

A re-assessment of reproductive anatomy and postfertilization development in the systematics of *Grateloupia* (Halymeniales, Rhodophyta)

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Abstract – The red algal family Halymeniaceae has been recently the subject of taxonomic revisions based strictly on molecular data. As a result, the number of genera ascribed to it has been decreasing and many generic definitions changed profoundly owing to inconsistencies in diacritical vegetative and particularly reproductive characters in standard literature. Reproductive uniformity within this family has been claimed since the late 19th century and is generally supported by recent authors. In this study we report on consistent significant differences in the architecture of carpogonial and auxiliary cell ampullae, as well as in early postfertilization events, among Mediterranean species currently assigned to the genus *Grateloupia* C. Agardh and provide new interpretations of these features. We recognize several distinct types of ampullae and postfertilization events that distinguish groups of species, these groups proving to be strongly supported by *rbcL* phylogenies. As a result we conclude that the genus *Grateloupia* as presently circumscribed should be segregated into multiple genera. In addition to *Grateloupia sensu stricto*, we resurrect *Dermocorynus* P.L. Crouan et H.M. Crouan, *Pachymeniopsis* Y. Yamada ex S. Kawabata, *Phyllymenia* J. Agardh and *Prionitis* J. Agardh, all of which have been subsumed in *Grateloupia* by previous authors. New genera based on our anatomical and *rbcL* results for *G. doryphora* (Montagne) M. Howe, *G. subpectinata* Holmes and *G. proteus* Kützinger will be described in subsequent papers.

Ampulla architecture / *Grateloupia* / Halymeniales / molecular phylogeny / postfertilization events / *rbcL* gene / reproduction / Rhodophyta / taxonomy

INTRODUCTION

Members of the Rhodophyta are known to have complex life histories and reproductive anatomies (Searles, 1980; Kraft, 1981). Consequently, traditional approaches to the taxonomy of this group have been based primarily on aspects of events involving carpogonial positioning, zygote formation and gonimoblast development (Kylin, 1930; Kraft & Woelkerling, 1981; Kraft & Robins, 1985). More recently, ultrastructural (e.g., Pueschel & Cole, 1982) and molecular-systematic (e.g., Yoon *et al.*, 2006; Le Gall & Saunders, 2007) investigations have brought additional information to bear on red-algal systematics and have resulted in much clarification of phylogenetic relationships within this ancient phylum. Nonetheless, reproductive anatomy and postfertilization development still remain important diagnostic characters for separating many genera and higher-level taxa

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of red algae (Kraft, 1977; Gargiulo *et al.*, 1986; Hommersand *et al.*, 1999; Ballantine *et al.*, 2003; Lin *et al.*, 2008). It is thus important that the implications of molecular data be married to detailed morphological and anatomical observations in order to advance knowledge of red-algal systematics generally and to promote practical and accurate identifications of species wherever possible on the basis of non-molecular observations.

Some families of “florideophyte” reds are particularly in need of these co-ordinated studies, few more so than the widespread and taxonomically challenging Halymeniaceae Bory (1828), in which so many thallus types are similarly foliose and the diagnostic features particularly cryptic or ambiguous. The family presently includes 23 genera (Kawaguchi *et al.*, 2004; De Clerck *et al.*, 2005b; Wilkes *et al.*, 2005; Hommersand *et al.*, 2010) and more than 250 species (Guiry & Guiry, 2012). Members of the Halymeniaceae are characterized by carpogonial branches and auxiliary cells borne in separate clusters of filaments called “ampullae” (Sjöstedt, 1926; Chiang, 1970; Hommersand & Fredericq, 1990).

The term “ampulla” was proposed by Sjöstedt (1926, p. 16, 70) to describe the general appearance of the basket-like arrangements of filaments in which the carpogonial branches and auxiliary cells are nested. He regarded the auxiliary-cell and carpogonial branches as meaning not the ampulla as a whole, but just the outwardly directed side-branch which includes or subtends either the auxiliary cell or the carpogonium. In his view the ampulla as a whole, including the primary filament and branches along with the auxiliary cell or carpogonium, fully corresponded to the homologous auxiliary-cell and carpogonial branches in the Dumontiacean genera (Gigartinales) *Dudresnaya* and *Thuretellopsis*, or *Acrosymphyton* (Acrosymphtales), in all of which the auxiliary-cell branches are unbranched and “accessory”, as are the carpogonial branches save for the pinnate laterals on a clearly percurrent primary axis in *Acrosymphyton*. Subsequent authors have followed Sjöstedt’s interpretation of ampullar structures in the Halymeniaceae, the result being that carpogonial branches are generally held to be two-celled and lateral and the auxiliary cell intercalary in the primary ampullar filament or, in some instances, the basal cell of a second- or third-order lateral (Kylin, 1930; Fritsch, 1952; Balakrishnan, 1961a; Kawabata, 1962; Kawabata, 1963; Chiang, 1970; Kawaguchi *et al.*, 2001; De Clerck *et al.*, 2005a, Lin *et al.*, 2008).

Reproductive uniformity within the assembly of halymeniacean genera has been claimed since the late 19th century (Berthold, 1884; Schmitz & Hauptfleisch, 1897) and largely supported by subsequent authors (Sjöstedt, 1926; Kylin, 1930, 1956; Balakrishnan, 1961b; Kawabata, 1963). For this reason vegetative, rather than reproductive, features came to be given greater weight in genus-level taxonomy in the second half of the 20th century (Kylin, 1956; Guiry & Irvine, 1974; Kraft, 1977). In contrast to this emphasis, however, Chiang (1970) proposed that the structure and the shape of the auxiliary cell ampullae can be valid characters in distinguishing genera, and even groups of species, in the Halymeniaceae and in organizing them phylogenetically, a perspective supported by a number of recent molecular-phylogenetic investigations. For example, published *rbcL* (the large subunit of the Rubisco operon) phylogenies have indicated the presence of five monophyletic groups at the generic or supra-generic level within the Halymeniaceae (Kawaguchi *et al.*, 2004; De Clerck *et al.*, 2005b) that are characterized by a particular combination of auxiliary-cell ampullar types as defined by Chiang (1970). In addition, the structure of carpogonial-branch ampullae is also thought have taxonomic value comparable to that of auxiliary-cell ampullae (Kawaguchi *et al.*, 2004).

Postfertilization development is not well documented in most of the members of the Halymeniaceae (Balakrishnan, 1960; Kraft, 1977), although a detailed study of the literature does reveal differences, especially in early events, both within and between genera (Sjöstedt, 1926; Kawabata, 1962; Kawabata, 1963; Chiang, 1970; Kraft, 1977; Guiry & Maggs, 1982; Maggs & Guiry, 1982; Gargiulo *et al.*, 1986; Kawaguchi, 1989, 1990, 1991, 1997), such as the number of cells of both carpogonial and auxiliary branches, the extension of carpogonial and auxiliary fusion cell, and the formation of pericarp.

Kylin (1937; 1956) segregated the family Halymeniaceae (as the Cryptonemiaceae) from other nonprocarpic families of the then Cryptonemiales on the basis of the direct origin of the connecting filaments from a generally enlarged carpogonium, without fusion of either the fertilized carpogonium itself or the connecting filaments with any other cell of the carpogonial branch system. This view was later supported by the observations of Balakrishnan (1961a; 1961b). However, fusion between the carpogonium and hypogynous cell, as well as in some instances additional ampullary cells, has been variously reported in members of this family (Kawabata, 1955, 1962, 1963; Chiang, 1970; Kraft, 1977; Maggs & Guiry, 1982; Gargiulo *et al.*, 1986; Kawaguchi, 1989, 1990; Kawaguchi *et al.*, 2001; De Clerck *et al.*, 2005a). Despite these claims, Womersley and Lewis (1994), in a monograph of the halymeniacean genera from southern and southwestern Australia, stated in their diagnosis of the family that "... connecting filaments developing from the fertilized carpogonium", and Saunders and Kraft (1996), when they proposed the substitution of the ordinal designation "Halymeniales" for the previously subsumed "Cryptonemiales" (Kraft & Robbins, 1985), wrote that "...multiple septate, branched connecting filaments [are issued] directly from the carpogonium".

Grateloupia is the largest genus in the Halymeniaceae, with 90 currently recognized species (Guiry & Guiry, 2012). It displays the broadest range of frond habits in the family, ranging from the finely pinnate *G. filicina* (J.V. Lamouroux) C. Agardh, through subdichotomous forms like *G. dichotoma* J. Agardh and *G. hawaiiiana* Dawson, to foliose blades like *G. lanceolata* (Okamura) Kawaguchi and *G. ovata* Womersley *et al.* Lewis. At present many of the species are poorly known or characterized.

Recently the genus has been thrown into a state of taxonomic flux by the removal of some members (Hommersand *et al.*, 2010; Kawaguchi *et al.*, 2002; Lee *et al.*, 1997), the addition of new species, the reinstatement of others that had been previously synonymized (Kawaguchi, 1990; Wang *et al.*, 2000; Kawaguchi *et al.*, 2001; Faye *et al.*, 2004; De Clerck *et al.*, 2005a), and the subsuming of whole genera once regarded as separate, such as *Pachymeniopsis* Y. Yamada *ex* S. Kawabata (Kawaguchi, 1997), *Prionitis* J. Agardh (Wang *et al.*, 2001), *Dermocorynus* H. Crouan *et* P. Crouan (Wilkes *et al.*, 2005) and *Phyllymenia* J. Agardh (De Clerck *et al.*, 2005b). With the exception of *Pachymeniopsis* (Kawaguchi 1997), all of the previous mergers were based almost exclusively on molecular evidence, being species of the above genera non monophyletic in published phylogenetic analyses. Reproductive structures and postfertilization stages are routinely reported as being homogeneous within *Grateloupia sensu lato* (Wang *et al.*, 2001; Kawaguchi *et al.*, 2004; De Clerck *et al.*, 2005b; Wilkes *et al.*, 2005).

Recently, however, two distinct types of auxiliary cell ampulla architectures have been revealed among the species of *Grateloupia* as presently circumscribed (Lin *et al.*, 2008). Ampullae consisting of three orders of filaments were observed in *G. taiwanensis* S.-M. Lin *et* H.-Y. Liang, with the auxiliary cell being the basal cell of a third-order filament, whereas ampullae composed of only

two orders of filaments were reported in *G. orientalis* S.-M. Lin *et* H.-Y. Liang and *G. ramosissima* Okamura, in both of which the first cell of a second-order filament functions as the auxiliary cell. According to *rbcL* phylogenies, *G. orientalis* is allied with the type species of *Grateloupia*, whereas *G. taiwanensis* clusters with a clade that includes the generitypes of *Phyllymenia*, *Prionitis* and *Pachymeniopsis*. In light of these observations, Lin and coworkers argued that a critical re-examination of the type species of the genera *Grateloupia*, *Phyllymenia*, *Prionitis* and *Pachymeniopsis* is highly desirable to clarify the generic and interspecific relationships among the species presently placed in *Grateloupia*. Furthermore, a review of the literature indicates that *Grateloupia* as currently circumscribed includes a number of species with still different patterns of gonimoblast development (Sjöstedt, 1926; Kawabata, 1962, 1963; Chiang, 1970; Guiry & Maggs, 1982; Maggs & Guiry, 1982; Gargiulo *et al.*, 1986; Kawaguchi, 1989, 1990, 1991, 1997; Lin *et al.*, 2008).

In the Mediterranean Sea *Grateloupia* includes ten species of both native [*G. filicina* (the generitype), *G. dichotoma*, *G. lanceola* (J. Agardh) J. Agardh, *G. proteus* Kützinger (including *G. cosentinii* Kützinger)] and introduced [*G. asiatica* Kawaguchi *et* Wang, *G. lanceolata*, *G. subpectinata* Holmes, *G. patens* (Okamura) Kawaguchi *et* H.W. Wang, *G. turuturu* Y. Yamada] species, as well as a species previously reported as *G. "doryphora"* (Montagne) M. Howe (Furnari *et al.*, 2003; Bárbara & Cremades, 2004; Verlaque *et al.*, 2005; Wilkes *et al.*, 2006). Several names have also been variously applied to Mediterranean populations and specimens, the taxonomic statuses of which are presently uncertain (Verlaque *et al.*, 2005), including *G. coriacea* Kützinger, *G. horrida* Kützinger (= *G. filicina* f. *horrida* (Kützinger) Børgesen), *G. filicina* var. *cylindricaulis* Solier in Castagne, *G. filicina* var. *simplex* Solier in Castagne, *G. fimbriata* Montagne, *G. gorgonioides* Kützinger, *G. neglecta* Kützinger.

In this paper we report on the anatomy of carpogonial and auxiliary-cell ampullae and postfertilization events for Mediterranean species of the genus *Grateloupia*. Included among these are the generitype, *G. filicina*, as well as *G. dichotoma*, *G. proteus* and *G. horrida*, all collected from their respective type localities, and also the *Grateloupia* species from the Straits of Messina that was previously reported as *G. "doryphora"* (De Masi & Gargiulo, 1982; Wilkes *et al.*, 2006). In addition we provide observations on the Mediterranean/Atlantic-European adventive *G. subpectinata*, a southern Australian species that until recently (Womersley & Lewis, 1994) was regarded as *G. filicina*. We also employ analyses of *rbcL* data for these and related species currently assigned to *Grateloupia* to place all of the Mediterranean species into a broader phylogenetic context.

MATERIALS AND METHODS

Anatomical observations

Samples used for anatomical studies are listed in Table 1. Preparations were made from formalin-preserved samples and/or from *exsiccata*. Voucher specimens are deposited in the phycological collection of the *Herbarium Messanaensis* of the University of Messina, Italy (MS, <http://sweetgum.nybg.org/ih/>).

