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Speciation in the exposed intertidal zone: the case of *Saccharina angustissima comb. nov.* & *stat. nov.* (Laminariales, Phaeophyceae)

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Abstract: *Saccharina latissima* is a perennial kelp with a circumboreal distribution from the North Pacific to the North Atlantic coasts. Our study clarified the taxonomy of the morphologically distinct *Saccharina latissima* forma *angustissima* (Collins) A.Mathieson from the low intertidal zone on exposed islands and ledges of Casco Bay, Maine, USA. To identify genetic divergence between the two morphotypes, *S. latissima* and *S. latissima* f. *angustissima*, we used a multilocus phylogenetic approach including nuclear-encoded internal transcribed spacer, mitochondrial *cox*1 and *cox3*, and plastid-encoded *rbc*L gene sequences. Genetic analysis of the individual markers and combined data set using SVDquartets resulted in p-distance values for all markers of $< 1\%$, suggesting low divergence between the two forms. However, there was as much or more genetic divergence between *S. latissima* and *S. latissima* f. *angustissima* as there were between other taxonomically accepted species of *Saccharina*. To investigate sexual compatibility between the two forms, we made reciprocal crosses of the gametophytes and observed sporophyte formation. All crosses were successfully grown to the juvenile sporophyte stage, suggesting that the two are reproductively compatible *in vitro*. It is unknown if the two populations freely hybridize in the field. Last, we compared wave action, the ecological factor most likely driving the unique morphology, at exposed sites with *S. latissima* f. *angustissima* and protected sites with *S. latissima*. The mean wave force at the exposed site was over 30 times higher in magnitude than at the protected site at 160.04 ± 32.58 N and 4.75 ± 6.75 N, respectively, during the summer. The significant differences in morphology, the lack of specimens with intermediate morphologies, and the results of a common garden experiment suggest that the morphological differences in *S. latissima* f. *angustissima* are heritable with a genetic basis. Therefore, on the basis of our molecular evidence coupled with ecological studies, we are elevating *S. latissima* f. *angustissima* (Collins) A.Mathieson to specific rank as *S. angustissima* (Collins) Augyte, Yarish & Neefus *comb. nov.* & *stat. nov.*

Key Words: Hybridization, Hydrodynamic forces, Laminariales, Phaeophyceae, *Saccharina angustissima*, Taxonomy

INTRODUCTION

Several studies on members in the order Laminariales (Phaeophyceae) have identified phenotypic plasticity as a driver of the great range of morphological variation seen in thallus shapes as well as macroalgal production and physiology when exposed to strong wave exposure (Gerard & Mann 1979; Sjtun & Fredriksen 1995; Hurd 2000; Blanchette *et al.* 2002; Fowler-Walker *et al.* 2006). In the rocky intertidal zone, macroalgae can adapt their shape and size to be more flexible and streamlined to lessen the hydrodynamic forces from breaking waves (Carrington 1990; Gaylord & Denny 1997; Boller & Carrington 2007). Typically, an increase in wave exposure has been linked to a decrease in kelp blade width (Philibert 1990; Fig. 1). Transplant experiments of kelps from exposed environments with rapid water movement to protected ones with slow flow can result in a shift in morphological development from narrow, thick, flat blades to wider, thinner, and more undulate blades (Sundene 1961; Gerard & Mann 1979; Koehl *et al.* 2008). Larger blade size maximizes surface area for light harvesting and nutrient uptake compared with narrower and thicker blade size reducing drag and preventing breakage when exposed to high water currents (Hurd *et al.* 2014). Furthermore, smooth macroalgal thalli experience lower drag than ruffled thalli but also lower photosynthetic rates (Carrington 1990). The strong selection pressure of water motion over small spatial scales has been shown to promote genetic and phenotypic divergence among kelp populations including the southern sea palm *Ecklonia arborea* (Areschoug) M.D.Rothman, Mattio & J.J.Bolton (Roberson & Coyer 2004) and the deep-water elk kelp *Pelagophycus porra* (Léman) Setchell (Miller *et al.* 2000).

Phenotypes are regulated by gene expression in response to certain environmental cues. Phenotypic plasticity may allow species to maximize their fitness to survive in a broader range of environments (Price *et al.* 2003; Auld *et al.* 2010). As selection acts on phenotypes, novel traits may emerge as adaptive responses to environmental cues. Populations may become genetically isolated, and produce fixed differences, causing a shift to a nonplastic phenotype (West-Eberhard 2005; Pfennig *et al.* 2010). The capacity of macroalgae to adjust the shape of their thalli to overcome various physical stressors is an important adaptive strategy allowing them to

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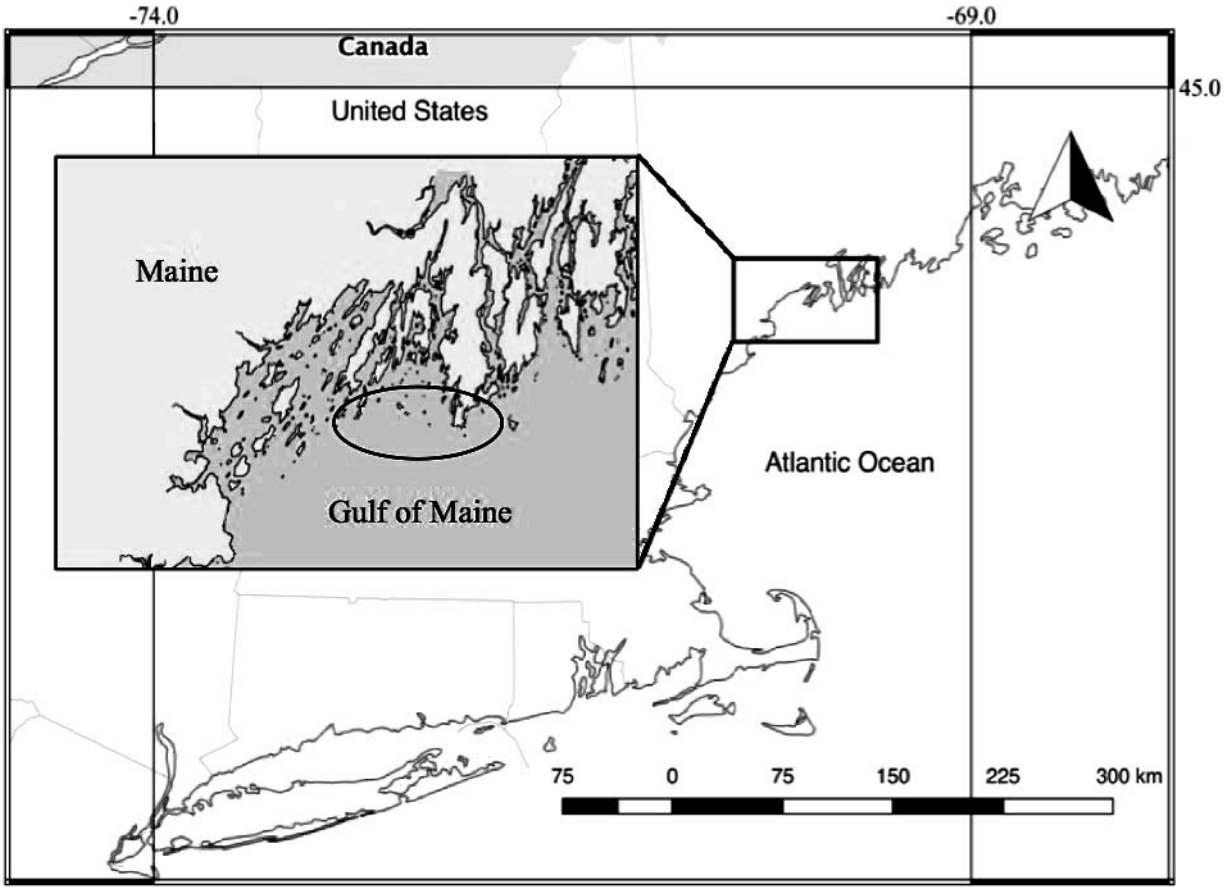

