MORPHOLOGICAL AND MOLECULAR ANALYSES OF *POLYSIPHONIA SENSU LATO* IN SOUTHERN CENTRAL AMERICA AND THE CARIBBEAN

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ABSTRACT

Molecular assisted identification using plastid-encoded rbcL and mitochondria-encoded COI loci identified 24 species of Polysiphonia sensu lato from 59 samples collected from Panama, Florida, and Caribbean Mexico. Morphological character states were examined and used to identify each sample at the rank of species. Phylogenetic relationships among these species were estimated using maximum likelihood analyses of rbcL and nuclear-encoded SSU sequence data. Panama samples include ten newly reported species, seven species for which the available morphological character states would not allow the positive application of names, and one new species, Polysiphonia nuda sp. nov. Florida and Caribbean Mexico samples include five species, two of which are previously unreported for Caribbean Mexico. Descriptions of species morphology and remarks on taxonomy and relationships are provided.
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CHAPTER ONE: Morphological and Molecular Assessment of *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae) in Panama
ABSTRACT

Only three species of *Polysiphonia* have been reported from the Caribbean and Pacific coasts of Panama. In contrast, 13 *Polysiphonia sensu lato* species are documented from the neighboring countries of Costa Rica and Colombia. Molecular assisted identification using plastid-encoded *rbcL* and mitochondria-encoded COI loci identified 19 species of *Polysiphonia sensu lato* from 43 samples collected from the Caribbean and Pacific coasts of Panama. Morphological character states were examined and used to identify each sample at the rank of species. Phylogenetic relationships among these species were estimated through maximum likelihood analyses of *rbcL* and nuclear-encoded SSU sequence data. Ten newly reported species and seven species for which available morphological character states would not allow the positive application of names were identified. A new species, *Polysiphonia nuda* sp. nov., is described from the Caribbean coast. A key to the Panamanian species, descriptions of species morphology and remarks on taxonomy and relationships are provided. These findings demonstrate that previous limited reports of *Polysiphonia* species from Panama resulted from a lack of study, rather than a lack of diversity, within the region.
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INTRODUCTION

*Polysiphonia* Greville (Rhodomelaceae) is one of the largest red algal genera with c. 200 currently recognized species (e.g. Kim *et al.* 2002). These species are common members of marine algal floras and have a nearly global distribution (e.g. Hollenberg 1968a, 1968b; Womersley 1979; Wynne 1998, 2009). *Polysiphonia* species exhibit a wide range of morphological variability, and this has led to much debate as to how species should be defined and classified. *Polysiphonia sensu lato* (s.l., “in the broad sense”) includes species that are predominantly placed in two genera: *Neosiphonia* M.S. Kim & I.K. Lee and *Polysiphonia*. A smaller number of *Polysiphonia s.l.* species are also placed in the genera *Boergeseniella* Kylin, *Bryocladia* F. Schmitz in Engler & Prantl, *Enelittosiphonia* Segi, and *Vertebrata* S.F. Gray.

Species of *Polysiphonia s.l.* are generally characterized by a delicate habit composed of filamentous, segmented main axes and branches. Axes are polysiphonous and are constructed of several cohering longitudinal columns of cells (pericentral cells) surrounding a central axis where the pericentral cells are the same length as cells of the central axis (Hine 1976; Littler & Littler 2000). Species are typically identified by variation among characters such as rhizoid attachment, number of pericentral cells, cortication, structure and arrangement of trichoblasts, origin of branches in relation to trichoblasts, tetraspore arrangement, structure and development of the spermatangial branches, and number of carpogonial branch cells (Abbott 1999; Hollenberg 1968a; Hollenberg & Norris 1977; Kapraun 1980a; Kapraun & Norris 1982; Kim *et al.* 2002; Schneider & Searles 1991). Stuercke & Freshwater (2008) examined North Carolina, USA species of *Polysiphonia s.l.* to determine which morphological characters have the same character state in all samples of the same species (i.e. consistent characters) and can therefore be utilized in the identification of *Polysiphonia s.l.* species. Twenty-two morphological characters
commonly used for identifying *Polysiphonia s.l.* species were examined. Five of the characters were observed to have consistent character states within species, and another six, although not perfectly consistent, were taxonomically useful in combination with other characters.

Molecular assisted identification (MAI) has also proven useful for discriminating species of *Polysiphonia s.l.* McIvor *et al.* (2001) demonstrated the utility of the plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*) for species identifications in their study of the *Neosiphonia harveyi* (J. Bailey) M.S. Kim, H.G. Choi, Guiry & G.W. Saunders (as *Polysiphonia harveyi* J. Bailey) invasion of the British Isles and Atlantic Europe. Cultured isolates of *N. harveyi* were successfully interbred to examine levels of morphological and *rbcL* DNA sequence variation within a single biological species. The single greatest observed intraspecific *rbcL* sequence divergence was 2.13% with most intraspecific comparisons differing by ≤ 1.3%. Interspecific sequence divergence was greater among species closely related to *N. harveyi* with values ranging from 4.05% to 13.10%. Kim *et al.* (2004) compared *rbcL* gene sequence data from *Polysiphonia morrowii* Harvey and found intraspecific variation to be 0.1-0.2%, further demonstrating low variability of *rbcL* sequences within the same species. Interspecific sequence divergence of species closely related to *P. morrowii* ranged from 3.07-14.12% (Kim *et al.* 2004). Kim & Yang (2005) also examined *rbcL* sequence divergence in species of *Polysiphonia*. Intraspecific *rbcL* sequence divergence was observed to be 0.03% and 0-0.1% for samples of *P. morrowii* and *Polysiphonia stricta* (Dillwyn) Greville, respectively. Interspecific *rbcL* sequence divergences of 2.6-14% were observed among species closely related to *P. morrowii* and *P. stricta* (Kim & Yang 2005). High interspecific variability combined with low intraspecific variability make *rbcL* a useful tool for helping to define *Polysiphonia* species.
Another form of MAI is DNA barcoding—“sequencing a short, diagnostic segment to discriminate between species” (Robba et al. 2006). The 5’ end of the mitochondria-encoded cytochrome c oxidase subunit I (COI) has been proposed as the barcoding locus for red algae. COI has several advantages for barcoding including limited recombination, low prevalence of indels, uniparental inheritance, and a relatively rapid rate of evolution (Hebert et al. 2003; Saccone et al. 1999; Saunders 2005). These characteristics allow for discrimination of closely related species as well as geographic groups within a species (Hebert et al. 2003; Robba et al. 2006; Saunders 2005). However, the utility of COI barcoding within Polysiphonia s.l. has not been established.

Other DNA sequence data can be used to determine the phylogenetic relationships of species within Polysiphonia s.l. The nuclear-encoded 18S rDNA (SSU) exhibits limited intraspecific sequence variation, making this locus appropriate for exploring taxonomic relationships above the species level. Choi et al. (2001) used a combination of morphological and SSU sequence data to evaluate the monophyly of Polysiphonia s.l. and resolved the included Polysiphonia s.l. species in three strongly supported clades referred to as the Polysiphonia, Neosiphonia, and “multipericentral” groups. The rbcL locus exhibits a rate of evolution faster than that of SSU, making it appropriate for determining species level relationships. The use of rbcL has been relatively common in recent phylogenetic studies of Polysiphonia s.l. (Kim et al. 2004, 2005; McIvor et al. 2001; Stuercke & Freshwater 2008, 2010). Thorough inference of phylogenetic relationships at different taxonomic levels within Polysiphonia s.l. can be made when both SSU and rbcL are analyzed (Stuercke 2006).

Studies of the marine algal flora (MAF) throughout much of Central America are limited in number and in Panama have included only opportunistic or brief concerted efforts (Dawson
Recent attempts to increase the knowledge of the MAF in Panama have included intensive sampling to better estimate biodiversity. These efforts have resulted in reports that indicate substantially more marine algal species than previously documented for the country (Wysor 2004; Wysor & De Clerck 2003; Wysor & Kooistra 2003; Wysor et al. 2000, 2009). This study is part of a greater collaborative effort to survey and inventory the MAF of the Caribbean and Pacific coasts of Panama.

The first report of *Polysiphonia* from Panama comes from Hollenberg in Taylor (1945) and was the result of opportunistic sampling while en route to and from the Galapagos. Hollenberg identified *Polysiphonia howei* Hollenberg from Isla Taboga in the Bay of Panama on the Pacific side of the country. Dawson used SCUBA to study marine algae in Panama for the first time in 1959 during a sampling cruise to Pacific Mexico and Central America (Earle 1972). Dawson’s Pacific Panama samples of *Polysiphonia* from Isla Brincancon in the Gulf of Chiriqui and Isla del Rey in the Gulf of Panama were identified by Hollenberg (1961) as *Polysiphonia flaccidissima* Hollenberg. Earle (1972) made several collecting trips to various sites along the Caribbean and Pacific coasts of Panama from 1965 to 1971. These visits resulted in reports of *P. howei* and a *Polysiphonia sp.* from the Caribbean localities of Colón and Holandés Cay, respectively. Earle (1972) also cited Hollenberg’s (in Taylor 1945) report of *P. howei* from Pacific Panama.

Although only three *Polysiphonia* species have been previously documented for Panama (Earle 1972; Hollenberg1961; Taylor 1945), 13 species have been reported from the neighboring countries of Colombia and Costa Rica (Dawson 1962; Hollenberg1961; Kapraun et al. 1983; Schnetter & Bula Meyer 1982; Taylor 1945, 1960). The low Panamanian species counts may
indicate limited biological diversity or a lack of study. Examination of the Panamanian flora is necessary to accurately determine *Polysiphonia s.l.* species numbers. The purpose of this study was to complete integrated molecular and morphological analyses of *Polysiphonia s.l.* in Panama. MAI and examination of morphology were used to identify species of *Polysiphonia s.l.* within Panamanian samples, and phylogenetic analyses of *rbcL* and SSU sequence data were used to determine the relationships among these species.

**MATERIAL AND METHODS**

**Collection**

*Polysiphonia* samples were collected from sites located along the Pacific and Caribbean coasts of Panama (Appendix 1; Fig. 1). Samples were collected from intertidal or subtidal substrates by snorkeling or SCUBA diving and dried in silica gel dessicant (Chase & Hills 1991). Samples were grouped into genetic species by MAI (see below; Clarkston & Saunders 2010; Yang *et al.* 2008), and identified to morphological species based on examination of morphological characters and use of taxonomic guides (Abbott 1999; Abbott & Hollenberg 1976; Adams 1991; Børgesen 1918; Dawes & Mathieson 2008; Dawson 1964; Hollenberg 1942, 1958, 1961, 1968a, 1968b; Hollenberg & Norris 1977; Kapraun unpublished manuscript; Kapraun 1977, 1980a; Kapraun & Norris 1982; Kapraun *et al.* 1983; Littler & Littler 2000; Schneider & Searles 1991; Sestchell & Gardner 1930; Taylor 1945, 1960; Womersley 1979, 2003). Permanent slide vouchers were made as in Tsuda & Abbott (1985) and deposited in the University of North Carolina-Wilmington (WNC) herbarium. Silica dried samples were deposited into the silica collection at the Center for Marine Science. New species vouchers were

**Morphological Data and Analysis**

Specimens and slides were observed using an Olympus SZH dissecting microscope (Olympus America Inc., Center Valley, PA, USA) and a Nikon Labophot-2 compound microscope (Nikon Inc., Melville, NY, USA). Images were captured using a Zeiss Axio Imager.Z1 compound microscope (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) fitted with an AxioCam MRc 5 camera system (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA), an Olympus BX41 compound microscope (Olympus America Inc., Center Valley, PA, USA) fitted with a Roper Scientific Photometrics® CoolSnap™ camera (Photometrics, Tucson, AZ, USA), or an Olympus BX60 compound microscope (Olympus America Inc., Center Valley, PA, USA) fitted with a SPOT™ RTKE Model 7.2 camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Species descriptions were written based predominantly on observations of specimens collected in this study and emphasize the morphological characters examined by Stuercke & Freshwater (2008). Character state information from the literature was included when not observed in these specimens.

**DNA Extraction and Sequencing**

DNA was extracted from specimens according to Hughey *et al.* (2001) with an additional cleaning step using the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Orange, CA, USA). SSU, *rbcL*, and COI were amplified following the basic PCR recipe outlined in Freshwater *et al.* (2005) but using GOTaq DNA polymerase and buffer (Promega, Madison, WI,
USA). Amplifications were performed in an MJ Research PTC-100™ (Watertown, MA, USA) or Eppendorf Mastercycler gradient (Hamburg, Germany) thermocycler. The thermocycling protocol followed Freshwater et al. (2000) but with 35 cycles of denaturing, annealing at 40, 45, or 50 °C, and extension for 90 s. Amplification products were cleaned with a Stratagene StrataPrep® PCR Purification Kit (Stratagene, La Jolla, CA, USA) and used as templates in sequencing reactions. Sequencing reactions were performed with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), cleaned using Sephadex® G-50 columns, and determined on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA USA). Sequences were edited and assembled using Sequencher™ (version 4.9, GeneCodes Corporation, Ann Arbor, MI, USA). Primers utilized in amplification and sequencing reactions are listed in Table 1.

An rbcL and COI sequence were each generated for as many samples as possible. These loci are appropriate for the examination of inter- and intraspecific relationships and were used to objectively assign samples to species (e.g. Millar & Freshwater 2005; Clarkston & Saunders 2010). SSU sequences were generated for only one sample per species because intraspecific sequence variation is minimal. Both rbcL and SSU sequence data were used to identify groupings of species (clades) within Polysiphonia s.l.

**Molecular Data Analyses**

Sequences of Polysiphonia generated in this study were combined with rbcL and SSU sequences available from GenBank as well as some from unpublished studies (Kelly & Freshwater unpublished). Each dataset included Rhodomelacean species as outgroup taxa. Sequences were aligned using MacClade (v.4, Maddison & Maddison 2000); SSU sequence data
were further aligned using the ClustalW multiple sequence alignment feature of the program Molecular Evolutionary Genetics Analysis (MEGA) (www.megasoftware.net; Kumar et al. 2008; Tamura et al. 2007). Characteristics of the DNA sequence data sets were determined using MacClade and PAUP (v.4, Swofford 2002).

MEGA was used to perform Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Neighbor Joining (NJ) cluster analyses on COI and rbcL sequence data for MAI. A p-distance model was used in all analyses. Maximum Likelihood (ML) analyses were performed on reduced rbcL (identical sequences removed) and SSU sequence data separately using the program Genetic Algorithm for Rapid Likelihood Inference (GARLI) (www.bio.utexas.edu/faculty/antisense/garli/Garli.html; Zwickl 2006). The ML analyses included ten separate searches from random starting trees that used default parameters, including 10000 generations without improving topology and allowing model estimation during the run. ML bootstrap values were based on 1003 (rbcL) and 1000 (SSU) replicates of the GARLI searches.

MOLECULAR RESULTS AND DISCUSSION

Molecular Assisted Identification

COI and rbcL data sets were used for MAI to objectively assign samples to species. The COI alignment consisted of 74 taxa and included 605 sites in the analysis, of which 248 (40.99%) were variable. The rbcL alignment consisted of 113 taxa and included 1081 sites in the analysis, of which 402 (37.19%) were variable. Nineteen species were resolved by UPGMA and NJ cluster analyses of rbcL sequences from 43 Panamanian Polysiphonia s.l. specimens (Fig. 2, only UPGMA cluster diagram shown). Species distinctions were based on inter- and intraspecific
rbcL sequence divergence values reported in Kim & Yang (2005), Kim et al. (2004), and McIvor et al. (2001). The greatest observed intraspecific sequence divergence in the Panama specimens was 1.3% in samples representing Polysiphonia binneyi Harvey. Panamanian samples morphologically representative of Polysiphonia subtilissima Montagne differ from North Carolina samples representative of P. subtilissima by 2.22-2.31%. These groups of samples are treated as two different species because they exceed the intraspecific range observed by McIvor et al. (2001).

UPGMA and NJ cluster analyses of COI sequence data from 26 Panamanian Polysiphonia s.l. specimens resolved 16 species (Fig. 3, only UPGMA cluster diagram shown). Fewer species were resolved using COI sequence data because, despite newly designed primers (Table 1), this locus did not amplify in samples representative of three species. Species distinctions were based on the 4.80% COI sequence divergence observed between P. subtilissima 1 (NC-24) and P. subtilissima 2 (PHYKOS-3271). The rbcL sequences for these species differed by 2.22% which is just beyond the range of intraspecific rbcL sequence divergence observed by McIvor et al. (2001). For these samples, a sequence divergence of 2.22% in the rbcL data corresponds to 4.80% sequence divergence in the COI data; the latter value was therefore used as the intraspecific-interspecific boundary of sequence divergence for the COI data. Comparatively, P. pentamera (PHYKOS-1995) and Polysiphonia sp. 1 (PHYKOS-3535) differ by 2.68% and 5.95% at the rbcL and COI loci, respectively. These values represent a lower limit of interspecific sequence divergence. Intraspecific sequence divergences of \( \leq 0.74\% \) and \( \leq 2.48\% \) at the rbcL and COI loci, respectively, were observed for samples of P. schneideri. Other useful comparisons are not available because of missing sequence data at the COI locus for some samples.
The use of COI as a DNA barcode for species identifications has been evaluated within various taxonomic groups of red algae. Previous studies report that COI has successfully identified cryptic species and displayed a relatively distinct break between inter- and intraspecific sequence divergences. The stochastic nature of DNA sequence evolution and variation in the age of species within evolutionary lineages, however, prevents absolute intra- and interspecific sequence divergence ranges for DNA barcoding. Nevertheless, the upper intraspecific/lower interspecific boundary divergences used in this study are similar to those observed by other red algal barcoding studies (Table 2).

**Molecular Systematics**

SSU and reduced *rbcL* data sets were used to infer phylogeny within *Polysiphonia s.l.* The SSU alignment consisted of 44 taxa and included 1602 sites in the analysis, 158 (9.86%) of which were variable. The reduced *rbcL* alignment consisted of 52 taxa and included 1334 sites in the analysis, 528 (39.58%) of which were variable. ML analyses of *rbcL* and SSU sequence data were performed to establish relationships among Panamanian species within *Polysiphonia s.l.* (Figs 4, 5). Both topologies resolve *Polysiphonia s.l.* as a polyphyletic group; this result is consistent with Choi *et al.* (2001).

The *rbcL* topology is similar to those recently published by Stuercke & Freshwater (2008, 2010) and includes a number of well-supported clades as well as clades and species with unresolved relationships. Two strongly supported clades (*rbcL* bootstrap values [rB] = 100) include species that have been considered *Polysiphonia sensu stricto* (s.s., “in the strict sense”). One contains *P. stricta* (Dillwyn) Greville, the generitype, *P. pacifica* Hollenberg, *P. morrowii* Harvey, *P. kapraunii* B. Stuercke & D.W. Freshwater, *P. atlantica* Kapraun & J.N. Norris, and
The other contains *P. scopulorum* Harvey, *P. scopulorum* var. *villum* (J. Agardh) Hollenberg, *P. subtilissima* Montagne, and an unidentified *Polysiphonia* species. A third strongly supported clade (rB100) includes species with numerous pericentral cells (7+), such as *P. aterrima* J.D. Hooker & Harvey and *P. fucoides* (Hudson) Greville, and has been referred to as the “multipercentral cell group” by Choi *et al.* (2001). This clade is positioned with strong support (rB99) sister to a large unsupported (rB<50) lineage including *Neosiphonia* and *Polysiphonia* species. Although *Neosiphonia* species are resolved in various positions within this large group, the majority are within a strongly supported clade (rB100) that includes *N. harveyi*, *N. sphaerocarpa* (Børgesen) M.S. Kim & I.K. Lee, *N. ferulacea* (Suhr ex J. Agardh) S.M. Guimarães & M.T. Fujii, *N. tongatensis* (Harvey ex Kützing) M.S. Kim & I.K. Lee, *P. strictissima* J.D. Hooker & Harvey, *P. forfex* Harvey, *P. bajacali* Hollenberg, *P. pseudovillum* Hollenberg, an unidentified species of *Neosiphonia*, and the newly described species *P. nuda* sp. nov.

