Fifty Seychelles warbler (Acrocephalus sechellensis) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds

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Table 1 Characteristics of five microsatellite loci in samples of black bream *(Acanthopagrus butcheri)* from nine water bodies in Western Australia and from the Gippsland Lakes in Victoria, south-eastern Australia. The Western Australian samples are the same as those used by Chaplin *et al*. (1998)

Locus	GenBank accession no.	Primer sequence (5′–3′)	Repeat unit*	T_a (°C)	Size range (bp)	No. of alleles	n	H_E	H_O
pAb1H1	AF284351	F: GGCTTTCATTTCCCCATTTGTG R: CACCTTTCTCCACGCCATAAA	$(TG)_{15}$	63	132–148	5	268	0.37	0.44
pAb2B7	AF284352	F: GGTGCGTGCATTGTTAATGTGT R: GATCTGCTTTCCTTTGACTCAGC	$(TG)_{24}$	65	98–128	14	274	0.70	0.72
pAb4D5	AF284353	F: ACCTCTTCATCTGCGTGACATCT R: GACAACACCCTCACTCAGCTGA	$(TG)_{60}$	54	199	1	50	0	0
pAb2A5	AF284354	F: AGTTACTTTCTCCAGAGTGGCGC R: GGCAACAGATAAGCACTGAGCATA	$(TG)_{19}$	63	105–119	7	273	0.56	0.62
pAb2D11	AF284355	F: CGGTCCAGTTTCACTCTGATGTT R: AACTGCTGTCATCGCCCTGTT	$(TG)_{15}$	65	106–110	4†	50	0.11	0.08

*determined from the sequenced insert; †polymorphic only within samples from the Gippsland Lakes. n, is the total number of individuals assayed per locus; T_a, is the optimal annealing temperature of each primer pair; H_E, is the expected heterozygosity, calculated as $1 - \Sigma(f_i^2)$, where f_i is the frequency of the ith allele; and H_O, is the observed heterozygosity.

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Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds

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The cooperatively breeding Seychelles warbler, *Acrocephalus sechellensis*, is a rare endemic of the Seychelles islands. By 1959, anthropogenic disturbance had pushed this species to the verge of extinction and only 26 individuals remained, confined to the island of Cousin. The population has since recovered and has been the focus of intense study since 1985 (e.g. Komdeur 1992; Komdeur *et al*. 1997).

We required a set of microsatellite markers to enable studies of mate choice, reproductive success and fitness. Genetic variability is relatively low within this species, possibly due to the recent population bottleneck. Consequently, many microsatellites had to be isolated and screened to provide sufficient polymorphic loci to enable parentage assignment and pedigree construction. We isolated 63 microsatellite loci from the Seychelles warbler and tested for their polymorphism in this and five other species of *Sylviidae*. We also examined the utility of a subset of these loci in 16 other passerine birds.

DNA was extracted following Bruford *et al*. (1998). A genomic library enriched for $(CA)_n$, $(GA)_n$ and $(TTTC)_n$ was prepared as described by Armour *et al*. (1994) using modifications suggested by Gibbs *et al*. (1997). DNA reactions were performed in a 10-μL volume containing 10–50 ng DNA, 1.0 μM of each primer, 0.2 mM of each dNTP, 0.05 units *Taq* DNA polymerase (Thermoprime Plus, Advanced Biotechnologies) and 1.0–2.0 mM $MgCl_2$ (Table 1) in 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween. Polymerase chain reaction (PCR) amplification was performed in a Hybaid Touchdown thermal cycler. Initially, a touchdown cycle was performed with a reaction profile of 95 °C for 3 min, then 94 °C for 30 s,

Table 1 Characterization of 50* polymorphic microsatellite loci from the Seychelles warbler (*Acrocephalus sechellensis*), *and* their polymorphism in five other members of the *Sylviidae* family