Fig. 1. Map of *Saccharina latissima* f. *angustissima* range with insert showing known distribution in Casco Bay in the Gulf of Maine, circled on insert. Insert from Nauticalchartsonline.com.

survive and exploit a range of niches (Roberson & Coyer 2004; Fowler-Walker *et al*. 2006; Wernberg & Vanderklift 2010). Intraspecific morphological variation in response to various environmental conditions is common in both macro- and microalgae (Blanchette 1997; Lürling 2003; Demes *et al*. 2009; Leliaert *et al*. 2014; Verbruggen *et al*. 2014).

The temperate kelp *Saccharina latissima* forms a large Pacific–Atlantic species complex with broad morphological and physiological plasticity with a strong genetic component (Bartsch *et al*. 2008). The center of evolutionary diversity of the genus *Saccharina* is in the northwest Pacific including Japan, Korea, and portions of the South China Sea (Bolton 2010). In the western North Atlantic, *S. latissima* is a common kelp, with its southern distributional limit in Long Island Sound (Egan & Yarish 1988).

Saccharina latissima forma *angustissima* (Collins) A.Mathieson is an endemic narrow-bladed morphotype found only in the most wave-exposed habitats of midcoastal Maine. It was first described by Frank S. Collins (1880) as *Laminaria agardhii* Kjellman, and was later amended as *Laminaria agardhii* forma *angustissima* (Collins 1911; Mathieson *et al*. 2008). The binomial *L. agardhii* has since been designated a synonym for *Laminaria saccarina* (Wilce 1965; Philibert 1990) and was finally reclassified as *S. latissima* (Lane *et al*. 2006). *Saccharina latissima* f. *angustissima* reportedly occurs in only two counties (Sagadahoc and Cumberland) of southern Maine spanning 8 nautical miles, on flat ledges and vertical cliffs exposed to strong surf (Fig. 1; Mathieson *et al*. 2008). The maximum tidal range in Casco Bay is 4.2 m. *Laminaria digitata* and *Alaria esculenta* are two other species that are found growing at the lower boundary of the *S. latissima* f. *angustissima* populations (Philibert 1990, personal observation). The upper boundary of the narrow-bladed kelp is set by extensive monospecific-stands of the red alga Irish moss (*Chondrus crispus*) and intermixed with the kelp beds are extensive populations of blue mussel (*Mytilus edulis*).

A 2-yr kelp aquaculture cultivation study was conducted using meiospores from *Saccharina latissima* f. *angustissima* at several open-water sites in coastal Maine (Augyte *et al*. 2017). Juvenile sporophytes were grown to maturity on long lines for 6 mo. The wave energy at both farm sites was low compared with what the kelp population experiences in the field. Furthermore, sporophytes of the common sugar kelp, *S. latissima*, with parental meiospores obtained from subtidal populations from Casco Bay, Maine, were grown alongside the *S. latissima* f. *angustissima* sporophytes and the resulting lengths and widths of the two morphologies were compared at harvest. The results of this common garden experiment showed that the sporophyte *S. latissima* f. *angustissima* blade retained its length-to-width ratio and did not become wide like the sporophytes of *S. latissima*.

Table 1. Morphological, ecological, and life-history trait comparison of broad-type *Saccharina latissima* (Egan & Yarish 1988, 1990; Lüning 1990; Bartsch *et al*. 2008; Lindeberg & Lindstrom 2010) and *S. latissima* f. *angustissima* based on measurements taken on 14 June 2013 and again on 8 October 2014 from Giant's Staircase, Bailey's Island, Harpswell, Maine and by Philibert (1990) and Mathieson & Dawes (2017).

	S. latissima	*S. latissima* f. *angustissima*
Blade length	~2 m, up to 3.5 m	180 cm (± 96 cm), up to 4.4 m
Blade width	20–30 cm	1.6 cm (± 0.7 cm), up to 5 cm
Blade thickness	moderately thin and undulate, with frequent rows of bullations or smooth	1.1 mm (± 0.25 mm), up to 1.5 mm, thick, flat, lacking bullations
Blade color	light to medium brown	dark brown
Haptera	large, ramified and fibrous, finely branched	coalescent with multiple stipes
Stipe	cylindrical, solid, flexible, usually < 15 cm, up to 50 cm	9 cm (± 7 cm), up to 36 cm
Mucilage ducts	absent in blade and stipe	absent in blade and stipe
Sporangia disposition	middle of the blade	middle of the blade
Sporogenesis	two peaks; spring and early autumn	one peak; early autumn
Depth	low intertidal to 30 m deep	low intertidal to shallow subtidal, from +0.5 m to −0.5 m relative to mean low water
Phenology	perennial in the Atlantic, annual in the Pacific Ocean. Blade can erode in summer, regrows in the fall. Or dies back in fall/winter and regrows in spring	blade erodes in winter, regrows in the spring
Habitat	protected to semiprotected	wave exposed
Distribution pattern	geographically widespread, circumpolar; Northwest and Northeast Pacific, Arctic Ocean, northeast and northwest Atlantic. Both cool temperate and polar coastal waters.	rare, restricted to flat promontories and islands in mid-coastal Maine, Casco Bay in cool temperate waters

Fig. 2. Representative specimens of the genus; *Saccharina latissima* f. *angustissima*, top, from exposed site, and *Saccharina latissima*, bottom, from wave-sheltered site.

Fig. 3. *Saccharina latissima* f. *angustissima* from habitat.

However, some characters, including thickness of the blade, were lost while ruffles formed on blade edges. These results confirmed that environmental cues alone were not wholly responsible for the unique morphology in *S. latissima* f. *angustissima* and suggested a genetic basis. Similarly, Philibert (1990) made preliminary observations on *S. latissima* f. *angustissima* and on the basis of habitat, morphology, and transplant experiments concluded that it was a genetically distinct form. His work further suggests that the two morphotypes remained distinct after growing in identical laboratory culture conditions for 15 wk.

