

# Circumscription of some Phylloporaceae (Gigartinales, Rhodophyta) from the Cape region, South Africa, based on molecular evidence

Suzanne Fredericq<sup>1</sup>, Robert J Anderson<sup>2</sup> & Juan M Lopez-Bautista<sup>1</sup>

<sup>1</sup>University of Louisiana at Lafayette, Louisiana, Lafayette, LA 70504-2451, USA

<sup>2</sup>Seaweed Research Unit, Marine and Coastal Management, Private Bag X2, Rogge Bay 8012, Republic of South Africa

E-mail: slf9209@louisiana.edu

**Key words:** biogeography, Gigartinales, ITS, life history, LSU rDNA, Namibia, phylogeny, Phylloporaceae, *rbcL*, Rhodophyta, South Africa, molecular systematics, taxonomy

## Abstract

The family Phylloporaceae is well represented in the Cape Peninsula region of South Africa with species possessing tetrasporoblasts and a bi-phasic life history placed in *Gymnogongrus*, and those with a heteromorphic tri-phasic life history and internal cystocarps in *Ahnfeltiopsis*. Six taxa have been recognized in the current literature: *Ahnfeltiopsis complicata*, *A. glomerata*, *A. intermedia*, *A. polyclada*, *A. vermicularis* and *Gymnogongrus dilatatus*. However, phylogenetic analyses inferred from the species' three sets of DNA sequences [the chloroplast-encoded *rbcL* gene, nuclear large-subunit ribosomal RNA gene (LSU rDNA), and internal transcribed spacer (ITS) regions of nuclear rDNA] indicate a lack of correlation between life history traits and traditional classification criteria. Both *rbcL* and ITS markers provide more adequate phylogenetic signals at the species level than does LSU rDNA. Four of the six species are recognized herein, with two species reduced to synonymy: *G. dilatatus* (including *A. glomerata*), *A. polyclada* (including *A. intermedia*), *A. vermicularis* and *A. complicata*. A critical revision of generic concepts is not made at this time and awaits completion of studies on species of the family worldwide. Different biogeographic scenarios are presented for the establishment of the extant South African Phylloporaceae.

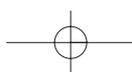
**Abbreviations:** LSU rDNA – large subunit ribosomal DNA, ITS – internal transcribed spacer, TBR – Tree-Bisection-Reconnection

## Introduction

The Phylloporaceae, together with the closely related family Gigartinaceae, form the Gigartinaceae-complex in the Gigartinales, one of Kylin's (1956) core orders. The Phylloporaceae is especially intriguing for comparison with the Gigartinaceae, as both families share anatomical/morphological features as well as ecological adaptations, presence of economically important phycocolloids (the gelling-strength kappa-type carrageenans), and an equally great number of species

with a similar distribution pattern worldwide. In contrast to the great diversity of cystocarp types that are present in the Gigartinaceae (Hommersand *et al.*, 1993), cystocarp morphology within the Phylloporaceae is routinely viewed as being less complex and of greater uniformity.

The families, however, differ dramatically in terms of their life histories, a feature long regarded as being of prime taxonomic importance in the Rhodophyta (Maggs, 1990; Guiry, 1987). The Phylloporaceae is unique within the Rhodophyta in that a single family exhibits four different life history types: 1) a tri-phasic isomorphic alternation of generations, 2) a tri-phasic heteromorphic alternation of generations in which gametophytes alternate with small free-living tetrasporophytes,



## PHYLLOPHORACEAE FROM SOUTH AFRICA

3) a bi-phasic type of life history lacking carposporophytes but with wart-like tetrasporophytes – called tetrasporoblasts or carpotetrasporophytes – that are parasitic on the female gametophytes (Newroth, 1971; Kapraun *et al.*, 1993; Masuda *et al.*, 1996), and 4) a direct type of life history involving only female gametophytes that apomictically produce carposporophytes, known to occur in some populations of taxa with a heteromorphic type of life history (Maggs, 1988; Masuda & Norris, 1994). Whereas the Gigartinaceae is characterized exclusively by an isomorphic alternation of generations, the four types of life histories mentioned above occur in the Phylloporaceae.

Life history type has figured prominently in the generic classification of the Phylloporaceae, more so than for any other red algal family. Until recently, the family comprised eight genera: typically strap-like, blade-like, or terete and bushy members, ie *Gymnogongrus* Martius, *Phyllophora* Greville, *Stenogramme* Harvey, *Ozophora* J. Agardh, *Petroglossum* Hollenberg, *Schottera* Guiry et Hollenberg; the parasite *Ceratocolax* Rosenvinge; and perhaps the rare and enigmatic *Besa* Setchell (Guiry & Garbary, 1990). Those species with life history patterns different from that of the generitype were subsequently transferred to other genera: some species of *Gymnogongrus* lacking tetrasporoblasts, but with internal cystocarps and heteromorphic life histories were transferred to *Ahnfeltiopsis* Silva et DeCew (Silva & DeCew, 1992; Masuda, 1993; Lewis & Womersley, 1994; Stegenga *et al.*, 1997); the heteromorphic species *Phyllophora trailii* Holmes et Batters was transferred to *Erythrodermis* Guiry et Garbary 1990, and the tetrasporoblastic species *P. truncata* (Pallas) A. Zinova was transferred to *Coccotylus* Wynne et Heine 1992, leaving in *Phyllophora* only those members undergoing an isomorphic alternation of generations. These eleven genera were mainly characterized on the basis of one to a few characters such as type of life history and position of reproductive structures, ie in specialized outgrowths (eg *Phyllophora*, *Ozophora*), or restricted to the medial line of branches (*Stenogramme*).

A preliminary *rbcl* gene sequence analysis of 38 taxa of Phylloporaceae (Fredericq & Ramírez,

1996) indicates that the family is in need of drastic generic recircumscription and that the current classification based on life history type can not be supported. In order to further test the relationship between systematics and life history type, the South African taxa from the Cape Peninsula region were investigated. Two genera and six species are reported for the region: *Gymnogongrus* Martens for bi-phasic species characterized with tetrasporoblasts and *Ahnfeltiopsis* Silva et DeCew for tri-phasic species with internal cystocarps and heteromorphic life histories. The species include *A. glomerata* (J. Agardh) Silva et DeCew, *A. intermedia* (Kylin) Stegenga, Bolton et Anderson, *A. polyclada* (Kützting) Silva et DeCew, *A. complicata* (Kützting) Silva et DeCew, *A. vermicularis* (C. Agardh) Silva et DeCew, and *G. dilatatus* (Turner) J. Agardh (Stegenga *et al.*, 1997).

Anderson & Bolton (1990) have elucidated the life histories of four of these six taxa with interesting results. They noted that some populations of *A. complicata* from Hondeklipbaai, Northern Cape Province, were similar in habit to more southern populations from Noordhoek, Western Cape Province, except that the former possessed tetrasporoblasts in contrast to immersed cystocarps in the latter. These initial observations have prompted us to further evaluate the taxonomic identity of the same taxa studied by Anderson & Bolton (1990) using gene sequencing tools, and to assess their phylogenetic relationships and biogeographic patterns in a world-wide context.

