

SYSTEMATICS OF GRACILARIOPSIS (GRACILARIALES, RHODOPHYTA) BASED ON *rbcl* SEQUENCE ANALYSES AND MORPHOLOGICAL EVIDENCE<sup>1</sup>

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A phylogeny has been inferred from parsimony and likelihood analyses of plastid *rbcl* DNA sequences for seven recognized and six undescribed species of *Gracilariopsis* (*Gp.*) (Gracilariales, Rhodophyta). New descriptions and illustrations of cystocarp morphology are provided for four *Gracilariopsis* species from North and South America. The generitype, *Gp. sjoestedtii* (Kylin) Dawson, is reinstated to include plants distributed from British Columbia to Pacific Baja California, and the name is corrected to *Gp. andersonii* (Grunow) Dawson. *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik is shown not to have a worldwide distribution but to be restricted to the vicinity of Peru. *Gracilariopsis costaricensis* is recognized with the provision that it may prove to be conspecific with *Gp. lemaneiformis*. *Gracilariopsis "lemaneiformis"* from North and South Carolina is described as a new species, *Gp. carolinensis* Liao et Hommersand sp. nov. *Gracilariopsis longissima* (Gmelin) Steentoft, Irvine et Farnham from Western Europe and the Mediterranean Sea and *Gp. tenuifrons* (Bird et Oliveira) Fredericq et Hommersand from the Caribbean Sea and Brazil are recognized. Entities that have been referred to *Gp. "lemaneiformis"* from China and Japan constitute an undescribed species that is related to *Gp. heteroclada* Zhang et Xia. An invasive species from the Gulf of California, Mexico, and South Australia that has been assigned to *Gp. "lemaneiformis"* is resolved in a clade that includes *Gp. longissima*. Four undescribed species are included in the molecular analyses. The systematics of *Gracilariopsis* is discussed in the light of the morphological and molecular evidence.

**Key index words:** biogeography; Gracilariaceae; Gracilariales; Gracilariopsis; invasive species; phylogeny; *rbcl*; Rhodophyta; systematics

A global phylogeny for the Gracilariaceae (Gracilariales) inferred from analyses of chloroplast-encoded *rbcl* sequences from over 140 gracilariacean taxa worldwide (Gurgel 2001) confirms the monophyly of the family and identifies three monophyletic clades, the first including *Melanthalia* and *Curdiea*, the second *Gracilariopsis* (*Gp.*), the third *Gracilaria* (*G.*) sensu lato (Abbott et al. 1991). Recognition of *Gracilariopsis* as a genus distinct from *Gracilaria* received strong support from the molecular studies of Bird et al. (1992, 1994), whose investigations of nuclear small subunit (SSU) rRNA demonstrated that species of *Gracilariopsis* form a clade that is widely separated from other members of the Gracilariaceae.

When Dawson (1949) monographed the Gracilariaceae from the eastern North Pacific Ocean he established a new genus, *Gracilariopsis*, to include algae having cystocarps with dome-shaped gonimoblasts composed of small densely staining cells that are never connected to the pericarp by tubular cells. In separating his new genus from *Gracilaria*, Dawson relied entirely on cystocarpic characters, most notably the absence of tubular cells, which he termed "nutritive filaments" (Dawson 1949). He designated *Gp. sjoestedtii* as the type species based on material first studied by Sjoestedt (1926) under the name *G. robusta* Setchell. This collection obtained by Kylin from Pacific Grove, California in 1922 became the basis of a new species, *G. sjoestedtii* Kylin (1930). Dawson noted that the spermatangia were borne in a continuous superficial layer in *G. sjoestedtii* and *G. costaricensis* Dawson; however, the five additional species he placed in *Gracilariopsis* either had spermatangia lining the walls of deep conceptacular pockets or the spermatangia were unknown. All seven species were characterized

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- Gibbs, S. P. 1981. The chloroplast endoplasmic reticulum: structure, function and evolutionary significance. *Int. Rev. Cytol.* 72:49–99.
- Jarosch, R. 1990. Lateral hydrodynamic effects of rotating cytoskeletal components as possible determining factors for cytomorphogenesis in plant cells. *Protoplasma* 157:38–51.
- Jenks, A. & Gibbs, S. P. 2000. Immunolocalisation and distribution of form II RUBISCO in the pyrenoid and chloroplast stroma of *Amphidinium carterae* and form I RUBISCO in the symbiont-derived plastids of *Peridinium foliaceum* (Dinophyceae). *J. Phycol.* 36:127–38.
- Jeon, K. W. 1999. Symbiogenesis of bacteria within amoeba. In Seckbach, J. [Ed.] *Enigmatic Microorganisms and Life in Extreme Environments*. Kluwer Academic Publishers, London, UK, pp. 587–98.
- Kowallik, K. V. 1992. Origin and evolution of plastids from chlorophyll a+c-containing algae: suggested ancestral relationship to red algal plastids. In Lewin, R. A. [Ed.] *Origins of Plastids*. Chapman and Hall, London, UK, pp. 223–63.
- Kowallik, K. V. 1999. Endosymbiose, ein Motor der Evolution. *Biol. Heute* 1:1–5.
- Kowallik, K. V., Stoebe, B., Schaffran, L., Kroth-Pancic, P. & Freier, U. 1995. The chloroplast genome of a chlorophyll a+c-containing alga, *Odontella sinensis*. *Plant Mol. Biol. Rep.* 13:336–42.
- Krammer, K. 2000. *The genus Pinnularia*. In Lange-Bertalot, H. [Ed.] *Diatoms of Europe*. Vol.1. A. R. G. Ganter Verlag KG, Rugell, Liechtenstein, 703 pp.
- Madigan, M. T., Martinko, J. M. & Parker, J. 2000. *Biology of Microorganisms*. 9th ed. Prentice Hall International Editions, Prentice Hall International Ltd., London, UK, 1175 pp.
- Pickett-Heaps, J. D., Tippit, D. H. & Andreozzi, J. A. 1978a. Cell division in the pennate diatom *Pinnularia*. I. Early stages in mitosis. *Biol. Cell.* 33:71–8.
- Pickett-Heaps, J. D., Tippit, D. H. & Andreozzi, J. A. 1978b. Cell division in the pennate diatom *Pinnularia*. II. Later stages in mitosis. *Biol. Cell.* 33:79–84.
- Reisser, W. 1990. Participation of algal cell wall surface structures in the formation of host-symbiont-interface of endocytobiotic systems. In Wiessner, W., Robinson, D. G. & Starr, R. C. [Eds.] *Experimental Phycology: Cell Walls and Surfaces, Reproduction and Photosynthesis*. Springer Berlin, Heidelberg, pp. 55–68.
- Schmid, A. M. 1994. Aspects of morphogenesis and function of diatom cell walls with implications for taxonomy. *Protoplasma* 181:43–60.
- Schmid, A. M. 2000. "Scattered cp-nucleoids" in diatoms explained: bacteria—inside the endoplasmic reticulum—pierce the plastids of *Pinnularia nobilis*. *J. Phycol.* 36:61–2.
- Schmid, A. M. 2001. Value of pyrenoids in the systematics of the diatoms: their morphology and ultrastructure. In Economou-Amilli, A. [Ed.] *Proceedings of the 16th International Diatom Symposium*, Athens, 2000, pp 1–32.
- Schmid, A. M. 2003. Endobacteria in the diatom *Pinnularia* (Bacillariophyceae). I. "Scattered ct-nucleoids" explained: DAPI-DNA complexes stem from exoplastidial bacteria boring into the chloroplasts. *J. Phycol.* 39:122–138.
- Schmid, A. M., Eberwein, R. & Hesse, M. 1996. Pattern morphogenesis in cell walls of diatoms and pollen grains: a comparison. *Protoplasma* 193:144–73.
- Schnepf, E. 1984. The cytological viewpoint of functional compartmentation. In Wiessner W., Robinson D. & Starr R. C. [Eds.] *Compartments in Algal Cells and their Interaction*. Springer Berlin, Heidelberg, pp.1–10.
- Sitte, P. 1981. Die Endosymbionten Hypothese - eine kritische Betrachtung zur Zell-Evolution. *Nova Acta Leopold.* 77:56 251:41–58.
- Studer, D., Michel, M. & Müller, M. 1989. High pressure freezing comes of age. *Scanning Microscopy* 3(Suppl.):253–69.
- Tschermak-Woess, E. 1953. Über auffallende Strukturen in den Pyrenoiden einiger Naviculoideen. *Österr. Bot. Z.* 100:160–78.
- Wordemann, L., McDonald, K. L. & Cande, Z. W. 1986. The distribution of cytoplasmic microtubules throughout the cell cycle of the centric diatom *Stephanopyxis turris*: their role in nuclear migration and positioning the mitotic spindle during cytokinesis. *J. Cell Biol.* 102:1688–98.

by the presence of terete axes and branches. In the years that followed, numerous species that lacked tubular nutritive cells were added to *Gracilariopsis*, some of which possessed superficial spermatangia and some with spermatangia borne in conceptacles.

Papenfuss (1967) compared cystocarp morphology in *Gp. sjoestedtii* from Sonoma County, California with that of *G. verrucosa* (Hudson) Papenfuss from southern England, at that time the generic type of *Gracilaria*. He found no fundamental difference in gonimoblast cell sizes in these two species, whereas tubular cells were present between the gonimoblasts and pericarp in some specimens and absent in others. He concluded that the presence or absence of tubular cells cannot be used to discriminate between the two genera, and he placed *Gracilariopsis* in synonymy under *Gracilaria*.

Abbott (1983) compared cystocarpic type and male topotype material of *G. sjoestedtii*, with cystocarpic and vegetative plants of *Cordylecladia andersonii* Grunow and *G. lemaneiformis* (Bory) Weber-van Bosse from Peru and concluded they were conspecific. *Gracilaria sjoestedtii* was reduced to synonymy under *G. lemaneiformis*. At the same time Abbott lectotypified *C. andersonii* with a plant from Santa Cruz collected by Anderson and deposited in the Grunow Herbarium at the Natural History Museum in Vienna (W). A new species, *G. papenfussii* Abbott, was established to contain *G. andersonii* sensu auct., non (Grunow) Kylin (1941, p. 21) and *Gp. andersonii* sensu Dawson (1949, p. 43, 1961, p. 216). The synonymy of *G. sjoestedtii* with *G. lemaneiformis* has received wide acceptance (e.g. Wynne 1998, Millar and Xia 1999, Littler and Littler 2000). *Gracilaria lemaneiformis* (sensu Abbott 1983) has been cited throughout the warm-temperate and tropical world either under *Gracilaria* or *Gracilariopsis*, including Indonesia (Weber-van Bosse 1928), Hawaii (Abbott 1999), West Africa (Price et al. 1988), Thailand (Lewmanomont 1994), India and Yemen (Silva et al. 1996), the South African west coast (Stegenga et al. 1997), China and Japan (Chang and Xia 1976, Xia 1985, Xia and Zhang 1999, Yoshida 1998), and western Europe (Bird et al. 1992, 1994). On the other hand, Bird and Oliveira (1986) stated that it is highly unlikely this species occurs naturally far from the geographic regions around its type locality and that records from the western Pacific, Indian and Atlantic Ocean should be regarded with care.

Fredericq and Hommersand (1989a,b) resurrected *Gracilariopsis* based on studies of *G. lemaneiformis* (sensu Abbott 1983) from California. In addition to the characters previously recognized by Dawson, they emphasized the feature that gonimoblast cells become linked to gametophytic cells in the floor of the cystocarp by means of secondary pit connections, while noting that multinucleate tubular cells are absent in the cystocarp and that the spermatangial parent cells are produced from superficial cortical cells (Fredericq and Hommersand 1990). Bouzon et al. (2000) commented that although spermatangial parent cells may be derived directly from cortical cells in *Gp. tenuifrons*, they can also issue from subtermi-

nal cells as in most other Gracilariaceae. Perceived variability of diagnostic features has led some authors to consider *Gracilariopsis* as indistinct from *Gracilaria* at the genus level (Gargiulo et al. 1992, Abbott 1995, 1999).

