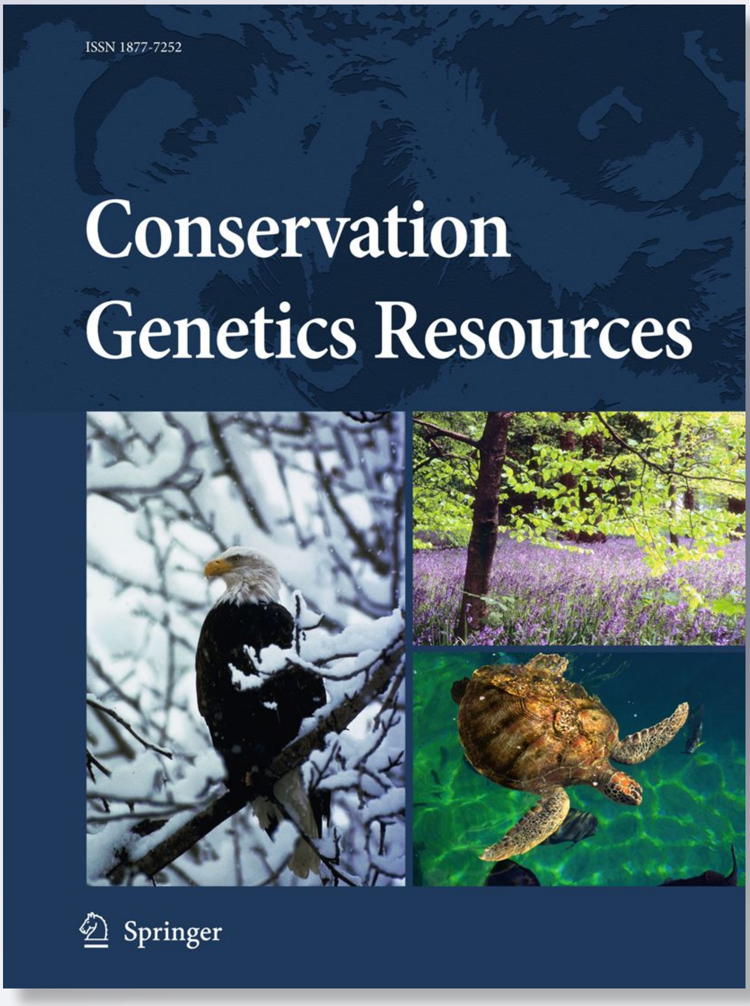
Isolation, characterization and predicted genome locations of ruff (Philomachus pugnax, *AVES) microsatellite loci*

Lindsay L. Farrell, Deborah A. Dawson, Gavin J. Horsburgh, Terry Burke & David B. Lank

Conservation Genetics Resources

ISSN 1877-7252

Conservation Genet Resour
DOI 10.1007/s12686-012-9639-0



Conservation Genet Resour
DOI 10.1007/s12686-012-9639-0

TECHNICAL NOTE

Isolation, characterization and predicted genome locations of ruff (*Philomachus pugnax,* AVES) microsatellite loci

**Lindsay L. Farrell · Deborah A. Dawson ·
Gavin J. Horsburgh · Terry Burke ·
David B. Lank**

Received: 26 March 2012 / Accepted: 28 March 2012

Abstract We identified 247 unique ruff (*Philomachus pugnax*) microsatellite sequences. Primer sets were designed from 102 selected loci and tested in 12–24 individuals from a captive population. Sequence homology was used to assign locations in the chicken (*Gallus gallus*) and/or zebra finch (*Taeniopygia guttata*) genome for the majority of these loci. Fifty-two loci were found to be polymorphic and 47 of these were typed in known families. Forty-six loci displayed Mendelian inheritance including *Ppu058*, which was confirmed to be Z-linked by the complete absence of any heterozygous females.

Keywords Lek · Male morphs · Microsatellite · Scolopacidae · Shorebird · Simple tandem repeat (STR)

We have isolated and characterized microsatellite markers for the ruff (*Philomachus pugnax*). The ruff is a lekking shorebird with an autosomal genetic polymorphism for male mating behaviour (Lank et al. 1995) and belongs to the Scolopacidae family. A proportion of the ruff microsatellites isolated will be of utility in closely related species (Primmer et al. 1996), including other species of Scolopacidae (n = 88, Sibley and Monroe 1990). Within this family, several species are of conservation concern, including the critically endangered spoon-billed sandpiper (*Eurynorhynchus pygmeus*; IUCN Red List, Birdlife International).

Blood was collected from captive ruff individuals at Simon Fraser University, Canada (population maintained by DBL since 1985), and stored in absolute ethanol. Genomic DNA was extracted using ammonium acetate (Nicholls et al. 2000).

Two ruff microsatellite-enriched libraries were created following Armour et al. (1994). For both libraries, genomic DNA from a single female ruff (Bird ID 6233) was digested with *Mbo*I, size selected (178–856 bp) and enriched for $(CA)_n$, $(CAG)_n$, $(GCC)_n$, $(TTTC)_n$ and their complements. The first library was additionally enriched for $(AT)_n$. Clones were sequenced in both directions using BigDye terminators and a consensus sequence created. We used BlastN 2.2.4 software (Altschul et al. 1997) to check for duplication of new and published ruff microsatellites (n = 9 published, Thuman et al. 2002). In total, 247 unique microsatellite sequences were identified and primers designed for 102 loci using PRIMER3 (Rozen and Skaletsky 2000; Tables 1, 2). Primers were designed for just three of the 104 sequences that contained only mononucleotide (A or T) repeats (*Ppu017*, *Ppu033* and *Ppu071*).

One hundred and two markers were tested in 12–24 known-sex individuals using multiplex PCR. Each 2-µl PCR contained approximately 10 ng of genomic DNA, 0.2 µM of each primer and 1 µl Qiagen Multiplex PCR Mix (Qiagen Inc.; Kenta et al. 2008). PCR amplification was performed using a DNA Engine Tetrad 2 Thermal Cycler (MJ Research, BioRad UK) with the profile: 15 min at 95 °C, followed by 35 cycles of 94 °C for 30 s, annealing temperature (Tables 1, 2) for 90 s, 72 °C for 1 min, and a final step of 60 °C for 10 min. Products were loaded on an ABI3730 Genetic Analyzer (Applied Biosystems) using ROX500 size standard and genotypes scored with GENEMAPPER v4.0

L. L. Farrell · D. A. Dawson · G. J. Horsburgh · T. Burke
Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Alfred Denny Building, Sheffield S10 2TN, UK

L. L. Farrell (✉) · D. B. Lank
Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada
e-mail: lfarrell@sfu.ca

Table 1 Characterization and predicted chromosome locations of 52 ruff (*Philomachus pugnax*) microsatellite loci

