

Diversity of Non-Geniculate Crustose Coralline Algae on San Juan Island, Washington

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Abstract

In the Northeastern Pacific Ocean, Corallinophycidae (Rhodophyta) sports two orders: Corallinales and Hapalidiales. This study focused on DNA-based identification of non-geniculate coralline algae on the rocky intertidal from San Juan Island, Washington. Here we recognized seven different species (*Boreolithothamnion phymatodeum*, *Chamberlainium tumidum*, *Lithophyllum impressum*, *Bossiella frondescens*, *Bossiella mayae*, *Bossiella exarticulata*, and *Corallina vancouveriensis*). Another was identified to the genus level (*Lithophyllum* sp. 1) and four to the ordinal level (Hapalidiales sp. 1, Hapalidiales sp. 2, Hapalidiales sp. 3, and Hapalidiales sp. 4). The result of this study showed that little is known about the diversity of coralline flora in San Juan Island despite their significant ecological role and abundant coverage in the rocky intertidal.

Introduction

Corallinophycidae (Rhodophyta) are one of the most ubiquitous macroalgae of the world and are very species rich (Manevelde et al., 2019). All orders of Corallinophycidae, except for the Rhodogorgonales, are called coralline algae due to their similar roles in the ecosystem (Townsend and Huisman 2018; Jeong et al., 2020). Coralline algae are distinctive in the florideophytes because of their calcification from calcite, intercalary meristems, roofed

conceptacles, and tetrasporocytes that experience zonate division (Hind and Saunders 2013; Silva and Johansen 1986). Historically, the evolutionary records of Corallinophycidae has been debated due to varying ideas of morphological characters that most accurately delineated different taxonomic groups. In fact, the orders within this subclass continue to change with frequent revisions of new species, genera, and subfamilies as they are described (Harvey et al., 2003; Hind et al., 2016; Jeong et al., 2021; Kato et al., 2011; Kim et al., 2007; Le Gall et al., 2010; Woelkerling et al., 1993). Molecular analyses are transforming our understanding of this essential group of red algae.

There are two orders from the Corallinophycidae subclass found within the northeastern Pacific Ocean: Corallinales and Hapalidales. There are discrete reproductive and anatomical features that separates the orders, notably the arrangement and division of tetrasporangia, position of sporangia, and the presence or absence of apical plugs in conceptacles (Bahia et al., 2015; Jeong et al., 2020; Le Gall et al., 2010; and Nelson et al., 2015).

The order Corallinales have conceptacles, apical plugs that are in uniporate sporangial, and tetrasporangia that are zonately divided. Corallinales are also the only order with members that are both non-geniculate and geniculate coralline algae (Bahia et al., 2015; Jeong et al., 2020; Le Gall et al., 2010 and Nelson et al., 2015). The controversy surrounding classification of Corallinales began in the 1970s and was based solely on morpho-anatomical grounds (Hind and Saunders 2013). Originally, genera that were geniculated and non-geniculated were placed together in two of the five subfamilies based on cell-cell linkage types, under the pretense that crustose forms were more closely related to some geniculated forms (Cahoon 1972). However, Johansen (1981) asserted that Corallinales consist of seven subfamilies where geniculated and non-geniculated forms are in separate lineages. Subsequently, the first molecular phylogenetic

survey of the group revealed that the first conceptualization of the subfamilies to be more accurate; geniculated and non-geniculated forms were not precisely monophyletic clades (Bailey and Chapman 1998).

The order Hapalidiales has only non-geniculate coralline algae. Hapalidiales are characterized as having zonately divided tetrasporangia, an apical plug, and multiporate sporangial conceptacles (Jeong et al., 2020; Nelson et al., 2015). Originally, Gray (1864) proclaimed Hapalidiaceae for a monotypic genus, *Hapalidium roseolum* Kütz (1843). Then Chamberlain (1983) determined that *Hapalidium roseolum* was a heterotypic synonym of the type species of *Mesobesia*, *Mesobesia membranacea* (Esper) J.V. Lamour. Woelkerling (1988) asserted that the family name Hapalidiaceae was legitimate and available for a family that included the genus *Melobesia*, although Hapalidiaceae was not recognized by subsequent authors. Later Hapalidiaceae was resurrected and emended in description by Harvey et al. (2003) as it was the oldest available name for the subfamilies Choreonematoideae, Austrolithoideae, and Melobesioideae.

Historically, coralline algae have been described mainly based on morpho-anatomical features (Manevelدت et al., 2019). Since the advent of large-scale DNA barcoding efforts, DNA sequencing has become the most important tool to assess phylogenetic relationships and diversity within coralline algae, albeit supplemented with morpho-anatomical investigations (Guiry 2012). The current consensus is that morpho-anatomical features traditionally used to identify coralline algae are unreliable at species level (Hernandez-Kantun et al., 2016). Species identification is onerous due to the simple morphologies, convergent evolution, phenotypic plasticity, and the often-lacking reproductive structures (Campbell et al., 1990; Deinhart et al., 2021; Woelkerling et al., 1993). The use of new molecular, phylogenetic, and morpho-anatomical techniques

highlighted an extreme underestimation of coralline diversity for all geographic regions at all taxonomic ranks below family level (Caragnano et al., 2020). The morpho-anatomical similarity that many coralline algae display has been speculated to be a result of either convergent evolution or speciation that has not yet been expressed morphologically/anatomically, or both phenomena acting synchronously (Maneveldt et al., 2017, Richards et al., 2017, Van der Merwe et al., 2015). With the use of molecular tools, evolutionary histories of coralline algae have and will be clarified to elucidate the diversity within this group.

Greater species diversity of coralline algae worldwide has been revealed through the implementation of an Integrated Taxonomic Approach (ITA, Maneveldt et al., 2017) paired with DNA sequencing of type material. ITA utilizes both DNA sequencing and morpho-anatomical studies of field collected specimens. Researchers using ITA have resolved the identity of many different species from many different ecosystems (Basso et al., 2015; Hernandez-Kantun et al., 2016; Maneveldt et al., 2019; Richards et al., 2018). This combined approach has permitted the correct application of phylogenetic names to coralline systematics (Gabrielson et al., 2011, 2018; Hind et al., 2014a, 2014b, 2015, 2016; Maneveldt et al., 2017, 2019; Richards et al., 2017; Sissini et al., 2014). However, controversy in the literature has proliferated regarding the application of morpho-anatomical characters to define genera of both geniculate and non-geniculate coralline algae (Bailey & Chapman 2002; Gabrielson et al., 2011; Hernandez-Kantun et al., 2015; Hernandez-Kantun et al., 2015; Hind et al., 2013; Hind and Saunders, 2013).

Contemporary studies assert that DNA barcoding is the only reliable form of identification, although morpho-metric data is still recorded for species descriptions (Hernandez-Kantun et al., 2015; Hind et al., 2016; Wade et al., 2023). In fact, Hind and Saunders (2013) specified that Neogoniolithoideae and Corallinodeae have no morpho-anatomical characters that

distinguish the two subfamilies. When characterizing the only non-geniculate taxon of Corallinodeae, *Crusticorallina*, Hind et al. (2016) corroborated the claim from Hind and Saunders (2013). The high diversity of coralline algae paired with the futility of delineating species by characterization of their morpho-anatomy results in a difficult task for taxonomic investigators of coralline algae (Bittner et al., 2011; Hernandez-Kantun et al., 2015; Hind and Saunders 2013; Hind et al., 2014a, b; Kato et al., 2011). The challenge is to attach correct Linnaean system names on the phylogeny.

In the northeast Pacific between the state of Washington and Vancouver Island, British Columbia, there is an archipelago of 172 islands and reefs called the San Juan Islands. San Juan Island is the largest island and located in the Salish Sea. The Haro Strait is on the west side and the San Juan Channel is on the east side. The oceanic currents flow from the Strait of Juan de Fuca and the Strait of Georgia, creating San Juan Island's notoriously strong currents. These currents are formed from the strong co-oscillation between the Straits of Juan de Fuca and Georgia (Mofjeld and Larsen 1984). There can be significant variations in the tides across the Strait of Juan De Fuca (Harris 1904; Parker 1977). The tidal currents tend to be rectilinear and flow parallel to the local channel or shoreline. The Haro Strait's tidal currents flow south to north, while the San Juan Channel flows north to south (Mofjeld and Larsen 1984). San Juan Island also has huge tidal swings between high and low tides greatly affecting its rocky intertidal regions. Freshwater circulation patterns play an important role in maintaining water quality in the San Juan Islands. The Fraser River is the largest river in British Columbia, Canada flowing into the Georgia Strait affecting the San Juan Islands. The freshwater input influences water temperature and salinity. The seasonality of the northeast Pacific also has considerable influence on temperature and salinity, as well as light intensity. San Juan Island has a temperate climate

with cool, wet winters and warm, dry summers; extremes in temperature and precipitation are unusual. The major environmental drivers of coralline distribution are reported to be correlated with temperature and water transmittance (Athanasiadis et al., 2004). Other small islands have noted an increase in coralline diversity where there is more rugosity or change in (micro)habitats (Mills et al., 2022), like the San Juan Islands.

Coralline algae is found intertidally to subtidally, and plays important functional roles in ecological systems across all latitudes and habitat types (McCoy and Kamenos 2015). The northeastern Pacific is noted to have a high number of endemic species of coralline algae (Hind et al., 2016). There are four endemic geniculate genera *Calliarthron* Manza (Gabrielson et al., 2011), *Chiharaea* (Martone et al., 2012), *Johansenia* (Hind and Saunders 2013), and *Lithothrix* J.E.Gray). There are two endemic non-geniculate genera *Crusticorallina* (K.R.Hind & P.W.Gabrielson 2016) and *Callilithophytum* (Adey et al., 2015), the obligate epiphyte on *Calliarthron tuberosum* (Postels & Ruprecht) Manza.

Multiple species of coralline algae in the order Hapalidiales have been reported in and around San Juan Island. Some of these genera have been part of a recent revision, including *Chamberlainium tumidum* and *Boreolithothamnion phymatodeum* (Foslie) P.W. Gabrielson, Maneveldt, Hughey & V. Peña (Caragnano et al., 2018; Gabrielson et al., 2023; van der Merwe et al., 2015). Gabrielson et al., 2018 reported *Melobesia marginata* (Setchell & Foslie), *Melobesia mediocris* (Foslie), *Mesophyllum conchatum* (Setchell & Foslie), *Mesophyllum vancouveriense* (Foslie), *Callilithophytum parcum* (Setchell & Foslie), *Neopolyporolithon reclinatu* (Kjellman), *Neopolyporolithon loculosum* (Foslie), *Boreolithothamnion phymatodeum* (Foslie) P.W. Gabrielson, Maneveldt, Hughey & V.Peña comb. nov, *Leptophytum lamelliocola* (Athanasiadis & W.H. Adey), *Leptophytum adeyi* (Steneck & R.T. Paine), *Leptophytum*

foecundum (Kjellman), *Leptophytum tenue* (Kjellman), *Clathomorpha circumscriptum* (Stromfelt), and *Clathomorpha parcum* (Setchell & Foslie).

