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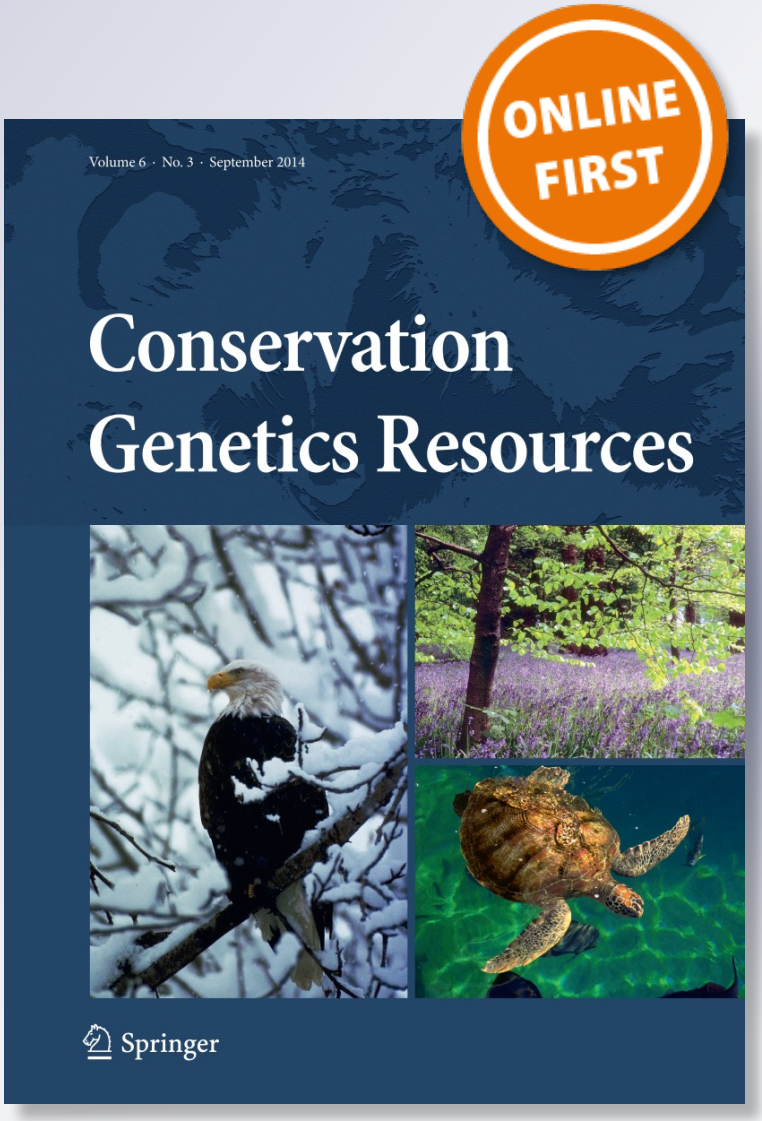

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MICROSATELLITE LETTERS

Isolation, characterization and cross amplification of eleven novel microsatellite loci for the hydrozoan coral *Millepora*

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Abstract Milleporidae are of high ecological and economic importance, as, together with the scleractinian corals, they belong to the main reef builders of tropical coral reefs. Coral reefs face severe threats mainly due to anthropogenic disturbance. Understanding their population structure and dynamics is crucial for any conservation effort. Here we report the first microsatellite loci for the Milleporidae. Eleven polymorphic markers were developed for the hydrozoan corals *Millepora dichotoma* from the Great Barrier Reef (Australia) and tested for amplification in *M. dichotoma* from the Red Sea (Egypt), as well as for *Millepora platyphylla* from the Pacific Ocean (Moorea, French Polynesia). All loci were variable with 4–15 alleles per locus. Nine loci were transferable between geographic regions and species. These are the first microsatellites for hydrozoan corals. They will provide valuable tools for characterizing the population structure and genetic diversity of the group thereby benefitting coral reef conservation.