Locus	EMBL accession number	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	$MgCl_2$ conc. (mM)	Product size† (bp)	Number of alleles/ number of individuals			Number of alleles/number of individuals tested in				
							SW	H_O	H_E	CRW	AW	GRW	EMW	WW
*Ase*2	AJ287385	$[(GAAA)_2GCAA]_3$	F: TTGACAGAGTGTTATTCAATGTG R: GAGCAGATAATAGACCTTGCT	60	1.5	97	2/7	0.71	0.50	1/6	0	1/3	3/2	1/2
*Ase*3	AJ287386	$(CA)_{14}CCA$	F: ACAGGTATGGCGCTCAAGTC R: CTGAATCTTACACAGGAGACCGT	60	1.5	101	3/7	0.86	0.60	1/6	4/4	1/3	1/2	1/2
*Ase*4	AJ287387	$(CA)_{11}$	F: TCTCCATCATCACCACAAAGC R: TTCCCATTGCCCTAGTTATTCCA	60	1.0	103	2/25	0.40	0.37	0	3/4	1/3	0	0
*Ase*5	AJ287388	$AAA(CA)_{12}AAA$	F: TGAAACAAAATGGGATGGTCC R: CCTTTCTCGGAACTGATTGCTT	61	1.0	110	1/7	0.00	0.00	1/6	1/4	1/3	1/2	2/2
*Ase*6	AJ287389	$(CA)_3G(CA)_{17}$	F: TAAAAGCCAGCAGTGGAGCC R: CGAGCTTGCAGGGTTTCCT	60	1.5	119	4/25	0.76	0.70	1/6	2/4	1/3	0	0
*Ase*7	AJ287390	$(CT)_{13}$	F: AATCAACTTCAAATGCTCACAG R: ACTACATGACTCCAGGCTCAG	60	1.5	123	2/7	0.83	0.53	2/6	1/4	2/3	1/2	0
*Ase*8	AJ287391	$(GT)_4TTT(GT)_7$	F: TACCTCTCCTTGGCTGAGCA R: CCAGCCCTAGCTGTTTCACC	TD	1.5	125	1/7	0.00	0.00	3/6	2/4	1/3	2/2	1/2
*Ase*9	AJ287392	$(CA)_{15}$	F: GACTGAAGTCCTTTCTGGCTTC R: CACCAGGAATACAAGTCCATTG	60	1.5	125	3/25	0.40	0.44	3/6	5/4	5/3	2/2	1/2
*Ase*10	AJ287393	$(CCTTCCCT)_7$	F: CATTGGGGTACTATGGAAAGACC R: TCCTGAGTGGAAGGAACATAGG	TD	1.5	127	3/25	0.64	0.56	9/5	0	1/3	1/2	0
*Ase*11	AJ287394	$(AC)_{14}$	F: TCCCCAAATCTCTCAATTCC R: AGTTCTAAGCCTGCCTGTGC	60	1.5	128	2/5	0.40	0.53	7/6	4/4	5/3	3/2	0
*Ase*12	AJ287395	$(CA)_{11}$	F: TCAAGGAAACACAACTACAGCC R: TTTCCTCACAGCCTTGACTG	60	1.5	128	1/7	0.00	0.00	4/6	4/4	1/3	3/2	2/2
*Ase*13	AJ287396	$(GT)_{11}$	F: TGTGCTCCTCTGCTTTCC R: CAGATGGCCAGTGTTAGTCC	62	1.5	132	3/25	0.52	0.54	5/5	7/4	1/3	2/2	1/2
*Ase*16	AJ276374	$(TCTCC)_{13}$	F: TCAGTTCCTGAGTAAATGTCTC R: TGAATTACCCCTAAATACCTG	58	1.5	155	4/7	100.0	0.70	5/6	0	6/3	1/2	0
*Ase*18	AJ276375	$(GT)_{12}$	F: ATCCAGTCTTCGCAAAAGCC R: TGCCCCAGAGGGAAGAAG	60	1.5	176	3/25	0.56	0.50	1/6	5/4	3/3	1/2	3/2
*Ase*19	AJ276376	$(CA)_4GA(CA)_5$	F: TAGGGTCCCAGGGAGGAAG R: TCTGCCCATTAGGGAAAAGTC	60	2.0	177	4/8	0.88	0.64	3/6	3/4	1/3	4/2	3/2
*Ase*20	AJ276377	$(CTTC/CTTT)_{10}$	F: TCTAAAGCTGCCTGCCAGAA R: GCGGTTGCAGTGGACTTG	TD	1.5	178	1/7	0.00	0.00	1/6	7/4	1/3	1/2	0
*Ase*21	AJ276378	$(CTTTT)_2CTC(TTTC)_8$	F: TTAGAACCATTTGATAGTTGCCAC R: ATGGGTTTCTTGGGGAAGAG	58	2.0	180	1/7	0.00	0.00	9/6	5/4	1/3	1/2	2/2
*Ase*22	AJ276379	$(GT)_{13}$	F: TGAACCATTGTCACCAACAC R: GCTTTAGTTCAGATGCCCAG	58	1.5	181	2/6	0.50	0.53	1/6	0	0	1/2	0
*Ase*25	AJ276382	$(GAAA)_{31}$	F: GATGGCTATATGCTTCAAATGC R: TTGAAAGCCTTAAAGTGGGA	58	1.5	187	5/25	0.76	0.74	1/6	6/4	0	2/2	0

