

I'm so *Ulva* it: Using *tufA* DNA barcoding for identification and contextualization of *Ulva* species in Salish Sea

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Abstract

Ulva is a genus of green algae, rapidly becoming relevant due to potential uses in the blue economy, in addition to potential biofuel and rare earth element uses. *Ulva* is characterized by its rapid growth sometimes causing harmful blooms, making it increasingly important to study this algae, particularly the understudied tubular forms. *Ulva* historically had been difficult to identify based on morphology alone. This project identified *Ulva* species in the greater San Juan Islands using DNA barcoding, comparing specimen morphological characteristics to what is typically described for the species, as well as using DNA data to contextualize samples into larger clades. From this, key morphological distinctions of identified species and potential distribution patterns relating to environmental and geographic factors are elucidated. In total, 12 species of *Ulva* were identified from 35 samples, with some samples matching traditional descriptions of named species while some differing from what is expected based on morphology alone. The species found included *Ulva compressa*, *Ulva flexuosa*, *Ulva expansa*, *Ulva californica*, *Ulva fenestrata*, *Ulva stenophylla*, *Ulva taeniata*, *Ulva intestinalis*, *Ulva prolifera*, *Ulva australis*, *Ulva linza*, and *Ulva torta*. In addition, 4 species of *Blidingia* were identified and treated as an outgroup. Not all of these species however have been tied to a type specimen and more sequencing efforts are needed to accurately quantify diversity of the San Juan region.

Introduction:

Ulva is a genus of green algae widely known for their fast growth ([Hiraoka et al 2020](#)), cosmopolitan nature ([Xia et al. 2023](#)), and potential for use in the blue economy ([Steinhagen et al. 2025](#)). There are many different uses for *Ulva*; relevant industries include pharmaceuticals, nutraceuticals, biofuels, and more ([Hofmann et al. 2024](#)). However, this green algae is also

known as a nuisance, becoming especially problematic in aquaculture and urban estuary settings ([Joniver et al. 2021](#)); blooms of *Ulva* have often been tied to increased nutrient runoff from land-use change ([Xu & Xu 2022](#)). As such, there is a global demand to understand the growth, life history, and identity of *Ulva* species.

Despite this, *Ulva* has been historically difficult to identify based on morphology, with species-specific identities changing constantly since the 20th century. Adding to the complication, *Ulva* morphologies are known to differ both inter- and intraspecifically based on microbial influence ([Spoerner et al 2012](#), [Ghaderiardakani et al 2017](#), [Singh et al. 2011](#), [van der Loos et al. 2022](#), [Wichard 2023](#)) and culture condition ([Zhang et al. 2013](#)). To resolve this issue, an increased effort has been placed on using molecular and genomic data to taxonomically characterize *Ulva* worldwide ([Mutizabal-Aroz et al. 2024](#), [Woo Lee et al. 2019](#), [Gabrielson et al. 2019](#)), and more recently, in the Northeast Pacific Ocean ([Hughey et al 2024](#), [Kuba et al. 2022](#)). However, most of these research efforts have focused on blade-forming and attached populations of *Ulva*, omitting other known morphologies and life-histories within the region. These include traditionally tubular species, such as *U. intestinales*, *U. flexuosa*, *U. torta*, and *U. prolifera*. Identification of tubular *Ulva* in particular is critical, as such species are known to play important ecological and economic roles in coastal communities. Bloom events of the tubular *U. prolifera*, for example, occur annually in eutrophic zones of the Yellow Sea, and have been cited as the largest macroalgal blooms in the world ([Liu et al. 2009](#)). These blooms sequester nutrients rapidly, outcompeting other photosynthetic organisms ([McGlathery 2001](#), [Xing et al. 2015](#)), interrupting marine anthropogenic activity, such as fishing and tourism ([Zhang et al 2025](#)), and often leading to hypoxic water conditions that disrupt local ecosystem structures ([Zhang et al. 2019](#)). From a bioeconomy standpoint, tubular *Ulva* is currently being investigated

for its potential to sequester rare earth elements ([Figueiredo et al 2022](#), [Edmundson et al. 2023](#)).

Ultimately, understanding the identification and diversity of tubular morphologies is an important next step in managing *Ulva* as a coastal resource and nuisance across the globe.

In this study, the morphological-genomic gap is addressed by sampling both tubular and bladed *Ulva* species from around the Salish Sea. Each sample was evaluated using DNA barcoding to identify species and contextualize them within the broader clade of *Ulva*. Key morphological distinctions of identified species and any potential distribution patterns relating to environmental and geographic variables were assessed. We predict that expanding the *Ulva* survey parameters to include tubular morphologies will expand the identified species known in the region; furthermore, we suggest that while morphological distinctions can be useful, molecular data is necessary to identify *Ulva* at the species level.

Methods and Materials:

Sample collection

Ulva samples were collected at sites around the Salish Sea (Figures 1-7[9]), focusing specifically on the San Juan Islands. Sites included two shellfish farms, one in Westcott Bay (Figure 6) and one in Samish Bay, as well as docks near Friday Harbor, and Friday Harbor Laboratories (Figure 4), and natural preserves such as Eagle Cove, Cattle Point, and Iceberg Point. At each site, when possible, at least one tubular and bladed sample was collected, with an emphasis on collecting tubular samples. In addition, some “filamentous” appearing forms or epizooic forms were found at sites and collected to expand on the potential habitats that different species may inhabit. Metadata from these collections included photographs, length measurements, coordinates, tidal height at time of collection, salinity of water, and substrate

characterization. Herbarium presses were made as voucher specimens for each sample and were submitted to the University of Washington herbarium for preservation.

