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Phylogenetic Systematics and Biogeography of the Gigartinaceae (Gigartinales, Rhodophyta) Based on Sequence Analysis of *rbc*L[1]

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Abstract

Recently Hommersand, Guiry, Fredericq and Leister (1993, *Hydrobiologia* 260/261 : 105–120) proposed a revised classification of the marine red algal family Gigartinaceae in which sixty-nine species were classified into four extant (*Chondrus* Stackhouse, *Gigartina* Stackhouse, *Iridaea* Bory, nom. cons., *Rhodoglossum* J. Agardh) and three reinstated (*Chondracanthus* Kützing, *Sarcothalia* Kützing, *Mazzaella* G. de Toni *f.*) genera based on developmental and morphological criteria. We have undertaken a preliminary study of the phylogenetic systematics and biogeography of the Gigartinaceae based on an independent data set derived from sequence analysis of *rbc*L, the gene that codes for the large subunit of RuBisCO. The topology of the *rbc*L tree, which contains 43 species, generally supports our recent systematic revision, while highlighting some taxonomic problems. '*Gigartina*' *alveata* occupies a basal position isolated from all other taxa. *Chondracanthus* forms a distinct clade with centers of speciation in East Asia and Pacific North America. The *Gigartina/Rhodoglossum* clade is primarily austral and appears to have originated in the southwestern Pacific Ocean. *Sarcothalia* is antiboreal. *Iridaea*, '*Gigartina*' *skottsbergii*, three *Mazzaella* clades, and *Chondrus* form a cluster partly localized in antiboreal waters but extending along Pacific South and North America to East Asia, with one species, *Chondrus crispus*, in the North Atlantic Ocean. We propose that ancestral taxa belonging to the Gigartinaceae originated along the eastern edge of Gondwanaland in the Mesozoic and spread around the perimeter of the supercontinent Pangea giving rise to present-day genera in more or less linear sequence, followed by secondary dispersal of some species.

Introduction

The marine red algal family Gigartinaceae contains approximately 100 species that are widely distributed in cold and warm temperate regions, as defined by Lüning (1990), in the Northern and Southern Hemispheres. At least four species have been recorded south of the Antarctic Convergence; however, none appears to be endemic to the Antarctic Region. No species has yet been reported from the Arctic Region, although *Chondrus crispus* reaches its boundary (Lüning 1990). Several species of *Chondracanthus* range into the tropics, but none is reported to be endemic to the Tropical Region.

Hommersand (1981) speculated that the major phylogenetic lines among the red algae (families or clusters of genera) originated during the Mesozoic and can be divided into two groups: those that evolved primarily in the Tethyan Ocean and those that evolved along the outer perimeter of Pangea and are primarily distributed along corresponding coastlines at the present time. The Gigartinaceae was cited as an example having this latter distribution. Species distributed along the islands of the West Wind Drift all appear to have originated in the

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vicinity of South America and to have been dispersed by ocean currents since the Miocene. In contrast, many of the endemic species found in the Cape Province, South Africa, and in Namibia belong to a group of taxa that appear to have originated in the antiboreal region of the Pacific Ocean. Hommersand (1986, fig. 2) speculated that such taxa were distributed through passageways between West and East Antarctica during warm periods in the Oligocene and Miocene when West Antarctica was little glaciated, and were subsequently rafted or dispersed by currents to southern Africa where they evolved into distinct species. According to this hypothesis, species of Gigartinaceae endemic to the temperate waters of southern Africa should have their closest affinities with species found in New Zealand and islands of the Campbell Plateau, or in southern Australia.

We here undertake a preliminary study of the phylogenetic systematics of the Gigartinaceae based on sequence analysis of *rbc*L, a gene that codes for the large subunit of RuBisCO, the primary enzyme of CO_2 fixation in photosynthesis. The results are analysed in comparison with the revised classification of the Gigartinaceae proposed by Hommersand *et al.* (1993) and with the biogeographic hypothesis outlined here.

Materials and Methods

Algal samples were desiccated in silica gel (Chase and Hills 1991) or air-dried and stored at −20 °C. Immediately before DNA extraction, the tissue was rehydrated in seawater and sorted to ensure monospecificity of the sample. Voucher specimens were fixed in 5% formalin/seawater and deposited in the Herbarium of the University of North Carolina. The location of the Type specimens we examined is given in brackets after the basionym, followed by an exclamation mark [!]. Herbarium abbreviations follow Holmgren *et al.* (1990).

Total cellular DNA was extracted from 1 to 5 g samples (damp weight) using procedures modified from Doyle and Doyle (1987). In general, best results were obtained when samples were ground at room temperature in CTAB (hexadecyltrimethylammonium bromide) extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% w/v CTAB, 2% w/v PVP-40 [polyvinylpyrrolidone-40]). After 15 min incubation in 20 mL of extraction buffer, samples were extracted with 15 mL of 1 : 1 Tris-buffered phenol : SEVAG (24 : 1 chloroform : isoamyl alcohol) for 10 min with gentle agitation, followed by a second extraction with 20 mL SEVAG for 30 to 40 min with vigorous agitation. The DNA was then precipitated by adding 2/3 volume ice-cold isopropyl alcohol and storing at −20 °C for 1−2 hours. Following precipitation, the DNA pellet was washed (70% ethanol, 10 mM ammonium acetate) and redissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, pH 8.0), before purification by cesium chloride-ethidium bromide centrifugation. The coding region of *rbc*L and spacer between *rbc*L and *rbc*S were amplified by temperature cycling with thermally stable DNA polymerase (Promega) and the synthetic oligonucleotides designated as amplification primers in Table I. The temperature-cycling protocol included 40 cycles of 1.5 min at 94 °C; 2 min at 37 °C; 3.0 min at 72 °C. In taxa in which nucleotide variation at the primer sites prevented proper annealing, partial amplification of *rbc*L was facilitated using internal primers.

