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Consistency of morphological characters used to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae): analyses of North Carolina, USA specimens

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Polysiphonia sensu lato (including *Neosiphonia*) is a genus within the red algal order Ceramiales that includes over 200 current species and is distributed throughout the world. The consistency of morphological characters commonly used to distinguish species was tested by analyses of these characters within eight North Carolina, USA, species of *P. sensu lato* objectively defined by *rbc*L sequence analysis. Examination of 22 characters found five to be consistent and useful for identifying species: number of pericentral cells, rhizoid–pericentral cell connection, relationship of lateral branches to trichoblasts, spermatangial axes development, and arrangement of tetrasporangia. Character states for the pattern and occurrence of trichoblasts and scar cells, the type of the holdfast, and formation of cicatrigenous branches may also help to strengthen the distinctions among species; although, some of these characters were not uniformly consistent. These morphological findings may be applicable to the study of *P. sensu lato* from other regions as well. The eight North Carolina species were resolved in a variety of positions within the *Polysiphonia sensu lato rbc*L tree, and integrated molecular and morphological analyses indicated that two, *Polysiphonia denudata* and *Polysiphonia urceolata*, have been historically misidentified.

KEY WORDS: Character consistency, Morphology, *Neosiphonia*, North Carolina, *Polysiphonia*, *rbc*L

INTRODUCTION

Polysiphonia Greville is one of the most speciose red algal genera (Segi 1951; Womersley 1979) and includes species distributed throughout the world (e.g. Hollenberg 1942, 1944; Womersley 1979; Kapraun 1980a; Yoon 1986). Greville (1824) proposed *Polysiphonia* to replace *Hutchinsia* C. Agardh (1817), a *nomen illegitimum*, based on its prior application to a group of cruciferous plants. Greville did not designate a generitype species when he transferred eight species to *Polysiphonia*. *Polysiphonia urceolata* (Dillwyn) Greville was later designated as the type species (Silva 1952; Silva *et al.* 1996), and recently this species has been subsumed under the earlier name *Polysiphonia stricta* (Dillwyn) Greville (Maggs & Hommersand 1993; Kim & Lee 1999; Kim *et al.* 2000). *Polysiphonia* has been conserved against *Vertebrata* Gray (1821), *Grammita* Bonnemaison (1822), and *Gratelupella* Bory (1823) (Silva 1952; Greuter *et al.* 1994). Additional details on the taxonomic history of *Polysiphonia* are presented by Kim *et al.* (2000).

Polysiphonia, when first described, included virtually all obviously segmented plants now placed in the Rhodomelaceae (Hollenberg 1942). However, Falkenberg (1901) restricted *Polysiphonia* to species with the following features: (1) main branches that are obviously polysiphonous, (2) branches that arise exogenously by more or less diagonal division of subapical cells before cutting off pericentral cells, (3) branches that are all basically alike and indeterminate, and (4) only one tetrasporangium in each fertile segment. Generally, the following characters need to be present in order to identify species: number of pericentral cells, origin of the rhizoid, origin of branches in relation to trichoblasts, arrangement of tetrasporangia, and position of the spermatangial branches (Hollenberg 1942, 1968a, b; Hollenberg & Norris 1977; Kapraun 1977a, 1980a; Kapraun & Norris 1982; Schneider & Searles 1991; Abbott 1999). The within-species consistency of these and other characters, however, has not been tested when species were objectively defined by nonmorphological characters.

Analyses of DNA sequence data have been incorporated in only a few *Polysiphonia* studies. Choi *et al.* (2001) analysed nuclear-encoded 18S rRNA gene sequences and morphological characters for 14 species of *Polysiphonia sensu lato* and other Rhodomelaceae. The resulting trees were used to examine the distribution of morphological character states among clades of species, but multiple samples of the same species were not included, so the within-species consistency of characters could not be assessed. Three studies have analysed chloroplast-encoded *rbc*L sequences from species of *P. sensu lato* (McIvor *et al.* 2001; Kim *et al.* 2004; Kim & Yang 2005). McIvor *et al.* (2001) integrated karyological, interbreeding, and sequence data in an analysis of the invasive species *Neosiphonia harveyi* (Bailey) Kim, Choi, Guiry & Saunders (as *Polysiphonia harveyi* Bailey). The combined breeding and sequence results allowed the estimation of the level of sequence divergence to be expected within and between species of *P. sensu lato*. The subsequent studies of Kim *et al.* (2004) and Kim & Yang (2005) further demonstrated the

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utility of *rbc*L sequence analyses for the identification of *Polysiphonia* species.

Fifteen species of *P. sensu lato* (including *Neosiphonia*) have been reported from the North Carolina coast (Williams 1948; Taylor 1960; Brauner 1975; Schneider 1976; Kapraun 1977a, 1980a; Kapraun & Searles 1990; Schneider & Searles 1991). These species have been the subject of field, culture, karyological, microspectrophotometric, and taxonomic studies (Kapraun 1977a, 1977b, 1978a, 1978b, 1993; Kapraun & Searles 1990; Kapraun & Dunwoody 2002), and consequently the species are relatively well differentiated; although, some taxonomic problems exist. This earlier research makes North Carolina species ideal candidates for the study of intraspecific and interspecific variability of morphological characters. The purpose of this study was to objectively define some of the North Carolina species of *P. sensu lato* using *rbc*L sequence data so that the consistency of characters that have been used in species identifications could be determined. An understanding of within-species character consistency is necessary before the evolutionary relationships of *P. sensu lato* can be fully understood.

MATERIAL AND METHODS

Collection

Polysiphonia samples were collected intertidally or subtidally by snorkeling or SCUBA from various locations in North Carolina, Florida, and Hawai'i and transported to the lab as fresh specimens in seawater or dried in silica gel desiccant (Chase & Hills 1991; Table 1). Specimens for morphological study were preserved in 5% formalin seawater solution buffered with borax, and voucher slides were made as described in Tsuda & Abbott (1985). Herbarium and permanent slide vouchers were deposited in the University of North Carolina Wilmington (WNC) herbarium, and the silica-dried and formalin-preserved samples were deposited into the silica and wet collections, respectively, at the Center for Marine Science. All herbarium abbreviations follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

DNA extraction and sequencing

DNA was extracted from specimens as in Hughey *et al.* (2001) with an additional cleaning step using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). The large subunit of the plastid-encoded ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) was amplified following the steps described in Freshwater *et al.* (2005). Amplification products were cleaned with either a QIAquick PCR purification kit or a GeneClean II kit (Q-Biogene, Irvine, CA, USA) and used as templates in Big Dye (v.3.1, Applied Biosystems, Foster City, CA, USA) sequencing reactions. Primers used for amplification and sequencing reactions were those published in Freshwater & Rueness (1994) and one primer designed specifically for this study (R-893 5′-GAATAAGTTGA(AG)TT(AT)CCIG-CAC-3′). Sequencing reactions were run on an ABI 3100 Genetic Analyzer (DNA Analysis Core Facility, CMS) and edited and assembled using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA).

Phylogenetic analyses

The 35 *Polysiphonia* and *Neosiphonia* sequences generated in this study were combined with 21 *rbc*L sequences available from GenBank (Table 1) and aligned using MacClade (v.4, Maddison & Maddison 2000). Three species, *Bostrychia simpliciuscula* Harvey ex J. Agardh, *Polyzonia elegans* Suhr, and *Wrightiella tumanowiczii* (Gatty ex Harvey) F. Schmitz, were included as outgroups because of their placement in other Rhodomelacean subfamilies (Hommersand 1963). The alignment was truncated to 1245 sites because the GenBank-derived sequences were incomplete at the 5′ and/or 3′ ends. Characteristics of the sequence data sets and models of nucleotide evolution were determined using MacClade, Modeltest (v.3.6, Posada & Crandall 1998), and PAUP* (v.4, Swofford 2002). Phylogenetic analyses of the sequence data sets were performed using PAUP*.

Maximum-likelihood and neighbor-joining distance analyses were performed using a GTR + G model of evolution and the following parameters derived from the Modeltest program: Base composition = (A: 0.3306, G: 0.0471, C: 0.1430, T: 0.4793), Nst = 6, rate matrix = (0.3341, 8.2159, 0.7204, 0.7670, 4.0433), gamma shape = 3.6010, proportion of invariable sites = 0. The maximum-likelihood analyses included 10 separate searches with random sequence addition using the tree bisection-reconnection (TBR) branch swapping algorithm, and the maximum-likelihood analysis was subjected to 500 replications of bootstrap resampling (random additions set to 1). The neighbor joining distance analysis was subjected to 5000 replications of bootstrap resampling. Parsimony analysis of the data was completed using a heuristic search of 10,000 random sequence additions with MULTREES, STEEPEST DESCENT, and TBR branch swapping options in effect. Parsimony bootstrap analyses consisted of 1000 replications of 10,000 random sequences additions.

Morphological data and analysis

Morphological characters examined were selected based on taxonomic keys for and descriptions of *Polysiphonia* species (Hollenberg 1942; 1968a, b; Abbott & Hollenberg 1976; Hollenberg & Norris 1977; Kapraun 1977a; Womersley 1979; Kapraun 1980a; Schneider & Searles 1991; Maggs & Hommersand 1993; Kim *et al.* 1994; Abbott 1999). Observations were made using an Olympus SZH dissecting microscope, a Zeiss Axio Imager.Z1 compound microscope, or an Olympus BH2 compound microscope, and images were captured using Spot RT and Zeiss Axio cam MRc5 digital camera systems. After an initial investigation some characters were determined to be too variable or difficult to score for all samples, and therefore the list of fully examined characters was narrowed to 22 (Table 2). Character states for the 22 characters examined in the 31 North Carolina samples were mapped onto a phylogenetic framework based on relationships inferred among these

samples in the *rbc*L analyses. North Carolina samples were identified to species based on examination of morphological characteristics and using the taxonomic keys and descriptions found in Kapraun (1977a, 1980a, unpublished observations), Schneider & Searles (1991), and Maggs & Hommersand (1993).

RESULTS AND OBSERVATIONS

Molecular analyses

A total of 34 unique *rbc*L sequences representing 54 ingroup and three outgroup specimens were analysed in this study. The analysed alignment was 1245 base pairs in length; 482 sites (38.7%) were variable and 380 sites (30.5%) were parsimony informative. Parsimony, neighbor-joining distance and maximum-likelihood analyses were completed for this data set. The three analyses varied in the exact relationships of closely related species within some clades, but conflict observed within these clades was not supported by bootstrap analyses. The overall relationships of species were the same in all three analyses, and therefore only the maximum-likelihood *rbc*L topology with bootstrap results from all three methods is shown in Fig. 1. The 31 North Carolina samples sequenced in this project were resolved in eight separate highly supported clades representing individual species (Fig. 1).

Character assessment

Twenty-two characters were examined in the 31 North Carolina *Polysiphonia* and *Neosiphonia* samples (Table 2). The character states for each sample (Table 3) were mapped onto a phylogenetic framework based on the relationships of these specimens resolved in the *rbc*L sequence analyses (Fig. 2).

Number of pericentral cells (character 1)

The number of pericentral cells was consistent within each North Carolina species and ranged from four to 13 (Table 3). The majority of the samples (28 of 31) had only four pericentral cells. The two samples of *Polysiphonia denudate sensu* Kapraun had six, and the only collected *Polysiphonia fucoides* sample had 12–13 pericentral cells. This character was determined by observations of transverse sections made through the main axes of specimens (Figs 3–5). Accurate pericentral cell counts can sometimes also be made by squashing main axes.