Current algal species delimitation is centered on molecular data combined with other supporting evidence including morphology, ecology, or physiology (Mann 2010; Balakirev *et al.* 2012; Leliaert *et al.* 2014). The cytochrome *c* oxidase subunit I (COI-5P) gene has been successfully used to test species boundaries and resolve groupings of mitochondrial haplotypes of various kelp populations (Lane *et al.* 2007; McDevit & Saunders 2009; Macaya & Zuccarello 2010; Marins *et al.* 2012). Similarly, the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) spacer has been used to resolve the phylogeny of members of class Phaeophyceae (Yoon *et al.* 2001; Fraser *et al.* 2009; Boo *et al.* 2011; Leliaert *et al.* 2014). Previous studies show that *Saccharina latissima* can easily hybridize other kelp genera including with *Eisenia arborea*, *Nereocystis leutkeana*, and *Lessoniopsis littoralis* (Druehl *et al.* 2005); however, after 20 wk, the sporophytes were stunted or morphologically deformed. Bolton *et al.* (1983) found a lack of prezygotic barriers with several species of *Saccharina* crossed from the Pacific and Atlantic Oceans, including with *Saccharina ochotensis* and *Saccharina longicruris*, before the latter was synonymized with *S. latissima* (McDevit & Saunders 2010). Because of the recent (15–35 mya) divergence events as well as similar reproductive phenology for genera of the Alariaceae, Laminariaceae, and

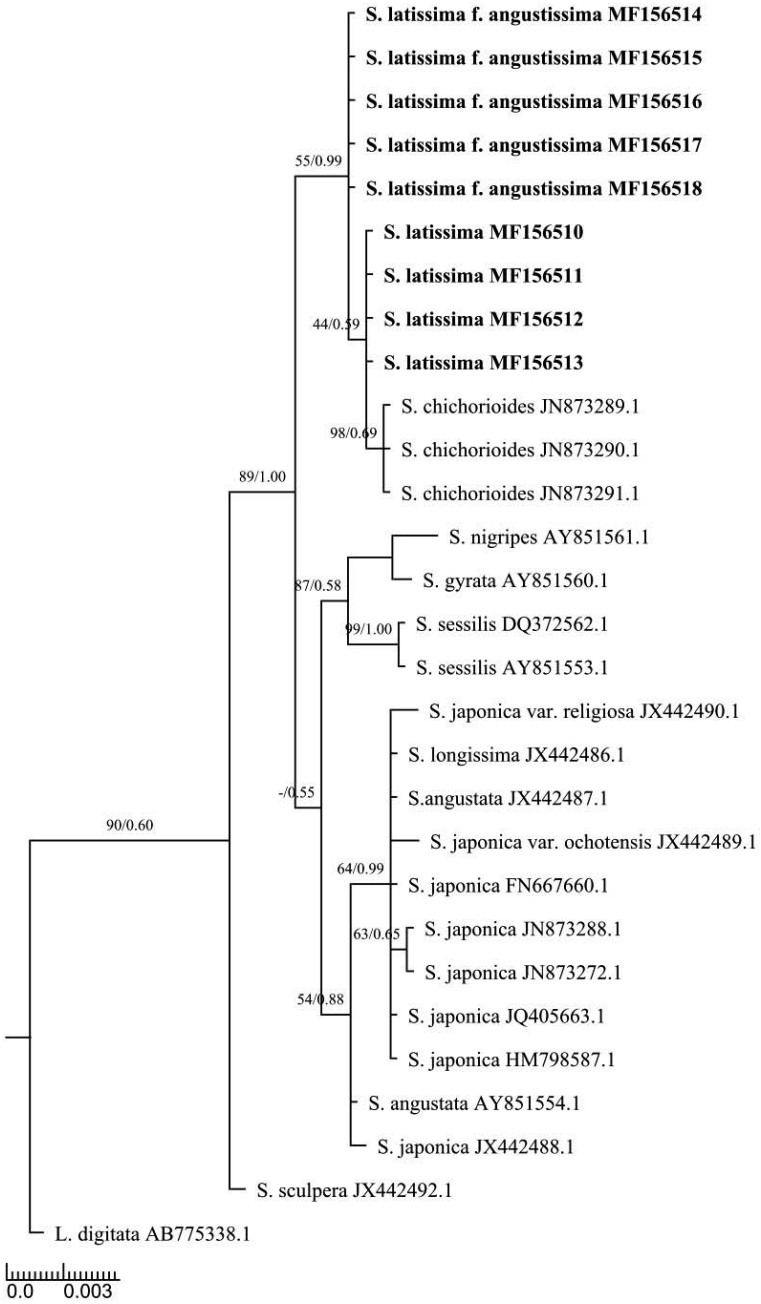

Fig. 4. Bayesian phylogenetic tree obtained with MrBayes on the basis of *rbc*L DNA sequences of *Saccharina* (alignment = 1017 bp in length). *Laminaria digitata* was used as an outgroup. Nodal supports are maximum likelihood (ML) bootstrap values ($\geq$ 50%; left) and Bayesian inference (BI) posterior probability ($\geq$ 0.50; right). Scale bar represents units of length in units of expected substitutions per site.

Lessoniaceae, intergeneric hybridization readily occurs among these family members. However, laboratory-produced kelp hybrids fail to yield viable meiosporangia on adult sporophytes (Lewis & Neushul 1995). As kelps easily hybridize among different genera, we hypothesized that our kelp would produce successful reciprocal crosses between the two forms.

The phenotypic differentiation of the narrow-bladed kelp along with strong physical isolation and potential reproductive isolation from the common sugar kelp could be driven by local adaptation in response to the drag forces imposed by waves. The small blade size of the *Saccharina latissima* f. *angustissima* population combined with its adaptation to these extreme conditions make it an ideal candidate for a rapidly evolving novelty. Therefore, the present study aimed to clarify the taxonomic position of *S. latissima* f. *angustissima* from southern Maine on the basis of (1) morphological measurements; (2) phylogenetic analyses of four common algal bar-code molecular markers; (3) an ecophysiological experiment testing sexual compatibility; and (4) quantification of hydrodynamic forces associated with the wave-exposed sites compared with sheltered sites. In

the present study, the high wave environment was hypothesized to be driving this unique kelp morphotype.

MATERIAL AND METHODS

Specimens were collected on 14 June 2013 and again on 8 October 2014 of *Saccharina latissima* f. *angustissima* from Giant's Staircase, Bailey's Island, Harpswell, Maine (43°43.38′N, 69°59.656′W) and regular *S. latissima* from Land's End, Bailey's Island, Harpswell, Maine (43°43.031′N, 70°0.288′W). Field-collected specimens were kept in plastic bags over ice and transported in a cooler to the UCONN Stamford Seaweed Biotechnology Laboratory for DNA extraction. Kelp specimens were measured for length and width of blades and stipes as well as thickness of blades. A small piece of each thallus was excised and placed in silica gel for DNA extraction. The specimens were then pressed onto acid-free herbarium paper and deposited into the UCONN George Stafford Torrey herbarium in Storrs, Connecticut USA (accession nos. CONN00209762–CONN00209779).

