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**Detecting no natural hybridization and predicting range overlap in *Saccharina angustata* and *Saccharina japonica***

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**Abstract**

Natural hybridization can play a significant role in evolutionary processes and influence the management and conservation strategies of species, however, this phenomenon is yet unknown in *Saccharina* kelps. *S. angustata* and two varieties of *S. japonica* (*S. japonica* var. *japonica* and *S. japonica* var. *diabolica*) partly overlap in the distribution along the pacific coast of Hokkaido, so they are good model system to learn about the hybridization and introgression among *Saccharina* species. Based on 13 highly variable nuclear microsatellites and a mitochondrial *COI* marker, we firstly assessed the genetic diversity levels of *S. angustata* and populations from Muroran to Shiranuka had the higher genetic diversity. Moreover, genetic diversity of *S. japonica* var. *japonica* is higher than that of *S. japonica* var. *diabolica*. There was poor genetic connectivity and limited interspecific hybridization among these closely related species. Ecological

niche models projected both *S. japonica* and *S. angustata* will expand northward under future climate scenarios, and the coast of Okhotsk Sea in Kamchatka Peninsula will be the future range overlap for these species. The interspecific hybridization and genetic diversity among these kelps provide insights for kelp farming practices as well as future conservation and management strategies of wild stocks.

**Key words:** *Saccharina*, genetic diversity, interspecific hybridization, range overlap, climate change

## 1. Introduction

*Saccharina* is a genus of cold temperature kelps and approximately twenty species are currently recognized, of which 12 are known to constitute the marine forest in north Japan (Kawashima, 2012; Lane et al., 2006; Yotsukura et al., 2010a). Particularly, the pacific coast of Hokkaido harbors a high diversity of *Saccharina* species (Kawashima, 2012), including two economically and ecologically important kelp species: *S. angustata* and *S. japonica* (*S. japonica* var. *japonica* and *S. japonica* var. *diabolica*) (Kawashima, 2012; Yotsukura et al., 2010a). These kelps build underwater forests that form the foundation of a diverse food web, and provide important ecosystem services such as carbon sequestration and nursery grounds for commercially important animals (e.g. fishes, Bertocci et al., 2015; abalone, Tegner and Dayton, 2000), as well as commodities for food and medical application (Tseng, 2001; Yotsukura et al., 2010b).

Hybridization is central in biology, particularly to provide a window into adaptive diversification and speciation (Arnold, 1997). Hybridization can generally result in several evolutionary outcomes, including genetic introgression (e.g. Wallace et al., 2004), genetic swarming of native species (e.g. Amanda and Gary, 2015), and the creation of new species (e.g. Neiva et al., 2017), but introgressive gene-flow is the most common outcome in seaweeds. Hybridization has been found to be prevalent in many seaweeds, for instance within congeneric species (e.g. *Alaria*, Kraan and Guiry 2000; *Fucus*, Coyer et al., 2007), even in members of the same family (Druehl et al. 2005). The best studied examples of natural hybridization are from *Fucus* (Wallace et al., 2004; Coyer et al., 2007), but the natural hybridization and introgression among *Saccharina* species is still unknown. Artificially hybridization has been widely reported in

*Saccharina*, because interspecific hybridization with heterosis have been used to produce elite cultivars, such as 901 (*S. longissima*×*S. japonica*)(Zhang et al., 2007), Rongfu (*S. japonica*× *S. latissima*)( Zhang et al., 2011), Dongfang No. 2 (*S. longissima*×*S. japonica*)(Li et al., 2007) and Dongfang No.3 (*S. longissima*×*S. japonica*)( Li et al., 2008). *S. angustata* and *S. japonica* partly overlap along the pacific coast of Hokkaido and are good model system to learn about natural hybridization and speciation in *Saccharina* species. These two sibling species can hybridize in the crossing tests under laboratory conditions (Druehl et al., 2005), however, whether there is natural hybridization and introgression among them still remain unclear and need further genetic evidence to support.

