

## Research article

Sandra C. Lindstrom\*, Matthew A. Lemay, Samuel Starko, Katharine R. Hind and Patrick T. Martone

# New and interesting seaweed records from the Hakai area of the central coast of British Columbia, Canada: Chlorophyta

<https://doi.org/10.1515/bot-2021-0038>

Received April 29, 2021; accepted July 7, 2021;

published online August 6, 2021

**Abstract:** Since 2011 we have been documenting seaweed diversity and abundance along a poorly studied area of the central coast of British Columbia, Canada. This first installment focuses on the Chlorophyta. To date, 42 species have been recorded, and we have obtained DNA sequences for most. Although most of these species reportedly have wide distributions along the west coast of North America, others appear to represent new northern or southern records or possible introductions, and a number have yet to be described. New southern limits are recorded for *Acrosiphonia sonderi*, *Acrosiphonia* sp., *Protomonostroma undulatum*, and *Ulva pouliotii* sp. nov., and new northern records are documented for *Ulva expansa*, *U. stenophylla*, and another undescribed species of *Ulva*. Among species of Cladophoraceae, we obtained a sequence only for *Cladophora opaca*, a Japanese species not previously recorded from North America, and an undetermined species of *Rhizoclonium*. We sequenced three species of *Derbesia*, none of which is *D. marina*, the currently recognized species

for this area; all three require taxonomic treatment. A sequence for a shell-boring species, which is tentatively identified as *Eugomontia sacculata*, was also obtained. These findings extend our understanding of the diversity and biogeography of green macroalgae in the northeast Pacific.

**Keywords:** British Columbia; Calvert Island; central coast; Chlorophyta; Hakai; taxonomy.

## 1 Introduction

As we gain a broader understanding of the impacts of climate change on species diversity and abundance, the importance of long-term data sets in documenting these changes has never been more relevant. This is especially true for seaweeds, which are responsive to environmental changes like marine heat waves (Harley and Paine 2009; Sanford et al. 2019; Weitzman et al. 2021), ocean acidification (Britton et al. 2019; Yoon et al. 2016) and species invasions (Davidson et al. 2015; Williams and Smith 2007). The creation of the Hakai Institute Calvert Island Field Station within the Hakai Lúxvbálís Conservancy of the Province of British Columbia has provided the base from which intensive studies, including long-term monitoring, of the central coast of British Columbia can be carried out.

The central coast of British Columbia runs from approximately the north end of Aristazabal Island at ~52° 49' N to the entrance to Queen Charlotte Strait at ~50°59' N. It does not include the offshore Haida Gwaii archipelago. Our study sites on Calvert Island are near 51°39' N, just north of Queen Charlotte Strait and near the southern end of Hecate Strait. Calvert is a coastal island between the open Pacific Ocean and the protected waters of the Inside Passage and coastal fjords extending inland to the Coast Range, or between Outer and Inner Pacific Marine Shelf Ecoregions (Zacharias et al. 1998). Zacharias et al. (1998) recognized the area as the Queen Charlotte Sound ecosystem; it is characterized by a wide, deep shelf with

---

\*Corresponding author: Sandra C. Lindstrom, Hakai Institute, Heriot Bay, BC V0P 1H0, Canada; and Current address: Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada,  
E-mail: [sandra.lindstrom@botany.ubc.ca](mailto:sandra.lindstrom@botany.ubc.ca). <https://orcid.org/0000-0001-5838-8595>

Matthew A. Lemay and Patrick T. Martone, Hakai Institute, Heriot Bay, BC V0P 1H0, Canada; and Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada, E-mail: [matt.lemay@hakai.org](mailto:matt.lemay@hakai.org) (M.A. Lemay), [patrick.martone@botany.ubc.ca](mailto:patrick.martone@botany.ubc.ca) (P.T. Martone)

Samuel Starko and Katharine R. Hind, Hakai Institute, Heriot Bay, BC V0P 1H0, Canada; Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada; and Department of Biology, University of Victoria, PO Box 1700 Station CSC, Victoria, BC V8W 2Y2, Canada,  
E-mail: [samuel.starko@gmail.com](mailto:samuel.starko@gmail.com) (S. Starko), [khind@uvic.ca](mailto:khind@uvic.ca) (K.R. Hind)

several large banks and channels, exposure to ocean waves and oceanic water intrusions, and is more oceanic and marine than the Vancouver Island Shelf to the south and Hecate Strait to the north.

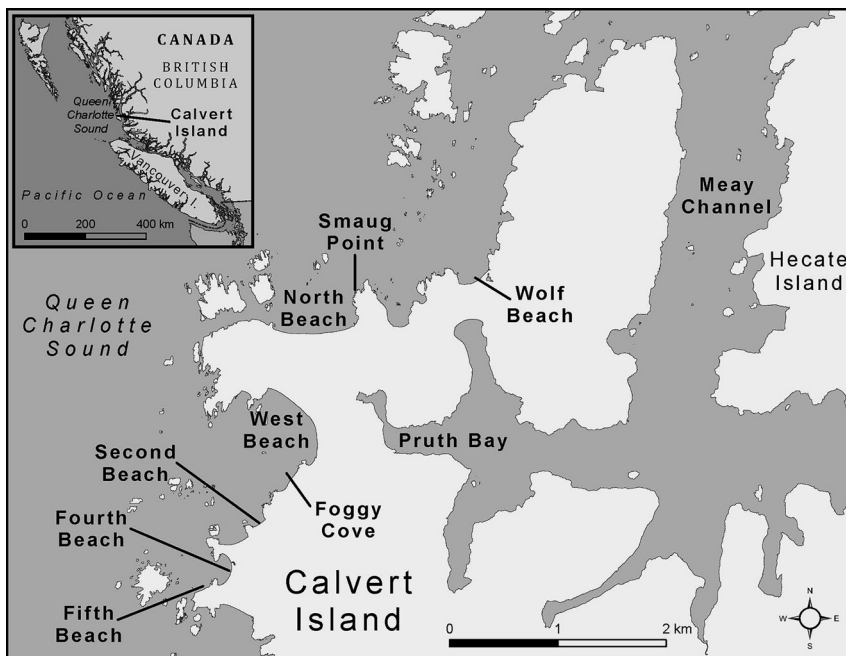
Physical processes, such as oceanography, are important in understanding the dynamics and history of any seaweed flora. Along this part of coastal British Columbia, the wind regime has two seasons, an upwelling-dominated season driven by northwesterly winds through Hecate Strait and Queen Charlotte Sound from April to September, and a downwelling-dominated season of southeasterly winds from September to April (Burd et al. 2019; Thomson 1981). Weak northerly winds in summer can lead to the formation of a clockwise gyre at the entrance to Queen Charlotte Sound around Goose Bank (Crawford et al. 1985; Sealy et al. 2013) and a shoreward, upwelling-driven flow in Moresby Trough (Hannah et al. 1991). These currents are illustrated by Jackson et al. (2015, Figure 1). The bifurcation of the Kuroshio current into the Alaska Current and the California Current usually occurs to the south of Calvert Island. Over the preceding 40 years, the strength of upwelling and downwelling due to these winds has increased (Foreman et al. 2011).

Conditions in the eastern North Pacific have changed significantly since 1988/89, including warming, declining temporal variance in the Aleutian low (Litzow et al. 2020), and suppression of storminess (Mundy et al. 2010). Cummins and Masson (2014) also noted long-term warming and freshening of the coastal waters of British Columbia. Whether these changes are having an effect on seaweed species along the coast remains to be determined.

Temperature in particular has long been recognized as the major factor affecting seaweed distributions (Lüning 1990; Setchell 1920). Also working on Calvert Island, Whalen et al. (Unpublished) noted changes in abundances and vertical distributions of many seaweed species, leading to complex changes in community and ecosystem dynamics, in response to the marine heat wave of 2014–2016.

The Calvert Island area has maintained a relatively stable coastal environment during the past 15,000 years. Perhaps unique along the northeast Pacific coast, the sea level at Calvert Island has remained mostly unchanged since the Last Glacial Maximum, unlike inland areas where sea level was elevated due to glacial loading, or Haida Gwaii where sea level was depressed due to a glacial forebulge effect (McLaren et al. 2014). Despite this, there is evidence that Calvert Island was glaciated during the last glacial maximum 18,000 years ago and again between 14,200 and 13,800 years BP (Eamer et al. 2017). However, Taylor et al. (2014) and others hypothesized that glaciers did not extend to the edge of the continental shelf in Queen Charlotte Sound, leaving open the possibility of a marine refugium in the area.

Although green seaweeds of the northeast Pacific have been studied intermittently since Setchell and Gardner's (1920) monograph, there has been no comprehensive coverage after Scagel (1966). Scagel et al. (1989) thoroughly updated the literature for British Columbia and adjacent regions, but it has been the series of *Keys to the Seaweeds and Seagrasses of Southeast Alaska, British Columbia, Washington, and Oregon* that have kept knowledge of that



**Figure 1:** Map showing location of Calvert Island along the central coast of British Columbia (inset) and major collecting sites on the northwest coast of Calvert Island.

flora up to date (Gabrielson and Lindstrom 2018). Here we describe the species of green seaweeds (Chlorophyta) that we have documented in the Hakai area of the central coast of British Columbia since 2011. This paper represents the first of three reports on seaweed diversity by phylum in this area and further updates the flora for the region as covered by Gabrielson and Lindstrom (2018). In addition to reporting new records, we also discuss species and species complexes that require taxonomic work, and describe one previously unnamed species.

## 2 Materials and methods

Specimens were collected during annual intertidal biodiversity surveys each summer since 2011 on the northwest corner of Calvert Island and vicinity (Figure 1), including subtidal collections in 2013 and 2017–2019, winter visits in 2014, 2015 and 2018, and Hakai Institute Bioblitzes in 2017, 2018, and 2019. There was no sampling in 2020 because of COVID-19 restrictions. Initial efforts focused on particular groups of interest to the investigators but, with the Bioblitzes, this effort was expanded to cover all observed seaweeds at many more sites and extending into the subtidal.

Specimens were initially identified following Gabrielson and Lindstrom (2018). A sample was removed from each individual and desiccated in silica gel or preserved in 95% ethanol for sequencing. Herbarium vouchers were prepared, and most of these specimens are deposited in the phycological herbarium of the Beaty Biodiversity Museum, University of British Columbia (UBC).

