

Research Article

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A molecular investigation of *Saccharina sessilis* from the Aleutian Islands reveals a species complex, necessitating the new combination *Saccharina subsessilis*

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Cryptic species complexes are increasingly recognized in phycological research, obscuring taxonomy and raising questions about factors influencing speciation. A recent exploration of kelp genetic diversity on Haida Gwaii, British Columbia revealed the existence of a new species, *Saccharina druehlii*, which is cryptic with *Saccharina sessilis*. This suggests that molecular investigations further north may be required to elucidate the taxonomy and evolutionary history of this lineage. Although, for several decades, *S. sessilis* was considered a single highly variable species, its taxonomy has been far from straightforward. In particular, *Hedophyllum subsessile* (Areschoug) Setchell is now recognized as a synonym of *S. sessilis* in North America, but as a growth form of *Saccharina bongardiana* in Far East Russia. To resolve this taxonomic confusion, we sequenced mitochondrial (CO1-5P) and nuclear (internal transcribed spacer) markers of *S. sessilis* populations from the Aleutian Islands, Alaska, USA. Interestingly, none of our sequences matched *S. sessilis* sensu stricto. Instead, CO1-5P sequences from populations in the central and eastern Aleutians matched exactly *S. druehlii* with increasing sequence divergence occurring westward. Samples from Attu, the western-most island, composed a genetic group that clearly represents Kjellman's concept of *Hafgygia bongardiana* f. *subsessilis* and is distinct enough from *S. druehlii* and *S. sessilis* to potentially constitute a distinct species. Therefore, *Saccharina subsessilis* comb. nov. is proposed for this entity. Our results suggest the existence of a species complex at the crown node of *S. sessilis* and thus further investigation of *Saccharina* in Alaskan waters should be conducted to reconstruct the evolutionary history of this fascinating lineage.

Key Words: Aleutian Islands; CO1-5P; DNA barcoding; kelp; phylogenetics; *Saccharina*; species complex; systematics

INTRODUCTION

Saccharina is a speciose kelp genus that is widespread throughout temperate regions of the northern hemisphere (Bolton 2010). Species of *Saccharina* play important foundational roles in nearshore ecosystems and have economic significance through both wild har-

vest and large-scale aquaculture operations (Bartsch et al. 2008). Like several other kelp genera, the taxonomic history of *Saccharina* is convoluted (Bartsch et al. 2008); convergent evolution and morphological plasticity have led to complications in using morphology to distinguish



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species (Bartsch et al. 2008, McDevit and Saunders 2010). More recently, molecular tools have uncovered cryptic species in some kelp genera, introducing another layer of complexity to the taxonomic challenges posed by kelps (e.g., McDevit and Saunders 2010, Tellier et al. 2011a, Saunders and McDevit 2014). For example, a cryptic sister species of *Lessonia nigrescens* with a different but overlapping geographical distribution was recently discovered (Tellier et al. 2011a)—a finding which may influence the way that these sympatric populations are managed (Tellier et al. 2011b). Furthermore, strong subspecies molecular structure has been reported in several genera (e.g., Miller et al. 2000: *Pelagophycus porra*, Lane et al. 2007: *Alaria marginata*, McDevit and Saunders 2010, Nielsen et al. 2016: *Saccharina latissima*). In some cases, wide divergences in molecular sequences suggest that additional species or subspecies should be described to reflect the genetic structure of morphospecies (e.g., Boo et al. 2011, Saunders and McDevit 2014, Augyte et al. 2018).

The common ancestor of modern *Saccharina* species likely originated in the north Pacific and spread to the Atlantic Ocean through the Arctic Ocean with high species diversity currently present on both sides of the Pacific (Bolton 2010). Recently, Saunders and McDevit (2014) conducted a survey of kelps on Haida Gwaii, British Columbia, and uncovered a cryptic sibling of *Saccharina sessilis* with enough sequence divergence to warrant recognition as a distinct species. Populations containing both sequences were found together on Haida Gwaii. Thus, Saunders and McDevit (2014) used this sequence divergence as evidence of speciation and described *S. druehlii*. The existence of this cryptic species in a northern glacial refugium suggests that further sequencing of northern populations may shed light on the diversification of this clade or uncover additional cryptic species.