Climate change has the potential to shift species distribution ranges as global warming increase (Chen et al., 2011; Hoegh-Guldberg and Bruno, 2010). Future projections for some kelp species was mainly conducted in the Atlantic Ocean (Assis et al. 2016; Assis et al. 2017; Assis et al. 2018a; Franco et al. 2018; Raybaud et al. 2013), but recently there are several related researches in the northwestern Pacific (Takao et al. 2015; Sudo et al., 2020; Zhang et al., 2019). Extinction at the southern distribution limits and poleward extension of the northern distribution limits paralleled climatic oscillations (Assis et al., 2018a; Assis et al., 2017; Neiva et al., 2012, 2014; Takao et al., 2015). For *Saccharina* species, ten years' monitoring survey showed *Saccharina*-dominated communities appear to be stable in the Muroran (southwestern Hokkaido) and Shimoda (Pacific coast of middle Honshu) regions (Terada et al., 2019). However, other reports predicted that the rising seawater temperature is likely to exceed the upper thermal tolerance limit of *Saccharina* kelp communities on the Pacific side of Hokkaido and northern Honshu (Borlongan et al., 2019; Gao and Agatsuma, 2014; Gao et al., 2017) and, thus, to lead to a northward shift of some *Saccharina* species (Sudo et al., 2020; Zhang et al., 2019). Overlap in distribution is necessary for hybridization, so climate-induced range overlap could influence interspecific hybridization (Krosby et al., 2015; Acevedo et al., 2012). However, whether the niche overlap of these two species in future will reduce or expand is still unknown.

The main aim of this study was to identify interspecific hybridization among closely

related species and provide genetic background for the management and conservation of *Saccharina* kelps. More specifically, our objectives were to 1) characterize the genetic diversity and structure of *S. angustata* and its closely related species based on thirteen highly polymorphic microsatellites and a mitochondrial marker *COI* (cytochrome oxidase I); 2) assess the levels of interspecific hybridization and introgression; 3) predict the impact of climate change on the range overlap among these two species until year 2100.

## **2. Materials and methods**

### **2.1 Sample collection and molecular markers**

We sampled four *S. angustata* populations (105 individuals), five *S. japonica* var. *japonica* populations (102 individuals) and two *S. japonica* var. *diabolica* populations (48 individuals) at the pacific coastline of Hokkaido (Fig.S1; Fig.1a). Detailed sampling information and morphologic characters were indicated in Table S1 and S2.

Extraction of total genomic DNA is described elsewhere (Zhang et al., 2015). The mitochondrial marker *COI* was used in this study, and the primers of *COI* and the PCR procedures were showed in Zhang et al. (2015). The 11 populations were genotyped with 13 polymorphic microsatellite loci (Zhang et al., 2019), of which the primer sequences are listed in Table S3. The PCR procedures were performed according to our previous protocols (Zhang et al., 2017).

### **2.2 Genetic diversity analysis**

The mitochondrial *COI* sequences were aligned and edited in BIOEDIT v. 7.1 (Hall, 1999). The number of segregating sites ( $S$ ), number of haplotypes ( $h$ ), haplotype diversity ( $Hd$ ), and nucleotide diversity ( $Pi$ ) were calculated in DNASP v.5 (Librado and Rozas, 2009). A median-joining network was constructed using NETWORK v.4.5.1.6 based on the *COI* sequences (Bandelt et al., 1999).

Scoring errors and null alleles in the microsatellites data were corrected with the software MICRO-CHECKER v.2.2 (Van Oosterhout et al., 2004). Tests of linkage disequilibrium were conducted for each population and each locus in GENEPOP v.4.1 with 20 batches and 5000 iterations per batch (Rousset, 2008), and the significance levels were adjusted with multiple comparisons following false discovery rate (FDR)

correction (Storey, 2002). The number of alleles observed ( $N_A$ ), private alleles ( $N_P$ ) as well as observed and expected heterozygosities ( $H_O$  and  $H_E$ , respectively) were estimated in GENALEX v.6.41 (Peakall and Smouse, 2006). Allelic richness ( $A_R$ ) across all loci was calculated in FSTAT 2.9.3.2 (Goudet, 1995) based on the minimal sample size with the rarefaction method.

### **2.3 Population structure analysis**

To investigate the population genetic structure and admixture among these three taxa, we conducted the Bayesian analysis in STRUCTURE 2.3.1 with the admixture model and correlated allele frequencies (Pritchard et al., 2000). To determine the optimal number of clusters ( $K$ ), ten independent analyses were performed for  $K = 1-10$  based on 1000 000 Markov chain Monte Carlo iterations following a burn-in period of 100 000 steps. The best  $K$  value was determined by the delta  $K$  ( $\Delta K$ ) method (Evanno et al., 2005) in STRUCTURE HARVESTER (Earl and Vonholdt, 2012). In addition, we calculated the average result over 10 runs to obtain the final admixture results, which were summarized in CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) and displayed with DISTRUCT 1.1 (Rosenberg, 2004).

