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Polysiphonia sensu lato (Ceramiales, Florideophyceae) species of Caribbean Panama including *Polysiphonia lobophoralis* sp. nov. and *Polysiphonia nuda* sp. nov.

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Abstract

Only two species of *Polysiphonia sensu lato* (predominately species of *Polysiphonia* and *Neosiphonia*) have been reported from Caribbean Panama. In contrast, 16 species are documented from the neighboring countries of Costa Rica and Colombia. Molecular-assisted identification using plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbc*L) and mitochondria-encoded cytochrome *c* oxidase subunit I gene (COI) loci identified 14 species from 38 samples collected along the Caribbean coast of Panama. Morphological character states were examined and used to identify each sample, and phylogenetic relationships among these species were estimated through maximum likelihood analyses of *rbc*L and nuclear-encoded SSU sequence data. *Neosiphonia ferulaceae*, *N. tongatensis*, *Polysiphonia binneyi*, *P. havanensis*, *P. macrocarpa*, *P. pseudovillum*, *P. schneideri*, *P. subtilissima*, and two species that could only be identified as *P. cf. sertularioides* are reported for the first time from Panama. *Polysiphonia pentamera*, a species only known from the eastern Pacific is reported for the first time in the Caribbean, and two new species, *P. lobophoralis* and *P. nuda* are described. A key to Caribbean Panama species, descriptions of species morphology and remarks on taxonomy and relationships are provided. These findings demonstrate that previous limited reports of *Polysiphonia sensu lato* species from Panama resulted from a lack of study, rather than a lack of diversity, within the region.

Keywords: Caribbean; molecular-assisted-identification; *Neosiphonia*; Panama; *Polysiphonia*.

Introduction

Studies of the marine algal flora throughout much of Central America are limited and in Panama have included only opportunistic or brief concerted efforts (Howe 1910, 1918, Lemoine 1929, Taylor 1929, 1941, 1942, 1945, Dawson and Beaudette 1959, Dawson 1960, Earle 1972). Recent attempts to increase knowledge of the marine algal flora 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 et al. 2000, 2009, Wysor and De Clerck 2003, Wysor and Kooistra 2003, Wysor 2004). This study is part of a greater collaborative effort to survey and inventory the Panamanian marine algal flora.

Polysiphonia Greville is one of the largest red algal genera, with approximately 200 currently recognized species (e.g., Kim et al. 2002, Guiry and Guiry 2012). These species are common members of marine algal floras and have a nearly global distribution (e.g., Hollenberg 1968a,b, Womersley 1979, Wynne 2009, 2011). *Polysiphonia* species have 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.*) includes species that are predominantly placed in two genera: *Neosiphonia* M.S. Kim *et* 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 *et* Prantl, *Enelittosiphonia* Segi, and *Vertebrata* S.F. Gray.

The first Panamanian report of *Polysiphonia* comes from G.J. Hollenberg in Taylor (1945) and was the result of opportunistic sampling by W.R. Taylor while en route to and from the Galapagos Islands. Hollenberg identified *Polysiphonia howei* Hollenberg from Isla Taboga in the Bay of Panama on the Pacific side of the country. In 1959, E.Y. Dawson used SCUBA to study marine algae in Panama for the first time 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 *P. 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 two *Polysiphonia* species have been previously documented for the Caribbean coast of Panama (Earle 1972), 16 species have been reported from the neighboring Caribbean coasts of Colombia and Costa Rica (Taylor 1960, Dawson 1962, Wellington 1974, Kapraun et al. 1983,

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Kemperman and Stegenga 1986, Soto and Ballantine 1986, Diaz-Pulido and Diaz-Ruiz 2003, Bernecker 2009). The low Panamanian species counts may indicate limited biological diversity or a lack of study.

Species of *Polysiphonia s.l.* 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 (Hollenberg 1968a, Hollenberg and Norris 1977, Kapraun 1980a, Kapraun and Norris 1982, Schneider and Searles 1991, Abbott 1999, Kim and Lee 1999). Stuercke and Freshwater (2008) examined many of these characters in North Carolinian (USA) species of *Polysiphonia s.l.* to determine which had consistent character states within species and were therefore particularly useful in their identification.

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 (*rbc*L) for species identifications in their study of the *Neosiphonia harveyi* (J. Bailey) M.S. Kim, H.G. Choi, Guiry *et* G.W. Saunders (as *Polysiphonia harveyi* J. Bailey) invasion of the British Isles and continental Atlantic Europe. Subsequent studies have continued to utilize this locus, establishing general ranges of inter- and intraspecific sequence variability and a baseline of publically available sequences for taxonomic studies (Kim et al. 2004, Kim and Yang 2005, Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011). Another form of MAI is DNA barcoding with sequences from the 5' end of the mitochondria-encoded cytochrome *c* oxidase subunit I (COI) gene (e.g., Hebert et al. 2003, Saunders 2005). Mamoozadeh and Freshwater (2011) demonstrated the utility of this locus for MAI in *Polysiphonia s.l.* COI was found to have greater sequence variability and a larger barcoding gap (e.g., Meier et al. 2008, Freshwater et al. 2010) than *rbc*L, but amplification and sequencing were also more difficult with currently available primers (Mamoozadeh and Freshwater 2011).

The phylogenetic relationships of *Polysiphonia s.l.* species have also been estimated through analyses of nuclear-encoded 18S rDNA (SSU) and *rbc*L sequence data (e.g., Choi et al. 2001, McIvor et al. 2001, Kim et al. 2004). SSU sequences are relatively conserved and analyses provide better resolution of relationships above the species level (Choi et al. 2001, Mamoozadeh and Freshwater 2011), whereas the more variable *rbc*L has proven especially useful for studying the relationships among species (e.g., Kim and Yang 2005, Stuercke and Freshwater 2010). Thorough inference of phylogenetic relationships at different taxonomic levels within *Polysiphonia s.l.* can be made when both SSU and *rbc*L are analyzed (Stuercke 2006).

The purpose of this study was to complete integrated molecular and morphological analyses of *Polysiphonia* and *Neosiphonia* species from the Caribbean coast of Panama. MAI and morphological examinations were used to identify species of *Polysiphonia* and *Neosiphonia* within Panamanian samples, and phylogenetic analyses of *rbc*L, and SSU sequence data were used to determine the relationships among these species.

Materials and methods

Collections, vouchers and morphological analyses

Polysiphonia samples were collected from intertidal or subtidal substrata by snorkeling or SCUBA diving at sites located along the Caribbean coast of Panama (Figure 1). Collection details are provided in the specimens studied section for each species and the Appendix 1 of Mamoozadeh and Freshwater (2011). Samples were dried in silica gel desiccant (Chase and Hills 1991) and deposited in the silica collection at the Center for Marine Science, University of North Carolina at Wilmington. Samples were grouped into genetic species by MAI (see below; Yang et al. 2008, Clarkston and Saunders 2010), and identified to morphological species based on examinations of morphological characters and use of taxonomic guides (Børgesen 1918, Setchell and Gardner 1930, Hollenberg 1942, 1958, 1961, 1968a,b, Taylor 1945, 1960, Dawson 1964, Abbott and Hollenberg 1976, Hollenberg and Norris 1977, Kapraun 1977, 1980a, Womersley 1979, 2003, Kapraun and Norris 1982, Kapraun et al. 1983, Adams 1991, Schneider and Searles 1991, Abbott 1999, Littler and Littler 2000, Dawes and Mathieson 2008, Kapraun unpublished manuscript). Permanent slide vouchers were made following Tsuda and Abbott (1985) and deposited in the University of North Carolina Wilmington Herbarium (WNC) and the United States National Herbarium (US). All herbarium abbreviations follow the Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp), and listed PHYKOS numbers refer to the accession number of samples in the PHYKOS database of Panamanian algae developed by Brian Wysor (2009).

Specimens and slides were observed and images captured as described in Mamoozadeh and Freshwater (2011). Species descriptions were written based predominantly on observations of specimens collected in this study and those at the United States National Herbarium (US), and emphasize the morphological characters examined by Stuercke and Freshwater (2008). Character state information from the literature was included when not observed in these specimens.

Molecular data analyses

DNA extraction, amplification and sequencing were performed following the methodology and using the primers described by Mamoozadeh and Freshwater (2011). Both *rbc*L and COI sequences were generated for as many samples as possible and used to objectively assign samples to species (e.g., Millar and Freshwater 2005, Clarkston and Saunders 2010). SSU sequences were generated for only one sample per species because intraspecific sequence variation was minimal. GenBank accession numbers are listed in the molecular voucher section of species treatments and Appendix 1 of Mamoozadeh and Freshwater (2011). Both *rbc*L and SSU sequence data were used to estimate phylogenetic

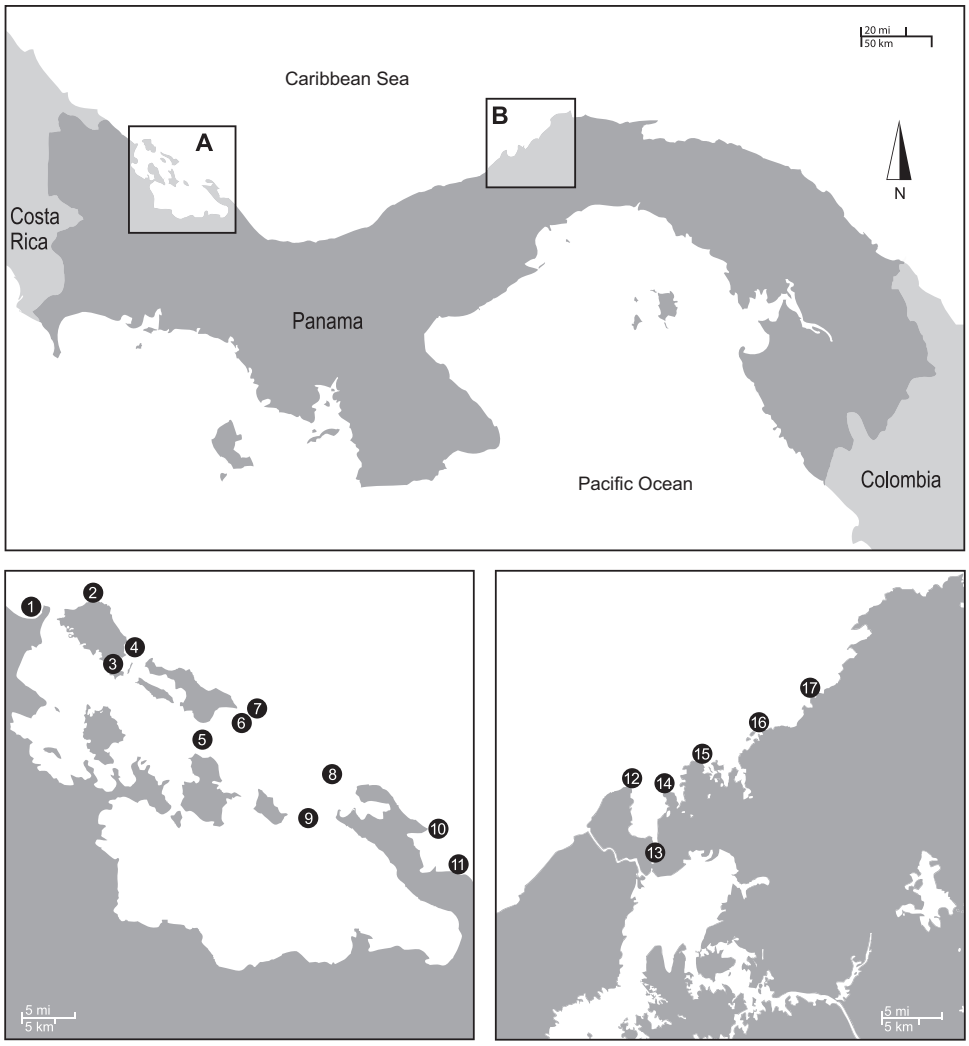


Figure 1 Caribbean Panama sampling sites.
(A) Bocas del Toro region: (1) Tervi Bight, 9.430°N 82.358°W; (2) Swan Cay, 9.451°N 82.299°W; (3) STRI Bocas Research Station, 9.351°N 82.257°W; (4) Flat Rock Beach, 9.380°N 82.238°W; (5) Crawl Cay, 9.245°N 82.134°W; (6) Cayos Zapatillas bayside, 9.258°N 82.049°W; (7) North of Cayos Zapatillas, 9.282°N 82.051°W; (8) Cayos Tigres, 9.214°N 81.932°W; (9) Canal del Tigre, 9.146°N 81.979°W; (10) South of Big Plantain Cay, 9.124°N 81.797°W; (11) Shark Hole Point, 9.067°N 81.783°W. (B) Collection sites in the Colón and Galeta regions: (12) West Limón Bay Jetty, 9.373°N 79.949°W; (13) Panama Canal North Ferry Terminus, 9.288°N 79.922°W; (14) Parque de Juventud, 9.367°N 79.904°W; (15) STRI Punta Galeta Research Station, 9.403°N 79.861°W; (16) Isla Naranjo Arriba, 9.445°N 79.784°W; (17) Punta Gorda, 9.483°N 79.733°W.

relationships and identify clades within *Polysiphonia s.l.* Sequences of *Polysiphonia* generated in this study were combined with *rbc*L and SSU sequences available from GenBank as well as some from unpublished studies (J. Kelly and D.W. Freshwater, unpublished). Sequences were aligned using MacClade software (v.4, Maddison and Maddison 2000); SSU sequence data were further aligned using the ClustalW multiple sequence alignment feature of the Molecular Evolutionary Genetics Analysis (MEGA) software (www.megasoftware.net; Tamura et al. 2007, Kumar et al. 2008).

MEGA was used to perform unweighted pair group method with arithmetic mean (UPGMA) and neighbor joining (NJ) cluster analyses on COI and *rbc*L sequence data for MAI. Simple mean distances (p-distances) were used in all MAI analyses. Maximum likelihood (ML) analyses were performed on reduced *rbc*L (identical sequences removed) and SSU sequence data separately using Genetic Algorithm for Rapid Likelihood Inference (GARLI) software (www.bio.utexas.edu/faculty/antisense/garli/Garli.html; Zwickl 2006) and methods outlined in Mamoozadeh and Freshwater (2011).

Results and discussion

Molecular-assisted identification

The COI alignment comprised 74 taxa and included 605 sites in the analysis, of which 248 (40.99%) were variable.