## Materials & Methods

Algal samples from the South African west coast and Namibia (Benguela Marine Province, Fig. 1) used in the molecular studies were desiccated in silica gel in the field. Silica gel-dried specimens and extracted DNA samples of species worldwide are deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at  $-20^{\circ}\text{C}$ . Voucher specimens and materials for morphological studies were fixed in 10 % Formalin/seawater and stored in 5 % Formalin/seawater or pressed as herbarium sheets, and deposited in the Herbarium of the University of Louisiana

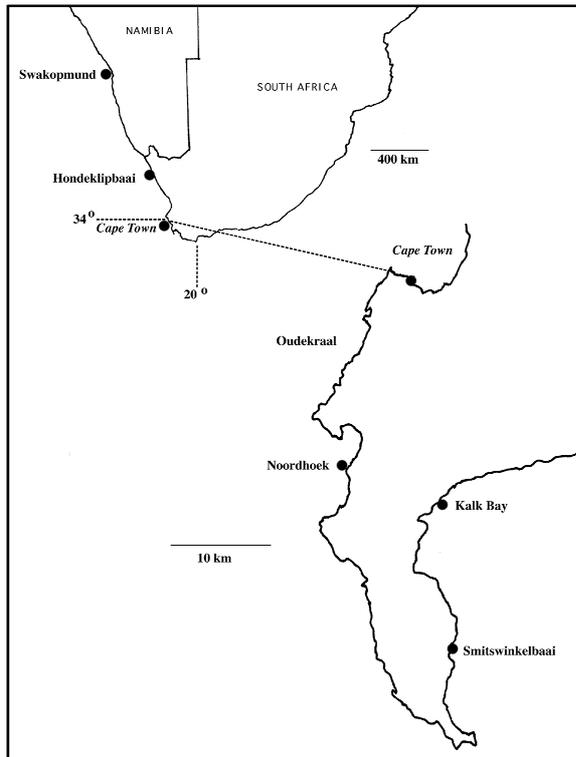


Figure 1. Map of Southern Africa and Cape Peninsula region.

at Lafayette (LAF) and in the Seaweed Research Unit, Marine Coastal Management in Rogge Bay, Republic of South Africa. Wherever possible, the same species selected for DNA sequence analysis have been included in the *rbcL*, ITS and LSU rDNA sequence analyses and are listed in the Appendix with their current names and *rbcL*<sup>+</sup>, LSU rDNA<sup>+</sup> and ITS<sup>#</sup> GenBank Accession Numbers.

DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA), or were submitted to a CTAB-Cesium Chloride DNA procedure (Freshwater & Rueness, 1994). Protocols for gene amplification and DNA sequencing are as described in Fredericq *et al.* (1999). The genetic markers selected to infer the phylogeny of the Phylloporaceae are 1) chloroplast-encoded *rbcL* (for list of primers see Freshwater & Rueness, 1994), 2) the nuclear-encoded large-subunit ribosomal RNA gene (LSU rDNA) (for list of primers see Freshwater *et al.*, 1999), and 3) the internal transcribed spacer regions (ITS) of nuclear ribosomal DNA (for list of

primers see Lindstrom *et al.*, 1996).

For gene amplification, 2  $\mu$ l of the resulting extractions were used as templates for a 50  $\mu$ l polymerase chain reaction consisting of 10  $\mu$ l 5M betaine, 6  $\mu$ l 10X PCR buffer (Perkin Elmer Corp.), 6  $\mu$ l 25 mM MgCl<sub>2</sub> solution, 8  $\mu$ l of 500mM dNTP stock, 2  $\mu$ l each of the appropriate primers at 10 mM, and 0.3-0.5  $\mu$ l Amplitaq<sup>®</sup> DNA Polymerase. Amplification conditions for both *rbcL*, LSU rDNA and ITS consisted of 4 minutes at 96 °C for denaturation, followed by 30 cycles of 60 seconds at 94 °C, 60 seconds at either 45 °C or 42 °C, and 90 seconds at 72 °C, with a final 10 minute extension cycle at 72 °C, and soak cycle at 4 °C. The amplification reactions were performed on a PE GeneAmp PCR system 9700 or 2400.

For automated gene sequencing, amplification products were cleaned of excess primer, enzyme and dNTPs by PEG precipitation. The sequences were determined over both strands using an ABI Prism 310 Genetic Analyzer or ABI 377 Automated Sequencer (PE Applied Biosystems, Foster City, CA) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA, USA). Reaction mixtures comprised 4  $\mu$ l Terminator Ready reaction mix with 4  $\mu$ l 5X buffer or 8 $\mu$ l Terminator Ready reaction mix, 1–2  $\mu$ l template, 3.2 pmol primer, and deionized water *q.s.* up to a total volume of 20  $\mu$ l. The cycle sequencing reactions were performed on a PE GeneAmp PCR system 9700 or 2400, for 25 cycles (96 °C for 10 seconds, rapid thermal ramp to 50 °C, 50 °C for 5 seconds, rapid thermal ramp to 60 °C, 60 °C for 4 minutes, rapid thermal ramp to 4 °C). Resulting products were then purified using Centri-Sep<sup>™</sup> spin columns (Princeton Separations P/N CS-901) following the manufacturer's instructions.

The generated sequence data were compiled and aligned with the software program Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and the contigs exported for phylogenetic analysis. Phylogenetic analyses of the *rbcL*, LSU rDNA and ITS sequence data were performed using the Minimum Evolution (Distance) Neighbor-Joining program with Kimura 2-parameter distances, and Maximum Parsimony algorithms available in the

