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SYSTEMATICS OF *GRATELOUPIA FILICINA* (HALYMENIACEAE, RHODOPHYTA), BASED ON *rbc*L SEQUENCE ANALYSES AND MORPHOLOGICAL EVIDENCE, INCLUDING THE REINSTATEMENT OF *G. MINIMA* AND THE DESCRIPTION OF *G. CAPENSIS* SP. NOV.[1]

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Grateloupia filicina (C. Agardh) Lamouroux, originally described from the Mediterranean Sea, has long been considered a textbook example of a marine red alga with a cosmopolitan distribution. An _rbc_L-based molecular phylogeny, encompassing samples covering the entire geographic distribution of the species, revealed a plethora of "cryptic" species, whereby the presence of genuine _G. filicina_ is limited to the Mediterranean basin. The phylogeny revealed a strong biogeographic imprint, with specimens from temperate regions resolved in clades composed of species inhabiting the same geographic region. Presence of widely divergent morphologies in the temperate clades indicated that several lineages have converged independently to a _G. filicina_-type morphology. Tropical representatives are resolved in a single clade with very uniform _G. filicina_-type morphology and pairwise sequence divergences that are lower than the average divergence observed in temperate lineages. This, combined with a lack of clear geographic structure among the tropical lineages, may indicate a more recent divergence with long-range dispersal capacities. Violations to the biogeographic signal in temperate lineages seemed to be due to either inadequate taxonomy or recent introductions. _Grateloupia minima_ P. & H. Crouan, a taxon placed in synonymy under _G. filicina_, is reinstated as a separate species distributed in the northeast Atlantic Ocean. _Grateloupia capensis_ sp. nov. is described to accommodate specimens from South Africa with a _G. filicina_-type morphology, and _G. filicina_ var. _luxurians_ is elevated to species status. Morphological and anatomical characters were put forward that support the distinctiveness of these three distinct species.

Key index words: **biogeography; cryptic diversity; _Grateloupia_; _Grateloupia filicina_; Halymeniaceae; molecular phylogeny; _rbc_L; systematics; taxonomy**

Abbreviations: **BI, Bayesian inference; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining**

The red algal genus *Grateloupia* (Halymeniaceae, Rhodophyta) is characterized by non-procarpic thalli in which auxiliary cells and two-celled carpogonial branches are situated in separate accessory branch systems, termed ampullae. The auxiliary cell ampullae of *Grateloupia* are simple, composed of a primary filament and two to three unbranched secondary filaments (Sjöstedt 1926, Kylin 1930, Chiang 1970, Kawaguchi et al. 2004). Investigations primarily based on comparative gene sequence analysis of the chloroplast-encoded large subunit of the RUBISCO gene (*rbc*L) have shown that other genera of the Halymeniaceae, characterized by identical ampullary structures, fall within a large *Grateloupia* clade, thereby strengthening the belief of Chiang (1970), that the nature of the auxiliary cell ampullae holds the key to a natural classification of the Halymeniaceae. Consequently, *Prionitis* and *Phyllymenia*, both characterized by *Grateloupia*-type auxiliary cell ampullae, have been merged in *Grateloupia*, making the genus by far the largest of the family (Wang et al. 2001, De Clerck et al. 2005). Vegetative characters

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such as overall habit, texture, relative presence of proliferations and midribs, cortex structure, and the location of reproductive structures are considered highly homoplasious and therefore are said to be of limited use at the generic level (Wang et al. 2001).

Apart from refining generic delineations, the abovementioned molecular studies indicated the presence of extensive cryptic diversity in the genus. Samples accredited to the same name, but from disjunct geographic areas, often belong to different genealogical lineages. De Clerck et al. (2005) demonstrated this for *Grateloupia dichotoma* from Europe and Brazil, and several western Pacific taxa (Wang et al. 2000, Kawaguchi et al. 2001, Faye et al. 2004), which at one stage were placed in synonymy with *G. filicina*, have been reinstated or described *de novo* if necessary. The latter species, originally described from the Gulf of Trieste, Adriatic Sea and subsequently reported from most cold temperate to tropical regions, is in dire need of a critical revision. As a general trend, virtually any *Grateloupia* species with a finely pinnate thallus has been considered as *G. filicina*. Specimens with slightly deviant morphologies were often described as infraspecific taxa regardless of geographic origin, leading to the description of several varieties and forms from most of the world's oceans (Table 1).

Here we report on the diversity in *G. filicina* observed from a molecular perspective. We used molecular sequence data of *rbc*L. Even though the analysis includes samples from all the world's oceans, the emphasis from a taxonomic point of view is placed on the temperate Atlantic representatives. Because of the large amount of tropical taxa reported or described (either validly or invalidly) from the tropical regions (Table 1), the correct identities of those taxa are dealt with in separate publications.

MATERIALS AND METHODS

Morphological analyses. Morphological observations were made on specimens preserved in a 5% formalin–seawater solution. Whole-mount and sectioned material was stained with

TABLE 1. Validly published *Grateloupia* species and infraspecific taxa associated with *G. filicina*, with indication of their original description, type locality, and current taxonomic status.

Name	Type locality	Comment
G. catenata Yendo, 1920:9	Japan	Reinstated by Wang et al. (2000)
G. concatenata Kützing, 1843:397	West Indies	Considered a synonym of *G. filicina* by Taylor (1960)
G. filicina (Lamouroux) C. Agardh, 1822:223		See Silva et al. (1996) for detailed nomenclatural notes
Delesseria filicina Lamouroux, 1813:125	Trieste, Italy	
G. filicina f. *cirrhosa* Børgesen, 1935: 54	Bombay, India	
G. filicina f. *horrida* (Kützing) Børgesen, 1935:53		
G. horrida Kützing, 1843:397	Palermo, Naples, Italy	
G. filicina f. *pectinata* Børgesen, 1935:53	Bombay, India	
G. filicina var. *conferta* Kützing, 1847:775	Java, Indonesia	
G. filicina var. *congesta* P. Crouan & H. Crouan *in* Schramm & Mazé, 1865:9	Basse-Terre, Guadeloupe	
G. filicina var. *cylindricaulis* Solier *in* Castagne, 1845:233	Ile de Riou, Marseille, Mediterranean France	
G. filicina var. *elongata* Kützing 1847:775	Java, Indonesia	
G. filicina var. *filiformis* (Kützing) P. Crouan & H. Crouan *in* Mazé & Schramm, 1878:155		Considered a separate species by Yokoya et al. (1993)
G. filiformis Kützing, 1849:731	Peru	
G. filicina var. *lomentaria* Howe, 1924:142	East Cliff, vicinity of Pei-Tai-Ho, China	Considered a synonym of *G. catenata* by Wang et al. (2000)
G. filicina var. *luxurians* A. Gepp & E. S. Gepp, 1906:259	Farm Cove, Sydney, Australia	
G. filicina var. *porracea* (Kützing) Howe, 1924:142		
G. porracea Kützing, 1843:397	West Indies	
G. filicina f. *prolongata* (J. Agardh) Tseng, 1936:42		Considered a separate species by Yoshida and Kawaguchi (1998).
G. prolongata J. Agardh, 1847:10	Pochutla, Pacific Mexico	
G. filicina var. *ramentacea* Montagne, 1836:322	Borders of the Seine, Atlantic France	
G. filicina var. *simplex* Solier in Castagne, 1845:233	Cap Croisette, Marseille, Mediterranean, France	
G. fimbriata Montagne, 1846:102	Algeria	Status uncertain
G. lancifera Montagne, 1856:433	Martinique	Synonym of *G. filicina* on the authority of J. Agardh (1876) and Taylor (1960)
G. minima P. Crouan & H. Crouan, 1867:142	Brest, Atlantic France	
G. pennatula (Pöppig) Kützing, 1847:24		
Sporochnus pennatula Pöppig *in* Sprengel, 1827:329	Cuba	Synonym of *G. filicina* on the authority of J. Agardh (1876) and Taylor (1960)
G. subpectinata Holmes, 1912:208	Japan	Considered a synonym of *G. filicina* by Okamura (1936) and Yoshida and Kawaguchi (1998) but reinstated by Faye et al. (2004)

aniline blue (1% w/v, acidified with 5% 1 N HCl) and mounted in Karo® syrup. Photographs were taken with a BX60 photomicroscope (Olympus, Melville, NY, USA) with a DMC Ie digital camera (Polaroid, Cambridge, MA, USA). Herbarium abbreviations follow Holmgren et al. (1990).

Molecular analyses. Sample information included in the molecular phylogenetic study is listed in Table 2. The geographic origin of the respective *G. filicina* specimens and allied taxa is represented in Figure 1. The DNA was extracted from silica gel-dried specimens or, if none was available, from herbarium material. Voucher specimens of the samples are deposited in the Ghent University Herbarium (GENT), the University of Louisiana at Lafayette (LAF), or the University of Santiago de Compostela (SANT). Sequences of *rbc*L were obtained as outlined by Gavio and Fredericq (2002) or De Clerck et al. (2005). Generated sequences were aligned manually in MacClade 4.0 (Maddison and Maddison 2000), and 28 previously published sequences were added to the data set. Additional sequences were carefully selected to cover the global phylogeny of the genus, ensuring a good representation of all major clades. Because of missing data at the 5′ and 3′ ends of the *rbc*L sequences, the first 107 and last 102 sites of 1467-bp gene were excluded from the analyses, leaving a total of 1259 bp.

Maximum parsimony (MP), neighbor joining (NJ), and maximum likelihood (ML) analyses were performed using PAUP 4.0b10 (Swofford 2002). MrBayes 3.0 (Huelsenbeck and Ronquist 2001) was used for Bayesian inference (BI). In MP analysis all characters and character changes were weighted equally. Heuristic searches, consisting of 500 replicates of random sequence additions, were performed with TBR and Multrees in effect. The MP bootstrap analysis consisted of 1000 replications of full heuristic searches. Before ML analysis, a hierarchical likelihood ratio test was performed in Modeltest 3.06 (Posada and Crandall 1998) to select the substitution model best fitting the data set. The parameters of the selected model were then fixed and used to analyze the data sets under NJ and ML, the latter using a heuristic search with 100 replicates of random sequence additions and TBR. The ML bootstrap analyses were not performed because of computational limitations. The optimal model selected for the *rbc*L data set was a general time reversible model with gamma distribution (GTR + G). The parameters estimated were as follows: nucleotide frequencies A = 0.3274; C = 0.1296; G = 0.2023; T = 0.3406; gamma distribution with shape parameter = 0.2895. Nucleotide substitution models for BI were calculated using MrModeltest (Nylander 2002). Posterior probabilities were calculated using a Metropolis-coupled Markow chain Monte Carlo approach with sampling according to the Metropolis-Hastings algorithm. The analysis used four chains, one cold and three incrementally heated. A single run consisted of 1 million generations that were sampled every 100th tree. Likelihood values reached a stable value after 5000 generations. To ensure that we included only trees after the chain had reached a stable ("burn-in") value, we fixed the burn-in for all analyses at 100,000 generations, which produced 9000 sampled trees and corresponding posterior probability distributions. Sequences were submitted to EMBL (see Table 2 for accession numbers) and the alignment, including the various trees, to TreeBASE (accession number S1243).