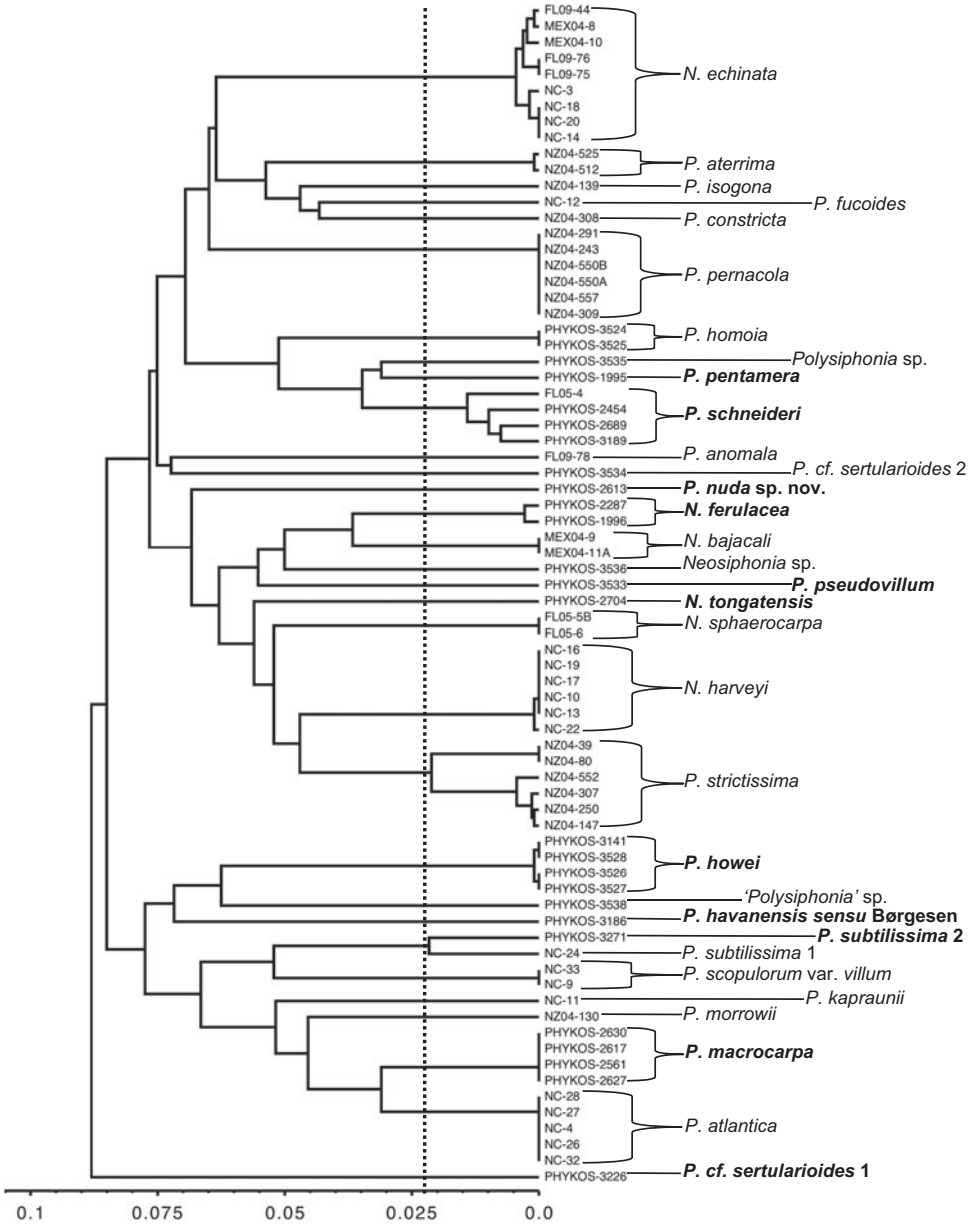


Figure 2 Unweighted pair group method with arithmetic mean COI cluster analysis for 74 *Polysiphonia s.l.* samples.
The 4.80% sequence divergence level is indicated by the vertical dashed line. Caribbean Panama species are shown in boldface type. *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

UPGMA and NJ cluster analyses of COI sequence data from 20 Caribbean Panama *Polysiphonia s.l.* specimens resolved 11 species (Figure 2, only UPGMA cluster diagram shown). 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 rationale behind this intraspecific variation cutoff value is discussed in Mamoozadeh and Freshwater (2011).

The *rbc*L alignment consisted of 113 taxa and included 1081 sites in the analysis, of which 402 (37.19%) were variable. Fourteen species were resolved by UPGMA and NJ cluster analyses of *rbc*L sequences from 29 Caribbean Panama *Polysiphonia s.l.* specimens (Figure 3, only UPGMA cluster diagram shown). Species distinctions were based on inter- and intraspecific *rbc*L sequence divergence values reported in previous studies (McIvor et al. 2001, Kim et al. 2004, Kim and Yang 2005, Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011). Sequence divergences below 1.3% are generally considered to represent intraspecific variation, and those greater than 2.13% interspecific variation. Determining the conspecific status of specimens with levels of variation between these values requires additional evidence. Intraspecific COI and *rbc*L sequence divergences are discussed in the remarks on each species.

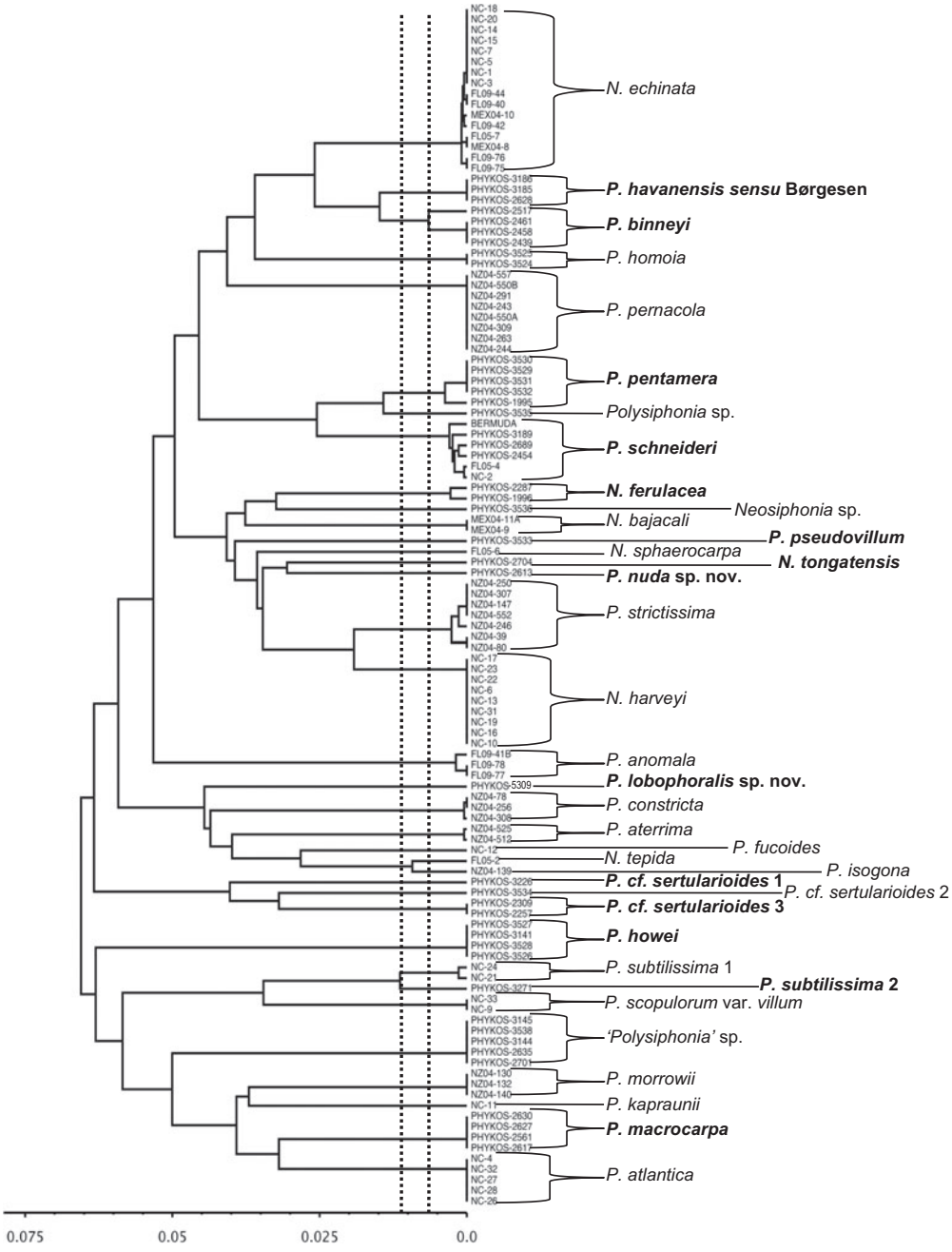


Figure 3 Unweighted pair group method with arithmetic mean *rbc*L cluster analysis for 113 *Polysiphonia s.l.* samples. The 2.13% and 1.3% sequence divergence levels are indicated by vertical dashed lines. Caribbean Panama species are shown in boldface type. *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

Molecular systematics

Reduced *rbc*L (only one of each identical sequence included) and SSU data sets were used to infer phylogeny within *Polysiphonia s.l.* (Figures 4 and 5). The reduced *rbc*L alignment consisted of 52 taxa and included 1334 sites in the analysis, 528 (39.6%) of which were variable. A general time-reversible model of molecular evolution was estimated as the best fit for these data. The SSU alignment consisted of 44 taxa and included 1602 sites in the analysis, 158 (9.86%) of which were variable. A general time reversible model of molecular evolution was also estimated as the best fit for these data.

The same general relationships and major clades were resolved in both the *rbc*L and SSU analyses, and these topologies are similar to those of previous studies (Stuercke and Freshwater 2010, Mamoozadeh and Freshwater 2011). These relationships include two strongly supported clades of *Polysiphonia sensu stricto* (*s.s.*) species that are resolved together as a monophyletic group in the SSU tree, and a well-supported clade of species with seven + pericentral cells

Figure 4 Maximum likelihood *rbc*L tree (lnL=-14603.23387; GTR+G+I model; model parameters: rates=1.7515, 7.8548, 3.2083, 1.6430, 25.4566, 1.000; base frequencies=0.3251, 0.1233, 0.1872, 0.3644; gamma shape=1.6251; proportion invariant sites=0.5705) for 50 *Polysiphonia s.l.* and two outgroup samples. Bootstrap proportion values for branches are shown for each node when >50. Caribbean Panama species are shown in boldface type. Vertical bars show: (A) *Polysiphonia sensu stricto* clades; (B) the "multipericentral cell" clade. Car., Caribbean; Pac., Pacific; NC, North Carolina; FL, Florida; OR, Oregon; *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

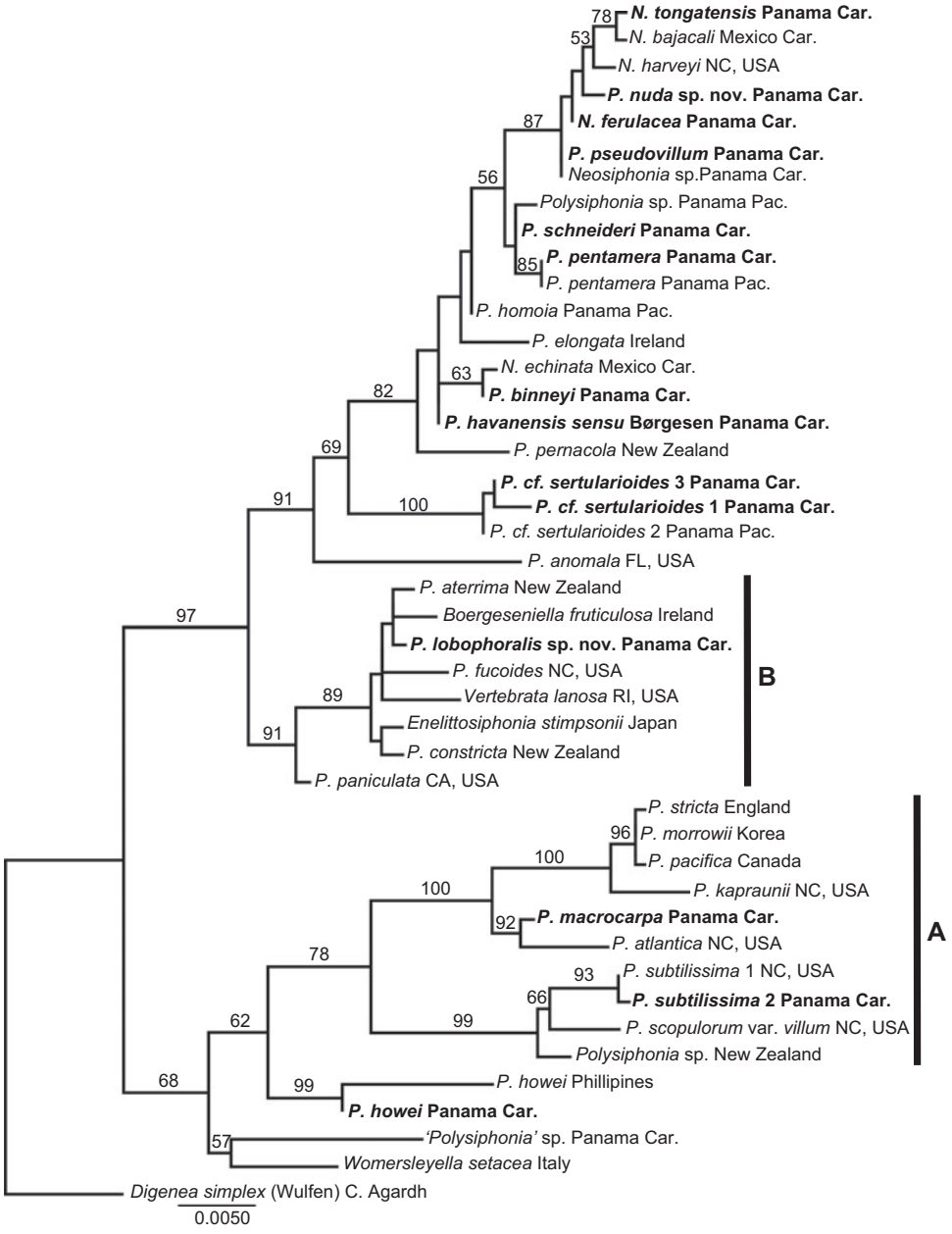


Figure 5 Maximum likelihood SSU tree (lnL=-4146.45008; GTR+G+I model; model parameters: rates=0.9061, 3.3954, 1.1248, 0.9080, 4.8508, 1.000; base frequencies=0.2608, 0.1958, 0.2705, 0.2729; gamma shape=0.7782; proportion invariant sites=0.8076) for 43 *Polysiphonia s.l.* and one outgroup samples. Bootstrap proportion values for branches are shown for each node when >50. Caribbean Panama species are shown in boldface type. Vertical bars show: (A) *Polysiphonia sensu stricto* clades; (B) the "multipericentral cell" clade. Car., Caribbean; Pac., Pacific; CA, California; NC, North Carolina; FL, Florida; OR, Oregon; RI, Rhode Island; *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

referred to as the "multipericentral cell group" by Choi et al. (2001). Relationships of the Caribbean Panama species are discussed in the remarks on each of them.

One problematic result of all recent DNA sequence analyses is the non-monophyly of species classified as *Polysiphonia* and *Neosiphonia*. Kim and Lee (1999) cited seven character states that distinguished *Neosiphonia*: i) trichoblasts and/or branches on every segment; ii) erect indeterminate branches developing from main axes (in contrast to their development from a prostrate axes); iii) rhizoids cut off from pericentral cells; iv) abundant trichoblasts; v) three-celled carpogonial branches; vi) spermatangia developing as furcations of trichoblasts; and vii) tetrasporangia in spiral series. No clades resolved by the current analyses (Figures 4 and 5) or in previous studies (Stuercke and Freshwater 2010, Mamoozadeh and Freshwater 2011) were also defined by these morphological

character states. Species are assigned to "*Neosiphonia*" in this study only when all observed morphological character states match those described for the genus, and with the understanding that this genus as currently described is untenable. Continuing molecular and morphological analyses of *Polysiphonia s.l.* by a number of research groups will lead to a natural classification of these species.