The general groupings of clades and species in the *rbcL* tree are also present in the SSU ML tree. Two clades of *Polysiphonia s.s.* species are strongly supported (SSU bootstrap values [sB] = 100 & 99) and there is moderate support (sB78) for their being resolved together in a single clade. A multipercentral cell group that includes species placed in the genera *Boergeseniella*, *Enelittosiphonia*, and *Vertebrata*, as well as *Polysiphonia* is well supported (sB91). This clade is strongly supported (sB97) as the sister taxon to a large clade comprised of *Neosiphonia* and *Polysiphonia* species. Unlike the *rbcL* tree, this latter group is well supported (sB91) in the SSU analysis. Relationships of the 19 Panamanian species included in these analyses will be discussed within the remarks for each species.
TAXONOMIC OBSERVATIONS AND REMARKS

Key to Panamanian species of *Polysiphonia sensu lato*

1. Pericentral cells 4.................................................................2
   Pericentral cells more than 4...............................................13

2. Rhizoids in open connection with pericentral cells ................3
   Rhizoids cut off from pericentral cells, pit plug present ................6

3. Lateral branches developing in the axils of trichoblasts ............4
   Lateral branches developmentally replacing trichoblasts .............5

4. Plants primarily erect, erect axes c. 175-225 µm in dia., spermatangial
   stichidia generally with 2 or 3 sterile tip cells ......................... *P. binneyi*
   Plants with extensive prostrate system, erect axes c. ≤ 110 µm in dia., spermatangial
   stichidia generally without sterile tip cells .......................... *P. havanensis sensu* Børgesen

5. Erect axes arising radially from prostrate axes, trichoblasts unbranched or with 1
   dichotomy ............................................................................. *P. subtilissima*
   Erect axes arising unilaterally from prostrate axes giving plants a dorsiventral habit,
   trichoblasts generally with 2 or 3 dichotomies ........................... *P. macrocarpa*

6. Trichoblasts and scar cells entirely lacking ........................... *P. nuda* sp. nov.
   Trichoblasts and scar cells present ...........................................7

7. Erect axes mostly < 70 µm in dia., prostrate axes mostly < 150 µm in dia. ..........8
   Erect axes mostly > 70 µm in dia., prostrate axes mostly > 150 µm in dia. ..........11

8. Lateral branches developmentally replacing trichoblasts ............ *P. pseudovillum*
   Lateral branches developing in the axils of trichoblasts ................9

9. Lateral branches with slight basal attenuation ......................... *P. cf. sertularioides* 1
   Lateral branches with prominent basal attenuation .....................10

10. Mid axis segments of erect axes > 1x as long as wide .............. *P. cf. sertularioides* 2
    Mid axis segments of erect axes ≤ 1x as long as wide ............... *P. cf. sertularioides* 3

11. Branchlets spindle-shaped, mid axis segments of erect axes < 1x as long as wide, erect axes
    mostly (150-) 250-350 µm in dia. ............................................. *N. ferulacea*
    Branchlets linear, mid axis segments of erect axes ≥ 1x as long as wide, erect axes mostly ≤ 200 µm in dia. ...............................12
12. Plants to 3 (-8) cm tall, erect axes moderately branched ...........................................*N. tongatensis*
   Plants to 1 cm tall, erect axes unbranched .................................................................*Neosiphonia sp.* 1

13. Pericentral cells ≥ 8 (occasionally 7 in *Polysiphonia sp.* 2) .....................................14
    Pericentral cells < 8 .......................................................................................................15

14. Pericentral cells (7-) 8, lateral branches developing in the axils of trichoblasts ...................
    .................................................................................................................................*Polysiphonia sp.* 2
    Pericentral cells 8-10+, lateral branches developmentally replacing trichoblasts, pericentral
    cells offset in some axes ...............................................................................................*P. howei*

15. Pericentral cells strictly 5 ..............................................................................................16
    Pericentral cells 5-7 .........................................................................................................*P. schneideri*

16. Trichoblasts and scar cells rare .....................................................................................17
    Trichoblasts and scar cells frequent ...............................................................................*Polysiphonia sp.* 1

17. Tetrasporangia in straight series ....................................................................................*P. homoia*
    Tetrasporangia in spiral series ......................................................................................*P. pentamera*

**Genus Neosiphonia**

*Neosiphonia ferulacea* (Suhr in J. Agardh) S.M. Guimarães & M.T. Fujii

in Guimarães et al. (2004, p. 165)

Figs 6-9

**Basionym:** *Polysiphonia ferulacea* Suhr in J. Agardh (1863, p. 980)

**Synonyms:** *Polysiphonia bharadwaja* Sreenivasa Rao (1967, p. 169)

**Description:** Plants to 4 (-15) cm tall, erect from discoid base with some branches
becoming decumbent and attached to substratum by rhizoids cut off from pericentral cells (Fig. 9);
sparsely to moderately branched in a dichotomous to alternate or irregular pattern (Fig. 6);
erect axes (150-) 250-350 µm in diameter, prostrate axes 225-375 µm in diameter; branchlets
distinctly spindle shaped (Figs 6, 7); mid axis segments of erect axes mostly to 0.5x as long as
wide, but even shorter near apices; cortication absent; main axes with 4 pericentral cells (Fig. 8);
branches replacing trichoblasts in development; trichoblasts short, to 90 µm in length, with several dichotomies, dense at apices (Fig. 7); scar cells not readily obvious, one per segment in ¼ spiral series; adventitious laterals absent; tetrasporangia not distending segments, in short spiral series in branch tips, 50-60 µm in diameter; spermatangial stichidia developing as a furcation of trichoblasts, 60x150 µm, with 1 or 2 sterile tip cells; cystocarps globose, to 250 µm in diameter, sessile or short stalked, with wide ostioles surrounded by ostiolar lip cells smaller than those of the pericarp below.

**Type Locality:** East coast of Mexico

**Other Sources:** Dawes & Mathieson 2008; as *Polysiphonia ferulacea*, Kapraun 1977, Kapraun & Norris 1982, Kapraun et al. 1983, Schneider & Searles 1991


**Molecular Vouchers:** GenBank accession numbers HM573574, HM573584 (rbcL); HM560645 (SSU); HM573512, HM573511 (COI).

**Remarks:** *Neosiphonia ferulacea* closely resembles *Polysiphonia sparsa* (Setchell) Hollenberg, but the latter species has spermatangial stichidia with no sterile tip cells and a more creeping and smaller habit (to 1 cm tall) (Abbott 1999; Hollenberg 1968a; Kapraun 1977). Segments are typically much shorter in *P. sparsa* than *N. ferulacea* but this character was found to be highly variable in Belize (as *P. ferulacea*) samples by Kapraun & Norris (1982).
Polysiphonia sparsa was described from Tahiti and is widely reported from the central and western Pacific (Abbott 1999; Hollenberg 1968a; Lobban & Tsuda 2003; Millar & Kraft 1993; Payri et al. 2000; Silva et al. 1987). Although N. ferulacea is also reported from the central and western Pacific, its type locality is Atlantic Mexico and the species is widely distributed in the Caribbean and tropical western Atlantic (Ganesan 1990; Kapraun & Norris 1982; Schneider & Searles 1991; Suárez 2005; Taylor 1960, 1969).

Neosiphonia ferulacea was collected at two different sites in the Galeta area of Caribbean Panama (Fig. 1). The rbcL and COI sequences generated from these samples are not identical but differ by only 0.56% and 0.50% respectively, well within the range of intraspecific divergence for these two loci (Figs 2, 3). ML analyses of rbcL and SSU sequence data resolve N. ferulacea within a strongly supported clade (rB100; sB87) that includes both Neosiphonia and Polysiphonia species (Figs 4, 5). Neosiphonia ferulacea is weakly allied in the rbcL tree (rB65) with a poorly characterized unidentified Neosiphonia specimen (PHYKOS-3536) collected from the Bocas del Toro region of Caribbean Panama. These species can be distinguished morphologically from the unidentified specimen, which has a smaller habit and axes dimensions, branches that are not spindle shaped, and mid axis segments of erect axes that are 1-1.5x as long as wide.

Neosiphonia tongatensis (Harvey in Kützing) M.S. Kim & I.K. Lee (1999, p. 280)

Figs 10-14

BASIONYM: Polysiphonia tongatensis Harvey in Kützing (1864, p. 14)

SYNONYMS: Polysiphonia aquamara I.A. Abbott (1947, p. 212)

Polysiphonia eastwoodae Setchell & N.L. Gardner (1930, p. 161)
Polysiphonia mollis sensu Hollenberg (1961, p. 359) [non P. mollis J.D. Hooker & Harvey (1847, p. 397)]

Polysiphonia mollis var. tongatensis (Harvey in Kützing) Hollenberg (1968, p. 69)

Polysiphonia snyderae Kylin (1941, p. 35)

**Description:** Plants to 1.5-3 (-8) cm tall, erect from a discoid base with some branches becoming decumbent and attached to substratum by rhizoids cut off from pericentral cells (Fig. 14); moderately branched in a dichotomous to subdichotomous pattern (Fig. 10); erect axes 50-150 µm in diameter, prostrate axes 125-250 µm in diameter; mid axis segments of erect axes predominantly 1-2x as long as wide; cortication absent; main axes with 4 pericentral cells (Fig. 13); branches replacing trichoblasts in development (Fig. 11); trichoblasts with several dichotomies, to 175 µm in length; scar cells one per segment in ¼ spiral series; adventitious laterals absent; tetratosporangia much distending segments, developing in spiral series, 40-80 µm in diameter; spermatangial stichidia developing as a furcation of trichoblasts (Fig. 12), 25-50x90-200 µm, with or without a single sterile tip cell; cystocarps ovoid, 300-350 µm in diameter.

**Type Locality:** Tonga, Western Pacific

**Other Sources:** As Polysiphonia tongatensis, Abbott 1999; as Polysiphonia eastwoodae, Kapraun et al. 1983; as Polysiphonia mollis sensu Hollenberg, Hollenberg 1961

**Specimens Studied:** Panama: WNC2009-s261 to s263 (PHYKOS-2704), STRI research station, Punta Galeta, Colón, B. Wysor, 21 May 2009.

**Molecular Vouchers:** GenBank accession numbers HM573570 (rbcL); HM560642 (SSU); HM573518 (COI).
REMARKS: Hollenberg (1961) synonymized *Polysiphonia tongatensis* “as interpreted by Segi 1951, p. 207, at least as to Mexican material” and *P. eastwoodae* Setchell & Gardner with *P. mollis* J.D. Hooker & Harvey. Womersley (1979) refutes this synonymy as the Hollenberg *P. mollis* material displays branches that replace trichoblasts in development whereas the type material has branches that develop laterally from the basal trichoblast cell. *Polysiphonia mollis sensu* Hollenberg is therefore regarded as distinct from *P. mollis* but is recognized as *N. tongatensis* by Abbott (1999).

Pacific material of *Neosiphonia tongatensis* is reported as having spermatangial stichidia that develop as a furcation of trichoblasts with or without 1-4 sterile tip cells (Abbott 1999; Hollenberg 1968a). Venezuelan material examined by Kapraun et al. (1983) (as *Polysiphonia eastwoodae*) is described as having spermatangial branches developing as a furcation of or developmentally replacing trichoblasts, with or without a single sterile tip cell. Spermatangial stichidia were not observed in the original descriptions of *N. tongatensis* (as *P. tongatensis*) and *P. eastwoodae* (Setchell & Gardner 1930). Further study is necessary to determine if these reports represent the same species.

Only one sample representative of *Neosiphonia tongatensis* was collected in this study (Figs 2, 3). ML analyses of *rbc*L and SSU sequence data resolve *N. tongatensis* within a strongly supported clade of predominantly *Neosiphonia* species (Figs 4, 5). *Neosiphonia tongatensis* appears most closely related to the newly described *Polysiphonia nuda* sp. nov. in the *rbc*L tree, but bootstrap support for this relationship is less than 50. *Polysiphonia nuda* sp. nov. can be distinguished from *N. tongatensis* by its lack of scar cells and trichoblasts. There is a moderate level of support (sB78) for a sister relationship between *N. tongatensis* and *P. bajacali*.
Hollenberg in the SSU tree. These species can be distinguished morphologically by *P. bajacali* having greater dimensions of erect axes (300-500 µm in diameter) and slight basal cortication.

*Neosiphonia sp. 1*

Figs 15-18

**Description:** Plants to 6 mm tall, with erect and prostrate portions; prostrate axes attached to substratum by rhizoids cut off from pericentral cells (Fig. 17); erect axes unbranched, 125-200 µm in diameter; prostrate axes 175-200 µm in diameter; mid axis segments of erect axes mostly 1-1.5x as long as wide (Fig. 18); cortication absent; main axes with 4 pericentral cells (Fig. 16); relationship of branches to trichoblasts unknown; trichoblasts with several dichotomies (Fig. 15); scar cells one per segment in ¼ spiral series; adventitious laterals lacking; reproductive structures unknown.

**Specimens Studied:** Panama: *WNC2010-s042-s043, (PHYKOS-3536)*, STRI Research Station, Bocas del Toro, D.W. Freshwater, 9 Jul 2008.

**Molecular Vouchers:** GenBank accession numbers HM573573 (*rbcL*); HM560649 (SSU); HM573525 (COI).

**Remarks:** In the absence of reproductive material, the basic morphological characteristics of *Neosiphonia sp. 1* could fit the description of a large number of species of *Polysiphonia s.l.* Several species are described as having no cortication, four pericentral cells, rhizoids cut off from pericentral cells, and scar cells every segment in a spiral pattern. These character states can be used to define species of *Neosiphonia.*

Only one sample representative of *Neosiphonia sp. 1* was collected in this study (Figs 2, 3). ML analyses of *rbcL* and SSU sequence data place *Neosiphonia sp. 1* within a strongly
supported clade of predominantly *Neosiphonia* species (Figs 4, 5). *Neosiphonia* sp. 1 appears as sister to *N. ferulacea* in the rbcL ML phylogeny, but this relationship is only weakly supported (rB65). *Neosiphonia* sp. 1 is morphologically similar to *N. ferulacea* by having 4 pericentral cells, no cortication, rhizoids cut off from pericentral cells, and scar cells every segment in a spiral pattern but differs in dimensions of habit, segment length, and erect and prostrate axis diameter. *Neosiphonia ferulacea* can also be distinguished from *Neosiphonia* sp. 1 by its spindle shaped branches. The SSU ML phylogeny shows *Neosiphonia* sp. 1 as most closely related to *Polysiphonia pseudovillum* (Fig. 5). These species have identical SSU sequence data, but this locus is not appropriate for species distinctions because of its highly conserved nature.

*Neosiphonia* sp. 1 and *P. pseudovillum* are similar in having four pericentral cells, no cortication, and rhizoids cut off from pericentral cells, but *P. pseudovillum* does not have a trichoblast or scar cell every segment.

**Genus Polysiphonia**

*Polysiphonia binneyi* Harvey (1853, p. 37)

Figs 19-24

**Description:** Plants to 3 (-15) cm tall, erect branches from a discoid base with some branches becoming decumbent and attached to substratum by rhizoids in open connection with pericentral cells; branching sparse to moderate in a dichotomous to subdichotomous pattern; erect axes 175-225 µm in diameter, prostrate axes 165-225 µm in diameter; branchlets basally attenuated towards point of attachment to main axis (Fig. 21); mid axis segments of erect axes mostly of slightly shorter length than width with segments getting increasingly shorter in upper portions of erect axes towards apices (Figs 19, 22); cortication absent; main axes with 4
pericentral cells (Fig. 20); branches forming in the axils of trichoblasts (Fig. 23); trichoblasts long, thin, and tangled when mature, typically with three dichotomies; scar cells one per segment in ¼ spiral series (Fig. 22); adventitious laterals lacking; tetrasporangia slightly distending segments, in long spiral series in upper branches, 55-80 µm in diameter (Fig. 24); spermatangial stichidia developing as a furcation of trichoblasts, 25-50x100-200 µm, with 2 or 3 sterile tip cells; cystocarps ovoid to globose, (90-) 250-420 µm in diameter short stalked, with wide ostioles.

**Type Locality:** Key West, Monroe County, Florida, USA

**Other Sources:** Dawes & Mathieson 2008, Kapraun et al. 1983, Schneider & Searles 1991, Taylor 1960


**Molecular Vouchers:** GenBank accession numbers HM573556, HM573555 (*rbcL*); HM560636 (SSU).

**Remarks:** Four samples of *Polysiphonia binneyi* were collected in this study (Fig. 2). Three of the four *rbcL* sequences generated for these samples are identical and the fourth differs by 1.30%, which is within the range of intraspecific *rbcL* sequence variation observed in other species of *Polysiphonia s.l.* (Kim et al. 2004; McIvor et al. 2001) but is relatively high.
considering that all four samples were collected contemporaneously from a Caribbean Panama
collection site near Colón.

Polysiphonia binneyi is associated with P. havanensis sensu Børge sen and P. echinata
Harvey in both the rbcL and SSU ML trees (Figs 4, 5). Polysiphonia binneyi is well supported
(rB93) as the sister species of P. havanensis sensu Børge sen in the rbcL analysis, but is weakly
supported (sB63) as sister to P. echinata in the SSU analysis. Polysiphonia binneyi closely
resembles P. havanensis sensu Børge sen, but the latter species can be distinguished by having a
distinct and extensive system of prostrate axes, main axes of lesser diameter (mostly less than
150 µm), and spermatangial stichidia with no sterile tip cells (Dawes & Mathieson 2008;
by having rhizoids that are cut off from pericentral cells, basal cortication, and abundant
adventitious laterals that give the species a coarse appearance.

Polysiphonia havanensis sensu Børge sen (1918, p. 266)
[non P. havanensis Montagne (1837, p. 352)]
Figs 25-29

Description: Plants to 3 (-9) cm tall, erect branches arising from a prostrate branching
system attached to the substratum by rhizoids in open connection with pericentral cells (Fig. 27);
branching moderate in a dichotomous to subdichotomous pattern (Fig. 25); erect axes 50-110 µm
in diameter, prostrate axes 150-170 µm in diameter; mid axis segments of erect axes vary from
0.75-2x as long as wide; cortication absent; main axes with 4 pericentral cells (Fig. 26); branches
forming in the axils of trichoblasts (Fig. 28); scar cells one per segment in ¼ spiral series;
adventitious laterals rare; tetrasporangia slightly distending upper segments, in spiral series, 50-
70 µm in diameter (Fig. 29); spermatangial stichidia developing as a furcation of trichoblasts,
30-60x80-300 µm, without sterile tip cells; cystocarps globose to subglobose, 250-300 µm in diameter, short stalked, with narrow ostioles.

**Type Locality:** Børgesen (1918) described specimens from Bovini Lagoon, St. Thomas and America Hill, St. John in the United States Virgin Islands. The type locality of *Polysiphonia havanensis* Montagne is Havana, Cuba.

