurgel et Fredericq subsp. *flabelliformis* (Figs 18-19, 25-28)**

Plocaria flabelliformis P. et H. Crouan in Schramm et Mazé, 1865: 21, as '*flabelliforme*'; Schramm & Mazé, 1866: 48; Mazé & Schramm, 1878: 224; Taylor, 1960: 450, as 'uncertain record'.

Gracilaria flabelliformis (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel in Gurgel et Fredericq, 2004: 155, fig. 2(D), as '*flabelliforme*'; Gurgel et al., 2004a: 162.

Sphaerococcus lacinulatus Kützinger, 1868: 30, pl. 87.

Gracilaria lacinulata (Kützinger) Piccone, 1886: 71 [non *G. lacinulata* (Vahl) Howe, 1920: 562; non *G. lacinulata* sensu Ortega, Godínez & Garduño-Solórzano, 2001: 124, as '*G. lacinulata* (Crouan' & Crouan) Piccone'].

Description: Algae 5-7 cm tall, 2-8 mm wide, 1.7-2.0 mm thick; composed of one axis to several axes arising from single holdfast. Main axis arising from a small discoid holdfast; basally a short cylindrical stipe, 2.0-20 mm long, 1 mm diameter; distally becoming compressed to flattened, strap-like, crassulaceous. Main axes not distinct beyond first or second order of branching. Branching pattern extremely variable, irregular, di-, tri-, poly-, and/or sub-dichotomous, sometimes opposite; concentrated at top, always in the same plane at the margins (Figs 18-19).

Cortex of 1-2 (-3) cell layers, outermost cells slightly radially elongated, 3.75-6.25 μm (mean = 5.2 μm) by 5.0-8.1 μm (mean = 7.5 μm), with a gradual transition towards the medulla (Figs 25-28); hair cells present (Fig. 28). Basal portion of main thallus producing several layers of squarish cells; cells mostly radially elongated, forming a cortex up to 21 cell layers thick. Medulla of 10-15 cell layers with medullary cells sizes and organization that resembles the microcystidiate condition (*sensu* Agardh 1901: 42, 52) (Figs 25, 27); medullary cells small, ellipsoid, 124.75-184.63 μm (mean = 157.2 μm) by 174.65-299.4 μm (mean=223.05 μm), with conspicuous cell walls (Figs 26-27).

Cystocarps prominent, constricted at base when mature; pericarp 350 μm thick, inner pericarp cells star-shaped (Fig. 29). Carposporophyte broad-based and pentagonal in longitudinal section. Floor of cystocarp composed of 4-6 layers of small darkly staining cells. Gonimoblasts composed of large, distinct radially elongated, thick-walled cells. Fusion cell often indiscernible from rest of gonimoblasts at mature stages of cystocarp development. Gonimoblast cells even-sized, forming small carposporangia filling the cystocarp cavity (Fig. 30). Presence of linear to ramified tubular nutritive cells connecting the gonimoblasts to the inner pericarpic cells and also to the cells of the floor of the cystocarp that are closer to the pericarp (Fig. 31). Tetrasporangia of variable size, cruciate to decussate-cruciate, immersed in an extended cortex, 40-47 μm thick. Tetrasporangial cortex composed of 3-5 cortical cell layers of radially elongated cells, with a lax organization. Sorus formation not evident; tetrasporangia on both sides of the blades (Fig. 32). Spermatangial thalli not observed.

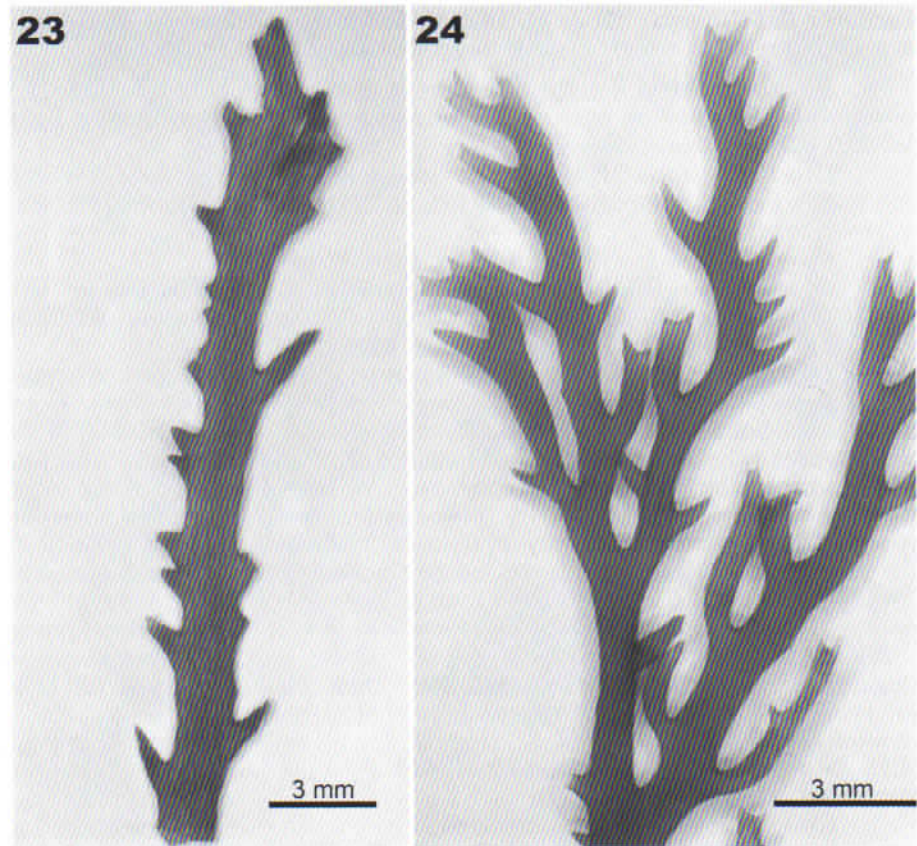
Lectotype: BM! (= "Coll. no 57, 1^{re} Serié" in Mazé & Schramm, 1878: 224; note: this is the first selection of a lectotype specimen for this taxon that we are aware of).

Type Locality: Vieux-Bourg, Moule, Guadeloupe, French West Indies.

Distribution: Florida; Guadeloupe; Venezuela; Brazil.

Habitat: intertidal to shallow subtidal; growing on rubble and on rocky shores, also growing in *Thalassia testudinum* beds.