Sequencing was carried out targeting a section of the chloroplast *tufA* gene and/or nuclear ribosomal ITS (Internal Transcribed Spacer) region (Table 1). These markers were used as they have previously been shown to have species-level resolution within the Chlorophyta (Lindstrom and Hanic 2005; Saunders and Kucera 2010). Samples were either submitted to the Canadian Centre for DNA Barcoding (CCDB) for DNA extraction, amplification and sequencing or handled in the Lindstrom or Martone laboratories following Lindstrom and Hanic (2005) or Saunders and Kucera (2010) protocols. Sequences were compared to accessions in GenBank and in the Barcode of Life Data system (BOLD; Ratnasingham and Hebert 2007). Pairwise distances between genotypes were calculated using MEGA version X (Kumar et al. 2018), and a maximum likelihood tree was generated using the same program.

## 3 Results

We sequenced fragments of the *tufA* gene and the ITS region in 69 Chlorophyta specimens, corresponding to 34 species (Table 2). An additional eight species have been identified morphologically but have yet to be sequenced; thus, the occurrence of these species at Hakai remains tentative: *Chaetomorpha linum* (O.F.Müller) Kützing, *Chaetomorpha melagonium* (F.Weber et Mohr) Kützing,

**Table 1:** Primers used in PCR amplifications and sequencing.

<i>TufA</i> primer	Primer sequence	Reference
TufGF4	5' GGNGCNGCNCAAATGGAYGG 3'	Saunders and Kucera (2010)
TufAFU	5' GCRAAACAAAGTTGGWGTGCCWG 3'	Lindstrom (Personal observation)
TufAR	5' CCTTCNCGAATMGCRAAWCGC 3'	Saunders and Kucera (2010)
TufARU	5' CRATCATYTTAACWCGRTCNC 3'	Lindstrom (Personal observation)
ITS primer		
ITS1 (F)	5' TCCGTAGGTGAACCTGCGG 3'	White et al. (1990)
ITS5 (F)	5' GGAAGTAAAAGTCGTAACAAGG 3'	White et al. (1990)
ITS4 (R)	5' TCCTCCGCTTATTGATATGC 3'	White et al. (1990)
JO6 (R)	5' ATATGCTTAAGTTCAGCGGGT 3'	Lindstrom et al. (1996)

*Cladophora columbiana* Collins, and *Cladophora sericea* (Hudson) Kützing belong to the Cladophoraceae, a family that is notoriously difficult to sequence (Saunders and Kucera 2010). *Percursaria percursa* (C.Agardh) Rose-ninge has a distinctive morphology; currently only a single species is recognized (O'Kelly et al. 2004a), and it is widely distributed on the west coast of North America from Alaska to California (Scagel et al. 1989). *Blidingia dawsonii* (Hollenberg et Abbott) S.C. Lindstrom, Hanic et Golden is a common epiphyte on limpets and is known from northern British Columbia to Baja California, Mexico (Lindstrom et al. 2006). *Kornmannia leptoderma* (Kjellman) Bliding and *Ulvaria* sp. were first collected only in 2021 and have yet to be sequenced. Below, we provide notes on records of sequenced specimens following the order of families in Gabrielson and Lindstrom (2018). A complete list of these species is found in Supplementary Table S1.

### 3.1 Family Bryopsidaceae

We obtained a *tufA* sequence from a single *Bryopsis* specimen collected in the low intertidal at an exposed point near Fifth Beach. It was one of only two specimens collected at Hakai. The sequence matched *Bryopsis corticulans* Setchell, a species originally described from Pacific Grove and Carmel Bay, California. Sequences from Hakai and Monterey Bay (Santa Cruz) were identical to each other but only 90% similar to *Bryopsis plumosa* (Hudson) C.Agardh, another species thought to occur in British Columbia but unverified. Records of *Bryopsis*, likely

**Table 2:** Sample information and collection data for sequenced specimens.

Species	Sample ID	Collection date	Location	Habitat	UBC accession	ITS accession	<i>tufA</i> accession
<i>Bryopsis corticulans</i>	PTM573	28 May 2013	Fifth Beach exposed	Low intertidal vertical bedrock	A89894	NA	MZ198525
<i>Derbesia</i> sp. 1	PTM1605	26 Jul 2017	Mouth of Kwakshua Channel	Subtidal 8.9 m, on rhodolith	A93242	NA	MZ198513
<i>Derbesia</i> sp. 2	SCL 16976	23 Jul 2017	Smaug Point	Low intertidal bedrock	NA	NA	MZ198516
<i>Derbesia</i> sp. 3	SCL 17069	26 Jul 2017	Foggy Cove	Subtidal 5.5 m	A94336	NA	MZ198517
<i>Codium fragile</i> subsp. <i>californicum</i>	SCL 17094	28 Jul 2017	Foggy Cove	Shallow subtidal	A94360	NA	MZ198518
<i>Codium setchellii</i>	SCL 17167	31 Jul 2017	West Beach islets	Subtidal	A94412	NA	MZ198519
<i>Cladophora opaca</i>	SCL 16768	25 May 2017	Fifth Beach	High intertidal vertical shaded bedrock	A94152	MZ153100	NA
<i>Rhizoclonium</i> sp.	PTM1728	13 Jun 2018	Fifth Beach	High intertidal bedrock	NA	MZ153098	NA
<i>Prasiola borealis</i>	SCL 17329	14 Jun 2018	North Beach	Supralittoral (in trees)	NA	NA	MZ198522
<i>Prasiola delicata</i>	PTM1537	29 May 2017	Little Wolf Beach	Supralittoral	A93588	NA	MZ198511
<i>Prasiola meridionalis</i>	PTM1538	29 May 2017	North Beach	Supralittoral	A93589	NA	MZ198512
<i>Monostroma</i> sp.	SCL 17330	14 Jun 2018	North Beach	Supralittoral	NA	NA	MZ198523
<i>Collinsiella tuberculata</i>	SCL 16806	27 May 2017	Meay Channel	Low intertidal cobble	A94178	MZ153104	MZ401486
	PTM1544	27 May 2017	North Beach	Mid intertidal bedrock	NA	MZ153097	NA
	SCL 17328	14 Jun 2018	North Beach	Mid intertidal bedrock edge of tidepool	NA	MZ153105	NA
<i>Eugomontia sacculata</i>	SCL 15669	12 Jun 2014	Head of Pruth Bay	Low intertidal mud, in cobble shell	NA	MZ153099	NA
	SCL 17343	15 Jun 2018	Meay Channel	Low intertidal cobble/pebble beach in shell	NA	MZ153106	NA
<i>Acrosiphonia arcta</i>	SCL14988	04 Jul 2012	North Beach	Low intertidal cobble in sand	A90440	MW939434	MW921441
	SCL15015	05 Jul 2012	Fifth Beach	High intertidal bedrock	A90471	MW939435	MW921442
	SCL15281	24 May 2013	Fifth Beach	Boulders	A90272	MW939436	MW921443
	SCL15685	14 Jun 2014	Fifth Beach	Mid intertidal bedrock	A90830	NA	MW921444
	SCL 15687	14 Jun 2014	Fifth Beach	High intertidal bedrock in sand	NA	MW939437	NA
	SCL 16821	28 May 2017	North Beach	Low intertidal rock in sand	A94190	MW939439	MZ401489
	SCL 16769	25 May 2017	Fifth Beach	High intertidal bedrock	A94153	MZ153101	NA
	SCL 16796	26 May 2017	North Beach	Low intertidal bedrock	A94172	MW939438	NA
	MAL 66	12 Aug 2011	Foggy Cove	Intertidal rock	NA	NA	MK930522
	MAL 528	21 May 2013	Foggy Cove	Intertidal rock	NA	NA	MK930520
<i>Acrosiphonia coalita</i>	SCL 14807	12 Aug 2011	Foggy Cove	Low intertidal boulder	NA	MW939440	MW921445
	SCL 15255	21 May 2013	Foggy Cove	Lower mid intertidal boulder/cobble	A90364	MW939441	NA
	SCL 16795	26 May 2017	North Beach	Low intertidal bedrock	A94171	MZ153102	MZ401491
	SCL 16819	28 May 2017	North Beach	Low intertidal rock in sand	A94188	MW939442	NA
	SCL 17030	25 Jul 2017	Fifth Beach exposed	High intertidal barnacles	A94300	MW939443	NA
	SCL 17423	28 Jun 2018	North Beach	Lower mid intertidal nearly vertical bedrock near sand	NA	MZ153108	MZ401488
	MAL 58	21 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930529
	MAL 424	19 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930528
	MAL 437	21 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930531
	MAL 523	19 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930525
<i>Acrosiphonia sonderi</i>	SCL 14987	04 Jul 2012	North Beach	Low intertidal cobble in sand	A90439	MW939444	MW921446

Table 2: (continued)