A multilocus approach was used to analyze the genetic variability of *Saccharina latissima* f. *angustissima* as compared with other *Saccharina* spp. DNA sequences from four loci were studied, including mitochondrial (*cox*1 = cytochrome oxidase subunit I and *cox*3 = cytochrome oxidase subunit III), nuclear (ITS = internal transcriber spacer 2 and 5.8S ribosomal RNA gene), and chloroplast (*rbc*L = large subunit of RuBisCo) markers.

Genomic DNA was extracted from dried kelp blade tissue following the protocol of the DNeasy plant mini kit (Qiagen, Hilden, Germany) with a slight modification of an initial soak in 400 µl of acetone for 10 min. Subsequently, the acetone was discarded and the tissue was air-dried. DNA amplification was done by polymerase chain reaction (PCR) following the protocol of Apli-Taq Master Mix (New England BioLabs). For *rbc*L, the primer pair KL2 and KL8 was used following the thermal profile by Lane *et al.* (2006). The nuclear ITS primers and thermal profile from Tai *et al.* (2001) were used with modifications as per Lane *et al.* (2006). Finally, for mitochondrial *cox*3, primers C3F34 and R20 were used according to Boo *et al.* (2011) following PCR thermal protocol as in Lee *et al.* (2009). For *cox*1, the CO1-5P region was amplified with the primer pair GAZR2 and GAZF2 (Lane *et al.* 2007) using the thermal profile by McDevit & Saunders (2009). PCR products were purified with a QIAquick PCR purification kit (Qiagen) and sequenced at the UCONN Biotechnology Core facility (Storrs, Connecticut USA).

Genome Compiler 2.2.86 (freely available program package, acquired by Twist Bioscience) was used to check and trim raw sequences. Additional sequences of the same genus were retrieved from the GenBank nucleotide database (Table S1). *Laminaria digitata* was added as an outgroup. Sequences were aligned with MUSCLE (Edgar 2004), as implemented in MEGA v6.06 (Tamura *et al.* 2013). Alignments were deposited in GenBank.

To assess the variation between the two morphotypes and other closely related species, each data set was compared using several techniques. First, pairwise uncorrected p-distances were estimated in MEGA v6.06 (Wang *et al.* 2014). Second, phylogenetic relationships were inferred using maximum likelihood and Bayesian approaches. Maximum likelihood analyses were performed in RAxML v8 (Stamatakis *et al.* 2008) with 10,000 bootstrap replicates. For each gene region, the best model for maximum likelihood was selected using MEGA v6.06 using the Akaike information criterion (with the fewest parameters). The best selected model was T92 + I for both ITS and *rbc*L, HKY + I for *cox*1, and TN93 + G for *cox*3. Bayesian inference was performed with MrBayes v3.2.4 (Huelsenbeck & Ronquist 2001) using 5 million generations and the selected models. The trees were sampled every 1000 generations. The initial 10,000 trees were discarded as burn-in, and the remaining trees were used to compute a consensus phylogeny and to determine posterior probability values at the nodes. Final phylogenies from RAxML and MrBayes data were displayed using FigTree v1.3.1 (Rambaut 2009). Last, a species-level phylogenetic analysis was performed using multilocus data under the coalescent model using SVDquartets in PAUP* 4.0 (Swofford 2002; Chifman & Kubatko 2014). We did this by combining data of *rbc*L and ITS and then again combining data from *cox*3 and *cox*1 sequences. All the sequences could not be used because the same specimen for one marker did not have data available for the other three markers.

A hybridization experiment was set up to test whether the two morphologically distinct forms were potentially reproductively isolated or if they could produce sporophytes. After collection of reproductive blades, spores released from sorus tissue were used to establish separate male and female gametophyte cultures that were used to make crosses using standard protocols following Redmond *et al.* 2014. The strips of sorus tissue were scraped gently and cleaned of epibionts by immersion in a dilute iodine solution, rinsed, and then wrapped in damp paper towels. The sorus tissue was stored overnight at 10°C in darkness. The following day, sorus tissue was reimmersed in sterile seawater to stimulate release of the meiospores. After removing the spent sori, the spore-filled seawater was filtered through 35–80-µm filters to remove potential contaminants and debris. Spore concentrations were determined with a hemocytometer under a compound microscope, and adjusted to a spore cell concentration of 4000–6000 cells ml^{-1}. These meiospores were allowed to settle onto cover slips left overnight in a settling chamber in Provasoli's enriched seawater (PES/2).

Kelp gametophytes were isolated and sexed on the basis of filament width, where female filament cells are larger than male cells, as well as the presence of oogonia and antheridia. These filaments were grown under 12:12 h light:dark at 10°C over a period of 3 mo, during which time the culture medium was changed biweekly and the filaments were periodically chopped up to increase biomass. The reciprocal and selfing crosses were made by mixing males and females of the two kelps into deep-well Petri dishes containing 250 ml of autoclaved seawater with 1/2PES nutrients. Trials were run in 10°C at 12:12 h light:dark cycles at 40 µmol photons m^{-2} s^{-1}. Final observations were made at 2 mo and presence or absence of sporophyte formation was recorded.

Maximum wave velocity and force comparisons of exposed and protected sides of ledges were quantified by deploying dynamometers (Bell & Denny 1994) at Bailey's

Table 2. Generic divergence in ITS/*cox*3/*rbc*L/*cox*1 sequences between *Saccharina* species. Each number indicates uncorrected p-distance (% divergence). Missing data are indicated with "—".

	S. latissima forma *angustissima*	*S. latissima*	*S. japonica*	*S. angustata*
S. latissima forma *angustissima*	0.65/0.16/0/0			
S. latissima	0.05/0.48/0.6/0.18	0		
S. japonica	1.54/3.31/0.88/4.52	1.54/3.01/0.37/4.52	0	
S. angustata	1.54/3.64/0.88/5.02	1.54/3.32/0.93	0/2.06/0/4.36	0
S. japonica var. *religiosa*	1.54/3.31/0.88/4.52	1.54/3.0/0.98/4.52	0/0/0.20/0	0/2.05/0.52/4.36
S. japonica var. *ochotensis*	1.54/3.32/1.08/4.52	1.54/3.01/0.93/4.52	0/0/0.2/0	0/2.06/ 0.41/4.36
S. longissimi	1.54/3.31/0.88/5.02	1.54/3.0/0.98/4.52	0/0/0.39/0	0/2.05/0.31/ 4.36
S. sessilis	3.028/—/0.83/6.15	4.37/—/1.03/6.15	4.34/—/0.88/6.15	4.37/—/0.52/ 5.81
S. sculpera	4.6/5.22/0.98/4.52	4.6/4.91/1.08/4.52	4.6/5.06/0.83/4.36	5.12/4.43/0.83/5.65
S. chicorioides	—/—/0.21/0.97	—/—/0.21/0.97	—/—/ 0.88/5.17	—/—/0.72/5.01
L. digitata	3.12/9.49/2.26/9.05	4.43/9.18/2.95/9.05	4.65/8.70/2.36/10.18	4.43/9.34/3.15/10.18