Population genetic differentiation was conducted with ARLEQUIN v.3.5 based on mitochondrial *COI* and microsatellite data (Excoffier and Lischer, 2010). The significance of the values was tested by 10 000 permutations for each pairwise comparison.

The phylogenetic tree was constructed based on Nei's standardized genetic distance  $D_a$  with microsatellites. An un-weighted pair-group method with an arithmetic average (UPGMA) algorithm was applied with 1 000 bootstrap replicates in POPTREE (Takezaki et al., 2010), and the tree topologies were visualized and adjusted in FIGTREE 1.4.2 (available at <http://tree.bio.ed.ac.uk/software/figtree/>).

### **2.4 Gene flow analysis**

We estimated contemporary migration rates (over the last several generations) between these populations and species using five independent runs of 3 000 000 iterations with 1 000 000 burn-in iterations (sampling every 2000) in BAYESASS v3.0.3 (Wilson and Rannala, 2003). The mixing parameters were adjusted to 0.30, 0.30 and

0.30 for migration rate, allele frequency and inbreeding coefficients, respectively.

## 2.5 Ecological niche modeling

Predictive distribution maps for *S. angustata* under past, present-day and future environmental conditions were constructed with correlative ENMs employing the maximum entropy algorithm in MAXENT v.3.3.3e (Phillips et al., 2017; Phillips and Dudik, 2004). Niche modeling was based on 24 occurrence records of *S. angustata* obtained from field observations, previous studies and online databases (Fig. S2, Table S4). Environmental conditions in the distributional range of this kelp were captured by 1 000 randomly chosen background points (also referred to as pseudo-absence locations) within 34° to 54° latitude and 127° to 161° longitude using the R package “raster” (Hijmans, 2015) (Fig. S3). Current and future environmental variables were downloaded from the Bio-ORACLE database (<http://www.oracle.urgent.be/index.html>, real values)(Assis et al., 2018b; Tyberghein et al., 2012) (Table S5).

To avoid overfitting the models to the occurrence records, we reduced the full set of 61 environmental variables. Long-term (yearly) mean and maximum Bio-ORACLE variables were excluded before variable selection given that no corresponding values were available for past or future conditions. Using the R package “MaxentVariableSelection” (Jueterbock et al., 2016), we selected a set of uncorrelated variables (Pearson’s product moment correlation < 0.9) with relative contribution scores > 5% and a betamultiplier (between 0.5 and 10) that resulted in a model of lowest AICc (sample-size-adjusted Akaike information criterion (Akaike, 1974)). In comparison with models selected by maximum AUC.Test (area under the receiver operating characteristic estimated from test data (Fielding and Bell, 1997)), models selected by minimum AICc better predict a species’ fundamental niche and better allow to transfer habitat suitability to novel environmental conditions (Jimenez-Valverde, 2012; Warren and Seifert, 2011; Warren et al., 2014). Model-overfitting was estimated by the difference between AUC values from test and training data (Warren and Seifert, 2011).

We projected habitat suitability for *S. angustata* with the settings and variables that characterized the model of highest performance (lowest AICc, Table S5). Projections

into the past and future differed from the present-day projection in the monthly mean ice thickness, monthly range of salinity, and the monthly minimum sea surface temperature (SST). Projections of the salinity and SST into the past, for 21kya (last glacial maximum, LGM) and 6kya (mid holocene, MH), were obtained from the PaleoMARSPEC data layers (Sbrocco, 2014). Ice thickness projections for the LGM and MH were developed following the methods of Assis et al. (2018b) using three distinct atmospheric-ocean general circulation models (CCSM4, IPSL-CM5A-LR, MIROC-ESM). Salinity, SST, and ice thickness projections into years 2050 and 2100 under representative concentration pathway (RCP) scenarios (Collins et al., 2013). RCP2.6 (low emissions peaking in 2010-2020), RCP6.0 (medium emissions peaking in 2080), and RCP8.5 (high emissions continuing to rise throughout the 21st century) were obtained from the Bio-ORACLE database (Assis et al., 2018b). All projections were based on complementary log-log (cloglog) output grids (Fithian et al., 2015) averaged over 10 replicated MAXENT runs.

