Diversity and revised classification of *Polysiphonia sensu lato* from the San Juan Islands based on morphology and molecular evidence

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Abstract

The large red algal group *Polysiphonia sensu lato* dominantly represents the Streblocladieae and Polysiphonieae tribes. The segregation of 12 genera under the Streblocladieae tribes further suggests the taxonomic update of this group. Specimens believed to be *Polysiphonia* were collected during low tide for this study at different locations throughout San Juan and Lopez Island and processed at Friday Harbor Laboratory (FHL) in June 2023. Initially, the fresh specimens were separated under a dissecting scope, selected for DNA extraction, and representative specimens were pressed between the herbarium sheets. The branching patterns, presence or absence of trichoblast cells, rhizoidal attachment, number of pericentral cells, and reproductive features were considered for anatomical study. A total of 36 specimens were sequenced, amplifying the *rbc*L gene, and sequenced data were aligned with worldwide representative species, and maximum likelihood (ML) was performed for the phylogenetic analysis. Our results revealed six species belonging to four genera. Four species, namely: *Eutrichosiphonia confusa* (Hollenberg) Savoie & G.W. Saunders; *Polysiphonia determinata* Hollenberg; *Vertebrata hendryi* (N.L.Gardner) Savoie & Saunders and *Savoiea robusta* (N.L.Gardner) M.J.Wynne was confirmed based on the anatomy and molecular phylogeny. Additionally, *Eutrichosiphonia* sp. and *Polysiphonia* sp. formed separate clades with their representatives, where *Polysiphonia* sp. had 2% genetic distance from *P. determinata*. *S. robusta* is a new record, and *Polysiphonia* sp. might be a new species. However, Multi-gene analysis with different localities might resolve the species identity. Finally, species belonging to *Polysiphonia sensu lato* within the San Juan Islands have been updated.

Keywords: Maximum likelihood; Molecular phylogeny Morphology; *rbc*L gene

Introduction

The red alga *Polysiphonia sensu lato*, belonging to the tribes Streblocladieae and Polysiphonieae, are globally distributed throughout coastal regions (Bustamante et al., 2021; Guiry and Guiry 2023). This group has been segregated based on morphological features using the following characteristics; pericentral cell number, rhizoid type, presence or absence of cortication, trichoblast arrangement, branching origin in association with the trichoblast, tetrasporangial arrangement, spermatangial branching, and carpogonial branch attachment (Hollenberg 1968, Kim and Lee 1999, Stuercke and Freshwater 2008). Approximately 300 species have been classified within *Polysiphonia sensu lato,* where more than 100 species of Streblocladieae and 185 species of *Polysiphonia* are currently taxonomically accepted (Guiry and Guiry 2023). Streblocladieae has been segregated into 12 genera, including the following; *Acanthosiphonia*, *Aiolocolax*, *Carradoriella*, *Eutrichosiphonia*, *Kapraunia*, *Lampisiphonia*, *Leptosiphonia*, *Melanothamnus*, *Savoiea*, *Streblocladia*, *Tolypiocladia*, and *Vertebrata* (Savoie and Saunders 2018, Bustamante et al., 2021; Guiry and Guiry 2023).

These algae form macroscopic clumps of turf that show unique filamentous thalli when observed under a light microscope (Díaz-Tapia et al. 2020; Hollenberg 1942). Cross-sections of the thalli show a main central cell that is surrounded in a verticillate pattern by at least four pericentral cells (Kapraun and Norris 1982, Stuercke and Freshwater 2008). As a group, *Polysiphonia* lacks morphological consistency, and identifying diagnostic features to distinguish them can be difficult. Due to the uncertainty in morphology, molecular phylogenetic analyses are imperative for the correct taxonomic classification of and resolving the ongoing uncertainty of *Polysiphonia sensu lato* (Savoie and Saunders 2018).

The Gabrielson and Lindstrom 2018 key describes 12 species distributed throughout Southeast Alaska, British Columbia, Washington, and Oregon. Of those 12 species, there are herbarium records for three species of Streblocladieae and Polysiphonieae in the San Juan Islands, two of which have four varieties listed within the islands. These records include the following species names: *Polysiphonia hyndryi* var. *deliquescens, P. hyndryi* var. *gardneri, P. hyndryi* var. *hendryi, P. hyndryi* var. *luxurians, P. pacifica* var. *delicatula, P. pacifica* var. *distans, P. pacifica* var. *gracilis, P. pacifica* var. *pacifica, and P. paniculata* (Table 1). The location that these specimens fit within the classification will be assessed in this study using molecular and morphological data given the current taxonomy, and the Gabrielson and Lindstrom Key will be updated.

Materials and Methods

Field collections

36 total specimens of *Polysiphonia sensu lato* were collected from Cattle Point, Eagle Cove, Deadman Bay, the FHL dock on San Juan Island, as well as Iceberg Point on Lopez Island, Washington, in June 2023 (Fig. 1). All specimens were kept in flow-through seawater tanks at ambient water temperature (~12 °C) until processed for molecular and morphological analyses. All *Polysiphonia* were reviewed using a Nikon SMZ800 to determine quick morphological differences. If differences in branching patterns, color, contrasting thallus length, rhizoidal attachment, or variations in pericentral cells were observed, they were segregated into unique petri dishes for DNA extraction and further morphological assessment.

DNA extraction, PCR amplification and sequencing

We pressed and submitted 36 voucher specimens to the University of Washington Burke Museum's herbarium (WTU), and preserved the remaining thalli in silica gel (Chase and Hills 1991, Freshwater et al. 1994) (Table S1). DNA was extracted from live material using a modified version of the Bioline Extract-PCR Kit (Bioline, Taunton, MA, USA). We incubated samples at 75°C for at least 30 minutes in 50 µL of extraction solution and then ground them using pellet pestles. After homogenization, samples were incubated at 75°C for an additional 10 minutes and then heated to 95°C for 10 minutes to end the enzymatic reaction. Cell debris was pelleted by centrifugation, and the supernatant was cleaned using the OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA) and diluted to 0.1% of its original concentration in dH_2O. Extractions were stored at -20°C (Taylor et al. 2017).

The *rbc*L-3P barcode or more complete *rbc*L were amplified using MyTaq HS Red Mix and primers F753/R*rbc*S-start and F57/R893 (Freshwater & Rueness 1994, Stuercke & Freshwater 2008) with the following PCR thermocycling protocol; an initial denaturation step at 95°C for 2:45 min, followed by 35 cycles of denaturation at 95°C for 15 s, primer annealing at 45°C for 15 s and primer extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. Amplified products were checked for yield and proper length using 1.2 % agarose gels stained with ethidium bromide, and successful amplifications were purified using ExoSAP (ThermoFisher Scientific, www.thermofisher.com) before being sequenced commercially by GENEWIZ (Azenta Life Sciences, Burlington, MA).

Sequence alignment and phylogenetic analysis

Sequencher (V. 5.4.6, Gene Codes Corp., Ann Arbor, MI, USA) was used to edit sequence reactions and build contigs. Resulting sequences were initially aligned using Muscle in Mega (V. 10.2.6, Kumar et al. 2016), and neighbor joining analysis was performed to determine the number of primary species hypotheses (PSH) in the data. Sequences of representative specimens from each PSH were chosen and aligned with related sequences from GenBank. Two separate alignments, one for Polysiphonieae and one for Streblocladieae, were compiled, and maximum likelihood (ML) analysis was used to build phylogenetic trees. ML analyses were performed using the General Time Reversal (GTR) + gamma + invariant sites. Support for clades was assessed through 500 replications of rapid bootstrap resampling, with a total of 7 threads.

Morphological observations

The distinct morphological characteristics found in this study for each specimen are described in Table 2. These characters were chosen based on Stuerke & Freshwater (2008), Díaz-Tapia et al. (2017), Savoie and Saunders (2019), and Bustamante et al. (2021). Microscopic images were captured using a Nikon SMZ800 dissection scope compound microscope and a Nikon Eclipse 50i with the Hayear HD 34MP 1080P camera attachment. After imaging the samples, they were semi-permanently mounted using a 50% Karo solution.

Results

Morphological analyses

Morphological characters were observed for each specimen collected in this study. These characters include: thallus length, branching patterns, trichoblast presence, pericentral cell number, rhizoid cell number, attachment and shape, male and female gametophyte structure, if

present, and tetrasporangial arrangement (Table 2). Not all features were observed for each specimen.

***Eutrichosiphonia confusa* (Hollenberg) Savoie & G.W.Saunders** (Fig. 2)

Description. Thallus 6-65 cm. Branches dense, radial, and alternate forming pinnacles in the apex region (Fig. 2a-b). Many spiraling trichoblasts at apex (Fig. 2e-f). 12 - 13 pericentral cells that are radially arranged (Fig. 2c, g-h). No rhizoids observed, but reported to be cut off from pericentral cells (Bustamante et al., 2021). Gametophytes not observed, but carpagonial branches reported to contain a five cell supporting complex and spermatangial branches reported to develop in first fircation of the trichoblasts (Bustamante et al., 2021). Tetrasporophytes not observed but reported to be arranged in spiral (Savoie and Saunders 2019) or straight (Bustamante et al., 2021).

BASIONYM: *Polysiphonia confusa* Hollenberg 1961, p. 350 (Marine red algae of Pacific Mexico, part 5: The genus *Polysiphonia. Pacific Naturalist,* 2: 345-375, 7 plates).

HOMOTYPIC SYNONYM: *Neosiphonia confusa* (Hollenberg) J.N.Norris.

HETEROTYPIC SYNONYMS: *Polysiphonia inconspicua* Hollenberg 1944 nom. illeg.

HOLOTYPE: US 61222 (Hollenberg 3285).

TYPE LOCALITY: Corona Del Mar, California, USA.

DISTRIBUTION: Santa Cruz, California, northern Baja California, Mexico. Samples in this study were collected from Cattle Point, San Juan Island, WA.

STUDIED MATERIALS: FHL23-13, FHL23-14.

