


Characterization of 12 polymorphic microsatellite markers in the sugar kelp *Saccharina latissima*

Cristina Paulino¹ · João Neiva¹  · Nelson C. Coelho¹ · Tânia Aires¹ · Núria Marbà² · Dorte Krause-Jensen^{3,4} · Ester A. Serrão¹

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Abstract *Saccharina latissima* is an ecologically and economically important kelp species native to the coastal regions of the Northern Hemisphere. This species has considerable phylogeographic structure and morphological plasticity, but lack of resolution of available genetic markers prevents a finer characterization of its genetic diversity and structure. Here, we describe 12 microsatellite loci identified in silico in a genomic library, and assess their polymorphism in three distant populations. Allelic richness at the species level was relatively high (5–23 alleles per locus), as was gene diversity within populations ($0.42 < H_E < 0.62$). In addition, individuals readily form distinct genotypic clusters matching their populations of origin. The variation detected confirms the great potential of these markers to investigate the biogeography and population dynamics of *S. latissima*, and to better characterize its genetic resources for the establishing farming industry.

Keywords Gene diversity · Genetic differentiation · Kelp · Microsatellite markers · *Saccharina latissima* · Phaeophyceae

Introduction

The sugar kelp *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl and G.W. Saunders (= *Laminaria saccharina*) is a cryptic perennial, canopy-forming, edible brown seaweed (Laminariales, Heterokontophyta) distributed throughout much of the Northern Hemisphere. Its vast range extends from the high Arctic to cold-temperate Iberia (NE) and New Jersey (NW) in the Atlantic, and to Central California (NE) and Korea (NW) in the Pacific (Müller et al. 2009). In addition to its ecological importance as a foundational species in lower intertidal and shallow subtidal environments (Steneck et al. 2002, Gutiérrez et al. 2011), *S. latissima* is cultivated/harvested for human consumption (Smale et al. 2013). It is prized for its sweet flavor, particularly in Asian markets (where it is sold with other species as “kombu”), but also in Europe and America, where sea farming is currently a growing enterprise (Peteiro et al. 2014).

The species *S. latissima* is morphologically and genetically polymorphic throughout its range, comprising regional ecotypes (e.g., now synonymized *Saccharina longicurvis* from Canada) and at least three distinct mtDNA phylogeographic lineages (McDevit and Saunders 2010). Where these overlap, such as along the Canadian Arctic and western Greenland, nuclear internal transcribed spacer (ITS) sequences are consistent with ongoing admixture, suggesting that *S. latissima* may correspond to an incipient species complex resulting from historical cycles of glacial isolation and interglacial admixture. The genetic markers available so far are, however, not suited to adequately assess gene flow across secondary contact zones, or intra-oceanic structure, highlighting the need for independent, more polymorphic, co-dominant markers such as microsatellites. Other applications for such markers include identifying regional hotspots of genetic diversity and endemism (i.e., refugia) to better forecast the consequences of

✉ João Neiva
jmneiva@ualg.pt

¹ Centro de Ciências do Mar, Universidade do Algarve, Gambelas, 8005-139 Faro, Portugal

² Department of Global Change Research, IMEDEA (CSIC-UIB), Esporles, Spain

³ Department of Bioscience, Aarhus University, Silkeborg, Denmark

⁴ Arctic Research Centre, Aarhus University, Aarhus, Denmark

ongoing climatic and environmental change (Müller et al. 2009; Moy and Christie 2012; Martínez et al. 2015), and characterizing available genetic resources, population dynamics, and relevant life-history traits of *S. latissima* for the establishing farming industry.

Given the above, we describe 12 microsatellite markers for *S. latissima* identified in silico following 454 pyrosequencing, and characterize their polymorphism and differentiation in three selected populations.

Material and methods

Four hundred fifty-four DNA shotgun pyrosequencing of *Saccharina latissima* was performed at Biocant (Cantanhede, Portugal). DNA was extracted from 100 mg of dried tissue from four individuals collected at Uummannaq (western Greenland) using a modified protocol of the commercial DNeasy Plant Mini (Qiagen, Hilden) DNA extraction kit (Snirc et al. 2010). Simple di- to hexanucleotide microsatellite loci were searched for, and primers designed, using WebSat (Martins et al. 2009). A subset of 22 promising loci (possessing high number of repeats, adequate anchoring sites for primers and close melting temperatures of primers) was amplified with labeled primers and evaluated for amplification

success, ease of scoring, and polymorphism, and 12 were eventually selected (Table 1) to screen three distant populations ($n=20$) from the NE Atlantic/Arctic distribution: Uummannaq (70.7 °N), Bergen (southwestern Norway, 60.4 °N), and Roscoff (northwest France, 48.7 °N).