Island, Harpswell, Maine. These simple devices, in conjunction with a mathematical model, provided direct measurements at the attachment position during the period of deployment and allowed for among-site estimates (Bell & Denny 1994; Robles *et al*. 2010). The dynamometers ($n = 3$ at each site) were attached to rocks at mean low water at two separate sites of Bailey's Island: at the very exposed site of Giant's Staircase with known populations of *Saccharina latissima* f. *angustissima*, and at the protected site of Land's End with known populations of *S. latissima*. Readings were taken over four consecutive low tides in late August 2015. Maximum water velocity (in meters per second) and drag force (measured in newtons) were calculated using the formulas provided in Bell & Denny (1994). Using R software 3.3.1 (R Development Core Team 2008), we ran a two-way analysis of variance to test the effects of location (exposed vs sheltered) and time (four low tide readings) on the amount of drag force as the response variable.

RESULTS

Morphological observations

Samples collected in June 2013 from Harpswell, Maine revealed that the narrow-bladed kelp is morphologically distinct from other common kelps in the western North Atlantic including *Saccharina latissima*, *Alaria esculenta*, and *Laminaria digitata*. Comparisons between the common *S. latissima* showed that *S. latissima* f. *angustissima* blades were on average over 10–20 times narrower (Table 1, Figs 2, 3). Measurements from 125 samples revealed that the average blade length was 180.0 ± 96.0 cm, blade width was 1.6 ± 0.7 cm, stipe length was 9.0 ± 7.0 cm, and blade thickness was 1.1 ± 0.3 mm). Besides being exposed to strong surf and high current velocities, this phenotype also grows in the very low intertidal zone where it is further exposed to desiccation stress during spring tides.

Phylogenetic analyses

For *rbc*L, alignments were based on a total of 1017 base pairs (bp) after trimming raw sequences. The consensus tree topology was identical between MrBayes (Fig. 4) and RAxML analyses. The tree branches were very short, providing little species-level resolution. The narrow form (*Saccharina latissima* f. *angustissima*) was sister to the broad form of *S. latissima* and to *Saccharina chichorioides* with low bootstrap support. Although the tree topology suggests that there is some divergence between the two forms of *S. latissima,* the p-distance was low at 0.6% (Table 2).

The two mitochondrial markers had congruent phylogenetic tree topologies. Both the *cox*3 (640 bp in length) and *cox*1 (619 bp in length) showed little divergence between the two morphologies and had p-distances of 0.48% and 0.18%, respectively (Table 2, Figs 5, 6). In the *cox*1 phylogenetic tree, the clade with high support (1) included the four species: *Saccharina coriacea*, *S. chichorioides*, *S. latissima*, and *S. latissima* f. *angustissima*. It is interesting to note that on the *cox*3 phylogenetic tree, one sequence of *S. latissima* (KM675818.1) from China appeared as sister to the *S. latissima* clade that included *S. latissima* f. *angustissima* and *S. coriacea*.

The nuclear ITS-based p-distances were low (0.05%) between the two forms of *Saccharina latissima* (Table 2, Fig. 7). The tree topology based on ITS sequences (404 bp in length) showed support for a clade that included both *S. latissima* forms. Interestingly, *Saccharina gyrata* (AF319021.1) and *Saccharina nigripes* (AY857893.2) were placed within that clade as well.

Results of the SVDquartets analysis (Fig. 8) reveal that the species tree of *rbc*L and ITS has moderate support for the clade of *S. latissima* and *S. latissima* f. *angustissima*. The tree based on mitochondrial data supports a clade composed of the two forms of *S. latissima* as well as *S. coriacea* (Fig. 9). However, it is interesting to note that some other taxonomically accepted *Saccharina* spp. are not well resolved in this tree. For example, in the *rbc*L + ITS tree (Fig. 8) there is moderate bootstrap support (53%) for a clade that shows low resolution for *Saccharina japonica*, *S. japonica* var. *ochotensis*, *Saccharina angustata*, *Saccharina longissima*, and *S. japonica* var. *religiosa* (Yotsukura *et al*. 2008). The second tree suggests a polytomy between the above-mentioned species in addition to *Saccharina diabolica* and *Saccharina longipedalis* (Fig. 9).

Hybridization

Successful crosses were completed between *Saccharina latissima* and *S. latissima* f. *angustissima*. Blades were observed

Table 2. Extended

S. religiosa	*S. ochotensis*	*S. longissima*	*S. sessilis*	*S. sculpera*	*S. chicorioides*	*L. digitata*
0						
0/0/0.39	0					
0/0/0.2/0	0/—/0.2/0	0				
4.34/—/0.88/6.15	4.34/—/1.08/6.15	4.34/ —/0.88/6.15	0			
4.6/5.05/0.88/5.65	4.6/5.06/0.93/5.01	4.6/5.05/0.82/4.36	5.64/—/1.18/5.48	0		
—/—/0.88/5.01	—/—/1.08/5.02	—/—/0.88/5.01	—/—/0.98/5.02	—/—/0.98/4.85	0	
4.65/8.83/3.05/10.18	4.65/8.7/3.34	4.65/8.83/3.15/10.18	1.81/ —/3.15/9.65	—/8.86/2.36/8.89	—/—/2.26/9.69	0

with all the reciprocal and selfed crosses. Juvenile kelp sporophytes were observed that grew $\sim$ 0.5 cm in length.

Wave energy comparisons

The maximum wave force recordings confirmed higher water velocities and forces affecting the kelps at the exposed vs the protected sites, with a significant relationship between drag force and location ($F_{1,22} = 179$, $P < 0.000$, Fig. 10). More than half of the data for the protected site were at 0 m s^{-1} at the time of measurement, meaning there was not enough water velocity to make an impact on the recording device. The exposed sites had mean water velocities of 18.12 $\pm$ 3.89 m s^{-1}, whereas the protected site mean was 2.22 $\pm$ 3.25 m s^{-1}. The mean force at the exposed site was over 30 times higher in magnitude than at the protected site at 160.04 $\pm$ 32.58 N and 4.75 $\pm$ 6.75 N, respectively.

DISCUSSION

Plasticity yields specific morphological polyphenic traits, multiple discreet phenotypes from a single genotype, and is a starting point for rapid evolution (Schlichting & Smith 2002). If, over time, a population subject to strong selection pressure becomes specialized and later becomes reproductively isolated, an ecotype may form that no longer maintains a plastic response (Schlichting & Smith 2002). This sort of divergent selection on ecological traits can result from sympatric populations that inhabit separate niches inside close geographic areas and over time become locally adapted (Rundle & Nosil 2005; Leliaert *et al*. 2014).