Table 1. List of specimens examined in the present paper

Current name	Collection information	Voucher information	GB accession number
<i>Grateloupia dichotoma</i> J. Agardh	Lake Ganzirri, Messina, Italy; 23.vii.2004	MS-PhL088-Gra036	JX070628
<i>Grateloupia dichotoma</i> J. Agardh	St. Hospice, 1841 (Golfe de Saint-Hospice, Beaulieu-sur-Mer, France)	LD22637 (type specimen, Herb. J. Agardh); LD22638 (sintype specimen, Herb. J. Agardh)	–
<i>Grateloupia dichotoma</i> J. Agardh	as <i>Sphaerococcus antibae</i> , Leg. Giraudy	LD22634 (Herb. J. Agardh)	–
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Muggia, Trieste, Italy; 19.vii.1995	MS-Gr001T; MS-Gr002T	–
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Livorno, Italy, 01.vii.2002; leg. G. Sartoni	MS-SG068; MS-SG069-Gra047	JX070629
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Rijeka, Fiume, Slovenia; 1861	HBG111/1786	–
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	S. Croce, Trieste, Italy; 24.VII.1967; leg. G. Giaccone	MS-35018-Gt02	–
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Savalletri, Brindisi, Italy; 1975	MS-SG234 (donation from Herb. University of Bari, Botany)	–
<i>Grateloupia filicina</i> (J.V. Lamouroux) C. Agardh	Trieste, Italy	HBG101/1786; HBG114/1786; HBG931/1786; HBG112/1786; HBG113/1786	–
<i>Grateloupia horrida</i> Kützinger	Licata, Agrigento, Italy; 17.vi.2002	MS-SG057-Gra037	–
<i>Grateloupia horrida</i> Kützinger	Napoli, Italy	L0105097	–
<i>Grateloupia horrida</i> Kützinger	Ercolano, Napoli, Italy; 20.04.1981	MS-35019-Gt01	–
<i>Grateloupia horrida</i> Kützinger	Posillipo, Napoli, Italy; 23.vi.2002	MS-SG065-Gra038	–
<i>Grateloupia horrida</i> Kützinger	S. Maria La Scala, Catania, Italy; 03.ii.2004	MS-SG138	–
<i>Grateloupia horrida</i> Kützinger	S. Maria La Scala, Catania, Italy; 09.iii.2004	MS-MB018; MS-SG142; MS-SG143	–
<i>Grateloupia horrida</i> Kützinger	S. Maria La Scala, Catania, Italy; 15.vii.2004	MS-MB045; MS-SG201-Gra021; MS-SG202-Gra022; MS-SG203-Gra023; MS-SG204; MS-SG205	–
<i>Grateloupia horrida</i> Kützinger	S. Tecla, Catania, Italy; 23.vii.2004	MS-MB055; MS-MB057; MS-SG224-Gra032; MS-SG225; MS-SG226-Gra033; MS-SG227-Gra034; MS-SG228; MS-SG229-Gra035; MS-MB052-Gra031; MS-MB054; MS-SG218; MS-SG219-Gra029; MS-SG220-Gra030; MS-SG221; MS-SG223	JX070627

Table 1. List of specimens examined in the present paper (*continued*)

<i>Current name</i>	<i>Collection information</i>	<i>Voucher information</i>	<i>GB accession number</i>
<i>Grateloupia horrida</i> Kützing	Villa S. Giovanni, Reggio Calabria, Italy; 03.v.2005	MS-MB064	–
<i>Grateloupia horrida</i> Kützing	Villa S. Giovanni, Reggio Calabria, Italy; 14.iv.2004	MS-SG101	–
<i>Grateloupia horrida</i> Kützing	Villa S. Giovanni, Reggio Calabria, Italy; 17.iii.2004	MS-SG161; MS-SG162; MS-SG163; MS-SG164; MS-SG165; MS-SG166; MS-SG167	–
<i>Grateloupia horrida</i> Kützing	Villa S. Giovanni, Reggio Calabria, Italy; 26.vii.2004	MS-MB046; MS-SG206- Gra024; MS-SG208; MS-SG207	–
<i>Grateloupia subpectinata</i> Holmes	Bass Strait, Victoria, Australia; 09.viii.1998, legit G.T. Kraft	MS-35063-Gt01	–
<i>Grateloupia proteus</i> Kützing	Ercolano, Napoli, Italy; 21.iv.1981	MS-35017-Gt01	–
<i>Grateloupia proteus</i> Kützing	S. Maria La Scala, Catania, Italy; vi.1983	MS-35017-Gt02	–
<i>Grateloupia proteus</i> Kützing	S. Maria La Scala, Catania, Italy; 17.x.2004	MS-MB051; MS-SG122; MS-SG215-Gra026; MS-SG216-Gra027; MS-SG217-Gra028	–
<i>Grateloupia proteus</i> Kützing	Stazzo, Catania, Italy; 23.vii.2004	MS-MB048; MS-PhL060- Gra025; MS-SG210; MS-SG211; MS-SG213; MS-SG214 Gra041	JX070626
<i>Grateloupia proteus</i> Kützing	Villa S. Giovanni, Reggio Calabria, Italy; vi. 1983	MS-35017-Gt03	–
<i>Grateloupia</i> sp.	Paradiso, Messina, 01.v.1982	MS-35016-Gt01	–
<i>Grateloupia</i> sp.	Posillipo, Napoli, Italy; 23/06/2002	MS-SG066-Gra019	–
<i>Grateloupia</i> sp.	Torre Faro, Messina, Italy; 22.iii.2004	MS-SG170-Gra018; MS-SG171; MS-SG172; MS-SG173; MS-SG174	–
<i>Grateloupia</i> sp.	Villa S. Giovanni, Reggio Calabria, Italy; 01.vii.1981	MS-35016-Gt02	–
<i>Grateloupia</i> sp.	Villa S. Giovanni, Reggio Calabria, Italy; 17.iii.2004	MS-SG146; MS-SG147; MS-SG148; MS-SG149; MS-SG150-Gra016; MS-SG151-Gra040; MS-SG152; MS-SG154; MS-SG155; MS-SG156; MS-SG157; MS-SG158; MS-SG159; MS-SG160; MS-SG169-Gra015; MS-MB026	AY651060

Hand-sections were squashed in 5N HCl and examined under the light microscope equipped with Nomarski interference optics (Aristoplan, Leitz). Photos were captured on a Wild-Heerbrugg MPS12 camera (Leica Microsystem). Negative films were scanned with a Nikon Coolscan LS-40ED and processed by Adobe® Photoshop® CS (v 8.0.1).

Molecular methods

Sequence data generated for the *rbcL* gene were submitted to GenBank. Accession numbers, together with collection information, are given in Table 1. In order to prevent errors in sorting of samples, each DNA isolation was performed from a single individual, a fragment of which was kept as a voucher either preserved in 4% formalin in seawater, dried in silica gel, or pressed as an herbarium sheet.

Freshly collected, silica-gel preserved or dried unformalized plants recovered from herbarium sheets were ground in liquid nitrogen. DNA was isolated as described in Manghisi *et al.* (2010). Undiluted DNA was used as template for PCR or diluted in sterile bidistilled water up to 1:20, depending on each template.

The *rbcL* gene was PCR-amplified from isolated DNA or from freshly released carpospores or tetraspores, as described in Morabito *et al.* (2005), as one to three overlapping fragments using primers as specified in the literature (Freshwater & Rueness, 1994; Wang *et al.*, 2000). PCR products were purified with the QIAquick® PCR purification kit (Qiagen spa, Italy) according to the manufacturer's instructions, or gel-purified (Saunders, 1993). The DNA was then ethanol precipitated (Sambrook *et al.*, 1989) and sent to an external company (MWG Biotech AG, Ebersberg, Germany) for DNA sequencing. Individual nucleotide sequences were assembled with the software ChromasPro (v. 1.41, Technelysium Pty Ltd), and a multiple sequence alignment was constructed in MacClade 4.08 for MacOSX (Maddison & Maddison, 2000).

Additional *rbcL* sequences from species of *Grateloupia* and *Yonagunia* Kawaguchi *et Masuda* were downloaded from GenBank (Benson *et al.*, 2008, browsed 08 May 2008). All phylogenetic analyses were performed in PAUP* 4b10 for the Macintosh (Swofford 2002), MrBayes 3.1.2 (serial version for the Macintosh and MPI versions for Unix clusters; Ronquist & Huelsenbeck, 2003; Altekar *et al.*, 2004) and PhyML 3.0 (online version, <http://atgc.lirmm.fr/phyml>; Guindon & Gascuel, 2003).

An initial alignment, including 234 halymeniacean and four outgroup sequences and 1260 nucleotide positions, was subjected to neighbor-joining (NJ) distance analysis under a general time-reversible model (GTR, Lanave *et al.*, 1984) in PAUP* to identify species groups. The resulting tree was used to prepare a second alignment for subsequent phylogenetic analyses with 62 sequences (56 ingroup) by the exclusion of duplicate or similar sequences (poor quality sequences, i.e., those missing more than 30% of data, were also removed). Different models of nucleotide substitutions were tested in Modeltest 3.7 (Posada & Crandall, 1998; Posada & Buckley, 2004) on the second alignment. The model of nucleotide substitution was GTR+I+G, selected in accordance with the Akaike Information Criterion without using branch lengths as parameters.

Likelihood analyses were performed under a heuristic search. Ten random sequence- addition replicates with tree bisection and reconnection (TBR) were analyzed, with all minimal trees (MulTrees) saved in PAUP*. The steepest

descent option in the branch-swapping procedure was not used because of an unfixed bug in the current beta version of PAUP* (<http://paup.csit.fsu.edu/problems.html>). Bootstrap resampling was performed to estimate robustness of the internal nodes (Felsenstein, 1985) based on 1000 replicates in PhyML, with a GTR+I+G substitution model (with all parameters estimated during the search), starting from a BIONJ tree (Gascuel, 1997) with subtree pruning and regrafting (SPR) as the branch-swapping algorithm.

For bayesian inference (BI) the dataset was partitioned according to codon positions and the prior probability distribution for the site-specific rates in the phylogenetic model was set as “variable”. Six different analytical strategies were assessed combining the covarion-like model (Huelsenbeck, 2002) with the GTR+G model of sequence evolution and unlinking parameters among partitions (shape, statefreq, revmat, tratio, switchrates, brlens). Each analysis consisted of two parallel runs, each run using four chains, one cold and three incrementally heated (temp = 0.05 in analysis D, temp = 0.10 in others). A single run consisted of ten million generations that were sampled every 1000th tree. After the runs were completed, likelihood values for the two runs were plotted against the number of generations to estimate an appropriate burn-in for each analysis. Only trees saved during the stationary phase of the runs were used to calculate a majority-rule consensus tree and the corresponding posterior-probability distribution. Bayes factors were used to compare all six analytical strategies and were calculated according to Kass and Raftery (1995), using the harmonic mean of the likelihood values of the MCMC samples during the stationary phase of the runs. According to Bayes factors: a) the covarion-like model is not favored if parameter estimation is not unlinked among partitions; b) unlinking parameter estimation is favored, both with or without the covarion option, even without unlinking branch lengths; c) unlinking branch lengths among partitions is favored both with or without covarion; d) the covarion-like model is favored if the parameter estimation is unlinked among partitions, both unlinking branch lengths or not. Therefore, the selected model was the GTR+G combined with the covarion-like, unlinking the estimation of all parameters among partitions.

In all phylogenetic analyses unrooted trees were constructed and subsequently rooted with reference to the outgroup taxa.

RESULTS

Anatomical observations

***Grateloupia filicina* (J.V. Lamouroux) C. Agardh** 1822:223, generitype species of *Grateloupia* C. Agardh 1822: 221. **Figs 1-12**

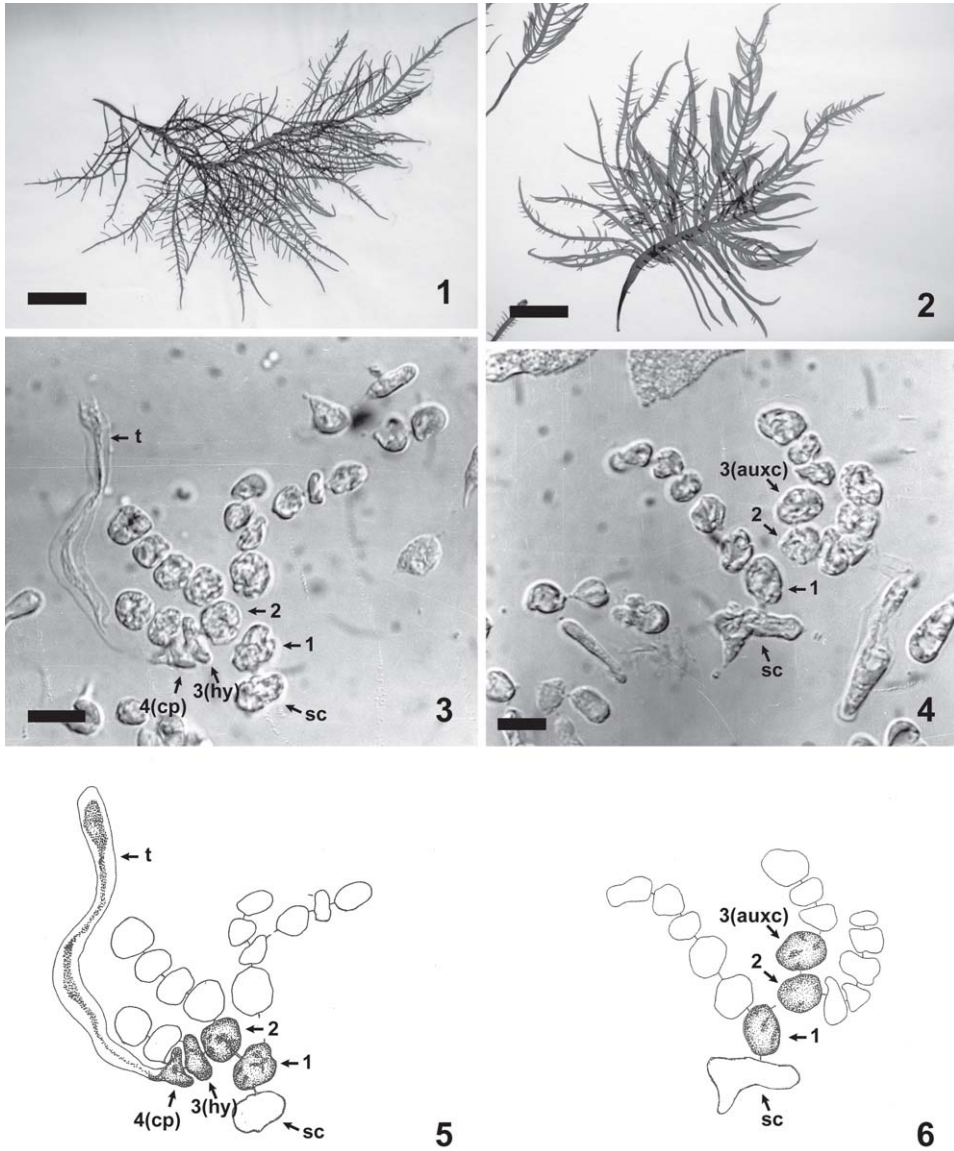
Basionym = *Delesseria filicina* J.V. Lamouroux 1813: 125.