Saccharina sessilis (formerly *Hedophyllum sessile*) and *S. druehlii* from Haida Gwaii are unique among species of *Saccharina* in that they lack a stipe at maturity, and haptera emerge directly from the blade (Widdowson 1965, Saunders and McDevit 2014). Although a small stipe is present in young individuals, it is lost through development as the blade grows to fuse with its holdfast (Widdowson 1965). The timing of this transition from stiped to stipeless has caused substantial taxonomic confusion. In particular, one entity, originally described as *Hafgygia bongardiana* f. *subsessilis* by Areschoug (1883), then as *Laminaria bongardiana* f. *subsessilis* by Kjellman (1889), and finally raised to species status by Setchell (1901) as *Hedophyllum subsessile*, can have a broad cuneiform

base that looks much like a stipe, raising the blade above the substratum. The variation in forms of this entity, however, has led to differing taxonomic interpretations by Western and Russian literature. In Western literature, *Hedophyllum subsessile* is recognized as a synonym for an early life-stage of *S. sessilis* (Widdowson 1965). While in the Russian literature, *Saccharina bongardiana* f. *subsessilis* is recognized as a distinct form of *S. bongardiana* (Selivanova et al. 2007, Klochkova et al. 2009). In this study, we sought to clarify the taxonomic placement of *Hedophyllum subsessile* (Areschoug) Setchell.

MATERIALS AND METHODS

Hedophyllum subsessile (Areschoug) Setchell was originally distinguished by means of two morphological characteristics (Saunders 1901, Setchell 1901): 1) a cordate to even reniform, stipe-like blade base and 2) necrosis of the central portion of the blade, leaving two separated partial blades, each borne at the extremity of a thickened basal margin that resembles a running rootstock (as described by Areschoug 1883). We collected *S. sessilis* specimens from the Aleutian Islands, many of which matched this description. Sampling occurred along a longitudinal gradient: Attu Island (n = 3), Hawadax Island (formerly Rat Island) (n = 1), Little Tanaga Island (n = 1), and Akutan Island (n = 1) (Fig. 1).

We sequenced the mitochondrial (CO1-5P) DNA barcoding locus of all specimens and used two methods to distinguish species: phylogenetic distance and Generalized Mixed Yule Coalescent (GMYC) automated species delimitation. The nuclear marker internal transcribed spacer (ITS) was also sequenced for one individual from Attu Island and compared to previously published data.

Genomic DNA was extracted from ~5 mg of dried thallus ground in liquid nitrogen using a NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. The extracted DNA was stored at -20°C and used to amplify CO1-5P.

Primer pairs for amplification and sequencing of CO1-5P gene were as follows: C1F101 (5'-CCA CHA AYC AYA AAG ATA TWG G-3')-C1R942 (5'-CCH CAN GTA AAC ATR TGR TGA GCC-3'), which were designed for this study and GazF2-GazR2 (Lane et al. 2007). The polymerase chain reaction (PCR) amplification was carried out according to the protocol of Lee et al. (2009), and products were checked by gel electrophoresis in 0.8% agarose. PCR products were purified using Exo-SAP PCR Clean-Up Mix (MGMED, Seoul, Korea) according to the manufacturer's

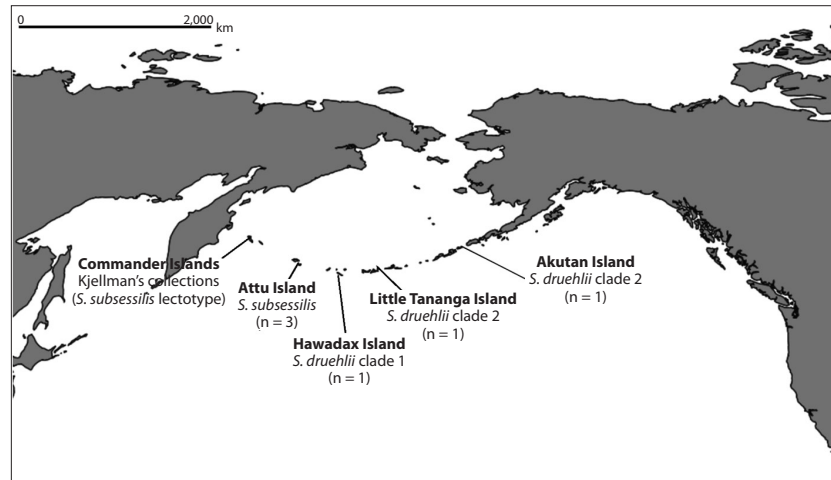


Fig. 1. Map of the Aleutian Islands indicating sampling sites for this study as well as the location of Kjellman's original collection, including the lectotype specimen A-823620 in Uppsala.

instructions and then sent for commercial sequencing (Genotech, Daejeon, Korea). Sequences of the forward and reverse strands were determined for all taxa, and the electropherograms were edited using the program Chromas v.1.45 (McCarthy 1998) and checked manually.

