**Title:** Microsatellite locus development in the seaweed *Plocamium* sp.

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## Supplemental Material

## Methods

*Sample collection*

We collected *Plocamium* sp. thalli along transects perpendicular to the shore at 3m depth intervals using SCUBA in April 2017 at "East Litchfield" Island, the unofficial name of a small islet off the northeast corner of Litchfield Island, and in May to June 2018 at Laggard Island, both near Palmer Station on Anvers Island (64° 46' S, 64° 03' W; see supplement to Shilling *et al.* 2021 for map). Upon return to station, thalli were inspected for reproductive structures (Figure S1). Tetrasporangial sori and carposporophytes were easily identified, but male gametophytes were not observed. All thalli were photographed, and a small piece was preserved in silica. We removed carposporophytes from the female gametophytes before preservation in silica as the carposporophytes contain the diploid carpospores.

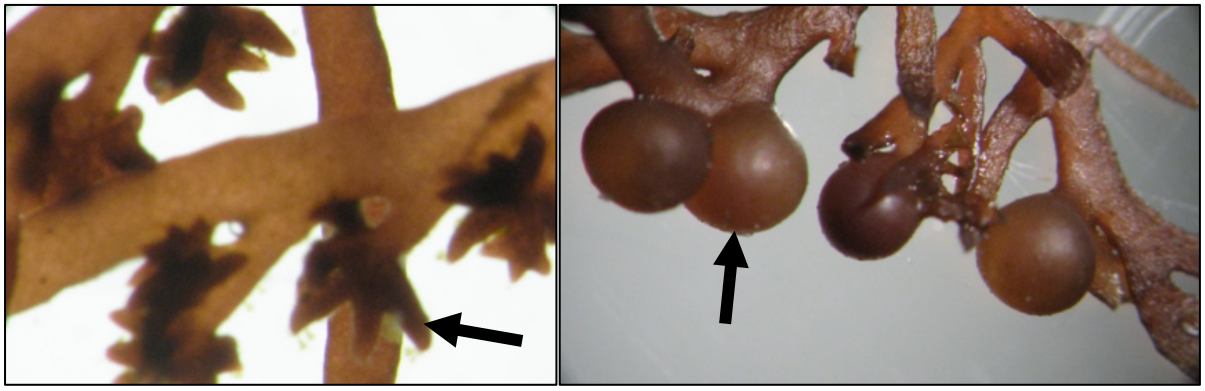

**Figure S1**. Reproductive structures on Antarctic *Plocamium* sp. Left: Arrow points at tetrasporangial sorus found on a tetrasporophyte. Right: Arrow points at a cystocarp which consists of haploid female tissue covering the diploid carposporophyte generation and is found on female gametophytes.

*Microsatellite library enrichment and identification of putative loci*

Four samples collected between February and April 2016 from different sites within 3.5km of Palmer Station were used to develop microsatellite loci commercially at Ecogenics GmbH (Balgach, Switzerland). We identified putative loci from the SSR-enriched library and followed Schoebel *et al*. (2013). We used MSATCOMMANDER 1.0.8-beta (Faircloth 2008) to design primers for dinucleotide and trinucleotide repeat motifs separately. A minimum of eight repeats was selected and the following primer melting temperatures ($T_m$): minimum of 50°C, optimum of 55°C, and maximum of 60°C. We also searched for tetranucleotides, but since we identified enough loci with di- and trinucleotides, these were not used. For dinucleotides, we identified 802 sequences with eight or more repeats, 351 of those had primers assigned, and 119 were potentially duplicated in the library. For trinucleotides, we identified 516 sequences with eight or more repeats, 270 of those had primers assigned, and 75 were potentially duplicated in the library. We had 232 potential loci with dinucleotide repeat units and 195 potential loci with trinucleotide repeat units.

We used the R code provided by Schoebel *et al*. (2013) in R version 3.5.2 (R Core Team 2019) to combine the primer and microsatellite sequences into one file. For the dinucleotides, after merging the files we had 222 unique reads left. After removing duplicated forward and reverse primer sequences, we had 169 unique reads left. For trinucleotides, after merging the files we had 189 unique reads left. After removing duplicated forward and reverse primer sequences, we had 147 unique reads left. We, then, combined the files with unique reads.

We calculated the absolute difference between the forward and reverse $T_m$ for each primer pair and sorted from smallest (0°C) to largest (3.58°C). We, then, sorted the putative loci by the forward penalty score, reverse penalty score, and by the pair penalty score. Lastly, we

calculated and sorted the ratio (absolute difference between penalty scores divided by the pair penalty) from smallest to largest to ensure that the difference between the forward and reverse penalties was as small as possible. We chose the top 60 loci from each of those five categories and combined them in one file. We ranked the 182 loci through the combined score from all five categories.

Finally, before ordering primers, we conducted a BLAST search in Geneious Prime 2020.0.5 (Biomatters, Ltd., Auckland, New Zealand) using the SSR-enriched library to ensure that only one primer pair was binding to the same locus, no primer pair was binding to more than one locus, and repeat regions were not within the primers. A total of 50 putative loci were screened using four female gametophytes and three tetrasporophytes. For 10 loci that produced bands for all samples on agarose gels and produced reliable patterns on the capillary sequencer, we performed fragment analysis of all samples at the Heflin Center for Genomic Sciences at UAB.