Table 1 *Continued*

Locus	EMBL accession number	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	$MgCl_2$ conc. (mM)	Product size† (bp)	Number of alleles/ number of individuals			Number of alleles/number of individuals tested in				
							SW	H_O	H_E	CRW	AW	GRW	EMW	WW
*Ase*26	AJ276383	$(CTC)_3(TC)_{12}$	F: GCTGGCCTTGCAAAAACTTC R: AACACCTCCCTGTCCCTGC	60	1.5	203	1/7	0.00	0.00	1/6	5/4	1/3	2/2	2/2
*Ase*27	AJ276384	$(TTTC)_{16}$	F: TTAACATTGCATGCTCCTGC R: AGTCAAGGTACAGGCTAGATAGCC	60	1.0	204	4/25	0.64	0.60	1/6	1/4	3/3	2/2	1/2
*Ase*29	AJ276386	$(AC)_7TTTG(AC)_6$	F: GATCAGTTTGGAGACGTTTTCT R: ACAGAGCCATAAGGAATGTGC	62	1.5	207	2/7	0.14	0.14	1/6	1/4	1/3	1/2	2/2
*Ase*32	AJ276635	$(GT)_{13}(TCAC)_2(GT)_9$	F: AATGAGCAATACCATGACAGC R: GATCTTTCAGTCAGGAACAAGC	58	1.5	218	1/7	0.00	0.00	1/6	5/4	0	0	0
*Ase*33	AJ289865	$(AT)_{10}$	F: CTTTGGAATGCCAGGCTGCT R: TGCTGGAACCACAGGACTT	TD	1.5	220	1/7	0.00	0.00	1/6	4/4	2/3	1/2	1/2
*Ase*34	AJ276636	$(CT)_{11}$	F: GTTAATTCTTTTGGCCCTCAGC R: GGAGACACCACACCAATGC	60	1.5	220	1/7	0.00	0.00	3/5	1/4	3/3	4/2	3/2
*Ase*35	AJ276637	$(GT)_{10}$	F: GTCCTTGGTCCTTAGCATCTGT R: GCTCCTGTTGTTCTGGGAATAG	58	1.5	224	3/25	0.44	0.62	1/6	1/4	0	2/2	0
*Ase*36	AJ276638	$(TGTGG)_7$	F: AAGTTCCATGGGGTGAATGC R: GAGCGTGTTCCTCCAATTCC	60	1.5	225	2/5	0.20	0.20	1/6	1/4	1/3	1/2	0