RESULTS

rbc*L analysis.* In the 1259-bp alignment, including the four outgroup taxa (*C. luxurians*, *H. durvillei*, *H. floresia*, and *P. constrictus*), 399 characters were variable, of which 317 were parsimony informative. The alignment contained no insertions or deletions. Phylogenetic trees constructed with MP, ML, NJ, and BI were similar in overall topology (see TreeBASE accessing number S1243). Only the ML tree is shown in Figure 2. The MP and NJ trees revealed an identical topology, as did the ML and BI trees. Relative differences in the placement of certain lineages between the MP and ML are shown in Figure 3. Those differences related to clades that received little or no bootstrap support or posterior probabilities. The MP analysis resulted in 108 most parsimonious trees (1087 steps) differing only in the relative placement of the various Mediterranean *G. filicina* isolates and in the topology of the tropical *G. filicina* clade. The MP trees differed topologically from the single ML tree in the placement the *G. doryphora–schizophylla* clade. In the ML and BI analyses, the *G. doryphora–schizophylla* clade formed a monophyletic lineage sister to the northeast Atlantic *G. filicina* clade. The NJ and MP analyses retained the position of the northeast Atlantic *G. filicina* isolates as the sister group of the Pacific *Grateloupia* clade, but *G. doryphora* and *G. schizophylla* came out completely basal with respect to the other *Grateloupia* species. The uncertainty on the placement of *G. doryphora* and *G. schizophylla* is reflected in the low bootstrap values in all analyses (unresolved in both MP and NJ; 71 in BI). An additional topological difference was related to the position of the Mediterranean *G. filicina* samples and the *G. catenata–G. ramosissima* clade. The respective clades in the ML analysis were resolved, without bootstrap support, as two separate clades, sister to the remaining *Grateloupia* species. The MP and NJ analyses placed both clades as a monophyletic lineage, sister to the tropical *G. filicina* clade. An MP bootstrap consensus tree, however, showed that the position of those respective clades should be considered as unresolved. Only in the NJ analysis did this topology receive moderate support (71%–78% bootstrap support). Bayesian inference left the position of the respective clades unresolved.

The different phylogenetic analyses were unequivocal in that *G. filicina* samples were placed in several separate lineages. The Mediterranean isolates formed a well-supported clade with minimal sequence divergence (0–0.3%) that was either sister to or just basal to a predominantly tropical *G. filicina* clade. In the MP and NJ tree, the Mediterranean specimens grouped with *G. catenata* and *G. ramosissima*, both from the western Pacific Ocean. As discussed above, however, this grouping received no satisfactory support in any of the analyses. A large tropical clade of specimens traditionally named *G. filicina* was moderately supported in all analyses and revealed variable sequence divergence between the different samples, ranging from 0.1 to 5.7. Several clusters with nearly identical sequences could be discerned. Sequences were identical or showed only few differences among the Indian Ocean samples from Madagascar and Sri Lanka, Caribbean samples from Venezuela and the Dutch West Indies, samples from Florida (USA), and samples from Galveston and Port Aransas, Texas (USA) in the northwestern Gulf of

TABLE 2. List of species used in *rbc*L analysis with GenBank accession numbers.

Species	Location and collecting data	Accession no.	Source
Cryptonemia luxurians (C. Agardh) J. Agardh	Praia Rasa, Rio de Janeiro, Brazil	AF488813	Gavio and Fredericq 2002
Halymenia durvillei Bory	Beruwela, Sri Lanka	AY772020	De Clerck et al. 2005
Halymenia floresia (Clemente) C. Agardh	Illes Formigues, Palamos, Girona, Spain	AY772019	De Clerck et al. 2005
Polyopes constrictus (Turner) J. Agardh	Kommetjie, Cape Peninsula, South Africa	AF385642	Hommersand and Fredericq 2003
Grateloupia americana Kawaguchi & Wang	Pigeon Point, San Matio Co., California, USA	AY772037	De Clerck et al. 2005
G. asiatica Kawaguchi & Wang	Qingdao, Shadong Province, China	AY178763	Gavio and Fredericq 2002
G. asiatica Kawaguchi & Wang	Qingdao, Shadong Province, China	AY178762	Gavio and Fredericq 2002
G. belangeri (Bory) De Clerck	Yzerfonteyn, Western Cape Prov., South Africa	AY772027	De Clerck et al. 2005
G. catenata Yendo	Shinori, Hokadate, Hokkaido, Japan	AB038613	Wang et al. 2000
G. dichotoma J. Agardh	Lugo, Galicia, Spain	AY772031	De Clerck et al. 2005
G. dichotoma J. Agardh	Marataizes, Espiritu Santu, Brazil	AF488824	Gavio and Fredericq 2002
G. doryphora (Montagne) Howe	Playa de San Francisco, Lima, Peru	AF488817	Gavio and Fredericq 2002
G. filicina (Lamouroux) C. Agardh 1	Kommetjie, Cape Peninsula, South Africa, (S. Fredericq, 31.i.2001)	AJ868465	This study
G. filicina (Lamouroux) C. Agardh 2	Kommetjie, Cape Peninsula, South Africa, (O. De Clerck, 1.vi.2003)	AJ868466	This study
G. filicina (Lamouroux) C. Agardh 3	Yzerfonteyn, Western Cape Prov., South Africa, (O. De Clerck, 2.vi.2003)	AJ868467	This study
G. filicina (Lamouroux) C. Agardh 4	Audresselles, Nord-Pas de Calais, France, (E. Coppejans, 1.ix.1980)	AJ868468	This study
G. filicina (Lamouroux) C. Agardh 5	Boulogne sur Mer, Nord-Pas de Calais, France, (E. Coppejans, 16.ix.1978)	AJ868469	This study
G. filicina (Lamouroux) C. Agardh 6	Forte do Cão, Praia de Âncora, Portugal, (R. Araúa, 27.ix.2003)	AJ868470	This study
G. filicina (Lamouroux) C. Agardh 7	Aúlia, Esposende, Portugal, (R. Araúja, 28.ix.2003)	AJ868471	This study
G. filicina (Lamouroux) C. Agardh 8	Moledo, Caminha, Portugal, (R. Araúja, 30.ix.2003)	AJ868472	This study
G. filicina (Lamouroux) C. Agardh 9	Banyuls, France, (E. Coppejans, 25.vii.1976)	AJ868473	This study
G. filicina (Lamouroux) C. Agardh 10	Cala Aguafreida, Begur, Girona, Spain, (L. Lavelli, 1.v.2002)	AJ868474	This study
G. filicina (Lamouroux) C. Agardh 11	Roques Planes, Palamós, Spain, (N. Sánchez, 4.v.2004)	AJ868475	This study
G. filicina (Lamouroux) C. Agardh 12	Ponteleria, Sicily, Italy, (G. Furnari, 3.iv.2003)	AJ868476	This study
G. filicina (Lamouroux) C. Agardh 13	Quercianella, Italy	AB055470	Kawaguchi et al. 2001
G. filicina (Lamouroux) C. Agardh 14	Quercianella, Italy	AB055471	Kawaguchi et al. 2001
G. filicina (Lamouroux) C. Agardh 15	Livorno, Italy	AB055472	Kawaguchi et al. 2001
G. filicina (Lamouroux) C. Agardh 16	Coquina rocks, south of Marineland, Florida, USA, (P. W. Gabrielson, 8.iv.2001)	AJ868478	This study
G. filicina (Lamouroux) C. Agardh 17	Sebastian Inlet, Hutchinson Island, Florida, USA, (C. F. Gurgel, 14.ii.1999)	AJ868477	This study
G. filicina (Lamouroux) C. Agardh 18	Opunohu bay, Moorea, French Polynesia, (H. Verbruggen, 13.v.2002)	AJ868479	This study
G. filicina (Lamouroux) C. Agardh 19	Puppuka, Hawaii, Hawaiian Islands (J. M. Huisman, 12.v.2003)	AJ868480	This study
G. filicina (Lamouroux) C. Agardh 20	Punta Ceiba, Tabasco, Gulf of Mexico, Mexico, (C. F. Gurgel, 14.ii.1999)	AJ868481	This study
G. filicina (Lamouroux) C. Agardh 21	Sand Key Park, Sand Key, St. Petersburg, Florida, USA, (T. O. Cho, 19.ix.2002)	AJ868482	This study
G. filicina (Lamouroux) C. Agardh 22	Port Aransas, Texas, USA, (S. Fredericq, 1.ii.2000)	AJ868483	This study
G. filicina (Lamouroux) C. Agardh 23	Galveston, Texas, USA, (S. Fredericq, 1.vi.2001)	AJ868484	This study
G. filicina (Lamouroux) C. Agardh 24	Lae, Papua New Guinea, (E. Coppejans, 7.viii.1980)	AJ868485	This study
G. filicina (Lamouroux) C. Agardh 25	Dutch West Indies, (Y. de Jong)	AJ868486	This study
G. filicina (Lamouroux) C. Agardh 26	Dikwella, Sri Lanka	AY772029	De Clerck et al. 2005
G. filicina (Lamouroux) C. Agardh 27	Madrizavi Island, Los Roques, Venezuela, (C. F. Gurgel, 6.vi.1999)	AJ868487	This study
G. filicina (Lamouroux) C. Agardh 28	Tulér, Madagascar, (E. Coppejans & D. Douterlungne, 1.ix.2002)	AJ868488	This study
G. filicina var. *luxurians* Gepp & Gepp 29	Williamstown, Victoria, Australia, (J. West, 9.v.2003)	AJ868489	This study
G. filicina var. *luxurians* Gepp & Gepp 30	Perth, Western Australia, (J. M. Huisman, 10.x.2003)	AJ868490	This study
G. filicina var. *luxurians* Gepp & Gepp 31	Locmariaquer, Brittany, France, (G. Zuccarello, 20.v.2003)	AJ868491	This study
G. filicina var. *luxurians* Gepp & Gepp 32	Pontevedra, Tragove, Vilanova de Arousa, Spain, (I. Barbara, 4.v.2000)	AJ868492	This study
G. filiformis Kützing	Marataizes, Espiritu Santu, Brazil	AF488820	Gavio and Fredericq 2002
G. hawaiiana Dawson	Maui, Hawaii	AY772030	De Clerck et al. 2005
G. imbricata Holmes	Tsuyazaki, Fukuoka Pref., Japan	AB038607	Wang et al. 2000
G. livida (Harvey) Yamada	Muroran, Hokkaido, Japan	AF488815	Gavio and Fredericq 2002
G. longifolia Kylin	Yzerfonteyn, Western Cape Prov., South Africa	AY772023	De Clerck et al. 2005
G. phuquocensis Tanaka & Pham-Hoàng Hô	Kaalawai, Oahu, Hawaiian Islands	AY772022	De Clerck et al. 2005

Table 2. (Contd.)

Species	Location and collecting data	Accession no.	Source
G. ramosissima Okamura	Ho Ping Island, Keelung, North Taiwan	AF488810	Gavio and Fredericq 2002
G. schizophylla Kützing	Montemar, Chile	AF488825	Gavio and Fredericq 2002
G. schmitziana (Okamura) Kawaguchi & Wang	Shichirigahama, Kamakura, Kanagawa Pref., Japan	AB061398	Wang et al. 2001
G. subpectinata Holmes	Seto, Shirahama, Wakayama Pref., Japan	AB114208	Fay et al. 2004
G. turuturu Yamada	Muroran, Hokkaida, Japan	AF488820	Gavio and Fredericq 2002
G. turuturu Yamada	Roscoff, Brittany, France, (G. Zuccarello, 18.v.03)	AJ868493	This study
G. turuturu Yamada	Locmariaquer, Brittany, France, (G. Zuccarello, 20.v.03)	AJ868494	This study
G. turuturu Yamada	A Coruña, San Amaro, Ría de A Coruña, Spain, (I. Barbara, 9.xi.2003)	AJ868495	This study
Prionitis filiformis Kylin	Strawberry Hill, Lane Co., Oregon, USA, (M. H. Hommersand, 15/05/1999)	AJ868496	This study
Prionitis lyallii Harvey	Cambria, San Luis, Obispo Co., California, USA	AY772033	De Clerck et al. 2005

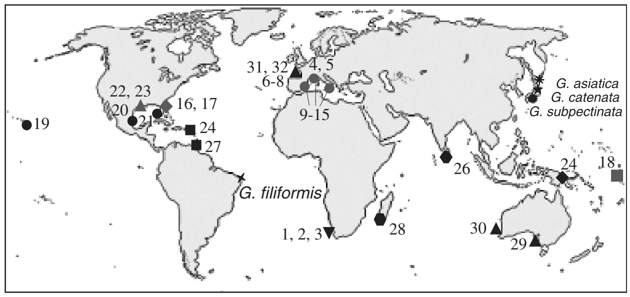

FIG. 1. World map showing sample locations of *Grateloupia filicina* and allied taxa used in this study. Different symbols denote dissimilar molecular signatures of the various samples as derived from *rbc*L gene sequences; the numbers associated with each symbol are from Table 2.

