MORPHOLOGICAL AND MOLECULAR DIFFERENTIATION OF *ULVA* SPP. (ULVOPHYCEAE, CHLOROPHYTA) AND *FUCUS* SPP. (PHAEOPHYCEAE, OCHROPHYTA) OF THE SAN JUAN ISLANDS, WA, US

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ABSTRACT

Marine macroalgae are foundation species that play a critical ecological role in coastal communities as primary producers in the ecosystem. Both *Ulva* and *Fucus* genera are vital in intertidal communities serving a food source and shelter for other organisms. Previous studies were limited, focusing only on morphological characteristics of these algal genera. This project aimed to identify the diversity of *Ulva* and *Fucus* species using an integrated approach of morphological and molecular analysis in the San Juan Islands, WA, to better understand defining characteristics of species and overall biodiversity. *Ulva* (Ulvophyceae) and *Fucus* (Phaeophyceae) specimens were collected from the lower, mid, and upper intertidal zones; each representative having different macroscopic morphological characteristics and collected in varying tidal zones. The *tuf*A and COI-5P loci were amplified for *Ulva* and *Fucus* specimens, respectively, then sequenced. Our study indicates that morphological assessment of these genera alone is not definitive. Molecular-based classification of proper *Ulva* and *Fucus* species identification are important to understand the biodiversity within coastal ecosystems.

Abbreviations: Maximum Likelihood (ML); Unweighted Pair Group Method with Arithmetic Mean (UPGMA); Operational Taxonomic Unit (OTU)

INTRODUCTION

The northeast Pacific Ocean, spanning from Southeast Alaska to Oregon, is characterized by a diverse community of marine algae including, 671 taxa and 284 genera (Gabrielson and Lindstrom 2018). The Washington coast has mixed semidiurnal tides that create zonation in the intertidal zone. Seasonality and physical factors further affect the diversity of the intertidal community and the interaction among resident organisms (Armos and Owen 2018).

Marine macroalgae are foundation species that play a critical ecological role in coastal communities as primary producers and habitat-defining organisms (Burke et al. 2011). *Ulva* species are important as components of biodiversity and bioindicators (Wolf et al. 2012). However, they have also been associated with the majority of blooms of free-floating green algae responsible for 'green tides' (Hayden and Waaland 2004, Duan et al. 2012, Guidone et al. 2013) since *Ulva* species can rapidly grow in nutrient rich habitats and have a high tolerance range for abiotic factors such as temperature and salinity (Hayden and Waaland 2004, Ismail and Mohamed 2017).

The eutrophication-driven green tides in shallow waters have a direct economic impact on coastal communities, and it is essential to know the identification of the species involved for bloom characterization and control (Duan et al. 2012, Wichard et al. 2015). However, in the case of *Ulva*, identification of species still represents a great challenge for scientists.

Ulva

The genus *Ulva* Linnaeus (1753) (Chlorophyta), or sea lettuce, is constituted of 85 taxonomically accepted species (Guiry and Guiry 2021) including those species previously placed in *Enteromorpha* (Hayden et al. 2003). This green algal genus is ubiquitous along coasts, rocky shores, and protected bays and estuaries, growing attached to substrata or found as drift (Hayden and Waaland 2004).

The morphological characterization of *Ulva* species has traditionally included both macro- and microscopic features. Macroscopic features include thallus shape, size, and presence or absence of marginal dentation. Cellular features that are key to identification include cellular shape and dimensions, number of pyrenoids, arrangement of the cells in regular or irregular patterns, and thallus thickness (Kazi et al. 2016, Gabrielson and Lindstrom 2018, Hughey et al. 2019). Although previous studies use these characters for identification, they can vary within species depending on thallus age, reproductive state, wave exposure, tidal factors, temperature, salinity, light, lifestyle, and biological factors such as herbivory (Wolf et al. 2012, Kazi et al. 2016). Also, the morphological plasticity of *Ulva* species results in a variety of forms and ecotypes. Therefore, the taxonomic status of species in this genus is still uncertain and difficult to assess (Wolf et al. 2012, Guidone et al. 2013, Kazi et al. 2016, Hughey et al. 2019).

Molecular analysis of *Ulva* spp. shows that their DNA contains valuable genetic information for understanding key aspects of their taxonomic and phylogenetic status and answering genomic questions that still remain (Hughey et al. 2019). It has been demonstrated molecularly that some

type-specimens of *Ulva* were synonyms (Hughey et al. 2019, Hughey et al. 2021a, Hughey et al. 2021b) even though morphologically they were identified as different species.

Recently, an integrated approach of morphological and molecular analyses has been recommended (Hayden and Waaland 2004, Duan et al. 2012, Guidone et al. 2013, Kazi et al. 2016, Hughey et al. 2019). However, Hayden and Waaland (2004) provided the only sequences from San Juan Island, and reported the specimens as *U. californica* and *U. lactuca.* This highlights the importance of using both types of analyses for robust species identification. In the San Juan Islands, the use of integrated analysis could provide genetic insights on species diversity and delimitation of different morphotypes.

The proper identification of *Ulva* species is essential to assess harmful environmental effects related to green tides (Guidone et al. 2013). In addition, it is important to understand their potential uses in pharmaceutical applications for drug development (Ismail and Mohamed 2017), as well as in biotechnological and industrial processes as bioremediators, biofuels, and food sources (Wichard et al. 2015).

Fucus

The genus *Fucus* Linnaeus (Ochrophyta) is constituted of 9 taxonomically accepted species (Guiry and Guiry 2021). It is found along the rocky intertidal zone of cold temperate water in the northern hemisphere (Bergström et al. 2005, Kucera and Saunders 2008, Neiva et al. 2012), and they typically grow attached to hard substrata (Neiva et al. 2012). In addition, *Fucus* species provide

shelter and food for various organisms such as invertebrate and fish species (Johannesson et al. 2012, Rinne et al. 2018, Davis et al. 2021).

Two major lineages of *Fucus* species are recognized. *Lineage 1* is considered to be constituted only of *F. distichus* (Coyer et al. 2006, 2011a, Kucera and Saunders 2008) even though Neiva et al. (2012) include *F. serratus* and *F. gardneri* as part of the *F. distichus* species complex. *F. gardneri* used to be part of this species complex, but it is currently a synonym for *F. distichus* (Guiry and Guiry 2021), and hybridization has been reported between *F. serratus* and *F. distichus* (Coyer et al. 2006, Jueterbock et al. 2016). *Lineage 2* comprises *F. vesiculosus*, *F. spiralis, F. ceranoides, F. cottonii* (Neiva et al. 2012), *F. virsoides,* and *F. radicans* (Coyer et al. 2011a).

Fucus species have a thick parenchymatous body characterized by flattened and dichotomous branches with a midrib. They also have discoid holdfast, and mature individuals have swollen receptacles at the frond tips (Graham et al. 2009). *Fucus* species have a large amount of morphological and physiological variation (Bergström et al. 2005, Johannesson et al. 2012, Neiva et al. 2012), and even though the physiological traits seem more genetically determined, they have also been linked to environmental factors (Johannesson et al. 2012, Rinne et al. 2018).

F. distichus is a dominant species in the mid and high intertidal zones of rocky shores where it is exposed to a wide range of environmental conditions such as desiccation, light, currents, wave action, and grazing and presents great phenotypic variation (Davis et al. 2021). The adaptation of *F. distichus* to the North Atlantic and Pacific coast conditions resulted in several morphologically

distinct and polyphyletic subspecies (Coyer et al. 2006, 2011b, Kucera and Saunders 2008, Jueterbock et al. 2016).

F. spiralis is a recently introduced species in the northeast Pacific Ocean and is restricted to the area around the USA and the Canadian border even though it is found along the eastern Atlantic shore in the northern hemisphere (Kucera 2010, Coyer et al. 2011a). In the eastern Pacific, *F. spiralis* is relatively rare and grows attached to substrata in the upper intertidal zone (Kucera and Saunders 2008).

In the Pacific, both *Fucus* species coexist. *F. distichus* tends to grow with similar morphologies and in niches characteristic of the Atlantic *F. spiralis, F. vesiculosus*, and their hybrids (Kucera and Saunders 2008). In a study conducted by Kucera & Saunders (2008), the researchers found that several specimens determined to be *F. spiralis* in the field were identified by both the DNA barcode and ITS data as *F. distichus*; these specimens often had classic *F. spiralis*-like morphologies. Specimens identified in the Pacific as *F. distichus* were not misidentified as *F. spiralis*. Finally, they found that *F. distichus* antheridia are significantly longer than those of *F. spiralis*, suggesting that it could be used as a distinctive character for these Pacific species.

Hybridizations between the various species and occurrence of intermediate genotypes in nature have been studied through molecular techniques (Coyer et al. 2011a), which makes *Fucus* species identification more challenging. Researchers have demonstrated that the implementation of DNA barcode analysis is effective to distinguish among the variants of *Fucus* that are morphologically difficult to identify in the field (Kucera and Saunders 2008, Kucera 2010). Clarifying the

morphological characterization of *F. spiralis* and *F. distichus* still represents a challenge in the eastern Pacific. An integrated approach in the San Juan Islands will help to determine if the distinctive characters and distributions associated with *F. distichus* and *F. spiralis* in the intertidal zone are supported by the DNA barcode analysis.

The objectives of this study were to determine the number and identity of *Ulva* and *Fucus* species present at three locations around the San Juan Islands, WA based on both molecular and morphological characterizations, and to assess potential morphological characteristics that can be used to identify these species in this region. Spatial and environmental variability in the presence of these two genera were examined to determine how intertidal position could also influence the morphology of the thallus structure and how they play a role in identification down to the species level.

MATERIALS AND METHODS

Sample Collection

Ulva and *Fucus* specimens were collected from the intertidal zone at multiple sites within the San Juan Islands, WA (Figure 1; Table 1). At each of these sampling locations, collections were made within the low, mid, and high intertidal zones (Table S1, Table S2). Specimens were chosen at each location based on varying morphologies identified in Gabrielson & Lindstrom (2018). At each of our sample locations, two algal specimens of representative morphologies identified in each intertidal zone were collected. Specimens were only collected if attached and not as drift.

Sampling was conducted at low tide in June 2021. The biological duplicates were collected and stored in the same sample bag and transported on ice back to the lab where they were placed into a water table until processed.

Table 1. Collection site information.

