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# Phylogeny, distribution and evolution of the filamentous Red Algae: *Polysiphonia* sensu lato (Rhodomelaceae, Rhodophyta)

조선대학교 대학원

생명과학과

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# 홍조류 붉은실속 (*Polysiphonia* sensu lato)의 계통, 분포 및 진화에 관한 연구

2016 년 2 월 25 일

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# ABSTRACT*

## Phylogeny, distribution and evolution of the filamentous Red Algae: *Polysiphonia* sensu lato (Rhodomelaceae, Rhodophyta)

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The genus *Polysiphonia* sensu lato is widely distributed in tropical, warm-temperature, cold-temperate, and subantarctic regions including mangrove habitats and estuaries. Our morphological and molecular analyses of 1088 specimens of *Polysiphonia* sensu lato (Tribe Polysiphonieae) collected from 156 different localities around 16 countries during twelve years (2003-2015) revealed that *Polysiphonia* sensu lato (Tribe Polysiphonieae) is composed of 17 genera (*Boergeseniella*, *Brongniartella*, *Bryocladia*, *Diplocladia*, *Dorsisiphonia*, *Enelittosiphonia*, *Hapterosiphonia*, *Lampisiphonia*, *Leptosiphonia*, *Lophosiphonia*, *Neosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Polyostea*, *Polysiphonia* sensu stricto, *Streblocladia*, *Tolypiocladia*, *Vertebrata*). Five of these genera were segregated as new genera (*Dorsisiphonia*, *Hapterosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Wilsonosiphonia*), two were enlarged (*Brongniartella*, *Leptosiphonia*), and one was resurrected

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(*Polyostea*) on the basis of diagnostic features and high bootstrap values of Maximum likelihood and posterior probabilities of Bayesian analyses. Also, 16 new species and 34 new combinations have been proposed among *Polysiphonia* sensu lato on the basis of morphological and molecular analyses of *rbc*L and *cox*1 locus. Moreover, *Neosiphonia* species have been segregated based on the three-celled carpogonial branches. Of them, a group of species having four pericentral cells and cortication, namely, *N. harveyi* complex is composed of six species. We sequenced genes from plastid (*rbc*L) and mitochondrial (*cox*1) genomes to examine the phylogeny, species status, biogeography, and evolution of specimens belonging to this complex collected worldwide. Our data strongly support two species within this complex: *N. harveyi* and "*P. strictissima".* The first species is composed of six genetic taxa described here as subspecies on the basis of DNA-based delimitation models. We also estimated the divergence time of these species using substitution rates of combined *rbc*L and *cox*1 data sets. We confirm that the centre of diversity and origin is the East Asia (Korea and Japan) and that these species were originated around 700000 years ago and their migration from the eastern Asia to the north Atlantic likely took place through an ice-free Arctic Ocean before the first widespread Pleistocene glaciations in Iceland. In contrast to these ideas, some polysiphonous species have been considered as invasive or introduced species and our study demonstrated the wide distribution of *Neosiphonia harveyi subsp. harveyi*, *Neosiphonia harveyi* subsp. *japonica*, *Neosiphonia echinata*, *Pterosiphonia arenosa*, and *Womersleyella indica* sp. nov.

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# I. INTRODUCTION

The family Rhodomelaceae, together with Dasyaceae and Delesseriaceae have been originated from a common ancestor within the Ceramiaceae (Choi et al. 2002). This family is supported phylogenetically by the ultimately sympodial branching of the gonimoblast after the first formed carposporangium (Phillips et al. 2000, Choi et al. 2002, Choi et al. 2008). This feature is considered diagnostic of the Rhodomelaceae (Choi et al. 2002). The Rhodomelaceae, including more than 130 genera, is the largest family of red algae. It is characterized by pericentral cells that are cut off in an alternating sequence such that, the last-formed is located opposite the first-formed (i.e. a rhodomelacean sequence). The sexual organs are typically borne on trichoblasts, which are exogenous branches arising from a subapical central-axial cell before pericentral cells are cut off, and a pericarp initial is usually present prior to fertilization (Maggs and Hommersand 1993, Choi et al. 2002). Two of the four largest genera in the Rhodophyta, *Polysiphonia* and *Laurencia*, belong to the Rhodomelaceae (Womersley 1979). The genus *Polysiphonia* Greville (1824) with a long and confused nomenclatural history has been considered as the core of the Family Rhodomelaceae (Segi 1951, Kim et al. 2000). The original name for species having features of *Polysiphonia* was proposed by C. Agardh (1817) as *Hutchinsia*, but this name was invalid as it was prior applied by Robert Brown to a group of cruciferous plants (Aiton 1812) in honor of Miss Ellen Hutchins who collected widely in Bantry Bay, Co. Cork, Ireland, in the early 1800s. The homonymy with *Hutchinsia* R. Brown was soon recognized and several substitute names were proposed independently. *Polysiphonia* Greville (1823) was conserved and the other considered as rejected names (Wynne 1986, Silva et al. 1996). The generitype of this genus was selected later by Silva (1952) on the basis of *Polysiphonia urceolata* (Lightfoot *ex* Dillwyn) Greville (1824), originally described as *Conferva urceolata* Dillwyn (1809). *P. urceolata* has been placed in synonymy with *Polysiphonia stricta* (Dillwyn) Greville by Maggs and Hommersand (1993).

## 1. History of the genera considered under synonymy of *Polysiphonia*

The recent review of the classification of red algal genera by Schneider and Wynne (2007) listed the following generic names placed in synonymy with the genus *Polysiphonia*: *Hutchinsia* C. Agardh (1817) nom. illeg. (non Aiton 1812), *Grammita* Bonnemaison (1822), *Dicarpella* Bory de St.-Vincent (1823), *Grateloupella* Bory de St.-Vincent (1823, as *Gratelupella*), *Girodia* Lestiboudois (1827), *Carradoria* C.F.P. Martius (1833) nom. illeg., *Grammitella* P. Crouan et H. Crouan (1848), *Polyostea* Ruprecht (1850), *Orcasia* Kylin (1941), *Carradoria* Kylin (1956) *nom. illeg*., *Carradoriella* P.C. Silva in Silva et al. (1996).

The genera *Grammita, Dicarpella*, *Grateloupella*, and *Girodia* were proposed to replace the illegitimate generic name *Hutchinsia* proposed by C.Agardh (1817). These genera were proposed without the designation of type species (generitype) for each of them (Bory de St.-Vincent 1823, Lestiboudois 1827, Bonnemaison 1822). All these genera are referring to *Polysiphonia* sensu stricto with its type *P. urceolata* after Silva et al. (1952) and Greuter et al. (1994). Although *Grammita* had priority over *Polysiphonia* for the invalid *Hutchinsia* C.Agardh, Greville (1824) objected to it because of the possibilities of confusion with the genus *Grammitis*, which had been used for one group of fungi and for another of ferns (Baten 1923, Tseng 1944).

The genus *Grammitella* was described by Crouan and Crouan (1848) as an intermediate genus closer to *Polysiphonia* (as *Grammita*) and *Rhytiphloea* based on the pericentral cells size which is having the same diameter with the cortical cells. Although Crouan and Crouan (1867) later transferred *Grammitella guernisaci* Crouan to *Dasya* in their Flora Finistere exisiccata, the genus *Grammitella* was synonymized with *Polysiphonia* by J. Agardh (1863) and confirmed by Kylin (1956) and Schneider and Wynne (2007).

The genus *Polyostea* was originally described by Donati (1750) without a designation of type species. Later, Ruprecht (1850) accepted this genus and described one new species (*P. gemmifera* Ruprecht) and transfer two known species to *Polyostea* (*P. bipinnata* (Postels et Ruprecht) Ru-

precht and *P. porphyroides* (Kützing) Ruprecht). Although Ruprecht (1850) knew about the priority of the other genera for the species of *Hutchinsia* C. Agardh (1817), Ruprecht (1850) preferred to use *Polyostea* only for the species which have the pericentral cells disposed in a similar way to connected bones of animals. Currently, *Polyostea gemmifera* and *Polyostea bipinnata* are considered synonymous of *Pterosiphonia bipinnata* (Postels et Ruprecht) Falkenberg.

The genus *Orcasia* was proposed by Kylin (1941) in his Californischen Rhodophyceen on the basis of *P. senticulosa* considering the endogenous origin of accessory branches as a diagnostic character. Kylin (1941) also placed *P. morrowii* in his new genus *Orcasia*. Kudo and Masuda (1988) rejected this new genus and treated both Kylin's species of *Orcasia* as members of *Polysiphonia*.

*Carradoria* was resurrected by Kylin (1956) based on the hyphae between the pericentral and axial cells as diagnostic feature and lectotypified the South African endemic species *C. virgata* (C.Agardh) Kylin as generitype. Although he attributed the name to Martius, he effectively created a homonym in accordance with Art. 48.1 by excluding the type species (Silva et al. 1996). Although *Carradoria* Kylin was considered to be a synonymous of *Polysiphonia* by Wynne (1986), Silva et al. (1996) proposed the new name *Carradoriella* P. C. Silva with its type *Carradoriella virgata* (C. Agardh) P.C. Silva to replace *Carradoria* Kylin (1956) and to distinguish from *Carradoria* Martius (1833).

## 2. History of the taxonomical changes and additions in *Polysiphonia*

After the description of *Polysiphonia* by Greville (1824), several new reports with bare descriptions for the species of this genus were made by Lyngbye (1819), Sprengel (1827), Montagne (1849), and Zanardini (1841). However, the monumental work of Kützing (1843, 1849) with remarkable figures listed and compiled 155 species of *Polysiphonia* in Kützing (1863) and 98 more in Kützing

(1864). The first systematic arrangement in *Polysiphonia* was made by Agardh (1863), who considered *Polysiphonia* as a diverse and speciose genus (Kim et al. 2000). Agardh (1863) divided this genus primarily on the basis of thallus size into four subgenera: *Ptilosiphonia*, *Herposiphonia*, *Oligosiphonia* and *Polysiphonia.* Then, the works of Suringar (1870), Kjellman (1883), Ardissone (1888), Hariot (1891), Holmes (1895), and De Toni (1895) increase the diversity of *Polysiphonia* by adding new reports and new descriptions.

The entire Rhodomelaceae were revised on the basis of vegetative and reproductive morphology by Schmitz (1889) including 41 genera, then Schmitz and Falkenberg (1897) including 78 genera, and finally by Falkenberg (1901) including 82 genera. These studies proposed that Rhodomelaceae is composed of the following tribes: Amansieae, Bostrychieae, Chondrieae, Herposiphonieae, Heterocladieae, Laurencieae, Lophothalieae, Polysiphonieae, Polyzonieae, Pterosiphonieae, Rhodomeleae, and the genus *Lophosiphonia*. Moreover, Falkenberg (1901) clearly delimited the genus *Polysiphonia* by having the following features: (1) at least the ultimate branches are evidently polysiphonous, (2) most of the branches arise exogenously by a more or less diagonal division of subapical cells before these have cut off pericentral cells, (3) all branches are essentially similar and indeterminate, and (4) only one tetrasporangium is borne normally in each segment.

Additionally, the following authors contribute with the description of new species or noteworthy species to the genus *Polysiphonia*: Howe (1914), Børgesen (1918, 1933), Baten (1923), Setchell and Gardner (1924, 1930), and Taylor (1937). A new arrangement in *Polysiphonia* was proposed by the following authors: (1) Kylin (1941) segregated the genus *Orcasia* based on *Polysiphonia senticulosa* Harvey, (2) Levring (1941) newly described the genus *Fernandosiphonia* Levring based on *F. unilateralis* Levring from Chile, and (3) Kylin (1956) resurrected the genera *Vertebrata* and *Carradoria* Kylin *non* Martius (known as *Carradoriella* P. Silva) and also proposed the genus *Boergeseniella* based on *Polysiphonia fruticulosa* (Wulfen) Sprengel.

The noteworthy works of *Polysiphonia* from the coast of Mexico and California by Hollenberg (1942, 1944, 1961, 1968), from Japan by Segi (1949, 1951, 1959, 1960), from Australia by Womersley (1979), from Korea by Yoon (1986), and from New Zealand by Adams (1994) clearly circumscribed the diversity of *Polysiphonia* in the Pacific Ocean with the recognition of 36 species in the Southern Pacific, 31 species in the Northeast Pacific and 36 species in the Northwest Pacific. This diversity was also expressed with the segregation of two new genera from *Polysiphonia* sensu lato: *Enellitosiphonia* Segi (1949) based on *P. hakodatensis* Yendo from Japan and *Womersleyella* Hollenberg (1967) based on *W. pacifica* Hollenberg from Pacific Islands. The diversity of *Polysiphonia* from the Western Atlantic was reported in the studies of Kapraun (1977, 1979), Kapraun and Norris (1982), and Kapraun and Rueness (1983) with the recognition of 20 species. The detailed diversity of Rhodomelaceae by Maggs and Hommersand (1993) recognized 25 species of *Polysiphonia* in Eastern Atlantic. The studies in the Indic Ocean were compiled by Silva et al. (1996) recognizing 60 species of *Polysiphonia* sensu lato.

### 3. History of the molecular studies in *Polysiphonia*

The molecular study of McIvor et al. (1999), using the large subunit of ribulose-1,5- bisphosphate carboxylase/oxygenase (*rbc*L) gene, concluded that the genus *Polysiphonia* is paraphyletic, consisting of four strongly supported lineages. Then, Kim and Lee (1999) segregated the genus *Neosiphonia* from *Polysiphonia* based on *Neosiphonia flavimarina* M.S. Kim et I.K. Lee from Korea. This genus was recognized and confirmed in the anatomical and molecular analyses of Choi et a. (2001). They also defined the multipericentral group which included the following four genera from the north Atlantic and northwest Pacific: *Boergeseniella* Kylin, *Enelittosiphonia* Segi, *Polysiphonia* Grev., and *Vertebrata* S.F. Gray.

McIvor et al. (2001) integrated karyological, interbreeding, and sequence data in an analysis of the invasive species *Polysiphonia harveyi* Bailey. Kim et al. (2004) and Kim and Yang (2005)

further demonstrated the utility of *rbc*L sequence analyses for the identification of *Polysiphonia* species. Stuercke and Freshwater (2008) examined many of the morphological characters used to distinguish *Polysiphonia* sensu lato species in an integrated molecular-morphological study. The subsequent studies of Stuercke and freshwater (2010), Mamoozadeh and Freshwater (2011, 2012), Bustamante et al. (2012, 2013a, 2013b, 2014a, 2014b, 2015a) and Kim and Kim (2014) characterized *Polysiphonia* sensu lato species in detailed morphology by describing new species and proposing new combinations with the support of molecular analyses. Finally, *Polysiphonia* sensu lato was arranged with the description of two more genera *Hapterosiphonia* D.E.Bustamante, B.Y.Won et T.O.Cho and *Lampisiphonia* H.G. Choi, Diaz-Tapia et Bárbara (Bárbara et al. 2013, Bustamante et al. 2015c). Currently, *Polysiphonia* sensu lato is composed of around 220 species widely distributed in marine environments (Guiry and Guiry 2015). The recent molecular studies divided *Polysiphonia* sensu lato into five strongly supported clades: *Hapterosiphonia*, *Lampisiphonia*, *Neosiphonia*, *Polysiphonia*, and the multipericentral group (Kim and Lee 1999, Choi et al. 2001, Bárbara et al. 2013, Bustamante et al. 2015c).

## 4. Objectives

The genus *Polysiphonia* Greville (1823) is one of the largest groups in the red algae. In *Polysiphonia* sensu lato around 900 species have been listed, of which 223 have been flagged as currently accepted taxonomically (Guiry and Guiry 2015). In the present study, we have collected 1088 polysiphonous specimens from 156 different localities around 16 different countries. We have reassessed these specimens based on detailed morphology and molecular phylogenetic analyses (1) to confirm the relationships among the different genera embedded in *Polysiphonia* sensu lato, (2) to evaluate species delimitation methods in species complex, (3) to determine the current distributional patterns of cosmopolitan species and (4) to infer mechanisms that gave rise to them, and (5) to calculate the possible evolutionary history. Here, we propose substantial taxonomical changes in *Polysiphonia* sensu lato with the segregation and resurrection of some genera and also with the

proposal of new combinations and new species, including detail descriptions of poorly known species.

The part 1 is showing in detail the vegetative and reproductive features found in *Polysiphonia* sensu lato. The consistent of these features to delimit species and genera in *Polysiphonia* sensu lato are discussed. Also, a general phylogenetic framework is provided.

The chapter 1 of the part 2 is proposing the segregation of *Hapterosiphonia* as a new genus from the Pacific Ocean based on detailed morphological and phylogenetic analyses of *rbc*L and *cox*1 sequences to include four taxa that are having in common paniculate branching pattern and rhizoids cutting off from pericentral cells with multicellular lobed terminations.

The chapter 2 of the part 2 is enlarging the circumscription of the genus *Leptosiphonia* based on detailed morphology and molecular evidence of *rbc*L and *cox1* locus to encompass *Leptosiphonia brodiei* comb. nov., *L. elongata* comb. nov., and *L. virgata* comb. nov. and also one new species *L. platensis*, which are having in common rhizoidal cells between pericentral and axial cells in the basal part and corticated axes.

The chapter 3 of the part 2 provides a reappraisal of the classification of some *Neosiphonia* specimens (characterized by having three-celled carpogonial branches and rhizoids cutting off the pericentral cells) based on phylogenetic analyses of their *rbc*L sequences. These analyses characterized 23 species in the genus *Neosiphonia* with the recognition of 9 new species and 9 new combinations.

The chapter 4 of the part 2 is proposing the resurrection of the genus *Polyostea* after reassessed specimens having apex spirally originated with bilateral phyllotaxy. These specimens, *Polyostea bipinnata* and *P. gracilis* comb. nov., were analyzed based on the detailed morphology and the phylogenetic relationships with other similar species by analyzing the *rbc*L and *cox*1sequences.

The chapter 5 of the part 2 provides a reassessment of the species embedded in the paraphyletic *Polysiphonia* sensu stricto based on anatomical observations and molecular analyses. The segregation of the new genera *Dorsisiphonia*, *Neostreblocladia,* and *Phillipsiphonia* are proposed to accommodate species with a wide phenotypic plasticity traditionally group in Polysiphonia sensu stricto.

The chapter 6 of the part 2 characterize morphologically and genetically samples of the generitype *Tolypiocladia*, namely, *T. glomerulata* and also *T. calodictyon* and examine their phylogenetic relationships among some members of the tribe Polysiphoniae by analyses of *rbc*L sequences.

The goals of the chapter 7 of the part 2 were the segregation of the new genus *Wilsonosiphonia* based on diagnostic anatomical feature (multicellular rhizoids produced from the distal end of pericentral cells) and molecular analyses (*rbc*L and *cox*1 markers), describe the new species *Wilsonosiphonia fujiiae*, and transfer *W. howei* from *Polysiphonia*.

The chapter 8 of the part 2 is providing a detail morphological analyses of polysiphonous specimens having in common numerous pericentral cells (multipericentral group) and also analyzing their phylogenetic relationship based on *rbc*L and *cox*1sequences. These analyses characterized the multipericentral group as a parphyletic group composed of six genera: *Boergeseniella, Brongniartella, Diplocladia, Enelittosiphonia, Polysiphonia,* and *Vertebrata.*

The chapter 9 of the part 2 is proposing the synonymy of the genus *Perrinia* with the genus *Diplocladia* and the new combination *Diplocladia ericoides* based on molecular analyses of *rbc*L and *cox*1 sequences and also on the lack of consistency of the features separating these genera.

The chapter 10 of the part 2 is issuing the taxonomy, phylogeny and distribution of *N. echinata* from the Southeast Asia to Western Atlantic as an epiphyte and introduced species, based on an integrated molecular-morphological study.

The chapter 11 of the part 2 provides the description of the new species *Womersleyella indica*, a widely distributed species from the coast of India to Panama. Further evidence is provided based on phylogenetic relationships with other species of *Womersleyella* and other polysiphonous groups using *rbc*L sequencing analyses.

The chapter 1 of the part 3 is providing DNA-species delimitation, biogeographic, phylogenetic, and evolutionary analyses to asses species boundaries, phylogenetic relationships, divergence rates leading to speciation, and times of origin of diversification of a group of species that are having in common four pericentral cells and cortication, namely the *Neosiphonia harveyi* complex.

# II. MATERIALS AND METHODS

## 1. Morphological examination

Anatomical observation were made from 1088 samples collected along 156 different localities around 16 countries (Table 1). The samples were preserved in 4–5% formalin/seawater for morphological examination and in silica gel for molecular analysis. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea. Materials were stained with 1% aqueous aniline blue acidified with 0.1% diluted HCl for microscopic observations. Photomicrographs were taken using an Olympus microscope (BX51TRF, Olympus, Tokyo, Japan) equipped with an Olympus DP71 camera. We selected 25 individuals from five tufts for the determination of quantitative characters, and calculated the means and standard deviations of these characters.

## 2. Molecular analyses

### 2.1. DNA extraction, amplification and sequencing

Genomic DNA was extracted from silica gel-dried samples by using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. We sequenced *rbc*L and *cox*1. Each gene was amplified by PCR using Bioneer reagents (Bioneer, Daejon, Korea) in the following reaction mixture: 2 μL of 10 mM dNTP mix; 2 μL of 10× reaction buffer; 1 μL of 5–10 μM forward and reverse primers and 0.2 μL of TOP DNA polymerase; and 3 μL of genomic DNA for a 20-μL reaction.

The PCR protocol for plastid *rbc*L consisted of an initial denaturing and enzyme activation step at 94℃ for 5 min, followed by 35 cycles each at 94℃ for 30 s (denaturation), 45℃ for 1 min (annealing), and 72℃ for 1 min (extension), and a final extension step at 72℃ for 10 min (Bustamante et al. 2012, 2013a). The *rbc*L locus was amplified using primers F7, F57, R753, F645, R1318, and RrbcS start (Freshwater and Rueness 1994, Table 2), and was purified with the PCR-

quick-spin™ PCR product purification kit (Intron Biotechnology Inc., Seongnam, Korea). Cycle sequencing was performed using the same primers for amplification (Table 2). The PCR protocol for mitochondrial *cox*1 consisted of an initial denaturing and enzyme activation step at 94℃ for 5 min, followed by 35 cycles each at 94℃ for 1min (denaturation), 45℃ for 1 min (annealing), and 72℃ for 1 min (extension), and a final extension step at 68℃ for 2min. The *cox*1 locus was amplified using primers COXI43F and COXI1549 (Geraldino et al. 2006), and was purified with the PCRquick-spin™ PCR product purification kit (Intron Biotechnology Inc., Seongnam, Korea). Cycle sequencing was performed using the same primers for amplification (Table 2).

The sequences for the forward and reverse strands were determined with an ABI Prism 3100 Genetic Analyzer (Life Technologies™, Seoul, Korea). New *rbc*L and *cox*1 sequences were obtained and deposited in EMBL/GenBank. These sequences and others obtained from GenBank were initially aligned with ClustalW (Thompson et al. 1994) and adjusted manually with MEGA5 software (Tamura et al. 2011).

### 2.2. Phylogenetic analyses

Three data sets were used for the phylogenetic analyses: 310 taxa for *rbc*L, 180 taxa for *cox*1 and 180 taxa for combined *rbc*L and *cox*1 data sets. All analyses on the individual and combined data sets were carried out with best-fit model of DNA substitution for maximum likelihood (ML) and Bayesian inference (BI). For the protein-coding data sets *rbc*L and *cox*1, partitioning by codon position and separate DNA-substitution models was chosen for each position. Phylogenetic and molecular evolutionary analyses for ML analysis were conducted with 1000 bootstrap replications in MEGA5 using the GTR+Γ+I model (Bustamante et al. 2012, 2013a). The BI analyses were performed with the program MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Two independent analyses, each consisting of four Markov chains, were run simultaneously for 6,000,000 generations, sampling every 1000 generations. Log likelihood and parameter values were assessed with

Tracers ver. 1.5 (Rambaut and Drummond 2009). A burn-in of 25% of saved trees was removed, and the remaining trees were used to calculate the Bayesian posterior probability values (Bustamante et al. 2014a, 2014b). ML and BI trees were edited with the program FigTree v1.3.1 (Rambaut 2009).

## 3. Biogeographic and evolutionary analyses

### 3.1. DNA-based species delimitations methods

We explored the two single-locus species delimitation methods using both the *cox*1 and *rbc*L data sets to assess species boundaries within the genus: automatic barcoding gap detection (ABGD) (Puillandre et al. 2012), statistical parsimony network analysis (SP) (Hart and Sunday2007), and the Generalized Mixed Yule Coalescent (GMYC) method. The ABGD method was tested via a web interface (ABGD web, http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). Before analysis, the model criteria were set as following: intraspecific variability (P) between 0.001 (Pmin) and 0.1 (Pmax), minimum gap width (X) of 0.1, Kimura-2-parameters and 50 screening steps. In addition, statistical parsimony networks of rbcL and *cox*1 data sets were generated in TCS 1.21 (Clement et al. 2000) with a maximum connection probability set at 95% statistical confidence. For the GMYC delimitation method, an ultrametric tree was constructed in BEASTv2.0.2 (Drummond et al. 2012), relying on the uncorrelated lognormal relaxed clock, the GTR+I+G, and a coalescent tree prior. Bayesian Markov chain Monte Carlo (MCMC) was run for 20 million generations, and trees and parameters sampled every 1000 generations. Log files were visualized in Tracers v1.5 (Rambaut and Drummond 2009) for assessing the stationary state of parameters on the basis of the value of estimate-effective sample size (ESS). After removing 25% of trees as burn-in, the remaining trees were used to generate a single summarized tree in TreeAnnotator v2.0.2 (part of the BEAST v2.0.2 package) as an input file for GMYC analyses. The GMYC analyses with a single threshold

model were performed in R (R Development Core Team, http://www.R-project.org) under the 'splits' package using 'gmyc' function (R-Forge, http://r-forge.r-project.org/projects/splits/).

### 3.2. Estimation of divergence time

The virtual lack of fossils impedes a precise calibration of the molecular clock for members of the Rhodomelaceae. We attempted to estimate the divergence time of our species by using substitution rates from the separation of *Neosiphonia* taxa with respect to the time of the emergence of the Isthmus of Panama (Zucarello and West 2002, Muangmai et al. 2014). Estimation of divergences times was based on the combined data set of rbcL and *cox*1 using BEAST v2.0.2 (Drummond et al. 2012). We set the criteria for BEAST analysis as following: the same partition and substitution model as used in phylogenetic analyses; unlinked site model and clock model with linked trees; and a Yule model as the tree priors. The rates used in the clock model were an uncorrelated lognormal relaxed clock for all data sets, with means rate of 0.043 substitutions site$^{-1}$(million years)$^{-1}$ for *rbc*L, and 0.058 substitutions site$^{-1}$ (million years)$^{-1}$ for *cox*1. We ran the MCMC analyses for 40 million generations, sampling every 5000 generations, and then determined the distribution and ESS of parameters using Tracers v1.5 (Rambaut and Drummond 2009). The initial 25% of saved trees was removed as the burn-in, and a maximum credibility tree based on the remainder was produced using TreeAnnotatorv2.0.2 (part of the BEAST v2.0.2 package). The time-calibrated tree with 95% highest posterior density was visualized and edited in FigTree v1.3.1 (Rambaut 2009).

### 3.3. Haplotype networks

Mean sequence divergence (number of differences) within and among populations was calculated using MEGA 5.0 (Tamura et al. 2011). Molecular diversity indices, such as number of haplotypes (h), polymorphic sites, transitions, transversions, and indels, were obtained using the program AR-

LEQUIN 3.1 (Excoffier et al. 2005). Values for haplotype diversity (Hd), nucleotide diversity (p), number of segregating sites (S), the mean number of pair-wise difference (k), and their corresponding variances were calculated in DNASP (Rozas et al.2003). As median-joining network approaches allow for incomplete reproductive isolation, recombination, multifurcation, and derived alleles (Posada and Crandall 2001), since its robustness can be compared to other network methods in simulation studies using known gene genealogies, we constructed a haplotype network implementing NETWORK 4.5.1 (Bandeltet al. 1999).

**Table 1.** Samples of *Polysiphonia* sensu lato collected from worldwide

| Country | Localities | Samples | Country | Localities | Samples |
|---|---|---|---|---|---|
| Argentina | 4 | 41 | Japan | 5 | 29 |
| Australia | 12 | 52 | Korea | 62 | 332 |
| Belize | 1 | 6 | Mexico | 1 | 14 |
| Brazil | 3 | 20 | Peru | 10 | 156 |
| Chile | 10 | 29 | South Afri- | 4 | 25 |
| Ecuador | 2 | 13 | Spain | 3 | 12 |
| India | 6 | 22 | UK | 11 | 89 |
| Indonesia | 7 | 34 | USA | 17 | 214 |

**Table 2.** Primer sequences used in amplification and sequencing reactions of *rbc*L and *cox*1

| Primer | Primer sequences | References |
|---|---|---|
| ***rbc*L** | | |
| F57 | GTAATTCCATATGCTAAAATGGG | Freshwater and Rueness (1994) |
| F645 | ATGCGTTGGAAAGAAAGATTCT | Freshwater and Rueness (1994) |
| F666 | CCTATATTCAATGGAAGCCGTA | This study |
| F7 | AACTCTGTAGAACGNACAAG | Freshwater and Rueness (1994) |
| F993 | GGTACTGTTGTAGGTAAATTAGAAGG | Freshwater and Rueness (1994) |
| R1150 | GCATTTGTCCGCAGTGAATAC | Freshwater and Rueness (1994) |
| R1381 | ATCTTTCGATAGATCTAAAG | Kim et al. (2010) |
| R753 | GCTCTTTCATACATATCTTCC | Freshwater and Rueness (1994) |
| Rrbcst | GTTCTTTGTGTTAATCTCAC | Freshwater and Rueness (1994) |
| ***cox*1** | | |
| C622F | CCTGTNTTAGCAGGWGCTATTACAATG | This study |
| C880R | ACAGTATACATATGATGNGCTCAAAC | This study |
| CerR1 | CCAAAAAATCAAAATARRTG | Saunders (2005) |
| COXI1549R | AGGCATTTCTTCAAANGTATGATA | Geraldino et al. (2006) |
| COXI43F | TCAACAAATCATAAAGATATTGGWACT | Geraldino et al. (2006) |
| COXI797F | GGTGGAGATCCTGTWTTATATCA | Yang et al. (2008) |
| COXI946R | GCAGCAGTAAAATAAGCACGAGT | Yang et al. (2008) |
| GazF1 | TCAACAAATCATAAAGATATTGG | Saunders (2005) |

# III. RESULTS AND DISCUSSION

## PART 1. SYSTEMATIC OF *POLYSIPHONIA* SENSU LATO (RHODOMELACEAE, RHODOPHYTA)

## Morphology and phylogenetic framework of *Polysiphonia* sensu lato

The studies of Falkenberg (1901), Hollenberg (1942), and Womersley (1979) have characterized *Polysiphonia* sensu lato as a group of the Rhodomelaceae having the following features: (1) radially symmetrical organization with all branches essentially similar and indeterminate; (2) subapical branches arising exogenously by diagonal division of subapical cells before formation of pericentral cells or from the basal cells of trichoblasts, and in many species from scar cells on lower parts of the thallus, endogenous branches may occur from prostrate branches in some species; and (3) tetrasporangium borne in series in the ultimate branchlets. The aim of this chapter is to give an overview of the morphology of *Polysiphonia* sensu lato. Generalities and particularities of the appearance of vegetative and reproductive structures are described and illustrated. Also, the consistency of these features to delimit species and genera in *Polysiphonia* sensu lato are discussed and a general phylogenetic framework is given.

### 1. Vegetative structures

#### 1.1. Nature of holdfast and prostrate development

This character is related to the way how plants of *Polysiphonia* sensu lato are attached to any surface and how it grows. Kapraun (1977) described three types of holdfast and prostrate development in *Polysiphonia*: (1) plants initially erect from a discoid base but forming secondary attachments with decumbent branches (Fig. 1A-B), (2) plants initially with a horizontal prostrate system derived from an erect apex (Fig. 1C), and (3) plants consisting of a horizontal prostrate system and apex, giving rise to erect exogenous branches (Fig. 1D-F). Stuercke and Freshwater (2008) find similar 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 shown to be consistent within a majority of species by Stuercke and Freshwater (2008). They also mentioned that this character might have a form

of environmental control. Our analyses shows that these character was consistent even at generic level, where the genus *Hapterosiphonia* having erect branches arising from a prostrate branching system was delimited from the genus *Lampisiphonia* which is having erect axes arising from a single basal holdfast (Bárbara et al. 2013, Bustamante et al. 2015c). The most common character state *Polysiphonia* sensu lato is the type (3), whereas the type (2) and (3) might be found in some genera as *Diplocladia* and *Polysiphonia* sensu stricto, respectively.

### 1.2. Branching pattern

The branching pattern is determined with the arrangement, position and frequency division of apical and subapical cells. This feature is considered as variable character and have been ranged from alternate to subdichotomous to irregular (Fig. 2A-E) (Stuercke and Freshwater 2008). Although this character is having a wide variation in *Polysiphonia* sensu lato, some species have shown consistency on their branching pattern as *P. dokdoensis* D.E. Bustamante, B.Y. Won et T.O. Cho where the erect axes with an alternating arrangement of unilateral branch pairs (Fig. 2F) is its diagnostic character (Bustamante et al. 2014a). The consistency of the branching pattern has also been confirmed at generic level. The spiral disposition of determinate branches in the genera *Boergeseniella* and *Diplocladia* (Fig. 2G-H) (Falkenberg 1901, Maggs and Hommersand 1993) and the paniculate branching pattern in the genus *Hapterosiphonia* (Fig. 2I) (Bustamante et al. 2015c) seem to be consistent characters to delimit these genera and distinguish them from other *Polysiphonia* sensu lato.

### 1.3. Branches origin

The origin of branches has been discussed in detail by Falkenberg (1901) distinguishing endogenous and exogenous origin of branches in Rhodomelaceae. Endogenous branches are originated from the axial cell after the pericentral cells have been developed and exogenous branches are originated when branch primordia cut off from subapical cells (Falkenberg 1901, Segi 1951). Endogenous branches are divided in two categories, normal endogenous branches (Fig. 3A) and adventi-

tious branches (Fig. 3B-D). The former is occurring at regular positions and the adventitious is occurring at irregular positions (Segi 1951). In *Polysiphonia* sensu lato, the most common endogenous branches are adventitious. Exogenous branches are also divided in two categories, normal (Fig. 3E) and cicatrigenous branches (Fig. 3F-H). The former is occurring near to the apical cells and the cicatrigenous branches are developing from the scar cells after the trichoblast have been shed. In *Polysiphonia* sensu lato, the most common exogenous branches are normal and it is considered as a distinguished feature from all *Polysiphonia* sensu lato (Falkenberg 1901, Hollenberg 1942).

### 1.4. Branches developmental types

Falkenberg (1901) distinguishes two morphological types of branches: (1) determinate branches (Fig. 2G-H) and (2) indeterminate branches (Fig. 2A, F, 3E). The former do not ordinarily give rise to further branches, whereas indeterminate branches has potentially unlimited growth. In *Polysiphonia* sensu lato, all branches are indeterminate with few exceptions. Our morphological analyses demonstrated that the genera *Boergeseniella, Bryocladia, Diplocladia,* and *Polyostea* are having determinate branches.

### 1.5. Apical cells

An apical cell in *Polysiphonia* sensu lato is a cell located at the tip of the axes that constantly divides leaving new cells behind (subapical cells) to build the axes. These apical cells show conspicuous size in some species (Fig. 4A-B) and not in others (Fig. 4C) (Stuercke and Freshwater 2008), and rarely some species might have exceedingly prominent apical cells (Fig. 4D) (Bustamante et al. 2013b). Apical cells might be divided obliquely (Fig. 4A) or transversally (Fig. 4B-D). Although the apical cell division has been used different genera and groups by Choi et al. (2001), Stuercke and Freshwater (2008) and Bustamante et al. (2014b) demonstrated that this character is useful to delimit species in *Polysiphonia* sensu lato.

### 1.6. Number of pericentral cells

The number of pericentral cells in *Polysiphonia* sensu lato has been shown to range from 4 to 24 (Stuercke and Freshwater 2008). The number of pericentral cells is usually quite constant if there are only four (Fig. 5A-B), but number becomes in general more variable if the number of pericentral cells is increased (Fig. 5C-H) among *Polysiphonia* sensu lato species (Falkenberg 1901, Womersley 1979, Hollenberg 1942). Fertile segments of tetrasporangial branches usually appear to have one or two more pericentral cells than have the vegetative branches because of the formation of cover cells by the longitudinal division of the fertile pericentral cell (Hollenberg 1942, Stuercke and Freshwater 2008). The number of pericentral cells has been considered as synapomorphic feature by Choi et al. (2001). *Neosiphonia* and *Polysiphonia* sensu stricto are generally having four pericentral cells (Kim and Lee 1999, Bustamante et al. 2012) and *Hapterosiphonia*, *Lampisiphonia*, and multipericentral group are having more than 5 pericentral cells (Choi et al. 2001, Bárbara et al. 2013, Bustamante et al. 2015c).

### 1.7. Shape of pericentral cells

Womersley (1979) pointed out that the form of the pericentral cells in *Polysiphonia* sensu lato varies from isodiametric near the branch apices (Fig. 6A) to distinctly elongate (Fig. 6B) below in most species. Womersley (1979) also noticed that in some species the pericentral cells always remain radially elongate (Fig. 6C), and other species the pericentral cells attain their greatest length in mid parts of the thallus of most larger species (Fig. 6D). Womersley (1979) concluded that in many species the shape of pericentral cells can vary markedly depending on size, rate of growth, and probably on the conditions under which the plant is growing. Thus, this character is lacking of consistency to distinguish species.

### 1.8. Cortication

Cortication in *Polysiphonia* sensu lato is originated with cells cutting off outwardly from the corners or sides of pericentral cells, and these first cortical cells extend and divide to form a complete

cover to the pericentral cells. In some species the cortication may become several cells thick and compact (Womersley 1979). Cortication (Fig. 7A-B) was considered as a consistent character to delimit species by Stuercke and Freshwater (2008) and to delimit genera by Bustamante et al. (2015c). Cortication has been related with the nature of holdfast and prostrate development (Stuercke and Freshwater 2008, Bustamante et al. 2015c). Thus, species attached by prostrate systems lack of cortication, whereas species attached by a single basal holdfast has light or compact cortication.

### 1.9. Trichoblasts

Trichoblasts look like hairs in *Polysiphonia* sensu lato and they are originated exogenously from the subapical cells (Hollenberg 1942). They are almost always nonpigmented, seldom unbranched with typically two to several furcations, and are generally lost from segments further from the apices (Womersley 1979, Stuercke and Freshwater 2008). Trichoblasts are deciduous structures leaving scar cells after they have shed on some or all segments of the thallus (Stuercke and Freshwater 2008). Trichoblasts have been reported to be abundant in some species (Fig. 8A-B), whereas in others this feature is exceedingly rare (Fig. 8C), and in some cases they are absent (Fig. 4A-B) or occur in connection with reproductive structures (Kim et al. 2000, Stuercke and Freshwater 2008, Bustamante et al. 2013b).

### 1.10. Relationship of lateral branches to trichoblasts

The relationship between lateral branches and trichoblasts has four character states (Hollenberg 1942, Stuercke and Freshwater 2008). These character states are considered to involve the lateral branch having some sort of connection with trichoblasts: (1) branches developing independently of trichoblasts (Fig. 9A), (2) branches replacing trichoblasts (Fig. 9B), (3) branches developing at the axils of trichoblasts (Fig. 9C), and (4) lateral branches developing laterally from the basal trichoblast cell (Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2012). Stuercke and Freshwater (2008) proposed that the relationship of lateral branches and trichoblasts is determined when

trichoblasts had dropped off before branch initiation by looking at the first axial cell of each lateral branch. A second pit connection that led to the trichoblasts will be present at the distal end of these cells when branches formed in the axil of the trichoblasts. If the branch replaced the trichoblasts, then this second pit connection will be absent.

### 1.11. Scar cells

Scar cells have been described as the basal cells of a trichoblast that remains embedded between pericentral cells after the trichoblast has been shed (Womersley 1979, Stuercke and Freshwater 2008). The occurrence and pattern of scar cells have been referred together with trichoblast in most taxonomic keys for *Polysiphonia* because scar cells are developmentally derived from trichoblasts (Womersley 1979, Hollenberg 1968, Stuercke and Freshwater 2008). Scar cells might produce lateral branches in some species of *Polysiphonia* sensu lato and they are named as cicatrigenous branches (Hollenberg 1942). Stuercke and Freshwater (2008) found that species having scar cells disposed in spiral series produce cicatrigenous branches, whereas species that did not have a scar cell pattern did not show these branches. Bustamante et al. (2014a, 2015a) also demonstrated that the position of scar cells respect to the pericentral cells is a consistent character to delimited species in *Polysiphonia* sensu stricto. They distinguished that scar cells might be developed between the distal ends of two pericentral cells (Fig. 10A-B) or that scar cells might be placed in the space between the segments (Fig. 10C-D).

### 1.12. Rhizoid-pericentral cell connection

There are two potential character states for how rhizoids are connected to pericentral cells: (1) rhizoids are in open connection with pericentral cells when their cytoplasm are in continuous or directly connected with the pericentral cells (Fig. 11A-B) and (2) rhizoids are cut off from pericentral cells when they are separate cells but pit-connected (Fig. 11C-D) (Hollenberg 1942, Segi 1951, Womersley 1979, Stuercke and Freshwater 2008). Although this character shows consistency at specific level (Stuercke and Freshwater 2008), current studies have demonstrated the consistency of

this character higher than specific level because species having rhizoids cutting off pericentral cells belong to *Hapterosiphonia*, *Lampisiphonia*, multipericentral group, and *Neosiphonia*, whereas species having open connected rhizoids only belong to *Polysiphonia* sensu stricto (Choi et al. 2001, Mamoozadeh and Freshwater 2012, Bustamante et al. 2012, 2013a, 2015c).

### 1.13. Rhizoidal-pericentral cell position

The location of origin of the rhizoids on a mature pericentral cell was proposed as a useful character to distinguish species by Hollenberg (1968), whether the rhizoid arises on the distal end, on the proximal end, or from the centre. Stuercke and Freshwater (2008) noticed in *Polysiphonia* sensu lato that species with pit-connected rhizoids were typically attached at the proximal end of pericentral cells; whereas species with open-connected rhizoids were generally extended from the middle of the pericentral cell. Bustamante et al. (2015c) demonstrate that species in *Polysiphonia* sensu lato has rhizoids arisen only on the proximal end (Fig. 12A) and centre of pericentral cells (Fig. 12B), whereas other groups out of *Polysiphonia* sensu lato are having rhizoids arisen on the distal end of pericentral cells (Fig. 12C).

### 1.14. Rhizoidal terminations

The shape of rhizoidal terminations in *Polysiphonia* sensu lato has been frequently reported as rounded, blunt, thread, or lobed (Yoon 1986, Stuercke and Freshwater 2008), but this character is lacking of consistency for delimit species. Recently, Bustamante et al. (2015c) demonstrated the multicellularity of rhizoidal terminations as consistent character to delimit genera in *Polysiphonia* sensu lato. Species having unicellular rhizoidal terminations (Fig. 13A) belong to multipericentral group, *Neosiphonia*, and *Polysiphonia* sensu stricto, whereas species having multicellular rhizoidal terminations (Fig. 13B) belong to *Hapterosiphonia* or *Lampisiphonia*.

## 2. Reproductive structures

### 2.1. Number of cells in the carpogonial branches

The pericarp forms before fertilization in Rhodomelaceae (Hommersand 1963), making it difficult to observe the number of carpogonial branch cells (Stuercke and Freshwater 2008). Thus, the number of cells in the carpogonial branches has not been considered often in the descriptions of the species of *Polysiphonia* sensu lato (Hollenberg 1942, Womersley 1979, Stuercke and Freshwater 2008). Carpogonial branches in *Polysiphonia* sensu lato have been reported to be typically composed of four cells and rarely of three cells (Fig. 14A-D) (Stuercke and Freshwater 2008). Kim and Lee (1999) and Choi et al. (2001) found that carpogonial branches with three cells were the main morphological difference distinguishing *Neosiphonia* from other species of *Polysiphonia* sensu lato. Subsequent studies have confirmed this character state as a consistent character among species of *Neosiphonia* (Bustamante et al. 2013a, 2013b).

### 2.2. Shape of cystocarps

The cystocarps of species of *Polysiphonia* sensu lato have been reported to differ slightly in shape (Fig. 15). Stuercke and Freshwater (2008) observed that shape of cystocarps might range from globose to ovate to oval to urceolate or any combination of these shapes. Although several studies have characterized species based on the shape of cystocarps (Hollenberg 1942, Segi 1951, Womersley 1979), Stuercke and Freshwater (2008) considered this character as lacking of consistency to delimit species in *Polysiphonia* sensu lato.

### 2.3. Development of spermatangial axes

The development of spermatangial branches has two character states: (1) spermatangia that replace the whole trichoblasts (Fig. 16A-B) and (2) spermatangia developed on a furcation of trichoblast (Fig. 16C-D) (Choi et al. 2001, Stuercke and Freshwater 2008). The development of spermatangial branches has been used in previous studies as a reliable character for species designation (Hollenberg 1942, 1968, Segi 1951, Womersley 1979). Although Choi et al. (2001) proposed this character

as consistent to delimit groups in *Polysiphonia* sensu lato, subsequent studies have shown that the development of spermatangial branches is useful to delimit species in each group of *Polysiphonia* sensu lato (Bustamante et al. 2013b, 2015b). The number of sterile cells present in the tips of spermatangial branches has been considered a useful character to identify species in *Polysiphonia* sensu lato (Stuercke and Freshwater 2008). However, recent studies have shown that these will depend on the maturity of the spermatangia because cells that appear to be sterile at the tip of developing spermatangial branches will often become spermatangial mother cells in the mature spermatangia (Stuercke and Freshwater 2008, Kim and Kim 2014).

### 2.4. Arrangement of tetrasporangia

The arrangement of tetrasporangia shows two character states: (1) tetrasporangia arranged in spiral series (Fig. 17A-B) where tetrasporangia appeared to form from different pericentral cells in each successive segment and (2) tetrasporangia arranged in straight series (Fig. 17C-D) where tetrasporangia appeared to form in the same pericentral cell in each segment (Stuercke and Freshwater 2008). In some species, the pericentral cells of short series of straight tetrasporangia shift every couple of segments giving slightly spiral or spiral-straight appearance (Fig. 17E) (Stuercke and Freshwater 2008, Bustamante et al. 2015c). Although this character has been considered a good character for distinguishing groups and genera in *Polysiphonia* sensu lato by Choi et al. (2001), Stuercke and Freshwater (2008, 2010) and Bustamante et al. (2013b, 2015c) demonstrated that this character is useful to delimit species in each genera of *Polysiphonia* sensu lato.

### 2.5. Number of tetrasporangia per fertile segment

In *Polysiphonia* sensu lato, fertile segments in tetrasporangia branches may appear to have one extra pericentral cell from the normal vegetative number, owing to the formation of two cover cells from the one sporangial mother cell (Womersley 1979). Some species also may have two extra pericentral cells, owing the formation of four cover cells, two from each of the sporangial mother cell. Thus, some species might have one or two tetrasporangia per fertile segment. Bustamante et

al. (2012) considered this character as consistent to delimit species instead to delimit genera (Womersley 2003, Diaz-Tapia and Bárbara 2013).

Although the studies in *Polysiphonia* sensu lato based on morphology have used the characters above mentioned to distinguish among species and genera, the present study consider the following features as consistent to delimit species and genera in *Polysiphonia* sensu lato: the nature of holdfast and prostrate development (Bárbara et al. 2013, Bustamante et al. 2015c), the number of pericentral cells (Bustamante et al. 2012), cortication (Bustamante et al. 2015c), trichoblasts abundance (Kim et al. 2002), relationship of lateral branches to trichoblasts (Stuercke and Freshwater 2008), scar cells position (Bustamante et al. 2014a, 2014b), rhizoids-pericentral cell connection and position (Bustamante et al 2015c), multicellularity of rhizoidal terminations (Bustamante et al. 2015c), number of cells in the carpogonial branches (Kim and Lee 1999), development of spermatangial branches (Mamoozadeh and Freshwater 2012), and arrangement of tetrasporangia (Mamoozadeh and Freshwater 2012). The variation of these characters shows *Polysiphonia* sensu lato as a heterogeneous group composed of several species and genera (paraphyletic group (Stuercke and Freshwater 2008)). The current studies in *Polysiphonia* sensu lato are demonstrating the consistency of these characters and also building a natural classification system based on the evolutionary relationships of their species and genera (Stuercke and Freshwater 2008).

### 3. Phylogenetic framework

Our phylogenetic analyses revealed that *Polysiphonia* sensu lato (tribe Polysiphoieae) is composed of 17 genera (*Boergeseniella, Brongniartella, Bryocladia, Diplocladia, Dorsisiphonia, Enelittosiphonia, Hapterosiphonia, Lampisiphonia, Leptosiphonia, Neosiphonia, Neostreblocladia, Phillipsiphonia, Polyostea, Polysiphonia* sensu stricto, *Streblocladia, Tolypiocladia, Vertebrata*). These genera are monophyletic clades or some are grouped in bigger paraphyletic groups as the multipericentral group (*Boergeseniella, Brongniartella, Diplocladia, Enelittosiphonia, Vertebrata*)

and *Polysiphonia* sensu stricto 2 clade (*Bryocladia*, *Dorsisiphonia, Neostreblocladia, Phillipsiphonia*) (Fig. 19). Five genera were segregated as new genera (*Dorsisiphonia*, *Hapterosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Wilsonosiphonia*), two enlarged (*Brongniartella*, *Leptosiphonia*), and one was resurrected (*Polyostea*) on the basis of diagnostic features and high bootstrap values of Maximum likelihood and posterior probabilities of Bayesian analyses. The taxonomy and systematic of these genera will be discussed in detail in the following chapters. Also, 16 new species and 34 new combinations have been proposed among *Polysiphonia* sensu lato on the basis of morphological and molecular analyses of *rbc*L and *cox*1 locus.

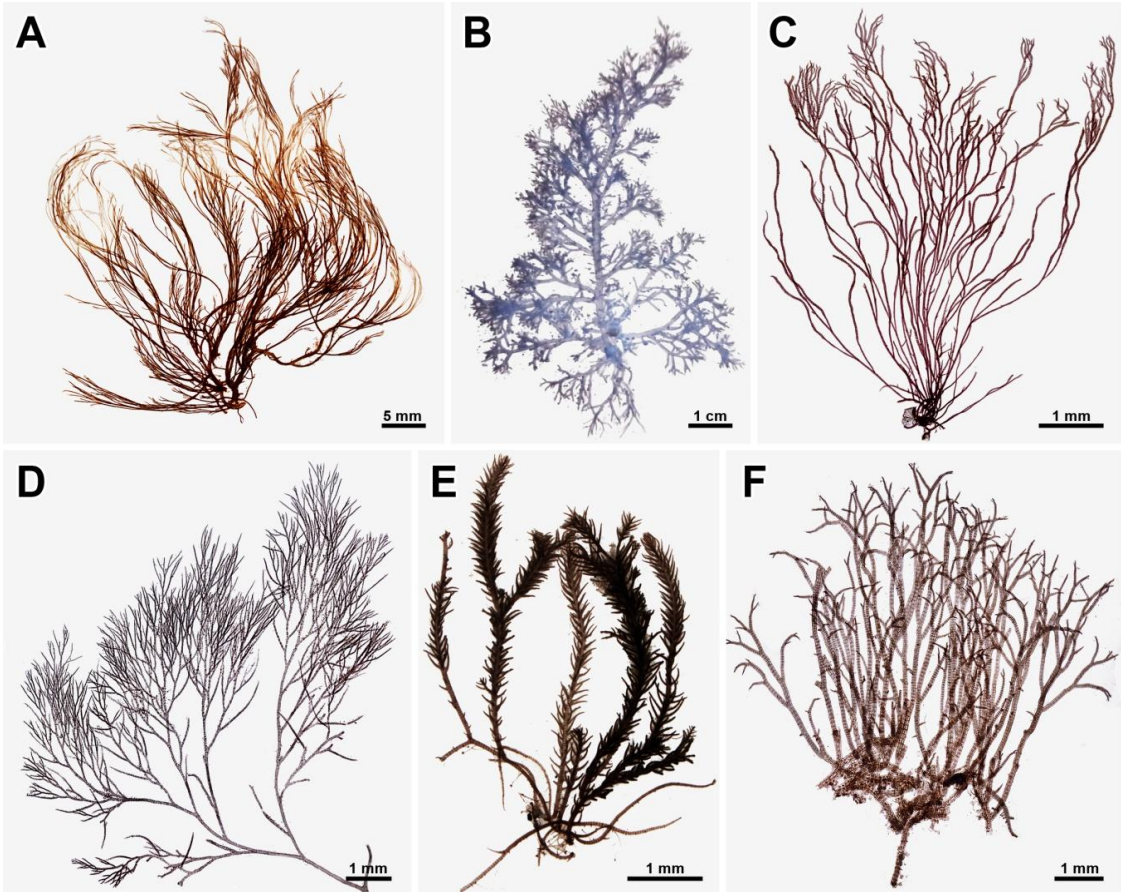


**Fig. 1.** Habit in species of *Polysiphonia* sensu lato showing the three different natures of holdfast and prostrate development. (A-B) Plants initially erect from a discoid base but forming secondary attachments with decumbent branches. (C) Plants initially with a horizontal prostrate system derived from an erect apex. (D-F) Plants consisting of a horizontal prostrate system and apex, giving rise to erect exogenous branches.

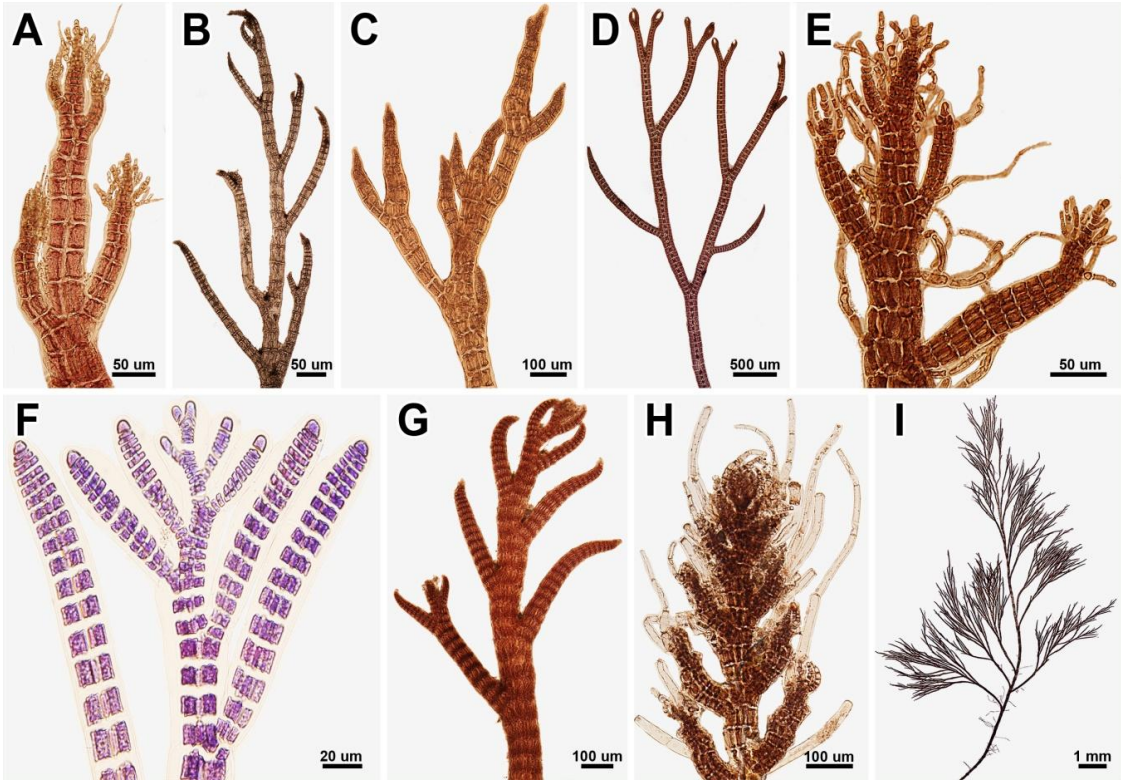


**Fig. 2.** Types of branching pattern in *Polysiphonia* sensu lato. (A) Trichotomous. (B) Subdichotomous and alternated unilateral branches pairs. (C) Irregular. (D) Dichotomous. (E) Alternate. (F) Alternated unilateral branches pairs.(G) Alternated determinate branches. (H) Spiral determinate branches. (I) Paniculate branching pattern.

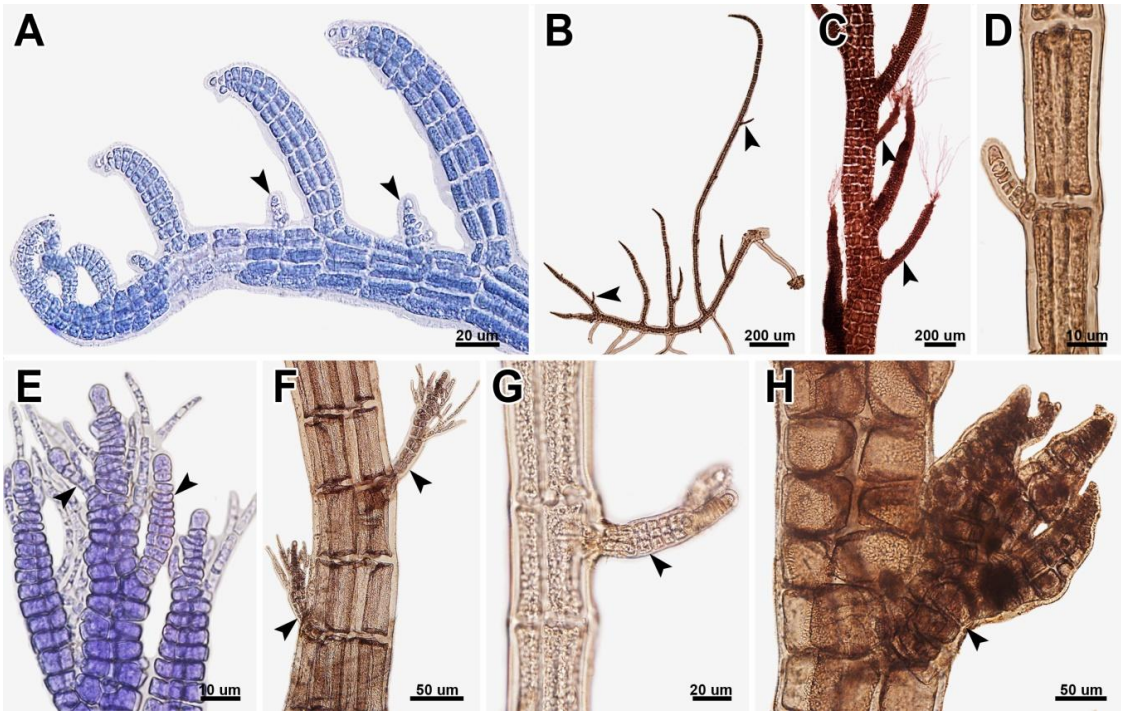


**Fig. 3.** Types of branching origin in *Polysiphonia* sensu lato. (A) Normal endogenous branches. (B-D) Adventitious branches. (C) Normal exogenous branches. (F-H) Cicatrigenous branches. Arrowheads indicated branches.

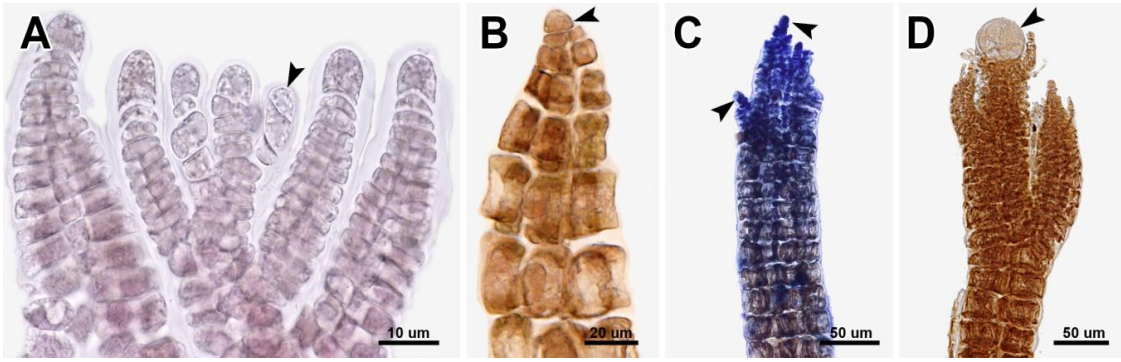


**Fig. 4.** Size of apical cell in *Polysiphonia* sensu lato. (A-B) Conspicuous apical cells. (C) Non conspicuous apical cells. (D) exceedingly prominent apical cells. Arrowheads apical cells.

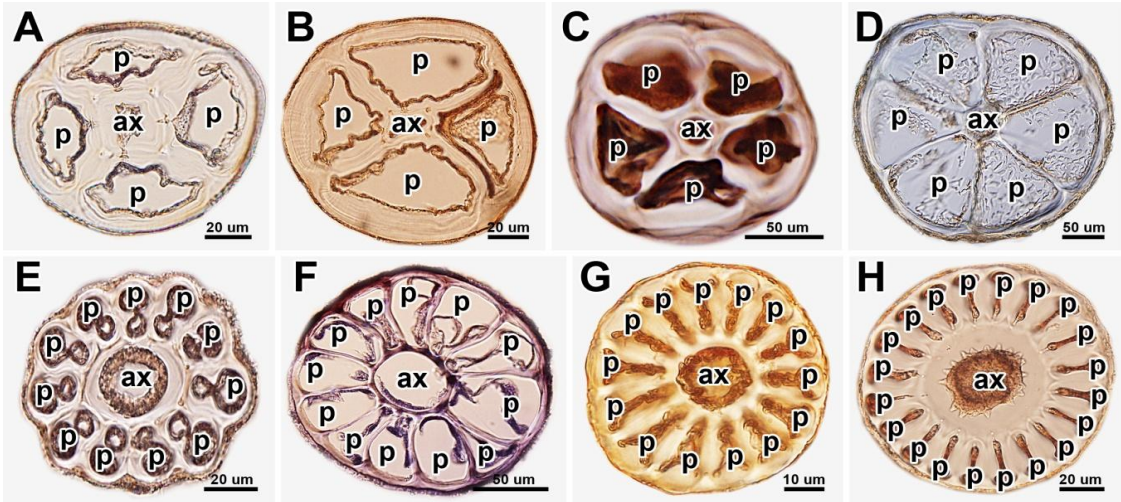


**Fig. 5.** Cross section of the thallus in *Polysiphonia* sensu lato with (A-B) four, (C) five, (D) six, (E) 11, (F) 12, (G) 14, and (H) 20 pericentral cells. ax: axial cell, p: pericentral cell.

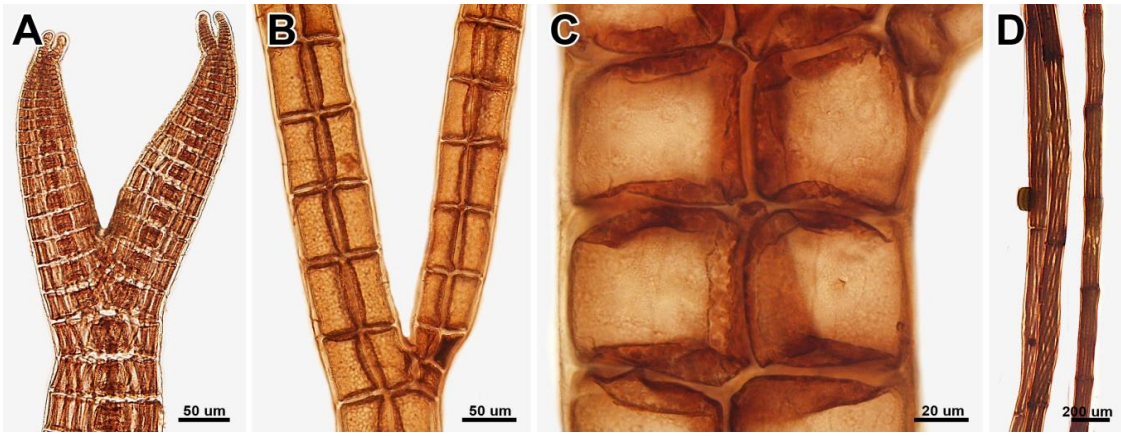


**Fig. 6.** Shape of pericentral cells in *Polysiphonia* sensu lato. (A) Isodiametric. (B) Distinctly elongate. (C) Radially elongate. (D) Greatest length.

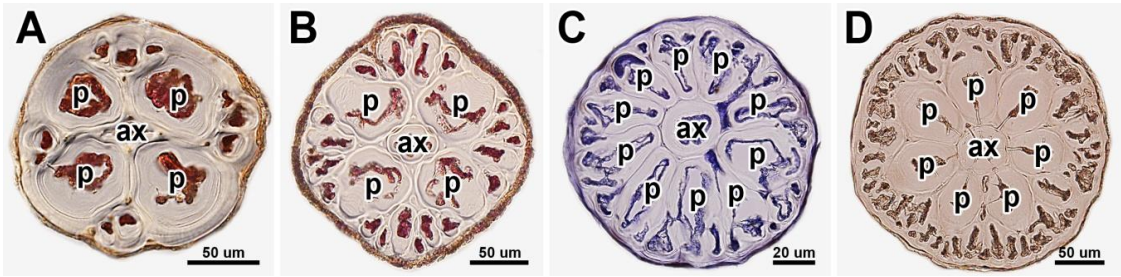


**Fig. 7.** Cross section of the thallus in *Polysiphonia* sensu lato with (A-B) four, (C) ten, and (D) six pericentral cells surrounded by cortical cells. ax: axial cell, p: pericentral cell.


**Fig. 8**. Trichoblasts in *Polysiphonia* sensu lato. (A-B) Abundant trichoblasts. (C) Rare trichoblasts**.**

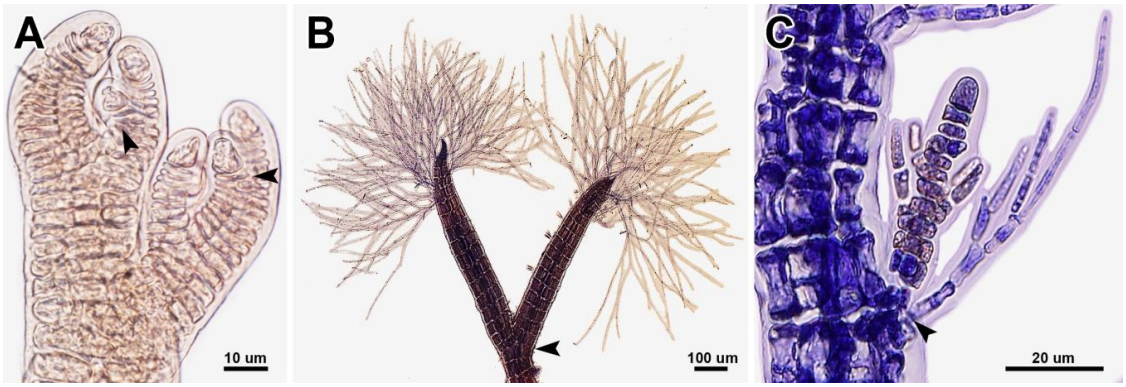


**Fig. 9.** Relationship between lateral branches and trichoblasts in *Polysiphonia* sensu lato. (A) Branches developing independently of trichoblasts. (B) Branches replacing trichoblasts. (C) Branches developing at the axils of trichoblasts. Arrowheads indicated lateral branches.

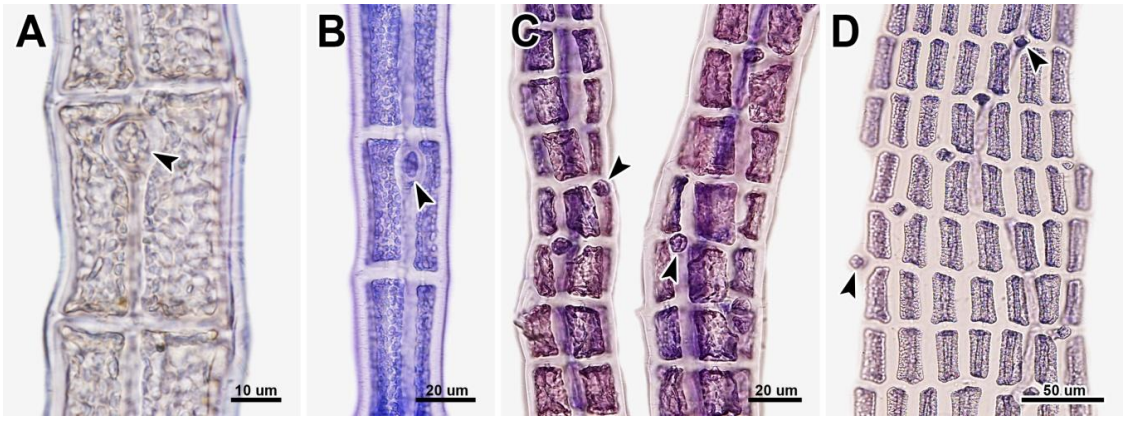


**Fig. 10.** Position of scar cells respect to the pericentral cells in *Polysiphonia* sensu lato. (A-B) Scar cells developed between the distal ends of two pericentral cells. (C-D) Scar cells placed in the space between the segments. Arrowheads indicated scar cells.

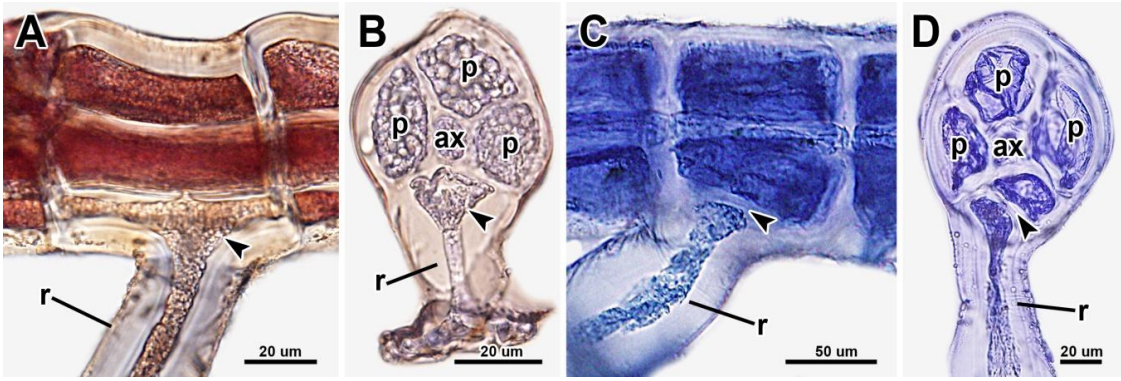


**Fig. 11.** Rhizoids-pericentral cell connections (arrowheads) in *Polysiphonia* sensu lato. (A-B) Rhizoids in open connection with pericentral cells. (C-D) Rhizoids cutting off pericentral cells. ax: axial cell, p: pericentral cell, r: rhizoids.

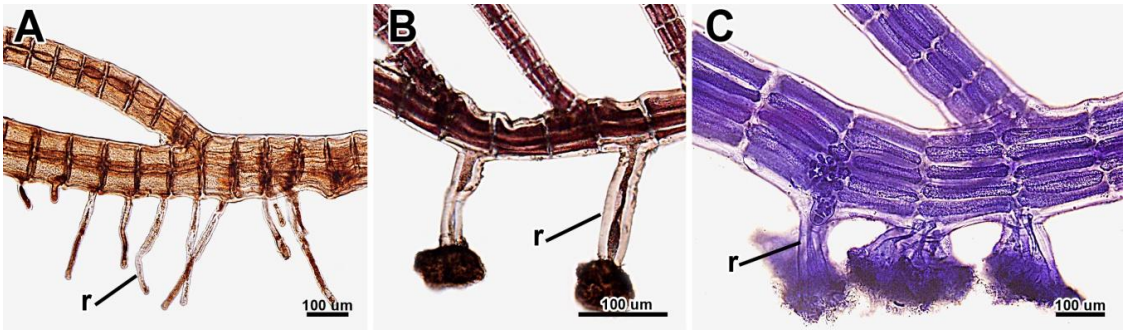


**Fig. 12.** Rhizoids-pericentral cell position in *Polysiphonia* sensu lato. (A) Rhizoids arisen on the proximal end of pericentral cells. (B) Rhizoids arisen from the centre of pericentral cells. (C) Rhizoids arisen on the distal end of pericentral cells. r: rhizoids.

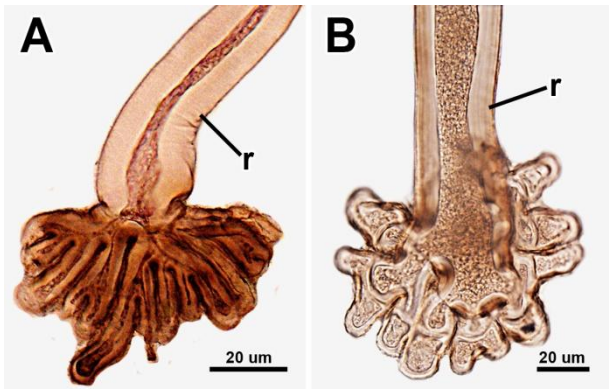


**Fig. 13.** Rhizoids-pericentral cell position in *Polysiphonia* sensu lato. (A) Rhizoids arisen on the proximal end of pericentral cells. (B) Rhizoids arisen from the centre of pericentral cells. (C) Rhizoids arisen on the distal end of pericentral cells. r: rhizoids.

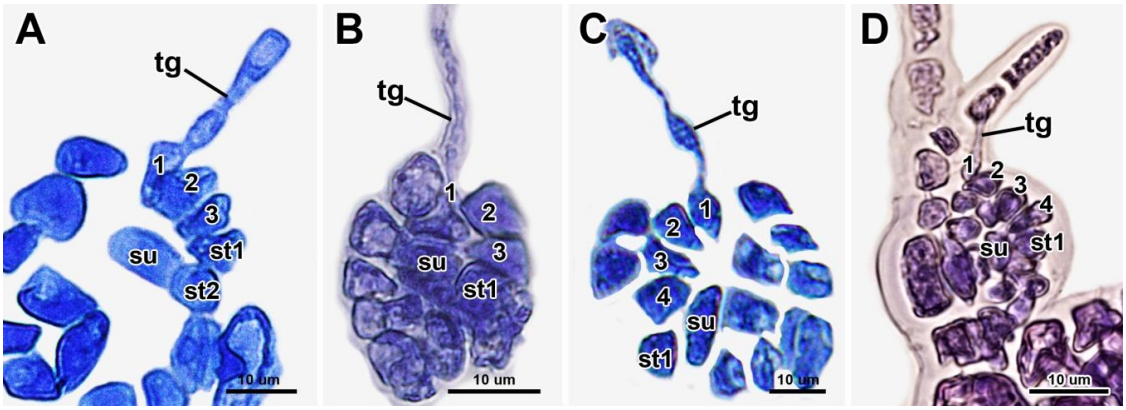


**Fig. 14.** Number of cells in the carpogonial branches in *Polysiphonia* sensu lato. (A-B) Three-celled carpogonial branch. (C-D) Four-celled carpogonial branch. tg: trichogyne, st: sterile cell, su: supporting cell.

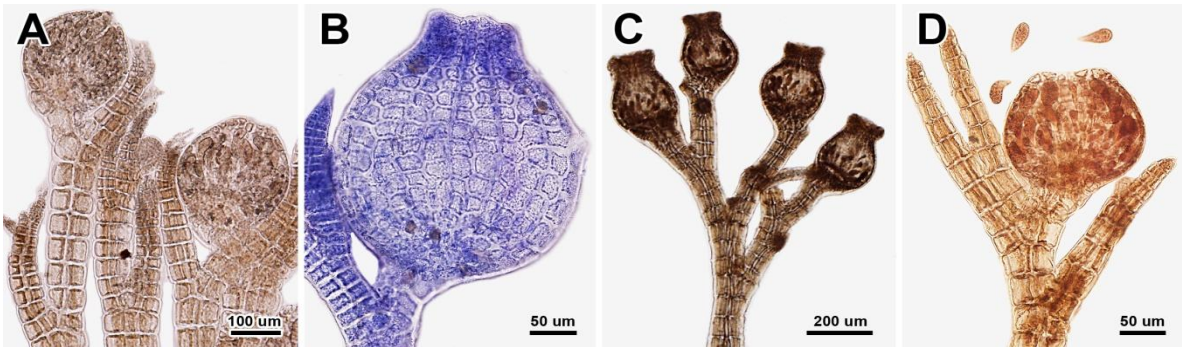


**Fig. 15.** Shapes of cystocarps in *Polysiphonia* sensu lato. (A) Oval. (B) Ovoid. (C) Urceolate. (D) Non-urceolate.

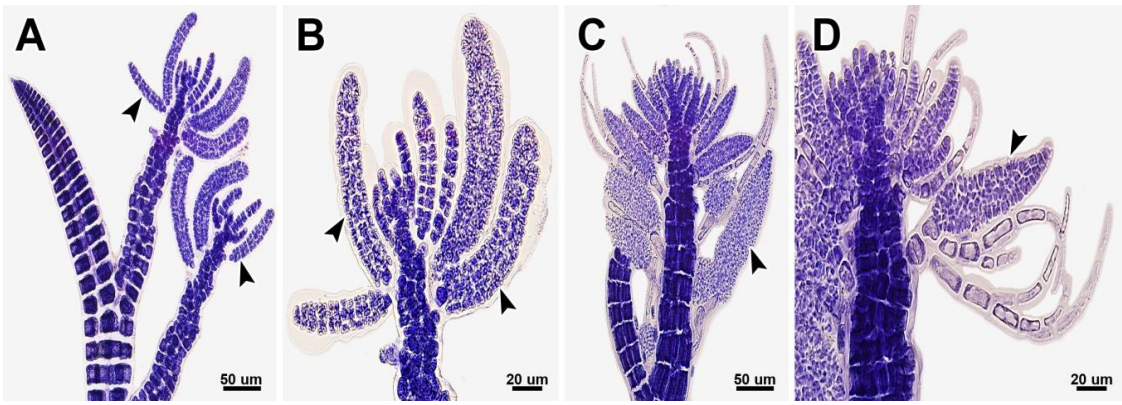


**Fig. 16.** The development of spermatangial branches in *Polysiphonia* sensu lato. (A-B) Spermatangia (arrowheads) replacing the whole trichoblasts. (C-D) Spermatangia (arrowheads) developed on a furcation of trichoblast.

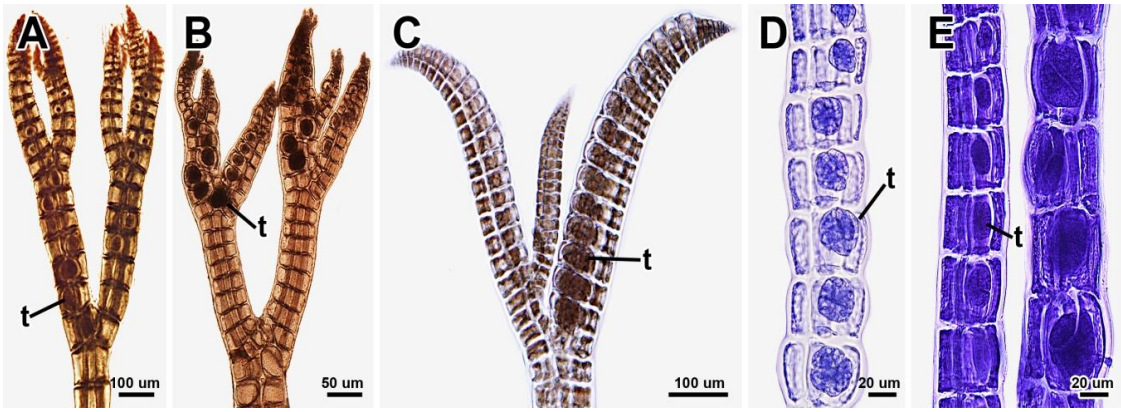


**Fig. 17.** The arrangement of tetrasporangia in *Polysiphonia* sensu lato. (A-B) Tetrasporangia (t) arranged in spiral series. (C-E) Tetrasporangia (t) arranged in straight series.

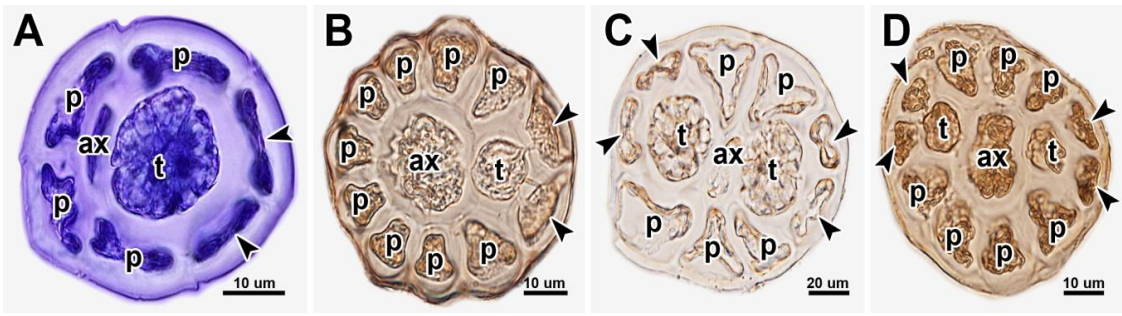


**Fig. 18.** Number of tetrasporangia per fertile segment in *Polysiphonia* sensu lato. (A-B) A single tetrasporangium (t) per fertile segment. (C-D) Two tetrasporangia(t) per fertile segment. Arrow-heads: cover cells, ax: axial cell, p: pericentral cell.

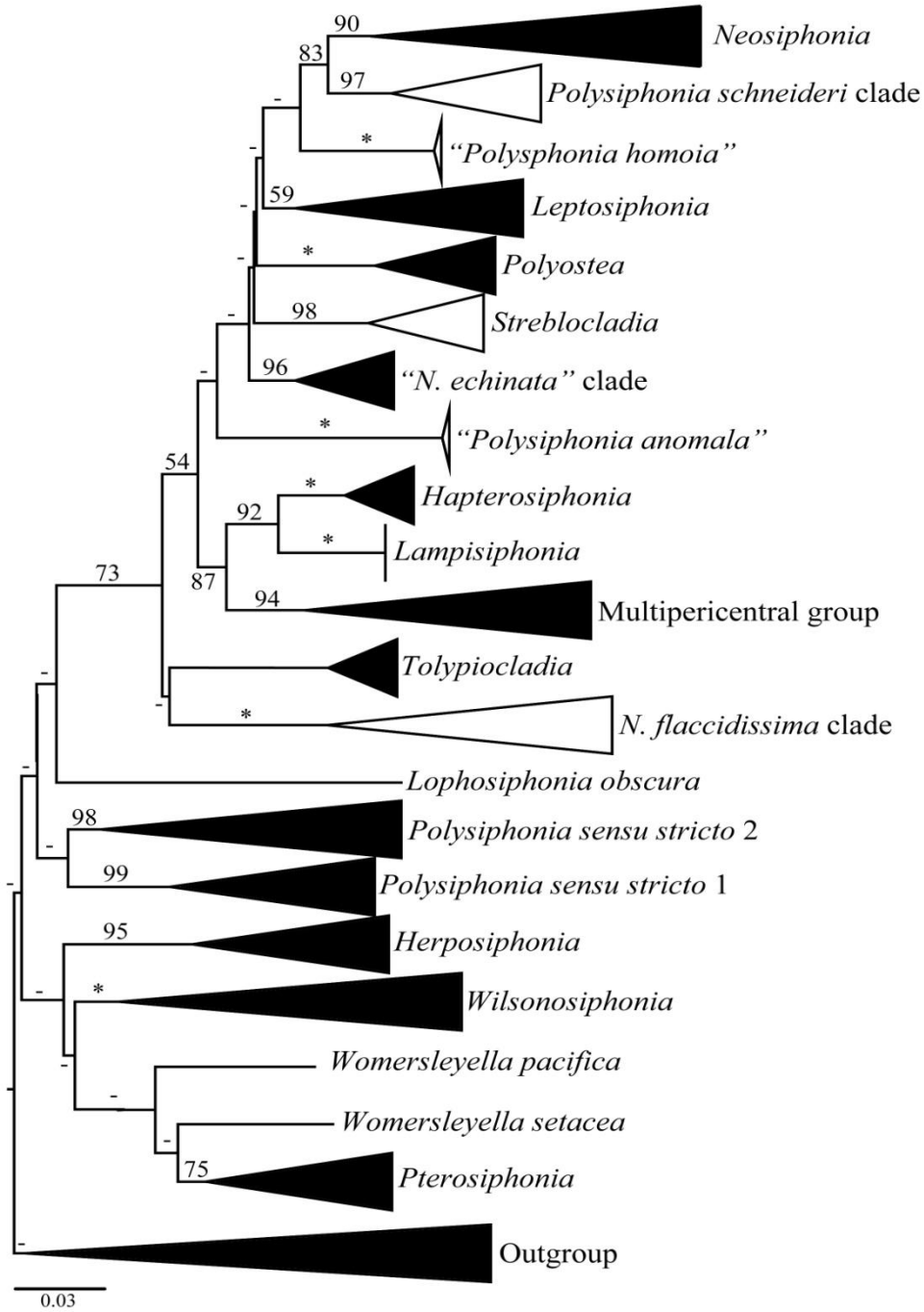


**Fig. 19.** Phylogenetic tree based on ML analysis on the concatenated *rbc*L and *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50. BS values of <50% are indicated by hyphens (-). BS values of 100% are indicated by asterisks (*). Black collapsed clades represent clade to be discussed in the following chapters.

# PART 2. TAXONOMY AND PHYLOGENY OF *POLYSIPHONIA* SENSU LATO (RHODOMELACEAE, RHODOPHYTA)

## CHAPTER 1. Taxonomy and phylogeny of the genus *Hapterosiphonia* gen. nov. (Rhodomelaceae, Rhodophyta)

*Polysiphonia* sensu lato is a large and cosmopolitan group with more than 200 species, whose taxonomy has not yet been fully resolved (Kim et al. 2000). *Polysiphonia* sensu lato is characterized by rhizoids in open or close connection with pericentral cells, axial cells surrounded by pericentral cells, three or four-celled carpogonial branches, spermatangial branches developed from trichoblasts, and tetrasporangia arranged in straight or spiral series. *Polysiphonia* sensu lato is composed of several genera, but the following heterogeneous genera are currently recognized on the basis of molecular analyses: *Boergeseniella* Kylin, *Bryocladia* F. Schmitz, *Enelittosiphonia* Segi, *Lampisiphonia* H.G. Choi, Díaz-Tapia et Bárbara, *Leptosiphonia* Kylin, *Neosiphonia* M.S. Kim et I.K. Lee, *Polysiphonia* Grev., *Streblocladia* F. Schmitz, and *Vertebrata* S.F. Gray (Kim and Lee 1999, Choi et al. 2001, Mamoozadeh and Freshwater 2012, Bárbara et al. 2013, Bustamante et al. 2015a).

Recently, molecular studies of small-subunit ribosomal DNA (SSU rDNA) and plastid-encoded *rbc*L have demonstrated the presence of four main groups of taxa in *Polysiphonia* sensu lato: *Neosiphonia*, *Lampisiphonia*, a multipericentral group, and *Polysiphonia* sensu stricto (Kim and Lee 1999, Choi et al. 2001, Mamoozadeh and Freshwater 2011, Bárbara et al. 2013). Kim and Lee (1999) segregated *Neosiphonia* from *Polysiphonia* based on rhizoids cut off from the pericentral cells, procarps with a three-celled carpogonial branch, spermatangial branches arising on trichoblast furcations, and tetrasporangia arranged in a spiral series. Choi et al. (2001) split *Polysiphonia* sensu lato into three clades: the "*Polysiphonia*" group, the "*Neosiphonia*" group and the "multipericentral" group. Of these, the multi-pericentral group (including species of *Boergeseniella*, *Enelittosiphonia*, *Polysiphonia*, and *Vertebrata*) were recognized by having numerous pericentral cells, rhizoids cut off from the pericentral cells, spermatangia arising on trichoblast furcations, and tetrasporangia arranged in a spiral series. Recently, Norris (2014) proposed *Neosiphonia* sect. *Multisiphonia* [type species *N. johnstonii* (Setchell et N.L. Gardner) J.N. Norris] for taxa with five or

more pericentral cells. Earlier, Bárbara et al. (2013) had described *Lampisiphonia* to include species with multicellular rhizoids, absence of trichoblasts, and presence of a compact basal cortication. *Polysiphonia* sensu stricto is characterized by ecorticate axes, rhizoids in open connection with the pericentral cells, procarps with a four-celled carpogonial branch, spermatangial branches replacing the trichoblasts, and a straight arrangement of tetrasporangia (Kim et al. 2000, Bustamante et al. 2014a)

In the present study, we collected polysiphonous specimens with rhizoids cut off from the pericentral cells and with multicellular lobed terminations, which had been previously reported as *Neosiphonia confusa* (Hollenb.) J.N. Norris, *N. paniculata* (Mont.) J.N. Norris, and *P. tapinocarpa* Suringar. These species were collected in the vicinity of their type localities in the North and Southeast Pacific coast and their classification critically reassessed based on detailed morphological study and phylogenetic analyses of their *rbc*L and *cox*1 sequences. We here describe a new genus, *Hapterosiphonia,* to include these three taxa, in addition to *Polysiphonia sabulosia* B. Kim et M.S. Kim (on the basis of *rbc*L sequence analysis and observation of its isotype).

## 1. Morphological analyses

***Hapterosiphonia* D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho gen. nov.**

**Type species:** *Hapterosiphonia paniculata* (Montagne) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho

**Diagnosis:** Plants prominent, forming large tufts predominantly attached to rock surfaces. Thalli composed of an extensive and entangled prostrate system, and interwoven indeterminate erect axes. Erect axes composed of 5–12 pericentral cells, ecorticate throughout, and densely and radially branched in an alternate pattern, forming panicles. Adventitious branches present. Lateral branches arising in the axils of trichoblasts. Trichoblasts delicate, deciduous, numerous, 1–3 times forked,

elongate. Conspicuous scar cells present. Rhizoids cut off from the pericentral cells, with lobed, multicellular terminations. Procarps positioned laterally and subapically, each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial branches arising from trichoblast fork, cylindrical, sometimes with sterile tip. Tetrasporangia tetrahedral, either in straight or spiral arrangement. A single tetrasporangium is produced on each fertile segment.

**Etymology:** "*Hapterosiphonia*" refers to the complex pattern of rhizoidal (haptera) terminations.

**Key to species of *Hapterosiphonia* gen. nov.**

1. Pericentral cells 5-6 ..... ***Hapterosiphonia sabulosia***
1. Pericentral cells 8-12 ..... 2
    2. Thalli with small filaments and short lateral branches on the main tetrasporangial axes ..... ***Hapterosiphonia confusa***
    2. Thalli with very long filaments .....3
3. Robust plants ..... ***Hapterosiphonia paniculata subsp. paniculata***
3. Delicate and soft plants ..... ***Hapterosiphonia paniculata subsp. tapinocarpa***

***Hapterosiphonia paniculata* (Montagne) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho subsp. *paniculata* comb. nov. (Fig. 21-22)**

**Basionym:** *Polysiphonia paniculata* Montagne.

**Homotypic synonyms:** *Neosiphonia paniculata* (Mont.) J.N. Norris.

**Heterotypic synonyms:** *Polysiphonia californica* Harvey.

**Type locality:** Peru.

**Distribution:** Peru and Chile (Fig. 20).

**Specimens examined:** CUK6464, CUK6469, CUK6476 (Atacama, Caldera, Antofagasta, Chile collected by T.O.C., Aug. 20 2008), CUK6514, CUK6518, CUK6523, CUK6524, CUK6525, CUK6526, CUK6540 (Lagunillas, Pisco, Ica, Peru collected by T.O.C. and D.E.B., Aug. 27 2008), CUK6550, CUK6557 (Callao, Lima, Peru collected by T.O.C and D.E.B., Aug. 30 2012), CUK6649 (Punta Veleros, Los Organos, Piura collected by T.O.C. and D.E.B. Sep. 04 2008), CUK8233, CUK8234 (Punta Zorritos, Talara, Piura collected by T.O.C. and D.E.B. Jul. 02 2012), CUK8251, CUK8337, CUK8341, CUK8346, CUK8361, CUK8370, CUK8383, CUK8388, CUK8393, CUK8399 (Lagunillas, Pisco, Ica, Peru collected by T.O.C and D. E. B. Jul. 02 2012), CUK8407, CUK8408, CUK8409 (Callao, Lima, Peru collected by T.O.C and D.E.B., Jul. 06 2012), CUK13681 (Callao, Lima, Peru collected by T.O.C and D.E.B., Feb. 08 2013)

**Vegetative morphology:** Specimens were collected from Peru and Chile. Plants are prominent, robust, 3–16 cm in height (Fig. 21A), dark red to brown in color, and associated with other species. Thalli form large tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of an erect and prostrate system, with the erect system composed of interwoven indeterminate axes (Fig. 21B). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 2–7 axial cells. They are densely and radially branched in an alternate pattern forming panicles every 3–6 axial cells. Apical cells are prominent, 7.34 ± 1.96 μm × 6.39 ± 1.2 μm in size, and transversely divided (Fig. 21C). Young erect axes are alternately disposed, curved in the direction of the main axes, and have short segments. Older segments of erect axes are larger, normally 428.24 ± 54.67 μm and rarely 932.09 ± 30.80 μm in length, and 298.21 ± 28.84 μm in diameter, normally 0.7 times broader than long (L:D 1.44 ± 0.24), commonly twisted (Fig. 21F), and infrequently branched. Trichoblasts are delicate, deciduous, numerous, 1–3 times forked, and 153.03 ± 35.01 μm long, rarely >278 μm, and arising on each segment near the apical cells (Fig. 21D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, reaching 12.10 ± 3.13 μm × 11.95 ± 1.69 μm, and developed each two in spiral to irregular series in

the space between segments (Fig. 21E). Each segment is completely ecorticated along the thallus and composed of 9-12 pericentral cells (Fig. 21G-J). Adventitious branches are present. Lateral branches arise in the axils of trichoblasts. The prostrate system is extensive and entangled, with axial segments 164.16 ± 11.33 μm in length and 164.99 ± 6.06 μm in diameter, broader than long (L:D 0.99 ± 0.08). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and cut off from the pericentral cells (Fig. 21K), 526.96 ± 215.24 μm in length, and 30.47 ± 6.95 μm in diameter. Rhizoids are unicellular in younger stages, but produce lobed, multicellular terminations when mature (Fig. 21L-N).

**Reproductive morphology:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 22A). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 22B). Cystocarps are alternately disposed (Fig. 22C), and are globose when mature (Fig. 22D), 240.43 ± 56.10 μm in height and 300.30 ± 61.62 μm in diameter. In male gametophytes, spermatangial branches are clustered at the apices of erect axes (Fig. 22E), and develop on a trichoblast furcation in each axial segment (Fig. 22F–G). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell (Fig. 22G). In tetrasporangial plants (Fig. 22H), tetrasporangia are tetrahedral and 34.33 ± 5.16 μm × 35.78 ± 4.10 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 22I–K). The development of tetrasporangia follows a straight arrangement but the position of the pericentral cells shifts every couple of segments giving slightly spiral appearance (Fig. 22J–K). The ninth or tenth pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment.

**Habitat:** Plants grow from the intertidal to subtidal zone, forming extensive tufts. They are found attached to rocks in sheltered to wave-exposed areas. Tufts are usually wide, long, and very robust, and are associated with other species such as *Centroceras clavulatum*, *Chondracanthus chamissoi*, and *Neosiphonia japonica.*

**Remarks:** *Hapterosiphonia paniculata* subsp. *paniculata* was originally described as *Polysiphonia paniculata* by Montagne (1842) and recently transferred to *Neosiphonia* sect. *Multisiphonia* by Norris (2014). *Hapterosiphonia paniculata* subsp. *paniculata* is characterized by having a robust habit, 10-12 long and sometimes spirally twisted pericentral cells, and a paniculate branching pattern (Montagne 1842, Dawson et al. 1964). Our specimens from Peru and Chile correspond to the original description. *Hapterosiphonia paniculata* subsp. *paniculata* is similar to three *Polysiphonia* sensu lato species (*Polysiphonia anisogona* J.D. Hook. et Harv.; *P. tenuistriata* J.D. Hook et Harv.; *P. curta* Montag.) in having numerous and twisted pericentral cells and abundant trichoblasts. However, *P. anisogona* and *P. tenuistriata* differ from *Hapterosiphonia paniculata* subsp. *paniculata* by their flaccid and delicate texture (Hooker and Harvey 1845) and by subdichotomous and irregular branching patterns (Hooker and Harvey 1845). *Polysiphonia curta* is also distinguished from *Hapterosiphonia paniculata* subsp. *paniculata* by its small habit (≤5 cm tall) and by having 15-20 pericentral cells and cystocarps apically disposed (Montagne 1843, Bustamante and Ramírez 2009). Our *Hapterosiphonia paniculata* subsp. *paniculata* has been well documented from the Peruvian and Chilean coast (Montagne 1842, Howe 1914, Dawson et al. 1964, Ramírez and Santelices 1991). The result of our present study confirms the distribution of *Hapterosiphonia paniculata* subsp. *paniculata* for the northern coast of Peru (Piura) to the northern coast of Chile (Antofagasta) (Fig. 20).

***Hapterosiphonia paniculata* (Montagne) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho subsp. *tapinocarpa* (Suringar) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho comb. nov. (Fig. 23-24)**

**Basionym:** *Polysiphonia tapinocarpa* Suringar.

**Type locality:** Japan.

**Distribution:** Japan and Korea (Fig. 20).

**Specimens examined:** CUK4120, CUK4130, CUK4131, CUK4132, CUK4133, CUK4134, CUK4135 (Minamiarao, Kumamoto, Kyushu, Japan collected by T.O.C., Apr. 18 2008), CUK12236 (Gwakji, Aewol-eup, Jeju-si, Korea collected by T.O.C. and D.E.B., May 30 2014).

**Vegetative morphology:** All specimens were collected from Kyushu, Japan. Plants are prominent, 3–8 cm in height (Fig. 23A), dark red to brown in color, and not associated with other filamentous species. Thalli form large tufts predominantly attached to rocky substrata in the intertidal zone and are composed of a prostrate and erect system with a flaccid texture (Fig. 23A). The erect system is composed of interwoven, delicate indeterminate axes that arise exogenously from the prostrate axes at intervals of 2–9 axial cells. They are densely and radially branched in an alternate pattern forming panicles every 3–10 axial cells (Fig. 23B). Apical cells are prominent, 6.18 ± 0.77 μm × 6.25 ± 0.42 μm in size, and transversely divided (Fig. 23C). Young erect axes are alternately disposed (Fig. 23C), curved in the direction of the main axes, and have short segments. Older segments of erect axes are larger, 220.06 ± 59.64 μm in length and 94.89 ± 8.93 μm in diameter, 0.43 times broader than they are long (L:D 2.31 ± 0.58), and infrequently branched (Fig. 23F). Trichoblasts are delicate, deciduous, numerous (Fig. 23C), 1–3 forked, and 47.50 ± 13.52 μm long, rarely >120 μm, and arising on each segment near the apical cells (Fig. 23D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, reaching 9.29 ± 1.38 μm × 12.36 ± 1.78 μm, and developed in a spiral series in the space between segments (Fig. 23E). Each segment is completely ecorticated and composed of 7–10 pericentral cells (Fig. 23H–I). Adventitious branches are present (Fig. 23G). Lateral branches arise in the axils of trichoblasts (Fig. 23C). The prostate system is extensive and entangled. Segments of the prostrate axes are 212.84 ± 20.41 μm in length and 83.89 ± 11.11 μm in diameter, 0.39 broader than they are long (L:D 2.59 ± 0.47). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells. They are cut off from the pericentral cells (Fig. 23J), 435.06 ± 190.40 μm in length, and 21.15 ± 4.60 μm in diame-

ter. Rhizoids are unicellular in younger stages, but form multicellular lobed terminations when mature (Fig. 23K-M).

**Reproductive morphology:** In tetrasporangial plants (Fig. 24A), tetrasporangia are tetrahedral and 27.87 ± 7.52 μm × 29.78 ± 7.67 μm in size. Tetrasporangial branches are swollen and sinuous and produce adventitious branches (Fig. 24B–C). The development of tetrasporangia follows a spiral arrangement (Fig. 24C–D). The sixth or seventh pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 24E–F). A single tetrasporangium is produced on each fertile segment. Female and male gametophytes were not found.

**Habitat:** Plants grow in the intertidal zone, forming extensive tufts. They are found attached to rocks in sheltered to wave-exposed areas. Tufts are usually broad, long, and very delicate, and not associated with other species.

**Remarks:** *Hapterosiphonia paniculata* subsp. *tapinocarpa* was originally described from Japan by Suringar (1867) as *Polysiphonia tapinocarpa.* It is characterized by having soft filaments, 8-10 twisted pericentral cells, and abundant trichoblasts (Suringar 1867, Segi 1951). Although Suringar (1867) observed cortication in the basal thallus part, Segi (1951) recognized this cortication as distorted siphons near the base. Our specimens from Korea and Japan correspond to the description of both Suringar and Segi. In this study, we report *Hapterosiphonia paniculata* subsp. *tapinocarpa* from Korea for the first time (Fig. 20). *Hapterosiphonia paniculata* subsp. *tapinocarpa* may be morphologically similar to *Enelittosiphonia stimpsonii* (Harvey) Kudo et Masuda and *P. howei* Hollenb. (known as *P. yonakuniensis* Segi) reported from Japan based on the presence of numerous pericentral cells, abundant trichoblasts, and tetrasporangia disposed in spiral series. However, *Enelittosiphonia stimpsonii* is distinguished from *Hapterosiphonia paniculata* subsp. *tapinocarpa* by being monoecious and by having branches arranged on the dorsal side of the parent axis (Segi 1949, 1951, Masuda et al. 1995), whereas *P. howei* has a dichotomous to subdichotomous branching pattern (Segi 1951, Mamoozadeh and Freshwater 2012).

The close relationship of *Hapterosiphonia paniculata* subsp. *paniculata* and *H. paniculata* subsp. *tapinocarpa* shows intraspecific variation and likely indicates allopatric populations separated after a long migration. The interconnection among the eastern Asia and the southwestern Pacific was confirmed by the wide distribution of *Polysiphonia morrowii* (Kim et al. 2004) and *Pterosiphonia arenosa* (unpublished data) in the coast of Korea and Peru-Chile. These subspecies are defined on the basis of the minimal morphological differences and the low genetic diversity (Mamoozadeh and Freshwater 2011).

***Hapterosiphonia confusa* (Hollenberg) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho comb. nov. (Fig. 25-26)**

**Basionym:** *Polysiphonia confusa* Hollenberg.

**Homotypic synonyms:** *Neosiphonia confusa* (Mont.) J.N. Norris.

**Heterotypic synonyms:** *Polysiphonia inconspicua* Hollenberg.

**Type locality:** Orange Co., near Corona del Mar, California, USA.

**Distribution:** California to Oregon, US (Fig. 26).

**Specimens examined:** CUK607, CUK609 (Lincoln beach, Oregon, USA collected by T.O.C., Jun 14 2003).

**Vegetative morphology:** All specimens were collected from Oregon, USA. Plants are prominent, 2.4–6 cm in height (Fig. 25A), dark-red to brown in color, and not associated with other species. Thalli form large tufts, predominantly attached to rocky substrata in the intertidal zone. Thalli are composed of an erect and prostrate system with a robust texture (Fig. 25A). The erect system is composed of interwoven indeterminate axes that are robust and arise exogenously from the pro-

strate axes at intervals of 2–12 axial cells. They are densely and radially branched in an alternate pattern forming panicles every 3–12 axial cells (Fig. 25B). Apical cells are prominent, 7.09 ± 0.73 μm × 6.57 ± 0.79 μm in size, and transversely divided (Fig. 25C). Young erect axes are alternately disposed, curved in the direction of the main axes, and have short segments (Fig. 25C). Older segments of erect axes are larger, 180.30 ± 150.52 μm in length and 90.95 ± 6.51 μm in diameter, being 0.5 times broader than they are long (L:D 1.99 ± 0.20), and infrequently branched (Fig. 25F). Trichoblasts are delicate, deciduous, numerous, 1–3 times forked, and 73.12 ± 25.54 μm long, and arising on each segment near the apical cells (Fig. 25D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, reaching 19.3 ± 1.51 μm × 21.17 ± 1.33 μm, and develop in a spiral series in the space between segments (Fig. 25E). Each segment is completely ecorticated along the thallus and composed of 9–12 pericentral cells (Fig. 25G–H). Adventitious branches are present. Lateral branches arise in the axils of trichoblasts. The prostrate system is extensive and entangled. Segments of the prostrate axes are 258.25 ± 16.22 μm in length and 194.16 ± 15.56 μm in diameter, 0.75 broader than they are long (L:D 1.33 ± 0.16). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells. They are cut off from the pericentral cells (Fig. 25I), 438.66 ± 217.16 μm in length, and 33.81 ± 5.70 μm in diameter. Rhizoids are unicellular in younger stages, but produce lobed, multicellular terminations when mature (Fig. 25J-L).

**Reproductive morphology:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 26A). Procarps are positioned laterally and subapically on erect axes, and are each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Cystocarps are globose when mature (Fig. 26B), 232.50 ± 57.61 μm in height, and 206.73 ± 40.84 μm in diameter. In tetrasporangial plants (Fig. 26C), tetrasporangia are tetrahedral and 36.96 ± 6.00 μm × 32.29 ± 6.82 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 26D). The development of tetrasporangia follows a straight arrangement but the position of the pericentral cells shifts every couple of segments giving slightly spiral appearance (Fig. 26D). The eighth or ninth

pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment. Male gametophytes were not found.

**Habitat:** Plants grow from the intertidal zone, forming extensive tufts. They are found in sheltered to wave-exposed areas and attached to rocks. Tufts of this species are usually wide, long, and very robust and are not associated with other species.

**Remarks:** *Hapterosiphonia confusa* was originally described by Hollenberg (1944) from California as *P. inconspicua* Hollenb. nom. illeg. and later renamed *P. confusa* by Hollenberg (1961). Recently, Norris (2014) transferred *P. confusa* to the genus *Neosiphonia* sect. *Multisiphonia*. *Hapterosiphonia confusa* is characterized by a robust and small habit, abundant trichoblasts, and 8-10 pericentral cells (Hollenberg 1944). Our specimens of *H. confusa* were collected from Oregon (Fig. 20) and correspond to the original description. *Hapterosiphonia confusa* is morphologically similar to *P. collinsii* Hollenb. and *P. hendryi* N. L. Gardner in having small habit, numerous pericentral cells, abundant trichoblasts, and main axes that are considerably branched (Hollenberg 1944). However, *P. collinsii* is distinct from *H. confusa* in having a creeping prostrate system (Hollenberg 1944), whereas *P. hendryi* has branches arranged in a corymbose pattern (Hollenberg 1961, Abbott and Hollenberg 1976). Our specimens of *H. confusa* have been well documented from California and Mexico by Hollenberg (1961) and Aguilar-Rosas et al. (2006).

***Hapterosiphonia sabulosia* (B. Kim et M.S. Kim) D.E. Bustamante, B.Y. Won, S. Fredericq et T.O. Cho comb. nov. (Fig. 27).**

**Basionym:** *Polysiphonia sabulosia* B. Kim et M.S. Kim.

**Holotype:** JNUB-JD120106-1

**Isotypes:** NIBR (NIBRAL0000138763-NIBRAL0000138765).

**Type locality:** Jongdal, Jeju, Korea.

**Description:** see Kim and Kim (2014) for general description. Fig. 27 shows its habit and rhizoidal morphology.

## 2. Phylogenetic analyses

We sequenced a 1,245-bp portion of *rbc*L and a 586-bp portion of *cox1* for *Hapterosiphonia paniculata* subsp. *paniculata*, *H. paniculata* subsp. *tapinocarpa*, *H. confusa*, and other *Polysiphonia* sensu lato species. In addition, an *rbc*L sequence for *P. sabulosia* was downloaded from GenBank (KF479248). Phylogenetic analyses of the *rbc*L locus placed the members of *Hapterosiphonia* gen. nov. as sister to the recently described genus *Lampisiphonia* (Fig. 28), with sequence divergences of 6.3% and 6.7% between them and supported by a bootstrap value of 97 and a posterior probability of 0.99. *Hapterosiphonia* gen. nov. is also diverging from the multipericentral group by 8.5% - 10.8% and associated to this group by a bootstrap value of 97 and a posterior probability of 1.00. On the other hand, phylogenetic analyses of the *cox1* locus placed the members of *Hapterosiphonia* gen. nov. as sister to the multi-pericentral group (Fig. 29) due to the absence of *Lampisiphonia* sequence, with sequence divergence ranging between 9.4% and 10.7% between them and supported by low bootstrap value and posterior probability. The sequence divergences among the species of *Hapterosiphonia* ranged from 1.1% to 2.6% for *rbc*L, and from 2.4% to 3.6% for *cox1*.

## 3. Discussion

Our *rbc*L and c*ox1* phylogenies placed *Hapterosiphonia confusa, H. paniculata* subsp. *paniculata*, *Hapterosiphonia paniculata* subsp. *tapinocarpa*, and *H. sabulosia* in a strongly supported clade.

The new genus, *Hapterosiphonia*, is characterized by rhizoids cut off from the pericentral cells and terminating in lobed, multicellular ends, by erect axes arising from an extensive and entangled prostrate system, a paniculate branching pattern, 8-12 pericentral cells, ecorticate axes, abundant trichoblasts and scar cells, procarps with four-celled carpogonial branches, spermatangial branches arising on a furcation of the trichoblast, and tetrasporangia arranged in either straight or spiral series. The results of our *rbc*L and *cox*1 sequence analyses support the taxonomic status of *Hapterosiphonia* as a new genus.

Among the features delineating *Hapterosiphonia*, lobed, multicellular rhizoidal terminations, and a paniculate branching pattern are the principal characters that separate this genus from others in *Polysiphonia* sensu lato and the tribe Polysiphonieae (Table 3). Although the shape of rhizoidal terminations in *Polysiphonia* sensu lato has been frequently reported as rounded, blunt, thread, or lobed (Yoon 1986, Stuercke and Freshwater 2008), the related features such as location of the rhizoidal origin, and whether the rhizoids are uni- or multicellular, had not been evaluated as taxonomically informative characters in *Polysiphonia* sensu lato. Unicellular rhizoids have been reported for most species in *Polysiphonia* sensu lato, whereas multicellular rhizoids have been observed in *Lampisiphonia iberica* Bárbara, Secilla, Díaz-Tapia et H. G. Choi and *Polysiphonia crassa* Okamura (Segi 1951, Yoon 1986, Bárbara et al. 2013). However, rhizoids of *Lampisiphonia iberica* are usually unicellular or occasionally become multicellular by the interpolation of an isodiametric cell at their place of origin on pericentral cells. Also, although some *Polysiphonia* sensu lato species, such as *N. baliana* D.E. Bustamante, B.Y. Won et T.O. Cho, *N. silvae* D.E. Bustamante, B.Y. Won et T.O. Cho, *N. ramirezii* D.E. Bustamante, B.Y. Won et T.O. Cho, *Polysiphonia dokdoensis* D.E. Bustamante, B.Y. Won et T.O. Cho, and *P. ulleungensis* D.E. Bustamante, B.Y. Won et T.O. Cho, have lobed rhizoidal terminations at matures stages, they are composed of a single cell only (Bustamante et al. 2013a, 2013b, 2014a, 2014b). The multicellular lobed termination in *Hapterosiphonia* is composed of multiple independent cells surrounding the terminations of long, tube-like rhizoids, and this pattern is unique for the genus *Hapterosiphonia.*

The branching pattern in *Polysiphonia* sensu lato has not been considered a taxonomic character at the species level because it ranges from alternate to subdichotomous to irregular, and it does not appear to be helpful for identifying species because its variation may be a result of the age of the plant or where the plant was growing (Stuercke and Freshwater 2008). However, some special branching patterns in *Polysiphonia* sensu lato have been recognized at the generic level, e.g., presence of dorsiventral secondary branches for *Streblocladia*, or determinate spiral laterals for *Bryocladia* (Schmitz and Falkenberg, 1897). The alternate disposition of panicles throughout the main axes (i.e., paniculate branching pattern) is observed as a consistent character state in *Hapterosiphonia*. This paniculate branching pattern may be one of the principal diagnostic characters for the identification of *Hapterosiphonia.*

*Hapterosiphonia paniculata* subsp. *paniculata*, *H. paniculata* subsp. *tapinocarpa* and *H. confusa*, can be distinguished from each other based on the habit of the thalli and tetrasporophyte. *H. paniculata* subsp. *paniculata* is characterized by robust and long filaments, and a very long series of unbranched tetrasporangial axes; *H. paniculata* subsp. *tapinocarpa* is characterized by soft and delicate filaments, and by the production of adventitious branches from the main tetrasporangial axes; and *H. confusa* is distinguished by robust and small filaments, and by the production of short lateral tetrasporangial branches from the main tetrasporangial axes. These three taxa resemble *P. isogona* Harv. and *P. subulifera* Harv. in their 8–13 pericentral cells, branches originating in the axils of the trichoblast, rhizoids cutting off from the pericentral cells, and spermatangial branches developed on a furcation of the trichoblast. *Polysiphonia isogona* and *P. subulifera* are distinguished from *H. paniculata* subsp. *paniculata*, *H. paniculata* subsp. *tapinocarpa* and *H. confusa* by having an irregular branching pattern (Maggs and Hommersand 1993, Womersley 2003). Moreover, *H. sabulosia* is distinguished from the other *Hapterosiphonia* by having 5-6 pericentral cells. Although *P. crassa* is the only species belonging to *Polysiphonia* sensu lato that has been reported with multicellular rhizoids (Yoon 1986), this species is distinguished from *Hapterosiphonia paniculata* subsp. *paniculata*, *H. paniculata* subsp. *tapinocarpa*, *H. confusa*, and *H. sabulosia* by hav-

ing an irregular branching pattern and cortication (Segi 1951, Yoon 1986). *Polysiphonia crassa* seems to be related in morphology to the genus *Lampisiphonia* but further molecular analysis may accurately define the relationship of *P. crassa*.

The results of our *rbc*L phylogenetic analyses revealed that *Hapterosiphonia* is closely related, with high support [93% for ML and 0.99 for Bayesian posterior probabilities (BPP)], to the recently described genus *Lampisiphonia* although there are clear differences in morphology. The genus *Lampisiphonia* is characterized by a combination of morphological features that include rhizoids cut off from the pericentral cells, 9–11 pericentral cells, cortication, exogenous branches, absence of trichoblasts, and tetrasporangia arranged in a straight series (Bárbara et al. 2013). *Lampisphonia* and *Hapterosiphonia* are distinguished based on the nature of holdfast and prostrate system, branching pattern, cortication, and presence of trichoblast. Additionally, in *Lampisiphonia*, the multicellularity of the rhizoids is confined to the place of rhizoid origin by having an isodiametric cell, whereas in *Hapterosiphonia*, it is restricted to their lobed terminations. Recently, Kim and Kim (2014) reported a new species named *Polysiphonia sabulosia* from Jeongdal, Jeju, Korea. Our phylogenetic analysis based on *rbc*L shows that *P. sabulosia* is closely related to our new genus *Hapterosiphonia* with 3.1-3.5% gene sequence divergence between it and other *Hapterosiphonia* species. Although Kim and Kim (2014) described *P. sabulosia* plants attached in the sand by unicellular rhizoids from prostrate axes, our observation of the isotype material (NIBRAL0000138763-NIBRAL0000138765 housed in National Institute of Biological Resources, Korea) revealed that *P. sabulosia* contains the diagnostic features of *Hapterosiphonia*: multicellular rhizoidal terminations and a paniculate branching pattern (Fig. 27). Thus, this species is also transferred to our new genus.

**Fig. 20.** Distribution of the genus *Hapterosiphonia* around the Pacific Ocean. *Hapterosiphonia paniculata* subsp. *paniculata* comb. nov. (sky blue), *Hapterosiphonia paniculata* subsp. *tapinocarpa* comb. nov. (blue), *Hapterosiphonia confusa* comb. nov. (yellow).

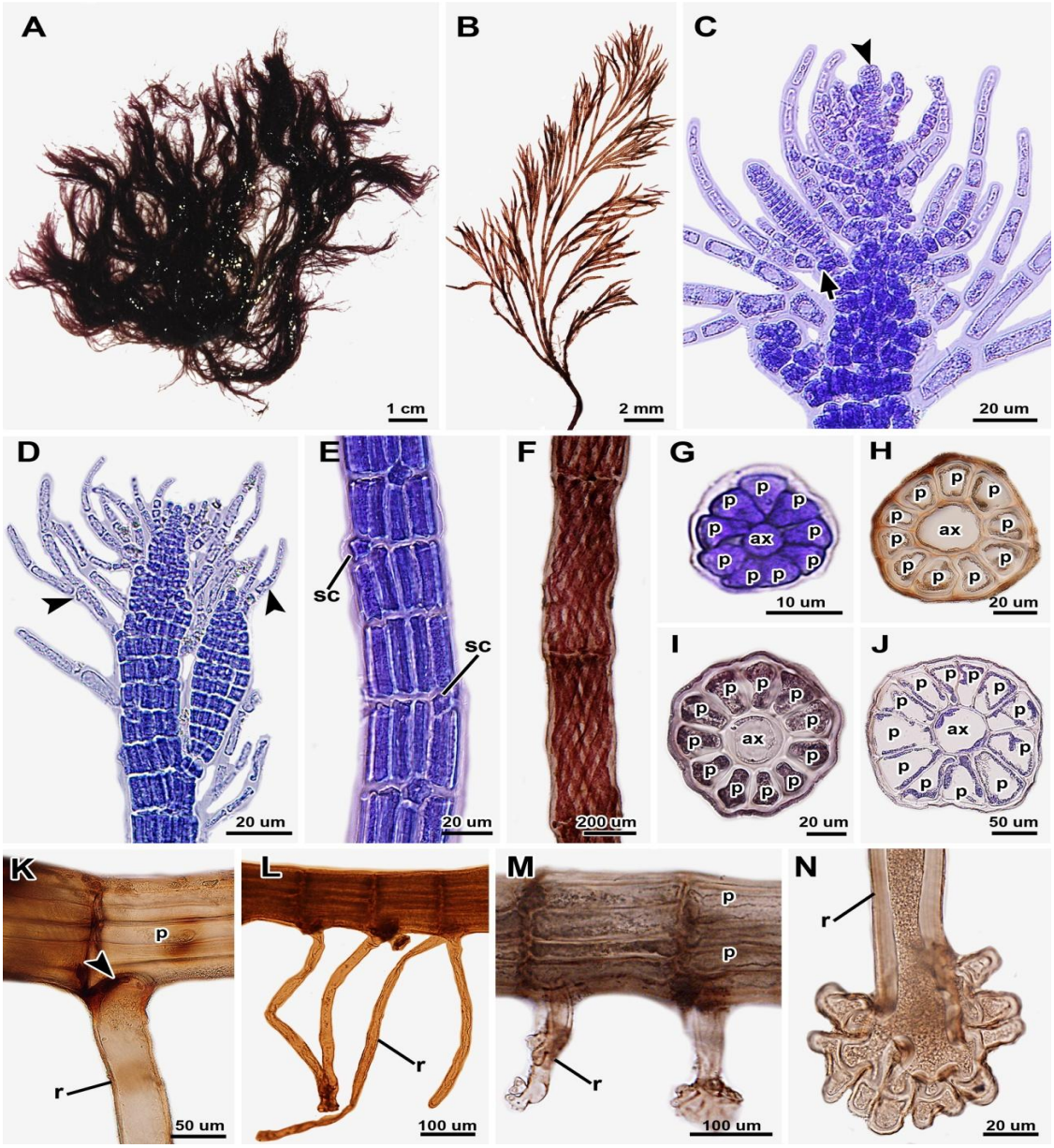


**Fig. 21.** Vegetative structures of *Hapterosiphonia paniculata* (Montagne) subsp. *paniculata* comb. nov. from Antofagasta, Chile. (A) Habit of vegetative thallus. (B) Erect axes showing paniculate branching pattern. (C) Apex of erect axis showing prominent apical cell (arrowhead) and branch (arrow) arising in the axil of a trichoblast. (D) Apical region with abundant trichoblasts (arrowheads). (E) Erect axis showing conspicuous scar cells (sc). (F) Axis showing long and twisted pericentral cells. (G–J) Cross-section views showing 9–12 pericentral cells (p) from apical (G), upper (H), middle (I), and lower (J) parts of thallus. (K) Rhizoid (r) cut off from proximal end of pericentral cell (p). (L) Young rhizoids with rounded termination. (M-N) Mature rhizoids with lobed, multicellular terminations (r).

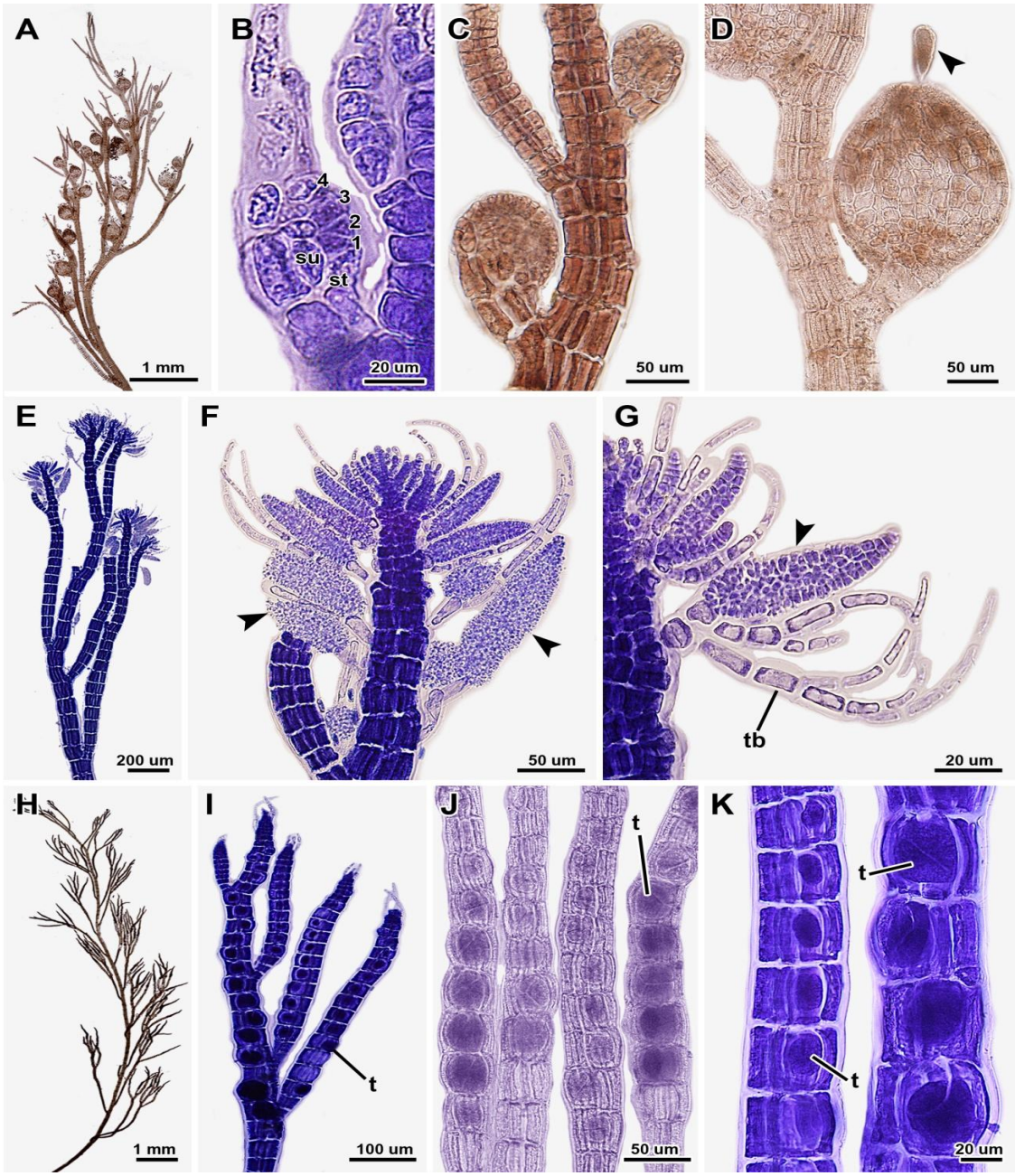


**Fig. 22.** Reproductive structures (CUK6540) of *Hapterosiphonia paniculata* (Montagne) subsp. *paniculata* comb. nov. from Ica, Peru. (A) Female thallus. (B) Procarp with four-celled carpogonial branch. (C–D) Young (C) and mature (D) cystocarps showing globose shape; and protruded, elongate carpospores (arrowhead). (E) Male thallus. (F) Spermatangial branches (arrowheads) clustered at apical region. (G) Mature spermatangial branch (arrowhead) arising from trichoblast (tb). (H) Tetrasporangial thallus. (I) Apical branches with tetrasporangia (t). (J–K) Branches of tetrasporangial thallus showing straight and spiral arrangements of tetrasporangia (t).

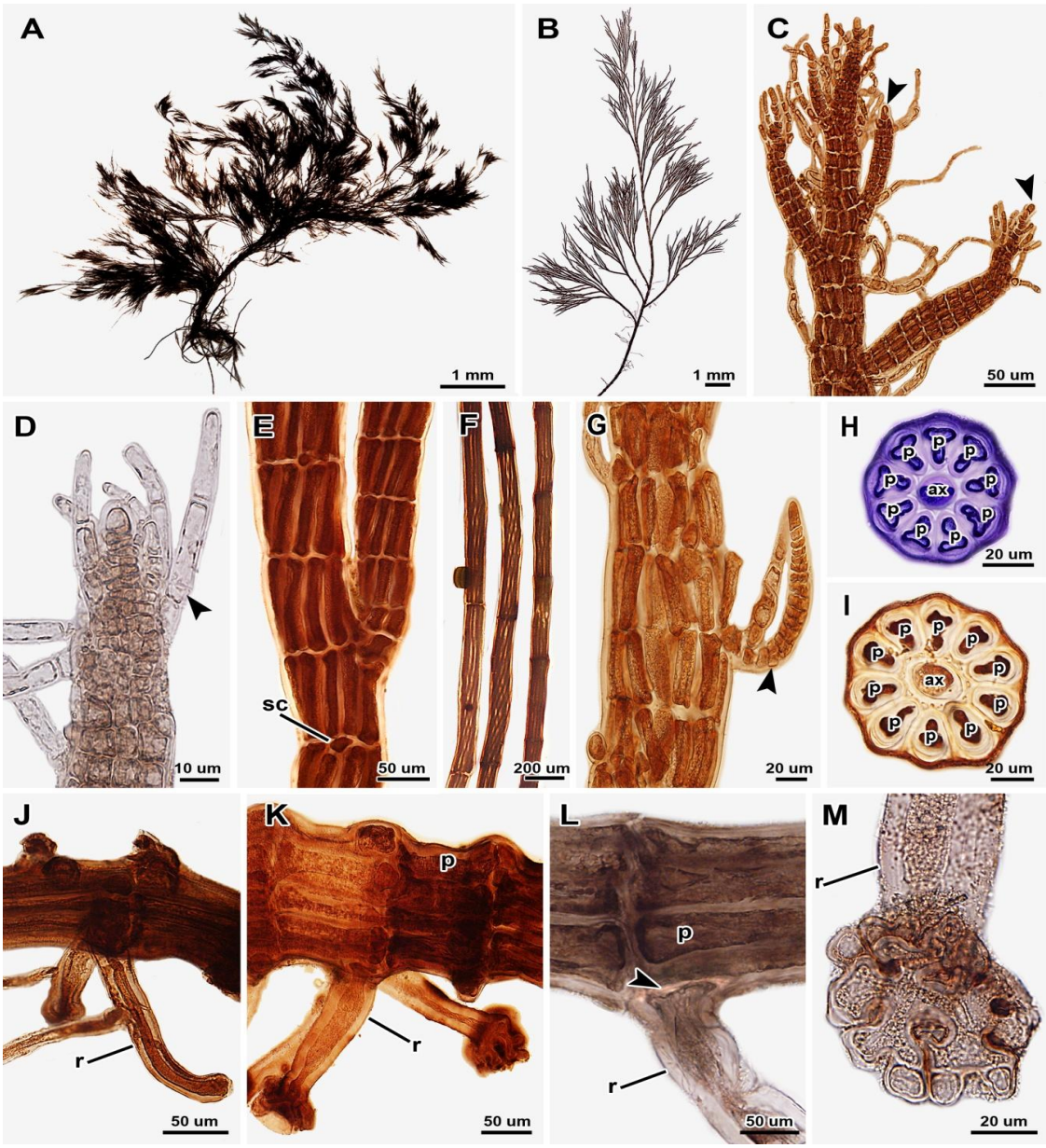


**Fig. 23.** Vegetative structures of *Hapterosiphonia paniculata* (Montagne) subsp. *tapinocarpa* comb. nov. from Kyushu, Japan. (A) Habit of vegetative thallus. (B) Erect axes showing paniculate branching pattern. (C) Apical region showing prominent apical cells (arrowheads). (D) Apex with trichoblasts (arrowhead). (E) Erect axis showing conspicuous scar cells (sc). (F) Axes showing long and twisted pericentral cells. (G). Basal part of thallus showing adventitious branch (arrowhead) developing from a trichoblast scar cell (tb). (H–I) Cross-section views of axes showing 9–10 pericentral cells (p) from middle (H) and lower (I) parts of thallus. (J) Rhizoid (r) cut off (arrowhead) from proximal end of pericentral cell (p). (K) Young rhizoids (r) with rounded termination. (L-M) Mature rhizoids (r) with lobed, multicellular terminations (r).

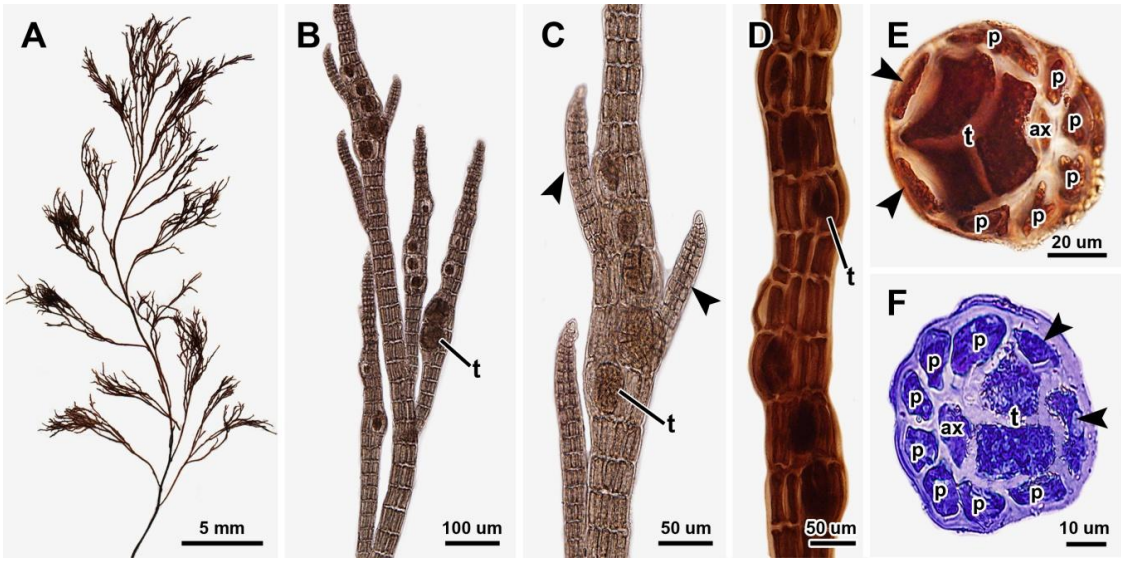


**Fig. 24.** Reproductive structures of *Hapterosiphonia paniculata* (Montagne) subsp. *tapinocarpa* comb. nov. from Jeju, Korea. (A) Tetrasporangial thallus. (B) Apical branches with tetrasporangia (t). (C) Upper part of tetrasporangial axes with adventitious branches (arrowheads). (D) Axis showing spiral arrangements of tetrasporangia (t). (E–F) Cross-section views of tetrasporangial segments with 6 or 7 pericentral cells (p) showing a single tetrasporangium (t) topped by two cover cells (arrowheads).

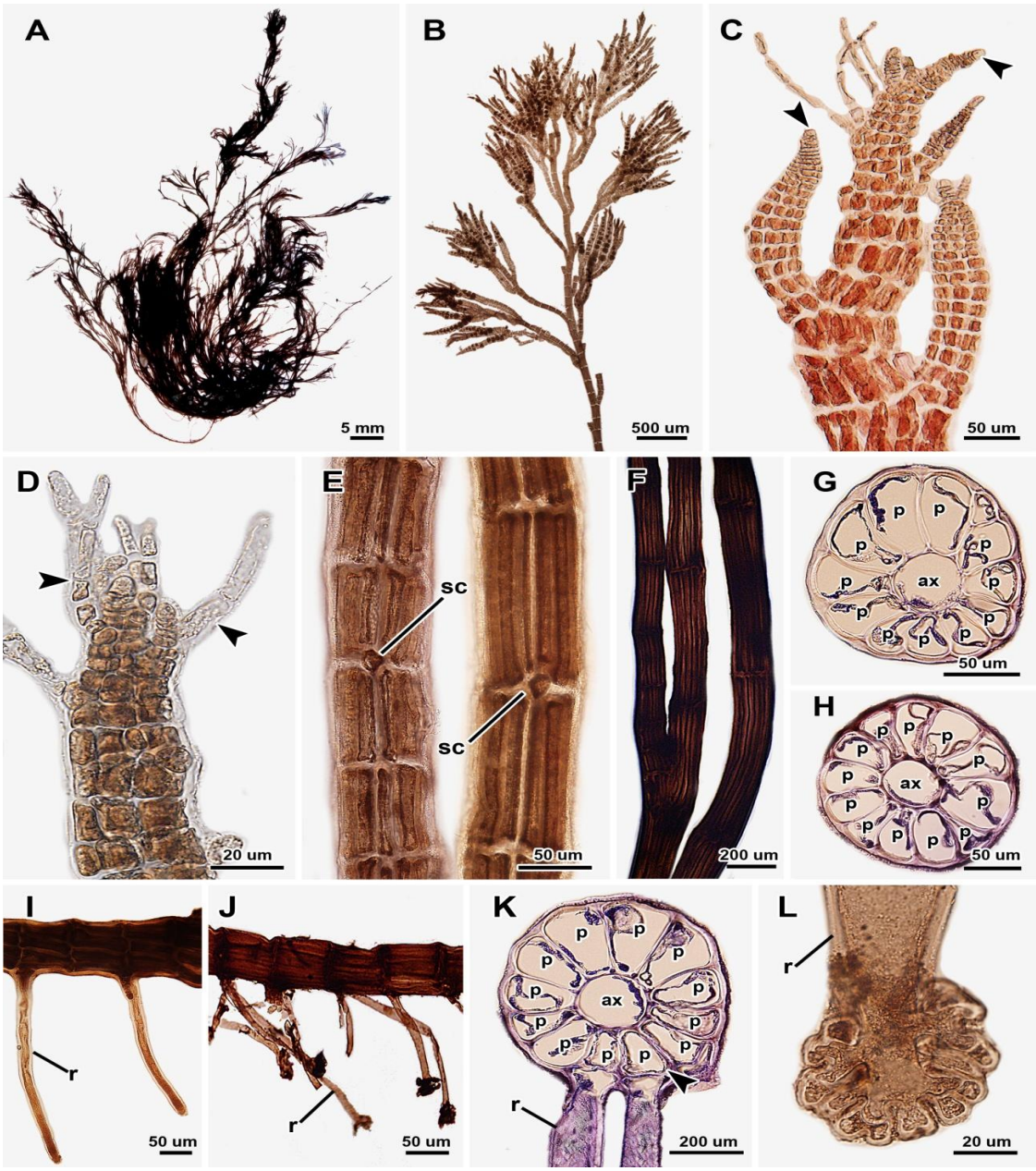


**Fig. 25.** Vegetative structures of *Hapterosiphonia confusa* (Hollenberg) comb. nov. from Oregon, USA. (A) Habit of vegetative thallus. (B) Erect axes showing paniculate branching pattern. (C) Apices showing prominent apical cells (arrowheads). (D). Apices with trichoblasts (arrowheads). (E) Erect axes showing conspicuous scar cells (sc). (F) Axes showing long pericentral cells. (G–H). Cross-section views of axes showing axial cell (ax) and 11–12 pericentral cells (p) from middle (G) and lower (H) parts of thallus. (I) Cross-section view of prostate axis showing rhizoids (r) cut off from pericentral cells (p), and axial cell (ax). (J) Young rhizoids (r) with rounded termination. (K-L) Mature rhizoids (r) with lobed, multicellular terminations.

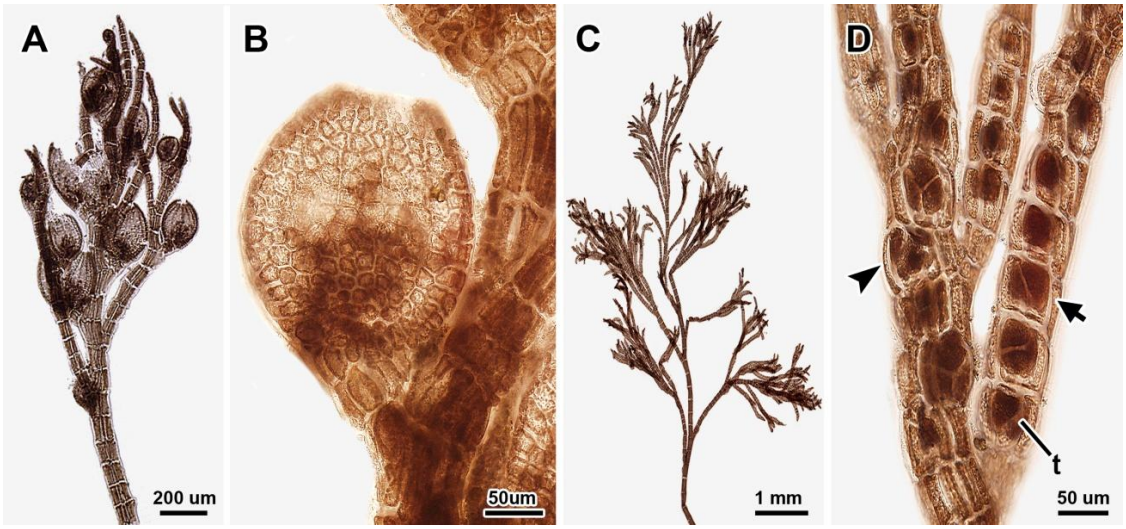


**Fig. 26.** Reproductive structures of *Hapterosiphonia confusa* (Hollenberg) comb. nov. from Oregon, USA. (A) Female thallus. (B) Mature, globose cystocarp. (C) Tetrasporangial thallus. (D) Branches of tetrasporangial thallus showing straight (arrow) and spiral (arrowhead) arrangements of tetrasporangia (t).

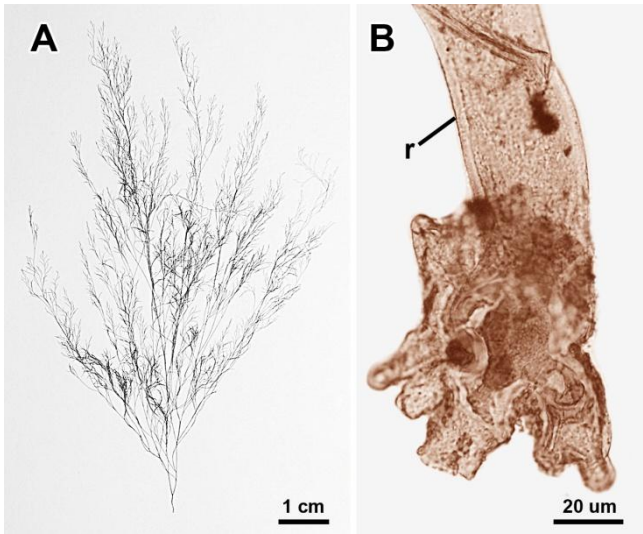


**Fig. 27**. Isotype (NIBRAL0000138763 as "*Polysiphonia sabulosia*") of *Hapterosiphonia sabulosia* (B. Kim et M.S. Kim) comb. nov. (A) Isotype specimen. (B) Multilobed and multicellular terminations of rhizoid (r) from *H. sabulosia* Isotype.

