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TECHNICAL NOTE

Isolation and characterization of microsatellite loci in two non-native hydromedusae in the San Francisco Estuary: *Maeotias marginata* and *Moerisia* sp.

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Abstract We characterized 10 new microsatellite markers in each of two species of hydromedusae, *Maeotias marginata* and *Moerisia* sp. Genetic diversity was estimated using 20–41 individuals collected from Suisun Marsh within the San Francisco Estuary, CA. Allelic richness ranged from 5–9 in *M. marginata* and 2–10 in *Moerisia* sp. with average expected heterozygosities of 0.71 and 0.57 respectively. One locus in *M. marginata and* two in *Moerisia* sp. deviated from Hardy–Weinberg equilibrium expectations, likely due to null alleles.

Keywords Hydrozoa · Jellyfish · Oligos · San Francisco Estuary

Two species of hydromedusae, *Maeotias marginata* and *Moerisia* sp., native to the Ponto-Caspian region, have become established in the San Francisco Estuary, CA (SFE). Non-native jellyfish and other hydroids can have severe effects on the ecosystems they invade as many are voracious predators, consuming large amounts of prey and disrupting planktivorous food webs (Purcell and Arai 2001). Additionally, jellyfish blooms are increasing globally (Mills 2001) and can directly affect fish populations by devouring massive quantities of eggs and larvae and decreasing fish survival through competition for resources (Purcell and Arai 2001; Purcell et al. 2001; Purcell 2003; Lynam et al. 2005). The brackish water hydrozomedusae studied herein are novel predators in the SFE and, thus, have an especially high likelihood of impacting this system and competing with juvenile fish (Moyle and Light 1996). Very little is known about the basic biology and life history of these invaders. Microsatellite markers characterized for these species will allow us to investigate population structure and genetic diversity, as well as determine reproductive strategies and life history characteristics of these potentially important non-natives.

We collected the medusae phase of *M. marginata* and *Moerisia* sp. from Suisun Marsh in the SFE. We extracted whole genomic DNA from bell tissue of *M. marginata* and entire individuals of *Moerisia* sp., due to their small size, using Qiagen's Gentra Puregene Kit protocol. Genetic Identification Services constructed, screened, and sequenced four libraries enriched with the following repeat motifs: (1) GATA, (2) CCAT and CTGT, (3) ATG, AAC, and ATT, and (4) a mix of all tetra-nucleotide repeats available, using pooled DNA from both species and according to the procedures of Meredith and May (2002). A total of 381 clones were sequenced.

We analyzed sequences using SEQUENCHER version 4.7 (Gene Codes Corporation) and used MREPS version 2.5 (Kolpakov et al. 2003) to identify repeat regions. Once repeat loci were located, we employed PRIMER 3 (Rozen and Skaletsky 2000) to create primer pairs flanking each region of interest. We then tested primer pairs on 2–10 individuals of each species to assess microsatellite amplification and level of polymorphism.

Some primer pairs worked best with Promega GoTaq Flexi DNA polymerase, while others worked better with Roche FastStart Taq DNA polymerase. The recipe for those with Promega Taq was 2 µl 5× Promega Buffer, 0.8 µl of 10 µM dNTPs, 0.6 µl of 25 µM Mg, 0.08 µl

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Table 1 Characterization of new microsatellite loci in hydromedusae from the San Francisco Estuary