One specimen from Attu Island (indicated with an asterisk in Table 1) was sequenced using high-throughput technology. Genomic DNA was extracted using a CTAB protocol (Rai et al. 2003). A DNA shotgun library was constructed using the Bioo NextFLEX rapid library preparation kit (Bioo Scientific Corporation, Austin, TX, USA) and sequenced using HiSeq 2500 at Genome Quebec

(McGill University, Montreal, Quebec, Canada). CO1-5P and ITS sequences were assembled to a reference sequence of *Saccharina nigripes* (GenBank No. FJ409198 and AY857894, respectively) using CLC Genomics Workbench 7.1.

In order to include the entire known range of haplotypes for *S. sessilis* and closely related species (*S. druehlii*, *S. nigripes*, *S. bongardiana*, *S. latissima*, and *Saccharina ochotensis*), all available CO1-5P and ITS reference sequences of these species were downloaded from BOLD (n = 190). Additional specimens of *Saccharina* spp. (n = 8) were sampled from Alaska and British Columbia, and

Table 1. Collection information and GenBank numbers for new CO1-5P sequences generated in this study

Species	Collection ID	UBC accession No.	Location	GenBank No.
<i>Saccharina dentigera</i>	ALEUT06_018	A93601	Tigalda Island, AK	MH327957
<i>S. dentigera</i>	SCL13510	A87446	Attu Island, AK	MH327949
<i>S. dentigera</i>	ALEUT06_389	No voucher ^a	Umnak Island, AK	MH327956
<i>S. druehlii</i>	SCL11522	A86968	Akutan Harbor, AK	MH327951
<i>S. druehlii</i>	SCL13659	A93118	Hawadax Island, AK	MH327954
<i>S. druehlii</i>	ALEUT07_325	A93121	Little Tanaga Island, AK	MH327961
<i>S. latissima</i>	SCL_BoulderPatch	No voucher ^b	Beaufort Sea, AK	MH327958
<i>S. nigripes</i>	SCL10295	A85185	Katmai Bay, AK	MH327953
<i>S. nigripes</i>	SCL11488	A86955	Akutan Harbor, AK	MH327952
<i>S. nigripes</i>	SCL12474	A93602	Cape Sitkinak, AK	MH327950
<i>S. sessilis</i>	SCL_SeppingsIs	No voucher ^c	Vancouver Island, BC	MH327959
<i>S. subsessilis</i>	SCL13477	A93120	Attu Island, AK	MH327960
<i>S. subsessilis</i>	ALEUT07_007	A93600	Attu Island, AK	MH327955
<i>S. subsessilis</i> *	SCL_AttuIs	A93599	Attu Island, AK	MH327848

*Also sequenced for ITS (GenBank No. MH257570).

^aCollected Jul 18, 2006 by Mandy Lindeberg.

^bCollected Aug 8, 2003 by Brenda Konar, Katrin Iken, and Casey Debenham.

^cCollected Apr 18, 2003 by Sandra C. Lindstrom.