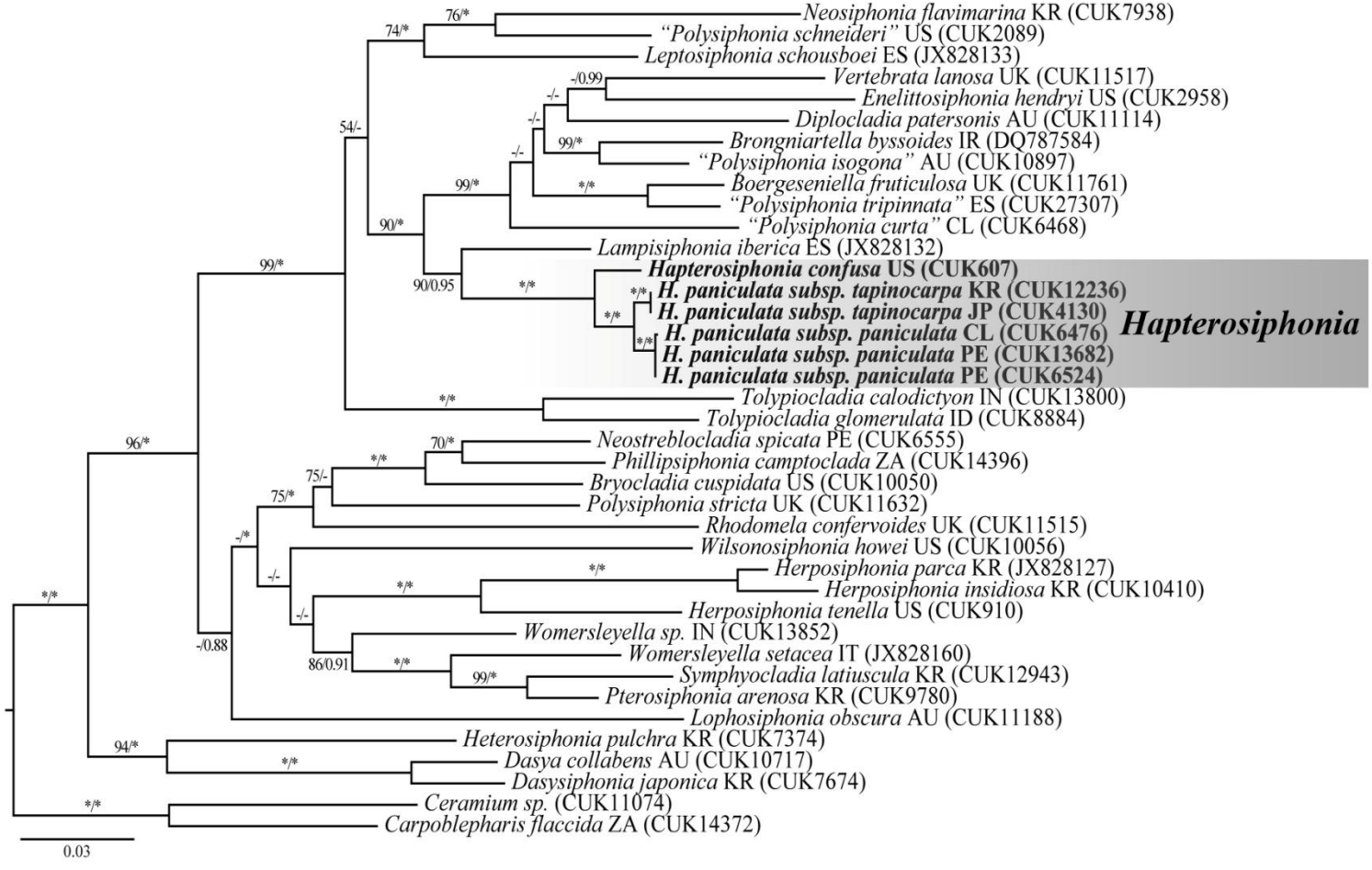


**Fig. 28.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

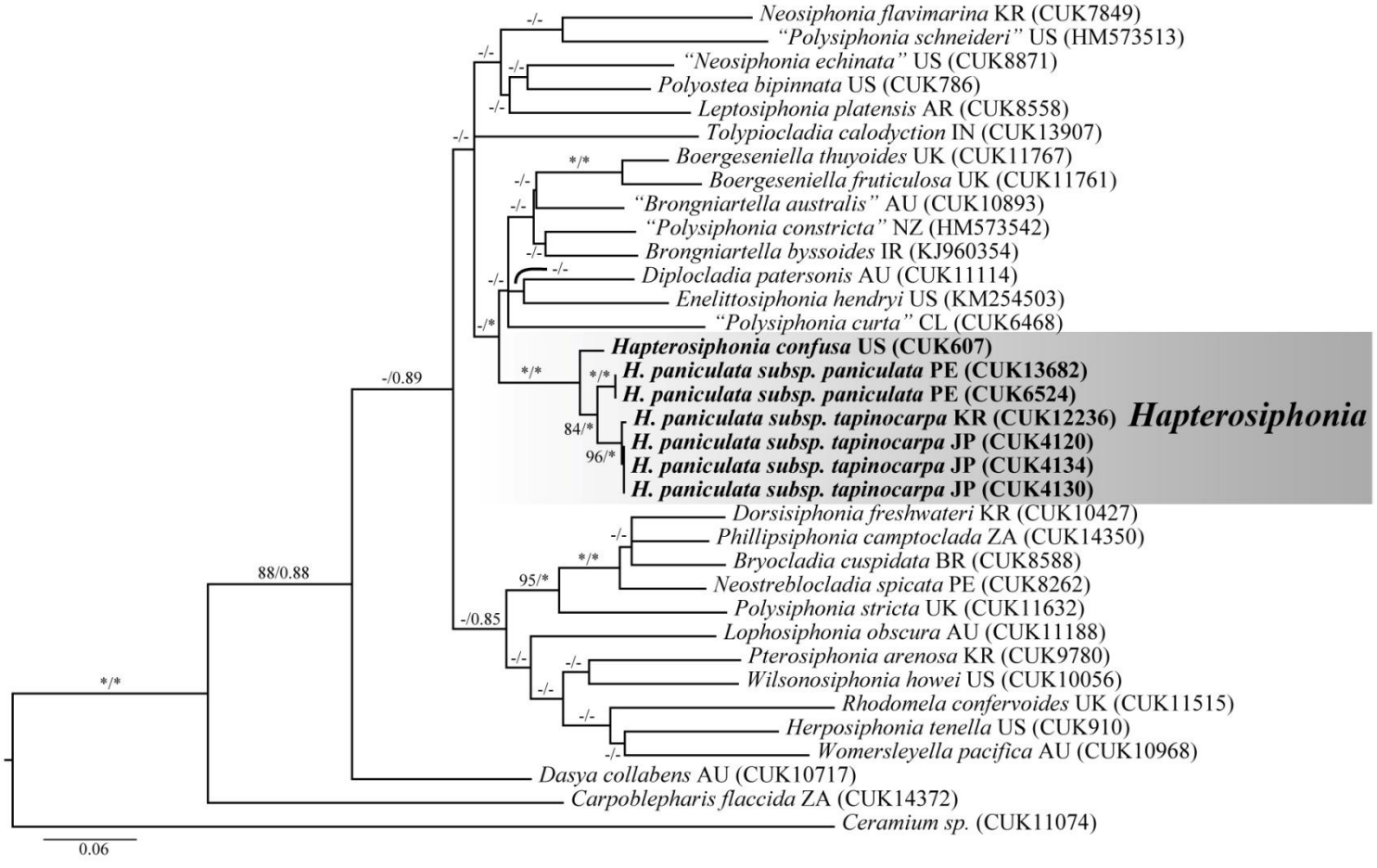


**Fig. 29.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 3.** Comparisons among genera belonging to *Polysiphonia* sensu lato and *Hapterosiphonia* gen. nov.

| **Features** | ***Hapterosiphonia*** | ***Boergeseniella*** | ***Bryocladia*** | ***Enelittosiphonia*** | ***Lampisiphonia*** | ***Leptosiphonia*** | ***Neosiphonia*** | ***Polysiphonia*** | ***Streblocladia*** | ***Vertebrata*** |
|---|---|---|---|---|---|---|---|---|---|---|
| Type species | *H. paniculata* | *Bo. fruticulosa* | *Br. cervicornis* | *E. stimpsonii* | *La. iberica* | *Le. schousboei* | *N. flavimarina* | *P. stricta* | *S. neglecta* | *V. lanosa* |
| Type locality | Lima, Peru | British Isles | Java | Japan | Spain | Biarritz, France | Bangpo, Korea | British Isles | South Pacific | Iceland |
| Pericentral cells | 8–12 | 8–12 | 6–12 | 7–11 | 9–11 | 12–16 | 4–9 | 4 | 4–8 | 17-23 |
| Branching pattern | Paniculate | Distichous (1/5 phyllotaxy) | Determinate spiral laterals | Dorsiventral | Pseudodichotomous | Alternate | Alternate | Pseudodichotomous to alternate | Dorsiventral secondary | Pseudodichotomous |
| Cortication | Absent | Present | Absent | Absent | Present | Present | Absent or present | Absent | Absent or present | Absent |
| Rhizoid connection | Cut off | Cut off | - | Cut off | Cut off | Cut off | Cut off | Open | - | Cut off |
| Rhizoids | Multicellular | Unicellular | Unicellular | Unicellular | Multicellular | Unicellular | Unicellular | Unicellular | Unicellular | Unicellular |
| Carpogonial branches | Four-celled | Four-celled | Four-celled | Four-celled | - | Four-celled | Three-celled | Four-celled | - | Four-celled |
| Spermatangia | Arising as primary branch | Replacing trichoblast | - | Arising as primary branch | - | Arising as primary branch | Arising as primary branch | Arising as primary branch or replacing trichoblast | Arising as primary branch or replacing | Replacing trichoblast |
| Tetrasporangia | Spiral or straight | Spiral | Straight | Spiral | Straight | Two per segment | Spiral or straight | Spiral or straight | Straight | Straight |
| References | This study | Maggs and Hommersand (1993) | Schmitz and Falkenberg (1897) | Segi (1949) | Bárbara et al. (2013) | Díaz-Tapia and Bárbara (2013) | Kim and Lee (1999) | Kim et al. (2000), this study | Schmitz and Falkenberg (1897) | Kim et al. (2002), this study |

## CHAPTER 2. Taxonomy and phylogeny of the genus *Leptosiphonia* (Rhodomelaceae, Rhodophyta)

The red-algal genus *Polysiphonia* sensu lato with around 220 species is widely distributed in tropical, warm-temperature, cold-temperate, and subantarctic regions including mangrove habitats and estuaries. *Polysiphonia* sensu lato can occur on rocks, epiphytic on other plants, or epizooic on various invertebrates and sea turtles (Hollenberg 1942, Womersley 2003, Bustamante et al. 2015b, 2015c, Guiry and Guiry 2015).

The original name for species having features of *Polysiphonia* was proposed by C. Agardh (1817) as *Hutchinsia*. *Hutchinsia* C. Agardh is a later homonym of *Hutchinsia* R. Brown described in Aiton (1812) (Greville 1823). The homonymy with *Hutchinsia* R. Brown was soon recognized and several substitute names were proposed independently. *Polysiphonia* Greville (1823) was conserved with the conserved type species *P. urceolata* (Dillwyn) Greville and the other considered as rejected names (Wynne 1986, Silva et al. 1996). The taxonomy of *Polysiphonia* sensu lato has suffered substantial changes since then. Falkenberg (1901) and Kylin (1956) proposed the transfer and segregation of several genera based on vegetative and reproductive morphology. One of these genera, *Carradoria* was resurrected by Kylin (1956) based on the hyphae between the pericentral and axial cells as diagnostic feature and lectotypified the South African endemic species *C. virgata* (C.Agardh) Kylin as generitype. Although he attributed the name to Martius, he effectively created a homonym in accordance with Art. 48.1 by excluding the type species (Silva et al. 1996). Although *Carradoria* Kylin was considered to be a synonymous of *Polysiphonia* by Wynne (1986), Silva et al. (1996) proposed the new name *Carradoriella* P. C. Silva with its type *Carradoriella virgata* (C. Agardh) P.C. Silva to replace *Carradoria* Kylin (1956) and distinguished from *Carradoria* Martius (1833).

Another genus segregated after the review of Falkenberg (1901) and Kylin (1956) was *Leptosiphonia* Kylin. This genus was described based on *Polysiphonia schousboei* Thuret (1876) from the

coast of France and Morocco (Díaz-Tapia and Barbara 2013). The two tetrasporangia per segment arranged in two longitudinal rows was the distinguishing feature considered by Falkenberg (1901) to include *P. schousboei* in the monotypic genus *Ophidocladus.* He pointed out that this generic assignment was tentative because *P. schousboei* clearly differed from *O. simpliciusculus* (P.L.Crouan et H.M.Crouan) Falkenberg. The former is distinguished by having radially organized thallus and branches mostly exogenous; whereas the latter is different by having dorsiventrally organized thallus and the exclusive formation of endogenous branches. The segregation of *P. schousboei* in the genus *Leptosiphonia* by Kylin (1956) has been widely accepted with the morphological characterization and lectotypification of *Leptosiphonia schousboei* (Thuret) Kylin (Díaz-Tapia and Bárbara 2013, Díaz-Tapia et al. 2014).

The recent phylogenetic studies in *Polysiphonia* sensu lato, based on molecular studies of nuclear-encoded 18S rDNA (SSU), mitochondrial-encoded COI, and plastid-encoded *rbc*L have revealed the presence of new taxa and the segregation of new genera. The taxa newly described in *Polysiphonia* sensu lato were a result of cryptic diversity or previous misidentifications (Stuercke and Freshwater 2010, Kim and Kim 2014, Bustamante et al. 2014a, 2014b, 2015a); whereas the establishment of the new genera was based on species having consistent vegetative features that clustered in monophyletic groups (Bárbara et al 2013, Bustamante et al. 2015c, see Chapter 4). These studies also have revealed that *Polysiphonia* sensu lato is currently composed of the following genera *Hapterosiphonia* D.E.Bustamante, B.Y.Won et T.O.Cho, *Lampisiphonia* H.G. Choi, Diaz-Tapia et Bárbara, *Neosiphonia* M.S. Kim et I.K. Lee, *Polysiphonia* Greville, and the multipericentral group (Bustamante et al. 2015c).

In the present study, we collected some polysiphonous specimens having in common rhizoidal cells between pericentral and axial cells in the basal part and cortication of the thallus. Three specimens previously reported as *Polysiphonia brodiei* (Dillwyn) Sprengel, *P. elongata* (Hudson) Sprengel and *P. virgata* were collected from the vicinity of their type localities in the eastern Atlantic and an unidentified species was collected from the southwestern Atlantic coast. After we re-

assessed these specimens, we enlarge the present circumscription of the genus *Leptosiphonia* based on the detailed morphology and molecular evidence of *rbc*L and *cox1* locus to encompass the following new combinations *Leptosiphonia brodiei* comb. nov., *L. elongata* comb. nov., and *L. virgata* comb. nov. and also one new species *L. platensis*.

## 1. Morphological analyses

***Leptosiphonia* Kylin emend. D.E. Bustamante, B.Y. Won et T.O. Cho.**

**Type species:** *Leptosiphonia schousboei* (Thuret) Kylin (1956).

**Lectotype locality:** Tanger, Morocco.

**Diagnosis:** Plants are prominent and form large tufts predominantly attached to rock surfaces or epizooic on the shell of some invertebrates. Thalli are composed of main indeterminate erect axes with interwoven lateral branch attached by prominent holdfast or reduced prostrate system. Erect axes are composed of polysiphonous axes with 4–15 pericentral cells per segment and with rhizoidal cells in the base and slight to dense cortication. Erect axes are densely and radially branched in pseudodichotomous to alternate to irregular pattern. Adventitious branches present. Lateral branches arise in the axils of trichoblasts or replacing them. Trichoblasts are scarce, delicate, deciduous, and 1–3 times forked. Conspicuous scar cells are present. Unicellular rhizoids are cut off from the proximal end of pericentral cells or cortical cells. Procarps are positioned laterally and subapically, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial branches arise from one fork of the trichoblasts or replacing them, cylindrical, sometimes with sterile tips. Tetrasporangia are formed singly or sometimes two per segment, tetrahedrally divided and arranged in straight or spiral series.

**Key to species of *Leptosiphonia***

1. Pericentral cells four ..... ***Leptosiphonia elongata***
1. Pericentral cells 6-15 ..... 2
    2. Spermatangial branches are clustered at the apices of erect axes and spirally disposed ..... ***Leptosiphonia brodiei***
    2. Spermatangial branchlets are arranged in two rows on the abaxial side of the branchlets ..... 3
3. 1-3 layers of rhizoidal cells ..... ***Leptosiphonia virgata***
3. Individual rhizoidal cells ..... ***Leptosiphonia platensis***

***Leptosiphonia brodiei* (Dillwyn) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 31-32)**

**Basionym:** *Conferva brodiei* Dillwyn (1809).

**Homotypic synonyms:** *Ceramium brodiei* (Dillwyn) C. Agardh, *Hutchinsia brodiei* (Dillwyn) Lyngbye, *Vertebrata brodiei* (Dillwyn) Kuntze, *Polysiphonia brodiei* (Dillwyn) Sprengel.

**Heterotypic synonyms:** *Polysiphonia penicillata* (C. Agardh) Sprengel, *Hutchinsia penicillata* C. Agardh, *Polysiphonia brodiei f. densa* Holmes et Batters, *Polysiphonia brodiei f. typica* Holmes et Batters.

**Type locality:** Bantry Bay, Co. Cork, Ireland

**Specimens examined:** CUK772 (Crescent city, California, USA collected by T.O.C, Jul. 01 2003), CUK10863, CUK10864 (Bellerive beach, Hobart, Tasmania, Australia collected by T.O.C and D.E.B, Mar. 22 2014), CUK11869 (Donaghadee, Rocky shore, Northern Ireland collected by T.O.C, May 08 2014).

**Vegetative structure:** Plants are prominent, 3-21 cm in height (Fig. 31A), and dark brownish to purplish-red in color. Thalli form large tufts predominantly attached to rock surfaces and epiphytic in intertidal zone. Thalli are composed of main indeterminate axes with a reduced or secondary prostrate system (Fig. 31A). The main erect axes are robust in texture, remaining distinct and forming few major laterals exogenously. They are densely and radially branched forming tufts in a spiral pattern in (Fig. 31B). Apical cells are prominent, 9.88 ± 1.19 μm × 7.39 ± 0.42 μm in size, and transversely divided (Fig. 31C) and subapical cells are sometimes rapidly becoming corticated. Young laterals axes are spirally and alternate disposed (Fig. 31C), very flexible and flaccid, and have short segments. Older segments of erect axes near to holdfast are 755.09 ± 256.68 μm in diameter but after the first branches are 420.32 ± 104.29 μm in length and 236.10 ± 21.17 μm in diameter, being 1.78 times broader than they are long [0.59 ± 0.13 (L/D)], and denudated or infrequently branched. Trichoblasts are delicate, deciduous, numerous, 1–4 times forked, and 283.50 ± 66.20 μm long, and arising on each segment near the apical cells (Fig. 31D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, reaching 9.31 ± 0.86 μm × 9.61 ± 1.06 μm, and developed in a spiral series in the space between segments (Fig. 31E). Each segment is composed of 6-8 pericentral cells (Fig. 31G-H). Segments also have 1-2 layers of rhizoidal cells between pericentral and axial cells covered by dense cortication (Fig. 31G-J).Adventitious branches present. Lateral branches arise in axils of trichoblasts (Fig. 31C). Holdfasts are prominent, 1257.91 ± 420.92 μm and composed of tightly clumped rhizoids produced from basal pericentral or cortical cells. The prostate system is reduced, entangled and produced from apical cells of major lateral axes (Fig. 31K). Segments of the prostrate axes are 96.62 ± 9.23 μm in length and 137.87 ± 13.51 μm in diameter, being 1.43 times broader than they are long [0.71 ± 0.08 (L/D)]. Rhizoids are also ventrally produced from the proximal end of the pericentral cells in prostrate axes (Fig. 31K). They are cut off from the pericentral cells (Fig. 31L), 486.96 ± 145.93 μm in length, and 20.85 ± 3.25 μm in diameter. Rhizoids are unicellular with multilobed terminations (Fig. 31M).

**Reproductive structure:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 32A). Procarps are positioned subapically and alternately on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 32B). Cystocarps are solitary (Fig. 32C-D) and urceolate in form when mature (Fig. 32D), 309.68 ± 122.71 μm in height and 345.96 ± 122.71 μm in diameter. In male gametophytes(Fig. 32E), spermatangial branches are clustered at the apices of erect axes and spirally disposed (Fig. 32F), and are developed on a furcation of the trichoblast in each segment of the axes (Fig. 32G). Each spermatangial branch is composed of spermatangia, and sometimes a single sterile tip cell (Fig. 32G). In tetrasporangial plants (Fig. 32H-I), tetrasporangia are tetrahedral and 35.65 ± 7.59 μm × 41.14 ± 7.33 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 32I). The development of tetrasporangia follows a spiral arrangement (Fig. 32J–K). One of the pericentral cells of the fertile segment is developed to a stalk cell, which will develop the tetrasporangia and the two cover cells (Fig. 32L-M). A single tetrasporangium is produced from a fertile segment (Fig. 32L-M).

**Habitat:** Plants grow from the high to the low intertidal zone, forming extensive tufts. They are found attached to rocks or epiphytic on corallines in sheltered to wave-exposed areas. Tufts are usually small, individual, and very robust, sometimes associated with other species such as *Neosiphonia harveyi*, *Polysiphonia strictissima*, and *Polysiphonia fucoides.*

**Remarks:** *Leptosiphonia brodiei* comb. nov. was originally described as *Conferva brodiei* Dillwyn (1809) from Ireland, but this species was subsequently transferred to *Ceramium*, *Hutchinsia*, *Vertebrata*, and finally established in *Polysiphonia brodiei* by Sprengel (1827). *Leptosiphonia brodiei* has been widely reported from the Northwestern Atlantic (Maggs and Hommersand 1993), Eastern Pacific (Abbott and Hollenberg 1976), and Western Pacific (Womersley 2003). This study confirm morphologically and molecularly the wide distribution of *L. brodiei* from the coast of Europe, California, and Australia (Fig. 30). Maggs and Hommersand (1993) already noticed that *L. brodiei* may have been spread by shipping based on the establishment of the Pacific populations near harbors. Our collections from these three areas having macroscopic gametophytic and tetrasporophytic

plants categorized *L. brodiei* into the last phase of the invasion process: "persistence" (Valentine et al. 2007, Bustamante et al. 2015b). Although the morphology of *Leptosiphonia brodiei* varies greatly accordingly to the environment (Maggs and Hommersand 1993, Díaz-Tapia et al. 2013a), it is characterized by having main prominent indeterminate erect axes attached by a prominent holdfast, numerous pericentral cells, heavy cortication, and its flaccid texture. The 6-8 pericentral cells heavy corticated in *L. brodiei* clearly separate it from other *Polysiphonia* sensu lato.

***Leptosiphonia elongata* (Hudson) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 33)**

**Basionym:** *Conferva elongata* Dillwyn (1809).

**Homotypic synonyms:** *Conferva elongata* Hudson, *Ceramium elongatum* (Hudson) Roth, *Hutchinsia elongata* (Hudson) C. Agardh, *Boryna elongata* (Hudson) Bory de Saint-Vincent, *Rhodomela elongata* (Hudson) Fries, *Polysiphonia elongata* (Hudson) Sprengel.

**Heterotypic synonyms:** *Polysiphonia denudata* f. fragilis Voronikh, *Neosiphonia elongata* (Hudson) Xiang Si-duan. (See Guiry and Guiry 2015 for the whole list).

**Type locality**: England

**Specimens examined:** CUK11765 (Forty Foot Beach, Ireland collected by T.O.C, May. 07 2014).

**Vegetative structure:** Plants are prominent, 10-23 cm in height (Fig. 33A), and dark brownish to purplish-red in color. Thalli form very large filaments predominantly attached to rock surfaces and epizooic in the intertidal zone. Thalli are composed of a single main erect axe of robust texture attached by a prominent holdfast (Fig. 33A). The erect system is composed of a very long indeterminate axe bearing laterals at irregular positions. These laterals branches are irregular branched 3 - 4 orders. Apical cells are inconspicuous, 6.78 ± 1.49 μm × 6.92 ± 0.77 μm in size, and transversely

divided (Fig. 33D) and subapical cells are rapidly becoming corticated (Fig. 33D-E). Young laterals axes are spirally disposed, very flexible and flaccid, and have short segments (Fig. 33B). Older segments of erect axes near to holdfast are 324.88 ± 51.62 μm in diameter but after the first adventitious branches 231.23 ± 39.52 μm in length and 260.91 ± 15.73 μm in diameter, being 1.13 times broader than they are long [0.89 ± 0.18 (L/D)], and denudated or infrequently branched (Fig. 33C). Trichoblasts are scarce, delicate, deciduous, 1–2 times forked, 75.69 ± 29.54 μm long, and arising on each segment near the apical cells (Fig. 33 D-E). Scar cells appear along the filament after the trichoblasts have been shed, reaching 13.69 ± 2.04 μm × 13.65 ± 2.45 μm, and arranged in irregular positions in the space between segments. Cicatrigenous branches are present (Fig. 33F). Each segment is composed of 4 pericentral cells (Fig. 33H-K). Segments have individual rhizoidal cells between pericentral cells and the axial cells covered by a heavy cortication (Fig. 33J-K). Adventitious branches are abundant at irregular positions of the whole axe. Lateral branches are replacing trichoblasts (Fig. 33D). Holdfasts are prominent, 355.82 ± 69.97 μm and composed of tightly clumped rhizoids produced from basal pericentral or cortical cells. Rhizoids are produced from the proximal end of the pericentral cells. They are cut off from the pericentral cells. Rhizoids are unicellular with multilobed terminations.

**Reproductive structure:** In tetrasporangial plants (Fig. 33L), tetrasporangia are tetrahedral, 51.04 ± 8.48 μm × 56.60 ± 10.33 μm in size, and developed in upper main axes or adventitious branches. Tetrasporangial branches are swollen and sinuous (Fig. 33M-O). The development of tetrasporangia follows a spiral arrangement (Fig. 33N-O). One of the pericentral cells of the fertile segment is developed to a stalk cell, which will develop the tetrasporangia and the two cover cells (Fig. 33P-Q). A single tetrasporangium is produced from a fertile segment (Fig. 33P-Q). Gametophytes were not found in this study.

**Habitat:** Plants grow from the high to the low intertidal zone, forming individual tufts. They are found attached to rocks or epizooic on shells in sheltered to wave-exposed areas. Tufts of this spe-

cies are very long, sometimes associated with other species such as *Neosiphonia harveyi* and *Polysiphonia fucoides.*

**Remarks:** *Leptosiphonia elongata* comb. nov. was originally described as *Conferva elongata* Hudson (1762) from England, but this species was subsequently transferred to *Ceramium*, *Hutchinsia*, *Boryna*, *Rhodomela*, and finally established in *Polysiphonia brodiei* by Sprengel (1827). *Leptosiphonia elongata* has been widely reported along the Northwestern Atlantic coast (Fig. 30) (Maggs and Hommersand 1993) and is characterized by having a main prominent indeterminate erect axe attached by a prominent holdfast, four pericentral cells, heavy cortication throughout, attenuated bases of branches, and flaccid texture (Maggs and Hommersand 1993). *Leptosiphonia elongata* is similar to *P. elongella* Harvey, *P. fibrata* (Dillwyn) Harvey and *P. fibrillosa* (Dillwyn) Sprengel, but *P. elongella* is distinguished from *L. elongata* by having bisporangia in spiral series, whereas *P. fibrata* and *P. fibrillosa* are different from *L. elongata* by having branches formed in axils of trichoblasts (Maggs and Hommersand 1993).

***Leptosiphonia platensis* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 34-35)**

**Diagnosis:** Thalli 3-12 cm tall, saxicolous, epizooic on shells, polysiphonous, composed of main indeterminate erect axes attached by a prominent holdfast with a reduced or secondary prostrate system. Erect axes with pseudodichotomous branching pattern, which are exogenously and replacing the trichoblasts. Trichoblast are scarce in vegetative thalli, but abundant and several times forked in gametophytes. Adventitious branches present. Axes have 7-12 pericentral cells. Basal parts of thalli having rhizoidal cells and heavy cortication. Rhizoids are unicellular and cutting off the proximal end of pericentral cells. Procarps bearing a four-celled carpogonial branches. Spermatangial branches arise from one fork of the trichoblasts. Tetrasporangia arranged in spiral series

**Holotype:** CUK8576.

**Type locality:** Mariano beach, Mar del Plata, Buenos Aires, Argentina; 37º58'43.11"S, 57º32'29.85"W; 09 July 2012.

**Etymology:** The specific epithet is derived from the locality of collection, Mar del Plata

**Specimens examined:** CUK8507, CUK8509, CUK8510 (Playa Parana, Puerto Madryn, Chubut, Argentina, collected by T.O.C. and D.E.B., Jul. 08 2012), CUK8558, CUK8562, CUK8563, CUK8564, CUK8574 (Mariano beach, Mar del Plata, Buenos Aires, Argentina collected by T.O.C and D.E.B., Jul. 09 2012).

**Vegetative structure:** Plants are prominent, 3-12 cm in height (Fig. 34A), and dark red to brown in color. Thalli form large tufts predominantly attached to rock surfaces or epizooic in the intertidal zone. Thalli are composed of main indeterminate erect axes attached by a prominent holdfast with a reduced or secondary prostrate system (Fig. 34B-C). The main erect axes are robust in texture, remaining distinct and forming few major laterals exogenously. They are densely and radially branched in a pseudodichotomous pattern every 4-10 axial cells, unusually 20 (Fig. 34D-E). Apical cells are prominent, 7.96 ± 1.21 μm × 9.21 ± 0.66 μm in size, and transversely divided (Fig. 34E). Young erect axes are pseudodichotomously disposed (Fig. 34E), curved in the direction of the main axes, and have short segments. Older segments of erect axes near to holdfast are 385.14 ± 23.01 μm in diameter but after the first branch are 97.93 ± 17.05 μm in length and 150.1 ± 46.02 μm in diameter, being 1.53 times broader than they are long [0.69 ± 0.16 (L/D)], twisted, and infrequently branched. Trichoblasts are delicate, deciduous, scarce in vegetative thalli but abundant in gametophytes, 1–2 times forked, 35.27 ± 11.76 μm long, and arising on each segment near the apical cells. Conspicuous scar cells are unusual. Segments along the thalli are composed of 7-12 pericentral cells (Fig. 34G-J). Older segments have individual rhizoidal cells between pericentral cells and the axial cells covered by a heavy cortication (Fig. 34K-L). Adventitious branches present in basal parts (Fig. 34F)**.** Lateral branches are replacing trichoblasts. Holdfasts are prominent (Fig. 34M), 634.04 ± 303.81 μm in diameter, and composed of tightly clumped rhizoids produced from basal

pericentral or cortical cells. The secondary prostate system is reduced, entangled and produced from apical cells of major lateral axes (Fig. 34N). Segments of the prostrate axes are 139.02 ± 17.29 μm in length and 175.08 ± 59.37 μm in diameter, being 1.26 times broader than they are long [0.86 ± 0.22 (L/D)]. Rhizoids are also ventrally produced from the proximal end of the pericentral cells in prostrate axes (Fig. 34N). They are cut off from the pericentral cells (Fig. 34O), 560.21 ± 262.14 μm in length, and 25.68 ± 6.19 μm in diameter. Rhizoids are unicellular with multilobed terminations (Fig. 34P).

**Reproductive structure:** In female gametophytes (Fig. 35A), erect axes are densely branched in the upper parts. Procarps are positioned in two rows along the abaxial side of the branchlets (Fig. 35B-C), and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 35D). Cystocarps are subapical (Fig. 35E) and globose in form when mature, 221.61 ± 39.76 μm in height and 172.14 ± 28.84 μm in diameter. In male gametophytes (Fig. 35F), spermatangial branchlets are arranged in two rows in a zigzag manner on the abaxial side of the branchlets (Fig. 35G-H), and are developed on a furcation of the trichoblast in each segment of the axes (Fig. 35I). Each spermatangial branch is composed of spermatangia and sometimes a single sterile tip cell. In tetrasporangial plants (Fig. 35J), tetrasporangia are tetrahedral and 37.79 ± 7.99 μm × 39.02 ± 8.64 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 35K-L). The development of tetrasporangia follows a spiral arrangement (Fig. 35L). One of the pericentral cells of the fertile segment is developed to a stalk cell, which will develop the tetrasporangia and the two cover cells (Fig. 35M-N). A single tetrasporangium is produced from a fertile segment (Fig. 35M-N).

**Habitat:** Plants grow from the high to the low intertidal zone, forming extensive tufts. They are found attached to rocks or epizooic on shells of invertebrates in sheltered to wave-exposed areas. Tufts of this species are usually wide, long, and very robust, and are associated with other species such as *Polysiphonia morrowii* and *Neosiphonia harveyi.*

**Remarks:** *Leptosiphonia platensis* is distributed along the Argentinean coast from Mar del Plata to Madryn Port. *L. platensis* is characterized by having main prominent indeterminate erect axes attached by a prominent holdfast or reduced prostrate system, numerous pericentral cells, heavy cortication in the basal parts of thalli, and robust texture. These features are similar with those reported to the Argentinean *L. brodiei* (as *P. brodiei*) by Borazo de Zaixo (2013) and *P. hassleri* by Taylor (1939). *Leptosiphonia platensis* is distinguished from *L. brodiei* by having individual rhizoidal cells, scarce trichoblasts, and cystocarps and spermatangial branchlets arranged in two rows along the abaxial side of the branchlets. Our molecular analyses support the morphological differences between these species (10.2% of sequence divergence for *rbc*L). *Leptosiphonia platensis* is distinguished from *P. hassleri* based on our morphological observations of the isotype UC949884 loaned from the University and Jepson Herbaria, Berkeley, by having more than 5 pericentral cells throughout (Taylor 1939). *Leptosiphonia platensis* is close related to *L. virgata* in our molecular analyses (1.8% sequence divergence), but *L. platensis* is distinguished by having individual rhizoidal cells, cortication only restricted to the basal part and secondary prostrate axes.

***Leptosiphonia virgata* (C. Agardh) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 36-37)**

**Basionym:** *Hutchinsia virgata* C. Agardh

**Homotypic synonyms:** *Hutchinsia virgata* C. Agardh, *Carradoria virgata* (C. Agardh) Kylin (1956), *Carradoriella virgata* (C. Agardh) P.C. Silva (1996), *Polysiphonia virgata* (C. Agardh) Sprengel.

**Heterotypic synonyms:** *Polysiphonia fuliginosa* Rudolphi.

**Type locality:** Cape of Good Hope, South Africa.

**Specimens examined:** CUK14397, CUK14406 (Hout Bay Harbour, Cape Town, Western Cape, South Africa collected by T.O.C. , Apr. 08 2015).

**Vegetative structure:** Plants are prominent, 14-28 cm in height (Fig. 36A-B), and dark red to brown in color. Thalli form large tufts predominantly attached to rock surfaces or epizooic in the intertidal zone. Thalli are composed of main indeterminate erect axes attached by a prominent holdfast (Fig. 36A-B). The main erect axes are robust in texture, remaining distinct and forming few major laterals exogenously. They are densely and radially branched in a pseudodichotomous to alternate pattern every 4-12 axial cells (Fig. 36C-D). Apical cells are prominent, 5.26 ± 0.89 μm × 6.24 ± 0.84 μm in size, and transversely divided (Fig. 36E). Young erect axes are pseudodichotomously disposed, curved in the direction of the main axes, and have short segments. Older segments of erect axes near to holdfast are 1.45 ± 0.63 mm in diameter but after the first branch are 98.14 ± 20.72 μm in length and 166.02 ± 38.46 μm in diameter, being 1.69 times broader than they are long [0.61 ± 0.15 (L/D)], twisted, and infrequently branched. Trichoblasts are delicate, deciduous, scarce, only occurring in fertile plants, 1–2 times forked, 48.74 ± 18.60 μm long, and arising on each segment near the apical cells. Conspicuous scar cells are unusual. Segments along the thalli are composed of 13-15 pericentral cells (Fig. 36G-H) and have 1-3 layers of rhizoidal cells between pericentral and axial cells covered by a dense cortication (Fig. 36I-J). Adventitious branches present in basal parts (Fig. 36F)**.** Lateral branches are replacing trichoblasts. Holdfasts are prominent (Fig. 36L-M), 2.44 ± 0.75 mm in diameter, and composed of tightly clumped rhizoids produced from basal pericentral or cortical cells. Rhizoids are unicellular with multilobed terminations.

**Reproductive structure:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 37A-B). Procarps are positioned in two rows along the abaxial side of the branchlets (Fig. 37C) and subapical (Fig. 37D), and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 37E). Cystocarps are ovoid to ellipsoid in form when mature, 182.23 ± 18.21 μm in height and 214.81 ± 14.15 μm in diameter. In tetrasporangial

plants (Fig. 37F), tetrasporangia are tetrahedral and 35.14 ± 7.38 μm × 35.64 ± 6.08 μm in size. Tetrasporangial branches are swollen (Fig. 37G-H). The development of tetrasporangia follows a straight arrangement (Fig. 37G-H). One of the pericentral cells of the fertile segment is developed to a stalk cell, which will develop the tetrasporangia and the two cover cells (Fig. 37I-J). A single tetrasporangium is produced from a fertile segment (Fig. 37I-J).

**Habitat:** Plants grow from the high to the low intertidal zone, forming long tufts. They are found attached to rocks or epizooic on shells of invertebrates in sheltered to wave-exposed areas. Tufts of this species are usually wide, long, and very robust, and are associated with other species such as *Strebloclad*ia *camptoclada*.

**Remarks:** *Leptosiphonia virgata* was initially described as *Hutchinsia virgata* by C. Agardh (1824) and subsequently it has been assigned to different genera, like *Polysiphonia* Greville (Sprengel 1827, Wynne 1986, Stegenga et al. 1997), *Tayloriella* Kylin (Papenfuss 1940b, Seagrief 1984), *Carradoria* Martius (Kylin 1956) and *Carradoriella* P. Silva (Silva et al. 1996). *Leptosiphonia virgata* is endemic to the Southeastern Atlantic coast (Wynne 1986, Silva et al. 1996, Rull Lluch 2002) and is characterized by having prominent indeterminate erect axes attached by a prominent holdfast, numerous pericentral cells, dense cortication in the basal parts of thalli, and robust texture. *Leptosiphonia virgata* is similar to *L. brodiei* but they are distinguished by the pericentral cells number. *L. virgata* has 12-16 pericentral cells, whereas *L. brodiei* only has 6-8 pericentral cells (Rull Llluch 2002).

## 2. Phylogenetic analyses

A 1,245-bp portion of the 1,467- bp *rbc*L (84.8%) and 1457-bp portion of the 1467-bp *cox1* (99%) were sequenced for *Leptosiphonia brodiei*, *L. elongata*, *L. platensis*, *L. virgata*, and other *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus (Fig.38) placed *Leptosiphonia*

sister to the clade composed by the genus *Neosiphonia* and "*P. schneideri*" (Fig. 38) with sequences divergence of 7.1% to 14.0% between them and supported by a high bootstrap value of 1 and by a posterior probability of 1. On the other hand, phylogenetic analyses of the *cox1* locus (Fig. 39) placed the members of *Leptosiphonia* sister to the clade composed by *P. arctica* and *P. echinata* with sequences divergence of 10.6% to 13.5% between them and with low support in ML (Maximum likelihood) and BI (Bayesian inference). *Leptosiphonia* is also distantly related to the clade composed by *Neosiphonia* and "*P. schneideri* clade" with sequences divergence of 14.2% to 17.7% for *cox*1. The sequence divergences among the members of *Leptosiphonia* are ranging from 1.9% to 12.5% for *rbc*L and 6.8% to 12.6% for *cox1*.

## 3. Discussion

The genus *Leptosiphonia* is characterized by having thallus radially organized, a main indeterminate erect axes attached by a prominent holdfast or a reduced prostrate system, 4-15 pericentral cells, rhizoidal cells in the base and dense cortication, exogenous indeterminate branches, delicate trichoblasts and conspicuous scar cells, unicellular rhizoids cutting off from the proximal end of pericentral cells or cortical cells, procarps with four-celled carpogonial branches, spermatangial branches arising on a furcation of the trichoblasts, and tetrasporangia arranged in straight or spiral series with one or two tetrasporangia per fertile segment. Among the features delineating the enlarged concept of *Leptosiphonia* for the present study are: (1) rhizoidal cells between axial and pericentral cells and (2) cortication (Table 4). *rbc*L and *cox*1 sequence analyses also supported its taxonomic status as a separate genus from *Polysiphonia* sensu lato.

The genus *Leptosiphonia* was described by Kylin (1956), based on *P. schousboei* which has two tetrasporangia per segment arranged in two longitudinal rows as diagnostic feature. This feature has been often reported in the tribes Amansieae, Lophothalieae, Rhodomeleae, and Pterosiphonieae, but rare in the tribe Polysiphoniae (Díaz-Tapia and Bárbara 2013). Among the Polysiphoniae, this

feature is not exclusive of *Leptosiphonia* and it has been reported in other genera: *Perrinia* Womersley and *Neosiphonia* (Womersley 2003, Bustamante et al. 2012). Although the consistency of this character to delimit genera in *Polysiphonia* sensu lato was recognized by Kylin (1956) and Díaz-Tapia and Bárbara (2013), Bustamante et al. (2012) demonstrated the consistency of this feature to delimit species in some genera as *Neosiphonia* (e.g. *N. peruviensis* D.E. Bustamante. B.Y. Won et T.O. Cho) and *Diplocladia* (e.g. *D. ericoides* (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho, see Chapter 9).

Only a few species in *Polysiphonia* sensu lato have been reported with rhizoidal tissue (Maggs and Hommersand 1993, Rull Lluch 2002), even though this feature has been reported extensively in some tribes of Rhodomelaceae (Chondriae, Heterocladiae, and Laurenciae) (Womersley 2003). Although the consistency of this character has not been evaluated before in *Polysiphonia* sensu lato, our morphological observations demonstrate that rhizoidal cells are restricted to the genus *Leptosiphonia*. The rhizoidal cells in *Leptosiphonia* are originated endogenously in the basal parts of the axes between the pericentral cells and axial cells. These rhizoidal cells may form 1-2 layers of thickness as in *Leptosiphonia brodiei* and *L. virgata*, or they may be individual rhizoidal cells pit connected with the pericentral and axial cells as in *L. elongata* and *L. platensis*.

The cortication was considered as a consistent character by Stuercke and Freshwater (2008) and confirmed with the description of the genera *Lampisiphonia* and *Hapterosiphonia* (Bárbara et al. 2013, Bustamante et al. 2015c). These studies concluded that species attached by prostrate systems lacked cortication, whereas species attached by a single basal holdfast had cortication. Although some species in *Leptosiphonia* developed a reduced or secondary prostrate system, the whole plant is attached by a corticated holdfast. The cortication in *Leptosiphonia* is originated by having an initial cortical cell outwardly and distally pit connected with its own pericentral cell, then this initial cortical cell is extended and forms primary and secondary pit connections with other cortical cells (Womersley 1979). This cortication pattern has been observed in the genus *Neosiphonia* and some multipericentral members (e.g. *P. fucoides*). The cortication in *Leptosiphonia* is several cells

thick in the base and distributed throughout the axes (e.g. *L. elongata*) or ecorticated near the apex (e.g. *L. brodiei, L. platensis,* and *L. virgata*) (Segi 1951, Womersley 1979). Although cortication and rhizoidal cells are likely found in other groups of *Polysiphonia* sensu lato, the combination of these features with unicellular rhizoids and four-celled carpogonial branches further strengthens the support of *Leptosiphonia* as a different taxon and our molecular analyses also confirm its status.

The molecular analysis of Choi et al. (2001) concluded that the endemic South African species *C. virgata* assigned to the monotypic genus *Carradoriella* might be resolved as sister genus of *Neosiphonia* or that *Neosiphonia* might be subsumed into *Carradoriella*. The genus *Carradoriella* (as *Carradoria* Kylin) based on *C. virgata* was proposed by Silva et al. (1996) as a "nomen novum", whereas *Leptosiphonia* Kylin, based on O*phidocladus schousboei* was segregated by Kylin (1956) as a different genus. Our phylogenetic analyses resolved *L. virgata* comb. nov. and *L. schousboei* in the same clade and based on the priority of *Leptosiphonia* over *Carradoriella.* The latter genus is placed under synonymy with *Leptosiphonia*. Our phylogenetic analyses (Fig. 38-39) also reveal that *Leptosiphonia* is a closely related genus with a high support in our *rbc*L (100% for ML and 1.0 for BPP) and *cox*1 (1.0 for BPP) analyses to the clade composed by the genus *Neosiphonia* and the clade composed by *P. amplacapilli* B.Kim et M.S.Kim, *P. morroides* B.Kim et M.S.Kim, *P. pentamera* Hollenberg, and *P. schneideri* B.Stuercke et D.W.Freshwater (labeled as *P. schneideri* clade in Fig. 19). Although *Leptosiphonia* is sister to these groups, the genus *Neosiphonia* is distinguished from *Leptosiphonia* by having a three-celled carpogonial branch (Kim and Lee 1999); whereas *P. schneideri* clade is distinguished by having ecorticated axes (Stuercke and Freshwater 2010, Kim and Kim 2014).

This study enlarges the present circumscription of the genus *Leptosiphonia* with the rhizoidal cells between axial and pericentral cells and the cortication as diagnostic generic level features to include the following new combinations: *L. brodiei, L. elongata,* and *L. virgata,* and one new species, namely, *Leptosiphonia platensis*. Masuda (1982) noticed that the taxonomy in Rhodomelaceae is ultimately based on the morphology of vegetative features, because of the close uniformity in the

development of reproductive organs and the postfertilization process. Our study attempted to resolve some of the heterogeneity in *Polysiphonia* sensu lato by emending the genus *Leptosiphonia* on the basis of consistent vegetative features: the rhizoidal cells between axial and pericentral cells and cortication. The *rbc*L and c*ox1* phylogenies reveal that *Leptosiphonia brodiei*, *L. elongata*, *L. platensis*, and *L. virgata* are in a strong and well supported clade (100% for ML, 1.0 for BPP). They were originally distributed along the western and eastern Atlantic coast, except by the recent introduction to the Pacific Ocean of *L. brodiei*.

**Fig. 30.** Distribution of the species of the enlarged genus *Leptosiphonia* around the Pacific and Atlantic Ocean. *Leptosiphonia brodiei* comb. nov. (blue), *Leptosiphonia elongata* comb. nov. (purple), *Leptosiphonia platensis* sp. nov. (sky blue), *Leptosiphonia virgata* comb. nov. (yellow).

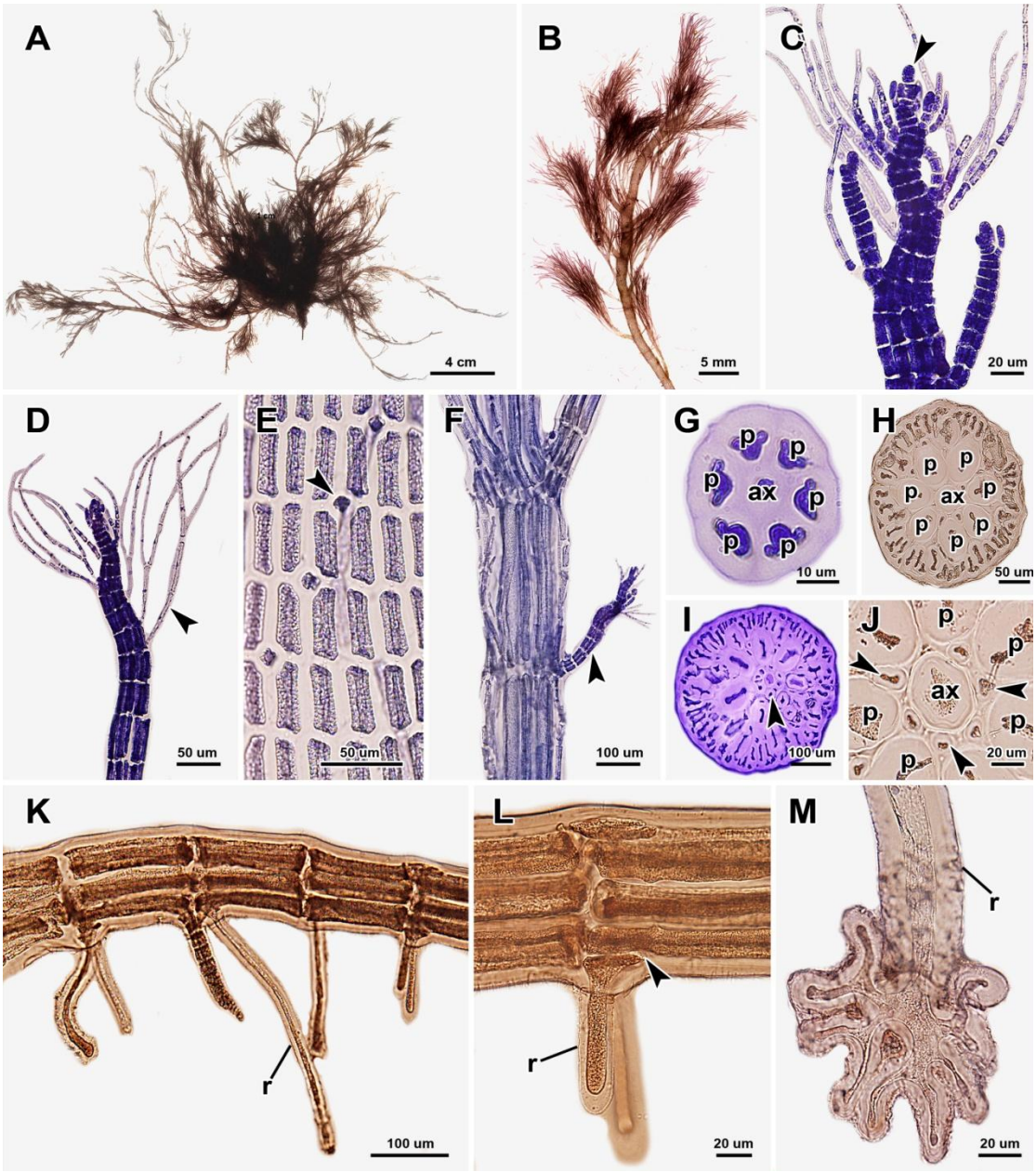


**Fig. 31.** Vegetative structures of *Leptosiphonia brodiei* comb. nov. (A) Habit of vegetative thallus. (B) Apex densely branched forming tufts in a spiral pattern. (C) Apex showing alternate branching pattern and prominent apical cells (arrowhead). (D) Apex showing abundant trichoblasts (arrowhead). (E) Scar cells (arrowhead) spirally disposed. (F) Cicatrigenous branches on basal erect axes (arrowhead). (G-J) Cross section views with 6 (G) and 7 (H) pericentral cells (p) showing dense cortication and rhizoidal cells (arrowhead). (K) Rhizoids (r) scattered and produced from prostrate axes. (L) Rhizoid (r) cut off from the proximal end of pericentral cell. (M) Unicellular terminations of rhizoid (r).

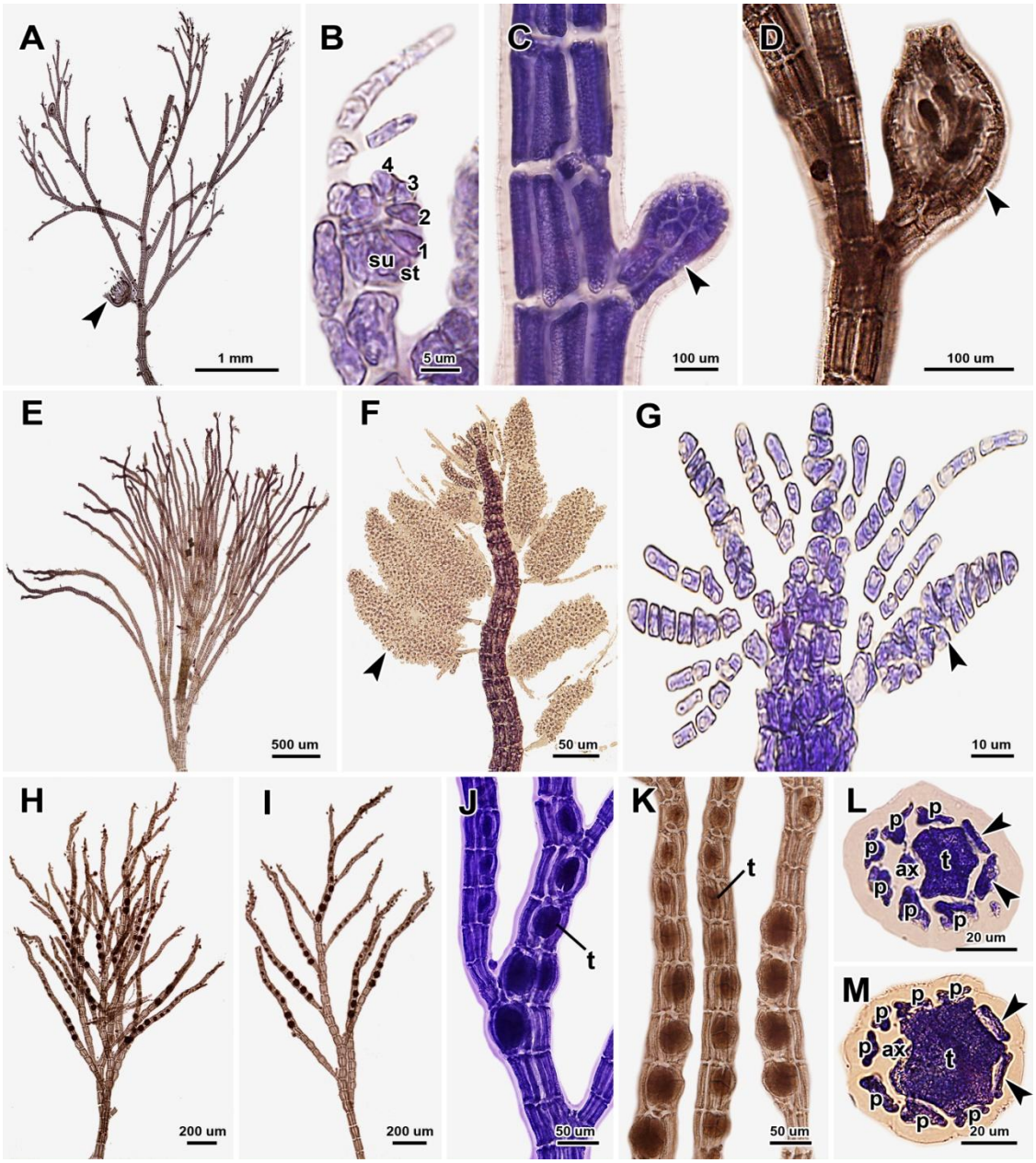


**Fig. 32.** Reproductive structures *Leptosiphonia brodiei* comb. nov. (A) Female thallus. (B) Procarp with four-celled carpogonial branch. (C–D) Young (C) and mature (D) cystocarps (arrowheads) showing urceolate shape. (E) Male thallus. (F) Spermatangial branches (arrowheads) clustered at apical region. (G) Mature spermatangial branch (arrowhead) arising from trichoblast (tb). (H) Tetrasporangial thallus. (I) Apical branches with tetrasporangia (t). (J–K) Branches of tetrasporangial thallus showing straight arrangement of tetrasporangia (t). (L-M) Cross-section with seven (L) and eight (M) pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

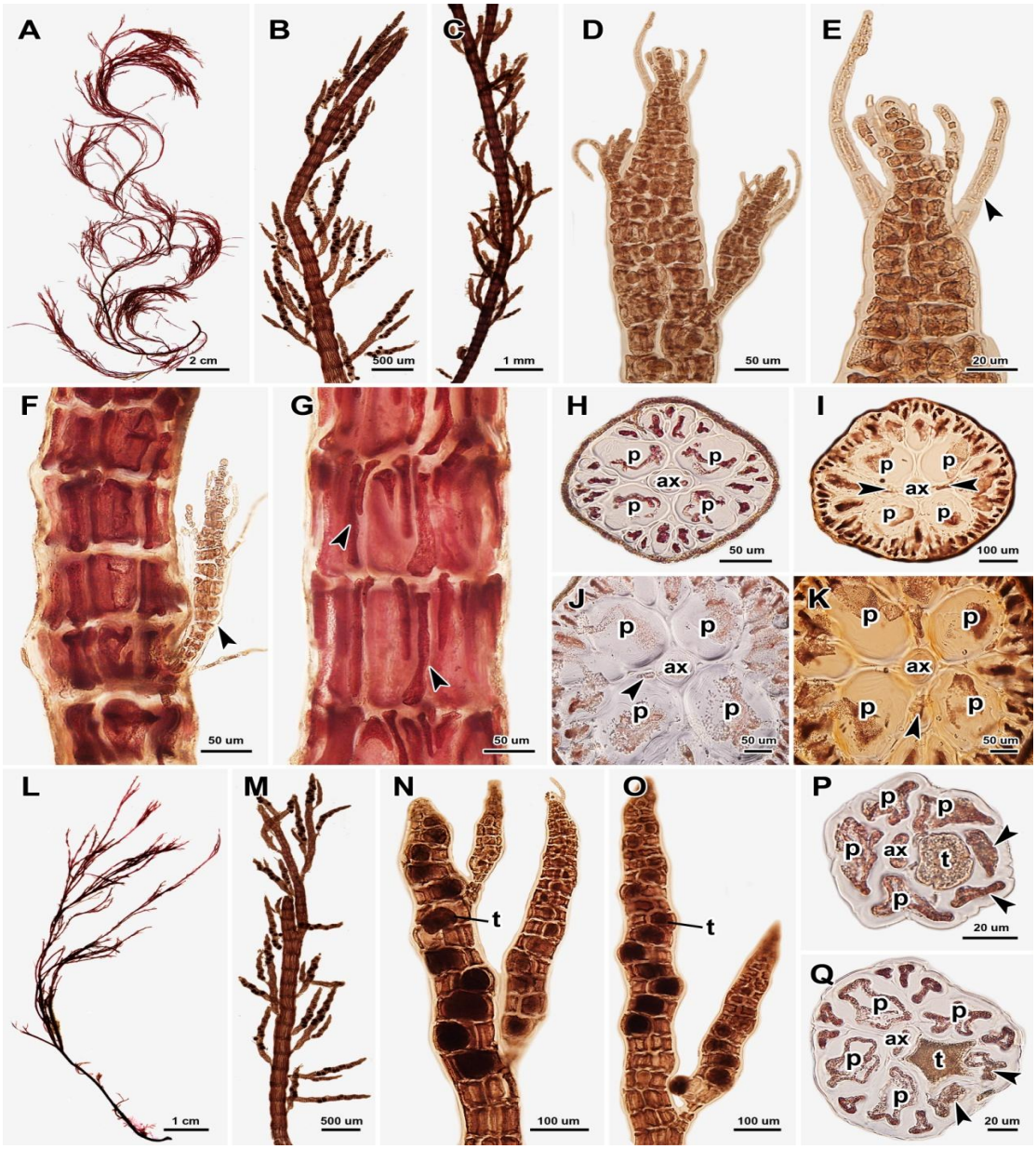


**Fig. 33.** Vegetative and reproductive structures of *Leptosiphonia elongata* comb. nov. (A) Habit of thallus. (B-C) Upper (B) and basal (C) part of the main erect axes. (D) Apices showing adventitious branches. (E) Apex showing scarce trichoblasts (arrowhead). (F) Erect axes showing cicatrigenous branches (arrowhead). (G) Erect axes showing cortication. (H-K) Cross section views of axes with 4 pericentral cells (p) from middle (H) and basal parts (I) showing cortication and rhizoidal cells (arrowhead) [ax, axial cell]. (L) Tetrasporangial thallus. (M) Tetrasporangia developed on adventitious laterals. (N-O) Apex showing spiral arrangements of tetrasporangia (t). (P–Q) Cross-section views of tetrasporangial segments with 5 pericentral cells (p) showing a single tetrasporangium (t) topped by two cover cells (arrowheads).

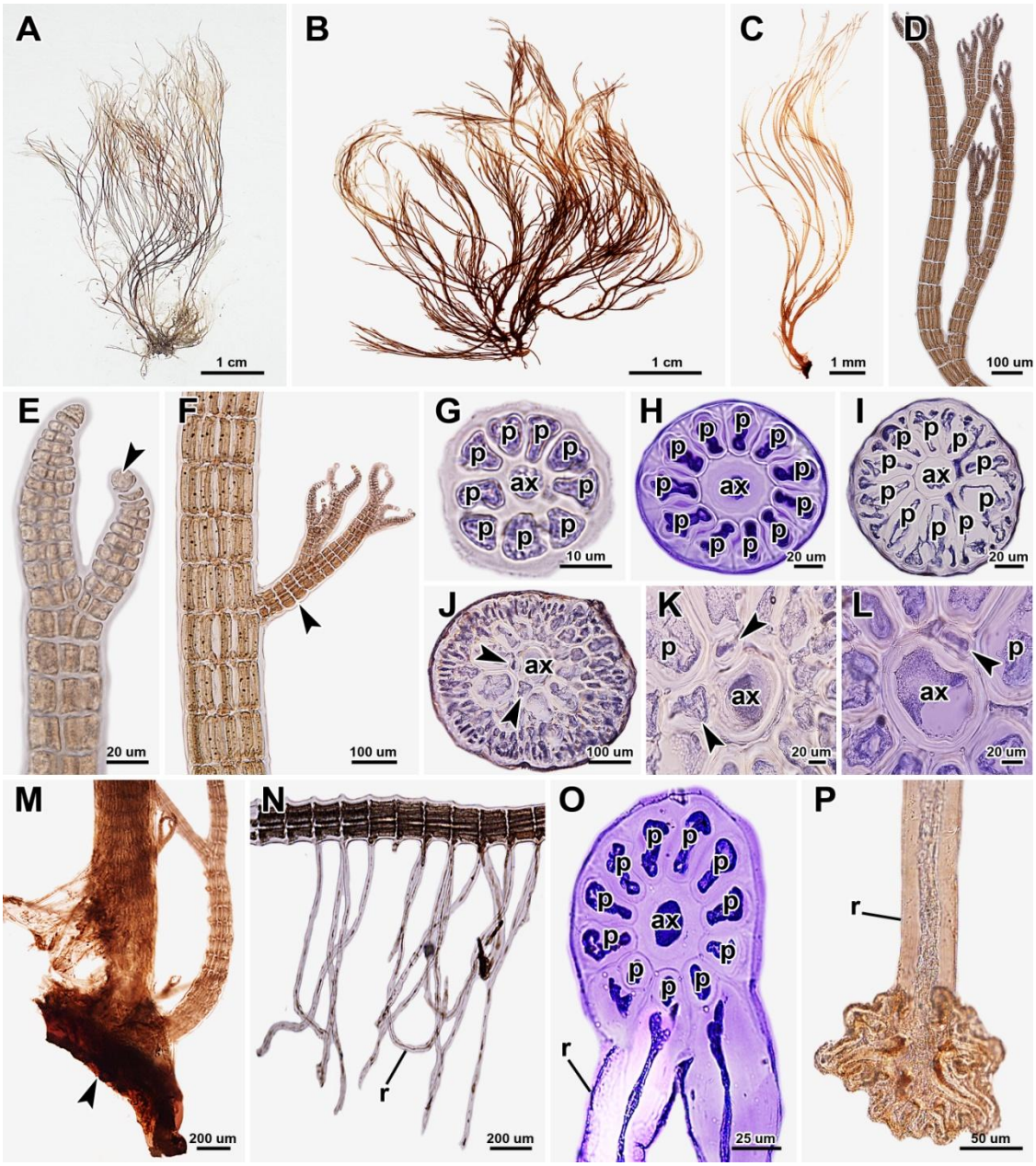


**Fig. 34.** Vegetative structures of *Leptosiphonia platensis* sp. nov. (A) Holotype specimen from Argentina. (B-C) Habit of vegetative plant showing the holdfast and extended erect axes. (D) Erect axes showing pseudodichotomous branching pattern. (E) Apices showing transversely (arrowhead) divided apical cells. (F) Erect axes showing adventitious branches (arrowhead). (G-L) Cross-section of axes with 9 (G) and 10 (H) pericentral cells (p) showing dense cortication and rhizoidal cells (arrowhead) (ax, axial cell). (M) Holdfast (arrowhead). (N) Rhizoids (r) scattered and produced from the secondary prostrate axes. (O) Cross-section of a prostrate axis showing rhizoids (r) cutting off pericentral cells (p). (P) Unicellular terminations of rhizoids (r).

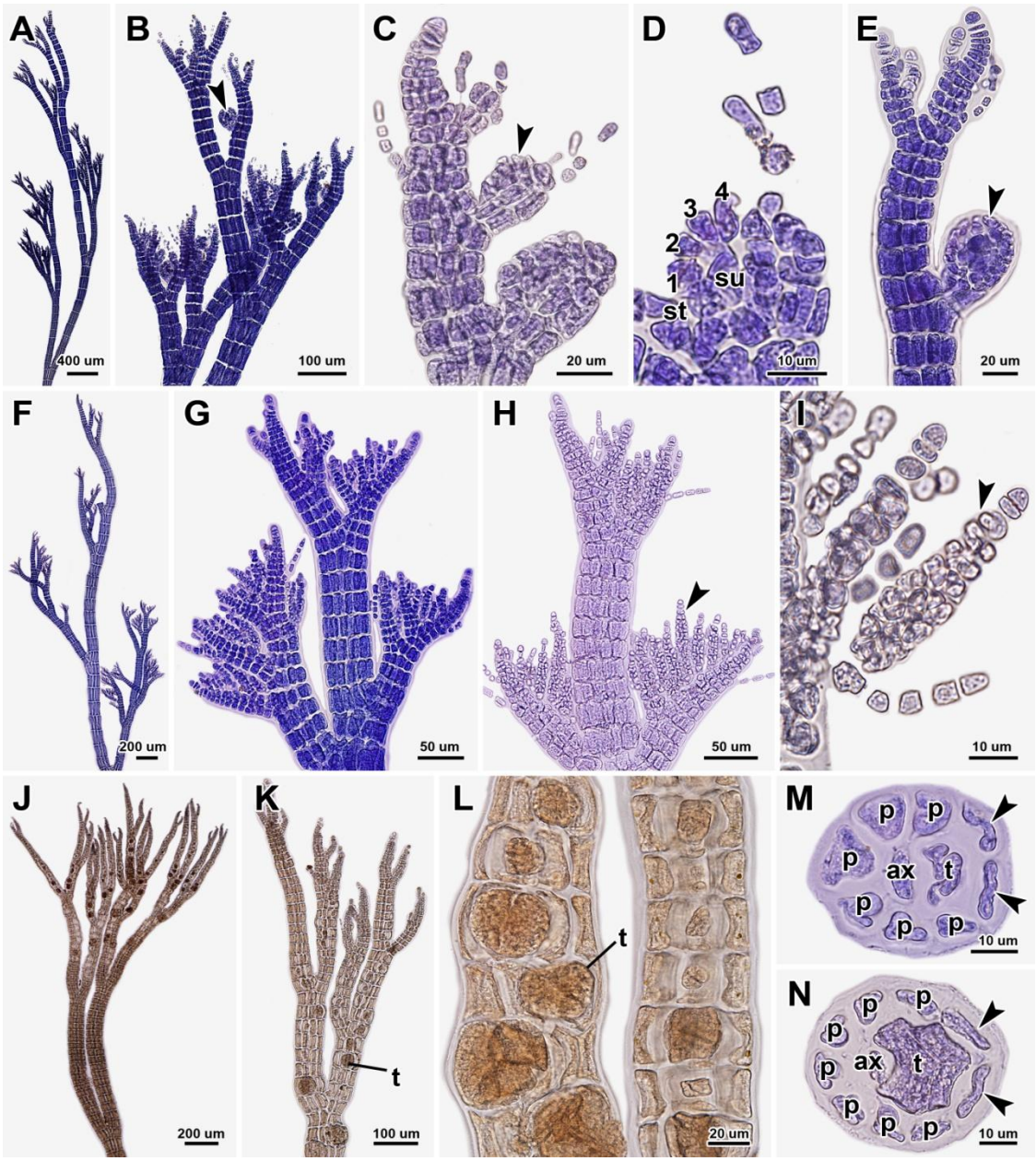


**Fig. 35.** Reproductive structures of *Leptosiphonia platensis* sp. nov. from Argentina. (A) Female thallus. (B-C) Upper part of female thallus showing subapical cystocarps (arrowhead). (D) Procarp with four-celled carpogonial branch. (E) Young cystocarps (arrowhead). (F) Male thallus. (G-H) Spermatangial branches (arrowheads) clustered at apical region. (I) Mature spermatangial branch (arrowhead) arising from trichoblast (tb). (J) Tetrasporangial thallus. (K) Apical branches with tetrasporangia (t). (L) Branches of tetrasporangial thallus showing straight and spiral arrangements of tetrasporangia (t). (M–N) Cross-section views of tetrasporangial segments with 7 (M) and 8 (N) pericentral cells (p) showing a single tetrasporangium (t) topped by two cover cells (arrowheads).

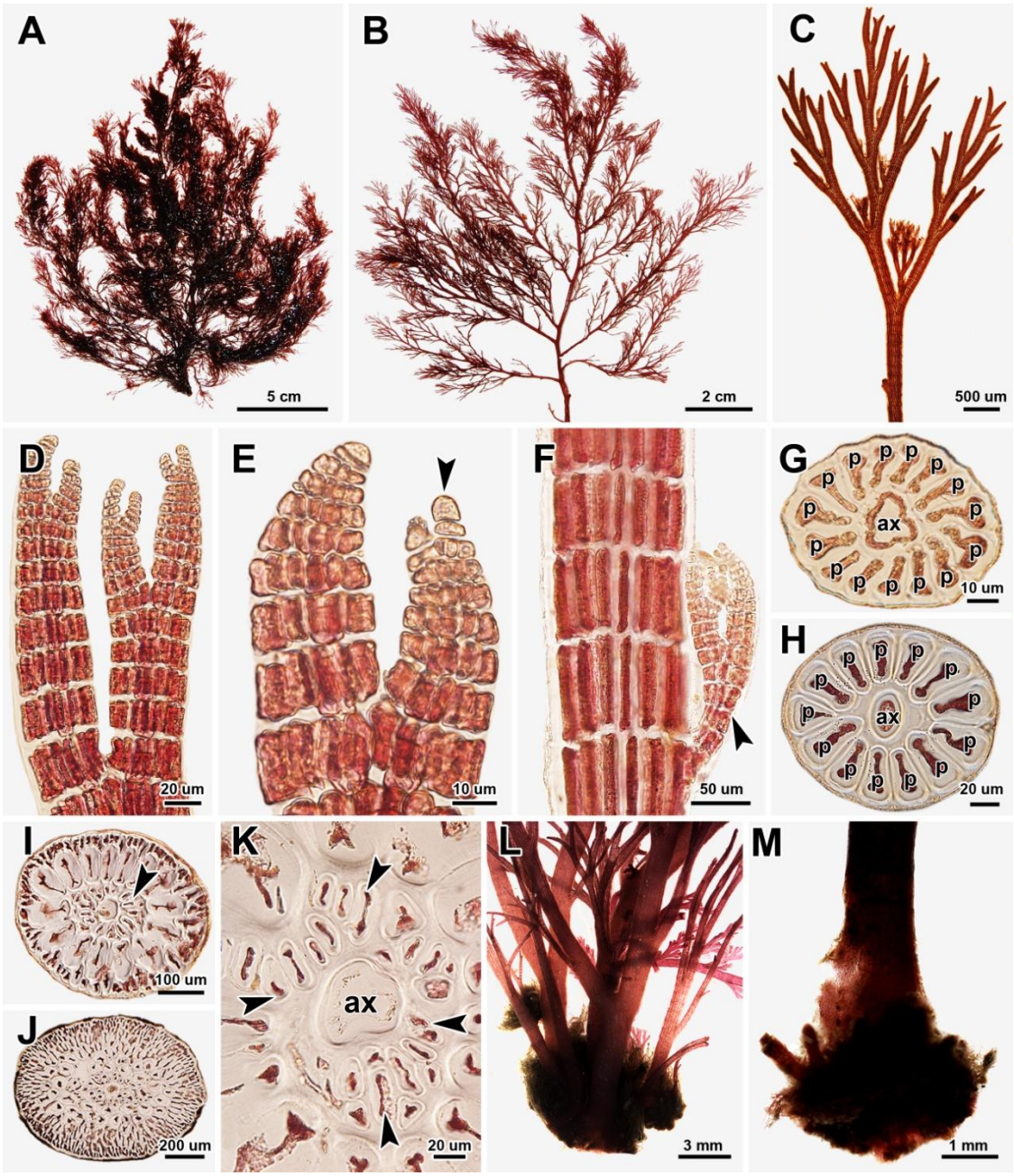


**Fig. 36.** Vegetative structures of *Leptosiphonia virgata* comb. nov. (A-B) Habit of vegetative plant showing the holdfast and extended erect axes. (C-D) Erect axes (C) and apex (D) showing pseudo-dichotomous branching pattern. (E) Apices showing transversely divided apical cells (arrowhead). (F) Erect axes showing adventitious branches (arrowhead). (G-K) Cross-section of axes with 15 (G) and 14 (H) pericentral cells (p) showing dense cortication and rhizoidal cells (arrowhead) (ax, axial cell). (L-M) Prominent holdfast with clumped rhizoids.

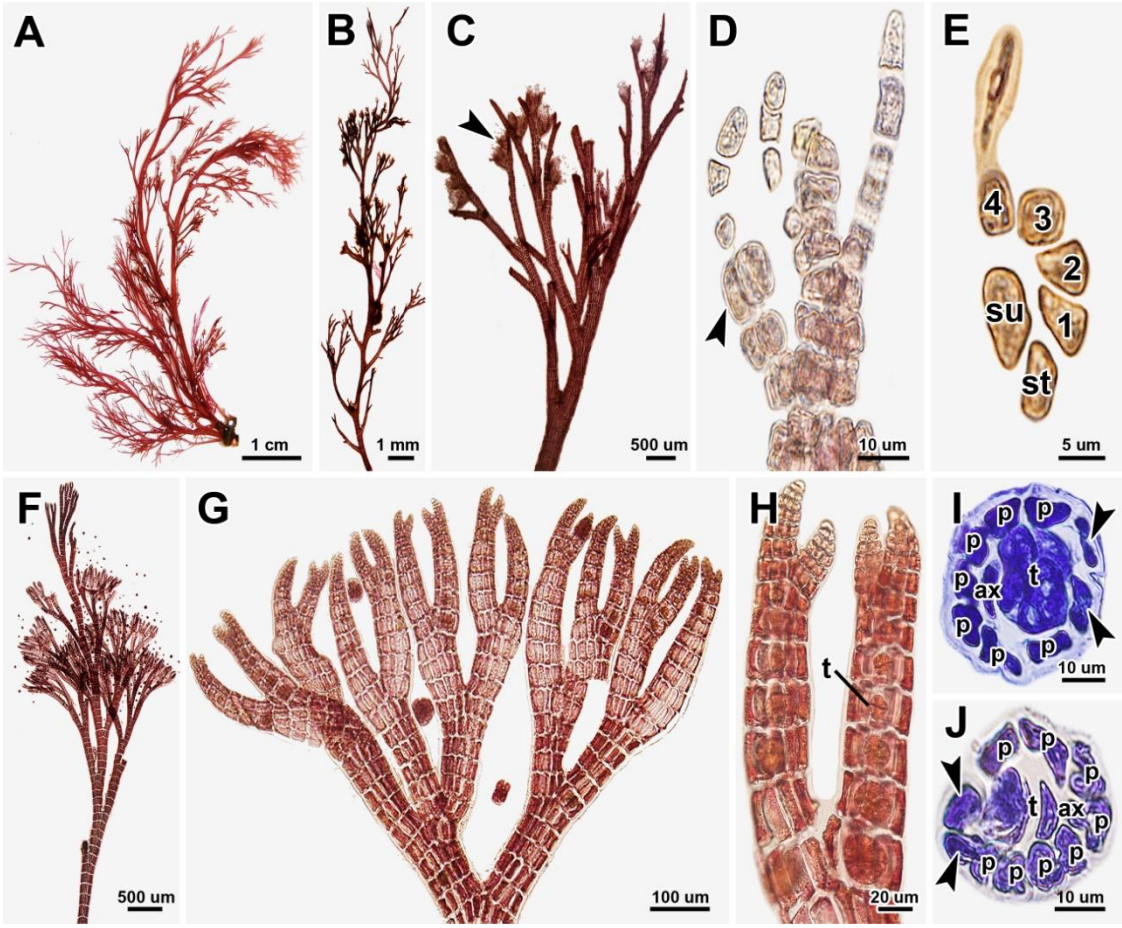


**Fig. 37.** Reproductive structures of *Leptosiphonia virgata* comb. nov. (A-B) Female thallus. (C) Upper part of female thallus showing subapical cystocarps (arrowhead). (D) Young procarp (arrowhead). (E) Procarp with four-celled carpogonial branch. (F) Tetrasporangial thallus. (G) Apical branches with tetrasporangia. (H) Branches of tetrasporangial thallus showing straight arrangements of tetrasporangia (t). (I–J) Cross-section views of tetrasporangial segments with 8 (I) and 9 (J) pericentral cells (p) showing a single tetrasporangium (t) topped by two cover cells (arrowheads).

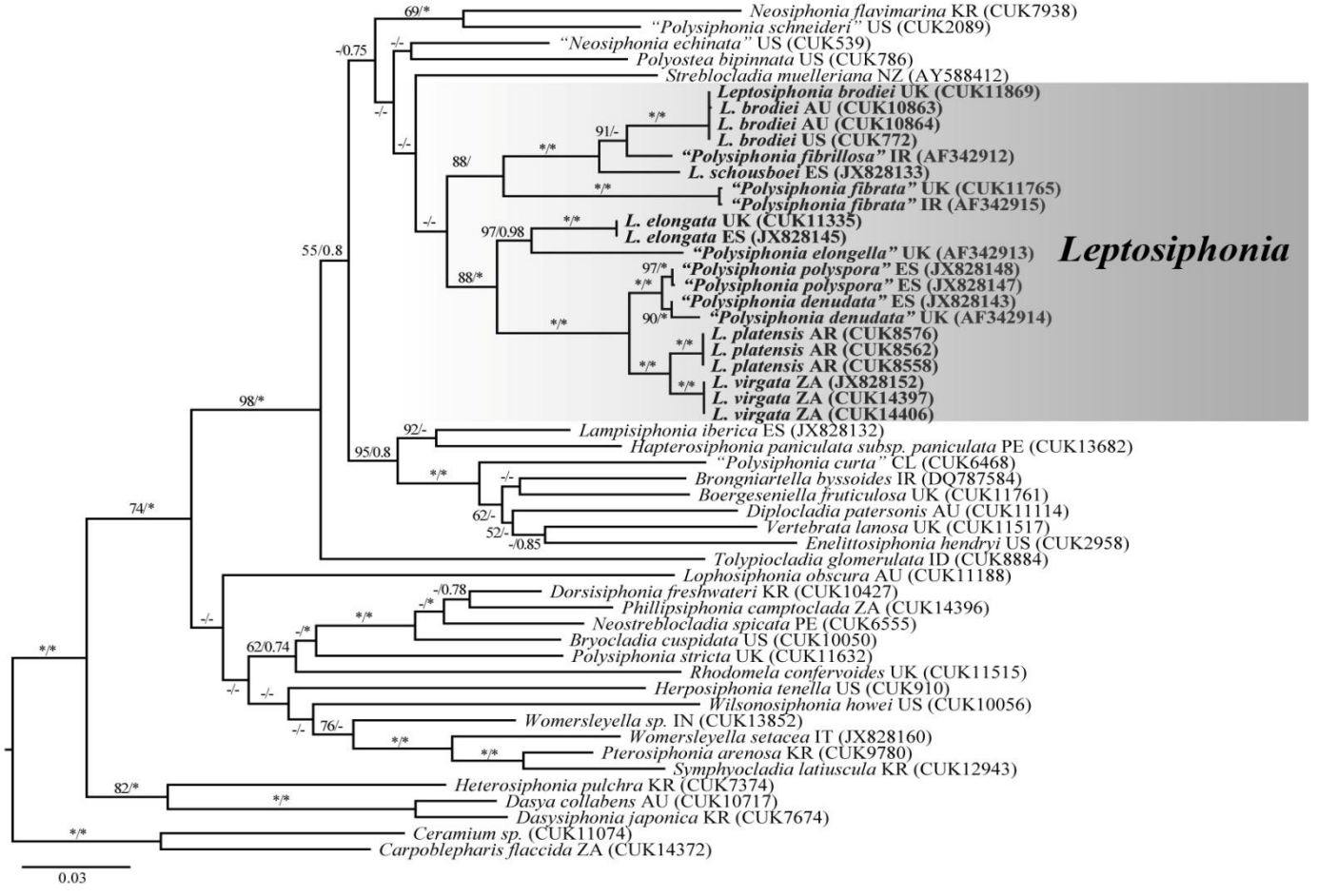


**Fig. 38.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

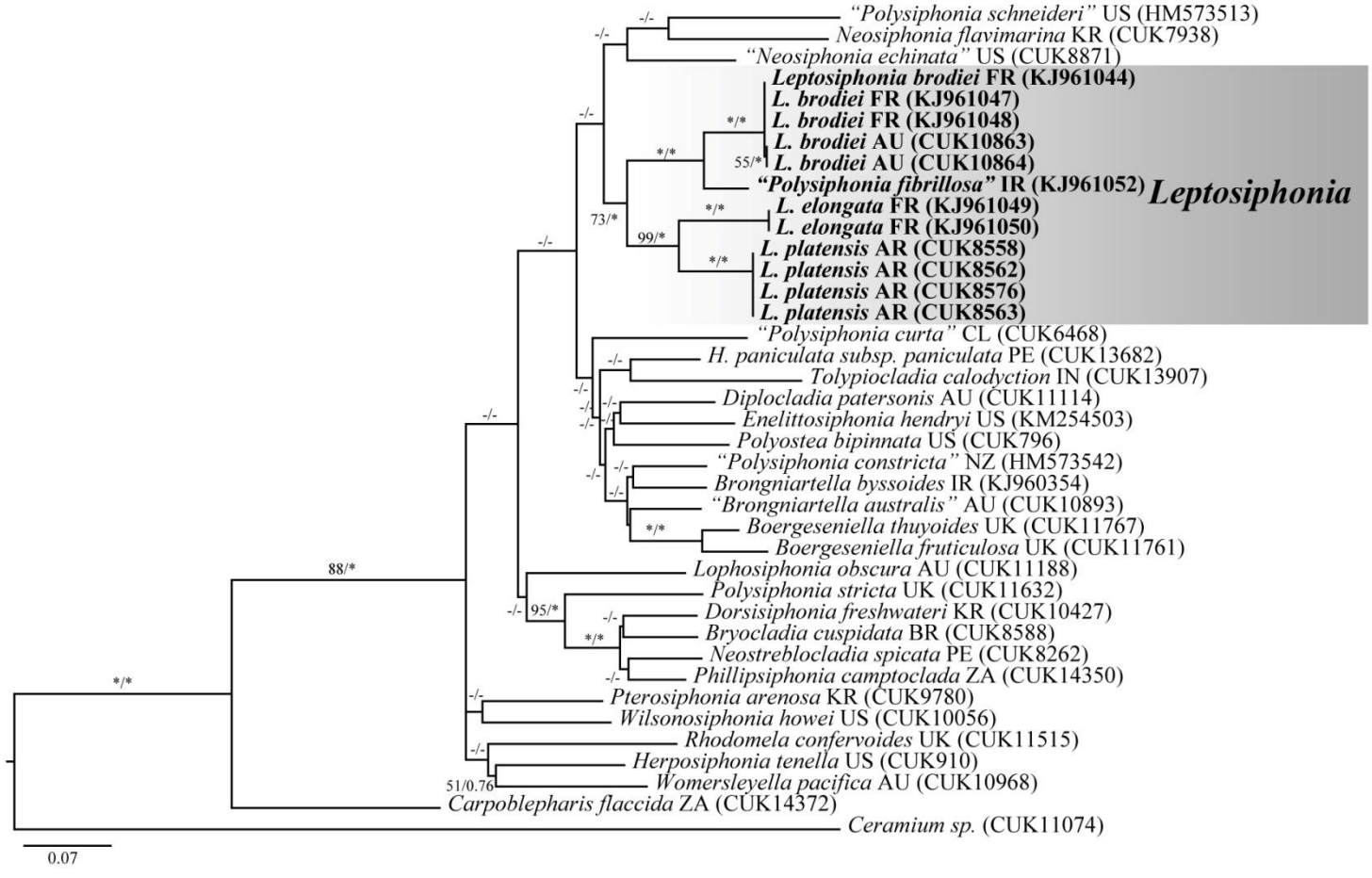


**Fig. 39.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 4.** Comparisons among genera belonging to *Polysiphonia* sensu lato and *Leptosiphonia*.

| Features | *Leptosiphonia* | *Boergeseniella* | *Bryocladia* | *Enelittosiphonia* | *Hapterosiphonia* | *Lampisiphonia* | *Neosiphonia* | *Polysiphonia* | *Streblocladia* | *Vertebrata* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Generitype** | *L. schousboei* | *Bo. fruticulosa* | *Br. cervicornis* | *E. stimpsonii* | *H. paniculata* | *La. iberica* | *N. flavimarina* | *P. stricta* | *S. neglecta* | *V. lanosa* |
| **Type localities** | Tanger, Morocco | British Isles | Java | Japan | Lima, Peru | Ría de A Coruña, Spain | Bangpo, Korea | Glamorgan, British Isles | South Pacific | Iceland |
| **Pericentral cells number** | 4–13 | 8–12 | 6–12 | 7–11 | 8–12 | 9–11 | 4–9 | 4 | 4–8 | 17-23 |
| **Branching pattern** | Alternate to pseudodichotomous | alternate-distichous | determinate spiral laterals | dorsiventral | paniculate | pseudodichotomous | alternate | pseudodichotomous to alternate | dorsiventral secondary branches | pseudodichotomously |
| **Rhizoidal cells** | present | absent | absent | Absent | absent | absent | absent | absent | absent | absent |
| **Cortication** | present | present | absent | Absent | absent | present | absent or present | absent | absent or present | absent |
| **Trichoblasts** | scarce | scarce | scarce | abundant | abundant | absent | abundant | scarce to abundant | scarce | absent |
| **Rhizoids connection** | cut off | cut off | cut off | cut off | cut off | cut off | cut off | open | - | cut off |
| **Rhizoids** | unicellular | unicellular | unicellular | unicellular | multicellular | multicellular | unicellular | unicellular | unicellular | unicellular |
| **Carpogonial branches** | four-celled | four-celled | four-celled | four-celled | four-celled | - | three-celled | four-celled | - | four-celled |
| **Spermatangia branches** | arising as primary branch or replacing | arising as primary branch or replacing | - | arising as primary branch | arising as primary branch | - | arising as primary branch | arising as primary branch or replacing | arising as primary branch or replacing | replacing trichoblast |
| **Tetrasporangia arrangement** | spiral or straight | spiral | straight | spiral | spiral or straight | straight | spiral or straight | spiral or straight | straight | straight |
| **References** | Díaz-Tapia et al. (2014), this study | Maggs and Hommersand (1993) | Schmitz and Falkenberg (1897) | Segi (1949) | Bustamante et al. (2015c) | Bárbara et al. (2013) | Kim and Lee (1999) | Kim et al. (2000) | Schmitz and Falkenberg (1897) | Kim et al. (2002) |

## CHAPTER 3. Taxonomy and phylogeny of the genus *Neosiphonia* (Rhodomelaceae, Rhodophyta)

The genus *Neosiphonia* Kim et Lee was segregated by Kim and Lee (1999) from *Polysiphonia* on the basis of the following morphological characters: the lateral branch initials, including the trichoblast initials, are produced on successive segments, erect indeterminate branches are developed from the main axes, rhizoids are separated from pericentral cells by a cross wall, trichoblasts are abundant, procarps bear a three-celled carpogonial branch, spermatangial branches arise from a branch of the trichoblasts, and tetrasporangia are arranged in spiral series (Kim and Lee 1999). Genetically, this genus was confirmed by Choi et al. (2001) based on small-subunit ribosomal DNA (SSU rDNA) and later by Mamoozadeh and Freshwater (2011) based on plastid-encoded *rbc*L.

Currently, the genus *Neosiphonia* has been widely recognized by Bustamante et al. (2012, 2013a, 2013b), Mamoozadeh and Freshwater (2012), Díaz-Tapia and Bárbara (2013), and Muangmai et al. (2014) with the proposal of new species and new combinations. Although the genus *Neosiphonia* is well segregated in the phylogenetic analyses of these studies, the features reported by Kim and Lee (1999) to delimit *Neosiphonia* are not consistent, except by the three cells in the carpogonial branches. *Neosiphonia* is the only genus having this character in *Polysiphonia* sensu lato.

In the present study, we collected polysiphonous specimens with three-celled carpogonial branches, which have been previously reported as *Polysiphonia* members. These species were collected from worldwide and some in the vicinity of their type localities and their classification is critically reassessed based on detailed morphological study and phylogenetic analyses of their *rbc*L sequences. We here characterized 22 species in *Neosiphonia*, being 9 new species and 9 new combinations.

## 1. Morphological analyses

***Neosiphonia* M.S. Kim et I.K. Lee**

**Holotype species:** *Neosiphonia flavimarina* M.S. Kim et I.K. Lee.

**Key to species of *Neosiphonia***

1. Habit composed of clumped rhizoids forming a holdfast-like structure and well-developed erect axes ..... 2
1. Habit composed by reduced or extended prostrate and erect axes ..... 7
    2. Axes having basal cortication ..... 3
    2. Axes ecorticated throughout ..... 4
3. Pericentral cells 7-8 ..... ***N. notoensis***
3. Pericentral cells 4 ..... ***N. strictissima***
    4. Tetrasporangia arranged in straight series..... ***N. sudafricana***
    4. Tetrasporangia arranged in spiral series ..... 5
5. Cicatrigenous branches spirally disposed and spermatangia branches developed on furcation of trichoblasts ..... ***N. apiculata***
5. Adventitious branches in basal parts and spermatangia branches replacing trichoblasts ..... 6
    6. Branches arranged in dichotomous pattern ..... ***N. gorgoniae***
    6. Branches arranged in subdichotomous pattern ..... ***N. pulvinata f. parvula***
7. Tetrasporangia arranged in straight series ..... 8
7. Tetrasporangia arranged in spiral series ..... 11
    8. Spermatangial branches developed on furcation of trichoblasts and always 4 pericentral cells ..... 9
    8. Spermatangial branches replacing trichoblasts and having 4-5 pericentral cells ..... 10
9. Branches arranged in pseudodichotomous pattern and abundant trichoblasts .... ***N. ecuatoriana***
9. Branches arranged in alternate pattern and scarce trichoblasts ..... ***N. tuticoriensis***

10. Lacking of adventitious and cicatrigenous branches and 5 pericentral cells throughout ..... ***N. baliana***

10. Adventitious branches and 4-5 pericentral cells ..... ***N. indonesiana***

11. Abundant trichoblasts spirally disposed ..... 12

11. Scarce trichoblasts ..... 17

12. Branches arranged in pseudodichotomous or dichotomous pattern ..... 13

12. Branches arranged in alternate pattern ..... 14

13. Branches arranged in pseudodichotomous pattern ..... ***N. coacta***

13. Branches arranged in dichotomous pattern ..... ***N. pseudovillum***

14. Robust axes with very short segments (L:D) and abruptly taper at the apices .....***N. sungminbooi***

14. Slender axes with longer axes (L:D) ..... ***N. yendoi***

15. Branches arranged in alternate pattern ..... 16

15. Branches arranged in pseudodichotomous or subdichotomous pattern ..... 19

16. Axes lacking of adventitious branches ..... 17

16. Axes with adventitious branches ..... 18

17. Spermatangial branches developing on furcation, apex with individual alternate axes ..... ***N. blandii***

17. Spermatangial branches developing on furcation and replacing trichoblasts, apex with corymbose disposition of branches, fertile segments in tetrasporophyte with four pericentral cells ..... ***N. mancorensis***

18. Prominent apical cells dome shaped and long series of tetrasporangia ..... ***N. infestans***

18. Exceedingly prominent apical cells ball shaped and 1 or two tetrasporangia per each segment ..... ***N. silvae***

19. Branches arranged in subdichotomous pattern ..... 20

19. Branches arranged in pseudodichotomous pattern ..... 21

20. Axes with cicatrigenous branches developed from scar cells ..... ***N. cockeri***

20. Axes with adventitious..... ***N. unguiformis***

21. Pericentral cells six ..... ***N. peruviensis***

21. Pericentral cells four ..... ***N. ramireziae***

***Neosiphonia apiculata*** **(Hollenberg) Masuda et Kogame (Fig. 40-41).**

**Basonym:** *Polysiphonia apiculata* Hollenberg.

**Type locality:** O'ahu, Hawaii.

**Distribution**: Pacific Islands, South-east Asia, Australia.

**Specimens examined:** CUK9066-CUK9067 (Inna beach, Sanur, Bali, Indonesia, collected by T.O.C. and D.E.B., Oct. 21 2012)**.**

**Vegetative morphology:** Plants are minute 0.2-1.1 cm high (Fig. 40A), purplish in color to colorless, and associated with other filamentous species. They form very small, entangled, and delicate tufts that are predominantly epiphyte on blades surfaces of plants. Thalli are composed of main indeterminate erect axes attached by clumped rhizoids forming a holdfast-like structure (Fig.40B). Erect axes are ecorticate throughout. They are densely and radially branched in an alternate to pseudodichotomous pattern at interval of 3-8 axial cells (Fig. 40C). Apical cells are prominent, dome shaped, 5.02 ± 0.87 μm × 5.19 ± 0.87 μm in size, and transversely divided. Young erect axes have short segments (Fig. 40C) and are having abruptly acute to apiculate branch apices. Older segments of erect axes are larger, normally 67.47 ± 10.29 μm in length and 84.65 ± 5.56 μm in diameter (L:D 0.80 ± 0.13) (Fig. 40D), and infrequently branched (Fig. 40B). Trichoblasts are delicate, deciduous, scarce, 1–4 times forked, 143.74 ± 20.35 μm in length, and arising on each segment near the apical cells. Conspicuous scar cells are 7.55 ± 1.52 μm x 8.06 ± 1.06 μm in size. Cicatrigenous branches are abundant and produced spirally. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 40E-F). Lateral branches re-

place trichoblasts. Rhizoids are clumped forming a holdfast-like structure on the basal part and radially produced around axes (Fig. 40G-H). Rhizoids are cutting off from pericentral cells, 82.67 ± 43.61 μm in length, and 19.85 ± 3.84 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 41A). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 41B). Cystocarps are irregularly disposed and globose when mature (Fig. 41C), 153.14 ± 25.92 μm in height and 191.63 ± 40.43 μm in diameter. In male gametophytes (Fig. 41D), spermatangial branches are clustered at the apices of erect axes (Fig. 41E), and develop on a trichoblast furcation in each axial segment (Fig. 41F). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 41G), tetrasporangia are tetrahedral and 49.80 ± 4.03 μm × 45.98 ± 2.80 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 41G–H). The development of tetrasporangia follows a spiral arrangement (Fig. 41H). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 41I). A single tetrasporangium is produced on each fertile segment (Fig. 41I).

**Habitat:** Plants grow forming small tufts from the intertidal zone. They are found attached to blades surfaces of *Zosteras sp.* in sheltered areas. Tufts are usually delicate and associated with other species such as *Ceramium sp*.

**Remarks:** *Neosiphonia apiculata* was originally described as *Polysiphonia apiculata* by Hollenberg (1968) and then transferred to *Neosiphonia* by Tani et al. (2003). The diagnostic character of this species is the abruptly acute to apiculate branch apices. This character was observed in materials from Hawai (Hollenberg 1968), Malaysia (Tani et al. 2003), and also in our samples from Indonesia. *N. apiculata* was transferred to *Neosiphonia* on the basis rhizoids cutting off from the

pericentral cells, production of lateral-branch initials from successive segments in a spiral arrangement, three-celled carpogonial branches, spermatangial trichoblasts with a sterile lateral, and spiraled tetrasporangia (Kim and Lee 1999, Tani et al. 2003).

***Neosiphonia baliana* D.E. Bustamante, B.Y. Won et T.O. Cho (Fig. 42-43).**

**Type locality:** Blue Lagoon beach, Padang Bai, Karangasem, Bali, Indonesia.

**Distribution**: Indonesia.

**Specimens examined:** CUK7937 (Blue Lagoon beach, Padang Bai, Karangasem, Bali, Indonesia, collected by T.O.C. and D.E.B., Apr. 27 2012)**.**

**Vegetative morphology:** Plants are found in the intertidal zone and epiphytic on Centroceras or in association with other filamentous species. Thalli are 0.5–1.7 cm high, dark reddish-brown, occurring in tufts with an entangled creeping system (Fig. 42A). Erect axes are 78.3 ± 20.1 μm in diameter, arising from prostrate axes (Fig. 42B). A transversely dividing apical cell is 11.8 ± 1.7 μm × 9.6 ± 1.3 μm in size (Fig. 42C). Adventitious branchlets are absent. Five pericentral cells are present and they lack cortication throughout (Fig.42D-E). Some pericentral cells remain fused (Fig. 42F). Segments of erect branches are 70.7 ± 20.2 μm long, 0.9 ± 0.2 in length/breadth ratio. The branching pattern is pseudodichotomous (Fig. 42B). Branching occurs at intervals of 4–25 (11.9 ± 6.3) axial cells in the upper part of main axes and at intervals of 13–29 (18.3 ± 4.6) axial cells in the lower part of main axes. Trichoblasts and scar cells are absent. Prostrate axes are 127.5 ± 9.8 μm in diameter and attached by unicellular rhizoids. Segments of prostrate axes are 85.5 ± 17.0 μm long, 0.7 ± 0.1 in length/breadth ratio. Rhizoids are 556.6 ± 136.7 μm in length, produced from the proximal end of pericentral cells (Fig. 42G), usually one or two per segment, cut off as separate cells from pericentral cells, and unicellular with multilobed tips (Fig. 42H)

**Reproductive morphology:** In male gametophytes (Fig. 43A), the spermatangia are developed from a basal cell arising in segments without production of trichoblasts (Fig. 43B). In tetrasporangial plants (Fig. 43C), tetrasporangia are tetrahedrally divided, interrupted, and 31.8 ± 6.7 μm × 44.1 ± 7.0 μm in size (Fig. 43D). The development of tetrasporangia follows a straight arrangement (Fig. 43D-F). Fertile segments have five or six pericentral cells (Fig. 43G-H). One tetrasporangium is produced from a single segment (Fig. 43G-H). Female plants were not found.

**Habitat:** Plants grow forming very small tufts from the intertidal zone. They are found attached to rocks in wave-exposed areas. Tufts are usually very delicate, and are associated with other species such as *Ceramium sp*.

**Remarks:** There are three *Neosiphonia/Polysiphonia* species that have the combined features of five pericentral cells and rhizoids cut off from pericentral cells*: N. polyphysa* (Kutz.) Skelton et South, *P. bifurcata* Hollenb., and *P. pentamera* Hollenb. (Hollenberg 1961, 1968b, Coppejans and Millar 2000, Millar and Prud'homme-van Reine 2005). Although *N. baliana* is similar to these species in the number of pericentral cells, it is distinguished from them by the straight arrangement of the tetrasporangia and by the spermatangia developed not from trichoblasts but from its basal cell. Although the development of spermatangia from basal cells has been reported in *Polysiphonia* sensu stricto and *Vertebrata* (Hollenberg 1942, Kim et al. 2002) and a straight arrangement of tetrasporangia has been known as one of typical characters in *Polysiphonia* sensu stricto (Choi et al. 2001, Mamoozadeh and Freshwater 2011), their occurrence in *N. baliana* is the first time that these features have been reported within the genus *Neosiphonia*. These features may be recognized as "independent comparative evidence" for *N. baliana* to be distinguished from all other species of *Neosiphonia* (Kim et al. 2000, Mamoozadeh and Freshwater 2011). Moreover, *N. baliana* is clearly distinguished from the 10 other *Neosiphonia/Polysiphonia* species in Indonesia listed in Bustamante et al. (2013b) by having five pericentral cells and a straight arrangement of tetrasporangia. The number of pericentral cells and the development of spermatangia and tetrasporangia have been

used as characters to recognize some groups in polysiphonous red algae (Hollenberg 1968a,b, Choi et al. 2001, Kim and Yang 2005).

***Neosiphonia blandii* (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 44).**

**Basonym:** *Polysiphonia blandii* Harvey.

**Type locality:** Brighton, Port Phillip, Victoria, Australia.

**Distribution**: China, Australia, and New Zealand.

**Specimens examined:** CUK11269 (Camp Cove, Watson Bay, Sydney, Australia collected by T.O.C. and D.E.B., Mar. 28 2014)**.**

**Vegetative morphology:** Plants are minute 0.2- 3 cm high (Fig. 44A), brownish to purplish in color, and associated with other filamentous species. They form very small, entangled, and robust tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system at intervals of 12-20 axial cells (Fig.44B). Erect axes are radially branched in an alternate pattern at intervals of 12-16 axial cells (Fig. 44C). Apical cells are inconspicuous, dome shaped, 6.23 ± 0.70 μm × 7.23 ± 0.87 μm in size, and transversely divided. Young erect axes have short segments (Fig. 44D) and are having acute branch apices (Fig. 44C-D). Older segments of erect axes are larger, normally 64.71 ± 8.34 μm in length and 75.24 ± 8.34 μm in diameter (L:D 0.87 ± 0.13) (Fig. 44E), and infrequently branched (Fig. 44E). Trichoblasts are delicate, deciduous, short, scarce, 1–3 times forked, 43.01 ± 11.70 μm in length, and arising on each segment near the apical cells. Scar cells are inconspicuous and 6.70 ± 0.22 μm × 6.36 ± 0.33 μm in size. Cicatrigenous branches are absent. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 44F-G). Straight lateral branches replace trichoblasts. The prostrate system is extensive and

entangled, with axial segments 77.72 ± 11.63 μm in length and 117.23 ± 9.87 μm in diameter (L:D 0.67 ± 0.15). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 44H), and cut off from the pericentral cells (Fig. 44I-J), 375.51 ± 133.99 μm in length, and 24.72 ± 5.00 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 44K).

**Habitat:** Plants grow forming very small tufts from the intertidal zone. They are found attached to rock surfaces in sheltered to wave-exposed areas. Tufts are usually robust and associated with other species such as *Neosiphonia sp*.

**Remarks:** *Neosiphonia blandii* was originally described as *Polysiphonia blandii* by Harvey (1862) from Australia. Our samples of *N. blandii* comb. nov. are in agreement with the descriptions of Harvey (1862), Womersley (1979, 2003), and Millar (1990). Although these studies cited several similar species to *N. blandii*, this species is distinguished from all of them by the combination of the following features: dark-red brown color, alternate branching pattern, ecorticate, and straight branches replacing trichoblasts. We are transferring this species to the genus *Neosiphonia* on the basis of our integrated morphological and molecular analyses. The rhizoids cutting off pericentral cells and the three-celled carpogonial branches are the diagnostic features that delineate *Neosiphonia*. Although reproductive structures were not found, the close connection of rhizoids in *N. blandii* and our molecular analyses ally *N. blandii* into the genus *Neosiphonia*. Thereby, the new combination is proposed. In our phylogenetic tree based on *rbc*L sequences, this species is closely related to *N. tongatensis*. These species are distinguished based on the branching pattern. *N. blandii* is having an alternate disposition of branches, whereas *N. tongatensis* is having a pseudodichotomous branching pattern (Kim et al. 2008).

***Neosiphonia coacta* (C.K. Tseng) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 45-46).**

**Basonym:** *Polysiphonia coacta* C.K. Tseng.

**Type locality:** Port Shelter, Hong Kong.

**Distribution**: Indic Ocean.

**Specimens examined:** CUK13051 (Songho beach, Songjimyeon, Haenamgun, Jeonllanamdo, Korea, collected by S.M.B. Oct. 25 2014)**.**

**Vegetative morphology:** Plants are minute 0.5-2.3 cm high (Fig. 45A-B), brownish to purplish in color, and associated with other terete species. They form entangled and delicate tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system at intervals of 14-22 axial cells. Erect axes are radially branched in a pseudodichotomous pattern at intervals of 14-20 axial cells (Fig. 45C-D). Apical cells are prominent, dome shaped, 7.57 ± 0.78 μm × 5.59 ± 0.35 μm in size, and transversely divided (Fig. 45E). Young erect axes have short segments (Fig. 45E). Older segments of erect axes are larger, normally 89.76 ± 11.86 μm in length and 63.63 ± 10.29 μm in diameter (L:D 1.43 ± 0.21), and infrequently branched (Fig. 45C). Trichoblasts are abundant delicate, deciduous, long, 1–3 times forked, 104.73 ± 43.27 μm in length, and arising on each segment near the apical cells (Fig. 45E-F). Scar cells are inconspicuous. Cicatrigenous branches are absent. Adventitious branches are present (Fig. 45G). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 45H). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 94.94 ± 4.36 μm in length and 96.13 ± 6.89 μm in diameter (L:D 1.01 ± 0.08). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 45I), and cut off from the pericentral cells (Fig. 45J-K), 441.68 ±

119.09 μm in length, and 23.91 ± 5.12 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 45L).

**Reproductive morphology:** In tetrasporangial plants (Fig. 46A), tetrasporangia are tetrahedrally divided and 31.92 ± 3.24 μm × 44.66 ± 5.35 μm in size (Fig. 46B). The development of tetrasporangia follows a spiral arrangement (Fig. 46C). Fertile segments have five pericentral cells (Fig. 46D). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 46E). A single tetrasporangium is produced on each fertile segment (Fig. 46E). Female and male plants were not found.

**Habitat:** Plants grow forming very small tufts from the intertidal zone. They are found attached to rock surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other species such as *Gelidium sp*.

**Remarks:** *Neosiphonia coacta* comb. nov. was originally described as *Polysiphonia coacta* by Tseng (1944) from Hong Kong. Our samples of *N. coacta* comb. nov. are in agreement with those of the original description. The only difference between the Tseng's specimens and ours is restricted to the branching pattern. *N. coacta* from Hong Kong is having a subdichotomous branching pattern, whereas *N. coacta* from Korea is having a pseudodichotomous branching pattern. This feature was proposed as inconsistent to delimit species in *Neosiphonia* (Stuercke and Freshwater 2008). We considered that *N. coacta* is showing plants with pseudodichotomous to subdichotomous disposition of branches. The wide distribution of *N. coacta* is confirmed from Honk Kong to the Korean coast. We are transferring *N. coacta* to the genus *Neosiphonia* on the basis of our integrated morphological and molecular analyses. Although female structures were not found, the close connection of rhizoids in *N. coacta* and our molecular analyses ally this species with the genus *Neosiphonia.* Thereby, the new combination is supported. In our phylogenetic tree based on *rbc*L sequences, *N. coacta* is closely related to the *N. harveyi* complex. *Neosiphonia coacta* is clearly distinguished from this species complex by lacking of cortication (Kim et al. 2006).

***Neosiphonia cockeri* (Hollenberg) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 47-48).**

**Basonym:** *Polysiphonia cokeri* Hollenberg.

**Type locality:** Lobos de Afuera, Peru.

**Distribution**: Northern Peru and Ecuador

**Specimens examined:** CUK6601 (Mancora, Piura, Peru, collected by T.O.C. and D.E.B. Sep. 01 2008), CUK6632 (Chocolatera, Las Salinas, Ecuador, collected by T.O.C. and D.E.B. Sep. 03 2008)**.**

**Vegetative morphology:** Plants are minute 4-10 cm high (Fig. 47A-B), brownish to purplish in color, and associated with other filamentous species. They form entangled and delicate tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system at irregular intervals (each 6-18 axial cells). Erect axes are radially branched in a subdichotomous to distichous pattern at intervals of 5-10 axial cells, rarely 17 (Fig. 47B-C). Apical cells are prominent, dome shaped, 7.21 ± 0.54 μm × 7.27 ± 0.53 μm in size, and transversely divided. Young erect axes have short segments, they are incurved and fusiform (Fig. 47D) and tapered about equally toward the base and apex (Fig. 47D). Older segments of erect axes are larger, normally 50.70 ± 5.93 μm in length and 91.86 ± 15.23 μm in diameter (L:D 0.57 ± 0.11), and infrequently branched (Fig. 47E). Trichoblasts are scarce, short, delicate, deciduous, 1–4 times forked, 62.83 ± 29.26 μm in length, and arising on each segment near the apical cells (Fig. 47D). Scar cells are inconspicuous and 9.00 ± 1.22 μm × 7.10 ± 0.94 μm in size (Fig. 47E). Cicatrigenous branches are present (Fig. 47F). Adventitious branches are present. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 47G). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 64.32 ± 7.82 μm in length and 132.37 ± 9.50

μm in diameter (L:D 0.49 ± 0.05). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 47H-I), and cut off from the pericentral cells (Fig. 47J), 275.30 ± 116.36 μm in length, and 41.03 ± 8.70 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature.

**Reproductive morphology:** In female gametophytes (Fig. 48A), erect axes are densely branched in the upper parts (Fig. 48B-C). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 48D). Cystocarps are alternate disposed and globose when mature, 206.65 ± 26.48 μm in height and 182.94 ± 17.89 μm in diameter. In male gametophytes (Fig. 48E), spermatangial branches are clustered at the apices of erect axes (Fig. 48F), and develop on a trichoblast furcation in each axial segment (Fig. 48G). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 48H), tetrasporangia are tetrahedral and 41.31 ± 6.55 μm × 41.26 ± 8.58 μm in size. Tetrasporangial branches are swollen and fusiform (Fig. 48I). The development of tetrasporangia follows a spiral arrangement (Fig. 48I). Fertile segments have four to five pericentral cells (Fig. 48J-K). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 48J-K). A single tetrasporangium is produced on each fertile segment (Fig. 48J-K).

**Habitat:** Plants grow forming very small tufts from the intertidal zone. They are found epiphyte on *Caulerpa* and also attached on rock surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other species such as *Sargassum sp.* and *Bryopsis sp.*

**Remarks:** *Neosiphonia cockeri* comb. nov. was originally described as *Polysiphonia cockeri* by Hollenberg (1958) on the basis of material collected by Coker in 1907 from the northern coast of Peru. Earlier, this species was reported without a taxonomical status as *Polysiphonia sp.* by Howe (1914) because of this material was scanty and sterile. The observations of the morphological features of our samples of *N. cockeri* are in agreement with those of Howe (1914) and Hollenberg

(1968). Although the description of Hollenberg (1958) reported *N. cockeri* with undeveloped trichoblasts which are represented by unicellular primordia, our specimens collected on the vicinity of the type locality show scarce trichoblasts which are leaving inconspicuous scar cells spirally disposed after they shed. Our study is characterizing this species on detail morphology and transferring it to the genus *Neosiphonia* on the basis of rhizoids cutting off the pericentral cells and the three-celled carpogonial branch. Our molecular analyses of *rbc*L sequences also supported the recognition of this species in *Neosiphonia*. *Neosiphonia cockeri* comb. nov. is reported for the first time form the coast of Ecuador. It shows the affinity of *N. cockeri* to low latitudes of the Pacific Coast.

***Neosiphonia ecuatoriana* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 49-50).**

**Diagnosis.** Thalli 0.6-3.1 cm tall, saxicolous, composed of indeterminate prostrate axes and indeterminate erect axes. Axes with four pericentral cells and ecorticate throughout. Erect axes with branches pseudodichotomously positioned, which are replacing the trichoblasts. Trichoblasts deciduous. Scar cells inconspicuous spirally disposed. Adventitious branches present. Rhizoids unicellular, in close connection with and produced from the proximal end of pericentral cells. Procarps bearing three celled carpogonial branches. Spermatangia branches developed on furcation of trichoblasts. Tetrasporangia arranged in straight series.

**Holotype**: CUK6631. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Chocolatera, Las Salinas, Ecuador (1° 24′ 21.47″ S, 79° 1′ 5.50″ W), collected T.O. Cho and D. E. Bustamante, Sep. 03 2008.

**Other specimens examined**. CUK6626, CUK6634 (Chocolatera, Las Salinas, Ecuador, collected by T.O.C. and D.E.B., Sep. 03 2008).

**Etymology**: The name "*ecuatoriana*" is derived from the country of collection.

**Distribution**: Ecuador

**Vegetative morphology:** Plants are minute 0.6-3.1 cm high (Fig. 49A-B), yellowish to purplish in color, and solitary. They form entangled and delicate tufts that are predominantly attached on rock surfaces of tide pools in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system at intervals of 6-14 axial cells. Erect axes are radially branched in a pseudodichotomous pattern at intervals of 3-12 axial cells (Fig. 49B-C). Apical cells are prominent, dome shaped, 5.88 ± 1.27 μm × 5.58 ± 1.07 μm in size, and transversely divided. Young erect axes have short segments (Fig. 49D) and tapered toward the apex (Fig. 49E). Older segments of erect axes are larger, normally 54.37 ± 5.74 μm in length and 101.65 ± 13.61 μm in diameter (L:D 0.54 ± 0.06), and infrequently branched (Fig. 49B). Trichoblasts are abundant, delicate, deciduous, long, 1–3 times forked, 40.59 ± 9.74 μm in length, and arising on each segment near the apical cells (Fig. 49E). Scar cells are inconspicuous (Fig. 49F). Cicatrigenous branches are absent. Adventitious branches are present (Fig. 49G). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 49H-I). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 49.28 ± 9.49 μm in length and 121.40 ± 17.28 μm in diameter (L:D 0.41 ± 0.06) (Fig. 49B). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 49J), and cut off from the pericentral cells (Fig. 49K), 126.24 ± 38.01 μm in length, and 34.10 ± 4.80 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 49L).

**Reproductive morphology:** In female gametophytes (Fig. 50A), procarps are positioned laterally and subapically on erect axes (Fig. 50B), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell. Cystocarps are globose when mature, 162.23 ± 9.98 μm in height and 153.22 ± 6.94 μm in diameter. In male gametophytes (Fig. 50C), spermatangial branches are clustered at the apices of erect axes (Fig. 50D-E), and develop on a trichoblast

furcation in each axial segment (Fig. 50D-E). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 50F-G), tetrasporangia are tetrahedral and 38.81 ± 6.63 μm × 47.16 ± 5.02 μm in size. Tetrasporangial branches are swollen and lanceolate (Fig. 50F-G). The development of tetrasporangia follows a straight arrangement (Fig. 50H). Fertile segments have four to five pericentral cells (Fig. 50I-J). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 50I-J). A single tetrasporangium is produced on each fertile segment (Fig. 50I-J).

**Habitat:** Plants grow forming very small tufts in the intertidal zone. They are found attached on rock surfaces of tide pools in sheltered to wave-exposed areas. Tufts are usually delicate and solitary.

**Remarks:** *Neosiphonia ecuatoriana* sp. nov. is newly described from Ecuador on the basis of morphological and molecular analysis. Seven *Neosiphonia*/*Polysiphonia* species have been previously reported from the Coast of Ecuador. Our new species, *N. ecuatoriana*, is distinguished from *Polysiphonia decussata*, *P. pacifica* var. *delicatula*, and *P. scopulorum* var. *villum* by having the rhizoids cutting off the pericentral cells (Hollenberg 1842, Kim and Yang 2005, Mamoozadeh and Freshwater 2011). *Neosiphonia ecuatoriana* sp. nov. is also different with *P. sertulariodes* and *N. simplex* by having the tetrasporangia arranged in straight series (Mamoozadeh and Freshwater 2012). The habit of *N. ecuatoriana* sp. nov. might be similar to *P. bifurcata* and *P. howei*, but *P. bifurcata* is different from *N. ecuatoriana* by having five pericentral cells and forcipate apices (Taylor 1945), whereas *P. howei* is distinguished by having 10-13 pericentral cells (Taylor 1945). The tetrasporangia arranged in straight series is an uncommon feature reported in the *Neosiphonia* clade and only *N. baliana* and *P. strictissima* has been reported with this. *N. ecuatoriana* sp. nov. is distinguished from *N. baliana* by having four pericentral cells, whereas it is distinguished from *P. strictissima* by lacking of cortical cells (Adams 1994). The fertile segment in the tetrasporangia of *Neosiphonia*/*Polysiphonia* species usually has one more pericentral cell that in vegetative segments (Falkenberg 1901, Hollenberg 1942). The fertile segment of *N. ecuatoriana* sp. nov. is having the

same number as vegetative segments. Although this features has not been evaluated as consistent by previous studies, we reported the same number of pericentral cells in vegetative and fertile segments only in *N. cockeri* and *N. ecuatoriana.* These two species are distinguished on the tetrasporangia arrangement and the branching pattern. *N. cockeri* is having tetrasporangia arranged in spiral series and subdichotomous to distichous branching pattern, whereas *N. ecuatoriana* is having straight series and pseudodichotomous branches. Our study is characterizing this species on detail morphology and placed in the genus *Neosiphonia* on the basis of rhizoids cutting off the pericentral cells and the three-celled carpogonial branch. Our molecular analyses of *rbc*L sequences also supported the recognition of this species in *Neosiphonia*. *Neosiphonia ecuatoriana* sp. nov. is reported in the coast of Ecuador showing affinity to low latitudes environments of the Pacific Coast.

***Neosiphonia gorgoniae* (Harvey) S.M. Guimarães et M.T. Fujii (Fig. 51-52).**

**Basonym:** *Polysiphonia gorgoniae* Harvey.

**Type locality:** Key West, Florida, U.S.A.

**Distribution**: Western Atlantic.

**Specimens examined:** CUK8595 (Praia Domigas Dias, Ubatuba, Sao Paulo, Brazil, collected by T.O.C. and D.E.B. Jul. 11 2012)**.**

**Vegetative morphology:** Plants are 0.5-4.1 cm high (Fig. 51A-B), brownish in color, and associated with other filamentous species. They form entangled and delicate tufts that are predominantly epiphyte on big plants in the intertidal zone. Thalli are composed of main indeterminate erect axes attached by clumped rhizoids forming a holdfast-like structure. Erect axes are radially branched in a dichotomous pattern at intervals of 8-12 axial cells (Fig. 51B-C). Apical cells are prominent, dome shaped, 6.43 ± 0.67 μm × 6.16 ± 1.03 μm in size, and transversely divided.

Young erect axes have short segments, they are incurved (Fig. 51C) and tapered toward the apex. Older segments of erect axes are larger, normally 104.44 ± 33.45 μm in length and 161.42 ± 66.50 μm in diameter (L:D 0.70 ± 0.16), and infrequently branched (Fig. 51B). Trichoblasts are scarce, short, delicate, deciduous, 1–3 times forked, 26.99 ± 2.82 μm in length, and arising on each segment near the apical cells of gametophytes. Scar cells are very inconspicuous and 5.49 ± 0.42 μm × 5.63 ± 0.28 μm in size. Cicatrigenous branches are present (Fig. 51F). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 51D-E). Lateral branches replace trichoblasts. Rhizoids are clumped forming a holdfast-like structure on the basal part and radially produced around the axes (Fig. 51F). Rhizoids are cutting off from the proximal end of pericentral cells (Fig. 51G), 348.51 ± 104.06 μm in length, and 26.19 ± 4.48 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes (Fig. 52A-B), erect axes are densely branched in the upper parts and covered with trichoblasts (Fig. 52C). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 52D). Cystocarps are alternate disposed and globose when mature, 230.27 ± 47.18 μm in height and 195.92 ± 54.02 μm in diameter (Fig. 52E). In male gametophytes (Fig. 52F-G), spermatangial branches are clustered at the apices of erect axes (Fig. 52H), and replacing the trichoblast in each axial segment (Fig. 52E). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. Tetrasporophyte were not found

**Habitat:** Plants grow forming tufts in the intertidal zone. They are found often epiphyte on *Gracilaria sp, Padina sp.* and *Codium sp.* thallus in sheltered areas. Tufts are usually delicate and associated with other species such as *Bostrychia sp. and Polysiphonia howei*.

**Remarks:** *Neosiphonia gorgoniae* was originally described as *Polysiphonia gorgoniae* by Harvey (1853) from Florida and then transferred by Guimarães et al. (2003) to the genus *Neosiphonia* on

the basis of the three-celled carpogonial branch. This species has been widely reported in the Western Atlantic from Florida to the Brazil (Dawes and Mathieson 2008). The morphological observations of our samples of *N. gorgoniae* are in agreement with those reports of this species around the Western Atlantic (Harvey 1853, Guimarães et al. 2003)

***Neosiphonia indonesiana* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 53-54).**

**Diagnosis.** Thalli 0.7-4.2 cm tall, epiphyte, composed of indeterminate prostrate axes and indeterminate erect axes. Axes with four to five pericentral cells and ecorticate throughout. Erect axes with branches pseudodichotomously positioned, which are replacing the trichoblasts. Trichoblasts deciduous almost absent. Scar cells inconspicuous. Rhizoids unicellular, in close connection with and produced from the proximal end of pericentral cells. Procarps bearing three-celled carpogonial branches. Spermatangia branches developed adaxially and replacing trichoblasts. Tetrasporangia arranged in straight series.

**Holotype**: CUK8950. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Geger, Nusadua, Bali, Indonesia (8° 48′ 07″ S, 115 °14 ′31″ E), collected by T.O. Cho and D. E. Bustamante, Oct. 21 2012 .

**Other specimens examined**. CUK9072 (Padang Bai, Karangasem, Bali, Indonesia, collected by T.O.C. and D.E.B., Oct. 22 2012).

**Etymology**: The name "*indonesiana*" is derived from the country of collection.

**Distribution**: Indonesia

**Vegetative morphology:** Plants are 0.7-4.2 cm high (Fig. 53A), brownish to purplish in color, and solitary. They form entangled and delicate tufts that are predominantly epiphyte on other big seaweeds in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system at irregular intervals. Erect axes are radially branched in a pseudodichotomous pattern at intervals of 4-9 axial cells, rarely 2 (Fig. 53B-C). Apical cells are prominent, dome shaped, 5.76 ± 0.87 μm × 5.05 ± 0.70 μm in size, and transversely divided. Young erect axes have short segments (Fig. 53C) and tapered toward the apex. Older segments of erect axes are larger, normally 91.56 ± 13.83 μm in length and 116.98 ± 17.72 μm in diameter (L:D 0.80 ± 0.18), and infrequently branched (Fig. 53B). Trichoblasts are very scarce, almost absent, delicate, deciduous, short, 1–2 times forked, and often in gametophytes. Scar cells are inconspicuous (Fig. 53D). Cicatrigenous branches are absent. Adventitious branches are absent. Each segment is completely ecorticated along the thallus and composed of four to five pericentral cells (Fig. 53E-F). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 80.39 ± 10.48 μm in length and 168.04 ± 14.18 μm in diameter (L:D 0.48 ± 0.09). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 53G), and cut off from the pericentral cells (Fig. 53H-I), 225.62 ± 36.14 μm in length, and 34.93 ± 9.43 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature.

**Reproductive morphology:** In female gametophytes (Fig. 54A), procarps are positioned laterally and subapically on erect axes (Fig. 54B), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 54C). Cystocarps are elongate when mature (Fig. 54D), 217.54 ± 30.23 μm in height and 151.04 ± 15.22 μm in diameter. In male gametophytes (Fig. 54E), spermatangial branches are clustered adaxially at the apices of erect axes (Fig. 54G-H), and replacing trichoblasts at intervals of each two axial segment (Fig. 54G-H). In tetrasporangial plants (Fig. 54I), tetrasporangia are tetrahedral and 37.70 ± 5.68 μm × 34.09 ± 4.49 μm in size. Tetrasporangial branches are swollen and linear (Fig. 54J-K). The development of tetrasporangia follows a straight arrangement (Fig. 54K). Fertile segments have five pericentral cells (Fig. 54L-

M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 54L-M). A single tetrasporangium is produced on each fertile segment (Fig. 54L-M).

**Habitat:** Plants grow forming tufts in the intertidal zone. They are found epiphyte on other big seaweeds as *Gracilaria sp.* and also attached on soft surfaces as ropes in sheltered areas. Tufts are usually delicate and solitary.

**Remarks:** *Neosiphonia indonesiana* sp. nov. is newly described from the Indonesian coast based on detail morphology and molecular analyses. The diagnostic features delimiting this as new species in *Neosiphonia* and *Polysiphonia* sensu lato are the 4-5 pericentral cells, tetrasporangia arranged in straight series, and spermatangial branches developed adaxially and replacing the trichoblasts. There are twelve species having at least five pericentral cells reported from worldwide. Of them, *N. beaudettei*, *P. forcipata*, *P. homoia*, *P. incompta*, and *P. pentamera* are distinguished from *N. indonesiana* by having tetrasporangia arranged in spiral series, whereas *N. porrecta*, *P. guadalupensis*, and *P. kowiensis* are different from *N. indonesiana* by having cortication. Also, *P. abscissoides* is distinguished from *N. indonesiana* by having spermatangial branches developed on the furcation of trichoblasts., *P. bifurcata* by having forcipate apices, P. gracilis by having saccate rhizoids, and *P. johnstonii* by having one or two tetrasporangia per segment. Our phylogenetic analyses related *N. indonesiana* to *N. gorgoniae* and *N. baliana*. *N. gorgoniae* is distinguished from *N. indonesiana* by having only four pericentral cells and rhizoids clumped in a holdfast-like structure, whereas *N. baliana* is distinguished by having only five pericentral cells throughout. The placement of *N. indonesiana* sp. nov. in *Neosiphonia* was based on the three-celled carpogonial branches and rhizoids cutting off pericentral cells.

***Neosiphonia infestans* (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 55).**

**Basonym:** *Polysiphonia infestans* Harvey

**Heterotypic synonym:** *Polysiphonia zostericola* Lucas

**Type locality:** King George Sound, Western Australia.

**Distribution**: Australia, New Zealand, Indonesia and Vietnam

**Specimens examined:** CUK8952 (Geger, Nusadua, Bali, Indonesia collected by T.O.C. and D.E.B. Oct. 21 2012)**.**

**Vegetative morphology:** Plants are minute 0.2-0.8 cm high (Fig. 55A-B), reddish to purplish in color, and associated with other filamentous species. They form entangled and delicate tufts that are predominantly attached on rock surfaces or epiphyte on bigger plants in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from an extended prostrate system. Erect axes are radially branched in an alternate to irregular pattern at intervals of 6-20 axial cells (Fig. 55C). Apical cells are prominent, dome shaped, 6.38 ± 0.81 μm × 6.57 ± 0.97 μm in size, and transversely divided. Young erect axes have short segments, they are incurved and fusiform (Fig. 55C) and tapered about equally toward the base and apex (Fig. 55C). Older segments of erect axes are larger, normally 53.30 ± 10.86 μm in length and 54.15 ± 4.75 μm in diameter (L:D 1.01 ± 0.29), and infrequently branched (Fig. 55B, E). Trichoblasts are scarce, short, delicate, deciduous, 1–2 times forked, 48.31 ± 13.17 μm in length, and arising on each segment near the apical cells (Fig. 55C). Scar cells are inconspicuous (Fig. 55D). Adventitious branches are present. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 55F-G). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 67.49 ± 6.45 μm in length and 64.64 ± 4.58 μm in diameter (L:D 1.04 ± 0.10). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 55H), and cut off from the pericentral cells (Fig. 55I-J), 307.27 ± 175.81 μm in

length, and 15.56 ± 1.97 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 55K), tetrasporangia are tetrahedral and 37.53 ± 3.49 μm × 40.80 ± 3.33 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 55L). The development of tetrasporangia follows a spiral arrangement (Fig. 55L-I). Fertile segments have four to five pericentral cells (Fig. 55N). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 55N). A single tetrasporangium is produced on each fertile segment (Fig. 55N).

**Habitat:** Plants grow forming very small tufts from the intertidal zone. They are found epiphyte on bigger plants as *Sargassum sp.* and *Codium* and also attached on rock surfaces in sheltered areas. Tufts are usually delicate and associated with other species such as *Neosiphonia indonesiana* and *Ceramium sp*.

**Remarks:** *Neosiphonia infestans* comb. nov. was originally described as *Polysiphonia infestans* by Harvey (1955) and then this species has been widely reported from the coast of Australia by Millar and Kraft (1993) and Womersley (1979, 2003). Although female gametophytes of *N. infestans* were not found in the present study, the rhizoids cutting off from pericentral cells and our phylogenetic analyses support the placement of this species in *Neosiphonia*. Thereby, the new combination *N. infestans* is confirmed.

***Neosiphonia mancorensis*** **D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 56-57).**

**Diagnosis.** Thalli 0.2-0.9 cm tall, saxicolous, composed of indeterminate extended prostrate axes and reduced indeterminate erect axes. Axes with four pericentral cells and ecorticate throughout. Erect axes with branches alternate positioned, which are replacing the trichoblasts. Trichoblasts deciduous. Scar cells conspicuous. Rhizoids unicellular, in close connection with and produced

from the proximal end of pericentral cells. Procarps bearing three-celled carpogonial branches. Spermatangia branches developed on furcation and replacing trichoblasts. Tetrasporangia arranged in spiral series.

**Holotype**: CUK6587. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Punta Sal, Tumbes, Peru (3° 58′ 59.46″ S, 80° 59′ 10.99″ W), collected by T.O. Cho and D. E. Bustamante, Sep. 01 2008.

**Other specimens examined**. CUK6597-1 (Máncora, Piura, Peru collected by T.O.C. and D.E.B., Sep. 01 2008).

**Etymology**: The name "*mancorensis*" is derived from the beach of collection.

**Distribution**: Northern Peru.

**Vegetative morphology:** Plants are minute 0.2-0.9 cm high (Fig. 56A), brownish to reddish in color, and solitary. They form entangled and delicate tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate reduced erect axes arisen exogenously from an extended prostrate system at intervals of 4-6 axial cells, sometimes 10 (Fig. 56B). Erect axes are radially branched in an alternate pattern at intervals of 6-7 axial cells and sometimes 9. Apical cells are prominent, dome shaped, 7.57 ± 1.20 μm × 7.94 ± 1.37 μm in size, and transversely divided. Young erect axes have short segments and tapered toward the apex (Fig. 56C). Older segments of erect axes are larger, normally 34.57 ± 52.15 μm in length and 52.15 ± 4.76 μm in diameter (L:D 0.67 ± 0.15), and infrequently branched (Fig. 56B, D). Trichoblasts are scarce, delicate, deciduous, short, 69.63 ± 22.32 μm in length, and 1–2 times forked. Scar cells are conspicuous spirally disposed and 6.88 ± 1.04 μm × 6.08 ± 1.59 μm in size (Fig. 56D). Cicatrigenous branches are absent. Adventitious branches are absent. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 56E-F). Lateral branches

replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 61.50 ± 6.65 μm in length and 77.05 ± 15.01 μm in diameter (L:D 0.82 ± 0.15) (Fig. 56 B). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 56G), and cut off from the pericentral cells (Fig. 56H-I), 289.97 ± 112.98 μm in length, and 36.62 ± 8.33 μm in diameter. Rhizoids are unicellular, bifurcate (Fig. 56G), and produce lobed termination when mature.

**Reproductive morphology:** In female gametophytes (Fig. 57A), procarps are positioned laterally and apically on erect axes, and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 57B-C). Cystocarps are ovoid when mature (Fig. 57D), 281.13 ± 31.20 μm in height and 224.25 ± 23.49 μm in diameter. In male gametophytes (Fig. 57E), spermatangial branches are radially clustered at the apices of erect axes (Fig. 57F), and replacing or developed on a furcation of trichoblasts at each axial segment (Fig. 57G-H). In tetrasporangial plants (Fig. 57I), tetrasporangia are tetrahedral and 22.80 ± 6.51 μm × 25.18 ± 7.50 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 57J). The development of tetrasporangia follows a spiral arrangement (Fig. 57J). Fertile segments have four pericentral cells (Fig. 57K). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 57K). A single tetrasporangium is produced on each fertile segment (Fig. 57K).

**Habitat:** Plants grow forming tufts in the intertidal zone. They are found attached on soft surfaces and also sometimes epiphyte on *Padina sp.* in sheltered areas. Tufts are usually delicate and solitary, but these plants might be associated with other filaments as *Ceramium sp*.

**Remarks:** *Neosiphonia mancorensis* sp. nov. is newly described from the Peruvian coast based on detail morphology and molecular analyses. The diagnostic features delimiting *N. mancorensis* as new species in *Neosiphonia* are the four pericentral cells, four pericentral cells in fertile segments of tetrasporophyte, and spermatangial branches developed on a furcation and replacing the trichoblasts. Although the four pericentral cells is a very common feature reported in *Neosiphonia* the four pericentral cells in fertile segments of tetrasporophyte is a character that is not usually present.

Falkenberg (1901), Hollenberg (1942) and Womersley (1979) reported that usually tetrasporophytes produce one extra fertile pericentral cell to develop the stalk cell, tetrasporangium, and cover cells, but *Neosiphonia mancorensis* sp. nov. is lacking of this extra pericentral cell. This feature has also been reported in *N. cockeri* and *N. ecuatoriana*, species which are similar in habit to *N. mancorensis* sp. nov. *Neosiphonia cockeri* is distinguished from *N. mancorensis* by having subdichotomous to distichous branching pattern, cicatrigenous branches and extended erect axes; whereas *N. ecuatoriana* is different from *N. mancorensis* by tetrasporangia arranged in straight series and extended erect axes. The spermatangial branches developed from a furcation of trichoblasts and replacing trichoblasts have been often reported in different species but not in the same plants (Hollenberg 1942). This feature was considered as consistent character by Hollenberg (1942) to separate species and also by Kim and Lee (1999) to be delimit the genus *Neosiphonia*. *Neosiphonia mancorensis* sp. nov. is having these two character states in the same plants and thereby, refuse the consistency of this feature to delimit species in *Neosiphonia*.

***Neosiphonia notoensis* (Segi) M.S. Kim et I.K. Lee (Fig. 58-59).**

**Basonym:** *Polysiphonia notoensis* Segi.

**Homotypic synonym:** *Polysiphonia notoensis* Segi.

**Heterotypic synonym:** *Polysiphonia teradomariensis* M. Noda, *Polysiphonia japonica* var. *teradomariensis* (M. Noda) H.Y. Yoon, *Neosiphonia teradomariensis* (M. Noda) M.S. Kim et I.K. Lee.

**Type locality:** Shibagak, Noto Province, Japan.

**Distribution**: Korea, Japan, China.

**Specimens examined:** CUK12848 (Daejinhang, Daejindong, Donghae, Gangwondo, Korea collected by T.O.C. and D.E.B. Aug. 01 2014)**.**

**Vegetative morphology:** Plants are large 3.3-10.8 cm high (Fig. 58A), brownish in color, and solitary. They form entangled and robust tufts that are predominantly attached to rocks in the intertidal zone. Thalli are composed of main indeterminate erect axes arisen from clumped rhizoids forming a holdfast-like structure. Erect axes are radially branched in an alternate pattern (Fig. 58B-C). Apical cells are inconspicuous, dome shaped, 5.59 ± 0.80 μm × 4.44 ± 0.62 μm in size, and transversely divided. Young erect axes have short segments, they are incurved, lanceolate, and abruptly acute apices (Fig. 58C-D). Older segments of erect axes are larger, normally 53.95 ± 43.24 μm in length and 422.99 ± 51.92 μm in diameter (L:D 0.22 ± 0.09), and infrequently branched (Fig. 58A). Trichoblasts are abundant, long, delicate, deciduous, 1-4 times forked, 148.06 ± 41.27 μm in length, and arising on each segment near the apical cells (Fig. 58E). Scar cells are very conspicuous and 9.90 ± 1.33 μm × 8.69 ± 1.14 μm in size (Fig. 58F). Adventitious branches are abundant and distributed throughout the main axes (Fig. 58B). Each segment is composed of six to nine pericentral cells and ecorticate in upper and middle parts of axes, but corticated in the basal part of the thallus (Fig. 58G-J). Lateral branches replace trichoblasts. Rhizoids are clumped forming a holdfast-like structure on the basal part and 728.90 ± 138.46 μm in diameter (Fig. 58K). Rhizoids are cutting off from pericentral cells (Fig. 58L), 395.63 ± 67.11 μm in length, and 37.67 ± 9.47 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes (Fig. 59A), erect axes are densely branched in the upper parts and covered with trichoblasts and adventitious branches (Fig. 59B). Procarps are positioned alternately and subapically on erect axes (Fig. 59C), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell. Cystocarps are alternate disposed and globose when mature, 495.95 ± 118.79 μm in height and 422.54 ± 93.20 μm in diameter (Fig. 59D). In male gametophytes (Fig. 59E), spermatangial branches are clustered at the apices of erect axes (Fig. 59F), and developed on a furcation of trichoblast in each axial segment (Fig. 59G). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 59H), tetrasporangia are tetrahedral and 59.80 ± 10.85

μm × 50.02 ± 10.68 μm in size. Tetrasporangial branches are swollen and lanceolate (Fig. 59I). The development of tetrasporangia follows a spiral arrangement (Fig. 59J). Fertile segments have seven to nine pericentral cells (Fig. 59K-L). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 59K-L). A single or two tetrasporangia is produced on each fertile segment (Fig. 59K-L).

**Habitat:** Plants grow forming tufts in the intertidal zone. They are found often attached on rock surfaces in sheltered areas. Tufts are usually robust, rigid, and solitary but sometimes it might be associated with other species such as *Neosiphonia japonica* and *N. yendoi*.

**Remarks:** *Neosiphonia notoensis* was originally described as *Polysiphonia notoensis* by Segi (1951) from the coast of Japan and then Kim and Lee (1999) transfer this species to genus *Neosiphonia*. *Neosiphonia notoensis* is very similar in morphology to *N. teradomariensis* by having several pericentral cells and cortication in the basal part. *N. teradomariensis* was described by Noda (1971) from the coast of Japan as *Polysiphonia teradomariensis.* Our morphological analyses in *N. notoensis* did not find any consistent character to distinguish with *N. teradomariensis*. Moreover, our phylogenetic relationships demonstrated that the *rbc*L sequence labeled as *N. teradomariensis* (JX828136) by Bárbara et al. (2013) is identical with our materials of *N. notoensis.* Thereby, we conclude that *N. notoensis* and *N. teradomariensis* are referring to the same species and based on the principle of priority (Article 11, International Code of Nomenclature) *N. teradomariensis* is designated under synonymy with *N. notoensis.*

***Neosiphonia peruviensis*** **D.E. Bustamante, B.Y. Won et T.O. Cho (Fig. 60-61).**

**Type locality:** Lagunillas, Pisco, Ica, Peru.

**Distribution**: Peru and Chile.

**Specimens examined:** CUK6474 (Caldera, Atacama, Chile collected by T.O.C., Aug. 12 2008), CUK6510, CUK6516, CUK6520 (Lagunillas, Pisco, Ica, Peru collected by T.O.C. and D.E.B., Aug 27 2008)**.**

**Vegetative morphology:** Plants are found in tidal zones and attached to rocks in association with another filamentous species. Thalli are 1-5 cm high (Fig. 60A), red-brown in color, occurring in dense tufts with an entangled prostrate system and erect axes and usually forming mats on rocks. Erect axes are 203 ± 5 μ m in diameter (Fig. 60B) and arise from prostrate axes. Adventitious branchlets arise from scar cells in basal and older segments of the main axes (Fig. 60C). The apex has a prominent apical cell 13 ± 1 μm × 12 ± 1 μm with oblique divisions (Fig. 60D). There are six completely ecorticate pericentral cells through the thalli (Fig. 60F-G). Trichoblasts arise on each segment near the apex, 160 ± 51 μm long, deciduous, delicate, numerous and one or two times forked (Fig. 60D). Conspicuous scar cells appear along the filament after trichoblasts have been shed, reaching 12 ± 2 μm × 9 ± 1 μm at basal filaments (Fig. 60E). Segments of erect branches are 104 ± 9 μm in length, and are 1.9 times broader than long (L:D 0.5 ± 0.04). Prostrate axes are 338 ± 23 μm in diameter and attached to the surface of rock by unicellular rhizoids. Segments of prostrate axes are 252 ± 11 μm in length and are 1.3 times broader than long (L:D 0.7 ± 0.1). The branching pattern is alternate (Fig. 60B) but pseudodichotomous near the apex (Fig. 60G). Branching takes place at intervals of 6-11 (8 ± 2) axial cells on main axes and 6-16 (9 ± 2) cells on the lateral axes. Rhizoids are 290 ± 137 μm in length, usually one or two per segment (Fig. 60I), and are cut off as separate cells from the proximal ends of pericentral cells (Fig. 60J).

**Reproductive morphology:** In female plants (Fig. 61A), cystocarps measure 347 ± 85 μm × 330 ± 84 μm, and are ovoid to globose in shape (Fig. 61B-C). They have a reduced ostiole and short pedicel (Fig. 61C). In tetrasporangial plants (Fig. 61D), tetrasporangia are 118 ± 17 μm × 106 ± 13 μm in size, with one or two per segment (Fig. 61E-F). The development of tetrasporangia is in a spiral series (Fig. 61G-H) with up to three successively maturing sporangia. Tetrasporangia develop be-

low the ultimate pseudodichotomous apex, swollen, distorted and interrupted (Fig. 61G-I). The male gametophyte was not found in this study.

