Development and characterization of microsatellite loci for the haploid-diploid red seaweed *Gracilaria vermiculophylla*

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Microsatellite loci are popular molecular markers due to their resolution in distinguishing individual genotypes. However, they have rarely been used to explore the population dynamics in species with biphasic life cycles in which both haploid and diploid stages develop into independent, functional organisms. We developed microsatellite loci for the haploid-diploid red seaweed *Gracilaria vermiculophylla*, a widespread non-native species in coastal estuaries of the Northern hemisphere. Forty-two loci were screened for amplification and polymorphism. Nine of these loci were polymorphic across four populations of the extant range with two to eleven alleles observed. Mean observed and expected heterozygosities ranged from 0.265 to 0.527 and 0.317 to 0.387, respectively. Overall, these markers will aid in the study of the invasive history of this seaweed and further studies on the population dynamics of this important haploid-diploid primary producer.

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INTRODUCTION

In the last decade, genetic approaches to answering evolutionary and ecological questions have become less expensive and more easily applied to non-model species (Allendorf *et al.* 2010; Guichoux *et al.* 2011). Microsatellites, or tandem repeats of one to six nucleotides, are popular molecular markers due to their resolution in distinguishing individual genotypes (Selkoe & Toonen 2006) and their ability to describe patterns of population connectivity across landscapes (Manel *et al.* 2003) and seascapes (Galindo *et al.* 2006). Much of the literature focuses on organisms with single free-living diploid stages (i.e., animals and higher plants). Yet, there are many species with both haploid and diploid stages in the same life cycle in which both ploidies undergo somatic development and live as independent, functional organisms.

Theory predicts that selection should favor either diploidy or haploidy (Mable & Otto 1998), Hughes and Otto (1999) demonstrated the maintenance of both haploid and diploid stages when the two stages occupy different ecological niches. However, there are relatively few empirical tests of these alternative hypotheses (but see Destombe *et al.* 1992; Thornber & Gaines 2004; Guillemin *et al.* 2013), and for isomorphic species in which ploidy is not easily identified through morphological traits, molecular markers will be essential to address them. These same markers can additionally be used to understand connectivity and demographic history in haploid-diploid populations. Among marine haploid-diploid macroalgae, relatively few microsatellites have been developed to address any of these issues (but see Table 1).

Understanding the consequences of biphasic life cycles and land- or seascape features on population structure is particularly relevant in light of the increasing

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24 frequency of biological introductions. There are numerous examples of widespread, and
25 putatively invasive species, that have free-living haploid and diploid stages, including
26 macroalgae (e.g., *Asparagopsis* spp.; Andreakis *et al.* 2007), ferns (e.g., *Lygodium* spp.;
27 Lott *et al.* 2003) and mosses (e.g., *Campylopus introflexus*; Schirmel *et al.* 2010).
28 Macroalgae, or seaweeds, account for approximately 20% of the world's introduced
29 marine species (Andreakis & Schaffelke 2012) and a subset of these invasions are by
30 species that are exploited in their native range, either for the phycocolloid industry or as
31 food products (Williams & Smith 2007).

32 The red seaweed *Gracilaria vermiculophylla* (Omhi) Papenfuss is native to the
33 northwest Pacific and, in the last 30-40 years, has spread throughout high to medium
34 salinity estuaries of the eastern North Pacific (Saunders 2009), the western North Atlantic
35 (Byers *et al.* 2012) and the eastern North Atlantic (Weinberger *et al.* 2008; Guillemin *et*
36 *al.* 2008a). *G. vermiculophylla* transforms the ecosystems into which it is introduced
37 through negative impacts on native species (e.g., direct competition, Hammann *et al.*
38 2013), the addition of structural complexity to soft-bottom systems (e.g., Nyberg *et al.*
39 2009, Wright *et al.* 2014) and the alteration of community structure, species interactions
40 and detrital pathways (e.g., Byers *et al.* 2012). Previous studies of the population genetics
41 of *G. vermiculophylla* focused on the mitochondrial gene *cytochrome b oxidase I* (Kim *et*
42 *al.* 2010, Gulbransen *et al.* 2012), but mitochondrial genetics do not necessarily predict
43 the population genetics of the nuclear genome and cannot assess patterns of ploidy and
44 mating system. Thus, we developed nine polymorphic microsatellite loci for *G.*
45 *vermiculophylla*.