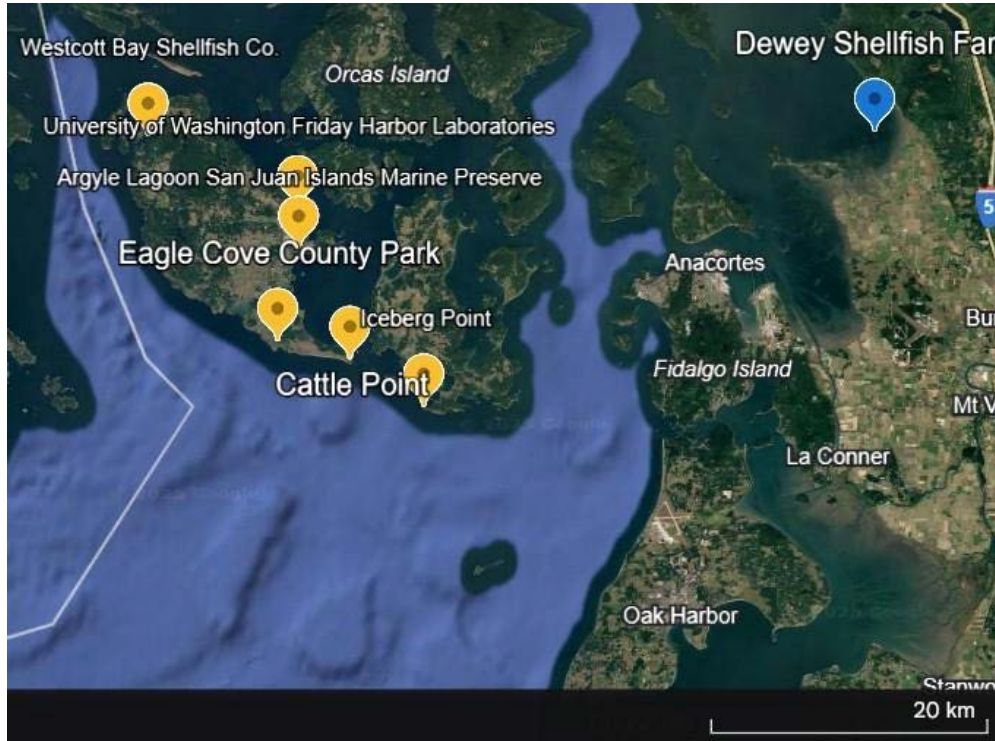


Figure 1: Collection sites in greater Salish Sea

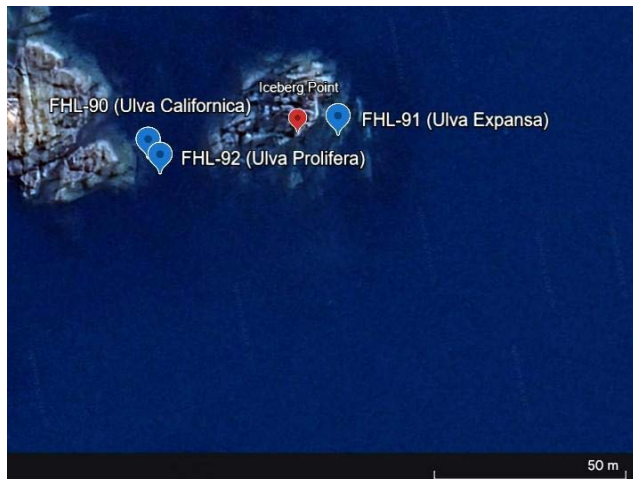


Figure 2: Iceberg Point (Lopez Island) Ulva Collection Site.

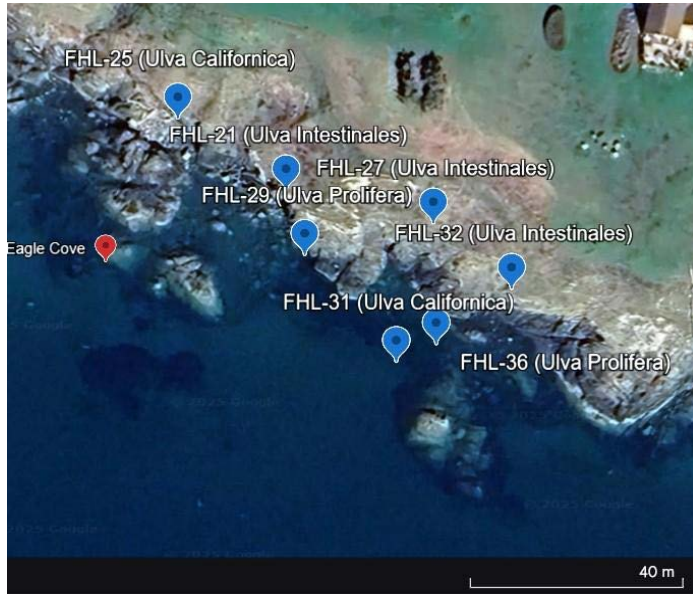


Figure 3: Eagle Cove Ulva Collection Site.