**Remarks:** *Neosiphonia peruviensis* is recognized by the following characteristics: rhizoids cutting off from the proximal ends of pericentral cells by cross walls, six pericentral cells, ecorticate axes through thallus, abundant trichoblasts near the apex, prominent scar cells and spiral arrangement of tetrasporangia. Although Bustamante et al. (2012) did not observe a three-celled carpogonial branch, which is one of the main key characters for *Neosiphonia*, their morphological evidence has shown that *N. peruviensis* is highly likely a member of *Neosiphonia* based on the morphology of rhizoids, trichoblasts and tetrasporangial arrangement. *rbc*L molecular analysis also indicates that *N. peruviensis* is placed in genus *Neosiphonia.* The number of pericentral cells and cortication may be used as significant diagnostic features to recognize each species of *Neosiphonia* (Guimarães et al. 2004, Mamoozadeh and Freshwater 2011). The number of pericentral cells typically remains constant in species with four of these cells, but numbers tend to vary within species as the number of pericentral cells increases (Hollenberg 1942, Womersley 1979, Womersley 2003, Stuercke and Freshwater 2008). The number of pericentral cells in *Neosiphonia* ranges from 4 to 9 (Segi 1951, Hollenberg 1958, Kim and Lee 1999, Guimarães et al. 2004, Mamoozadeh and Freshwater 2011). Three species in *Neosiphonia* are similar to *N. peruviensis* by having more than 5 pericentral cells *N. notoensis*, *N. porrecta*, and *N. tepida.* The number of their pericentral cells varies within each species from five to nine, and their pericentral cells produce cortical cells. *N. peruviensis* differs from *N. porrecta* and *N. notoensis* in having ecorticate pericentral cells. *N. peruviensis* also differs from *N. tepida* in having ecorticate pericentral cells and in having six pericentral cells through the thallus. Our molecular phylogenetic analyses using *rbc*L gene sequences revealed sufficient sequence divergence between *N. peruviensis* and other *Neosiphonia* species previously reported to classify our as a natural entity. Thus, *N. peruviensis* is situated in an independent and well supported clade embedded in *Neosiphonia*.

***Neosiphonia pseudovillum* (Hollenberg) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 62-63).**

**Basonym:** *Polysiphonia pseudovillum* Hollenberg.

**Type locality:** North Isl., Johnston Isla, Johnston Atoll, Central Pacific.

**Distribution**: Pacific Island and Western Atlantic

**Specimens examined:** CUK8756 (Praia do Meio, Natal, Brazil collected by T.O.C. and D.E.B. Jul. 17 2012).

**Vegetative morphology:** Plants are minute 0.5-2.6 cm high (Fig. 62A-B), reddish to purplish in color, and associated with other filamentous species. They form entangled and delicate tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from a reduced prostrate system at intervals of 1-6 axial cells. Erect axes are radially branched in a pseudodichotomous pattern at intervals of 14-24 axial cells. Apical cells are prominent, dome shaped, 7.15 ± 0.50 μm × 6.36 ± 0.90 μm in size, and transversely divided. Young erect axes have short segments, they are incurved and fusiform (Fig. 62C) and tapered about equally toward the base and apex (Fig. 62C). Older segments of erect axes are larger, normally 70.01 ± 6.10 μm in length and 50.61 ± 4.53 μm in diameter (L:D 1.40 ± 0.20), and infrequently branched (Fig. 62B). Trichoblasts are abundant, long, delicate, deciduous, 1–3 times forked, 176.30 ± 40.58 μm in length, and arising on each segment near the apical cells (Fig. 62D). Scar cells are inconspicuous and 4.90 ± 0.58 μm × 4.71 ± 0.90 μm in size (Fig. 62E). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 62F-G). Adventitious branches are present (Fig. 62H). Lateral branches replace trichoblasts. The prostrate system is reduced and entangled, with axial segments 68.62 ± 4.70 μm in length and 84.40 ± 11.43 μm in diameter (L:D 0.83 ± 0.18). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 62I), and cut off from the pericentral cells (Fig. 62J-K), 264.05

± 71.13 μm in length and 28.50 ± 6.99 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 63A), tetrasporangia are tetrahedral and 35.21 ± 7.95 μm × 29.10 ± 6.73 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 63B). The development of tetrasporangia follows a slightly spiral arrangement (Fig. 63C). Fertile segments have five pericentral cells (Fig. 63D-E). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 63D-E). A single tetrasporangium is produced on each fertile segment (Fig. 63D-E).

**Habitat:** Plants grow forming small tufts from the intertidal zone. They are found attached on rock surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other species such as *Bryocladia cuspidata* and *Polysiphonia howei*.

**Remarks:** *Neosiphonia pseudovillum* comb. nov. was described by Hollenberg (1968) from Johnston Atoll as *Polysiphonia pseudovillum*. This species has been widely reported in the Western Atlantic by Schneider and Searles (1991), Mamoozadeh and Freshwater (2011), and Wynne (2011). Mamoozadeh and Freshwater (2011) retained this species in *Polysiphonia* because lateral branches or trichoblasts are not produced each segment and thallus does not develop from a solid disc of rhizoids. Recent studies have demonstrated that the only consistent features in *Neosiphonia* are the three-celled carpogonial branch in combination with rhizoids cutting off from pericentral cells. Although we did not find female gametophyte of *N. pseudovillum*, the rhizoids connection and our phylogenetic relationships show this species as a good member of *Neosiphonia.*

***Neosiphonia pulvinata f. parvula*** **(Heydrich ) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 64-65).**

**Basonym:** *Polysiphonia pulvinata f. parvula*.

**Type locality:** Papua New Guinea.

**Distribution**: Indonesia

**Specimens examined:** CUK7137 (Banna, Donggala, Palu, Sulawesi, Indonesia collected by T.O.C. Jun. 19 2010).

**Vegetative morphology:** Plants are minute 0.4-0.7 cm high (Fig. 64A-B), reddish to purplish in color and sometimes colorless, and solitary. They form entangled and delicate tufts that are predominantly attached on rock, corals and solid surfaces from the intertidal to subtidal zone. Thalli are composed of main indeterminate erect axes attached by clumped rhizoids forming a holdfast-like structure. Erect axes are radially branched in a subdichotomous pattern at intervals of 3-9 axial cells (Fig. 64B-C). Apical cells are prominent, dome shaped, 5.50 ± 0.90 μm × 5.01 ± 0.58 μm in size, and transversely divided (Fig. 64E). Young erect axes have short segments, they are incurved and tapered toward the apex (Fig. 64E). Older segments of erect axes are larger, normally 118.64 ± 16.78 μm in length and 107.12 ± 23.24 μm in diameter (L:D 1.15 ± 0.25), and infrequently branched (Fig. 64B). Trichoblasts are scarce, short, delicate, deciduous, 1–2 times forked, 42.71 ± 10.73 μm in length, and arising on each segment near the apical cells (Fig. 64E). Scar cells are conspicuous and 3.97 ± 0.70 μm × 4.79 ± 0.86 μm in size (Fig. 64F). Cicatrigenous branches are developed from scar cells and they are spirally disposed (Fig. 64G). Adventitious branches are present (Fig. 64H). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 64I-J). Lateral branches replace trichoblasts. Rhizoids are clumped forming a holdfast-like structure on the basal part (Fig. 64K). Rhizoids are cutting off from pericentral cells (Fig. 64K), 58.47 ± 23.87 μm in length, and 18.43 ± 4.53 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 64L).

**Reproductive morphology:** In tetrasporangial plants (Fig. 65A-B), tetrasporangia are tetrahedral and 20.20 ± 2.25 μm × 31.62 ± 2.75 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 65C-D). The development of tetrasporangia follows a spiral arrangement (Fig. 65C-D). Fertile

segments have five pericentral cells (Fig. 65E-F). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 65E-F). A single tetrasporangium is produced on each fertile segment (Fig. 65E-F).

**Habitat:** Plants grow forming small tufts from the intertidal to subtidal zone. They are found attached on rock, coral and solid surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and solitary.

**Remarks:** *Neosiphonia pulvinata f. parvula* comb. nov. was originally described as *P. pulvinata f. parvula* by Heidrich (1892) when trying to distinguish from *P. pulvinata* described by Sprengel (1827). Our samples of *N. pulvinata f. parvula* are in agreement whit those of the original description (Kützing 1863, Heidrich 1892). Our phylogenetic analyses in combination with the following morphological features: rhizoids clumped in a holdfast-like structure, rhizoids cutting off from pericentral cells, and tetrasporangia arranged in spiral series: further support the recognition of *N. pulvinata f. parvula* as a *Neosiphonia* member. In our phylogenetic tree, *N. pulvinata f. parvula* is close related to *N. unguiformis* from India, but the latter is distinguished from *N. pulvinata f. parvula* by having a plant composed of prostrate and erect axes.

***Neosiphonia ramireziae*** **D.E. Bustamante, B.Y. Won et T.O. Cho (Fig. 66-67).**

**Type locality:** Lagunillas, Pisco, Ica, Peru.

**Distribution**: Peru, Australia, and Florida.

**Specimens examined:** CUK6511, CUK 6515, CUK 6520 (Lagunillas, Pisco, Ica, Peru collected by T.O.C. and D.E.B. Aug. 08 2008), CUK8269, CUK8357, CUK8360 (Lagunillas, Pisco, Ica, Peru collected by T.O.C. and D.E.B. Jul. 05 2012), CUK10052-54 (Sand Key Park, Gulf Boulevard, Clearwater, Florida, USA collected by T.O.C., Aug. 07 2013), CUK11221-2 (Bare Island, La Pe-

rouse, Sydney, Australia collected by T.O.C. and D.E.B., Mar. 28 2014), CUK11265, CUK11267 (Camp Cove, Watson Bay, Sydney, Australia collected by T.O.C. and D.E.B., Mar. 28 2014).

**Vegetative morphology:** Plants grow in the intertidal zone on rocks in association with other filamentous species. Thalli are 2.4 to 5 cm high (Fig. 66A), red-brown in color and occur in dense tufts (Fig. 66B) consisting of a limited prostrate system and well-developed erect filaments. Erect axes are 95.3 ± 9.8 μm in diameter (Fig. 66B) and arise endogenously from prostrate axes (Fig. 66C). Apices have a prominent apical cell, 8.2 ± 1.4 μm × 6.9 ± 1 μm in size (Fig. 66D). Trichoblasts are 22.7 ± 8.2 μm in length, deciduous, delicate, and scarce, produced from each segment near the apex, and once or twice forked (Fig. 66E). Inconspicuous scar cells are 11.8 ± 3.9 μm × 11.9 ± 3.9 μm, visible on the surface after shedding of trichoblasts (Fig. 66F). Adventitious branchlets arise on basal and older segments of the main axes as well as on its dichotomies. Four pericentral cells remain completely ecorticated throughout the thallus (Fig. 66G-H). Segments of erect branches measure 136.4 ± 43.6 μm in length and are 0.7 broader than long (L:D 1.4 ± 0.4). Prostrate axes are 168.5 ± 11.1 μm in diameter and are attached on the surface of rocky substrata by numerous unicellular rhizoids located in the proximal and distal parts of the pericentral cells. Segments of prostrate axes are 279.4 ± 24.8 μm in length and 0.6 broader than long (L:D 1.7 ± 0.2). Branching pattern is dichotomous in the main axes (Fig. 66B) but alternate near the apex, showing a pseudofastigiate appearance (Fig. 66B). Branching points occur at intervals of 5-14 (8.4 ± 2.6) axial cells in main axes and 6-11 (7.3 ± 1.5) cells in lateral axes replacing trichoblasts. Rhizoids are 362.1 ± 139.9 μm in length, cut off as separate cells from any position by pericentral cells (Fig. 66I) usually more than one per segment, and terminating in digitate tips (Fig. 66J).

**Reproductive morphology:** In female plants (Fig. 67A), cystocarps are narrowly globose, ovate, 152 ± 50 μm × 175 ± 57 μm in size, scattered on branchlets (Fig. 67B), and produced on short pedicels. Procarps consist of three-celled carpogonial branches (Fig. 67C-D). In male plants (Fig. 67E), spermatangial branches each arise from the basal cell of a forked trichoblast (Fig. 67F). In tetrasporangial plants (Fig. 67G), the fertile segments comprise 5 pericentral cells (Fig. 67H). Te-

trasporangia are tetrahedral, swollen, distorted, and 59.8 ± 13 μm × 87.8 ± 18.4 μm in size. A single tetrasporangium is produced per single segment (Fig. 67I). Tetrasporangia are spirally arranged (Fig. 67J) with successively maturing sporangia.

**Remarks:** *Neosiphonia ramireziae* is recognized by the presence of erect filaments arising from limited prostrate filaments, numerous rhizoids cut off from irregular positions of pericentral cells by a cross wall, 4 pericentral cells throughout, ecorticate axes, scarce trichoblasts, inconspicuous scar cells, procarps with three-celled carpogonial branches, spermatangia arising from the basal cell of trichoblasts, and a spiral arrangement of tetrasporangia. Four *Neosiphonia* species have been reported from the Pacific temperate coast of South America: *N. flaccidissima*, *N. peruviensis*, *N. savatieri*, and *N. sphaerocarpa*. Although these *Neosiphonia* species co-occur with our new species in the Pacific temperate coast of South America, they are morphologically distinct from *N. ramireziae*. *N. flaccidissima* reported from Lima, Peru, is distinguished by having branches arising in connection with trichoblasts, indistinct main axes, and abundant trichoblasts (Hollenberg 1942, 1968, Dawson et al. 1964). *N. peruviensis* described from Ica, Peru, has 6 pericentral cells and 1 or 2 tetrasporangia per segment (Bustamante et al. 2012). *N. savatieri* reported from Isla de Pascua, has a rhizoidal cluster and abundant trichoblasts (Hariot 1891, Santelices and Abbott 1987, Kim 2005). *N. sphaerocarpa*, reported from Lima and Talara, Peru, is distinguished by having creeping prostrate filaments and abundant trichoblasts with 3-4 dichotomies (Børgesen 1918, Dawson et al. 1964, Hollenberg 1968, Hollenberg and Norris 1977, Guimãraes et al. 2004, Mamoozadeh and Freshwater 2011). Our recent collections of *N. ramireziae* from the coast of Australia and Florida show the wide distribution of this species in the Pacific and Atlantic coast suggesting this species as invasive red algae. Further studies on the basis of phylogeographic assumptions might clarify the origin and distributional patterns in *N. ramireziae.*

***Neosiphonia silvae*** **D.E. Bustamante, B.Y. Won et T.O. Cho (Fig. 68-69).**

**Type locality:** Lagunillas, Pisco, Ica, Peru.

**Distribution**: Peru, Australia, and Florida.

**Specimens examined:** CUK7926, CUK7976 (Geger, Nusadua, Bali, Indonesia collected by T.O.C. and D.E.B. Apr. 26 2012).

**Vegetative morphology:** Plants are found in the intertidal zone and epiphytic on *Centroceras*. Thalli are 1.0–2.9 cm tall, dark reddish brown, and occur in tufts with an entangled creeping system (Fig. 68A). Erect axes are 67.2 ± 6.4 μm in diameter, arising from prostrate axes (Fig. 68B). Acute apices have prominent apical cells (8.3 ± 1.8 μm × 8.6 ± 2.5 μm) with a dome shape and transverse division (Fig. 68C-D). Apical cells are also exceedingly prominent with a ball shape, 32.2 ± 13.3 μm × 34.5 ± 10.8 μm (Fig. 68C-D). Adventitious branchlets are absent. Axes have four pericentral cells and lack cortication throughout (Fig. 68E). Segments of erect branches are 42.1 ± 4.2 μm long, 0.63 ± 0.07 in length/breadth ratio. Small determinate lateral branches arise in an alternate pattern (Fig. 68B) along the polysiphonous axes replacing the trichoblasts. Branching occurs at intervals of 4–10 (7.2 ± 1.8) axial cells in main axes. Trichoblasts arise from each segment near the apex, 104.6 ± 12.5 μm long. They are deciduous, delicate, numerous, and one or two times branched (Fig. 68F). Conspicuous scar cells appear along the filament after a trichoblast is lost, reaching 12.1 ± 2.0 μm × 9.3 ± 1.5 μm in size (Fig. 68G). Prostrate axes are 93.4 ± 6.1 μm in diameter and attached by unicellular rhizoids. Segments of prostrate axes are 78.5 ± 6.5 μm in length, 0.9 ± 0.1 in length/ breadth ratio. Axes branch at intervals of 5–12 (8.2 ± 2.0) axial segments in main axes. Rhizoids are 395.9 ± 224.7 μm long, produced from the proximal end of pericentral cells, usually one per segment, unicellular with multilobed tips, and cut off as separate cells from pericentral cells (Fig. 68H).

**Reproductive morphology:** In tetrasporangial plants (Fig. 69A), tetrasporangia are tetrahedrally divided and 32.5 ± 9.9 μm × 30.9 ± 9.8 μm in diameter (Fig. 69B). Normally, only one tetrasporangium is found on a single segment of each branch. Sometimes, there are two tetrasporangia on a branch (Fig. 69C-D). Fertile segments have five pericentral cells (Fig. 69E). Male and female plants were not found in this study.

**Remarks:** *Neosiphonia silvae* is distinguished by having atypical and prominent apical cells that are almost four times larger than normal apical cells. This character will aid in species identification. *Neosiphonia silvae* resembles many other previously reported *Neosiphonia/Polysiphonia* species in having four pericentral cells and rhizoids cut off from pericentral cells: *N. ferulacea*, *N. infestans*, *N. pulvinata* var. *parvula*, *N. sertularioides*, *P. flexicaulis*, *P. mollis*, and *P. tenerrima*. However, *N. ferulacea* can be distinguished from *N. silvae* by the discoid base and with some branches becoming decumbent (Mamoozadeh and Freshwater 2012); *N. sertulariodes* and *N. infestans* by the dichotomous and fastigiated branching, respectively (Womersley 2003, Nam and Kang 2012); *P. flexicaulis* and *P. mollis* by the presence of cortication (Harvey 1853, Womersley 1979); and *N. pulvinata* var. *parvula* by the high frequency of dichotomous branches (Heydrich 1892). Also, *N. silvae* is clearly distinguished from all these Indonesian species by having one or two tetrasporangia distributed on only a single segment of each branch.

***Neosiphonia strictissima* (J.D. Hooker et Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 70-71).**

**Basonym:** *Polysiphonia strictissima* J.D. Hooker et Harvey

**Type locality:** New Zealand.

**Distribution:** Australia and New Zealand.

**Specimens examined:** CUK10963-65 (Falmouth, Tasmania, Australia collected by T.O.C. and D.E.B. Mar. 23 2014), CUK10997, CUK11003 (Scamander, Tasmania, Australia collected by T.O.C. and D.E.B. Mar. 23 2014).

**Vegetative morphology:** Plants are 3.1-8.8 cm high (Fig. 70A), reddish to purplish in color, and solitary. They form entangled and delicate tufts that are predominantly attached on rock or epiphyte on blades of big seaweeds from the intertidal to subtidal zone. Thalli are composed of main indeterminate erect axes attached by clumped rhizoids forming a holdfast-like structure. Main erect axes are radially branched in a pseudodichotomous pattern at irregular intervals (Fig. 70B-C). Apical cells are prominent, dome shaped, 5.77 ± 1.38 μm × 5.1 ± 0.68 μm in size, and transversely divided (Fig. 70C). Young erect axes have short segments, they are incurved and tapered toward the apex (Fig. 70C). Older segments of erect axes are larger but with pericentral cells in square shape (Fig. 70D), normally 139.49 ± 12.62 μm in length and 165.25 ± 18.22 μm in diameter (L:D 0.86 ± 0.15), and frequently branched (Fig. 70A-B). Trichoblasts are abundant, short, delicate, deciduous, 1–3 times forked, 63.55 ± 21.28 μm in length, and arising on each segment near the apical cells (Fig. 70C). Scar cells are very conspicuous (Fig. 70D). Cicatrigenous branches are developed from scar cells and they are spirally disposed and 5.77 ± 1.38 μm × 5.10 ± 0.68 μm in size (Fig. 70I). Each segment is composed of four pericentral cells and ecorticate in upper and middle parts of axes, but corticated in the basal part of the thallus (Fig. 107E-H). Lateral branches arise on a furcation of trichoblasts. Rhizoids are clumped forming a holdfast-like structure on the basal part (Fig. 70J). Rhizoids are cutting off from pericentral cells (Fig. 70K-M), 438.35 ± 123.93 μm in length, and 29.47 ± 7.78 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes (Fig. 71A), erect axes are densely branched in the upper parts and covered with trichoblasts. Procarps are positioned subapically on erect axes (Fig. 71A-B), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 71C). Cystocarps are globose when mature, 302.46 ± 23.22 μm in height

and 265.44 ± 33.53 μm in diameter (Fig. 71D). In male gametophytes (Fig. 71E), spermatangial branches are clustered at the apices of erect axes (Fig. 71F), and developed on a furcation or replacing the trichoblasts in each axial segment (Fig. 71G-H). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 71I-J), tetrasporangia are tetrahedral and 44.79 ± 10.42 μm × 44.14 ± 7.04 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 71I-J). The development of tetrasporangia follows a spiral arrangement (Fig. 71K-L). Fertile segments have five pericentral cells (Fig. 71M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 71M). A single tetrasporangium is produced on each fertile segment (Fig. 71M).

**Habitat:** Plants grow forming tufts from the intertidal to subtidal zone. They are found attached on rock and highly epiphyte on blades and thallus of seaweeds as *Grateloupia sp.* and *Codium sp.* in sheltered to wave-exposed areas. Tufts are usually delicate and solitary.

**Remarks:** *Neosiphonia strictissima* comb. nov. was originally described by Hooker and Harvey (1845) from the coast of New Zealand. This species has been widely reported from the coast of Australia and New Zealand (Adams 1994, Mamoozadeh and Freshwater 2011). The first molecular analyses of *N. strictissima* was done by Mamoozadeh and Freshwater (2011). Although their phylogenetic analyses placed this species in the genus *Neosiphonia*, the transfer were not proposed. Our integrated morphological and molecular analyses support *N. strictissima* in the genus *Neosiphonia*. Morphologically, this species is having the two diagnostic features of *Neosiphonia* members: the three-celled carpogonial branches and rhizoids cutting off the pericentral cells and genetically this species is embedded in *Neosiphonia* and close related to *N. japonica* complex. *N. strictissima* and the *N. japonica* complex have been considered as cryptic sibling species by McIvor et al (2001), but Kim and Yang (2006) considered that his species are distinguished on the basis of habit and morphological features. Although Adams (1994) reported *N. strictissima* with spermatangia developed in a furcation of trichoblast and tetrasporangia arranged in straight series, our study

shows that *N. strictissima* is having the two types of spermatangia development and tetrasporangia arranged in spiral series.

***Neosiphonia sudafricana*** **D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 72-73).**

**Diagnosis.** Thalli 0.4-1.8 cm tall, epiphyte on delesserioid, composed of indeterminate reduced prostrate axes and indeterminate erect axes. Axes with four pericentral cells and ecorticate throughout. Erect axes with branches pseudodichotomously positioned, which are not in relationship with trichoblasts. Rhizoids unicellular, in close connection with and produced from the proximal end of pericentral cells. Reproductive structures adaxially disposed. Procarps bearing three-celled carpogonial branches. Spermatangia branches replacing trichoblasts. Tetrasporangia arranged in spiral series.

**Holotype**: CUK2760. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Mzamba, Eastern Cape, South Africa (31° 6′ 28.65″ S, 30° 10′ 36.39″ E), collected by T.O. Cho, Aug. 21 2005.

**Etymology**: The name "*sudafricana*" is derived from the country of collection.

**Vegetative morphology:** Plants are minute 4-10 cm high (Fig. 72A), brownish to purplish to colorless, and solitary. They form entangled and very delicate tufts that are predominantly epiphyte on delesserioid plants in the intertidal zone. Thalli are composed of interwoven and indeterminate erect axes arisen exogenously from a reduced prostrate system at irregular intervals (Fig. 72B). Erect axes are radially branched in a pseudodichotomous pattern at irregular intervals of axial cells (Fig. 72B-C). Apical cells are prominent, dome shaped, 6.36 ± 0.62 μm × 6.78 ± 0.48 μm in size, and transversely divided (Fig. 72C). Young erect axes have short segments and tapered toward the apex (Fig. 95C). Older segments of erect axes are larger, normally 88.57 ± 3.89 μm in length and

58.15 ± 6.57 μm in diameter (L:D 1.54 ± 0.20), and infrequently branched (Fig. 72B, D). Trichoblasts and scar cells are absent. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 72E-F). Lateral branches not in relationship with trichoblasts. The prostrate system is reduced and entangled, with axial segments 55.55 ± 3.99 μm in length and 110.94 ± 5.24 μm in diameter (L:D 0.50 ± 0.04) (Fig. 72B). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 72G), and cut off from the pericentral cells (Fig. 72H), 173.21 ± 44.73 μm in length, and 33.56 ± 2.42 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature.

**Reproductive morphology:** In female gametophytes (Fig. 73A), procarps are positioned laterally and adaxially on apices of erect axes (Fig. 73B), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell. Cystocarps are ovoid when mature (Fig. 73D), 192.71 ± 39.09 μm in height and 180.03 ± 38.94 μm in diameter. In male gametophytes (Fig. 73D), spermatangial branches are adaxially produced at the apices of erect axes at intervals of each two axial cells (Fig. 73D), and replacing trichoblasts (Fig. 73E-F). In tetrasporangial plants (Fig. 73G), tetrasporangia are tetrahedral and 40.86 ± 3.97 μm × 36.44 ± 3.19 μm in size. Tetrasporangial branches are swollen and linear (Fig. 73H-I). The development of tetrasporangia follows a straight arrangement (Fig. 73H-I). Fertile segments have five pericentral cells (Fig. 73J). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 73J). A single tetrasporangium is produced on each fertile segment (Fig. 73J).

**Habitat:** Plants grow forming very small tufts in the intertidal zone. They are found epiphyte on delesserioid plants and soft surfaces in sheltered areas. Tufts are usually delicate and solitary, but these plants might be associated with other filaments as *Streblocladia camptoclada*.

**Remarks:** *Neosiphonia sudafricana* sp. nov. is newly described on the basis of detail morphology and molecular analyses. The diagnostic features in *N. sudafricana* are the four pericentral cells, the adaxial disposition of the reproductive structures, and tetrasporangia arranged in straight series.

The reproductive structures adaxially disposed has been considered diagnostic to delimit the genus *Strebloclad*ia (Schmitz and Falkenberg 1897), but recently Phillips (2010) proposed that several groups are sharing this character because it might be a synapomorphy or developed independently more than once. This feature has been observed in other three *Neosiphonia*: *N. baliana*, *N. collabens*, and *N. indonesiana*. *N. baliana* and *N. indonesiana* are distinguished from *N. sudafricana* by having five pericentral cells, whereas *N. collabens* is different from *N. sudafricana* by having 6 pericentral cells and clumped rhizoids (Diaz-Tapia and Bárbara 2013).

***Neosiphonia sungminbooi*** **D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 74-75).**

**Diagnosis.** Thalli 0.9-3.7 cm tall, saxicolous, composed of indeterminate extended prostrate axes and reduced indeterminate erect axes. Axes with four pericentral cells and ecorticate throughout. Erect axes with branches alternate positioned, which are replacing the trichoblasts. Trichoblasts deciduous. Scar cells conspicuous. Rhizoids unicellular, in close connection with and produced from the proximal end of pericentral cells. Procarps bearing three-celled carpogonial branches. Spermatangia branches developed on furcation and replacing trichoblasts. Tetrasporangia arranged in spiral series.

**Holotype**: CUK9707. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Udodeungdae, Udomyeon, Jeju, Korea (33° 29′ 48.26″ N, 126° 58′ 6.47″ W), collected by T.O. Cho and D. E. Bustamante, Apr. 26 2013.

**Other specimens examined**. CUK12031(Gwakji, Aewoleup, Jeju, Korea collected by T.O.C. and D.E.B., May. 30 2014), CUK12233 (Gapado, Daejeongeub, Seagwiposi, Jeju, Korea collected by T.O.C. and D.E.B., May. 29 2014).

**Etymology**: The name "*sungminbooi*" is in honor of Professor Sung Min Boo, for his valuable contributions to the understanding of the systematics and biodiversity of the Marine Flora in Korea.

**Distribution**: Korea and Japan.

**Vegetative morphology:** Plants are 0.9-3.7 cm high (Fig. 74A), brownish to blackish in color, and solitary. They form entangled and rigid tufts that are predominantly attached on rock surfaces in the intertidal zone. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended prostrate system at irregular intervals (Fig. 74B). Erect axes are radially branched in alternate to pseudodichotomous pattern at irregular intervals (Fig. 74C-D). Apical cells are inconspicuous, dome shaped, 5.28 ± 1.11 μm × 7.04 ± 1.03 μm in size, and transversely divided (Fig. 74C). Young erect axes have short segments and tapered abruptly toward the apex (Fig. 74C-D). Older segments of erect axes are larger with pericentral cells in square shape (Fig. 74G), normally 210.14 ± 47.44 μm in length and 99.60 ± 19.99 μm in diameter (L:D 0.43 ± 0.06 (L/D)], and infrequently branched (Fig. 74B, D). Trichoblasts are abundant, delicate, deciduous, short, 1–3 times forked, 90.17 ± 28.13 μm in length, and arising on each segment near the apical cells (Fig. 74D). Scar cells are inconspicuous and 8.49 ± 0.88 μm × 8.23 ± 0.99 μm in size (Fig. 74D). Cicatrigenous branches present (Fig. 74F). Adventitious branches are present. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 74H). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 322.11 ± 152.78 μm in length and 207.67 ± 90.11 μm in diameter (L:D 1.52 ± 0.23). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 74I), and cut off from the pericentral cells (Fig. 74J), 240.75 ± 37.78 μm in length, and 26.33 ± 1.32 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 74K).

**Reproductive morphology:** In female gametophytes (Fig. 75A), procarps are positioned laterally and subapically on erect axes (Fig. 75B-D), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 75C). Cystocarps are ovoid when mature

(Fig. 96D), 204.14 ± 29.41 μm in height and 217.42 ± 31.52 μm in diameter (Fig. 75E). In tetrasporangial plants (Fig. 75F), tetrasporangia are tetrahedral and 34.11 ± 7.23 μm × 41.24 ± 7.93 μm in size. Tetrasporangial branches are swollen and lanceolate (Fig. 75G-I). The development of tetrasporangia follows a spiral arrangement (Fig. 75G-I). Fertile segments have five pericentral cells (Fig. 75J-K). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 75J-K). A single tetrasporangium is produced on each fertile segment (Fig. 75J-K)

**Habitat:** Plants grow forming turfs in the intertidal zone. They are found attached on rocks in sheltered to wave-exposed areas. Turfs are usually rigid, robust, and solitary, but these plants might be associated with other species as *Champia sp.* and *Centroceras gasparrini*.

**Remarks:** *Neosiphonia sungminbooi* sp. nov. is newly described on the basis of detail morphology and molecular analyses. This species has as diagnostic features the abruptly tapered apices, wide cell walls, and very short segments. *Neosiphonia sungminbooi* sp. nov. has been previously reported from the coast of Japan as *Polysiphonia kampsaxii* sensu Segi (1951) and from the coast of Korea as *Neosiphonia simplex* sensu Lee (2008). *Polysiphonia kampsaxii* was described from Bushire, Iran by Børgesen (1939) having as diagnostic features the dark brown color, robust thallus, very thick cell walls, and long-living trichoblasts (Børgesen 1939); whereas *N. simplex* was described from California by Hollenberg (1942) having as diagnostic features the short segments and nearly black color of the plants. *Neosiphonia sungminbooi* sp. nov. is similar with these species by the dark brown color and wide segments, but it is clearly distinguished from *P. kampsaxii* by the abruptly tapered apices, deciduous trichoblasts, and small habit. *P. kampsaxii* is a large plant of around 10 cm with smooth apices (Børgesen 1939). Also, *Neosiphonia sungminbooi* sp. nov. is distinguished from *N. simplex* by having a prominent and very long trichoblasts and rigid and robust filaments. *N. simplex* is having trichoblasts primordia remained as unicellular cells and delicate filaments (Hollenberg 1942). The abruptly tapered apices relate *Neosiphonia sungminbooi* sp. nov. with *N. apiculata* and *N. notoensis. N. apiculata* is distinguished from *N. sungminbooi* by

having clumped rhizoids forming a holdfast-like structure, whereas *N. notoensis* is different from *N. sungminbooi* by having more than four pericentral cells.

***Neosiphonia tuticorinensis* (Børgesen) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 76-77).**

**Basonym:** *Polysiphonia tuticorinensis* Børgesen.

**Type locality:** Hare Island, Tuticorin, Tamil Nadu, India.

**Distribution**: Indic Ocean

**Specimens examined:** CUK13834, CUK13836 (Pudumatam, Ramanathapuram, Tamil Nadu, India, collected by T.O.C. and D.E.B., Feb. 10 2015).

**Vegetative morphology:** Plants are 0.2-0.9 cm high (Fig. 76A), reddish to purplish in color, associated with other filamentous species. Plants form entangled and delicate tufts that are predominantly attached on rock, corals, solid surfaces or epiphyte on blades from the intertidal to subtidal zone. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended prostrate system. Erect axes are radially branched in an alternate pattern at intervals of 3-12 axial cells (Fig. 76B-C) forming corymbose apex. Apical cells are prominent, dome shaped, 5.61 ± 0.87 μm × 5.41 ± 0.67 μm in size, and transversely divided (Fig. 76D). Young erect axes have short segments, they are incurved and tapered toward the apex (Fig. 76C). Older segments of erect axes are larger with pericentral cells in square shape, normally 89.96 ± 7.95 μm in length and 138.30 ± 25.65 μm in diameter (L:D 0.67 ± 0.12), and branched (Fig. 76A). Trichoblasts are abundant, short, delicate, deciduous, 1–3 times forked, 30.11 ± 6.94 μm in length, and arising on each segment near the apical cells (Fig. 106E). Scar cells are inconspicuous and 5.89 ± 1.19 μm × 7.17 ± 1.23 μm in size (Fig. 76E). Adventitious branches are present. Each segment is completely

ecorticated along the thallus and composed of four pericentral cells (Fig. 76G). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 53.71 ± 3.17 μm in length and 79.31 ± 5.98 μm in diameter (L:D 0.68 ± 0.07) (Fig. 76A). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 76G), and cut off from the pericentral cells (Fig. 76G-H), 260.24 ± 22.63 μm in length, and 25.04 ± 4.56 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 76I).

**Reproductive morphology:** In female gametophytes (Fig. 77A), procarps are positioned laterally and subapically on erect axes (Fig. 77B-D), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 77C). Cystocarps are ovoid and urceolate when mature (Fig. 77D), 168.82 ± 24.88 μm in height and 162.62 ± 16.92 μm in diameter. In male gametophytes (Fig. 77E), spermatangial branches are radially clustered at the apices of erect axes (Fig. 77F), and developed on a furcation of trichoblasts (Fig. 77G). In tetrasporangial plants (Fig. 77H-I), tetrasporangia are tetrahedral and 29.77 ± 4.38 μm × 27.37 ± 2.94 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 77J-K). The development of tetrasporangia follows a spiral arrangement (Fig. 77J-K). Fertile segments have five pericentral cells (Fig. 77L-M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 77L-M). A single tetrasporangium is produced on each fertile segment (Fig. 77L-M)

**Habitat:** Plants grow forming small tufts from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades of *Grateloupia sp.* in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other filamentous species as *Centroceras sp.* and *Spyridia sp.*

**Remarks:** *Neosiphonia tuticorinensis* comb. nov. was originally described as *Polysiphonia tuticoriensis* from the coast of India by Børgesen (1938). This species was described as different species by having large cells around the ostiole of cystocarps (Børgesen 1938). We do not consider this

character as diagnostic in *N. tuticoriensis* because this character has been commonly reported in *Polysiphonia* sensu lato. The absence of adventitious branches in combination with the common *Neosiphonia* features present in *N. tuticoriensis* separate it from other *Neosiphonia*. Moreover, the corymbose disposition of apical branches also separate from other *Neosiphonia*. This species is transferred to Neosiphonia by the presence of the three-celled carpogonial branches and the rhizoids cutting off from pericentral cells (Kim and Lee 1999). In our molecular analyses, *N. tuticoriensis* was closely related to *N. collabens*, but the latter is distinguished from the former by having ramysimpodial branching pattern (Phillips 2010, Diaz-Tapia and Bárbara 2013)

***Neosiphonia unguiformis* (Børgesen) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 78-79).**

**Basonym:** *Polysiphonia unguiformis* Børgesen.

**Type locality:** Shingly Island, Tamil Nadu, India.

**Distribution**: India

**Specimens examined:** CUK13801 (Mandapam beach, Vedalai, Ramanathapuram, Tamil Nadu, India collected by T.O.C. and D.E.B., Feb. 09 2015), CUK13832, CUK13833 (Pudumatam, Ramanathapuram, Tamil Nadu, India collected by T.O.C. and D.E.B., Feb. 10 2015), CUK13954, CUK13955 (Kanyakumari beach, Kanyakumari, Tamil Nadu, India collected by T.O.C. and D.E.B., Feb. 12 2015)

**Vegetative morphology:** Plants are 0.3-1.2 cm high (Fig. 78A), reddish to brownish in color, associated with other filamentous species. Plants form entangled and delicate tufts that are predominantly attached on rock, corals, solid surfaces or epiphyte on blades from the intertidal to subtidal zone. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously

from a reduced prostrate system at irregular intervals. Erect axes are radially branched in an alternate to subdichotomous pattern forming corymbose apex at intervals of 2-7 axial cells (Fig. 78B-C). Apical cells are prominent, dome shaped, 4.93 ± 1.03 μm × 5.09 ± 1.12 μm in size, and transversely divided (Fig. 78D). Young erect axes have short segments, they are incurved and tapered toward the apex. Older segments of erect axes are larger, normally 130.35 ± 15.20 μm in length and 101.50 ± 13.90 μm in diameter (L:D 0.79 ± 0.13), and infrequently branched(Fig. 78B). Trichoblasts are scarce, short, delicate, deciduous, 1–2 times forked, 32.63 ± 8.82 μm in length, and arising on each segment near the apical cells (Fig. 78E). Scar cells are inconspicuous. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 78E-F). Adventitious branches are present (Fig. 78G). Lateral branches replace trichoblasts. The prostrate system is reduced and entangled, with axial segments 59.39 ± 9.44 μm in length and 68.54 ± 4.11 μm in diameter (L:D 0.87 ± 0.13). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 78I), and cut off from the pericentral cells (Fig. 78J), 307.36 ± 49.04 μm in length, and 20.16 ± 2.97 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 78K).

**Reproductive morphology:** In female gametophytes (Fig. 79A), procarps are positioned laterally and subapically on erect axes (Fig. 79A), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 79B-C). Cystocarps are ovoid when mature (Fig. 79D), 160.93 ± 18.12 μm in height and 153.63 ± 22.15 μm in diameter. In male gametophytes (Fig. 79E), spermatangial branches are radially clustered at the apices of erect axes (Fig. 79F), paired and developed on a furcation of trichoblasts (Fig. 79G). In tetrasporangial plants (Fig. 79H), tetrasporangia are tetrahedral and 30.53 ± 2.96 μm × 38.09 ± 3.21 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 79I). The development of tetrasporangia follows a spiral arrangement (Fig. 79I-J). Fertile segments have five pericentral cells (Fig. 79K). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 79K). A single tetrasporangium is produced on each fertile segment (Fig. 79K)

**Habitat:** Plants grow forming small tufts from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades of *Grateloupia sp.* and leaves of sea grass in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other filamentous species as *Centroceras sp.*, *Laurencia sp*., and *Spyridia sp.*

**Remarks:** *Neosiphonia unguiformis* comb. nov. was originally described from the coast of India by Børgesen (1937). This species was distinguished from other *Polysiphonia* sensu lato by having branches disposed several times pseudodichotomous (Børgesen 1937). Although our samples are showing the branches disposed several times subdichotomous to sometimes alternate, the other morphological features are in agreement with the original description. We are transferring this species to *Neosiphonia* based on the three-celled carpogonial branches and rhizoids cutting off pericentral cells. The placement in *Neosiphonia* is also supported by our phylogenetic analyses of the *rbc*L sequences. This also analyses also show that *N. unguiformis* is closely related to *N. pulvinata f. parvula*, but the latter is distinguished from the former by having clumped rhizoids forming a holdfast-like structure and thinner filaments.

***Neosiphonia yendoi* (Segi) M.S. Kim et I.K. Lee (Fig. 80-82).**

**Basonym:** *Polysiphonia yendoi* T.Segi.

**Type locality:** Japan.

**Homotypic synonyms:** *Polysiphonia yendoi* T.Segi.

**Heterotypic synonyms:** *Polysiphonia obsoleta* Segi, *Polysiphonia cystophyllicola* Noda, *Polysiphonia latiovalis* Noda.

**Distribution**: Japan, China, Korea, and Russia.

**Specimens examined:** CUK6679 (Sinheung beach, Cheongsando, Cheongsanmyeon, Wando, Jeollanamdo, Korea, collected by T.O.C., Aug. 19 2009), CUK7418 (Gunnaeri, Wando, Jeollanamdo, Korea, collected by T.O.C. and D.E.B., Jun. 16 2011), CUK7568 (Gampohang, Gampoeub, Gyeongjusi, Gyeongsangbugdo, Korea, collected by T.O.C. and D.E.B., Dec. 14 2011), CUK9178 (Anin beach, Gangdong-myeon, Gangneung, Gangwondo, Korea, collected by T.O.C. and D.E.B., Nov. 14 2012), CUK9706 (Udodeungdae, Udomyeon, Jeju Island, Korea, collected by T.O.C. and D.E.B., Apr. 26 2013), CUK9909 (Chujahang, Chujado, Chujamyeon, Jeju Island, Korea, collected by T.O.C. and D.E.B., Jun. 24 2013), CUK12819 (Hajodae, Hyeonbukmyeon, Yangyang, Gangwondo, Korea, collected by T.O.C. and D.E.B., Jul. 31 2014), CUK12864 (Yeonjiri, Uljineup, Uljin, Gyeongsangbukdo, Korea, collected by T.O.C. and D.E.B., Aug. 01 2014)

**Vegetative morphology:** Plants are 1.4-4.7 cm high (Fig. 80A-E), reddish to brownish in color, associated with other filamentous species. Plants form entangled and delicate tufts that are predominantly attached on rock, corals, solid surfaces or epiphyte on blades from the intertidal to subtidal zone. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from a reduced prostrate system. Erect axes are radially branched in an alternate to pseudodichotomous pattern at intervals of 6-8 axial cells, forming corymbose apex (Fig. 80A-81A). Apical cells are prominent, dome shaped, 4.73 ± 0.82 μm × 4.99 ± 0.60 μm in size, and transversely divided. Young erect axes have short segments, they are incurved and tapered opposite to the apex (Fig. 81B). Older segments of erect axes are larger, normally 69.74 ± 9.63 μm in length and 97.46 ± 22.87 μm in diameter (L:D 0.77 ± 0.18), and infrequently branched (Fig. 81C). Trichoblasts are abundant, large, delicate, deciduous, 1–3 times forked, 97.14 ± 41.28 μm in length, and arising on each segment near the apical cells (Fig. 81B). Scar cells are conspicuous, spirally disposed, and 11.22 ± 1.51 μm × 7.56 ± 1.95 μm in size (Fig. 81C). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 81D-E). Adventitious branches are present (Fig. 81F). Lateral branches replace trichoblasts. The prostrate system is reduced and entangled, with axial segments 115.90 ± 11.24 μm in length and 122.36 ± 8.83 μm in diameter (L:D 1.05 ±

0.13). Rhizoids are ventrally produced from the proximal end of the pericentral cells (Fig. 81G), and cut off from the pericentral cells (Fig. 81H-I), 268.15 ± 109.31 μm in length, and 32.24 ± 6.73 μm in diameter. Rhizoids are unicellular and produce lobed termination when mature (Fig. 81J).

**Reproductive morphology:** In female gametophytes (Fig. 82A-B), procarps are positioned laterally and subapically on erect axes (Fig. 82C), and are composed of a supporting cell bearing a three-celled carpogonial branch and a basal sterile cell (Fig. 82D). Cystocarps are ovoid when mature (Fig. 82E), 234.31 ± 25.04 μm in height and 217.45 ± 28.52 μm in diameter. In male gametophytes (Fig. 82F), spermatangial branches are radially clustered at the apices of erect axes (Fig. 82G-H), and developed on a furcation of trichoblasts (Fig. 82H). In tetrasporangial plants (Fig. 82I), tetrasporangia are tetrahedral and 38.17 ± 5.29 μm × 36.11 ± 7.61 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 82J). The development of tetrasporangia follows a spiral arrangement (Fig. 82J-K). Fertile segments have five pericentral cells (Fig. 82L-M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 82L-M). A single tetrasporangium is produced on each fertile segment.

**Habitat:** Plants grow forming small tufts widely distributed from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades of *Grateloupia sp.* and leaves of sea grass in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other filamentous species as *Neosiphonia japonica*, *N. decumbens*, and *N. harlandii.*

**Remarks:** *Neosiphonia yendoi* was originally described by Segi (1951) from Japan as *Polysiphonia yendoi.* This species has been widely reported from the coast of Japan, Korea, and China (Segi 1951, Lee and Kang 2001, Nam and Kang 2012). Our morphological and molecular study demonstrated that the species *N. elongella*, *N. sertulariodes*, *N. simplex*, *N. sphaerocarpa*, and *N. tongatensis* reported from the Korean coast sensu Nam and Kang (2012) should be named as *N. yendoi.* The molecular comparisons among these taxa show less that 0.6% for *rbc*L of sequences diver-

gence. Thereby, *N. yendoi* is a wide distributed species along the Korean coast showing a wide phenotypic plasticity (Fig. 80 A-E).

## 2. Phylogenetic analyses

A 1,301-bp portion of the 1,467- bp *rbc*L (88.7%) was sequenced for 22 species of *Neosiphonia* and other *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus placed the monophyletic genus *Neosiphonia* sister to the clade composed by *P. amplacapilli*, *P. morroides*, *P. pentamera*, *P. schneideri* ("*P. schneideri* clade") with sequences divergence of 8.4% to 10.7% between them and supported by a bootstrap value of 78 and a posterior probability of 0.99 (Fig. 83). The sequence divergences among all the species of *Neosiphonia* are ranging from 2.1% between *N. ramireziae* and *N. peruviensis* to 9.4% between *N. collabens* and *N. cockeri* for *rbc*L.

## 3. Discussion

Our *rbc*L phylogenies reveal that the 22 species characterized in this study are well supported as *Neosiphonia* members (Fig. 83). The morphological observations of these species agree with the features of *Neosiphonia*. The three-celled carpogonial branches and the rhizoids cutting off the pericentral cells result in consistent characters to place these species in *Neosiphonia*. Also, these characters are useful to separate *Neosiphonia* from *Polysiphonia* sensu lato. Among the 22 species reported in the present study nine are new species (three already published) and nine are new combinations. These species were distinguished on the basis of diagnostic features at specific level as the nature of holdfast and prostrate system, number of pericentral cells, branching pattern, abundance of trichoblasts, tetrasporangia arrangement, adventitious branches origin, number of cells per fertile segment in the tetrasporophyte, and spermatangia development (Table 5). The distinction of species in our phylogenetic analyses for *rbc*L followed the threshold proposed by Mamoozadeh and

Freshwater (2011), who established sequence divergences below 1.3 % represent intraspecific variation and those greater than 2.13 % represent interspecific variation in *Polysiphonia* sensu lato. Our study suggests *Neosiphonia* as a wide distributed genus with a high diversity of species especially in the eastern Indic Ocean, evolutionary studies might clarify the origin of this genus.

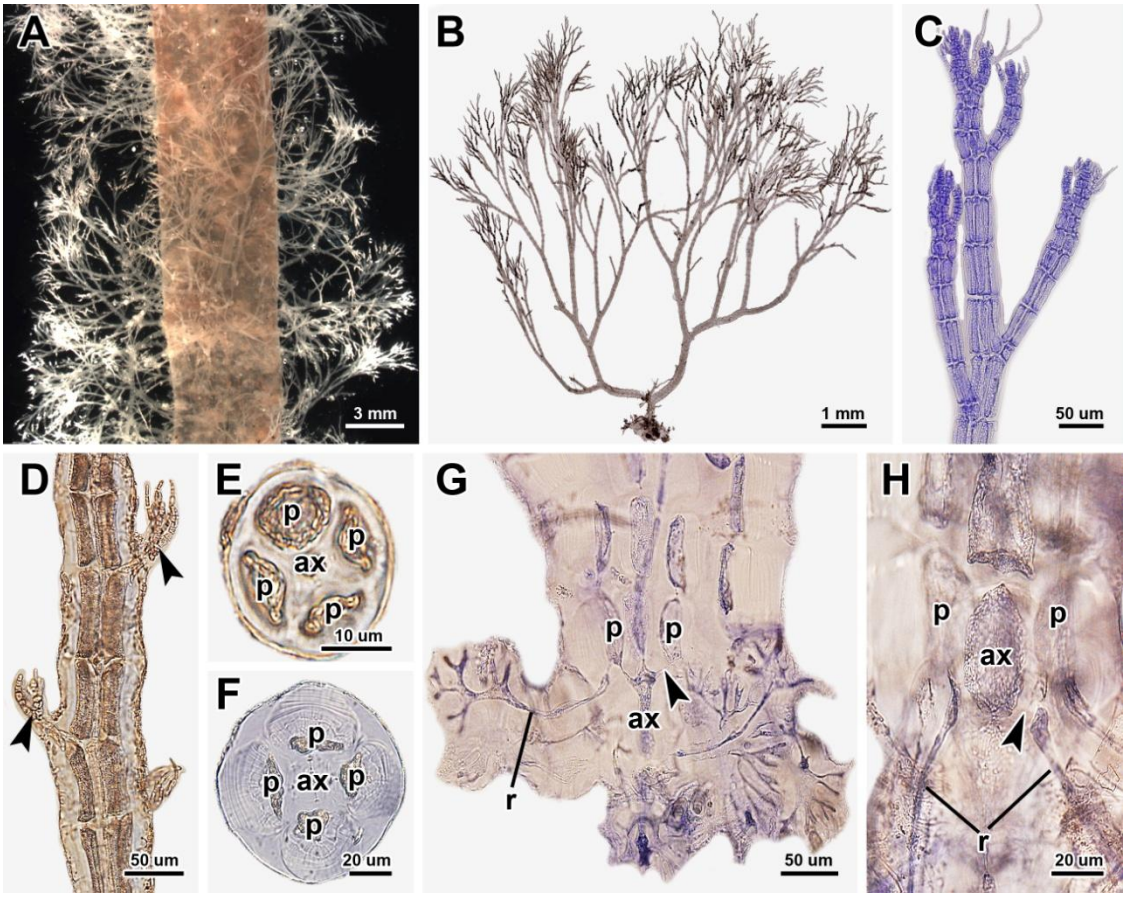


**Fig. 40.** Vegetative structures of *Neosiphonia apiculata.* (A) Plants epiphyte on *Zostera sp.* (B) Habit of vegetative plant showing the holdfast and extended erect axes. (C) Apex showing alternate to pseudodichotomous branching pattern. (D) Erect axes showing cicatrigenous branches (arrowhead) spirally disposed. (E-F) Cross-section of axes with four pericentral cells (p) from the middle (E) and basal part (F) (ax, axial cell). (G) Basal part of axes showing clumped unicellular rhizoids (r) cutting off (arrowhead) from pericentral cells (p). (H) Rhizoids (r) cutting off from (arrowhead) proximal end of pericentral cell (p).

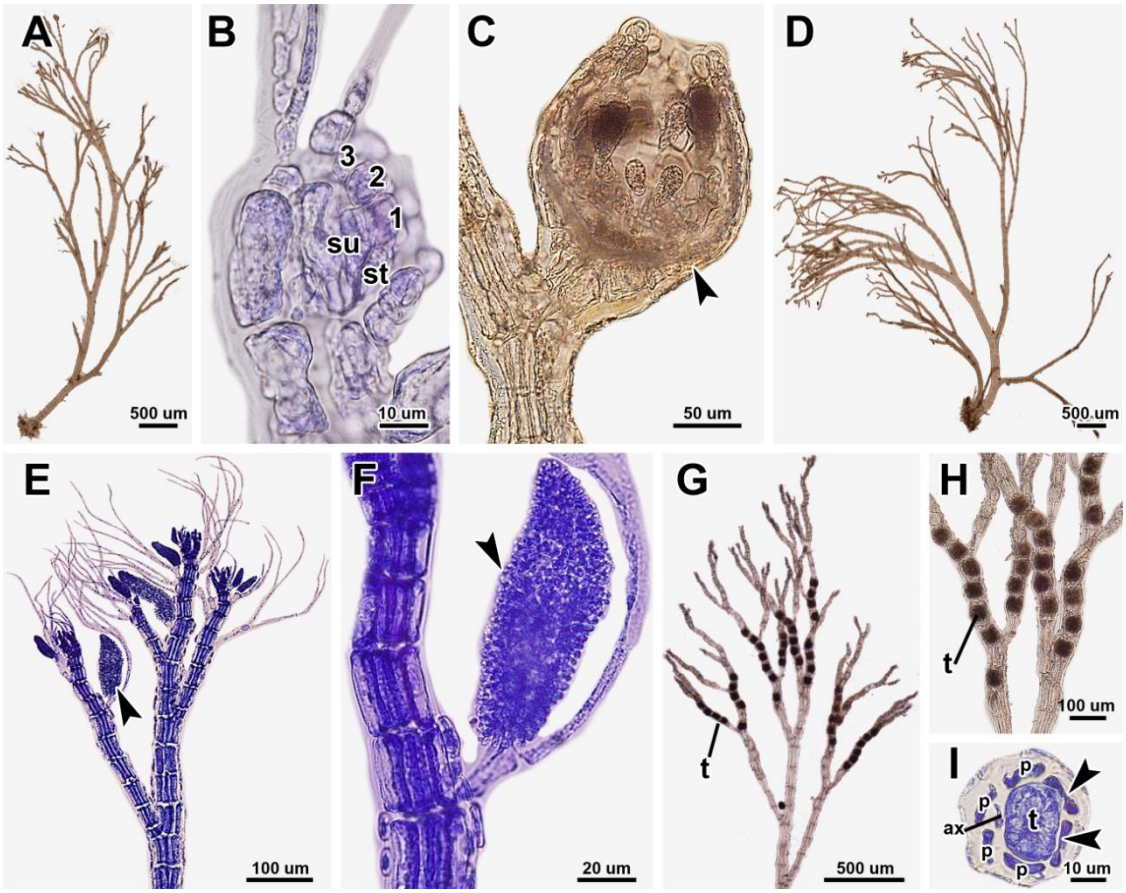


**Fig. 41.** Reproductive structures of *Neosiphonia apiculata*. (A) Female thallus. (B) Procarp with three-celled carpogonial branch (1-3, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (C) Mature cystocarp (arrowhead) with globose shape. (D) Male thallus. (E) Spermatangial branches (arrowhead) clustered at apical region. (F) Mature spermatangial branch (arrowhead) arising from trichoblast. (G) Tetrasporangial thallus. (H) Branches of tetrasporangial thallus showing spiral arrangement of tetrasporangia (t). (I) Cross-section views of tetrasporangial segments with 5 pericentral cells (p) showing a single tetrasporangium (t) topped by two cover cells (arrowheads).

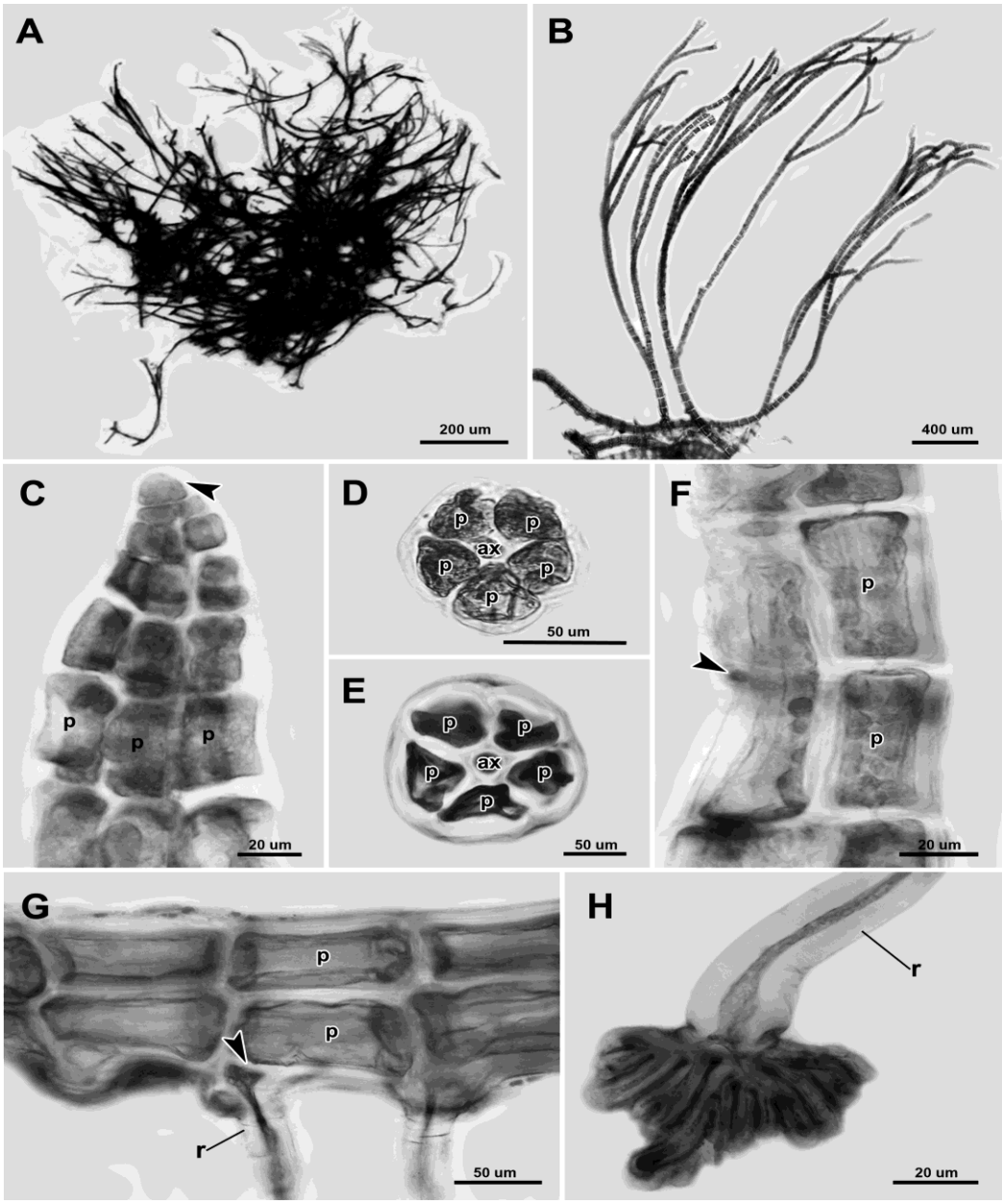


**Fig. 42.** Vegetative structures of *Neosiphonia baliana.* (A) Holotype. (B) Habit of vegetative plant showing pseudodichotomous branching pattern. (C) Apical portion showing a prominent apical cell without trichoblasts below it. (D-E) Cross sections views of upper (D) and lower (E) parts of thallus. (F) Middle part of thallus showing pericentral cells unusually fused (arrowhead). (G) Rhizoids produced from the proximal end of pericentral cells by a wall (arrowhead). (H) Unicellular rhizoid having multilobed tips. (ap, apical cell; ax, axial cell; p, pericentral cell; r, rhizoid).

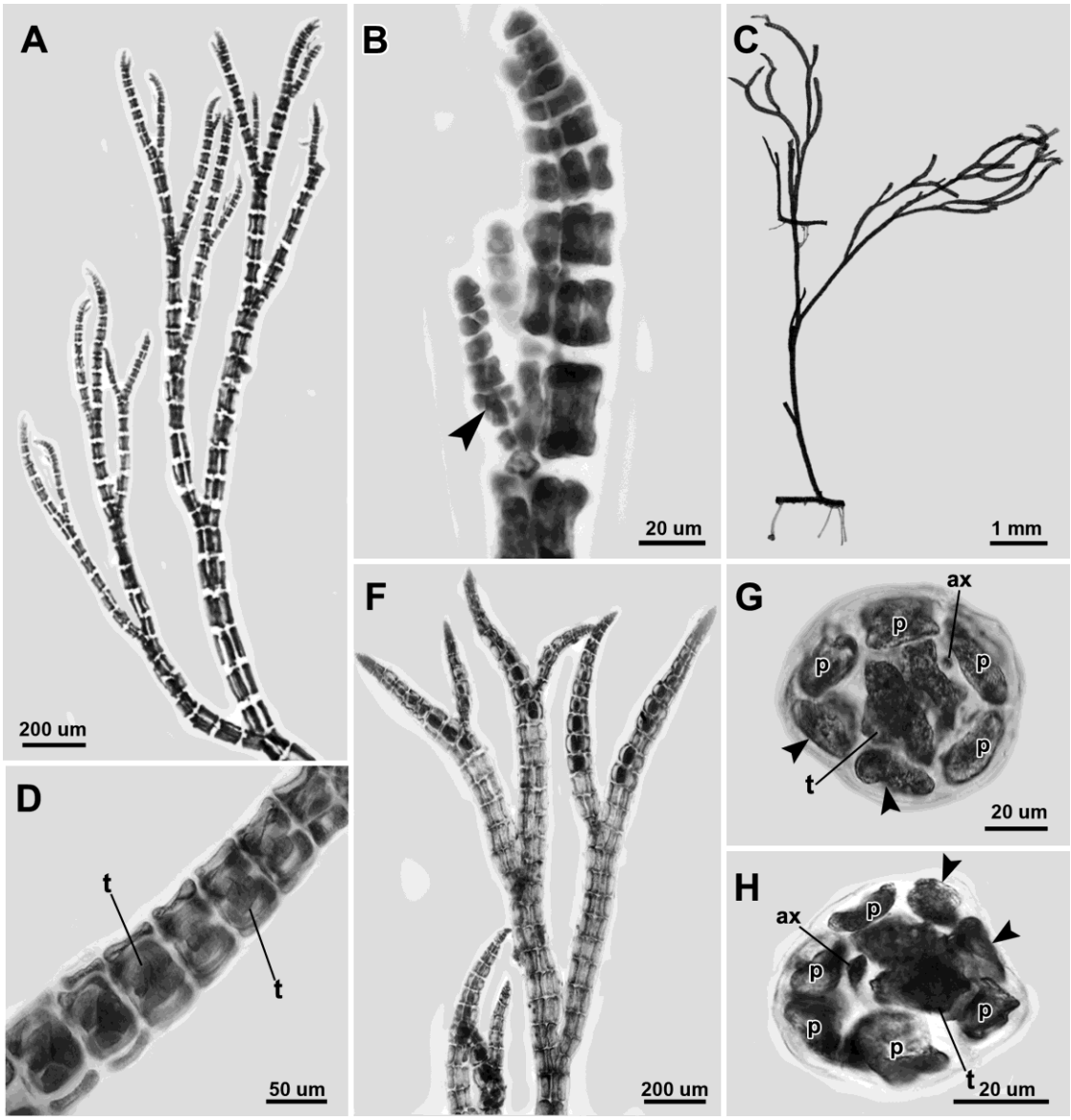


**Fig. 43.** Reproductive structures of *Neosiphonia baliana*. (A) Male plant. (B) Young spermatangia developed from one basal cell (arrowhead). (C) Tetrasporangial plant. (D) Segments of tetrasporangial thallus showing straight arrangement and one tetrasporangium in each segment. (F) Upper part of tetrasporangial thallus showing straight series of tetrasporangia with pseudodichotomous apex. (G-H) Cross section view of fertile segment showing 5 or 6 pericentral cells and tetrasporangia rounded by cover cells (arrowheads). (ax, axial cell; p, pericentral cell; t, tetrasporangium).

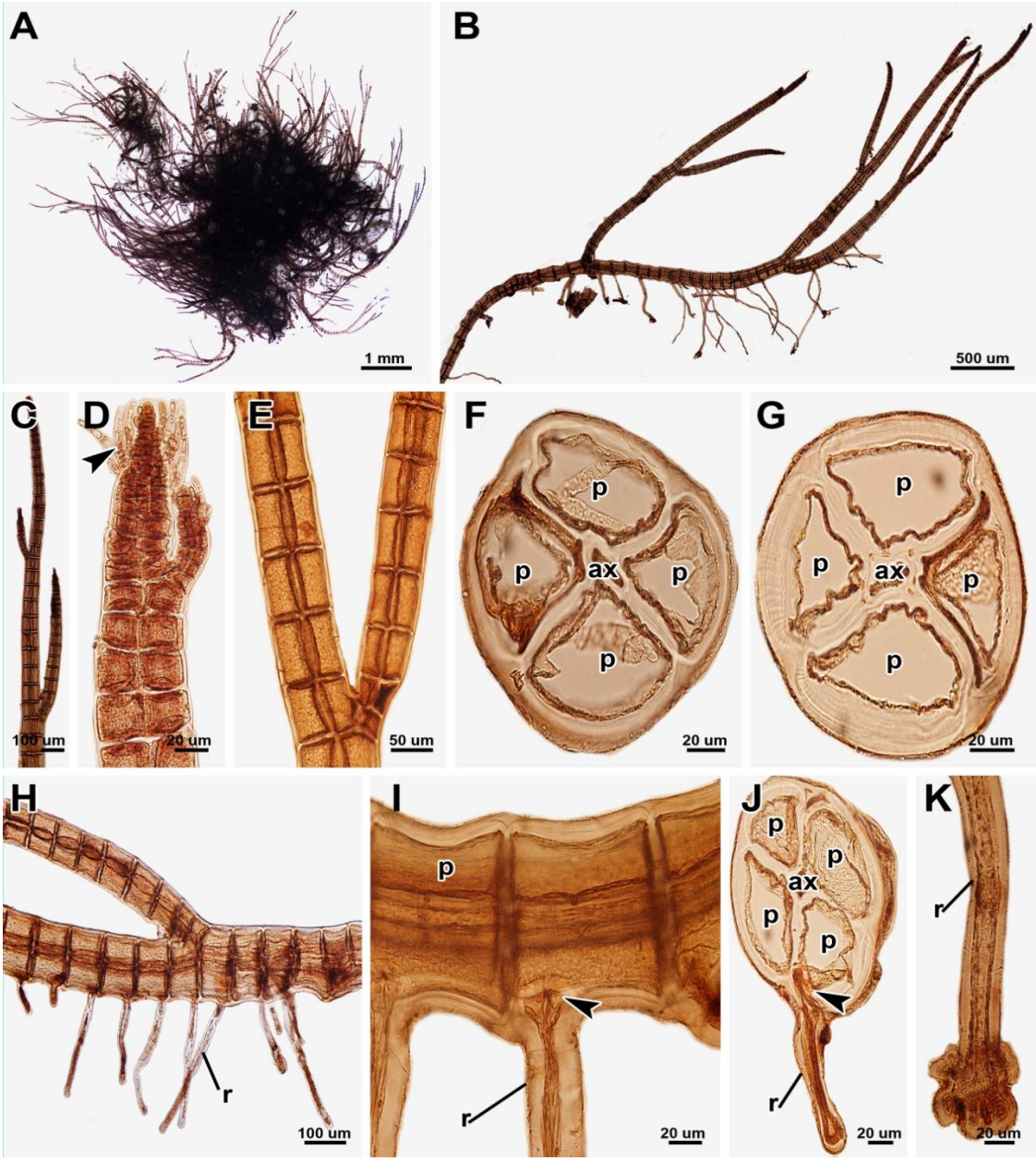


**Fig. 44.** Vegetative structures of *Neosiphonia blandii* comb. nov. (A-B) Habit of vegetative plant showing the extended prostrate and erect axes. (C) Erect axes with an alternate branching pattern. (D) Apices showing trichoblasts (arrowhead). (E) Inconspicuous scar cells (arrowhead) spirally disposed in erect axes. (F-G) Cross-sections showing four pericentral cells (p) of erect (F) and prostrate (G) axes (ax, axial cell). (H) Rhizoids (r) scattered and produced from prostrate axes. (I) Rhizoid (r) cutting off (arrowhead) from proximal end of pericentral cell (p). (J) Cross-section of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (K) Unicellular terminations of rhizoids (r).

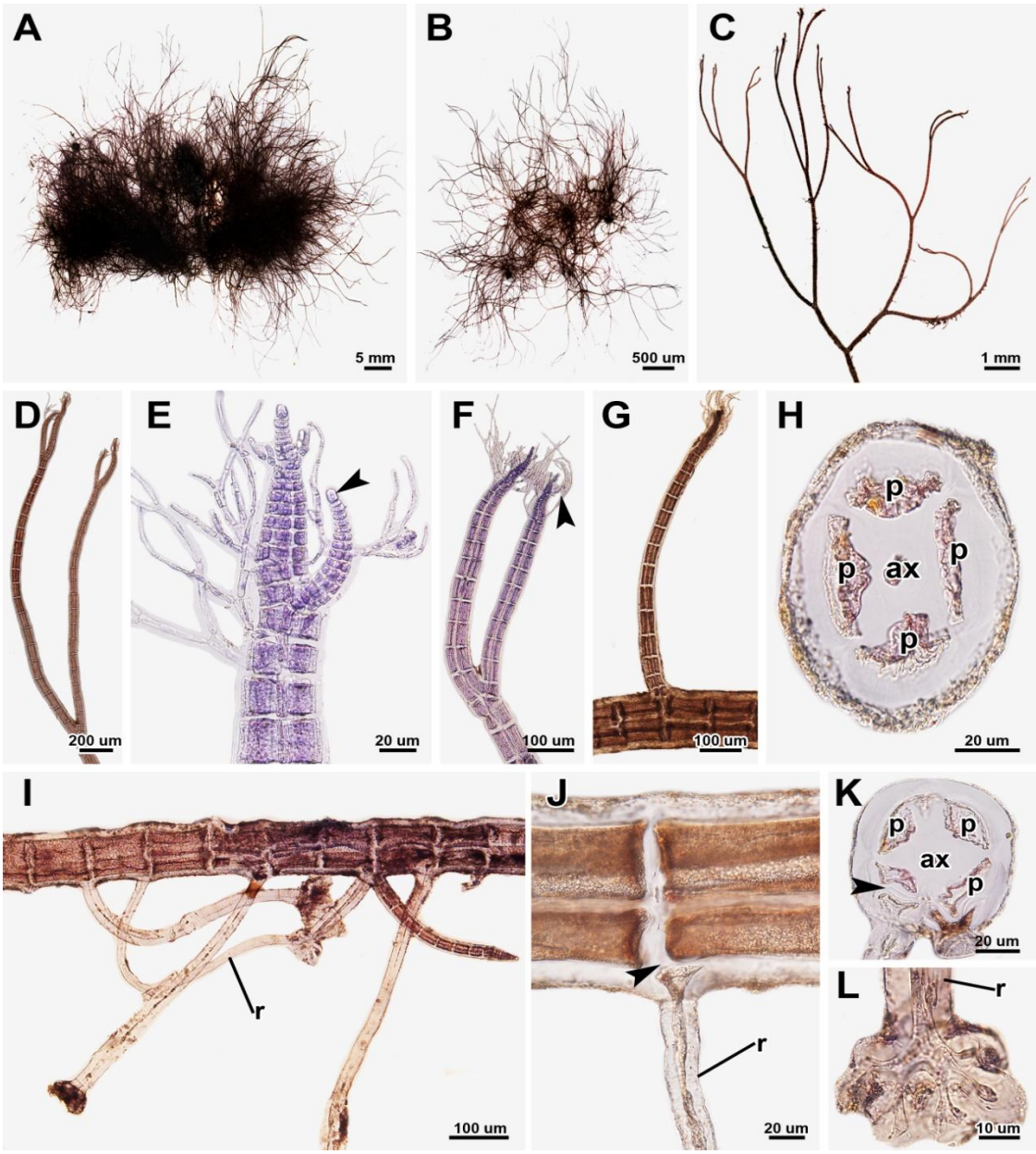


**Fig. 45.** Vegetative structures of *Neosiphonia coacta* comb. nov. (A-B) Tuft and habit of vegetative plant showing the extended prostrate and erect axes. (C-D) Erect axes with pseudodichotomous branching pattern. (E) Apices showing prominent and dome shaped apical cells (arrowhead). (F) Apices showing abundant trichoblasts (arrowhead). (G) Adventitious branch (arrowhead) on prostrate axes. (H) Cross-section view showing four pericentral cells (p) (ax, axial cell). (I) Rhizoids (r) scattered and produced from prostrate axes. (J) Rhizoid (r) cutting off (arrowhead) from proximal end of pericentral cell (p). (K) Cross-section of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (L) Unicellular terminations of rhizoids (r).

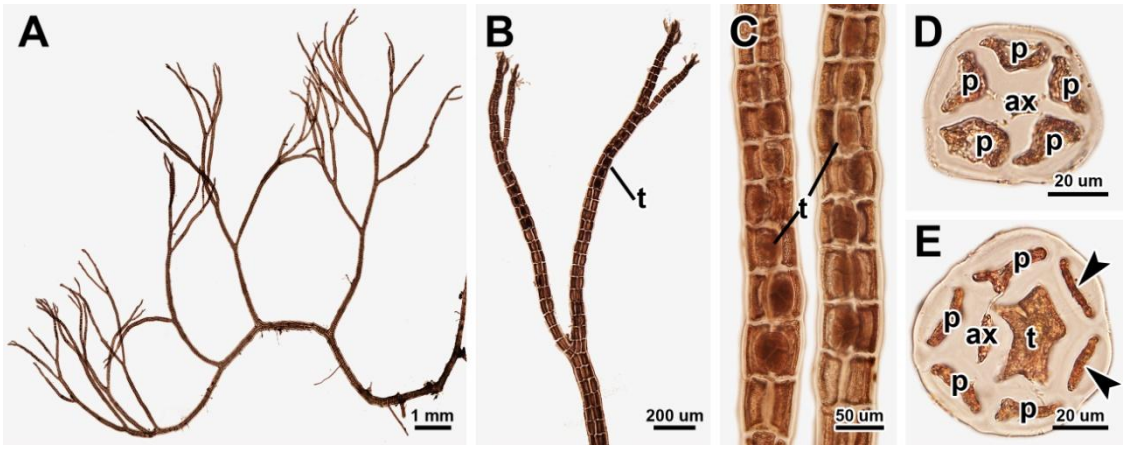


**Fig. 46.** Reproductive structures of *Neosiphonia coacta* comb. nov. (A) Tetrasporangial plant. (B) Apical branch showing subapical tetrasporangia (t). (C) Apical branch showing tetrasporangia (t) in spiral series. (D-E) Cross-sections with five pericentral cells (p) in the fertile segment (D) and also showing a tetrasporangium (t) rounded by cover cells (arrowheads) (E).

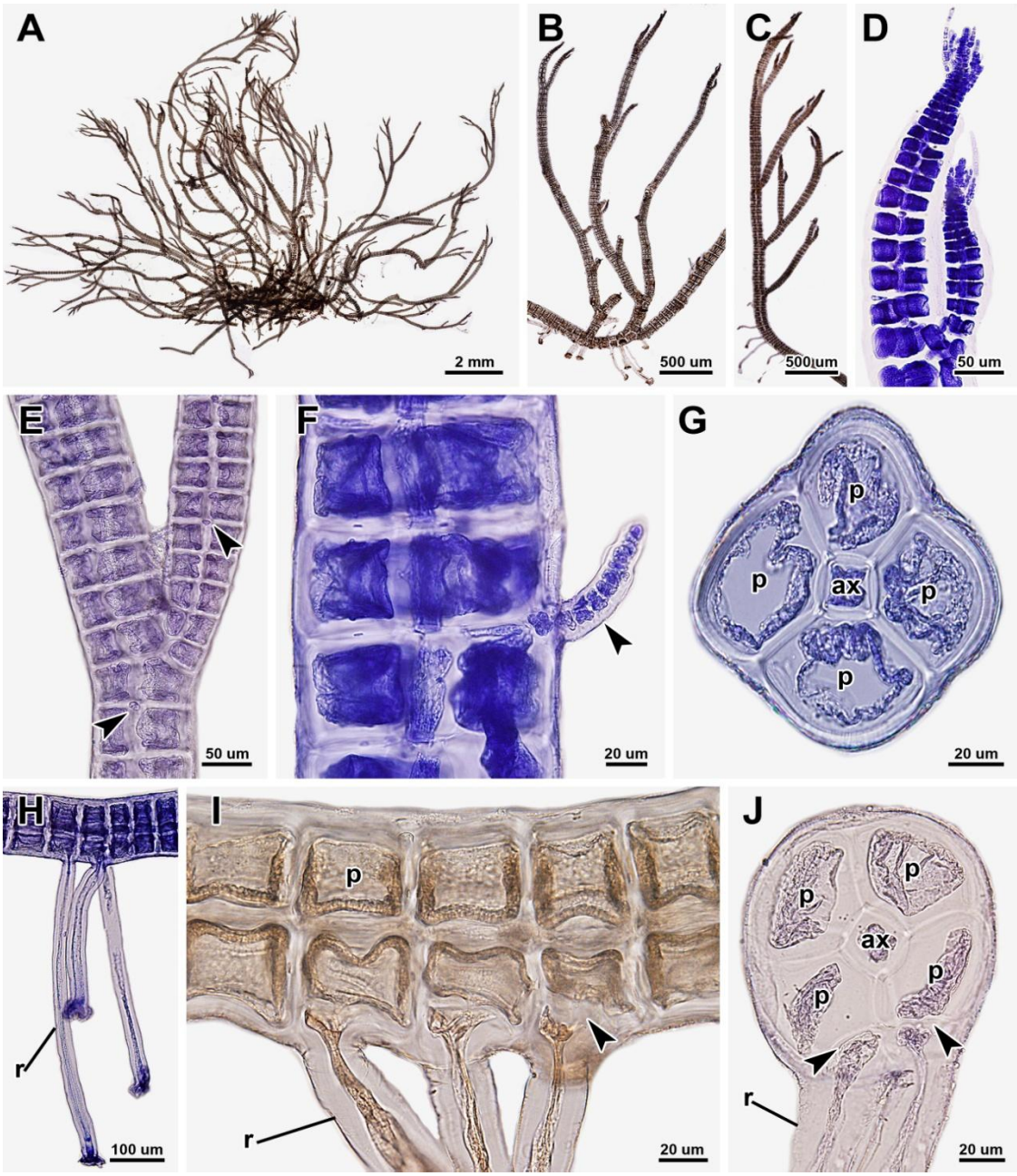


**Fig. 47.** Vegetative structures of *Neosiphonia cockeri* comb. nov. (A-C) Habit of vegetative plant showing the extended prostrate and erect axes. (D) Young erect axes with subdichotomous to distichous branching pattern. (E) Scar cells (arrowhead) spirally disposed in erect axes. (F) Erect axis showing cicatrigenous branch (arrowhead). (G) Cross-sections view showing four pericentral cells (p) (ax, axial cell). (H) Long rhizoids (r) scattered and produced from prostrate axes. (I) Rhizoids (r) cutting off from the proximal end of pericentral cells (p). (J) Cross-section view of a prostrate axis showing rhizoids (r) cutting off from pericentral cells (p).

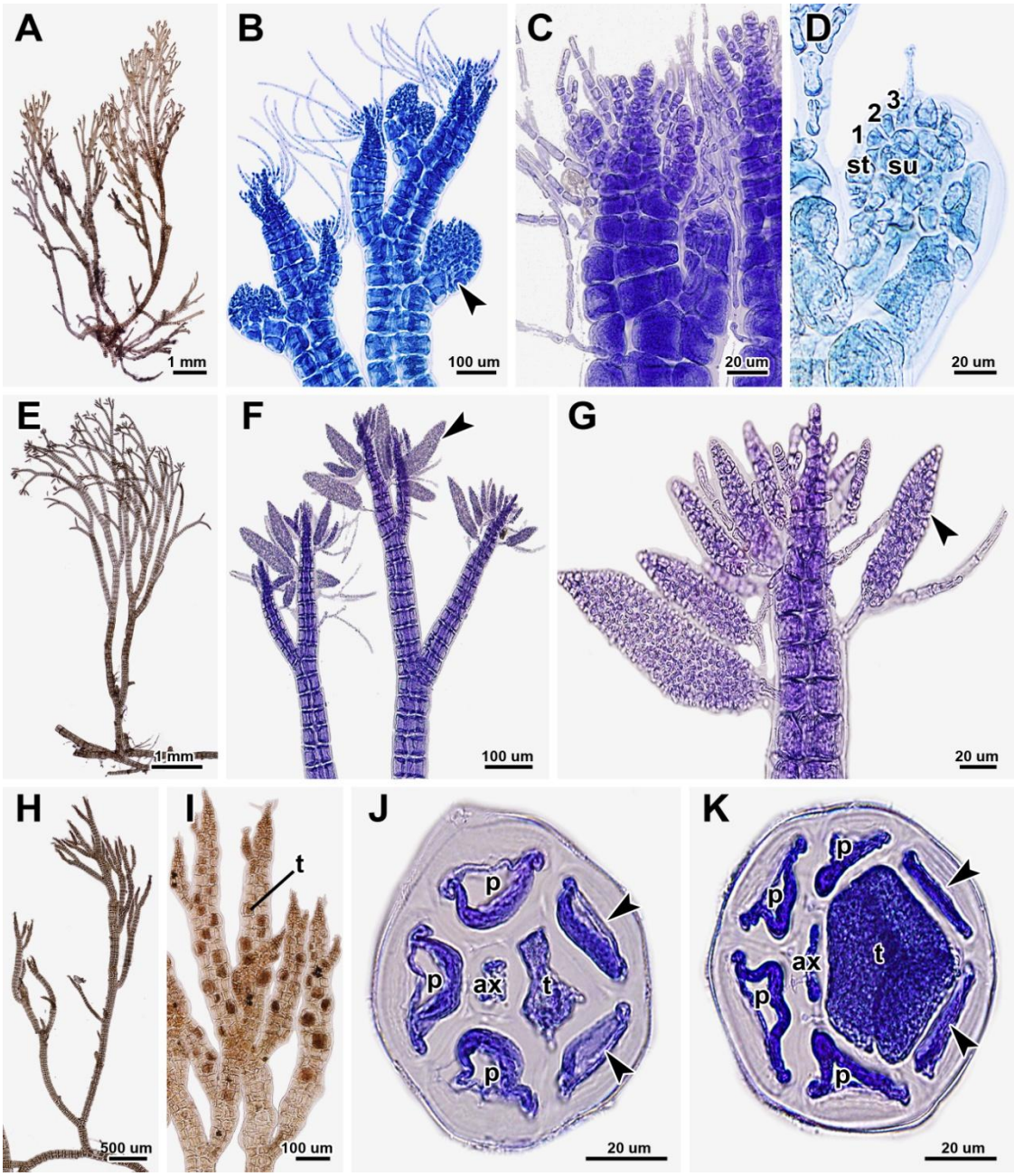


**Fig. 48.** Reproductive structures of *Neosiphonia cockeri* comb. nov. (A) Female plant. (B-C) Upper part of female thallus showing subapical cystocarps (arrowhead). (D) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (E) Male plant. (F) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (G) Spermatangial branches (arrowhead) arising from trichoblast. (H) Tetrasporangial thallus. (I) Apical branches showing tetrasporangia (t) in spiral series. (J-K) Cross-section with four (J) and five (K) pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

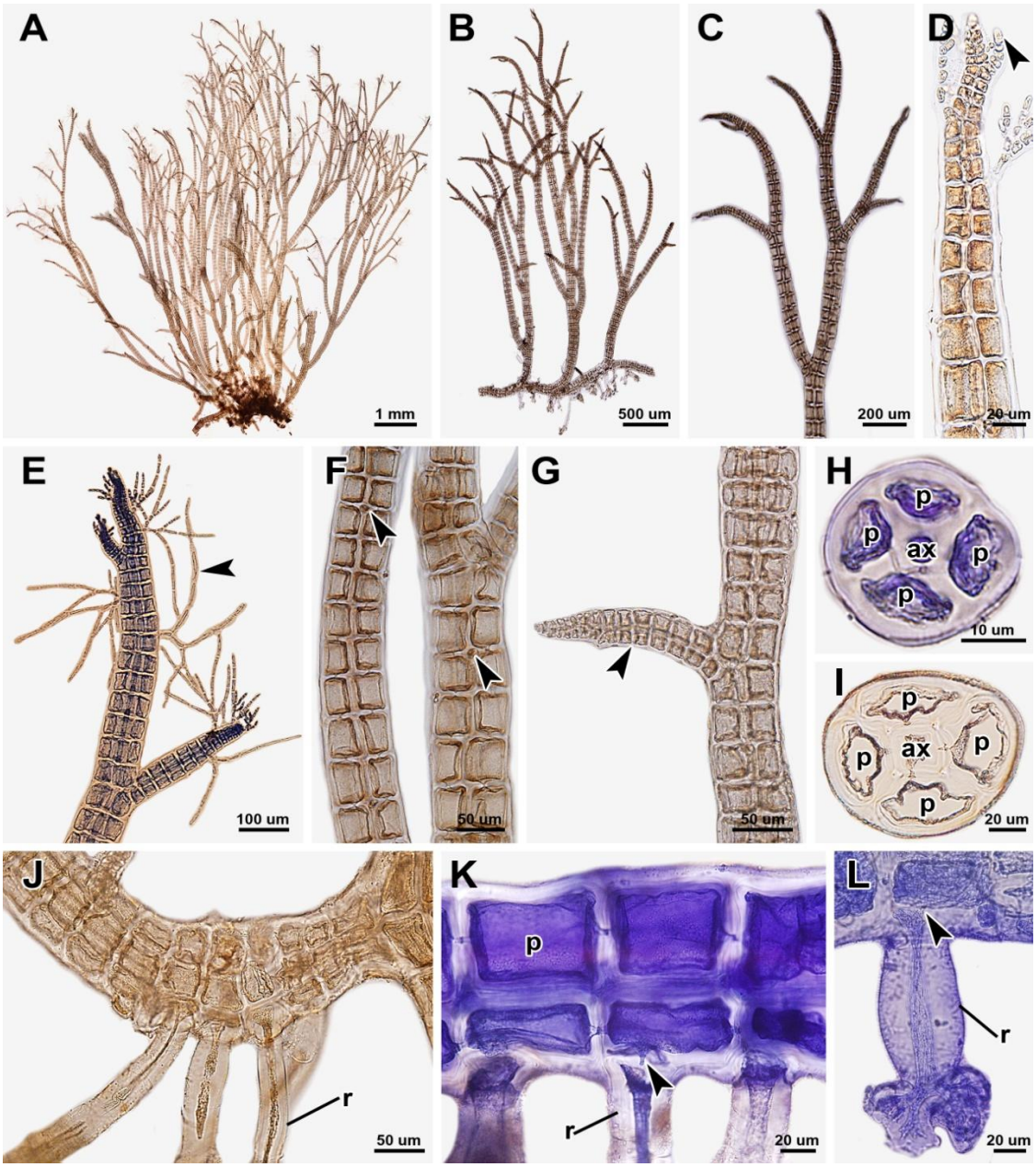


**Fig. 49.** Vegetative structures of *Neosiphonia ecuatoriana* sp. nov. (A-B) Habit of vegetative plant showing the extended prostrate and erect axes. (C) Erect axes with pseudodichotomous branching pattern. (D) Apices showing prominent and dome shaped apical cells (arrowhead). (E) Apex showing abundant trichoblasts (arrowhead). (F) Scar cells (arrowhead) spirally disposed in erect axes. (G) Erect axis showing an adventitious branch (arrowhead). (H-I) Cross-section views showing four pericentral cells (p) (ax, axial cell). (J) Rhizoids (r) scattered and produced from prostrate axes. (K) Rhizoids (r) cutting off from the proximal end of pericentral cells (p). (L) Unicellular terminations of rhizoid (r).

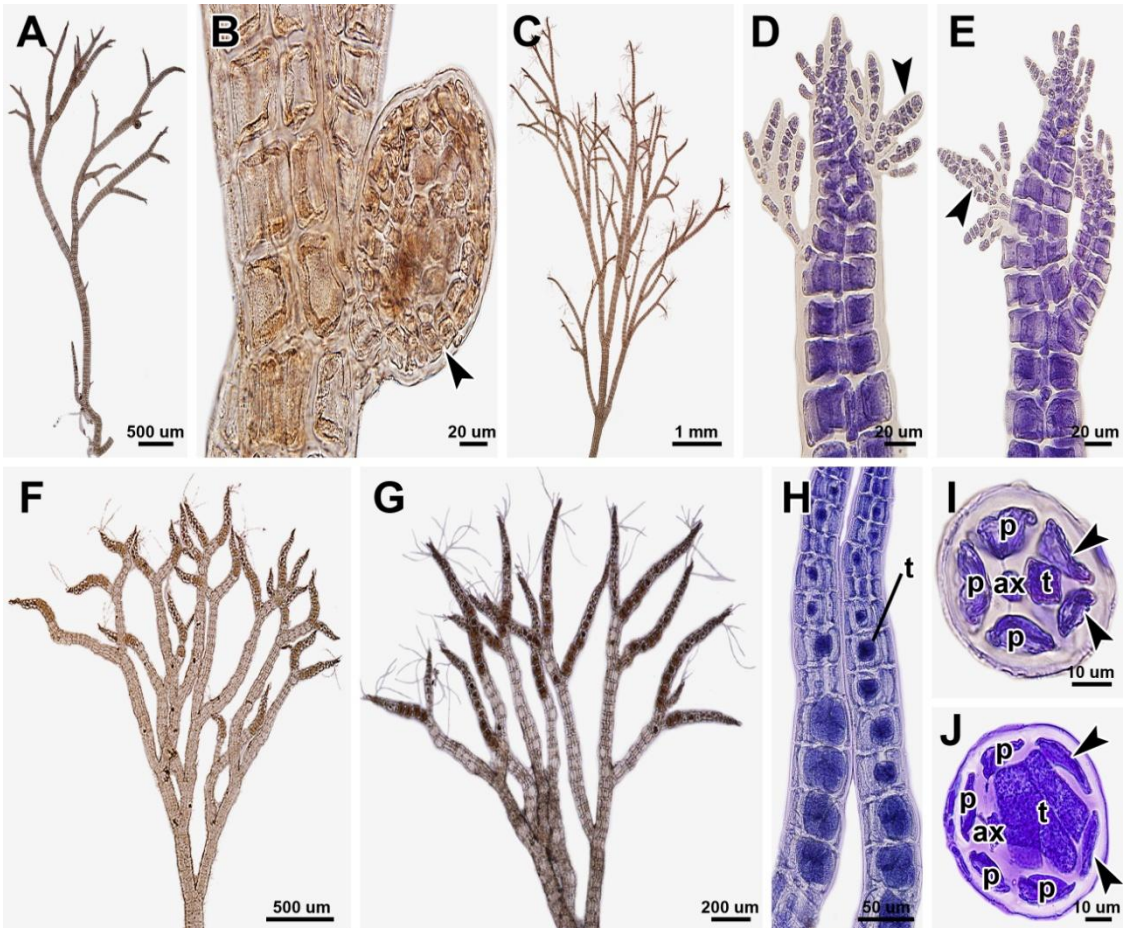


**Fig. 50.** Reproductive structures of *Neosiphonia ecuatoriana* sp. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps (arrowhead). (C) Male plant. (D-E) Spermatangial branches (arrowhead) clustered at the apices of erect axes and arising from trichoblast. (F-G) Tetrasporangial thallus. (H) Apical branches showing tetrasporangia (t) in straight series. (I-J) Cross-section with four (I) and five (J) pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

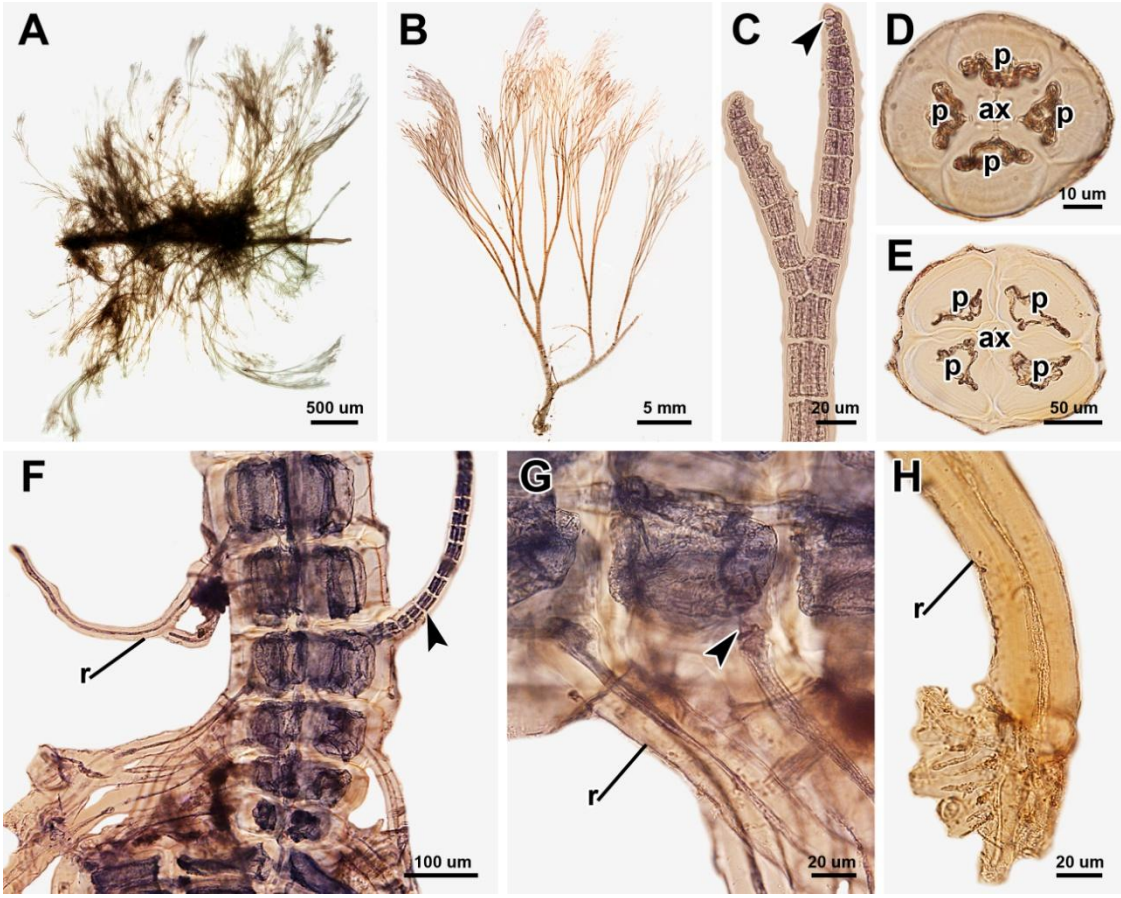


**Fig. 51.** Vegetative structures of *Neosiphonia gorgoniae*. (A) Plants epiphyte on *Gracilaria sp.* (B) Habit of vegetative plant showing the very reduced prostrate and extended erect axes. (C) Apex showing prominent apical cells (arrowhead) lacking of trichoblasts. (D-E) Cross-section of axes with four pericentral cells (p) from the middle (D) and basal part (E) (ax, axial cell). (F) Basal part of axes showing clumped unicellular rhizoids (r) and cicatrigenous branches (arrowhead) (G) Rhizoids (r) cutting off (arrowhead) from pericentral cells (p). (H) Unicellular terminations of rhizoid (r).

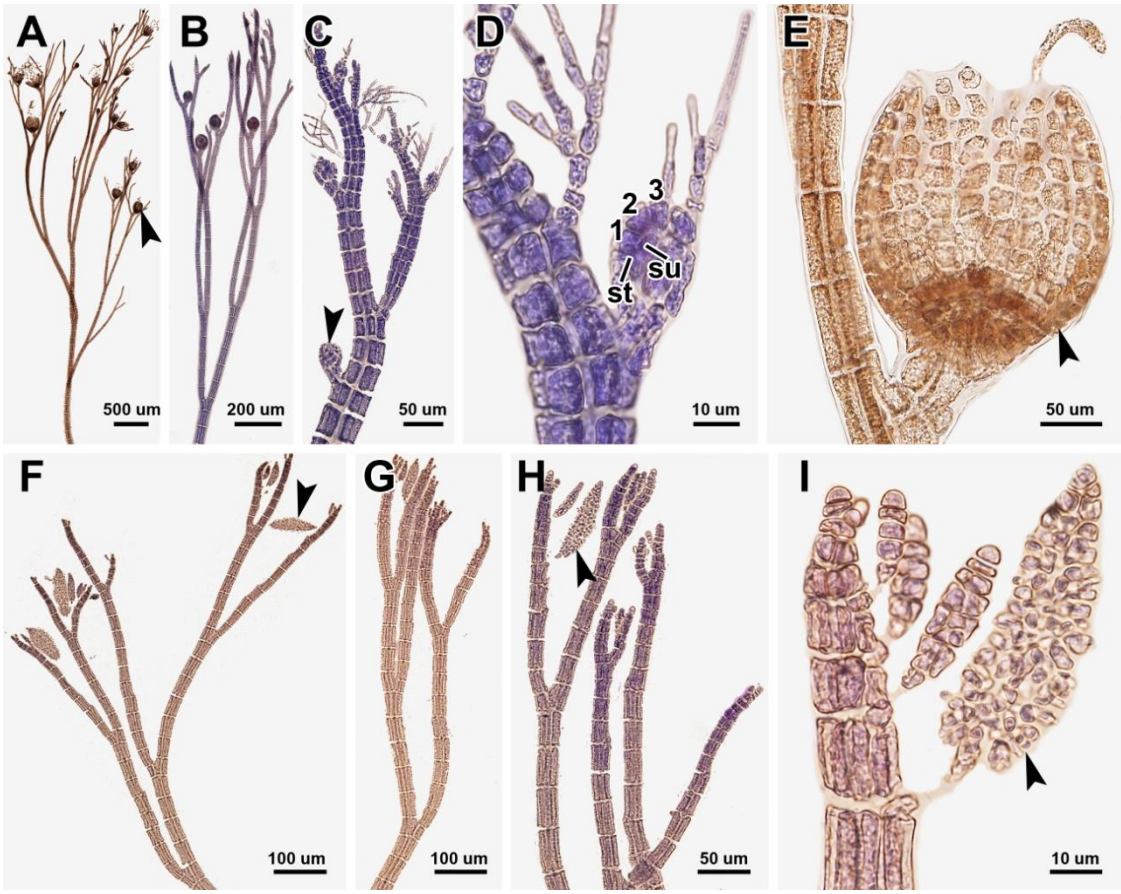


**Fig. 52.** Reproductive structures of *Neosiphonia gorgoniae*. (A-B) Female plant. (C) Upper part of female thallus showing subapical cystocarps (arrowhead). (D) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (E) Mature cystocarp (arrowhead) showing globose shape. (F-G) Male plant. (H) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (I) Spermatangial branches (arrowhead) replacing trichoblast.

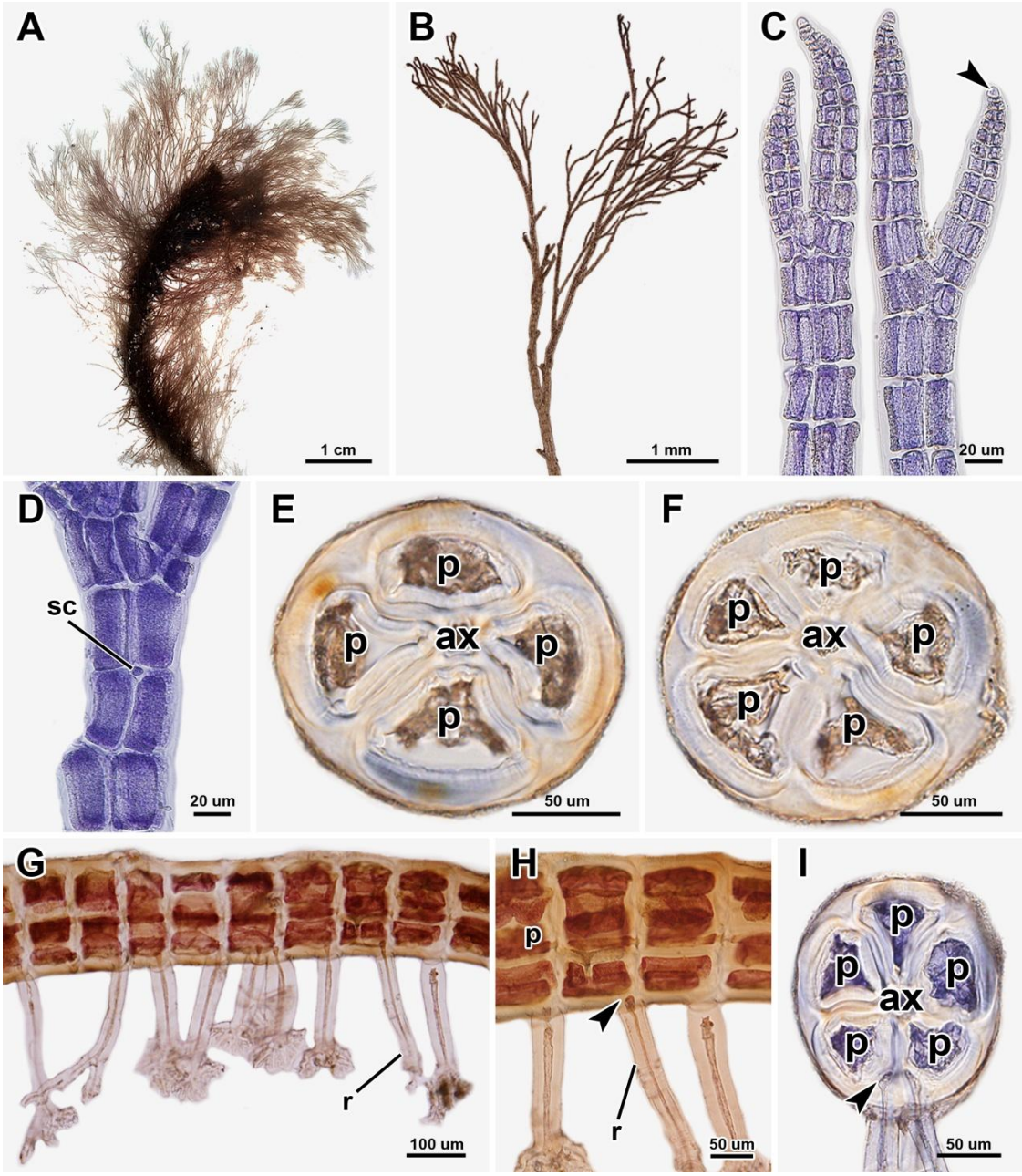


**Fig. 53.** Vegetative structures of *Neosiphonia indonesiana* sp. nov. (A) Plants epiphyte on *Gracilaria sp.* (B) Habit of vegetative plant showing the extended erect axes. (C) Apex with pseudodichotomous branching pattern and prominent and dome shaped apical cells (arrowhead). (D) Erect axes showing scar cells (arrowhead). (E-F) Cross-section views showing four (E) and five (F) pericentral cells (p) (ax, axial cell). (G) Rhizoids (r) scattered and produced from prostrate axes. (H) Rhizoids (r) cutting off (arrowhead) from the proximal end of pericentral cells (p). (I) Cross-section of a prostrate axis showing rhizoids (r) cutting off (arrowhead) from pericentral cells (p).

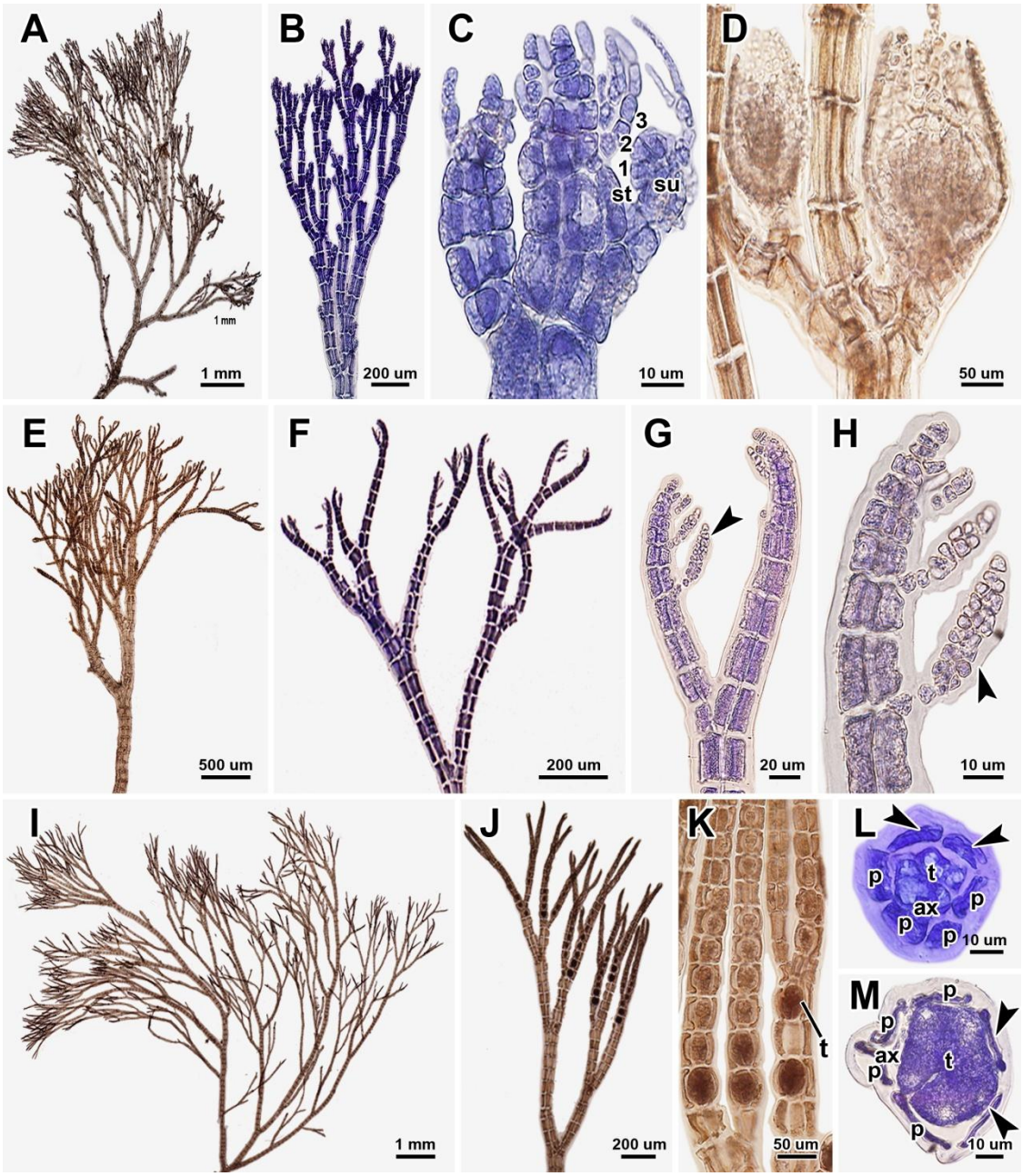


**Fig. 54.** Reproductive structures of *Neosiphonia indonesiana* sp. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Mature cystocarp showing globose shape. (E-F) Male plant. (G-H) Spermatangial branches (arrowheads) replacing trichoblast and adaxially developed. (I-J) Tetrasporangial thallus. (K) Apical branches showing tetrasporangia (t) arranged in spiral series. (L-M) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

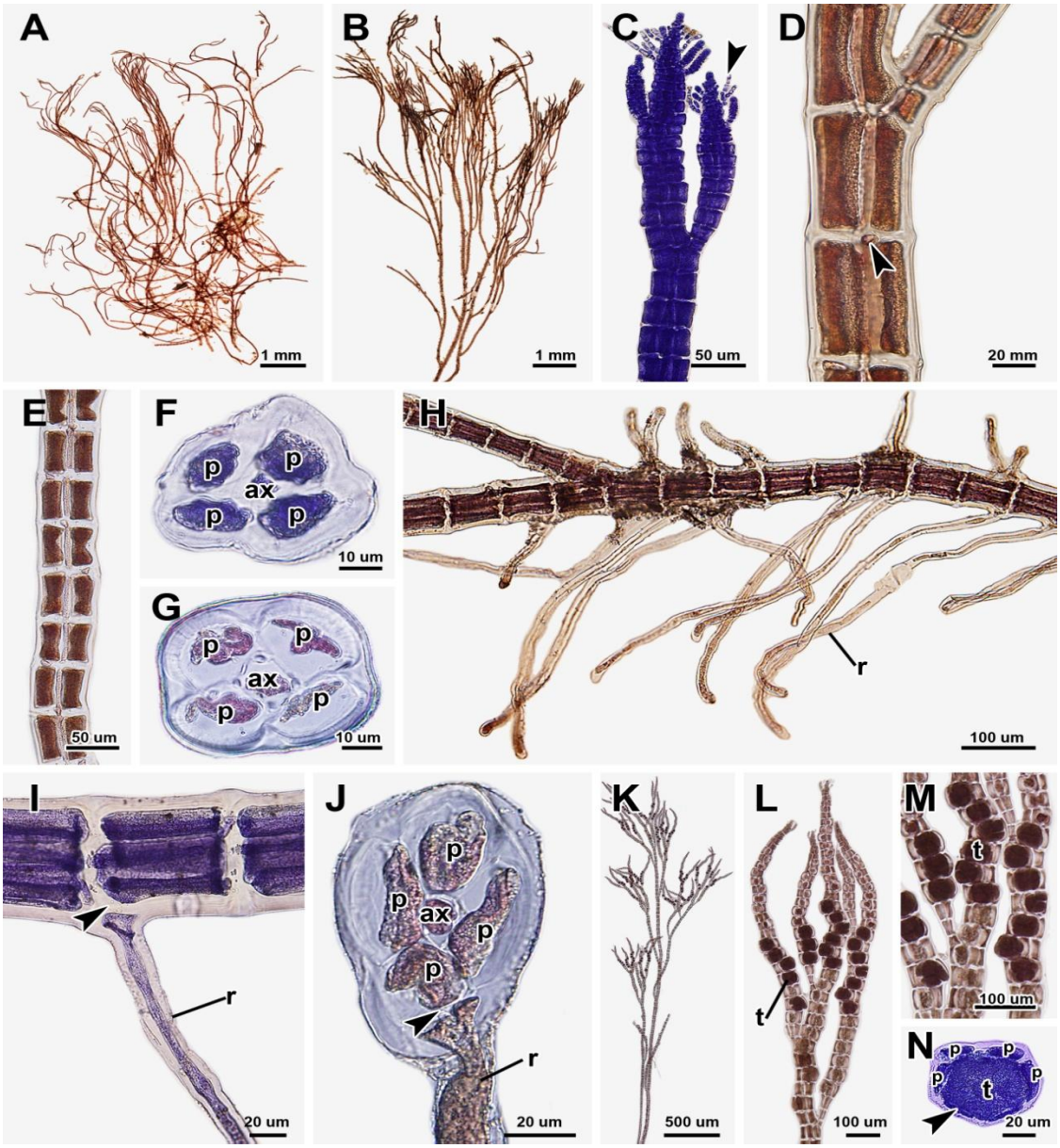


**Fig. 55.** Vegetative and reproductive structures of *Neosiphonia infestans* comb. nov. (A-B) Habit of vegetative plant showing the entangled prostrate and erect axes. (C) Apex with alternate branching pattern and short trichoblasts (arrowhead). (D-E) Scar cells (arrowhead) spirally disposed in erect axes. (F-G) Cross-sections view showing four pericentral cells (p) from middle (F) and basal part (G) (ax, axial cell). (H) Rhizoids (r) scattered and produced from prostrate axes. (I) Rhizoids (r) cutting off (arrowhead) from the proximal end of pericentral cells (p). (J) Cross-section of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (K) Tetrasporangial thallus. (L-M) Apical branches showing tetrasporangia (t) in spiral series. (N) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

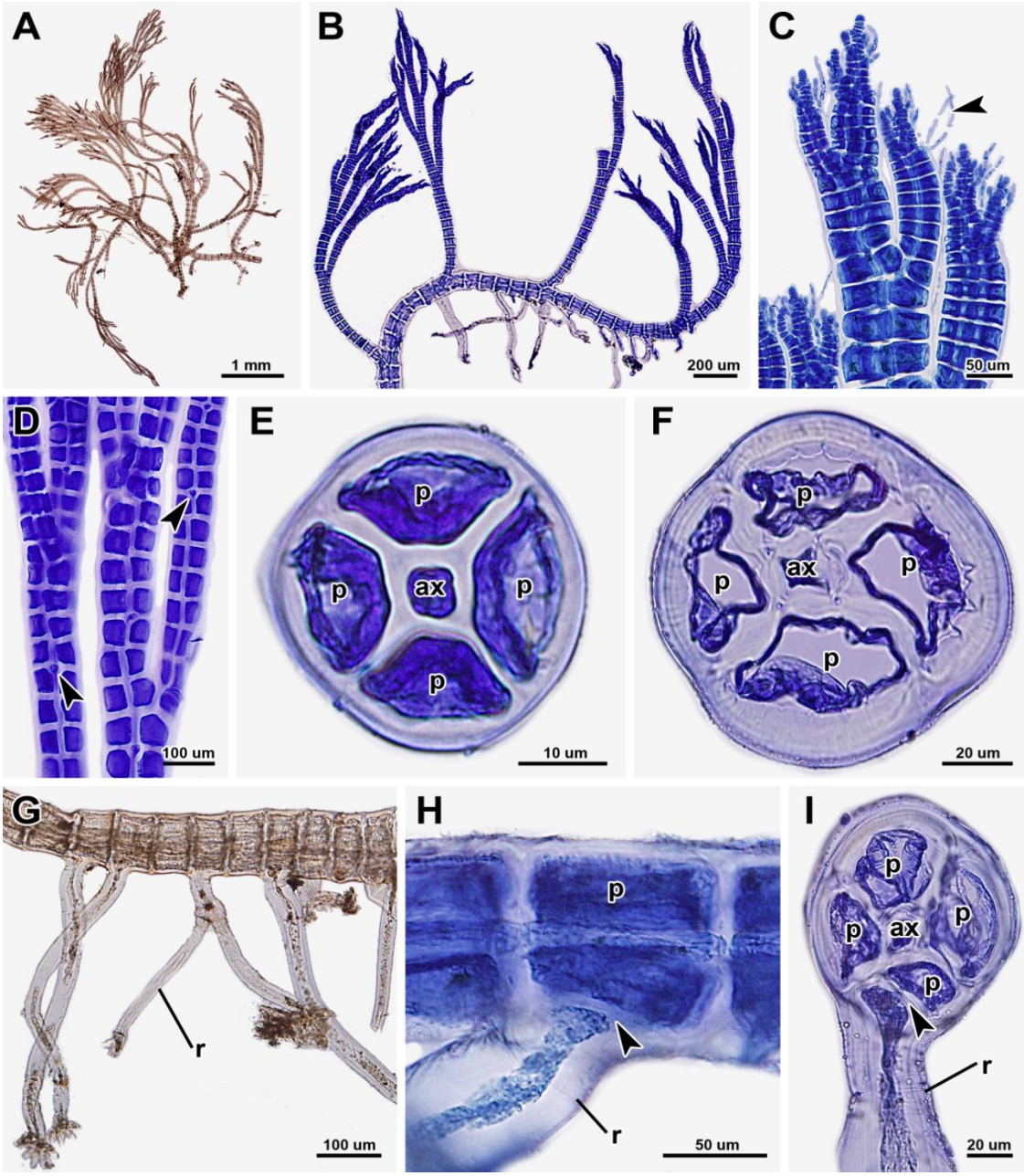


**Fig. 56.** Vegetative structures of *Neosiphonia mancorensis* sp. nov. (A-B) Habit of vegetative plant showing the extended prostrate axes and reduced erect axes. (C) Apex with alternate branching pattern and abundant trichoblasts (arrowhead). (D) Erect axes showing scar cells (arrowhead). (E-F) Cross-section views showing four pericentral cells (p) from the erect (E) and prostrate axes (F) (ax, axial cell). (G) Rhizoids (r) scattered and produced from prostrate axes. (H) Rhizoid (r) cutting off (arrowhead) from the proximal end of pericentral cells (p). (I) Cross-section of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

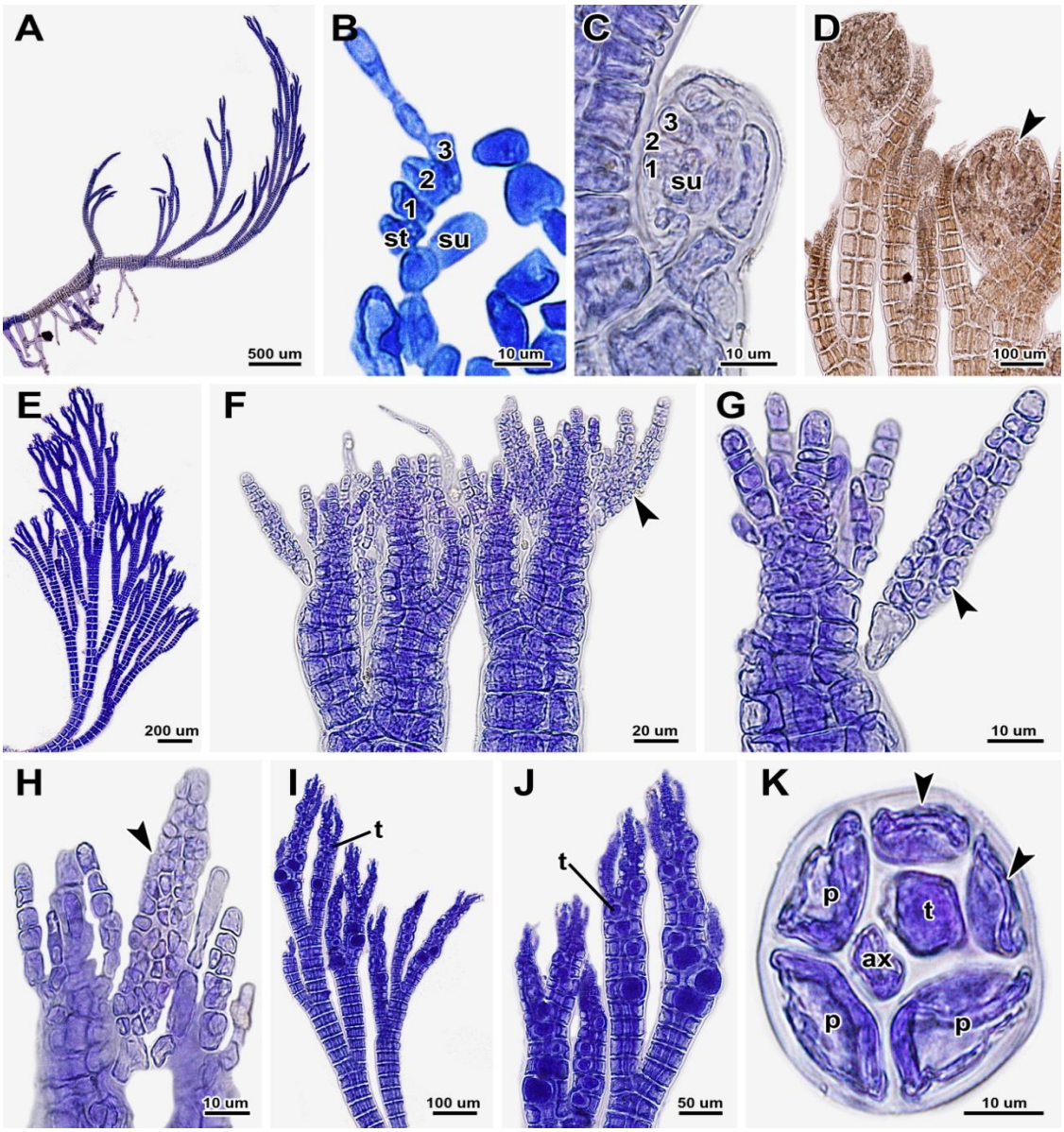


**Fig. 57.** Reproductive structures of *Neosiphonia mancorensis* sp. nov. (A) Female plant. (B-C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Upper part of female thallus showing apical cystocarps (arrowhead) in globose shape. (E) Male plant. (F) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (G-H) Spermatangial branches (arrowhead) replacing trichoblast (G) and arising from trichoblasts. (I) Tetrasporangial thallus. (J) Apical branches showing tetrasporangia (t) in spiral series. (K) Cross-section with four pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

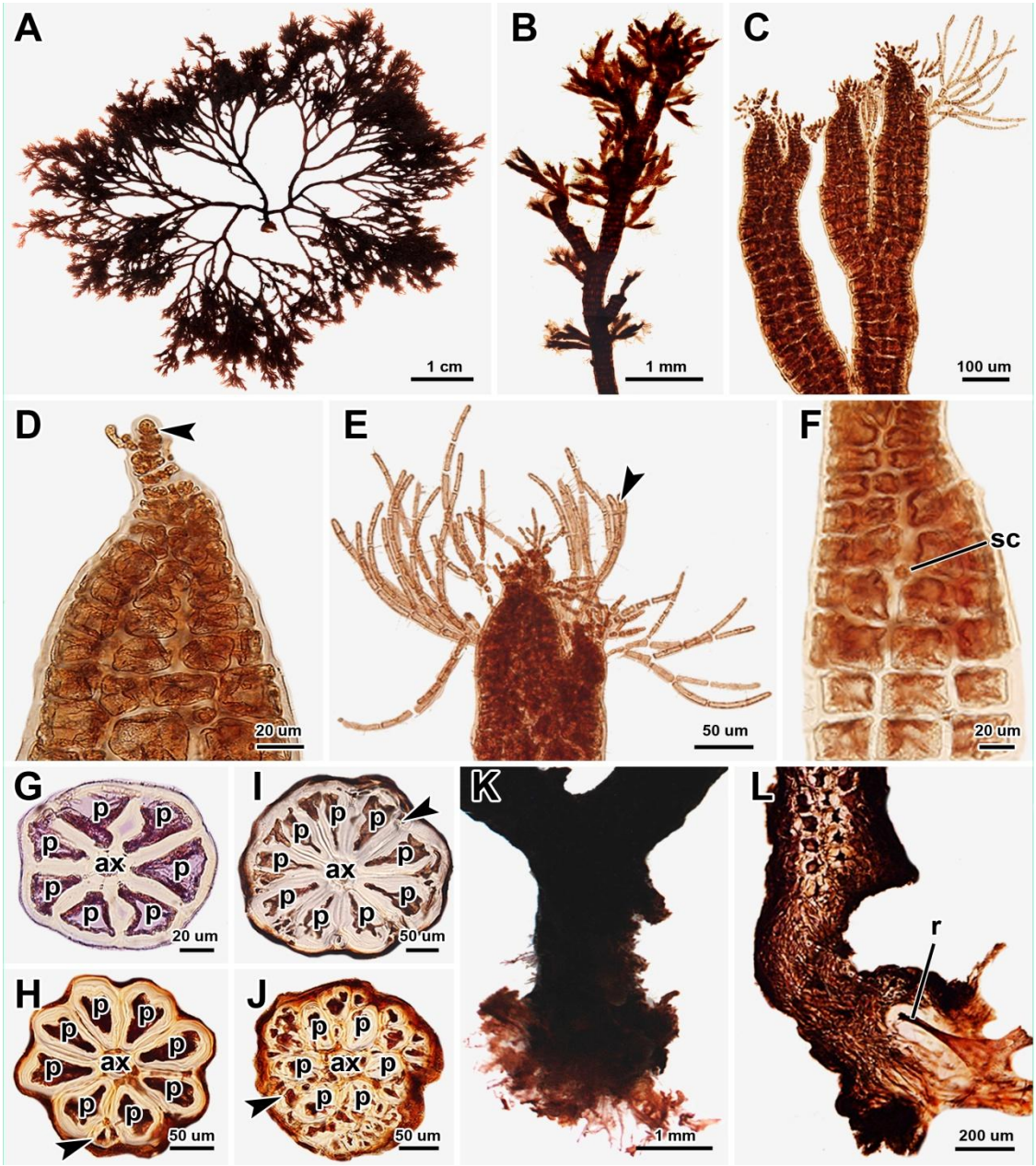


**Fig. 58.** Vegetative structures of *Neosiphonia notoensis*. (A) Habit of vegetative plant showing the holdfast and an extended erect axes. (B) Erect axes densely branched. (C) Apex with alternate branching pattern. (D) Apex showing inconspicuous apical cells (arrowhead). (E) Apex showing abundant trichoblasts. (F) Erect axes showing scar cells (sc). (G-J) Cross-section views showing 6-9 pericentral cells (p) and cortication (arrowheads) (ax, axial cell). (K) Holdfast formed by clumped rhizoids. (L) Rhizoid (r) cutting off from pericentral cells or cortical cells.

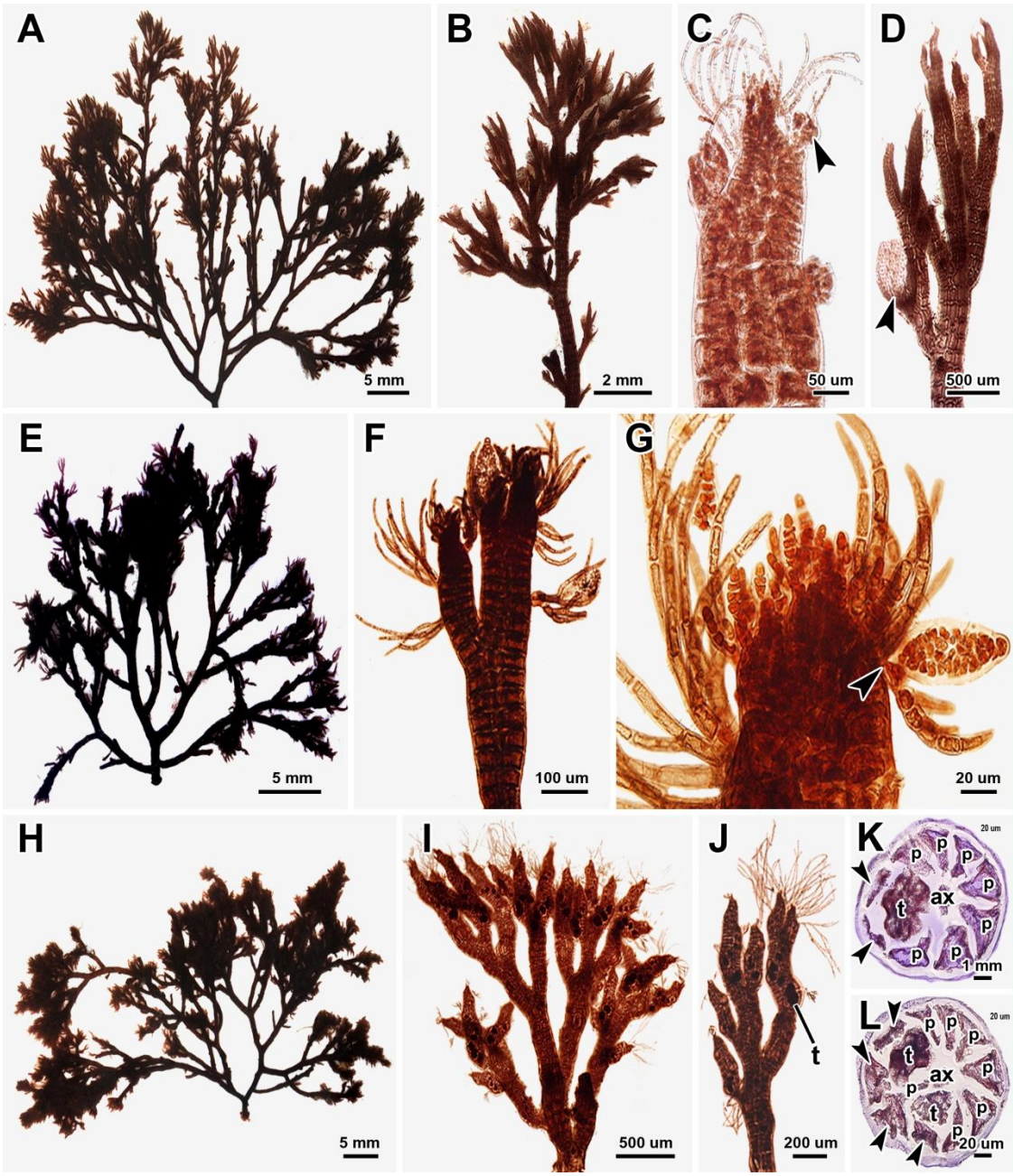


**Fig. 59.** Reproductive structures of *Neosiphonia notoensis*. (A-B) Female plant. (C-D) Upper part of female thallus showing subapical cystocarps (arrowhead) in globose shape. (E) Male plant. (F) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (G) Spermatangial branches arising from trichoblasts. (H) Tetrasporangial thallus. (I-J) Apical branches showing tetrasporangia (t) in spiral series. (K-L) Cross-section views with 8 (K) and 9 (L) pericentral cells (p) showing a tetrasporangium (t) (K) and two tetrasporangia (L) rounded by cover cells (arrowheads).

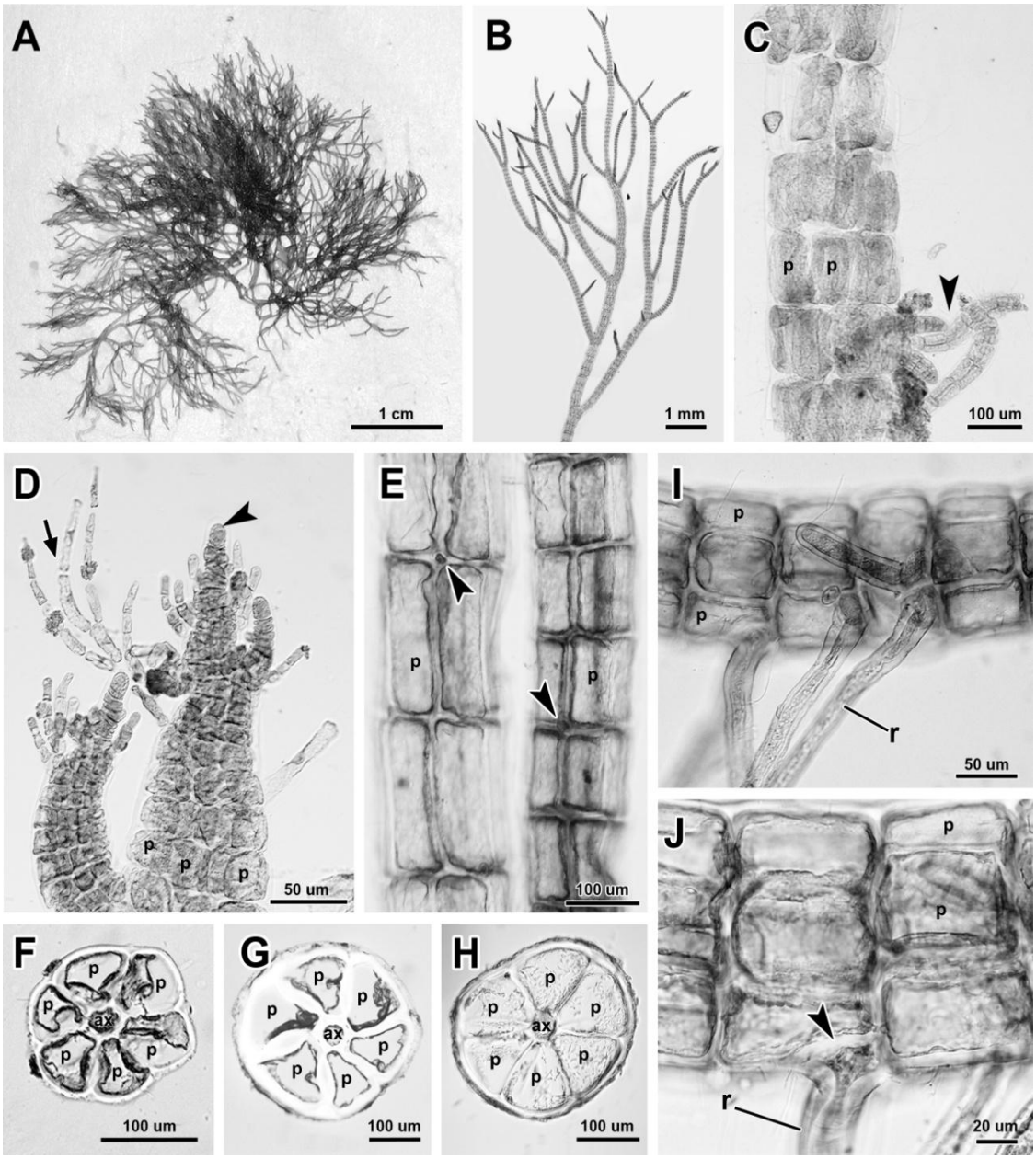


**Fig. 60.** Vegetative structures of *Neosiphonia peruviensis.* (A) Holotype specimen from Peru. (B) Habit of vegetative plant showing alternate branching pattern. (C) Portion of erect axis with exogenous adventitious branchlets (arrow) (p, pericentral cell). (D) Apical part with trichoblasts (tb) and an apical cell (ap) (p, pericentral cell). (E) Middle part of thallus with scar cells (sc) (p, pericentral cell). (F-H) Cross section views of upper (F), middle (G), and basal (H) parts of thallus (ax, axial cell) (p, pericentral cell). (I) Prostrate axis with 2 rhizoids (r) per segment. (J) Rhizoids (r) produced from the proximal ends of pericentral cells by a wall (arrow) (p, pericentral cell).

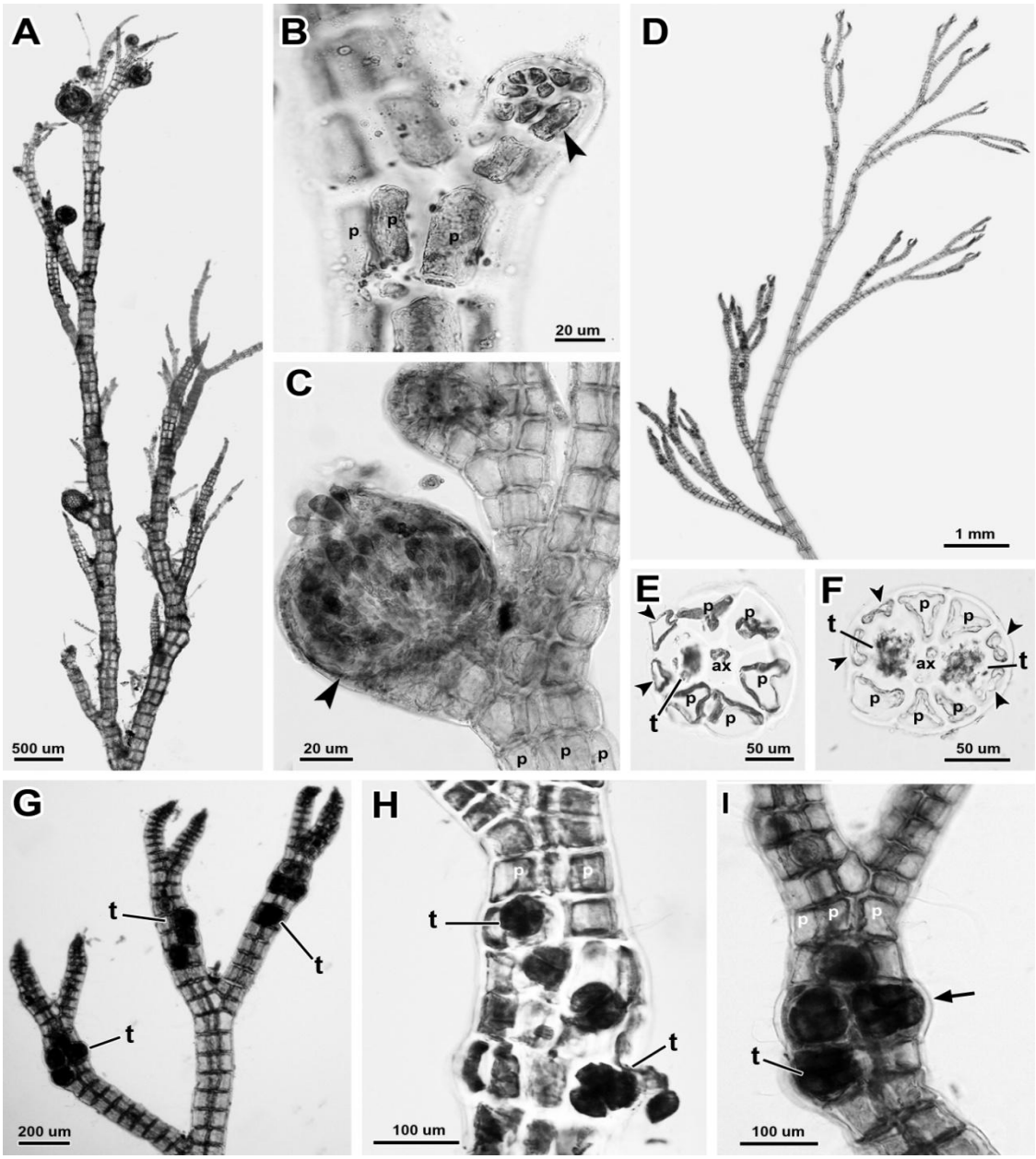


**Fig. 61.** Reproductive structures of *Neosiphonia peruviensis.* (A) Female plant. (B) A young cystocarp (arrow). (C) Mature cystocarp on a short-stalked pedicel (arrow) (p, pericentral cell). (D) Tetrasporangial plant. (E) Cross sectional view showing one tetrasporangium (t) rounded by cover cells (arrowheads) (ax, axial cell; p, pericentral cell). (F) Cross sectional view showing two tetrasporangia rounded by cover cells (arrowheads) (ax, axial cell; p, pericentral cell). (G) Upper part of tetrasporangial thallus showing tetrasporangia (t) located below the apex. (H) Upper part of tetrasporangial thallus showing spiral series of tetrasporangia (t) (p, pericentral cell). (I) Segment of tetrasporangial thallus with two tetrasporangia (arrow) (p, pericentral cell; t, tetrasporangium)

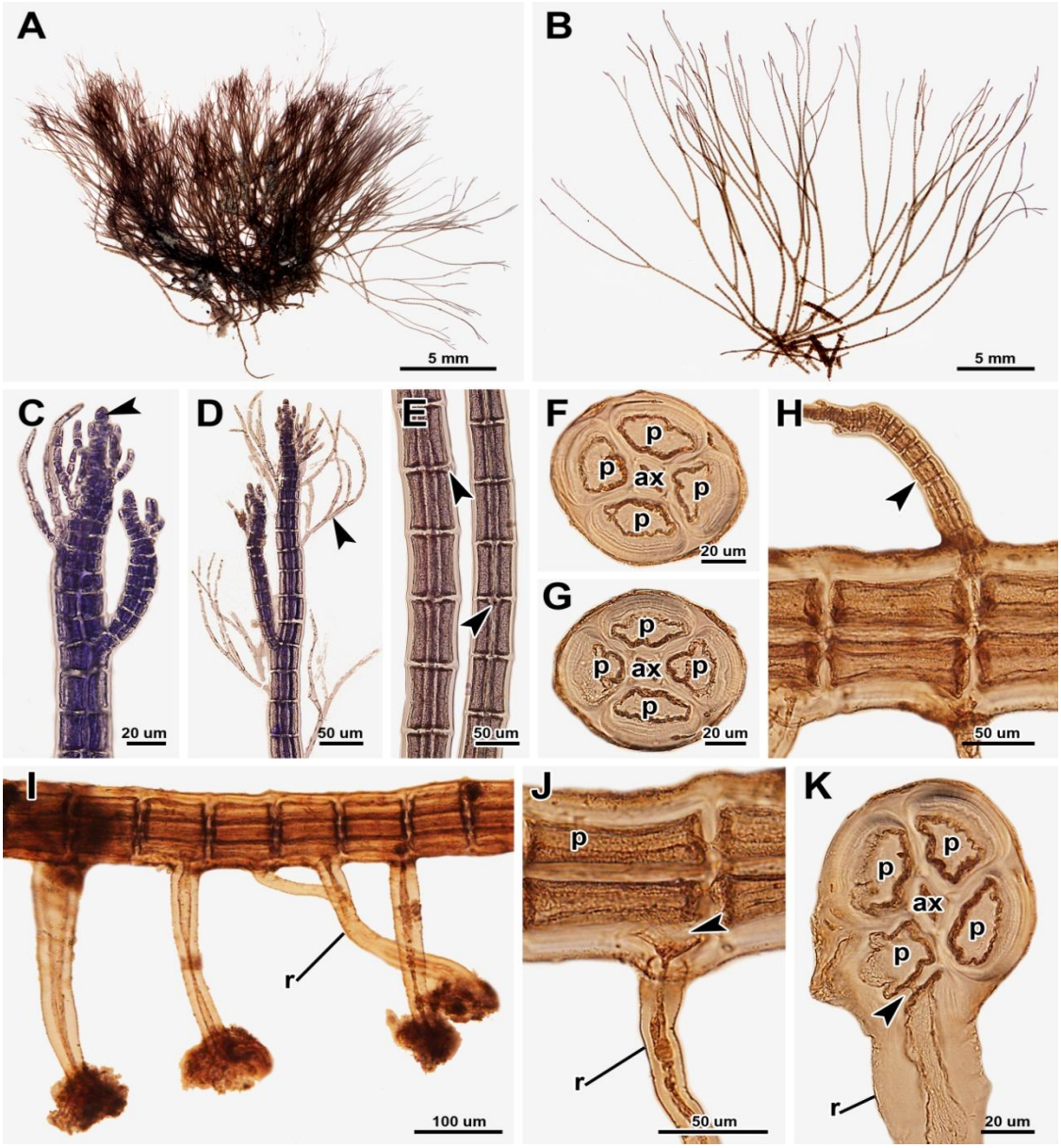


**Fig. 62.** Vegetative structures of *Neosiphonia pseudovillum* comb. nov. (A-B) Habit of vegetative plant showing the reduced prostrate axes and long erect axes. (C) Erect axes with pseudodichotomous branching pattern and prominent apical cells (arrowhead). (D) Apices showing abundant trichoblasts (arrowhead). (E) Inconspicuous scar cells (arrowheads) spirally disposed in erect axes. (F-G) Cross-sections view showing four pericentral cells (p) from the erect (F) and erect (G) axes (ax, axial cell). (H) Prostrate axis showing an adventitious branch (arrowhead). (I) Rhizoids (r) scattered and produced from prostrate axes. (J) Rhizoids (r) cutting off from the proximal end of pericentral cells (p). (K) Cross-section of a prostrate axis showing rhizoids (r) cutting off (arrowhead) from pericentral cells (p).