PCR amplifications were performed in 15 µL reaction mixtures containing ±10 ng of template DNA, 1× buffer, 1.5–2.5 mM of MgCl₂ (Table 1), 0.28 mM of dNTPs (Bioline), 0.1–0.3 µM of each primer pair with 5' end of forward primer fluorescently labeled, and 1 U of GoTaq G2 Flexi DNA Polymerase (Promega). PCR reactions were performed in a ABI 2720 Thermal Cycler (Applied Biosystems) and included an initial denaturation step (95 °C, 5 min), 35 cycles of 95 °C for 30 s, T_a °C for 30 s (Table 1) and 72 °C for 40 s, and a final extension step (72 °C, 20 min; 5 min for SLN314). Amplified fragments were run in an ABI PRISM 3130xl automated DNA sequencer (Applied Biosystems) at CCMAR, Portugal. Alleles were manually scored in STRand (Toonen and Hughes, 2001) using the GeneScan 500 LIZ size standard (Applied Biosystems), and binned using MsatAllele (Alberto 2009).

GENETIX v4 (Belkhir et al. 1996) was used to test for potential linkage disequilibrium (LD) of alleles and to estimate the total number of alleles or allelic richness

Table 1 Characterization of 12 microsatellite loci in *Saccharina latissima*, including locus name, GenBank accession number, primer sequences, repeat motif, annealing temperature (T_a) and magnesium concentration (MgCl₂) used in PCR, and size range of the alleles

Locus name	Genbank accession	Primer sequences (5'-3')	Repeat motif	T_a (°C)	MgCl ₂ (mM)	Size range (bp)
SLN314	KT723013	F: CTGTGTGTGTTGTCGTACATCG R: GGATTCTTATTTGAGGGAGGG	(TAC) ₁₁	58	2.0	235–302
SLN319	KT723014	F: CGAAGGAAGTGAATGACAACAA R: GGTAGTTACGGATTGCGACAAG	(ACA) ₁₀	56	2.0	378–433
SLN32	KT723015	F: GAGAAAACATGCCAGGTCTA R: GTATCGCTGTACCCTCCTCCT	(CAG) ₁₁	57	2.0	222–280
SLN320	KT723016	F: TACGATGGTTTATGGGTTAGGG R: AGCGAACAACGAAGCAACTAAT	(TGT) ₁₃	56	2.0	210–241
SLN34	KT723017	F: ACGAAGTGCTAATAATGTGCCG R: GAGATAGCCCGACCACTGC	(AGC) ₁₀	56	2.0	183–319
SLN35	KT723018	F: GCGTATGAACAAATGACCGTA R: TGTGAGTTCCTTTCTGTGAGC	(CTG) ₁₁	56	2.0	343–372
SLN36	KT723019	F: CGAGACTTTTGGGTAGATTTCG R: CGCCTGCCTCTTGTCTAAGTA	(AGT) ₁₉	57	2.0	264–315
SLN510	KT723020	F: CCGTCTATGGCGAGAAAGAGAT R: ATCTTACCTGGGCACTTGCTTT	(ACACA) ₁₃	58	2.0	242–339
SLN511	KT723021	F: ATGTCCTGACCTGACCTACAGC R: AATTCTGTGAACATTCGGGAGT	(ACCTT) ₁₉	54	2.5	366–400
SLN54	KT723022	F: GTGGTTGCTGTTGTTGCTGT R: CGAATAAAGACAAATCGGCTG	(ATATC) ₁₁	54.5	1.5	298–337
SLN58	KT723023	F: GCGAAGAAACGAGGTTACAT R: CTGGGTTTGTGCGAGTGTGAT	(GCAAG) ₈	55	2.0	153–173
SLN62	KT723024	F: ACAAAGCGTTCTCAACCGAT R: CGACACCTACACAATACGAAA	(TATACA) ₆	55	2.0	164–281

(A), expected (H_E) and observed (H_O) heterozygosities and inbreeding (F_{IS}) in each population. Polymorphic information content (PIC) was calculated using Cervus v3 (<http://www.fieldgenetics.com/pages/home.jsp>). Population differentiation was inspected using a factorial correspondence analysis (FCA) based on individual multilocus genotypes.