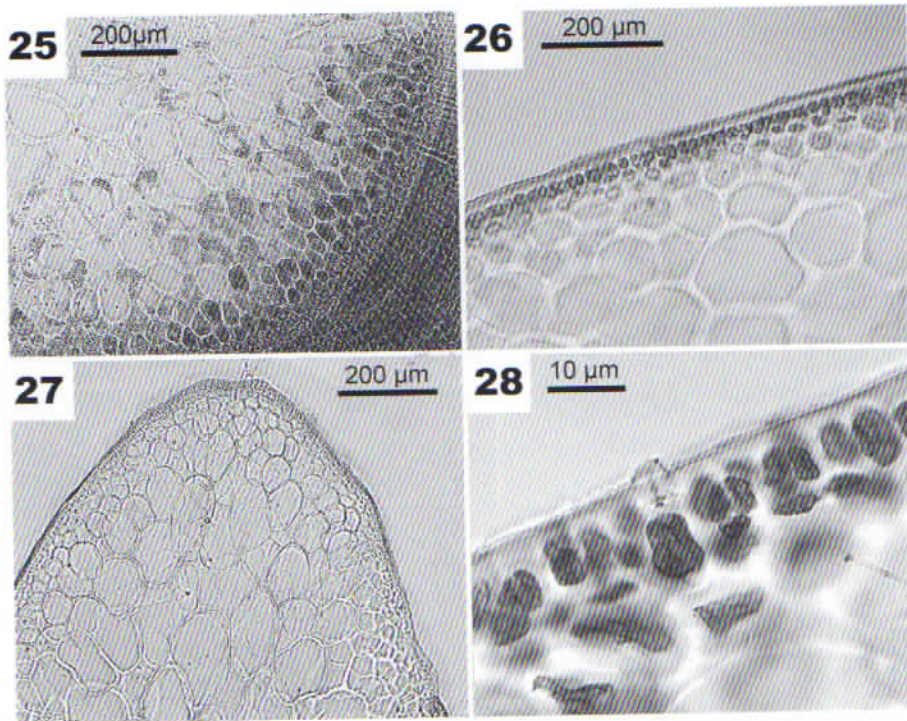
Specimens studied: USA - Florida: Walton Rocks [27°20'17"N; 80°13'59"W], Saint Lucie Co., coll. S. Fredericq, J.N. Norris & C.F.D. Gurgel, *s.n.*,



Figs 23-24. *Gracilaria flabelliformis* subsp. *aionana*: showing characteristic spine-like branchlets along thallus margin, and also (24) the subdichotomous branching pattern of upper branches.

11 Apr 1998 (#LAF-04-11-98-01-01-02), and coll. C.F.D. Gurgel, *s.n.* (cystocarpic), 03 Jul 1998 (#LAF-07-03-98-01-01); and Riomar reefs [27°38'40"N; 80°21'08"W], North Hutchinson Is., Vero Beach Co., coll. C.F.D. Gurgel, *s.n.*, 07 Jul 1998 (#LAF-07-07-98-01). **Venezuela** - Estado Falcón: Playa Barranquita, coll. C.F.D. Gurgel, J.E. Conde & C.S. Carmona, *s.n.*, 14 Jul 1999 (#LAF-07-14-99-01); Buchuaco, in *Thalassia testudinum* beds [11° 59.21'N; 69° 49.16'W], Peninsula Paraguaná, coll. C.F.D. Gurgel, J.E. Conde & C.S. Carmona, #FG-22, 13 Jul 1999 (#US Alg. Coll.-208958; #LAF-07-13-99-03-01); and Isla Margarita, La Restinga, coll. D.F. Kapraun, #FG-67, 21 May 1998 (#US Alg. Coll.-208956; #US Alg. Coll.-208957; #LAF-5-21-98-1). **Brazil** - Estado da Bahia: Arakakai, coll. J.M.C. Nunes, *s.n.*, 09 Mar 2001 (#ALCB-57517; #ALCB-57530); and Mucugê, Arraial d'Ajuda, coll. J.M.C. Nunes, *s.n.*, 12 Mar 2001 (#ALCB-57521).

Remarks: Since the generic names *Gracilaria* and *Plocaria* are feminine, the correct ending of the species name should be *-is*, and the spelling is therefore corrected to '*flabelliformis*' (Greuter *et al.*, 2000, *ICBN*: Art. 23.5).



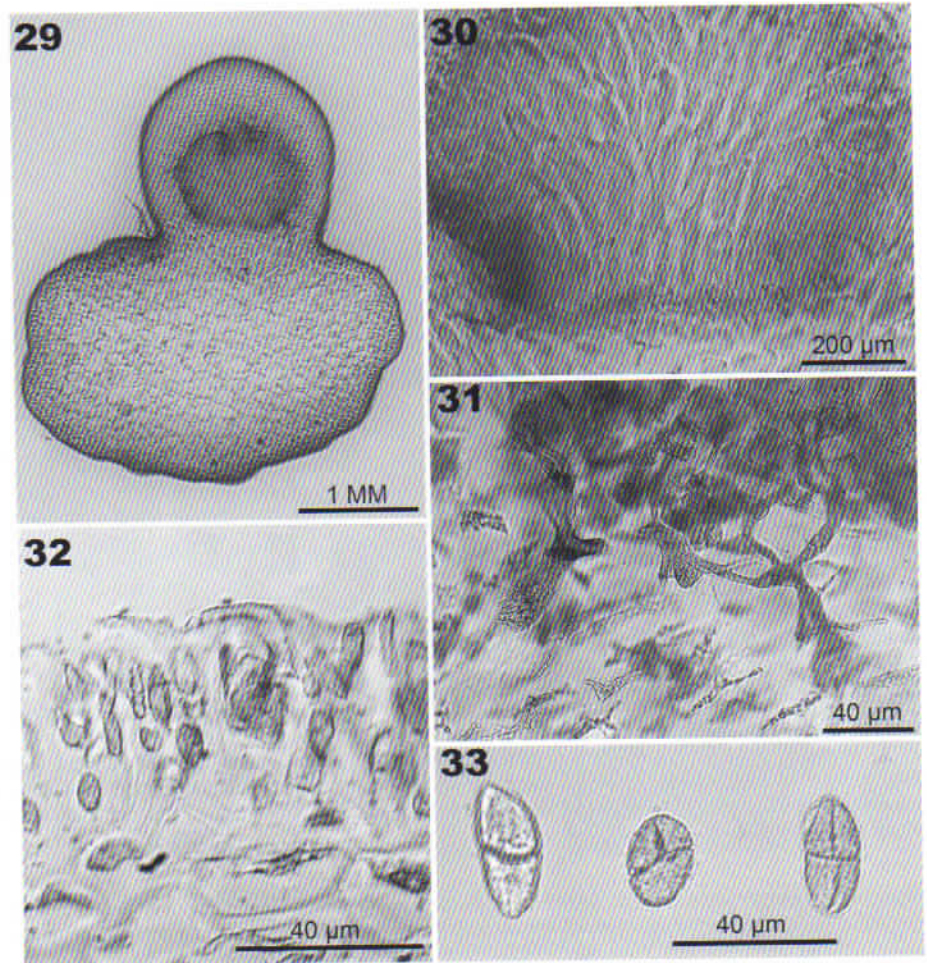
Figs 25-28. *Gracilaria flabelliformis* subsp. *flabelliformis*: Transverse sections. **25**: Cortical development close to base of main axis. **26**: Gradual cortex to medulla transition at terminal region of the thallus. **27**: Medulla composed of thick-walled, more or less uniformly sized cells. **28**: Cortex with hair cell.