**Other Sources:** Børgesen 1918, Kapraun 1977, Schneider & Searles 1991


**Molecular Vouchers:** GenBank accession numbers HM573554 (*rbcL*); HM560641 (SSU); HM573522 (COI).

**Remarks:** This species as interpreted by Børgesen (1918) is distinct from the species as originally described by Montagne (1837). Ardré (1970) and Kützing (1863) indicate that *Polysiphonia havanensis* Montagne was originally described as having spiraled pericentral cells in segments of older axes and lacking trichoblasts. No spiraling of pericentral cells, numerous conspicuous trichoblasts, and scar cells every segment in a spiral pattern are reported for *P. havanensis sensu* Børgesen (Børgesen 1918; Kapraun 1977; Schneider & Searles 1991). Reproductive structures are unknown for *P. havanensis* Montagne as both the original and Kützing’s 1863 description include only sterile thalli. Børgesen indicates that while most specimens observed in his 1918 study were sterile, a few specimens displayed tetradsporangia
scattered in upper axes, occurring singly or as a few together, with intermittent sterile segments. Potential differences in reproductive structures between the two entities are therefore unknown. *Polysiphonia havanensis sensu* Børgesen is a well-described taxon in need of a validly published species name.

Two samples of *Polysiphonia havanensis sensu* Børgesen were collected from the Bocas del Toro region and another sample from near the Panama Canal mouth on the Caribbean coast. Only one COI sequence was generated from these samples (Fig. 3), but the *rbcL* sequences for all three samples were identical (Fig. 2). Phylogenetic analyses of *rbcL* and SSU sequence data indicate that this species is closely related to *P. binneyi* and *P. echinata* (Figs. 4, 5). However, these species are easily distinguished from one another morphologically (see remarks for *P. binneyi*).

It has been suggested that *P. havanensis sensu* Børgesen is possibly synonymous with *P. sertularioides* (Grateloup) J. Agardh (Kapraun 1977; Schneider & Searles 1991). These species are clearly distinct from one another as the former has rhizoids that remain in open connection with pericentral cells and the latter has rhizoids that are cut off from pericentral cells by a pit connection (Athanasiadis 1987; Børgesen 1918; Womersley 1979, 2003). The distinction between these two species is supported by ML phylogenies based on *rbcL* and SSU sequence data as *P. havanensis sensu* Børgesen is distantly related to *P. sertularioides* (Figs 4, 5).

**Polysiphonia homoia** Setchell & N.L. Gardner (1930, p. 162)

*Fig* 30-35

**Description:** Plants to 1 (-6) cm tall, erect axes arising from prostrate axes attached to substratum by rhizoids cut off from pericentral cells (Fig. 34); sparsely to moderately branched
in a dichotomous to subdichotomous pattern; erect axes (60-) 100-220 µm in diameter, prostrate axes 280-310 µm in diameter; apices bi- to multifurcate (Figs 30, 31); mid axis segments of erect axes vary from 0.5-1.5x as long as wide (Fig. 35) with segments typically shorter than wide near apices (Figs 30, 31); cortication absent; main axes with 5 pericentral cells (Fig. 33); branches developing independently of trichoblasts; trichoblasts and scar cells rare; adventitious laterals absent; tetrasporangia moderately distending segments, in extremely long (to 65 segments) straight series, 95-113 µm in diameter (Fig. 32); spermatangial stichidia and cystocarps unknown.

**Type Locality:** Isla Guadalupe, Mexico

**Other Sources:** Setchell & Gardner 1930, Taylor 1945


**Molecular Vouchers:** GenBank accession numbers HM573553 (rbcL); HM560653 (SSU); HM573507 (COI).

**Remarks:** *Polysiphonia homoia* was originally described by Setchell & Gardner (1930) as having rhizoids that are “nonseptate”. Whether this refers to an open rhizoidal connection with pericentral cells or continuous, uninterrupted cytoplasm throughout the length of the rhizoid is unclear. As all other morphological characters fit the original description of *P. homoia*, the Panama samples are identified as this species. *Polysiphonia homoia* was recently included in a web resource of Pacific Panama marine algae by Littler & Littler (2010). These samples are described as having rhizoids that are cut off from pericentral cells (Littler & Littler 2010).
Hollenberg (1968b) described Hawaiian specimens of *P. homoia* as having rhizoids cut off from pericentral cells, however, these specimens displayed tetrasporangia developing in spiral series. As the original description of *P. homoia* describes tetrasporangia developing in straight series, it seems likely that the Hollenberg specimens, also studied by Abbott (1999), are a species other than *P. homoia*.

*Polysiphonia homoia* could potentially be confused with *P. bifurcata* Hollenberg, *P. guadalupensis* Setchell & N.L. Gardner, or *P. pentamera* Hollenberg, which also have five pericentral cells, but these species have tetrasporangia developing in spiral series (Abbott 1999; Hollenberg 1961, 1968b; Setchell & Gardner 1930). Two specimens of *Polysiphonia homoia* were collected in this study (Figs 2, 3). ML analyses of *rbcL* and SSU sequence data resolve *P. homoia* as an independent lineage with little to no support for its topological position (Figs 4, 5).

*Polysiphonia howei* Hollenberg in W.R. Taylor (1945, p. 302)

Figs 36-41

SYNONYMS: *Neosiphonia howei* (Hollenberg) Skelton & G.R. South (2007, p. 188)

*Polysiphonia rhizoidea* Meñez (1964, p. 217)

*Polysiphonia yonakuniensis* Segi (1951, p. 257)

*Lophosiphonia obscura sensu* Weber v. Bosse 1923 [non *L. obscura* (C. Agardh) Falkenberg in F. Schmitz & Falkenberg (1897, p. 460)]

DESCRIPTION: Plants to 2 (-5) cm tall, erect branches arising from a prostrate branching system (Fig. 36) attached to substratum by rhizoids cut off from pericentral cells; branching sparse to moderate in a dichotomous to subdichotomous pattern (Fig. 38); young erect axes at first strongly arched toward prostrate axis (Fig. 40); erect axes 100-150 μm in diameter, prostrate
axes (75-) 125-150 µm in diameter; mid axis segments of erect axes predominantly 0.5-1x as long as wide; cortication absent; main axes with 8-10 (-12) pericentral cells that tend to shift to offset positions across segments (Fig. 37); central axial cell distinctly enlarged; branches replacing trichoblasts in development; trichoblasts with distinctly short basal cell, dense at apices when present (Fig. 41); scar cells present and obvious, variable in pattern and frequency (Fig. 37); adventitious laterals common; tetrasporangia in long spiral series in mid to upper branch segments, slightly distending segments, (40-) 50-65 µm in diameter (Fig. 39); spermatangial stichidia reported to both replace trichoblasts and develop as a furcation of trichoblasts (see remarks); cystocarps globose to ovoid, 120-360 µm in diameter.

TYPE LOCALITY: Whale Cay, Berry Island, Bahamas.


MOLECULAR VOUCHERS: GenBank accession numbers HM573543 (rbcL); HM560656 (SSU); HM573520, HM573521 (COI).

REMARKS: Samples of Polysiphonia howei were collected from a variety of locations around Bocas del Toro. All rbcL sequences generated from these samples were identical and variation among COI sequences for these samples ranged from 0 to 0.17% (Figs 2, 3).
*Polysiphonia howei* is resolved as an independent lineage at the base of the *Polysiphonia s.l.* rbcL ML tree (Fig. 4). It occupies a position basal to a *Polysiphonia s.s.* clade in the SSU ML tree (Fig. 5), but support for the positions of *P. howei*, *Polysiphonia sp. 3*, and *Womersleyella setacea* (Hollenberg) R.E. Norris in this topology are weak. A second *P. howei* specimen from the Phillipines is resolved together with the Panamanian *P. howei* specimen in the SSU tree (sB99, Fig. 5). However, the branch length between these specimens suggests that they are not conspecific, and is a reflection of the morphological variation that is also reported in this species.

Development of spermatangial stichidia appears to be variable within samples identified as *P. howei*. Gametophytic structures were not described in Hollenberg’s original description of the species in Taylor (1945). In a later study of samples identified as *P. howei* from the central and western tropical Pacific Ocean, Hollenberg predominantly observed single spermatangial stichidia developmentally replacing trichoblasts but occasionally also pairs, with each stichidium developing on the primary trichoblast bifurcation, and neither condition displayed sterile tip cells (Hollenberg 1968b). Abbott (1999) observed similar spermatangial stichidia development in Hawaiian samples identified as *P. howei* but made no mention regarding sterile tip cell presence or absence. Spermatangial stichidia are reported as developmentally replacing trichoblasts, with or without sterile tip cells, in the southern Caribbean (Kapraun *et al.* 1983). In contrast, Dawes & Mathieson (2008) report that spermatangial stichidia develop as a furcation of trichoblasts in Florida specimens, and this condition is also reported for North Carolina specimens tentatively assigned to *P. howei* (Hollenberg 1958); no mention regarding sterile tip cell presence or absence was made in either study. Development of spermatangial stichidia was not observed in this study.

The diverse states of spermatangial stichidia structure reported for specimens identified as *Polysiphonia howei* suggest that perhaps more than one species has been identified under this
name. Diversity within the species is also suggested by the number of pericentral cells reported for samples identified as *P. howei*. Hollenberg originally described the species as having 10-12 pericentral cells (Hollenberg in Taylor 1945). This number was also observed in specimens examined by Abbott (1999) and Kapraun *et al.* (1983). Hollenberg’s 1968b description of central western Pacific samples describes them as having 8-10 pericentral cells; Kapraun (1980a) also observed this number in North Carolina specimens. Samples in this study exhibit 8-10 (-11) pericentral cells. Further morphological and genetic analyses of a wide array of *P. howei* samples are needed to determine its exact character states and geographic distribution.

*Lophosiphonia obscura* (C. Agardh) Falkenberg in F. Schmitz & Falkenberg has been historically confused with *P. howei* (Hollenberg 1958, 1968b; Taylor 1945). The endogenous origin of erect branches (branches arise from the central axis subsequent to pericentral cell formation) distinguishes species of *Lophosiphonia* from species of *Polysiphonia*, whose species have branches of exogenous origin (branches arise from subapical cell division prior to pericentral cell formation) (Hollenberg 1942). *Polysiphonia howei* also closely resembles *P. exilis* Harvey, but the latter species can be distinguished by having pericentral cells arranged in non-shifting longitudinal rows and young branches that are perpendicular to, and not arching towards, the prostrate axis (Hollenberg 1968b).

Skelton & South (2007) transferred *Polysiphonia howei* to the genus *Neosiphonia*, whose members can be characterized by an erect habit originally developing from a solid disc of rhizoids and by having spermatangial stichidia developing as a furcation of trichoblasts, sometimes with one or two sterile tip cells (Kim & Lee 1999). In contrast, Hollenberg (1945, 1968b) describes a creeping habit and spermatangial stichidia that developmentally replace trichoblasts for *P. howei*. Members of *Neosiphonia* also exhibit 4-9 pericentral cells; *P. howei*
was originally described as having 10-12 pericentral cells. In ML phylogenies based on SSU and rbcL sequence data, samples identified as *P. howei* are distantly related to species of *Neosiphonia* (Figs 4, 5). For these reasons, *P. howei* is not recognized as belonging in *Neosiphonia* and is retained in *Polysiphonia*.

*Polysiphonia macrocarpa* (C. Agardh) Sprengel (1827, p. 350)
[non *P. macrocarpa* Harvey 1836, p. 206]
Figs 42-46

**Basionym:** *Hutchinsia macrocarpa* C. Agardh (1824, p. 157)

**Description:** Plants to 1-3 cm tall, composed predominantly of entangled erect axes attached to the substratum by short decumbent sections that have rhizoids in open connection with pericentral cells (Fig. 43); highly branched in a subdichotomous pattern below, alternate near apices (Fig. 42); erect axes 30-70 (-100) µm in diameter; mid axis segments of erect axes predominantly 2-3x as long as wide (Fig. 46); cortication absent; main axes with 4 pericentral cells (Fig. 45); branches replacing trichoblasts in development; trichoblasts usually abundant near apices, long and delicate, to 500 µm in length, commonly with 2 or 3 dichotomies (Fig. 44); scar cells present and obvious, but not present every segment; adventitious laterals occasionally present, linear in shape; tetrasporangia in straight series; cystocarps slightly urceolate, distinctly enlarged; spermatangial stichidia unknown.

**Type Locality:** Port-au-Prince, Haiti (see remarks)

**Other Sources:** Agardh 1824, Sprengel 1827

**Specimens Studied:** *P. macrocarpa* (Agardh) Sprengel 1827, Panama: *WNC2009-s215 to s219 (PHYKOS-2561)*, Punta Gorda, Colón, D.W. Freshwater, 20 May 2009; *WNC2009-s250*

**Molecular Vouchers:** GenBank accession numbers HM573545 (rbcL); HM560632 (SSU); HM573538 (COI).

**Remarks:** The type locality of *Hutchinsia macrocarpa* C. Agardh is described as “In mari Antillarum ad ‘Port au Pray’” (Agardh 1824). Kapraun & Norris (1982) noted that while other type localities are Latinized in C. Agardh’s *Systema Algarum* (Agardh 1824), the type locality for *H. macrocarpa* is left in French with quotation marks, possibly suggesting a quandary with the locality name. It seems likely that this name might refer to Port-au-Prince or Port-de-Paix, Haiti in the Greater Antilles, giving *H. macrocarpa* a Caribbean type locality. *Hutchinsia macrocarpa* was later transferred to *Polysiphonia* by Sprengel (1827) as *P. macrocarpa* (C. Agardh) Sprengel, a species that is distinct from *P. macrocarpa* Harvey, which was described from Portstewart, Miltown Malbay, Ireland (Harvey 1836). The latter species has historically reported been from the western Atlantic (e.g. Børgesen 1918; Taylor 1960). The
occurrence of these heterotypic homonyms led Kapraun & Norris (1982) to propose a new name, *P. atlantica* Kapraun & J.N. Norris, for *P. macrocarpa* Harvey. This has left *P. macrocarpa* (C. Agardh) Sprengel as a potentially overlooked name for species reported from the tropical western Atlantic.

In the present study, four Panamanian samples were initially identified as *Polysiphonia atlantica* based on morphological character states that fit the description of this taxon. However, cluster analyses of *rbcL* and COI sequence data clearly show that the Panamanian samples are genetically distinct from offshore North Carolina samples that are also morphologically identified as *P. atlantica* (Figs 2, 3). The two groups of samples differ by 6.38% at the *rbcL* locus and 6.28% at the COI locus. This is clearly beyond the ≤ 2.13% (predominantly ≤ 1.3%) intraspecific *rbcL* sequence divergence that has been observed in previous studies of *Polysiphonia* and better agrees with interspecific *rbcL* sequence divergence values of 3.07% to 14.12% observed for other species within the genus (Kim & Yang 2005; Kim *et al.* 2004; McIvor *et al.* 2001). The distinction between these two groups of samples is also apparent in *rbcL* and SSU ML phylogenies (Figs 4, 5).

The only morphological differences observed between these two groups of samples include the presence of trichoblasts in all Panamanian samples but only some North Carolina samples, greater observed height in some Panamanian samples (3 cm) than in North Carolina samples (2 cm), and consistently longer segment length in the North Carolina samples. Trichoblast presence is not a reliable species identifier as this character is described as variable within North Carolina *Polysiphonia atlantica* (Kapraun 1977; Stuercke & Freshwater 2008; this study). A small height difference and slight variation in segment length are also not

Morphological comparisons were also made among specimens from Panama, inshore and offshore North Carolina, and others identified as *Polysiphonia atlantica* or *P. macrocarpa* in WNC and US. No morphological distinction was observed in the vegetative morphologies of these samples. Reproductive structures were not observed in Panamanian and some US samples. US samples from Cuba displayed tetrasporangia developing in straight series and urceolate cystocarps; inshore North Carolina samples displayed these same character states as well as spermatangial stichidia developmentally replacing trichoblasts and with no sterile tip cells (Kapraun 1977, 1980a; this study). Offshore North Carolina samples also displayed tetrasporangia developing in straight series but no gametophytic structures were present.

Schneider & Searles (1991) observed that offshore North Carolina samples identified as *Polysiphonia macrocarpa* Harvey in Schneider (1976) differed greatly from inshore North Carolina samples identified as *P. atlantica* but did not seem to fit any other species description. In contrast to the offshore *P. atlantica* specimens of Stuercke & Freshwater (2008), these offshore samples had spirally arranged tetrasporangia and therefore represent a different species.

Panamanian samples are given the name *Polysiphonia macrocarpa* (C. Agardh) Sprengel based on proximity to the supposed type locality of Haiti. Although these samples fit the morphological descriptions of *Hutchinsia macrocarpa* and *P. macrocarpa* (C. Agardh) Sprengel (Agardh 1824; Sprengel 1827), these early descriptions are vague and do not include all morphological characters needed to identify *Polysiphonia* species. Further morphological and genetic studies are required to confirm this taxonomic placement. Examination of reproductive...
structures may reveal morphological differences between the genetically distinct North Carolina and Panama species.

*Polysiphonia nuda sp. nov.*

Figs 47-52


**Diagnosis:** Thallus ecorticate, with erect branches arising from a prostrate branching system. Erect portion highly branched. Rhizoids cut off from pericentral cells. Pericentral cells four. Trichoblasts and scar cells absent. Tetrasporangia arranged in straight series. Gametophytic structures not observed.

**Description:** Plants to 7 mm tall, forming dense tufts, erect portions arising from prostrate axes attached to substratum by rhizoids cut off from pericentral cells (Fig. 50); rhizoids with digitate, unicellular tips (Fig. 49); highly branched in a dichotomous to subdichotomous pattern (Figs 47, 48); erect axes 70-140 µm in diameter, prostrate axes 120-180 µm in diameter; mid axis segments of erect axes 0.5-1x as long as wide; cortication absent; main axes with 4 pericentral cells (Fig. 51); trichoblasts and scar cells absent; adventitious laterals absent; tetrasporangia slightly distending segments, in long straight series (Fig. 52); spermatangial stichidia and cystocarps unknown.

**Type Locality:** Parque de Juventud, Calle Primero, Colón, Panama

MOLECULAR VOUCHERS: GenBank accession numbers HM573571 (rbcL); HM560648 (SSU); HM573517 (COI).

REMARKS: Polysiphonia nuda is distinguished from other species of Polysiphonia by the combination of the following character states: main axes with 4 pericentral cells, rhizoids cut off from pericentral cells, tetrasporangia that develop in straight series, and the absence of trichoblasts and scar cells. While several species of Polysiphonia are described as having four pericentral cells, rhizoids cut off from pericentral cells, and tetrasporangia in straight series, the complete lack of scar cells and trichoblasts prevents P. nuda from fitting any other available species description. Polysiphonia pacifica Hollenberg and P. senticulosa Harvey, with type localities of Santa Cruz, CA, USA and Orcas Island, WA, USA, respectively, are described as having four pericentral cells, tetrasporangia in straight series, and lacking or exceedingly rare trichoblasts and scar cells, but both species have rhizoids that are in open connection with pericentral cells (Hollenberg 1942 (as Polysiphonia pungens Hollenberg), 1961; Hollenberg & Norris 1977; Womersley 2003).