46

47 MATERIALS AND METHODS

48 A library of contigs for *G. vermiculophylla* was generated using the 454 next-
49 generation sequencing platform (Cornell University Life Sciences Core Laboratory
50 Center) from a single individual collected from Charleston, SC, USA. For library
51 preparation, DNA was extracted using CTAB (Eichenberger et al. 2000) and library
52 construction followed Hamilton et al. (1999). Dimeric to hexameric microsatellite repeats
53 were identified with the program MSATCOMMANDER, ver 1.0.8 (Faircloth 2008) and
54 primers were designed using PRIMER 3 (Rozen and Skalesty 2000) for contigs with at
55 least four sequences present in the library and motifs with less than 5 repeats.
56 Bioinformatics of these sequences was facilitated by the APE package (Paradis *et al.*
57 2004) in *R* (R Core Team 2014).

58 Total genomic DNA was isolated using 120 µL of a 10% Chelex solution
59 (BioRad Laboratories, Hercules, CA, USA) in which approximately 1 cm of dried algal
60 tissue was heated at 95°C for 30 minutes and vortexed intermittently (Walsh *et al.* 1991).
61 Loci were amplified on a thermocycler (BioRad) as follows: 10 µL final volume, 2 µL of
62 DNA template, 0.5 units of GoTAQ Flexi-DNA Polymerase (Promega), 1X buffer, 250
63 µM of each dNTP, 1.5 mM of $MgCl_2$, 150 nM of fluorescently-labeled forward primer,
64 100 nM of unlabeled forward primer and 250 nM of unlabeled reverse primer. The PCR
65 program included 2 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at
66 72°C, and a final 5 min at 72°C. One µL of each PCR product was added to 10 µL of
67 loading buffer containing 0.35 µL of size standard (GeneScan500 Liz; Applied
68 Biosystems, Foster City, CA, USA). Samples were electrophoresed on an ABI 3130xL
69 genetic analyzer equipped with 36 cm capillaries (Applied Biosystems). Alleles were

70 scored manually using GENEMAPPER ver. 4 (Applied Biosystems) and allele sizes were
71 binned with TANDEM software (Matschiner & Saltzburger 2009; Krueger-Hadfield *et*
72 *al.* 2013).

73 We screened a total of 42 primer pairs for amplification and polymorphism in *G.*
74 *vermiculophylla* (Table 2). For the amplifiable loci that also showed polymorphism (nine
75 total, see Results and Discussion), we verified single locus genetic determinism (SGLD)
76 in a subset of known haploid gametophytes (n = 28) and diploid tetrasporophytes (n = 30)
77 collected at Elkhorn Slough, CA, USA (Table 3, Supplementary Figure 1). Elkhorn
78 Slough was the only population for which ploidy was determined by reproductive
79 structures and for which we had known haploids and diploids for genotyping. Loci were
80 in SLGD if known haploids produced a single allele and diploids produced either one or
81 two alleles in their homozygous or heterozygous state, respectively.

82 The frequency of null alleles was estimated in the haploid subpopulation from
83 Elkhorn Slough as well as diploid tetrasporophytes for each of the four populations
84 (Table 3). It is possible to calculate the null allele frequency directly in the haploids based
85 on the number of non-amplification events, after discounting technical errors. For diploid
86 tetrasporophytes, we used a maximum likelihood estimator (ML-NullFreq: Kalinowski &
87 Taper 2006). However, Krueger-Hadfield *et al.* (2013) demonstrated a strong bias in the
88 maximum likelihood estimators of null allele frequency when macroalgal populations do
89 not undergo random mating.

90 Next, we screened loci for short allele dominance (Wattier *et al.* 1998). The
91 presence of short allele dominance is rarely tested during microsatellite development,
92 even though it does result in artificial heterozygote deficiencies. In contrast to null

93 alleles, primer binding is successful, but the larger allele is not amplified due to the
94 preferential amplification of the smaller allele. Wattier *et al.* (1998) demonstrated an
95 analytical method to detect short allele dominance. If a regression of allele-specific F_{is}
96 (inbreeding coefficient) statistics on allele size reveals a significant negative slope, then
97 short allele dominance may be expected. We determined three to four allele size classes
98 per locus and performed linear regressions in *R* (R Core Team 2014).