Our studies showed *Sphaerococcus lacinulatus* Kützing (1868; basionym of *Gracilaria lacinulata* (Kützing) Piccone 1886) to be a synonym of *G. flabelliformis*. The illustration of the habit by Kützing (1868: pl. 87, fig. a) agrees with the phenotypic variation of *G. flabelliformis* (cf. Fig. 19), as does the cystocarp illustrated by Kützing (1868: pl. 87, fig. d) which is identical to that found in our specimens of *G. flabelliformis* (cf. Figs 29-30).

Among the specimens studied in the molecular-based phylogenies, three distinct phenotypes recognized were found to comprise the same species. Two of these were distinct from *G. flabelliformis* subsp. *flabelliformis*, and we conclude, based on morphological and molecular analyses, that they are new subspecies, and accordingly describe them below.

***Gracilaria flabelliformis* subsp. *aionana* Gurgel, Fredericq et J. Norris subsp. nov.** (Figs 20, 23-24, 29-33)

Latin diagnosis: *Thalli usque ad 30 cm altae, axis principalis 1.0-2.2 mm altus, compressus omnino; ramificatio plano unico, usque ad 5-6 ordines; subdichotoma aut alterna; rami sparsim distributa secus axem principalem super hapteron parvum. Axis principalis indistinctus ultra ordinem secundum ramificationis. Ramificatio prolifer, distributa aequaliter secus thallum, leviter constricta basi, ad initium ultra*



Figs 29-33. *Gracilaria flabelliformis* subsp. *aionana*. 29-32: Transverse sections. **29**: Mature cystocarp and medullary organization. **30**: Carposporophyte, long internal gonimoblast cells with thick cell walls, and small gametophytic cells at floor of cystocarp. **31**: Ramified tubular nutritive cells at base of carposporophyte; scale bar = 40 µm **32**: Tetrasporic cortex. **33**: Released tetrasporangia with various zonation patterns, bisporangia, decussate, cruciate.

partem medium thalli; rami terminantes in bifurcationem subdichotomum. Ramuli terminales restricti ad margines, parvi, spiniformes, abundantes, divisi alterni ad oppositi in plano unico obtegentes paginam integram marginum solum, emittentes aspectum dentatum exiguum.

Description: Algae slender and up to 30 cm tall, main axis 1.0-2.2 mm wide, compressed throughout; branching only in one plane, up to 5-6 orders, subdichotomous or alternate; branches sparsely distributed along main axis above a small holdfast. Main axis indistinct beyond second order of branching. Branching proliferous, evenly distributed along thallus, slightly constricted at base, starting

beyond middle portion of thallus; branches terminating in subdichotomous bifurcation (Fig. 20). Terminal branchlets restricted to the margins, small, spine-like, abundant, alternately to oppositely divided in one plane and covering the entire side of the margins only, giving a slight dentate appearance (Figs 23-24).

Epithet: In celebration for her 85th birthday, we name this taxon to honor Dr. Isabella Aiona Abbott (University of Hawaii at Manoa). We choose *aionana*, for her Hawaiianized-Chinese name, Aiona, and wish to express our appreciation for her extensive phycological contributions, including marine floras of Hawai'i (Abbott, 1999; Abbott & Huisman, 2004) and California (Abbott & Hollenberg, 1976), and her contributions to Hawaiian ethnobotany and uses of seaweeds (e.g., Abbott, 1992, 1996).

Holotype: #US Alg. Coll.-208953 (not fixed in Formalin), coll. C.F.D. Gurgel, J.E. Conde and C.S. Carmona, #FG-29, 13 Jul 1999; GenBank *rbcL* sequence = AY049337. **Isotypes:** #LAF-07-13-99-04-02 (5 herbarium sheets, & specimens liquid-preserved in Formalin).

Type Locality: Puerto Escondido, Península Paraguaná, Estado Falcón, Venezuela.

Distribution: Estado Falcón, Venezuela.

Habitat: Low intertidal, protected from wave exposure.

***Gracilaria flabelliformis* subsp. *simplex*, Gurgel, Fredericq et J. Norris subsp. nov. (Figs 21-22)**

Latin diagnosis: *Thalli compressi aut complanati omnino plus subtilesque, breviores minus quam G. subsp. flabelliformis; usque ad 10 cm alti, 1-4 mm lati, compositi axium principalium indistinctorum angustorum; ramificatio subdichotoma in plano unico solum, exorientium parvo haptero. Rami sparsi secus axem principalem, ramuli aut spinae dentiformes absentes secus margines. Apices terminantes gradatim acute.*

Description: Algae compressed or flattened throughout and more delicate, smaller and less wide than *G. flabelliformis* subsp. *flabelliformis*; up to 10 cm tall and 1-4 mm wide, composed of indistinct narrow main axes, branching subdichotomous only in one plane, arising from small holdfast. Branches sparse along main axis, branchlets or teeth-like spines along margins absent. Apices terminating in gradually sharper tips.

Epithet: The species name, *simplex* (L.) (Brown 1956), refers to the simple habit composed of only sub-dichotomously branched flat axes with branches gradually tapering toward the apices (Figs 21-22).

Holotype: #US Alg. Coll.-208954 (not fixed in Formalin), coll. C.F.D. Gurgel, J.E. Conde and C.S. Carmona, #FG-36, 14 Jul 1999 (Fig. 22); GenBank *rbcL* sequence = AY049343. **Isotypes:** #LAF-07-14-99-01-03 (13 specimens on 4 sheets).