Species	Sample ID	Collection date	Location	Habitat	UBC accession	ITS accession	<i>tufA</i> accession
	SCL 15315	27 May 2013	North Beach	Low intertidal small boulder/cobble in sand	A90350	MW939445	MW921447
<i>Acrosiphonia</i> sp.	SCL 15882	17 Jun 2015	Meay Channel	Low intertidal boulder/cobble in sand	NA	MW939446	MW921448
	SCL 15883	17 Jun 2015	Meay Channel	Low intertidal boulder/cobble in sand	NA	NA	MW921449
	SCL 16799	27 May 2017	Meay Channel	Low intertidal cobble	NA	MZ153103	NA
<i>Proto-monostroma undulatum</i>	MAL 445	18 Mar 2015	Foggy Cove	Intertidal rock	NA	MZ153096	MZ401485
<i>Urospora neglecta</i>	SCL 15258	21 May 2013	Foggy Cove	High intertidal boulder	A90365	NA	MW921451
	SCL 17354	16 Jun 2018	Smaug Point	High intertidal plate	NA	MZ153107	MZ401487
<i>Urospora wormskioldii</i>	SCL 15260	21 May 2013	Foggy Cove	Lower mid intertidal cobble	A90357	NA	MW921452
<i>Blidingia marginata</i>	SCL 17365	16 Jun 2018	Pruth Bay	On piling near high tide level	NA	NA	MZ198524
<i>Blidingia</i> sp.	MAL 457	19 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930524
	MAL 468	19 Mar 2015	Foggy Cove	Intertidal rock	NA	NA	MK930526
<i>Ulva compressa</i>	SCL 15722	16 Jun 2014	Pruth Bay	Low intertidal boulder	A90773	MW939384	NA
<i>Ulva expansa</i>	SCL 16360	08 Jun 2016	Meay Channel	Low intertidal	A92863	NA	MW921453
	SCL 17155	31 Jul 2017	Kelpie Point	Subtidal, on shell	A94402	MW939385	MW921454
	SCL 17219	01 Aug 2017	North Beach	Subtidal	A94440	MW939386	MZ401490
	SCL 17318	12 Jun 2018	Foggy Cove	Low intertidal boulder	NA	NA	MZ198521
<i>Ulva fenestrata</i>	SCL 15328	27 May 2013	North Beach	Mid intertidal rock	A90347	MW939387	MW921455
	SCL 15727	16 Jun 2014	Pruth Bay	Drift	A90778	MW939388	NA
<i>Ulva intestinalis</i>	SCL 15373	28 May 2013	Second Beach	Supralittoral bedrock seepage area	A90299	NA	MW921456
	SCL 15644	20 Feb 2014	Fifth Beach	Above high intertidal pool	A90958	MW939389	MW921457
	SCL 16778	25 May 2017	Fourth Beach	Mid intertidal boulder in sand	A94160	NA	MZ198515
<i>Ulva linza</i>	SCL 14731	13 Aug 2011	North Beach	Low intertidal cobble	A89104	MW939390	MW921458
	SCL 15727	16 Jun 2014	Pruth Bay	Unattached in low intertidal	A90778	NA	MW921459
	SCL 15904	19 Jun 2015	North Beach	Low intertidal rock in sand	A92824	MW939391	MW921460
	SCL 17317	12 Jun 2018	Foggy Cove	Low intertidal boulder	NA	NA	MZ198520
<i>Ulva pouliotii</i>	SCL 14726	13 Aug 2011	North Beach	Supralittoral pool	A89091	MW939392	MW921461
<i>Ulva prolifera</i>	SCL 14717	12 Aug 2011	Pruth Bay	Low intertidal wood	A89124	MW939398	MW921465
<i>Ulva</i> sp.	SCL 17065	27 Jul 2017	North Beach	Low intertidal rock in sand	A94332	MW939399	MW921466
<i>Ulva stenophylla</i>	PTM1670	04 Aug 2017	Choked Pass	Subtidal 5 m, on seagrass	A93302	NA	MZ198514
<i>Ulva "torta"</i>	SCL 14718	12 Aug 2011	Pruth Bay	Mid intertidal mud	A89078	MW939400	MW921467
<i>Ulvaria obscura</i> var. <i>blyttii</i>	PTM414	22 May 2013	Wolf Beach	Shallow subtidal boulder	A90361	MW939401	MW921468

GenBank accession numbers are given for ITS and *tufA* sequences deposited there. Voucher specimens for most are deposited in the UBC Beaty Biodiversity Museum and can be searched for there (<https://herbweb.botany.ubc.ca/herbarium/search.php?Database=algae>) by collector number. NA, not available.

*B. corticulans*, extend north to Sea Otter Sound in Southeast Alaska (UBC A69717).

We obtained three distinct *tufA* sequences for specimens of *Derbesia*, which suggests cryptic diversity within the genus. Two were for the gametophytic Halicystis phase, and one was for the sporophytic phase. A gametophyte from a subtidal *Nereocystis* bed on boulders had a sequence 100% identical to an undetermined *Derbesia* specimen

from the Prince Rupert area (HQ610251) and 99% similar to specimens from Bamfield (KM255028) and Haida Gwaii (KM254998, KM255030). A sporophyte from low intertidal bedrock was only 98% similar to these specimens. Neither specimen was more than 94% similar to *D. marina* (Lyngbye) Solier from the northwestern Atlantic whereas PTM1605 (Figure 2), a gametophyte, was 96% similar to NW Atlantic *D. marina* but only 93% similar to the other Hakai

specimens. The only name that might apply to any of these species is *Derbesia pacifica* Jao (1937). The type specimen (Jao 1273B) was collected as an epiphyte on *Calliarthron tuberosum* in the upper subtidal of Turn Island, San Juan Islands, Washington, Sept 1935. The whereabouts of Jao's type specimen is unknown. In contrast, the type locality of *D. marina* is the Faroe Islands (Silva et al. 1996).

### 3.2 Family Codiaceae

*TufA* sequences were also obtained for both *Codium fragile* subsp. *californicum* (J.Agardh) Maggs et J.Kelly and *C. setchellii* N.L. Gardner. We did not find *C. Ritteri* Setchell et N.L. Gardner, which has only been reported as far south as Campania I., BC at 53.18° N 129.54° W (Gabrielson and Lindstrom 2018).

### 3.3 Family Cladophoraceae

We obtained a sequence of the ITS region for a *Cladophora* specimen from shaded, vertical, high intertidal bedrock at Fifth Beach (Figure 3). This sequence was identical to the sequence of *Cladophora opaca* Sakai from Lake Hiruga, Japan (Hayakawa et al. 2012). Sakai's original material (SAP 029181) was collected 13 April 1948 on the Sea of Japan coast at Kamome-jima, Esashi, Hokkaido, Japan—almost due west of Hakodate (Sakai 1964: 62). This specimen was coarser than other specimens we identified as *C. sericea* in the Hakai area (Figure 4). These latter specimens, collected from a variety of habitats (Meay Channel site, low intertidal rock in sand between Fourth and Fifth Beaches, and high intertidal tidepool at the exposed Fifth Beach site), did not sequence; however, they were morphologically similar to a specimen of *C. sericea* from Southeast Alaska sequenced by Bakker et al. (1995). *Cladophora opaca* is the second species of Japanese origin found on Calvert I. (Lindstrom 2018), possibly reflecting recent origins related to marine debris from the Great Tohoku Earthquake and Tsunami of 2011.

As noted above, we were unable to obtain a sequence for *C. columbiana*. This common mid to high intertidal species, usually associated with tidepools, was originally described from material collected near Port Renfrew, British Columbia. Given the similarity of that area to Hakai, as well as its proximity, it is highly likely the same species occurs in both areas.

We also did not obtain sequences for *Chaetomorpha linum* or *C. melagonium*. Boedeker et al. (2016, 2017) showed that the genus *Chaetomorpha* appears to be monotypic: the



Figure 2: *Derbesia* (Halicystis phase, UBC A93242), subtidal on a rhodolith, mouth of Kwakshua Channel, 26 Jul 2017.



Figure 3: *Cladophora opaca* (UBC A94152), high intertidal vertical bedrock, Fifth Beach, 25 May 2017. Scale bar = 2 cm.



Figure 4: *Cladophora sericea* (UBC A94181), low intertidal, Meay Channel site, 27 May 2017. Scale bar = 5 cm.

type species, *C. melagonium*, occurs at the base of a branch including subclades with other species of *Chaetomorpha* and with the type species of *Cladophora* and *Rhizoclonium*. Although ITS sequences are slightly divergent (<0.5%) between northeast Atlantic *C. melagonium* (type locality: Varberg, Sweden) and other regions (northwest Atlantic, Arctic and North Pacific), the authors chose to maintain a single species. We identified a different, morphologically highly variable species as *Chaetomorpha linum*. Boedeker et al. (2016) showed that northeast Pacific specimens variously identified as *C. aerea* (Dillwyn) Kützing, *C. brachygona* Harvey, *C. californica* Collins and *C. linum* had LSU fragment sequences at least 99.5% similar to each other. Of these, *C. linum* is the oldest name. Contemporary specimens from North Sea and Baltic Sea sites in Germany (with the Danish type locality between them) also had sequences at least 99.5% similar to these, suggesting it is reasonable to identify our material as *C. linum*.

We obtained an ITS sequence from a single specimen of *Rhizoclonium* (PTM1728) collected from the high intertidal at Fifth Beach. This was 96% similar to an unidentified species of *Rhizoclonium* from China (GenBank KC914570) and 95% similar to a specimen identified as *R. hieroglyphicum* (C.Agardh) Kützing, possibly from the UK, but also 96% similar to *Chaetomorpha melagonium* according to GenBank BLAST. It is clear that significant work remains to be done on the taxonomy of this group before we can identify the Hakai specimen to species.

### 3.4 Family Prasiolaceae

The *tufA* sequence from a *Prasiola* specimen collected above the supralittoral beneath a tree canopy was at least 99.6% similar to *P. furfuracea* (Mertens ex Hornemann) Trevisan (LN877826) of Heesch et al. (2016) and *P. borealis* M.Reed of Moniz et al. (2014) and Garrido-Benavent et al. (2017). Since *P. borealis* has type localities of Unalaska and Kodiak Islands, Alaska, whereas *P. furfuracea*, which is the older name, has a type locality of Denmark, we follow AlgaeBase (Guiry and Guiry 2021) and identify our specimen(s) as *P. borealis*. Both species are distinguished by cells occurring in groups; they may or may not be infected with a fungus, which turns them black.

SCL 17330 and PTM1538 were 100% identical to *P. stipitata* Suhr ex Jessen (KF993452) of Moniz et al. (2014) and most GenBank *P. meridionalis* Setchell et N.L. Gardner, also of Moniz et al. (2014). *Prasiola stipitata* was described from various northern European localities (Baltic, near Sandwieck, Slesvig; Ireland; Norway)

whereas *P. meridionalis* was originally collected at Neah Bay, Washington. The sequence of *P. “meridionalis”* from Bodega Marine Lab, CA (KF993444) was only 95% similar to other *P. meridionalis* sequences, suggesting that California populations represent a different species. As suggested by its name, European *P. stipitata* can have a long stipe whereas local specimens usually have a short, broad stipe. Therefore, for the present, we continue to use the name *P. meridionalis* for our species, as does AlgaeBase (Guiry and Guiry 2021).

PTM1537 was identified as *P. delicata* Setchell et N.L. Gardner. This sequence was 100% identical to specimens identified as this species from northeast Hokkaido, Japan (KT354070, Sutherland et al. 2016), northeast Vancouver Island (KF993454, Moniz et al. 2014), Prince Rupert area (HQ610263, Saunders and Kucera 2010), and Petersburg, Alaska (KY029020, Garrido-Benavent et al. 2017). The species, originally described from near the upper tidemark on rocky islets near Sitka, Alaska (Setchell and Gardner 1920), is distinguished by its small stature and thin blade. Sequences of this species were no more than 96% similar to other species of *Prasiola*.

We did not observe any specimens of *Rosenvingiella* or *Rosenvingiellopsis*, despite the area falling within range of their species (Scagel et al. 1989).