Figure 4: Friday Harbor Labs Ulva Collection Site.

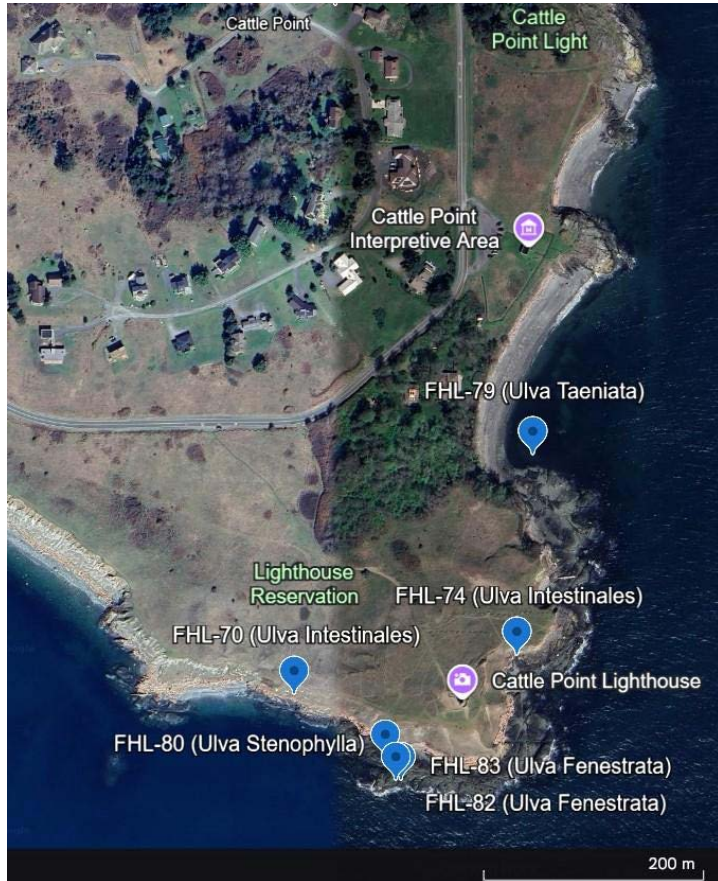


Figure 5: Cattle Point Ulva Collection Site.



Figure 6: Westcott Bay Ulva Collection Sites.



Figure 7: Argyle Lagoon Ulva Collection Sites.



Figure 8: Westcott “filamentous” *U. torta*.

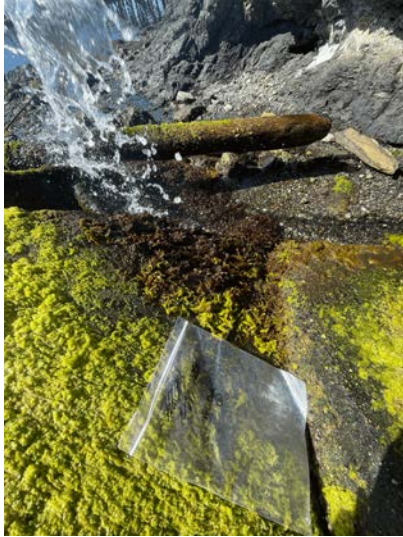


Figure 9: Friday Harbor labs outflow pipe showing tubular *U. intestinalis*.

DNA Extraction, Amplification, and Sequencing

DNA extraction generally followed the protocols of [Kuba et al. \(2022\)](#) and Taylor et al. (2017), using a Bioline Extract-PCR Kit and One-Step PCR Inhibitor Removal Kit (Zymo Research). Small samples (2 mm or less) of collected specimens were placed in a tube of Nuclease-free water (35 μ L) and frozen. After thawing, 15 μ l of Extract-PCR kit enzymatic solution were added to each sample tube which were then incubated at 75°C for at least 15 minutes before manually grinding with a pestle and re-incubating for another 15-30 minutes. The tubes were then transferred to a heat block set to 95°C to trigger enzyme deactivation. Debris was removed by centrifuging samples at 15000 xg for 1 minute and removing supernatant (~50 μ l). The supernatants were diluted by 1:5 or 1:10, and stored at -20°C before use in amplifications.

PCR amplification of the *tufA* locus—a plastid-encoded gene used in characterization of *Ulva*—was accomplished using the Bioline MyTaq HS Red Mix kit following the standard conditions outlined in Kuba et al. (2022) and [Fama et al. \(2002\)](#); samples were denatured for

three minutes at 95°C, and then underwent 35 cycles of denaturing at 95°C for 15 seconds, an annealing step at 45°C for 15 seconds, and an extension step at 72°C for 1 min, followed by a final extension at 72°C for an additional five minutes. After confirming amplification with gel-electrophoresis, Exo-Sap (Thermo Fisher Scientific, Waltham, MA, USA) was added to samples for PCR product clean-up. Selected samples were then sent to Genewiz for DNA sequencing (Azenta Life Sciences, South Plainfield, NJ, USA).

In Geneious, sequence files for both the forward and reverse reactions of the *tufA* locus were aligned using De Novo Assemble, resulting in a consensus sequence. The frame was then adjusted to eliminate any stop codons, allowing the sequence to read as accurately as possible. The sequence was then copied and entered into the NCBI (National Center for Biotechnology Information) BLAST search, where the DNA sequence is compared to the most similar sequences in order to help determine the original sample species by DNA similarity comparison. Edited sequences were then submitted to the BOLD (Barcode of Life Data Systems) project.