***Eutrichosiphonia* sp. (Montagne) D.E.Bustamante & T.O.Cho** (Fig. 3)

Description. Erect thalli can be 3-16 cm tall (Fig. 3a), and have a radial alternate branching pattern (Fig. 3b). Adventitious branches observed (Fig. 3d). Trichoblasts arising from apical regions. This collection shows 10 pericentral ecorticate cells (Fig. 3e), though reportedly they can contain anywhere from 9-12 (Bustamante et al., 2021). Rhizoids not clearly observed (Fig. 3f). Female gametophytes not observed for this specimen. Spermatangial branches arising from trichoblasts (Fig. 3c). Tetrasporophytes not observed.

BASIONYM: *Polysiphonia paniculata* (Montagne), *Troisième centurie de plantes cellulaires exotiques nouvelles…,* p. 254, pl.2, fig. 2 (1842).

HOMOTYPIC SYNONYMS: *Neosiphonia paniculata* (Montagne) J.N.Norris 2014. *Vertebrata paniculata* (Montagne) Kuntze 1891.

HETEROTYPIC SYNONYMS: *Polysiphonia californica* Harvey 1853.

TYPE LOCALITY: Chile and Peru; (Index Nominum Algarum).

DISTRIBUTION: Atlantic Islands: Salvage, Europe: Adriatic Sea, Black Sea, France (Mediterranean), Sardinia, N. America: Alaska, British Columbia, California, Gulf of California, Mexico, Oregon, Washington, S. America: Chile, Peru, Temperate S. America, Middle East: Turkey. The sample in this study was collected from Iceberg Point, Lopez Island, WA.

STUDIED MATERIALS: FHL23-102.

***Polysiphonia determinata* Hollenberg 1942** (Fig. 4)

Description. Thallus 5-5.5 cm tall (Fig. 4a, g). Branches dense, alternate, radial, forming pinnacles at apex (Fig. 4a, g). Adventitious branches observed every 2-3 segments along the main axis. Cross sections show 4 pericentral ecorticate cells (Fig. 4d). Rhizoids are not present

on this specimen. Tetrasporangial branches are also arranged in a slight spiral, with one spore per fertile segment (Fig. 4b). Male and female gametophytes were not observed.

HOLOTYPE: US 2814.

TYPE LOCALITY: Santa Cruz, California.; (Silva 1979: 331).

DISTRIBUTION: N. America: Alaska, Baja California Sur (Gulf), British Columbia, California, Mexico, Oreon, Washington, C. America: El Salvador, S. America: Chile, SE Asia: Philippines. Samples in this study were collected at Friday Harbor Laboratory docks, San Juan Island, WA.

STUDIED MATERIALS: FHL23-83, FHL23-104.

***Polysiphonia* sp.** (Fig. 5)

Description. Thallus 3-4.2 cm tall. Fragile appearance with dichotomous branching (Fig. 5b). Adventitious branches spiraling along the primary axis every 7 segments (Fig. 5f, SD1). Rhizoids are attached to pericentral cells, unicellular with lobed termination (Fig. 5c, f). Cross sections showing 4 ecorticate pericentral cells. Trichoblasts absent (Fig. 5e). Female and male gametophytes not observed. Tetrasporangial segments not observed.

Habit. Found approximately 130 feet beneath the surface water in Mosquito Pass. Epilithic.

DISTRIBUTION: This sample was collected from Mosquito Pass on FHL Kittiwake (~130 ft. depth).

STUDIED MATERIALS: FHL23-101.

***Savoiea robusta* (N.L.Gardner) M.J. Wynne** (Fig. 6)

Description. Thallus 3-11.5 cm tall, showing dense, alternating indeterminate branching (Fig. 6a-c). Branches strongly arched (Fig. 6f). Rhizoids unicellular with lobed termination (SD1). Cross sections showing 12 - 15 pericentral cells (Fig. 6g, SD1). Trichoblasts are absent.

Tetrasporangia spirals with one spore every fertile segment (SD1). Female gametophyte displaying globose cystocarp (Fig. 6c-e). Male gametophyte not observed.

BASIONYM: *Pterosiphonia robusta* N.L.Gardner Univ. Calif. Publs Bot. 14: 102, pls 26-29, 1927.

HETEROTYPIC SYNONYMS: *Polyostea robusta* (N.L.Gardner) Savoie & G.W.Saunders 2016: 928.

HOLOTYPE: UC 266542.

TYPE LOCALITY: USA: California: San Mateo Co.: Moss Beach; (Gardner 1927: 102).

DISTRIBUTION: Asia: Kuril Islands (Nagai 1941). Samples in this study were collected from Eagle Cove, San Juan Island, WA and Deadman Bay, San Juan Island, WA.

STUDIED MATERIALS: FHL23-57, FHL23-60, FHL23-61, FHL23-62, FHL23-63, FHL23-64, FHL23-65, FHL23-67, FHL23-69, FHL23-70, FHL23-71 FHL23-81.

Vertebrata hendryi (N.L.Gardner) **Savoie & Saunders** (Figs. 7-9)

Description. Thallus 2-4.5 cm tall (Fig. 7a). Alternate branching (Fig. 7d). 11- 13 ecorticate pericentral cells (Fig. 8a-e). Trichoblasts present in spiral arrangement at each apical region (Fig. 7b-c). Unicellular rhizoids separated from pericentral cells with lobed terminations (Fig. 7e-f, Fig. 8c). Female gametophytes grow in alternating patterns along branches, with ovate to globose shape (Fig. 9a, e-g). Male gametophytes were not observed. Tetrasporangial branches spiral with one spore per fertile segment (Fig. 9b-c).

BASIONYM: *Polysiphonia hendryi* N.L. Gardner 1927, p. 101, pl. 24, figs 1, 2, pl. 25 (New Rhodophyceae from the Pacific coast of North America. VI. University of California Publications in Botany, 14: 99–138, pls 20–36).

HETEROTYPIC SYNONYMS: *Polysiphonia collinsii* Hollenberg, *P. gardneri* Kylin.

HOLOTYPE: UC 296607.

TYPE LOCALITY: Santo Domingo, Baja California, Mexico.

DISTRIBUTION: Reported from Alaska to Mexico (Abbott & Hollenberg, 1976). Samples in this study were collected from Eagle Cove, San Juan Island, WA, Deadman Bay, San Juan Island, WA, and Friday Harbor Laboratory docks, San Juan Island, WA.

STUDIED MATERIALS: FHL23-66, FHL23-68, FHL23-82, FHL23-84, FHL23-85, FHL23-86, FHL23-87, FHL23-88, FHL23-89, FHL23-90, FHL23-91, FHL23-92.

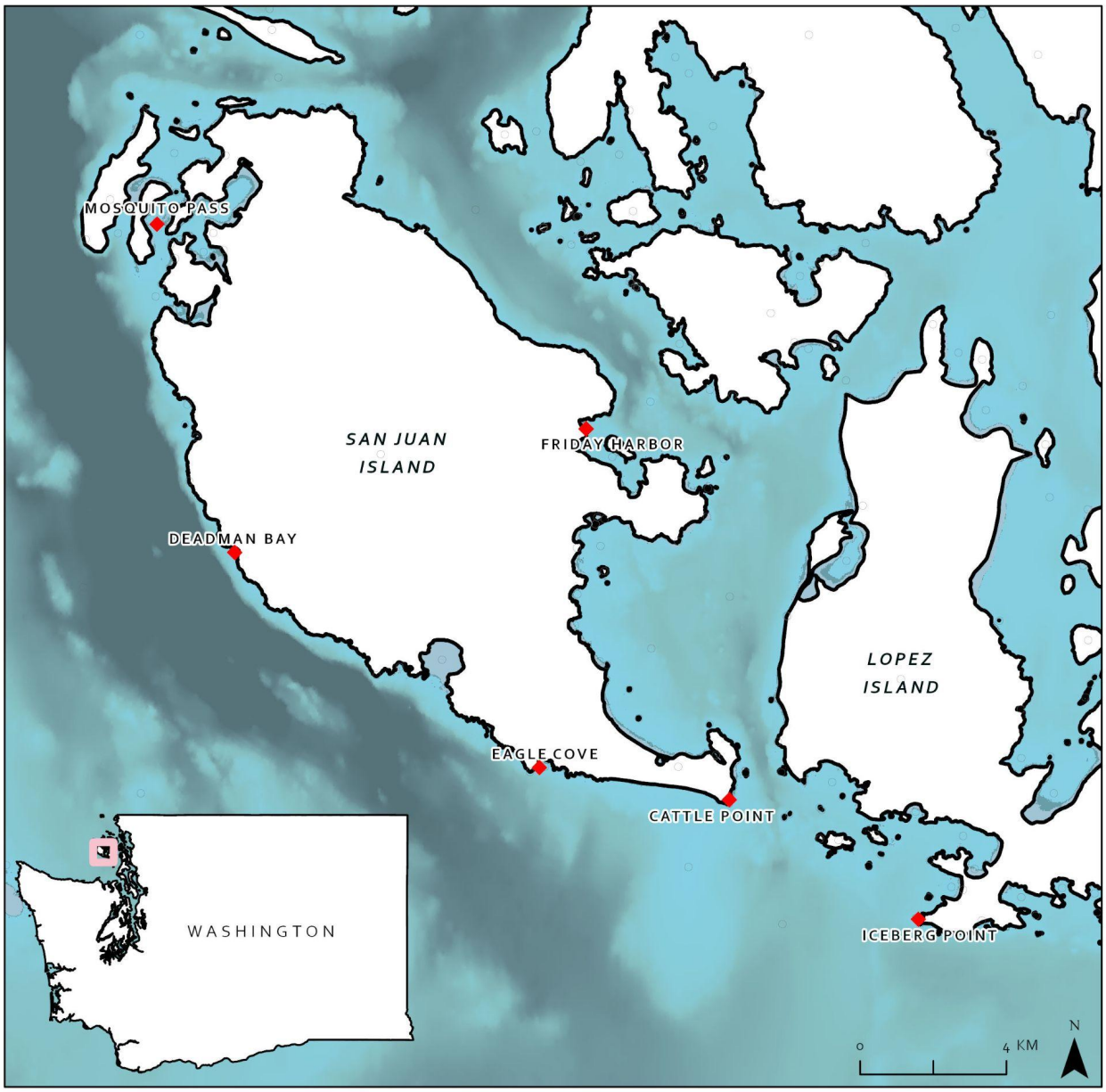

Fig. 1. Map showing the locations of sample collections. Cattle Point collections made on June 14, 2023 (FHL23-58, 59). Eagle Cove collections made on June 15, 2023 (FHL23-16 through FHL23-34). Deadman Bay collections made on June 21, 2023 (FHL23-81, 82). Friday Harbor Laboratory dock collections made on June 22, 2023 (FHL23-83 through FHL23-92). Mosquito Pass collections made on June 29, 2023 (FHL23-101). Iceberg Point collections made on July 3, 2023 (FHL23-102 through FHL23-109). Figure created using ArcGIS Pro 3.1.0.