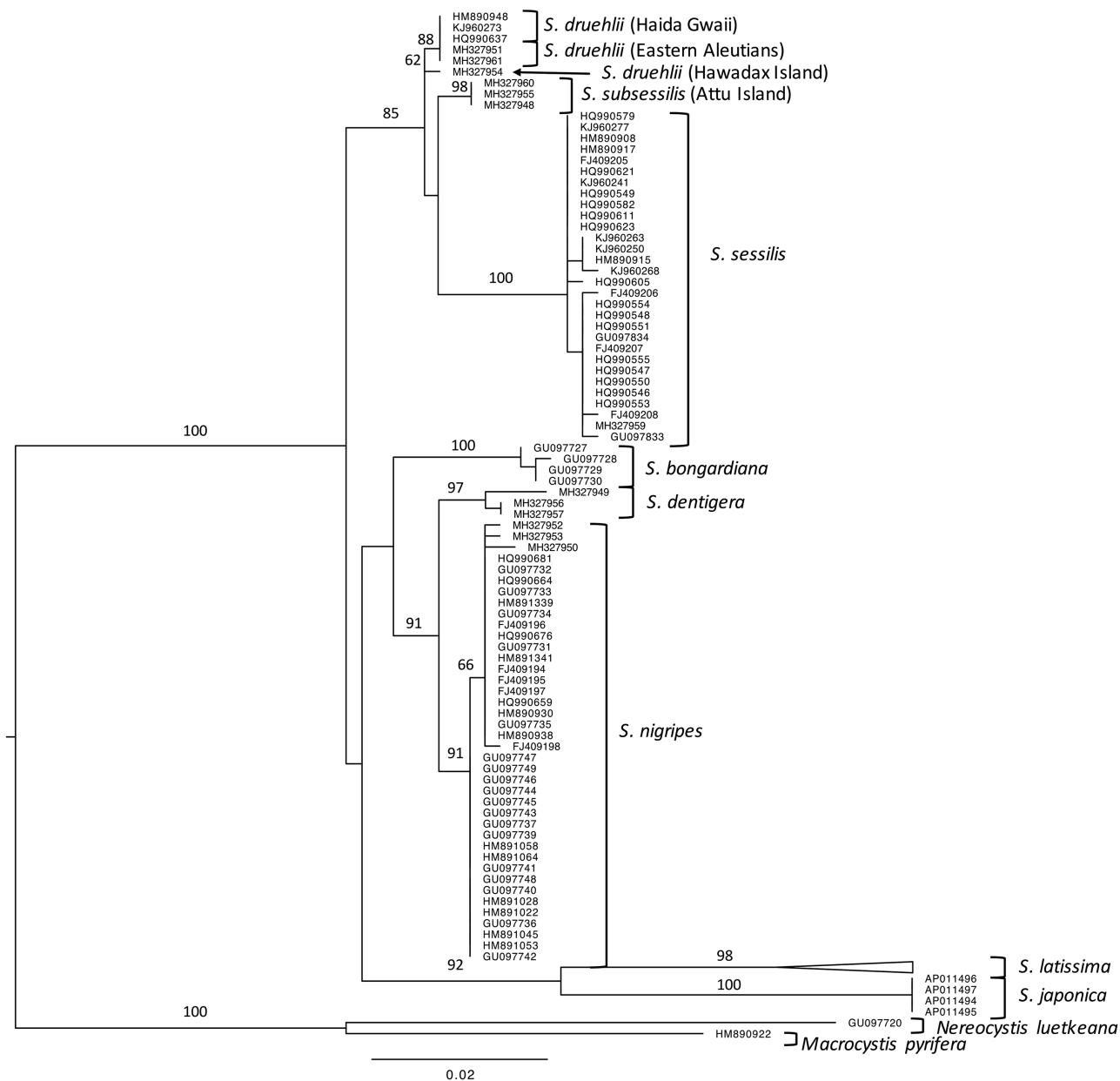


Fig. 2. Maximum likelihood trees for CO1-5P of *Saccharina* spp., made using RAxML. Node labels indicate bootstrap support, and only support values >50 are shown.

CO1-5P was sequenced (Table 1, Supplementary Table S1). Alignments were generated using Multiple Alignment using Fast Fourier Transform (MAFFT) (Kato et al. 2002), and phylogenetic analyses were performed in RAxML (Stamatakis 2014). GMYC analysis was performed by constructing ultrametric trees in BEAST (Bouckaert et al. 2014) and using the “splits” package in R (v. 3.1.2; Foundation for Statistical Computing, Vienna, Austria) (Fujisawa et al. 2013).

RESULTS AND DISCUSSION

Three specimens, including two with distinctive wedge-shaped bases, were found to match, with minimal sequence divergence (0.0-0.3%), Saunders and McDevit’s collections of *S. druehlii* from Haida Gwaii. Other specimens from Attu Island (n = 3), our farthest west sampling (Fig. 1), were identical in sequence and found to form a distinct clade with both CO1-5P and ITS. Although Attu