Three additional species have been recognized in recent years: *Gp. tenuifrons* (Bird et Oliveira) Fredericq et Hommersand (Fredericq and Hommersand 1989b), *Gp. heteroclada* (Zhang et Xia) Zhang et Xia in Abbott et al. (1991) from Hainan Is., China, and *Gp. longissima* (S. G. Gmelin) Steentoft, Irvine et Farnham (Steentoft et al. 1995) from the southern British Isles, France, and Spain. *Gracilariopsis heteroclada* was originally described as a species of *Gracilaria* by Zhang and Xia (1988), a later homonym of *G. heteroclada* (Montagne) J. Feldmann & G. Feldmann, a Mediterranean species. As Hurtado-Ponce and Liao (1998) have shown, *Gp. heteroclada* Zhang et Xia in Abbott et al. (1991) should be treated as a nomen novum in accordance with Art. 58.1b in the St. Louis Code (Greuter et al. 2000). The distribution of *Gracilariopsis* was extended to Western Europe by Fredericq and Hommersand (1989b). The European species was subsequently characterized morphologically by Steentoft et al. (1995). *Gracilariopsis longissima* (S. G. Gmelin) Steentoft, Irvine et Farnham (Steentoft et al. 1995) is the first *Gracilariopsis* species described based on eastern Atlantic material. It was established upon *Fucus longissimus* S. G. Gmelin (1768) with the selection of a specimen from Kent in the Dillenius collection in the Fielding-Druce Herbarium at the University of Oxford as the neotype. Nucleotide sequences of nuclear-encoded SSU rRNA demonstrated that the European populations were distinct from plants referred to *Gp. lemaneiformis* from Pacific North America (Bird et al. 1992). *Gracilariopsis* species have been the focus of several genetic and biochemical studies (Goff and Coleman 1988, Bhattacharya et al. 1990, Scholfield et al. 1991, Kapraun 1993, Kapraun et al. 1993, Goff et al. 1994, Belloirin et al. 2002).

In the present study we investigate the molecular phylogeny of seven recognized species of *Gracilariopsis* and six species that are either incorrectly assigned to *Gp. lemaneiformis* or are undescribed. We examined cystocarp development in three species presently placed in *Gp. lemaneiformis*, namely the type species from Peru, the generic type species *Gp. sjoestedtii* (= *Gp. andersonii*) from California, *Gp. costaricensis*; and a new species, *Gp. carolinensis*, from North Carolina. The significance of our phylogenetic and morphological observations on the classification and biogeography of *Gracilariopsis* is discussed. Cystocarp development is also illustrated for the first time in *Gp. costaricensis*.

#### MATERIALS AND METHODS

Algal samples used in molecular studies were desiccated in silica gel, air dried, or preserved in 95% alcohol in the field. Voucher specimens and materials for morphological studies were fixed and stored in 5% formalin/seawater or pressed as herbarium sheets and deposited in the Herbarium of the University of Louisiana at Lafayette (LAF) and/or the Herbarium of the University of North Carolina at Chapel Hill (NCU). Herbarium abbreviations follow Holmgren et al. (1990). Hand sec-

tions were prepared using a double-edged platinum-chrome razor blade from samples bleached in the light in 5% formalin and rinsed in deionized water and partly dried. Sections were stained with aceto-iron-hematoxylin-chloral hydrate (Wittmann 1965), destained under the microscope with 45% glacial acetic acid, and mounted in 50% Hoyer's mounting medium (Stevens 1981). Photographs were prepared with a photomicroscope (Carl Zeiss Inc., Thornwood, NY, USA) and T-Max 100 black and white (Eastman Kodak Co., Rochester, NY, USA). Digital images were edited and assembled in plates using Photoshop v.5.0 (Adobe Systems Inc., San Jose, CA, USA).

Silica gel-dried specimens and extracted DNA samples were deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at  $-20^{\circ}\text{C}$ . The DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) or were submitted to a CTAB-cesium chloride DNA procedure (Freshwater and Ruess 1994). Plastid-encoded *rbcL* was selected to infer a phylogeny for *Gracilariopsis*. PCR and sequencing primers used in this study were *rbcL* start, F7, F57, F492, F577, F753, F993, R753, R1381, and *RbcS* start as listed in Freshwater and Ruess (1994) and Hommersand et al. (1994). Protocols for gene amplification, automated sequencing, and alignment are identical to those given in Lin et al. (2001).

Partial *rbcL* sequences were produced from 20 recently collected samples of *Gracilariopsis*. *Melanthalia obtusata*, *Cordia coriacea* from New Zealand, and *C. crassa* from Australia were chosen as outgroup taxa based on their close phylogenetic relationship with the ingroup in global searches of the Gracilariaceae (data not shown). The use of partial *rbcL* sequences in red algae has been shown to produce stable topologies due to the even distribution of informative characters and homoplasies across the length of the sequence (Freshwater et al. 1995). Sequences and the sequence alignment were deposited in GenBank (Benson et al. 1994). Information regarding sample site, date of collection, collector's name, percentage of *rbcL* gene sequenced, and GenBank accession numbers are listed in Table 1.

All phylogenetic analyses were performed with PAUP\* v.4.0 beta 10 (Swofford 2002) for Macintosh. Phylogenetic trees were generated by maximum likelihood (ML) and maximum parsimony (MP) methods. Because the first 40 bp were missing in many sequences, the phylogenetic analyses were restricted to the last 1427 bp of the *rbcL* gene in both the MP and ML analyses. Parameters used in the ML analysis were obtained using the software Modeltest v. 3.0 (Posada and Crandall 1998) to compare different models of DNA substitutions in a hierarchical hypothesis-testing framework to select a base substitution model that best fit the sequence data. The optimal ML model found was the general time reversible model with invariable sites and gamma distribution (GTR+I+G). The parameters were as follows: assumed nucleotide frequencies A = 0.3144; C = 0.1404; G = 0.2138; T = 0.3313; substitution rate matrix A-C substitutions = 1.6828, A-G = 5.3578, A-T = 1.2693, C-G = 1.4901, C-T = 13.0529, G-T = 1.0; proportion of sites assumed to be invariable = 0.5338; and rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.4582. MP trees were inferred from heuristic searches of 5000 replications of random sequence addition using only the phylogenetically informative characters, unordered, under the Fitch criterion of equal weights for all substitutions (Fitch 1971), tree bisection reconnection, saving multiple trees (MULTREES) but holding 20 trees at each step, and STEEPEST DESCENT. Support for all nodes for all trees was assessed by bootstrap analysis (Felsenstein 1985) on the data set using 3000 replicates for MP and 200 replicates for ML, as implemented in PAUP\*. Bootstrap proportion values are abbreviated as BP.

When presented, the range of *rbcL* divergence values within and among species was calculated using uncorrected percentages (% total number of pair-wise substitutions divided by the total number of base pairs sequenced).

## RESULTS

Among the 1427 bp in the *rbcL* data set, 989 bp (69.3%) were identical, 438 bp (30.7%) vary at least

once, and 270 bp (19%) were phylogenetically informative. No insertion or deletion mutations were found in the *rbcL* sequences produced in this study, allowing for unambiguous alignment of all sequences. Tree lengths of 100,000 randomly generated trees for the data set had a skewed distribution ( $g_1 = -1.31$ ,  $p < 0.01$ ), indicating the presence of nonrandom structures and phylogenetic signal in the data set (Hillis and Huelsenbeck 1992).

MP produced four equally most parsimonious trees of 591 steps, with one most parsimonious tree shown in Figure 1. The topology of the MP (Fig. 1) and ML (Fig. 2) phylogenies differed from each other only in the position of *Gp. longissima* from England and *Gp. sp.* from Venezuela. The relative position of *Gp. sp.* from Venezuela, *Gp. longissima* from England, and *Gp. "lemaniformis"* from Namibia lacked or received low bootstrap support, and the phylogenetic placement of these taxa is not resolved.

*Gracilariopsis heteroclada* was the most basal species in all four most parsimonious trees (Fig. 1), but its basal position received no bootstrap support. In the ML tree (Fig. 2) it is basal and sister to the species from China, Japan, and Venezuela. *Gracilariopsis heteroclada* exhibited the highest level of intragenetic uncorrected pair-wise sequence divergence values (5.6%–7.51%). The close relationship between *Gp. lemaniformis* from Peru and *Gp. costaricensis* was strongly supported (MP: BP = 94%; ML: BP = 81%), and this clade in turn formed a strongly supported group with *Gp. carolinensis* in both the MP (Fig. 1, BP = 92%) and ML (Fig. 2, BP = 80%) trees.

The topological position of *Gp. andersonii* did not receive bootstrap support. *Gracilariopsis tenuifrons* and *Gp. sp.* from the Gulf of Mexico formed a strongly supported clade (BP = 95% and 82% in MP and ML trees, respectively), and the genetic distance between these two species is small, with a *rbcL* sequence divergence of 1.14%. Ten substitutions contributed to this pair-wise base pair difference. Five are phylogenetically noninformative (in the whole data set context), three are nonsynonymous substitutions in the first codon position, the fourth is a nonsynonymous substitution in the second codon position, and the fifth is a synonymous substitution in the third codon position. The genetic divergence among four Caribbean *Gp.* aff. *panamensis* specimens (two of the sequences were not included in the alignment) ranged from 0.07% to 0.22%. The pair-wise base distance between *Gp. lemaniformis* and *Gp. carolinensis* was 2.54%, whereas that between *Gp. lemaniformis* and *Gp. costaricensis* was only 0.63%. The pair-wise base distance between *Gp. lemaniformis* and *Gp. andersonii* was 4.49% and that between *Gp. andersonii* and *Gp. longissima* from Europe ranged from 4.44% to 4.84%. The genetic distances between the *rbcL* sequences obtained for specimens referable to *Gp. longissima* from Italy and England were 1.42% to 1.47% and ranged from 2.73% to 2.80% between the English and Namibian haplotypes. Two samples referred to *Gp. "lemaniformis"* from Pacific Mexico and South Australia differed by 4.76% to

TABLE 1. List of species, their collection information, and the *rbcL* GenBank accession numbers followed by *rbcL* fraction (in %) sequenced. All sequences were newly generated.

Species	Collection data	GenBank Accession number and % sequenced
<i>Curdiea coriacea</i> (Hook. et Harv.) J. Agardh	Doubtless Bay, New Zealand, coll. W. Nelson, 01.xii.1993	AY049425, 66.5%
<i>Curdiea crassa</i> Millar	Bongin Bongin Bay, North of Sydney, NSW Australia; coll. A. Millar & P. Richards; 18.ii.1994	AY049427, 98.1%
<i>Gracilariopsis andersonii</i> (Grunow) Dawson	Pigeon Point, San Mateo Co., California, USA; coll. M.H. & F.H. Hommersand; 20.v.1992	AY049413, 94.2%
<i>Gracilariopsis andersonii</i> (Grunow) Dawson	Seal Rock, Lincoln Co., Oregon, USA; coll. S. Fredericq; 15.v.1999	AY049414, 96.4%
<i>Gracilariopsis costaricensis</i> Dawson	South end, Playa Tamarindo, Nicoya Peninsula, Guanacaste, Costa Rica; coll. D.T. Talbot & D.W. Freshwater; 17.iii.1999	AY049423, 98.4%
<i>Gracilariopsis carolinensis</i> Liao et Hommersand, sp. nov.	Kure Beach, Fort Fisher, NC, USA; coll. D.W. Freshwater; 14.iv.1991	AY049412, 96.7%
<i>Gracilariopsis heteroclada</i> (Zhang et Xia) Zhang et Xia in Abbott	Dapdap, Bulusan, Luzon, Philippines; coll. S.M. Lin, 22.iv.1998	AY049411, 91.1%
<i>Gracilariopsis lemneiformis</i> (Bory) Dawson, Acleto et Foldvik	Topotype, Yacilla, Paita, Piura, Peru; coll. C. Acleto & R. Zuniga; 03.iii.1994	AY049415, 97.6%
<i>Gracilariopsis "lemneiformis"</i>	Tosa Bay, Shikiku I., Japan; coll. M. Ohono, D.B. Largo & J. Rebello, leg. L. Liao; 11.ix.1992	AY049419, 97.8%
<i>Gracilariopsis "lemneiformis"</i>	Qingdao, Shandong Prov., China; coll. M.H. Hommersand; 23.iv.1994	AY049421, 65%
<i>Gracilariopsis "lemneiformis"</i>	Bahía de Las Animas, Gulf of California, Mexico; aquaculture; leg. J. Zertuche-González; vi.1998	AY049416, 41.1%
<i>Gracilariopsis "lemneiformis"</i>	Lake Butler, Robe, S. Australia, Australia; coll. H.B.S. Womersley; 03.iii.1995	AY049422, 97.8%
<i>Gracilariopsis "lemneiformis"</i>	Swakopsmund, Namibia; coll. M.H. Hommersand; 06.vii.1993	AY049410, 98.2%
<i>Gracilariopsis longissima</i> (Stackhouse) Irvine, Steentoft et Farnham	Cadiz, Spain; coll. J.R. Andria Gonzalez; s.d	AY130244, 97.5%
<i>Gracilariopsis longissima</i> (Stackhouse) Irvine, Steentoft et Farnham	off Sandfoot Castle, Portland Harbour, Dorset, England; coll. Wm. Farnham & M. Steentoft; 30.viii.1992; sample provided by C. Bird	AY049420, 97.3%
<i>Gracilariopsis</i> aff. <i>panamensis</i> (Taylor) Dawson	Fort Randolph, Colon City, Panama; coll. B. Wysor; 26.iii.1998; sequence used in the phylogenetic analyses	AY049405, 97.1%
<i>Gracilariopsis</i> aff. <i>panamensis</i> (Taylor) Dawson	Los Francisky Is., Los Roques Archipelago, Venezuela; coll. C.F.D. Gurgel; 04.vii.1999; specimen no. 1	AY049408, 98.4%
<i>Gracilariopsis</i> aff. <i>panamensis</i> (Taylor) Dawson	Los Francisky Is., Los Roques Archipelago, Venezuela; coll. C.F.D. Gurgel; 04.vii.1999; specimen no. 2, sequence used in the phylogenetic analyses	AY049409, 98.4%
<i>Gracilariopsis</i> aff. <i>panamensis</i> (Taylor) Dawson	La Encrucijada, Peninsula Paraguana Panama, Falcon State, Venezuela; coll. C.F.D. Gurgel, J.E. Conde & C. Carmona; 13.vii.1999	AY049407, 93.3%
<i>Gracilariopsis</i> sp.	La Vela de Coro, Falcon State, Venezuela; coll. C.F.D. Gurgel, J. E. Conde & C. Carmona; 14.vii.1999	AY049309, 96.7%
<i>Gracilariopsis</i> sp.	2 miles West of Anton Lizardo, Vera Cruz area, Mexico; coll. C.F.D. Gurgel; ii.1999	AY049406, 80.2%
<i>Gracilariopsis tenuifrons</i> (Bird et Oliveira) Fredericq et Hommersand	Ilet Caret, Guadeloupe, French West Indies; coll. A. Renoux; 2.xii.1993	AY049418, 97.8%
<i>Gracilariopsis tenuifrons</i> (Bird et Oliveira) Fredericq et Hommersand	Arya Peninsula, Sucre, Venezuela, coll. D.W. Freshwater; v.1990	AY049417, 82.4%
<i>Melanthalia obtusata</i> (Labillardière) J. Agardh	Warrnambool, Victoria, Australia; coll. M.H. Hommersand; 13.vii.1995	AY049431, 99%