*Ase*37	AJ276639	$(AC)_9$	F: TAATTCATGGAGAAGCCCAG R: TCAAAACAACAGTTTTCACAGC	58	1.5	226	3/25	0.32	0.37	2/6	1/4	0	4/2	0
*Ase*38	AJ276640	$(CA)_{15}$	F: ATCCGAGAACCCAATCACTT R: GCAGCATTACAGTCTCAAAGAAC	58	2.0	226	2/4	0.50	0.43	0	3/4	1/3	3/2	1/2
*Ase*40	AJ276642	$(GT)_{10}$	F: CACTGCTCCAGGCACTCTG R: TCCAAGGCACACAAAGGTG	58	1.5	230	1/7	0.00	0.00	3/6	3/4	1/3	1/2	1/2
*Ase*42	AJ276644	$(GT)_4(AT)_6(GT)_8(AT)_2$	F: CATGGGTAGGTTGGGATGTC R: AGGTGAGGGTATGCAAACATG	62	1.5	243	2/25	0.32	0.27	1/6	1/4	4/3	1/2	2/2
*Ase*43	AJ276645	$(TA)_3(CA)_8(TA)_5$	F: ATTGTGTGGGATTTGCAT R: TTGCTGTGCAGTTTGCTTTT	TD	1.5	250	1/7	0.00	0.00	2/6	3/4	1/3	1/2	2/2
*Ase*44	AJ276646	$(GT)_{18}$	F: TTCCCGTAATTATGACCTCTCTTG R: ACCAGAACTTGTTGTCTGGGAG	TD	1.5	250	1/7	0.00	0.00	1/6	4/4	3/3	1/2	2/2
*Ase*46	AJ276775	$(TG)_{13}$	F: CTGGCTGTATCTTGGTGTGC R: CAGTGTTTTAGGTCTCCTGCTG	62	1.5	265	3/25	0.24	0.48	1/6	2/4	1/3	1/2	1/2
*Ase*47	AJ276776	$(CA)_{10} \ldots (CA)_4$	F: GATCACATTTGGCATTTACTGAT R: ACTCTTTAGGGCAAGGCACT	TD	1.5	267	1/7	0.00	0.00	4/6	0	1/3	1/2	2/2
*Ase*48	AJ276777	$(CCTTCT)_6$	F: TTTATTTCCTGGACTGGAACAATC R: GAACATTGGGCTACTGGGC	58	1.0	270	4/25	0.56	0.53	7/5	7/4	5/3	3/2	0
*Ase*49	AJ276778	$(AC)_{10}$	F: CCCCTGAAGTGTCCAACG R: ACTTTCCCAGCACATCTTGC	58	1.5	272	2/7	0.00	0.26	1/6	2/4	1/3	1/2	2/2
*Ase*50	AJ276779	$(CA)_{12}$	F: CTGTGGAATGCTGTCTGGC R: ATGGACTCCCGTCTAACTTGC	60	1.5	272	1/7	0.00	0.00	1/6	6/3	2/3	2/2	2/2

