AN INTEGRATED TAXONOMIC ASSESSMENT OF NORTH CAROLINA *POLYSIPHONIA* (CERAMIALES, RHODOPHYTA) SPECIES

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ABSTRACT

The rhodomelacean genus *Polysiphonia* Greville (Ceramiales, Rhodophyta) contains approximately 200 species that are distributed throughout the world's oceans. *Polysiphonia* species have been defined by the number of pericentral cells, presence or absence of cortication, nature and arrangement of trichoblasts, origin of branches in relation to trichoblasts, origin of attachment rhizoids, nature of spermatangial branches, number of carpogonial branch cells and arrangement of tetrasporangia. Most systematic studies of *Polysiphonia* have had a physiological, karyological, morphological, or reproductive basis, but very few have explored molecular characters. In this study, relationships among North Carolina *Polysiphonia* species were investigated using an integrated taxonomic approach. Thirty-one *Polysiphonia* samples were collected from various coastal and offshore environments. Analyses of *rbc*L were used to objectively delimit species collected in North Carolina and the consistency of 22 morphological characters used to identify *Polysiphonia* species was examined within eight molecularly defined species clades. Phylogenetically informative morphological characters for North Carolina *Polysiphonia* species included the number of pericentral cells, rhizoid-pericentral cell connection, relationship of lateral branches to trichoblasts, spermatangial axes development, and arrangement of tetrasporangia. The molecular and morphological analyses were used to identify eight North Carolina species, which were found to represent *Neosiphonia harveyi*, *Polysiphonia atlantica*, *P. breviarticulata*, *P. denudata sensu* Kapraun, *P. fucoides*, *P. scopulorum* var. *villum*, *P. subtilissima*, and *P. urceolata sensu* Kapraun. Both *rbc*L and SSU analyses were used to determine the relationship of North Carolina *Polysiphonia* species to those collected elsewhere, and these analyses resolved four major clades within *Polysiphonia sensu lato*. The character

states of characters found to be consistent within species were mapped on the *rbc*L and SSU trees to determine if there were phylogenetically informative morphological characters for the different molecularly defined clades. The clade containing the generitype, *P. stricta*, was supported by seven morphological characters considered to be taxonomically significant. Two of the resolved major clades had little morphological support, but these clades are probably comprised of heterogeneous mixtures of distinct species whose relationships could not be confidently resolved based upon the limited number of taxa investigated.

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DEDICATION

I would like to dedicate this thesis to my undergraduate mentor, Dr. Karla J. McDermid, who introduced me to the wonderful world of algae. Without her support and encouragement my interest in this amazing field would not have developed. She is an excellent teacher, role model and friend. Her ability to balance everyday life and her professional life has been inspiration to me.

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INTRODUCTION

Rhodophyta, or red algae, are a diverse group of eukaryotes including 4000 species. They are characterized by chlorophyll *a*, unstacked thylakoids in plastids, plastids containing the accessory pigments phycoerythrin, phycocyanin, and allophycocyanin arranged in phycobilisomes, the lack of chloroplast endoplasmic reticulum, pit connections between cells in filamentous genera and the absence of flagellated cells (Woelkerling, 1990; Lee, 1999). Red algae are predominately marine and have been reported in tropical, temperate, and cold-water regions. They are also found at greater depths (200 m) than any other algal class perhaps because of their accessory pigments (Lee, 1999).

Rhodophyta have traditionally been placed in a single class, the Rhodophyceae, containing two subclasses, Bangiophycidae and Florideophycidae. These two subclasses contain six and 19 orders, respectively (Woelkerling, 1990; Saunders & Hommersand, 2004). The dichotomy between subclasses Bangiophycidae and Florideophycidae is supported by nuclear conditions, plastid number, shape and location, as well as, differing patterns of cell division, pit connection, thallus complexity, sexual reproduction, and tetrasporangia (Ragan *et al.*, 1994).

There has been much debate upon the separation of these two subclasses because none of these morphological characteristics are absolute. Molecular analyses resolved the Florideophycidae as a monophyletic group, whereas Bangiophycidae is more likely polyphyletic (Freshwater *et al.*, 1994; Ragan *et al.*, 1994; Saunders & Bailey, 1997; Harper & Saunders, 2001a, b). The most recent work on higher red algal classification took a contemporary taxonomic perspective, by combining fossil and molecular evidence with recent phylogenetic knowledge, and established 25 orders (Saunders & Hommersand, 2004).

Ceramiales (Florideophycidae) exhibit filamentous growth and are generally delicate in form. Shared characteristics among these plants include four-celled carpogonial branches, and auxiliary cells that are cut off after fertilization and borne on the supporting cell of the carpogonial branch (Hommersand & Fredericq, 1990; Lee, 1999). Within this order there are four families, Ceramiaceae, Delesseriaceae, Dasyaceae, and Rhodomelaceae. It has been estimated that more half of the red algal species belong to the family Rhodomelaceae (Abbott, 1999).

Rhodomelaceae is comprised of members that differ greatly in vegetative structure but are uniform in reproductive development (Scagel, 1953; Dawson, 1966). Species within Rhodomelaceae are uniaxial and polysiphonious in structure. Rhodomelaceae differ from other families in the Ceramiales by the formation of pericentral cells in an alternating sequence (Scagel, 1953; Hommersand, 1963; Dawson, 1966). Other shared characteristics of this family are the 1) number of pericentral cells (four to 24); 2) presence of trichoblasts, either persistent and pigmented, which are typically associated with vegetative plants, or deciduous and colorless, which are typically associated with reproductive plants; 3) usually four-celled but sometimes three-celled carpogonial branches; 4) cystocarps with a distinct pericarp that forms before fertilization; and 5) tetrasporangia that generally form on pericentral cells (Scagel, 1953; Hommersand, 1963; Stegenga *et al*., 1997; Abbott, 1999; Womersley, 2003).

The focus of this study was the rhodomelaceaen genus *Polysiphonia* Greville, *nom. cons*., which comprises one of the largest red algal genera (Womersley, 1979; Choi *et al*. 2001) and includes species distributed throughout the world (e.g. Hollenberg, 1942; Kapraun, 1980a; Kapraun & Norris, 1982; Abbott, 1999; Kim *et al*., 2002).

Greville (1824) erected *Polysiphonia* for the plants named *Hutchinsia* C. Agardh (1817), a *nomen illegitimum* based on its prior application to a group of cruciferous plants. Greville did not designate a type 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 taxonomists have adopted the oldest available name, *P. stricta* (Dillwyn) Greville for *P. urceolata* (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).

Jacob Agardh (1863) divided *Polysiphonia* into four subgenera, *Pterosiphonia, Herposiphonia, Oligosiphonia,* and *Polysiphonia,* based on thallus size and listed 119 species in total (Kim & Lee, 1999; Kim *et al.*, 2000). Three of the four previously described subgenera are recognized today within Rhodomelaceae, but at the generic level (Kim & Lee, 1999). Hollenberg (1942, 1968a, b) separated species of *Polysiphonia* from the Pacific into two groups with no taxonomic designation, Oligiosiphonia for species that had four pericentral cells and Polysiphonia for species with more than four pericentral cells.

Taxonomy of *Polysiphonia*

When first described, *Polysiphonia* included virtually all obviously segmented plants now placed in Rhodomelaceae (Hollenberg, 1942). However, Falkenberg (1901) restricted *Polysiphonia* to species with the following diagnostic 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 is borne in each segment. *Polysiphonia* species

differ in the number of pericentral cells, cortication, nature and arrangement of trichoblasts and scar cells, origin of branches in relation to trichoblasts, attachment of the rhizoid, nature of the spermatangial branches, number of carpogonial branch cells, and arrangement of tetrasporangia (Hollenberg, 1942, 1968a, b; Hollenberg & Norris, 1977; Kapraun, 1980a; Kapraun & Norris, 1982; Schneider & Searles, 1991; Abbott, 1999; Kim *et al.*, 2002).

Generally, the following characters of *Polysiphonia* need to be present in order to properly identify species: number of pericentral cells, origin of the rhizoid, origin of branches in relation to trichoblasts, arrangement of the tetrasporangia, and nature of the spermatangial branches (Hollenberg, 1942, 1968a, b; Hollenberg & Norris, 1977; Kapraun, 1977, 1980a; Kapraun & Norris, 1982; Schneider & Searles, 1991; Abbott, 1999). Roughly 450 *Polysiphonia* species have been described since Greville erected the genus in 1824, although only about 190 of these are currently accepted (Kim *et al*., 2002).

Life History of *Polysiphonia*

Polysiphonia displays a triphasic life history, which includes a haploid gametophytic phase and two diploid sporophytic phases (Searles, 1980). Most Rhodomelaceae, including *Polysiphonia*, are dioecious. The male plants produce antheridial sori that either replace the whole trichoblast or form as a furcation of a trichoblast (e.g. Womersley, 1979). The spermatia lack flagella and are delivered to the egg cell (carpogonium) through water motion. The auxiliary cell is cut off from the supporting cell once the spermatia fertilize the carpogonium. The diploid nucleus is then transferred from the carpogonium to the auxiliary cell either by direct fusion (Yamanouchi, 1906; Kylin 1923; Broadwater & Scott, 1982) or a connecting cell (Hommersand & Fredericq, 1990). After transfer of the diploid nucleus, the auxiliary cell divides to form the

gonimoblast initial that will further develop and form the carposporangia. The carposporangia will release diploid carpospores that will develop into the diploid tetrasporophytic stage. The tetrasporophyte produces haploid tetraspores through meiosis that germinate and develop into the gametophytes (Yamanouchi, 1906; Searles, 1980; Lee, 1999).

Molecular Analyses of *Polysiphonia*

Many studies on *Polysiphonia* have been ecological, karyological, or reproductive (e.g. Kapraun, 1977, 1979; Kapraun & Searles, 1990; Kapraun, 1993); however, very few have explored molecular characters (Choi *et al*., 2001; McIvor *et al*., 2001; Kim *et al.*, 2004; Kim & Yang, 2005). Choi et al. (2001) examined relationships among *Polysiphonia* based on 28 anatomical features and small-subuint ribosomal DNA (SSU rDNA) sequence data for 25 ceramialean algae, including 14 species of *Polysiphonia*. *Polysiphonia sensu lato* was found to be paraphyletic, and divided among three clades supported by both anatomical and molecular data. Species in the three clades shared different combinations of four morphological features: the number of pericentral cells, origin of the rhizoid, arrangement of the tetrasporangia, and the number of cells in the carpogonial branch. The first clade was identified as "Type *Polysiphonia urceolata* and additional species of the genus *Polysiphonia*," species within this group had four pericentral cells, open connected rhizoids, four carpogonial branch cells, and tetrasporangia arranged in a straight series. Species of the second group, "multipercentral group including the genus *Vertebrata*," had more than five pericentral cells, pit connected rhizoids, four carpogonial branch cells, and tetrasporangia arranged in a spiral series. The last group, "*Neosiphonia* group," had four pericentral cells, pit connected rhizoids, three carpogonial branch cells, and tetrasporangia arranged in a spiral series.

McIvor et al. (2001) looked more specifically at one species within the genus *Polysiphonia*. They focused on *Polysiphonia harveyi*, which is an alien species in the British Isles and Atlantic Europe, and used sequences for the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) in conjunction with karyological and interbreeding data to examine the origin of this species in Europe. Their results indicated that *P. harveyi* originated from the northwestern Pacific and that there are at least two separate invasive lineages. The results of the *rbc*L analyses also revealed that *Polysiphonia* was paraphyletic, but in contrast to Choi *et al*. (2001), the examined species were resolved in two clades reflecting biogeographic rather than morphological differences.

Kim *et al.* (2004) used a combination of molecular and morphological data to identify *P. morrowii* and demonstrate its recent introduction to the southeastern Pacific Ocean (Chile). *Polysiphonia morrowii* is a common intertidal species in the northwest Pacific Ocean, but *rbc*L analyses resolved the sequence data from *Polysiphonia* species from Chile to be identical to the sequence data from samples of *P. morrowii* found in Korea. This species has also recently been introduced to the Mediterranean (Curiel *et al.*, 2002).

In the most recent molecular study of *Polysiphonia* species, Kim & Yang (2005) used *rbc*L analyses to determine relationships among varieties of *P. pacifica* and closely related species. *Polysiphonia pacifica* has been reported to have five varieties in the Pacific and this species is also morphologically similar to *P. stricta* and *P. morrowii*. Kim & Yang (2005) collected samples of *P. stricta*, *P. morrowii*, and two varieties of *P. pacifica* and used both morphological and molecular analyses to identify the species. The sequence data of the two varieties of *P. pacifica* were identical even though there were obvious morphological differences. In contrast, *Polysiphonia pacifica*, *P. stricta* and *P. morrowii* were resolved in a well supported

clade but were found to be distinct from one another despite their similar morphological characteristics.

Recent Taxonomic Reassessment of *Polysiphonia*

Kim and Lee (1999) carefully examined the vegetative and reproductive characters of several species of *Polysiphonia* from Korea and found that these species could be divided into two groups. They proposed a new genus *Neosiphonia* for one species group based on the following characteristics: lateral branch initials, including the trichoblats initials, produced on successive segments; erect indeterminate branches that develop from the main axes; rhizoids separated from pericentral cells by a cross wall; abundant trichoblasts; three-celled carpogonial branches; spermatangial branches that arise from a branch of the trichoblasts, and tetrasporangia arranged in a spiral series (Kim & Lee, 1999).

Polysiphonia, although similar in appearance, differs from *Neosiphonia* by having determinant and indeterminate branches that arise from an extensive creeping base, rhizoids that are in open connection with the pericentral cells, scarce or lacking trichoblasts, four-celled carpogonial branches, lateral branch initials that give rise to spermatangial branches and tetrasporangia that are in a straight series (Kim & Lee, 1999). Cystocarp shape may also separate the two genera, as they are commonly urceolate in *Polysiphonia* but are typically globose in *Neosiphonia* (Kim & Lee, 1999).

The type species designated for *Neosiphonia* is *N. flavimarina* Kim & Lee. Kim & Lee (1999) transferred eleven Korean species of *Polysiphonia* to *Neosiphonia* and ten of these changes have been accepted (Guiry *et al*., 2006). Since the separation of *Neosiphonia* from *Polysiphonia* several publications have been published accepting this new classification (Choi *et*

al., 2001; Masuda *et al*., 2001; Abbott *et al.*, 2002). Currently, *Neosiphonia* is comprised of 19 species (Guiry *et al*., 2006).

Objectives

Fifteen species of *Polysiphonia* have been reported from the North Carolina coast (Williams, 1948; Taylor, 1960; Brauner, 1975; Kapraun, 1977, 1980a; Schneider & Searles, 1991). In this study, the relationships of North Carolina *Polysiphonia* species were examined using an integrated taxonomic approach. The objectives of this study were to 1) objectively define North Carolina species of *Polysiphonia* using *rbc*L sequence data; 2) explore the consistency of characters that have been used in species identifications within the molecularly defined North Carolina species; 3) use *rbc*L and SSU analyses to compare the relationships of North Carolina species within *Polysiphonia sensu lato*; 4) determine shared character states for the *Polysiphonia* species clades resolved in the molecular analyses, and 5) identify the collected North Carolina species using both morphological and molecular data.

MATERIALS AND METHODS

Collection

Polysiphonia samples were collected from various locations in North Carolina, Florida and Hawai'i (Table 1). Samples were collected either intertidally or subtidally by snorkeling or SCUBA and were transported to the lab as fresh specimens in seawater or dried in silica gel desiccant (Chase & Hills, 1991). Specimens for morphological study were preserved in 5% formalin seawater solution buffered with borax and voucher slides were made as described in

Table 1. Collection information and loci sequenced for Rhodmelaceae investigated in this study.

Sample	Species	Collection location	*rbc*L	SSU
Poly NC-1	*Polysiphonia breviarticulata* (C. Agardh) Zanardini	Sneades Ferry-New River Inlet area, Onslow Co., NC 11.VII.03, Coll: D.W. Freshwater & F. Montgomery	x	
Poly NC-2	*P. denudata* (Dillwyn) Greville ex Harvey in W.J. Hooker *sensu* Kapraun (1977, 1980a)	Cassamir Wreck (WR2), Onslow Bay, NC 02.VII.04, Coll: D.W. Freshwater & K. Johns	x	x
Poly NC-3	*P. breviarticulata*	CORMP Site OB-1, Onslow Bay, NC 19.VII.04, Coll: D.W. Freshwater & K. Johns	x	
Poly NC-4	*P. atlantica* Kapraun & J.N. Norris	CORMP Site OB-27, Onslow Bay, NC 30.VIII.04, Coll: D.W. Freshwater	x	
Poly NC-5	*P. breviarticulata*	CORMP Site OB-27, Onslow Bay, NC 12.I.05, Coll: J. Souza & J. Dorton	x	x
Poly NC-6	*Neosiphonia harveyi* (J. Bailey) M.S. Kim, H.G. Choi, Guiry & G.W. Saunders	Banks Channel, floating dock, New Hanover Co., NC 30.I.05, Coll: D.W. Freshwater & B. Stuercke	x	x
Poly NC-7	*P. breviarticulata*	CORMP Site OB-27, Onslow Bay, NC 12.I.05, Coll: J. Souza & J. Dorton	x	
Poly NC-9	*P. scopulorum* var. *villum* (J. Agardh) Hollenberg	South Masonboro Inlet Jetty, New Hanover Co., NC 04.V.05, Coll: D.F. Kapraun, D.W. Freshwater & B. Stuercke	x	
Poly NC-10	*N. harveyi*	South Masonboro Inlet Jetty, New Hanover Co., NC 04.V.05, Coll: D.F. Kapraun, D.W. Freshwater & B. Stuercke	x	
Poly NC-11	*P. 'urceolata'* (Lightfoot) Greville *sensu* Kapraun (1977, 1980a)	South Masonboro Inlet Jetty, New Hanover Co., NC 04.V.05, Coll: D.F. Kapraun, D.W. Freshwater & B. Stuercke	x	x
Poly NC-12	*P. fucoides* (Hudson) Greville	Drift, South Masonboro Inlet Jetty, New Hanover Co., NC 04.V.05, Coll: D.F. Kapraun, D.W. Freshwater & B. Stuercke	x	x
Poly NC-13	*N. harveyi*	South Masonboro Inlet Jetty, New Hanover Co., NC 04.V.05, Coll: D.F. Kapraun, D.W. Freshwater & B. Stuercke	x	x
Poly NC-14	*P. breviarticulata*	Banks Channel (Site NH-M), New Hanover Co., NC 11.V.05, Coll: D.W. Freshwater	x	
Poly NC-15	*P. breviarticulata*	Ludens Creek, New Hanover Co., NC, Site NH-J 11.V.05, Coll: D.W. Freshwater, B. Stuercke & K. Braly	x	
Poly NC-16	*N. harveyi*	Ludens Creek, New Hanover Co., NC, Site NH-J 11.V.05, Coll: D.W. Freshwater, B. Stuercke & K. Braly	x	
Poly NC-17	*N. harveyi*	Bogue Sound, Corkey's house, Carteret Co., NC 26.III.05, Coll: D.W. Freshwater	x	

Poly NC-18	*P. breviarticulata*	Bogue Sound, Corkey's house, Carteret Co., NC 26.III.05, Coll: D.W. Freshwater	x	x
Poly NC-19	*N. harveyi*	Bogue Sound, Corkey's house, Carteret Co., NC 26.III.05, Coll: D.W. Freshwater	x	
Poly NC-20	*P. breviarticulata*	Bogue Sound, W. of Camp Morehead Pier, Carteret Co., NC 26.XII.03, Coll: D.W. Freshwater	x	x
Poly NC-21	*P. subtilissima* Montagne	Neuse River at Oriental, Pamlico Co., NC 00.IX.03, Coll: R. Peterson	x	
Poly NC-22	*N. harveyi*	South Masonboro Inlet Jetty, New Hanover Co., NC 19.V.05, Coll: D.W. Freshwater, B. Stuercke & K. Braly	x	
Poly NC-23	*N. harveyi*	Wrightsville Beach, New Hanover Co., NC 19.V.05, Coll: B. Stuercke	x	x
Poly NC-24	*P. subtilissima*	Snow's Cut Park, New Hanover County, NC 22.V.05, Coll: B.Stuercke & J.B. Landry	x	
Poly NC-25	*P. breviarticulata*	CORMP Site OB-27, Onslow Bay, NC 12.V.05, Coll: J. Souza		
Poly NC-26	*P. atlantica*	CORMP Site OB-27, Onslow Bay, NC 12.V.05, Coll: J. Souza, D. Wells & S. Hall	x	
Poly NC-27	*P. atlantica*	CORMP Site OB-27, Onslow Bay, NC 09.VI.05, Coll: D.W. Freshwater & J. Souza	x	
Poly NC-28	*P. atlantica*	CORMP Site OB-27, Onslow Bay, NC 09.VI.05, Coll: D.W. Freshwater & J. Souza	x	
Poly NC-29	*P. breviarticulata*	Bogue Sound, East end of Emerald Isle, Carteret Co., NC 23.VI.05, Coll: D.W. Freshwater	x	
Poly NC-30	*P. denudata sensu* Kapraun (1977, 1980a)	Green buoy, Mouth of North River, Carteret Co., NC 23.VI.05, Coll: D.W. Freshwater	x	x
Poly NC-31	*N. harveyi*	Buoy at ICW to New Topsail and little Topsail inlets, Pender Co., NC, Site PC-O 07.VII.05, Coll: D.W. Freshwater, B. Stuercke & R. Hammer	x	
Poly NC-32	*P. atlantica*	CORMP site OB-3, Onslow Bay, NC 11.VII.05, Coll: D.W. Freshwater & B. Stuercke	x	x
Poly NC-33	*P. scopulorum* var. *villum*	South Masonboro Inlet Jetty, New Hanover Co., NC 22.VII.05, Coll: D.W. Freshwater, R. York, K. Braly, R. Hammer & B. Stuercke	x	x
Poly HI-1	*P. subtilissima*	Reed's Bay, Hilo, Hawai'i 17.V.04, Coll: B. Stuercke	x	

Poly FL-1	*Polysiphonia* sp.	Sebastian Inlet State Park, Indian River Co., FL 26.II.05, Coll: D.W. Freshwater, R. York, K. Braly, R. Hammer & B. Stuercke	x	
Poly FL-2	*Polysiphonia* sp.	Sebastian Inlet State Park, Indian River Co., FL 26.II.05, Coll: D.W. Freshwater, R. York, K. Braly, R. Hammer & B. Stuercke	x	
Poly FL-4	*P. denudata sensu* Kapraun (1977, 1980a)	Sebastian Inlet State Park, Indian River Co., FL 26.II.05, Coll: D.W. Freshwater, R. York, K. Braly, R. Hammer & B. Stuercke	x	
P-LLG025*	*Polysiphonia* sp.	Mira Bay, N.S., Canada 01.IX.04, Coll: G.W. Saunders	x	
P-GO420*	*P. lanosa* (Linnaeus) Tandy	Pt. Lepreau, N.B., Canada 07.X.95, Coll: G.W. Saunders	x	
P-GWS2657*	*P. stricta* (Dillwyn) Greville	Letete Pt., N.B., Canada 14.III.05, Coll: G.W. Saunders	x	
Dig sim	*Digenia simplex* (Wulfen) C. Agardh	Mabibi, 40 km N. of Sodwana Bay, KwaZulu-Natal, SA 13.II.01, Coll: D.W. Freshwater	x	x
Herp ten	*Herposiphonia tenella* (C. Agardh) Nägeli	Old Topsail creek (site PC-0), Pender Co., NC 07.VII.05, Coll: D.W. Freshwater, B. Stuercke & R. Hammer	x	

* samples were ground material sent by G.W. Saunders

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 (CMS). All herbarium abbreviations follow Holmgren *et al.* (1990).

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) and the nuclear-encoded small-subunit ribosomal DNA (SSU rDNA) were amplified in reactions containing 10-50 ng template DNA; 80μM each dNTP; 1 mM $MgCl_2$; 0.2μM each amplification primer; and Hot Star Taq DNA polymerase and buffer per manufacturer's suggestion (Qiagen, Valencia, CA, USA). The thermocycling protocol for amplifications consisted of a denaturing and enzyme activation step of 95ºC for 15 min, followed by 30 cycles of 94ºC for 30 sec; 50ºC for 30 sec; 72ºC for 1 min 30 sec (with a 0.2ºC /sec ramp between the annealing and extension steps), completed by a final extension step of 72ºC for 5 min. Amplification products were cleaned using a Qiagen 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 are listed in Table 2. 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).

Table 2. Primers used for amplifying and sequencing *rbc*L and SSU genes in Rhodomelaceae species.

Label	Primer sequence
***rbc*L**	
F-*rbc*L start[1]	5'-ATG TCT AAC TCT GTA GAA G-3'
F-57[1]	5'-GTA ATT CCA TAT GCT AAA ATG GG-3'
F-577[1]	5'-GTA TAT GAA GGT CTA AAA GG-3'
F-753[1]	5'-GGA AGA TAT GTA TGA AAG AGC-3'
R-753[1]	5'-GCT CTT TCA TAC ATA TCT TCC-3'
R-893[2]	5'-GAA TAA GTT GA(AG) TT(AT) CC6 GCA C-3'
R-1150[1]	5'-GCA TTT GTC CGC AGT GAA TAC C-3'
R-1381[1]	5'-ATC TTT CCA TAG ATC TAA AGC-3'
R-*rbc*S start[1]	5'-GTT CCCT TGT GTT AAT CTC AC-3'
SSU	
G-01[3]	5'-CAC CTG GTT GAT CCT GCC AG-3'
G-03[3]	5'-GTC TGG TGC CAG CAG CCG CGG-3'
G-04[3]	5'-CAG AGG TGA AAT TCT TGG AT-3'
G-08[3]	5'-GAA CGG CCA TGC ACC ACC ACC-3'
G-07[3]	5'-ATC CTT CTG CAG GTT CAC CTA C-3'
G-10[3]	5'-CCG CGG CAG CTG GCA CCA GAC-3'
Cer-A[4]	5'-TGC CAG TGG (AT)AT ATG CTT GTC-3'
Cer-D[4]	5'-GCA AGT CTG GTG CCA GCA G-3'
Cer-G[4]	5'-AGC CTG CGG CTT AAT TTG AC-3'
Cer-E[4]	5'-CTA TTA TTC CAT GCT AAT GTA TTC-3'
Cer-H[4]	5'-TAA CCA GAC AGA TCA CTC CAC-3'
Cer-J[4]	5'-TCT CCT TCC TCT AAG TGA TAA-3'

[1]Freshwater and Rueness (1994)

[2] Stuercke & Freshwater (unpublished)

[3] Saunders and Kraft (1994)

[4] Freshwater (unpublished)

Phylogenetic Analyses

The *Polysiphonia* sequences generated in this study were combined with *rbc*L and SSU sequences available from GenBank and aligned using MacClade (v.4, Maddison & Maddison, 2000). Rhodomelacean outgroup species used for *rbc*L and SSU analyses were *Herposiphonia tenella* (C. Agardh) Nägeli and *Digenia simplex* (Wulfen) C. Agardh, respectively. The outgroup species were chosen based on preliminary phylogenetic analyses including multiple rhodomelacean species. 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*.

A total of 58 *rbc*L sequences were examined in this study, 19 of which were obtained from GenBank. Twenty-five sequences were not included in the final analyses because they were identical to at least one other sequence in the alignment. The final *rbc*L alignment consisted of sequences representing 32 ingroup specimens, including 17 previously published *Polysiphonia* *rbc*L sequences (Table 3). Red algal *rbc*L sequences are 1467 base pairs in length, but the sequences from GenBank were incomplete at the 5' and/or 3' ends; for this reason the analyzed alignment was truncated to 1245 sites.

Maximum-likelihood and neighbor-joining distance analyses were performed using a GTR + I + G model of evolution and using the following parameters derived from the Model Test program: Base = (0.3202, 0.1330, 0.2050), Nst = 6, Rmat = (1.7618, 5.5415, 3.2465, 1.1408, 19.6785), Rates = gamma, Shape = 1.5957, Pinvar = 0.5437. The maximum-likelihood analyses included 10 separate searches with random sequence addition using the tree bisection-reconnection (TBR) branch swapping algorithm and subjected to 100 rounds of bootstrap

Table 3. Source of additional *rbc*L and SSU sequence data included in the molecular analyses.

Species and authority	*rbc*L accession no.	SSU accession no.	References[a]
Boergeseniella fruticulosa (Wulfen) Kylin		AF427526	1
Enelittosiphonia stimpsonii (Harvey) Kudo & Masuda		AF427527	1
Neosiphonia japonica (Harvey) M.S. Kim & I.K. Lee		AF427528	1
Neosiphonia savatieri (Hariot) M.S. Kim & I.K. Lee		AF203885	4
Polysiphonia akkeshiensis Segi	AF342901		2[b]
Polysiphonia brodiaei (Dillwyn) Sprengel	AF342916		2
Polysiphonia denudata (Dillwyn) Greville ex Harvey	AF342914		2
Polysiphonia elongata (Hudson) Sprengel	AF342911	AF427529	2, 1
Polysiphonia elongella Harvey	AF342913		2[c]
Polysiphonia fibrata (Dillwyn) Harvey	AF342915		2
Polysiphonia fibrillosa (Dillwyn) Sprengel	AF342912		2
Polysiphonia forfex Harvey	AF342910		2
Polysiphonia fucoides (Hudson) Greville		AF427531*	1
Polysiphonia harveyi Bailey	AF342906	AF427530*	2, 1
Polysiphonia japonica Harvey	AF342904		2
Polysiphonia lanosa (Linnaeus) Tandy		AF203886	4
Polysiphonia morrowii Harvey	AY396034	AF427532	3,1
Polysiphonia nigra (Hudson) Batters		AF427534	1
Polysiphonia pacifica Hollenberg	AY396036	AF427533	3,1
Polysiphonia paniculata Montagne	AY396041	AY617144	3,5
Polysiphonia scopulorum Harvey	AY396039		3
Polysiphonia stricta (Dillwyn) Greville		AF427535	1
Polysiphonia strictissima J.D. Hooker & Harvey	AF342908		2
Polysiphonia virgata (C. Agardh) Sprengel		AF427536	1
Womersleyella setacea (Hollenberg) R.E. Norris		AF427537	1

[a] 1, Choi et al. (2001); 2, McIvor et al. (2001); 3, Kim et al. (2004); 4, Phillips et al. (2000); 5, Zuccarello et al. (2004)

[b] Regarded as *Neosiphonia akkeshiensis* (Harvey) M.S. Kim & I.K. Lee, Kim & Lee (1999)

[c] Regarded as *Neosiphonia elongella* (Harvey) M.S. Kim & I.K. Lee, Kim & Lee (1999)

* Accession numbers were switched in GenBank, accession numbers and species are correct in this publication

resampling (random additions set to 1). Distance analyses were subjected to 5000 rounds of bootstrap resampling. Parsimony analysis of the data was completed using a heuristic search of 15000 random sequence additions with MULTREES, STEEPEST DESCENT and TBR branch swapping options in effect. Parsimony bootstrap analyses consisted of 1000 replications of 1000 random sequences additions.

Twenty-nine SSU sequences were investigated in this study, 16 of which were obtained from GenBank. Six sequences were not included in the final analyses because they were identical to at least one other sequence in the alignment. The final alignment consisted of sequences representing 22 ingroup specimens, including 12 previously published *Polysiphonia* and rhodomelaceae SSU sequences (Table 3). The 1861 aligned nucleotide positions of SSU were edited to exclude missing data at the beginning and end of the GenBank sequences and those generated in this study. Thirty-seven sites within the truncated sequence alignment were excluded because of alignment uncertainties resulting in an alignment of 1595 sites that was used in the final phylogenetic analyses.

Maximum-likelihood and neighbor-joining distance analyses were performed using a HKY + I + G model of evolution and using the following parameters derived from the Model Test program: Base = (0.2570, 0.2006, 0.2656), Nst = 2, TRatio = 2.0577, Rates = gamma, Shape = 0.7038, Pinvar = 0.752. The maximum-likelihood analyses included 10 separate searches with random sequence addition using TBR branch swapping and were subjected to 100 rounds of bootstrap resampling (random additions set to 10). Distance analyses were subjected to 5000 rounds of bootstrap resampling. Parsimony analysis of the data was completed using a branch and bound search using the default settings followed by bootstrapping (1000 replications).

Morphological Data and Analysis

Morphological characters examined were selected based upon taxonomic keys for, and descriptions of *Polysiphonia* species (Hollenberg, 1942, 1968a, b; Abbott & Hollenberg, 1976; Hollenberg & Norris, 1977; Kapraun, 1977; 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 characters was narrowed to 22 (Table 4). Observations were made on all 22 characters for the 31 North Carolina samples. These results led to the investigation of 11 of these 22 characters for rhodomelacean species that had previously been reported as *Polysiphonia* and *Polysiphonia* species with sequence data in GenBank, as well as the samples collected and sequenced in this study from Florida and Hawai'i. Morphological data for species with GenBank sequences was gathered from the literature (Hollenberg, 1942; Hollenberg, 1968a, c; Abbott & Hollenberg, 1976; Kudo & Masuda, 1986; Lawson & John, 1987; Schneider & Searles, 1991; Kudo & Masuda, 1992; Maggs & Hommersand, 1993; Adams, 1994; Kim *et al*., 1994; Masuda *et al*., 1995; Stegenga *et al*., 1997; Abbott, 1999; Kim *et. al*, 2000; Littler & Littler, 2000; Choi *et al*., 2001; Kim *et al*., 2002; Womersley, 2003; De Clerk *et al*., 2005; Kapraun, unpublished MS).

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

Table 4. Characters and character states observed for the North Carolina *Polysiphonia* samples. Characters that were used in further investigation of Rhodomelaceae samples from GenBank, FL, HI and Canada were indicated by an '*'.

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	Nature of apical cell: conspicuous (0), inconspicuous (1)
10	Apical cell division: transverse (0), oblique (1)
11*	Nature of 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), not spiral (2)
18*	Scar cells producing lateral branches: 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)

the *rbc*L and SSU analyses. Character states for the 11 characters used to describe all of the species in this study, including the GenBank species, Canada, Florida, Hawai'i and North Carolina samples, were traced directly onto the *rbc*L and SSU trees. North Carolina samples were identified to species based on examination of morphological characteristics and using the taxonomic keys and descriptions found in Kapraun (1977, 1980a, unpublished MS), Schneider & Searles (1991), and Maggs & Hommersand (1993).

RESULTS

Molecular Analyses

*rbc*L Analyses

A total of 32 ingroup *rbc*L sequences representing 57 specimens were analyzed in this study. The final sequence alignment was 1245 base pairs in length; 463 sites (37.2%) were variable and 367 sites (29.5%) were phylogenetically informative. Parsimony, neighbor joining distance and maximum likelihood analyses were completed for this data set.

Parsimony analysis resulted in 226 equal length trees of 1397 steps (CI = 0.364, RI = 0.671). The maximum likelihood and neighbor joining trees were not among this set of trees but they were within four and five steps of the parsimony trees, respectively. There was variation among the three analyses 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 Figure 1.