Table 1. List of described Strebloclad ieae and Polysiphonieae species and varieties from the Gabrielson and Lindstrom Key (2018) including their recorded presence in the San Juan Islands, WA, USA.

Species Name	Presence in San Juan Islands
Melanothamnus eastwoodiae	Not listed
Polyostea bipinnata	Not present
P. hamata	Not listed
P. robusta	Not listed
Polysiphonia brodiei	Not present
P. hendryi var. *deliquescens*	Present
P. hendryi var. *gardneri*	Present
P. hendryi var. *hendryi*	Present
P. hendryi var. *luxurians*	Present
P. macounii	Not Present
P. pacifica var. *delicatula*	Present
P. pacifica var. *determinata*	Not present
P. pacifica var. *distans*	Present
P. pacifica var. *disticha*	Not present
P. pacifica var. *gracilis*	Present
P. pacifica var. *pacifica*	Present
P. paniculata	Present
P. senticulosa	Not listed
P. stricta	Not listed
P. villum	Not listed

Table 2. Morphological characters observed for San Juan and Lopez Island Samples

Species	Thallus length (cm)	Thallus Branching	Trichoblast	Rhizoid	Pericentral Cells	Female Gametophyte	Male Gametophyte	Tetrasporangia
Eutrichosiphonia confusa	6-6.5	Dense, radial, alternate, forms pinnacles	Present at apex	Not observed	12-13, ecorticate	Not observed	Not observed	Not observed
Eutrichosiphonia sp.	3	Radial, alternate, possibly pseudo dichotomous	Present at apex	Not observed	10-11, ecorticate	Not observed	Spermatia observed in first fircation of trichoblast	Not observed
Polysiphonia determinata	5-5.5	Alternate, radial, forms pinnacles	Absent	Not observed	4, ecorticate	Not observed	Not observed	Arranged in spiral, 1 spore per fertile cell
Polysiphonia sp.	3-4.2	Fragile, dichotomous	Absent	Lobed, unicellular	4, ecorticate	Not observed	Not observed	Not observed
Savoiea robusta	3-11.5	Dense, strongly arched, alternate, indeterminate	Absent	Lobed, unicellular	12-15, ecorticate	Globose in shape, 1 carpospore per branch	Not observed	Arranged in spiral, 1 spore per fertile cell
Vertebrata hendryi	2-4.5	Alternate, radial	Absent	Attached to pericentral cell, lobed, unicellular	11-13, ecorticate	Globose in shape, carpospores arranged alternately	Not observed	Arranged in spiral, 1 spore per fertile cell

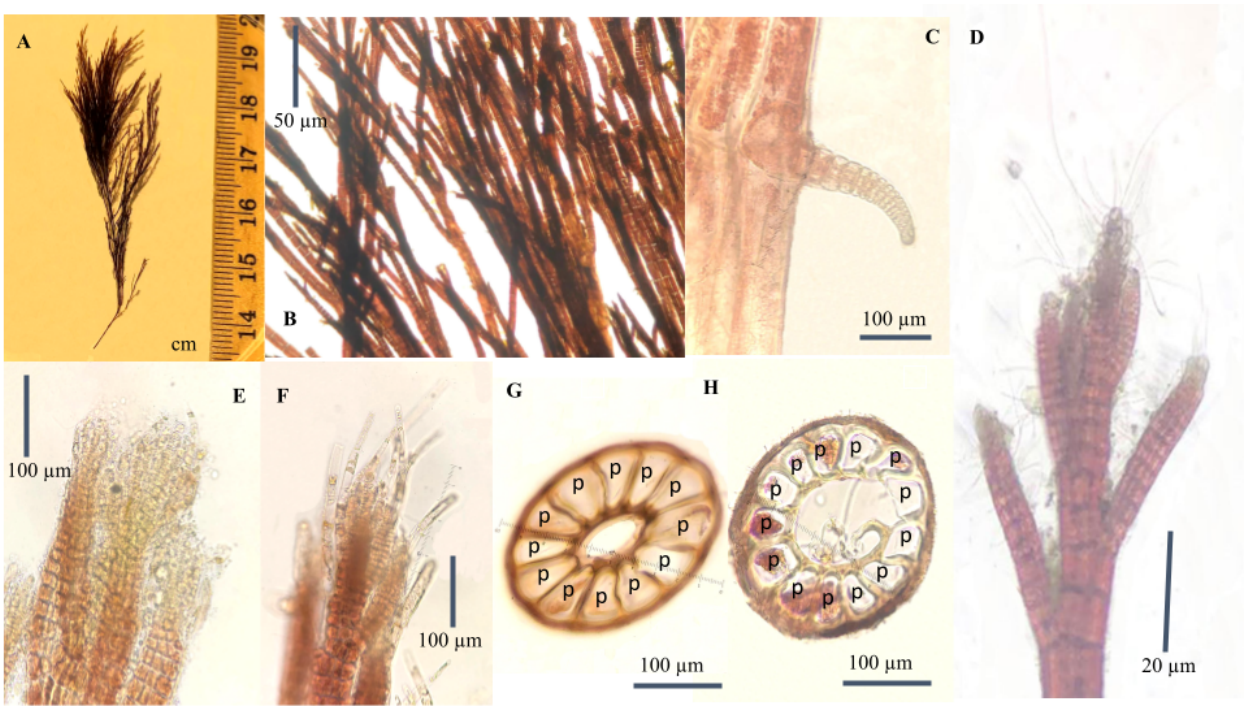

Fig. 2. Vegetative structures of *Eutrichosiphonia confusa* *(Voucher: FHL23-58, 59)*; A. Thallus showing alternate branching. B. Upper part of the thallus. C. Middle of thallus showing new lateral branch. D-F. Apical and subapical cells showing trichoblasts elongating from tips. G-H. Cross section at the base of the main axis for FHL23-13 (G), FHL23-14 (H) consists of 12-13 pericentral cells.

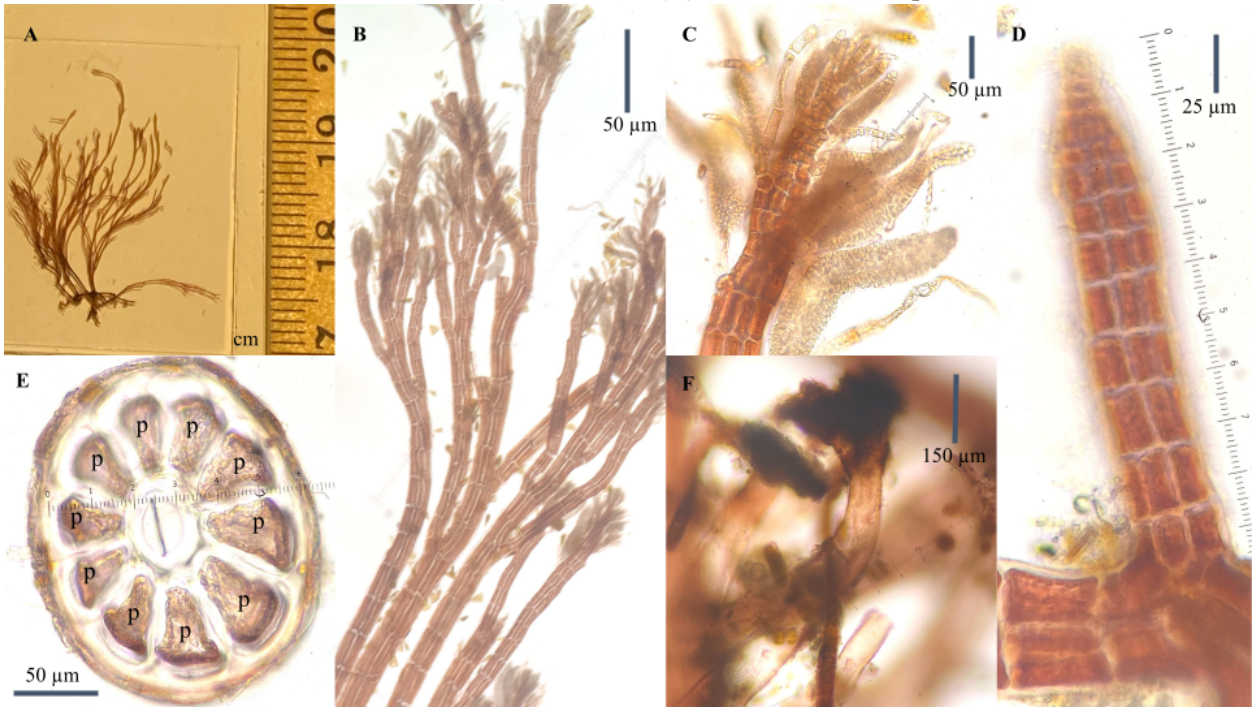

Fig. 3. Vegetative and reproductive structures of *Eutrichosiphonia* sp. *(Voucher: FHL23-102)*; A. Thallus showing thin radial branches. B. Upper part of the thallus showing spermatia. C. Apical area of the thallus showing spermatia and trichoblasts. D. Middle part of the thallus showing a new lateral branch. E. 10 pericentral cells taken from the main axis. F. Multicellular rhizoids.