Location Name	Site ID	Exposure Level	Long/Lat	Sample Day
Iceberg Point, Lopez Island	IP	High Exposure	48.42˚, -122.90˚	25-June-2021
Cattle Point, San Juan Island	CP	Mid Exposure	48.45˚, -122.96˚	27-June-2021
Friday Harbor Lab Beach	FHLB	Low Exposure	48.55˚, -123.01˚	29-June-2021

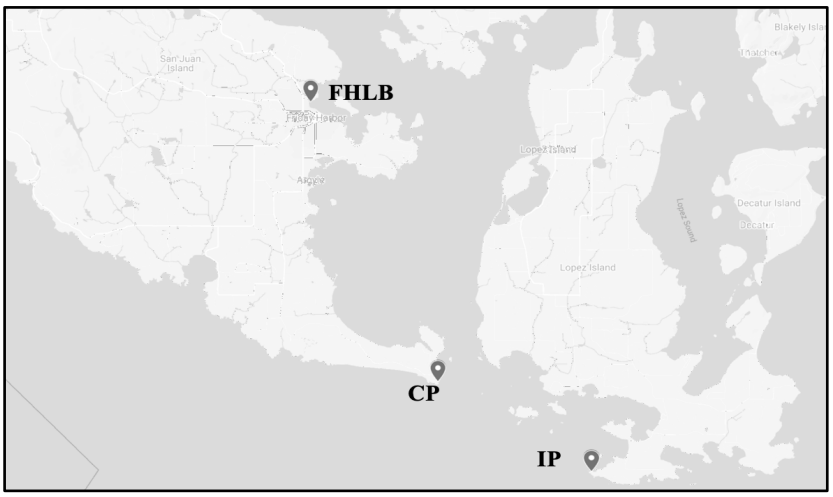

Figure 1. Map of sampling sites around the inner bay between San Juan Island and Lopez Island, Washington, US. Sampling locations include: Iceberg Point (IP): 48.42˚, -122.90˚; Cattle Point (CP): 48.45˚, -122.96˚; and Friday Harbor Lab Beach (FHLB): 48.55˚, -123.01˚.

Morphological Assessment

Each specimen was examined in the laboratory, for macroscopic and microscopic morphological characteristics (Tables S3 - TableS6). Information regarding the thallus type (blade or tube), thallus size, and branching pattern were recorded. Cellular features of each algal specimen were recorded based on fresh tissue samples. Cell arrangement, shape, size, chloroplast shape, location, and pyrenoid number were identified using an *Olympus BH2* compound microscope. Digital images of these cellular features were taken using a *Samsung Galaxy A52*. Tentative specimen identifications based on morphological characters were made using Gabrielson & Lindstrom (2018). A small part of the thallus (~ 0.5 cm^2) was removed for DNA extraction and the remainder was mounted on herbarium paper for a voucher. All voucher specimens were deposited in the University of Washington's Herbarium (WTU).

DNA Extraction, Amplification, and Sequencing

DNA was extracted from specimens using a Bioline Extract-PCR Kit (Bioline, Taunton, MA, USA) and protocol of Taylor et al. (Taylor et al. 2017) with small modifications. Approximately 0.5 cm^2 of healthy tissue was removed from the thallus, chopped into small pieces, and incubated at 75°C in 50 µL Extract-PCR kit enzymatic solution for 1-20 h. Tissue from *Fucus* specimens was taken from the proximal lamina, whereas tissue from *Ulva* specimens was taken from the distal blade margins. The cellular debris from *Ulva* and *Fucus* specimens were pelleted by centrifugation. The supernatant of each *Fucus* specimen was filtered using the OneStep PCR

Inhibitor Removal Kit (Zymo Research) following the manufacturer's protocol. After extraction, samples were diluted 1:10 and stored for PCR.

The COI-5P and *tuf*A gene loci were amplified for *Fucus* and *Ulva* specimens respectively. PCR was performed using primers GAZF2 (5' -CCA ACC AYA AAG ATA TWG GTA C -3') and GAZR2 (5' -GGA TGA CCA AAR AAC CAA AA -3') from Saunders (2005) for *Fucus* spp. and TUFGF4 (5' -GGI GCN GCN CAA ATG GAY GG -3') and TUFAR (5' -CCT TCI CGA ATM GCR AAW CGC -3') from Fama et al. (2002) for *Ulva* spp. Cycling conditions were as follows: an initial denaturing step of 95˚C for 2:45 min, followed by 35 cycles of 95˚C for 15 sec, 45˚C for 15 sec, and 72˚C for 1 min, with a final extension at 72˚C for 4 min. PCR products were enzymatically cleaned using Exo-Sap (Thermo Fisher Scientific, Waltham, MA, USA) and sent to Genewiz for sequencing.

Phylogenetic Analysis

40 *tuf*A sequences of representative *Ulva* specimens and 8 COI-5P sequences of representative *Fucus* specimens were analyzed. Additionally, 34 *Ulva* spp. and 17 *Fucus* spp. sequences were downloaded from GenBank and all sequences were aligned using the Sequencher 5.4 software package (GeneCodes Corp., Ann Arbor, MI, USA), then further corrected and trimmed by eye.

Sequences from this study were initially analyzed using the Unweighted pair group method with arithmetic mean (UPGMA) analysis for each genus to look at initial grouping patterns in our data. Maximum likelihood (ML) analysis was executed using MEGA 7.0.26 (Kumar et al. 2016). The

optimal molecular phylogenetic tree and associated bootstrap values were heuristically searched through this method.

RESULTS

Ulva

Based on molecular analyses of *tuf*A sequences, we grouped our *Ulva* specimens into four (Operational Taxonomic Units) OTUs: *U. "prolifera", U. fenestrata, U. expansa,* and *U. "californica"* (Figure 4a-d; Table 2a-d). Each of these taxonomic groups were supported with a 94 to 100 bootstrap value. Similar to the UPGMA analysis, *Ulva* specimens assembled into four basic groups (Figure 3). The grouping of *U. "prolifera"* assembled together with a bootstrap value of 88 (Figure 3). The holotype of both *U. fenestrata* (Accession #MK456404) and *U. expansa* (Accession #MH731007) were included in the ML analysis, supporting these specimen groupings. The holotype *U. fenestrata* assembled with other *U. fenestrata* sequences with a bootstrap value of 100 (Figure 3). Similarly, specimens grouped as *U. expansa* assembled with the holotype with a bootstrap value of 100 (Figure 3). A definite *U. lactuca* specimen was included (Accession #MANCO56-13) to differentiate between *U. fenestrata* and *U. lactuca* sequences due to recent changes in species identification. Specimens that were molecularly grouped as *U. "californica"* are grouped together with a bootstrap value of 86 (Figure 3).

Ulva species identified by morphological analysis differed in spatial distribution and tidal location. Specimens identified potentially as *U. californica, U. linza,* and *U. australis* were identified at all

three sites (Table S1). *U. australis, U. californica, U. lactuca,* and *U. linza* were the only species identified morphologically from the low intertidal zone.

Specimens found within each *Ulva* group shared general macroscopic and microscopic characteristics, varying slightly in blade morphology. A significant number of the *Ulva* specimens collected and identified as *U. linza* had the molecular identification of *U. "prolifera"* (Figure 4a; Table 2a). This potential molecular group is characterized generally by unbranched and lanceolate distromatic blades with smooth and ruffled margins that lead into a tubular, one cell thick base. Variation in thallus morphology included broad blades (17UA and 17UB) and narrowing/cuneate blades (20UA, 20UB, 24UA) (Figure 4a; Table 2a). Specimens that were moleculary grouped as *Ulva fenestrata* had similar morphological characteristics to the *U. "prolifera"* group and also varied in thallus structure (Figure 4b; Table 2b). Blade shape included broad (1UA, 12UA), cuneate (6UA), obovate (15A), and oblanceolate (15UB) (Figure 4b; Table 2b). Specimens grouped molecularly as *U. fenestrata* included numerous morphological identifications of *U. californica, U. lactuca,* and *U. australis.* The molecular grouping of *U. expansa* included various potential species from morphological identification (Figure 4c; Table 2c). Specimens included in this group were characterized by branched and unbranched thalli with either lobed or ovate blades. Finally, specimens grouped molecularly as *U. "californica"* were characterized by similar morphologies to other groups (Figure 4d; Table 2d).

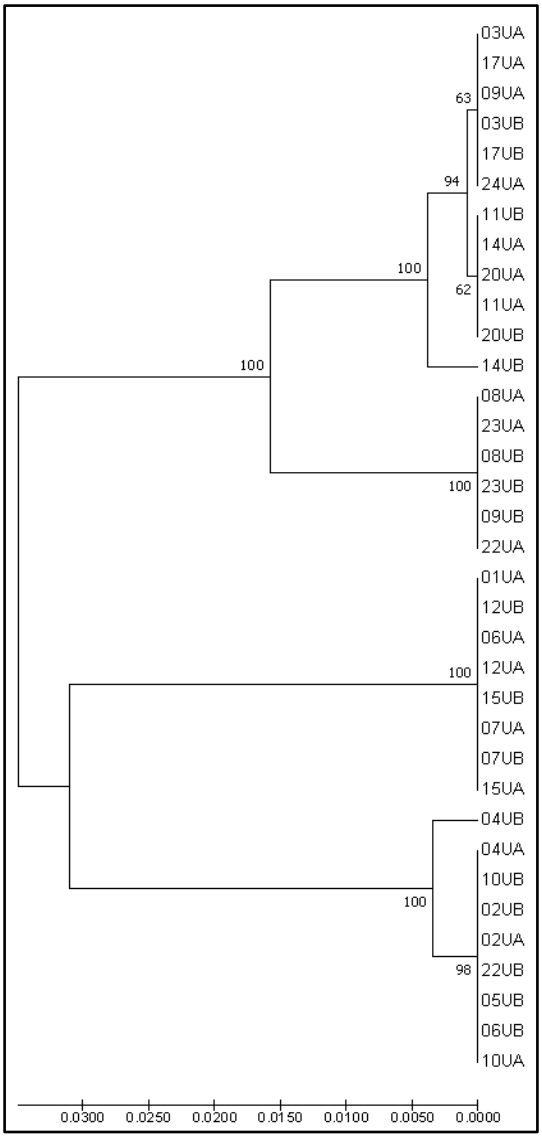

Figure 2. UPGMA phylogram of *tuf*A sequences showing evolutionary relationships of specimens of *U. "prolifera"* (03UA-14UB), *U. "californica"* (08UA-22UA), *U. fenestrata* (01UA-15UA), and *U. expansa* (04UB-10UA). Bootstrap supports based on 500 replicates are cited at the nodes. Evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