99 To provide preliminary assessment of the genotypic and genetic diversity one can
100 gain from these loci, we genotyped diploid tetrasporophytes only from one native and
101 three non-native populations of *G. vermiculophylla* (Table 3). Diploids were identified
102 based either on reproductive phenology (Elkhorn) or microsatellite genotype (after
103 assuring SLGD) if at least one locus was heterozygous (Akkeshi, Fort Johnson and
104 Nordstrand, Table 3).

105 We calculated expected allelic richness using rarefaction in order to account for
106 differences in sample size (HP-Rare; Kalinowski 2005). Observed (H_O) and expected
107 heterozygosities (H_E) were calculated using GenAlEx, ver. 6.501 (Peakall & Smouse
108 2006; Peakall & Smouse 2012). Tests for Hardy-Weinberg equilibrium and *F*-statistics
109 were performed in FSTAT, ver. 2.9.3.2 (Goudet 1995). F_{is} was calculated for each locus
110 and over all loci according to (Weir & Cockerham 1984) and significance (at the adjusted
111 nominal level of 0.001) was tested by running 10000 permutations of alleles among
112 individuals within samples. We also tested for linkage disequilibrium in each population
113 using GENEPOP, ver. 4.2.2 (Rousset 2008), with 1000 permutations followed by
114 Bonferroni correction for multiple comparisons (Sokal & Rohlf 1995).

115

116 RESULTS AND DISCUSSION

117 Of the 42 loci screened, 16 did not amplify for *G. vermiculophylla* even after
118 several PCR modifications (Table 2). Of the remaining 26 loci, four loci exhibited multi-
119 peak profiles and were discarded from further use, 13 loci were considered
120 monomorphic, and nine loci showed polymorphism. The nine polymorphic loci exhibited
121 SLGD in which known haploids always exhibited one allele.

122 The frequency of null alleles was zero at all loci except Gverm_1803 and
123 Gverm_2790 in which the frequencies were both 0.019 in the haploids at Elkhorn Slough
124 (Supplementary Table 1). The only evidence of null alleles in the diploids from Elkhorn
125 Slough was at locus Gverm_1803, with a maximum likelihood estimated frequency of
126 0.115. The discrepancy between the haploid and diploid estimates is likely due to
127 assumptions underlying the maximum likelihood estimators implemented in software like
128 HP-Rare (Kalinowski 2005), such as random mating. The higher frequencies of null
129 alleles (0.115-0.207) in the Akkeshi diploid subpopulation were most likely driven by a
130 violation of these assumptions as well, though empirical estimates in haploid
131 subpopulations are warranted. Nevertheless, the low frequency of null alleles and lack of
132 evidence for short-allele dominance (all regression *p*-values were > 0.2, Supplemental
133 Table 2), suggest that observed heterozygote deficiencies using these loci will be due to
134 the mating system or spatial substructuring (Guillemin *et al.* 2008b; Krueger-Hadfield *et*
135 *al.* 2011; 2013).

136 The nine polymorphic markers described genetic variability in four populations
137 sampled across the extant distribution of *G. vermiculophylla*. Overall, there was little
138 evidence for linkage disequilibrium after Bonferroni correction (Supplementary Figure

139 2). Additionally, allelic diversity was comparable among the one native and three non-
140 native sites we sampled, but F_{is} varied considerably (summary in Table 4; per locus
141 statistics in Supplementary Table 3). Together, these results suggest that unique
142 demographic and evolutionary processes could be operating between native and non-
143 native ranges and within each population, but more detailed sampling is needed to
144 address these patterns.

145 In summary, we have developed and characterized microsatellite markers for the
146 haploid-diploid red seaweed *G. vermiculophylla*. These loci have the resolution to
147 distinguish individual thalli and will aid studies on the invasive history of *G.*
148 *vermiculophylla*, as well as the evolutionary ecology of rapidly spreading populations and
149 mating system shifts in organisms that have biphasic life cycles with free-living haploid
150 and diploid stages (i.e., macroalgae, ferns, mosses and some fungi).

151

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Table 1. Studies in which both the haploid and diploid stages of seaweeds and mosses were investigated to reveal patterns in genetic structure and mating system.