Locus	Sp.	GenBank Accession no.	Primer sequence (5′–3′) forward and reverse	Primer 5′ label	*N*	Repeat motif	Annealing temp °C (Taq used)	*A* (size range in bp)	H_O	H_E	HWE_{pv}
MmaG105	MA	GQ281247	F: AACGGATCCACCACTAGCAC R: TTTGTTCATTGTTCGTTTGTGA	PET	26	$(TACA)_{35}$	58 (P)	9 (280–367)	0.92	0.83	0.85
MmaG107-2	MA	GQ281248	F: GGTAGAACTCTGGATACATGCAA R: TCTATGCTAACTGGGATGGAGA	PET	27	$(TCAG)_9…(GTCT)_{21}$	56 (F)	5 (213–234)	0.85	0.74	0.80
MmaG108	MA	GQ281249	F: ACTCCGCTCGTCTGTCAGTT R: GAGTCCGTGGCGAAGTTATG	VIC	30	$(AGTC)_6…(CAGT)_{16}$	60 (P)	5 (171–184)	0.83	0.76	0.08
MmaG137-2	MA	GQ281250	F: CATCAGCAAGACGAAGGTGA R: GCGCCGCATATTATGTAACC	6-FAM	20	$(AGAT)_8$	60 (P)	5 (140–201)	0.60	0.58	1.00
MmaG139	MA	GQ281251	F: AATTGTCCACCCTCAGTTCG R: TACTCTGCCAGACTGCTTGC	VIC	25	$(AGAC)_{14}$	58 (f)	5 (374–398)	0.92	0.80	0.87
MmaG142	MA	GQ281252	F: TGGAACAGGTTGAACGACTG R: AAACTGGTCAGGGATGTTCG	NED	36	$(ATAG)_{13}$	TD[a] (F)	6 (210–230)	0.64	0.66	0.16
MmaG154-1	MA	GQ281253	F: GACGTACGTGCGAAGTACCC R: AGAGACGGACCGACTGAAGA	NED	20	$(TCTG)_{15}…(CTGT)_{18}$	60 (P)	6 (227–308)	0.90	0.80	0.39
MmaG155	MA	GQ281254	F: GATGGCTTCTCGTACATGACC R: TACTGAGCAGGCGTACATGG	PET	41	$(ATCTTT)_4$	TD[a] (F)	4 (303–329)	0.56	0.54	0.90
MmaG157	MA	GQ281255	F: TGAGTCCCGACTTGAAGTGA R: GTTTTCCCAGTCACGACGTT	VIC	30	$(ACA)_{19}$	50 (F)	5 (209–581)	0.60	0.77	0.00*
MmaG177	MA	GQ281256	F: TGTTTGGAAAACCGAAAAGC R: ACAAGCAAGCCATGCACATA	6-FAM	25	$(AGAT)_{13}$	50 (F)	4 (250–262)	0.60	0.61	0.52
MmoG109	MO	GQ281257	F: CAATACATTCGCAAATAAACAAACT R: CCCCGCTGTATTGGTGTTA	PET	24	$(AAC)_4$	50 (P)	3 (270–292)	0.04	0.12	0.02
MmoG115	MO	GQ281258	F: CCATTGTGCTGCAACGTATT R: ATTGTTACTTTTCGTTGTTATGTCCT	NED	25	$(ACA)_{16}…(AAC)_4$	50 (P)	10 (237–263)	0.64	0.60	0.50
MmoG123	MO	GQ281259	F: AGGTGCTTCTTGTGGGATTCT R: ACCAACAGGAAACGAACCAA	PET	22	$(TTG)_{20}$	50 (P)	6 (311–346)	0.73	0.72	0.73
MmoG133	MO	GQ281260	F: TCATCATGACGCCACTTGTT R: TGATGTTGATCGTGGTTGTTTT	6-FAM	25	$(AAAC)_9$	50 (P)	4 (307–329)	0.64	0.53	0.66
MmoG138	MO	GQ281261	F: TTTGCACGAATGTTTGGTGT R: CCTCATGCTTAAGTGGTGCTC	VIC	28	$(AGAAGGAA)_2$	58 (F)	4 (173–183)	0.64	0.69	0.04
MmoG167	MO	GQ281262	F: AGCTTCATTTGGACGCAAAG R: ACATTTTGTCGGCGATTACC	VIC	24	$(AAC)_{23}$	58 (F)	8 (336–367)	0.50	0.87	0.00*
MmoG181	MO	GQ281263	F: CGAAGCTAGCTAATGAAATGACC R: GCTCTTATGGATGGGTTTGC	VIC	29	$(AAC)_{17}$	50 (F)	5 (277–303)	0.76	0.73	0.21