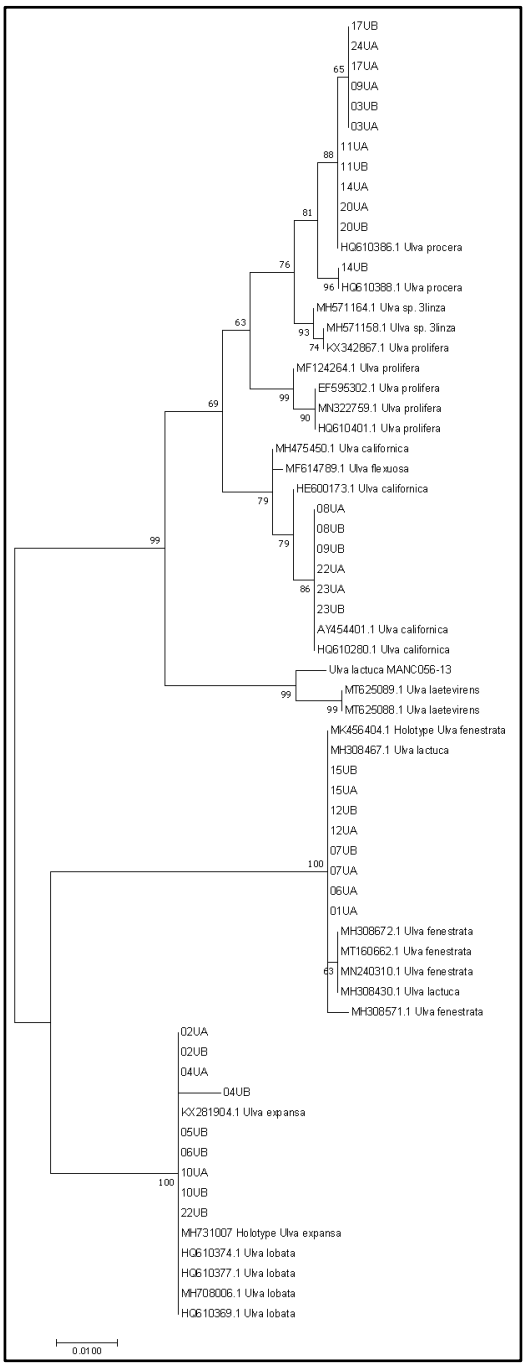

Figure 3. Maximum Likelihood phylogram of *tuf*A sequences showing evolutionary relationships of specimens of *U. "prolifera"*, *U. "californica", U. fenestrata,* and *U. expansa*. Bootstrap supports based on 500 replicates are cited at the nodes. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model.

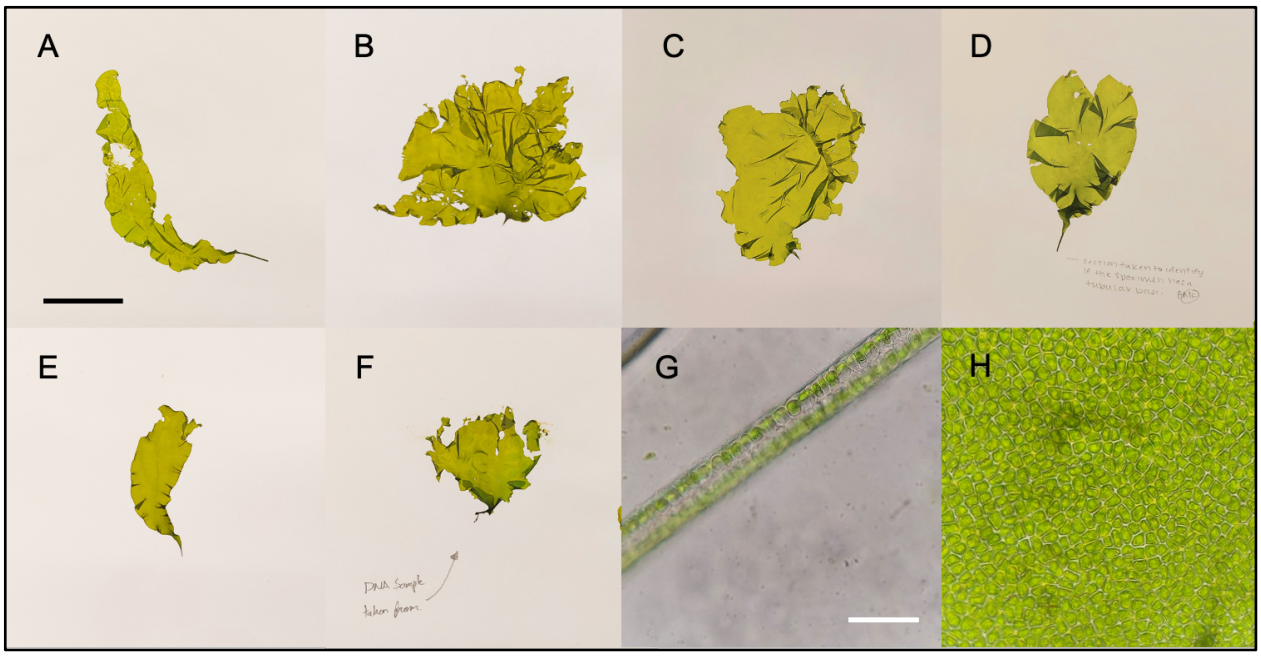

Figure 4a. *Ulva "prolifera"* morphological characteristics: (A) FHL-03UA unbranched and lanceolate; (B) FHL-17UA smooth, flat, and broad distally; (C) FHL-17UB smooth, flat, and broad distally; (D) FHL-20UA cuneate blade with a tubular base; (E) FHL-20UB narrow cuneate blade ruffled throughout; (F) FHL-24UA cuneate blade that twists towards tubular base; (G) FHL-03UA cross sectional view of distromatic blade; (H) FHL-03UB surface view. Horizontal black bar equals 5cm (A-F); white bars equal 2.5µm (G, H).

Table 2a. Characteristics of *Ulva "prolifera"* that have been previously identified as defining characteristics found in the Pacific Northwest according to Gabrielson and Lindstrom (2018).

Specimen	Morphological Description	Morphological Identification	Gabrielson and Lindstrom Morphological Description	Molecular Identification

03UA	Unbranched, lanceolate, ruffled margins, smooth with tubular base; irregular cell arrangement; polygonal cells with rounded corners; mostly 1 pyrenoid; chloroplast cup-shaped and peripheral	*U. linza*	(1) Tubular throughout; (2) Cells in longitudinal rows, plastid filling surface view; (4) Plant broader with 1-5 pyrenoids; (5) Branched (abundant or not) or unbranched, tubular or distally compressed. Cells with 1 (2) pyrenoids, basally rectangular and distally quadrate, longitudinal rows	*Ulva "prolifera"*
03UB		*U. linza*		
09UA		*U. linza*		
11UA		*U. linza*		
11UB		*U. linza*		
14UA		*U. linza*		
14UB		*U. linza*		
17UA		*U. linza*		
17UB		*U. linza*		
20UA		*U. linza*		
20UB		*U. linza*		
24UA		*U. linza*		

			inconspicuous in older growth.	

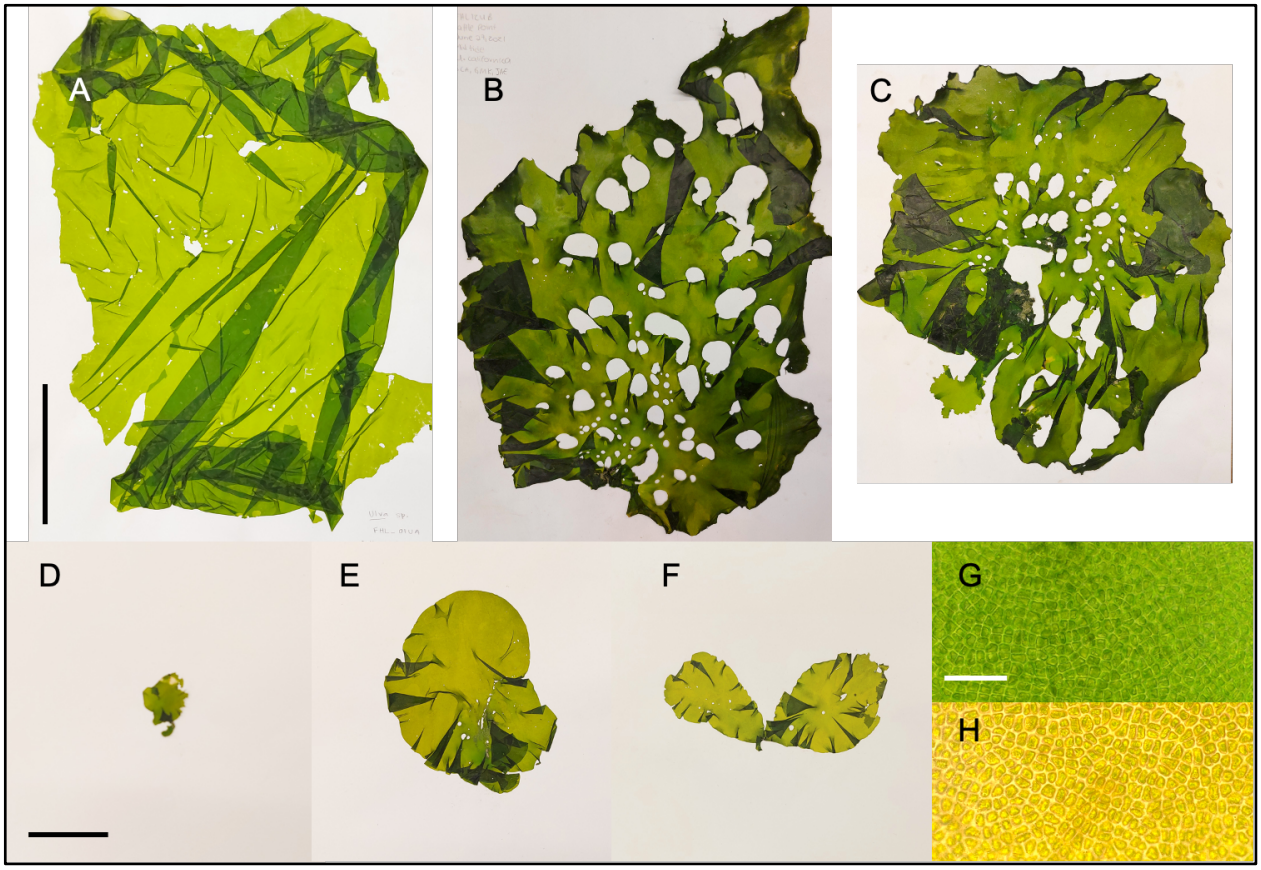

Figure 4b. *Ulva fenestrata* morphological characteristics: (A) FHL-01UA unbranched broad blade with smooth margin; (B) FHL-12UA large, thick blade with many perforations and smooth, ruffled margin; (C) FHL-12UB large, thick blade with many perforations and smooth, ruffled margin; (D) FHL-06UA unbranched cuneate blade with smooth margin; (E) FHL-15UA smooth, obovate, minimally ruffled blade; (F) FHL-15UB smooth, oblanceolate, minimally ruffled blade; (G) FHL-

06UA surface view; (H) FHL-15UB surface view. Vertical black bar equals 10cm (A-C), horizontal black bar equals 5cm (D-F); white bar equals 2.5µm (G, H).

Table 2b. Characteristics of *Ulva fenestrata* that have been previously identified as defining characteristics of the two species found in the Pacific Northwest according to Gabrielson and Lindstrom (2018).