	Phylum	**Species**	**Marker**	**Type of study**
Sosa *et al.* (1998)	Rhodophyta	*Gelidium arbuscula*	Isozymes	Genetic structure and mating system
Sosa *et al.* (1998)	Rhodophyta	*Gelidium canariensis*	Isozymes	Genetic structure and mating system
Engel *et al.* (1999)	Rhodophyta	*Gracilaria gracilis*	Microsatellites	Paternity analyses and dispersal
van der Velde *et al.* (2001)	Bryophyta	*Polytrichum formosum*	Microsatellites	Paternity analyses and dispersal
van der Strate *et al.* (2002)	Chlorophyta	*Cladophoropsis membranacea*	Microsatellites	Shorescape structure and mating system
Engel *et al.* (2004)	Rhodophyta	*Gracilaria gracilis*	Microsatellites	Shorescape structure and mating system
Guillemin *et al.* (2008)	Rhodophyta	*Gracilaria chilensis*	Microsatellites	Genetic structure, mating system and comparisons between natural and farmed populations
Szövényi *et al.* (2009)	Bryophyta	*Sphagnum lescurii*	Microsatellites	Paternity analyses and dispersal
Alström-Rapaport *et al.* (2010)	Chlorophyta	*Ulva intenstinalis*	Microsatellites	Genetic structure and mating system
Krueger-Hadfield *et al.* (2011)	Rhodophyta	*Chondrus crispus*	Microsatellites	Genetic structure and mating system
Krueger-Hadfield *et al.* (2013)	Rhodophyta	*Chondrus crispus*	Microsatellites	Shorescape structure and mating system
Krueger-Hadfield *et al.* (2015)	Rhodophyta	*Chondrus crispus*	Microsatellites	Paternity analyses and dispersal

Table 2. Characteristics of 42 microsatellite loci developed for *Gracilaria vermiculophylla*: Acc. No. = genbank accession number; locus; motif; primer sequences; Profile: one- or multi-locus genetic determinism, no amp. indicates non-amplification; allele range; avg. error: TANDEM (Matschiner & Saltzburger 2009) rounding errors for each microsatellite locus (the authors of TANDEM suggest that good loci have an average rounding error which is below 10% of the repeat size); *Ntall* = total number of alleles.

Locus	**Acc. No.**	**Motif**	**Primer sequence**	**Profile**	**Allele Range**	**Tandem Avg. Error**	N_{tall}
Gverm_5276	########	$(AC)_{10}$	F: GGAGAGCAGCACGTTTTAGG R: CTGCTTAGTTCCACGATCGAC	one	282-316	0.14	11
Gverm_6311	########	$(AG)_9$	F: GCGTCATTCCACTGAATGTG R: GATGAACCTCAATGCCTCGT	one	203-223	0.17	6
Gverm_8036	########	$(AC)_{12}$	F: GCCCTTTTAAGGATGCAACA R: GGGGTAAACGACCACAGAGA	one	213-251	0.14	5
Gverm_3003	########	$(AG)_{11}$	F: CATCTTGCTTCCTTGCTTCC R: TTGAAAGCCGGAATTTATCG	one	198-230	0.11	4
Gverm_1203	########	$(AAG)_8$	F: CTCCTGGTGCACAAGCAATA R: ACATTCTGCGCACCTTTCTT	one	284-308	0.12	4
Gverm_1803	########	$(AC)_{11}$	F: GCGTGCACGATGTCTACACT R: GACAGCAACAAGTGGGGTTT	one	352-356	0.07	3
Gverm_804	########	$(AAG)_8$	F: TGTAGGATTGCTCTCCTGGTG R: CAGGCTGGCCAAAATAACAT	one	182-188	0.16	3
Gverm_10367	########	$(AG)_8$	F: GCTGAGAAATGAAGCGAAGG R: GCAAACCTGCCTTGTTTGTT	one	198-200	0.07	2
Gverm_2790	########	$(AATGC)_5$	F: GAACAATGCGGGAAAACATT R: GGAAGAGGCTCAAAAGCAGA	one	262-267	0.16	2