The four markers used in this study indicate some level of separation between the broad form of *Saccharina latissima* and the narrow *S. latissima* f. *angustissima*; all of the phylogenetic trees indicate as much or more divergence between the two as there are between other species of taxonomically accepted *Saccharina* spp. For example, the ITS marker shows no divergence between *S. latissima* and *S. latissima* f. *angustissima*, but this is expected as other studies report as few as five to seven bp differences for *S. latissima* and closely related species (Lane *et al*. 2006, 2007). Yotsukura *et al*. (2008, 2010) also show low divergence between *S. angustata* and *S. longissima* from the ITS region.

McDevit & Saunders (2009) report 3–6% divergence within *Saccharina* on the basis of the mitochondrial COI marker. In this study, only 0.18% divergence was observed between *S. latissima* and *S. latissima* f. *angustissima* for the mitochondrial markers used. Although these values are low, in cases with divergent selection and local adaptation, neutral markers can be uninformative for the assessment of rapid phenotypic divergence (Nosil *et al*. 2009, Leliaert *et al*. 2014). All phylogenetic trees, including the ones generated with SVDquartets, provide support for a close relationship between *S. latissima* and *S. latissima* f. *angustissima*, whereas other taxonomically accepted species in the genus show similar levels of divergence. With enough time, we can expect to see substantially more genetic divergence between the two populations.

Furthermore, the unique population/ecotype is not geographically widespread and intermediate morphologies are not evident between the distinct populations. The range boundaries of *Saccharina latissima* f. *angustissima* are clearly delimited to a few wave-facing ledges and islands in Casco Bay (this study; Mathieson *et al*. 2008; Mathieson & Dawes 2017). The extreme example is Bailey's Island, where on the side that faces incoming swells, dense low intertidal kelp beds of *S. latissima* f. *angustissima* are found, whereas on the protected side, only the typical *S. latissima*. Furthermore, there are some noticeable differences in the timing of sporogenesis—for *S. latissima* it occurs in both spring and summer, whereas it has only been observed in the fall for *S. latissima* f. *angustissima*.

In fact, the entities are persistently morphologically distinct and in a common garden experiment, *Saccharina latissima* f. *angustissima* stayed true to its parental population, exhibiting traits adapted to a wave-bashed environment even when grown in calm conditions (Augyte *et al*. 2017). The narrow morphotype was conserved over two consecutive growing seasons for the duration of the cultivation study (Augyte *et al*. 2017). In subsequent years, as farmers continue to grow it, they provide feedback on the differences observed in texture, thickness, and taste (S. Redmond, personal communication). For example, significant differences were found between the average length-to-width ratios ($P < 0.001$) of the two cultivated kelp; *S. latissima* f. *angustissima* was at 85.89 $\pm$ 20.23–56.14 $\pm$ 5.02 cm, whereas *S. latissima* was 9.54 $\pm$ 2.24–12.9 $\pm$1.10 cm for the two

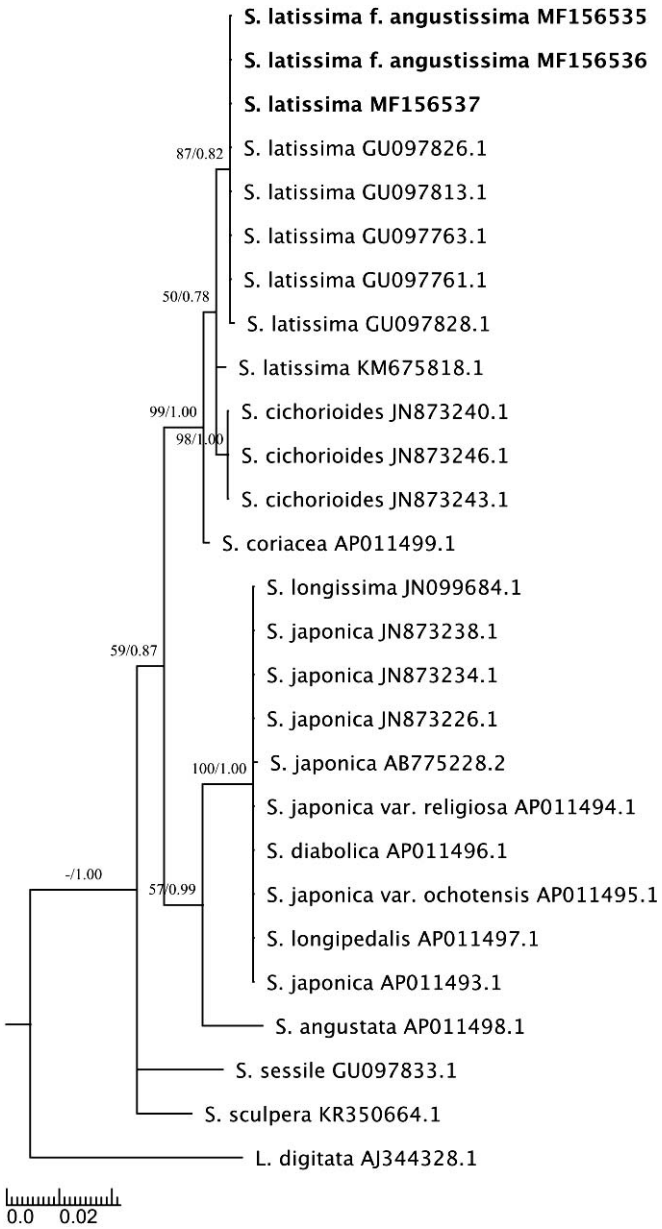

Fig. 5. Phylogenetic topology based on sequences of the mitochondrial *cox*1 marker obtained with MrBayes. DNA sequences of *Saccharina* (alignment = 619 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values (≥ 50%; left) and Bayesian inference (BI) posterior probability (≥ 0.50; right). Scale bar represents units of length in units of expected substitutions per site.