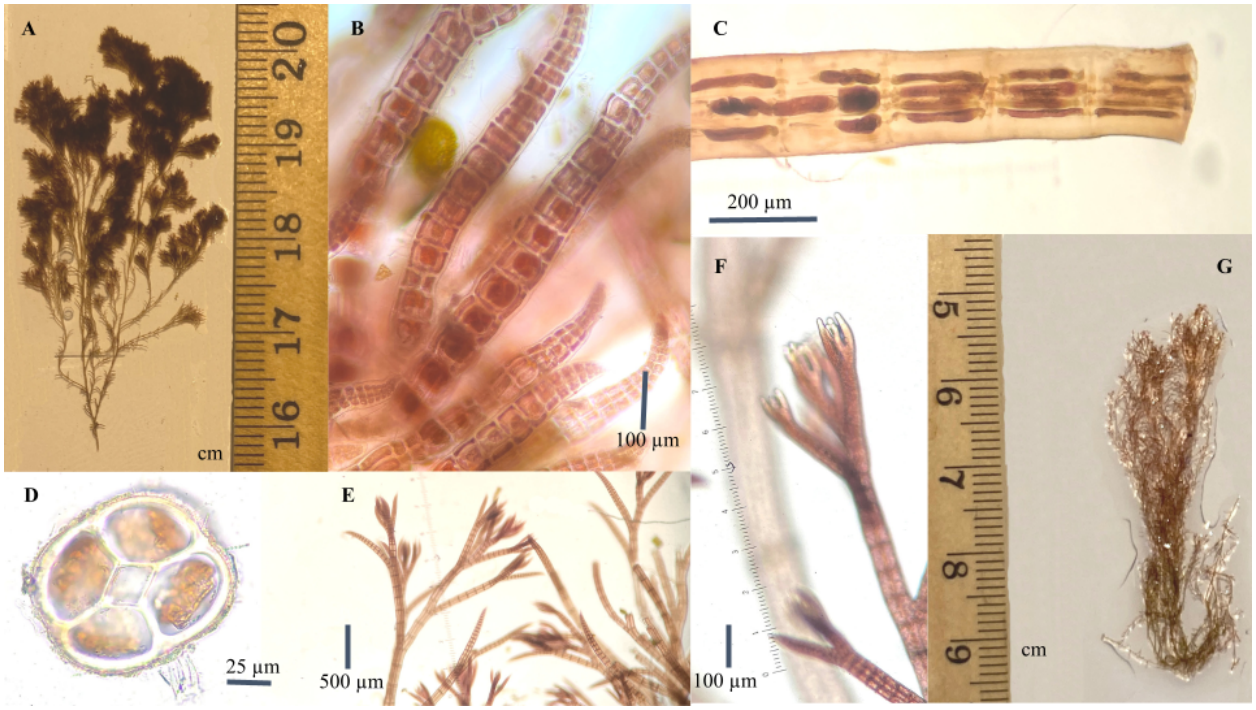

Fig. 4. Vegetative and reproductive structures of *Polysiphonia determinata* *(Voucher: FHL23-83, 104)*; A. Entire specimen. B. Tetrasporangia contained within the tetrasporophyte. C. Cells of the thallus. D. 4 pericentral cells. E. Apical region showing alternate branching. F. Apical region. G. Entire specimen.

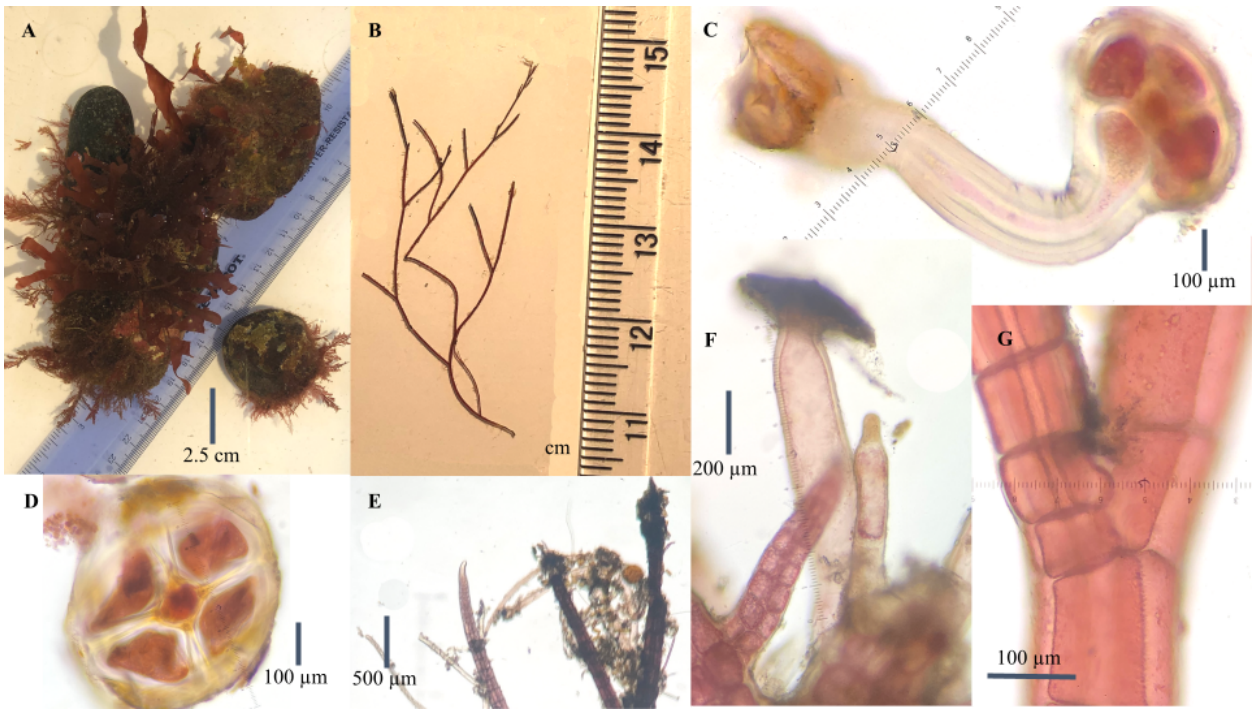

Fig. 5. Vegetative structures of *Polysiphonia* sp. *(Voucher: FHL23-101)*; A. Specimen habit. B. Thallus. C. Pericentral cells attached to rhizoid. D. 4 pericentral cells. E. Apical region showing rhizoidal growth. F. Rhizoid. G. Thallus region showing dichotomous branching.

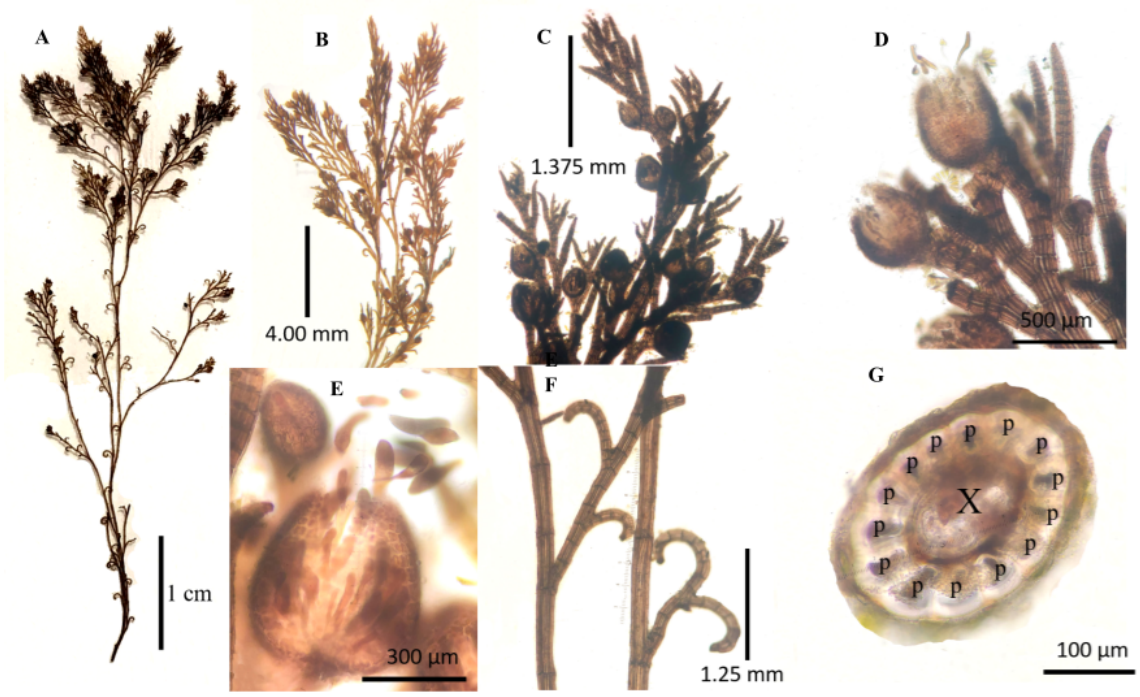

Fig. 6. Vegetative and reproductive structures of *Savoiea robusta* *(Voucher: FHL23-34, 57)*; A. Thallus showing alternate and dichotomous branching. B. Upper part of the thallus. C. Upper part of the thallus with cystocarps. D-E. Mature carposporophytes (2N) showing released carpospores (2N) from the pericarp (1N). F. Thallus contains arched branches from both the main and lateral axis. G. Cross section at the base of the main axis consists of 14 pericentral cells.