<table>
<tr><th>Specimen</th><th>Morphological Description</th><th>Morphological Identification</th><th>Morphological Description</th><th>Molecular Identification</th></tr>
<tr><td>01UA</td><td rowspan="8">Unbranched, smooth broad blade, no marginal teeth; irregular cell arrangement; rectangular cells with curved edges; chloroplast cup-shaped and peripheral; 1 pyrenoid</td><td>U. californica</td><td rowspan="8">No description provided in Abbott & Hollenberg (1976) or Gabrielson & Lindstrom (2018)</td><td rowspan="8">Ulva fenestrata</td></tr>
<tr><td>06UA</td><td>U. lactuca</td></tr>
<tr><td>07UA</td><td>U. australis</td></tr>
<tr><td>07UB</td><td>U. australis</td></tr>
<tr><td>12UA</td><td>U. lactuca</td></tr>
<tr><td>12UB</td><td>U. californica</td></tr>
<tr><td>15UA</td><td>U. lactuca</td></tr>
<tr><td>15UB</td><td>U. californica</td></tr>
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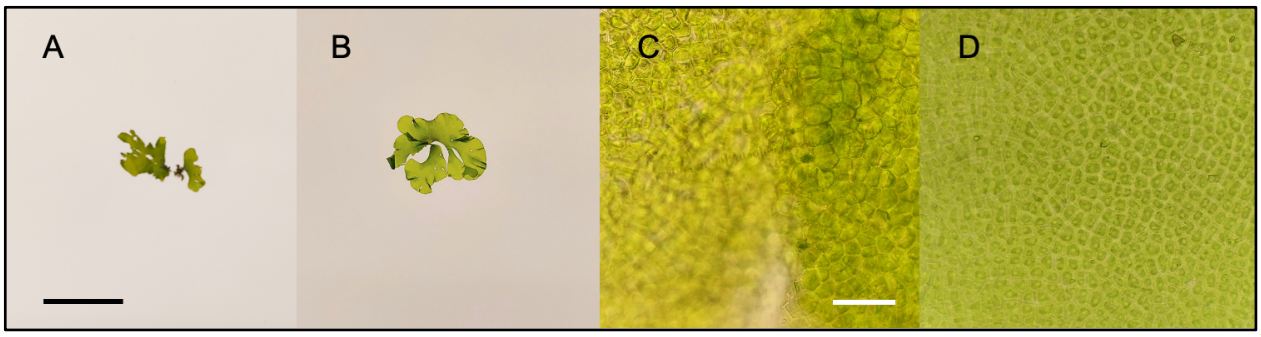

Figure 4c. *Ulva expansa* morphological characteristics: (A) FHL-02UA unbranched, inconspicuously lobed blade with minimally ruffled margin; (B) FHL-10UA unbranched, ovate blade with smooth margin; (C) FHL-02UA surface view; (D) FHL-10UA surface view. Horizontal black bar equals 5cm (A, B); white bar equals 2.5µm (C, D).

Table 2c. Characteristics of *Ulva expansa* that have been previously identified as defining characteristics of the two species found in the Pacific Northwest according to Abbott and Hollenberg (1976).

Specimen	Morphological Description	Morphological Identification	Abbott Morphological Description	Molecular Identification
02UA	Branching at base of thallus, smooth minimally ruffled; polygonal cells	*U. lactuca*	(1) Blades mostly over 6 cm tall at maturity; usually not in dense groups	*Ulva expansa*
02UB		*U. lactuca*		
06UB		*U. lactuca*		
10UA		*U. lactuca*		

	with curved edges; chloroplast cup-shaped and peripheral; 1-2 pyrenoids		(2) Cells in middle of blade anticlinally elongated (3) Blades not digitate; if divided, with portions narrowed toward base (4) Blades orbicular or less than 3 times as long as broad (8) Blades neither stiff nor deeply divided, often much ruffled (9) Blades entire, orbicular or irregularly	
05UB		*U. rigida* complex		
04UA	Unbranched, lobed blades; irregular cell arrangement, polygonal cells with curved edges; peripheral chloroplast; 1 pyrenoid	*U. lobata*		
04UB		*U. lobata*		
10UB	Unbranched, smooth, ruffled margins;	*U. australis*		

	irregular cell arrangement, polygonal cells with curved edges; peripheral chloroplast; 1 pyrenoid		expanded, commonly free-floating in quiet water	
22UB	Unbranched, smooth, thin blade, broad distally and narrow proximally; irregular cell arrangement, polygonal cells with curved edges; peripheral chloroplast; 1 pyrenoid	*U. californica*		

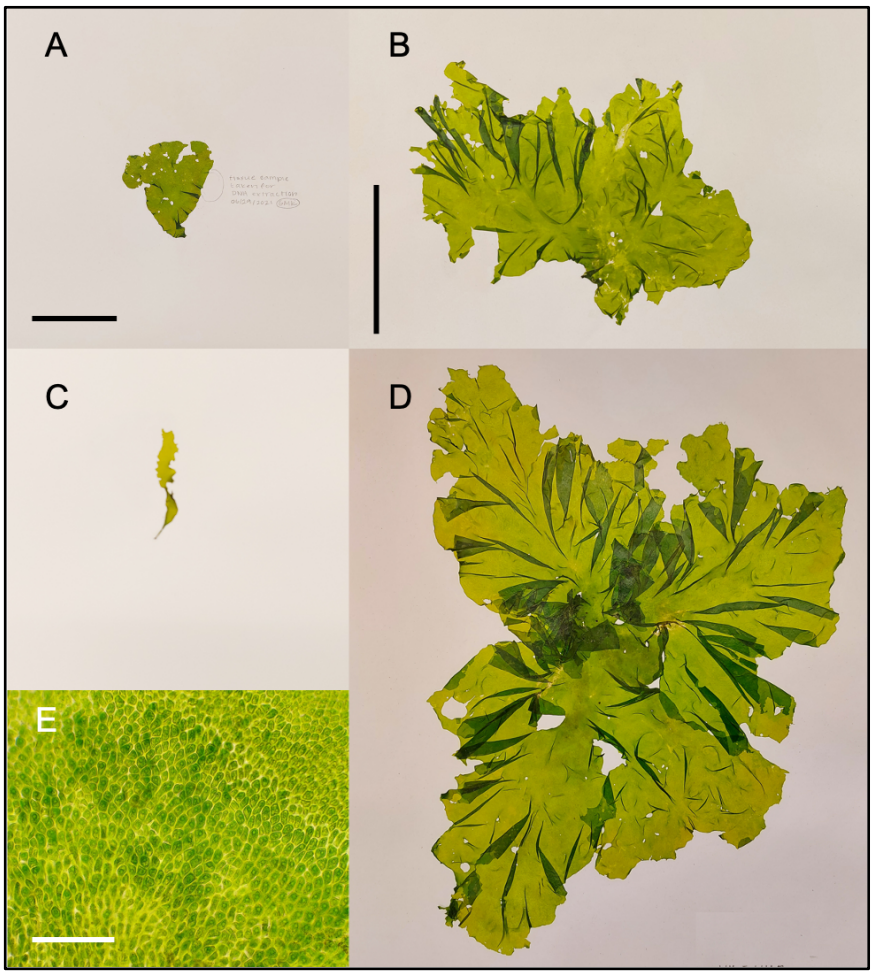

Figure 4d. *Ulva "californica"* morphological characteristics: (A) FHL-08UA unbranched, cuneate blade with smooth margin; (B) FHL-23UB unbranched, ovate blade with small discoid holdfast; (C) FHL-09UB unbranched lanceolate blade with smooth, ruffled margin and discoid holdfast; (D) FHL-22UA large blade thicker in the center than at the ruffled margin; (E) FHL-09UB surface view. Vertical black bar equals 10cm (B, D), horizontal black bar equals 5cm (A, C); white bar equals 2.5µm.

Table 2d. Characteristics of *Ulva "californica"* that have been previously identified as defining characteristics found in the Pacific Northwest according to Gabrielson and Lindstrom (2018).

Specimen	Morphological Description	Morphological Identification	Gabrielson and Lindstrom Morphological Description	Molecular Identification
08UA	Unbranched, ruffled, smooth margins; irregular cell arrangement, polygonal cells with curved edges; cup-shaped chloroplast; 1 pyrenoid	*U. californica*	(1) Bladelike, not tubular; (7) Plant flat throughout; (8) Margin not serrate; (9) Cunate, oblanceolate, lanceolate, linear, or lobed, usually with few perforations; (11) Not lobed or divided; (12) Cunate to oblanceolate,	*Ulva "californica"*
08UB		*U. californica*		
09UB	Unbranched, smooth, lanceolate blade, no marginal teeth; irregular	*U. linza*		

	cell arrangement, polygonal cells with curved edges; cup-shaped chloroplast; 1 pyrenoid		usually wider towards the apex of uniform thickness. Cells angular with rounded corners in surface view, usually with 1 pyrenoid. Occurring in mid to high intertidal, sometimes endophytic, common.	
22UA	Large unbranched thallus, thicker near center, minimally ruffled; irregular cell arrangement, polygonal cells with curved edges; cup-shaped chloroplast; 1 pyrenoid	*U. lobata*		
23UA	Broad distally,	*U. australis*		

23UB	narrow proximally, ruffled throughout; discoid holdfast; irregular cell arrangement, polygonal cells with curved edges; peripheral chloroplast; 1 to multiple pyrenoids	*U. australis*		

Fucus

Based on COI-5P sequences, we sorted our *Fucus* specimens into two OTUs. These two taxonomic groups were supported by a bootstrap value of 100. (Figure 5). Type specimens of *F. distichus* and *F. spiralis* were not identified for this phylogram. Distinct groupings of *Fucus* specimens were supported with bootstrap values of 87 and 100, respectively (Figure 6).

Both *Fucus* species were identified at mid and low exposure sites, but only *F. distichus* was identified morphologically at the high exposure site (Table S1). These species were identified at mid and high intertidal zones; *F. distichus* was the only one collected from the low intertidal zone. These specimens all were characterized by many pyrenoids per cell, dichotomous branching, and irregular cell distribution (Appendix C). Despite these similarities, specimens varied in their morphological and cellular characteristics. The presence of caecostomata, pores, cryptostomata, and receptacle morphology vary between specimens (Table 3; Appendix C).

According to Gabrielson and Lindstom (2018), the only two species that are found in the San Juan Islands are *F. distichus* and *F. spiralis. F. distichus* was identified morphologically possessing no cryptostomata, a non-conspicuous midrib, and typically with no caecostomata (Figure 7, Table 3). *F. spiralis* was morphologically identified by a conspicuous midrib and typically possessing cryptostomata with the presence of hairs (Table 3).

