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# Taxonomic notes on Caribbean *Neosiphonia* and *Polysiphonia* (Ceramiales, Florideophyceae): five species from Florida, USA and Mexico

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## Abstract

Molecular-assisted identification using plastid-encoded *rbc*L and mitochondrion-encoded COI loci identified five species of *Polysiphonia sensu lato* from 16 Florida and Caribbean Mexico samples. Morphological character states were examined and used to identify these species as *Neosiphonia bajacali* comb. nov., *N. echinata* comb. nov., *N. sphaerocarpa*, *N. tepida*, and *Polysiphonia anomala*. Descriptions are provided and the phylogenetic relationships of the five species were determined through maximum likelihood analyses of *rbc*L and nuclear-encoded SSU sequence data. *Neosiphonia bajacali* and *N. echinata* had a combination of character states described for *Neosiphonia*: rhizoids cut-off from pericentral cells, lateral branch or trichoblast initials on every segment in a spiral pattern, tetrasporangia in spiral series, and spermatangial stichidia developing as bifurcations of trichoblasts, and these new combinations are proposed. Examination of *N. echinata*, *P. fracta* and North Carolina specimens identified as *P. breviarticulata* revealed no significant morphological differences. *Polysiphonia fracta* is proposed as a synonym of *N. echinata* and the presence of *P. breviarticulata* within the western Atlantic is questioned. This is the first report of *N. bajacali* from the Caribbean and the first report of *N. echinata* from Caribbean Mexico.

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

## Introduction

*Polysiphonia* Greville (Rhodomelaceae) is a large (ca. 200 species) red algal genus whose species have a nearly global distribution. Species of *Polysiphonia sensu lato* (*s.l.*) have a delicate habit composed of filamentous main axes and branches that are segmented and polysiphonous. A wide range of morphological variability occurs among the many species of *Polysiphonia s.l.* This variation has led to much debate on the classification of species within the genus. To revise the classification of *Polysiphonia s.l.*, Kim et al. (2000) suggested the use of ''independent comparative evidence'' to determine which morphological characters are relevant to species identification. The recent coupling of morphological characters with DNA sequence data is helping establish an accurate classification of *Polysiphonia* based on natural relationships.

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

The 5′ end of the mitochondrion-encoded cytochrome *c* oxidase subunit I gene (COI) is another region of DNA that is employed for MAI. Previous studies of red algae have shown COI to be successful in distinguishing closely related and cryptic species as well as geographic groups within a species (Saunders 2005, Robba et al. 2006, Yang et al. 2008, Clarkston and Saunders 2010, Le Gall and Saunders 2010). The utility of COI barcoding within *Polysiphonia s.l.* has yet to be established.

Phylogenetic relationships among *Polysiphonia s.l.* species can also be determined using molecular data. The nuclear-encoded 18S rDNA (SSU) gene has limited intraspecific sequence variation and provides better resolution of taxonomic relationships above the species level. Analyses of SSU sequence data showed that *Polysiphonia s.l.* is polyphyletic with respect to other genera (Choi et al. 2001). The *rbc*L locus has a faster rate of evolution than SSU allowing greater resolution of species-level relationships. The use of *rbc*L to determine species relationships has been common in recent studies of *Polysiphonia s.l.* (McIvor et al. 2001, Kim et al. 2004, 2005, Stuercke and Freshwater 2008, 2010). Phylogenetic relationships within *Polysiphonia s.l.* can be thoroughly evaluated when analyses of SSU and *rbc*L are compared with one another (Stuercke 2006).

Stuercke and Freshwater (2008) examined many of the morphological characters used to distinguish *Polysiphonia s.l.* species in an integrated molecular-morphological study. Little to no intraspecific variation was found in the character states for pericentral cell number, rhizoid-pericentral cell connection, lateral branch-trichoblast relationship, spermatangial axis development, tetrasporangial arrangement, trichoblasts/scar cell occurrence and pattern, holdfast type, and cicatrigenous branch formation. The genus *Neosiphonia* M.-S. Kim *et* I.K. Lee was segregated from *Polysiphonia* based on a combination of character states including the following from those listed above: rhizoids that are cut-off from pericentral cells, spirally arranged trichoblasts or scar cells on every segment, three-celled carpogonial branches, spermatangial axes developing as a bifurcation of trichoblasts, and spirally arranged tetrasporangia (Kim and Lee 1999). *Polysiphonia s.l.* now includes species that are predominantly placed in *Neosiphonia* or *Polysiphonia*, although less speciose genera are also included.

Study of the Caribbean marine algal flora has resulted in reports of at least 28 distinct species of *Polysiphonia s.l.* (e.g., Wynne 1998). Early reports of *Polysiphonia s.l.* by Harvey (1853) and Børgesen (1918), described several species from Florida, USA and the West Indies, respectively. More recent reports are the result of floristic assessments made throughout the Caribbean (e.g., Taylor 1929, 1941, 1942, 1945, 1960, 1969, Almodovar and Ballantine 1983, Ballantine and Aponte 1997, Littler and Littler 2000, Mateo-Cid et al. 2006). Caribbean studies specific to *Polysiphonia s.l.* include reports of species from Belize (Kapraun and Norris 1982), Colombia and Venezuela (Kapraun et al. 1983).

The purpose of this study was to complete integrated molecular and morphological analyses of *Polysiphonia s.l.* samples collected from Caribbean Mexico and Florida. Examination of morphology and MAI using *rbc*L and COI sequence data were used to identify four *Neosiphonia* and one *Polysiphonia* species within samples while phylogenetic analyses of *rbc*L and SSU sequence data were used to determine the relationships of these species.

## Materials and methods

### Collections and vouchers

Samples of *Neosiphonia* and *Polysiphonia* were collected from intertidal and subtidal substrata in Caribbean Mexico and Florida by snorkeling (Appendix 1). 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-Wilmington. Examinations of morphological characters and taxonomic guides were used to identify species (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, 1980, 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, D. Kapraun unpublished manuscript). Specimens were stained with aniline blue solution (Millar and Wynne 1992) and permanent slide vouchers made following Tsuda and Abbott (1985) for deposit in the University of North Carolina Wilmington (WNC) herbarium. Additional specimens from the United States National Herbarium (US) were also studied. All herbarium abbreviations follow the Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

### Morphological data

Specimens and slides were observed using an Olympus SZH dissecting microscope (Olympus America Inc., Center Valley, PA, USA) and a Nikon Labophot-2 compound microscope (Nikon Inc., Melville, NY, USA). Images were captured using a Zeiss Axio Imager.Z1 compound microscope fitted with an AxioCam MRc 5 camera system (Carl Zeiss Microimaging Inc., Thornwood, NY, USA) or an Olympus BX41 compound microscope fitted with a Roper Scientific Photometrics® CoolSnap™ camera (Photometrics, Tucson, AZ, USA). Species descriptions were written based on observations of specimens collected in this study and at the US, and with information from the literature included for character states that were not directly observed.

### DNA extraction and sequencing

DNA was extracted from specimens according to Hughey et al. (2001) with an additional cleaning step using the *One-Step*™ PCR Inhibitor Removal Kit (Zymo Research, Orange, CA, USA). SSU, *rbc*L, and COI were amplified following the basic PCR recipe outlined in Freshwater et al. (2005) but using GOTaq DNA polymerase and buffer (Promega, Madison, WI, USA). The thermocycling protocol followed Freshwater et al. (2000) but with 35 cycles of denaturing, annealing at 40, 45, or 50°C, and an elongation period of 90 s. Amplification products were cleaned with a Stratagene StrataPrep® PCR Purification Kit (Stratagene, La Jolla, CA, USA) and used as templates in BigDye® Terminator v3.1 sequencing reactions (Applied Biosystems, Foster City, CA, USA). Sequences were edited and assembled using Sequencher™ (version 4.9, GeneCodes Corporation, Ann Arbor, MI, USA). Primers utilized in amplification and sequencing reactions are listed in Table 1.

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

### Molecular data analyses

Sequences of *Neosiphonia* and *Polysiphonia* generated in this study were combined with *rbc*L and SSU sequences available from GenBank and with some from unpublished studies (J. Kelly and D.W. Freshwater unpublished) and initially aligned using MacClade (v.4, Maddison and Maddison 2000). SSU sequence data were also aligned using the ClustalW multiple sequence alignment feature of the program Molecular Evolutionary Genetics Analysis (MEGA) (www.megasoftware.net; Tamura et al. 2007, Kumar et al.

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

| Locus/primer name | Primer sequence | References |
|---|---|---|
| *rbc*L | | |
| FrbcLstart | 5′-ATGTCTAACTCTGTAGAAG-3′ | Freshwater and Rueness (1994) |
| F87 | 5′-GGATCCTAAYTACGTAAYTAAAG-3′ | This study |
| F577 | 5′-GTATATGAAGGTCTAAAAGG-3′ | Freshwater and Rueness (1994) |
| F753 | 5′-GGAAGATATGTATGAAAGAGC-3′ | Freshwater and Rueness (1994) |
| R893 | 5′-GAATAAGTTGARTTWCCIGCAC-3′ | Stuercke and Freshwater (2008) |
| R1415 | 5′-CTACRAAGTCAGCTGTATCTG-3′ | This study |
| RrbcSstart | 5′-GTTCCTTGTGTTAATCTCAC-3′ | Freshwater and Rueness (1994) |
| SSU | | |
| A.2 | 5′-AGACTAAGCCATGCAAGTGC-3′ | D.W. Freshwater (unpublished) |
| A.3 | 5′-ACA(AT)CGAAACTGCGAATGG-3′ | D.W. Freshwater (unpublished) |
| CerD | 5′-GCAAGTCTGGTGCCAGCAG-3′ | Hommersand et al. (2005) |
| CerE | 5′-CTATTATTCCATGCTAATGTATTC-3′ | Hommersand et al. (2005) |
| CerG | 5′-AGCCTGCGGCTTAATTTGAC-3′ | Hommersand et al. (2005) |
| CerH | 5′-TAACCAGACAGATCACTCCAC-3′ | Hommersand et al. (2005) |
| CerJ | 5′-TCTCCTTCCTCTAAGTGATAA-3′ | Hommersand et al. (2005) |
| GO1 | 5′-CACCTGGTTGATCCTGCCAG-3′ | Saunders and Kraft (1994) |
| GO4 | 5′-CAGAGGTGAAATTCTTGGAT-3′ | Saunders and Kraft (1994) |
| GO7 | 5′-ATCCTTCTGCAGGTTCACCTAC-3′ | Saunders and Kraft (1994) |
| GO8 | 5′-GAACGGCCATGCACCACCACC | Saunders and Kraft (1994) |
| COI | | |
| CerR1 | 5′-CCAAAAAATCAAAATARRTG-3′ | Saunders (unpublished) |
| GazF1 | 5′-TCAACAAATCATAAAGATATTGG-3′ | Saunders (2005) |
| GHalF | 5′-TCAACAAATCATAAAGATATYGG-3′ | Saunders (2008) |
| Mam1R | 5′-CCICCWCCIGCWGGATCAA-3′ | This study |
| Mam2R | 5′-GTATTAAAATTWCKATCWGTTA-3′ | This study |

2008) followed by manual adjustment. Characteristics of the DNA sequence data sets were determined using MacClade and PAUP (v.4, Swofford 2002). MEGA was used to perform Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Neighbor Joining (NJ) cluster analyses on COI and *rbc*L sequence data for MAI. Simple mean distances (p-distances) were used in all MAI analyses. ML analyses were performed on reduced (identical sequences removed) *rbc*L and SSU sequence data separately using the program Genetic Algorithm for Rapid Likelihood Inference (GARLI) (www.bio.utexas.edu/faculty/antisense/garli/Garli.html; Zwickl 2006). The ML analyses included 10 separate searches from random starting trees that used default parameters, including 10,000 generations without improving topology and allowing model estimation during the run (models available from the second author). GARLI was also used to perform a total of 1003 ML bootstrap (BS) replicates on the *rbc*L dataset and 1000 ML BS replicates on the SSU dataset.