## PHYLLOPHORACEAE FROM SOUTH AFRICA

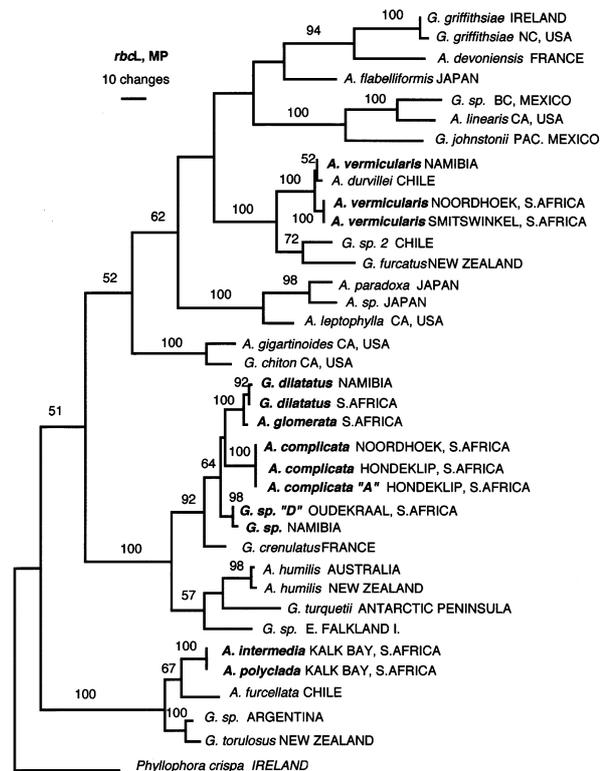
computer program PAUP (v.4.0b4a\*: Swofford 2000). Sequencher inserted gaps in the ITS and LSU rDNA contig alignments. Gaps were treated as a "fifth base". Parsimony heuristic searches, designed to increase the likelihood of swapping within the "island" of trees leading to the most parsimonious solution (Maddison, 1991), were performed. They consisted of 100 random stepwise additions, MULPARS (but holding only five trees at each step) and Tree-Bisection-Reconnection (TBR) swapping algorithm until swapping was complete. The searches were done on each data set under the criterion of equal weights for all substitutions. Consistency (CI) and retention (RI) indices (Kluge & Farris, 1989) were calculated excluding uninformative characters. Support for nodes of parsimony trees was determined by calculating bootstrap proportion values (BP) (Felsenstein, 1981) based on 100 resamplings of the heuristic searches done with random stepwise additions, MULPARS and TBR. Support for nodes in the Neighbor-Joining trees was based on 100 resamplings of Neighbor-Joining searches done with Kimura 2-parameter distances.

## Results

*RbcL* sequences were generated from 36 taxa of *Gymnogongrus* and *Ahnfeltiopsis*, ITS sequences from 33 taxa, and LSU rDNA sequences from 35 taxa; all three data sets included in addition the outgroup *Phyllophora crispa*, the type species of the Phylloporaceae (Fig.s 2–7).

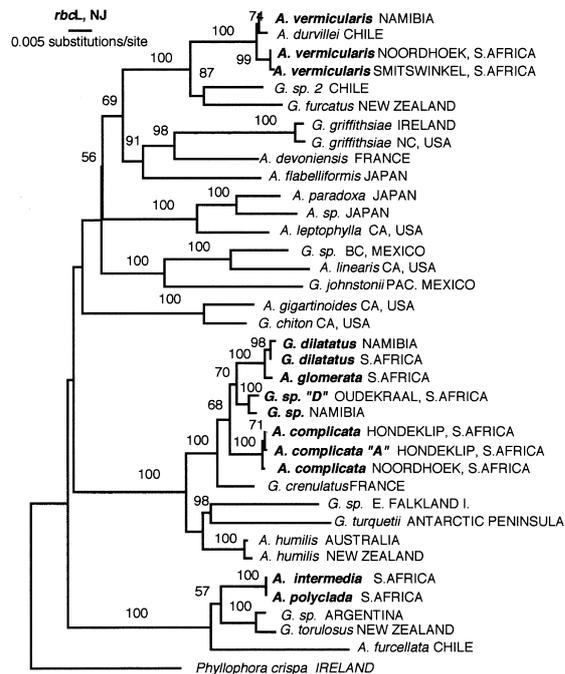
The *rbcL* sequence alignment consisted of 1,467 sites, but, because information was missing for the 5' ends of many sequences, the first 50 sites were excluded from the analyses. The analyzed data matrix included 1,417 sites total of which 340 were parsimony informative (24 %). The ITS sequence alignment consisted of a matrix that included 1,075 total and 412 parsimony informative sites (38 %). The LSU rDNA sequence alignment included 1,300 sites and 60 parsimony informative sites (4.6 %).

Parsimony and distance analyses obtained from multiple heuristic searches of the *rbcL*, ITS and LSU rDNA sequence alignments are each present-



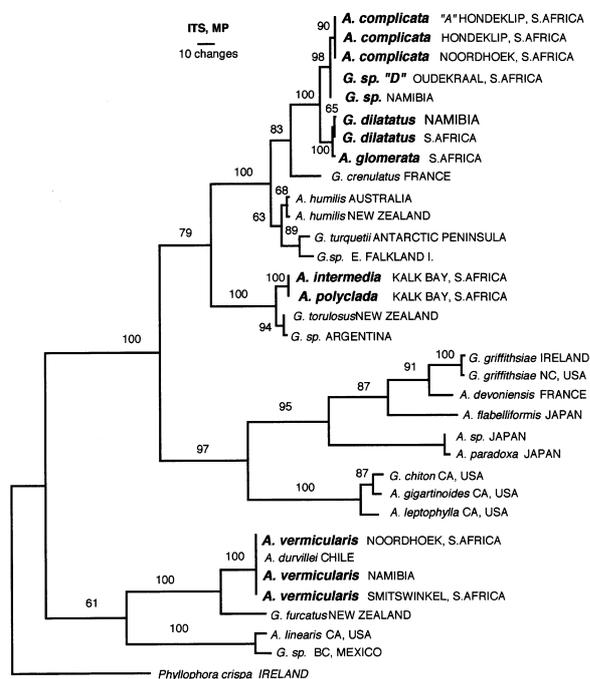
Figures 2. *RbcL* tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change. One of 7 equally most parsimonious trees. Tree length = 942 steps, CI = 0.47, RI = 0.78. Informative characters = 340 out of 1,417 included sites (24 %).

ed in a phylogenetic tree (Fig.s 2–7). Parsimony analysis of the *rbcL* sequence data set resulted in a strict consensus tree of seven equally minimal length trees of 942 steps, CI = 0.47, and RI = 0.78 with one of the trees shown in Fig. 2; a distance tree is shown in Fig. 3. Parsimony analysis of the ITS data set resulted in a strict consensus tree of 52 equally minimal trees of 1,116 steps, CI = 0.66, and RI = 0.86, with one tree shown in Fig. 4; a distance tree is shown in Fig. 5. Parsimony analysis of the LSU rDNA data set resulted in a strict consensus tree of 1,328 equally minimal trees of 126 steps, CI = 0.61, and RI = 0.85, with one tree shown in Fig. 6; a distance tree is shown in Fig. 7. Results of these three data sets are discussed together, listing bootstrap values (BP) from the *rbcL* (Maximum Parsimony [MP], Distance NJ) and ITS trees in the sequence: *rbcL* (Fig.s 2, 3) / ITS (Fig.s 4, 5) / LSU rDNA (Fig.s 6, 7). Only boot-



Figures 3. *RbcL* tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change.

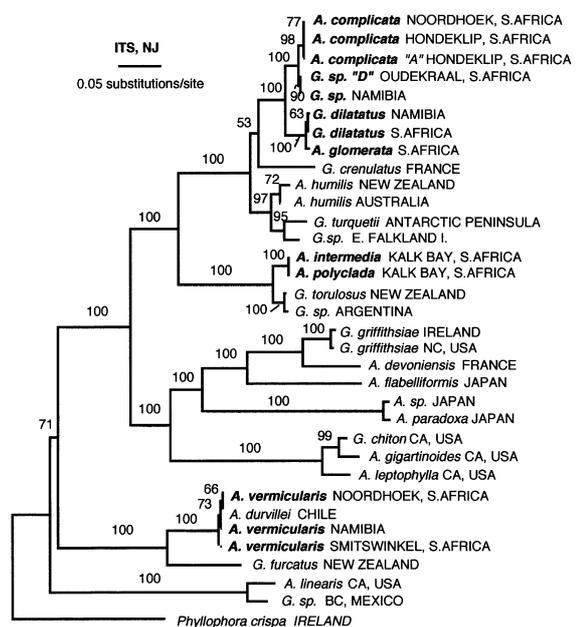
Neighbour-Joining Distance tree (Kimura-2 parameter)



Figures 4. ITS tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change.