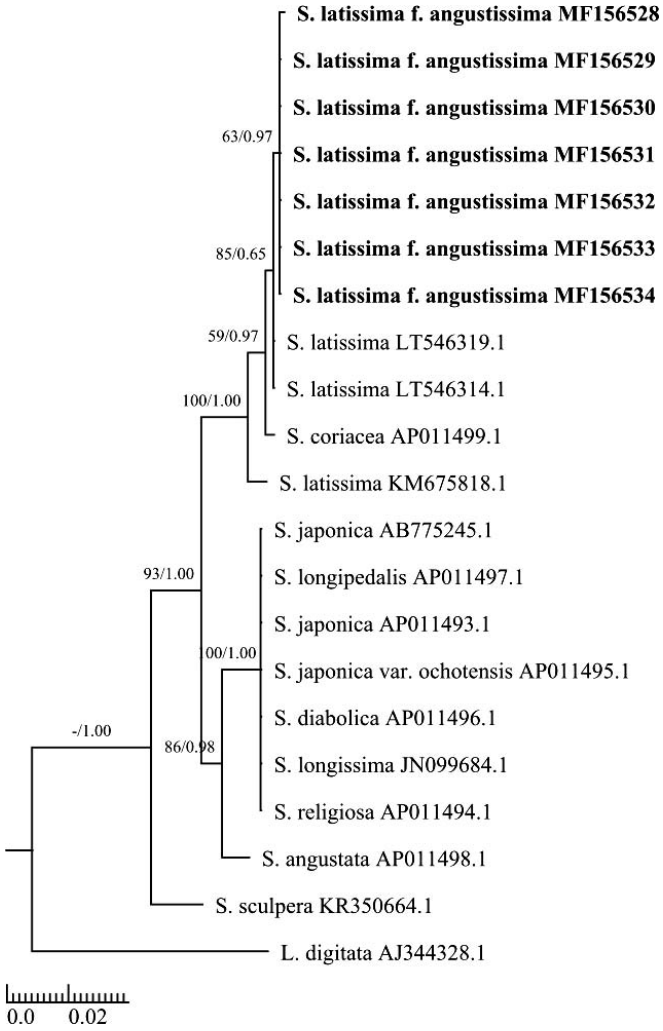

Fig. 6. Phylogenetic topology based on sequences of the mitochondrial *cox*3 marker obtained with MrBayes. DNA sequences of *Saccharina* (alignment = 640 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values (≥ 50%; left) and Bayesian inference (BI) posterior probability (≥ 0.50; right). Scale bar represents units of length in units of expected substitutions per site.

farms, respectively (Augyte *et. al.* 2017). This research shows that some of the traits enhancing fitness on the wave-exposed coast are heritable, with a genetic basis for the observed morphotype.

Our combined morphological and molecular analyses and results of the common garden cultivation study (Augyte *et al.* 2017) provide evidence that *Saccharina latissima* f. *angustissima* adapted to an extreme habitat with high exposure to oceanic swells, has undergone some level of speciation, and has a genetic basis driving its unique morphology. These results further suggest that the differences in environment, specifically the extreme habitat niche, have led to barriers in gene flow between the *S. latissima* f. *angustissima* population and other *S. latissima* populations. These prezygotic barriers to gene-flow have allowed the unique kelp to diverge, colonize, and persist in the new habitat. Consequently, the molecular evidence presented in this study, coupled with ecological and hybridization studies, provide support to elevate *S. latissima* f. *angustissima* (Collins) A.Mathieson to the new status designation of *Saccharina angustissima* (Collins) Augyte, Yarish & Neefus *comb. nov.* & *stat. nov.*

The fact that the two forms can successfully hybridize under laboratory conditions is another indication that the two are closely related, yet not necessarily conspecific. Many previous studies demonstrated that kelp easily hybridize with congeners and even with members of the same family (Lewis & Neushul 1995; Kraan & Guiry 2000; Liptack & Druehl 2000; Druehl *et al.* 2005). Furthermore, without genetic

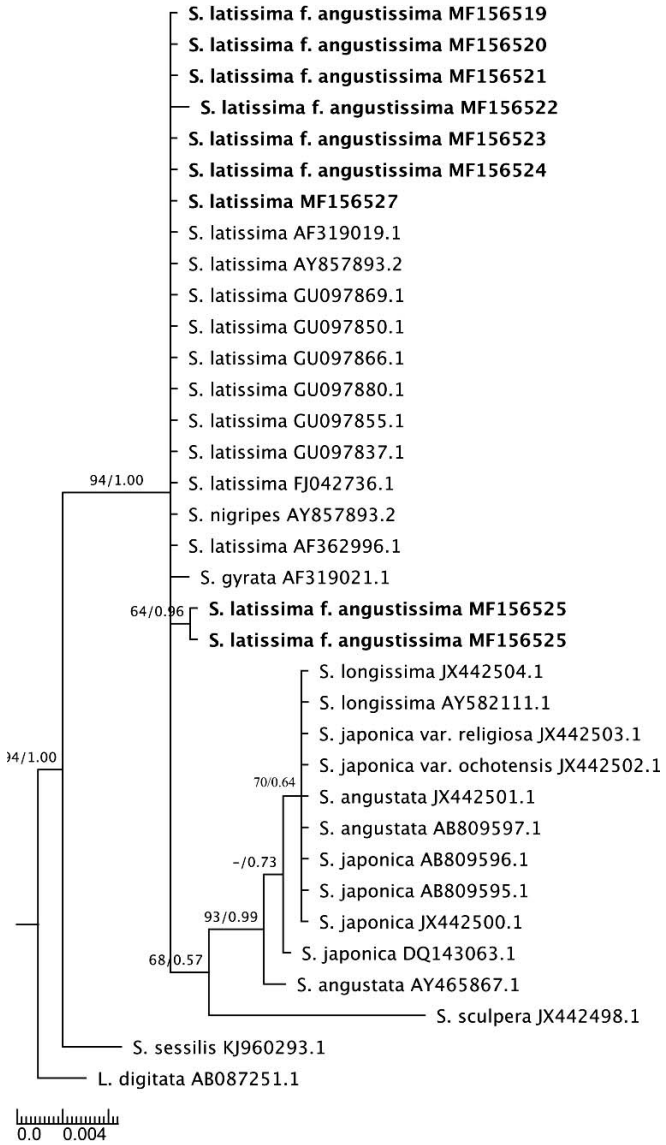

Fig. 7. Phylogenetic topology based on sequences of the nuclear internal transcriber spacer(ITS) region obtained with MrBayes. DNA sequences of *Saccharina* (alignment = 404 bp in length). Nodal supports are maximum likelihood (ML) bootstrap values ($\geq$ 50%; left) and Bayesian inference (BI) posterior probability ($\geq$ 0.50; right). Scale bar represents units of length in units of expected substitutions per site.

testing, it is impossible to elucidate whether the blades observed in this experiment were sporophytes from hybridization or the products of parthenogenesis, androgenesis, or apogamy (Liptack & Druehl 2000). Although few kelp hybrids have been observed in natural populations, many kelp species occur sympatrically, suggesting that in the field, prezygotic barriers exist to preserve species integrity (Tellier *et al*. 2011). On the ledges where *Saccharina angustissima* occurs, the timing of sexual reproduction is similar to that of *S. latissima*, with peak sorus production occurring in the fall (Philibert 1990). The critical reproductive barrier then is the different wave-exposure types corresponding to the two morphotypes.