Select sequences were chosen to represent our samples and were compared to other known sequences from a previously developed dataset from [Dibner et al 2025](#). The sequences that were chosen were representative of the twelve species found within this study *Ulva compressa*, *Ulva flexuosa*, *Ulva expansa*, *Ulva californica*, *Ulva fenestrata*, *Ulva stenophylla*, *Ulva taeniata*, *Ulva intestinalis*, *Ulva prolifera*, *Ulva australis*, *Ulva linza* and *Ulva torta*. The sequences for each sample were aligned using MUSCLE (citation??) and then checked for any base pair differences. Any differences between sample base pairs were checked for error or significant difference before a tree was generated. A RAxML (Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014 May 1;30(9):1312-3) tree was made using Geneious prime version 2025.1.3. The tree was run

with the nucleotide model GTR GAMMA, using the rapid bootstrapping algorithm, run with 1000 bootstraps. The partitioning was DNA, codon1 = 1-781/3, DNA, codon2 = 2-781/3, and DNA, codon3 = 3-781/3.

Results

The *tufA* sequences of 35 samples were used to create a UPGMA clustering tree which revealed 12 distinct species, as well as 4 more distant *Blidingia* species that were treated as an outgroup (see Fig 10). Of species groups identified, 4 were distinctly monostromatic, and tubular, including 6 of the *U. torta* samples taken from 4 different sites, 5 of the *U. intestinales* samples taken from 3 different sites, 5 of the *U. flexuosa* samples taken from 2 different sites, and 1 of the *U. compressa*. Furthermore, 2 tubular specimens (FHL25-25, and 31) had *tufA* sequences that matched that of *U. californica*, a traditionally blade-forming species. Blade-forming *Ulva* were identified as outlined in Kuba et al. 2022, collecting 4 samples of both *U. prolifera* and *U. californica* at 3 different sites, as well as 2 samples of *U. fenestrata* and 1 sample of *U. expansa* at 1 site each. Additionally, 4 other blade-forming species were identified, including 1 sample each of *U. australis*, *fenestrata*, *stenophylla*, and *linza*.

The comparison between our data and older data resulted in the comparison of type specimens to 12 select specimens. FHL25-083 *Ulva fenestrata* matched the type specimen, as they were sister to one another. FHL25-091 *Ulva expansa* was also closely related to the type specimen for this species as it was one clade different. FHL25-010 *Ulva australis* matches other specimens of the same species. FHL25-074 *Ulva intestinalis* is far away from the one other *U. intestinalis* species in this analysis, as it is many clades away.

Table 1: correlating site to species morphology and environment

Site	Species Found	Morphology	Environment
Eagle Cove, Friday Harbor, WA	<i>Ulva intestinalis</i>	Tubular and wrinkled	Upper intertidal on cliffside
	<i>Ulva californica</i>	Filamentous and tubular, growing as mat	Upper intertidal on cliffside
	<i>Ulva prolifera</i>	Bladed	Epizootic (on limpet), Lower Intertidal
Cattle Point, Friday Harbor, WA	<i>Ulva intestinalis</i>	Tubular thin	high upper tidal in crevice, low salinity (3-6 ppt)
	<i>Ulva fenestrata</i>	Bladed	Epizootic (on barnacle and chiton), lower intertidal
	<i>Ulva taeniata</i>	Bladed	Lower intertidal region

	<i>Ulva stenophylla</i>	Bladed	Lower intertidal in tide pool on a rock
	<i>Ulva intestinales</i>	Tubular wide	Higher upper intertidal on rock
Iceberg Point, Lopez Island, WA	<i>Ulva californica</i>	Bladed	Epizootic (on barnacle)
	<i>Ulva expansa</i>	Filamentous	Lower intertidal growing on limpet
Friday Harbor Labs, San Juan Island, WA	<i>Ulva prolifera</i>	Bladed	Lower intertidal found on Egregia
	<i>Ulva torta</i>	Tubular, Wide	On tire in Friday Harbor Labs
Westcott Bay, San Juan Island, WA	<i>Ulva flexuosa</i>	Tubular, thin	In sand/mud lower intertidal

	<i>Ulva torta</i>	Tubular, Thin	On tree log in upper intertidal region
Argyle Lagoon, San Juan Island, WA	<i>Ulva torta</i>	Tubular, thin	On the sand
	<i>Ulva flexuosa</i>	Filamentous	Tubular, thin

Figure 10: UPGMA tree showing interspecies differences in morphology/broad classification