Only one sample of Polysiphonia nuda was collected from Panama. This sample appears as a distinct entity in cluster analyses of rbcL and COI sequence data (Figs 2, 3). In ML trees generated from SSU and rbcL sequence data, P. nuda is placed within a clade of predominantly Neosiphonia species (Figs 4, 5). Species of the genus Neosiphonia are characterized by having lateral branch or trichoblast initials every segment, tetrasporangia in spiral series, and abundant trichoblasts (Kim & Lee 1999); the absence of these character states in P. nuda distinguish it from Neosiphonia. Polysiphonia nuda appears most closely related to N. tongatensis in the rbcL
ML phylogeny, but this relationship is not supported by the SSU data. *Neosiphonia tongatensis* has scar cells one per segment in ¼ spiral series, abundant trichoblasts, and tetrasporangia developing in spiral series.

*Polysiphonia pentamera* Hollenberg (1968, p. 204)

Figs 53-58

**DESCRIPTION:** Assurgent erect branches to 3 cm tall, arising from an extensive prostrate system to 3 cm long, attached to substratum by rhizoids cut off from pericentral cells (Fig. 56); branching sparse to moderate in a subdichotomous pattern (Fig. 53); erect axes 175-250 (-300) μm in diameter, prostrate axes 175-300 μm in diameter; mid axis segments of erect axes mostly 0.5x as long as wide (Fig. 58); cortication absent; main axes with 5 pericentral cells (Fig. 57); branches forming laterally from the basal trichoblast cell (Figs 54, 55); trichoblasts long, thin, and tangled when mature, to 300 μm in length, with several dichotomies; scar cells not at every segment and sometimes rare; adventitious laterals infrequent, mostly linear in shape; tetrasporangia in long spiral series, 55 μm in diameter; spermatangial stichidia and cystocarps unknown.

**TYPE LOCALITY:** Eniwetok Atoll, Marshall Islands

**OTHER SOURCES:** Abbott 1999, Hollenberg 1968b

**SPECIMENS STUDIED:** Panama: WNC2009-s152 to s154 (PHYKOS-1995), STRI research station, Punta Galeta, Colón, B. Wysor & L. Sargent, 14 May 2009; WNC2009-s183 to s186 (PHYKOS-3529), Isla Planito near Coiba, Veraguas, B. Wysor & J. Alden, 15 Jan 2008; WNC2009-s187 to s192 (PHYKOS-3530), Bahia Honda near El Barranco, Veraguas, B. Wysor & J. Alden, 15 Jan 2008; WNC2009-s193 to s198 (PHYKOS-3531), Bahia Honda near El Barranco,

**Molecular Vouchers:** GenBank accession numbers HM573563, HM573564 (rbcL); HM560644, HM560643 (SSU); HM573510 (COI).

**Remarks:** Hollenberg’s original description of *Polysiphonia pentamera* indicates that branches arise “in connection with trichoblasts” but does not describe the exact relationship (Hollenberg 1968b). The origin of lateral branches is a character that has been previously treated as having three possible states: branches develop independently of trichoblasts, branches replace trichoblasts, and branches develop in the axils of trichoblasts (e.g. Stuercke & Freshwater 2008). Panamanian *P. pentamera* exhibits a fourth character state where lateral branches develop laterally from the basal trichoblast cell rather than in an axillary position immediately distal to the trichoblasts. This character state is also observed in *P. schneideri* Stuercke & Freshwater (Stuercke & Freshwater 2010; this study) and *P. mollis* J.D. Hooker & Harvey (Womersley 1979).

Five samples of *Polysiphonia pentamera* were collected in this study (Fig. 2). Four were collected from Pacific sites and one from a Caribbean site. All four Pacific specimens (PHYKOS-3529, -3550, -3531, and -3532) share the same rbcL sequence, which differs from the Caribbean *P. pentamera* rbcL sequence by only 0.74%. Investigation of COI divergence between these Pacific and Caribbean samples was not possible because COI sequences could not be generated for the Pacific specimens. ML phylogenies based on rbcL and SSU sequence data (Figs 4, 5) place *P. pentamera* in a clade with two other species that have 5-7 pericentral cells: *P. schneideri*, which has 5-7 pericentral cells, and an unidentified species of *Polysiphonia*
(PHYKOS-3535), which has strictly five pericentral cells. *Polysiphonia schneideri* differs from *P. pentamera* in predominantly having 5-7 pericentral cells and tetrasporangia that develop in straight series. The unidentified species of *Polysiphonia* differs from *P. pentamera* by having scar cells every segment in spiral series in erect axes near the apices and segments that are mostly 1x as long as wide. *Polysiphonia pentamera* also closely resembles *P. homoia*, but the latter species has tetrasporangia that develop in straight series.

**Polysiphonia pseudovillum** Hollenberg (1968, p. 73)

Figs 59-63

**Description:** Plants to 5 mm tall, erect branches arising from a prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Fig. 62); moderately branched in a subdichotomous pattern (Fig. 59); erect axes 60-70 µm in diameter, prostrate axes 70-100 µm in diameter; mid axis segments of erect axes mostly 1x as long as wide or only slightly longer (Fig. 61); cortication absent; main axes with 4 pericentral cells; branches replacing trichoblasts in development; trichoblasts to 420 µm in length, with only a few dichotomies (Fig. 60); scar cells common but highly variable in frequency, mostly every few segments in no regular pattern on both erect and prostrate axes; adventitious laterals occasionally present, mostly linear in shape; tetrasporangia in long slightly spiral series (Fig. 63); spermatangial stichidia and cystocarps unknown.

**Type Locality:** Johnston Atoll, Central Pacific

**Other Sources:** Abbott 1999, Hollenberg 1968a, Kapraun 1980a, Schneider & Searles 1991

MOLECULAR VOUCHERS: GenBank accession numbers HM573568 (rbcL); HM560650 (SSU); HM573524 (COI).

REMARKS: This specimen closely fits the description of Polysiphonia pseudovillum as originally described from the central Pacific by Hollenberg (1968a), however, some slight differences were observed. Polysiphonia pseudovillum is described as having erect branches to 1-2.7 mm in height and 40-60 µm in diameter, prostrate branches to 60 µm in diameter, and scar cells mostly one per segment on both erect and prostrate axes. The specimen observed in this study had erect branches to 5 mm in height and 60-70 µm in diameter, prostrate branches 70-100 µm in diameter, and scar cells common but with no regular frequency or pattern on both erect and prostrate axes. Gametophytic structures were not observed. As all available morphological characters fit the description of P. pseudovillum, the specimen examined in this study is identified as such.

Only one sample of Polysiphonia pseudovillum was collected in this study (Figs 2, 3). In ML phylogenies generated from rbcL and SSU sequence data, P. pseudovillum is placed within a clade of predominantly Neosiphonia species (Figs 4, 5). The Panamanian P. pseudovillum sample has four pericentral cells, rhizoids cut off from pericentral cells, and tetrasporangia that develop in spiral series, all of which are characteristics of the genus Neosiphonia (Kim & Lee 1999). However, in contrast to Neosiphonia species (Kim & Lee 1999), the Panamanian P. pseudovillum does not produce lateral branch or trichoblast initials every segment in a spiral pattern, nor does it appear that the thallus originally develops from a solid disc of rhizoids.
**Polysiphonia schneideri** B. Stuercke & D.W. Freshwater 2010 (in press)

Figs 64-71


**Description:** Plants to 3 cm (-15) tall, erect from discoid base with some branches becoming decumbent and forming a prostrate system attached to substratum by rhizoids cut off from pericentral cells (Fig. 65); moderately to highly branched in a dichotomous pattern (Fig. 64); erect axes 50-275 µm in diameter, prostrate axes 250-450 µm in diameter; mid axis segments of erect axes vary from 0.75-1.25x as long as wide; cortication absent; main axes with 5 or 6 (-7) pericentral cells; branches forming in the axils of trichoblasts or occasionally laterally from the basal trichoblast cell (Figs 68-70); trichoblasts long, thin, and tangled when mature, to 375 µm in length, with several dichotomies; scar cells not at every segment and sometimes rare; adventitious laterals lacking; tetrasporangia in long straight series, moderately distending segments, 55-65 (-85) µm in diameter (Fig. 67); spermatangial stichidia developing as a furcation of trichoblasts, 60-80x300 µm, with or without 1-3 sterile tip cells (Fig. 66); cystocarps globose, (200-) 350-470 µm in diameter, with narrow ostioles, short stalked with stalk cells noticeably larger than pericentral cells of axes (Fig. 71).

**Type Locality:** Wrightsville Beach, New Hanover County, North Carolina, USA


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MOLECULAR VOUCHERS: GenBank accession numbers HM573566, HM573565, GU385837 (rbcL); HM560629 (SSU); HM573516, HM573513, HM573515 (COI).

REMARKS: Polysiphonia schneideri is a newly described western Atlantic species (Stuercke & Freshwater 2010) that previously had been misidentified as Polysiphonia denudata (Dillwyn) Greville & Harvey, a species originally described from Southampton, England. True P. denudata has a single basal holdfast composed of a solid disc of rhizoids, basal cortication, trichoblasts one per segment in a spiral pattern, and tetrasporangia developing in spiral series (Maggs & Hommersand 1993). Polysiphonia schneideri has an initial basal holdfast with secondarily decumbent branches, no cortication, trichoblasts or scar cells not present every segment and in no particular pattern, and tetrasporangia developing in straight series (Stuercke & Freshwater 2010). These morphological characters were observed in all samples examined in this study. Panama records support the distribution of P. schneideri in the southern Caribbean as previously noted by Kapraun et al. (1983, as P. denudata; Stuercke & Freshwater 2010).

Three samples of Polysiphonia schneideri were collected in this study (Figs 2, 3). Sequences of P. schneideri samples from Panama and also North Carolina, Florida, and Bermuda are not identical but differ by only 0.093-0.74% at the rbcL and 1.87-3.34% at the COI locus. The rbcL values are within the range of intraspecific sequence variation observed in other
species of *Polysiphonia s.l.* (e.g. McIvor et al. 2001) and most likely reflect the wide geographic range of the species. ML phylogenies based on *rbcL* and SSU sequence data place *P. schneideri* in a clade with *P. pentamera* and *Polysiphonia sp.* 1 (Figs 4, 5). *Polysiphonia pentamera* is similar to *P. schneideri* in having rhizoids cut off from pericentral cells, branches that develop laterally from the basal trichoblast cell, and scar cells not at every segment but differs by having tetrasperangia in spiral series and strictly five pericentral cells. *Polysiphonia sp.* 1 is similar to *P. schneideri* in having rhizoids cut off from pericentral cells but differs by having strictly five pericentral cells and scar cells one per segment.

*Polysiphonia cf. sertularioides* (Grateloup) J. Agardh (1863, p. 969) 1

Figs 72-77

**Basionym:** *Ceramium sertularioides* Grateloup (1806)

**Synonyms:** *Polysiphonia flaccidissima* Hollenberg (1942, p. 783)


**Description:** Plants to 1 cm tall, erect branches arising from a limited prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Fig. 75); highly branched in an alternate pattern (Fig. 72); erect axes 40-70 µm in diameter, prostrate axes 110-150 µm in diameter; branchlets slightly basally attenuated (Fig. 73); mid axis segments of erect axes mostly 2x as long as wide (Fig. 76); cortication absent; main axes with 4 pericentral cells; branches forming in the axils of trichoblasts (Fig. 77); trichoblasts short, to 70 µm in length, mostly simple or with 1 dichotomy; scar cells one per segment in ¼ spiral series; adventitious laterals absent; tetrasperangia in moderately long spiral series, greatly distending
segments, (58-) 73-88 µm in diameter (Fig. 74); spermatangial stichidia and cystocarps unknown.


**Specimens Studied:** Panama: *WNC2009-s563 to s565 (PHYKOS-3226)*, Crawl Cay, Bocas del Toro, Diaz-Pulido & Riosmena, 28 Aug 2009.

**Molecular Vouchers:** GenBank accession numbers HM573546 (*rbc*L); HM560647 (SSU); HM573519 (COI).

**Remarks:** Samples representing three genetic species fit the morphological concept of *Polysiphonia sertularioides* (Grateloup) J. Agardh and are therefore referred to as *P. cf. sertularioides* 1 (PHYKOS-3226), *P. cf. sertularioides* 2 (PHYKOS-3534), and *P. cf. sertularioides* 3 (PHYKOS-2257 and PHYKOS-2309). Cluster analyses of *rbc*L and COI sequence data show that these species are clearly distinct from one another (Figs 2, 3). The *rbc*L sequence divergence values between the three are: 8.60% *P. cf. sertularioides* 1–*P. cf. sertularioides* 2; 7.77% *P. cf. sertularioides* 1–*P. cf. sertularioides* 3; and 6.38% *P. cf. sertularioides* 2–*P. cf. sertularioides* 3. These values are beyond the (predominantly ≤ 1.3%) intraspecific *rbc*L sequence divergence that has been observed in previous studies of *Polysiphonia s.l.* and agree better with interspecific *rbc*L sequence divergence values of 3.07% to 14.12% observed for other species within the genus (Kim & Yang 2005; Kim *et al.* 2004; McIvor *et al.* 2001). The cryptic diversity revealed by the *rbc*L and COI sequence data is apparent in the more conserved SSU gene as well (Fig. 5). Phylogenetic analyses of *rbc*L and SSU sequences resolve the three species in a strongly supported clade (*rB100; sB100*) that
occupies a basal position within the large clade of *Polysiphonia* and *Neosiphonia* species (Figs 4, 5).

Slight morphological differences are apparent between the three genetic species of *Polysiphonia cf. sertularioides* but are not sufficient to determine which species if any is true *P. sertularioides*. *Polysiphonia cf. sertularioides* 1 has branchlets that are slightly basally attenuated and segments that are mostly longer than wide. *Polysiphonia cf. sertularioides* 2 has branchlets that are more prominently basally attenuated and segments that are mostly longer than wide. *Polysiphonia cf. sertularioides* 3 has branchlets that are more prominently basally attenuated and segments that are mostly as long as or shorter than wide. Segment length is not an independently reliable character to determine species (Curiel *et al.* 2002; Kim *et al.* 1994, 2004; Stuerke & Freshwater 2008).

*Polysiphonia sertularioides* was originally described from Sète, France and is widely reported throughout the Mediterranean Sea and Pacific, Indian, and Eastern Atlantic Oceans (Adams 1991; Athanasiadis 1987; Gómez Garreta *et al.* 2001; John *et al.* 2004; Lobban & Tsuda 2003; Silva *et al.* 1996; Womersley 1979). This species has also been reported in the tropical western Atlantic, specifically Cuba and Venezuela (Ganesan 1990; Suárez 2005). *Neosiphonia flaccidissima* (Hollenberg) M.S. Kim & I.K. Lee 1999, which is currently regarded as a taxonomic synonym of *P. sertularioides*, was originally described from Laguna Beach, Orange County, CA, USA and is widely reported from the Pacific, Gulf of Mexico, Caribbean, and western Atlantic, specifically Venezuela, Colombia, and Belize (Abbott 1999; Abbott & Hollenberg 1976; Ballantine & Aponte 2005; Hollenberg 1968a; Hollenberg & Norris 1977; Kapraun & Norris 1982; Kapraun *et al.* 1983; Littler & Littler 2000; Wynne 2009). The synonymy of these species names gives *P. sertularioides* a near global distribution so its
presence in Panama is not unexpected. However, the cryptic diversity revealed by analyses of three different loci indicates that the status of \textit{P. sertularioides} as a single, widely distributed species needs further investigation.

Hollenberg defined several varieties of \textit{Polysiphonia flaccidissima} including \textit{P. flaccidissima} var. \textit{decimera} Hollenberg 1968a, \textit{P. flaccidissima} var. \textit{iki} Hollenberg 1968a, \textit{P. flaccidissima} var. \textit{lopi} Hollenberg 1968a, and \textit{P. flaccidissima} var. \textit{smithii} Hollenberg 1942 (Abbott & Hollenberg 1976; Hollenberg 1942, 1968a). \textit{Polysiphonia flaccidissima} var. \textit{smithii} is currently regarded as a taxonomic synonym of \textit{P. sertularioides}. The other varieties exhibit some combination of lateral branch frequency and prostrate branch development that do not fit the Panama samples.

\textit{Polysiphonia cf. sertularioides} (Grateloup) J. Agardh (1863, p. 969) 2

\textbf{Figs 78-83}

\textbf{BASIONYM:} \textit{Ceramium sertularioides} Grateloup (1806)

\textbf{SYNONYMS:} \textit{Polysiphonia flaccidissima} Hollenberg (1942, p. 783)


\textbf{DESCRIPTION:} Plants to 5 mm tall, erect branches arising from a limited prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Fig. 82); highly branched in an alternate pattern (Fig. 78); erect axes 40-50 \(\mu\text{m}\) in diameter, prostrate axes 60-100 \(\mu\text{m}\) in diameter; branchlets prominently basally attenuated (Fig. 79); mid axis segments of erect axes mostly 2-2.5x as long as wide (Fig. 81); cortication absent; main axes with 4 pericentral cells (Fig. 80); branches forming in the axils of trichoblasts (Fig. 83); trichoblasts
long, to 185 µm in length, simple; scar cells one per segment in $\frac{1}{4}$ spiral series; adventitious laterals absent; reproductive structures unknown.


**Specimens Studied:** Panama: WNC2009-s175 to s180 (PHYKOS-3534), Isla Afuera, Veraguas, B. Wysor & J. Alden, 16 Jan 2008.

**Molecular Vouchers:** GenBank accession numbers HM573547 (*rbcL*); HM560652 (SSU); HM573509 (COI).

**Remarks:** See remarks for *P. cf. sertularioides* (Grateloup) J. Agardh 1.
adventitious laterals absent; tetrasmorangia in moderately long spiral series, moderately
distending segments (Fig. 90); spermatangial stichidia and cystocarps unknown.

**Other Sources:** As *P. sertularioides*, Adams 1991, Womersley 1979; as *P.

**Specimens Studied:** Panama: *WNC2009-s159, s160 (PHYKOS-2257)*, West Limón Bay
Jetty, Punta Toro, Colón, D.W. Freshwater & N. Mamoozadeh, 17 May 2009; *WNC2009-s567 to
s569 (PHYKOS-2309)*, West Limón Bay Jetty, Punta Toro, Colón, D.W. Freshwater & N.
Mamoozadeh, 17 May 2009.

**Molecular Vouchers:** GenBank accession numbers HM573548 (*rbcL*); HM560646
(SSU).

**Remarks:** See remarks for *P. cf. sertularioides* (Grateloup) J. Agardh 1.

**Polysiphonia subtillissima** Montagne 1840: 199

**Figs 91-95**

**Synonyms:** *Polysiphonia subtillissima* var. *westpointensis* Harvey (1853, p. 45)

*Polysiphonia angustissima* Kützing (1864, p. 17)

**Description:** Plants to 4 (-15) cm tall, erect branches arising radially from a prostrate
branching system attached to substratum by rhizoids in open connection with pericentral cells
(Fig. 94); highly branched in a dichotomous pattern (Fig. 91); erect axes 30-100 µm in diameter,
prostrate axes 80-150 µm in diameter; mid axis segments of erect axes mostly 1-3x as long as
wide; cortication absent; main axes with 4 pericentral cells (Fig. 93); branches replacing
trichoblasts in development; trichoblasts long and slender, to 850 µm in length, typically with up
to one dichotomy (Fig. 92); scar cells present and obvious, variable in pattern and frequency; adventitious laterals occasionally present, mostly linear in shape; tetrasporangia greatly distending segments, in long straight series; spermatangial stichidia developmentally replacing trichoblasts, 30-50×120-265 μm, without sterile tip cells (Fig. 95); see remarks for cystocarp structure.