Taxonomic observations and remarks

Key to Caribbean Panama species of *Polysiphonia* and *Neosiphonia*

1. Pericentral cells, four 2
   Pericentral cells, more than four 11
2. Rhizoids in open connection with pericentral cells..... 3
   Rhizoids cut off from pericentral cells..... 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 diameter, spermatangia generally with two or three sterile tip cells .....*P. binneyi*
   Plants with extensive prostrate system, erect axes *c.* ≤110 µm in diameter, spermatangia generally without sterile tip cells .....*P. havanensis sensu* Børgesen
5. Erect axes arising radially from prostrate axes, trichoblasts unbranched or with one dichotomy .... *P. subtilissima*
   Erect axes arising unilaterally from prostrate axes giving plants a dorsiventral habit, trichoblasts generally with two or three 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 diameter, prostrate axes mostly <150 µm in diameter. 8
   Erect axes mostly >70 µm in diameter, prostrate axes mostly >150 µm in diameter. 10
8. Lateral branches developmentally replacing trichoblasts ..... *P. pseudovillum*
   Lateral branches developing in the axils of trichoblasts 9
9. Lateral branches with slight basal attenuation, mid-axis segments of erect axes >1× as long as wide .... *P. cf. sertularioides* 1
   Lateral branches with prominent basal attenuation, mid-axis segments of erect axes ≤1× as long as wide..... *P. cf. sertularioides* 3
10. Branchlets spindle-shaped, mid-axis segments of erect axes <1× as long as wide, erect axes mostly (150–) 250–350 µm in diameter. .....*N. ferulacea*
    Branchlets linear, mid-axis segments of erect axes ≥1× as long as wide, erect axes mostly ≤200 µm in diameter. .....*N. tongatensis*
11. Pericentral cells, ≥7..... 12
    Pericentral cells, five to six (occasionally seven in tetrasporophytic specimens)..... 13
12. Pericentral cells, seven to eight, lateral branches developing in the axils of trichoblasts .... *P. lobophoralis* sp. nov.
    Pericentral cells, eight to ten or more, lateral branches developmentally replacing trichoblasts, pericentral cells, offset in some axes.....*P. howei*
13. Pericentral cells, strictly five, tetrasporangia in spiral series..... *P. pentamera*
    Pericentral cells, (5–) 6 (–7), tetrasporangia in straight series.....*P. schneideri*

Genus *Neosiphonia*

Neosiphonia ferulacea (Suhr ex J. Agardh) S.M. Guimarães *et* M.T. Fujii in Guimarães et al. (2004, p. 165) (Figures 6–9)

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

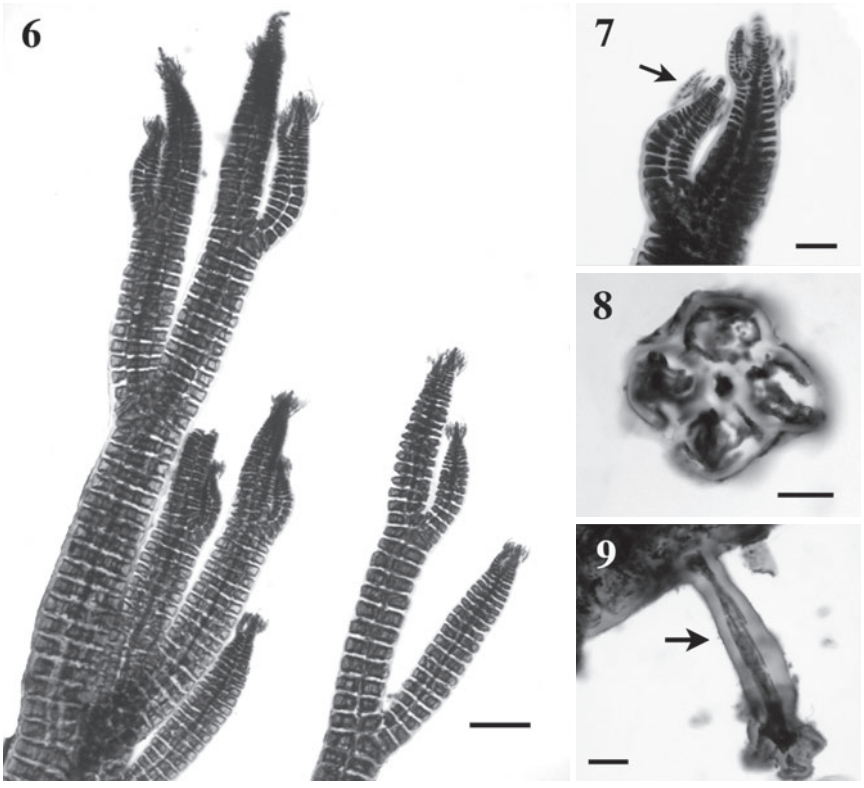
Synonyms *Polysiphonia bharadwajae* Rao (1967, p. 169).

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 (Figure 9); sparingly to moderately branched in a pseudodichotomous to alternate or irregular pattern (Figure 6); erect axes (150–) 250–350 µm in diameter, prostrate axes 225–375 µm in diameter; branchlets somewhat to distinctly spindle shaped (Figures 6 and 7); mid-axis segments of erect axes mostly 0.5× as long as wide, but shorter near apices; cortication absent; main axes with four pericentral cells (Figure 8); branches replacing trichoblasts in development; trichoblasts short, to 90 µm in length, with several dichotomies, dense at apices (Figure 7); scar cells not readily obvious, one per segment in ¼ spiral series; adventitious laterals absent; tetrasporangia not distending segments, in spiral series in branch tips, 50–60 µm in diameter; spermatangial branches developing as furcations of trichoblasts, 60×150 µm, with one or two 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.

Syntype localities East coast of Mexico; North America; Guadeloupe; Australia; Marquesas Islands; Hawaiian Islands.

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

Specimens studied Panama: *WNC2010-s040 (PHYKOS-1996),* West Limón Bay Jetty, Punta Toro, Colón, N. Mamoozadeh, 17 May 2009; *WNC2009-s155 to s157 (PHYKOS-1996)*, STRI research station, Punta Galeta, Colón, B. Wysor and L. Sargent, 14 May 2009; *WNC2010-s039,*



Figures 6–9 *Neosiphonia ferulacea.*
(6) Habit of erect axes, PHYKOS-2287, scale=0.2 mm, WNC2009-s166. (7) Apical portion of erect axes bearing short trichoblasts (arrow), PHYKOS-2287, scale=50 µm, WNC2010-s046. (8) Cross section of branch axis showing central axial cell and four pericentral cells, PHYKOS-2287, scale=50 µm, WNC2010-s039. (9) Prostrate axis showing a rhizoid (arrow) that is cut off from the pericentral cell, PHYKOS-2287, scale=50 µm, WNC2010-s047.

s046, s047 (PHYKOS-2287), West Limón Bay Jetty, Punta Toro, Colón, N. Mamoozadeh, 17 May 2009; *WNC2009-s166, s167 (PHYKOS-2287)*, STRI research station, Punta Galeta, Colón, B. Wysor and L. Sargent, 14 May 2009.

Molecular vouchers GenBank accession numbers HM573574, HM573584 (*rbc*L); HM560645 (SSU); HM573512, HM573511 (COI).

Remarks Skelton and South (2007) cited *Neosiphonia ferulacea* as a new combination, evidently unaware that Guimarães and Fujii had already proposed this combination (Guimarães et al. 2004). Previous authors have discussed the similarity between *N. ferulaceae*, *N. hawaiiensis* (Hollenberg) M.S. Kim *et* I.A. Abbot (type locality Hawai'i), and *Polysiphonia sparsa* (Setchell) Hollenberg (type locality Tahiti) (e.g., Hollenberg 1968a, Kapraun 1977, Abbott 1999), and Skelton and South (2007) included the latter two as synonyms of *N. ferulaceae*.

Neosiphonia ferulacea closely resembles *N. hawaiiensis* and *Polysiphonia sparsa*, but *Polysiphonia sparsa* has a more creeping and smaller habit (to 1 cm tall) than *N. ferulacea* or *N. hawaiiensis* (Hollenberg 1968a, Kapraun 1977, Abbott 1999). *N. hawaiiensis* differs from *N. ferulacea* in its divaricate branching and lack of fully percurrent axes (Hollenberg 1968a). Hollenberg also notes the contrast between the larger ovoid spermatangia of *N. hawaiiensis* and the smaller cylindrical spermatangia reported for *N. ferulacea*. Other possible differences between *N. ferulacea* and these two species are segments that are somewhat longer in *N. hawaiiensis* and typically much shorter in *P. sparsa* (Hollenberg 1968a, Kapraun 1977, Kapraun and Norris 1982). Segment length was variable in *N. ferulacea* samples from Belize (as *P. ferulacea*, Kapraun and Norris 1982), and this variation was also present in Caribbean Panama specimens, making absolute distinctions based on this character difficult. Similarly, differences in the number of spermatangial sterile tip cells have been reported for these three species (Hollenberg 1968a, Kapraun and Norris 1982), but the intraspecific consistency of this character has been questioned (e.g., Skelton and South 2007, Stuercke and Freshwater 2008).

The classification of these taxa is further confounded by the absence of both a holotype specimen and distinct type locality. The east coast of Mexico is often cited as the type locality for *Neosiphonia ferulacea* (Hollenberg 1968a, Kapraun and Norris 1982, Kapraun et al. 1983), but this is just the first of the syntype localities listed by J. Agardh (1863), which also include North America, Guadeloupe, Australia, Marquesas Islands, and Hawaiian Islands. This wide geographic

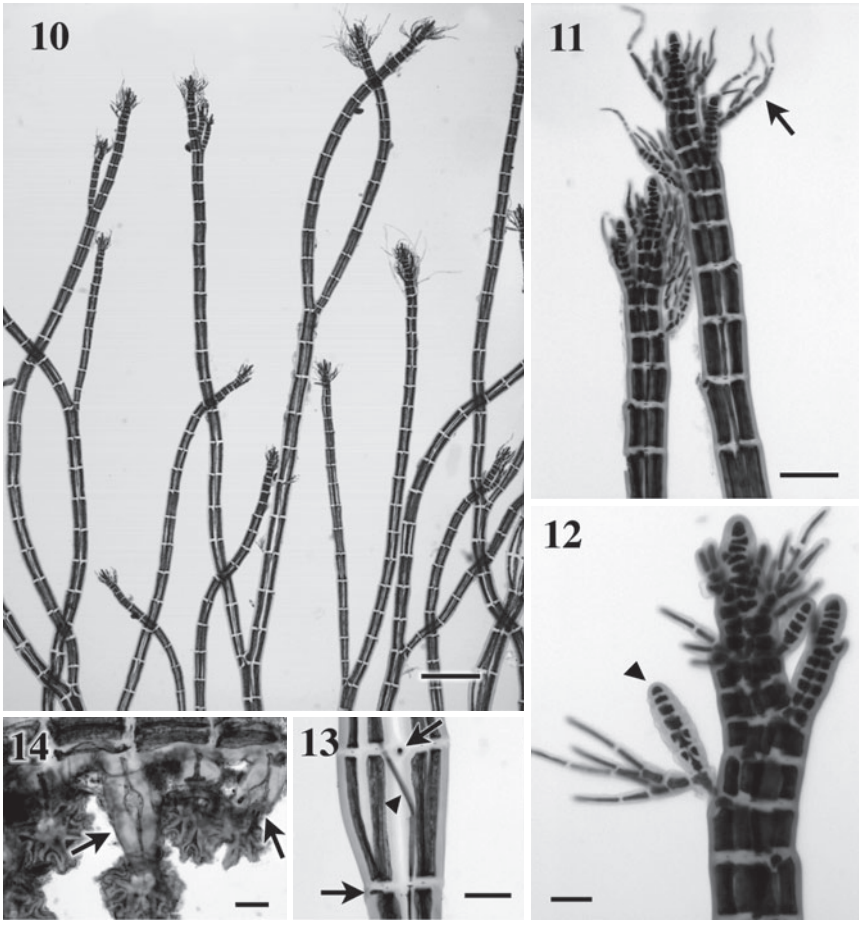
distribution increases the likelihood that *N. ferulacea* as described by J. Agardh includes multiple species. *N. ferulacea, N. hawaiiensis* and *Polysiphonia sparsa* are maintained as distinct species in this treatment based on their combinations of morphological differences and geographic distributions. Although *N. ferulacea* has been reported from the central and western Pacific, this name has been applied mostly to specimens from the Caribbean and tropical western Atlantic (Taylor 1960, 1969, Kapraun and Norris 1982, Kapraun et al. 1983, Ganesan 1990, Schneider and Searles 1991, Suárez 2005).

Neosiphonia ferulacea was collected at two different sites in the Galeta area of Caribbean Panama (Figure 1). The *rbc*L and COI sequences generated from these samples were not identical, but differed by only 0.56% and 0.50%, respectively, which is well within the range of intraspecific divergence for these two loci (Figures 2 and 3). ML analyses of *rbc*L and SSU sequence data resolved *N. ferulacea* within a strongly supported clade (rB100; sB87) that included both *Neosiphonia* and *Polysiphonia* species (Figures 4 and 5). *Neosiphonia ferulacea* is weakly allied in the *rbc*L 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 by the unidentified species' smaller habit and axis dimensions, branches that are not spindle-shaped, and mid-axis segments of erect axes that are 1–1.5× as long as wide.

Neosiphonia tongatensis **(Harvey in Kützing) M.S. Kim *et* I.K. Lee (1999, p. 280) (Figures 10–14)**

Basionym *Polysiphonia tongatensis* Harvey in Kützing (1864, p. 14).

Synonyms *Polysiphonia aquamara* I.A. Abbott (1947, p. 212); *Polysiphonia eastwoodiae* Setchell *et* N.L. Gardner (1930, p. 161); *Polysiphonia mollis sensu* Hollenberg (1961, p. 359) [non *P. mollis* J.D. Hooker *et* Harvey (1847, p. 397)]; *Polysiphonia mollis* var. *tongatensis* (Harvey in Kützing) Hollenberg (1968a, p. 69); *Polysiphonia snyderae* Kylin (1941, p. 35).



Figures 10–14 *Neosiphonia tongatensis*.
(10) Habit of erect axes, PHYKOS-2704, scale=0.20 mm, WNC2009-s262. (11) Apical portion of erect axes bearing trichoblasts (arrow), PHYKOS-2704, scale=50 µm, WNC2009-s262. (12) Branch apex with immature spermatangial branch (arrowhead) developing as a furcation of the trichoblast, PHYKOS-2704, scale=20 µm, WNC2009-s263. (13) Portion of erect axis squashed to show central axial cells (arrowhead), four pericentral cells per segment, and scar cells (arrows), PHYKOS-2704, scale=50 µm, WNC2009-s262. (14) Prostrate axis with rhizoids (arrows) cut off from the pericentral cells, PHYKOS-2704, scale=50 µm, WNC2009-s263.

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 (Figure 14); moderately branched in a dichotomous to subdichotomous pattern (Figure 10); erect axes 50–150 µm in diameter, prostrate axes 125–250 µm in diameter; mid-axis segments of erect axes predominantly 1–2× as long as wide; cortication absent; main axes with four pericentral cells (Figure 13); branches replacing trichoblasts in development; trichoblasts with several dichotomies, to 175 µm in length; scar cells one per segment in ¼ spiral series (Figures 11 and 13); adventitious laterals absent; tetrasporangia much distending segments, developing in spiral series, 40–80 µm in diameter; spermatangial branches developing as furcations of trichoblasts (Figure 12), 25–50×90–200 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 eastwoodiae*, Kapraun et al. 1983; as *Polysiphonia mollis sensu* Hollenberg, Hollenberg 1961; as *Polysiphonia tongatensis*, Schneider and Searles 1997, Abbott 1999.

Specimens studied Panama: *WNC2009-s261 to s263 (PHYKOS-2704)*, STRI research station, Punta Galeta, Colón, B. Wysor, 21 May 2009; *WNC2012-s020 (PHYKOS-3914)*, Shark Hole Point, Península Valiente, Bocas del Toro, B. Wysor, 06 Aug 2010.

Molecular vouchers GenBank accession numbers HM573570 (*rbc*L); 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. eastwoodiae* Setchell *et* Gardner (type locality Islas Revillagigedo) with *P. mollis* J.D. Hooker *et* Harvey (type locality Tasmania). Womersley (1979) refuted 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 was recognized as *Neosiphonia tongatensis* by Abbott (1999). Schneider and Searles (1997) studied isotype specimens of *N. tongatensis* and provided a detailed discussion of its taxonomy, especially its relationship with *P. eastwoodiae*, which they cited as a synonym.

Pacific material of *Neosiphonia tongatensis* reportedly has spermatangial branches that develop as furcations of trichoblasts with or without one to four sterile tip cells (Hollenberg 1968a, Abbott 1999). Venezuelan material examined by Kapraun et al. (1983) (as *Polysiphonia eastwoodiae*) was described as having spermatangial branches developing as furcations of, or developmentally replacing, trichoblasts, with or without a single sterile tip cell. Spermatangial branches were not included in the original descriptions of *N. tongatensis* (as *P. tongatensis*) and *P. eastwoodiae* (Setchell and Gardner 1930). Further study is necessary to determine if these reports represent the same species.