### 3.5 Family Gomontiaceae

Although our *Monostroma* specimen had a *tufA* sequence 99.85% similar to specimens identified as *M. grevillei* (Thuret) Wittrock from the NW Atlantic (HQ610253, HQ610255, HQ610258, HQ610259—the only differences being apparent misreads near the ends of the sequences), this specimen also had an ITS sequence 99.5% similar to NW Pacific *M. angicava* Kjellman but only 96.6% identical to NE Atlantic *M. grevillei*. The type locality of *M. grevillei* is Finistère, Bretagne, France (Burrows 1991) and that of *M. angicava* is Finnmark, Norway (Kjellman 1883). Since the identity of NW Pacific *M. angicava* with a Norwegian type specimen has not been confirmed, we are left with identifying our species as *Monostroma* sp. for now. Hakai specimens were encountered seasonally, in spring up to the first week of June but not in all years.

*Collinsiella tuberculata* Setchell et N.L. Gardner represents another species of Gomontiaceae (O’Kelly et al. 2004b), a family with a shell-boring, polyrhizoidal Codium (sporophyte) phase. Our specimens, from the edge of mid intertidal pools at North Beach (Figure 5), had ITS sequences identical to those of O’Kelly et al. (2004b) from southern BC. The species is known to occur from Northern



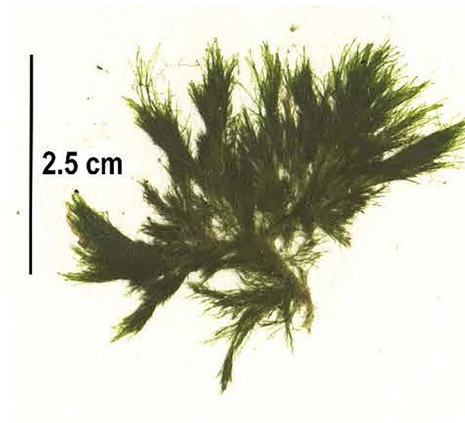
**Figure 5:** *Collinsiiella tuberculata*, along edge of mid intertidal pool, North Beach, 2 Jun 2019.

Southeast Alaska (Klokachef Island) to Central California (Pacific Grove) on the Pacific coast of North America (Scagel et al. 1989).

We also obtained ITS sequences for a shell-inhabiting species in this family. The two sequences were essentially identical, the single base-pair difference being a possible misread. These sequences did not show significant similarity to other sequences in GenBank. Both were from bivalve shells (clam and cockle) from low intertidal sandy/muddy habitats in Meay Channel and Pruth Bay (both protected sites, Figure 1). The presence of *Gomontia* in the region has not been confirmed by culture or molecular studies (Gabrielson and Lindstrom 2018) so this entity is tentatively identified as *Eugomontia sacculata* Kornmann.

### 3.6 Family Ulotrachaceae

Four species of *Acrosiphonia* have been confirmed by sequencing: *A. arcta* (Dillwyn) Gain, *A. coalita* (Ruprecht) Scagel, Garbary, Golden et M.W. Hawkes, *A. sonderi* (Kützing) Kornmann sensu Saunders and Kucera (2010), and an undescribed species. *Acrosiphonia arcta* (Figure 6) represents the Pacific form of the species, for which the ITS differed by 0.3% from North Atlantic specimens; we consider this insufficient to recognize separate species. ITS sequences of *A. coalita* differed by 0.4% from specimens from northern California, the type locality, again insufficient to warrant distinct species. Both *Acrosiphonia* sp. (Figure 7) and *A. sonderi* (Figure 8) are more common in western Alaska (Lindstrom, Personal observation); the Hakai specimens represent southern and eastern limits of both species. Whereas *A. arcta* and *A. coalita* (Figures 9 and



**Figure 6:** *Acrosiphonia arcta* (UBC A90272), low intertidal boulder, Fifth Beach, 24 May 2013. Scale bar = 2.5 cm.



**Figure 7:** *Acrosiphonia* sp. (SCL 15882), low intertidal small boulder, Meay Channel site, 17 Jun 2015. Scale bar = 2.5 cm.

11) were widespread at outer coast rocky sites (the former throughout the intertidal, the latter mostly at lower elevations), *A. sonderi* was collected only on low intertidal rock scoured by sand at North Beach (Figure 10). In contrast, *Acrosiphonia* sp. was collected in the low intertidal only at the protected Meay Channel site (Figure 12). While we are only beginning to understand the ecological diversity within this genus, it is noteworthy that these four species mostly occupy distinct niches around Calvert Island.

*TufA* and ITS sequences of *Protomonostroma undulatum* (Wittrock) K.L.Vinogradova represent a southern record of the species in the northeast Pacific. The species has also been collected in Alaska (Table 3) and at Triple Island, northern BC (Golden and Garbary 1984, as *Monostroma*). Our Hakai specimen, collected 15 Mar 2018, represented only the basal portion of the thallus, suggesting that the blade had already disintegrated, post-reproduction.





**Figure 8:** *Acrosiphonia sonderi* (UBC A90439), low intertidal cobble in sand, North Beach, 04 Jul 2012. Scale bar = 5 cm.



**Figure 9:** *Acrosiphonia coalita* (UBC A90364), lower mid intertidal boulder/cobble, Foggy Cove, 21 May 2013. Scale bar = 5 cm.

Sequences of both *Urospora neglecta* (Kornmann) Lokhorst *et* Trask (*tufA* and ITS) and *U. wormskioldii* (Mertens *ex* Hornemann) Rosenvinge (*tufA*) were also obtained. *Urospora penicilliformis* was not recorded from the area despite Hanic (2005) recording it as the most common *Urospora* in the upper littoral from southern California to Homer, Alaska.



**Figure 10:** *Acrosiphonia sonderi*, low intertidal boulder, North Beach, 5 Jun 2019.



**Figure 11:** *Acrosiphonia coalita*, low intertidal small boulder, Foggy Cove, 29 May 2017.

### 3.7 Family Kornmanniaceae

We obtained *tufA* sequences for two *Blidingia* species. One was collected from near high tide level on a piling of the Pruth Bay dock; this specimen was identified as *B. marginata* (J.Agardh) P.J.L.Dangeard *ex* Bliding based on recent studies by Steinhagen *et al.* (2021). The second species was collected from Foggy Cove; it was identical to an undetermined *Blidingia* species from Korea (GenBank MK992087 *et al.*) and possibly represents another introduced species from Asia (see *Cladophora opaca* above). As noted previously, we tentatively identified other specimens



**Figure 12:** *Acrosiphonia* sp., low intertidal small boulder, Meay Channel site, 27 May 2017.

as *B. dawsonii* based on morpho-anatomy, but were unable to sequence them.

Specimens of *Kormmannia leptoderma* were first observed in 2021 as epiphytes on old, disintegrating blades of *Phyllospadix* spp.

### 3.8 Family Ulvaceae

We confirmed the occurrence of 10 species of *Ulva* at Hakai based on sequence data (Figures 13–23): *Ulva compressa* Linnaeus, *U. expansa* (Setchell) Setchell et N.L. Gardner, *U.*

*fenestrata* Postels et Ruprecht (formerly misidentified as *U. lactuca* Linnaeus), *U. intestinalis* Linnaeus, *U. linza* Linnaeus, *U. prolifera* O.F.Müller, *U. stenophylla* Setchell et N.L. Gardner, *U. “torta”* (Mertens) Trevisan, and two unnamed species, one of which we describe below (quotation marks indicate further work on the species is required). Two species (*U. compressa* and *U. “torta”*) were found only in protected waters whereas the other species occurred primarily on at least partially exposed shores. One of the undescribed species (SCL 17065; Figure 21) is the same species as a specimen from Humboldt Bay, CA, based on similarity to an ITS sequence (GenBank AY422520) and a specimen from Montara, CA, based on identity with a *tufA* sequence (GenBank KM255024). Our North Beach specimens, collected 27 July 2017 on low intertidal rock in sand, had cells  $\sim 12 \times 20 \mu\text{m}$ , in irregular rows, and with 1–3 pyrenoids; no other specimens in GenBank were more than 97% similar. Pairwise distances between species for both ITS and *tufA* sequences can be found in Table 4. Intra-specific variation, when more than one specimen in a species was sequenced, was 0.80% for *U. expansa* ITS, and averaged 0.19% for *U. expansa tufA* and 0.08% for *U. linza tufA*.

The *tufA* phylogeny of Hakai specimens of *Ulva* (Figure 24) was congruent with other recently published phylogenies of *Ulva* spp. (e.g., Melton and Lopez-Bautista 2021). Hakai specimens separated into two distinct groups. *Ulva compressa*, *U. expansa*, *U. fenestrata*, and *U. intestinalis* comprised one group (we did not obtain a *tufA* sequence for *U. compressa*, but its ITS sequence—data not shown—clearly

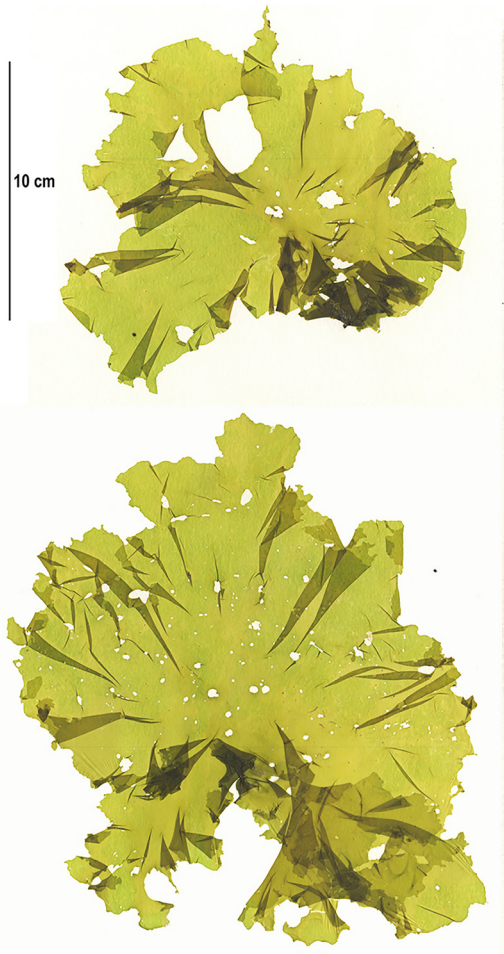
**Table 3:** Sequenced non-Hakai specimens referred to in the text, including collection data.