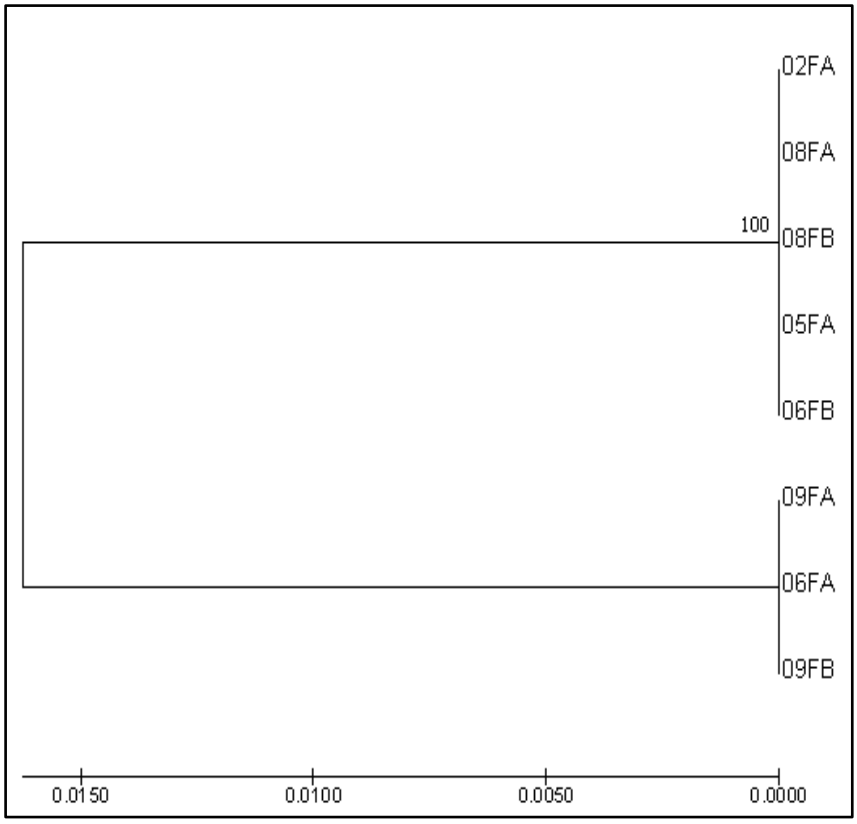

Figure 5. UPGMA phylogram of COI-5P sequences showing evolutionary relationships of specimens of *F. distichus* and *F. spiralis*. Bootstrap supports based on 500 replicates are cited at the nodes. Evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

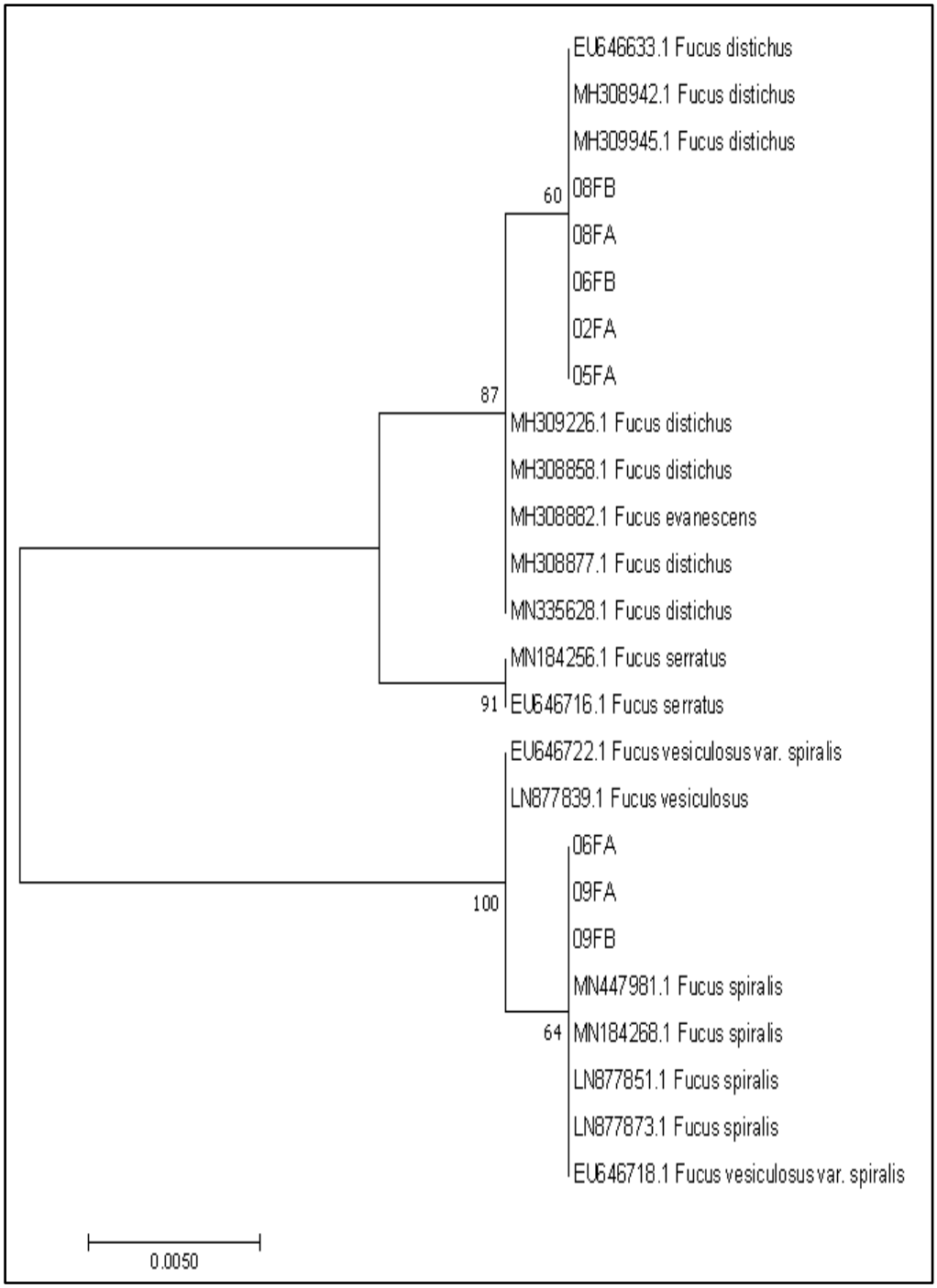

Figure 6. Maximum Likelihood phylogram of COI-5P sequences showing evolutionary relationships of specimens of *F. distichus* and *F. spiralis.* Bootstrap supports based on 1000 replicates are cited at the nodes. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model.

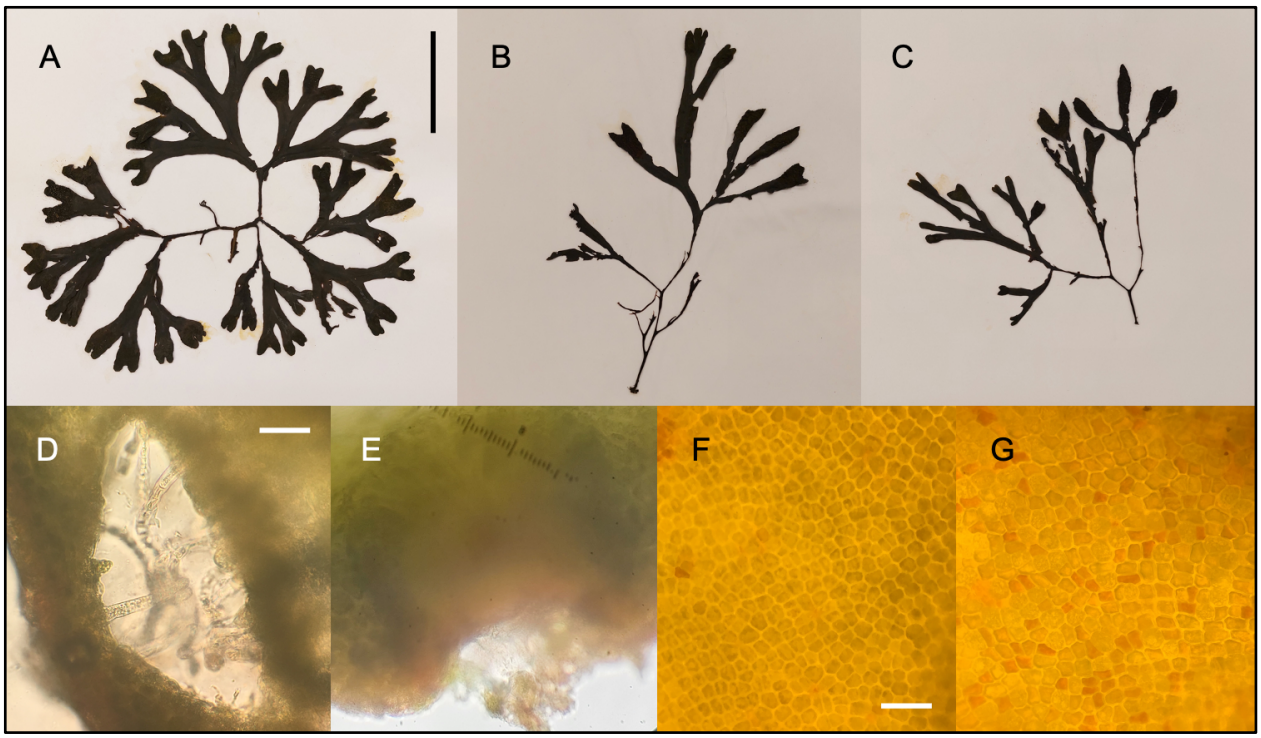

Figure 7. *Fucus distichus* (A, D, F) and *Fucus spiralis* (B, C, E, G) morphological characteristics: (A) FHL-06FB dichotomous branching pattern with flattened receptacles, and a conspicuous midrib which erodes in older growth; (B) FHL-09FA dichotomous branching pattern with flattened receptacles and a lamina that feels smooth but has prominent bumps throughout, and erodes in older growth; (C) FHL-09FB dichotomous branching pattern with flattened receptacles and a lamina that feels smooth, eroding in older growth; (D) FHL-06FB cryptostomata; (E) FHL-09FA cryptostomata; (F) FHL-06FB surface view; (G) FHL-09FB surface view. Vertical black bar equals 10cm (A-C), horizontal white bar equals 2.5µm (D-G).

Table 3. Characteristics of *Fucus* spp. that have been previously identified as defining characteristics of the two species found in the Pacific Northwest according to Gabrielson and Lindstrom (2018).