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	MI	T_m (°C)	PCR T_a (°C)	n_1	n_2	*A*	Exp allele size (bp) ǂ	Obs. allele size (ruff 6233) & range (bp) ¥	H_O	H_E	*p*HWE*
Ppu001	HE616911	1	1	52975585	$1.00e^{-07}$	$(TAGA)_{12}$	HEX	F: ACCAGGCTTCTTCCCTCTGGA	494	Y	64.65	56	24	24	4	197	(275, 279)	0.62	0.57	0.12
	3C02		1A	50776302	$3.30e^{-27}$			R: TGAAACTTCACATTTTGGGGATGA			64.67						275–287			
Ppu003	HE616913	1	1	122413141	$5.60e^{-61}$	$(CTAT)_{11}$	HEX	F: CAGGATTGCTTTGGCTGGAG	337	Y	63.13	56	24	23	4	274	(366, 371)	0.65	0.70	0.30
	3E04		1	13670975	$1.20e^{-27}$			R: AGCATGTAGTGCTTCAGTTATTTAGATGC			63.18						367–373			
Ppu004	HE616914	1	2	89244831	$9.50e^{-79}$	$(TCTA)_{10}$	6-FAM	F: TTGGCACCAATAGTTGCCTCA	646	N	60.8	56	24	10	3	230	NT	0.10	0.59	$0.11^{\S}$
	3E05		2	92082626	$8.80e^{-84}$			R: CCTCTTCAGAGGAACAAAGCAAGA			62.9						192–233			
Ppu005	HE616915	1	8	22771586	$8.00e^{-108}$	$(TC)_5$	6-FAM	F: GGAGCAATGTGATACCACTAAGGACTG	480	Y	65.13	56	24	24	2	227	(221, 221)	0.12	0.11	1.00
	3H04		8	19350034	$4.40e^{-73}$			R: CTCCTGACCTCCACCGCAAC			65.42						216–220			
Ppu006	HE616916	1	5	17130526	$2.70e^{-53}$	$(GT)_9$	6-FAM	F: TGGAAGTGGAAGGAGGTCTGTG	534	Y	63.90	56	24	22	2	250	(245, 251)	0.31	0.48	0.06
	4A08		5	16041469	$8.10e^{-70}$			R: TCCACTCAGGTGCAGGCTTC			63.80						241–246			
Ppu007	HE616917	1	3	76776326	$4.00e^{-27}$	$(GT)_5$	6-FAM	F: GCCAGAGTAGCAACAGTCAGTGC	577	Y	63.20	56	24	13	2	293	(294, 294)	0.38	0.40	1.00
	4C09		3	76352543	$4.50e^{-52}$			R: CCTATTCATGTCTCCAAGTTCAATCC			63.66						281–290			
Ppu008	HE616918	1	–	–	–	$(AC)_{12}$	6-FAM	F: GAAGTTCCTCTTACCAATTTGCTTGC	427	Y	64.41	56	24	24	3	300	(299, 299)	0.20	0.19	1.00
	4D01							R: TGACCTGCTGGTACTCCACCAC			64.68						298–302			
Ppu009	HE616919	1	4	23020195	$5.90e^{-64}$	$(AC)_{12}$	HEX	F: TCTTTATGATGCTATTTGAGGGTTTGG	436	Y	64.36	56	24	24	12	226	(436-436)	0.95	0.89	1.00
	4D05		4	29737180	$3.70e^{-46}$			R: AATGCCACTGCACCAGAAGTAGTC			63.91						317–474			
Ppu010	HE616920	1	4	6796049	$6.90e^{-17}$	$(GT)_{12}$	HEX	F: GGGAGCTCAGGGATGCAGTG	530	N	65.55	56	24	23	5	371	NT	0.17	0.71	$0.00^{\S}$
	4D09		4A	2561871	$2.20e^{-37}$			R: GATGTGAACTACCTGCAAATCCACAG			65.06						221–233			
Ppu011	HE616921	1	3	57218799	$3.40e^{-63}$	$(AC)_5$	HEX	F: CGCACATCTGCTGTTGAGAAATC	371	Y	64.29	56	24	21	3	222	(223, 223)	0.57	0.51	1.00
	4F08		3	49883043	$6.40e^{-43}$			R: TGGACTGAAGGTGACTATTCTGCTG			63.99						216–223			
Ppu012	HE616922	1	3	51033809	$4.90e–^{03}$	$(AC)_6$	6-FAM	F: TGCAAGGCCAGGTAGAAACAAG	495	Y	63.69	56	24	23	2	219	NT	0.69	0.48	0.19^{+}
	4G03		3	56310381	$1.20e^{-32}$			R: ACCAAGGCATTACTGTGTTGGAAG			63.55						214–222			
Ppu013	HE616923	1	3	18786830	$1.40e^{-61}$	$(AG)_6$	HEX	F: ACATGCTCCTCTTCCATTTGCAG	542	Y	65.17	56	24	17	2	230	(229, 229)	0.35	0.47	0.50
	4H10		3	18163845	$3.80e^{-63}$			R: TGCTCCATGGAATCAAACATGG			64.86						223–228			
Ppu014	HE616924	1	–	–	–	$(GT)_{10}$	6-FAM	F: CAACCCCATCTCCTGGCTTTT	431	Y	64.61	56	24	24	6	201	(214, 220)	0.62	0.72	0.19
	5A10		24	3112345	$1.20e^{-30}$			R: CAGCTCGGTACATTGGTGCTTG			65.07						194–204			
Ppu015	HE616925	1	2	19888521	$9.80e^{-27}$	$(AC)_5$	HEX	F: GGTCCAGTTCTGTGTGCCAGTTT	693	Y	64.51	56	24	20	3	248	(247, 247)	0.40	0.51	0.65
	5B12b		2	22065196	$1.70e^{-54}$			R: TGACTTTGGAGGTTGTTACTTATTGTTGTC			64.80						244–278			
Ppu016	HE616926	1	1	166535076	$1.10e^{-22}$	$(TC)_{12}$	HEX	F: TCAGGCAGTGGGACTAGATGATTG	584	Y	64.74	56	24	24	6	299	NT	0.62	0.62	0.05
	5E02		1	65490386	$5.70e^{-13}$			R:TCAAAGACTTCTGCAAAGTTATTCTTCTAAGC			65.15						212–227			
Ppu017	HE616927	1	4	52883524	$6.00e^{-06}$	$(T)_{14}$	HEX	F: GTTGGCCTGGACTCCGTCTG	414	Y	65.16	56	24	10	2	223	(227, 227)	0.10	0.52	$0.02^{\S}$
	6F12		–	–	–			R: GTGCTACTGAAATCGTCTGATGTTGG			65.42						207–218			
Ppu018	HE616928	1	2	61061152	$1.90e^{-28}$	$(AGAT)_{13}$	HEX	F: TGCCTTCTTACTTTCTCAATATTTGTGG	536	Y	63.74	56	24	24	8	250	(250, 258)	0.79	0.78	0.04
	6G09		2	71904153	$4.90e^{-38}$			R: AGAGATACAGTAAGCTTCGTATGACAGACAC			63.51						242–270			
Ppu019	HE616929	2	3	84720681	$7.60e^{-10}$	$(AC)_{11}$	HEX	F: TAACCCACGAGTGGCTCTG	407	Y	59.84	56	24	20	5	150	(151, 154)	0.45	0.69	$0.02^{\S}$
	24A01		–	–	–			R: GCTACTGGGTGCTGTTACTTCC			60.19						145–156			
Ppu020	HE616930	2	11	19600964	$3.10e^{-20}$	$(GT)_{13}$	HEX	F: TCCTGTCCTGTCCTTGGAAC	367	Y	60.09	56	24	23	7	246	(243, 245)	0.52	0.44	1.00
	24B05		11	71134	$6.70e^{-25}$			R: GCGGTATTTCTGGCCTAGC			59.82						245–302			
Ppu021	HE616931	2	1	156510069	$3.90e^{-57}$	$(CTAT)_{12}$	6-FAM	F: AAAGCTTGTAAGCTCTAAGCAATACC	791	Y	59.92	56	24	15	5	319	(321, 321)	0.40	0.70	$0.04^{\S}$
	24C05		1	61096046	$4.00e^{-81}$			R: AGGCTATTGACACTTCACAAAGG			59.70						312–329			
Ppu022	HE616932	2	2	75106465	$8.90e^{-20}$	$(ATAGAT)_9$	6-FAM	F: TGAATGCATGAATTAGGTAGTGG	722	Y	59.02	56	24	23	7	297	(296, 296)	0.91	0.80	0.61
	24C08		2	79772128	$1.20e^{-37}$			R: GGGAAACATCATGCAACAAC			58.83						267–302			