*DNA extraction*

The 2016 *Plocamium* sp. samples were all previously identified as tetrasporophytes through the presence of tetrasporangial sori. Total genomic DNA was extracted from 10-15 mg of dried thallus using the Qiagen DNeasy® Plant Mini kit. We followed the manufacturer's protocols except the final elution in which we used 50 µL of autoclaved Milli-Q water. For the 2017 and 2018 *Plocamium* sp. samples, we extracted total genomic DNA using the Machery-Nagel Nucleospin® Plant II kit. We followed the manufacturer's protocol except for the lysis step which was done at room temperature for one hour and the final elution where we used 100 µL of autoclaved Milli-Q water (see Krueger-Hadfield *et al.* 2013).

*Protocol for PCR amplification using unlabeled primers*

PCRs were performed with a total volume of 20 µL: 2 µL of DNA, 250 nM of each primer, 1X Promega green GoTaq® Flexi buffer, 2 mM of $MgCl_2$, 250 µM of each dNTP, 0.5 units of Promega GoTaq® Flexi DNA Polymerase, and the remaining volume using autoclaved Milli-Q water with the following program: 95 ºC for 2 min, followed by 40 cycles of 95 ºC for 30s, $T_m$ for 30s, and 72 ºC for 30s, with a final extension at 72 ºC for 5 min (see Table S1 for the $T_m$). PCR products were visualized by gel electrophoresis in 1.5% agarose gels stained with GelRed (Biotium, Fremont, CA, USA). Only primer pairs which produced 1 band in all gametophytes or 1-2 bands in all tetrasporophytes in the expected size range were retained.

*Protocol for PCR amplification using labeled primers*

We used the same PCR program, but with a PCR mix of a final total volume of 10 µL: 2 µL of DNA, 100 nM of labeled forward primer, 150 nM of unlabeled forward primer, and 250 nM of unlabeled reverse primer, 1X Promega clear GoTaq® Flexi buffer, 2 mM of $MgCl_2$, 250 µM of each dNTP, 0.5 units of Promega GoTaq® Flexi DNA Polymerase, and the remaining volume using autoclaved Milli-Q water. When samples had low amplification, the PCR protocol was further adjusted by adding 0.02 µg/µL of bovine serum albumin (BSA) and 400nM each of labeled forward and unlabeled reverse primers.

*Protocol for duplex and multiplex PCR amplification*

We combined the following loci into multiplexes using the same concentrations as outlined above without BSA: Multiplex 1 – Pc_16 (NED, 250 nM labeled forward and unlabeled

reverse), Pc_21 (6FAM, 300 nM labeled forward and unlabeled reverse), and Pc_36 (VIC/HEX, 350 nM labeled forward and unlabeled reverse); Duplex 1 – Pc_27 (VIC/HEX, 350 nM labeled forward and unlabeled reverse) and Pc_29 (6FAM, 350 nM labeled forward and unlabeled reverse); and Duplex 2 – Pc_47 (6FAM, 350 nM labeled forward and unlabeled reverse) and Pc_49 (VIC, 350 nM labeled forward and unlabeled reverse).

*Fragment analysis*

We used two ladders to perform fragment analysis (see *Ladder calibration* below). When using GeneScan 500 LIZ (Applied Biosystems, Foster City, CA, USA), 1 µL of PCR product was added to 9.7 µL of HiDi formamide (Applied Biosystems) and 0.35 µL of GS 500 LIZ. When using SM594 (Mauger *et al.* 2012), we added 1 µL of PCR product to 9.5 µL of HiDi formamide and 0.5 µL of SM594. We used Geneious Prime (Biomatters, Ltd., Auckland, New Zealand) to score raw allele sizes and TANDEM (Matschiner & Salzburger 2009) to assign bins (see also Krueger-Hadfield *et al*. 2013). Pc_05 and Pc_09 were amplified in simplex (due to their different $T_m$) and submitted as a poolplex for fragment analysis. Multiplex 1 and duplex 1 were each submitted for fragment analysis without the addition of further loci. Duplex 2 and Pc_40 which was amplified in simplex (since it did not work in a multiplex with Pc_47 and Pc_49) were submitted as a poolplex for fragment analysis.

*Marker calibration*

Applied Biosystems fluorescent dyes (6-FAM, VIC, NED) were initially ordered from ThermoFisher Scientific (USA), but subsequent replacements were ordered from Eurofins Genomics (Louiseville, KY, USA) for 6-FAM and HEX (replacement of Applied Biosystems

VIS dye). Forward primers with the 6-FAM dye showed no shift in fragment length on the capillary sequencer. For loci Pc_27 and Pc_36, there was a 0.7 and 0.8 base pair (bp) shift, respectively. All subsequent allele calls were shifted by 0.7 and 0.8 when scoring using HEX.

*Ladder calibration*

For the ladder calibration, 140 samples across all markers encompassing the entire allelic range were used to determine differences between GS500 LIZ and SM549. There were shifts from 3 – 4.3 bp when using SM594. Generally, smaller fragment lengths had a larger difference between the two ladders, whereas larger fragment lengths had a smaller difference. Samples analyzed with GeneScan 500 LIZ (Applied Biosystems, Foster City, CA, USA) were adjusted by subtracting an average of 3.6 bp for subsequent analyses.