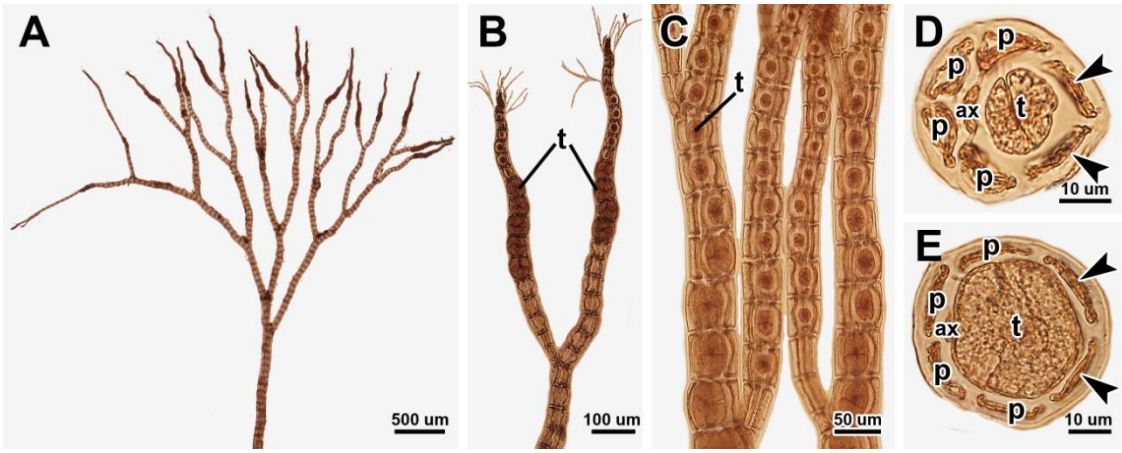


**Fig. 63.** Reproductive structures of *Neosiphonia pseudovillum* comb. nov. (A) Tetrasporangial thallus. (B-C) Apical branches showing tetrasporangia (t) arranged in spiral series. (D-E) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

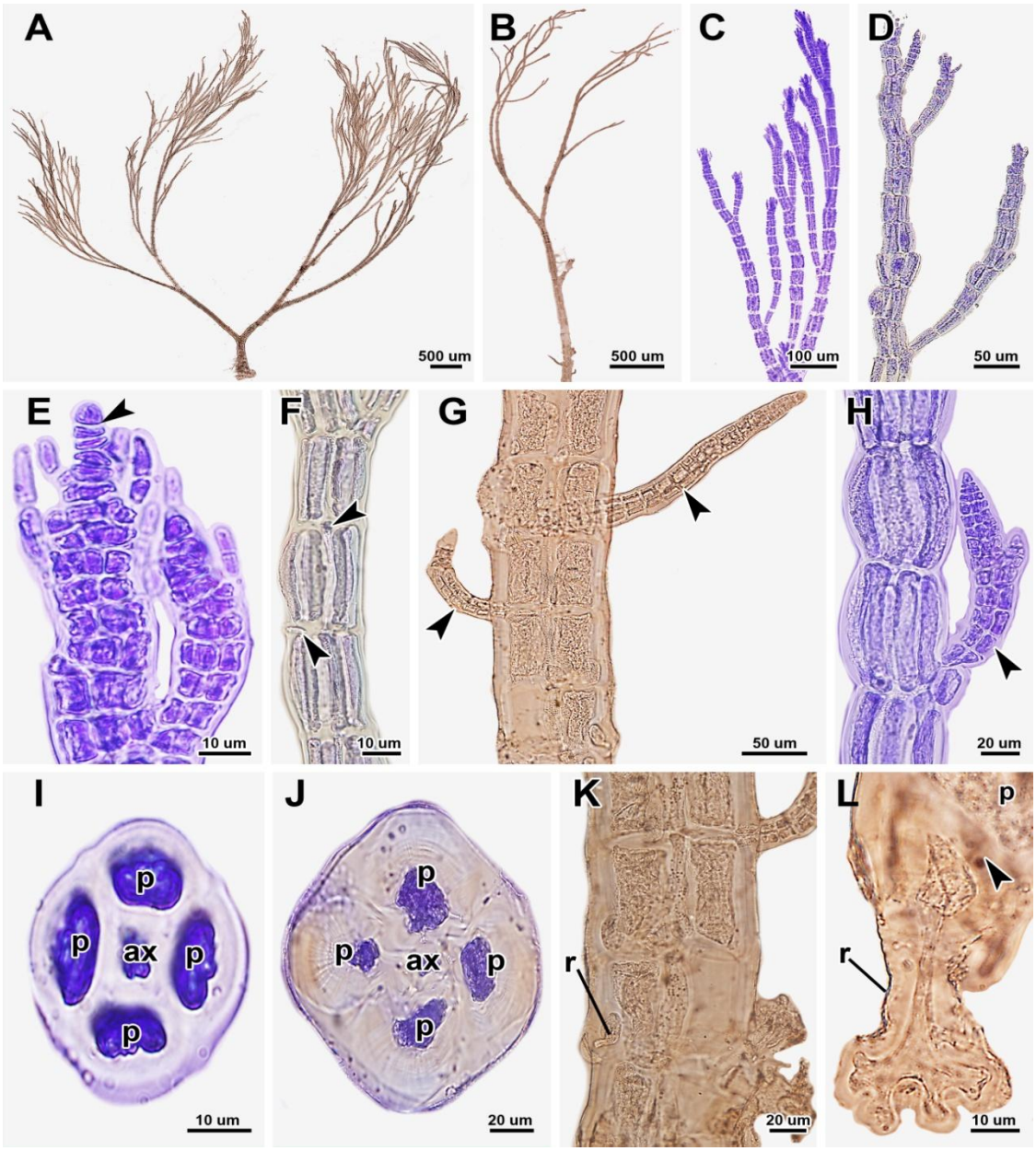


**Fig. 64.** Vegetative structures of *Neosiphonia pulvinata f. parvula* comb. nov. (A-B) Habit of vegetative plant showing the reduced prostrate axes and long erect axes. (C-D) Erect axes with subdichotomous branching pattern. (E) Apices showing prominent apical cells (arrowhead) and abundant trichoblasts. (F) Inconspicuous scar cells (arrowheads) spirally disposed in erect axes. (G) Erect axis showing cicatrigenous branch (arrowhead). (H) Erect axis showing an adventitious branch (arrowhead). (I-J) Cross-sections view showing four pericentral cells (p) from the middle (I) and basal part (J) of axes (ax, axial cell). (K) Rhizoids (r) cutting off from the proximal end of pericentral cells. (L) Unicellular rhizoid (r) cutting off (arrowhead) from pericentral cells.

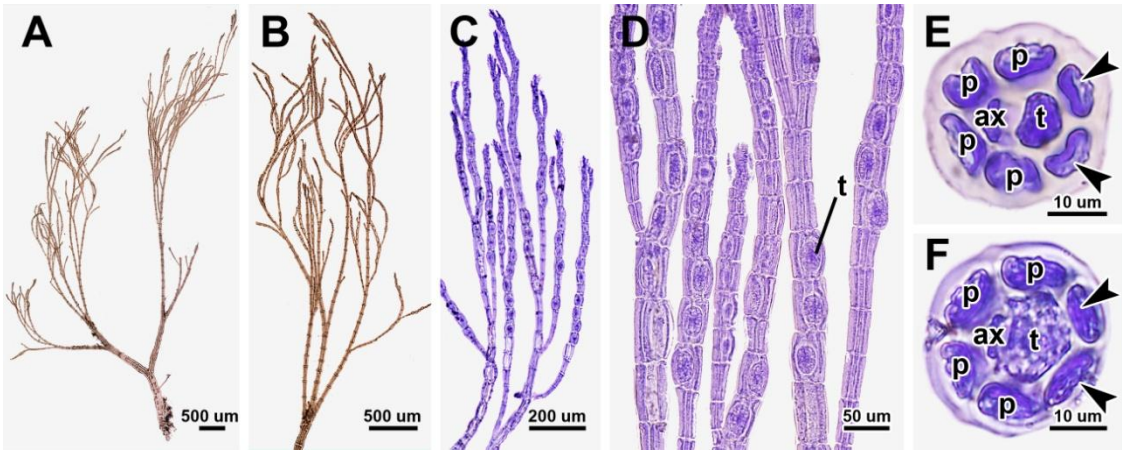


**Fig. 65.** Reproductive structures of *Neosiphonia pulvinata f. parvula* comb. nov. (A-B) Tetrasporangial thallus. (C-D) Apical branches showing tetrasporangia (t) arranged in spiral series. (E-F) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

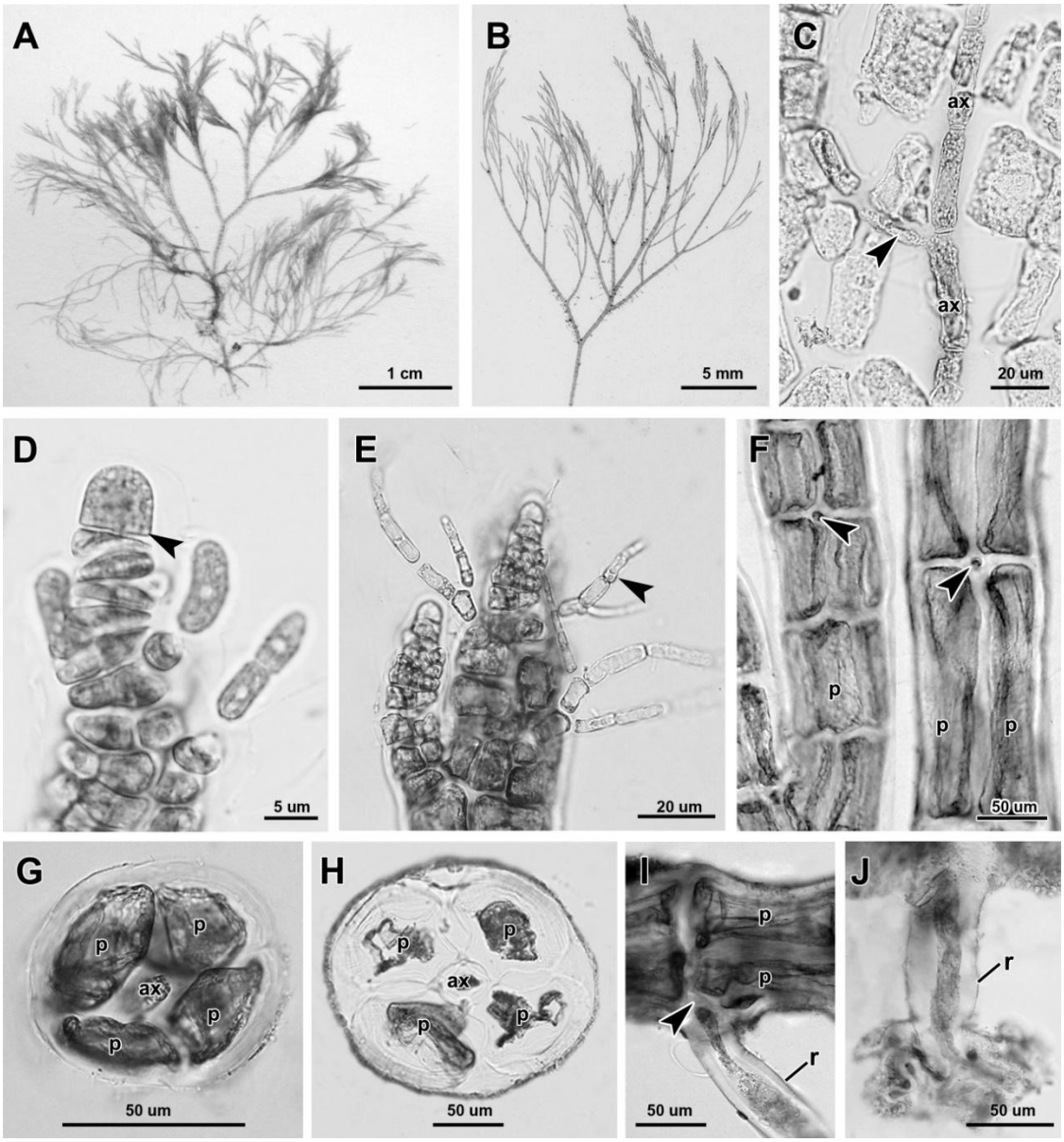


**Fig. 66.** Vegetative structures of *Neosiphonia ramireziae.* (A) Holotype specimen from Lagunillas, Pisco, Peru. (B) Habit of vegetative plant showing dichotomous branching pattern and pseudofastigiate appearance. (C) Axial filament (arrowhead) endogenously developed from main axial cells (ax). (D) Apical region of thallus showing a prominent apical cell with transverse division (arrowhead) of subapical cell. (E) Apical region with trichoblasts. (F) Middle part of thallus with scar cells (sc). (G-H) Cross section views of upper (G) and basal (H) parts of thallus (ax: axial cell), (p: pericentral cell). (I) Rhizoid (r) cut off from pericentral cell (p) by a wall (arrow). (J) Unicellular rhizoid with digitate tips.

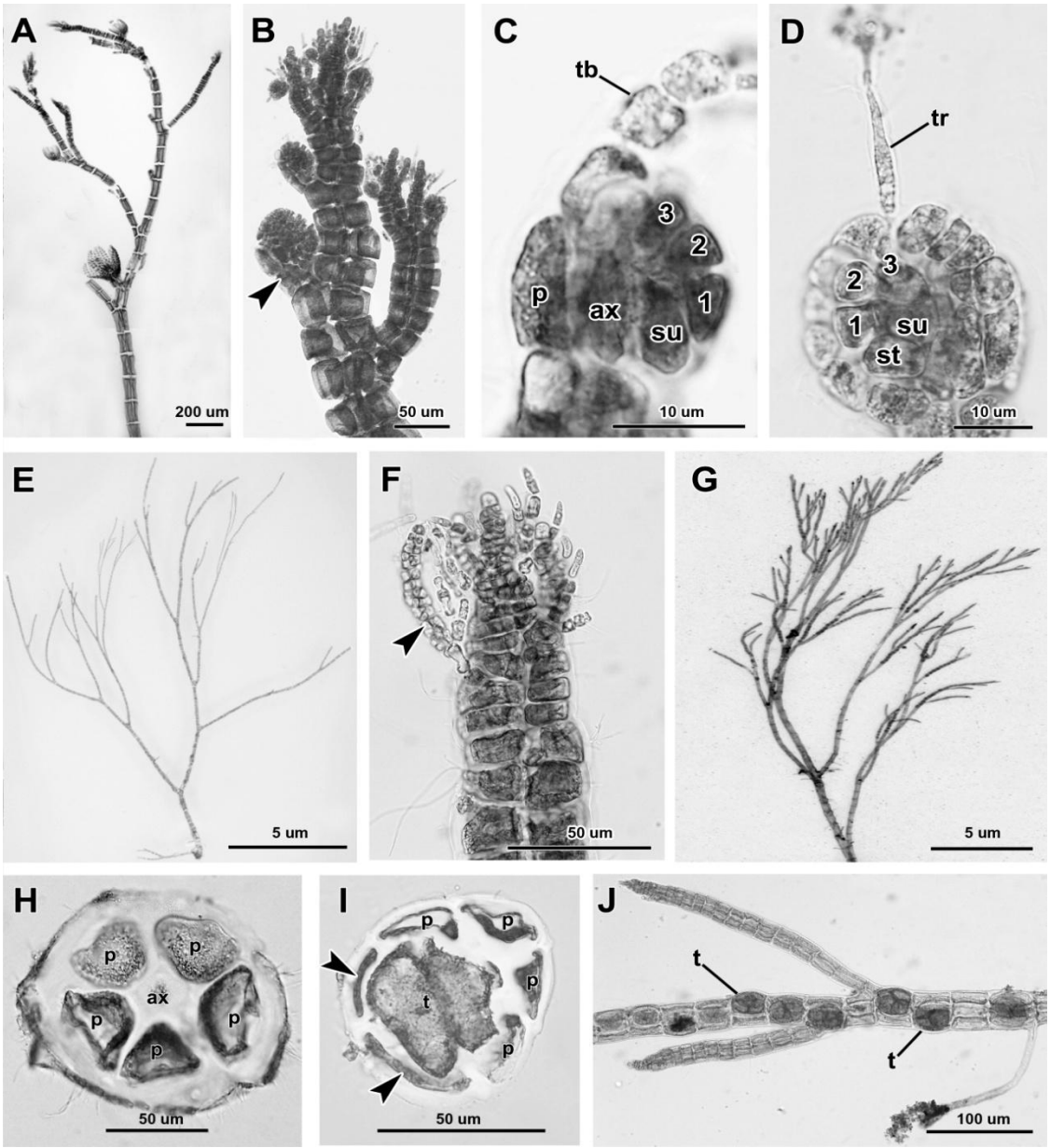


**Fig. 67.** Reproductive structures of *Neosiphonia ramireziae*. (A) Female gametophyte bearing cystocarps. (B) Apical part of thallus with cystocarps (arrowhead) at various stages of development. (C-D) Young (C) and mature procarp (D) with a three-celled carpogonial branch and trichogyne (tr), (ax: axial cell), (1-3: sequence of carpogonial branch cells), (p: pericentral cell), (su: supporting cell). (E) Male gametophyte. (F) Apex showing spermatangial branch (arrowhead) developed on the basal cell of trichoblast (tb). (G) Tetrasporangial plant. (H-I) Cross section view of fertile segment (H) showing 5 pericentral cells, and mature stage (I) showing one tetrasporangium (t) surrounded by cover cells (arrows). (J) Upper part of tetrasporangial thallus showing interrupted and spiral series of mature tetrasporangia (t),

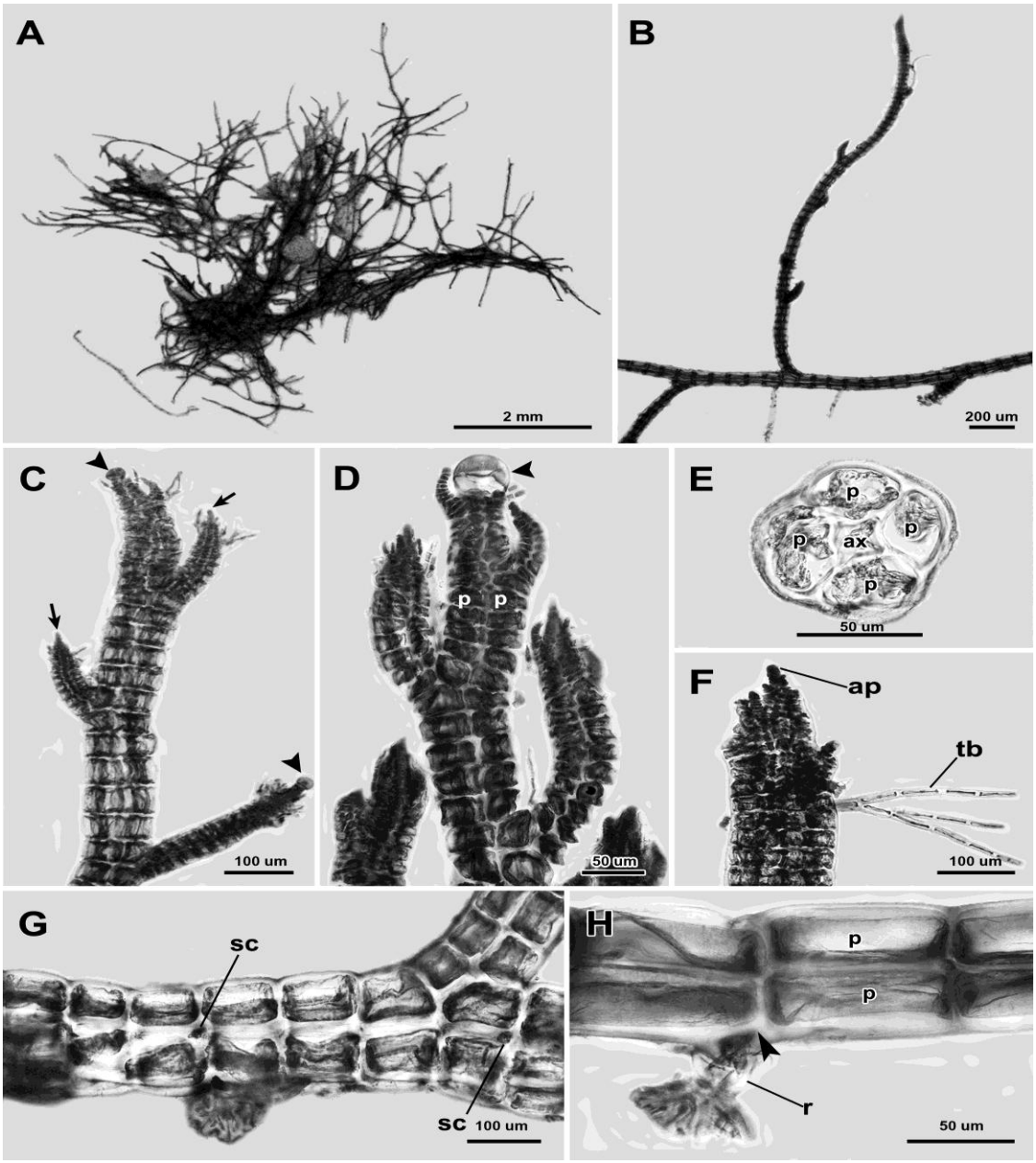


**Fig. 68.** Vegetative structures of *Neosiphonia silvae*. (A) Holotype. (B) Habit of vegetative plant showing erect branch arising from prostrate filament. (C) Portion of erect axis showing alternate branching pattern, prominent apical cells (arrow), and exceedingly prominent apical cells (arrowhead). (D) Apical region showing the exceedingly prominent apical cell (arrowhead). (E) Cross section of thallus showing 4 pericentral cells. (F) Apical region showing trichoblasts and prominent apical cells. (G) Prostrate part of thallus with scar cells. (H) Rhizoid produced from the proximal end of pericentral cells by a wall (arrow). (ap, apical cell; ax, axial cell; p, pericentral cell; r, rhizoid; sc, scar cells; tb, trichoblast)

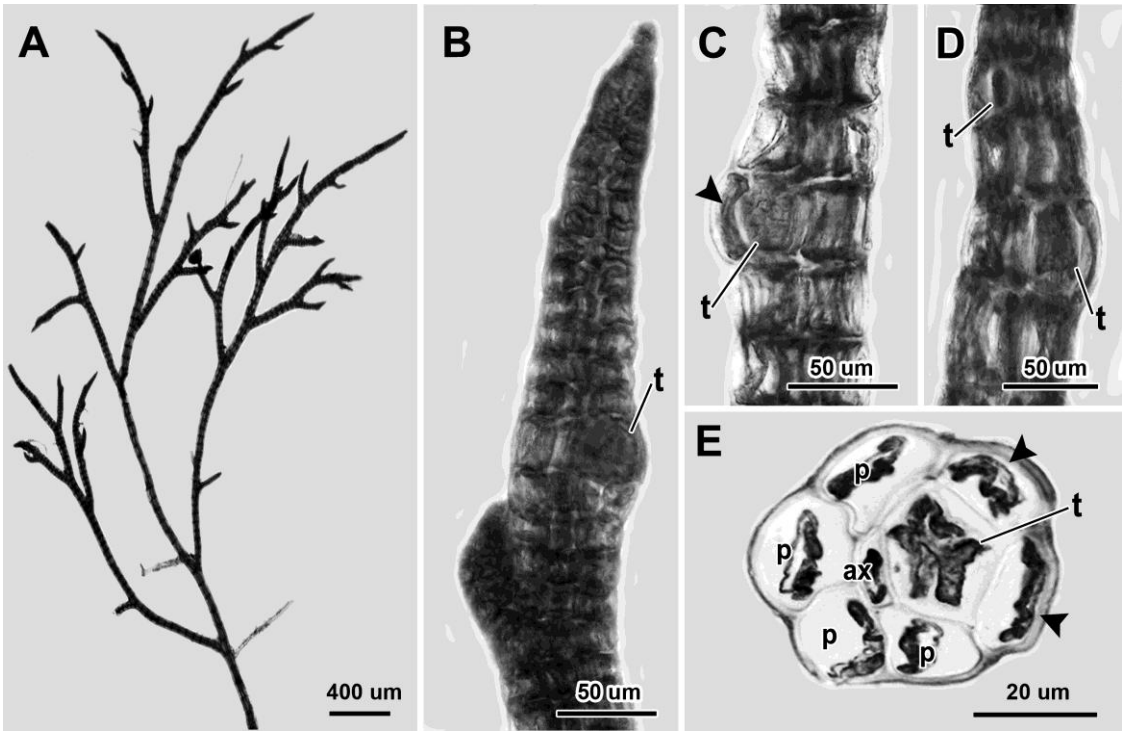


**Fig. 69.** Reproductive structures of *Neosiphonia silvae*. (A) Tetrasporangial plant. (B-C) Filaments showing one tetrasporangium surrounded by cover cells (arrowhead), near to the apex (B) and in middle part of thallus (C). (D) Upper part of tetrasporangial thallus showing two tetrasporangia. (E) Cross section showing one tetrasporangium and cover cells (arrowheads). (p, pericentral cells; t, tetrasporangium).

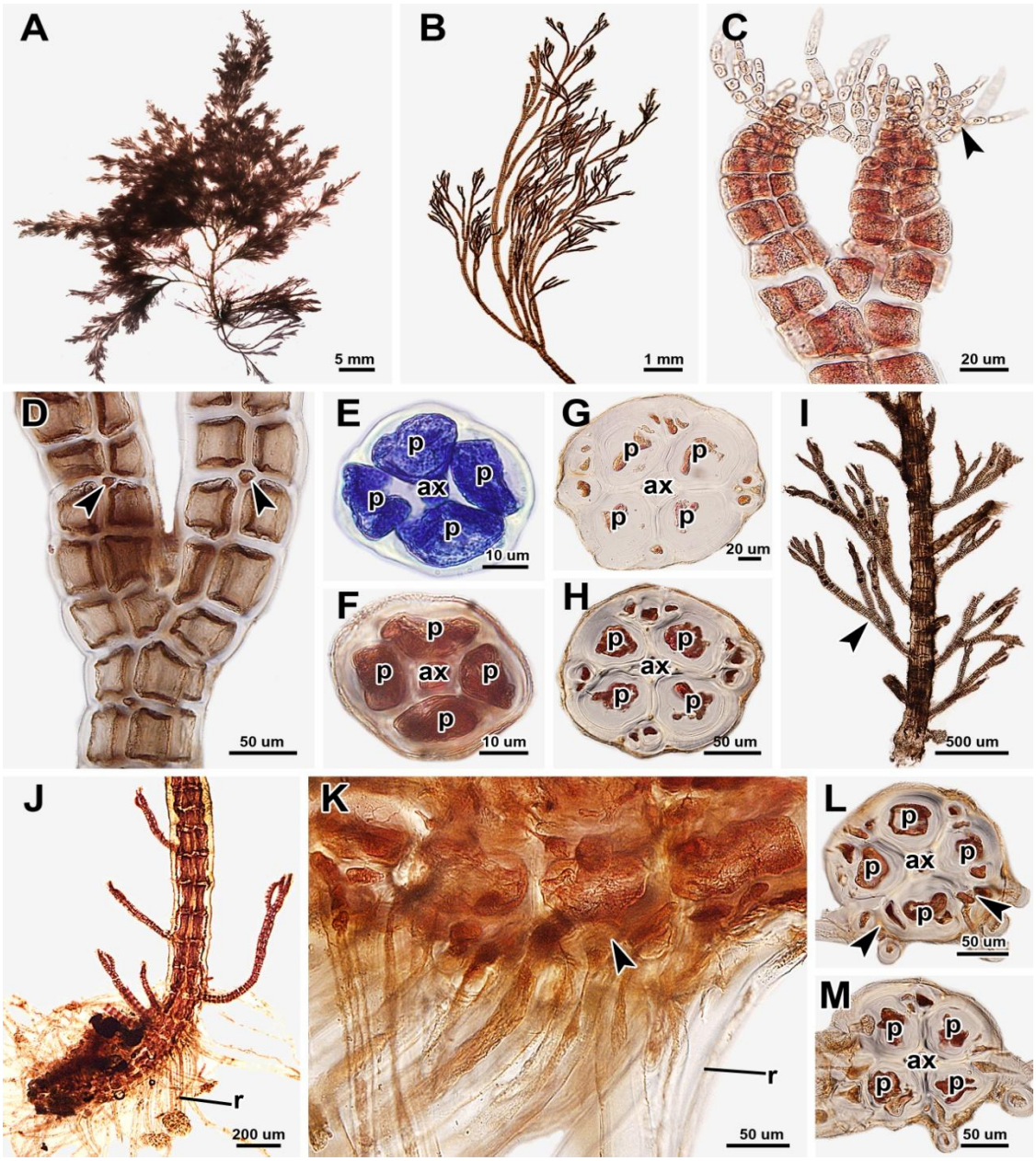


**Fig. 70.** Vegetative structures of *Neosiphonia strictissima* comb. nov. (A) Habit of vegetative plant showing well-developed erect axes. (B) Erect axes with pseudodichotomous to dichotomous branching pattern. (C) Apices showing abundant trichoblasts (arrowhead). (D) Conspicuous scar cells (arrowhead) disposed in erect axes. (E-H) Cross-section views showing four pericentral cells (p) and cortication from apical (E), middle (F), and basal part (G-H) (ax, axial cell). (I) Erect axis showing mature cicatrigenous branches (arrowhead). (J) Clumped rhizoids (r) on the basal part of erect axes. (K) Rhizoids (r) cutting off from pericentral cells (p). (L-M) Cross-section views of prostrate axes showing rhizoids (r) cutting off (arrowhead) from pericentral cells (p).

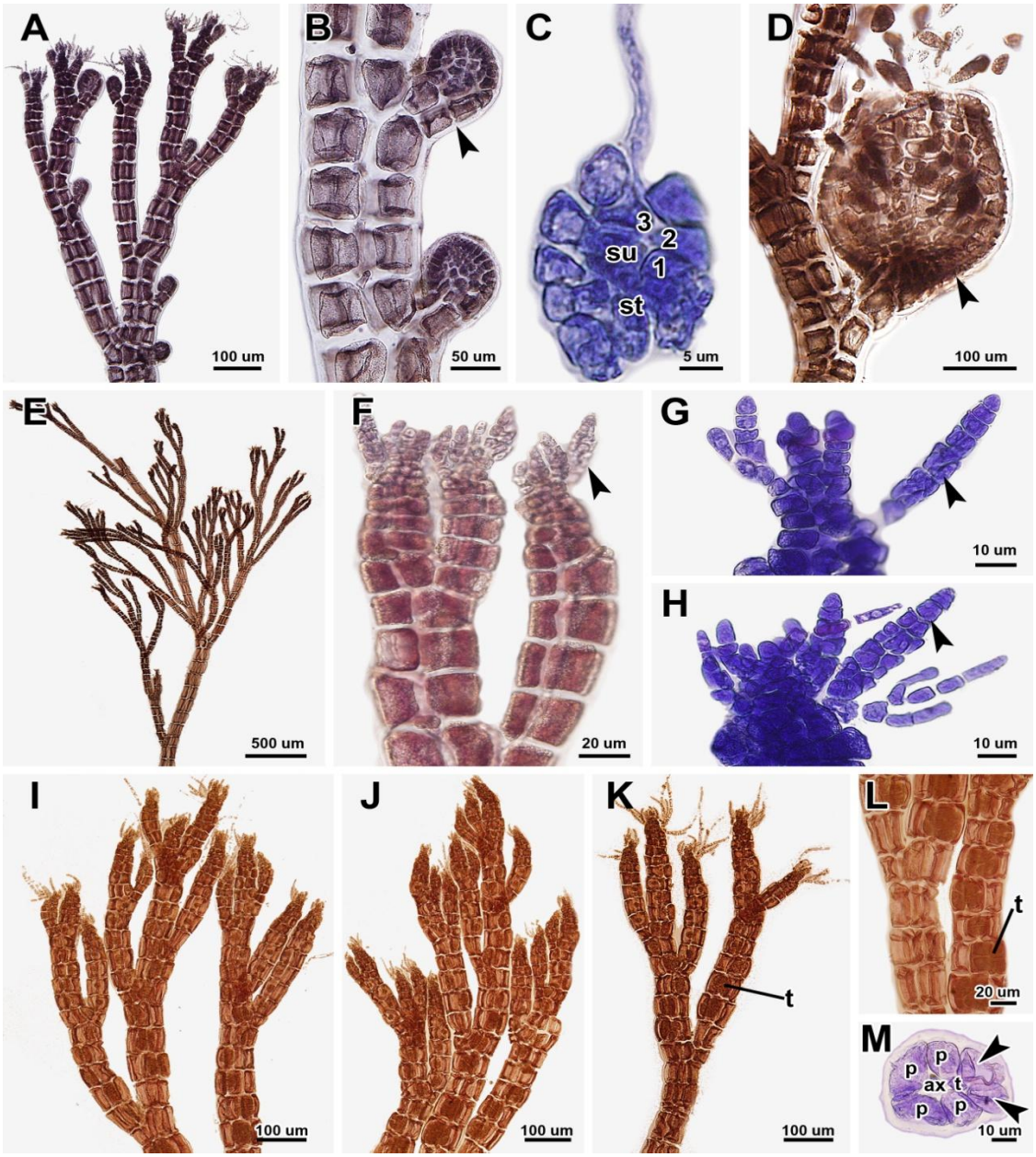


**Fig. 71.** Reproductive structures of *Neosiphonia strictissima* comb. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Mature cystocarp showing globose shape. (E) Male plant. (F) Spermatangial branches (arrowhead) clustered on the apex. (G-H) Spermatangia branches (arrowhead) replacing trichoblast (G) and developed on trichoblast (H). (I-J) Tetrasporangial thallus. (K-L) Apical branches showing tetrasporangia (t) in spiral series. (M) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

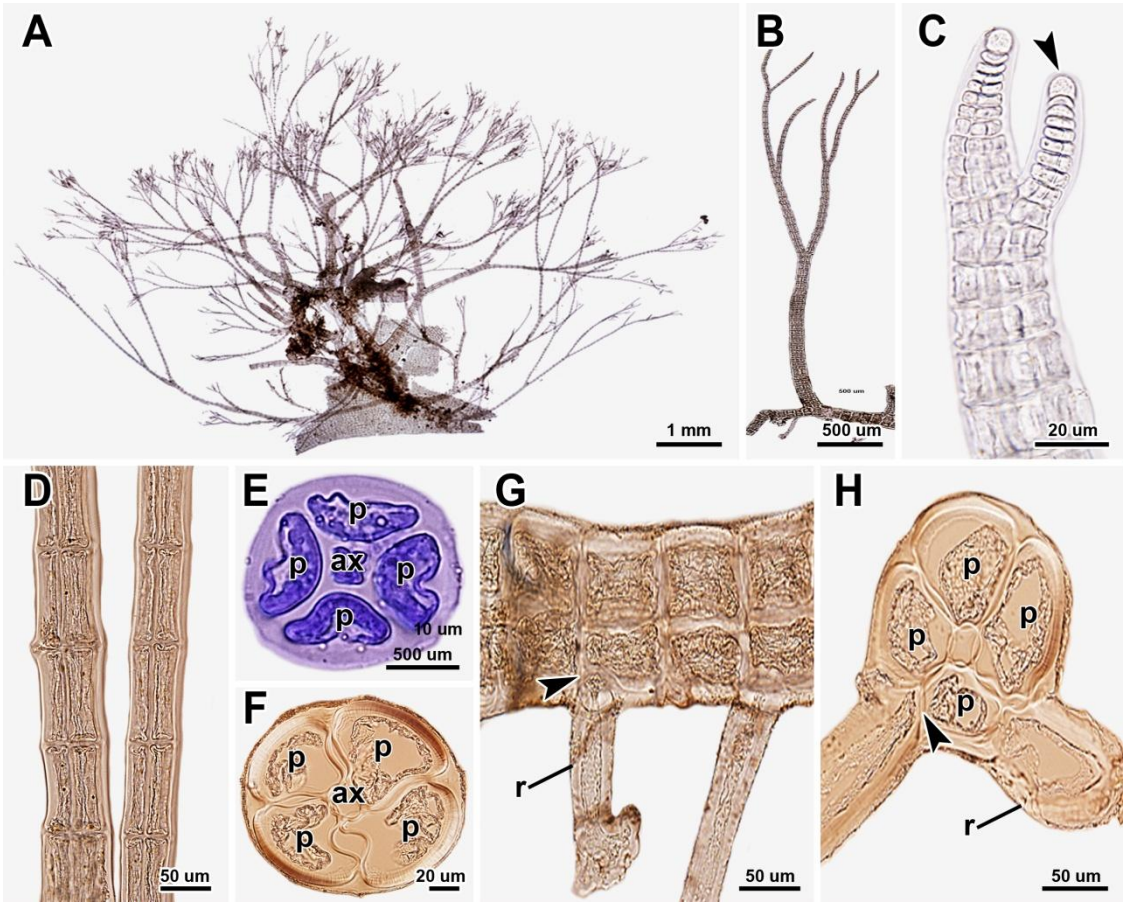


**Fig. 72.** Vegetative structures of *Neosiphonia sudafricana* sp. nov. (A) Habit of vegetative plant showing the reduced prostrate axes and long erect axes. (B) Erect axes with dichotomous to pseudodichotomous branching pattern. (C) Prominent and dome shaped apical cells (arrowhead) transversally divided. (D) Old erect axes showing lacking of scar cells. (E-F) Cross-section views showing four pericentral cells (p) from erect and prostrate axes (ax, axial cell). (G) Rhizoids (r) scattered and produced from proximal end of pericentral cells. (H) Cross-section of a prostrate axis showing rhizoids (r) cutting off (arrowhead) from pericentral cells (p).

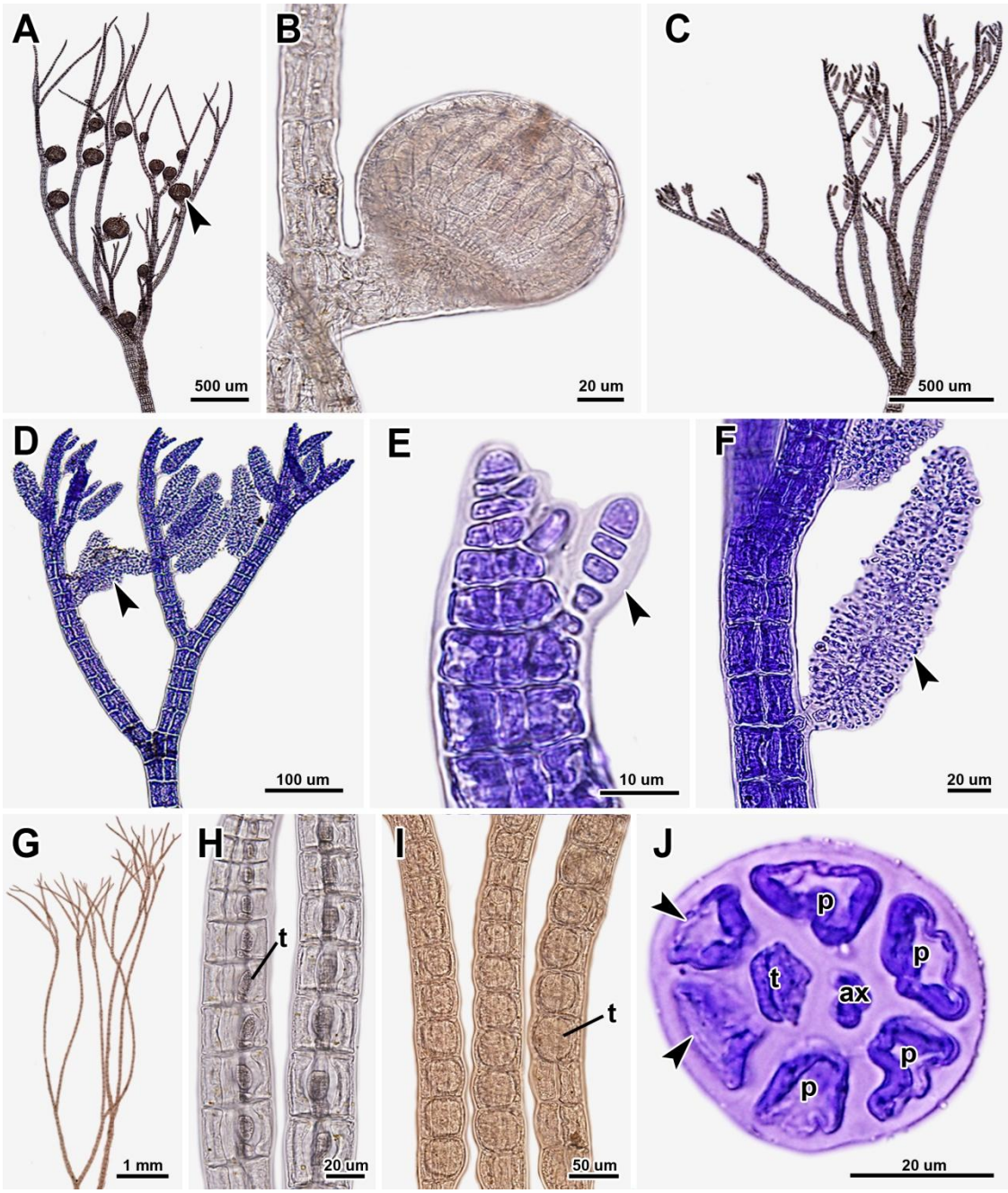


**Fig. 73.** Reproductive structures of *Neosiphonia sudafricana* sp. nov. (A) Female plant showing subapical cystocarps adaxially disposed. (B) Mature cystocarp showing globose shape. (C) Male plant. (D) Spermatangial branches (arrowheads) adaxially disposed. (E-F) Spermatangial branches replacing trichoblast. (G) Tetrasporangial thallus. (H-I) Apical branches showing tetrasporangia (t) arranged in straight series. (J) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

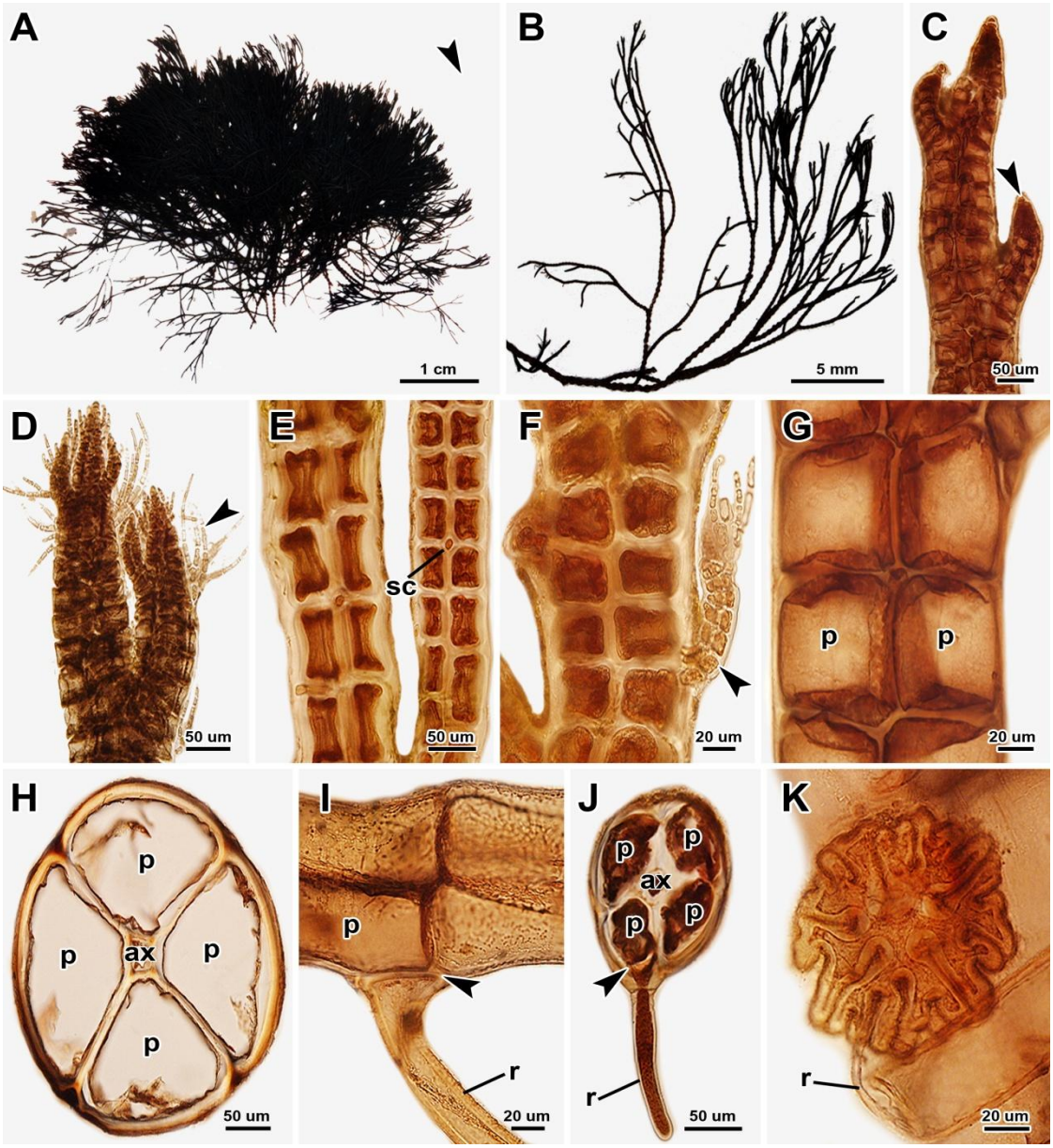


**Fig. 74.** Vegetative structures of *Neosiphonia sungminbooi* sp. nov. (A) Habit of vegetative plant. (B) Plants showing the extended prostrate axes and erect axes. (C) Inconspicuous and dome shaped apical cells (arrowhead) transversally divided. (D) Apex showing abundant and short trichoblasts. (E) Erect axes with scar cells (sc). (F) Erect axes showing cicatrigenous branches (arrowhead). (G) Old axes showing short segments. (H) Cross-section view showing four pericentral cells (p) (ax, axial cell). (I) Rhizoids (r) cutting off (arrowhead) from the proximal end of pericentral cells. (J) Cross-section of a prostrate axis showing rhizoids (r) cutting off (arrowhead) from pericentral cells (p). (K) Unicellular terminations of rhizoid (r).

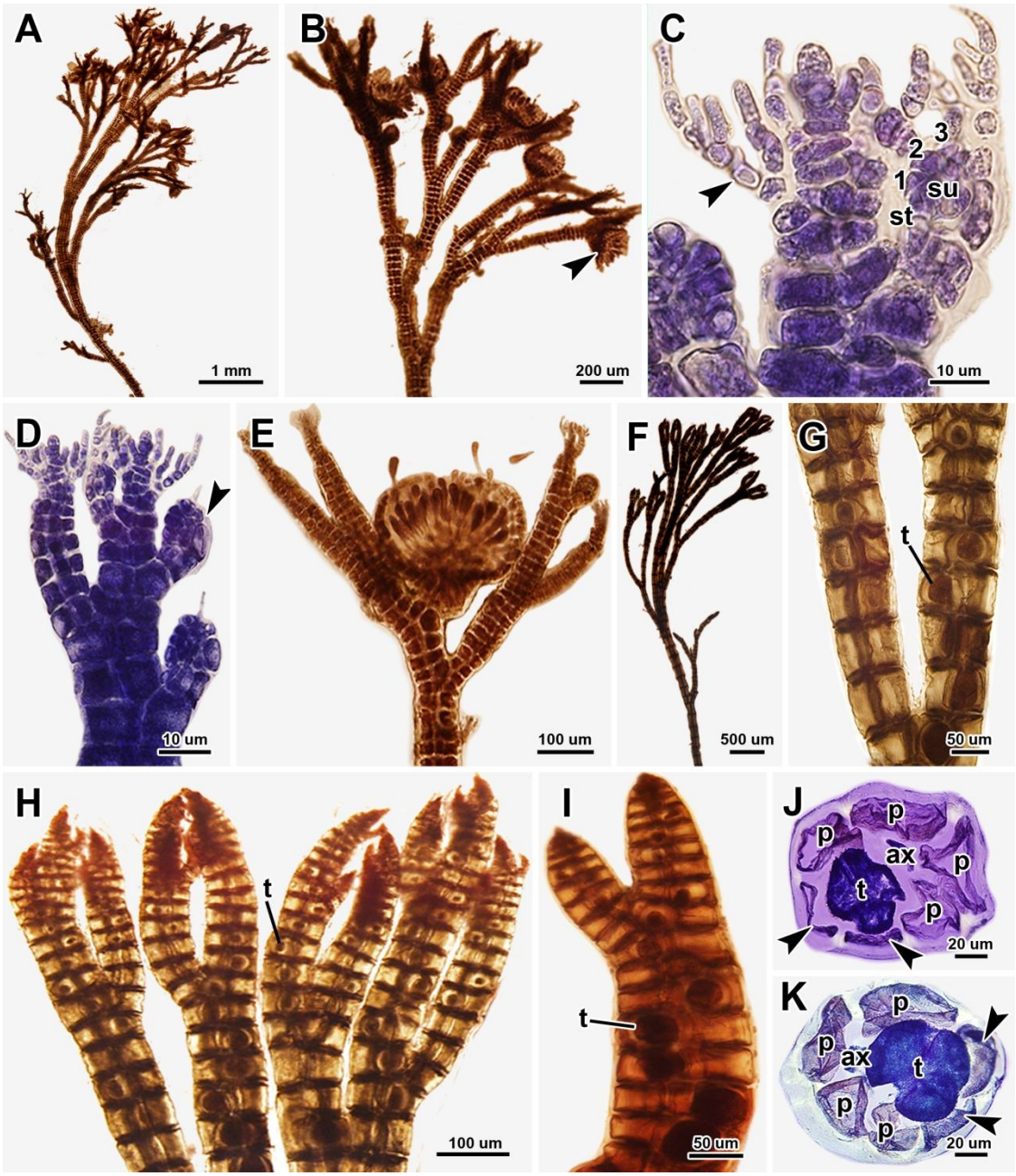


**Fig. 75.** Vegetative structures of *Neosiphonia sungminbooi* sp. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps (arrowhead). (C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Young cystocarp (arrowhead). (E) Mature cystocarps showing globose shape. (F) Tetrasporangial thallus. (G-I) Apical branches showing tetrasporangia (t) arranged in spiral series. (J-K) Cross-section with four pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

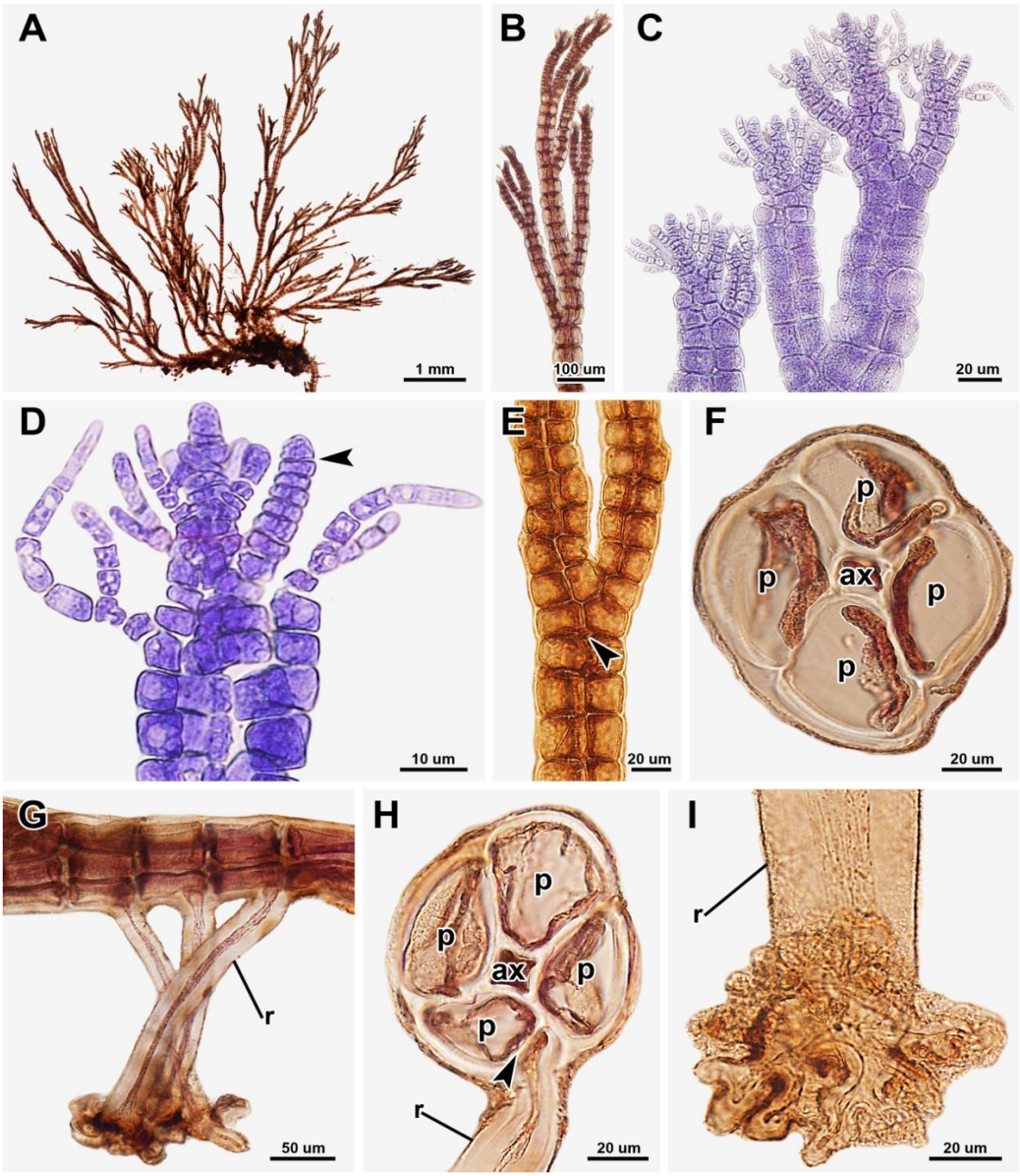


**Fig. 76.** Vegetative structures of *Neosiphonia tuticoriensis* comb. nov. (A) Habit of vegetative plant showing the extended prostrate and erect axes. (B-C) Apex with Alternate branching pattern and abundant trichoblasts. (D) Apex showing prominent apical cells transversally divided (arrowhead). (E) Erect axes showing scar cells (arrowhead). (F) Cross-section view showing four pericentral cells (p) (ax, axial cell). (G) Rhizoids (r) scattered and produced from proximal end of pericentral axes. (H) Cross-section of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (I) Unicellular terminations of rhizoid (r).

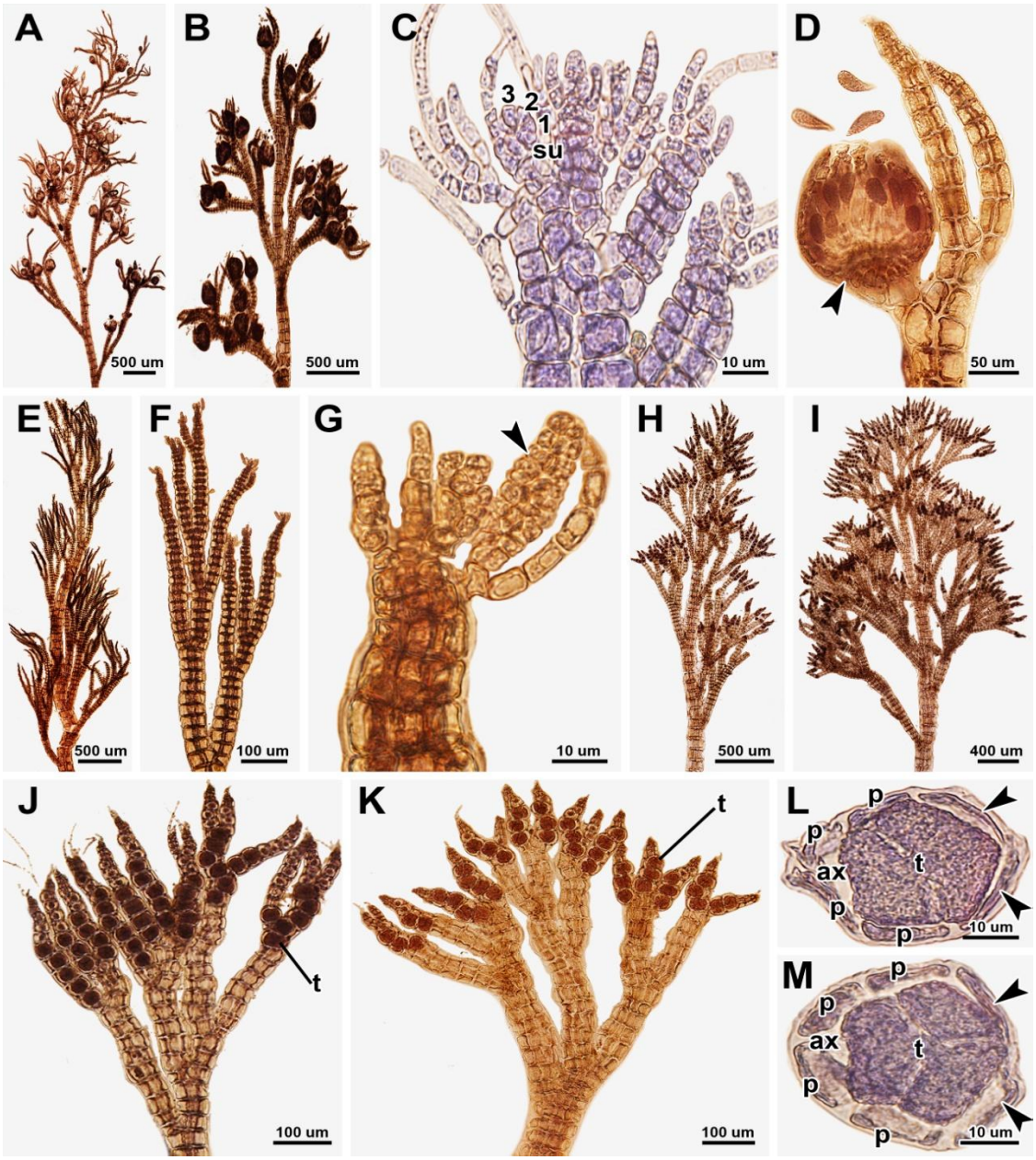


**Fig. 77.** Reproductive structures of *Neosiphonia tuticoriensis* comb. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Mature cystocarp (arrowhead) showing globose shape. (E) Male plant. (F) Spermatangial branches clustered at the apices of erect axes. (G) Spermatangial branches (arrowhead) arising from trichoblasts. (H-I) Tetrasporangial thallus. (J-K) Apical branches showing tetrasporangia (t) arranged in straight series. (L-M) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

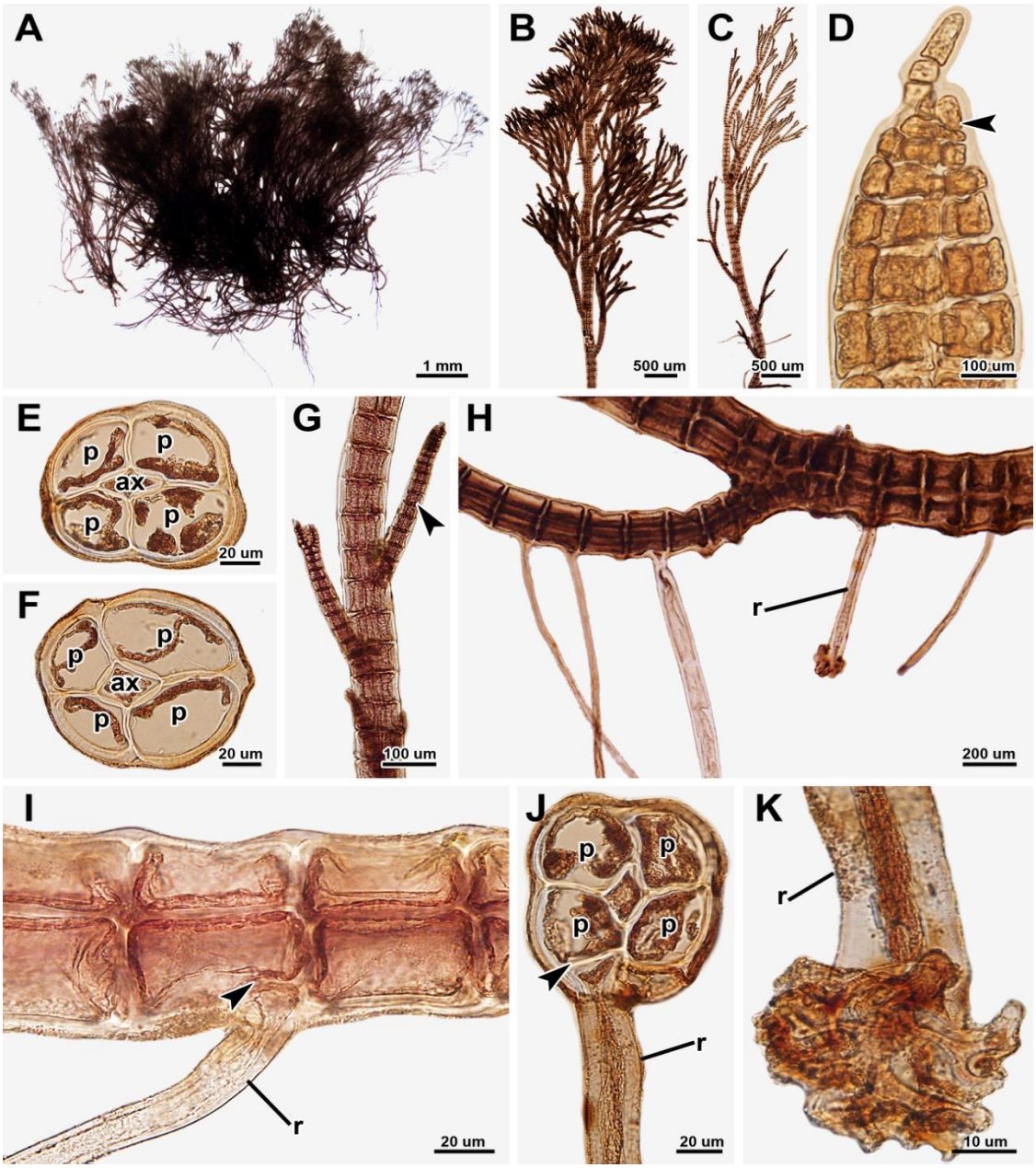


**Fig. 78.** Vegetative structures of *Neosiphonia unguiformis*. (A) Habit of vegetative plant. (B) Erect axes densely branched. (C) Apex with alternate to subdichotomous branching pattern. (D) Apex showing inconspicuous apical cells transversally divided (arrowhead). (E-F) Cross-section view showing four pericentral cells (p) (ax, axial cell). (G) Erect axes showing adventitious branches (arrowhead). (H) Rhizoids (r) scattered and produced from proximal end of pericentral axes. (I) Rhizoid (r) cutting off (arrowhead) from the proximal end of pericentral cells (p). (J) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (K) Unicellular terminations of rhizoid (r).

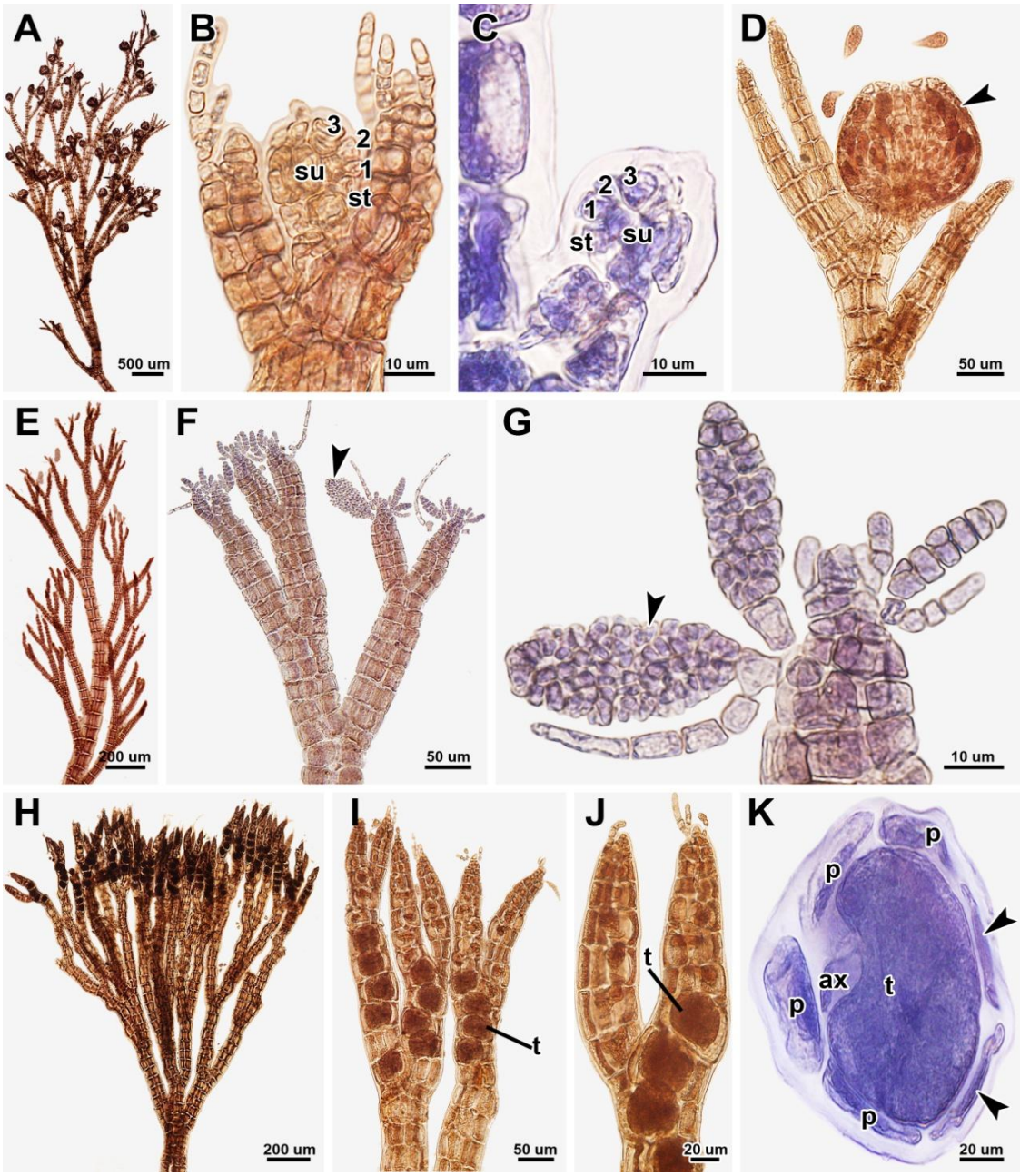


**Fig. 79.** Reproductive structures of *Neosiphonia unguiformis*. (A) Female plant. (B-C) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Mature cystocarps (arrowhead) with globose shape. (E) Male plant. (F) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (G) Paired spermatangial branches (arrowhead) arising from trichoblasts. (H) Tetrasporangial thallus. (I-J) Apical branches showing tetrasporangia (t) in spiral series. (K) Cross-section with four pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

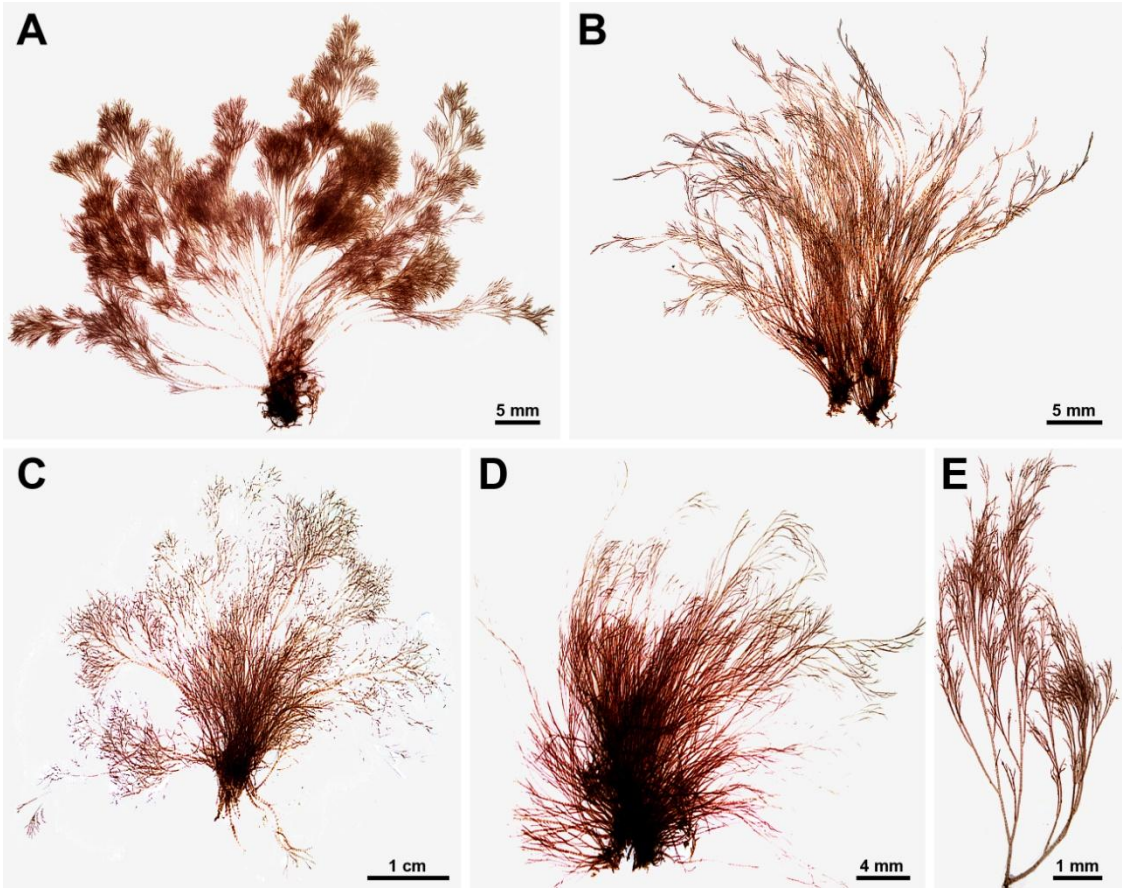


**Fig. 80.** Habit of *Neosiphonia yendoi*. (A-E) Plants showing a wide phenotypic plasticity.

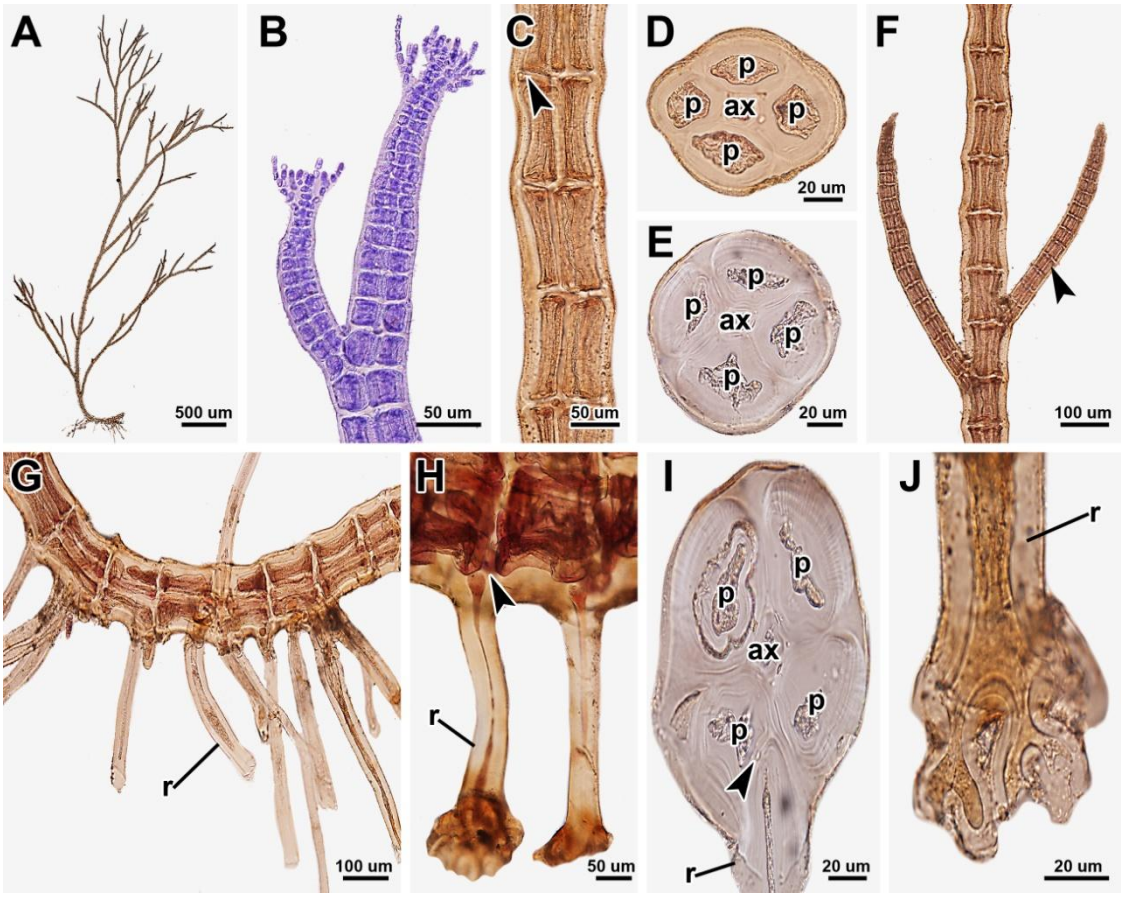


**Fig. 81.** Vegetative structures of *Neosiphonia yendoi*. (A) Erect axes arisen from reduced prostrate axes. (B) Apex showing abundant trichoblast. (C) Erect axes showing inconspicuous scar cells (arrowhead). (D-E) Cross-section views showing four pericentral cells (p) from the middle (D) and basal (E) part of axes (ax, axial cell). (F) Erect axes showing adventitious branches (arrowhead). (G) Rhizoids (r) scattered and produced from proximal end of pericentral axes. (H) Rhizoid (r) cutting off (arrowhead) from the proximal end of pericentral cells (p). (I) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (J) Unicellular terminations of rhizoid (r).

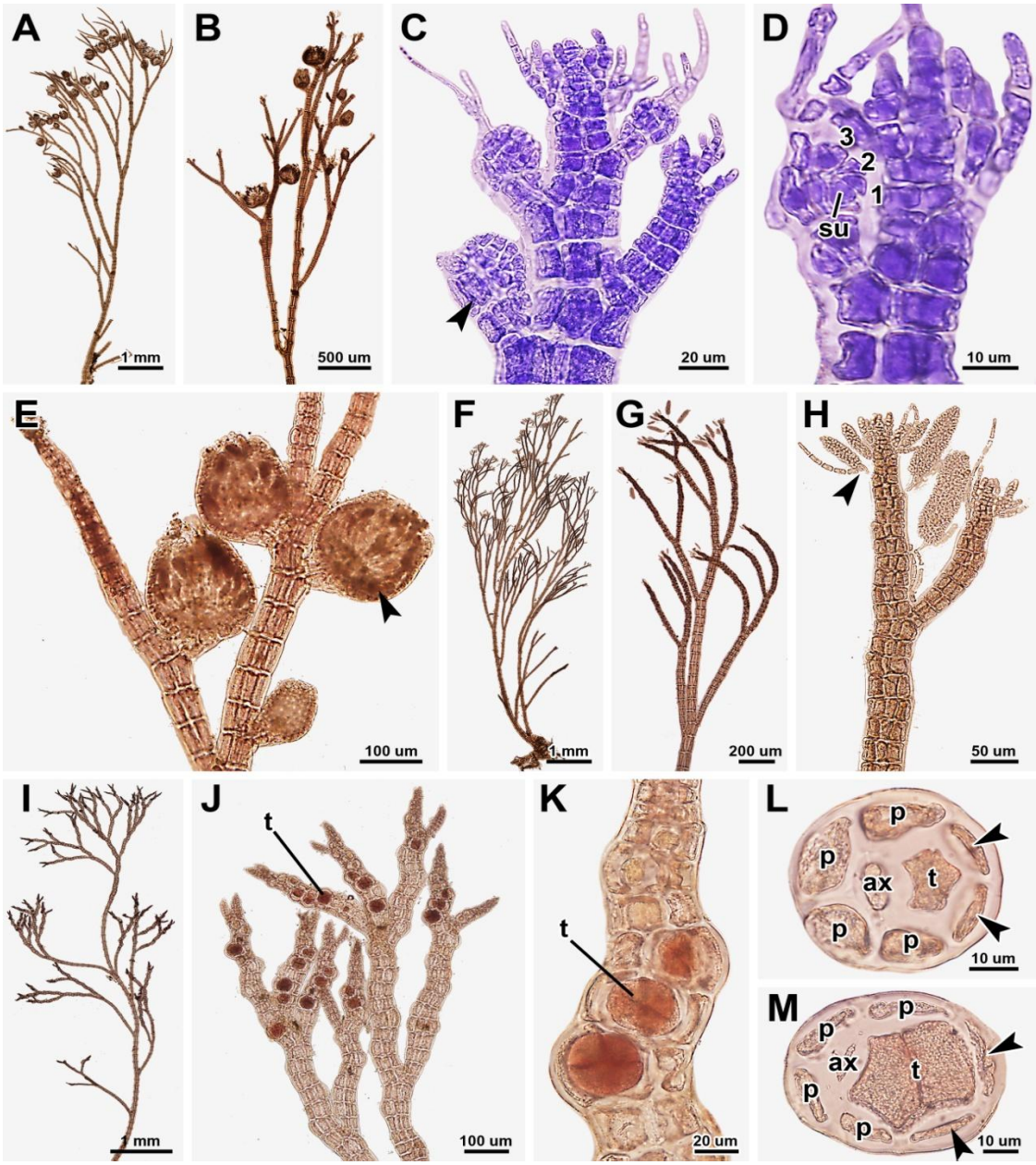


**Fig. 82.** Reproductive structures of *Neosiphonia yendoi*. (A) Female plant. (B-C) Upper part of female thallus showing subapical cystocarps. (D) Procarp with a three-celled carpogonial branch (1-3, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (E) Mature cystocarps (arrowhead) with globose shape. (F) Male plant. (G) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (H) Spermatangial branches (arrowhead) arising from trichoblasts. (I) Tetrasporangial thallus. (J-K) Apical branches showing tetrasporangia (t) in spiral series. (L-M) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

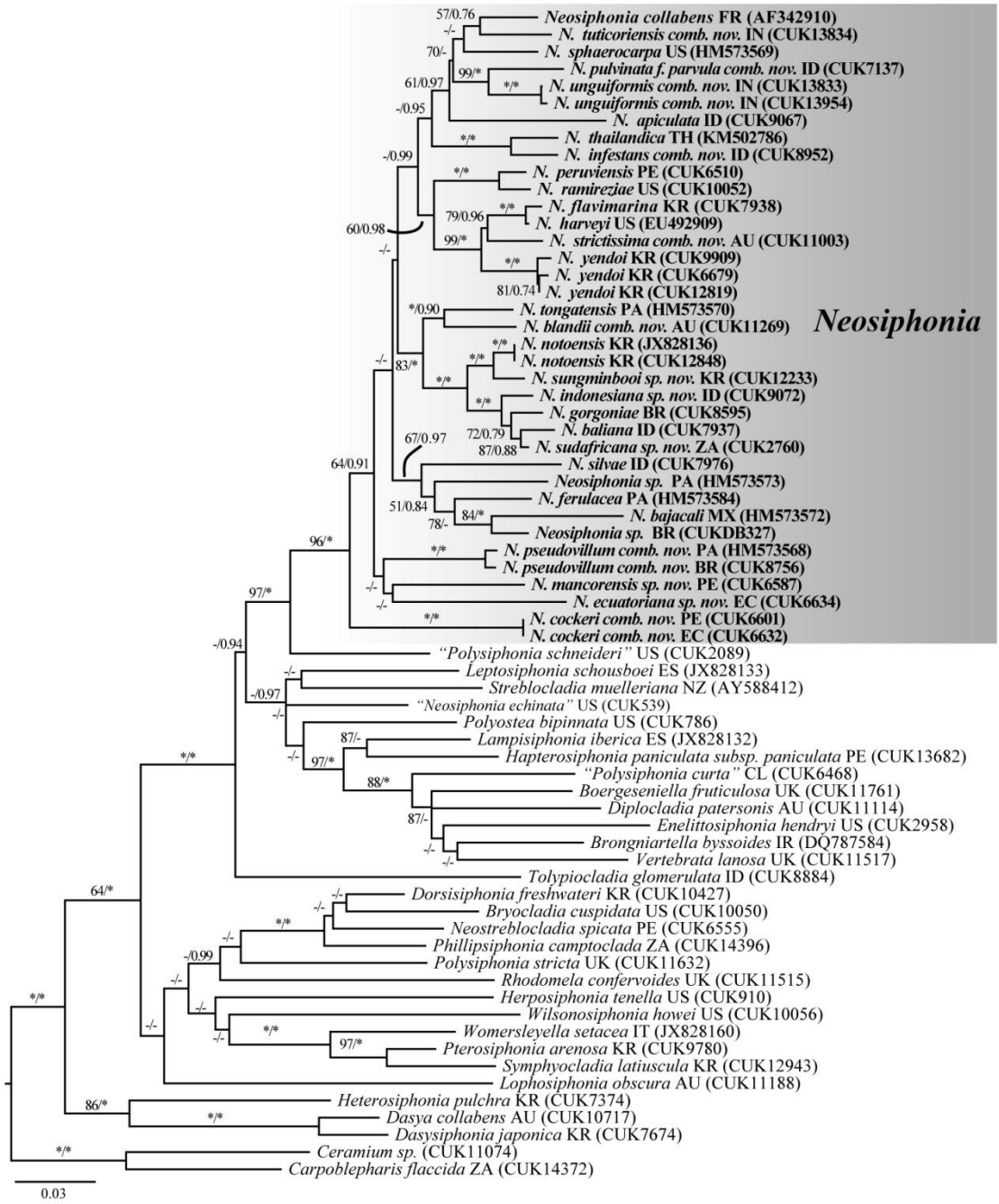


**Fig. 83.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 5.** Comparisons among species of *Neosiphonia*

| Species | Number of PC | Nature of habit | Tetrasporangia | Tetrasporangia per segment | PC in fertile segment | Spermatangia | Trichoblasts | Branching pattern | Branches type |
|---|---|---|---|---|---|---|---|---|---|
| *N. apiculata* | 4 | Holdfast | Spiral | 1 | 5 | Furcation | Abundant | Alternate | Cicatrigenous |
| *N. baliana* | 5 | Prostrate | Straight | 1 | 5 - 6 | Replace | Absent | Pseudodichotomous | Lacking |
| *N. blandii* | 4 | Prostrate | Spiral | 1 | - | - | Scarce | Alternate | Lacking |
| *N. coacta* | 4 | Prostrate | Spiral | 1 | 5 | - | Abundant | Pseudodichotomous | Adventitious |
| *N. cockeri* | 4 | Prostrate | Spiral | 1 | 4 - 5 | Furcation | Scarce | Subdichotomous | Cicatrigenous |
| *N. ecuatoriana* | 4 | Prostrate | Straight | 1 | 4 - 5 | Furcation | Abundant | Pseudodichotomous | Adventitious |
| *N. gorgoniae* | 4 | Holdfast | - | 1 | - | Replace | Scarce | Dichotomous | Adventitious |
| *N. indonesiana* | 4 - 5 | Prostrate | Straight | 1 | 5 | Replace | Absent | Pseudodichotomous | Adventitious |
| *N. infestans* | 4 | Prostrate | Spiral | 1 | 5 | - | Scarce | Alternate | Adventitious |
| *N. mancorensis* | 4 | Prostrate | Spiral | 1 | 4 | Replace and furcation | Scarce | Alternate | Lacking |
| *N. pulvinata f. parvula* | 4 | Holdfast | Spiral | 1 | 5 | - | Scarce | Subdichotomous | Adventitious |
| *N. peruviensis* | 6 | Prostrate | Spiral | 2 | 6 - 7 | - | Scarce | Pseudodichotomous | Adventitious |
| *N. pseudovillum* | 4 | Prostrate | Spiral | 1 | 5 | - | Abundant | Dichotomous | Adventitious |
| *N. sudafricana* | 4 | Holdfast | Straight | 1 | 5 | Replace | Absent | Dichotomous | Lacking |
| *N. ramireziae* | 4 | Prostrate | Spiral | 1 | 5 | Furcation | Scarce | Pseudodichotomous | Adventitious |

**Table 5.** *Continue*

| Species | Number of PC | Nature of habit | Tetrasporangia | Tetrasporangia per segment | PC in fertile segment | Spermatangia | Trichoblasts | Branching pattern | Branches type |
|---|---|---|---|---|---|---|---|---|---|
| *N. silvae* | 4 | Prostrate | Spiral | 1 | 5 | - | Scarce | Alternate | Adventitious |
| *N. notoensis* | 7 - 8 | Holdfast | Spiral | 2 | 8 - 9 | Furcation | Abundant | Pseudodichotomous | Adventitious |
| *N strictissima* | 4 | Holdfast | Spiral | 1 | 5 | Replace and furcation | Abundant | Dichotomous and pseudodichotomous | Cicatrigenous |
| *N. sungminbooi* | 4 | Prostrate | Spiral | 1 | 5 | - | Abundant | Alternate to pseudodichotomous | Cicatrigenous |
| *N. tuticoriensis* | 4 | Prostrate | Straight | 1 | 5 | Furcation | Scarce | Alternate | Adventitious |
| *N. unguiformis* | 4 | Prostrate | Spiral | 1 | 5 | Furcation | Scarce | Subdichotomous | Adventitious |
| *N. yendoi* | 4 | Prostrate | Spiral | 1 | 5 | Furcation | Abundant | Alternate | Adventitious |

## CHAPTER 4. Taxonomy and phylogeny of the genus *Polyostea* (Rhodomelaceae, Rhodophyta)

The genus *Polysiphonia* Grev. (1823) with a long and confused nomenclatural history has been considered as the core of the Family Rhodomelaceae (Segi 1951, Kim et al. 2000). The original name for species having features of *Polysiphonia* was proposed by C. Agardh (1817) as *Hutchinsia*, but this name was invalid as it was prior applied by Robert Brown to a group of cruciferous plants in honor of Miss Ellen Hutchins (Aiton and Aiton 1812, Kim et al. 2000). The homonymy with *Hutchinsia* R. Brown was soon recognized and several substitute names were proposed independently. *Polysiphonia* Grev. (1823) was conserved and the other considered as rejected names (Wynne 1986, Silva et al. 1996). The generitype of this genus was selected later by Silva (1952) on the basis of *Polysiphonia urceolata* (Lightf. *ex* Dillwyn) Grev. (1824), originally described as *Conferva urceolata* Dillwyn (1809). *P. urceolata* has been placed in synonymy with *Polysiphonia stricta* (Dillwyn) Grev. by Maggs and Hommersand (1993).

The recent review of the classification of red algal genera by Schneider and Wynne (2007) listed the following generic names placed in synonymy with the genus *Polysiphonia*: *Polyostea* Donati (1750), *Hutchinsia* C. Agardh (1817) *nom. illeg.* (non Aiton and Aiton 1812), *Grammita* Bonnem. (1822), *Dicarpella* Bory de St. Vincent (1823), *Grateloupella* Bory de St. Vincent (1823, as *Gratelupella*), *Girodia* T. Lestib. (1827), *Carradoria* Mart. (1833) *nom. illeg.*, *Grammitella* P. Crouan et H. Crouan (1848), *Orcasia* Kylin (1941), *Carradoria* Kylin (1956) *nom. illeg.*, *Carradoriella* P.C. Silva in Silva et al. (1996). Donati (1750) is considered the first to introduce a scientific system based on strictly logical scheme in phycology and recognize genera based on reproductive structures (Ruprecht 1850). Although Donati (1750) originally described the genus *Polyostea* based on the pericentral cells of axes disposed in a “bones-connected” appearance, a type species was not designated. Later, Ruprecht (1850) recognized this genus without a type species designation and described one new species namely, *Polyostea gemmifera* Rupr., and transfer two species from *Po-*

*lysiphonia* to *Polyostea*, namely *Ps. bipinnata* (Postels et Rupr.) Rupr. and *Ps. porphyroides* (Kütz.) Rupr. Although Ruprecht (1850) knew about the nomenclatural problems and priority of *Polysiphonia*, he did not reject *Polysiphonia* as a valid genus but preferred using Donati's genus.

Currently, *Polyostea gemmifera* and *Polyostea bipinnata* are considered synonymous of *Pterosiphonia bipinnata* (Postels et Rupr.) Falkenb. The genus *Pterosiphonia* was described by Falkenberg in Schmitz and Falkenberg (1897) on the basis of *Pt. cloiophylla* (C.Agardh) Falkenb. and the following diagnostic characters: erect axes with alternately distichous branches of determinate or indeterminate growth and flattened to strongly compressed. Although, the consistency of *Pterosiphonia* Falkenb. has been confirmed molecularly by the recent studies of Kim et al. (2012), some *Pterosiphonia* species as *Pt. bipinnata* and *Pt. gracilis* have not been evaluated yet.

In the present study, we collected some polysiphonous specimens having in common apex spirally originated with bilateral phyllotaxy of determinate or indeterminate growth. These specimens, *Pterosiphonia bipinnata* and *Pt. gracilis* Kylin, were collected from the North Pacific. We reassessed these specimens based on the detailed morphology and the phylogenetic relationships with other similar species by analyzing the *rbc*L and *cox*1 sequences. Here, we propose the resurrection of the genus *Polyostea* and the new combination *Polyostea gracilis.*

## 1. Morphological analyses

***Polyostea* Donati**

**Type species:** *Polyostea bipinnata* (Postels et Rupr.) Rupr.

**Diagnosis:** Plants prominent, forming large tufts predominantly attached to rock surfaces. Thalli are composed of prostrate and erect system. Erect axes are composed of 8-13 pericentral cells, ecorticate throughout, and densely and radially branched in subdistichous pattern with bilateral

phyllotaxy of determinate or indeterminate laterals. Branches not in relation with trichoblast. Adventitious branches present. Trichoblasts absent or deciduous. Rhizoids cut off from pericentral cells with unicellular terminations. Tetrasporangia tetrahedral, straight arrangement. Single tetrasporangium produced from each segment.

**Key to species of *Polyostea***

1. Pericentral cells 10-13, basal branches hooked ..... ***Polyostea bipinnata***
1. Pericentral cells 8, basal branches straight ..... ***Polyostea gracilis***

***Polyostea bipinnata* (Postels et Rupr.) Rupr (Fig. 85)**

**Basionym:** *Polysiphonia bipinnata* Postels et Rupr.

**Homotypic synonyms:** *Polysiphonia bipinnata* Postels et Rupr., *Pterosiphonia bipinnata* Falkenb.

**Heterotypic synonyms:** *Polyostea gemmifera* Rupr., *Polysiphonia californica* var. *plumigera* Harv., *Polysiphonia robusta* N.L.Gardner, *Pterosiphonia robusta* N.L.Gardner, *Polysiphonia robusta* var. *inermis* N.L.Gardner.

**Type locality:** Kamchatka, Russia

**Distribution:** North Pacific (Fig. 84)

**Specimens examined:** CUK786, CUK796 (Pescadero, California, USA collected by T.O.C. and B.Y.W., 03 Jul. 2003), CUK2951, CUK2953, CUK2954, CUK2960--63, CUK2965, CUK2996 (Outer point, Juneau, Alaska, USA collected by T.O.C., 10 Jul. 2006), CUK2974, CUK2984, CUK2989 (Point Brown, Kruzof, USA, collected by T.O. Cho, 13 Jul. 2006).

**Vegetative morphology:** Plants are prominent, robust, 5.4 to 12.9 cm high, and darkish-red in colour (Fig. 85A). Thalli form large tufts predominantly attached to rock surfaces in the intertidal

zone. Thalli are composed of reduced prostrate and robust erect system. The erect system is composed of interwoven and indeterminate main axes and arises exogenously from the prostrate axes. Main erect axes are prominent and are repeatedly branched by indeterminate and determinate branches, becoming denuded near to the base (Fig. 85B). Erect axes are radially branched in irregular pattern for 3-5 orders with bilateral phyllotaxy of determinate or indeterminate laterals (Fig. 85B-C). Apical cells are prominent, dome shaped, 7.3 ± 1.4 μm × 7.1 ± 1.1 μm in size, and transversely divided (Fig. 85C). Young determinate branches are abundant, formed by short segments, arising in an almost distichous pattern (bilateral phyllotaxy), and 1-3 orders of branches (Fig. 85C). Older segments of erect axes are larger, branches 227.7 ± 180.5 μm long and 103.3 ± 28.4 μm in diameter (L:D 2.22 ± 1.56), infrequently branched but with some recurved branchlets (Fig. 85D), forming congenital fusions of proximal parts in short laterals with main axes (Fig. 85E), and pericentral cells left twisted. Trichoblasts are absent or deciduous (Fig. 85C). Each segment is completely ecorticated along the thallus and composed of 10-13 pericentral cells (Fig. 85F-G). Adventitious branches are present. The prostrate system is reduced. Segments of the prostrate system are 56.7 ± 13.8 μm long and 67.1 ± 10.6 μm in diameter (L:D 0.86 ± 0.22). Rhizoids are ventrally produced from the centre or the proximal end of pericentral cells (Fig. 85H). They are cut off from pericentral cells (Fig. 85I). Rhizoids are unicellular and 384.4 ± 124.8 μm long and 22.9 ± 4.2 μm in diameter with multilobed terminations (Fig. 85J).

**Reproductive morphology:** In tetrasporangial plants (Fig. 85K), tetrasporangia are tetrahedrally divided, developed on main axes or on determinate branches (Figs 85L-M), and are 51.3 ± 22.3 μm × 48.1 ± 19.7 μm in size. Tetrasporangial branches are swollen, linear, and distorted (Fig. 85M). Tetrasporangia are arranged in interrupted straight series (Fig. 85L-M). The fertile segments have 9-11 pericentral cells. The fertile pericentral cell is developed as a stalk cell, which will develop a tetrasporangium and two presporangial cover cells (Fig. 85N). One tetrasporangium is produced on each segment (Fig. 85N).

**Habitat:** Plants grow in the intertidal zone, forming tufts. They are attached on rocks in sheltered sites. Tufts of this species are usually very robust and are associated with other filamentous species like *Ps. gracilis* and *Neosiphonia japonica*.

***Polyostea gracilis* (Kylin) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 86)**

**Basionym:** *Pterosiphonia gracilis* Kylin.

**Homotypic synonyms:** *Pterosiphonia gracilis* Kylin.

**Type locality:** Washington

**Distribution:** Northwestern Pacific (Fig. 84)

**Specimens examined:** CUK 2956, CUK2959 (Pescadero, California, USA collected by T.O.C. and B.Y.W., 03 Jul. 2003).

**Vegetative morphology:** Specimens were collected from Alaska. Plants are prominent, delicate, 3.8 to 10.1 cm high, and darkish-red to almost colorless (Fig. 86A). Thalli form large tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of prostrate and delicate erect system. The erect system is composed of interwoven and indeterminate main axes and arises exogenously from the prostrate axes. Main erect axes are prominent and are repeatedly branched by indeterminate and determinate branches, becoming denuded near to the base (Fig. 86B-C). Erect axes are radially branched in irregular pattern for 3-5 orders with bilateral phyllotaxy of determinate or indeterminate laterals (Fig. 86A-C). Apical cells are prominent, sharp, 14.9 ± 1.9 μm × 5.4 ± 0.8 μm in size, and transversely divided (Fig. 86D). Young determinate branches are abundant, formed by short segments, arising in an almost distichous pattern (bilateral phyllotaxy), and 1-3 orders of branches (Fig. 86E). Older segments of erect axes are larger, 60.4 ± 12.7 μm long and 51.8 ± 4.7 μm in diameter (L:D 1.16 ± 0.21), infrequently branched, forming congenital fu-

sions of proximal parts in short laterals with main axes (Fig. 86F), and pericentral cells left twisted (Fig. 86G). Trichoblasts are absent (Fig. 86D-E). Each segment is completely ecorticated along the thallus and composed of 8 pericentral cells (Fig. 86H-I). Adventitious are present (Fig. 86J). The prostrate system is reduced. Segments of the prostrate system are 162.6 ± 91.6 μm long and 63.1 ± 8.5 μm in diameter (L:D 2.53 ± 1.2). Rhizoids are secondary and ventrally produced from the centre or the proximal end of pericentral cells (Fig. 86K-L). They are cut off from pericentral cells (Fig. 86M). Rhizoids are unicellular and 218.4 ± 104.4 μm long and 22.9 ± 2.2 μm in diameter with multilobed terminations.

**Habitat:** Plants grow in the intertidal zone, forming tufts. They are attached on rocks in sheltered sites. Tufts of this species are usually delicate and are associated with other filamentous species like *Ps. bipinnata* and *Neosiphonia japonica*.

## 2. Phylogenetic analyses

A 1,245-bp portion of the 1,467- bp *rbc*L (84.8%) and 586-bp portion of the 1467-bp *cox1* (40%) were sequenced for *Polyostea bipinnata*, *Polyostea gracilis* and other *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus placed *Polyostea* sister to the clade composed by *Leptosiphonia*, *Neosiphonia*, and "*P. schneideri* clade" with sequences divergence of 9.2% to 11.6% between them and supported by a bootstrap value of 100 and a posterior probability of 1 (Fig. 87). *Polyostea* is also diverging from *Pterosiphonia* by 13.5% to 14.3%. On the other hand, phylogenetic analyses of the *cox1* locus placed the members of *Polyostea* sister to the clade composed by *N. echinata* and *Leptosiphonia* with sequences divergence of 11% to 13.4% between them and low support of bootstrap and posterior probability values (Fig. 88). *Polyostea* is also diverging from *Pterosiphonia* by 15.8% to 16.0%. The sequence divergences between *Polyostea bipinnata* and *Polyostea gracilis* are 5.9% for *rbc*L and 7.7% for *cox1*.

## 3. Discussion

The genus *Polyostea* was described by Donati (1750) without the designation of a generitype. Although the genus *Polyostea* was placed under synonymy with *Polysiphonia* by Silva (1952), earlier Ruprecht (1850) recognized *Polyostea* and also the genus *Polysiphonia* as two different and valid genera. Here, we are resurrecting *Polyostea* as a different genus embedded into *Polysiphonia* sensu lato on the basis of morphological and molecular analyses. The genus *Polyostea* is having the following diagnostic features: (1) apex spirally originated with bilateral phyllotaxy of determinate or indeterminate laterals, (2) ecorticated axes, and (3) congenital fusions of proximal parts in short laterals with main axes. The results of our *rbc*L and *cox*1 sequence analyses support the generic status of the resurrected *Polyostea* as a consistent genus in *Polysiphonia* sensu lato with a high support (100% of bootstrap in ML and 1.0 in Bayesian posterior probability for *rbc*L) .

Among the features delineating the genus *Polyostea*, bilateral phyllotaxy, ecorticated axes, and congenital fusions, are the principal characters that separate *Polyostea* from others in *Polysiphonia* sensu lato (Table 6). The bilateral phyllotaxy of the branches in the genus *Polyostea* is due to secondary torsion and Uwai and Masuda (1999) defined it as the branches on either side that undergo torsion in opposite directions. This character related *Polyostea* to *Boergeseniella* Kylin and *Tayloriella* Kylin (Maggs and Hommersand 1993, Uwai and Masuda 1999) but *Boergeseniella* is distinguished from *Polyostea* by having corticated axes (Maggs and Hommersand 1993) and *Tayloriella* is distinguished by having monosiphonous laterals (Wynne 1985). The ecorticated axes and congenital fusion of lateral to the axes related *Polyostea* to *Pterosiphonia* (Schmitz and Falkenberg 1987) but *Pterosiphonia* is different by having multicellular rhizoids on the distal end of pericentral cells.

The resurrected *Polyostea* is composed of *Polyostea bipinnata* and *Ps. gracilis.* The generitype of the resurrected genus, namely *Ps. bipinnata*, was assigned on the present study because Donati (1750) and Ruprecht (1850) did not propose a type species for this genus. *Polyostea bipinnata* was originally described as *Polysiphonia bipinnata* by Postels and Ruprecht (1840), then transferred to

*Polyostea* by Ruprecht (1850), and later transferred to *Pterosiphonia bipinnata* by Falkenberg (1901). It is characterized by having 10-13 pericentral cells, bilateral phyllotaxy, and recurved branchlets (hooked branches). Our specimens collected from Alaska and California (Fig. 84) correspond in morphology to those of the type locality (Ruprecht 1850, Klochkova et al. 2009) and those of previous reports from California and Oregon (Abbott and Hollenberg 1976, Gabrielson et al. 2006). *Polyostea bipinnata* is similar to *Polysiphonia decipiens* Mont. and *Po. senticulosa* Harv. by having hooked branches, but *Po. decipiens* is distinguished from *Ps. bipinnata* by having seven pericentral cells (Adams 1994), whereas *Po. senticulosa* is distinguished by having four pericentral cells and rhizoids open connected to pericentral cells (Kim and Nam 2014). *Polyostea bipinnata* has been well documented from the North Pacific (Abbot and Hollenberg 1976, Gabrielson et al. 2006) and our study confirms its wide distribution in the northwestern Pacific (Fig. 84).

*Polyostea gracilis* was originally described as *Pterosiphonia gracilis* by Kylin (1925). It is characterized by having eight pericentral cells, bilateral phyllotaxy, and sharp apical cells. The last feature related *Polyostea gracilis* to *Polysiphonia morrowii* Harv., but *P. morrowii* is distinguished by having four pericentral cells (D'Archino et al. 2013). Our specimens from Alaska correspond in morphology with *Polyostea gracilis* and their particular habit clearly distinguishes from other *Polysiphonia* sensu lato. *Polyostea gracilis* has only been reported from Washington (Gabrielson et al. 2006). Our study reported *Pt. gracilis* from Alaska and confirmed its restricted distribution in the northwestern Pacific. *Polyostea bipinnata* and *Ps. gracilis* are distinguishing each other on the basis of the pericentral cells number, apical cells shape and basal branches type. *Ps. bipinnata* is having 10-13 pericentral cells, dome shape apical cells, and recurved basal branches; whereas *Ps. gracilis* is having only 8 pericentral cells, sharp apical cells, and straight basal branches.

The results of our *rbc*L and *cox*1 phylogenetic analyses revealed that *Polyostea* is closely related, with high support [100% for ML and 1.0 for Bayesian posterior probabilities (BPP)], to the clade composed by the species *N. echinata* (Harv.) N.Mamoozadeh et Freshwater, *Po. binneyi* Harv., *Po. havanensis* Mont., and *Po. homoia* Setch. et N.L.Gardner (Fig. 87-88). These species are clearly

distinguished from the species of *Polyostea* by having four pericentral cells (Mamoozadeh and Freshwater 2011). Moreover, our phylogenetic analyses positioned *Polyostea* as a separated genus in *Polysiphonia* sensu lato. *Polyostea* is clearly distinguished from all the genera of *Polysiphonia* sensu lato by the combination of bilateral phyllotaxy, ecorticated axes and congenital fusion. Thus, *Polysiphonia* sensu lato is currently composed by *Hapterosiphonia*, *Lampisiphonia*, multipericentral group, *Neosiphonia*, *Polyostea*, and *Polysiphonia* sensu stricto (Bustamante et al. 2015c).

**Fig. 84.** Distribution of the species of the resurrected genus *Polyostea* around the Northern Pacific Ocean. *Polyostea bipinnata* (blue) and *Polyostea gracilis* comb. nov. (yellow).

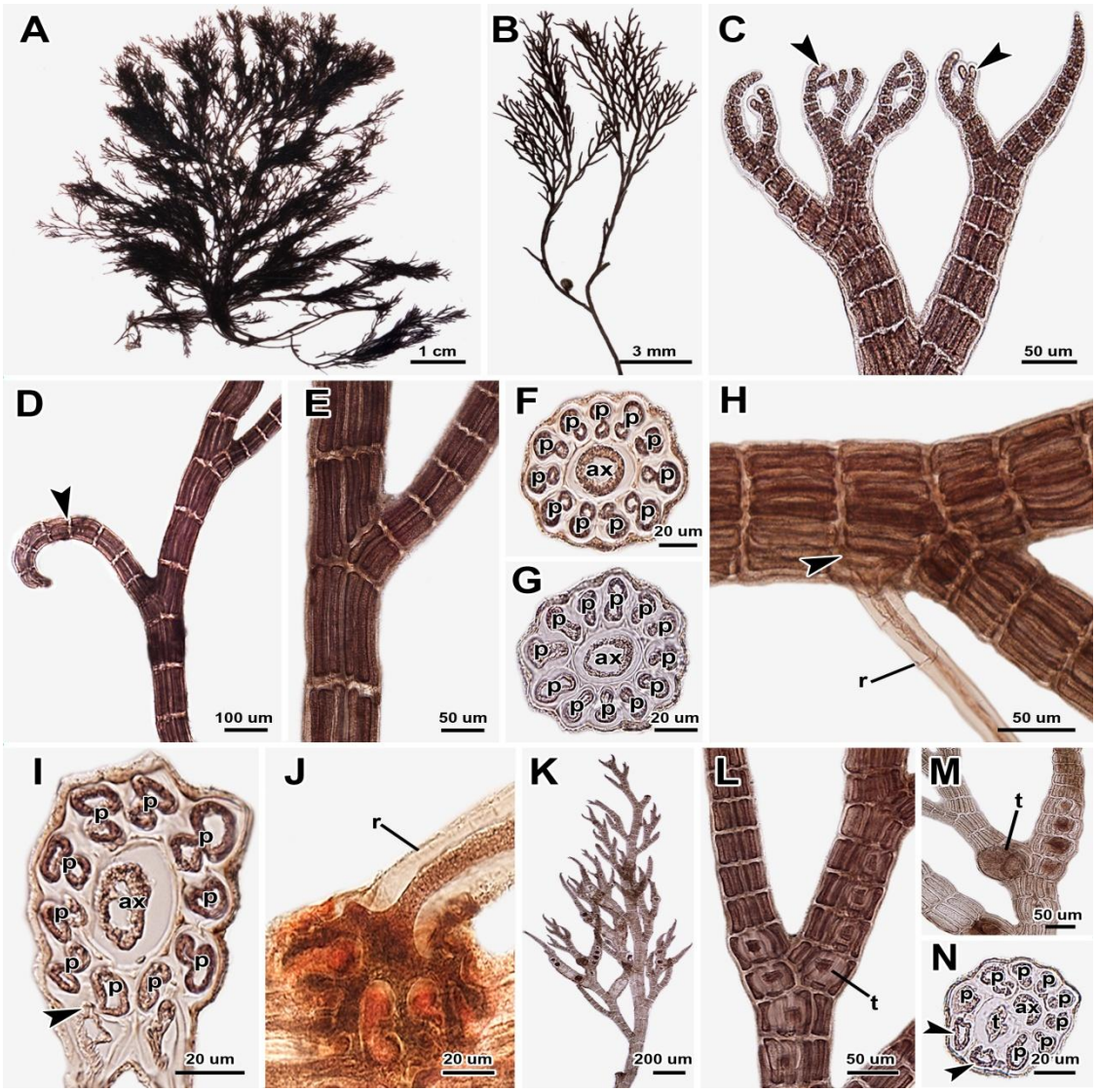


**Fig. 85.** Vegetative and reproductive structures of *Polyostea bipinnata*. (A) Habit of vegetative plant showing the reduced prostrate axes and robust erects axes. (B) Main axes with determinate branches in the apex and denudated in the base. (C) Apices showing the apical cells (arrowhead) and bilateral phyllotaxy. (D) Old axes showing recurved branchlets (arrowhead). (E) Old axes showing congenital fusions. (F-G) Cross section views of erect (F) and prostrate (G) axes showing 11-12 pericentral cells (p) (ax: axial cell). (H) Reduced prostrate axes bearing a rhizoid (r) cutting off from the centre of pericentral cells (arrowhead). (I) Cross-section view of prostrate axes showing rhizoids cutting off (arrowhead) from pericentral cells (p) (ax: axial cell). (J) Multilobed and unicellular terminations of rhizoid (r). (K) Tetrasporangial plant. (L-M) Upper part of thallus showing tetrasporangia (t) in straight series in the main axes and determinate branches. (N) Cross section view of fertile segment showing one tetrasporangium (t) surrounded by cover cells (arrowheads) (ax: axial cell, p: pericentral cell).

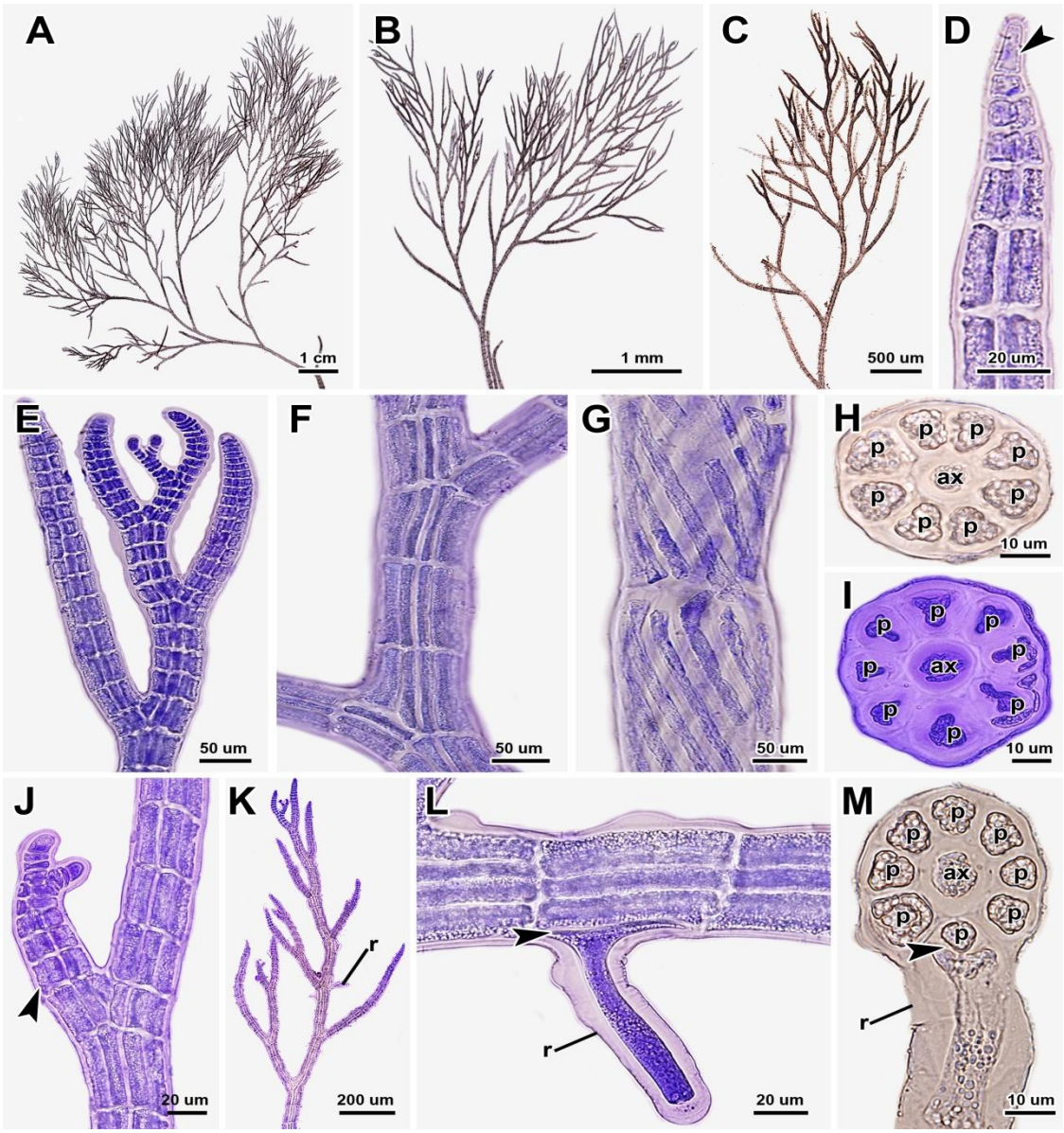


**Fig. 86.** Vegetative and reproductive structures of *Polyostea gracilis* comb. nov. (A) Habit of vegetative plant showing the reduced prostrate axes and robust erects axes. (B-C) Main axes with determinate branches in the apex and denudated in the base. (D) Apex showing sharp apical cells (arrowhead). (E) Apex showing alternate branches with bilateral phyllotaxy. (F) Old axes showing congenital fusions. (G) Old axes showing pericentral cells left twisted. (H-I) Cross section views of erect (H) and prostrate (I) axes showing 8 pericentral cells (p) (ax: axial cell). (J) Erect axes showing adventitious branches (arrowhead). (K) Prostrate axes showing secondary rhizoids (r). (L) Reduced prostrate axes bearing a rhizoid (r) cutting off from the proximal end of pericentral cells (arrowhead). (M) Cross-section view of prostrate axes showing a rhizoid cutting off (arrowhead) from pericentral cells (p) (ax: axial cell).

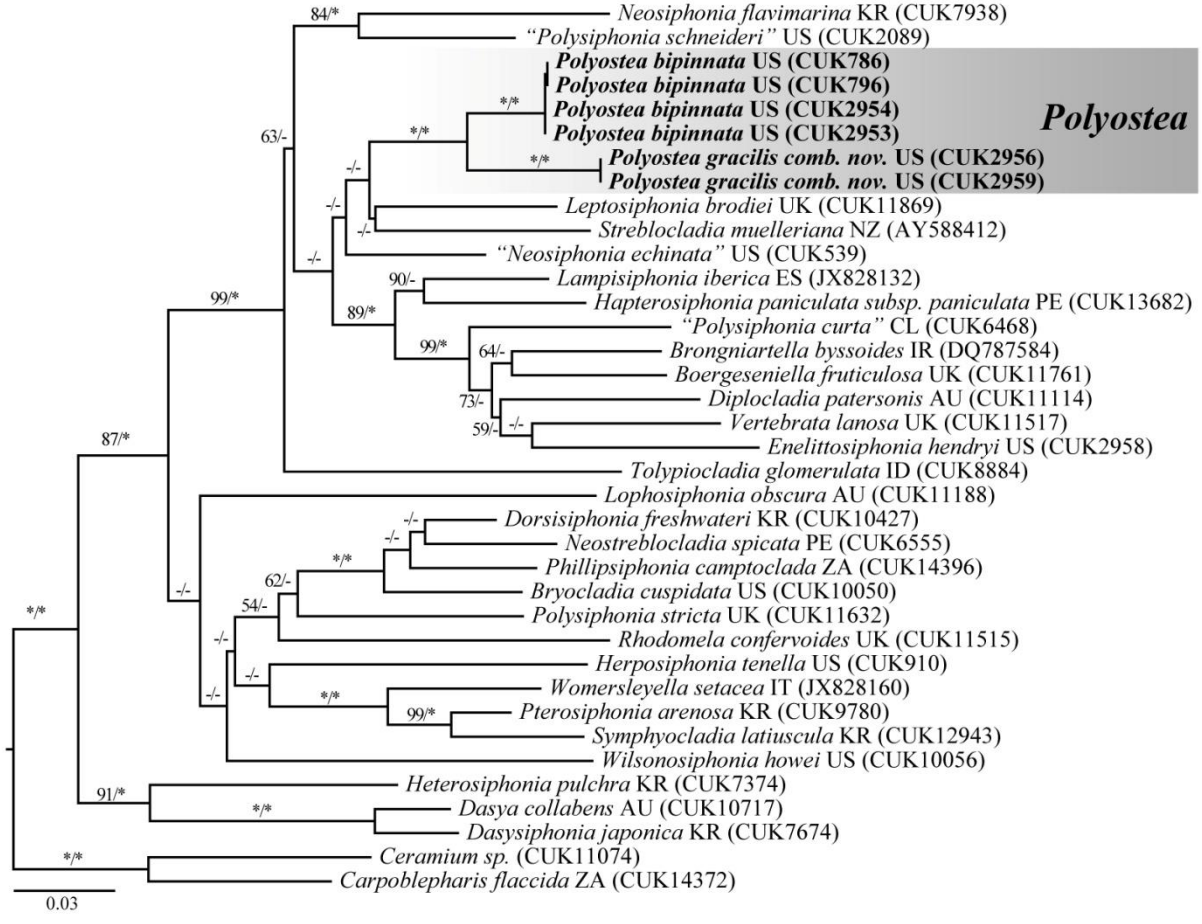


**Fig. 87.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

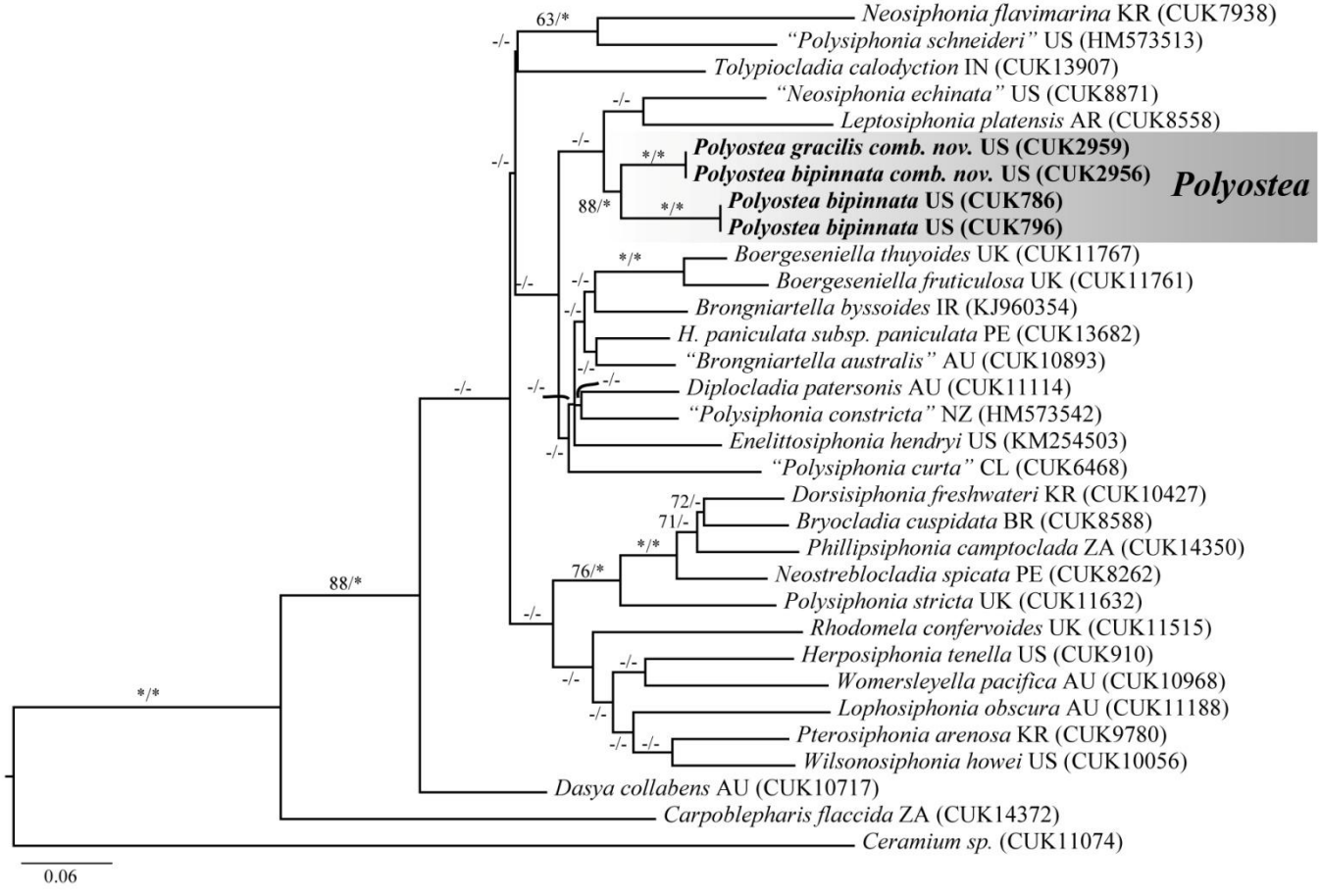


**Fig. 88.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 6.** Comparisons among genera belonging to *Polysiphonia* sensu lato and *Polyostea*.

| Species | *Polyostea* | *Boergeseniella* | *Bryocladia* | *Diplocladia* | *Enelittosiphonia* | *Hapterosiphonia* | *Lampisiphonia* | *Neosiphonia* | *Polysiphonia* | *Vertebrata* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Type species** | *Ps. bipinnata* | *Bo. fruticulosa* | *Br. cervicornis* | *D. patersonis* | *E. stimpsonii* | *H. paniculata* | *La. iberica* | *N. flavimarina* | *Po. stricta* | *V. lanosa* |
| **Type localities** | Kamchatka, Russia | British Isles | Java | Victoria, Australia | Japan | Lima, Peru | Spain | Bangpo, Korea | Glamorgan, British Isles | Iceland |
| **Pericentral cells** | 8--13 | 8–12 | 6–12 | 7–13 | 7–11 | 8–12 | 9–11 | 4–9 | 4 | 17-23 |
| **Branching pattern** | Spiral with bilateral phyllotaxy | Alternate-distichous | Determinate spiral laterals | Needle-like determinate branches | Dorsiventral | Paniculate | Pseudodichotomous | Alternate | Pseudodichotomous to alternate | Pseudodichotomously |
| **Cortication** | Absent | Present | Absent | Absent | Absent | Absent | Present | Absent or present | Absent | Absent |
| **Trichoblasts** | Absent | Scarce | Scarce | Scarce to abundant | Abundant | Abundant | Absent | Abundant | Scarce to abundant | Absent |
| **Rhizoids connection** | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Open | Cut off |
| **Rhizoids** | Unicellular | Unicellular | Unicellular | Unicellular | Unicellular | Multicellular | Multicellular | Unicellular | Unicellular | Unicellular |
| **Tetrasporangia** | Straight | Spiral | Straight | Spiral | Spiral | Spiral or straight | Straight | Spiral or straight | Spiral or straight | Straight |
| **References** | Abbott and Hollenberg (1976), this study | Maggs and Hommersand (1993) | Schmitz and Falkenberg (1897) | Womersley (2003), this study | Segi (1949) | Bustamante et al. (2015c) | Bárbara et al. (2013) | Kim and Lee (1999) | Kim et al. (2000) | Kim et al. (2002) |

## CHAPTER 5. Taxonomy and phylogeny of the genus *Polysiphonia* sensu stricto (Rhodomelaceae, Rhodophyta)

The recent studies in *Polysiphonia* sensu lato have reported that this group is composed of around 220 species which are distributed in the following heterogeneous genera: *Hapterosiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho, *Lampisiphonia* H.G. Choi, Diaz-Tapia et Bárbara, multipericentral group, *Neosiphonia* M.S. Kim et I.K. Lee, *Polysiphonia* sensu stricto, and *Wilsonosiphonia* (Choi et al. 2001, Bárbara et al. 2013, Bustamante et al. 2015c, see Chapter 4).

The detailed examination of the generitype of *Polysiphonia*, *P. stricta* (Dillwyn) Greville by Kim et al. (2000) shows that *Polysiphonia* consist of prostrate and erect ecorticate axes with four pericentral cells, rhizoids in open connection with the pericentral cells, a four-celled carpogonial branch, spermatangial branches replacing the whole trichoblast, and tetrasporangia arranged in straight series (Kim et al. 2000, Bustamante et al. 2015a). This characterization was outstanding to understand the wide circumscription of the heterogeneous features in vegetative and reproductive structures and also to notice the long and confused nomenclatural history of species labeled as *Polysiphonia* (Kim et al. 2000).

Phylogenetic tree based on Bayesian analysis from plastid-encoded *rbc*L revealed that *Polysiphonia* sensu stricto is composed of 17 species which are distributed in two main clades. One of these clades is containing the generitype *P. stricta*, whereas the other is composed of different genera (Bustamante et al. 2015a). Although thirteen new species have been described recently in *Polysiphonia* sensu lato (Stuercke and Freshwater 2010, Kim and Kim 2014, Bustamante et al. 2013, 2014a, 2014b, 2015a), molecular analyses have demonstrated that only five of them are situated in *Polysiphonia* sensu stricto (Bustamante et al. 2015a).

The goals of the present study were to reassess specimens embedded in *Polysiphonia* sensu stricto based on anatomical observations and molecular analyses. We also provide evidence of their

phylogenetic relationship with other polysiphonous groups by analyses of *rbc*L and *cox*1 sequences. Here, we segregate the new genera *Dorsisiphonia*, *Neostreblocladia*, and *Phillipsiphonia* to accommodate the paraphyletic relationships among species previously identified in *Polysiphonia* and *Streblocladia*.

## 1. Morphological analyses

Our molecular analyses have shown that species characterized by having ecorticated thalli and rhizoids in open connection from pericentral cells are clustered under two main clades traditionally labeled as *Polysiphonia* sensu stricto (Mamoozadeh and Freshwater 2012, Bustamante et al. 2015a). These clades are composed by *Bryocladia*, true *Polysiphonia* sensu stricto, three new genera and two species of uncertain position which are widely distributed (Fig. 89).

**Key to genera**

1. Rami-sympodial branching pattern .....2
1. Dorsiventral or spiral branching pattern .....3
    2. Pericentral cells four ..... ***Phillipsiphonia***
    2. Pericentral cells 6-13 ..... ***Neostreblocladia***
3. Erect axes arisen from prostrate axes in strictly dorsiventral disposition ..... ***Dorsisiphonia***
3. Erect axes arisen from prostrate axes in spiral disposition ..... 4
    4. Pericentral cells four, simple adventitious branches ..... ***Polysiphonia* sensu stricto**
    4. Pericentral cells eight, adventitious laterals on determinate branches ..... ***Bryocladia***

***Bryocladia* F.Schmitz**

**Type species:** *Bryocladia cervicornis* (Kützing) F.Schmitz.

***Bryocladia cuspidata* (J. Agardh) De Toni (Fig. 90-91)**

**Basionym**: *Polysiphonia cuspidata* J. Agardh.

**Homotypic synonym:** *Polysiphonia cuspidata* J. Agardh.

**Type locality**: Vera Cruz, Mexico.

**Distribution**: Tropical and subtropical western Atlantic.

**Specimens examined:** CUK10046, CUK10050 (Sand Key Park, Clearwater, Florida, USA collected by T.O.C, Aug. 08 2013).

**Vegetative morphology:** Plants were attached on rock surface in the intertidal zone. Thalli form reduced tufts, 1.5 to 7.0 cm high, and darkish-red in colour (Fig. 90A). They are composed of prostrate and erect systems with robust texture (Fig. 90B). The erect system is composed of interwoven and indeterminate main axes that arise exogenously from the prostrate axes (Fig. 90A). Main erect axes are prominent which are covered by needle-like determinate branches (Fig. 90B-C). Erect axes are radially branched in irregular pattern. Apical cells are inconspicuous (Fig. 90D), 6.21 ± 0.8 μm × 5.7 ± 0.72 μm in size, and transversely divided. Trichoblasts are very delicate, numerous, 1-2 times forked, 81.6 ± 36.6 μm long, arise on each segment, and scarce on determinate branches (Fig. 90E-F). Scar cells are inconspicuous. Cicatrigenous branches are absent. Needle-like determinate branches are abundant, formed by short segments, arising spirally, and bearing adventitious branches (Fig. 90D-G). Indeterminate laterals of erect axes are 74.4 ± 13.3 μm long and 207.2 ± 18.5 μm in diameter (L:D 0.4 ± 0.06). Each segment is completely ecorticated along the thallus

and composed of eight pericentral cells (Fig. 90I-K). Adventitious branchlets are present on the main filaments (Fig. 90H) and determinate branches. The prostrate system is extended (Fig. 90L-M) and forming entangled stolones (Fig. 90B). Segments of the prostrate system are 52.3 ± 5.9 μm long and 93.1 ± 8.0 μm in diameter (L:D 0.6 ± 0.1). Rhizoids are ventrally produced from the centre or the proximal end of pericentral cells (Fig. 90L). They are in open connection to pericentral cells (Fig. 90N). Rhizoids are unicellular and 538.9 ± 285.5 μm long and 26.5 ± 5.3 μm with multi-lobed terminations (Fig. 90O).

**Reproductive morphology:** In male gametophytes (Fig. 91A), spermatangial branches are clustered at the apices of determinate branches axes (Fig. 91B-C), and replacing the whole trichoblast in each axial segment (Fig. 91C). Each spermatangial branch is composed of spermatangia, and lacking of sterile tip cell (Fig. 91C).

**Habitat:** Plants grow in the intertidal zone, forming small and individual tufts. They are attached on rocks in sheltered sites. Tufts of this species are usually very flaccid and are highly covered with epiphytes and other filamentous species.

**Remarks:** The neddle-like branches have been reported also in *Diplocladia* Kylin and *Perrinia* Womersley. However, *Bryocladia* is distinguished from them by having adventitious laterals growing on the neddle-like branches and rhizoids in open connection to pericentral cells.

***Dorsisiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho gen. nov.**

**Type species:** *Dorsisiphonia freshwateri* (D.E. Bustamante, B.Y. Won et T.O. Cho) D.E. Bustamante, B.Y. Won et T.O. Cho.

**Diagnosis:** Plants diminutive, forming small tufts predominantly attached to rock surfaces. Thalli are composed of an extensive and entangled prostrate system, and interwoven indeterminate erect

axes. They are disposed in a strictly dorsiventral disposition. Axes composed of 4 pericentral cells, ecorticate throughout. Adventitious branches present. Lateral branches arising in the axils of trichoblasts. Trichoblasts delicate, deciduous, small, 1–3 times forked, elongate. Conspicuous scar cells. Rhizoids open connected to the pericentral cells, with lobed and unicellular terminations. Procarps positioned laterally and subapically, each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial branches replacing trichoblast, cylindrical, sometimes with sterile tip. Tetrasporangia tetrahedral arranged in straight series. A single tetrasporangium is produced on each fertile segment.

**Etymology**: "*Dorsisiphonia*" refers to the strictly dorsiventral disposition of prostrate and erect axes.

**Key to species of *Dorsisiphonia* gen. nov.**

1. Tetrasporangia arranged in straight series and slightly spiral ..... ***D. freshwateri***
1. Tetrasporangia arranged in straight series ..... 2
    2. Subdichotomous branching pattern of erect axes with adventitious lateral branches alternate arranged ..... ***D. parvula***
    2. Irregular branching pattern of erect axes with adventitious lateral branches irregular arranged ..... 3
3. Erect axes densely branched in upper parts ..... ***D. utricularis***
3. Erect axes scarcely branched ..... ***D. villum***

***Dorsisiphonia freshwateri* (D.E. Bustamante, B.Y. Won et T.O. Cho) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 92-93)**

**Basionym**: *Polysiphonia freshwateri* (D.E. Bustamante, B.Y. Won et T.O. Cho).

**Homotypic synonym:** *Polysiphonia freshwateri* (D.E. Bustamante, B.Y. Won et T.O. Cho).

**Type locality**: Yeonji-ri, Uljin-eup, Uljin-gun, Gyeonsangbuk-do, Korea.

**Distribution**: Korea.

**Specimens examined:** CUK10427 (Yeonji-ri, Uljin-eup, Uljin-gun, Gyeonsangbuk-do, Korea collected by T.O.C, 05 Oct. 2013).

**Vegetative morphology:** Plants are diminutive, 0.3–1.4 cm high (Fig. 92A), and purplish red in color. They form small tufts and are predominantly attached to rocks of the intertidal zone. Thalli are composed of prostrate and erect systems with flaccid texture and arranged in strictly dorsiventral disposition (Fig. 92I). The erect system is composed of interwoven indeterminate axes. Erect axes are delicate and rise endogenously from the prostrate axes mostly at intervals of 2–6 axial cells (Fig. 92B-C). They are composed of four pericentral cells, ecorticate throughout, and are densely and radially branched in an alternate to subdichotomous pattern every 3–12 axial cells (Fig. 92C-F). Young erect axes are slightly curved in the direction of the main axes and have short segments. Older segments of erect axes are 47.16 ± 10.50 μm long and 29.50 ± 4.92 μm in diameter, being 0.6 times broader than long (L:D 1.6 ± 0.3) (Fig. 92C). Apical cells are prominent, 4.75 ± 1.05 μm long and 5.95 ± 0.94 μm in diameter, and transversely divided (Fig. 92E). Adventitious branches present (Fig. 92D). Lateral branches are replacing trichoblasts (Fig. 92C-D). Trichoblasts are delicate, deciduous, numerous, 1–3 times forked, and 85.39 ± 42.43 μm long, rarely longer than 176 μm, and arise on every segment near the apical cells (Fig. 92E,F). After the trichoblasts have been shed, conspicuous scar cells appear among the four pericentral cells along the filament, reaching 9.45 ± 2.8 μm × 8.43 ± 1.08 μm in size, (Fig. 92H) in erect axes of the tetrasporophyte and gametophyte. The prostate system is reduced and entangled. Segments of the prostrate axes are 30.73 ± 6.28 μm long and 33.10 ± 4.22 μm in diameter, being as broad as they are long (L:D 0.93 ± 0.21)Rhizoids are produced from the center or proximal end of the pericentral cells. They are in open connection with the pericentral cells. Rhizoids are unicellular with multilobed terminations, and are 11.13 ± 3.78 μm in diameter and 100.07 ± 81.87 μm long (Fig. 92I-K).

**Reproductive morphology:** In female plants, erect axes are densely branched in the upper parts (Figs 93A-B). The fertile segment cuts off five pericentral cells. Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell (Fig. 93C), and with two lateral sterile cells. Cystocarps are globose when mature (Fig. 93D), 181.41 ± 23.44 μm high and 146.69 ± 23.59 μm in diameter. In tetrasporangial plants (Fig. 93E), tetrasporangia are tetrahedrically divided and 27.44 ± 5.01 μm × 28.31 ± 3.75 μm in size. Tetrasporangial branches are swollen and sinuous. The development of tetrasporangia follows a straight or a spiral arrangement (Fig. 93F-H), both arrangements in different or in the same axes (Fig. 93H). The fifth pericentral cell is developed to a stalk cell, which will develop a tetrasporangium and two cover cells (Fig. 93I). One tetrasporangium is produced from a single segment (Fig. 93I). Male gametophytes were not found.

**Habitat:** Plants grow from the intertidal zone to the subtidal area, forming tufts. They were mainly found attached to rocks in wave-exposed areas. Tufts of this species are usually small and very flaccid, and are monospecific.

**Remarks:** *Polysiphonia* sensu stricto members have been characterized by having tetrasporangia arranged in straight series; however, *D. freshwateri* comb. nov. resembles the following four members of the group in having tetrasporangia arranged in spiral series: *P. caespitosa* (Pocock) Hollenb.; *P. decussata*; *P. devoniensis*; and *P. scopulorum*. Morphologically, *D. freshwateri* comb. nov. is most closely similar to *P. decussata* from California, described by Hollenberg (1942). *Polysiphonia decussata* is distinguished from *D. freshwateri* comb. nov. by having cicatrigenous branches, and trichoblasts decussate and alternately arranged, with scar cells placed two segments apart (Hollenberg 1942). *Polysiphonia caespitosa* from the Atlantic Ocean is distinguished from *D. freshwateri* comb. nov. by having an extended prostrate system of robust texture, and an apex bearing endogenous young branches (Díaz-Tapia and Bárbara 2013). *Polysiphonia devoniensis* from the UK and *P. scopulorum* from Australia differ from *D. freshwateri* comb. nov. because tetrasporangia are arranged in spiral series (Maggs and Hommersand 1993, Womersley 2003).

***Dorsisiphonia parvula* (Suhr ex Kützing) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 94-95)**

**Basionym:** *Polysiphonia parvula* Suhr ex Kützing.

**Homotypic synonyms:** *Polysiphonia parvula* Suhr ex Kützing, *Herposiphonia parvula* (Suhr ex Kützing) De Toni.

**Type locality:** Canary Islands.

**Distribution**: Canary Islands and Indic Ocean.

**Specimens examined:** CUK13853 (Seneeyappa, Tharuga, Ramanathapuram, Tamil Nadu, India collected by T.O.C and D.E.B, 10 Feb. 2015).

**Vegetative morphology:** Plants are diminutive, flaccid, 2.5-5 mm in height (Fig. 94A), and red to purplish in color. Thalli form small tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of an erect and prostrate system arranged in a strictly dorsiventral disposition (Fig.94A-B), with the erect system composed of interwoven indeterminate axes (Fig. 94A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 2–5 axial cells. They are radially branched in an alternate to subdichotomous pattern (Fig. 94C-D). Apical cells are prominent, 7.35 ± 0.9 μm × 6.39 ± 0.9 μm in size, and transversely divided (Fig. 94D). Young erect axes are lacking of branches, slightly curved in the direction of the prostrate axes (Fig. 94J), and have short segments. Older segments of erect axes are larger, normally 46.9 ± 7.7 μm in length, and 33.9 ± 2.7 μm in diameter (L:D 1.4 ± 0.2) (Fig. 94F-H), and infrequently branched. Trichoblasts are delicate, deciduous, numerous, 1–3 times forked, and 57.7 ± 18.6 μm long, and arising on each segment near the apical cells (Fig. 94E). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, and developed in spiral to irregular series in the space between segments (Fig. 94F). Each segment is completely ecorticated along the thallus and composed of 4 pericentral cells (Fig. 94I). Adventitious branches are present (Fig. 94G). Later-

al branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 45.8 ± 5.9 μm in length, and 52.99 ± 3.1 μm in diameter (L:D 0.87 ± 0.12) (Fig. 94A-B, J). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 94K), 105.7 ± 27.5 μm in length, and 12.1 ± 0.97 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 94L).

**Reproductive morphology:** In tetrasporangial plants (Fig. 95A), tetrasporangia are tetrahedral and 25.31 ± 2.7 μm × 29.4 ± 2.1 μm in size. Tetrasporangial branches are swollen and linear (Fig. 95B). The development of tetrasporangia follows a straight arrangement (Fig. 95B). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the three cover cells (two presporangial and one postsporangial). A single tetrasporangium is produced on each fertile segment (Fig. 95C-D).

**Habitat**. Plants grow from the intertidal to subtidal zone, forming small tufts. They are found attached to rocks in sheltered to wave-exposed areas. Tufts are usually inconspicuous, flaccid, and are associated with other species such as *Centroceras.*

**Remarks:** *D. parvula* comb. nov. has been considered an illegitimate species because it is a later homonym of *P. parvula* (C. Agardh) Montagne (Silva et al. 1996). In order to distinguish them, we transfer *Polysiphonia parvula* Suhr ex Kützing to *Dorsisiphonia*. *D. parvula* comb. nov. has been reported mainly from the Canary Islands, but reports from Sri Lanka (Silva et al. 1996) expand its distribution to the Indic Ocean. Our study is describing it for the first time from the Indian coast.