Table 1 continued

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	MI	T$_m$ (°C)	PCR T$_a$ (°C)	n_1	n_2	*A*	Exp allele size (bp) ǂ	Obs. allele size (ruff 6233) & range (bp) ¥	H$_O$	H$_E$	*p*HWE*
Ppu023	HE616933	2	7	29587083	4.70e^{-13}	(AGAGAGGAG AAGAGAGA)$_7$	6-FAM	F: CAGCTATGAGGTTACCAGAGAGG	592	Y	59.44	56	24	21	11	294	(287, 287)	0.23	0.78	0.00§
	33C07		7	3954280	5.80e^{-52}			R: GCCAGGGCAGAGACATAAAG			59.84						287–406			
Ppu024	HE616934	1	13	9775634	1.80e^{-12}	(TCTA)$_7$	HEX	F: GGAAACCTTCCCATCAACAG	294	Y	59.38	56	24	17	9	138	(135, 157)	0.82	0.86	0.38
	63A05		–	–	–			R: GAAGGGATGCATGGTTGG			59.85						122–161			
Ppu025	HE616935	1	1	24109016	2.20e^{-18}	(AC)$_{17}$	6-FAM	F: GATCCAGACTGCCTAAACAGC	421	Y	58.95	56	24	14	7	348	(332, 346)	0.85	0.87	0.72
	63C04		–	–	–			R: GCATCACAAATGCAACTTCAG			59.34						331–346			
Ppu027	HE616937	1	7	15230709	1.20e^{-28}	(GAAA)$_8$	6-FAM	F: TGTTAGCAGGCTGATGTGTG	480	Y	58.44	56	24	24	10	301	NT	0.58	0.72	0.54§
	63D02		7	19299021	6.60e^{-43}			R: TCCTGTGAGCTGTTAATTCTGAG			58.66						277–320			
Ppu028	HE616938	1	1	130142524	2.50e^{-12}	(TGAT)$_6$	HEX	F: CCTGAACCATTAGTTTACTTGCTG	266	Y	59.28	56	24	24	3	182	(185, 185)	0.58	0.58	0.44
	63D04		1	108220670	2.30e^{-14}			R: GCACCAGAACTGCCACATAG			59.32						185–193			
Ppu029	HE616939	1	10	9185554	1.50e^{-50}	(GT)$_{10}$	HEX	F: AGGGTATTGTTGGAGAAATGG	567	Y	57.92	56	24	18	2	166	(168, 168)	0.11	0.35	0.34§
	63G02		10	7745116	1.10e^{-62}			R: CTAACCTGGATGGCTGTTTG			58.23						168–170			
Ppu030	HE616940	1	2	120354672	2.30e^{-77}	(GT)$_{11}$	HEX	F: CAGGCTTAACACTCTTTCTTCC	713	Y	57.32	56	24	21	3	136	(136, 136)	0.90	0.62	0.21^{+}
	64A02		2	122215464	1.30e^{-110}			R: CTCGTTGGTCATAATTTGAGG			57.23						131–140			
Ppu031	HE616941	1	13	1071128	2.30e^{-25}	(GT)$_{10}$	6-FAM	F: TGATTCTTATTAGGATTATTTGATGC	437	Y	57.14	56	24	17	3	321	(321, 321)	0.11	0.11	1.00
	64C01		13	16024608	8.90e^{-41}			R: TGAGGACTGTGGTTTAAGAGC			57.07						320–325			
Ppu032	HE616942	1	2	29908799	6.50e^{-10}	(AC)$_{18}$	6-FAM	F: CATTTCTTGTTGTGATTAATAGTCTCC	461	Y	58.69	56	24	19	8	262	(258, 260)	0.57	0.77	0.04§
	65A08		2	49636829	1.70e^{-05}			R: TAAGAGGTTGCCAGGTTGTG			58.77						250–271			
Ppu033	HE616943	1	8	30604045	1.80e^{-07}	(G)$_{12}$	HEX	F: AGTTGTTGAGGCACCATCC	491	Y	58.50	56	24	24	3	156	NT	0.95	0.55	0.00^{+}
	65A09		8	27910568	9.00e^{-27}			R: GATCATCTAGTCTAACCATCAACTCTG			58.85						135–162			
Ppu034	HE616944	1	10	12101849	6.40e^{-37}	(AAT)$_6$	HEX	F: CTCCATGGACCAGAAATGAG	548	Y	58.07	56	24	15	2	136	(132, 132)	0.00	0.12	0.04§
	67A09		10	10268721	3.20e^{-56}			R: CCACCCTTCATATTGACTCG			58.02						132–149			
Ppu035	HE616945	1	3	104574877	2.00e^{-14}	(GT)$_9$	6-FAM	F: AAGCTTGTCTTCTGGAATGAAGCAG	576	N	64.40	56	24	22	2	235	NT	1.0	0.51	0.00§+
	4H11		3	104595407	7.90e^{-37}			R: TTGAACGTTTGGGACAGGTGAC			64.33						178–214			
Ppu036	HE616946	1	10	4306840	2.10e^{-33}	(GT)$_7$	6-FAM	F: AGACCCGGGTGTTCAAGGTG	673	Y	64.53	64	12	12	3	203	(200, 204)	0.50	0.42	1.00
	4A01		10	895537	1.20e^{-46}			R: TTTCCCAGCATGACATACATTGC			64.15						200–208			
Ppu037	HE616947	1	26	2193405	1.10e^{-19}	(GT)$_6$	HEX	F: CTCTTGTGGTACCTGGAAGAGGTG	454	Y	63.43	63	12	12	2	234	(236, 236)	0.33	0.29	1.00
	6A11		26	1368934	3.30e^{-50}			R: TCCATATTTATTACAGCCCAGAAGACC			63.55						235–237			
Ppu038	HE616948	1	2	98252724	1.70e^{-42}	(GAAA)$_5$	HEX	F: CATGACTACCTATCGAATCCTCTTTGG	422	Y	64.21	64	12	12	3	279	NT	0.33	0.30	1.00
	3D04		2	101346526	4.10e^{-22}			R: TTAATATGGCAGCCTTACCTAACGAAAC			64.38						276–283			
Ppu039	HE616949	1	1	52147383	1.50e^{-73}	(TGAT)$_6$	6-FAM	F: GCAACTGCTGCACTCCCAAC	563	Y	64.30	64	12	12	2	193	(194, 194)	0.58	0.50	1.00
	3E11		1A	49840927	1.30e^{-52}			R: CTTGCCATTCAGGTTAAGTACACTTCC			64.23						190–194			
Ppu040	HE616950	1	5	2524315	1.20e^{-59}	(GT)$_9$	HEX	F: CTCCTGGCTGCGTTGTTCTG	600	Y	64.38	64	12	12	3	207	(207, 209)	0.25	0.23	1.00
	4G04		5	321716	1.90e^{-58}			R: GGAACGATGTGGGTTACTTCCAG			63.96						209–213			
Ppu041	HE616951	1	11	10001495	6.50e^{-56}	(AC)$_9$	HEX	F: TGATTTTCCGAAACAAGTTTTAATCG	614	Y	63.39	63	12	12	2	173	(173, 173)	0.50	0.46	1.00
	6D06		11	15593478	1.90e^{-71}			R: AGCAGACCGGAGAAGCAACA			63.40						172–174			
Ppu042	HE616952	1	11	9474078	1.0e^{-139}	(TTTC)$_{48}$	HEX	F: TGGTCCCTTCCAACTCTAGAAA	413	Y	60.10	60	12	11	2	377	(127, 127)	0.18	0.17	1.00
	3A02		11	15017303	3.80e^{-07}			R: TTGTTCACATTGAGAAGTTGATGAC			60.44						360–363			
Ppu043	HE616953	1	2	50681577	8.30e^{-54}	(GT)$_7$	HEX	F: CTCTCCAGGTTGCCTAAAGTCTATGG	255	N	64.51	64	12	12	2	186	NT	0.16	0.15	1.00
	5A12		2	33590280	2.10e^{-05}			R: GACTTTGGTAGGTCTTCTGGTTCCTG			64.54						185–187			
Ppu046	HE616956	1	4	41459422	1.90e^{-138}	(GT)$_{10}$	6-FAM	F: TCGTCTGATTTGTATTGTTCTT	306	Y	55.22	56	12	12	3	170	(177, 177)	0.58	0.62	0.29
	3C03		4	37989476	2.20e^{-42}			R: TGACACACAGGTTTGGAA			55.34						173–180			

