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

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Abstract

The family Phyllophoraceae 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 Phyllophoraceae.

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

Introduction

The Phyllophoraceae, together with the closely related family Gigartinae, form the Gigartinae-complex in the Gigartinales, one of Kylin’s (1956) core orders. The Phyllophoraceae is especially intriguing for comparison with the Gigartinae, 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 Gigartinae (Hommersand et al., 1993), cystocarp morphology within the Phyllophoraceae 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 Phyllophoraceae 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,
3) a bi-phasic type of life history lacking carposporophytes but with wart-like tetrarospophytes – called tetrarospoblasts or carpotetrarospophytes – 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 Phyllophoraceae.

Life history type has figured prominently in the generic classification of the Phyllophoraceae, 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, i.e. 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 tetrarospoblasts, 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 tetrarospoblastic 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, i.e. in specialized outgrowths (e.g. Phyllophora, Ozophora), or restricted to the medial line of branches (Stenogramme).

A preliminary rbcL gene sequence analysis of 38 taxa of Phyllophoraceae (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 tetrarospoblasts 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 (Kützing) Silva et DeCew, A. complicata (Kützing) 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 tetrarospoblasts 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 °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 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+, LSU rDNA+ and ITS# 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 & Ruiness, 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 Phyllophoraceae are 1) chloroplast-encoded rbcL (for list of primers see Freshwater & Ruiness, 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 µl of the resulting extractions were used as templates for a 50 µl polymerase chain reaction consisting of 10 µl 5M betaine, 6 µl 10X PCR buffer (Perkin Elmer Corp.), 6 µl 25 mM MgCl₂ solution, 8 µl of 500mM dNTP stock, 2 µl each of the appropriate primers at 10 mM, and 0.3-0.5 µl Amplitaq® 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 µl Terminator Ready reaction mix with 4 µl 5X buffer or 8µl Terminator Ready reaction mix, 1–2 µl template, 3.2 pmol primer, and deionized water q.s. up to a total volume of 20 µl. 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™ 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
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 (Figs. 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-
ed in a phylogenetic tree (Figs. 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, RI = 0.78. Informative characters = 340 out of 1,417 included sites (24 %).

Figure 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 %).
Figure 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)

Figure 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%).

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 Hondklelipbaai (1.6% /
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 topological 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. griffithsiæ, A. devoniensis*) and from the eastern Pacific coast of Mexico and the USA, and Japan.

*Ahnfeltiopsis 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 Phyllophoraceae; 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

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**Figure 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%).

**Figure 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).
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 *rbc*L, 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 *rbc*L 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. *Rbc*L, 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 from 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 *rbc*L, 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 *rbc*L and LSU rDNA analyses in the Delesseriaceae (Ceramiales) indicated that LSU rDNA was more
useful at estimating phylogenetic relationships among tribal lineages than did *rbcL* (Lin *et al.*, 2001).

The difficulty in resolving phylogenetically sound relationships in the Phyllophoraceae 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 Phyllophoraceae worldwide should become more evident.

**Biogeography of the South African Phyllophoraceae**

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 Phyllophoraceae 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 Phyllophoraceae 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) Phyllophoraceae in this study.

There are two main biogeographic patterns emerging for the west coast South African Phyllophoraceae 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 with 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 south 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 reflect-
ing length and time of genetic separation remaining higher among New Zealand and S. Australian taxa in accordance with Hommersand’s (1986) biogeographic model. As in studies of the closely related Gigartinaceae (Hommersand et al., 1994), the phylogenetic trees inferred from sequence analysis of three molecular datasets encompassing members of the Phyllophoraceae 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 Phyllophoraceae, 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 (e.g. 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* (Kützing) Silva et DeCew 1992: 577
*Chondrus complicatus* Kützing 1849: 737
Type locality: Cape of Good Hope, South Africa.

3) *Ahnfeltiopsis polyclada* (Kützing) Silva et DeCew 1992: 578
*Chondrus? polycladus* Kützing 1849: 737
Type locality: Kalk Bay, Cape of Good Hope, South Africa.
Synonym: *Ahnfeltiopsis intermedia* (Kylin) Stegenga, Bolton et Anderson 1997
*Gymnogongrus intermedius* Kylin 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

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**References**


Appendix

Species selected for DNA sequence analysis are listed below with their current names and rbcL, LSU rDNA and ITS GenBank Accession Numbers.

Gymnogongrus chiton (Howe) Silva Crissie Field, San Francisco, CA, USA, coll. MH Hoomersand, 23.xii.1992 (U21748, AF3885613, AF388656)

Ahnfeltiopsis complicata (Kützing) Silva et DeCew, Noordhoek, Cape Peninsula, South Africa, coll. RJ Anderson, 27.i.1994 (U21735, AF388594, AF388633)

Ahnfeltiopsis complicata (Kützing) Silva et DeCew, Kalk Bay, Cape Peninsula, South Africa, coll. RJ Anderson, 8.i.1994, subtidal (AF388555, AF388591, AF388632)

Ahnfeltiopsis 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)

Ahnfeltiopsis complicata (Kützing) Silva et DeCew (as sp. "D"), Oudekraal, Cape Peninsula, South Africa, coll. RJ Anderson, 5.i.1994 (AF388553, AF388587, AF388629)

Ahnfeltiopsis complicata (Kützing) Silva et DeCew, Swakopmund, Namibia, coll. MH Hoomersand, 7.vii.1993 (AF388554, AF388592, AF388630)

Gymnogongrus crenulatus (Turner) J. Agardh, Ile Verte, Roscoff, Brittany, France, coll. MH Hoomersand, 22.vi.1993 (U22299, AF388520, AF388651)

Ahnfeltiopsis devoniensis (Greville) Silva et DeCew, Ile Verte, Roscoff, Brittany, France, coll. J Cabioch, 22.vi.1993 (U21607, 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 Hoomersand (U21750, AF388586, AF388622)

Ahnfeltiopsis dureillei (Bory) Silva et DeCew, Isla Negra, Prov. San Antonio, Chile, coll. S Fredericq & ME Ramírez, 23.i.1994 (U21606, AF388506, AF388635)

Ahnfeltiopsis 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)

Ahnfeltiopsis furcellata (C. Agardh) Silva et DeCew, Navidad, C. Chile, coll. S Fredericq & ME Ramírez, 17.i.1995 (AF388562, AF388650)

Ahnfeltiopsis gigartinoidea (J. Agardh) Silva et DeCew, Pigeon Point, California, USA, coll. MH Hoomersand, 21.xii.1992 (U21740, AF3885614, AF388649)

Ahnfeltiopsis glomerata (J. Agardh ) Silva et DeCew, Oudekraal, Cape Peninsula, South Africa, 5.i.1994, coll. RJ Anderson (AF388552, AF388596, AF388634)

Gymnogongrus griffithiae (Turner) Martius, Ireland, Bishop’s Quarter Beach, Co. Clare, Ireland, coll. MD Guiry, 9.iv.1993 (AF388567, AF3885618, AF388653)


Ahnfeltiopsis humilis (Lindauer) Lewis et Womersley, Victoria, S. Australia, s.d., coll. MD Guiry (U21737U, AF3885610, AF388638)

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