The number of pericentral cells in *P. sensu lato* has been shown to range from 4 to 24 (Dawson 1966; Schneider & Searles 1991; Maggs & Hommersand 1993; Stegenga *et al.* 1997; Abbott 1999; Womersley 2003). The number of pericentral cells typically remains constant in species with four pericentral cells, but this number tends to vary within species as the number of pericentral cells increases (Hollenberg 1942; Womersley 1979). In segments that have tetrasporangia, the appearance of an extra pericentral cell from the vegetative number of pericentral cells has been reported because of the formation of cover cells by the longitudinal division of the fertile pericentral cell (Hollenberg 1942; Womersley 1979). Kim *et al.* (2000), however, found that the fertile axes of *P. stricta* did have five rather than four pericentral cells prior to the division leading to cover cells.

Rhizoid–pericentral cell connection (character 2)

This character (Figs 6, 7) has been described in the literature as each rhizoid being an extension of the pericentral cell (open connection) or each rhizoid is cut off from the pericentral cell as a separate cell (pit connected; Hollenberg 1968b; Womersley 1979; Kapraun 1980a; Schneider & Searles 1991; Maggs & Hommersand 1993; Abbott 1999). The connection between rhizoids and pericentral cells was determined for all but one North Carolina sample, and this character was found to be consistent. There also was a distinct separation in the tree between samples with open-connected and those with pit-connected rhizoids.

This character is not always easy to determine because the rhizoid must be observed at the proper angle to accurately identify the character state. Hollenberg (1968a) suggested that the location of origin of the rhizoid on a pericentral cell may also be used to characterize species, whether the rhizoid arises on the distal end, on the proximal end, or from the center. This detail was not recorded in this study; however, pit-connected rhizoids were typically attached at the proximal end of pericentral cells; whereas, the open-connected rhizoids generally extended from the middle of the pericentral cell. The consistency of the rhizoid–pericentral cell connection character within the North Carolina species makes it ideal for species identifications and exploring their relationships.

Cortication (character 3)

The presence or absence of cortication was determined for all but three North Carolina samples in this study. This character was consistent in all but one species, *Polysiphonia breviarticulata*, where cortication was present in three, absent in five, and undetermined in one of the samples examined (Table 3). There appears to be some relationship between the presence or absence of cortication and the mode of attachment in the North Carolina samples. Species with specimens that were attached by a prostrate branching system were ecorticate throughout, while those that were attached by a single basal holdfast had cortication. Cortication may also become more obvious as the plant matures, and the absence of cortication in some studied North Carolina samples may be age related.

Kudo & Masuda (1986) found that the level of cortication varied based on whether the plants of *Polysiphonia japonica* and *Polysiphonia akkeshiensis* were cultured and if the cultured plants were forming reproductive structures. They also observed weakly developed cortical cells in the lower segments of the main axis of reproductive plants in wild populations.

Relationship of lateral branches to trichoblasts (character 4)

The relationship of lateral branches and trichoblasts (Figs 8–10) was not observed in all samples, but where this

Table 1. Collection information, sample number/source, and GenBank accession numbers for Rhodomelaceae investigated in this study. Identical accession numbers indicate specimens with identical sequences.

Species	Sample number/source	Collection location, date, and collector	Accession number
Neosiphonia harveyi (Bailey) Kim, Choi, Guiry & Saunders[1]	McIvor *et al.* (2001)	Wilmington, NC, USA 01.vii.98, DW Freshwater	AF342906
	McIvor *et al.* (2001)	Skerries, Dublin, Ireland 30.viii.92, CA Maggs	AF342898
	NC-06	Banks Channel, New Hanover Co., NC, USA 34°11.785′N, 77°48.515′W, 30.i.05, DW Freshwater & B Stuercke	EU492909
	NC-10	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 04.v.05, DF Kapraun, DW Freshwater & B Stuercke	EU492909
	NC-13	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 04.v.05, DF Kapraun, DW Freshwater & B Stuercke	EU492909
	NC-16	Ludens Creek, New Hanover Co., NC, USA 34°10.131′N, 77°49.755′W, 11.v.05, DW Freshwater, B Stuercke & K Braly	EU492909
	NC-17	Bogue Sound, Corkey's house, Carteret Co., NC, USA 34°43.427′N, 76°49.749′W, 26.iii.05, DW Freshwater	EU492909
	NC-19	Bogue Sound, Corkey's house, Carteret Co., NC, USA 34°43.427N, 76°49.749′W, 26. iii.05, DW Freshwater	EU492909
	NC-22	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 19.v.05, DW Freshwater, B Stuercke & K Braly	EU492909
	NC-23	Wrightsville Beach, New Hanover Co., NC, USA 34°12.618′N, 77°47.805′W, 19.v.05, B Stuercke	EU492909
	NC-31	Howard's Channel, Topsail inlet, Pender Co., NC, USA 34°21.151′N, 77°38.857′W, 07.vii.05, DW Freshwater, B Stuercke & R Hammer	EU492909
Neosiphonia japonica (Harvey) Kim & Lee[2]	McIvor *et al.* (2001)	Akkeshi, Hokkaido, Japan 24.vi.93, K Kogame	AF342901
	McIvor *et al.* (2001)	Oshoro, Hokkaido, Japan 01.vii.93, T Abe	AF342902
	McIvor *et al.* (2001)	Shimoda, Honshu, Japan 05.ix.93, CA Maggs	AF342903
'*Neosiphonia*' *elongella* (Harvey) Kim & Lee[3]	McIvor *et al.* (2001)	Pwllheli, Cardigan, Wales 20.viii.98, CA Maggs	AF342913
Polysiphonia atlantica Kapraun & JN Norris	NC-04	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 30.viii.04, DW Freshwater	EU492910
	NC-26	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 12.v.05, J Souza, D Wells & S Hall	EU492910
	NC-27	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 09.vi.05, DW Freshwater & J Souza	EU492910
	NC-28	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 09.vi.05, DW Freshwater & J Souza	EU492910
	NC-32	CORMP site OB-3, 5-mile ledge, Onslow Bay, NC, USA 34°06.129′N, 77°45.049′W, 11.vii.05, DW Freshwater & B Stuercke	EU492910
Polysiphonia breviarticulata (C. Agardh) Zanardini	NC-01	Sneads Ferry-New River Inlet area, Onslow Co., NC, USA 34°33.021′N, 77°20.358′W, 11.vii.03, DW Freshwater & F Montgomery	EU492911
	NC-03	CORMP Site OB-1, Onslow Bay, NC, USA 34°18.639′N, 77°03.010′W, 19.vii.04, DW Freshwater & K Johns	EU492911
	NC-05	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 12.i.05, J Souza & J Dorton	EU492911
	NC-07	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 12.i.05, J Souza & J Dorton	EU492911
	NC-14	Banks Channel, New Hanover Co., NC, USA 34°11.785′N, 77°48.515′W, 11.v.05, DW Freshwater	EU492911
	NC-15	Ludens Creek, New Hanover Co., NC, USA 34°10.131′N, 77°49.755′W, 11.v.05, DW Freshwater, B Stuercke & K Braly	EU492911
	NC-18	Bogue Sound, Corkey's house, Carteret Co., NC, USA 34°43.427′N, 76°49.749′W, 26.iii.05, DW Freshwater	EU492911
	NC-20	Bogue Sound, Corkey's house, Carteret Co., NC, USA 34°43.427′N, 76°49.749′W, 26.xii.03, DW Freshwater	EU492911
	NC-25	CORMP Site OB-27, Onslow Bay, NC, USA 33°59.646′N, 77°21.144′W, 12.v.05, J Souza	not sequenced
	NC-29	Bogue Sound, East end of Emerald Isle, Carteret Co., NC, USA 34°40.974′N, 76°55.463′W, 23.vi.05, Coll: D.W. Freshwater	EU492911

Table 1. Continued

Species	Sample number/source	Collection location, date, and collector	Accession number
Polysiphonia brodiei (Dillwyn) Sprengel	McIvor *et al.* (2001)	Portaferry, Down, N. Ireland 20.iii.98, CA Maggs	AF342916
Polysiphonia denudata (Dillwyn) Greville ex Harvey	McIvor *et al.* (2001)	Plymouth, Devon, England 30.ix.98, F Bunker	AF342914
P. '*denudata*' *sensu* Kapraun[4]	NC-02	Cassamir Wreck (WR2), Onslow Bay, NC, USA 33°57.878′N, 77°01.715′W, 02.vii.04, DW Freshwater & K Johns	EU492912
	NC-30	Mouth of North River, Carteret Co., NC, USA 34°42.511′N, 76°36.936′W, 23.vi.05, DW Freshwater	EU492912
	FL-04	Sebastian Inlet, Indian River Co., FL, USA 26.ii.05, Freshwater, R York, K Braly, R Hammer & B Stuercke	EU492912
Polysiphonia elongata (Dillwyn) Harvey	McIvor *et al.* (2001)	Fanad, N. Donegal, Ireland 17.v.98, CA Maggs	AF342911
Polysiphonia fibrata (Dillwyn) Harvey	McIvor *et al.* (2001)	Marble Hill, N. Donegal, Ireland 05.viii.93, CA Maggs	AF342915
Polysiphonia fibrillosa (Dillwyn) Sprengel	McIvor *et al.* (2001)	Marble Hill, N. Donegal, Ireland 05.viii.93, CA Maggs	AF342912
Polysiphonia forfex Harvey	McIvor *et al.* (2001)	Biarritz, Aquitaine, SW France 15.vii.99, CA Maggs	AF342910
Polysiphonia fucoides (Hudson) Greville	NC-12	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 04.v.05, DF Kapraun, DW Freshwater & B Stuercke	EU492913
Polysiphonia lanosa (Linnaeus) Tandy	GO420	Pt. Lepreau, New Brunswick, Canada 07.x.95, GW Saunders	EU492914
Polysiphonia morrowii Harvey	Kim *et al.* (2004)	Dolsando, Yeosu, Korea	AY396034
Polysiphonia pacifica Hollenberg	Kim *et al.* (2004)	Seal Rock, Oregon, USA	AY396036
Polysiphonia paniculata Montagne	Kim *et al.* (2004)	Seno Otway, Punta Arenas, Chile	AY396041
Polysiphonia scopulorum Harvey	Kim *et al.* (2004)	Devil's Punchbowl, Oregon, USA	AY396039
P. scopulorum var. *villum* (J. Agardh) Hollenberg	NC-09	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 04.v.05, DF Kapraun, DW Freshwater & B Stuercke	EU492915
	NC-33	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 22.vii.05, DW Freshwater, R York, K Braly, R Hamner & B Stuercke	EU492915
Polysiphonia stricta (Dillwyn) Greville	Kim & Yang (2005)	Flambourough, Yorkshire, England	AY958167
	GWS2657	Letete Pt., New Brunswick, Canada 14.iii.05, GW Saunders	EU492916
Polysiphonia strictissima Hooker & Harvey	McIvor *et al.* (2001)	Titahi Bay, Wellington, New Zealand 03.v.93, W Nelson	AF342908
Polysiphonia subtilissima Montagne	NC-21	Neuse River, Oriental, Pamlico Co., NC, USA ix.03, R Peterson	EU492917
	NC-24	Snow's Cut, New Hanover Co., NC, USA 34°03.510′N, 77°54.008′W, 22.v.05, B Stuercke & JB Landry	EU492918
	HI-01	Reed's Bay, Hilo, Hawai'i, Hawaiian Islands 17.v.04, B Stuercke	EU492919
Polysiphonia '*urceolata*' (Lightfoot) Greville *sensu* Kapraun[4]	NC-11	South Masonboro Inlet Jetty, New Hanover Co., NC, USA 34°10.800′N, 77°49.355′W, 04.v.05, DF Kapraun, DW Freshwater & B Stuercke	EU492920
Polysiphonia sp.	Kim *et al.* (2004)	Las Cruses, Central Chile	AY396038
Polysiphonia sp.	Kim & Yang (2005)	Oshoro, Hokkaido, Japan	AY958168
Bostrychia simpliciuscula Harvey ex J. Agardh[5]	Zuccarello *et al.* (2002)	Sydney, New South Wales, Australia	AF458710
Polyzonia elegans Suhr		Palm Beach, KwaZulu-Natal, South Africa 07.ii.01, DW Freshwater	EU492921
Wrightiella tumanowiczii (Gatty ex Harvey) Schmitz		CORMP site Anchor Ledge, Onslow Bay, NC, USA 33°44.426′N, 77°14.486′W, 30.vi.04, DW Freshwater & M Bailey	EU492922