*Null allele frequencies*

For the gametophytes, null allele frequencies were determined from thalli that did not amplify at a given locus after several amplification attempts to ensure there were no technical errors during PCR. For tetrasporophytes, the maximum likelihood estimator as implemented in ML-NullFreq (Kalinowski & Taper 2006) was used.

*Short allele dominance*

Short allele dominance was tested following Wattier *et al*. (1998). We included tetrasporphytes identified through reproductive structures and through multilocus genotypes (MLGs) by having at least one heterozygous locus to encompass a larger allelic range for each locus. For each locus, allelic size classes were determined, and their respective $F_{IS}$ values were

157 calculated in GenAlEx 6.5 (Peakall & Smouse 2006, Peakall & Smouse 2012). We tested for
158 short allele dominance using linear regression in base R. However, for five loci, there were either
159 not enough size classes due to a small allelic range or some of the size classes were
160 monomorphic from which $F_{IS}$ could not be calculated (Table S3).
161
162 *Gametophyte to tetrasporophyte ratios*
163 The binomial law was used to estimate the probability of detecting gametophyte to
164 tetrasporophyte ratios deviating from the null hypothesis of √2:1. If all life cycle stages had
165 equivalent survival and fecundity rates, we would expect a gametophyte to tetrasporophyte ratio
166 of √2:1 (Destombe *et al.* 1989, Thornber & Gaines 2004). This ratio is driven by a difference in
167 costs for producing spores and gametes and by the inherent cost to tetrasporophytes of producing
168 males as only females produce offspring (Thornber & Gaines 2004).
169
170 *Population genetic summary statistics*
171 We calculated ploidy diversity ($P_{HD}$) following Krueger-Hadfield *et al*. (2019) using $\frac{1-x}{0.59}$.
172 As $P_{HD}$ approaches 1, the ratio of gametophytes to tetrasporophytes is closer to √2:1. As $P_{HD}$
173 approaches 0, one stage dominates a population. In *Plocamium* sp., this indicates a
174 tetrasporophytic bias.
175 Next, we created a gametophyte (haploid) and a tetrasporophyte (diploid) data set for
176 each site for all subsequent analyses (Table S4). We investigated the likelihood of a repeated
177 multilocus genotype (MLG) to originate from a separate sexual event by calculating $P_{sex}$ using
178 GenClone 2.0 (Arnaud-Haond & Belkhir 2007). If $p > 0.05$, repeated MLGs are from separate
179 sexual events and if $p < 0.05$, repeated MLGs are ramets of the same genet. We then calculated

genotypic richness ($R$) following Dorken & Eckert (2001). We used rarefaction to estimate allelic richness ($A_E$) and private allelic richness ($P_A$) on the smallest sample size in gametophytes (N=9 alleles, or genes) using HP-RARE (Kalinowski 2005). We used $A_E$ for each locus to rank them from most to least polymorphic and plotted this against the proportion of unique genotypes (Figure S2) using ggplot2 (Wickham 2016) in R. We calculated unbiased expected heterozygosity ($H_E$) in GenAlEx. For gametophytes, we adjusted the unbiased $H_E$ by a factor of (2N-1)/(2N-2) (Engel *et al.* 2004). For tetrasporophytes, we calculated observed heterozygosity ($H_O$) in GenAlEx and the inbreeding coefficient ($F_{IS}$) using FSTAT 2.9.4 (Goudet 1995). We tested for significance using 1000 permutations.

**Results**

*Summary of locus characteristics*

We tested a total of 50 loci of which 34 did not amplify across all seven individuals on the initial test on agarose gel. For 16 loci that amplified well on agarose, we ordered a labeled forward primer. Five loci had multi-peak profiles following fragment analysis and were removed from subsequent analyses (Table S1). While Pc_04 looked promising, alleles were often separated by 1 bp, suggesting problems with amplification or scoring. Pc_04 was removed from subsequent analyses. Ten polymorphic microsatellite loci were ultimately retained and used for further analyses.

*Null allele frequencies*

Overall null alleles were not detected (Table S2). One locus, Pc_21, had one thallus that did not amplify in the gametophytes after repeated attempts. There were three loci in the

tetrasporophytes that showed evidence of null alleles based on maximum likelihood. The maximum likelihood estimator used by Kalinowski & Taper (2006) assumes random mating and previous studies have found similar discrepancies between direct estimates in gametophytes and those using maximum likelihood in the tetrasporophytes when populations are not mating at random (e.g., Krueger-Hadfield *et al*. 2013, Kollars *et al*. 2015).

*Repeated MLGs*

Repeated MLGs were found at both sites for tetrasporophytes (one at Laggard which was repeated twice, and two at "East Litchfield" which were each repeated once) and gametophytes (three at Laggard of which two were repeated once and one which was repeated three times, and two at "East Litchfield" which were each repeated once). The *p*-value for $P_{sex}$ was larger than 0.05 for all repeated MLGs except for one tetrasporophyte pair at Laggard which had a *p*-value of 0.003. Therefore, this was the only repeated MLG that was considered as a ramet of the same genet.