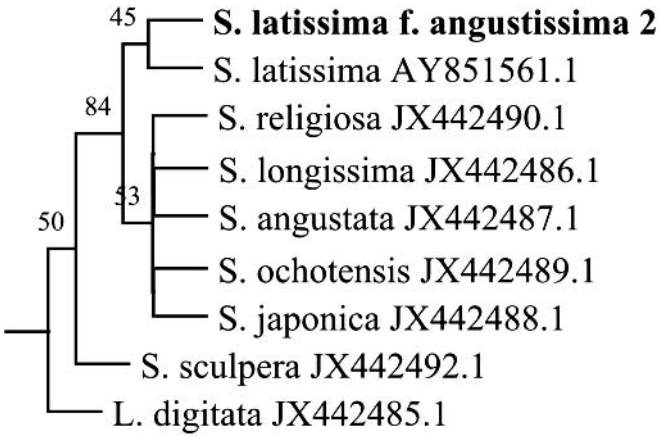

Fig. 8. SVDquartets bootstrap consensus species tree of 13 *Saccharina* taxa and 1 outgroup (*L. digitata*) based on *rbc*L and ITS markers; a total of 1422 bp made by evaluating 20,000 random quartets performing bootstrapping, with 10,000 replicates, with seed at 475,839. Bootstrap support values are indicated on tree nodes.

Macroalgal photosynthesis and nutrient uptake are profoundly influenced by wave motion (Kregting *et al*. 2013) and considerable variation in wave energies is found even along short segments of coastline (Robles *et al*. 2010). Water flow rates and wave velocities experienced by macroalgae in the subtidal zone are greatly reduced compared with the forces experienced on exposed rocky coasts (Gaylord & Denny 1997). The known distribution for *S. angustissima* is the low intertidal zone on islands and flat horizontal platforms or ledges severely affected by breaking waves (Mathieson *et al*. 2008; Mathieson & Dawes 2017). As expected, we found large differences in wave forces at the exposed sites compared with the protected sites. In stormy

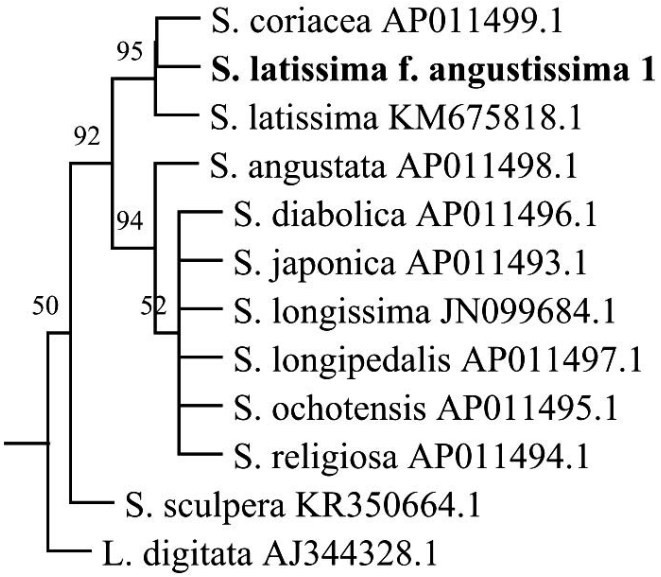

Fig. 9. SVDquartets bootstrap consensus species tree based on 11 *Saccharina* taxa and 1 outgroup (*L. digitata*) was built with sequences from *cox*3 and *cox*1 markers, for a total of 1259 bp made by evaluating 20,000 random quartets performing bootstrapping, with 10,000 number of replicates, with seed at 475,839. Bootstrap support values are indicated on tree nodes.

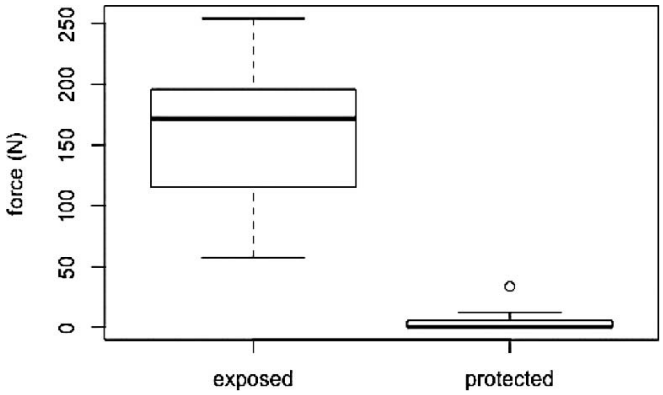

Fig. 10. Average forces experienced at two sites with varying degrees of wave exposure. Mean maximum wave forces (N) recorded at two sides of Bailey's Island, the exposed site (Giant's Stairs) and the protected site (Land's End) over four consecutive low tides.

conditions in the surf zone, seaweeds may experience flow rates of 25 m s^{-1} and accelerations of > 400 m s^{-2} (Harder *et al*. 2006). Maximum wave forces have been measured for the stipe of the large (> 4 m) kelp *Durvillaea antarctica* (Chamisso) Heriot of around 300 N and water velocities of 30 m s^{-2} (Stevens *et al*. 2002). Forces of 101–234 N were documented over the course of a few tidal cycles. It is also interesting to note that measurements were taken on relatively calm days in the summer in August. In the winter, sites with high wave exposure would be expected to have greater water forces and velocities compared with days in the summer (Blanchette 1997). Similarly, the wave velocity recordings at our site during the low wave energy season were underestimates for the differences in flow speed among sites, as we would expect higher wave exposure during the fall season or during storms. In the winter, the forces are so great that the blades can be torn off from their stipes (personal observations).

It is still unclear why this unique population does not take up residence on numerous other sites along the coast with similar exposure habitats, especially since *Saccharina latissima* has a circumboreal distribution, whereas *S. angustissima* is only known to span a radius of 8 nautical miles in Maine. As part of future work, we will utilize microsatellite markers to examine population-level divergences and identify potential hybridization events in field populations.

TAXONOMIC TREATMENT

The formal combination and new status is presented below:

Saccharina angustissima (Collins) Augyte, Yarish & Neefus *comb. nov.* & *stat. nov.*

BASIONYM: *Laminaria agardhii* forma *angustissima* Collins, *Phycotheca Boreali-Americana,* D: LXXXIII (1905). Also: Collins, *Rhodora* 8: 108 (1906).

HOMOTYPIC SYNONYM: *Saccharina latissima* forma *angustissima* (Collins) A.Mathieson in Mathieson *et al*. (2008).

LECTOTYPE (DESIGNATED HERE): Phycotheca Boreali-Americana no. LXXXIII (1905), NY No. 02243824 collected by Frank Collins, 18 July 1903.

ISOTYPES: NY No. 02243826, 02243815.

TYPE LOCALITY: Bailey Island, Casco Bay, Maine, USA. 43°43.38′N, 69°59.656′W.

DESCRIPTION: Blade length up to 4.5 m, strictly narrow, 1–5 cm wide, arising from hapterous base, usually coalesced with multiple terete stipes of 9 cm in length, up to 36 cm long. Thalli are annuals with peak sorus production in October through late November. GenBank accession numbers: MF156514–MF156536.

HABITAT: Dense beds found on flat, rocky ledges and vertical cliffs within the low intertidal to shallow subtidal zones (+0.5 to –0.5 m) of very exposed coast and offshore islands. Rare; distribution spans 8 nautical miles.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/17-40.1.s1.

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