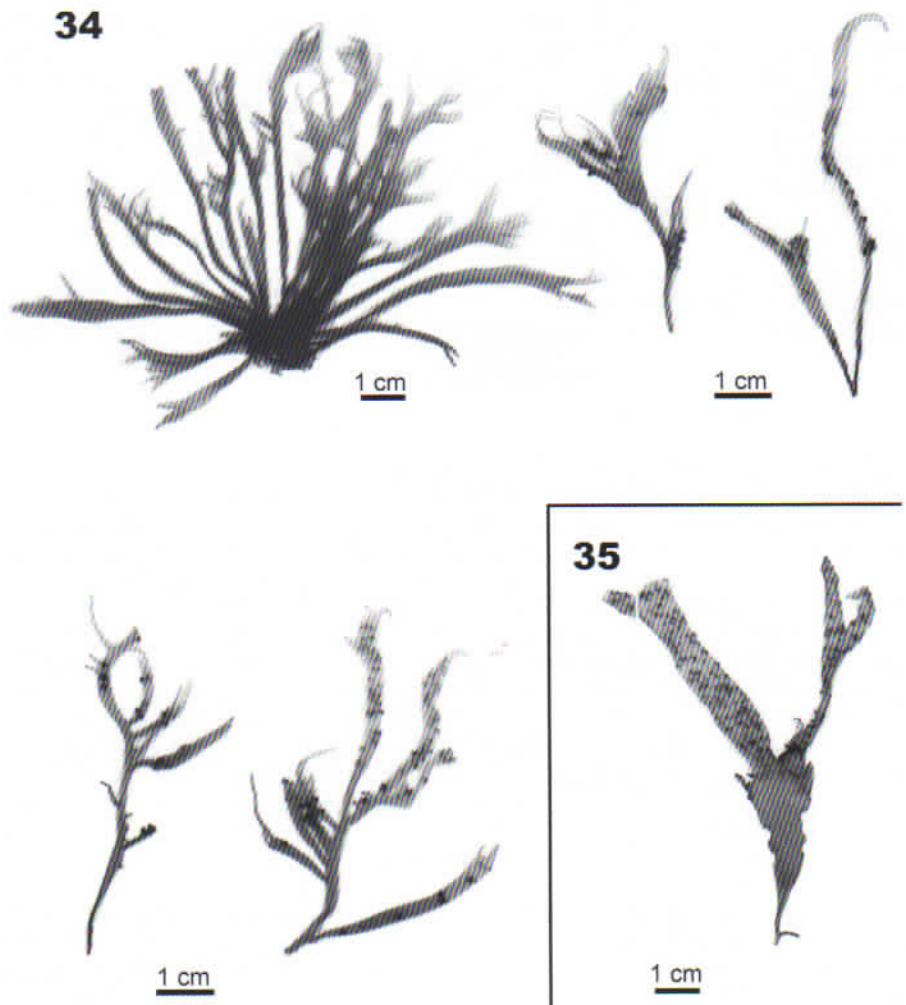
Type Locality: Playa Barranquita, Estado Falcón, Venezuela.

Distribution: México (southwestern Gulf of Mexico); Venezuela; Brazil.

Habitat: Intertidal to shallow subtidal in both, protected and moderately exposed environments.

Paratypes studied: **México** – Campeche Bay, Champoton area [19° 17.67'N; 90° 46.26' W], Estado Campeche, Gulf of Mexico, coll. C.F.D. Gurgel, #FG-119, 14 Feb 1999 (#US Alg. Coll.-208955, two specimens; Fig. 21). **Brazil** – Estado da Bahia, Itacimirim, coll. C.F.D. Gurgel, *s.n.*, 05 Nov 2002 (#LAF-05-11-02-02-03, 5 specimens on single sheet).

Remarks: *Gracilaria flabelliformis* subsp. *simplex* is characterized by the smaller size and width of its strap-like axes, and sparse, sub-dichotomous branching in one plane (Figs 21-22).

Gracilaria isabellana* Gurgel, Fredericq et J. Norris nom. nov. (Figs 34-42)Fucus lacinulatus* Vahl, 1802: 39.*Gracilaria lacinulata* (Vahl) Howe in Britton et Millspaugh, 1920 [26 June]: 562 [non *G. lacinulata* (Kützinger) Piccone, 1886: 71].*Gracilaria lacinulata* (Vahl) Børgesen, 1920 [23 October]: 379, Nr. [fig.] 366 [non *G. lacinulata* (Kützinger) Piccone, 1886:71].

Figs 34-35. *Gracilaria isabellana*. 34-35: Liquid-preserved specimens from Venezuela showing phenotypic variation. 35: Habit of a typical specimen that closely resembling the lectotype specimen of *Fucus lacinulatus* Vahl (C!).

G. lacinulata sensu Ortega, Godínez & Garduño-Solórzano, 2001: 124, as '*G. lacinulata* (Crouan & Crouan) Piccone' [non *G. lacinulata* (Kützinger) Piccone, 1886: 71].

Gracilaria foliifera sensu Taylor 1960:446 (*pro parte*); Ortega, Godínez & Garduño-Solórzano, 2001: 124 [non *G. foliifera* (Forsskål) Børgesen, 1932: 7].

Description: Algae erect, composed of compressed, linear, oblong or lanceolate main axes and branches; displaying a wide range morphological variation in size and apex shape; up to 12 cm long, up to 1.8 cm broad, and up to 1.4 mm thick; varying in color from light-green to yellow-green and olive-green to dark brown; main axis narrow at base, forming a very short, ± terete stipe above a small round cushion-shaped holdfast. Thalli simple or irregular, di- to trichotomously branched in one plane; blades sometimes twisted. Branchlets simple, arising from margins only, irregularly distributed, occasionally secund; usually concentrated at top of major axes; sometimes compressed at base; with rounded, sometimes bent, apices. Cortex 1-2 cell layers thick; cortical cells of variable size, mostly anticlinally elongated, 12.5-31.25 µm (mean = 21.0 µm) by 7.5-18.5 µm (mean = 13.0 µm). Hair cells and gland cells rare. Abrupt transition from a central medullary layer of 3(-5) large, thick-walled medullary cells, 197.6–516.0 µm (mean = 328.7 µm) by 101.3–247.0 µm (mean = 175.4 µm) to an outer cortical layer of 1-2 small cortical cells (Figs 41-42); medullary cells compressed in same plane as the thallus, and rich in floridean starch.

Cystocarps conspicuous, protruding on both sides of blade, spherical, dark-purple to black, constricted at base; in longitudinal section 675.0–872.0 µm (mean = 780.5 µm) tall by 1.0–1.075 mm (mean=1.041 mm) diameter. Pericarp

