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Isolation and characterization of nine microsatellite markers for the red alga *Corallina officinalis*

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Abstract

We report the development of nine polymorphic microsatellite markers for *Corallina officinalis* (Linnaeus, 1758), a calcifying intertidal red alga and important ecosystem engineer spread along the North East Atlantic. Characterization and analysis of loci were made using 15 individuals of *C. officinalis* from populations in Iceland and the UK. The average number of alleles per locus was 3.78 (range 2–6) and mean of gene diversity was 0.58 (range 0.38–0.77). The set of microsatellites developed here will provide a useful molecular tool for population genetic and conservation studies.

Keywords *Corallina officinalis* · Genetic diversity · Microsatellites · Red algae · Marginal population

Introduction

Within the red algae, Corallinales (Rhodophyta) is one of the most species rich orders [1, 2]. Given their role as bioengineers and of their sensitivity to climate change and ocean acidification, species in the Corallinales are of great ecological relevance in various marine, wave-exposed littoral and sublittoral habitats [3, 4]. They are also abundant components in fossil and sub-fossil records, thus representing valuable indicators of paleoenvironmental conditions and providing important information to contextualize ongoing climatic changes [5–8].

Corallina officinalis Linnaeus, 1758 (Corallinales, Rhodophyta) is an abundant and widespread calcified geniculate coralline alga on rocky shores in the North Atlantic [9–11]. The distribution of *C. officinalis* along NE Atlantic shores extends from northern Spain and the Azores, in the south, to northern Norway, Iceland and southern Greenland, in the north [10, 12]. The dense turfs created by this species

provide shelter in hydrodynamically stressful habitats for many invertebrates [13]. Moreover, *C. officinalis* contributes to carbon dioxide fluxes within the ocean, thus playing a critical role in the carbon cycle of coastal marine ecosystems [14].

Despite its ecological importance, genetic studies for this species are limited [15]. These have used mitochondrial and nuclear sequence data for barcoding, taxonomy, mitogenomics and phylogeny [2, 9, 10, 16–19]. Only very recently have population genetic patterns of *C. officinalis* been investigated [20, 21]. Specifically, novel SNP markers were developed and used to assess population structure based on five locations throughout the NE Atlantic distribution of the species and, at finer spatial scale, along UK shores. Here, with the aim of providing new useful tools using methods previously unutilised for this group, we developed and characterized nine polymorphic microsatellite markers for the red algae *C. officinalis*.

Methods

All samples were hand-collected during low tide from rocky substrate. Tissue was stored in silica drying crystals prior to extraction of DNA. Genomic DNA was extracted from eight individuals, four collected in Iceland (Stafnesviti 63°58'06.5"N 22°45'03.0"W and Stafnes 63°58'28.1"N 22°45'13.8"W) and four in the UK (Margate 51.393200°N

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Table 1 Characterization of nine microsatellite loci in two *Corallina officinalis* populations

Locus name	Primer sequences 5'-3'	Repeat motif	Size range (bp)	Ta (°C)	Iceland			UK				
					A	H _E	H _O	F _{IS}	A	H _E	H _O	F _{IS}
Coroff_1036	F-TCGATACGCACACAAACAGC R-ATCGGGCGCATATATGTTCCAC	(AGC) ₇	235–254	56	2	0.375	0.001	1*	3	0.561	0.143	0.778*
Coroff_1258	F-ATATTTGGGTGCCAGAAGC R-CCGACACCCATAAATCTCCC	(GA) ₁₃	105–121	56	4	0.492	0.375	0.333	2	0.408	0.001	1*
Coroff_1640	F-GCCCGGTATCATAATGGG R-GCTCCAGTATTCGGCTAC	(GA) ₁₅	142–145	56	2	0.375	0.000	1*	3	0.663	0.143	0.812**
Coroff_2347	F-CTCAAGTGAAGGCGGAAG R-GAGTACTTTGGCCACATGCC	(GTGC) ₇	175–646	56	6	0.687	0.5	0.333	5	0.622	0.143	0.8**
Coroff_2797	F-TAAGAAGCGATCCGGTCGAG R-CGCTTACGCTCTCAGTTTG	(CA) ₁₅	194–226	56	4	0.648	0.375	0.475*	4	0.684	0.571	0.238
Coroff_3261	F-CCAGCCTAAAGAAAGTTGCGG R-GACTGATAAAATCGACGGCGG	(CCG) ₈	224–246	56	5	0.734	0.875	-0.126	3	0.561	0.571	0.059
Coroff_7707	F-CCCTGGTCACGAACGAAAAC R-AAGACTTGACCGAGGACAGC	(TGC) ₁₀	77–97	56	4	0.68	0.875	-0.225	3	0.612	0.857	-0.333
Coroff_8333	F-CGTTTCGATATGCGTCCCAC R-TCTCTGTATGACCAACGGC	(AG) ₂₀	169–197	56	5	0.422	0.25	0.461*	5	0.765	0.429	0.5*
Coroff_11062	F-CGGTTAGGTAGCCTTGGGAG R-TTCGCCCTCAGAAAATGCG	(ACAA) ₁₁	211–239	56	4	0.539	0.375	0.364	4	0.541	0.143	0.769**