Table 1 continued

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	MI	T_m (°C)	PCR T_a (°C)	n_1	n_2	A	Exp allele size (bp) ǂ	Obs. allele size (ruff 6233) & range (bp) ¥	H_O	H_E	pHWE*
Ppu047	HE616957	1	6	28674879	$1.60e^{-103}$	$(TC)_{10}$	HEX	F: TGCAGCTTTAATTGCAACAGCTAATC	496	Y	64.66	64	12	12	3	291	(290, 292)	0.58	0.62	0.21
	3B01		6	27151910	$1.60e^{-61}$			R: AGCGCTCAGGTCTGAATGAGTTC			64.35						288–292			
Ppu048	HE616958	1	1	4758111	$2.20e^{-70}$	$(TC)_{10}$	HEX	F: TGCAGCATTCTTCGCAGCTA	659	Y	63.22	63	12	12	4	225	(226, 230)	0.50	0.53	0.45
	5C08		1A	3982690	$2.70e^{-75}$			R: AACACACTGAGCGTCGTTTTATCA			63.09						223–231			
Ppu049	HE616959	1	1	163634502	$3.00e^{-08}$	$(AG)_{12}$	6-FAM	F: AACTTCAAAGACTTCTGCAAAGTTATTCTTC	522	Y	63.61	63	12	12	10	389	(368, 376)	0.75	0.87	0.05
	6B02		1	65490386	$2.10e^{-14}$			R: TGAACTTACACTGGTGAACTAACTTTCTCTC			63.48						280–366			
Ppu050	HE616960	1	4	64774867	$4.20e^{-09}$	$(AC)_7$	6-FAM	F: TCAGCACTGAAACTGAGGAAATTATTG	640	N	63.80	63	12	12	4	276	NT	0.16	0.23	0.13
	6F01		4	41075858	$2.00e^{-58}$			R: GAGCATTCCTCCCGCTGTG			64.26						225–275			
Ppu051	HE616961	1	3	2063655	$3.10e^{-41}$	$(AC)_{10}$	HEX	F: CCCGTCAGCGAATATAAGAGCAG	593	N	64.26	64	12	10	3	198	NT	0.20	0.24	0.04
	3F11		3	26505233	$1.60e^{-53}$			R: GTTGGGTGTTCCTGCTGACG			64.02						192–204			
Ppu052	HE616962	1	4	38001317	$2.30e^{-02}$	$(GT)_6$	6-FAM	F: TTGCACGTGTCCTTAGCTTGC	641	N	63.67	63	12	12	3	238	NT	0.08	0.23	$0.05^{\S}$
	4H06		–	–	–			R: AATGCTTGTGGGTGGCAATG			64.11						238–242			
Ppu057	HE616967	1	6	20079681	$3.70e^{-21}$	$(AG)_8$	6-FAM	F: TGCAGTGCAATGTGTGTGACC	564	Y	64.28	64	12	8	8	360	(329, 358)	1.00	0.90	0.82
	3F07		6	18076829	$8.50e^{-28}$			R: CCTGCTGTGAAATCTACCCATCC			64.07						328–381			
Ppu058 (Z-linked)	HE616968	1	Z	6052811	$1.30e^{-27}$	$(GT)_{14}$	6-FAM	F: AGTAGCTGCCAATCCACAGG	477	Y	60.28	60	12	6 (M)	6	224	(227, 227)	0.85	0.87	0.06
	4C06		Z	37214802	$1.30e^{-45}$			R: TCTCCTGCTTGGCCTCTTT			60.08			6 (F)	6		221–233	0.00	0.00	–
Ppu059	HE616969	1	1	121754800	$2.20e^{-51}$	$(GT)_8$	HEX	F: TCTACTGAGCTCACAGAAACAAAGGAAC	331	Y	64.36	63	12	9	2	263	(262, 262)	0.33	0.50	$0.49^{\S}$
	4F01		1	12955004	$2.60e^{-34}$			R: CTGACTCATGATGCCTCATCTCG			64.42						263–265			
Ppu060	HE616970	1	1	136476610	$6.10e^{-27}$	$(GT)_7$	6-FAM	F: TGAATGCAAATACAGCATCAGTGAG	493	N	63.51	64	12	9	2	214	NT	0.44	0.36	1.00^{+}
	5C04		1	67624179	$1.80e^{-14}$			R: TTGTGATGCACAAGTCTTTCAAGG			63.60						213–214			

Ŧ The location of each microsatellite sequence was assigned in the chicken (v 2.1, May 2006 ENSEMBL release) and zebra finch (December 2011 ENSEMBL Release 65) based on sequence homology (see Dawson et al. 2006, 2007). Of these 52 polymorphic loci characterized, 50 could be assigned a location in the chicken genome and 46 in the zebra finch genome.

n_1 Number of individuals tested in a captive population. n_2 Number of individuals amplified and genotyped. *A* Number of alleles observed, *M* male, *F* female. H_O observed heterozygosity (calculated from n, using CERVUS v3.0). H_E expected heterozygosity (calculated from n, using CERVUS v3.0), *HWE* Hardy–Weinberg equilibrium

§ Deficiency of heterozygotes observed; $^{+}$ excess of heterozygotes observed, determined by Chi squared test (two tailed $p < 0.05$). Deficiencies and excesses of heterozygotes are likely attributable to non-random population structure caused by captive breeding

p_{HWE} calculated from a maximum of 12 individuals, using GENEPOP v.4.0 (Rousset 2008)

MI Mendelian inheritance displayed when genotyped in a pedigree of 64 families, consisting of both parents and 5–33 offspring per family; *Y* yes, *N* no

ǂ Expected allele size based on the sequenced clone allele of the female ruff individual (Bird ID 6233) from which the genome library was created

¥ the allele sizes presented in parentheses are those of the female ruff individual (Bird ID 6233) from which the genomic library was created, *NT* not tested

* HWE for all loci was assessed in 12 individuals, which may contain second order relatives (half-sibs and closer relatives were removed)

