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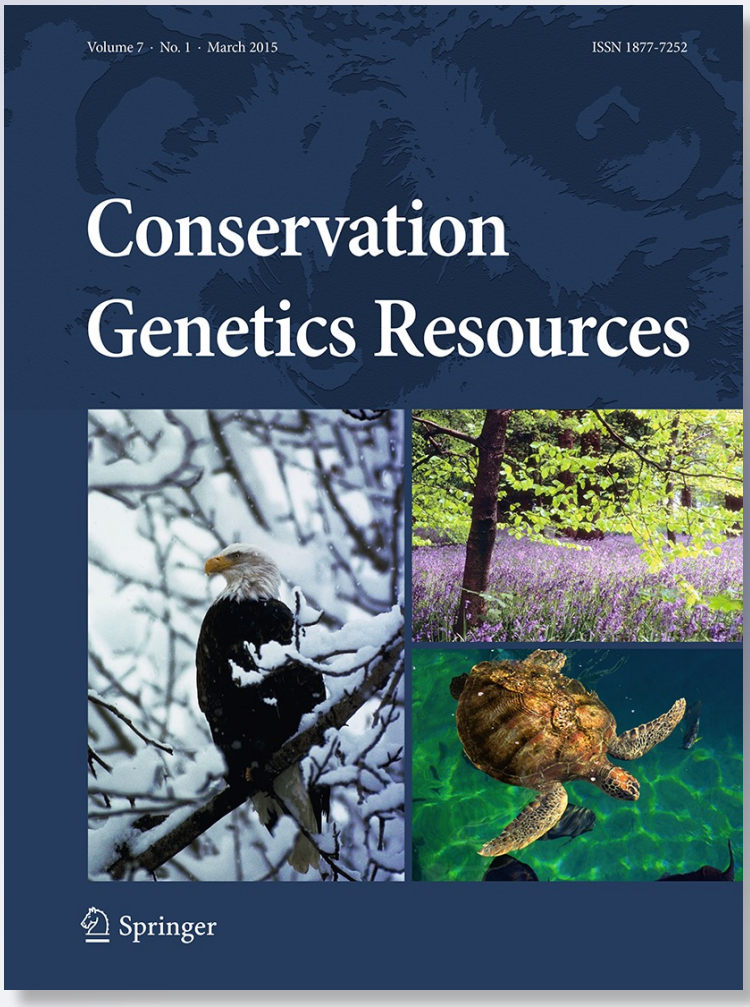

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MICROSATELLITE LETTERS

An evaluation of primers for microsatellite markers in Scarlet Macaw (*Ara macao*) and their performance in a Peruvian wild population

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Abstract Primer pairs were designed for 41 di-nucleotide microsatellite loci identified from across the full genome of the Scarlet Macaw (*Ara macao*). We present the best 30 polymorphic loci with 5–22 alleles, 3–14 effective alleles and expected heterozygosities of 0.669–0.930. These markers will facilitate population genetic and conservation genetic studies on macaws.

Keywords Parrot · Macaw · Population genetics · Conservation genetics · Microsatellite

Nearly 30 % of the 398 parrot species (*Psittaciformes*) are classified as threatened (critically endangered, endangered, or vulnerable) according to IUCN RedList 2014. The majority of the parrots are secondary cavity nesters hence mostly affected by habitat loss and other human disturbances. They are very popular birds in aviculture and also threatened by the illegal pet-trade.

Macaws are large and colorful neotropic parrots that remain poorly understood in the wild. At least three species of macaws have already gone extinct in the wild with 16 species remaining (1 Critically Endangered, 3 Endangered, 3 Vulnerable, 1 Near Threatened, and 8 Least Concern). In this study we used the Scarlet Macaw (*Ara macao*), which is considered of Least Concern, as a model for its close endangered relatives. We report here the development of 30 microsatellite markers for genetic tagging and population genetics studies. We tested the markers on a wild population from the southeastern Peruvian Amazon. Blood samples were collected from macaw nestlings and captured and released adults from natural and artificial nests (Olah et al. 2014), with the blood stored in 70 % Ethanol and on FTA cards and extracted using general salting out protocol and DNAzol.

Potential target loci were identified from the first de novo genome assembly for the Scarlet Macaw (Seabury et al. 2013—version SMACv1.1) using PHOBOS (v3.3.12) (http://www.rub.de/spezzoo/cm/cm_phobos.htm) to detect genome-wide microsatellite loci (STR). Initially, 41 autosomal di-nucleotide candidates with at least 20 repeat units were selected. We used the program iQDD (v3.1) (Meglécz et al. 2014) to design primers for these loci with PCR product lengths of 90–300 base pairs.