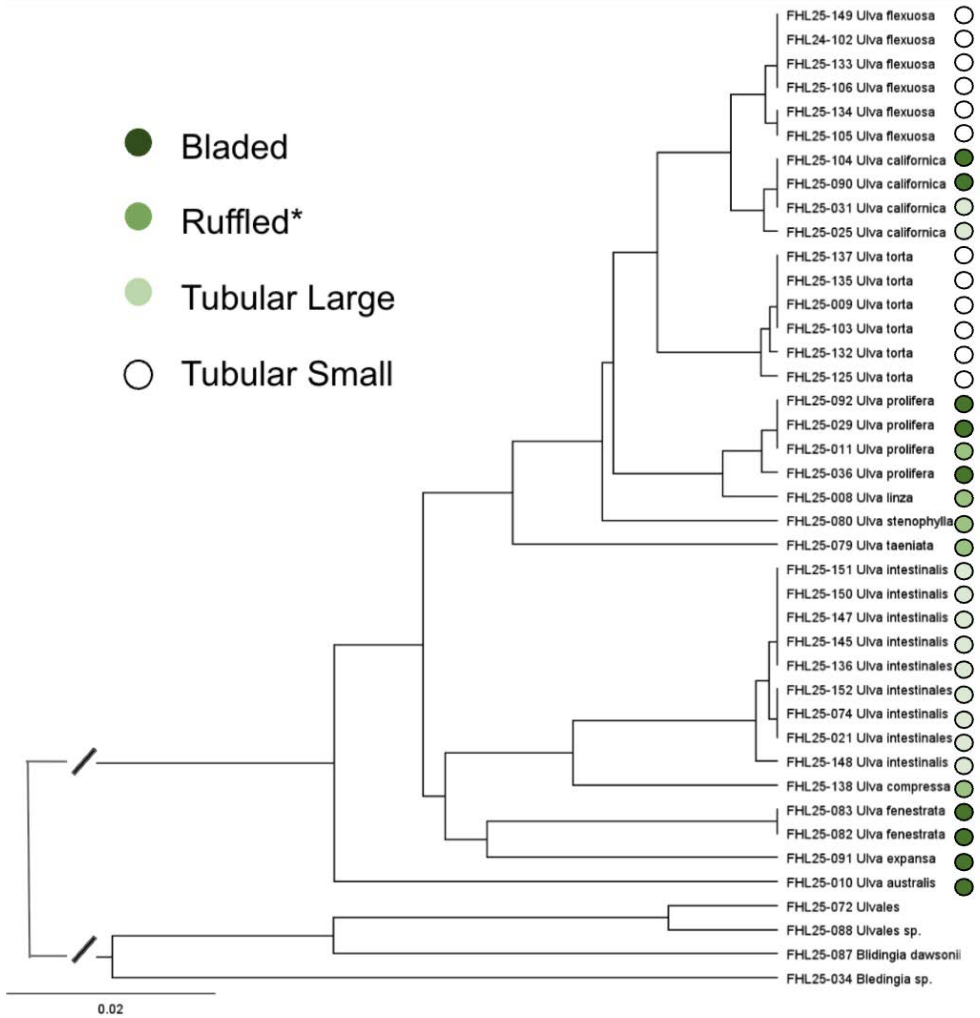

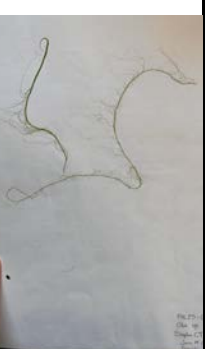






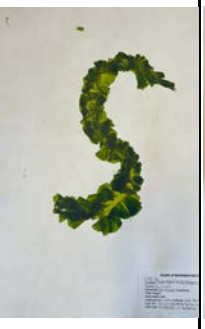





Figure 11: UPGMA tree showing interspecies differences in morphology and broad classification. *=Ruffled refers to blades with distinct edges, otherwise referred to as undulate.

Table 2: Correlating species found to herbarium label.

					
FHL25-105 <i>U. flexuosa</i> Argyle Lagoon	FHL25-009 <i>U. torta</i> Samish Bay	FHL25-074 <i>U. intestinalis</i> Cattle Point	FHL25-011 <i>U. prolifera</i> Samish bay	FHL25-083 <i>U. fenestrata</i> Cattle Point	FHL25-091 <i>U. expansa</i> Iceberg Point
					
FHL25-090 <i>U. californica</i> Iceberg Point	FHL25-138 <i>U. compressa</i> Westcott Bay	FHL25-080 <i>U. stenophylla</i> Cattle Point	FHL25-079 <i>U. taeniata</i> Cattle Point	FHL25-010 <i>U. australis</i> Samish Bay	FHL25-008 <i>U. linza</i> Samish Bay