Table 2 Additional ruff microsatellite loci with assigned predicted genome locations but that were abandoned from further testing

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	T_m (°C)	PCR T_a (°C)	n_1	n_2	*A*	Exp allele size (bp) ¥	Obs allele size (bp)	Marker status
Ppu002	HE616912	1	–	–	–	(GAAA)$_{44}$	HEX	F: GAAGCTGAGATTCCCATATAATCACT	538	60.23	56	24	21	11	384	313–378	Unreliable poor reverse primer
	3D09							R: AACATACAACACCTCTCTCTCTTTCTTT		60.45							
Ppu026	HE616936	1	1	39405325	$3.90e^{-19}$	(AC)$_{10}$	6-FAM	F: CTTTGTCATTATGTAGGGTCCTG	414	57.70	56	24	23	1	294	291	Monomorphic
	63C05		1A	36868616	$5.40e^{-43}$			R: GCTGCCACTTACTGAAATCTG		57.65							
Ppu044	HE616954	1	–	–	–	(TGTT)$_6$	HEX	F: TCAGGCACATTACATTATTCACTATCTATG	543	61.77	61	12	11	1	193	195	Monomorphic
	3E10		–	–	–			R: CCACCCTCCTAGCAAACACC		61.83							
Ppu045	HE616955	1	2	140526702	$6.60e^{-70}$	(AC)$_{10}$	HEX	F: TCATTTCTCCTCCTAACGCTGAAG	561	63.12	63	12	11	1	326	324	Monomorphic
	4D02		2	141544149	$7.10e^{-61}$			R: ACTGTCCTTGCATCTTCCTCTCC		63.22							
Ppu053	HE616963	1	Z	32113785	$4.30e^{-84}$	(AC)$_{10}$	6-FAM	F: TTGGCACCAATAGTTGCCTCAT	407	63.81	56	12 (M)	8	1	233	230–238	Very poor amplification
	3H05		Z	59515109	$9.00e^{-49}$			R: CCTCTTCAGAGGAACAAAGCAAGA		63.32		12 (F)		1		230	
Ppu054	HE616964	1	8	15987120	$2.00e^{-25}$	(GT)$_5$	6-FAM	F: GCACCGCAGAAGTTGATAAG	505	58.53	56	24	7	3	284	322–338	Very poor amplification
	65F04		8	11832348	$7.50e^{-48}$			R: CTGAGGTGCTCATGGTTACAG		58.38							
Ppu055	HE616965	1	1	196624265	$8.80e^{-13}$	(GAAA)$_{14}$	6-FAM	F: TGGAGCTTAACATCTACAAATGC	375	58.47	56	24	2	2	336	276–283	Very poor amplification
	65H06		1	85953917	$7.90e^{-12}$			R: TTGGCTTTCTCTTATCCATCAC		58.33							
Ppu056	HE616966	1	22	690245	$1.00e^{-22}$	(AC)$_8$	HEX	F: CCTCTGGCAAATACTCAATGC	305	59.72	56	24	4	2	143	249–283	Very poor amplification
	66F02		22	1590011	$1.50e^{-06}$			R: CACTGGAAAGGTCAGGAAGC		59.84							
Ppu064	HE616974	1	5	50276059	$4.20e^{-135}$	(AC)$_5$	6-FAM	F: TGTTGCTGTTGTTGTCGTGGTC	357	64.41	56	12	11	1	248	245	Monomorphic
	5H06		5	48965965	$5.20e^{-43}$			R: GAGTGCTCTGCACCCTTCACC		64.58							
Ppu070	HE616980	1	5	36514685	$5.70e^{-62}$	(AT)$_5$	6-FAM	F: TTCTCCTCACCCTCCTCTAGCATTTAG	401	65.17	56	24	21	1	198	198	Monomorphic
	6E05		5	33970154	$1.80e^{-44}$			R: GCGCATGGCTTTATACCATATTTCC		65.41							
Ppu071	HE616981	1	2	17900039	$3.50e^{-61}$	(T)$_{10}$	6-FAM	F: AAATCTGAACCAGAAATAATATAGTCAATCC	585	61.42	56	24	1	1	241	241	Monomorphic
	4G02		2	19900285	$1.20e^{-43}$			R: TGAAGTGTTCTCCACTTCTTTGATG		61.87							
Ppu079	HE616989	1	4	39272313	$2.10e^{-19}$	(GA)$_7$	HEX	F: AGCTGAATGAACATCTGCATATTAGAACAC	324	64.72	56	24	17	1	245	245	Monomorphic
	5G10B		–	–	–			R: GTTGGGCGGAGAATGGACTG		65.12							
Ppu083	HE616993	1	9	11757764	$7.10e^{-96}$	(GT)$_5$	6-FAM	F: CTGGGTGTTGTGCCTTCAGC	562	64.15	60	12	11	1	212	213	Monomorphic
	4G10		9	12079437	$5.70e^{-92}$			R: TCCATCAAGAACTCATTAGCTTAGTAGCC		64.16							
Ppu086	HE616996	1	Z	72933854	$2.90e^{-76}$	(GT)$_5$	6-FAM	F: TCTGCAGTTGGAAGGCTGTAA	524	60.95	60	6 (M)	6	1	196	196	Monomorphic
	6B07		Z	22314343	$1.00e^{-81}$			R: TCTTGATGTTGCCAAAATATACATACA		60.81		6 (F)	6	1		196	
Ppu092	HE617002	1	6	14776618	$1.90e^{-55}$	(AT)$_5$	HEX	F: AAAGGAGTTCCCTGGCACAGTTT	606	64.44	56	24	24	1	280	279	Monomorphic
	6C11		6	12562519	$1.30e^{-67}$			R: CGGCACAATTAATACTACCGGCTTA		64.15							
Ppu095	HE617005	1	9	13341088	$2.00e^{-71}$	(TC)$_5$	6-FAM	F: GGTGTGTGTTTCTGTCTCCCTCT	504	61.87	56	24	19	1	247	244	Monomorphic
	5C07		9	13825337	$1.70e^{-35}$			R: ATGGGAGTTATGCACACATCAAA		61.51							
Ppu101	HE617011	1	Z	14416326	$7.60e^{-82}$	(GT)$_5$	6-FAM	F: TTCACTTACACTTGAGGATGGTTG	367	60.44	60	6 (M)	6	1	205	205	Monomorphic
	5F03		Z	45558110	$6.10e^{-33}$			R: AAACTAAACTACTGCTGGGCATTG		60.88		6 (F)	6	1		205	
Ppu104	HE617014	1	5	54678545	$1.10e^{-70}$	(GT)$_5$	HEX	F: CCCAATGTGCAGAAACCCTCAC	471	66.02	56	24	23	1	246	243	Monomorphic
	6H07		5	53923720	$6.40e^{-46}$			R: TGTGAAAGCCCTTGAAGGCATC		65.74							