Supplemental Figures and Table from Main Text and Supplemental Materials

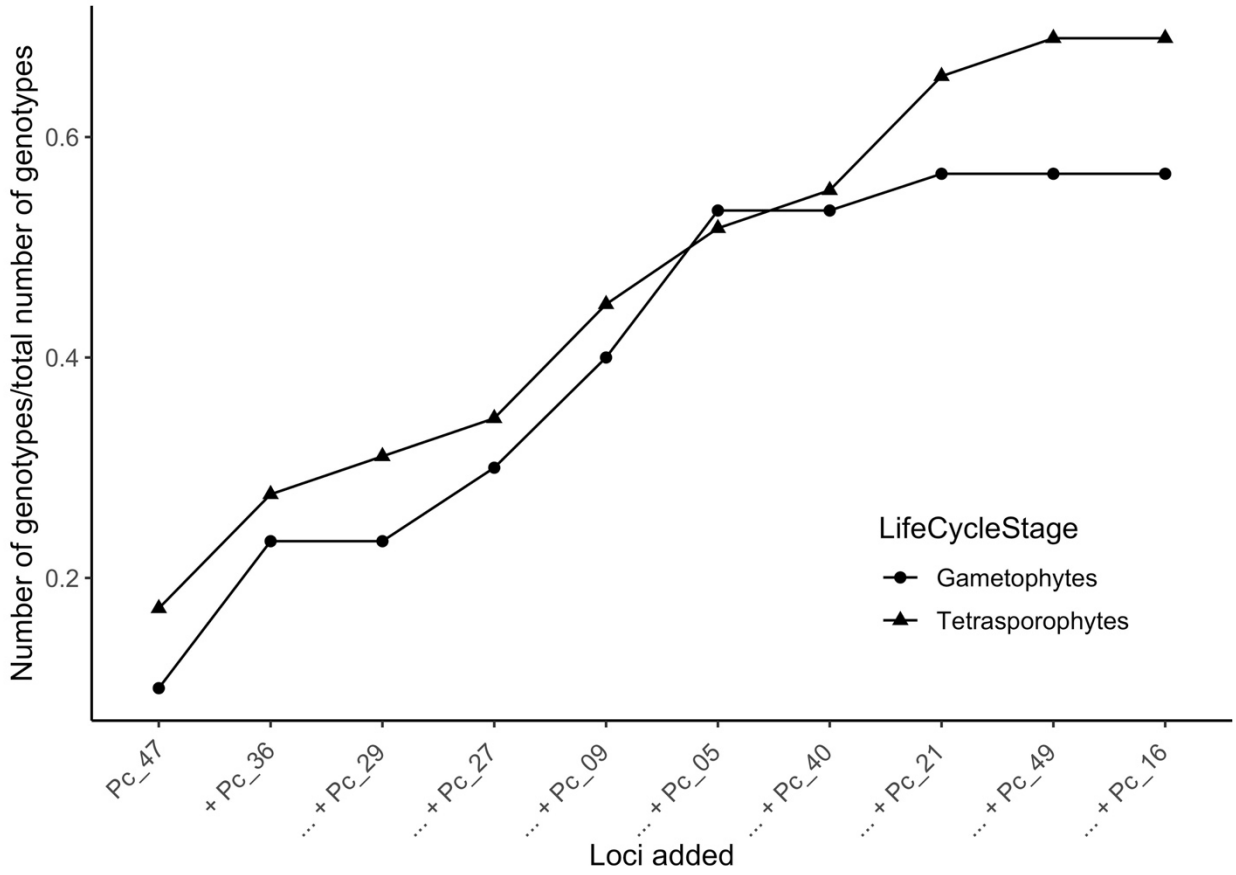


Figure S2. The proportion of unique genotypes identified in gametophytes and tetrasporophytes of *Plocamium* sp. when adding microsatellite loci from most polymorphic to least polymorphic (based on allelic richness in tetrasporophytes).

**Supplemental Table S1** Microsatellite locus information for Antarctic *Plocamium* sp. Locus name, motif, and primer sequences are given for all loci tested. The fluorescent dye and annealing temperature ($T_m$) are given for 16 labeled primers tested. The allele size range and total number of unique alleles are reported for samples from "East Litchfield" (N=149) and Laggard (N=47) identified as tetrasporophytes either through reproductive structures (tetrasporangial sori) or, if thalli were vegetative, by having a multilocus genotype with at least one heterozygous locus (the latter were included in this table to better represent the full allele range of the markers). (a) Loci used for fragment analysis. (b) 1 bp difference between alleles – locus removed. (c) Multipeak profiles observed during fragment analysis – loci removed. (d) No amplification in initial amplification tests using agarose gels.