M13 PCR tags were attached to the forward primers (5′–3′: TGT AAA ACG ACG GCC AGT) and we amplified all loci individually. PCR products were multiplexed using different fluorescent tags (Electronic Supplementary Material) and genotyped on an ABI 3130XL sequencer (Applied Biosystem) with the size standard GS500 (-250) LIZ. We used a negative and a positive control for each genotyping run. We dropped three loci that failed to amplify as a single locus.

Electronic supplementary material The online version of this article (doi:10.1007/s12686-014-0317-2) contains supplementary material, which is available to authorized users.

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Table 1 Population statistics for microsatellite markers in Scarlet Macaw (*Ara macao*) and their cross transferability to Red-and-green Macaw (*Ara chloropterus*)

Locus name	Scarlet Macaw (*Ara macao*)								Red-and-green Macaw (*Ara chloropterus*)						
	N	Size range (bp)	No. alleles	No. effective alleles	H_O	H_E	*P*	Freq. null alleles (Oosterhout)	N	Size range (bp)	No. alleles	No. effective alleles	H_O	H_E	*P*
SCMA01	40	161–197	16	8.84	0.70	0.89	0.001*	0.106[a]	9	169–193	9	5.79	1.00	0.83	0.469
SCMA02	40	268–300	14	10.46	0.95	0.90	0.725	−0.025	9	282–292	6	4.63	1.00	0.78	0.622
SCMA04	39	251–277	14	6.84	0.82	0.85	0.079	0.024	9	267–281	6	4.38	0.78	0.77	0.688
SCMA05	40	205–235	12	7.64	0.88	0.87	0.767	−0.008	9	211–219	5	3.06	0.78	0.67	0.154
SCMA06	40	265–305	17	6.91	0.85	0.86	0.902	0.006	9	279–301	6	4.05	0.78	0.75	0.855
SCMA07	40	276–302	9	5.19	0.83	0.81	0.234	−0.010	9	298–306	5	3.77	0.56	0.73	0.652
SCMA08	40	287–307	12	8.56	0.63	0.88	0.007*	0.148[a]	9	287–301	6	3.77	0.89	0.73	0.578
SCMA09	40	112–136	11	5.95	0.85	0.83	0.793	−0.008	9	116–128	5	3.45	0.78	0.71	0.414
SCMA10	40	176–230	15	10.39	0.28	0.90	0.000*	0.342[a]	9	186–186	Monomorphic				
SCMA11	40	265–307	12	7.98	0.68	0.87	0.001*	0.114[a]	9	281–297	9	7.36	0.89	0.86	0.540
SCMA12	40	280–308	13	7.57	0.85	0.87	0.766	0.012	9	278–300	9	5.59	1.00	0.82	0.861
SCMA13	40	268–296	12	5.29	0.88	0.81	0.956	−0.040	9	258–286	7	2.89	0.89	0.65	1.000
SCMA14	40	222–250	11	7.73	0.93	0.87	0.441	−0.033	9	220–236	4	2.05	0.67	0.51	0.895
SCMA15	40	275–309	16	7.14	0.80	0.86	0.061	0.033	9	277–297	4	2.35	0.67	0.57	0.815
SCMA16	40	223–254	13	8.38	0.95	0.88	0.991	−0.040	9	234–246	7	5.40	0.78	0.81	0.076
SCMA17	40	261–289	8	3.54	0.75	0.72	0.624	−0.014	9	265–287	6	3.86	0.89	0.74	0.862
SCMA19	40	270–300	15	8.84	0.95	0.89	0.987	−0.036	9	276–302	9	5.59	0.78	0.82	0.588
SCMA20	40	107–143	11	7.08	0.35	0.86	0.000*	0.291[a]	9	129–143	6	3.52	0.44	0.72	0.037
SCMA21	40	176–200	11	4.98	0.78	0.80	0.992	0.017	9	178–196	6	4.38	0.89	0.77	0.221
SCMA22	40	114–160	18	12.36	1.00	0.92	0.842	−0.045	9	122–146	7	5.06	0.89	0.80	0.870
SCMA25	40	265–293	8	3.02	0.63	0.67	0.660	0.017	9	270–272	2	1.98	0.00	0.49	0.003*
SCMA26	40	210–240	13	6.97	0.88	0.86	0.446	−0.008	9	224–236	7	4.76	0.89	0.79	0.711
SCMA27	40	211–245	14	10.09	0.95	0.90	0.933	−0.028	9	211–241	11	8.10	1.00	0.88	0.886
SCMA28	40	280–330	22	14.35	0.95	0.93	0.871	−0.012	9	292–318	7	3.86	0.67	0.74	0.513
SCMA29	40	238–256	8	5.04	0.73	0.80	0.246	0.047	9	241–255	7	5.06	1.00	0.80	0.784
SCMA30	40	214–246	15	7.51	0.95	0.87	0.935	−0.049	9	212–242	8	4.38	0.89	0.77	0.932
SCMA31	40	141–159	9	7.39	0.90	0.86	0.911	−0.021	9	135–165	9	5.40	0.78	0.81	0.299
SCMA32	38	181–211	12	7.72	0.84	0.87	0.381	0.017	9	175–185	4	2.79	0.89	0.64	0.282
SCMA33	40	174–206	15	8.82	0.85	0.89	0.044	0.018	9	166–200	5	2.75	0.67	0.64	0.740
SCMA34	40	159–183	12	6.99	0.88	0.86	0.489	−0.013	9	159–177	6	4.63	0.67	0.78	0.470
SCMA35	40	286–308	10	7.60	0.88	0.87	0.762	−0.005	9	274–284	2	1.91	0.56	0.48	0.613
SCMA37	40	208–220	7	4.71	0.83	0.79	0.553	−0.022	9	216–224	5	3.95	0.56	0.75	0.238
SCMA38	40	215–249	13	7.75	0.45	0.87	0.000*	0.237[a]	9	227–229	2	1.12	0.11	0.10	0.860

