Constancea 83, 2002
University and Jepson Herbaria
P.C. Silva Festschrift

Characterization and Phylogenetic Position of the Red Alga *Besa papillaeformis* Setchell: An Example of Progenetic Heterochrony?

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Land's End: Gilbert Munger (ca. 1870). With permission of M.D. Schroeder and North Point Gallery

ABSTRACT

In order to elucidate the taxonomic placement of the enigmatic red alga *Besa papillaeformis* Setchell, fragments from topotype material collected by W.A. Setchell in 1912 were sectioned for morphological observation, and processed for chloroplast−encoded *rbc*L sequencing. The morphological and molecular analyses confirm that *Besa* is a member of the Phyllophoraceae, Gigartinales, in the *Ahnfeltiopsis paradoxa*/*Mastocarpus* clade. The multiple diminutive axes of *B. papillaeformis*, each up to 0.4 mm tall borne on a fleshy−cartilaginous epilithic crust, typically resemble the erect juvenile stages of the Phyllophoraceae, as well as the reproductive papillae of *Mastocarpus*. Heterochrony, in particular progenesis — the process by which sexual maturity is accelerated relative to somatic development — is inferred as the mechanism for the evolutionary origin of *Besa*, in which perennating crusts give rise to uprights that become reproductive while remaining miniaturized. The isomorphic Besa−type life cycle encompasses proterandrous monoecy (and possibly self−fertilization), and is one more example of the

wide range of life history types characteristic of the Phyllophoraceae.

INTRODUCTION

Besa papillaeformis is an enigmatic red alga described by Setchell in 1912 from cystocarpic specimens collected at Land's End, San Francisco, California. The new genus was diagnosed as being an epiphyte or hemiparasite, thallus elongate−papillate or clavate, simple or slightly lobed, bright red, with internal cystocarps composed of clusters of gonimoblast cells separated by large vegetative cells nearly completely filling the thallus interior. An ostiole was lacking and male structures and tetrasporangia were not seen (Setchell 1912: 236, pl. 25, figs. 5–6). The species was characterized by fleshy−cartilaginous fronds, each up to 0.45 mm high and 0.38 mm broad, growing epiphytically or as hemiparasites in groups or scattered on crusts that resemble *Hildenbrandia*. Abbott and Hollenberg (1976) later added that the cartilaginous crusts may reach a width of up to 3 cm and consist of firmly adjoining, erect filaments bearing several upright axes, each not taller than 1 mm. They made no mention of the possibility of *Besa papillaeformis* being an epiphyte or hemiparasite, so presumably they recognized the crust as being part of the alga rather than as a basiphyte.

B. papillaeformis has only been reported, besides the type locality, from Pacific Grove and Cypress Point (Monterey Co., California) growing on rocks in the intertidal to subtidal (to 14 m) (Abbott and Hollenberg 1976).

Morphological information is scant; originally placed in the Gigartinaceae by Setchell, the genus was subsequently transferred to the Phyllophoraceae by Kim (1976) upon examination of the type specimen of *B. papillaeformis* (UC 173542) which showed a relatively large sterile cell on the first cell of the carpogonial branch and the absence of enveloping tissue surrounding gonimoblast filaments. Spermatangial and tetrasporangial structures were unknown until DeCew reported them in Setchell's topotype material (DeCew and West 1981); in a footnote DeCew and West (1981) reported that “Re−examination of the type material of *Besa papillaeformis* Setchell and Gardner, the type of the genus, reveals that the tetrasporophyte and gametophyte are isomorphic (DeCew, unpublished observations). We believe that *Besa* belongs to the Gigartinaceae due to the filamentous nature of the medulla”. In contrast, McCandless et al. (1982) supported its transfer to the Phyllophoraceae based on polysaccharide chemistry. Guiry and Garbary (1990) concurred that *Besa* may belong in the Phyllophoraceae.

Besides *B. papillaeformis*, two other species of *Besa* have been described. *Besa stipitata* Hollenberg &Abbott was described from Monterey Bay, California, and has also been recorded from the San Juan Islands, Washington (Hollenberg and Abbott 1968, Abbott and Hollenberg 1976) where it is likewise a rare species growing subtidally on rocks. A third species, *Besa gracilis* Yamada was transferred to *Ahnfeltia* by Yamada and Mikami (Mikami 1965) and to *Ahnfeltiopsis* by Masuda (1993). In these three species a fleshy crust produces diminutive axes that bear internal cystocarps lacking carpostomes.