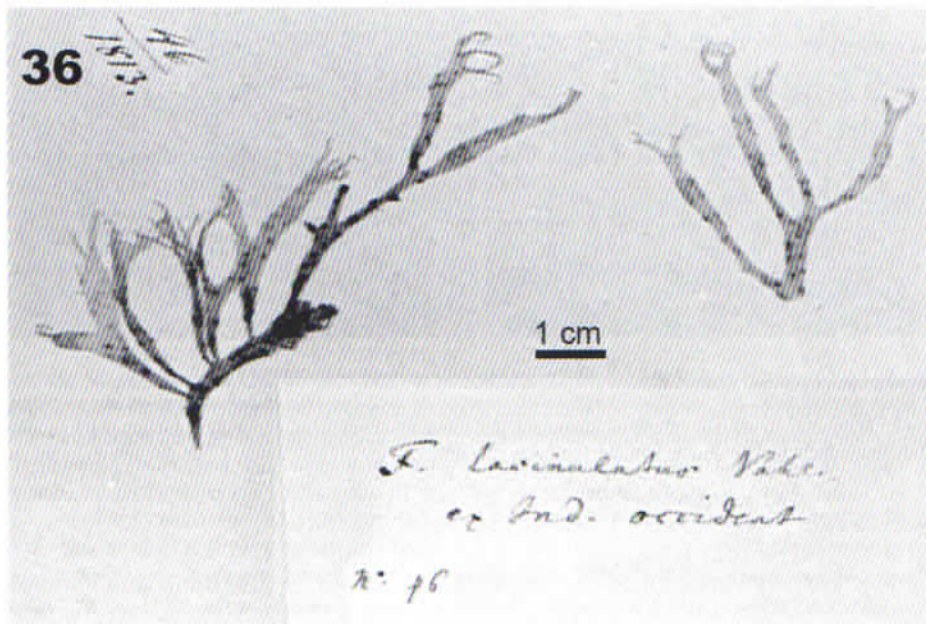
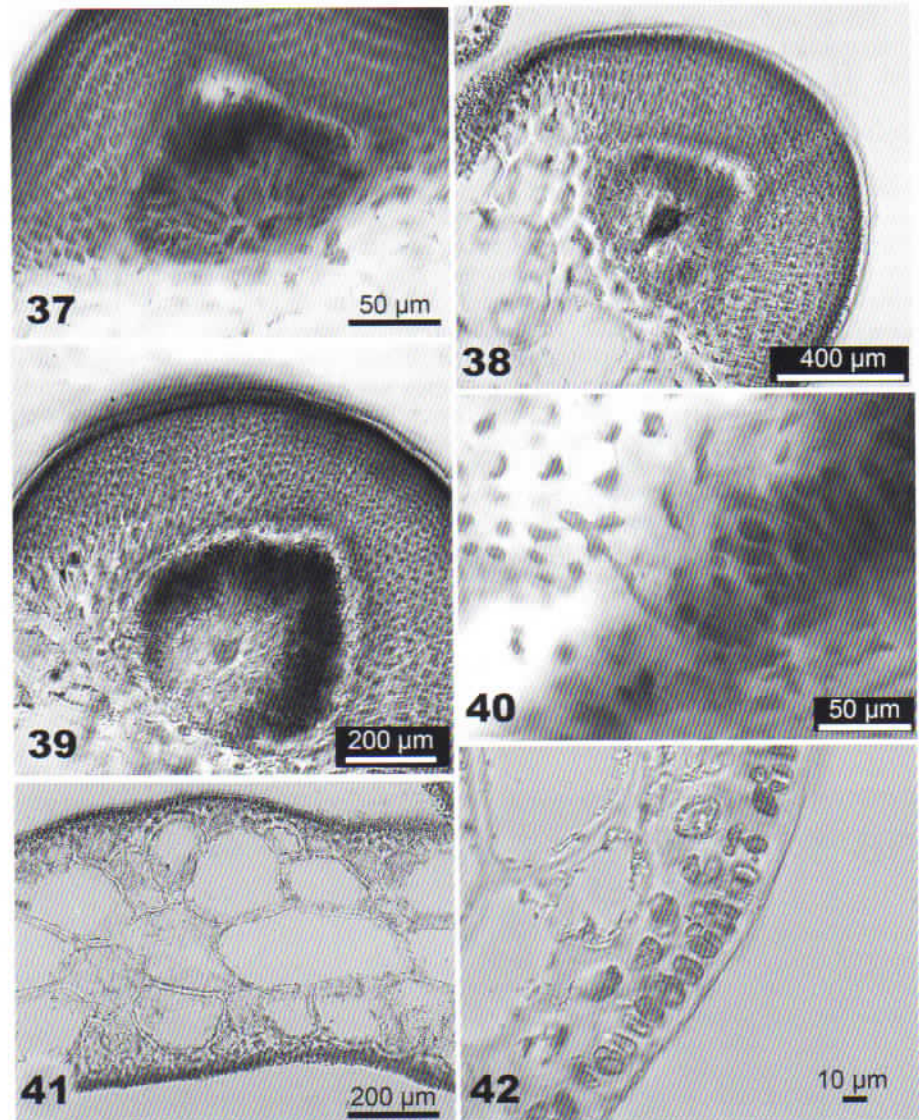


Fig. 36. *Fucus lacinulatus* Vahl. 36: Specimens in Vahl Herbarium (C!).



Figs 37-42. *Gracilaria isabellana*. **37-40**: Transections of cystocarps, (**37-39**) showing the conspicuous globose fusion cell. **40**: Tubular nutritive filaments connecting the gonimoblast to the pericarp. **41-42**: Transection of the thallus, showing the abrupt transition from the large, thick-walled medullary cells to the much smaller cells of the cortex.

175 μm thick, composed of (9-) 10-13 cell layers (Figs 37-39). Inner gonimoblasts composed of large, radially elongated cells around a conspicuous fusion cell (Figs 37-39), 117.5 μm by 37.50 μm. Nutritive tubular cells present (Fig. 40). Spermatangia and tetrasporangia not found.

Epithet: We chose the name *isabellana*, after Isabella, the given name of Isabella Aiona Abbott. On this occasion, we wish to recognize her contributions to our understanding of the taxonomy of economic seaweeds, particularly *Gracilaria*, and her encouragement to others to help elucidate this important genus.

Lectotype: *Fucus lacinulatus* Vahl: C! (= Børgesen, 1920: 379, Nr. [fig.] 366).

Type Locality: St. Croix, U.S. Virgin Islands.

Distribution: U.S. Virgin Islands; Venezuela.

Habitat: Low intertidal to shallow subtidal; growing on rubble and on rocky shores, often in wave-exposed habitats.

Specimens studied: *U.S. Virgin Islands* – “Ind. Occident.” [probably St. Croix], no. 46, dated: 1813 (topotype; C!). *Venezuela* – La Encrucijada, Península Paraguaná, Estado Falcón, coll. C.F.D. Gurgel, J.E. Conde and C.S. Carmona, #FG-19, 13 Jul 1999; GenBank *rbcL* sequence = AY049332 (#US Alg. Coll.-208966; #LAF-7-13-99-1); Playa Barranquilla, Estado Falcón, coll. C.F.D. Gurgel, s.n., GenBank *rbcL* sequence = AY049344, 14 Jul 1999 (#US Alg. Coll.-208967; #LAF-7-14-99-2).

Remarks: Ortega, Godínez & Garduño-Solórzano (2001: 124, as “*Gracilaria lacinulata* (Crouan et Crouan) Piccone”) noted *G. lacinulata* (Kützting) Piccone (1886) is the earliest use of the name, “*lacinulata*”, in the genus *Gracilaria*. Therefore *G. lacinulata* (Vahl) Howe (1920) is a later homonym (Greuter *et al.*, 2000, ICBN: Art. 53.1). Taxonomically and molecularly distinct (Gurgel *et al.*, 2004a: 162, tabl. 1, fig. 1; Gurgel *et al.*, 2004b: 2, tabl. 1, fig. 3, as ‘*G. lacinulata*’), this species is given a replacement name (Greuter *et al.*, 2000, ICBN: Arts. 7.3 & 33.3).

Although *G. isabellana* is a remarkably plastic species, specimens in Vahl’s Herbarium (C!), including the lectotype, *Fucus lacinulatus* (Børgesen, 1932), closely match the specimens recently collected in Venezuela. The distinct features of *G. isabellana* are its more or less strap-like, sometimes bifurcated main axes, with irregular branches sometimes close to the base, along the upper margins, and/or scattered on the upper portion of thallus, and its green to dark brown color.