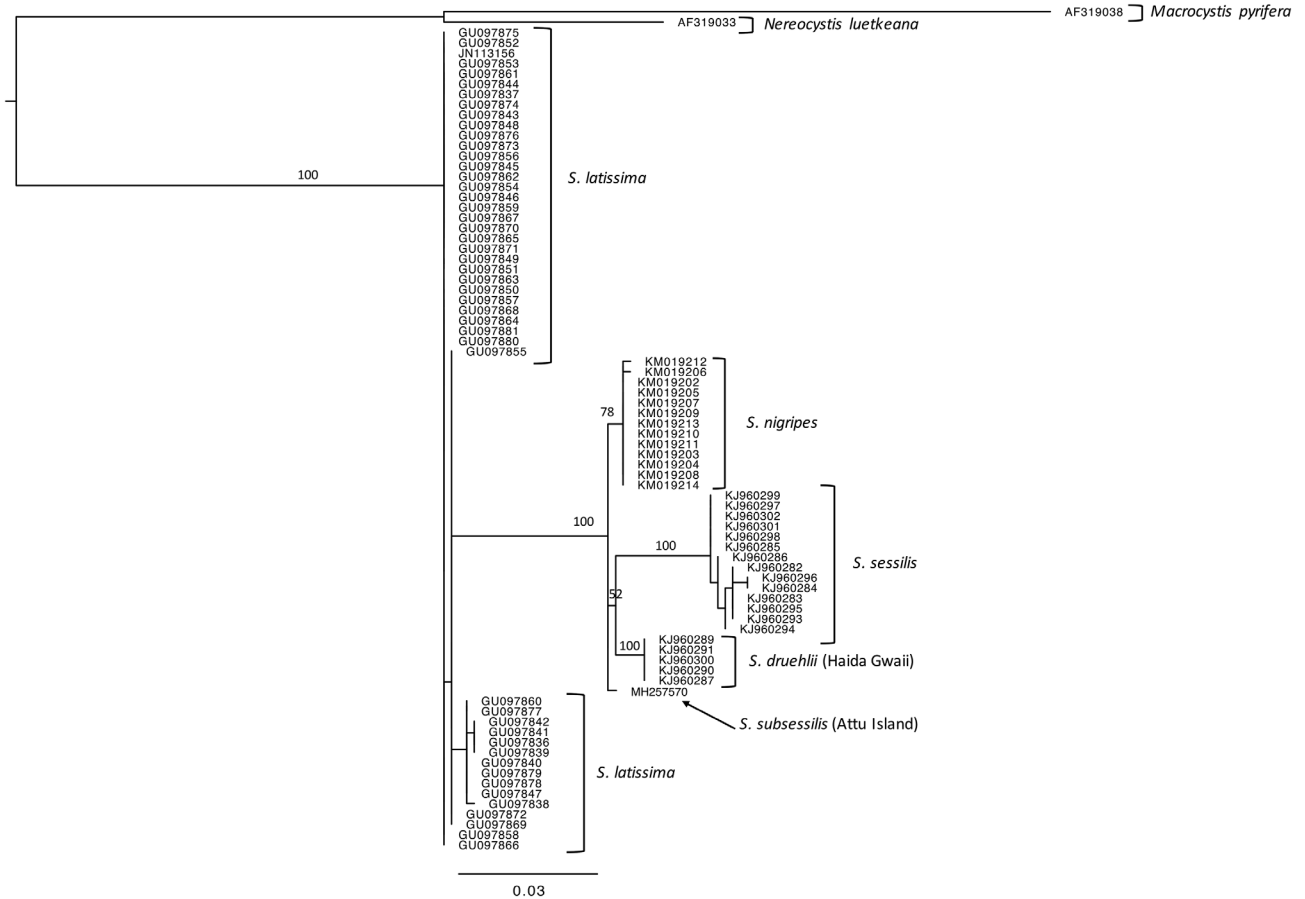


Fig. 3. Maximum likelihood trees for internal transcribed spacer of *Saccharina* spp., made using RAxML. Node labels indicate bootstrap support, and only support values >50 are shown.

sequences were only ~0.8% divergent in CO1-5P from samples collected from other islands, monophyly was unsupported for both markers (Figs 2 & 3). Instead, the best tree from maximum likelihood analysis of the CO1-5P alignment suggests that *S. druehlii* and Attu sequences are paraphyletic, only forming a clade with the inclusion of *S. sessilis*. Furthermore, the genetic distance in

CO1-5P was greater than the intraspecific variation of all currently described species except *S. latissima* (Table 2), which is likely to require further taxonomic revision due to its unusual genetic variation (McDevit and Saunders 2010). The best tree produced from ITS data also supports paraphyly of *S. druehlii* and Attu specimens, and the Attu specimens were more similar in sequence to *S.*

Table 2. Genetic distance (CO1-5P) between species of *Saccharina*

Species	Sample size ^a	Nearest neighbour (NN)	Distance to NN ^b	Max-intra difference ^b
<i>Saccharina subsessilis</i>	3	<i>S. druehlii</i>	0.79	0
<i>S. druehlii</i>	6	<i>S. subsessilis</i>	0.79	0.30
<i>S. sessilis</i>	29	<i>S. subsessilis</i>	1.64	0.62
<i>S. nigripes</i>	39	<i>S. dentigera</i>	0.91	0.30
<i>S. bongardiana</i>	4	<i>S. nigripes</i>	2.13	0.30
<i>S. dentigera</i>	3	<i>S. nigripes</i>	0.91	0.66
<i>S. latissima</i> Global ^c	104	<i>S. ochotensis</i>	4.24	1.06
<i>S. latissima</i> NA ^c	20	<i>S. ochotensis</i>	4.33	0.46

^aAll sequences from BOLD and this study.

^bExpressed as percent sequence divergence.