Table 1 *Continued*

Locus	EMBL accession number	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	$MgCl_2$ conc. (mM)	Product size† (bp)	Number of alleles/ number of individuals			Number of alleles/number of individuals tested in				
							SW	H_O	H_E	CRW	AW	GRW	EMW	WW
*Ase*51	AJ276780	$(CA)_{12}$	F: AATTCCCCTAGACAGGCAGC R: TCACTGGAGAGCCAAATTCC	60	1.5	277	1/7	0.00	0.00	1/6	7/4	2/3	2/2	1/2
*Ase*52	AJ276781	$(CA)_9(CA)_5$	F: TCTTAGCCTGCACTCATTTCA R: CAGTCACCGTAAGTTCATAGGC	60	1.5	278	1/7	0.00	0.00	1/6	2/4	1/3	1/2	1/2
*Ase*53	AJ276782	$(CTT)_{22}$ $(CTCCTT)_{10}$	F: ATGGAGAATTCTGGGTGCTG R: CCCAATAATGAGGTAACACCAA	60	1.5	285	2/7	0.43	0.54	1/6	8/4	0	0	0
*Ase*55	AJ276784	$(GT)_9$	F: GTGTGGACTCTGGTGGCTC R: TCCCAAAGCACTCAAACTAGG	62	1.5	292	1/7	0.00	0.00	1/6	6/4	2/3	2/2	2/2
*Ase*56	AJ276785	$(GT)_{18}$	F: TTCACTGAGAAGTGAGAATGTG R: GTCCTTGATTGATTACAGGCT	60	1.5	298	3/25	0.44	0.40	5/6	5/4	2/3	3/2	0
*Ase*57	AJ276786	$(AC)_{14}$	F: GCAAGTGCAGATGTTTCCCT R: CCAAAGCAGGACAATGCTG	TD	1.5	299	1/7	0.00	0.00	6/6	3/4	4/3	1/2	0
*Ase*58	AJ276787	$(CTTTTT)_{27}$	F: ATTCCAGGGATTGGGCAG R: CTCAAAGCGAAATTGAGCAGT	60	1.0	311	5/25	0.76	0.76	1/6	7/4	5/3	4/2	1/2
*Ase*60	AJ276789	$(GT)_9GG(GT)_8$	F: CATGAAAAGGAACTCTCCAGC R: TTCCATCTCTGTTCTACTGCG	62	1.5	353	1/7	0.00	0.00	0	5/4	4/3	1/2	3/2
*Ase*61	AJ276790	$(GAAAAA)_{13}$	F: AGGATTTTTAATGGGATATACACATCTG R: AGCCACATTTTAGCCCACAG	54	2.0	369	2/5	0.40	0.36	0	0	3/3	0	0
*Ase*62	AJ276791	$(CT)_2(GT)_8$	F: TCGCCAGGTCGTGTGTAGTC R: CAAAACCGTGTCGGGGAG	58	1.5	372	1/7	0.00	0.00	1/6	1/4	1/3	2/2	0
*Ase*63	AJ276792	$(GAGAAA)_8(GA)_7$	F: TTTGGGGTTTAGGAATAGCAGA R: GGCTTCAGCCTGAGAAAGTC	60	1.0	400	2/7	0.29	0.26	2/6	8/4	2/3	4/2	1/2
*Ase*64	AJ276793	$(AGGG)_9$ $(ATGG)_{12}$	F: CCACCTTTCATACTGGGGAG R: TTCAGCCAGTCAGTGTAGCC	TD	1.5	412	2/8	0.50	0.40	7/6	1/4	3/3	1/2	1/2

*An additional 13 loci were monomorphic in all species tested (EMBL accession numbers: AJ287384, AJ287397, AJ287398 AJ276380, AJ276381, AJ276385, AJ276387, AJ276634, AJ276641, AJ276643, AJ276647, AJ276783, AJ276788).

†Size in cloned allele.

SW, Seychelles warbler, *Acrocephalus sechellensis*; CRW, clamorous reed warbler, *Acrocephalus stentoreus australis* (M. Berg, personal communication); AW, aquatic warbler, *Acrocephalus paludicola* (P. Hedrich, personal communication); GRW, great reed warbler, *Acrocephalus arundinaceus* (B. Hansson, personal communication); EMW, European marsh warbler, *Acrocephalus palustris* (B. Hansson, personal communication); WW, willow warbler, *Phylloscopus trochilus* (B. Hansson, personal communication).

T_a, annealing temperature; TD, Touchdown cycle; H_O, observed heterozygosity; H_E, expected heterozygosity; 0, no product detected.