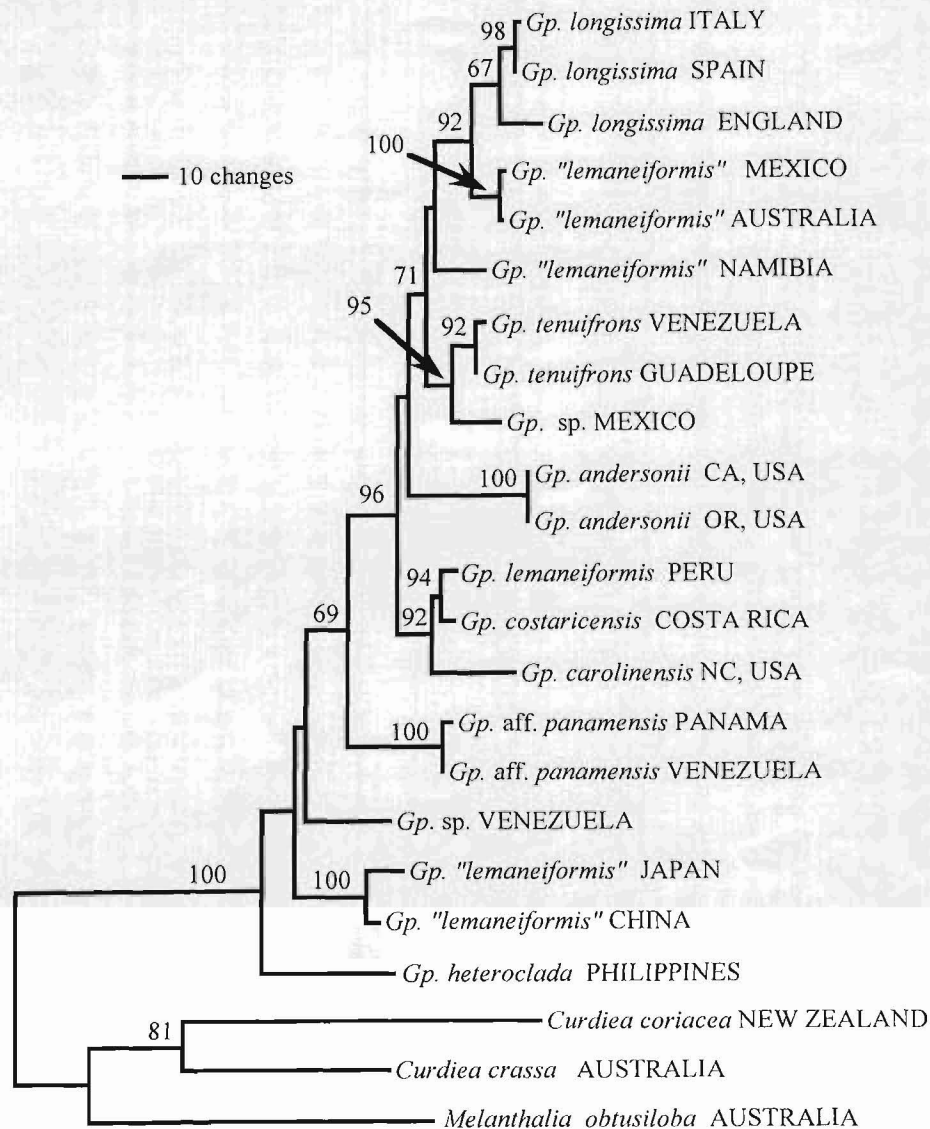


FIG. 1. One of four MP trees resulting from MP analysis of *tbl*L sequences from 22 samples of *Gracilariopsis* ( $L = 591$ ,  $CI = 0.58$ ,  $RI = 0.68$ ). Bootstrap support based on 3000 replicates is shown above the nodes. *Curdiea* and *Melanthalia* species were selected as out-group taxa.

5.24% when compared with the true *Gp. lemaniformis* from Peru.

1. *Gracilariopsis lemaniformis* (Bory) Dawson, Acleto et Foldvik (1964: 59, pl. 56, fig. A (as *lemanaeformis*))

Plants cylindrical up to 100 cm tall, consisting of one to few irregularly branched indeterminate axes from a discoid holdfast (Fig. 3a); axes, 0.5 mm diameter at the base, broadening to 1.3 mm diameter and tapering toward the apices, sparsely irregularly branched, the branches up to 30 cm or more long and resembling the main axes, sometimes with a few shorter proliferous laterals; spermatangia superficial in indefinite sori; cystocarps scattered over the axes and branches, domoid, slightly constricted at the base and often rostrate at maturity (Fig. 4a), pericarp composed of a single layer of darkly staining anticlinally

oriented surface cells and 7 to 9 subsurface layers below extending to 14 to 18 layers at the apex next to the ostiole (Fig. 4b); the lowermost cell layers separating into individual cell files due to stretching in the mature pericarp, especially in the ostiolar region (Fig. 4c); gonimoblasts pedicellate, attached to the cystocarp floor by palisade-like cells, approximately 200  $\mu\text{m}$  broad (Fig. 4d), gonimoblast mass generally spherical, approximately 500  $\mu\text{m}$  broad (Fig. 4d), composed of sterile pseudo-parenchymatous cells that give rise to straight chains of darkly staining carposporangial initials that are transformed distally into chains of carposporangia approximately 450  $\mu\text{m}$  long (Fig. 4e); gonimoblasts attached to the vegetative floor of the cystocarp by means of ovoid to irregularly shaped conjuctor cells arising individually from basal gonimoblast cells (Fig. 4f).

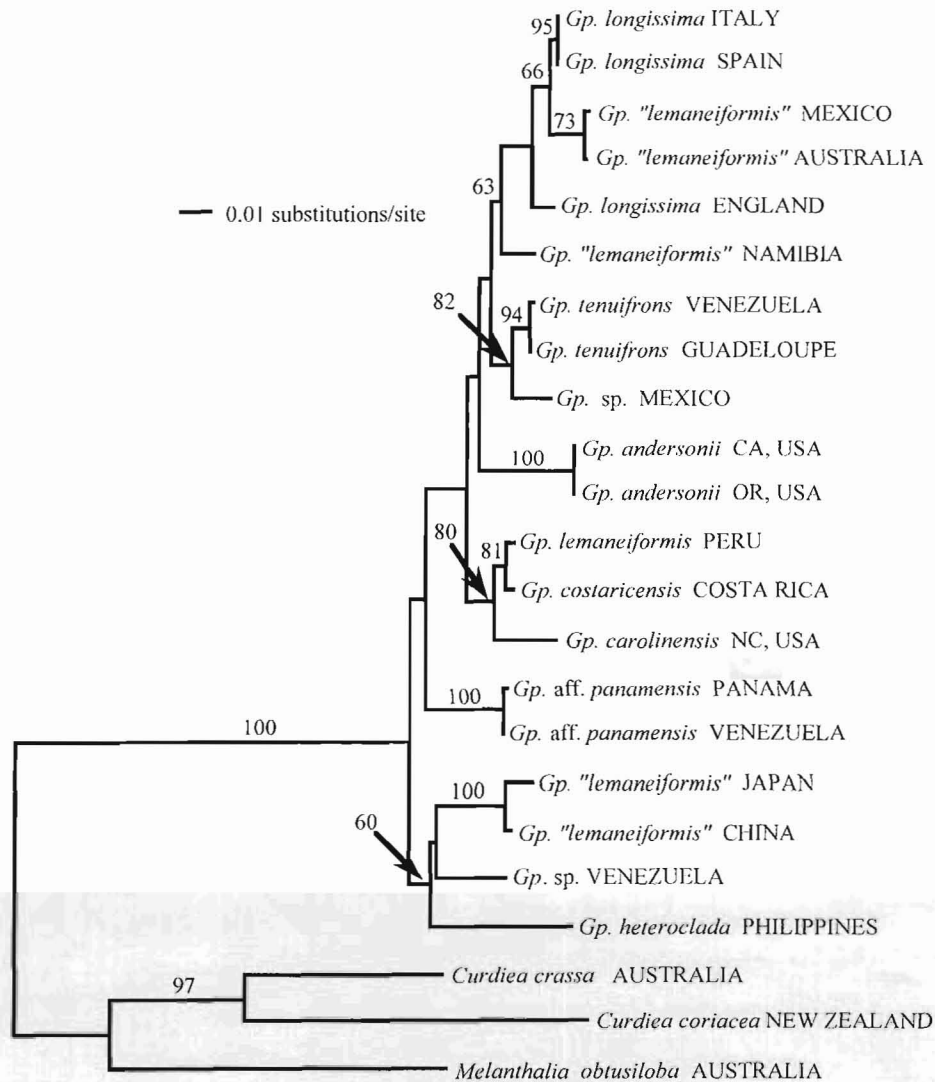


FIG. 2. ML tree with a  $-\log$  likelihood of 5813.83796 calculated using the GTR+I+G model of evolution and the same data set as in Figure 1. Bootstrap support based on 200 replicates is shown above the nodes.

*Basionym:* *Gigartina lemaniformis* Bory 1828:151 (as *lemaniformis*).

*Holotype:* An 1823 collection by Dumont d'Urville made during the "La Coquille" expedition, deposited in the Bory herbarium (PC). (See Howe, 1914, pl. 52, Fredericq and Hommersand 1989b, Fig. 1.)

*Type locality:* Paita, coast of Peru near 5° south latitude.