^c“Global” includes samples from around the world, while “NA” includes only samples from North America.

nigripes (0.8%) than to *S. druehlii* (1.12%) (Supplementary Table S2). Although these conflicting genetic signals are likely a consequence of poor resolving power with the ITS locus, they could also be attributed to incomplete lineage sorting (e.g., Maddison and Knowles 2006, Pollard et al. 2006), possibly indicating rapid speciation, or may even suggest hybrid speciation (e.g., Joly et al. 2009). We also identified three specimens that were similar, but genetically distinct from *S. nigripes*. These specimens were presumed to be members of *Saccharina dentigera*, which was described from the nearby Commander Islands but has never been sequenced for CO1.

GMYC analyses using CO1-5P suggested the lumping of the populations from Attu and *S. druehlii* into a single species (Supplementary Fig. S1). However, the lack of a definitive species concept for the kelps (Bolton 2010) makes the decision on how to taxonomically treat these entities especially difficult, particularly given that both ITS and CO1-5P indicated divergence between populations and similar divergence to *S. dentigera* and *S. nigripes* (Table 2), which are currently recognized as distinct. The magnitude of divergence between and within clades suggests that these populations may be part of a species complex (Saunders 2005, Lane et al. 2007), requiring further sampling and sequencing to elucidate species groupings. As we now discuss, lumping these entities into a single species would require a taxonomic demotion of *S. druehlii* (making it a junior synonym of *S. subsessilis*); thus, we choose to be conservative and recognize both as distinct. We acknowledge, however, that given our results, future work may synonymize these species under *S. subsessilis*, if monophyly is supported with comprehensive sampling of more genes and / or populations.

Our results demonstrate that three distinct entities make up what, prior to Saunders and McDevit (2014), had been called *S. sessilis*. While *S. sessilis* sensu stricto likely has a wide distribution, *S. druehlii* is a northern species that reaches its southern limit on Haida Gwaii (Supplementary Fig. S2). Additionally, a third clade is present on Attu, in the western Aleutians. This suggests that a geographical cline may separate *S. druehlii* from this third, western clade. This biogeographical pattern is further supported by the sequence from Hawadax Island, the furthest west sample matching *S. druehlii*, that showed divergence from all other populations (0.2% from other *S. druehlii* samples) (Fig. 2). However, the Hawadax Island sequence was distinct from other *S. druehlii* in base-pair differences that were not shared with *S. subsessilis*. Together, our molecular data suggest that Setchell (1901) was correct in recognizing *Hedophyllum subsessile* as

distinct from *H. sessile*. Thus, placements of *H. subsessile* into *S. bongardiana* f. *subsessilis* by Russian literature and into *S. sessilis* by Western literature, were both incorrect. Instead, it is necessary to resurrect the species epithet *subsessile* and make the combination *Saccharina subsessilis* for this entity:

***Saccharina subsessilis* (Areschoug) Starko & S. C. Lindstrom**

Basionym. *Hafgygia bongardiana* f. *subsessilis* Areschoug 1883, p. 5.

Homotypic synonyms. *Laminaria bongardiana* f. *subsessilis* Kjellman 1889, p. 43; *Hedophyllum subsessile* (Areschoug) Setchell 1901, p. 122.

Possible heterotypic synonym. *Streptophyllum spirale* (Yendo) Miyabe & Nagai in Nagai 1941, p. 96 [basionym: *Hedophyllum spirale* Yendo 1903, p. 165, pl. VI, figs 1-7 (excl. f. *kamtshatkensis*)]. Type locality: Shimushu Island, Northern Kurile Island, Russia.

Saccharina subsessilis (Areschoug) Starko & S. C. Lindstrom was originally described by Areschoug (1883, p. 5) from material collected by Kjellman on Bering Island between Aug 14 and 19, 1879. Areschoug included two other forms of *Hafgygia bongardiana*: f. *normalis* and f. *furcata*. Forma *subsessilis* was distinguished by the blades developing directly out of the top of the conglobated haptera. Although Areschoug mentioned specimens from both Bering Island and Sitka, this clearly refers to *H. bongardiana* sensu lato. Since Areschoug names Ruprecht specifically in addition to Kjellman, it is likely that the Sitka specimens represent material examined by Ruprecht (Kjellman did not visit Sitka; Ruprecht worked up material collected there by Mertens, Kastalsky, and Wosnesensky and illustrated by Postels) (Lindstrom 2009). We hereby designate A-823620 in Uppsala (UPS) as the lectotype of *H. bongardiana* f. *subsessilis* (Fig. 4). This specimen is one of two of this form collected by Kjellman on Bering Island between Aug 14 and 19, 1879 and deposited in the Uppsala herbarium. We consider the other specimen in UPS (A823624) to be a syntype.