Table 2 Cross-species utility of 15 Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci in 21 other passerine birds

		Number of alleles/Number of individuals tested (n = 4 unless stated)														
Family*	Species	*Ase*8	*Ase*9	*Ase*13	*Ase*18	*Ase*19	*Ase*29	*Ase*34	*Ase*37	*Ase*40	*Ase*42	*Ase*43	*Ase*46	*Ase*48	Ase55	*Ase*56
Maluridae	Superb fairy-wren, *Malurus cyaneus*	0	1	1	0	0	1	1	0	1	0	1	1	0	0	0
Pomatostomidae	White-browed babbler, *Pomatostomus superciliosus*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Laniidae	Loggerhead shrike, *Lanius ludovicianus*	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0
Corvidae	Azure-winged magpie, *Cyanopica cyana*	0	1	1	1	0	0	1	0	0	1	1	1	0	1	1
Cinclidae	White-throated dipper, *Cinclus cinclus*	0	1	1	1	0	1	1	0	1	1	1	1	0	1	0
Sturnidae	European starling, *Sturnus vulgaris*	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Certhiidae	Winter wren, *Troglodytes troglodytes*	7/6	2/6	2/6	5/6	3/6	5/6	2/6	3/6	2/5	—	3/6	3/6	—	5/6	6/6
Paridae	Blue tit, *Parus caeruleus*	0	1	1	1	0	1	1	0	0	1	1	0	0	1	0
Paridae	Long-tailed tit, *Aegithalos caudatus*	—	—	—	1	—	1	1	16/680	1	1	0	1	0	—	—
Hirundinidae	Sand martin, *Riparia riparia*	0	1	1	0	1	1	1	0	1	1	1	1	1	1	0
Pycnonotidae	White-spectacled bulbul, *Pycnonotus xanthopygos*	0	1	1	1	1	1	1	0	1	0	1	1	0	1	0
Zosteropidae	Seychelles grey white-eye, *Zosterops modestus*	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0
Sylviidae	Aquatic warbler, *Acrocephalus paludicola*	2/4	5/4	7/4	5/4	3/4	1/4	1/4	1/4	3/4	1/4	3/4	2/4	7/4	6/4	5/4
Sylviidae	Sedge warbler, *Acrocephalus schoenobaenus*	—	1/8	—	16/40	—	—	—	—	—	5/8	1/8	—	—	—	—
Sylviidae	European marsh warbler, *Acrocephalus palustris*	2/2	2/2	2/2	1/2	4/2	1/2	4/2	4/2	1/2	1/2	1/2	1/2	3/2	2/2	3/2
Sylviidae	Great reed warbler, *Acrocephalus arundinaceus*	1/4	5/4	1/4	3/4	1/4	1/4	3/4	0	1/4	4/4	1/4	1/4	5/4	2/4	2/4
Sylviidae	Clamourous reed warbler, *Acrocephalus stentoreus australis*	3/6	3/6	5/5	1/6	3/6	1/6	3/5	2/6	3/6	1/6	2/6	1/6	7/5	1/6	5/6
Sylviidae	**Seychelles warbler, *Acrocephalus sechellensis***	**1/7**	**3/25**	**3/25**	**4/25**	**4/8**	**2/7**	**1/7**	**3/25**	**1/7**	**2/25**	**1/7**	**3/25**	**4/25**	**1/7**	**3/25**
Sylviidae	Willow warbler, *Phylloscopus trochilus*	1/2	1/2	1/2	3/2	3/2	2/2	3/2	0	1/2	2/2	2/2	1/2	0	2/2	0
Nectariniidae	Seychelles sunbird, *Nectarinia dussumieri*	0	1	1	1	0	1	1	0	1	1	1	0	0	1	1
Passeridae	Seychelles fody, *Fodia sechallarum*	0	1	1	1	0	1	1	0	1	1	1	0	1	1	0
Fringillidae	European greenfinch, *Carduelis chloris*	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1
	Number of species tested for amplification	20	21	20	21	20	21	21	21	21	21	22	21	20	20	20
	% of species in which a product was amplified	50	95	100	90	55	86	100	38	86	90	95	80	52	90	50
	Number of species tested for variability	7	8	7	8	7	7	7	6	7	7	8	7	5	7	6
	% of species (tested for variability) with ≥3 alleles	29	50	43	75	86	14	57	67	29	29	25	14	100	29	83

*Following Sibley & Monroe (1990), except Seychelles warbler which follows Komdeur (1992).