***Dorsisiphonia utricularis* (Zanardini) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 96-97)**

**Basionym:** *Polysiphonia utricularis* Zanardini.

**Homotypic synonyms:** *Polysiphonia utricularis* Zanardini.

**Type locality:** Red Sea.

**Distribution:** Red sea and Indic Ocean.

**Specimens examined:** CUK8953 (Geger, Bali, Indonesia, collected by T.O.C and D.E.B, 12 Oct. 2012).

**Vegetative morphology:** Plants are diminutive, flaccid, 0.6–2.8 cm in height (Fig. 96A), and red to purplish in color. Thalli form small tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of an erect and prostrate system arranged in a strictly dorsiventral disposition (Fig.96A-C), with the erect system composed of interwoven indeterminate axes (Fig. 96B). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 2–8 axial cells. They are radially branched in an irregular pattern (Fig. 96C). Apical cells are prominent, 7.9 ± 0.5 µm × 7.3 ± 1.3 µm in size, and transversely or obliquely divided (Fig. 96D-E). Young erect axes are lacking of branches, slightly curved in the direction of the prostrate axes (Fig. 96C), and have short segments. Older segments of erect axes are larger, normally 34.5 ± 4.2 µm and 29.3 ± 4.2 µm in diameter (L:D 1.2 ± 0.3) (Fig. 96G), and infrequently branched. Trichoblasts are delicate, deciduous, short, 1–3 times forked, and 194.65 ± 99.93 µm long, and arising on each segment near the apical cells (Fig. 96F). Conspicuous scar cells appear along the filament after the trichoblasts have been shed and developed irregular series in the space between segments (Fig. 96G). Each segment is completely ecorticated along the thallus and composed of 4 pericentral cells (Fig. 96H-I). Adventitious branches are present. Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 32.92 ± 3.5 µm in length and 42.36 ± 3.03 µm in diameter (L:D 0.78 ± 0.09) (Fig. 96B-C). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 96J-L), 239.2 ± 71.6 µm in length, and 21.6 ± 6.97 µm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 96K).

**Reproductive morphology:** In female plants, erect axes are branched in the upper parts (Figs 97A-B). The fertile segment cuts off five pericentral cells. Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell (Fig. 97C), and with two lateral sterile cells. Cystocarps are globose when mature (Fig. 97D), 194.6 ± 8.27 μm high and 197.88 ± 12.70 μm in diameter. In tetrasporangial plants (Fig. 97E), tetrasporangia are tetrahedral and 22.74 ± 6.96 μm × 24.86 ± 6.35 μm in size. Tetrasporangial branches are swollen and linear (Fig. 97F-G). The development of tetrasporangia follows a straight arrangement (Fig. 97F-G). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 97H-I).

**Habitat**. Plants grow from the intertidal, forming extended tufts. They are found attached to rocks in sheltered areas. Tufts are usually conspicuous, flaccid, and are monotypic.

**Remarks:** *D. utricularis* comb. nov. has been reported from the Red sea, but reports from Sri Lanka (Silva et al. 1996) expand its distribution to the Indic Ocean. Our study is describing it for the first time from the Indonesian coast.

***Dorsisiphonia villum* (J. Agardh) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 98)**

**Basionym:** *Polysiphonia villum* J.Agardh.

**Homotypic synonyms:** *Polysiphonia villum* J. Agardh, *Lophosiphonia villum* (J. Agardh) Setchell et N.L. Gardner.

**Type locality:** Tropical coast of Mexico.

**Distribution:** Pacific, Atlantic and Indian Ocean.

**Specimens examined:** CUK6616 (Yacila, Paita, Piura, Peru, collected by T.O.C and D.E.B, 02 Sep. 2008), CUK8556, CUK8560, CUK8571 (Mariano Beach, Mar del Plata, Argentina by T.O.C and D.E.B, 09 Jul. 2012).

**Vegetative morphology:** Plants are diminutive, delicate, 0.6–1.8 cm in height (Fig. 98A), and red to purplish in color. Thalli form very small tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of an erect and prostrate system arranged in a strictly dorsiventral disposition (Fig.98A-B), with the erect system composed of individual indeterminate axes (Fig. 98B). Erect axes are ecorticate throughout and arise exogenously from the prostrate axes at intervals of 2–8 axial cells, but commonly each 4 (Fig. 98B). Apical cells are prominent, 8.33 ± 1.51 μm × 6.97 ± 0.9 μm in size, and transversely (Fig. 98C). Young erect axes are lacking of branches, slightly curved in the direction of the prostrate axes, and have short segments. Older segments of erect axes are larger, normally 54.67 ± 6.24 μm in length and 25.99 ± 2.92 μm in diameter, normally 0.7 times broader than long (L:D 2.12 ± 0.28) (Fig. 98D, G), and infrequently branched. Trichoblasts are delicate, deciduous, short, 1–3 times forked, and 142.29 ± 50.30 μm long, and arising on each segment near the apical cells (Fig. 98D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed and developed irregular series in the space between segments. Each segment is completely ecorticated along the thallus and composed of 4 pericentral cells (Fig. 98E-F). Adventitious branches are abundant (Fig. 98G-H). Lateral branches replace trichoblasts. The prostrate system is extensive and entangled, with axial segments 102.10 ± 11.75 μm in length and 99.45 ± 9.20 μm in diameter (L:D 1.04 ± 0.15) (Fig. 98B). Rhizoids are ventrally produced from the centre or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 98I), 116.27 ± 31.62 μm in length, and 49.96 ± 10.14 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 98I).

**Reproductive morphology:** In female plants, erect axes are branched in the upper parts. The fertile segment cuts off five pericentral cells. Procarps are positioned laterally and subapically on erect axes (Fig. 98J), and are composed of a supporting cell bearing a four-celled carpogonial branch, a

basal sterile cell (Fig. 98K), and with two lateral sterile cells. Cystocarps are globose when mature (Fig. 98L), 314.78 ± 19.34 μm high and 276.84 ± 16.33 μm in diameter. In tetrasporangial plants, tetrasporangia are tetrahedral and 23.99 ± 5.64 μm × 25.10 ± 5.59 μm in size. Tetrasporangial branches are swollen and linear (Fig. 98M). The development of tetrasporangia follows a straight arrangement (Fig. 98M-N). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 98O).

**Habitat**. Plants grow forming small tufts from the intertidal zone,. They are found attached to rocks in sheltered to wave-exposed areas. Tufts are usually inconspicuous, delicate, and are monotypic.

**Remarks:** *D. villum* comb. nov. has been widely reported from tropical areas. Our study is recording it for the first time from the Peruvian and Argentinean coast. *Polysiphonia scopulorum* has been widely described and four varieties were described by Hollenberg (1986a). Our molecular analyses demonstrated that one of these varieties *P. scopulorum var. villum* belongs to the new genus *Dorsisiphonia* as a different species. This species has been confirmed molecularly by Mamoozadeh and Freshwater (2011) from North Carolina. On the basis of molecular analysis of plastid-encoded *rbc*L, *D. freshwateri* is closely related to *D. villum* (4.7% of divergence). *Dorsisiphonia villum* from the western Atlantic is distinguished from *D. freshwateri* comb. nov. by having an extensive prostrate system and tetrasporangia arranged exclusively in straight series (Hollenberg 1968, Mamoozadeh and Freshwater 2011).

***Neostreblocladia* D.E. Bustamante, B.Y. Won et T.O. Cho, gen. nov.**

**Type species:** *Neostreblocladia spicata* (M.A. Howe) D.E. Bustamante, B.Y. Won et T.O. Cho.

**Diagnosis:** Plants prominent, forming large and individual tufts predominantly attached to rock surfaces. Thalli composed of a reduced and entangled prostrate system, and interwoven indeterminate erect axes. Axes composed of 7-12 pericentral cells, ecorticate throughout. Adventitious branches present. Lateral branches not in connection of trichoblasts. Trichoblasts and scar cells absent. Rhizoids open connected to the pericentral cells, with lobed and unicellular terminations. Procarps positioned axially and subapically, each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial branches replacing trichoblast and cylindrical. Tetrasporangia tetrahedral arranged in straight series. A single tetrasporangium is produced on each fertile segment.

**Etymology**: "*Neostreblocladia*" refers to a new genus segregated from *Streblocladia*.

**Key to species of *Neostreblocladia* gen. nov.**

1. Reduced prostrate system and 9-13 pericentral cells ..... ***N. spicata***
1. Extended prostrate system (stolones) and 6-9 pericentral cells ..... ***N. thwaitesii***

***Neostreblocladia spicata* (M.A. Howe) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 99-100)**

**Basionym:** *Streblocladia spicata* M.A.Howe.

**Homotypic synonyms:** *Streblocladia spicata* M.A.Howe.

**Type locality:** Peru.

**Distribution:** Peru.

**Specimens examined:** CUK6505 (Lagunillas, Pisco, Peru, collected by T.O.C and D.E.B, 27 Aug. 2008), CUK6550 (Punta Hermosa, Lima, Peru, collected by T.O.C. and D.E.B, 30 Aug. 2008), CUK8262 (Lagunillas, Pisco, Peru, collected by T.O.C and D.E.B, 05 Jul. 2012).

**Vegetative morphology:** Plants are prominent, robust, 6.5–16 cm in height (Fig. 99A), and dark red to black in color. Thalli form large and individual tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of entangled indeterminate erect axes arisen from a reduced prostrate system (Fig.99A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at irregular intervals (Fig. 99A) . They are densely and radially branched in an alternate pattern (Fig. 99B-C) forming cluster of determinate branches originated in a rami-sympodial disposition of laterals (Fig. 99D). Apical cells are prominent, sharp pointed, 8.49 ± 2.76 μm × 6.10 ± 1.12 μm in size, and transversely divided (Fig. 99E). Young erect axes are lacking of cluster of branches and have short segments (Fig. 99F). Older segments of erect axes are larger, normally 72.29 ± 8.73 μm in length and 178.95 ± 9.36 μm in diameter (L:D 0.41 ± 0.05) (Fig. 99D, G), and infrequently branched (Fig. 99G-H). Trichoblasts and scar cells are absent (Fig. 99E-F). Each segment is completely ecorticated along the thallus and composed of 7-13 pericentral cells (Fig. 99E-F). Adventitious branches are abundant as individual branches or forming clusters (Fig. 99G-H). Lateral branches not in connection with trichoblasts. The prostrate system is reduced and entangled, with axial segments 62.17 ± 9.03 μm in length and 120.51 ± 16.78 μm in diameter, (L:D 0.53 ± 0.11) (Fig. 99K). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 99L-N), 572.37 ± 178.08 μm in length, and 34.23 ± 6.16 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 99O).

**Reproductive morphology:** In female plants (Fig. 100A), erect axes are branched in the upper parts. Procarps are positioned axially and subapically on determinate branches (Fig. 100B,D), and

are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell (Fig. 100C), and with two lateral sterile cells. Cystocarps are globose when mature, 182.34 ± 21.34 μm high and 178.42 ± 25.12 μm in diameter. In male gametophytes (Fig. 100E), spermatangial branches are clustered at the apices of erect axes (Fig. 100F) in a rami-sympodial disposition, and replacing the trichoblast (Fig. 100G). Each spermatangial branch is composed of spermatangia, and sometimes terminates in a single sterile tip cell. In tetrasporangial plants (Fig. 100H), tetrasporangia are tetrahedral and 25.96± 5.11 μm × 21.74 ± 5.04 μm in size. Tetrasporangial branches are swollen and linear (Fig. 100I). The development of tetrasporangia follows a straight arrangement (Fig. 100I-J). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 100K).

**Habitat**: Plants grow forming large and individual tufts from the intertidal zone,. They are found attached to rocks in wave-exposed areas. Tufts are usually wide, long, and very robust, and are associated with other species such as *Centroceras clavulatum*, *Chondracanthus chamissoi*, and *Neosiphonia japonica.*

**Remarks:** *Neostreblocladia spicata* comb. nov. was originally described by Howe (1914) as *Streblocladia spicata* on the basis of the rami-sympodial branches. Phillips (2010) pointed out that this species and also *S. camptoclada* might not be *Streblocladia* members by lacking of cortication and by being geographically distant from the *Streblocladia* sensu stricto group (New Zealand). Our morphological and molecular analyses confirm the endemic distribution of *Neostreblocladia spicata* in the central coast of Peru and its segregation from *Streblocladia.* Although *Neostreblocladia spicata* is having determinate needle-like branches that related to *Bryocladia* (Schmitz 1987). It is distinguished from *Bryocladia* by the rami-sympodial branches.

***Neostreblocladia thwaitesii* (Harvey ex J. Agardh) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 101-102)**

**Basionym:** *Polysiphonia thwaitesii* Harvey ex J. Agardh.

**Homotypic synonyms:** *Bryocladia thwaitesii* (Harvey ex J. Agardh) De Toni, *Vertebrata thwaitesii* (Harvey ex J.Agardh) Kuntze.

**Type locality:** Sri Lanka.

**Distribution:** Sri Lanka, India.

**Specimens examined:** CUK13691 (Mahapalipuram, Chennai, Tamil Nadu, India, collected by T.O.C and D.E.B, 07 Feb. 2015), CUK13697 (Puducherry Beach, Puducherry, Chennai, Tamil Nadu, collected by T.O.C. and D.E.B, 08 Feb. 2015).

**Vegetative morphology:** Plants are prominent, robust, 7.5–14.2 cm in height (Fig. 101A), and dark red to black in color. Thalli form small and individual turfs predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of entangled indeterminate erect axes arisen from an extended prostrate system (Fig.101B). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at irregular intervals (Fig. 101B) . They are densely and radially branched in a continuous and irregular pattern (Fig. 101B-C) forming cluster of determinate branches originated in a rami-sympodial disposition of laterals (Fig. 101D-E). Apical cells are prominent, sharp pointed, 8.13 ± 2.09 μm × 13.34 ± 4.25 μm in size, and transversely divided (Fig. 101E). Young erect axes are lacking of cluster of branches and have short segments. Older segments of erect axes are larger, normally 48.8 ± 15.5 μm in length and 161.24 ± 48.80 μm in diameter (L:D 0.30 ± 0.09) (Fig. 101G), and infrequently branched. Trichoblasts and scar cells are absent (Fig. 101F). Each segment is completely ecorticated along the thallus and composed of 5-9 pericentral cells (Fig. 101H-I). Adventitious branches are abundant as individual branches or forming clusters (Fig. 101J-K). Lateral branches not in connection with trichoblasts. The prostrate system is

extended growing as stolones (Fig. 101B, L), with axial segments 60.48 ± 11.08 μm in length and 87.52 ± 11.83 μm in diameter (L:D 0.70 ± 0.14) (Fig. 101K). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 101L-N), 546.45 ± 244.34 μm in length, and 21.27 ± 3.91 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 101O).

**Reproductive morphology:** In tetrasporangial plants (Fig. 102A-B), tetrasporangia are tetrahedral and 16.20 ± 5.63 μm × 22.66 ± 2.60 μm in size. Tetrasporangial branches are swollen and linear (Fig. 102C-D). The development of tetrasporangia follows a straight arrangement (Fig. 102I-J). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 102E-F).

**Habitat**. Plants grow forming large turfs from the intertidal zone. They are found attached to rocks and invertebrates in wave-exposed areas. Turfs are usually wide, small, and very robust, and are associated with other species of *Centroceras.*

**Remarks:** *Neostreblocladia thwaitesii* comb. nov. was originally described by Harvey in J. Agardh (1863) as *Polysiphonia thwaitesii* and then transferred to *Bryocladia* by De Toni (1903) on the basis of determinate branches. This species has been widely reported from the Indic Ocean (Coppejans et al. 2009). Although *Neostreblocladia thwaitesii* is showing determinate branches, our morphological analyses found also the rami-sympodial branches and close connection of rhizoids. Thereby, we proposed the transfer of *Neostreblocladia thwaitesii* from *Bryocladia* on the basis of morphological and molecular analyses.

***Phillipsiphonia*** **D.E. Bustamante, B.Y. Won et T.O. Cho gen. nov.**

**Type species:** *Phillipsiphonia camptoclada* (Montagne) D.E. Bustamante, B.Y. Won et T.O. Cho.

**Diagnosis:** Plants prominent, forming extended turfs predominantly attached to rock surfaces. Thalli composed of an extended and entangled prostrate system, and interwoven indeterminate erect axes. Axes composed of 4 pericentral cells, ecorticate throughout. Adventitious branches present. Lateral branches not in connection of trichoblasts. Trichoblasts absent. Rhizoids open connected to the pericentral cells, with lobed and unicellular terminations. Procarps positioned axially and subapically, each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial branches replacing trichoblast and cylindrical. Tetrasporangia tetrahedral arranged in straight series. A single tetrasporangium is produced on each fertile segment.

**Etymology**: The name "*Phillipsiphonia*" is in honor of Louise Phillips, for her valuable contributions to the understanding of the systematics of Rhodomelaceae, specially the genus *Strebloclad*ia.

***Phillipsiphonia camptoclada* (Montagne) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 103-104)**

**Basionym:** *Polysiphonia camptoclada* Montagne.

**Homotypic synonyms:** *Polysiphonia camptoclada* Montagne, *Strebloclad*ia *camptoclada* (Montagne) Falkenberg.

**Heterotypic Synonym:** *Orcasia pulla* R.H. Simons.

**Type locality:** Peru.

**Distribution:** Peru, Chile, Argentina, Namibia, South Africa.

**Specimens examined:** CUK6424, CUK6428 (Antofagasta, Chile, collected by T.O.C and D.E.B, 17 Aug. 2008), CUK6467 (Tocopilla, Antofagasta, Chile, collected by T.O.C and D.E.B, 20 Aug. 2008), CUK6486 (Playa Chica, Quintay, Valpariso, Chile, collected by T.O.C and D.E.B, 22 Aug.

2008), CUK6556 (Punta Hermosa, Lima, Peru, collected by T.O.C. and D.E.B, 30 Aug. 2008), CUK6599 (Mancora, Piura, Peru, collected by T.O.C. and D.E.B, 01 Sep. 2008), CUK8347 (Lagunillas, Pisco, Peru, collected by T.O.C and D.E.B, 05 Jul. 2012), CUK8407 (Barranco, Lima, Peru, collected by T.O.C and D.E.B, 06 Jul. 2012), CUK8408, CUK8409 (La Punta, Callao, Peru, collected by T.O.C and D.E.B, 06 Jul. 2012), CUK8571 (Mariano Beach, Mar del Plata, Argentina by T.O.C and D.E.B, 09 Jul. 2012), CUK14350 (Columbine Nature Reserve, West Coast Peninsula, Western Cape, South Africa, collected by T.O.C, 07 Apr. 2015), CUK14396, CUK14404 (Hout Bay Harbour, Cape Town, Western Cape, South Africa, collected by T.O.C., 08 Apr. 2015).

**Vegetative morphology:** Plants are prominent, robust, 5.8–14.5 cm in height (Fig. 103A), and dark red to purplish in color. Thalli form large turfs predominantly attached to rock surfaces and shells in the intertidal zone. Thalli are composed of entangled indeterminate erect axes arisen from an extended prostrate system (Fig.103A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 3–6 axial cells (Fig. 103A) . They are densely and radially branched in an alternate pattern (Fig. 103B-C) forming cluster of determinate branches originated in a rami-sympodial disposition of laterals (Fig. 103D). Apical cells are prominent, dome shaped, 7.71 ± 0.89 μm × 5.89 ± 0.29 μm in size, and transversely divided (Fig. 103D). Young erect axes are lacking of cluster of branches and have short segments (Fig. 103D). Older segments of erect axes are larger, normally 138.35 ± 32.88 μm in length and 180.78 ± 28.67 μm in diameter(L:D 0.77 ± 0.13) (Fig. 103E-F), and infrequently branched (Fig. 103A). Trichoblasts and scar cells are very scarce (Fig. 103F). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 103G-H). Adventitious branches are scarce. Lateral branches not in connection with trichoblasts. The prostrate system is reduced and entangled, with axial segments 100.33 ± 18.84 μm in length and 151.73 ± 45.32 μm in diameter, (L:D 0.69 ± 0.12) (Fig. 103I). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 103I-J), 173.44 ± 26.60 μm in length,

and 87.60 ± 16.04 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 103K).

**Reproductive morphology:** In female plants (Fig. 104A), erect axes are densely branched in the upper parts. Procarps are positioned axially and subapically on determinate branches (Fig. 104B-C), and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell, and with two lateral sterile cells. Cystocarps are globose when mature, 290.63 ± 54.61 μm high and 226.39 ± 59.17 μm in diameter (Fig. 104C). In male gametophytes (Fig. 104D), spermatangial branches are clustered at the apices of erect (Fig. 104E), and replacing the trichoblast (Fig. 104F). Each spermatangial branch is composed of spermatangia, and lacking of sterile tip cell. In tetrasporangial plants (Fig. 104G), tetrasporangia are tetrahedral and 46.02 ± 9.44 μm × 28.87 ± 4.85 μm in size. Tetrasporangial branches are swollen and linear (Fig. 104H). The development of tetrasporangia follows a straight arrangement (Fig. 104H). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 104I-J).

**Habitat**. Plants grow forming large turfs from the intertidal zone. They are found attached to rocks in and shells in sheltered to wave-exposed areas. Turfs are usually wide and robust, and are associated with other species such as *Centroceras clavulatum*, *Chondracanthus chamissoi*, and *Neosiphonia japonica.*

**Remarks:** *Phillipsiphonia camptoclada* comb. nov. was originally described by Montagne (1837) as *Polysiphonia camptoclada* from the Peruvian coast and then transferred to *Streblocladia* by Falkenberg (1901) on the basis of the rami-sympodial branches. Phillips (2010) noticed that this species might be different from *Streblocladia* because of the absence of cortication. Our morphological and molecular analyses confirm the segregation of this new genus from *Streblocladia* and its distribution is restricted to the southern hemisphere (Peru, Chile, Argentina, South Africa). Phil-

lips (2010) also noticed that *Phillipsiphonia camptoclada* comb. nov. might originated in the Pacific coast of South America and then distributed to South Africa.

***Polysiphonia* Greville**

**Holotype species:** *Polysiphonia urceolata* (Lightfoot ex Dillwyn) Greville

**Key to species of *Polysiphonia* sensu stricto**

1. Diminutive plants lower than 2 cm ..... 2
1. Larger plants ..... 4
    2. Lacking of trichoblasts and scar cells ..... ***P. ulluengensis***
    2. Scar cells produced in the distal position of pericentral cells ..... 3
3. Scar cells producing cicatrigenous branches ..... ***P. koreana***
3. Scar cells not producing cicatrigenous branches ..... ***P. dokdoensis***
    4. Sharply pointed apex..... ***P. morrowii***
    4. Dome pointed apex ..... 5
4. Pseudodichotomous-alternate branches, spermatangia with 2-5 sterile cells ..... ***P. stricta***
5. Alternate branches, spermatangia with 1-3 sterile cells ..... ***P. pacifica***

***Polysiphonia dokdoensis* D.E. Bustamante, B.Y. Won et T.O. Cho (Fig. 105-106).**

**Type locality:** Sado, Dokdori, Korea.

**Distribution**: Endemic to the Korean coast.

**Specimens examined:** CUK9492 (Sado, Dokdo-ri, Ulleung-eup, Ulleung-gun, Gyeonsangbuk-do, Korea, collected by T.O.C, Apr. 22 2013).

**Vegetative morphology:** Plants are diminutive, 1.4 – 2.7 cm high (Fig. 105A), red brownish in color, epiphytic or in association with other filamentous species. Thalli form small tufts predominantly attached to and entangled with calcareous organisms like *Corallina*. Thalli are composed of prostrate and erect systems with flaccid texture (Fig. 105B). The prostrate system is reduced and attached by ventral rhizoids (Fig. 105C). Segments of prostrate axes are 34.52 ± 7.66 μm in length and 29.21 ± 3.01 μm in diameter, 0.8 times broader than long (L:D 1.21 ± 0.4). The erect system has radial indeterminate and decumbent axes (Fig. 105B-C). Erect axes arise endogenously from prostrate axes at intervals of (2-) 3-4 axial cells (Fig. 105B) frequently becoming decumbent and producing secondary rhizoids (Fig. 105C). Erect axes are densely branched with a pair of unilateral branches borne on one side of the axis alternating with a pair on the other side (Fig. 105D-E). Young erect axes have short segments (Fig. 105E) and are slightly curved in the direction of the apex of prostrate axes while older axes have larger and often twisted segments (Fig. 105F). Older segments of erect axes are 100.07 ± 92.84 μm in length and 27.10 ± 13.70 μm in diameter at the third branching point from basal part, being 0.27 times broader than long (L:D 3.69 ± 2.88), and are composed of a slender axial cell with 4 pericentral cells ecorticated throughout (Fig. 105G-H). Apices have prominent, transversely divided apical cells of 6.86 ± 1.30 μm × 9.20 ± 1.50 μm in size. Branching occurs at intervals of 4-6 axial cells. Adventitious branches are present in the prostrate system. Trichoblasts and scar cells are absent on sterile erect axes, and developed between the distal ends of pericentral cells, but not in the space between segments, of tetrasporophyte erect axes (Fig. 105I). Rhizoids produced one per segment from the centre or proximal ends of ventral pericentral cells of prostrate axes. They are in open connection with pericentral cells (Fig. 105J-K). Rhizoids are unicellular with multilobed terminations (Fig. 105J), and 24.75 ± 11.23 μm in diameter and 300.50 ± 191.57 μm long.

**Reproductive morphology:** In tetrasporangial plants (Fig. 106A), the development of tetrasporangia is in a straight series (Fig. 106A-D). Tetrasporangia are tetrahedral and 40.99 ± 3.52 μm × 39.40 ± 2.93 μm in size. Tetrasporangial branches are sinuous and interrupted (Fig. 106D). The

fertile segments have the 4 pericentral cells and a 5th cell as a stalk, which develops into the tetrasporangia and the two cover cells (Fig. 106E). One tetrasporangium is produced from a single segment (Fig. 106E). Female and male gametophytes were not found.

**Habitat:** *Polysiphonia dokdoensis* grows as tufts in tide pools and from the intertidal zone to subtidal area. They are found in sheltered to wave-exposed areas, attached on rocks inside tide pools. Tufts of this species are usually small, flaccid, delicate, and epiphytic on *Corallina* or in association with other filamentous species.

**Remarks:** Kim and Lee (1996) reported specimens collected from Daesambudo as "*P. atlantica*" based on the examination of field and laboratory cultured materials. They identified "*P. atlantica"* from Korea based on the flaccid texture, well developed prostrate system, small height, urceolate cystocarps, and 1-2 sterile cells on spermatangial branches. However, this plant is distinguished from the authentic *P. atlantica* described from Europe by the pairs of unilateral branches borne on one side of the axis alternating with pairs on the other side (Bustamante et al. 2014a). Although branching patterns range from alternate to subdichotomous to irregular for some *Polysiphonia* sensu stricto species, this feature has not been considered to be consistent and useful for identifying species (Stuercke and Freshwater 2008) because of its intraspecific variation. However, the alternating arrangement of unilateral branch pairs in "*P. atlantica"* sensu Kim and Lee from Korea is very distinctive from branching patterns in other *Polysiphonia* species and it is a constant character state in all plants examined. *"Polysiphonia atlantica"* from Korea reported by Kim and Lee (1996) may be recognized as *Polysiphonia dokdoensis* and this distinctive branching pattern is one of the useful characters for recognizing our new species (Bustamante et al. 2014a).

*Polysiphonia dokdoensis* is morphologically identical to "*P. atlantica"* sensu Kim and Lee, even though the scar cell between two pericentral cells was not recognized by Kim and Lee (1996). Although the existence of scar cells has been reported by most previous taxonomic studies of *Polysiphonia* (Hollenberg 1942, Maggs and Hommersand 1993, Womersley 2003), their position has not

been described in detail. Most of the previous studies showed that, within *Polysiphonia* sensu stricto, scar cells in *P. scopulorum*, *P. subtilissima*, and *P. rudis* were placed in the space between segments surrounded by four pericentral cells (Hollenberg 1968, Adams 1991). The placement of scar cells among four pericentral cells is a common character state among species of *Polysiphonia*, and the unusual placement of scar cells between two pericentral cells within a segment in *P. dok-doensis* distinguishes it well from other *Polysiphonia* species and is another useful character for recognizing our new species (Bustamante et al. 2014a).

***Polysiphonia koreana* D.E. Bustamante, B.Y. Won et T.O. Cho. (Fig. 107-108).**

**Type locality:** Jukdo, Ulleung-gun, Korea.

**Distribution**: Endemic to the Korean coast.

**Specimens examined:** CUK9556 (Jukdo, Ulleung-eup, Ulleung-gun, Gyeonsangbuk-do, Korea, collected by T.O.C, Apr. 23 2013).

**Vegetative morphology:** Plants are diminutive, 0.8–1.8 cm high (Fig. 107A), purplish red in color, and associated with other filamentous species. Thalli form small tufts and are predominantly attached to rock surfaces of tidal pools. Thalli are composed of prostrate and erect systems with flaccid texture (Fig. 107B). The prostate system is reduced and entangled. Segments of the prostrate axes are 26.59 ± 4.89 μm long and 33.65 ± 3.32 μm in diameter, being 1.3 times broader than long (L:D 0.81 ± 0.21). The erect system is composed of interwoven indeterminate axes. Erect axes (Fig. 107B) are delicate and rise endogenously from the prostrate axes at regular intervals of 3–8 axial cells. They are composed of four pericentral cells, ecorticate throughout, and are densely and radially branched in an alternate to subdichotomous pattern every 3–8 axial cells (Fig. 107B-E). Young erect axes are slightly curved in the direction of the main axes and are formed by short segments. Older segments of erect axes are 46.55 ± 23.44 μm long and 42.58 ± 13.52 μm in diameter,

being 0.9 times broader than long (L:D 1.12 ± 0.48). Apical cells are prominent, 5.12 ± 0.92 μm × 4.52 ± 0.82 μm in size, and transversely divided (Fig. 107D). Trichoblasts and scar cells are absent in sterile erect axes, and are developed between the distal ends of the pericentral cells, but not in the space between the segments in the erect axes of the tetrasporophytes (Fig. 107F). Cicatrigenous branches are developed from scar cells and are scarce (Fig. 107G). Rhizoids are produced from the center or proximal end of the pericentral cells. They are in open connection with the pericentral cells. Rhizoids are unicellular with multilobed terminations, and are 16.20 ± 5.39 μm in diameter and 88.08 ± 39.30 μm long (Fig. 107H-I).

**Reproductive morphology:** In female plants, erect axes are densely branched in the upper parts (Fig. 108A). The fertile segment cuts off five pericentral cells. Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell, and two lateral sterile cells. Cystocarps are ovoid to urceolate when mature (Fig. 108B-C), 198.81 ± 23.69 μm high and 154.25 ± 29.16 μm in diameter. In tetrasporangial plants (Fig. 108D), tetrasporangia are tetrahedral and 30.37 ± 5.12 μm × 30.58 ± 6.70 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 108E).The development of the tetrasporangia follows a straight arrangement (Fig. 108F). The fifth pericentral cell is developed to a stalk cell, which will develop a tetrasporangium and two cover cells (Fig. 108G). One tetrasporangium is produced from a single segment (Fig. 108G). Male gametophytes were not found.

**Habitat:** Plants grow from the intertidal zone to the subtidal area, forming tufts. They were found attached to rocks inside tidal pools in sheltered to wave-exposed areas. Tufts of this species are usually small and very flaccid, and were found associated with other filamentous species.

**Remarks:** *Polysiphonia koreana* resembles seven species belonging to *Polysiphonia* sensu stricto in having a diminutive habit. Among these seven species, *P. atlantica*, *P. decussata* Hollenb., *P. devoniensis* Maggs et Hommers., and *P. macrocarpa* (C. Agardh) A.Spreng. differ from *P. koreana* in having abundant trichoblasts and scar cells (Hollenberg 1942, Maggs and Hommersand 1993,

Mamoozadeh and Freshwater 2012, Díaz-Tapia and Bárbara 2013); *P. scopulorum* differs from *P. koreana* in having a rigid texture (Womersley 2003); and *P. ulluengensis* differs from *P. koreana* in having an extensive prostrate system and oblique apical cell division (Bustamante et al. 2014b). Morphologically, *P. koreana* is most closely similar to the recently described *P. dokdoensis* from Dokdo, Korea by Bustamante et al. (2014a), which was previously identified as *P. atlantica* by Kim and Lee (1996). The two species are similar in having a diminutive habit, which is common in several *Polysiphonia* sensu stricto members, and also in the unusual placement of scar cells between two pericentral cells. The latter morphological character was considered useful for distinguishing *P. dokdoensis* from other *Polysiphonia* species; however, *P. koreana* sp. nov. also shows this feature. *Polysiphonia koreana* sp. nov. is distinguished from *P. dokdoensis* by the development of cicatrigenous branches from the scar cells, and by the alternate branching pattern (Bustamante et al. 2014a). On the basis of molecular analysis of the plastid-encoded *rbc*L, *P. koreana* sp. nov. is also most closely related to *P. dokdoensis*. This phylogenetic relationship might be related to have the placement of scar cells not in the distal ends of the pericentral cells but in the space between segments. However, father sampling of different populations and species might confirm its phylogenetic significance.

***Polysiphonia morrowii* Harvey (Fig. 109-110).**

**Type locality:** Hakodate, Japan.

**Distribution**: Worldwide distributed to temperate oceans.

**Specimens examined:** CUK5035 (Songjeong Beach, Haeundae-gu, Busan, Korea, collected by T.O.C., Feb. 23 2008), CUK8559, CUK8575 (Mariano Beach, Mar del Plata, Argentina by T.O.C and D.E.B, 09 Jul. 2012), CUK12461, CUK12464 (Chuja Island, Jikgudo, Chujamyeon, Jeju, Korea, collected by T.O.C., Jun. 28 2014).

**Vegetative morphology:** Plants are large 5-19 cm high (Fig. 109A), blackish red to purplish in color, and associated with other filamentous species. They form long and entangled tufts and are predominantly epiphyte or attached to rock surfaces of tide pools. Thalli are composed of entangled indeterminate erect axes arisen from an extended prostrate system (Fig. 109A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at irregular intervals. They are densely and radially branched in an alternate pattern each 1-4 axial cells, but normally 3 (Fig. 109B-C). Apical cells are prominent, sharply pointed, 9.09 ± 3.15 μm × 5.09 ± 1.95 μm in size, and transversely divided (Fig. 109D). Young erect axes have short segments (Fig. 109C-D). Older segments of erect axes are larger, normally 70.75 ± 8.56 μm in length and 70.46 ± 5.08 μm in diameter in the middle of the axes [1.01 ± 0.15 (L/D)] and 823.25 ± 33.54 μm in length and 103.23 ± 23.12 at base [7.98 ± 0.32\(L/D)] (Fig. 109E), and infrequently branched (Fig. 109B). Trichoblasts and scar cells are very scarce. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 109F-G). Adventitious branches are recurved. Lateral branches not in connection with trichoblasts. The prostrate system is extended and entangled, with axial segments 61.30 ± 25.52 μm in length and 80.22 ± 39.61 μm in diameter, broader than long [1.00 ± 0.60 (L/D)]. Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 109I-J), 224.26 ± 86.77 μm in length, and 26.07 ± 4.77 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In male gametophytes (Fig. 110A), spermatangial branches are clustered at the apices of erect axes (Fig. 110B), and replacing the trichoblast (Fig. 110C). Each spermatangial branch is composed of spermatangia and 5-8 sterile cells. In tetrasporangial plants (Fig. 110D-E), tetrasporangia are tetrahedral and 44.54 ± 10.10 μm × 33.86 ± 7.86 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 110F). The development of tetrasporangia follows a straight arrangement (Fig. 110G). The fertile segment is composed of five or six pericentral cells (Fig. 110H-I). The fertile pericentral cell develops as a stalk cell, which will cut off the tetraspo-

rangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 110I-J).

**Habitat:** Plants grow forming large tufts from the intertidal zone. They are found attached to rocks in sheltered to wave-exposed areas or epiphyte on other filamentous brown algae. Tufts are usually long and robust, and are associated with other species such as *Neosiphonia japonica*, *Sargassum muticum*, and *Symphyocladia* sp.

**Remarks:** *Polysiphonia morrowii* is considered an invasive species. Recently, new studies have reported this species from Argentina (Croce and Parodi 2014, Raffo et al. 2014), New Zealand (D'Archino et al 2013), Mediterranean (Geoffroy et al. 2012), and Chile (Kim et al. 2004) on the basis of molecular analyses.

***Polysiphonia pacifica* Hollenberg (Fig. 111).**

**Type locality:** Santa Cruz, California.

**Distribution**: Eastern coast of the Pacific ocean.

**Specimens examined:** CUK693 (Seal Rock, Oregon, USA, collected by T.O.C., Jun. 20 2003).

**Vegetative morphology:** Plants are large 3-8 cm high (Fig. 111A), blackish red to purplish in color, and associated with other filamentous species. They form long and entangled tufts and are predominantly attached to rock surfaces of tide pools. Thalli are composed of entangled indeterminate erect axes arisen from a reduced prostrate system (Fig.111A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at irregular intervals. They are densely and radially branched in an alternate pattern with corymbose tips at intervals of 1-4 axial cells (Fig. 111B-C). Apical cells are prominent, dome shaped, 4.88 ± 0.55 μm × 4.90 ± 0.88 μm in size, and transversely divided (Fig. 111D). Young erect axes have short segments (Fig. 111C-D). Older segments of

erect axes are larger (Fig. 111E), normally 177.03 ± 51.38 μm in length and 97.80 ± 12.24 μm in diameter [1.85 ± 0.42 (L/D)] (Fig. 111D, G), and infrequently branched. Trichoblasts and scar cells are absent. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 111G-H). Adventitious branches are recurved (Fig. 111F). Lateral branches not in connection with trichoblasts. The prostrate system is reduced and entangled, with axial segments 65.76 ± 14.93 μm in length and 91.37 ± 7.55 μm in diameter, broader than long [0.68 ± 0.16 (L/D)]. Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells, 84.27 ± 17.56 μm in length, and 31.79 ± 3.79 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 111J), tetrasporangia are tetrahedral and 35.41 ± 5.96 μm × 32.13 ± 6.73 μm in size. Tetrasporangial branches are swollen and linear (Fig. 111H). The development of tetrasporangia follows a straight arrangement (Fig. 111K). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 111L-M).

**Habitat:** Plants grow forming large tufts from the intertidal zone. They are found attached to rocks in wave-exposed areas. Tufts are usually long and robust, and are associated with other species such as *Hapterosiphonia confusa*.

**Remarks:** Kim and Yang (2005) characterized this species by observing the type material from Smithsonian Institution, National Museum of Natural History, Washington DC (US) herbarium and concluded that some of the varieties of *P. pacifica* described by Hollenberg (1942) represent the same species. Although *Polysiphonia pacifica* has been reported in the northeastern and southeastern Pacific coast, our study only reported this species from the northeastern Pacific ocean.

***Polysiphonia stricta* Hollenberg (Fig. 112).**

**Homotypic Synonyms:** *Conferva stricta* Mertens ex Dillwyn, *Ceramium strictum* (Mertens ex Dillwyn) Poiret, *Hutchinsia stricta* (Dillwyn) C. Agardh.

**Heterotypic synonyms:** *Conferva urceolata* Lightfoot ex Dillwyn, *Conferva patens* Dillwyn, *Hutchinsia urceolata* (Lightfoot ex Dillwyn) Lyngbye, *Polysiphonia urceolata* (Lightfoot ex Dillwyn) Greville, *Hutchinsia comosa* C. Agardh, *Hutchinsia roseola* C. Agardh, *Polysiphonia formosa* Suhr, *Polysiphonia patens* (Dillwyn) Harvey, *Polysiphonia roseola* (C. Agardh) Fries, *Polysiphonia pulvinata* Liebmann, *Polysiphonia urceolata* f. *roseola* (C. Agardh) J. Agardh, *Polysiphonia urceolata* f. *comosa* (C. Agardh) J. Agardh, *Polysiphonia urceolata* f. *formosa* (Suhr) J. Agardh, *Hutchinsia abyssina* Lyngbye, *Polysiphonia urceolata* f. *typica* Kjellman, *Polysiphonia urceolata* f. *pulvinata* Kylin, *Polysiphonia spiralis* L.Batten.

**Type locality:** Glamorgan (Swansea), UK.

**Distribution**: North Atlantic coast.

**Specimens examined:** CUK5134 (Seal Rock, Oregon, USA, collected by T.O.C., Aug. 12 2007), CUK11632 (Berwick-upon-tweed, Magadalene Fields Golf Club, Rocky shore, England, collected by T.O.C., May 06 2014), CUK11868 (Donaghadee, Rocky shore, Northern Ireland, collected by T.O.C., May 08 2014).

**Vegetative morphology:** Plants are large 4-10 cm high (Fig. 112A), blackish red to purplish in color, and associated with other filamentous species. They form long and entangled tufts and are predominantly epiphyte or attached to rock surfaces of tide pools. Thalli are composed of entangled indeterminate erect axes arisen from prostrate system (Fig. 112A). Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at irregular intervals (Fig. 112A) . They are densely and radially branched in an alternate to pseudodichotomous pattern with corymbose tips (Fig. 112B-E). Apical cells are prominent, dome shaped, 6.86 ± 0.36 μm × 10.24 ± 0.85 μm in

size, and transversely divided (Fig. 112D). Young erect axes have short segments (Fig. 112C-D). Older segments of erect axes are larger, normally 127.16 ± 35.47 μm in length and 65.60 ± 15.23 μm in diameter [2.03 ± 0.66 (L/D)] (Fig. 112F-G), and infrequently branched (Fig. 112F). Trichoblasts and scar cells are very scarce. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 112H-I). Adventitious branches are abundant and recurved (Fig. 112F,J). Lateral branches not in connection with trichoblasts. The prostrate system is extended and entangled, with axial segments 75.23 ± 14.54 μm in length and 82.12 ± 6.41 μm in diameter, broader than long [1.01 ± 0.21 (L/D)]. Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells, 123.21 ± 21.12 μm in length, and 18.21 ± 6.34 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 112K), tetrasporangia are tetrahedral and 44.54 ± 10.10 μm × 33.86 ± 7.86 μm in size. Tetrasporangial branches are swollen and linear (Fig. 112M-N). The development of tetrasporangia follows a straight arrangement (Fig. 112M-N). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 112O-P).

**Habitat:** Plants grow forming large tufts from the intertidal zone. They are found attached to rocks in sheltered to wave-exposed areas. Tufts are usually long and delicate, and are associated with other species such as *Polysiphonia fucoides*.

**Remarks:** The detail morphological characterization by Kim et al. (2000) of generitype *Polysiphonia stricta* clarified the taxonomy of species referred to *Polysiphonia* sensu stricto. This group consist of species having prostrate and erect ecorticate axes spirally disposed, four pericentral cells, rhizoids in open connection with the pericentral cells, a four-celled carpogonial branch, spermatangial branches replacing the whole trichoblast, and tetrasporangia arranged in straight series (Bustamante et al. 2015a).

***Polysiphonia ulleungensis* D. E. Bustamante, B. Y. Won et T. O. Cho (Fig. 113-114)**

**Type locality:** Sadong-ri, Ulleung-gun, Korea.

**Distribution**: Endemic to the Korean coast.

**Specimens examined:** CUK9483 (Sadong-ri, Ulleung-eup, Ulleung-gun (Ulleung Island), Gyeonsangbuk-do, Korea, collected by T.O.C, Apr. 21 2013).

**Vegetative morphology:** Plants are diminutive, 0.9–1.8 cm high (Fig. 113A), blackish red in color, and associated with other filamentous species. They form small tufts and are predominantly attached to rock surfaces of tide pools. Thalli are composed of prostrate and erect system (Fig. 113B). Prostate system is extensive, entangled, and decumbent with rigid texture. Segments of prostrate axes are 85.45 ± 7.56 µm long and 61.36 ± 5.45 µm in diameter, being 0.7 times broader than long (L:D 1.4 ± 0.18). The erect system is composed of interwoven indeterminate axes. Erect axes are slender (Fig. 113E), delicate, and arise endogenously from prostrate axes at intervals of 1–4 (3.24 ± 1.39) axial cells (Fig. 113B). They are composed of 4 pericentral cells (Fig. 113G, H), ecorticate throughout and radially and regularly branched 1-3 orders in an alternate pattern every 2–3 axial cells (Fig. 113C). Adventitious branches are absent. Young erect axes are slightly curved in the direction of the apices of prostrate axes (Fig. 113B) and have short segments (Fig. 113D). Older segments of erect axes are 70.15 ± 43.67 µm long and 35.76 ± 10.71 µm in diameter, being 0.5 times broader than long (L:D 1.98 ± 1.02). Apical cells are prominent, 6.78 ± 1.85 µm long and 6.32 ± 1.04 µm wide, and obliquely or transversely divided (Fig. 113F). Trichoblasts are absent in vegetative thalli. Rhizoids are ventrally produced from the center or proximal end of pericentral cells of prostrate axes. They are in open connection with pericentral cells, unicellular with multilobed terminations, and 33.33 ± 12.8 µm in diameter and 180.68 ± 87.50 µm long (Fig. 113I, J).

**Reproductive morphology:** Erect axes of female gametophytes are densely branched distally and bear small, scarce trichoblasts (Fig. 114A, B). Procarps are positioned laterally and subapically on

erect axes and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 114C, D). Cystocarps are elongate, slightly urceolate (Fig. 114E), 270.01 ± 72.73 μm high, and 200.79 ± 42.43 μm in diameter. Spermatangial branches of male gametophytes are clustered at the apices of erect axes, replace the whole trichoblast, develop one to several segments apart (Fig. 114F) and they have 2–4 sterile tip cells when mature (Fig. 114G). Tetrasporangial plants were not found.

**Habitat:** Plants grow from the intertidal to subtidal zones. They were found in sheltered to wave-exposed areas, and attached to rocks inside tide pools. Tufts of *P. ulleungensis* were entangled with filamentous algae like *Antithamnion*, *Ceramium*, and other *Polysiphonia* species.

**Remarks:** *Polysiphonia ulleungensis* is morphologically most similar to *P. atlantica. Polysiphonia atlantica* was originally described from Ireland as *P. macrocarpa* Harvey nom. illeg. by Harvey (1836) and later renamed as *P. atlantica* by Kapraun and Norris (1982). Maggs and Hommersand (1993) described in detail the morphology of *P. atlantica* from the British Isles, and European specimens were sequenced by Bárbara et al. (2013) and Díaz-Tapia (2013). *Polysiphonia atlantica* has been reported from the northeastern Atlantic, western Atlantic, and Korea (Kapraun 1977, Yoon 1986, Maggs and Hommersand 1993, Kim and Lee 1996, Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011, Nam and Kang 2012, Bárbara et al. 2013, Díaz-Tapia 2013). However, these records apparently involve four species, as it is explained below. Although our *P. ulleungensis* is similar to the European *P. atlantica* in having a diminutive habit and the typical features for the *Polysiphonia* sensu stricto members, the former is morphologically distinguished from the latter by the scarce trichoblasts and 2–4 sterile tip cells in the spermatangia.

*Polysiphonia atlantica* was initially reported and described from Korea by Yoon (1986), and additional detailed morphological descriptions were provided by Kim and Lee (1996) and Nam and Kang (2012). Kim and Lee (1996) reported the plants collected from Daesambudo as "*P. atlantica*" on the basis of field and laboratory cultured materials. However, these plants are distinguished

from *P. atlantica* described from Europe by paired branches being borne on one side of the axis and then a pair on the other side. *Polysiphonia atlantica* sensu Kim and Lee (1996) has not been included in molecular analyses, and it may represent a different species of *Polysiphonia* sensu stricto. *Polysiphonia atlantica* sensu Nam and Kang (2012) was collected from the eastern and southern coasts of Korea. It is distinguished from European *P. atlantica* by the scarce trichoblasts and 2–4 sterile tip cells in the spermatangia. *Polysiphonia atlantica* sensu Nam and Kang differs from *P. atlantica* sensu Kim and Lee (1996) in having an alternate branching pattern. *Polysiphonia atlantica* sensu Nam and Kang resembles *P. ulleungensis* based on the morphology of vegetative, male, and female thalli, although it has been reported with a dichotomous branching pattern that seems to be alternate in the tetrasporophyte (see Fig. 18H in Nam and Kang 2012). We recognize the *Polysiphonia atlantica* from Korea reported by Nam and Kang (2012) as *P. ulleungensis*.

**Species of uncertain status belonging to *Polysiphonia***

***Polysiphonia scopulorum* Harvey (Fig. 115-116)**

**Homotypic synonyms:** *Vertebrata scopulorum* (Harvey) Kuntze, *Lophosiphonia scopulorum* (Harvey) Womersley.

**Type locality:** Rottnest Island, Western Australia.

**Distribution:** Worldwide distributed.

**Specimens examined:** CUK11071 (Bales beach, Kangaroo Island, Australia, collected by T.O.C and D.E.B, 26 Mar. 2014).

**Vegetative morphology:** Plants are diminutive, delicate, 0.6–2.4 cm in height (Fig. 115A-B), and red to purplish in color. Thalli form small tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of entangled indeterminate erect axes arisen from an extended pro-

strate system (Fig. 115B) spirally disposed. Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 1–6 axial cells, usually 4 (Fig. 115C) . They are densely and radially branched in irregular pattern (Fig. 115B). Apical cells are inconspicuous, dome shaped, 6.71 ± 0.77 μm × 5.52 ± 1.35 μm in size, and transversely divided (Fig. 115D). Young erect axes have short segments (Fig. 115D). Older segments of erect axes are larger, normally 62.79 ± 14.45 μm in length and 42.75 ± 4.54 μm in diameter, (L:D 1.50 ± 0.43) (Fig. 115E), and infrequently branched. Trichoblasts are delicate, deciduous, short, 1–3 times forked, and 104.65 ± 56.53 μm long, and arising on each segment near the apical cells (Fig. 115D). Conspicuous scar cells appear along the filament after the trichoblasts have been shed and developed in spiral series in the space between segments (Fig. 115E). Scarce cicatrigenous branches. Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 115F-G). Adventitious branches are scarce (Fig. 115H). Lateral branches are replacing trichoblasts. The prostrate system is reduced and entangled, with axial segments 42.49 ± 8.78 μm in length and 56.99 ± 5.24 μm in diameter (L:D 0.75 ± 0.16) (Fig. 115I). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 115J-K), 339.47 ± 156.51 μm in length, and 18.69 ± 3.51 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 115K).

**Reproductive morphology:** In female plants (Fig. 116A), erect axes are scarcely branched. Procarps are positioned laterally and subapically on indeterminate branches (Fig. 116C-D), and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell, and with two lateral sterile cells (Fig. 116B). Cystocarps are globose when mature. In tetrasporangial plants (Fig. 116E), tetrasporangia are tetrahedral and 34.02 ± 3.59 μm × 36.94 ± 8.23 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 116G). The development of tetrasporangia follows a straight arrangement (Fig. 116F), but the position of the pericentral cells shift every couple of segments giving slightly spiral appearance (Fig. 116G). The fertile pericentral cell devel-

ops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 116H-J).

**Habitat**. Plants grow forming small tufts from the intertidal zone. They are found attached to rocks in sheltered to wave-exposed areas. Turfs are usually delicate, and are associated with other species such as *Polysiphonia strictissima.*

**Remarks:** *Polysiphonia scopulorum* has been widely described Womersley (1979). Four varieties described by Hollenberg (1968) based on minimum morphological differences. Our study confirm that one of these varieties has a new species status. Thereby, the other varieties need to be confirmed on the basis of combined morphological and molecular data.

***Polysiphonia peruana* D. E. Bustamante, B. Y. Won et T. O. Cho sp. nov. (Fig. 117-118)**

**Holotype:** CUK8402. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type Locality:** Lagunillas, Pisco, Peru, collected by T.O.C and D.E.B, 05 Jul. 2012**.**

**Etymology:** The name *"peruana"* is derived from the country of collection**.**

**Vegetative morphology:** Plants are diminutive, delicate, 0.1–0.4 cm in height (Fig. 117A-B), and red to purplish in color. Thalli form small tufts predominantly attached to rock surfaces in the intertidal zone. Thalli are composed of entangled indeterminate erect axes arisen from a reduced prostrate system (Fig. 117B) spirally disposed. Erect axes are ecorticate throughout, and arise exogenously from the prostrate axes at intervals of 1–3 axial cells (Fig. 117B) . Main erect axes are densely and radially branched in perfect alternate pattern by determinate branches (Fig. 117C). Apical cells are conspicuous, dome shaped, 4.65 ± 0.84 μm × 4.38 ± 0.82 μm in size, and transversely divided (Fig. 117D). Young erect axes have short segments (Fig. 117E). Older segments of erect

axes are larger, normally 38.61 ± 9.50 μm in length and 40.65 ± 4.79 μm in diameter (L:D 0.96 ± 0.25) (Fig. 117F-H), and frequently branched. Trichoblasts are delicate, deciduous, short, 1–2 times forked, and 49.23 ± 11.26 μm long, and arising on each segment near the apical cells of gametophytes (Fig. 117E). Conspicuous scar cells appear along the filament after the trichoblasts have been shed, reaching 5.64 ± 0.26 μm × 5.82 ± 0.24 μm, and developed in spiral series in the space between segments (Fig. 117F), scar cells are sometimes shed leaving conspicuous hollows (Fig. 117G). Cicatrigenous branches are present (Fig. 117H). Each segment is completely ecorticated along the thallus and composed of four pericentral cells (Fig. 117I-J). Lateral branches are replacing trichoblasts. The prostrate system is reduced and entangled, with axial segments 32.01 ± 5.46 μm in length and 49.03 ± 3.80 μm in diameter (L:D 0.66 ± 0.12) (Fig. 117B). Rhizoids are ventrally produced from the center or the proximal end of the pericentral cells, and in open connection from the pericentral cells (Fig. 117K), 87.08 ± 16.44 μm in length, and 15.34 ± 3.73 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 117L).

**Reproductive morphology:** In female plants (Fig. 118A), erect axes are densely branched with alternate determinate branches. Procarps are positioned laterally and subapically on indeterminate branches (Fig. 118C-D), and are composed of a supporting cell bearing a four-celled carpogonial branch, a basal sterile cell, and with two lateral sterile cells (Fig. 118B). Cystocarps are elongate when mature (Fig. 118D), 258.52 ± 18.74 μm high and 164.23 ± 7.63 μm in diameter. In male gametophytes (Fig. 118E), spermatangial branches are clustered at the apices of determinate branches and main axes in branches alternate disposition (Fig. 118E), and replacing the trichoblast (Fig. 118F). Each spermatangial branch is composed of spermatangia, and lacking of sterile tip cell. In tetrasporangial plants (Fig. 118G), tetrasporangia are tetrahedral and 23.72 ± 5.98 μm × 20.28 ± 3.23 μm in size. Tetrasporangial branches are swollen and linear (Fig. 118G). The development of tetrasporangia follows a straight arrangement (Fig. 118H). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells. A single tetrasporangium is produced on each fertile segment (Fig. 118I).

**Habitat:**. Plants grow forming small tufts from the intertidal zone. They are found attached to rocks in sheltered to wave-exposed areas. Turfs are usually delicate, diminutive, and monotypic.

**Remarks:** The alternate disposition of determinate branches in *Polysiphonia peruana* sp. nov. resemble the disposition of determinate branches in *Pterosiphonia.* Our new species is clearly distinguished form *Pterosiphonia* by having only four pericentral cells and unicellular rhizoids open connected from pericentral cells. *Polysiphonia peruana* sp. nov. is also distinguished from all other *Polysiphonia* by having the alternate determinate branches.

## 2. Phylogenetic analyses

We sequenced a 1314-bp portion of *rbc*L and a 520-bp portion of *cox1* for all members of *Polysiphonia* sensu stricto and other of *Polysiphonia* sensu lato species. In addition, three partial sequences of *rbc*L and *cox*1 for *P. atlantica, P. kapraunii*, and *P. macrocarpa* was downloaded from GenBank. Phylogenetic analyses of the independent *rbc*L and *cox*1 locus revealed the same topology of the *rbc*L (Fig. 119) and concatenated *rbc*L and *cox*1 tree (Fig. 120). This concatenated analysis shows two main clades with high support values in bootstrap for Maximum likelihood (100) and in posterior probability for Bayesian inference (1). The first clade corresponds to the true *Polysiphonia* sensu stricto where the generitype *P. stricta* is embedded and the second clade is composed of *Bryocladia*, *Dorsisiphonia* gen. nov., *Neostreblocladia* gen. nov., and *Phillipsiphonia* gen. nov. They are having in common rhizoids and pericentral cells in open connection. The sequence divergence among the members of these genera and the true *Polysiphonia* sensu stricto is ranging 8.7-14.6 % for *cox*1 and 5.9-12.2 % for *rbc*L.

## 3. Discussion

Our phylogenetic analyses obtained from combined data of markers for plastidial (*rbc*L) and mitochondrial (*cox*1) genomes confirm two sister clades in *Polysiphonia* sensu stricto (Bustamante et al. 2015a). The true clade of *Polysiphonia* sensu stricto is the monophyletic clade where the generitype *P. stricta* is embedded (Fig. 119-120). This clade is composed of nine species that are characterized by having prostrate and erect axes spirally disposed with four pericentral cells ecorticated throughout, rhizoids in open connection with the pericentral cells, a four-celled carpogonial branch, spermatangial branches replacing the whole trichoblast, and tetrasporangia arranged in straight series (Kim et al. 2000, Bustamante et al. 2015a). The recent studies of Stuercke and Freshwater (2010) and Bustamante et al. (2014a, 2014b) have extensively characterized this clade with the description of three new species: *P. dokdoensis* D.E. Bustamante, B.Y. Won et T.O, *P. kapraunii* B. Stuercke et D.W. Freshwater, and *P. ulluengensis* D.E. Bustamante, B.Y. Won et T.O. Moreover, our tree based on the plastid-encoded *rbc*L shows that *P. orbigniana* Kützing from Peru is also confirmed in *Polysiphonia* sensu stricto.

The clade sister to the true *Polysiphonia* sensu stricto is a paraphyletic clade composed of three genera (i.e. *Bryocladia* F.Schmitz, *Polysiphonia*, and *Streblocladia* F.Schmitz) dispersed in five different subclades. The first subclade labeled as the new genus *Dorsisiphonia*, based on *D. freshwateri* comb. nov., is composed by other four new combinations (*D. parvula*, *D. subtilissima*, *D. utricularis*, and *D. villum*) that previously were reported as *Polysiphonia* members. *Dorsisiphonia* gen. nov. is having the same features of *Polysiphonia* sensu stricto, except by the disposition of the prostrate and erect axes in a strictly dorsiventral pattern. This disposition is originated when the initial exogenous erect axes are always arisen from the same position of the axial cell and developed among the same pericentral cells on different segments. This character has been reported in the genera *Enelittosiphonia* Segi and *Lophosiphonia* Falkenberg as a consistent character to segregate them from *Polysiphonia* sensu lato (Falkenberg 1901, Segi 1949) and it was confirmed by molecular analyses (see Chapter 8). Earlier, the consistency of this character was pointed out by

Setchell and Gardner (1903) transferring *Dorsisiphonia villum* (as *P. villum*) to *Lophosiphonia villum*, but Hollenberg (1968) reject it and reduced this species as a variety of *P. scopulorum*. *Lophosiphonia* is distinguished from *Dorsisiphonia* by having six pericentral cells (see Chapter 7). The strictly dorsiventral pattern in combination with four ecorticated pericentral cells and open-connected rhizoids distinguish *Dorsisiphonia* gen. nov. from other genera in *Polysiphonia* sensu lato.

The second subclade is composed by *Polysiphonia peruana* from Peru and *P. scopulorum* from Australia. Although our phylogenetic analyses closely related these species to the genus *Bryocladia*, our morphological observations did not find the diagnostic feature to transfer these species to *Bryocladia* and also they are not showing any diagnostic features to segregate these species as a different genus in *Polysiphonia* sensu lato. Although, further collections of close related species to these pseudo *Polysiphonia* would likely resolve their taxonomical status, we provisionally retain in *Polysiphonia* and described *Polysiphonia peruana* as a new species on the basis of its determinate branches alternately and spirally disposed throughout the main axes.

Another subclade in the paraphyletic group of our concatenated tree correspond to the genus *Bryocladia*. This genus has as diagnostic features spirally disposed needle-like branches with endogenous laterals and rhizoids open connected to pericentral cells (Schmitz 1897). *Bryocladia* is composed of five species currently accepted (Guiry and Guiry 2015). The morphology of *B. cuspidata* correspond to the diagnostic characters of *Bryocladia* and it was supported in our molecular analyses.

The last two subclades are composed of species identified as *Streblocladia* members. The diagnostic feature in *Streblocladia* was initially named as dorsiventral secondary branches by Falkenberg (1901) and renamed as rami-sympodial branches by Phillips (2010). The rami-sympodial branching is formed when an apical portion is pushed aside by an endogenous lateral branch and then it becomes known as a pseudolateral, then this process is repeated several times with each new

pseudolateral branches (Phillips 2010). Phillips (2010) reviewed type materials of the species of *Strebloclad*ia and concluded that although these species are sharing this diagnostic feature, there are significant morphological and biogeographical differences that cluster the species of *Strebloclad*ia in three main groups that could have generic status (Phillips 2010). The first group is composed by the generitype *S. glomerulata* and *S. muelleriana*. They are having in common a heavy cortication and rhizoids cutting off from pericentral cells and are found in New Zealand and subantartic islands. Phillips (2010) and Mamoozadeh and Freshwater (2011) demonstrated that these species, which are composing the *Strebloclad*ia sensu stricto, are related to the emended genus *Leptosiphonia* on the basis of 18S rRNA and plastid-encoded *rbc*L. The second group is only composed by *S. collabens* that is distributed in the northern hemisphere and already was transferred to *Neosiphonia* as *Neosiphonia collabens* on the basis of the three-celled carpogonial branches and rhizoids cutting off pericentral cells (Díaz-Tapia and Bárbara 2013). The last group is composed by *S. camptoclada*, *S. spicata*, *S. corymbirifera*, *S. atrata*, and *S. tenuissima*, which are ecorticated species and distributed between South America and South Africa. Our study sequenced specimens of *S. camptoclada* and *S. spicata* collected from the type localities in Peru and confirmed that these species are distantly related to *Neosiphonia* or *Strebloclad*ia sensu stricto (Bustamante et al. 2015c) by having rhizoids in open connection to pericentral cells. Rhizoids-pericentral cells connections is character that has shown consistency to segregate several genera in *Polysiphonia* sensu lato (Choi et al. 2001, Stuercke and Freshwater 2008). Thereby, we segregate these species from *Strebloclad*ia to accommodate them on their own genus. *Strebloclad*ia *camptoclada* is transferred to *Phillipsiphonia* and *S. spicata* is transferred to *Neostrebloclad*ia. Although these new genera are similar by having rami-sympodial branches, open connection of rhizoids, and lacking of cortication, they are distinguished by the number of pericentral cells. *Phillipsiphonia* is having only four pericentral cells, whereas *Neostrebloclad*ia is having 9-13 pericentral cells (Howe 1914, Dawson et al. 1964).

Our study attempted to solve the paraphyly in *Polysiphonia* sensu stricto by recognizing the clade where the generitype *P. stricta* is embedded as the true *Polysiphonia* sensu stricto, also by

segregating the three new genera *Dorsisiphonia*, *Phillipsiphonia*, and *Neostreblocladia*, and finally by recognizing the generic status *Bryocladia* (Table 7) on the basis diagnostic morphological features and supported by molecular analyses.

**Fig. 89.** Distribution of the genera belonging to the paraphyletic group of *Polysiphonia* sensu stricto. *Bryocladia cuspidata* (green), *Dorsisiphonia* (red), *Neostreblocladia* (yellow), *Phillipsiphonia* (blue), and *Polysiphonia* sensu stricto (sky blue).

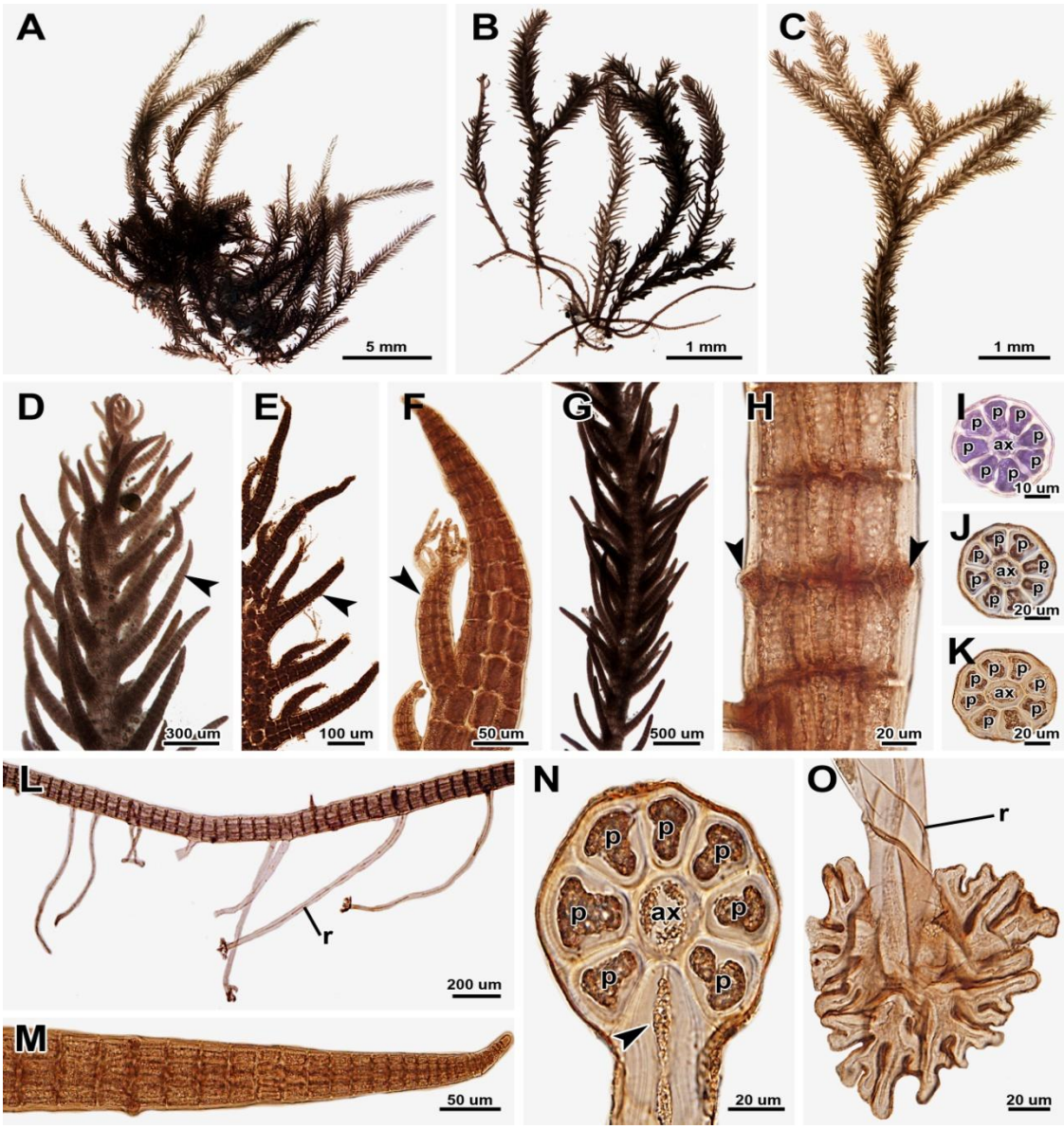


**Fig. 90.** Vegetative structures of *Bryocladia cuspidata*. (A) Habit of vegetative thallus. (B) Erect axes arising exogenously from the prostrate axes (stolones). (C) Erect main axes irregularly branched. (D) Apex showing needle-like branches (arrowhead). (E) Adventitious laterals arisen on needle-like branches (arrowhead). (F) Short trichoblasts developed on apex of adventitious laterals (arrowhead). (G) Determinate needle- like branches on the basal part of main axes. (H) Primordia (arrowheads) of adventitious lateral branches on main axes. (I-K) Cross section views of axes showing 7 pericentral cells (p) from apex (I), middle (J), and basal part (K) [ax, axial cell]. (L) Long rhizoids (r) scattered in prostrate axes. (M) Apex of prostrate axes showing a stolone (N) Cross section showing rhizoids in open connection with pericentral cells (p) (arrowhead). (O) Unicellular terminations of rhizoids.

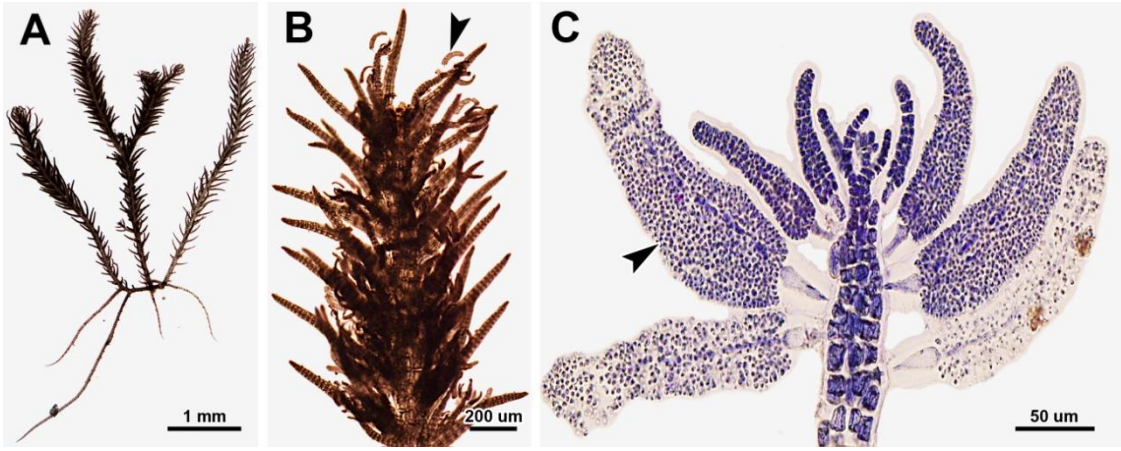


**Fig. 91.** Reproductive structures of *Bryocladia cuspidata*. (A) Male plant. (B) Apex showing spermatangial branches (arrowhead) arisen on the apices of needle-like branches. (C) Spermatangial branches (arrowhead) clustered at apical region and replacing trichoblasts.

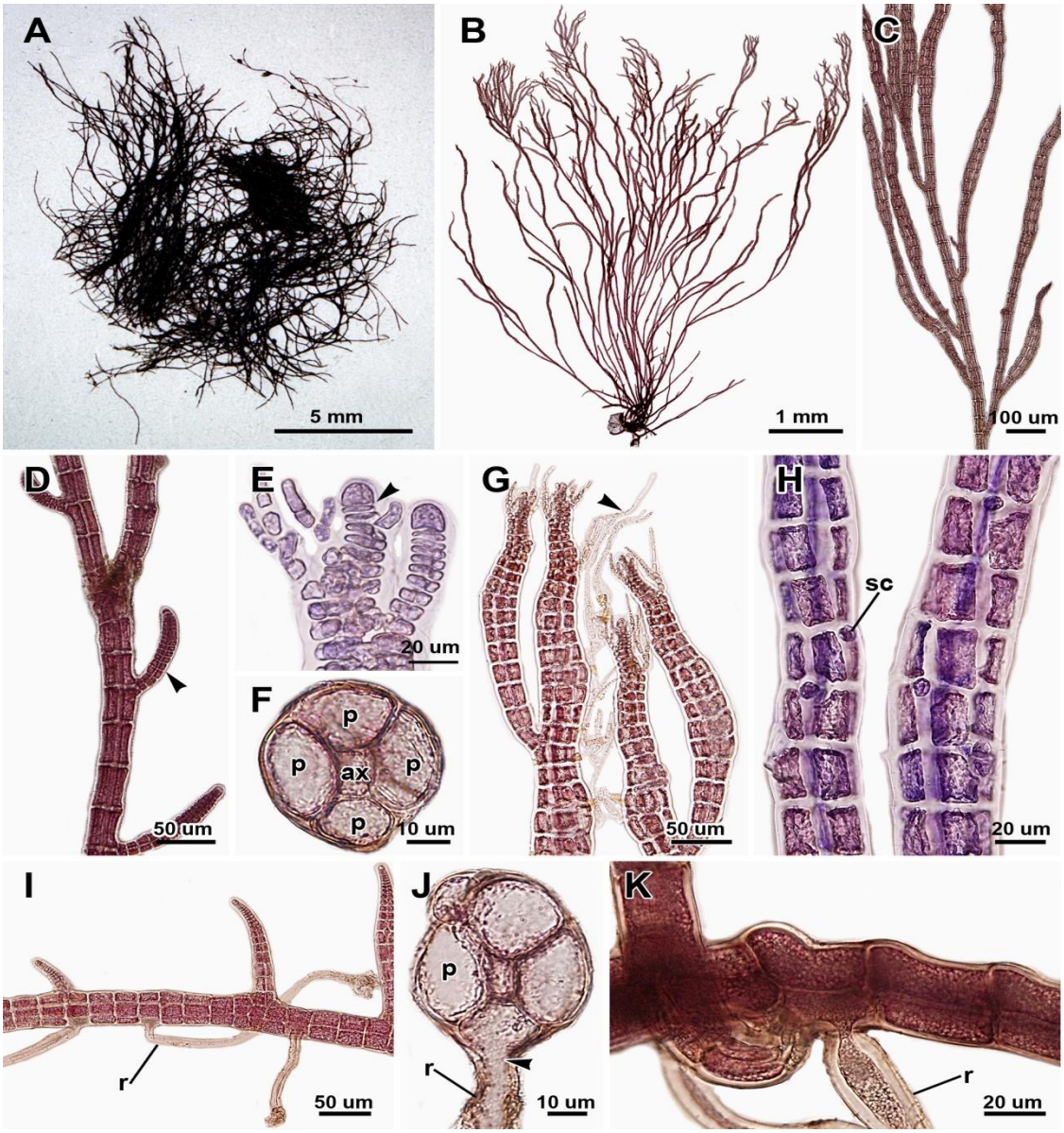


**Fig. 92.** Vegetative structures of *Dorsisiphonia freshwateri* comb. nov. (A) Holotype specimen from Yeonji-ri, Uljin, Korea. (B) Habit of vegetative plant showing the reduced prostrate and extended erect axes. (C) Erect axes showing subdichotomous to alternate branching pattern. (D) Axes showing young adventitious lateral branches (arrowhead). (E) Apices showing transversely (arrowhead) divided apical cells. (F) Cross-section of an axis showing four pericentral cells (p) (ax, axial cell). (G) Apex showing abundant trichoblasts (arrowhead). (H) Erect axes showing conspicuous scar cells (sc) placed among four pericentral cells. (I) Rhizoids (r) scattered and produced from prostrate axes. (J) Cross-section of a prostrate axis showing rhizoid (r) in open connection with pericentral cells (p). (K) Rhizoid (r) showing open connection with the center of the pericentral cell (p).

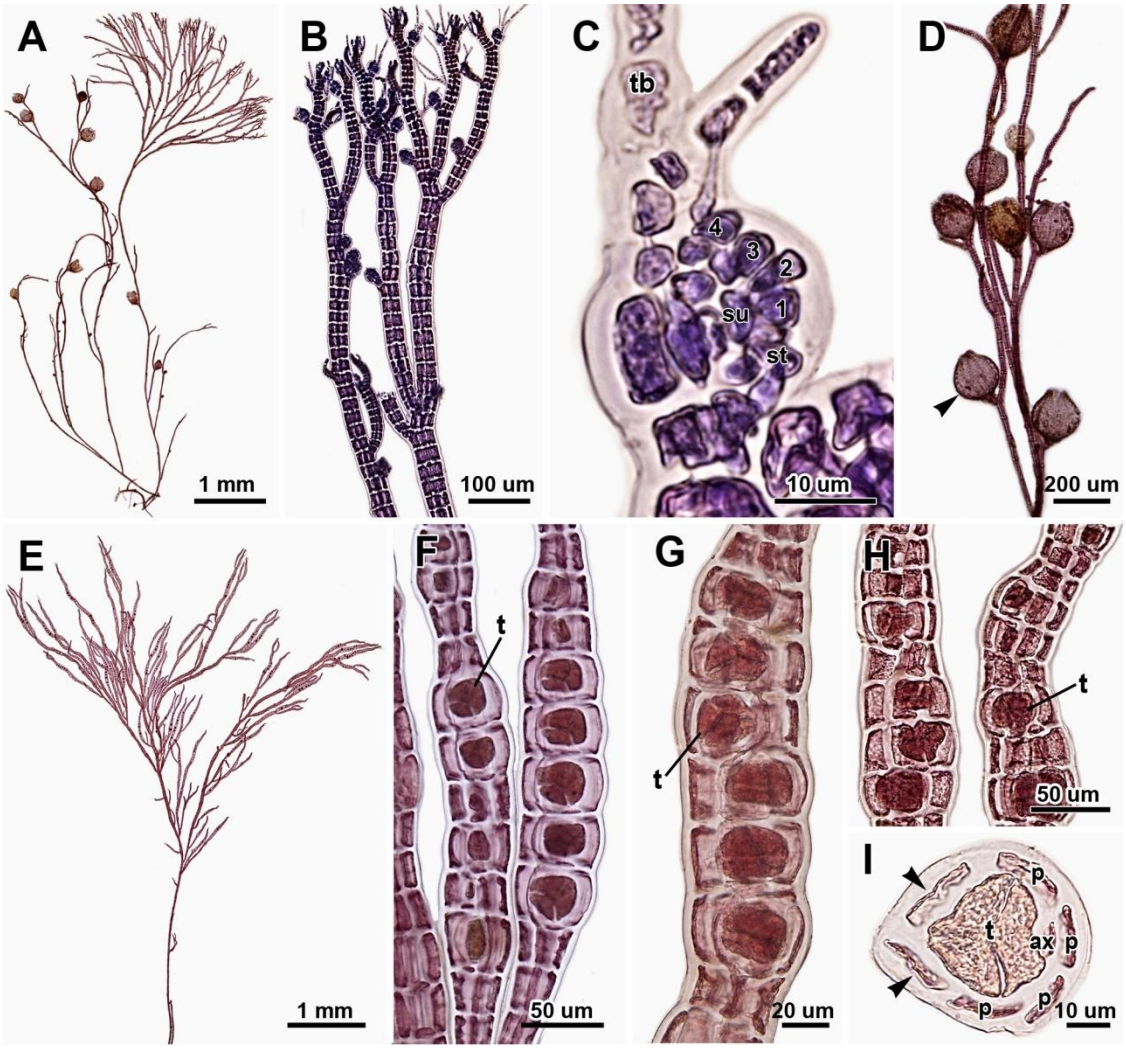


**Fig. 93.** Reproductive structures of *Dorsisiphonia freshwateri* comb. nov. (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (D). Mature cystocarp showing globose shape. (E) Tetrasporangial plant. (F) Apical branches showing straight arrangement of tetrasporangia (t). (G) Apical branch showing straight series interrupted by spiral series of tetrasporangia (t). (H) Apical branches showing spiral arrangement of tetrasporangia (t). (I) Cross-section showing a tetrasporangium (t) rounded by cover cells (arrowheads) (p, pericentral cells).

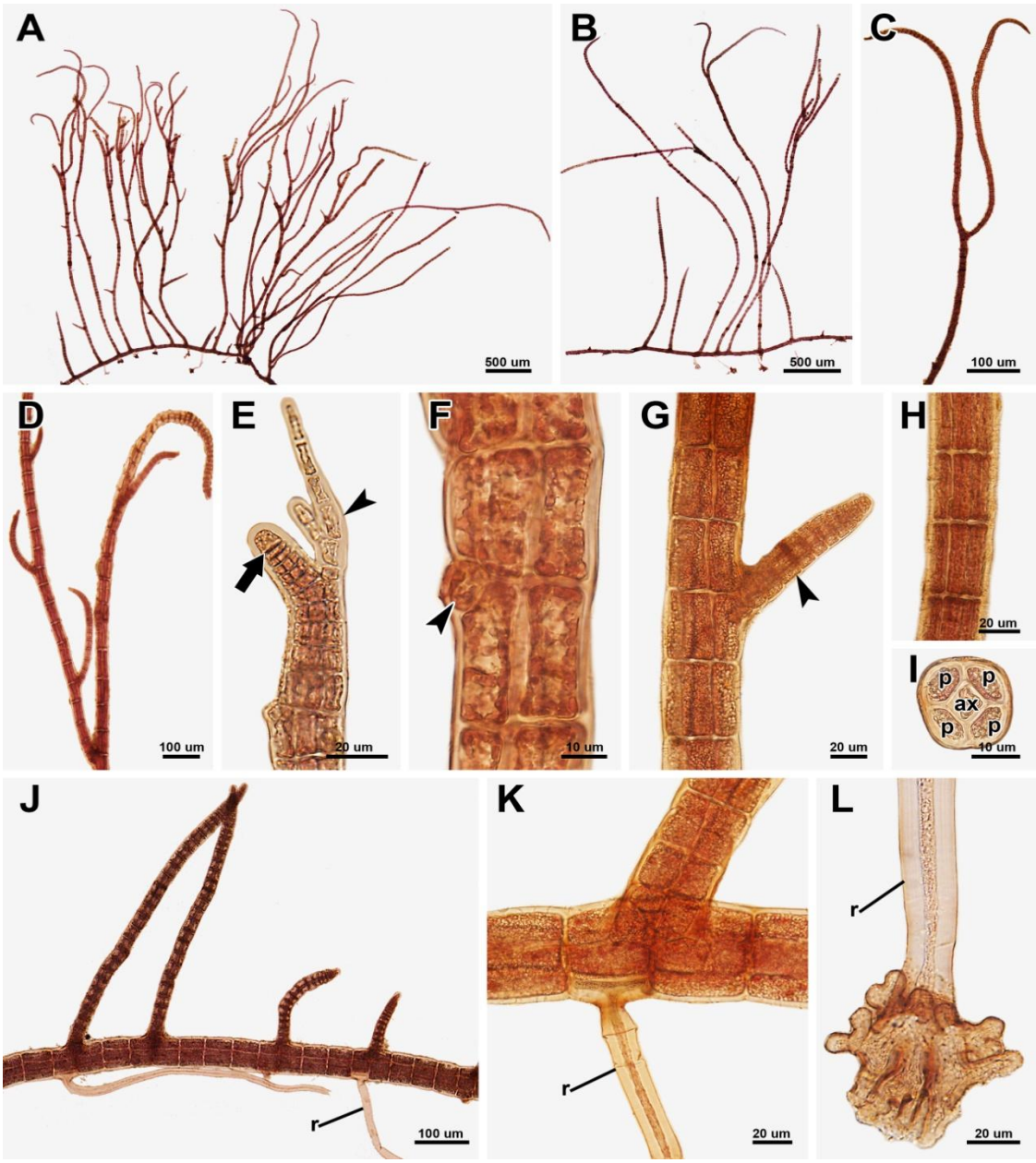


**Fig. 94.** Vegetative structures of *Dorsisiphonia parvula* comb. nov. (A) Habit of vegetative thallus. (B) Erect axes arising exogenously from the prostrate axes in strictly dorsiventral disposition. (C) Erect main axes dichotomous branched. (D) Erect main axes with laterals alternately disposed. (E) Short trichoblasts (arrowhead) developed on apices with a prominent apical cell (arrow). (F) Conspicuous scar cells. (G) Adventitious lateral branches. (H) Old filament of erect axes. (I) Cross section views of axes showing 4 pericentral cells (p) [ax, axial cell]. (J) Long rhizoids (r) scattered in prostrate axes. (K) Rhizoid (r) cut off from the centre of pericentral cells. (L) Unicellular terminations of rhizoids (r).

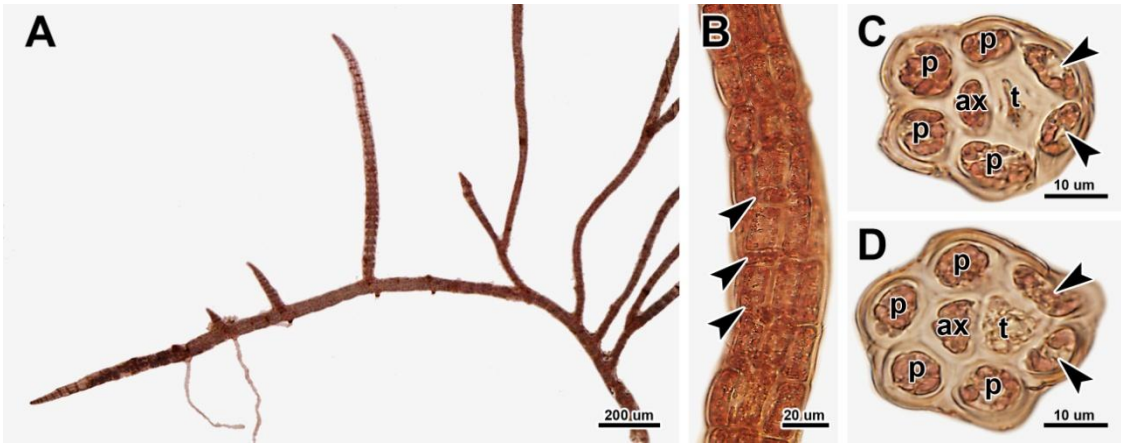


**Fig. 95.** Reproductive structures of *Dorsisiphonia parvula* comb. nov. (A) Tetrasporangial plant. (B) Apical branches showing straight arrangement of tetrasporangia and the post sporangial cover cells (arrowhead). (C-D) Cross section view of tetrasporangial segments with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

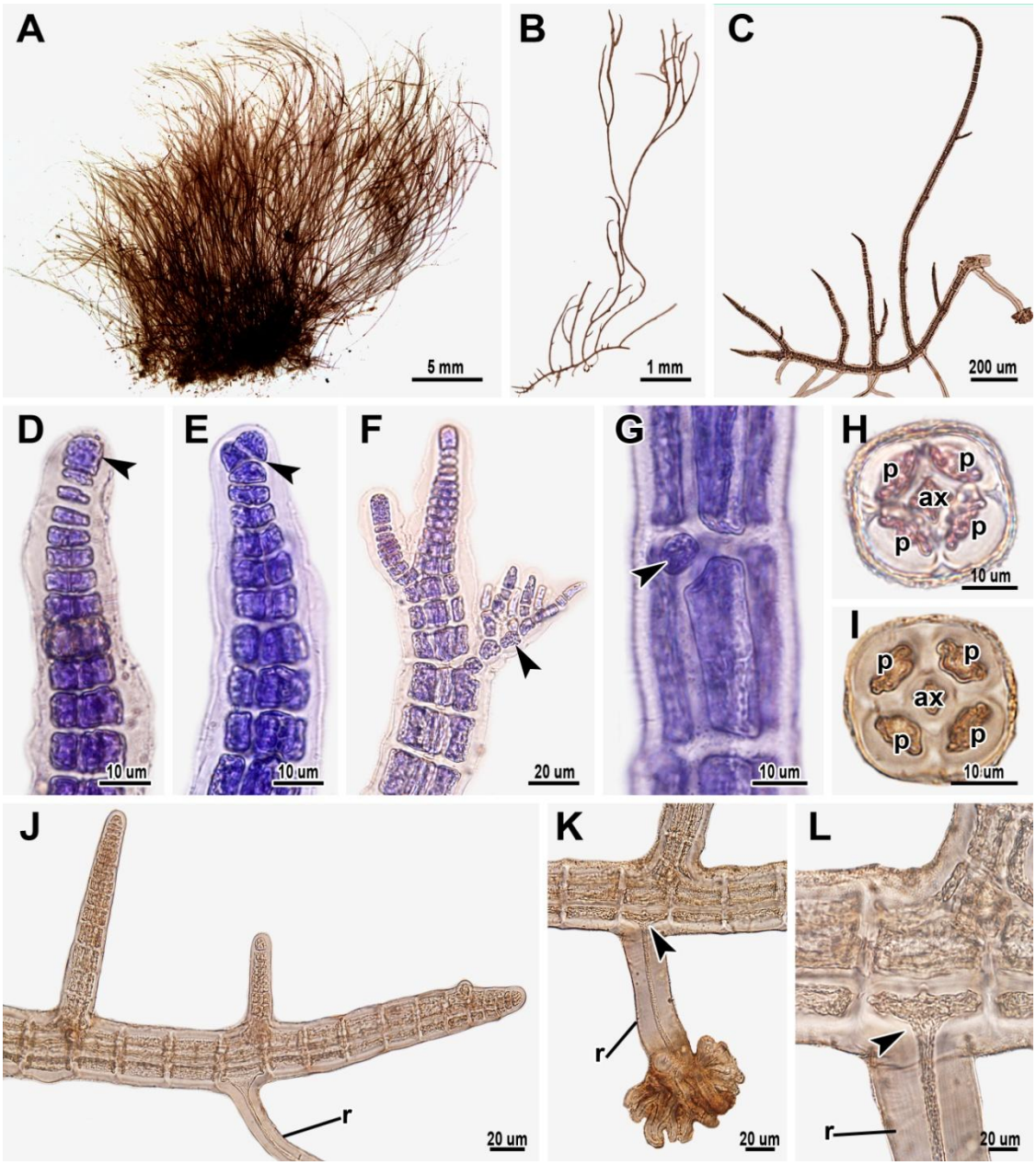


**Fig. 96.** Vegetative structures of *Dorsisiphonia utricularis* comb. nov. (A) Habit of vegetative thallus. (B-C) Erect axes arising exogenously from the prostrate axes in strictly dorsiventral disposition. (D-E) Apices showing prominent apical cells (arrowheads) transversally (D) and obliquously divided (E). (F) Apices showing short trichoblasts (arrowhead) subapical. (G) Conspicuous scar cells. (H-I) Cross section views of axes showing 4 pericentral cells (p) from middle (H) and basal parts (I) [ax, axial cell]. (J) Rhizoid (r) scattered in prostrate axes. (K-L) Rhizoids (r) cut off from the centre of pericentral cells with unicellular terminations.

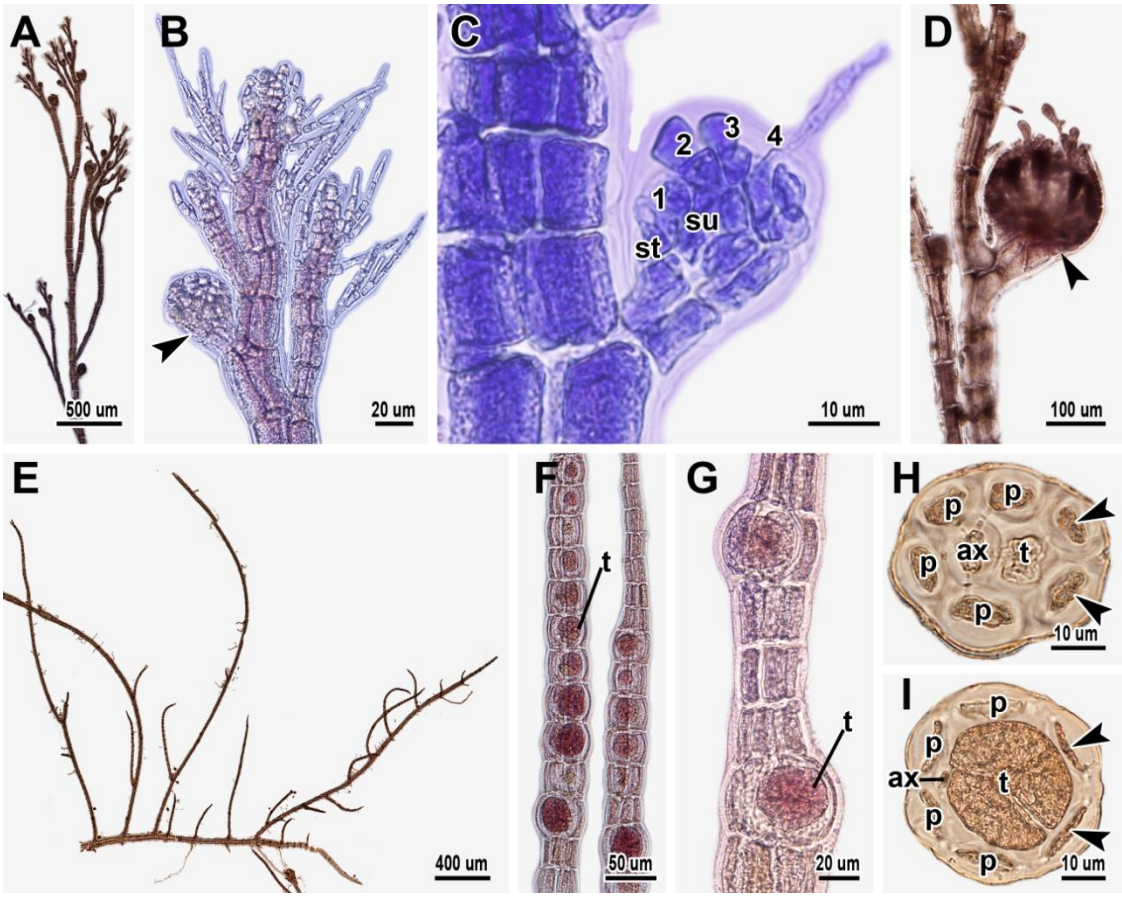


**Fig. 97.** Reproductive structures of *Dorsisiphonia utricularis* comb. nov. (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (D). Mature cystocarp showing globose shape. (E) Tetrasporangial plant. (F) Apical branches showing straight arrangement of tetrasporangia (t). (G) Apical branch showing interrupted series of tetrasporangia (t). (H-I) Cross-section showing a tetrasporangium (t) rounded by cover cells (arrowheads) (p, pericentral cells).

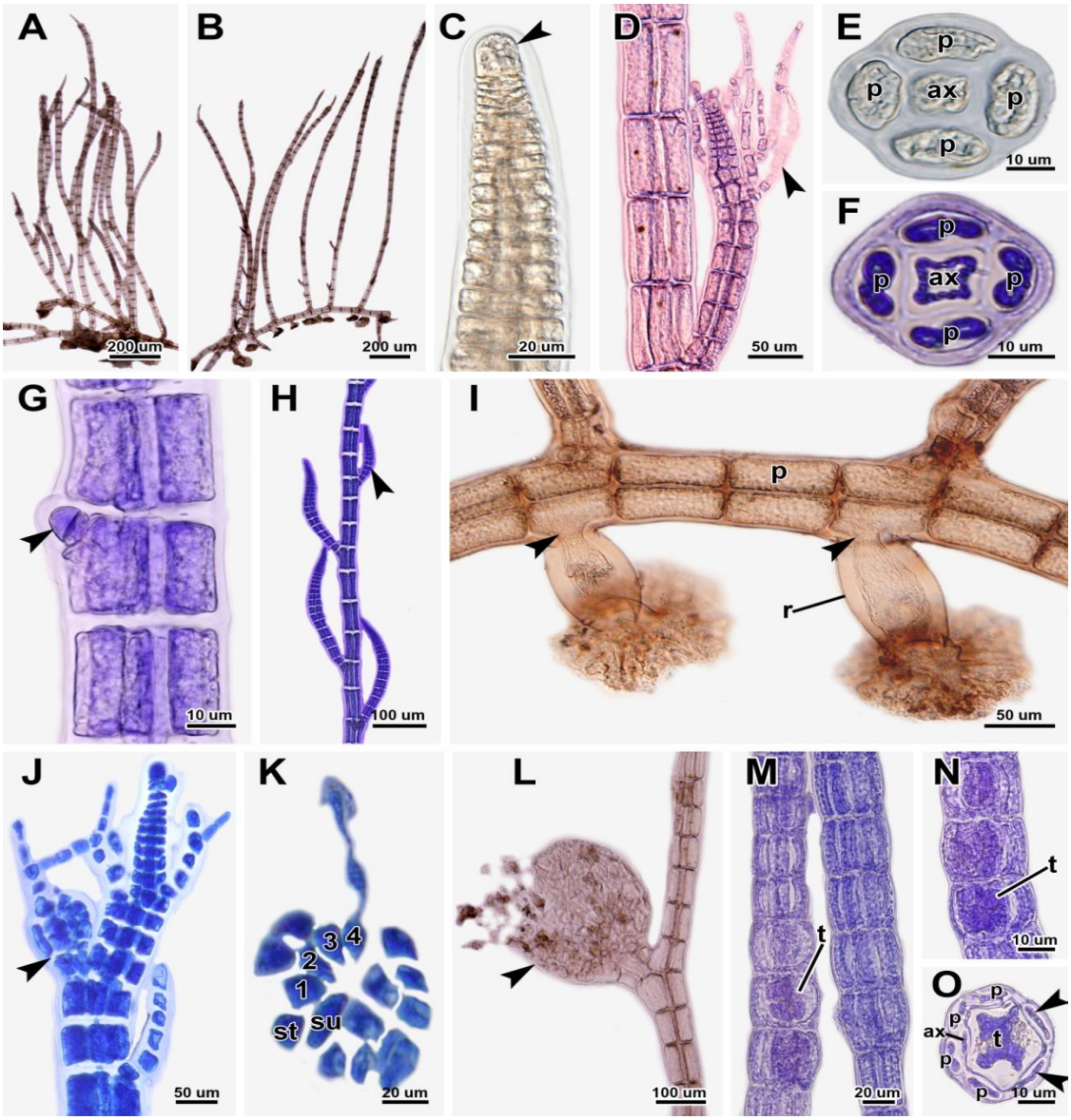


**Fig. 98.** Vegetative and reproductive structures of *Dorsisiphonia villum* comb. nov. (A) Habit of vegetative thallus. (B) Erect axes arising exogenously from the prostrate axes in strictly dorsiventral disposition. (C) Apices showing prominent apical cells (arrowhead) transversally divided. (D) Axes showing young adventitious lateral branches with abundant trichoblasts. (E-F) Cross section views of axes showing 4 pericentral cells (p) from middle (E) and basal parts (F) [ax, axial cell]. (G-H) Erect axes showing young (G) and mature (H) adventitious laterals. (I) Rhizoids (r) in open connection (arrowhead) from pericentral cells (p), scattered and produced from prostrate axes. (J) Upper part of female thallus showing subapical cystocarps. (K) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: st, basal sterile cell; su, supporting cell). (L). Mature cystocarp (arrowhead) showing globose shape. (M) Apical branches showing straight arrangement of tetrasporangia (t). (O) Cross-section showing a tetrasporangium (t) rounded by cover cells (arrowheads) (p, pericentral cells).

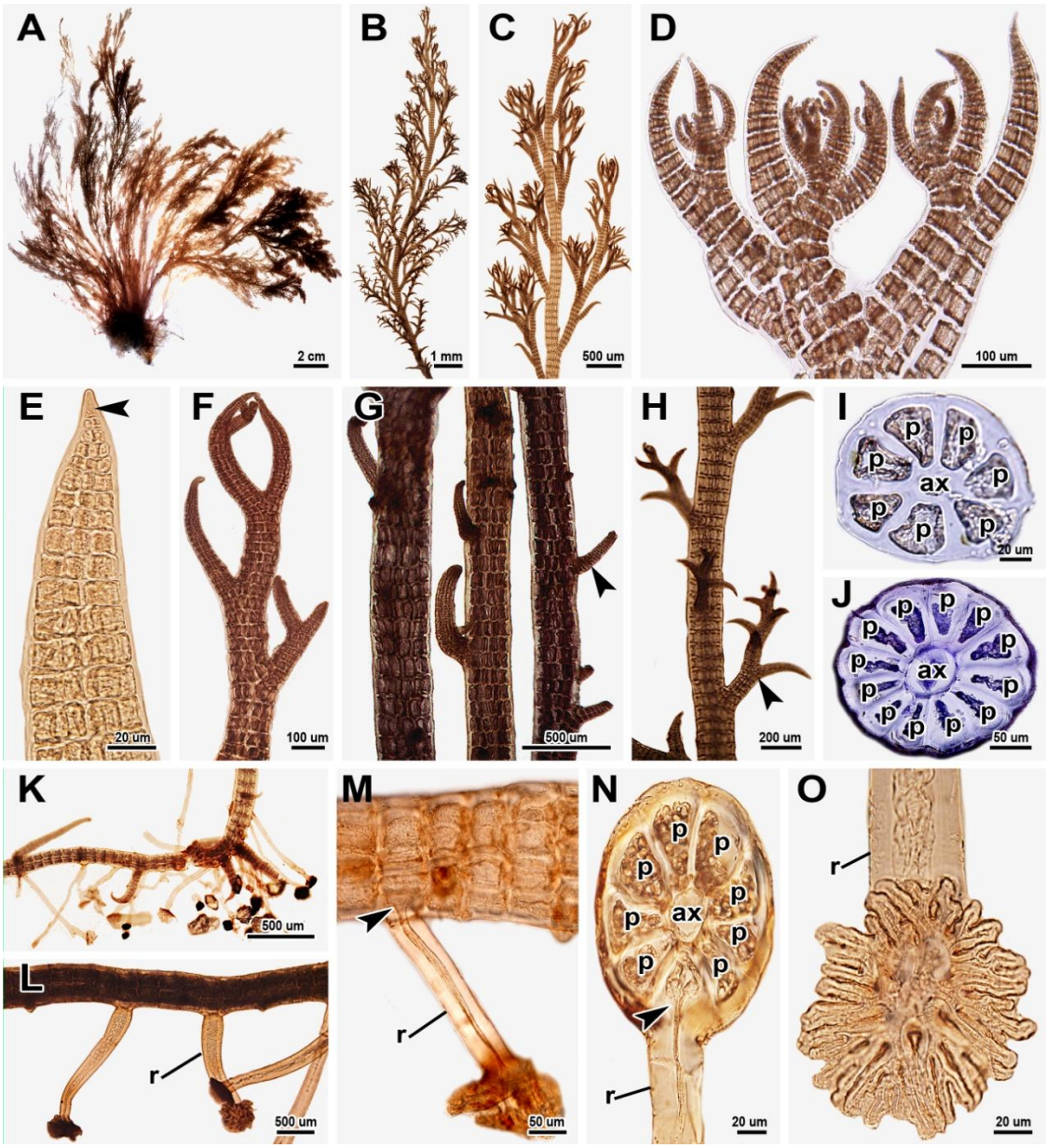


**Fig. 99.** Vegetative structures of *Neostreblocladia spicata* comb. nov. (A) Habit of vegetative plant showing the reduced prostrate and extended erect axes. (B-C) Erect axes showing alternate branching pattern. (D) Apices showing ramisympodial disposition of laterals. (E) Apices showing transversely (arrowhead) divided sharp-pointed apical cells (arrowhead). (F) Apex showing young branches. (G-H) Old axes showing abundant adventitious branches (arrowheads). (I-J) Cross-section of an axis showing 7 (I) and 13 (J) pericentral cells (p) (ax, axial cell). (K-L) Rhizoids (r) scattered and produced from prostrate axes. (M) Rhizoid (r) showing open connection with the centre of the pericentral cell (p). (N) Cross-section of a prostrate axis showing rhizoid (r) in open connection with pericentral cells (p). (O) Unicellular terminations of rhizoids (r).

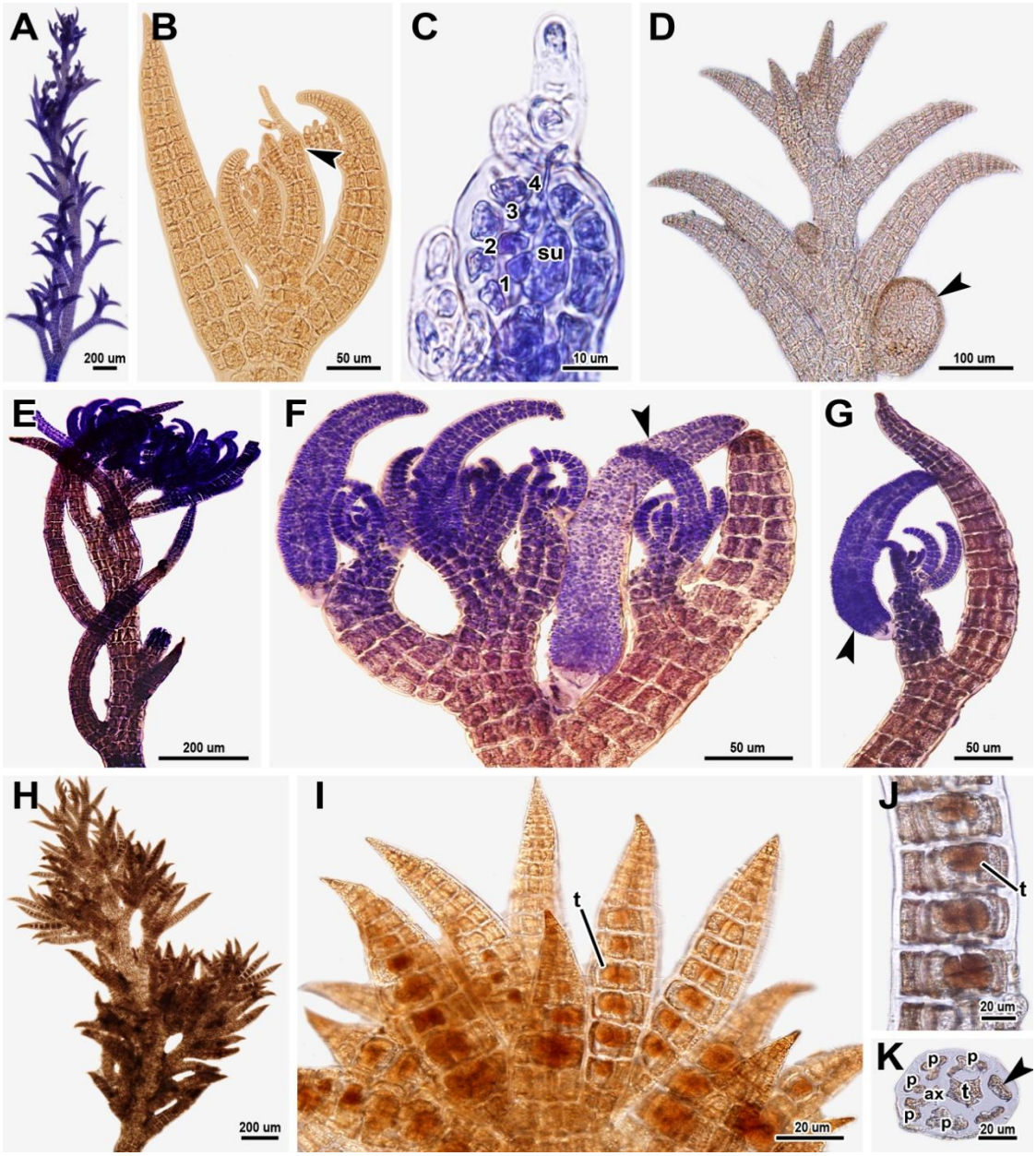


**Fig. 100.** Reproductive structures of *Neostreblocladia spicata* comb. nov. (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps (arrowhead). (C) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (D). Young cystocarp showing globose shape. (E) Male plant. (F) Spermatangial branches (arrowhead) clustered at the apices of erect axes in a ramisympodial disposition (G) Spermatangial branches (arrowhead) replacing trichoblasts. (H) Tetrasporangial plant. (I-J) Apical branches showing straight arrangement of tetrasporangia (t). (K)

Cross-section showing a tetrasporangium (t) rounded by cover cells (arrowheads) (p, pericentral cells).

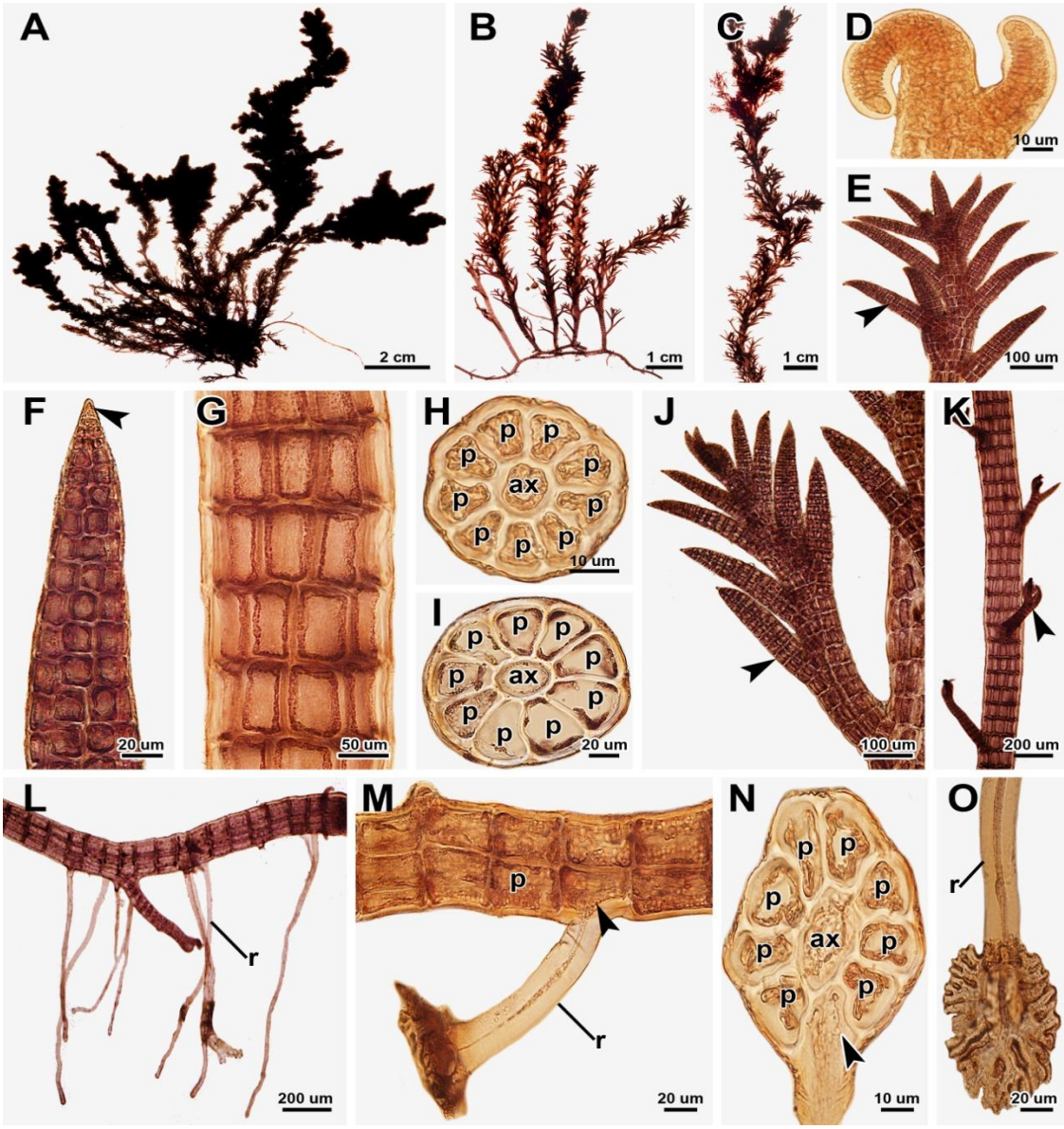


**Fig. 101.** Vegetative structures of *Neostreblocladia thwaitesii* comb. nov. (A-B) Habit of vegetative plant showing the extended prostrate and erect axes. (C) Erect axes showing abundant clustered branches. (D) Apices showing initial ramisympodial disposition of laterals. (E) Apex showing clustered laterals (arrowhead). (F) Apex showing apical cells (arrowhead) transversely divided and sharp-pointed. (G) Old Axes lacking of scar cells. (H-I) Cross-sections of axes showing 9 pericentral cells (p) (ax, axial cell). (J-K) Old axes showing abundant adventitious branches with clustered laterals (arrowheads). (L) Long rhizoids (r) scattered and produced from prostrate axes. (M) Rhizo-

id (r) showing open connection from the centre of the pericentral cell (p). (N) Cross-section of a prostrate axis showing rhizoid (r) in open connection with pericentral cells (p). (O) Unicellular terminations of rhizoids (r).

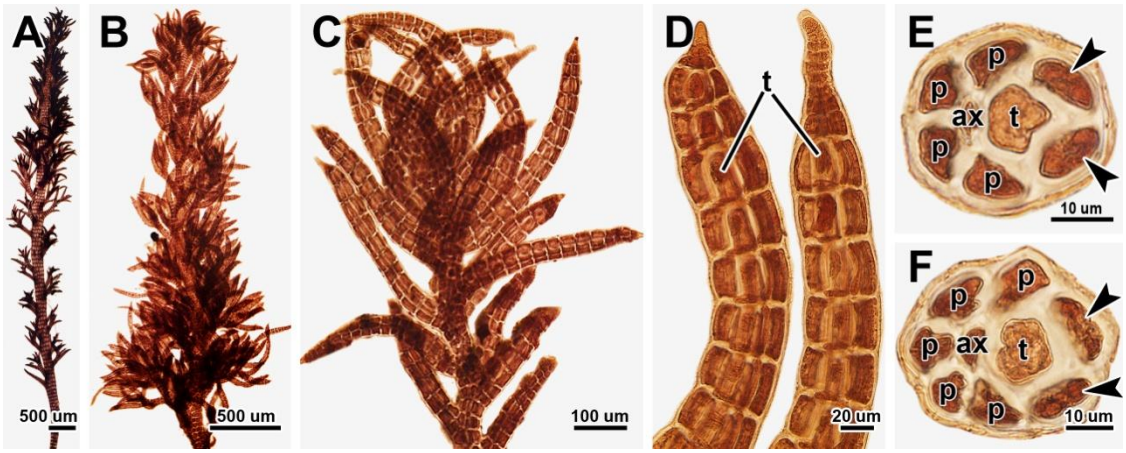


**Fig. 102.** Reproductive structures of *Neostreblocladia thwaitesii* comb. nov. (A-C) Tetrasporangial plant. (D) Apical branches showing straight arrangement of tetrasporangia (t). (E-F) Cross-section view of tetrasporangial segments with five (E) and six (F) pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

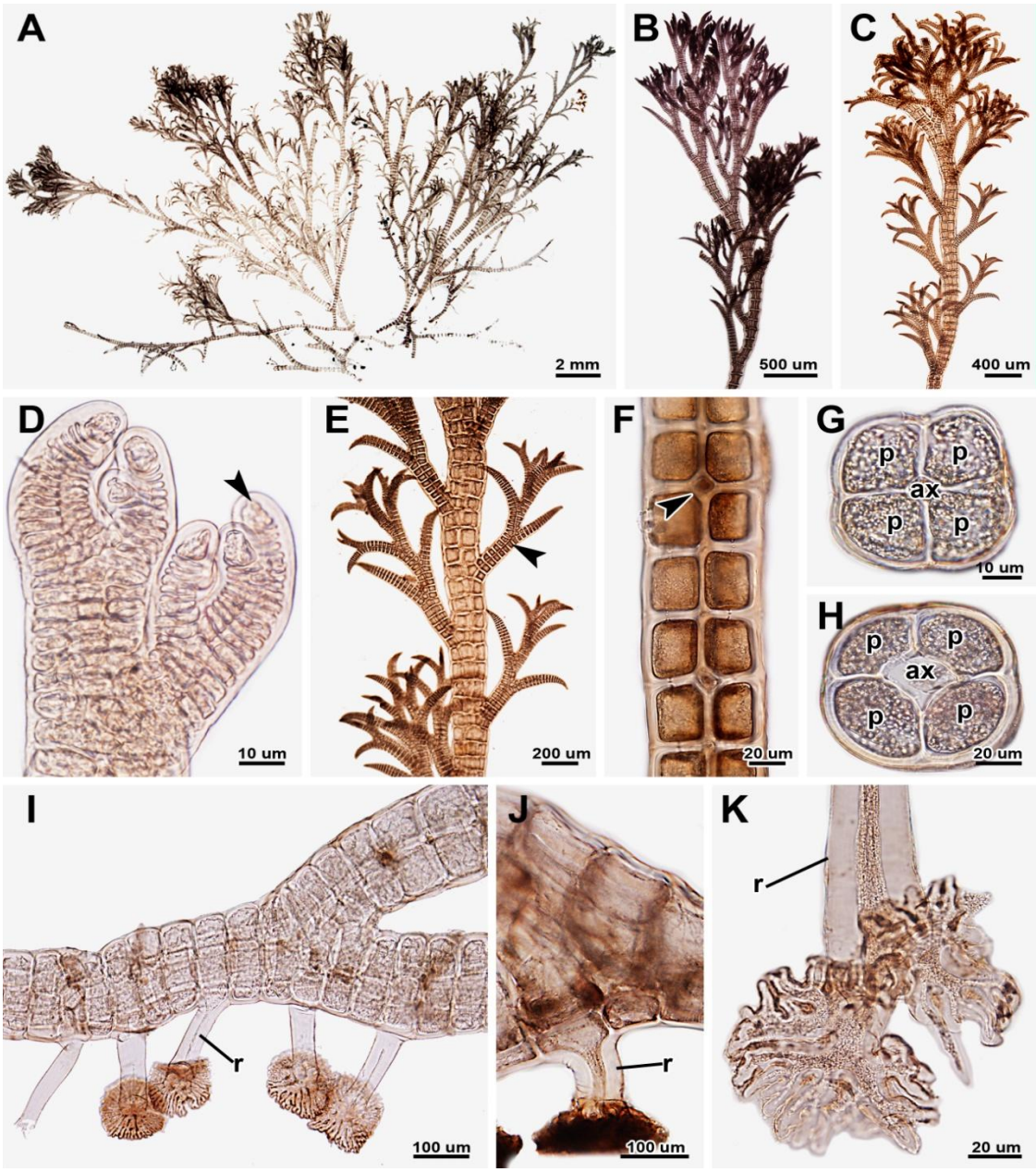


**Fig. 103.** Vegetative structures of *Phillipsiphonia camptoclada* comb. nov. (A) Habit of vegetative plant showing the extended prostrate and erect axes. (B-C) Erect axes showing abundant clustered branches spirally disposed. (D) Apices showing initial ramisympodial disposition of laterals and apical cells (arrowhead) transversely divided and dome shaped. (E) Apex showing clustered laterals (arrowhead). (F) Old Axes showing scarcely scar cells. (G-H) Cross-sections of axes showing four pericentral cells (p) (ax, axial cell). (I) Rhizoids (r) scattered and produced from prostrate axes. (J) Rhizoid (r) showing open connection from the centre of the pericentral cell (p). (K) Unicellular terminations of rhizoids (r).

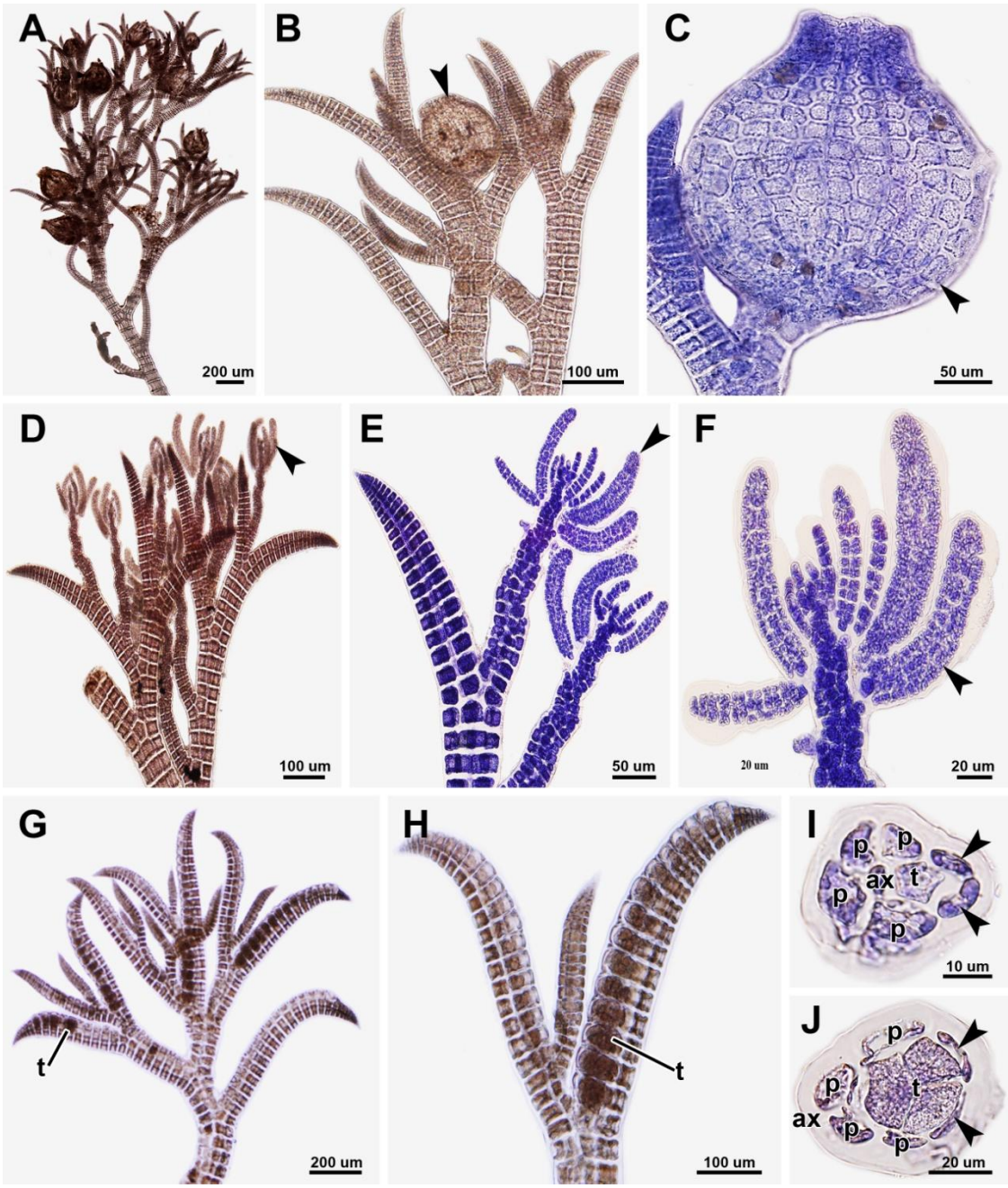


**Fig. 104.** Reproductive structures of *Phillipsiphonia camptoclada* comb. nov. (A) Female gametophyte. (B-C) Upper part of female thallus showing subapical cystocarps (arrowhead). (D) Male plant. (E) Spermatangial branches (arrowhead) clustered at the apices. (F) Spermatangial branches (arrowhead) replacing trichoblasts. (G) Tetrasporangial plant. (H) Apical branches showing straight arrangement of tetrasporangia (t). (I-J) Cross-section views with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

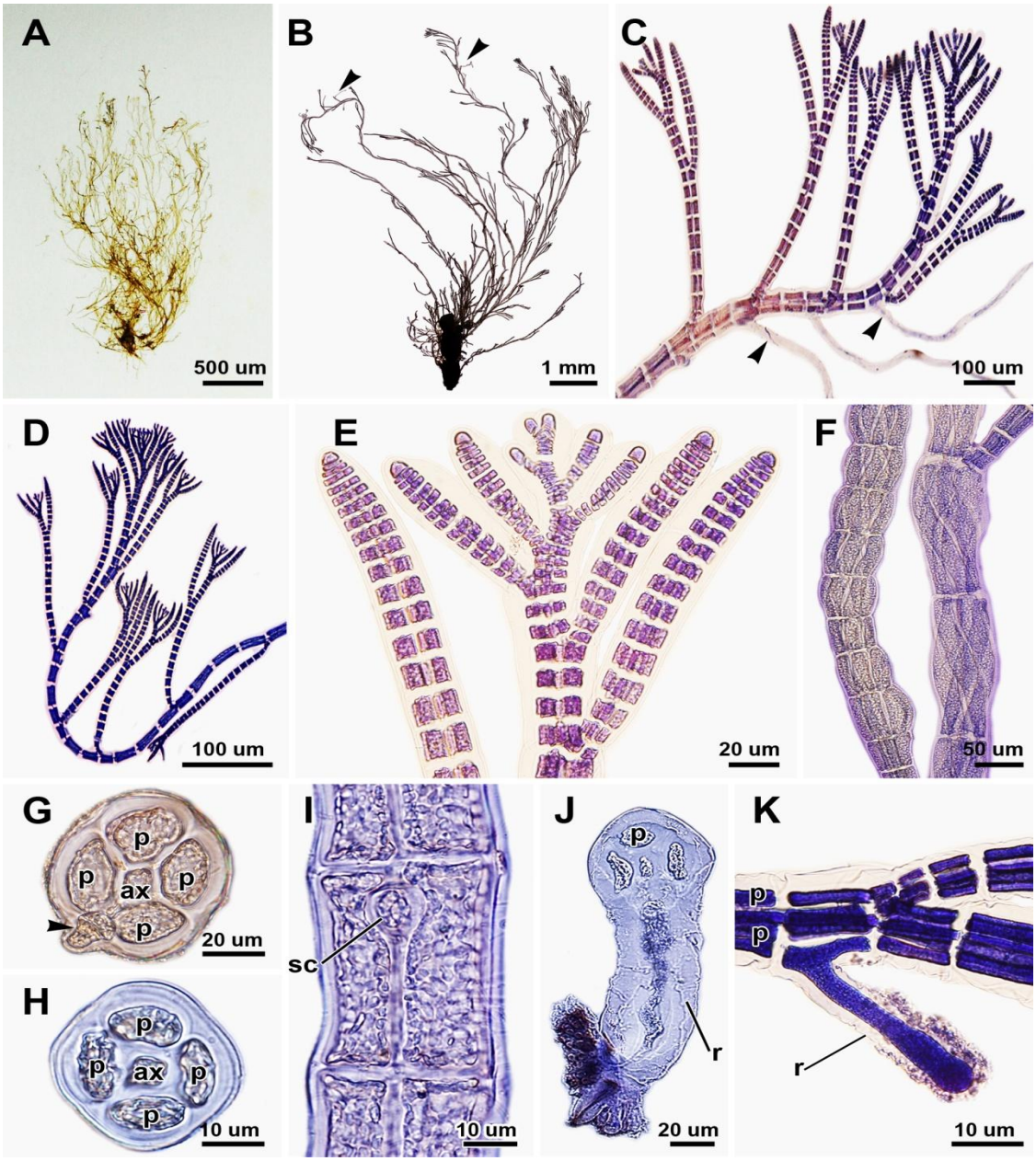


**Fig. 105.** Vegetative structures of *Polysiphonia dokdoensis.* (A) Holotype specimen from Dokdo, Korea. (B) Habit of vegetative plant showing prostrate axes attached to *Corallina* and erect axes (arrowheads). (C) Decumbent axes producing long rhizoids (arrowheads). (D) Apex showing alternate arrangement of unilateral branch pairs. (E) Apex showing branching pattern and prominent apical cells. (F) Axes showing short and long twisted pericentral cells. (G-H) Cross section showing four pericentral (p) cells in prostrate axis with initial endogenous branch (arrowhead) (G) and in erect axis (H). (I) Erect axis with scar cell (sc) placed between two pericentral cells. (J) Rhizoid (r) terminating in multilobed tips. (K) Rhizoid (r) in open connection to pericentral cell.

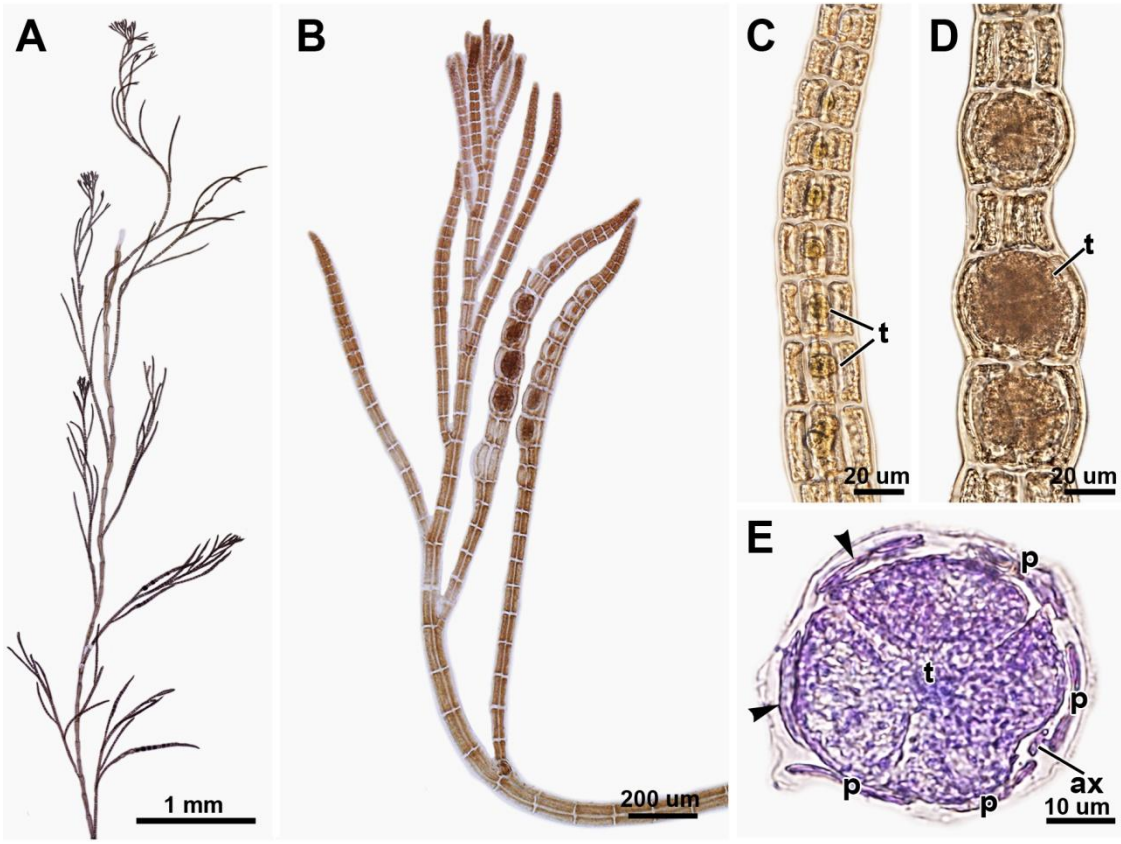


**Fig. 106.** Reproductive structures of *Polysiphonia dokdoensis* sp. nov. (A) Tetrasporangial plant. (B) Tetrasporangial plant showing alternate arrangement of unilateral branch pairs. (C-D) Apical axes showing straight arrangement of continuous (C) and interrupted (D) series of tetrasporangia. (E) Cross section with five pericentral cells (p) showing one tetrasporangium (t) surrounded by cover cells (arrowheads) (ax, axial cell).

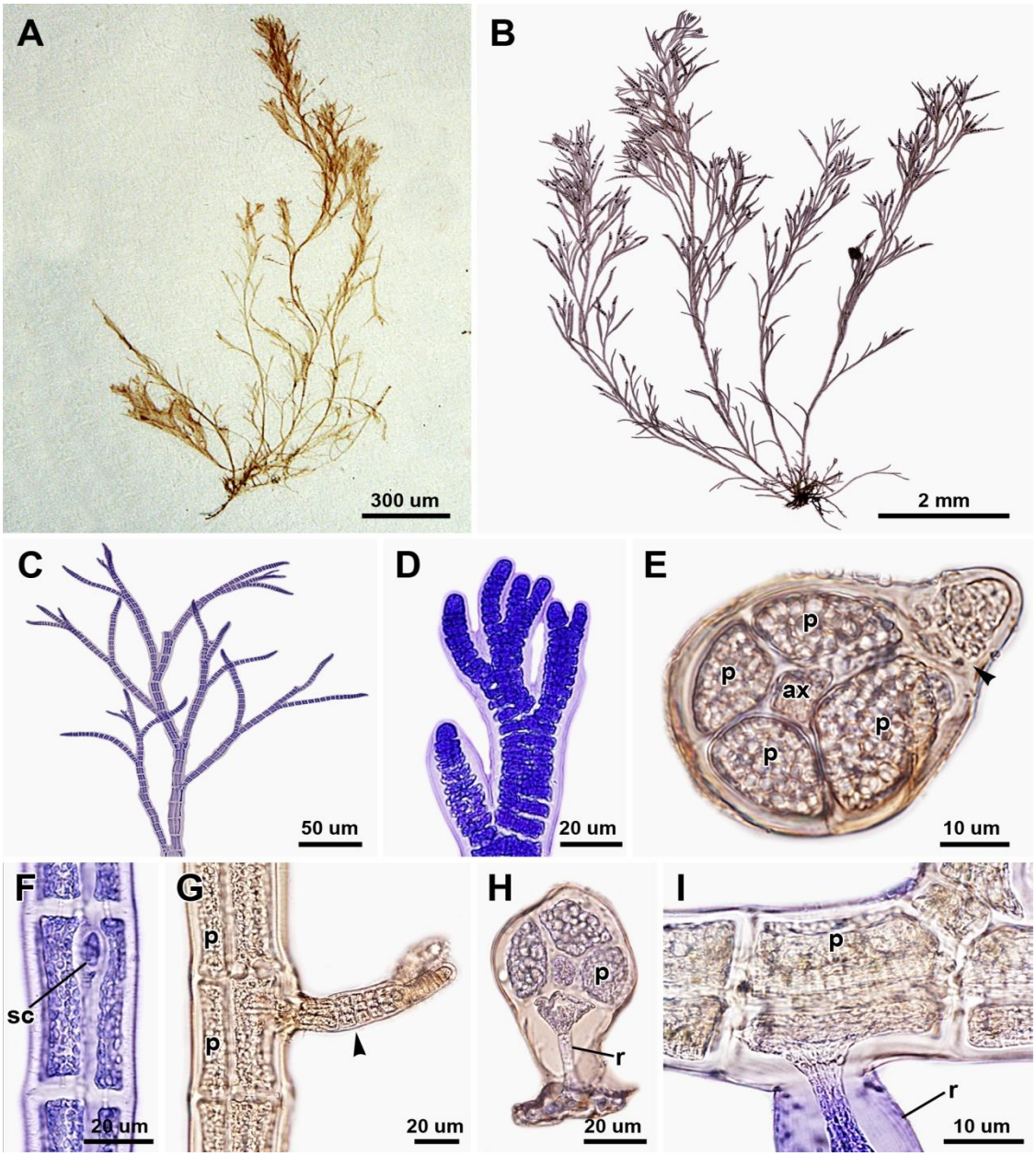


**Fig. 107.** Vegetative structures of *Polysiphonia koreana*. (A) Holotype specimen from Jukdo, Ulleungdo, Korea. (B) Habit of vegetative plant showing the reduced prostrate system and extended erect axes. (C) Apex showing subdichotomous to alternate branching pattern. (D) Apices showing transversely (arrowhead) divided apical cells without trichoblasts. (E) Cross-section of axis with initial endogenous branch (arrow) (ax, axial cell; p, pericentral cell). (F) Erect axis showing prominent scar cells (sc) placed between two pericentral cells. (G) Erect axis showing cicatrigenous branch (arrowhead). (H) Unicellular rhizoid terminated in multilobed tip. (I) Rhizoid (r) in open connection with the proximal end of the pericentral cell (p).

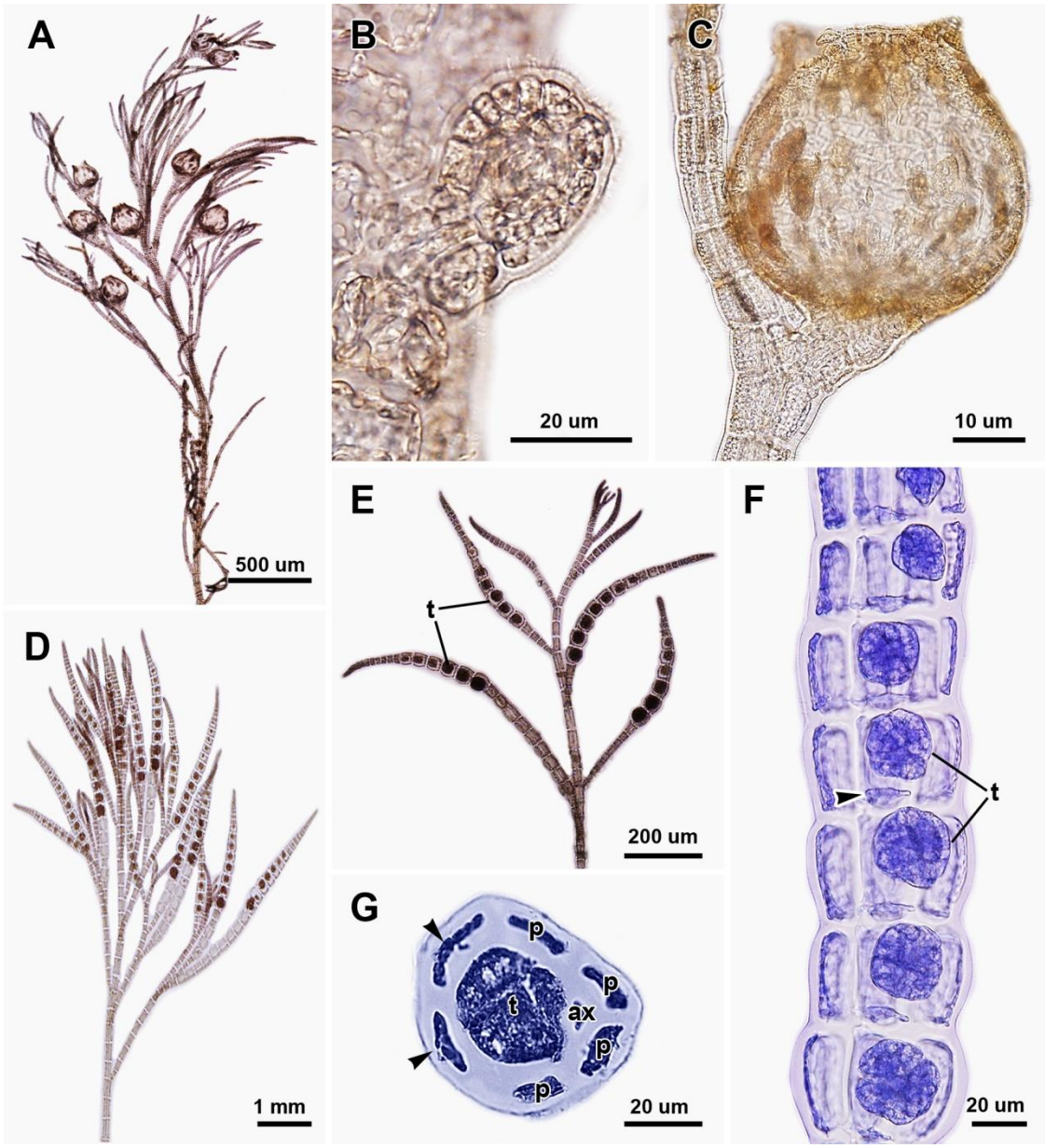


**Fig. 108.** Reproductive structures of *Polysiphonia koreana*. (A) Female gametophyte. (B) Young cystocarp. (C) Mature cystocarp showing slightly urceolate shape. (D) Tetrasporangial plant. (E) Apical branches showing straight arrangement of tetrasporangia (t). (F) Apical axis showing tetrasporangia (t) and stalk cells (arrowhead). (G) Cross-section showing a tetrasporangium (t) rounded by cover cells (arrowheads) (p, pericentral cells).

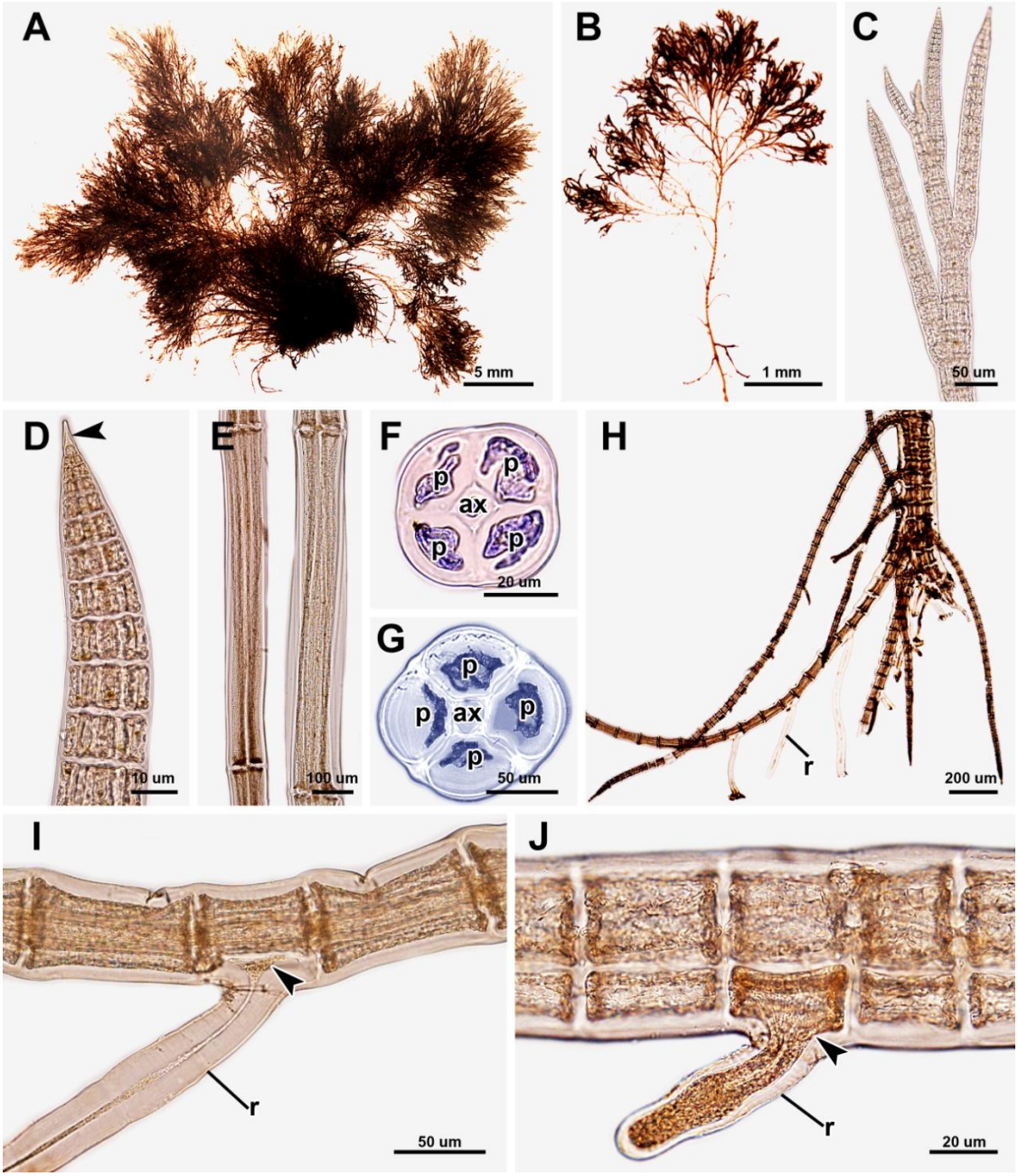


**Fig. 109.** Vegetative structures of *Polysiphonia morrowii*. (A) Habit of vegetative plant showing the extended prostrate system and erect axes. (B-C) Apex showing irregularly alternate branching pattern. (D) Apices showing transversely divided and sharp pointed (arrowhead) apical cells. (E) Erect axes showing very long pericentral cells. (F-G) Cross-section views of erect and prostrate axes (ax, axial cell; p, pericentral cell). (H) Rhizoids (r) scattered and produced from prostrate axes. (I-J) Unicellular rhizoids (r) in open connection with the proximal end of the pericentral cells.