Kjellman (1889, p. 43) recognized the form as *Laminaria bongardiana* f. *subsessilis* (Areschoug) Kjellman in his treatise on the marine algae of the Bering Sea collected on the Vega expedition. Kjellman noted that f. *subsessilis* was the smallest of the forms of *L. bongardiana* and showed affinity with *L. sessilis* Agardh in that the stipe was almost indiscernible, if not lacking; the haptera emerging from the thick cuneiform base of the blade directly.

Setchell (1901, p. 122) raised the form to specific rank when he made the new combination, *Hedophyllum sub-*



Fig. 4. Representative specimens of *Saccharina subsessilis* UBC A93120, Chichagof Harbor, Attu Island (A), *Saccharina druehlii* clade 1 UBC A93118, Hawadax Island (B), and *Saccharina druehlii* clade 2 UBC A93121, Little Tanaga Island (C). Scale bar represents: C, 5 cm.

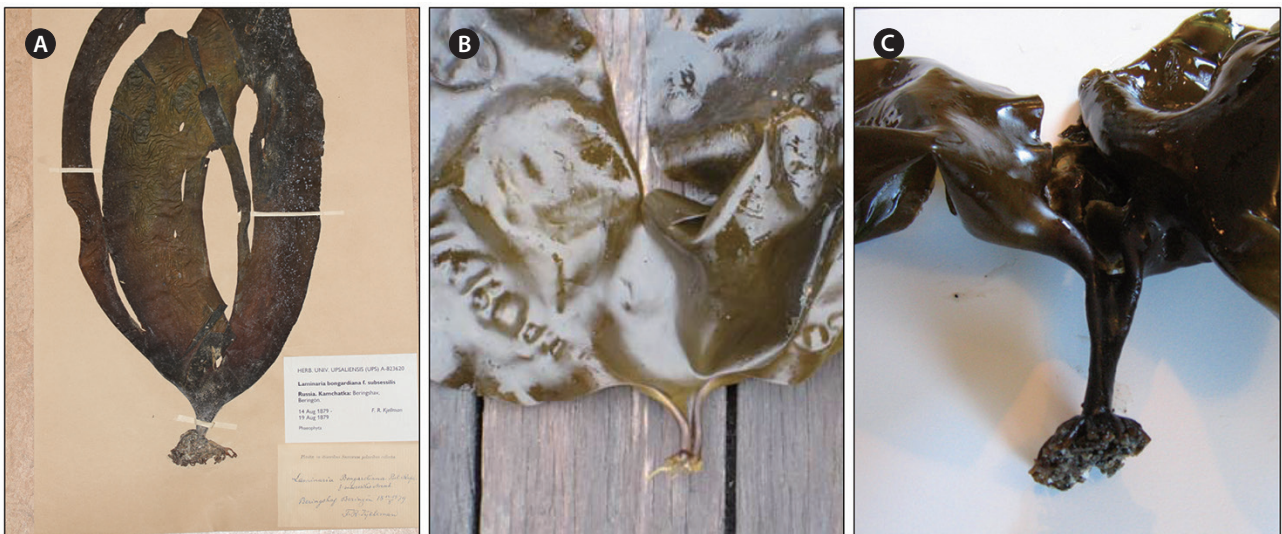


Fig. 5. The lectotype of *Saccharina subsessilis* (Uppsala A-823620) in the Uppsala Herbarium (A) and specimens from Attu Island (B & C). Note the stipe-like base where the blade meets the holdfast, the thickened margin, and the splitting of the blade down the middle.

sessile (Areschoug) Setchell. He provided a more thorough description of what he thought was the same species, based on collections from Amaknak Island, Unalaska Bay (this was likely what we recognize here as *S. druehlii*). Widdowson (1965, p. 1410) reduced *H. subsessile* to a synonym of *H. sessile*. Widdowson had looked for the type of *Hafgygia bongardiana* f. *subsessilis* in Stockholm (S). Although Areschoug retired to Stockholm, he held faculty status at UPS prior to his retirement, and Kjellman, on whose specimens Areschoug based his new form, was also on the faculty at UPS (Wynne 2006) so it is unclear why Widdowson looked for the specimens of the species in Stockholm rather than UPS. Widdowson did mention the specimens in UPS, citing a personal communication from R. F. Scagel. He stated, "Although it seems likely that these specimens were the basis of Areschoug's descriptions, there is no concrete evidence of this." This is hardly surprising in that the detailed protocols associated with typification were not developed until the twentieth century. Claiming "Areschoug's material could not be found," Widdowson then selected as neotype the material in UC that Setchell had examined in raising *Hafgygia bongardiana* f. *subsessilis* to specific rank. As noted above, given the geographical patterns observed in our study, Widdowson's neotype is likely to be *S. druehlii* rather than *S. subsessilis*. However, according to the International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code) (2012) "9.7. [a] neotype (McNeill et al. 2012) is a specimen or illustration selected to serve as nomenclatural type if no original material is extant, or as long as it is missing." Since the original material on which the name *S. subsessilis* is based exists, Widdowson's neotype can be disregarded in relation to the nomenclature of either *S. subsessilis* or *S. druehlii*. Thus, *S. druehlii* remains a valid name for the entity present in the eastern Aleutians and on Haida Gwaii, and we recognize *S. subsessilis* as a species from the western Aleutians and Commander Islands that may or may not extend further west into Russia (Selivanova et al. 2007, Klochkova et al. 2009) (Supplementary Fig. S2).