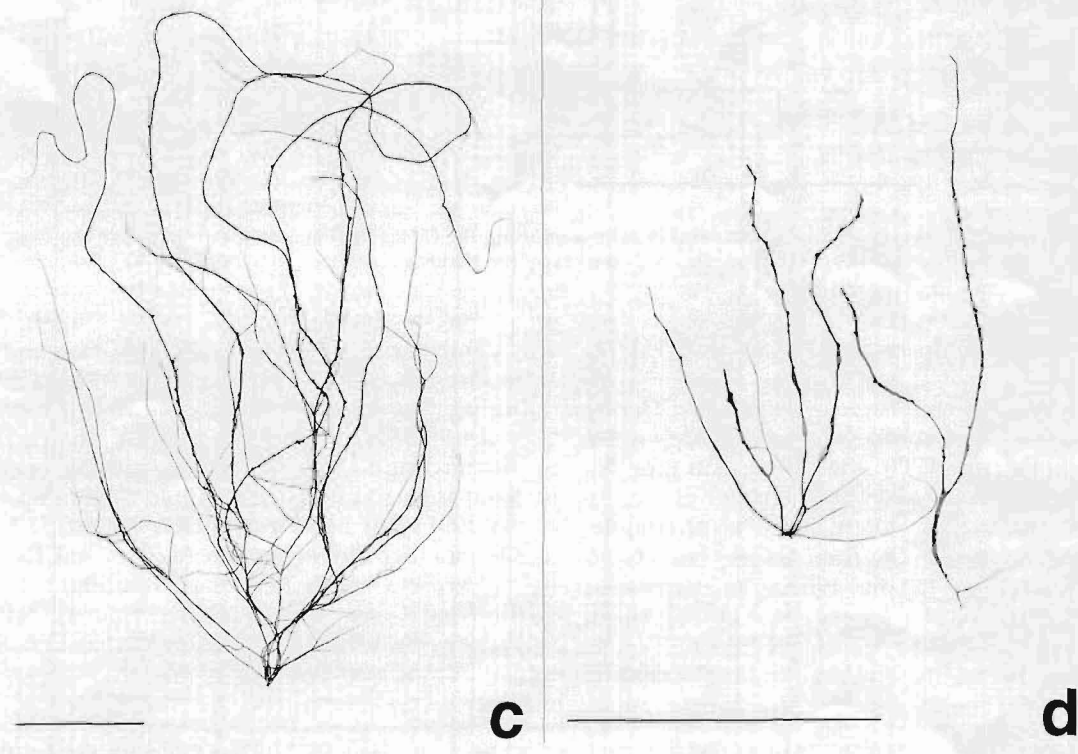
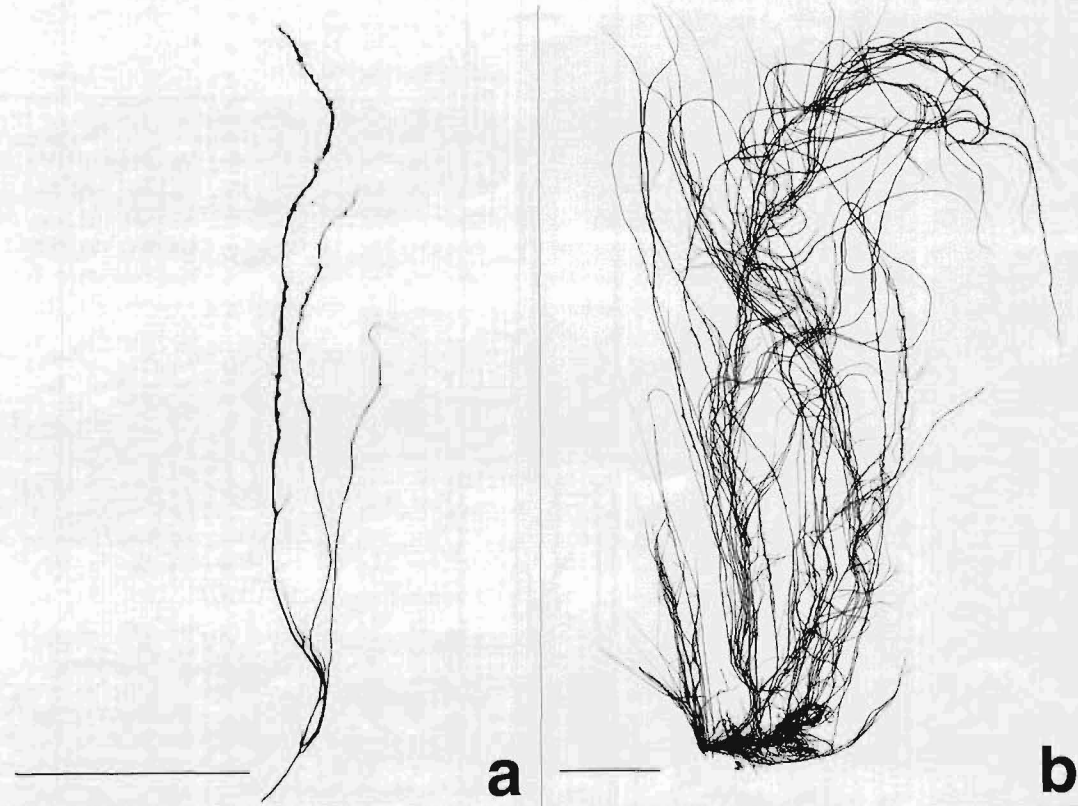
*Nomenclatural synonyms:* *Gracilaria lemaniformis* (Bory) Weber-van Bosse 1928:435, fig. 176 (as *lemaniformis*), Abbott 1983; *Cordylecladia lemaniformis* (Bory) Howe 1914:128, pl. 52 (as *lemaniformis*).

*Taxonomic synonyms:* *Cordylecladia andersonii* Grunow in Piccone 1886:62 (pro parte, see Abbot 1983, fig. 4, right); *Gracilaria lichenoides* sensu Hariot 1889:70.

*Distribution:* Piura, Peru to Prov. Antofagasta, Chile (Ramírez and Tapia 1991). Some records for this species from as far south as Chilöe, Chile (Ramírez and Santelices 1991) may be *Gracilaria chilensis* Bird, McLachlan et Oliveira (See Bird et al. 1986, Ramírez and Tapia 1991).

*Specimens examined:* Yacila, Paita, Piura, Peru, 03.iii.1994, leg. C. Acleto and R. Zuniga 2355; Playa Primavera, Lobitos, Paita, Piura, Peru, 20.ii.1967, leg. C. Acleto 1204.

FIG. 3. Habits of cystocarpic plants of *Gracilariopsis*. Scale, 5 cm. (a) *Gp. lemaniformis* (NCU) Playa Primavera, Lobitos, Paita, Peru, 20.ii.1967, leg. C. Acleto 1204. (b) *Gp. andersonii* (NCU) Greyhound Rock, Ano Nuevo, San Mateo Co., California, 17.vii.1966, leg. M. H. Hommersand. (c) *Gp. carolinensis* (US) type specimen, Coquina rocks, Kure Beach, New Hanover Co., North Carolina, 28.x.1981, leg. M. H. Hommersand. (d) *Gp. costaricensis* (LAM) isotype specimen, dredged from bay bottom, Puerto Parker, Bahía Santa Elena, Costa Rica, 22.i.1938, leg. Crocker 3, Zaca Expedition.





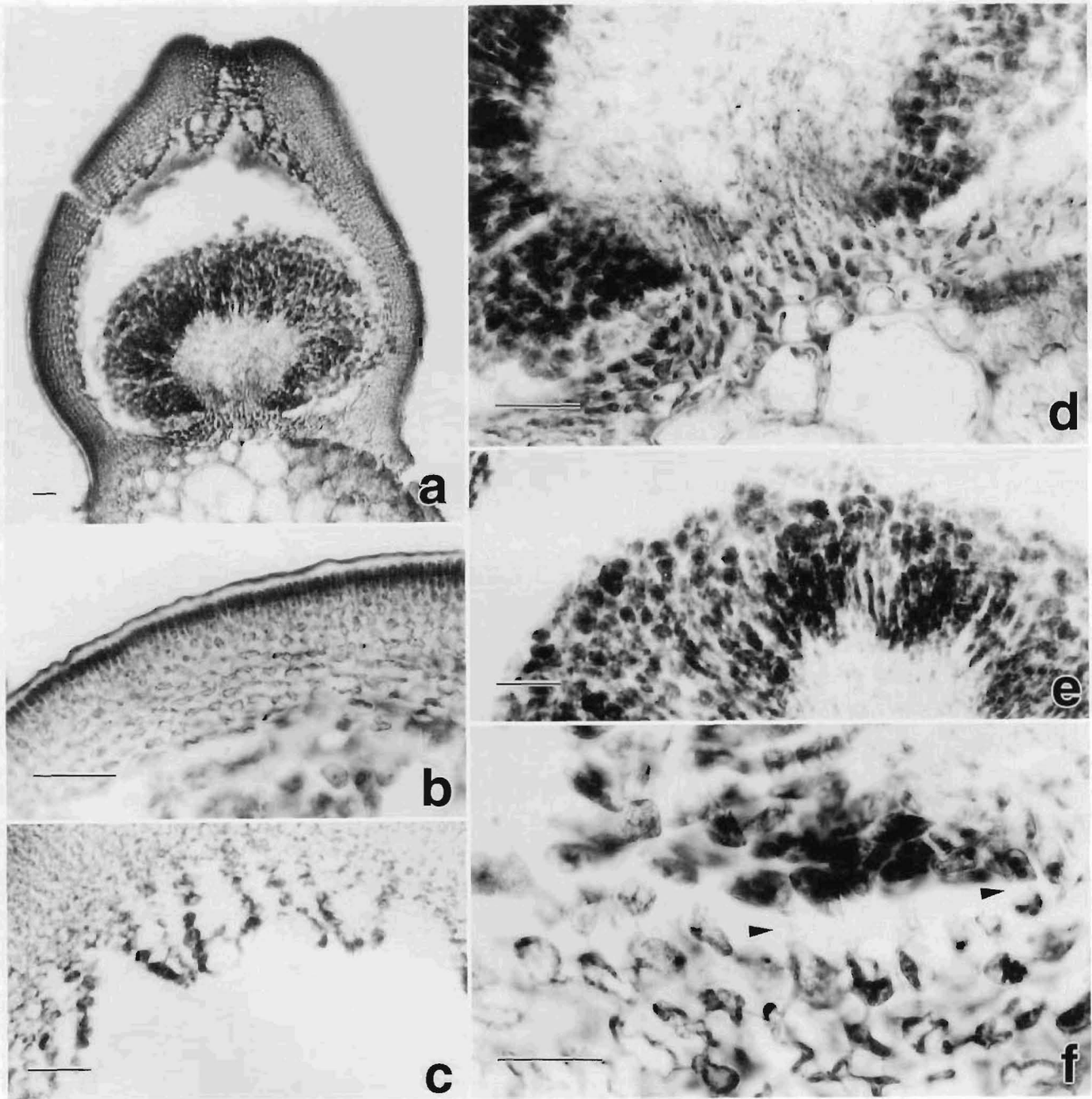


FIG. 4. *Gracilariopsis lemaneiformis*, based on cystocarpic plants from Yacila, Paita, Piura, Peru. 03.iii.1994, leg. C. Acleto & R. Zuñiga 2355. (a) Median section of mature cystocarp. Scale, 100  $\mu$ m. (b) Cell layers in the pericarp. Scale, 100  $\mu$ m. (c) Cells in the lower layer of the pericarp showing separation into separate cell files. Scale, 100  $\mu$ m. (d) Enlarged view of base of gonimoblasts seen in a. Scale, 100  $\mu$ m. (e) Carposporangial chains at periphery of gonimoblasts. Scale, 100  $\mu$ m. (f) Basal part of gonimoblasts showing conjuncture cells forming secondary pit connections (arrowheads) with cells in the cystocarp floor. Scale, 50  $\mu$ m.

2. *Gracilariopsis andersonii* (Grunow) Dawson  
1949, p. 43

Plants up to 2 m tall, yellowish brown, reddish brown, or reddish purple, consisting of few to many axial branches growing from a primary discoid holdfast or from associated prostrate stoloniferous branches (Fig. 3b); erect branches cylindrical, 0.5 to 1.5 (3.5) mm in di-

ameter, irregularly and sparingly branched, sometimes bearing numerous short proliferous branchlets (Fig. 3b); spermatangia in a continuous surface layer covering major branches; cystocarps dome-shaped, without a prominent beak, markedly constricted at the base (Fig. 5a); pericarp with a single layer of palisade-like surface cells and two to three layers of ovoid cells be-