Mexico. A single clade consisting of samples from the Gulf Mexico as well as of a single specimen from Hawaii differed in the fact that the samples were derived from highly disjunct localities. Sequence divergence between these clusters was typically between 3% and 4% with a maximum of 5.7% (Table 3). Although the terminal clades received very high support, the relationships between the various predominantly geographically defined clades remained largely unresolved. The position of the northeast Atlantic *G. filicina* specimens was unresolved but was clearly distinct from all clades that go under the same name. The South African isolates clustered with *G. belangeri* and *G. longifolia*, two other species from South Africa. Sequences of *G. filicina* var. *luxurians* were identical, regardless of geographic origin. This variety of *G. filicina*, known to be introduced in Europe, formed the sister group of *G. subpectinata* from Japan and is well embedded in an entirely western Pacific clade consisting of *G. phuquocensis* and *G. turuturu*. Likewise, *G. filicina* specimens from South Africa were well resolved in a clade composed only of South African species, *G. longifolia* and *G. belangeri*.

Morphological observations. The phylogeny, presented in Figure 2, called for several taxonomic changes with respect to the names attributed to *G. filicina* samples from region other than the Mediterranean basin. In the present study, three taxa were dealt with from taxonomic morphological perspective.

Grateloupia minima **P. Crouan & H. Crouan (1867, p.142)**

Figures 4–6

Nomenclature: Because the northeast Atlantic species traditionally attributed to *G. filicina* prove to be only distantly related to genuine *G. filicina* specimens from the Mediterranean Sea, both entities should not go under the same name. *Grateloupia minima* P. Crouan & H. Crouan is the obvious candidate for the correct name of northeast Atlantic lineage. The species first appeared as a nomen in a list of algae from the Finistère region (Brittany, France) by the Crouan brothers (1860, p. 369) and was only later

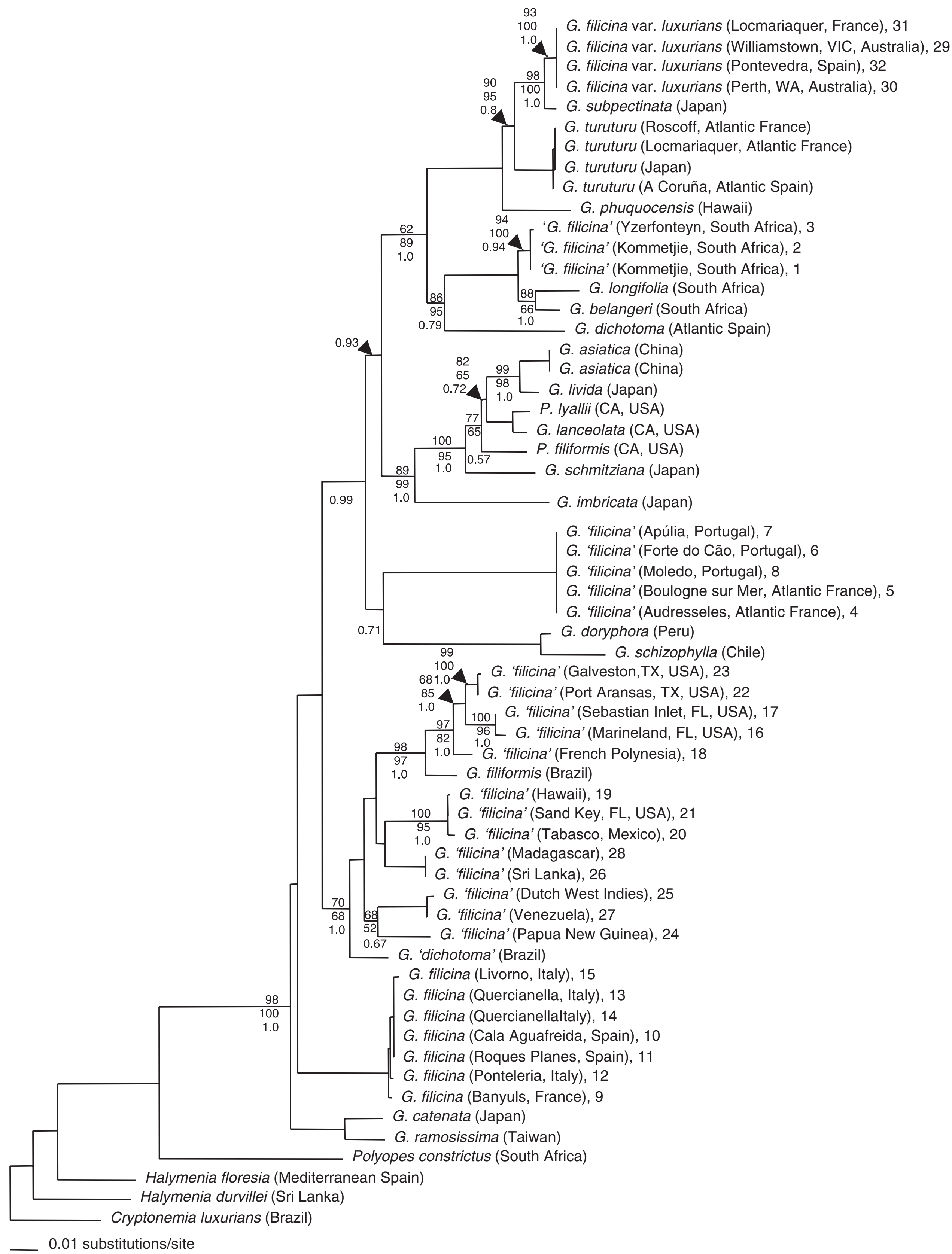

FIG. 2. ML tree with a –log likelihood of 7430.39308 calculated using the GTR + G model of evolution. Bootstrap support based on MP shown above the nodes; bootstrap support resulting from NJ and posterior probabilities resulting from BI are shown below the nodes; nodes receiving maximum support in all three analyses are indicated by an asterisk.

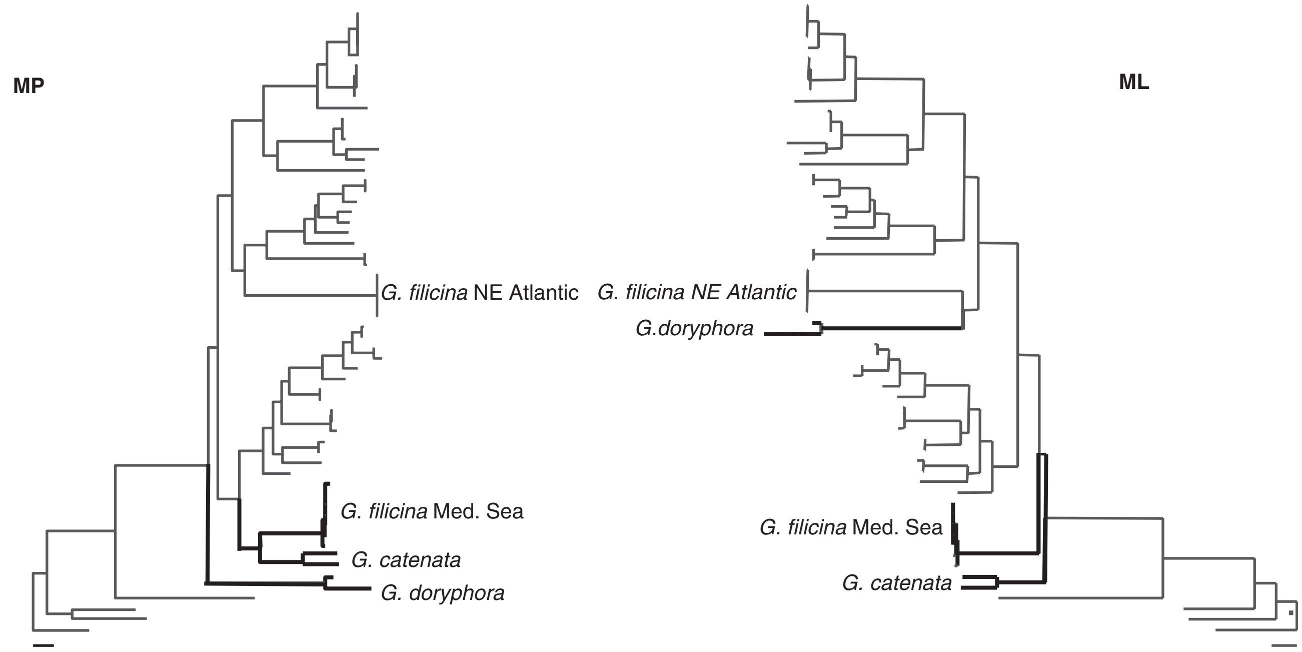

FIG. 3. One of 108 MP trees (1087 steps) versus the ML tree, indicating the differences in respective the topologies.

(Crouan and Crouan 1867) formally described based on material collected from Saint-Marc in the bay of Lannion, Brittany (Fig. 4A). More recently, *G. minima* was relegated to a variety status of *G. filicina* by Cabioch and Giraud (1982). The new combination, however, was technically invalid, failing to meet the provisions of ICBN Article 33.3 (Greuter et al. 2000). More commonly, *G. minima* has been considered a synonym rather than a variety of *G. filicina* (Irvine and Farnham 1983, Hardy and Guiry 2003, Guiry and Nic Dhonncha 2004).

Selected specimens examined: France: Nord-Pas de Calais, Audresselles, Pointe du Nid de Corbet (E. Coppejans, ix.1980, GENT HEC 4829); Audresselles, Pointe du Nid de Corbet (O. De Clerck & F. Leliaert, 14.ix.2003, GENT ODC 980); Boulogne, Digue Nord (E. Coppejans, 1.xi.1977, GENT HEC 3445); Wimereux, Fort de Croix (E. Coppejans, 16.ix.1982, GENT HEC 5150). Brittany, Roscoff (J. Cabioch, .ix.1992, UNC s.n.); Brittany, Roscoff (J. Cabioch & E. Coppejans, 15.vii.2004, GENT HEC 15295). Ireland: Clare Island, County Mayo (M. Guiry, 22.vi.1990, UNC #047). Portugal: Caminha, Moledo (R. Araújo, 30.ix.2003, GENT ODC 984). Esposende, Apúlia (R. Araújo, 28.ix.2003, GENT ODC 983); Praia de Âncora, Forte do Cão (R. Araújo, 27.ix.2003, GENT ODC 982). Spain: A Coruña, Playa de Gandario, Bergondo (I. Bárbara, 21.vi.2001, SANT-Algae 13429); A Coruña, Ensena de Lourido, Sada (I. Bárbara, 7.ii.2004, SANT-Algae 15060-15062); A Coruña, Cabo Prioriño Chico, Ferrol, Ría de Ferrol (J. Cremades, 16.iv.1991, SANT-Algae 13029); A Coruña: Esteiro, Ría de Muros e Noia (I. Bárbara, 8.v.2001, SANT-Algae 13416); Asturias: Playa del Bozo (I. Bárbara, 8.viii.1998, SANT-algae 13256); Lugo, Puerto de Nois (I. Bárbara, 27.vi.1995, SANT-Algae 13726); Lugo, Playa de Toxido, Ría de Vivero, (I. Bárbara & P. Díaz, 10.ix.2002, SANT-Algae 13971); Lugo, Punta del Castro, Ría del Barquero, O Vicedo (I. Bárbara, 10.ix.1998, SANT-Algae 13445); Pontevedra, Santa Tegra, A Guarda (I. Bárbara & J. Cremades, 10.iv.1997, SANT-Algae 9253).

Distribution and ecology: Widely distributed in the northeast Atlantic Ocean from Portugal to southern England and extending into northern Wales and the Irish coast (Irvine and Farnham 1983, Hardy and Guiry 2003). The southern border of the distribution range is at present less well-defined. *Grateloupia filicina* was reported from Morocco (Dangeard 1949, Gayral 1958) as well as from most of the West African coast (Lawson and John 1987), but the absence of material suitable for molecular analyses precludes a more precise statement on the taxonomic affinities of these specimens. The southernmost specimens included in this study, attributable to *G. minima*, are from northern Portugal.