In order to further characterize and elucidate the taxonomic placement of *Besa papillaeformis*, fragments of material collected by Setchell on February 1, 1912 (UC173490) were newly sectioned for morphological observation, and sequenced for chloroplast−encoded *rbc*l. The *rbc*L sequence was included in a large *rbc*L dataset of both Gigartinaceae (Hommersand et al. 1994, 1999, unpubl. data) and Phyllophoraceae worldwide (Fredericq and Ramírez 1996, Fredericq et al. 2002, unpubl. data). The algae are listed as they are currently known in the literature or for which existing names are available. New combinations are not provided in this study.

MATERIALS AND METHODS

Fragments of Setchell's 1912 air−dried herbarium specimens of *Besa papillaeformis* from the type locality were hand−sectioned with a razor blade, stained with 1% aniline blue and mounted in 30% karo/distilled water on microscope slides. Nine photographic slides made by Dr. T.C. DeCew were borrowed from UC.

Other algal samples used in molecular studies were desiccated in silica gel or air−dried in the field. Specimens and extracted DNA samples were deposited in the Seaweed Laboratory at the University of Louisiana at Lafayette and stored at −20° C. Species selected for DNA sequence analysis are listed with their current names and *rbc*L GenBank Accession Numbers in Table 1. PCR and sequencing *rbc*L primers used in this study are listed in Freshwater and Rueness (1994) and Hommersand et al. (1994).

For gene amplification, 2 µl of the resulting extractions was 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 *rbc*L 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 (Hillis et al. 1996). The sequences were determined over both strands using an ABI Prism 3100 Genetic Analyzer (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 2.5X 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 sec., rapid thermal ramp to 60°C, 60°C for 4 min, 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 Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Phylogenetic analyses were performed using the Maximum Parsimony algorithms available in the computer program PAUP (v.4.0b10*, Swofford 2002). Parsimony heuristic searches, designed to increase the likelihood of swapping within the ‘island’ of trees leading to the most parsimonious solution consisted of 5000 random stepwise additions, MULPARS (but holding only 5 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 1989) were calculated excluding uninformative characters. Support for nodes (Felsenstein 1985) of parsimony trees was assessed by calculating 5000 bootstrap resamplings of the heuristic searches based on random stepwise additions, MULPARS and TBR.

RESULTS

Multiple unbranched uprights arise from fleshy cartilaginous crustose bases (Figs. 1–2). Each erect upright is unbranched or with few irregular branches, cylindrical (Fig. 2) or irregularly globose (Figs. 1, 3–4) and is shorter than 1 mm in length. Some cystocarpic uprights are topped by a large ostiolar region (Fig. 5). A longitudinal section through the crust and the uprights reveals that the boundary between them is not abrupt (Figs. 7, 10). Cells composing the crust are isodiametric except directly beneath the uprights, where they are vertically elongated (Figs. 8, 9). Crustal filaments are uniform in diameter and close fitting with little intercellular spaces (Figs. 8, 9), with cells from different cell files linked by secondary pit connections. Uprights are initiated from the surface of the crust as dome−shaped bulges (Fig. 10). These dome−shaped structures consist of vertically elongated dedifferentiated apical cells and their derivatives (Figs. 11–12). The medulla is variously and loosely compact, composed of longitudinal filaments slightly radiating thallus outward (Figs. 6–7, 10, 11–14). As the upright expands in width, the intercellular spaces enlarge.

Actively growing surface cells divide in half by longitudinal concavo−convex division (Figs. 11, 12). In addition, in rapidly growing upright thalli, successive transverse divisions may occur between longitudinal divisions resulting in multiple unbranched segments between branch−bearing segments (Fig. 13). Below the surface of the erect thallus and throughout the medullary region, intercalary cells cut off conjunctor cells laterally which fuse with neighboring cells and

form secondary pit connections (Figs. 11–14, 15, 16). A conjunctor cell is initiated as a bulge in the middle or towards the base of an intercalary cell and fuses horizontally or obliquely with a neighboring intercalary cell. The conjunctor cells range in shape from fusiform extensions to hemispherical cells. Secondarily formed vegetative cells in turn may also cut off conjunctor cells (Figs. 11–14). The recipient cell typically possesses a lateral extension formed by the flow of cytoplasm into the fused conjunctor cell (Fig. 14).