The order of Corallinales hosts just as many species as Hapalidiales. This includes *Bossiella*, the only genera with species that are both non-geniculate and geniculate. There are also three reported species of the geniculated genera of *Corallina*. Gabrielson et al. (2018) reports four species of *Crusticorallina*, which was noted to be previously passing under the name *Pseudolithophyllum*. There are also multiple species of *Lithophyllum* reported. *Chamberlainium tumidum* (Foslie) and *Chamberlainium decipiens* (Foslie) previously holding the name *Spongites* are also reported for the northeast Pacific. Gabrielson et al. (2018) cautioned using morphological methods to delineate between any of these species, and emphasized that there is continual taxonomic reform within these two orders.

This study will focus on identifying non-geniculate coralline algae on the rocky intertidal from San Juan Island, Washington. Here we will investigate the diversity of non-geniculate crustose coralline algae around San Juan Island within the rocky intertidal zone by sampling at six different sites around the island. The San Juan Islands and greater Washington area have been reported to have *Lithophyllum impressum*, *Boreolithothamnion phymatodeum*, *Chamberlainium tumidum*, *Chamberlainium decipiens*, *Neopolyporolithon reclinatu*, *Leptophytum adeyi*, *Mesophyllum vancouveriense*, *Lithothamnion conchatum*, *Crusticorallina painei*, *Crusticorallina adhaerens*, *Crusticorallina nootkan*, *Crusticorallina muricata*, *Bossiella mayae* and *Bossiella exarticulata* (Gabrielson et al., 2018; Gabrielson et al., 2023; Steneck et al., 2019). We will authenticate these claims by DNA sequencing and comparison to type specimens.

Materials and Methods

Specimen Collection and Preparation

We examined specimens we collected in the rocky intertidal in San Juan Island by hammer and chisel at five different locations: Reuben Tarte County park, Deadman's Bay, Friday Harbor Laboratories' shore trail, Cattle Point, and Eagle Cove (Figure 1). Additional subtidal specimens were collected at a sixth site by dredge at Mosquito Pass. Specimens were photographed *in situ*, collected, and then photographed again, *ex situ*. Specimens were air-dried for storage in the University of Washington Burke Museum herbarium.



Figure 1. Collection sites on San Juan Island, Washington, USA.

DNA Sequences and Phylogenetic Analyses

For each specimen, a small portion of the thallus was cleaned of epiphytes then crushed using a pestle for DNA extraction. Total genomic DNA was extracted from specimens using a Mytaq Extract PCR kit (Bioline) and incubated at 75 °C for 10 minutes. Each sample was crushed again then incubated for 10 additional minutes. Each sample was then heated at 95 °C for 10 minutes. The samples were then centrifuged for 3 minutes and diluted $\frac{1}{50}$ of their original concentrations in dH₂O. Some specimens required greater or lesser dilutions for successful PCR. Extractions were stored at -20 °C. PCR reaction mixtures followed the Bioline protocol and contained 4.25µL dH₂O, 6.25µL MyTaq 2x Mix and 0.5µL of the primers F753 and R893 at a concentration of 10 µM. The 3' end of the chloroplast gene *rbcL* (*rbcL*-3P, roughly 660 base pairs), which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase was used for species delimitation and identification. The PCR thermocycling profile included an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturing at 95 °C for 15 seconds, annealing at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. PCR products were sent to Genewiz (Azenta Life Sciences, Seattle, Washington) for sequencing.

Sequences of *rbcL* were aligned and analyzed independently using GeneiousPro 11.0.5. GenBank sequences of other non-geniculate crustose coralline algae that were a close match were added to the alignment based on BLAST searches (Basic Local Alignment Search tool). The sequences were aligned using the MUSCLE plugin in GeneiousPro 11.0.5. Bayesian Inference was completed for each alignment for 1,000,000 generations with trees sampled every 100 generations, and the first 1,000 trees were burned using the MrBayes3.1.2 plugin in GeneiousPro 11.0.5. then visualized in FigTree v1.4.4 (Edgar 2004; Ronquist and Huelsenbeck 2003).

Morpho-anatomical Analyses

The preparation of specimens for scanning electron microscopy (SEM) followed Richards et al. (2017). Terminology and measurements of vegetative and reproductive features followed Adey and Adey (1973) and Hernandez-Kantun et al. (2015). The thallus anatomical terminology and morphological terminology followed as described by Chamberlain (1990) and Woelkerling et al. (1993).

Results

We successfully sequenced 38 of the 46 specimens curated and recognized seven different species (*Boreolithothamnion phymatodeum*, *Chamberlainium tumidum*, *Lithophyllum impressum*, *Bossiella frondescens*, *Bossiella mayae*, *Bossiella exarticulata*, and *Corallina vancouveriensis*; Table 1; Figures 2-14). Another was recognized to the genus level (*Lithophyllum* sp. 1; Table 1; Figure 14, 15) and four to the ordinal level (Hapalidiales sp. 1, Hapalidiales sp. 2, Hapalidiales sp. 3, and Hapalidiales sp. 4; Table 1; Figure 14, 16).

Habitat and biogeographical affinities for each species is based solely on DNA-sequenced specimens. The two specimens identified as *Boreolithothamnion phymatodeum* were found at Cattle Point and Friday Harbor Laboratories's Shore trail (Figure 17). The eight specimens identified as *Chamberlainium tumidum* were found at Reuben Tarte County Park, Deadman's Bay, Eagle Cove, Cattle Point. All five specimens identified as *Lithophyllum impressum* were collected at Deadman's Bay, while *Lithophyllum* sp. 1 was collected subtidally at Mosquito Pass. The only specimen of *Bossiella frondescens* was collected at Cattle Point. *Bossiella mayae* specimens were collected from Deadman's Bay, Eagle Cove, Cattle Point and Friday Harbor Laboratories's Shore trail, and *Bossiella exarticulata* was collected from just Deadman's Bay. The only specimen of *Corallina vancouveriensis* that we curated was from

Cattle Point. Hapalidiales sp. 1 was found at Deadman's Bay, Reuben Tarte County Park, and Mosquito Pass. Hapalidiales sp. 2 was found at Reuben Tarte County Park. Hapalidiales sp. 3 was found at Eagle Cove. Hapalidiales sp. 4 was found at Friday Harbor Laboratories's Shore trail and Reuben Tarte County Park.

Twelve species of coralline algae, representing seven genera, were observed across six sites on San Juan Island (Figure 17). Four species were recorded in the low tidal zone, five species were recorded in the mid tidal zone, two species were recorded in the low-mid tidal zone, two species were recorded in the subtidal zone, and one species were recorded in a cave (Table 1). The most abundant species was *Chamberlainium tumidum*, which was 22% of the total 38 specimen count. The least abundant species was *Lithophyllum* sp. 1, *Bossiella exarticulata*, *Corallina vancouveriensis*, *Bossiella frondescens* and Hapalidiales sp. 3, which each make up 3% of the total specimen count. Hapalidiales sp. 4 and *Boreolithothamnion phymatodeum* each made up 5%, Hapalidiales sp. 2 accounted for 8%, *Bossiella mayae* and *Lithophyllum impressum* each made up 13%, and finally Hapalidiales sp. 1 made up 19%.

DNA sequence divergence was recorded based on the pairwise alignment generated by Maximum likelihood (ML) methods in RAxML. Sequence divergence between the *Boreolithothamnion phymatodeum* reported in this study and its isotype was 0%. Sequence divergence between *Chamberlainium tumidum* reported in this study and its isolectotype was 0.4%. The *Lithophyllum impressum* reported in this study also had 0% sequence divergence compared to its type specimen. *Bossiella frondescens*, *Bossiella mayae*, and *Corallina vancouveriensis* reported in this study had 0% sequence divergence to their respective isolectotype, holotype and isolectotype. Sequence divergence between the *Bossiella exarticulata* reported in this study and its holotype was 0.2%. Sequence divergence between *Lithophyllum* sp.

1 and the next closest related *Lithophyllum* species was 8.7-13.4%. Sequence divergence between Hapalidales sp. 1 and the next closest related Hapalidales species was 2.8-16.6%. Sequence divergence between Hapalidales sp. 2 and the next closest related Hapalidales species was 13.6-17.1%. Sequence divergence between Hapalidales sp. 3 and the next closest related Hapalidales species was 9.3-15.7%. Lastly, sequence divergence between Hapalidales sp. 4 and the next closest related Hapalidales species was 7.6-13.3%.

No morpho-metric data was taken from the SEM images. However, any notable morpho-anatomical features seen were captured and presented here (Figure 2-13).

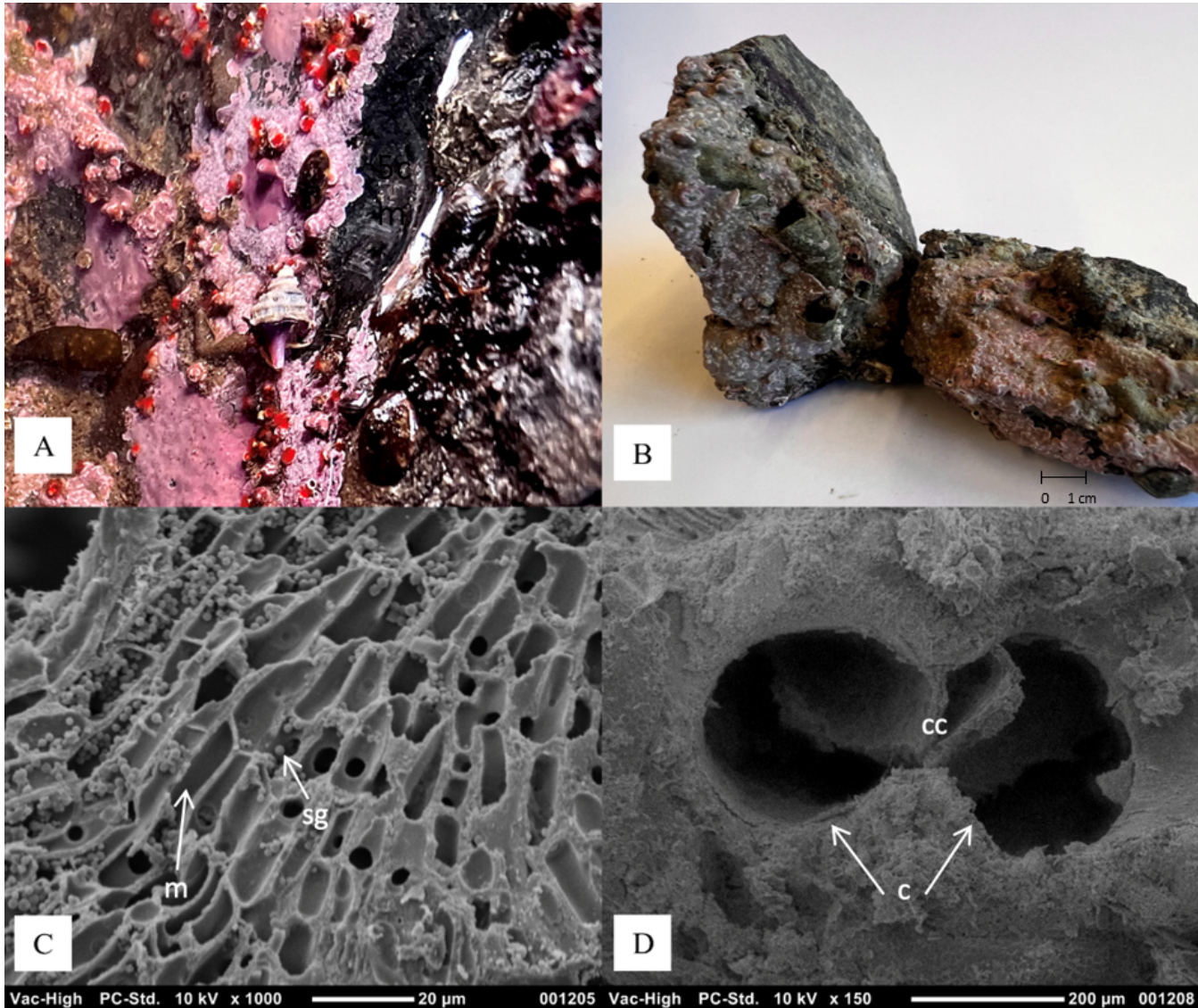


Figure 2. CCA-35. (A) Epilithic *Boreolithothamnion phymatodeum* at Friday Harbor

Laboratories's Shore Trail. (B) Herbarium dried specimen. (C) A cross section of the inner thallus shows medullary filaments (m) and floridean starch grains (sg). (D) A buried tetrasporangia conceptacle (c) with a central columella (cc).