Keywords Genetic diversity · Microsatellites · Milleporidae · Population genetics · Reef-building cnidarians

Mainly due to anthropogenic disturbances coral reefs are declining at a rapid rate. To understand and potentially counteract this development an increasing number of studies focus on genetic diversity, a key potential to adapt to environmental changes. While reef building hexacorallia (scleractinia) have been the subject of several molecular investigations, the equally important Milleporidae have so far not received much attention. This was mainly due to the lack of suitable genetic markers. Here we report the first polymorphic microsatellites for *Millepora dichotoma* and *Millepora platyphylla*.

Microsatellite sequences were isolated from *M. dichotoma* (Great Barrier Reef, Australia) using the reporter genome protocol (Leese et al. 2008) (Mille01, Mille 02), as well as 454 sequencing on a 454 GS junior platform (Mille03–Mille11). Total genomic DNA was obtained using a DNAzol extraction (Maier et al. 2001) for the reporter genome protocol and with a Qiagen Blood and Tissue Kit for 454 Sequencing. The extracted DNA was checked for presence of zooxanthellae using a chloroplast genotyping protocol (Santos et al. 2004) prior to library preparation. The uncontaminated 454 sequence library contained 199,823 sequences and was searched for microsatellites with the Phobos plugin 3.3.11 (http://www.rub.de/spezzoo/cm/cm_phobos.htm). The most suitable sequences for primer development were extracted using an R script (available on request). All primers were developed

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Table 1 Polymorphic microsatellite loci for *Millepora dichotoma* and *M. platyphylla*

Locus name	Primer sequence 5′–3′	Repeat motive	Annealing temperature (°C)	Site	# Ind.	# Alleles (size range)	Ho	He
Mille_01	Ft: AGGAACAAAGAAATGAACAAAGT	$(ACTCCAGTC)_3$ $(ACCG)_9$ $(ACTG)_3$	51	GB	21	8 (215–259)	**1.000**	**0.776**
	R: TGATCTCTTTGATGATTCTTTCA			RS	10	4 (215–247)	0.500	0.684
				FP	10	2 (211–215)	**1.000**	**0.526**
				Total	41	10 (211–259)		
Mille_02*	Ft: AGTCATCCTTAGATTATGTTTTCT	$(AG)_{39}$	58	GB	21	9 (238–274)	**0.429**	**0.717**
	R: AGGCATTCTGACCACGTGAT			RS	10	n.a.	–	–
				FP	10	n.a.	–	–
				Total	21	9 (238–274)		
Mille_03	Ft: ACGGTGAAATTGGAGAAGCTG	$(TCTA)_{15}$	58	GB	21	7 (191–215)	1.000	0.801
	R: GGATCAGAGATGATGCACCTGT			RS	9	6 (199–223)	0.778	0.797
				FP	9	8 (193–225)	0.889	0.889
				Total	39	15 (191–225)		
Mille_04	Ft: TCTGAGGGACTGCTTCTATTTGTC	$(AAAT)_6$	58	GB	21	3 (175–183)	0.810	0.556
	R: GGTCCTGTCACTGTGCACTT			RS	10	3 (171–179)	0.500	0.589
				FP	10	2 (179–183)	0.300	0.268
				Total	41	4 (171–183)		
Mille_05	F: GTTCTGCGCAGGGTCAAATC	$(ATC)_{10}$	58	GB	21	4 (218–233)	**0.714**	**0.628**
	Rt: TCCAGGAGTCTAGTTGCACA			RS	10	4 (218–227)	0.700	0.784
				FB	10	2 (242–254)	**1.000**	**0.526**
				Total	41	7 (218–254)		
Mille_06	F: AGGATGGTGTTAAGAAGGACGG	$(AAT)_9$	58	GB	21	4 (155–170)	0.714	0.661
	Rt: GCTGCTGCAACTAAATAATCGC			RS	10	2 (161–164)	**1.000**	**0.526**
				FP	10	3 (155–164)	**1.000**	**0.574**
				Total	41	5 (155–170)		
Mille_07	Ft: GCAGTGTGTGGAGAGACGAA	$(GAT)_{11}$	58	GB	21	3 (251–272)	0.381	0.333
	R: CGTCGGTCAACCATACCACA			RS	10	4 (266–275)	1.000	0.695
				FP	10	2 (251–260)	**1.000**	**0.526**
				Total	41	6 (251–275)		
Mille_08*	Ft: TCTGCTGAAACCTCCAGTGG	$(AAT)_9$	60	GB	21	4 (115–133)	0.476	0.524
	R: ATACGAACAGAAACCCTCGGG			RS	10	1 (115)	–	–
				FP	10	2 (115–130)	**1.000**	**0.526**
				Total	41	4 (115–133)		
Mille_09	F: TTGGGAAACAGAAAGCCCT	$(AAC)_8$	58	GB	21	5 (198–216)	0.714	0.791
	Rt: GCTTGGGGTTAGAAAGTTCC:			RS	8	5 (177–216)	0.500	0.667
				FP	10	1 (210)	–	–
				Total	39	7 (177–216)		
Mille_10*	F: ATCCCTGCCGCTATACATG	$(CATA)_{28}$	60	GB	20	8 (231–267)	0.800	0.821
	Rt: AGATGTTGCGTGACTACCA			RS	10	n.a.	–	–
				FP	10	n.a.	–	–
				Total	20	8 (231–267)		
Mille_11	F: ACAGGGAAAGTAGAACAAAGG	$(AAGG)_8$	58	GB	21	5 (157–177)	0.714	0.705
	Rt: TGCAGACCTCACTTCCTTT			RS	10	2 (165–167)	**1.000**	**0.526**
				FP	10	2 (157–161)	0.100	0.100
				Total	41	6 (157–177)		