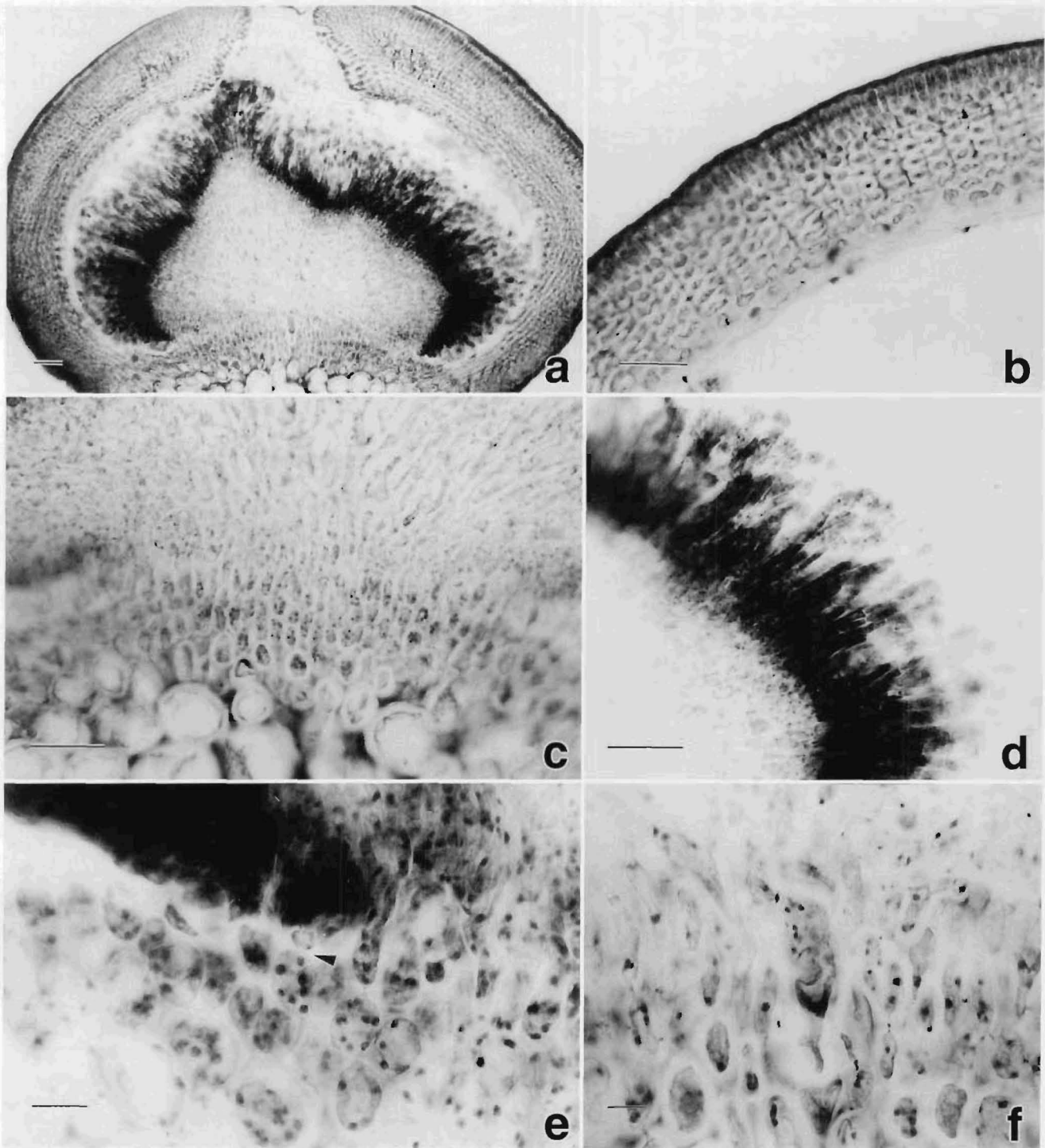


FIG. 5. *Gracilariopsis andersonii*, based on cystocarpic plants from Pigeon Point, San Mateo County, California, 20.v.1992, leg. M. H. Hommersand. (a) Median section of mature cystocarp. Scale, 100  $\mu$ m. (b) Cell layers of pericarp. Scale, 100  $\mu$ m. (c) Basal portion of gonimoblasts. Scale, 100  $\mu$ m. (d) Darkly staining carposporangial initials. Scale, 100  $\mu$ m. (e) Conjunctor cell (arrowhead) forming secondary pit connection with multinucleate cell in floor of cystocarp. Scale, 20  $\mu$ m. (f) Degenerating fusion cell embedded in cystocarp floor. Scale, 20  $\mu$ m.

low, followed by six to eight layers of regularly arranged horizontally elongate inner cells that are stretched laterally in mature cystocarps (Fig. 5b); gonimoblasts with a broad base, approximately 1.1

mm across (Fig. 5, a and c); gonimoblast mass somewhat convoluted (Fig. 5a) and bearing carposporangial initials in dense compact chains composed of two to three filamentous cells attached end to end,

each cell approximately 50  $\mu\text{m}$  long, 10  $\mu\text{m}$  wide, bearing regular carposporangial chains distally (Fig. 5d); gonimoblast cells attached to the vegetative floor of the cystocarp by a single secondary pit connection formed by ovoid to slightly hemispherical conjuctor cells (Fig. 5e); fusion cell persistent in mature cystocarps (Fig. 5, a and f), tubular nutritive cells absent.

*Basionym:* *Cordylecladia andersonii* Grunow in Piccone, 1886, p. 62 (pro parte).

*Holotype:* A collection made by C. L. Anderson and deposited in the Grunow herbarium in W. (See Abbott, 1983, fig. 4, left.)

*Type locality:* Santa Cruz, California.

*Taxonomic synonyms:* *Gracilaria sjoestedtii* Kylin 1930: 55, figs. 40 E-F, 41 A-D, 43 A-B; *Gracilaria robusta* sensu Sjoestedt non Setchell (Sjoestedt 1926: 51-64, figs. 31 F-G, 33 B-F, 34 A-B, 38 A, 39 A, 40 A, 41 C-D); *Gracilariopsis sjoestedtii* (Kylin) Dawson (1949: 40-42, pl. 15, fig. 10; pl. 16, figs 5-8; pl. 17, figs 1-9; pl. 18, fig. 4; 1961: 218, pl. 10, fig. 14, pl. 11, fig. 10, pl. 23); *Gracilaria lemneiformis* (non [Bory] Weber van Bosse 1928) Abbott (1983, fig. 4, left); *Gracilariopsis lemneiformis* (non [Bory] Dawson, Acleto et Foldvik 1964) Fredericq and Hommersand (1989b, fig. 2, 5-6). (For additional synonyms see Smith 1944.)

*Note on the nomenclature:* (from P. C. Silva). Kylin (1941, p. 21) noted that mixed specimens and collections cited in the protologue of *Cordylecladia andersonii* Grunow were referable to two different species. Because the specimens from Peru, collected by Marcacci and by Winterfeldt, were referable to a species that already had a name, *Cordylecladia lemneiformis* (Bory) Howe, Kylin designated the collections made by Anderson in California as type material ("Originalexamplare"). Howe (1914, p. 129) effectively lectotypified *Cordylecladia andersonii* with the California plant, and later Abbott (1983, p. 563, fig. 4) explicitly lectotypified the Grunow name with a specimen in Grunow's herbarium (at W) collected by Anderson at Santa Cruz, California. Kylin had available to him only two collections that had been distributed as *C. andersonii*, both from southern California: Phytotheca Boreali-Americana no. 839, collected at La Jolla by Mrs. E. Snyder; and P. B.-A. 1498 collected at San Pedro by Miss S. P. Mionks. Both of these collections are referable to a southern California-Baja California species that is distinct from the northern C. L. Anderson's species whose correct name is now *Gp. andersonii* (Grunow) Dawson (1949, p. 43). The southern species was re-described as *Gp. papenfussii* by Abbott (1983, p. 562), who designated P. B.-A. no. 839 (UC) as the type collection. Art. 7.4 of the Botanical Code covers Dawson's combination: "A new name formed from a previously published legitimate name (stat. nov., comb. nov.) is, in all circumstances, typified by the type of the basionym, even though it may have been applied erroneously to a taxon now considered not to include that type."

### 3. *Gracilariopsis carolinensis* Liao et Hommersand, sp. nov.

*Thalli usque ad 2 m alti, purpurascens vel bruneoli ad ruber rosi, affixi per hapteron parvum discoideum, saepeque secundariter per ramos curtos prostratos rhizomatososque orientes prope basem axis principalis, axes teretes, 0.5-2 mm diam., saepe parte ramosi ad pauciores, ramificatio irregularis, radialis saepeque breviprolicifica, gradualim angustata in acutiapices sed non constricta infra, cortex 4-6-stromaticus cellularum pigmentosarum; spermatangia superficialia formata in soros continuos hyalinos super amplas partes corticis externi; cystocarpia tholiformia constrictionibus indistinctis basi vel interdum constrictionibus absentibus; pericarpium monostrato cellularum superficialium atrotinctarum in vallo 2-3 strataque cellularum ovoidearum infra post 5-6 strata cellularum elongatarum, cellulae duplo longiores quam latae; gonimoblasti latibasi, usque ad 450  $\mu\text{m}$  diam., massa gonimoblasti irregulariter convoluta vel lobata ferens initia carposporangiorum curta ca. 45  $\mu\text{m}$  long. ferentium rectas catenas carposporangiorum distalis; gonimoblasti affixi ad cellulas vegetativas per synapses secundas formatas elongatis cellulis conjunctivis natis filis ramosis descendentibus; tetrasporangia ellipsoidea ad subglobosa, divisa cruciatim, 25-27.5  $\mu\text{m}$  diam. 32.5-47.5  $\mu\text{m}$  long., dispersa in corticem externum.*

Thalli up to 2 m tall, purplish or brownish to rosy red, attached by a small primary discoid holdfast, and often secondarily attached by short prostrate rhizomatous branches arising near the base of the main axis (Fig. 3c); axes terete, 0.5-2 mm diameter, often sparsely branched to a few orders, branching irregular, radial, and often short proliferous, tapering gradually to acute apices and slightly tapered but not constricted below (Fig. 3c); cortex composed of 4-6 layers of pigmented cells; spermatangia superficial, formed in continuous hyaline sori extending over large portions of the outer cortex; cystocarp dome-shaped with indistinct basal constrictions or constrictions sometimes absent (Fig. 6, a and b); pericarp with a single layer of darkly staining surface cells in palisade arrangement and 2-3 layers of ovoid cells below followed by 5-6 layers of stretched cells twice as long as broad (Fig. 6c); gonimoblasts broad-based, up to 450  $\mu\text{m}$  in diameter, the gonimoblast mass irregularly convoluted or lobed and bearing short, loosely arranged carposporangial initials approximately 45  $\mu\text{m}$  long bearing straight carposporangial chains distally (Fig. 6d); gonimoblasts attached to vegetative cells by means of secondary pit connections formed by elongate conjuctor cells borne on descending branched filaments (Fig. 6e); tetrasporangia ellipsoid to subglobose, cruciately divided, 25-27.5  $\mu\text{m}$  diameter 32.5-47.5  $\mu\text{m}$  long, scattered in the outer cortex.

*Holotype:* a female specimen collected by Max H. Hommersand on 28 November 1981 and deposited at US (US 204321).

*Isotype:* a female plant as above deposited at NCU.

*Type locality:* attached to a coquina-type limestone reef and partly buried in sand, Kure Beach, Fort Fisher, New Hanover Co., North Carolina.

*Taxonomic synonyms:* *Gracilaria confervoides* var. *longissimus* (Harvey 1853:108) *Gracilariopsis sjoestedtii* (Dawson 1953); *Gracilaria sjoestedtii* (Taylor 1960, Schneider 1976,