The species is most frequently encountered on bedrock or smaller stones and pebbles from upper intertidal to lower intertidal pools but may well extend in the subtidal to –12 m. The hildenbrandioide life strategy makes the thalli resistant to periodic sand covering. At least in the northern part of its distribution range the species shows a marked seasonality, with the crustose phase overwintering in winter and young erect axes being formed from January until May onward (Irvine and Farnham 1983, personal observations).

Habit: The thallus is composed of a dark blackish crust from which several erect axes, up to 2–4 (–10) cm high, arise. Specimens from the northern part of the distribution range (Great Britain and the

TABLE 3. Uncorrected pairwise distances of the *rbc*L gene of tropical "*Grateloupia filicina*" specimens compared with selected temperate taxa.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	15	16	18	19	20
1	"*G. filicina*" Galveston, TX, USA																				
2	"*G. filicina*" Port Aransas, TX, USA	0.08																			
3	"*G. filicina*" Sebastian Inlet, FL, USA	1.35	1.27																		
4	"*G. filicina*" Marineland, FL, USA	1.59	1.50	0.35																	
5	"*G. filicina*" Tabasco, Mexico	4.45	4.37	4.21	3.87																
6	"*G. filicina*" St. Petersburg, FL, USA	4.98	4.98	4.55	4.30	0.49															
7	"*G. filicina*" Hawaii, USA	4.37	4.29	4.21	3.87	0.32	0.00														
8	"*G. filicina*" Madagascar	4.13	4.06	4.06	3.89	3.26	3.93	3.10													
9	"*G. filicina*" Sri Lanka	4.13	4.06	4.06	3.89	3.26	3.93	3.10	0.00												
10	"*G. filicina*" French Polynesia	1.35	1.27	1.75	2.02	4.53	4.99	4.45	3.90	3.90											
11	"*G. filicina*" Venezuela	4.69	4.61	4.69	4.31	4.21	5.28	3.97	3.34	3.34	4.77										
12	"*G. filicina*" Dutch West Indies	4.77	4.69	4.77	4.39	4.45	5.72	4.21	3.42	3.42	4.85	0.24									
13	"*G. filicina*" Papua New Guinea	4.21	4.13	3.97	3.78	3.74	3.90	3.66	3.50	3.50	4.13	2.94	3.18								
14	"*G. dichotoma*" Brazil	4.21	4.13	4.13	3.95	3.97	4.17	3.65	3.42	3.42	4.05	2.78	2.94	3.18							
17	*G. filiformis* Brazil	2.30	2.22	2.86	2.99	4.37	4.33	4.29	3.34	3.34	2.30	4.05	4.13	4.21	3.65						
15	*G. filicina* Italy	5.56	5.48	5.48	5.01	5.48	5.91	5.32	5.72	5.72	5.88	5.08	5.24	5.64	4.92	5.56					
16	"*G. filicina*" Kommetjie, South Africa	7.15	7.08	6.76	6.97	7.63	6.87	7.31	7.24	7.24	7.23	7.31	7.31	7.08	7.00	6.76	6.92				
18	*G. asiatica*	7.23	7.15	7.31	7.23	7.47	6.33	7.15	7.15	7.15	7.31	7.07	6.99	7.23	6.91	6.59	7.15	7.15			
19	*G. catenata*	5.88	5.80	5.96	5.81	5.08	5.20	4.77	5.64	5.64	6.04	4.92	5.00	5.33	4.69	5.64	5.08	8.11	7.78		
20	*G. filicina* var. *luxurians*	6.65	6.57	6.89	7.11	6.89	6.18	6.57	6.97	6.97	6.97	6.97	7.13	6.97	6.41	6.17	6.96	6.00	7.13	7.52	
21	"*G. filicina*" Moleda, Portugal	8.42	8.34	8.02	7.72	7.55	6.10	7.23	7.87	7.87	8.26	7.63	7.86	7.71	7.31	7.63	7.39	7.79	7.23	8.10	8.49

White, tropical–tropical distances; light gray, tropical–temperate distances; dark gray, temperate–temperate distances.

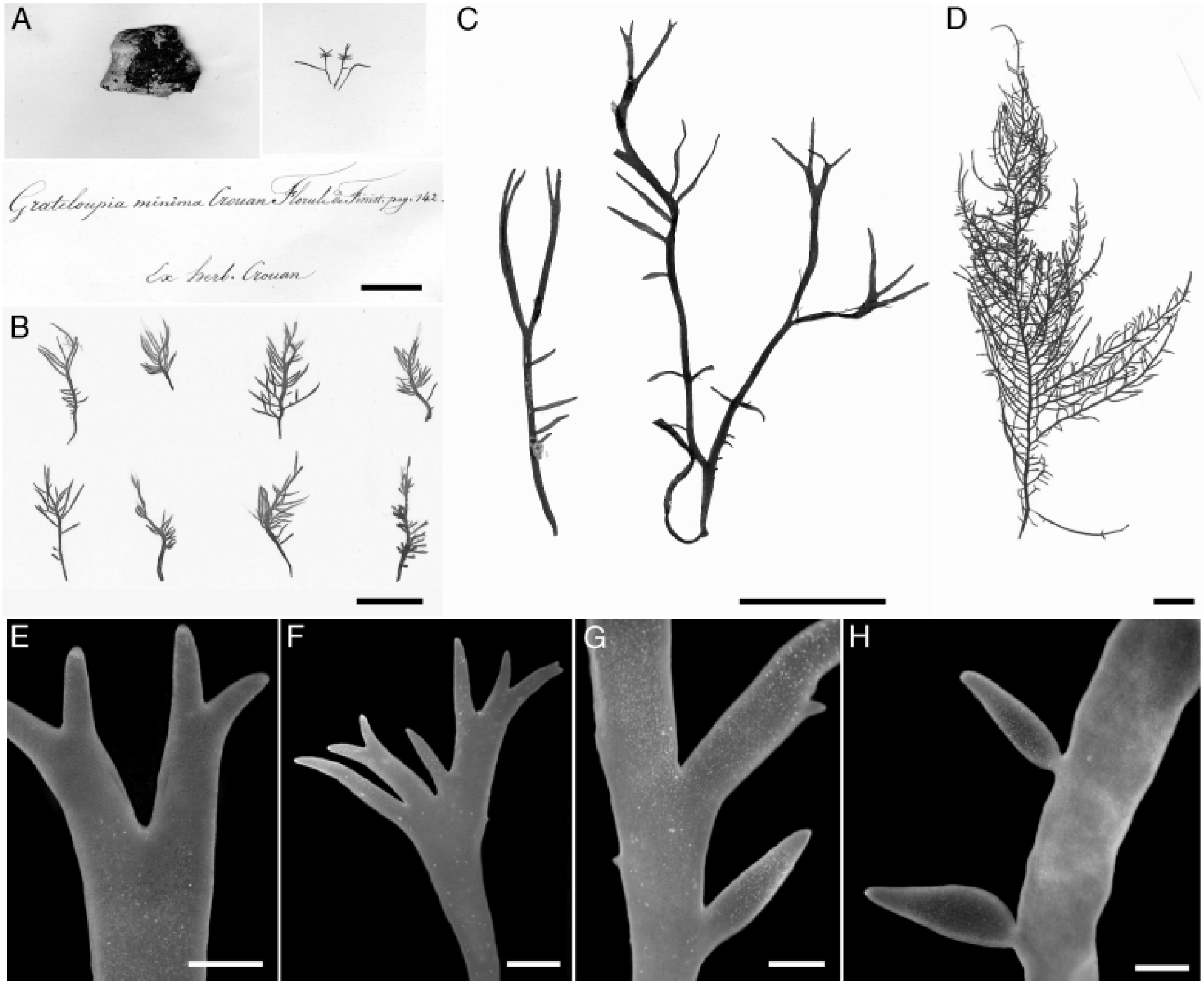

FIG. 4. Type and external morphology of *Grateloupia minima* P. Crouan & H. Crouan. (A) The holotype of *G. minima* consisting of the extensive crustose base and some separated erect axes. Scale bar, 1 cm. (B, C) Typical growth forms of *G. minima* composed of predominantly dichotomously branched axes and lateral pinnules (B = SANT–Algae 13416; C = ODC 980). Scale bar, 1 cm. (D) Habit of a large specimen from the southern part of the distribution range (SANT–Algae 13726). Scale bar, 1 cm. (E) Detail of a bifurcating apex. Scale bar, 0.5 mm. (F) Detail of an antler-like apex. Scale bar, 1 mm. (G) Detail of a dichotomously branched axis with pinnules developing from the margins. Scale bar, 0.5 mm. (H) Typical fusiform lateral pinnules. Scale bar, 0.5 mm.

Channel) only rarely attain a height of more than 6–7 cm, with most axes from a single population usually not exceeding 4 cm in height (Fig. 4, B–D). Some growth forms, even though fully reproductive, consist of an extensive crust with sparse erect axes not higher than 1 cm.The axes are terete to compressed. Even in compressed thalli, the apical and proximal parts are terete to subterete. Compressed axes measure 0.8–1 (1.5) mm wide and 200–300 µm thick. Erect thalli may be either simple or two to four times dichotomously branched (Fig. 4G). Spindle-shaped marginal proliferations or pinnules are absent in young or weakly developed thalli but are usually present when the plants mature (Fig. 4H). Proliferations develop from the margins of the thallus and measure 200 µm in diameter and 0.4 to 3 mm long. The thallus apices are often bifurcate to antler-like (Fig. 4, E and F). The thallus has a firm texture and is blackish purple in color or greenish translucent when fading.

Vegetative structure: Each crust (Fig. 5A) consists of straight densely packed filaments up to 15 cells long (Fig. 5B). The individual cells are isodiametric and no larger than 5 µm in diameter. The erect axes are composed of multiple axial filaments, with each axial cell cutting off a single periaxial cell outwardly immediately below the apex (Fig. 5C). The periaxial cells and their derivatives divide further to form fascicles of cortical cells. The primary cortical filament is up to nine cells long. Secondary and higher order filaments are formed in an abaxial position from every consecutive cell by means of longitudinal concavo-convex divisions followed by transverse division. Axial and inner cortical cells become directly pit