[1] McIvor *et al.* (2001) sequences published as *Polysiphonia harveyi* Bailey.
[2] McIvor *et al.* (2001) sequences published as *Polysiphonia akkeshiensis* Segi and *Polysiphonia japonica* Harvey.
[3] Sequence published as *Polysiphonia elongella* Harvey.
[4] As described by Kapraun (1977a, 1980a) for the western Atlantic.
[5] Zuccarello *et al.* (2002) sequence published as *Bostrychia tenuissima* King & Puttock.

character was observed, there was consistency within the North Carolina species (Table 3). Four of the eight species had samples with lateral branches replacing trichoblasts. *Polysiphonia denudata sensu* Kapraun and *P. breviarticulata* both had samples where lateral branches formed in the axil of the trichoblasts. The lateral branch was independent of the trichoblasts in *Polysiphonia scopulorum* var. *villum* and *Polysiphonia subtilissima*.

This character has been used in many descriptions of *Polysiphonia* (Hollenberg 1942; Abbott & Hollenberg 1976;

Table 2. Characters and character states observed for the North Carolina *Polysiphonia sensu lato* samples.

Character no.	Character description and character states
1	number of pericentral cells: four (0), five to seven (1), exceeding eight (2)
2	rhizoid–pericentral cell connection: open (0), pit connected (1)
3	cortication: present (0), absent (1)
4	relationship of lateral branches to trichoblasts: branches replacing trichoblasts (0), branches forming in axil of trichoblasts (1), branches independent of trichoblasts (2)
5	adventitious laterals: present (0), absent (1)
6	shape of adventitious laterals: linear (0), lanceolate (1), triangular (2)
7	vegetative unpigmented trichoblasts: present (0), absent (1)
8	number of segments between trichoblasts: every segment (0), not every segment (1)
9	apical cell: conspicuous (0), inconspicuous (1)
10	apical cell division: transverse (0), oblique (1)
11	holdfast: Thallus erect, arising from single basal holdfast (0), erect branches initially arising from basal rhizoids, sometimes becoming prostrate (1), erect branches arising from prostrate branching system (2)
12	branching pattern: alternate (0), subdicotomous (1), irregular (2), simple (3), secund (4)
13	shape of ultimate branches: linear (0), lanceolate (1), fractiflexus (2)
14	shape of ultimate branch apices: acute (0), narrowly acute (1), widely acute (2)
15	frequency of branching: not highly branched (0), highly branched (1), moderately branched (2)
16	scar cells: present (0), absent (1)
17	scar cell pattern: none (0), spiral (1)
18	scar cells producing lateral branches (cicatrigenous branching): present (0), absent (1)
19	shape of cystocarp: globose (0), subglobose (1), obovate (2), ovate (3), ovatus latibasis (4), ovalis (5), urceolate (6)
20	development of spermatangial axes: replacing whole trichoblasts (0), forming as a furacation of trichoblasts (1)
21	number of sterile cells present on spermatangial branch: none (0), none to one (1), two or more (2)
22	arrangement of tetrasporangia: in a straight series (0), in a spiral series (1)

Kapraun 1977a; Womersley 1979, 2003; Schneider & Searles 1991; Maggs & Hommersand 1993), but the way this character has been identified has not been clear. Ideally, early stages of branch formation, before trichoblasts have detached from the thallus and the branch–trichoblast relationship is lost, can be observed, but these stages are often absent because of the deciduous nature of the trichoblasts in many species. In the current study, the relationship of lateral branches and trichoblasts was determined when trichoblasts had dropped off before branch initiation by looking at the first axial cell of each lateral branch. A second pit connection that led to the trichoblasts was present at the distal end of these cells when branches formed in the axil of the trichoblasts (Figs 8, 9). If the branch replaced the trichoblasts, then this second pit connection was absent (Fig. 10). It is still necessary to observe this character in young portions of thalli, as the residual pit connection that leads to the trichoblasts becomes indistinct over time.

Adventitious laterals (characters 5 and 6)

Hollenberg (1942) considered all branches in *Polysiphonia* to be adventitious because they do not form in constant positions in regular intervals with respect to the segments. Kudo & Masuda (1986) in their study on *P. japonica* and *P. akkeshiensis* observed two types of adventitious laterals: branches that originated endogenously from the axial cell of lower segments and branches that originated exogenously from scar cells. Adventitious laterals that arise from scar cells are referred to as cicatrigenous (Hollenberg 1942). Branches were considered adventitious in the present study if they did not originate from lateral branch initials produced as part of the growing apices.

Adventitious laterals were observed in 20 of the 31 North Carolina samples and were present in six of the eight North Carolina species (Table 3). This character was consistent within all but *N. harveyi* and *P. denudate sensu* Kapraun, where adventitious laterals were present in seven of the nine samples and one of three samples, respectively. The majority of *N. harveyi* plants producing tetrasporangia also were producing laterals; however, this correlation was not absolute.

The shape of adventitious laterals was variable within all North Carolina species (Table 3). In general, the shape of adventitious laterals varied from linear to lanceolate, with one sample displaying a triangular shape. This character also seemed to vary based on the developmental stage of the adventitious branch. Younger adventitious branches tended to be lanceolate and became more linear as they matured. This character was too variable to be used to distinguish species.

Trichoblasts (characters 7 and 8)

Trichoblasts are almost always nonpigmented, seldomly unbranched with typically two to four furcations, and are generally lost from segments further from the apices (Womersley 1979). Abbott (1999) described trichoblasts in *Polysiphonia* as being deciduous and leaving scar cells on some or all segments. Trichoblasts have been reported to be abundant in some species, whereas in others this feature is exceedingly rare, and in some cases they occur only in connection with reproductive structures (Hollenberg 1942; Hollenberg & Norris 1977; Kim & Lee 1996; Kim *et al.* 2000).

Trichoblasts were observed in all but three of the North Carolina samples, but the abundance of trichoblasts varied among the samples in this study. Trichoblasts were generally abundant in samples of *N. harveyi*, *P. breviarticulata*, *P. fucoides*, and *P. urceolata sensu* Kapraun; whereas, samples of the remaining four species had

relatively few trichoblasts. The three samples that lacked trichoblasts were within *Polysiphonia atlantica* and *P. scopulorum* var. *vilium*, two of the species that displayed a low abundance of trichoblasts. In addition to the frequency of trichoblasts, there appeared to be a relationship between plants that are reproductive and the presence of trichoblasts. Two of the three samples that lacked trichoblasts and only 2 of the 28 samples that had tichoblasts were not reproductive.

Hollenberg (1942) stated that trichoblasts in *Polysiphonia* typically arise in definite positions with one trichoblast per segment. The two character states that have been described in the literature were whether trichoblasts were present on every segment or whether there were naked segments between trichoblasts (Hollenberg 1968a, b; Womersley 1979; Stegenga *et al.* 1997).

The number of segments between trichoblasts was consistent within each of the eight North Carolina species and displayed the same pattern when mapped on molecular trees as the arrangement of tetrasporangia. Plants that had trichoblasts or scar cells on every segment also had tetrasporangia arranged in a spiral series. Conversely, plants that lacked trichoblasts or scar cells on every segment had tetrasporangia arranged in a straight series. Kapraun (1977a) had previously observed this same relationship of trichoblasts and tetatrasporangia in nine North Carolina *Polysiphonia* species, and studies of other species have also found the same relationship (Hommersand 1963; Kim *et al.* 2000).

Apical cells (characters 9 and 10)

All but one North Carolina sample showed conspicuous apical cells, and all the samples in this study displayed transverse apical divisions (Table 3). *Polysiphonia fucoides* was the only North Carolina species to have inconspicuous apical cells, and this character was difficult to observe because the tips of this plant were crowded with trichoblasts and epiphytes. Only one *P. fucoides* sample, consisting of only one specimen, was collected, and apical cells may be more conspicuous in other specimens.

All examined North Carolina samples showed transverse apical divisions, in contrast to the report of oblique apical divisions in some species studied by Choi *et al.* (2001). All of the species resolved in their ''*Polysiphonia* group'' had oblique apical cell divisions as well as one sample within their ''*Neosiphonia* group.'' It is not clear how this character has been identified previously, and this may be the reason for the difference in the current observations and those of Choi *et al.* (2001).

Apical cell division was defined in the current study by the division of only the apical cell and not the cells below. Slightly oblique divisions of apical cells were sometimes observed, but clearly transverse divisions were also present on the same thalli. Strongly oblique divisions were often observed in the cells immediately below the apical cell, and nonuniform expansion of these subtending cells during formation of the apical cells of lateral initials tends to displace apical cells, giving the appearance of oblique apical divisions.

Nature of the holdfast (character 11)

Kapraun (1977a) described three types of holdfasts and prostrate development in *Polysiphonia*: (1) plants initially erect from a discoid base but forming secondary attachments with decumbent branches, (2) plants initially with a horizontal prostrate system derived from an erect apex, and (3) plants consisting of a horizontal prostrate system and apex, giving rise to erect exogenous branches. In the current study, samples were split into three categories: (1) thallus erect, arising from a single basal holdfast; (2) erect branches initially arising from basal rhizoids, sometimes becoming prostrate; and (3) erect branches arising from a prostrate branching system.