Gverm_10883	########	$(AAG)_7$	F: TCCCATCAGCCAACAGTAGA R: GCAGAGCTTGGTAGGCATTC	one	-	-	1
Gverm_7969	########	$(ATC)_6$	F: CGATCCTTCCTCCTGTGGTA R: AATTGGGATACGCAATACGG	one	-	-	1
Gverm_13240	########	$(ACG)_8$	F: AACACATTGCTTCCGTTCTTG R: CTGCGAAGCACAAGTGATGT	one	-	-	1
Gverm_2178	########	$(ACG)_6$	F: ATTTGCACCGGTAAAACTGG R: GGCTGTCATGCAAGATGATG	one	-	-	1
Gverm_12220	########	$(AC)_7$	F: TGACTCGAGGAGTGCAGATG R: CTTTTGCCAGCAATGCAATA	one	-	-	1
Gverm_10926	########	$(AC)_7$	F: CTTTTGCCAGCAATGCAATA R: TGACTCGAGGAGTGCAGATG	one	-	-	1
Gverm_10612	########	$(AAG)_7$	F: GCAGAGCTTGGTAGGCATTC R: GGCAACACCATTGGACTCTT	one	-	-	1
Gverm_12990	########	$(AC)_7$	F: GGGCGTAGAGAGCTGAAAGA R: TCGCCGTTTTTCTCCTACAC	one	-	-	1
Gverm_9808	########	$(AG)_{10}$	F: GCCTGTTCCTCATCTTTTGG R: GCGACAGAAGAGGCGACTTA	one	-	-	1
Gverm_10115	########	$(AG)_8$	F: CAGGGGCTACTCACCTTCAC R: GTGTGTCTTGATCCGCTGTG	one	-	-	1
Gverm_10134	########	$(AC)_8$	F: CCGAAAGATTAGCGATCCAC R: CTCCCCCTCTTGGTTTTGTT	one	-	-	1
Gverm_871	########	$(AG)_9$	F: ACACGGGTCTCATGTTCCTC R: AGGAGCGCAGTCCAAGTAAA	one	-	-	1
Gverm_3707	########	$(AG)_{10}$	F: ACGACTCACGGGTTGTTTTT R: AAGCAGACCAGCACATTTCA	one	-	-	1

Gverm_4346	########	$(AC)_8$	F: ATCGCTCTTCTTCGGCTACA R: AGGGATGACTCACCAAGTGC	multi	-	-	-
Gverm_263	########	$(ACG)_9$	F: CACATTGCTTCCGTTCTTGTT R: TGAGTTCGTCGTCACGATTC	multi	-	-	-
Gverm_12453	########	$(AG)_{13}$	F: GAAGACTGACCGGAATCTGC R: ACAATCAACACGCAGCTCAG	multi	-	-	-
Gverm_13408	########	$(AAG)_9$	F: GGGAACGCATATCTTTGTGG R: GATACGGGAACAGCGTTTGT	multi	-	-	-
Gverm_8854	-	$(AAAT)_5$	F: TGCGCAGGAAAGGGTTAATA R: ATCCCTCGTGATAGGCAAAT	no amp.	-	-	-
Gverm_6659	-	$(AC)_6$	F: TGGTACGTGATCCCAGTGTG R: CATCGTGCTTCACCACATTC	no amp.	-	-	-
Gverm_85	-	$(ACC)_6$	F: AAAGTGGAGGCAGCTATGGA R: TCCCCAATGAACTCATGACA	no amp.	-	-	-
Gverm_5509	-	$(AC)_9$	F: ACTTTGCACCTTTGCACCTT R: GTTGGAATGGTTCTGCGATT	no amp.	-	-	-
Gverm_269	-	$(ACG)_9$	F: CACATTGCTTCCGTTCTTGTT R: TGAGTTCGTCGTCACGATTC	no amp.	-	-	-
Gverm_3258	-	$(AC)_9$	F: AACTGGAGCTTGGGATGCTA R: CCGTCTCTGTTTCTTTCTAGTGC	no amp.	-	-	-
Gverm_3883	-	$(ACAG)_{20}$	F: ATCGAGATATTTACACGCAACA R: AGAGGGCAGTTAGATTGTCTGA	no amp.	-	-	-
Gverm_5516	-	$(AAC)_8$	F: AGTTGTAACAGCGGGAAACG R: CCCTGTTGCGAATCTTCTGT	no amp.	-	-	-
Gverm_6564	-	$(AAC)_{13}$	F: CCCTAAGCTCTGCCATTGTC R: TGCGCTGTAACAAGAAGAAGA	no amp.	-	-	-