The successive concavo−convex division pattern characterizing vegetative growth also occurs in the formation of spermatangial parent cells (Figs. 15–19). A vegetative cortex can be readily distinguished from a spermatangial cortex, in that in the latter secondary pit connections do not form in the distal−most six cells of a filament (Fig. 16), whereas in the former, secondary pit connections form nearly to the surface. Spermatangial parent cells are elongated and occur in pairs (Figs. 16–19) and each cuts off a spermatangium (Fig. 19). Spermatangia were found on the surface of uprights not bearing procarps and on the surface of uprights bearing procarps (Fig. 21), suggesting that the life history involves proterandrous monoecy. Multiple young unfertilized procarps are scattered in the cortical region of the uprights (Figs. 20–24). They are darkly staining and each consists of a supporting cell bearing a three−celled carpogonial branch. The supporting cell typically enlarges, with the first cell of the carpogonial branch becoming lens−shaped and lying directly above the supporting cell. The second cell is small and cut off at right angles, and bears a small carpogonium with the tip extending in a club−shaped trichogyne (Figs. 23–24). The first cell of the carpogonial branch bears a rounded sterile cell distally. All cells of unfertilized or abortive carpogonial branches typically enlarge (Fig. 22) before reverting to vegetative growth.

Fertilization was not observed. The earliest presumed post−fertilization stage is the initiation of gonimoblast filaments that grow in all directions amongst the medullary cells (Fig. 25) in the vicinity of degenerating procarps. The region immediately surrounding the supporting/auxiliary cells and small rounded gonimoblast filaments is not transformed cytologically into a specialized nutritive tissue (Figs. 27–28). The gonimoblast filaments continue spreading across the medulla (Fig. 29) and the medullary cells are initially regular in shape when there are few gonimoblasts; however, they become progressively more irregular as more gonimoblasts are cut off (Fig. 28). The auxiliary cell seemingly degenerates quickly and becomes undistinguishable from normal vegetative cells. Gonimoblasts may form chains of 4 or 5 cells long before linking to a neighboring medullary cell (Fig. 29). Fusiform conjunctor cells extending from gonimoblast cells fuse with vegetative cells (Fig. 29). The small chains of gonimoblasts become carposporangial initials and are indistinguishable from one another (Figs. 27–28). As portions of the gonimoblast cells continue to fuse with vegetative cells and as carposporangia increase in size, the vegetative cells stretch and become stellate to accommodate cytocarp expansion. Pit plugs among clusters of gonimoblasts may become dislodged to one side, resulting in partial fusion of cells in the same gonimoblast filament (Fig. 29, top). Although it may seem that secondary crops of carposporangia arise from vegetative cells, all carposporangia arise from primary gonimoblast filaments (Fig. 30). Gonimoblasts (Figs. 27, 28) and carposporangia (Fig. 26, 31) all mature at the same time and are the same size. Vegetative cells in old cystocarps eventually collapse in diameter (Figs. 30–31). Cystocarps occupy half or one third of the interior of an upright, and are surrounded by a multilayer cortex. There is no distinct boundary between the vegetative medulla and the carposporangial region. Secondarily formed vegetative filaments are not formed around the developing gonimoblasts. Carposporangia are apparently released simultaneously as a result of the disintegration of secondary pit connections surrounding the carposporophytic network. The carposporophytic network is excised and released in its entirety through a terminal ostiolar region (Fig. 32).

*Rbc*L sequences were generated from 51 representatives of the Phyllophoraceae in addition to two Gigartinaceae, the sister group of the Phyllophoraceae. The *rbc*L alignment consisted of 1467 base pairs but because information was missing for the 5' ends of many sequences the first 39 sites were excluded from the analyses. The analyzed data matrix included a total of 1428 sites of which 437 were parsimony−informative (30.6%). The parsimony analysis obtained from multiple heuristic searches is presented in a phylogenetic tree (Fig. 33) and resulted in a consensus tree of 10 equally most parsimonious trees of 1722 steps, CI = 0.36, and RI = 0.68.