Type locality: Trieste, Adriatic Sea (Italy).

Specimens examined: see Table 1.

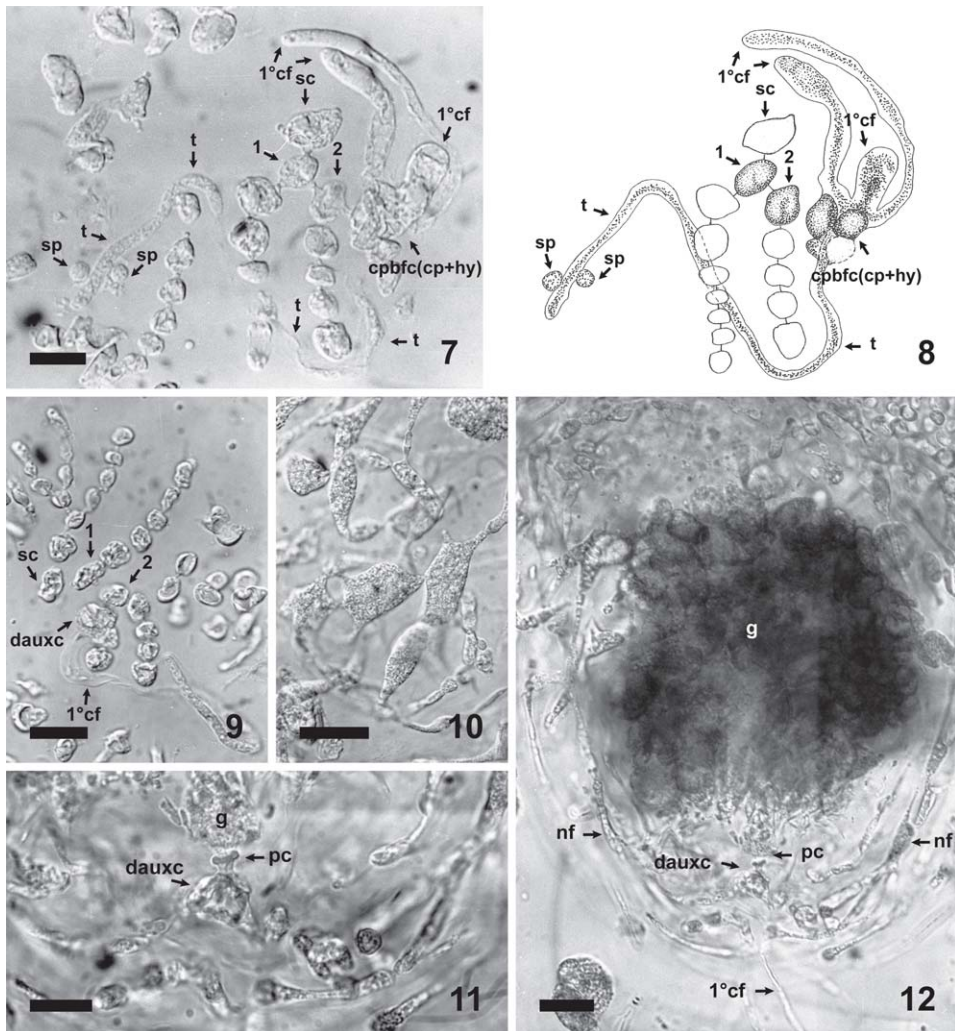
The thallus arising from a discoid holdfast is formed by pinnate erect fronds tapering at ends, mainly compressed, mucilaginous but firm, reddish to blackish purple, sometimes greenish (Figs 1, 2).

The carpogonial branch is 4-celled (Figs 3, 5), slightly bent, and borne on a supporting cell ($12.2 \pm 0.8 \times 18.5 \pm 0.6 \mu\text{m}$). The branch consists of a trapezoidal carpogonium ($12.1 \pm 0.7 \times 11.5 \pm 0.4 \mu\text{m}$) with a long trichogyne, a rectilinear



Figs 1-6. *Grateloupia filicina*. **1.** Herbarium specimen from Trieste, Italy (MS-35018-Gt02). **2.** Herbarium specimen from Livorno, Italy (MS-SG069). **3.** Carpogonial branch. **4.** Auxiliary-cell branch. **5, 6.** Interpretative drawings of Figs 3 and 4: cells of carpogonial and auxiliary-cell branches are shaded and numbered. Scale bars: 1.5 cm in Figs 1 and 2; 15 μ m in Figs 3 and 4. Abbreviations: auxc, auxiliary cell; cp, carpogonium; hy, hypogynous cell; sc, supporting cell.

hypogynous cell ($11.3 \pm 0.6 \times 5.2 \pm 0.9 \mu\text{m}$) with a two- or three-celled sterile lateral branch, an ovoid subhypogynous cell ($12.3 \pm 0.8 \times 13.4 \pm 1 \mu\text{m}$) with a four- or five-celled sterile lateral branch, and a subtending ovoid cell ($11.9 \pm 0.7 \times 13.3 \pm 0.9 \mu\text{m}$) bearing a 5-7-celled sterile, occasionally branched, lateral branch (Figs 3, 5). The



Figures 7-12. *Grateloupia filicina*. **7.** Carpegonial ampulla after fertilization; note fusion between carpegonium and hypogynous cell and primary connecting filaments arising from it; supporting cell and cells one and two of carpegonial branch with their respective 2nd order branches are still recognizable; two spermatia are stuck to a very long thrycogynous. **8.** Interpretative drawing of Fig. 7; cells of carpegonial branch and auxiliary branch are shaded and numbered. **9.** Auxiliary-cell ampulla after diploidization; note the primary connecting filament fusing with the auxiliary cell without forming a pit-connection; supporting cell and cells one and two of auxiliary branch with their respective 2nd and 3rd order branches are still recognizable. **10-12.** Mature cystocarp. **10.** Detail of nutritive filaments constituting the pericarp. **11.** Basal portion of cystocarp with diploidized auxiliary cell and gonimoblast. **12.** General view of mature cystocarp. Scale bars: 15 μ m in Fig. 7; 25 μ m in Figs 9, 11; 50 μ m in Figs 10, 12. Abbreviations: 1°cf, primary connecting filament; cpbfc(cp+hy), fusion cell made of carpegonium and hypogynous cell; dauxc, diploidized auxiliary cell; g, gonimoblast; nf, nutritive filaments; pc, pit connection; sc, supporting cell; sp, spermatium; t, thrycogyne.

trichogyne points to the thallus surface. The sterile branches of the carpogonial branch cells curve towards the thallus surface to form an ampulla.

The auxiliary cell ($14.1 \pm 0.7 \times 13.8 \pm 0.9 \mu\text{m}$) is the terminal cell of a 3-celled branch (Figs 4, 6) borne on an intercalary inner-cortical cell ($12.1 \pm 0.9 \times 18.0 \pm 0.8 \mu\text{m}$). All the cells of the auxiliary branch, including the auxiliary cell itself, are ovoid and bear a simple 3-6-celled lateral branch (Figs 4, 6). The laterals curve towards the thallus surface to form an ampulla similar to that of the carpogonial branch. The auxiliary-cell branch is also curved so that the auxiliary cell lies in the center of the ampulla.

After presumed fertilization, the carpogonium and the hypogynous cell fuse and directly produce two or three primary connecting filaments that are unbranched and non-septate (Figs 7, 8). When a connecting filament approaches an auxiliary cell, its apical region expands and fuses to the subapical part of the auxiliary cell (Fig. 9). This filament then either ceases growth or continues on from the point of its juncture to the auxiliary cell to presumably effect further diploidizations.

The diploidized auxiliary cell cuts off a single gonimoblast initial towards the thallus surface (Fig. 11). At the same time, several nutritive filaments are produced by the auxiliary cell and also directed toward the thallus surface (Fig. 12). These filaments consist initially of elongated cells ($132.1 \pm 26.8 \times 6.2 \pm 0.8 \mu\text{m}$) that swell ($92.3 \pm 30.7 \times 35.2 \pm 12.3 \mu\text{m}$) and contain dense refractive contents (Fig. 10). The diploidized auxiliary cell is always recognizable during gonimoblast development by virtue of its greater size.

The young gonimoblast is composed of two or more synchronously maturing clusters of cells derived from the gonimoblast initial, spores of the outermost cluster maturing and released first as those of the second and subsequent gonimolobes successively reach mature size. Almost all the cells of the gonimolobes are converted basipetally into carposporangia. The mature cystocarp is sub-spherical and $940 \pm 200 \mu\text{m}$ in diameter (Fig. 12). A thin, non-consolidated pericarp is formed by the nutritive filaments arising from the diploidized auxiliary cell, these mixed with 3-5 layers of elongate medullary filaments (Fig. 12). Mature carposporangia are $17.7 \pm 2.0 \times 13.6 \pm 2.6 \mu\text{m}$. The cystocarp is deeply immersed in the thallus and an ostiole is present from an early stage.

***Grateloupia proteus* Kützinger 1843: 397.**

Figs 13-24

Type locality: Mediterranean Sea.

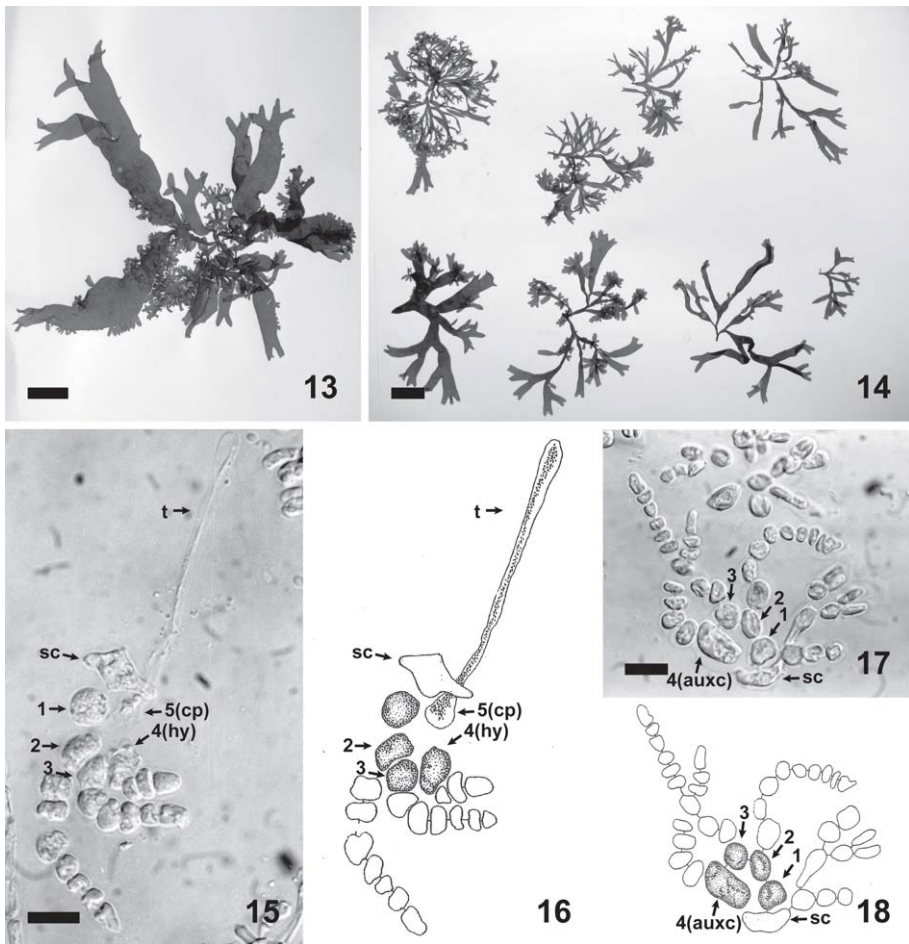
= *Grateloupia cosentinii* Kützinger 1849: 732 (Zanardini 1871; Balakrishnan 1961a)

Type locality: Sicily, Italy.

Specimens examined: see Table 1.

The thallus arising from a basal disc is formed by a short stipe from which dichotomously divided fronds arise, compressed, firm, reddish to blackish purple; proliferations in all directions are frequent especially in old plants (Figs 13, 14).