Ecological niche modeling projections of *S. japonica* (including four varieties) have been predicted in our previous research (Zhang et al., 2019). Furthermore, we display the geographic overlap between the suitable range of *S. japonica* and *S. angustata*.

### 3. Results

#### 3.1 Genetic characteristic of three *Saccharina* species

For microsatellites, the MICROCHECKER analysis did not identify genotyping errors that could have been due to stuttering and large allele dropout. Linkage disequilibrium (LD) tests indicated that no pair of loci was significance after false discovery rate (FDR) correction. Among the *S. angustata* populations, the mean number of alleles across loci ( $N_A$ ) varied from  $4.85 \pm 0.71$  for Sa4 (one population from Kushiro) to  $8.08 \pm 1.21$  for Sa3 (one population from Hidaka) (Table 1). The number of private alleles ( $N_P$ ) was highest ( $1.69 \pm 1.21$ ) in the Sa1 population and lowest ( $0.23 \pm 0.23$ ) in the Sa4 population. All diversity estimates were higher for *S. angustata* populations Sa1, Sa2 and Sa3 ( $H_O$ : 0.38-0.52;  $H_E$ : 0.57-0.66;  $A_R$ : 4.42-5.00) as compared with population Sa4 ( $H_O$ : 0.37;  $H_E$ : 0.47;  $A_R$ : 3.31; Table 1). Out of the three studied species, Sa (*S. angustata*) has highest estimation in  $N_A$  (12.85),  $N_P$  (8.31) and  $A_R$  (10.48). Only heterozygosity was



higher in Sj (*S. japonica* var. *japonica*) than in Sa ( $H_O$ : 0.44;  $H_E$ : 0.64)(Table 1). Genetic diversity indices were lowest in Sd (*S. japonica* var. *diabolica*) ( $H_O$ : 0.20-0.22;  $H_E$ : 0.20-0.22;  $A_R$ : 1.87-2.02).

Estimates of genetic diversities based on the mitochondrial marker *COI* are list in Table 1. Nucleotide diversity ( $\pi$ ) in the four Sa populations ranged from 0.00014 to 0.00055 and haplotype diversity ( $H_d$ ) ranged from 0.20 to 0.54. Population Sa4 showed lower genetic diversity than other three populations. Sj had higher diversity estimates ( $H_d$ : 0.73;  $\pi$ : 0.00135) than Sa ( $H_d$ : 0.38;  $\pi$ : 0.00036) and Sd ( $H_d$ : 0.04;  $\pi$ : 0.00030).

### 3.2 Haplotype network

There are 14 haplotypes (H13-H26; GenBank accession number: MT757623-MT757636) in *S. angustata* populations and 12 haplotypes (H1-H12; GenBank accession number: KT963115-KT963117; KT963119-KT963120; MT757637-MT757642; MK227355) in *S. japonica* populations (Table S6). In total, 4 haplotypes (H1, H8, H10, H13) were shared among at least two populations (Fig. S4). However, *S. angustata* and *S. japonica* did not share any haplotype. The most common haplotype, H1, was shared by 92 individuals and accounted for 62.58% of all *S. japonica* samples (Fig. S4), but was not found with any of the *S. angustata* samples. H13 was the most frequent haplotype (78.64%) in all *S. angustata* populations but was absent from the *S. japonica* populations. H8 was shared by Sj1 and Sj3, and H10 was shared by Sj1 and Sj2. The median-joining network separated the 26 haplotypes into two major haplogroups, which were corresponding to these two species. Haplotypes H1 and H13 were separated by 54 mutation steps (Fig. S4).

### 3.3 Genetic structure

The UPGMA tree indicates that the *S. angustata* populations were clearly distinct from the seven populations of *S. japonica*, and that the two *S. japonica* var. *diabolica* populations had diverged from other five *S. japonica* var. *japonica* populations (Fig. 1b). The metric delta K peaked at K=2, indicating that it was the highest hierarchical degree (Fig. S5; delta K=3321.93). In agreement with the UPGMA tree results, Bayesian clustering of the eleven populations also indicated two distinct genetic groups: one composed of four *S. angustata* populations (Sa1, Sa2, Sa3 and Sa4), the other of the