Venezuelan specimens tentatively identified by Ganesan (1989: 85) as ‘*G. lacinulata* (Vahl) Howe Prox.’, were recently shown to be *G. intermedia* J. Agardh subsp. *ganesana* Gurgel, Fredericq *et* J. Norris (2004a: 169). In Venezuela, *G. isabellana* differs from *G. intermedia* subsp. *ganesana* in being smaller, and having an irregular, more proliferous branching pattern and variously shaped apices.

DISCUSSION

A strong geographic correlation exists among the six clades found in the *Gracilaria sensu stricto* lineage that was focused on in this study (Figs 1-2). These results are similar to what Gurgel & Fredericq (2004) found using a larger dataset. This biogeographic pattern, where distinct clades are composed of taxa from geographically isolated regions, supports the hypothesis that the Gracilariaceae dispersion, followed by local speciation, is a common pattern of evolution.

Eleven of the fifteen species in the ingroup herein studied have at least some parts of the thallus that are foliose and with spermatangial conceptacles of the *textorii*-type, at least for the ones where this reproductive character has been documented. The other four, *G. bursa-pastoris*, *G. cervicornis*, *G. sp.* from Brazil, and *G. sp.* from the Philippines, are not markedly flattened but compressed species. This most derived *Gracilaria* lineage also contains two South African sister

species, *G. beckeri* (J. Agardh) Papenfuss (1952:175) and *G. capensis* F. Schmitz ex Mazza (1907: 134). One of these was once placed in the genus *Tylopus* J. Agardh (1876: 428), as *T. capensis* (Schmitz ex Mazza) Papenfuss (1940: 220), and the other in *Tyleiophora* J. Agardh (1890: 35), as *T. beckeri* J. Agardh (1890: 36) for having nemathecial tetrasporangia. It was later observed by Dawson (1949) that several Pacific taxa displayed different degrees of tetrasporangial nemathecial formation, and *Tyleiophora* was subsequently considered to be synonym of *Gracilaria* by Papenfuss (1952). Our molecular results do not support the monophyly of *Tyleiophora*, thus supporting Papenfuss' taxonomic opinion. The phylogenetic relationship of *Gracilaria textorii* (Suringar) De Toni (1895: 27), the type species for *G.* subgen. *Textoriella* Yamamoto (1978, 1984) received little (PP = 70%, Fig. 2) to no support (BV < 50%, Fig. 1) as was also noted in global phylogenies (Gurgel & Fredericq, 2004). However, both methods placed *G. textorii* as the most basal taxon to the Indo-Pacific – South African clade (Figs 1-2).

Different species with similar habits may be found throughout the subtropics and tropics, and this has caused frequent misidentifications. For example, *Gracilaria textorii*, originally described from material collected in Japan by Suringar (1868: 259, as *Sphaerococcus textorii*), is considered the most difficult flat species of *Gracilaria* to identify due to its great morphological variation within and outside Japan (Yamamoto, 1984). Many of the forms found in Japan (Yamamoto, 1978) overlap with those reported in California (Abbott & Hollenberg, 1976), Pacific Mexico (Dawson, 1949, 1961) and the Gulf of California (Dawson, 1949; Norris, 1985: 132). Subsequently, similar looking forms have resulted in *G. textorii* being reported from several places in southeastern Asia and Australia (Millar, 1997) and Venezuela (Rodríguez de Ríos, 1986). We detected (Figs 1-2) the presence of another distinct flat species of *Gracilaria* in the Philippines that was previously identified as "*G. textorii*" (Silva *et al.*, 1987) but represents a distinct species of *Gracilaria*. Because the amount of material obtained from the Philippine species in this study was too small, a precise taxonomic identification or a reliable species description was not possible at this time. Further, when four recent collections, including specimens with a "*G. textorii*-like" appearance, from the western Atlantic were studied, none of them were phylogenetically similar to *G. textorii* from Japan, and it was concluded they represented four new species (Gurgel *et al.*, 2004a). Thus, the report of "*G. textorii*" in the Caribbean (Rodríguez de Ríos, 1986) is also questionable (Ganesan, 1989).

Gracilaria apiculata - Other than in a checklist (Wynne, 1998) and in Gurgel & Fredericq (2004; Gurgel *et al.*, 2004a), this name has not been considered for the Caribbean marine flora since the original, short but validly published, description made by the brothers, Pierre Louis Crouan and Hippolyte Marie Crouan (in Schramm & Mazé, 1865; Schramm & Mazé, 1866; Mazé & Schramm, 1878), French botanists and pharmacists. Our results recognized two consistently different phenotypes, one matching type material of *G. apiculata* (Figs 7-8) and identified using the type method. The other phenotype was determined to be a new distinct subspecies, *G. apiculata* subsp. *candelabrifformis* (Figs 5-6).

The close phylogenetic relationship based on molecular data found among *G. apiculata*, *G. cervicornis* and *G. sp.* from Brazil (Figs 1-2) is also reflected in similarities of their habit morphologies. Habit morphologies of both *G. apiculata* subsp. *apiculata* and *G. sp.* from Brazil are somewhat similar to *G. cervicornis* (Turner) J. Agardh (1852: 604), the most commonly used species name for the northwestern Atlantic Gracilariaceae flora. In fact, *G. apiculata* subsp. *apiculata* has been identified as '*G. cervicornis*' in the past (e.g. López-Bautista & Kapraun, 1995) and their entire lineage can be morphologically recognized as the

"*G. cervicornis* complex". Despite the low bootstrap values obtained for this clade in the MP result (BV = 68), this lineage is well supported in the Bayesian analysis (PP = 99%) and in a more global analysis (BV = 85%; Gurgel & Fredericq, 2004). *Gracilaria apiculata* subsp. *apiculata* and *G. sp.* from Brazil are even more morphologically similar to the concept of *G. ferox* J. Agardh (1852: 592), which has been considered a synonym of *G. cervicornis* (Oliveira *et al.*, 1983). Taxonomic problems surrounding similar looking forms of *G. cervicornis*, *G. ferox*, and *G. domingensis* Sonder ex Kützinger (1869: 8) were addressed by Oliveira *et al.* (1983) in which the most reliable characters used to separate the former two species from the latter are the type of spermatangial arrangement and position of cystocarps on the thallus. Oliveira *et al.* (1983) merged *G. ferox* into *G. cervicornis*, thus recognizing only two species to encompass diverse phenotypes whose species limits in the Western Atlantic had overlapping morphologies. Our results suggest that there are more taxa currently included in the "*G. cervicornis*-*G. domingensis* complex", and only a thorough molecular survey of distinct phenotypes and forms from this complex will resolve the systematics of this group. Molecular comparisons of recently collected topotype material, along with morphological comparisons with type specimens, will be critical in order to elucidate the correct name and taxonomy of each distinct taxon, especially those with similar or overlapping morphologies. Our preliminary data suggest that *G. ferox* might be resurrected to encompass the distinct taxa represented here as "*G. sp.* from Brazil" and that, based on published morphological evidences (Oliveira *et al.*, 1983), *G. domingensis* might be a member of the genus *Hydropuntia* instead of *Gracilaria*. However, all these hypotheses remain to be tested.