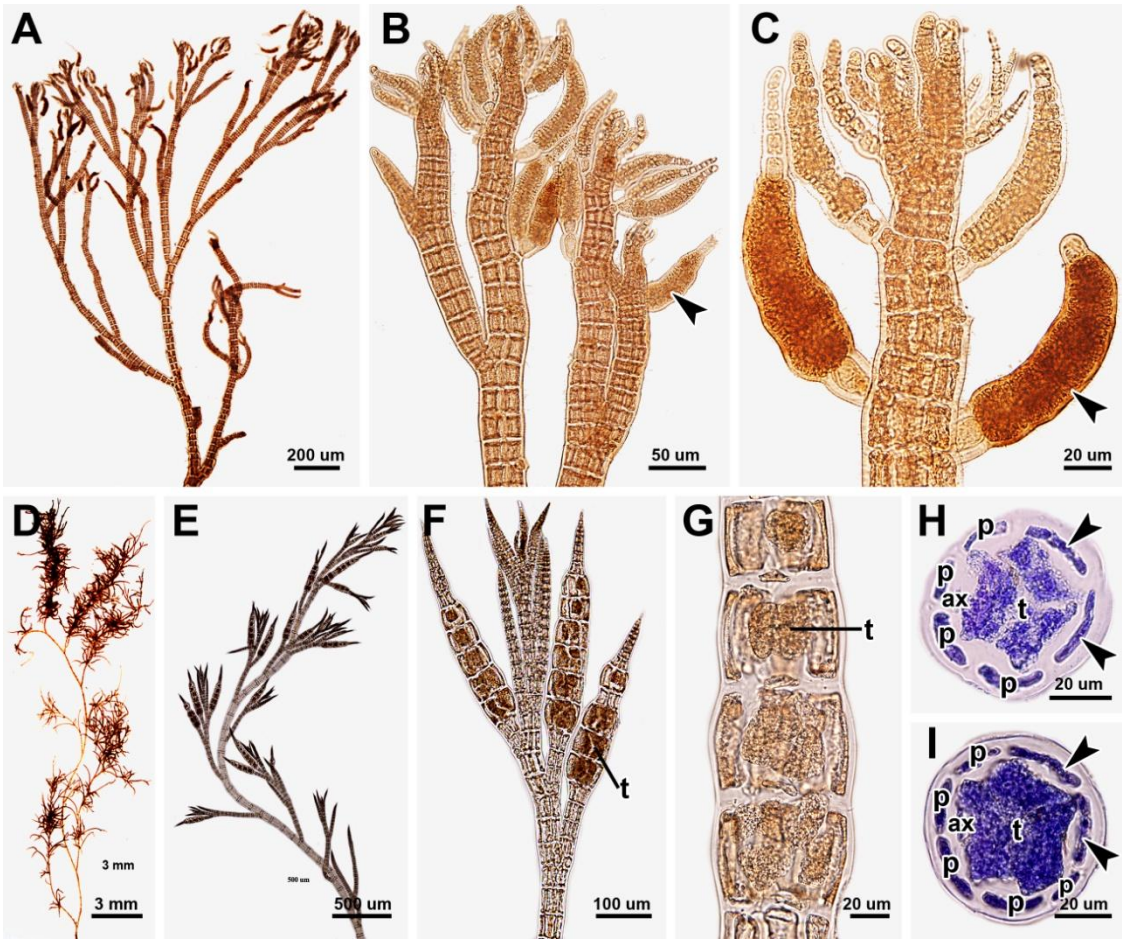


**Fig. 110.** Reproductive structures of *Polysiphonia morrowii*. (A) Male plant. (B) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (C) Spermatangial branches (arrowhead) replacing trichoblasts. (D-E) Tetrasporangial plant. (F-G) Apical branches showing straight arrangement of tetrasporangia (t). (H-I) Cross-section views with five (H) and six (I) pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

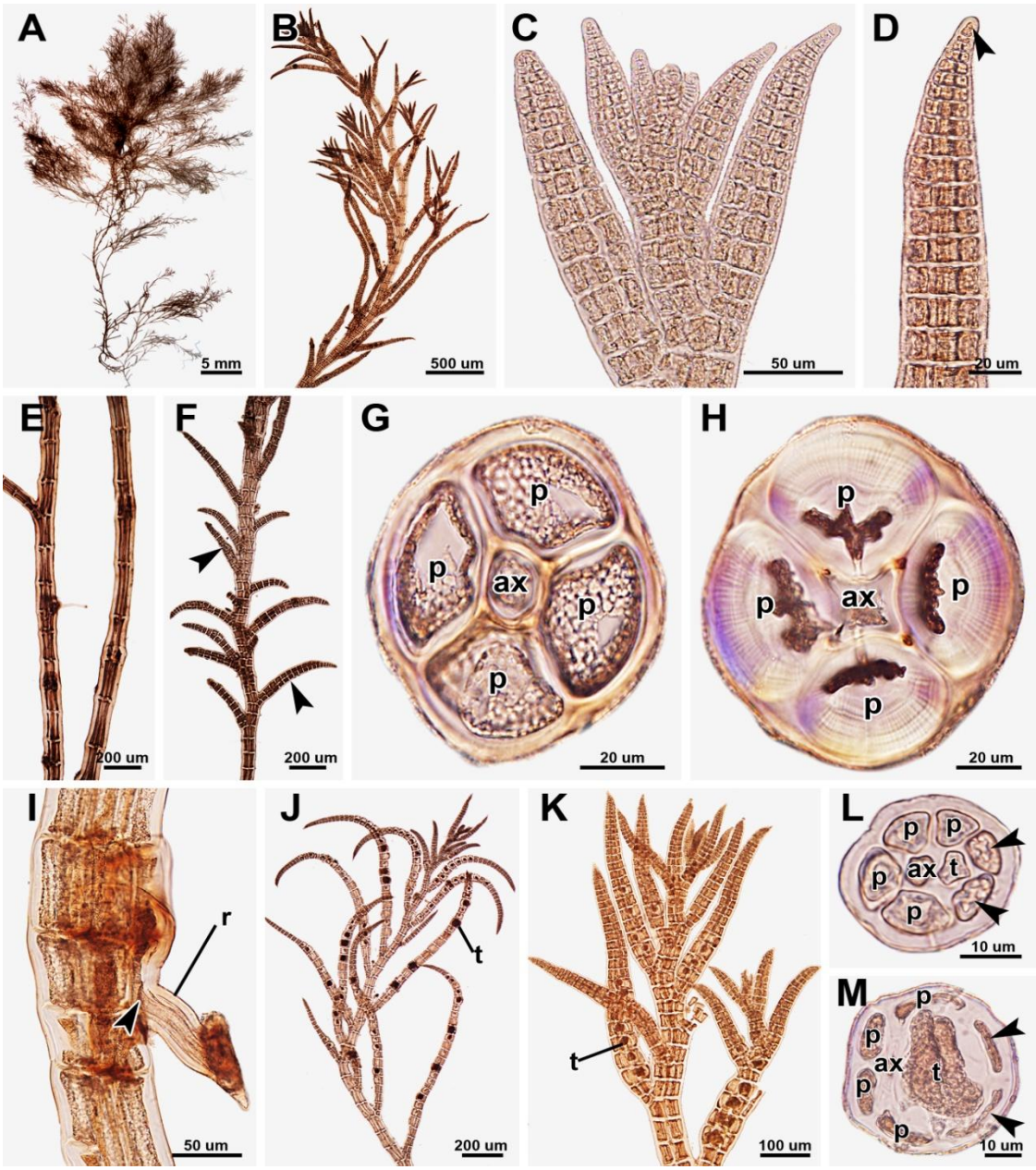


**Fig. 111.** Vegetative and reproductive structures of *Polysiphonia pacifica***.** (A) Habit of vegetative plant showing the extended prostrate system and erect axes. (B-C) Apex showing alternate branching pattern. (D) Apices showing transversely divided and dome shaped (arrowhead) apical cells. (E) Erect axes showing long pericentral cells. (F) Axes showing recurved adventitious branches (arrowheads). (G-H) Cross-section views of erect and prostrate axes (ax, axial cell; p, pericentral cell). (I) Rhizoid (r) in open connection (arrowhead) with pericentral cell. (J) Tetrasporangial plant. (K) Apical branches showing straight arrangement of tetrasporangia (t). (L-M) Cross-section views with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

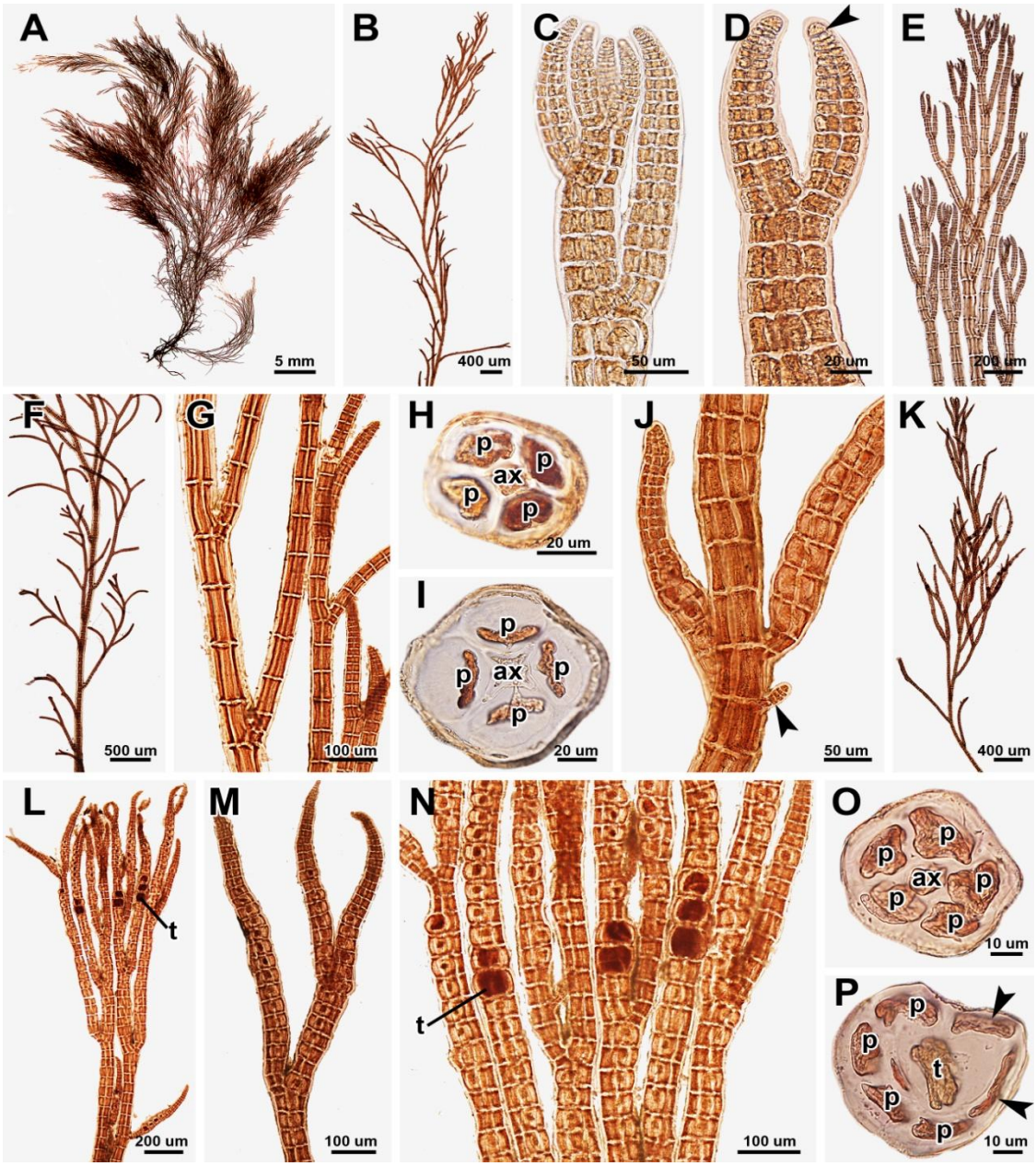


**Fig. 112.** Vegetative and reproductive structures of *Polysiphonia stricta*. (A) Habit of vegetative plant showing the extended erect axes. (B-E) Apex showing alternate (B-C) to pseudodichotomous (D) branching pattern and corymbose at the tips (E). (F) Axes showing basal recurved branches (arrowheads). (G) Erect axes showing long pericentral cells. (H-I) Cross-section views of erect and prostrate axes (ax, axial cell; p, pericentral cell). (J) Axes showing endogenous branches. (K) Tetrasporangial plant. (L-N) Apical branches showing straight arrangement of tetrasporangia (t). (O-P) Cross-section views with five pericentral cells (p) (O) showing a tetrasporangium (t) rounded by cover cells (arrowheads) (P).

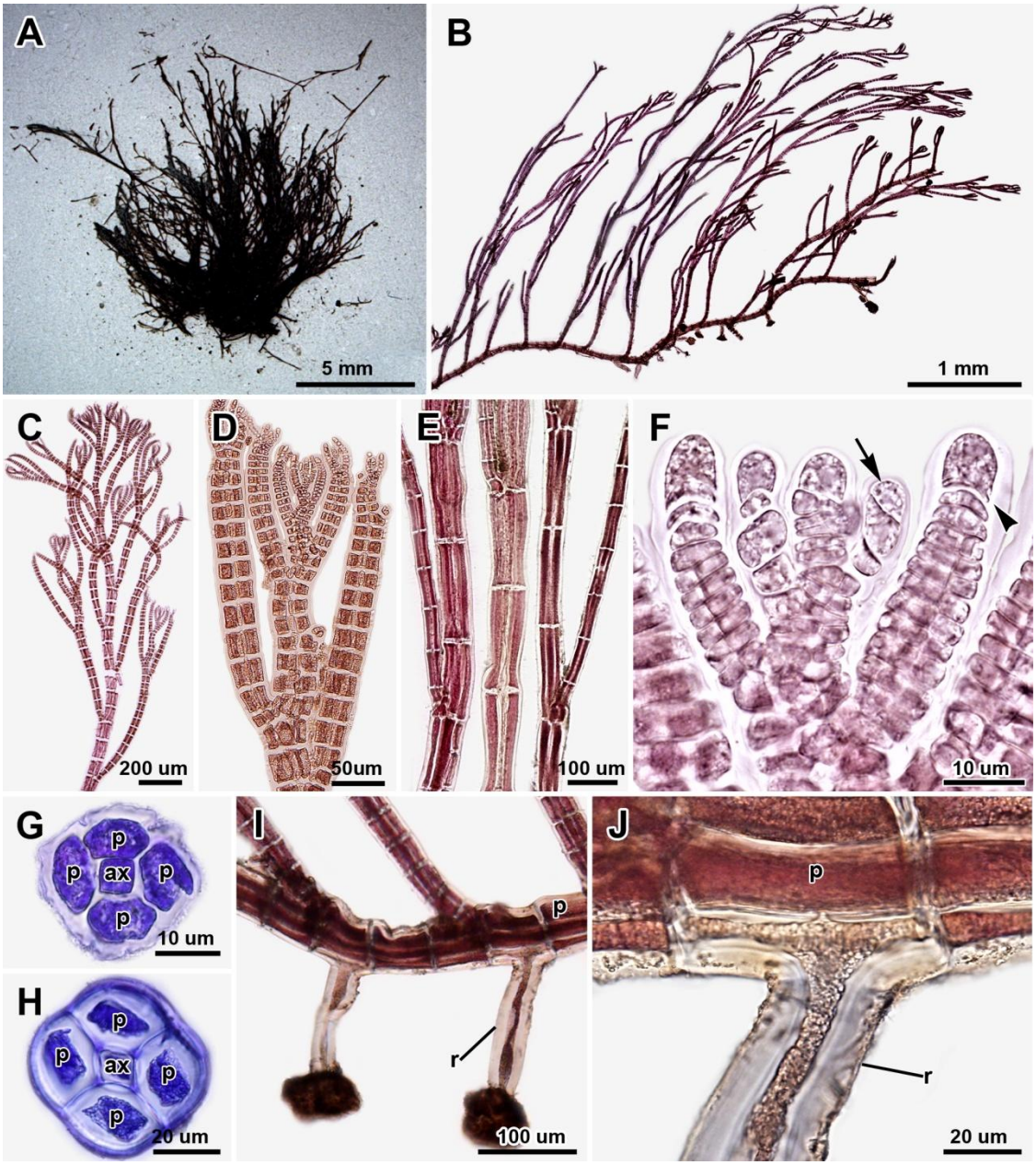


**Fig. 113.** Vegetative structures of *P. ulleungensis* sp. nov. (A) Holotype specimen from Sado, Dokdo-ri, Ulleung Island, Korea. (B) Habit of vegetative plant showing the extended prostrate axes and regularly branched erect axes. (C) Thallus with an alternate branching pattern. (D) Young erect axes showing short segments with alternate laterals and without trichoblasts . (E) Lower part of erect axes showing long segments without scar cells. (F) Apex showing obliquely (arrow) and transversely (arrowhead) divided apical cells. (G–H) Cross section views of erect axes (G) and prostrate axes (H) (ax: axial cell, p: pericentral cell). (I–J) Rhizoids (r) showing open connection to pericentral cells (p).

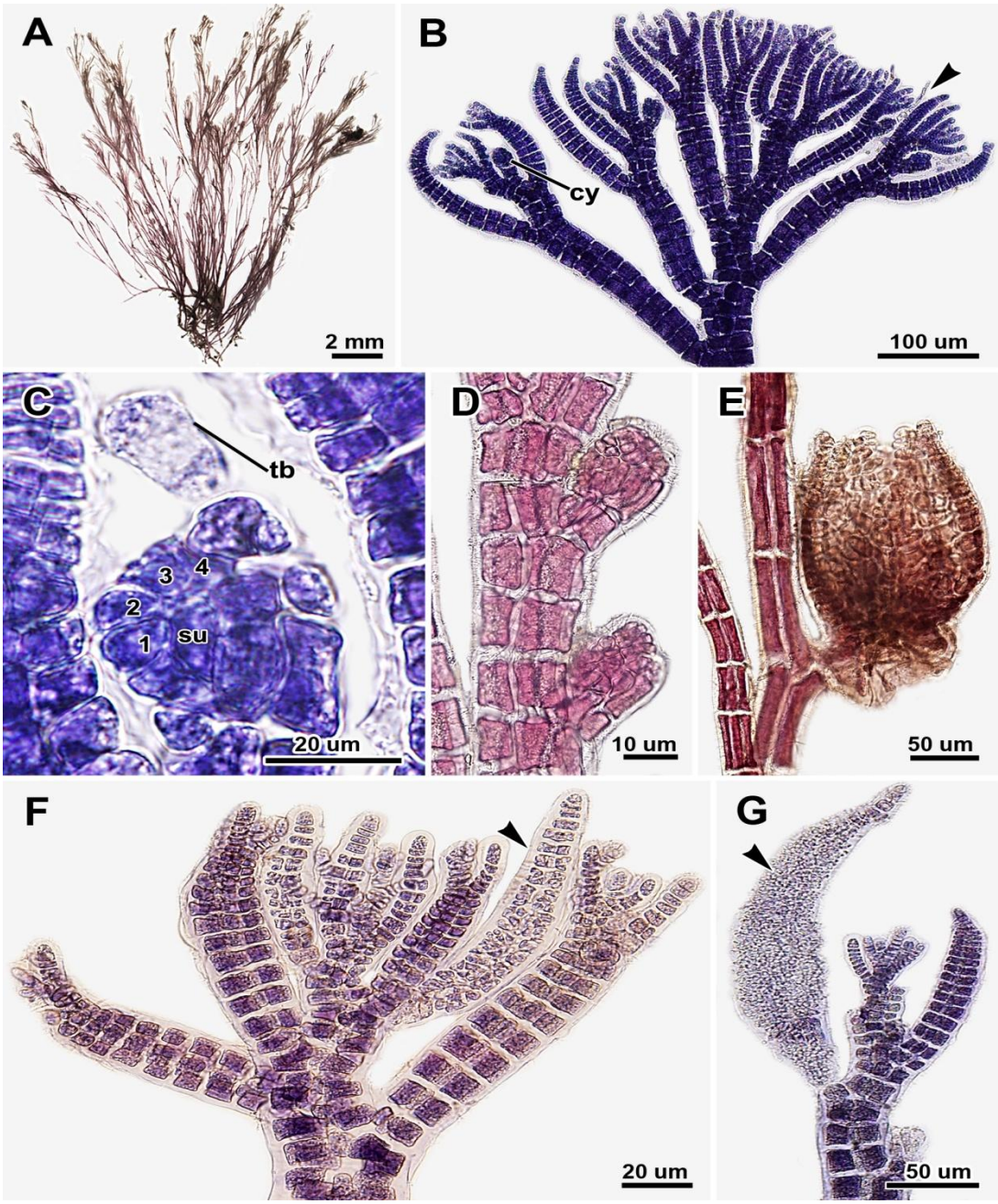


**Fig. 114.** Reproductive structures of *P. ulleungensis* sp. nov. (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps (cy) in densely branched axes with scarce trichoblast (arrowhead). (C) Procarp with a four-celled carpogonial branch (1-4: sequence of carpogonial branch cells, su: supporting cell). (D) Young cystocarps. (E) Mature cystocarp showing slightly urceolate shape. (F) Young spermatangial branch (arrowhead) replacing trichoblast. (G) Mature spermatangial branch having spermatangia.

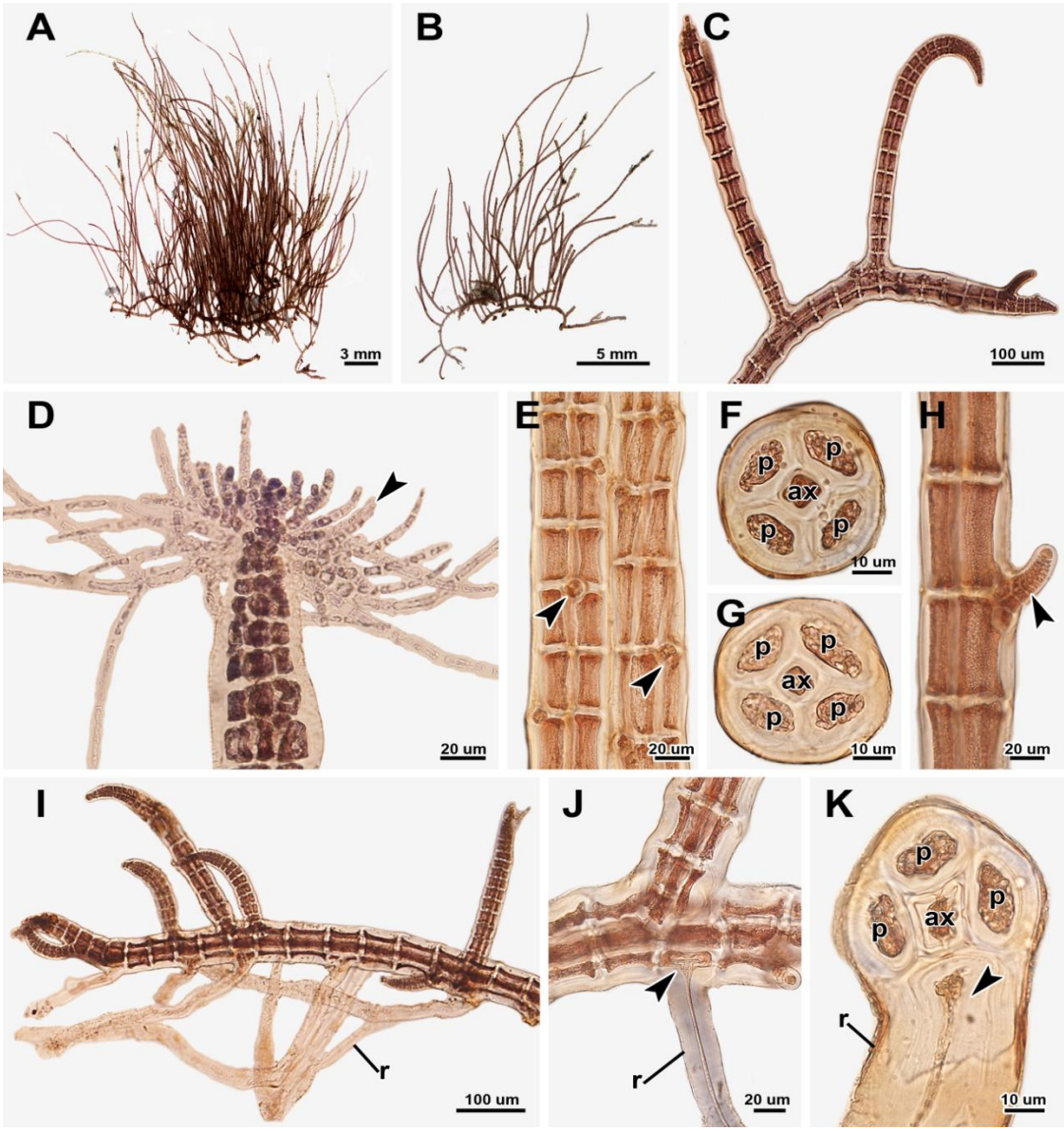


**Fig. 115.** Vegetative structures of *"Polysiphonia scopulorum"*. (A-B) Habit of vegetative plant showing the extended prostrate and erect axes. (C) Young erect axes recurved to prostrate axes. (D) Apices showing abundant and long trichoblasts (arrowhead). (E) Scar cells (arrowhead) spirally disposed in erect axes. (F-G) Cross-sections showing four pericentral cells (p) of erect (F) and prostrate (G) axes (ax, axial cell). (H) Adventitious branch (arrowhead) on basal erect axes. (I) Long rhizoids (r) scattered and produced from prostrate axes. (J) Rhizoid (r) showing open connection from the centre of the pericentral cell (p). (K) Cross-section of a prostrate axis showing rhizoid (r) in open connection with pericentral cells (p).

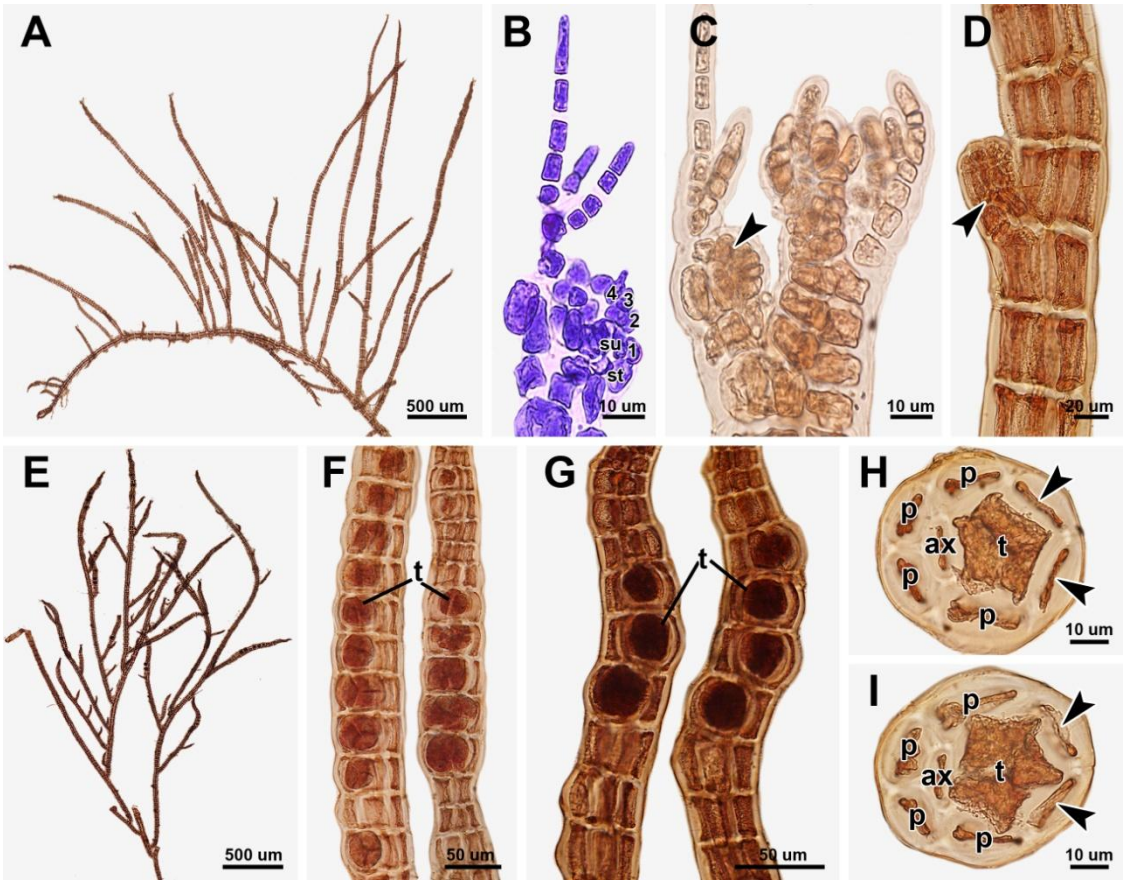


**Fig. 116.** Reproductive structures of *"Polysiphonia scopulorum"*. (A) Female gametophyte. (B) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (C) Upper part of female thallus showing subapical cystocarps. (D). Young cystocarp. (E) Tetrasporangial plant. (F) Apical branches showing straight arrangement of tetrasporangia (t). (G) Apical branch showing slightly spiral appearance of tetrasporangia (t). (H-I) Cross-sections with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

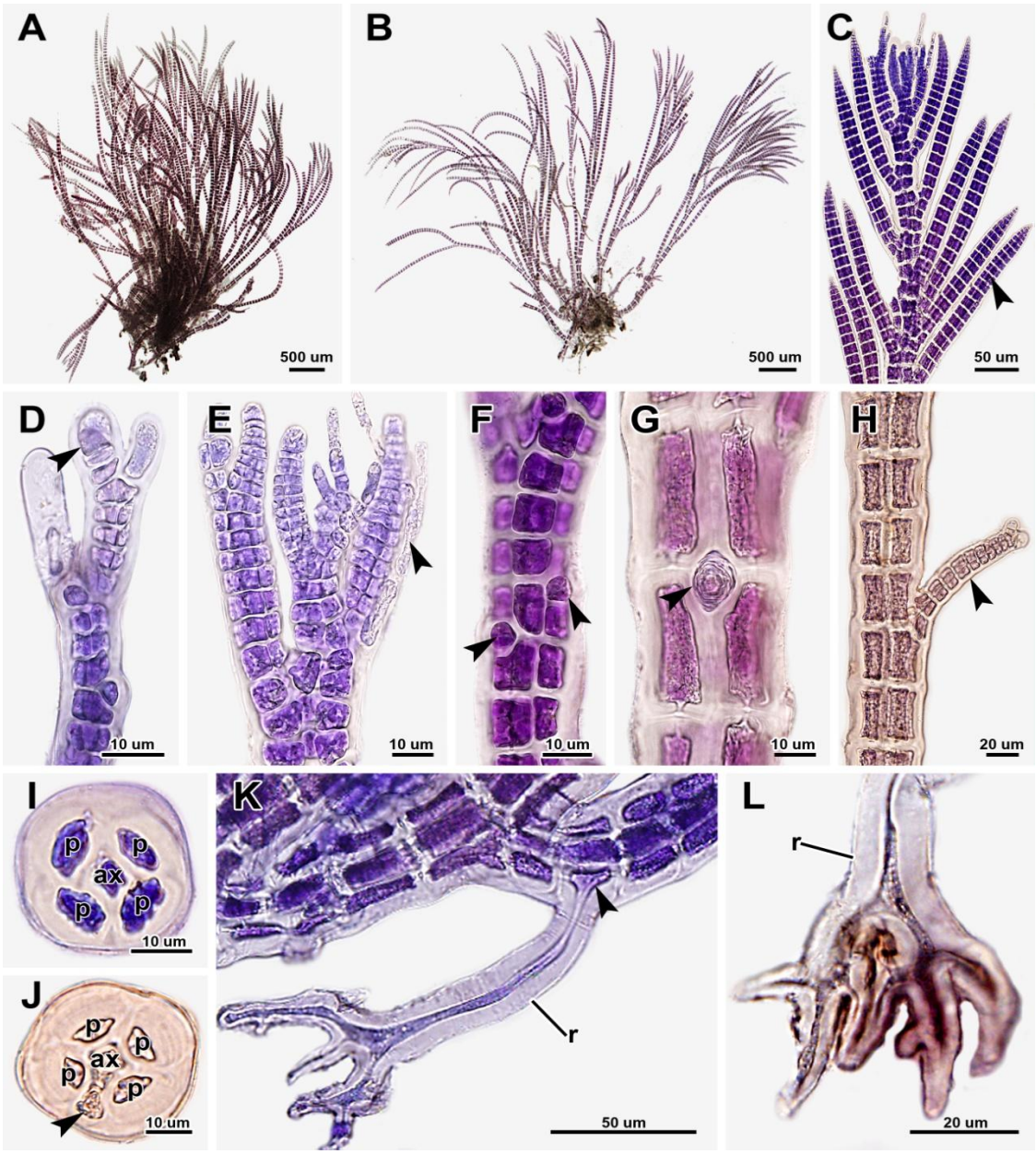


**Fig. 117.** Vegetative structures of *"Polysiphonia peruana* sp. nov.". (A-B) Habit of vegetative plant showing the reduced prostrate and extended erect axes. (C) Apex showing perfect alternate branching pattern by determinate branches. (D) Apices showing prominent apical cells (arrowhead). (E) Apex showing short trichoblasts (arrowhead). (F) Scar cells (arrowhead) spirally disposed in erect axes. (G) Erect axis showing a hollow (arrowhead). (H) Erect axis showing cicatrigenous branch (arrow). (I-J) Cross-sections showing four pericentral cells (p) of erect (I) and prostrate (J) axes (ax, axial cell). (K) Rhizoid (r) showing open connection from the centre of the pericentral cell (p). (L) Unicellular terminations of rhizoid (r).

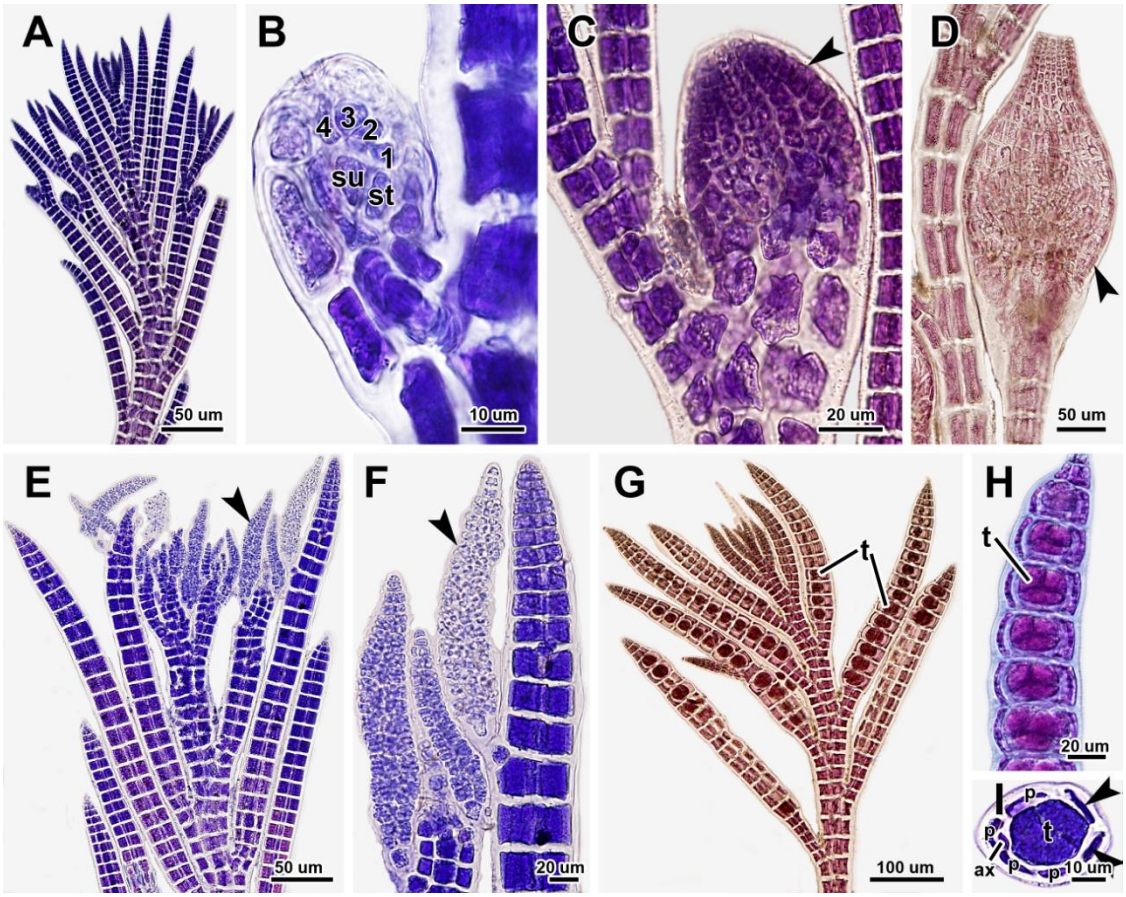


**Fig. 118.** Reproductive structures of *"Polysiphonia peruana* sp. nov.*"*. (A) Upper part of female thallus showing subapical cystocarps. (B) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (C-D). Young (C) and mature (D) cystocarps showing an elongate shape. (E) Spermatangial branches (arrowhead) clustered at the apices of erect. (F) Spermatangial branches (arrowhead) replacing trichoblasts. (G-H) Apical branches showing straight arrangement of tetrasporangia (t). (I) Cross-section with five pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

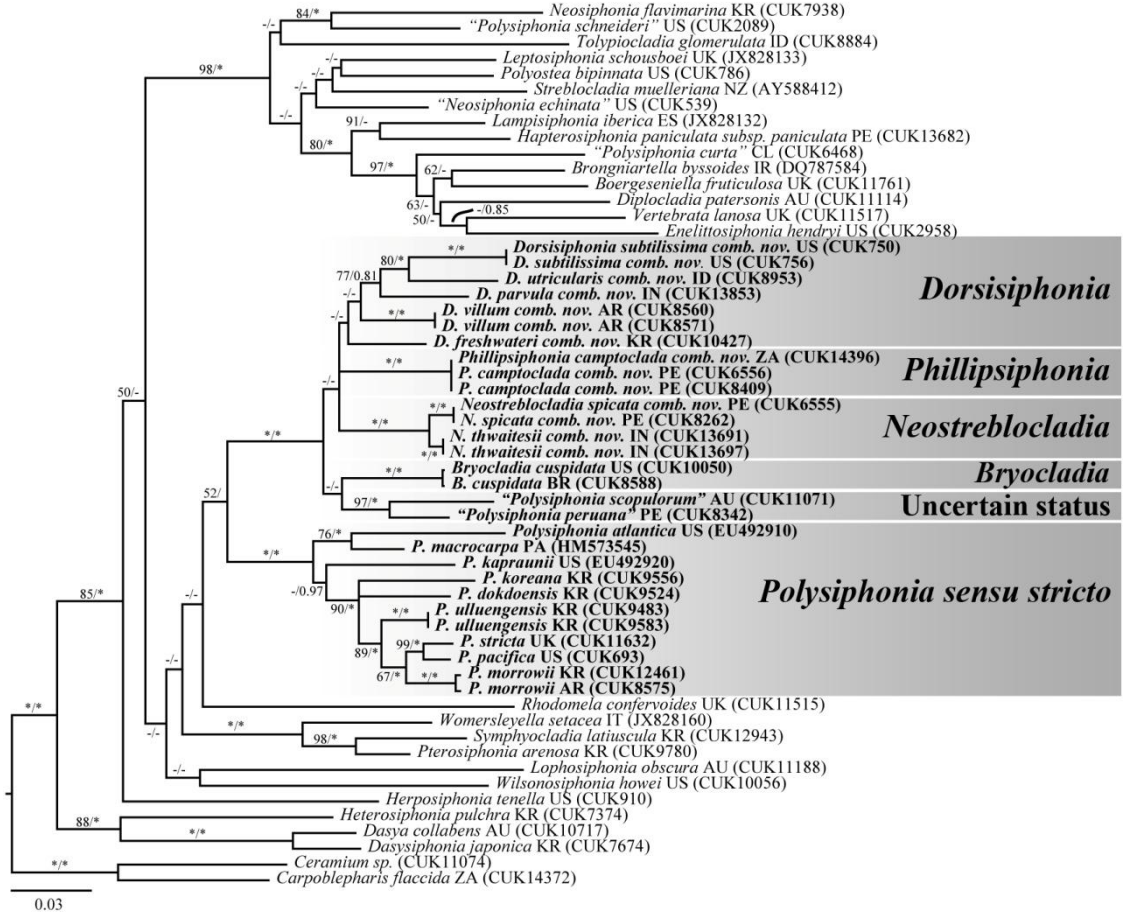


**Fig. 119.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

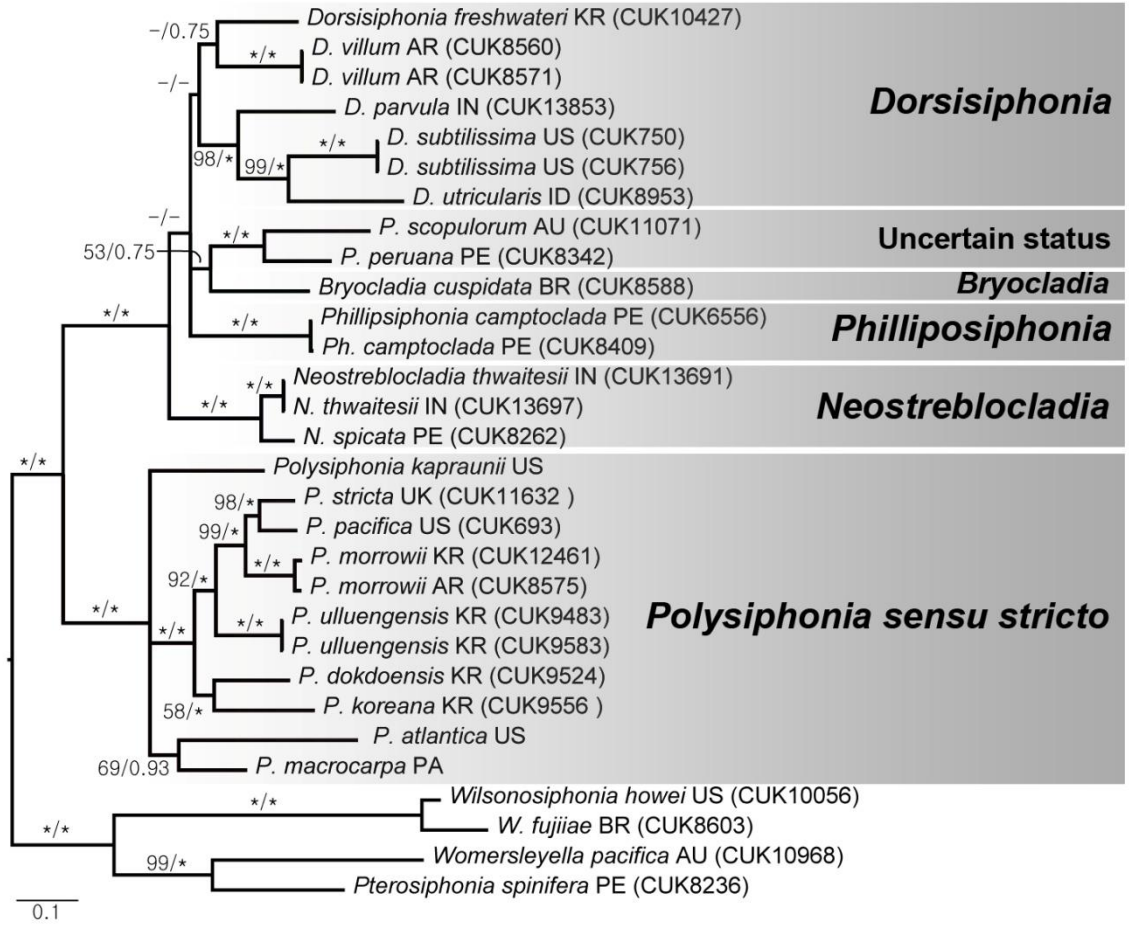


**Fig. 120.** Phylogenetic tree based on Bayesian analyses of the concatenated *rbc*L and *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 7.** Comparisons among the genera embedded in *Polysiphonia* sensu stricto.

| **Features** | ***Bryocladia*** | ***Dorsisiphonia* gen. nov.** | ***Neostreblocladia* gen. nov.** | ***Phillipsiphonia* gen. nov.** | ***Polysiphonia* sensu stricto** |
|---|---|---|---|---|---|
| Pericentral cells number | 6-12 | 4 | 6-13 | 4 | 4 |
| Trichoblasts | Scarce | Scarce to abundant | Absent | Absent | Scarce to abundant |
| Branching pattern | Spiral determinate branches | Strictly dorsiventral | Ramy-sympodial | Ramy-sympodial | Radial |
| Adventitious branches | On determinate branches | Simple | Simple | Simple | Simple |
| References | Schmitz and Falkenberg (1897), this study | This study | This study | This study | Kim et al. (2000), this study |

## CHAPTER 6. Taxonomy and phylogeny of the genus *Tolypiocladia* (Rhodomelaceae, Rhodophyta)

The genus *Tolypiocladia* Schmitz was segregated by Schmitz and Falkenberg (1897) on the basis of *Tolypiocladia glomerulata* (C.Argardh) F .Schmitz. The genus *Tolypiocladia* is characterized by having monopodial plants, indeterminate axes clothed with many exogenous determinate branchlets, spine-like branches on the distal end of exogenous determinate branches, unbranched trichoblasts, four pericentral cells, spermatangial branches replacing trichoblasts, four-celled carpogonial branches, and one tetrasporangium per fertile segment. *Tolypiocladia* is composed of four species *Tolypiocladia calodictyon* (Harvey ex Kützing) P.C.Silva from Tonga, *T. condensata* (Weber-van Bosse) P.C.Silva from Sansibar, *T. glomerulata* from western Australia, and *T. penningtonensis* Womersley from south Australia. They are distributed along the subtropical to tropical areas in western Pacific and Indian Oceans (Silva et al. 1996, Guiry and Guiry 2015).

The genus *Tolypiocladia* was established into the tribe Polysiphoniae (Schmitz and Falkenberg 1897) and it was described with a close relationship in morphology to *Bryocladia* F.Schmitz by having determinate branches spirally disposed along the main axes (Schmitz and Falkenberg 1897, Falkenberg 1901). Although species of *Tolypiocladia* have been widely reported along the subtropical to tropical areas in western Pacific and Indian Oceans (Silva et al. 1996, Coppejans and Millar 2000, Womersley 2003, Skelton and South 2007, Nunes et al. 2014), the phylogenetic relationships of this genus among the members of the tribe *Polysiphoniae* have not been confirmed yet.

Samples of the generitype *Tolypiocladia*, namely, *T. glomerulata* and also *T. calodictyon* have been collected in the Indian Ocean (Indonesia and India), we characterize these samples morphologically and molecularly, and examine their phylogenetic relationships among some members of the tribe Polysiphoniae by analyses of *rbc*L sequences.

## 1. Morphological analyses

***Tolypiocladia* F.Schmitz**

**Type species:** *Tolypiocladia glomerulata* (C. Agardh) F.Schmitz.

**Keyto species of *Tolypiocladia***

1. Dwarf and robust plants, spongy consistency, apical cells lightly sharp shaped, and unbranched trichoblast deciduous ..... ***Tolypiocladia calodictyon***
1. Long and delicate plants, greasy consistency, apical cells dome shape, and unbranched trichoblasts persistent ..... ***Tolypiocladia glomerulata***

***Tolypiocladia calodictyon* (Harvey ex Kützing) P.C. Silva (Fig. 121-122)**

**Basionym:** *Polysiphonia calodictyon* Harvey ex Kützing

**Homotypic synonyms:** *Polysiphonia calodictyon* Harvey ex Kützing, *Roschera calodictyon* (Harvey ex Kützing) Weber-van Bosse.

**Heterotypic synonyms:** *Roschera africana* Sonder.

***Type locality:*** Tonga.

**Distribution**: Pacific Islands and Indian Ocean.

**Specimens examined:** CUK13800, (Mandapam beach, Vedalai, Ramanathapuram, Tamil Nadu, India, collected by T.O.C. and D.E.B., Feb. 09 2015), CUK13907 (Kancharnkuti, Keelarai, Tamil Nadu, India collected by T.O.C. and D.E.B., Feb. 11 2015).

**Vegetative morphology:** Plants are 1.1-3.7 cm high (Fig. 121A-C), brownish to yellowish in color, associated with other filamentous species. Plants form very entangled and robust tufts that are pre-

dominantly attached on rock and solid surfaces or epiphyte on brown seaweeds from the intertidal. Thalli are monopodial and composed of interwoven and indeterminate extended erect axes (Fig. 121A) clothed with abundant determinate branchlets (Fig. 121B-C). These determinate branchlets arise exogenously from the erect axes radially disposed at intervals of 1 axial cell (Fig. 121D-E). The segments of determinate branchlets are 47.65 ± 9.72 μm in length and 121.79 ± 14.62 μm in diameter (L:D 0.39 ± 0.05) (Fig. 121B-D). The distal ends of the exogenous determinate branchlets is forming a cluster of spine-like branches after divided 1-3 times (Fig. 121D-E). Older segments of erect axes are larger, normally 89.36 ± 28.43 μm in length and 136.32 ± 30.45 μm in diameter (L:D 0.74 ± 0.46), and lightly unbranched (Fig. 121G). Apical cells are prominent, lightly sharp shaped, 6.22 ± 0.67 μm × 6.94 ± 0.55 μm in size, and transversely divided (Fig. 121F). Trichoblasts are scarce, short, delicate, deciduous, unbranched, 185.17 ± 61.96 μm in length, and arising on each segment near the apical cells of spine-like branches. Each segment is completely ecorticated along the thallus and composed of 4 pericentral cells (Fig. 121G-I). Adventitious branches are absent. Lateral branches replace trichoblasts. Rhizoids are scattered from cluster of spine-like branches and produced from the distal end of the pericentral cells (Fig. 121J). Rhizoids are in open connection with pericentral cells (Fig. 121K), 241.87 ± 82.60 μm in length, and 35.64 ± 10.99 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 121L).

**Reproductive morphology:** In tetrasporangial plants (Fig. 122A), tetrasporangia are tetrahedral and 35.79 ± 3.96 μm × 33.33 ± 3.77 μm in size. Tetrasporangial branches are swollen, spine-like shaped, determinate and composed of 1-3 tetrasporangia (Fig. 122B-C). The development of tetrasporangia follows a spiral arrangement (Fig. 122C). Fertile segments have five pericentral cells (Fig. 122D). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the three cover cells (Fig. 122D). A single tetrasporangium is produced on each fertile segment (Fig. 122D).

**Habitat:** Plants grow forming small tufts of spongy consistency from the intertidal zone. They are found attached on rock, solid surfaces or epiphyte on brown seaweeds as *Padina sp.* in sheltered to

wave-exposed areas. Tufts are usually robust and associated with other filamentous species as *Womersleyella sp.* and *Spyridia sp.*

**Remarks:** *Tolypiocladia calodictyon* has been described originally as *Polysiphonia calodictyon* Harvey in Kützing (1864) from Tonga in the western Pacific Ocean. *Tolypiocladia calodictyon* has been widely reported from the coast of the Indic Ocean and we reported this species from the coast of India for the first time. The spongy consistency, densely branched in the distal part of determinate branches, and deciduous trichoblasts in *T. calodictyon* distinguished this species from other members in *Tolypiocladia*.

***Tolypiocladia glomerulata* (C. Agardh) F. Schmitz (Fig. 123-124).**

**Basionym:** *Hutchinsia glomerulata* C. Agardh.

**Homotypic synonyms:** *Hutchinsia glomerulata* C. Agardh, *Polysiphonia glomerulata* (C. Agardh) Sprengel, *Vertebrata glomerulata* (C. Agardh) Kuntze, *Roschera glomerulata* (C. Agardh) Weber-van Bosse.

**Heterotypic synonyms:** *Sphacelaria cupressina* Harvey, *Polysiphonia calacantha* Harvey, *Polysiphonia inflata* G.Martens

**Type locality:** Shark Bay, Western Australia.

**Distribution**: Australia and Indic Ocean.

**Specimens examined:** CUK8884, (Donggala, Sulawesi, Indonesia,, collected by T.O.C. and D.E.B., Oct. 17 2012).

**Vegetative morphology:** Plants are 0.8-4.9 cm high (Fig. 123A-C), brownish to yellowish in color, associated with other filamentous species. Plants form very entangled and delicate tufts that are

predominantly attached on rock or epiphyte on succulent seaweeds from the intertidal. Thalli are monopodial (Fig. 123A) and composed of interwoven and indeterminate extended erect axes clothed with abundant determinate branchlets (Fig. 123B-C). These determinate branchlets arise exogenously from the erect axes radially disposed at intervals of 1 axial cell. The segments of determinate branchlets are 20.01 ± 2.77 μm in length and 57.81 ± 3.91 μm in diameter (L:D 0.35 ± 0.05). The distal ends of the exogenous determinate branchlets is forming a cluster of spine-like branches after divided 1-3 times (Fig. 123F-G). Older segments of erect axes are larger, normally 58.35 ± 3.15 μm in length and 87.49 ± 3.67 μm in diameter (L:D 0.67 ± 0.05), and lightly unbranched (Fig. 123H). Apical cells are prominent, dome shaped, 4.50 ± 0.79 μm × 4.47 ± 0.63 μm in size, and transversely divided (Fig. 123D). Trichoblasts are abundant, short, delicate, persistent, unbranched, 100.16 ± 37.49 μm in length, and arising on each segment near the apical cells of spine-like branches (Fig. 123E). Each segment is completely ecorticated along the thallus and composed of 4 pericentral cells (Fig. 123I-J). Adventitious branches are absent. Lateral branches replace trichoblasts. Rhizoids are scattered from cluster of spine-like branches (Fig. 123K) and produced from the distal end of the pericentral cells. Rhizoids are in open connection with pericentral cells (Fig. 123L), 136.87 ± 31.10 μm in length, and 10.04 ± 1.43 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 124A), tetrasporangia are tetrahedral and 44.61 ± 5.40 μm × 45.65 ± 4.93 μm in size. Tetrasporangial branches are swollen, spine-like shaped, determinate and composed of 1-3 tetrasporangia (Fig. 124B-C). The development of tetrasporangia follows a spiral arrangement (Fig. 124B-C). Fertile segments have five pericentral cells. The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the three cover cells. A single tetrasporangium is produced on each fertile segment.

**Habitat:** Plants grow forming small tufts of spongy consistency from the intertidal zone. They are found attached on rock, solid surfaces but predominantly epiphyte on succulent seaweeds as *Kappaphycus alvarezii* in sheltered, wave-exposed areas, and culture farms. Tufts are usually delicate

and associated with other filamentous species as *Neosiphonia echinata, Ceramium sp,* and *Antithamnion sp.*

**Remarks:** *Tolypiocladia glomerulata* has been described originally as *Hutchinsia glomerulata* C.Agardh by Agardh (1824) from Shark Bay, in the western coast of Australia. *Tolypiocladia glomerulata* has been widely reported from the coast of the Indic, Pacific and Atlantic Ocean (Silva et al. 1996, Lobban and Tsuda 2003, Atmadja and Prud'homme van Reine 2012, Nunes et al. 2014). Our study is the first reporting this species on the basis of molecular analyses of plastid-encoded *rbc*L. The greasy consistency, densely branched in the distal part of determinate branches, and persistent trichoblasts in *T. glomerulata* distinguished this species from other members in *Tolypiocladia*.

## 2. Phylogenetic analyses

A 1,394-bp portion of the 1,467- bp *rbc*L (95.0%) was sequenced for *Tolypiocladia calodictyon* and *Tolypiocladia glomerulata* and other *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus placed genus *Tolypiocladia* sister to the clade composed by *Hapterosiphonia, Lampisiphonia, Leptosiphonia,* multipericentral group, *Neosiphonia,* and "*Polysiphonia schneideri*" clade with sequences divergence ranging from 11.1% to 17.0% between them and supported by a bootstrap value of 98 and a posterior probability of 1 (Fig. 125). *Tolypiocladia.* The sequence divergences between *Tolypiocladia calodictyon* and *Tolypiocladia glomerulata* are 7.9% for *rbc*L.

### 3. Discussion

The tribe Polysiphoniae is characterized by having radial branches on all sides of axes, determinate and indeterminate branches, prominent apical cells, trichoblasts and laterals produced spirally from successive segments, dioecious gametophytes, procarps and spermatangial branches produced from trichoblasts, and single tetrasporangia per segment (Schmitz 1897, Falkenberg 1901, Womersley 2003). The genus *Tolypiocladia* have been established in the tribe Polysiphoniae by having determinate laterals branches spirally disposed throughout indeterminate branches and ecorticated axes (Womersley 2003). Our molecular analyses based on plastid-encoded *rbc*L confirmed the genus *Tolypiocladia* embedded in the tribe Polysiphoniae (Fig. 125). Although Schmitz and Falkenberg (1897) established *Tolypiocladia* in a close relationship to the genus *Bryocladia* by having determinate branches spirally disposed, our phylogenetic analyses revealed that *Tolypiocladia* is related to the clade composed by *Hapterosiphonia*, *Lampisiphonia*, *Leptosiphonia*, multipericentral group, and *Neosiphonia* (Fig. 125). Morphologically, all these groups are different to *Tolypiocladia* and there is no any consistent feature that justified the close phylogenetic relationship among *Tolypiocladia* and these genera. *Hapterosiphonia*, *Lampisiphonia*, *Leptosiphonia*, multipericentral group, and *Neosiphonia* are having rhizoids cutting off from pericentral cells as feature that cluster them, whereas the genus *Tolypiocladia* is having rhizoids open connected to pericentral cells. Open connection of rhizoids has been widely reported in the species of the paraphyletic *Polysiphonia* sensu stricto (see Chapter 5), where the members of genus *Bryocladia* are embedded. However, the distant relationship between *Tolypiocladia* and *Polysiphonia* sensu stricto (including *Bryocladia*) might suggest that open connection of rhizoids and pericentral cells is a convergent character that evolved separately in *Tolypiocladia* and *Polysiphonia* sensu stricto (Martone et al. 2009).

Morphologically, *Tolypiocladia* is similar to *Bryocladia* and *Diplocladia* in external appearance by having determinate branches spirally arranged along the main axes, but they are distinguished from *Tolypiocladia* by having more than four pericentral cells (Schmitz and Falkenberg 1897, Falkenberg 1901). *Bryocladia* is also distinguished from *Tolypiocladia* by having endogenous

branches developed from exogenous determinate branches (Schmitz and Falkenberg 1897, Womersley 2003), whereas *Diplocladia* is also different from *Tolypiocladia* by having close connection of rhizoids and pericentral cells (Womersley 2003). The distant relationship in our phylogenetic analyses among these similar genera might suggest that the determinate branches is also a convergent feature that evolved independently in these three genera (Martone et al. 2009).

The two species reported in the present study are *Tolypiocladia glomerulata* and *T. calodictyon* collected in the Indian Ocean (Indonesia and India). These species are distinguished each other by differences on the habit, texture, apical cells shape, and trichoblasts nature. *Tolypiocladia glomerulata* is having very long and delicate filaments, greasy consistency, apical cells dome shaped, and persistent trichoblasts; whereas, *T. calodictyon* is having dwarf and robust plants, spongy consistency, sharp apical cells, and deciduous trichoblasts. The results of our *rbc*L phylogenetic analyses revealed that *Tolypiocladia glomerulata* and *T. calodictyon* are closely related, with high support [99% for ML and 0.99 for Bayesian posterior probabilities (BPP)].

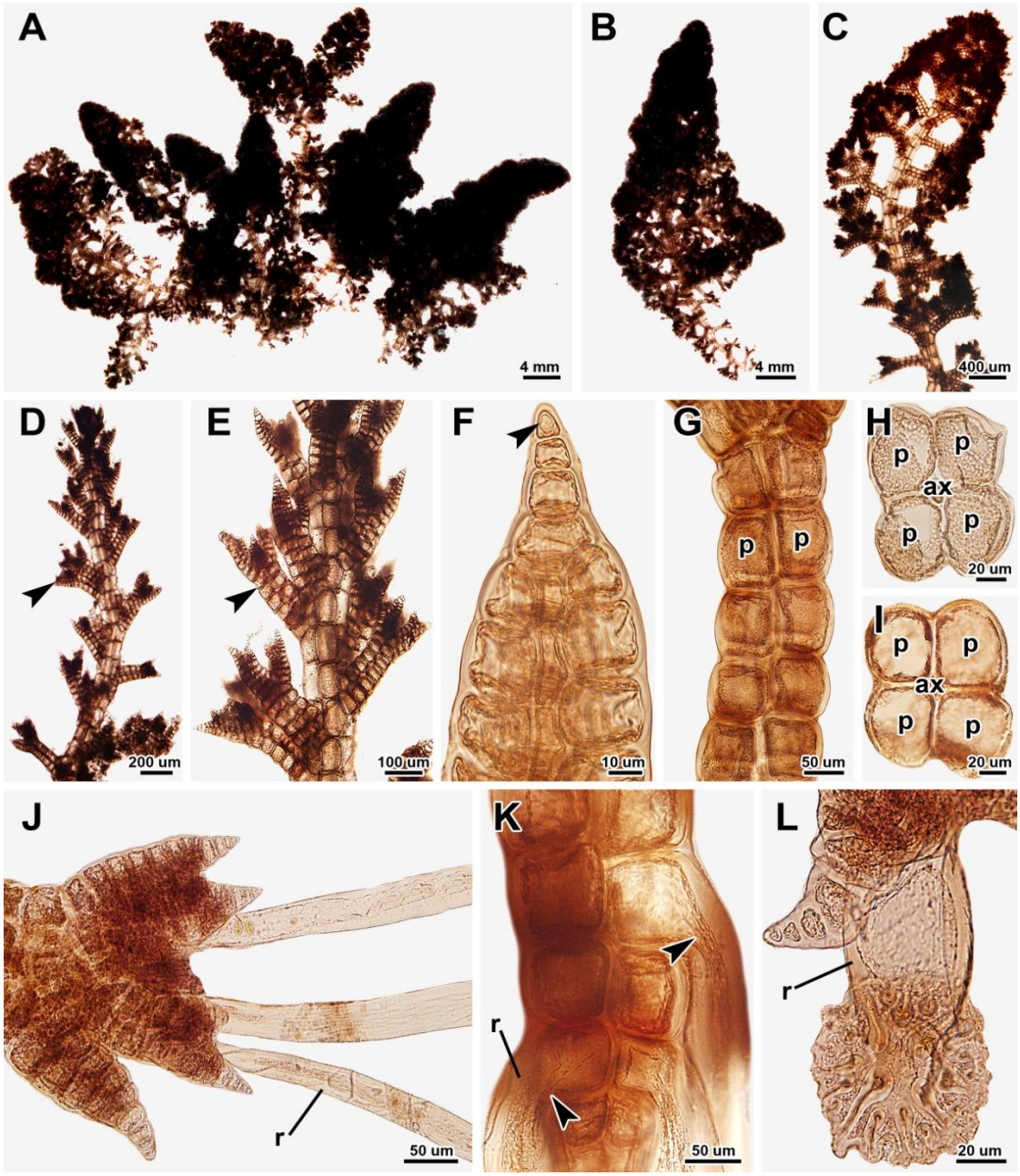


**Fig. 121.** Vegetative structures of *Tolypiocladia calodictyon.* (A) Habit of vegetative plant. (B-C) Erect axes showing spongy consistency and dense branchlets. (D-E) Axes showing exogenous branchlets divided into spine-like branches (arrowhead). (F) Apex showing prominent apical cells (arrowhead) transversally divided. (G) Old axes lacking of cortication and scar cells. (H-I) Cross-section views showing 4 pericentral cells (p) from the spine-like branches (H) and main axes (I) (ax, axial cell). (J) Rhizoids (r) scattered on the spine-like branches. (K) Rhizoids (r) produced in open connection (arrowhead) from pericentral cells (p). (L) Unicellular terminations of rhizoid (r).

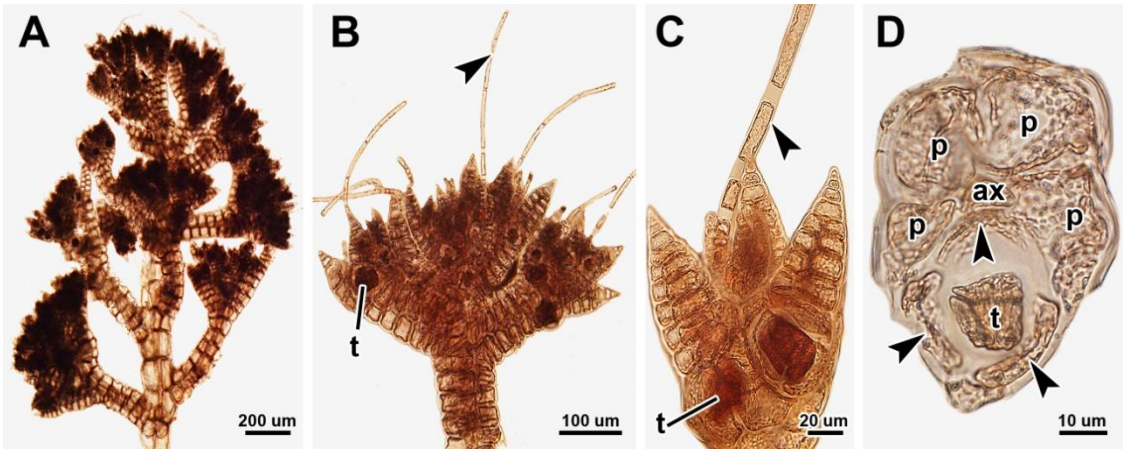


**Fig. 122.** Reproductive structures of *Tolypiocladia calodictyon*. (A) Tetrasporangial thallus. (B) Branchlets having tetrasporangial spine-like determinate branches showing swollen tetrasporangia (t). (C) Spine-like determinate branches showing one tetrasporangia (t) per segment and unbranched trichoblasts (arrowhead). (D) Cross-section with 5 pericentral cells (p) showing a tetrasporangium (t) rounded by three cover cells (arrowheads).

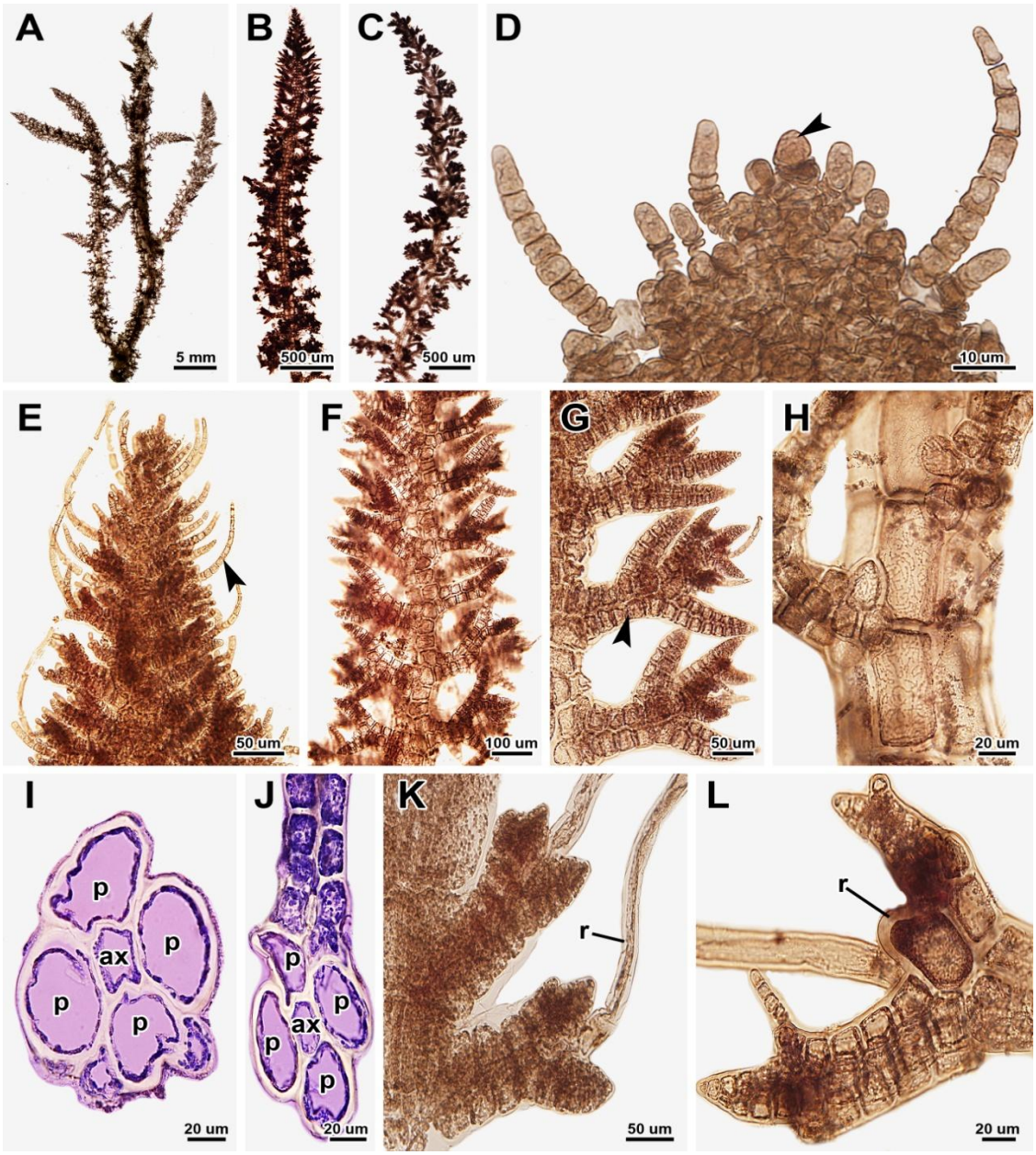


**Fig. 123.** Vegetative structures of *Tolypiocladia glomerulata.* (A) Habit of vegetative plant. (B-C) Erect axes showing dense branchlets. (D) Apex showing prominent apical cells (arrowhead) transversally divided. (E) Apex showing numerous unbranched trichoblasts (arrowhead). (F-G) Axes showing exogenous branchlets composed of determinate short spine-like branches (arrowhead). (H) Old axes lacking of cortication and scar cells. (I-J) Cross-section views showing 4 pericentral cells (p) from the spine-like branches (I) and main axes (J) (ax, axial cell). (K) Rhizoids (r) scattered on the spine-like branches. (L) Rhizoids (r) produced in open connection from pericentral cells (p).

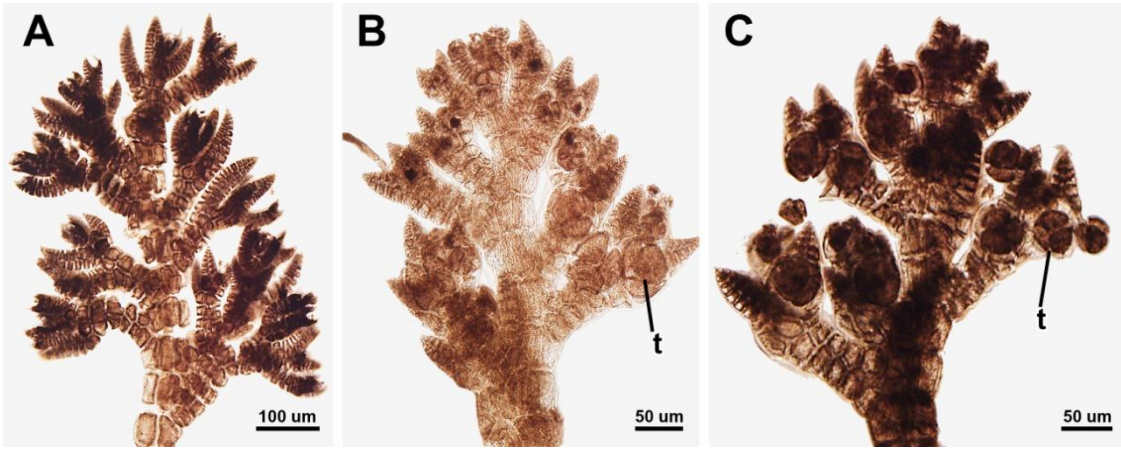


**Fig. 124.** Reproductive structures of *Tolypiocladia glomerulata*. (A) Tetrasporangial thallus. (B) Branchlets having tetrasporangial spine-like determinate branches showing swollen tetrasporangia (t). (C) Spine-like determinate branches showing one tetrasporangia (t) arranged in spiral series.

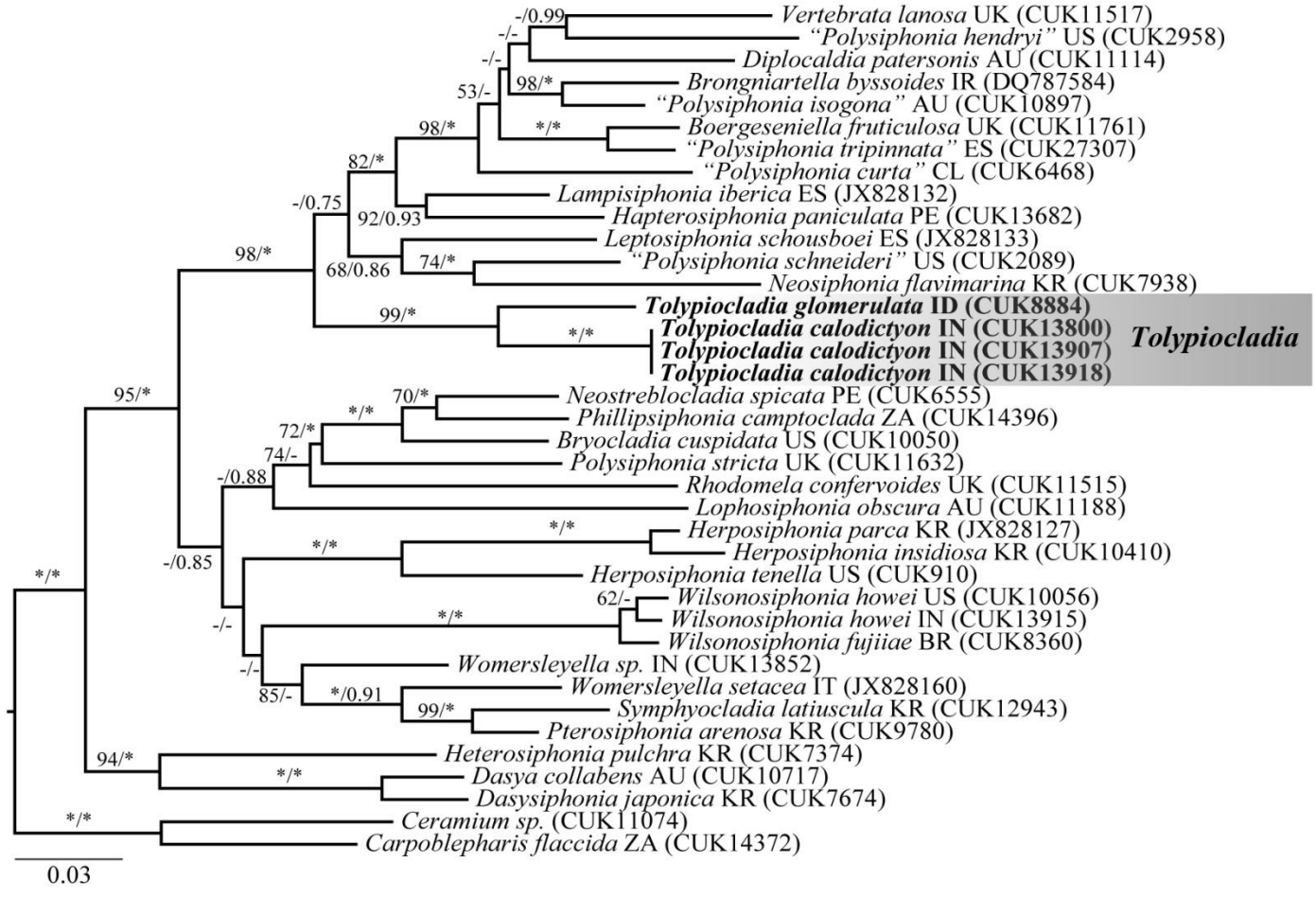


**Fig. 125.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

## CHAPTER 7. Taxonomy and phylogeny of the genus *Wilsonosiphonia* gen. nov. (Rhodomelaceae, Rhodophyta)

*Polysiphonia* sensu lato is composed of several heterogeneous genera (Bustamante et al. 2015a, 2015b), but only the following five main groups have been demonstrated molecularly: *Hapterosiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho, *Lampisiphonia* H.G. Choi, Diaz-Tapia et Bárbara, multipericentral group, *Neosiphonia* M.S. Kim et I.K. Lee, and *Polysiphonia* sensu stricto (Choi et al. 2001, Mamoozadeh and Freshwater 2011, Bárbara et al. 2013, Bustamante et al. 2015b).

*Polysiphonia howei* Hollenberg was described by Hollenberg in Taylor (1945) based on samples collected from Whale Cay, Berry Is., Bahamas (Western Atlantic). Additional collections from Tutuila Island (Samoa), Isla Taboga (Pacific Panama), and Bahía Cabita (Colombia) were also listed in the original description. *Polysiphonia howei* has also been reported based on morphology from the Pacific Islands (Taylor 1950), Japan (Segi 1951), Brazil (Joly 1957), Western Atlantic (Taylor 1960), Australia (Lewis 1984), and Indic ocean (Silva et al. 1996). *Polysiphonia howei* is currently a wide-distributed species and only Sherwood *et al.* (2010) and Mamoozadeh and Freshwater (2011) confirmed the Pacific and Western Atlantic reports on the basis of molecular studies with nuclear, mitochondrial, and plastidial markers. Although *Polysiphonia howei* shows some distinctive features to be considered in *Polysiphonia* sensu lato, the molecular analyses demonstrated that the taxonomical status of *Polysiphonia howei* in this group is still inconsistent (Mamoozadeh and Freshwater 2012).

The goals of the present study were to reassess specimens identified as *P. howei* collected from Western Atlantic (Florida), Southwestern Atlantic (Brazil) and Indic Ocean (India) based on anatomical observations and molecular analyses. We also provide evidence of their phylogenetic relationship with other polysiphonous species by analyses of *rbc*L and *cox*1 sequences. Here, we describe a new genus *Wilsonosiphonia* based on *W. fujiiae* sp. nov. and also propose the new combination *W. howei* composed of two subspecies *W. howei subsp. howei* and *W. howei subsp. indica.*

## 1. Morphological analyses

***Wilsonosiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho gen. nov.**

**Description:** Plants are minute forming extended turfs predominantly attached to rock surfaces. Thalli are composed of extensive and entangle creeping system and interwoven indeterminate prostrate and erect axes. Pericentral cells of 8-14 pericentral cells. Ecorticate axes are sparse to moderate branched in a dichotomous to subdichotomous pattern. Adventitious branches present. Lateral branches are replacing trichoblast. Trichoblasts are delicate, deciduous, numerous, several times forked, and long. Conspicuous scar cells. Rhizoids cut off the pericentral from the distal position of mature pericentral cells, with multicellular terminations in axonomorphus shape (taproot shape). Tetrasporangia are tetrahedral and disposed in spiral series. One or two tetrasporangia are produced from a single segment.

**Type species:** *Wilsonosiphonia fujiiae* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov.

**Etymology:** "*Wilsonosiphonia*" is in honor of Professor D. Wilson Freshwater, for his valuable contributions to the understanding of the systematics of *Polysiphonia* sensu lato from the Western Atlantic.

***Wilsonosiphonia fujiiae* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 127-128)**

**Diagnosis*:*** Thalli 1-3 cm tall, saxicolous, polysiphonous, composed of an extensive creeping system, with indeterminate prostrate and erect axes. Axes with 10-13 pericentral cells and ecorticate throughout. Erect axes with dichotomous to subdichotomous branches, which are exogenously and replacing the trichoblasts. Trichoblast numerous and several times forked. Scar cells conspicuous and placed among pericentral cells. Adventitious branches present. Rhizoids multicellular, cutting

off in the distal end of pericentral cells. Tetrasporangia arranged in spiral series with one or two per segment.

**Holotype*:*** CUK8603, collected 10 July 2012, deposited in the herbarium of Chosun University, Korea (CUK).

**Isotypes:** CUK8585, CUK 8589, CUK 8590, collected 10 July 2012, deposited in the herbarium of Chosun University, Korea (CUK).

**Type locality:** 23° 25' 11.47" S 45° 3' 38.86" W; intertidal, attached to rocks, Praia Domingas Dias, Ubatuba, São Paulo, Brazil.

**Etymology:** "*fujiiae*" is in honor of Professor Mutue Toyota Fujii, for his valuable contributions to the understanding of the systematics of Rhodomelaceae from Brazil.

**Vegetative structure:** Plants are minute, 1-3 cm in high (Fig. 127A), dark red to brown in color, and mainly associated to *Bostrychia*. Thalli are forming extended turfs predominantly attached to rock surfaces of intertidal zone. Thalli are composed of prostrate and erect system with robust texture (Fig. 127B-C). Prostate system is composed of extensive and entangled indeterminate axes. Segments of prostrate axes are 80.15 ± 17.08 μm in length and 82.88 ± 12.75 μm in diameter, being as broader as long (L:D 0.97 ± 0.12). The erect system is composed of interwoven indeterminate axes. Erect axes are robust and arisen exogenously from the prostrate axes at intervals of 2, 4 or 6 axial cells. Young erect axes are strongly curved toward the direction of the prostrate axes and have short segments. Older segments of erect axes are wide, 49.07 ± 3.38 μm long and 78.17 ± 3.84 μm in diameter, being 1.6 times broader than long (L:D 0.63 ± 0.05), shifted, and infrequently branched. Adventitious branches present. Lateral branches are replacing the whole trichoblast. Apical cells are prominent, 8.23 ± 1.58 μm × 10.10 ± 0.75 μm in size, and transversely divided (Fig. 127E). Axes are composed of 10-13 pericentral cells, ecorticate throughout (Fig. 127F-G), and sparse to moderate branched every 4 or 16-32 axial cells in dichotomous to subdichotomous

pattern (Fig. 127D). Trichoblasts are having a short basal cell, delicate, numerous, several times forked, and 385.54 ± 73.52 μm long, rarely over 543.4 μm and arise on each segment near the apical cells but persistent subapically (Figs 127H-J). Conspicuous scar cells appear along the filament after trichoblast have been shed, reaching 11.34 ± 1.69 μm × 11.39 ± 0.93 μm , and developed in a variable pattern series in the space between segments (Fig. 127I). Rhizoids are ventrally produced from the distal end of mature pericentral cells (Fig. 127J). They are cutting off the pericentral cells (Fig. 127K). Rhizoids are unicellular in younger stages but when mature they have multicellular terminations in axonomorphus shape (taproot shape) (Fig. 127L), and 18.50 ± 1.84 μm in diameter and 138.19 ± 50.52 μm long.

**Reproductive structure:** In tetrasporangial plants (Fig. 128A), tetrasporangia are tetrahedral and 38.22 ± 1.95 μm × 41.67 ± 3.58 μm in size. Tetrasporangial branches are slightly swollen and sinuous (Fig. 128B-C). The development of tetrasporangia is arranged in spiral series from upper part of axes (Fig. 128C). The fertile segment has 9 or 10 pericentral cells and the fertile pericentral cell developed to a stalk cell, which will develop the tetrasporangia and the two cover cells (Fig. 128D-F). A single (Fig. 128D-E) or two tetrasporangia (Fig. 128E) is produced on each segment.

**Habitat:** Plants grow in the intertidal zone, forming extensive turfs. They are found attached on rocks in sheltered to wave-exposed areas. Turfs are usually wide, extensive, very robust, and in association with *Bostrychia*.

***Wilsonosiphonia howei* (Hollenberg) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 129-131)**

**Basionym*:*** *Polysiphonia howei* Hollenberg in Taylor (1945)

**Homotypic synonym*:*** *Neosiphonia howei* (Hollenberg) Skelton et G.R.South in Skelton and South (2007).

**Heterotypic synonyms*:*** *Polysiphonia yonakuniensis* Segi in Segi (1951); *Polysiphonia rhizoidea* Meñez in Meñez (1964).

**Specimens observed:** CUK6205, collected in Belize 10 May 2005; CUK10056, collected 07 August 2013 from Sand Key Park, Gulf Boulevard, Florida; CUK13915 collected 11 February 2015 from Thankachi Madam, Rameswaram, Tamil Nadu, India.

**Type locality*:*** Whale Cay, Berry Island, Bahamas

**Distribution*:*** Bermuda, Florida, North Carolina, Mexico and Caribbean Islands (Taylor 1960).

**Vegetative structure:** Plants are minute, 1.3-3.2 cm in high (Fig. 129A, 131A), dark red to brown in color, and mainly associated to *Ceramium*. Thalli are composed of prostrate and erect system with robust texture (Fig. 129B). Prostate system is composed of indeterminate axes and is extensive and entangle. Segments of prostrate axes are 90.39 ± 10.71 μm in length and 89.02 ± 6.92 μm in diameter, being as broader as long (L:D 1.02 ± 0.12). The erect system is composed of interwoven indeterminate axes. Erect axes are robust and arisen exogenously from the prostrate axes at intervals of 2, 4, or 6 axial cells. Young erect axes are strongly curved toward the direction of the prostrate axes and have short segments (Fig. 129J). Older segments of erect axes are wide, 64.13 ± 7.77 μm long (Fig. 129G) and 74.22 ± 9.07 μm in diameter, being 1.2 times broader than long (L:D 0.87 ± 0.16), shifted, and infrequently branched (Fig. 129D). Adventitious branches present. Lateral branches are replacing the whole trichoblast. Apical cells are prominent, 8.59 ± 0.77 μm × 6.99 ± 0.66 μm in size, and transversely divided (Fig. 129F). Axes are composed of 8-13 pericentral cells, ecorticate throughout (Fig. 129F-G, 131C-D), and sparse to moderate branched every 4-28 axial cells in dichotomous to subdichotomous pattern (Fig. 129C, 131B). Trichoblasts are having a short basal cell, delicate, numerous, 321.12 ± 71.48 μm long, rarely over 521.3 μm, and arise on each segment near the apical cells where they are persistent (Fig. 131E-F). Conspicuous scar cells appear along the filament after trichoblast have been shed, reaching 15.81 ± 1.61 μm × 16.37 ± 0.94 μm , and developed in a variable pattern series in the space between segments (Fig. 129H, 131G).

Rhizoids are scattered (Fig. 129I) and ventrally produced from the distal end of mature pericentral cells (Fig. 129J, 131I). They are cutting off the pericentral cells (Fig. 129K, 131I). Rhizoids are unicellular in younger stages but when mature they have multicellular terminations in axonomorphus shape (taproot shape) (Fig. 131K), and 24.07 ± 2.86 μm in diameter and 152.34 ± 55.24 μm long.

**Reproductive structure:** In tetrasporangial plants (Fig. 130A, 131K), tetrasporangia are tetrahedral and 26.43 ± 5.09 μm × 24.54 ± 5.26 μm in size. Tetrasporangial branches are slightly swollen and sinuous (Fig. 130B-C). The development of tetrasporangia is arranged in spiral series from middle to upper part of axes (Fig. 130C, 131L-M). The fertile segment has 9 or 10 pericentral cells and the fertile pericentral cell developed to a stalk cell, which will develop the tetrasporangia and the two cover cells. A single is produced on each fertile segment (Fig. 130D-F, 131L-O).

**Habitat.** Plants grow in the intertidal zone, forming turfs. They are found attached on rocks in sheltered to wave-exposed areas. Turfs are robust, and in association with *Ceramium.*

## 2. Phylogenetic analyses

A 1,245-bp portion of the 1,467- bp *rbc*L (84.8%) and 1333-bp portion of the 1467-bp *cox1* (90%) were sequenced for *Wilsonosiphonia fujiiae*, *W. howei* and other *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus placed *Wilsonosiphonia* gen. nov. sister to the genus *Herposiphonia* (Fig. 132) with sequences divergence of 10% and 11.4% between them and supported by a bootstrap value of 58. *Wilsonosiphonia* gen. nov. is also diverging from other *Polysiphonia* sensu lato by over than 11.2 %. On the other hand, phylogenetic analyses of the *cox1* locus placed the members of *Wilsonosiphonia* gen. nov. sister to the clade composed by *Womersleyella*, *Herposiphonia*, and *Polysiphonia* sensu lato (Fig. 133) with sequences divergence of 12% and 15.6% between them and high supported by a bootstrap value of 100 and a posterior probability of 1. The sequence divergences between *W. fujiiae* and *W. howei* are 2.3% for *rbc*L and 3.1% for *cox1*.

## 3. Discussion

The new genus *Wilsonosiphonia*, based on *W. fujiiae* sp. nov., is characterized by having erects axes arisen from an extensive and entangle creeping system, 8-14 pericentral cells, ecorticate axes, subdichotomous to dichotomous branching pattern, exogenous indeterminate branches, abundant trichoblasts, conspicuous scar cells, rhizoids cutting off from the distal end of pericentral cells with multicellular terminations in axonomorphus shape (taproot shape), and tetrasporangia arranged in spiral series. The diagnostic feature segregating *Wilsonosiphonia* from *Polysiphonia* sensu lato is the location of rhizoids in the distal end of pericentral cells. The combination of this character with the exogenous indeterminate branches, and multicellular terminations of rhizoids in axonomorphus shape (taproot shape) separates this new genus from other polysiphonous groups. *rbc*L and *cox1* sequence analyses also supported its taxonomic status as a new genus.

The location of origin of the rhizoids on a mature pericentral cell was proposed as a useful character state to distinguish species by Hollenberg (1968), whether the rhizoid arises on the distal end, on the proximal end, or from the center. In previous and the present studies, species having the rhizoids originated on the proximal end and from the center of pericentral cells were restricted to *Polysiphonia* sensu lato (*i.e. Hapterosiphonia*, *Lampisiphonia*, *Neosiphonia*, multipericentral group, and *Polysiphonia* sensu stricto) (Stuercke and Freshwater 2008, Bárbara et al. 2013, Bustamante et al. 2015c), whereas rhizoids located on the distal end of pericentral cells were restricted to the genus *Herposiphonia, Pterosiphonia, Womersleyella* and our new genus *Wilsonosiphonia.* The location of the rhizoids on pericentral cell is here confirmed as an adequate and consistent character to distinguish taxa higher than species level in polysiphonous species.

The origin of branches has been discussed in detail by Falkenberg (1901) distinguishing endogenous and exogenous origin of branches in Rhodomelaceae. Exogenous origins of branches are present in *Polysiphonia* sensu lato species, whereas endogenous origin of branches has been reported exclusively in *Lophosiphonia* (Hollenberg 1942, Mamoozadeh and Freshwater 2012). Fal-

kenberg (1901) also distinguished two developmental types of branches: determinate branches (present in *Herposiphonia* and *Pterosiphonia*) and indeterminate branches (present in *Polysiphonia* sensu lato) (Hollenberg 1942). Based on the origin of the branches, *Wilsonosiphonia* has exogenous origin of branches and rarely adventitious branches (*i.e.* branches with endogenous origin) and based on the development of branches, *Wilsonosiphonia* only shows indeterminate branches.

The unicellular or multicellular rhizoids are characters states that have recently been evaluated as consistent at genus level in *Polysiphonia* sensu lato with the description of the new genus *Hapterosiphonia* (Bustamante et al. 2015c). Unicellular rhizoids have been restricted to *Neosiphonia*, multipericentral group, and *Polysiphonia* sensu stricto; whereas multicellular rhizoids have been reported in *Hapterosiphonia* and *Lampisiphonia* (Kim and Lee 1999, Choi et al. 2001, Bárbara et al. 2013, Bustamante et al. 2015c). Multicellular rhizoids have also been reported in the following genera: *Herposiphonia*, *Pterosiphonia*, and *Womersleyella* (Schmitz 1889, Hollenberg 1956, Hoffmann and Santelices 1997). These multicellular rhizoids are characterized by having a multilobed shape among these genera. The multicellular rhizoids in *Wilsonosiphonia* are different from these genera by having an axonomorphus shape (taproot shape).

Although the new genus *Wilsonosiphonia* has similarities with the genus *Neosiphonia* and the multipericentral group, they are distinguished from *Wilsonosiphonia* by having unicellular rhizoids and the origin of rhizoids on the proximal end or from the center (Bustamante et al. 2013a, Diaz-Tapia and Bárbara 2013) (Table 8). *Wilsonosiphonia* also resembles to *Lampisiphonia* and *Hapterosiphonia*. However, *Lampisiphonia* is distinguished by having a compact cortication and tetrasporangia arranged in straight series, whereas *Hapterosiphonia* is distinguished by having a paniculate branching pattern (Bárbara et al. 2013, Bustamante et al. 2015c) (Table 8). Our morphological and phylogenetic analyses reveal that *Wilsonosiphonia* is a close related genus with a high support in our *rbc*L (88% for ML and1.0 for BPP) and *cox1* analyses to the genus *Herposiphonia*, but *Herposiphonia* is distinguished by having determinate and indeterminate branches distichously arranged, scarce trichoblasts, and tetrasporangia in straight series (Nägeli 1846, Schmitz 1889, Kylin

1956). *Wilsonosiphonia* has been related to the genus *Lophosiphonia* Falkenberg (Mamoozadeh and Freshwater 2012) because *Lophosiphonia obscura* (C. Agardh) Falkenberg were incorrectly cited with similar number of pericentral cells to *W. howei* (Taylor 1945, Hollenberg 1958, 1968b). Silva *et al.* (1996) clarified that the number of pericentral cells in *L. obscura* is 5-7 rather than 11-18 as noticed by Falkenberg (1901). Our sample of *L. obscura* from Australia differs from *Wilsonosiphonia* members by having only 6 pericentral cells and exclusively endogenous origin of branches (Mamoozadeh and Freshwater 2012, Díaz-Tapia and Bárbara 2013). Our molecular analyses confirmed *Lophosiphonia* and *Wilsonosiphonia* as two different and distantly related genera with 10.3-12.8% of sequence divergence in *rbc*L locus.

The present study is establishing the new genus *Wilsonosiphonia* with the description of a new species *Wilsonosiphonia fujiiae* and a new combination *W. howei.* The generitype of the new genus, namely, *Wilsonosiphonia fujiiae* sp. nov. is characterized by having a robust habit, 10-13 ecorticated pericentral cells, dichotomous to subdichotomous branching pattern, and abundant and persistent trichoblasts subapically disposed . This species was initially reported as the Brazilian *Polysiphonia howei* by Joly (1957) from Itapeva, and later from São Paulo to Pernambuco, Brazil by Guimarães et al. (2004). Although our specimens from Brazil corresponded to the original description of Taylor (1945) and are very similar with our collections of *W. howei* from Belize, Florida, and India, *W. fujiiae* is distinguished from the *W. howei* by having two tetrasporangia per fertile segment.

*Wilsonosiphonia howei* was originally described by Hollenberg as *Polysiphonia* species on the basis of the distal location of rhizoids from Whale Cay, Berry Is., Bahamas (Taylor 1945) and then it was transferred to *Neosiphonia* by Skelton and South (2007), but Mamoozadeh and Freshwater (2012) retained in *Polysiphonia* because of the distant relationship to species of *Neosiphonia* in SSU and *rbc*L phylogenies. *W. howei* is characterized by having robust habit, 8-13 ecorticated pericentral cells, dichotomous to subdichotomous branching pattern, and abundant trichoblasts apically disposed. Although *W. howei* has been widely reported, only Mamoozadeh and Freshwater

(2012) confirmed it from the vicinity of its type locality in the western Atlantic. Further genetic analyses of a wide array of reports of *W. howei* are needed to determine its exact geographic distribution (Mamoozadeh and Freshwater 2012). Gametophytic structures in *W. fujiiae* and *W. howei* were not found in the present study although Guimarães et al. (2004) reported female plants in *W. fujiiae* (as the Brazilian *Polysiphonia howei*) having a four-celled carpogonial branch from Espírito Santo, and Hollenberg (1958, 1968) reported in samples of *W. howei* (as *P. howei*) from the Pacific and western Atlantic Ocean spermatangial branches replacing and developed from a furcation of trichoblasts.

Mamoozadeh and Freshwater (2012) pointed out that the diverse states in the number of pericentral cells perhaps suggest more than one species identified under "*Polysiphonia howei*". *Wilsonosiphonia howei* was originally described having 10-12 pericentral cells (Taylor 1945), but Mamoozadeh and Freshwater (2012) characterized *W. howei* (as *P. howei*) from Panama with 8-10 pericentral cells and our study reported *W. howei* from Belize, Florida, and India having 10-14 pericentral cells. The number of pericentral cells is usually quite constant if there are only four, but number becomes in general more variable if the number of pericentral cells is increased (Falkenberg 1901, Hollenberg 1942). Although the variable number of pericentral cells between the Panamanian reports and our samples of *W. howei*, our *rbc*L and *cox1* analyses (% sequences divergence) demonstrate that these samples correspond to the same species.

*Wilsonosiphonia fujiiae* from Brazil and *W. howei* from Belize and Florida (Fig. 126) are showing a high genetic differentiation (2.6% for *rbc*L). This differentiation has been reported in other marine organisms between the Western and the Southwest Atlantic (Sarver et al. 1998, Tourinho et al. 2012) and it was explained as the result of the outflow of the Amazon River in the Atlantic Ocean (George 2005). Currently, the Amazon River discharges about one-fifth of the world's freshwater runoff into the Atlantic (Curtin 1986a, 1986b), which causes an alteration of salinity and sediment discharge up to 500 km seaward and 30 m depth (Rocha 2003). These form a barrier of low salinity between the Western and the Southwest Atlantic (Tourinho et al. 2012).

Hommersand (1986) pointed out that species similarity between Indo West Pacific and the West Atlantic is due to these species were originated in the Indo West Pacific and then distributed to the West Atlantic by way of the South equatorial and Guiana currents. The allopatric populations of *W. howei* from Florida and India suggest that they likely follow this distribution pattern. Comparisons of these allopatric populations of *W. howei* show 1.6% of divergence for *rbc*L and no morphological differences, indicating cryptic (Maggs et al. 2007) and intraspecific diversity (Mamoozadeh and Freshwater 2012) between them. The degree of genetic divergence between these populations is similar to that between distinct subspecies of other *Polysiphonia* sensu lato that have been previously described (Bustamante et al. 2015a, 2015b). Based on low genetic diversity and the geographic isolation of the two populations of *W. howei*, the taxonomic status of these populations are determined with the proposal of the following two subspecies:

*Wilsonosiphonia howei subsp. howei* D.E. Bustamante, B.Y. Won et T.O. Cho stat. nov. (Fig. 129-130)

Basionym*: Polysiphonia howei* Hollenberg in Taylor (1945)

Type locality*:* Whale Cay, Berry Island, Bahamas

*Wilsonosiphonia howei subsp. indica* D.E. Bustamante, B.Y. Won et T.O. Cho subsp. nov. (Fig. 131)

Holotype*:* CUK13915, collected 11 February 2015, deposited in the herbarium of Chosun University, Korea (CUK).

Type locality: 9° 18' 33.49" N 79° 16' 39.19" E; intertidal, attached to rocks, Thankachi Madam, Rameswaram, Tamil Nadu, India.

Etymology: the subspecies "*indica*" make reference to the country of collection.

Our study attempted to resolve some of the heterogeneity in *Polysiphonia* sensu lato and some polysiphonous genera with the segregation of two species into the new genus *Wilsonosiphonia*. The *rbc*L and c*ox1* phylogenies reveal that *Wilsonosiphonia fujiiae* and *W. howei* are in a strong and well supported clade (100% for ML, 1.0 for BPP).

**Fig. 126.** Distribution of the genus *Wilsonosiphonia* around the Western Atlantic Ocean and the Indic Ocean. *Wilsonosiphonia fujiiae* sp. nov. (sky blue), *Wilsonosiphonia howei* subsp. *indica* comb. nov. (blue), and *Wilsonosiphonia howei* subsp. *howei* comb. nov. (yellow).

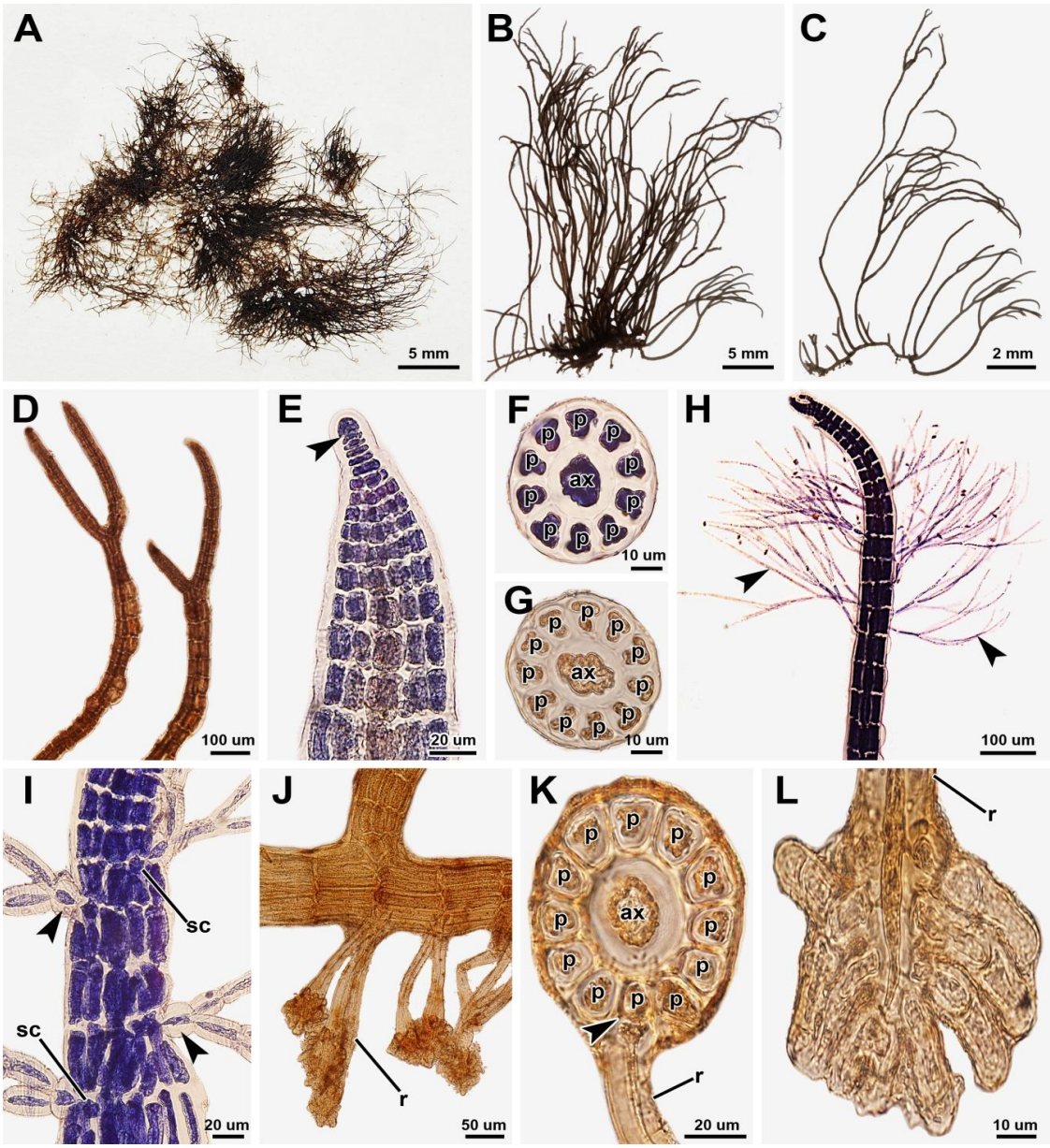


**Fig. 127**. Vegetative structures of *Wilsonosiphonia fujiiae* sp. nov. (A) Voucher of the specimen from Ubatuba, São Paulo, Brazil. (B) Habit of vegetative plant. (C) Erect axes arising exogenously from the prostrate axes. (D) Apex showing the dichotomous to subdichotomous branching pattern. (E) Apices showing transversely (arrowhead) divided apical cells. (F-G) Cross section views of axes showing 10-11 pericentral cells (p) from middle (F) and basal part (G) [ax, axial cell]. (H) Apical part of erect axes showing abundant and long trichoblasts (arrowhead). (I) Erect axes showing the prominent basal cell of trichoblasts (arrowhead) and conspicuous scar cells (sc). (J) Rhizoids (r) scattered and ventrally produced from the distal end of pericentral cells. (K) Rhizoid (r) cutting off (arrow) pericentral cells (p). (L) Multicellular terminations of rhizoid (r).

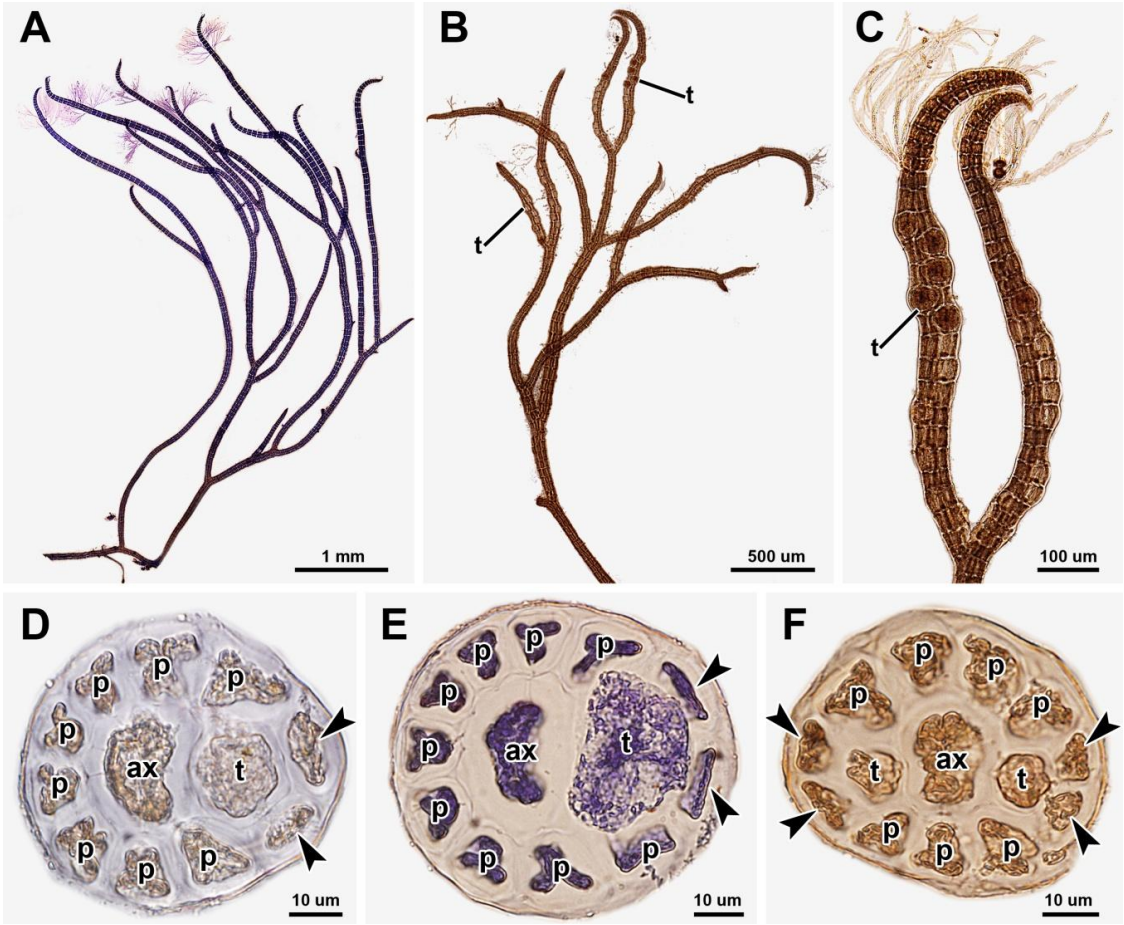


**Fig. 128.** Reproductive structures of *Wilsonosiphonia fujiiae* sp. nov. (A) Tetrasporangial plant. (B) Apical branches of tetrasporangial plant showing the subapically disposed tetrasporangia (t). (C) Apical branches showing tetrasporangia (t) arranged in spiral series. (D-E) Cross-section view showing a single tetrasporangium (t) per segment having 9 (D) and 10 (E) pericentral cell (p) rounded by cover cells (arrowhead). (F) Cross-section view showing two tetrasporangia (t) per segment rounded by cover cells (arrowhead).

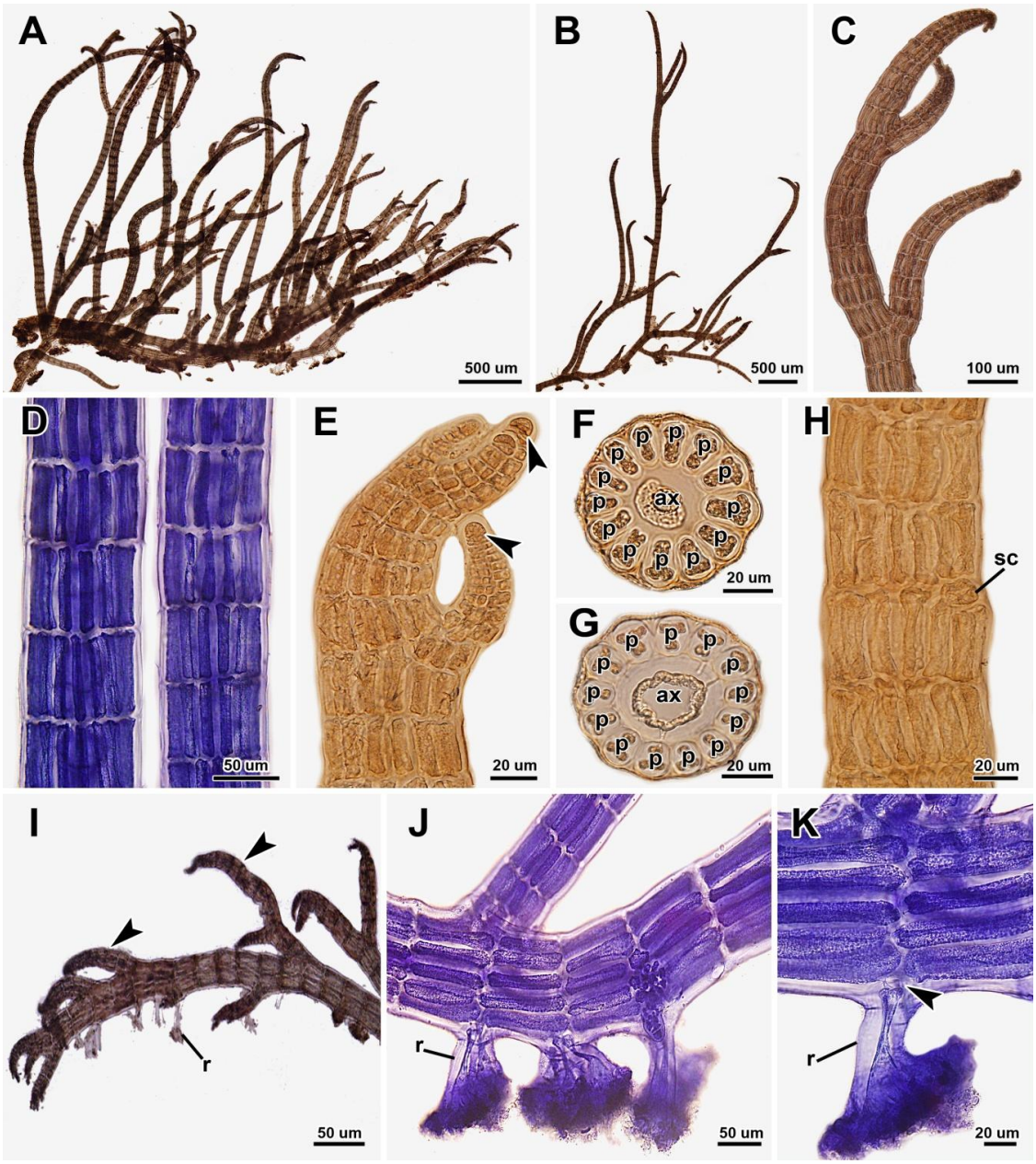


**Fig. 129**. Vegetative structures of *Wilsonosiphonia howei subsp. howei*. (A) Habit of vegetative plant. (B) Erect axes arising exogenously from the prostrate axes. (C) Apex showing the subdichotomous branching pattern. (D) Basal erect axes showing wide older segments. (E) Apices showing transversely (arrowhead) divided apical cells. (F-G) Cross section views of axes showing 13 pericentral cells (p) from middle (F) and basal part (G) [ax, axial cell]. (H) Erect axes showing conspicuous scar cells (sc). (I) Rhizoids (r) scattered in prostrate axes with young erect axes strongly curved (arrows). (J) Rhizoids (r) ventrally produced from distal end of pericentral cells. (K) Rhizoid (r) cutting off pericentral cells (p).

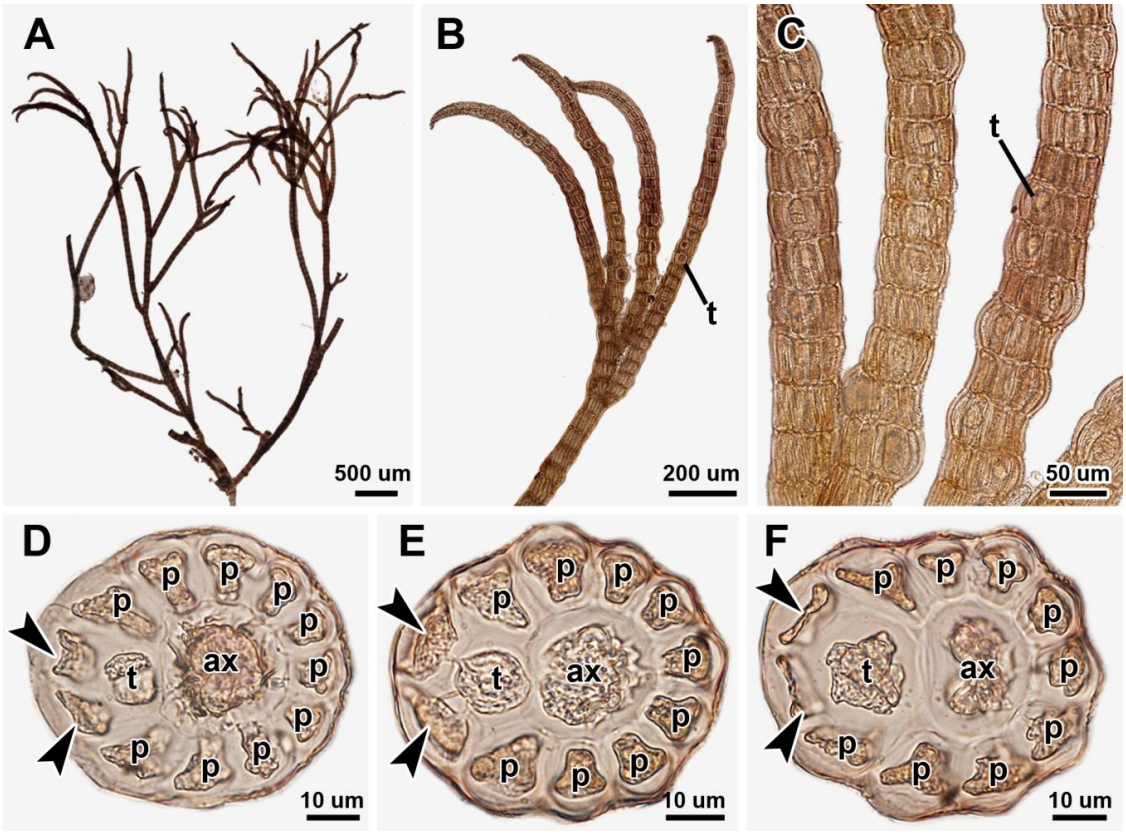


**Fig. 130.** Reproductive structures of *Wilsonosiphonia howei subsp. howei*. (A) Tetrasporangial plant. (B) Apical branches of tetrasporangial plant showing the subapically disposed tetrasporangia (t). (C) Apical branches showing tetrasporangia (t) arranged in spiral series. (D-F) Cross-section view showing a single tetrasporangium (t) having 10-11 pericentral cells (p) rounded by cover cells (arrowhead).

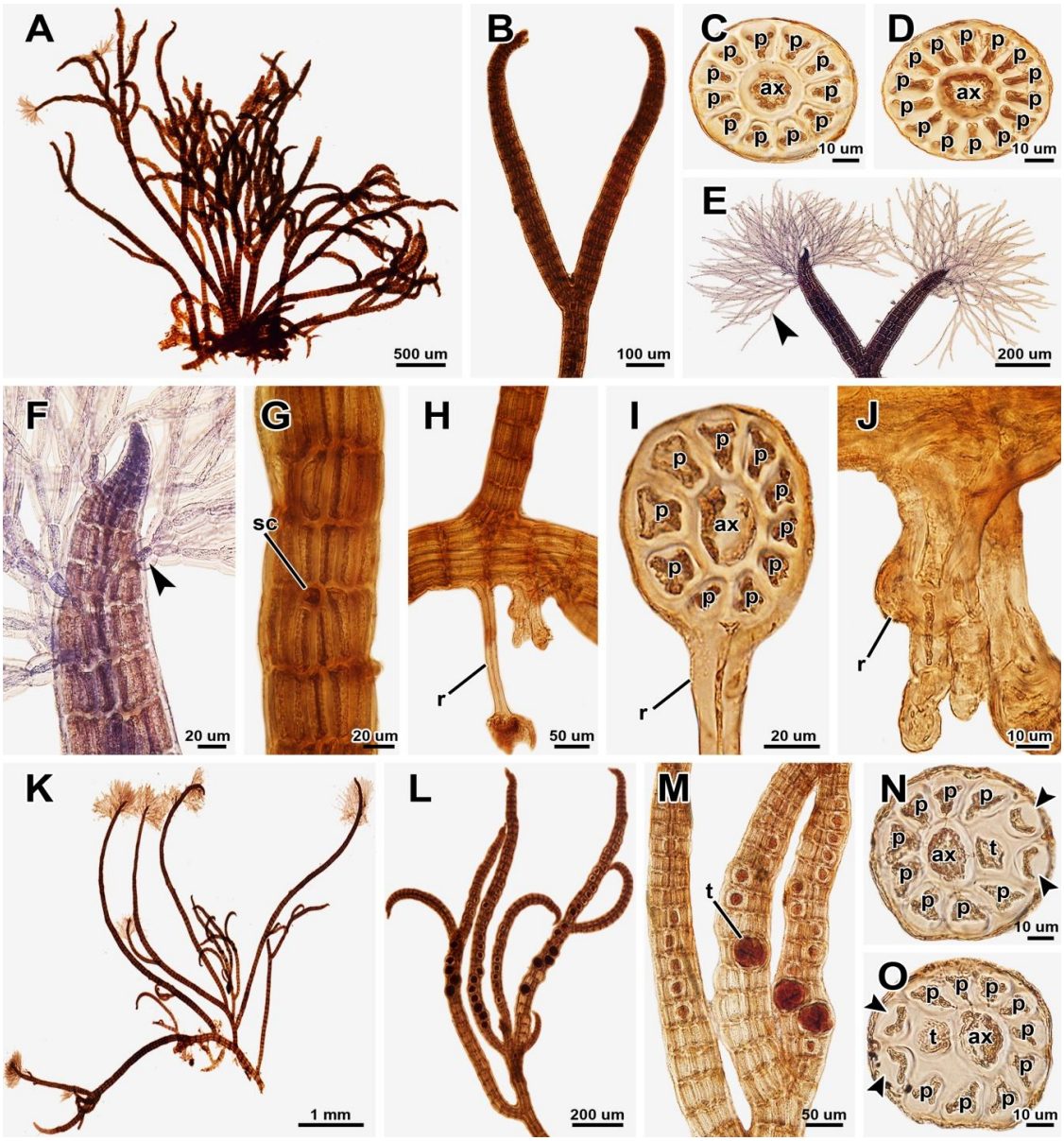


**Fig. 131.** Vegetative and reproductive structures of *Wilsonosiphonia howei subsp. indica.* (A) Habit of vegetative plant. (B) Apex showing the dichotomous branching pattern. (C-D) Cross section views of axes showing 11 pericentral cells (p) from middle (C) and 14 pericentral cells from basal part (D) [ax, axial cell]. (E) Apical part of erect axes showing abundant and long trichoblasts (arrowhead). (F) Erect axes showing the prominent basal cell of trichoblasts (arrowhead). (G) Erect axes showing conspicuous scar cells (sc). (H) Rhizoids (r) ventrally produced from distal end of pericentral cells. (I) Rhizoid (r) cutting off pericentral cells (p). (J) Multicellular terminations of rhizoid (r). (K) Tetrasporangial plant. (L) Apical branches of tetrasporangial plant showing the subapically disposed tetrasporangia. (M) Apical branches showing tetrasporangia (t) arranged in spiral series. (N-O) Cross-section view showing a single tetrasporangium (t) having 9-10 pericentral cells (p) rounded by cover cells (arrowhead).

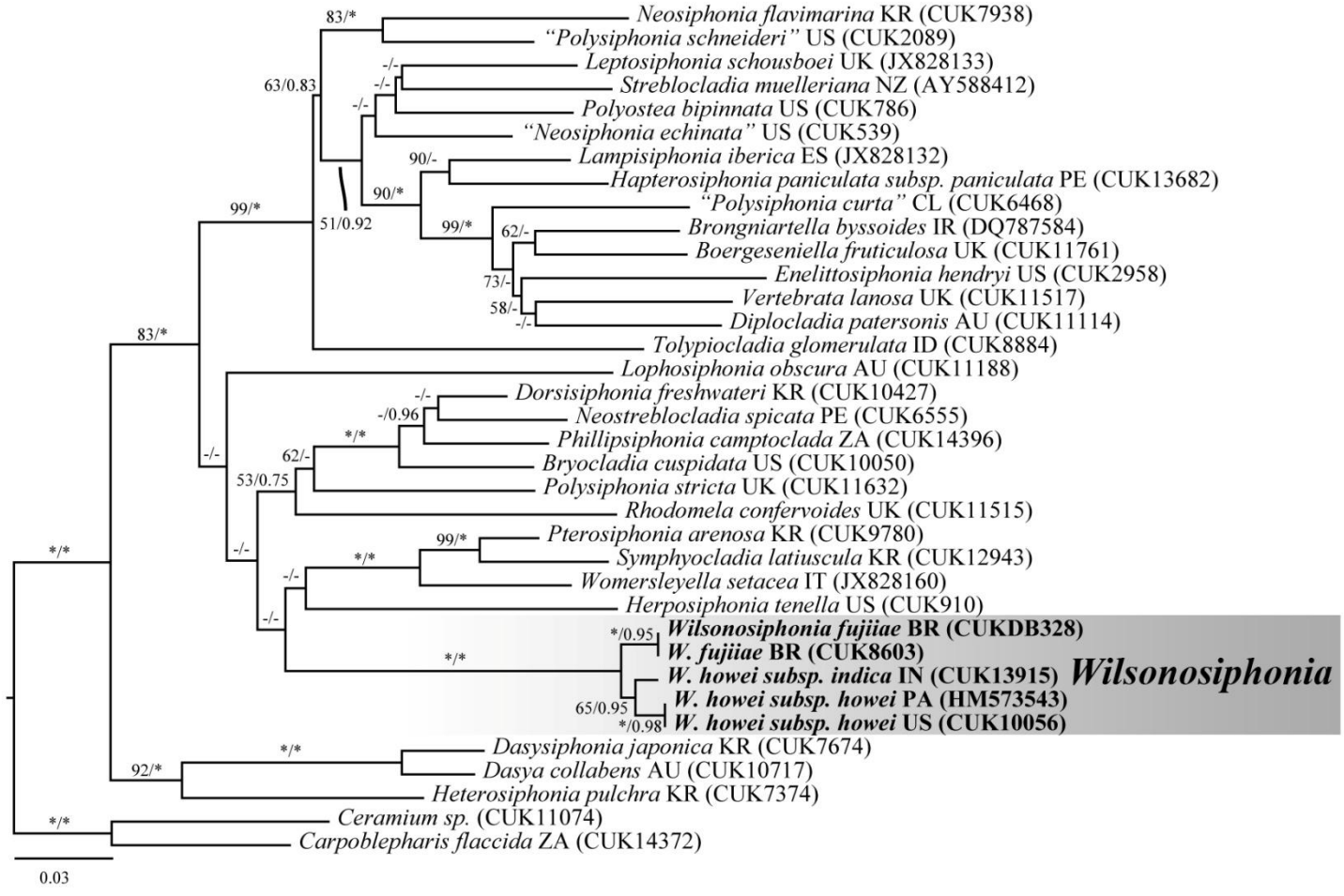


**Fig. 132.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

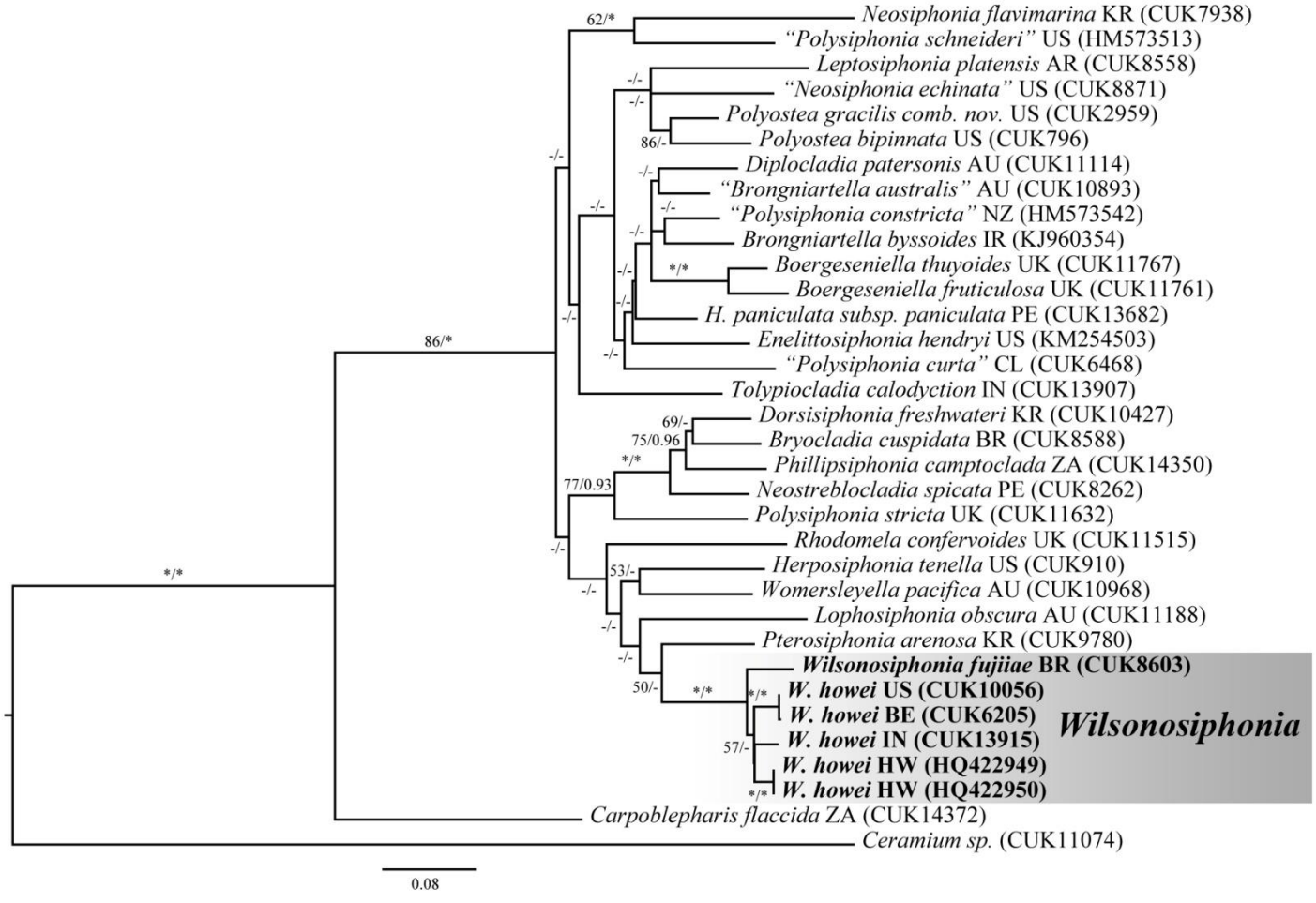


**Fig. 133.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 8.** Comparisons among genera belonging to *Polysiphonia* sensu lato and *Wilsonosiphonia* gen. nov.

| | ***Wilsonosiphonia* gen. nov.** | ***Herposiphonia*** | ***Neosiphonia*** | ***Lampisiphonia*** | ***Lophosiphonia*** | **Multipericentral group** | ***Polysiphonia* sensu stricto** |
|---|---|---|---|---|---|---|---|
| **Exogenous origin of branches** | Present | Present | Present | Present | Absent | Present | Present |
| **Endogenous origin of branches** | Present | Present | Present | Present | Exclusively | Present | Present |
| **Determinate branches** | Absent | Present | Absent | Absent | Present | Absent | Absent |
| **Indeterminate branches** | Present | Present | Present | Present | Present | Present | Present |
| **Rhizoids** | Multicellular | Multicellular | Unicellular | Multicellular | Unicellular | Unicellular | Unicellular |
| **Origin of rhizoids** | Distal | Distal | Proximal or center | Proximal | Proximal or center | Proximal | Proximal or center |
| **Pericentral cells number** | 8 - 14 | 6 - 18 | 4 - 9 | 10 - 13 | 6 | 8 - 14 | 4 |
| **References** | This study | Nägeli (1846), Schmitz (1889) | Kim and Lee (1999) | Bárbara et al. (2013) | Schmitz and Falkenberg (1897), this study | Choi et al. (2001) | Bustamante et al. (2014b) |

## CHAPTER 8. Taxonomy and phylogeny of the multipericentral group: genera *Boergeseniella*, *Brongniartella*, *Enelittosiphonia*, *"Polysiphonia"*, and *Vertebrata* (Rhodomelaceae, Rhodophyta)

The genus *Polysiphonia* was described by Greville (1823) and around 220 species have been reported from worldwide (Kim et al. 2000, Guiry and Guiry 2013). Choi et al. (2001) analyzed *Polysiphonia* sensu lato species identifying three main groups based on molecular and anatomical features. Of them, the multipericentral group was characterized mainly by having axes with more than 5 pericentral cells, rhizoids cutting off from pericentral cells, and procarps bearing four-celled carpogonial branch. Genetically, recent studies have demonstrated that the multipericentral group is comprised of the following heterogeneous genera: *Boergeseniella*, *Enelittosiphonia*, *Polysiphonia*, and *Vertebrata* (Choi et al. 2001, Mamoozadeh and Freshwater 2011, Kim and Kim 2014, Bustamante et al. 2015c). Although recently described genus *Lampisiphonia* and *Hapterosiphonia* are showing features that related them to the multipericentral group, the molecular analyses of Barbara et al. (2013) and Bustamante et al. (2015c) demonstrated the segregation of this species from the multipericentral group and *Polysiphonia* sensu lato by having multicellular rhizoids.

The current five genera recognized in the multipericentral group defined this group as one of the most heterogeneous. These genera are showing different diagnostic features that separate among them and among other genera in *Polysiphonia* sensu lato. Choi et al. (2001) noticed that one approach to dealing with the taxonomy in the multipericentral group might be to sink all the species of the multipericentral group into the genus *Vertebrata*, but they concluded that a thorough systematic investigation of the genera and species of this group is necessary prior to formal taxonomic proposals.

In the present study, we collected some polysiphonous specimens having in common more than numerous pericentral cells. Most of these specimens were collected from the vicinity of their type localities around Australia, Chile, England, Peru, and USA. We reassessed these specimens based

on the detailed morphology and the phylogenetic relationships with other similar species by analyzing the *rbc*L and *cox*1 sequences.

## 1. Morphological analyses

Our analyses confirmed that the multipericentral group is composed of the following six genera characterized by the combination of diagnostic features.

**Key to genera in the paraphyletic multipericentral group**

1. More than five pericentral cells and axes completely corticated throughout ..... ***Boergeseniella***
1. More than five pericentral cells and axes ecorticated throughout ..... 2
    2. Branching pattern strictly dorsiventral ..... ***Enelittosiphonia***
    2. Branching pattern spiral ..... 3
3. Needle-like determinate branches spirally disposed ..... ***Diplocladia***
3. Indeterminate branches spirally disposed ..... 4
    4. Apex lacking of trichoblasts ..... ***Vertebrata***
    4. Apex with abundant trichoblasts ..... 5
5. Dispostion of trichoblasts following the arrangement of origin of the second pericentral cell respect to the first pericentral cell ..... ***Brongniartella***
5. Deciduous trichoblasts leaving prominent scar cells ..... "***Polysiphonia***"

***Boergeseniella* Kylin**

**Type species:** *Boergeseniella fruticulosa* (Wulfen) Kylin

***Boergeseniella fruticulosa* (Wulfen) Kylin (Fig. 134-135).**

**Basonym:** *Fucus fruticulosus* Wulfen.

**Homotypic synonyms:** *Pterosiphonia fruticulosa* (Wulfen) L. Batten, *Fucus fruticulosus* Wulfen, *Polysiphonia fruticulosa* (Wulfen) Sprengel, *Rytiphlaea fruticulosa* (Wulfen) Harvey.

**Heterotypic synonyms:** *Boergeseniella fruticulosa* var. *wulfenii* Bornet, *Polysiphonia fruticulosa* var. *wulfenii* (Roth) Bornet, *Ceramium wulfeni* Roth, *Polysiphonia wulfenii* (Roth) J. Agardh, *Polysiphonia martensiana* Kützing, *Polysiphonia comosa* Kützing, *Polysiphonia cymosa* Kützing, *Polysiphonia pycnophloea* Kützing, *Polysiphonia comatula* Kützing, *Polysiphonia humilis* Kützing.

**Distribution**: Northeastern Atlantic

**Specimens examined:** CUK11607, CUK11609 (Berwick-upon-tweed, Magadalene Fields Golf Club, Rocky shore, England, collected by T.O.C., May. 05 2014), CUK11705, CUK11720, CUK11725, CUK11761 (Forty Foot Beach, Ireland collected by T.O.C., May. 07 2014), CUK11804, CUK11808, CUK11818 (Donaghadee, Rocky shore, Northern Ireland collected by T.O.C., May. 08 2014).

**Vegetative morphology:** Plants are 3.6-8.4 cm high (Fig. 134A), reddish to brownish to yellowish in color, associated with other filamentous species. Plants form entangled and robust tufts that are predominantly attached on rock, corals, solid surfaces or epiphyte on brown seaweeds from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended and entangled prostrate system at irregular intervals. Erect axes are composed of determinate and indeterminate axes radially branched in an irregularly spiral to alternate-distichous pattern at intervals of 3-7 axial cells and complanate near apex (bilateral phylotaxy) (Fig. 134B-D). Apical cells are inconspicuous, dome shaped, 5.33 ± 0.66 μm × 6.28 ± 0.74 μm in size, and transversely divided (Fig. 134E). Young erect axes have short segments, they are incurved and tapered toward the apex and alternated disposed. Older segments of erect axes are larger, normally

153.60 ± 58.62 μm in length and 441.96 ± 243.69 μm in diameter (L:D 0.44 ± 0.18), and infrequently branched (Fig. 134A). Trichoblasts are scarce, short, delicate, deciduous, 1–2 times forked, 110.24 ± 73.26 μm in length, and arising on each segment near the apical cells (Fig. 134E-F). Each segment is completely corticated along the thallus and composed of 9-11 pericentral cells (Fig. 134G-I), which are having a triangular shape in longitudinal view (Fig. 134J). Adventitious branches are present and have corkscrew shape when mature throughout the main axes. Lateral branches replace trichoblasts. The prostrate system is reduced and entangled, with axial segments 235.50 ± 43.41 μm in length and 675.83 ± 152.13 μm in diameter (L:D 0.36 ± 0.10). Rhizoids are clustered and ventrally produced from the proximal end of the pericentral cells (Fig. 134K), and cut off from the pericentral cells (Fig. 134L-M), 428.37 ± 120.77 μm in length, and 16.51 ± 2.16 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 135A, tetrasporangia are tetrahedral and 28.24 ± 3.92 μm × 23.09 ± 4.81 μm in size. Tetrasporangial branches are swollen, lanceolate, and alternate disposed (Fig. 135B-C). The development of tetrasporangia follows a spiral and straight arrangement (Fig. 135C). Fertile segments have nine pericentral cells (Fig. 135D). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 135D). A single tetrasporangium is produced on each fertile segment (Fig. 135D).

**Habitat:** Plants grow forming small tufts from the intertidal zone. They are found attached on rock, coral, solid or epiphyte on brown seaweeds in sheltered to wave-exposed areas. Tufts are usually robust and associated with other filamentous species as *Polysiphonia fucoides* and *P. Boergeseniella thuyoides.*

**Remarks:** The species of *Boergeseniella* were distinguished from other *Polysiphonia* sensu lato by the arrangement of trichoblasts and branches which is spiral with bilateral phyllotaxy and apically compressed (Maggs and Hommersand 1993). *Boergeseniella fruticulosa* was originally described from Northwestern Atlantic by Wulfen (1789), and then transferred to several genera and finally

segregated to its own genus by Kylin (1956). *Boergeseniella fruticulosa* is very similar in external morphology to *Boergeseniella thuyoides*, but *B. fruticulosa* is distinguished from *B. thuyoides* by having the pericentral cells bigger in diameter than cortical cells in the basal part of axes and corkscrew shape of adventitious branches when mature throughout main axes.

***Boergeseniella thuyoides* (Harvey) Kylin (Fig. 136).**

**Basonym:** *Polysiphonia thuyoides* Harvey.

**Homotypic synonyms:** *Polysiphonia thuyoides* Harvey, *Rytiphlaea thuyoides* (Harvey) Harvey, *Vertebrata thuyoides* (Harvey) Kuntze, *Pterosiphonia thuyoides* (Harvey) Batters.

**Type locality:** Milltown Malbay, Co. Clare, Ireland

**Distribution**: Northeastern Atlantic

**Specimens examined:** CUK11721, CUK11767 (Forty Foot Beach, Ireland collected by T.O.C., May. 07 2014).

**Vegetative morphology:** Plants are 5.4-9.6 cm high (Fig. 136A, K), reddish to brownish in color, associated with other filamentous species. Plants form entangled and robust tufts that are predominantly attached on rock, corals, solid surfaces or epiphyte on brown seaweeds from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended and entangled prostrate system at irregular intervals (Fig. 136A, K). Erect axes are composed of determinate and indeterminate axes radially branched in an irregularly spiral to alternate-distichous pattern at intervals of 3 axial cells and complanate near apex (bilateral phylotaxy) (Fig. 136B-D). Apical cells are inconspicuous, dome shaped, 5.12 ± 0.71 μm × 5.16 ± 0.63 μm in size, and transversely divided (Fig. 136E). Young erect axes have short segments, they are incurved and tapered toward the axes and alternated disposed (Fig. 136F). Older segments of erect axes are

larger, normally 93.17 ± 11.59 μm in length and 209.99 ± 15.39 μm in diameter (L:D 0.45 ± 0.06), and infrequently branched (Fig. 136A). Trichoblasts are scarce, short, delicate, deciduous, 1–2 times forked, 44.94 ± 10.20 μm in length, and arising on each segment near the apical cells (Fig. 136F). Each segment is completely corticated along the thallus and composed of 9-10 pericentral cells (Fig. 136G-I). Adventitious branches are present and branched several times when mature throughout the main axes (Fig. 136J). Lateral branches replace trichoblasts. The prostrate system is reduced and entangled (Fig. 136K), with axial segments 91.37 ± 19.89 μm in length and 219.51 ± 23.99 μm in diameter (L:D 0.42 ± 0.08). Rhizoids are clustered and ventrally produced from the proximal end of the pericentral cells (Fig. 136L), and cut off from the pericentral cells (Fig. 136M), 339.94 ± 177.19 μm in length, and 29.19 ± 9.10 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 136N).