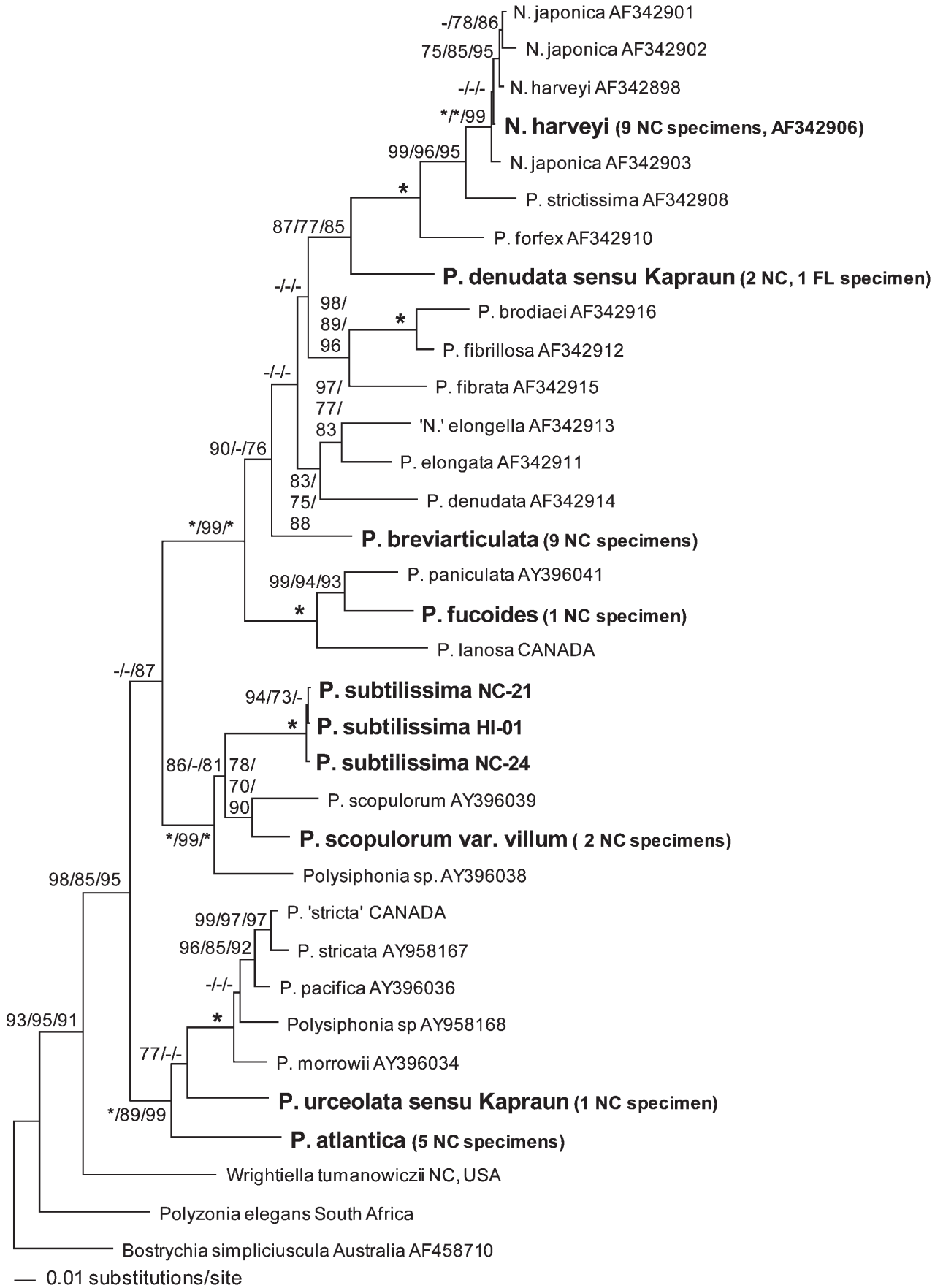
The nature of the holdfast was determined in 28 of the 31 samples examined and found to be consistent in seven of the eight species (Table 3). *Polysiphonia breviarticulata* varied in the nature of the holdfast with samples in this species having all three character states. The variation seen in this species may be a result of the samples being collected from a variety of locations and environments; whereas, samples of all other species were collected either from the same location or from similar environments. Further study is needed to determine if the nature of the holdfast is consistent within a majority of species or is under some form of environmental control.

Branching pattern, shape and frequency (characters 12, 13, and 15)

Branching pattern, shape of ultimate branches, and frequency of branching were variable characters for the samples in this study (Table 3). The branching patterns of the samples were consistent in only half of the North Carolina species. The species that displayed variation had character states that ranged from alternate to subdicotomous to irregular. The most variation in character states was observed in species that contained a large number of samples. Those species with a small number of samples tended to be more consistent, making it difficult to separate out sample size and variation for this character.

The shape of the ultimate branch varied in all of the North Carolina species. The most consistency was observed in *P. denudata sensu* Kapraun and *P. subtillissima*, in which branches only varied from linear to lanceolate. The variation in this character may be a result of the age of the plant or where the plant was growing and does not appear to be helpful for identifying species.

Frequency of branching in North Carolina species appeared consistent in half of the species, but the consistency observed might be a result of sampling size since these species were represented by at most three samples. The remaining four North Carolina species each had one sample that did not agree with the other samples found within these species. The samples in disagreement were either found as an epiphyte or growing in distinctly different environments than the other samples. For example, in the samples of *P. denudata sensu* Kapraun, one sample was growing on a buoy within a sound; whereas, the other sample was collected from a wreck at a depth of 36 m.


N. japonica AF342901
-/78/86
75/85/95
N. japonica AF342902
-/-/-
N. harveyi AF342898
//99
N. harveyi (9 NC specimens, AF342906)
99/96/95
N. japonica AF342903
*
P. strictissima AF342908
87/77/85
P. forfex AF342910
P. denudata sensu Kapraun (2 NC, 1 FL specimen)
-/-/-
98/
89/
96
*
P. brodiaei AF342916
P. fibrillosa AF342912
-/-/-
97/
77/
83
P. fibrata AF342915
'N.' elongella AF342913
90/-/76
P. elongata AF342911
83/
75/
88
P. denudata AF342914
/99/
P. breviarticulata (9 NC specimens)
99/94/93
P. paniculata AY396041
*
P. fucoides (1 NC specimen)
P. lanosa CANADA
-/-/87
94/73/-
P. subtilissima NC-21
*
P. subtilissima HI-01
86/-/81
78/
70/
90
P. subtilissima NC-24
P. scopulorum AY396039
/99/
P. scopulorum var. villum (2 NC specimens)
Polysiphonia sp. AY396038
98/85/95
99/97/97
P. 'stricta' CANADA
96/85/92
P. stricata AY958167
-/-/-
P. pacifica AY396036
93/95/91
*
Polysiphonia sp AY958168
77/-/-
P. morrowii AY396034
P. urceolata sensu Kapraun (1 NC specimen)
*/89/99
P. atlantica (5 NC specimens)
Wrightiella tumanowiczii NC, USA
Polyzonia elegans South Africa
Bostrychia simpliciuscula Australia AF458710
0.01 substitutions/site

Apices of ultimate branches (character 14)

All observed apices were acute to some degree. Only four out of the eight species were consistent for this character (Table 3). Kim & Lee (1999) described a difference between the branch apices of *Polysiphonia* and *Neosiphonia* species. The apices of *Neosiphonia* they called round-pointed; whereas, in *Polysiphonia* they refer to the apices as sharp-pointed. In the current study, apices that were termed narrowly acute would fall under the Kim & Lee (1999) *Polysiphonia* type; whereas, those that were widely acute would better match their *Neosiphonia* type. However, the shape of ultimate branch apices was not consistent for samples within the North Carolina species. The differences between narrowly acute, acute, and widely acute apices may be subtle and subjective, making this character a difficult one to score. The North Carolina species that contained only one or two samples were consistent, but this was probably more a reflection of sample size than the nature of the character.

Scar cells (characters 16, 17, and 18)

Womersley (1979) described scar cells as the basal cell of a trichoblast that remains embedded between the pericentral cells after the trichoblast has been shed (Figs 11, 12). Because scar cells are developmentally derived from trichoblasts, the occurrence and pattern of both have been referred to together in most taxonomic keys for *Polysiphonia* (Hollenberg 1968b; Abbott & Hollenberg 1976; Womersley 1979, 2003; Schneider & Searles 1991; Abbott 1999). Scar cells typically are persistent but do vary in their conspicuousness based on their location away from apices (Hollenberg 1942; Womersley 1979).

Scar cells were found in all but two of the 31 examined samples, and these two samples also lacked trichoblasts (Table 3). Both samples were within multiple sample species where all other samples had scar cells. When scar cells were present, their pattern was consistent in all species (Table 3). Three species had scar cells in a spiral pattern; the remaining five species displayed no distinct pattern. The presence or absence of scar cells was not useful in distinguishing among North Carolina species, but the pattern of these scar cells was found to be informative. Scar cell pattern of North Carolina species showed a correlation with the arrangement of tetrasporangia. Species that had tetrasporangia arranged in a straight series had scar cells that were not arranged in a specific pattern; whereas, the species that had tetrasporangia arranged in a spiral series also had scar cells similarly arranged.

The production of branches from scar cells (Fig. 13) could be scored with certainty in 23 of the 31 samples, and, where scored, it was consistent within the species (Table 3). Two species comprised of multiple samples had branches that arose from scar cells. The remaining species had no scar cells with branches, or this character could not be confidently determined from the available material. The presence or absence of scar cells producing lateral branches, also known as cicatrigenous branching (Hollenberg 1942), was correlated with the scar cell pattern. Species that had scar cells in a spiral pattern had branches that were arising from them, whereas species that did not have a scar cell pattern also did not have cicatrigenous branches.

Fertile material (characters 19, 20, 21, and 22)

Species within the Rhodomelaceae show a wide variety of vegetative types but are surprisingly uniform in reproductive development (Dawson 1966). In this family, cystocarps form a distinct pericarp before fertilization (Scagel 1953; Maggs & Hommersand 1993; Womersley 2003). Thirteen of the 31 North Carolina samples studied included female gametophytes with cystocarps (Table 3). The cystocarps of *Polysiphonia* species have been reported to differ slightly in shape. The shape of cystocarps observed in the North Carolina samples ranged from globose to ovate to oval to urceolate or any combination of these shapes.

The variation in these shapes among all the North Carolina samples made it impossible to determine a shape that is species specific. Only cystocarps containing carposporangia were included in these observations to reduce any variation in shape because of cystocarp maturity. Part of the variation seen may be a result of sample preparation, both in preserving the sample and in preparing the sample for microscopic work. Further observations using fresh material are needed to determine how much of the observed variation is due to sample preparation.

Spermatangia have been reported to develop on modified trichoblasts, either arising from the entire trichoblast primordium or subtended by a trichoblast branch (Abbott & Hollenberg 1976; Schneider & Searles 1991; Maggs & Hommersand 1993; Womersley 2003). Only seven North Carolina samples had spermatangia present, making it difficult to determine the consistency of this character within species. The development of spermatangial axes was consistent in the one species where six of the nine samples contained male gametophytes.

Spermatangial development (Figs 14, 15) has been used in previous studies as a reliable character for species designation (Hollenberg & Norris 1977; Womersley 1979, 2003; Schneider & Searles 1991; Maggs & Hommersand 1993; Choi *et al.* 2001). Choi *et al.* (2001) also considered spermatangial development to be a diagnostic character for their groups of species. Their ''*Polysiphonia* group'' had spermatangia that replaced the whole trichoblast; whereas, spermatangia developed on a trichoblast furcation in the other two groups of species that they recognized.

The number of sterile cells present at the tips of spermatangial branches has been considered to be a useful character in species identification (Kim *et al.* 1994; Kim & Lee 1996). This character may be difficult to determine

←

Fig. 1. Maximum likelihood *rbc*L tree (lnL = −10596.06117) for 54 *P. sensu lato* and three outgroup samples. Bootstrap proportion values for branches are shown from distance, parsimony, and maximum-likelihood analyses, respectively. * represents bootstrap values of 100%, and – represents values <70%.

Table 3. Data matrix of character states of anatomical characters for North Carolina *Polysiphonia sensu lato* samples explored in this study. Morphological characters determined to be useful in identification are marked by an asterisk.