The North Carolina samples sequenced in this project were resolved in eight separate positions (*Polysiphonia* spp. A-H) representing individual species. Species A, which contained

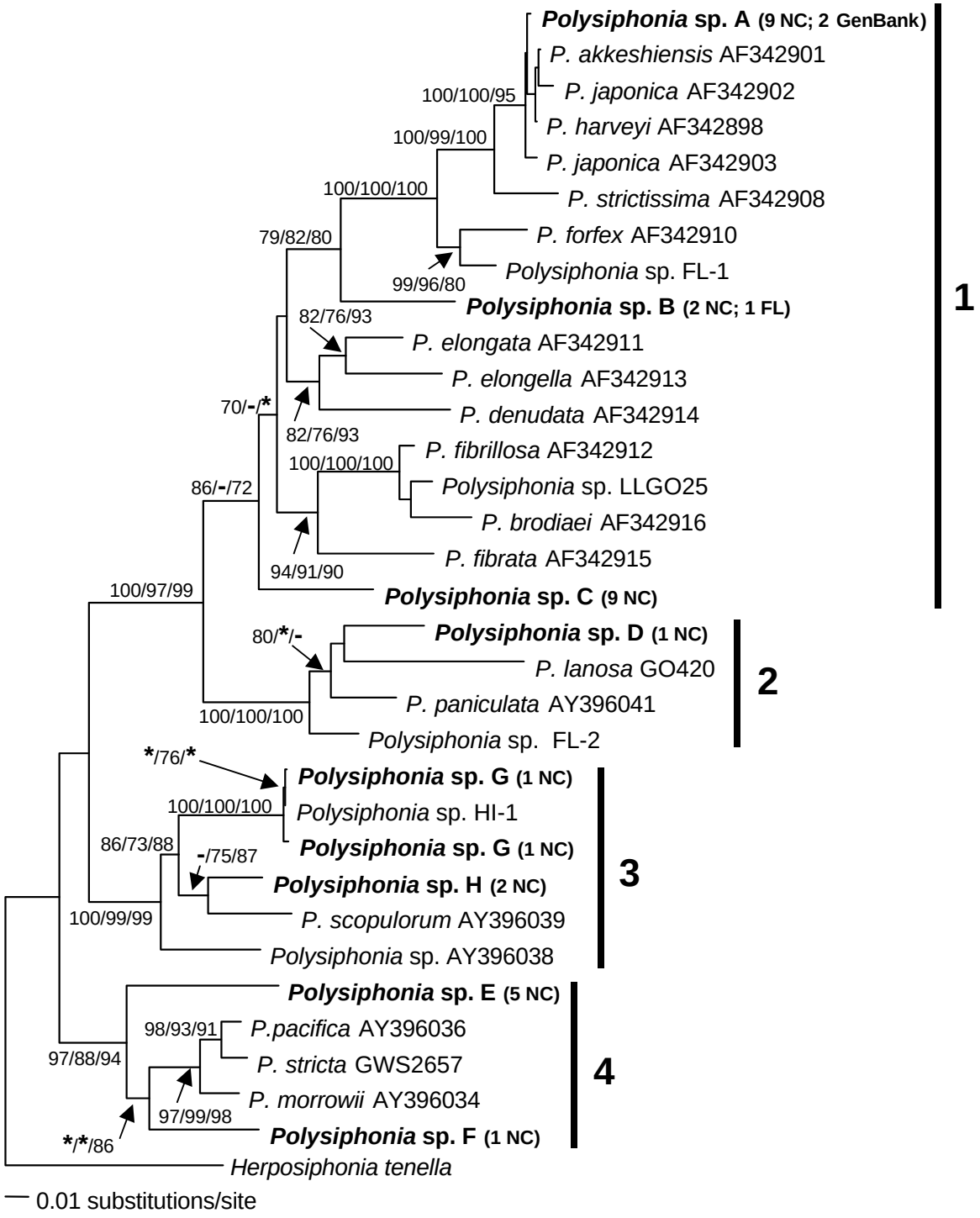

Figure 1. Maximum likelihood *rbc*L tree (lnL = -8798.2035) for 32 *Polysiphonia sensu lato* and one outgroup sample. Bootstrap proportion values for branches are shown from distance, parsimony and maximum likelihood analyses, respectively. '*' represents bootstrap values 50% < but < 70%, and '-' or no values represents values < 50%. Major clades of species indicated by 1-4.

nine North Carolina samples (Poly NC-6, 10, 13, 16, 17, 19, 22, 23 and 31) and two GenBank samples (*Polysiphonia japonica* AF342904 and *P. harveyi* AF342906), grouped with sequences from *Polysiphonia akkeshiensis*, *P. harveyi*, and *P. japonica*. Species B, comprised of two North Carolina samples (Poly NC-2 and 30) and one Florida sample (Poly FL-4), was resolved with moderate bootstrap support (distance (D) 79, parsimony (P) 82 and maximum likelihood (M) 80 bootstrap proportion values) in an isolated position sister to clades containing *Polysiphonia akkeshiensis*, *P. harveyi*, *P. japonica*, *P. strictissima*, *P. forfex*, and *Polysiphonia* sp. FL-1.

The nine North Carolina samples (Poly NC-1, 3, 5, 7, 14, 15, 18, 20, and 29) of species C occupied a position within the *rbc*L tree with no strong bootstrap support associating it with another closely related species or clade. Species D, with only one North Carolina sample (Poly NC-12), was in a highly supported clade (D100, P100, M100) with four other species. The exact relationships of the species within this clade were different in all three phylogenetic analyses but there was little or no support for any of these inner relationships.

Two North Carolina samples, Poly NC-21 and Poly NC-24, and one Hawai'i sample (Poly HI-1) formed species G that was moderately supported (D86, P73, M88) to be sister to a variably supported clade (D < 50, P75, M87) containing *P. scopulorum* and species H, which was made up of two other North Carolina samples (Poly NC-9 and 33). The final two North Carolina species, E and F, were resolved with moderate to strong support (D97, P88, M94) in a clade with *Polysiphonia pacifica*, *P. stricta*, and *P. morrrowii*. The actual relationships of these two species with *Polysiphonia pacifica*, *P. stricta*, and *P. morrrowii* however is not as well resolved and only received moderate support in the maximum likelihood analyses (M86).

SSU Analyses

The final SSU alignment included 22 ingroup sequences representing 28 specimens and 1595 sites. There were 58 coded insertion-deletion (indel) mutations in the alignment that were analyzed, with the largest indel for any sequence containing only four contiguous sites. Three regions totaling 37 sites were excluded from analyses because they could not be confidently aligned. Of the 1595 sites analyzed, 184 (11.5%) were variable and 130 (8.2%) were phylogenetically informative. Parsimony, neighbor joining distance and maximum likelihood analyses were completed for these data.

A branch and bound parsimony analysis resulted in six most parsimonious trees (L = 263, CI = 0.620, RI = 0.839). The maximum likelihood tree was included within the set of most parsimonious trees, whereas the neighbor joining tree was one step longer than the most parsimonious solutions. The overall relationships among the specimens in all three analyses were similar, with only a few closely related specimens displaying some conflict in topological position between the different analyses. The observed topological conflicts were unsupported by bootstrap analyses. The maximum likelihood SSU topology with the bootstrap values for all three methods appended is shown in Figure 2.

The same eight North Carolina species resolved in the *rbc*L analyses were also recovered in the SSU analyses. Species A, comprised of three North Carolina samples (Poly NC-6, 13 and 23) and three GenBank samples (*Polysiphonia harveyi* AF427530, *Neosiphonia japonica* AF427528 and *N. savatieri* AF203885), was resolved sister to species B (Poly NC-2 and 30) but with less than 70 % bootstrap support for this relationship. Species A, B, C, and the clade containing *Polysiphonia elongata* and *P. virgata* formed a highly supported clade (D100, P100, M100). *Polysiphonia nigra* and species D (composed of one North Carolina sample Poly NC-12

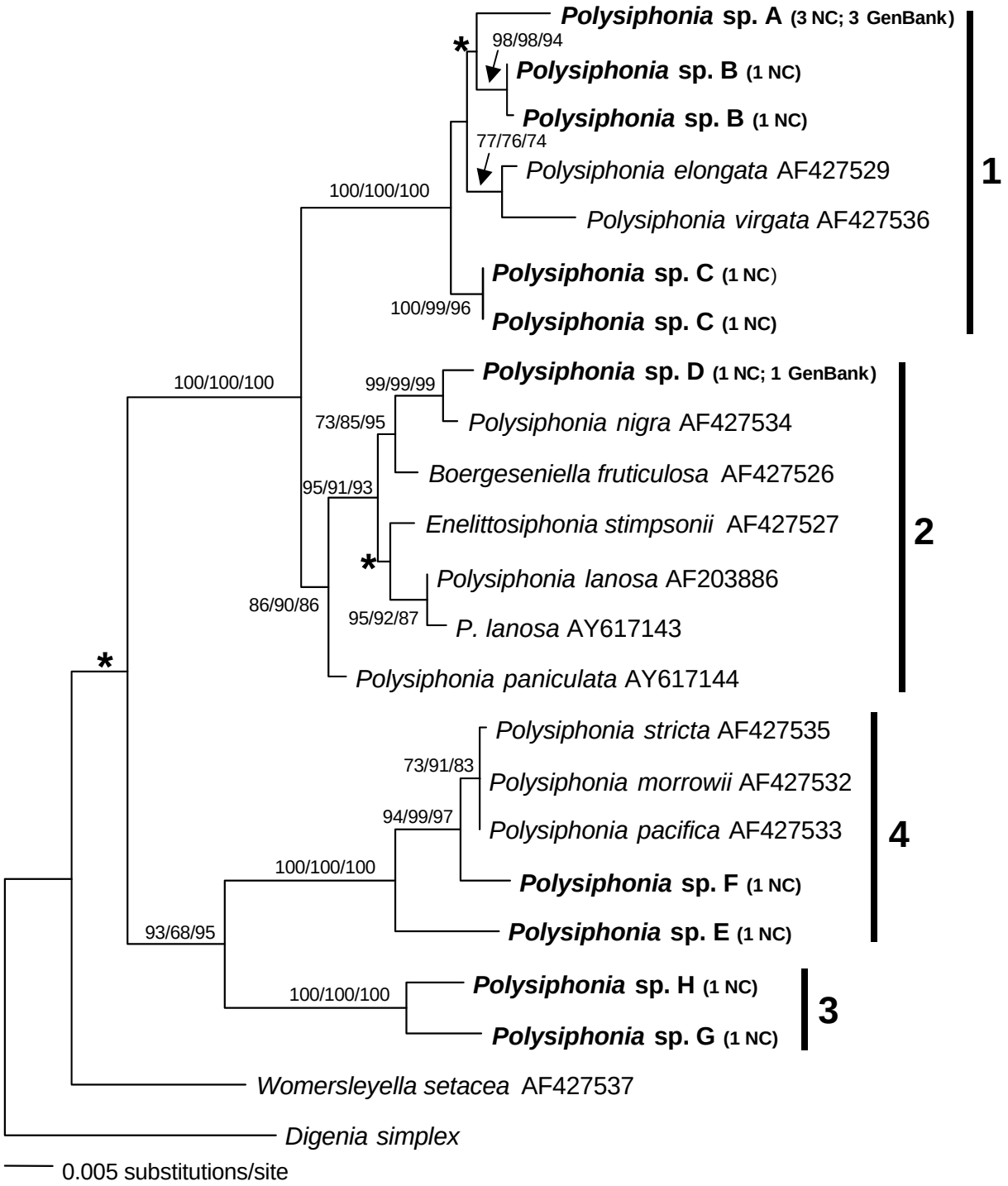

Figure 2. Maximum likelihood SSU tree (lnL = -4103.33878) for 22 *Polysiphonia sensu lato* and one outgroup sample. Bootstrap proportion values for branches are shown from distance, parsimony and maximum likelihood analyses, respectively. '*' represents bootstrap values 50% < but < 70%, and no values represents values < 50%. Major clades of species indicated by 1-4.

and one GenBank sample *P. fucoides* AF427534) were resolved sister to one another with strong bootstrap support (D99, P99, M99). Species E and F showed the same relationship seen in the *rbc*L analysis but with more support for the exact relationship of these two species to the *Polysiphonia pacifica*, *P. stricta*, and *P. morrrowii* clade. Species G and H were grouped together with 100% bootstrap support in all analyses.

Both *rbc*L and SSU topologies resolved the same four major clades, despite differences in the identification and number of specimens and species included in the analyses. The four major clades in the SSU tree had moderate to high bootstrap support (Figure 2), where as the major clades in the *rbc*L tree received less bootstrap support (Figure 1). Major clade one contained species A, B and C, as well as other *Polysiphonia* and *Neosiphonia* species and the bootstrap support for this clade was strong (D 100, P 100, M 100) in the SSU analyses and poor to moderate (D 86, P <50, M 72) in the *rbc*L analyses. Major clade two included species D, *P. nigra*, *P. lanosa*, *P. paniculata*, *Boergeseniella fruticulosa* and *Enlittosiphonia stimpsonii* and received strong (D 100, P 100, M 100) support in the *rbc*L analyses and moderate to strong (D 86, P 90, M 86) support in SSU analyses. Major clade three contained species G, H and other *Polysiphonia* species and it was strongly supported in both analyses (SSU: D 100, P 100, M 100; *rbc*L: D 100, P 99, M 99). The final clade, major clade four, included species E, F, *P. pacifica*, *P. morrowii* and *P. stricta* with strong (D 100, P 100, M 100) support in the SSU analyses and moderate to strong (D 97, P 88, M 94) in the *rbc*L analyses.

Morphological Analyses of North Carolina Samples

Twenty-two characters were used to examine the 31 North Carolina *Polysiphonia* samples (Table 4). The character states for each sample (Table 5) were mapped onto a phylogenetic framework

Table 5. Data matrix of character states of anatomical characters for the North Carolina *Polysiphonia* samples investigated in this study.

	Characters																					
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Poly NC-1	0	1	1	?	0	0	0	0	0	0	?	1/2	?	?	?	0	1	?	?	?	?	1
Poly NC-2	1	1	1	1	1	?	0	1	0	0	1	1/2	0/1	1	0	0	0	1	0/2	?	?	?
Poly NC-3	0	1	1	1	0	0	0	0	0	0	2	0/1	0	0	1	0	1	0	?	?	?	1
Poly NC-4	0	0	1	0	1	?	1	?	0	0	2	0	1	1	0	1	?	?	?	?	?	0
Poly NC-5	0	1	0	1	0	0	0	0	0	0	1	0	1/2	0	1	0	1	0	3	?	?	1
Poly NC-6	0	1	?	0	1	?	0	0	0	0	0	0/1	0	0	1	0	1	0	1/3	1	1	?
Poly NC-7	0	1	0	1	0	0/1	0	0	0	0	2	1	1	0	1	0	1	0	?	1	0	1
Poly NC-9	0	0	1	2	1	?	0	1	0	0	2	0/4	1	1	2	0	0	1	?	?	?	0
Poly 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
Poly NC-11	0	0	1	0	0	1	0	1	0	0	2	0	0/1/2	1	1	0	0	1	0	?	?	0
Poly NC-12	2	1	0	0	0	0/1	0	1	1	0	1	0	0/1	0	1	0	1	?	3/5	?	?	?
Poly 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
Poly NC-14	0	?	1	1	0	1	0	0	0	0	?	0	1	0	1	0	1	0	?	?	?	1
Poly NC-15	0	1	1	1	0	0	0	0	0	0	0	0	1/2	0	1	0	1	0	0/5	?	?	1
Poly 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
Poly NC-17	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0/3/6	?	?	1
Poly NC-18	0	1	?	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	?	?	?	1
Poly NC-19	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	?	?	?	1
Poly NC-20	0	1	0	1	0	1	0	0	0	0	?	0	0/1	1	1	0	1	0	3/6	?	?	1
Poly NC-21	0	0	1	0	0	2	0	1	0	0	2	0	0/1	1	0	0	0	1	?	?	?	0
Poly 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	?
Poly NC-23	0	1	?	0	1	?	0	0	0	0	0	0	0	0	0	0	1	?	?	?	?	?
Poly NC-24	0	0	1	0	0	0	0	1	0	0	2	0	0/1	1	0	0	0	1	?	?	?	?
Poly NC-26	0	0	1	?	1	?	1	?	0	0	2	0	0/1	0	0	0	?	?	?	?	?	?
Poly NC-27	0	0	1	0	1	?	0	1	0	0	2	0	0/1	1	2	0	0	1	?	?	?	0
Poly NC-28	0	0	1	0	1	?	0	1	0	0	2	0	0/1	1	0	0	0	1	?	?	?	?
Poly NC-29	0	1	1	1	0	0	0	0	0	0	2	0	0/1	0	0	0	1	0	?	?	?	1
Poly NC-30	1	1	1	1	1	?	0	1	0	0	1	0/1	0/1	1	1	0	0	?	?	?	?	0

Poly NC-31	0	1	0	0	0	0/1	0	0	0	0	0	0	0	2	1	0	1	?	4	1	1	1
Poly NC-32	0	0	1	0	1	?	0	1	0	0	2	0	0	1	0	0	0	1	?	?	?	0
Poly NC-33	0	0	1	2	1	?	1	?	0	0	2	3	?	2	2	1	?	?	?	?	?	?

based on the relationships of these specimens resolved in both the *rbc*L and SSU sequence analyses.

Number of Pericentral Cells (Character 1)

The number of pericentral cells in North Carolina *Polysiphonia* samples ranged from four to thirteen (Figure 3). Most of the samples collected had only four pericentral cells (28 samples out of 31, Figure 4). This character was found to be consistent within all eight North Carolina species. Species A, C, E, F, G, and H had samples with four pericentral cells. The two samples in species B had six pericentral cells. The only sample collected for species D had 12-13 pericentral cells.

Rhizoid-Pericentral Cell Connection (Character 2)

The connection between the rhizoid and the pericentral cells was determined for all but one sample (Figures 5 and 6). This character showed consistency within each North Carolina species and the monophyletic group composed of species A, B, C and D was also consistent with samples displaying rhizoids that were pit connected to the pericentral cells. The four remaining species had rhizoids in open connection to the pericentral cells.

Cortication (Character 3)

Presence or absence of cortication was a consistent character in all but one of the North Carolina species (Figure 7). In species C, both character states were observed with most samples being ecorticate throughout. Cortication was absent in five out of the eight North Carolina species.

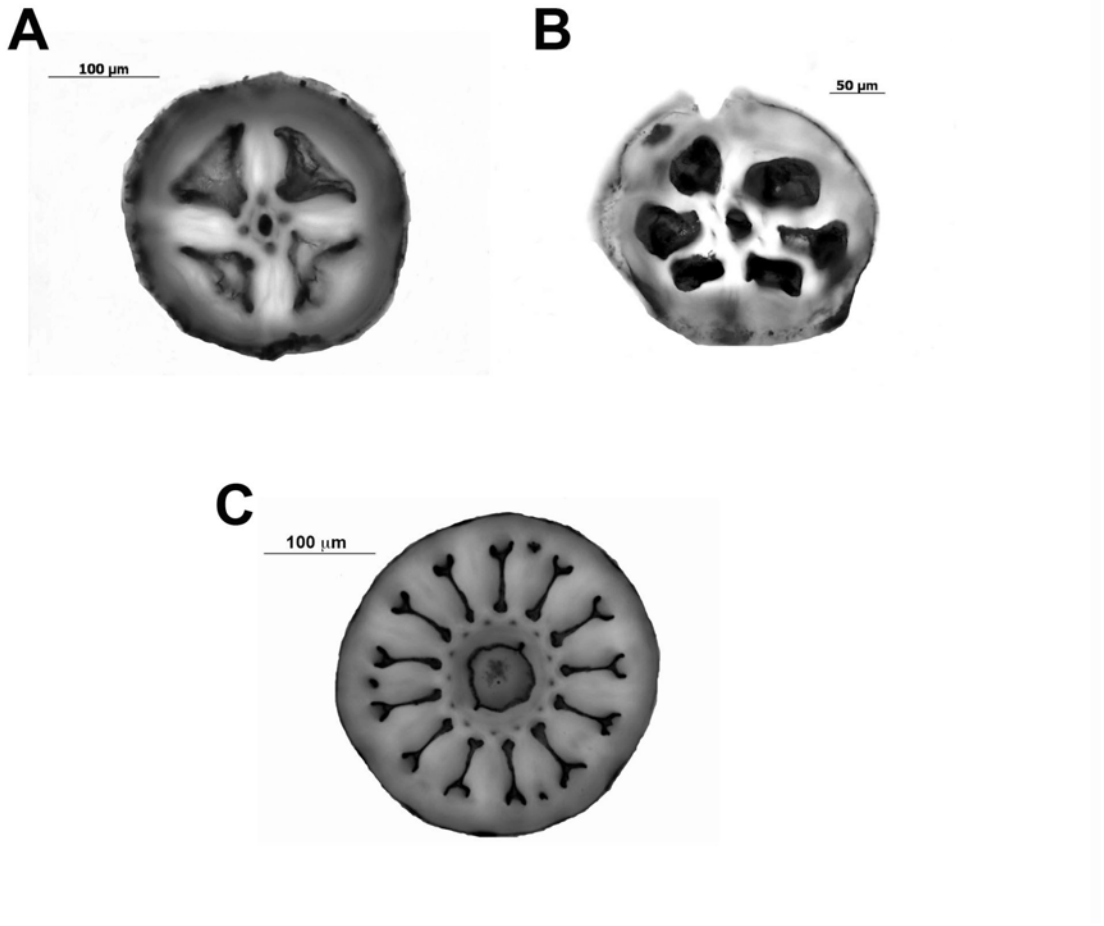

Figure 3. Cross-sections of branch axes showing the central axil cell and surrounding pericentral cells. A. Sample having 4 pericentral cells, *Polysiphonia breviarticulata*, Poly NC-5. B. Sample having 5-7 pericentral cells, *P. denudata sensu* Kapraun, Poly NC-2. C. Sample having more than eight pericentral cells, *P. fucoides*, Poly NC-12.

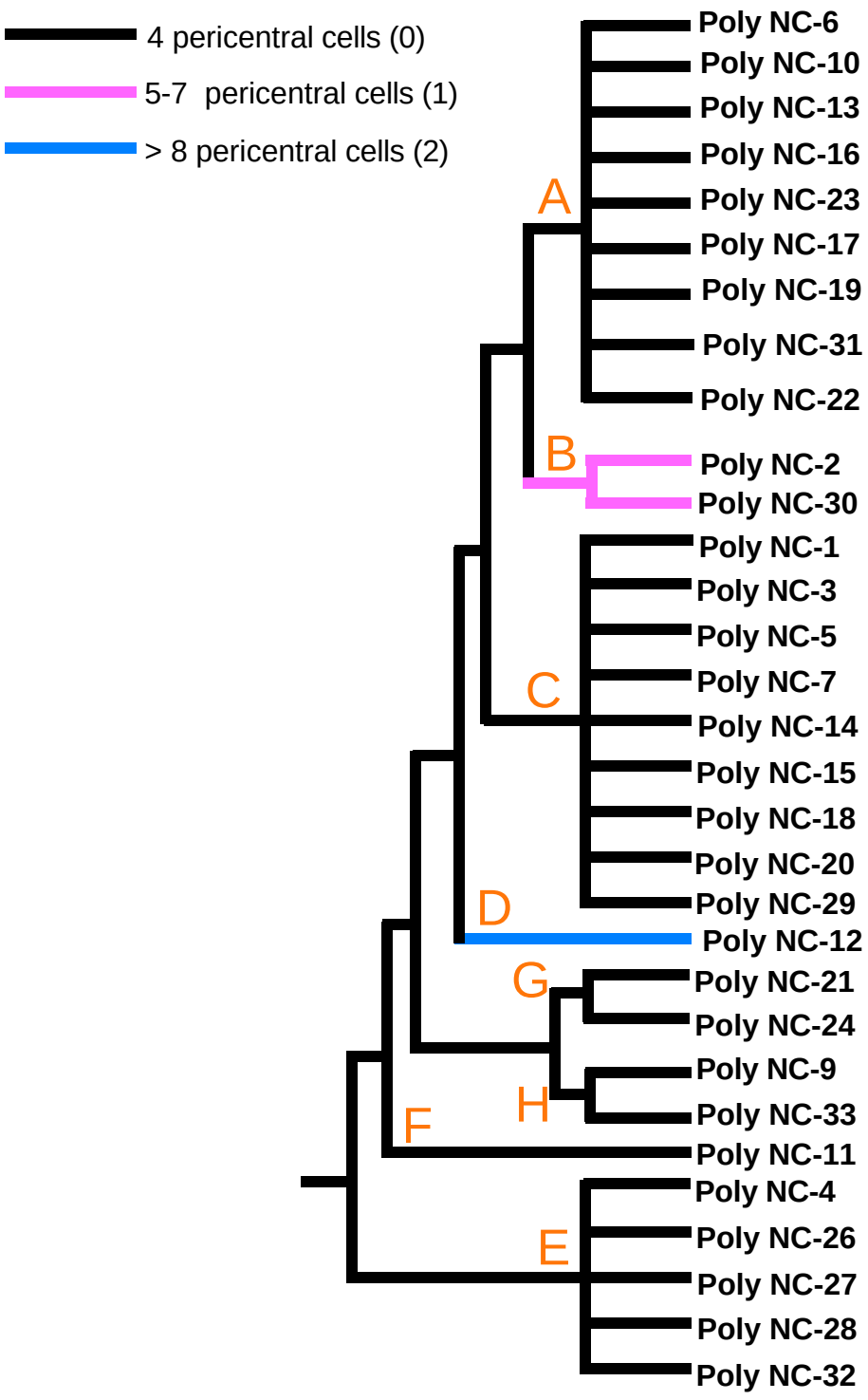

Figure 4. Number of pericentral cells mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

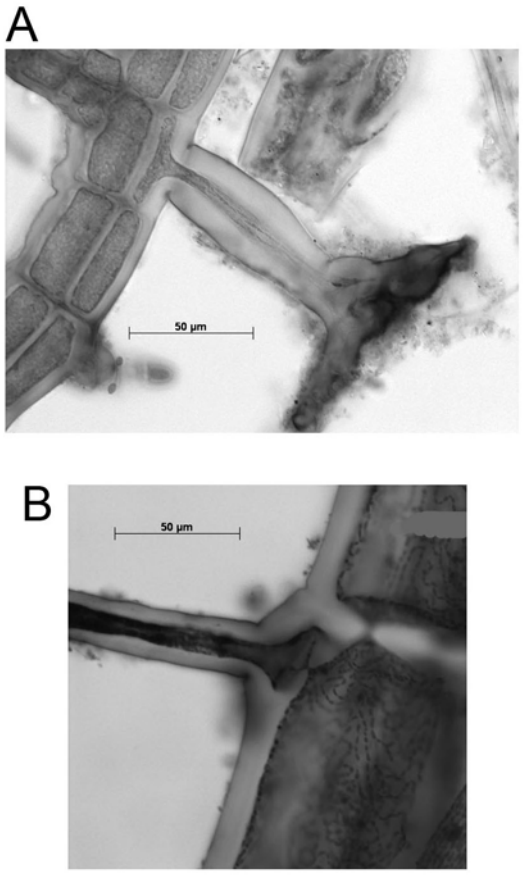

Figure 5. Connection between rhizoids and pericentral cells as seen in North Carolina *Polysiphonia* samples. A. Sample with an open connection between rhizoid and pericentral cell, *P. atlantica*, Poly NC-4. B. Sample that has rhizoids that are cut off from the pericentral cell (i.e. pit connected), *P. denudata sensu* Kapraun, Poly NC-2.

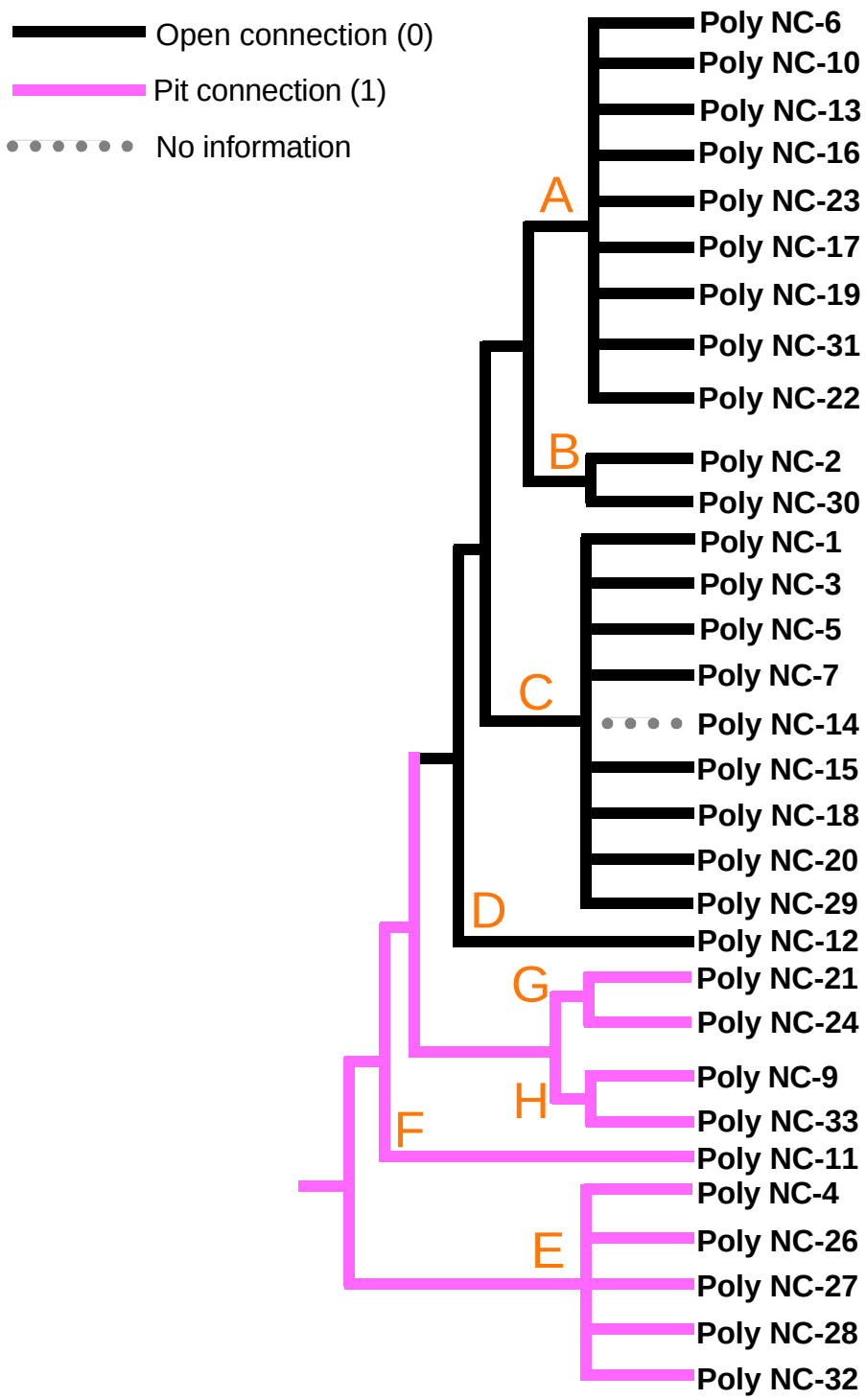

Figure 6. Rhizoid-pericentral cell connection was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

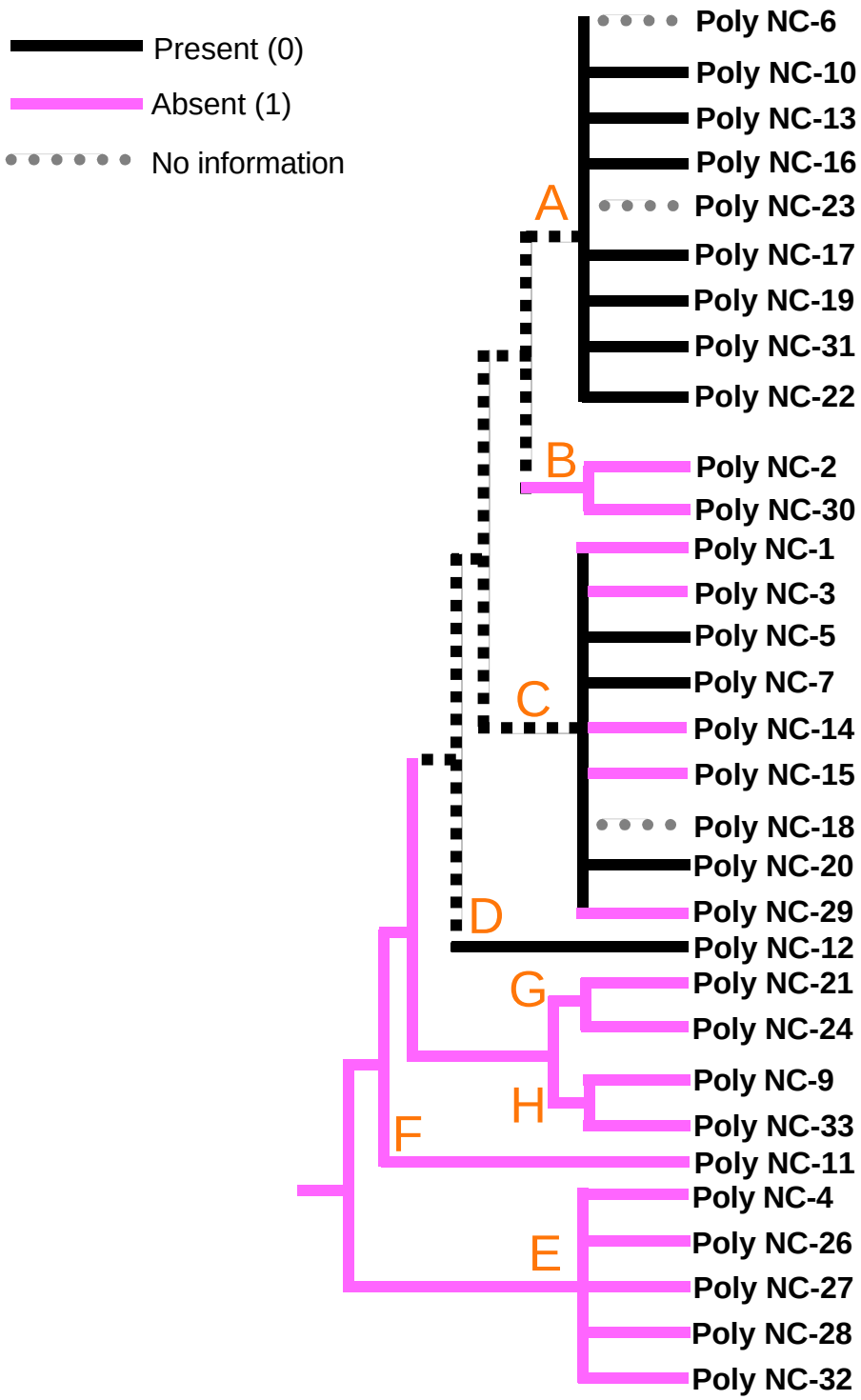

Figure 7. The presence or absence of cortication was mapped upon a cladogram depicting the relationships of 31 North Carolina Polysiphonia samples based upon rbcL and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

Relationship of Lateral Branches to Trichoblasts (Character 4)

The relationship of lateral branches and trichoblasts was not observed in all samples (Figures 8 and 9). In the samples where this character was observed there was consistency within the North Carolina species (Figure 9). Five out of the eight species had samples with lateral branches replacing the trichoblasts. Species B and C both had samples where lateral branches formed in the axil of the trichoblasts. The lateral branch was independent of the trichoblasts in species H.

Adventitious laterals (Characters 5 and 6)

The presence or absence of adventitious laterals was observed to be consistent in all but one North Carolina species (Figure 10). Species A had two samples that did not have adventitious laterals present, whereas the remaining seven samples did have adventitious laterals. Adventitious laterals were present in five out of the eight North Carolina species. The shape of adventitious laterals was variable within all of the North Carolina species (Figure 11). In general, the shape of adventitious laterals varied from linear to lanceolate with one sample displaying a triangular shape.