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

Genus *Polysiphonia*

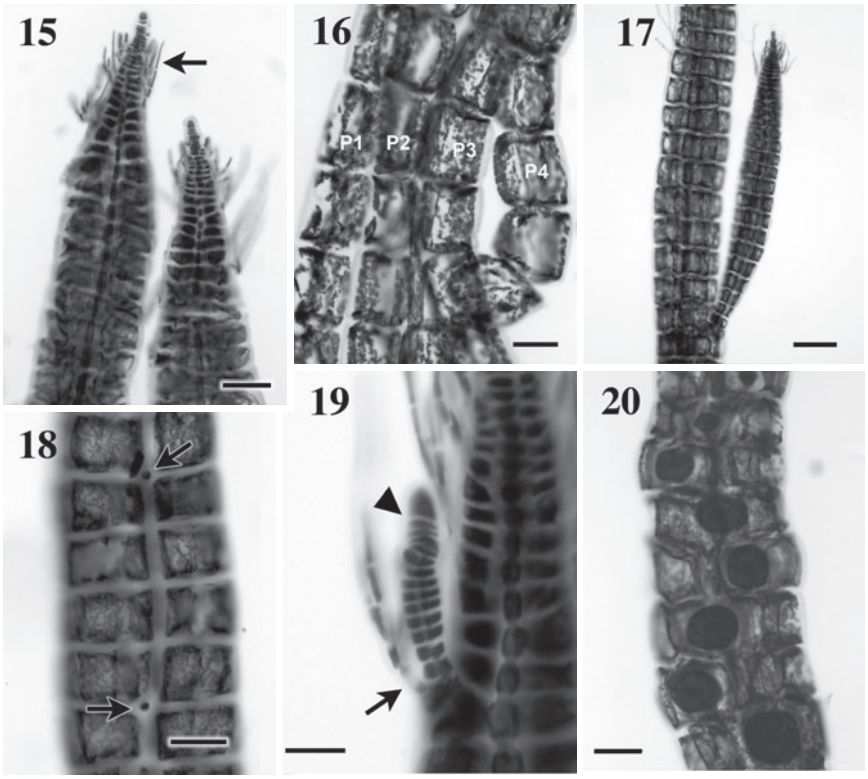
Polysiphonia binneyi Harvey (1853, p. 37) (Figures 15–20)

Description Plants to 3 (–15) cm tall, erect branches arising 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 (Figure 17); mid-axis segments of erect axes mostly slightly shorter than wide, with segments becoming increasingly short in upper portions of erect axes towards apices (Figures 15 and 18); cortication absent; main axes with four pericentral cells (Figure 16); branches forming in the axils of trichoblasts (Figure 19); trichoblasts long, thin, and tangled when mature, typically with three dichotomies; scar cells one per segment in ¼ spiral series (Figure 18); adventitious laterals lacking; tetrasporangia slightly distending segments, in spiral series in upper branches, 55–80 µm in diameter (Figure 20); spermatangial branches developing as furcations of trichoblasts, 25–50×100–200 µm, with two or three 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 Taylor 1960, Kapraun et al. 1983, Schneider and Searles 1991, Dawes and Mathieson 2008.

Specimens studied Panama: *WNC2009-s222 to s224* (*PHYKOS-2439*) and *WNC2009-s213*, *s214* (*PHYKOS-2458*), Isla Naranjo Arriba, Colón, L. Sargent, 19 May 2009; *WNC2009-s207 to s209* (*PHYKOS-2461*) and *WNC2009-s220, s221* (*PHYKOS-2517*), Isla Naranjo Arriba, Colón, S. Schmitt, 19 May 2009.



Figures 15–20 *Polysiphonia binneyi*.
(15) Apices of erect axes bearing trichoblasts (arrow), PHYKOS-2458, scale=50 µm, WNC2009-s213. (16) Portion of erect axis squashed to show four pericentral cells per segment (P1–P4), PHYKOS-2439, scale=50 µm, WNC2009-s223. (17) Lateral branch showing basal attenuation, PHYKOS-2517, scale=0.10 mm, WNC2009-s220. (18) Mid-axis segments of erect branch showing scar cells (arrows), PHYKOS-2461, scale=50 µm, WNC2009-s207. (19) Apical portion of main axis showing lateral branch (arrowhead) developing in the axil of a trichoblast (arrow), PHYKOS-2517, scale=20 µm, WNC2009-s220. (20) Reproductive branch displaying spiral series of tetrasporangia, PHYKOS-2439, scale=50 µm, WNC2009-s222.

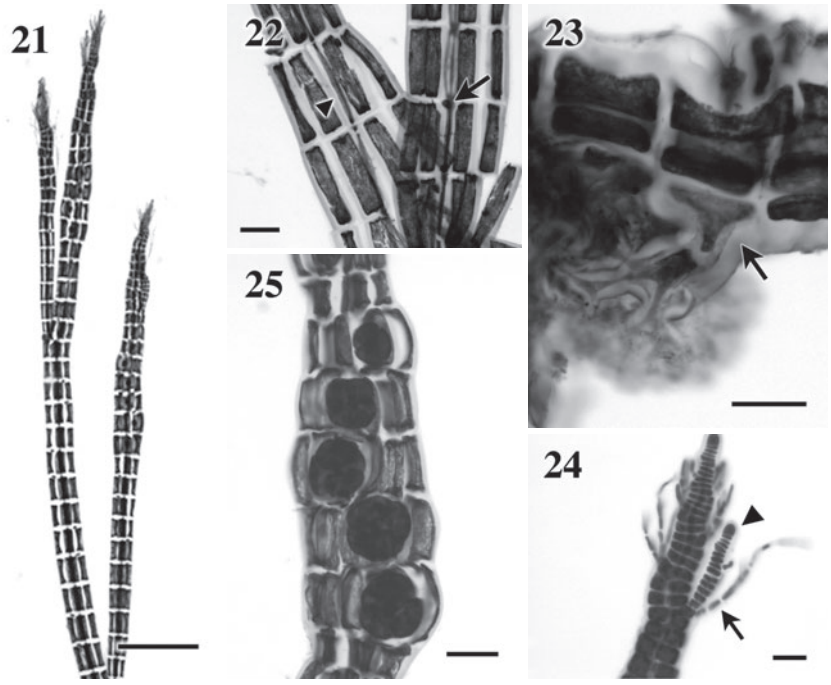
Molecular vouchers GenBank accession numbers HM573556, HM573555 (*rbc*L); HM560636 (SSU).

Remarks Four samples of *Polysiphonia binneyi* were collected in this study (Figure 3). Three of the four *rbc*L sequences generated for these samples were identical, and the fourth differed by 1.30%, which is within the range of intraspecific *rbc*L sequence variation observed in other species of *Polysiphonia s.l.* (McIvor et al. 2001, Kim et al. 2004) but relatively high considering that all four samples were collected contemporaneously from a Caribbean Panama collection site near Colón. The COI locus did not amplify for any of the collected specimens.

Polysiphonia binneyi was associated with *P. havanensis sensu* Børgesen and *Neosiphonia echinata* Harvey in both the *rbc*L and SSU ML trees (Figures 4 and 5). *Polysiphonia binneyi* was well supported (rB93) as the sister species of *P. havanensis sensu* Børgesen in the *rbc*L analysis, but was weakly supported (sB63) as sister to *N. echinata* in the SSU analysis. *Polysiphonia binneyi* closely resembles *P. havanensis sensu* Børgesen, but the latter species can be distinguished by its distinct and extensive system of prostrate axes, main axes of lesser diameter (mostly less than 150 µm), and spermatangial branches with no sterile tip cells (Kapraun et al. 1983, Schneider and Searles 1991, Dawes and Mathieson 2008). *Polysiphonia echinata* differs from *P. binneyi* in its rhizoids, which are cut off from pericentral cells, basal cortication, and abundant adventitious laterals that give the species a coarse appearance.

Polysiphonia havanensis sensu Børgesen (1918, p. 266) [non *P. havanensis* Montagne (1837, p. 352)] (Figures 21–25)

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 (Figure 23); branching moderate in a dichotomous to subdichotomous pattern (Figure 21); erect axes 50–110 µm in diameter, prostrate axes 150–170 µm in diameter; mid-axis segments of erect axes vary from 0.75–2× as long as wide; cortication absent; main axes with 4 pericentral cells (Figure 22); branches forming in the axils of trichoblasts (Figure 24); 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 (Figure 25); spermatangial branches developing as furcations



Figures 21–25 *Polysiphonia havanensis sensu* Børgesen.
(21) Habit of erect axes, PHYKOS-2628, scale=0.20 mm, WNC2009-s256. (22) Portion of erect axis squashed to show central axial cells (arrowhead), four pericentral cells per segment, and scar cells (arrow), PHYKOS-3185, scale=50 µm, WNC2009-s570. (23) Prostrate axis with rhizoid that is in open connection with the pericentral cell (arrow), PHYKOS-2628, scale=50 µm, WNC2009-s256. (24) Apical portion of main axis showing lateral branch (arrowhead) developing in the axil of a trichoblast (arrow), PHYKOS-3185, scale=20 µm, WNC2009-s570. (25) Reproductive branch displaying spiral series of tetrasporangia, PHYKOS-2628, scale=50 µm, WNC2009-s256.

of trichoblasts, 30–60×80–300 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 and Searles 1991.

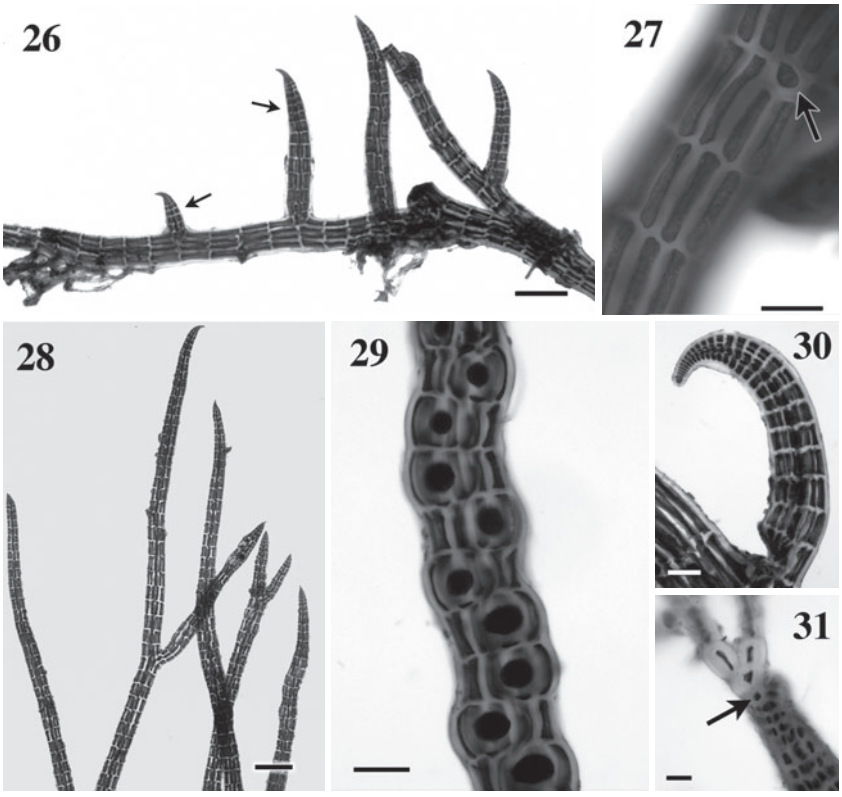
Specimens studied Panama: *WNC2009-s256 (PHYKOS-2628)*, West Limón Bay Jetty, Punta Toro, Colón, B. Wysor, 20 May 2009; *WNC2009-s570, s572 (PHYKOS-3185)*, Cayos Zapatillas, Bocas del Toro, B. Wysor and D.W. Freshwater, 25 Aug, 2009; *WNC2009-s571, s573* (*PHYKOS-3186)*, Cayos Zapatillas, Bocas del Toro, B. Wysor and D.W. Freshwater, 25 Aug, 2009.

Molecular vouchers GenBank accession numbers HM573554 (*rbc*L); 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) indicated 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 have been reported for *P. havanensis sensu* Børgesen (Børgesen 1918, Kapraun 1977, Schneider and Searles 1991). Reproductive structures are unknown for *P. havanensis* Montagne as both the original and Kützing's 1863 description include only sterile thalli. Børgesen indicated that whereas most specimens observed in his 1918 study were sterile, a few specimens displayed tetrasporangia 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. *P. 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 (Figure 2), but the *rbc*L sequences for all three samples were identical (Figure 3). Phylogenetic analyses of *rbc*L and SSU sequence data indicated that this species is closely related to *P. binneyi* and *Neosiphonia echinata* (Figures 4 and 5). However, these species are easily distinguished from one another morphologically (see remarks for *P. binneyi*).

It has been suggested that *Polysiphonia havanensis sensu* Børgesen may be synonymous with *P. sertularioides* (Grateloup) J. Agardh (Kapraun 1977, Schneider and Searles 1991). These species are clearly distinct from one another as



Figures 26–31 *Polysiphonia howei*.
(26) Habit image showing erect axes (arrows) arising from prostrate axis, PHYKOS-3528, scale=0.20 mm, WNC2009-s577. (27) Mid-axis segments of erect branch showing scar cell (arrow) and pericentral cells that are shifted to offset positions across segments, PHYKOS-3527, scale=40 µm, WNC2009-s556. (28) Habit of erect axes, PHYKOS-3528, scale=0.20 mm, WNC2009-s576. (29) Reproductive branch displaying spiral series of tetrasporangia, PHYKOS-3527, scale=50 µm, WNC2009-s556. (30) Young erect branch strongly arched toward prostrate axis, PHYKOS-3526, scale=50 µm, WNC2009-s558. (31) Apical part of erect axis showing distinctly short basal cell of trichoblast (arrow), PHYKOS-3141, scale=20 µm, WNC2010-s041.

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 pit connections (Børgesen 1918, Womersley 1979, 2003, Athanasiadis 1987). The distinction between these two species was supported by ML phylogenies based on *rbc*L and SSU sequence data as *P. havanensis sensu* Børgesen was distantly related to *P. sertularioides* (Figures 4 and 5).

Polysiphonia howei Hollenberg in W.R. Taylor (1945, p. 302) (Figures 26–31)

Synonyms *Neosiphonia howei* (Hollenberg) Skelton *et* G.R. South (2007, p. 188); *Polysiphonia rhizoidea* Meñez (1964, p. 217); *Polysiphonia yonakuniensis* Segi (1951, p. 257); *Lophosiphonia obscura sensu* Weber van Bosse 1923 [non *L. obscura* (C. Agardh) Falkenberg in F. Schmitz *et* Falkenberg (1897, p. 460)].

Description Plants to 2 (–5) cm tall, erect branches arising from a prostrate branching system (Figure 26) attached to the substratum by rhizoids cut off from pericentral cells; branching sparse to moderate in a dichotomous to subdichotomous pattern (Figure 28); young erect axes at first strongly arched toward prostrate axis (Figure 30); erect axes 100–150 µm in diameter, prostrate axes (75–) 125–150 µm in diameter; mid-axis segments of erect axes predominantly 0.5–1× as long as wide; cortication absent; main axes with 8–10 (–12) pericentral cells that tend to shift to offset positions across segments (Figure 27); central axial cell distinctly enlarged; branches replacing trichoblasts in development; trichoblasts with distinctly short basal cell, dense at apices when present (Figure 31) but infrequent to absent on many specimens; scar cells present and obvious, variable in pattern and frequency (Figure 27); adventitious laterals common; tetrasporangia in spiral series in mid to upper branch segments, slightly distending segments, (40–) 50–65 µm in diameter (Figure 29); spermatangial branches reported to replace trichoblasts and develop as furcations of trichoblasts (see remarks); cystocarps globose to ovoid, 120–360 µm in diameter.

Type locality Whale Cay, Berry Islands, Bahamas.

Other sources Taylor 1945, Hollenberg 1958, 1968b, Kapraun 1980a, Kapraun et al. 1983, Schneider and Searles 1991, Abbott 1999, Dawes and Mathieson 2008.

Specimens studied Panama: *WNC2010-s041, s074, s075 (PHYKOS-3141)*, Cayos Tigres, Bocas del Toro, B. Wysor, 25 Aug 2009; *WNC2009-s558, s559 (PHYKOS-3526)*, Flat Rock Beach, Isla Colón, Bocas del Toro, D.W. Freshwater, 17 Jan 2007; *WNC2009-s556, s557 (PHYKOS-3527)*, Flat Rock Beach, Isla Colón, Bocas del Toro, D.W. Freshwater, 17 Jan 2007; *WNC2009-s576, s577 (PHYKOS-3528)*, Swan Cay, Isla Colón, Bocas del Toro, M. Albis, 19 Jul 2008.