Figure 3. CCA-03. (A) Epilithic Hapalidales sp. 1 at Reuben Tarte County Park. (B) Herbarium dried specimen. (C) Floridean starch grains (sg) are stored in the perithallium cells. Secondary pit connections (sp) are also present among the filaments and calcium carbonate (cc) are stored within the cell walls.

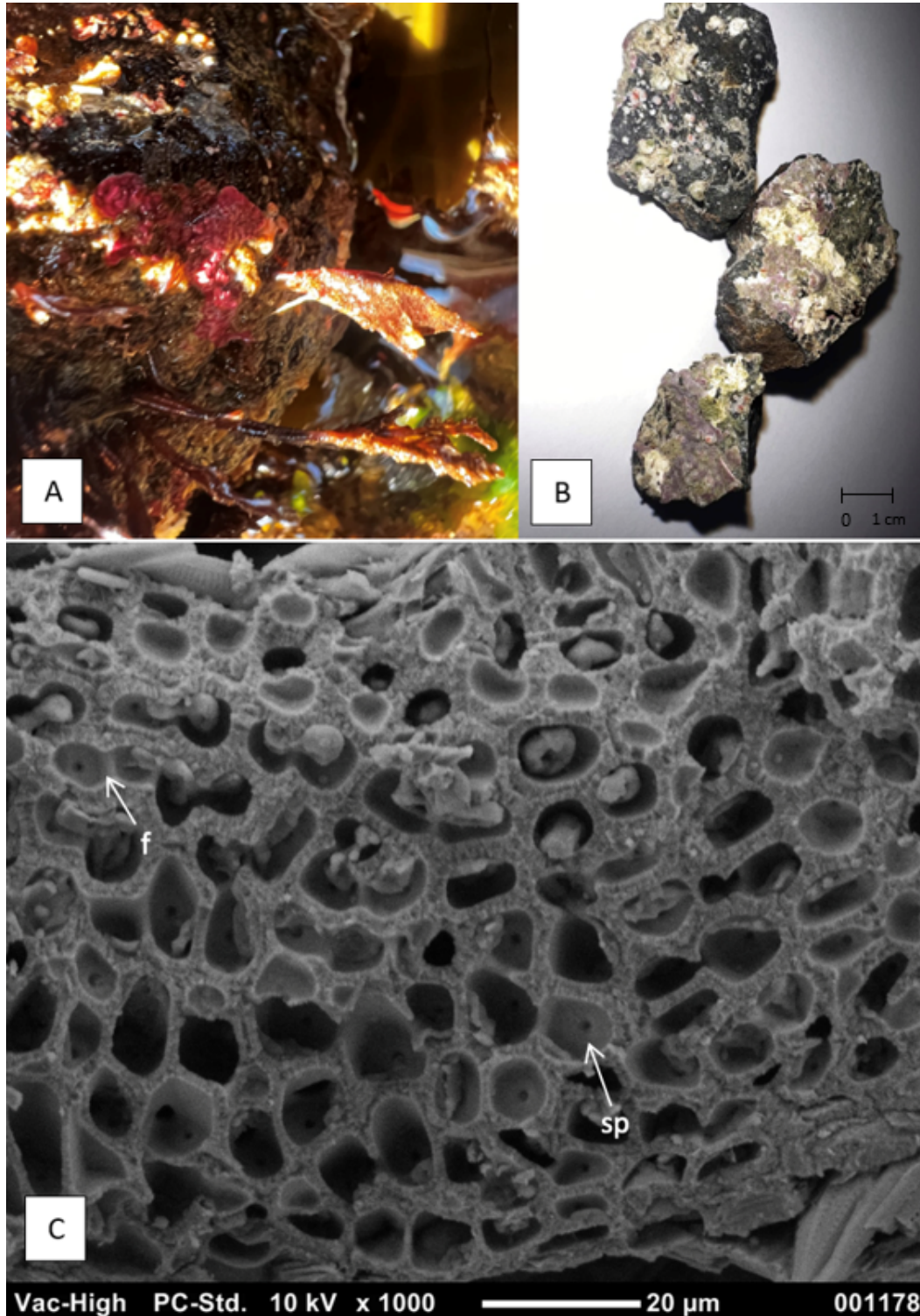


Figure 4. CCA-10. (A) Epilithic Hapalidales sp. 2 at Reuben Tarte County Park. (B) Herbarium dried specimen. (C) A cross section of the inner thallus reveals secondary pit connections (sp) among the filaments and fusions (f) between cells of adjacent medullary filament.

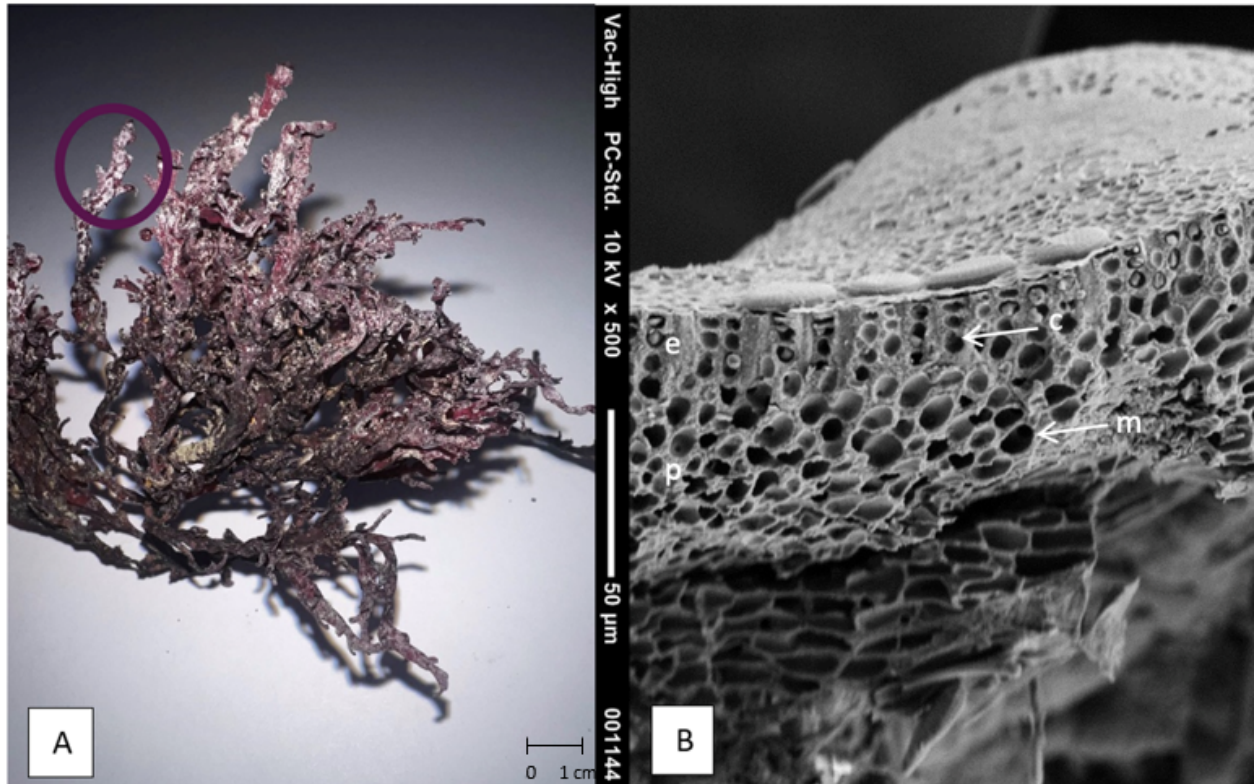


Figure 5. FHL-23_25. (A) Epiphytic Hapalidales sp. 3 on fleshy red algae (herbarium dried specimen) collected from Eagle Cove. (B) Transverse fracture of the monomerous constructed thallus shows the perithallium (p) and epithallium (e), along with the medullary filaments (m) curving upward to form cortical filaments (c).