## Results and discussion

### Molecular assisted identification

The *rbc*L MAI alignment consisted of 113 taxa and included 1081 sites in the analysis, of which 402 (37.19%) were variable. Five species from 16 Florida and Caribbean Mexico samples were resolved in UPGMA and NJ cluster analyses of *rbc*L sequence data (Figure 1, only UPGMA cluster diagram shown). Species distinctions were based on values of inter- and intraspecific *rbc*L sequence divergence from previous studies of *Polysiphonia s.l.* (McIvor et al. 2001, Kim et al. 2004, Kim and Yang 2005). The COI MAI alignment consisted of 74 taxa and included 605 sites in the analysis, of which 248 (40.99%) were variable. Four species from 10 Florida and Caribbean Mexico samples were resolved in UPGMA and NJ cluster analyses of COI sequence data; this locus did not amplify in some samples (Figure 2, only UPGMA cluster diagram shown). Species distinctions were based on the 4.80% COI sequence divergence between samples identified as *Polysiphonia subtilissima* 1 (NC-24) and *P. subtilissima* 2 (PHYKOS-3271). These samples differed by 2.22% in the *rbc*L data, which slightly exceeds the range of intraspecific *rbc*L sequence divergence observed in previous studies of *Polysiphonia s.l.* (McIvor et al. 2001). A sequence divergence of 2.22% in the *P. subtilissima rbc*L data corresponds to 4.80% sequence divergence in the COI data; the latter value was therefore used as the maximum intraspecific barcoding gap value (Meier et al. 2008) in the COI data. Values of intraspecific sequence divergence are discussed in the remarks on each species.

### Molecular systematics

Phylogenetic relationships of Florida and Caribbean Mexico species within *Polysiphonia s.l.* were determined through ML analyses of SSU and reduced *rbc*L sequence data


**Figure 1** 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. Florida and Caribbean Mexico species are shown in boldface type. *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

(Figures 3 and 4). The specific relationships among these species are discussed later in the remarks on each species. The reduced *rbc*L alignment consisted of 52 taxa and included 1334 sites in the analysis, 528 (39.58%) of which were variable. The *rbc*L topology contains several strongly supported clades as well as groups of clades and species with unresolved relationships. Near the base of the topology are two well-supported clades (*rbc*L bootstrap values [rB]=100) that contain species typically regarded as *Polysiphonia sensu stricto* (*s.s.*). These species all have four pericentral cells, rhizoids in open connection with the pericentral cells, tetrasporangia in straight series, and spermatangial stichidia that developmentally replace trichoblasts. Species in these clades include the generitype, *Polysiphonia stricta* (Dillwyn) Greville. Another well-supported clade (rB100) contains a group of species referred to as the ''multipericentral cell group''


**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. Florida and Caribbean Mexico species are shown in boldface type. *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

by Choi et al. (2001). These species all have 7+ pericentral cells and rhizoids cut-off from pericentral cells. The majority of species in the *rbc*L ML topology are placed within a large unsupported (rB<50) group of species and variously supported clades that contain both *Neosiphonia* and *Polysiphonia* species. Although species of *Neosiphonia* are scattered throughout the topology, a strongly supported clade (rB100) of predominantly *Neosiphonia* species is apparent within this large grouping.

The SSU alignment consisted of 44 taxa and included 1602 sites in the analysis, 158 (9.86%) of which were variable. The major clades and species resolved in the *rbc*L topology were also resolved in the SSU topology. Two clades containing *Polysiphonia s.s.* species are strongly supported (SSU bootstrap values [sB]=99 and 100) and received moderate support (sB78) for resolution as a monophyletic group. Another well-supported (sB91) clade contains ''multipericentral cell'' species of multiple genera including *Polysipho-*

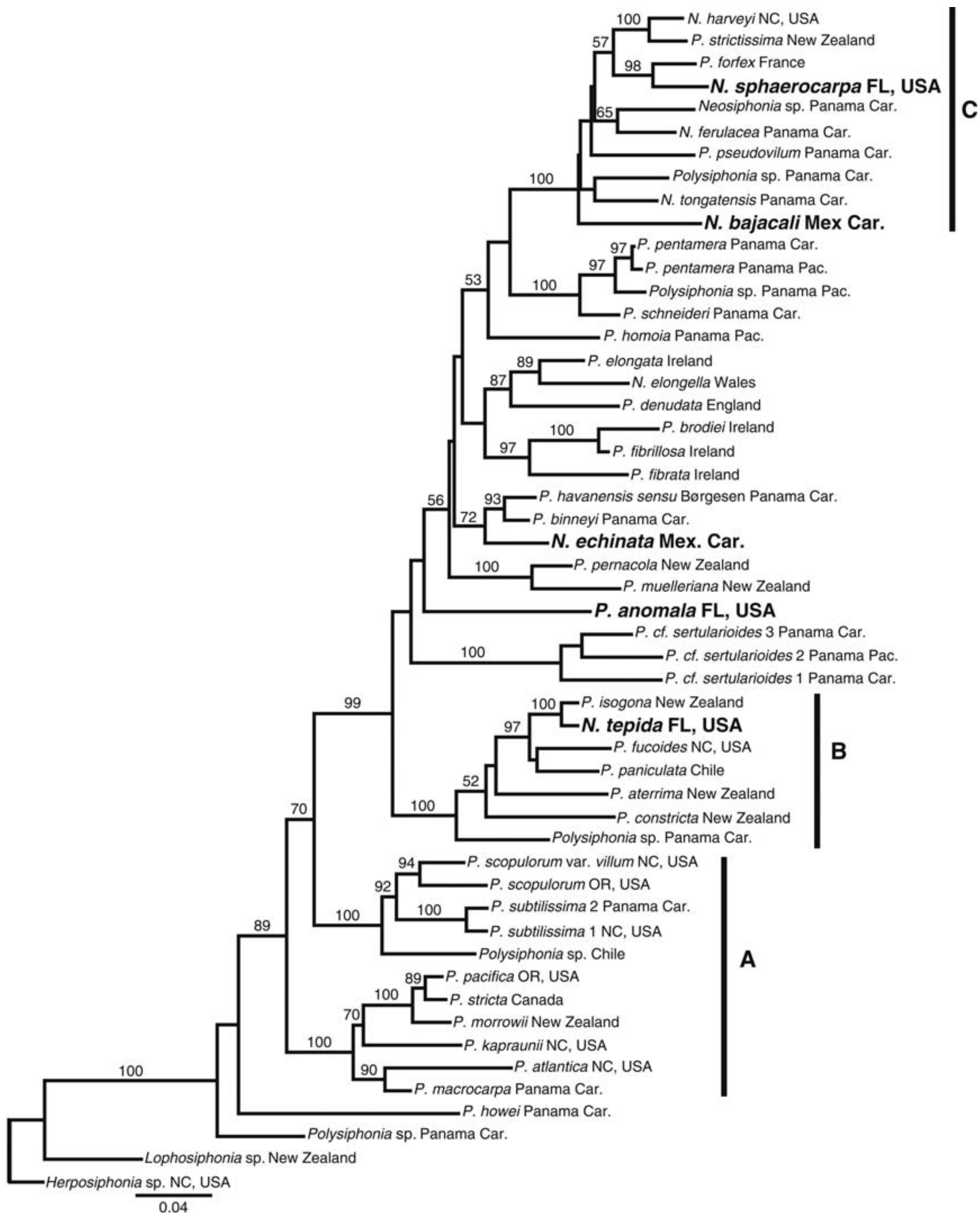



**Figure 3** Maximum likelihood *rbc*L tree (lnL=–14603.23387) for 50 *Polysiphonia s.l.* and two outgroup species. Bootstrap proportion values for branches are shown for each node when >50. Florida and Caribbean Mexico species are shown in boldface type.
Vertical bars show: A) *Polysiphonia sensu stricto* clades; B) the ''multipericentral cell'' clade; C) predominately *Neosiphonia* clade. Car., Caribbean; Pac., Pacific; NC, North Carolina; FL, Florida; OR, Oregon; *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

*nia*, *Boergeseniella* Kylin, *Enelittosiphonia* Segi, and *Vertebrata* S.F. Gray. The majority of species are contained within a large, strongly supported (sB91) grouping of species and clades that include both *Neosiphonia* and *Polysiphonia* species. A clade of predominantly *Neosiphonia* species is contained within this large grouping and received moderate support (sB87).