Trichoblasts (Characters 7 and 8)

Vegetative unpigmented trichoblasts were seen in all but three North Carolina samples. The three samples that lacked trichoblasts were found in species E and H, where there was some inconsistency in this character (Figure 12). The remaining six species displayed consistency among all the samples. The number of segments between trichoblasts was a very consistent character within the North Carolina *Polysiphonia* samples (Figure 13). Two of the eight species

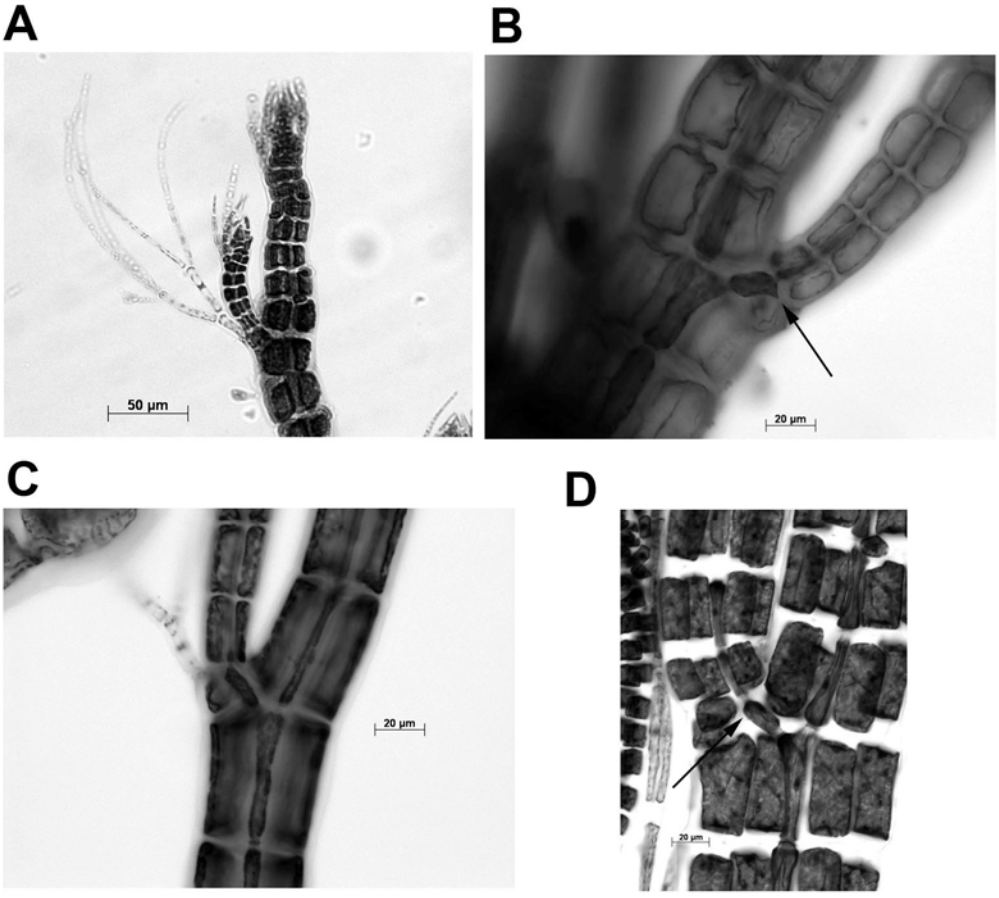

Figure 8. The relationship of lateral branches and trichoblasts in *Polysiphonia* samples showing that branches either replace or form in the axil of trichoblasts. A. Tip of a *P. breviarticulata* showing the lateral branch forming in the axil of the trichoblasts, Poly NC-5. B. Close up of the axil filament leading to the lateral branch, arrow pointing to the first axil cell of the lateral branch with an extra pit connection that led to a trichoblasts, *P. breviarticulata*, Poly NC-15. C. *Polysiphonia breviarticulata* sample with a focus on the axial filament leading to the lateral branch, showing branch forming in axil of trichoblasts, Poly NC-5. D. Example of branch replacing trichoblasts, arrow pointing to first axial cell in the lateral branch with no extra pit connection, P. *'urceolata' sensu* Kapraun, Poly NC-11.

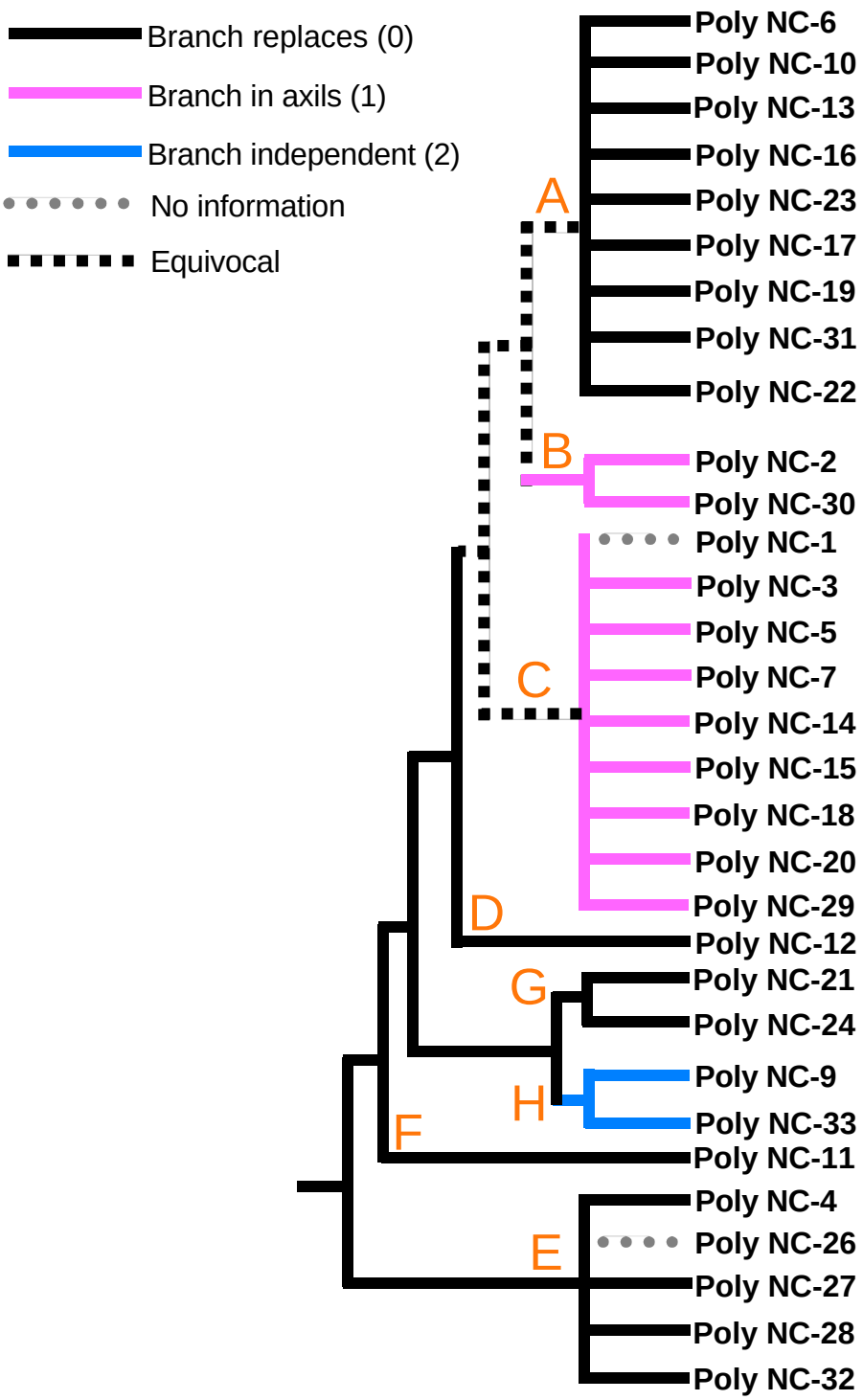

Figure 9. Relationship of the lateral branches to trichoblasts was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

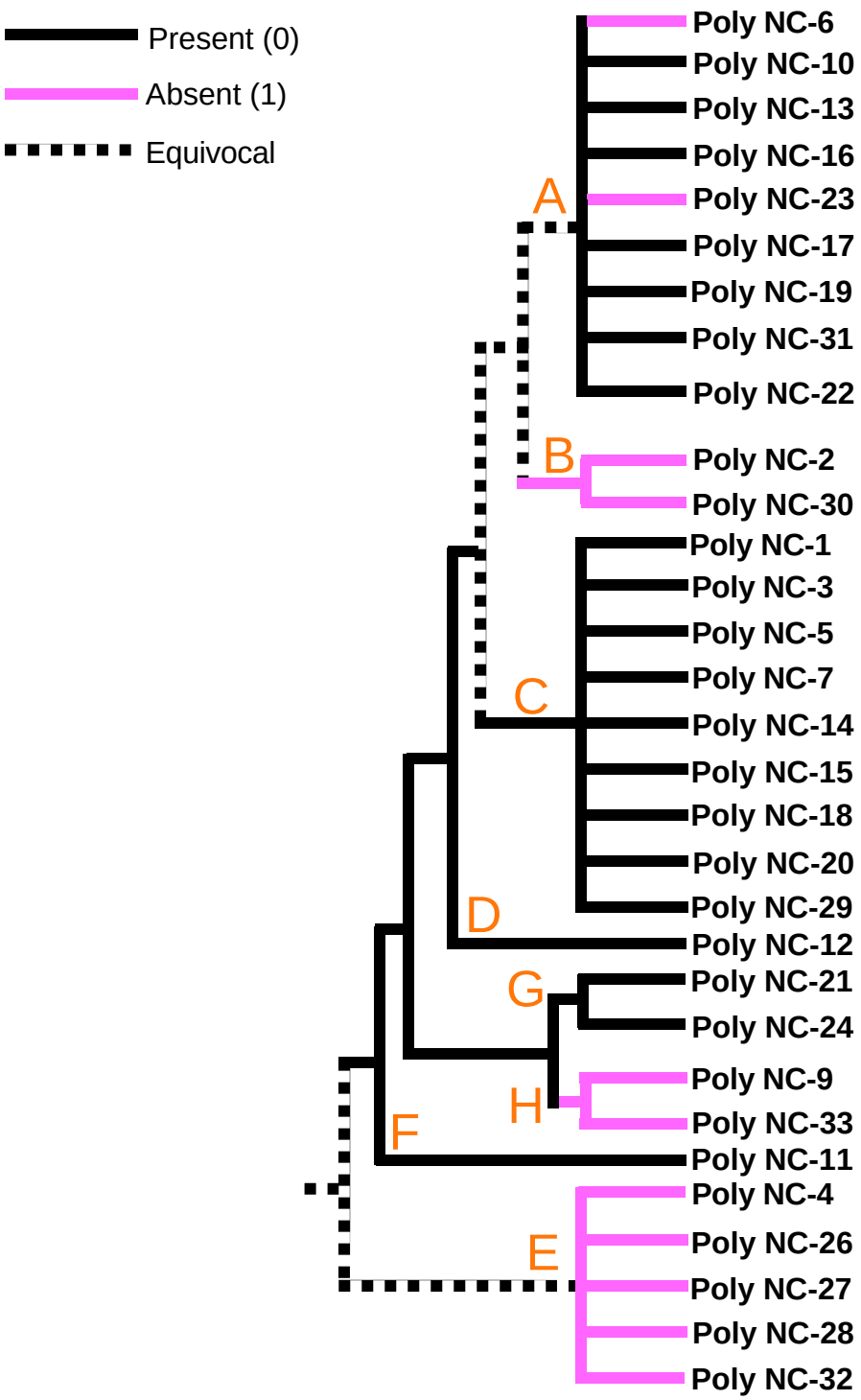

Figure 10. The presence or absence of adventitious laterals was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

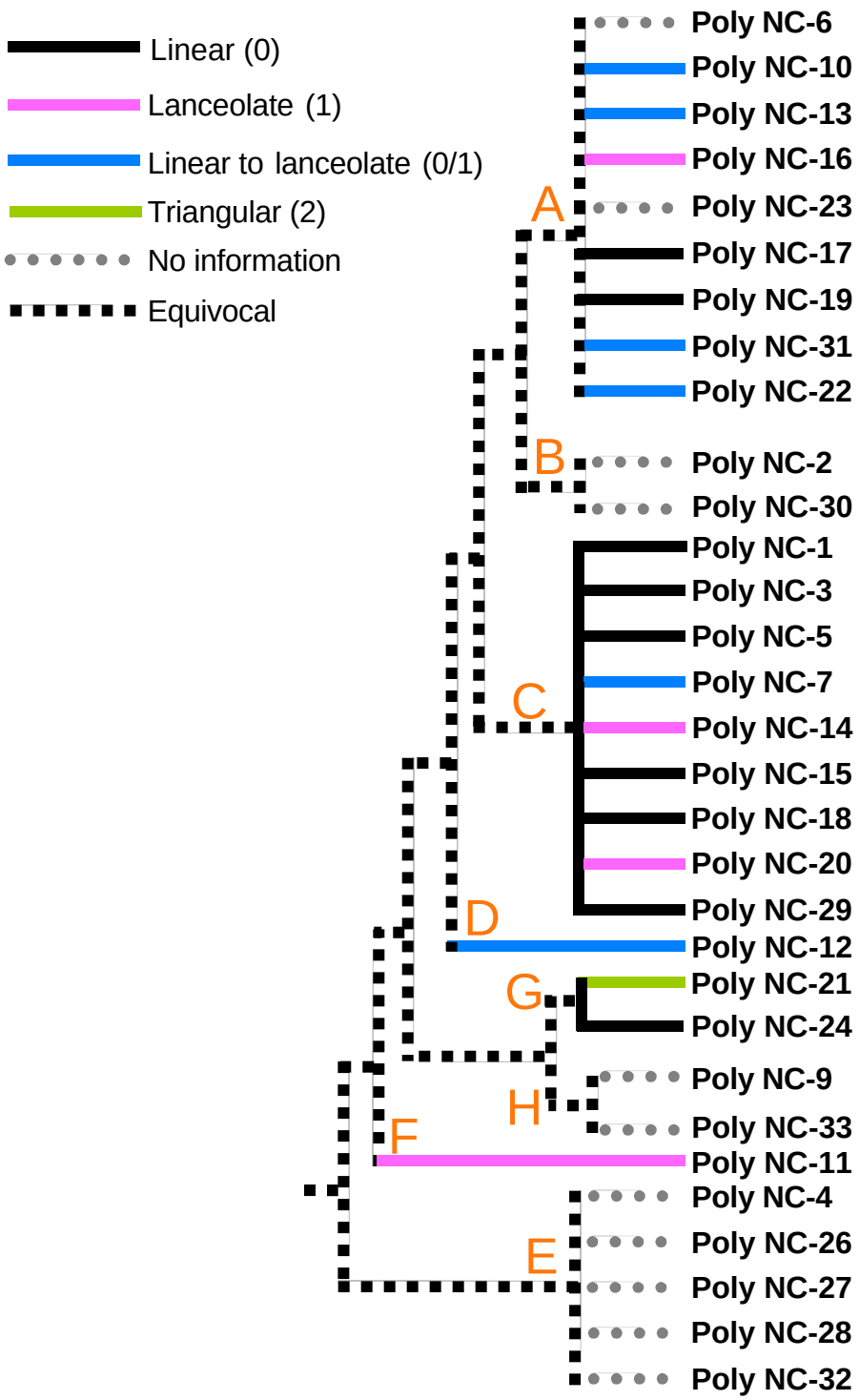

Figure 11. The shape of adventitious laterals was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

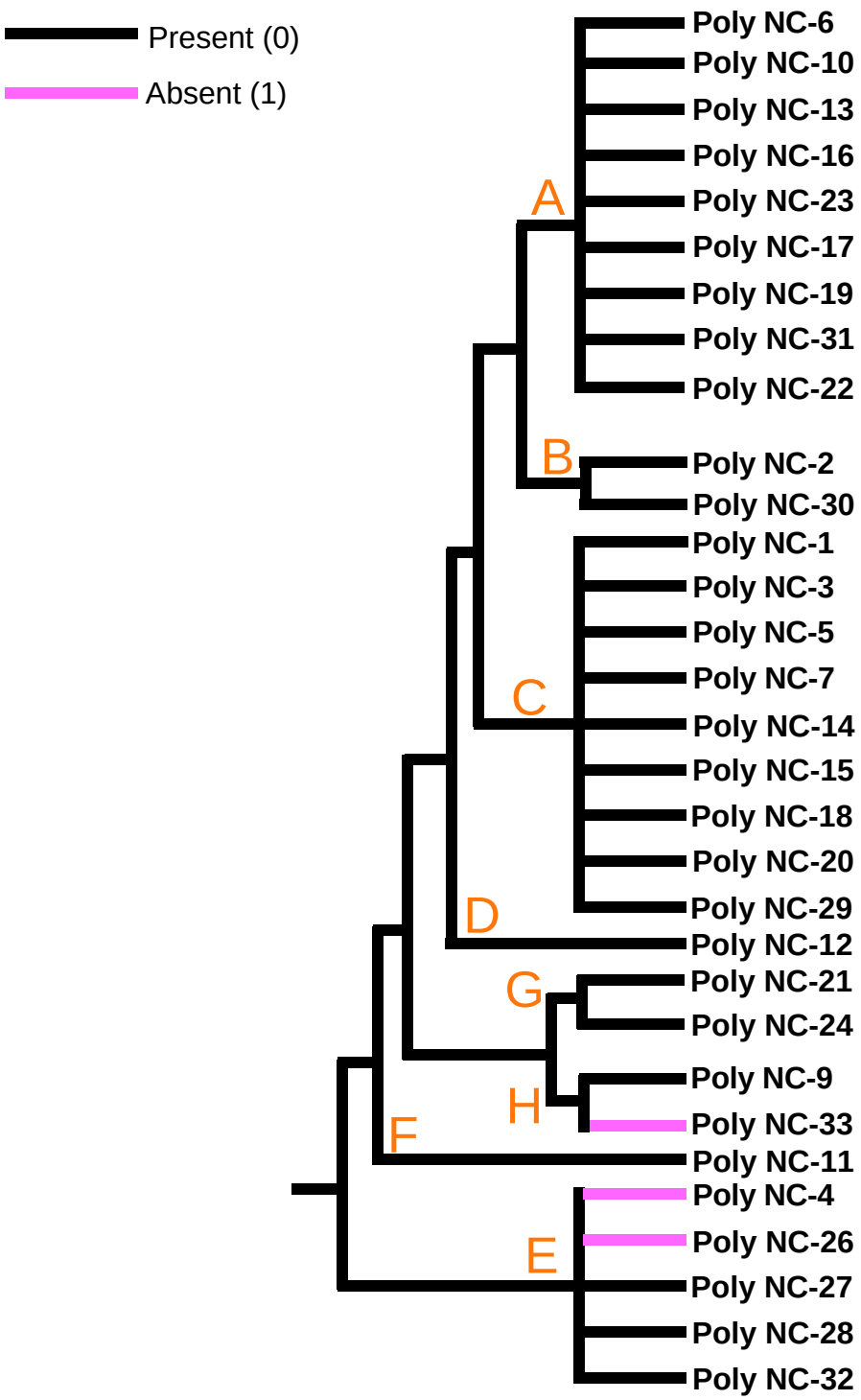

Figure 12. The presence or absence of vegetative unpigmented trichoblasts was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

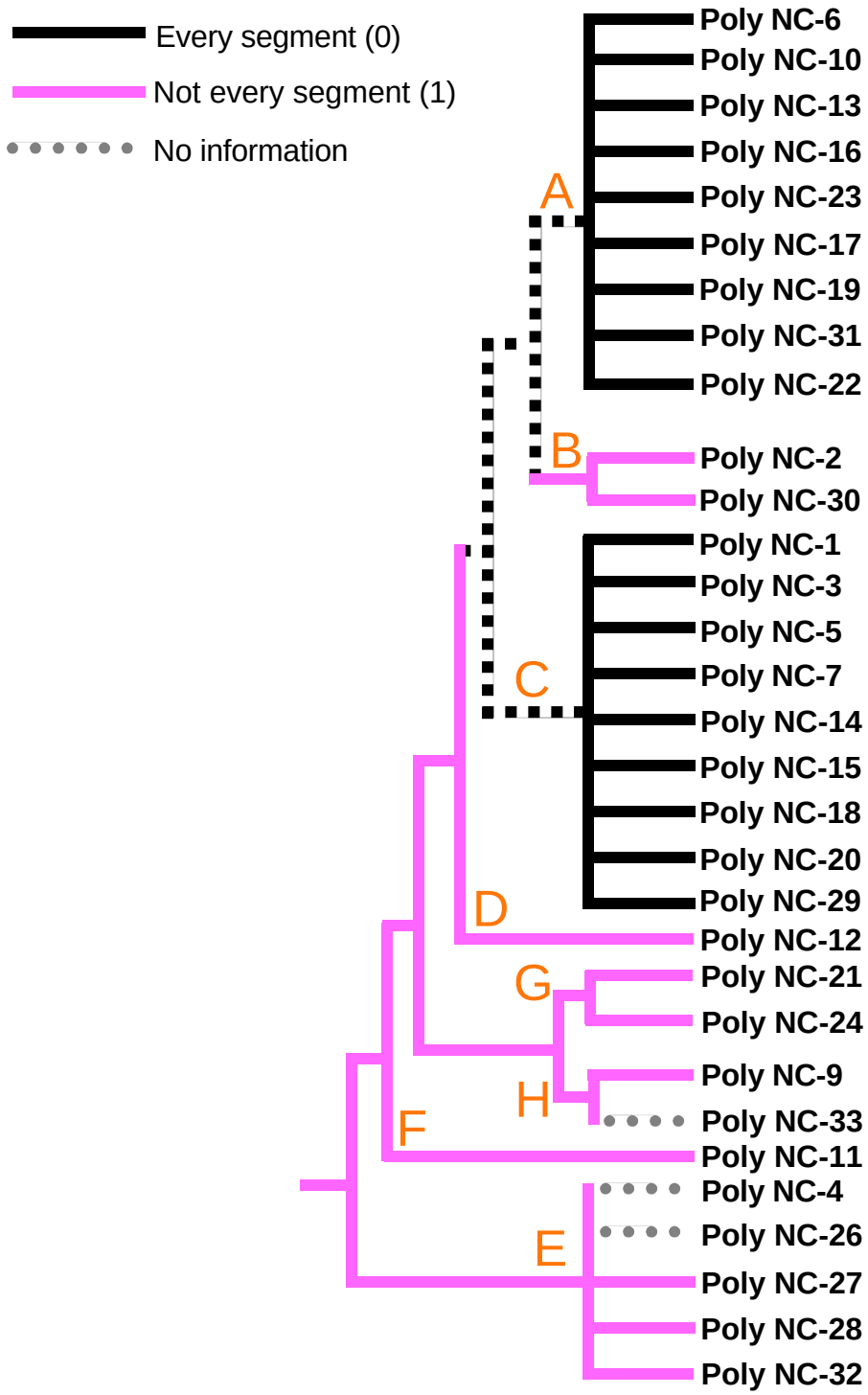

Figure 13. The number of segments between trichoblasts was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

had trichoblasts every segment. This character could not be determined for three out the 31 samples studied.

Apical Cells (Characters 9 and 10)

All but one North Carolina sample showed conspicuous apical cells (Figure 14) and all the samples in this study displayed transverse apical divisions (Figure 15).

Nature of the Holdfast (Character 11)

The nature of the holdfast was determined in 28 out of the 31 samples examined, and found to be consistent in seven of the eight species (Figure 16). Species C varied in the nature of the holdfast with samples in this species having all three-character states: thallus erect, arising from a single basal holdfast; erect branches initially arising from a basal holdfast, sometimes becoming prostrate, and erect branches arising from a prostrate branching system.

Branching Pattern, Shape and Frequency (Characters 12, 13 and 15)

Branching pattern was a variable character for the samples in this study. Only four out of the eight species were consistent for this character but two of these four species were represented by only one sample (Figure 17). Species A, B, C and H included samples that varied in branching pattern. The shape of ultimate branches was also inconsistent between samples within the North Carolina species (Figure 18). The frequency of branching was found to be consistent in only half the North Carolina species (Figure 19). Species A, B, C, and E included at least one sample that did not agree with the other samples in the clades.

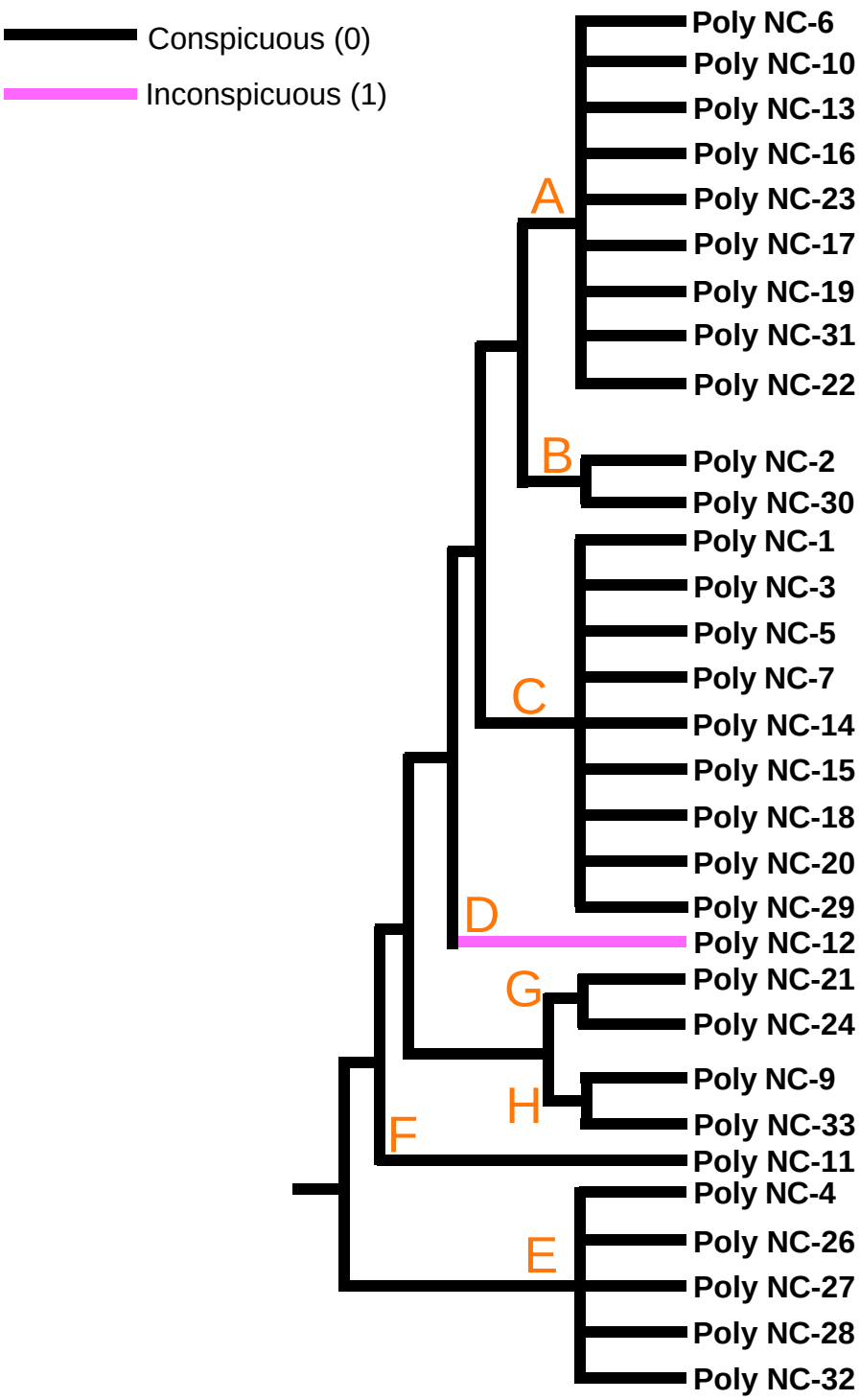

Figure 14. The nature of the apical cells was mapped upon a cladogram depicting the relationship of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

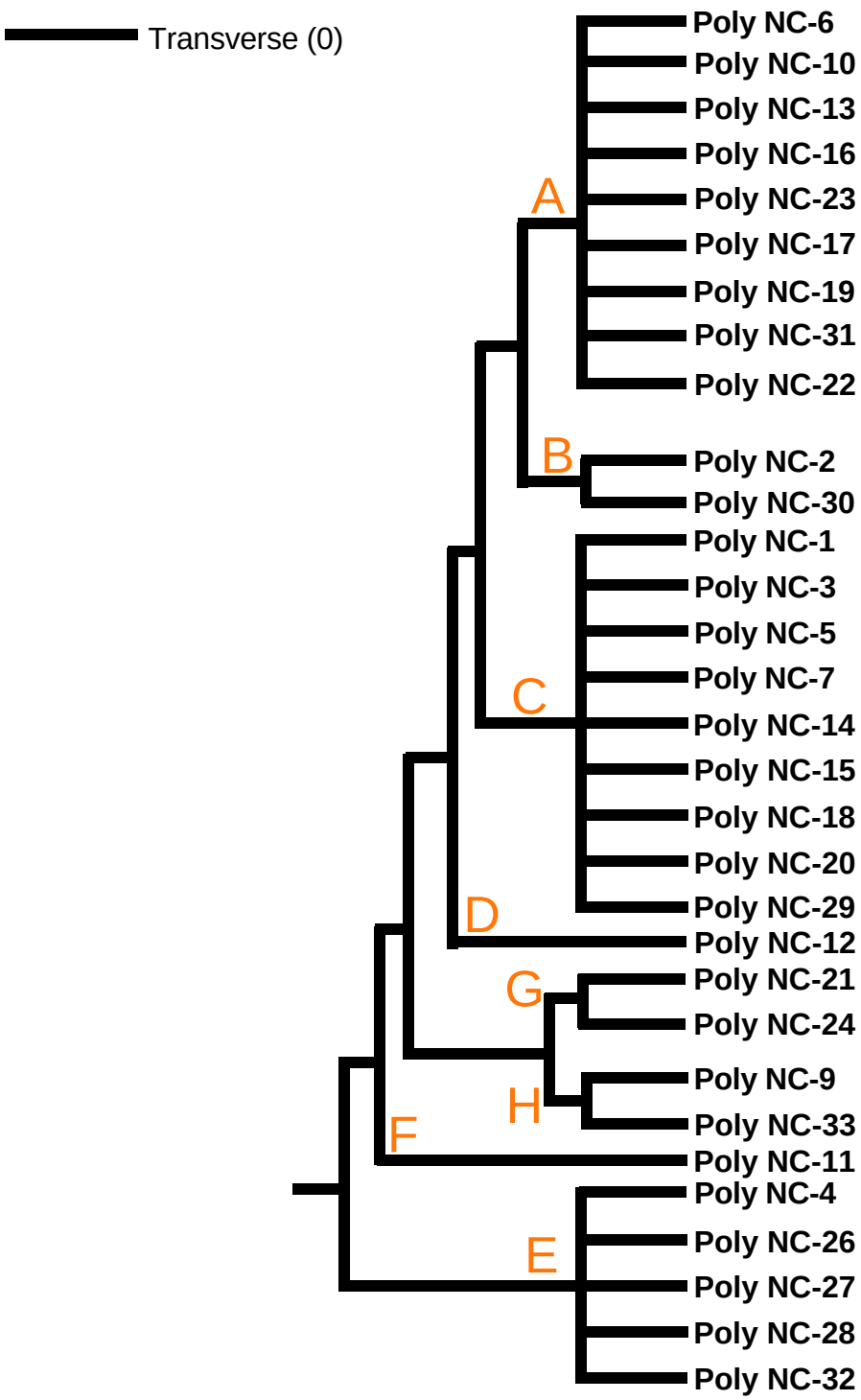

Figure 15. The apical cell divisions was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letter A-H.

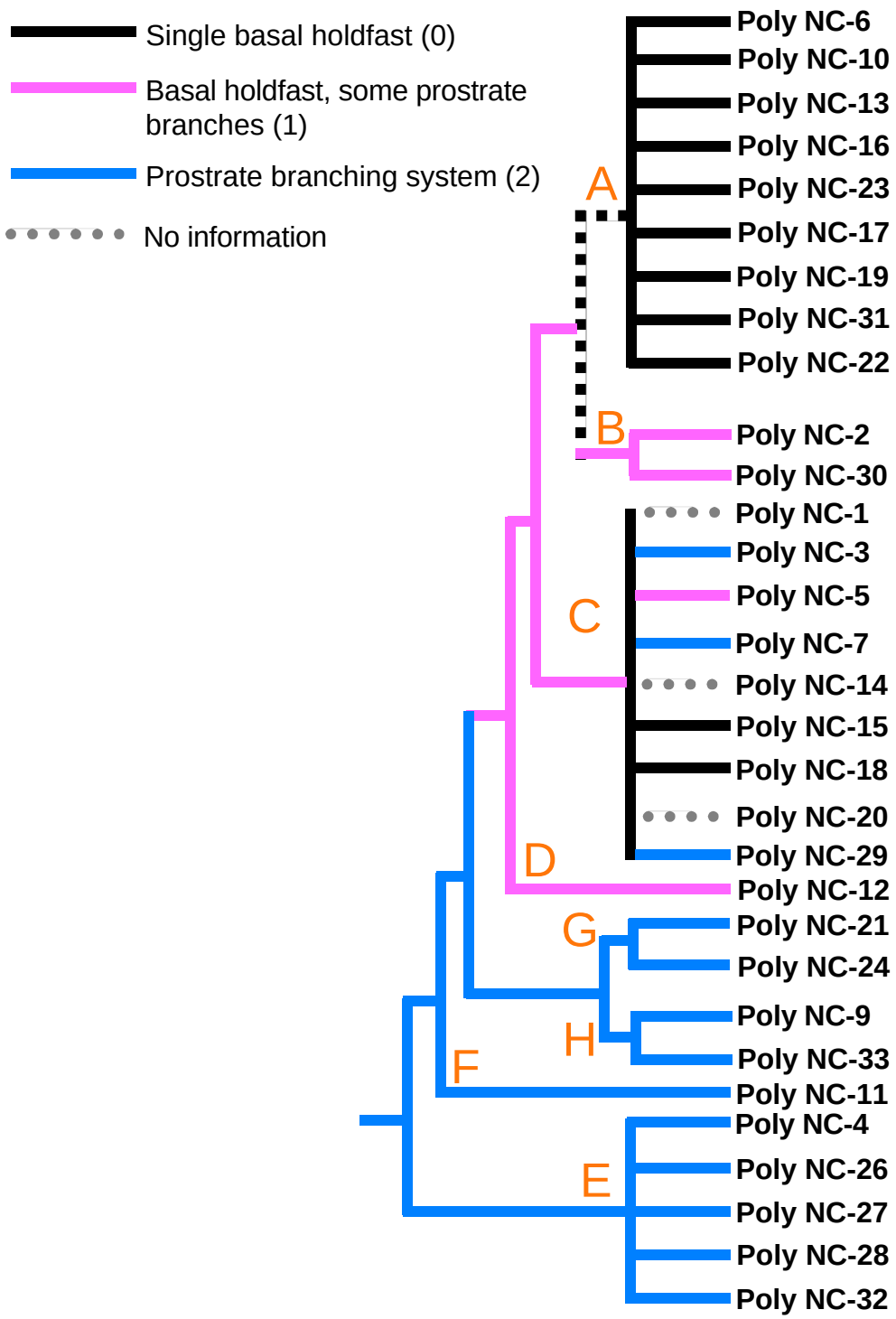

Figure 16. The nature of the holdfast was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letter A-H.

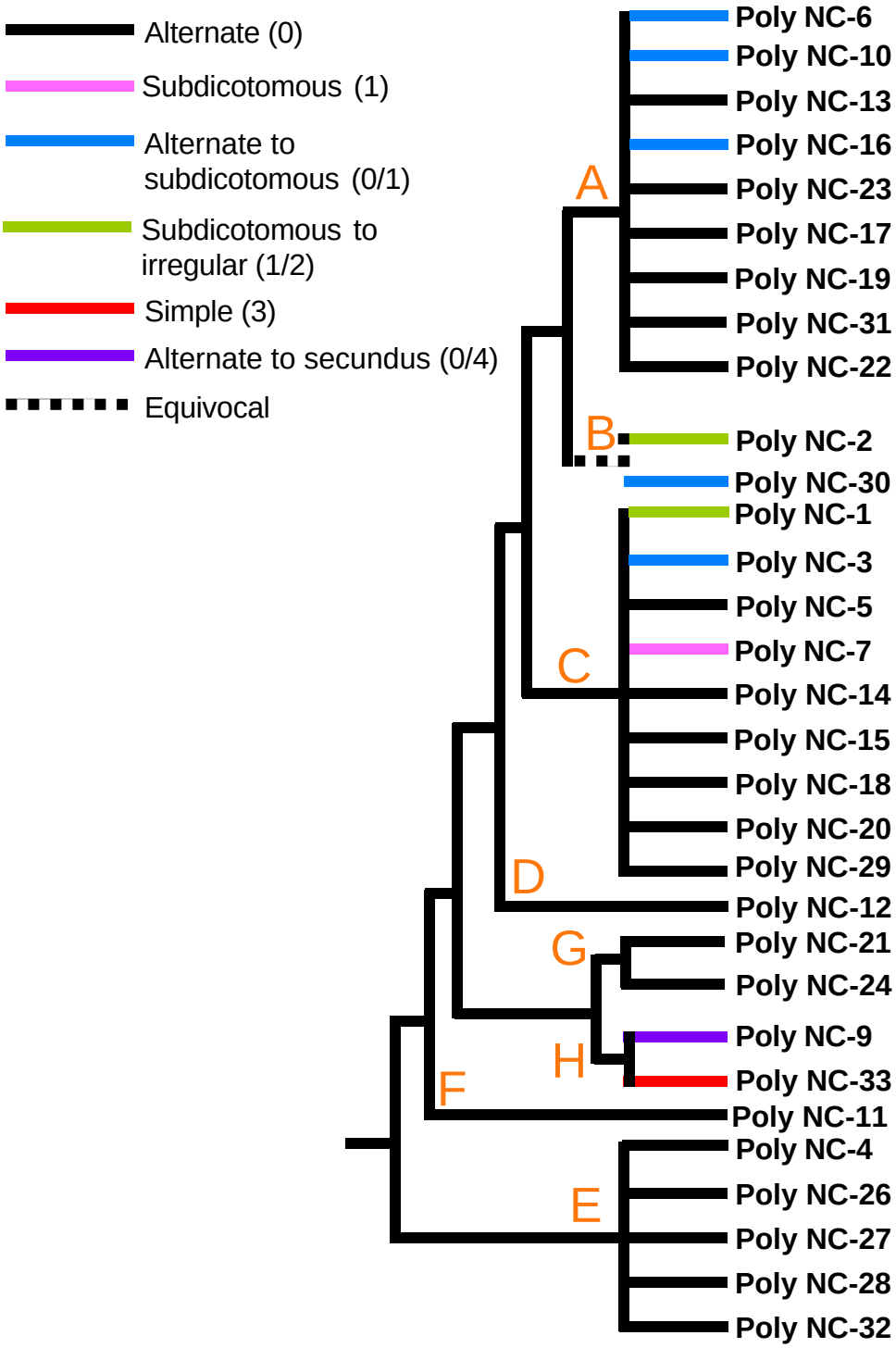

Figure 17. The branching pattern was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letter A-H.