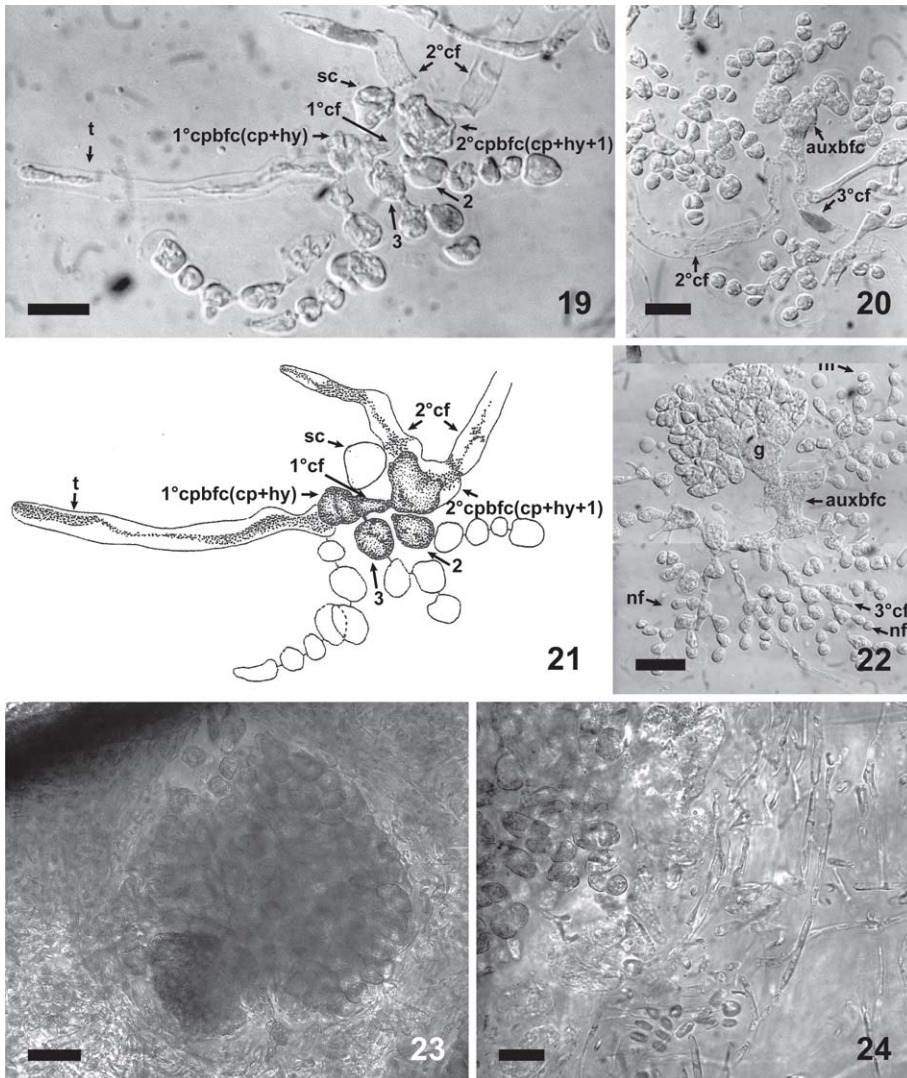
The carpogonial branch is 5-celled (Figs 15, 16) and borne on an inner cortical supporting cell ($28.2 \pm 0.9 \times 15.3 \pm 0.7 \mu\text{m}$). It consists of a small carpogonium ($10.5 \pm 0.6 \times 12.4 \pm 0.8 \mu\text{m}$) with a long trichogyne, a hypogynous cell ($11.4 \pm 0.8 \times 14.3 \pm 0.5 \mu\text{m}$) bearing a three- or four-celled sterile filament, an ovoid subhypogynous cell ($13.5 \pm 0.5 \times 12.3 \pm 0.7 \mu\text{m}$) with a 4-6-celled sterile filament, a subtending ovoid cell ($13.4 \pm 0.2 \times 12.7 \pm 0.9 \mu\text{m}$) bearing a 7-12-celled unbranched sterile filament, and a large rounded refractive basal cell ($15.6 \pm 0.6 \times 14.3 \pm 0.6 \mu\text{m}$) without sterile filaments (Figs 15-16). The branch is distally curved so that the carpogonium lies adjacent to the large basal cell. The tricho-



Figs 13-18. *Grateloupia proteus*. **13**. Herbarium specimens from Villa San Giovanni, Italy (MS-35017-Gt03). **14**. Herbarium specimens from S. Maria La Scala (MS-35017-Gt02). **15**. Carpo-gonial ampulla. **17**. Auxiliary-cell ampulla. **16**, **18**. Interpretative drawings of Figs 15, 17; cells of carpo-gonial branch and auxiliary-cell branch are shaded and numbered. Scale bars: 2 cm in Figs 13, 14; 20 μ m in Figs 15, 17. Abbreviations: auxc, auxiliary cell; cp, carpo-gonium; hy, hypogynous cell; sc, supporting cell.

gyne is directed straight to, or sometimes curved to various degrees toward, the thallus surface. The sterile branches of each cell of the carpo-gonial branch curve towards the thallus surface to form an ampulla.

The auxiliary cell ($28.2 \pm 0.8 \times 13.8 \pm 0.9 \mu$ m) is the terminal cell of a 4-celled branch (Figs 17, 18) borne on an intercalary inner-cortical cell ($25.8 \pm 0.9 \times 14.9 \pm 0.8 \mu$ m). Cells 2-4 of the auxiliary branch, including the auxiliary cell itself, have a simple or sparingly branched, 4-12-celled lateral (Figs 17, 18). The laterals curve towards the thallus surface to form an ampulla similar to the carpo-gonial branch. The auxiliary-cell branch curves so that the auxiliary cell is central in the ampulla.



Figs 19-24. *Grateloupia proteus*. **19.** Carpogonial ampulla after fertilization; note fusion between carpogonium and hypogynous cell and primary connecting filaments arising from it and contacting the first cell of the carpogonial branch; supporting cell and cells two and three of carpogonial branch with their respective branches are still recognizable. **20.** Auxiliary-cell ampulla after diploidization; the secondary connecting filament fuses with the auxiliary cell without forming a pit-connection; note the pit connection between the tertiary connecting filament and the auxiliary-cell fusion cell. **21.** Interpretative drawing of Fig. 19. **22.** Auxiliary ampulla with an extended fusion complex and initial gonimoblast; branched nutritive filaments, mostly downwardly directed, produced by the auxiliary fusion cell are recognizable; note tertiary connecting filaments issuing from nutritive filaments. **23, 24.** Mature cystocarp. **23.** General view of mature cystocarp. **24.** Detail of elongate medullary filaments forming the thin pericarp. Scale bars: 20 μ m in Figs 19, 20; 35 μ m in Figs 22, 24; 70 μ m in Fig. 23. Abbreviations: 1°cpbfc(cp+hy) = carpogonial branch fusion cell made of carpogonium and hypogynous cell; 2°cf = secondary connecting filament; 2°cpbfc(cp+hy+1) = carpogonial branch fusion cell made of carpogonium, hypogynous and 1st cell of the carpogonial branch; sc, supporting cell; t, trichogyne.

After presumed fertilization, the carpogonium enlarges and fuses with its hypogynous cell (Figs 19, 21). The resulting fusion cell forms a short projection (a 1° connecting filament) that extends towards and fuses with the large basal cell of the carpogonial branch. This secondary fusion cell can produce one or two secondary connecting filaments that are separated from it by a pit-connected cross-wall. The secondary connecting filaments are unbranched and non-septate.

When a connecting filament approaches an auxiliary cell, its terminal region expands and directly fuses to the sub-apical portion of the auxiliary cell (Fig. 20). This filament either stops or continues growing to effect further diploidizations. In addition, tertiary connecting filaments can be cut off from sub-apical or lateral parts of the auxiliary cell, but in these cases they are separated by a pit-connection (Fig. 20).

After being contacted by a connecting filament, the auxiliary cell expands and fuses with adjacent ampullary cells to form an extended fusion complex (Figs 20, 22). Branched nutritive filaments, mostly downwardly directed, are produced by the auxiliary fusion cell and consist of rounded cells, $8.7 \pm 0.3 \mu\text{m}$ in diameter, with dense refractive contents. Additional filaments, morphologically similar to the connecting ones, can develop from the terminal cells of nutritive branches, which continue to elongate instead of dividing (Fig. 22). Simultaneously with the production of nutritive filaments, the auxiliary fusion cell cuts off a single gonimoblast initial (Fig. 22) toward the thallus surface. The young gonimoblast is composed of two or more gonimolobes derived from the gonimoblast initial. Almost all the cells of gonimoblast are converted basipetally into carposporangia. The mature cystocarp is subspherical and $342 \pm 94 \mu\text{m}$ in diameter (Fig. 23). It is surrounded by 3-5 layers of elongate medullary filaments that contribute to the formation of a thin pericarp augmented proximally with a few nutritive filaments (Fig. 24). Mature carposporangia are $10.7 \pm 1.2 \times 7.6 \pm 2.4 \mu\text{m}$. The cystocarp is deeply immersed in the thallus and an ostiole is present from an early stage.

***Grateloupia dichotoma* J. Agardh 1842: 103.**

Figs 25-35

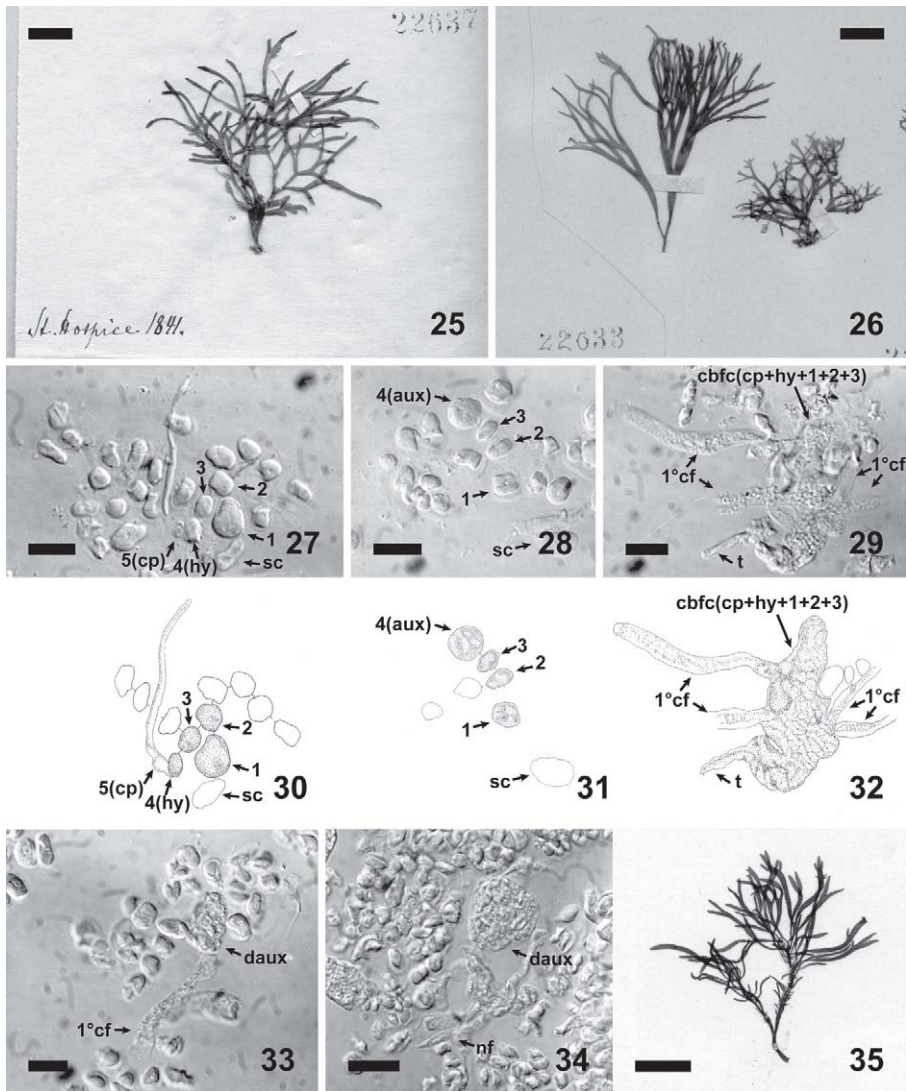
Type locality: Nice and Mediterranean France coasts.

Specimens examined: see Table 1.

The thallus arising from a basal disc is formed by a short stipe from which dichotomously divided fronds arise, mainly compressed, terete in young portions, firm, reddish to blackish purple (Figs 25, 26, 35).

Carpogonial branches are 5-celled and consist of a small carpogonium ($10.1 \pm 0.6 \times 11.4 \pm 0.4 \mu\text{m}$) with a long trichogyne, a hypogynous cell ($12.1 \pm 0.5 \times 13.4 \pm 0.7 \mu\text{m}$) bearing a three- or four-celled sterile lateral branch, an ovoid subhypogynous cell ($12.5 \pm 0.4 \times 13.8 \pm 0.6 \mu\text{m}$) with a four- or five-celled sterile lateral branch, a subtending ovoid cell ($13.5 \pm 0.6 \times 14.8 \pm 0.7 \mu\text{m}$) bearing a four- or five-celled unbranched sterile lateral branch and a basal cell ($15.5 \pm 0.3 \times 16.1 \pm 0.5 \mu\text{m}$) without laterals (Figs 27, 30). The carpogonial branch is distally curved so that the carpogonium lies adjacent to the large basal cell. The trichogyne is directed straight to, or sometimes curved to various degrees towards, the thallus surface. The sterile branches of the carpogonial branch curve toward the thallus surface to form an ampulla.

The auxiliary cell ($15.3 \pm 0.5 \times 15.1 \pm 0.7 \mu\text{m}$) is the terminal cell of a 4-celled branch (Figs 28, 31). All the cells of the auxiliary-cell branch, including the auxiliary cell itself, bear a simple or sparingly branched lateral (Figs 28, 31), all of which curve toward the thallus surface to form an ampulla similar to that of the carpogonial branch.