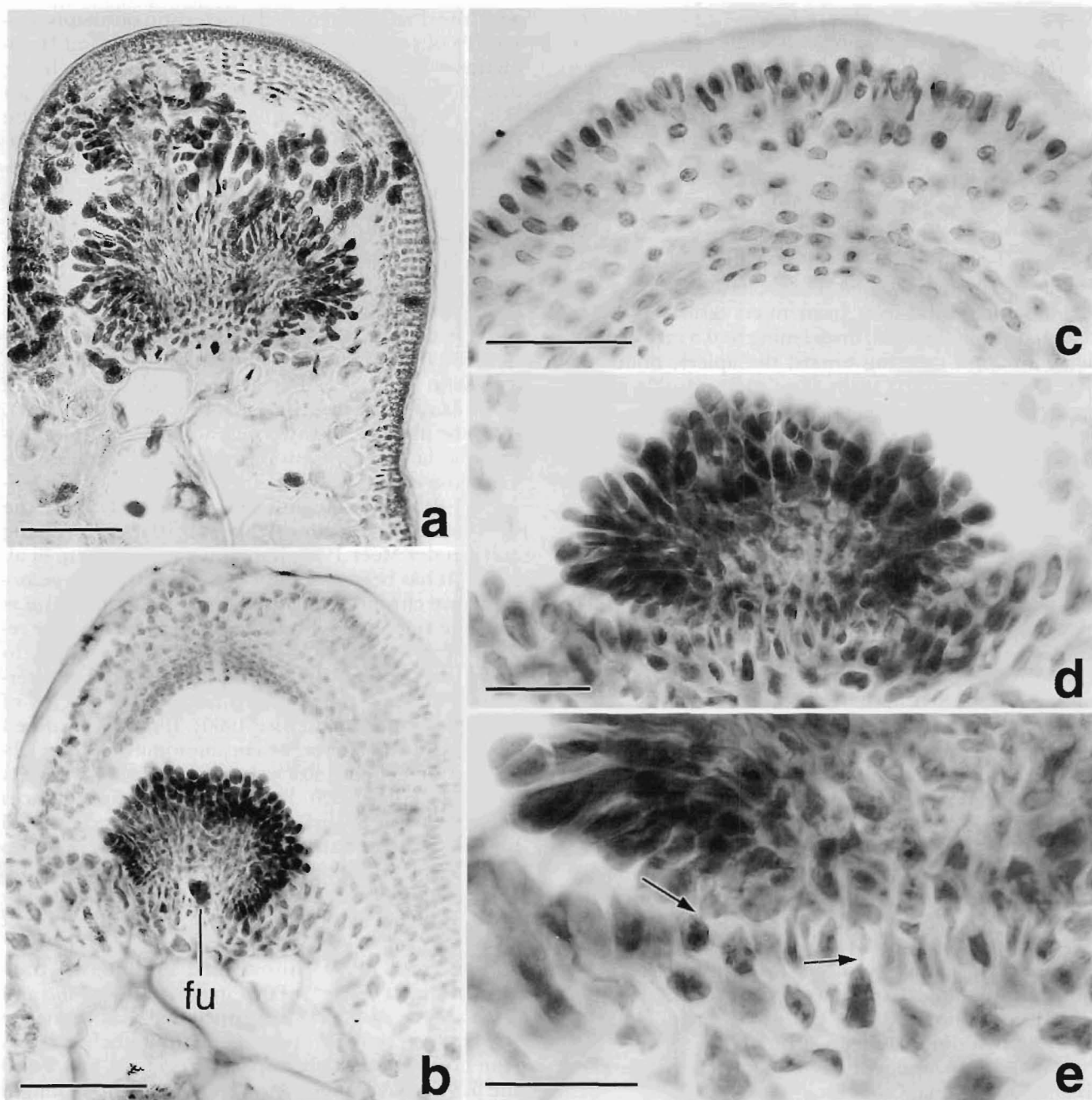


FIG. 7. *Gracilariopsis costaricensis* (NCU), based on cystocarpic plants from Playa Tamarindo, Nicoya Peninsula, Guanacaste, Costa Rica, leg. D. T. Talbot & D. W. Freshwater, 17.iii.1999. (a) Tangential section of mature cystocarp. Scale, 100  $\mu\text{m}$ . (b) Median section of mature cystocarp showing prominent fusion cell. Scale, 100  $\mu\text{m}$ . (c) Tangential section of young cystocarp. Scale, 50  $\mu\text{m}$ . (d) Basal portion showing conjuncture cells forming secondary pit connections (arrows) with multinucleate cell in floor of cystocarp. Scale, 20  $\mu\text{m}$ .

ences from the generic type species of *Gracilariopsis*, *Gp. sjoestedtii* (= *Gp. andersonii*) from California (4.49%) and from *Gp. carolinensis* from North Carolina (2.54%), similarly to the results obtained by Goff et al. (1994). *Gracilariaceae* phylogenies based on *rbcL* sequences suggest that haplotypes with values above 2% sequence divergence correspond to taxonomic species established upon morphological characters (Gurgel,

personal observations). The opposite may not be true, however, because species with less than 2% pair-wise sequence divergence are not necessarily conspecific and can sometimes be distinguished based on morphological criteria. Additional data may be required in evaluating the status of taxa that show little genetic divergence. The phylogenetic position of the group encompassing *Gp. lemaneiformis*/*Gp. costaricensis* and

*Gp. carolinensis* received strong bootstrap support in both ML and MP analyses, suggesting that they are indeed related. Cystocarpic material of *Gp. lemaneiformis* from the type locality corresponded closely to the sterile type specimen illustrated by Howe (1914, pl. 52) and Fredericq and Hommersand (1989b, fig. 1).

Our molecular data suggests that *Gp. lemaneiformis* and *Gp. costaricensis* may be conspecific, corresponding to distinct forms of a single species. However, *Gracilariopsis costaricensis* is generally shorter and narrower than *Gp. lemaneiformis* from Peru. The cystocarps are domoid and rostrate in both species (our data, Bird and Oliveira 1986). On the other hand, spermatangial sori are said to be produced in anastomosing longitudinal patches separated by extensive sterile cortex in *Gp. costaricensis* (Dawson 1949, Bird and Oliveira 1986) and to be superficial in indefinite sori in *Gp. lemaneiformis* (Dawson et al. 1964). The reported distribution of *Gp. costaricensis* from Jalisco, Mexico to Costa Rica places this species within the Eastern Pacific tropical region characterized by average water temperatures of 26 to 27° C and large yearly variations in temperature of 15 to 32° C (Lüning 1990). In contrast, *Gp. lemaneiformis* known from Paita, Peru to Taital, Prov. Antofagasto, Chile lies within the warm-temperate region of coastal upwelling provided by the Humboldt Current with average temperatures ranging from 15 to 25° C. It is unclear at this point whether the plant known in Peru and Chile as *Gp. lemaneiformis* comprises one or more species. The reliability of morphological and molecular characters for separating all the taxonomic entities in the complex will require an extensive investigation of samples throughout their geographic range. In the absence of detailed information it seems best to continue recognizing *Gp. costaricensis* in addition to *Gp. lemaneiformis*.

In agreement with the phylogenetic analyses, an evaluation of similarities and differences in cystocarp morphology among the three North and South American species formerly placed in *Gp. lemaneiformis* also suggests that *Gp. lemaneiformis* and *Gp. costaricensis* stand closer to *Gp. carolinensis* than to *Gp. andersonii*. The largest cystocarps of *Gp. lemaneiformis*, *Gp. costaricensis*, and *Gp. carolinensis* are narrow based with indistinct pericarp constrictions at the point of thallus insertion compared with the usually broader cystocarps of *Gp. andersonii* with well-defined constrictions at the base. Correspondingly, the gonimoblasts are not as massive in the three former species as those seen in large cystocarps of *Gp. andersonii*. Cystocarps in *Gracilariopsis* mature at different stages in their development and may continue to enlarge and fruit after the first release of carposporangia. For example, the cystocarp of *Gp. andersonii* illustrated by Bird and Oliveira (1986, as *Gp. lemaneiformis*) appears to represent an early fruiting stage of a young cystocarp. It is clear that the morphology must be compared over the full cycle of cystocarp development to yield useful diagnostic characters.

Five haplotypes cluster with high bootstrap support in the MP tree (BP = 92%) but are unsupported in the ML tree, namely *Gp. longissima* from England, Spain, and It-

aly and *Gp. "lemaneiformis"* from the west coast of the Gulf of California and Australia. *Gracilariopsis "lemaneiformis"* from Namibia (Stegenga et al. 1997) is basal to this clade but without bootstrap support in the MP tree and with weak support (BP = 63%) in the ML tree. In view of the lack of congruence in the MP and ML analyses and low bootstrap support, the relationship between Atlantic and Mediterranean specimens placed in *Gp. longissima* should be investigated further using a more rapidly evolving molecular marker, such as nuclear internal transcribed spacer sequences (Famá et al. 2000). The Mediterranean Sea is known for a high degree of endemism, and the Strait of Gibraltar may serve as a barrier promoting the geographic isolation and speciation of these two populations. Plants belonging to the "*longissima*" group have apparently been introduced recently into the Gulf of California, Mexico, and South Australia. The Mexican plants may have been recorded by Dawson (1949) as a slender variant of *Gp. sjoestedtii* (see Bird and Oliveira 1986, p. 318), or they may correspond to more recent introductions. Seasonal biomass densities and agar strengths and yields have been investigated for the plant sequenced here under the name *Gp. "lemaneiformis"* from the Gulf of California where commercial exploitation of this resource has begun (Pacheco-Ruiz et al. 1999). Male, female, and tetrasporangial stages have been described and illustrated for a similar plant recorded as *Gp. "lemaneiformis"* from the harbor and fishing village at Lake Butler, Robe, South Australia, where it is thought to be an adventive species (Womersley 1996, fig. 8, A-F). The origin of this invasive species in the Gulf of California and South Australia is unknown. Millar and Xia (1999) noted that their record of *Gp. lemaneiformis* from Norfolk Island might have resulted from an anthropogenic introduction event. The plant we sequenced from Swakopmund, Namibia that was recorded as *Gp. "lemaneiformis"* by Stegenga et al. (1997) is not to be confused with the more widespread *verrucosus*-type *Gracilaria* studied by Bird et al. (1994) from Luderitz, Namibia. The phylogenetic distance of the Namibian *Gracilariopsis* compared with that of the northwestern Atlantic specimens is sufficient to suggest that the Namibian plant is an undescribed species.

*Gracilariopsis tenuifrons* from Guadeloupe (French West Indies) and Venezuela and *Gp. sp.* from Mexico form a well-supported clade in MP (BP = 95%) and ML trees (BP = 82%). *Gracilariopsis tenuifrons* (Bird et Oliveira) Fredericq et Hommersand was originally described by Bird and Oliveira (1986) as a narrow freely branched species of *Gracilaria* possessing a well-developed cortex, subcortical cells in radial files, and few large medullar cells. The spermatangial cortex was distinct in having spermatangia interrupted by scattered cortical cells.

*Gracilariopsis heteroclada* from the Philippines, *Gp. "lemaneiformis"* from China and Japan, *Gp. sp.* from Venezuela, and *Gp. aff. panamensis* from Panama and Venezuela appear to form a basal group in the ML tree, but this clade is not well supported (BP = 60%). *Gracilariopsis heteroclada* Zhang et Xia in Abbott et al. (1991) was originally described as a species of *Gracilaria*

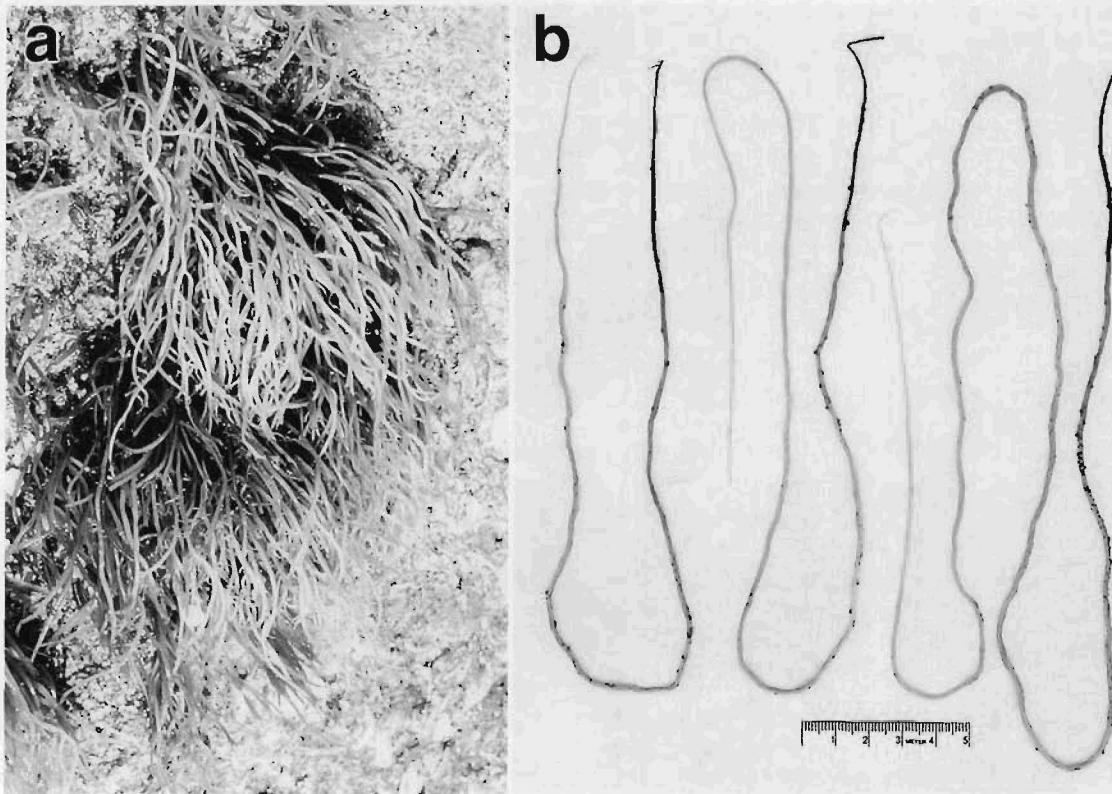


FIG. 8. *Gracilariopsis* aff. *panamensis* (LAF). Habit of cystocarpic plants from La Encrucijada, Peninsula Paraguana, Venezuela, leg. C. F. D. Gurgel, 13.vii.1999. (a) Habit of specimens in the field. (b) Herbarium-mounted specimens.

from Hainan, China, (Zhang and Xia 1988, Xia and Zhang 1999, as *G. bailinae*) and is widely distributed in the Philippines (Hurtado-Ponce and Liao 1998). *Gracilariopsis* "*lemaniformis*" is reported from China (Xia 1985, Xia and Zhang 1999, fig. 34) and from Shikoku Island in southern Japan (Yoshida 1998, as *Gracilaria lemaniformis*). This species has slender branches and greenish main axes, unlike *Gp. chorda* Holmes (1896), which is thicker with reddish axes and main branches (Chirapart et al. 1994). Chirapart et al. (1994) treated their plants from Shikoku Island as new to the Japanese flora.