## Taxonomic observations and remarks

### Genus *Neosiphonia*

### *Neosiphonia bajacali* (Hollenberg) N.R. Mamoozadeh *et* D.W. Freshwater, comb. nov. (Figures 5–8)

**Basionym** *Polysiphonia bajacali* Hollenberg (1961, p. 347).

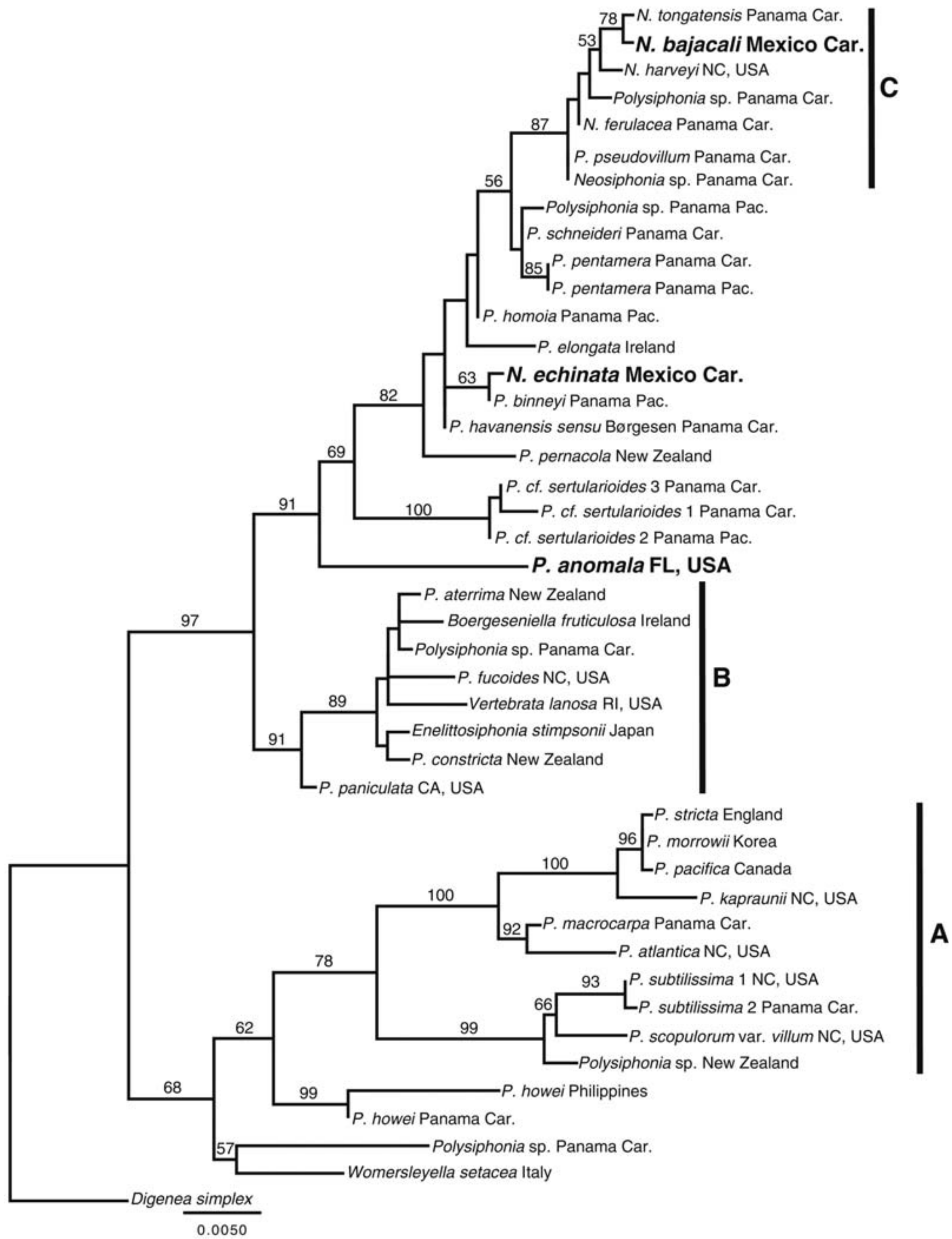
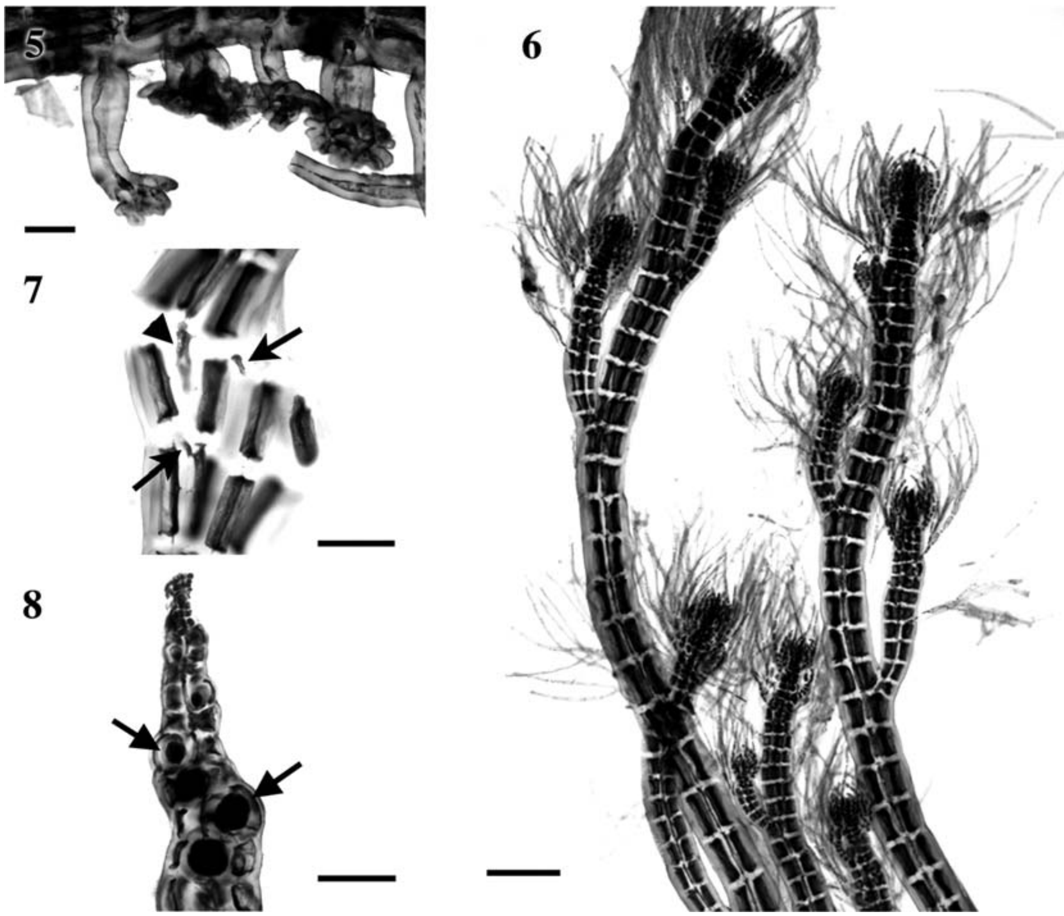



**Figure 4** Maximum likelihood SSU tree (lnL=−4146.45008) for 43 *Polysiphonia s.l.* and one outgroup species.
Bootstrap proportion values for branches are shown for each node when >50. Florida and Caribbean Mexico species are shown in boldface type. Vertical bars show: A) *Polysiphonia sensu stricto* clades; B) the ''multipericentral cell'' clade; C) predominately *Neosiphonia* clade. Car., Caribbean; Pac., Pacific; CA, California; NC, North Carolina; FL, Florida; OR, Oregon; RI, Rhode Island; *P.*, *Polysiphonia*; *N.*, *Neosiphonia*.

**Description** Plants to 3 (–6) cm tall, chiefly erect from limited basal prostrate axes attached to the substratum by rhizoids that are cut-off from pericentral cells (Figure 5); highly branched; branching subdichotomous to alternate (Figure 6); erect axes (150–) 200–300 μm in diameter, prostrate axes (175–) 225–325 μm in diameter; branchlets basally attenuated; mid axis segments of erect axes mostly 1× as long as wide; light cortication present in some mature prostrate axes; main axes with four pericentral cells (Figure 7); branches replacing trichoblasts in development; trichoblasts with several dichotomies, to 750 μm in length; scar cells one per segment in 1/4 spiral series; adventitious laterals rare; tetrasporangia greatly distending segments, developing in short spiral series (Figure 8); spermatangial stichidia




**Figures 5–8** *Neosiphonia bajacali* comb. nov.
(5) Prostrate axis with rhizoids that are cut-off from the pericentral cells, MEX04-09, scale=0.10 mm, WNC2010-s061. (6) Habit of erect axes, MEX04-09, scale=0.20 mm, WNC2010-s061. (7) Portion of erect axis flattened to show four pericentral cells per segment, central axial cell (arrowhead), and scar cells (arrows), MEX04-09, scale=0.10 mm, WNC2010-s061. (8) Reproductive branch displaying short spiral series of tetrasporangia (arrows), MEX04-11A, scale=0.10 mm, WNC2010-s056.

developing as a furcation of trichoblasts, without sterile tip cells; cystocarps urceolate to globose.

**Type locality** Isla Guadalupe, Baja California.

**Other sources** Abbott and Hollenberg 1976, Hollenberg 1961.

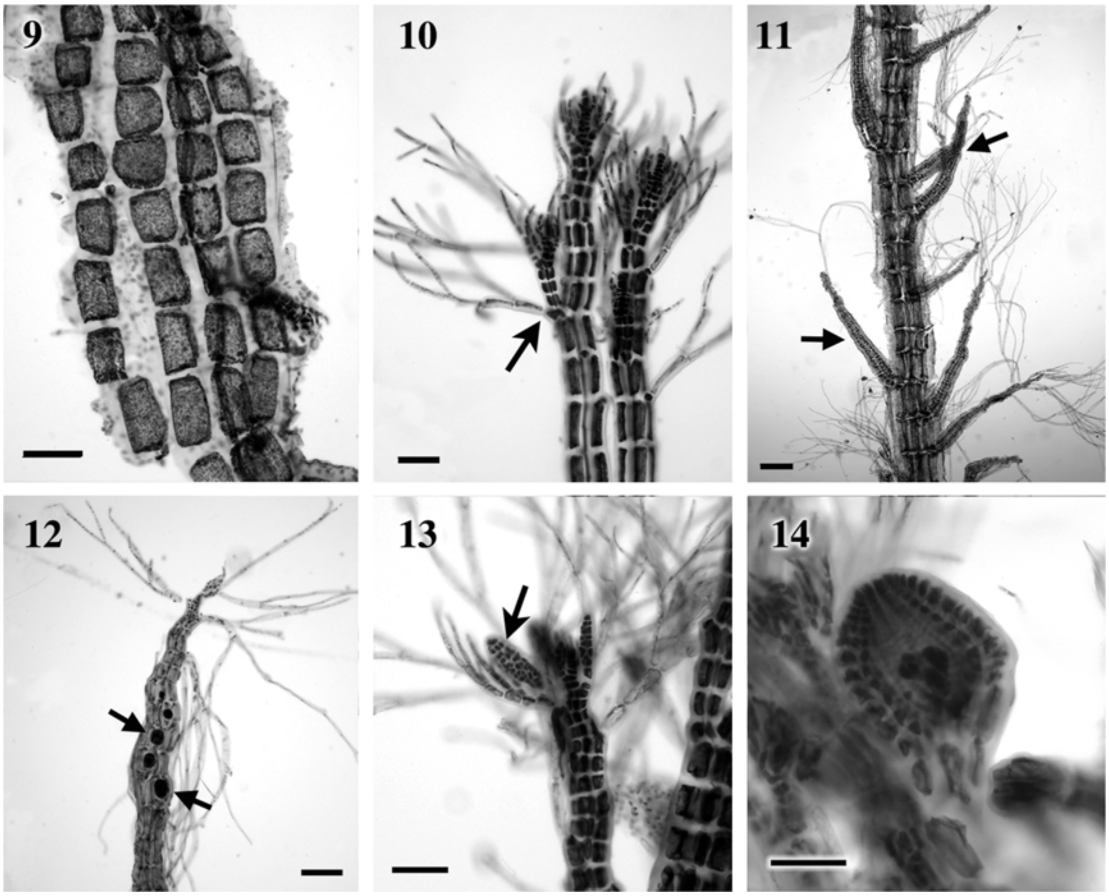
**Specimens studied** Mexico: *US-42350*, Isotype, extreme south tip of Isla Guadalupe, 18 Dec 1949, E.Y. Dawson; *WNC2010-s055 to s57 (MEX04-11A)*, Blue Bay Marina, North of Cancun, Yucatan, D.W. Freshwater, 29 Feb 2004; *WNC2009-s58 to s061 (MEX04-09)*, Blue Bay Marina, North of Cancun, Yucatan, D.W. Freshwater, 29 Feb 2004.

**Molecular vouchers** GenBank accession numbers HM573572 (*rbc*L); HM560659 (SSU); HM573526 (COI).

**Remarks** Hollenberg (1961) originally described *Neosiphonia bajacali* (as *Polysiphonia bajacali*) as ecorticate but in a later description indicated that light basal cortication may be present (Abbott and Hollenberg 1976). Samples in this study infrequently displayed cortication in limited portions of some mature prostrate axes. The original and subsequent descriptions of *N. bajacali* do not indicate whether rhizoids are in open connection or are cut-off from pericentral cells (Hollenberg 1961, Abbott and Hollenberg 1976, Stewart 1991). Samples in this study had rhizoids cut-off from pericentral cells (Figure 5).

*Neosiphonia bajacali* displays a combination of character states unique to *Neosiphonia*. These include rhizoids cut-off from pericentral cells, lateral branch or trichoblast initials one per segment in a spiral pattern, tetrasporangia developing in spiral series, and spermatangial stichidia developing as furcations of trichoblasts.