Results and discussion

Four hundred fifty-four pyrosequencing produced a total of 214,127 quality reads between 40 and 640 bp, of which 22,454 (10.5 %) contained at least one simple di- to hexanucleotide microsatellite with at least 4 repeats. All 12 microsatellite loci selected successfully amplified in the three surveyed populations. Consistent LD across populations was not detected in any loci. Globally, the number of alleles per locus (A) ranged from 5 to 23 (averaging 12.42), and within populations, it ranged from 1 to 13. Polymorphic information content (PIC) was rather high for all loci, ranging from 0.452 to 0.939 in the pooled dataset. Polymorphism within populations was also appreciable, with mean expected heterozygosity (H_E) ranging between 0.48 and 0.62 and mean A ranging between 4 and 6.25 alleles per locus. Significant homozygote excess was observed in all populations in at least some loci (Table 2). These disequilibria were locus- and population-specific, however, and thus were more likely to reflect sampling artifacts or null alleles rather than local differences in mating

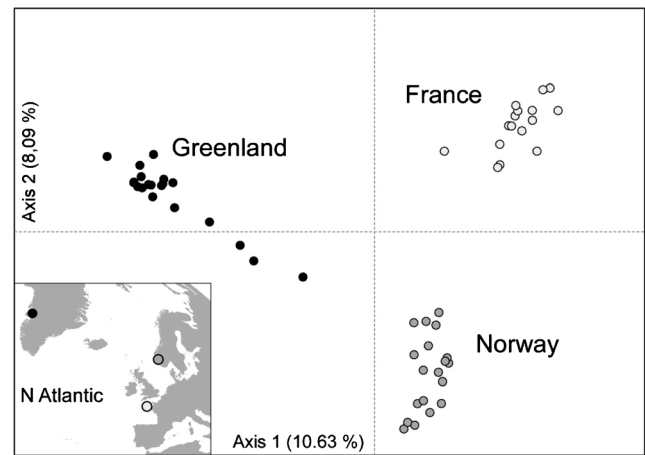


Fig. 1 FCA plot of *Saccharina latissima* based on individual multilocus genotypes

systems or population dynamics. Indeed, MICRO-CHECKER (Van Oosterhout et al. 2004) identified null alleles at loci SLN34 (Uummanaq, Bergen) and SLN35 (Uummanaq). Allelic compositions across populations were sufficiently different so that individual multilocus genotypes formed clearly discontinuous and coherent clusters according to geography (Fig. 1).

The levels of intra- and interpopulation polymorphism in this preliminary survey confirm the usefulness of these markers to characterize population diversity and structure of *S. latissima* at multiple spatial scales, and their potential use to identify and manage genetic resources (e.g., selection of germplasm banks) for cultivation purposes.

Table 2 Polymorphism of microsatellites, including number of alleles (A), expected (H_E) and observed (H_O) heterozygosities, polymorphic information content (PIC), and inbreeding coefficients (F_{IS}) for each locus and population ($n = 20$) of *Saccharina latissima*

Locus	Global ($n = 60$)			Uummanaq				Bergen				Roscoff			
	A	H_E	PIC	A	H_E	H_O	F_{IS}	A	H_E	H_O	F_{IS}	A	H_E	H_O	F_{IS}
SLN314	19	0.885	0.875	5	0.569	0.450	0.214	10	0.819	0.650	0.211*	8	0.782	0.650	0.173
SLN319	7	0.531	0.499	4	0.572	0.550	0.039	5	0.665	0.600	0.101	1	–	–	–
SLN32	17	0.887	0.876	5	0.591	0.550	0.071	10	0.753	0.750	0.004	9	0.864	0.700	0.194*
SLN320	5	0.630	0.591	4	0.645	0.700	–0.088	2	0.450	0.550	–0.229	1	–	–	–
SLN34	14	0.810	0.795	2	0.328	0.100	0.701*	13	0.925	0.368	0.608*	6	0.812	0.750	0.078
SLN35	10	0.737	0.701	3	0.381	0.111	0.714*	6	0.637	0.650	–0.021	4	0.460	0.350	0.244
SLN36	23	0.942	0.939	9	0.849	0.750	0.119	8	0.845	0.800	0.054	11	0.880	1.000	–0.141
SLN510	15	0.758	0.733	3	0.344	0.200	0.424*	5	0.605	0.650	–0.076	13	0.917	1.000	–0.094
SLN511	8	0.703	0.656	3	0.179	0.063	0.659*	3	0.540	0.550	–0.020	3	0.304	0.350	–0.157
SLN54	10	0.828	0.807	5	0.673	0.700	–0.041	4	0.610	0.550	0.101	4	0.594	0.600	–0.011
SLN58	6	0.514	0.452	3	0.337	0.200	0.413	3	0.347	0.400	–0.156	3	0.099	0.100	–0.013
SLN62	15	0.573	0.560	2	0.262	0.200	0.240	3	0.189	0.200	–0.063	12	0.882	1.000	–0.138
All loci	12.42	0.739	0.707	4.00	0.478		0.206*	6.00	0.615		0.093*	6.25	0.549		0.014

*Significant departures (permutation test, $n = 1000$)

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