seven *S. japonica* populations. These two genetic clusters were consistent with prior morphological assignment. A second hierarchical level of genetic division was detected with  $K=3$  (Fig. S5;  $\Delta K=399.66$ ), indicating that *S. japonica* var. *diabolica* and *S. japonica* var. *japonica* separated into two distinct subgroups, thus supporting the three taxa (Sa, Sj and Sd). Admixture among the different taxa was very limited (Fig. 1b). Each population showed high probabilities (admixture coefficient,  $Q > 0.94$ ) to belong to each of the three corresponding clusters identified in the admixture analysis. Sj1 ( $Q: 0.94$ ) and Sj2 ( $Q: 0.96$ ) showed little admixture with *S. japonica* var. *diabolica*. Similarly, Sa2 ( $Q: 0.96$ ) and Sa3 ( $Q: 0.96$ ) showed little admixture with *S. japonica* var. *japonica*. Also the remaining populations showed high admixture coefficient ( $Q > 0.99$ ), which suggests absence of introgression among the taxa.

#### **3.4 Genetic differentiation and gene flow**

Based on the mitochondrial marker *COI*, the pairwise  $F_{ST}$  values between the four *S. angustata* populations and the seven *S. japonica* populations exceeded 0.97 ( $P < 0.01$ ). The  $F_{ST}$  values between populations of the same species was lower ( $F_{ST} < 0.8$ ) (Fig. S6a). Similarly, the microsatellite-based  $F_{ST}$  values between two species populations ( $F_{ST} > 0.25$ ,  $P < 0.05$ ) was higher than the intraspecific  $F_{ST}$  values (Fig. S6b). Both markers showed significant genetic differentiation between *S. japonica* and *S. angustata*.

Group  $F_{ST}$  values (where all populations of a single taxon were grouped) showed significant genetic differentiation between group Sa and Sd based on mitochondrial *COI* ( $F_{ST}: 0.993$ ) (Fig. S6c) and microsatellite data ( $F_{ST}: 0.467$ ) (Fig. S6d), suggesting a deep phylogenetic divergence between the *S. japonica* var. *diabolica* and *S. angustata*. Group Sj and Sd showed less genetic divergence at the *COI* marker ( $F_{ST}: 0.074$ ) (Fig. S6c), but high genetic divergence at the microsatellite markers ( $F_{ST}: 0.299$ ) (Fig. S6d).

The Bayesian inference of contemporary migration rates ( $m$ ) was lower than 0.05 among the three taxa (Sa, Sj, Sd) (Fig. 1c), suggesting that there was restricted gene flow among species. Similarly, recent gene flow among all populations was generally lower than 0.1 (Fig. 2; Table S7). Nevertheless, gene flow were detected in the two pairs of conspecific and geographically proximate populations. The estimated migration rate from Sj2 to Sj1 were 0.1644, and the reciprocal migration rates between

Sa2 and Sa3 were also exceeded 0.1 (Fig. 2; Table S7).

### **3.5 Ecological niche modeling of *S. angustata***

The model with the lowest AIC<sub>c</sub> (566) was constructed with a beta-multiplier of 2.5 (Fig. S7) and five uncorrelated environmental variables (Table S5). Salinity (Salinity.Range) was the most important variable (30.75% model contribution, Table S5) in discriminating suitable from non-suitable habitats. Sea surface temperature (Temperature.Min), ice thickness (Ice.thickness.Mean) and nitrate had contributions of 23.60%, 23.65% and 15.03%, respectively. Cloud cover (Cloud.cover.Max) had the lowest contribution of 6.96% (Table S5). Kelp habitat suitability was negatively correlated with increasing values of all five environmental variables (Fig. S8).

An Average AUC.Test value of 0.92 (Table S5) suggests that the present-day model could discriminate well between presence and absence sites. The low AUC.Diff value of 0.03 (Table S5) suggests that the model was not overfit to the occurrence locations and, thus, suitable to be transferred to past and future climate conditions (Warren and Seifert 2011).

The habitat suitability projections for present-day conditions (Fig.S9) matched well the set of occurrence records (Fig. S2). The ENM predictions to the LGM show that the kelp populations may have only been present on the west coast of the Japan Sea and the Pacific side of northern Japan (Fig.S9). In the mid-Holocene (6 Kya), the melting of ice and opening of the Japan Sea allowed the kelp population to expand and colonize the north coast of the Japan Sea and the north coast of Yellow-Bohai Sea (Fig.S9). The future projections show that this kelp will find new suitable habitat along the coast of the Okhotsk Sea, and that most existing populations will not be impacted under continued global warming (Fig.S9). However, the northern coast of Honshu in Japan is projected to become less suitable, particularly under the scenario of highest emissions (RCP8.5) in year 2100 (Fig. S9).