Two samples of *Neosiphonia bajacali* were collected from Cancun, Mexico (Figures 1, 2). *Neosiphonia bajacali* is resolved within a strongly supported (rB100; sB87) clade of predominantly *Neosiphonia* species in ML analyses of *rbc*L and SSU sequence data (Figures 3, 4). *Neosiphonia bajacali* is most closely related to *N. tongatensis* in the SSU phylogeny, but this relationship received only moderate support




**Figures 9–14** *Neosiphonia echinata* comb. nov.
(9) Portion of erect axis flattened to show four pericentral cells per segment, FL09-40, scale=100 μm, WNC2009-s112. (10) Apical part of main axis showing a lateral branch developing in the axil of a trichoblast (arrow), FL05-07, scale=50 μm, WNC2010-s085. (11) Habit showing adventitious laterals (arrows) arising every segment to every few segments, FL09-42, scale=0.20 mm, WNC2009-s119. (12) Reproductive branch displaying long spiral series of tetrasporangia (arrows), FL09-42, scale=100 μm, WNC2009-s118. (13) Branch apex with spermatangial stichidium developing as a furcation of the trichoblast (arrow), MEX04-8, scale=50 μm, WNC2010-s054. (14) Main axis bearing a short-stalked cystocarp, MEX04-10, scale=50 μm, WNC2010-s062.

(sB78) and is not present in the *rbc*L phylogeny. *Neosiphonia bajacali* and *N. tongatensis* share many character states but can be distinguished by a smaller diameter of erect and prostrate axes (to 150 μm and 250 μm, respectively), no cortication, and segments 1–2× as long as wide in *N. tongatensis*.

### ***Neosiphonia echinata*** **(Harvey) N. Mamoozadeh *et* D.W. Freshwater, comb. nov. (Figures 9–14)**

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

**Synonym** *Polysiphonia fracta* Harvey (1853, p. 38).

**Misapplied name** *Polysiphonia breviarticulata sensu* Stuercke and Freshwater.

**Description** Plants to 3 cm tall, entirely erect from single basal holdfast of rhizoids cut-off from pericentral cells; main axes sparsely branched in a subdichotomous pattern; erect main axes (275–) 375–500 μm in diameter, basal axes (375–) 500–625 (–750) μm in diameter; mid axis segments of erect axes mostly 0.5–1× as long as wide; light to moderate cortication present in some mature prostrate axes and at the bases of older adventitious laterals; main axes with four pericentral cells (Figure 9); branches forming in the axils of trichoblasts (Figure 10); trichoblasts with several dichotomies, to 500–800 μm in length; scar cells one per segment in 1/4 spiral series; adventitious laterals abundant, every segment to every few segments on main axes giving plants a coarse appearance (Figure 11), linear in shape, variable in length from 0.5 to 1.5 mm long; tetrasporangia little distending segments, developing in short spiral series (Figure 12); spermatangial stichidia developing as furcations of trichoblasts, without sterile tip cells (Figure 13); cystocarps globose to subglobose, on short stalk (Figure 14).

**Type locality** Key West, Monroe County, Florida, USA.

**Other sources** Harvey 1853, Taylor 1960, Dawes and Mathieson 2008.

**Specimens studied** *Neosiphonia echinata* (Harvey) N. Mamoozadeh *et* D.W. Freshwater, Florida: *US-66789* (slide number *US-3323, 3324*), Isotype, Key West, Monroe County, W.H. Harvey, Feb 1850; *US-22409*, West Summerland Key, Monroe County, J. Brunson, 21 Mar 1976; *US-22404*, Alligator Point, Franklin County, H.J.H., 16 Apr 1950; *US-22403*, Alligator Point, Franklin County, H.J.H., 21 Mar 1950; *WNC2009-s112* to *s115 (FL09-40)*, Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; *WNC2009-s117* to *s119 (FL09-42)*, Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; *WNC2009-s123* to s127 (FL09-44), Lake Surprise, Key Largo, Monroe County, N. Mamoozadeh, 09 Mar 2009; *WNC2009-s137* to *140 (FL09-75)*, KML Mangrove, Long Key, Monroe County, N. Mamoozadeh, 10 Mar 2009; *WNC2009-s141* to s144 (FL09-76), KML Mangrove, Long Key, Monroe County, N. Mamoozadeh, 10 Mar 2009; *WNC2010-s085 (FL05-7)*, West Summerland Key, Monroe County, B. Stuercke, 28 Feb 2005; Mexico: *WNC2010-s051* to *s054 (MEX04-8)*, Blue Bay Marina, Cancun, D.W. Freshwater, 29 Feb 2004; *WNC2010-s062* to *s064 (MEX04-10)*, Blue Bay Marina, Cancun, D.W. Freshwater, 29 Feb 2004; North Carolina: WNC2005-s039 (NC-1), Snead's Ferry, New River Inlet, Onslow County, D.W. Freshwater and F. Montgomery, 11 Jul 2005; *WNC2005-s053, s054 (NC-3)*, CORMP Site OB-1, Onslow Bay, D.W. Freshwater and K. Johns, 19 Jul 2004; *WNC2005-s001, s059 (NC-5)*, CORMP Site OB-27, Onslow Bay, J. Souza and J. Dorton, 02 Jan 2005; *WNC2005-s067 (NC-7)*, CORMP Site OB-27, Onslow Bay, J. Souza and J. Dorton, 12 Jan 2005; *WNC2005-s026, s104 (NC-14)*, Bank's Channel (Site NH-M), New Hanover County, D.W. Freshwater, 11 May 2005; *WNC2005-s031 (NC-18)*, Bogue Sound, Corkey's house, Carteret County, D.W. Freshwater, 26 Mar 2005; *WNC2005-s008 (NC-20)*, Bogue Sound, Corkey's House, Carteret County, D.W. Freshwater, 26 Dec 2003; *WNC2005-s124, s127 (NC-25)*, CORMP Site OB-27, J. Souza, 12 May 2005; *Polysiphonia fracta* Harvey, Florida: *US-66791* (slides *US-3320* to *3322*), Isotype, Key West, Monroe County, W.H. Harvey, Feb 1850; Texas: *US-2329*, near Aransas Pass, Corpus Christi, E.Y. Dawson, 12 Dec 1957; Bahamas: *US-14293*, Eleuthra, M.E. Hay, 10 May 1981; *US-14294*, Eleuthra, M.E. Hay, 08 May 1981; *Polysiphonia hapalacantha* Harvey, Florida: *US-66793* (slides *US-3325, 3326*), Isotype, Key West, Monroe County, W.H. Harvey, Feb 1850; Puerto Rico: *US-202615*, Boquerón, Cabo Rojo, M. Díaz-Piferrer, 26 Feb 1964; *US-40250*, Boquerón, Cabo Rojo, M. Díaz-Piferrer, 26 Feb 1964; *US-78248*, Boquerón, Cabo Rojo, M. Díaz-Piferrer, 26 Feb 1964.

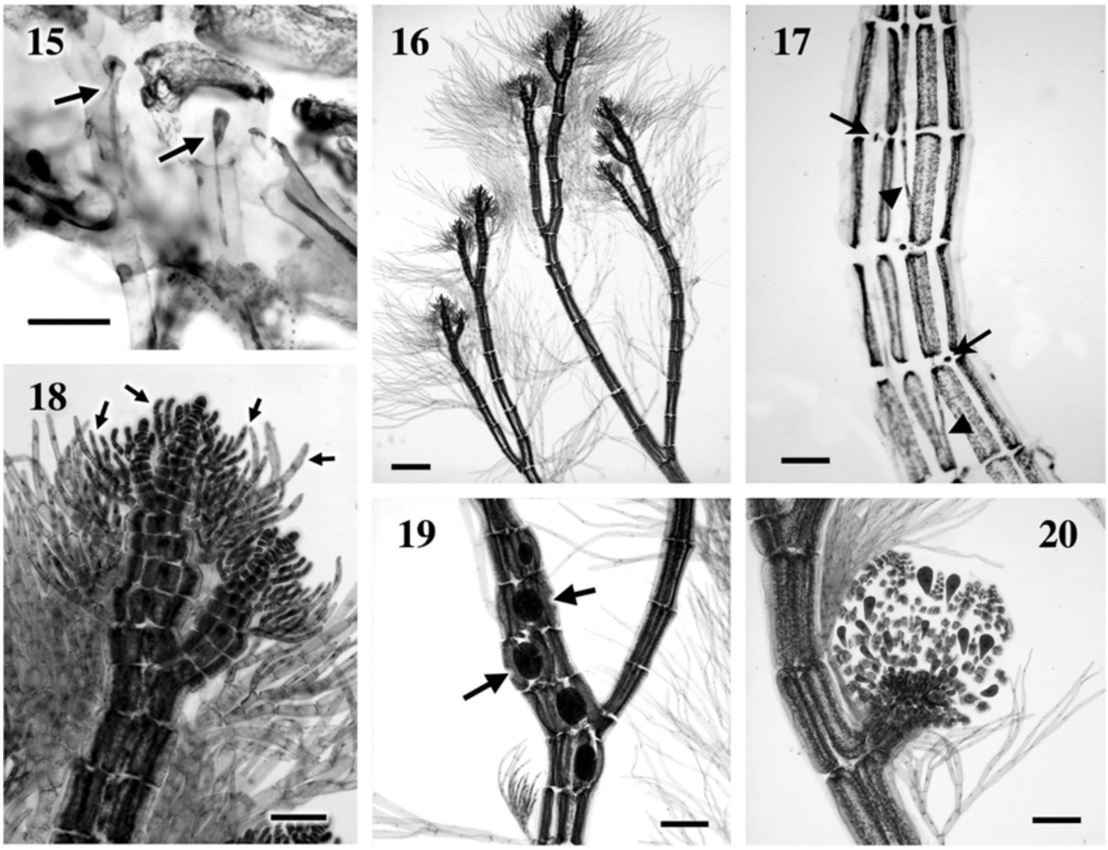
**Molecular vouchers** GenBank accession numbers HM573561, HM573559, HM573558, HM573560, HM573557 (*rbc*L); HM560658 (SSU); HM573503, HM573506, HM573504, HM573505 (COI).

**Remarks** Harvey (1853) originally described *Neosiphonia echinata* (as *Polysiphonia echinata*), *P. fracta* Harvey, and *P. hapalacantha* Harvey from Key West, Florida, USA. A report and description of *P. breviarticulata* (C. Agardh) Zanardini were also included among these original species descriptions (Harvey 1853). These species all have erect thalli with four pericentral cells, segment length-width ratios generally $\leq 1$, and numerous adventitious laterals that often give the plants a coarse appearance. The character state combination of rhizoids cut-off from pericentral cells, spermatangia developing as a furcation of trichoblasts, tetrasporangia in spiral series, and trichoblasts or scar cells in a spiral pattern on every segment observed in the specimens of *N. echinata* studied is characteristic of the genus *Neosiphonia*. This same combination of character states may be present in *P. breviarticulata* and *P. hapalacantha*, but these characters were not all observed in the specimens available for study and recent descriptions do not include this information (Taylor 1960, Athanasiadis 1987, Dawes and Mathieson 2008), making it impossible to determine whether new combinations are warranted for these species as well.

Harvey's original description of *Polysiphonia hapalacantha* included no remarks on its similarity to *Neosiphonia echinata*, *P. fracta*, or *P. breviarticulata*. *P. hapalacantha* has traditionally been distinguished from these species by its adventitious laterals to 2–4 mm in length. The type material of *P. hapalacantha* at US is in poor condition, and an assessment of the species from this material was not possible. Material at US identified as *P. hapalacantha* from Puerto Rico displayed a habit consistent with the original species description and seemed to exemplify true *P. hapalacantha*. These specimens showed longer adventitious laterals that gave the specimens a more lax and less coarse habit than *N. echinata* and *P. fracta*, allowing these species to be distinguished.