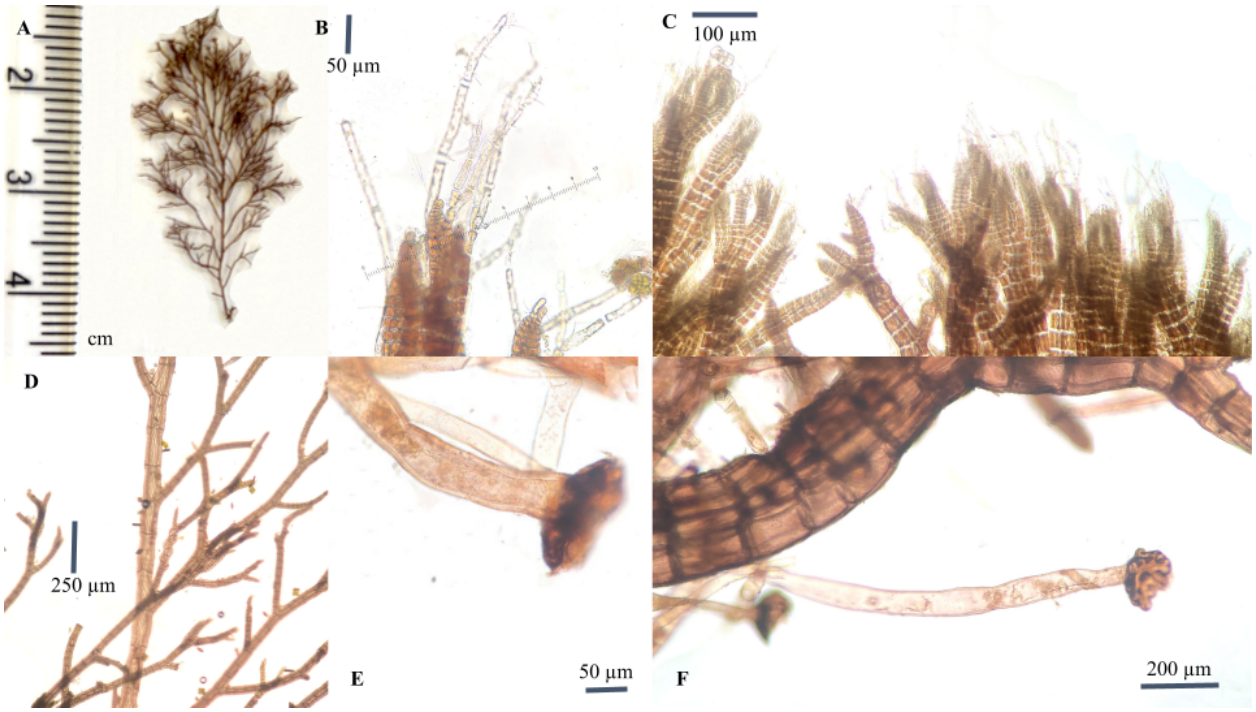

Fig. 7. Vegetative structures of *Vertebrata hendryi* *(Voucher: FHL23-66, 68, 87, 106, 109)* ; A. Erect axis alternate branching. B-C. Apical region showing multinucleated trichoblast structures. D. Thallus showing dichotomous branching. E-F. Rhizoids.

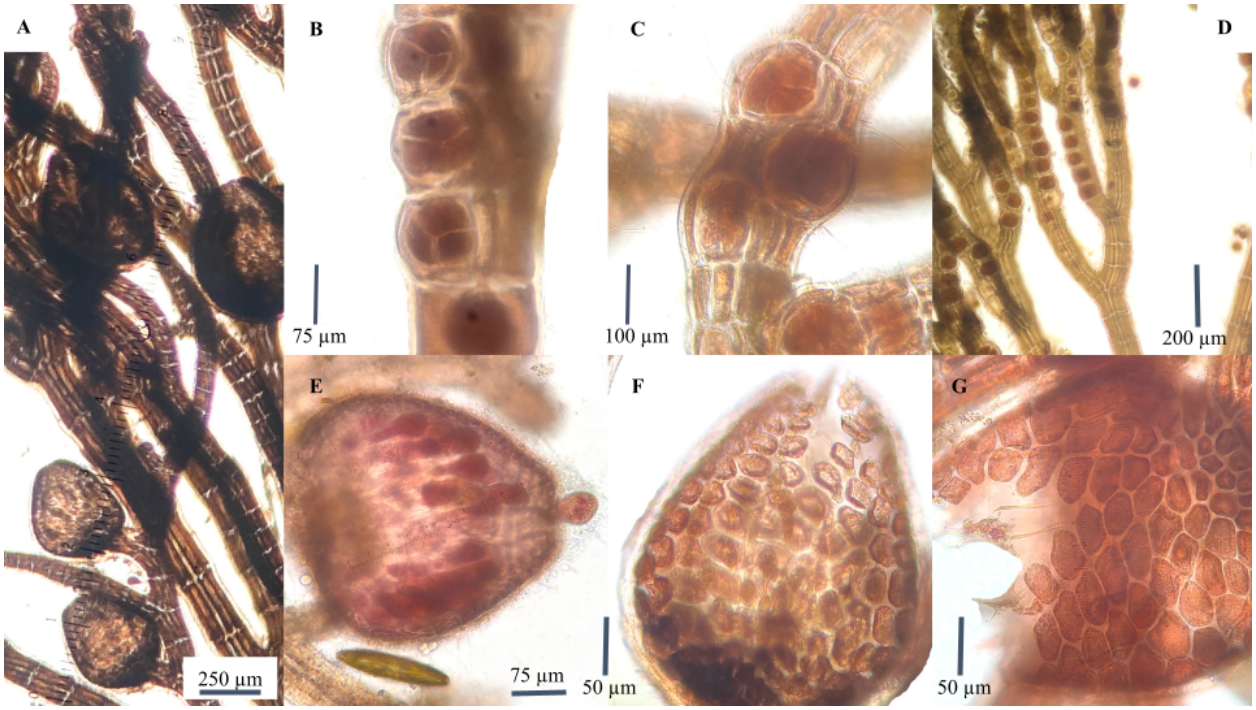

Fig. 8. Reproductive structures of *Vertebrata hendryi* *(Voucher: FHL23-85, 86, 91, 103, 109)*; A. Thallus showing pericarps of the female reproductive structure. B-C. Tetrasporangia with tetrahedral tetraspores beginning to spiral down the thallus. D. Thallus showing tetrasporangia. E. Mature globulose cystocarp (2N) showing carpospores (2N) with one protruding, surrounded by the pericarp (1N). F-G. Female reproductive structures found on carposporophyte.

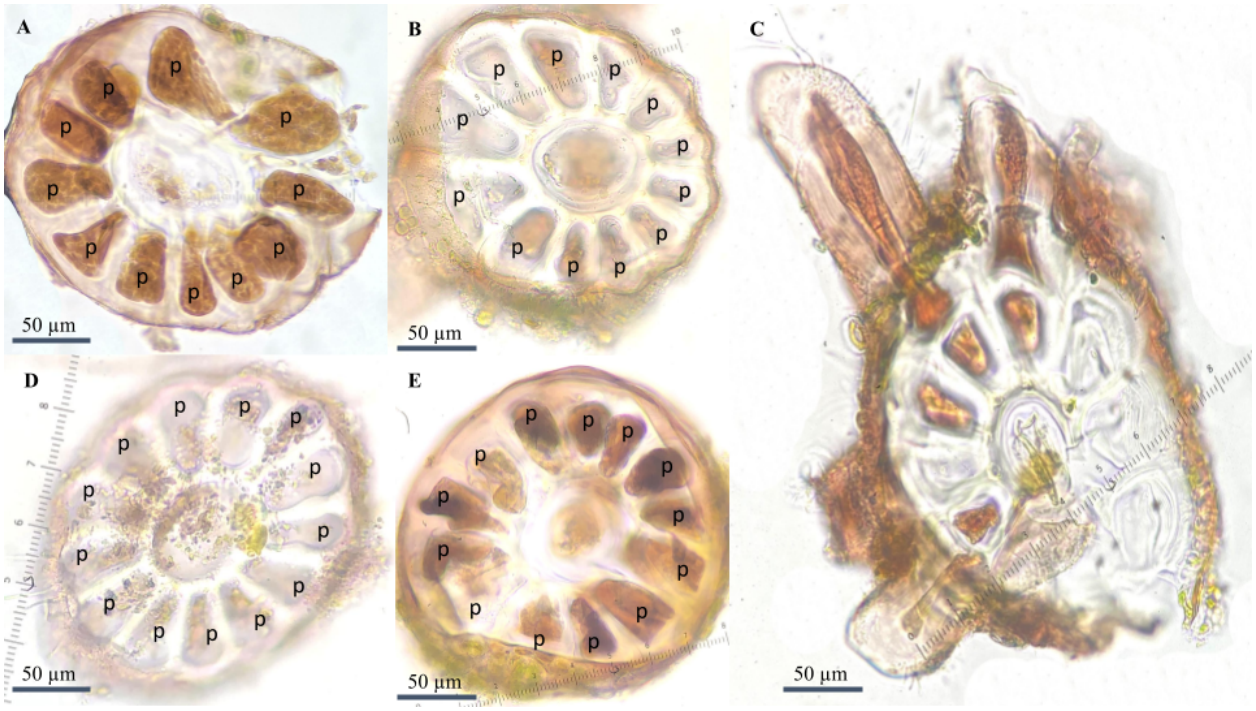

Fig. 9. Pericentral cells of *Vertebrata hendryi* *(Voucher: FHL23-66, 85, 103, 109)*; A. 11 pericentral cells *(Voucher: FHL23-66)*. B. 12 pericentral cells *(Voucher: FHL23-85)*. C. Rhizoids detached from pericentral cells *(Voucher: FHL23-103)*. D-E. 13 pericentral cells *(Voucher: FHL23-109)*.

Molecular analyses

A total of 36 *rbc*L specimens were sequenced, and 98 rbcL representative specimens were downloaded from GenBank and aligned in MEGA X using Muscle. Both neighbor joining and maximum likelihood were performed through 500 replications of rapid bootstrap resampling, with a total of 7 threads, where Nei Tamura was selected for neighbor joining (Díaz-Tapia et al. 2020, Kim and Yang 2005). Four species, namely: *Eutrichosiphonia confusa* (Hollenberg) Savoie & G.W. Saunders; *Polysiphonia determinata* Hollenberg; *Vertebrata hendryi* (N.L.Gardner) Savoie & Saunders and *Savoiea robusta* (N.L.Gardner) M.J. Wynne was confirmed based on molecular phylogeny. Additionally, *Eutrichosiphonia* sp. and *Polysiphonia* sp. formed separate clades with their representatives, where *Polysiphonia* sp. had 2% genetic distance from *P. determinata*.