### **3.6 Geographic overlap of suitable ranges**

Suitable versus non-suitable habitat conditions are based on threshold values that best reflected the species' contemporary distribution limits (*S. japonica*: 0.6 and *S. angustata*: 0.6). During the LGM, range overlap of both kelps was in the west coast of

Japan Sea and the Pacific coast of northern Honshu and southern Hokkaido (Fig.3). Both kelp populations expanded far beyond the species' current limit in the mid-Holocene (6 Kya), so their overlapping range is predicted to increase significantly (Fig.3). Range overlap increase along the coast of Hokkaido and decrease in the northern coast of Honshu in all future climate scenarios (Fig.3). Both kelp populations are predicted to shift northward and colonize the new habitat in Okhotsk Sea coast in future, and therefore both species obtain the newly overlapping range in Okhotsk Sea coast in Kamchatka Peninsula.

## 4. Discussion

### 4.1 Genetic diversity

Previous studies indicated that genetic diversity center of wild *S. japonica* may be on the coast of Oshima peninsula in Hokkaido (Zhang et al., 2015; 2019). *S. japonica* var. *japonica* mainly distributed along the coast of Oshima peninsula (Yotsukura, et al., 2010a), and our study suggested that all the *S. japonica* var. *japonica* populations presented the highest genetic diversity. *S. japonica* is an important economic seaweed, and widely cultivated in Japan, Korea and China, and its wet weight production was 11 448 250 t in 2018 (<http://www.fao.org/fishery/statistics/global-aquaculture-production>). Therefore, the wild *S. japonica* var. *japonica* resources might make important contributions to improve the cultivated *S. japonica*.

*S. japonica* var. *diabolica* inhabits only along eastern coast of Hokkaido (Nemuro and Kushiro) (Kawashima, 2012) and far away from *S. japonica* var. *japonica* (Fig. S1). *S. japonica* var. *diabolica* have poor connectivity with other the *S. japonica* var. *japonica* populations possibly due to the habitat discontinuity (Table S7). Therefore, *S. japonica* var. *diabolica* in Nemuro and Kushiro should be regarded as separate management and conservation units.

However, unlike *S. japonica*, which is widely cultivated in Japan, Korea and China, *S. angustata* is harvested from wild stocks (Iida 1996; Borlongan et al., 2019). Our study firstly assessed the genetic diversity level of *S. angustata* populations and revealed that Sa2 and Sa3 had high genetic diversity (e.g.  $N_p$ ,  $A_R$ ) (Table 1). We inferred that the diversity center of *S. angustata* might be from Muroran to Shiranuka (eastern part of

the Pacific coast in Hokkaido). Under the most extreme emission scenario RCP8.5 in 2100, suitable habitat from Muroran to Shiranuka might disappear (Fig.S9). Preserving genetic diversity of *S. angustata* from Muroran to Shiranuka should be a priority for the management of natural stocks.

#### **4.2 Limited interspecific gene flow**

Gene flow between these two closely related species is restricted, as was observed also in other brown seaweeds (Ardehed et al., 2016; Bergstrom et al., 2005; Pereyra et al., 2013). Several investigators reported that gene flow was influenced by several factors, including reproductive strategy (e.g. seaweed, Engel et al., 2005), habitat discontinuity (e.g. mangrove, Binks et al., 2019), ocean current (e.g. seaweed, Li et al., 2017; Hu et al., 2012) and historical factors (e.g. seagrass, Alberto et al., 2008). In our study, the poor connectivity among *S. angustata* and *S. japonica* populations may be explained by two factors. Firstly, the swimming duration of both kelps' zoospores is less than 24 hours, and its gamete disperse less than 3.5 meters (Balakirev et al., 2012; Hasegawa, 1962), thus limiting the possibility of hybridization. Secondly, *S. japonica* and its varieties inhabits sublittoral zone (5-11 m depth), and *S. angustata* inhabits the upper sublittoral zone (2-4 m depth) (Balakirev et al., 2012; Kawashima, 2012). Therefore, differences in the spatial arrangement of their populations might result from the poor connectivity, as has been shown for two cryptic species of *Lessonia nigrescens* (Gonzalez et al., 2012). In addition, the spatial proximity and habitat discontinuity of two species on local scales may limit contact and further natural hybridization (Tellier et al., 2011; Binks et al., 2019).