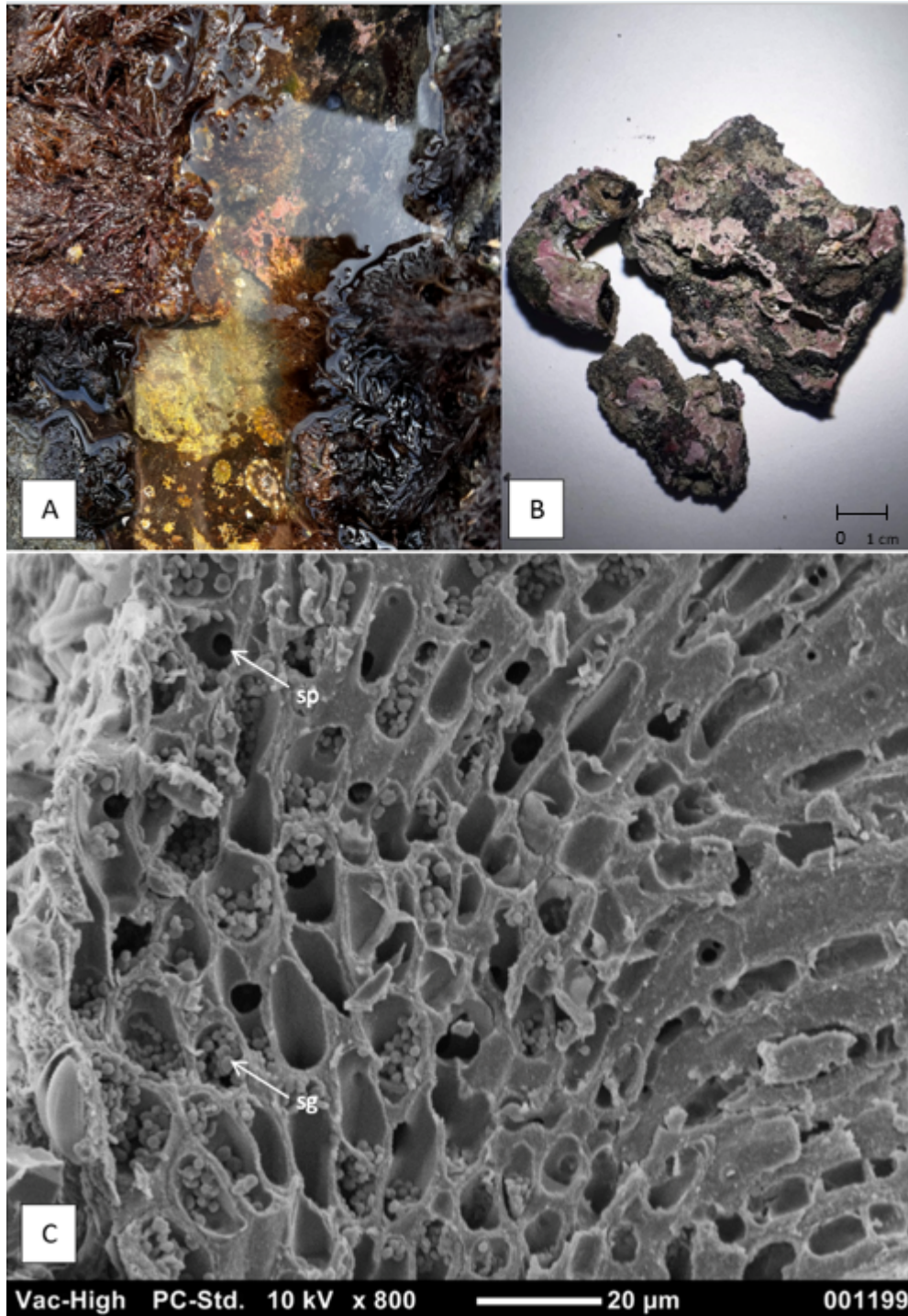


Figure 6. CCA-27. (A) Epilithic Hapalidales sp. 4 at Friday Harbor Laboratories's Shore trail. (B) Herbarium dried specimen. (C) Transverse fraction shows secondary pit connections (sp) are present along with floridean starch grains (sg).

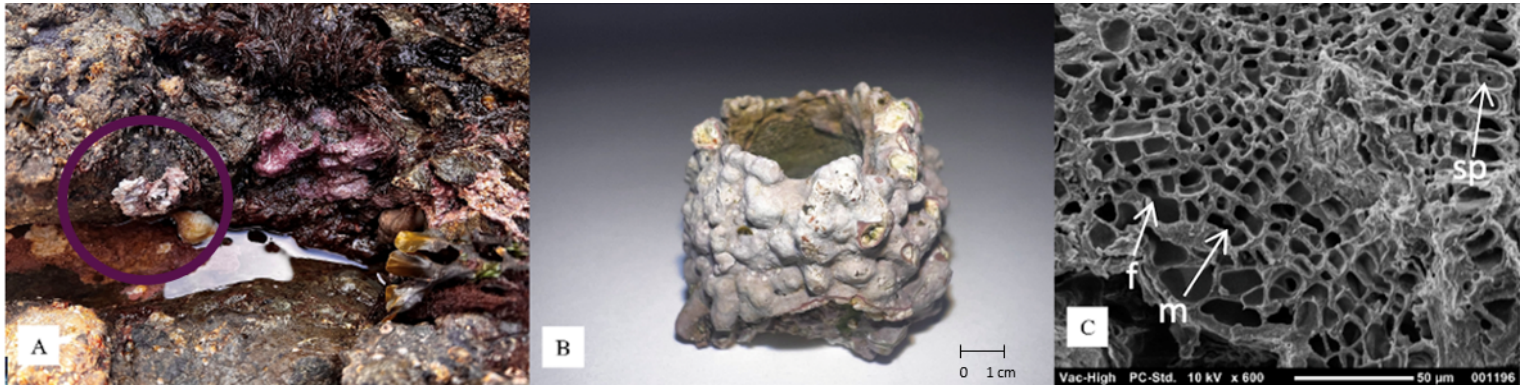


Figure 7. FHL-23_26. (A) Epizoic *Chamberlainium tumidum* at Friday Harbor Laboratories's Shore trail. (B) Herbarium dried specimen. (C) A cross section of the inner thallus shows fusions (f) between cells of adjacent medullary filaments (m) and present secondary pit connections (sp).

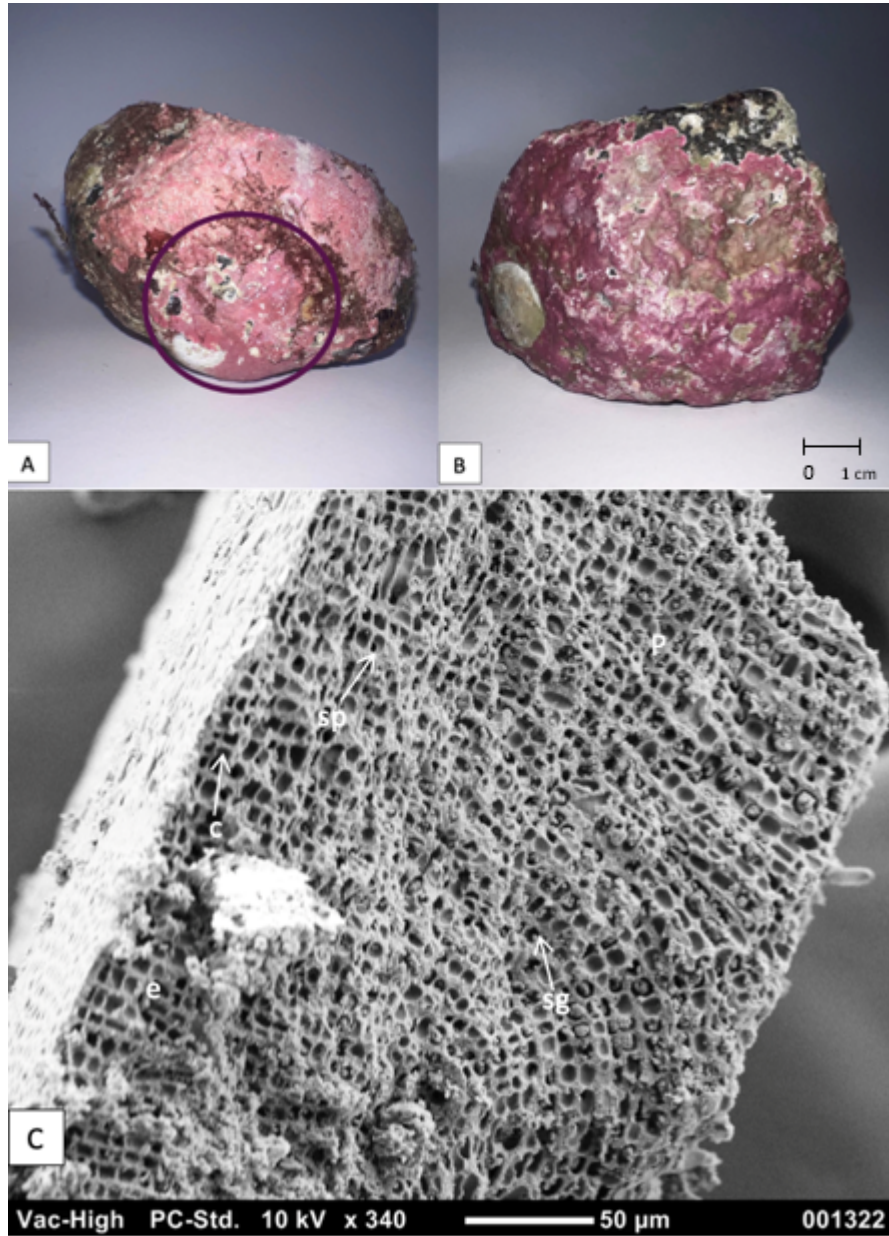


Figure 8. CCA 32 (A) CCA 42 (B). *Lithophyllum* sp. 1 (herbarium dried specimens) collected from Mosquito Pass. (C) Transverse fracture of the monomerous constructed thallus shows the perithallium (p) and epithallum (e), along with cortical filaments (c), secondary pit connections (sp) and floridean starch grains (sg).

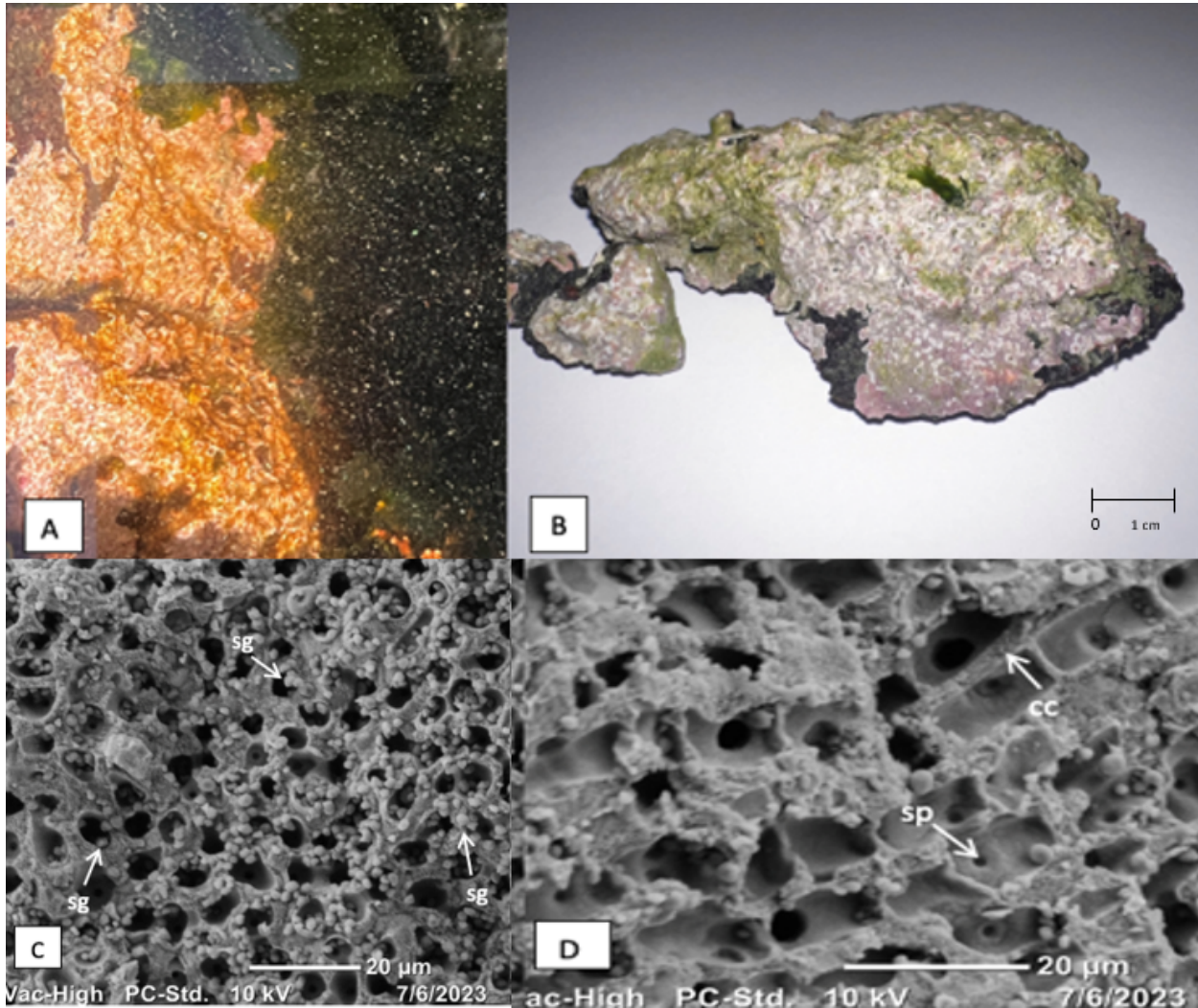


Figure 9. CCA-17. (A) Epilithic *Lithophyllum impressum* at Deadman's Bay in tidepool. (B) Herbarium dried specimen. (C) Floridean starch grains (sg) stored in the perithallium cells. (D) Vertical fracture of the peripheral region of the crust shows secondary pit connections (sp) present among the filaments and calcium carbonate (cc) stored within the cell walls.