Amplified fragments were prepared for sequencing by a modification of the primer annealing protocol of S. Rehner (pers. comm.). To the amplification reaction solution a 1/20 volume of 50% v/v slurry of nitric acid-treated 325-mesh powdered flint glass and 3× volume 6M NaI solution saturated with Na_2SO_3 were added and mixed thoroughly. After incubation for 15 min at room temperature the solution was spun down and the pellet rinsed by resuspending 3 times in wash buffer (70% ethanol, 10 mM ammonium acetate). After the final wash, DNA was eluted from the glass fines by resuspension of the pellet in TE buffer and incubation at 65 °C for 5 min. For primer annealing a solution of 0.25 pmol glass powder-purified template, 3 pmol primer, 2 µl 5× Sequenase buffer and dH_20 to a total volume of 10 µL, was heated for 3 min at 95 °C before snap chilling in an ice-water slurry. Dideoxynucleotide chain termination sequencing (Sanger *et al.* 1977) was done using Sequenase 2.0 (US Biochemical, Inc.) and primers listed in Table I.

Table I. Synthetic primers used for sequencing *rbc*L in red algae. 'F' and 'R' represent primers for the forward and reverse strand respectively, while the following numbers designate the position of the terminal 5' nucleotide site on the forward strand.

F-rbcL start:	5'-TGTGTTGTCGACATGTCTAACTCTGTAGAAG-3' (forward amplification primer)
F-57:	5'-GTAATTCCATATGCTAAAATGGG-3' (secondary amplification primer)
F-321:	5'-GATATCGATTTATTTGAAGAAGG-3'
F-492:	5'-CGTATGGATAAATTTGGTCG-3'
F-577:	5'-GTATATGAAGGTCTAAAAGGTGG-3'
F-753:	5'-GGAAGATATGTATGAAAGAGC-3'
F-939:	5'-TTCCGTGTAATTTGTAAGTGG-3'
F-993:	5'-GGTACTGTTGTAGGTAAATTAGAAGG-3'
F-1237:	5'-CCAGATGGTATTCAAGCAGGTGC-3'
R-rbcS start:	5'-TGTGTTGCGGCCGCCCTTGTGTTAGTCTCAC-3' (reverse amplification primer)
R-1381:	5'-ATCTTTCCATAGATCTAAAGC-3'
R-1150:	5'-GCATTTGTCCGCAGTGAATACC-3'
R-753:	5'-GCTCTTTCATACATATCTTCC-3'
R-406:	5'-CATATCTTCTAAGCGTAAAGC-3'

Sequence data were analyzed using PAUP 3.1.1 (Phylogenetic Analysis Using Parsimony, Swofford 1993) and MacClade 3.01 (Maddison and Maddison 1992). Due to the large number of taxa included in the analysis and limitations of software, heuristic searches were necessary. Searches were based upon the method of Qiu *et al.* (1993) and done under the Fitch criterion (equal weights for all substitutions; Fitch 1971) using 555 random sequence additions, STEEPEST DESCENT, MULPARS (but permitting only five trees be held at each step) and NNI (nearest-neighbor interchange). Trees found in these random searches were then used as starting points for further searches with MULPARS and TBR (tree bisection-reconnection) until swapping was complete. As a measure of internal support, decay indices, representing the number of steps less parsimonious than minimal at which branches were no longer resolved, were determined based on strict consensus analysis of cladograms found by relaxing parsimony sequentially, one step at a time (Bremer 1988) up to five steps.

Results

Forty-five complete or partial *rbc*L sequences representing forty-three species of Gigartinaceae and two outgroup taxa (*Phyllophora crispa*, the type species of the Phyllophoraceae and *Mastocarpus stellatus*, the type species of the Petrocelidaceae) were produced and analysed in this study (see Appendix). A previous analysis of 92 red algal sequences carried out by Freshwater (1993) established the estimated range of error for *rbc*L sequence data to be < 5 incorrectly designated base-pairs (bp) per sequence or 0.3%. In that study, the Gigartinaceae consistently formed a monophyletic clade in trees resolved at 5 steps less parsimonious than minimal trees ($d > 5$), and the Gigartinaceae-complex (Petrocelidaceae, Phyllophoraceae, Gigartinaceae) was distinct.

Previous studies have found the length of *rbc*L in red algae to be 1467 base pairs with no insertions or deletions (Valentin and Zetsche 1989, Kostrzewa *et al.* 1990, Freshwater 1993, Freshwater and Rueness 1994, Freshwater *et al.* 1994). Likewise, in this study insertions and deletions were absent, allowing for the unambiguous alignment of all sequences. Though both *rbc*L and the spacer region between *rbc*L and *rbc*S were amplified, only sequence data from *rbc*L was used in this analysis. Preliminary studies demonstrated that disagreements between the forward and reverse strands are well within the estimated range of error due to methodology. Accordingly, only sequences of the forward strand were produced in most taxa. Sequences have been deposited in GenBank (Bilofsky and Burks 1988) under the accession numbers given in the Appendix.

Prior to analysis, the data set was truncated to include the 1133 nucleotide positions between bp 135 and bp 1269. For many taxa within the Gigartinaceae amplification with the internal F-57 primer (Table I) was required and sequence data for the start of *rbc*L is not available. Likewise, in the majority of *Mazzaella* species internal primers for sequencing the 3' terminus of *rbc*L did not work well and all sequences were truncated to reduce the disparity between the amount of data included for each taxon. Parsimony analysis resulted in nine minimal trees of length = 1239, consistency index (CI) = 0.38, and retention index (RI) = 0.69 (Fig. 1).

In this truncated data set there were 285 informative characters spread evenly throughout the analyzed sequence (Table II). The incidence of homoplasy, as reflected in CI and RI values is also evenly distributed across the data set (Table II). Uniformity in the distribution of informative and homoplastic characters appears to be characteristic of red algal *rbc*L data (Freshwater *et al.* 1994) and allows for the derivation of consistent topologies when partial sequences are analyzed. It was found that once 70% of complete sequences had been reached, the inclusion of additional sequence data did not change the topological position of a taxon in the *rbc*L tree.

Minimal tree topologies generally support the genera recognized by Hommersand *et al.* (1993). Two poorly understood taxa currently placed in *Gigartina*, '*G.*' *alveata* and '*G.*' *skottsbergii* that were treated as *incertae sedis* occupy isolated positions. *Chondracanthus* and *Gigartina* are both well-supported assemblages. *Gigartina*, which is primarily a southern hemisphere genus, and the closely related monotypic *Rhodoglossum* form a monophyletic clade ($d > 5$). Though all minimal solutions place *Rhodoglossum* within the *Gigartina* clade, a basal position of *Rhodoglossum* requires only a one-step penalty in parsimony. *Chondracanthus* is resolved as a strongly supported monophyletic group ($d > 5$) sister to the *Gigartina/Rhodoglossum* clade, though their sister relationship is only moderately supported (d2).