Table 2 continued

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	T_m (°C)	PCR T_a (°C)	n_1	n_2	*A*	Exp allele size (bp) ¥	Obs allele size (bp)	Marker status
Ppu105	HE617015	1	22	1024052	7.60e^{-12}	$(GT)_6$	6-FAM	F: AAATTAGCATAAAGACGGAGAAGTTGC	304	63.14	60	12	10	1	249	251	Monomorphic
	6H11		–	–	–			R: GCCTTCTTGCTGTATTCAGGTGAG		63.56							
Ppu138	HE617048	2	5	4932892	1.00e^{-11}	$(ATAG)_4$	HEX	F: TTGGATATTCTGCCGAGATG	531	58.67	56	24	15	1	257	284	Monomorphic
	36B04		5	2549845	7.60e^{-42}			R: GGGAAGCTTGGGATTCTATG		58.60							
Ppu140	HE617050	2	18	5211094	4.30e^{-105}	$(AC)_6$	HEX	F: TAGTAGCCAATCGGGTGAGC	713	60.24	56	24	23	1	256	255	Monomorphic
	21A01		18	9810117	7.00e^{-85}			R: AGCCAGCTGGAGTAGTGTGTG		60.52							
Ppu148	HE617058	2	12	13482713	1.70e^{-106}	$(AC)_7$	6-FAM	F: TGGCTTGTGCAAAGTAGATTTC	756	59.41	56	24	23	1	340	341	Monomorphic
	64A03		12	13993026	1.20e^{-132}			R: TCAGGATGACTCCAGACGTG		59.82							
Ppu164	HE617074	2	1	178594445	5.20e^{-62}	$(TC)_5$	6-FAM	F: CTGCTCATCTCTCCTTTCTTCC	609	59.60	56	24	21	1	329	331	Monomorphic
	64B02		1	51091377	1.50e^{-69}			R: CTGGAAAGGCTGAAATCCTG		59.81							
Ppu172	HE617082	2	3	110633741	2.10e^{-48}	$(GA)_6$	HEX	F: CAGGAAGAGGTGAGCTGGAG	305	60.13	56	24	23	1	155	155	Monomorphic
	66G11		3	110357795	4.50e^{-52}			R: CTAAGGCACACAGCTGAACG		59.66							
Ppu181	HE617091	1	Z	42994287	7.20e^{-05}	$(GT)_{10}$	6-FAM	F: TTGGAGGGTTCTTTGGGTTG	677	62.15	56	12 (M)	4	1	262	265	Monomorphic
	63D03		Z	7124071	2.80e^{-41}			R: AGCTGCAATTGATGCCAGTG		62.35		12 (F)		0	–	–	
Ppu199	HE617109	2	10	19602417	1.90e^{-35}	$(GT)_5$	HEX	F: TGCTGAAATCCATTCTACTAACG	557	58.47	56	24	4	1	148	148	Monomorphic
	67D10		10	18002891	7.10e^{-39}			R: CCTATTAATTGCACACAAATTGC		58.63							
Ppu221	HE617131	1	26	1257327	9.90e^{-22}	$(GT)_8$	6-FAM	F: GCGAGACATCTGAATCTCCTC	468	58.98	56	24	18	1	356	357	Monomorphic
	63C10		26	273083	2.10e^{-60}			R: TGCTCTGCACTTGTGTAACG		58.65							
Ppu242	HE617152	2	1	191524107	7.30e^{-12}	$(GT)_7$	6-FAM	F: GATCAGGTTGTGAGCAGCAG	323	59.58	56	24	5	1	315	126	Monomorphic
	67C06		1	81416718	5.20e^{-18}			R: TTCTTCTGGAGCCCTCTCTG		59.67							
Ppu062	HE616972	1	9	15940752	3.40e^{-98}	$(AC)_7$	HEX	F: TCAGTGTTTCTACTTGGATGGAATGG	400	64.50	60	12	0	–	243	–	Failed to amplify
	5E09		9	16851809	1.40e^{-37}			R: TGTGGAATTAAAGTTCAACCCATTAGC		64.28							
Ppu067	HE616977	1	–	–	–	$(AGAAG)_{55}$	6-FAM	F: CTGACTGCTGCCTGCCTCTC	606	64.27	56	12 (M)	0	–	509	–	Failed to amplify
	4G09		Z	43145669	3.00e^{-25}			R: GGTGTCACCTCCTATACCTACATGCTC		63.79		12 (F)					
Ppu068	HE616978	1	Z	6212608	4.20e^{-80}	$(AGAT)_{11}$	6-FAM	F: CTTGCCAAAGGGCAAGGTG	669	63.57	56	12 (M)	0	–	263	–	Failed to amplify
	3E09		Z	37374037	1.20e^{-32}			R: GGCAGAAGCAAGAAACAACAGAAC		63.96		12 (F)					
Ppu080	HE616990	1	1	60285776	6.90e^{-13}	$(GA)_7$	HEX	F: TTTCAGATGTGATTGGTGTTCAGC	570	63.36	56	24	0	–	447	–	Failed to amplify
	6H10		1A	58008408	3.00e^{-19}			R: CCACAGCTCTGCCTCTGTTAATATG		63.49							
Ppu085	HE616995	1	–	–	–	$(GT)_6$	HEX	F: TGCCCAGGGAAGTGGTTG	450	63.12	60	12	0	–	203	–	Failed to amplify
	6A05		2	31937001	6.90e^{-09}			R: AGTCCAACCATCAACCTAACTCTGAC		63.02							
Ppu093	HE617003	1	3	106292912	4.20e^{-60}	$(TAGA)_6$	HEX	F: AGCCATTTGCTTAATCCTACCTTCTG	813	63.93	60	12	0	–	230	–	Failed to amplify
	5C11		3	106323359	7.50e^{-40}			R: ATGGGAGGCCATGCAACAC		64.27							
Ppu107	HE617017	1	1	188022341	3.40e^{-68}	$(TTGTT)_9$	6-FAM	F: CTTTGGACATGCCAGCAGGA	316	65.04	56	24	0	–	215	–	Failed to amplify
	6F04B		1	77944747	4.20e^{-09}			R: CACAGACACTTCTTTAACAGTTATTCCAACC		64.61							
Ppu121	HE617031	1	6	33702631	7.60e^{-25}	$(AC)_5$	HEX	F: TTCCCTAACCAGCAATTAGCTATC	593	60.02	56	24	0	–	256	–	Failed to amplify
	65E06		6	32662952	1.80e^{-33}			R: ACGAGAGGCCAAATATGGTG		59.96							