Specimen	Cryptostomata	Hairs	Caecostomata	Conspicuous Midrib	Wings on Receptacles	Tidal Zone	Morphological Identification	Molecular Identification
02FA	-	-	-	-	-	High	*F. distichus*	*F. distichus*
05FA	No	No	Yes	No	No	High	*F. distichus*	*F. distichus*
06FA	No	No	Yes	Yes	No	High	*F. distichus*	*F. spiralis*
06FB	Yes	Yes	No	Yes	No	High	*F. spiralis*	*F. distichus*
08FA	No	No	No	No	No	Mid	*F. distichus*	*F. distichus*
08FB	No	No	No	Yes	No	Mid	*F. distichus*	*F. distichus*
09FA	Yes	Yes	Yes	Yes	Yes	High	*F. spiralis*	*F. spiralis*
09FB	No	No	No	Yes	No	High	*F. spiralis*	*F. spiralis*

DISCUSSION

Ulva

Ulva specimens of the San Juan Islands are not distinct based on their placement in the intertidal zone or sample site. Of the four distinct OTUs found, *U. fenestrata* and *U. expansa* are not described in Gabrielson and Lindstrom (2018). However, *U. expansa* is also not previously described in the northeast Pacific by Abbot (1976). Previously, sequences identified as "*U. lobata"* grouped within the same clade as the holotype of *U. expansa* (Hughey et al. 2019), similar to our findings (Figure 3). Recently, *U. lactuca* was identified in the northeast Pacific (Hayden and Waaland 2004), contrary to previous findings (Tanner 1986). Phylogenetic analyses revealed that *U. fenestrata* identified within this region included *U. lactuca* and may be conspecific with *U. fenestrata* (Hayden and Waaland 2004). In the current study, *U. lactuca* specimens from GenBank group within the same clade as *U. fenestrata* holotype suggests that *U. lactuca* were misidentified (Figure 3.)

Genetic analysis to better understand species diversity is critical, especially for coastal species with high phenotypic plasticity as within the genus *Ulva* (Blomster et al. 2002, Wolf et al. 2012, J. R. Hughey et al. 2021). Initially, members of this genus were identified and described based on their seemingly simplistic morphology; these characteristics may be variable in response to environmental stressors (Hayden and Waaland 2004). *Ulva* spp. collected from the San Juan Islands were identified potentially as six species using morphological characteristics only; however, they were separated molecularly into four distinct groups (Figure 2; Figure 3).

Of the four OTUs seen in the *Ulva* barcode tree (Figure 2), macroscopic characteristics including thallus shape varied across and within each group, reflective of phenotypic plasticity of this genus (Table 2a-d). Distinct differences in blade morphology were not identified in relation to sampling location or intertidal zone (Table S1; Table S3). Changes in *Ulva* morphology can be induced by salinity (Russell et al. 1997, Gao et al. 2016), bacterial communities (Matsuo et al. 2003, Spoerner et al. 2012, Wichard et al. 2015), ambient temperature (Gao et al. 2016), and eutrophication (Valiela et al. 1977). Moreover, closely related species can appear morphologically identical (Hayden and Waaland 2004), making identification based solely on morphological characteristics impractical.

When compared to other *Ulva* barcodes in Genbank, it was determined that the four OTUs consistently grouped with four different species, with no interspecific variation (Figure 3). Holotype specimen sequences were found for both *U. expansa*, and *U. fenestrata*, linking those OTUs to those species. Though no holo- or haplotype sequence for *U. californica* was found, Accessions from Genbank for this species consistently grouped with the same OTU with a boostrap value greater than 80. Specimens labelled as *U. linza*, *U. procera*, and *U. prolifera* all grouped with the fourth OTU. This suggests that the specimens from this OTU were part of this LPP complex described by Cui et al (2018). Though there was no *tuf*A sequence that could link Cui et al 2018 specimens to our own, the *tuf*A sequence taken from the complete genome of an *U. prolifera* in Genbank did (Accession number KX342867.1). Since this specimen was retrieved from the Yellow Sea off the coast of Qingdao, China, where other *U. prolifera* specimens were found to have matching sequences to the *U. prolifera* holotype (Personal communication, Dr. Wilson Freshwater); this OTU was given the tentative label of *U. "prolifera"*.

Analysis of *Ulva* spp. using morphological descriptions within the Gabrielson and Lindstrom (2018) key differed from their molecular characterizations due to their phenotypic plasticity (Table 2a and 2d). Dichotomous choices are sometimes subjective, for example, referring to thallus margins as either minimally or somewhat ruffled. Some dichotomies contradicted the molecular results at other points in the identification of species. All morphologies molecularly grouped with *U. prolifera* had a blade-like thallus with a tubular base although *U. prolifera* and the now synonymized *U. procera* (Cui et al. 2018) are described as tubular throughout in Gabrielson and Lindstrom (2018). The blade-like thallus with the tubular base morphology only led to *U. linza* within Gabrielson & Lindstrom (2018) key.

The morphological and molecular analysis differed due to the sequence dichotomies and lack of proper characterization within Gabrielson and Lindstrom (2018), as well as incomplete taxonomic representation of *Ulva* spp. Even though *U. fenestrata* and *U. expansa* are not included in the key, they made up nearly half of the molecularly analyzed specimens. Based on these results and similar published findings (Guidone et al. 2013, Ismail and Mohamed 2017, Hughey et al. 2019, J. R. Hughey et al. 2021), it is likely that molecular analysis is currently the most reliable technique for correct identification and application of species names, specifically in the genus *Ulva*. It should also be noted that molecular identification can still be misleading. Missidentification of lectotype and holotype material caused by interspecific morphological overlap has led to a complex amalgamation of correctly and incorrectly identified sequences within public databases such as Genbank (Hughey et al. 2019). When using these sequences, it is thus up to the user to fact check their accuracy.

Fucus

In this study, we identified the coexistence of *F. spiralis* and *F. disticus* in the intertidal zone of the San Juan Islands based on their morphological identification (Gabrielson and Lindstrom 2018). However, the distinctive characteristics of each species were not consistent among the specimens (Table 3). According to Gabrielson and Lindstrom (2018) the absence of caecostomata, a prominent midrib, and the presence of conspicuous cryptostomata are main characteristics that distinguished *F. spiralis*; however, cryptostomata with hairs was seen in two of the specimens (06FB and 09FA) and only one of them consistently grouped molecularly with *F. 'spiralis'* (09FA). On the other hand, an individual without it (09FB) was molecularly associated with *F. 'spiralis'* (Table 3). In addition, based on morphological analysis, the midrib is not a distinct characteristic for morphological identification supported with the molecular information provided by the DNA barcode analysis (Figure 6). Therefore, despite these similarities, specimens varied in their morphological and cellular characteristics. The presence of caecostomata, pores, cryptostomata, and receptacle morphology vary between specimens (Table 3; Appendix C).

In the northeast Pacific, *F. spiralis* is found in the upper intertidal, higher than where *F. distichus* is found, which is in the mid and upper intertidal zone (Kucera and Saunders 2008, Gabrielson and Lindstrom 2018). This is consistent with the zonation of our specimens (Table 3). However, if both can be found in the upper intertidal zone, the location of the species is not a distinctive characteristic for species identification. In addition, Kucera and Saunders (2008) explained that there is a zone between the high and mid intertidal zones, in which *Fucus* specimens exhibit morphologies of both species (Figure 7).

Even though both especies present phenotypic plasticity and the inconsistencies in morphological characteristics they exhibit, which make their identification difficult, there is no evidence of hybridization between *F. spiralis* and *F. distichus* that could explain the intermediate morphology of the distinctive characters (Kucera and Saunders 2008, Gabrielson and Lindstrom 2018). Therefore, the use of DNA barcode analysis reveals information about their relationship with other species and provides molecular support for species delimitation (Figure 6). Kucera and Saunders (2008) demonstrated for the first time the effectiveness of DNA barcode analysis to distinguish brown algae species, and it is the only study that analyzes *F. distichus* and *F. spiralis* morphologically and molecularly.

These results expand upon the morphological complexity of *Ulva* and *Fucus* and elaborate on the importance of molecular-based analyses for species identification of these genera. Molecular analysis of *Ulva* specimens was limited by the lack of *tuf*A sequence data available. Previous studies have focused on *rbc*L, ITS, or both sequences for molecular identification. Due to this discrepancy, comparisons with type sequences were not always available. For more robust morphological and molecular analysis, larger sample sizes from each collection site should be considered; the results of this study were limited due to small sample sizes. Brown algal DNA extraction is historically difficult due to polysaccharide and phenolic compounds in the cell wall (Pandey et al. 1996; Koonjul et al. 1999). Without specific optimization of DNA extraction for *Fucus*, sample size was limited. Multiple transects for *Fucus* and *Ulva* collections with random selection of specimens from each intertidal zone would provide a more complete census of algal species and a better characterization of collection sites.

Our results highlight the importance of using an integrated approach for species identification, especially with genera that present high phenotypic plasticity such as *Ulva* and *Fucus*. A taxonomical and morphological revision supported by up-to-date molecular analyses should be considered in the Gabrielson and Lindstrom key (2018). Future studies should target multiple loci for both genera in order to better characterize species and compare results with other studies or type specimens.

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SUPPLEMENTAL TABLES AND FIGURES:

Supplemental Table S1. List of *Ulva* taxa that DNA sequences were generated for this study and collection information. Iceberg Point (IP) (48.42˚,-122.89˚); Cattle Point (CP) (48.45˚, -122.96˚); Friday Harbor Lab Beach (FHLB) (48.55˚, -123.01).

FHL Number	Species	Region	Geographic Location	Determined by	Date Collected	Tidal Location
FHL_01UA	*U. californica*	SJI	Friday Harbor Lab Beach	GMK	24-June-2021	Mid
FHL_02UA	*U. lactuca*	LI	Iceberg Point	BCA	25-June-2021	Low
FHL_02UB	*U. lactuca*	LI	Iceberg Point	GMK	25-June-2021	Low
FHL_03UA	*U. linza*	LI	Iceberg Point	BCA	25-June-2021	Low
FHL_03UB	*U. linza*	LI	Iceberg Point	JAE	25-June-2021	Low
FHL_04UA	*U. lobata*	LI	Iceberg Point	BCA	25-June-2021	Mid

FHL_04UB	*U. lobata*	LI	Iceberg Point	GMK	25-June-2021	Mid
FHL_05UA	*U. rigida complex*	LI	Iceberg Point	BCA	25-June-2021	Mid
FHL_05UB	*U. rigida complex*	LI	Iceberg Point	GMK	25-June-2021	Mid
FHL_06UA	*U. lactuca*	LI	Iceberg Point	JAE	25-June-2021	High
FHL_06UB	*U. lactuca*	LI	Iceberg Point	GMK	25-June-2021	High
FHL_07UA	*U. australis*	LI	Iceberg Point	BCA	25-June-2021	Mid
FHL_07UB	*U. australis*	LI	Iceberg Point	JAE	25-June-2021	Mid
FHL_08UA	*U. californica*	LI	Iceberg Point	GMK	25-June-2021	High
FHL_08UB	*U. californica*	LI	Iceberg Point	BCA	25-June-2021	High