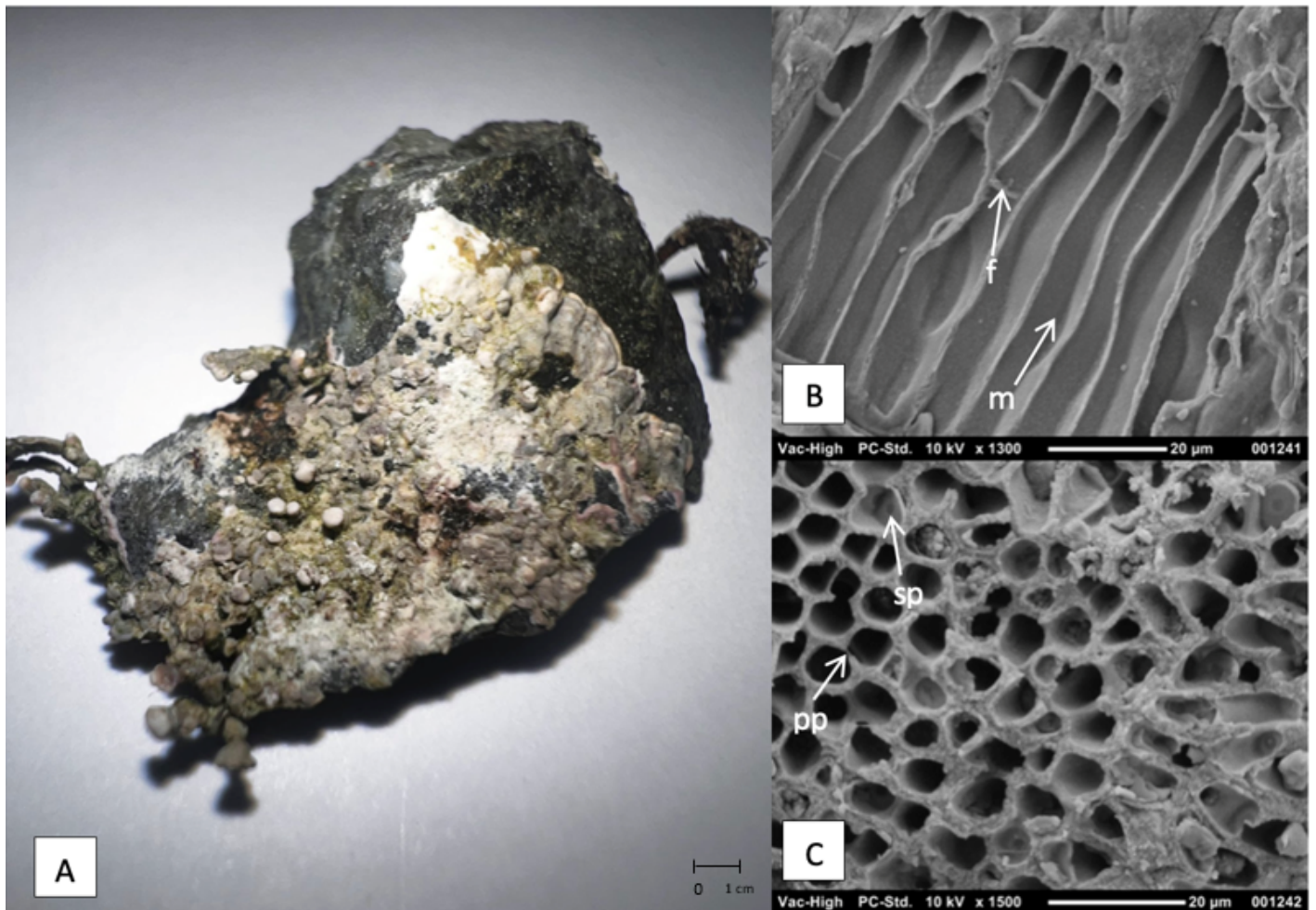


Figure 10. FHL-23_06. (A) Epilithic *Bossiella frondescens* (herbarium dried specimen) collected at Cattle Point. (B) A cross section of the inner thallus shows fusions (f) between cells of adjacent medullary filament, which forms rectangular, elongated cells (m). (C) Transverse fraction of thallus shows both primary (pp) and secondary pit (sp) connections.

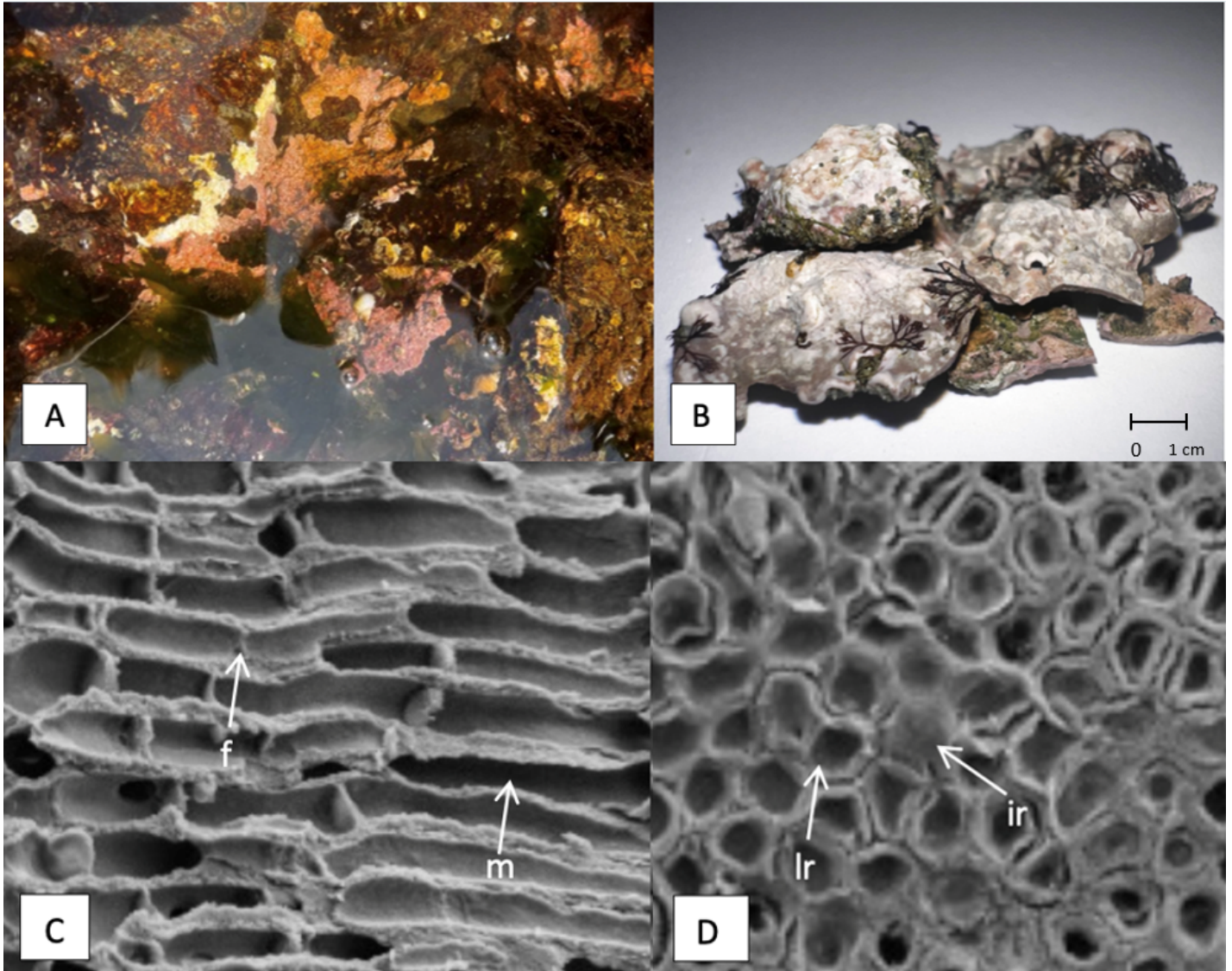


Figure 11. FHL-23_22. (A) Epilithic *Bossiella mayae* at Friday Harbor Laboratories's Shore Trail in a tidepool. (B) Herbarium dried specimen. (C) A cross section of the inner thallus shows fusions (f) between cells of adjacent medullary filament, which forms rectangular, elongated cells (m). (D) A surface view of polygonal epithelial cells with both intact (ir) and lost (lr) roofs.

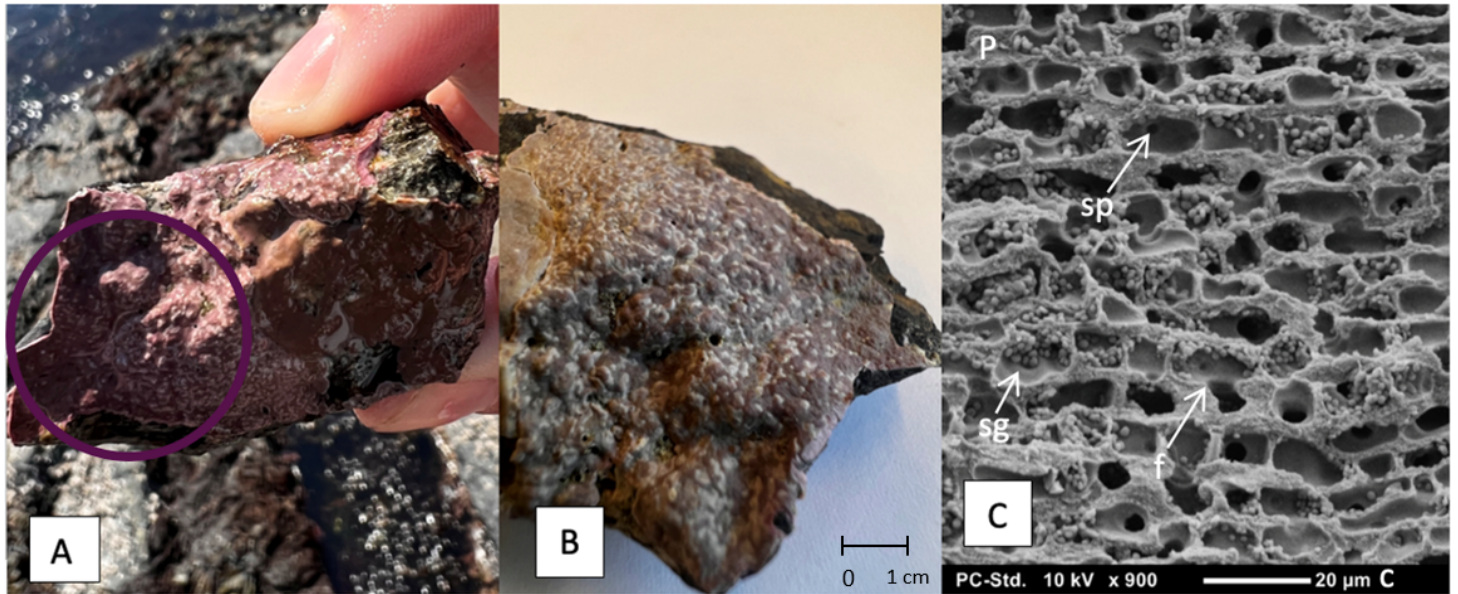


Figure 12. CCA-24b. (A) Epilithic *Bossiella exarticulata* at Deadman's Bay. (B) Herbarium dried specimen. (C) A cross section of the perithallium (p) region shows fusions (f) between cells of adjacent medullary filaments, secondary pit (sp) connections, and floridean starch grains (sg).