Table 2 continued

Locus	EMBL acc. no. & clone name	Lib	CH chr ZF chr	Chicken chr loc. Ŧ Zebra finch chr loc. Ŧ	E-value in Gga E-value in ZF	Repeat motif	Fluoro label (F)	Primer sequence 5′–3′	Seq. length (bp)	T_m (°C)	PCR T_a (°C)	n_1	n_2	A	Exp allele size (bp) ¥	Obs allele size (bp)	Marker status
Ppu125	HE617035	1	1	73188968	$5.70e^{-30}$	$(AC)_5$	HEX	F: TCTATGCCTTAAATCCTATTTACAAAC	401	57.68	56	24	0	–	199	–	Failed to amplify
	65F08		1A	61734639	$4.60e^{-28}$			R: CAGCACTGTCATTGTTCTGC		57.98							
Ppu129	HE617039	1	3	31663341	$1.40e^{-36}$	$(AC)_7$	HEX	F: ACACCCACTATTTGTGAAGAGC	460	58.25	56	24	0	–	159	–	Failed to amplify
	66E12		3	31383766	$2.10e^{-47}$			R: GCTCTCCTTGTGCTTGTTTG		58.65							
Ppu134	HE617044	1	6	2107306	$6.80e^{-54}$	$(AG)_6$	HEX	F: TTGTGTCATTTGGATGCCTTAG	808	60.00	56	24	0	–	166	–	Failed to amplify
	66C09		6	6728242	$8.60e^{-34}$			R: GCTGGAGGGAAGCTTGTTC		59.94							
Ppu150	HE617060	1	15	3838997	$3.70e^{-39}$	$(CA)_6$	HEX	F: GATCGGAGCCTTCTTACCTG	307	58.89	56	24	0	–	232	–	Failed to amplify
	65C11		15	994445	$9.40e^{-23}$			R: CTGGCACTAATGTGTAGTCGTG		58.39							
Ppu154	HE617064	1	2	146180882	$5.00e^{-28}$	$(CA)_5$	HEX	F: ATCACACGTGCACCAAACAC	561	60.50	56	24	0	–	131	–	Failed to amplify
	66A01		2	147316308	$3.30e^{-42}$			R: GAGGTTTATGGTAATGAAGAAGTTGC		60.59							
Ppu155	HE617065	1	4	91803651	$7.00e^{-18}$	$(CA)_{10}$	HEX	F: GATCTAAGTTTCCTGGGATTGC	326	59.09	56	24	0	–	154	–	Failed to amplify
	66A03		4	68748861	$8.00e^{-30}$			R: GCTCCAAGCTCTTAGTCATGG		59.09							
Ppu161	HE617071	1	4	32471914	$7.90e^{-44}$	$(CA)_8$	6-FAM	F: ACTGATGGCAGAGCAGATTG	621	58.99	56	24	0	–	287	–	Failed to amplify
	67B07		4	12229126	$4.00e^{-86}$			R: TTTCTCTTCCTGCTAAATCCAAC		58.94							
Ppu163	HE617073	1	1	104968881	$7.50e^{-27}$	$(CA)_9$	6-FAM	F: GCCAGTGAGTAGTGGGTTCC	441	59.58	56	24	0	–	284	–	Failed to amplify
	67F11		1	112179239	$9.90e^{-18}$			R: AATAGCAAGCTGCATCACAAAG		59.56							
Ppu168	HE617078	1	2	89221409	$1.40e^{-56}$	$(CTTC)_{10}$	6-FAM	F: TCAGCATTGCCTTTACCTTTC	721	59.35	56	24	0	–	358	–	Failed to amplify
	65H12		2	92057916	$1.10e^{-68}$			R: TCCAGGGTTACAAACAAGAGC		59.23							
Ppu170	HE617080	1	2	70828859	$7.30e^{-27}$	$(CTTTCT)_8$	HEX	F: AAATATGTGAAATTGGTCCAACAG	856	59.21	56	24	0	–	175	–	Failed to amplify
	66E10		2	39737798	$2.10e^{-80}$			R: AACTTTCCAACTGTTTCAGTTCC		58.73							
Ppu173	HE617083	1	1	58206176	$1.50e^{-39}$	$(GA)_8$	6-FAM	F: ACTTGGGCAGAACCAGAATG	507	60.11	56	24	0	–	319	–	Failed to amplify
	67G04		1A	56094038	$2.30e^{-80}$			R: AGAGTATGCTGGGCTTGTGG		60.28							
Ppu184	HE617094	1	1	7368828	$1.30e^{-09}$	$(GT)_6$	HEX	F: GGGTTACAGCTGTTTGTATTTCC	206	58.99	56	24	0	–	132	–	Failed to amplify
	64E01		1A	6252519	$7.00e^{-13}$			R: AAACTGTAAGTTATTCCAAAGGAACC		59.35							
Ppu236	HE617146	1	3	97005426	$4.00e^{-29}$	$(GT)_6$	6-FAM	F: AACTCACCATATGTGCCAAGG	398	59.87	56	24	0	–	263	–	Failed to amplify
	66F08		–	–	–			R: CCAAGACATGCACTGTGTCC		60.16							
Ppu240	HE617150	1	–	–	–	$(GT)_{10}$	6-FAM	F: CCCAGGCTAGGAGCTTTG	338	58.54	56	24	0	–	259	–	Failed to amplify
	67A12		1A	48527357	$6.20e^{-23}$			R: TCTCATGTACTGTGATGTCTACTGTG		58.41							

Ŧ The location of each microsatellite sequence was assigned in the chicken (v 2.1, May 2006 ENSEMBL release) and zebra finch (December 2011 ENSEMBL Release 65) based on sequence homology (see Dawson et al. 2006, 2007). Of these 50 additional loci tested, 45 could be assigned a location in the chicken genome and 46 in the zebra finch genome.

n_1 Number of individuals tested in a captive population. n_2 Number of individuals amplified and genotyped. *A* Number of alleles observed, *M* male, *F* female

¥ Expected allele size based on the sequenced clone allele of the female ruff individual (Bird ID 6233) from which the genomic library was created