**Type Locality:** Cayenne, French Guiana


**Specimens Studied:** Panama: *WNC2009-s560 to s562 (PHYKOS-3271)*, STRI research station, Bocas del Toro, Bocas del Toro, D.W. Freshwater, 03 Sept 2009; North Carolina: *WNC2005-s009, s117, s118*, Neuse River, Oriental, Pamlico County, R. Peterson, 03 Sept 2003; *WNC2005-s035, s036, s122, s123*, Site CF-J, Snow’s Cut Park, Carolina Beach, New Hanover County, B. Stuercke, 22 May 2005.

**Molecular Vouchers:** GenBank accession numbers HM573575 (*rbcL*); HM560635 (*SSU*); HM573528 (*COI*).

**Remarks:** Womersley (1979, 2003) identified southern Australian material of *Polysiphonia subtilissima* as having spermatangial stichidia developmentally replacing trichoblasts with 4-6 sterile tip cells. This differs from New Zealand material of *P. subtilissima* identified by Adams (1991) as having spermatangial stichidia developing as a furcation of trichoblasts with presence of sterile tip cells not reported. Both of these southwestern Pacific descriptions differ from material of *P. subtilissima* from the tropical western Atlantic, which is described as having spermatangial stichidia developmentally replacing trichoblasts and without
sterile tip cells (Kapraun 1980b, unpublished manuscript; this study). Cystocarp structure for tropical western Atlantic specimens of *P. subtilissima* is predominantly unknown but is reported as urceolate and to 225 µm in diameter in specimens from Brazil examined by Oliveira (1969) and ovoid to urceolate, short stalked, and with a wide ostiole in specimens from St. Croix and Venezuela examined by Kapraun (unpublished manuscript). These descriptions appear similar to cystocarps reported as urceolate from New Zealand (Adams 1991) and ovoid to slightly urceolate and short stalked from southern Australia (Womersley 1979).

The varying descriptions of spermatangial stichidia reported for specimens identified as *Polysiphonia subtilissima* suggest that perhaps more than one species has been identified under this name. Southern Australian and New Zealand material not only differ from each other, but both differ from material identified as *P. subtilissima* from the tropical western Atlantic. Womersley (1979) identified southern Australian material as *P. subtilissima* based on examination of type material for the species. He further commented on the morphological similarity between *P. subtilissima* and *P. urceolata* (Lightfoot in Dillwyn) Greville, *P. abscissa* J.D. Hooker & Harvey, and *P. pacifica* Hollenberg and indicated that these taxa must be closely related and may truly represent only one or two species. Spermatangial stichidia with 3-7 sterile tip cells are reported as developmentally replacing trichoblasts for *P. urceolata* (as *Polysiphonia stricta* (Dillwyn) Greville [Maggs & Hommersand 1993]) and developmentally replacing the trichoblast with “one to several” sterile tip cells for *P. pacifica* (Hollenberg 1942). A satisfactory description of spermatangial stichidia development could not be found for *P. abscissa*.

Only one Panamanian *Polysiphonia subtilissima* specimen was collected in this study (Figs 2, 3), and although it clusters with North Carolina *P. subtilissima* specimens, divergences of 2.22-2.31% and 4.80% are observed in the *rbcL* and COI sequence data, respectively. This is
only slightly beyond the \( \leq 2.13\% \) (predominantly \( \leq 1.3\% \)) intraspecific \textit{rbcL} sequence divergence that has been observed in previous studies of \textit{Polysiphonia} (Kim \textit{et al.} 2004; McIvor \textit{et al.} 2001) but is within the range of interspecific COI sequence divergence observed in previous studies of other red algal species (Clarkston & Saunders 2010; Le Gall & Saunders 2010; Robba \textit{et al.} 2006; Yang \textit{et al.} 2008). No morphological differences were observed between the two groups of samples, suggesting the possible presence of cryptic species. Reproductive structures were not present in the Panamanian sample and tetrasporangia were observed in only one North Carolina sample. Examination of a complete set of reproductive characters may reveal differences between these groups of samples that distinguish them as separate morphological species.

ML phylogenies based on \textit{rbcL} and SSU sequence data place \textit{Polysiphonia subtilissima} in a clade with \textit{P. scopulorum} Harvey (Figs 4, 5). Both species have four pericentral cells, rhizoids in open connection with pericentral cells, branches that replace trichoblasts, and tetrasporangia that develop in straight series. \textit{Polysiphonia scopulorum} can be distinguished from \textit{P. subtilissima} by its smaller habit (to 1.5 cm in height), erect branches that are simple or sparsely branched, and growing on rocky substrate in strictly marine environments (Hollenberg 1968a). \textit{Polysiphonia subtilissima} also closely resembles \textit{P. atlantica}, but the latter can be distinguished by having erect branches developing unilaterally from prostrate axes to give plants a dorsiventral habit. \textit{Polysiphonia atlantica}, along with \textit{P. stricta} and \textit{P. pacifica}, is distantly related to \textit{P. subtilissima} and is resolved in a separate clade of \textit{Polysiphonia s.s.} species.

\textbf{Polysiphonia sp. 1}

Figs 96-99
DESCRIPTION: Plants with erect and prostrate axes attached to substratum by rhizoids cut off from pericentral cells (Figs 97, 98); erect axes 150-170 µm in diameter, prostrate axes 220-270 µm in diameter; mid axis segments of erect axes mostly 1x as long as wide or slightly shorter (Fig. 99); cortication absent; main axes with 5 pericentral cells (Fig. 96); relationship of branches to trichoblasts unknown; trichoblasts with several dichotomies; scar cells variable in pattern and frequency, one per segment in 1/5 spiral series in erect axes near apices but less common below; reproductive structures unknown.


MOLECULAR VOUCHERS: GenBank accession numbers HM573562 (rbcL); HM560651 (SSU); HM573508 (COI).

REMARKS: Limited material was available for Polysiphonia sp. 1 and only those characters included in the above description were observed. Several species of Polysiphonia are described as having five pericentral cells. These include P. denudata, P. schneideri, P. johnstonii Setchell & N.L. Gardner, P. guadalupensis Setchell & N.L. Gardner, P. abscissoides Womersley, P. bifurcata, P. homoia, and P. pentamera. Polysiphonia denudata and P. schneideri mostly exhibit six pericentral cells in erect and prostrate axes. Polysiphonia johnstonii has erect branches to 1 mm in diameter and segments mostly 1.5x as long as wide. Polysiphonia guadalupensis has four or five pericentral cells, segments that are 0.5x as long as wide or shorter, and basal cortication. Polysiphonia abscissoides has four or five pericentral cells, segments 8-10x as long as wide in some erect portions, and lateral branches that sometimes form conspicuous hooks. Polysiphonia bifurcata has five pericentral cells but entirely lacks trichoblasts and scar cells. Polysiphonia pentamera or P. homoia are species names that could
potentially fit *Polysiphonia sp.* 1, but these names are applied to other specimens in this study that clearly fit original and subsequent descriptions of these species. Both *P. pentamera* and *P. homoia* are described as having trichoblasts and scar cells that are infrequent; upper portions of erect axes in *Polysiphonia sp.* 1 have scar cells every segment in spiral series. It seems possible that *Polysiphonia sp.* 1 could represent a new species, but too few morphological characters were observed in the available material to determine if it is unique.

Only one sample representative of *Polysiphonia sp.* 1 was collected in this study (Figs 2, 3). ML analysis of *rbcL* sequence data resolves *Polysiphonia sp.* 1 within a strongly supported clade (rB100) of *Polysiphonia* species that have 5-7 pericentral cells, but this relationship is not supported in the SSU data (Figs 4, 5). *Polysiphonia sp.* 1 appears most closely related to *P. pentamera* in the *rbcL* ML phylogeny, with this relationship receiving strong support (rB97) (Fig. 4). Both of these species have five pericentral cells, but trichoblasts and scar cells in *P. pentamera* are infrequent. Cluster analyses show these species as differing by 2.68-2.87% at the *rbcL* and 6.0% at the COI loci. These values are slightly beyond those observed for intraspecific sequence divergence in previous studies (e.g McIvor *et al.* 2001; Clarkston & Saunders 2010).

**Polysiphonia sp. 2**

Figs 100-105

**DESCRIPTION:** Plants to 3 mm tall, erect branches arising from an extensive prostrate branching system attached to substratum by rhizoids cut off from the middle of pericentral cells (Figs 100, 101, 105); moderately branched at wide angles in an alternate to irregular pattern; erect axes 90-120 µm in diameter, prostrate axes 110-150 µm in diameter; mid axis segments of erect axes mostly 0.5x as long as wide; cortication absent; main axes with (7-) 8 pericentral cells
(Fig. 102); branches forming in the axils of trichoblasts (Fig. 104); trichoblasts to 240 µm in length, typically with 2 or 3 dichotomies; scar cells present and obvious, variable in pattern and frequency but mostly every few segments; adventitious laterals occasionally present, linear in shape; tetrasporangia moderately distending segments, in short spiral series, 70-78 µm in diameter (Fig. 103); spermatangial stichidia and cystocarps unknown.

**Specimens Studied:** Panama: *WNC2009-2574, s575 (PHYKOS-3537)*, Tervi Bight, Bocas del Toro, S. Fredericq, 13 Jul 2008.

**Molecular Vouchers:** GenBank accession numbers HM573551 (*rbcL*); HM560657 (SSU).

**Remarks:** While most species of *Polysiphonia* that have higher numbers of pericentral cells typically display a variable and relatively wide range of these cells, *Polysiphonia sp. 2* appears to have eight, sometimes seven, pericentral cells. Other species of *Polysiphonia sensu lato* that have no cortication, branches forming in the axils of trichoblasts, rhizoids cut off from pericentral cells, tetrasporangia in spiral series, and seven or eight pericentral cells include *Neosiphonia tepida* (Hollenberg) S.M. Guimarães & M.T. Fujii, *P. foetidissima* Cocks & Bornet, *P. nigra* (Hudson) Batters, and *P. paniculata* Montagne. However, *N. tepida* is larger (to 8 cm in height), segments of erect axes 1-2x as long as wide, rhizoids cut off from the proximal ends of pericentral cells, and narrow branch angles (Hollenberg 1958). Original and subsequent descriptions of *N. tepida* clearly fit another sample (FL05-2) included in phylogenetic analyses of this study. *Polysiphonia foetidissima* has a larger habit (to 11 cm in height), erect axes 20-40 µm in diameter, segments of erect axes 1-2x as long as wide, trichoblasts every segment, and branches with slight to marked basal attenuation (Maggs & Hommersand 1993). *Polysiphonia nigra* has a larger habit (to 30 cm in height), major axes 200-300 µm in diameter, and mostly 9-
13 pericentral cells that are spirally twisted (Maggs & Hommersand 1993). *Polysiphonia paniculata* has a larger habit (to 25 cm in height), ultimate branches with basal attenuation, segments of erect axes 2-4x as long as wide, and mostly 10-12 pericentral cells (Hollenberg & Norris 1977). Descriptions of *P. paniculata* clearly fit another sample included in phylogenetic analyses of this study.

Other possible species names that have the aforementioned character states include *Polysiphonia decipiens* Montagne, *P. isogona* J.D. Hooker & Harvey, and *P. confusa* Hollenberg. *Polysiphonia decipiens* has lateral branches that are occasionally hooked, and trichoblasts every segment (Womersley 1979). *Polysiphonia isogona* has rhizoids cut off from the proximal ends of pericentral cells, segments extending to 8x as long as wide in erect axes, and mostly 9 or 10 pericentral cells (Womersley 1979). Descriptions of *P. isogona* clearly fit another sample included in phylogenetic analyses of this study. *Polysiphonia confusa* has erect axes with segments 1-2.5x as long as wide, rhizoids cut off from the proximal ends of pericentral cells, branches that are attenuated at both ends, and mostly 8-10 pericentral cells (Abbott 1999; Abbott & Hollenberg 1976; Dawson 1964). *Polysiphonia decipiens* and *P. isogona* are both described as having habits much greater in height than that observed in *Polysiphonia sp. 2*.

*Polysiphonia sp. 2* may represent either a new species or an unusual variant of one of the above species. Only one sample of *Polysiphonia sp. 2* epiphytic on *Lobophora* J. Agardh was collected in this study (Figs 2, 3). ML analysis of *rbcL* sequence data resolves *Polysiphonia sp. 2* within a strongly supported clade (rB100) of *Polysiphonia s.l.* species that have ≥ 7 pericentral cells and are referred to as the “multipericentral cell group” (Choi et al. 2001) (Fig. 3). Placement within the multipericentral cell group is also well supported in the SSU ML phylogeny (sB89) (Fig. 4). Other species resolved in the clade differ from *Polysiphonia sp. 2* by
the character states listed above or by having a significantly greater number of pericentral cells (as in *P. fucoides* (Hudson) Greville), rhizoids in open connection with pericentral cells (as in *P. aterrima* J.D. Hooker & Harvey), or branches with marked basal attenuation (as in *P. constricta* Womersley).

**Incertae Sedis**

*‘Polysiphonia sp.’ 3*

Figs 106-111

**DESCRIPTION:** Plants to 0.8-1 cm tall, erect axes arising from a prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Fig. 109); branching sparse to moderate in an alternate pattern; apices of erect branches slightly arched toward prostrate axis (Figs 106, 111); erect axes 100-125 µm in diameter but widening to 175 µm in upper portions, giving branches an elongated clavate shape, prostrate axes (75-) 100-150 µm in diameter; mid axis segments of erect axes mostly 1x as long as wide and abruptly shifting to 0.5x as long as wide or less in upper portions (Figs 107, 108); cortication absent; main axes with 4 pericentral cells (Fig. 110); central axial cell distinctly enlarged; relationship of branches to trichoblasts unknown; trichoblasts with several dichotomies; scar cells variable in pattern and frequency, mostly every few segments but one per segment in ¼ spiral series in some portions; adventitious laterals common, linear in shape (Fig. 108); reproductive structures unknown.

**SPECIMENS STUDIED:** Panama: WNC2009-s239 to s241 (PYHKOS-2701), STRI Research Station, Punta Galeta, Colón, B.Wysor, 21 May 2009; WNC2009-s242 to s244 (PHYKOS-2635), Punta Gorda, Colón; WNC2010-s067, s068 (PHYKOS-3144), Cayos Zapatillas, Bocas
Molecular Vouchers: GenBank accession numbers HM573544 (rbcL); HM560655 (SSU); HM573523 (COI).

Remarks: The unique clavate shape of erect axes in ‘Polysiphonia sp.’ 3 is produced by a simultaneous increase in axis diameter and decrease in segment length, and distinguishes this species from the others collected. ‘Polysiphonia sp.’ 3 may represent a new species, but this is difficult to determine in the absence of reproductive structures because character states of its basic vegetative morphology (four pericentral cells; rhizoids cut off from pericentral cells; no cortication; variable scar cell pattern and frequency) are shared by many species.

Five samples of ‘Polysiphonia sp.’ 3 were collected from sites in both the Bocas del Toro and Galeta regions along the Caribbean coast of Panama (Figs 2, 3). These samples all share the same rbcL and COI sequence. ‘Polysiphonia sp.’ 3 is resolved as an independent lineage at the base of Polysiphonia s.l. in the rbcL ML analysis (Fig. 4). It is positioned as sister to Womersleyella setacea (Hollenberg) R.E. Norris in the SSU ML tree (Fig. 5) but with weak support (sB57). The resolved position of ‘Polysiphonia sp.’ 3 in these analyses not only supports its distinctness from other Polysiphonia s.l. species, but also suggest that it may not be part of this group.

Conclusion

Nineteen species of Polysiphonia s.l. (ten new reports, seven uncertainly identified, and one new species) were recovered from samples obtained along the Pacific and Caribbean coasts of Panama. This number is significantly greater than the two named Polysiphonia species.
previously reported for Panama and better reflects the *Polysiphonia* species reports from the neighboring countries of Colombia and Costa Rica. It seems likely that further collection of *Polysiphonia s.l.* samples, especially from the Pacific coast, would yield even higher species counts and help clarify the several taxonomic issues raised in this study.

**REFERENCES**


Harvey W.H. 1836. Algae. In J.T. Mackay (Ed.), Flora hibernica comprising the Flowering Plants Ferns Characeae Musci Hepaticae Lichenes and Algae of Ireland arranged according to the natural system with a synopsis of the genera according to the Linnaean System (pp. 157-254). William Curry Jun and Company, Dublin, Ireland.


Table 1. Primer sequences utilized in amplification and sequencing reactions of *rbcL*, SSU, and COI.

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<th>Label</th>
<th>Primer Sequence</th>
<th>Reference</th>
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**Table 2.** COI sequence divergences reported in previous studies of red algae.

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<th>Interspecific Sequence Divergence (%)</th>
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<td>3.2-16.1</td>
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Fig. 1. Map of Caribbean and Pacific Panama sampling sites for collection of Polysiphonia s.l. species. A. Collection sites in the Bocas del Toro region. B. Collection sites in the Colón and Galeta region. C. Collection sites offshore of Isla Coiba.
Fig. 2. Unweighted Pair Group Method with Arithmetic mean rbcL cluster analysis for 113 Polysiphonia s.l. samples. The 2.13% and 1.3% sequence divergence levels are indicated by vertical dashed lines. Panamanian species are shown in boldface type.
Fig. 3. Unweighted Pair Group Method with Arithmetic mean COI cluster analysis for 74 *Polysiphonia* s.l. samples. The 4.80% sequence divergence level is indicated by the vertical dashed line. Panamanian species are shown in boldface type.
Fig. 4. Maximum likelihood rbcL tree (lnL= −14603.23387) for 50 Polysiphonia s.l. and two outgroup samples. Bootstrap proportion values are shown for each node when >50. Panamanian species are shown in boldface type.
Fig. 5. Maximum likelihood SSU tree (lnL = – 4146.45008) for 43 Polysiphonia s.l. and one outgroup samples. Bootstrap proportion values for branches are shown for each node when >50. Panamanian species are shown in boldface type.
Figs 6-9. Morphology of *Neosiphonia ferulacea*. **Fig. 6.** Habit of erect axes, PHYKOS-2287, scale = 0.2 mm, WNC2009-s166. **Fig. 7.** Apical portion of erect axes bearing short trichoblasts, PHYKOS-2287, scale = 50 µm, WNC2010-s046. **Fig. 8.** Cross section of branch axis showing central axial cell and four pericentral cells, PHYKOS-2287, scale = 50 µm, WNC2010-s039. **Fig. 9.** Prostrate axis showing a rhizoid that is cut off from the pericentral cell, PHYKOS-2287, scale = 50 µm, WNC2010-s047.
Figs 10-14. Morphology of *Neosiphonia tongatensis*. Fig. 10. Habit of erect axes, PHYKOS-2704, scale = 0.20 mm, WNC2009-s262. Fig. 11. Apical portion of erect axes bearing trichoblasts, PHYKOS-2704, scale = 50 µm, WNC2009-s262. Fig. 12. Branch apex with immature spermatangial stichidium (arrowhead) developing as a furcation of the trichoblast, PHYKOS-2704, scale = 20 µm, WNC2009-s263. Fig. 13. Portion of erect axis flattened to show central axial cells (arrowhead), four pericentral cells per segment, and scar cells (arrows), PHYKOS-2704, scale = 50 µm, WNC2009-s262. Fig. 14. Prostrate axis with rhizoids that are cut off from the pericentral cells, PHYKOS-2704, scale = 50 µm, WNC2009-s263.
Figs 15-18. Morphology of Neosiphonia sp. 1. **Fig. 15.** Apical portion of erect axis bearing a single trichoblast, PHYKOS-3536, scale = 50 µm, WNC2010-s042. **Fig. 16.** Portion of erect axis flattened to show four pericentral cells per segment and scar cells (arrows), PHYKOS-3536, scale = 50 µm, WNC2010-s043. **Fig. 17.** Prostrate axis with rhizoid that is cut off from the pericentral cell, PHYKOS-3536, scale = 20 µm, WNC2010-s042. **Fig. 18.** Mid axis segments of erect branch showing scar cells (arrows), PHYKOS-3536, scale = 50 µm, WNC2010-s042.
**Figs 19-24.** Morphology of *Polysiphonia binneyi*.  
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### Appendix 1.