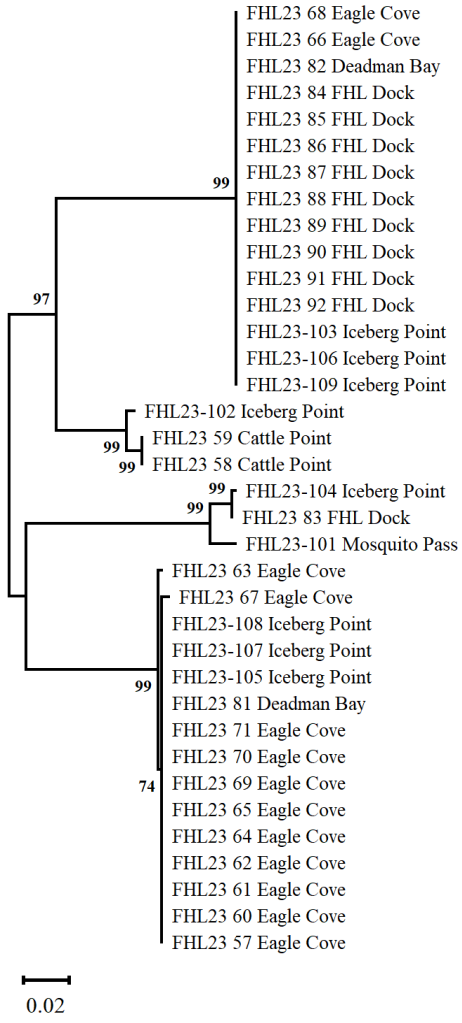

Fig 10. Phylogenetic tree based on maximum likelihood (ML) analysis of *rbc*L-3P sequences. Values along branches are bootstrap supports. A total of 36 sequences were performed in 5 sampling points (Cattle Point, Deadman Bay, Eagle Cove, FHL Dock, and Mosquito Pass on San Juan Island, WA, and Iceberg Point on Lopez Island, WA). ML result formed 4 major branches.

The phylogenetic tree of the barcoding gene rbcL-3P formed four major branches with 99 % bootstrap support (Fig. 10). In addition, FHL23-102 formed a sister clade with the specimens from Cattle Point (FHL23-59 and FHL23-58). Lastly, the specimen from Mosquito Pass (FHL23-101) formed a sister clade with FHL23-104 and FHL23-83 from different locations (Fig 10).

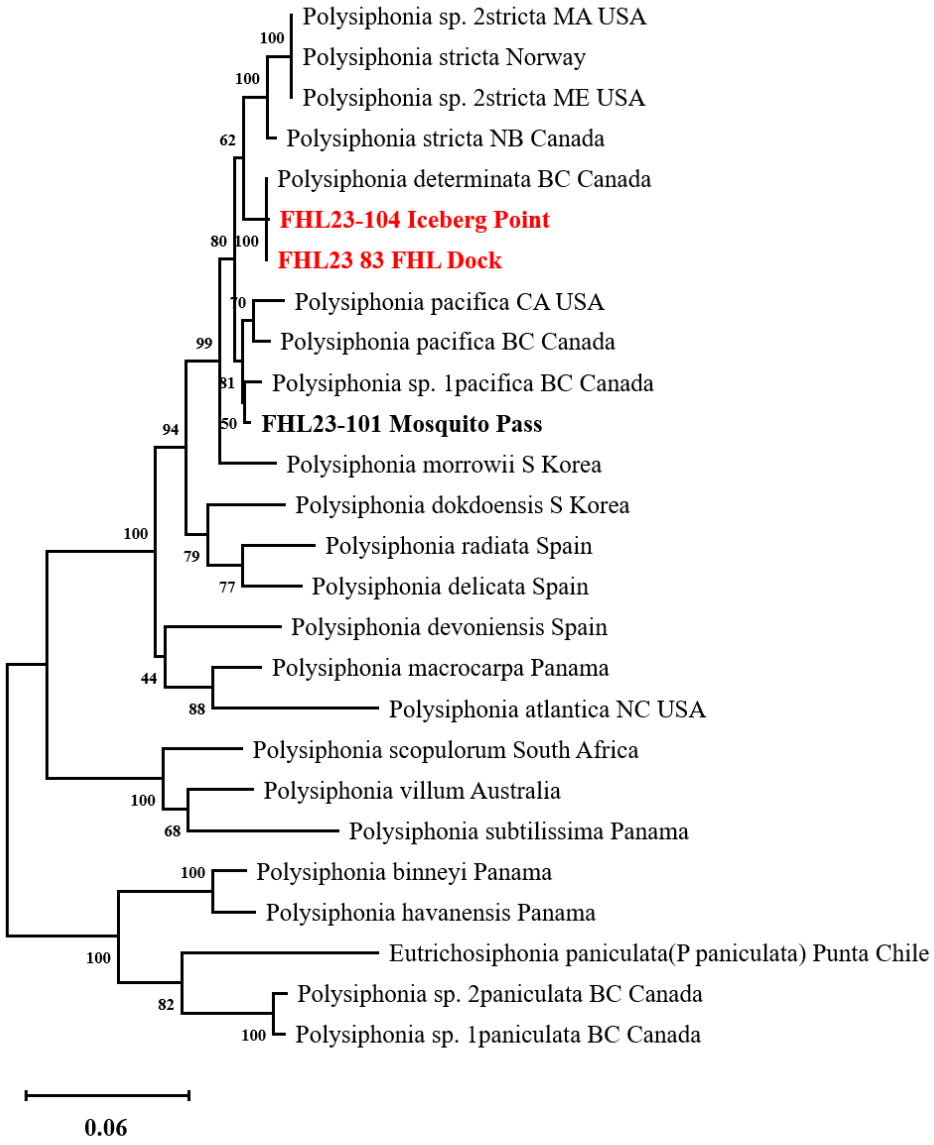

Fig. 11. Phylogenetic tree based on the ML using *rbc*L of the *Polysiphonia sensu lato* (Polysiphonieae tribes). Values along branches are bootstrap supports. The red color indicates the same taxa within the clade. Bold indicates a new *Polysiphonia* sp. in a separate clade.

In the Polysiphonieae tribes, specimens from Iceberg Point (FHL23-104) and FHL Dock (FHL23-83) matched the *Polysiphonia determinata* from British Columbia (BC), Canada (Fig. 11). *P. determinata* forms a sister clade with *P. stricta*. Furthermore, the Mosquito Pass material (FHL23-101) clustered with *Polysiphonia* sp.1 from British Columbia, Canada, with lower bootstrap values (50*%)* (Fig. 11). Savoie and Saunders (2019) named *Polysiphonia* sp.1 (*pacifica*) with uncertain identity as it was

not a consistent match to any of the described varieties of this species or to any other species reported in the British Columbia flora.

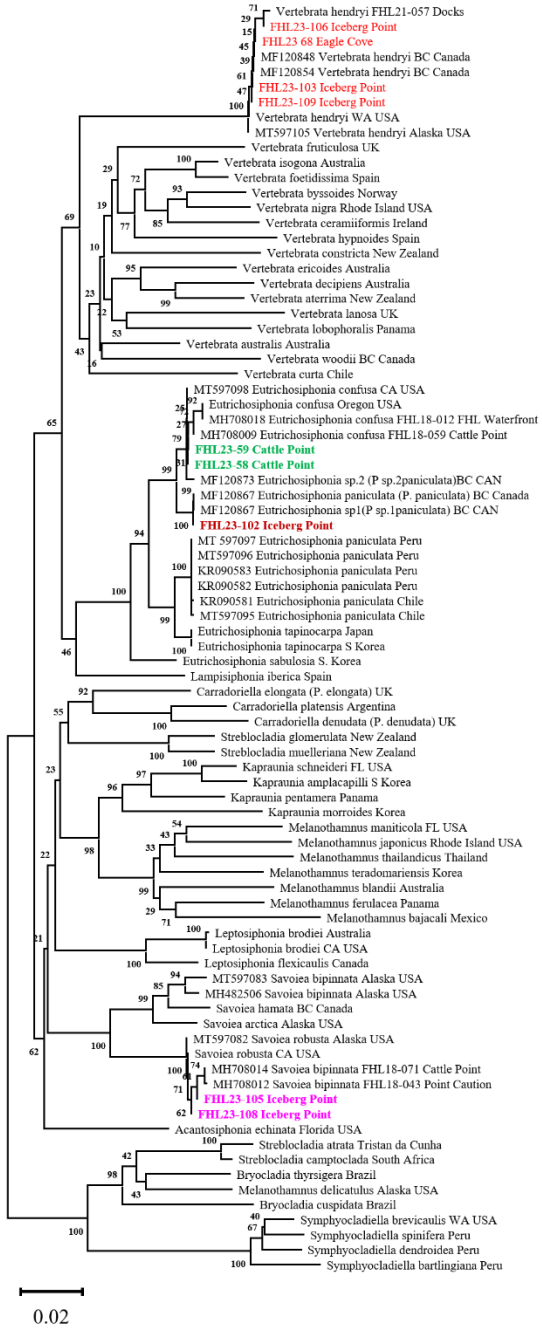

Fig. 12. Phylogenetic tree of neighbor joining among *Polysiphonia sensu lato* (Streblocladieae) using *rbc*L with the representative species. Values along branches as bootstrap supports. The colors indicated different genera in the present study.