Molecular vouchers GenBank accession numbers HM573543 (*rbc*L); HM560656 (SSU); HM573520, HM573521 (COI).

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

Development of spermatangial branches appears to be variable within samples identified as *Polysiphonia 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 most frequently observed single spermatangial branches developmentally replacing trichoblasts but occasionally also pairs, with each spermatium developing on the primary trichoblast bifurcation, and neither condition with sterile tip cells (Hollenberg 1968b). Abbott (1999) observed similar spermatangial branch development in Hawaiian samples identified as *P. howei* but made no mention of sterile tip cell presence or absence. Spermatangial branches are reported as developmentally replacing trichoblasts, with or without sterile tip cells, in the southern Caribbean (Kapraun et al. 1983). In contrast, Dawes and Mathieson (2008) reported that spermatangial branches develop as furcations of trichoblasts in Florida specimens, and this condition is also reported for North Carolinian specimens tentatively assigned to *P. howei* (Hollenberg 1958); no mention of sterile tip cell presence or absence was made in either study. Development of spermatangial branches was not observed in our study.

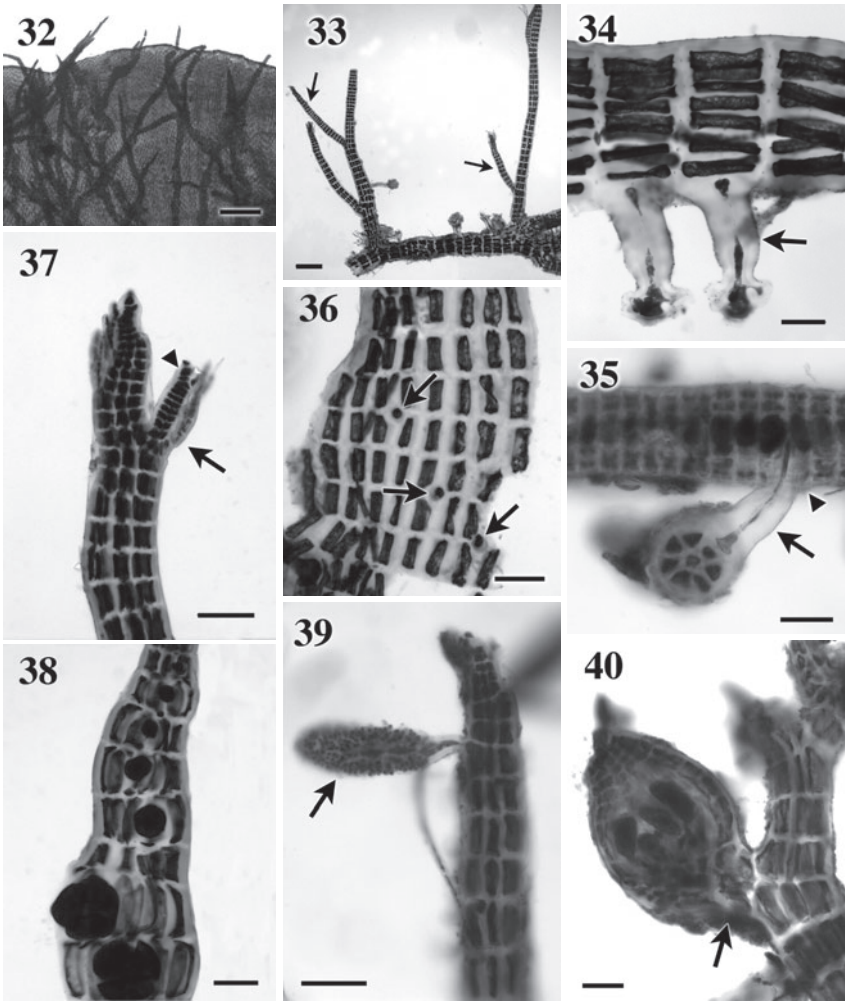
The diverse states of spermatangial branch structure reported for specimens identified as *Polysiphonia howei* suggest that perhaps more than one species has been identified under this name. Diversity within the species is also suggested by the number of pericentral cells reported for samples identified 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 and western Pacific samples describes them as having eight to ten pericentral cells; Kapraun (1980a) also observed this number in North Carolinian specimens. Samples in this study had 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 *et* Falkenberg has been historically confused with *Polysiphonia howei* (Taylor 1945, Hollenberg 1958, 1968b) due to the number of pericentral cells in *L. obscura* being incorrectly cited as 11–14 rather than 5–7 (see Silva et al. 1996 for further discussion). The exclusive endogenous origin of erect branches (branches arising from the central axis subsequent to pericentral cell formation) also distinguishes species of *Lophosiphonia* from many species of *Polysiphonia*, the majority of which have branches of exogenous origin (branches arising from subapical cell division prior to pericentral cell formation) (Hollenberg 1942). Specimens of *P. howei* examined in this study had trichoblasts/lateral branch primordia that were cut off from subapical cells prior to pericentral cell formation (exogenous), but the quick development of pericentral cells in this species makes the observation of this character difficult. *P. howei* also closely resembles *P. exilis* Harvey, but the latter species can be distinguished by its arrangement of pericentral cells in non-shifting longitudinal rows and young branches that are perpendicular to, and not arching towards, the prostrate axis (Hollenberg 1968b).

Skelton and 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 spermatangial branches developing as furcations of trichoblasts, sometimes with one or two sterile tip cells (Kim and Lee 1999). In contrast, Hollenberg (1945, 1968b) described a creeping habit in *P. howei* and spermatangial branches that developmentally replace trichoblasts. Members of *Neosiphonia* also have four to nine pericentral cells; *P. howei* was originally described as having 10–12 pericentral cells. In ML phylogenies based on SSU and *rbc*L sequence data, samples identified as *P. howei* were distantly related to species of *Neosiphonia* (Figures 4 and 5). For these reasons, *P. howei* is not recognized here as belonging to *Neosiphonia* and is retained in *Polysiphonia*.

Polysiphonia lobophoralis sp. nov. (Figures 32–40)

Diagnosis *Thallus usque ad 3 mm altus, ecorticatus, ramis erectis extense systemate ramificanti prostrato exorientibus. Axes erecti simplices aut parce ramose; axes prostrati ramosi moderate ad angulos latos. Rami facientes in axillas trichoblastorum. Rhizoidea medio cellularum pericentralium abscissa. Cellulae pericentrales septem usque ad octo. Trichoblasti et cellulae-cicatrices variabiles ordinatione. Tetrasporangia in seriebus spiralibus disposita. Spermatia crescendia ut pote furcam trichoblastorum, sine cellulis*



Figures 32–40 *Polysiphonia lobophoralis* sp. nov.
(32) Habit image of part of holotype specimen showing axes growing on *Lobophora*, US#217938 (PHYKOS-3899), scale=0.50 mm. (33) Habit image showing erect (arrows) and prostrate axes, PHYKOS-5309, scale=0.20 mm, WNC2009-s574. (34) Prostrate axis with rhizoids (arrow) that are cut off from the middle of pericentral cells, PHYKOS-5309, scale=50 µm, WNC2009-s574. (35) Transverse section through prostrate axis with seven pericentral cells and a rhizoid (arrow) cut off from one pericentral cell and penetrating into the *Lobophora* thallus (arrowhead), PHYKOS-3895, scale=50 µm, WNC2012-s023. (36) Portion of erect axis sqashed to show eight pericentral cells per segment and scar cells (arrows), PHYKOS-5309, scale=50 µm, WNC2009-s575. (37) Apical portion of main axis showing lateral branch (arrowhead) developing in the axil of a trichoblast (arrow), PHYKOS-5309, scale=50 µm, WNC2009-s575. (38) Reproductive branch displaying spiral series of tetrasporangia, PHYKOS-5309, scale=50 µm, WNC2009-s574. (39) Spermatangial branch (arrow) with no sterile tip cells developing as a furcation of the trichoblast, Holotype US#217938 (PHYKOS-3899). (40) Short stalked (arrow), elliptical cystocarp, Holotype US#217938 (PHYKOS-3899).

sterilibus apicalibus. Cystocarpia brevipedicellata, elliptica usque ad ovata, ostiolis angustis.

Thallus to 3 mm tall, ecorticate, with erect axes arising from an extensive prostrate branching system. Erect axes simple to sparingly branched; prostrate axes moderately branched at wide angles. Branches forming in the axils of trichoblasts. Rhizoids cut off from middle of pericentral cells. Pericentral cells seven to eight. Trichoblasts and scar cells variable in pattern. Tetrasporangia arranged in spiral series. Spermatia developing as furcations of trichoblasts, without sterile tip cells. Cystocarps short, stalked, elliptical to ovate, with narrow ostioles.

Type specimens Holotype deposited in US Algal Collection #217938 (=*PHYKOS-3899*). Epiphyte on *Lobophora*, south of Big Pantain Cay, Península Valiente, Bocas del Toro, Caribbean coast of Panama, 9.12376° N, 081.79743° W, 06 Aug 2010, Coll. D.W. Freshwater. Herbarium sheet and permanent slides. Isotype in WNC (WNC33415). Permanent slides.

Etymology The epithet "*lobophoralis*" is a play of words on the genus *Lobophora* and the greek "phorein" equals "to carry," i.e., *Lobophora* carries the new species of *Polysiphonia*, as all specimens collected to date have

been growing epiphytically on *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira.

Description Plants to 3 mm tall, erect branches arising from an extensive prostrate branching system (Figures 32 and 33) attached to substratum by rhizoids cut off from the middles of pericentral cells (Figures 34 and 35); erect axes simple to sparingly branched, prostrate axes 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 0.5× as long as wide (Figures 33 and 34); cortication absent; main axes with (7–) 8 pericentral cells (Figures 35 and 36); branches forming in the axils of trichoblasts (Figure 37); trichoblasts to 240 µm in length, typically with two or three dichotomies; scar cells present and obvious, variable in pattern and frequency, but mostly every few segments; adventitious laterals occasionally present, linear; tetrasporangia moderately distending segments, in spiral series, 70–78 µm in diameter (Figure 38); spermatangial branches developing as furcations of trichoblasts, without sterile tip cells (Figure 39); observed fertile trichoblasts sparingly branched; cystocarps elliptical to ovate, short stalked, with narrow ostioles (Figure 40).

Specimens studied Panama: *WNC2009-s574, s575 (PHYKOS-5309* or *TFP08-177)*, Tervi Bight, Bocas del Toro, S. Fredericq, 13 Jul 2008. *WNC33416* and *WNC33418 (PHYKOS-3891; 3918)*, South of Big Pantain Cay, Península Valiente, Bocas del Toro, D.W. Freshwater, 06 Aug 2010. *WNC33417, WNC2012-s023, s024 (PHYKOS-3895)*, off Shark Hole Point, Península Valiente, Bocas del Toro, D.W. Freshwater, 06 Aug 2010. *WNC33419 (PHYKOS-4002)*, Canal del Tigre, Bocas del Toro, D.W. Freshwater, 08 Aug 2010. *WNC33420 (PHYKOS-4036)*, North of Cayo Zapatilla, Bocas del Toro, D.W. Freshwater, 08 Aug 2010.

Molecular vouchers GenBank accession numbers HM573551 (*rbc*L); HM560657 (SSU).

Remarks *Neosiphonia tepida* (Hollenberg) S.M. Guimarães *et* M.T. Fujii, *P. foetidissima* Cocks *et* Bornet, *P. nigra* (Hudson) Batters, and *P. paniculata* Montagne are 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. However, *N. tepida* is larger (to 8 cm tall), segments of erect axes 1–2× 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. *P. foetidissima* has a larger habit (to 11 cm tall), erect axes 20–40 µm in diameter, segments of erect axes 1–2× as long as wide, trichoblasts apparently on every segment, and branches with slight to marked basal attenuation (Maggs and Hommersand 1993). *P. nigra* has a larger habit (to 30 cm tall), major axes 200–300 µm in diameter, and mostly 9–13 pericentral cells that are spirally twisted (Maggs and Hommersand 1993). *Polysiphonia paniculata* has a larger habit (to 25 cm tall), ultimate branches with basal attenuation, segments of erect axes 2–4× as long as wide, and mostly 10–12 pericentral cells (Hollenberg and 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 *et* Harvey, and *P. confusa* Hollenberg. *Polysiphonia decipiens* has lateral branches that are occasionally hooked and trichoblasts on every segment (Womersley 1979). *Polysiphonia isogona* has rhizoids cut off from the proximal ends of pericentral cells, segments extending to 8× as long as wide in erect axes, and mostly nine or ten 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 1–2.5× as long as wide, rhizoids cut off from the proximal ends of pericentral cells, branches that are attenuated at both ends, and mostly eight to ten pericentral cells (Dawson 1964, Abbott and Hollenberg 1976, Abbott 1999). *Polysiphonia decipiens* and *P. isogona* are both described as having habits much taller than that of *P. lobophoralis*.

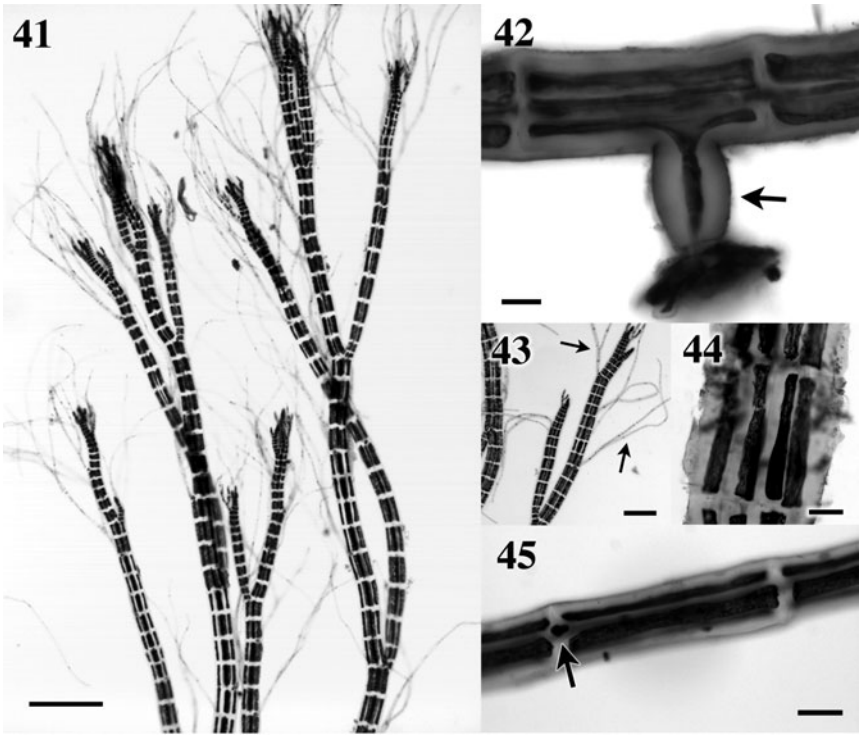
The first *Polysiphonia lobophoralis* specimen recognized as a new species was collected by S. Fredericq from Tervi Bight near Isla Colon in July 2008. Six additional samples were collected during August 2010 visits to four other sites in the Bocas del Toro area. Despite focused searches of various macroalgae for epiphytic *Polysiphonia* species, *P. lobophoralis* specimens were only found growing epiphytically on *Lobophora variegata*.

Sequences of *rbc*L were generated for three of the collected specimens (PHYKOS-3899 [Holotype]; -4002; 5309), and they were found to be identical. ML analysis of *rbc*L sequence data resolves *Polysiphonia lobophoralis* 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) (Figure 4). Placement within the multipericentral cell group was also well supported in the SSU ML phylogeny (sB89) (Figure 5). Other species resolved in the clade differed from *P. lobophoralis* in the character states listed above or in 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 *et* Harvey), or branches with marked basal attenuation (as in *P. constricta* Womersley).