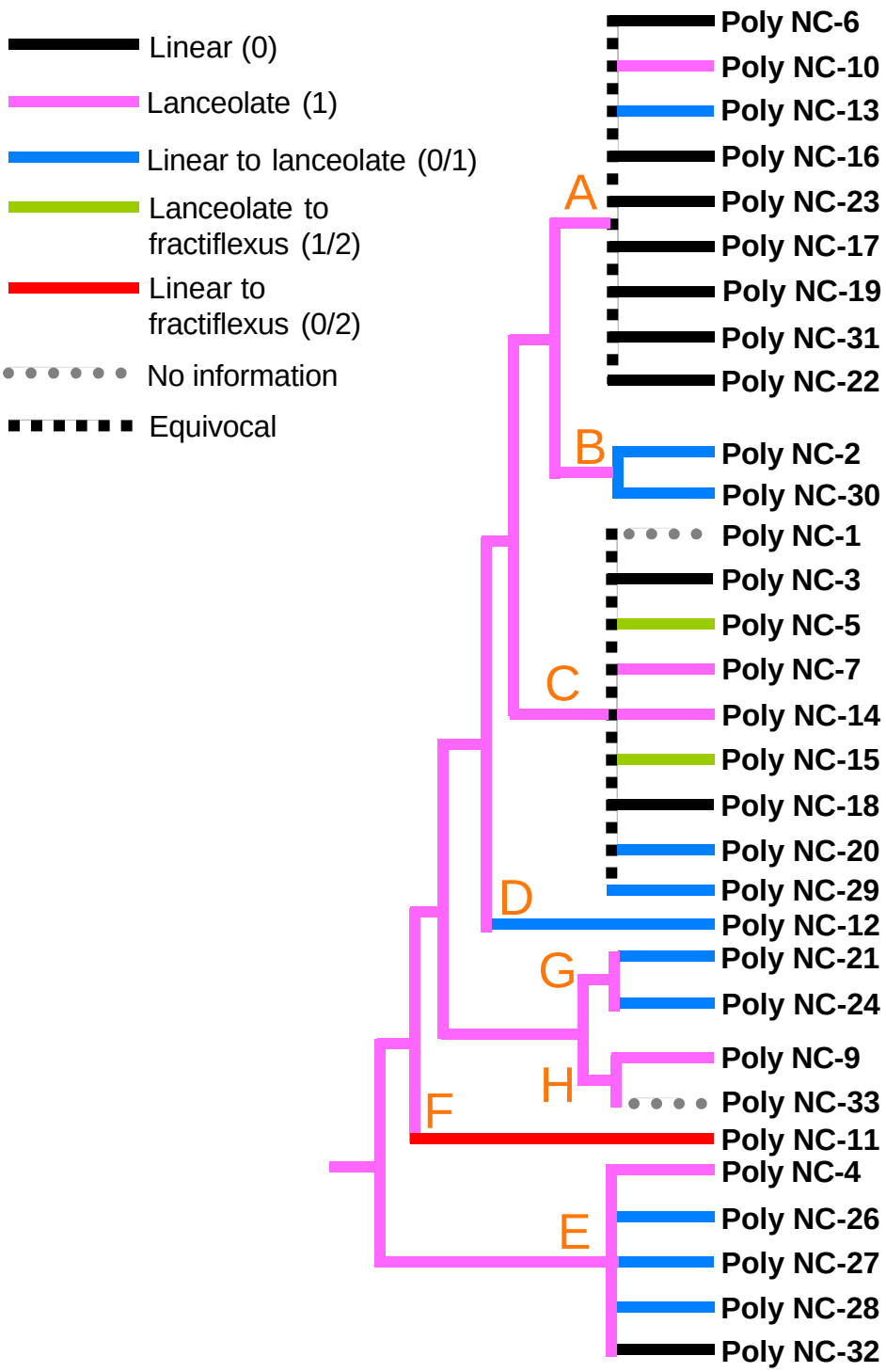

Figure 18. The shape of the ultimate branches was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letter A-H.

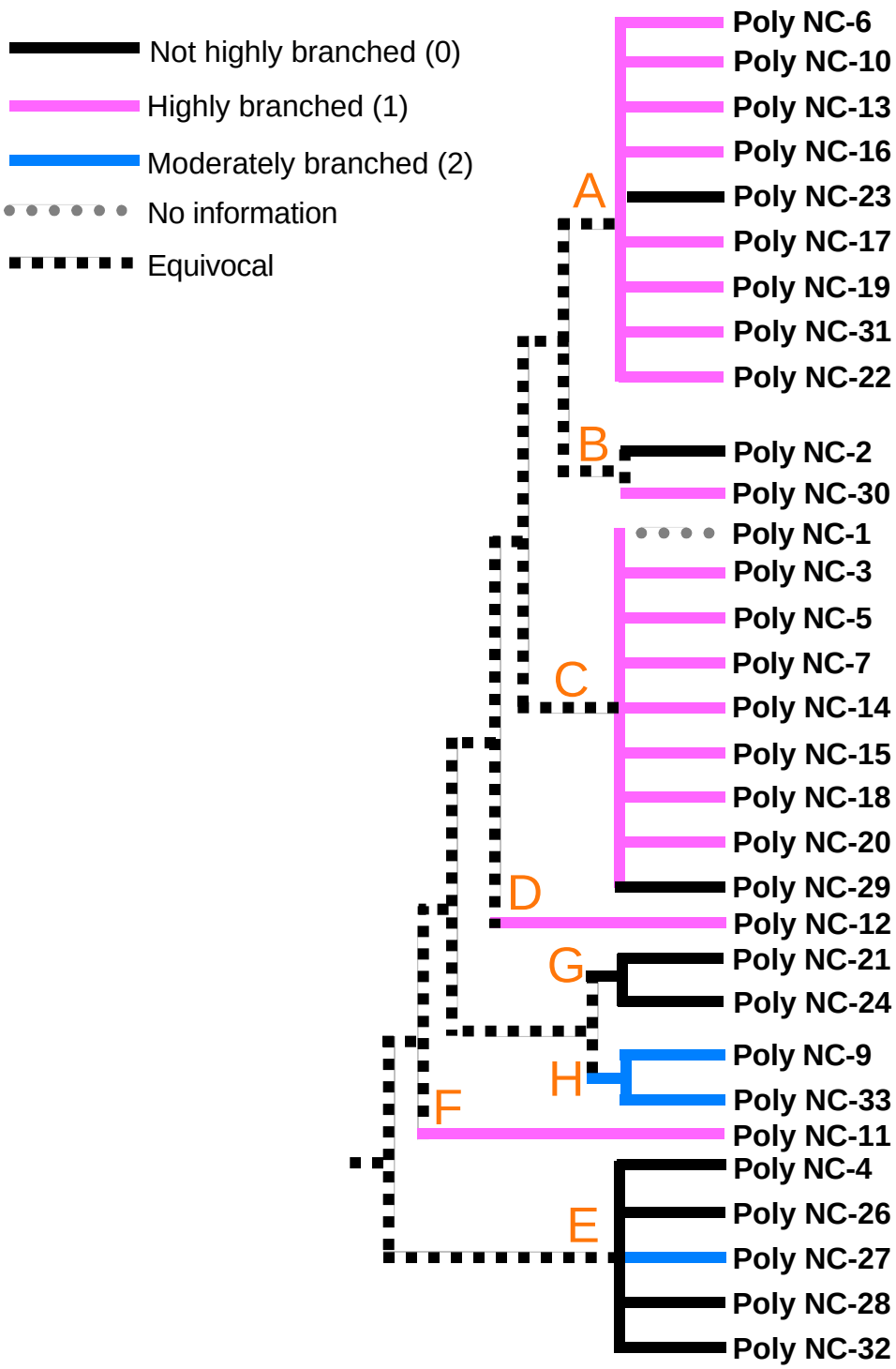

Figure 19. The frequency of branching was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letter A-H.

Apices of Ultimate Branches (Character 14)

All observed apices were acute to some degree. Only three out of the eight species were consistent for this character (Figure 20). All samples in species B and G were scored as narrowly acute while apices observed in the one sample examined from species D were acute.

Scar cells (Characters 16, 17, and 18)

Scar cells were present in all but two of the 31 examined samples and this character was consistent in six of the eight species (Figure 21). When scar cells were present their pattern was also consistent in all species (Figures 22 and 23). Three species had scar cells in a spiral pattern; the remaining five species displayed no distinct pattern. The production of branches from scar cells could be scored with certainty in 23 of the 31 samples, and where scored it was consistent within the species (Figure 24). Two species comprised of multiple samples had braches that arose from the scar cells. The remaining species had no scar cells with branches or this character could not be confidently determined from the available material.

Fertile Material (Characters 19, 20, 21, and 22)

Characteristics of reproductive structures were examined when fertile material was present in samples. Thirteen of the 31 samples studied included female gametophytes with cystocarps (Figure 25). No obvious pattern in cystocarp shape was found for the species. Cystocarp shape was variable not only within species but also within samples. Only seven samples included spermatangial plants and six of these were from species A (Figure 26). Within this species all the samples had spermatangial branches occupying one furcation of the trichoblasts (Figure 27). All

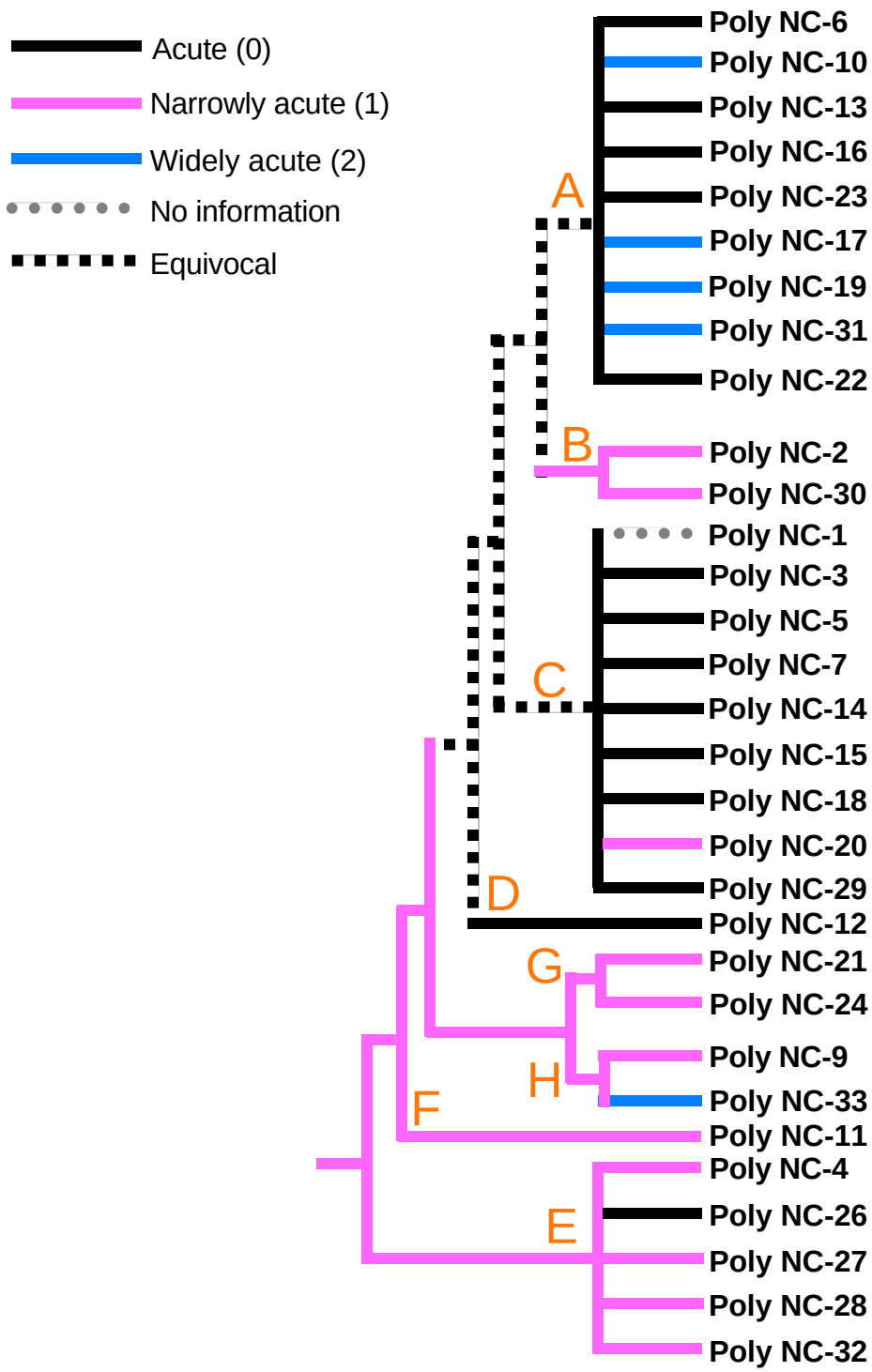

Figure 20. The shape of ultimate branch apices was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

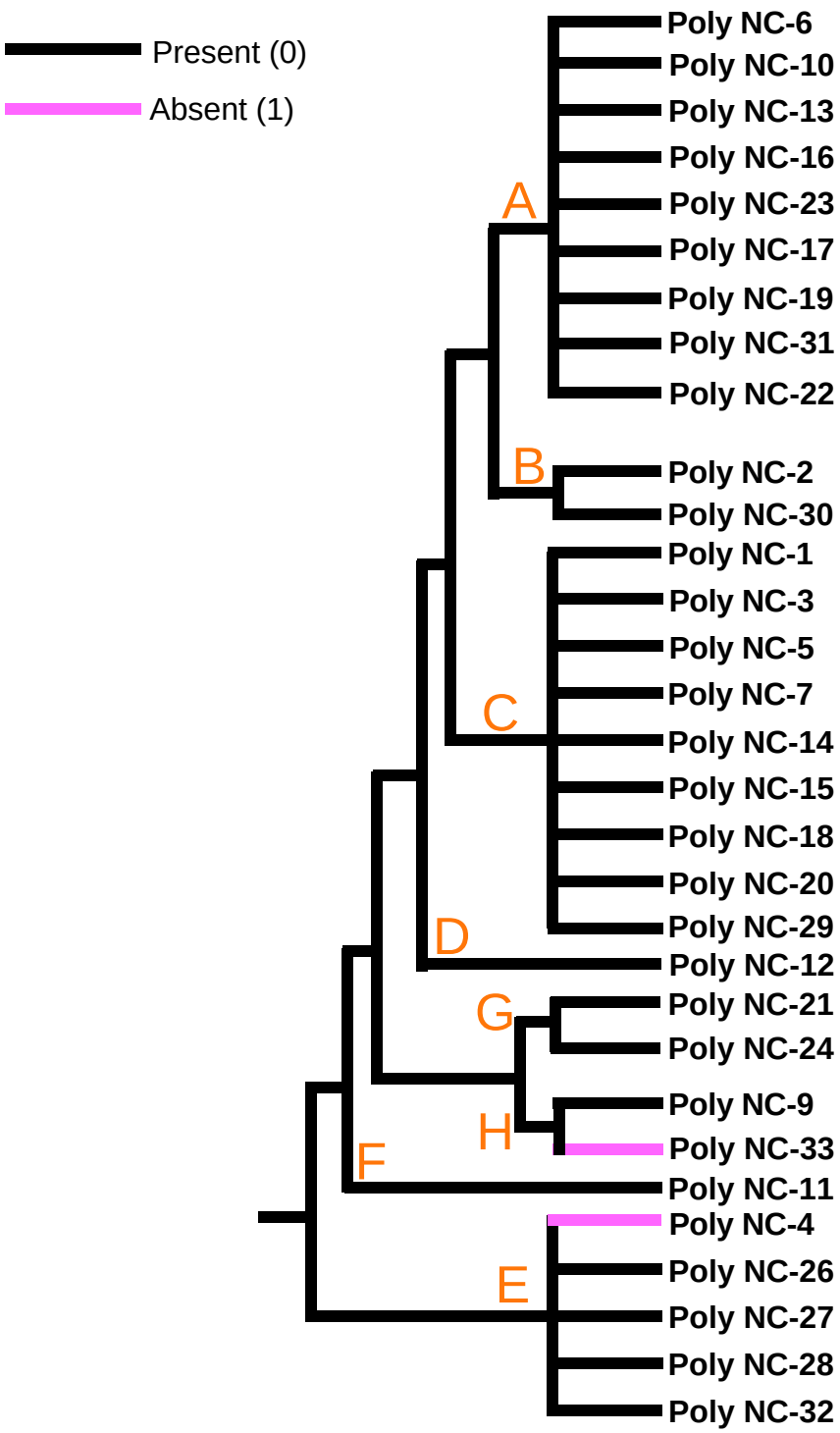

Figure 21. The presence and absence of scar cells was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

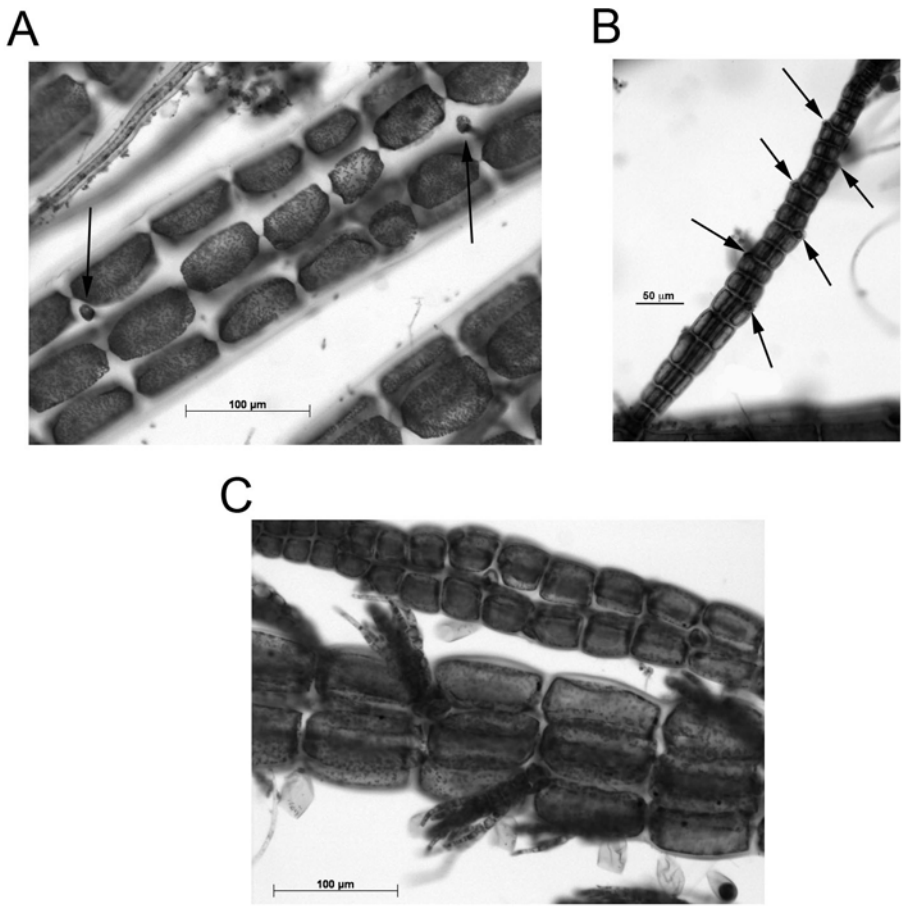

Figure 22. Pattern of scar cells and cicatrigenous branching observed in *Polysiphonia* samples. A. *Polysiphonia denudata sensu* Kapraun with no scar cell pattern, Poly NC-2. B. Branch of a *P. breviarticulata* with a distinct pattern, arrows pointing to scar cells in a spiral series, Poly NC-15. C. Main axes with adventitious laterals arising from scar cells (cicatrigenous branching), *P. breviarticulata*, Poly NC-18.

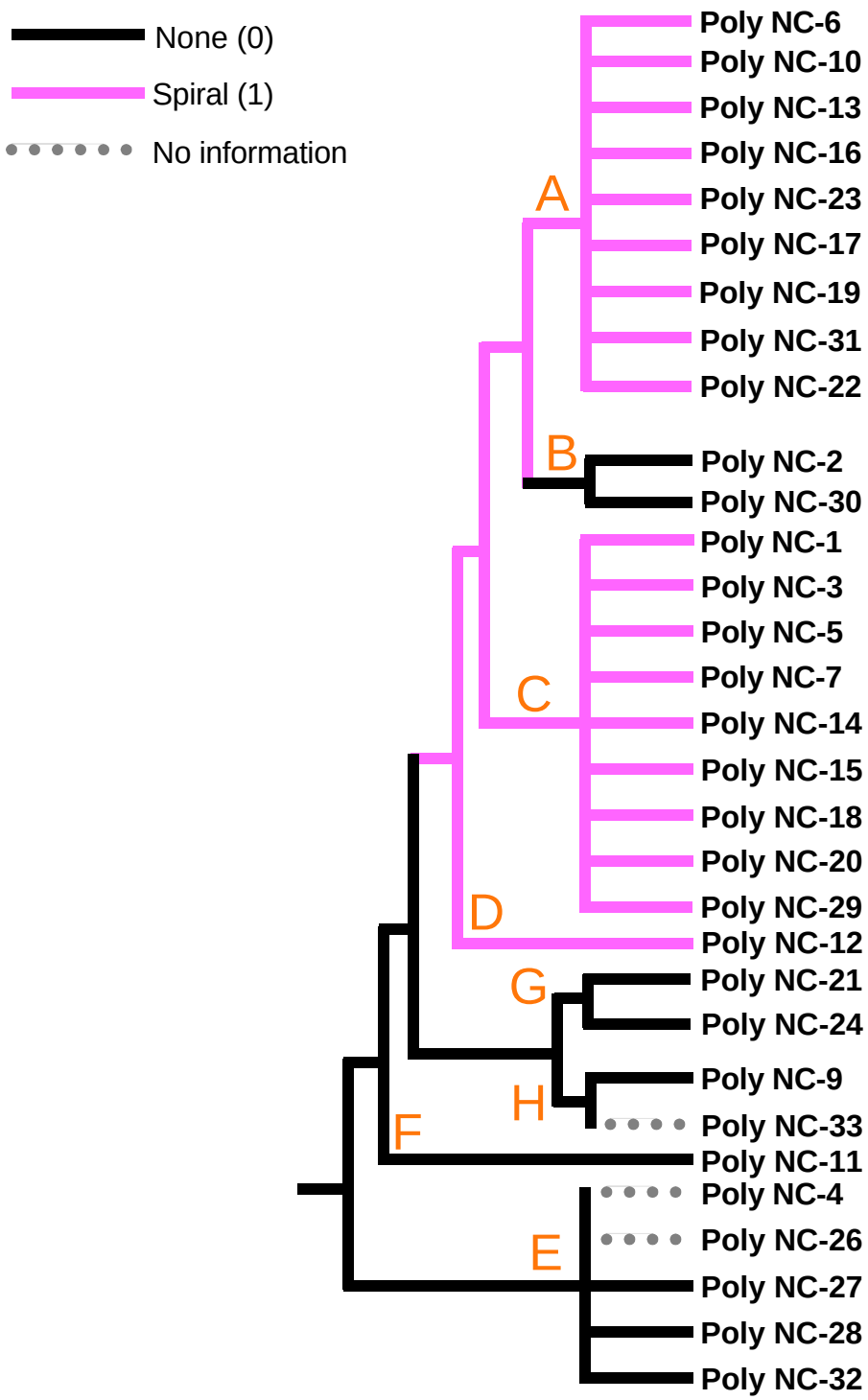

Figure 23. The pattern of the scar cells was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

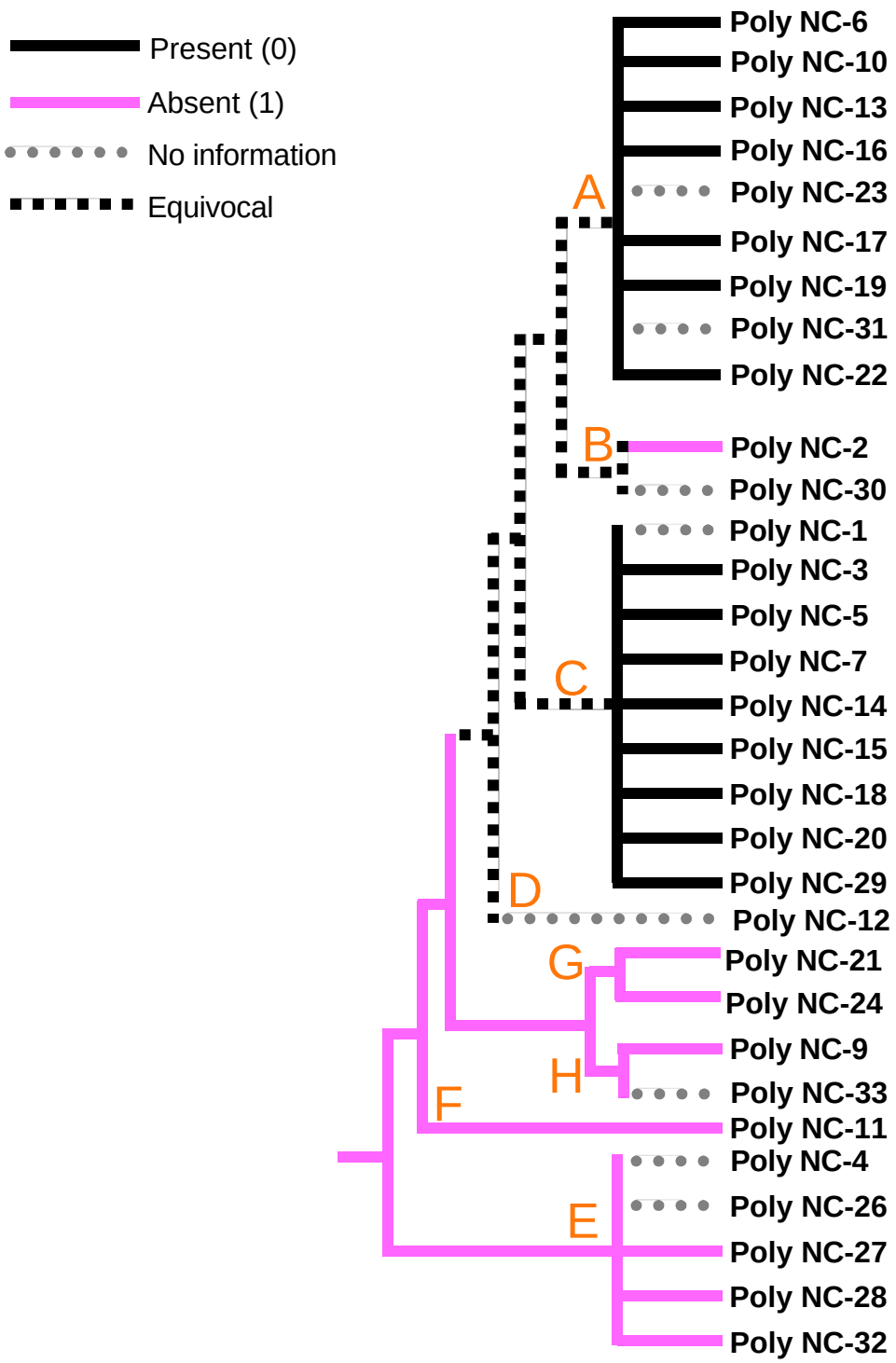

Figure 24. The production of lateral branches by scar cells was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

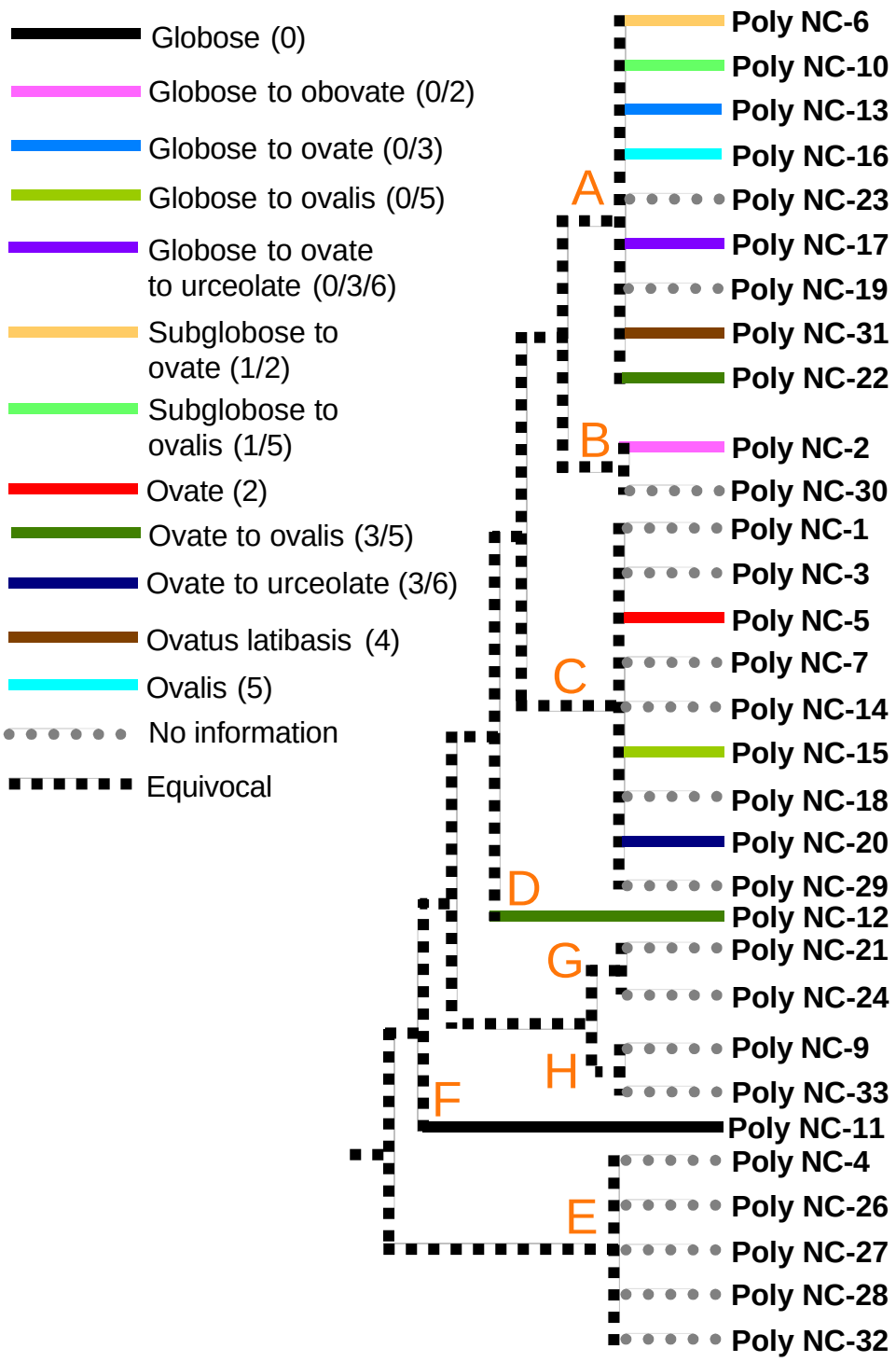

Figure 25. Cystocarp shape was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

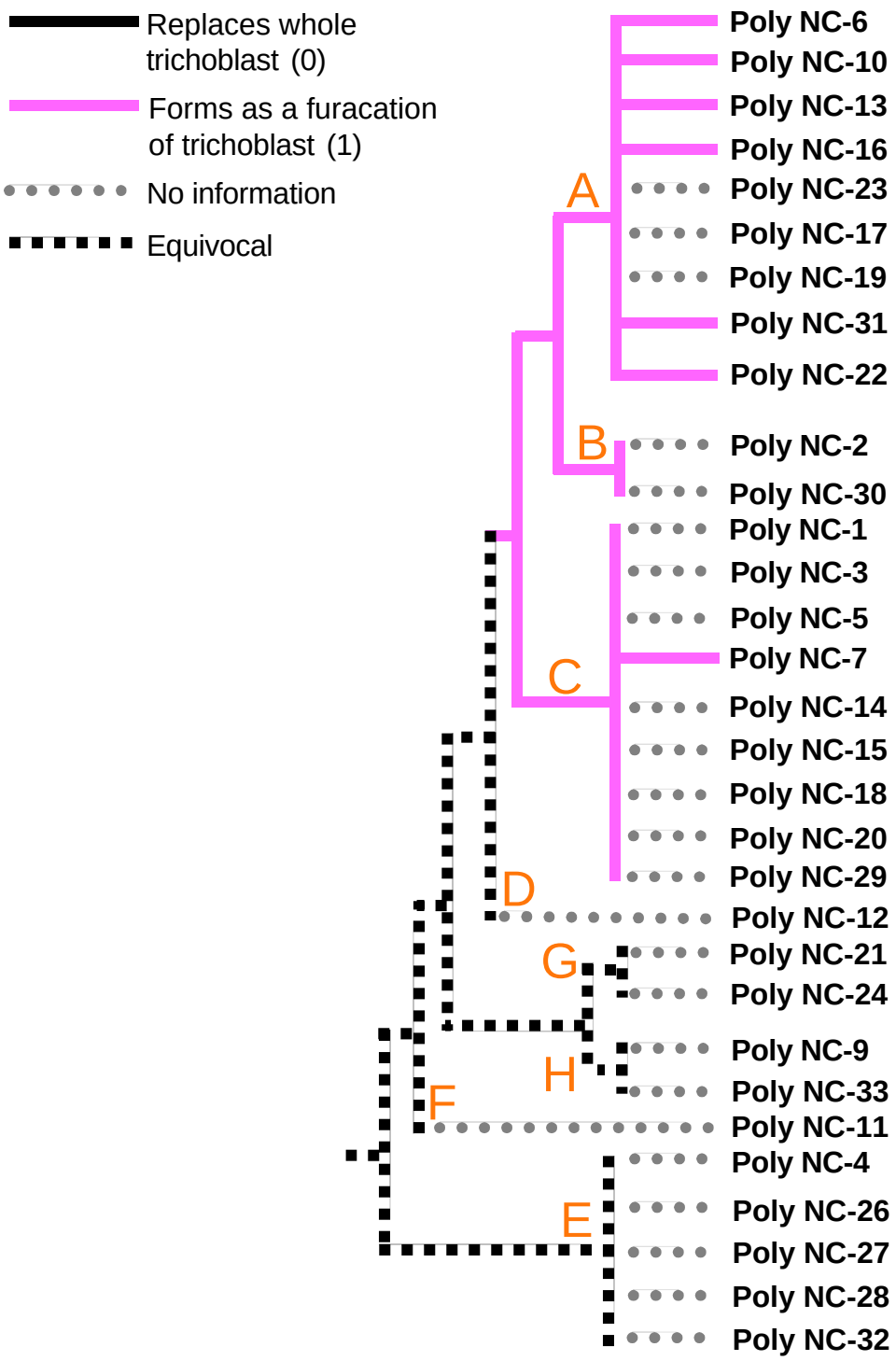

Figure 26. Development of spermatangial axes was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

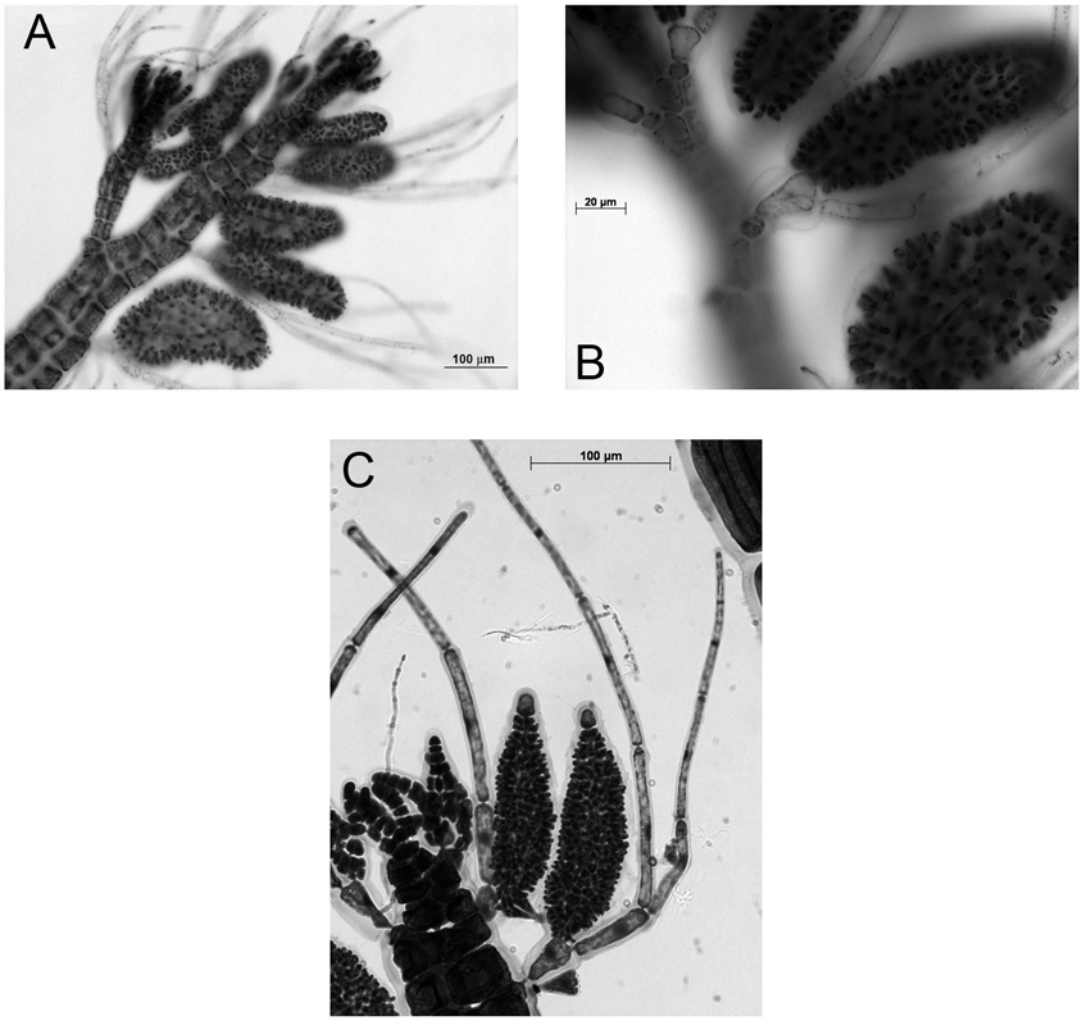

Figure 27. Spermatangial axes development observed in *Polysiphonia* samples. A. Multiple spermatangia at *P. breviarticulata* branch tips, with no sterile cells present, Poly NC-25. B. Close up of spermatangia, showing spermatangia developing as a furcation of trichoblast, *P. breviarticulata*, Poly NC-25. C. Spermatangia developing as a furcation of trichoblast, with one sterile cell at tip, *Neosiphonia harveyi*, Poly NC-6.

samples with spermatangial plants were also found to have one or no sterile cells present at the tips of spermatangial branches (Figure 28). The lack of spermatangial plants makes it difficult to establish if the character states for these characters are consistent within the species.