The distinctness of westernmost Aleutian Island material from more easterly collections is perhaps hardly surprising. The 1,600 km island arc is composed of clusters of island groups separated by increasingly wider and deeper passages from east to west (Stabeno et al. 2005). Lindstrom et al. (2015) observed western Aleutian *Pyropia taeniata* occurred in the mid to high intertidal in contrast to the low intertidal habitat of more easterly populations, but only three species of foliose Bangiales from there had a single unique nucleotide in their *rbcl*.

sequences. However, other species of red algae appear to show more uniqueness in the western Aleutian Islands (Lindstrom unpublished data).

Although our sampling was limited to only a few sites, there are possible morphological differences between *S. subsessilis* and *S. druehlii*. In general, the stipe-like base is much more pronounced and robust in *S. subsessilis* than in *S. druehlii*. In these specimens, the base of the blade narrows and becomes canaliculate like a stipe, and older individuals form splits in their blades as they widen in a semicircular fashion. Setchell (1901) suggested that the samples that he inspected (which are likely to be *S. druehlii*) could be indistinguishable from *S. sessilis* after their second year of development. Indeed, the collections by Saunders and McDevit (2014) are not conspicuously distinct from collections of *S. sessilis*, and in our samples, the stipe-like base of *S. druehlii* was either less evident or fully absent in larger individuals. However, the base of the blade was much wider in these larger samples than would be typical of *S. sessilis*, and blades tended to split in a similar manner to *S. subsessilis*. The striking morphology of samples from Attu (Figs 4 & 5) suggest that *S. subsessilis* is much more distinguishable from *S. sessilis* than *S. druehlii*, however, and differences between these species may become even more apparent as individuals age and grow. Saunders and McDevit (2014) also suggested that *S. druehlii* was strongly bullate in morphology as an adult. Because none of our collections corroborate this description, it appears that bullation is a population level morphological feature rather than one at the level of species. Instead, reassessment of their images of *S. druehlii* on the Barcode of Life Database (BOLD) suggest that the base of their samples, like ours, may be morphologically distinct from the rest of the blade (BOLD accession No.: MACRO3473-12, MACRO3474-12). Future work should assess the morphology of the blades (especially basal regions) prior to sequencing specimens believed to be *S. sessilis*, *S. druehlii*, or *S. subsessilis*. In doing so, we may be able to determine a way to distinguish these entities in the field in locations in which they overlap.

CONCLUSION

Regardless of how this species complex came to be, it is clear from this study and others (e.g., Nielsen et al. 2016) that *Saccharina* serves as an interesting case study into the dynamics of speciation in kelps. Considering our inability to pose a definitive species concept for this group, we would stand to gain much from intensively

studying the structure of key genera on both global and local scales. Future work should continue to investigate the phylogenetic structure of populations of *Saccharina* to reconstruct the evolutionary history of the lineage and incorporate morphological, biogeographical and ecological characters to better understand the factors differentiating genetic species. In doing so, we may not only determine how best to taxonomically treat the species complex presented in this study but also learn more about drivers of speciation in brown algae in general.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. List of published sequences used in this study (<http://e-algae.org>).

Supplementary Table S2. Genetic distance (ITS) between species of *Saccharina* (<http://e-algae.org>).

Supplementary Fig. S1. Generalized Mixed Yule Coalescent analysis using COI-5P gene tree. Ultrametric tree was constructed in BEAST using a HKY model of evolution and strict clock priors. Prior choice (relaxed clock, random local clock) did not influence the resulting topology or species clustering (<http://e-algae.org>).

Supplementary Fig. S2. A map showing the known and putative distributions of *Saccharina sessilis*, *S. subsessilis*, and *S. druehlii* (<http://e-algae.org>).

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