Polysiphonia macrocarpa (C. Agardh) Sprengel (1827, p. 350) [non *P. macrocarpa* Harvey 1836, p. 206] (Figures 41–45)

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

Description Plants to 1–3 cm tall, composed predominantly of entangled erect axes arising from prostrate axes that have rhizoids in open connection with pericentral cells (Figure 42); highly branched in a subdichotomous pattern below,



Figures 41–45 *Polysiphonia macrocarpa*.
(41) Habit of erect axes, PHYKOS-2617, scale=0.1 mm, WNC2009-s253. (42) Prostrate axis with rhizoid (arrow) that is in open connection with the pericentral cell, PHYKOS-2561, scale=20 µm, WNC2009-s215. (43) Apical part of erect axis bearing trichoblasts (arrows), PHYKOS-2561, scale=50 µm, WNC2009-s218. (44) Portion of erect axis squashed to show four pericentral cells per segment, PHYKOS-2561, scale=50 µm, WNC2009-s215. (45) Mid-axis segments of erect branch showing scar cells (arrow) and elongated pericentral cells, PHYKOS-2627, scale=50 µm, WNC2009-s258.

alternate near apices (Figure 41); erect axes 30–70 (–100) µm in diameter; mid-axis segments of erect axes predominantly 2–3× as long as wide (Figure 45); cortication absent; main axes with four pericentral cells (Figure 44); branches replacing trichoblasts in development; trichoblasts usually abundant near apices, long and delicate, to 500 µm long, commonly with two or three dichotomies (Figure 43); scar cells present and obvious (Figure 45), but not present on every segment; adventitious laterals occasionally present, linear; tetrasporangia in straight series; cystocarps slightly urceolate, distinctly enlarged; spermatangial branches unknown.

Type locality Port-au-Prince, Haiti (see remarks).

Other sources C. Agardh 1824, Sprengel 1827.

Specimens studied *Polysiphonia macrocarpa* (C. Agardh) Sprengel, Panama: *WNC2009-s215 to s219 (PHYKOS-2561)*, Punta Gorda, Colón, D.W. Freshwater, 20 May 2009; *WNC2009-s250 to s254, s566 (PHYKOS-2617),* Punta Gorda, Colón, S. Schmitt, 20 May 2009; *WNC2009-s257 to s259 (PHYKOS-2627)*, Punta Gorda, Colón, S. Schmitt, 20 May 2009; *WNC2009-s255, s260 (PHYKOS-2630)*, Punta Gorda, Colón, D.W. Freshwater, 20 May 2009; Cuba: *US-2507 to 2515*, Batabano, Havana Province, E.Y. Dawson, 7 May 1949; Florida: *US-2501 to 2506*, South of Dinner Key, Biscayne Bay, Miami-Dade County, Miami, E.Y. Dawson, 4 May 1949; *P. atlantica* Kapraun *et* Norris, North Carolina: *WNC-8793*, floating dock, Wrightsville Beach, New Hanover County, D.F. Kapraun, 19 Aug 1976; *WNC*-14579, floating dock, Wrightsville Beach, New Hanover County, D.F. Kapraun, 8 Sept 1976; *WNC2005-s037*, CORMP site OB-27, Onslow Bay, B. Stuercke, 12 May 2005; *WNC2005-s057, s058*, CORMP site OB-27, Onslow Bay, B. Stuercke, 30 Aug 2004; *WNC2005-s137, s138*, CORMP site OB-27, Onslow Bay, B. Stuercke, 9 Jun 2005.

Molecular vouchers GenBank accession numbers HM573545 (*rbc*L); HM560632 (SSU); HM573538 (COI).

Remarks The type locality of *Hutchinsia macrocarpa* C. Agardh is described as "In mari Antillarum ad 'Port au Pray'" (C. Agardh 1824). Kapraun and Norris (1982) noted that while other type localities are Latinized in C. Agardh's *Systema Algarum* (C. Agardh 1824), the type locality for *H. macrocarpa* is left in French with quotation marks, possibly suggesting a quandary concerning 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. *H. 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 been reported historically from the western Atlantic (e.g., Børgesen 1918, Taylor 1960). The occurrence of these heterotypic homonyms led Kapraun and Norris (1982) to propose a new name, *P. atlantica* Kapraun *et* 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 *rbc*L and COI sequence data clearly showed that the Panamanian samples were genetically distinct from offshore North Carolinian samples that were also identified morphologically as *P. atlantica* (Figures 2 and 3). The two groups of samples differed by 6.38% at the *rbc*L locus and 6.28% at the COI locus. This is clearly beyond the ≤2.13% (predominantly ≤1.3%) intraspecific *rbc*L sequence divergence that has been observed in previous studies of *Polysiphonia* and better agrees with interspecific *rbc*L sequence divergence values of 3.07%–14.12% observed for other species within the genus (McIvor et al. 2001, Kim et al. 2004, Kim and Yang 2005). The distinction between these two groups of samples is also apparent in *rbc*L and SSU ML phylogenies (Figures 4 and 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 Carolinian samples, greater observed height in some Panamanian samples (3 cm) than in North Carolinian samples (2 cm), and consistently longer segment length in the North Carolinian samples. Trichoblast presence is not a reliable species identifier as this character is described as variable within North Carolinian *Polysiphonia atlantica* (Kapraun 1977, Stuercke and Freshwater 2008, this study). A small height difference and slight variation in segment length are also not independently reliable characters (Kim et al. 1994, 2004, Curiel et al. 2002, Stuercke and Freshwater 2008).

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 herbaria. No distinction was observed in the vegetative morphologies of these samples. Reproductive structures were not observed in Panamanian or some US samples. US samples from Cuba had tetrasporangia developing in straight series and urceolate cystocarps; inshore North Carolinian samples had these same character states, as well as spermatangial branches developmentally replacing trichoblasts and with no sterile tip cells (Kapraun 1977, 1980a, this study). Offshore North Carolinian samples also had tetrasporangia developing in straight series but no gametophytic structures were present.

Schneider and Searles (1991) observed that offshore North Carolinian samples identified as *Polysiphonia macrocarpa* Harvey in Schneider (1976) differed greatly from inshore North Carolinian 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 and 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 (C. 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 Carolinian and Panamanian species.

Polysiphonia nuda sp. nov. (Figures 46–51)

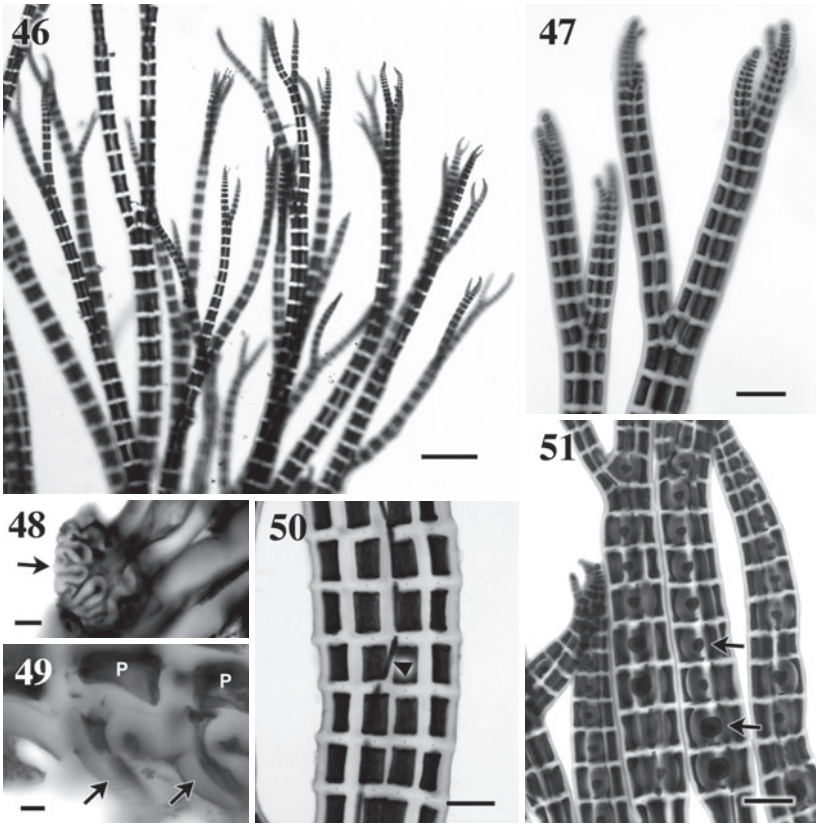
Diagnosis Thallus ecorticatus, ramis erectis systemate ramificanti prostrato exorientibus. Pars erecta maxime ramose, dichotoma usque ad subdichotoma. Rhizoidea cellulis pericentralibus abscissa. Cellulae pericentrales quattuor. Trichoblasti et cellulae-cicatrices absentes. Tetrasporangia in seriebus rectis disposita. Structurae sexuales non observatae.

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

Type specimens Holotype deposited in US Algal Collection #211334 (=*PHYKOS-2613*) including US Algal Collection slides nos. –8611 thru –8614. Growing intertidally on synthetic plastic among rubble, Parque de Juventud, Calle Primero, Colón, Caribbean coast of Panama, 9°22.004′ N, 079°54.259′ W, 20 May 2009, Coll. Brian Wysor. Isotype in WNC, WNC32156. Herbarium sheets and permanent slides.

Etymology From the Latin adjective *nuda*, meaning nude or bare, referring to the lack of vegetative trichoblasts and scar cells in this species.

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 (Figure 49); rhizoids with digitate, unicellular tips (Figure 48); highly branched in a dichotomous to subdichotomous pattern (Figures 46 and 47); erect axes 70–140 µm in diameter, prostrate axes 120–180 µm in diameter; mid-axis segments of erect axes 0.5–1× as long as wide; cortication absent; main axes with four pericentral cells (Figure 50); trichoblasts and scar cells absent; adventitious laterals absent; tetrasporangia slightly distending segments, in long straight series (Figure 51); spermatangial branches and cystocarps unknown.



Figures 46–51 *Polysiphonia nuda* sp. nov.
(46) Habit of erect axes, PHYKOS-2613, scale=0.20 mm, US#211334. (47) Apical portion of erect axes lacking trichoblasts, PHYKOS-2613, scale=50 µm, US#211334. (48) Rhizoid with digitate, non-multicellular ends (arrow), PHYKOS-2613, scale=20 µm, US#211334. (49) Prostrate axis with rhizoids (arrow) that are cut off from the pericentral cells (P), PHYKOS-2613, scale=20 µm, US#211334. (50) Portion of erect axis squashed to show central axial cells (arrowhead), four pericentral cells per segment, and lack of scar cells, PHYKOS-2613, scale=50 µm, WNC32156. (51) Reproductive branches displaying straight series of developing tetrasporangia (arrows), PHYKOS-2613, scale=50 µm, US#211334.

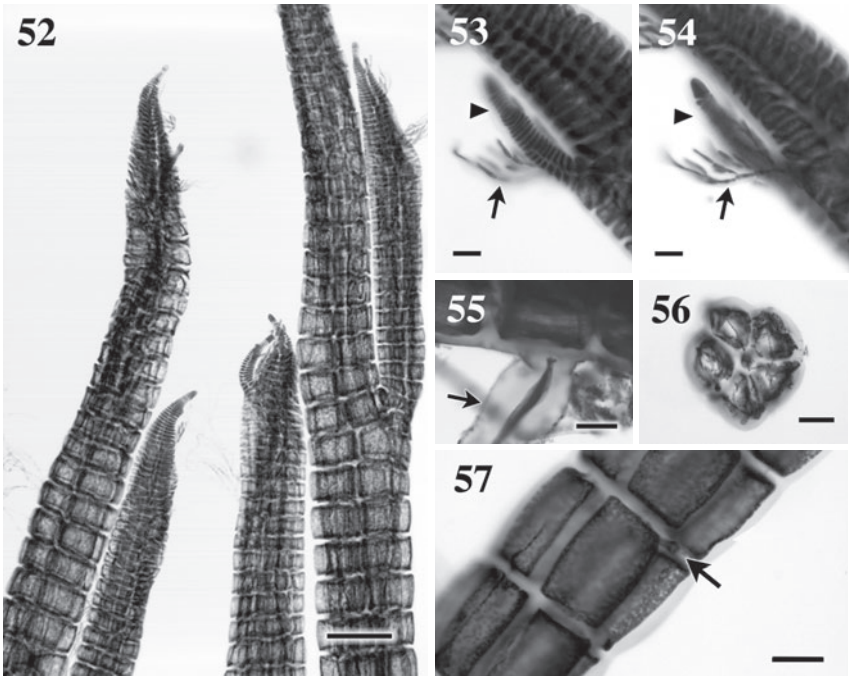
Molecular vouchers GenBank accession numbers HM573571 (*rbc*L); HM560648 (SSU); HM573517 (COI).

Remarks *Polysiphonia nuda* is distinguished from other species of *Polysiphonia* by a combination of the following character states: main axes with four pericentral cells, rhizoids cut off from pericentral cells, tetrasporangia that develop in straight series, and the absence of trichoblasts and scar cells. Although 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 *P. pungens* Hollenberg), 1961, Hollenberg and 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 *rbc*L and COI sequence data (Figures 2 and 3). In ML trees generated from *rbc*L and SSU sequence data, *P. nuda* was placed within a clade of predominantly *Neosiphonia* species (Figures 4 and 5). Species of the genus *Neosiphonia* are characterized by having lateral branch or trichoblast initials on every segment, tetrasporangia in spiral series, and abundant trichoblasts (Kim and Lee 1999); the absence of these character states in *P. nuda* distinguishes it from *Neosiphonia*. *P. nuda* appears most closely related to *N. tongatensis* in the *rbc*L ML phylogeny, but this relationship is not supported by the SSU data. *N. tongatensis* has one scar cell per segment, scar cells in ¼ spiral series, abundant trichoblasts, and tetrasporangia developing in spiral series.

Polysiphonia pentamera Hollenberg (1968, p. 204) (Figures 52–57)

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 (Figure 55); branching sparse to moderate in a subdichotomous pattern (Figure 52); erect axes 175–250 (–300) µm in diameter,



Figures 52–57 *Polysiphonia pentamera*.
(52) Habit of erect axes, PHYKOS-3529, scale=0.10 mm, WNC2009-s185. (53) Apical part of main axis showing lateral branch (arrowhead) forming to the side of a trichoblast (arrow), PHYKOS-3529, scale=20 µm, WNC2009-s184. (54) Apical part of main axis showing foreground of lateral branch from Figure 53 (arrowhead) forming to the side of a trichoblast (arrow), PHYKOS-3529, scale=20 µm, WNC2009-s184. (55) Prostrate axis with rhizoid (arrow) cut off from the pericentral cell, PHYKOS-3531, scale=50 µm, WNC2009-s195. (56) Cross section of branch axis showing central axial cell and five pericentral cells, PHYKOS-3529, scale=50 µm, WNC2009-s184. (57) Mid-axis segments of erect branch showing scar cell (arrow), PHYKOS-3532, scale=50 µm, WNC2009-s171.

prostrate axes 175–300 µm in diameter; mid-axis segments of erect axes mostly 0.5× as long as wide (Figure 57); cortication absent; main axes with five pericentral cells (Figure 56); branches forming laterally from the basal trichoblast cell (Figures 53 and 54); trichoblasts long, thin, and tangled when mature, to 300 µm long, with several dichotomies; scar cells not occurring at every segment and sometimes rare (Figure 57); adventitious laterals infrequent, mostly linear; tetrasporangia in spiral series, 55 µm in diameter; spermatangial branches and cystocarps unknown.

Type locality Eniwetok Atoll, Marshall Islands.

Other sources Hollenberg 1968b, Abbott 1999.

Specimens studied Panama: *WNC2009-s152 to s154 (PHYKOS-1995)*, STRI research station, Punta Galeta, Colón, B. Wysor and L. Sargent, 14 May 2009; *WNC2009-s183 to s186 (PHYKOS-3529)*, Isla Planito near Coiba, Veraguas, B. Wysor and J. Alden, 15 Jan 2008; *WNC2009-s187 to s192 (PHYKOS-3530)*, Bahía Honda near El Barranco, Veraguas, B. Wysor and J. Alden, 15 Jan 2008; *WNC2009-s193 to s198 (PHYKOS-3531)*, Bahía Honda near El Barranco, Veraguas, B. Wysor and J. Alden, 15 Jan 2008; *WNC2009-s168 to s174 (PHYKOS-3532)*, Isla Afuera, Veraguas, B. Wysor and J. Alden, 16 Jan 2008; Vietnam: *US-66525* (slide number *US-2543*), Ile de Tre, Nhatrang, E.Y. Dawson, 6 Feb 1953.