Species	Sample ID	Collection date	Location	Habitat	UBC accession	ITS accession	<i>tufA</i> accession
<i>Protomonostroma undulatum</i>	SCL 10583	15 Jun 2003	Shaw Island, AK 59.01° N 153.38° W	Intertidal	A86066	MW939447	NA
	SCL 13717	12 Jun 2008	Avatanak I., AK 54.08° N 165.38° W	Low intertidal, on <i>Mastocarpus</i>	A93898	MZ413899	NA
	SCL 14934	07 Jun 2012	Knoll Head, AK 59.63° N 153.51° W	Mid intertidal	A91334	MW939448	MW921450
<i>Ulva pouliotii</i> sp. nov.	Golden s/n	20 Feb 2005	Triple I., BC 54.29° N 130.88° W	High intertidal pool	NA	MW939393	MW921462
	Golden s/n	26 Apr 2005	Triple I., BC 54.29° N 130.88° W	High intertidal pool	NA	MW939394	NA
	SCL 11892	23 May 2005	Perevalnie Pass, AK 58.64° N, 152.37° W	High intertidal pool	NA	MW939395	NA
	SCL 13467	04 Jun 2008	Chichagof Hbr, AK 52.93° N 173.27° E	High intertidal bedrock	NA	MW939396	MW921463
	SCL 15161	01 Sep 2012	East Sanak I., AK 54.39° N 162.54° W	High intertidal small pool	A90118	MW939397	MW921464

GenBank accession numbers are given for ITS and *tufA* sequences deposited there. Voucher specimens for most are deposited in UBC and can be searched for there (<https://herbweb.botany.ubc.ca/herbarium/search.php?Database=algae>) by collector number. NA, not available.



**Figure 13:** *Ulva expansa* (UBC A94440), subtidal, North Beach, 1 Aug 2017. Scale bar = 10 cm.



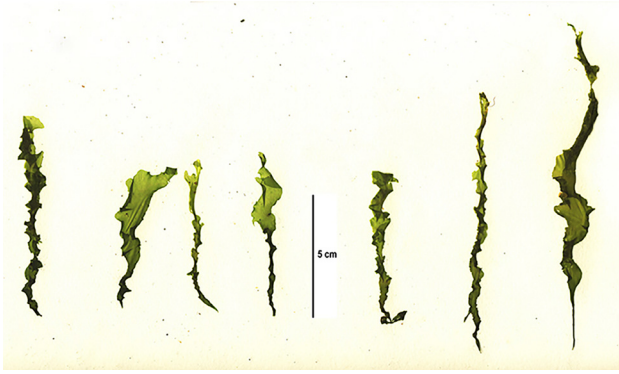
**Figure 14:** *Ulva fenestrata* (UBC A90347), mid intertidal rock, North Beach, 27 May 2013. Scale bar = 10 cm.

place it in this clade as do analyses by others). The second clade included the remaining species: *U. linza*, *U. prolifera*, *Ulva* sp., *U. torta*, *U. pouliotii* and *U. stenophylla*.



**Figure 15:** *Ulva intestinalis* (UBC A94160), mid intertidal boulder in sand, Fourth Beach, 25 May 2017. Scale bar = 10 cm.

Identification of specimens in the closely related *Ulva linza-procera-prolifera* clade is especially difficult due to morphological plasticity and misapplied names in published sequence databases. Cui et al. (2018) clarified the application of the name *U. prolifera* by examining populations from the type locality in Denmark, including sequencing. The ITS sequence of our Hakai specimen of this species differed by <0.5% from the Danish material.



**Figure 16:** *Ulva linza* (UBC A89104), low intertidal cobble, North Beach, 13 Aug 2011. Scale bar = 5 cm.

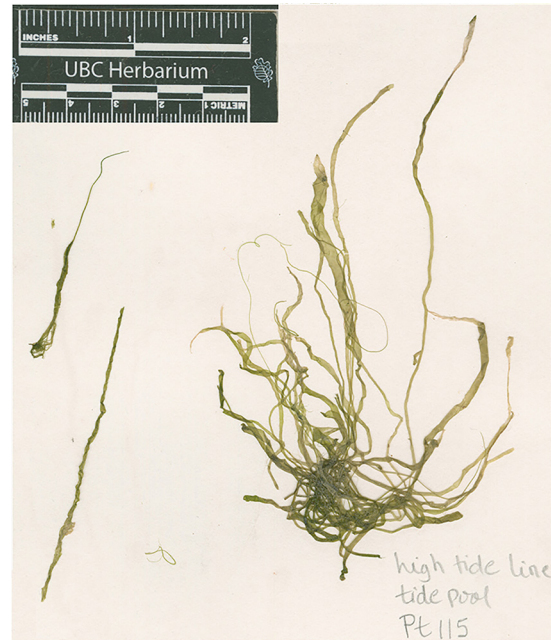


**Figure 17:** *Ulva pouliotii* (SCL 13467), high intertidal bedrock, Chichagof Harbor, Attu Island, Alaska, 4 Jun 2008. Scale bar = 5 cm.



**Figure 18:** *Ulva pouliotii* (UBC A89091), supralittoral pool, North Beach, 13 Aug 2011. Scale bar = 5 cm.

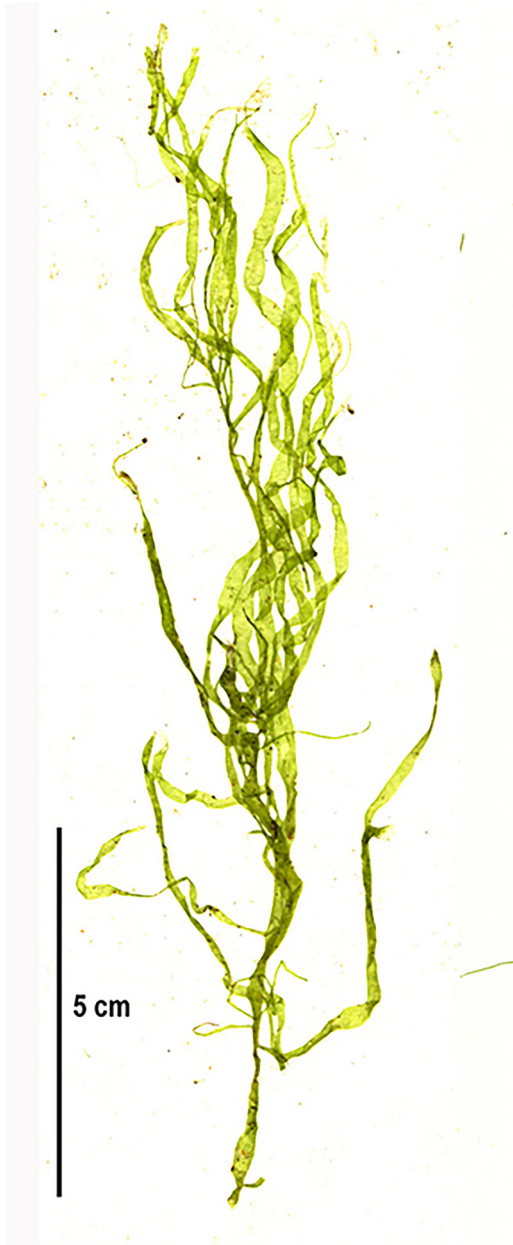
Differences between our specimens of *U. linza* (Figure 16) and *U. prolifera* (Figure 20) were 0.5 and 0.8% for ITS and *tufA*, respectively, indicating a much closer relationship between these two species than between any other pair of species (Table 4).



**Figure 19:** Holotype of *Ulva pouliotii* (UBC A90118), high intertidal small pool, eastern tip of Sanak Island, 1 Sep 2012. Scale bar = 5 cm.

Our specimens of *U. expansa* were identified originally as *U. lobata* (Kützinger) Harvey, based on identity with sequences in BOLD and GenBank. However, we now know that *U. lobata* is a synonym of *U. lactuca*, and published sequences of *U. "lobata"* are identical to *U. expansa* (Hughes et al. 2018, 2019). Local specimens of *U. expansa* (as *U. lobata*) had been distinguished by deeply lobed or divided blades, narrowing to a cuneate, crisped and often twisted base, with mature blades conspicuously thicker in the center than at the margins (Gabrielson and Lindstrom 2018). These features clearly apply to *U. expansa*, which was originally distinguished by its deeply ruffled margins and thickened center (Setchell and Gardner 1920). According to Setchell and Gardner (1920), the blades are attached to rock in the low intertidal zone for only a short time before becoming detached and increasing greatly in size while floating freely. This characterization fits the material of this species from Hakai (SCL 17318 was found attached to low intertidal boulders whereas SCL 17219 was collected, presumably unattached, from the subtidal). While *U. expansa* can form green tides in California (Hughes et al. 2018), only a few thalli have been observed at Hakai, and these only since 2016. Specimens are rarely perforate (Figure 13), in contrast to *U. fenestrata* (Figure 14), which commonly has holes, and *U. expansa* is usually a darker green and can appear stiffer than *U. fenestrata*.

The *Ulva* sp. from a high intertidal pool at the North Beach site was identical to sequences of specimens from other outer coast high intertidal pools from northern



**Figure 20:** *Ulva prolifera* (UBC A89124), low intertidal wood, Pruth Bay, 12 Aug 2011. Scale bar = 5 cm.

British Columbia to the western tip of the Aleutian Islands (Table 3). The Hakai collection (Figure 18) represents a southern limit for this species, which we describe below as new to science. We have been aware of this species since the 1970s, when culture work by Larry Golden indicated that a high intertidal tidepool species superficially resembling *U. intestinalis* lacked the typical early germination pattern of that species; rather, it more closely resembled that of *U. californica/scagelii* (Golden, pers. comm.). We describe the species below:



**Figure 21:** *Ulva* sp. (UBC A94332), low intertidal rock in sand, North Beach, 27 Jul 2017. Scale bar = 10 cm.

### 3.8.1 *Ulva pouliotii* sp. nov. Golden et S.C. Lindstrom

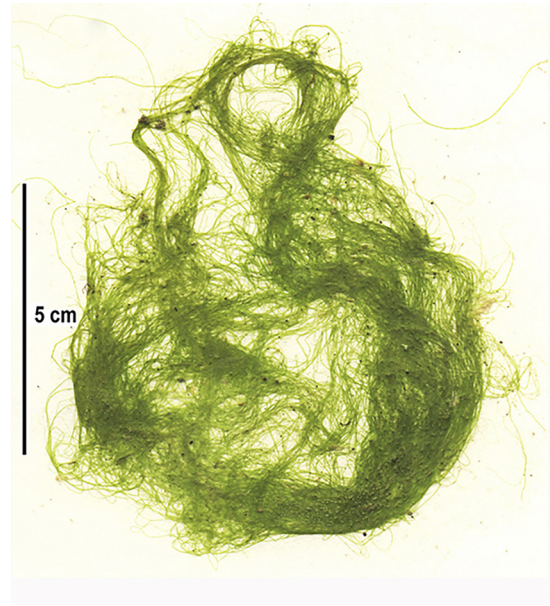
Thallus tubular, to 100 mm in length, 1 to 15+ mm in diameter (initially so thin as to appear filamentous, becoming wider as the season progresses). Cells less than 20  $\mu\text{m}$  on a side, rectangular in surface view, initially arranged in regular ranks and files but becoming disordered as the season progresses. Cells at base of thallus often lacking rhizoidal processes. Pyrenoids one per cell. Eyespot indistinct. Zygotes and zoospores have the *Ulva californica*-type germination pattern, with development of a branched prostrate system before initiation of the upright. Occurring in high intertidal pools on granite and schist from early winter to late summer, depending on locality (Figures 17–19).