Collection information, sample number/source, and GenBank accession numbers for Rhodomelaceae examined in this study. Identical accession numbers indicate specimens with identical sequences.

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<td>01 Nov 2004, DW Freshwater &amp; M Hommersand</td>
<td>GU385832</td>
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<td>FL05-7 West Summerland Key, Monroe Co., FL, USA</td>
<td>28 Feb 2005, B Stuercke</td>
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<td>Polysiphonia echinata  Harvey</td>
<td>FL09-40 Lake Surprise, Key Largo, Monroe Co., FL, USA</td>
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<td>FL09-42 Lake Surprise, Key Largo, Monroe Co., FL, USA</td>
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<td>MEX04-8</td>
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<td>MEX04-10</td>
<td>Blue Bay Marina, Cancun, Yucatan, Mexico</td>
<td>29 Feb 2004, DW Freshwater</td>
<td>HM573557, HM573505</td>
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**Polysiphonia elongata** (Hudson) Sprengel
3 McIvor et al. (2001)
Fanad, N. Donegal, Ireland
17 May 1998, CA Maggs
AF342911

**Polysiphonia fibrata** (Dillwyn) Harvey
3 McIvor et al. (2001)
Marble Hill, N. Donegal, Ireland
05 Aug 1993, CA Maggs
AF342915

**Polysiphonia fibrillosa** (Dillwyn) Sprengel
3 McIvor et al. (2001)
Marble Hill, N. Donegal, Ireland
05 Aug 1993, CA Maggs
AF342912

**Polysiphonia forfex** Harvey
3 McIvor et al. (2001)
Biarritz, Aquitaine, France
15 Jul 1999, CA Maggs
AF342910

**Polysiphonia fucoides** (Hudson) Greville
3 NC-12 South Masonboro Inlet Jetty, New Hanover Co., NC, USA
04 May 2005, DF Kapraun, DW Freshwater & B Stuercke
EU492913, HM560627, HM573496

**Polysiphonia havane**nsis sensu Børgeesen
2 PHYKOS-2628 West Limón Bay Jetty, Punta Toro, Colón, Panama
20 May 2009, B Wysor
HM573554, HM560641

**Polysiphonia homoia** Setchell & Gardner
PHYSKOS-3185 Cayos Zapatillas, Bocas del Toro, Panama
25 Aug 2009, B Wysor & DW Freshwater
HM573554

**Polysiphonia howei** Hollenberg
Talisoy, Vivac Catanduanes, Phillipines
14 May 1988, J West
AY237282

**Polysiphonia howei** Hollenberg
2 PHYKOS-3141 Cayos Tigres, Bocas del Toro, Panama
25 August 2009, B Wysor
HM573543, HM560656, HM573520

**Polysiphonia howei** Hollenberg
PHYSKOS-3526 Flat Rock Beach, Isla Colón, Bocas del Toro, Panama
17 Jan 2007, DW Freshwater
HM573543, HM573521
**Polysiphonia isogona** Harvey

PHYKOS-3527 Flat Rock Beach, Isla Colón, Bocas del Toro, Panama 17 Jul 2008, DW Freshwater

PHYKOS-3528 Swan Cay, Isla Colón, Bocas del Toro, Panama 19 Jul 2008, M Albis

**Polysiphonia kapraunii** Stuercke & Freshwater

PHYKOS-2617 Punta Gorda, Colón, Panama 20 May 2009, DW Freshwater

**Polysiphonia macrocarpa** (Agardh) Sprengel

PHYKOS-2561 Punta Gorda, Colón, Panama 20 May 2009, DW Freshwater

**Polysiphonia morrowii** Harvey

PHYKOS-2627 Punta Gorda, Colón, Panama 20 May 2009, S Schmitt

**Polysiphonia muelleriana** Agardh

NZ04-139 Curio Bay, South Island, New Zealand 28 Oct 2004, DW Freshwater & M Hommersand

**Polysiphonia nuda** sp. nov.

PHYKOS-2613 Parque de Juventud, Calle Primero, Colón, Panama 20 May 2009, B Wysoz

**Polysiphonia pacifica** Hollenberg

PHYKOS-1995 STRI research station, Punta Galeta, Colón, Panama 14 May 2009, B Wysoz & L Sargent

**Polysiphonia paniculata** Montagne

Kim et al. (2004) Seno Otway, Punta Arenas, Chile AY396041

Zuccarello et al. (2004) Four Mile Beach, Santa Cruz, CA, USA AY617144

**Polysiphonia pentamera** Hollenberg

PHYKOS-2630 Punta Gorda, Colón, Panama 20 May 2009, DW Freshwater

**Polysiphonia pentamera** Hollenberg

PHYKOS-1995 STRI research station, Punta Galeta, Colón, Panama 14 May 2009, B Wysoz & L Sargent

**Polysiphonia pacifica** Hollenberg

Choi et al. (2001) Bradys Beach, Bamfield, B.C., Canada 29 Apr 1998, J Warneboldt & JT Harper AF427533

Kim et al. (2004) Seal Rock, OR, USA AY396036

Kim et al. (2004) Seno Otway, Punta Arenas, Chile AY396041
Polysiphonia pernacola Adams

NZ04-243 Paua Beach, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand
HM573576
HM573495

NZ04-244 Paua Beach, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand
HM573576

NZ04-263 Oban, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand
HM573576

NZ04-291 Ulva Island near Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand
HM573576
HM560637
HM573495

Polysiphonia pseudovillum Hollenberg

2PHYKOS-3533 Flat Rock Beach, Isla Colón, Bocas del Toro, Panama
17 Jul 2008, DW Freshwater
HM573568
HM560650
HM573524

Polysiphonia schneideri Stuercke & Freshwater

BERMUDA Tucker’s Bay, Harrington Sound, Bermuda
17 Mar 2009, CW Schneider & CE Lane
GU385836

FL05-04 Sebastian Inlet, Indian River County, FL, USA
26 Feb 2005, B Stuercke
HM573567
HM573514

NC-2 Cassamir Wreck (WR2), Onslow Bay, NC, USA
02 Jul 2004, DW Freshwater & K Johns
EU492912

2PHYKOS-2454 Panama Canal north ferry terminus, Colón, Panama
19 May 2009, K Larson & L McCann
HM573566
HM560629
HM573516

PHYKOS-2689 STRI research station, Punta Galeta, Colón, Panama
21 May 2009, S Schmitt
HM573565
HM573513

PHYKOS-3189 Cayos Zapatillas, Bocas del Toro, Panama
25 Aug 2009, DW Freshwater
GU385837
HM573515

Polysiphonia scopulorum Harvey

3Kim et al. (2004)

Devil’s Punchbowl, OR, USA
AY396039
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<td><strong>Polysiphonia cf. sertularioides</strong> (Grateloup) Agardh 1</td>
<td>2PHYKOS-3226 Crawl Cay, Bocas del Toro, Panama 28 Aug 2009, Diaz-Pulido &amp; Riosmena</td>
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<td><strong>Polysiphonia cf. sertularioides</strong> (Grateloup) Agardh 2</td>
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<td>NZ04-147 Lonnekers Nugget, Stewart Island, New Zealand 29 Oct 2004, DW Freshwater &amp; M Hommersand</td>
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<td>NZ04-246 Paua Beach, Stewart Island, New Zealand 01 Nov 2004, DW Freshwater &amp; M Hommersand</td>
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<td>NZ04-250 Paua Beach, Stewart Island, New Zealand 01 Nov 2004, DW Freshwater &amp; M Hommersand</td>
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<td><strong>Polysiphonia strictissima</strong> Hooker &amp; Harvey</td>
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<td>NC-24 Snow’s Cut Park, New Hanover Co., NC, USA 22 May 2005, B Stuercke &amp; JB Landry</td>
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<td>13 Jul 2008, S Fredericq</td>
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<td>PHYKOS-3145</td>
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<td>²PHYKOS-3538</td>
<td>Swan Key, Isla Colón, Bocas del Toro, Panama</td>
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<td>14 Jul 2007, DW Freshwater</td>
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<td>Polysiphonia sp.</td>
<td>⁴NZ04-515</td>
<td>Mataikona, Castle Point, North Island, New Zealand</td>
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<td>16 Nov 2004, DW Freshwater &amp; M Hommersand</td>
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<td>Polysiphonia sp.</td>
<td>³Kim et al. (2004)</td>
<td>Las Cruses, Central Chile</td>
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<td>Vertebrata lanosa (Linnaeus) Christensen</td>
<td>Zuccarello et al. (2004)</td>
<td>Nahant, Providence, Providence Co., RI, USA</td>
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<tr>
<td>Womersleyella setacea (Hollenberg) Norris</td>
<td>³Choi et al. (2001)</td>
<td>NUIG Marine Algal Culture Collection, Italy</td>
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1. Sequence published as *Polysiphonia elongella* Harvey.
2. Sequence used in ML analysis of *rbcL* and SSU data.
3. Sequence used in ML analysis of *rbcL* data.
4. Sequence used in ML analysis of SSU data.
CHAPTER TWO: Taxonomic notes on five species of *Polysiphonia sensu lato* (Ceramiales, Florideophyceae) from the Caribbean
ABSTRACT

At least 28 distinct Polysiphonia sensu lato species have been reported from the Caribbean. These reports are mostly found in species checklists that have been developed for localities within the Caribbean. Molecular assisted identification using plastid-encoded rbcL and mitochondria-encoded COI loci identified five species of Polysiphonia sensu lato from 16 Florida and Caribbean Mexico samples. Morphological character states were examined and used to identify each sample at the rank of species. Descriptions are provided and the phylogenetic relationships of the five species determined through maximum likelihood analyses of rbcL and nuclear-encoded SSU sequence data. These integrated molecular and morphological analyses have resulted in two new reports for Caribbean Mexico, the transfer of five Polysiphonia species to Neosiphonia, and the placement of one species in synonymy.
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INTRODUCTION

*Polysiphonia* Greville (Rhodomelaceae) is a large red algal genus including nearly 450 species names, of which nearly 200 are currently accepted (e.g. Kim *et al.* 2002). Species of *Polysiphonia sensu lato* (s.l., “in the broad sense”) are globally common and are characterized by a delicate habit composed of filamentous main axes and branches that are segmented and polysiphonous. *Polysiphonia s.l.* species are typically identified by their rhizoid attachment, number of pericentral cells, cortication, nature and arrangement of trichoblasts, origin of branches in relation to trichoblasts, tetraspore arrangement, nature of the spermatangial branches, and number of carpogonial branch cells (Abbott 1999; Hollenberg 1968a; Hollenberg & Norris 1977; Kapraun 1980; Kapraun & Norris 1982; Kim *et al.* 2002; Schneider & Searles 1991).

A wide range of morphological variability is encountered among the many *Polysiphonia s.l.* species. This has led to much debate as to the classification of species within the genus. The genus *Neosiphonia* M.-S. Kim & I.K. Lee was segregated from *Polysiphonia* by Kim & Lee (1999) so that *Polysiphonia s.l.* now includes species that are predominantly placed in *Neosiphonia* or *Polysiphonia*, although less speciose genera are also included. To revise the classification of *Polysiphonia s.l.* species, Kim *et al.* (2000) suggested the use of “independent comparative evidence” to determine which morphological characters are relevant to species identification. The recent coupling of morphological characters with DNA sequence data is helping establish a better classification of *Polysiphonia* species based on natural relationships.

Molecular assisted identification (MAI) through use of the plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*) has proven useful for discriminating species of *Polysiphonia s.l.* Previous studies of *rbcL* sequence data in species of *Polysiphonia s.l.* have observed comparatively low intraspecific and high interspecific sequence
divergences, with values ranging from 0-1.3% (with an instance of 2.13%) and 2.6-14.12%, respectively (Kim & Yang 2005; Kim et al. 2004; McIvor et al. 2001). A relatively distinct break between intra- and interspecific sequence variation makes rbcL a useful tool for aiding species identifications.

Another form of MAI utilizes the 5’ end of the mitochondria-encoded cytochrome c oxidase subunit I (COI) for barcoding, which involves the use of a short diagnostic segment of DNA for species discrimination. COI has several advantages for barcoding including limited recombination, low prevalence of indels, uniparental inheritance, and a relatively rapid rate of evolution (Hebert et al. 2003; Saccone et al. 1999; Saunders 2005). Previous studies of red algae have shown COI to be successful in the discrimination of closely related and cryptic species as well as geographic groups within a species (Clarkston & Saunders 2010; Le Gall & Saunders 2010; Robba et al. 2006; Saunders 2005; Yang et al. 2008). The utility of COI barcoding within Polysiphonia s.l. has yet to be established.

Phylogenetic relationships among Polysiphonia s.l. species can also be determined using molecular data. The relatively slow rate of evolution among nuclear-encoded 18S rDNA (SSU) genes limits intraspecific sequence variation and makes this locus appropriate for exploring taxonomic relationships above the species level. Analyses of SSU sequence data showed that Polysiphonia s.l. was polyphyletic with respect to other genera (Choi et al. 2001). The rbcL locus exhibits a rate of evolution faster than that of SSU, making it appropriate for examining species level relationships, and the use of rbcL to determine species relationships has been common in recent studies of Polysiphonia s.l. (Kim et al. 2004, 2005; McIvor et al. 2001; Stuercke & Freshwater 2008, 2010). Phylogenetic relationships within Polysiphonia s.l. can be
thoroughly evaluated when analyses of SSU and \textit{rbcL} are compared with one another (Stuercke 2006).

Study of the Caribbean marine algal flora (MAF) has been relatively common and has resulted in reports of at least 28 distinct \textit{Polysiphonia s.l.} species (e.g. Wynne 1998). Early reports of \textit{Polysiphonia s.l.} come from Harvey (1853) and Børgesen (1918), who described several species from Florida, USA and the West Indies, respectively. More recent reports are the result of MAF assessment from expeditions made throughout the Caribbean (e.g. Almodovar & Ballantine 1983; Ballantine & Aponte 1997; Littler & Littler 2000; Mateo-Cid \textit{et al.} 2006; Taylor 1929, 1941, 1942, 1945, 1960, 1969). These studies were not focused exclusively on \textit{Polysiphonia s.l.} species but rather attempted to assess all encountered flora. Caribbean studies specific to \textit{Polysiphonia s.l.} include reports of species from Belize (Kapraun \textit{et al.} 1983) and Colombia and Venezuela (Kapraun & Norris 1982).

The purpose of this study was to complete integrated molecular and morphological analyses of \textit{Polysiphonia s.l.} samples collected from Caribbean Mexico and Florida. Examination of morphology and MAI using \textit{rbcL} and COI sequence data were used to identify species of \textit{Polysiphonia s.l.} within samples while phylogenetic analyses of \textit{rbcL} and SSU sequence data were used to determine the relationships of these species.

\textbf{MATERIAL AND METHODS}

\textbf{Collection}

Samples of \textit{Polysiphonia} were collected from intertidal and subtidal substrates in Caribbean Mexico and Florida by snorkeling (Appendix 1). Samples were dried in silica gel dessicant (Chase & Hills 1991) and deposited in the silica collection at the Center for Marine

**Morphological Data and Analysis**

Specimens and slides were observed using an Olympus SZH dissecting microscope (Olympus America Inc., Center Valley, PA, USA) and a Nikon Labophot-2 compound microscope (Nikon Inc., Melville, NY, USA). Images were captured using a Zeiss Axio Imager.Z1 compound microscope (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) fitted with an AxioCam MRc 5 camera system (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) or an Olympus BX41 compound microscope (Olympus America Inc., Center Valley, PA, USA) fitted with a Roper Scientific Photometrics® CoolSnap™ camera (Photometrics, Tucson, AZ, USA). Observations of specimens collected in this study were used to write species descriptions that emphasize the morphological characters examined by Stuercke & Freshwater (2008), and use supplementary information from the literature for character states that were not observed.
DNA Extraction and Sequencing

DNA was extracted from specimens according to Hughey et al. (2001) with an additional cleaning step using the OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Orange, CA, USA). SSU, rbcL, and COI were amplified following the basic PCR recipe outlined in Freshwater et al. (2005) but using GOTaq DNA polymerase and buffer (Promega, Madison, WI, USA). Amplifications were performed in an MJ Research PTC-100™ (Watertown, MA, USA) or Eppendorf Mastercycler gradient (Hamburg, Germany) thermocycler. The thermocycling protocol followed Freshwater et al. (2000) but with 35 cycles of denaturing, annealing at 40, 45, or 50 °C, and an elongation period of 90 s. Amplification products were cleaned with a Stratagene StrataPrep® PCR Purification Kit (Stratagene, La Jolla, CA, USA) and used as templates in BigDye® Terminator v3.1 sequencing reactions (Applied Biosystems, Foster City, CA, USA). Reactions were cleaned by spinning through Sephadex® G-50 columns, and run on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA USA). Sequences were edited and assembled using Sequencher™ (version 4.9, GeneCodes Corporation, Ann Arbor, MI, USA). Primers utilized in amplification and sequencing reactions are listed in Table 1.

An rbcL and COI sequence were each generated for as many samples as possible. These loci are appropriate for the examination of inter- and intraspecific relationships and were used as barcodes to objectively assign samples to species (e.g. Millar & Freshwater 2005; Clarkston & Saunders 2010). An SSU sequence was generated for one sample per species. Both rbcL and SSU sequence data were used to identify groupings of species (clades) within Polysiphonia s.l.
Molecular Data Analyses

Sequences of *Polysiphonia* generated in this study were combined with *rbcL* and SSU sequences available from GenBank as well as some from unpublished studies (Kelly & Freshwater unpublished) and initially aligned using MacClade (v.4, Maddison & Maddison 2000). SSU sequence data were also aligned using the ClustalW multiple sequence alignment feature of the program Molecular Evolutionary Genetics Analysis (MEGA) (www.megasoftware.net; Kumar *et al.* 2008; Tamura *et al.* 2007) followed by further manual adjustment. Characteristics of the DNA sequence data sets were determined using MacClade and PAUP (v.4, Swofford 2002). COI and *rbcL* data sets were used for MAI/DNA barcoding to objectively assign samples to species. The COI alignment consisted of 74 taxa and included 605 sites in the analysis, of which 248 (40.99%) were variable. The *rbcL* alignment consisted of 113 taxa and included 1081 sites in the analysis, of which 402 (37.19%) were variable. SSU and reduced (identical sequences removed) *rbcL* data sets were used for Maximum Likelihood (ML) analyses to infer phylogeny within *Polysiphonia s.l.* The SSU alignment consisted of 44 taxa and included 1602 sites in the analysis, 158 (9.86%) of which were variable. The *rbcL* alignment consisted of 52 taxa and included 1334 sites in the analysis, 528 (39.58%) of which were variable.