One of 52 equally most parsimonious trees. Gaps are treated as 'fifth' base. Tree length = 1,116 steps, CI = 0.66, RI = 0.86. Informative characters = 412 out of 1,075 included sites (38 %). Informative characters = 412 out of 1,075 included sites (38.3 %).

PHYLLOPORACEAE FROM SOUTH AFRICA



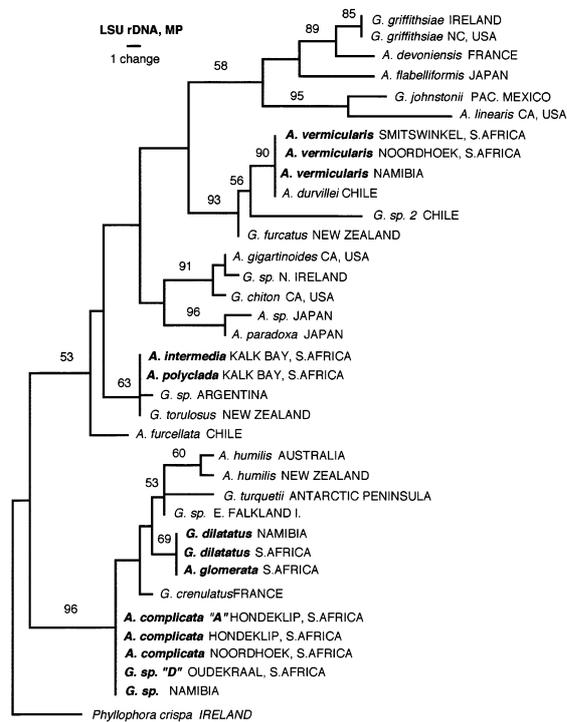
Figures 5. ITS tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change.

Neighbour-Joining Distance tree (Kimura-2 parameter)

strap values >50 from analyses of *Gymnogongrus* and *Ahnfeltiopsis* and the outgroup taxon are included.

The South African taxa were dispersed among various clades in the analyses of the three data sets. Members of a group of *A. complicata*, *G. dilatatus* and *A. glomerata* were consistently nested in a same clade showing strong bootstrap support. The cystocarpic *A. glomerata* is conspecific with *G. dilatatus*, as sequence divergence between it and *G. dilatatus* from Namibia (0.43 % / 0.33 % / 0.08 %) and South Africa (0.29 % / 0.29 % / 0.0 %) is minimal; the sequence divergence among *G. dilatatus* is 0.15 % / 0.11 % / 0.08 %. The tetrasporoblastic specimen of *A. complicata* from Hondeklipbaai is sufficiently genetically identical to the cystocarpic specimen from Noordhoek (the sequence divergence of *rbcL* = 0.07 %, of ITS = 0.0 % and of LSU rDNA = 0.0 %); however, an entity from Namibia and a deep-water population from Oudekraal are more closely related to one another (0.36 % / 0.0 % / 0.0 % sequence divergence) than to an intertidal population from Noordhoek (1.6 % / 0.31 % / 0.0 % sequence divergence) and

## PHYLLOPORACEAE FROM SOUTH AFRICA

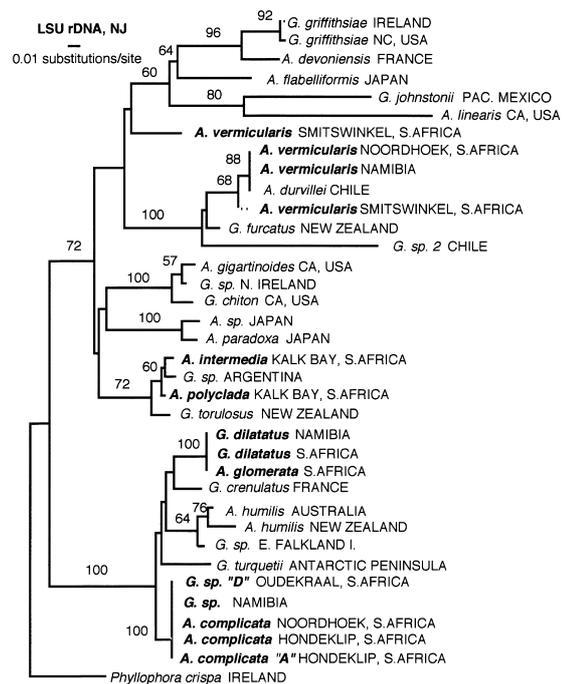


Figures 6. LSU rDNA tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change.

One of 1,328 equally most parsimonious trees inferred from LSU rDNA. Gaps are treated as 'fifth' base. Tree length = 126 steps, CI = 0.61, RI = 0.85. Informative characters = 60 out of 1,300 included sites (4.6 %).

Hondklipbaai (1.6 % / 0.31 % / 0.0 % sequence divergence). This strongly supported clade (BP 90, 100 / 100, 100 / 96, 100) also consistently contained *G. crenulatus* from Europe, *A. humilis* from Australia and New Zealand, *G. turquetii* from the Antarctic Peninsula, *G. antarcticus* from the Antarctic Peninsula, and an unnamed species from E. Falkland I. These taxa were basal to the more derived topological position of the western South African and Namibian assemblage containing the sister taxa *A. complicata*-*G. dilatatus* / *A. glomerata*.

A strongly supported clade (BP 100, 100 / 100, 100 / 93, 100) included the three collections of *A. vermicularis*, *A. durvillei* from Horcon, central Chile, *G. furcatus* from New Zealand and an unnamed species from Coquimbo, central Chile, that also goes under the name *A. durvillei*. The three populations of *A. vermicularis* were identical, but could vary from 1–4 steps from their topolog-



Figures 7. LSU rDNA tree. Bootstrap proportion values (>50 %) are shown above the nodes. Branch lengths are proportional to the amount of sequence change.

Neighbour-Joining Distance tree (Kimura-2 parameter)

ical position relative to one another if *A. durvillei* was included. They were identical if *A. durvillei* was removed from the analysis. When included, *G. furcatus* from New Zealand clustered with the unnamed species from Coquimbo, Chile. The large clade including *A. vermicularis* / *A. durvillei* is also sister to species from the Atlantic (*G. griffithsiae*, *A. devoniensis*) and from the eastern Pacific coast of Mexico and the USA, and Japan.