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

Harvey's reports of *Polysiphonia breviarticulata* in Key West, Florida, USA and Vera Cruz, Mexico seem to be the first reports of this species in the western Atlantic (Harvey 1853). Agardh (1824) originally described *P. breviarticulata* from the Adriatic Sea as *Hutchinsia breviarticulata* C. Agardh. Prior to Harvey (1853), *P. breviarticulata* was known only from the Adriatic and the Mediterranean. In his description of *P. breviarticulata*, Harvey noted that the Florida material appeared more robust and with more lateral branches than Mediterranean material of *P. breviarticulata* (Harvey 1853). Kützing (1863) described and illustrated a specimen identified as *P. breviarticulata*, from Vera Cruz, Mexico that may be the same Liebman specimen that Harvey

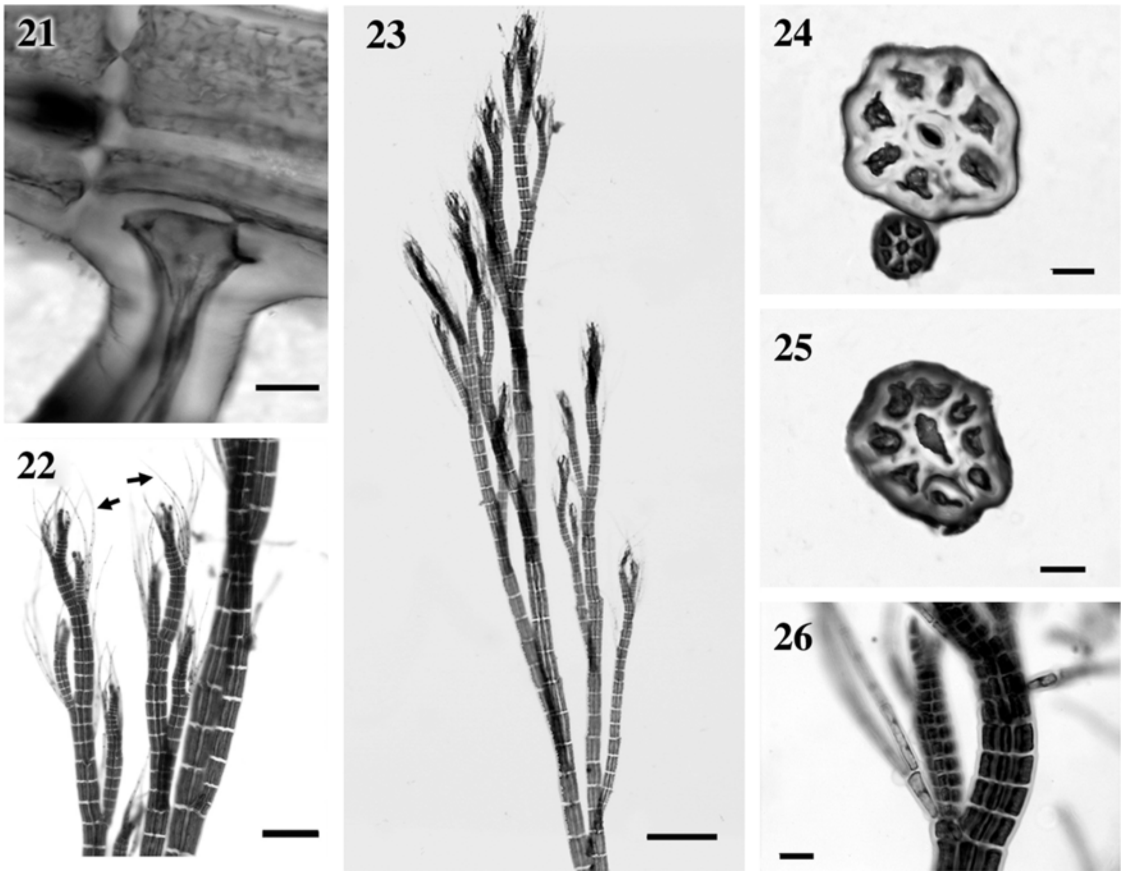



**Figures 15–20** *Neosiphonia sphaerocarpa*.
(15) Prostrate axis with rhizoids (arrows) that are cut-off from the pericentral cells, FL05-06, scale=0.10 mm, WNC2010-s083. (16) Habit of erect axes, FL05-5B, scale=0.20 mm, WNC2010-s073. (17) Portion of erect axis flattened to show four pericentral cells per segment, central axial cells (arrowheads), and scar cells in spiral pattern (arrows), FL05-5B, scale=0.10 mm, WNC2010-s071. (18) Apical portion of erect axis bearing many trichoblasts (arrows), FL05-5B, scale=50 μm, WNC2010-s071. (19) Reproductive branch displaying long spiral series of tetrasporangia (arrows), FL05-5B, scale=0.10 mm, WNC2010-s073. (20) Main axis bearing cystocarp (flattened during preparation) on short stalk, FL05-5B, scale=0.10 mm, WNC2010-s072.

studied. It differs in branching pattern and segment size from the Adriatic *P. breviarticulata* that Kützing also described and illustrated (Kützing 1863).

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

This conclusion is supported by cluster analyses of *rbc*L and COI sequence data. Four samples of *Neosiphonia echinata* were collected from the Florida Keys and two from Caribbean Mexico (Figures 1, 2). Samples identified as *Polysiphonia breviarticulata* by Stuercke and Freshwater (2008) differed from Florida and Mexico samples of *N. echinata* by 0.093–0.19% and 0.50–1.09% in the *rbc*L and COI sequence




**Figures 21–26** *Neosiphonia tepida*.
(21) Prostrate axis with rhizoid cut-off from the pericentral cell, FL05-02, scale=20 μm, WNC2010-s077. (22) Apical portion of erect axes bearing trichoblasts (arrows), FL05-02, scale=0.10 mm, WNC2010-s077. (23) Habit of erect axes, FL05-02, scale=0.20 mm, WNC2010-s077. (24) Cross-sections of young and mature branch axes showing central axial cell and seven pericentral cells, FL05-02, scale=20 μm, WNC2010-s079. (25) Cross-section of mature branch axis showing central axial cell and eight pericentral cells, FL05-02, scale=20 μm, WNC2010-s079. (26) Apical part of main axis showing a lateral branch developing in the axil of a trichoblast, FL05-02, scale=20 μm, WNC2010-s077.

data, respectively. These values are well within the range of intraspecific sequence divergence observed in previous studies for these two loci (e.g., McIvor et al. 2001, Yang et al. 2008). ML analysis of *rbc*L sequence data resolves *N. echinata* within a large unsupported clade (rB<50) of species and variously supported clades of *Polysiphonia s.l.* (Figure 3). This placement is also supported in the SSU ML phylogeny (Figure 4). The *rbc*L ML phylogeny places *N. echinata* as sister to *P. havanensis sensu* Børgesen and *P. binneyi* Harvey, with this relationship receiving weak support (rB72). These species are all similar in having four pericentral cells, but the latter two have rhizoids in open connection with pericentral cells.

### *Neosiphonia sphaerocarpa* (Børgesen) M.S. Kim *et* Lee (1999, p. 280) (Figures 15–20)

**Basionym** *Polysiphonia sphaerocarpa* Børgesen (1918, p. 321).

**Description** Plants to 2.5 cm tall, erect from basal rhizoids cut-off from pericentral cells (Figure 15), with some branches becoming decumbent and attached to substratum by rhizoids; moderately to highly branched in a subdichotomous pattern (Figure 16); erect axes 75–150 μm in diameter, prostrate axes 225–325 μm in diameter; mid axis segments of erect axes mostly (1–) 1.5–2× as long as wide; cortication lacking; main axes with four pericentral cells (Figure 17); branches replacing trichoblasts in development; trichoblasts long, to 875 μm in length, with several dichotomies, dense at apices (Figure 18); scar cells one per segment in 1/4 spiral series (Figure 17); adventitious laterals absent; tetrasporangia greatly distending segments, in long spiral series near branch tips (Figure 19), 70–90 μm in diameter; spermatangial stichidia developing as furcations of trichoblasts, 40–60×150–180 (–290) μm, with or without one sterile tip cell; cystocarps globose, short stalked (Figure 20), 250–375 μm in diameter.

**Type locality** St. Thomas, Virgin Islands.

**Other sources** Dawes and Mathieson 2008; as *Polysiphonia sphaerocarpa*, Abbott 1999, Børgesen 1918, Hollenberg 1968a, Kapraun 1977, Kapraun and Norris 1982, Kapraun et al. 1983, Schneider and Searles 1991, Taylor 1960.

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

**Molecular vouchers** GenBank accession numbers HM573569 (*rbc*L); HM573527 (COI).

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

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

***Neosiphonia tepida* (Hollenberg) S.M. Guimarães *et* M.T. Fujii in Guimarães et al. (2004, p. 171)**
**Figures 21–26**

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

**Synonyms** *Polysiphonia taylorii* Hollenberg *ex* Williams (1949, p. 694); *Polysiphonia flabellulata sensu* Meñez (1964, p. 219) [*non P. flabellulata* Harvey (1860, p. 330)].

**Description** Plants to 1.5 cm tall, erect branches arising from a prostrate branching system attached to substratum by rhizoids cut-off from pericentral cells (Figure 21); highly branched in an alternate to subdichotomous pattern (Figures 22, 23); erect axes 50–75 μm in diameter, prostrate axes 125–175 μm in diameter; mid axis segments of erect axes mostly 1.5× as long as wide; cortication lacking; main axes with 7–8 pericentral cells (Figures 24, 25), occasionally 5–6 in immature axes; branches forming in the axils of trichoblasts (Figure 26); trichoblasts to 620 μm in length, typically with 2–3 dichotomies; scar cells obvious, mostly every three to four segments and in no particular pattern; adventitious laterals absent; tetrasporangia scattered or in short to long straight or slightly spiral series, 50–95 μm in diameter; spermatangial stichidia developing as a furcation of trichoblasts, 60–80×250 μm, without sterile tip cells; cystocarps subglobose to urceolate, 160 μm in diameter, stalked, with wide ostioles.

**Type locality** Beaufort, Carteret County, North Carolina, USA.

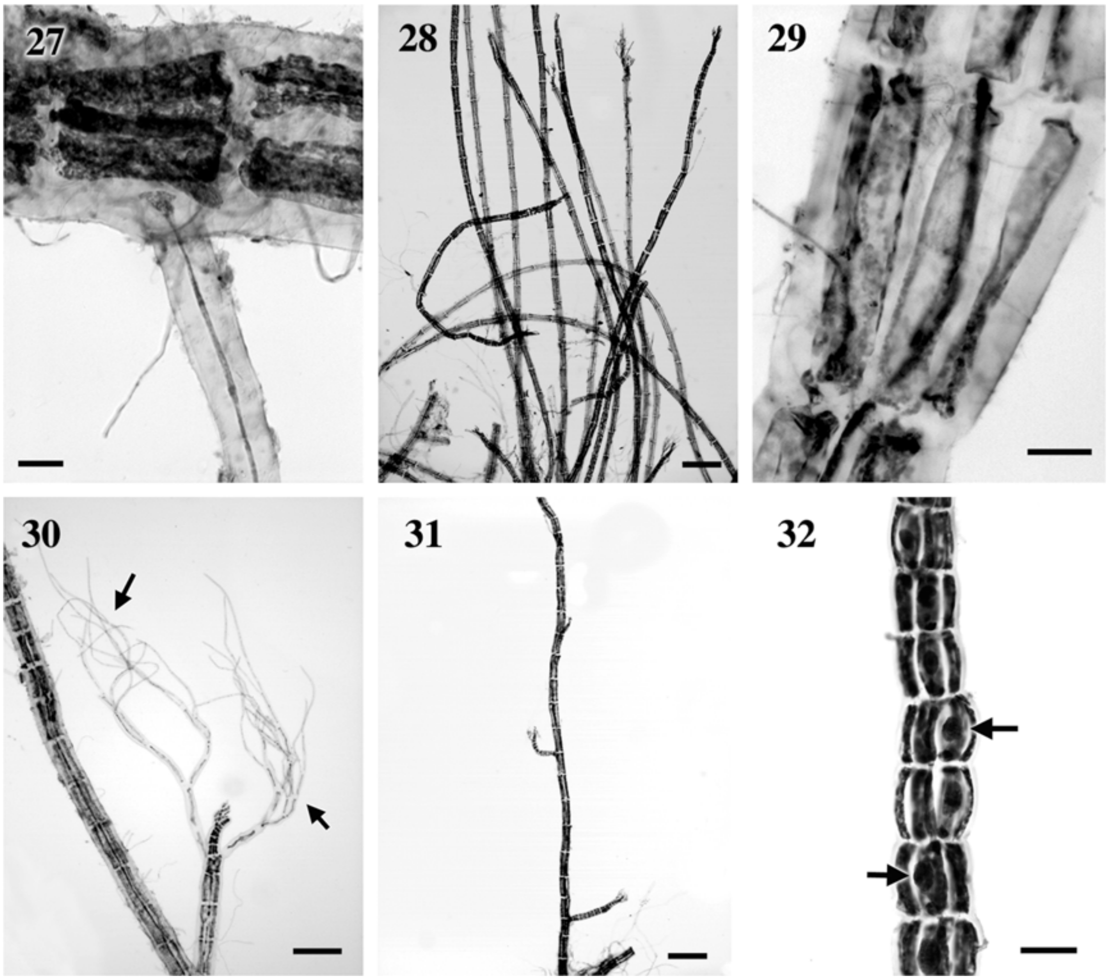
**Other sources** Dawes and Mathieson 2008; as *Polysiphonia tepida*, Abbott 1999, Hollenberg 1958, Hollenberg 1968b, Kapraun 1977, Kapraun 1980, Schneider and Searles 1991, Taylor 1960; as *Polysiphonia flabellulata*, Meñez 1964.