Besa papillaeformis consistently clustered with strong bootstrap support in the clade containing *Ahnfeltiopsis paradoxa*, *A. leptophylla* and *Mastocarpus*.

DISCUSSION

Besa papillaeformis has the following morphological features that are characteristic of the Phyllophoraceae: the first cell of the carpogonial branch bears a sterile cell; cystocarps are internal; spermatangial parent cells are elongated; the medulla consists of isodiametric cells that may stretch becoming locally filiform; multiple secondary pit connections form between vegetative cells (e.g., Maggs 1990). Although we did not find tetrasporangia, DeCew had previously examined fragments from the same material we studied and made photographic slides of squashed papillae showing cruciately divided tetraspongia (data not shown). Unfortunately, it could not be determined with confidence whether the tetrasporangia are in a catenate series, as in most genera of the Phyllophoraceae, or positioned singly in an intercalary position, as in *Mastocarpus*, a genus transferred from the Gigartinaceae initially to the Petrocelidaceae, and then to the Phyllophoraceae. If tetrasporangia of *Besa papillaeformis* are indeed borne in uprights, then the tetrasporophyte is not the heteromorphic crust that is characteristic of many Phyllophoraceae.

It is not known whether the crustose base arises from the germination and expansion of a single spore, or whether it instead is the result of sporeling coalescence from several individuals (Maggs and Cheney 1990) that may produce genetically polymorphic chimeras. Coalescence of neighboring crusts has been demonstrated for several Phyllophoraceae and it is a adaptive phenomenon in red algae (summarized in Santelices et al. 1999).

In mature thalli of the Phyllophoraceae medullas are typically composed of isodiametric cells, but in juvenile thalli they may consist of slender, elongated cells. Such cells may stretch in length (as in *Mastocarpus*), or expand in width (as in most other Phyllophoraceae). In *B. papillaeformis* the presence of both elongated and isodiametric cells in the same part of the medulla is common. Medullary and cortical cells do not cut off secondary filaments but throughout the thallus they produce multiple elongated or hemispherical conjunctor cells that fuse with neighboring cells establishing secondary pit connections. Likewise, gonimoblast conjunctor cells fuse to vegetative cells that do not become cytologically modified into a nutritive tissue (for a review of this process throughout the red algae see Hommersand and Fredericq 1990). These medullary cells become stellate due to fusion of multiple conjunctor cells and subsequent stretching to accommodate cystocarp expansion. Because secondary production of carposporangia from medullary cells was not seen, it is unlikely that the gonimoblasts originate apomictically, as they often do in the family (e.g., Maggs 1988, Maggs et al. 1992). Reproduction in *Besa papillaeformis* is assumed to be sexual on the basis of the presence of spermatangia and tetrasporangia, even though stages showing gonimoblast initials cut off from auxiliary cells were not seen.

Analysis of *rbc*L sequences shows *B. papillaeformis* to be nested, with strong support, in a clade composed of *Ahnfeltiopsis paradoxa* from Japan, an unnamed species from Japan, and *A. leptophylla* from California; this clade in turn is sister to *Mastocarpus*. Morphological characters consistent with the grouping based on *rbc*L analysis include a basal, perennial crust characterized by tightly adhering isodiametric cell files with numerous secondary pit connections, and similarity of diminutive uprights with erect juvenile stages and marginal papillae (see DeCew and West 1981, Guiry and West 1983, Masuda 1987, Masuda et al. 1997).

One of the processes postulated to play an important role in evolution is heterochrony, the phenomenon of change in the relative rate or timing of specific developmental events in a descendant relative to its ancestor (Gould 1977, McKinney and McNamara 1991, Friedman and Carmichael 1998, Li and Johnston 2000). Relatively simple developmental or genetic changes can give rise to significant phenotypic alterations in descendants (Gould 1977, 1992; Alberch et al. 1979). Heterochronic processes involve six possible mechanisms (summarized in Porras and Muñoz 2000); one category encompasses paedomorphosis, the retention of the ancestral juvenile shape in the descendant adult (Gould 1977, Guerrant 1988). Two processes of paedomorphosis that result in traits produced by truncated development are progenesis, in which sexual maturity is accelerated relative to somatic development, and neoteny, in which somatic development slows and is overtaken by normal sexual maturity (see Gould 1977, Alberch et al. 1979, McKinney and McNamara 1991, Porras and Muñoz 2000). Both progenesis and neoteny are relatively simple processes that may play a major role in morphological evolution (Gould 1977). References to heterochrony in algae are scant (Feldmann 1952, Cabioch 1969, Magne 1972, Mann 2000). Yet, these processes may be much more widespread in algae than are currently appreciated. We envision the