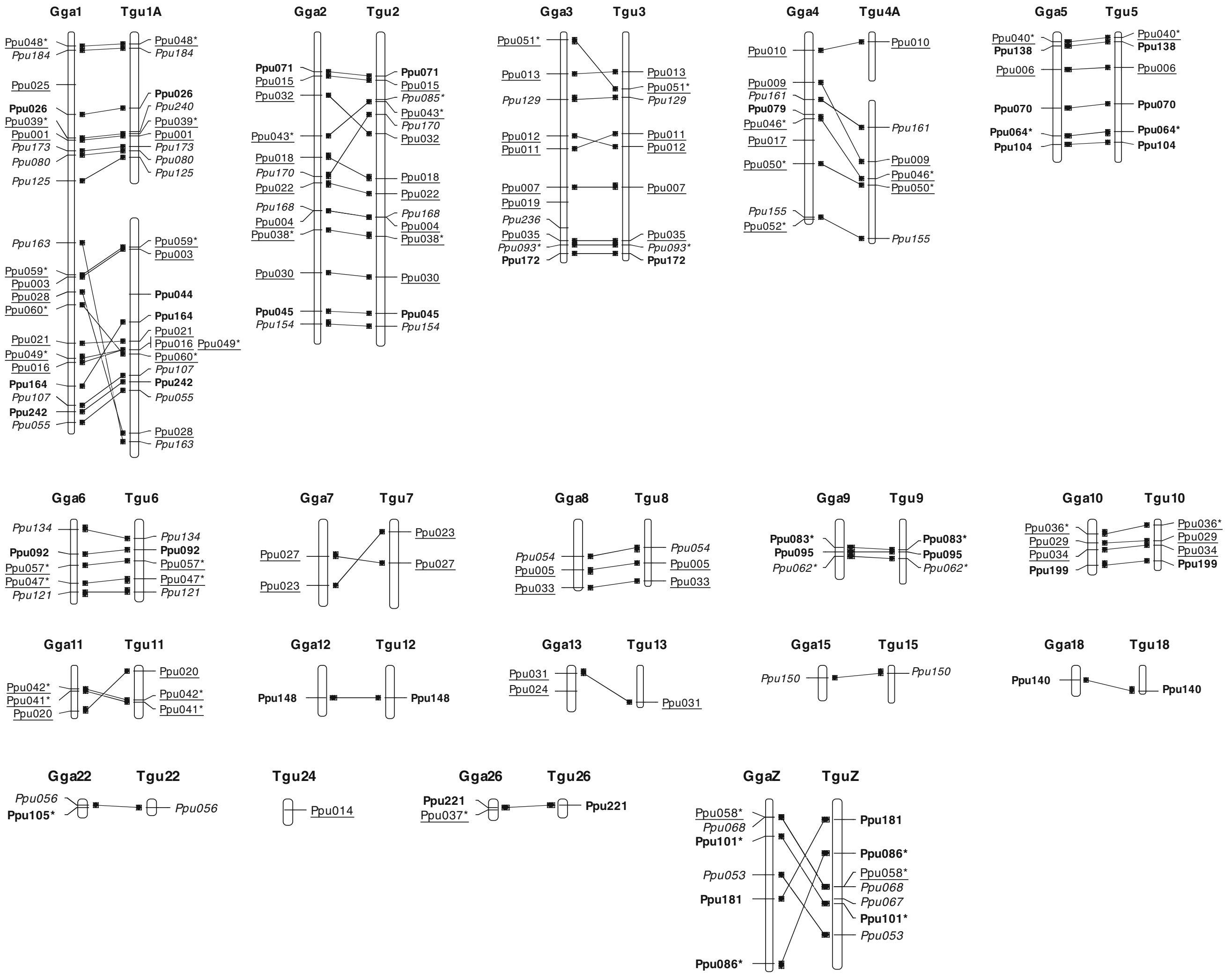


Fig. 1 Chromosomal locations in the chicken and zebra finch genomes of 102 ruff microsatellite loci. Locations were assigned as in Table 1. Gga, chicken (*Gallus gallus*) chromosome; Tgu, zebra finch (*Taeniopygia guttata*) chromosome; *Underlined* loci are polymorphic, loci listed in **bold** were monomorphic (*polymorphism assessed in 12 individuals; all other loci were tested in 24 individuals); Loci listed in *italics* failed to amplify a product or amplified a stutter/unreliable product (Table 2) and the loci in plain text have not been tested for amplification or polymorphism

software (Applied Biosystems). Observed and expected heterozygosities were calculated using CERVUS v3.0 (Kalinowski et al. 2007; Table 1). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP v.4.0 (Rousset 2008). All loci were assessed for Hardy–Weinberg equilibrium and linkage disequilibrium in captive individuals, selected to avoid parent–offspring relationships and full and half sibs. Polymorphic loci were suspected of Z-linkage if no females were heterozygous. Mendelian inheritance was assessed in 64 families, consisting of both parents and 5–33 offspring per family, comprising 381 known-sex individuals. Any Z-linked loci were tested for HWE in males only.

Of the 102 markers tested, 23 were monomorphic, 27 failed to amplify or amplified a stuttery/unreliable product, and 52 were polymorphic (Tables 1, 2). Five polymorphic autosomal loci (*Ppu001*, *Ppu003*, *Ppu009*, *Ppu010* and *Ppu016*) amplified a product >70 bp larger than the cloned sequence but were all inherited in a Mendelian fashion (Table 1). In total, 47 of the 52 polymorphic loci were checked for Mendelian inheritance, which was confirmed for all except *Ppu002* (Tables 1, 2).

A high proportion of sequences (85 %) could be assigned locations in both the zebra finch and chicken genomes based on sequence homology (following Dawson et al. 2006, 2007). Of the 102 newly-isolated ruff microsatellites, 95 could be assigned to a chromosome location in the chicken, 92 in the zebra finch and 87 in both chicken and zebra finch (Fig. 1). Only two loci could not be assigned a chromosomal location in either chicken or zebra finch (*Ppu002* and *Ppu008*, Tables 1, 2). There was no statistical difference in the number of sequences that could be assigned a location to each genome (Fisher's two-tailed Exact test $p = 0.66$). One locus (*Ppu058*) assigned to both the zebra finch and chicken Z chromosomes (Fig. 1), was homozygous in all of the 127 females genotyped, but heterozygous in 27 of the 140 males typed, confirming its sex-linked status (Fisher's two-tailed Exact test $p < 0.001$).

Twelve autosomal loci, assessed in 12 individuals, showed significant deviation from Hardy–Weinberg equilibrium ($p < 0.05$) or a deficiency of heterozygotes (Table 1). Three groups of loci showed evidence of linkage disequilibrium ($p < 0.05$; *Ppu010–Ppu016, Ppu036–Ppu038–Ppu041–Ppu042–Ppu051–Ppu052*, *Ppu037–Ppu040*; Table 1), however following FDR correction (Benjamini and Hochberg 1995) no *p*-values were significant. All loci in each group were assigned to different chromosomes, except *Ppu041–Ppu042*, which are closely neighbouring on chromosome 11 in chickens and zebra finches, and therefore may be physically linked (Table 1, Fig. 1). The deviation from Hardy–Weinberg equilibrium and linkage disequilibrium displayed by some groups of loci is probably due to the non-random structure of our captive population. However, 46 of the 47 loci checked displayed a pattern consistent with Mendelian inheritance (Tables 1, 2).

The microsatellite loci developed during this study are suitable for the analysis of parentage and population structure and will be used to construct a linkage map for the ruff. The utility of these loci in other shorebird species can be predicted based on the BLAST E-value recorded from a comparison of their sequences similarity with the chicken genome (Küpper et al. 2008, but see also Dawson et al. 2010). The sequences of three loci (*Ppu004, Ppu005* and *Ppu030*) displayed particularly high sequence homology to both the genetically distant chicken and zebra finch (BLAST E-values < E-70; Table 1). These loci are therefore expected to amplify in a wide range of species, including those of conservation interest. Additionally, if required, these specific loci would be the most suitable to develop into conserved markers, which would further enhance their cross-species utility (Dawson et al. 2010). Any primer sets designed as a consensus among all three species (ruff, zebra finch and chicken) are expected to display the highest cross-species utility.

Acknowledgments This work was performed at the NERC Biomolecular Analysis Facility–Sheffield supported by the UK Natural Environmental Research Council. The captive ruff colony was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC; to DBL) and LLF was supported by an NSERC studentship.

References

Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped blast and psi-blast: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Armour JAL, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. Hum Mol Genet 3:599–605

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289–300

Dawson DA, Burke T, Hansson B et al (2006) A predicted microsatellite map of the passerine genome based on chicken–passerine sequence similarity. Mol Ecol 15:1299–1320

Dawson DA, Åkesson M, Burke T, Pemberton JM, Slate J, Hansson B (2007) Gene order and recombination in homologous regions of the chicken and a passerine bird. Mol Biol Evol 24:1537–1552

Dawson DA, Horsburgh GJ, Küpper C et al (2010) New methods to identify conserved microsatellite loci and develop primer sets of high utility—as demonstrated for birds. Mol Ecol Res 10: 475–494

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Kenta T, Gratten J, Hinten G, Slate J, Butlin RK, Burke T (2008) Multiplex SNP-SCALE: a cost-effective medium-throughput SNP genotyping method. Mol Ecol Res 8:1230–1238

Küpper C, Burke T, Székely T, Dawson DA (2008) Enhanced cross-species utility of conserved microsatellite markers in shorebirds. BMC Genomics 9:502–522

Lank DB, Smith CM, Hanotte O, Burke T, Cooke F (1995) Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. Nature 378:59–62

Nicholls JA, Double MC, Rowell DM, Magrath D (2000) The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). J Avian Biol 31:165–176

Primmer CR, Møller AP, Ellegren H (1996) A wide-range survey of cross-species microsatellite amplification in birds. Mol Ecol 5:365–378

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Bioinformatics methods and protocols: methods in molecular biology, pp 365–386. Humana Press, Totowa, NJ

Sibley CG, Monroe BL (1990) Distribution and taxonomy of birds of the world. Yale University Press, New Haven, CT

Thuman K, Widemo F, Piertney SB (2002) Characterization of polymorphic microsatellite DNA markers in the ruff (*Philomachus pugnax*). Mol Ecol Notes 2:276–277