**Specimens studied** Florida: *WNC2010-s77* to *s79 (FL05-02)*, Sebastian Inlet, Indian River County, B. Stuercke, 26 Feb 2005.

**Molecular voucher** GenBank accession number HM573552 (*rbc*L).

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

Only one sample of *Neosiphonia tepida* was collected from Sebastian Inlet, Indian River County, Florida, USA. COI and SSU sequence data could not be generated for this sample. Cluster analyses of the *N. tepida rbc*L sequence show it to be similar to that for *Polysiphonia isogona* Harvey




**Figures 27–32** *Polysiphonia anomala*.
(27) Prostrate axis with a rhizoid cut-off from the pericentral cell, FL09-41B, scale=20 μm, WNC2010-s048. (28) Habit of erect axes, FL09-78, scale=0.20 mm, WNC2009-s149. (29) Portion of erect axis flattened to show four pericentral cells per segment, FL09-41B, scale=25 μm, WNC2010-s048. (30) Apex of lateral branch bearing trichoblasts (arrows), FL09-41B, scale=0.10 mm, WNC2010-s049. (31) Main axis showing development of adventitious laterals, FL09-77, scale=0.20 mm, WNC2009-s145. (32) Reproductive branch displaying long spiral series of tetrasporangia (arrows), FL09-77, scale=50 μm, WNC2009-s145.

(Figure 1), with sequences differing by only 1.85%. This is comparable to the maximum intraspecific *rbc*L sequence divergence of ≤2.13% (predominantly ≤1.3%) that has been observed in previous studies of *Polysiphonia* (McIvor et al. 2001). *Neosiphonia tepida* is resolved in a strongly supported clade (rB100) of multipericentral cell species in ML analysis of *rbc*L sequence data (Figure 3). *Neosiphonia tepida* is sister to *P. isogona* in this tree, with the relationship receiving strong support (rB100).

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

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

### Genus *Polysiphonia*

### *Polysiphonia anomala* Hollenberg (1968, p. 59) (Figures 27–32)

**Description** Plants small, to 5 mm tall, forming dense tangled mats, erect determinate branches arising from an indeterminate prostrate branching system attached to the substratum by rhizoids cut-off from pericentral cells (Figure 27); erect axes simple or with limited subdichotomous to irregular branching (Figure 28); erect axes 40–50 (–80) μm in diameter, prostrate axes 80–100 μm in diameter; mid axis segments of erect axes mostly 2.5–3× as long as wide; cortication lacking; main axes with four pericentral cells (Figure 29); relationship of branches to trichoblasts unknown; trichoblasts with several dichotomies, long and delicate, to 900 μm in length (Figure 30); scar cells obvious, variable in pattern and frequency; adventitious laterals frequent to occasionally present, linear in shape (Figure 31); tetrasporangia slightly distending segments, in long spiral series (Figure 32), 45 μm in diameter; spermatangial stichidia development unknown; cystocarps ovoid to slightly urceolate, short stalked, 120–140 μm in diameter.

**Type locality** Bikini Atoll, Marshall Islands.

**Other sources** Abbott 1999, Dawes and Mathieson 2008, Hollenberg 1968a.

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

**Molecular vouchers** GenBank accession numbers HM573549, HM573550 (*rbc*L); HM560654 (SSU); HM573502 (COI).

**Remarks** Hollenberg originally described *Polysiphonia anomala* with scar cells occurring one per segment in 1/4 spiral series on both prostrate and erect axes (Hollenberg 1968a). Hollenberg also noted that a very similar specimen collected by E.Y. Dawson from Lake Surprise, Key Largo, Florida (*US-2355* to *2357*) closely resembled *P. anomala* and was therefore identified as such by Hollenberg in the original description of the species. Hollenberg observed slight differences between Dawson's Florida samples and the Pacific holotype of the species, however, including scar cells that do not occur regularly one per segment on prostrate branches and tetrasporangia that occur in much longer series in the Florida specimen. This same scar cell pattern was observed in all other Florida samples examined in this study. Tetrasporangia were also observed occurring in long spiral series in *WNC2009-s145* to *s147*. Scar cell pattern alone is not a reliable species identifier as this character is often variable within species (Stuercke and Freshwater 2008); it is unclear whether length of tetrasporangial series can be independently used to distinguish species.

Three samples of *Polysiphonia anomala* were collected from Lake Surprise and Long Key, Monroe County, Florida, USA in this study (Figures 1, 2). Two of the three *rbc*L sequences generated for these samples are identical and the third differs by only 0.37%, which is well within the range of intraspecific *rbc*L sequence variation observed in previous studies of *Polysiphonia s.l.* (e.g., McIvor et al. 2001). Only one COI sequence was generated from these specimens. ML analyses of *rbc*L and SSU sequence data resolve *P. anomala* as an independent lineage with variable levels of support (rB<50 and 56; sB91 and 69) for its topological position (Figures 3, 4).

## Conclusion

Four species of *Neosiphonia* and one species of *Polysiphonia* were identified in 16 samples from Florida and Caribbean Mexico. This is the first report of *Neosiphonia bajacali* in the Caribbean and of *N. echinata* from Caribbean Mexico. Samples of the other three species do not represent new reports from their collection sites, but help confirm their known distributions. Further study of Caribbean *Neosiphonia* and *Polysiphonia* is necessary to determine the true generic status of the species reported for this widespread area. It also seems likely that more than 29 of the nearly 200 *Neosiphonia/Polysiphonia* species are present in this diverse region.

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**Appendix 1** Collection information, sample number/source, and GenBank accession numbers for Rhodomelaceae examined in this study; identical accession numbers indicate specimens with identical sequences.