situation in *Besa papillaeformis* in which precocious sexual maturation has been evolutionarily fixed in a putative morphological juvenile stage as the possible consequence of progenesis.

It has already been suggested (Ardré 1978, Maggs 1988, Fredericq and Ramírez 1996) that in the Phyllophoraceae tetrasporoblasts, which occur in taxa that are monoecious, are seemingly the result of self−fertilization. It is also well accepted that self−pollination in higher plants in geographically or ecologically marginal environments assures reproductive success in habitats where pollinators are scarce; an alternative hypothesis is that selfing arises as a by−product in marginal environments where selection may favor individuals that can complete reproduction early, in advance of impending stress (Runions and Geber 2000). One way of achieving early reproduction is through a decrease in developmental time, both of individual organs and of whole organisms. In the case of *Besa*, the decrease of developmental time would thus be at the level of the juvenile upright. Progenesis is closely related to the r−strategy in adaptation (Gould 1977) and viewed as an adaptive strategy to varying environmental conditions (Masuyama 1996). Progenesis, like apomixis might enable populations to survive in ecologically marginal environments, such as close to the distribution limit of a species.

The Phyllophoraceae contains more variety in life−history patterns than any other family in the Rhodophyta: some species have an alternation of generations in which tetrasporophytes and gametophytes are vegetatively similar, with carposporophytes parasitic on gametophytes—so−called triphasic alternation of isomorphic generations; others have a triphasic alternation of heteromorphic generations, with the tetrasporophyte being a crust; others have a a biphasic cycle in which the zygote gives rise not to a carposporophyte, but to a filamentous structure that produces tetraspores—the tetrasporoblast; others have a biphasic cycle in which female gametophytes produce carposporophytes apomictically (e.g., Maggs 1990). Life history type has traditionally been considered an important character at the level of genus. Eleven genera have been characterized mainly on the basis of one to a few characters such as type of life history and position of reproductive structures (e.g., Guiry and Garbary 1990).

Those species with life history patterns different from that of the generitype have been transferred to other genera: some species of *Gymnogongrus* lacking tetrasporoblasts but with internal cystocarps and heteromorphic life histories were transferred to *Ahnfeltiopsis* (Silva and DeCew 1992); the heteromorphic species *Phyllophora traillii* Holmes &Batters was transferred to *Erythrodermis* (Guiry and Garbary 1990), and the tetrasporoblastic species *P. truncata* (Pallas) A. Zinova was transferred to *Coccotylus* (Wynne and Heine 1992), leaving in *Phyllophora* only those members undergoing an alternation of isomorphic generations.

However, phylogenies inferred from DNA sequence analysis do not support classifications of the Phyllophoraceae based on life history patterns. In *Gymnogongrus devoniensis* both heteromorphic and direct−type populations have been found Maggs et al. (1992). The populations that differed in life history showed 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. Fredericq and Ramírez (1996) and Fredericq et al. (2002) have shown that species with parasitic tetrasporoblasts were genetically more closely related to certain cystocarpic members than to other tetrasporoblastic species. A recircumscription of the genera in the Phyllophoraceae that does not place weight on life history characters will be addressed in forthcoming papers (Fredericq et al., in prep).

The *Besa*−type life cycle encompasses isomorphy and proterandrous monoecy (and self−fertilization?), and is one more example of the wide range of life history types characteristic of the Phyllophoraceae. At this time we cannot ascertain whether *Besa* is a form genus (such as *Ahnfeltiopsis*, *Coccotylus*, and *Erythrodermis*), or a taxonomically valid genus. DNA sequences of additional species, namely *Besa stipitata* Hollenberg &Abbott from California and Washington, USA, *B. gracilis* Yamada from Japan, and *Gymnogongrus crustiformis* Dawson (1961: 248. pl. 24, fig. 2) described from Oaxaca, Pacific Mexico, and illustrated in Abbott and Hollenberg (1976, fig. 452) for southern California specimens, are urgently needed. These species all share an expanded fleshy−cartilaginous crustose base bearing diminutive, simple, erect, cylindrical and papillate axes, and internal cystocarps lacking carpostomes.

