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MICROSATELLITE LETTERS

Characterization of 22 microsatellite loci for conservation genetic studies of an endemic anemonefish, *Amphiprion latezonatus*

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Island endemic species are of particular conservation concern as they exhibit a range of vulnerable traits that greatly increases their risk of extinction (McKinney 1997). The wideband anemonefish, *Amphiprion latezonatus*, is endemic to a range of 4° of latitude (Rushworth et al. 2011) in the subtropical waters off the Eastern Australian coast, with populations occurring at Lord Howe Island (LHI), Norfolk Island, and scattered locations off the east Australian coast between the Sunshine Coast and South-West Rocks. They are hosted by only two species of anemones, *Entacmaea quadricolor* and *Heteractis crispa* (Scott and Malcolm 2011). Though there have been several ecological and ornamental aquaculture studies on *A. latezonatus* (e.g., Scott and Malcolm 2011; Rushworth et al. 2011), none have examined gene flow, population genetic structure, or genetic diversity of this species. This study describes the development of 22 polymorphic microsatellite markers for *A. latezonatus* using 454 shotgun pyrosequencing on a 454 GS FLX Titanium (Roche) at the Biosciences Core Laboratory (BCL), at the King Abdullah University of Science and Technology (KAUST) in Saudi Arabia.

Genomic DNA was extracted using a Qiagen Gentra Puregene (Qiagen, Doncaster, Australia) extraction protocol including RNAse treatment. DNA was shotgun sequenced on 50 % of a 454 GS FLX Titanium plate in the KAUST BCL. Resulting sequences (totalling 1,636,335 reads, average read length 399.5 bp) were screened for microsatellite loci as per van der Meer et al. (2012). This process identified 45,228 microsatellite loci (within 2.76 % of sequences obtained); PCR primers were successfully designed for 5,318 (0.3 %) of loci found. Of these, directly-labelled forward primers (FAM, NED, or VIC) were synthesised for 24 loci and grouped into 4 multiplex reactions of 6 loci per multiplex (Table 1). Loci were tested for amplification success and specificity; genotypes were generated as per van der Meer et al. (2012). Primer pairs for 22 loci reliably amplified products of the expected size without additional products and were polymorphic, representing seven dimer, six trimer, six tetramer, and three pentamer simple sequence repeat (SSR) loci.

Directly labelled multiplex reactions were used to genotype the 22 loci in 36 *A. latezonatus* individuals from LHI. Number of alleles, observed heterozygosity, expected heterozygosity, conformation to Hardy–Weinberg Equilibrium (HWE), polymorphic information content (PIC), and presence of null alleles were calculated as per van der Meer et al. (2012) (Table 1).

Fifteen of the 22 loci were in HWE after FDR correction (Benjamini and Hochberg 1995) (Table 1). Less than 5 % (10 of 231 locus pairs) displayed significant linkage disequilibrium after FDR correction: Al1/Al16, Al1/Al22,

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Table 1 Details for 22 *Amphiprion latezonatus* microsatellite loci