The five Southern Hemisphere species of *Sarcothalia* included in this study are resolved into two separate strongly ($d > 5$) to well-supported (d4) clades. Forced monophyly of these species results in a > 100-step penalty in parsimony. Sequence divergence values, which range from 1.6% to 4.0% within clades and 7.4% to 9.4% between clades, also emphasize the distinctness of the two clades (Table III). By contrast, forced monophyly of '*S.*' *decipiens* and '*S.*' *scutellata* with *Iridaea* imposes only a one-step penalty. Another species, *Gigartina atropurpurea*, which had been placed in *Sarcothalia* in Hommersand *et al.* (1993), is here returned to *Gigartina*. Forced monophyly of *G. atropurpurea*

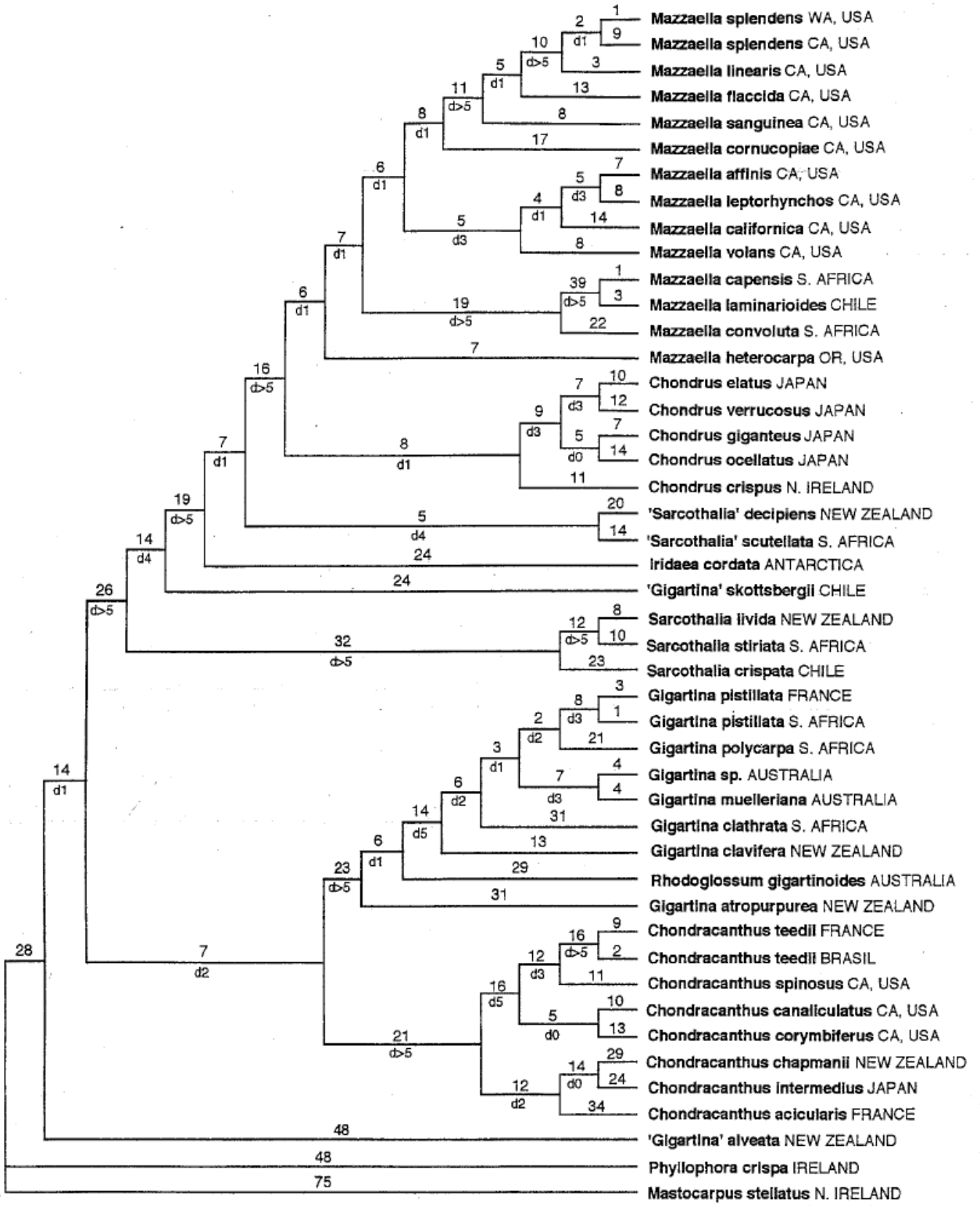

Fig. 1. One of nine most parsimonious trees of length = 1239, CI = 0.38, RI = 0.69 for 43 Gigartinaceae and two outgroup species. Estimated branch lengths are given above and decay indices below the branches.

with *Sarcothalia* imposes a 26-step penalty in parsimony.

Sarcothalia, '*Gigartina*' *skottsbergii*, *Iridaea cordata* and '*Sarcothalia*' form a series of lineages basal to the *Chondrus* and *Mazzaella* clades. Examination of Table III reveals that divergence values between clades range from 5.4% to 11.3% for clades at the bottom of the chart up to *Sarcothalia*, whereas the range is generally smaller (2.8% to 7.6%) for clades above *Sarcothalia*. Forced monophyly of *Iridaea* with *Sarcothalia* imposes a > 100-step penalty.

Table II. Distribution of informative characters (percent of total), steps (percent of total), consistency index (CI) and retention index (RI) across the *rbc*L sequence data set for 45 Gigartinacean taxa.

Nucleotide sites	Informative characters (%)	Steps (%)	CI	RI
136– 362	18	18	0.39	0.67
363– 588	21	21	0.39	0.72
589– 814	22	22	0.36	0.67
815–1040	17	19	0.33	0.67
1041–1268	22	20	0.42	0.72

As currently circumscribed, *Mazzaella* contains three distinct clades. The Pacific North American clade which includes the type species, *M. californica*, is moderately supported (d3), while the North American clade that includes *M. splendens* (Table IV) and the austral clade that includes *M. capensis* are strongly supported (d > 5). The sister relationships of these three *Mazzaella* clades along with the topological positions of *M. cornucopiae* and *M. heterocarpa* in minimal trees is not well resolved.