Given are the locus name (NCBI accession # KM 458768 to KM 458778, Online Resource), primer sequences, repeat motive, annealing temperature, field site (GB—Great Barrier Reef, Australia, RS—Red Sea, Egypt, FP—French Polynesia), the number of individuals genotyped (# Ind.), the number of alleles found (# alleles), and the observed (Ho) and expected heterozygosity (He). Significant deviations from Hardy–Weinberg equilibrium: bold, heterozygote deficiencies: underlined. t = M13-tailed Primer (M13 sequence: CAC GAC GTT GTA AAA CGA) * primer deviates from the standard-PCR protocol: only 35 cycles no DMSO

in Geneious vers. 6.1.6 (Biomatters, New Zealand) using the Primer3 plugin and NetPrimer (PremierBiosoft, USA). A fluorescent-dye labeled M13-tail was added (Schuelke 2000). PCR amplifications were done in a 10 µl volume containing 1 µl (10×) reaction buffer, 2 mM dNTPs, 0,5 pmol tailed primer, 2 pmol untailed and fluorescently-labeled M13 primer, 0.5 µl DMSO (excluded for primers with * see Table 1), 0.5 U Hotmaster Taq-Polymerase (5U/µl,), 1 µl of 1:20–1:40 diluted template DNA and deionized water to volume with the following parameters: 95 °C 5 min, 40 cycles at 94 °C 30 s, 51/58/60 °C (see Table 1) 30 s, 65 °C 30 s, final extension 65 °C 5 min. Locus specificity was ensured by testing all loci with *Symbiodinium* DNA of clades A, B, C, and D (http://www.advancedaquarist.com/2011/4/aafeature). Fragment size analyzes was conducted with a LICOR 4300 automated sequencer and microsatellite bands were scored using the software SAGA^{GT} (Licor Biosciences GmbH, USA). The number of alleles for each locus and site as well as the observed and expected heterozygosity were calculated with ARLEQUIN vers. 3.5.1.2 (Excoffier et al. 2005).

Eleven loci (NCBI accession numbers: KM 458768 to KM 458778, Online Resources) could be successfully and reliably amplified in *M. dichotoma* samples from Australia (N = 21) (Table 1). Nine of them were transferable to *M. dichotoma* samples from the Red Sea (N = 10) as well as *M. platyphylla* (N = 10) from the Pacific Ocean (Table 1). The number of observed alleles ranged from 4 to 15 alleles with an average of 7 alleles per locus. Deviations from Hardy–Weinberg Equilibrium (HWE) were found in several loci however, heterozygote deficiencies were only found once (Mille 2) (Table 1). These first microsatellites characterized for *M. dichotoma* and *M. platyphylla* will provide very useful tools for estimating genotypic diversity, population dynamics and connectivity and will thereby benefit reef conservation efforts.

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