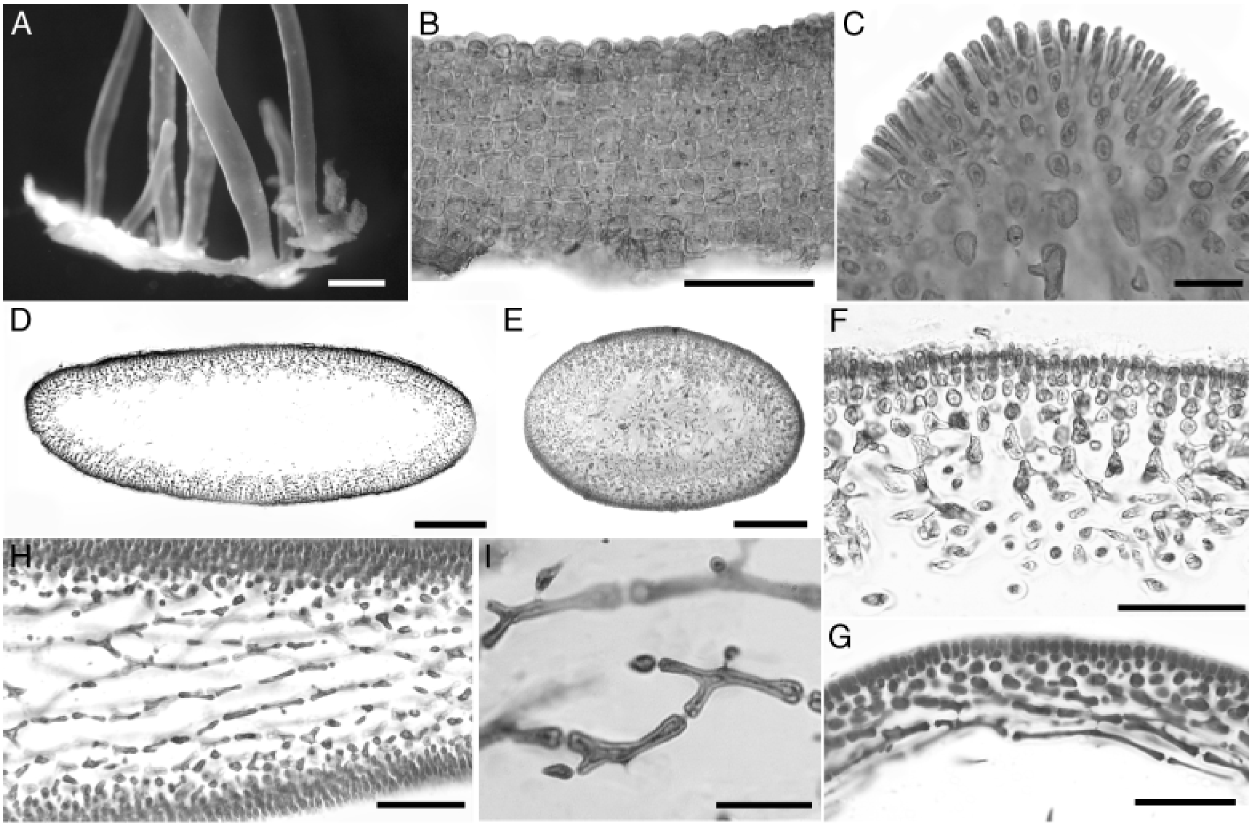

Fig. 5. Vegetative anatomy of *Grateloupia minima* P. Crouan & H. Crouan. (A) Detail of basal portion of a thallus with a prostrate crust from which several erect axes arise. Scale bar, 1 mm. (B) Transverse section of crustose base showing straight, anticlinal filaments composed of small isodiametric cells. Scale bar, 40 μm. (C) Longitudinal section of apical region with axial filaments cutting off cortical fascicles towards the periphery. Scale bar, 10 μm. (D) Transverse section of compressed axis in the median thallus part. Scale bar, 40 μm. (E) Transverse section of axis just above the base. Scale bar, 40 μm. (F) Detail of cortex in transverse section. Scale bar, 40 μm. (G) Longitudinal section of cortex at thallus height corresponding to F. Scale bar, 40 μm. (H) Paradermal section of pinnule revealing inner cortical cells becoming pit connected to adjacent cells. Scale bar, 40 μm. (I) Detail of inner cortical cells forming small conjunctor cells. Scale bar, 10 μm.

connected with neighboring cells, resulting in typical x-shaped cells characterized by four (five to six) pit connections (Fig. 5, F–H). Secondary pit connections are formed by small conjunctor cells that fuse with neighboring cells (Fig. 5I). Intercalary cell divisions are absent throughout. Instead, cells elongate considerably in a longitudinal periclinal direction. The pattern of medulla development seems quite fixed, resulting in a rather regular network of inner medullary cells composed of highly stretched x-shaped medullary cells. Thin multicellular rhizoidal filaments, running predominantly parallel to the long axis of the thallus, are formed from medullary as well as from inner cortical cells. Rhizoidal filaments have the capacity to branch and become secondarily pit connected to other rhizoidal cells or cells lining the inner cavity of the thallus (Fig. 5F). The central thallus cavity remains nearly completely free of filaments in the median thallus parts (Fig. 5D). Distinctive stellate cells, derived from axial and inner cortical cells due to the extensive formation of secondary pit connections, are infrequent. Toward the base of the thallus, outer cortical filaments undergo several transverse divisions, resulting in straight anticlinal filaments of small isodiametric cells up to six to seven cells long. The central cavity becomes dense by filaments but does not become entirely filled by them (Fig. 5E).

Reproductive structures: Thalli are dioecious. In female thalli, carpogonial branches and auxiliary cells are formed in separate, narrow, flask-shaped ampullae, located in the inner cortex (Fig. 6B). Inner cortical cells cut off toward the thallus surface a single cell that divides to form a primary ampullary filament. Carpogonial ampullae are characterized by a primary filament, six to eight cells long. Typically, the first cell bears an unbranched secondary filament up to five to six cells long. An additional secondary filament may develop from the cell adjacent to the supporting cell, but more frequently only a single secondary filament is produced. The supporting cell, typically the third cell of the primary filament, bears a two-celled carpogonial branch directed toward the thallus surface. The hypogynous cell cuts off a short secondary filament, three to four cells long.

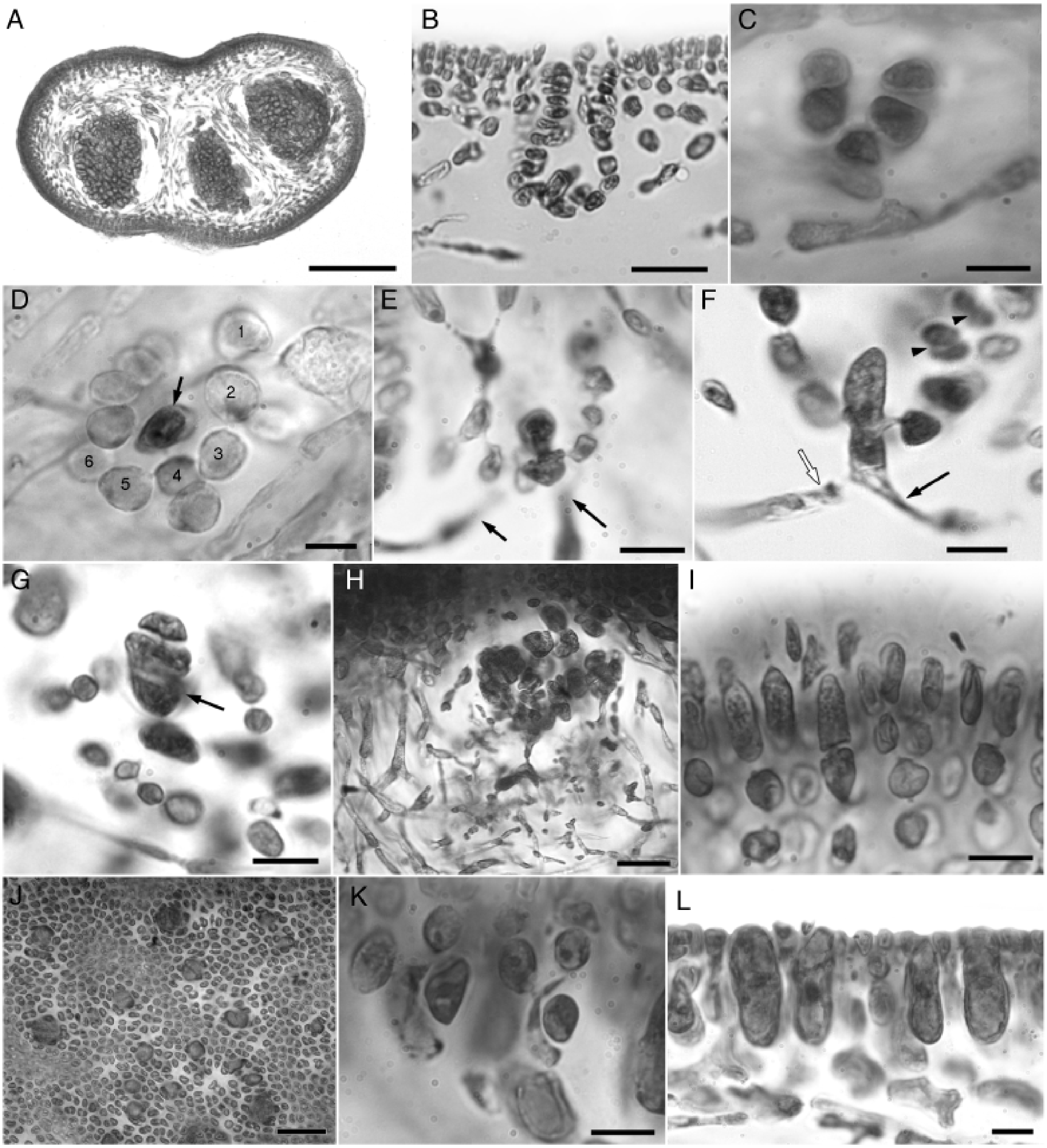

FIG. 6. Reproductive anatomy of *Grateloupia minima* P. Crouan & H. Crouan. (A) Transverse section through axis of female plant showing three mature cystocarps. Scale bar, 300 μm. (B) Lateral view of mature auxiliary ampulla with the distinctly elongated auxiliary cell at the base of the ampulla. Scale bar, 20 μm. (C) A young stage in the formation of an ampulla revealing a basal cell cut off from an inner cortical cell, which bears two branches of equal length. Scale bar, 5 μm. (D) A later stage of an auxiliary cell ampulla, with the auxiliary cell (arrow) being the basal cell of a secondary ampullary filament developed from the fourth cell of the primary ampullary filament. Scale bar, 5 μm. (E) Detail of a fertilized carpogonium which has fused with the hypogynous cell; two connecting filaments (arrows) emerge from the fusion product. Scale bar, 5 μm. (F) Detail of a diploidized and still undivided auxiliary cell with an incoming (black arrow) and outgoing (white arrow) connecting filament; cells of the ampullary filaments have begun to divide (arrow heads); derivatives of ampullary cells form bead-like filaments. Scale bar, 5 μm. (G) Development of young gonimoblasts, with the auxiliary cell (arrow) bearing a two-celled gonimoblast filament. Scale bar, 5 μm. (H) Transverse section of a maturing carposporophyte subtended by an auxiliary cell that has fused with adjacent cells of the ampullary filament. Scale bar, 20 μm. (I) Transverse section of a male plant showing elongated outer cortical cells cutting of spermatia. Scale bar, 5 μm. (J) Surface view of cruciately divided tetrasporangia interspersed among outer cortical cells. Scale bar, 100 μm. (K) Detail of inner cortical cells cutting off tetrasporangial parent cells by means of a pronounced concavoconvex division. Scale bar, 5 μm. (L) Lateral view of mature tetrasporangial parent cell before meiotic division. Scale bar, 5 μm.

The carpogonium is rather small and conical and bears a long trichogyne that reaches the surface. Auxiliary cell ampullae (Fig. 6B) resemble the carpogonial ampullae but are generally more robust. Because of the elusive nature of the early stages of ampullary development, the exact sequence of cell formation could not be fully determined. In all cases, however, an ampullary initial is cut off adventitiously from an inner cortical cell, which develops into a primary filament.

A secondary filament is formed from the first cell, as shown in Figure 6C. The primary ampullary filament may be 15 cells long, with one to four secondary filaments arising from the first to the fifth cell. Secondary filaments are 7–10 cells long and generally unbranched. Occasionally, however, filaments may branch once or twice. The auxiliary cell is usually the first cell of a secondary filament issuing from the third or fourth cell of the primary filament (Fig. 6D). Upon fertilization the carpogonium fuses with the hypogynous cell, and two robust, unbranched, and tube-like connecting filaments are produced from the carpogonial fusion cell (Fig. 6E). Direct fusion of a portion of the incoming connecting filament with an auxiliary cell results in the marked elongation of the latter that will then cut off a single gonimoblast initial that goes on dividing toward the thallus surface (Fig. 6, F and G). Upon diploidization of the auxiliary cell by this incoming connecting filament, the connecting filament continues its course as an outgoing filament that is pit connected at the base of the auxiliary cell. Several gonimolobe initials are produced from the gonimoblast initial by concave divisions. Cells of the ampullary filaments undergo periclinal divisions even before the gonimoblast initial is cut off, but no globular masses of cells are formed (Fig. 6F). Instead, the ampullary filaments and derivatives transform into bead-like filaments loosely enveloping the developing gonimoblasts. Inner cortical cells in the vicinity of the developing gonimoblasts cut off numerous multicellular inwardly growing filaments (Fig. 6H). Mature gonimoblasts, 250 μm in diameter (Fig. 6A), consist of a compact mass of angular carpospores, 10–25 μm each. Carpospores are released through an ostiole.