Monophyly of the Japanese *Chondrus* species is moderately supported (d3) but the basal position of the Atlantic species, *C. crispus*, is only weakly supported (d1). Though the position of *Chondrus* basal to *Mazzaella* is poorly resolved (d1), the close relationship of these two genera within the Gigartinaceae is strongly supported (d > 5).

Discussion

Significance of rbcL *for the classification of the Gigartinaceae*

The topology of the cladogram based on *rbc*L sequence analysis generally supports the taxonomic revision of the Gigartinaceae proposed by Hommersand *et al.* (1993) which was founded upon an assessment of morphological characters. The molecular data highlight potential taxonomic problems and point out instances of misidentification and taxonomic error. Each of the families (Phyllophoraceae, Petrocelidaceae and Gigartinaceae) that make up the Gigartinaceae-complex are well-defined morphologically (Guiry and Garbary 1990) and in preliminary studies form monophyletic clades based on *rbc*L sequence analysis that are resolved at > 5 steps less parsimonious than minimal trees (Freshwater 1993). We therefore selected the type species of *Mastocarpus*, *M. stellatus* (Petrocelidaceae) and *Phyllophora*, *P. crispa* (Phyllophoraceae) as outgroup species in this study.

There is a strong possibility that '*Gigartina*' *alveata*, which occupies a basal position in Figure 1, represents

Table III. Range of *rbc*L sequence divergence values (%) for species within and between clades shown in Figure 1. Within clade values are shown in bold type.

	Chondrus	*M. splendens*	*M. californica*	*M. capensis*	'*Sarcothalia*'	*Iridaea*	'*G.' skottsbergii*	*Sarcothalia*	*Rhodoglossum Gigartina*	*Chondracanthus*	'*G.' alveata*
Chondrus	**1.9– 3.2**										
M. splendens	2.8– 5.9	**0.4– 4.2**									
M. californica	3.3– 5.4	2.8– 4.5	**1.3– 2.4**								
M. capensis	5.2– 7.6	5.1– 7.4	4.4– 7.2	**0.4– 5.5**							
'*Sarcothalia*'	3.5– 5.9	4.1– 6.3	4.5– 5.8	5.4– 8.1	**3.0**						
Iridaea	4.5– 5.8	5.0– 6.5	5.5– 6.0	6.4– 7.9	4.0– 4.2	–					
'*G.' skottsbergii*	5.1– 6.9	5.8– 7.0	5.3– 6.4	5.8– 7.0	5.0– 5.8	5.6	–				
Sarcothalia	7.2–10.4	6.9– 9.4	7.5– 9.8	7.9–10.5	7.4– 9.4	7.7– 9.1	5.6–6.0	**1.6– 4.0**			
Rhodoglossum/ Gigartina	7.2–10.9	7.6–11.0	6.5–10.4	6.8–10.8	7.3– 9.7	8.1–10.1	5.4–7.8	7.4–10.7	**1.9–5.9**		
Chondracanthus	8.2–10.9	8.4–10.5	7.7–10.6	7.8–11.3	7.4– 9.6	8.1– 9.5	6.0–8.0	7.5–11.1	5.9–9.0	**1.0–6.8**	
'*G.' alveata*	9.4–10.6	10.2–11.0	9.3–10.3	10.5–11.0	10.1–10.2	9.8	8.3	9.9–10.9	7.1–8.2	7.4–9.9	

a new genus. Zollner (1977) observed that, whereas the pattern of development of primary filaments seen in '*G.*' *alveata* is common to all members of the Gigartinaceae, no other species studied formed so few secondary filaments. Secondary filaments develop around the fertilized auxiliary cell; however, the envelope produced is unlike any seen in other Gigartinaceae (Hommersand, pers. obs.). Tetrasporangial plants are rare in nature; however the tetrasporangia are borne in raised sori that are released by excision, much as in *Gigartina*.

Gigartina forms a monophyletic clade ($d > 5$) sister to *Chondracanthus*. In *Chondracanthus* the female reproductive structures are borne on ordinary branches, pinnules or papillae, whereas they are typically formed on secondary branches, pinnules or papillae in *Gigartina*. During cystocarp development, the gonimoblasts in both genera penetrate the envelope filaments, linking to them by secondary pit connections and tubular cells to form a placenta. Whereas tetrasporangia are transformed from cells in primary cortical filaments in *Chondracanthus*, in *Gigartina* they are formed progressively at the boundary between cortex and medulla, mostly in secondary filaments (Hommersand *et al.* 1993). *Gigartina* is primarily a genus of the Southern Hemisphere, whereas *Chondracanthus* is widespread, with a present center of distribution in the North Pacific Ocean. A few species of *Chondracanthus (C. tenellus*, *C. intermedius*, *C. acicularis*, and *C. teedii*) extend into the tropics. The plant referred to in Hommersand *et al.* (1993) as *Gigartina chapmanii* from New Zealand belongs in *Chondracanthus*. The type specimen in the British Museum is sterile; however, analysis of new material of this species establishes that it is most closely related to *C. intermedius* in the *acicularis* group. It is here transferred to *Chondracanthus* (Table IV).

Rhodoglossum possesses a unique tetrasporangial configuration in which the tetrasporangia are generated by apical division of cortical cells and form files that radiate inwardly from the thallus surface (Edyvane and Womersley 1993). Even so, this monotypic genus clusters with *Gigartina* in the *rbc*L tree. It is resolved basally along with *Gigartina atropurpurea*, a species with maculate tetrasporangial sori that was incorrectly placed in *Sarcothalia* by Hommersand in Hommersand *et al.* (1993). The gonimoblast filaments in both unite to form an intricate network, the nature of which is not yet clear. *Gigartina atropurpurea* resembles the plant called *Gigartina radula* in Australia, and the two may be identical. The remaining species of *Gigartina* form a distinct clade characterized by tetrasporangia formed progressively at the boundary between the cortex and medulla with the tetraspores typically released by excision of the entire sorus. This cluster includes a plant that has long been referred to as *Gigartina* '*radula*' in South Africa but which is distinct from *G. radula* (Esper) J. Agardh, a species originally described as coming from Australia (See, however, Parkinson 1981). Our material corresponds to the holotype of *Gigartina polycarpa*, and we hereby reinstate that species in the South African flora (Table IV).