Figs 25-35. *Grateloupia dichotoma*. **25.** Type specimen from Herbarium "J. Agardh" (22637, St. Hospice, France). **26.** Specimen from Herbarium "J. Agardh" (22634, St. Hospice, France). **27-34.** From syntype specimen from Herbarium "J. Agardh" (22638, St. Hospice, France). **27.** Carpogonial branch. **28.** Auxiliary-cell branch. **29.** Carpogonial ampulla after presumed fertilization; note fusion between carpogonial branch cells and primary connecting filaments arising from it; trichogyne is still recognizable. **30-32.** Interpretative drawings of Figs 27-29, respectively; cells of carpogonial branch and auxiliary-cell branch are shaded and numbered. **33.** Auxiliary-cell ampulla after diploidization; note the primary connecting filament fusing with the auxiliary cell without forming a pit-connection. **34.** Auxiliary-cell ampulla after diploidization; note branched nutritive filaments, mostly upwardly directed, produced by the auxiliary cell after diploidization and enveloping the young gonimoblast. **35.** Specimen from Lake Ganzirri (MS-PhL088). Scale bars: 1 cm in Figs 25, 26, 35; 20 μ m in Figs 27-29, 33; 40 μ m in Fig. 34. Abbreviations: 1°cf = primary connecting filament; auxc, auxiliary cell; cp, carpogonium; cbfc(cp+hy+1+2+3) = carpogonial branch fusion cell made of carpogonium, hypogynous and 1st to 3rd cells of the carpogonial branch; hy, hypogynous cell; sc, supporting cell; t, trichogyne.

After presumed fertilization, the carpogonium, hypogynous cell and all the other cells of the carpogonial branch fuse (Figs 29, 32). The resulting fusion cell forms two or more primary connecting filaments that are separated by a pit-connected cross wall. Connecting filaments are unbranched and non-septate.

The terminal region of the connecting filament expands and fuses sub-apically to the auxiliary cell (Fig. 33), where it either terminates or continues on from the point of fusion to effect further diploidizations. Auxiliary cells do not form a fusion cell with adjacent ampullary cells (Fig. 34). Upwardly directed, branched nutritive filaments are subsequently produced by the auxiliary cell, which also cuts off a single gonimoblast initial toward the thallus surface. The young gonimoblast is composed of two or more gonimolobes derived from the gonimoblast initial.

***Grateloupia horrida* Kützinger** 1843: 397, pl. 76 I.

Figs 36-44

= *Grateloupia filicina* (J.V. Lamouroux) C. Agardh f. *horrida* (Kützinger) Børgesen 1935: 53.

Type locality: Gulf of Naples, Palermo, Sicily, Italy

Specimens examined: see Table 1.

The thallus arising from a discoid holdfast is formed by simple to sub-dichotomous erect fronds tapering at ends, mainly terete, sometimes swollen, compressed in proximal part, with frequent proliferations pinnate to irregular; the thallus has a firm texture and is blackish purple or rarely greenish (Fig. 36).

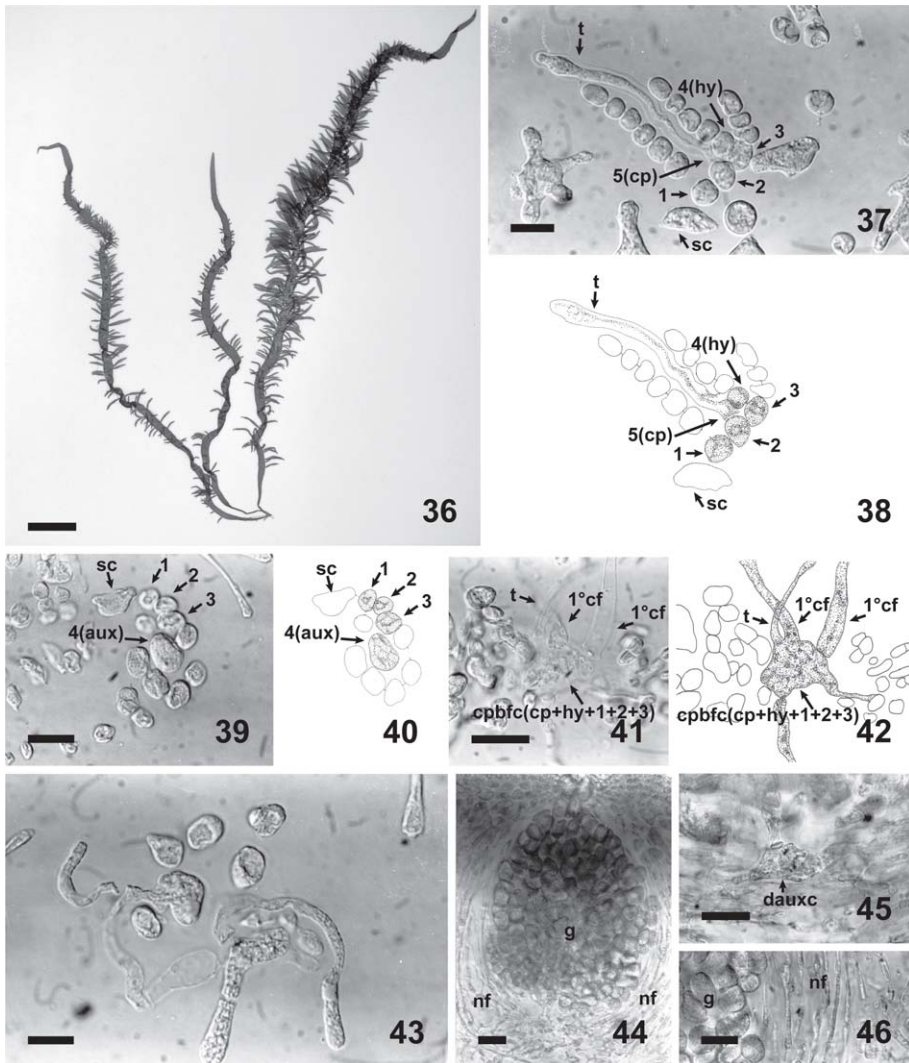
The carpogonial branch is 5-celled and consists of a small carpogonium ($11.1 \pm 0.4 \times 13.4 \pm 0.5 \mu\text{m}$) with a long trichogyne, a hypogynous cell ($13.2 \pm 0.2 \times 14.3 \pm 0.1 \mu\text{m}$) bearing a four- or five-celled sterile lateral branch, an ovoid subhypogynous cell ($13.3 \pm 0.1 \times 14.2 \pm 0.3 \mu\text{m}$) with a 3-6-celled sterile lateral branch, a subtending ovoid cell ($13.4 \pm 0.5 \times 14.3 \pm 0.5 \mu\text{m}$) bearing a 6-10-celled unbranched sterile lateral, and a basal cell ($13.8 \pm 0.4 \times 14.9 \pm 0.3 \mu\text{m}$) without laterals (Figs 37, 38). The carpogonial branch is distally bent. The trichogyne is directed straight to, or sometimes curved to various degrees towards, the thallus surface. The sterile branches of the carpogonial branch curve toward the thallus surface to form an ampulla.

The auxiliary cell ($15.2 \pm 0.4 \times 15.2 \pm 0.7 \mu\text{m}$) is the terminal cell of a 4-celled branch. All the cells of the auxiliary branch, including the auxiliary cell itself, have a simple or sparingly branched lateral (Figs 39, 40), all of which curve toward the thallus surface to form an ampulla similar to that of the carpogonial branch.

After presumed fertilization, the carpogonium, hypogynous cell and all the other cells of the carpogonial branch fuse (Figs 41, 42). The resulting fusion cell forms two or more primary connecting filaments that are separated from it by a pit-connected cross wall. Connecting filaments are unbranched and non-septate.

When a connecting filament approaches an auxiliary cell its terminal region expands and fuses to it directly (Fig. 43), following which it either terminates or grows on to effect further diploidizations. In addition, tertiary connecting filaments can be cut off from other parts of the auxiliary cell, but in these cases they are basally pit-connected.

The auxiliary cell does not form a fusion cell with adjacent ampullary cells (Fig. 45). Upwardly directed, several branched nutritive filaments are then produced by the auxiliary cell, the filaments consisting of elongate cells, $91.3 \pm 12.0 \times 3.9 \pm 1.2 \mu\text{m}$, with dense refractive contents (Figs 44, 46). At the same time, the auxiliary cell cuts off a single gonimoblast initial from its upper side. The young



Figs 36-46. *Grateloupia horrida*. **36.** Herbarium specimens from S. Maria La Scala, Italy (MS-35019-Gt01). **37.** Carpogonial ampulla. **38.** Interpretative drawing of Fig. 37; cells of carpogonial branch are shaded and numbered. **39.** Auxiliary-cell ampulla. **40.** Interpretative drawing of Fig. 39; cells of auxiliary-cell branch are shaded and numbered. **41.** Carpogonial ampulla after fertilization; note the fusion between carpogonial branch cells and primary connecting filaments arising from it; trichogyne is still recognizable. **42.** Interpretative drawing of Fig. 41; carpogonial branch fusion cell is shaded. **43.** Auxiliary-cell ampulla after diploidization; note the primary connecting filament fusing with the auxiliary cell without forming a pit-connection, and the secondary connecting filament issuing from diploidized auxiliary cell with a pit connection. **44-46.** Mature cystocarp. **44.** General view. **45.** Basal part of cystocarp anchored to the diploidized auxiliary cell. **46.** Detail of nutritive filament cells and medullary filaments forming the pericarp. Scale bars: 2 cm in Fig. 36; 20 μ m in all others. Abbreviations: 1 $^{\circ}$ cf = primary connecting filament; auxc, auxiliary cell; cp, carpogonium; cpbfc (cp+hy+1+2+3) = carpogonial branch fusion cell made of carpogonium, hypogynous and 1 $^{\text{st}}$ to 3 $^{\text{rd}}$ cells of the carpogonial branch; dauxc = diploidized auxiliary cell; g = gonimoblast; hy, hypogynous cell; nf = nutritive filaments; sc, supporting cell; t, trichogyne.

gonimoblast is composed of two or more gonimolobes consisting almost entirely of basipetally maturing carposporangia. Mature cystocarps are sub-spherical, $450 \pm 380 \mu\text{m}$ in diameter (Fig. 44), and surrounded by a thin pericarp of 3-5 layers of medullary filaments, these augmented by filaments derived from ampullary cells (Figs 44, 46). Mature carposporangia are $19.8 \pm 2.0 \times 20.1 \pm 2.3 \mu\text{m}$. The cystocarp is non-ostiolate and deeply immersed in the thallus.

***Grateloupia subpectinata* Holmes 1912: 208, pl. 1.**

Figs 47-56

Type locality: Japan.

= *Grateloupia luxurians* (A. Gepp et E. Gepp) R.J. Wilkes, L.M. McIvor et Guiry 2005: 58 (Verlaque et al., 2005).

= *Grateloupia filicina* var. *luxurians*. A. Gepp et E. Gepp 1906:259.

Type locality: Farm Cove, Sydney, New South Wales.

Specimens examined: see Table 1.

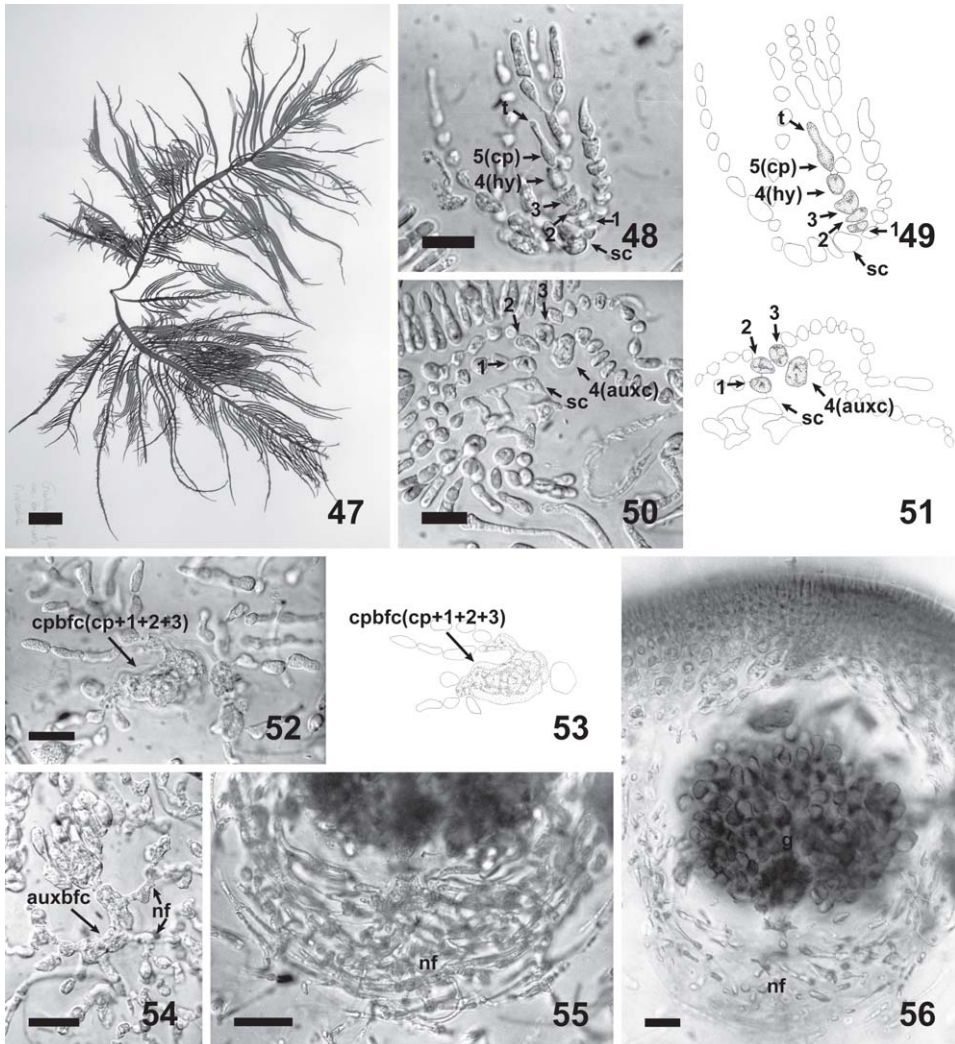
The thallus arising from a basal disc is formed by large pinnate erect fronds with occasional proliferations in all directions, mainly compressed, soft to mucilaginous becoming cartilaginous when old, reddish to brownish red (Fig. 47).