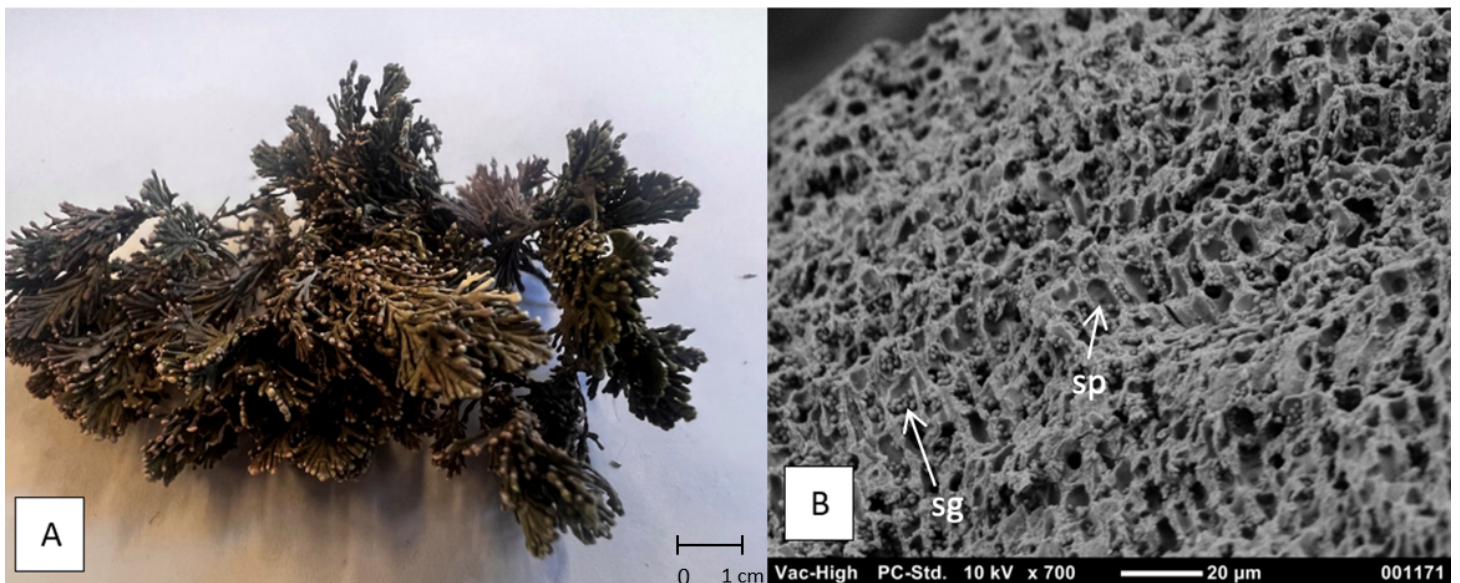


Figure 13. FHL-23_07. (A) *Corallina vancouveriensis* (herbarium dried specimen) collected at Cattle Point. (B) A transverse fraction shows secondary pit (sp) connections and floridean starch grains (sg).

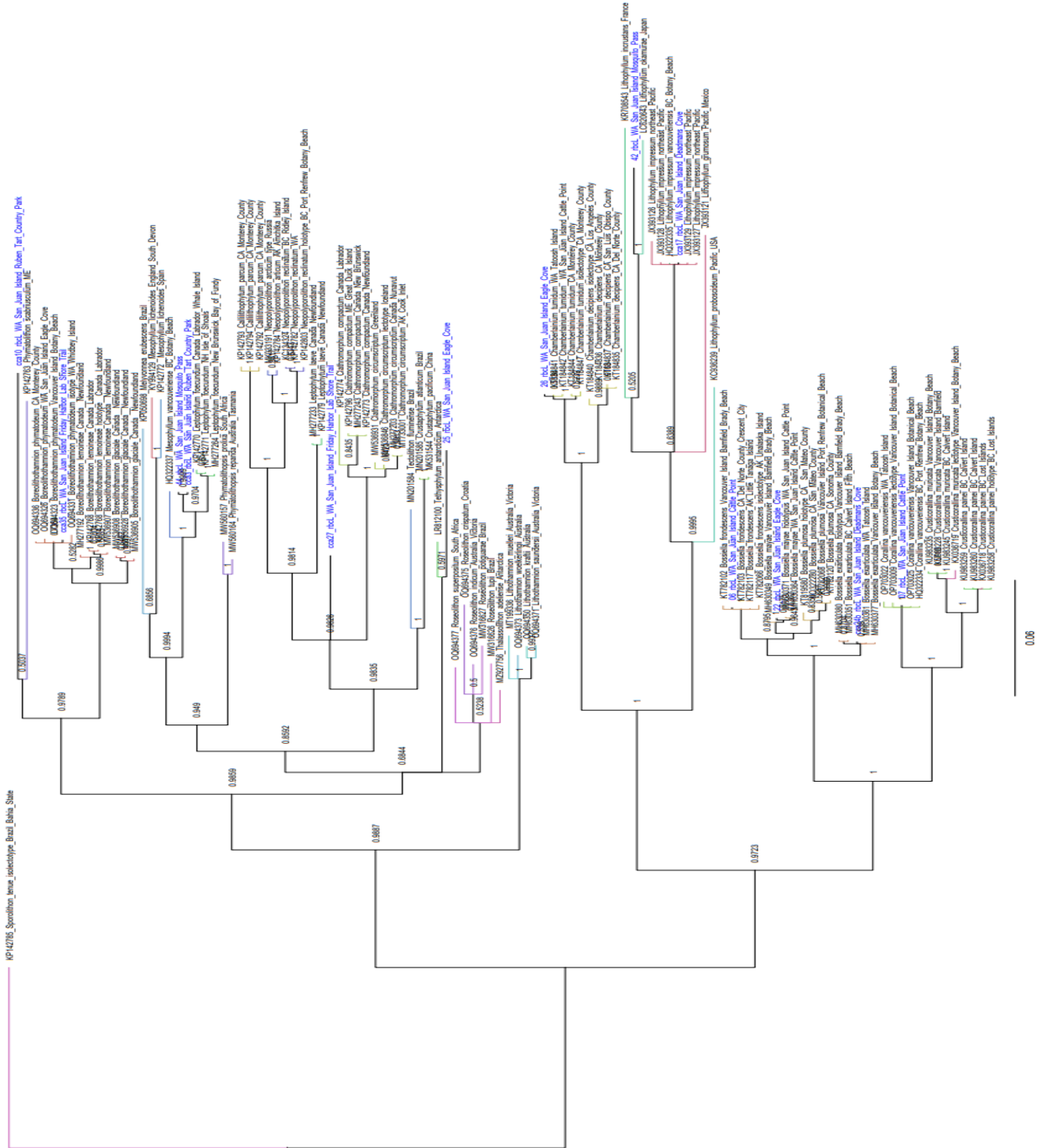


Figure 14. Phylogenetic tree based on Bayesian analysis of *rbcL* sequences. Nodes show posterior probability. The taxa reported in this study are in blue text. [FHL.pdf](#)

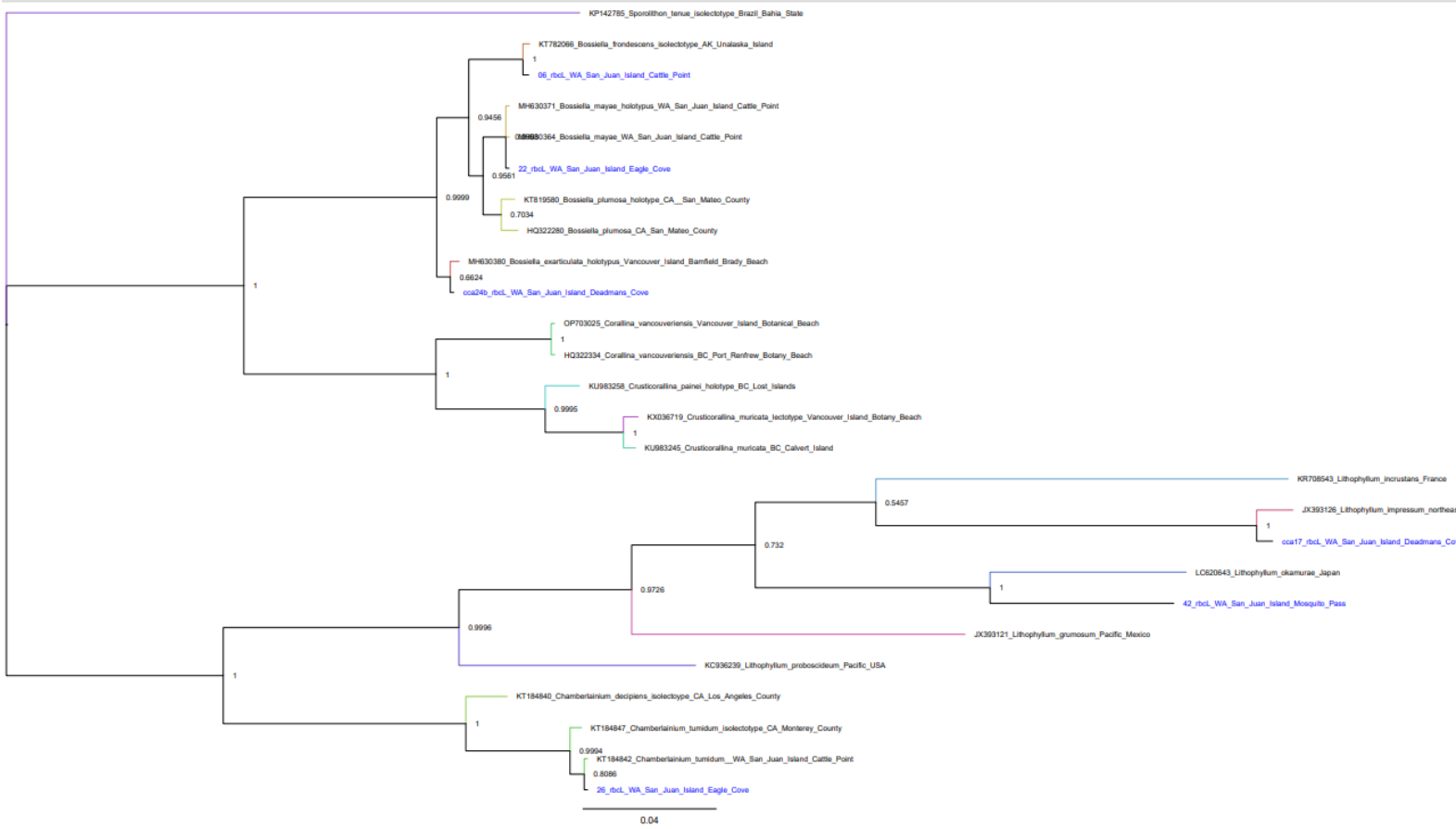


Figure 15. Phylogenetic tree based on Bayesian analysis of *rbcL* sequences of Corallinales.