The arrangement of tetrasporangia was consistent in all species that included multiple tetrasporic samples (Figures 29 and 30). Species A and C both had samples with tetrasporangia in a spiral. A straight series of tetrasporangia was seen in species B, E, F, G and H.

Morphological Analyses of All Species Included in Study

Eleven of the 22 morphological characters found in Table 4 were used to examine all of the species of Rhodomelaceae included in this study. The character states for each species (Table 6) were mapped onto cladograms resulting from phylogenetic analyses of both the *rbc*L and SSU sequence data.

Number of Pericentral Cells (Character 1)

All of the samples included in this study had pericentral cells that were within the known range for *Polysiphonia*. All species in major clades three and four had four pericentral cells (Figures 31 and 32). Clade two was comprised of species that had a highly elevated number of pericentral cells (more than eight). All three character states were observed in clade one. Species with a slightly elevated number of pericentral cells (five to seven) were resolved in clade one scattered among species that had four pericentral cells.

Rhizoid-Pericentral Cell Connection (Character 2)

All species in major clade one had rhizoids that were cut off from pericentral cells with a pit connection between the rhizoid and the pericentral cells (Figures 33 and 34). All species in major

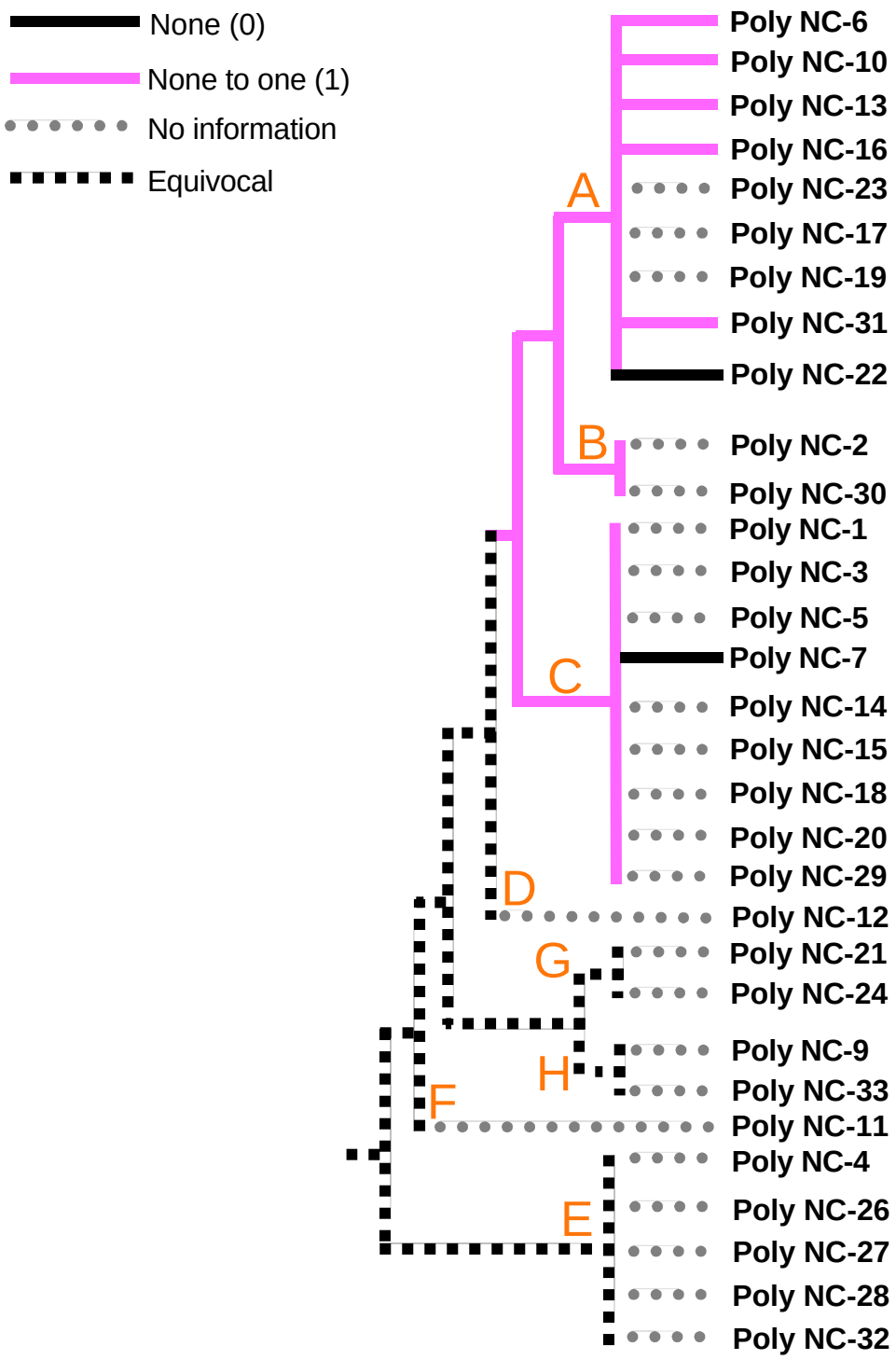

Figure 28. The number of sterile cells present on spermatangial branches was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

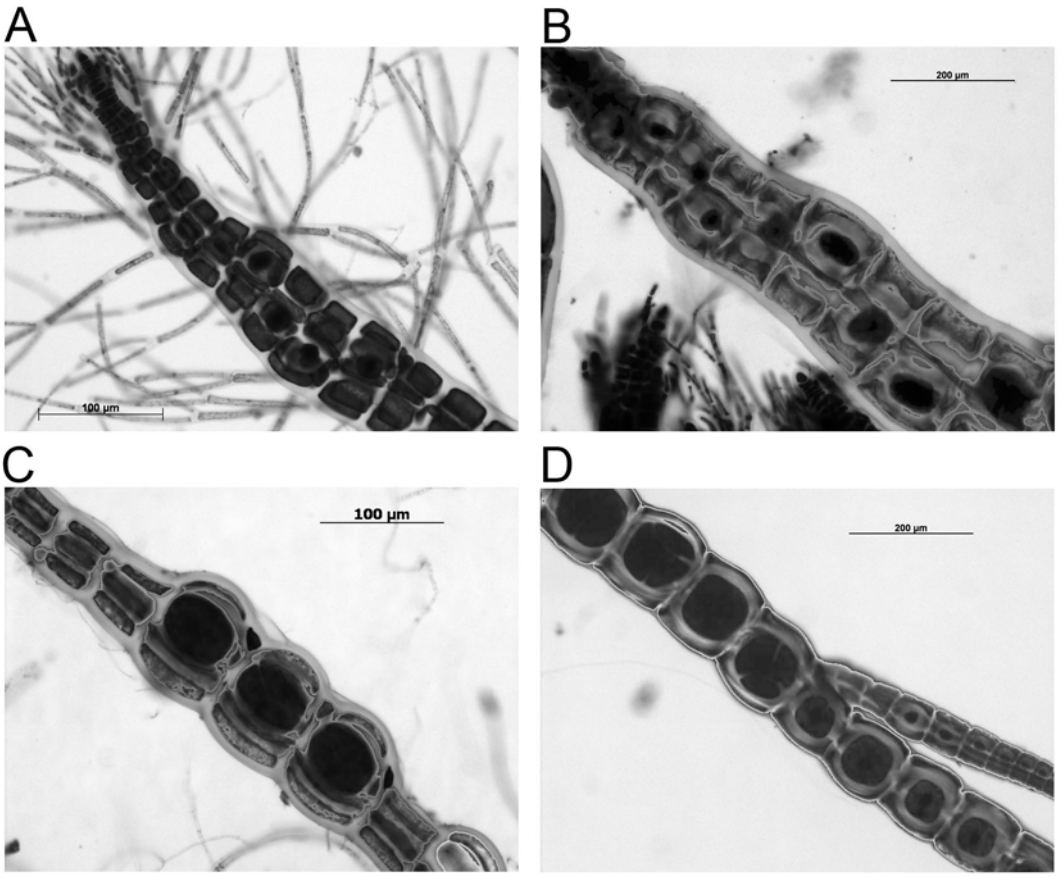

Figure 29. Arrangement of tetrasporangia observed in *Polysiphonia* samples. A. Tetrasporangia arranged in a spiral series, *P. breviarticulata*, Poly NC-7. B. *Neosiphonia harveyi* with spirally arranged tetrasporangia, Poly NC-16. C. Tetrasporangia arranged in a short, straight series, *Polysiphonia scopulorum* var. *villum*, Poly NC-9. D. *Polysiphonia denudata sensu* Kapraun displaying a long, straight series of tetrasporangia, Poly NC-30.

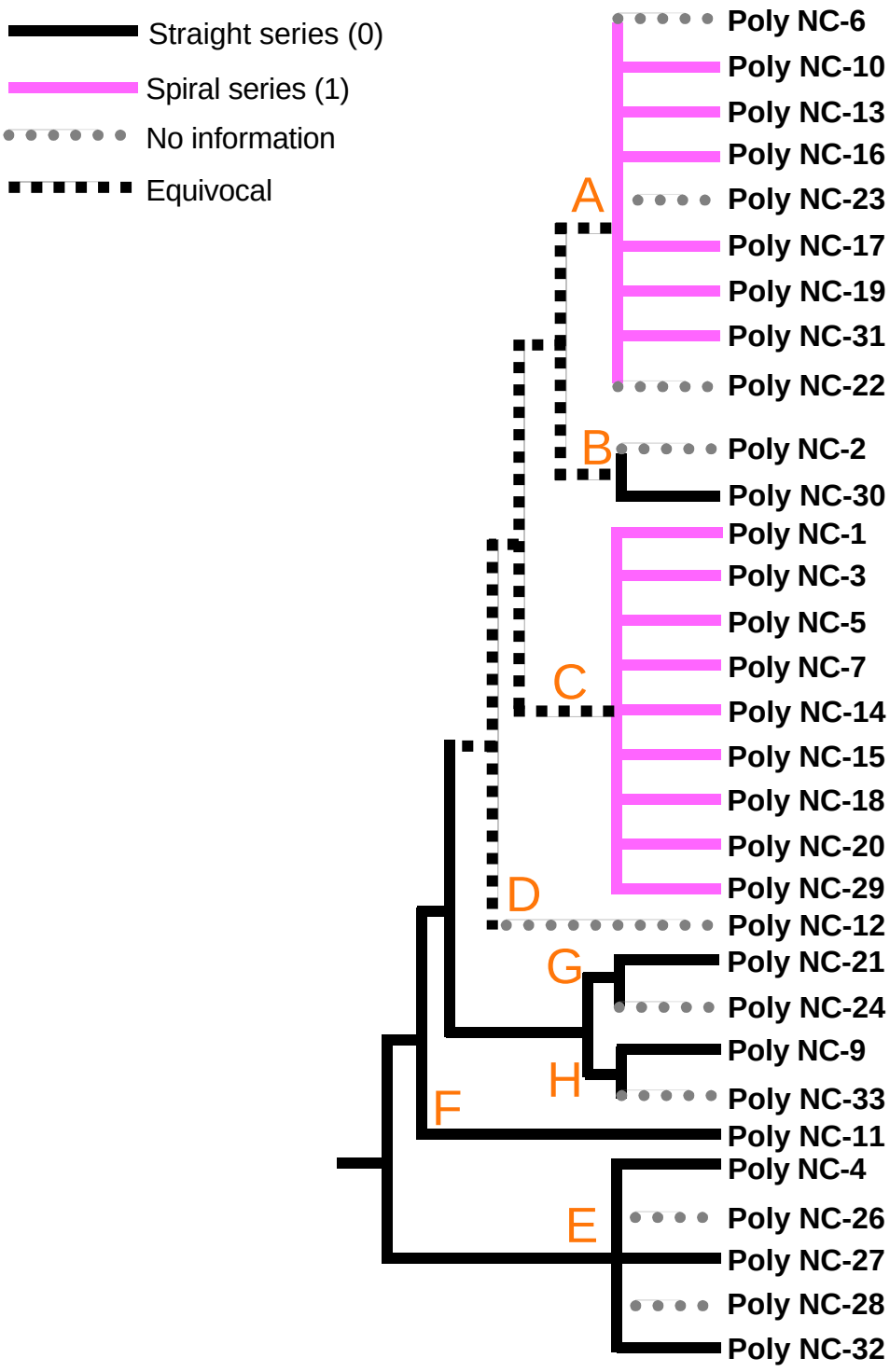

Figure 29. The arrangement of tetrasporangia was mapped upon a cladogram depicting the relationships of 31 North Carolina *Polysiphonia* samples based upon *rbc*L and SSU sequence analyses. The eight North Carolina species are indicated by letters A-H.

Table 6. Data matrix of character states for anatomical characters for all rhodomelacean species investigated in this study. Character numbers follow those in Table 4.

	Characters											
Species	1	2	3	4	8	11	16	17	18	20	22	References[a]
Boergeseniella fruticulosa	2	?	0	0	0	1	1	?	1	1	1	1
Digenia simplex	2	?	0	?	?	0	?	?	?	?	1	2, 3, 4,
Enelittosiphonia stimpsonii	2	1	1	?	1	2	?	?	?	1	1	5[b], 6
Herposiphonia tennue	2	1	1	?	1	2	0	1	?	0	1	7,8
Neosiphonia akkeshiensis	0	1	0	0	0	0	0	?	0	1	1	9
N. japonica	0	1	0	0	0	0	0	?	0	1	1	9
N. savatieri	0	1	1	2	0	1	0	1	?	1	1	10[c],11
Polysiphonia brodiaei	1	1	0	1	0	1	0	1	?	1	1	1,12,13,14
P. denudata	1	1	0	1	0	1	0	1	?	1	1	1,14
P. elongata	0	1	0	0	0	0/1	0	1	0	0/1	1	1,14
P. elongella	0	?	0	0	0	0	0	1	0	?	1	1
P. fibrata	0	1	0/1	1	0	2	0	1	?	1	1	1
P. fibrillosa	0	1	0	1	0	1	0	1	0	1	1	1
P. forfex	1	1	0	0	0	0	0	?	0	0	1	13
P. fucoides	2	1	0	0	0	2	0	1	0	1	1	1
P. harveyi	0	1	0	0	0	0	0	1	0	1	1	15
P. lanosa	2	0	1	2	1	1	1	?	?	0	1	1,16
P. morrowii	0	0	1	?	1	2	?	?	?	0	0	17,18
P. nigra	2	1	1	1	1	1	0	?	?	0/1	1	1
P. pacifica	0	0	1	?	1	2	0	0	1	0	0	19, 20
P. paniculata	2	1	1	1	0	2	0	1	?	0	1	20
P. scopulorum	0	0	1	2	1	2	0	1	0	0	0/1	11, 12, 13
P. stricta	0	0	1	0	1	2	0	0	1	0	0	1, 21
P. strictissima	0	?	0	?	?	1	?	?	?	1	1	12
P. virgata	2	?	0	?	?	0	1	?	?	?	0	22
Womersleyella setacea	0	1	1	?	0	2	0	1	?	?	1	10[d]

[a] 1, Maggs & Hommersand (1993); 2, Lawson & John (1987); 3, Littler & Littler (2000); 4, De Clerk *et al.* (2005); 5, Masuda *et al.* (1995); 6, Choi *et al.* (2001); 7, Hollenberg (1968c); 8, Schneider & Searles (1991); 9, Kudo & Masuda (1986) ;10, Hollenberg (1968a); 11, Abbott (1999); 12, Adams (1994); 13, Womersley (2003); 14, Kapraun (unpublished MS); 15, Stuercke (unpublished); 16, Kim *et al.* (2002); 17, Kudo & Masuda (1992); 18, Kim *et al.* (1994); 19, Hollenberg (1942); 20, Abbott & Hollenberg (1976); 21, Kim *et al.* (2000); 22, Stegenga *et al.* (1997)

[b] as *Polysiphonia stimpsonii* Harvey

[c] as *Polysiphonia savatieri* Hariot

[d] as *Polysiphonia setacea* Hollenberg

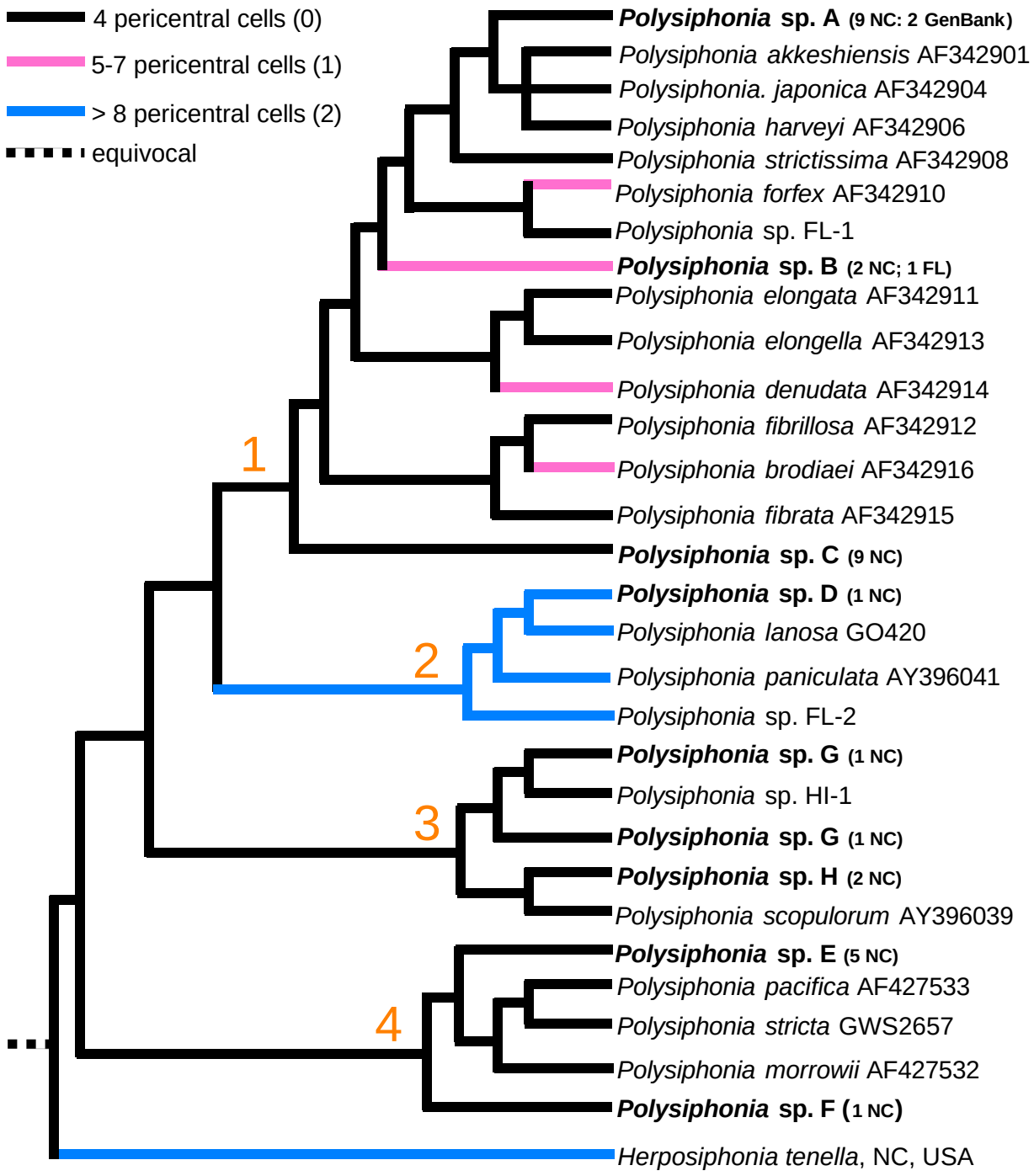

Figure 31. Number of pericentral cells was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

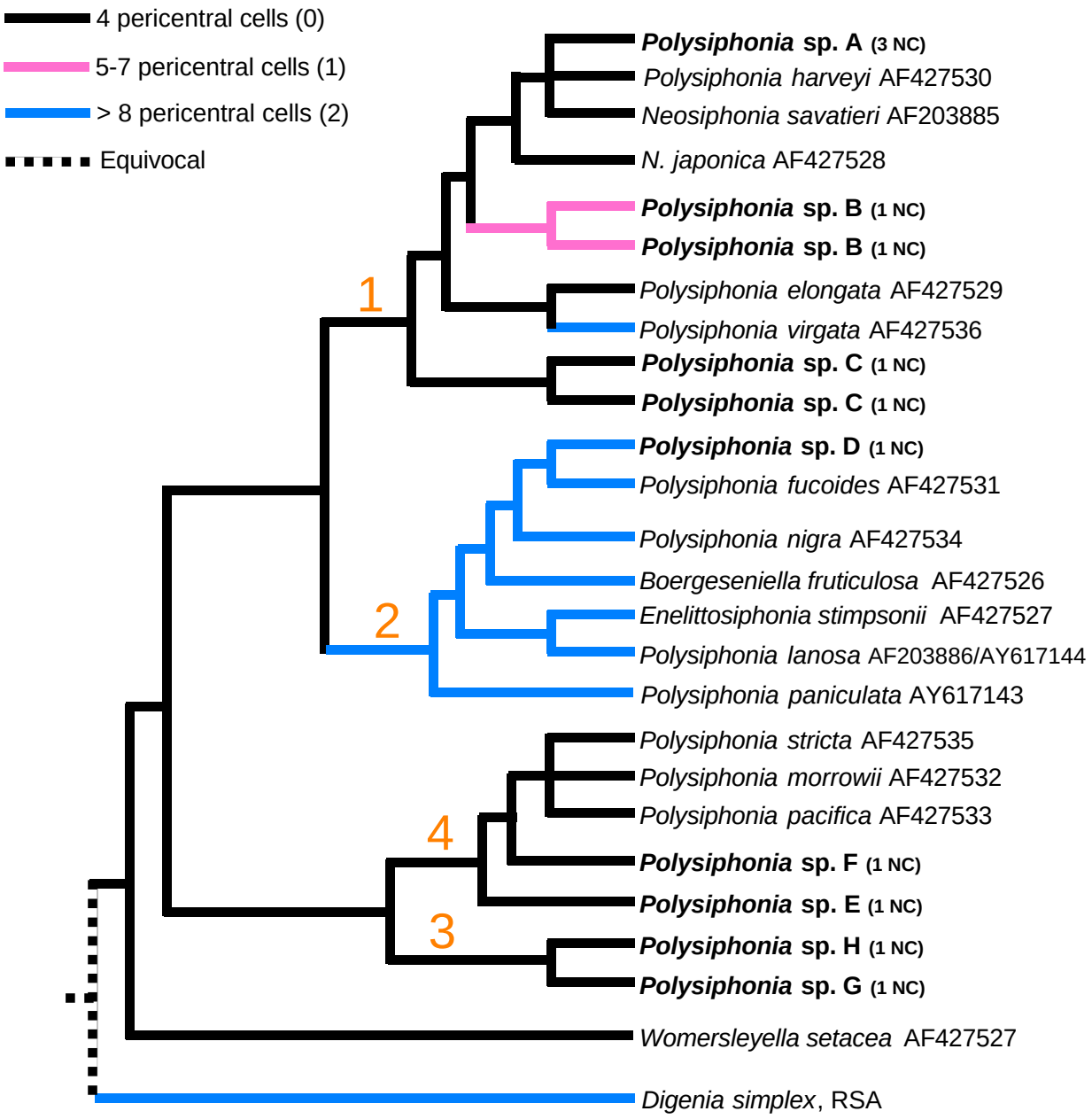

Figure 32. Number of pericentral cells was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

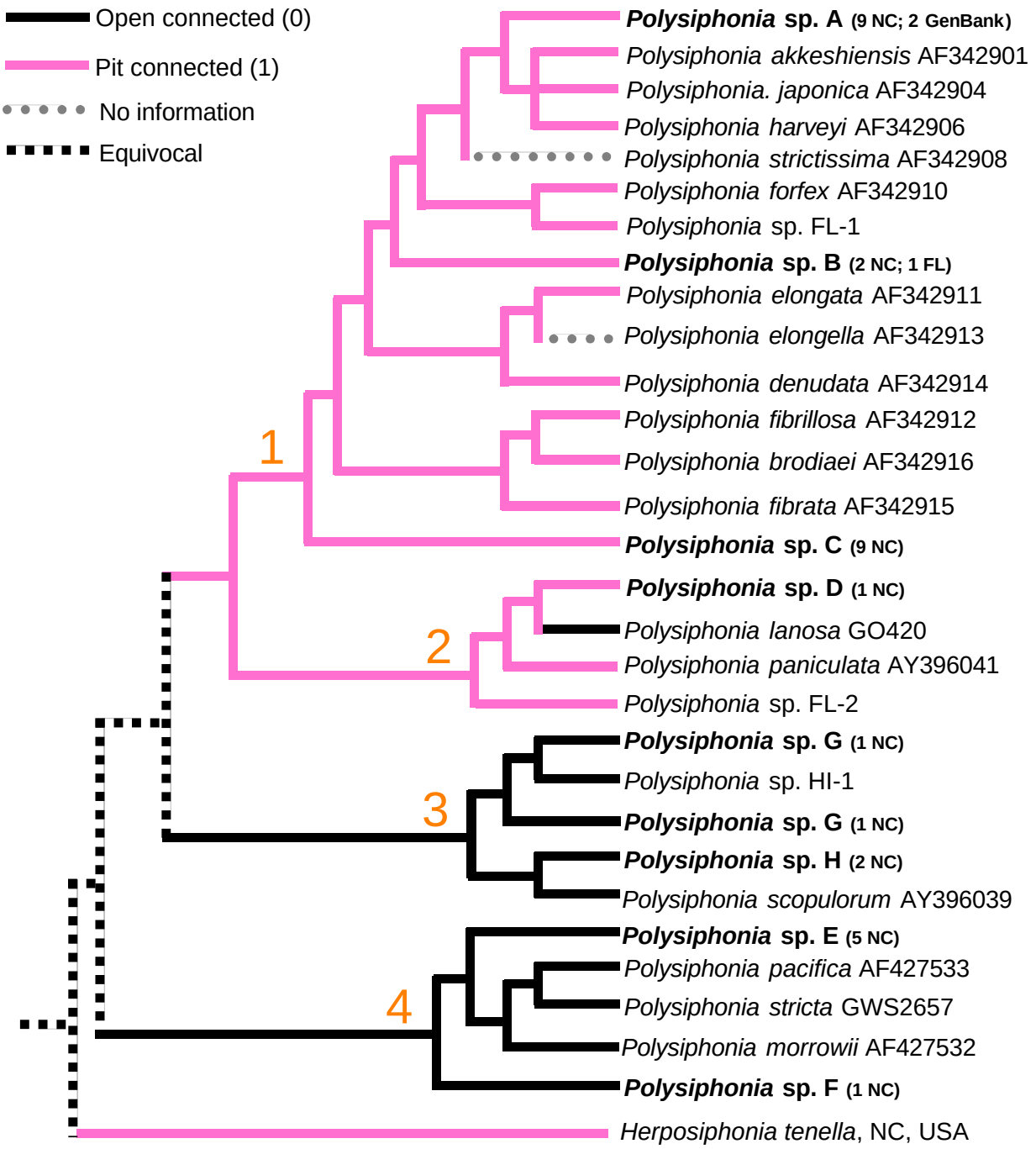

Figure 33. Rhizoid-pericentral cell connection was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

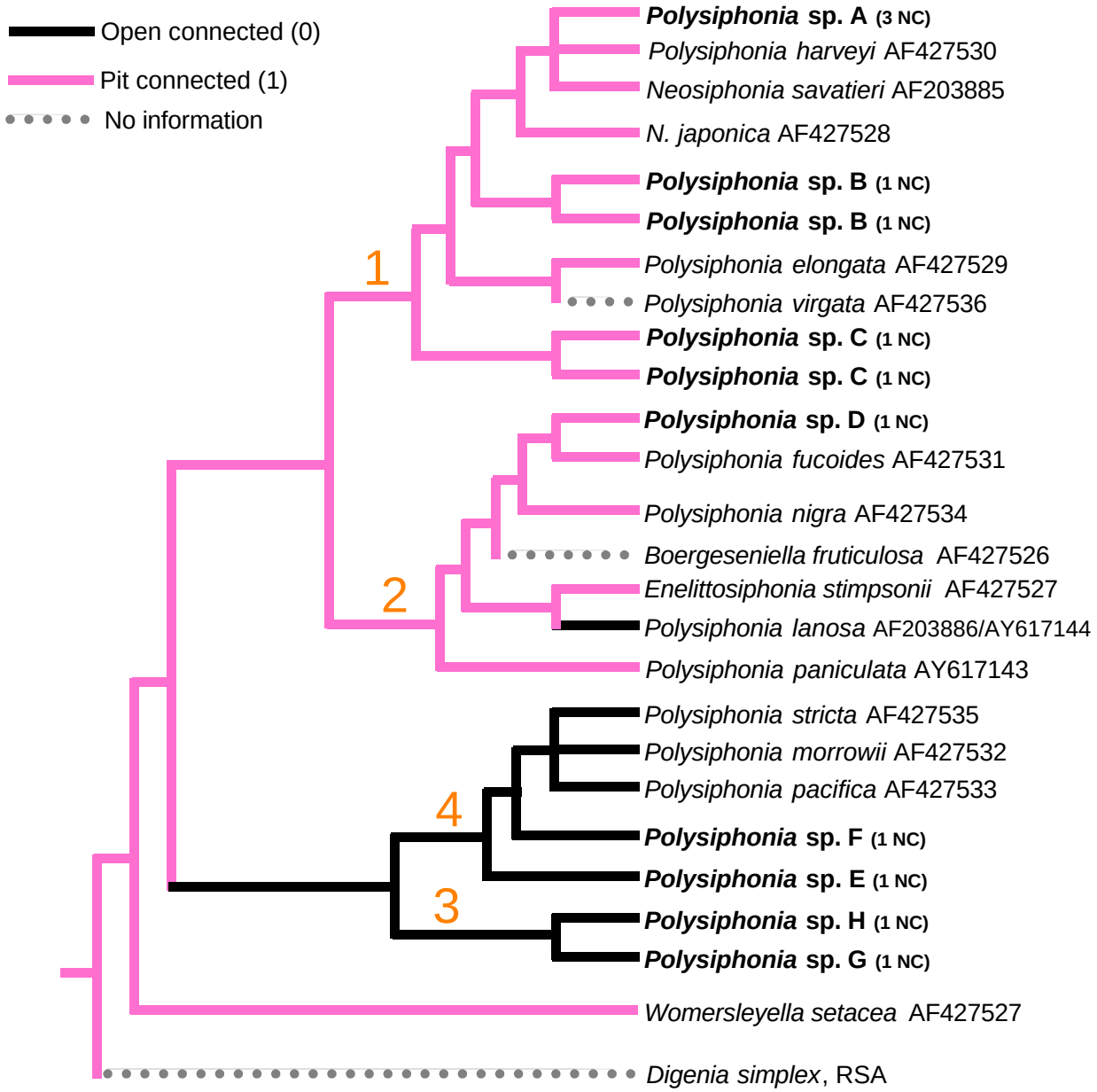

Figure 34. Rhizoid-pericentral cell connection was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

clades three and four had an open connection between the rhizoids and the pericentral cells. The majority of included species that were resolved in major clade two had pit-connected rhizoids, but *Polysiphonia lanosa* had open connected rhizoids, making this character inconsistent within this clade.

Cortication (Character 3)

Presence or absence of cortication displayed consistency in two of the four major clades in the molecular trees (Figures 35 and 36). Major clades three and four included species that were ecorticate throughout. Both character states were present in major clades one and two. Most species in major clade one had cortication.

Relationship of Lateral Branch and Trichoblasts (Character 4)

The relationship of lateral branches and trichoblasts was found to vary in all major clades except clade four, but information on this character was not available for all clade four species (Figures 37 and 38). All species resolved in clade three had lateral branches that replaced trichoblasts except for *Polysiphonia scopulorum* and species H, which had lateral branches that developed independently from trichoblasts. Species resolved in major clades one and two had lateral branches that developed independently from trichoblasts, in the axil of trichoblasts, or replaced trichoblasts.