**Holotype:** UBC A90118, SCL 15161, high intertidal pool, eastern tip of Sanak Island, Alaska (54.39° N 162.54° W), 01 Sep 2012 (Figure 19). *TufA* sequence (GenBank MW921464), ITS sequence (GenBank MW939397).



**Figure 22:** *Ulva stenophylla* (UBC A93302), subtidal seagrass, Choked Pass, 4 Aug 2017. Scale bar = 10 cm.

**Etymology:** Named for a Canadian Coast Guard mechanic, Paul Pouliot, killed in 1978 by a rogue wave just a few feet from the tidepool at Triple Island, northern BC, where this species was first recognized.



**Figure 23:** *Ulva torta* (UBC A89078), mid intertidal mud, head of Pruth Bay, 12 Aug 2011. Scale bar = 5 cm.

**Known geographic distribution:** Central coast of British Columbia to Attu Island, Aleutian Islands, Alaska. This species may be more widely distributed and has likely been overlooked because of its morphological similarity to *U. intestinalis*. Additional specimen records, including sequences, can be found in Table 3.

**Reproduction (observations by Larry Golden, pers. comm.):** Alternation of isomorphic generations. Anisogametes are biflagellate, produced on separate thalli, and weakly photo-positive (eyespot indistinct, dull red). Female gametes are 7–10  $\mu\text{m}$  long; male gametes, 5–7  $\mu\text{m}$  long, with a reduced chloroplast. Size can vary to some extent depending on culture conditions. The gametes settle quickly. Asexual biflagellate zoospores are more common than other reproductive cells. They are of similar size to the female gamete. Quadriflagellate zoospores also occur occasionally; they can reach 12  $\mu\text{m}$  or more in length.

**Comments:** *Ulva pouliotii* superficially resembles *U. intestinalis* in its tubular habit. It differs from that species because it usually lacks basal rhizoids (present in *U. intestinalis*), its cells are almost always arranged in orderly rows (sometimes present in *U. intestinalis*), and its maximum cell size is larger (20 vs 15  $\mu\text{m}$ ). Thalli of *U. pouliotii* do not achieve the overall dimensions of those of *U. intestinalis*: 150 mm long versus 500 mm long, and 15 mm wide versus 60 mm wide. Its eyespot is much fainter than that of *U. intestinalis*.

**Table 4:** Matrix of pairwise distances between species of *Ulva* analyzed in this study (distances averaged when more than one specimen of a species was compared).

<i>U. compressa</i>	<i>U. expansa</i>	<i>U. fenestrata</i>	<i>U. intestinalis</i>	<i>U. linza</i>	<i>U. pouliotii</i>	<i>U. prolifera</i>	<i>Ulva</i> sp.	<i>U. stenophylla</i>	<i>U. torta</i>
<i>U. compressa</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>U. expansa</i>	0.0788		0.0518	0.0556	0.0681	0.0696	0.0645	0.0642	0.0614
<i>U. fenestrata</i>	0.0687	0.0524		0.0576	0.0789	0.0836	0.0743	0.0739	0.0670
<i>U. intestinalis</i>	0.0790	0.0970	0.0877		0.0615	0.0529	0.0542	0.0491	0.0534
<i>U. linza</i>	0.0814	0.0743	0.0641	0.0856		0.0268	0.0083	0.0199	0.0362
<i>U. pouliotii</i>	0.0740	0.0775	0.0622	0.0973	0.0272		0.0154	0.0173	0.0343
<i>U. prolifera</i>	0.0801	0.0800	0.0707	0.0894	0.0048	0.0282		0.0116	0.0319
<i>Ulva</i> sp.	0.0642	0.0647	0.0559	0.0954	0.0346	0.0179	0.0359		0.0312
<i>U. stenophylla</i>	NA	NA	NA	NA	NA	NA	NA	NA	0.0439
<i>U. torta</i>	0.1036	0.1205	0.0888	0.1305	0.0605	0.0686	0.0686	0.0611	NA

Lower left: ITS; upper right: *tufA*. NA, not available.

In contrast to *U. intestinalis*, which initiates the upright thallus as a uniseriate filament from a filamentous base, *U. pouliotii* forms a prostrate disc from which the upright thallus is initiated.

Although both species occur in upper intertidal pools, *U. intestinalis* more commonly occurs in freshwater seepage areas in the upper intertidal. *Ulva pouliotii* reaches peak abundance in spring in northern British Columbia, about the time *U. intestinalis* first makes its annual appearance on the shore. *Ulva intestinalis* reaches its maximum abundance during the summer, by which time *U. pouliotii* has mostly disappeared.

It is worth noting several species of *Ulva* not yet found at Hakai. This includes the introduced species *Ulva*

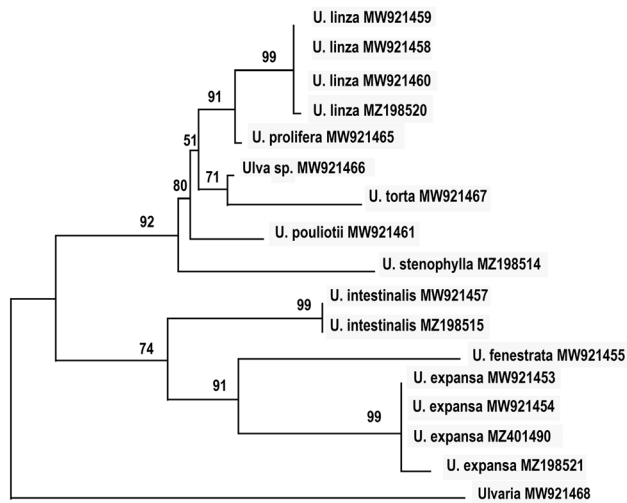
*australis* Areschoug (previously identified as *U. pertusa* Kjellman), which has been recorded from numerous sites in southern BC. Nor have we confirmed the occurrence of *Ulva californica* Wille.

### 3.8.2 Other Ulvaceae

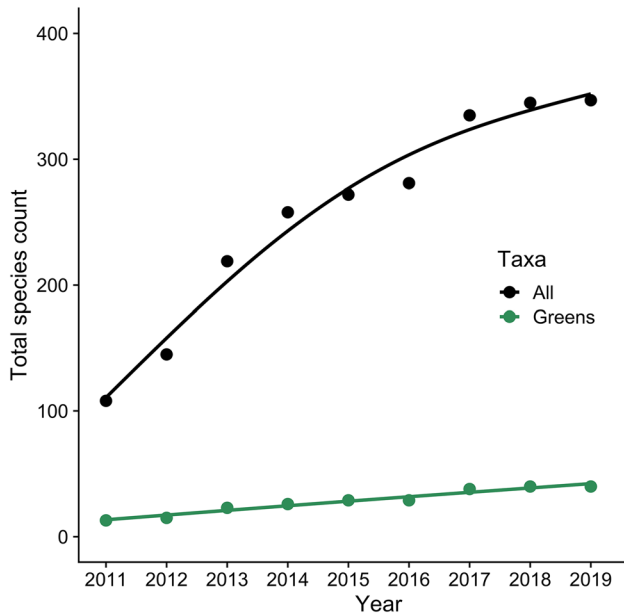
In addition to the species of *Ulva*, we found a species of *Ulvaria*, which we identify as *U. obscura* var. *blyttii* (Areschoug) Bliding, following Gabrielson and Lindstrom (2018). Work has yet to be done confirming the identity of our species with northeast Atlantic *Ulva obscura* Kützting (type locality: Biarritz, France) or *Ulva blyttii* Areschoug (type locality: Finnmark, Norway), both of which are older names than *Ulvaria splendens* (Ruprecht) K.L.Vinogradova (type locality: western Aleutian Islands). An undescribed species of *Ulvaria*, which has been recorded from southern (Bamfield: KM255011) and northern BC (Lucy Island: SCL 13133), was collected at Hakai for the first time in 2021. Further work on this species is required before it can be described as new.

## 4 Discussion

It is with humility that we provide a floristic account of the benthic marine green macroalgae of the Hakai area. Even after nine years of collecting, we continue to discover species not seen previously. Figure 25 shows the accumulation of species records for all seaweeds, greens, browns and reds, over that period. Although our visits were during “summer” months, they have varied from late May (in 2013 and 2017) to mid August (in 2011). Until Bioblitz efforts in 2017, we had only sampled subtidal regions during one



**Figure 24:** Maximum likelihood tree of *tufA* sequences from Hakai *Ulva* specimens. Numbers above the branches are bootstrap values. The phylogenetic tree was run using the GTR + I + G model of nucleotide evolution. Scale bar = 0.010 substitutions.



**Figure 25:** Species accumulation curve for all (green, brown and red) seaweeds observed during this study. Green line shows species accumulation for green algae.

visit in 2013; both years showed slightly more species than the smooth line in Figure 25 would predict. Moreover, while we began by sampling only moderately exposed sites, we included protected sites beginning in 2014, adding more species. During 2015–2016, the area was impacted by a marine heat wave (Di Lorenzo and Mantua 2016), and relatively fewer species were added. There remains a significant number of species that we expect to find but we have yet to record. Indeed, we have documented fewer than half of the 97 or so species of green seaweeds currently recognized to occur in British Columbia (Gabrielson and Lindstrom 2018). This suggests that our list of species for this area will continue to grow.

In addition to new southern records, *Acrosiphonia sonderi*, *Acrosiphonia* sp., *Protomonostroma undulatum*, and *Ulva poulitii*, and new northern records, *Ulva expansa*, *U. stenophylla*, and another undescribed species of *Ulva*, we noted the occurrence of species requiring further taxonomic study in *Blidingia*, *Chaetomorpha*, *Cladophora*, *Derbesia*, *Eugomontia*, *Monostroma*, and *Rhizoclonium*. The identity of *Cladophora opaca* and *Blidingia* sp. with Asian specimens suggests recent introductions rather than isolated populations that persisted in glacial refugia.