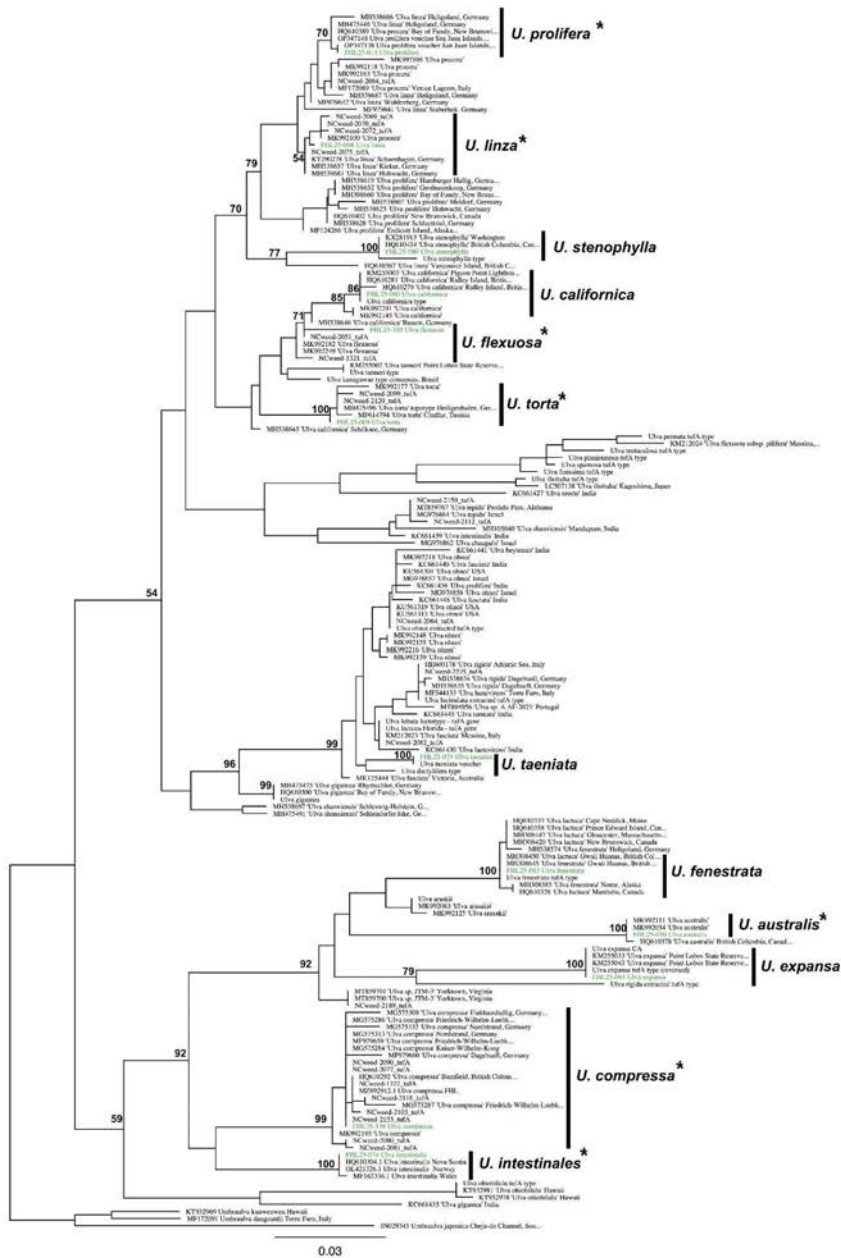


Figure 12 : Maximum-likelihood tree built using RAxML in Geneious Prime using a rapid-hill climbing algorithm. Support for nodes was determined by building a consensus tree from 1000 bootstraps. Asterisks denote species names for which the *tufA*-type sequences are unavailable for comparison, and thus cannot be definitively identified as such species.

Discussion

Twelve species of *Ulva* were identified across 7 different sites (fig. 10, 11, 12). Of these, 5 were successfully matched to type specimens; *U. stenophylla*, *U. californica*, *U. fenestrata*, *U. taeniata*, and *U. expansa*. *Ulva torta* matched the topotype. There were some species that do not have a type specimen or the type specimen does not have a *tufA* sequenced; these specimens are *U. prolifera*, *U. linza*, *U. flexuosa*, *U. australis*, *U. compressa*, and *U. intestinales*.

Samples of *U. prolifera* and *U. linza* were both placed in the broad ‘LPP’ clade consisting of other specimens deemed as *U. prolifera*, *procera*, and *linza*, with 79% support (fig. 12). *U. prolifera* collected was largely bladed, often with ruffled margins, not matching traditional descriptions of the species that identify it as tubular (Gabrielson & Lindstrom 2018). However, previous surveys of *Ulva* in the region have found bladed *U. prolifera* with ITS-2 sequences with only 1-2 base pair differences from designated epitype specimen, indicating that the species may have broad phenotypic plasticity despite little genetic difference (Kuba et al. 2022). Furthermore, the *tufA* sequences for our *U. prolifera* were found to be an identical match to that of *U. prolifera* from the previous surveys of the region (not seen in tree, OP347129.1), and seen as a close match to that of those in the tree as well (OP347138). While the *U. prolifera* collected did not have extreme intraspecies genetic variation between samples and sites, one specimen from Eagle Cove (Fig.3) (FHL25-036) had noticeably wider and larger blades than others, which had thinner blades. All samples were distally-compressed, with slight tubes forming near points of attachment. FHL25-029 and FHL25-036 from Eagle Cove were found growing epizootically in the mid-intertidal, on limpets and tube-worms respectively, whereas FHL25-092 from Iceberg point was found in the lower-intertidal, growing epiphytically on

Egregia menziesii (fig 2). FHL25-011 from Samish Bay was found growing on netting that covered beds of *Rhuditapes phillipinarum*, or Manilla clam, alongside *U. torta*, *U. linza*, and *U. australis*.

U. linza is traditionally described as often having hollow-tubular blade margins that are monostromatic; while FHL25-008 did not have this morphology, it still generally follows descriptions of *U. linza* with near-tubular morphology at the base and broad blades. FHL25-008 is resolved with little to no support (54%) within a sub-clade of specimens identified as *Ulva 'linza'*, *Ulva 'procera'*, and *Ulva prolifera* that is part of the larger LPP clade of specimens that are currently most accurately named *U. prolifera* (Kuba et al., 2022). However, as discussed in Dibner et al. 2025, the identity of species within the LPP clade has been long debated (Kuba et al 2022, Dibner et al. 2025). Additionally, the highest (99.76%) matching *tufA* sequences to that of FHL25-008 in GenBank are from specimens named both *U. prolifera* and *U. linza*. Interestingly, some of the top matches are to species from China with tubular morphologies, previously termed *Enteromorpha prolifera* (AC: MZ561599). Type specimens for both *U. prolifera* and *U. linza* are unavailable, and thus while broad classification of our samples in this clade are supported, more work is needed to definitively apply species-specific taxonomic labels.