Table 1 continued

Locus name	Scarlet Macaw (*Ara macao*)							Red-and-green Macaw (*Ara chloropterus*)					
	N	Size range (bp)	No. alleles	No. effective alleles	H_O	H_E	*P*	Freq. null alleles (Oosterhout)	N	Size range (bp)	No. alleles	No. effective alleles	H_O	H_E	*P*
SCMA40	40	292–300	5	3.38	0.68	0.70	0.623	0.026	9	288–300	5	4.63	1.00	0.78	0.175
SCMA41	40	292–318	13	6.90	0.85	0.86	0.994	0.006	9	284–308	8	5.40	0.56	0.81	0.295
SCMA43	38	89–127	13	7.10	0.32	0.86	0.000*	0.309[a]	8	97–125	4	2.51	0.25	0.60	0.019
SCMA44	40	280–308	13	7.57	0.85	0.87	0.766	0.012	9	278–300	9	5.59	1.00	0.82	0.861
SCMA46	39	152–188	14	8.01	0.67	0.88	0.002*	0.118[a]	9	160–180	8	5.79	0.89	0.83	0.433

Number of samples (N), Observed (H_O) and Expected (H_E) Heterozygosity, probability value from Hardy–Weinberg equilibrium test (P) calculated by GenAlEx 6.5 (Peakall and Smouse 2012)

All loci were scored using Geneious R6

* Significant Hardy–Weinberg disequilibrium ($P < 0.01$)

[a] Presence of a null allele calculated by MicroChecker (v2.2.3) (Van Oosterhout et al. 2004)

To test the suitability of the remaining markers we genotyped a total of 86 samples that included 7 family groups. For population genetic analysis we analyzed a subset of 40 unrelated individuals. We only included one sample per nest (excluding siblings) and if a sample was available from parents we excluded offspring of that pair. Consideration of the family groups confirmed mendelian inheritance, but indicated null alleles at eight loci. A MicroChecker (v2.2.3) (Van Oosterhout et al. 2004) analysis on the 40 sample set indicated departures from the Hardy–Weinberg equilibrium (HWE) and null alleles at those same eight loci. However, all of the other 30 loci fit expectation under HWE assumptions (Table 1). These 30 loci exhibited 5–22 alleles, 3–14 effective alleles and expected heterozygosities of 0.669–0.930 (Table 1). Checks for linkage disequilibrium in GenePop (v4.2) (http://genepop.curtin.edu.au) revealed that <5 % of all combinations showed potential departures from equilibrium.

We tested the cross species transferability of our new primers on a closely related species, the Red-and-green Macaw (*Ara chloropterus*), where all but one marker resulted to be polymorphic (Table 1). We also tested the primers on a distant relative, the Swift Parrot (*Lathamus discolor*, N = 12), but only seven loci were found polymorphic (2–11 alleles, 2–6 effective alleles and expected heterozygosities of 0.278–0.840).

Our panel of 30 microsatellite markers will be highly suitable for conservation genetics studies of Scarlet Macaw, other macaws, and probably other neotropic parrot species. Furthermore, ready expansion to 38 loci is possible by re-designing primers for the additional eight loci that exhibited null alleles.

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