(a)

| Locus | Motif | Primer Sequence | Dye | $T_m$ (ºC) | Allele size range (bp) | Total alleles |
|---|---|---|---|---|---|---|
| Pc_05 | GCT | F: GTCGTTGATGTCTAGCGTGC<br>R: ATGGATGTGGAGTCCGATCG | VIC | 53 | 225-240 | 3 |
| Pc_09 | CT | F: GGTCTAACGGCCTTGTGTTG<br>R: CCGGTTGTGAGTAAGTTGCC | NED | 59 | 151-185 | 8 |
| Pc_16 | GA | F: CGATGCCGCAAAGACTACAG<br>R: TACAAGACCTGGTAGTGGCG | NED | 56 | 266-276 | 4 |
| Pc_21 | TC | F: ATTCATAGGCCCACTCGTCC<br>R: CAGGCACCGACAAAGCTTAC | 6-FAM | 56 | 283-303 | 2 |
| Pc_27 | ACC | F: TCCACTACCACCGCTGATG<br>R: TCACGTCGGCTAAGGGTAAG | VIC or HEX | 56 | 281-290 | 3 |
| Pc_29 | AC | F: CCTCCATCCCTTAACCTACCG<br>R: GGAAGCGGGAGAATTTGGTG | 6-FAM | 56 | 210-220 | 3 |
| Pc_36 | ACC | F: ACCATCACGCTATCATTGCG<br>R: AGCGAAACATGAACGGGAAG | VIC or HEX | 56 | 193-247 | 7 |
| Pc_40 | AC | F: GAAAGCGGGAGATGTGAAGG<br>R: ACCTGCAACGAACAAACCTG | NED | 56 | 148-210 | 5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pc_47 | AGC | F: ATCAACGGGTGCTGTCAAAG<br>R: CTGACAAGTGTGCCAAACCG | 6-FAM | 56 | 232-352 | 18 |
| Pc_49 | GTC | F: TTGAAACGTGCCCACTTGTC<br>R: AACGAGTACTGGCGGAAGTG | VIC | 56 | 263-287 | 3 |

(b)

| **Locus** | **Motif** | **Primer Sequence** | **Dye** | **$T_m$ (ºC)** |
|---|---|---|---|---|
| Pc_04 | CTC | F: AACAACACAGCAGCCAAGTC<br>R: CGGAACATGACGGAACAAGG | 6-FAM | 53 |

(c)

| **Locus** | **Motif** | **Primer Sequence** | **Dye** | **$T_m$ (ºC)** |
|---|---|---|---|---|
| Pc_02 | CTT | F: CTCCAGGTCAGCTCTACGTC<br>R: TGGTGGAAGTGGAGGATTGG | NED | 53 |
| Pc_25 | AT | F: TGGGCATAGTCGGGATGATG<br>R: GAAAGATTGCGGGTGTGTCC | VIC | 56 |
| Pc_38 | CT | F: GTAGTTCGGATGGTGTTGGC<br>R: GTAGGCAGCTTTCACACACC | NED | 56 |
| Pc_39 | CT | F: TGCCTCTCGGTAGCCTTATG<br>R: AGCCAAACTACCCACCTTCC | 6-FAM | 56 |
| Pc_44 | AT | F: CGCCATGAAATCAACGTTCTC<br>R: AACACTGCTGCTGTATGAGG | NED | 56 |

(d)

| **Locus** | **Motif** | **Primer Sequence** |
|---|---|---|
| Pc_01 | AGG | F: AGGTTGATACGGGAAGAGGC<br>R: CCTCCTCCTGAACTCTACGC |
| Pc_03 | GAC | F: CAGATTCCGACGATGGCAAC<br>R: ATCGGAGCAGGGTCATGATC |
| Pc_06 | ACC | F: GTTTAGCCGTCGTTGTAGGC<br>R: TGTGAGAGTGGAAAGAGGCC |
| Pc_07 | ACG | F: GAGATACCCGGACGTAGAGC<br>R: AAACTTTCGCACGGGTTCTG |

| | | |
|---|---|---|
| Pc_08 | AGC | F: AACTGGACGAGACCTCCAAC |
| | | R: AGGACTGTGATGGAGGCATC |
| Pc_10 | AC | F: GCTCCTGTTTCACACCTTCG |
| | | R: TCCAACACTGCCTTGCTTTG |
| Pc_11 | AC | F: GATACACCAGAGTTGCACGC |
| | | R: CACCAGGTGCGTTTATGTCC |
| Pc_12 | TTG | F: TCAGTCACTCAGCGGCTATC |
| | | R: TTGACTACCTCCTTCACCGC |
| Pc_13 | CCG | F: TATCTCTGCTCGACATGGCC |
| | | R: GGCTTTCAGAATGGCTCGAC |
| Pc_14 | AT | F: GCAACACACGACTCTGACTG |
| | | R: GAGCCTTCCATGTTTCAGGC |
| Pc_15 | TG | F: GTTCCTTGCCATGAGATGCC |
| | | R: TGCCAAAGATGTCCAAAGCG |
| Pc_17 | GT | F: TGCTGTCTCCTCTCGTGATG |
| | | R: TGGAGAGGAGAGCGATGTTC |
| Pc_18 | AGG | F: ATAGACACGCACCTTCCTCC |
| | | R: CATGCAGTGTCTCCTCAACG |
| Pc_19 | AT | F: ACGAGGGTGCACTACTAAGG |
| | | R: ACATTAGTGCGCAACGTCAG |
| Pc_20 | CTT | F: AGCAGTCGATCCTTGGTCTG |
| | | R: ACGACGAAGCATGCAAGAAG |
| Pc_22 | TA | F: AGTGTAGAGTGCAGCGACAG |
| | | R: TAGATGGCCCGACTGTTAGC |
| Pc_23 | AGG | F: GATCTCGGCGTGTACACAAC |
| | | R: CTTCCGAAGAGCTGTGCAAG |
| Pc_24 | CT | F: GGCTTCGAATCAAGTCAGGC |
| | | R: GTCCAAGAAGTTCACGTCGG |
| Pc_26 | TTG | F: AGAATGTGATGCTCGAACGC |
| | | R: CCGTGGGCTGCAATGAATAG |
| Pc_28 | TCTA | F: AGCTCGGTGTACTGATGGAG |
| | | R: ATCCAGGCTCCTTAACCCTG |