We continue to look for evidence among DNA sequences of seaweeds that show signatures of refugial populations, namely the occurrence of private haplotypes

that are otherwise unknown for a species. However, finding such haplotypes requires a large number of DNA sequences, which we have for very few species. Among green algae, a private *tufA* haplotype was observed in an *U. intestinalis* specimen from Second Beach, where a single transversion separated this haplotype from the nine other sequences available from northern Washington to Southeast Alaska and from Ireland. In *Acrosiphonia arcta*, a private ITS haplotype (a transition) was found in three specimens from boulder near sand habitat at North Beach compared to three Hakai specimens from bedrock and 18 other sequences from other northeast Pacific and North Atlantic sites.

With continued global warming, especially as exemplified by the recent marine heat wave(s), and increased marine traffic, we expect the Hakai flora to continue to change. Green algae are among some of the most easily transported marine species so we need to remain vigilant to invaders. The discovery in this study of two species (*Cladophora opaca* and *Blidingia* sp.) previously known only in Asia highlights the reality of cryptic species invasions; however, neither of these species was identified among specimens originating in the Tohoku area of Japan and arriving on northeast Pacific shores as Japanese tsunami marine debris (Hanyuda et al. 2018). In a companion study to this one (Whalen et al. Unpublished), we demonstrated that, although most seaweed populations shifted downslope towards reduced abiotic stress during the marine heat wave of 2014–2016, different species were impacted differently, leading to complex changes in community and ecosystem dynamics, and green algae were among the least affected. The present study documents current diversity and provides a baseline for future monitoring in an evolving environment.

**Acknowledgments:** We thank Hakai Institute, Tula Foundation, particularly Eric Peterson and Christina Munck, for inviting us to Calvert Island and hosting us there. We also thank the students and others who helped us with these collections, notably Laura Anderson, Kyra Janot, Luran Liggan and the divers who participated in the 2017 Bioblitz and collected seaweeds for us: Neha Acharya-Patel, Aaron Galloway, Kyle Hall, Andy Lamb, Derek van Mannen, Zach Monteith, Angelene Olsen, Gustav Paulay, Ondine Pontier, Tanya Prinzing, and Matt Whalen. We thank Keith Holmes for preparing the map in Figure 1. Communications with Jeffery Hughey and Charley O’Kelly are gratefully acknowledged.



**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** We are grateful to Emilie Lindstrom† and the Tula Foundation for providing funds for sequencing and other laboratory expenses. The Tula Foundation also covered all field expenses, including travel. Additional funding was provided by NSERC Discovery grants (RGPIN 2014-06288, 2019-06240) to PTM.

**Conflict of interest statement:** The authors declare no conflicts of interest regarding this article.

## References

- Bakker, F.T., Olsen, J.L., and Stam, W.T. (1995). Evolution of nuclear rDNA ITS sequences in the *Cladophora albida/sericea* clade (Chlorophyta). *J. Mol. Evol.* 40: 640–651.
- Boedeker, C., Leliaert, F., and Zuccarello, G.C. (2016). Molecular phylogeny of the Cladophoraceae (Cladophorales, Ulvophyceae), with the resurrection of *Acrocladus* Nägeli and *Willeella* Børegesen, and the description of *Lurbica* gen. nov. and *Pseudorhizoclonium* gen. nov. *J. Phycol.* 52: 905–928.
- Boedeker, C., Leliaert, F., and Zuccarello, G.C. (2017). Genetic diversity and biogeography in *Chaetomorpha melagonium* (Ulvophyceae, Cladophorales) based on internal transcribed spacer (ITS rDNA) sequences. *Bot. Mar.* 60: 319–325.
- Britton, D., Mundy, C.N., McGraw, C.M., Revill, A.T., and Hurd, C.L. (2019). Responses of seaweeds that use CO<sub>2</sub> as their sole inorganic carbon source to ocean acidification: differential effects of fluctuating pH but little benefit of CO<sub>2</sub> enrichment. *ICES (Int. Counc. Explor. Sea) J. Mar. Sci.* 76: 1860–1870.
- Burd, B., Jackson, J., Thomson, R., and Holmes, I. (2019). Northern West Coast of Canada. In: Sheppard, C. (Ed.), *World seas: an environmental evaluation. Europe, the Americas and West Africa*, 2nd ed., Vol. 1. Amsterdam: Elsevier, pp. 333–361.
- Burrows, E.M. (1991). *Seaweeds of the British Isles. Chlorophyta*, Vol. 2. London: British Museum (Natural History).
- Crawford, W.R., Huggett, W.S., Woodward, M.J., and Daniel, P.E. (1985). Summer circulation of the waters in Queen Charlotte sound. *Atmos.-Ocean* 23: 393–413.
- Cui, J., Monotilla, A.P., Zhu, W., Takano, Y., Shimada, S., Ichihara, K., Matsui, T., He, P., and Hiraoka, M. (2018). Taxonomic reassessment of *Ulva prolifera* (Ulvophyceae, Chlorophyta) based on specimens from the type locality and Yellow Sea green tides. *Phycologia* 57: 692–704.
- Cummins, P.F. and Masson, D. (2014). Climatic variability and trends in the surface waters of coastal British Columbia. *Prog. Oceanogr.* 120: 279–290.
- Davidson, A.D., Campbell, M.L., Hewitt, C.L., and Schaffelke, B. (2015). Assessing the impacts of nonindigenous marine macroalgae: an update of current knowledge. *Bot. Mar.* 58: 55–79.
- Di Lorenzo, E. and Mantua, N. (2016). Multi-year persistence of the 2014/15 North Pacific marine heatwave. *Nat. Clim. Change* 6: 1042–1047.
- Eamer, J.B.R., Shugar, D.H., Walker, I.J., Lian, O.B., Neudorf, C.M., and Telka, A.M. (2017). A glacial readvance during retreat of the Cordilleran Ice Sheet, British Columbia central coast. *Quat. Res.* 87: 468–481.
- Foreman, M.G.G., Pal, B., and Merryfield, W.J. (2011). Trends in upwelling and downwelling winds along the British Columbia shelf. *J. Geophys. Res.* 116: C10023.
- Gabrielson, P.W. and Lindstrom, S.C. (2018). *Keys to the seaweeds and seagrasses of Southeast Alaska, British Columbia, Washington, and Oregon*. Phycological Contribution No. 9. Hillsborough, North Carolina: Phycold.
- Garrido-Benavent, I., Pérez-Ortega, S., and de los Ríos, A. (2017). From Alaska to Antarctica: species boundaries and genetic diversity of *Prasiola* (Trebouxiophyceae), a foliose chlorophyte associated with the bipolar lichen-forming fungus *Mastodia tessellata*. *Mol. Phylogenet. Evol.* 107: 117–131.
- Golden, L. and Garbary, D. (1984). Studies on *Monostroma* (Monostromataceae, Chlorophyta) in British Columbia with emphasis on spore release. *Jpn. J. Phycol.* 32: 319–332.
- Guiry, M.D. and Guiry, G.M. (2021). *AlgaeBase*. Galway: World-Wide Electronic Publication, National University of Ireland, <http://www.algaebase.org> (Accessed 22 Mar 2021).
- Hanic, L.A. (2005). Taxonomy, gamete morphology and mating types of *Urospora* (Ultrichales, Chlorophyta) in North America. *Phycologia* 44: 183–193.
- Hannah, C.G., LeBlond, P.H., Crawford, W.R., and Budgell, W.P. (1991). Wind-driven depth averaged circulation in Queen Charlotte Sound and Hecate Strait. *Atmos.-Ocean* 29: 712–736.
- Hanyuda, T., Hansen, G.I., and Kawai, H. (2018). Genetic identification of macroalgal species on Japanese tsunami marine debris and genetic comparisons with their wild populations. *Mar. Pollut. Bull.* 132: 74–81.
- Harley, C.D.G. and Paine, R.T. (2009). Contingencies and compounded rare perturbations dictate sudden distributional shifts during periods of gradual climate change. *Proc. Natl. Acad. Sci. Unit. States Am.* 106: 11172–11176.
- Hayakawa, Y., Ogawa, T., Yoshikawa, S., Ohki, K., and Kamiya, M. (2012). Genetic and ecophysiological diversity of *Cladophora* (Cladophorales, Ulvophyceae) in various salinity regimes. *Phycol. Res.* 60: 86–97.
- Heesch, S., Pažoutová, M., Moniz, M.B.J., and Rindi, F. (2016). Prasiolales (Trebouxiophyceae, Chlorophyta) of the Svalbard Archipelago: diversity, biogeography and description of the new genera *Prasionella* and *Prasionema*. *Eur. J. Phycol.* 51: 171–187.
- Hughey, J.R., Maggs, C.A., Mineur, F., Jarvis, C., Miller, K.A., Shabaka, S.H., and Gabrielson, P.W. (2019). Genetic analysis of the Linnean *Ulva lactuca* (Ulvales, Chlorophyta) holotype and related type specimens reveals name misapplications, unexpected origins, and new synonymies. *J. Phycol.* 55: 503–508.
- Hughey, J.R., Miller, K.A., and Gabrielson, P.W. (2018). Mitogenome analysis of a green tide forming *Ulva* from California, USA confirms its identity as *Ulva expansa* (Ulvaceae, Chlorophyta). *Mitochondrial DNA B* 3: 1302–1303.
- Jackson, J.M., Thomson, R.E., Brown, L.N., Willis, P.G., and Borstad, G.A. (2015). Satellite chlorophyll off the British Columbia coast, 1997–2010. *J. Geophys. Res.* 120: 4709–4728.