**Habitat:** Plants grow forming small tufts from the intertidal zone. They are found attached on rock, coral, solid or epiphyte on brown seaweeds in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other filamentous species as *Boergeseniella fruticulosa* and *N. harveyi.*

**Remarks:** *Boergeseniella thuyoides* was originally described as *Polysiphonia* by Harvey (1836) and finally transferred to *Boergeseniella* by Kylin (1956). *Boergeseniella thuyoides* is similar in morphology to *P. fucoides* and *B. fruticulosa*. *Polysiphonia fucoides* is different from *B. thuyoides* by lacking of cortication, whereas *B. fruticulosa* is also distinguished by having 10-12 pericentral cells (Maggs and Hommersand 1993).

***Brongniartella* Bory**

**Type species:** *Brongniartella byssoides* (Goodenough et Woodward) F. Schmitz

***Brongniartella australis* (C. Agardh) F. Schmitz (Fig. 137).**

**Basonym:** *Cladostephus australe* C.Agardh.

**Homotypic synonyms:** *Cladostephus australe* C. Agardh, *Griffithsia australis* (C. Agardh) C. Agardh, *Polysiphonia australis* (C. Agardh) J. Agardh, *Lophothalia australis* (C. Agardh) J. Agardh, *Vertebrata australis* (C. Agardh) Kuntze .

**Heterotypic synonyms:** *Bindera cladostephus* Decaisne, *Polysiphonia cladostephus* Montagne, *Polysiphonia byssoclados* Harvey, *Bindera australis* (C. Agardh) Trevisan, *Brongniartella australis* f. *recurva* Parsons.

**Type locality:** Geographe Bay, Cape Leeuwin, or King George Sound.

**Distribution**: Australia and New Zealand

**Specimens examined:** CUK10893 (Sorelle, Tasmania, Australia, collected by T.O.C and D.E.B., Mar. 22 2014), CUK10914-2 (Swansea, Tasmania, Australia, collected by T.O.C and D.E.B., Mar. 23 2014).

**Vegetative morphology:** Plants are 2.5-7.9 cm high (Fig. 137A), brownish in color to purple, and solitary. Plants form entangled and delicate tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously and from an extended and entangled prostrate system at irregular intervals. Erect axes are composed of indeterminate axes radially branched in irregular alternate pattern at irregular intervals (Fig. 137A) and clothed by with branched rhodoplastic trichoblasts (Fig. 137B-D). Apical cells are conspicuous, dome shaped, and transversely divided (Fig. 137E). Young erect axes have short segments, they are incurved and tapered toward the apex. Older segments of erect axes are larger, normally 761.28 ± 118.80 μm in length and 924.02 ± 53.34 μm in diameter (L:D 0.83 ± 0.15), and infrequently branched and denudated (Fig. 137A). Trichoblasts are abundant,

long, robust, persistent throughout main axes, usually upwardly curved to recurved, basally branched 3-4 times, 1046.12 ± 250.69 μm in length, and arising on each segment in opposite direction to the formation of the second pericentral cells respect to the first pericentral cell, and covering the apical cells (Fig. 137E-F). Each segment is ecorticated along the thallus and composed of 7 pericentral cells (Fig. 137H-I). Adventitious branches are present (Fig. 137G). Lateral branches replace trichoblasts. The prostrate system is extended and entangled and lightly covered by persistent trichoblasts (Fig. 137A, J). Rhizoids are scattered and radially produced from the proximal end of the pericentral cells (Fig. 137I). Rhizoids cut off from the pericentral cells (Fig. 137K-L). Rhizoids are unicellular and produce lobed terminations when mature.

**Habitat:** Plants grow forming very small tufts from the intertidal. They are found attached on rock, coral or solid surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and solitary.

**Remarks:** *Brongniartella* is featured with persistent trichoblasts spirally disposed throughout the axes. The two current accepted members in this genus are *Brongniartella byssoides* and *Br. australis* (Parsons 1980, Guiry and Guiry 2015). Although Parsons (1980) placed these species in the genus *Brongniartella*, he reported (1) the presence of a fifth or extra cell lateral to the basal cell of the carpogonial branch and (2) two connecting cells formed immediately after fertilization in *Br. australis* as interesting differences. Our phylogenetic analyses placed these species distantly related each other, suggesting that the persistent trichoblasts throughout the axes is not a consistent character to delimit this genus. In contrast, the extra cell to the basal cell in the caporgonial branch and the two connecting cell might reveal consistency to delimit the generitype *Br. byssoides* and *Br. australis* in separate taxon above the rank of species. Although, the significance of the extra cell on the carpogonial branch in *Br. australis* was not confimed by Parsons (1980), our phyogenetic analyses separate this genus from *Brongniartella*, indicating consistency of this feature. Also, the position of second pericentral cell in relation to first pericentral cell opposite to the spiral of the trichoblasts seems to delimit *Br. australis* as a different genus in the multipericentral group. However, the lack of support in our phylogenetic analyses suggest that more studies on the basis of reproductive ma-

terial should be done to confirm the taxonomical status of "*Br. australis*". Although *Brongniartella* genus was characterized in the tribe Lophothalieae by having non-adhederent walls at branching in trichoblasts (Womersley 2003), our current analyses demonstrate that *Brongniartella* is embedded in the tribe Polysiphonieae.

***Brongniartella byssoides* (Goodenough et Woodward) F. Schmitz (Fig. 138).**

**Basonym:** *Fucus byssoides* Goodenough et Woodward.

**Homotypic synonyms:** *Fucus byssoides* Goodenough et Woodward, *Conferva byssoides* (Goodenough et Woodward) Smith, *Conferva byssoides* (Goodenough et Woodward) F. Weber et Mohr, *Ceramium byssoides* (Goodenough et Woodward) C. Agardh, *Hutchinsia byssoides* (Goodenough et Woodward) C. Agardh, *Polysiphonia byssoides* (Goodenough et Woodward) Greville, *Dasyclonia byssoides* (Goodenough et Woodward) J.E. Gray, *Lophothalia byssoides* (Goodenough et Woodward) J. Agardh.

**Heterotypic synonyms:** *Brongniartella elegans* Bory, *Polysiphonia solierii* J. Agardh, *Polysiphonia lyngbyei* Kützing, *Polysiphonia bangi* Kützing.

**Type locality:** Christchurch, Hampshire.

**Distribution**: Northeastern Atlantic.

**Specimens examined:** CUK15428 (Portsmouth, Southsea, England, collected by T.O.C., May 03 2014).

**Vegetative morphology:** Plants are 5-11.2 cm high (Fig. 138A), brownish in color to purple, and associated to filaments. Plants form entangled and robust tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate

erect axes arisen exogenously and from an extended and entangled prostrate system in spiral series. Erect axes are composed of indeterminate axes radially branched of first-order bearing 2-3 orders of spiral branches (Fig. 138A) and clothed by with branched rhodoplastic trichoblasts (Fig. 138B-D). Apical cells are conspicuous, dome shaped, 5.65 ± 0.67 μm × 5.83 ± 0.59 μm in size, and transversely divided (Fig. 138E). Young erect axes have short segments, and completely covered by trichoblasts. Older segments of erect axes are larger, normally 123.03 ± 11.74 μm in length and 166.04 ± 11.85 μm in diameter (L:D 0.75 ± 0.09), and denudated (Fig. 138A). Trichoblasts are abundant, long, robust, persistent throughout main axes, usually upwardly curved, basally branched 1-4 times, 220.80 ± 64.81 μm in length, and following the arrangement of origin of the second pericentral cell respect to the first pericentral cell (Fig. 138E-F). Each segment is ecorticated along the thallus and composed of 6-7 pericentral cells (Fig. 138H-I). Adventitious branches are absent (Fig. 138G). Lateral branches are borne in the furcation trichoblasts. The prostrate system is extended and entangled and lightly covered by persistent trichoblasts (Fig. 138A, J), with axial segments 139.61 ± 14.55 μm in length and 233.78 ± 32.24 μm in diameter (L:D 0.61 ± 0.12). Rhizoids are scattered and radially produced from the proximal end of the pericentral cells (Fig. 138I). 1-4 rhizoids cut off from the pericentral cells (Fig. 138K-L). Rhizoids are unicellular and produce lobed terminations when mature.

**Habitat:** Plants grow forming very long and large tufts from the intertidal. They are found attached on rock, coral or solid surfaces in sheltered to wave-exposed areas. Tufts are usually robust and associated to other filaments species as *Polysiphonia fucoides* and *Dasya sp.*

**Remarks:** The generitype of the genus *Brongniartella*, *Br. byssoides*, has been found to show clear morphological differences with *Br. australiss* to be considered both in the same genus. Parsons (1980) distinguished *Br. australis* from *Br. byssoides* by having (1) a fifth or extra cell lateral to the basal cell of the carpogonial branch, (2) two connecting cells formed immediately after fertilization, and (3) the position of second pericentral cell in relation to first pericentral cell opposite to the spiral of the trichoblasts. Our study also found that *Br. byssoides* is different from *Br. australis* by

having lateral branches of 2-3 order. These significant differences between *Br. australis* and *Br. byssoides* support the distant relationship in our phylogenetic analyses based on *rbc*L and *cox*1 sequences and also the separation of *Br. australis* from the genus *Brongniartella*. It also suggest the inconsistency of the persistent trichoblasts as a diagnositc character of the genus *Brongniartella.* Although *Brongniartella* genus was characterized in the tribe Lophothalieae by having non-adhederent walls at branching in trichoblasts (Womersley 2003), our current analyses demonstrate that *Brongniartella* is embedded in the tribe Polysiphonieae. *Br. byssoides* was embedded in the clade composed by *P. isogona*, *P. fucoides*, and *P. anisogona*. Our morphological analyses found the dispostion of trichoblasts following the arrangement of origin of the second pericentral cell respect to the first pericentral cell is a common feature among these species. The grouping of these species was support by our phylogentic analyses of *rbc*L (100% for ML and 1.0 for BI) and *cox*1 (65% for ML). These results suggest the recognition of these clade as a different genera in multipericentral group. Thereby, we enlarge the concept of *Brongniartella* to encompass *P. isogona*, *P. fucoides*, and *P. anisogona*.

***Brongniartella fucoides* (Hudson) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 139-140).**

**Basonym:** *Conferva fucoides* Hudson.

**Homotypic synonyms:** *Conferva fucoides* Hudson, *Polysiphonia nigrescens var. fucoides* (Hudson) Harvey, *Polysiphonia urceolata f. fucoides* (Hudson) J.Agardh**,** *Polysiphonia fucoides* (Hudson) Greville.

**Heterotypic synonyms:** *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, *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* J.Agardh, *Polysiphonia nigrescens f. pectinata* (C.Agardh) J.Agardh, *Polysiphonia nigrescens f. flaccida* (Areschoug) Kylin.

**Type locality:** York, England.

**Distribution**: Northern Atlantic.

**Specimens examined:** CUK11324, CUK11329, CUK11374 (Portsmouth, Southsea, England, collected by T.O.C., May 03 2014), CUK11380, CUK11382, CUK11403 (Sussex heritage coast, England, collected by T.O.C., May 03 2014), CUK11613 (Berwick-upon-tweed, Magadalene Fields Golf Club, Rocky shore, England, collected by T.O.C and D.E.B., May 06 2014), CUK11808 (Donaghadee, Rocky shore, Northern Ireland, collected by T.O.C and D.E.B., May 08 2014).

**Vegetative morphology:** Plants are 4.1-13.3 cm high (Fig. 139A), reddish to brownish to blackish in color, associated with other filamentous species. Plants form entangled, terete, and robust tufts that are predominantly attached on rock and solid surfaces or epiphyte on other brown seaweeds from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended and very entangled prostrate system at irregular intervals. Erect axes are composed of indeterminate axes radially branched in an alternate to distichous-alternate pattern at intervals of 2-7 axial cells but often each 3 axial cells (Fig. 139B-C). Apical cells are prominent, dome shaped, 6.89 ± 1.45 μm × 6.41 ± 0.69 in size, and transversely divided (Fig. 139D). Young erect axes have short segments, they are lanceolate and tapered to the apex (Fig. 139D). Older segments of erect axes are larger, normally 104.07 ± 8.24 μm in length and 115.94 ± 6.26 μm in diameter (L:D 0.90 ± 0.09), and densely branched (Fig. 139B). Trichoblasts are abundant, long, delicate, deciduous, 1–3 times forked, 156.17 ± 70.33 μm in length, and arising on each segment near

the apical cells (Fig. 139D) following the arrangement of origin of the second pericentral cell respecto to the first pericentral cell. Scar cells are prominent and formed after trichoblasts have been shed. Each segment is ecorticated along the thallus and composed of 14-18 pericentral cells (Fig. 139E-F), sometimes the basal part are lightly corticated (Fig. 139F-G). Adventitious branches are present (Fig. 139H-I). Lateral branches replace trichoblasts. The prostrate system is extended (Fig. 139J) and very entangled, with axial segments 57.55 ± 8.23 μm in length and 78.47 ± 5.19 μm in diameter (L:D 0.73 ± 0.11). Rhizoids are scattered and ventrally produced from the proximal end of the pericentral cells (Fig. 139J), and cut off from the pericentral cells (Fig. 139K-L), 456.71 ± 143.27 μm in length, and 21.62 ± 2.79 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 139M).

**Reproductive morphology:** In female gametophytes (Fig. 140A), erect axes are densely branched at alternate pattern (Fig. 140A). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 140B-C). Cystocarps are irregularly disposed and globose when mature (Fig. 140D), 259.02 ± 37.62 μm in height and 222.89 ± 22.94 μm in diameter. In tetrasporangial plants (Fig. 140E), tetrasporangia are tetrahedral and 42.67 ± 6.69 μm × 46.66 ± 7.02 μm in size. Tetrasporangial branches are swollen and linear (Fig. 140F-G). The development of tetrasporangia follows a spiral and straight arrangement (Fig. 140G). Fertile segments have 7-8 pericentral cells (Fig. 140H-I). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 140H-I). A single tetrasporangium is produced on each fertile segment (Fig. 140H-I).

**Habitat:** Plants grow forming large tufts very common and wide distributed from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades of *Grateloupia sp.* in sheltered to wave-exposed areas. Tufts are usually robust and associated with other filamentous species as *Boergeseniella thuyoides*, *N. harveyi*, and *Vertebra lanosa.*

**Remarks:** *Brongniartella fucoides* was originally described as *Conferva fucoides* by Hudson (1762) from the England coast. This species has been transferred to several genera and finally established in *Polysiphonia* by Greville (1824). The present study is proposing the new combination *Brongniartella fucoides* based on trichoblasts following the arrangement of origin of the second pericentral cell respecto to the first pericentral cell. *Brongniartella fucoides* has been commonly confused with *Boergeseniella thuyoides* by having a similar branching pattern, these species are distinguished by the cortication degree. *Brongniartella fucoides* is having a light cortication on the base of the axes, whereas *Boergeseniella thuyoides* is having the cortication throughout the whole axes (Maggs and Hommersand 1993).

***Brongniartella isogona*** **(Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 141-142).**

**Basonym:** *Polysiphonia isogona* Harvey.

**Homotypic synonyms:** *Polysiphonia isogona* Harvey**.**

**Heterotypic synonyms:** *Polysiphonia comoides* Harvey, *Polysiphonia amoena* Sonder, *Polysiphonia neglecta* Harvey ex J. Agardh, *Lophosiphonia neglecta* (Harvey) De Toni, *Polysiphonia compacta* Lucas**.**

**Type locality:** Blind Bay, Cook Straits, New Zealand.

**Distribution**: Australia and New Zealand.

**Specimens examined:** CUK10896, CUK10897, CUK10898 (Sorelle, Tasmania, Australia, collected by T.O.C. and D.E.B., Mar. 23 2014), CUK10956, CUK10957 (Lagoon Beach, Tasmania, Australia, collected by T.O.C. and D.E.B., Mar. 23 2014) CUK10998, (Scamander, Tasmania, Australia, collected by T.O.C. and D.E.B., Mar. 23 2014), CUK11266 (Camp Cove, Watson Bay, Sydney, Australia, collected by T.O.C., Mar. 28 2014).

**Vegetative morphology:** Plants are 2-12 cm high (Fig. 141A), reddish to purplish to blackish in color, associated with other filamentous species. Plants form entangled and delicate tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from a reduced and very entangled prostrate system at irregular intervals (Fig. 141B). Erect axes are composed of indeterminate axes radially branched in an alternate pattern at intervals of 5-8 axial cells but often each 6 axial cells (Fig. 141B-D). Apical cells are prominent, dome shaped, 5.60 ± 0.75 μm × 4.74 ± 0.70 in size, and transversely divided (Fig. 141E). Young erect axes have short segments, they are linear and tapered to the apex (141E-F). Older segments of erect axes are larger, normally 80.72 ± 6.73 μm in length and 66.90 ± 11.46 μm in diameter (L:D 1.24 ± 0.25), and infrequently branched (Fig. 141B-C). Trichoblasts are abundant, long, delicate, deciduous, 1–3 times forked, 105.25 ± 22.88 μm in length, and arising on each segment near the apical cells (Fig. 141F) following the arrangement of origin of the second pericentral cell respecto to the first pericentral cell. Conspicuous scar cells appear along the filament after trichoblasts have been shed, reaching 10.07 ± 1.46 μm × 8.35 ± 1.87 μm, and developed in spiral series in the space between segments (Fig. 141G). Cicatrigenous branches are present (Fig. 141H). Each segment of the axes is ecorticated along the thallus and composed of 8-10 pericentral cells (Fig. 141I-J). Lateral branches arise in a furcation of trichoblasts (Fig. 141F). The prostrate system is reduced (Fig. 141B) and very entangled, with axial segments 73.03 ± 13.17 μm in length and 73.03 ± 13.17 μm in diameter (L:D 0.77 ± 0.14). Rhizoids are scattered and ventrally produced from the proximal end of the pericentral cells (Fig. 141K), and cut off from the pericentral cells (Fig. 141L-N), 179.52 ± 73.15 μm in length, and 22.08 ± 6.51 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes (Fig. 142A), erect axes are densely branched at alternate pattern (Fig. 142A-B). Procarps are positioned laterally and subapically on erect axes (Fig. 142B), and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 142C). Cystocarps are irregularly disposed and globose when mature (Fig.

142D), 259.02 ± 37.62 μm in height and 222.89 ± 22.94 μm in diameter. In male gametophytes (Fig. 142E), spermatangial branchlets are clustered in the apical part of axes (Fig. 142F-G), and are borne on furcation of the trichoblast in each segment of the axes (Fig. 142H). Each spermatangial branch is composed of spermatangia and sometimes a single sterile tip cell. In tetrasporangial plants (Fig. 142I), tetrasporangia are tetrahedral and 42.67 ± 6.69 μm × 46.66 ± 7.02 μm in size. Tetrasporangial branches are swollen and lanceolate (Fig. 142J-K). The development of tetrasporangia follows a spiral arrangement (Fig. 142K). Fertile segments have 7-9 pericentral cells (Fig. 142L-M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 142L-M). A single tetrasporangium is produced on each fertile segment (Fig. 142L-M).

**Habitat:** Plants grow forming large tufts very common and wide distributed from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades in sheltered to wave-exposed areas. Tufts are delicate and associated with other filamentous species as *N. japonica, Polysiphonia strictissima,* and *N. ramireziae.*

**Remarks:** *Brongniartella isogona* has been widely reported from the coast of New Zealand and Australia (Womersley 2003, Nelson 2012). The molecular analyses of Mamoozadeh and Freshwater (2012) confirmed *Brongniartella isogona* as a member of the multipericentral group. Although *Br. isogona* has been reported from other coast (e.g. Chile, Bustamante and Ramirez 2009), molecular analyses have not confirmed their wide distribution. *Br. isogona* and *P. anisogona* are very similar but *P. anisogona* is distinguished by having the branching intervals each 4-10 axial cells and very long pericentral cells (>1mm). *Polysiphonia anisogona* has been described from Cape Horn, Falkland Islands by Hooker and Harvey (1845) and it was reported as "*P. isogona"* by Bustamante and Ramirez (2009) from the coast of Chile. Our phylogenetic analyses distinguished these species by 5.4% in sequence divergence.

***Enelittosiphonia*** Segi

**Type species:** *Enelittosiphonia hakodatensis* (Yendo) Segi

**Currently accepted name for the type species**: *Enelittosiphonia stimpsonii* (Harvey) Kudo et Masuda.

***Enelittosiphonia hendryi* (N.L. Gardner) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 143).**

**Basonym:** *Polysiphonia hendryi* N.L. Gardner.

**Homotypic synonyms:** *Polysiphonia hendryi* N.L. Gardner.

**Type locality:** Santo Domingo, Baja California, Mexico.

**Distribution**: Northeastern Pacific

**Specimens examined:** CUK757 (Port Townsend, Washington, USA, collected by T.O.C and B.Y.W., Jun. 27 2003), CUK2958 (Outer point, Juneau, Alaska, USA, collected by T.O.C., Jul. 10 2006).

**Vegetative morphology:** Plants are 3.5-10.6 cm high (Fig. 143A), reddish in color to colorless, associated with other filamentous species. Plants form entangled and delicate tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously and strictly dorsiventrally from an extended and entangled prostrate system at intervals of 3-4 axial cells and rarely of 6 (Fig. 143A-B, J). Erect axes are composed of indeterminate axes branched in a pair of unilateral branches borne on one side of the axis alternating with a pair on the other side pattern at intervals of 3-4 axial cells (Fig. 143B). Apical cells are conspicuous, dome shaped, 5.05 ± 0.78 μm × 5.18 ± 0.82 μm in size, and transversely divided (Fig. 143C). Young erect axes have short segments, they are incurved and

tapered toward the apex. Older segments of erect axes are larger, normally 76.30 ± 28.86 μm in length and 88.79 ± 16.88 μm in diameter (L:D 0.84 ± 0.22) (Fig. 143D), and infrequently branched (Fig. 143A). Trichoblasts are abundant, long, delicate, deciduous, 1-3 times forked, 371.38 ± 137.60 μm in length, and arising on each segment near the apical cells (Fig. 143C). Conspicuous scar cells appear along the filament after trichoblasts have been shed, reaching 10.57 ± 1.50 μm × 10.22 ± 1.14 μm , and developed in irregular series in the space between segments (Fig. 143E). Each segment is ecorticated along the thallus and composed of 9-11 pericentral cells (Fig. 143F-G). Adventitious branches are present. Lateral branches replace trichoblasts. The prostrate system is extended (Fig. 143J) and entangled, with axial segments 132.14 ± 25.52 μm in length and 117.50 ± 17.82 μm in diameter (L:D 1.17 ± 0.35). Rhizoids are scattered and sometimes clustered and ventrally produced from the proximal end of the pericentral cells (Fig. 143H), and cut off from the pericentral cells (Fig. 143I), 222.24 ± 108.36 μm in length, and 21.97 ± 3.83 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In tetrasporangial plants (Fig. 143J), tetrasporangia are tetrahedral and 22.03 ± 5.68 μm × 25.38 ± 5.91 μm in size. Tetrasporangial branches are swollen and sinuous. The development of tetrasporangia follows a spiral and straight arrangement (Fig. 143K-L). Fertile segments have ten pericentral cells (Fig. 143M). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 143M). A single tetrasporangium is produced on each fertile segment (Fig. 143K-M).

**Habitat:** Plants grow forming small tufts from the intertidal. They are found attached on rock, coral or solid surfaces in sheltered to wave-exposed areas. Tufts are usually delicate and associated with other filamentous species as *Polyostea bipinnata*.

**Remarks:** *Enelittosiphonia hendryi* comb. nov. was originally described as *Polysiphonia* member by Gardner (1927) from the coast of Mexico. The present study is transferring this species to the genus *Enelittosiphonia* based on the strictly dorsiventral origin of indeterminate erect axes respect

to prostrate axes. Although the monoecious plants were reported as criterion for discrimination in the genus *Enelittosiphonia*, our results did not confirm the consistency of this character due to gametophytes were not found in the present study. Moreover, our molecular analyses of *rbc*L sequences demonstrated the consistency of this character and the close relationship between *Enelittosiphonia hendryi* and the generitype of the genus, namely, *E. stimpsonii*. *E. stimpsonii* is distinguished from *E. hendryi* comb. nov. by having 8-10 pericentral cells (Segi 1949, Masuda et al. 1995).

***Polysiphonia*** Greville

**Type species:** *Polysiphonia urceolata* (Lightfoot ex Dillwyn) Greville

**Currently accepted name for the type species:** *Polysiphonia stricta* (Dillwyn) Greville

Although the following species are showing different features from those of the type species of *Polysiphonia* and should be referred as a different genus, we characterized them as *Polysiphonia* sensu lato species. Further analyses will resolve their taxonomical position in *Polysiphonia* sensu lato.

***Polysiphonia curta*** **Montagne (Fig. 144-145).**

**Type locality:** Peru.

**Distribution**: Chile and Peru

**Specimens examined:** UC1883969 (Holotype, Peru, collected by D'Orbigny), CUK6424, CUK6425 (Antofagasta, Chile, collected by T.O.C., Aug. 17 2008), CUK6443 (Caleta Razuri, Antofagasta, Chile, collected by T.O.C., Aug. 19 2008), CUK6448, CUK6449 (El Lagarto Island, Antofagasta, Chile, collected by T.O.C., Aug. 19 2008), CUK6465, CUK6466, CUK6468 (Tocopilla, Antofagasta, Chile, collected by T.O.C., Aug. 20 2008), CUK6491 (Playa Chica, Quintay,

Valparaiso, Chile, collected by T.O.C., Aug. 22 2008), CUK6497, CUK6498 (Valparaiso, Chile, collected by T.O.C., Aug. 23 2008).

**Vegetative morphology:** Plants are 0.5-3.2 cm high (Fig. 144A), reddish to greenish in color, not associated with other filamentous species. Plants form entangled and robust tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from an extended and entangled prostrate system at intervals 5-6 axial cells (Fig. 144B). Erect axes are composed of indeterminate axes radially branched in a subdichotomous pattern at intervals of 5-7 axial cells but usually 6 axial cells (Fig. 144B). Apical cells are prominent, dome shaped, 5.82 ± 0.90 μm × 5.77 ± 0.72 in size, and transversely divided (Fig. 144C-D). Young erect axes have short segments, they are incurved and tapered opposite to the apex (Fig. 144B, D). Older segments of erect axes are larger, normally 100.99 ± 27.67 μm in length and 92.48 ± 14.72 μm in diameter (L:D 1.12 ± 0.35) (Fig. 144E), and infrequently branched (Fig. 144B). Trichoblasts, scar cells and cicatrigenous branches are absent. Each segment is ecorticated along the thallus and composed of usually 17 pericentral cells but sometimes ranging from 13 to 22 (Fig. 144F-G). Adventitious branches are present. Lateral branches are not in connection with trichoblasts. The prostrate system is extended (Fig. 144H) and entangled, with axial segments 67.72 ± 4.43 μm in length and 135.57 ± 6.41 μm in diameter (L:D 0.50 ± 0.04). Rhizoids are scattered and ventrally produced from the proximal end of the pericentral cells (Fig. 144H), and cut off from the pericentral cells (Fig. 144I-J), 313.70 ± 86.03 μm in length, and 13.40 ± 2.19 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In female gametophytes, erect axes are densely branched in the upper parts (Fig. 145A). Procarps are positioned apically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Cystocarps are irregularly disposed and urceolate to ovoid when mature (Fig. 145B), 244.67 ± 25.56 μm in height and 188.49 ± 18.35 μm in diameter. In male gametophytes (Fig. 145C), spermatangial branchlets are clustered

in the apical part of axes (Fig. 145D), and are replacing the trichoblast in each segment of the axes (Fig. 145E). Each spermatangial branch is composed of spermatangia and sometimes a single sterile tip cell. In tetrasporangial plants (Fig. 145F), tetrasporangia are tetrahedral and 27.33 ± 3.85 μm × 27.71 ± 2.90 μm in size. Tetrasporangial branches are swollen and linear (Fig. 145G-H). The development of tetrasporangia follows a spiral and straight arrangement (Fig. 145G-H). Fertile segments have 10-12 pericentral cells (Fig. 145I-J). The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 145I-K). A single tetrasporangium is produced on each fertile segment (Fig. 145I-J).

**Habitat:** Plants grow forming tufts from the intertidal to subtidal zone. They are found attached on rock and solid surfaces from the intertidal in sheltered to wave-exposed areas. Tufts are usually robust and solitary.

**Remarks:** Montagne (1843) described *Polysiphonia curta* Montagne (Holotype UC1883969 growing near to *Trematocarpus fragilis* (C.Agardh) De Toni from the Peruvian Coast, and cited "*Polysiphonia polymorpha* Montagne" as synonymous of *P. curta* (Montagne 1839). Montagne (1839) reported various species with the epithet "*polymorpha"* or "*polymorphum"*, as synonymous of *P. fastigiata* Greville from Cobija, but any of them have Montagne's authority. Thus, "*Polysiphonia polymorpha* Montagne" is considered an invalid name. Furthermore, the binomial *Polysiphonia polymorpha* was used earlier by Duby (1830), as *Polysiphonia polymorpha* (Linnaeus) Duby (Guiry and Guiry 2015). Then, *Polysiphonia curta* does not have any accepted synonym Montagne (1843). *Polysiphonia curta* is one of only five species in multipericentral group with more than 15 pericentral cells from worldwide. One of these other species *V. lanosa*, the only member of genus *Vertebrata* currently accepted (Guiry and Guiry 2015), is very similar with *P. curta*, but *P. curta* is distinguished from *V. lanosa* by the lower number of pericentral cells and the pseudodichotomous branches (Kim et al. 2002, Bustamante and Ramírez 2009). *P. fucoides*, from York, England, and *P. opaca*, from Adriatic Sea, differ from *P. curta* by the presence of trichoblast (Greville 1824, Maggs and Hommersand 1993). *P. fucoides* is also different in having cortication. *P. indigena*

from California, USA, is different from *V. curta* by having the tetrasporangia only straightly arranged and the strictly dichotomous branching pattern (Hollenberg 1944, Hollenberg 1958). Although, *P. curta* is distributed from the Pacific Temperate Coast of South America, *P. curta* remained inconspicuous since Montagne (1943)'s description (Bustamante and Ramírez 2009). It might be due to misidentifications with other similar species like *P. confusa* Hollenberg or *P. paniculata* Montagne also distributed in this area (Dawson et al. 1964, Ramírez and Santelices 1991, Hoffman and Santelices 1997). Both of them are distinguished from *P. curta* by the presence of abundant trichoblast and 8-12 pericentral cells through the thallus (Howe 1914, Dawson et al. 1964, Sentíes 1995).

***Polysiphonia decipiens* Montagne (Fig. 146-148).**

**Heterotypic synonyms:** *Polysiphonia frutex* Harvey, *Polysiphonia fuscescens* Harvey, *Polysiphonia nigrita* Sonder, *Polysiphonia rytiphlaeoides* J.D. Hooker et Harvey, *Polysiphonia caespitula* Sonder, *Polysiphonia cancellata* Harvey, *Vertebrata nigfita* (Sonder) Kuntze.

**Type locality:** Toud Island (Warrior Islet), Torres Strait, Australia.

**Distribution**: Australia and New Zealand.

**Specimens examined:** CUK10914-1 (Swansea, Tasmania, Australia, collected by T.O.C and D.E.B., Mar. 23 2014), CUK10933 (Lagoon Beach, Tasmania, Australia, collected by T.O.C and D.E.B., Mar. 23 2014).

**Vegetative morphology:** Plants are 3.3-11.6 cm high (Fig. 146A, 147A), reddish to brownish in color, not associated with other filamentous species. Plants form entangled, terete, and robust tufts that are predominantly attached on rock and solid surfaces from the intertidal. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from a reduced and entangled prostrate system at irregular intervals (Fig. 14A). Erect axes are composed of indeterminate

axes radially branched in irregular to alternate pattern at intervals of 5-11 axial cells but often each 5-6 axial cells (Fig. 146B-D, 147B-D). Apical cells are inconspicuous, dome shaped, 5.34 ± 1.21 μm × 5.98 ± 1.89 in size, and transversely divided (Fig. 146E, 147E). Young erect axes have short segments, they are lanceolate and tapered opposite to the apex. Older segments of erect axes are larger, normally 86.94 ± 43.56 μm in length and 372.04 ± 175.75 μm in diameter (L:D 0.23 ± 0.04), and infrequently branched (Fig. 146B-C). Trichoblasts are scarce, long, delicate, deciduous, 1–2 times forked, 185.53 ± 63.00 μm in length, and arising on each segment near the apical cells (Fig. 146E, 147J). Conspicuous scar cells appear along the filament after trichoblasts have been shed, reaching 20.16 ± 2.31 μm × 19.43 ± 2.26 μm, and developed in irregular series in the space between segments. Cicatrigenous branches are present (Fig. 146G). Each segment is ecorticated along the thallus and composed of 7-8 pericentral cells (Fig. 146G-H, 147H-I). Adventitious branches are present (Fig. 146I, 147G). Lateral branches replace trichoblasts and some developed a prominent hook (Fig. 146C, F). The prostrate system is reduced and entangled, with axial segments 51.62 ± 5.03 μm in length and 161.25 ± 15.05 μm in diameter (L:D 0.32 ± 0.05). Rhizoids are scattered and ventrally produced from the proximal end of the pericentral cells (Fig. 146J, 146K), and cut off from the pericentral cells (Fig. 146K-L, 147L-M), 265.74 ± 130.06 μm in length, and 33.09 ± 10.86 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature (Fig. 146M).

**Reproductive morphology:** In female gametophytes (Fig. 148A), erect axes are densely branched at irregular to alternate pattern (Fig. 148B). Procarps are positioned laterally and subapically on erect axes, and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 148C). Cystocarps are irregularly disposed and ovoid when mature (Fig. 148D), 340.43 ± 15.96 μm in height and 366.88 ± 63.49 μm in diameter. In tetrasporangial plants (Fig. 148E), tetrasporangia are tetrahedral and 52.37 ± 9.74 μm × 54.41 ± 8.82 μm in size. Tetrasporangial branches are swollen and lanceolate (Fig. 148F-H). The development of tetrasporangia follows a spiral arrangement (Fig. 148F-H). Fertile segments have 7 pericentral cells (Fig. 148I-J).

The fertile pericentral cell develops as a stalk cell, which will cut off the tetrasporangia and the two cover cells (Fig. 148K). A single or two tetrasporangia are produced on each fertile segment (Fig. 148I-J).

**Habitat:** Plants grow forming large tufts from the intertidal to subtidal zone. They are found epiphyte on brown seaweeds like *Marginariella boryana* and *Cystophora sp.* or attached to solid surfaces in sheltered to wave-exposed areas. Tufts are usually robust and solitary, sometimes these plants are epiphyte by *Acrosorium sp.*

**Remarks:** *Polysiphonia decipiens* was described by Montagne (1942) from the coast of Australia. *Polysiphonia frutex, P. fuscescens, P. nigrita, P. rytiphlaeoides, P. caespitula, P. cancellata,* and *V. nigrita* are species in synonymy with *Polysiphonia decipiens. Polysiphonia decipiens* was characterized by having mainly 7 and rarely 8 pericentral cells (Womersley 2003) and having variation in size and robustness and also variation in pericentral cells diameter, length and shape (Fig. 146-147). Womersley (2003) concluded that these morphological variation is correlated with ecological conditions and names placed in synonymy (e.g. *P. cancellata, P. fuscescens, P. frutex*) represent only ecological forms or genetic variation at subspecific level and our molecular analyses demonstrate that ecotypes of 7 pericentral cells (Fig. 146) and 8 pericentral cells (Fig. 147) belongs to the same species. The diagnostic features of *P. decipiens* are the 7-8 pericentral cells and the prominent hooked branches. *P. decipiens* was embedded in the clade composed by the generitype *D. patersonis*, *D. ericoides* and *P. aterrima. P. decipiens* is lacking of deteminate neddle-like branches and its transfer to *Diplocladia* is hard to conclude, although it is having 7-8 pericentral cells and two tetrasporangia per segment species as *D. patersonis* and *D. ericodes* are having respectevely.

***Vertebrata* S.F. Gray**

**Type species:** *Vertebrata fastigiata* S.F. Gray

**Currently accepted name for the type species:** *Vertebrata lanosa* (Linnaeus) T.A. Christensen

***Vertebrata lanosa* (Linnaeus) T.A. Christensen (Fig. 149-150).**

**Basonym:** *Fucus lanosus* Linnaeus.

**Homotypic synonyms:** *Fucus lanosus* Linnaeus, *Polysiphonia lanosa* (Linnaeus) Tandy.,

**Heterotypic synonyms:** *Conferva omissa* Gunnerus, *Ceramium fastigiatum* Roth, *Hutchinsia fastigiata* (Roth) C. Agardh, *Vertebrata fastigiata* S.F. Gray, *Polysiphonia fastigiata* (Roth) Greville.

**Type locality:** Iceland.

**Distribution**: Widely reported along the northern Atlantic.

**Specimens examined:** CUK11451-CUK11455 (Fishguard, Lower Town, Wales, collected by T.O.C., May 04 2014), CUK11514, CUK11517, CUK11530 (Aberystwyth, Rocky shore, Wales, collected by T.O.C., May 04 2014), CUK11549, CUK11561, CUK11567 (Whitley Bay, England, collected by T.O.C., May 05 2014), CUK11622 (Berwick-upon-tweed, Magadalene Fields Golf Club, Rocky shore, England, collected by T.O.C., May 06 2014), CUK11833-CUK11834, CUK11843 (Donaghadee, Rocky shore, Northern Ireland, collected by T.O.C., May 08 2014).

**Vegetative morphology:** Plants are 3.8-8.4 cm high (Fig. 149A), reddish to blackish in color, associated with other filamentous species. Plants form entangled and rigid tufts that are predominantly epiphyte on *Ascophyllum nodosum* and *Fucus vesiculosus*. Thalli are composed of interwoven and indeterminate extended erect axes arisen exogenously from a reduced, highly branched, and very entangled prostrate system. Erect axes are composed of indeterminate axes radially branched in a dichotomous pattern at intervals of 8-12 axial cells (Fig. 149B-C). Apical cells are prominent, dome shaped, 7.96 ± 0.67 μm × 7.28 ± 1.23 in size, and transversely divided (Fig. 149D). Young

erect axes have short segments, they are linear and tapered to the apex (Fig. 149D-E). Older segments of erect axes are larger (Fig. 149F), normally 40.60 ± 4.07 μm in length and 102.35 ± 6.84 μm in diameter (L:D 0.40 ± 0.05), and unfrequently branched (Fig. 149B-C). Trichoblasts, scar cells, and cicatrigenous branches are absent. Each segment of the axes is ecorticated along the thallus and composed of 17-22 pericentral cells (Fig. 149G-H). Lateral branches are not associated to trichoblasts. The prostrate system is reduced, highly entangled, and with axial segments 35.13 ± 3.94 μm in length and 72.89 ± 5.99 μm in diameter (L:D 0.49 ± 0.08) (Fig. 149I-J). Rhizoids are scattered and ventrally produced from the proximal end of the pericentral cells (Fig. 149J), and cut off from the pericentral cells (Fig. 149K), 148.58 ± 80.77 μm in length, and 23.20 ± 7.81 μm in diameter. Rhizoids are unicellular and produce lobed terminations when mature.

**Reproductive morphology:** In male gametophytes (Fig. 150A), spermatangial branchlets are clustered in the apical part of axes (Fig. 150B-C), and are replacing the trichoblast in each segment of the axes (Fig. 150D). Each spermatangial branch is composed of spermatangia and sometimes a single sterile tip cell.

**Habitat:** Plants grow forming large tufts very common and wide distributed from the intertidal to subtidal zone. They are found attached on rock, coral, solid or epiphyte on blades in sheltered to wave-exposed areas. Tufts are delicate and associated with other filamentous species as *N. japonica, Polysiphonia strictissima,* and *N. ramireziae.*

**Remarks:** The genus *Vertebrata* was originally described by Gray (1821) based on *V. fastigiata* S.F. Gray, which has been placed in synonymy with *P. lanosa* (Tandy 1931) and then it was considered as a synonymous of the genus *Polysiphonia* (Kim et al. 2002). *Vertebrata* was resurrected by Kylin (1956) and distinguished *Vertebrata* from *Polysiphonia* by lacking of trichoblasts and cortication. Christensen (1967) later proposed the combination *Vertebrata lanosa* (Linnaeus) T. Christensen as a necessary consequence of accepting Kylin's concept of *Vertebrata* (Kim et al. 2002). Thereby, *Vertebrata* S.F.Gray is a nom. rej. vs. *Polysiphonia* Greville 1823 (nom. cons.); but the

generitype is considered to be used if it represents a genus other than *Polysiphonia* (Guiry and Guiry 2015). The molecular analyses of Choi et al. (2001) proposed that one approach to dealing with taxonomy in the multipericentral group might be to sink all species into the single genus *Vertebrata*, but a thorough systematic investigation of the genera and species of this group is necessary prior to formal taxonomic proposals. Our morphological analyses determine that *Vertebrata* is a monotypic with a unique combination of characters. The wide variation of features and the distant genetic relationship (over 10% of sequence divergence for *rbc*L and over 12% for *cox*1) among species of the multipericentral group suggest that *Vertebrata* should be considered as distinct genus in the multipericentral group. Recently, Mamoozadeh and Freshwater (2011), Kim and Kim (2014), and Bustamante et al. (2015c) have considered the genus *Vertebrata*, with its unique species *V. lanosa*, as a separate genus in their phylogenies.

## 2. Phylogenetic analyses

A 1,245-bp portion of the 1,467- bp *rbc*L (84.8%) and 1426-bp portion of the 1467-bp *cox*1 (97%) were sequenced for 14 species embedded in the multipericentral group and other from *Polysiphonia* sensu lato species. Phylogenetic analyses of the *rbc*L locus confirmed six genera embedded in the multipericentral group *Boergeseniella*, *Brongniartella*, *Diplocladia*, *Enelittosiphonia*, *Polysiphonia*, and *Vertebrata* (Fig. 151). The multipericentral group is sister to the clade composed by the genera *Hapterosiphonia* and *Lampisiphonia* (Fig. 151) with sequences divergence over 8.8% among them and supported by a bootstrap value of 97 and a posterior probability of 0.99. On the other hand, phylogenetic analyses of the *cox1* locus placed the genera of multipericentral group sister to *Hapterosiphonia* (Fig. 152) with sequences divergence of 12% and 15.6% between them and supported by a bootstrap value of 54%. The sequence divergences between the genera of multipericentral group are 6.3% to 10.2% *rbc*L and over 10.4% for *cox1*.

## 3. Discussion

The synapomorphic features of more than five pericentral cells and rhizoids cutting off pericentral cells defined those genera embedded in the multipericentral group (Choi et al. 2001). Traditionally, *Boergeseniella*, *Enelittosiphonia*, *Polysiphonia*, and *Vertebrata* have been confirmed in the multipericentral group on the basis of plastidial and nuclear markers (Choi. et al. 2001, Mamoozadeh and Freshwater 2012). In addtion, our morphological and molecular analyses have demonstrated that the multipericentral group is composed of two more genera *Brongniartella* and *Diplocladia* (Table 9). These six genera embedded in the multipericentral group are showing a unique combination of diagnostic features that support their recognition as separate genera into this paraphyletic multipericentral group. The genetic distance among these genera is over 8% of sequence divergence for *rbc*L, whereas is over 10% of divergence for *cox*1. It supports the morphological differences among these genera.

The molecular analyses of Choi et al. (2001) proposed that one approach to dealing with taxonomy in the multipericentral group might be to sink all species into the single genus *Vertebrata*, but a thorough systematic investigation of the genera and species of this group is necessary prior to formal taxonomic proposals. Our molecular analyses based on the phylogenetic relationships of plastidial and mitochondrial markers suggest that the multipericentral group is composed of several genera highly diverged and characterized by having consistent morphological features that distinguish one from others.

*Boergeseniella* is delimited by having the following combination of features: cortication, clustered rhizoids, scarce trichoblast, and alternate-distichous branches with bilateral phylotaxy. Our phylogenetic analyses revelead that this clade is composed of three species: *Boergeseniella fruticulosa*, *Bo. thuyoides*, and *P. tripinnata*. Although *P. tripinnata* presents similar morphological traits to species of *Boergenseniella,* Díaz-Tapia and Bárbara (2013) did not propose the combination due to the lacking of cortication in *P. tripinnata*. Our phylogeny for *cox*1 and *rbc*L with high support

for the Bayesian analyses and Maximum likelihood suggest that *P. tripinnata* should be transfered to the genus *Boergenseniella.*

In our phylogenetic analyses, *Brongniartella byssoides* become embedded into the clade composed by *P. anisogona, P. nigra, P. fucoides, P. isogona,* and *P. foetidissima*. This monophyletic clade is high supported by bootstrap values (100%) and posterior probabilities (1.0). Morphologically, *Brongniartella byssoides, P. anisogona, P. fucoides,* and *P. isogona* are having in common the position of second pericentral cell in relation to first pericentral cell following the spiral of the trichoblasts. Thereby, *Brongniartella* should be enlarged to encompass these species and the following new combinations are prosposed: *Br. anisogona* (J.D.Hooker et Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov., *Br. fucoides*(Hudson) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov., and *Br. isogona* (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. Another group having these arrengement of pericentral cells and trichoblasts is the genus *Hapterosiphonia,* but the enlarged *Brongniartella* is distinguished by lacking of multicellular rhizoids (Bustamante et al. 2015c). Although *Brongniartella* genus was characterized in the tribe Lophothalieae by having non-adhederent walls at branching in trichoblasts (Womersley 2003), our current analyses demonstrate that *Brongniartella* is embedded in the tribe Polysiphonieae.

*Diplocladia* is defined by having needle-like determinate branches spirally disposed throughout the axes (see Chapter 9). In our phylogenetic analyses, *Diplocladia* is closely related to the clade composed by "*P. aterrima*" and "*P. decipiens*". Morphologically, these two species are lacking of determinate needle-like branches spirally disposed and the only character in common among these taxa and *Diplocladia* are the very short segments of the axes. The consistency of this feature has not been confirmed. One approach to dealing with the taxonomy of these taxa might be sink "*P. aterrima*" and "*P. decipiens*" into the genus *Diplocladia*, but a further systematic investigation of these species is necesary prior to formal taxonomic proposals.

*Enelittosiphonia* is delimited by having erect axes originated exogenously in a strictly dorsiventral pattern from prostrate axes. The transfer of *P. hendryi* to *Enelittosiphonia* is well supported by molecular and morphological analyses. The monotypic genus *Vertebrata* is delineated with axes lacking of trichoblasts and cortication and restricted to northern Atlantic. Although *Polysiphonia lobophoralis* was allied to *Vertebrata lanosa* in our phylogenetic analyses, the presence of trichoblasts and spermatangial branches arisen in a furcation of trichoblasts in *P. lobophoralis* (Mamoozadeh and Freshwater 2012), and also the low support in our phylogenetic analyses reject the transfer of this species to the genus *Vertebrata*. Moreover, *Polysiphonia curta* is very similar in morphology to *Vertebrata lanosa*, but they are distantly related in phylogeny of *rbc*L and *cox*1. These three taxa seem to have the status of genus based on molecular data, but the lacking of diagnostic features to delimit them as separate genera in the multipericentral group and very low support in our phylogenetic analyses confirm the paraphyly of "*Polysiphonia*" in the multipericentral group. *Polysiphonia curta* will be retained in "*Polysiphonia*" until further morphological and molecular analyses with exhaustive sampling clarify its taxonomical status.

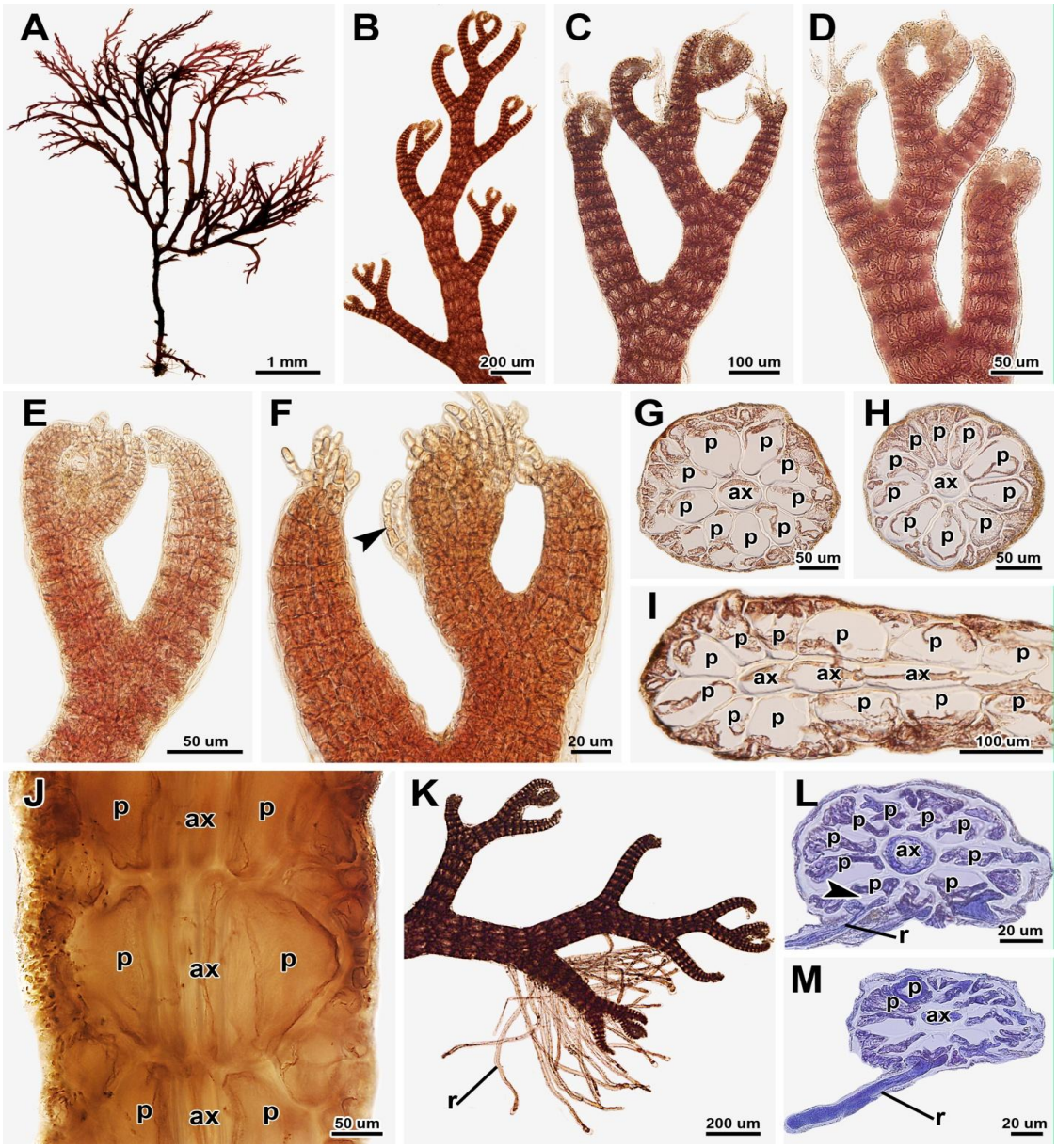


**Fig. 134.** Vegetative structures of *Boergeseniella fruticulosa*. (A) Habit of vegetative plant. (B) Erect axes showing the alternate-distichous branching pattern. (C-D) Apex showing complanate branches. (E) Apex showing inconspicuous apical cells. (F) Apex showing abundant trichoblasts. (G-H) Cross-section views showing 9-10 pericentral cells (p) from the middle (G) and basal (H) part of axes (ax, axial cell). (I) Cross section view showing a longitudinal section of a lateral branch. (J) Longitudinal section view showing triangular pericentral cells (p). (K) Rhizoids (r) clustered and produced from prostrate axes. (L) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (M) Unicellular terminations of rhizoid (r).

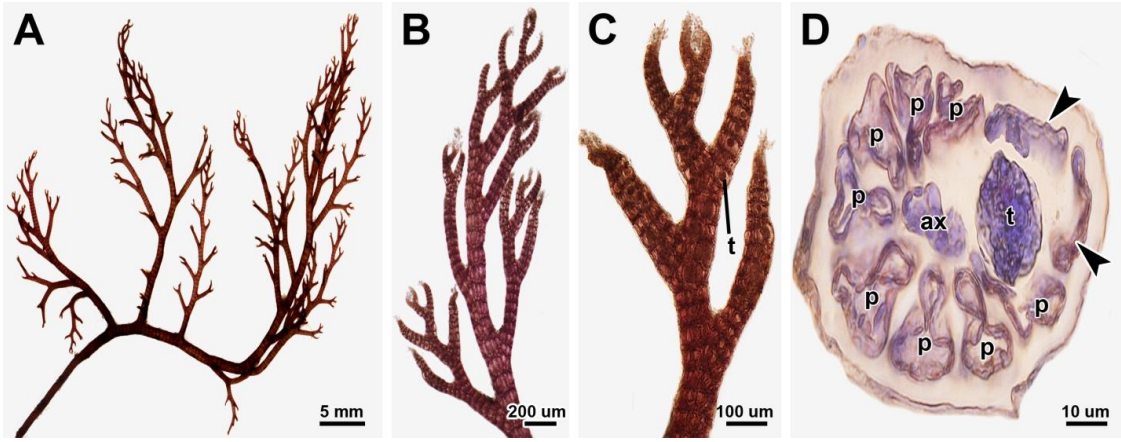


**Fig. 135.** Reproductive structures of *Boergeseniella fruticulosa*. (A) Tetrasporangial thallus. (B) Apex showing tetrasporangial branches with alternate-distichous disposition (C) Apical branches showing tetrasporangia (t) in spiral and straight series. (K) Cross-section with nine pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

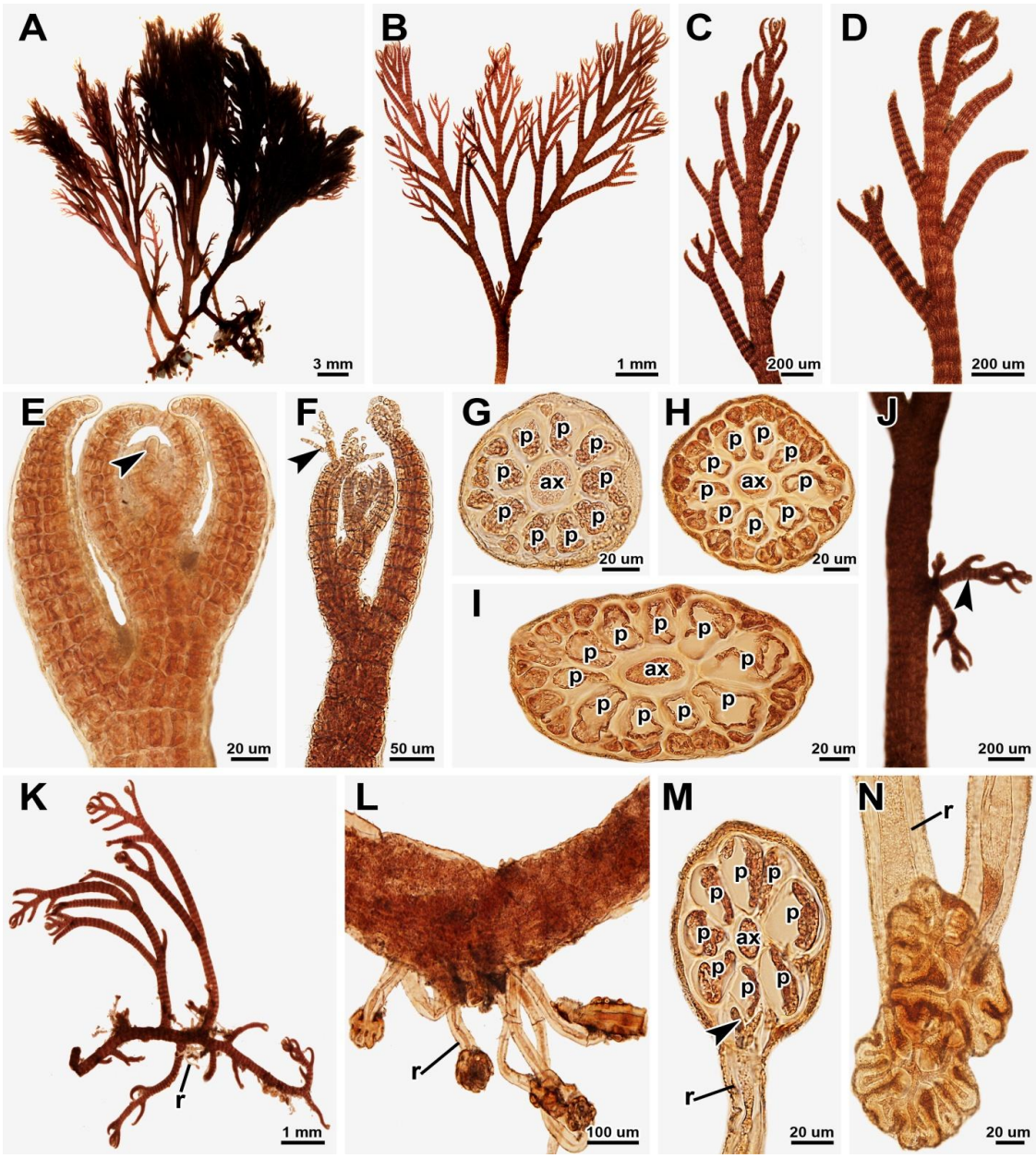


**Fig. 136.** Vegetative structures of *Boergeseniella thuyoides*. (A) Habit of vegetative plant. (B) Erect axes showing the alternate-distichous branching pattern. (C-D) Apex showing complanate branches. (E) Apex showing inconspicuous apical cells. (F) Apex showing abundant trichoblasts. (G-I) Cross-section views showing 9-10 pericentral cells (p) from the apex (G), middle (H) and basal (I) part of axes (ax, axial cell). (J) Erect axes showing adventitious branches (arrowhead). (K) Extended prostrate axes with rhizoids (r). (L) Rhizoids (r) clustered and produced from prostrate axes. (M) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (N) Unicellular terminations of rhizoid (r).

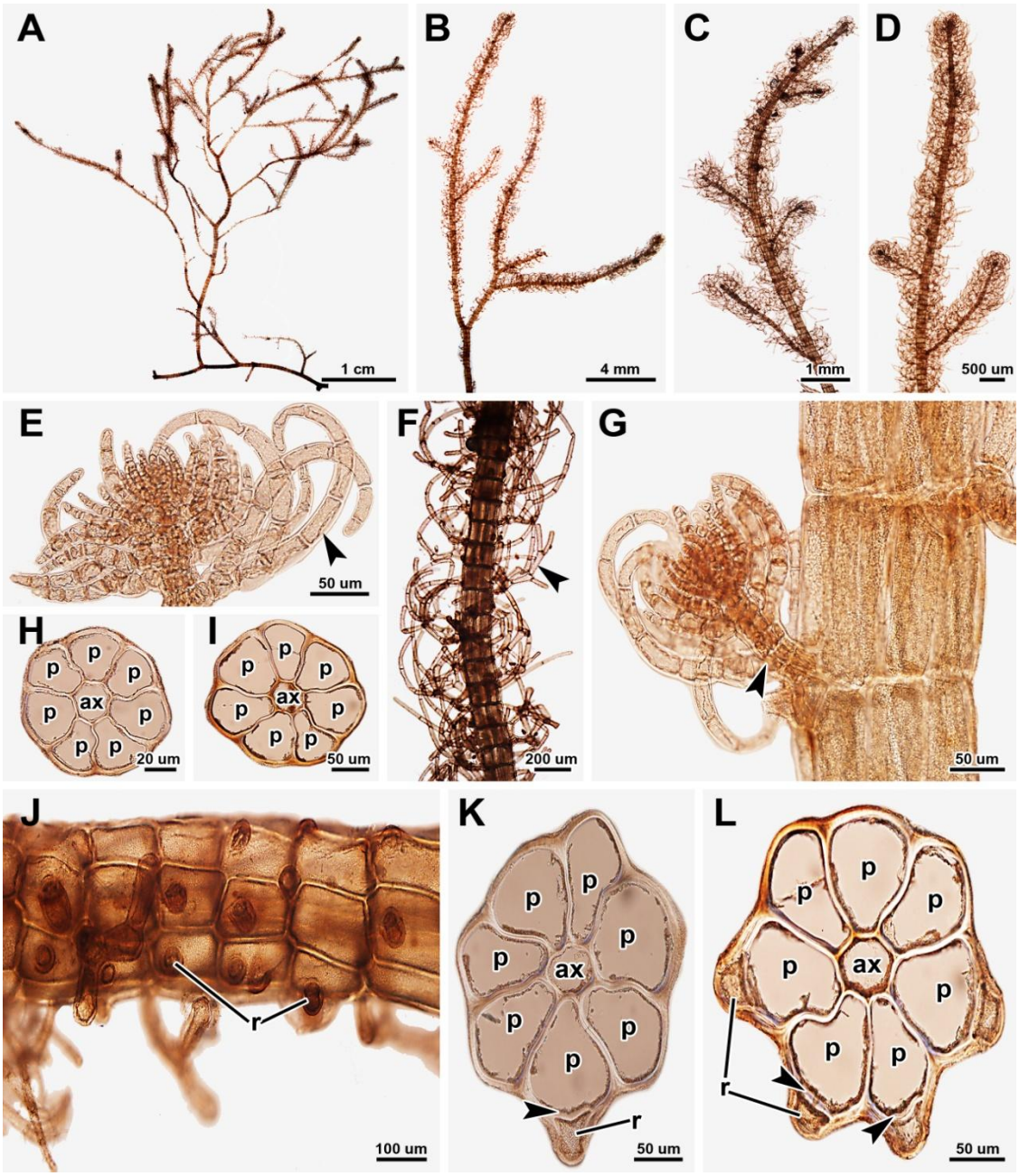


**Fig. 137.** Vegetative structures of *"Brongniartella australis"*. (A) Habit of vegetative plant showing the extended prostrate and erect axes. (B-D) Erect axes with an irregular to alternate branching pattern. (E) Apices showing abundant and persistent trichoblasts (arrowhead). (F) Basal axes showing persistent trichoblasts. (G) Axes showing adventitious branch (arrowhead). (H-I) Cross-sections showing seven pericentral cells (p) of erect (H) and prostrate (I) axes (ax, axial cell). (J) Rhizoids (r) primordia scattered and produced from prostrate axes. (K-L) Cross-section views of prostrate axes showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

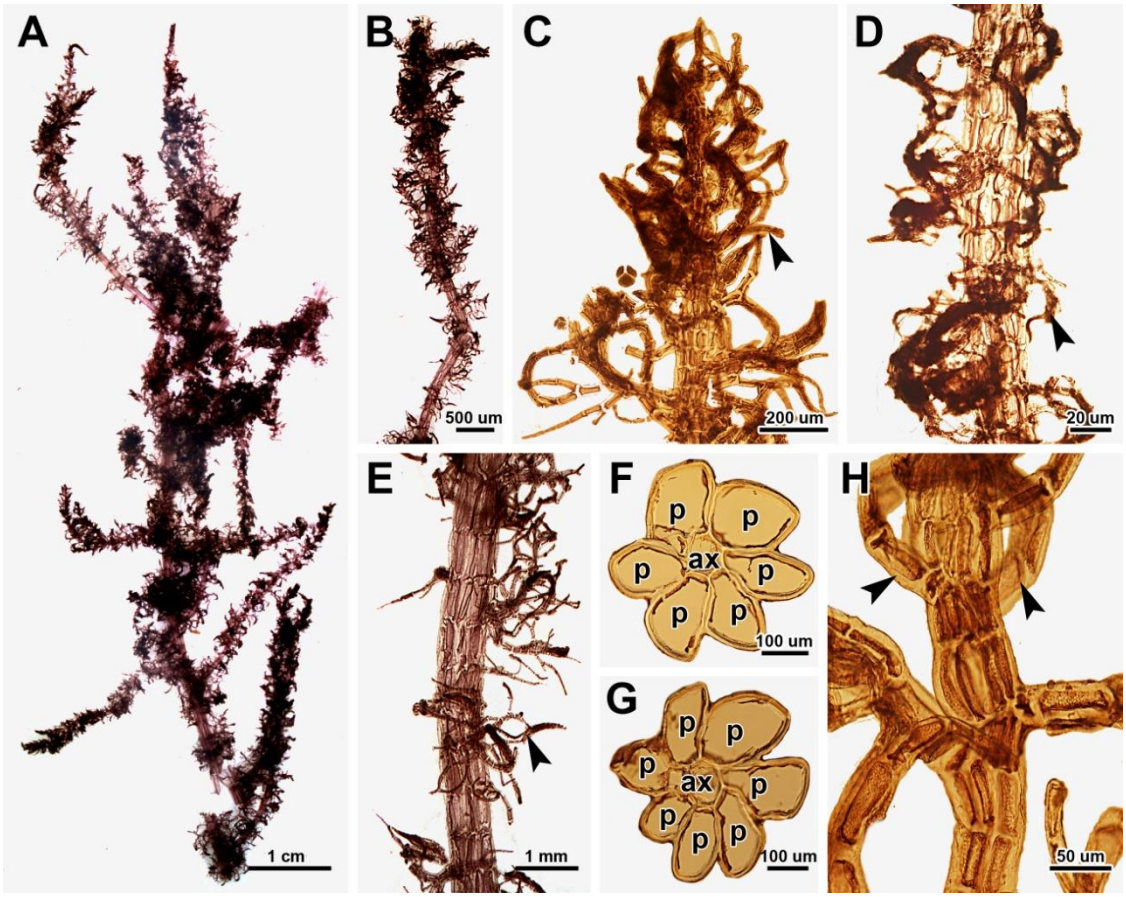


**Fig. 138.** Vegetative structures of *Brongniartella byssoides*. (A) Habit of vegetative plant. (B) Erect axes clothed by abundant persistent trichoblasts. (C-D) Apex (C) and middle part of axes (D) clothed by abundant trichoblasts (arrowhead). (E) Denudated basal part of axes showing scarse trichoblasts (arrowhead). (F-G) Cross-section views showing 6-7 pericentral cells (p) from the middle (E) and basal (F) part of erect axes (ax, axial cell). (H) Trichoblasts (arrowhead) arisen each segment in spiral disposition.


**Fig. 139.** Vegetative structures of *Brongniartella fucoides* comb. nov. (A) Habit of vegetative plant. (B) Erect axes showing irregular branching pattern. (C) Apex showing complanate branches alternate-distichous disposed. (D) Apex showing abundant trichoblasts (arrowhead). (E-F) Cross-section views showing 14-18 pericentral cells (p) from the middle (E) and basal (F) part of axes (ax, axial cell). (G) Longitudinal section view showing prominent axial cells. (H-I) Erect axes showing adventitious branches (arrowhead). (J) Rhizoids (r) scattered and produced from prostrate axes. (K) Rhizoids (r) produced from the proximal end of pericentral cells (arrowhead) . (L) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (M) Unicellular terminations of rhizoid (r).

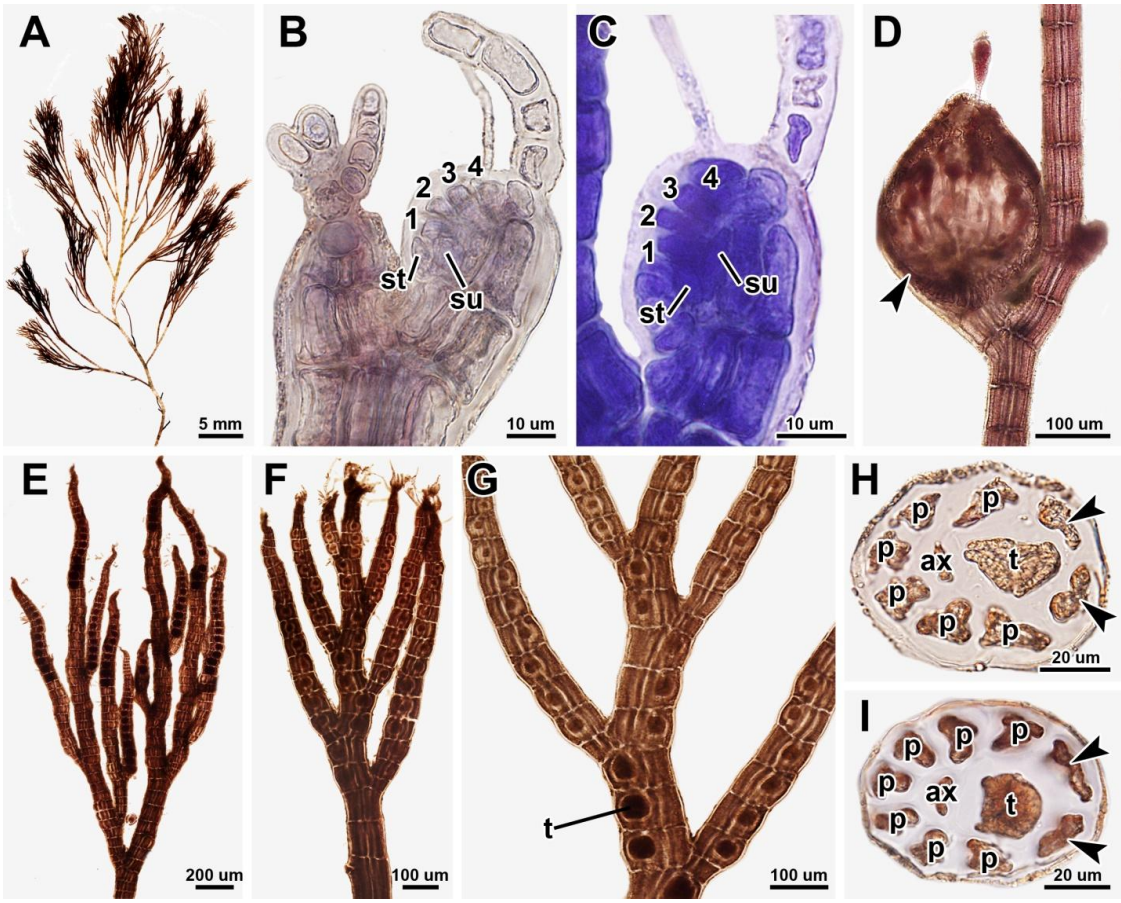


**Fig. 140.** Reproductive structures of *Brongniartella fucoides* comb. nov. (A) Female gametophyte. (B-C) Procarps with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (D) Mature cystocarp (arrowhead) showing ovoid shape. (E) Tetrasporangial plant. (F-G) Apical branches showing spiral and straight arrangement of tetrasporangia (t). (H-I) Cross-section views showing one tetrasporangia (t) per fertile segment rounded by cover cells (arrowheads) (p, pericentral cells).

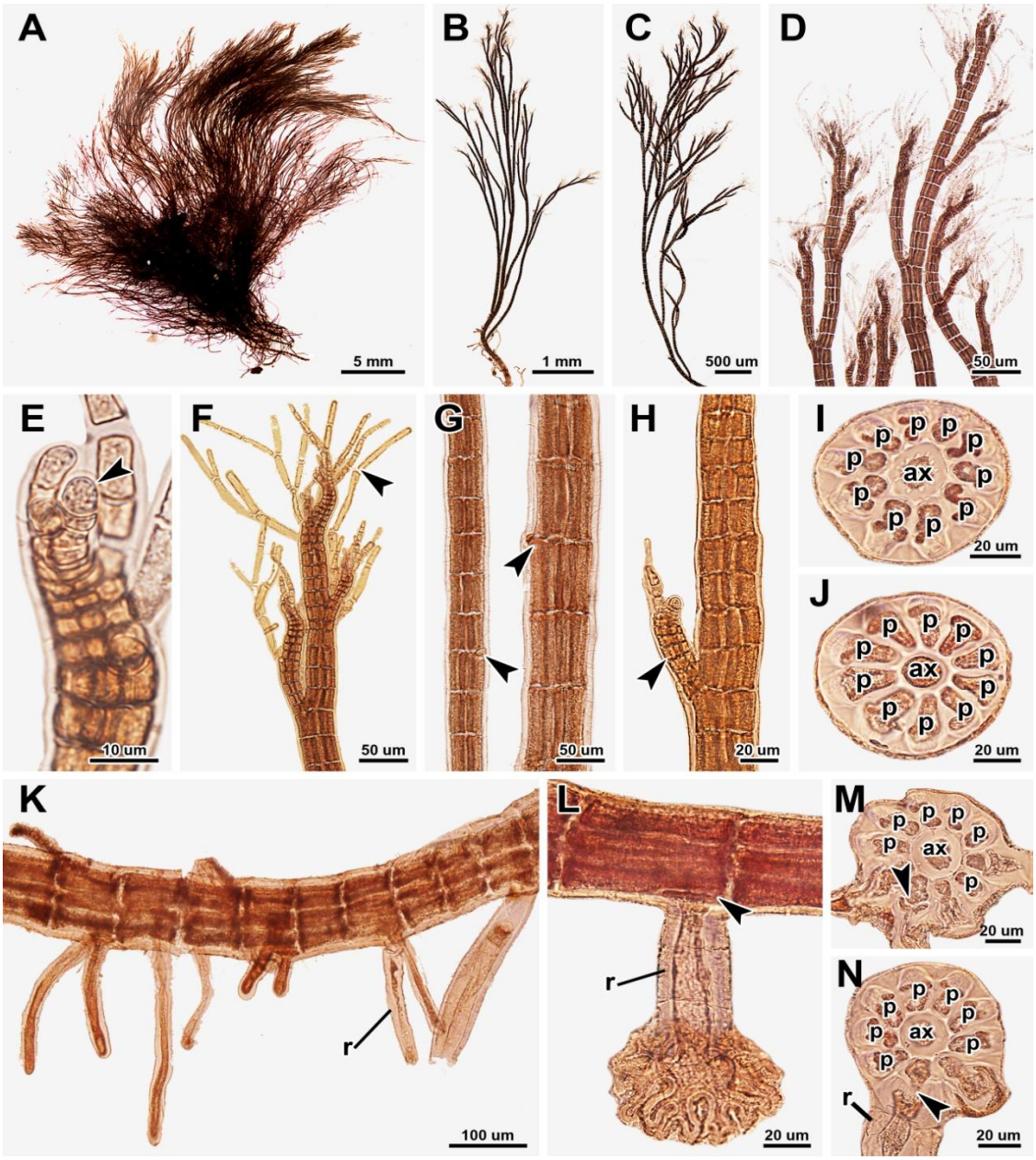


**Fig. 141.** Vegetative structures of *Brongniartella isogona* comb. nov. (A) Habit of vegetative plant. (B-D) Erect axes showing alternate branching pattern. (E) Apex showing prominent apical cell (arrowhead). (F) Apex showing abundant trichoblasts (arrowhead). (G) Axes showing prominent scar cells (arrowhead). (H) Axes showing cicatrigenous branches (arrowhead). (I-J) Cross-section views showing 10 pericentral cells (p) from the middle (I) and basal (J) part of axes (ax, axial cell). (K) Rhizoids (r) scattered and produced from prostrate axes. (L) Unicellular rhizoids (r) produced from the proximal end of pericentral cells (arrowhead) . (M-N) Cross-section views of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

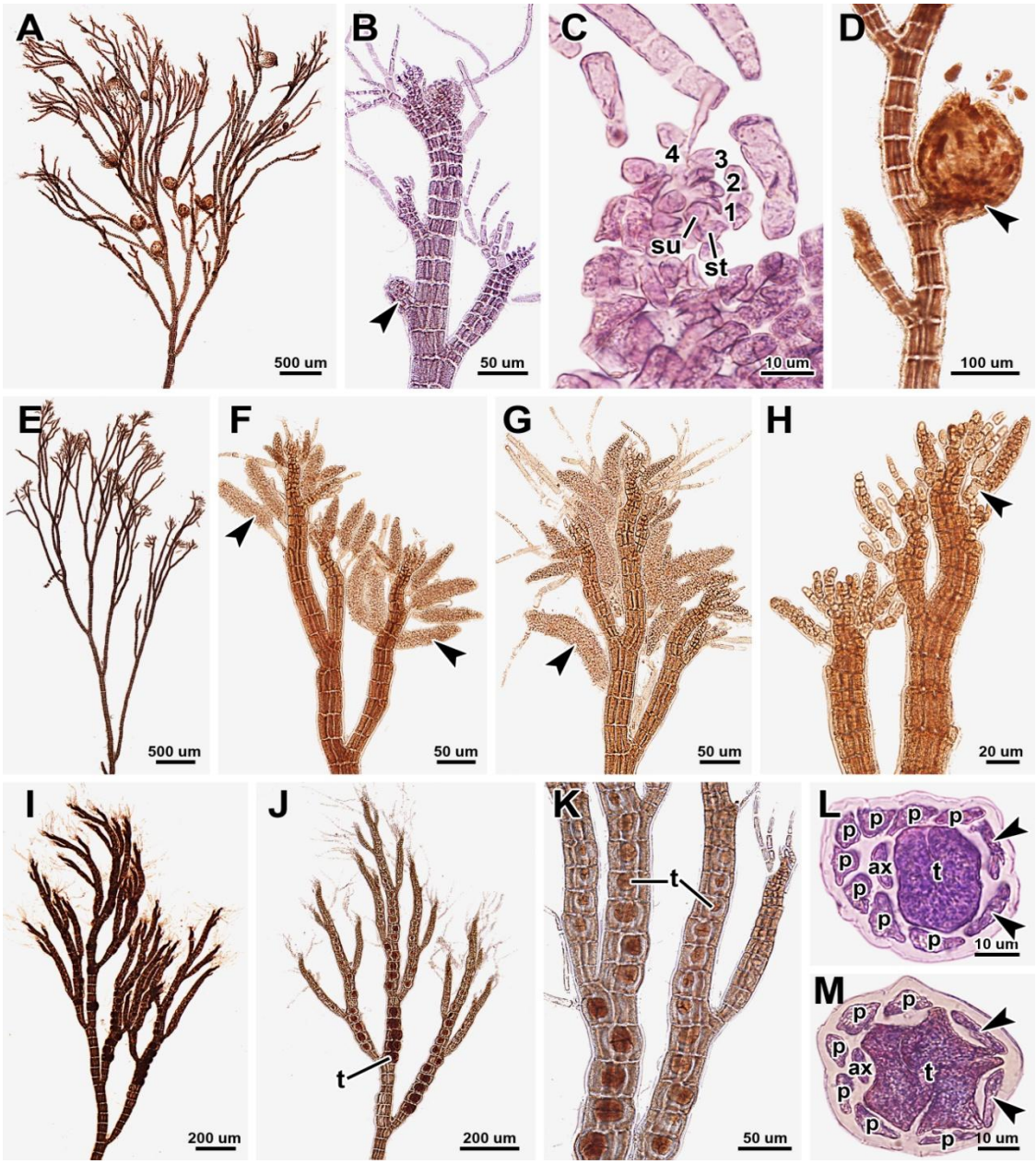


**Fig. 142.** Reproductive structures of *Brongniartella isogona* comb. nov. (A) Female plant. (B) Upper part of female thallus showing subapical cystocarps. (C) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells; st, basal sterile cell; su, supporting cell). (D) Mature cystocarps (arrowhead) with globose shape. (E) Male plant. (F-G) Spermatangial branches (arrowhead) clustered at the apices of erect axes. (H) Spermatangial branches (arrowhead) arising from trichoblasts. (I) Tetrasporangial thallus. (J-K) Apical branches showing tetrasporangia (t) in spiral series. (L-M) Cross-section with 7-9 pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

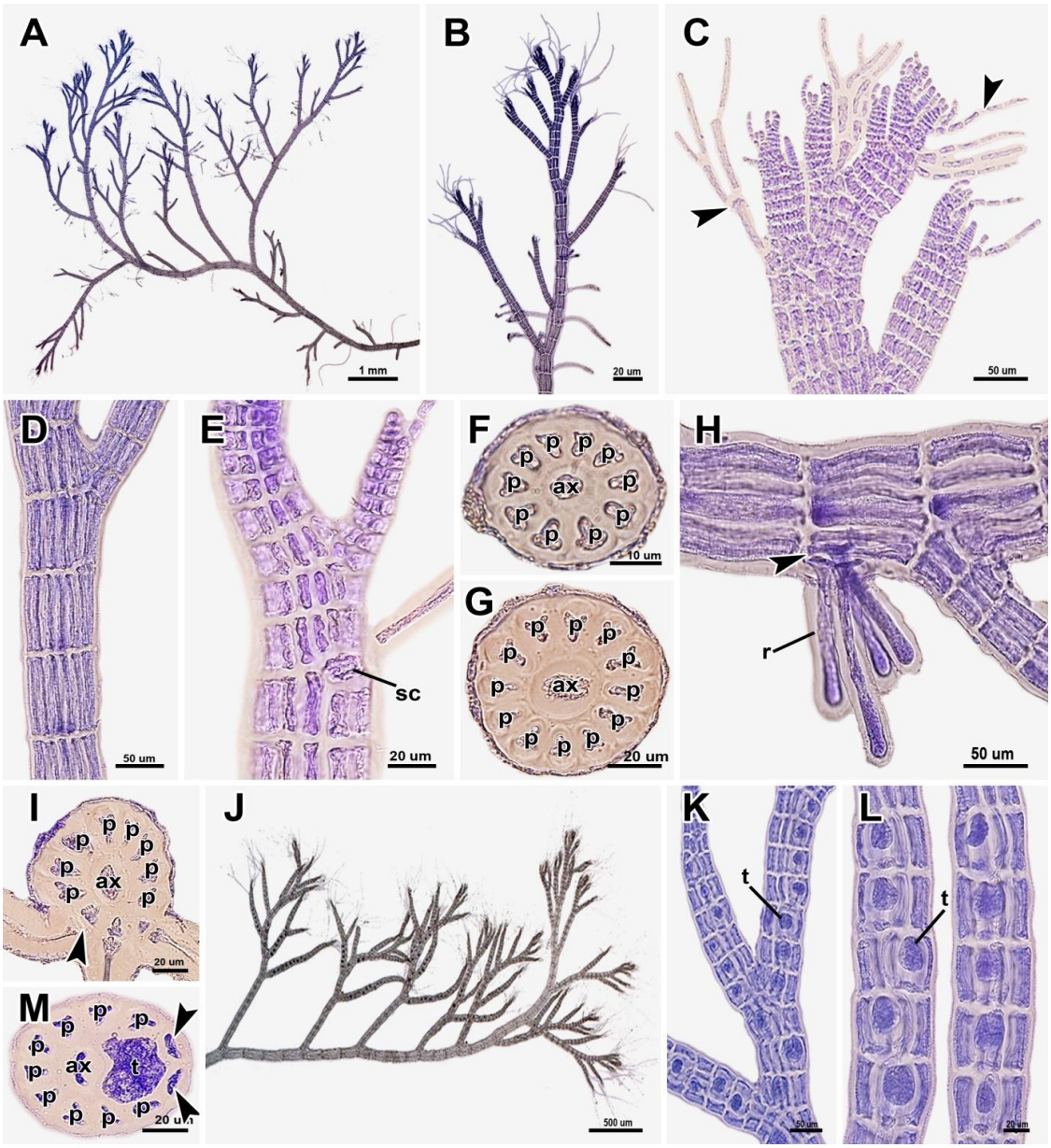


**Fig. 143.** Anatomical structures of *Enelittosiphonia hendryi* comb. nov. (A) Habit of vegetative plant. (B) Erect axes showing a pair of unilateral branches borne on one side of the axis alternating with a pair on the other side. (C) Apex showing abundant trichoblasts. (D) Old axes. (E) Prominent scar cells (sc). (F-G) Cross-section views showing 10-12 pericentral cells (p) (ax, axial cell). (H) Rhizoids (r) clustered and produced from the proximal end of pericentral cells. (I) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (J) Tetrasporangial thallus. (K-L) Apical branches showing tetrasporangia (t) in spiral and straight series. (M) Cross-section view with 10 pericentral cells (p) showing a tetrasporangium (t) rounded by cover cells (arrowheads).

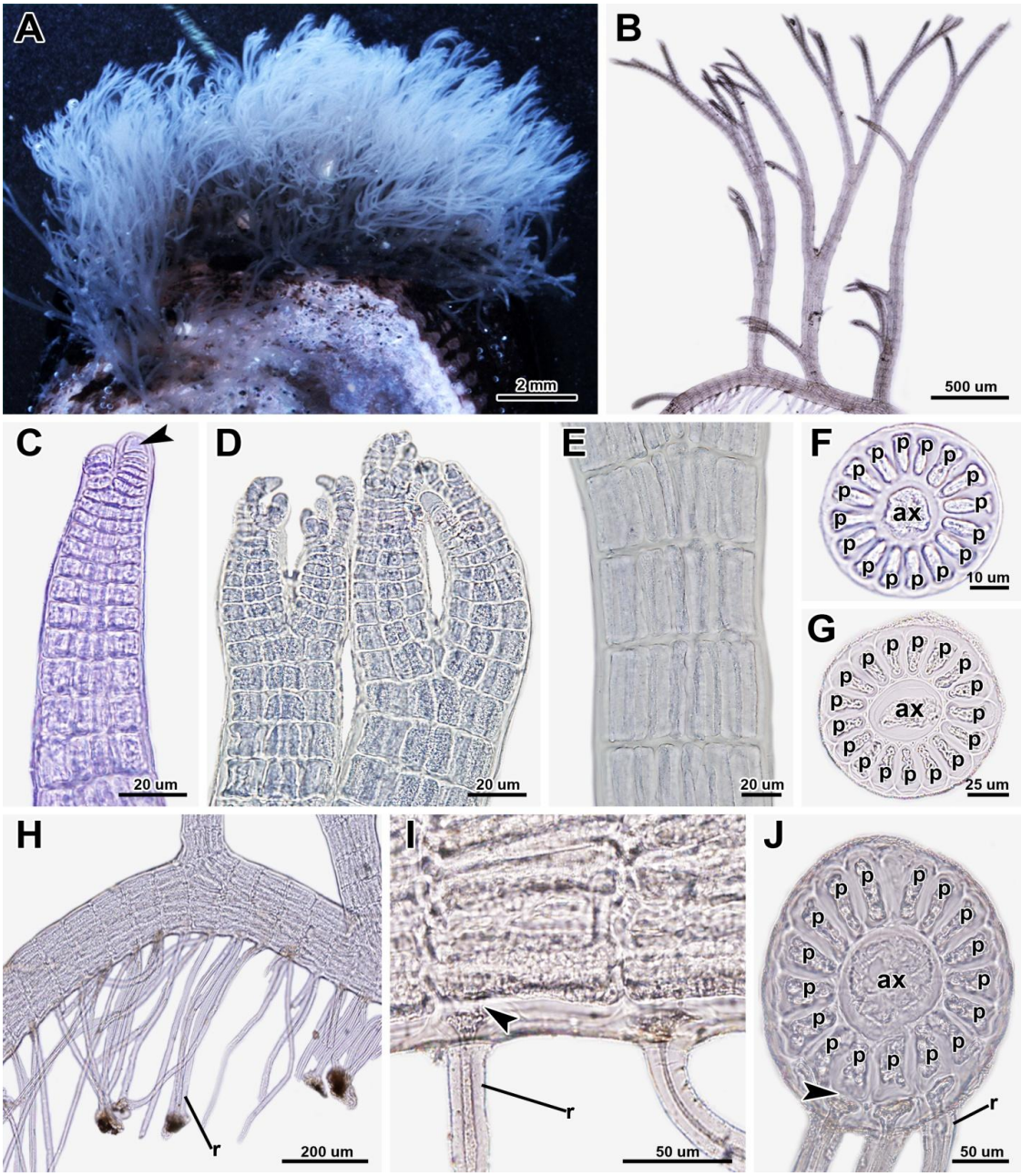


**Fig. 144.** Vegetative structures of *Polysiphonia curta*. (A) Habit of vegetative plant. (B) Plant showing pseudodichotomous branching pattern. (C) Apex showing prominent apical cells (arrowhead). (D) Apex lacking of trichoblasts. (E) Old axes lacking of cortication. (F-G) Cross-section views showing 16-17 pericentral cells (p) from the middle (F) and basal (G) part of axes (ax, axial cell). (H) Rhizoids (r) scattered and produced from prostrate axes. (I) Rhizoids (r) produced from the proximal end of pericentral cells (arrowhead) . (J) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

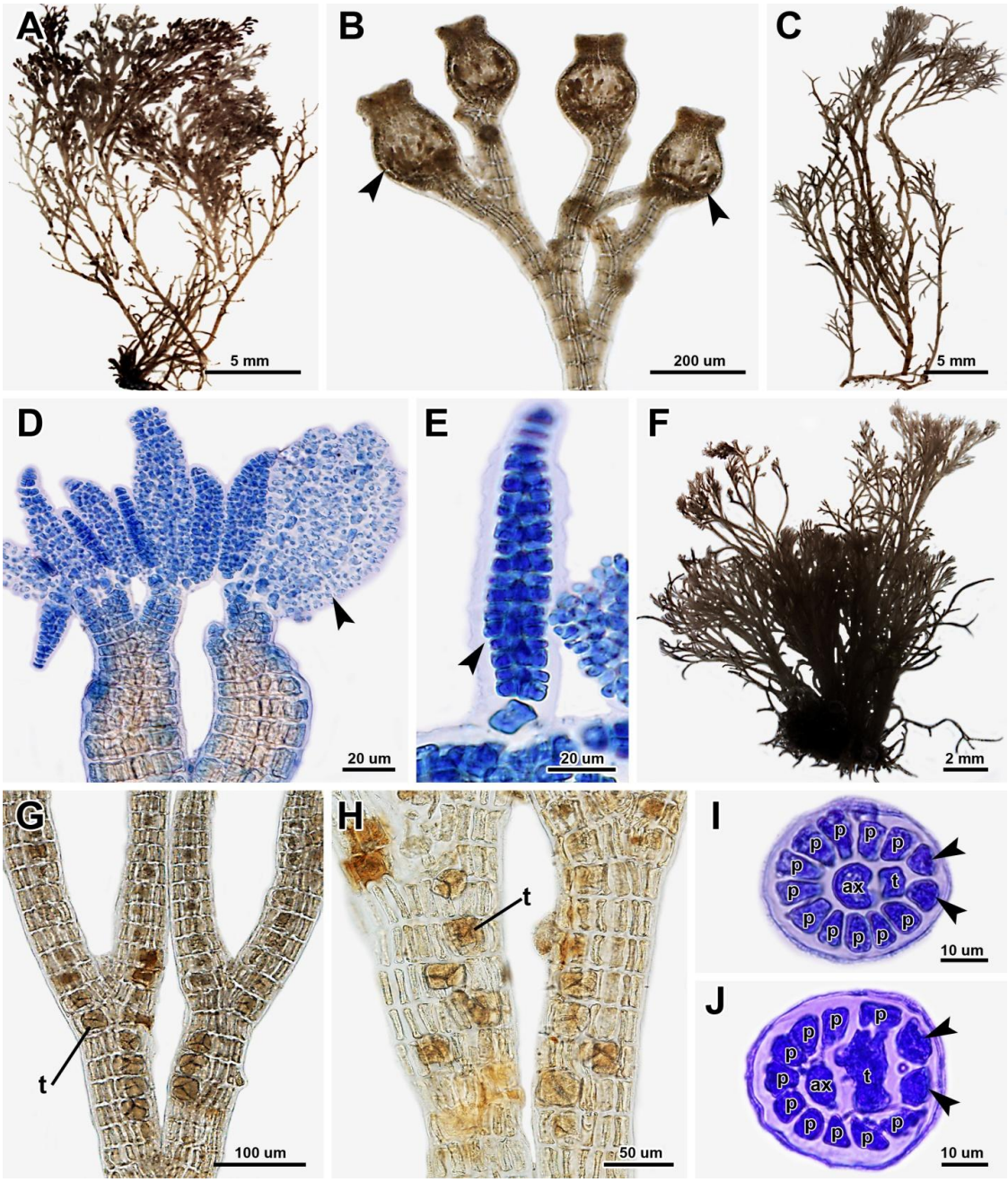


**Fig. 145.** Reproductive structures of *Polysiphonia cancellata*. (A) Female gametophyte. (B) Mature urceolate cystocarp. (C) Male gametophyte. (D) Apex showing spermatangial branches (arrowhead) clustered on the apices of erect axes. (E) Spermatangial branches (arrowhead) replacing trichoblasts. (F) Tetrasporangial plant. (G-H) Apical branches showing spiral and straight arrangement of tetrasporangia (t). (I-J) Cross-section views showing 10-11 pericentral cells showing a tetrasporangium (t) rounded by cover cells (arrowheads).

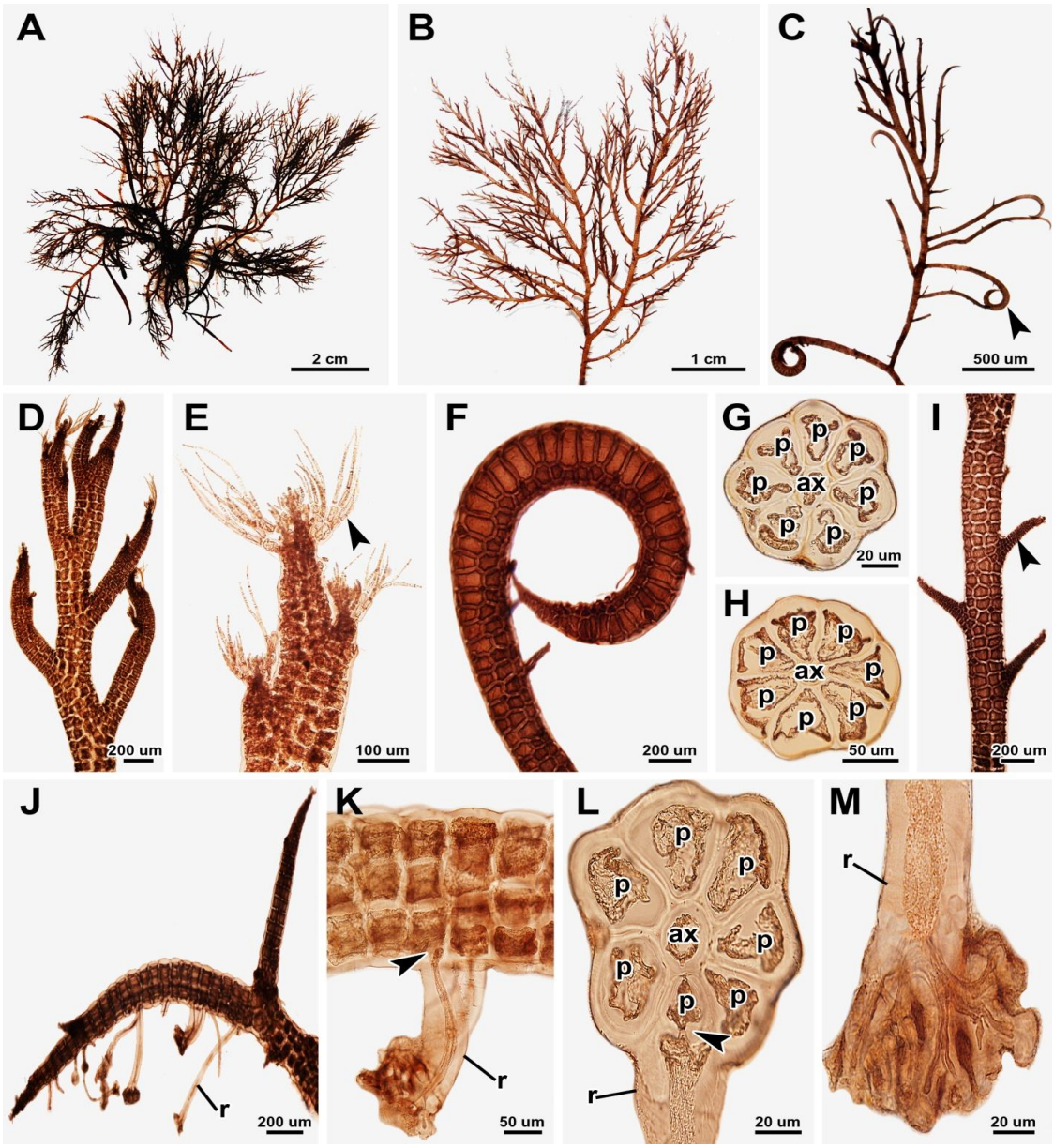


**Fig. 146.** Vegetative structures of *Polysiphonia decipiens.* (A-B) Habit of vegetative plant. (C) Erect axes showing the irregular branching pattern and hooked laterals (arrowhead). (D) Apex showing alternate branches. (E) Apex showing abundant trichoblasts (arrowhead). (F) Apical part of hooked branches. (G-H) Cross-section views showing 7 pericentral cells (p) from the middle (G) and basal (H) part of axes (ax, axial cell). (I) Erect axes showing adventitious branches (arrowhead). (J) Rhizoids (r) scattered and produced from prostrate axes. (K) Rhizoids (r) produced from the proximal end of pericentral cells (arrowhead) . (L) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p). (M) Unicellular terminations of rhizoid (r).

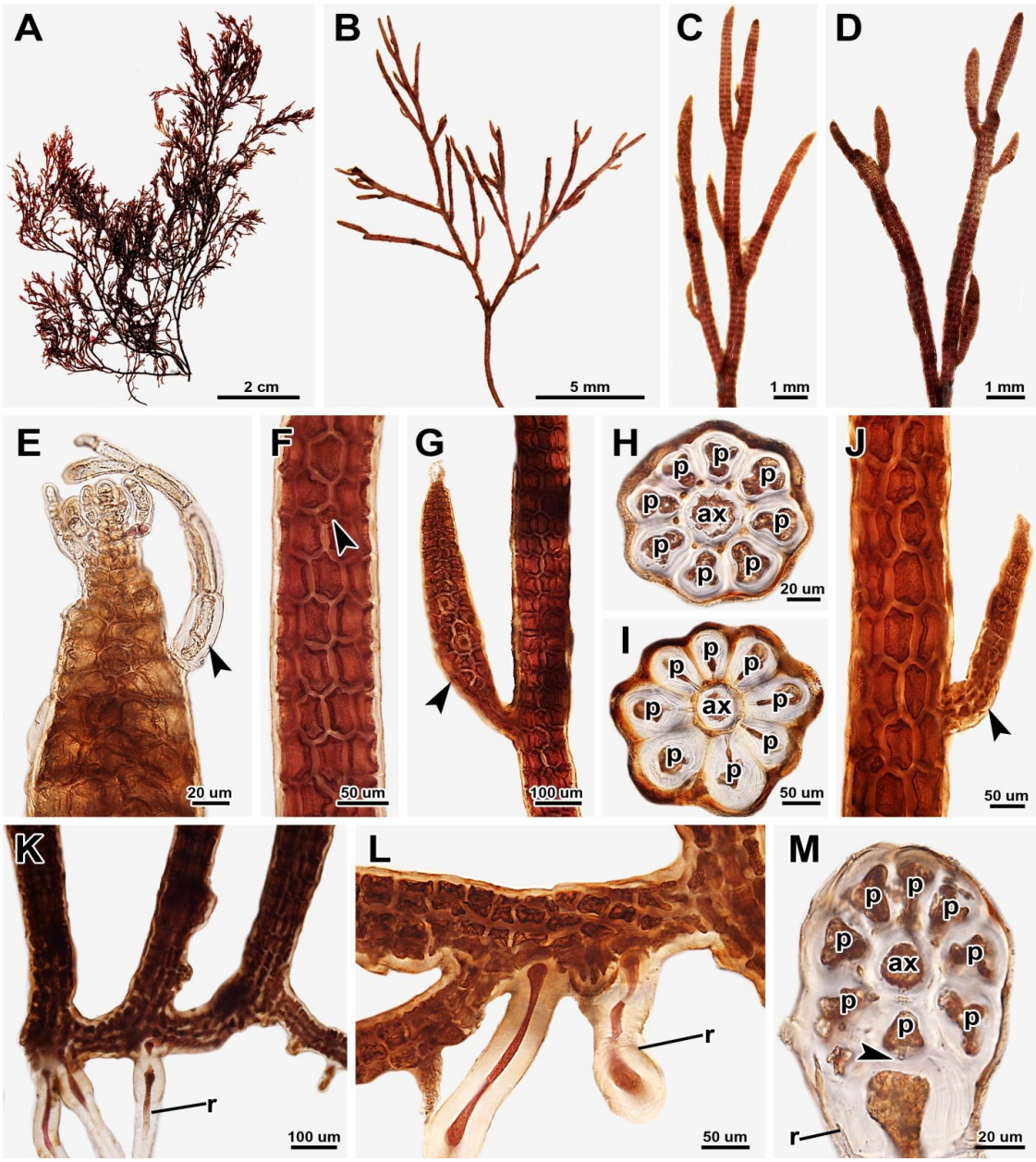


**Fig. 147.** Vegetative structures of *Polysiphonia cancellata*. (A) Habit of vegetative plant. (B) Erect axes showing the irregular branching pattern. (C-D) Apex showing laterals irregular disposed. (E) Apex showing scarce trichoblasts (arrowhead). (F) Erect axes showing prominent scar cells (arrowhead). (G) Erect axes showing a cicatrigenous branch (arrowhead). (H-I) Cross-section views showing 8 pericentral cells (p) from the middle (H) and basal (I) part of axes (ax, axial cell). (J) Erect axes showing adventitious branches (arrowhead). (K-L) Rhizoids (r) scattered and produced from prostrate axes. (M) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

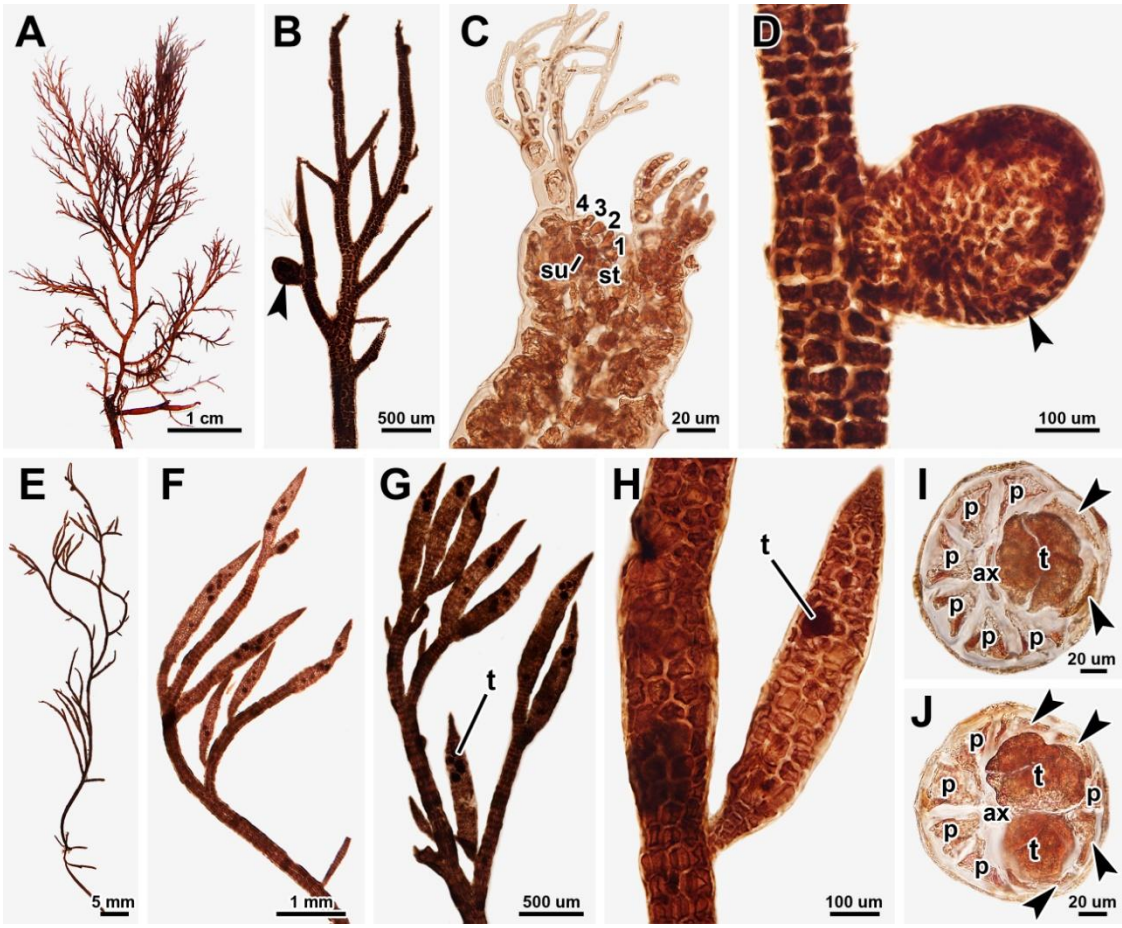


**Fig. 148.** Reproductive structures of *Polysiphonia decipiens* (A) Female gametophyte. (B) Upper part of female thallus showing subapical cystocarps (arrowhead). (C) Procarp with a four-celled carpogonial branch (1-4, sequence of carpogonial branch cells: tb, trichoblast; st, basal sterile cell; su, supporting cell). (D). Young cystocarp showing globose shape. (E) Tetrasporangial plant. (F-G) Apical branches showing spiral arrangement of tetrasporangia (t). (H) Lateral branches bearing tetrasporangia (t) in spiral series (I-J) Cross-section views showing one (I) and two (J) tetrasporangia (t) per fertile segment rounded by cover cells (arrowheads) (p, pericentral cells).

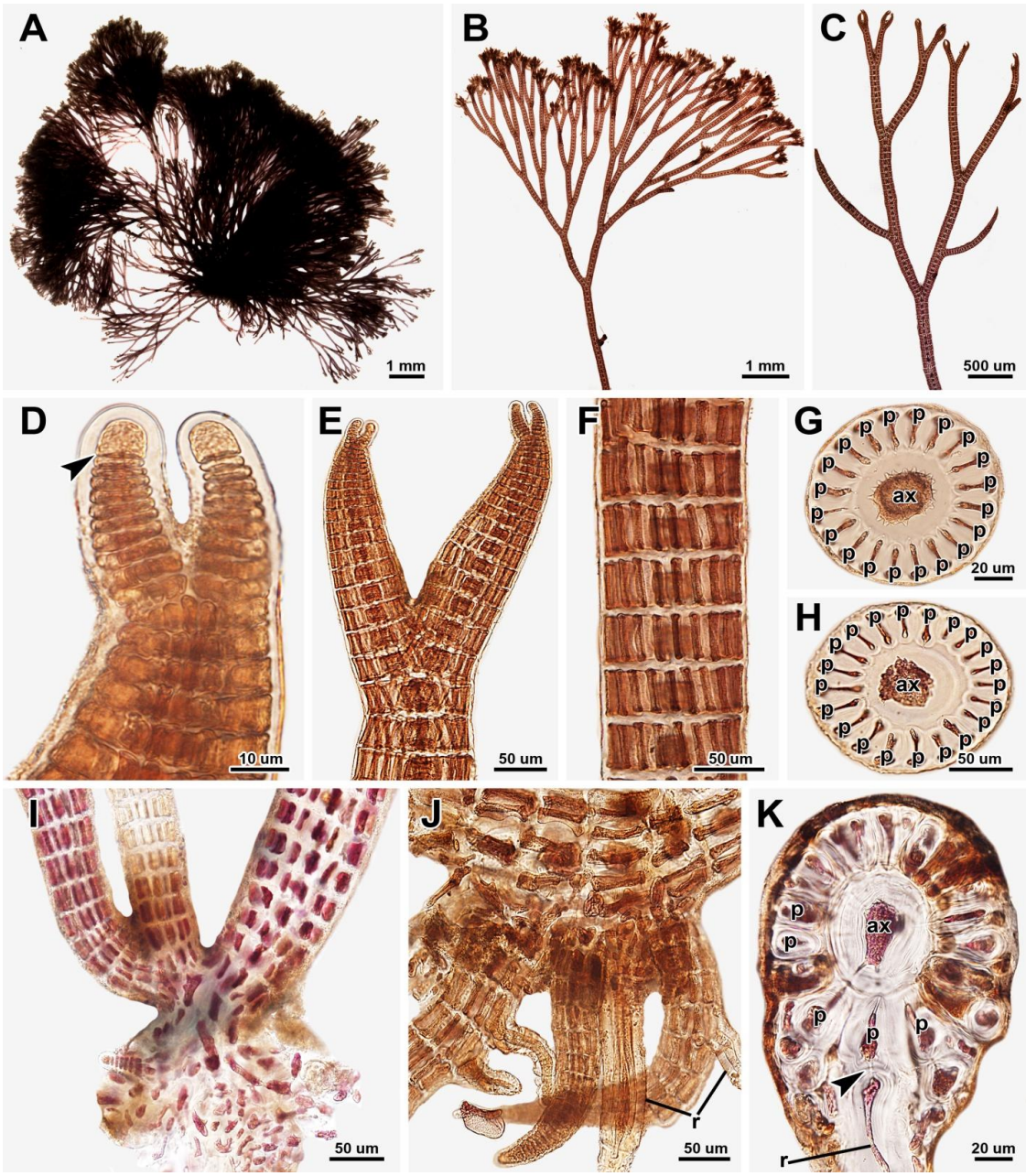


**Fig. 149.** Vegetative structures of *Vertebrata lanosa*. (A) Habit of vegetative plant. (B-C) Erect axes showing dichotomous branching pattern. (D) Apex showing prominent apical cells (arrowhead) transversally divided. (E) Apex lacking of trichoblasts. (F) Old axes lacking of cortication and scar cells. (G-H) Cross-section views showing 20-21 pericentral cells (p) from the middle (G) and basal (H) part of axes (ax, axial cell). (I) Rhizoids (r) clustered on the basal part of axes. (J) Secondary rhizoids (r) clustered and produced from prostrate axes. (K) Cross-section view of a prostrate axis showing rhizoid (r) cutting off (arrowhead) from pericentral cells (p).

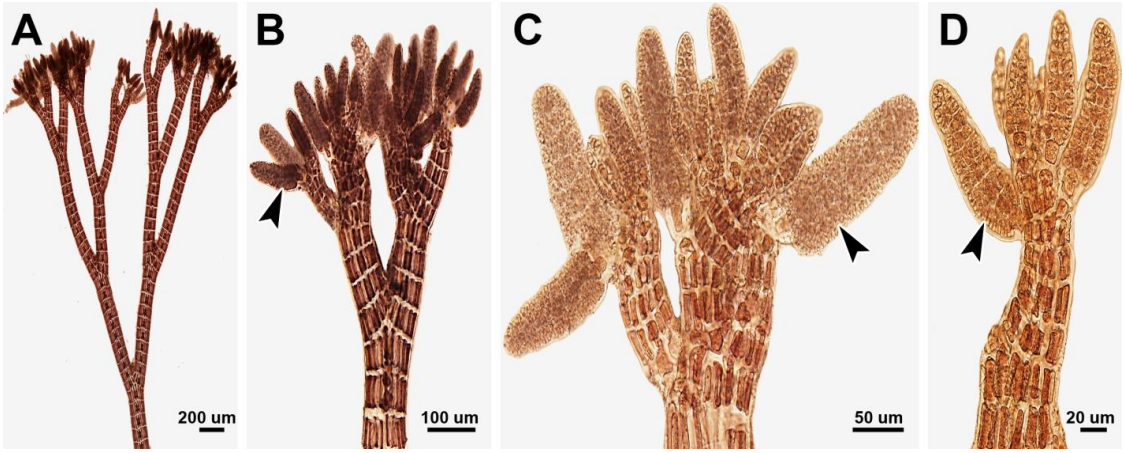


**Fig. 150.** Reproductive structures of *Vertebrata lanosa*. (A) Male gametophyte. (B-C) Apex showing spermatangial branches (arrowhead) clustered on the apices of erect axes. (D) Spermatangial branches (arrowhead) replacing trichoblasts.

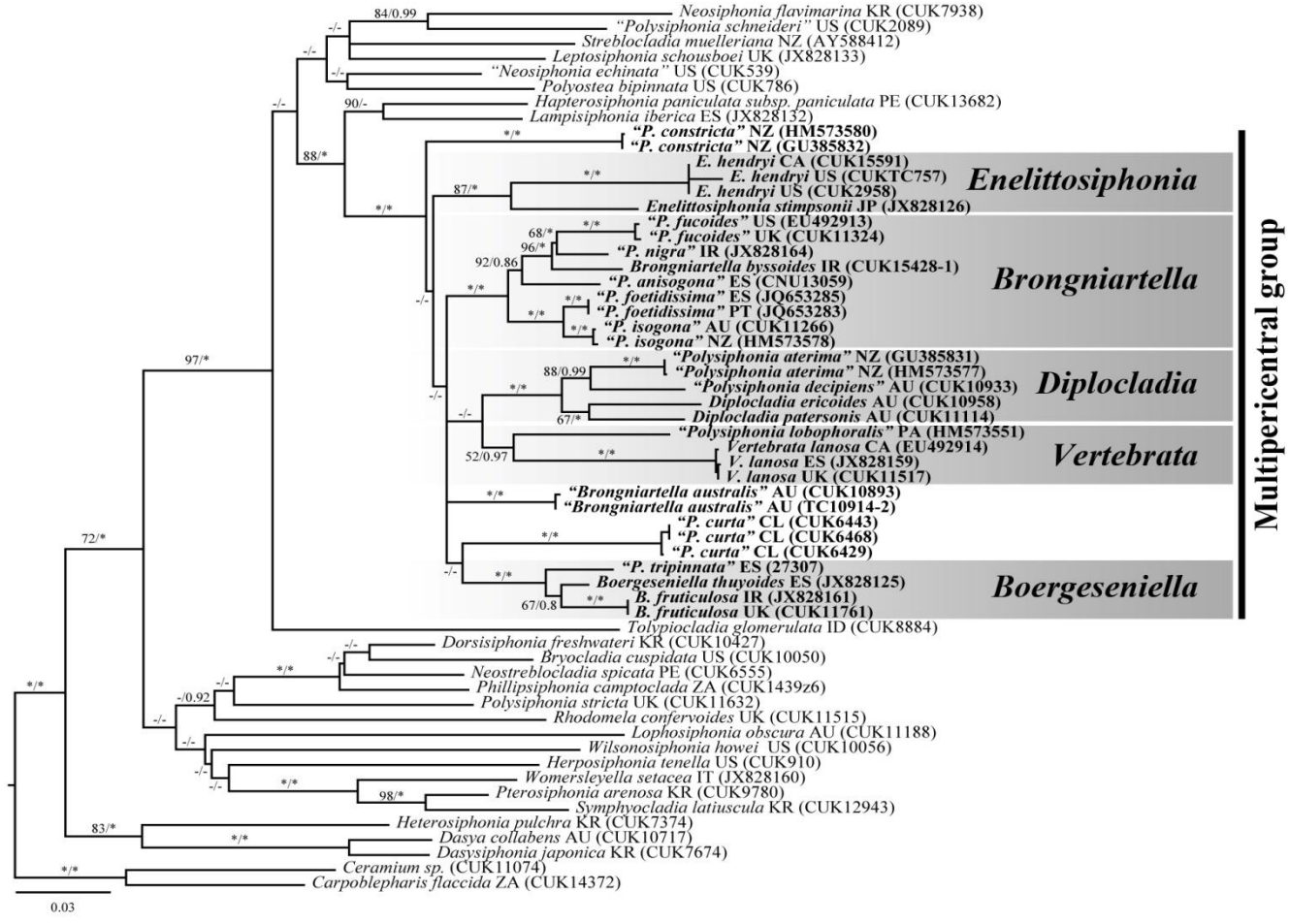


**Fig. 151.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

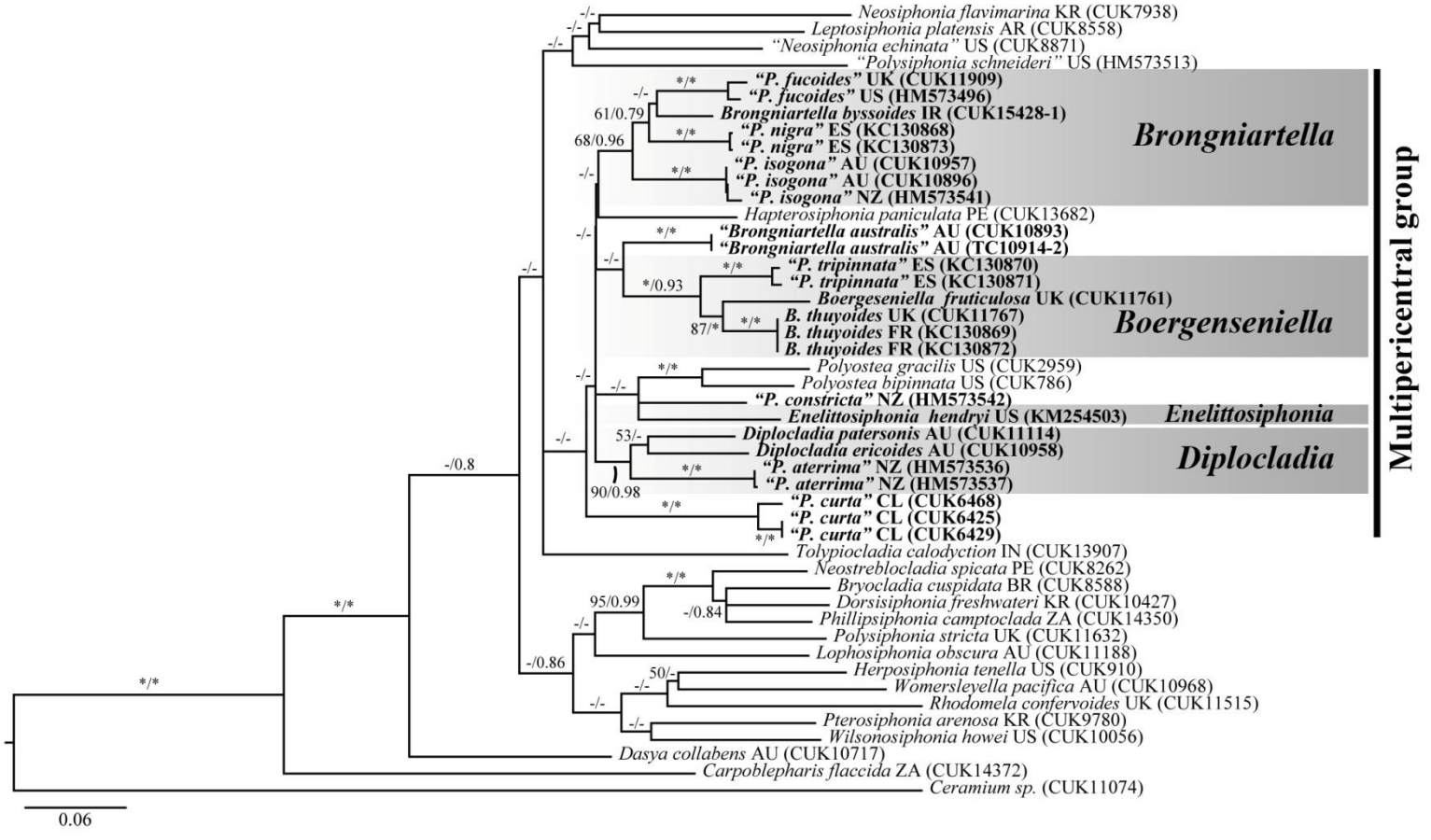


**Fig. 152.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 9.** Comparisons among the genera embedded in the multipericentral group.

| **Features** | ***Boergeseniella*** | ***Brongniartella*** | ***Diplocladia*** | ***Enelittosiphonia*** | ***"Polysiphonia"*** | ***Vertebrata*** |
|---|---|---|---|---|---|---|
| Cortication | Present | Absent | Absent | Absent | Absent | Absent |
| Trichoblasts | Present | Present | Present | Present | Present | Absent |
| Branching pattern | Alternate-distichous (bilateral phyllotaxy) | Irregular | Radial determinate branches | Strictly dorsiventral | Alternate to irregular | Dichotomous |
| Rhizoids disposition | Clustered | Scattered | Scattered | Scattered | Scattered | Scattered or clustered |
| References | Maggs and Hommersand (1993), this study | Parsons (1980), this study | Womersley (2003), this study | Segi (1949), this study | Kim et al. (2000), this study | Kim et al. (2002), this study |

## CHAPTER 9. Taxonomy and phylogeny of the multipericentral group: genus *Diplocladia* (Rhodomelaceae, Rhodophyta)

*Polysiphonia* sensu lato is composed of around 220 species widely distributed in marine environments (Guiry and Guiry 2015). The recent molecular studies divided *Polysiphonia* sensu lato into five strongly supported clades: *Hapterosiphonia* D.E.Bustamante, B.Y.Won et T.O.Cho, *Lampisiphonia* H.G. Choi, Diaz-Tapia et Bárbara, *Neosiphonia* M.S. Kim et I.K. Lee, *Polysiphonia* sensu stricto Greville, and the multipericentral group (Choi et al. 2001, Bárbara et al. 2013, Bustamante et al. 2015b, 2015c). This multipericentral group was defined originally by Choi et al. (2001) and included the following four genera from the north Atlantic and northwest Pacific: *Boergeseniella* Kylin, *Enelittosiphonia* Segi, *Polysiphonia* Grev., and *Vertebrata* S.F. Gray. These genera are sharing the important synapomorphic feature of more than five pericentral cells, and the included species variously share other key anatomical features with *Neosiphonia* and *Polysiphonia* sensu stricto (Choi et al. 2001). The evolutionary study of the Ceramiales by Yang (2007) sequenced the type species of the genus *Brongniartella* Bory de Saint-Vincent and the molecular analysis of *Polysiphonia* sensu lato by Kim and Kim (2014) and Bustamante et al. (2015c) showed that *Brongniartella* was embedded into the multipericentral group.

The monotypic genus *Diplocladia* was described by Kylin (1956) on the basis of *D. patersonis* from the Australian coast and the monotypic genus *Perrinia* was described by Womersley (2003) on the basis of *P. ericoides* from Tasmania. These two genera have in common the presence of needle-like branches spirally disposed and have been closely related in morphology to the genus *Bryocladia* F.Schmitz (Schmitz 1897). Although the morphological similarities among these three genera are evident (Abbott and Hollenberg 1976, Womersley 2003), their phylogenetic relationships have not been confirmed.

In the present study, we collected some polysiphonous specimens having in common needle-like branches. These specimens, *Diplocladia patersonis* (Sonder) Kylin and *Perrinia ericoides* (Harvey)

Womersley, were collected from the vicinity of their type localities in the Australian coast. We reassessed these specimens based on the detailed morphology and the phylogenetic relationships with other similar species by analyzing the *rbc*L and *cox*1 sequences. Here, we propose the synonymy of the genus *Perrinia* with the genus *Diplocladia* and the new combination *Diplocladia ericoides.*

## 1. Morphological analyses

### *Diplocladia* Kylin

**Diagnosis:** Plants prominent, forming reduced tufts predominantly attached to rock surfaces. Thalli composed of an entangled prostrate basal system or stolones, interwoven main erect axes bearing indeterminate laterals, and all branches covered by needle-like determinate branches. Erect axes composed of 7–13 pericentral cells, ecorticate throughout, and determinate laterals densely and spirally disposed with 1-4 orders. Adventitious branches present in main axes but not in determinate branches. Lateral branches arising in connection with trichoblasts. Trichoblasts delicate, deciduous, prominent. Rhizoids cut off from the pericentral cells, with lobed unicellular terminations. Procarps positioned laterally on determinate branches, each composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell. Spermatangial replacing the whole trichoblast (Womersley 2003). Tetrasporangia tetrahedral, in spiral arrangement, single or paired per segment.

**Type species:** *Diplocladia patersonis* (Sonder) Kylin

**Key to species of *Diplocladia***

1. Pericentral cells 7, one single tetrasporangium per fertile segment ..... ***Diplocladia patersonis***
1. Pericentral cells 10-13, two tetrasporangia per fertile segment ..... ***Diplocladia ericoides***

***Diplocladia patersonis* (Sonder) Kylin (Fig. 154-155)**

**Basionym:** *Polysiphonia patersonis* Sonder.

**Homotypic synonyms:** *Polysiphonia patersonis* Sonder, *Polysiphonia spinosissima* Harvey, *Vertebrata patersonis* (Sonder) Kuntze, *Brongniartella spinosissima* (Harvey) Falkenberg, *B. patersonis* (Sonder) De Toni.

**Type locality:** Cape Paterson, Victoria, Australia.

**Distribution:** Australia (Fig. 153).

**Specimens examined:** CUK 11114 (Kingscote, Kangaroo Island, Australia collected by T.O.C and D.E.B, Mar. 26 2012).

**Vegetative morphology:** Plants were attached on rock surface in the intertidal zone. Thalli form reduced tufts, 1.7 to 6.1 cm high, and darkish-red in colour (Fig. 154A). They are composed of prostrate and erect systems with delicate texture. The erect system is composed of interwoven and indeterminate main axes and arises exogenously from the prostrate axes (Fig. 154A). Main erect axes are prominent which are covered by needle-like determinate branches (Fig. 154B). Erect axes are radially branched in irregular pattern for 4-6 orders. Apical cells are prominent, 5.9 ± 3.9 μm × 5.5 ± 4.5 μm in size, and transversely divided (Fig. 154C). Trichoblasts are delicate, numerous, 1-2 times forked, 305.5 ± 123.4 μm long, arise on each segment, and prominent on determinate branches (Fig. 154C-D). Cicatrigenous branches are absent. Needle-like determinate branches are abundant, formed by short segments, arising spirally, and 1-3 orders of branches (Fig. 154E-G). Indeterminate laterals of erect axes are 325.7 ± 38.3 μm long and 352.7 ± 26.8 μm in diameter (L:D 0.1 ± 0.2). Each segment is completely ecorticated along the thallus and composed of seven pericentral cells (Fig. 154H-I). Adventitious branchlets are scarce and arise from the main filaments, but absent in the determinate branches. The prostrate system is extended (Fig. 154J) and forming entangled stolones. Segments of the prostrate system are 54.5 ± 4.9 μm long and 83.5 ± 6.7 μm in

diameter (L:D 1.5 ± 0.7). Rhizoids are ventrally produced from the centre or the proximal end of pericentral cells (Fig. 154K-L). They are cut off from pericentral cells (Fig. 154M-N). Rhizoids are unicellular and 296.3 ± 84.8 μm long and 21.0 ± 5.7 μm with multilobed terminations (Fig. 154O).

**Reproductive morphology:** In tetrasporophytes (Fig. 155A), tetrasporangia are tetrahedrally divided, developed on needle-like determinate branches (Fig. 155B-C), and are 31.9 ± 6.7 μm × 32.5 ± 7.6 μm in size. Tetrasporangial branches are swollen, sinuous, and distorted (Fig. 155D). Tetrasporangia are arranged in interrupted spiral series (Fig. 155D). The fertile segments have 6 pericentral cells. The fertile pericentral cell is developed as a stalk cell, which will develop a tetrasporangium and two presporangial cover cells (Fig. 155E-F). One tetrasporangium is produced on each segment (Fig. 155E-F). Although female and male plants were not found, Womersley (2003) characterized in detail these reproductive features.

**Habitat:** Plants grow in the intertidal zone, forming tufts. They are attached on rocks in sheltered sites. Tufts of this species are usually very flaccid and are highly covered with epiphytes and other filamentous species.

***Diplocladia ericoides* (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho comb. nov. (Fig. 156)**

**Basionym**: *Polysiphonia ericoides* Harvey in Hooker and Harvey.

**Homotypic synonyms**: *Polysiphonia ericoides* Harvey, *Bryocladia ericoides* (Harvey) F. Schmitz.

**Type locality**: Tasmania, Australia.

**Distribution**: Australia and New Zealand (Adams 1994, Womersley 2003) (Fig. 153).

**Specimens examined:** CUK 10958 (Lagoon Beach, Tasmania, Australia collected by T.O.C and D.E.B, Mar. 23 2012).

**Vegetative morphology:** Plants were attached on rock surface in the intertidal zone. Thalli form reduced tufts, 2.1 to 5.9 cm high, and darkish-red in colour (Fig. 156A). They are composed of reduced prostrate and robust erect systems. The erect system is composed of interwoven and indeterminate main axes and arises exogenously from the prostrate axes. Main erect axes are prominent which are covered by abundant needle-like determinate branches (Fig. 156B-C), becoming denuded near to the base (Fig. 156B). Erect axes are radially branched in irregular pattern for 2-3 orders. Apical cells are prominent, 7.2 ± 1.1 μm × 6.1 ± 1.3 μm in size, and transversely divided (Fig. 156D). Trichoblasts are delicate, twice forked, 104.8 ± 27.7 μm long, arise on each segment (Fig. 156D), and prominent on determinate branches. Cicatrigenous branches are absent. Needle-like determinate branches are abundant, formed by short segments, arising spirally, and 1-3 orders of branches (Fig. 156E-F). Indeterminate laterals of erect axes are 313.8 ± 101.3 μm long and 421.9 ± 121.4 μm in diameter (L:D 1.73 ± 0.67 ). Each segment is completely ecorticated along the thallus and composed of 10-13 pericentral cells (Fig. 156G-H). Adventitious branchlets are scarce and arise from the main filaments, but absent in the determinate branches. The prostrate system is reduced (Fig. 156I) and forming short stolones. Segments of the prostrate system are 68.9 ± 31.9 μm long and 163.8 ± 44.1 μm in diameter (L:D 0.5 ± 0.2). Rhizoids are ventrally produced from the centre or the proximal end of pericentral cells (Fig. 156J). They are cut off from pericentral cells (Fig. 156K). Rhizoids are unicellular and 1251.2 ± 850.5 μm long with multilobed terminations (Fig. 156L).

**Reproductive morphology:** In female plants (Fig 156M), procarps are formed laterally and subapical on the needle-like determinate branches (Fig. 156N-O) and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 156Q). Cystocarps are globose to ovate when mature, 321.4 ± 101.1 μm high and 231.4 ± 154.9 μm in diameter. Although male plants and tetrasporophytes were not found, Womersley (2003) characterized in detail these reproductive features.

**Habitat:** Plants grow in the intertidal zone, forming tufts. They are attached on rocks in sheltered sites. Tufts of this species are usually very robust and are associated with other filamentous species.

## 2. Phylogenetic analyses

We sequenced a 1442-bp portion of *rbc*L (98.3%) and 1394-bp portion of *cox*1 (95.0%) for material of *Diplocladia ericoides* and *D. patersonis* from Australia. These sequences were aligned with additional sequences of specimens belonging *to Polysiphonia* sensu lato downloaded from GenBank. We used *Lophosiphonia* sp. (GU385835) and *Herposiphonia* sp. (GU385834) as outgroups for our *rbc*L alignment and *Womersleyella pacifica* (CUK10968) and *Polysiphonia ulluengensis* (CUK9483) for our *cox*1 alignment. These phylogenetic analyses showed that both *D. patersonis* and *D. ericoides* (as *Perrinia ericoides*) were embedded on the multipericentral clade (Fig. 157-158) and close related to "*P. aterrima*" from New Zealand diverging 5.5% to 6.1% for *rbc*L and 8.4% to 9.0% for *cox*1 . The sequence divergences between *D. patersonis* and *D. ericoides* were 5.6% for *rbc*L and 6.4% for *cox*1.

## 3. Discussion

Our *rbc*L and *cox*1 phylogenies revealed the very close relationship of the monotypic genera *Diplocladia* Kylin (1956) and *Perrinia* Womersley (2003) on the basis of new material collected from the vicinity of their type localities. Our phylogenetic analyses resolved together *P. ericoides* and *D. patersonis* in a well supported clade and based on the priority of *Diplocladia* over *Perrinia*, *Perrinia* is placed under synonymy with *Diplocladia* and the new combination *D. ericoides* is proposed. Although the genus *Perrinia* was distinguished from *Diplocladia* by Womersley (2003) in having 13-15 pericentral cells, scarce trichoblasts, and paired tetrasporangia; our morphological and molecular analyses have confirmed these characters as inconsistent to delimit these genera. Womersley

(2003) also considered that the lacking of endogenous branches on the determinate branches is a distinguishing feature to transfer *Diplocladia ericoides* from *Bryocladia* to its own genus. *Bryocladia* is having endogenous branches growing on the determinate branches (Schmitz and Falkenberg 1897). Our morphological analyses found scarce adventitious branches arising from the main axes but absent on the determinate branches. This support the segregation of *D. ericoides* from *Bryocladia* but not to its own genus, instead it confirms its placement in the genus *Diplocladia*, a genus which is also lacking of adventitious branches growing on the determinate branches.

Among the features delineating *Diplocladia*, the needle-like branches spirally disposed without adventitious branches in combination with the rhizoids cutting off from pericentral cells are the principal characters that separate this genus from other multipericentral group members and other *Polysiphonia* sensu lato (Table 10). The needle-like branches have also been reported in other polysiphonous genera of Rhodomelaceae (e.g. *Bryocladia*, *Streblocladia* F.Schmitz, *Tolypiocladia* F.Schmitz) (Schmitz and Falkenberg 1897). It shows that the needle-like branches is a convergent character state that likely segregate different genera in combination with other features as cortication (e.g. *Streblocladia* in (Phillips 2010)), pericentral cells number (e.g. *Tolypiocladia* in Schmitz and Falkenberg (1897)), and adventitious branches and rhizoids-pericentral cells connection (e.g. *Bryocladia*, see chapter 5).

Although the connection of rhizoids with pericentral cells has not been evaluated as a consistent feature to delimitate genera bearing needle-like branches (Womersley 2003), this character has shown to be consistent in segregating other polysiphonous genera (e.g. *Polysiphonia* sensu stricto is having open connection, whereas *Neosiphonia* is having close connection) (Mamoozadeh and Freshwater 2011, Bustamante et al. 2012, 2015c). Our morphological and molecular analysis have demonstrated that species having needle-like branches lacking of adventitious branches in combination with rhizoids cutting off from pericentral cells are embedded in the multipericentral group (e.g. genus *Diplocladia*), whereas those in combination with rhizoids in open connection with peri-

central cells are close related to *Bryocladia* in the paraphyletic *Polysiphonia* sensu stricto (see chapter 5).

The genus *Diplocladia* is composed of the generitype *D. patersonis* and the new combination *D. ericoides*. *Diplocladia patersonis* was originally described as *Polysiphonia patersonis* by Sonder (1855) from Victoria, Australia and later transferred to *Diplocladia* by Kylin (1956). *Diplocladia patersonis* has as diagnostic characters: seven pericentral cells, ecorticated throughout, and abundant trichoblasts spirally disposed (Womersley 2003, this study). In addition to these features, Womersley (2003) also considered the differentiation of indeterminate branches and short determinate branches as well defined characters. This species is restricted only to the Australian coast (Womersley 2003).

*Diplocladia ericoides* comb. nov. was originally described as *Polysiphonia ericoides* by Hooker and Harvey (1847) from Tasmania, then transferred to *Bryocladia* by F.Schmitz in Falkenberg (1901) and later positioned on its own genus, namely *Perrinia*, by Womersley (2003). The diagnostic characters of *D. ericoides* are 13-15 pericentral cells, ecorticated throughout, and usually two tetrasporangia paired per segment (Womersley, this study). This species is restricted to Australia (Fig. 152) and New Zealand (Adams 1994, Womersley 2003). Although *Diplocladia ericoides* and *D. patersonis* are similar in habit and external morphology, they are distinguished each other by the pericentral cells number, trichoblast abundance, and number of tetrasporangia per segment. *D. patersonis* has always seven pericentral cells, abundant trichoblasts, and single tetrasporangia per segment; whereas *D. ericoides* has 10-13 pericentral cells, scarce trichoblasts, and usually two tetrasporangia per segment. *Diplocladia ericoides* and *D. patersonis* are similar with other species of the genus *Bryocladia*, but the species of *Diplocladia* are distinguished by having determinate branches lacking of adventitious and rhizoids cutting off from the center or the proximal end of pericentral cells. This morphological differentiation is well supported in our phylogenetic analyses with the placement of species with open connected rhizoids in a close relationship to *Polysiphonia*

sensu stricto (e.g. genus *Bryocladia*) and species with close connected rhizoids embedded in the multipericentral group (e.g. genus *Diplocladia*) (Fig. 157-158).

The results of our *rbc*L and *cox*1 phylogenetic analyses revealed that *Diplocladia* is closely related, with high support [100% in ML and 1 in BI for *rbc*L and 90% in ML and 0.91 in BI for *cox*1], to *Polysiphonia aterrima* J.D.Hooker et Harvey. However, the latter is distinguished from the species of the genus *Diplocladia* by lacking of determinate branches spirally disposed (Adams 1994, Womersley 2003).

Choi et al. (2001) grouped several genera in the multipericentral group with the synapomorphic features of more than five pericentral cells and rhizoids cutting off pericentral cells. Our morphological analyses revealed that the genus *Diplocladia* is sharing these features and our phylogenetic analyses included *Diplocladia* into the multipericentral group and confirming the complex paraphyly of this group. Thus, the multipericentral group is currently composed of six genera: *Boergeseniella, Brongniartella, Diplocladia*, *Enelittosiphonia*, *Polysiphonia*, and *Vertebrata*.

**Fig. 153.** Distribution of the species of the genus *Diplocladia* from Australia. *Diplocladia patersonis* (blue) and *Diplocladia ericoides* comb. nov. (yellow).

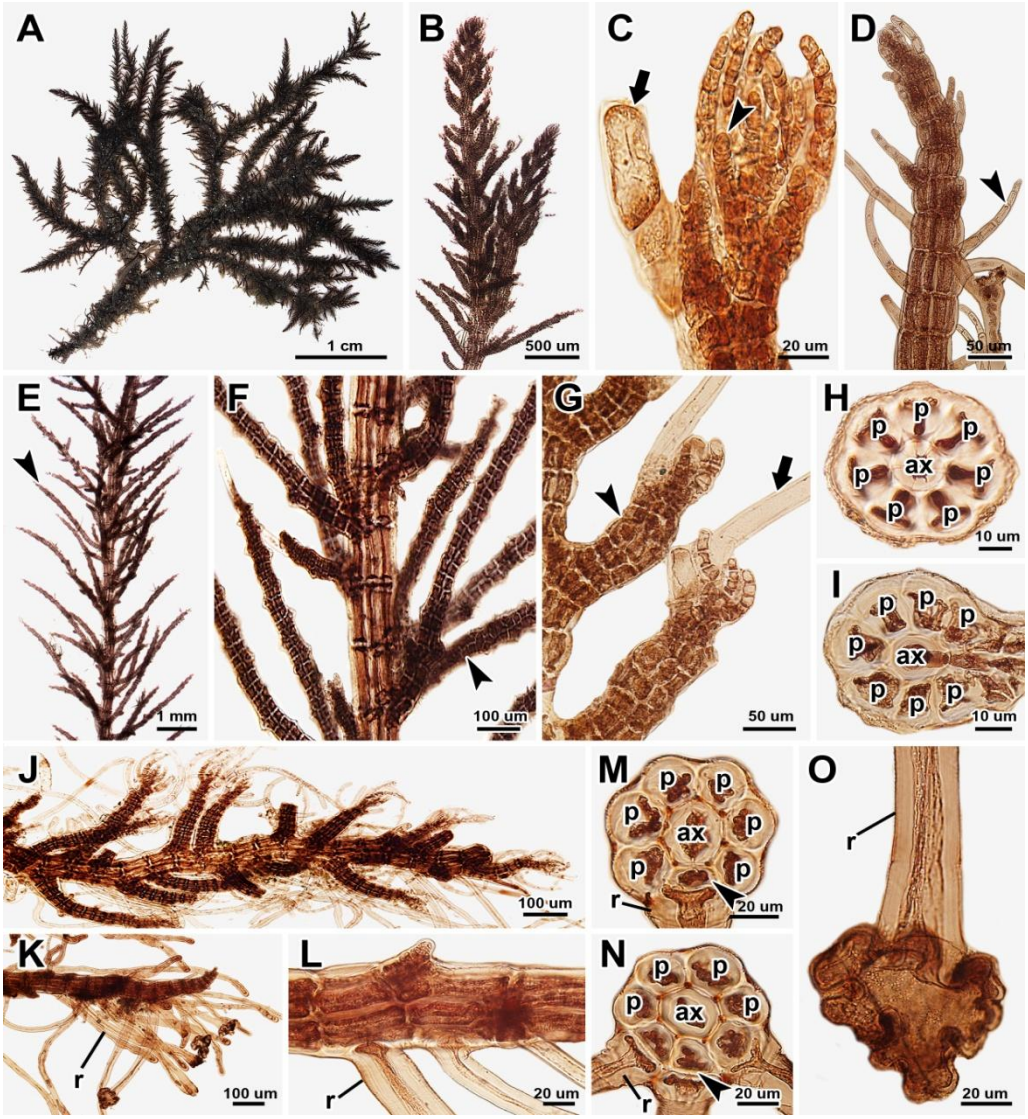


**Fig. 154.** Vegetative structures of *Diplocladia patersonis*. (A) Habit of vegetative plant showing the extended prostrate axes and erects axes. (B) Upper part of thallus showing the main axes covered by needle-like determinate branches. (C) Apices showing the apical cells transversely divided (arrow) and a prominent trichoblast (arrowhead). (D) Young erect axes showing short segments with abundant trichoblasts. (E) Lower part of main axes showing needle-like determinate branches. (F) Main axes showing determinate laterals (arrowhead) with 2-3 order of branching. (G) Needle-like determinate branches without branching (arrowhead) having prominent trichoblasts (arrow). (H-I) Cross section views of erect axes (H) and prostrate axes (I) (ax: axial cell), (p: pericentral cell). (J) Extended prostrate axes bearing needle-like determinate branches. (K) Rhizoids (r) scattered and produced from prostrate axes. (L) Rhizoid (r) cut off from pericentral cell. (M-N) Cross-section views of prostrate axes showing rhizoids (r) cutting off from pericentral cells (arrowhead) (ax: axial cell, p: pericentral cell). (O) Multilobed and unicellular terminations of rhizoid (r).