The Number of Segments Between Trichoblasts (Character 8)

The *rbc*L and SSU trees both showed the same patterns of consistency for this character (Figures 39 and 40). The species resolved within major clades three and four did not have trichoblasts

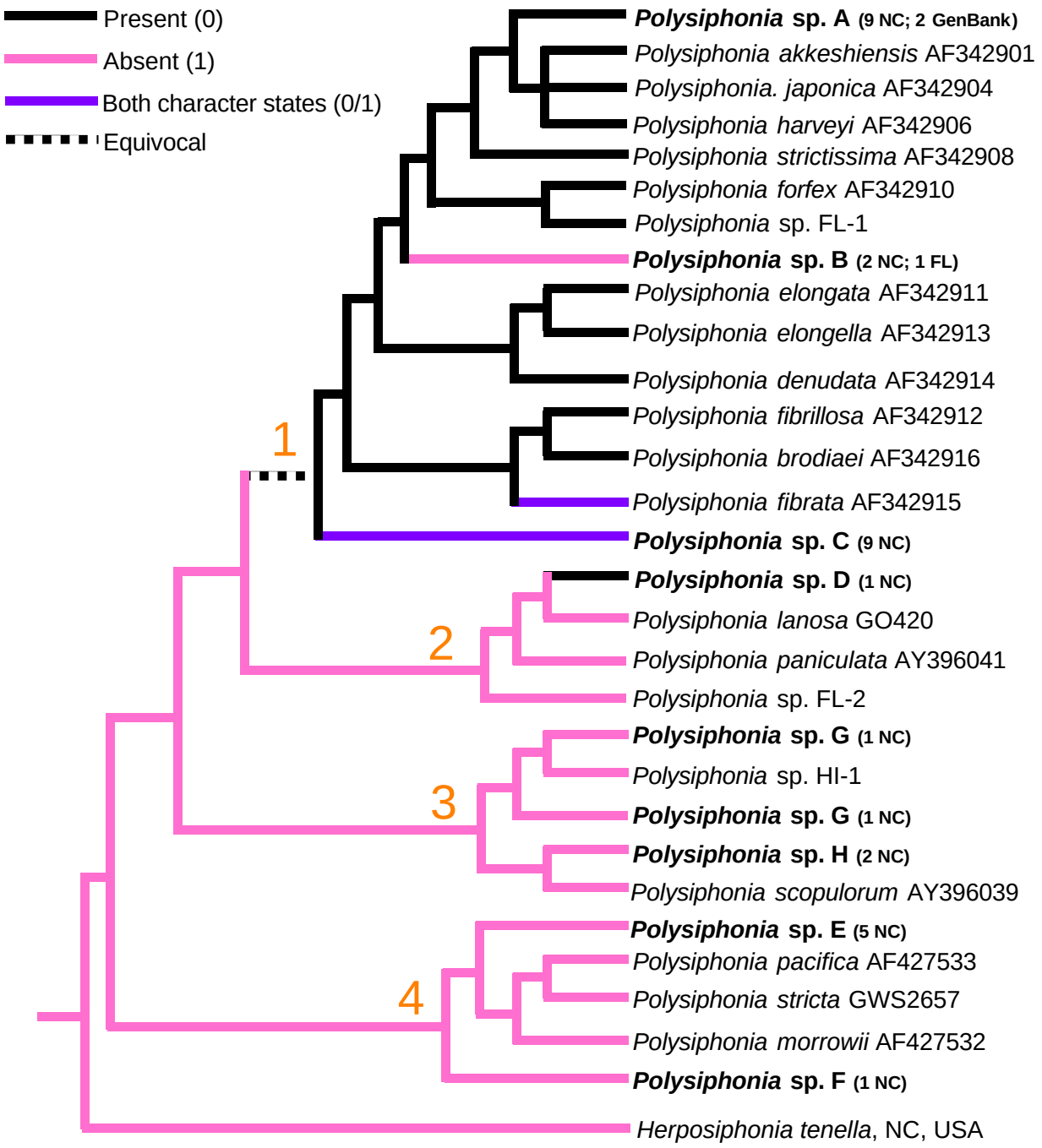

Figure 35. The presence or absence of cortication was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

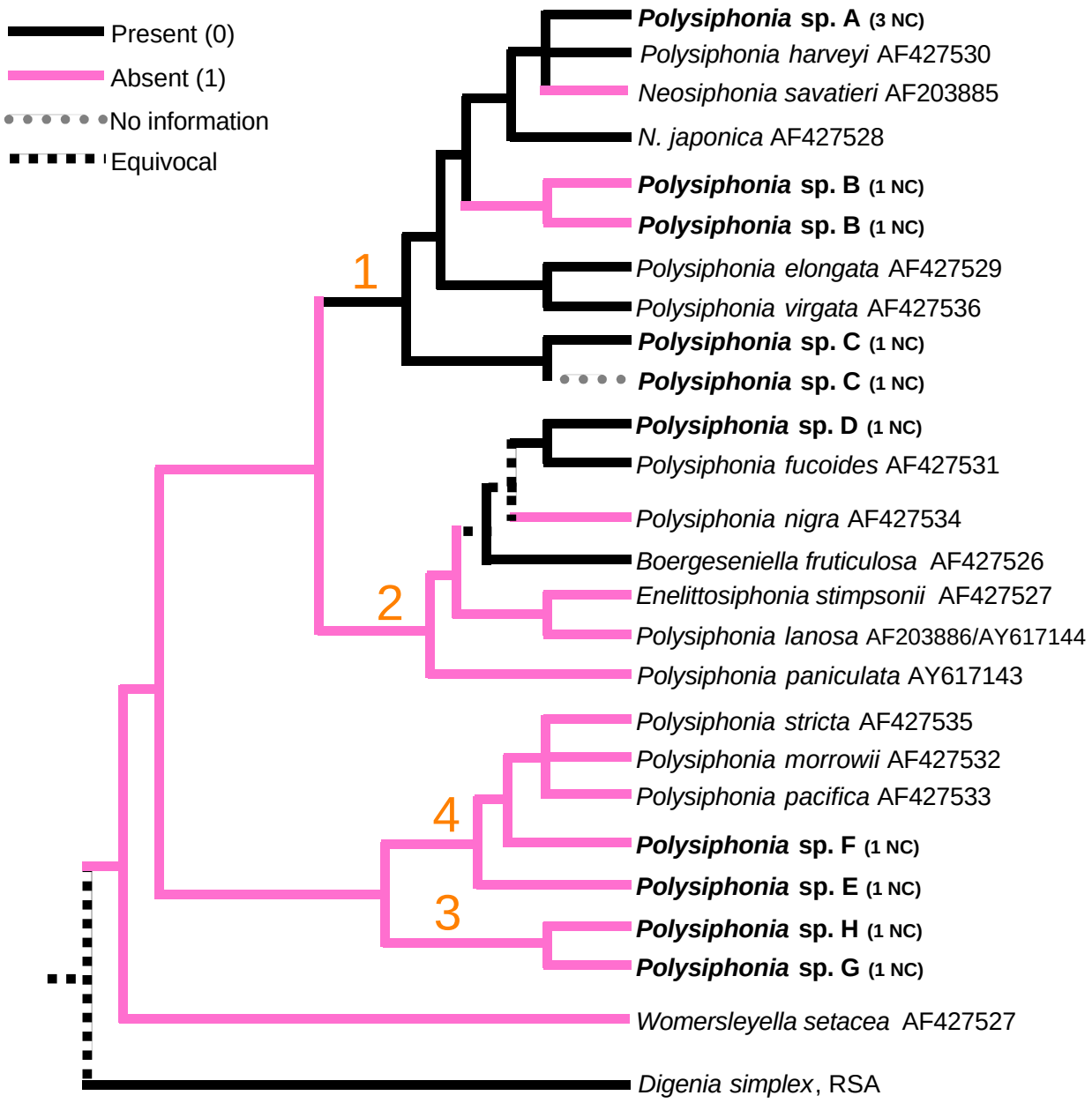

Figure 36. The presence or absence of cortication was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

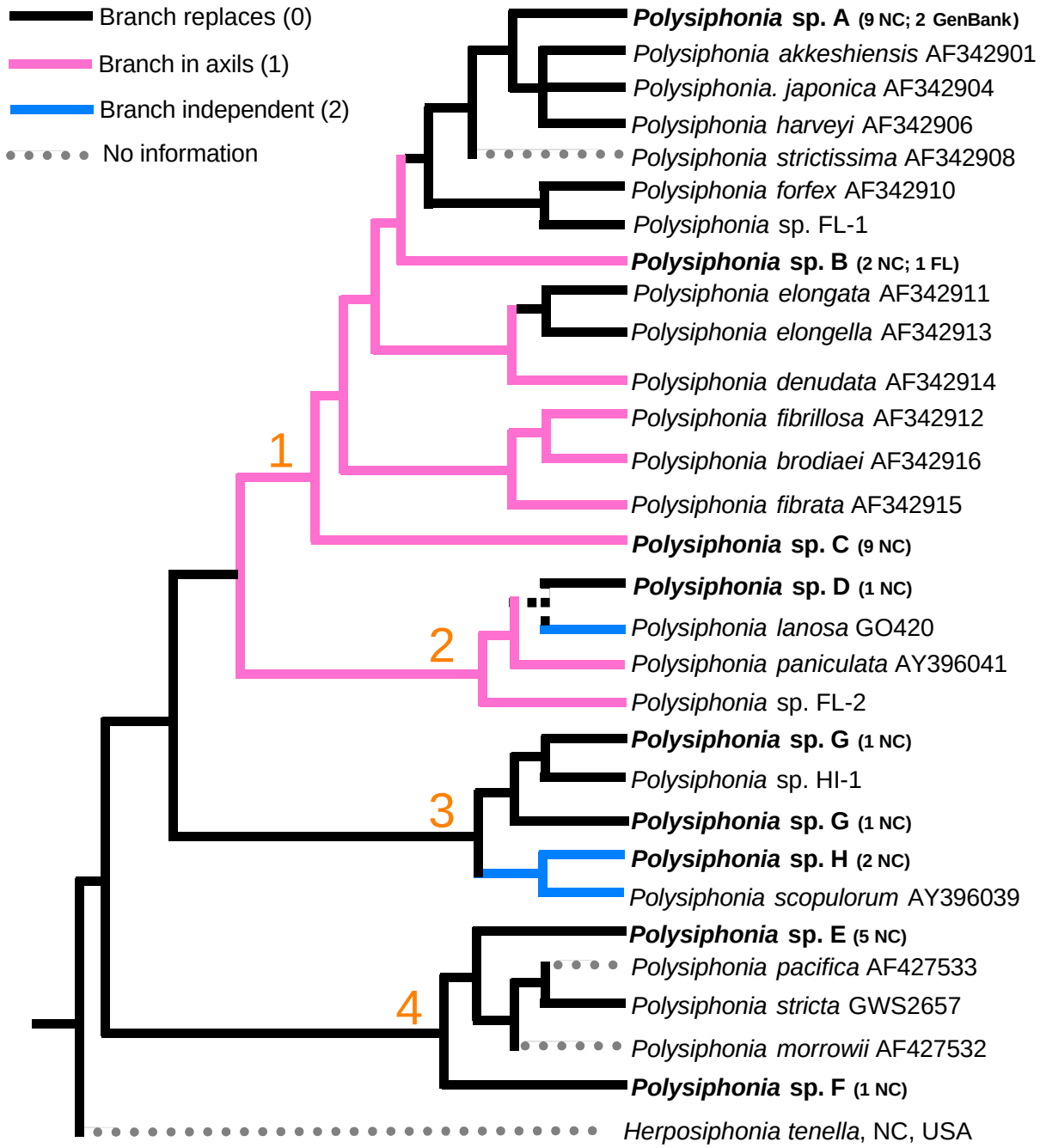

Figure 37. The relationship of lateral branches to trichoblasts was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

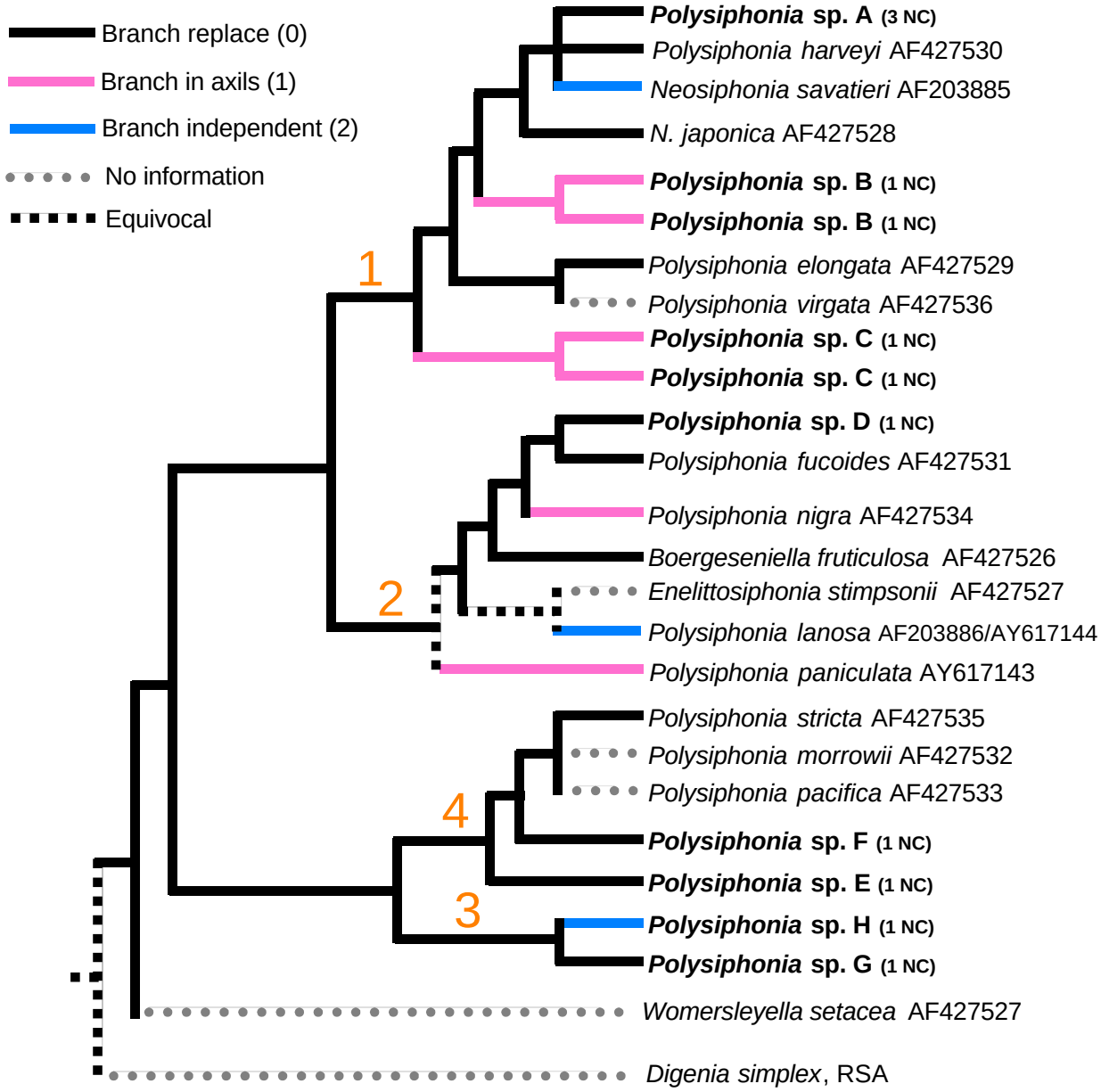

Figure 38. The relationship of lateral branches to trichoblasts was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

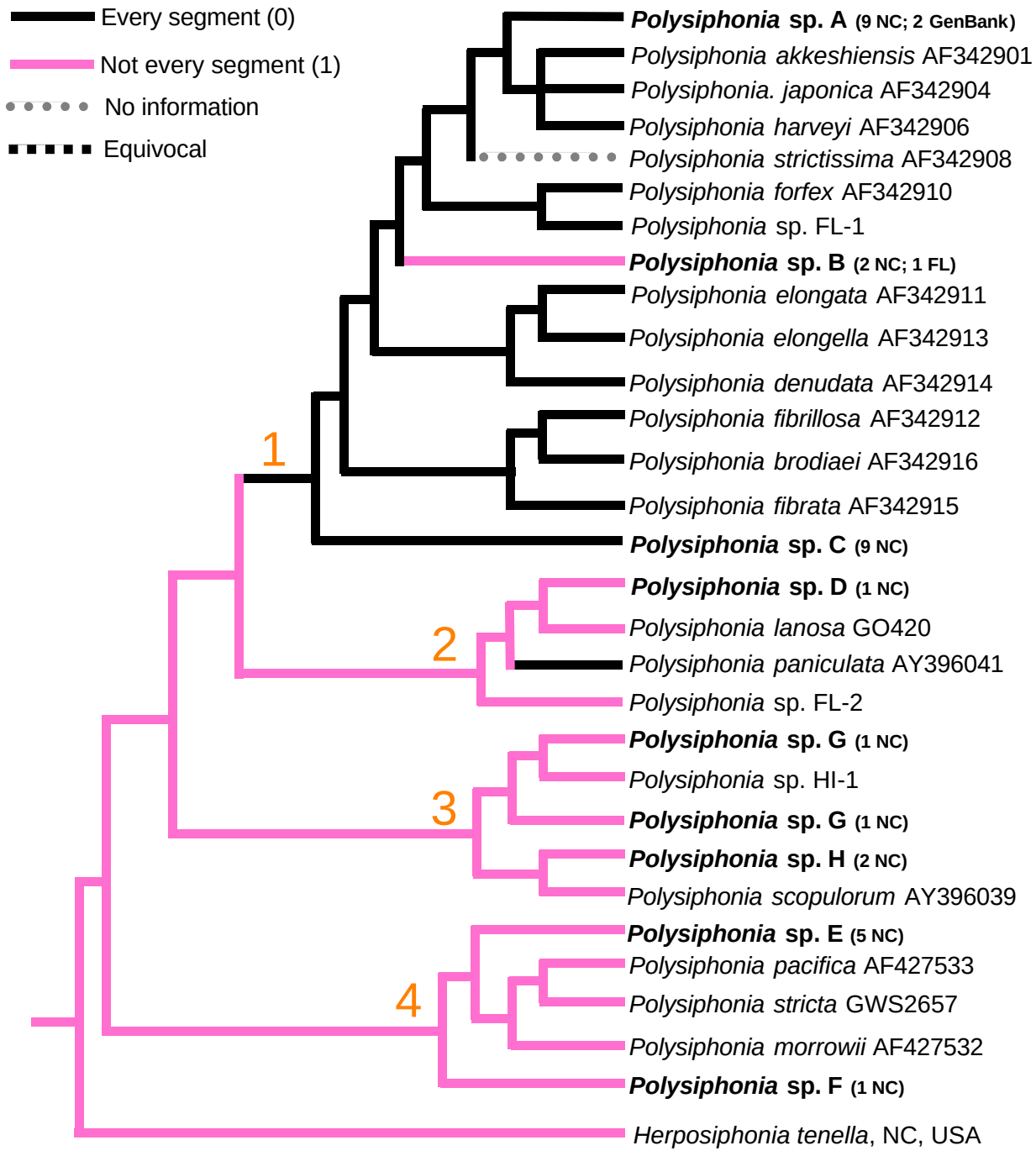

Figure 39. The number of segments between trichoblasts was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

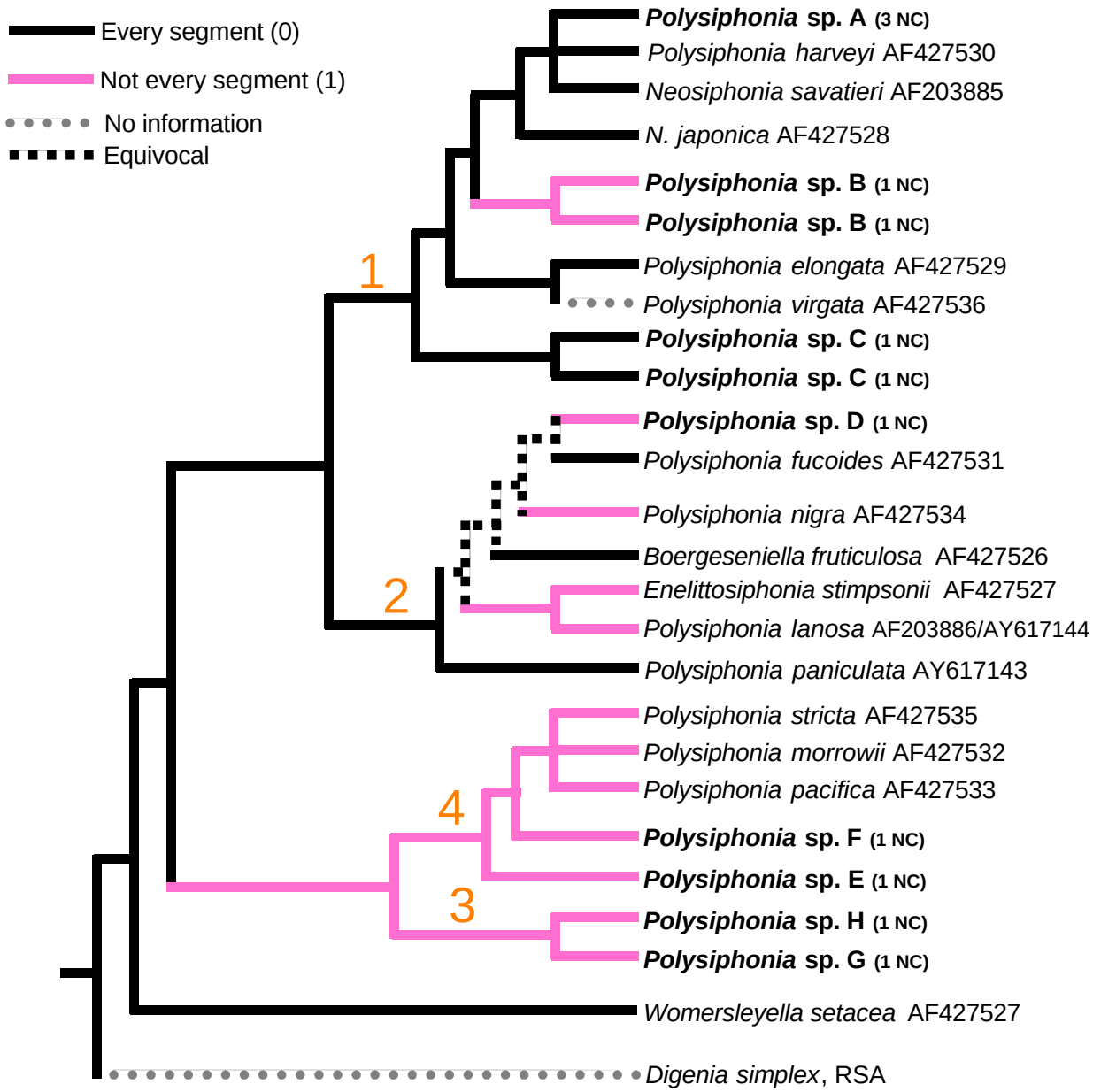

Figure 40. The number of segments between trichoblasts was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

every segment. Species in major clade one had trichoblasts on every segment, except for species B, which did not have trichoblasts every segment. Both character states were found among species resolved in major clade two.

The Nature of the Holdfast (Character 11)

Major clades three and four were consistent for the nature of the holdfast in the *rbc*L and SSU trees (Figures 41 and 42) with all species having a prostrate branching system. Clades one and two in both analyses showed variation in the character state among the species within the clades.

Scar Cells (Characters 16, 17 and 18)

Scar cells were present in the majority of included species (Figures 43 and 44). *Polysiphonia lanosa*, *P. virgata* and *Boergeseniella fruticulosa* were the only species without scar cells. This character was variable in two of the North Carolina species, E and H, that were resolved in clades three and four. Scar cells were reported to be present in all other species resolved in these clades.

The same overall pattern was seen when character states for the pattern of scar cells were mapped on the *rbc*L and SSU trees (Figures 45 and 46). Clades three and four comprised species that displayed no scar cell pattern, with the exception of *Polysiphonia scopulorum* in clade three, which had scar cells in a spiral series. All species in clades one and two had scar cells in a spiral pattern, except for species B located within clade one that displayed no scar cell pattern.

The production of lateral branches from scar cells displayed a similar pattern when mapped on the molecular trees (Figures 47 and 48). Clades three and four included species that lacked scar cells that produced lateral branches, with the exception of *Polysiphonia scopulorum*.

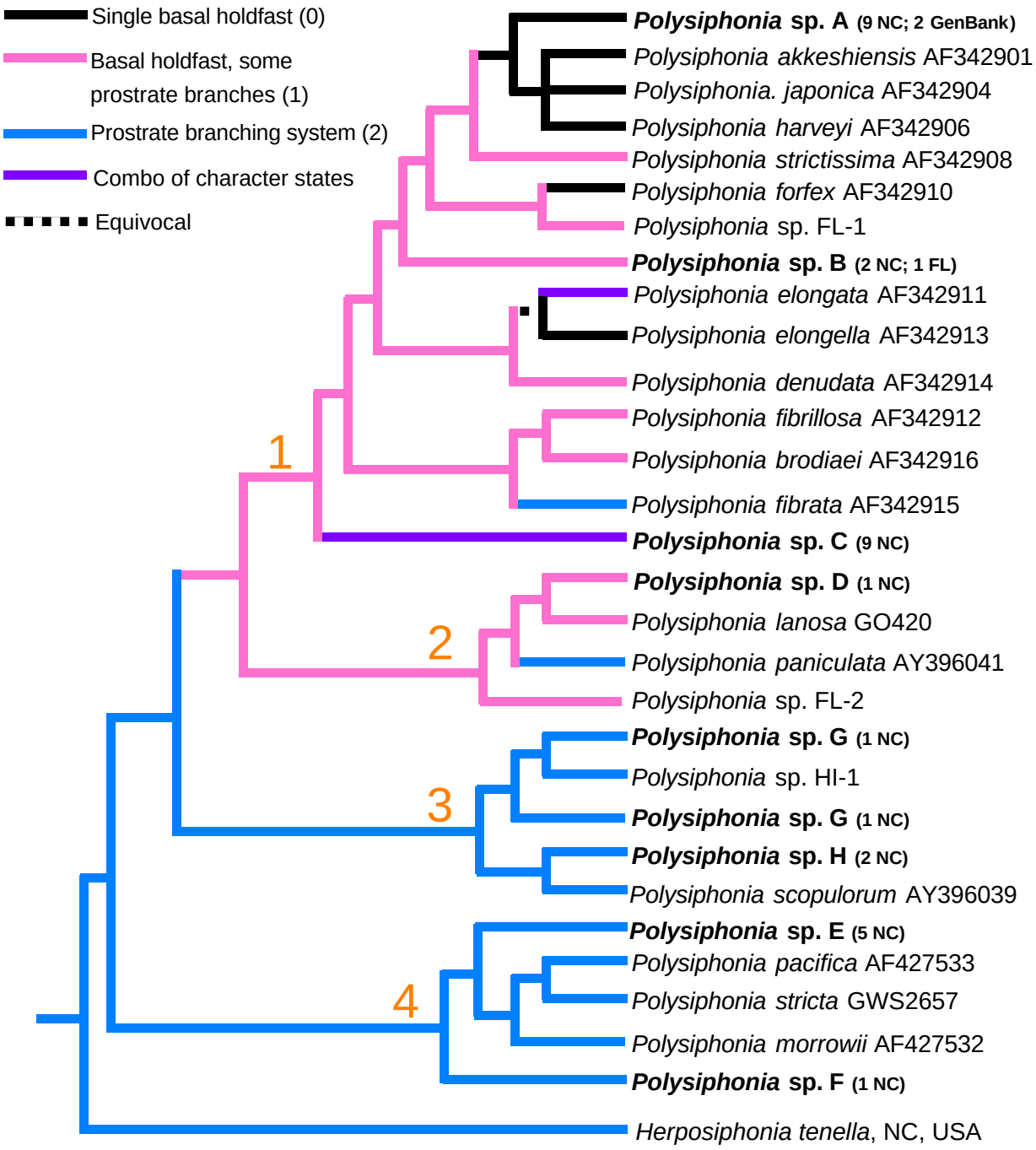

Figure 41. The nature of the holdfast was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

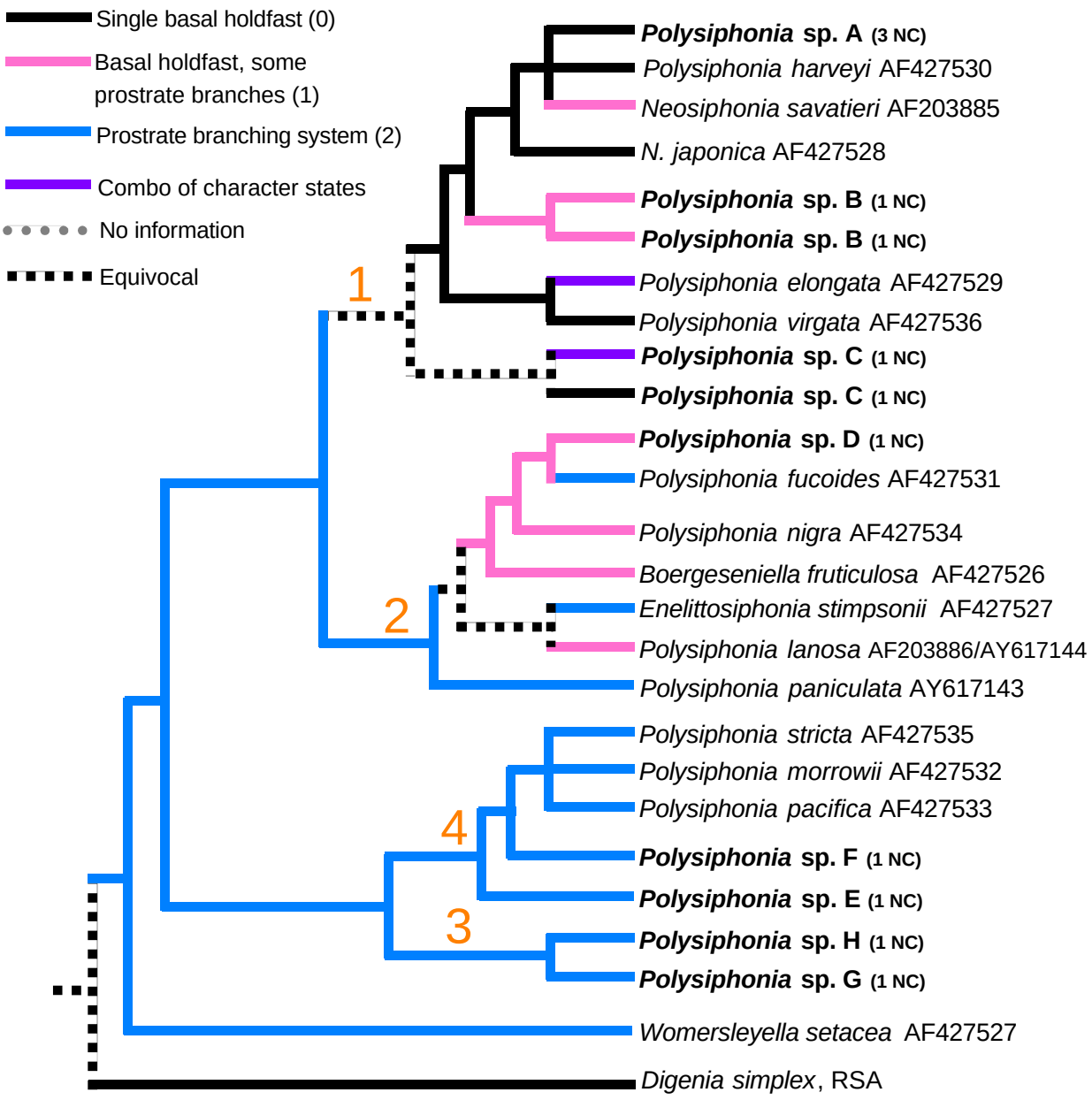

Figure 42. The nature of the holdfast was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

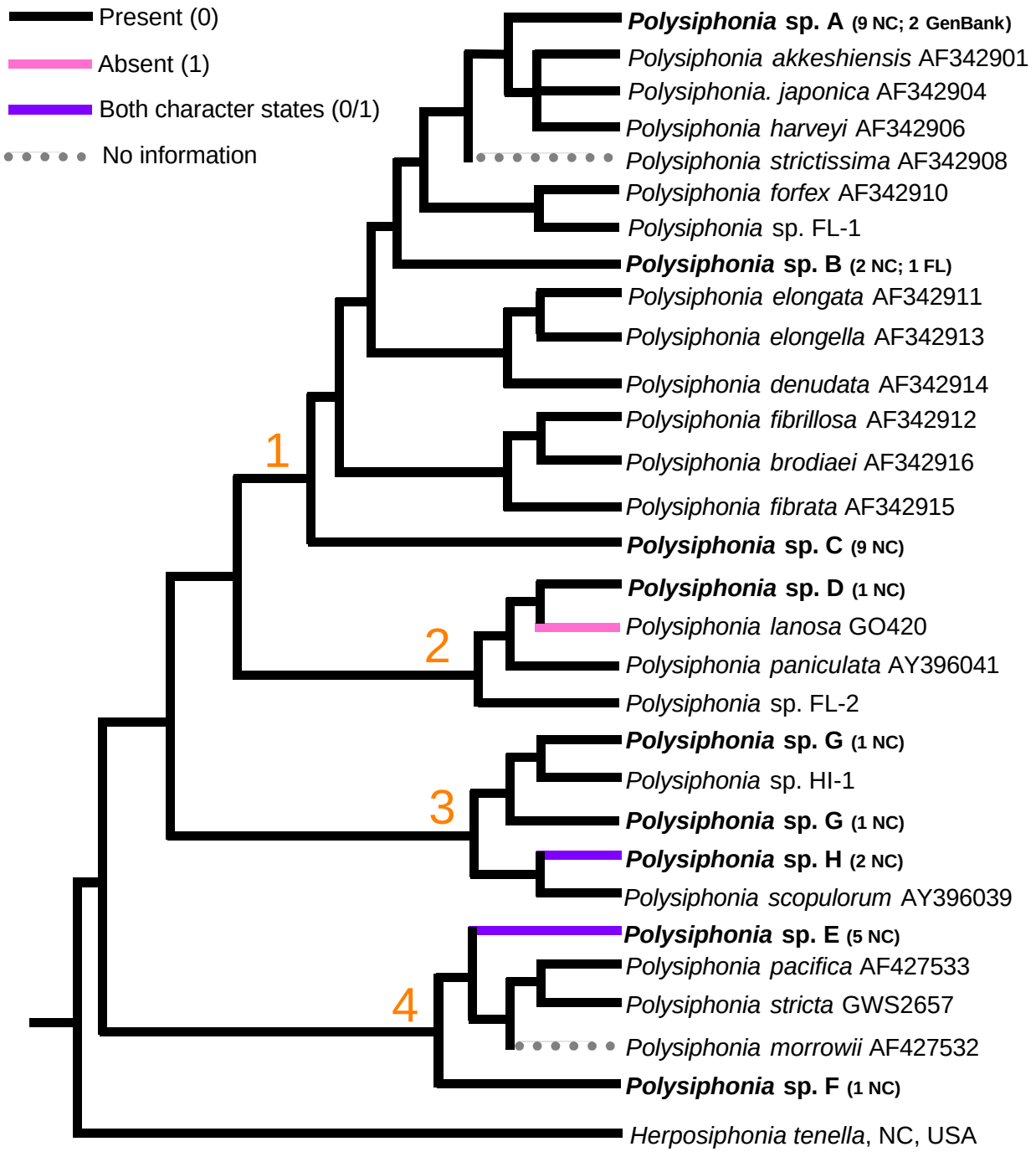

Figure 43. The presence and absence of scar cells was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