*Gracilariopsis panamensis* (Taylor) Dawson (1949) was originally described as a species of *Gracilaria* by Taylor (1945: 231, pl. 76, figs. 1–4) from the Pacific coast of Panama where it has a recorded range from Costa Rica to the Galapagos Islands, Ecuador. A species found in the Caribbean Sea from Panama and Venezuela that is morphologically similar to *Gp. panamensis* is a common member of the upper subtidal zone in sandy habitats (Gurgel, personal observation). Plants of *Gp.* aff. *panamensis* are characteristically yellow in color except at the base where dense patches may be pinkish to dark brown. Usually several distinct thalli grow closely together forming clusters of long cylindrical entangled axes. Individual thalli may be branched (Fig. 8a) or unbranched (Fig. 8b). Occasionally, a few short hook-like branchlets are

formed. These occur mostly near the apices but sometimes in the middle portion of entangled axes where they may link to adjacent thalli to form clusters. Plants of exposed shores (e.g. La Encrucijada, Venezuela) are thicker and seldom ramified, whereas those from calm protected bays and *Thalassia testudinum* Koenig beds (e.g. Francisky Is., Los Roques Archipelago) are thinner and more delicate and may be more highly branched with frequent terminal hooked branchlets. The Caribbean plant is not as tall as *Gp. panamensis* Taylor, a species that reaches up to 165 cm in length. It also displays a range of morphological variability not reported for *Gp. panamensis*. Unlike the Caribbean plant, *Gp. panamensis* has two cortical cell layers and the subcortex contains several layers of larger cells. Dawson (1953) detected a tropical *Gracilariopsis* species in the southern Caribbean, which he considered to be close to, but distinct from, *Gp. sjoestedtii*. This species may be *Gp.* aff. *panamensis*, identified in this study. *Gracilariopsis* aff. *panamensis* may have passed under the name *Gp. lemaniformis* in floristic surveys during the past two decades. The Caribbean plant may represent a new species and will be the subject of further study. Should it prove to be genetically distinct but, nonetheless, related to *Gp. panamensis*, the rise of the Panama Isthmus could have been the vicariant event responsible for its isolation and subsequent speciation (Haug and Tiedemann 1998).

*Gracilariopsis* comprises a monophyletic group in the Gracilariaceae (Bird et al. 1992, 1994, Gurgel 2001). Diagnostic characters that have defined *Gracilariopsis* in the past, namely, superficial spermatangia, cystocarps lacking tubular cells, and gonimoblasts linked by secondary pit connections to modified gametophytic cells in the floor of the cystocarp were confirmed for the taxa studied. Species diversity among the character-poor species of *Gracilariopsis* has been underestimated in the eastern Pacific and western Atlantic Oceans and generally throughout the world. Even in the absence of species descriptions and diagnostic species characters, the molecular evidence presented here suggests a pattern in the biogeography of species assigned to *Gracilariopsis*. The ancestral forms in the genus, although still unknown, may have originated in the IndoPacific Ocean, as suggested by the apparent basal position of *Gp. heteroclada* in our trees and in other molecular studies (Bellorin et al. 2002). *Gracilariopsis* aff. *panamensis* may be a species belonging to this group of early diverged lineages that reached the eastern Pacific in central and South America and spread to the Caribbean Sea. There appears to be an American cluster that includes the true *Gp. lemaneiformis* and *Gp. costaricensis* together with *Gp. carolinensis* in the western Atlantic Ocean. *Gracilariopsis tenuifrons* and *Gp. sp.* from the Gulf of Mexico form a separate Western Atlantic group. Finally, *Gp. longissima* from Europe and the Mediterranean Sea comprises a group that includes an invasive species reported as *Gp. "lemaneiformis"* from the Gulf of California and also from South Australia. *Gp. "lemaneiformis"* from Namibia may also belong to this Western Atlantic assemblage.

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- Abbott, I. A. 1983. Some species of *Gracilaria* (Rhodophyta) from California. *Taxon* 32:561–4.
- Abbott, I. A. 1995. A decade of species of *Gracilaria* (sensu lato). In Abbott, I. A. [Ed.] *Taxonomy of Economic Seaweeds* Vol. V. California Sea Grant College Program, La Jolla, CA. pp. 185–94.
- Abbott, I. A. 1999. *Marine red Algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu. 477 pp.
- Abbott, I. A., Zhang, J. & Xia, B. 1991. *Gracilaria mixta* sp. nov. and other western Pacific species of the genus (Rhodophyta: Gracilariaceae). *Pacif. Sci.* 45:12–27.
- Bellorin, A. M., Oliveira, M. C. & Oliveira, E. C. 2002. Phylogeny and systematics of the marine algal family Gracilariaceae (Gracilariales, Rhodophyta) based on small subunit rDNA and ITS sequences of Atlantic and Pacific species. *J. Phycol.* 38: 551–63.
- Benson, D. A., Boguski, M., Lipman, D. J. & Ostell, J. 1994. GenBank. *Nucleic Acids Res.* 22:3441–4.
- Bhattacharya, D., Elwood, H. J., Goff, L. J. & Sogin, M. L. 1990. Phylogeny of *Gracilaria lemaneiformis* (Rhodophyta) based on sequence analysis of ITS small subunit ribosomal RNA coding region. *J. Phycol.* 26:181–6.
- Bird, C. J., van der Meer, J. P. & McLachlan, J. 1982. A comment on *Gracilaria verrucosa* (Huds.) Papenf. (Rhodophyta: Gigartinales). *J. Mar. Biol. Assoc. U.K.* 62:453–9.
- Bird, C. J., McLachlan, J. & Oliveira, E. C. 1986. *Gracilaria chilensis* sp. nov. (Rhodophyta, Gigartinales), from Pacific South America. *Can. J. Bot.* 64:2928–34.
- Bird, C. J. & Oliveira, E. C. 1986. *Gracilaria tenuifrons* sp. nov. (Gigartinales, Rhodophyta), a species from the tropical western Atlantic with superficial spermatangia. *Phycologia* 25:313–20.
- Bird, C. J., Ragan, M. A., Critchley, A. T., Rice, E. L. & Gutell, R. R. 1994. Molecular relationships among Gracilariaceae (Rhodophyta): further observations on some undetermined species. *Eur. J. Phycol.* 29:195–202.
- Bird, C. J. & Rice, E. L. 1990. Recent approaches to the taxonomy of the Gracilariaceae (Gigartinales, Rhodophyta) and the *Gracilaria verrucosa* problem. *Hydrobiologia* 204/205:111–8.
- Bird, C. J., Rice, E. L., Murphy, C. A. & Ragan, M. A. 1992. Phylogenetic relationships in the Gracilariales (Rhodophyta) as determined by 18S rDNA sequences. *Phycologia* 31:510–22.
- Bory de Saint-Vincent, J. B. G. M. 1828. Cryptogamie In Duperrey, L. I. [Ed.] *Voyage autour du monde . . . "La Coquille" . . .* Bertrand, Paris, pp. 97–200, pls. 1–39.
- Bouzon, Z. L., Miguens, F. & Oliveira, E. C. 2000. Male gametogenesis in the red algae *Gracilaria* and *Gracilariopsis* (Rhodophyta, Gracilariales). *Cyptog. Algal.* 21:33–47.
- Chang, C. F. & Xia, B. 1976. Studies on Chinese species of *Gracilaria*. *Stud. Mar. Sinica* 11:91–163 (in Chinese).
- Chirapart, A., Ohno, M. & Yamamoto, H. 1994. Occurrence of a different *Gracilaria* in Japan. In Abbott, I. A. [Ed.] *Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species*. Vol. IV. California Sea Grant College, University of California, La Jolla, CA, pp. 119–24.
- Dawson, E. Y. 1949. Studies of northeast Pacific Gracilariaceae. *Alan Hancock Foundation, Publ., Occ. Pap.* 7:1–105, 25 pls.
- Dawson, E. Y. 1953. On the occurrence of *Gracilariopsis* in the Atlantic and Caribbean. *Bull. Torrey Bot. Club* 80:314–6.
- Dawson, E. Y. 1961. Marine red algae of Pacific Mexico. Part 4. Gigartinales. *Pacif. Nat.* 2:191–343.
- Dawson, E. Y., Acleto, C. & Foldvik, N. 1964. The seaweeds of Peru. *Böih. Nova Hedw.* 13:1–111, 81 pls.
- Famá, P., Olsen, J. L., Stam, W. T. & Procaccini, G. 2000. High levels of intra- and inter-individual polymorphisms in the rDNA ITS1 of *Caulerpa racemosa* (Chlorophyta). *Eur. J. Phycol.* 35:349–56.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–91.
- Fitch, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. *Syst. Zool.* 20:406–16.
- Fredericq, S. & Hommersand, M. H. 1989a. Proposal of the Gracilariales, ord. nov. (Rhodophyta) based on an analysis of the reproductive development of *Gracilaria verrucosa*. *J. Phycol.* 25: 213–27.
- Fredericq, S. & Hommersand, M. H. 1989b. Comparative morphology and taxonomic status of *Gracilariopsis* (Gracilariales, Rhodophyta). *J. Phycol.* 25:228–41.
- Fredericq, S. & Hommersand, M. H. 1990. Diagnoses and key to the genera of the Gracilariaceae (Gracilariales, Rhodophyta). *Hydrobiologia* 204/205:173–8.