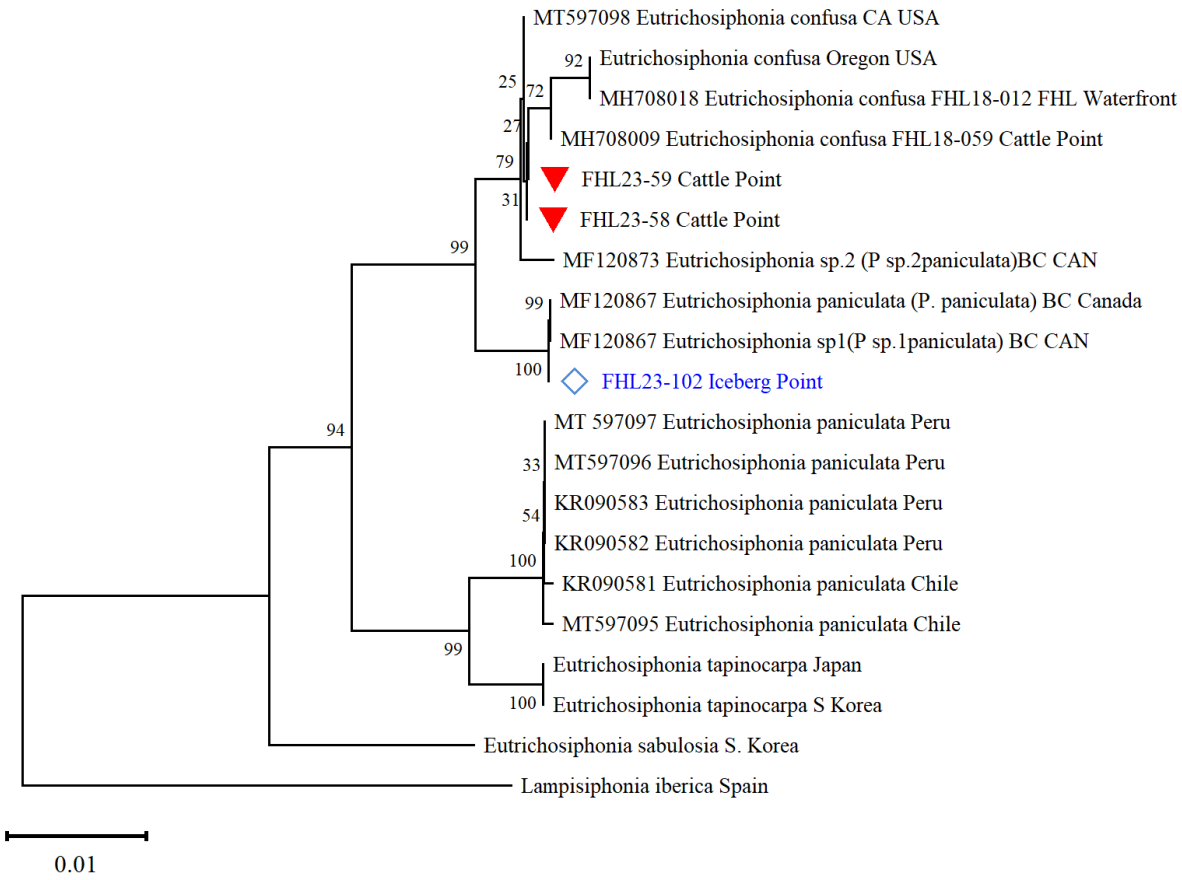

Fig. 13. Phylogenetic analysis of the subtree of neighbor joining among the *Eutrichosiphonia* spp. (Streblocladieae) using *rbc*L with the representative species. Values along branches as bootstrap supports. The colors and shapes indicated different genera in the present study.

The multi-pericentral genera were transferred to the Streblocladieae tribe. Both neighbor joining and maximum likelihood well supported our collections into three separate genera, namely: *Vertebrata*, *Eutrichosiphona*, and *Savoiea* (Figs. 12–14). Furthermore, the Iceberg Point collection (FHL23-102) formed a 100% supported clade with an unknown Eutrichosiphonia sp. (Fig. 13).

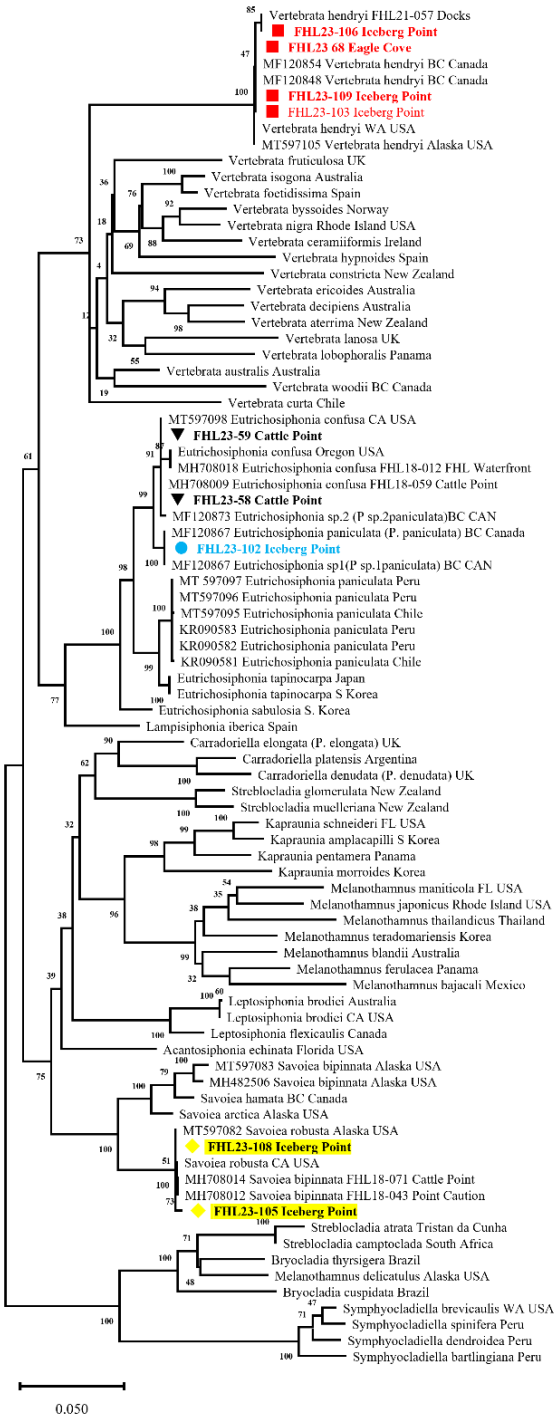

Fig. 14. Phylogenetic tree of ML among *Polysiphonia sensu lato* (Strebloclad**i**eae) using *rbc*L with the representative species. Values along branches as bootstrap supports. The colors and shapes indicated different genera in the present study.

Updated Gabrielson and Lindstrom Key

These phylogenetic and morphological analyses, along with information collected from Algaebase.com, have revealed that three species from the Gabrielson and Lindstrom Key are present in the San Juan Islands (Table 2). Eight species names have been given, and one new species, *Eutrichosiphonia confusa*, has been added to the San Juan Islands' flora (Table 3).

Table 3. Updated names of Streblocladieae and Polysiphonieae species in the Gabrielson and Lindstrom 2018 Key.

2018 Key Names	Current Classification
Melanothamnus eastwoodiae	*Polysiphonia eastwoodiae*
Polyostea bipinnata	*Savoiea bipinnata*
P. hamata	*Savoiea hamata*
P. robusta	*Savoiea robusta*
Polysiphonia brodiei	*Leptosiphonia brodiei*
P. hendryi var. *hendryi*	*Vertebrata hendryi*
P. hendryi var. *luxurians*	Requires further study
P. pacifica var. *determinata*	*Polysiphonia determinata*
P. pacifica var. *pacifica*	*Polysiphonia pacifica*
P. paniculata	*Eutrichosiphonia sp. (= Eutrichosiphonia* sp. *1, paniculata sensu* Savoie and Saunders, 2019)

Discussion

A total of six *Polysiphonia sensu lato* species belonging to four genera were separated based on morphological and molecular evidence in the San Juan Islands. Each genus formed a separate group with significant genetic distance (Figs. 10–14). The Streblocladieae tribe was recently separated based on both multinucleated trichoblast cells and more than six pericentral cells. This reduced the Polysiphonieae to include species of the lineage corresponding to *Polysiphonia*

sensu stricto (Diaz-Tapia et al. 2017, Savoie and Saunders 2019; Bustamante et al. 2021). *Polysiphonia sensu stricto* has four pericentral cells and rhizoids that are continuations of the pericentral cells. *P. determinata* from British Columbia, Canada, of that group, matched specimens from Iceberg Point (FHL23-104) and FHL Dock (FHL23-83) both morphologically by containing 4 pericentral cells with attached rhizoids and molecularly with 100.0 % bootstrap values (Fig. 11). However, morphologically, FHL23-104 was densely branched in the apical region, and its main axis was macroscopically indistinct, whereas the main axis of FHL23-83 was less branched, delicate, and soft (Fig. 4). Gabrielson and Lindstrom (2018) note that Key's description of *P. pacifica* var. *determinata* corresponds to our collected *P. determinata*. Another *Polysiphonia* specimen (FHL23-101) that was collected from Mosquito Pass, San Juan Island, made a sister clade (bootstrap = 50*%)* with *Polysiphonia* sp.1 (*pacifica*). The identity of these specimens does not match the described varieties of *P. pacifica* or any other species reported in the British Columbia flora (Savoie and Saunders 2019). Morphologically, FHL23-101 was similar to *P. pacifica*, but molecularly, it formed a separate clade (see phylogenetic tree, Savoie and Saunders 2019). In addition, *Polysiphonia* sp. (FHL23-101) showed 2.00% and 2.19% genetic distance from the FHL23-83 and FHL23-104 samples, respectively. Morphologically, this species is different from *P. pacifica* and *P. determinata*. All specimens were collected during low tide in the upper intertidal zone except FHL23-101, which was collected at approximately 20.0 m depth during a dredge trip in Mosquito Pass. This Mosquito Pass specimen is likely a new species of *Polysiphonia*; however, multi-gene analysis is needed to confirm its true identity.

FHL23-58 and FHL23-59 found at Cattle Point, San Juan Island, WA, were determined to be most similar to those previously described in Oregon and the San Juan Islands (Figs. 12–14). These specimens have dense, radial, and alternate branches forming pinnacles in the apex region

(Fig. 2a–b), many spiraling trichoblasts at the apex (Fig. 2e–f), and 12–13 pericentral cells that are radially arranged (Fig. 2c, g–h). One specimen collected showed no trichoblasts in the apical regions, with unknown organismal branching instead (Fig. 4d), but this is believed to be due to the specimen's health. FHL23-58 and FHL23-59 correspond most closely with the topotype of Santa Cruz, CA, which is approximately 600 km away from the type locality of *E. confusa* in Corona del Mar, CA (Savoie and Saunders 2019).