If *Besa* is proved to be generically distinct, the genus may include the heteromorphic *A. paradoxa*, an unnamed species from Japan, and perhaps *A. leptophylla* from California, and be the sister group of *Mastocarpus*. It will be interesting to find out whether *Besa*, being isomorphic, is basal in that assemblage, in analogy to the *Phyllophora* clade that contains both isomorphic (*Phyllophora*), heteromorphic ("*Erythrodermis*") and tetrasporoblastic ("*Coccotylus*") members, and in which *Phyllophora* has a basal topology. Furthermore, culture studies of new field collections of *Besa papillaeformis* and allied species should shed light on whether or not their expanded crustose bases are the result of spore coalescence.

ACKNOWLEDGEMENTS

We dedicate this paper to Dr. Paul Silva in honor of his 80th birthday. We thank D. Moe for critically reviewing the manuscript, and our colleagues, R. Anderson, J. Cabioch, J. Estevez, M. Guiry, M. Hommersand, G. Kraft, C. Maggs, W. Nelson, J. Norris, N. Phillips, M.E. Ramírez, D. Rodriguez, J. Rueness, B. Wysor, M. Yoshizaki, for sending vouchers used in this study. This study was supported by NSF grant DEB9903900 to SF.

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Table 1. List of species, their collection information, and the *rbc*L GenBank accession numbers followed by *rbc*L fraction (in %) sequenced.

Entity	Collection data	GenBank #	Source
Gymnogongrus chiton (Howe) Silva	Crissy Field, San Francisco, California, USA, coll. M.H. Hommersand, 23.xii.1992	U21749 97.6%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus chiton (Howe) Silva	Bahia Colnett, Baja California, Mexico, coll. M.H. Hommersand, 3.vii.1996	AY135160 98.0%	This paper
Ahnfeltiopsis concinna (J. Agardh) Silva & DeCew	Susaki, Shimoda−shi, Shizuoka Pref., Honshu, Japan, Coll. T. Tanaka, 17.vi.1985	U22301 96.3%	Fredericq and Ramírez 1996
			This paper