Table IV. Nomenclatural and taxonomic data: new combinations and reinstated species relating to Gigartinaceae.

Chondracanthus chapmanii (J. D. Hooker *et* Harvey) Fredericq, *comb. nov.* (New Zealand).
Basionym: *Gigartina chapmanii* J. D. Hooker *et* Harvey in Harvey, Algae in J. D. Hooker, *Bot. Antarctic Voyage, Pt. 2, Flora Novae-Zelandiae*, p. 251, pl. 119, B. 1855. [BM!].

Gigartina polycarpa (Kützing) Setchell *et* Gardner 1933:295 (Cape Province, South Africa).
Basionym: *Mastocarpus polycarpus* Kützing *Sp. Alg.*, p. 733. 1849; *Tab. Phyc.* 17, pl. 44, misprinted *polycystis*. 1867. [L 938, 344 ... 360!].

Mazzaella convoluta (Areschoug *ex* J. Agardh) Hommersand *comb. nov.* (Cape Province, South Africa).
Basionym: *Gigartina convoluta* Areschoug *ex* J. Agardh, *Lunds Univ. Årsskr.* Afd. 2, 35:32. 1899. [Isotype LD 23870!].

Mazzaella sanguinea (Setchell *et* Gardner) Hommersand *comb. nov.* (Pacific North America).
Basionym: *Iridophycus sanguineum* Setchell *et* Gardner, *Proc. Nat. Acad. Sci. USA*, 23:172. 1937. [Lectotype UC 507, 503!].

Mazzaella splendens (Setchell *et* Gardner) Fredericq *comb. nov.* (Pacific North America).
Basionym: *Iridophycus splendens* Setchell *et* Gardner, *Proc. Nat. Acad. Sci. USA*, 23:170. 1937. [Lectotype UC 539565!].

Sarcothalia is characterized by thallus dimorphism with the gametophytes proliferously branched or spiny and the tetrasporophytes smooth. The gonimoblasts consist of multinucleate, interconnected inner cells and gonimoblast filaments that displace a compact envelope and link to it by terminal tubular cells. Tetrasporangia are borne in secondary filaments that radiate from a common center and form circular sori. Although only three species were investigated, this genus is well supported by *rbc*L sequence analysis. In Hommersand *et al.* (1993) the diagnosis of *Sarcothalia* was relaxed to include species with less specialized cystocarps and tetrasporangia borne in circular sori that do not radiate from a common center. Two such species, '*Sarcothalia*' *scutellata* from South Africa and '*S.*' *decipiens* from New Zealand form a second clade in the *rbc*L tree that is clearly separate from true *Sarcothalia* and is sister to *Iridaea*. As in *Iridaea*, the gonimoblasts do not form an internal network of expanded cells; however, unlike in *Iridaea* the tetrasporangia are formed in secondary filaments borne in immersed sori, rather than in primary cortical filaments. These observations call for new morphological studies.

'*Gigartina*' *skottsbergii* occupies an isolated position in the *rbc*L tree between *Sarcothalia* and *Iridaea*. Placed under *incertae sedis* in Hommersand *et al.* (1993), this

species may comprise a new genus. It possesses a unique combination of characters that distinguishes it from all other Gigartinaceae. The thallus is umbilicate, attached from the lower face by a short stipe and secondary haptera. The carpogonial branches and cystocarps are borne on stalked papillae with the gonimoblasts surrounded by broad, weakly defined envelopes. Tetrasporangia are formed in secondary filaments in the center of the medulla, much as in *Chondrus*.

The range of divergence values given in Table III appear to identify a cluster of related taxa that include: *Iridaea*, the *Mazzaella* clades, *Chondrus* and, perhaps, *Gigartina skottsbergii*. Basal to *Chondrus* and the *Mazzaella* clades, *Iridaea* is distinguished by the presence of a massive envelope surrounding the young gonimoblasts and tetrasporangia borne in primary cortical filaments in superficial sori. The ontogeny of the vegetative medullary filaments resembles that of *Mazzaella*. New information may help clarify the phylogenetic systematics of this pivotal genus.

A clade is identified that includes *Mazzaella* and *Chondrus*. In both genera the cystocarp lacks a compact envelope and diffuse, filiform gonimoblast filaments penetrate between vegetative filaments and attach to them either by secondary pit connections (*Chondrus*) or by terminal tubular cells (*Mazzaella*). Based upon differences in tetrasporangial development, the three clades in *Mazzaella* could either comprise subgenera or be recognized as separate genera. In the group containing the type species, *M. californica*, tetrasporangia are transformed from primary cortical cells, or are borne in secondary filaments originating from inner cortical cells. In the remaining taxa, tetrasporangia are formed only in secondary filaments originating from inner cortical or medullary cells. Secondary filaments are formed around the developing gonimoblasts in all species of *Mazzaella*; however, they are rapidly consumed and inconspicuous in the austral clade that includes *M. capensis*.

The Southern Hemisphere clade containing *M. capensis* could be identified with *Iridophycus* Setchell *et* Gardner 1936, p. 470 based on *Iridaea capensis* J. Agardh, 1847, p. 85. *Mazzaella capensis* is identical to *M. laminarioides* in the *rbc*L tree within the limits of sequence resolution. Also recorded from Gough Island (Chamberlain 1965), it is likely that *M. laminarioides* originated in South America and was distributed to Gough Island and the western Cape, South Africa, perhaps by the Benguela current in comparatively recent times. If this interpretation is correct, *Mazzaella laminarioides*, based on *Iridaea laminarioides* Bory 1828, p. 105, pl. 11, fig. 1, takes precedence over *M. capensis* based on *Iridaea capensis* J. Agardh 1847, p. 85, pl. 1.

Hommersand, in Hommersand *et al.* 1993, had transferred the plant known as *Iridaea convoluta* (Areschoug *ex* J. Agardh) Hewitt 1960 in South Africa to *Sarcothalia*. This was an error based on a misidentification of a form of the tetrasporophyte of *Sarcothalia stiriata*. Our present material corresponds to an isotype of *Gigartina convoluta* Areschoug *ex* J. Agardh and possesses cystocarp and tetrasporangial characters that are very similar to those found in *Mazzaella capensis*. Accordingly, this species is transferred to *Mazzaella* (Table IV), while bearing in mind that it may ultimately reside in *Iridophycus*. Sequence analysis of *rbc*L shows that *Mazzaella convoluta* is well separated from *M. capensis* (Fig. 1).