FHL25-080 from Cattle Point (fig.5) matched perfectly to other samples in GenBank from the Northeast Pacific labeled *U. stenophylla*, and had a morphology that matched that general descriptions in the key; thalli grew attached in long, undulate or ruffled blades that tapered towards the end. 70% of generated bootstrap trees placed FHL25-081 *U. stenophylla* as a closely-nested sister to the LPP clade, and 100% of tree builds matched it to the type-specimen from Monterey, CA. The find is significant, as it was not identified in previous surveys of bladed *Ulva* at Cattle Point, performed in 2022. Additionally, the species is believed to be rare at these

latitudes, usually occurring in warmer climates (Gabrielson et al., 2018). Our specimen was found in a tide pool in the mid-intertidal region; while the water temperature was not taken, tide-pools are known to have significantly warmer water than the ocean water, and may play an important role in species distribution patterns in a changing climate ([Bermejo et al., 2023](#)).

Samples FHL25-025, 031, 090, and 104 found at both Eagle Cove([fig. 3](#)) and Iceberg Point([fig.2](#)) were closely identified to *Ulva californica* in GenBank, and had very little intraspecific variation as observed in our Neighbor-Joining consensus tree. Additionally, the selected representative (FHL25-090) matched to the type specimen from La Jolla, CA in 86% of RAxML trees constructed, and to a broad clade of multiple *tufA* sequences labeled ‘*U. californica*’ in 85% of trees constructed as well. *U. californica* is common throughout the Northeast Pacific and was one of the four species identified in Kuba et al. (2022). FHL25-090 and FHL25-104 from Iceberg Point and Argyle Lagoon respectively followed traditional morphological descriptions of *U. californica*, with broad oblanceolate-type thalli construction. FHL25-090 was found growing epizoidally on barnacles in the low-to-mid intertidal region. *U. californica* is also known to grow epi- and endophytically on larger algae or seagrass as well. However, FHL25-025 and FHL25-031 from Eagle Cove had noticeably different morphologies, taking on a monostromatic-tubular shape similar to that of *U. intestinalis* (see Fig. 13). Noticeably, it was found growing in a similar habitat alongside *U. intestinalis*, in pools near freshwater seeps in the high-upper intertidal. While it is possible that environmental factors (substrate, nutrients, salinity, microbiome) led to changes in the morphology of *Ulva californica*, it’s important to note that the *tufA* sequences returned for these samples had poor quality scores and read length (see BOLD database). There have been no reported cases of true tubular *U.*

californica cited in the literature. Thus, these identifications are still questionable and warrant further evaluation before definitive taxonomic identification.



Figure 13: *Ulva californica* samples from Eagle Cove, displaying tubular morphology.

Samples identified as *Ulva flexuosa* (FHL25–102, 105, 106, 133, 134, and 149) and *Ulva torta* (FHL25-009, 103, 125, 132, 135, and 137) were the only thin-tubular specimens identified, and generally followed morphological descriptions traditionally ascribed to such species names. While largely indistinguishable in the water, species that matched to *U. flexuosa* in the phylogeny were less branched and slightly wider than those identified as *U. torta*, which appears filamentous and highly branched. *Ulva torta* and *Ulva flexuosa* were also found in largely the same locations as one another: Argyle Lagoon(fig. 7), the docks in Friday Harbor(fig. 4), and the shellfish farm at Wescott Bay(fig. 6); *U. torta* was also found on clam-beds in Samish Bay. Interestingly, all locations where these species have been found in this survey are significantly influenced by anthropogenic activity. *U. torta* does not have a *tufA* type-sequence that was identifiable or tied to our selected sample (FHL25-009). However, 100% of trees constructed placed it within a sub-clade of other non-type species referred to as *U. torta*. *Ulva flexuosa* also has no available *tufA*-type sequence, as well as a much less clearly defined subclade; 75% of

trees constructed placed samples identified as *U. flexuosa* it in a broader clade with other species defined as *U. flexuosa* as well as *U. californica*. *U. flexuosa* is also the subject of current taxonomic revision, based on surveys of the species from tropical regions carried out by O’Kelly (O’Kelly et al. 2010). Thus, Guiry suggests that the application of the ‘*flexuosa*’ name to populations in temperate regions is likely inappropriate (Guiry & Guiry 2025). This highlights the need for further revision of *Ulva* with tubular morphologies, including both *Ulva torta* and *flexuosa*.