*Abnfeltiopsis intermedia* and *A. polyclada* clustered together with strong bootstrap support in a separate clade, and the two are conspecific. *A. intermedia* and *A. polyclada* are sister to *A. furcellata* from Chile which in turn is sister to *G. torulosus* from New Zealand, and a closely related unnamed species from Argentina.

The number of taxa included in these analyses was insufficient to resolve the generic concepts in the Phylloporaceae; however, the present circumscription of many of them is called into question and will be addressed in forthcoming papers. While most of the taxa identified here received

strong to moderate support in bootstrap replication analyses, the topological position of some species and genera remains equivocal.

## Discussion

Because the Phyllophoraceae exhibits such a wide range of life history types, each with its distinctive ecological adaptations, the family provides a model system for investigating the phylogenetic importance of life history traits as they relate to classification in this family and the Rhodophyta in general. Maggs *et al.* (1992) demonstrated that both heteromorphic and direct-type populations of *A. devoniensis* (as *G. devoniensis*) have almost identical DNA sequences in the RuBisCo spacer, suggesting that a high degree of genetic differentiation is not necessarily involved in the development of the direct type of life history. The results of our study likewise indicate that there is no high degree of genetic differentiation involved in the production of cystocarps versus tetrasporoblasts. Based on parsimony analyses of three molecular data sets, type of life history thus cannot be used as the character to separate the species from South Africa in either *Gymnogongrus* or *Ahnfeltiopsis*.

The South African west coast Phyllophoraceae have been well characterized morphologically by Stegenga *et al.* (1997). From the genetic similarity of the tetrasporoblastic *G. dilatatus* and the cystocarpic *A. glomerata* in both the *rbcL*, ITS and LSU rDNA sequence analyses, *A. glomerata* should be merged under *G. dilatatus*. As was already noted by Anderson & Bolton (1990) and Stegenga *et al.* (1997), the tetrasporoblastic and cystocarpic entities of *A. complicata* are identical in habit. In the *rbcL* sequence analyses, a specimen from Namibia was more closely related genetically to a specimen (sp. "D") from Oudekraal, Western Cape Province, than to other specimens from Oudekraal, Noordhoek (Western Cape Province) or Hondklipbaai (Northern Cape Province). *A. complicata* and *G. dilatatus* are closely related taxa. Anderson (pers. obs.) noted that *A. intermedia* grows directly above *A. polyclada* in mid-intertidal associated with fresh-water runoff in the type locality of Kalk Bay. *RbcL*, ITS and LSU rDNA

sequence analyses all indicate that the two species are conspecific. *A. intermedia* most likely is a stunted, miniature version of *A. polyclada* as a result of living in a stressful environment.

One entity going under the name *A. durvillei* from Horcon, central Chile, is virtually identical to *A. vermicularis* from South Africa and Namibia in all analyses, only differing with one another by 1–4 base pairs total. A re-examination of the lectotype of *A. durvillei* (Bory de Saint-Vincent) Silva et DeCew from Paita, Peru is called for, as several separate species are likely included under this name. If the type specimen corresponds to the Horcon material, then *Sphaerococcus vermicularis* C. Agardh 1817 would have taxonomic precedence over *Polyides durvillei* Bory de Saint Vincent 1828 [1826–1829].

The molecular analyses inferred from sequences of a chloroplast-encoded gene and two nuclear markers were largely congruent, providing a robust hypothesis of relationships. The two internal transcribed spacers of the nuclear ribosomal repeat, ITS-1 and ITS-2 and 5.8S ribosomal DNA (rDNA) are very highly conserved at population and species levels in a great number of organisms (Baldwin *et al.*, 1995; review in Hershkovitz & Zimmer, 1996). In red algae, this region accumulates mutations at a rapid rate, providing an amount of sequence variation suitable for comparisons at the species and subspecies levels in red algae (eg Goff *et al.*, 1994; Lindstrom *et al.*, 1996). Likewise, in this study of Phyllophoraceae, ITS sequence analysis has proven useful at estimating phylogenetic relationships among lineages at the same taxonomic levels as *rbcL*, with parsimony informative sites of 38 % versus 24 %, respectively. In contrast, the LSU rDNA data set contained only 4.6 % of parsimony informative sites, and hence the phylogenetic signal of LSU rDNA is not adequate to resolve most species relationships in this family. For other red algae, an analysis of LSU rDNA sequences from 13 species of red algae classified in 11 orders suggested that this gene may be useful in studies of higher-level relationships of red algae (Freshwater *et al.*, 1999). A comparison of *rbcL* and LSU rDNA analyses in the Delesseriaceae (Ceramiales) indicated that LSU rDNA was more

## PHYLLOPHORACEAE FROM SOUTH AFRICA

useful at estimating phylogenetic relationships among tribal lineages than did *rbcl* (Lin *et al.*, 2001).

The difficulty in resolving phylogenetically sound relationships in the Phylloporaceae has arisen because studies to date have relied on single or a few morphological characters that for the most part ignored ontogeny. By integrating a more extensive set of morphological characters rooted in an ontogenetic framework, with three sets of molecular data, the evolutionary histories of the Phylloporaceae worldwide should become more evident.

### *Biogeography of the South African Phylloporaceae*

Molecular-based phylogenies of marine red algae living today have started to shed light on possible paleo-biogeographical scenarios that help to explain extant distributions (eg Hommersand *et al.*, 1994, Lindstrom *et al.*, 1996; Fredericq & Ramírez, 1996; Fredericq *et al.*, 1999; Oppen *et al.*, 1995; Lin *et al.*, 2001; Hommersand & Fredericq, 2002). The ability to differentiate between the historic and recent biogeographic distribution patterns of the extant Phylloporaceae can only be enhanced from rigorous phylogenetic analyses encompassing many sequence data from a great number of taxa worldwide.

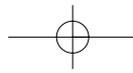
When investigating the antarctic species of the Phylloporaceae and their nearest relatives based on *rbcl* sequence analysis, Fredericq & Ramírez (1996) reached a conclusion of proposed pathways of migration and distribution of taxa as similar to the one presented for the Gigartinaceae by Hommersand *et al.* (1994) and first proposed by Hommersand in 1986. This conclusion is reinforced herein with the inclusion of more South African west coast and Namibian (Benguela Marine Province) Phylloporaceae in this study.

There are two main biogeographic patterns emerging for the west coast South African Phylloporaceae with separate distributions having taken place at three different times. One biogeographic pattern inferred from parsimony analysis involves three clades:

- 1) one group (*G. dilatatus*/*A. glomerata*, *A. complicata*) has its closest relationships with antiboreal S. Australian/New Zealand/Antarctic/Falkland Islands species. In accordance to Hommersand's model, these taxa have a southern hemisphere origin and were distributed from eastern Australasia to South Africa and South America across the antiboreal Pacific Ocean when there was an ice-free passage through western Antarctica at the end of the Cenozoic (Ciesielski *et al.*, 1982). The antarctic species occur basally, and one species (*G. crenulatus*) reached western Europe by amphitropical distribution.
- 2) a second group likewise comprises *A. polyclada*/*A. intermedia*, New Zealand, Argentinian and Chilean species. It is interesting to note that *A. polyclada* (as *G. polycladus*) has been reported from Tristan da Cunha in the mid-South Atlantic (Stegenga *et al.*, 1997), an island group lying at the subtropical convergence and thousands of km from the South African shore, and if the taxonomic identity is correct, may have been established there via long range dispersal from the South African west coast.
- 3) a third group encompasses *A. vermicularis*, and other species from Chile and New Zealand. In all three groups, the temperate S. Australian/New Zealand species are basal to the temperate western South African taxa.