FHL_09UA	*U. linza*	LI	Iceberg Point	JAE	25-June-2021	High
FHL_09UB	*U. linza*	LI	Iceberg Point	GMK	25-June-2021	High
FHL_10UA	*U. lactuca*	LI	Iceberg Point	JAE	25-June-2021	High
FHL_10UB	*U. australis*	LI	Iceberg Point	BCA	25-June-2021	High
FHL_11UA	*U. linza*	SJI	Cattle Point	JAE	27-June-2021	Low
FHL_11UB	*U. linza*	SJI	Cattle Point	GMK	27-June-2021	Low
FHL_12UA	*U. lactuca*	SJI	Cattle Point	GMK	27-June-2021	Mid
FHL_12UB	*U. californica*	SJI	Cattle Point	BCA	27-June-2021	Mid
FHL_13UA	*U. australis*	SJI	Cattle Point	JAE	27-June-2021	Mid

FHL_13UB	*U. linza*	SJI	Cattle Point	BCA	27-June-2021	Mid
FHL_14UA	*U. linza*	SJI	Cattle Point	GMK	27-June-2021	Mid
FHL_14UB	*U. linza*	SJI	Cattle Point	JAE	27-June-2021	Mid
FHL_15UA	*U. lactuca*	SJI	Cattle Point	JAE	27-June-2021	High
FHL_15UB	*U. californica*	SJI	Cattle Point	GMK	27-June-2021	High
FHL_16UA	*U. linza*	SJI	Cattle Point	BCA	27-June-2021	High
FHL_16UB	*U. linza*	SJI	Cattle Point	JAE	27-June-2021	High
FHL_17UA	*U. linza*	SJI	Cattle Point	BCA	27-June-2021	High
FHL_17UB	*U. linza*	SJI	Cattle Point	GMK	27-June-2021	High

FHL_18UA	*U. lactuca*	SJI	FHL Beach	JAE	29-June-2021	Low
FHL_18UB	*U. lactuca*	SJI	FHL Beach	GMK	29-June-2021	Low
FHL_19UA	*U. lactuca*	SJI	FHL Beach	BCA	29-June-2021	Low
FHL_19UB	*U. lactuca*	SJI	FHL Beach	JAE	29-June-2021	Low
FHL_20UA	*U. linza*	SJI	FHL Beach	GMK	29-June-2021	Low
FHL_20UB	*U. linza*	SJI	FHL Beach	BCA	29-June-2021	Low
FHL_21UA	*U. linza*	SJI	FHL Beach	JAE	29-June-2021	Mid
FHL_21UB	*Ulva sp.*	SJI	FHL Beach	BCA	29-June-2021	Mid
FHL_22UA	*U. lobata*	SJI	FHL Beach	JAE	29-June-2021	Mid

FHL_22UB	*U. californica*	SJI	FHL Beach	GMK	29-June-2021	Mid
FHL_23UA	*U. australis*	SJI	FHL Beach	BCA	29-June-2021	High
FHL_23UB	*U. australis*	SJI	FHL Beach	GMK	29-June-2021	High
FHL_24UA	*U. linza*	SJI	FHL Beach	JAE	29-June-2021	High
FHL_24UB	*U. linza*	SJI	FHL Beach	GMK	29-June-2021	High

Supplemental Table S2. List of *Fucus* taxa that DNA sequences were generated for this study and collection information. Iceberg Point (IP) (48.42˚,-122.89˚); Cattle Point (CP) (48.45˚, -122.96˚); Friday Harbor Lab Beach (FHLB) (48.55˚, -123.01).

FHL Number	Species	Region	Geographic Location	Identification	Date Collected	Tidal Location
FHL_01FA	*F. distichus*	LI	Iceberg Point	JAE	25-June-2021	Mid
FHL_01FB	*F. distichus*	LI	Iceberg Point	GMK	25-June-2021	Mid

FHL_02FA	*F. distichus*	LI	Iceberg Point	GMK	25-June-2021	High
FHL_02FB	*F. distichus*	LI	Iceberg Point	JAE	25-June-2021	High
FHL_03FA	*F. spiralis*	SJI	Cattle Point	BCA	27-June-2021	Mid
FHL_03FB	*F. distichus*	SJI	Cattle Point	GMK	27-June-2021	Mid
FHL_04FA	*F. spiralis*	SJI	Cattle Point	GMK	27-June-2021	Mid
FHL_04FB	*F. distichus*	SJI	Cattle Point	BCA	27-June-2021	Mid
FHL_05FA	*F. distichus*	SJI	Cattle Point	JAE	27-June-2021	High
FHL_05FB	*F. distichus*	SJI	Cattle Point	BCA	27-June-2021	High
FHL_06FA	*F. distichus*	SJI	Cattle Point	GMK	27-June-2021	High
FHL_06FB	*F. spiralis*	SJI	Cattle Point	JAE	27-June-2021	High

FHL_07FA	*F. spiralis*	SJI	FHL Beach	BCA	29-June-2021	Low
FHL_07FB	*F. spiralis*	SJI	FHL Beach	GMK	29-June-2021	Low
FHL_08FA	*F. distichus*	SJI	FHL Beach	JAE	29-June-2021	Mid
FHL_08FB	*F. distichus*	SJI	FHL Beach	GMK	29-June-2021	Mid
FHL_09FA	*F. spiralis*	SJI	FHL Beach	BCA	29-June-2021	High
FHL_09FB	*F. spiralis*	SJI	FHL Beach	JAE	29-June-2021	High

Table S3 (appendix A). Macroscopic morphological features of *Ulva* specimens analyzed.

OTU	Species or species complex	Overall blade morphology	Branching pattern/ Average # branches (cm)	Average holdfast diameter (mm)	Thallus texture and marginal edge appearance	Longest length, longest width (cm)
F	*U. californica*	Broad	Unbranched	Not present	Smooth, no marginal teeth	N/A
A	*U. lactuca*	Slightly lobed; minimally	Branching at thallus base	0.5	Smooth, minimally	4.1, 1.9

		ruffled			ruffled	
A	*U. lactuca*	Minimally lobed;	Branching at thallus base	0.4	Smooth, minimally ruffled	3.26, 2.1
B	*U. linza*	Lanceolate	Unbranched	Not present	Smooth; ruffled	10.3, 3.2
B	*U. linza*	Lanceolate	Unbranched	Not present	Smooth; ruffled	6.7, 3
C	*U. lobata*	Highly lobed	Unbranched	1.1	Smooth, thicker thallus center	24.5, 6.8
C	*U. lobata*	Lobed	Unbranched	Not present	Smooth, thicker thallus center	17, 23
D	*U. rigida* complex	Primarily branched	Branching at thallus base	0.2	Smooth; toothed microscopic margin	4.76, 8.83
D	*U. rigida* complex	Primarily branched	Branching at thallus base	0.3	Smooth toothed microscopic	13.5, 6.5

					margin	
A	*U. lactuca*	Ceneate	Unbranched	N/A	Smooth	4.5, 3
A	*U. lactuca*	Ovate	Unbranced	Not present	Smooth	1.8, 1.6
E	*U. australis*	Ruffled margins	Unbranched	0.2	Smooth without a toothed margin	5, 4.7
E	*U. australis*	Ruffled margins	Unbranched	Not present	Smooth; ruffled	12, 13
F	*U. californica*	Smooth margins	Unbranched	0.5	Smooth; ruffled	6, 3.8
F	*U. californica*	Ruffled; Lanceolate	Unbranched	Not present	Smooth; ruffled	6, 4.1
B	*U. linza*	Lanceolate	Unbranched	Not present	N/A	6, 3.5
B	*U. linza*	Lanceolate	Unbranched	N/A	Smooth; no marginal teeth	6, 1.3
A	*U. lactuca*	Ovate	Unbranched	Not present	Smooth, no marginal teeth	5.5, 3

E	*U. australis*	Ruffled	Unbranched	Not present	Smooth; ruffled	3, 6.7
B	*U. linza*	Lanceolate	Unbranched	Not present	Smooth; ruffled	12.5, 1.8
B	*U. linza*	Lanceolate	Unbranched	Not present	Smooth; ruffled	12, 2.6
A	*U. lactuca*	Large branches; ruffled near stipes	Unbranched	2	Smooth; no marginal teeth	33, 21
F	*U. californica*	Ruffled margins with perforations	Unbranched	2	Smooth; no marginal teeth	40, 32
E	*U. australis*	Orbiculate	Unbranched	Not present	Smooth; minimally ruffled	9, 9
B	*U. linza*	Lanceolate; tubular base	Unbranched	Not present	Smooth; ruffled	10.5, 11
B	*U. linza*	Lanceolate; tubular base	Unbranched	Not present	Smooth; ruffled especially near stipe/holdfast	

B	*U. linza*	Lanceolate	Unbranched	N/A	Smooth; ruffled	11, 1.2
A	*U. lactuca*	Obovate	unbranched	Not present	Smooth; minimally ruffled	12, 12
F	*U. californica*	Oblanceolate	Unbranched	3	Smooth; minimally ruffled	8, 4
B	*U. linza*	Ruffled, twisting in the main axis, narrow, lanceolate	Unbranched	Not present	Ruffled; smooth, narrow	9.2, 1.2
B	*U. linza*	Lanceolate; ruffled	unbranched	Not present	Smooth; ruffled	6, 0.5
B	*U. linza*	Flat, broad distally	Unbranched	Not present	Smooth; minimally ruffled	11.5, 10.5
B	*U. linza*	Flat, broad distally	Unbranched	Not present	Smooth; minimally ruffled	9.5, 7.5

A	*U. lactuca*	Orbiculate	Unbranched	Not present	Smooth; minimally ruffled	14, 7
A	*U. lactuca*	Orbiculate	Unbranched	0.5	Smooth; no marginal teeth	8.2, 9
A	*U. lactuca*	Broad blade; deeply lobed	Unbranched	5	Smooth; no marginal teeth	22, 10
A	*U. lactuca*	Orbiculate	unbranched	1	Smooth; minimal ruffling	26, 16
B	*U. linza*	Cuneate blade; tubular base	Unbranched	3	Smooth; very thin blade	10, 7
B	*U. linza*	Narrow blade; cunate	Unbranched	Not present	Smooth, thin blade; ruffled margins	15, 2.7
B	*U. linza*	Obovate	Unbranched	<1	Smooth; ruffled margins	8.5, 3
G	*Ulva sp.*	Ovade	Unbranched	Not present	Smooth; ruffled margins	9, 5.5

C	*U. lobata*	Broad	Unbranched	Not present	Smooth; Thick center; thin margins.	27, 17
F	*U. californica*	Broad, thin; narrow proximally	Unbranched	1.5	Smooth and thin; tattered margins	25, 17
E	*U. australis*	Broad distally; narrow proximally	Unbranched	1.5	Smooth; ruffled	9, 10
E	*U. australis*	Ovate	Unbranched	0.5	Smooth; ruffled Margins near the end	10.5, 15.8
B	*U. linza*	Cuenate; ruffled thallus	Unbranched	1	Smooth; ruffled	7, 6
B	*U. linza*	Cuneate; tubular base	Unbranched	1.5	Smooth; marginally ruffled near base	7.8, 2.7

Appendix B. Microscopic morphological features of *Ulva* specimens analyzed.