Carpogonial branches are 5-celled and consists of a small carpogonium ($8.3 \pm 0.2 \times 11.4 \pm 0.6 \mu\text{m}$) with a long trichogyne, a rounded hypogynous cell ($10.5 \pm 0.3 \times 11.4 \pm 0.7 \mu\text{m}$), an ovoid subhypogynous cell ($13.2 \pm 0.5 \times 12.2 \pm 0.6 \mu\text{m}$), a subtending ovoid cell ($13.2 \pm 0.5 \times 10.7 \pm 0.4 \mu\text{m}$) and a slightly compressed basal cell ($11.3 \pm 0.7 \times 10.2 \pm 0.9 \mu\text{m}$). All cells of the carpogonial branch, save for the carpogonium itself, bear up to three orders of lateral branches (Figs 48-49). The trichogyne is directed straight to, or sometimes arches towards, the thallus surface. The sterile branches of each cell of the carpogonial branch curve towards the thallus surface to form an ampulla.

The auxiliary cell is the terminal cell of a curved 4-celled branch. All the cells of the auxiliary-cell branch, including the auxiliary cell itself, produce a simple or sparingly branched 5-12-celled lateral (Figs 50-51). All laterals grow towards the thallus surface to form an ampulla similar to that of the carpogonial branch.

After presumed fertilization, the carpogonium fuses with all the cells of the carpogonial branch (Figs 52, 53). The resulting fusion cell produces two or more connecting filaments that are separated by a pit-connected cross wall. Connecting filaments are unbranched and non-septate.

When a connecting filament approaches an auxiliary cell, its apical region swells and fuses directly to the sub-apical part of the auxiliary cell, where it terminates or continues growing to effect further diploidizations. The auxiliary cell expands after fusing with a connecting filament and forms a laterally extended fusion complex by the incorporation of adjacent ampullary cells (Fig. 54). Branched nutritive filaments arise from the fusion cell, the filaments more visible in basal portions of the developing cystocarp (Figs 54, 55) and consisting of elongate cells with dense refractive contents. At the same time the fusion cell cuts off a single gonimoblast initial toward the thallus surface. The young gonimoblast is composed of two or more gonimolobes, most cells of which mature basipetally into carposporangia. The mature cystocarp is sub-spherical, $185.0 \pm 10.2 \times 215.0 \pm 10.8 \mu\text{m}$ in diameter (Fig. 56) and surrounded by a thin pericarp of 3-5 layers of elongate medullary cells augmented by a few filaments derived from the ampullary cells. Mature carposporangia reach $17.3 \pm 0.8 \times 15.1 \pm 0.6 \mu\text{m}$ in diameter. The cystocarp is ostiolate from early development and deeply immersed in the thallus.



Figs 47-56. *Grateloupia subpectinata*. **47.** Herbarium specimens from Victoria, Australia (MS-35063-Gt01). **48.** Carpogonial ampulla. **49.** Interpretative drawing of Fig. 48; cells of carpogonial branch are shaded and numbered. **50.** Auxiliary-cell ampulla. **51.** Interpretative drawing of Fig. 50; cells of auxiliary-cell branch are shaded and numbered. **52.** Carpogonial ampulla after fertilization; note fusion between carpogonial branch cells. **53.** Interpretative drawing of Fig. 52; carpogonial branch fusion cell is shaded. **54.** Auxiliary-cell ampulla after diploidization. **55, 56.** Mature cystocarp. **55.** Basal part of cystocarp with branched, mostly downwardly directed nutritive filaments. **56.** General view of a cystocarp. Scale bars: 2 cm in Fig. 47; 20 μ m in all others. Abbreviations: auxc, auxiliary cell; auxbfc = auxiliary branch fusion cell; cp, carpogonium; cpbfc(cp+hy+1+2+3) = carpogonial branch fusion cell made of carpogonium, hypogynous and 1st to 3rd cells of the carpogonial branch; g = gonimoblast; hy, hypogynous cell; nf = nutritive filaments; sc, supporting cell; t, trichogyne.

The species of *Grateloupia* previously reported as *G. "doryphora"* (De Masi and Gargiulo 1982; Wilkes *et al.*, 2006).

Specimens examined: see Table 1.

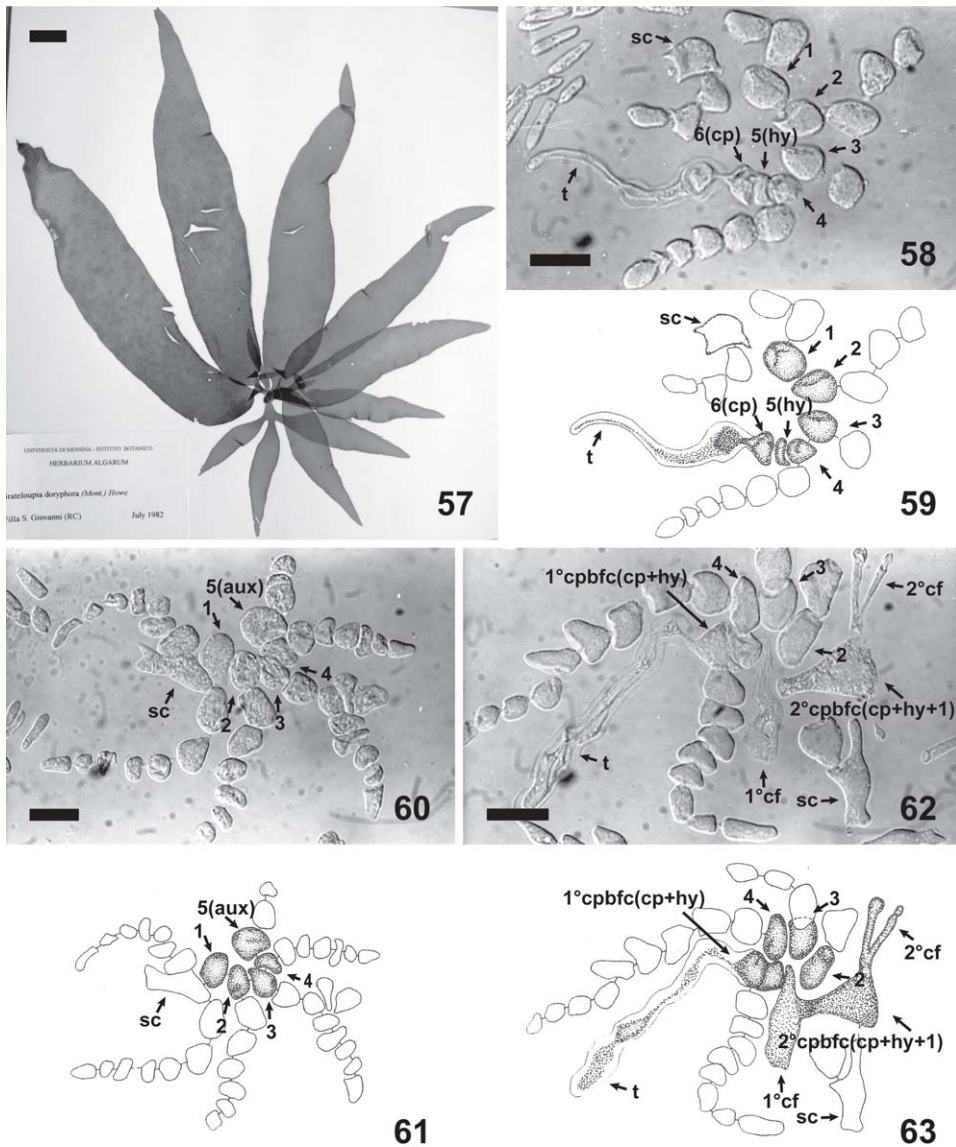
The thallus is made by lanceolate foliose blades arising from a discoid holdfast, membranaceous, lubricous, increasing in thickness in old plants, reddish purple to yellow-brownish in color, branched dichotomously to palmately and complanate with proliferations in old plants (Fig. 57).

The carpogonial branch is 6-celled and consists of a carpogonium ($12.1 \pm 0.4 \times 8.2 \pm 0.4 \mu\text{m}$) with a long trichogyne, a hypogynous cell ($11.4 \pm 0.3 \times 6.2 \pm 0.5 \mu\text{m}$) bearing a three- or four-celled sterile lateral branch, an ovoid subhypogynous cell ($10.5 \pm 0.7 \times 10.2 \pm 0.3 \mu\text{m}$) with a 4-6-celled sterile filament, this subtended in turn by three large rounded refractive cells ($13.1 \pm 1.4 \times 13.6 \pm 1.7 \mu\text{m}$) bearing sterile laterals (Figs 58, 59). The carpogonial branch is distally bent so that the carpogonium lies adjacent to the large basal cell. The trichogyne is directed straight to, or sometimes curves to various degrees towards, the thallus surface. The sterile laterals of the carpogonial branch curve towards the thallus surface to form an ampulla.

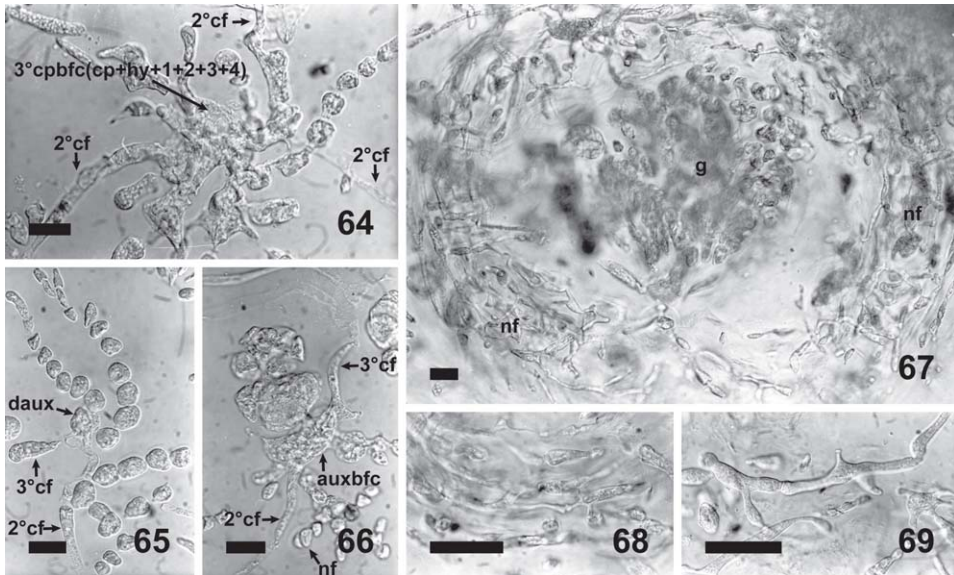
The auxiliary cell is the terminal cell of a 5-celled branch. All the cells of the auxiliary branch, including the auxiliary cell itself, produce a simple or sparingly branched, 5-12-celled lateral (Figs 60, 61). All laterals of the auxiliary-cell branch curve towards the thallus surface to form an ampulla similar to that of the carpogonial branch. The auxiliary-cell branch is also curved, placing the auxiliary cell in a central position.

After presumed fertilization, the carpogonium enlarges and fuses with its hypogynous cell. The resulting fusion cell (1° carpogonial fusion cell) forms a short primary connecting filament that extends toward and fuses with the basal cell of the carpogonial branch (Figs 62, 63). This fusion product (2° carpogonial fusion cell) produces secondary connecting filaments that move towards auxiliary cells. Meanwhile, the carpogonial fusion cell expands contacting all the other cells of the carpogonial branch, resulting in a large fusion cell (3° carpogonial fusion cell) from which other secondary connecting filaments can rise (Fig. 64). Connecting filaments are unbranched and non-septate.

When a connecting filament approaches an auxiliary cell, its apical region swells and fuses directly with the auxiliary cell (Fig. 65), where it either terminates or continues growing to effect further diploidizations. Additional connecting filaments can then arise sub-apically or laterally from the auxiliary cell at other than the initial fusion site, although these are pit-connected where they originate. The auxiliary cell expands laterally after presumed diploidization through incorporation of adjoining ampullary cells on both sides, forming an irregularly contoured fusion complex (Fig. 66). Branched nutritive filaments are directed both upwardly and downwardly from the fusion cell. Those that are downwardly directed consist of rounded cells, $4.6 \pm 0.3 \mu\text{m}$ in diameter, with dense refractive contents, whereas those directed toward the thallus surface are composed of elongate and lobed cells. At the same time, the auxiliary fusion cell cuts off a single gonimoblast initial toward the thallus surface (Fig. 66). The young carposporophyte is composed of two or more gonimolobes of synchronously developing, basipetally maturing carposporangia. The mature cystocarp is sub-spherical, $350.0 \pm 32.0 \mu\text{m}$ in diameter (Fig. 67), and surrounded by 3-5 layers of medullary filaments (Fig. 68) that contribute to the formation of a thin pericarp together with few filaments of elongate cells derived from the ampullary cells (Fig. 69). Mature carposporangia are $15.6 \pm 0.3 \mu\text{m}$ in diameter, and the ostiolate cystocarp is deeply immersed in the thallus.