Ahnfeltiopsis concinna (J. Agardh) Silva & DeCew	Guerrero, Pac. Mexico, coll. D. Rodriguez, s.d.	AY135161 92.8%	
Gymnogongrus crenulatus (Turner) J. Agardh	Ile Verte, Roscoff, Brittany, France, coll. M.H. Hommersand, 22.vi.1993	U22299 96.7%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus crenulatus (Turner) J. Agardh	Williamstown, Victoria, Australia, coll. G.T. Kraft, 28.iv.95	AY135157 99.5%	This paper
Ahnfeltiopsis devoniensis (Greville) Silva & DeCew	Ile Verte, Roscoff, Brittanny, France, coll. J. Cabioch, 22.vi.1993	U21697 98.0%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus dilatatus (Turner) J. Agardh	Oudekraal, Cape Peninsula, South Africa, coll. R.J. Anderson, 5.i.1994 (tetrasporoblastic)	U21748 94.0%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus dilatatus (Turner) J. Agardh [incl. *A. glomerata*]	Oudekraal, Cape Peninsula, South Africa, coll. R.J. Anderson, 5.i.1994 (cystocarpic)	AF388552 100%	Fredericq et al. 2002
Ahnfeltiopsis divaricata (Holmes) Masuda	Kaalawei, Oahu, Hawaii, USA, coll. N. Phillips 13.iv.2001	AY35163 96.7%	This paper
Ahnfeltiopsis durvillei (Bory) Silva & DeCew	Isla Negra, Prov. San Antonio, Chile, coll. S. Fredericq and M.E. Ramírez, 23.ii.1994	U21696 97.8%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis flabelliformis (Harvey) Masuda	Toji Beach, Chiba, Japan. Coll. C. A. Maggs, 26.v.1996	AF388571 98.0%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus furcatus (Hooker f. & Harvey) Kützing	Timary Port, New Zealand, coll. W. Nelson, 17.x.1993	U22335 93.2%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis furcellata (C. Agardh) Silva & DeCew	Navidad, C. Chile, coll. S. Fredericq and M.E. Ramírez, 17.i.1995	AF388562 94.2%	Fredericq et al. 2002
Ahnfeltiopsis gigartinoides (J. Agardh) Silva & DeCew	Pigeon Point, California, USA, coll. M.H. Hommersand, 21.xii.1992	U21740 98.0%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis gigartinoides (J. Agardh) Silva & DeCew	Seabrook, Oregon, USA, coll. S. Fredericq 17.v.1999	AY135158 98.0%	This paper
Gymnogongrus griffithsiae (Turner) Martius	Praia de Peruibe, Sao Paulo State, Brasil, coll. M. Cordeiro Marino, 28.i.1994	AY135158 98.4%	This paper
Ahnfeltiopsis humilis (Lindauer) Lewis & Womersley	Victoria, S. Australia, s.d., coll. M.D. Guiry	U22306 96.8%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahfeltiopsis humilis (Lindauer) Lewis & Womersley	Pilot's Beach, Dunedin, New Zealand, coll. W.A. Nelson, 6.xi.1993	AF388566 96.8%	Fredericq et al. 2002
Gymnogongrus johnstonii (Setchell & Gardner) Dawson	Puerto Escondido, Papanoa, Guerrero, Pac. Mexico, coll. M. Cordeiro Marino, 26.x.1993	U21749 98.6%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis leptophylla (J. Agardh) Silva & DeCew	Pigeon Point, California, USA, coll. M.H. Hommersand, 21.xii.1992	U21742 96.7%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis linearis (C. Agardh) Silva & DeCew	Pigeon Point, California, USA, coll. M. H. Hommersand, 21.xii.1992	U21741 97.8%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus martinensis Setchell & Gardner	La Butadon, Baja California, Mexico, coll. M.H. Hommersand, 6.vii.1996	AY135162 93.1%	This paper
Ahnfeltiopsis paradoxa (Suringar) Masuda	Kominato, Chiba, Japan, coll. M. Yoshizaki	AF388568 98.2%	Fredericq et al. 2002