Six species of *Mazzaella* from Pacific North America were formerly placed in *Iridaea*. Four of them (*M. heterocarpa*, *M. cornucopiae*, *M. flaccida*, and *M. linearis*) were transferred to *Mazzaella* in Hommersand *et al.* (1993), and two (*M. splendens* and *M. sanguinea*) are transferred here (Table IV). *Mazzaella heterocarpa*, which has broad cystocarps of varying diameter is poorly resolved in the *rbc*L tree and presently occupies a basal position sister to the *Chondrus* clade (Fig. 1). Our material of *Mazzaella cornucopiae* corresponds to the type of *Iridophycus parksii* Setchell *et* Gardner 1937, as illustrated by Abbott (1971). The specimen of *Mazzaella sanguinea* is a tetrasporangial plant that agrees well with the cystocarpic lectotype of *Iridophycus sanguinea*. It is distinguished by the red-brown color of dried herbarium material and by the presence of broad, short-celled secondary filaments in the medulla of both sterile and fertile plants. *Mazzaella flaccida*, a species that is difficult to distinguish from *M. splendens* is clearly separated ($d > 5$) in the *rbc*L tree. Our material was collected in the upper intertidal, was greenish-yellow in color and the tetrasporophyte was bordered by a clear, non-sporangial margin, as described by Abbott (1971). The collection of *Mazzaella splendens* from Washington corresponded in habit to that of *Iridaea cordata* var. *cordata* sensu Abbott (1971), that of *M. splendens* from California to that of *Iridaea cordata* var. *splendens* sensu Abbott (1971), and that of *M. lineare* to *Iridaea lineare* as illustrated by Abbott (1971). These three entities are not well resolved in the *rbc*L analysis (Fig. 1). We have examined the holotype (LE) of *Mazzaella lilacina*, which was kindly sent by P. Perestenko, and have confirmed that it belongs in *Mazzaella*; however, we are unable, at present, to establish its identity with one of the presently recognized species.

Significance of rbc*L to the biogeography of the Gigartinaceae*

The present-day distribution of taxa belonging to the seven genera of the Gigartinaceae is shown on a world

map in Hommersand *et al.* (1993, fig. 41) and on a −40 Ma Lambert equal-area projection map here (Fig. 2). Were the distribution of the clades resolved in the *rbc*L tree (Fig. 1) to be plotted on a map of Pangea as it appeared in the Mesozoic, most would lie in a continuous circle extending from the western South Pacific Ocean (eastern edge of Gondwanaland) to the western edge of the North Pacific Ocean (eastern edge of Laurasia). The sequence would be '*Gigartina*' alveata, *Gigartina/Rhodoglossum*, *Sarcothalia*, '*Gigartina*' *skottsbergii*, *Iridaea*, *Mazzaella laminarioides*-group, *Mazzaella californica*-group, *Mazzaella splendens*-group, and *Chondrus*. (*Chondrus* appears at the top of the tree in some cladograms one step less parsimonious than minimal.) *Chondracanthus* could have originated somewhere along the eastern edge of Gondwanaland; however, its present center of distribution appears to be East Asia with a second center in Pacific North and South America, and with two species, *C. acicularis* and *C. teedii*, widespread in the Atlantic Ocean. The data in the *rbc*L tree favor on easterly distribution of taxa belonging to *Chondracanthus* from East Asia to Pacific North America and from there to the Atlantic Ocean. The possibility that species of *Chondracanthus* belonging to the *teedii*