Species	Sample no.	Morphological characters and character states																					
		1*	2*	3	4*	5	6	7*	8*	9	10	11*	12	13	14	15	16*	17*	18*	19	20*	21	22*
Neosiphonia harveyi	NC-06	0	1	?	0	1	?	0	0	0	0	0	0/1	0	0	1	0	1	0	1/3	1	1	?
	NC-10	0	1	0	0	0	0/1	0	0	0	0	0	0/1	1	2	1	0	1	0	1/5	1	1	1
	NC-13	0	1	0	0	0	0/1	0	0	0	0	0	0	0/1	0	1	0	1	0	0/3	1	1	1
	NC-16	0	1	0	0	0	1	0	0	0	0	0	0/1	0	0	1	0	1	0	5	1	1	1
	NC-17	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0/3/6	?	?	1
	NC-19	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	?	?	?	1
	NC-22	0	1	0	0	0	0/1	0	0	0	0	0	0	0	0	1	0	1	0	3/5	1	0	?
	NC-23	0	1	?	0	1	?	0	0	0	0	0	0	0	0	0	0	1	?	?	?	?	?
	NC-31	0	1	0	0	0	0/1	0	0	0	0	0	0	0	2	1	0	1	?	4	1	1	1
Polysiphonia atlantica	NC-04	0	0	1	0	1	?	1	?	0	0	2	0	1	1	0	1	?	?	?	?	?	0
	NC-26	0	0	1	?	1	?	1	?	0	0	2	0	0/1	0	0	0	?	?	?	?	?	?
	NC-27	0	0	1	0	1	?	0	1	0	0	2	0	0/1	1	2	0	0	1	?	?	?	0
	NC-28	0	0	1	0	1	?	0	1	0	0	2	0	0/1	1	0	0	0	1	?	?	?	?
	NC-32	0	0	1	0	1	?	0	1	0	0	2	0	0	1	0	0	0	1	?	?	?	0
Polysiphonia breviarticulata	NC-01	0	1	1	?	0	0	0	0	0	0	?	1/2	?	?	?	0	1	?	?	?	?	1
	NC-03	0	1	1	1	0	0	0	0	0	0	2	0/1	0	0	1	0	1	0	?	?	?	1
	NC-05	0	1	0	1	0	0	0	0	0	0	1	0	1/2	0	1	0	1	0	3	?	?	1
	NC-07	0	1	0	1	0	0/1	0	0	0	0	2	1	1	0	1	0	1	0	?	1	0	1
	NC-14	0	?	1	1	0	1	0	0	0	0	?	0	1	0	1	0	1	0	?	?	?	1
	NC-15	0	1	1	1	0	0	0	0	0	0	0	0	1/2	0	1	0	1	0	0/5	?	?	1
	NC-18	0	1	?	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	?	?	?	1
	NC-20	0	1	0	1	0	1	0	0	0	0	?	0	0/1	1	1	0	1	0	3/6	?	?	1
	NC-29	0	1	1	1	0	0	0	0	0	0	2	0	0/1	0	0	0	1	0	?	?	?	1
Polysiphonia denudata sensu Kapraun	NC-02	1	1	1	1	1	?	0	1	0	0	1	1/2	0/1	1	0	0	0	1	0/2	?	?	?
	NC-30	1	1	1	1	1	?	0	1	0	0	1	0/1	0/1	1	1	0	0	?	?	?	?	0
	FL-04	1	1	1	1	0	0	0	1	0	0	1	0	0/1	1	2	0	0	1	?	?	?	?
Polysiphonia fucoides	NC-12	2	1	0	0	0	0/1	0	1	1	0	1	0	0/1	0	1	0	1	?	3/5	?	?	?
Polysiphonia scopulorum var. *villum*	NC-09	0	0	1	2	1	?	0	1	0	0	2	0/4	1	1	2	0	0	1	?	?	?	0
	NC-33	0	0	1	2	1	?	1	?	0	0	2	3	?	2	2	1	?	?	?	?	?	?
Polysiphonia subtilissima	NC-21	0	0	1	2	0	2	0	1	0	0	2	0	0/1	1	2	0	0	1	?	?	?	0
	NC-24	0	0	1	?	0	0	0	1	0	0	2	0	0/1	1	2	0	0	1	?	?	?	?
	HI-01	0	0	1	2	0	0	0	1	0	0	2	0	0/1	1	2	0	0	1	?	?	?	?
Polysiphonia urceolata sensu Kapraun	NC-11	0	0	1	0	0	1	0	1	0	0	2	0	0/1/2	1	1	0	0	1	0	?	?	0

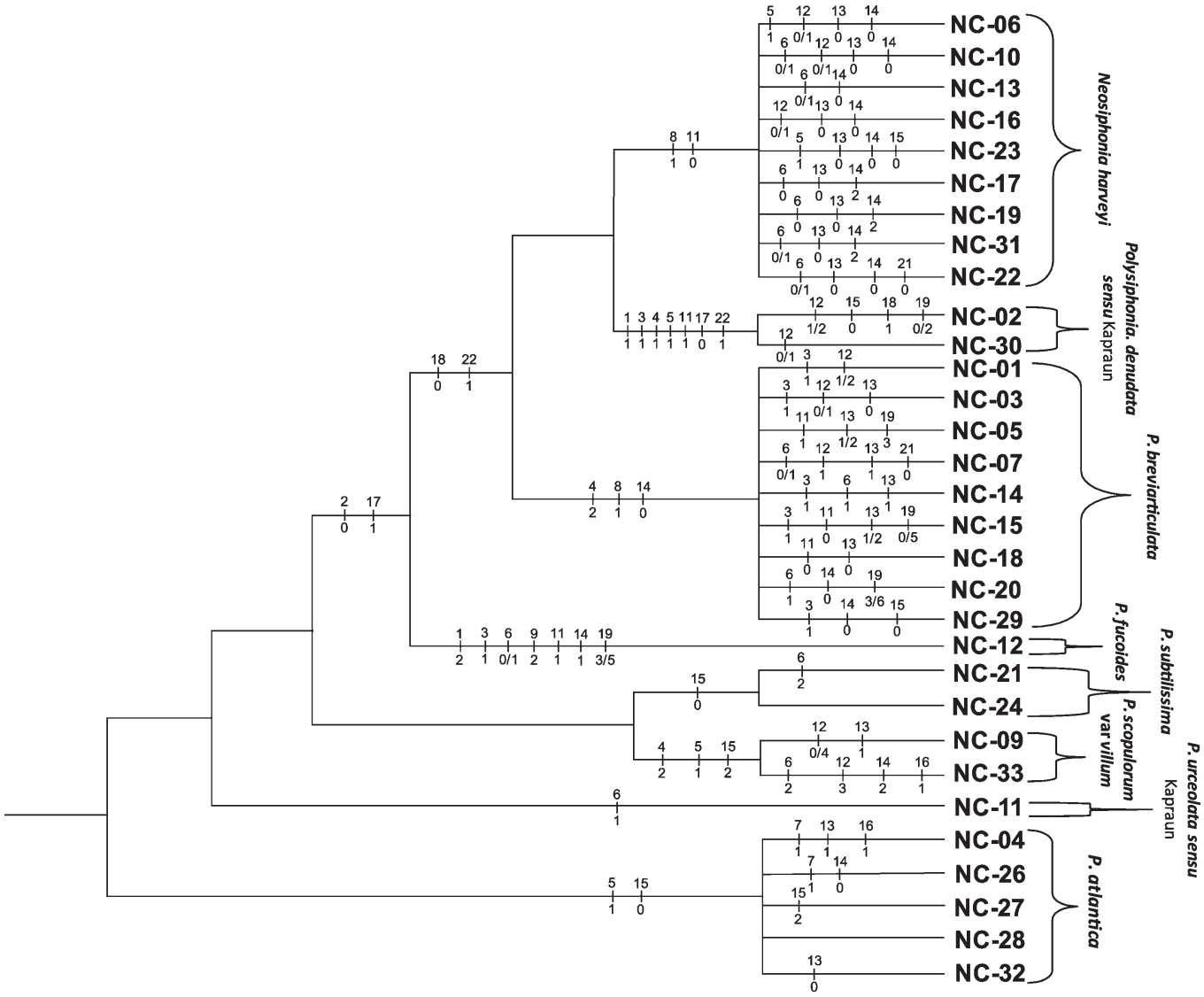


Fig. 2. North Carolina *P. sensu lato* character state changes mapped on a Fig. 1–based topology of the studied specimens. Vertical bars represent character state changes. Character numbers (Table 2) are shown above bars, and new character states (Table 3) are shown below bars.

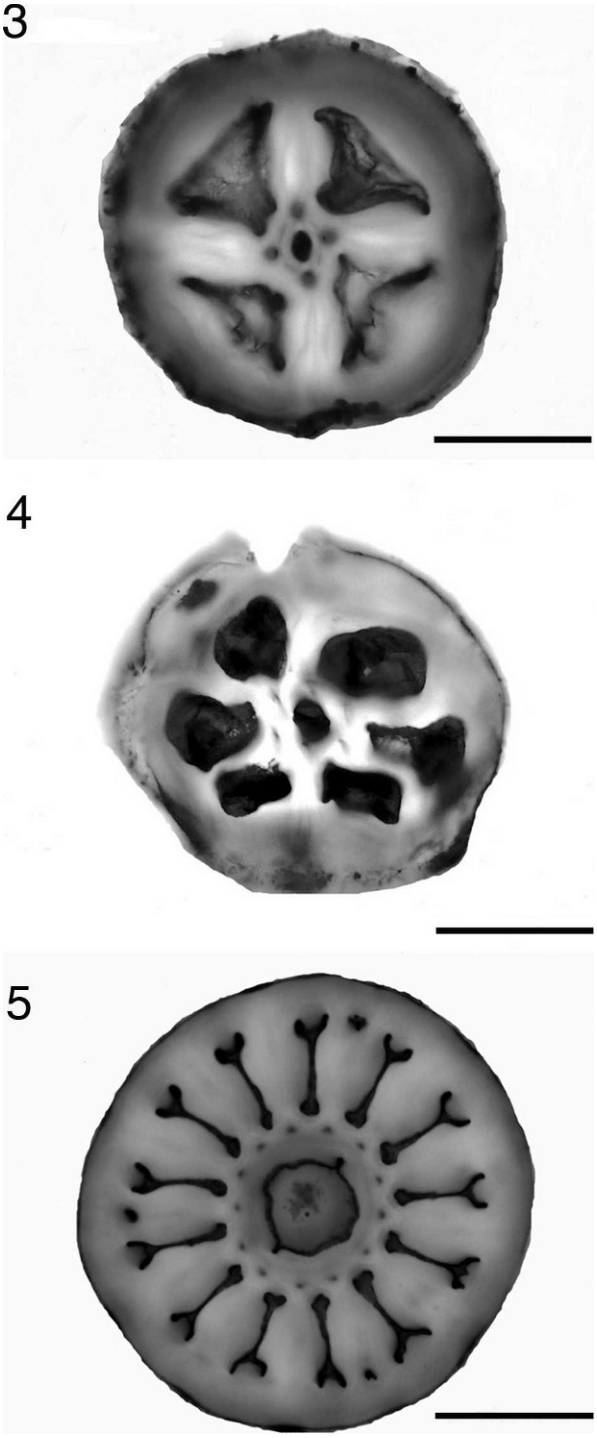
because the number of sterile cells present will depend on the maturity of the spermatangia. Cells that appear to be sterile at the tip of developing spermatangial branches will often become spermatangial mother cells in the mature spermatangia. Examination of this character in the North Carolina samples was not informative because of the lack of spermatangial plants.