Male plants are characterized by small inconspicuous spermatangial sori. Cortical cells bearing spermatia are usually elongate and cut off one or two teardrop-shaped spermatia, 2–3 μm wide and 4–5 μm long, by oblique divisions (Fig. 6I). Tetrasporangia are scattered over the entire thallus, except in the basal parts. They are interspersed among outer cortical cells (Fig. 6J). Tetrasporangial initials are cut off laterally from inner cortical cells by means of a pronounced concavoconvex division (Fig. 6, K and L). Tetrasporangial parent cells enlarge considerably in a longitudinal direction and reach their full size before undergoing a meiotic division (Fig. 6L). This coincides with a pronounced elongation of the adjacent cortical cells. Mature tetrasporangia are cruciately divided, measuring 18–25 × 40–45 μm.

***Grateloupia capensis* De Clerck sp. nov.**
Figures 7 and 8

Axes erecti usque ad 20 (–30 cm) alti, pinnatim usque ad irregulariter ramose, ramificatio praecipue in partes proximales thalli, axes terminales elongati non ramose; axes complanati linearesque, 6–15 (–25) mm lati, 200–500 μm crassi, gradatim angustati versus apicem simplicem; proliferationes marginales abundantes, leviter usque ad non constricta prope basim, 700–1000 μm diam., 5–10 (–30) mm longae; proliferationes superficiales abundantes in partes medianas thalli; cortex initio 5–6 cellularum crassus, spissescens usque ad 12 strata crassa in partes medianas thalli; cellulae corticales externae elongatae, 2–3 μm latae usque ad 10 μm longae; cavitas interna plenans filamentis intertextis cellulis stellatis in partibus medianis thalli. Gametophyti dioecii, producentes structuras reproductivas super thallum omnino, praeter partem basalem; ampullae carpogoniales compositae fili primarii et 2–3 filorum secundorum et fili carpogonialis bicellularis; ampullae auxiliaries filo primario 2–4 et 3–4 filis secundis, cellula auxiliaris valde ovalis ubi matura; gonimoblasti maturi usque ad 150 μm diam. involuti involucro magnopere oriundo cellulis corticalibus internis. Spermatia non visa. Tetrasporangia dispersa pagina thalli omnino praeter partem basalem, elongata, cruciatim divisa, 13–18 μm lata, 35–40 μm longa.

Upright axes of a rather firm texture, to 20 (–30) cm high, pinnately to irregularly branched, with branching concentrated in the proximal parts of the thallus, and with long unbranched terminal axes; axes complanate and linear, 6–15 (–25) mm wide and 200–500 μm thick, tapering gradually toward a simple apex; marginal proliferations abundant, slightly to unconstricted at the base, 700–1000 μm in diameter, and to 5–10 (–30) mm long; surface proliferations abundant in the median parts of the thallus; cortex initially 5–6 cells thick, becoming up to 12 cell layers thick in the median and basal thallus parts; outer cortical cells elongate, 2–3 μm wide and to 10 μm long; the inner cavity becoming gradually filled with intertwining filaments and stellate cells in the median thallus parts. Gametophytes dioecious, producing reproductive structures over the entire thallus, except in the basal portion; carpogonial ampullae composed of a primary filament and 2–3 secondary filaments, and a two-celled carpogonial branch; auxiliary cell ampullae with a primary filament and 3–4 secondary filaments, the auxiliary cell markedly oval when mature; mature gonimoblasts to 150 μm in diameter, enveloped by an involucrum largely derived from inner cortical cells. Spermatia not observed. Tetrasporangia scattered over the entire thallus surface except in the basal portion, elongate, cruciately divided, 13–18 μm wide and 35–40 μm long.

Holotype: South Africa, Western Cape Province, Kommetjie (O. De Clerck, 1.vi.2003, GENT ODC 924) ; isotypes in BOL and LAF.

Additional specimens examined: South Africa, Western Cape Province: Cape of Good Hope (E. Coppejans, 7.xi.1995, GENT HEC 10860); False Bay, Buffels Bay (F. Leliaert, 24.i.1997, GENT FL 110); False Bay, Glencairn (O. De Clerck, 3.v.2000, GENT

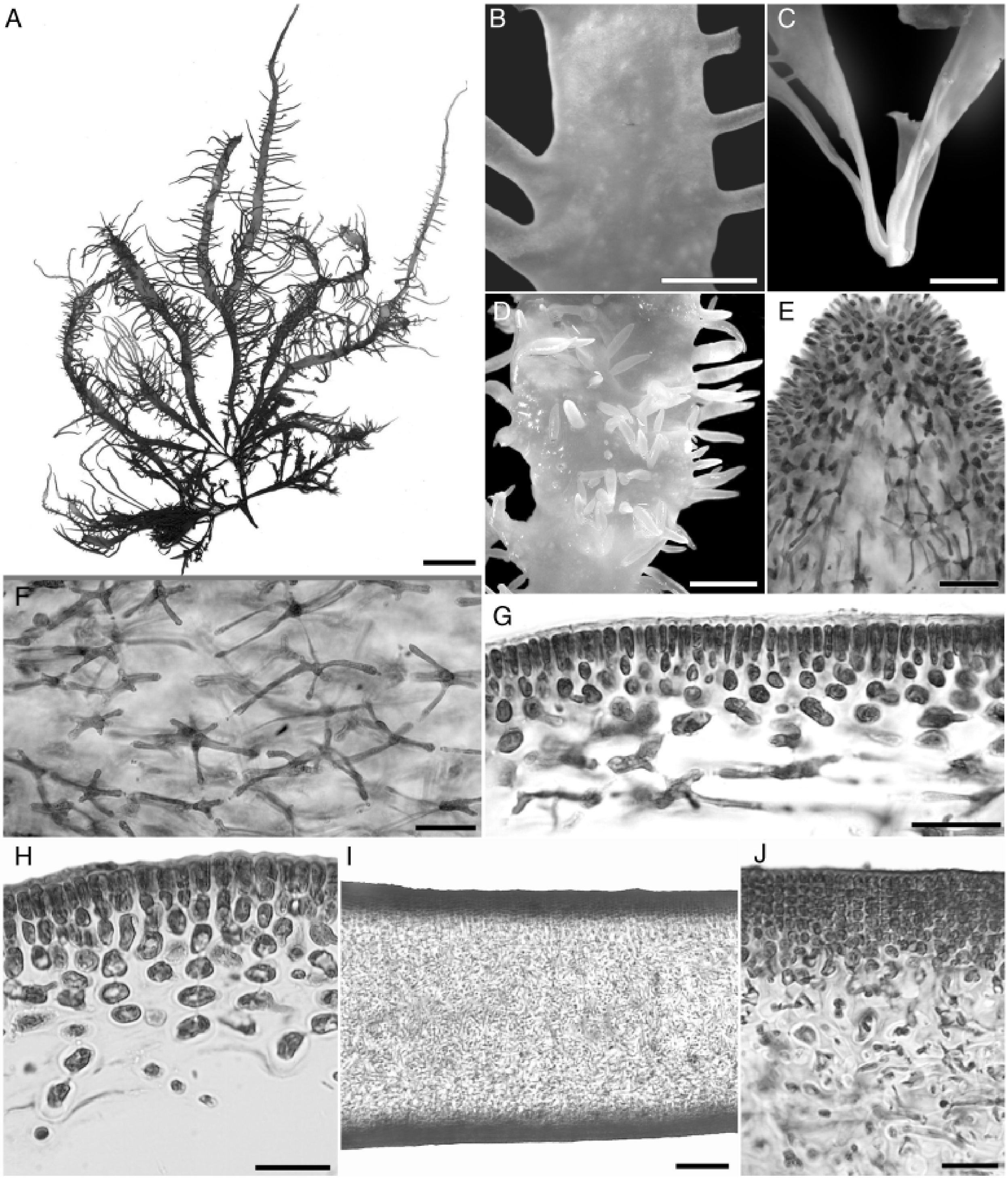

FIG. 7. Habit and vegetative morphology of *Grateloupia capensis* sp. nov. (A) Holotype of *G. capensis* from Kommetjie, South Africa (GENT ODC 924). Scale bar, 2 cm. (B) Detail of axis in distal thallus part showing base of lateral pinnules. Scale bar, 5 mm. (C) Detail of basal thallus part with subterete axes gradually expanding into flattened axes. Scale bar, 5 mm. (D) Surface view of axis in median thallus part with abundant proliferations arising from thallus surface. Scale bar, 5 mm. (E) Longitudinal section of pinnule apex showing the irregular pattern of inner cortical and medullary cells. Scale bar, 25 µm. (F) Detail of inner cortical cells near apex with each of the cells forming multiple secondary pit connections resulting in a stellate appearance. Scale bar, 25 µm. (G) Longitudinal section of cortex showing gradual transition between highly elongated inner cortical cells and small isodiametric outer cortical cells. Scale bar, 25 µm. (H) Transverse section of cortex at same height as G. Scale bar, 25 µm. (I) Transverse section of axis near base of thallus revealing an inner cavity completely filled with rhizoidal filaments. Scale bar, 100 µm. (J) Detail of cortical cell layers composed of anticlinal rows of filaments in the proximal thallus parts in transverse section. Scale bar, 25 µm.

ODC 863); False Bay, St. James (F. Leliaert, 4.xii.1996, GENT FL 97); Kommetjie (E. Coppejans, x.1971, GENT HEC 1582), (F. Leliaert, 16.xi.1996, GENT FL 12); Paternoster (E. Coppejans, x.1971, GENT HEC 1583); Pringle Bay (F. Leliaert, 17.xi.1996, GENT FL 69); Yzerfontein (O. De Clerck, 24.xi.1999, GENT ODC 829), (O. De Clerck, 9.xi.1999, ODC 847).

Distribution and ecology: Known from the Western Cape Province as far north as Namibia (Rull Lluch 2002) and extending eastward at least as far as Port Alfred (Stegenga et al. 1997). *Grateloupia capensis* is a common to abundant species, predominantly found in shallow mid to high intertidal pools.

Habit: The thallus, reaching a height of 20 and occasionally even 30 cm, is of a rather firm texture, yellowish-green in color, and is attached by a discoid holdfast from which several erect axes arise (Fig. 7A). The axes are complanate and linear, 6–15 (–25) mm wide and 200–500 μm thick, except near the basal parts and the pinnules where the axes are terete to subterete (Fig. 7, B and D). The thallus is initially pinnately branched, but this pattern becomes obscured in older plants when they become five to seven times irregularly dichotomously branched, with the branching concentrated in the proximal thallus parts and with relatively long terminal axes (up to 12 cm). Axes taper gradually toward a simple apex. Marginal proliferations are abundant and unconstricted to slightly constricted near the base, 700–1000 μm in diameter and to 5–10 (–30) mm long (Fig. 7B). The lateral pinnules remain usually unbranched and do not produce second-order proliferations. Surface proliferations are usually abundant and mainly concentrated in the median parts of the thallus (Fig. 7D).

Vegetative structure: The thallus is composed of multiple axial filaments, with each axial cell cutting off a single periaxial cell toward the outside of the thallus immediately below the apex (Fig. 7E). Periaxial cells and their derivatives divide further to form fascicles of cortical cells that are easily observed in longitudinal sections of the cortex (Fig. 7G). The primary cortical filaments are up to 12 cells long. Secondary and higher order filaments are formed in an abaxial position from every consecutive cell by means of longitudinal concavoconvex divisions. The axial and inner cortical cells become directly pit connected with neighboring cells in a highly irregular manner, with each cell bearing as much as five to six (to eight) pit connections. The inner cortex is therefore highly irregular, with abundant stellate cells (Fig. 7, E and F). Intercalary divisions are absent throughout. Thin multicellular rhizoidal filaments, running predominantly parallel to the long axis of the thallus, are formed from the inner cortical cells (Fig. 7H). Inner filaments have the capacity to branch and become secondarily pit connected to other rhizoidal cells or to cells lining the inner cavity of the thallus. The central thallus cavity remains relatively free of filaments in the upper thallus parts only. In the median and proximal thallus parts, the central cavity becomes increasingly filled with rhizoidal filaments imbedded in a thick matrix (Fig. 7I). Toward the base of the thallus, outer cortical filaments undergo several transverse divisions, resulting in anticlinal filaments of small isodiametric cells up to six to eight cells long (Fig. 7, I and J).