Table 1 continued

Locus	Sp.	GenBank Accession no.	Primer sequence (5′–3′) forward and reverse	Primer 5′ label	*N*	Repeat motif	Annealing temp °C (Taq used)	*A* (size range in bp)	H_O	H_E	HWE_{pv}
MmoG183	MO	GQ281264	F: ACAAAGTTTAGGTGAACTGTGCTC R: ACGCCAGTGTGGTATGTGAT	6-FAM	29	$(TA)_9$	50 (P)	2 (147–149)	0.24	0.22	1.00
MmoG194	MO	GQ281265	F: TTTGCGCACGTATGACAAAT R: TGAGGTATTTTGTTGAAGGTTGG	VIC	24	$(AATG)_8…(TGAA)_5$	50 (P)	6 (376–436)	0.79	0.71	0.94
MmoG196	MO	GQ281266	F: TCTTACAACTTTGCCCACGA R: CATGCCCGCCACAAT	PET	24	$(CAGA)_4$	50 (F)	4 (147–340)	0.04	0.49	0.00*

Shown are locus name, species (Sp.) (MA, *Maeotias marginata*; MO, *Moerisia* sp.), GenBank accession number, forward and reverse primer sequences, primer dye label, repeat motif, PCR annealing temperature and Taq polymerase used (P = Promega, F = Roche FastStart), number alleles observed (*A*) and allele size range, observed and expected heterozygosities (H_O and H_E, respectively), and *P* values for tests for deviation from Hardy–Weinberg Equilibrium (HWE_{pv})

[a] *TD* Touch down PCR conditions: 95°C for 5 min, 15 cycles of 94°C for 30 s, 65°C for 30 s (decreasing by 1°C with each cycle), and 72°C for 1 min, 15 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min, and hold at 10°C

* Significant after Bonferroni correction

Promega Taq, and 0.4–0.7 µl of each 1 µM primer. The recipe for PCRs run with FastStart Taq was 1 µl 10× FastStart Buffer (with $MgCl_2$), 0.8 µl of 10 µM dNTPs, 0.3 µl FastStart Taq, and 0.4–0.7 µl of each 1 µM primer, with the exception of primers MmoG167 which were used at 5 µM. For all reactions, enough water was added to bring the total reaction volume up to 10 µl. All PCRs were performed using a Bio-Rad DNA Engine Dyad thermal cycler using the following cycling parameters: 95°C for 5 min, and 30 cycles of 94°C for 30 s, various annealing temperatures for 30 s (See Table 1), and 72°C for 1 min, followed by 60°C for 45 min, and held at 10°C. Loci were screened by diluting PCR products 1:1 with 98% formamide loading buffer, denaturing at 95°C for 3 min, and separating PCR products on a 5% denaturing polyacrylamide gel at 50 W. We visualized the gel by overlaying with SYBR-Green-agarose following the protocol of Rodzen et al. (1998) and scanning with a GE Healthcare Fluorimager 595.

Ten for each species of the 71 loci screened were polymorphic and well-resolved in the initial screening. These loci were then characterized with additional individuals to bring the total to a minimum of 20 individuals screened (Table 1). This characterization was completed by adding a 5′ fluorescent label (NED, VIC, and PET from Applied Biosystems, 6-FAM from Integrated DNA Technologies) to the forward primer. One ml of labeled PCR product was added to 8.8 µl of highly deionized formamide (Gel Company) and 0.2 µl of LIZ600 size standard (ABI) and run on an ABI 3130xl Genetic Analyzer. We used GENEMAPPER version 4.0(ABI) to visualize and score fragments. None of the loci amplified for both species.

Expected and observed heterozygosities were calculated using GENETIC DATA ANALYSIS (Lewis and Zaykin 2001) and Genepop (Raymond and Rousset 1995) was used to evaluate for Hardy–Weinberg equilibrium (HWE) using Fisher's exact test with 100,000 permutations and removal of missing data. Summary of the microsatellite loci are presented in Table 1. Number of alleles per locus ranged from 5–9 in *M. marginata* and 2–10 in *Moerisia* sp. with average expected heterozygosities of 0.71 and 0.57, respectively. One locus in *M. marginata* and two in *Moerisia* sp. deviated from HWE expectations. This is likely due to the presence of null alleles since observed heterozygosities were lower than expected for all three loci.

The microsatellite loci described here will be used to conduct future genetic studies investigating the invasion biology of these hydrozoans.

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