Figs 57-63. *Grateloupia* sp. (previously reported as *G. 'doryphora'*). **57.** Herbarium specimens from Villa San Giovanni, Italy (MS-35016-Gt02). **58.** Carpogonial ampulla. **59.** Interpretative drawing of Fig. 58; cells of carpogonial branch are shaded. **60.** Auxiliary-cell ampulla. **61.** Interpretative drawing of Fig. 60; cells of auxiliary-cell branch are shaded. **62.** Carpogonial ampulla soon after presumed fertilization; note the carpogonium enlarging and fusing with its hypogynous cell, the resulting fusion cell forming a short projection further fusing with the large basal cell of the carpogonial branch and the basal fusion cell producing more connecting filaments. **63.** Interpretative drawing of Fig. 62; carpogonial branch fusion cell is shaded. Scale bars: 2 cm in Fig. 57; 20 μ m in all others. Abbreviations: 1°cf = primary connecting filament; 1°cpbfc(cp+hy) = carpogonial branch fusion cell made of carpogonium and hypogynous cell; 2°cpbfc(cp+hy+1) = carpogonial branch fusion cell made of carpogonium, hypogynous and 1st cell of the carpogonial branch; auxc, auxiliary cell; cp, carpogonium; hy, hypogynous cell; sc, supporting cell; t, trichogyne.



Figs 64-69. **64.** *Grateloupia* sp. (previously reported as *G. "doryphora"*). Carpogonial ampulla after fertilization; note the large fusion cell from which more connecting filaments arise. **65.** Auxiliary-cell ampulla after diploidization; note the connecting filament fusing apically to the auxiliary cell without formation of a pit-connection. **66.** Carpogonial ampulla after fertilization; note the fusion complex, branched nutritive filaments, and the gonimoblast initial. **67-69.** Mature cystocarp: **67.** General view. **68.** Detail of the pericarp with medullary filaments. **69.** Detail of the pericarp with nutritive filaments deriving from the ampullary cells. Scale bars: 20 μ m.

DNA phylogenies

Maximum likelihood analysis of the *rbcL* alignment resulted in two equally likely trees (ln likelihood = -12084.52) differing only in minor details in the position of *Prionitis filiformis* Kylin (GenBank AJ868496, Oregon USA). One of the ML trees is shown with bootstrap proportion values and bayesian posterior probabilities superimposed at the internal branches (Fig. 70).

Within the large *Grateloupia sensu lato* group a number of highly supported subgroups emerge: a) a *G. lanceolata* group – fully supported in all analyses and including species from Japan and Korea (also introduced in California and the Mediterranean Sea) and an isolate of the "*Grateloupia* sp." collected along the southern Italian coast that was previously reported as *G. "doryphora"*;

b) a *G. americana* group – this (94/1.00 of bootstrap values and bayesian posterior probabilities, respectively) composed of species from East Asia and Pacific North America, including *G. americana* Kawaguchi *et* H.W. Wang from California and *G. asiatica* from China (also present in Japan and introduced in Thau Lagoon, France);

c) a *G. subpectinata* group – (96/1.00), comprised of species from Australia, New Zealand, Japan, Korea, Hawaiian Islands, including *G. subpectinata* from Australia (also introduced in Atlantic Europe and the Mediterranean) and *G. turuturu* from Japan (also introduced in North Atlantic, both in the USA and Europe, and in the Mediterranean);

d) a *G. belangeri* group – a fully supported suite of South African species, including *G. belangeri* (Bory) De Clerck, Gavio, Fredericq, Cocquyt *et* Coppejans;

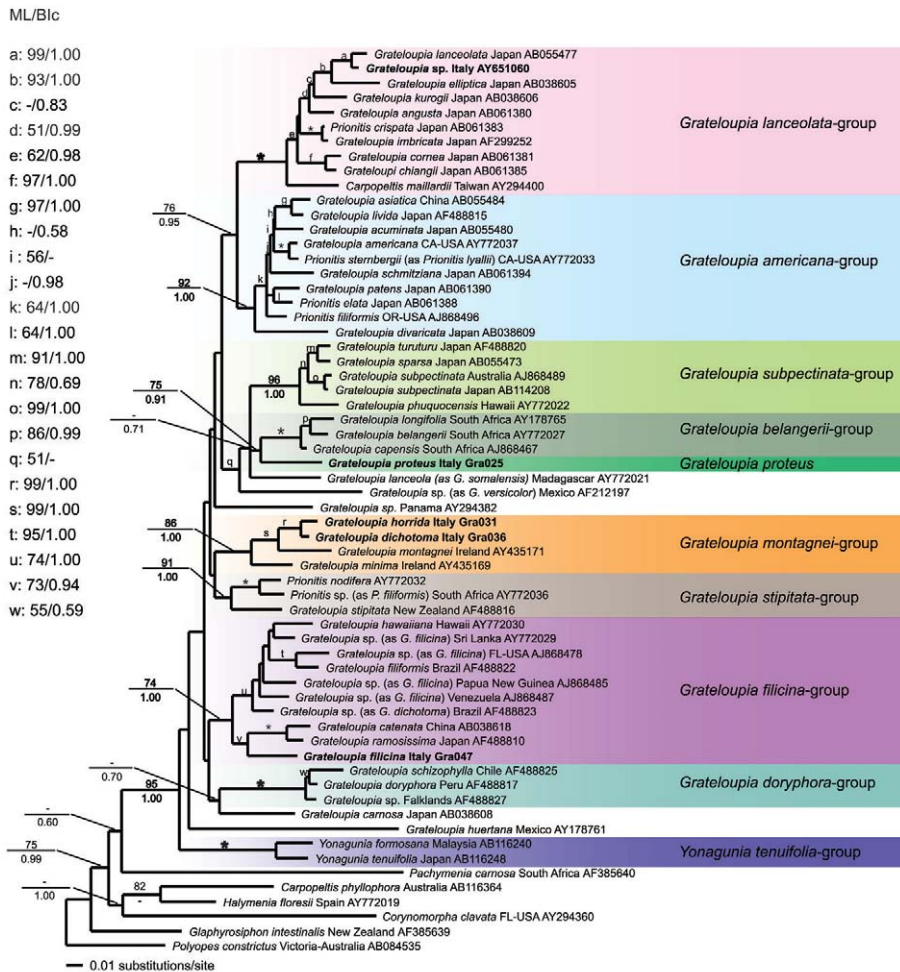


Fig. 70. One of two estimated Maximum Likelihood phylograms (ln L = -12084.52122) with support estimates inferred from bootstrap proportion values and Bayesian posterior probabilities superimposed at internal branches; branches with 100% support in all analyses are marked with an asterisk. Support values at nodes resolving *rbcL* groups presented in the discussion are in bold. Sequences generated in the present study are indicated in bold.

e) a *G. montagnei* group – this (84/1.00) composed of species from the Mediterranean and Northeastern Atlantic, namely *G. montagnei* (P. Crouan et H. Crouan) R.J. Wilkes, L.M. McIvor et Guiry from Ireland, *G. dichotoma* and *G. horrida* from Italy and *G. minima* P. Crouan et H. Crouan from Ireland;

f) a *G. stipitata* group – this (88/1.00) including *G. stipitata* J. Agardh from New Zealand, *Prionitis nodifera* (Hering) E.S. Barton and *Prionitis* sp. (as “*filiformis*”), the latter two both from South Africa;

g) a *G. filicina*-group – this (72/1.00) including *G. filicina*, the generitype of *Grateloupia*, from Italy, in a subgroup (60/64/0.94) with *G. catenata* Yendo from

China (Japan) and *G. ramosissima* Okamura from Japan, the three allied with a sister group including tropical and subtropical species with a “*G. filicina*” or “*G. dichotoma*” morphology;

h) a *G. doryphora* group – this clade with full support in all analyses and including *G. doryphora* from Peru, *G. schizophylla* Kützting from Chile, and an undescribed *Grateloupia* sp. from the Falklands Islands; and

i) a *Yonagunia tenuifolia* group – this fully supported in all analyses and including the two species of the genus *Yonagunia* Kawaguchi *et* Masuda.

Deeper relationships within the genus are generally not supported (Fig. 70), with the exception of moderately indicated alliances of the *G. lanceolata*-group with the *G. americana*-group (78/0.95) and of the *G. belangeri*-group with *G. proteus* (75/0.91). Further relationships are weakly supported only in bayesian analyses, including an association of the *G. doryphora*-group with *G. carinosa* Y. Yamada *et* Segawa (–/0.70) and the *G. belangeri*-group with *G. proteus*, the *G. subpectinata*-group and *G. lanceola* (–/0.71). The sister relationship of *Yonagunia* with *Grateloupia*, as presently circumscribed, is resolved but lacked significant support.

DISCUSSION

Most past researches have overlooked or downplayed differences in ampullar systems within the Halymeniaceae, ignoring their genuine diversity. As we interpret them, carpogonial branches are composed not simply of a carpogonium and hypogynous cell, but consist of a carpogonium that is the terminal cell of a primary filament of which the number of cells can differ between species, as is epitomized in the genus *Grateloupia* (Figs 71–73). We regard the earlier interpretations of Berthold (1884) [for *G. consentinii* (= *G. proteus*) and *G. dichotoma*, both from Gulf of Naples] and later of Schmitz and Hauptfleisch (1897) [for *G. filicina*], as actually more correct than Sjöstedt's, for they regarded the carpogonium as the terminal cell of a primary filament of four or five cells, with the subtending cells, including the hypogynous cell, producing upwardly directed laterals which converge distally to form the ampulla. Our observations on carpogonial ampullae of the same entities support these early findings (Figs 71, 73). Moreover, Balakrishnan's (1980) interpretation of the terminal position of the carpogonium on a three- or four-celled primary filament in *Isabbottia* M.S. Balakrishnan and *Norrissia* M.S. Balakrishnan accords with those of early authors. A four-celled carpogonial branch is also reported in the Australian-endemic *Zymurgia* (Lewis & Kraft, 1992).

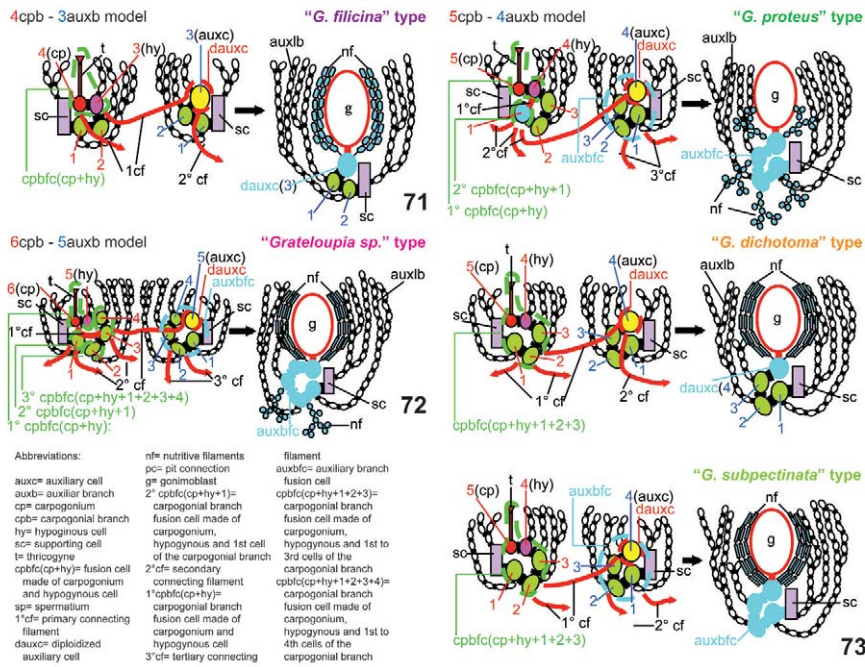
Similar considerations apply to the halymeniaceous auxiliary-cell ampulla. A terminal position of the auxiliary cell in a primary filament was reported by Lewis and Kraft (1992) for *Zymurgia*, and a similar configuration was claimed by Lewis (in Lewis & Kraft, 1992) for Australian “*G. filicina*” (presumably *G. subpectinata*), in which the auxiliary cell was said to terminate a curved four-celled primary ampullary filament. Lewis & Kraft (1992) also reported that the auxiliary cell and other cells of the auxiliary-cell ampulla each produced a secondary lateral branch towards the surface that converged distally to form the flask-like filament complex in *Zymurgia*. Terminal auxiliary cells are also reported in *Isabbottia* (Balakrishnan, 1980), a genus that, like *Zymurgia* and

Norrissia, has non-ampullar carpogonial branches. Our observations on the architecture of auxiliary-cell ampullae are in agreement with these interpretations for all species that we have studied and, furthermore, we find that in primary auxiliary-cell branches the number of cells changes from species to species but is always exactly one cell less than the corresponding carpogonial-branch length (Figs 71-73).

On our interpretation, it is possible to make sense of the relationships among genera of the Halymeniaceae from both an anatomical and a molecular perspective. *Zymurgia*, *Isabbottia* and *Norrissia* are unique in having auxiliary-cell ampullae typical of the remainder of the Halymeniaceae, but differ from the other genera by the absence of ampullae around their three- or four-celled carpogonial branches (Balakrishnan, 1980; Lewis & Kraft, 1992). Their carpogonial branches differ from those of the other genera, apart from the number of cells, only in the absence of lateral branches generated by the cells proximal to the carpogonium. Although this is a striking difference, the implications of molecular analyses (Saunders *et al.*, 2004) clearly indicate that these genera are basal clades of a monophyletic Halymeniaceae.