Reproductive structures: Thalli are dioecious. Female gametophytes are characterized by carpogonial branches and auxiliary cells formed in separate, narrow, flask-shaped ampullae, positioned in the inner cortex (Fig. 8, B and C). Carpogonial ampullae are characterized by primary filaments six to eight cells long. Typically, the first and the second cell bear an unbranched secondary filament up to five to nine cells long (Fig. 8B). An additional secondary filament may develop from the cell adjacent to the supporting cell, but more frequently only a single secondary filament is produced. The supporting cell, the third cell of the primary filament, bears a two-celled carpogonial branch directed toward the thallus surface. The hypogynous cell may cut off a short secondary filament, three to four cells long, but in some instances such a filament was not observed. The carpogonium is rather small and conical and bears a long trichogyne that reaches the surface. Auxiliary cell ampullae are in principle identical to the carpogonial ampullae but are generally more robust (Fig. 8, C and D). The primary ampullary filament may be up to 13 cells long, with one to four secondary filaments arising from the first to the fifth cell. Secondary filaments are six to nine cells long and normally unbranched. The auxiliary cell is usually the first cell of a secondary filament, cut off from the third or fourth cell of the primary filament. Postfertilization stages were not observed. Fusion of a connecting filament with an auxiliary cell results in a marked elongation of the latter that will then cut off a single gonimoblast initial toward the thallus surface (Fig. 8, E and F). Cells of the ampullary filaments undergo periclinal divisions even before the gonimoblast initial is cut off, but no globular masses of cells are formed. Instead, the ampullary filaments transform into bead-like structures, loosely enveloping the developing gonimoblasts (Fig. 8F). Inner cortical and medullary cells in the vicinity of the developing gonimoblasts cut off numerous multicellular inwardly growing filaments, which will connect by means of secondary pit connections to the derivatives of the ampullary filaments (Fig. 8H). Mature gonimoblasts, 90–150 μm in diameter (Fig. 8G), consist of a compact mass of angular carpospores, 10–25 μm each. Carpospores are released through an ostiole. Male plants were not observed.

Tetrasporangia are scattered over the entire thallus surface, except in the basal parts. The tetrasporangial parent cells are cut off laterally from inner cortical cells and expand outwardly (Fig. 8I). When reaching their definite size, the parent cells undergo a meiotic

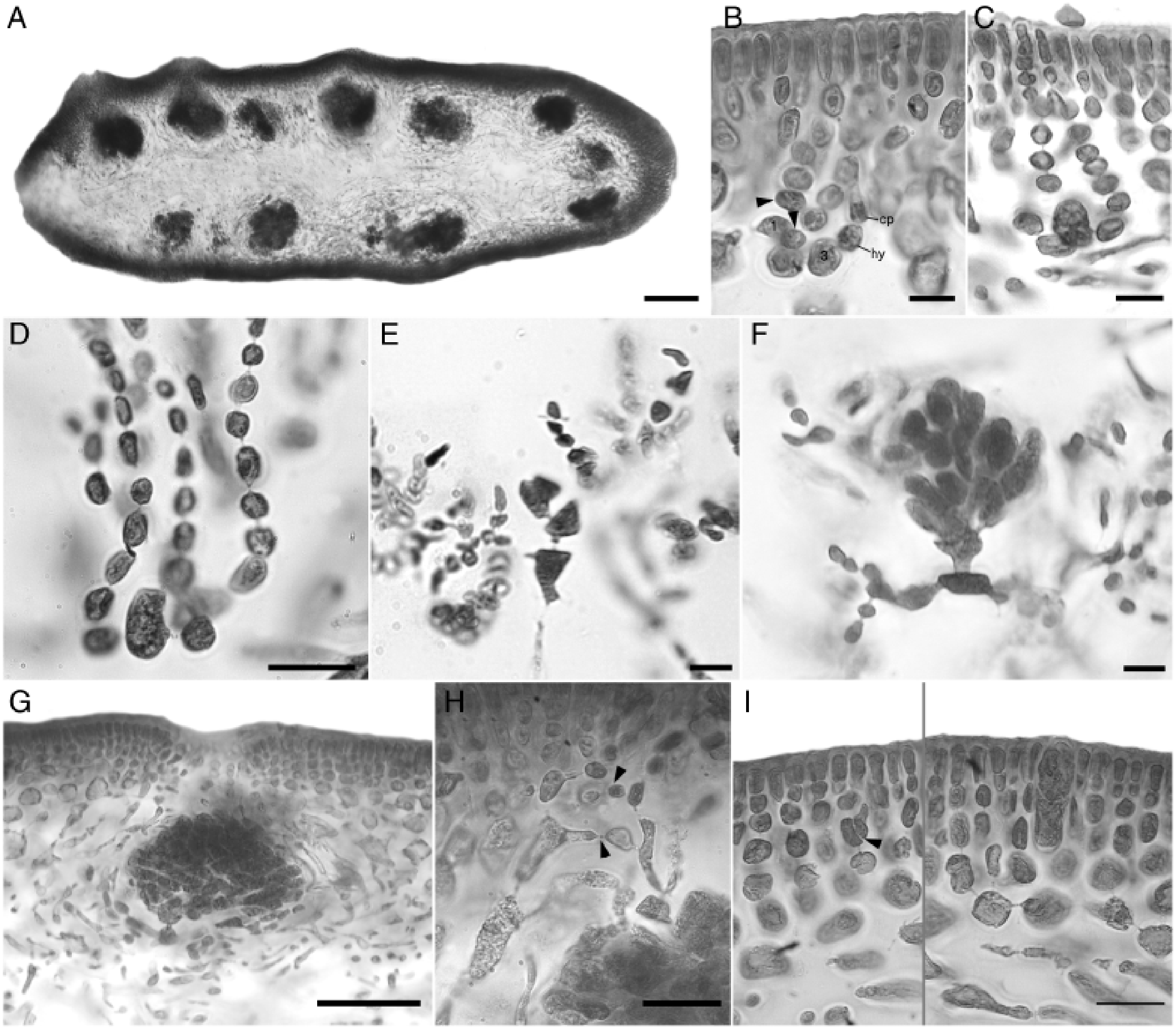

FIG. 8. Reproductive morphology of *Grateloupia capensis* sp. nov. (A) Transverse section of a fertile axis, showing numerous mature cystocarps, deeply imbedded in the thallus. Scale bar, 100 µm. (B) Lateral view of carpogonial ampulla with the primary ampullary filament (numbered cells) bearing secondary filaments (arrowheads) on the first and second cell, and the two-celled carpogonial branch (hy, hypogenous cell; cp, carpogonium) on the third cell. Scale bar, 5 µm. (C, D) Lateral views of auxiliary cell ampullae with elongated auxiliary cells positioned at the base of the ampullae. Scale bar, 10 µm. (E) Stage in gonimoblast development showing connecting filaments, auxiliary cell bearing single gonimoblast initial and the first gonimolobe initial. Scale bar, 10 µm. (F) Auxiliary cell fused with neighboring cells and gonimoblast initial bearing additional gonimolobe initials. Scale bar, 10 µm. (G) Detail of mature cystocarp showing compact mass of carpospores surrounded by an involucrum primarily derived from medullary cells. Scale bar, 50 µm. (H) Detail of secondary pit connection establishment between ampullary cell derivatives with neighboring cortical and medullary cells (arrow heads). Scale bar, 10 µm. (I) Detail of cortex bearing cruciately divided tetrasporangia; tetrasporangial initials cut off by concavoconvex division from cortical cells three to four cell layers below surface (arrow head). Scale bar, 20 µm.

division, resulting in a cruciately divided tetrasporangium, measuring 13–18 × 35–40 µm. The cortical filaments giving rise to the tetrasporangia are typically more elongate than ordinary vegetative filaments.

***Grateloupia luxurians* (A. Gepp & E. S. Gepp) De Clerk & Gavio**
Figure 9

Basionym: Grateloupia filicina var. luxurians A. Gepp & E. S. Gepp, Journal of Botany 44:259.

Holotype: Farm Cove, Sydney, New South Wales, Australia (A. H. S. Lucas, vii.1901, Lucas nr. 6 in BM 530322) (Fig. 9A).

Selected specimens examined: England, Hampshire, Haling Island, Longshore Harbour (R. Fletcher, 28.iii.2002, LAF B081). Isle of Wight, Bembridge (E. Coppejans, 9.iv.1977, GENT HEC 2900) (Fig. 9B).

Distribution and ecology: Grateloupia luxurians was originally described from Sydney harbor and is known to extend in Australia from Cottesloe, Western Australia

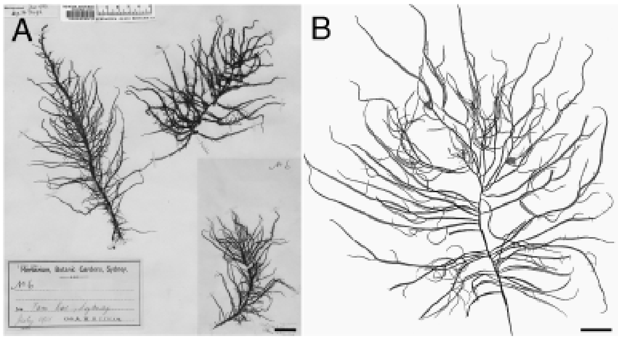

FIG. 9. Type and habit of *Grateloupia luxurians*. (A) Holotype of *G. luxurians* from Farm Cove, Sydney, Australia (BM530322). Scale bar, 2 cm.(B) Habit of a typical specimen collected in Bembridge, Isle of Wight (HEC 2900). Scale bar, 2 cm.

to Wybury Head in Queensland (Womersley and Lewis 1994). It was first reported from Europe by Farnham and Irvine (1968), who recorded the alga from the Portsmouth area, England. More recently the species was reported from Spain (Casares Pascual and Seoane Camba 1988, López Rodríguez et al. 1991) and Brittany (Cabioch et al. 1997). According to Verlaque (2001), the species is also introduced in the Thau Lagoon in Mediterranean France, a region renown for its introduced species. For the morphology and anatomy, we refer to detailed treatments of the species (Table 4) by Irvine and Farnham (1983), Womersley and Lewis (1994), and Cabioch et al. (1997).