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| *Boergeseniella fruticulosa* (Wulfen) Kylin | Choi et al. (2001)[4] | Spiddal Co., Galway, Ireland<br>14 May 1998, M.S. Kim | | AF427526 | |
| *Digenea simplex* (Wulfen) Agardh | Dig sim[4] | Mabibi, north of Sodwana Bay, KwaZulu-Natal, South Africa<br>13 Feb 2001, D.W. Freshwater | | HM560626 | |
| *Enelittosiphonia stimpsonii* (Harvey) Kudo *et* Masuda | Choi et al. (2001)[4] | Akkeshi, Hokkaido, Japan<br>09 May 1999, H.S. Yoon and S.M. Boo | | AF427527 | |
| *Herposiphonia* sp. | NC-1[3] | ICW, Pender Co., NC, USA<br>07 Jul 2005, B. Stuercke, R. Hamner and D.W. Freshwater | GU385834 | | |
| *Lophosiphonia* sp. | NZ04-133[3] | Curio Bay, South Island, New Zealand<br>28 Oct 2004, D.W. Freshwater and M. Hommersand | GU385835 | | |
| *Neosiphonia bajacali* (Hollenberg) Mamoozadeh *et* Freshwater | MEX04-9[2] | Blue Bay Marina, Cancun, Yucatan, Mexico<br>29 Feb 2004, D.W. Freshwater | HM573572 | HM560659 | HM573526 |
| | MEX04-11A | Blue Bay Marina, Cancun, Yucatan, Mexico<br>29 Feb 2004, D.W. Freshwater | HM573572 | | HM573526 |
| *Neosiphonia echinata* (Harvey) Mamoozadeh *et* Freshwater | FL05-7 | West Summerland Key, Monroe Co., FL, USA<br>28 Feb 2005, B. Stuercke | HM573561 | | |
| | FL09-40 | Lake Surprise, Key Largo, Monroe Co., FL, USA<br>09 Mar 2009, N. Mamoozadeh | HM573559 | | |
| | FL09-42 | Lake Surprise, Key Largo, Monroe Co., FL, USA<br>09 Mar 2009, N. Mamoozadeh | HM573558 | | |
| | FL09-44 | Lake Surprise, Key Largo, Monroe Co., FL, USA<br>09 Mar 2009, N. Mamoozadeh | HM573559 | | HM573503 |
| | FL09-75 | KML Mangrove, Long Key, Monroe Co., FL, USA<br>10 Mar 2009, N. Mamoozadeh | HM573560 | | HM573506 |
| | FL09-76 | KML Mangrove, Long Key, Monroe Co., FL, USA<br>10 Mar 2009, N. Mamoozadeh | HM573560 | | HM573506 |
| | MEX04-8[2] | Blue Bay Marina, Cancun, Yucatan, Mexico<br>29 Feb 2004, D.W. Freshwater | HM573561 | HM560658 | HM573504 |
| | MEX04-10 | Blue Bay Marina, Cancun, Yucatan, Mexico<br>29 Feb 2004, D.W. Freshwater | HM573557 | | HM573505 |
| *Neosiphonia elongella* (Harvey) Kim *et* Lee[1] | McIvor et al. (2001)[3] | Pwllheli, Cardigan, Wales<br>20 Aug 1998, C.A. Maggs | AF342913 | | |
| *Neosiphonia ferulacea* (Suhr *ex* Agardh) Guimarães *et* Fujii | PHYKOS-1996[2] | STRI Research Station, Punta Galeta, Colón, Panama<br>14 May 2009, B. Wysor and L. Sargent | HM573574 | HM560645 | HM573512 |
| | PHYKOS-2287 | West Limón Bay Jetty, Punta Toro, Colón, Panama<br>17 May 2009, N. Mamoozadeh | HM573584 | | HM573511 |
| *Neosiphonia harveyi* (Bailey) Kim, Choi, Guiry *et* G.W. Saunders | NC-6[2] | Banks Channel, New Hanover Co., NC, USA<br>30 Jan 2005, D.W. Freshwater and B. Stuercke | EU492909 | HM560628 | |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| | NC-10 | South Masonboro Inlet Jetty, New Hanover Co., NC, USA<br>04 May 2005, D.F. Kapraun, D.W. Freshwater and B. Stuercke | EU492909 | | HM573499 |
| | NC-13 | South Masonboro Inlet Jetty, New Hanover Co., NC, USA<br>04 May 2005, D.F. Kapraun, D.W. Freshwater and B. Stuercke | EU492909 | | HM573499 |
| | NC-16 | Ludens Creek, New Hanover Co., NC, USA<br>11 May 2005, D.W. Freshwater, B. Stuercke and K. Braly | EU492909 | | HM573499 |
| | NC-17 | Bogue Sound, Corkey's House, Carteret Co., NC, USA<br>26 Mar 2005, D.W. Freshwater | EU492909 | | HM573499 |
| | NC-19 | Bogue Sound, Corkey's House, Carteret Co., NC, USA<br>26 Mar 2005 D.W. Freshwater | EU492909 | | HM573499 |
| | NC-22 | South Masonboro Inlet Jetty, New Hanover Co., NC, USA<br>19 May 2005, D.W. Freshwater, B. Stuercke and K. Braly | EU492909 | | HM573500 |
| | NC-23 | Wrightsville Beach, New Hanover Co., NC, USA<br>19 May 2005, B. Stuercke | EU492909 | | |
| | NC-31 | Howard's Channel, Topsail Inlet, Pender Co., NC, USA<br>07 Jul 2005, D.W. Freshwater, B. Stuercke and R. Hamner | EU492909 | | |
| *Neosiphonia sphaerocarpa* (Børgesen) Kim *et* Lee | FL05-5B | KML, Long Key, Monroe Co., FL, USA<br>27 Feb 2005, B. Stuercke | | | HM573527 |
| | FL05-6[3] | KML, Long Key, Monroe Co., FL, USA<br>27 Feb 2005, B. Stuercke | HM573569 | | HM573527 |
| *Neosiphonia tepida* (Hollenberg) Guimarães *et* Fujii | FL05-2[3] | Sebastian Inlet, Indian River Co., FL, USA<br>26 Feb 2005, B. Stuercke | HM573552 | | |
| *Neosiphonia tongatensis* (Harvey *ex* Kützing) Kim *et* Lee | PHYKOS-2704[2] | STRI Research Station, Punta Galeta, Colón, Panama<br>21 May 2009, B. Wysor | HM573570 | HM560642 | HM573518 |
| *Neosiphonia* sp. | PHYKOS-3536[2] | STRI Research Station, Bocas del Toro, Panama<br>09 Jul 2008, D.W. Freshwater | HM573573 | HM560649 | HM573525 |
| *Polysiphonia anomala* Hollenberg | FL09-41B[2] | Lake Surprise, Key Largo, Monroe Co., FL, USA<br>09 Mar 2009, N. Mamoozadeh | HM573549 | HM560654 | |
| | FL09-77 | KML Mangrove, Long Key, Monroe Co., FL, USA<br>10 Mar 2009, N. Mamoozadeh | HM573550 | | |
| | FL09-78 | KML Mangrove, Long Key, Monroe Co., FL, USA<br>10 Mar 2009, N. Mamoozadeh | HM573550 | | HM573502 |
| *Polysiphonia aterrima* Hooker *et* Harvey | NZ04-512[2] | Mataikona, Castle Point, North Island, New Zealand<br>16 Nov 2004, D.W. Freshwater and M. Hommersand | GU385831 | HM560638 | HM573537 |
| | NZ04-525 | Mataikona, Castle Point, North Island, New Zealand<br>16 Nov 2004, D.W. Freshwater and M. Hommersand | HM573577 | | HM573536 |
| *Polysiphonia atlantica* Kapraun *et* Norris | 2NC-4 | CORMP Site OB-27, Onslow Bay, NC, USA<br>30 Aug 2004, D.W. Freshwater | EU492910 | HM560631 | HM573539 |
| | NC-26 | CORMP Site OB-27, Onslow Bay, NC, USA<br>12 May 2005, J. Souza, D. Wells and S. Hall | EU492910 | | HM573539 |
| | NC-27 | CORMP Site OB-27, Onslow Bay, NC, USA<br>09 Jun 2005, D.W. Freshwater and J. Souza | EU492910 | | HM573539 |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| | NC-28 | CORMP Site OB-27, Onslow Bay, NC, USA<br>09 Jun 2005, D.W. Freshwater and J. Souza | EU492910 | | HM573539 |
| | NC-32 | CORMP Site OB-3, Onslow Bay, NC, USA<br>11 Jul 2005, D.W. Freshwater and B. Stuercke | EU492910 | | HM573539 |
| *Polysiphonia binneyi* Harvey | PHYKOS-2439 | Isla Naranjo Arriba, Colón, Panama<br>19 May 2009, L. Sargent | HM573556 | | |
| | PHYKOS-2458 | Isla Naranjo Arriba, Colón, Panama<br>19 May 2009, L. Sargent | HM573556 | | |
| | PHYKOS-2461 | Isla Naranjo Arriba, Colón, Panama<br>19 May 2009, S. Schmitt | HM573556 | | |
| | PHYKOS-2517[2] | Isla Naranjo Arriba, Colón, Panama<br>19 May 2009, S. Schmitt | HM573555 | HM560636 | |
| ''*Polysiphonia breviarticulata*'' *sensu* Stuercke *et* Freshwater | NC-1 | Snead's Ferry, New River Inlet, Onslow Co., NC, USA<br>11 Jul 2003, D.W. Freshwater and F. Montgomery | EU492911 | | |
| | NC-3 | CORMP Site OB-1, Onslow Bay, NC, USA<br>19 Jul 2004, D.W. Freshwater and K. Johns | EU492911 | | HM573497 |
| | NC-5 | CORMP Site OB-27, Onslow Bay, NC, USA<br>12 Jan 2005, J. Souza and J. Dorton | EU492911 | | |
| | NC-7 | CORMP Site OB-27, Onslow Bay, NC, USA<br>12 Jan 2005, J. Souza and J. Dorton | EU492911 | | |
| | NC-14 | Bank's Channel (Site NH-M), New Hanover Co., NC, USA<br>11 May 2005, D.W. Freshwater | EU492911 | | HM573498 |
| | NC-15 | Ludens Creek (Site NH-J), New Hanover Co., NC, USA<br>11 May 2005, D.W. Freshwater, B. Stuercke and K. Braly | EU492911 | | |
| | NC-18 | Bogue Sound, Corkey's House, Carteret Co., NC, USA<br>26 Mar 2005, D.W. Freshwater | EU492911 | | HM573498 |
| | NC-20 | Bogue Sound, Corkey's House, Carteret Co., NC, USA<br>26 Dec 2003, D.W. Freshwater | EU492911 | | HM573498 |
| *Polysiphonia brodiei* (Dillwyn) Sprengel | McIvor et al. (2001)[3] | Portaferry, Down, Ireland<br>20 Mar 1998, C.A. Maggs | AF342916 | | |
| *Polysiphonia constricta* Womersley | NZ04-78 | Shag Point, South Island, New Zealand<br>26 Oct 2004, D.W. Freshwater and M. Hommersand | GU385832 | | |
| | NZ04-256 | Paua Beach, Stewart Island, New Zealand<br>01 Nov 2004, D.W. Freshwater and M. Hommersand | GU385832 | | |
| | NZ04-308[2] | Ulva Island near Stewart Island, New Zealand<br>03 Nov 2004, D.W. Freshwater and M. Hommersand | HM573580 | HM560639 | HM573542 |
| *Polysiphonia denudata* (Dillwyn) Greville *ex* Harvey | McIvor et al. (2001)[3] | Plymouth, Devon, England<br>30 Sep 1998, F. Bunker | AF342914 | | |
| *Polysiphonia elongata* (Hudson) Sprengel | McIvor et al. (2001)[3] | Fanad, N. Donegal, Ireland<br>17 May 1998, C.A. Maggs | AF342911 | | |
| | Choi et al. (2001)[4] | Spiddal Co., Galway, Ireland<br>13 Mar 1998, M.S. Kim | | AF427529 | |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| *Polysiphonia fibrata* (Dillwyn) Harvey | McIvor et al. (2001)[3] | Marble Hill, N. Donegal, Ireland<br>05 Aug 1993, C.A. Maggs | AF342915 | | |
| *Polysiphonia fibrillosa* (Dillwyn) Sprengel | McIvor et al. (2001)[3] | Marble Hill, N. Donegal, Ireland<br>05 Aug 1993, C.A. Maggs | AF342912 | | |
| *Polysiphonia forfex* Harvey | McIvor et al. (2001)[3] | Biarritz, Aquitaine, France<br>15 Jul 1999, C.A. Maggs | AF342910 | | |
| *Polysiphonia fucoides* (Hudson) Greville | NC-12[2] | South Masonboro Inlet Jetty, New Hanover Co., NC, USA<br>04 May 2005, D.F. Kapraun, D.W. Freshwater and B. Stuercke | EU492913 | HM560627 | HM573496 |
| *Polysiphonia havanensis sensu* Børgesen | PHYKOS-2628[2] | West Limón Bay Jetty, Punta Toro, Colón, Panama<br>20 May 2009, B. Wysor | HM573554 | HM560641 | |
| | PHYKOS-3185 | Cayos Zapatillas, Bocas del Toro, Panama<br>25 Aug 2009, B. Wysor and D.W. Freshwater | HM573554 | | |
| | PHYKOS-3186 | Cayos Zapatillas, Bocas del Toro, Panama<br>25 Aug 2009, B. Wysor and D.W. Freshwater | HM573554 | | HM573522 |
| *Polysiphonia homoia* Setchell *et* Gardner | PHYKOS-3524 | Isla Afuera, Veraguas, Panama<br>16 Jan 2008, B. Wysor and J. Alden | HM573553 | | HM573507 |
| | PHYKOS-3525[2] | Isla Afuera, Veraguas, Panama<br>16 Jan 2008, B. Wysor and J. Alden | HM573553 | HM560653 | HM573507 |
| *Polysiphonia howei* Hollenberg | Phillips (2006)[4] | Talisoy, Vivac Catanduanes, Phillipines<br>14 May 1988, J. West | | AY237282 | |
| | PHYKOS-3141[2] | Cayos Tigres, Bocas del Toro, Panama<br>25 August 2009, B. Wysor | HM573543 | HM560656 | HM573520 |
| | PHYKOS-3526 | Flat Rock Beach, Isla Colón, Bocas del Toro, Panama<br>17 Jan 2007, D.W. Freshwater | HM573543 | | HM573521 |
| | PHYKOS-3527 | Flat Rock Beach, Isla Colón, Bocas del Toro, Panama<br>17 Jul 2008, D.W. Freshwater | HM573543 | | HM573521 |
| | PHYKOS-3528 | Swan Cay, Isla Colón, Bocas del Toro, Panama<br>19 Jul 2008, M. Albis | HM573543 | | HM573520 |
| *Polysiphonia isogona* Harvey | NZ04-139[3] | Curio Bay, South Island, New Zealand<br>28 Oct 2004, D.W. Freshwater and M. Hommersand | HM573578 | | HM573541 |
| *Polysiphonia kapraunii* Stuercke *et* Freshwater | NC-11[2] | South Masonboro Inlet Jetty, New Hanover Co., NC, USA<br>04 May 2005, D.F. Kapraun, D.W. Freshwater and B. Stuercke | EU492920 | HM560630 | HM573501 |
| *Polysiphonia macrocarpa* (C. Agardh) Sprengel | PHYKOS-2561 | Punta Gorda, Colón, Panama<br>20 May 2009, D.W. Freshwater | HM573545 | | HM573538 |
| | PHYKOS-2617 | Punta Gorda, Colón, Panama<br>20 May 2009, S. Schmitt | HM573545 | | HM573538 |
| | PHYKOS-2627[2] | Punta Gorda, Colón, Panama<br>20 May 2009, S. Schmitt | HM573545 | HM560632 | HM573538 |
| | PHYKOS-2630 | Punta Gorda, Colón, Panama<br>20 May 2009, D.W. Freshwater | HM573545 | | HM573538 |
| *Polysiphonia morrowii* Harvey | Choi et al. (2001)[4] | Sachon, Korea<br>08 Mar 1997, M.S. Kim | | AF427532 | |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| | NZ04-5[3] | Onuku, Akaroa Harbor, South Island, New Zealand<br>23 Oct 2004, D.W. Freshwater and M. Hommersand | HM573583 | | |
| | NZ04-130 | Curio Bay, South Island, New Zealand<br>28 Oct 2004, D.W. Freshwater and M. Hommersand | HM573579 | | HM573540 |
| | NZ04-132 | Curio Bay, South Island, New Zealand<br>28 Oct 2004, D.W. Freshwater and M. Hommersand | HM573579 | | |
| | NZ04-140 | Curio Bay, South Island, New Zealand<br>28 Oct 2004, D.W. Freshwater and M. Hommersand | HM573579 | | |
| *Polysiphonia muelleriana* J. Agardh | Fujii et al. (2006)[3] | Fiordland, Thompson Sound, Deas Cove, New Zealand<br>03 Oct 2000, S. Wing and N. Goebel | AY588412 | | |
| *Polysiphonia pacifica* Hollenberg | Choi et al. (2001)[4] | Bradys Beach, Bamfield, B.C., Canada<br>29 Apr 1998, J. Warneboldt and J.T. Harper | | AF427533 | |
| | Kim et al. (2004)[3] | Seal Rock, OR, USA | AY396036 | | |
| *Polysiphonia paniculata* Montagne | Kim et al. (2004)[3] | Seno Otway, Punta Arenas, Chile | AY396041 | | |
| | Zuccarello et al. (2004)[4] | Four Mile Beach, Santa Cruz, CA, USA | | AY617144 | |
| *Polysiphonia pentamera* Hollenberg | PHYKOS-1995[2] | STRI Research Station, Punta Galeta, Colón, Panama<br>14 May 2009, B. Wysor and L. Sargent | HM573563 | HM560644 | HM573510 |
| | PHYKOS-3529[2] | Isla Planito near Coiba, Veraguas, Panama<br>15 Jan 2008, B. Wysor and J. Alden | HM573564 | HM560643 | |
| | PHYKOS-3530 | Bahia Honda near El Barranco, Veraguas, Panama<br>15 Jan 2008, B. Wysor and J. Alden | HM573564 | | |
| | PHYKOS-3531 | Bahia Honda near El Barranco, Veraguas, Panama<br>15 Jan 2008, B. Wysor and J. Alden | HM573564 | | |
| | PHYKOS-3532 | Isla Afuera, Veraguas, Panama<br>16 Jan 2008, B. Wysor and J. Alden | HM573564 | | |
| *Polysiphonia pernacola* Adams | NZ04-243 | Paua Beach, Stewart Island, New Zealand<br>01 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | HM573495 |
| | NZ04-244 | Paua Beach, Stewart Island, New Zealand<br>01 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | |
| | NZ04-263 | Oban, Stewart Island, New Zealand<br>01 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | |
| | NZ04-291[2] | Ulva Island near Stewart Island, New Zealand<br>02 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | HM560637 | HM573495 |
| | NZ04-309 | Ulva Island near Stewart Island, New Zealand<br>03 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | HM573495 |
| | NZ04-550A | Muritai, Wellington Harbor, North Island, New Zealand<br>17 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | HM573495 |
| | NZ04-550B | Muritai, Wellington Harbor, North Island, New Zealand<br>17 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | HM573495 |
| | NZ04-557 | Muritai, Wellington Harbor, North Island, New Zealand<br>17 Nov 2004, D.W. Freshwater and M. Hommersand | HM573576 | | HM573495 |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| *Polysiphonia pseudovillum* Hollenberg | PHYKOS-3533[2] | Flat Rock Beach, Isla Colón, Bocas del Toro, Panama 17 Jul 2008, D.W. Freshwater | HM573568 | HM560650 | HM573524 |
| *Polysiphonia schneideri* Stuercke *et* Freshwater | BERMUDA | Tucker's Bay, Harrington Sound, Bermuda 17 Mar 2009, C.W. Schneider and C.E. Lane | GU385836 | | |
| | FL05-04 | Sebastian Inlet, Indian River County, FL, USA 26 Feb 2005, B. Stuercke | HM573567 | | HM573514 |
| | NC-2 | Cassamir Wreck (WR2), Onslow Bay, NC, USA 02 Jul 2004, D.W. Freshwater and K. Johns | EU492912 | | |
| | PHYKOS-2454[2] | Panama Canal North Ferry Terminus, Colón, Panama 19 May 2009, K. Larson and L. McCann | HM573566 | HM560629 | HM573516 |
| | PHYKOS-2689 | STRI Research Station, Punta Galeta, Colón, Panama 21 May 2009, S. Schmitt | HM573565 | | HM573513 |
| | PHYKOS-3189 | Cayos Zapatillas, Bocas del Toro, Panama 25 Aug 2009, D.W. Freshwater | GU385837 | | HM573515 |
| *Polysiphonia scopulorum* Harvey | Kim et al. (2004)[3] | Devil's Punchbowl, OR, USA | AY396039 | | |
| *Polysiphonia scopulorum var. villum* (J. Agardh) Hollenberg | NC-9 | South Masonboro Inlet Jetty, New Hanover Co., NC, USA 04 May 2005, D.F. Kapraun, D.W. Freshwater and B. Stuercke | EU492915 | | HM573535 |
| | NC-33[2] | South Masonboro Inlet Jetty, New Hanover Co., NC, USA 22 Jul 2005, D.W. Freshwater, R. York, K. Braly, R. Hamner and B. Stuercke | EU492915 | HM560633 | HM573535 |
| *Polysiphonia cf. sertularioides* (Grateloup) J. Agardh 1 | PHYKOS-3226[2] | Crawl Cay, Bocas del Toro, Panama 28 Aug 2009, G. Diaz-Pulido and R. Riosmena | HM573546 | HM560647 | HM573519 |
| *Polysiphonia cf. sertularioides* (Grateloup) J. Agardh 2 | PHYKOS-3534[2] | Isla Afuera, Veraguas, Panama 16 January 2008, B. Wysor and J. Alden | HM573547 | HM560652 | HM573509 |
| *Polysiphonia cf. sertularioides* (Grateloup) J. Agardh 3 | PHYKOS-2257[2] | West Limón Bay Jetty, Punta Toro, Colón, Panama 17 May 2009, D.W. Freshwater and N. Mamoozadeh | HM573548 | HM560646 | |
| | PHYKOS-2309 | West Limón Bay Jetty, Punta Toro, Colón, Panama 17 May 2009, D.W. Freshwater and N. Mamoozadeh | HM573548 | | |
| *Polysiphonia stricta* (Dillwyn) Greville | Choi et al. (2001)[4] | Flamborough, England 16 Jul 1998, M.S. Kim | | AF427535 | |
| | [3]GWS2657 | Letete Pt., New Brunswick, Canada 14 Mar 2005, G.W. Saunders | EU492916 | | |
| *Polysiphonia strictissima* Hooker *et* Harvey | NZ04-39 | Shag Point, South Island, New Zealand 25 Oct 2004, D.W. Freshwater and M. Hommersand | HM573582 | | HM573530 |
| | NZ04-80 | Shag Point, South Island, New Zealand 26 Oct 2004, D.W. Freshwater and M. Hommersand | HM573582 | | HM573530 |
| | NZ04-147 | Lonnekers Nugget, Stewart Island, New Zealand 29 Oct 2004, D.W. Freshwater and M. Hommersand | GU385835 | | HM573534 |
| | NZ04-246 | Paua Beach, Stewart Island, New Zealand 01 Nov 2004, D.W. Freshwater and M. Hommersand | HM573581 | | |
| | NZ04-250 | Paua Beach, Stewart Island, New Zealand 01 Nov 2004, D.W. Freshwater and M. Hommersand | GU385833 | | HM573532 |