Locus	Repeat motif	Primer sequence 5′-3′	Size range (bp)	N	N_a	H_O	H_E	pHWE	PIC	Genbank
Al1[1A]	$(CA)_{10}$	F:[FAM]TCCAACTGCTGCATCAAATC R:CCGAATCATTTTACCGCATT	80–86	36	4	0.583	0.560	0.945	0.822	KC193160
Al2[1B]	$(AT)_{10}$	F:[NED]ACCACGAATTGCTCTGAAGC R:CAGGGGTAGGAGGTAGAGGG	88–96[a]	36	3	0.389	0.545	0.231	0.782	KC193161
Al9[1C]	$(AGC)_{10}$	F:[VIC]GACGCTCGCTGATGTATGAA R:CACAGATGTCCCGTGTTTTG	99–117	36	7	0.694	0.642	0.7997	0.798	KC193167
Al21[1D]	$(CTTAT)_{10}$	F:[FAM]CATGGGTGCAGAGTGTTTGT R:GAACATTTCTGCCCTGTGGT	200–260	36	13	0.917	0.867	0.7883	0.868	KC193179
Al11[1E]	$(GAG)_{10}$	F:[NED]TCTCGGTCGAAACCTTTGTT R:ACACCTCCTGTCACCTCACC	230–248	36	6	0.833	0.749	0.7883	0.800	KC193169
Al17[1F]	$(ATCT)_{14}$	F:[VIC]CTGCAGGATCAACGAAGGTT R:TAACTGGAAGAGGTGGTGGG	202–254	36	11	0.972	0.827	0.262	0.873	KC193175
Al3[2A]	$(AC)_{12}$	F:[FAM]TGACCGTACTGTATGGACGC R:CCGTCGGTCTACCTGACAAT	94–98[a]	36	3	0.111	0.226	0.0016	0.622	KC193163
Al4[2B]	$(AC)_{11}$	F:[NED]CTATCAAATGGGAGCGGAAG R:GAGGGAAGAGGGAGAGGAGA	103–111	36	4	0.389	0.398	0.0513	0.573	KC193163
Al10[2C]	$(TGC)_{12}$	F:[VIC]CAACAGCAGTCACATGACGA R:ACAGCTTGGGCTCAGTCAAT	84–111	36	6	0.889	0.806	0.1568	0.837	KC193168
Al22[2D]	$(TTTCT)_{15}$	F:[FAM]TCAGGGAAATCCACAAGAGG R:GCCAACAGAAAATCCTGCAC	185–220	36	8	0.861	0.804	0.7997	0.743	KC193180
Al12[2E]	$(GTG)_{11}$	F:[NED]GGTTGCAGGTAGAGCAGAGG R:ATTCTGGTGCTCACTGCTGA	254–266	36	4	0.778	0.627	0.3486	0.752	KC193170
Al18[2F]	$(ATGG)_{10}$	F:[VIC]GCCTGACTGGAGGTTCTCAG R:AATGTGCCAAAGAGCCTGTC	220–244	36	4	0.639	0.542	0.7883	0.781	KC193176
Al5[3A]	$(AC)_{13}$	F:[FAM]TTAAACAACCTCCCTCGCAC R:GTCACCGAGTCCTGCTTACC	86–106	36	5	0.444	0.528	0.3486	0.666	KC193164
Al15[3C]	$(GACA)_{10}$	F:[VIC]GCCACAGCTAGCATTCCTTC R:TAGAGGAGGAAGAGGAGGGC	78–102[a]	36	6	0.333	0.538	<0.0001	0.784	KC193173
Al19[3D]	$(GATA)_{15}$	F:[FAM]CTCGATTTGCTGTTTCACGA R:TAATAGGAGCAGGAGCAGGC	278–330[a]	36	12	0.444	0.896	<0.0001	0.838	KC193177
Al13[3E]	$(AAT)_{14}$	F:[NED]ATCCCCTGCCACAAATGTAA R:TCTCCACCGGTTTAGAGTCG	297–324	36	8	0.722	0.746	0.7883	0.767	KC193171
Al23[3F]	$(GAATT)_{11}$	F:[VIC]ATGTGACGAAAGCAGGGAAA R:TGCACATTGAGAACATGCAA	220–240	36	4	0.583	0.575	0.7883	0.762	KC193181
Al7[4A]	$(AC)_{15}$	F:[FAM]CGAGTGTAACCGAGCTGTGA R:TGCACTACTTTCTGCCTGGA	96–108[a]	36	5	0.306	0.432	0.231	0.684	KC193165
Al8[4B]	$(CA)_{14}$	F:[NED]TGTCCAATTCTTGTCTCCGA R:CAGGTGAAGCTGTGTTTCCA	106–132	36	11	0.444	0.486	0.9292	0.520	KC193166
Al16[4C]	$(TCTT)_{13}$	F:[VIC]ACAGAGCCAGCTGCTAAAGG R:ATGGATTCCCCTCATCCTTC	98–130	36	6	0.722	0.781	0.0691	0.841	KC193174
Al20[4D]	$(TGGA)_{12}$	F:[FAM]TCGCGATACTTGCTGTGTTC R:TGATTGAACCAATGTGCGAT	291–315	36	6	0.528	0.578	<0.0001	0.698	KC193178
Al14[4E]	$(AGG)_{15}$	F:[NED]ACCGAACACCTTAAACACGC R:AGGGTTCAAGTGTCCCACTG	287–310	36	8	0.778	0.700	<0.0001	0.853	KC193172

N sample size, N_A number of alleles, H_o observed heterozygosity, H_E expected heterozygosity, *pHWE* Hardy–Weinberg equilibrium significance value at $P < 0.05$ after FDR correction, *PIC* polymorphic information content. [a]locus may have null alleles. [1A,B,C,D,E,F] *A. latezonatus* multiplex corresponding to group 1 with six loci (A,B,C,D,E and F)

Al4/Al14, Al5/Al15, Al8/Al16, Al13/Al15, Al13/Al21, Al14/Al22, Al16/Al22, and Al19/Al21. Null alleles were apparent in five loci due to homozygote excess, Al2, Al3, Al7, Al15, and Al19. High allelic richness (mean NA = 6.545 ± 0.627, range 3–13) and high levels of expected heterozygosity ($H_E = 0.630 \pm 0.036$, range 0.226–0.896) were displayed in these *A. latezonatus* loci. The mean PIC was 0.7575 providing a high level of discrimination between individuals, indicating these markers should be useful in population genetic and connectivity studies of *A. latezonatus*.

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