Our observations support the hypothesis that the auxiliary-cell and carpogonial ampullae are homologous structures, as posited by several authors (Kyllin, 1923; Sjöstedt, 1926; Norris, 1957; Lebednik, 1977). Our data clearly show that: a) both the carpogonium and auxiliary cell are terminal on “subsidiary” (*sensu* Kraft & Robins, 1985) filaments; b) the number of cells in the carpogonial and auxiliary branches may differ from species to species but is constant within a set range for any given species; and c), the number of cells in the primary auxiliary-cell branch is always one less than that of the carpogonial branch. This leads us to conclude that auxiliary cells occupy a position in auxiliary-cell branches homologous to that of hypogynous cells in carpogonial branches (Figs 71-73). We hypothesize that the auxiliary-cell branch evolved by modification of the carpogonial branch in the Halymeniaceae, such that the auxiliary cell is, in every respect, a cell converted from former hypogynous cells. If this be true, the hypogynous cell now functions as a generative auxiliary cell in what was formerly a carpogonial ampullar system in which it once played the role of a nutritive auxiliary cell, in line with Papenfuss’s (1966) view that generative auxiliary cells are derived from nutritive ones in many gigartinean/halymenialian groups.

Differences in early postfertilization events among entities currently assigned to the genus *Grateloupia* (Figs 71-73) seem to accord with molecular results showing a number of highly supported subgroups within a large *Grateloupia sensu lato* group (Kawaguchi *et al.*, 2004; De Clerck *et al.*, 2005b; Wilkes *et al.*, 2005; Lin *et al.*, 2008), although not all studies show exactly the same degree of relationship between them. We have found that deeper relationships receive very low or no support in analyses using only *rbcL* sequence data. In our opinion, the sister relationship of the *G. lanceolata*-group with the *G. americana*-group, as well as the association of the *G. doryphora*-group with *G. carnosa* and the *G. belangeri*-group with *G. proteus*, the *G. subpectinata*-group and *G. lanceola* should be considered provisional working hypotheses to be tested with more informative phylogenetic markers. The sister relationship between *Yonagunia* and *Grateloupia* as currently indicated by *rbcL* analyses is doubtful lacking any statistical support and a more conservative view is that *Yonagunia* represents a subgroup of the same rank of the others resolved within *Grateloupia sensu lato*. Such subgroups as have emerged from our analyses, however, are all solidly supported in our molecular trees and are clearly differentiated on the basis of reproductive anatomy and postfertilization development patterns.



Figs 71-73. Summarizing schemes of carpogonial and auxiliary branches and of postfertilization developments in groups within *Grateloupia sensu lato*. **71.** Model with a 4-celled carpogonial branch and a 3-celled auxiliary-cell branch ("*G. filicina*"-type). **72.** Model with a 6-celled carpogonial branch and a 5-celled auxiliary-cell branch ("*Grateloupia sp.*"-type, previously identified as *G. "doryphora"* from the Mediterranean Sea). **73.** Model with a 5-celled carpogonial branch and a 4-celled auxiliary-cell branch with correspondingly dissimilar postfertilization events: "*G. proteus*"-type, "*G. dichotoma*"-type, "*G. subpectinata*"-type.

CONCLUSIONS

The following groups are identified, and the diagnostic anatomical features common to the included species are given:

1) The *G. lanceolata*-group, which includes the western-Pacific species *G. lanceolata* Okamura [the generic type of *Pachymeniopsis* Kawabata], *G. kurogii* Kawaguchi, *Prionitis crispata* (Okamura) Kawaguchi, *G. angusta* (Okamura) Kawaguchi et H.W. Wang, *G. cornea* Okamura, *G. chiangii* Kawaguchi et H.W. Wang (as *Prionitis divaricata* (Okamura) Kawaguchi) and *G. elliptica* Holmes, as well as a population of *Grateloupia sp.* from the Straits of Messina previously reported as *G. "doryphora"* (De Masi & Gargiulo, 1982; Wilkes *et al.*, 2006). These species have 6-celled carpogonial branches and 5-celled auxiliary branches (6cpb-5auxb model), an extended carpogonial fusion cell, an extended auxiliary-cell fusion cell, and both upwardly and downwardly directed branched nutritive filaments (Fig. 72), as shown by the present observations and from literature (Kawaguchi, 1989, 1990, 1997).

2) The *G. subpectinata*-group, which includes species originally confined to the central, western and southern Pacific regions. The Australian specimens we

studied have a 5-celled carpogonial branch and 4-celled auxiliary branch (5cpb-4auxb model), an extended carpogonial fusion cell, an extended auxiliary-cell fusion cell, and upwardly directed branched nutritive filaments (Fig. 73). According to Kawabata, *G. turuturu* (1962, plate 1) and *G. sparsa* (Okamura) Chiang (1963, plate 20) show similar characteristics. For the other species in the group, as far as we know, useful observations are not available.

3) *G. proteus*, a species originally known from Sicily but recently also reported for Atlantic Spain [as *G. dichotoma* (De Clerck *et al.*, 2005a)]. It has a 5-celled carpogonial branch and 4-celled auxiliary-cell branch (5cpb-4auxb model), fusion of carpogonium and hypogynous cell with only the basal cell of carpogonial branch, an extended auxiliary-cell fusion cell, and upwardly and downwardly directed branched nutritive filaments (Fig. 73). According to De Clerck *et al.* (2005a, 2005b), *G. belangeri* (Bory de Saint-Vincent) De Clerck, Gavio, Fredericq, Cocquyt *et* Coppejans, the generitype species of *Phyllymenia* Setchell *et* N.L. Gardner, and *G. capensis* De Clerck have the same number of cells in the carpogonial and auxiliary-cell branches as *G. proteus*; they both cluster together in our *rbcL* phylogenies with *G. longifolia* Kylin and *G. proteus*. However, early postfertilization events were not observed in South-African species, so further investigations are needed to determine if they represent a separate group from *G. proteus* since molecular data could support either interpretation. We were not able to get useful information on *G. longifolia*.

4) *Grateloupia dichotoma* (data obtained from the syntype specimens from Herb. J. Agardh 22638-L) and *G. horrida*, from southern Italy, cluster with the Atlantic species *G. montagnei*, the generitype species of *Dermocorynus* P. Crouan *et* H. Crouan, and a species reported from Ireland by Wilkes *et al.* (2005) as *G. minima*. Other *rbcL* sequences for specimens from France, Portugal and Ireland not included in our final alignment but previously attributed to *G. minima* (De Clerck *et al.*, 2005a), belong to a different taxon from that of Wilkes *et al.* (2005) and are nearly identical with those of our *G. horrida* (0-5 bp, 0.00-0.48 %). Our specific attribution of Sicilian specimens to the latter is supported by detailed molecular and morpho-anatomical data on several populations from Southern Italy, including the type locality, and from comparison with original material in Kützing's herbarium (data not shown). The history and nomenclatural problems of the *G. dichotoma*-group will be reported in a subsequent paper. Both *G. dichotoma* and *G. horrida* have a 5-celled carpogonial branch and 4-celled auxiliary branch (5cpb-4auxb model), an extended carpogonial fusion cell, no fusion of the auxiliary cell with other cells, and upwardly directed branched nutritive filaments (Fig. 73). A similar suite of characters is present in the specimens reported as *G. minima* by De Clerck *et al.* (2005a). No data are available for "*G. minima*" *sensu* Wilkes *et al.* (2005). The observations on *G. montagnei* by Guiry and Maggs (1982) are in agreement with ours for post-fertilization events, but differ regarding the number of cells for the carpogonial and auxiliary branches. However, a more detailed study may be necessary to verify the cell number because it is not easy to determine these from the images in Guiry and Maggs (1982).

5) *Grateloupia filicina* from Italy, the generitype species of *Grateloupia*, clustered with species from all oceans. The samples of *G. filicina* from the type locality have a 4-celled carpogonial branch and 3-celled auxiliary branch (4cpb-3auxb model), carpogonial fusion limited to the hypogynous cell, no fusion of the auxiliary cell with other cells, and upwardly directed branched nutritive filaments (Fig. 71). The observations on *G. filicina* from Livorno by Kawaguchi *et al.* (2001) partially agree with ours on postfertilization development, although the number of

auxiliary-cell branch cells reported is different. However, we also examined material from Livorno and confirmed the same cell count as specimens of *G. filicina* from Trieste. *Grateloupia catenata* and *G. ramosissima* have similar postfertilization features, although the number of auxiliary-cell branch cells is verified only for the latter (Kawaguchi, 1989; Wang *et al.*, 2000). *Grateloupia filiformis*, *G. hawaiiiana* Dawson and some entities reported as *G. filicina* and *G. dichotoma*, which however deserve to be considered separate species according to *rbcL* data, await detailed morpho-anatomical and reproductive investigations.

We did not examine authentic material from the following groups indicated in our phylogenetic analyses, but on the basis of data from the literature, although limited and often not easy to interpret, we can hypothesize the reproductive model of some of the species included in our tree.

6) The *G. americana*-group includes *G. americana* [formerly *Prionitis lanceolata* (Harvey) Harvey, generic type species of *Prionitis*] and other Pacific species, namely *G. acuminata* Holmes, *G. asiatica* Kawaguchi *et* Wang, *G. divaricata* (Okamura) Kawaguchi, *G. elata* (Okamura) Kawaguchi *et* H.W. Wang, *G. livida* (Harvey) Y. Yamada, *G. patens* (Okamura) Kawaguchi *et* H.W. Wang, *G. schmitziana* (Okamura) Kawaguchi *et* H.W. Wang, *Prionitis filiformis* and *P. sternbergii* (C. Agardh) J. Agardh, with all *rbcL* sequences generated from topotype specimens. Species from this group show a 6-celled carpogonial branch and 5-celled auxiliary branch (6cpb-5auxb model), carpogonial fusion limited to the hypogynous cell, no fusion of the auxiliary cell with other cells, and downwardly directed branched nutritive filaments (Sjöstedt, 1926; Kawaguchi, 1989, 1991). However, *G. schmitziana* seems not to fit this model according to observation of Kawaguchi (1989) because fusions at both carpogonial and auxiliary-cell branches are more extensive.

7) The *Yonagunia*-group, including the species described from Asia, seems to have a 5-celled carpogonial branch and 4-celled auxiliary branch (5cpb-4auxb model), no fusion of the auxiliary cell with other cells, and mainly downwardly directed branched nutritive filaments (Kawaguchi & Nguyen, 1998; Kawaguchi *et al.*, 2004).

We were not able to obtain anatomical and reproductive data for the groups that include *G. doryphora* from Peru and *G. stipitata* from New Zealand, as well as for some species, namely *G. lanceola* (J. Agardh) J. Agardh, *G. carnosa* Yamada *et* Segawa, *G. huertana* Mateo-Cid, Mendoza-González *et* Gavio, and two undetermined species from Mexico and Panama, which do not cluster with the above mentioned groups.

A wholesale revision of reproductive features and postfertilization events in other species belonging to *Grateloupia sensu lato*, with an emphasis on specimens from type localities, is greatly needed to support or cast doubt on our interpretation on such characteristics. However, in the light of our data, the split of the genus *Grateloupia* as presently circumscribed into segregate genera by means of the resurrection of old names and the description of new ones appears to be well justified. We, therefore, propose the following genus reinstatements:

A) *Dermocorynus* P.L. Crouan *et* H.M. Crouan 1858: 69, generic type species *Dermocorynus montagnei* P.L. Crouan *et* H.M. Crouan 1858: 70.

B) *Pachymeniopsis* Y. Yamada *ex* S. Kawabata 1954: 67, generic type species *Pachymeniopsis lanceolata* (Okamura) Yamada *ex* S. Kawabata 1954: 67;

C) *Phyllymenia* J. Agardh 1848: 47, generic type species *Phyllymenia belangeri* (Bory de Saint-Vincent) Setchell *et* N.L. Gardner 1936: 473;

D) *Prionitis* J. Agardh 1851: 185, generic type species *Prionitis lanceolata* (Harvey) Harvey 1853: 197, pl. 27: fig. A.

As a result of the above reinstatements, the following new combinations are proposed:

a) *Dermocorynus dichotoma* (J. Agardh) Gargiulo, M. Morabito *et* Manghisi **comb. nov.**, basionym: *Grateloupia dichotoma* J. Agardh 1842: 103 - *Algae maris Mediterranei et Adriatici, observationes in diagnosis specierum et dispositionem generum*. Paris, Masson et Cie;

b) *Dermocorynus horrida* (Kützinger) Gargiulo, M. Morabito *et* Manghisi **comb. nov.**, basionym: *Grateloupia horrida* Kützinger 1843: 397, pl. 76.I - *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange*. Leipzig, F.A. Brockhaus.

c) *Phyllymenia capensis* (O. De Clerck) Gargiulo, M. Morabito *et* Manghisi **comb. nov.**, basionym: *Grateloupia capensis* O. De Clerck in De Clerck, Gavio, Fredericq *et* Copejans 2005: 402-405, figs 7, 8 - *Journal of Phycology* 41(2).

Further new combinations will likely be necessary, but we do not propose formal changes for species for which we have not had opportunity to study anatomical features.

Formal descriptions of new genera based on *G. doryphora* (Montagne) M. Howe, *G. subpectinata* Holmes and *G. proteus* Kützinger, although warranted, are left for a future publication.

Acknowledgements. The authors are greatly indebted to dr. Giusi Genovese for her appreciated support during the research, to prof. Gary W. Saunders for his encouragement and valuable suggestions during the preparation of the manuscript, and to prof. Gerald T. Kraft for his constructive and comprehensive review.

The Botanical Museum at Lund, Sweden, and the Nationaal Herbarium Nederland at Leiden, Netherlands are thankfully acknowledged for the permission to examine historical collections. Profs Gianfranco Sartoni and Gerald T. Kraft kindly provided specimens from Livorno and from Australia, respectively. Drawings of reproductive features from Salvatore Casella were greatly appreciated. The Section of Scientific Computing of C.E.C.U.M. at the University of Messina is thankfully acknowledged for the use of the Unix cluster.

This study was supported by grants from the University of Messina to G.G.

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