OTU	Species/ species complex	Cell shape	Cell diameter (μm)	Chloroplast shape/ location	Number of pyrenoids	Longest length, longest perpendicular width (μm)
F	*U. californica*	Mostly rectangular with curved edges; some polygonal	1.04	Cup shaped, peripheral	1	1.56, 1.04
A	*U. lactuca*	Polygonal with rounded corners	2.34	Cup shaped, whole cell	1	1.89, 1.34
A	*U. lactuca*	Polygonal with rounded corners	1.62	Peripheral, whole cell	1-2	2.34, 2.34
B	*U. linza*	Rectangular cells	1.3	Cup shaped	1	1.3, 1.3

B	*U. linza*	Rectangular cells	1.3	Cup shaped	1	1.3, 1.3
C	*U. lobata*	Polygonal with rounded corners	1.35	Whole cell	1	1.89, 2.16
C	*U. lobata*	Polygonal with rounded corners	1.04	Cup shaped, whole Cell	1	1.3, 1.04
D	*U. rigida complex*	Polygonal with rounded corners	1.56	Cup shaped	1-2	2.86, 1.56
D	*U. rigida complex*	Polygonal with rounded corners	1.08	Cup shaped	1	1.35, 1.56
A	*U. lactuca*	Polygonal with rounded corners	N/A	Cup shaped	1	1.3, 1.04

A	*U. lactuca*	Rectangular cells with rounded corners	0.78	Cup shaped	1	1.3, 0.78
E	*U. australis*	Polygonal with rounded corners	1.04	Peripheral, whole cell	1	1.04, 1.04
E	*U. australis*	Polygonal with rounded corners	1.04	Whole cell	1	1.04, 1.04
F	*U. californica*	Polygonale with rounded corners	1.04	Compressed to one side, some cup shaped	1	1.08, 1.08
F	*U. californica*	Polygonal with rounded corners	1.04	Cup shaped	1	1.3, 1.04
B	*U. linza*	Polygonal with	1.04	Cup shaped	1	1.04, 1.3

		rounded corners				
B	*U. linza*	Polygonal with rounded corners	1.35	Cup shaped	1	1.62, 135
A	*U. lactuca*	Polygonal with rounded corners	1.04	Cup shaped	1	1.3, 1.56
E	*U. australis*	Polygonal with rounded corners	1.56	Peripheral, whole cell	1	2.08, 1.56
B	*U. linza*	Polygonal with rounded corners	1.35	Cup shaped	1	1.62, 1.35
B	*U. linza*	Polygonal with rounded corners	0.78	Cup shaped, peripheral	1	0.78, 0.78

A	*U. lactuca*	Polygonal with rounded corners	1.04	Cup shaped, peripheral	1	1.56,1.04
F	*U. californica*	Polygonal with rounded corners	1.04	Cup shaped, peripheral	1	1.3, 1.04
E	*U. australis*	Polygonal with rounded corners	1.08	Peripheral	1	1.08, 1.35
B	*U. linza*	Polygonal with rounded corners	1.04	Cup shaped, peripheral	1	1.56, 1.04
B	*U. linza*	Polygonal with rounded corners	1.56	Cup shaped, Peripheral	1	1.56, 1.3
B	*U. linza*	Polygonal with	1.08	Cup shaped	1	1.08, 1.35

		rounded corners				
A	*U. lactuca*	Polygonal with rounded corners	1.35	Cup shaped	2-3	1.62, 1.35
F	*U. californica*	Polygonal with rounded corners	1.3	Peripheral	1 to multiple	1.3, 1.04
B	*U. linza*	Polygonal with rounded corners	0.78	Peripheral	1	1.04, 1.04
B	*U. linza*	Polygonal with rounded corners	1.35	Cup shaped, peripheral	1	1.89, 1.62
B	*U. linza*	Polygonal with rounded corners	1.04	Cup shaped, peripheral	1	1.3, 1.04

B	*U. linza*	Polygonal with rounded corners	1.04	Cup shaped, peripheral	1	1.3,1.56
A	*U. lactuca*	Polygonal with rounded corners	1.08	Cup shaped, peripheral	1	1.35, 1.08
A	*U. lactuca*	Polygonal with rounded corners	1	Peripheral	1	1, 1
A	*U. lactuca*	Polygonal with rounded corners	0.78	Peripheral	1+	1.04, 0.78
A	*U. lactuca*	Polygonal with rounded corners	1.08	Peripheral and Cup	1	1.35, 1.08
B	*U. linza*	Rectangular or	1.12	Peripheral	1+	1.12, 2.24

		polygonal with rounded corners				
B	*U. linza*	Polygonal with rounded corners	1.3	Peripheral	1	1.56,1.3
B	*U. linza*	Polygonal with rounded corners	0.81	Cup shaped, whole cell	1	0.81, 1.08
G	*Ulva sp.*	Polygonal with rounded corners	1.04	Peripheral, cup shaped	2+	1.3, 1.04
C	*U. lobata*	Polygonal with rounded corners	1.08	Cup shaped, whole cell	1	1.35, 1.08
F	*U. californica*	Rectangular with	1.5	Peripheral	1	1, 0.75

		rounded corners					
E	*U. australis*	Polygonal with rounded corners	1.04	Peripheral, cup shaped	1+	1.3, 1.04	
E	*U. australis*	Polygonal with rounded corners	1	Whole cell	1+	1.375, 0.875	
B	*U. linza*	Polygonal with rounded corners	1.35	Peripheral	N/A	1.62, 1.35	
B	*U. linza*	Polygonal with rounded corners	1.75	N/A	1+	1, 1.35	

Appendix C. Macroscopic morphological features of *Fucus* specimens analyzed.

OTU	Species or	Overall thallus	Average holdfast	Thallus texture	Longest length, width	Receptacles	Prominent Midrib?	Lamina Width,

	species complex	morphology	diameter (mm)	and marginal edge appearance	at base of receptacle (cm)	Wings?		Width from Midrib
A	*F. distichus*	Prominent midrib; no receptacles	0.5	Smooth; slimy	5.5, 1.1	N/A	N/A	N/A, N/A
A	*F. distichus*	No prominent midrib; Receptacles	4	Smooth; dull bumps on receptacles	8.8, 1.8	N/A	N/A	N/A, N/A
A	*F. distichus*	No prominent midrib; Receptacles	6	Smooth; dull bumps on receptacles	12.5, 1.4	N/A	N/A	N/A, N/A
A	*F. distichus*	No prominent midrib; Receptacles	3	Smooth; dull bumps on receptacle	11, 1	N/A	N/A	N/A, N/A

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B	*F. spiralis*	Receptacles without sterile wings	N/A	Prominent midrib	13, 1	No	Yes	1cm, 4mm
A	*F. distichus*	Receptacles that flatten distally; dull bumps on lamina	Not present	Smooth; dull bumps on receptacles	11.4, 1.4	No	No	1.2 cm, 3mm
B	*F. spiralis*	Receptacles newly developed and flattened	Not present	Smooth lamina with few bumps	13.6cm, 1.9cm	No	Yes	1.4cm, 5mm
A	*F. distichus*	Receptacles newly developed, rounded, flattened;	Not present	Smooth lamina with few bumps	13 cm, 2cm	No	No	1.2cm, 4mm

				Smooth and slimy with a midrib that becomes inconspicuous towards the ends or each branch.				
A	*F. distichus*	Smooth; small bumps	Not present		11cm, 1.5cm	No	No	1.5cm, 5mm
				Thick, smooth lamina, little bumps				
A	*F. distichus*	Smooth; small bumps	Not present		14.7cm, 1.7xm	No	No	1.7cm, 6mm
		Thick lamina around midrib in		Smooth and	17.3cm,			1.9cm,
A	*F. distichus*	distal areas;	4.5	ruffled	1.9cm	No	Yes	7mm

		eroded proximal lamina						
B	*F. spiralis*	Dichotomous branching; Flattened receptacles	3	smooth	, 2cm	No	Yes	1.2cm, 6mm
B	*F. spiralis*	Dichotomous branching; Flattened, elongated receptacles	6	Smooth	14.5, 3.2	Yes	Yes	
B	*F. spiralis*	Smooth branches	5	Smooth branches; No receptacles	10.1, 2.9	No	Yes	1.4cm, 6mm

A	*F. distichus*	Smooth branches	5.5	Smooth branches; Flattened receptacles	16, 1.5	No	No	1.5cm, 5mm
A	*F. distichus*	Smooth branches	Not present	Smooth branches; receptacles flat and blunt distally	21, 1.9	No	Yes	1.9cm, 7mm
B	*F. spiralis*	No prominent bumps; Flattened receptacles with irregular branching	5	Smooth, eroded lamina	24.5, 1.7	Yes	Yes	1.2cm, 5mm
B	*F. spiralis*	Flattened receptacles; Dichotomo	3	Smooth, eroded lamina	16.1, 1	No	Yes	9mm, 4mm

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branching

Appendix D. Microscopic morphological features of *Fucus* spp.

OTU	Species/ species complex	Cell arrangement	Cell shape, superficially	Cell diameter (µm)	Number of pyrenoids	Longest length, Longest perpendicular width (µm)
A	*F. distichus*	Irregular	Rectangular with rounded edges	1.3	Many	1.82, 1.82
A	*F. distichus*	Irregular	Rectangular with rounded edges	N/A	Many	1.35, 1.35
A	*F. distichus*	Irregular	Ovulate	3.9	Many	3.9, 1.3
A	*F. distichus*	Irregular	Rectangular with rounded edges	1.3	Many	1.82, 1.82
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.04	Many	1.56, 1.04

A	*F. distichus*	Irregular	Ovulate	1.45	Many	1.3, 1.3
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.5	Many	1.5, 1.5
A	*F. distichus*	Irregular	Polygonal with rounded edges	1.5	Many	1.5, 1.5
A	*F. distichus*	Irregular	Polygonal with rounded edges	1.08	Many	1.35, 1.08
A	*F. distichus*	Irregular	Polygonal with rounded edges	1.04	Many	1.56, 1.04
A	*F. distichus*	Irregular	Polygonal with rounded edges	1.47	Many	1.96, 1.14
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.08	Many	1.62, 1.35
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.04	Many	1.3, 1.3
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.35	Many	1.35, 1.35
A	*F. distichus*	Irregular	Polygonal with rounded edges	1.35	Many	1.89, 1.62

A	*F. distichus*	Irregular	Polygonal with rounded edges	1.35	Many	1.35, 1.0
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.56	Many	1.56,1.82
B	*F. spiralis*	Irregular	Polygonal with rounded edges	1.35	Many	1.89, 1.62