The second major biogeographic pattern is reflected in the clades that appear to have evolved from an antiboreal origin but with species continuing to migrate northward along the coast of Pacific South and North America and reaching east Asia and Europe via boreal distribution. The phylogenetic trees shed light on how, for example, *Gymnogongrus griffithsiae* may have become established into the north Atlantic. *G. griffithsiae* which clusters with E. Pacific and Japanese taxa could have reached Europe via boreal distribution, rather than by way of amphitropical distribution that characterizes the establishment of *G. crenulatus*.

The addition of more taxa worldwide in the molecular data sets will reveal whether the trend continues of % sequence divergence values reflecting length and time of genetic separation remain-



ing higher among New Zealand and S. Australian taxa in accordance with Hommersand's (1986) biogeographic model. As in studies of the closely related Gigartinales (Hommersand *et al.*, 1994), the phylogenetic trees inferred from sequence analysis of three molecular datasets encompassing members of the Phylloporaceae were essentially equivalent to a hypothesis of relationships inferred from an interpretation of biogeographic hypotheses.

### Taxonomic Conclusion

As shown earlier (Fredericq & Ramírez, 1996), species with parasitic tetrasporophytes were genetically more closely related to certain cystocarpic members (eg *G. dilatatus*-*A. glomerata*; *A. complicata* [tetrasporoblastic] – *A. complicata* [cystocarpic]) than to other tetrasporoblastic species worldwide, and this study reinforces the notion that in the Phylloporaceae, a classification based on life history type cannot be supported.

Based on congruence of phylogenetic analyses inferred from three molecular data sets, we recognize herein four species for the South African west Coast and Namibia. Material known as *Gymnogongrus corymbosus* J. Agardh which has been included under *A. glomerata* (see Papenfuss, 1943; Stegenga *et al.*, 1997) but distinguished as a separate entity by others (eg Wynne, 1986) has not been investigated.

- 1) *Gymnogongrus dilatatus* (Turner) J. Agardh 1851: 326  
*Fucus dilatatus* Turner 1819: 59  
Type locality: Cape of Good Hope, South Africa  
Synonym: *Ahnfeltiopsis glomerata* (J. Agardh) Silva et DeCew 1992: 578  
*Gymnogongrus glomeratus* J. Agardh 1849: 88  
Type locality: Cape of Good Hope, South Africa.
- 2) *Ahnfeltiopsis complicata* (Kützinger) Silva et DeCew 1992: 577  
*Chondrus complicatus* Kützinger 1867: 17, pl. 58: fig.s a–d  
Type locality: Cape of Good Hope, South Africa.

- 3) *Ahnfeltiopsis polyclada* (Kützinger) Silva et DeCew 1992: 578  
*Chondrus? polycladus* Kützinger 1849: 737  
Type locality: Kalk Bay, Cape of Good Hope, South Africa.  
Synonym: *Ahnfeltiopsis intermedia* (Kylín) Stegenga, Bolton et Anderson 1997  
*Gymnogongrus intermedius* Kylín 1938: 12  
Type locality: Kalk Bay, Cape of Good Hope, South Africa
- 4) *Ahnfeltiopsis vermicularis* (C. Agardh) Silva et DeCew 1992: 578  
*Sphaerococcus vermicularis* C. Agardh 1817: xvii. *Fucus vermicularis* Turner 1815-1819: 61, pl. 221  
Type locality: unknown

### Acknowledgements

This study was supported in part by a grant from the National Science Foundation (DEB-9903900) and from the Louisiana Board of Regents (BoR 1999-2000-RD-A-50) to SF.

We thank our colleagues listed in the Materials section, especially Max Hommersand, for collecting and sending vouchers used in this study. We also thank Valerie Vreeland and two anonymous reviewers for their valuable comments.

### References

- Agardh C (1817). *Species Algarum* 1(2). Lund, 169–398.
- Agardh J (1849). Algologiska bidrag. *Öfversigt af Kongl. [Svenska] Vetenskaps-Akademiens Förhandlingar* 6: 79–89.
- Agardh J (1851). Species genera et ordines Floridearum 2(1). Lund, 1–351.
- Anderson RJ & Bolton JJ (1990). Reproductive morphology and life histories of southern African *Gymnogongrus* species (Rhodophyta, Phylloporaceae). *Br. Phycol. J.* 25: 381–390.
- Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF & Donoghue MJ (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* 82: 247–277.
- Bory de Saint-Vincent JBGM (1826–1829). Cryptogamie. In: *Voyage autour du monde.... La Coquille, pendant les années 1822, 1823, 1824 et 1825*. LI Duperrey (Ed), Paris., pls. 97–200 (1828).
- Ciesielski P, Ledbetter M & Ellwood B (1982). The development of Antarctic glaciation and the Neogene paleoenvironment of the Maurice Ewing Bank. *Mar. Geol.* 29: 39–53.
- Felsenstein J (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.