*(Appendix 1 continued)*

| Species | Sample number/source | Collection location, date, and collector | Accession number | | |
|---|---|---|---|---|---|
| | | | *rbc*L | SSU | COI |
| | NZ04-307 | Ulva Island near Stewart Island, New Zealand<br>03 Nov 2004, D.W. Freshwater and M. Hommersand | GU385833 | | HM573531 |
| | NZ04-552[3] | Muritai, Wellington Harbor, North Island, New Zealand<br>17 Nov 2004, D.W. Freshwater and M. Hommersand | GU385833 | | HM573533 |
| *Polysiphonia subtilissima* Montagne 1 | NC-21[2] | Neuse River at Oriental, Pamlico Co., NC, USA<br>Sep 2003, R. Peterson | EU492917 | HM560634 | |
| | NC-24 | Snow's Cut Park, New Hanover Co., NC, USA<br>22 May 2005, B. Stuercke and J.B. Landry | EU492918 | | HM573529 |
| *Polysiphonia subtilissima* Montagne 2 | PHYKOS-3271[2] | STRI Research Station, Bocas del Toro, Panama<br>03 Sep 2009, D.W. Freshwater | HM573575 | HM560635 | HM573528 |
| *Polysiphonia* sp. | PHYKOS-2613[2] | Parque de Juventud, Calle Primero, Colón, Panama<br>20 May 2009, B. Wysor | HM573571 | HM560648 | HM573517 |
| *Polysiphonia* sp. | PHYKOS-3535[2] | Isla Afuera, Veraguas, Panama<br>16 Jan 2008, B. Wysor and J. Alden | HM573562 | HM560651 | HM573508 |
| *Polysiphonia* sp. | PHYKOS-3537[2] | Tervi Bight, Bocas del Toro, Panama<br>13 Jul 2008, S. Fredericq | HM573551 | HM560657 | |
| *Polysiphonia* sp. | PHYKOS-2635 | Punta Gorda West, Colón, Panama<br>20 May 2009, D.W. Freshwater | HM573544 | | |
| | PHYKOS-2701 | STRI Research Station, Punta Galeta, Panama<br>21 May 2009, B. Wysor | HM573544 | | |
| | PHYKOS-3144 | Cayos Zapatillas, Bocas del Toro, Panama<br>25 Aug 2009, D.W. Freshwater and B. Wysor | HM573544 | | |
| | PHYKOS-3145 | Cayos Zapatillas, Bocas del Toro, Panama<br>25 Aug 2009, D.W. Freshwater and B. Wysor | HM573544 | | |
| | PHYKOS-3538[2] | Swan Key, Isla Colón, Bocas del Toro, Panama<br>14 Jul 2007, D.W. Freshwater | HM573544 | HM560655 | HM573523 |
| *Polysiphonia* sp. | NZ04-515[4] | Mataikona, Castle Point, North Island, New Zealand<br>16 Nov 2004, D.W. Freshwater and M. Hommersand | | HM565963 | |
| *Polysiphonia* sp. | Kim et al. (2004)[3] | Las Cruses, Central Chile | AY396038 | | |
| *Vertebrata lanosa* (Linnaeus) Christensen | Zuccarello et al. (2004)[4] | Nahant, Providence, Providence Co., RI, USA | | AY617143 | |
| *Womersleyella setacea* (Hollenberg) Norris | Choi et al. (2001)[4] | NUIG Marine Algal Culture Collection, Italy<br>F. Rindi and M.D. Guiry | | AF427537 | |

[1] Sequence published as *Polysiphonia elongella* Harvey.
[2] Sequence used in ML analysis of *rbc*L and SSU data.
[3] Sequence used in ML analysis of *rbc*L data.
[4] Sequence used in ML analysis of SSU data.

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