Molecular vouchers GenBank accession numbers HM573563, HM573564 (*rbc*L); 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 has been previously treated as having three possible states: branches developing independently of trichoblasts, branches replacing trichoblasts, and branches developing in the axils of trichoblasts (e.g., Stuercke and Freshwater 2008). Panamanian *P. pentamera* has a fourth character state: lateral branches develop laterally from the basal trichoblast cell rather than in an axilary position immediately distal to the trichoblasts (Figures 53 and 54). This character state is also observed in *P. schneideri* Stuercke *et* Freshwater (Stuercke and Freshwater 2010, this study) and *P. mollis* J.D. Hooker *et* Harvey (Womersley 1979).

Skelton and South (2007) proposed the new combination *Neosiphonia polyphysa* (Kützing) Skelton *et* South for *Polysiphonia polyphysa* Kützing (type locality New Caledonia), and included *P. pentamera* as a synonym. This synonymy was based on their observation of specimens that lacked the forcipate apices characteristic of *N. polyphysa* (Skelton and South 2007, p. 189). Hollenberg (1968b) remarked on *P. polyphysa* being a species similar to *P. pentamera*, but cited its forcipate apices and exceedingly swollen pericentral

cells (the latter by quoting Kützing's "*valde inflates*") as differences. Kützing's (1863) complete statement, "*siphonibus inferioribus valde inflates*" more specifically states that it is the proximal pericentral cells that are exceedingly swollen. Neither swollen pericentral cells nor forcipate tips have been observed in Panamanian *P. pentamera* specimens. *P. pentamera* also exhibits three character states incompatible with its inclusion in *Neosiphonia*: trichoblasts that do not develop on every segment, trichoblasts not abundant, and a basal system of prostrate axes that give rise to erect axes.

Five samples of *Polysiphonia pentamera* were collected in this study (Figure 2). Four were collected from Pacific sites and one from a Caribbean site. All four Pacific specimens (PHYKOS-3529, -3550, -3531, and -3532) shared the same *rbc*L sequence, which differed from the Caribbean *P. pentamera rbc*L 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 *rbc*L and SSU sequence data (Figures 4 and 5) placed *P. pentamera* in a clade with two other species that have five to seven pericentral cells: *P. schneideri*, which has five to seven pericentral cells, and an unidentified species of *Polysiphonia* (PHYKOS-3535), which strictly has five pericentral cells. *Polysiphonia schneideri* differs from *P. pentamera* mainly in having five to seven pericentral cells and tetrasporangia that develop in straight series. The unidentified species of *Polysiphonia* differs from *P. pentamera* by having scar cells at every segment in spiral series in erect axes near the apices and segments that are mostly as long as they are 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) (Figures 58–62)

Description Plants to 5 mm tall, erect branches arising from a prostrate branching system attached to substratum by rhizoids cut off from pericentral cells (Figure 61); moderately branched in a subdichotomous pattern (Figure 58); erect axes 60–70 µm in diameter, prostrate axes 70–100 µm in diameter; mid-axis segments of erect axes mostly as long as they are wide or only slightly longer (Figure 60); cortication absent; main axes with four pericentral cells; branches replacing trichoblasts in development; trichoblasts to 420 µm in length, with only a few dichotomies (Figure 59); 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; tetrasporangia in slightly spiral series (Figure 62); spermatangia and cystocarps not observed.

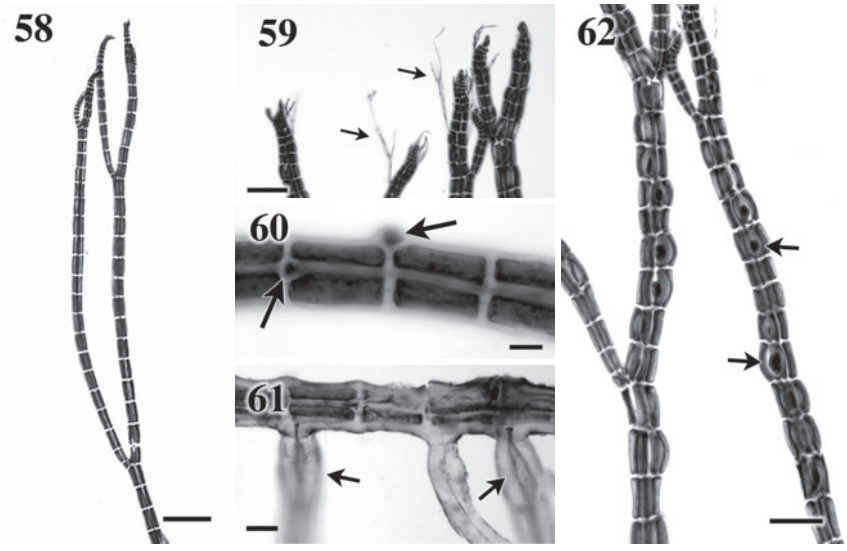
Type locality Johnston Atoll, Central Pacific.

Other sources Hollenberg 1968a, Kapraun 1980a, Abbott 1999, Littler and Littler 2000.

Specimens studied Panama: *WNC2010-s50 (PHYKOS-3533)*, Flat Rock Beach, Bocas del Toro, D.W. Freshwater, 17 Jul 2008.

Molecular vouchers GenBank accession numbers HM573568 (*rbc*L); HM560650 (SSU); HM573524 (COI).

Remarks This specimen best fits *Polysiphonia pseudovillum* as originally described from the central Pacific by Hollenberg



Figures 58–62 *Polysiphonia pseudovillum*.
(58) Habit of an erect axis, PHYKOS-3533, scale=0.20 mm, WNC2010-s050. (59) Apical part of erect axes bearing trichoblasts (arrow), PHYKOS-3533, scale=0.10 mm, WNC2010-s050. (60) Mid-axis segments of erect branch showing scar cells (arrows), PHYKOS-3533, scale=25 µm, WNC2010-s050. (61) Prostrate axis with rhizoids (arrows) that are cut off from pericentral cells, PHYKOS-3533, scale=50 µm, WNC2010-s050. (62) Reproductive branches with slightly spiral series of tetrasporangia (arrows), PHYKOS-3533, scale=0.10 mm, WNC2010-s050.

(1968a); however, some slight differences were observed. *P. 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 tall 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 in the Panamanian specimen, but spermatangial branches are reported to develop as furcations of trichoblasts and cystocarps are ovoid to slightly urceolate (Hollenberg 1968a, Abbott 1999). As the majority of available morphological character states 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 (Figures 2 and 3). In ML phylogenies generated from *rbc*L and SSU sequence data, *P. pseudovillum* was placed within a clade of predominantly *Neosiphonia* species (Figures 4 and 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 and Lee 1999). However, in contrast to *Neosiphonia* species (Kim and Lee 1999), the Panamanian *P. pseudovillum* does not produce lateral branch or trichoblast initials at every segment in a spiral pattern, nor does it appear that the thallus originally develops from a solid disc of rhizoids.

This is only the third report of *Polysiphonia pseudovillum* within the western Atlantic. Kapraun (1980a) included the species in his North Carolina flora based on a single specimen found growing epiphytically on pelagic *Sargassum* C. Agardh. Littler and Littler (2000) also record the species from Florida, but this report was not included in the floristic treatment of Dawes and Mathieson (2008). Additional study, including molecular data from central Pacific specimens is needed to clarify the distribution of this species.

Polysiphonia schneideri Stuercke *et* Freshwater (2010, p. 302) (Figures 63–70)

Misapplied names *Polysiphonia denudata sensu* Hoyt 1920, Taylor 1960, Dawes 1974, Kapraun 1977, 1980, Kapraun and Norris 1982, Kapraun et al. 1983, Schneider and Searles 1991, Littler and Littler 2000, Dawes and Mathieson 2008.

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 (Figure 64); moderately to highly branched in a dichotomous to subdichotomous pattern (Figure 63); erect axes 50–275 µm in diameter, prostrate axes 250–450 µm in diameter; mid-axis segments of erect axes vary from 0.75–1.25× as long as wide; cortication absent; main axes with five or six (to seven) pericentral cells; branches forming in the axils of trichoblasts or occasionally laterally to trichoblasts (Figures 67–69); trichoblasts long, thin, and tangled when mature, to 375 µm in length, with several dichotomies; scar cells not occurring at every segment and sometimes rare; adventitious laterals lacking; tetrasporangia in straight series, moderately distending segments, 55–65 (–85) µm in diameter (Figure 66); spermatangial branches developing as furcations of trichoblasts, 60–80×300 µm, with or without one to three sterile tip cells (Figure 65); cystocarps globose, (200–) 350–470 µm in diameter, with narrow ostioles, short stalked with stalk cells noticeably larger than pericentral cells of axes (Figure 70).

Type locality Wrightsville Beach, New Hanover County, North Carolina, USA.

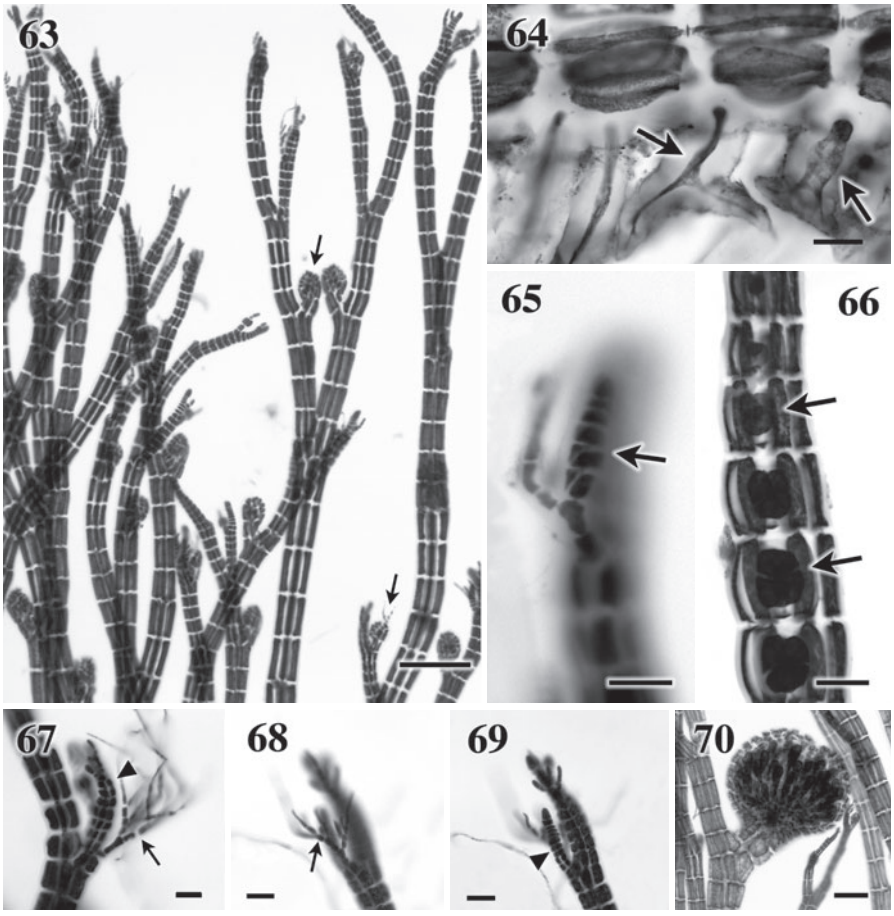
Other sources Stuercke and Freshwater 2010; as *Polysiphonia denudata*, Kapraun 1977, 1980a, Kapraun and Norris 1982, Kapraun et al. 1983, Schneider and Searles 1991, Dawes and Mathieson 2008, Stuercke and Freshwater 2008.

Specimens studied Panama: *WNC2009-s210 to s212 (PHYKOS-2454)*, Panama Canal North Ferry Terminus, Colón, K. Larson and L. McCann, 19 May 2009; *WNC2009-s364 (PHYKOS-2689)*, STRI research station, Punta Galeta, Colón, S. Schmitt, 21 May 2009; *WNC2009-s553 to s555 (PHYKOS-3189)*, Cayos Zapatillas, Bocas del Toro, D.W. Freshwater, 25 Aug 2009; Florida: *WNC2010-s082 (FL05-04)*, Sebastian Inlet, Indian River County, B. Stuercke, 26 Feb 2005.

Molecular vouchers GenBank accession numbers HM573566, HM573565, GU385837 (*rbc*L); HM560629 (SSU); HM573516, HM573513, HM573515 (COI).

Remarks *Polysiphonia schneideri* is a recently named western Atlantic species (Stuercke and Freshwater 2010) that previously had been misidentified as *P. denudata* (Dillwyn) Greville *et* 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 occurring one per segment in a spiral pattern, and tetrasporangia developing in spiral series (Maggs and Hommersand 1993). *Polysiphonia schneideri* has an initial basal holdfast with secondarily decumbent branches, no cortication, trichoblasts and scar cells not occurring at every segment and arranged in no particular pattern, and tetrasporangia developing in straight series (Stuercke and Freshwater 2010). These morphological characters were observed in all samples examined in this study. Panamanian records support the distribution of *P. schneideri* in the southern Caribbean as previously noted by Kapraun et al. (1983, as *P. denudata*, see Stuercke and Freshwater 2010).

Three samples of *Polysiphonia schneideri* were collected in this study (Figures 2 and 3). Sequences of *P. schneideri* samples from Panama and also North Carolina, Florida, and Bermuda were not identical but differed by only 0.093%–0.74% at the *rbc*L and 1.87%–3.34% at the COI locus. The *rbc*L values are within the range of intraspecific sequence variation observed in other species of *Polysiphonia s.l.* (e.g., McIvor et al. 2001) and



Figures 63–70 *Polysiphonia schneideri*.
(63) Habit of erect axes with immature cystocarps (arrows), PHYKOS-3189, scale=0.10 mm, WNC2009-s555. (64) Prostrate axis with rhizoids (arrows) that are cut off from the pericentral cells, PHYKOS-2689, scale=50 µm, WNC2009-s364. (65) Branch apex with immature spermatangial branch (arrow) developing as a furcation of the trichoblast, PHYKOS-2454, scale=20 µm, WNC2009-s211. (66) Reproductive branch with straight series of tetrasporangia (arrows), PHYKOS-2454, scale=50 µm, WNC2009-s212. (67) Apical portion of main axis showing a lateral branch (arrowhead) developing in the axil of a trichoblasts (arrow), PHYKOS-3189, scale=20 µm, WNC2009-s554. (68) Apical portion of main axis showing a lateral branch (out of focal plane below trichoblasts) forming to the side of a trichoblast (arrow), PHYKOS-3189, scale=20 µm, WNC2009-s554. (69) Apical portion of main axis showing background of lateral branch from Figure 68 (arrowhead) forming to the side of a trichoblast (out of focal plane above the lateral branch), PHYKOS-3189, scale=20 µm, WNC2009-s554. (70) Main axis bearing a cystocarp on a short stalk of enlarged cells, PHYKOS-3189, scale=0.10 mm, WNC2009-s554.