Gverm_7244	-	$(AAGG)_{20}$	F: CTGCATCAACACGATTACGC R: GATATGGGTGGACGAGTGCT	no amp.	-	-	-
Gverm_8378	-	$(AC)_{20}$	F: CCAACCTCTCCTCCTGTTTG R: TGTACGCTGCAATGCTGAAC	no amp.	-	-	-
Gverm_8448	-	$(ACC)_{8}$	F: AACTGGAACTGCAAACAATGG R: GCTTGTTGATGAGCCTGTTG	no amp.	-	-	-
Gverm_8535	-	$(AC)_{11}$	F: CGTACAGAATGGGGGATTTG R: GGAAGATGGATGTGCAGGTT	no amp.	-	-	-
Gverm_11521	-	$(AC)_{11}$	F: AAAGGTGGCATTGAGTCAGC R: TTCATTTGCCACTCTCTAAGCA	no amp.	-	-	-
Gverm_11615	-	$(ATCC)_{17}$	F: TGAAACGCCAATAAGTTTCTGTT R: CACTGTTAGACAACCCAGAAAGA	no amp.	-	-	-
Gverm_11818	-	$(AAGT)_{18}$	F: AAGGAAGAAGCATCACCTTCA R: TCGATTCTGATTGGTTAATAGCAA	no amp.	-	-	-

Table 3. Location of the four populations used to test for polymorphism in newly characterized microsatellite loci in *Gracilaria vermiculophylla*. The region (native or non-native), latitude, longitude, sampling date, collector* and ploidy determination (using reproductive phenology or microsatellite genotype) is provided.

Population	Region	Latitude	Longitude	Date	Collector	Ploidy determination
Akkeshi, Japan	Native	43.04774	144.9498	25Aug10, 31Jul12	NMK, KH, KM, AP, MS	µsat
Elkhorn Slough CA, USA	Non-native	36.50447	-121.4513	3Nov13	SAKH, BFK, TDK, BH	µsat, phenology
Fort Johnson SC, USA	Non-native	32.7513	-79.900	11Dec13	CEG	µsat
Nordstrand, Germany	Non-native	54.454571	8.874846	24Mar10	MH	µsat

* Collector abbreviations: AP: A. Pansch, NMK: N. M. Kollars, KH: K. Honda, KM: K. Momota, MS: M. Sato, SAKH: S. A. Krueger-Hadfield, BFK: B. F. Krueger, TDK: T. D. Krueger, BH: B. Hughes, CEG: C. E. Gerstenmaier, MH: M. Hammann

Table 4. Genetic features of four populations of *Gracilaria vermicuolphylla*, including: the sample size, N; the diploid genotypic richness, N_A, $\pm$ standard error (SE); mean allelic richness, A_E, based on the smallest sample size of 46 alleles (23 diploid individuals) $\pm$ SE; mean observed heterozygosity, H_O, $\pm$ SE; mean expected heterozygosity, H_E, $\pm$ SE; inbreeding coefficient, F_{is}, multilocus and per locus estimates (*, $p < 0.001$, adjusted nominal value).

Statistics	Akkeshi	Elkhorn Slough	Fort Johnson	Nordstrand
N	31	30	38	23
N_A	3.2 $\pm$ 0.5	2.2 $\pm$ 0.4	2.0 $\pm$ 0.2	1.9 $\pm$ 0.2
A_E	3.1 $\pm$ 0.4	2.2 $\pm$ 0.3	2.0 $\pm$ 0.2	1.9 $\pm$ 0.2
H_O	0.265 $\pm$ 0.060	0.311 $\pm$ 0.089	0.520 $\pm$ 0.110	0.527 $\pm$ 0.125
H_E	0.374 $\pm$ 0.079	0.317 $\pm$ 0.084	0.387 $\pm$ 0.077	0.352 $\pm$ 0.079
F_{is}	0.294 *	0.017	-0.350 *	-0.512 *
F_{is} per locus				
Gverm_5276	0.484 *	0.120	-0.209	-0.492
Gverm_6311	0.435 *	0.140	-0.267	-0.048
Gverm_8036	0.334	NA	-0.445 *	-0.217
Gverm_3003	0.529	-0.121	-0.138	-0.553
Gverm_1203	-0.15	-0.206	-0.310	-0.508
Gverm_1803	0.569 *	0.460	-0.696 *	NA
Gverm_804	-0.278	-0.206	-0.310	-0.508
Gverm_10367	-0.017	NA	NA	NA
Gverm_2790	NA	NA	NA	-0.913 *