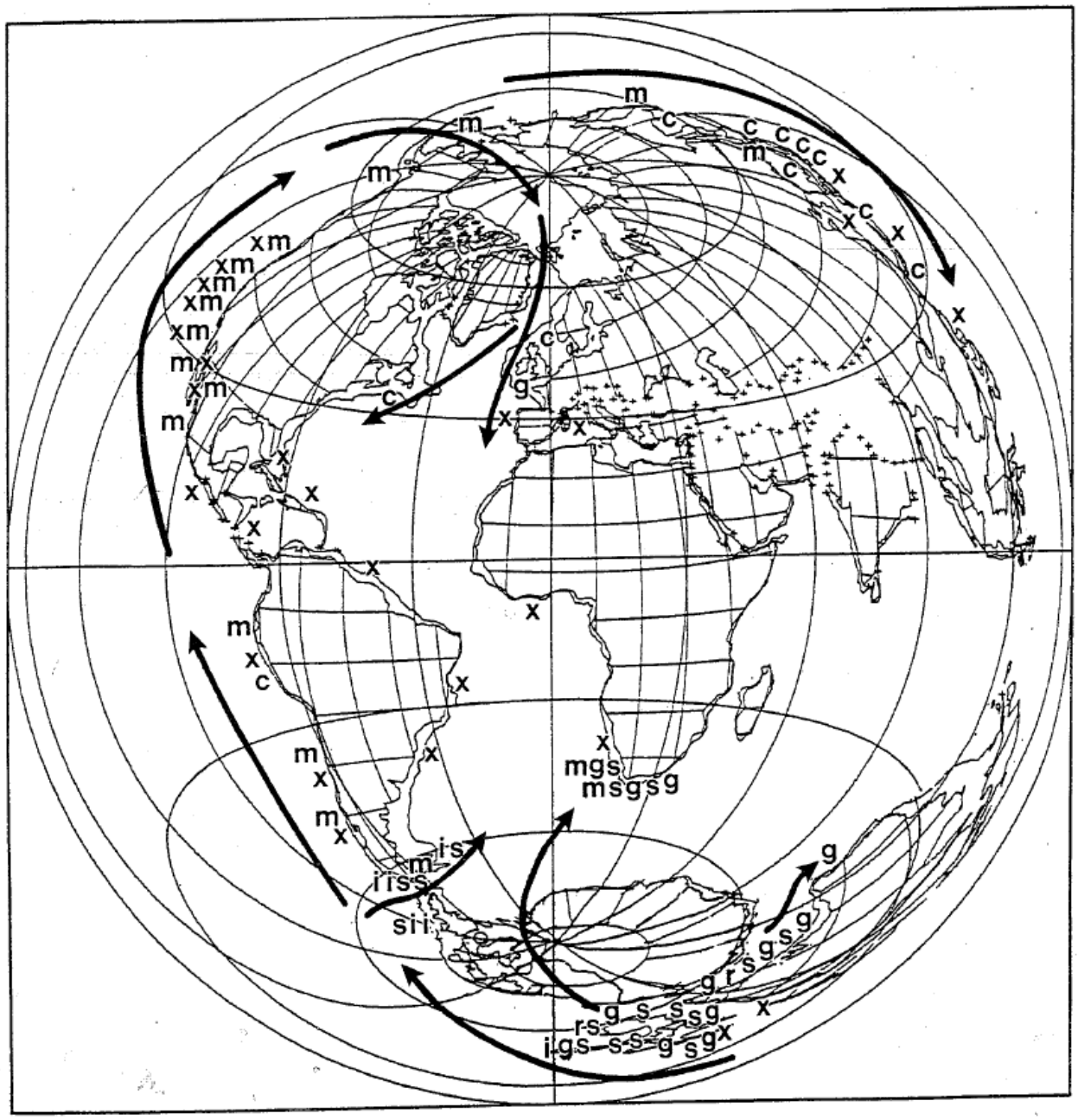

Fig. 2. Distribution and proposed pathway of migration of genera belonging to the Gigartinaceae plotted on a late Eocene (−40 Ma) Lambert equal-area map adapted from Smith and Briden (1977): *Gigartina* (g), *Rhodoglossum* (r), *Sarcothalia* (s), *Iridaea* (i), *Mazzaella* (m), *Chondrus* (c), and *Chondracanthus* (x). The number of letters correspond to the approximate number of species in each area.

group may have originated in the western Tethyan or Atlantic Ocean and spread westward to Pacific North and South America cannot, however, be ruled out. Accordingly, we have indicated the directional distribution of ancestral taxa belonging to the Gigartinaceae in Figure 2, except *Chondracanthus*.

The proposal by Hommersand (1986) that species of Gigartinaceae presently found in the Cape Province, South Africa, and Namibia originated on the Pacific Ocean side of Gondwanaland and were dispersed to South Africa through a passageway between western and eastern Antarctica is supported by the presence of species pairs distributed between New Zealand and South Africa. These include *Sarcothalia livida* (NZ)/*S. stiriata* (SA), '*Sarcothalia*' *decipiens* (NZ)/'*S.*' *scutellata* (SA) and, possibly, *Gigartina clavifera* (NZ)/*G. clathrata* (SA). Species pairs distributed between Australia and South Africa may have followed the same pathway. The *rbc*L data support the view expressed by Hommersand (1986) that *Gigartina pistillata* reached Atlantic Europe from South Africa by amphitropical distribution in recent times, rather than his later opinion (Hommersand 1990) that this species exhibits a Tethyan distribution.

A few species of *Iridaea* and *Sarcothalia* have been distributed by long distance dispersal along the islands of the West Wind Drift, but these seem to be the exception rather than the rule. The more general pattern is one of spread along continuous coastlines accompanied by gradual speciation.

The results of these preliminary studies suggest that *rbc*L sequence data provide a reliable indicator of phylogenetic and biogeographic tendencies in the Gigartinaceae. When more taxa have been sequenced and further developmental studies carried out, the possible congruence of phylogenetic and biogeographic trees can be assessed.

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Appendix: List of Species Examined and *rbc*L Accession Numbers in Genbank

Petrocelidaceae

Mastocarpus stellatus (Stackhouse) Guiry
Bally Castle, Co. Antrim, Northern Ireland, coll. C. A. Maggs, 20. i. 92. (U029920)

Phyllophoraceae

Phyllophora crispa (Hudson) Dixon
Spiddal, Co. Galway, Ireland, coll. M. D. Guiry, 7. iii. 93. (U02990)

Gigartinaceae

Chondracanthus acicularis (Roth) Fredericq *in* Hommersand *et al.* 1993
Île Verte, Roscoff, Brittany, France, coll. J. Cabioch, 9. iii. 93. (U02938)

Chondracanthus canaliculatus (Harvey) Guiry *in* Hommersand *et al.* 1993
Pigeon Point, San Mateo Co., California, coll. M. H. Hommersand, 20. v. 92. (U02939)

Chondracanthus chapmanii (J. D. Hooker *et* Harvey *in* Harvey) Fredericq *comb. nov.*
Island Bay, Wellington, New Zealand, coll. W. A. Nelson, 23. v. 93. (U02940)

Chondracanthus corymbiferus (Kützing) Guiry *in* Hommersand *et al.* 1993
Pigeon Point, San Mateo Co., California, coll. M. H. Hommersand, 21. xii. 92. (U02941)

Chondracanthus intermedius (Suringar) Hommersand *in* Hommersand *et al.* 1993
Tokawa, Choshi, Chiba Pref., Japan, Coll. M. Yoshizaki, 22. v. 93. (U02942)

Chondracanthus spinosus (Kützing) Guiry *in* Hommersand *et al.* 1993
Ramp, Crissie Field, Fort Point, San Francisco Co., California, coll. M. H. Hommersand, 23. xii. 92. (U02943)

Chondracanthus teedii (Roth) Kützing
Île Verte, Roscoff, Brittany, France, coll. J. Cabioch, 5. iii. 93. (U03024)

Chondracanthus teedii (Roth) Kützing
Praia de Peruibe, Itanhaém, Estado de São Paulo, Brazil, coll. M. Cordeiro-Marino, iv. 93. (U02945)

Chondrus crispus Stackhouse
Bally Castle, Co. Antrim, Northern Ireland, coll. C. A. Maggs, 20. i. 92. (U02984)

Chondrus elatus Holmes
Tokawa, Choshi, Chiba pref., Japan, coll. M. Yoshizaki, 22. v. 93. (U02985)

Chondrus giganteus Yendo
Tokawa, Choshi, Chiba pref., Japan, coll. M. Yoshizaki, 7. vi. 93. (U02986)

Chondrus ocellatus Holmes
Matsugahana, Amatsukominato, Awa Co., Chiba Pref., Japan, coll. M. Yoshizaki, 21. v. 93. (U02987)