Nodes show posterior probability. The taxa reported in this study are in blue text. [CL.pdf](#)

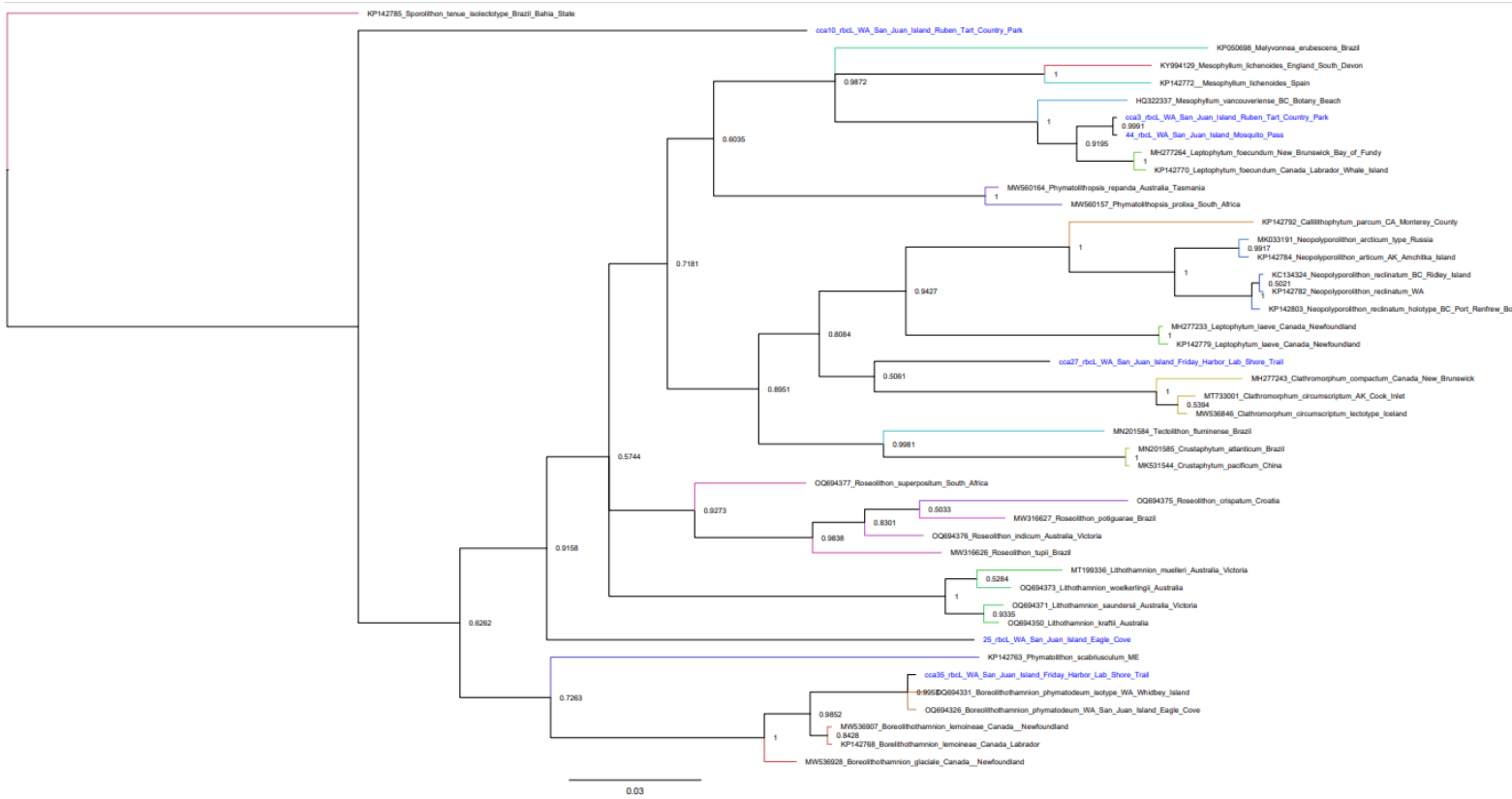


Figure 16. Phylogenetic tree based on Bayesian analysis of *rbcL* sequences of Hapalidales.

Nodes show posterior probability. The taxa reported in this study are in blue text. [HL.pdf](#)

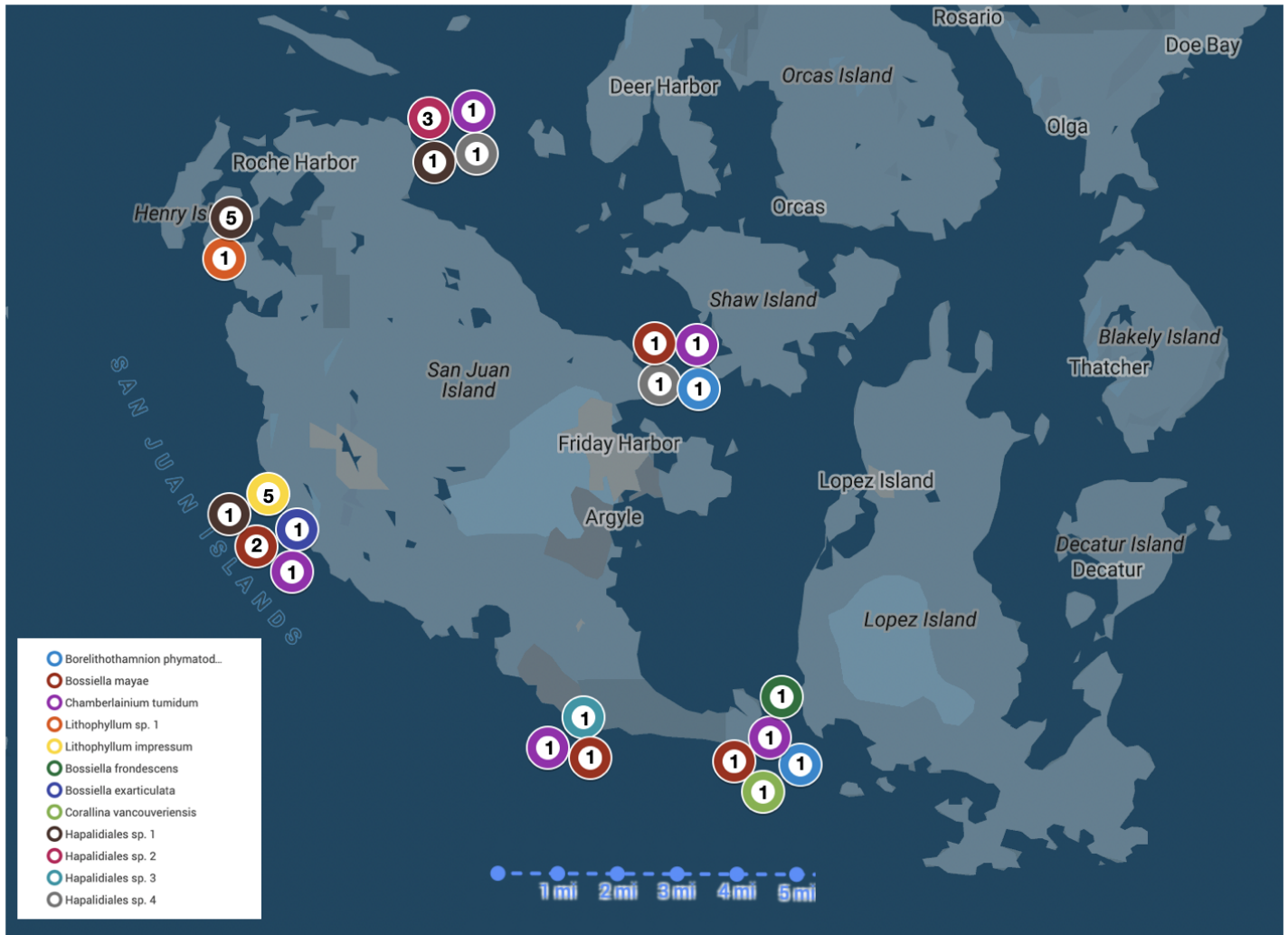


Figure 17. The number of each species at the six collection sites on San Juan Island, Washington.

Table 1. Specimens collected, herbarium number, location, habitat and habit.

Name	Herbarium number	Locations	Habitat	Habit
<i>Boreolithothamnion phymatodeum</i> (n=2)	CCA 35	Friday Harbor Laboratories's Shore trail	Rocky intertidal. Low-mid tide zone. Exposed.	Encrusting. Light pink. Bumpy.
	FHL23-09	Cattle Point	Epilithic on primary bedrock.	White tessellations. White on the perimeter of the crust.
<i>Chamberlainium tumidum</i> (n=8)	FHL23-26	Eagle Cove	Rocky shore. Mid-tide zone.	Encrusting. The gross morphology ranges from bumpy to smooth. White tessellations are smooth. Colors vary from light grayish purple to a light coral shade.
	CCA 04	Reuben Tarte County Park		
	CCA 22	Deadman's Bay		
	FHL23- 08 FHL23-05	Cattle Point		
	CCA 28 CCA 33 CCA 36A	Friday Harbor Laboratories's Shore trail		
<i>Lithophyllum impressum</i> (n=5)	CCA 17 CCA 14 CCA 15 CCA 19A CCA 24A	Deadman's Bay	Rocky intertidal. Low tide zone. Tidepool. Shaded by seagrasses, crevices, and caves.	Encrusting. Color varies from light orange pink to a purple. Bumpy. White tessellations. White on the perimeter of the crust.
<i>Bossiella frondescens</i> (n=1)	FHL23-06	Cattle Point	Rocky shore. Intertidal mid tide zone.	Large encrusting base to articulated branches. Dark Pink.
<i>Bossiella mayae</i> (n=5)	FHL-23-22	Eagle Cove	Rocky shore. Intertidal mid-tide zone.	Encrusting. Light pink. Bumpy.
	FHL-23-10	Cattle Point		