The arrangement of tetrasporangia (Figs 16–18) was a consistent character within North Carolina samples and species. Two of the eight species had samples with spiral tetrasporangia. The remaining species had tetrasporangia arranged in a straight series (Table 3). Tetrasporangia arranged in a spiral series appeared to form from different pericentral cells in each successive segment; whereas, tetrasporangia in straight series appeared to be forming from the same pericentral cell in each segment. Occasionally a short series of straight tetrasporangia will become offset from those in adjacent segments, resulting in a somewhat spiral or mixed spiral–straight appearance. However, observations of the pericentral cells in these specimens show that the whole segment has shifted, and the tetrasporangia are developing from the same pericentral cell.

Tetrasporangial arrangement has been considered a good character for species identifications and also for distinguishing groups of related species. Choi *et al.* (2001) found that only species in their ''*Polysiphonia* group'' possessed straight tetrasporangia; whereas, their ''*Neosiphonia* group'' and ''multipericentral group'' both had spiral tetrasporangia.

DISCUSSION

Taxonomic studies of *Polysiphonia* have used the following characters to distinguish between species: number of pericentral cells, position of trichoblasts and scar cells,

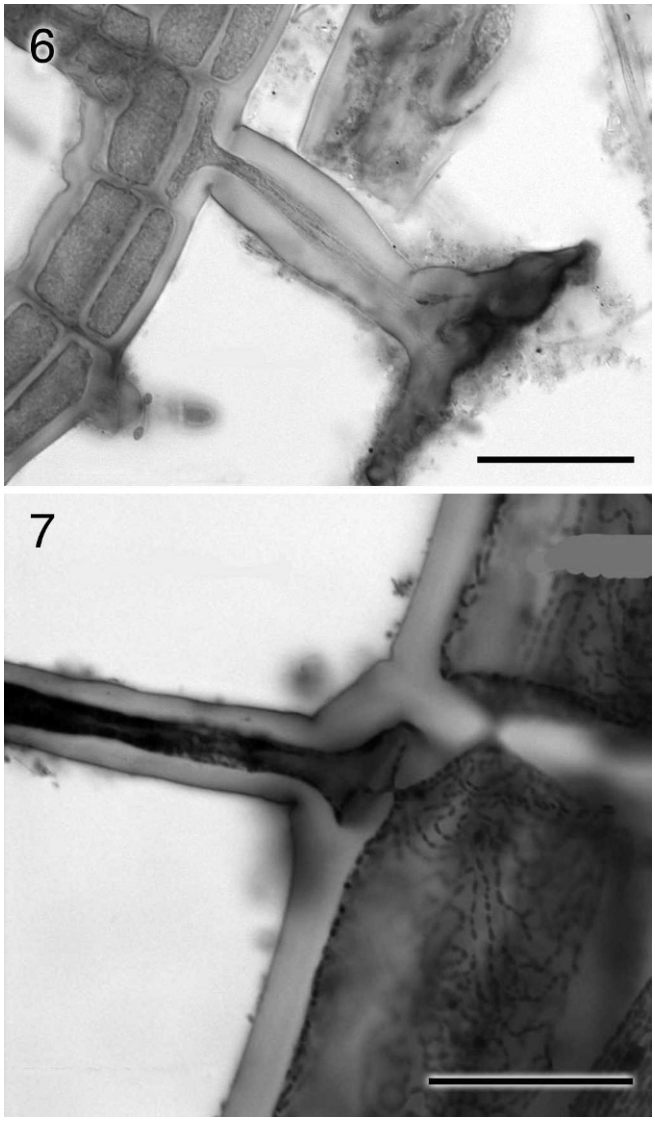


Figs 3–5. Cross sections of branch axes showing central axial cell and surrounding pericentral cells.

Fig. 3. Sample with four pericentral cells, *P. breviarticulata*, Poly NC-05, scale = 100 μm, WNC2005-S061.

Fig. 4. Sample with six pericentral cells (five to seven pericentral cell category), *P. denudata sensu* Kapraun, Poly NC-02, scale = 100 μm, WNC2005-S051.

Fig. 5. Sample with 13 pericentral cells (more than eight pericentral cells category), *P. fucoides*, Poly NC-12, scale = 100 μm, WNC2005-S097.

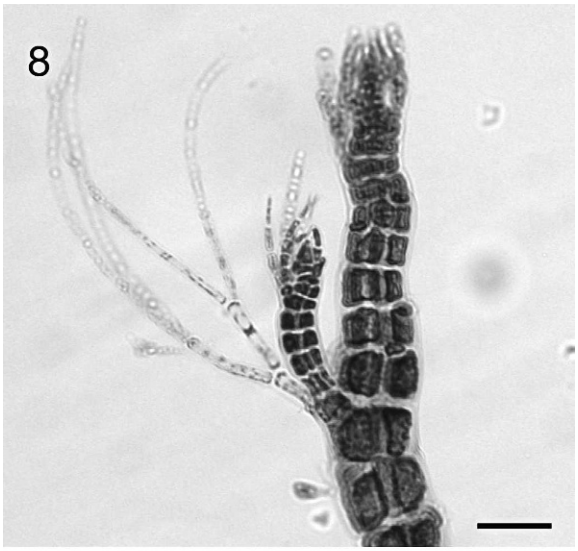


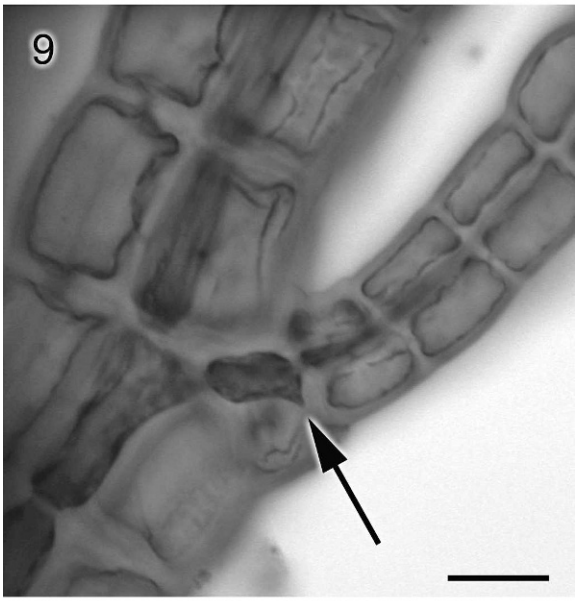
Figs 6, 7. Connections between rhizoids and pericentral cells.

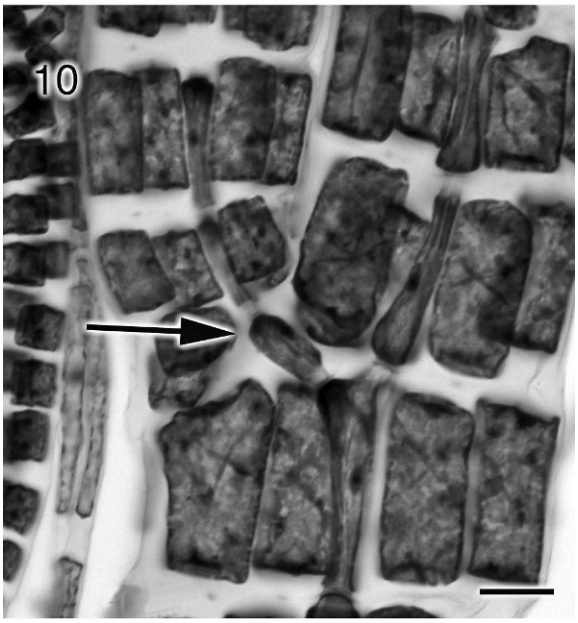
Fig. 6. Sample with an open connection between rhizoid and pericentral cell, *P. atlantica*, Poly NC-04, scale = 50 μm, WNC2005-S057.

Fig. 7. Sample with rhizoids that are cut off from the pericentral cell (i.e. pit connected), *P. denudata sensu* Kapraun, Poly NC-02, scale = 100 μm, WNC2005-S047.

origin of branches and their relationship to trichoblasts, position of the spermatangial branches in relation to tricoblasts, number of sterile cells on spermatangial branches, type of holdfast, point of origin of rhizoids, length/width (L/W) ratio of segments, extent of cortication, and number of carpogonial branch cells (Hollenberg 1942, 1968a; Hollenberg & Norris 1977; Womersley 1979; Kim *et al.* 1994; Kim & Lee 1996; Choi *et al.* 2001; Curiel *et al.* 2002). Of the 22 morphological characters observed in North Carolina *Polysiphonia* species, five were found to be consistent within the studied species and useful for their identification: number of pericentral cells; presence or absence of a pit connection between the rhozoid and its bearing pericentral cell; whether lateral branches develop independent of trichoblasts, in the axils of trichoblasts, or







replace them; whether spermatangia develop on a furcation of trichoblasts or replace them; and whether tetrasporangia are arranged in a spiral or straight series. The consistency of spermatiangial branch development was difficult to determine in this study because there were few samples with male specimens. However, it is considered to be consistent based on the limited sampling here, documentation of this character in the literature, and our finding that a previous report of multiple states for this character (Kapraun 1979) actually involved more than one species. The distinctions among species can be further strengthened by looking at additional characters, not all of which were uniformly consistent. These include the pattern and occurrence of trichoblasts and scar cells, the type of holdfast, and the formation of cicatrigenous branches.

Two morphological features that have not been discussed but that have been considered important were the L/W ratio of segments and the number of carpogonial branch cells (e.g. Hollenberg 1942; Womersley 1979; Kim & Lee 1999). Segment L/W ratios were variable within the species included in this study, even when the measurements were restricted to the base, middle, or tips of main axes (data not shown). These results are similar to those of Kim *et al.* (1994) and Curiel *et al.* (2002), who found that L/W ratios were too variable to identify species. Carpogonial branches of *P. sensu lato* species have been reported to be typically composed of four cells, but three-celled carpogonal branches have been observed (Hommersand & Fredericq 1990). Kim & Lee (1999) and Choi *et al.* (2001) found that carpogonial branches with three cells were a main morphological difference distinguishing *Neosiphonia* from *Polysiphonia* species. Specifically, the procarp structure in *Neosiphonia* includes a three-celled carpogonial branch with a two-celled and a single-celled sterile group also arising from the supporting cell (Kim & Lee 1999). In Rhodomelaceae, the pericarp forms before fertilization (Hommersand 1963), making it difficult to observe the number of carpogonial branch cells. The small number of female specimens observed in this study had cystocarps that were either too mature or did not stain in a manner allowing the determination of carpogonial branch cell number.