| | | |
|---|---|---|
| Pc_30 | AC | F: CACGTACTTGTAGCGCCTTC |
| | | R: CTCTTGTGATGGTGCTCAGC |
| Pc_31 | GT | F: TGTGCGATAACCTGTCATGC |
| | | R: TACTGCTGCTGTACAATGCG |
| Pc_32 | ACC | F: GGTTGGGTTGCTTGTCTTCG |
| | | R: TCATGGTTTGTGGCGTTTCG |
| Pc_33 | AAC | F: CATGGGATTCGAACCACAGC |
| | | R: GTGACAATACGATCACTGCAC |
| Pc_34 | CCT | F: GGAACTGCAACACCAAGCC |
| | | R: AAGAAGCGTGCGATGTTGAG |
| Pc_35 | TTG | F: GATCAGCAACACGACGAAGG |
| | | R: TGTCAGCTTTCAATCCACGG |
| Pc_37 | TTG | F: ACAAATTCGAGTTGGTGCCG |
| | | R: GTCTTTGAGCTGACGACGTC |
| Pc_41 | ACGC | F: CGCTTGCTTACAACCTCAGG |
| | | R: TCCACGCGAGATACTAACAAAC |
| Pc_42 | TG | F: TGGAGGCAGAGTCACCTTTC |
| | | R: AAAGCACACGTCTCACCTTG |
| Pc_43 | GGT | F: CCTTTCGCTCAAACCACG |
| | | R: TGTTGGTGAAGTGTGCGAAC |
| Pc_45 | AC | F: CACATATCCACTCGCACTCG |
| | | R: TGAGAGGAGTGAATGGGTGG |
| Pc_46 | CTG | F: GTCAGCCTCTACCCACGTC |
| | | R: TGGACTACATAGAACCGCCG |
| Pc_48 | GA | F: TACAAGACCTGGTAGTGGCG |
| | | R: TCCCGATTCTTCAGCACCTC |
| Pc_50 | AGG | F: TTTCGGAGCAGTTGTAGTGG |
| | | R: CTCAATCTCCACCCTCTCCG |

**Supplemental Table S2** Null allele frequencies for ten microsatellite loci in the Antarctic *Plocamium* sp. We calculated them directly for gametophytes and we used a maximum likelihood estimator for tetrasporophytes (Kalinowski & Taper 2006).

| Locus | "East Litchfield" | | Laggard | |
|---|---|---|---|---|
| | Gametophytes (N=9) | Tetrasporophytes (N=12) | Gametophytes (N=21) | Tetrasporophytes (N=17) |
| **Pc_05** | 0 | 0 | 0 | 0 |
| **Pc_09** | 0 | 0 | 0 | 0 |
| **Pc_16** | 0 | 0.281 | 0 | 0.218 |
| **Pc_21** | 0 | 0 | 0.048 | 0.394 |
| **Pc_27** | 0 | 0 | 0 | 0 |
| **Pc_29** | 0 | 0 | 0 | 0 |
| **Pc_36** | 0 | 0 | 0 | 0 |
| **Pc_40** | 0 | 0 | 0 | 0 |
| **Pc_47** | 0 | 0 | 0 | 0 |
| **Pc_49** | 0 | 0.275 | 0 | 0.190 |

**Supplemental Table S3** Results for short allele dominance of microsatellite markers developed for the Antarctic *Plocamium* sp. for samples from "East Litchfield" (N=149) and Laggard (N=47) identified as tetrasporophytes either through reproductive structures (tetrasporangial sori) or, if thalli were vegetative, by having a multilocus genotype which was heterozygous for one or more loci. The latter were included in this table to better represent the full allele range of the markers. Results of linear regression analysis of size class specific $F_{IS}$ values are shown.

| Locus | N of size classes | $R^2$ | *F* (DF) | *p-value* |
|---|---|---|---|---|
| **Pc_05** | 3 | *NA – some size classes were monomorphic* | | |
| **Pc_09** | 4 | -0.3561 | 0.2122 (1, 2) | 0.6903 |
| **Pc_16** | 3 | 0.531 | 3.264 (1, 1) | 0.3218 |
| **Pc_21** | NA | *NA – not enough size classes* | | |
| **Pc_27** | 3 | *NA – some size classes were monomorphic* | | |
| **Pc_29** | 3 | *NA – some size classes were monomorphic* | | |
| **Pc_36** | 3 | 0.6906 | 5.463 (1, 1) | 0.2574 |
| **Pc_40** | 3 | 0.2394 | 1.63 (1, 1) | 0.423 |
| **Pc_47** | 6 | 0.0319 | 1.165 (1, 4) | 0.3412 |
| **Pc_49** | 3 | *NA – some size classes were monomorphic* | | |