Chondrus verrucosus Mikami
Matsugahana, Amatsukominato, Awa Co., Chiba Pref., Japan, coll. M. Yoshizaki, 21. v. 93. (U02988)

'*Gigartina*' *alveata* (Turner) J. Agardh
Tauranga Bay, Northland, New Zealand, coll. W. A. Nelson, ii. 93. (U03422)

Gigartina atropurpurea (J. Agardh) J. Agardh
Island Bay, Wellington, New Zealand, coll. W. A. Nelson, 23. v. 93. (U03423)

Gigartina clathrata (Decaisne) Rabenhorst
Oudekraal, Cape Peninsula, South Africa, coll. J. Bolton, 28. ii. 93. (U03426)

Gigartina clavifera J. Agardh
Princess Bay, Wellington, New Zealand, coll. W. A. Nelson, 23. v. 93. (U03424)

Gigartina muelleriana Setchell *et* Gardner
Flinders Jetty, Victoria, Australia, coll. G. W. Saunders and G. T. Kraft, 10. ii. 93. (U03427)

Gigartina sp.
Flinders Jetty, Victoria, Australia, coll. G. W. Saunders and G. T. Kraft, 10. ii. 93. (U03428)

Gigartina pistillata (S. G. Gmelin) Stackhouse
Santec, Brittany, France, coll. J. Cabioch, 6. iv. 93. (U03429)

Gigartina pistillata (S. G. Gmelin) Stackhouse
Smitswinkel Bay, False Bay, Cape Peninsula, South Africa, coll. J. Bolton, 4. vi. 93. (U03430)

Gigartina polycarpa (Kützing) Setchell *et* Gardner
Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 24. ii. 93. (U03431)

'*Gigartina*' *skottsbergii* Setchell *et* Gardner
Playa de San Antonio, Bahía de Ancud, Chiloë, Chile, col. M. E. Ramírez, 14. iv. 93. (U03432)

Iridaea cordata (Turner) Bory
Hellerman rocks near Laggard I., Arthur Harbor, Anvers I., Antarctica, coll. R. L. Moe, 20. i. 88. (U02989)

Mazzaella affinis (Harvey) Hommersand *in* Hommersand *et al.* 1993
Pacific Grove, Monterey Co., California, coll. M. H. Hommersand, 2. i. 93. (U03081)

Mazzaella californica (J. Agardh) G. B. de Toni *f.*
Jalama Beach State Park, Santa Barbara Co., California, coll. M. H. Hommersand, 19. v. 92. (U03082)

Mazzaella capensis (J. Agardh) Fredericq *in* Hommersand *et al.* 1993
Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 24. ii. 93. (U03083)

Mazzaella convoluta (Areschoug *ex* J. Agardh) Hommersand *comb. nov.*
Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 23. ii. 93. (U03084)

Mazzaella cornucopiae (Postels *et* Ruprecht) Hommersand *in* Hommersand *et al.* 1993
S. side, Horseshoe Cove, Bodega Head, Sonoma Co., California, coll. M. H. Hommersand, 22. xii. 92. (U03377)

Mazzaella flaccida (Setchell *et* Gardner) Fredericq *in* Hommersand *et al.* 1993
Pigeon Point, San Mateo Co., California, coll. M. H. Hommersand, 21. xii. 92. (U03378)

Mazzaella heterocarpa (Postels *et* Ruprecht) Fredericq *in* Hommersand *et al.* 1993
Seal Rock, Lincoln Co., Oregon, coll. E. Henry, 24. v. 93. (U03379)

Mazzaella laminarioides (Bory) Fredericq *in* Hommersand *et al.* 1993
Quintay, Valparaiso, Chile, coll. M. E. Ramirez, 7. iv. 93. (U03380)

Mazzaella leptorhynchos (J. Agardh) Leister *in* Hommersand *et al.* 1993
Jalama Beach State Park, Santa Barbara Co., California, coll. M. H. Hommersand, 19. v. 92. (U03381)

Mazzaella linearis (Setchell *et* Gardner) Fredericq *in* Hommersand *et al.* 1993
S. side, Horseshoe Cove, Bodega Head, Sonoma Co., California, coll. M. H. Hommersand, 22. xii. 92. (U03383)

Mazzaella sanguinea (Setchell *et* Gardner) Hommersand *comb. nov.*
Drift, Horseshoe Cove, Bodega Head, Sonoma Co., California, coll. M. H. Hommersand, 22. xii. 92. (U03384)

Mazzaella splendens (Setchell *et* Gardner) Fredericq *comb. nov.*
Shannon Point, Skagit Co., Washington, coll. S. Linstrom, 11. v. 93. (U03382)

Mazzaella splendens (Setchell *et* Gardner) Fredericq *comb. nov.*
Pigeon Point, San Mateo Co., California, coll. M. H. Hommersand, 21. xii. 92. (U03385)

Mazzaella volans (C. Agardh) Fredericq *in* Hommersand *et al.* 1993
Pigeon Point, San Mateo Co., California, coll. M. H. Hommersand, 21. xii. 92. (U03386)

Rhodoglossum gigartinoides (Sonder) Edyvane *et* Womersley
Flinders Jetty, Victoria, Australia, coll. G. W. Saunders, 14. v. 93. (U02991)

Sarcothalia crispata (Bory) Leister *in* Hommersand *et al.* 1993
Playa San Antonio, Bahía de Ancud, Ancud, Chiloë, Chile, coll. M. E. Ramírez, 14. v. 93. (U03085)

Sarcothalia livida (Turner) Hommersand *in* Hommersand *et al.* 1993
Island Bay, Wellington, New Zealand, coll. W. A. Nelson, 23. v. 93. (U03087)

Sarcothalia stiriata (Turner) Leister *in* Hommersand *et al.* 1993
Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 24. ii. 93. (U03089)

'*Sarcothalia*' *decipiens* (J. D. Hooker *et* Harvey) Hommersand *in* Hommersand *et al.* 1993
Muritai, Wellington Harbor, New Zealand, coll. W. A. Nelson, 4. v. 93. (U03086)

'*Sarcothalia*' *scutellata* (Hering) Leister *in* Hommersand *et al.* 1993
Kommetjie, Cape Peninsula, South Africa, coll. J. Bolton, 23. ii. 93. (U03088)

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