DISCUSSION

Grateloupia, with at present over 50 species recognized, is by far the most species-rich genus of the red algal family Halymeniaceae (Kraft 1977, Gavio and Fredericq 2002, Guiry and Nic Dhonncha 2004). Species boundaries, based on morphological discontinuities, the typological species concept, have been considered problematic because of substantial intraspecific or even within-individual variation in gross morphology (Ardré and Gayral 1961, Irvine and Farnham 1983, Cabioch et al. 1997). The generitype *G. filicina* (Lamouroux) C. Agardh, characterized by a finely pinnate morphology, was originally described from Trieste in the Adriatic Sea but has been reported from nearly all tropical to cold-temperate regions, making it one of the most widespread species of red algae. Even though the (semi)cosmopolitan nature of most red algae is seriously questioned (Kain and Norton 1990), *G. filicina* was until recently still regarded as a prime candidate for a potentially cosmopolitan species (Kraft 1992, Saunders and Kraft 1996). The recent description of *G. asiatica* and the reinstatement of *G. catenata* and *G. subpectinata*, all from the western Pacific Ocean (Wang et al. 2000, Kawaguchi et al. 2001, Faye et al. 2004), questioned the cosmopolitan nature of the species. The *rbc*L-based phylogeny presented in this study, including specimens covering the entire distribution range, demonstrates that the various geographically disjunct populations hitherto attributed to *G. filicina* do not constitute a single monophyletic lineage. The phylogeny confirms the initial results by Kawaguchi et al. (2001), where the western Pacific species, *G. asiatica*, is only distantly related to the Mediterranean *G. filicina* despite remarkable morphological similarity. Inclusion of additional *G. filicina* specimens from temperate and tropical regions spanning the entire geographic range reveals that cryptic diversity is much more prevalent than previously anticipated. So far, specimens attributable to *G. filicina* have only been collected in the Mediterranean Sea based on comparative sequence data. All other specimens were resolved in different genealogical lineages. Several recent studies dealing with Rhodophyta or marine diversity in general have highlighted that in relatively simple organisms, characterized by a limited number of reliable diagnostic characters, the true diversity is likely to be underestimated using a classical morphological–anatomical approach (Knowlton 2000, Wattier and Maggs 2001, Zuccarello and West 2002, Zuccarello et al. 2002, Ciniglia et al. 2004). The phenomenon whereby independent evolutionary lineages are attributed to the same taxonomic species, coined the ‘‘low-morphology problem’’ by van Oppen et al. as early as 1996, is more than likely a widespread trend in algae, thereby rendering formal descriptive taxonomy more difficult (van der Strate et al. 2002).

As in other notoriously difficult red algal genera, careful morphological and anatomical observation in *Grateloupia* has resulted in characters that prove to be diagnostic for the various genealogical lineages (Wang et al. 2000, Kawaguchi et al. 2001, Gavio 2002, Faye et al. 2004, Mateo-Cid et al. 2005, this study). Interestingly, those characters predominantly relate either

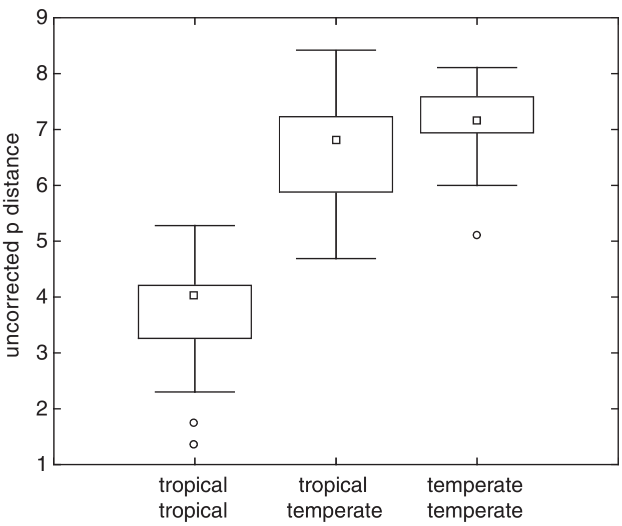

FIG. 10. Pairwise uncorrected distances of tropical–tropical, tropical–temperate, and temperate–temperate specimens (see Table 3). The central squares indicate median values, the boxes are the 25% percentiles, the whiskers refer to the non-outlier range, and dots represent outliers. Analysis of variance and subsequent Tukey HD (honest significant difference) tests indicate that the difference between tropical–tropical distances versus tropical–temperate and temperate–temperate distances is significant ($P < 0.001$).

to general habit, external morphology, or vegetative anatomy. Reproductive structures appear remarkably homogenous within *Grateloupia*, despite an enormous variety in growth forms ranging from delicately foliose (e.g. *G. acuminata* Holmes), over foliose and leathery (*G. lanceolata* (Okamura) S. Kawaguchi), to stiff and divaricate (most species formerly in the genus *Prionitis*), to extremely rigid and nearly woody (*Prionitis nodifera* Hering). Consistent with earlier observations (Sjöstedt 1926, Kylin 1930, Balakrishnan 1954, 1961, Chiang 1970), the morphology of the ampullary structures in *Grateloupia* is very simple, consisting of a primary filament and two to four unbranched secondary filaments, forming narrow flask-shaped ampullae. The only diagnostic reproductive character observed by Kawaguchi et al. (2001) to differentiate *G. asiatica* from *G. filicina* is the shape of the mature auxiliary cell (i.e. oval or markedly elongate). It remains to be determined, however, if such a difference represents a stable character and not different developmental phases before the diploidization of the auxiliary cell. Postfertilization stages seem to be uniform as well, with the fertilized carpogonium producing several connective filaments that will fuse with nearby auxiliary cells. Each connective filament continues the process of diploidization to other auxiliary cells, resulting in the production of single gonimoblast initials cut off toward the thallus surface. Earlier authors (Kylin 1930, Balakrishnan 1961) have denied the presence of a fusion cell between the carpogonium and the hypogynous cell. More recently, however, the existence of a fusion cell has been demonstrated convincingly in several taxa of the Halymeniaceae (Chiang 1970, Kraft 1977, Kawaguchi 1989, Kawaguchi et al. 2001,this study), thereby confirming the initial observations of Kawabata (1955).

The species discussed here can be identified based on a combination of characters relating to external morphology, vegetative morphology, and anatomy (Table 4). The growth pattern is essentially in agreement with the observations by Kylin (1930, Fig. 9, A–D) for *G. filicina*, based on material collected in the Mediterranean Sea, whereby the cells of several axial filaments cut off a single initial each, developing in a cortical fascicle toward the periphery of the thallus. The primary cortical filament produces higher order filaments by means of longitudinal concavoconvex divisions from every consecutive cell. Axial and inner cortical cells become directly pit connected by small conjuctor cells that fuse with neighboring cells. The extent to which secondary pit connections are formed, however, differs substantially between different species. In *G. minima*, usually no more than two secondary pit connections are formed, resulting in typically x-shaped cells and a very regular construction of the inner cortex because secondary pit connections are restricted to neighboring cells. Inner cortical cells of *G. capensis* produce multiple secondary pit connections, not restricted to cells in each other's immediate vicinity, resulting in a highly irregular inner cortex, which contains numerous stellate cells even in the subapical parts of the thallus. Similarly to *G. minima*, the inner cortex in *G. filicina* is highly regular near the apices, and the initial structure of the inner cortex is likewise decisive for the loosely constructed medulla in the median thallus parts (personal observation). The tendency to form secondary pit connections is believed to govern the extent to which the central cavity of the filaments becomes filled with medullary and rhizoidal filaments or retains a more lax construction. Further observations on species with widely varying morphologies (e.g. *Prionitis*-type, *foliose*-type, and *filicina*-type) should clarify if the patterns observed are congruent with the relationships based on the molecular phylogeny.

Biogeography. A strong geographic imprint can be detected in the phylogeny, with most temperate representatives of *G. filicina* resolved in clades consisting entirely of species from the same geographic area. Isolates from South Africa form a well-supported clade with *G. longifolia* and *G. belangeri*, two species from South Africa. Likewise, *G. asiatica*, *G. subpectinata*, and *G. catenata*, all of them until recently regarded as *G. filicina*, are related to different clades consisting of western Pacific species. *Grateloupia luxurians*, originally described from Sydney, Australia, also conforms to this pattern if one accepts that the specimens from the Atlantic coasts of Europe represent an introduction that dates back at least to the first half of the 20th century (Farnham and Irvine 1968, Irvine and Farnham 1983, Casares Pascual and Seoane Camba 1988, López Rodríguez et al. 1991, Cabioch et al. 1997). The other example where a strong biogeographic signal seems to be lacking is in the Japanese *G. turuturu* known to be introduced and invasive in most of the Atlantic Ocean (Farnham and Irvine 1973, Villalard-Bohnsack and Harlin 1997, 2001, Maggs and Stegenga 1999, Gavio and Fredericq 2002, Araújo et al. 2003).

Specimens collected in tropical regions of all major oceans show a different pattern. They do form a single monophyletic clade, indicating they have evolved from a common ancestor. Contrary to the temperate representatives, a clear geographic structure is lacking, with specimens from the individual clades present in the Caribbean Sea in several cases being sister to specimens from either the Pacific Ocean or Indian Ocean. This lack of geographic structure coincides with significantly lower molecular divergence values of *rbc*L. Values of interspecific divergence in the genus *Grateloupia* usually vary from 5% to 10%, with the odd divergence as low as 1.5% or 2.8% (Wang et al. 2001, Gavio and Fredericq 2002, Mateo-Cid et al. 2005). The tropical *Grateloupia* representatives show a distinctly lower divergence than their temperate counterparts, with values generally not exceeding 5% (Fig. 10). It is possible that temperate and tropical lineages evolve at different rates. The lack of geographic structure and the morphological uniformity, however, are more indicative of a more recent divergence combined with good dispersal capacities. Taxon sampling at this stage is too limited to fully appreciate the distribution of the various

TABLE 4. Comparison of diagnostic features between temperate Atlantic *Grateloupia* species.

	G. filicina	*G. capensis*	*G. luxurians*[a]	*G. minima*
Habit	Discoid holdfast and erect axes, to 9–10 cm high	Discoid holdfast and erect axes, to 30 cm high	Discoid holdfast and erect axes, to 40–70 cm	Extensive crust and erect axes, no higher than 2–4 (– 10) cm
Branching pattern	Main axes percurrent, simple to dichotomous	Pinnate to irregularly dichotomous	Once or twice pinnate	Simple to 2–4 times dichotomously branched
Axes	Compressed, 2–3 mm wide and to 1300 µm thick	Complanate, 6–15 (–25) mm wide and 200–500 µm thick	Compressed, to 5–10 mm wide and 1–3 mm thick	Terete to compressed, 0.8–1 (1.5) mm wide and 200–300 µm thick
Texture	Mucilaginous but not gelatinous	Mucilaginous but firm	Soft and mucilaginous	Firm
Marginal proliferations	Numerous, constricted at the base, producing second-order proliferations	Numerous, unconstricted to slightly constricted at the base, simple	Numerous, constricted at the base, simple	Common, constricted at the base, simple
Surface proliferations	Occasional	Common to abundant	Occasional to common	Absent
Thickness of cortex (outer cortex)	5–8 cells thick (2–4 cells)	6–10 cells thick (4–6 cells)	5–8 cells thick (2–3 cells)	5–7 cells thick (3–4 cells)
Medullary structure	Very laxly constructed	Filaments and stellate cells abundant	Laxly constructed	Hollow to very laxly constructed
Tetrasporangia	20–25 × 45–50 µm	13–18 × 35–40 µm	13–16 × 30–40 µm	18–25 × 40–45 µm
Mature auxiliary cell	Conspicuously elongate, much larger than ampullary cells	Oval, larger than ampullary cells	Oval, larger than ampullary cells	Oval, larger than ampullary cells
Mature cystocarp	150–200 µm	90–150 µm in diameter	120 µm to over 300 µm in diameter	to 250 µm in diameter
Geographical distributions	Mediterranean Sea	South Africa	Australia, northeast Atlantic Ocean	Northeast Atlantic Ocean
References	Kawaguchi et al. 2001; personal observation	Stegenga et al. 1997; this study	Irvine and Farnham 1983; Womersley and Lewis 1994; Cabioch et al. 1997	This study

[a]Dimensions of the thallus are those as observed in European specimens, which appear to be more robust than the Australian specimens.

tropical lineages. A sample from Hawaii, for example, is shown to have a nearly identical sequence to specimens from the southern Gulf of Mexico. A recent cryptic introduction could explain this observation. Alternatively, the species could have been dispersed naturally to the Hawaiian Archipelago before the closure of the Isthmus of Panama, only 3.1 Ma B.P. (Coates and Oblando 1996). The fact that records of *G. filicina* in Hawaii date back to at least 1880 (Abbott 1999) makes a recent introduction less likely. However, without more extensive sampling and data from herbarium samples, it is impossible to prove either scenario. At least in Florida (USA) several lineages coexist, indicating that sympatric distribution patterns as observed in *Bostrychia* (Zuccarello et al. 2002) are possible. Additional in-depth morphological and molecular studies of *Grateloupia* taxa are called for to resolve and reconstruct the biogeographic histories of the genus worldwide.

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