Figure 14: showing larger tubes not usually characteristic of *U. torta*

The other truly-tubular *Ulva* identified in the survey was identified as *Ulva intestinalis* (FHL25-021, 032, 070, 074, 136, 145, 147, 148, 150, 151), which had markedly different morphology and habitat than those identified as *U. torta* and *U. flexuosa*. Thalli of these samples were wider, unbranched and wrinkled, and often associated with freshwater seeps. At both Cattle Point (fig.5) and Eagle Cove (fig.3), the samples at both locations were found in the upper-intertidal or even higher in pools on the cliffside of bluffs where freshwater came through cracks, and salinity was found to be as low as 3 ppt. At Wescott Bay, *U. intestinalis* was found on the

upper-intertidal region of mudflats near a freshwater creek, alongside *U. torta* and *U. flexuosa*, likely experiencing ranging salinities throughout the tidal cycle; water in the bay was found to be 34 ppt while the creek was 11 ppt. *U. intestinalis* was also found growing at both freshwater and saltwater outflow pipes at Friday Harbor Laboratory, as well as attached to tires on the dock with a salinity of ~32 ppt. One of the potential benefits of being tubular and wide is that it reduces the surface area compared to the relative volume, meaning that there is slower water loss, which would be advantageous in intertidal regions where thalli are prone to high light, temperature and few water exchanges. There was very little intraspecies variation between sequences identified as *U. intestinalis*, and no morphological distinctions. *Ulva intestinalis* does not have an available type specimen or *tufA* type sequence. However, 100% of trees constructed placed our selected specimen (FHL25-136) within a clade of other species identified as *U. intestinalis*, including a topotype specimen (AC: MF162336.1) from Wales. Like *U. flexuosa*, surveys carried out by O’Kelly suggest that the name ‘*intestinalis*’ may be inaccurately applied to species between tropical, sub-tropical and temperate climates based on morphology, and thus warrant further taxonomic review (O’Kelly et al. 2010, Guiry & Guiry 2025). The *U. intestinalis* collected in this study are some of the only specimens found growing at such low salinities, making it a species of interest in wastewater treatment and other potential freshwater applications.

92% of trees constructed placed *U. intestinalis* in a broad clade alongside *U. fenestrata*, *australis*, *expansa*, and *compressa*. Within this clade, 92% of trees grouped *U. fenestrata*, *australis* and *expansa* within a separate subclade, while 99% of trees grouped *U. compressa* in a separate subclade from this. Both of our *U. fenestrata* (FHL25-082 and FHL25-083) specimens were found at Cattle Point (*fig.5*) in the lower intertidal region. These species were found living

epizootically on both a chiton and a barnacle respectively, and sequences returned very little intraspecific variation. Furthermore, 100% of trees constructed placed our selected sequence (FHL25-083) in a clade alongside the *tufA*-type sequence for *U. fenestrata* in the database used by Dibner et al. (2025) and Hughey et al. (2024), lending support to our identification. *Ulva fenestrata* had somewhat ruffled margins, and was notably not in the Gabrielson key for specimens in the region, warranting further taxonomic work to identify the specimen (Gabrielson & Lindstrom 2018). Only one bladed specimen of *U. australis* was identified, collected from Samish Bay. While no type specimen or *tufA*-type sequence is available for *U. australis*, it was matched in 100% of trees constructed to other specimen referred to as ‘*australis*’ from British Columbia (AC: HQ610378). 79% of trees constructed placed FHL25-091 in a clade alongside the *tufA*-type specimen sequence for *Ulva expansa*. Unlike *U. fenestrata* or *australis*, *U. expansa* was previously described in the survey conducted by Kuba et al. (2022) and is traditionally described as being large blades. However, our sample was found growing epizootically on a limpet, with very thin oblate shaped blades.

Only one sample (FHL25-138) identified as *Ulva compressa* was found at the Westcott Bay (*fig. 6*) site in the lower intertidal region, growing partially or fully attached to sand and mud. While the taxonomy of *U. compressa* is currently under review, as no available *tufA*-type sequence has been published, 99% of trees constructed placed our sequence in a broad clade of *tufA* sequences associated with the name ‘*compressa*’ including one sequence (AC: MZ892912.1) collected from Friday Harbor in previous surveys . *Ulva gigantea* is known as a synonym of *U. compressa*, but this classification is currently being reviewed. As described in the Gabrielson & Lindstrom (2018) key *U. compressa* is “usually branched and cylindrical near the

base, however compressed and broadened distally.” This description also seems to match our sample of *U. compressa* from Westcott Bay (Table 2)

Sample FHL25-079 collected at Cattle Point (*fig.5*) had an identical match to the *tufA* sequence for the type specimen of *U. taeniata* from Monterey CA, a relationship supported in every tree constructed (AC: OR723826.1). The specimen collected appeared to have a “spiral” like formation with widely-ruffled edges, a description supported by the key (Gabrielson & Lindstrom 2018).

Further analysis needs to be done to confirm the morphology of each *Ulva* species. In addition, more collections and sequencing around the San Juan Island could be beneficial to including more species and seeing morphology changes based on site. One of the mistakes made in the analysis is that some of our collection data was not *Ulva* as our GenBank Blast data result ended up back as *Blidingia* for (FHL25–72, 34, 87, 88). *Blidingia* was growing alongside *Ulva* and looked very similar to our tubular *Ulva* collection samples. Further research is needed for determining differences in the field between *Blidingia* and tubular *Ulva* samples.

This survey analyzing *tufA* sequence data from specimens around the Salish Sea has expanded on previous work conducted at Friday Harbor by identifying a more diverse *Ulva* population. Additionally, the survey supports the findings of Kuba et al. (2022), that while morphological features (i.e. such as bladed vs. tubular forms) help to narrow down taxonomic identification, it is nearly impossible to reach species-level identification without molecular support. Further work on *Ulva* taxonomy should focus on molecular characterization of tubular morphologies that are currently poorly classified and misidentified on a global scale.

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Conflict of Interest:

The authors declare no conflict of interest.

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