- Jao, C.C. (1937). New marine algae from Washington. Pap. Mich. Acad. Sci. Arts Lett. 22: 99–121.
- Kjellman, F.R. (1883). The algae of the Arctic Sea. K. - Sven. Vetenskapsakademiens Handl. 20: 1–350.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35: 1547–1549.
- Lindstrom, S.C. (2018). An undescribed species of putative Japanese *Pyropia* first appeared on the central coast of British Columbia, Canada, in 2015. Mar. Pollut. Bull. 132: 70–73.
- Lindstrom, S.C. and Hanic, L.A. (2005). The phylogeny of North American *Urospora* (Ulotrichales, Chlorophyta) based on sequence analysis of nuclear ribosomal genes, introns and spacers. Phycologia 44: 194–201.
- Lindstrom, S.C., Olsen, J.L., and Stam, W.T. (1996). Recent radiation of the Palmariaceae (Rhodophyta). J. Phycol. 32: 457–468.
- Lindstrom, S.C., Hanic, L.A., and Golden, L. (2006). Studies of the green alga *Percursaria dawsonii* (= *Blidingia dawsonii* comb. nov., Kornmanniaceae, Ulvales) in British Columbia. Phycol. Res. 54: 40–56.
- Litzow, M.A., Hunsicker, M.E., Bond, N.A., Burke, B.J., Cunningham, C.J., Gosselin, J.L., Norton, E.L., Ward, E.J., and Zador, S.G. (2020). The changing physical and ecological meanings of North Pacific Ocean climate indices. Proc. Natl. Acad. Sci. Unit. States Am. 117: 7665–7671.
- Lüning, K. (1990). *Seaweeds: their environment, biogeography, and ecophysiology*. New York: John Wiley and Sons, Inc.
- McLaren, D., Fedje, D., Hay, M.B., Mackie, Q., Walker, I.J., Shugar, D.H., Eamer, J.B.R., Lian, O.B., and Neudorf, C. (2014). A post-glacial sea level hinge on the central Pacific coast of Canada. Quat. Sci. Rev. 97: 148–169.
- Melton, J.T., III and Lopez-Bautista, J.M. (2021). Diversity of the green macroalgal genus *Ulva* (Ulvophyceae, Chlorophyta) from the East and Gulf Coast of the United States based on molecular data. J. Phycol. 57: 551–568.
- Moniz, M.B.J., Guiry, M.D., and Rindi, F. (2014). *tufA* phylogeny and species boundaries in the green algal order Prasiolales (Trebouxiophyceae, Chlorophyta). Phycologia 53: 396–406.
- Mundy, P.R., Allen, D.M., Boldt, J.L., Bond, N.A., Dressel, S., Farley, E.V., Jr., Hanselman, D.H., Heifetz, J., Hopcroft, R.R., Janout, M.A., et al. (2010). Status and trends of the Alaska current region, 2003–2008. In: McKinnell, S.M. and Dagg, M.J. (Eds.), *Marine ecosystems of the North Pacific Ocean, 2003–2008*, Vol. 4. PICES Special Publication, pp. 142–195.
- O’Kelly, C.J., Wysor, B., and Bellows, W.K. (2004a). Phylogenetic position of *Bolbocoleon piliferum* (Ulvophyceae, Chlorophyta): evidence from reproduction, zoospore and gamete ultrastructure, and small subunit rRNA gene sequences. J. Phycol. 40: 209–222.
- O’Kelly, C.J., Wysor, B., and Bellows, W.K. (2004b). *Collinsiella* (Ulvophyceae, Chlorophyta) and other ulotrichalean taxa with shell-boring sporophytes form a monophyletic clade. Phycologia 43: 41–49.
- Ratnasingham, S. and Hebert, P.D.N. (2007). BOLD: the barcode of life data system. Mol. Ecol. Notes 7: 355–364, www.barcodinglife.org.
- Sakai, Y. (1964). The species of *Cladophora* from Japan and its vicinity. Sci. Pap. Inst. Algol. Res. Fac. Sci. Hokkaido Univ. 5: 1–104, +17 pls.
- Sanford, E., Sones, J.L., García-Reyes, M., Goddard, J.H.R., and Largier, J.L. (2019). Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. Sci. Rep. 9: 4216.
- Saunders, G.W. and Kucera, H. (2010). An evaluation of *rbcL*, *tufA*, *UPA*, *LSU* and *ITS* as DNA barcode markers for the marine green macroalgae. Cryptogam. Algal. 31: 487–528.
- Scagel, R.F. (1966). *Marine algae of British Columbia and northern Washington, Part I: Chlorophyceae (Green Algae)*. National Museum of Canada Bulletin 207. Ottawa: National Museum.
- Scagel, R.F., Gabrielson, P.W., Garbary, D.J., Golden, L., Hawkes, M.W., Lindstrom, S.C., Oliveira, J.C., and Widdowson, T.B. (1989). *A synopsis of the benthic marine algae of British Columbia, southeast Alaska, Washington and Oregon*. Phycological Contribution no. 3. Vancouver: University of British Columbia, Reprinted 1993.
- Sealy, S.G., Carter, H.R., Thomson, R.E., and Morgan, K.H. (2013). Movements of Ancient Murrelet family groups to northern Vancouver Island, British Columbia. Northwest. Nat. 94: 209–226.
- Setchell, W.A. (1920). The temperature interval in the geographical distribution of marine algae. Science 52: 187–190.
- Setchell, W.A. and Gardner, N.L. (1920). Phycological contributions I. Univ. Calif. Publ. Bot. 7: 279–324, pls 21–31.
- Silva, P.C., Basson, P.W., and Moe, R.L. (1996). Catalogue of the benthic marine algae of the Indian Ocean. Vol. 79. Univ. Calif. Publ. Bot., 79: xiv–1259.
- Steinhagen, S., Düsedau, L., and Weinberger, F. (2021). DNA barcoding of the German green supralittoral zone indicates the distribution and phenotypic plasticity of *Blidingia* species and reveals *Blidingia cornuta* sp. nov. Taxon 70: 229–245.
- Sutherland, J.E., Miyata, M., Ishikawa, M., and Nelson, W.A. (2016). *Prasiola* (Prasiolales, Trebouxiophyceae) in Japan: a survey of freshwater populations and new records of marine taxa. Phycol. Res. 64: 110–117.
- Taylor, M.A., Henty, I.L., and Pak, D.K. (2014). Deglacial ocean warming and marine retreat of the Cordilleran Ice Sheet in the North Pacific Ocean. Earth Planet Sci. Lett. 403: 89–98.
- Thomson, R.E. (1981). *Oceanography of the British Columbia Coast*, Vol. 56. Ottawa: Canadian Special Publication of Fisheries and Aquatic Sciences.
- Weitzman, B., Konar, B., Iken, K., Coletti, H., Monson, D., Suryan, R., Dean, T., Hondolero, D., and Lindeberg, M. (2021). Changes in rocky intertidal community structure during a marine heatwave in the northern Gulf of Alaska. Front. Mar. Sci. 8: 556820.
- Whalen, M.A., Starko, S., Lindstrom, S.C., and Martone, P.T. Extreme warming restructures habitat distribution and productivity along local gradients in stress and biodiversity. Ecol. Lett., Unpublished, <https://doi.org/10.22541/au.162192715.58945198/v1>.
- White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, J., Smitsky, J., and White, T. (Eds.). *PCR protocols: a guide to methods and applications*. San

Diego, California: Academic Press, pp. 315–322, <https://doi.org/10.1016/b978-0-12-372180-8.50042-1>.

- Williams, S.L. and Smith, J.E. (2007). A global review of the distribution, taxonomy, and impacts of introduced seaweeds. *Annu. Rev. Ecol. Syst.* 38: 327–359.
- Yoon, H.S., Nelson, W., Lindstrom, S.C., Boo, S.M., Pueschel, C., Qui, H., and Bhattacharya, D. (2016). Rhodophyta. In: Archibald, J.M., et al. (Eds.). *Handbook of the protists*. Springer International Publishing Switzerland, pp. 1–45, [https://doi.org/10.1007/978-3-319-32669-6\\_33-1](https://doi.org/10.1007/978-3-319-32669-6_33-1).
- Zacharias, M.A., Howes, D.E., Harper, J.R., and Wainwright, P. (1998). The British Columbia marine ecosystem classification: rationale, development, and verification. *Coast. Manag.* 26: 105–124.

**Supplementary Material:** The online version of this article offers supplementary material (<https://doi.org/10.1515/bot-2021-0038>).

## Bionotes



**Sandra C. Lindstrom**  
Hakai Institute, Heriot Bay, BC V0P 1H0, Canada  
Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada  
[sandra.lindstrom@botany.ubc.ca](mailto:sandra.lindstrom@botany.ubc.ca)  
<https://orcid.org/0000-0001-5838-8595>

Sandra C. Lindstrom is an adjunct professor in the Department of Botany, University of British Columbia. She has studied the flora of the northeast Pacific for nearly 50 years, describing many new species and documenting their geographic distributions and phylogenetic relationships.



**Matthew A. Lemay**  
Hakai Institute, Heriot Bay, BC V0P 1H0, Canada  
Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada  
[matt.lemay@hakai.org](mailto:matt.lemay@hakai.org)

Matthew A. Lemay is a Research Scientist at the Hakai Institute in British Columbia, Canada. His interests are in the use of genomic approaches to study biodiversity. Key areas of research include DNA barcoding, marine microbial ecology, and the use of environmental DNA to quantify the biodiversity of marine ecosystems.



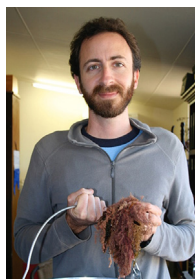
**Samuel Starko**  
Hakai Institute, Heriot Bay, BC V0P 1H0, Canada  
Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada  
Department of Biology, University of Victoria, PO Box 1700 Station CSC, Victoria, BC V8W 2Y2, Canada  
[samuel.starko@gmail.com](mailto:samuel.starko@gmail.com)

Samuel Starko was a doctoral student at the University of British Columbia during these field surveys. He is currently a postdoctoral fellow at the University of Victoria. His research focuses on understanding how marine species respond to environmental drivers over ecological and evolutionary timescales.



**Katharine R. Hind**  
Hakai Institute, Heriot Bay, BC V0P 1H0, Canada  
Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada  
Department of Biology, University of Victoria, PO Box 1700 Station CSC, Victoria, BC V8W 2Y2, Canada  
[khind@uvic.ca](mailto:khind@uvic.ca)

Katharine R. Hind is a Senior Laboratory Instructor at the University of Victoria. She completed a PhD and post-doctoral studies examining the evolutionary relationships of coralline algae using molecular phylogenetic techniques. She continues to use DNA barcoding to identify and describe new species and more recently incorporates the use of historical DNA to apply accurately species names. Katy teaches courses in evolution, biodiversity, and marine botany at the University of Victoria and the Bamfield Marine Sciences Centre on Vancouver Island.



**Patrick T. Martone**  
Hakai Institute, Heriot Bay, BC V0P 1H0, Canada  
Department of Botany, University of British Columbia, #3156 – 6270 University Blvd., Vancouver, BC V6T 1Z4, Canada  
[patrick.martone@botany.ubc.ca](mailto:patrick.martone@botany.ubc.ca)

Patrick T. Martone is a marine phycologist and professor in the Botany Department at the University of British Columbia. His research program focuses on seaweed diversity, ecology, biomechanics, and evolution. He teaches algae courses at UBC and the Bamfield Marine Sciences Centre.