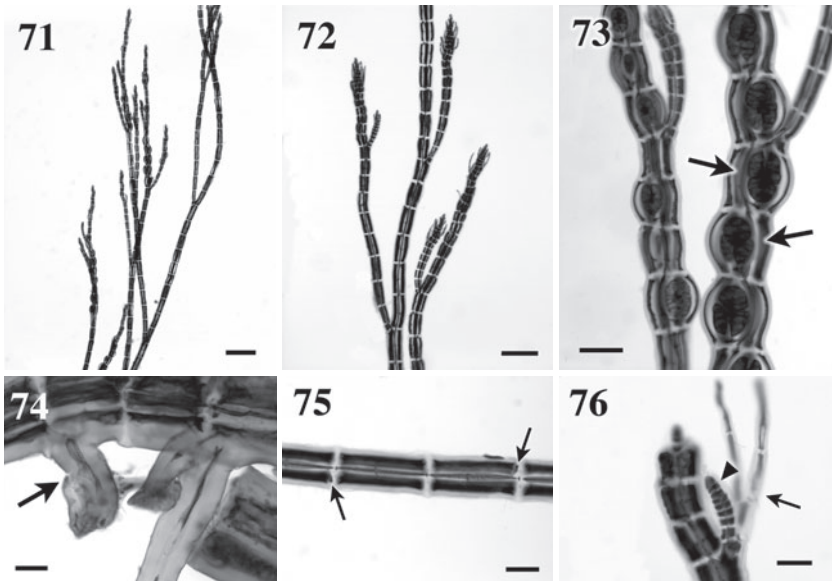
most likely reflect the wide geographic range of the species. ML phylogenies based on *rbc*L and SSU sequence data placed *P. schneideri* in a clade with *P. pentamera* and *Polysiphonia* sp. 1 (Figures 4 and 5). *Polysiphonia pentamera* is similar to *P. schneideri* in having rhizoids cut off from pericentral cells, branches that develop laterally from the basal trichoblast cell, and scar cells not occurring at every segment, but differs in having tetrasporangia 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 in strictly having five pericentral cells, and scar cells one per segment.

Polysiphonia cf. sertularioides (Grateloup) J. Agardh (1863, p. 969) (Figures 71–83)

Basionym *Ceramium sertularioides* Grateloup (1806).

Synonyms *Polysiphonia flaccidissima* Hollenberg (1942, p. 783), *Neosiphonia flaccidissima* (Hollenberg) M.S. Kim *et* I.K. Lee (1999, p. 279).

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 (Figures 74 and 81); highly branched in an alternate pattern (Figures 71 and 77); erect axes 40–70 µm in diameter, prostrate axes 80–150 µm in diameter; branchlets slightly to prominently basally attenuated (Figures 72 and 78); mid-axis segments of erect axes mostly 0.5–2× as long as wide (Figures 75 and 80); cortication absent; main axes with four pericentral cells; branches forming in the axils of trichoblasts (Figures 76 and 82); trichoblasts short to long, to 215 µm in length, mostly simple or with 1 dichotomy; scar cells occurring one per segment in ¼ spiral series;



Figures 71–76 *Polysiphonia cf. sertularioides* 1.
(71) Habit of erect axes, PHYKOS-3226, scale=0.2 mm, WNC2009-s563. (72) Erect axes bearing lateral branches with slight basal attenuation, PHYKOS-3226, scale=0.10 mm, WNC2009-s563. (73) Reproductive branches with spiral series of tetrasporangia (arrows), PHYKOS-3226, scale=50 µm, WNC2009-s564. (74) Prostrate axis with rhizoids (arrow) that are cut off from the pericentral cells, PHYKOS-3226, scale=20 µm, WNC2009-s563. (75) Mid-axis segments of erect branch showing scar cells (arrows) and elongated pericentral cells (twice as long as wide), PHYKOS-3226, scale=50 µm, WNC2009-s564. (76) Apical part of main axis showing a lateral branch (arrowhead) developing in the axil of a trichoblast (arrow), PHYKOS-3226, scale=50 µm, WNC2009-s563.

adventitious laterals absent; tetrasporangia in spiral series, moderately to greatly distending segments, (58–) 73–88 µm in diameter (Figures 73 and 83); spermatangial branches and cystocarps unknown.

Other sources As *Polysiphonia sertularioides*, Womersley 1979, Adams 1991; as *P. flaccidissima*, Hollenberg 1942, 1961, 1968a, Abbott and Hollenberg 1976, Hollenberg and Norris 1977, Kapraun and Norris 1982, Kapraun et al. 1983, Abbott 1999.

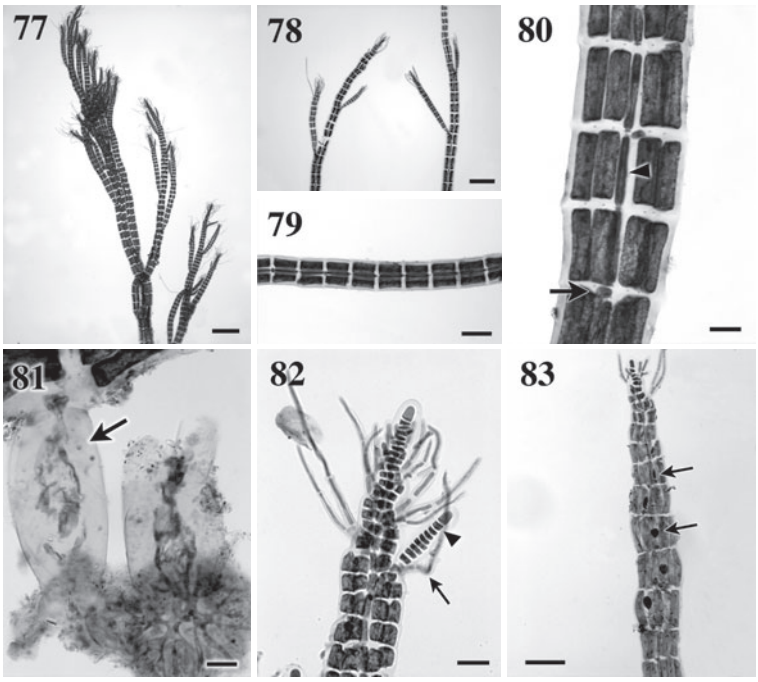
Specimens studied Panama: *WNC2009-s563 to s565 (PHYKOS-3226)*, Crawl Cay, Bocas del Toro, Diaz-Pulido and Riosmena, 28 Aug 2009; *WNC2009-s159, s160 (PHYKOS-2257),* West Limón Bay Jetty, Punta Toro, Colón, D.W. Freshwater and N. Mamoozadeh, 17 May 2009; *WNC2009-s567 to s569 (PHYKOS-2309)*, West Limón Bay Jetty, Punta Toro, Colón, D.W. Freshwater and N. Mamoozadeh, 17 May 2009.

Molecular vouchers GenBank accession numbers HM573546, HM573548 (*rbc*L); HM560646, 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 from Caribbean Panama), *P. cf. sertularioides* 2 (PHYKOS-3534 from Pacific Panama), and *P. cf. sertularioides* 3 (PHYKOS-2257 and PHYKOS-2309 from Caribbean Panama). The previous morphological description encompasses the character state variation seen in all three species, but the included images are of the two species collected from the Caribbean.

Cluster analyses of *rbc*L and COI sequence data showed that these species were clearly distinct from one another (Figures 2 and 3). The *rbc*L sequence divergence values between the three were: 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 ≤2.13% (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%–14.12% observed for other species within the genus (McIvor et al. 2001, Kim et al. 2004, Kim and Yang 2005). The cryptic diversity revealed by the *rbc*L and COI sequence data was apparent in the more conserved SSU gene as well (Figure 5). Phylogenetic analyses of *rbc*L and SSU sequences resolved the three species in a strongly supported clade (rB100; sB100) that occupied a basal position within the large clade of *Polysiphonia* and *Neosiphonia* species (Figures 4 and 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*



Figures 77–83 *Polysiphonia cf. sertularioides* 3.
(77) Habit of erect axes, PHYKOS-2257, scale=0.20 mm, WNC2009-s160. (78) Erect axes bearing lateral branches with prominent basal attenuation, PHYKOS-2257, scale=0.1 mm, WNC2009-s159. (79) Mid-axis segments of erect branch showing stout pericentral cells (0.5–1.0× as long as wide), PHYKOS-2309, scale=50 µm, WNC2009-s569. (80) Portion of erect axis squashed to show central axial cells (arrowhead), four pericentral cells per segment, and scar cells (arrow), PHYKOS-2309, scale=20 µm, WNC2009-s569. (81) Prostrate axis with rhizoids (arrow) cut off from pericentral cells and having unicellular digitate ends, PHYKOS-2309, scale=20 µm, WNC2009-s569. (82) Apical part of main axis showing lateral branch (arrowhead) developing in the axil of a trichoblast (arrow), PHYKOS-2309, scale=20 µm, WNC2009-s567. (83) Reproductive branch with spiral series of developing tetrasporangia (arrows), PHYKOS-2309, scale=50 µm, WNC2009-s567.

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. Previous studies have shown that segment length may be a variable character within species (Kim et al. 1994, 2004, Curiel et al. 2002, Stuercke and Freshwater 2008), and additional samples will be required to determine if the differences in segment length and attenuation of branch bases are consistent.

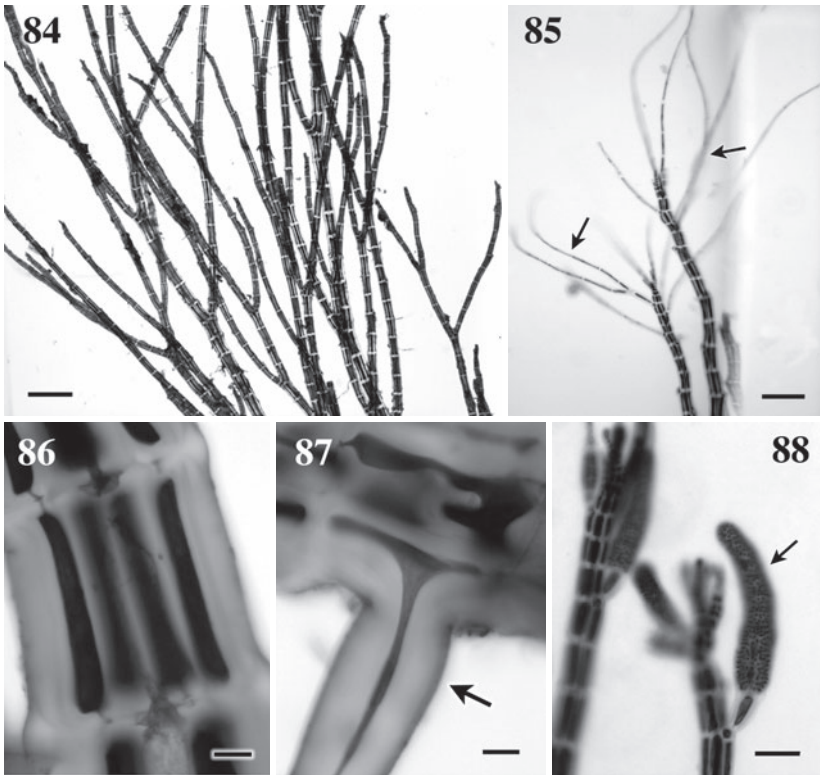
Polysiphonia sertularioides was originally described from Sète, France and is widely reported throughout the Mediterranean Sea and Pacific, Indian, and eastern Atlantic Oceans (Womersley 1979, Athanasiadis 1987, Adams 1991, Silva et al. 1996, Gómez Garreta et al. 2001, Lobban and Tsuda 2003, John et al. 2004). 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 *et* I.K. Lee, 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 (Hollenberg 1968a, Abbott and Hollenberg 1976, Hollenberg and Norris 1977, Kapraun and Norris 1982, Kapraun et al. 1983, Abbott 1999, Littler and Littler 2000, Ballantine and Aponte 2005, 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 *P. sertularioides* as a single, widely distributed species needs further investigation.

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

Polysiphonia subtilissima Montagne (1840, p. 199) (Figures 84–88)

Synonyms *Polysiphonia subtilissima* 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



Figures 84–88 *Polysiphonia subtilissima*.
(84) Habit of erect axes, PHYKOS-3271, scale=0.20 mm, WNC2009-s560. (85) Apical parts of erect axes bearing trichoblasts (arrows), PHYKOS-3271, scale=0.10 mm, WNC2009-s561. (86) Portion of erect axis squashed to show four pericentral cells per segment, PHYKOS-3271, scale=20 µm, WNC2009-s560. (87) Prostrate axis with a rhizoid (arrow) in open connection with the pericentral cell, PHYKOS-3271, scale=20 µm, WNC2009-s562. (88) Branch apex with spermatangial branch (arrow) developmentally replacing trichoblasts, with no sterile tip cells, PHYKOS-3271, scale=50 µm, WNC2009-s561.

to substratum by rhizoids in open connection with pericentral cells (Figure 87); highly branched in a dichotomous pattern (Figure 84); erect axes 30–100 µm in diameter, prostrate axes 80–150 µm in diameter; mid-axis segments of erect axes mostly 1–3× as long as wide; cortication absent; main axes with 4 pericentral cells (Figure 86); branches replacing trichoblasts in development; trichoblasts long and slender, to 850 µm in length, typically with up to one dichotomy (Figure 85); scar cells present and obvious, variable in pattern and frequency; adventitious laterals occasionally present, mostly linear; tetrasporangia greatly distending segments, in straight series; spermatangial branches developmentally replacing trichoblasts, 30–50×120–265 µm, without sterile tip cells (Figure 88); see remarks for cystocarp structure.

Type locality Cayenne, French Guiana.

Other sources Hollenberg 1968a, Womersley 1979, Kapraun et al. 1983, Adams 1991, Schneider and Searles 1991, Abbott 1999, Dawes and Mathieson 2008.

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 (*rbc*L); HM560635 (SSU); HM573528 (COI).

Remarks Womersley (1979, 2003) identified southern Australian material of *Polysiphonia subtilissima* as having spermatangial branches developmentally replacing trichoblasts and with 4–6 sterile tip cells. This differs from New Zealand material of *P. subtilissima* described as having spermatangial branches developing as furcations of trichoblasts and with no sterile tip cells (Adams 1991). Both of these southwestern Pacific descriptions differ from material of *P. subtilissima* from the tropical western Atlantic, which is described as having spermatangial branches developmentally replacing trichoblasts and without sterile tip cells (Kapraun 1980b, Kapraun unpublished manuscript, this study). Cystocarp structure for tropical western Atlantic specimens of *P. subtilissima* is mostly 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 D.F. 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 branches 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 *et* Harvey, and *P. pacifica* Hollenberg, and indicated that these taxa must be closely related and may truly represent only one or two species. Spermatangial branches with 3–7 sterile tip cells are reported as developmentally replacing trichoblasts in *P. urceolata* [as *P. stricta* (Dillwyn) Greville (Maggs and Hommersand 1993)] and developmentally replacing the trichoblast with "one to several" sterile tip cells in *P. pacifica* (Hollenberg 1942). A satisfactory description of spermatangial branch development could not be found for *P. abscissa*.

Only one Panamanian *Polysiphonia subtilissima* specimen was collected in this study (Figures 2 and 3), and although it clusters with North Carolinian *P. subtilissima* specimens, divergences of 2.22%–2.31% and 4.80% are observed in the *rbc*L and COI sequence data, respectively. This is only slightly beyond the ≤2.13% (predominantly ≤1.3%) intraspecific *rbc*L sequence divergence that has been observed in previous studies of *Polysiphonia* (McIvor et al. 2001, Kim et al. 2004) but is within the range of interspecific COI sequence divergence observed in previous studies of other red algal species (Robba et al. 2006, Yang et al. 2008, Clarkston and Saunders 2010, Le Gall and Saunders 2010). 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 Carolinian 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 *rbc*L and SSU sequence data placed *Polysiphonia subtilissima* in a clade with *P. scopulorum* Harvey (Figures 4 and 5). Both species had four pericentral cells, rhizoids in open connection with pericentral cells, branches that replaced trichoblasts, and tetrasporangia that developed in straight series. *Polysiphonia scopulorum* can be distinguished from *P. subtilissima* by its smaller habit (to 1.5 cm in height), erect branches that are simple or sparsely branched, and growing on rocky substrata in strictly marine environments (Hollenberg 1968a). *Polysiphonia subtilissima* also closely resembles *P. atlantica*, but the latter can be distinguished by having erect branches developing unilaterally from prostrate axes to give plants a dorsiventral habit. *Polysiphonia atlantica*, along with *P. stricta* and *P. pacifica*, is distantly related to *P. subtilissima* and is resolved in a separate clade of *Polysiphonia s.s.* species.

Conclusions

Two *Neosiphonia* and 12 *Polysiphonia* species were recovered from samples obtained along the Caribbean coast of Panama. These collections represent 13 new reports from this coast, including two new species, *P. lobophoralis* and *P. nuda*. This number of species is significantly greater than the one named *Polysiphonia* previously reported for Caribbean 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, will yield even higher species counts for Panama and help clarify the several taxonomic issues raised in this study.

Acknowledgements

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