MEGA was used to perform Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Neighbor Joining (NJ) cluster analyses on COI and *rbcL* sequence data for MAI/DNA barcoding. A p-distance model was used in all analyses. ML analyses were performed on *rbcL* and SSU sequence data separately using the program Genetic Algorithm for Rapid Likelihood Inference (GARLI) (www.bio.utexas.edu/faculty/antisense/garli/Garli.html; Zwickl 2006). The ML analyses included ten separate searches from random starting trees that
used default parameters, including 10000 generations without improving topology and allowing model estimation during the run. GARLI was also used to perform a total of 1003 ML bootstrap (BS) replicates on the rbcL dataset and 1000 ML BS replicates on the SSU dataset.

MOLECULAR RESULTS AND DISCUSSION

Molecular Assisted Identification

Five species from 16 Florida and Caribbean Mexico samples were resolved in UPGMA and NJ cluster analyses of rbcL sequence data (Fig. 1, only UPGMA cluster diagram shown). Values of inter- and intraspecific rbcL sequence divergence from previous studies of Polysiphonia s.l. were used for species distinctions (Kim & Yang 2005; Kim et al. 2004; McIvor et al. 2001). Four species from ten Florida and Caribbean Mexico samples were resolved in UPGMA and NJ cluster analyses of COI sequence data (Fig. 2, only UPGMA cluster diagram shown). Fewer species were resolved in this analysis because the COI locus would not amplify in some samples. Species distinctions were based on the 4.80% COI sequence divergence between samples identified as Polysiphonia subtilissima 1 (NC-24) and P. subtilissima 2 (PHYKOS-3271). These samples differed by 2.22% in the rbcL data, which is just beyond the range of intraspecific rbcL sequence divergence observed in previous studies of Polysiphonia s.l. (McIvor et al. 2001). A sequence divergence of 2.22% in the P. subtilissima rbcL data corresponds to 4.80% sequence divergence in the COI data; the latter value was therefore used as the intraspecific-interspecific break level of sequence divergence in the COI data. In comparison, P. pentamera (PHYKOS-1995) and Polysiphonia sp. 1 (PHYKOS-3535) differ by 2.68% and 5.95% at the rbcL and COI loci, respectively. These values represent a lower limit of interspecific sequence divergence. Other useful comparisons are not available because of missing
sequence data at the COI locus for some samples. Values of intraspecific sequence divergence are discussed in the remarks of each species.

**Molecular Systematics**

The phylogenetic relationships of Florida and Caribbean Mexico species within *Polysiphonia s.l.* were determined through ML analyses of SSU and reduced *rbcL* sequence data (Figs 3, 4). The specific relationships among these species are discussed later in the remarks for each species. The *rbcL* topology contains several strongly supported clades as well as groups of clades and species with unresolved relationships. Near the base of the topology are two well-supported clades (*rbcL* bootstrap values [rB] = 100) including species typically regarded as *Polysiphonia sensu stricto* (s.s., “in the strict sense”). These species all have four pericentral cells and rhizoids in open connection with the pericentral cells. Species in these clades include *Polysiphonia pacifica* Hollenberg, *P. stricta* (Dillwyn) Greville, the generitype, *P. morrowii* Harvey, *P. kapraunii* B. Stuercke & D.W. Freshwater, *P. atlantica* Kapraun & J.N. Norris, *P. macrocarpa* (C. Agardh) Sprengel, *P. scopulorum* var. *villum* (J. Agardh) Hollenberg, *P. scopulorum* Harvey, and *P. subtilissima* Montagne. Another well-supported clade (rB100) contains a group of species referred to as the “multipercentral cell group” by Choi et al. (2001). These species all have 7+ pericentral cells and rhizoids cut off from pericentral cells. Species in this clade include *P. isogona* Harvey, *Neosiphonia tepida* (Hollenberg) S.M. Guimarães & M.T. Fujii, *P. fucoides* (Hudson) Greville, *P. paniculata* Montagne, *P. aterrima* J.D. Hooker & Harvey, *P. constricta* Womersley, and *Polysiphonia sp. 2.*

The majority of species in the *rbcL* ML topology are placed within a large unsupported (rB<50) group of species and variously supported clades that contain both *Neosiphonia* and
Polysiphonia species. Although species of Neosiphonia are scattered throughout the topology, a strongly supported clade (rB100) of predominantly Neosiphonia species is apparent within this large grouping. Species in this clade include N. harveyi (J. Bailey) M.S. Kim, H.G. Choi, Guiry & G.W. Saunders, P. strictissima J.D. Hooker & Harvey, P. forfex Harvey, N. sphaerocarpa (Børgesen) M.S. Kim & I.K. Lee, Neosiphonia sp. 1, N. ferulacea (Suhr ex J. Agardh) S.M. Guimarães & M.T. Fujii, P. pseudovillum Hollenberg, P. nuda sp. nov., N. tongatensis (Harvey ex Kützing) M.S. Kim & I.K. Lee, and N. bajacali (Hollenberg) N. Mamoozadeh & D.W. Freshwater.

The major clades and species resolved in the rbcL topology are also resolved in the SSU topology. Two clades containing Polysiphonia s.s. species are strongly supported (SSU bootstrap values [sB] = 99 and 100) and received moderate support (sB78) for being resolved as a monophyletic group. Another well-supported (sB91) clade contains “multipericentral cell” species of multiple genera including Polysiphonia, Boergeseniella Kylin, Enelittosiphonia Segi, and Vertebrata S.F. Gray. The majority of species are contained within a large, strongly supported (sB91) grouping of species and clades that include both Neosiphonia and Polysiphonia species. A clade of predominantly Neosiphonia species is contained within this large grouping and received moderate support (sB87).

TAXONOMIC OBSERVATIONS AND REMARKS

Genus Neosiphonia

Neosiphonia bajacali (Hollenberg) N. Mamoozadeh & D.W. Freshwater, comb. nov.

Figs 5-8
Basionym: Polysiphonia bajacali Hollenberg (1961, p. 347)

Description: Plants to 3 (-6) cm tall, chiefly erect from limited basal prostrate axes attached to the substratum by rhizoids that are cut off from pericentral cells (Fig. 7); highly branched; branching in a subdichotomous to alternate pattern (Fig. 5); erect axes (150-) 200-300 µm in diameter, prostrate axes (175-) 225-325 µm in diameter; branchlets basally attenuated; mid axis segments of erect axes mostly 1x as long as wide; light cortication present in some mature prostrate axes; main axes with four pericentral cells (Fig. 6); branches replacing trichoblasts in development; trichoblasts with several dichotomies, to 750 µm in length; scar cells one per segment in ¼ spiral series; adventitious laterals rare; tetrasporangia greatly distending segments, developing in short spiral series (Fig. 8); spermatangial stichidia developing as a furcation of trichoblasts, without sterile tip cells; cystocarps urceolate to globose.

Type Locality: Isla Guadalupe, Baja California

Other Sources: Abbott & Hollenberg 1976, Hollenberg 1961


Molecular Vouchers: GenBank accession numbers HM573572 (rbcL); HM560659 (SSU); HM573526 (COI).

Remarks: Hollenberg (1961) originally described Neosiphonia bajacali (as Polysiphonia bajacali) as ecorticate but in a later description indicates that light basal cortication may be present (Abbott & Hollenberg 1976). Samples in this study infrequently displayed cortication in limited portions of some mature prostrate axes. The original and subsequent descriptions of N.
bajacali do not indicate whether rhizoids are in open connection or are cut off from pericentral cells (Abbott & Hollenberg 1976; Hollenberg 1961; Stewart 1991). Samples in this study have rhizoids that are cut off from pericentral cells (Fig. 7).

*Neosiphonia bajacali* displays a combination of character states unique to *Neosiphonia*. These include having four pericentral cells, rhizoids cut off from pericentral cells, lateral branch or trichoblast initials one per segment in a spiral pattern, tetrasporangia developing in spiral series, and spermatangial stichidia developing as a furcation of trichoblasts. Original and subsequent descriptions of the following species of *Polysiphonia* indicate that they also exhibit these character states and are proposed as new combinations below:

   
   Basionym: *Polysiphonia acuminata* N.L. Gardner (1927, p. 100)
   
   Type locality: White’s Point near San Pedro, Los Angeles County, CA, USA
   

   
   Basionym: *Polysiphonia beaudettei* Hollenberg (1961, p. 348)
   
   Type locality: Isla Grande, Guerrero, Mexico

   
   Basionym: *Polysiphonia mollis* J.D. Hooker & Harvey (1857, p. 43)
Type locality: Georgetown, Tasmania

Descriptions of *P. masonii* Setchell & Gardner and *P. savatieri* Hariot indicate that these species also exhibit the above character states. *Polysiphonia masonii* and *P. savatieri* were placed into synonymy with *P. japonica* Harvey as a new variety of the latter species, *P. japonica* var. *savatieri* (Hariot) Yoon (Yoon 1986). *Polysiphonia japonica* has since been transferred to the genus *Neosiphonia* (Kim & Lee 1999), however, the transfer of its varieties has not been effected. The new combination for this variety is proposed as follows:


Basionym: *Polysiphonia japonica* var. *savatieri* (Hariot) Yoon (1986, p. 34)

Type Locality: Yokosuka, Kanagawa Prefecture, Japan

Two samples of *Neosiphonia bajacali* were collected from Cancun, Mexico (Figs 1, 2). *Neosiphonia bajacali* is resolved within a strongly supported (rB100; sB87) clade of predominantly *Neosiphonia* species in ML analyses of *rbc*L and SSU sequence data (Figs 3, 4). *Neosiphonia bajacali* is most closely related to *N. tongatensis* in the SSU phylogeny, but this relationship received only moderate support (sB78) and is not present in the *rbc*L phylogeny. *Neosiphonia bajacali* and *N. tongatensis* share many character states but can be distinguished by a smaller diameter of erect and prostrate axes (to 150 µm and 250 µm, respectively), no cortication, and segments 1-2x as long as wide in *N. tongatensis*.

*Neosiphonia sphaerocarpa* (Børgesen) M.S. Kim & Lee (1999, p. 280)

Figs 9-14

Basionym: *Polysiphonia sphaerocarpa* Børgesen (1918, p. 321)
DESCRIPTION: Plants to 2.5 cm tall, erect from basal rhizoids cut off from pericentral cells (Fig. 12), with some branches becoming decumbent and attached to substratum by rhizoids; moderately to highly branched in a subdichotomous pattern (Fig. 9); erect axes 75-150 µm in diameter, prostrate axes 225-325 µm in diameter; mid axis segments of erect axes mostly (1-) 1.5-2x as long as wide; cortication lacking; main axes with 4 pericentral cells (Fig. 11); branches replacing trichoblasts in development; trichoblasts long, to 875 µm in length, with several dichotomies, dense at apices (Fig. 10); scar cells one per segment in ¼ spiral series (Fig. 11); adventitious laterals absent; tetrarosporangia greatly distending segments, in long spiral series near branch tips (Fig. 13), 70-90 µm in diameter; spermatangial stichidia developing as a furcation of trichoblasts, 40-60x150-180 (-290) µm, with or without 1 sterile tip cell; cystocarps globose, short stalked (Fig. 14), 250-375 µm in diameter.

TYPE LOCALITY: St. Thomas, Virgin Islands


SPECIMENS STUDIED: Florida: WNC2010-s71 to s73 (FL05-5B), Keys Marine Laboratory, Long Key, Monroe County, B. Stuercke, 27 Feb 2005; WNC2009-s83, s84 (FL05-6), Keys Marine Laboratory, Long Key, Monroe County, B. Stuercke, 27 Feb 2005.

MOLECULAR VOUCHERS: GenBank accession numbers HM573569 (rbcL); HM573527 (COI).

REMARKS: Neosiphonia sphaerocarpa is part of a greater complex of Polysiphonia s.l. species that share several morphological character states, potentially leading to species misidentifications. These species include N. tongatensis (Harvey in Kützing) M.S. Kim & I.K.
Lee, *N. bajacali* Hollenberg, *Polysiphonia acuminata* N.L. Gardner, *P. beaudettei* Hollenberg, *P. japonica* var. *savatieri* (Hariot) Yoon, *P. masonii* Setchell & N.L. Gardner, and *P. mollis* J.D. Hooker & Harvey. These species are all described as having four pericentral cells, rhizoids cut off from pericentral cells, scar cells one per segment in $\frac{1}{4}$ spiral series, tetrasporangia developing in spiral series, and spermatangial stichidia developing as a furcation of trichoblasts (Abbott & Hollenberg 1976, Hollenberg 1961, Hollenberg & Norris 1977, Setchell & Gardner 1930, Yoon 1986). These character states, among others, are used to define members of the genus *Neosiphonia*. *Neosiphonia sphaerocarpa* is distinguished from the aforementioned species by a combination of the following characters: lack of a central percurrent axis, smaller habit (typically $\leq 1.5$ cm), smaller dimensions for erect and prostrate axes, lack of cortication, ultimate and fertile branches that are not basally attenuated, segments mostly longer than wide (but not typically more than 2x as long as wide), and a subdichotomous branching pattern.

Two samples of *Neosiphonia sphaerocarpa* were collected from Long Key, Monroe County, FL, USA (Figs. 1, 2). Only one *rbcL* sequence was generated from these samples, but the COI sequences for both samples were identical. SSU sequence data could not be generated for this species. *Neosiphonia sphaerocarpa* is placed within a strongly supported clade (rB100) of predominantly *Neosiphonia* species in the *rbcL* ML phylogeny (Fig. 3). This topology shows *N. sphaerocarpa* as most closely related to *Polysiphonia forfex* Harvey with this relationship receiving strong support (rB98). These species are similar in having rhizoids cut off from pericentral cells, scar cells every segment in spiral series, branches replacing trichoblasts, and tetrasporangia in spiral series, but *P. forfex* has (5-) 6 (-7) pericentral cells, segments shorter than wide, basal cortication, and spermatangial stichidia that developmentally replace trichoblasts.
**Neosiphonia tepida** (Hollenberg) S.M. Guimarães & M.T. Fujii et al. (2004, p. 171)

Figs 15-20

**Basionym:** *Polysiphonia tepida* Hollenberg (1958, p. 65)

**Synonyms:** *Polysiphonia taylorii* Hollenberg ex Williams (1949, p. 694)

*Polysiphonia flabellulata sensu* Meñez (1964, p. 219) [non *P. flabellulata* Harvey (1860, p. 330)]

**Description:** Plants to 1.5 cm tall, erect branches arising from a prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Fig. 17); highly branched in an alternate to subdichotomous pattern (Figs 15, 16); erect axes 50-75 µm in diameter, prostrate axes 125-175 µm in diameter; mid axis segments of erect axes mostly 1.5x as long as wide; cortication lacking; main axes with 7-8 pericentral cells (Figs 19, 20), occasionally 5-6 in immature axes; branches forming in the axils of trichoblasts (Fig. 18); trichoblasts to 620 µm in length, typically with 2-3 dichotomies; scar cells obvious, mostly every three to four segments and in no particular pattern; adventitious laterals absent; tetrarosporangia scattered or in short to long straight or slightly spiral series, 50-95 µm in diameter; spermatangial stichidia developing as a furcation of trichoblasts, 60-80x250 µm, without sterile tip cells; cystocarps subglobose to urceolate, 160 µm in diameter, stalked, with wide ostioles.

**Type Locality:** Beaufort, Carteret County, North Carolina, USA


**Specimens Studied:** Florida: *WNC2010-s77 to s79 (FL05-02)*, Sebastian Inlet, Indian River County, B. Stuercke, 26 Feb 2005.
**Molecular Vouchers:** GenBank accession number HM573552 \((rbcL)\).

**Remarks:** Hollenberg (1958) originally described *Neosiphonia tepida* (as *Polysiphonia tepida*) as having tetrasporangia in slightly spiral series but in a later publication describes tetrasporangia as developing in straight series (Hollenberg 1968b). This later description agrees with descriptions of *P. tepida* provided by Abbott (1999), Meñez (1964, as *Polysiphonia flabellulata* Harvey), and Schneider & Searles (1991). Tetrasporangia were not observed in this study but are listed in the above description as developing in straight or slightly spiral series according to both of Hollenberg’s observations.

Only one sample of *Neosiphonia tepida* was collected from Sebastian Inlet, Indian River County, FL, USA. COI and SSU sequence data could not be generated for this sample. Cluster analyses of *rbcL* sequence data show *N. tepida* as similar to *Polysiphonia isogona* Harvey (Fig. 1), with their sequences differing by only 1.85%. This is comparable to the maximum intraspecific *rbcL* sequence divergence of \(\leq 2.13\%\) (predominantly \(\leq 1.3\%\)) that has been observed in previous studies of *Polysiphonia* (McIvor *et al.* 2001). *Neosiphonia tepida* is resolved in a strongly supported clade (rB100) of multipericentral cell species in ML analysis of *rbcL* sequence data (Fig. 3). *Neosiphonia tepida* is sister to *P. isogona* in this tree, with the relationship receiving strong support (rB100).

*Neosiphonia tepida* and *Polysiphonia isogona* are identical in having rhizoids cut off from pericentral cells, scar cells that are variable in pattern and frequency, spermatangial stichidia developing as a furcation of trichoblasts, and subglobose cystocarps. If true *N. tepida* has tetrasporangia in spiral series, then this character state is also shared. *Polysiphonia isogona* can be distinguished from *N. tepida* by having erect axes to 250 (-300) \(\mu m\) in diameter, segments 2-4 (-6)x as long as wide in mid portions of erect axes, spermatangular branches with 1-3(-5)
sterile tip cells, and branches that develop to the side of trichoblasts (Adams 1991; Womersley 1979, 2003).

*Neosiphonia tepida* and *Polysiphonia isogona* may also differ in pericentral cell number. Hollenberg’s original description of *N. tepida* describes the species as “mostly with 7-8 pericentral cells” (Hollenberg 1958). Whether the term “mostly” indicates that fewer or more pericentral cells were observed is uncertain. Harvey did not indicate the number of pericentral cells in his original description of *P. isogona* (Harvey in Hooker 1855), but this species is described as having 9-10 (~12) pericentral cells by Adams (1991) and (8-) 9-10 pericentral cells by Womersley (1979). Womersley also remarked that this species may rarely have seven pericentral cells, as several samples collected from southern Australia appeared identical to *P. isogona* except for this difference in pericentral cell number and were therefore considered “unusual variants” of the species (Womersley 1979). He further commented that comparison of *P. isogona* and *N. tepida* (as *P. tepida*) is required. Specimens examined in this study strictly exhibit no more than 8 pericentral cells. Thorough comparison of type material is necessary in order to clarify the status of both species.

**Genus Polysiphonia**

*Polysiphonia anomala* Hollenberg (1968, p. 59)

Figs 21-26

**Description:** Plants small, to 5 mm tall, forming dense tangled mats, erect determinate branches arising from an indeterminate prostrate branching system attached to the substratum by rhizoids cut off from pericentral cells (Fig. 23); erect axes simple or with limited subdichotomous to irregular branching (Fig. 21); erect axes 40-50 (~80) µm in diameter, prostrate
axes 80-100 µm in diameter; mid axis segments of erect axes mostly 2.5-3x as long as wide; cortication lacking; main axes with four pericentral cells (Fig. 25); relationship of branches to trichoblasts unknown; trichoblasts with several dichotomies, long and delicate, to 900 µm in length (Fig. 24); scar cells obvious, variable in pattern and frequency; adventitious laterals frequent to occasionally present, linear in shape (Fig. 22); tetrasporangia slightly distending segments, in long spiral series (Fig. 26), 45 µm in diameter; spermatangial stichidia development unknown; cystocarps ovoid to slightly urceolate, short stalked, 120-140 µm in diameter.