Although the morphological character analyses were restricted to North Carolina species, the findings of this study can probably be applied in studies of *P. sensu lato* from other regions. Further investigations of character

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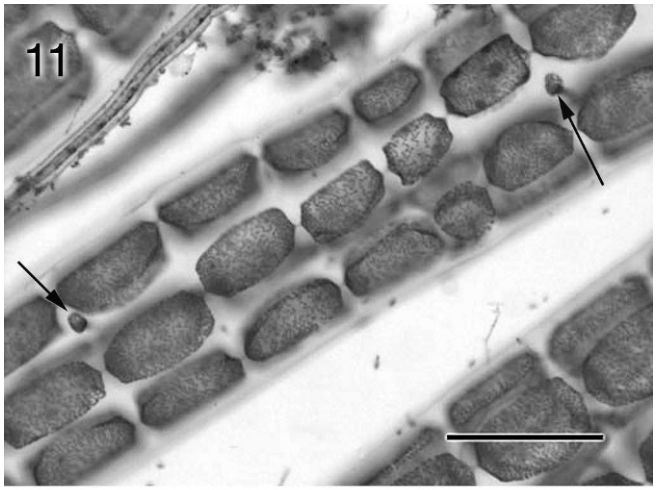
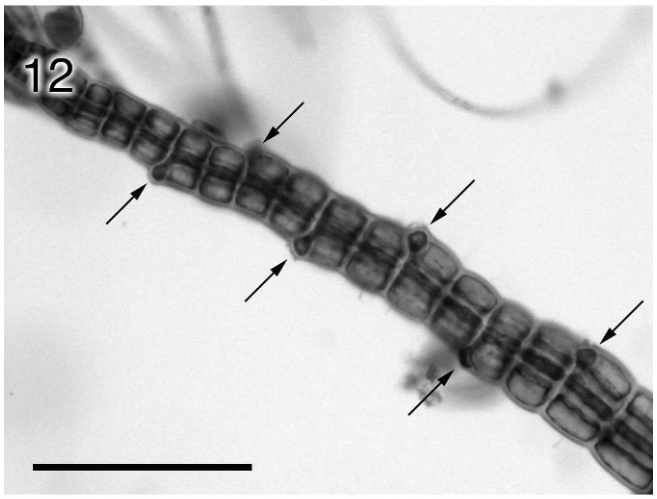
Figs 8–10. Relationship of lateral branches and trichoblasts in *Polysiphonia* samples showing that branches either replace or form in the axil of trichoblasts.

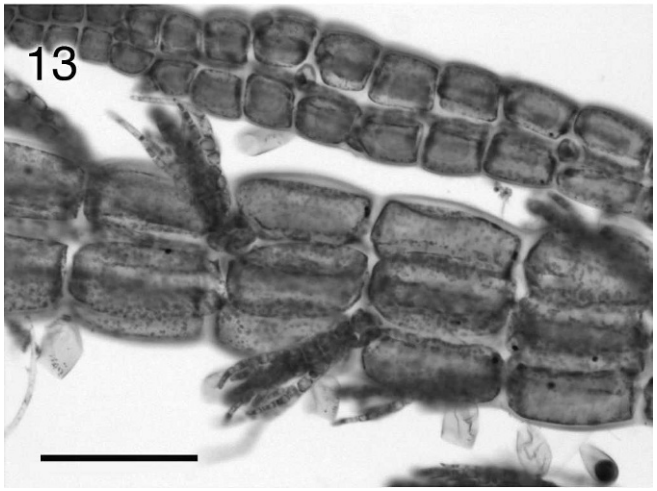
Fig. 8. Apex of main axis showing lateral branch forming in axil of trichoblast, *P. breviarticulata*, Poly NC-05, scale = 50 μm, WNC2005-S059.

Fig. 9. Close-up of axial cell filament leading to lateral branch, showing first axial cell of lateral branch with pit connection (arrow) to basal (scar) cell of trichoblast, *P. breviarticulata*, Poly NC-15, scale = 20 μm, WNC2005-S027.

Fig. 10. Example of branch replacing trichoblast, showing first axial cell of lateral branch (arrow) with no extra pit connection, *P. urceolata sensu* Kapraun, Poly NC-11, scale = 20 μm, WNC2005-S089.





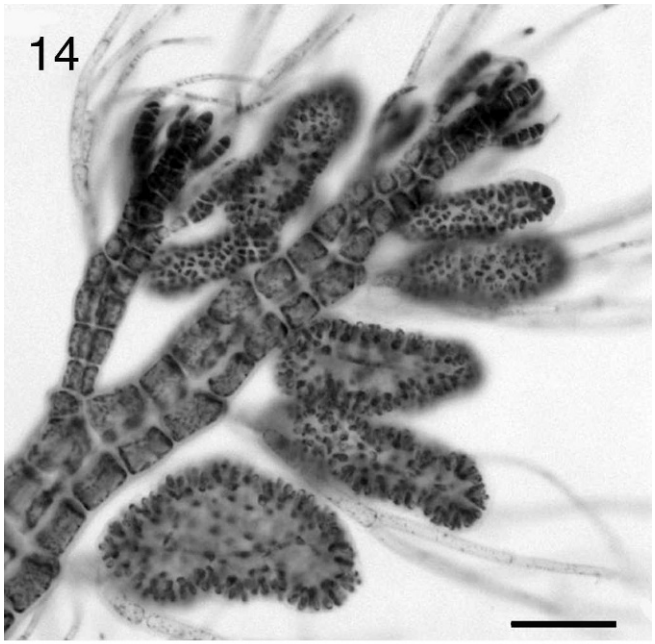


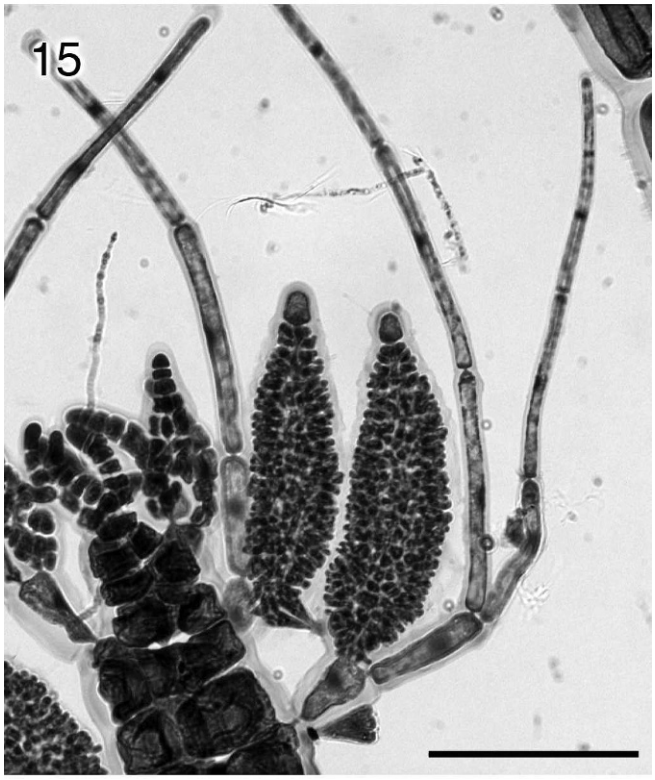
Figs 11–13. Patterns of scar cells and cicatrigenous branching observed in *Polysiphonia* samples.

Fig. 11. *Polysiphonia denudata sensu* Kapraun with scar cells (arrows) showing no pattern, Poly NC-02, scale = 100 μm, WNC2005-S049.

Fig. 12. Branch showing scar cells (arrows) in spiral series, *P. breviarticulata*, Poly NC-15, scale = 50 μm, WNC2005-S027.

Fig. 13. Main axis with adventitious laterals arising from scar cells (cicatrigenous branching), *P. breviarticulata*, NC-18, scale = 100 μm, WNC2005-S031.



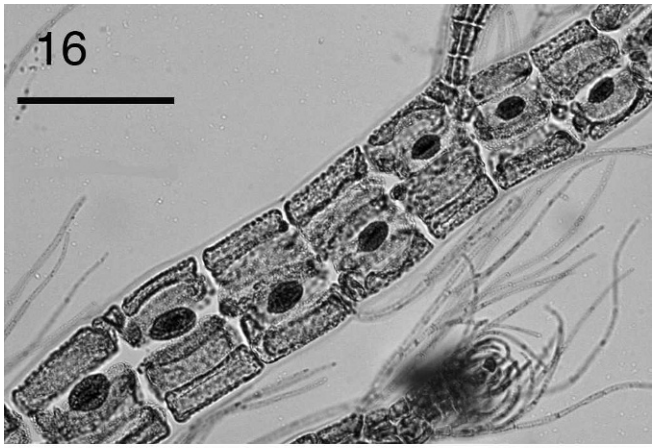


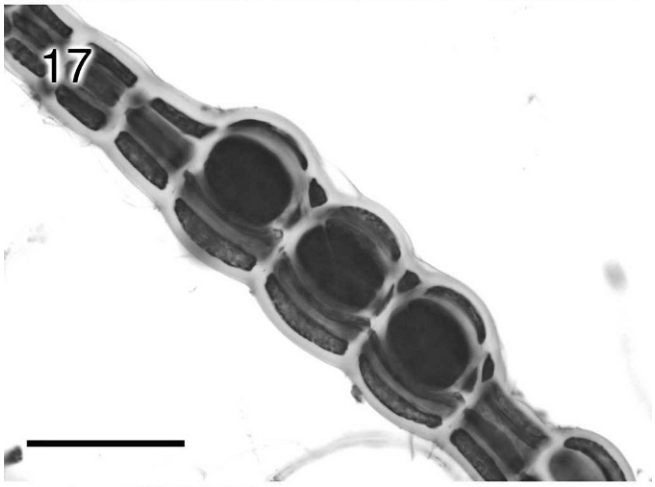
Figs 14, 15. Spermatangial axes development observed in *Polysiphonia* samples.

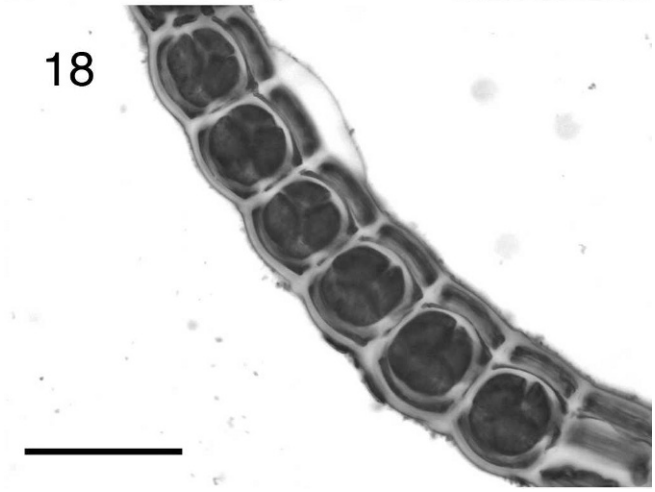
Fig. 14. Branch apex with spermatangia replacing a furcation of trichoblasts; no sterile cells present at apices of spermatangia, *P. breviarticulata*, Poly NC-07, scale = 50 μm, WNC2005-S067.

Fig. 15. Spermatangia replacing a furcation of trichoblasts; one sterile cell at each apex, *N. harveyi*, Poly NC-06, scale = 100 μm, WNC2005-S063.

consistency in objectively defined species are needed to verify these results. A complete understanding of within-species character consistency is needed to fully understand the evolutionary relationships of *P. sensu lato* species.







Figs 16–18. Arrangement of tetrasporangia observed in *Polysiphonia* samples.

Fig. 16. Tetrasporangia arranged in spiral series, *P. breviarticulata*, Poly NC-25, scale = 100 μm, WNC2005-S127.

Fig. 17. Tetrasporangia arranged in short, straight series, *P. scopulorum* var. *villum*, Poly NC-09, scale = 100 μm, WNC2005-S072.

Fig. 18. Reproductive branch displaying long, straight series of tetrasporangia, *P. subtilissima*, Poly NC-21, scale = 100 μm, WNC2005-S118.

Taxonomic consideration and phylogenetic relationships of North Carolina species

Integrated molecular and morphological analyses of eight North Carolina *P. sensu lato* species found that two have been historically misidentified and require additional study. *Polysiphonia denudata* was originally described from Southampton, England, and northeast Atlantic specimens exhibit the following character states: basal cortication present, numerous trichoblasts that are found on every segment, thalli arising from single basal holdfasts, and tetrasporangia arranged in a spiral series (Maggs & Hommersand 1993). In contrast, the species identified as *P. denudata* from the southeastern United States is reported to have no to slight cortication, trichoblasts that are not on every segment, thalli that initially arise from a basal holdfast but sometimes becoming prostrate, and tetrasporangia in straight series (Kapraun 1977a, 1980a; Schneider & Searles 1991). Three of these characters are ones determined to be useful for species identifications in this study.