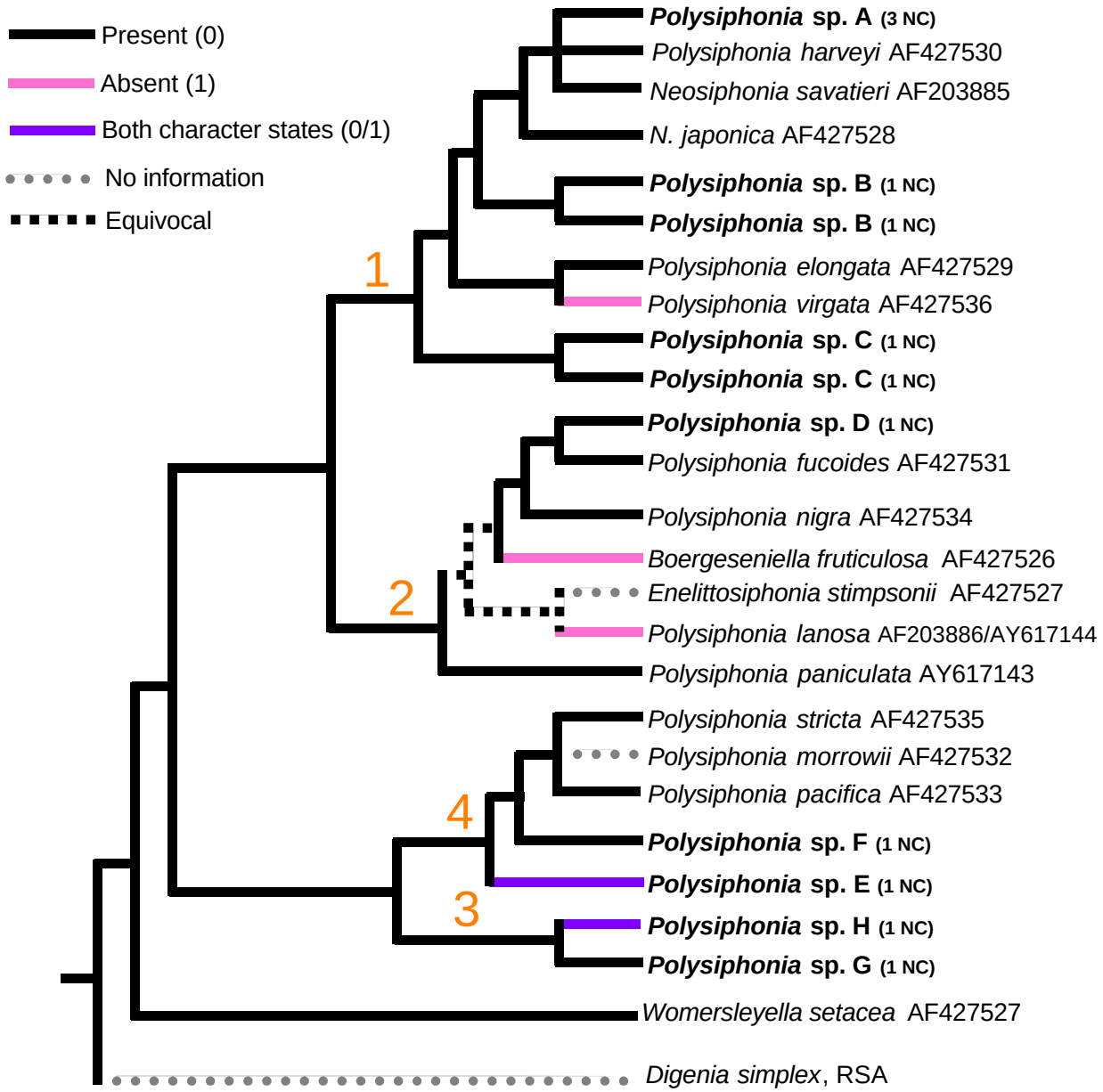

Figure 44. The presence and absence of scar cells was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

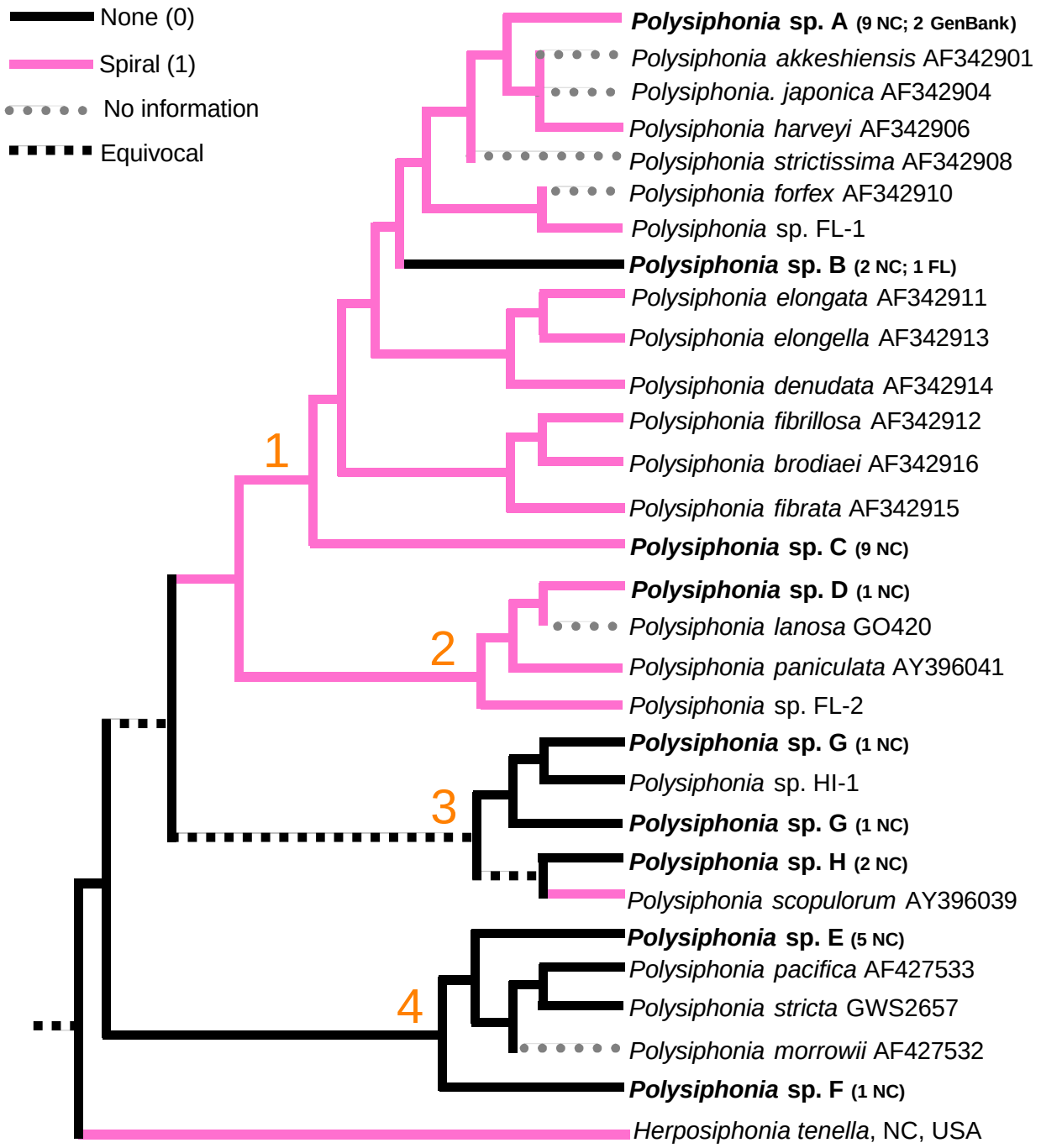

Figure 45. The pattern of scar cells was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

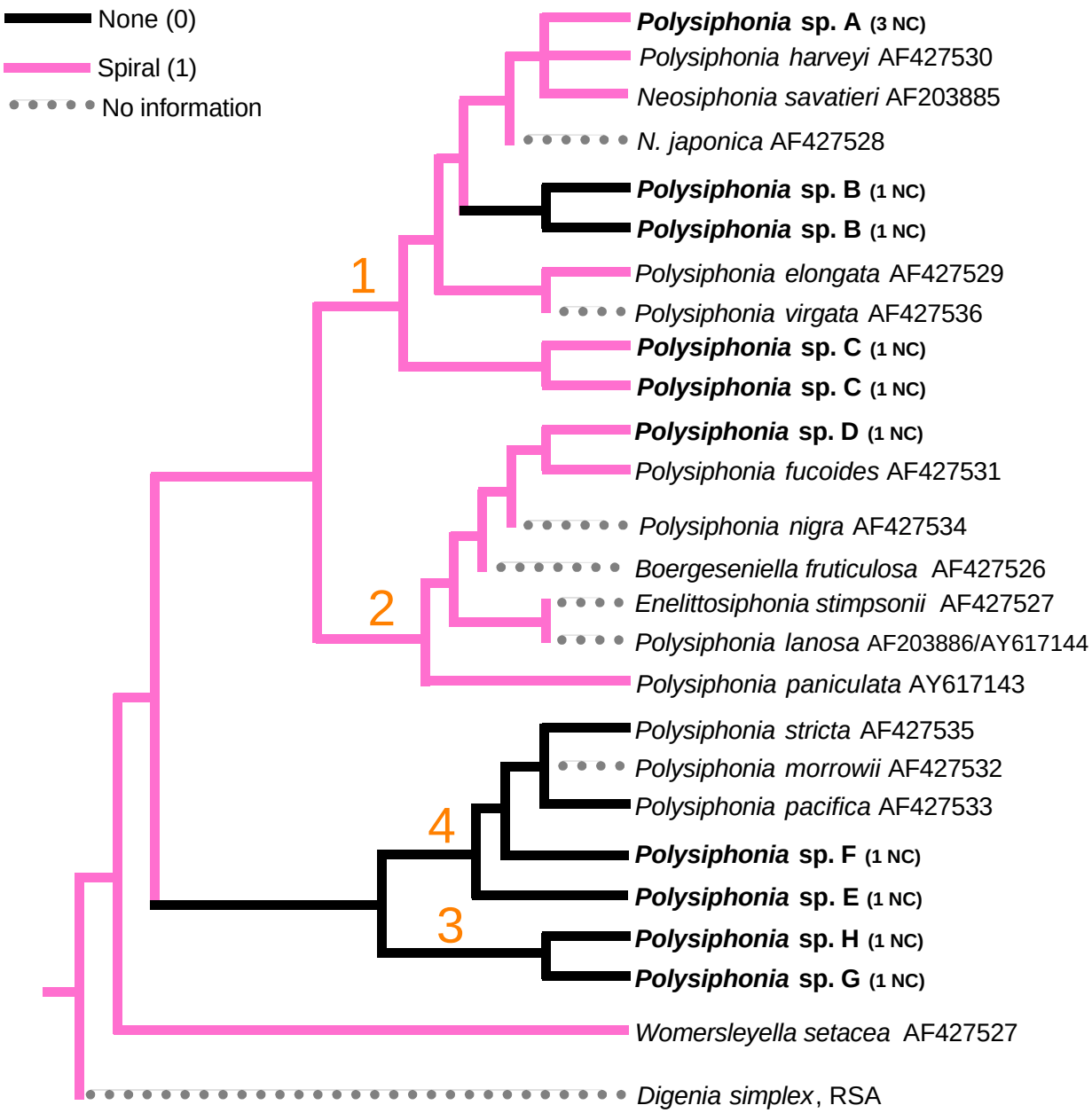

Figure 46. The pattern of scar cells was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

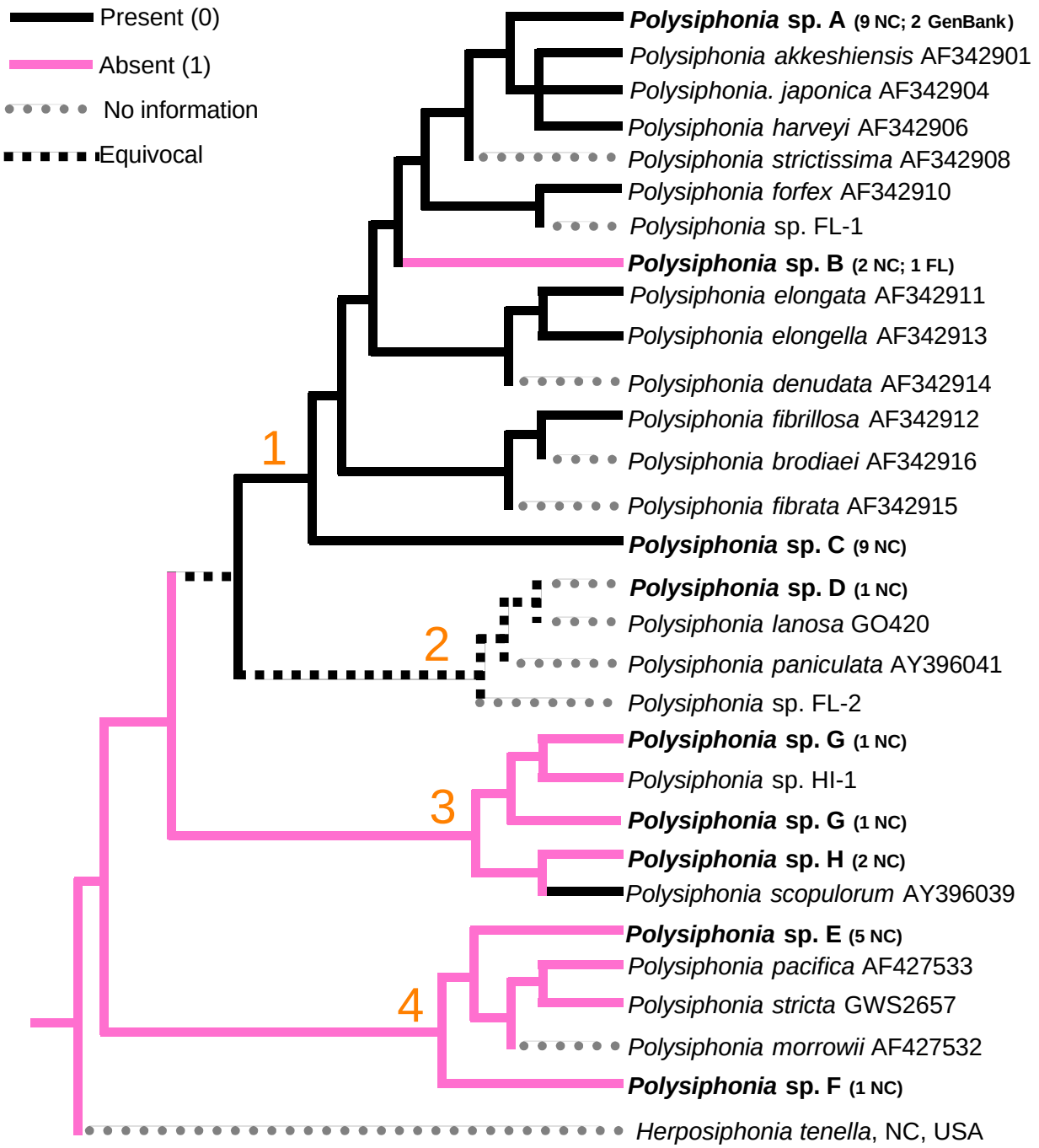

Figure 47. The production of lateral branches by scar cells was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

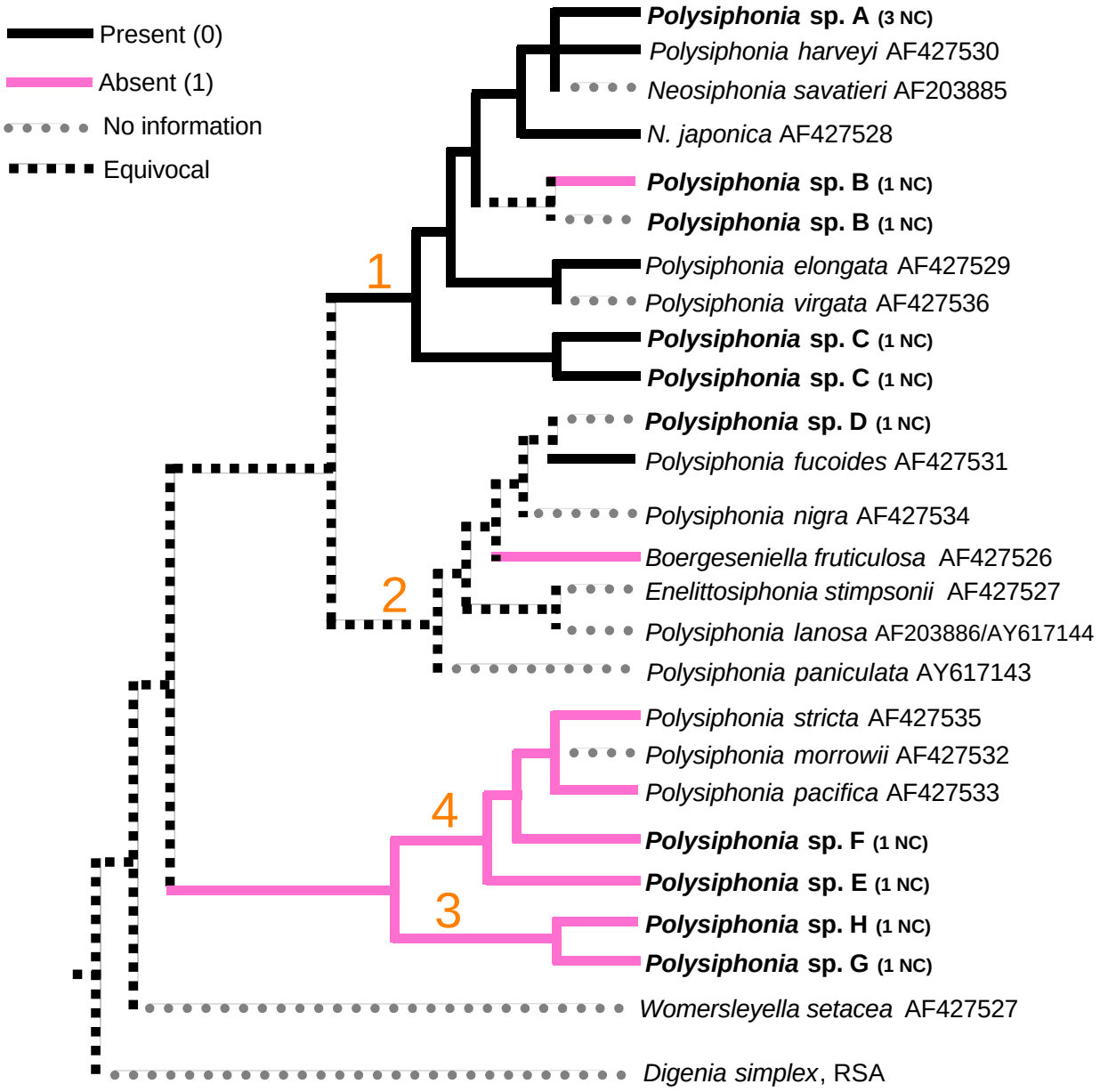

Figure 48. The production of lateral branches by scar cells was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

Clade two was inconsistent for this character, while species in clade one had scar cells with lateral branches except for species B.

Spermatangial Axes Development (Character 20)

The development of the spermatangial axes was not observed in all of the specimens collected for this study, but information was available in the literature for all the other species included in the molecular analyses (Figures 49 and 50). Species in clades three and four for which information was available had spermatangial axes that replaced the whole trichoblasts. Both character states were present in species resolved in clade two. The majority of species in clade one had spermatangia that formed as a furcation of the trichoblasts, but spermatangia are reported to replace trichoblasts in *Polysiphonia forfex*, and both character states are reported for *P. elongata*.

Tetrasporangial Arrangement (Character 22)

Clades two and four resolved in the molecular analyses were consistent for this character (Figures 51 and 52). Species in clade two all had tetrasporangia in a spiral series, while species in clade four had tetrasporangia in a straight series. Species resolved in clade three also had tetrasporangia in a straight series except for *Polysiphonia scopulorum*, which is reported to display both character states. Similarly, all species in clade one except for *P. virgata* and species B had tetrasporangia in a spiral series.

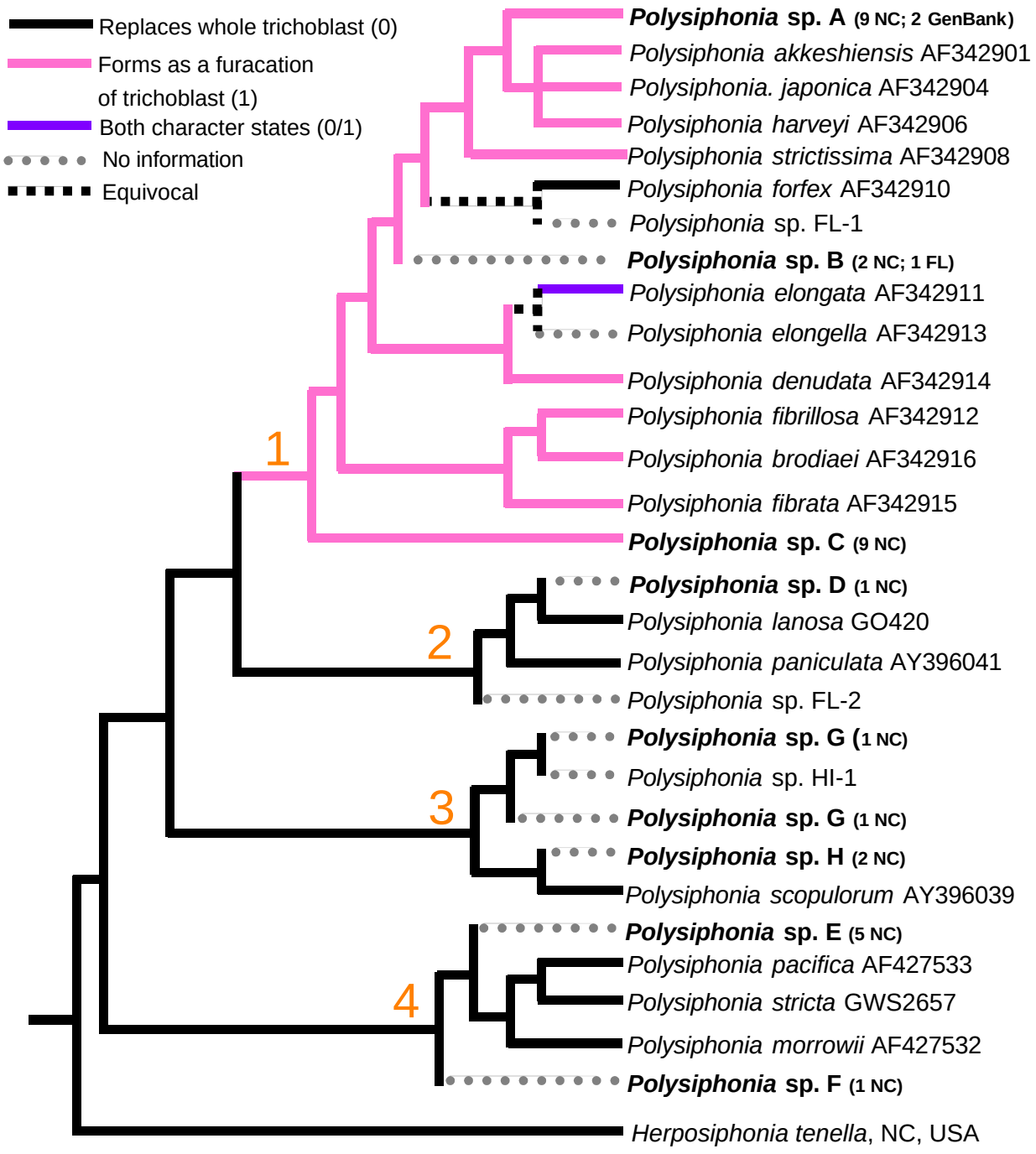

Figure 49. Development of spermatangial axes was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

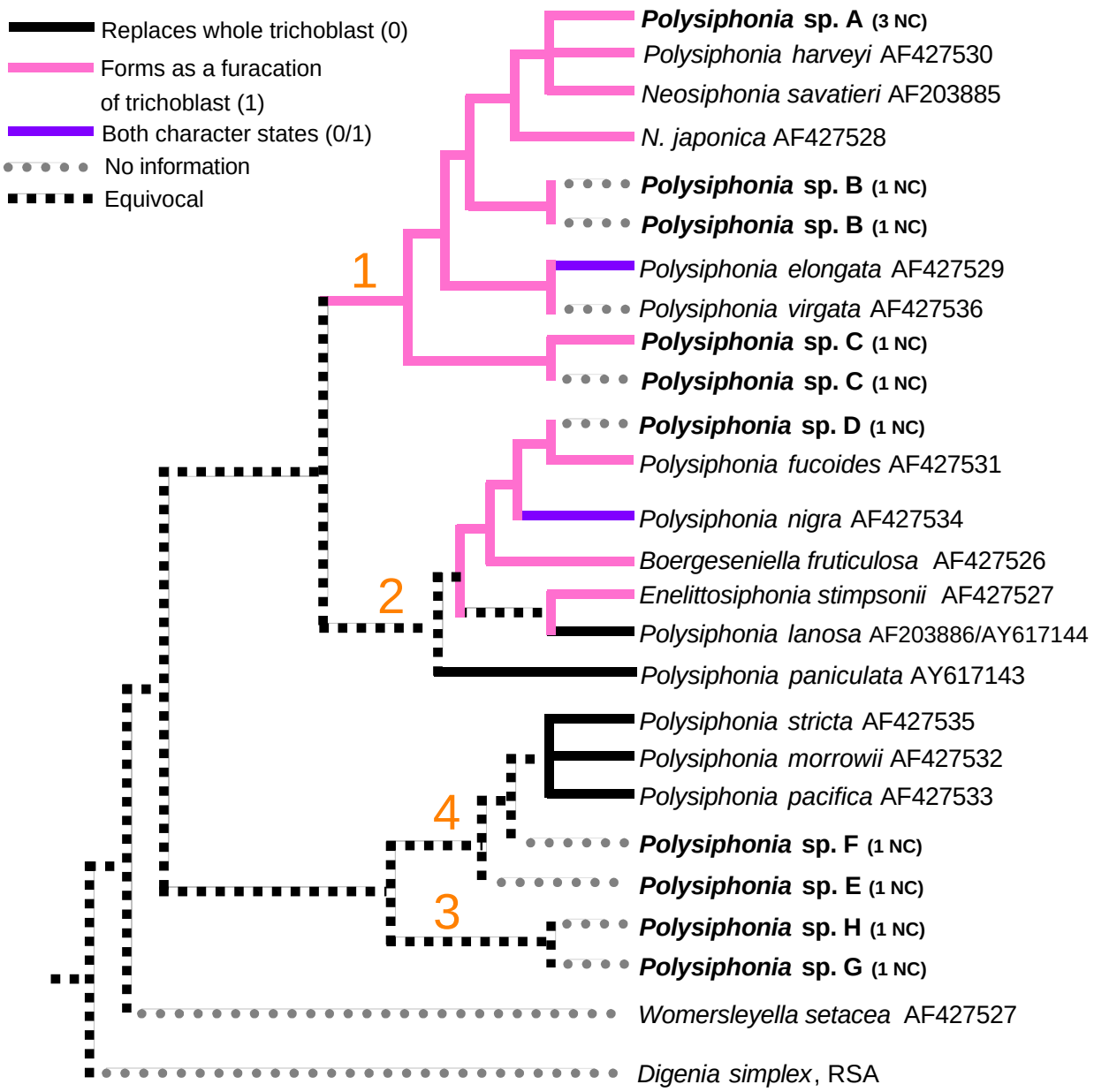

Figure 50. Development of spermatangial axes was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

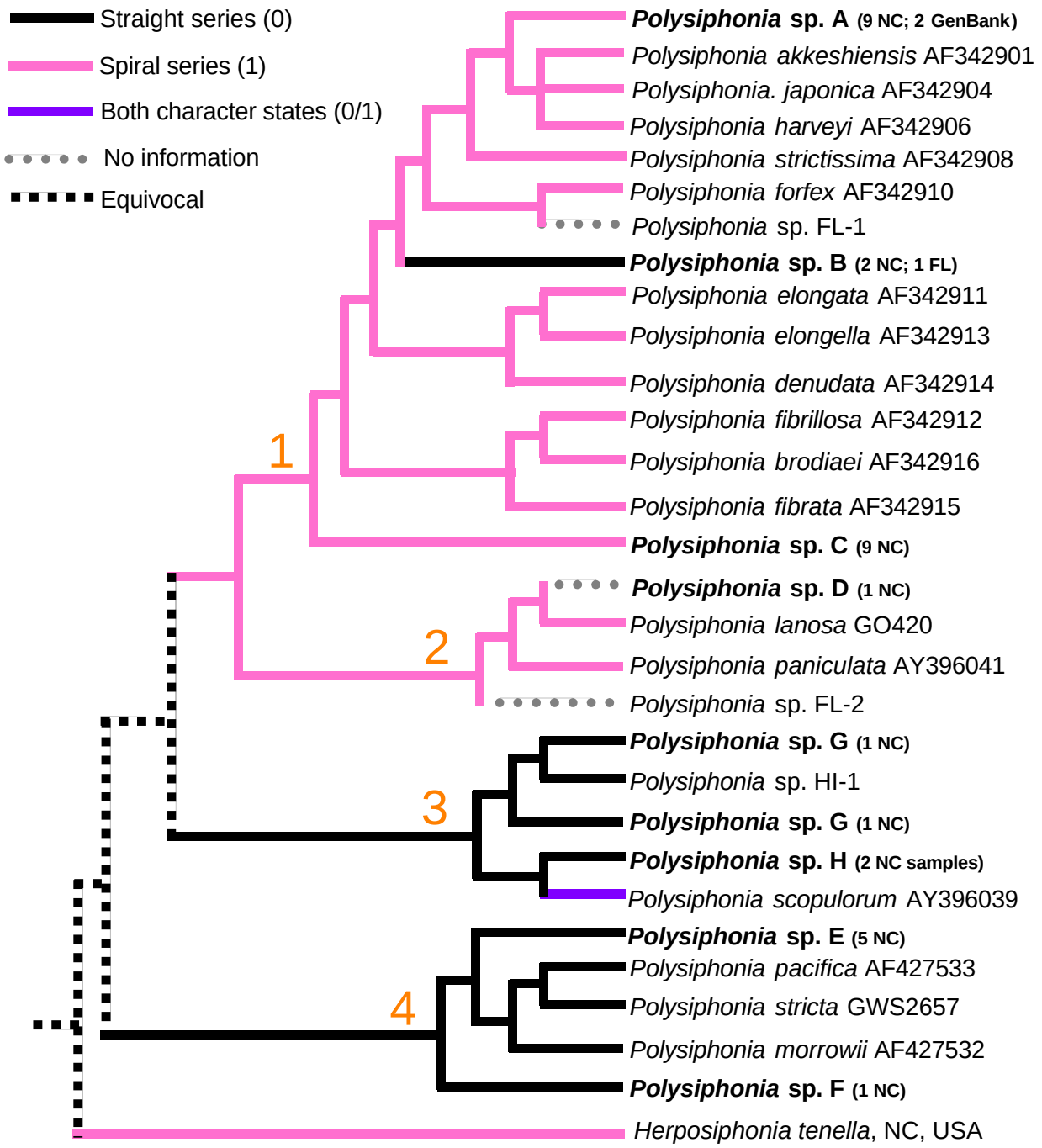

Figure 51. The arrangement of tetrasporangia was mapped upon a cladogram resulting from analyses of *rbc*L sequence data for 33 Rhodomelaceae. Major clades are represented by numbers 1-4.

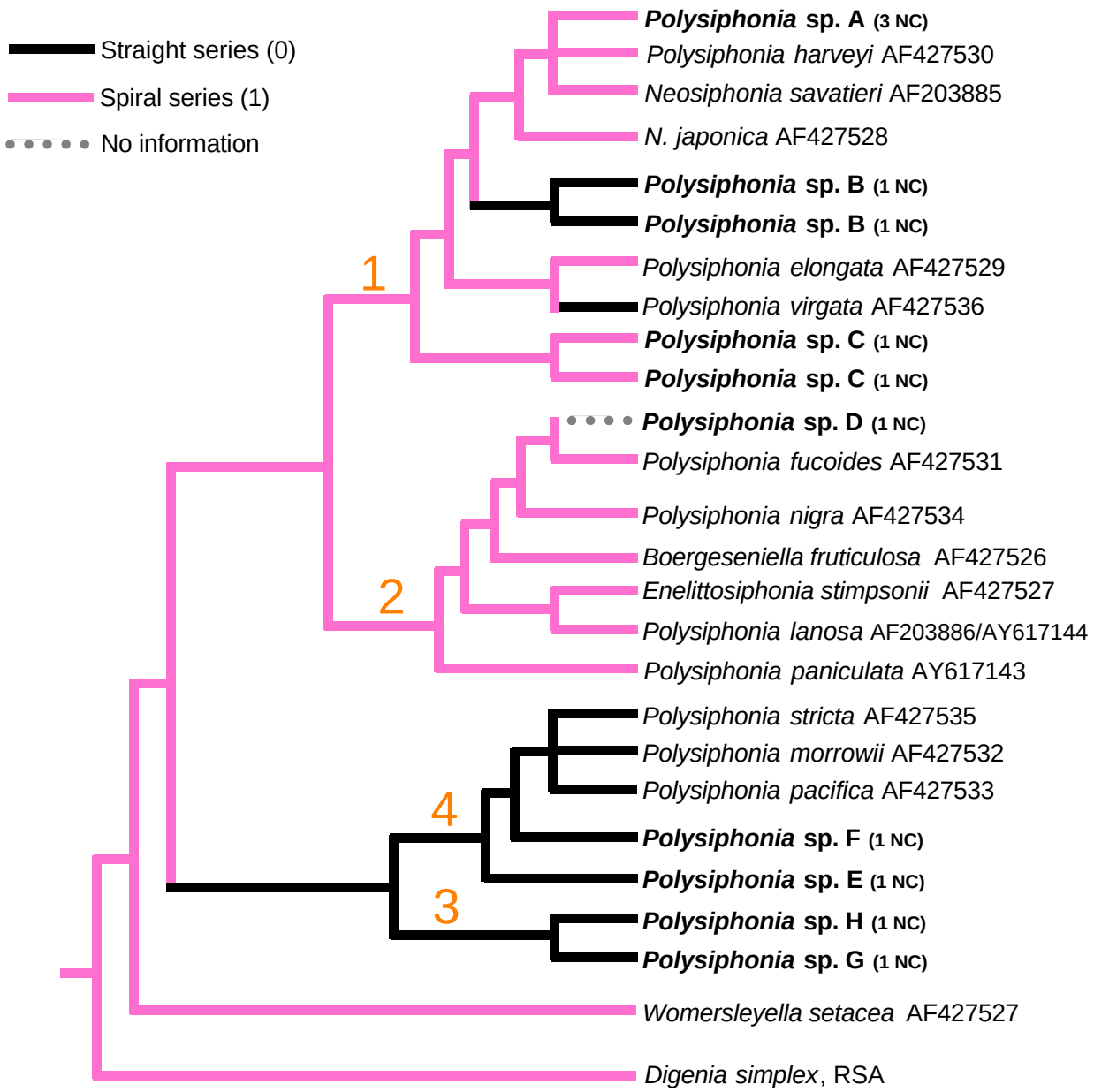

Figure 52. The arrangement of tetrasporangia was mapped upon a cladogram resulting from analyses of SSU sequence data for 23 Rhodomelaceae. Major clades are represented by numbers 1-4.

North Carolina Species Identifications

Species A

Neosiphonia harveyi (Bailey) M.S. Kim, H.G. Choi, Guiry, & G.W. Saunders
Type: TCD
Type locality: Stonington, Connecticut, U.S.A.
Basionym: *Polysiphonia harveyi* Bailey, 1848. *American Journal of Science & Arts,* 6: 38.
Synonyms: *Polysiphonia harveyi* var. *arietina*; *Polysiphonia nova-angliae* W.R. Taylor; *Polysiphonia harveyi* J. Bailey; *Polysiphonia havanensis* var. *insidiosa* J. Agardh; *Polysiphonia insidiosa* (J. Agardh) P.L. Crouan & H.M. Crouan *nom. illeg*.; *Polysiphonia argentinica* W.R. Taylor.