Ahnfeltiopsis polyclada (Kützing) Silva & DeCew	Kalk Bay, Cape Peninsula, South Africa, coll. R.J. Anderson, 11.ii.1994	AF388584 97.9%	Fredericq et al. 2002
Ahnfeltiopsis polyclada (Kützing) Silva & DeCew [incl. *A. intermedia*]	Kalk Bay, Cape Peninsula, South Africa, coll. R.J. Anderson, 11.ii.1994	AF388558 96.7%	Fredericq et al. 2002
Gymnogongrus torulosus (Hooker f. & Harvey) Schmitz	Cable Bay, Doubtless Bay, New Zealand, coll. W. Nelson, 30.xi.1993	U22336 96.3%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus torulosus (Hooker f. & Harvey) Schmitz	Mar del Plata, Argentina, coll. J. Estevez, 28.iv.1999	AF388561 96.8%	Fredericq et al. 2002
Gymnogongrus turquetii Hariot	Bahía Fildes, King George I., S. Shetland Is, Antarctic Peninsula, coll. S. Fredericq and M.E. Ramírez, 13.ii.1994	U27019 96.9%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Ahnfeltiopsis vermicularis (C. Agardh) Silva & DeCew	Swakopmund, Namibia, coll. M.H. Hommersand, 7.vii.1993.	U22300 96.5%	Fredericq and Ramírez 1996 Fredericq et al. 2002
Gymnogongrus sp.	Murroran, Hokkaido, Japan, coll. S. Fredericq, 6.ix.1993	AF388569 98.2%	Fredericq et al. 2002
Gymnogongrus sp.	Rookery Bay, E. Falkland Island, coll. S. Fredericq and M.H. Hommersand	AF388563 100%	Fredericq et al. 2002
Gymnogongrus sp.	La Herradura, Coquimbo, Chile, coll. S. Fredericq and M.E. Ramírez, 19.i.1995	AF388564 97.7%	Fredericq et al. 2002
Gymnogongrus sp.	Strangford Lough, Co. Down, N. Ireland, coll. C.A. Maggs, 15.xii.1992	AY135170 98.3%	This paper
Besa papillaeformis Setchell	Topotype, Land's End, San Francisco, California, USA, coll. W.A. Setchell #6485, 1.ii.1912, UC172490	AY135156 62.8%	This paper
Gigartina pistillata (S.G. Gmelin) Stackhouse	Santec, Brittany, France, coll. J. Cabioch, 6.iv.1993	U03429 90%	Hommersand et al. 1994, 1999 Hommersand and Fredericq 2002
Mastocarpus papillatus (C. Agardh) Kützing	Horsehoe Cove, Bodega Bay, California, USA, coll. M.H. Hommersand, 22.x.1992	AY135154 100%	This paper
Mastocarpus pacificus (Kjellman) Perestenko	Oshoro, Hokkaido, Japan, coll. S. Fredericq and K. Kogame, 5.ix.1992	AY135155 98.3%	This paper
Mastocarpus stellatus (Stackhouse) Guiry	Bally Castle, Co. Antrim, Ireland, coll. C.A. Maggs, 20.i.1992	U02992 100%	Fredericq and Ramírez 1996
Ozophora clevelandii (Farlow) Abbott	Fort Point, San Francisco, California, USA, coll. J.A. West, 12.iii.1994	U21851 97.9%	Fredericq and Ramírez 1996
Ozophora norrissii Abbott	Washington, USA, coll. B. Wysor	AY135164 83.4%	This paper
Petroglossum pacificum Hollenberg	Isla Negra, Prov. San Antonio, C. Chile, coll. S. Fredericq and M.E. Ramírez, 26.i.1994	U22337 99.3%	Fredericq and Ramírez 1996
Petroglossum pacificum Hollenberg	La Bocca, Navidad, C. Chile, coll. S. Fredericq and M.E. Ramírez, 17.i.1995	AY135167 96.7%	This paper
Phyllophora crispa (Hudson) Dixon	Spiddall, Co. Galway, Ireland, coll. M.D. Guiry, 7.iii.1993	U02990 96.8%	Fredericq and Ramírez 1996 Fredericq et al. 2002
			This paper

Phyllophora heredia (Clemente) J. Agardh	Le Trez Hir, Brittanny, France, coll. J. Cabioch, 27.02.1994,	AY135165 97.9%	
Phyllophora pseudoceranoides (Gmelin) Newroth & Taylor	Spidall, Co. Galway, Ireland, coll. M.D. Guiry, 7.iii.1993	U22307 97.9%	Fredericq and Ramírez 1996
Phyllophora traillii Holmes ex Batters [*Erythrodermis traillii* (Holmes ex Batters) Guiry & Garbary]	Strangford Narrows, Co. Down, N. Ireland, UK, coll. C.A. Maggs, 17.i.1994	U21852 97.8%	Fredericq and Ramírez 1996
Phyllophora truncata (Pallas) Zinova [*Coccotylus truncatus* (Pallas) Wynne & Heine]	Drobak, Norway, coll. J. Rueness, 10.iv.1994	AY135167 97.9%	This paper
Sarcothalia crispata (Bory) Leister	Playa San Antonio, Bahía de Ancud, Chiloé, Chile, coll. M.E. Ramírez, 14.v.1993	U03085 99%	Hommersand et al. 1994, 1999
Schottera nicaeensis (Lamx. ex Duby) Guiry & Hollenberg	France, 2.viii.1993, coll. M.D. Guiry	U22309 97.7%	Fredericq and Ramírez 1996
Stenogramme californica Harvey	Pigeon Point, California, USA, coll. M.H. Hommersand, 21.xii.1992	U27020 99.3%	Fredericq and Ramírez 1996 (as *S. interrupta*)
Stenogramme interrupta (C. Agardh) Montagne	Brittanny, France, coll. M.H. Hommersand	AY135168 97.3%	This paper
Stenogramme rhodymenioides Joly & Alveal	Horcón, Prov. Valparaiso, Chile, coll. S. Fredericq and M.E. Ramírez, 27.ii.1994	AY135169 97.3%	This paper