**TYPE LOCALITY:** Bikini Atoll, Marshall Islands

**OTHER SOURCES:** Abbott 1999, Dawes & Mathieson 2008, Hollenberg 1968a

**SPECIMENS STUDIED:** Marshall Islands: *US-48521* (slide number *US-1100*), Holotype, Amen Island, Bikini Atoll, G.J. Hollenberg, 07 July 1948; Florida: *WNC2009-s145 to s147 (FL09-77)*, mangrove near Keys Marine Laboratory, Long Key, Monroe County, N. Mamoozadeh, 10 March 2009; *WNC2009-s148 to s151 (FL09-78)*, mangrove near Keys Marine Laboratory, Long Key, Monroe County, N. Mamoozadeh, 10 March 2009; *WNC2010-s048, s-49 (FL09-41B)*, Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 March 2009; *US-66362* (slide numbers *US-2352 to 2354*), Panama City, Bay County, M.L. Jones, 17 Jan 1958; *US-2355 to 2357*, Along highway near NW side of lagoon, Lake Surprise, Key Largo, Monroe County, E.Y. Dawson, 28 May 1949.

**MOLECULAR VOUCHERS:** GenBank accession numbers HM573549, HM573550 *(rbcL)*; HM560654 *(SSU)*; HM573502 *(COI)*.

**REMARKS:** Hollenberg’s original description of *Polysiphonia anomala* describes scar cells as occurring one per segment in ¼ spiral series on both prostrate and erect axes (Hollenberg 1968a). Hollenberg also noted that a very similar specimen collected by E.Y. Dawson from Lake
Surprise, Key Largo, FL (US-2355 to 2357) closely resembled *P. anomala* and was therefore identified as such by Hollenberg in the original description of the species. Hollenberg observed slight differences between Dawson’s Florida samples and the Pacific holotype of the species, however, including scar cells that do not occur regularly one per segment on prostrate branches and tetrasperangia that occur in much longer series in the Florida specimen. This same scar cell pattern was observed in all other Florida samples examined in this study. Tetrasperangia were also observed as occurring in long spiral series in *WNC2009-s145 to s147*. Scar cell pattern alone is not a reliable species identifier as this character is often variable within species (Stuercke & Freshwater 2008); it is unclear whether length of tetrasperangial series can be independently used to distinguish species.

Three samples of *Polysiphonia anomala* were collected from Lake Surprise and Long Key, Monroe County, FL, USA in this study (Figs. 1, 2). Two of the three *rbc*L sequences generated for these samples are identical and the third differs by only 0.37%, which is well within the range of intraspecific *rbc*L sequence variation observed in previous studies of *Polysiphonia s.l.* (e.g. McIvor et al. 2001). Only one COI sequence was generated from these specimens. ML analyses of *rbc*L and SSU sequence data resolve *P. anomala* as an independent lineage with no (rB<50) or strong (sB91) support, respectively, for its topological position (Figs 3, 4).

*Polysiphonia echinata* Harvey (1853, p. 38)

Figs 27-32

**SYNONYMS:** *Polysiphonia fracta* Harvey (1853, p. 38)

**MISAPPLIED NAMES:** *Polysiphonia breviarticulata sensu* Stuercke & Freshwater 2008
DESCRIPTION: Plants to 3 cm tall, entirely erect from single basal holdfast of rhizoids that are cut off from pericentral cells; main axes sparsely branched in a subdichotomous pattern; erect main axes (275-) 375-500 µm in diameter, basal axes (375-) 500-625 (-750) µm in diameter; mid axis segments of erect axes mostly 0.5-1x as long as wide; light to moderate cortication present in some mature prostrate axes and at the base of older adventitious laterals; main axes with four pericentral cells (Fig. 28); branches forming in the axils of trichoblasts (Fig. 29); trichoblasts with several dichotomies, to 500-800 µm in length; scar cells one per segment in ¼ spiral series; adventitious laterals abundant, every segment to every few segments on main axes giving plants a coarse appearance (Fig. 27), linear in shape, variable in length from 0.5-1.5 mm long; tetrasporangia little distending segments, developing in short spiral series (Fig. 30); spermatangial stichidia developing as a furcation of trichoblasts, without sterile tip cells (Fig. 31); cystocarps globose to subglobose, on short stalk (Fig. 32).

TYPE LOCALITY: Key West, Monroe County, FL, USA

OTHER SOURCES: Harvey 1853, Dawes & Mathieson 2008

SPECIMENS STUDIED: P. echinata Harvey, Florida: US-66789 (slide number US-3323, 3324), Isotype, Key West, Monroe County, W.H. Harvey, Feb 1850; US-22409, West Summerland Key, Monroe County, J. Brunson, 21 Mar 1976; US-22404, Alligator Point, Franklin County, H.J.H., 16 Apr 1950; US-22403, Alligator Point, Franklin County, H.J.H., 21 Mar 1950; WNC2009-s112 to s115 (FL09-40), Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; WNC2009-s117 to s119 (FL09-42), Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; WNC2009-s123 to s127 (FL09-44), Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; WNC2009-s137 to 140 (FL09-75), KML Mangrove, Long Key, Monroe County, N. Mamoozadeh, 10 Mar 2009;
MOLECULAR VOUCHERS: GenBank accession numbers HM573561, HM573559, HM573558, HM573560, HM573557 (rbcL); HM560658 (SSU); HM573503, HM573506, HM573504, HM573505 (COI).

REMARKS: Harvey (1853) originally described *Polysiphonia echinata* Harvey, *P. fracta* Harvey, and *P. hapalacantha* Harvey from Key West, FL. A report and description of *P. breviarticulata* (C. Agardh) Zanardini were also included among these original species descriptions (Harvey 1853). These species of *Polysiphonia* all have a habit that is erect from basal rhizoids which are cut off from pericentral cells, four pericentral cells, branches developing in the axils of trichoblasts, tetrasporangia in spiral series, and spermatangial stichidia developing as a furcation of trichoblasts and are distinct from other species of *Polysiphonia* in having numerous adventitious laterals that give the plants a coarse appearance.

Harvey’s original description of *Polysiphonia hapalacantha* included no remarks on similarity to *P. echinata*, *P. fracta*, or *P. breviarticulata*. *Polysiphonia hapalacantha* has traditionally been distinguished from these species by having adventitious laterals to 2-4 mm in length. Type material of *P. hapalacantha* at US was scarce and disfigured and an assessment of the species from this material was not possible. Material at US identified as *P. hapalacantha* from Puerto Rico displayed a habit consistent with the original species description and seems to exemplify true *P. hapalacantha*. These specimens had longer adventitious laterals that gave the specimens a more lax and less coarse habit than *P. echinata* and *P. fracta*, allowing these species to be distinguished.

Harvey (1853) indicated that while *Polysiphonia echinata* closely resembled *P. fracta*, a more robust habit and adventitious laterals that were shorter, more abundant, and “more equally inserted on all sides of the branches” could distinguish *P. echinata*. Examination of type material
for *P. echinata* and *P. fracta* suggests that these two names represent one morphological species as no satisfactory difference was observed. It seems possible that Harvey’s material of *P. fracta* is simply a diminutive form of *P. echinata*; this possibility was also suggested by Kapraun (unpublished manuscript). Although both names were introduced in the same publication, *P. echinata* appears to be more widely utilized in species reports and is therefore conserved.

Harvey’s report of *Polysiphonia breviarticulata* in Key West, FL seems to be the first report of this species in the western Atlantic (Harvey 1853). Agardh (1824) originally described *P. breviarticulata* from the Adriatic Sea as *Hutchinsia breviarticulata* C. Agardh. Prior to Harvey (1853), *P. breviarticulata* was known only from the Adriatic and the Mediterranean. In his description of *P. breviarticulata*, Harvey noted that the Florida material appeared more robust and with more adventitious laterals than Mediterranean material of *P. breviarticulata* (Harvey 1853).

The next report of *Polysiphonia breviarticulata* in the western Atlantic comes from Kapraun & Searles (1990) who identified this as the species of an intense macro-algal bloom along the coast of North Carolina. Kapraun & Searles’ (1990) description of North Carolina *P. breviarticulata* appears identical to descriptions and material of Mexico and Florida *P. echinata* except for the presence of basal attenuation in the lateral branches of *P. breviarticulata* specimens. Examination of North Carolina specimens identified as *P. breviarticulata* by Stuercke & Freshwater (2008) revealed basal attenuation to be a variable character state that is present in some branchlets, particularly ones that are tetrasporangial, but not others. The basal attenuation observed in tetrasporangial branchlets could be the result of mid axis segments that are distended due to the presence of tetrasporangia and in turn give basal segments a slightly constricted appearance. No other morphological difference was observed between North
Carolina material of *P. breviarticulata* examined by Stuercke & Freshwater (2008) and Florida and Mexico material of *P. echinata*. North Carolina material in WNC labeled as ‘*P. echinata*, 01 Dec 1982, Masonboro Bay’ most likely represents material published under the name *P. breviarticulata* in Kapraun & Searles (1990), who indicated that their initial material of *P. breviarticulata* was collected “in the sound behind Masonboro Island in December 1982”. This material, apart from basal attenuation in some branchlets, particularly ones that are tetrasporangial, also appears indistinguishable from Florida and Mexico specimens. Based on type localities and morphology, it seems likely that North Carolina material previously identified as *P. breviarticulata* by Stuercke & Freshwater (2008) and perhaps Kapraun & Searles (1990) actually represents *P. echinata*.

This conclusion is supported by cluster analyses of *rbcL* and COI sequence data. Four samples of *Polysiphonia echinata* were collected from the Florida Keys and 2 from Caribbean Mexico (Figs. 1, 2). Samples identified as *P. breviarticulata* by Stuercke & Freshwater (2008) differ from Florida and Mexico samples of *P. echinata* by 0.093-0.19% and 0.50-1.09% in the *rbcL* and COI sequence data, respectively. These values are well within the range of intraspecific sequence divergence observed in previous studies for these two loci (e.g. McIvor et al. 2001; Yang et al. 2008). ML analysis of *rbcL* sequence data resolves *P. echinata* within a large unsupported clade (rB<50) of species and variously supported clades of *Polysiphonia s.l.* species (Fig. 3). This placement is also supported in the SSU ML phylogeny (Fig. 4). The *rbcL* ML phylogeny places *P. echinata* as sister to *P. havanensis sensu* Børgesen and *P. binneyi* Harvey with this relationship receiving weak support (rB72). These species are all similar in having four pericentral cells, but the latter two species have rhizoids in open connection with pericentral cells.
CONCLUSION

Five species of *Polysiphonia s.l.* were identified in 16 samples from Florida and Caribbean Mexico. Two of these species, *Neosiphonia bajacali* and *P. echinata*, represent new reports for Caribbean Mexico. The other three species do not represent new reports for their respective geographic region but their presence helps support their known species distribution. Further intensive study of Caribbean *Polysiphonia s.l.* species is necessary to determine the true generic status of the species reported for this widespread area. It also seems likely that more than 28 of the nearly 200 *Polysiphonia s.l.* species are present in this diverse and extensive region.

REFERENCES


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Fig. 1. Unweighted Pair Group Method with Arithmetic mean rbcL cluster analysis for 113 Polysiphonia s.l. samples. The 2.13% and 1.3% sequence divergence levels are indicated by vertical dashed lines. Panamanian species are shown in boldface type.
Fig. 2. Unweighted Pair Group Method with Arithmetic mean COI cluster analysis for 74 Polysiphonia s.l. samples. The 4.80% sequence divergence level is indicated by the vertical dashed line. Panamanian species are shown in boldface type.
Fig. 3. Maximum likelihood rbcL tree ($\ln L = -14603.23387$) for 50 Polysiphonia s.l. and two outgroup samples. Bootstrap proportion values are shown for each node when $>50$. Panamanian species are shown in boldface type.
Fig. 4. Maximum likelihood SSU tree (lnL = –4146.45008) for 43 Polysiphonia s.l. and one outgroup samples. Bootstrap proportion values for branches are shown for each node when >50. Panamanian species are shown in boldface type.
Figs 5-8. Neosiphonia bajacali. **Fig. 5.** Habit of erect axes, MEX04-09, scale = 0.20 mm, WNC2010-s061. **Fig. 6.** Portion of erect axis flattened to show four pericentral cells per segment, central axial cell (arrowhead), and scar cells (arrows), MEX04-09, scale = 0.10 mm, WNC2010-s061. **Fig. 7.** Prostrate axis with rhizoids that are cut off from the pericentral cells, MEX04-09, scale = 0.10 mm, WNC2010-s061. **Fig. 8.** Reproductive branch displaying short spiral series of tetrasporangia, MEX04-11A, scale = 0.10 mm, WNC2010-s056.
Figs 9-14. *Neosiphonia sphaerocarpa*. Fig. 9. Habit of erect axes, FL05-5B, scale = 0.20 mm, WNC2010-s073. Fig. 10. Apical portion of erect axis bearing many trichoblasts, FL05-5B, scale = 50 µm, WNC2010-s071. Fig. 11. Portion of erect axis flattened to show four pericentral cells per segment, central axial cells (arrow), and scar cells in spiral pattern, FL05-5B, scale = 0.10 mm, WNC2010-s071. Fig. 12. Prostrate axis with rhizoids that are cut off from the pericentral cells, FL05-06, scale = 0.10 mm, WNC2010-s083. Fig. 13. Reproductive branch displaying long spiral series of tetrasporangia, FL05-5B, scale = 0.10 mm, WNC2010-s073. Fig. 14. Main axis bearing cystocarp on short stalk, FL05-5B, scale = 0.10 mm, WNC2010-s072.
Figs 15-20. *Neosiphonia tepida*. **Fig. 15.** Habit showing erect axes, FL05-02, scale = 0.20 mm, WNC2010-s077. **Fig. 16.** Apical portion of erect axes bearing trichoblasts, FL05-02, scale = 0.10 mm, WNC2010-s077. **Fig. 17.** Prostrate axis with rhizoid that is cut off from the pericentral cell, FL05-02, scale = 20 µm, WNC2010-s077. **Fig. 18.** Apical part of main axis showing a lateral branch developing in the axil of a trichoblast, FL05-02, scale = 20 µm, WNC2010-s077. **Fig. 19.** Cross sections of young and mature branch axes showing central axial cell and seven pericentral cells, FL05-02, scale = 20 µm, WNC2010-s079. **Fig. 20.** Cross section of mature branch axis showing central axial cell and eight pericentral cells, FL05-02, scale = 20 µm, WNC2010-s079.
Figs 21-26. *Polysiphonia anomala*. **Fig. 21.** Habit showing erect axes, FL09-78, scale = 0.20 mm, WNC2009-s149. **Fig. 22.** Main axis showing development of adventitious laterals, FL09-77, scale = 0.20 mm, WNC2009-s145. **Fig. 23.** Prostrate axis with a rhizoid that is cut off from the pericentral cell, FL09-41B, scale = 20 µm, WNC2010-s048. **Fig. 24.** Apex of lateral branch bearing trichoblasts, FL09-41B, scale = 0.10 mm, WNC2010-s049. **Fig. 25.** Portion of erect axis flattened to show four pericentral cells per segment, FL09-41B, scale = 25 µm, WNC2010-s048. **Fig. 26.** Reproductive branch displaying long spiral series of tetrasporangia, FL09-77, scale = 50 µm, WNC2009-s145.
Figs. 27-32. Morphology of *Polysiphonia echinata*. **Fig. 27.** Habit showing adventitious laterals arising every segment to every few segments, FL09-42, scale = 0.20 mm, WNC2009-s119. **Fig. 28.** Portion of erect axis flattened to show four pericentral cells per segment, FL09-40, scale = 100 µm, WNC2009-s112. **Fig. 29.** Apical part of main axis showing a lateral branch developing in the axil of a trichoblast (arrow), FL05-07, scale = 50 µm, WNC2010-s085. **Fig. 30.** Reproductive branch displaying long spiral series of tetrasporangia, FL09-42, scale = 100 µm, WNC2009-s118. **Fig. 31.** Branch apex with spermatangial stichidium developing as a furcation of the trichoblast (arrow), MEX04-8, scale = 50 µm, WNC2010-s054. **Fig. 32.** Main axis bearing a short-stalked cystocarp, MEX04-10, scale = 50 µm, WNC2010-s062.
### Appendix 1. Collection information, sample number/source, and GenBank accession numbers for Rhodomelaceae examined in this study. Identical accession numbers indicate specimens with identical sequences.

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**Polysiphonia sp.**

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- **NC-23** Wrightsville Beach, New Hanover Co., NC, USA 19 May 2005, B Stuercke
- **NC-31** Howard’s Channel, Topsail inlet, Pender Co., NC, USA 07 Jul 2005, DW Freshwater, B Stuercke & R Hamner

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- **NC-28** CORMP Site OB-27, Onslow Bay, NC, USA 09 Jun 2005, DW Freshwater & J Souza
- **NC-32** CORMP Site OB-3, Onslow Bay, NC, USA 11 Jul 2005, DW Freshwater & B Stuercke

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<td>Brady's Beach, Bamfield, B.C., Canada</td>
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Polysiphonia pernacola Adams

**NZ04-243**
Paua Beach, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand

**NZ04-244**
Paua Beach, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand

**NZ04-263**
Oban, Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand

**NZ04-291**
Ulva Island near Stewart Island, New Zealand
01 Nov 2004, DW Freshwater & M Hommersand

**NZ04-309**
Ulva Island near Stewart Island, New Zealand
02 Nov 2004, DW Freshwater & M Hommersand

**NZ04-550A**
Muritai, Wellington Harbor, North Island, New Zealand
17 Nov 2004, DW Freshwater & M Hommersand

**NZ04-550B**
Muritai, Wellington Harbor, North Island, New Zealand
17 Nov 2004, DW Freshwater & M Hommersand

**NZ04-557**
Muritai, Wellington Harbor, North Island, New Zealand
17 Nov 2004, DW Freshwater & M Hommersand

Polysiphonia pseudovillum Hollenberg

**PHYKOS-3533**
Flat Rock Beach, Isla Colón, Bocas del Toro, Panama
17 Jul 2008, DW Freshwater

Polysiphonia schneideri Stuercke & Freshwater

**BERMUDA**
Tucker’s Bay, Harrington Sound, Bermuda
17 Mar 2009, CW Schneider & CE Lane

**FL05-04**
Sebastian Inlet, Indian River County, FL, USA
26 Feb 2005, B Stuercke

**NC-2**
Cassamir Wreck (WR2), Onslow Bay, NC, USA
02 Jul 2004, DW Freshwater & K Johns

**PHYKOS-2454**
Panama Canal north ferry terminus, Colón, Panama
02 Jul 2004, DW Freshwater & K Johns

**PHYKOS-2689**
STRI research station, Punta Galeta, Colón, Panama
19 May 2009, K Larson & L McCann

**PHYKOS-3189**
Cayos Zapatillas, Bocas del Toro, Panama
19 May 2009, K Larson & L McCann

Polysiphonia scopulorum Harvey

**Kim et al. (2004)**
Devil’s Punchbowl, OR, USA

**AY396039**
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1. Sequence published as *Polysiphonia elongella* Harvey.
2. Sequence used in ML analysis of *rbcL* and SSU data.
3. Sequence used in ML analysis of *rbcL* data.
4. Sequence used in ML analysis of SSU data.