Gracilaria apiculata subsp. *candelabriformis*, though having a distinct stout upper thallus (Figs 5-6), could possibly be confused with some forms of *G. flabelliformis*, especially when the species occur together (sympatric), under the same environmental conditions. *Gracilaria apiculata* subsp. *candelabriformis* could in the past have been confused with the misconception of "*G. mammillaris*" (*sensu* Taylor, 1960). However, the taxonomy of *Gracilaria mammillaris* (Montagne) Howe (1918: 515) was recently elucidated with recently collected specimens (Gurgel *et al.* 2004a), and it is a deepwater species from the Gulf of Mexico and Caribbean.

***Gracilaria flabelliformis*.** *Plocaria flabelliformis* was also described by the brothers P. and H. Crouan (in Schramm & Mazé, 1865, as '*flabelliforme*') but no holotype was designated. Our results reveal that this taxon is one of the most morphologically variable species of *Gracilaria* in the tropical western Atlantic, and because of that and despite its common occurrence, *G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel has not been recognized by earlier investigators. Our taxonomic identification was based on the fact that the most common phenotype of *G. flabelliformis* subsp. *flabelliformis* (Fig. 19) matches the type material perfectly (Fig. 18). Taylor (1960: 447, as '*Plocaria flabelliforme*') placed *G. flabelliformis* and twenty-five other species names in synonymy with "*G. foliifera*", a species described for plants originally collected in the Red Sea. The name *G. foliifera* var. *angustissima* was commonly cited for the eastern coast of the USA (e.g. Taylor, 1957), until the northwestern Atlantic populations were recognized as a species, *G. tikvahiae* McLachlan (1979: 19; note: *G. foliifera* var. *angustissima* (Harvey) W. Taylor was listed as a "synonym" [basonym: *G. multipartita* var. β *angustissima* Harvey, 1853: 107]). *Gracilaria tikvahiae* is another morphologically variable species whose molecular and morphological variation has been recently investigated across its geographical range (Gurgel *et al.*, 2004c). *Gracilaria foliifera* (Forsskål) Børgesen (1932: 7) is now believed to be restricted

to the Red Sea (type locality) and nearby regions (Guiry & Freamhainn, 1986; Gargiulo *et al.*, 1992). Nevertheless, some authors still apply the name to Atlantic species of *Gracilaria* (e.g. Bellorin *et al.*, 2002, as '*G. foliifera* var. *angustissima*'). Recently, Wynne (1998, as '*Plocaria flabelliformis*') tentatively placed *G. flabelliformis* in synonymy with another poorly understood species, "*G. lacinulata*" as reported from the western Atlantic by Ganesan (1989). However recent studies showed that "*G. lacinulata*" *sensu* Ganesan (1989: 85) was a distinct taxon and it was described as *G. intermedia* subsp. *ganesana* Gurgel, Fredericq *et* J. Norris (2004a).

Together with *Gracilaria occidentalis* (Børgesen) Bodard (1965: 874) and *G. ornata* Areschoug (1854: 351), *G. flabelliformis* forms a clade with high support (BP = 88, PP = 100). Phylogenetically, *G. flabelliformis* is distinct from all species with which it has been confused, i.e. *G. mammillaris*, *G. multipartita* (Clemente) Harvey (1846: pl. 15), *G. tikvahiae* (including "*G. foliifera* var. *angustissima*"), and *G. lacinulata*. At first look, the habit of *G. flabelliformis* subsp. *aionana* appears to be somewhat morphologically similar to some morphs from the *G. cervicornis*-complex due to the profusion of branchlets arising from a distinct main axis. However, the dentate-like margin is unique to *G. flabelliformis* subsp. *aionana* and readily separates them.

***Gracilaria isabellana*.** There has been so much taxonomic and nomenclature confusion surrounding the western Atlantic "*G. lacinulata*", that its morphological and molecular distinctions and its nomenclature quagmire are elucidated herein. *Gracilaria lacinulata* (Kützing) Piccone (1886) was found to be a synonym of *G. flabelliformis*. A later homonym, *G. lacinulata* (Vahl) Howe (1920), was found to be taxonomically different from *G. lacinulata* (Kützing) Piccone (basonym: *Sphaerococcus lacinulatus* Kützing 1868). Therefore it was herein given a replacement name, *G. isabellana*, and its taxonomy and morphology described.

This study has characterized and established *G. apiculata*, *G. flabelliformis* and *G. isabellana* as common species in the tropical Western Atlantic marine flora. Despite the already large number of described *Gracilaria sensu stricto* species (*sensu* Gurgel & Fredericq, 2004), the genus is still far more species-rich than previously recognized. The general lack of distinct diagnostic characters and often overlapping species boundaries has brought much confusion to the taxonomy of the genus *Gracilaria*, and the re-circumscription of *G. apiculata* and *G. flabelliformis* laid down in this study was only possible with the guidance provided by informative molecular-based *rbcL* phylogenies. Molecular techniques have shown in this and other studies (e.g. Gurgel & Fredericq, 2004; Gurgel *et al.*, 2003a,b, 2004a,b) to help resolve many systematic questions in *Gracilaria sensu stricto* at the species level. So far, *rbcL*-based phylogenies helped to identify eight new species (Gurgel *et al.*, 2003a, b; 2004a) and five new subspecies (Gurgel *et al.*, 2004a; and herein), and to recognize that there are more than two other distinct taxa in the *G. cervicornis*-*G. domingensis* complex. The fact that *G. sp.* from Brazil with a "*G. ferox* morphology" is a distinct entity suggests that *G. ferox* may also be a discrete taxon to be resurrected. More extensive collections and further studies are needed to address these questions.