	CCA11 CCA16	Deadman's Bay	Submerged in the tidepool.	White tessellations. White on the perimeter of the crust.
	CCA30	Friday Harbor Laboratories's Shore trail		
<i>Bossiella exarticulata</i> (n=1)	CCA 24b	Deadman's Bay	Shaded inside a cave. Mid-tide zone.	Encrusting. Purple. Bumpy. White tessellations. White on the perimeter of the crust.
<i>Corallina vancouveriensis</i> (n=1)	FHL-23-07	Cattle Point	Rocky intertidal. Low tide zone.	Geniculate. Pink.
<i>Lithophyllum</i> sp. 1 (n=1)	CCA 42 CCA 38	Mosquito Pass	Subtidal zone.	Encrusting. Varying colors from light pink to purple. Bumpy. White tessellations.
Hapalidiales sp. 1 (n=7)	CCA 3	Reuben Tarte County Park	Rocky intertidal. Mid-tide zone. Shaded. Subtidal zone	Encrusting. Dark purple. Lumpy with white coloration on top of the bumps.
	CCA 25	Deadman's Bay		
	CCA 39 CCA 41 CCA 44 CCA 45 CCA 46	Mosquito Pass		
Hapalidiales sp. 2 (n=3)	CCA 10 CCA 2	Reuben Tarte County Park	Rocky shore. Intertidal and	Encrusting. Color varies from bright dark

	CCA 6		exposed. Low-mid tide zones.	pink to purple. Bumpy. White tessellations.
Hapalidiales sp. 3 (n=1)	FHL-23-25	Eagle Cove	Epiphyte. Low tide zones.	Encrusting. Pink. Bumpy. White tessellations.
Hapalidiales sp. 4 (n=2)	CCA 27	Friday Harbor Laboratories's Shore trail	Submerged in the tidepool. Low tide zone.	Encrusting. Pink. Smooth.
	CCA 4	Reuben Tarte County Park		

Discussion

Assignment of names

The progressing evaluation of coralline algae using DNA sequence data has clarified relationships within the large class of Corallinophycidae that morpho-metric data alone could not differentiate. Our modest sampling effort illuminated the diversity of non-geniculated coralline algae in San Juan Island. The least ambiguous method to link historical names and recently collected specimens to type material is to compare DNA sequences from both of these sources (Hughey et al., 2011). Using this method, we are confident that the names *Boreolithothamnion phymatodeum*, *Chamberlainium tumidum*, *Bossiella mayae*, *Bossiella exarticulata*, and *Lithophyllum impressum* are correctly applied to the newly collected algae. Partial *rbcL* sequences were identical to comparable lengths of the newly collected specimens and differ from all other sequenced species. Here we report six Corallinales species (*Chamberlainium tumidum*, *Lithophyllum impressum*, *Bossiella mayae*, *Bossiella exarticulata*, *Bossiella frondescens* and *Corallina vancouveriensis*) and one to the genus level (*Lithophyllum* sp. 1). Within the order Hapalidiales one species was identified (*Boreolithothamnion phymatodeum*) and four to the

ordinal level (Hapalidiales sp. 1, 2, 3, and 4). Of the fourteen previously reported non-geniculated species, five were identified in this study (*Boreolithothamnion phymatodeum*, *Chamberlainium tumidum*, *Bossiella mayae*, *Bossiella exarticulata*, and *Lithophyllum impressum*). The two geniculated species (*Bossiella frondescens* and *Corallina vancouveriensis*) have also been previously reported for the northeast Pacific.

Corallinales

Chamberlainium tumidum (Foslie) Caragnano, Foetisch, Maneveldt & Payri

The type locality of *Chamberlainium tumidum* is Monterey, California, USA. The range is reported to be near Sitka, Alaska to Monterey, California. In this study, DNA sequence validated the presence of *Chamberlainium tumidum* at all five intertidal collection sites.

Bossiella mayae P.W. Gabrielson, K.R. Hind, Martone, & C.P. Jensen

The type locality of *Bossiella mayae* is Cattle Point, San Juan Island, WA, USA. The range is reported to be Gwaii Haanas, BC, Canada south to Mill Creek Beach, CA. In this study, DNA sequence validated the presence of *Bossiella mayae* in Friday Harbor Laboratories's Shore trail, Deadman's Bay, Eagle Cove, and Cattle Point in San Juan Island. Similar to other reports, this epilithic algae was collected in the mid to low intertidal zone on rock exposed to the ocean and submerged in tidepools.

Bossiella exarticulata K.R. Hind, Martone, C.P. Jensen, & P.W. Gabrielson

The type locality of *Bossiella exarticulata* is Brady's Beach blowhole, Bamfield, Vancouver Island, BC, Canada. The range is reported to be Gwaii Haanas, BC, Canada south to

CCA's of SJI

Mill Creek Beach, CA. In this study, DNA sequence validated the identity of *Bossiella exarticulata* in Deadman's Bay. This algae was collected in a shaded cave.

Lithophyllum impressum Foslie

The type locality of *Lithophyllum impressum* is Port Renfrew, Vancouver Island, British Columbia, Canada. The distribution is reported to be southeast Alaska to the Puget Sound and the Strait of Juan de Fuca; this includes San Juan Islands. In this study, DNA sequences validated the presence of *Lithophyllum impressum*; this algae was only found in Deadman's Bay. The specimens of *Lithophyllum impressum* collected in Deadman's Bay were found in the low intertidal zone similar to other reports. All of the specimens collected were found shaded by other algae.

***Lithophyllum* sp. 1** Philippi 1837

The subtidal dredge at Mosquito Pass produced two more specimens of *Lithophyllum* with identical *rbcL* sequences. The genus *Lithophyllum* is very speciose; there are currently 127 taxonomically accepted species (Guiry & Guiry 2022). Further genetic information is necessary in order to pursue the identification of these cryptic species.

Hapalidiales

Hapalidiales sp. 1, 2, 3, and 4 W.A. Nelson, J.E. Sutherland, T.J. Farr & H.S. Yoon 2015

Extensive morphological and phylogenetic analysis support the recognition of Hapalidiales as a distinct order and not a suborder of Corallinales (Gabrielson et al., 2023). We sequenced and reported *Boreolithothamnion phymatodeum*, one of the best understood species of the Hapalidiales, and four distinct Hapalidiales to the ordinal level. DNA sequence divergence indicates distinction between Hapalidiales sp. 1, 2, 3, and 4 and all other sequenced species. More genetic information is necessary in order to pursue the identification of these cryptic algae.

In addition, within the last eight years, researchers proposed multiple new genera (*Amphithallia* (Athanasiadis, 2017), *Carlskottsbergia* (Athanasiadis, 2019a), *Capensia* (Athanasiadis, 2019b), *Melyvonnea* (Athanasiadis & Ballantine, 2014), *Phragmope* (Athanasiadis, 2020), *Perithallus* (Athanasiadis, 2021), *Printziana* (Athanasiadis, 2021), *Sunesonia* (Athanasiadis, 2021), and *Thallis* (Athanasiadis, 2021)) recognized as Hapalidiales based only on morpho-anatomical metrics. No genus has its generitype species sequenced; we have no knowledge of the phylogenetic relationship among these genera or to other described genera in the Hapalidiales. Intensive work using molecular analysis is critical to resolving this order, not only for the four species of Hapalidiales in San Juan Island, but globally.

Boreolithothamnion phymatodeum (Foslie) P.W. Gabrielson, Maneveldt, Hughey & V. Peña

The type locality of *Boreolithothamnion phymatodeum* is Whidbey Island, Washington, USA. The distribution is north to Calvert Island, British Columbia, Canada and south to San Nicholas Island, Ventura, California. This study confirmed the presence of *Boreolithothamnion phymatodeum* in San Juan Island at two locations: Cattle Point and Friday Harbor Laboratories's Shore trail. This species is noted to be epilithic and epizoic on barnacles and most common in the

low intertidal and subtidal to 15 meters of depth. The specimens collected at Cattle Point and Friday Harbor Laboratories's Shore trail were noted to be in exposed mid to low intertidal zone.

“Leptophytum foecundum” Kjellman, W.H. Adey

Type locality of *“Leptophytum foecundum”* is the Kara Sea (East Coast of Novaja Zemlya). The distribution of this alga includes: Alaska, Canada, Russia, Greenland, Iceland, Maine, and New Hampshire. In past reports, this genus has also been described on the Pacific coast of North America (Athanasiadis et al., 2004). However, recent studies report an inconsistent distribution range. In this study, Hapalidiales sp. 1 (CCA 3 and FHL_44) had the closest relation to *“Leptophytum foecundum”*. Interestingly, *“Leptophytum foecundum”* did not clade with their generitype, *Leptophytum laeve* W.H. Adey. The taxonomic and/or nomenclatural status of *Leptophytum* and the specimens CCA-03 and FHL-23_44 is unresolved and requires further investigation.

Mesophyllum vancouveriense (Foslie) Steneck & R.T. Paine 1986

Type locality of *Mesophyllum vancouveriense* is Port Renfrew (Port San Juan), Vancouver Island, and is noted to be epiphytic on Laminaria holdfasts, epizoic on limpets, and epilithic. The distribution range of *Mesophyllum vancouveriense* is the Strait of Juan de Fuca, Tatoosh Island, Washington to Hedley Island, British Columbia, Canada. This alga has been noted to be very common at Tatoosh Island, Washington. The genus *Mesophyllum* has a wide distribution and currently has 60 species globally (Athanasiadis et al., 2004). Five species are reported in the northeast Pacific with a range between the Aleutian Islands and southern California: *M. aleuticum* Lebednik, *M. vancouveriense* (including *Lithohamnion phymatodeum* f.

aquilonium), *M. conchatum*, *M. lamellatum* and *M. crassiusculum* (Foslie) Lebednik, (including *Lithothamnion aculeiferum*). However, the relationships between the five species of *Mesophyllum* reported in the northeast Pacific have only been described using vegetative and reproductive characters. A phylogenetic analysis of the genus is needed as well as the greater *Mesophyllum-Leptophytum-Synarthrophyton* complex found within the Hapalidales. This would further clarify the relationships of CCA-33 and FHL-23_44.

Conclusion

The results of this study highlight how little is known about the diversity of San Juan Island's coralline flora, despite their important ecological role and dominant coverage in the rocky intertidal. Understanding the diversity of coralline algal communities on San Juan Island could improve the comprehension of ecosystem processes and community dynamics of temperate coastlines. The relationships of coralline algae will continue to unfold as more type and historical specimens are sequenced for comparison to DNA of algae from contemporary collections. Any taxonomic statement should be validated with genomic evidence and identified in junction to the type specimen when possible. Employing different genes, like the mitochondrial gene cytochrome c oxidase I (CO1) or the plastid gene of photosystem II protein D1 (psbA) for the coralline algae in this collection, especially for *Lithophyllum* sp. 1 and Hapalidales sp. 1, 2, 3, and 4 could prove to be more diagnostic. It is possible that there are more species of coralline algae in San Juan Island than reported here. Not all of the genera of coralline algae that has been reported in the northeast Pacific were collected in this study. We urge future collectors to sample with a greater coverage of the island in the intertidal and subtidal to obtain a broader geographical and more complete (micro) habitat representation. Morpho-metric data was

not implemented, although we advise future phycologists to record morphological information if possible. This study is a demonstration of the benefit to DNA-based identification in surveying coralline diversity. This is also the start to better understanding this dominant, ecologically significant, and overlooked group of ecosystem engineers.

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