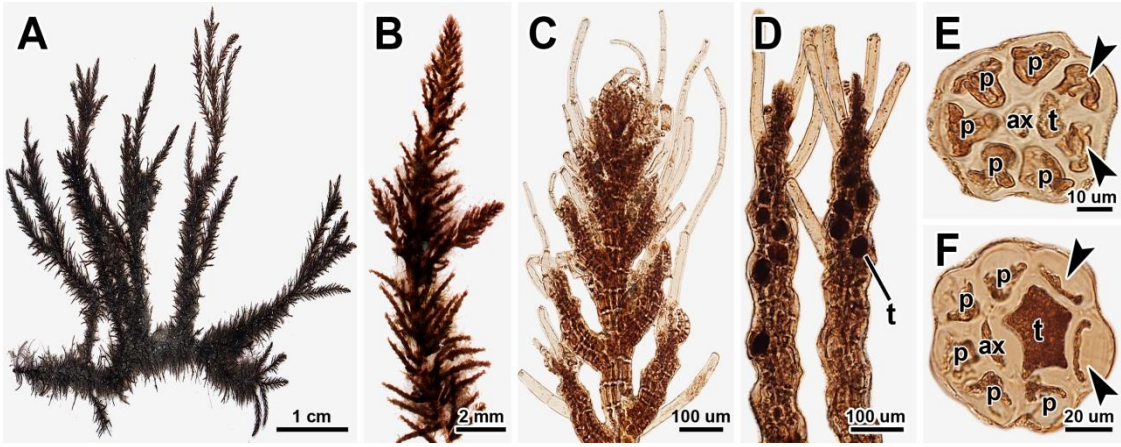


**Fig. 155.** Reproductive structures of *Diplocladia patersonis*. (A) Tetrasporangial plant. (B-C) Upper part of thallus showing the main axes covered by needle-like determinate branches bearing tetrasporangia. (D) Tetrasporangial determinate branches showing interrupted and spiral series of mature tetrasporangia (t). (E-F) Cross section view of fertile segment showing one (E) young and (F) mature tetrasporangia (t) surrounded by cover cells (arrows) (ax: axial cell, p: pericentral cell).

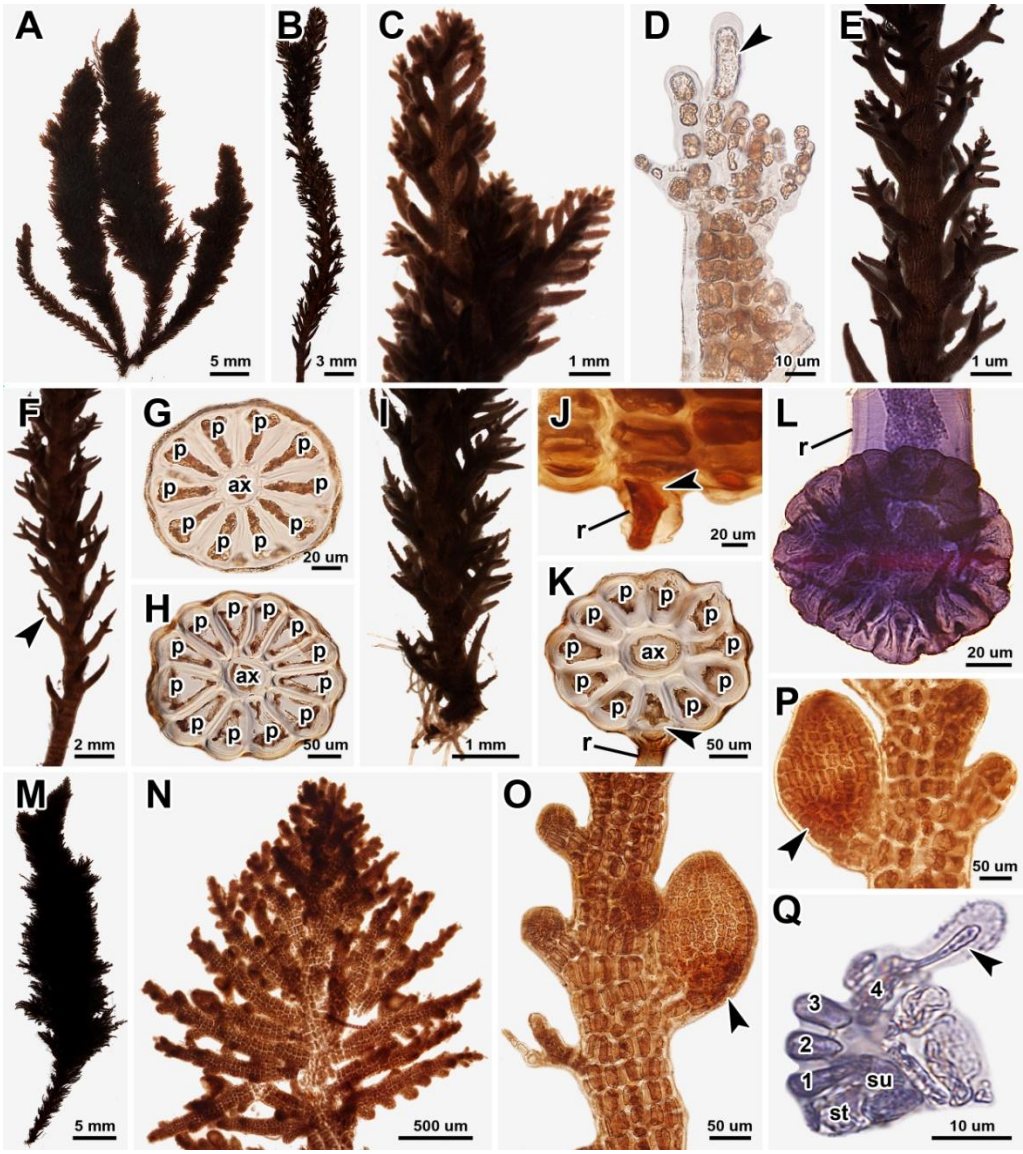


**Fig. 156.** Vegetative and reproductive structures of *Diplocladia ericoides* comb. nov. (A) Habit. (B) Main axes covered by needle-like branches. (C) Upper part of thallus showing the main axes covered by needle-like determinate branches. (D) Apices showing the apical cells and a prominent trichoblast (arrowhead). (E-F) Main axes showing determinate laterals (arrowhead) with 1-3 order of branching. (G-H) Cross section views of erect axes showing 10-12 pericentral cells (ax: axial cell), (p: pericentral cell). (I) Reduced prostrate axes bearing needle-like determinate branches. (J) Rhizoid (r) cutting off (arrowhead) from the proximal end of pericentral cells. (K) Cross-section view of prostrate axes showing a rhizoid (r) cutting off from pericentral cells (arrowhead). (L) Multilobed and unicellular terminations of rhizoid (r). (M) Female gametophyte. (N) Apical part of thallus with lateral cystocarps. (O-P) Young and mature cystocarps (Q) Procarp with a four-celled carpogonial branch and trichogyne (arrowhead) (1-4: sequence of carpogonial branch cells, st: sterile cell, su: supporting cell).

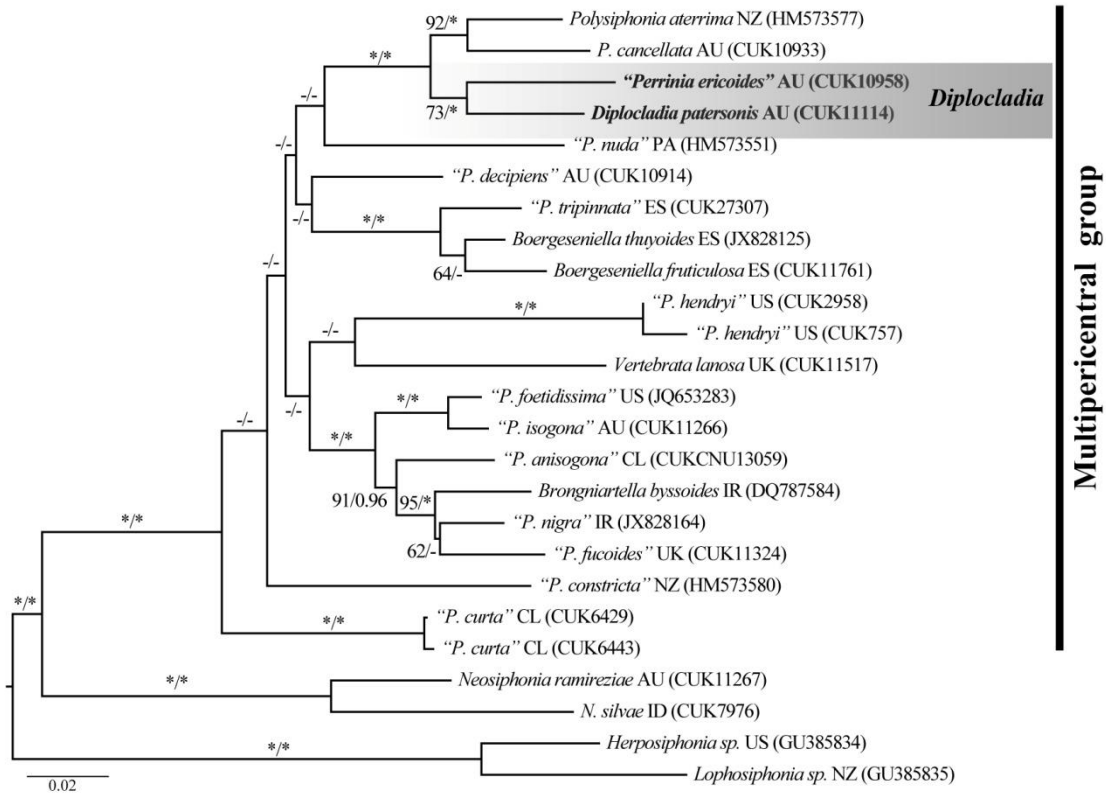


**Fig. 157.** Phylogenetic tree based on ML analysis of *rbc*L sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (AU, Australia; CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

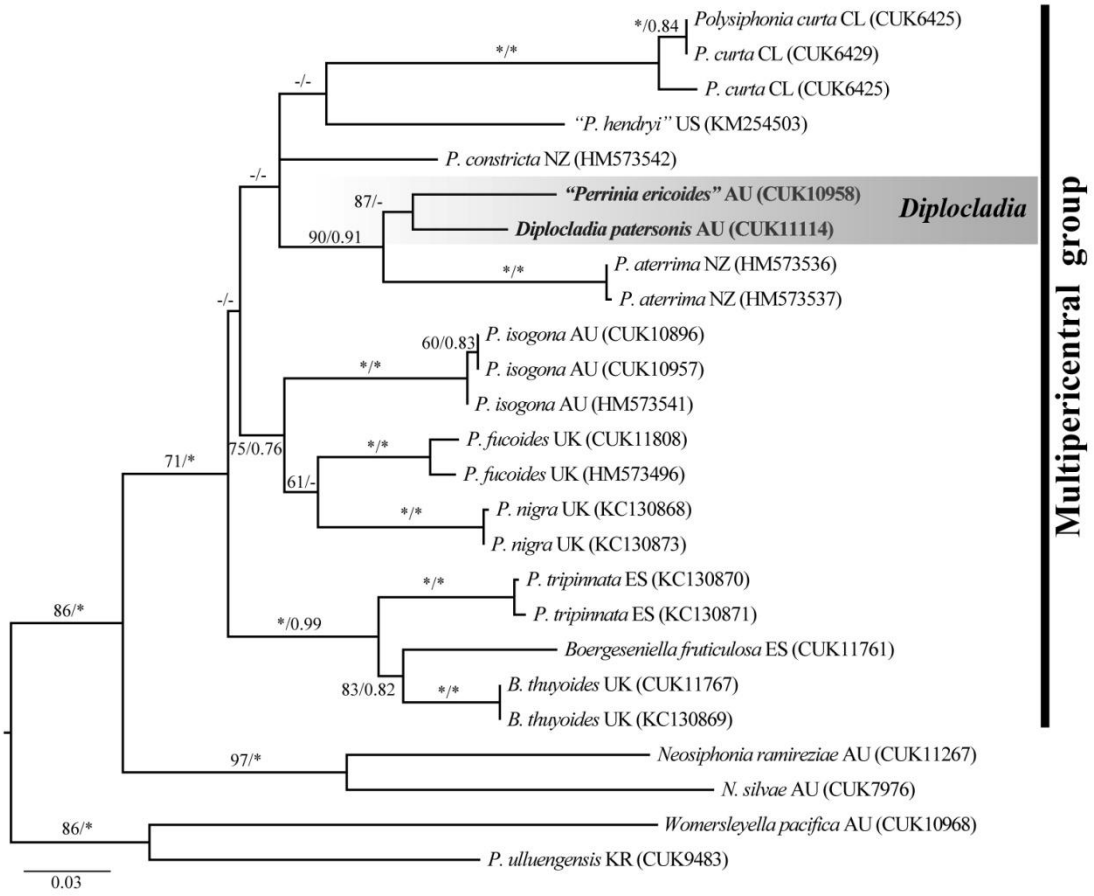


**Fig. 158.** Phylogenetic tree based on ML analysis of *cox*1 sequences. Values above branches denote maximum likelihood bootstrap values (BS) in % > 50/Bayesian posterior probabilities (BPP) > 0.75. BS values of <50% and BPP values of <0.75 are indicated by hyphens (-). BS values of 100% and BPP values of 1.00 are indicated by asterisks (*) (AU, Australia; CL, Chile; JA, Japan; KR, Korea; MX, Mexico; NZ, New Zealand; PA, Panama; PE, Peru; ES, Spain; US, United States).

**Table 10.** Comparisons among genera belonging to *Polysiphonia* sensu lato and *Diplocladia*.

| Species | *Diplocladia* | *Boergeseniella* | *Bryocladia* | *Enelittosiphonia* | *Haterosiphonia* | *Lampisiphonia* | *Leptosiphonia* | *Neosiphonia* | *Polysiphonia* | *Strebloclaadia* | *Vertebrata* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type species** | *D. patersonis* | *Bo. fruticulosa* | *Br. cervicornis* | *E. stimpsonii* | *H. paniculata* | *La. iberica* | *Le. Schousboei* | *N. flavimarina* | *P. stricta* | *S. neglecta* | *V. lanosa* |
| **Pericentral cells** | 7–13 | 8–12 | 6–12 | 7–11 | 8–12 | 9–11 | 12–16 | 4–9 | 4 | 4–8 | 17-23 |
| **Branching pattern** | Needle-like determinate branches | Alternate-distichous | Determinate spiral laterals | Dorsiventral | Paniculate | Pseudodichotomous | Alternate | Alternate | Pseudodichotomous to alternate | Dorsiventral secondary branches | Pseudodichotomously |
| **Cortication** | Absent | Present | Absent | Absent | Absent | Present | Present | Absent or present | Absent | Absent or present | Absent |
| **Trichoblasts** | Scarce to abundant | Scarce | Scarce | Abundant | Abundant | Absent | Abundant | Abundant | Scarce to abundant | Scarce | Absent |
| **Branch origin** | In the axils of trichoblasts | Replacing trichoblast | Not associated with trichoblast | - | In the axils of trichoblasts | Not associated with trichoblast | In the axils of trichoblasts | In the axils of trichoblasts | Replacing trichoblast | - | Not associated with trichoblast |
| **Rhizoids connection** | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Cut off | Open | - | Cut off |
| **Rhizoids** | Unicellular | Unicellular | Unicellular | Unicellular | Multicellular | Multicellular | Unicellular | Unicellular | Unicellular | Unicellular | Unicellular |
| **Carpogonial branches** | Four-celled | Four-celled | Four-celled | Four-celled | Four-celled | - | Four-celled | Three-celled | Four-celled | - | Four-celled |
| **Spermatangia branches** | Replacing trichoblast | Arising as primary branch or replacing | - | Arising as primary branch | Arising as primary branch | - | Arising as primary branch | Arising as primary branch | Arising as primary branch or replacing | Arising as primary branch or replacing | Replacing trichoblast |
| **Tetrasporangia** | Spiral | Spiral | Straight | Spiral | Spiral or straight | Straight | Straight | Spiral or straight | Spiral or straight | Straight | Straight |
| **References** | Womersley (2003), this study | Maggs and Hommersand (1993) | Schmitz and Falkenberg (1897) | Segi (1949) | Bustamante et al. (2015c) | Bárbara et al. (2013) | Díaz-Tapia and Bárbara (2013) | Kim and Lee (1999) | Kim et al. (2000) | Schmitz and Falkenberg (1897) | Kim et al. (2002) |

# CHAPTER 10. Taxonomy and phylogeny of the "*Neosiphonia echinata*" clade (Rhodomelaceae, Rhodophyta)

As an expected and inexorable result of human activity, alterations in biodiversity have impacted terrestrial, freshwater, and marine communities (Carlton 2009). Shipping was the single largest vector for marine species introductions in North America (Ruiz and Carlton 2003). Although the two primary subvectors (ballast water and hull fouling) are responsible for most shipping invasions worldwide, it remains a challenge to distinguish or quantify the relative importance of these subvectors (Ruiz and Carlton 2003). It has been estimated that, on any one day, more than 3000 species may be in motion around the world in the ballast of ocean-going vessels (Carlton and Geller 1993, Carlton 1996). Also, it has been reported that more than 50% of the species introduced via hull fouling are filamentous (Williams and Smith 2007). Kaluza et al. (2010) showed that the highest number of journeys (over 5000) of cargo ships bigger than 10000 gross tonnes were along the routes western Atlantic-Mediterranean-Indonesia and western Atlantic-Pacific Ocean-Indonesia.

The genus *Polysiphonia* Greville is one of the largest in the Rhodophyta (Guiry and Guiry 2014). *Neosiphonia* was segregated from *Polysiphonia* by Kim and Lee (1999). *Neosiphonia/Polysiphonia* species are widely distributed worldwide. To date, 12 *Neosiphonia/Polysiphonia* species have been reported from Indonesia (Silva et al. 1996, Atmadja and Prud'homme van Reine 2012, Bustamante et al. 2013b).

*Neosiphonia echinata* (Harvey) N. Mamoozadeh et D.W. Freshwater was originally described as *Polysiphonia echinata* Harvey from Key West, Monroe County, Florida by Harvey (1853) and then transferred to the genus *Neosiphonia* by Mamoozadeh and Freshwater (2011). *Neosiphonia echinata* is considered to be restricted to the western Atlantic: North Carolina (Mamoozadeh and Freshwater 2011), South Carolina (Kapraun and Searles 1990), Florida (Taylor 1960), Mexico (Mamoozadeh and Freshwater 2011), and Texas (Wynne 2009). It was reported in bloom proportions along

200 km of coastline from North Carolina to South Carolina (Kapraun and Searles 1990, Schneider and Searles 1991). Recently, Stuercke and Freshwater (2008) found small localized blooms of *N. echinata* with wide seasonal and annual variations in North Carolina.

Unidentified *Neosiphonia* specimens were collected from Sulawesi, Indonesia as an epiphyte on *Kappaphycus alvarezii* (Weber-van Bosse) Doty ex P.C.Silva in a culture farm in 2012. In this study, based on an integrated molecular-morphological study, we report that *Neosiphonia echinata* is an introduced species on the coast of Indonesia Southeast Asia and that its distribution now extends to the South Pacific Ocean.

## 1. Morphological analyses

***Neosiphonia echinata* (Harvey) N. Mamoozadeh et D.W. Freshwater (Fig. 160-161)**

**Basionym:** *Polysiphonia echinata* Harvey 1853: 38

**Synonym:** *Polysiphonia fracta* Harvey1853: 38

**Type locality:** Key West, Monroe County, Florida

**Distribution:** North Carolina, South Carolina, Florida, Cuba, and Mexico in the Western Atlantic and Indonesia in the South Pacific (Fig. 159).

**Specimens examined:** CUK 8872 (Donggala, Sulawesi, Indonesia collected by T.O.C and D.E.B, Sep. 06 2012), CUK 8882-8883 (Donggala, Sulawesi, Indonesia collected by T.O.C and D.E.B, Oct. 17 2012), CUK 8886 (Donggala, Sulawesi, Indonesia collected by T.O.C and D.E.B, Oct 17 2012), CUK 8896 (Donggala, Sulawesi, Indonesia, collected by T.O.C and D.E.B, Oct. 20 2012).

**Vegetative morphology:** Plants were epiphytic on *Kappaphycus alvarezii* and attached on ropes in its culture farm. Thalli form tufts, 1 to 5 cm high (Fig. 160A), and darkish-red in colour. They are composed of prostrate and erect systems with delicate texture. The prostrate system is extended and entangled. Segments of the prostrate system are 94.5 ± 38.2 μm long and 87.7 ± 34.5 μm in diameter (L:D 1.1 ± 0.1). The erect system is composed of interwoven and indeterminate main axes highly branched above. Main erect axes are prominent (Fig. 160B) and arise exogenously from the prostrate axes. They are composed of four pericentral cells, ecorticated throughout (Fig. 160C-D), and radially branched in a subdichotomous to alternate pattern every 4-17 axial cells (Fig. 160B). Branches arise in connection with trichoblasts (Fig. 160E). Pericentral cells of young erect axes are sometimes "dextrogyre" twisted and are formed by short segments (Fig. 160F). Older segments of erect axes are 52.8 ± 3.3 μm long and 49.4 ± 11.4 μm in diameter (L:D 1.1 ± 0.2 ). Apical cells are prominent, 5.8 ± 1.1 μm × 4.8 ± 0.7 μm in size, and transversely divided (Fig. E). Trichoblasts are delicate, numerous, more than twice forked, 96.5 ± 38.4 μm long, and arise on each segment (Fig. 160G). When trichoblasts are shed, they leave conspicuous scar cells among the four pericentral cells, which are spirally arranged along the axes; they are 7.3 ± 1.7 μm × 5.8 ± 1.3 μm in size (Fig. 160H). Cicatrigenous branches are abundant and developed from scar cells. Adventitious branchlets arise from the main filaments throughout the whole thallus, are sometimes branched, with abundant trichoblasts, and are very narrow at the base (Fig. 160E-J). Rhizoids are ventrally produced from the proximal end of pericentral cells (Fig. 160K). They are cut off from pericentral cells. Rhizoids are unicellular and 204.3 ± 70.7 μm long with multilobed terminations (Fig. 160L).

**Reproductive morphology:** In female plants (Fig. 161A), procarps are formed on the basal parts of modified trichoblasts (Fig. 161B-C) and are composed of a supporting cell bearing a four-celled carpogonial branch and a basal sterile cell (Fig. 161C-D). Cystocarps are globose to ovate when mature, 177 ± 13.4 μm high and 186.7 ± 1.9 μm in diameter (Fig. 161E). Carpospores are 50.3 ± 9.7 μm long (Fig. 161E). In tetrasporophytes (Fig. 161F), tetrasporangia are tetrahedrally divided

and are 59.8 ± 13 μm × 87.8 ± 18.4 μm in size. Tetrasporangial branches are swollen, sinuous, and distorted (Fig. 161G). Tetrasporangia are arranged in interrupted spiral series (Fig. 161G). The fertile segments have 5 pericentral cells. The fifth pericentral cell is developed as a stalk cell, which will develop a tetrasporangium and three cover cells (two presporangial and one post sporangial; Fig. 161H-I). One tetrasporangium is produced on each segment (Fig. 161I).

**Habitat**: Plants grow in the intertidal zone, forming tufts. They are attached on *Kappaphycus alvarezii* and the ropes of culture farm in sheltered sites. Tufts of this species are usually very flaccid and are found associated with other filamentous species.

## 2. Phylogenetic analyses

We sequenced a 1442-bp portion of the 1467-bp *rbc*L (98.3%) for material of *N. echinata* from Indonesia and U.S.A. These sequences were aligned with additional *N. echinata* sequences downloaded from GenBank and used 1150-bp for analysis. We used *Lophosiphonia* sp. (GU385835) and *Herposiphonia* sp. (GU385834) as outgroups. Based on *rbcL*, the phylogenetic analyses showed that both samples of *N. echinata* from Indonesia were in the same clade as *N. echinata* from the western Atlantic (Fig. 162). The sequence divergence between Indonesian material and western Atlantic material was 0.7%.

A 1394-bp of *cox*1 was also sequenced for *N. echinata* from Indonesia. These sequences were aligned with additional *N. echinata* sequences downloaded from GenBank and used 521-bp (Fig. 163). We used *Herposiphonia secunda* f. *tenella* (KF671171, KF671179) as outgroups. Phylogenetic analyses based on *cox*1 showed again that samples of *N. echinata* from Indonesia were included in a clade with *N. echinata* from the western Atlantic. The sequence divergence between Indonesian and western Atlantic materials was 3.5%.

## 3. Discussion

Our specimens from Indonesia correspond in their habit, vegetative morphology, and reproductive structures to the original description of *Neosiphonia echinata* from Florida (Harvey 1853, Kapraun and Searles 1990). Chloroplast *rbc*L and mitochondrial *cox*1 genes show that *N. echinata* specimens from Indonesia are placed within the *N. echinata* clade from Florida. For a long time, *N. echinata* in the western Atlantic has been misidentified as *P. breviarticulata*, which was originally described as *Hutchinsia breviarticulata* Agardh (1824) from the Adriatic Sea (Kapraun and Searles 1990, Stuercke and Freshwater 2008, Mamoozadeh and Freshwater 2011). However, based on type localities and morphology, material from Florida and North Carolina previously identified as *P. breviarticulata* represents *N. echinata* (Mamoozadeh and Freshwater 2011). Also, our material from Indonesia appears identical to *N. echinata* from Florida based on the morphology of rhizoids, trichoblasts, adventitious branchlets, and the tetrasporangia arrangement. The absence of cortication is the only remarkable difference. However, examination of North Carolina and Florida specimens by Mamoozadeh and Freshwater (2011) revealed moderate cortication only in some mature prostrate axes and at the bases of older adventitious laterals. Accordingly, Stuercke and Freshwater (2008) reported the presence or absence of cortication in North Carolina materials of *N. echinata* (as *P. breviarticulata*) and determined this character as inconsistent for *N. echinata*. The differences in cortication between western Atlantic and Indonesian material of *N. echinata* may be related differences in the type of substratum or also age related (Stuercke and Freshwater 2008). Western Atlantic material was attached on solid substrata or was epiphytic on *Zostera* (Schneider and Searles 1991, Stuercke 2006, Stuercke and Freshwater 2008), whereas our Indonesian material occurred in small tufts on *Kappaphycus alvarezii* and the ropes of culture farm.

An introduced species is often defined as a species introduced beyond its native range by human activities, and successfully established. However, an invasive species is an introduced species that is ecologically and/or economically harmful (Bodouresque and Verlaque 2002, Williams and Smith 2007, Richardson et al. 2011). *Neosiphonia echinata* (as *P. breviarticulata*) has been reported as

the main species in an intense macroalgal bloom along the coast of North Carolina (Kapraun and Searles 1990), and appeared to be a common species from North Carolina to Mexico in the western Atlantic (Kapraun and Searles 1990, Mamoozadeh and Freshwater 2011). Although its distribution has been considered to be restricted to the western Atlantic (Mamoozadeh and Freshwater 2011), we report *N. echinata* from Southeast Asia for the first time in this study. *Neosiphonia echinata* may be added as an introduced species to the Indonesian marine flora, extending its distribution to Southeast Asia. This introduced status might turn in an invasive stage unless the infestation of *N. Echinata* can cause economic or ecological harm in the cultivation of *Kappaphycus*. Because of the interconnections between Southeast Asia and the western Atlantic by shipping, the occurrence of *N. echinata* in Indonesia may be associated with the two primary subvectors for marine species introduction: hull fouling and ballast water, and also aquaculture (Ruiz and Carlton 2003, Hewitt et al. 2007). Although Indonesia and the western Atlantic are almost 10000 nautical miles away from each other, the interconnections between these realms are provided by shipping. Keller et al. (2011) concluded that any important ecosystem is connected to most active global ports by no more than two ship voyages, and to all global ports by no more than five voyages. Keller et al. (2011) also noticed that invasive species might use ports as 'stepping-stones' in a sequence of invasions in which an invader spreads from its endemic area to other ports that become the origins for further spread. Although there are no reports of *N. echinata* from other places to confirm its introduction pathway, the two most common ship routes might account for its wide distribution: 1) the western Atlantic-Mediterranean-Indonesia route by having a high number of major ports (Kaluza et al. 2010) and 2) the western Atlantic-Pacific Ocean-Indonesia route by having the Panama channel as a big major stepping-stone. The second route might explain the wide distribution of *N. echinata* from Bahamas to North Carolina in the western Atlantic (Mamoozadeh and Freshwater 2011) However, future research on the basis of new collections and phylogeographic assumptions are needed to confirm the origin and distribution patterns of this species. Especially, the occurrence and proliferation of *N. echinata* in Indonesia may be associated with the aquaculture of *Kappaphycus alvarezii*, which is a kappa carrageenan-producing seaweed commercially cultivated in the

tropics and is also colloquially called “cottonii” (Hurtado et al. 2001, 2006, Chavanich et al. 2010). *Neosiphonia/Polysiphonia* epiphytes create small, elevated pores or ‘goose bumps’ on the surface that are actually sites of penetration from the cortical to the medullary layers of the host plant. Although these filamentous species might be introduced by shipping, the relocation of infected seedlings of *K. alvarezii* to new cultivation areas has been a vector for rapid dispersal (Ask and Azanza 2002, Hurtado et al. 2006). The infestation of *Neosiphonia/Polysiphonia* epiphytes is persistent rather than periodic (Ask 1999, Ask and Azanza 2002) and may contribute to the dissemination of *N. echinata* and a number of *Neosiphonia/Polysiphonia* epiphytes around Indonesia.

Eight species of *Neosiphonia/Polysiphonia* reported from Indonesia are similar to *N. echinata* from Indonesia in having 4 pericentral cells. Of them, *N. ferulacea, P. pulvinata* var. *parvula,* and *P. sertulariodes* are distinguished from *N. echinata* by lacking adventitious laterals (Heydrich 1892, Guimarães et al. 2004, Mamoozadeh and Freshwater 2012). *Neosiphonia silvae* differs from *N. echinata* by having a prominent apical cell that is almost four times larger than normal apical cells (Bustamante et al. 2013b). *Polysiphonia infestans* is distinguished by having laterals which replace trichoblasts (Womersley 2003). *Polysiphonia mollis* differs by having a cluster of rhizoids (Womersley 2003). *Polysiphonia tenerrima* is distinguished by having a dorsiventral habit (Fig. 7 in Afonso-Carrillo and Rojas-González 2004).

Our molecular phylogenetic analyses using *rbc*L and *cox*1 locus sequences revealed low sequence divergence (below 0.7% in *rbc*L and 3.5% in *cox*1) among Indonesia, U.S.A., and Mexico populations of *N. echinata*. Our molecular sequence data suggest smaller diversity than was previously recognized among species of *Neosiphonia*. Mamoozadeh and Freshwater (2011) proposed that sequence divergences below 1.3 % for *rbc*L locus are generally considered to represent intraspecific variation, and those greater than 2.13 % for *rbc*L and 4.8% for CO1 represent interspecific variation in *Polysiphonia* sensu lato. The populations from the two oceans seem to be clearly differentiated haplotypes but without a consistent morphological differentiation. It might suggest a intraspecific cryptic relationship which can be confirmed with future evolutionary studies. We con-

firm that the *N. echinata* is an introduced species in Indonesia and that its distribution now extends to the South Pacific Ocean based on an integrated molecular-morphological study.

**Fig. 159.** Distribution of *Neosiphonia echinata* from the coasts of Florida and Indonesia.

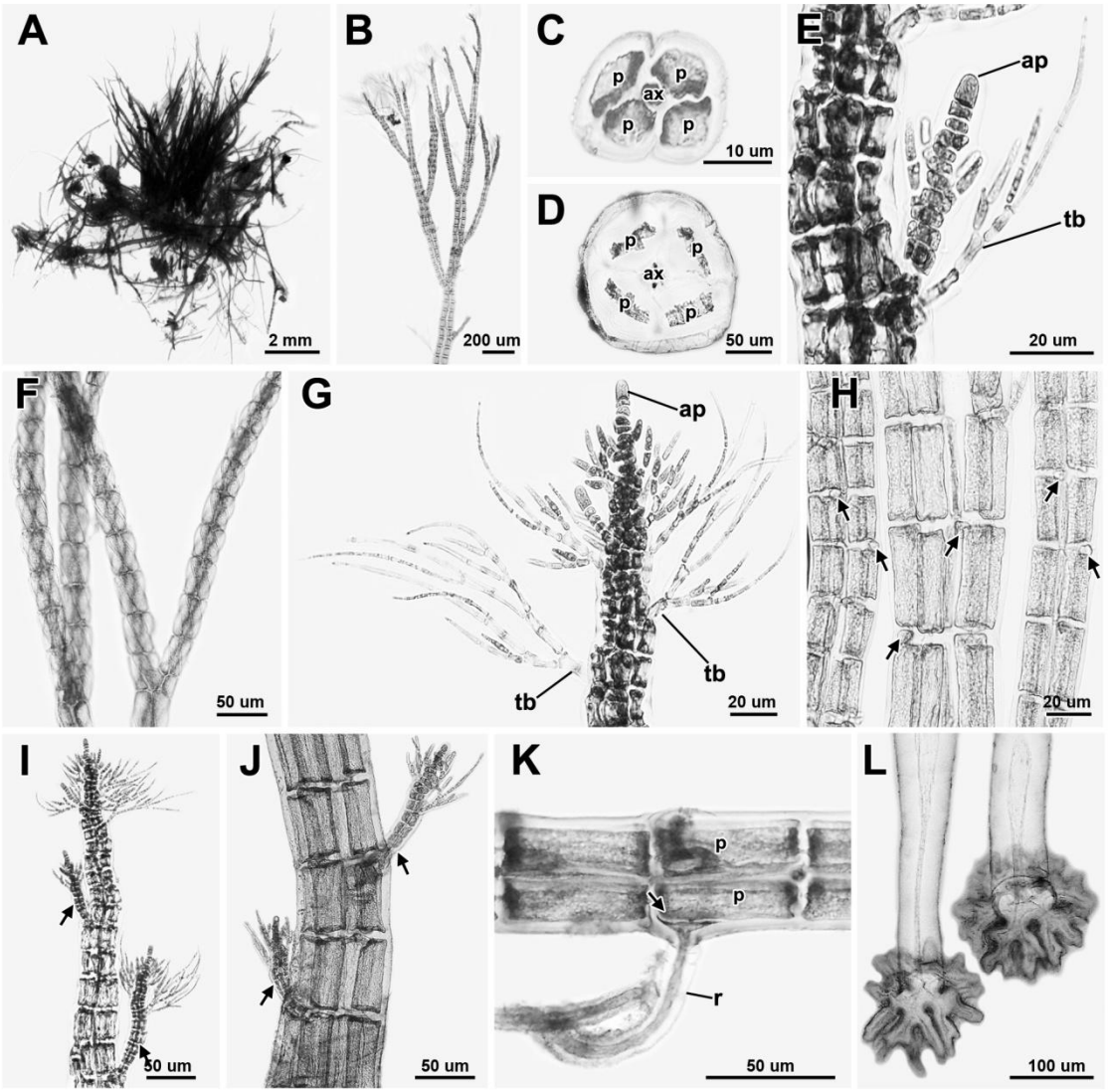


**Fig. 160.** Vegetative structures of *Neosiphonia echinata.*(A) Specimen from Dongala, Sulawesi, Indonesia. (B) Habit of vegetative thallus showing subdichotomous to alternate branching pattern. (C) Cross-section of upper part of thallus (ax: axial cell, p: pericentral cells). (D) Cross section of basal part of thallus. (E) Lateral branch arising in connection with trichoblast (tb) (ap: apical cell). (F) Upper part of thallus with pericentral cells "dextrogyre" twisted. (G) Apex of thallus with long trichoblasts (tb) and apical cell (ap). (H) Middle part of thallus with numerous scar cells (arrows). (I) Adventitious laterals (arrows) arising from main axes. (J) Cicatrigenous branches (arrows) arising from scar cells. (K) Rhizoid (r) cut off from the proximal end of pericentral cell (arrow). (L) Unicellular rhizoids with lobed terminations.

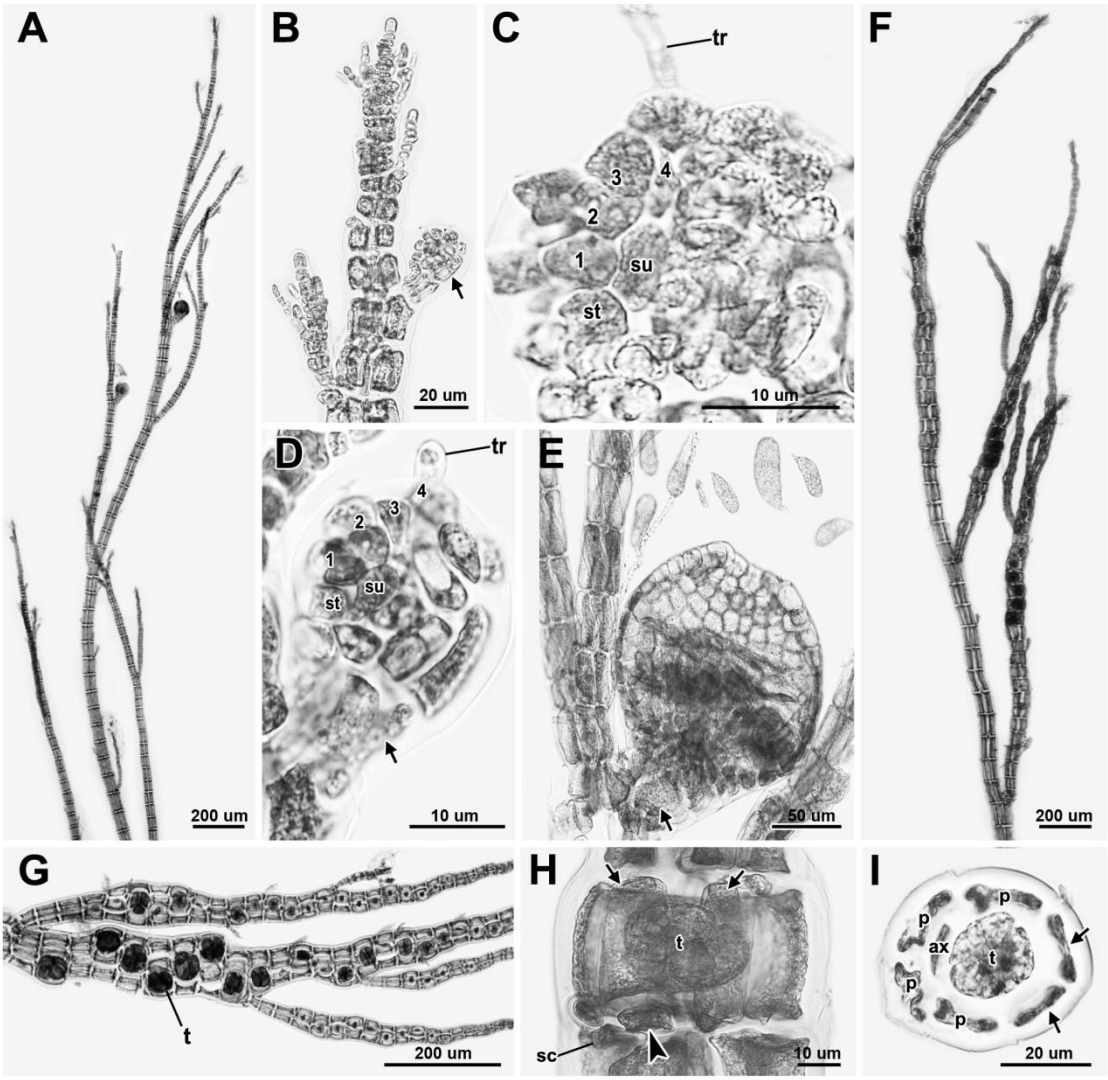


**Fig. 161.** Reproductive structures of *Neosiphonia echinata*. (A) Female thallus. (B) A young cystocarp (arrow) on the subapical part of the thallus. (C-D) Young procarp composed of four-celled carpogonial branch (carpogonial branch: 1-4, su: supporting cell, st: sterile cell, tr: trichogyne). (E) Globose cystocarp on a short-stalked pedicel (arrow). (F) Tetrasporangial thallus. (G) Upper part of tetrasporangial thallus showing spiral series of tetrasporangia (t). (H) Surface view of segment with a tetrasporangium (t) surrounded by two presporangial cover cells (arrows) and one postsporangial cover cell (arrowhead) (sc: scar cell). (I) Cross-section of tetrasporangium (t) surrounded by presporangial cover cells (arrows) (ax: axial cell, p: pericentral cells).

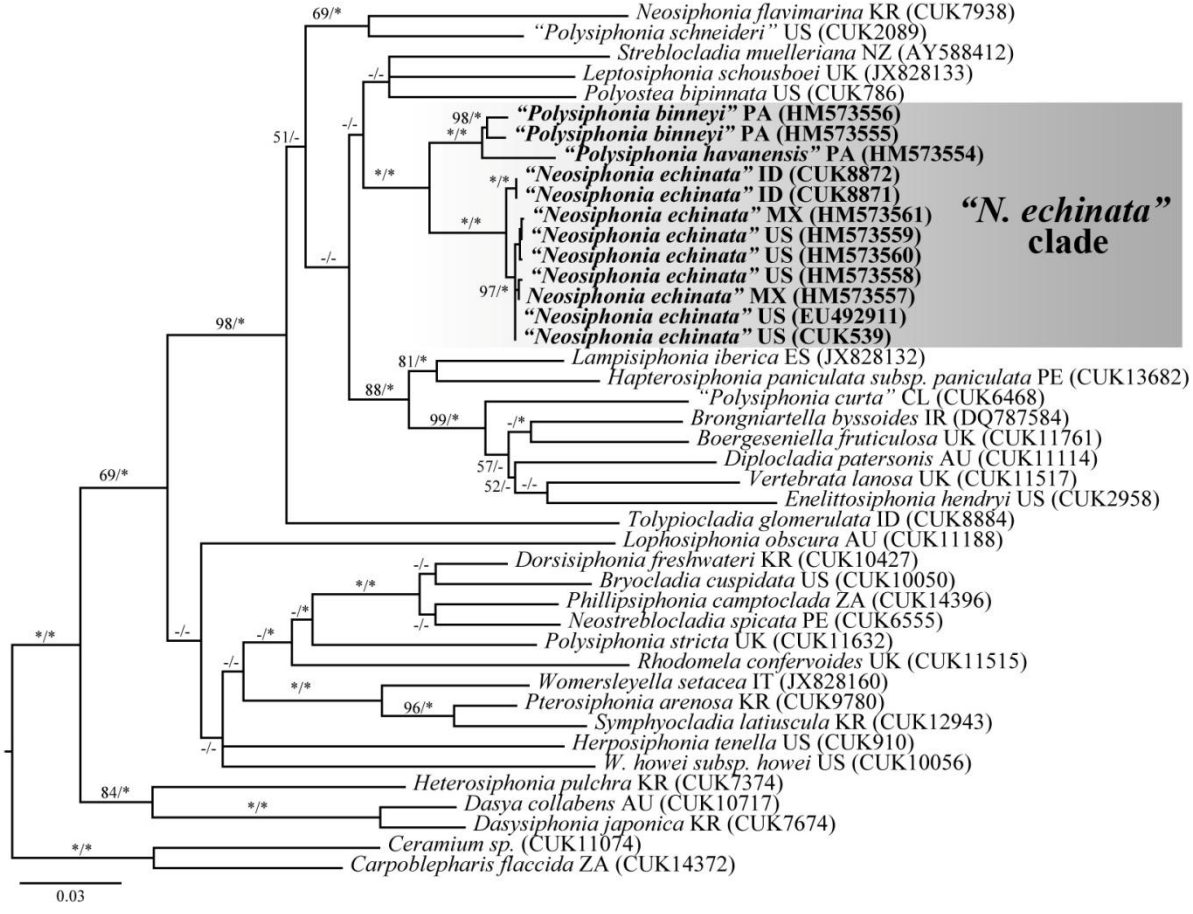


**Fig. 162.** Phylogenetic tree for *Neosiphonia echinata* and relatives, derived from maximum likelihood (ML) using plastid-encoded *rbc*L sequence data. Bootstrap proportion values (BS; >50%) for ML (left) and Bayesian posterior probabilities (BPP; >0.75) for Bayesian analysis (right) are shown at the nodes. Values lower than 50% (BS) or 0.75 (BPP) are indicated by hyphens (-). Values of 100% (BS) or 1.00 (BPP) are indicated by asterisks (*). Scale bar = 0.02 nucleotide substitutions/site

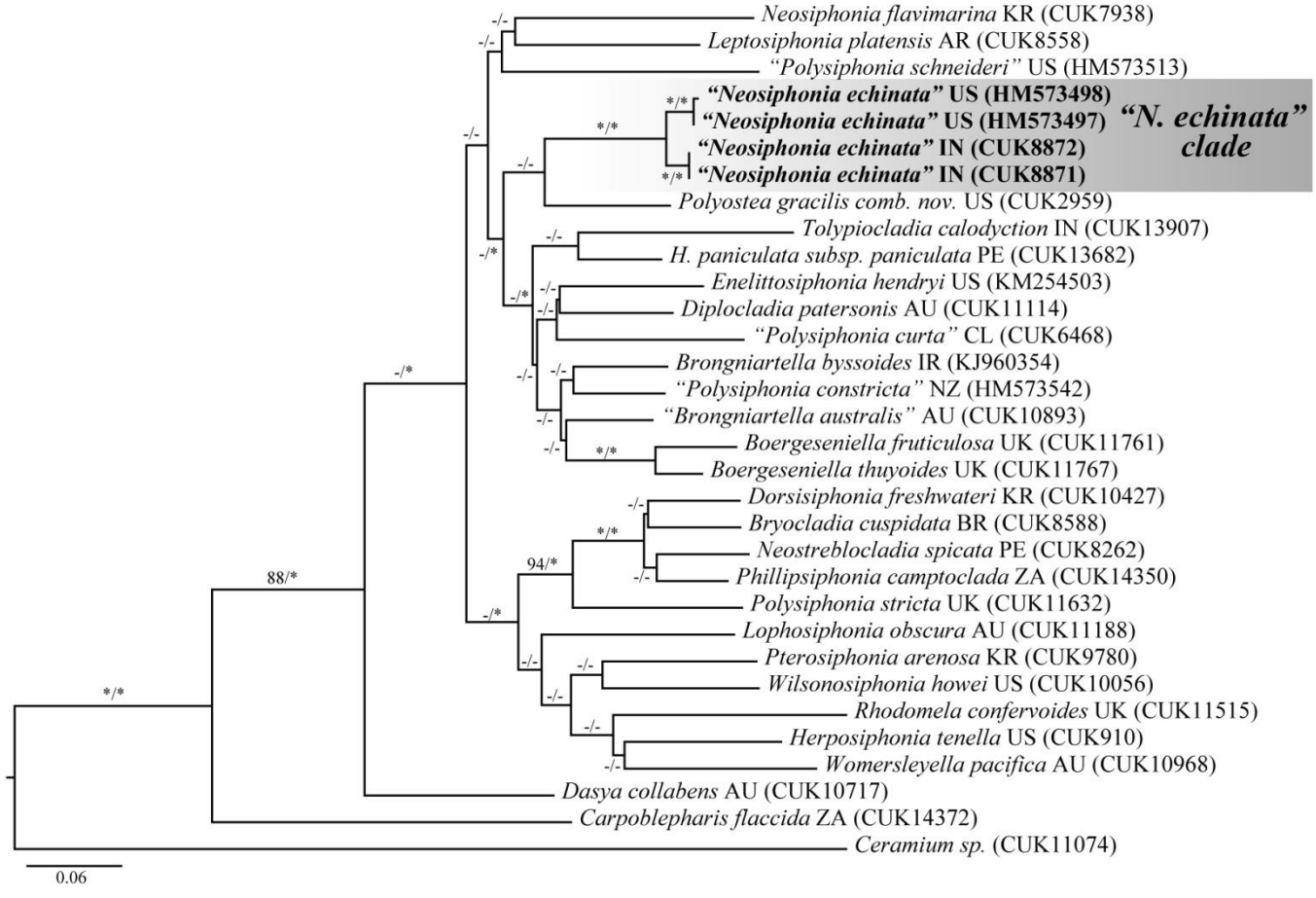


**Fig. 163.** Phylogenetic tree based on maximum likelihood using mitochondrial-encoded *cox*1 sequences. Values at nodes = Maximum likelihood bootstrap (BS) values >50% /Bayesian posterior probabilities (BPP) values > 0.75. Values lower than 50% (BS) or 0.75 (BPP) are indicated by hyphens (-). Values of 100% (BS) or 1.00 (BPP) are indicated by asterisks (*). Scale bar = 0.05 nucleotide substitutions/site

## CHAPTER 11. Taxonomy and phylogeny of *Womersleyella indica* sp. nov. (Rhodomelaceae, Rhodophyta)

The genus *Womersleyella* Hollenberg was described by Hollenberg (1967) on the basis of *W. pacifica* Hollenberg from Oahu, Hawaii. *Womersleyella* is characterized by having (1) indeterminate prostrate system, (2) absence of emergent trichoblasts on prostrate axes and presence of non-emergent branches, (3) upright branches determinate, (4) multicellular rhizoidal terminations, (5) four or five pericentral cells, (6) exogenous branches in one-fourth or one-fifth spiral sequence, and (7) one tetrasporangia per segment (Hollenberg 1967, Norris 1992). The establishment and relationship of *Womersleyella* with other polysiphonous groups has been confirmed molecularly by Choi et al. (2001) based on small-subunit ribosomal DNA (SSU rDNA) and subsequent studies of Bárbara et al. (2013) and Bustamante et al. (2015c) sequenced additional species of *Womersleyella* based on the plastid-encoded *rbc*L.

Currently, only four taxa has been recognized in *Womersleyella* from worldwide: *W. kwazuluensis* R.E.Norris, *W. pacifica*, *W. pacifica* var. *minor* Hollenberg, and *W. setacea* (Hollenberg) R.E.Norris. *Womersleyella kwazuluensis* has been described in detail morphology by Norris (1992) and was only restricted to Kwazulu, Natal. *W. pacifica*, and *W. pacifica* var. *minor* were studied by Hollenberg (1967) and restricted them to the Hawaiian Islands. In contrast, *W. setacea* originally described as *P. setacea* by Hollenberg (1967) from Oahu, Hawaii, has been widely distributed and considered as an invasive species (Rindi et al. 1999, Nikolic et al. 2010, Cebrian and Rodrighuez-Prieto 2012). *W. setacea* has been reported in the Pacific, Atlantic, and Indic Ocean (Hollenberg 1968, Rojas-Gonzales and Afonso-Carrilo 2000, Cebrian and Rodrighuez-Prieto 2012), but it is considered native to tropical areas of these oceans (Nikolic et al. 2010). Although *W. setacea* has been widely reported, only Bárbara et al. (2013) confirmed molecularly this species from Italy.

Recently, we have collected unidentified polysiphonous specimens from India, that in morphology share the features of *Womersleyella* species. We describe these unidentified samples as a new species, namely *Womersleyella indica* sp. nov. on the basis of vegetative and reproductive morphology. We also provide further evidence on its phylogenetic relationships with other species of *Womersleyella* and other polysiphonous groups using *rbc*L sequencing analyses.

## 1. Morphological analyses

***Womersleyella indica* D.E. Bustamante, B.Y. Won et T.O. Cho sp. nov. (Fig. 165-166)**

**Diagnosis.** Thalli 0.4-1.8 cm tall, saxicolous, composed of indeterminate prostrate axes and indeterminate erect axes. Axes with four pericentral cells and ecorticate throughout. Erect axes with branches irregular positioned, which are replacing the trichoblasts. Trichoblasts deciduous. Scar cells conspicuous spirally disposed. Adventitious branches present. Rhizoids multicellular, in close connection with and produced from the distal end of the pericentral cells. Tetrasporangia arranged in spiral series.

**Holotype**: CUK13852. Voucher specimens were deposited in the herbarium of Chosun University (CUK), Korea.

**Type locality**: Seneeyappa, Tharuga, Ramanathapuram, Tamil Nadu, India (9° 15′ 48.40″ N, 78° 55′ 17.69″ E); coll. T.O. Cho and D. E. Bustamante; February 10, 2015, (Fig. 164).

**Other specimens examined**. CUK15249 (Kanyakumari beach, Kanyakumari, Tamil Nadu, India collected by T.O.C. and D.E.B., Feb. 12 2015).

**Etymology**: The name "*indica*" is derived from the country of collection.

**Vegetative morphology**. Plants are diminutive, 0.4-1.8 cm high (Fig. 165A), and purplish red in color. They form small tufts and are predominantly attached to rocks of the intertidal zone. Thalli

are composed of prostrate and erect systems with flaccid texture (Fig. 165B). The prostate system is extended, entangled and composed of indeterminate axes (Fig. 165B-D). Segments of the prostrate axes are 86.31 ± 6.03 μm long and 62.85 ± 4.17 μm in diameter, being 0.7 times broader than long [1.38 ± 0.14 in length/diameter (L/D)] (Fig. 165C-D). The erect system is composed of interwoven indeterminate axes (Fig. 165B). Erect axes are delicate and arise exogenously from the prostrate axes mostly at intervals of 1–3 axial cells (Fig. 165C-D). Young erect axes are straight (Fig. 165C) or curved (Fig. 165D) in the direction of prostate axes and have short segments. Older segments of erect axes are wide, 81.39 ± 11.19 μm long and 43.20 ± 5.03 μm in diameter, being 0.5 times broader than long (1.92 ± 0.39 in L/D), shifted, and infrequently branched. Lateral branches are replacing the whole trichoblast. Apical cells are prominent, 7.49 ± 1.22 μm × 5.46 ± 1.17 μm in size, and transversely divided (Fig. 165E). Trichoblasts are deciduous, delicate, numerous, several times forked, and 30.94 ± 11.78 μm long (Fig. 165E). Conspicuous scar cells appear along the filament after trichoblast have been shed, reaching 11.67 ± 1.85 μm × 11.34 ± 2.01 μm , and developed in spiral series in the space between segments (Fig. 165F). Axes are composed of four pericentral cells sometimes slightly oblique, ecorticate throughout (Fig. 165G-I), and sparse branched every 1-16 axial cells in irregular pattern. Adventitious branches present (Fig. 165J). Rhizoids are ventrally produced from the distal end of mature pericentral cells (Fig. 165K-L). They are cutting off the pericentral cells (Fig. 165M). Rhizoids are unicellular in younger stages but when mature they have lobed multicellular terminations (Fig. 165M), and 20.46 ± 2.42 μm in diameter and 91.86 ± 74.06 μm long.

**Reproductive morphology**: In tetrasporangial plants (Fig. 166A-B), tetrasporangia are tetrahedrically divided and 29.45 ± 5.20 μm × 34.33 ± 5.59 μm in size. Tetrasporangial branches are swollen and sinuous (Fig. 166C). The development of tetrasporangia follows a spiral arrangement (Fig. 166D). The fertile segment has four or five pericentral cells and the fertile pericentral cell developed to a stalk cell, which will develop a tetrasporangium and two cover cells (Fig. 166E-F). A

single tetrasporangium is produced on each segment (Fig. 166E-F). Female and male gametophytes were not found.

**Habitat:** Plants grow forming tufts from the intertidal to upper subtidal zone. They were mainly found attached to rocks in sheltered areas. Tufts of this species are usually small, very flaccid, and monospecific.

## 2. Phylogenetic analyses

We sequenced a 1395-bp portion of the 1467-bp *rbc*L (95.1%) for *Womersleyella indica* sp. nov. and other *Womersleyella* and polysiphonous species. These sequences were aligned with additional *Polysiphonia* sensu lato sequences downloaded from GenBank. We used the Ceramiaceae *Carpoblepharis flaccida* (CUK14372) and *Ceramium sp*. (CUK11074) as outgroups. Phylogenetic analyses placed *W. indica* sp. nov. in very close relationship with "*Polysiphonia sp."* (HM573544) and as sister to *W. setacea*. (JX828160) and *W. pacifica* (CUK10968) (Fig. 167). The sequence divergences comparisons among *W. indica* sp. nov. with *W. setacea* and *W. pacifica* were 4.7% and 5.9% respectively.

## 3. Discussion

In the present study, we describe for the first time *Womersleyella indica* sp. nov. from India, based on morphological and molecular evidence. This new species is recognized as a *Womersleyella* member by having four pericentral cells, thalli ecorticated throughout, rhizoids cutting off the distal position of pericentral cells, multicellular rhizoidal terminations, and tetrasporangia arranged in spiral series. *Womersleyella indica* sp. nov. is further characterized by having indeterminate erect

axes, adventitious branches, and four to five pericentral cells in the fertile segments of tetrasporangial branches. The results of *rbc*L sequence analyses supported this taxonomic placement.

Falkenberg (1901) distinguished two developmental types of branches: (1) determinate branches that do not ordinarily give rise to further branches and (2) indeterminate branches that has potentially unlimited growth. The indeterminate prostrate branches and determinate erect branches has been reported widely among all species of *Womersleyella* (Hollenberg 1968, Norris 1992). *Womersleyella indica* sp. nov. is the only species in this genus having indeterminate erect branches (Fig. 165B), confirming that the erect axes in species of *Womersleyella* might show the two developmental types of branches.

The multicellular rhizoidal terminations has been considered as consistent character to delimit genera in *Polysiphonia* sensu lato with the description of *Hapterosiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho and *Wilsonosiphonia* D.E. Bustamante, B.Y. Won et T.O. Cho (Bustamante et al. 2015c, see Chapter 7). The multicellular rhizoidal terminations was a common character among *Herposiphonia* Nägeli, *Pterosiphonia* Falkenberg , *Wilsonosiphonia*, and *Womersleyella* (Bustamante et al. 2015c, see Chapter 7) and our phylogenetic analyses confirmed the relationship among these genera by grouping them in a well supported clade with a high bootstrap and posterior probability (Fig. 167). However, *Herposiphonia* is distinguished from *Womersleyella* by having determinate and indeterminate branches distichously arranged at regular positions, *Pterosiphonia* is different from *Womersleyella* by having proximal coalescence of lateral branches and main axes, and *Wilsonosiphonia* is distinguished from *Womersleyella* by having 8 to 14 pericentral cells (Schmitz 1889, Hoffmann et Santelices 1997, see Chapter 7).

The new species *Womersleyella indica* is similar in external morphology to the other members of the genus, but *W. kwazuluensis* is distinguished from *W. indica* by having determinate erect axes, *W. pacifica* and *W. pacifica* var. *minor* are different from *W. indica* by having five pericentral cells, and *W. setacea* is different from *W. indica* by lacking of adventitious branches (Table 11).

Molecular-assisted identification through use of the plastid-encoded *rbc*L has proven useful for discriminating species of *Polysiphonia* sensu lato with sequence divergences below 1.3 % representing intraspecific variation and those greater than 2.13 % representing interspecific variation (Mamoozadeh and Freshwater 2011, Bustamante et al. 2014a, 2014b). Our molecular phylogenetic analyses revealed that *W. indica* was previously reported from Panama as *Polysiphonia* sp. (HM573544) by Mamoozadeh and Freshwater (2011). These specimens are showing very low sequence divergence (0.3%). It suggests that *W. indica* is having a wide distribution between the Indian Ocean and the Western Atlantic as an introduced species. Bustamante et al. (2015b) proposed that the introduction of species among the Indian Ocean and Western Atlantic is due to human activities as a consequence of ballast water and hull fouling. Our phylogenetic analyses also revealed that *Womersleyella indica* was sister to the clade composed by *W. pacifica* and *W. setacea* confirming the status of this genus as a well supported clade and in close relationship to the genus *Pterosiphonia* (Fig. 167)

Although *W. setacea* has been widely characterized on the basis of morphological and laboratory cultured materials (Rindi et al. 1999, Schneider and Lane 2007, Nikolic et al. 2010, Wynne 2011, Cebrian and Rodriguez-Prieto 2012), there is no genetic studies that confirm the wide distribution and evaluate the invasiveness of *W. setacea* in the western Atlantic and other oceans. Thus, the reports of *Womersleyella setacea* from the western Atlantic (Schneider and Lane 2007, Wynne 2011) likely refer to *W. indica.* Currently, the distribution of *W. setacea* was confirmed from the Mediterranean sea by Bárbara et al. (2013), whereas this study is confirming the wide geographic distribution of *W. indica* in the Indian Ocean and Western Atlantic.

**Fig. 164.** Distribution of the *Womersleyella indica* sp. nov. from the Western Atlantic and Indic Ocean.

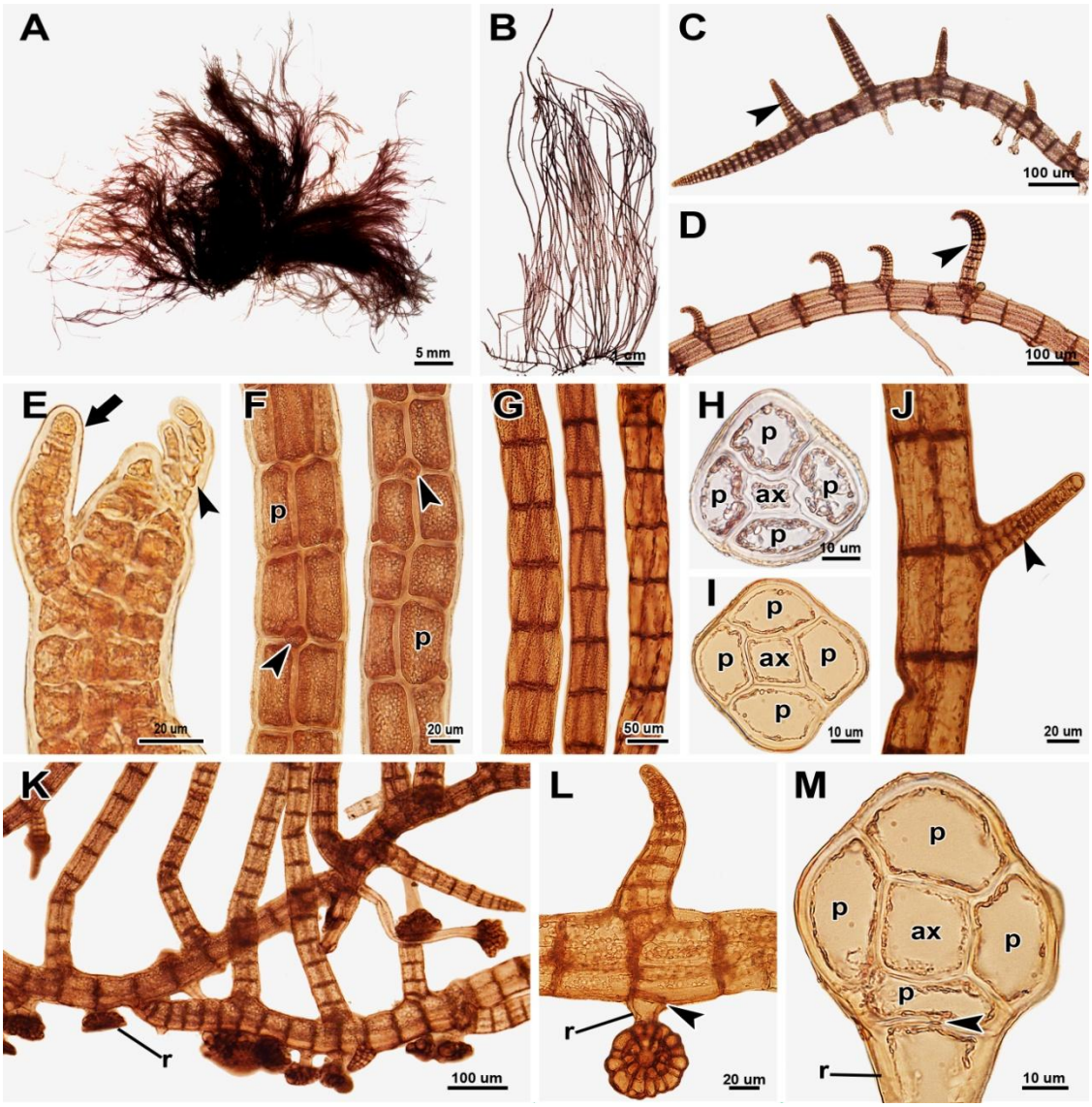


**Fig. 165.** Vegetative structures of *Womersleyella indica* sp. nov. (A). Holotype specimen from India. (B). Habit of vegetative plant showing the indeterminate prostrate and indeterminate erect axes. (C-D). Prostrate axes showing young erect axes straight (C) or curved (D) in the direction of prostate axes. (E). Apices showing transversely divided apical cells (arrow) and trichoblasts (arrowhead). (F) Erect axis showing prominent scar cells (arrowheads) (p, pericentral cells). (G) Erect axis showing pericentral cells slightly oblique (H-I). Cross-section of erect axis (H) and prostrate axis (I) showing four pericentral cells (p) (ax, axial cell). (J). Erect axis showing young adventitious lateral branches (arrowhead). (K) Rhizoids (r) scattered and produced from prostrate axes. (L). Rhizoid (r) cutting off from the distal position of pericentral cells (arrowhead). (M). Cross-section of a prostrate axis with rhizoid (r) cutting off (arrowhead) from pericentral cells (p) (ax, axial cell).

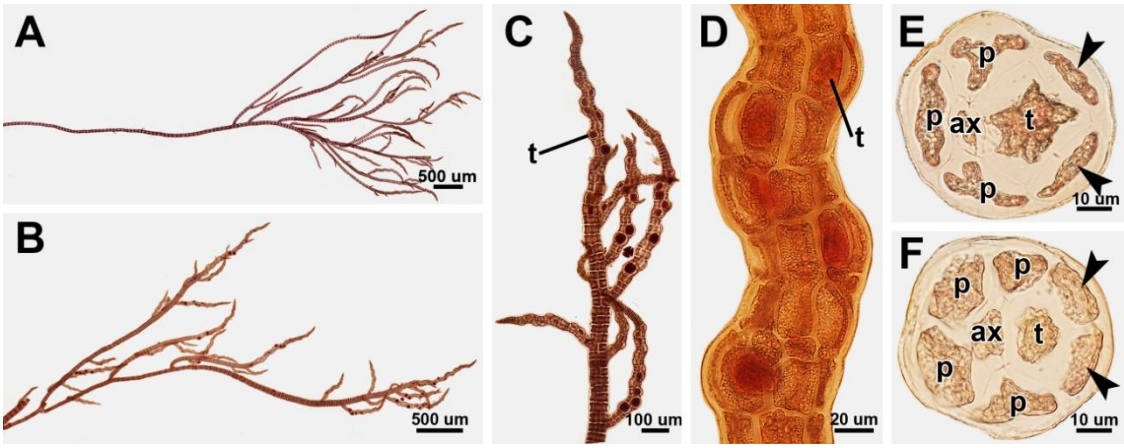


**Fig. 166.** Reproductive structures of *Womersleyella indica* sp. nov. (A) Tetrasporangial plant. (B). Erect tetrasporangial branches arisen from prostrate axes. (C). Apical branch showing tetrasporangia (t) developed in main adventitious axes. (D). Apical branch showing spiral arrangement of tetrasporangia (t). (E-F). Cross-section showing four (E) and five (F) pericentral cells (p) in fertile segment with a tetrasporangium (t) rounded by cover cells (arrowheads) (ax, axial cells).

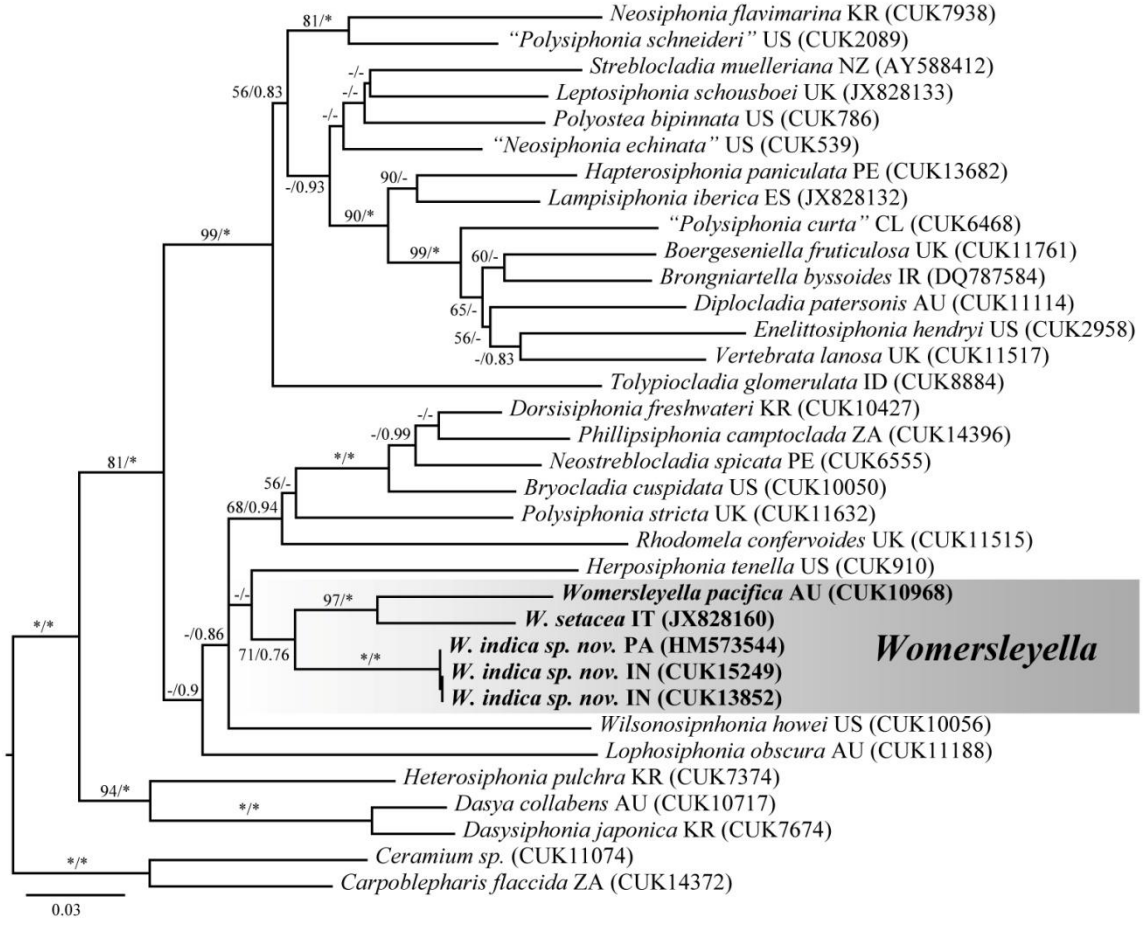


**Fig. 167.** Phylogenetic tree derived from maximum likelihood (ML) using plastid-encoded *rbc*L sequence data. Bootstrap proportion values (BS; >50%) for ML (left) and Bayesian posterior probabilities (BPP; >0.75) for Bayesian analysis (right) are shown at the nodes. Values lower than 50% (BS) or 0.75 (BPP) are indicated by hyphens (-). Values of 100% (BS) or 1.00 (BPP) are indicated by asterisks (*). Scale bar = 0.02 nucleotide substitutions/site.

**Table 11.** Morphological comparisons among species of *Womersleyella*.

| **Features** | ***W. indica* sp. nov.** | ***W. kwazulu-ensis*** | ***W. pacifica*** | ***W. pacifica* var. *minor*** | ***W. setacea*** |
|---|---|---|---|---|---|
| Prostrate branches development | Indeterminate | Indeterminate | Indeterminate | Indeterminate | Indeterminate |
| Erect branches development | Indeterminate | Determinate | Determinate | Determinate | Determinate |
| Pericentral cells number | Four | Four | Five | Five | Four |
| Adventitious branches | Present | Absent | Absent | Absent | Absent |
| References | This study | Norris (1992) | Hollenberg (1967) | Hollenberg (1967) | Hollenberg (1968) |

# PART 3. BIOGEOGRAPHY AND EVOLUTION.

## CHAPTER 1. Biogeography and evolution of the *Neosiphonia harveyi* complex.

Species are one of the fundamental units of biology (Mayr1982, De Queiroz 2005, 2007), and there is a common evolutionary idea: speciation results from isolation of populations by interrupted gene flow, resulting in divergence due to selection and drift, and ultimately in separately evolving metapopulation lineages (Coyne and Orr 2004, Leliart et al. 2014). Given the problems of species delimitation in algae using morphology (e.g. morphological plasticity and the lack of adequate diagnostic characters) or sexual compatibility (e.g. the lack of adequate methods to detect reproductive isolation), molecular data are becoming the standard for delimiting species and testing their traditional boundaries (Muangmai et al 2014, Leliaert et al. 2014, Verbruggen 2014). DNA-based species-delimitation analysis methods are often used for evaluating true species diversity among genera of marine macroalgae (Zuccarello and West 2003, Payo et al. 2012, Zucarello et al. 2015)

The cosmopolitan genus *Polysiphonia* sensu lato is a rhodomelacean red algal genus and is composed of heterogeneous genera. One of these genera is the genus *Neosiphonia* M.S. Kim *et* I.K. Lee. This genus was segregated by Kim and Lee (1999) on the basis of rhizoids cut off from the pericentral cells, procarps with a three-celled carpogonial branch, spermatangial branches arising on trichoblast furcations, and tetrasporangia arranged in a spiral series (Kim and Lee 1999, Bustamante et al. 2015c). *Neosiphonia* is commonly encountered in the intertidal zone of tropical and temperate waters around the world (Kim and Yang 2006, Bustamante et al. 2012). The taxonomy of *Neosiphonia* have been clarify in recent studies by the recognition of three-celled carpogonial branches as an exclusive diagnostic feature (Kim and Kim 2014, Bustamante et al. 2015a). In this genus, a group of species having four pericentral cells and cortication has been labeled as the *N. japonica* complex (Kim and Yang 2006). This species complex have been easily wrong identified among them and it is composed of the following species: *Neosiphonia decumbens* (Segi) M.S. Kim et I.K. Lee, *N. flavimarina* M.S. Kim et I.K. Lee, *N. japonica* (Harvey) M.S. Kim et I.K. Lee, *N. harlandii* (Harvey) M.S. Kim et I.K. Lee, *N. harveyi* (J.Bailey) M.S. Kim, H.G. Choi, Guiry et G.W. Saunders, and *Polysiphonia strictissima* J.D. Hooker et Harvey (Kudo and Masuda 1986,

Yoon 1986, McIvor et al. 2001, Kim and Yan 2006). This species complex were treated as conspecific and cryptic siblings species by McIvor et al (2001), but Kim and Yang (2006) concluded that this group of species is not composed of cryptic species because they can be distinguished based on the combination of characters relating to habit and vegetative morphology. Although Mamoozadeh and Freshwater (2011) proposed that sequence divergences below 1.3 % for *rbc*L locus represent intraspecific variation and those greater than 2.13 % for *rbc*L and 4.8% for COI represent interspecific variation in *Polysiphonia* sensu lato, earlier Kim and Yang (2006) justified the low *rbc*L sequence divergence (0.08% to 0.24%) among *N. decumbens*, *N. flavimarina*, *N. japonica*, and *N. harlandii* as recently diverged species which might have a recent most common ancestor.

In this study, we have used DNA-species delimitation methods, phylogeographic and phylogenetic inferences based on two molecular markers: the Cytochrome oxidase subunit I gene (COI) from the mitochondrial genome and the large subunit of the RUBISCO gene (*rbc*L) from the chloroplast genome in species having four pericentral cells and cortication collected worldwide, to asses species boundaries, to examine phylogenetic relationships, to explore cryptic diversity, estimate the divergence rates leading to speciation and to determine the approximate times of origin of diversification.

## 1. Results

### 1.1. Phylogenetic analyses

We succesfully obtained the partial sequences of two genes, and our final aligments comprised 1154 base pairs (bp) of *rbc*L and 500 bp of cox1, including samples of *Neosiphonia* species, including some published sequences downloaded from genbank. The 1154 bp of *rbc*L were comprised of 26.8% variable sites and the 500 bp of the cox1 gene of 37.0% variable sites. The genetic distance of *Neosiphonia harveyi* complex. Among the plastid and mitochondrial markers, the *cox*1 appears to be more variable than the *rbc*L. The most adequate DNA substitution models for each marker was calculated and the GTR+Γ+I model was selected. The topologies of the phylogenetic trees were completely topologically congruent for the two individual markers and the concatenated data set, but only the phylogenetic tree based on BI and the combined data set is shown (Fig. 182). ML and BI trees from the combined data set indicated monophyly of *N. decumbens, N. flavimarina*, *N. harlandii*, *N. harveyi, N. japonica, N. nipponica,* and *N. strictissima*. *N. strictissima* was recovered as sister to the remaining species. *N. japonica* was sister to the clade composed by *N. harlandii* and *N. decumbens*, whereas *N. flavimarina* and *N. harveyi* are sister. Each genetic species was morphologically characterized to confront the those features proposed by Kim and Yang (2006) (Fig. 169-181). There was no any diagnostic feature to characterize this genetic species. The genetic species *N. decumbens, N. flavimarina, N. harlandii*, *N. nipponica,* and *N. strictissima* were restricted to the northern hemisphere, whereas *N. harveyi* and *N. japonica* were widely distributed in both hemispheres (Fig. 168).

### 1.2. Delimiting *Neosiphonia* species

Three different methods for species delimitation (GMYC, ABGD, and SP) were used for taxa of the *N. harveyi* complex and *N. strictissima* based on the separate *rbc*L and *cox*1 data sets. *rbc*L marker supports only two main species for all species-delimitation methods, whereas *cox*1 marker

supports seven putative species for GMYC ($L_{GMYC}$= 572.928 > $L_0$= 561.209, *P*=0.01), 12 for ABGD (P=0.001), and six for SP (Fig. 182, Table 12). The results obtained from all species-delimitations methods in *N. harveyi* complex and *N. strictissima* based on *cox*1 were incongruent with the phylogenetic analyses.

### 1.3. Estimation of divergence time and phylogeographic analyses in *Neosiphonia*

The divergence time of our species was inferred on the basis of substitution rates of the *rbc*L and *cox*1 genes. The mutation rates of *rbc*L and *cox*1 obtained by this study from *Neosiphonia ramirezieae* collected from both sides of the Panama Strait 0.043 substitutions site$^{-1}$(million years)$^{-1}$ for *rbc*L, and 0.058 substitutions site$^{-1}$ (million years)$^{-1}$ for *cox*1. The divergence time in the phylogeny was estimated from the combined *rbc*L and *cox*1 data sets (Fig. 183, Table 13). The origins of the genus *Neosiphonia* ocurred approximately in the Middle Eocene (39.4-51.2 MYA). Diversification of the *N. harveyi* complex and *N. strictissima* was around the Middle Miocene (12.4-16.2 MYA), whereas the timings of divergences of the taxa of the *N. harveyi* complex were early Pliocene (3.6-4.6 MYA).

## 2. Discussion

### 2.1. Phylogenetic analyses

Our phylogenetic analyses obtained from combined data of markers for plastidial and mitochondrial genomes show that the genus *Neosiphonia* is a big monophyletic lineage (Fig. 182). It suggests that the three-celled carpogonial branch is an exclusive and consistent character to delimit the genus *Neosiphonia* (Kim and Lee 1999, Bustamante et al. 2015c). The status of all species is maintained with high support values. Our results also confirmed the monophyly of the group composed by species having four pericentral cells and cortication. We showed that this corticated group con-

tained multiple lineages (Fig. 182) which are showing a wide phenotypic plasticity being morphologically indistinguishable (Fig. 169-181). However, the lineages *N. flavimarina*, *N. harlandii*, and *N. nipponica* might likely be distinguished based on vegetative morphology as pointed by Kim and Yang (2006). *N. flavimarina* is likely having ultimate branchlets abundant, short, obtuse, and spur-like in several orders with a pair borne on one side of the axis alternating with a pair on the other side (Kim and Yang 2006); *N. harlandii* might be recognized by distinct main axes with numerous cicatrigenous branchlets near apex (Segi 1951); and *N. nipponica* is sometimes lacking of conspicuous main axis (Segi 1951). Generally, all lineages in morphology are having conspicuous main axis with variable branching pattern (alternate to pseudodichotomous to irregular to dichotomous and/or trichotomous) and scarce to abundant cicatrigenous branches throughout. Although these characters are not consistent to differentiate these lineages, Kim and Yang (2006) proposed them as segregating characters. We confirm the wide phenotypic plasticity among these lineages and the inconsistency of these features to delimit them as different species (McIvor et al. 2001, Stuercke and Freshwater 2010). Such morphological variation is likely driven by environmental conditions (Fraser et al. 2010).

The high support for each lineage indicates that these lineages should be regarded as species (Fig. 182), as they meet the criteria of phylogenetic species (Martin and Zucarello 2012). Similar findings were made by Kim and Yang (2006), but earlier Kudo and Masuda (1986) demonstrated incompletely isolated breeding groups among some of these lineages, suggesting that they cannot be defined on the basis of biological species concept. Moreover, the sequences divergences among these lineages excepting *P. strictissima* for *rbc*L and COI are lower than those values for intraspecific variation proposed by Mamoozadeh and Freshwater (2011) and the divergence among these lineages including *P. strictissima* are greater than those for interspecific variation.

### 2.2. Delimiting *Neosiphonia* species

Our DNA-based species delimitation analyses with two different markers (*rbc*L and COI) and three algorithmic techniques (GMYC, ABDG, and statistical parsimony) have demonstrated distinct lineages within the group of species with four pericentral cells and cortication (Fig. 182). Delimited species based on *rbc*L clearly identified two species, whereas delimitation based on COI provided a greater number of "putative genetic species" and yielded similar species to those determined on the basis of well-supported clades in our phylogenetic analyses (Fig. 182). These results support and are in accordance with Payo et al. (2013), Silberfeld et al. (2013) and Muangmai et al. (2014). The two species based on *rbc*L and the greater number based on COI provides an incongruent species delimitation boundaries. These differences are likely to be influenced by the different mutation rates of these two genetic markers as pointed out by Muangmai et al. (2014). The substitutions rates in *Neosiphonia* for *rbc*L and COI showed that the mitochondrial genes evolved 6.8-9.7 times faster than the plastid gene and it is 1.8-2.5 times faster than in other Rhodomelaceae (e.g. *Bostrychia,* Muangmai et al. 2014). The faster mutation rate in *Neosiphonia* might explain the higher degree of species diversity in this polysiphonous group (i.e. Guiry and Guiry (2015) listed 220 species in *Polysiphonia* sensu lato) than in *Bostrychia* (i.e. Guiry and Guiry (2015) listed 37 species in *Bostrychia* sensu lato). Kim and Yang (2006) recognized these corticated lineages as recently diverged species, but uncertainty about species boundaries is inevitable in recently diverged lineages (Leliaert et al. 2014) because speciation is a process and not a single event in time (Hey and Pinho 2012). The greater variability of COI is more suitable for detecting young divergence events (Muangmai and Zucarello 2014). Although the three DNA-based delimitation-methods for COI are not in agreement, our GMYC and ABGD model provide a better resolution of the multiple lineages present in our data (Fig. 182).

On the basis of our phylogenetic analyses, low genetic divergence, wide phenotypic plasticity, incomplete reproductive isolation (Kudo and Masuda 1986), and DNA-based species delimitation methods; the species complex with species having four pericentral cells and cortication is com-

posed of two species, namely *N. harveyi* and *P. strictissim*a, which are showing a wide phenotypic plasticity and the lineages embedded into *N. harveyi* are considered at subspecific rank. We propose the following combinations:

***Neosiphonia harveyi* subsp. *decumbens*** (Segi) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 169)

Basionym: *Polysiphonia decumbens* Segi (1951)

***Neosiphonia harveyi* subsp. *flavimarina*** (Kim et Lee) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 170)

Basionym: *Neosiphonia flavimarina* Kim and Lee (1999)

***Neosiphonia harveyi* subsp. *harlandii*** (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 171)

Basionym: *Polysiphonia harlandii* Harvey (1860)

***Neosiphonia harveyi* subsp. *harveyi*** (Bailey) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 172)

Basionym: *Polysiphonia harveyi* Bailey (1848).

***Neosiphonia harveyi* subsp. *japonica*** (Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 173-179)

Basionym: *Polysiphonia harveyi* Harvey (1857).

***Neosiphonia harveyi* subsp. *nipponica*** (Segi) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 180)

Basionym: *Polysiphonia nipponica* Segi (1951).

The present study is also proposing the following new combination based on the three-celled carpogonial branches in *P. strictissima*.

***Neosiphonia strictissima*** (J.D. Hooker et Harvey) D.E. Bustamante, B.Y. Won et T.O. Cho **comb. nov.** (Fig. 181)

Basionym: *Polysiphonia strictissima* J.D. Hooker et Harvey (1845).

In addition, the morphospecies *N. savatieri* (CUK9705, CUK9736 and CUK9761) was immersed in the *N. harveyi* subsp. *japonica* clade (% mean divergence). Although *N. savatieri* was characterized in detail by Kim (2005) and she pointed out the very small habit, ecorticated axes, and endogenous horizontal branches as diagnostic features, our molecular analyses demonstrate that these features are not consistent to consider *N. savatieri* as a different species. Earlier Yoon (1986) and Kudo and Masuda (1986) suggested the close relationship of *N. savatieri* with *N. japonica* on the basis of analyses of life history stages in laboratory culture. Thus, we propose the synonymy of *N. savatieri* with *N. harveyi* subsp. *japonica.* Our results also show that *N. harveyi* subsp. *japonica* is widely distributed in the northern and southern hemispheres of Atlantic and Pacific Ocean, whereas *N. harveyi* subsp. harveyi is only restricted to the Atlantic Ocean (Kim and Yang 2006). *N. harveyi* subsp. *decumbens*, *N. harveyi* subsp. *flavimarina*, and *N. harveyi* subsp. *nipponica* are distributed in the northwestern Pacific and north Atlantic (McIvor et al. 2001). The distribution *N. harveyi* subsp. *harlandii* and *N. strictissima* is likely to be restricted to the northwestern and southwestern Pacific, respectively. We have provided the first report of *N. harveyi* subsp. *flavimarina* in western Atlantic, *N. harveyi* subsp. *nipponica* in USA, and *N. harveyi* subsp. *japonica* in the Pacific coast of South America.

### 2.3. Estimation of divergence time and phylogeographic analyses in *Neosiphonia*

Our analysis of the divergence time of the genus *Neosiphonia* (Fig. 183) was calculated from substitution rate based on combined data set (*rbc*L and COI) and revealed that the most common ancestor of the *Neosiphonia* species was in Eocene (45.3 ± 5.9 MYA) (Fig. 183, Table 13). This ancestor was the first in showing the three-celled carpogonial branch and its origin is related to the first major global decline in temperature of water in the Tertiary (Savin 1977, Lunning 1990). The rapid temperature drops in this Eocene/Oligocene boundary event generated cool-adapted marine organisms (Benson et al. 1984, Gradstein et al. 2004) and also enhanced the evolution of temperate seaweed groups (Lunning 1990). Vegetative features have been conserved over long periods of time in Rhodomelaceae (Zucarello and West 2003, Muangmai et al. 2014). Our results confirmed the morphological stability (around 45 MYA) of the three-celled carpogonial branches in *Neosiphonia*.

The cortication in species with four pericentral cells in the genus *Neosiphonia* occurred for first time around 14.3 ± 1.9 MYA in the most common ancestor of *N. harveyi* and *N. strictissima* (Fig. 183) and has appeared later than other Rhodomelaceae (e.g. Muangmai et al. (2014) suggested 25 MYA for corticated species of *Bostrychia*). This derived character appeared in the second major temperature drop during the global glaciations in the Tertiary (15-10 MYA) (Fig. 183). This event generated the cooling of higher latitudes and drove speciation in cold water groups (e.g. North Pacific *Laminaria* species, Stam et al. (1988)). The most common ancestor of the *N. harveyi* were originated 4.1 ± 0.5 MYA and of *N. strictissima* were 3.0 ± 0.4 MYA. In the species *N. harveyi*, the oldest subspecies originated was *N. harveyi* subsp. *japonica* (1000000 years ago) and the youngest was *N. harveyi* subsp. *harveyi* (200000 years ago). These subspecies have recently originated during the fluctuations of temperature of late Pliocene and early Pleistocene (Fig. 183).

Our phylogeographic analyses in *N. harveyi* (Fig. 184-186) revealed that (1) the Korean and Japanese populations are genetically highly differentiated, (2) the ice age in the late Pleistocene and the associated marine tectonic configuration had a profound effect on biogeographical patterns of

*N. harveyi*, through the establishment of a glacial refugia for population survival during that glacial cycle, (3) the north Atlantic populations are showing a significant genetic differentiation, and (4) specimens from eastern Pacific just lack of a significant genetic substructure.

According to our sequence data, the highest genetic diversity of *N. harveyi* occurs around Japan and Korea (Fig. 168, 186, Table 13), suggesting the north-eastern Pacific as the most likely geographic origin (McIvor et al. 2001). The north Atlantic specimens of *N. harveyi* are showing a significant genetic diversity by having all *N. harveyi* subspecies, except *N. harveyi* subsp. *harlandii*. This suggests a genetic connectivity between the eastern Asia and North Atlantic. The subspecies *N. harveyi subsp. decumbens*, *N. harveyi subsp. flavimarina*, *N. harveyi subsp. japonica*, and *N. harveyi subsp. nipponica* were originated around 700000 years ago and their migration from the eastern Asia to the north Atlantic likely took place through an ice-free Arctic Ocean before the first widespread Pleistocene glaciations in Iceland (Einarsson et al. 1967, Lunning 1990). In contrast, the low genetic diversity and the wide distribution of *N. harveyi* subsp. *japonica* in the northern and southern hemisphere and the distribution of *N. harveyi* subsp. *harveyi* in the Atlantic Ocean likely suggest that they were introduced by human activities as a consequence of ballast water and hull fouling (Bustamante et al. 2015b).

**Fig. 168.** Sampling locations of the *Neosiphonia harveyi* complex specimens in Argentina, Australia, Chile, Japan, Korea, Peru, Spain, United Kingdom, and USA, Colors refers to each of the subspecies that are composing this species complex (*N. harveyi* subsp. *decumbens*, green; *N. harveyi* subsp. *flavimarina*, orange; *N. harveyi* subsp. *harlandii*, red; *N. harveyi* subsp. *harveyi*, purple; *N. harveyi* subsp. *japonica*, blue; *N. harveyi* subsp. *nipponica*, yellow)

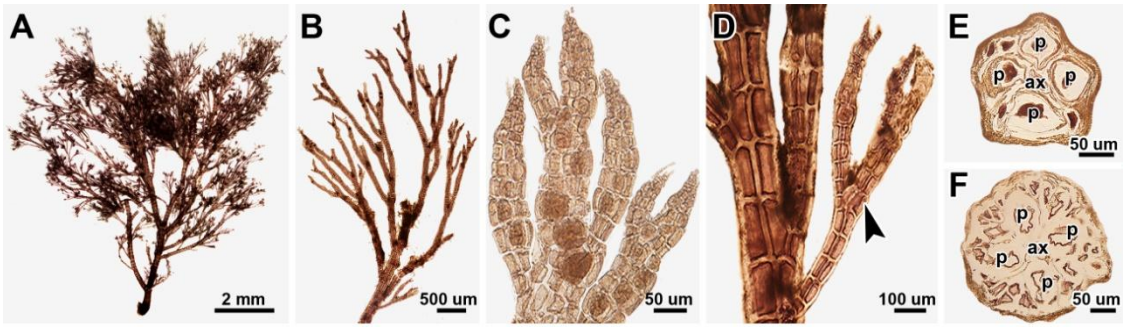


**Fig. 169.** Anatomical structures of *N. harveyi* subsp. *decumbens*. (A) Habit of the plant. (B-C) Erect axes showing pseudodichotomous (B) to alternate (C) branching pattern. (D) Erect axis showing a cicatrigenous branch (arrowhead). (E-F) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

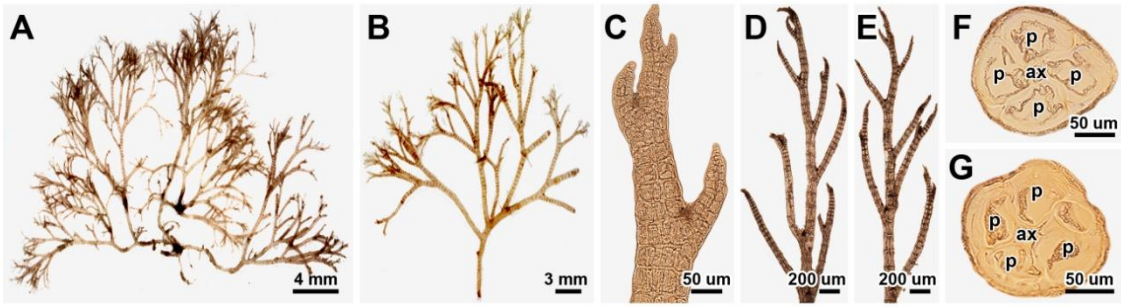


**Fig. 170.** Anatomical structures of *N. harveyi* subsp. *flavimarina*. (A) Habit of the plant. (B-C) Erect axes showing pseudodichotomous (B) to alternate (C) branching pattern. (D-E) Apex showing axes branched with a pair of unilateral branches borne on one side of the axis alternating with a pair on the other side. (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

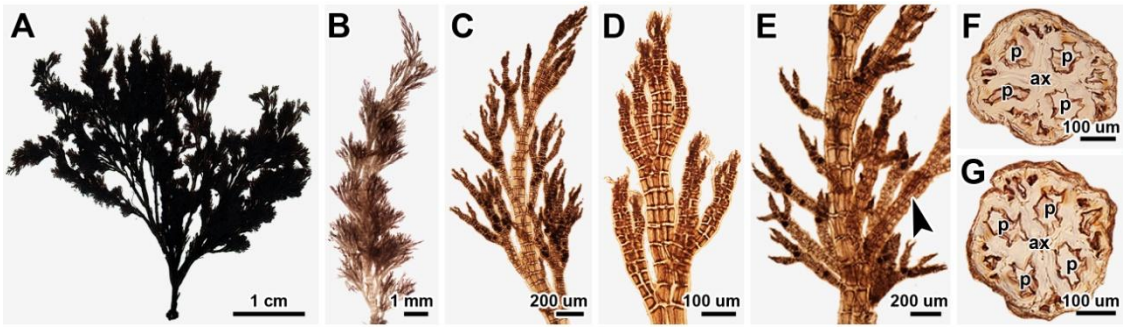


**Fig. 171.** Anatomical structures of *N. harveyi* subsp. *harlandii*. (A) Habit of the plant. (B) Erect axes densely branched. (C-D) Erect axes showing pseudodichotomous (C) branching pattern bearing dichotomous and trichotomous branches (D). (E) Erect axis showing abundant cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

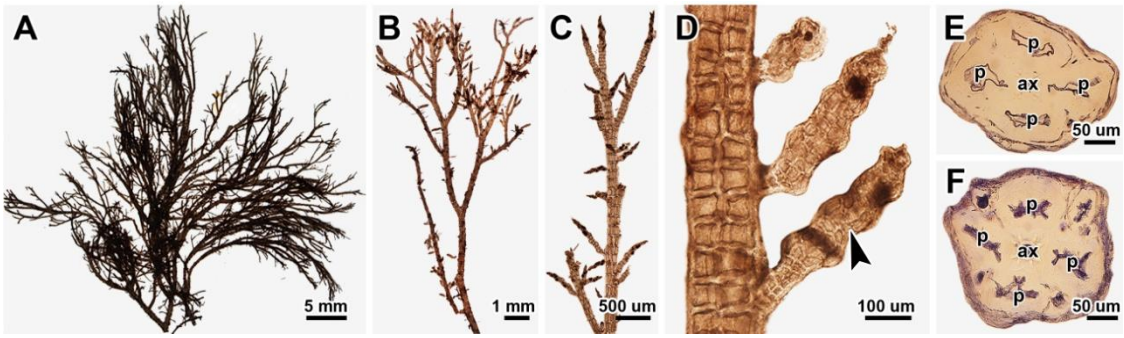


**Fig. 172.** Anatomical structures of *N. harveyi* subsp. *harveyi*. (A) Habit of the plant. (B) Erect axes showing pseudodichotomous to alternate branching pattern. (C-D) Erect axes showing cicatrigenous branches (arrowhead) throughout. (E-F) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

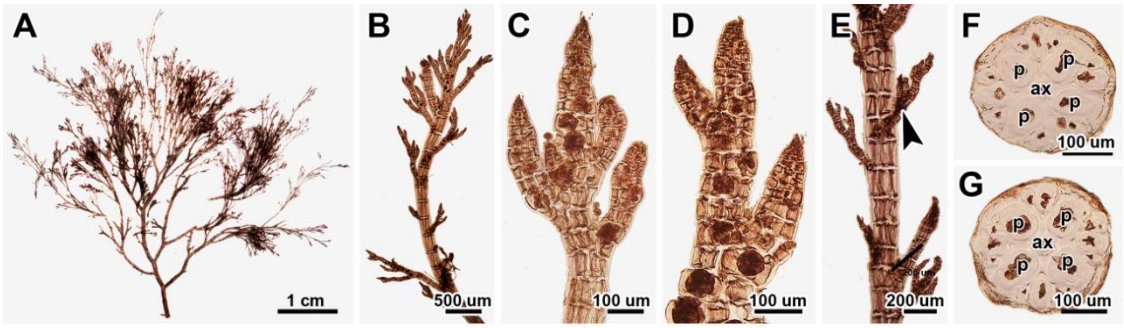


**Fig. 173.** Anatomical structures of *N. harveyi* subsp. *japonica* from United Kingdom. (A) Habit of the plant. (B) Erect axes scarcely branched. (C-D) Erect axes showing irregular to alternate branching pattern. (E) Erect axes showing cicatrigenous branches (arrowhead) spirally disposed. (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

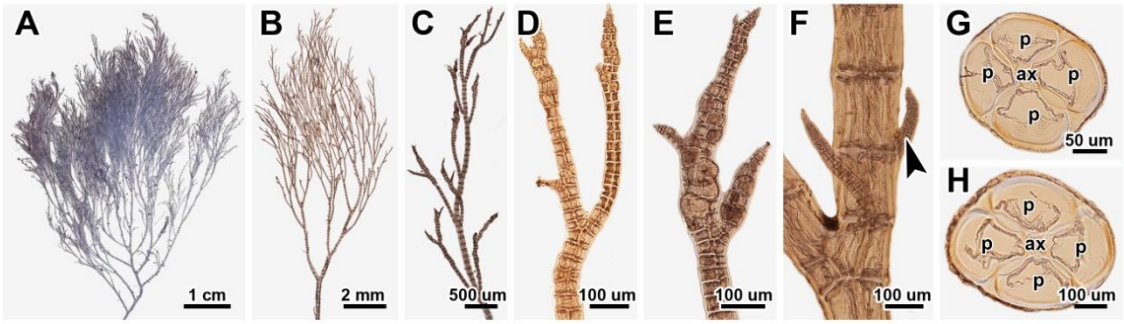


**Fig. 174.** Anatomical structures of *N. harveyi* subsp. *japonica* from United States. (A) Habit of the plant. (B) Apex densely branched. (C) Erect axes scarcely branched. (D-E) Erect axes showing dichotomous (D) to pseudodichotomous (E) branching pattern. (F) Erect axes showing cicatrigenous branches (arrowhead) spirally disposed. (G-H) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

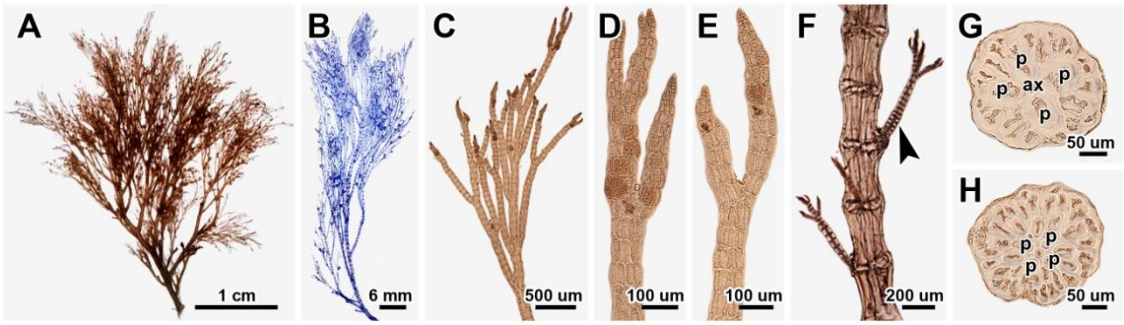


**Fig. 175.** Anatomical structures of *N. harveyi* subsp. *japonica* from Peru. (A) Habit of the plant. (B-C) Apex densely branched. (D-E) Erect axes showing alternate to pseudodichotomous branching pattern. (F) Erect axes showing cicatrigenous branches (arrowhead) spirally disposed. (G-H) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

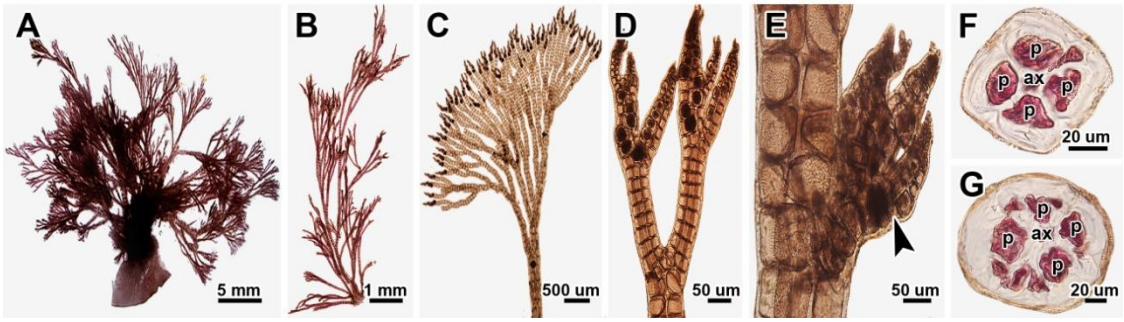


**Fig. 176.** Anatomical structures of *N. harveyi* subsp. *japonica* (previously known as *N. savatierii*) from Korea. (A-B) Habit of the plant. (C) Erect axes showing the apex densely branched. (D) Apex showing alternate branching pattern with trichotomous branches. (E) Erect axes showing cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

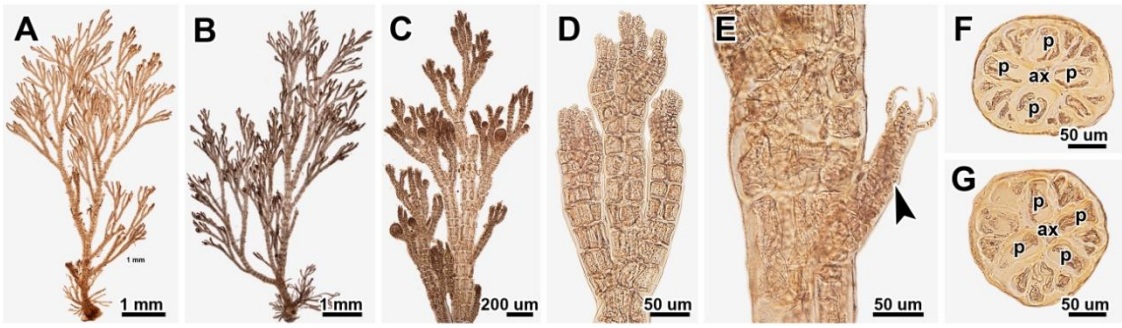


**Fig. 177.** Anatomical structures of *N. harveyi* subsp. *japonica* from Korea. (A-B) Habit of the plant. (B) Erect axes showing the apex densely branched. (C-D) Apex showing alternate branching pattern. (E) Erect axes showing cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

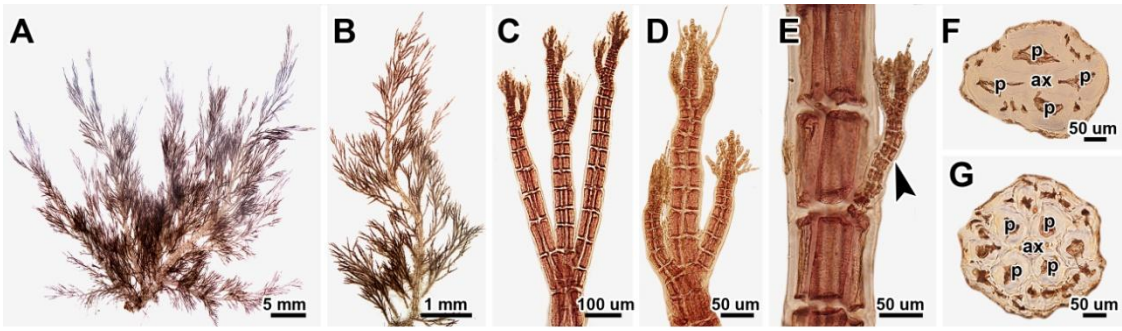


**Fig. 178.** Anatomical structures of *N. harveyi* subsp. *japonica* from Australia. (A) Habit of the plant. (B) Erect axes showing the apex densely branched. (C-D) Apex showing pseudodichotomous branching pattern with trichotomous branches. (E) Erect axes showing cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

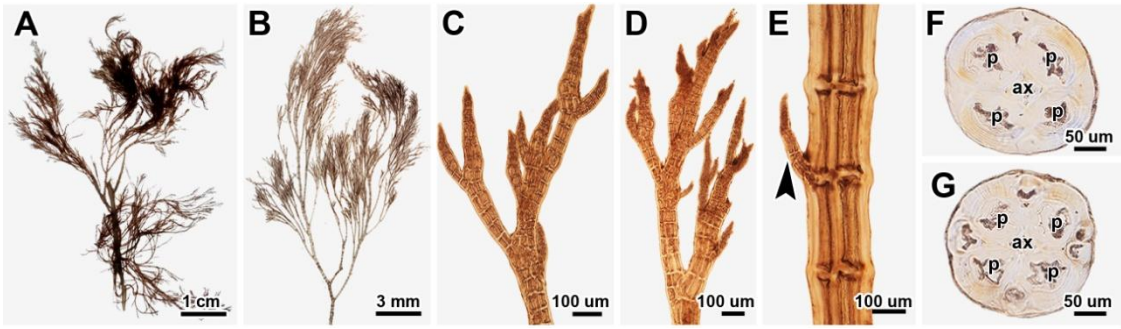


**Fig. 179.** Anatomical structures of *N. harveyi* subsp. *japonica* from Japan. (A) Habit of the plant. (B) Erect axes showing the apex densely branched. (C-D) Apex showing irregular branching pattern. (E) Erect axes showing cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

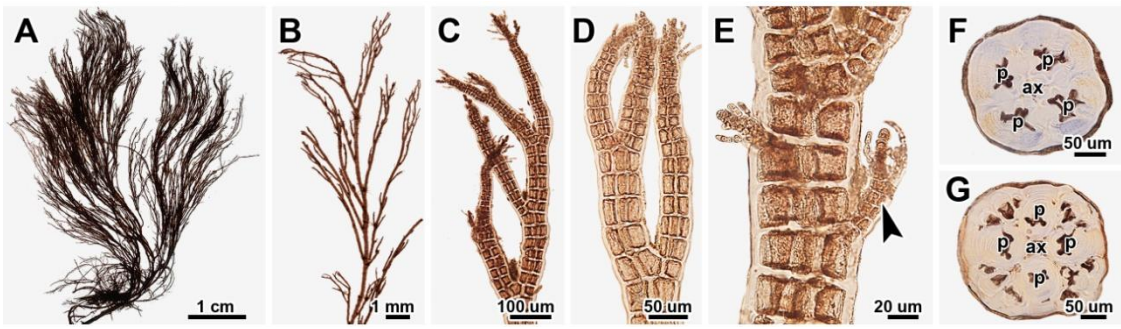


**Fig. 180.** Anatomical structures of *N. harveyi* subsp. *nipponica* from Japan. (A) Habit of the plant. (B) Erect axes showing the apex scarcely branched. (C-D) Apex showing irregular branching pattern. (E) Erect axes showing cicatrigenous branches (arrowhead). (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

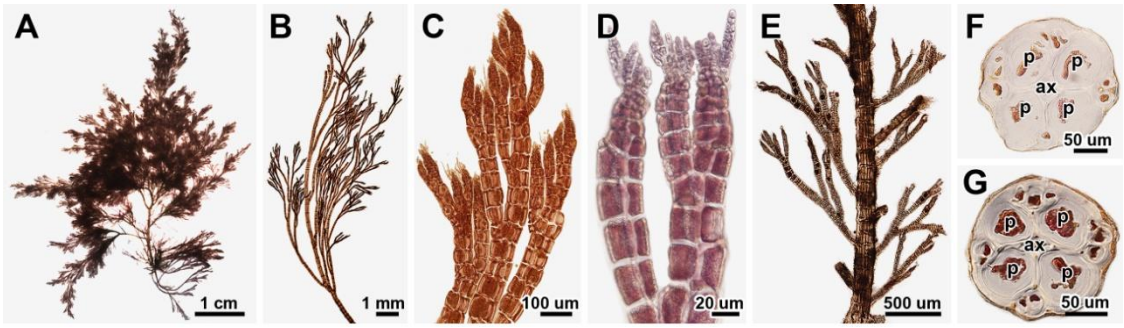


**Fig. 181.** Anatomical structures of *N. strictissima* from Australia. (A) Habit of the plant. (B) Erect axes showing the apex densely branched. (C-D) Apex showing pseudodichotomous to alternate branching pattern. (E) Erect axes showing abundant cicatrigenous branches. (F-G) Cross-section views of middle and basal part of the axes (ax, axial cell; p, pericentral cell).

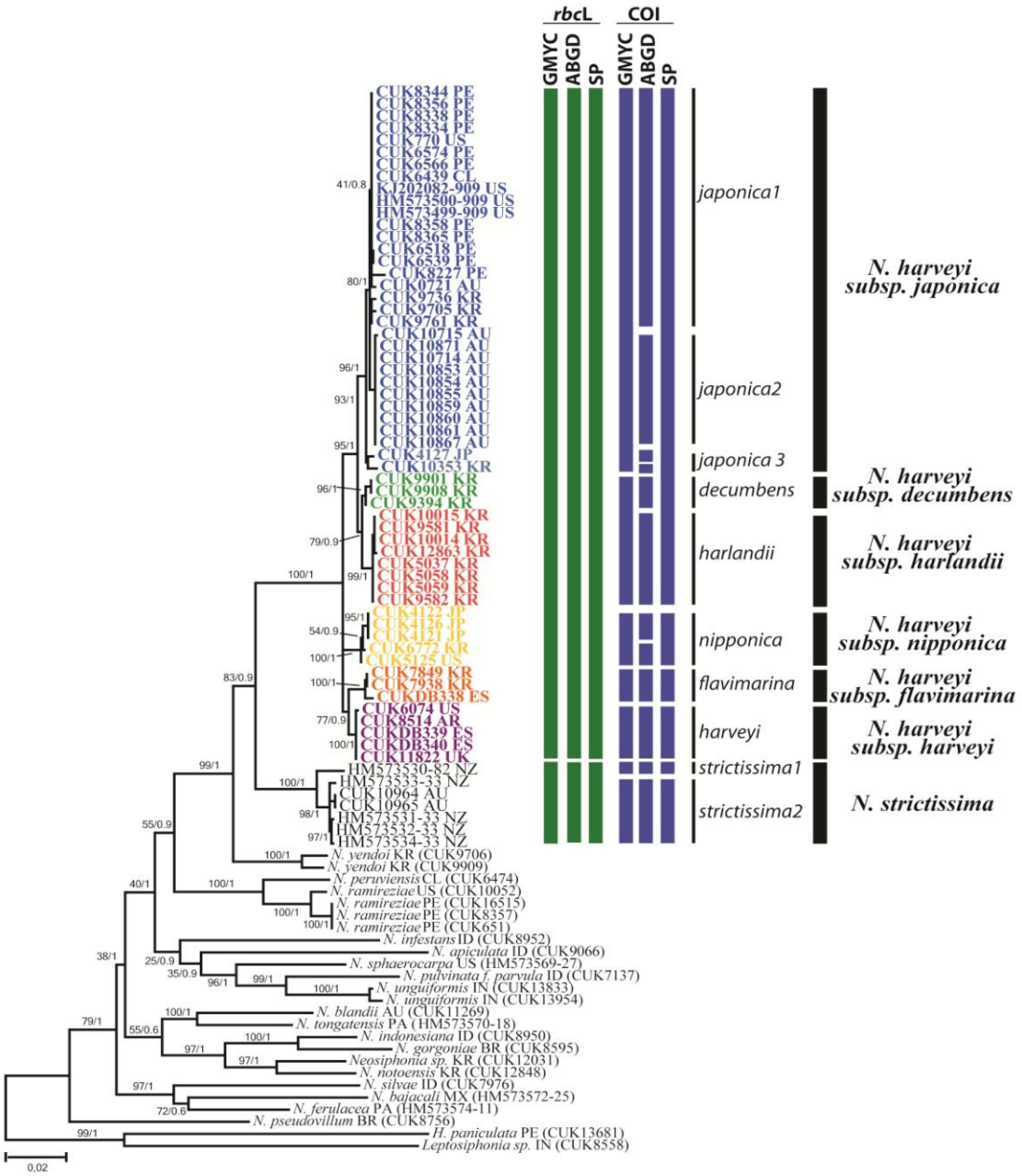


**Fig. 182.** Phylogenetic tree inferred from Maximum likelihood analyses of the combined data set of rbcL and COI for taxa of the *Neosiphonia harveyi* complex and some *Neosiphonia* species. Support values at each node are bootstrap values from ML (left) and Bayesian posterior probability (right). The results of three species delimitation methods: GMYC model, the ABGD, and statistical parsimony (SP), based on *rbc*L (green bars) and COI (blue bars), are indicated at the right edge of the tree. The thin black bars indicate the species obtained from congruent results of phylogenetic analyses and thick black bars indicate the category of subspecies.

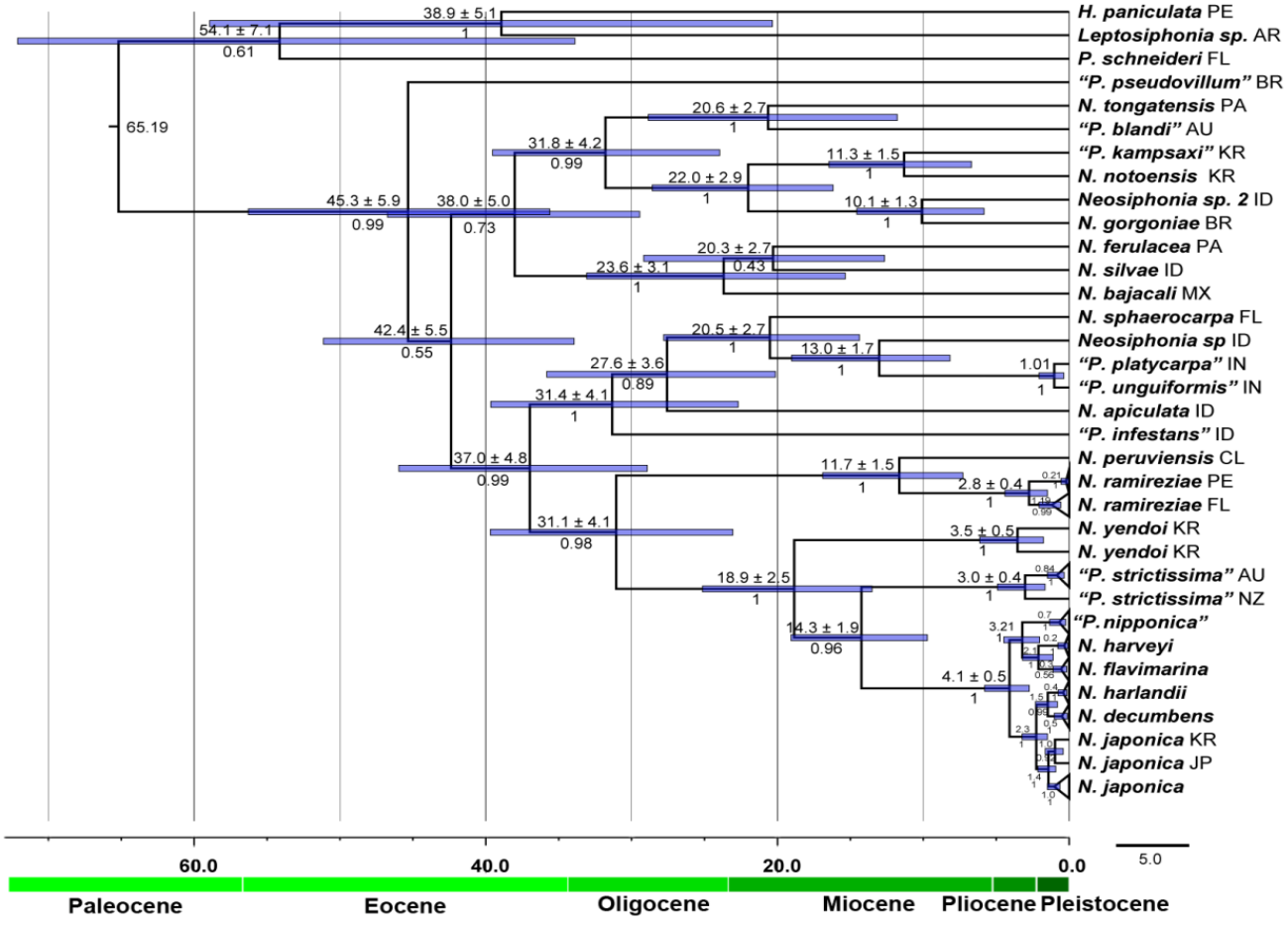


**Fig. 183.** Bayesian tree for taxa from the *Neosiphonia harveyi* complex and some *Neosiphonia* species reconstructed using BEAST under a relaxed clock model of the combined data set of rbcL and COI. Bars show 95% highest posterior densities of divergence dates. Mean dates followed by ± standard deviation (above bars) and Bayesian posterior probabilities (below bars). Scale bar are in million years.

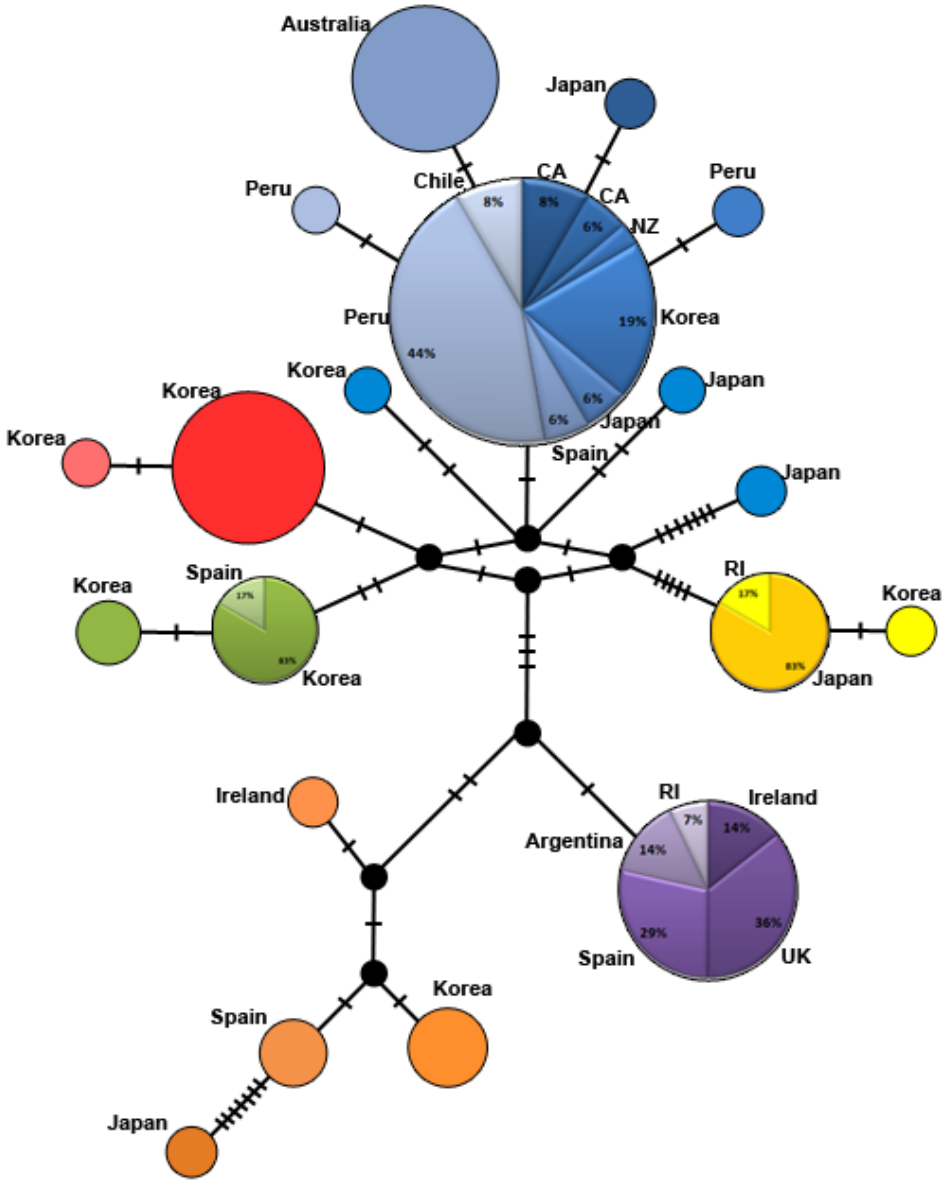


**Fig. 184.** *rbc*L haplotype network for taxa of the *Neosiphonia harveyi* complex obtained from the Network analyses. Colors represent the different species: *N. harveyi* subsp. *decumbens*, green; *N. harveyi* subsp. *flavimarina*, orange; *N. harveyi* subsp. *harlandii*, red; *N. harveyi* subsp. *harveyi*, purple; *N. harveyi* subsp. *japonica*, blue; *N. harveyi* subsp. *nipponica*, yellow. The size of the circles is proportional to the number of individuals sampled belonging to that haplotype. Small black circles represent inferred missing or extinct haplotypes and small lines represent mutational steps between haplotypes.

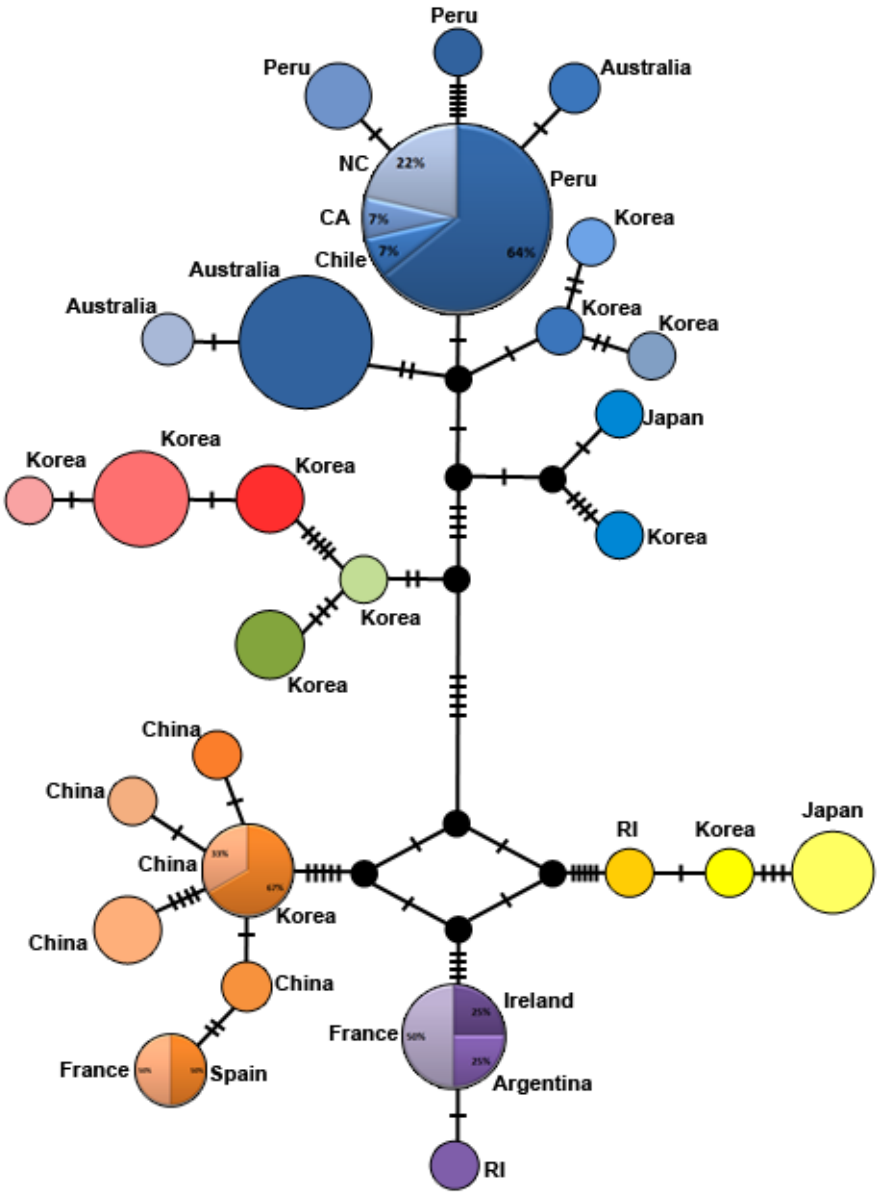


**Fig. 185.** COI haplotype network for taxa of the *Neosiphonia harveyi* complex obtained from the Network analyses. Colors represent the different species: *N. harveyi* subsp. *decumbens*, green; *N. harveyi* subsp. *flavimarina*, orange; *N. harveyi* subsp. *harlandii*, red; *N. harveyi* subsp. *harveyi*, purple; *N. harveyi* subsp. *japonica*, blue; *N. harveyi* subsp. *nipponica*, yellow. The size of the circles is proportional to the number of individuals sampled belonging to that haplotype. Small black circles represent inferred missing or extinct haplotypes and small lines represent mutational steps between haplotypes.


**Fig. 186.** Worldwide distribution of for taxa of the *Neosiphonia harveyi* complex based on *rbc*L data. Each pie chart shows the proportion of subspecies and their haplotypes. *N. harveyi* subsp. *decumbens*, green; *N. harveyi* subsp. *flavimarina*, orange; *N. harveyi* subsp. *harlandii*, red; *N. harveyi* subsp. *harveyi*, purple; *N. harveyi* subsp. *japonica*, blue; *N. harveyi* subsp. *nipponica*, yellow. Empty circles represent the absence of this complex.

**Table 12.** Number of putative species based on separate *rbc*L and COI data sets for three different species-delimitations methods.

| **Markers** | ***rbc*L** | | | **COI** | | |
|---|---|---|---|---|---|---|
| Species-delimitations methods | GMYC | ABGD | SP | GMYC | ABGD | SP |
| Number of putative species | 2 | 2 | 2 | 7 | 12 | 6 |

**Table 13.** Timings of divergences of the most common ancestors of the taxa in *Neosiphonia*, the *N. harveyi* complex, and *N. strictissima.*

| **Taxa** | **Timings of diver-gences (MYA)** |
|---|---|
| *Neosiphonia* | 39.4-51.2 |
| *N. strictissima* | 2.6-3.4 |
| *N. harveyi* complex | 3.6-4.6 |
| *N. harveyi* subsp. *japonica* comb. nov. | 1 |
| *N. harveyi* subsp. *decumbens* comb. nov. | 0.5 |
| *N. harveyi* subsp. *nipponica* comb. nov. | 0.7 |
| *N. harveyi* subsp. *harlandii* comb. nov. | 0.4 |
| *N. harveyi* subsp. *flavimarina* comb. nov. | 0.3 |
| *N. harveyi* subsp. *harveyi* comb. nov. | 0.2 |

# IV. CONCLUDING REMARKS

The family Rhodomelaceae is a monophyletic group and the ultimately sympodial branching of the gonimoblast after the first formed carposporangium is considered diagnostic feature for this family (Choi et al. 2002). According to Guiry and Guiry (2015), Rhodomelaceae is composed of fourteen tribes. Our study focuses on the study of the *Polysiphonia* sensu lato, which is placed in the tribe Polysiphoniae. Our phylogenetic analyses based on mithochondria-encoded *cox*1 and plastid-enconded *rbc*L revealed that *Polysiphonia* sensu lato (= tribe Polysiphonieae) is sister to the clade composed by the tribes Herposiphonieae and Pterosiphonieae (Schmitz and Falkenberg 1897, Falkenberg 1901) and the genera *Wilsonosiphonia* (a candidate for a new tribe) and *Womersleyella*. Moreover, the genus *Brongniartella* (tribe Brongniartelleae), composed by *B. byssoides* and *B. australis* (Parsons 1980), was placed into the multipericentral group, suggesting that Brongniartelleae is embedded in the tribe Polysiphonieae. Also, the genus *Streblocladia* (tribe Streblocladieae) based on the generitype *Streblocladia glomerulata* (Kylin 1956, Phillips 2010), resolved sister to the clade composed by *Leptosiphonia*, *Neosiphonia*, and *Polyostea*, indicating that Streblocladieae is also embedded in Polysiphonieae. In addition, the genus *Lophosiphonia* has been placed to an unnamed artificial group by Falkenberg (1901) and extensive discussion and proposals about its relationsip in Rhodomelaceae has been proposed (Kylin 1956, Maggs and Hommersand 1993, Womersley 2003, Díaz-Tapia and Bárbara 2013). Recent works did not clarify the taxonomic position of the *Lophosiphonia* group within the family Rhodomelaceae (Díaz-Tapia and Bárbara 2013), but our phylogenetic analyses of *rbc*L and *cox*1 locus revealed that *Lophosiphonia* based on the generitype *L. obscura* is embedded in the tribe Polysiphonieae. On the other hand, the genus *Womersleyella*, placed in the Polysiphonieae (Hollenberg 1967), was resolved paraphyletic with low support to the tribe Pterosiphonieae, suggesting that *Womersleyella* is not a member of the tribe Polysiphonieae. Thereby, Polysiphonieae is an extensive tribe composed of the following genera: *Boergeseniella*, *Brongniartella*, *Bryocladia*, *Diplocladia*, *Dorsisiphonia*, *Enelittosiphonia*, *Hapterosiphonia*, *Lampisiphonia*, *Leptosiphonia*, *Lophosiphonia*, *Neosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Polyostea*, *Polysiphonia* sensu stricto, *Streblocladia*, *Tolypiocladia*, *Vertebrata* (Fig. 19).

Our *rbc*L and c*ox*1 phylogenies placed the new genus *Hapterosiphonia* based on *H. paniculata* in a strongly supported clade. Among the features delineating *Hapterosiphonia,* lobed, multicellular rhizoidal terminations, and a paniculate branching pattern are the principal characters that separate this genus from others in *Polysiphonia* sensu lato and the tribe Polysiphonieae.

The present taxonomical circumscription of the genus *Leptosiphonia* was enlarged with the rhizoidal cells between axial and pericentral cells and the cortication as diagnostic generic level features to include the following new combinations: *L. brodiei, L. elongata,* and *L. virgata,* and one new species, namely, *Leptosiphonia platensis. rbc*L and c*ox1* phylogenies reveleaed *Leptosiphonia* as a monoplyletic genus with strong and well support originally distributed along the western and eastern Atlantic coast.

Our *rbc*L phylogenies reveal that our 22 species colleted from worldwide are well supported as *Neosiphonia* members. The three-celled carpogonial branches and the rhizoids cutting off the pericentral cells result in consistent characters to place these species in *Neosiphonia*. Among the 22 species, six are new species and nine are new combinations. The distinction of species in our phylogenetic analyses for *rbc*L followed the threshold proposed by Mamoozadeh and Freshwater (2011), who established sequence divergences below 1.3 % represent intraspecific variation and those greater than 2.13 % represent interspecific variation in *Polysiphonia* sensu lato. Our study suggests *Neosiphonia* as a wide distributed genus with a high diversity of species especially in the eastern Indic Ocean.

The resurrected genus *Polyostea* is composed of *Polyostea bipinnata* and *Ps. gracilis.* Our phylogenetic analyses positioned *Polyostea* as a separated genus in *Polysiphonia* sensu lato. *Polyostea* is clearly distinguished from all the genera of *Polysiphonia* sensu lato by the combination of bilateral phyllotaxy, ecorticated axes, and congenital fusion.

The new genera *Dorsisiphonia*, *Phillipsiphonia*, and *Neostreblocladia* has been segregated from the paraphyletic *Polysiphonia* sensu stricto on the basis of morphological diagnostic features and

molecular analyses. *Dorsisiphonia* is widely distributed, whereas *Phillipsiphonia* and *Neostreblocladia* are only restricted to the southern hemisphere. The true clade of *Polysiphonia* sensu stricto, where the generitype *P. stricta* is embedded, is monophyletic and worldwide distributed.

Our molecular analyses based on plastid-encoded *rbc*L confirmed the genus *Tolypiocladia* embedded in the tribe Polysiphoniae. The distant relationship between *Tolypiocladia* and *Polysiphonia* sensu stricto (including *Bryocladia*) might suggest that open connection of rhizoids and pericentral cells is a convergent character that evolved separately in *Tolypiocladia* and *Polysiphonia* sensu stricto.

The segregation of two species, previously identified as *Polysiphonia howei*, into the new genus *Wilsonosiphonia* resolve some of the heterogeneity in *Polysiphonia* sensu lato. The new genus *Wilsonosiphonia* was established with the description of a new species *Wilsonosiphonia fujiiae* and a new combination *W. howei*. The diagnostic features segregating *Wilsonosiphonia* from *Polysiphonia* sensu lato is the location of rhizoids in the distal end of pericentral cells and multicellular terminations of rhizoids in axonomorphus shape (taproot shape). The *rbc*L and c*ox1* phylogenies revealed strong and well support.

Our morphological and molecular analyses have demonstrated that the multipericentral group is composed the following genera *Boergeseniella, Brongniartella, Diplocladia, Enelittosiphonia, "Polysiphonia", and Vertebrata*. These six genera embedded in the multipericentral group are showing a unique combination of diagnostic features that support their recognition as separate genera into this paraphyletic multipericentral group. The genetic distance among these genera is over 8% of sequence divergence for *rbc*L, whereas is over 10% of divergence for *cox*1.

The detail morphological and molecular analyses of the species *Neosiphonia echinata* and *Womersleyella indica* collected from the western Atlantic and Indic Ocean suggest that these polysiphonous species are introduced species that extent their original distribution. Because of the worldwide interconnections by shipping, the occurrence of these species along distant realms may

be associated with the two primary subvectors for marine species introduction: hull fouling and ballast water.

On the basis of our phylogenetic analyses, low genetic divergence, wide phenotypic plasticity, incomplete reproductive isolation, and DNA-based species delimitation methods; the species complex with species having four pericentral cells and cortication in the genus *Neosiphonia* is composed of two species, namely *N. harveyi* and *N. strictissim*a and the lineages embedded into *N. harveyi* are considered at subspecific rank. This analyses revelaed that the most common ancestor of the *Neosiphonia* species was in Eocene (45.3 ± 5.9 MYA) confirming the morphological stability (around 45 MYA) of the three-celled carpogonial branches in *Neosiphonia*. Cortication in *Neosiphonia* appeared in the second major temperature drop during the global glaciations in the Tertiary (15-10 MYA). In the species *N. harveyi*, the oldest subspecies originated was *N. harveyi* subsp. *japonica* (1000000 years ago) and the youngest was *N. harveyi* subsp. *harveyi* (200000 years ago). The highest genetic diversity of *N. harveyi* occurs around Japan and Korea, suggesting the north-eastern Pacific as the most likely geographic origin. The north Atlantic specimens of *N. harveyi* are showing a significant genetic diversity suggesting a genetic connectivity between the eastern Asia and North Atlantic. In contrast, the low genetic diversity and the wide distribution of *N. harveyi* in the northern and southern hemisphere and the likely suggest that they were introduced by human activities as a consequence of ballast water and hull fouling.

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# ABSTRACT*

## 홍조류 붉은실속 (*Polysiphonia* sensu lato)의 계통, 분포 및 진화에 관한 연구

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붉은실속 (*Polysiphonia*) sensu lato 는 열대, 온대, 한대지역에서 남극에 가까운 지역, 그리고 맹그로브 (mangrove)가 서식하고 있는 담해수 지역까지도 매우 광범위하게 분포하고 있는 그룹이다. 2003 년부터 2015 년까지 총 16 개국, 156 개의 채집지로부터 채집된 1088 개의 붉은실속 (*Polysiphonia*) sensu lato 샘플들을 형태적, 분자적 연구를 수행한 결과 이들은 총 15 개의 속으로 분류되었다 (*Boergeseniella*, *Bryocladia*, *Diplocladia*, *Dorsisiphonia*, *Hapterosiphonia*, *Lampisiphonia*, *Leptosiphonia*, *Neosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Polyostea*, *Polysiphonia* sensu stricto, *Streblocladia*, *Tolypiocladia*, *Vertebrata*, *Wilsonosiphonia*). 이 중 형태학적 특징들과 분자분석 방법의 최대우도추정법 (Maximum likelihood)과 베이시안 네트워크 분석 (Bayesian analyses)의 높은 bootstrap value 에 기반하여 *Dorsisiphonia*, *Hapterosiphonia*, *Neostreblocladia*, *Phillipsiphonia*, *Wilsonosiphonia* 5 개의 속이 신속으로 추가되었고, 식별형질이 명확하지 않았던 *Leptosiphonia*

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속의 형질들을 추가하여 속의 범위를 더 광범위하게 한정하였고, *Polystea* 속을 다시 부활시켰다. 따라서, *Polysiphonia* sensu lato 그룹 내에서 총 16 개의 신종과 31 개의 new combination 을 제안하였다. *Neosiphonia* 속은 3 개의 세포로 구성된 조과사를 가진다는 점에서 *Polysiphonia* 속으로부터 분리되었다. 그 중, 4 개의 주심세포와 피층세포를 가지는 6 개의 *Neosiphonia* 종들은 *Neosiphonia harveyi* complex 그룹으로 보고 되어왔다. 본 연구에서는 형태적으로 종을 구분하는 뚜렷한 형질은 나타나지 않지만 유전적인 염기서열 사이에서는 뚜렷이 구분되는 이 종들의 분류학적 위치를 규명하고 그 종들의 진화를 연구하기 위해 전 세계에서 채집된 *Neosiphonia harveyi* complex 에 속하는 표본들을 엽록체의 *rbc*L 유전자와 미토콘드리아의 *cox*1 유전자로 염기서열을 분석하였다. 그 결과, 종 수준으로는 *N. harveyi* 와 "*P. strictissima*" 두 종이 *Neosiphonia harveyi* complex 내에 존재하는 것으로 분석되었다. 유전적으로 6 개의 분류군으로 구성되었던 *N. harveyi* 는 DNA-based delimitation models 분석방법에 의해 아종 (subspecies)의 범위로 한정하였다. 또한, *rbc*L 과 *cox*1 의 염기서열 자료의 치환비율을 이용하여 이들 종들이 계통지리학적으로 언제, 어디에서 기원하였는지 알아보기 위하여 divergence time 을 측정해 본 결과, 이 종들은 Iceland 에서 홍적세 빙기가 널리 퍼지기 이전인 무빙극 시대였던 약 700,000 년 전에 동아시아 (한국과 일본)로부터 기원하여 북대서양으로 이동을 한 것으로 분석되었다. 이러한 결과에 반해, 몇몇 *Polysiphonia* 와 같은 종들은 외래종이나 도입종으로써 인식되어 왔고 본 연구에서 *Neosiphonia haveryi subsp. harveyi*, *Neosiphonia harveyi subsp. japonica*, *Neosiphonia echinata*, *Pterosiphonia arenosa*, *Womersleyella indica* sp. nov. 는 전 세계적으로 널리 분포한다는 것을 입증하였다.

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