Locus name, primer sequence, motif repetition, size range, PCR annealing temperature (Ta)

A number of alleles, H_E expected heterozygosity; H_O observed heterozygosity, F_{IS} inbreeding coefficient

*Significant values at p < 0.05 and ** at p < 0.01

01.383820°E) using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) in accordance with the manufacturer's instructions. Extracted DNA was sent to Ecogenics GmbH molecular marker services (Zurich-Schlieren, Switzerland) to isolate and design microsatellite markers. Genomic DNA size-selected fragments were enriched for simple sequence repeat (SSRs) content by using magnetic streptavidin beads and biotin-labeled CT, GT, AGG, GGT GATA and GTAT repeat oligonucleotides. The SSR-enriched library was analyzed on an Illumina MiSeq platform using the Nano 2×250 v2 format. After assembly, 2444 contigs or singlets contained a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least ten repeat units. Out of 1129 microsatellite candidates, 20 were designed and tested. Out of these, nine pairs of primers showed polymorphism. The identified, polymorphic primers were further characterized using the eight and seven *C. officinalis* individuals from Icelandic and UK populations respectively (Table 1). The 10 µL volume PCR mix contained ± 10 ng of DNA, 0.3 µM of each primer labelled with a fluorescent marker, 200 µM dNTPs, 1.0 µl of 10× PCR Buffer with 15 mM MgCl₂ and 0.5 U of HotStar Taq. Cycling conditions consisted of an initial denaturing step of 15 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s at 56 °C annealing temperature, 45 s at 72 °C, and a final elongation step at 72 °C for 30 min. The allele sizes were determined on an ABI3730 (Applied Biosystems), using GeneScan™-500 LIZ Size Standard. Alleles were scored manually using STRand [22]. GENETIX software version 4.05 [23] was used to estimate observed (HO) and expected (HE) heterozygosities. The Hardy–Weinberg expectation (HWE) and linkage disequilibrium were tested using Genepop 4.2 [24] and the resulting p values were Bonferroni-corrected for multiple comparisons ($p < 0.0016$).

Results and discussion

All microsatellites described in this study were polymorphic (Table 1). The number of alleles per locus ranged between 1–6 and 1–5 for the populations from Iceland and the UK, respectively. For the Icelandic populations, expected and observed heterozygosities ranged from 0.37 to 0.69 and from 0.25 to 0.88 respectively (Table 1) while for the UK populations, values ranged from 0.41 to 0.77 and from 0 to 0.86 (Table 1). The highest genetic diversity was found in the UK population, with Coroff_8333 being the most informative locus. The lowest genetic diversity values were given by Coroff_1036 and Coroff_1640 and by Coroff_1258 for Iceland and UK populations respectively. For all loci pairs, we tested for linkage disequilibrium between them, using 1000 replicates and for two pairs (Coroff_2347–Coroff_3261; Coroff_1640–Coroff_7707) there was no significant

deviation after Bonferroni correction. Significant deviation from HWE was found for Coroff_11062 in the UK population.

Relatively high values of observed heterozygosity recently reported for SNP markers [20] suggest polyploidy in *C. officinalis*. It is important to note that potential polyploidy could significantly affect our ability to reliably utilise codominant scoring and thus interpretation of peak patterns [25].

Most SNP markers recently developed for *C. officinalis* show significant departures from HWE [20]; in our study, significant deviation was limited to one locus, highlighting the value of the set of microsatellites developed here to investigate ecological and evolutionary dynamics of this intertidal bioengineer associated with habitat contraction and environmental change.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent For this type of study, informed consent is not required.

Research involving human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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