Species A was identified by the following the keys of Kapraun (1977, 1980a) and Maggs & Hommersand (1993). This species fits the description of *Neosiphonia harveyi* (as *P. harveyi*) found in Kapraun (1977, 1980a) and Schneider & Searles (1991), except for conflict in the character of lateral branches (character 4). The descriptions of *P. harveyi* in Maggs & Hommersand (1993) and Kapraun (unpublished MS) agreed with characters found for the North Carolina species A. Analyses of molecular sequence data also resolved this species within a clade that included samples identified as *P. harveyi*, *P. japonica*, *P. akkeshiensis*, and *P. strictissima*. *Polysiphonia japonica* is now regarded as *Neosiphonia japonica* and *P. akkeshiensis* is considered a synonym of *N. japonica*.

Species B

Polysiphonia denudata *sensu* Kapraun (1977, 1980a).
Type: Unknown
Type locality: Unknown

Species B was identified by the following the keys of Kapraun (1977, 1980a), Schneider & Searles (1991) and Maggs & Hommersand (1993). The various descriptions of *Polysiphonia denudata* found in these references, however, did not agree in the character states of cortication (character 3), number of segments between trichoblasts (character 8), nature of holdfast (character 11), and arrangement of tetrasporangia (character 22). The Maggs & Hommersand (1993) description of *P. denudata* states that this species has basal cortication, numerous trichoblasts found on every segment, thalli arising from single basal holdfast, and tetrasporangia arranged in a spiral series. Species B in this study lacked cortication, had few trichoblasts with bare segments between trichoblasts, thalli that attached with a basal holdfast but had some prostrate attachments, and tetrasporangia arranged in a straight series. The character states for species B agreed with the descriptions of Kapraun (1977, 1980a), which disagreed with the description of Maggs & Hommersand (1993). The molecular analyses of sequence data resolved this species sister to clades containing *P. harveyi*, *P. japonica*, *P. strictissima*, and *P. forfex*, instead of the clade containing *P. denudata* from the type locality. Species B was therefore regarded as *P. denudata sensu* Kapraun because it differs morphologically and molecularly from the typical *P. denudata*.

Species C

Polysiphonia breviarticulata (C. Agardh) Zanardini
Type: unknown
Type locality: Adriatic (al Lido)
Basionym: *Hutchinsia breviarticulata* C. Agardh
Synonyms: *Hutchinsia breviarticulata* C. Agardh; *Polysiphonia chrysoderma* Kützing; *Polysiphonia physartra* Kützing

Species C was identified by the following the key of Schneider & Searles (1991). The descriptions of Schneider & Searles (1991), Kapraun & Searles (1990) and Kapraun (unpublished MS) agreed with the character states found for species C. *Polysiphonia breviarticulata* was not reported in North Carolina until 1982 and remained inconspicuous until a bloom occurred in 1988 (Kapraun & Searles, 1990). Analyses of sequence data resolved this species in an isolated clade, sister to clades including species A and B.

Species D

Polysiphonia fucoides (Hudson) Greville
Neotype: BM-K, in Herb. Lightfoot
Type locality: British Isles (Cornubia & Exmouth in Devonia)
Basionym: *Conferva fucoides* Hudson
Synonyms: *Conferva fucoides* Hudson; *Conferva nigrescens* Hudson; *Ceramium violaceum* Roth; *Conferva atrorubens* Wahlenberg; *Hutchinsia violacea* var. *nigrescens* (Hudson) C. Agardh; *Hutchinsia violacea* (Roth) C. Agardh; *Hutchinsia nigrescens* (Hudson) Lyngbye; *Hutchinsia nigrescens* var. *pectinata* C. Agardh; *Ceramium violaceum* var. *nigrescens* (Hudson) Wahlenberg; *Polysiphonia violacea* (Roth) Sprengel; *Polysiphonia nigrescens* (Hudson) Greville *ex* Harvey; *Polysiphonia atropurpurea* Moore *ex* Harvey; *Polysiphonia nigrescens* var. *flaccida* Areschoug; *Polysiphonia senticosa* Suhr *ex* Kützing; *Polysiphonia nigrescens* f. *senticosa* (Kützing) J.Agardh; *Polysiphonia nigrescens* f. *protensa* J. Agardh; *Polysiphonia nigrescens* f. *fucoides* (Hudson) J.Agardh; *Polysiphonia nigrescens* f. *pectinata* (C.Agardh) J.Agardh; *Polysiphonia nigrescens* f. *flaccida* (Areschoug) Kylin

Species D was identified by the following the key of Kapraun (1980a), Schneider & Searles (1991) and Maggs & Hommersand (1993). In Kapraun (1980a) and Schneider & Searles (1991) species D keys out to *Polysiphonia nigrescens*, which is a synonym of *P. fucoides* and the key of Maggs & Hommersand (1993) lead to this species. The descriptions found in all three

taxonomic keys agree with the character states of species D. The SSU analyses showed that sequence data from species D was identical to sequence data of *P. fucoides* from Ireland. Molecular analyses of sequence data resolved this species with other species with a highly elevated number of pericentral cells (eight or more).

Species E

Polysiphonia atlantica Kapraun & J.N. Norris
Type: TCD
Type locality: Port Stewart, Miltown, Mal Bay, County Claire, Ireland
Basionym: *Polysiphonia atlantica* Kapraun & J.N. Norris
Synonyms: *Polysiphonia macrocarpa* Harvey, *nom. illeg.*

Species E was identified by the following the key of Kapraun (1980a), Schneider & Searles (1991) and Maggs & Hommersand (1993). This species fits the descriptions of *Polysiphonia atlantica* in Schneider & Searles (1991) and Maggs & Hommersand (1993). Kapraun's (1980a) description of *P. atlantica* (as *P. macrocarpa*) also fits this species. Molecular analyses of sequence data resolved this species sister to a clade containing species of *P. pacifica*, *P. stricta*, and *P. morrowii*.

Species F

North Carolina *Polysiphonia urceolata* *sensu* Kapraun (1977, 1979, 1980)
Type: Unknown
Type locality: Unknown

Species F was identified by the following the key of Kapraun (1980a), Schneider & Searles (1991) and Maggs & Hommersand (1993). Two of the three taxonomic keys identified

this species as *Polysiphonia urceolata* (Kapraun, 1980a; Schneider & Searles, 1991), whereas the other keyed to *P. stricta* (Maggs & Hommersand, 1993). The current taxonomic status of *Polysiphonia urecolata* is that it is regarded as a synonym of *P. stricta* (Maggs & Hommersand, 1993). The descriptions and keys in these references differed in one important morphological feature. Maggs & Hommersand (1993) state that *P. stricta* had spermatangia that replaced the trichoblasts, whereas Kapraun (1980a) and Schneider & Searles (1991) found that *P. urceolata* had spermatangia that form as a furcation of the trichoblasts. In this study, however no male gametophytes were found for species F.

Analyses of sequence data resolved species F in a position very distinct from the English *P. stricta*, but sister to the clade containing *P. stricta*, *P. pacifica* and *P. morrowii*. The morphological, physiological and molecular results show that North Carolina *P. urecolata* is not the same species as *P. stricta*.

Species G

Polysiphonia subtilissima Montagne
Holotype: PC
Type locality: Cayenne, French Guiana
Basionym: *Polysiphonia subtilissima* Montagne
Synonyms: *Polysiphonia angustissima* Kützing

Species G was identified by following the keys of Kapraun (1980a) and Schneider & Searles (1991). The descriptions in both of these references agreed with the morphological data gathered for species G. *Polysiphonia subtilissima* has been reported as a brackish water species and all samples collected in this study were from brackish-water environments. Molecular

analyses of sequence data showed this species to be sister to a clade containing samples identified as of *P. scopulorum*.

Species H

Polysiphonia scopulorum var. *villum* (J. Agardh) Hollenberg
Type: Unknown
Type locality: "ad littus americae tropicae' (Mexico) (Silva, Basson & Moe 1996: 545)
Basionym: *Polysiphonia villum* J. Agardh
Synonyms: *Polysiphonia villum* J. Agardh; *Lophosiphonia villum* (J. Agardh) Setchell & N.L. Gardner

Species H was identified by the following the keys of Hollenberg (1968a) and Schneider & Searles (1991). This species fits the description of *Polysiphonia scopulorum* var. *villum* found in Schneider & Searles (1991), except for conflict in the character of lateral branches (character 4). The description states that lateral branches replace trichoblasts but investigation of this species in this study found lateral branches to be independent of trichoblasts. Molecular analyses of sequence data placed this species sister to *P. scopulorum* from the northwest Pacific. Hollenberg (1968a) stated that *P. scopulorum* var. *villum* differed from *P. scopulorum* var. *scopulorum* by the number of segments between erect branches.

DISCUSSION

Morphological and molecular analyses distinguished eight species of North Carolina *Polysiphonia* in this study. Kapraun (1977) collected nine of the 13 *Polysiphonia* species recorded from North Carolina at that time. Later work reported that 15 species of *Polysiphonia*

had been collected along the North Carolina coast (Table 7; Schneider & Searles, 1991). Molecular and morphological analyses are discussed in detail for the species collected in this study, as well as the taxonomic implications for the included North Carolina species.

Molecular Analyses

Molecular analyses of *Polysiphonia* species have been limited (Choi *et al.*, 2001; McIvor *et al.*, 2001; Kim *et al.*, 2004; Kim & Yang, 2005). Choi *et al.* (2001) found in their SSU analyses that *Polysiphonia* was paraphyletic and that the clades in their molecular trees were strongly supported by specific morphological characters. McIvor *et al.* (2001) and Kim *et al.* (2004) used molecular techniques to verify introductions of alien species. McIvor *et al.* (2001) used *rbc*L sequence data to determine the origin of *Polysiphonia harveyi* in Atlantic Europe and their molecular trees resolved two distinct clades of *Polysiphonia* species that had a biogeographic signal. Kim *et al.* (2004) used *rbc*L data to confirm the taxonomic identification of *P. morrowii* from Chile, as well as the introduction of this species from the northwestern Pacific. In the most recent study, Kim & Yang (2005) used molecular techniques to confirm taxonomic identification of *Polysiphonia pacifica* varieties and closely related species. Their molecular trees showed that the *P. pacifica, P. stricta* and *P. morrowii* were separate distinct species and that the two varieties of *P. pacifica* had identical sequence data despite obvious morphological differences.

Comparing the Choi *et al.* (2001) SSU and McIvor *et al.* (2001) *rbc*L trees reveals no agreement in the resolution of species or clades, which is most likely, a result of the difference in sampling. In contrast, trees resulting from *rbc*L and SSU analyses in the current study had the same general topology. Although not perfectly complimentary, there was greater taxon overlap in these analyses than that between the Choi *et al.* (2001) and McIvor *et al.* (2001) studies. In the

Table 7. List of *Polysiphonia* species reported for North Carolina. Taxonomic status as reported in Algaebase (Guiry *et al.*, 2006), regarded as current unless noted otherwise.

Species	Williams (1948)	Taylor (1960)	Brauner (1976)	Kapraun (1977)	Kapraun (1980a)	Schnider & Searles (1991)	Kapraun (pers. com.)	Present study
Polysiphonia atlantica	x[a]	x[a]		x[a]	x[a]	x		x
P. breviarticulata						x	x	x
P. denudata		x	x	x	x	x	x	x
P. ferulacea Suhr ex J. Agardh				x	x	x		
P. flaccidissima[1]			x		x	x		
P. harveyi[2]			x	x	x	x	x	x
P. havanensis Montagne	x	x	x	x	x	x		
P. howei Hollenberg	x	x	x		x	x		
P. nigrescens[3]		x		x	x	x		x[c]
P. opaca (C. Agardh) Moris & De Notaris							x	
P. pseudovillum Hollenberg					x	x		
P. scopulorum var. villum			x[b]			x		x
P. sphaerocarpa[4]			x	x	x	x	x	
P. subtilissima		x			x	x	x	x
P. tepida Hollenberg		x		x	x	x		
P. urceolata[5]	x			x	x	x	x	x

[1] syn. of *Polysiphonia sertularioides* (Grateloup) J. Agardh

[2] syn. of *Neosiphonia harveyi* (J. Bailey) M.S. Kim, H.G. Choi, Guiry & G.W. Saunders

[3] syn. of *Polysiphonia fucoides*

[4] syn. of *Neosiphonia sphaerocarpa* (Børgesen) M.S. Kim & I.K. Lee

[5] syn. of *Polysiphonia stricta* but see discussion in present study

[a] reported as *Polysiphonia macrocarpa*

[b] reported as *Polysiphonia scopulorum*

[C] reported as *Polysiphonia fucoides*

present study, the four major molecular clades that were shown in the *rbc*L and SSU trees did not have a biogeoraphic signal but appeared to be defined by specific morphological characters.

The reason for sequencing both genes was to molecularly delimit species boundaries, and to better understand the specific relationships among North Carolina *Polysiphonia*, as well as their relationships to other species. SSU has been shown to reveal higher-level interactions, such as generic or familial relationships, in Ceramiales (Phillips *et al.*, 2000; Choi *et al.*, 2002; Phillips, 2002), whereas *rbc*L has been more commonly used to distinguish species-level relationships (McIvor *et al.*, 2001; Kim *et al.*, 2004; Kim & Yang, 2005). The relationships of species in major clade four in this study verify these taxonomic level differences. SSU sequences of the closely related species, *P. pacifica, P. stricta* and *P. morrowii*, were nearly identical and poorly distinguished in the SSU tree, whereas in the *rbc*L tree these same taxa were obviously different species. McIvor *et al.* (2001) also showed how *rbc*L was used to determine species level relationships when they measured sequence divergence within species and between species in their study. They found that there was less sequence divergence within species (< 2.1%) than there was between species where the highest amount (13%) was observed between their two biogeographic clades.

Morphological Characters

The current study examined morphological characters that have been classically used to distinguish *Polysiphonia* species in conjunction with molecular sequence data to determine which morphological character can be used to determine species.

Number of Pericentral Cells (Character 1)

The number of pericentral cells in *Polysiphonia* has been shown to range from four to twenty-four (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, however 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 normal number of vegetative 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.

The North Carolina samples collected for this study fell within the range of pericentral cells reported for *Polysiphoina*, with 28 out of 31 samples having four pericentral cells and the remaining three having a more elevated number but less than 24. This character was consistent within each North Carolina species, with all samples in each of the species having the same number of pericentral cells. Species B and D both had elevated numbers of pericentral cells but they were not sister to one another in the tree, indicating that changes in pericentral cell number have occurred multiple times in the evolution of *Polysiphonia sensu lato*. This is further supported when the number of pericentral cells was mapped upon trees including all species (Figures 31 and 32). The character state of having five to seven pericentral cells appeared multiple times within major clade one. The state of having eight or more pericentral cells occured in both major clades one and two. Although all species in major clade two share this

character state as coded, there was a wide range of pericentral cell numbers (eight to 24 for the included species) within this category.

Rhizoid-Pericentral Cell Connection (Character 2)

This character has been described in the literature as the rhizoids being an extension of pericentral cell (open connection) or rhizoids cut off from the pericentral cell becoming a separate cell (pit connected; Hollenberg, 1968b; Womersley, 1979; Kapraun, 1980a; Schneider & Searles, 1991; Maggs & Hommersand, 1993; Abbott, 1999). Hollenberg (1968a) suggested that the point of origin of the rhizoid may also be used to characterize species, with rhizoids that arise on the distal end of the pericentral cell, on the proximal end of the pericentral cell or from the center of the pericentral cell.

The rhizoidal connection was a very consistent character for the North Carolina species sampled. There was a distinct separation in the tree between samples with open connected rhizoids and those with pit connected. This character was not always easy to determine because it required observation of the rhizoid at the right angle to properly identify the character state. 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. A clear separation of character states was also observed when this character was mapped on the molecular trees that included all the species used in this study. Only species in major clade two were inconsistent for this character with *Polysiphonia lanosa* having open connected rhizoids while they were pit connected in the other species resolved in this clade. Kim *et al*. (2002) stated that open connected rhizoids are not common in most species of *Polysiphoina* and hypothesized that the open connected rhizoids in *P. lanosa* may be a result of the epiphytic nature of this plant

on a specific fucoid and its ability to absorb photosynthate from its host. Turner & Evans (1977, 1978), however, showed that large-scale translocation of photoassimilates in the fucoid host, and between the fucoid host and the *Polysiphoina* epiphyte did not exist, and that *P. lanosa* would only be able to extract photoassimilatory products from host cells in direct contact with the rhizoid.

The clear separation of samples with pit-connected rhizoids from those with open connections in the overall species tree, as well as the consistency of this 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. Cortication was both present and absent in the nine samples of species C, which is one of the three species with the most samples. Species A, also containing nine samples, was consistent for this character suggesting that increased sample size is not the reason for the inconsistency seen in species C. The variation within this species could, however, have something to do with the nature of the holdfast. There appears to be some relationship between the presence or absence of cortication and the mode of attachment. 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 be related to the age of the plant in that as the plant matures the cortication becomes more obvious. The lack of cortication in some of the North Carolina samples may be age related.

Cortication was consistently absent in major clades three and four in the molecular trees including all species. The remaining two clades were composed of species that had either the

presence or absence of cortication. The relationship between cortication and the mode of attachment was not as clear when more species were examined. Most species with a strictly prostrate branching system were ecorticated throughout with the exception of *P. fibriata* and *P. fuccoides*, which both had the presence of cortication. Species that initially were attached by a single basal holdfast but sometimes displayed prostrate branching varied in the presence or absence of cortication, whereas species that arose from a single basal holdfast all had the presence of cortication.

Kudo & Masuda (1986) found that the level of cortication varied based on whether the plants of *P. japonica* and *P. 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. This may be a reason for the variation observed in this study.

Relationship of Lateral Branches to Trichoblasts (Character 4)

This character has been used in many descriptions of *Polysiphonia* (Hollenberg, 1942; Abbott & Hollenberg, 1976; Kapraun, 1977; Womersley, 1979; Schneider & Searles, 1991; Maggs & Hommersand, 1993; Womersley, 2003). The way this character has been identified, however, has not been clear. Ideally, one is able to observe early stages of branch formation before trichoblasts have detached from the thallus and the branch-trichoblast relationship is lost, however, 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 axial filaments of the main branch and those in the lateral branches. The first axial cell of the lateral

branch after the forked axial cell in the main branch was the site of determining whether branches replaced or formed in the axil of the trichoblasts. An extra pit connection that lead to the trichoblasts was present at the distal end of the first axial cell of the lateral branch when branches formed in the axil of the trichoblasts (Figure 8b). If the branch replaced the trichoblasts then this extra pit connection was absent (Figure 8d). 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. This character was consistent within each of the North Carolina species, and therefore it should be useful in species identification.

The consistency observed within species prompted the investigation of this character between species. The relationship of lateral branches to trichoblasts was not consistent within the four major clades identified in the molecular analyses. Major clades one, two and three had species where branches replaced trichoblasts, branches formed in the axil of trichoblasts, or the branches were independent of the trichoblasts.

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 *Polysiphonia 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 21 out of the 31 North Carolina samples. This character was consistent within all but species A. The inconsistency observed in this species appeared to have some correlation with other characters used in this study. The first character used to explain the inconsistency was the reproductive status of the plant. The two samples in species A that did not have adventitious laterals present also lacked tetrasporangia, however, further investigation revealed plants that produced adventitious laterals but were not tetrasporic. The same comparison was made for plants that were cystocarpic and spermatangial. No congruent trends were found, therefore, there does not appear to be any trend associated with the plant being fertile and the presence of adventitious laterals. A comparison between the arrangement of tetrasporangia and the production of adventitious laterals was made for the collected North Carolina samples. Most samples that produced straight tetrasporangia did not produce adventitious laterals, whereas the samples with spiral tetrasporangia did produce adventitious laterals.

The shape of the adventitious laterals was not a consistent character in the North Carolina samples, so it was not explored in the molecular trees including all species used in this study. Each North Carolina species showed a wide range of shapes from linear to lanceolate to triangular. 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 non-pigmented, 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, while 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 species A, C, D and F, whereas samples of the remaining four species had relatively few trichoblasts. The three samples that lacked trichoblasts were within the species that displayed a low abundance of trichoblasts. In addition to the frequency of trichoblasts, there appears to be a relationship between plants that are reproductive and the presence of trichoblasts. The three samples that lacked trichoblasts were not reproductive, although one appeared to have been previously fertile. This sample had pericentral cells that were displaced in the same manner as those that still have tetrasporangia attached and therefore was considered to have been tetrasporic. Out of the 28 samples that had trichoblasts present, only two were not reproductive and they were resolved in species that displayed a low abundance of trichoblasts. The age of the plant, as well as the condition of the plant may also effect the production of trichoblasts. The lack of variation seen in the North Carolina species did not warrant the exploration of this character for all the species included in this study.

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 every segment or 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 (Figures 39 and 40). 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 every segment had tetrasporangia arranged in a straight series. Kapraun (1977) 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 relationships (Hommersand, 1963; Kim *et al.*, 2000).

The relationship of the pattern of the trichoblasts to the arrangement of the tetrasporangia was not as strong in the overall species trees. The pattern of trichoblasts was consistent in major clades three and four, whereas species in clades one and two had trichoblasts every segment or several naked segments between trichoblasts. *Polysiphonia lanosa, P. nigra* and *Enelittosiphonia fruticulosa* lacked trichoblasts every segment but had spiral tetrasporangia. Despite the inconsistence of the character states observed in the major clades, this character was consistent within species and should be useful for species identifications.

Apical Cells (Characters 9 and 10)

Species D was the only North Carolina species to have inconspicuous apical cells. The apical cells were hard to observe because the tips of this plant were crowded with trichoblasts and epiphytes. This species only contained one sample and there was only one plant within this sample. The results of this character may have been specific to the plant and the apical cells may have been more conspicuous in other plants.

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 non-uniform 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. The characters pertaining to the apical cell were not traced onto the overall species tree because of the subjective nature of the character states.

Nature of the Holdfast (Character 11)

Kapraun (1977) described three types of prostrate development observed 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 described for all but three North Carolina samples. These three samples either had poor material or not enough material to make the correct designation of the nature of the holdfast. Overall, only one species showed inconsistency of this character, but the samples within this species shared the same character state within the other species. The variation seen in species C may be a result of where the samples were collected. The samples of all other species were either collected from the same location or similar environments. Samples in species C were collected from a variety of locations and 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. The other trend that was apparent was the relationship of basal cortication to the nature of the holdfast, which has already been addressed.

The investigation of this character in the molecular trees that included all species used in this study showed that only major clades three and four were consistent with all species displaying strictly prostrate branching development. The other two major clades displayed all three character states and did not show species specific trends. This character was useful for the identification of species and in combination with other characters may provide some information on the relationships of species.

Branching Pattern, Shape and Frequency (Characters 12, 13 and 15)

The branching patterns of the *Polysiphonia* 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. Increasing the number of samples in species D, E, F, and G may result in the same patterns seen in the more variable species A, B, C and H. This character may be more environmentally than genetically determined and therefore was not one of the characters included in the analyses of all the species used in this study.

The shape of the ultimate branch varied in all of the North Carolina species. At first glance, species B and G appear to be consistent for this character but all these samples are only consistent in varying from linear to lanceolate. Samples in these species had some ultimate branches that were linear while others were lanceolate; this character is inconsistent for all samples in this study. 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; however, the consistency observed in species D, G, H and F might be a result of sampling size, since these species contain at the most two samples. Species A, B, C and E 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 instance, in species B one sample was found growing on a buoy within a sound while the other sample was found on a wreck at a depth of 117 feet. The designation of the character states may also be subjective and therefore this character was not included in the overall species analyses.

Apices of Ultimate Branches (Character 14)

Kim & Lee (1999) described a difference between the branch apices of *Polysiphonia* and *Neosiphonia* species. The apices of *Neosiphonia* were round-pointed, whereas in *Polysiphonia*

the apices were 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* apices 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. Scar cells are typically persistent but do vary in their conspicuousness based on their location away from apices (Hollenberg, 1942; Womersley, 1979).

The only two North Carolina samples that did not have scar cells also lacked trichoblasts. Both these samples were within multiple sample species where all other samples had scar cells. The nearly uniform presences of scar cells and within species variability when the character is not constant make it non-informative for species identifications or determining species relationships.

The presence or absence of scar cells may not have proved to be a species-specific character, but the pattern of these scar cells was found to be informative. Scar cell pattern was consistent within the North Carolina species and correlated with the arrangement of tetrasporangia. Species that had straight tetrasporangia had scar cells that were not arranged in a

specific pattern, whereas the species that had spiral tetrasporangia also had spiral scar cells. In the overall species tree, the same relationship of tetrasporangia and scar cells was observed. Major clades one and three showed some inconsistency in the character states for this character.

Scar cells and trichoblasts occurrence and pattern have been referred to together in most taxonomic keys for *Polysiphonia* (Hollenberg, 1968b; Womersley, 1979; Abbott & Hollenberg, 1976; Schneider & Searles, 1991; Abbott, 1999; Womersley, 2003). Scar cells are developmentally derived from trichoblasts so they are not independent and should not be considered separate characters.

The presence or absence of scar cells producing lateral branches, also known as cicatrigenous branches (Hollenberg, 1942), was a consistent character within the North Carolina *Polysiphonia* species and was correlated with the previous character of 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. This pattern was also seen when the character states were traced on the molecular trees containing all the species used in this study.

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). The cystocarps of *Polysiphonia* species are all similar in their makeup but they have been reported to differ slightly in shape. The shape of cystocarps observed in the North Carolina samples ranged from globose to ovate to ovalis 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, and therefore this character was not explored in the molecular trees containing all the species used in this study. Only cystocarps containing carpospores were included in these observations to reduce any variation in shape with a basis in cystocarp maturity. Part of the variation seen may be a result of sample preparation, both in preserving the sample, as well as, 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 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 has been used in previous studies as a reliable character for species designation (Hollenberg & Norris, 1977; Womersley, 1979; Schneider & Searles, 1991; Maggs & Hommersand, 1993; Choi *et al.*, 2001; Womersley, 2003). Choi *et al.* (2001) also considered its character states to be diagnostic for groups of species. Their "*Polysiphonia* group" had spermatangia that replaced the whole trichoblast, while spermatangia developed on a trichoblast furcation in the other two groups they recognized. In the current study, spermatangial development traced on the molecular trees that included all species examined showed that major clades three and four had spermatangia that replaced the whole trichoblast, while the other two clades were inconsistent in the character states.

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 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 was a consistent character within North Carolina samples and species. Two out of the eight species had samples with spiral tetrasporangia. The remaining species had tetrasporangia arranged in a straight series. The tetrasporangia that formed a spiral series appear to form from different pericentral cells in each successive segment whereas the ones in the straight series seem to be forming from the same pericentral cell in each segment.

Choi *et al*. (2001) found that only species in their "*Polysiphonia* group" possessed straight tetrasporangia. Their "*Neosiphonia* group" and "multipericentral group" both had spiral tetrasporangia. In the current study, three of the four major clades included species that were also studied by Choi *et al.* (2001). Species within their "*Neosiphonia* group" were resolved within major clade one but not all species in this clade displayed spiral tetrasporangia. Choi *et al.* (2001) recognized that some variation exsisted in their "*Neosiphonia* group" because *Polysiphonia virgata* displays straight tetrasporangia. In major clade one, *P. virgata* and species B were in conflict with the other species for the character states observed. Major clade two included species that were found within the Choi *et al.* (2001) "multipericentral group" and there was no disagreement in character states observed. Their "*Polysiphonia* group" species were resolved with species within major clade four in this study, which all had straight tetrasporangia.

Taxonomically Important Morphological Characters

Previous taxonomic studies on *Polysiphonia* used the following characters to determine between species: number of pericentral cells; position of trichoblasts and scar cells; origin of branches and their relationship to trichoblasts; nature of the antheridial branches; nature and origin of the holdfast; point of origin of rhizoids; length/width (L/W) ratio of segments; nature 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). After thorough investigation of the 22 morphological characters the following five characters have been found useful in defining *Polysiphonia* species in the present study: number of pericentral cells, rhizoid-pericentral cell connection, relationship of lateral branches to trichoblasts, spermatangial axes development, and arrangement of tetrasporangia. The pattern and occurence of trichoblasts and scar cells, the nature of the holdfast, and formation of cicatrigenous branches may also help in determining between species but may not provide much information on their own.

Two morphological features that were not pursued in this study but have been considered important were the L/W ratio of segments and the number of carpogonial branch cells. An investigation of L/W ratios was carried out, but it was not included in the final results because no conclusive trends were found. The L/W ratios of segments at the base, middle and tips of the main axes varied within a species. Kim *et al.* (1994) and Curiel *et al.* (2002) found the same result in their studies and determined that the L/W ratios were too variable to be used to identify species.

The carpogonial branches of *Polysiphonia* 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) found that carpogonial branches with three cells were a main morphological difference between species in *Neosiphonia* and *Polysiphonia*. 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 the current study, no conclusive observations were made of this character. In Rhodomelaceae, the pericarp forms before fertilization (Hommersand, 1963) making it very difficult to observe the number of carpogonial branch cells. The material in this study either was not stained properly or the material was too mature to determine the number of carpogonial branch cells. This character is one that has been used to separate genera and is considered useful, but was not included in this study.

Taxonomic Implications

Species A: *Neosiphonia harveyi*

Neosiphonia harveyi is the current name for the species formerly identified as *Polysiphonia harveyi*. The transfer to *Neosiphonia* was proposed by Choi *et al.* (2001) based on molecular analyses and morphological characters. *Polysiphonia harveyi*, *Neosiphonia japonica* and *N. savatieri* were found to have identical SSU sequences and 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 split 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.

The morphological character states of species A in the present study agree with the generic description of *Neosiphonia* by Kim & Lee (1999). The *rbc*L and SSU sequence data generated in the current study also agreed with this classification, and resolved this species within clades comprised of other currently recognized *Neosiphonia* species. The molecular and morphological data therefore support the classification of this species as *Neosiphonia harveyi*.

Species B: *Polysiphonia denudata sensu* Kapraun

The identification of species B as *P. denudata sensu* Kapraun needs further taxonomic attention. Southampton, England, is the type locality for *P. denudata*. The keys and descriptions used to identify this species did not agree with important taxonomic character states (Kapraun, 1977, 1980a; Schneider & Searles, 1991; Maggs & Hommersand, 1993). Descriptions of *P. denudata* from Britain include the following character states: presences of basal cortication, numerous trichoblasts found on every segment, thalli arising from single basal holdfast, and tetrasporangia arranged in a spiral series (Maggs & Hommersand, 1993). Speciemens of *P. denudata sensu* Kapraun in the present study did not share these character states, although they keyed to *P. denudata* in taxonomic keys specific to North Carolina and agreed with the descriptions found in these taxonomic references (Kapraun, 1977, 1980a; Schneider & Searles, 1991). *Polysiphonia*

denudata sensu Kapraun was not resolved in the clade containing *P. denudata* from the type locality by analyses of *rbc*L (Figure 1). Kapraun (1978) considered *Polysiphonia denudata* to be a warm temperature species because of its response to culture conditions, the growth and reproductive limitations observed within its North American range, and its geographic distribution. These findings suggested that *P. denudata* may consist of ecologically different populations through its extensive latitudinal range. The disagreement seen in the taxonomic references, as well as molecular data and results from field and culture studies indicate that *P. denudata sensu* Kapruan is not conspecific with *P. denudata* TYPE and, therefore, requires further investigation for proper taxonomic identification.

Species C: *Polysiphonia breviarticulata*

Polysiphonia breviarticulata is an introduced species that was first reported in North Carolina in 1982 and 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). In the present study, the samples within species C fit the descriptions of Kapraun & Searles (1990), Schneider & Searles (1991) and Kapraun (unpublished MS). This species has been identified by its abundant adventitious laterals and enlarge ostiolar lip cells. *Polysiphonia breviarticulata* has also been reported to vary in size. Three of the nine samples of this species were cystocarpic and the presence of enlarged ostiolar lip cells was observed. All of the samples collected in this study had adventitious laterals and they also varied greatly in the size of plants. One of the samples was collected from a bloom that took place in the intracoastal waterway near Snead's Ferry at 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 be repeated. This species was collected from numerous sites in North Carolina and can be found from intertidal creeks in the states sounds to deep offshore waters.

Species D: *Polysiphonia fucoides*

This taxon has been reported to reach its southern limit of distribution in the western Atlantic in the Carolinas. Kapraun (1977) verified the occurrence of this taxon in the Wilmington, North Carolina area but the reports of this species in South Carolina have been questioned (Schneider & Searles, 1991). *Polysiphonia fucoides* has been found to be quite variable in morphology throughout its geographic range (Schneider & Searles, 1991; Maggs & Hommersand, 1993).

The descriptions of *P. fucoides* report that lateral branches replace the trichoblasts (Kapraun, 1980a; Schneider & Searles, 1991; Maggs & Hommersand, 1993), however this character state was deceiving 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 has also 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 SSU sequence generated in this study was identical to that of a *P. fucoides* sample collected from an area near the type locality supporting the conspecificity of the western Atlantic taxon.

Species E: *Polysiphonia atlantica*

Kapraun & Norris (1982) abandoned the name *P. 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

(1977) 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 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 this species differed from the inshore *P. atlantica* in important morphological characters (Schneider & Searles, 1991). Schneider (1976) described spiral tetrasporangia and numerous adventitious laterals for the offshore species, but Schneider & Searles (1991) later decided that these samples from offshore were not the same species as the inshore populations of *P. atlantica*. The 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 some of these species suggested by the morphological data.

Species F: North Carolina *Polysiphonia urceolata sensu* Kapraun

Kapraun (1979) found morphological and physiological differences among three north Atlantic populations of *P. urecolata*. North Carolina *P. urceolata* formed spermatangia on a furcation of the trichoblasts and rarely produced sterile cells, New England *P. urceolata* spermatangia sometimes formed on a furcation but they had a number of sterile cells present, and Norwegian *P. urceolata* 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 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. The samples collected in the present study were equivalent to the *P. urceolata* described in Kapraun's (1979) study.

Polysiphonia urceolata is currently considered a synonym of *P. stricta*. The sequence data collected in this study, as well as previous morphological and physiological data, provides evidence that the North Carolina *P. 'urceolata'* differs from the taxon described as *P. stricta*, and should not be referred to as *P. stricta*. Further study is needed in order to determine the best name for this North Carolina species.

Species G: *Polysiphonia subtilissima*

Polysiphonia subtilissima has been reported as a brackish-water species (Schneider & Searles, 1991; Abbott, 1999), and the samples collected in study were all found in brackish water environments. Male gametophytes have only been observed 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 conspecificty 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.

Species H: *Polysiphonia scopulorum* var. *villum*

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

Overall Conclusion

The morphological and molecular results showed that eight distinct species of *Polysiphonia sensu lato* were collected in this study. The *rbc*L and SSU analyses resolved four major clades within *Polysiphonia sensu lato* (Figures 1 and 2). These four molecularly defined clades were also supported to some degree by taxonomically important morphological characters. Species in major clades one and two showed the greatest variation in morphological character states compared to the species in clades three and four. Clades three and four in the SSU analyses formed a single well-supported clade with the exception of the parsimony analysis (D93, P68, M95). These two clades did not form a single clade in the *rbc*L trees but there was no support for

this different topological arrangement. I feel that clades three and four should be regarded as *Polysiphonia sensu stricto* based on the shared morphological character states observed within these clades.

Major clade two was variable in eight of the 11 morphological characters assessed in this study. The character states that were consistent in clade two were the highly elevated number of pericentral cells (> eight) and the spiral arrangement of tetrasporangia and scar cells. The variation observed in morphological characters within this clade suggests that it may contain multiple distinct species groups. Multiple generic names have been applied to different species in clade two and further species sampling is needed in order to determine which if any names should be used to classify these species groups.

Major clade one diplayed the most variation in morphological character states, with pit connected rhizoids being the only shared character state for these species. Within clade one there are a number of smaller well-supported clades that were consistent for additional morphological character states. The clade comprised of species *P. fibrillosa*, *P. brodiaei*, and *P. fibrata*, for example, shared charater states for eight of the 11 assessed characters. Similar to major clade two, clade one contains species from more than one currently recognized genus, with species of *Neosiphonia* also resolved within this group.

Choi *et al*. (2001) showed in their integrated study of *Polysiphonia sensu lato* that this was not a monophyletic genus. They used a limited number of species and only sequenced one gene but found *Polysiphonia sensu lato* to be polyphyletic. The current study incorporated two genes and increased the taxon sampling, and these results support the polyphyletic status of *Polysiphonia* as currently circumscribed. Additional molecular and morphological analyses will

be needed in order to develop a natural classification system based on the evolutionary relationships of species within *Polysiphonia sensu lato*.

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