241 **Supplemental Table S4** Multilocus genotypes (MLGs) using ten microsatellite markers for gametophytes and tetrasporophytes of the
242 Antarctic *Placamium* sp. identified through reproductive structures. Samples were collected from different transects at different
243 depths.

| Transect | Depth (m) | Pc_05 | | Pc_09 | | Pc_16 | | Pc_21 | | Pc_27 | | Pc_29 | | Pc_36 | | Pc_40 | | Pc_47 | | Pc_49 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tetrasporophytes at "East Litchfield" (N=12) | | | | | | | | | | | | | | | | | | | |
| 2 | 5 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 214 | 214 | 154 | 154 | 268 | 271 | 275 | 275 |
| 2 | 8 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 313 | 275 | 275 |
| 2 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 290 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 310 | 275 | 275 |
| 2 | 17 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 2 | 17 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 220 | 193 | 193 | 154 | 154 | 271 | 271 | 000 | 000 |
| 2 | 20 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 319 | 275 | 275 |
| 3 | 8 | 240 | 240 | 151 | 151 | 000 | 000 | 303 | 303 | 281 | 281 | 218 | 218 | 214 | 214 | 154 | 154 | 271 | 313 | 275 | 275 |
| 3 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 313 | 275 | 275 |
| 3 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 290 | 290 | 220 | 220 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 290 | 218 | 220 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| | | Gametophytes at "East Litchfield" (N=9) | | | | | | | | | | | | | | | | | | | |
| 1 | 11 | 240 | | 157 | | 270 | | 283 | | 281 | | 218 | | 199 | | 154 | | 271 | | 275 | |
| 2 | 11 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 2 | 11 | 240 | | 151 | | 270 | | 303 | | 281 | | 220 | | 193 | | 154 | | 271 | | 275 | |
| 2 | 14 | 240 | | 151 | | 270 | | 303 | | 290 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 2 | 14 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 3 | 8 | 237 | | 151 | | 270 | | 303 | | 281 | | 220 | | 193 | | 154 | | 310 | | 275 | |
| 3 | 8 | 240 | | 151 | | 270 | | 303 | | 281 | | 220 | | 193 | | 154 | | 271 | | 275 | |
| 3 | 8 | 240 | | 151 | | 270 | | 303 | | 290 | | 220 | | 193 | | 154 | | 271 | | 275 | |
| 3 | 17 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 295 | | 275 | |
| | | Tetrasporophytes at Laggard (N=17) | | | | | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 1 | 17 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 1 | 20 | 237 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 290 | 218 | 220 | 193 | 193 | 154 | 154 | 271 | 310 | 275 | 275 |
| 1 | 23 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 263 | 275 |
| 1 | 29 | 225 | 225 | 159 | 185 | 268 | 268 | 303 | 303 | 284 | 284 | 210 | 210 | 232 | 232 | 208 | 208 | 343 | 343 | 263 | 263 |
| 2 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 000 | 000 | 281 | 290 | 218 | 220 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 2 | 11 | 237 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 2 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 283 | 283 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 307 | 310 | 275 | 275 |
| 2 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 148 | 154 | 271 | 271 | 275 | 275 |
| 2 | 14 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 2 | 20 | 240 | 240 | 151 | 151 | 270 | 270 | 283 | 283 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 295 | 275 | 275 |
| 2 | 26 | 237 | 240 | 151 | 157 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 220 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 11 | 240 | 240 | 151 | 151 | 270 | 270 | 303 | 303 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 295 | 310 | 275 | 275 |
| 3 | 20 | 240 | 240 | 151 | 151 | 270 | 270 | 283 | 283 | 281 | 281 | 218 | 220 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 23 | 240 | 240 | 151 | 151 | 270 | 270 | 283 | 283 | 281 | 281 | 218 | 218 | 193 | 193 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 23 | 240 | 240 | 151 | 157 | 270 | 270 | 283 | 283 | 281 | 281 | 218 | 218 | 193 | 214 | 154 | 154 | 271 | 271 | 275 | 275 |
| 3 | 26 | 225 | 225 | 159 | 183 | 268 | 268 | 283 | 283 | 284 | 284 | 210 | 210 | 232 | 232 | 204 | 208 | 343 | 343 | 263 | 263 |

Gametophytes at Laggard (N=21)