#### **4.3 Climate-induced range overlap in Kamchatka Peninsula**

Suitable range of temperate seaweeds are expected to poleward shift under ongoing climate change (e.g., Jueterbock et al., 2013; Müller et al., 2009; Neiva et al., 2014). Similarly, our future projections indicate that both *S. japonica* and *S. angustata* will expand northward, and the coast of Okhotsk Sea in Kamchatka Peninsula will be the future range overlap for these two closely related species (Fig.3). In the present, even these two species overlaps in pacific coast of Hokkaido and northern Honshu, we find that there was poor genetic connectivity and limited interspecific hybridization

between two species due to the limited dispersal ability of zoospores. Therefore, we presumed that there will be limited interspecific gene flow between these two species in the Kamchatka Peninsula.

As previous study reported, Okhotsk Sea is a major diversity hot-spot of Laminariales (Bolton, 2010), but only a few systematic and phylogenetic studies were reported along the Okhotsk Sea coast (Selivanova et al., 2007). Sudo et al (2020) did not project the future range shifts and abundance of seaweeds along colder climate regions, such as the eastern coast of the Kamchatka Peninsula, but they thought this area might be a potential refuge for these studied seaweeds. In our projections, the coast of Kamchatka Peninsula was predicted to provide suitable habitat conditions for *S. japonica* and *S. angustata* (Fig.3), and we inferred the coast of Okhotsk Sea might be a new climate refugia for warm-temperature seaweeds, especially some seaweeds in northern Japan.

#### **4.4 Loss of suitable habitats of *S. angustata***

The southwest of the Japan Sea and the Pacific coastlines of Hokkaido and Honshu were two glacial refugia for *S. angustata* during the LGM (Fig.S9). Compared to distribution projections into the past (LGM and MH), habitats that were suitable along the southwest of the Japan Sea (near the Vostok Bay in Russia) have already become unsuitable (Fig.S9). *S. angustata* was dominant species along the Vostok Bay in 1990s, but has become rare in the recent 2000s under the influence of global climate change and other local anthropogenic factors (Kozhenkova, 2009).

The coast of Hokkaido and the northern coast of Honshu facing the Pacific Ocean represent the southern distribution limits of *S. angustata* (Kawashima, 2012). Our Niche Models predict that ongoing climate change has slight impact on the southern distribution limits of this kelp. However, under the most extreme emission scenario RCP8.5, the coast of northern Honshu is predicted to be no longer suitable in 2100 (Fig.S9). Hence, northern Honshu should become an important kelp protection area.

#### **5 Conclusion**

*S. angustata* and *S. japonica* can hybridize in the crossing tests under laboratory conditions, but we did not detect natural hybridization among them. Our future

projections indicate that both species will expand northward, and obtain newly overlapping along the coast of Okhotsk Sea in Kamchatka Peninsula. Due to limited dispersal ability of zoospores, both species might have no propensity to hybridize in future niche overlap. Our study firstly reveal the genetic diversity levels of *S. angustata* populations and infer that its diversity center might be from Muroran to Shiranuka. The interspecific hybridization and intraspecific genetic diversity are noteworthy in view of future kelp farming practices and it should also be taken into account in future conservation and management strategies of wild stocks.

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#### Figures:

**Figure 1** Collecting sites and morphology of three different taxa (a), genetic structure based on STRUCTURE analysis and UPGMA tree (b) and estimation of contemporary gene flow between three taxa (c). Numbers above/below arrows represent migration rates and confidence interval (in Brackets).

**Figure 2** Contemporary gene flow between 13 geographic proximate populations and contemporary gene flow between pair populations above 0.04 are shown. Population locations are shown in the map. The thickness of arrow is scaled according to the values.

**Figure 3** Projected range overlap for *Saccharina angustata* and *Saccharina japonica* for the Last Glacial Maximum (LGM; 21kya), Mid-Holocene (MH; 6 kya), present time and future (2050s and 2100s) with different climate scenarios (RCP26, RCP60 and RCP85).