In 2019, Savoie and Saunders separated the genus *Eutrichosiphonia* using both morphological and molecular data. They found that *Eutrichosiphonia* was consistent in the following features: having greater than four pericentral cells; multicellular digitate rhizoids that are cut off from pericentral cells; abundant trichoblasts on every segment in a spiral pattern; obvious persistent scar cells; ecorticate axes; and tetrasporangia in spiral series. Furthermore, *Polysiphonia confusa* and *P. sabulosia* were transferred to *Eutrichosiphonia* as *E. confusa* and *E. sabulosia*. *E. paniculata* and *E. tapinocarpa* were also separated on the basis of phylogenetic analyses and morphological observations from Peru, Japan, and South Korea (Bustamante et al., 2021). Based on the *rbc*L analysis, *E. confusa* formed a sister clade with *Eutrichosiphonia* sp. 1 and *Eutrichosiphonia* sp. 2 (Savoie and Saunders 2019). The collections of *E. paniculata* from Peru and Chile have since been placed into a separate sister clade with *E. tapinocarpa.* In addition, *E. paniculata* on the Peruvian coast was also confirmed with the type materials. However, the type locality collected specimens of *E. paniculata* from Peru show an incongruence in taxonomic position with those found in California based on the *rbc*L gene sequence (Díaz-Tapia et al. 2017, Bustamante et al., 2021).

FHL-23-102, collected from Iceberg Point, Lopez Island, WA, was determined to be identical to *E. paniculata* specimens from the Peruvian coast (Bustamante et al., 2021). This specimen showed the following morphological characteristics: 10–11 pericentral cells, radially arranged pericentral cells on the main and lateral axes, multicellular digitate haptera, and conspicuous scar cells (Fig. 3). The Peruvian specimen has the following morphological characteristics: 10–11 pericentral cells, radially arranged pericentral cells, multicellular digitate haptera, and spermatangial arrangement. The determination of *FHL-23-102* is also supported by herbarium specimens documented from British Columbia that share a common clade (Fig. 4). Even though *P. paniculata* was recently reclassified as *E. paniculata*, it is possible that they are genetically different due to divergence or biogeographical speciation. Collecting specimens from a variety of locations for genetic analyses might resolve the identity of *Eutrichosiphonia* sp. for the Gabrielson and Lindstrom 2018 Key. Based on the molecular evidence, none of the *Eutrichosiphonia* species in this region represent a genuine *E. paniculata*. This data leads to the conclusion that these specimens have either been misidentified as *E. confusa* or that their taxonomic position needs to be resolved.

The globally distributed genus *Vertebrata* is the sister clade of *Eutrichosiphonia* in the Streblocladieae tribe, and representatives have been described from all regions (Diaz-Tapia et al., 2017). This genus shares morphological characteristics in that it has multinucleated trichoblast cells and greater than six pericentral cells (DiazTapia et al., 2017). We collected *V. hendryi* (N.L.Gardner) Savoie & G.W.Saunders from Deadman Bay, Eagle Cove, FHL Dock, and Iceberg Point. These specimens shared the morphological characteristics of 11–13 pericentral cells and multinucleated trichoblast cells (Figs. 7, 9), and they were 100.0 % supported by bootstrap values (Figs. 10, 12, 14). In addition, the molecular data produced in this study are fully

supported by the previous collection in the San Juan Islands, as well as from Washington, Alaska, and British Columbia (Figs. 12, 14).

The reassessment of the Pterosiphonieae tribe, emphasizing the Northeast Pacific taxa, was recently described by Savoie and Saunders (2016). This included the description of the new genus *Polyostea* Ruprecht. Three taxa from *Pterosiphonia* and one species from *Polysiphonia* were transferred to this genus. However, Ruprecht clearly proposed *Polyostea* as a replacement name for *Polysiphonia* ("*Polysiphonia = Polyostea"*). Later, Wynne (2018) referred to this genus as *Savoie* and transferred four taxa as *S. arctic*a, *S. bipinnata*, *S. hamata,* and *S. robusta*. This genus was separated by considering the following shared morphology: 4–14 ecorticate pericentral cells; absent trichoblasts; cylindrical axes; unicellular rhizoids cut off from pericentral cells; tetrahedral tetrasporangia; and globular to ovoid cystocarps (Savoie and Saunders 2016; Wynne 2018).

Our representative species of *Savoiea* were collected from Deadman Bay, Eagle Cove, and Iceberg Point. Through analyses, they were confirmed to be *S. robusta* (Postels & Ruprecht) M.J. Wynne. They shared the following morphological characters: 10–15 ecorticate pericentral cells; no trichoblasts; radially arranged tetrasporangia; and ovoid cystocarps. Our specimens strongly resembled the topotypic specimens from California as well as those from Alaska. *S. robusta* has previously been misidentified with *S. bipinnata* (see phylogenetic tree). In 2018, Gabrielson and Lindstrom collected this alga from the Salish Sea, Haida Gwaii, and Prince Rupert in British Columbia. They described the following morphological characters: cystocarps ovoid to pyriform, 350–450 µm in diameter, with narrow ostioles. *S. bipinnata* has also been confirmed in Haida Gwaii and Prince Rupert, British Columbia, mentioning cystocarps globular to barrel-shaped,

400–600 µm in diameter, with wide ostioles (Gabrielson and Lindstrom 2018), showing variation in these morphological characteristics biogeographically.

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Supplemental Information

Supplemental Table 1. Showing 36 voucher specimens submitted to the University of Washington Burke Museum's herbarium (WTU). This is currently a living document and will be updated with WTU voucher ID and Genbank accession number. Living Document.

#	Sample ID	Field ID	Species	Date	Location	WTU Number	Accession Number
1	FHL23-57	FHL-AI-34/21/16	*Savoiea robusta*	15-Jun	Eagle Cove		
2	FHL23-58	FHL-AI-13	*Eutrichosiphonia confusa*	14-Jun	Cattle Point		
3	FHL23-59	FHL-AI-14	*Eutrichosiphonia confusa*	14-Jun	Cattle Point		
4	FHL23-60	FHL-AI-17	*Savoiea robusta*	15-Jun	Eagle Cove		
5	FHL23-61	FHL-AI-18	*Savoiea robusta*	15-Jun	Eagle Cove		
6	FHL23-62	FHL-AI-22/19	*Savoiea robusta*	15-Jun	Eagle Cove		
7	FHL23-63	FHL-AI-20	*Savoiea robusta*	15-Jun	Eagle Cove		
8	FHL23-64	FHL-AI-23	*Savoiea robusta*	15-Jun	Eagle Cove		
9	FHL23-65	FHL-AI-24	*Savoiea robusta*	15-Jun	Eagle Cove		
10	FHL23-66	FHL-AI-25	*Vertebrata hendryi*	15-Jun	Eagle Cove		
11	FHL23-67	FHL-AI-26	*Savoiea robusta*	15-Jun	Eagle Cove		
12	FHL23-68	FHL-AI-27	*Vertebrata hendryi*	15-Jun	Eagle Cove		
13	FHL23-69	FHL-AI-29	*Savoiea robusta*	15-Jun	Eagle Cove		
14	FHL23-70	FHL-AI-32	*Savoiea robusta*	15-Jun	Eagle Cove		
15	FHL23-71	FHL-AI-33	*Savoiea robusta*	15-Jun	Eagle Cove		
16	FHL23-81	FHL-AI-37	*Savoiea robusta*	21-Jun	Deadman Bay		
17	FHL23-82	FHL-AI-38	*Vertebrata hendryi*	21-Jun	Deadman Bay		
18	FHL23-83	FHL-AI-35	*Polysiphonia determinata*	22-Jun	FHL Dock		
19	FHL23-84	FHL-AI-36	*Vertebrata hendryi*	22-Jun	FHL Dock		
20	FHL23-85	FHL-AI-39	*Vertebrata hendryi*	22-Jun	FHL Dock		
21	FHL23-86	FHL-AI-40	*Vertebrata hendryi*	22-Jun	FHL Dock		
22	FHL23-87	FHL-AI-41	*Vertebrata hendryi*	22-Jun	FHL Dock		
23	FHL23-88	FHL-AI-42	*Vertebrata hendryi*	22-Jun	FHL Dock		
24	FHL23-89	FHL-AI-43	Vertebrata hendryi	22-Jun	FHL Dock		
25	FHL23-90	FHL-AI-44	Vertebrata hendryi	22-Jun	FHL Dock		
26	FHL23-91	FHL-AI-45	Vertebrata hendryi	22-Jun	FHL Dock		
27	FHL23-92	FHL-AI-46	Vertebrata hendryi	22-Jun	FHL Dock		
28	FHL23-101	FHL-AI-47	Polysiphonia sp. (pacifica)	29-Jun	Mosquito Pass		
29	FHL23-102	FHL-AI-48	Eutrichosiponia sp.	3-Jul	Iceberg Point		
30	FHL23-103	FHL-AI-49	Vertebrata hendryi	3-Jul	Iceberg Point		
31	FHL23-104	FHL-AI-50	Polysiphonia determinata	3-Jul	Iceberg Point		
32	FHL23-105	FHL-AI-51	Savoiea robusta	3-Jul	Iceberg Point		

33	FHL23-106	FHL-AI-52	Vertebrata hendryi	3-Jul	Iceberg Point
34	FHL23-107	FHL-AI-53	Savoiea robusta	3-Jul	Iceberg Point
35	FHL23-108	FHL-AI-54	Savoiea robusta	3-Jul	Iceberg Point
36	FHL23-109	FHL-AI-55	Vertebrata hendryi	3-Jul	Iceberg Point

Supplemental Data File 1. All data collected during this study can be found by visiting the following website: Polysiphonia Project Google Drive, or by using the QR code below.