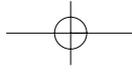
## PHYLLOPHORACEAE FROM SOUTH AFRICA

- Fredericq S & Ramírez ME (1996). Systematic studies of the antarctic species of the Phylloporaceae (Gigartinales, Rhodophyta) based on *rbcl* sequence analysis. *Hydrobiologia* **326/327**: 137–143.
- Fredericq S, Hommersand MH & Freshwater DW (1999). Observations on the phylogenetic systematics and biogeography of the Solieriaceae (Rhodophyta, Gigartinales) inferred from *rbcl* sequences and morphological evidence. *Hydrobiologia* **398/399**: 25–38.
- Freshwater DW & Rueness J (1994). Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcl* nucleotide sequence analysis. *Phycologia* **33**: 187–194.
- Freshwater DW, Fredericq S & Bailey CJ (1999). Characteristics and utility of nuclear-encoded large-ribosomal subunit gene sequences in phylogenetic studies of red algae. *Phycol. Res.* **47**: 33–38.
- Goff LJ, Moon DA & Coleman AW (1994). Molecular delineation of species and species relationships in the red algal agaro-phytes *Gracilariopsis* and *Gracilaria* (Gracilariales). *J. Phycol.* **24**: 521–537.
- Guiry MD (1987). The evolution of life history types in the Rhodophyta: an appraisal. *Cryptog. Algal.* **8**: 1–12.
- Guiry MD & Garbary GJ (1990). A preliminary phylogenetic analysis of the Phylloporaceae, Gigartinaceae and Petrocelidaceae (Rhodophyta) in the North Atlantic and North Pacific. In: *Evolutionary Biogeography of the Marine Algae of the North Atlantic*. Garbary DJ & South GR (Eds), Springer-Verlag, Berlin, 265–290 pp.
- Hershkovitz MA & Zimmer EA (1996). Conservation patterns in angiosperm rDNA ITS2 sequences. *Nucleic Acids Res.* **24**: 2857–2867.
- Hommersand MH (1986). The biogeography of the South African marine red algae: a model. *Bot. mar.* **24**: 257–270.
- Hommersand MH, Guiry MD, Fredericq S & Leister GL (1993). New perspectives in the taxonomy of the Gigartinaceae (Gigartinales). *Hydrobiologia* **260/261**: 105–120.
- Hommersand MH, Fredericq S & Freshwater DW (1994). Phylogenetic systematics and biogeography of the Gigartinaceae (Gigartinales, Rhodophyta) based on sequence analysis of *rbcl*. *Bot. mar.* **37**: 193–203.
- Hommersand MH & Fredericq S (this publ.). Biogeography of the red seaweeds of the South African west coast: a molecular approach. *Proc. Int. Seaweed Symp.* **17**.
- Kapraun DE, Dutcher JA, Bird KT & Capecchi MF (1993). Nuclear genome characterization and carrageenan analysis of *Gymnogongrus griffithsiae* (Rhodophyta) from North Carolina. *J. Appl. Phycol.* **5**: 99–107.
- Kluge AG & Farris JS (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Syst. Zool.* **38**: 7–25.
- Kützing FT (1849). *Species Algarum*. Leipzig, 922 pp.
- Kützing FT (1867). *Tabulae phycologicae* Vol. 17. Nordhausen [III+] 20 pp., 100 pp.
- Kylin H (1938). Verzeichnis einiger Rhodophyceen von Südafrika. *Lunds Univers. Årsskr.*, N. Avd. 2, **34**(8): 1–26.
- Kylin H (1956). *Die Gattungen der Rhodophyceen*. Gleerups, Lund. 673 pp.
- Lewis JA & Womersley HBS (1994). Family Phylloporaceae Nägeli 1947:248. In: *The Marine Benthic Flora of Southern Australia. Part IIIA Bangiophyceae and Florideophyceae*, Womersley HBS (Ed), Austr. Biol. Resources Study, Canberra. 259–270 pp.
- Lin S-M, Fredericq S & Hommersand MH (2001). Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbcl* sequences, including the Phycodryioideae, subfam. nov. *J. Phycol.* **37**: 000–000.
- Lindstrom SC, Olsen JL & Stam WT (1996). Recent radiation of the Palmariaceae (Rhodophyta). *J. Phycol.* **32**: 457–468.
- Maddison WP (1991). The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* **40**: 315–328.
- Maggs CA (1988). Intraspecific life history variability in the Florideophycidae (Rhodophyta). *Bot. Mar.* **31**: 465–490.
- Maggs CA (1990). Taxonomy of phylloporoid algae: the implications of life history. *Hydrobiologia* **204/205**: 119–124.
- Maggs CA, Douglas SE, Fenety J & Bird CJ (1992). A molecular and morphological analysis of the *Gymnogongrus devoniensis* (Rhodophyta) complex in the North Atlantic. *J. Phycol.* **28**: 214–232.
- Masuda M (1993). *Ahnfeltiopsis* (Gigartinales, Rhodophyta) in the western Pacific. *Jap. J. Phycol.* **41**: 1–6.
- Masuda M & Norris RE (1994). Introduction to Section IV. *Ahnfeltiopsis* (Rhodophyta, Phylloporaceae). In: *Taxonomy of Economic Seaweeds Vol. IV*, Abbott IA (Ed), Calif. Sea Grant Program, La Jolla. 149–158 pp.
- Masuda M, Kogame K & Guiry MD (1996). Life history of *Gymnogongrus griffithsiae* (Phylloporaceae, Gigartinales) from Ireland: implications for life history interpretation in the Rhodophyta). *Phycologia* **35**: 421–434.
- Newroth PR (1971). Studies on the life histories in the Phylloporaceae. I. *Phyllophora truncata* (Rhodophyceae, Gigartinales). *Phycologia* **10**: 345–354.
- Oppen, MJH van, Draisma SGA, Olsen JL & Stam WT (1995). Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: Evidence from nuclear rDNA ITS sequences. *Mar. Biol.* **123**: 179–188.
- Papenfuss GF (1943). Notes on South African algae II. *J. S. African Bot.* **9**: 79–92.
- Silva PC & DeCew TC (1992). *Ahnfeltiopsis*, a new genus in the Phylloporaceae (Gigartinales, Rhodophyceae). *Phycologia* **31**: 576–580.
- Stegenga H, Bolton JJ & Anderson RJ (1997). Seaweeds of the South African West Coast. *Contr. Bolus Herbarium* **18**: 655 pp.
- Swofford DL (2000). PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). version 4.0. Sinauer, Sunderland, MA.
- Turner D (1819). *Fuci*. Vol. 4. London, 153 + 7 pp., pl. 197–258.
- Wynne MJ (1986). Report on a collection of benthic marine algae from the Namibian coast (southwestern Africa). *Nova Hedwigia* **43**: 311–355.
- Wynne MJ & Heine JN (1992). Collections of marine red algae from St. Matthew and St. Lawrence Islands, the Bering Sea. *Nova Hedwigia* **55**: 55–97.

## Appendix

Species selected for DNA sequence analysis are listed below with their current names and *rbcL*<sup>†</sup>, LSU rDNA<sup>‡</sup> and ITS<sup>#</sup> GenBank Accession Numbers.