- Freshwater, D. W., Fredericq, S. & Hommersand, M. H. 1995. A molecular phylogeny of the Gelidiales (Rhodophyta) based on analysis of plastid *rbcL* nucleotide sequences. *J. Phycol.* 31:616–32.
- Freshwater, D. W. & Rueness, J. 1994. Phylogenetic relationship of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33:187–94.
- Gargiulo, G. M., de Masi, F. & Tripodi, G. 1992. Morphology, reproduction and taxonomy of the Mediterranean species of *Gracilaria* (Gracilariaceae, Rhodophyta). *Phycologia* 31:53–80.
- Gmelin, S. G. 1768. *Historia fucorum*. Academia Scientiarum, St. Petersburg. [XIII] 239 I 6 pp., 35 pls. [IA, IB, IIA, IIB, III–XXXIII].
- Goff, L. J. & Coleman, A. W. 1988. The use of plastid DNA restriction endonuclease patterns in delineating red algal species and populations. *J. Phycol.* 24:357–68.
- Goff, L. J., Moon, D. A. & Coleman, A. W. 1994. Molecular delineation of species and species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariaceae). *J. Phycol.* 30:521–37.
- Greuter, W., McNeill, J., Barrie, F. R., Burdet, H. M., Demoulin, V., Filgueiras, T. S., Nicolson, D. H., Silva, P. C., Skog, J. E., Treharne, P., Turland, N. J. & Hawksworth, D. L. 2000. *International Code of Botanical Nomenclature (St. Louis Code)*. Koeltz, Königstein. [Regnum Veg. 138] xviii + 474 pp.
- Gurgel, C. F. D. 2001. *Phylogeny, Taxonomy and Biogeography of the Gracilariaceae (Gracilariaceae, Rhodophyta) with Emphasis on Taxa from the Western Atlantic*. Ph.D. Dissertation. University of Louisiana at Lafayette, UMI microform no. 3029835, Bell & Howell-Proquest Information and Learning Press, Ann Arbor, MI. 273 pp.
- Hariot, P. A. 1889. *Algues, Mission scientifique du Cap Horn 1882–1883*. Vol. V. Botanique. Gauthier-Villars et fils, Paris, pp. 3–109, pls. 1–9.
- Harvey, W. H. 1853. *Nereis boreali-americana*. Part II. Rhodospereae. *Smithsonian Contributions to Knowledge* 5. 258 pp., pls. 13–36.
- Haug, G. H. & Tiedemann, R. 1998. Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. *Nature* 393:673–6.
- Hillis, D. M. & Huelsenbeck, P. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *J. Hered.* 83:189–95.
- Holmes, E. M. 1896. New marine algae from Japan. *J. Linn. Soc. Lond. Bot.* 31:248–60, pls. 7–12.
- Holmgren, P. K., Holmgren, N. H. & Barnett, L. 1990. *Index Herbariorum. Part I: The Herbaria of the World*, 8th ed. International Association of Plant Taxonomy, New York Botanical Garden, Bronx, New York, x + 693 pp. [Regnum vegetabile vol. 120]
- Hommersand, M. H., Fredericq, S. & Freshwater, D. W. 1994. Phylogenetic systematics and biogeography of Gigartinales (Gigartinales, Rhodophyta) based on sequence analysis of *rbcL*. *Bot. Mar.* 37:193–203.
- Howe, M. A. 1914. The marine algae of Peru. *Mem. Torrey Bot. Club.* 15:1–185, 66 pls.
- Hurtado-Ponce, A. Q., & Liao, L. 1998. The genus *Gracilariopsis* (Rhodophyta, Gracilariaceae) in the Philippines: morphological and taxonomic confirmations. *Philipp. Scient.* 35:141–51.
- Kapraun, D. F. 1980. *An Illustrated Guide to the Benthic Marine Algae of Coastal North Carolina. I. Rhodophyta*. University of North Carolina Press, Chapel Hill, vii + 206 pp.
- Kapraun, D. F. 1993. Karyology and cytophotometric estimation of nuclear DNA content variation in *Gracilaria*, *Gracilariopsis* and *Hydropuntia* (Gracilariaceae, Rhodophyta). *Eur. J. Phycol.* 28:253–60.
- Kapraun, D. F., Dutcher, J. A. & Freshwater, D. W. 1993. Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariaceae and Gelidiales. *Hydrobiologia* 260/261:679–88.
- Kylin, H. 1930. Über die Entwicklungsgeschichte der Florideen. *Lunds Univ. Årsskr.*, N. F. Afd. 2, 23:1–104.
- Kylin, H. 1941. Californische Rhodophyceen. *Lunds Univ. Årsskr.*, N. F. Afd. 37:1–55.
- Lewmanomont, K. 1994. The species of *Gracilaria* from Thailand. In Abbott, I. A. [Ed.] *Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species*. Vol. IV. California Sea Grant College, University of California, La Jolla, CA, pp. 135–48.
- Lin, S. M., Fredericq, S. & Hommersand, M. H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbcL* sequences, including the Phycodroideae, subfam. nov. *J. Phycol.* 37:881–99.
- Littler, D. S. & Littler, M. M. 2000. *Caribbean Reef Plants: An Identification Guide to the Reef Plants of the Caribbean, Bahamas, Florida and Gulf of Mexico*. Offshore Graphics Inc., Washington, DC, 542 pp.
- Lüning, K. 1990. *Seaweeds. Their Environment, Biogeography, and Ecology*. John Wiley & Sons, New York, 527 pp.
- Millar, A. J. & Xia, B. 1999. The genera *Gracilaria* and *Gracilariopsis* from Norfolk Island, Southwestern Pacific. In Abbott, I. A. [Ed.] *Taxonomy of Economic Seaweeds*. Vol. VII. I. California Sea Grant College Program, La Jolla, CA, pp. 113–9.
- Pacheco-Ruiz, I., Zertuche-González, J. A., Correa-Díaz, F., Arellano-Carbajal, F. & Chee-Barragan, A. 1999. *Gracilariopsis lemaneiformis* beds along the west coast of the Gulf of California, Mexico. *Hydrobiologia* 398/399:509–14.
- Papenfuss, G. F. 1967. Notes on algal nomenclature. V. Various Chlorophyceae and Rhodophyceae. *Phykos* 5:95–105.
- Piccone, A. 1886. *Alghie del viaggio di circumnavigazione della vettura Pisani*. R. Istituto Sordo-muti, Genova, 97 pp., 2 pls.
- Posada, D. & Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.
- Price, J. H., John, D. M. & Lawson, G. W. 1988. Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. IV. Rhodophyta (Florideae) 2. Genera G. *Bull. Br. Mus. (Nat. Hist.) Bot.* 18:195–273.
- Ramírez, M. E. & Santelices, B. 1991. *Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica*. Monografías Biológicas no. 5. Fac. Cienc. Biol., Pontificia Univ. Católica Chile, 437 pp.
- Ramírez, M. E. & Tapia, L. 1991. *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto & Foldvik en el norte de Chile (Rhodophyta, Gracilariaceae). *Rev. Chil. Hist. Nat.* 64:323–30.
- Schneider, C. W. 1976. Spatial and temporal distributions of benthic marine algae on the continental shelf of the Carolinas. *Bull. Mar. Sci. Gulf Caribb.* 26:133–51.
- Schneider, C. W. & Searles, R. B. 1991. *Seaweeds of the Southeastern United States. Cape Hatteras to Cape Canaveral*. Duke University Press, Durham & London, xiv + 553 pp.
- Scholfeld, C. I., Gacesa, P., Price, J. H., Russell, S. J. & Bhoday, R. 1991. Restriction fragment length polymorphism of enzymically-amplified small-subunit rRNA-coding regions from *Gracilaria* and *Gracilariopsis* (Rhodophyta)—a rapid method for assessing “species” limits. *J. Appl. Phycol.* 3:329–34.
- Silva, P. C., Basson, P. W. & Moe, R. L. 1996. *Catalogue of the Marine Algae of the Indian Ocean*. University of California publ. Bot., Vol. 79. University of California Press, Berkeley, 1259 pp.
- Sjoestedt, L. G. 1926. Floridean studies. *Acta Univ. Lund, N. F., Avd.* 2. 22:241–323.
- Smith, G. M. 1944. *Marine Algae of the Monterey Peninsula, California*. Stanford University, Stanford, CA, 622 pp., 98 pls.
- Stentoft, M., Irvine, L. M. & Farnham, W. F. 1995. Two terete species of *Gracilaria* and *Gracilariopsis* (Gracilariaceae, Rhodophyta) in Britain. *Phycologia* 34:113–27.
- Stengena, H., Bolton, J. J. & Anderson, R. J. 1997. *Seaweeds of the South African West Coast*. Contr. Bolus Herbarium Nr. 18, 655 pp.
- Stevens, R. B. 1981. *Mycology Guidebook*. University of Washington Press, Seattle, 712 pp.
- Swofford, D. L. 2002. *PAUP\*: Phylogenetic Analysis Using Parsimony (and Other Methods)*. Version 4.0, beta release version 10. Sinauer Associates, Sunderland, Massachusetts.
- Taylor, W. R. 1945. Pacific marine algae of the Allan Hancock expeditions to the Galapagos Islands. *Allan Hancock Pacif. Exped.* 12: [3]. iv + 528 pp.
- Taylor, W. R. 1960. *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*. University of Michigan Press, Ann Arbor, xi + 870 pp.
- Weber-van Bosse, A. 1928. Liste des algues du Siboga, IV. Rhodophyceae, Part 3. Gigartinales et Rhodymeniales. *Siboga Exp. Monogr.* 59d:393–533.
- Wiseman, D. R. & Schneider, C. W. 1976. Investigations of the marine algae of South Carolina. I. New records of Rhodophyta. *Rhodora* 78:516–24.
- Wittmann, W. 1965. Aceto-iron-haematoxylin-chloral hydrate for chromosome staining. *Stain Technol.* 40:161–4.
- Womersley, H. B. S. 1996. *The Marine Benthic Flora of the Southern Australia. Part IIIB.—Gracilariaceae, Rhodymeniales, Corallinales and*



- Bonnemaisoniales*. Australian Biological Resources Study, Canberra, 392 pp.
- Wynne, M. J. 1998. A checklist of the benthic marine algae of the tropical and subtropical western Atlantic: first revision. *Nova Helv.* 116: 1-155.
- Xia, B. 1985. *Gracilaria* from China: key, list and distribution of the species. In Abbott, I. A. & Norris, J. N. [Eds.] *Taxonomy of Economic Seaweeds*. Vol. I. California Sea Grant College Program, La Jolla, CA, pp. 71-6.
- Xia, B. & Zhang, J. 1999. *Flora Algarum Marimum Sinicarum. Tomus II, Rhodophyta. No. V. Ahnfeltiales, Gigartinales, Rhodymeniales*. Florarum Cryptogamarum Sinicarum Academiae Sinicae Edita, Science Press, 201 pp., XI pls. (in Chinese).
- Yoshida, T. 1998. *Marine Algae of Japan*. Uchida-Rokakuho, Tokyo, 25 + 1222 pp. (in Japanese).
- Zhang, J. & Xia, B. 1988. On two new *Gracilaria* (Gigartinales, Rhodophyta) from South China. In Abbott, I. A. [Ed.] *Taxonomy of Economic Seaweeds with Reference to some Pacific and Caribbean Species*. Vol. II. California Sea Grant College, University of California, La Jolla, CA, pp. 131-36, figs. 1-17.
- Zhang, X. & van der Meer, J. P. 1988. A genetic study on *Gracilaria sjoestedtii*. *Can. J. Bot.* 66:2022-6.

## RELATIONSHIP BETWEEN PRESENCE OF A MOTHER CELL WALL AND SPECIATION IN THE UNICELLULAR MICROALGA *NANNOCHLORIS* (CHLOROPHYTA)<sup>1</sup>

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The cell division mechanisms of seven strains from six species of *Nannochloris* Naumann were analyzed and compared with those of three species of *Chlorella* Beijerinck and *Trebouxia erici* Ahmadjian using differential interference microscopy and fluorescence microscopy. *Nannochloris bacillaris* Naumann divides by binary fission and *N. coecoides* Naumann divides by budding. Distinct triangular spaces or mother cell walls were found in the dividing autosporangia of the other five strains from four species of *Nannochloris*, three species of *Chlorella*, and *T. erici*. In an attempt to infer an evolutionary relationship between nonautosporic and autosporic species of *Nannochloris*, we constructed a phylogenetic tree of the actin genes using seven strains from six species of *Nannochloris*, three species of *Chlorella*, and *T. erici*. *Nannochloris* species were polyphyletic in the Trebouxiophyceae group. Two nonautosporic species of *N. bacillaris* and *N. coecoides* were monophyletic and positioned distally. Moreover, to determine their phylogenetic position within the Trebouxiophyceae, we constructed phylogenetic tree of 18S rRNA genes adding other species of Trebouxiophyceae. *Nannochloris* species were polyphyletic in the Trebouxiophyceae and appeared in two different lineages, a *Chlorella*–*Nannochloris* group and a *Trebouxia*–*Choricystis* group. The nonautosporic species, *N. bacillaris* and *N. coecoides*, and three autosporic species of *Nannochloris* belonged to the *Chlorella*–*Nannochloris* group. *Nannochloris bacillaris* and *N. coecoides* were also monophyletic and positioned distally in the phylogenetic tree of 18S rRNA genes. These results suggest that autospore formation is the ancestral mode of cell division in *Nannochloris* and that nonautospore formation mechanisms, such as binary fission and budding, evolved secondarily.

**Key index words:** 18S rRNA; actin gene; autospore formation; binary fission; budding; *Chlorella*; Chlorophyta; molecular phylogeny; *Nannochloris*; *Trebouxia*

**Abbreviations:** ME, minimum evolution; MP, maximum parsimony; TBR, tree bisection reconnection

Cell division is one of the most important and fundamental processes in all living organisms. The plant cell is enclosed by a rigid cell wall. New daughter cells are generated in the space that is enclosed by the wall of the mother cell. Terrestrial plants multiply by phragmoplast formation, whereas some algae multiply by autospore formation. Because protoplast division and daughter cell wall formation occur in the mother cell wall, the daughter cells are enclosed by the mother cell wall during autospore formation.

The presence of a mother cell wall is a central issue in the taxonomy of *Nannochloris*. According to Naumann's (1921) original description of the type species *N. bacillaris*, this alga propagates by binary fission. The second species described in the genus, *N. coecoides*, was also reported to undergo binary fission. Naumann emphasized that autospore formation did not occur within this genus (Krienitz et al. 1996). Several marine species were later added to the genus (Butcher 1952, Droop 1955, Jeffrey 1961, Thomas 1966). However, autospore formation was observed subsequently in most marine species and freshwater *N. coecoides* (SAG 251-1) by EM (Sarokin and Carpenter 1982, Brown and Elfman 1983, Menzel and Wild 1989). Menzel and Wild (1989) insisted that *Nannochloris* was characterized by autospore formation and transferred the autospore forming *Nannochlorium eucaryotum* Wilhelm et al. to the genus *Nannochloris* as *N. eucaryotum* (Wilhelm et al.) Menzel et Wild. However, Shimada et al. (1993) reisolated *N. bacillaris* and verified Naumann's (1921) original findings. Krienitz et al. (1996) insisted that discussions of whether autospore formation was a feature of *Nannochloris* were meaningless, because the conclusions were based on a misconception of the genus *Nannochloris*. Indeed, they transferred the autospore forming "*N. coecoides* SAG251-1" to the genus *Choricystis* (Skuja) Fott and redesignated it as *C. minor*. Huss et al. (1999) suggested that the revised name "*Nannochloris eucaryotum*" denoted by Menzel and Wild was untenable and that the original genus name "*Nannochlorium*" should be maintained. In addition, Woess (1999) insisted that *N. coecoides* (CCAP251/6), which was established by Hibberd (1981), was not a *Nannochloris* species but instead was *Marvania geminata* Hindák (Hindák 1976), which reproduces by budding.

Currently, it seems that the genus *Nannochloris* contains dubious species that could probably be trans-

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