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11 | 240 | | 157 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 310 | | 275 | |
| 1 | 14 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 1 | 14 | 240 | | 151 | | 270 | | 283 | | 281 | | 220 | | 193 | | 154 | | 310 | | 275 | |
| 1 | 17 | 240 | | 151 | | 270 | | 283 | | 281 | | 218 | | 214 | | 154 | | 271 | | 275 | |
| 1 | 17 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 1 | 23 | 240 | | 157 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 1 | 26 | 225 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 1 | 29 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 310 | | 275 | |
| 2 | 11 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 2 | 20 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 214 | | 154 | | 295 | | 275 | |
| 2 | 20 | 240 | | 151 | | 270 | | 283 | | 281 | | 218 | | 193 | | 154 | | 271 | | 275 | |
| 2 | 23 | 240 | | 151 | | 270 | | 0 | | 281 | | 218 | | 214 | | 154 | | 310 | | 275 | |
| 2 | 23 | 240 | | 151 | | 270 | | 303 | | 281 | | 218 | | 214 | | 154 | | 271 | | 275 | |
| 3 | 11 | 237 | | 151 | | 270 | | 303 | | 281 | | 218 | | 214 | | 154 | | 271 | | 275 | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 20 | 240 | 151 | 270 | 303 | 281 | 218 | 193 | 154 | 271 | 275 |
| 3 | 20 | 225 | 171 | 268 | 303 | 284 | 210 | 232 | 204 | 346 | 263 |
| 3 | 20 | 240 | 151 | 270 | 283 | 281 | 218 | 193 | 154 | 307 | 275 |
| 3 | 23 | 240 | 151 | 270 | 303 | 281 | 220 | 193 | 154 | 271 | 275 |
| 3 | 23 | 240 | 151 | 270 | 303 | 281 | 218 | 214 | 154 | 271 | 275 |
| 3 | 26 | 225 | 159 | 268 | 303 | 284 | 210 | 232 | 204 | 349 | 263 |
| 3 | 26 | 240 | 151 | 270 | 283 | 281 | 218 | 193 | 154 | 271 | 275 |

**Supplemental Table S5** Summary statistics for ten polymorphic microsatellite loci developed in the Antarctic *Plocamium* sp. and analyzed in the gametophytic and tetarsporophytic subpopulations of two sites along the WAP. N, number of samples; $A_E$ and $P_A$, mean and private allelic richness (using smallest sample size in gametophytes – 9); $H_E^A$, unbiased expected heterozygosity in gametophytes adjusted by a factor of (2N-1)/(2N-2); $H_E$, unbiased expected heterozygosity; $H_O$, observed heterozygosity $F_{IS}$, inbreeding coefficient. * $p < 0.0025$ (with $p$-adjusted to 0.0025 for significance)

| | **"East Litchfield"** | | | | | | | | **Laggard** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Gametophytes (N=9) | | | Tetrasporophytes (N=12) | | | | | Gametophytes (N=21) | | | Tetrasporophytes (N=17) | | | | |
| **Locus** | $A_E$ | $P_A$ | $H_E^A$ | $A_E$ | $A_P$ | $H_E$ | $H_O$ | $F_{IS}$ | $A_E$ | $A_P$ | $H_E^A$ | $A_E$ | $A_P$ | $H_E$ | $H_O$ | $F_{IS}$ |
| **Pc_05** | 2.0 | 0.6 | 0.236 | 1.0 | 0.0 | - | - | - | 2.3 | 0.8 | 0.347 | 2.3 | 1.3 | 0.358 | 0.176 | 0.515 |
| **Pc_09** | 2.0 | 0.3 | 0.236 | 1.0 | 0.0 | - | - | - | 2.5 | 0.9 | 0.356 | 2.5 | 1.5 | 0.323 | 0.235 | 0.277 |
| **Pc_16** | 1.0 | 0.0 | - | 1.0 | 0.0 | - | - | - | 1.7 | 0.7 | 0.185 | 1.7 | 0.7 | 0.214 | - | 1.000 |
| **Pc_21** | 2.0 | 0.0 | 0.236 | 1.0 | 0.0 | - | - | - | 2.0 | 0.0 | 0.404 | 2.0 | 1.0 | 0.484 | - | 1.000* |
| **Pc_27** | 2.0 | 1.0 | 0.413 | 1.9 | 0.5 | 0.290 | 0.167 | 0.436 | 1.7 | 0.7 | 0.185 | 2.2 | 0.8 | 0.314 | 0.118 | 0.632 |
| **Pc_29** | 2.0 | 0.3 | 0.590 | 1.9 | 0.2 | 0.290 | 0.167 | 0.436 | 2.4 | 0.7 | 0.351 | 2.5 | 0.8 | 0.399 | 0.235 | 0.418 |
| **Pc_36** | 2.0 | 1.0 | 0.236 | 2.0 | 0.2 | 0.507 | 0.500 | 0.015 | 2.7 | 1.7 | 0.566 | 2.5 | 0.7 | 0.437 | 0.294 | 0.333 |
| **Pc_40** | 1.0 | 0.0 | - | 1.0 | 0.0 | - | - | - | 1.7 | 0.7 | 0.185 | 2.1 | 1.1 | 0.271 | 0.118 | 0.573 |
| **Pc_47** | 3.0 | 0.7 | 0.443 | 2.9 | 1.7 | 0.435 | 0.500 | -0.158 | 3.6 | 1.3 | 0.615 | 3.1 | 1.8 | 0.490 | 0.235 | 0.528 |
| **Pc_49** | 1.0 | 0.0 | - | 1.0 | 0.0 | - | - | - | 1.7 | 0.7 | 0.185 | 1.8 | 0.8 | 0.258 | 0.059 | 0.778 |

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