British *P. denudata* and *P. denudata sensu* Kapraun were also distinct in *rbc*L sequence analyses (Fig. 1). McIvor *et al.* (2001) generated a sequence for a *P. denudata* specimen from Plymouth, England, that is resolved in a clade with British specimens of *P. elongata* and *Neosiphonia elongella*. Sequences of *P. denudata sensu* Kapraun from two North Carolina and one Florida specimens were identical and resolved in an isolated position within the *rbc*L tree. Kapraun (1978a) considered North Carolina *P. denudata* (= *P. denudata sensu* Kapraun) to have a warm temperate floristic affinity because of its response to culture conditions, the growth and reproductive limitations observed within its North American range, and its geographic distribution. He consequently suggested that *P. denudata* may consist of ecologically different populations through its extensive latitudinal range. The current molecular and morphological analyses indicate that *P. denudata sensu* Kapruan is a distinct species from British *P. denudata* and not simply an ecological variant.

A second species needing taxonomic attention is *P. urecolata sensu* Kapruan. *Polysiphonia urceolata*, originally described from England and designated as the lectotype of the genus *Polysiphonia* (Silva 1952), was placed in synonymy under *P. stricata* by Maggs & Hommersand (1993). Kapraun (1979) found morphological and physiological differences among three north Atlantic populations of *P. urecolata*. North Carolina *P. urceolata* (= *P. urecolata sensu* Kapruan) formed spermatangia on a furcation of the trichoblasts and rarely produced sterile cells, New England *P. urceolata* spermatangia sometimes formed on a furcation but had a number of sterile cells present, and Norwegian *P. urceolata* (= *P. stricta*) spermatangia replaced trichoblasts and produced one to four sterile cells. The North Carolina isolate also differed physiologically from the New England and Norway isolates. The latter two responded to temperature regimes 5° cooler than the North Carolina isolate and despite longer incubation times never achieved the vegetative development seen in the North Carolina isolate. Although Kapraun referred to the North Carolina isolate as *P. urceolata*, he indicated that this identification was complicated by the number of varietal forms described

for the species (Kapraun 1977a) and that the status of the North Carolina isolate as an ecotype of *P. urceolata* or a distinct species remained to be determined (Kapraun 1979).

The sequence data collected in this study, as well as previous morphological and physiological data (Kapraun 1979, 1980a, b), indicate that North Carolina *P. urecolata sensu* Kapruan is a distinct species from *P. stricta*. Analyses of *rbc*L sequences resolve both *P. stricta* and *P. urecolata sensu* Kapruan in a well-supported clade of *Polysiphonia* species; however, these two species are well separated and clearly distinct within this clade (Fig. 1).

There are 992 species names listed for *Polysiphonia* in AlgaeBase, but only 230 of these names are considered to be current (Guiry & Guiry 2007). A review of the 230 currently accepted names has not revealed a morphological match for either *P. denudata sensu* Kapraun or *P. urecolata sensu* Kapruan, and consequently these two species require new names and descriptions that are being prepared for another publication.

Polysiphonia harveyi is currently regarded as *N. harveyi*, and this transfer was proposed by Choi *et al.* (2001) based on molecular analyses and morphological characters. *Polysiphonia harveyi*, *Neosiphonia japonica*, and *Neosiphonia savatieri* were found to have identical SSU sequences and to share a number of morphological character states, including four pericentral cells, pit-connected rhizoids, three-celled carpogonial branches, development of spermatangial axes on a furcation of the trichoblasts, and tetrasporangia arranged in a spiral series (Choi *et al.* 2001). Kim & Lee (1999) proposed the segregation of *Neosiphonia* from *Polysiphonia* based on these same morphological characters, and they transferred 11 species in Korea that were previously regarded as *Polysiphonia* to *Neosiphonia*. The genus *Neosiphonia* has been generally accepted, and several papers have been published using this new classification (Choi *et al.* 2001; Masuda *et al.* 2001; Abbott *et al.* 2002; Kim & Abbott 2006). The reassessment of species in Malaysia (Masuda *et al.* 2001), Vietnam (Abbott *et al.* 2002), and Hawai'i (Kim & Abbott 2006) has resulted in the transfer of seven additional species to *Neosiphonia* so that the genus is currently comprised of 19 species. Morphological and molecular sequence data generated for *N. harveyi* in this study agreed with Kim & Lee's (1999) *Neosiphonia* classification and resolved this species within clades comprised of other currently recognized *Neosiphonia* species.

Polysiphonia breviarticulata is considered to be a Mediterranean species with its type locality in the Adriatic Sea, and until a 1982 collection in North Carolina, this species was unreported in the western Atlantic (Kapraun & Searles 1990). *Polysiphonia breviarticulata* remained inconspicuous until the late spring of 1988, when it was reported in bloom proportions along 200 km of the North Carolina and South Carolina coasts (Kapraun & Searles 1990). One of the samples in the current study was collected from a bloom that took place in the intracoastal waterway near the mouth of the New River. Small *P. breviarticulata* blooms of restricted geographic range continue to occur in North Carolina waters, although the extensive bloom of 1988 has not been repeated.

Distinguishing characteristics of *P. breviarticulata* are its abundant adventitious laterals and enlarged ostiolar lip cells. All the samples collected in this study had adventitious laterals, and in samples that were cystocarpic we observed the presence of enlarged ostiolar lip cells. This species has been reported to vary in size, from 3 to 12 cm in height, and the specimens collected in this study also varied greatly in size. This species was collected from numerous sites in North Carolina and can be found from intertidal creeks in the state's sounds to deep offshore waters.

Polysiphonia fucoides was originally described from the British Isles and is morphologically variable throughout its geographic range (Schneider & Searles 1991; Maggs & Hommersand 1993). It is reported to reach its western Atlantic southern distribution limit in the Carolinas. Kapraun (1977a) verified the occurrence of *P. fucoides* in southeastern North Carolina, but reports of this species in South Carolina have been questioned (Schneider & Searles 1991).

The descriptions of *P. fucoides* report that lateral branches replace trichoblasts (Kapraun 1980a; Schneider & Searles 1991; Maggs & Hommersand 1993), but this character state was difficult in the North Carolina sample observed in this study. There were apparent branches that formed in the axils of the trichoblasts, but further investigation revealed that these were adventitious laterals and not primary laterals. This species also has been reported to have adventitious laterals forming in the axils of primary branches (Maggs & Homnmersand 1993), and this character state was observed in the current study. The *rbc*L data resolved *P. fucoides* in a highly supported and distinct clade with two species of *Polysiphonia*, *P. paniculata* and *P. lanosa*, that also have numerous pericentral cells (10–12 and 12–24, respectively; Fig. 1).

Kapraun & Norris (1982) abandoned the name *Polysiphonia macrocarpa* Harvey, which was widely applied to this neotropical species because it was a later homonym of *P. macrocarpa* (C. Agardh) Sprengel, and chose the new name, *P. atlantica*, for this widespread species. Kapraun (1977a) collected specimens of *P. atlantica* in North Carolina from floating docks and jetties during the summer months and reported that this species reached maximum growth from June through September. In the current study, samples of this species were only collected offshore. There have been previous reports of *P. atlantica* in North Carolina's offshore waters (Schneider 1976), but these specimens differed from the inshore *P. atlantica* specimens in important morphological characters (Schneider & Searles 1991). Schneider (1976) described spiral tetrasporangia and numerous adventitious laterals for his offshore collections, but Schneider & Searles (1991) later decided that these samples did not represent the same species as the inshore populations of *P. atlantica*. The offshore samples collected in the present study share the same character states reported for the inshore *P. atlantica*, with the exception of male gametophytes, which were not seen.

Kim & Lee (1996) reported that *P. atlantica* shared many important morphological features with other species of *Polysiphonia*, such as *P. subtilissima*, *P. abscissa*, *P. morrowii*, *P. pacifica*, and *P. pungens*. Analyses of the sequence data generated in the current study resolved *P.*

atlantica within a strongly supported clade including *P. stricta*, *P. pacifica*, and *P. morrowii* and supports the proposed relationships between these species suggested by the morphological data.

Polysiphonia subtilissima has been reported as a brackish-water species (Kapraun 1980b; Schneider & Searles 1991; Abbott 1999), and all the samples collected in this study were found in brackish-water environments. Male gametophytes have been observed only in cultures (Schneider & Searles 1991), and the samples collected in the present study also lacked spermatangia. Analyses of *rbc*L sequences from the North Carolina samples and one collected in Hawai'i determined that these samples represented the same species and suggested that it has a widespread distribution. However, Schneider & Searles (1991) stated that there was some question about the conspecificity of this taxon throughout its reported distribution because samples collected in Brazil differed in their spermatangial axes development from cultured North Carolina samples. The Brazil plants had spermatangia that developed as a furcation of the trichoblasts (Oliveira 1969); whereas, the North Carolina isolate had spermatangia that replaced the trichoblasts (Kapraun 1980b). The morphological differences observed suggest that taxa identified as *P. subtilissima* may need further examination.

Hollenberg (1968a) recognized four varieties of *P. scopulorum* that were described from Pacific specimens, including *P. scopulorum* var. *villum*. The sequence data generated in this study showed that the western Atlantic *P. scopulorum* var. *villum* was a distinct species from the northwest Pacific *P. scopulorum*. The northwest Pacific sample was collected as part of a study assessing the introduction of another *Polysiphonia* to Chile (Kim *et al.* 2004), but it was not clear which of Hollenberg's varieties this specimen represents. In order for the exact relationship of the Pacific and western Atlantic *P. scopulorum* var. *villum* to be understood, more analyses of Pacific *P. scopulorum* and its varieties need to be completed.

Morphological and molecular analyses conducted in this study distinguished eight of the 15 reported species of North Carolina *P. sensu lato*. Previous studies have reported a similar finding; for example, Kapraun (1977a) collected only nine of the 13 *Polysiphonia* species recorded from North Carolina at that time over the four years of his study. Seasonal and annual variations in North Carolina *Polysiphonia* species have been previously reported. *Polysiphonia ferulacea* was collected only during the latter years of Kapraun's intensive studies of North Carolina *Polysiphonia* species (Kapraun 1977b, 1978a, b), and *Polysiphonia howei* was only later collected by Kapraun (1980a); although, it had been previously reported from nearshore hard-bottom (Williams 1948) and inshore waters (Brauner 1975). Similarly, the introduced species *P. breviarticulata* was seldomly collected after its initial discovery in North Carolina waters until a widespread 1988 bloom of this species received regional attention because of its effect on the local fishing and tourist industries (Kapraun & Searles 1990). Although small localized blooms of *P. breviarticulata* continue to occur periodically, and it was encountered frequently in this study, the species shows wide seasonal and annual variations. Consistent seasonal collecting from a variety of habitats will be required to completely sample North Carolina's *P. sensu lato* species.

Our findings were in agreement with Choi *et al.* (2001) who found in their SSU analyses that *Polysiphonia* was paraphyletic and that the clades in their molecular trees were strongly supported by specific morphological characters. Additional molecular and morphological analyses will be needed in order to develop a natural classification system based on the evolutionary relationships of species within *P. sensu lato*.

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