—, sample not tested; 0, no reliable product; 1, product visualized on agarose gel (not tested for variability).

annealing temperature X for 45 s, 72 °C for 45 s for two cycles each at X = 60 °C, 57 °C, 54 °C, 51 °C then 25 cycles at X = 48 °C, followed by 72 °C for 5 min. To optimize the PCR amplification of the loci found to be polymorphic, further PCRs consisted of one cycle at 95 °C for 3 min then 35 cycles at 94 °C for 1 min, annealing temperature (Table 1) for 30 s, 72 °C for 45 s, followed by 72 °C for 5 min. For the cross-species amplifications, a touchdown cycle was performed as above.

PCR products were visualized on a 0.8% agarose gel stained with ethidium bromide. When testing for polymorphism, PCR products were run on 6% polyacrylamide gels and visualized by staining with silver (Promega) or by autoradiography (after PCR with one of the primers end-labelled with [γ^{33}P]-dATP; Sambrook *et al*. 1989).

We developed primers for 63 microsatellites, of which 50 were polymorphic in at least one of the tested species of *Sylviidae* (Table 1). Thirty loci were polymorphic, displaying up to five alleles, in a test panel of up to 25 unrelated Seychelles warblers. There was no significant difference at any locus between the observed and expected heterozygosity, though these comparisons were of limited power.

All 50 loci found to be polymorphic in the Sylviidae were tested for polymorphism in six unrelated individuals of the winter wren, *Troglodytes troglodytes* (M. Berg, personal communication). Fifteen of the loci that were also found to be polymorphic in the winter wren were selected and tested for utility in 16 other species, representing 15 passerine families (Table 2; following Sibley & Monroe 1990).

The high proportion of loci found to be polymorphic in the other *Sylviidae* will reduce or eliminate the need to develop new primers for future studies of these species. The cross-species amplification suggests that, after further testing, many of the primers presented here may also be useful for detecting polymorphic loci in other passerine families (Table 2).

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Variable microsatellite loci in red swamp crayfish, *Procambarus clarkii*, and their characterization in other crayfish taxa

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The red swamp crayfish, *Procambarus clarkii*, is a temperate freshwater crayfish native to the south-eastern United States. It is heavily exploited as a fishery product and is used widely in aquaculture. Its economic importance led to widespread introductions on four continents. The species has been used extensively in laboratory studies, but studies of its population biology in the wild have been rare (Huner 1988). Previous population work using allozymes found low levels of genetic variation in two *Procambarus* species, including *P. clarkii* (Busack 1988). We developed two microsatellite libraries for *P. clarkii* (f. Cambaridae) from which 23 variable microsatellite loci were optimized. The 18 clearest markers were tested in representative taxa of the other two crayfish families (Parastacidae and Astacidae), as well as two cambarid species in Orconectes and one congeneric species; characterization is reported here.

Genomic DNA was extracted from frozen (–80 °C) tail muscle of a red swamp crayfish (Putah Creek, Yolo County California) using the Tris sodium chloride EDTA sodium dodecyl sulphate (SDS) (TNES)-urea buffer extraction protocol (Asahida *et al*. 1996) with the following modifications. Approximately 200 mg tissue were added to 700 µL extraction buffer, containing 4 M urea and 0.5% SDS, and 0.035 mg Proteinase K. After overnight incubation (37 °C), samples were extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). DNA was precipitated with 0.3 M sodium acetate pH 5.3 in a final ethanol concentration of 67%. The pellet was washed in 70% ethanol, air or vacuum dried, and resuspended in Tris low EDTA (TLE) buffer (10 mM tris + 0.1 mM EDTA, pH 8.0). Two subgenomic libraries were created by Genetic Identification Services (Chatsworth, CA) by partially digesting whole genomic DNA with a mixture of the following restriction enzymes: *Bsr*BR1,