- Gymnogongrus chiton* (Howe) Silva Crissie Field, San Francisco, CA, USA, coll. MH Hommersand, 23.xii.1992 (†U21748, ‡AF3885613, #AF388656)
- Abnfeltiopsis complicata* (Kützing) Silva et DeCew, Noordhoek, Cape Peninsula, South Africa, coll. RJ Anderson, 27.i.1994 (†U21735, ‡AF388594, #AF388633)
- Abnfeltiopsis complicata* (Kützing) Silva et DeCew, Hondeklip Bay, Northern Cape Peninsula, South Africa, coll. RJ Anderson, 8.ii.1994, subtidal (†AF388555, ‡AF388591, #AF388632)
- Abnfeltiopsis complicata* (Kützing) Silva et DeCew (as sp. "A"), Hondeklip Bay, Northern Cape Peninsula, South Africa, coll. RJ Anderson, 9.i.1994, subtidal (†AF388556, ‡AF388590, #AF388630)
- Abnfeltiopsis complicata* (Kützing) Silva et DeCew (as sp. "D"), Oudekraal, Cape Peninsula, South Africa, coll. RJ Anderson, 5.i.1994 (†AF388553, ‡AF388587, #AF388629)
- Abnfeltiopsis complicata* (Kützing) Silva et DeCew, Swakopmund, Namibia, coll. MH Hommersand, 7.vii.1993 (†AF388554, ‡AF388592, #AF388630)
- Gymnogongrus crenulatus* (Turner) J. Agardh, Ile Verte, Roscoff, Brittany, France, coll. MH Hommersand, 22.vi. 1993 (†U22299, ‡AF388520, #AF388651)
- Abnfeltiopsis devoniensis* (Greville) Silva et DeCew, Ile Verte, Roscoff, Brittany, France, coll. J Cabioch, 22.vi. 1993 (†U21697, ‡AF3885617, #AF388652)
- Gymnogongrus dilatatus* (Turner) J. Agardh, Oudekraal, Cape Peninsula, South Africa, coll. RJ Anderson, 5.i.1994 (†AF388551, ‡AF388588, #AF388623)
- Gymnogongrus dilatatus* (Turner) J. Agardh, Swakopmund, Namibia, 5.vii.1993, MH Hommersand (†U21750, ‡AF388586, #AF388622)
- Abnfeltiopsis durvillei* (Bory) Silva et DeCew, Isla Negra, Prov. San Antonio, Chile, coll. S Fredericq & ME Ramírez, 23.ii. 1994 (†U21696, ‡AF3885606, #AF388635)
- Abnfeltiopsis flabelliformis* (Harvey) Masuda, Toji Beach, Chiba, Japan., coll. CA Maggs 26.v.1996 (†AF388571, ‡AF388598, #AF388646)
- Gymnogongrus furcatus* (Hooker f. et Harvey) Kützing, Timary Port, New Zealand, coll. W Nelson, 17.x. 1993 (†U22335, ‡AF3885602, #AF388637)
- Abnfeltiopsis furcellata* (C. Agardh) Silva et DeCew, Navidad, C. Chile, coll. S Fredericq & ME Ramírez, 17.i. 1995 (†AF388562, ‡AF3885605)
- Abnfeltiopsis gigartinoidea* (J. Agardh) Silva et DeCew, Pigeon Point, California, USA, coll. MH Hommersand, 21.xii. 1992 (†U21740, ‡AF3885614, #AF388649)
- Abnfeltiopsis glomerata* (J. Agardh) Silva et DeCew, Oudekraal, Cape Peninsula, South Africa, 5.i.1994, coll. RJ Anderson (†AF388552, ‡AF388596, #AF388634)
- Gymnogongrus griffithsiae* (Turner) Martius, Ireland, Bishop's Quarter Beach, Co. Clare, Ireland, coll. MD Guiry, 9.iv.1993 (†AF388567, ‡AF3885618, #AF388653)
- Gymnogongrus griffithsiae* (Turner) Martius, Fort Macon Jetty, North Carolina, USA, coll. MH Hommersand, 19.ix.1993 (†U22305, ‡AF3885619, #AF388654)
- Abnfeltiopsis humilis* (Lindauer) Lewis et Womersley, Victoria, S. Australia, s.d., coll. MD Guiry (†U21737U, ‡AF3885610, #AF388638)
- Abnfeltiopsis humilis* (Lindauer) Lewis et Womersley, Pilot's Beach, Dunedin, New Zealand, coll. WA Nelson, 6.xi.1993 (†AF388566, ‡AF3885611, #AF388639)
- Abnfeltiopsis intermedia* (Kylin) Stegenga, Bolton et Anderson, Kalk Bay, Cape Peninsula, South Africa, coll. RJ Anderson, 11.ii.1994 (†AF388557, ‡AF388584, #AF388625)
- Gymnogongrus johnstonii* (Setchell et Gardner) Dawson, Puerto Escondido, Papanao, Guerrero, Pac. Mexico, coll. M Cordeiro Marino, 26.x.1993 (†U21749, ‡AF3885615)
- Abnfeltiopsis leptophylla* (J. Agardh) Silva et DeCew, Pigeon Point, CA, USA, coll. MH Hommersand 21.xii.1992 (†U21742, #AF388655)
- Abnfeltiopsis linearis* (C. Agardh) Silva et DeCew, Pigeon point, CA, USA, coll. MH Hommersand, 21.xii.1992 (†U21741, ‡AF3885600, #AF388650)
- Abnfeltiopsis paradoxa* (Suringar) Masuda, Kominato, Chiba, Japan, coll. M Yoshizaki (†AF388568, ‡AF388597, #AF388648)
- Abnfeltiopsis polyclada* (Kützing) Silva et DeCew, Kalk Bay, Cape Peninsula, South Africa, coll. RJ Anderson, 11.ii.1994 (†AF388558, ‡AF388595, #AF388628)
- Gymnogongrus torulosus* (Hooker f. et Harvey) Schmitz, Cable Bay, Doubtless Bay, New Zealand, coll. W Nelson, 30.xi.1993 (†U22336, ‡AF3885603, #AF388636)
- Gymnogongrus turquetii* Hariot, Bahía Fildes, King George I., S. Shetland Is, Antarctic Peninsula, coll. S Fredericq & ME Ramírez, 13.ii.1994 (†U27019, ‡AF3885610, #AF388642)
- Abnfeltiopsis vermicularis* (C. Agardh) Silva et DeCew, Smitswinkel Bay, False Bay, Cape Peninsula, South Africa, coll. RJ Anderson, 20.iii.1994 (†AF388560, ‡AF388589, #AF388624)
- Abnfeltiopsis vermicularis* (C. Agardh) Silva et DeCew, Hondeklip Bay, Northern Cape Peninsula, South Africa, coll. RJ Anderson, 8.ii.1994 (†AF388559, ‡AF388593, #AF388627)
- Abnfeltiopsis vermicularis* (C. Agardh) Silva et DeCew, Swakopmund, Namibia, coll. MH Hommersand, 7.vii.1993 (†U22300, ‡AF388585, #AF388626)
- G. sp.* Murroran, Hokkaido, Japan, coll. S Fredericq, 6.ix.1993 (†AF388569, ‡AF388599, #AF388647)
- G. sp. 2*, La Herradura, Coquimbo, Chile, coll. S Fredericq & ME Ramírez, 19.i.1995 (†AF388564, ‡AF3885608, #AF388641)
- G. sp.*, Rookery Bay, E. Falkland Island, coll. S Fredericq & MH Hommersand, 4.i.1998 (†AF388563, ‡AF3885601, #AF388643)
- G. sp.* Mar del Plata, Argentina, coll. J Estevez, 28.iv.1999 (†AF388561, ‡AF3885604, #AF388644)
- G. sp.*, La Bufadora, Punta Banda, Pacific Baja California, Mexico, coll. J Hughey, 6.vii.1996 (†AF388570, ‡AF3885609, #AF388645)
- G. sp.*, Strangford Lough, Co. Down, N. Ireland, coll. CA Maggs, 15.xii.1992 (†AF3885616)
- Phyllophora crispa* (Hudson) Dixon, Spiddall, Co. Galway, Ireland, coll. MD Guiry, 7.iii.1993 (†U02990, ‡AF388583, #AF388621)



PHYLLOPHORACEAE FROM SOUTH AFRICA