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- Appendix.** List of the species, collection location, collector(s) and date, and GenBank accession number and *rbcL* DNA sequences (percent) in this study. Author abbreviations: *FG* = Carlos F. D. Gurgel; *SF* = Suzanne Fredericq; *JN* = James N. Norris; *JC* = Jesus Eloy Conde; *CC* = Carlos Carmona-Suárez.
- G. apiculata* subsp. *apiculata* J. Agardh. La Vela de Coro, Estado Falcón, Venezuela, coll. FG, JC & CC, #FG-10, 14 Jul 1999 (AY049329, 98.1%)
- G. apiculata* subsp. *apiculata* J. Agardh. La Encrucijada, Peninsula Paraguana, Estado Falcón, Venezuela, coll. FG, JC & CC, #FG-20, 13 Jul 1999 (AY049333, 98.1%)
- G. apiculata* subsp. *apiculata* J. Agardh. Laguna Madre, Punta Piedras, Ciudad Modero, Estado Tamaulipas, México, coll. J. Lopez-Bautista, #FG-149, 31 Jan 1992 (AY049352, 98.6%)
- G. apiculata* subsp. *apiculata* J. Agardh. Playa Barranquita, Estado Falcón, Venezuela, coll. FG, JC & CC, #FG-35, 14 Jul 1999 (AY049342, 99.1%)
- G. apiculata* subsp. *candelabriformis* subsp. nov. Playa Barranquita, Estado Falcón, Venezuela, coll. FG, JC & CC, #FG-34, 14 Jul 1999 (AY049341, 98.8%)
- G. beckeri* (J. Agardh) Papenfuss. Sharks Bay, Port Alfred, South Africa, coll. M.H. Hommersand, #FG-193, 19 Jul 1993 (AY049377, 96.3%)
- G. bursa-pastoris* (Gmelin) Silva. Anse de Caro, Brittany, France, #FG-191, 03 Jul 2000 (AY049375, 93.3%)
- G. bursa-pastoris* (Gmelin) Silva. Dorset, Chesil Fleet Narrows, England, coll. W. Farnham, #FG-189, 27 Jul 2000 (AY049374, 86%)
- G. bursa-pastoris* (Gmelin) Silva. Italy, coll. E. Cecere, *s.n.*, 25 Jul 1994 (AY049376, 91.6%)
- G. bursa-pastoris* (Gmelin) Silva. Lagoon basin, Mar Piccolo, Taranto, Italy, coll. E. Cecere, #FG-188, 02 Apr 1994 (AY049373, 97.5%)
- G. capensis* Schmitz *ex* Mazza. Sharks Bay, Port Alfred, South Africa, coll. M.H. Hommersand, *s.n.*, 19 Jul 1993 (AY049378, 96.5%)
- G. cervicornis* (Turner) J. Agardh. Conn Beach, N. Hutchinson Is., Indian River Co., FL, USA, 27°39'23"N; 80°21'24"W, on underwater concrete structures, coll. FG, *s.n.*, 09 Jul 1998 (AY049369, 89.7%)
- G. cervicornis* (Turner) J. Agardh. Sandspur Camping Area, 24°39'N; 81°15'W, Bahia Honda (State Park), Big Pine Key, Florida Keys, FL, USA, coll. FG, *s.n.*, 16 Sep 1998 (AY049366, 95.9%)
- G. cervicornis* (Turner) J. Agardh, specimen #1. Higgins Beach, Key West, Florida Keys, FL, USA, coll. FG, *s.n.*, 17 Sep 1998 (AY049365, 95.6%)
- G. cervicornis* (Turner) J. Agardh, specimen #2. Higgins Beach, Key West, Florida Keys, FL, USA, coll. FG, *s.n.*, 17 Sep 1998 (AY049368, 94.8%)
- G. cervicornis* (Turner) J. Agardh, specimen #3. Higgins Beach, Key West, Florida Keys, FL, USA, coll. FG, *s.n.*, 17 Sep 1998 (AY049367, 95.6%)
- G. chilensis* Bird, McLachlan et Oliveira. Playa Charya, Coquimbo, Chile, coll. SF, *s.n.*, 19 Jan 1995 (AY049396, 98.2%)
- G. curtissiae* J. Agardh. Mangue Lloroso, Peninsula Paraguana, Venezuela, coll. FG, JC & CC, #FG-33, 13 Jul 1999 (AY049327, 98.4%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel *in* Gurgel et Fredericq subsp. *flabelliformis*, specimen #1. Walton Rocks, St. Lucie Co., FL, USA, [27°20'17"N; 80°13'59"W], coll. FG, SF & JN, *s.n.*, 03 Jul 1998 (AY049355, 79.4%)

- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel in Gurgel et Fredericq subsp. *flabelliformis*, specimen #2. Walton Rocks, St. Lucie Co., FL, USA, [27°20'17"N; 80°13'59"W], coll. FG, SF & JN, *s.n.*, 03 Jul 1998 (AY049354, 95.4%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel in Gurgel et Fredericq subsp. *flabelliformis*. Isla Margarita, La Restinga, Venezuela, coll. D.F. Kapraun, #FG-67, 21 May 1998 (AY049345, 98.6%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel in Gurgel et Fredericq subsp. *flabelliformis*. Buchuaco, Peninsula Paraguana, Venezuela, in *Thalassia testudinum* beds, coll. FG, JC & CC, #FG-22, 13 Jul 1999 (AY049334, 96.4%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel subsp. *aionana* Gurgel, Fredericq et J. Norris subsp. nov. Puerto Escondido, Peninsula Paraguaná, Estado Falcón, Venezuela, protected from wave exposure, coll. FG, JC & CC, #FG-29, 13 Jul 1999, (AY049337, 93.1%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel subsp. *simplex* Gurgel, Fredericq et J. Norris subsp. nov. Champoton Area, Campeche Bay, Mexico [19°17.67'N; 90°46.26'W], coll. FG, #FG-119, 14 Feb 1999, (AY049347, 98.4%)
- G. flabelliformis* (P. et H. Crouan in Schramm et Mazé) Fredericq et Gurgel subsp. *simplex* Gurgel, Fredericq et J. Norris subsp. nov. Playa Barranquita, Estado Falcón, Venezuela, coll. FG, JC & CC, #FG-36, 14 Jul 1999, (AY049343, 98.5%)
- G. gracilis* (Hudson) Irvine, Steentoft et Farnham. Ile Verte, Roscoff, Brittany, France, coll. J. Cabioch, *s.n.*, 22 Jun 1993 (AY049399, 98%)
- G. gracilis* (Hudson) Irvine, Steentoft et Farnham. W. Angle Bay, Wales, UK, coll. M. & F. Hommersand, *s.n.*, 22 Jul 1997 (AY049400, 98%)
- G. multipartita* (Clemente) Harvey. Carantec, Brittany, France, coll. J. Cabioch, #FG-131, 22 Jun 1993 (AY049317, 87.2%)
- G. occidentalis* (Børgesen) Bodard. Offshore Louisiana, Gulf of Mexico, USA, coll. SF, #FG-153, 26 May 2000 (AY049322, 98.6%)
- G. ornata* Areschoug. Fort Randolph, Caribbean Panama, coll. B. Wysor, #BW-1221, #FG-164, 26 Feb 1999 (AY049318, 92.9%)
- Gracilaria* sp. Praia Rasa, Búzios City, Rio de Janeiro, Brazil, coll. FG, #FG-92, 12 Dec 1998 (AY049371, 98.6%)
- Gracilaria* sp. Ho Pin Deo. Taiwan, coll. Lin S.-M., #FG-163, 09 Jul 1994 (AY049316, 97.5%)
- Gracilaria* sp. (= *G. aff. textorii*). Bulusan, South Luzon, Philippines, coll. Lin S.M., #FG-49, 21 Apr 1998 (AY049384, 93.2%)
- G. spinulosa* (Okamura) Chang et Xia. Taiwan, coll. Lin S.-M., #FG-111, 11 May 1998 (AY049395, 93.3%)
- G. textorii* (Suringar) De Toni. Gobogahana, Japan, #FG-169, 10 Jul 1994 (AY049325, 97.5%)
- G. vieillardii* Silva. Taiwan, coll. Lin S.M., #FG-112, 22 May 1998 (AY049394, 95.5%)