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MICROSATELLITE LETTERS

Characterization of microsatellite markers for *Saxicola* species

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Abstract We characterized 28 unique African stonechat (*Saxicola torquata*) microsatellite loci. Seventeen loci characterized in 24 unrelated *Saxicola torquata axillaris* individuals sampled at Mount Meru, Tanzania displayed 2–26 alleles per locus and observed heterozygosities ranged from 0.29 to 0.92. Heterozygous females and sequence similarity suggested all 17 loci were autosomal. All markers also successfully amplified in nine different species ranging from Europe to Asia, including three endemic Island species. These microsatellite markers will be useful to assess the genetic diversity of the large and widely distributed genus *Saxicola*, a group comprising 11 recognized species with evidences of cryptic diversification. Several species show a narrow range distribution and are of conservation concern.

Keywords Microsatellite · Passerine · Predicted genome locations · *Saxicola torquata* · Sequence tandem repeats (STR) · Songbird

Here, we present a new set of microsatellite markers isolated from a songbird, the African stonechat (*Saxicola torquata axillaris*), which will be useful to assess genetic diversity for conservation efforts in other *Saxicola* species. An Illumina paired-end library was created using 1 μg of genomic DNA extracted from a female *S. t. axillaris* (AR02F) by following the standard protocol of the SureSelect Library Prep Kit, ILM (Agilent Technologies Inc. Santa Clara, California). DNA sequencing was conducted using a MiSeq Benchtop Sequencer (Illumina Inc., San Diego, California).

Primer sets were designed for 28 unique microsatellite sequences (EMBL accession numbers: HG798924–HG798951) using PRIMER3 v0.4.0 (Rozen and Skaletsky 2000). Blood samples were obtained from 24 unrelated

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Table 1 Microsatellite loci for the *Saxicola* species: (a) primer sequences and characteristics of 21 autosomal African stonechat (*Saxicola torquata axillaris*) microsatellite loci, (b) cross amplification of 16 *Saxicola torquata axillaris* microsatellite loci for ten *Saxicola* species

Locus	EMBL accession number and clone name	Repeat motif	Primer sequence (5′–3′)	T_m (°C)	n	Exp. allele size (bp)	Obs. allele size range and genotype of AR02F[¥] (bp)	N_A	H_E	H_O	$_P$HWE	Est. null allele freq.
(a)												
Stor01	HG798924	$(ATCC)_{16}$	F:[HEX]CTCATCCTCTGCTTCCATCTG	59.96	24	194	181–209	19	0.81	0.85	0.63	0.02
	STC10640		R:GATCAACTGATGTCATCCATGC	60.36			193, 205					
Stor02	HG798925	$(GT)_{10}$	F:[6FAM]TGGGCTCAAATTGAACTGTG	59.69	23	198	194–202	6	0.64	0.63	0.14	0.01
	STC10944		R:CTTTGTGCTGCTGCTTTCAC	59.79			198					
Stor03	HG798926	$(ATCC)_{13}$	F:[HEX]AACTGGAGGTCCAGGCATC	60.06	22	161	159–183	15	0.92	0.84	1.00	−0.05
	STC12196		R:GAGTGGCCTGTGTGTGGAG	60.31			159, 171					
Stor04	HG798927	$(ATGTT)_{21}$	F:[HEX]TCCTAAATGCGACATTGCAC	59.69	24	199	151–207	19	0.85	0.89	0.05	0.02
	STC13188		R:GCAAGGTAAATTTGTCTTCTTGTG	59.27			191, 197					
Stor05	HG798928	$(AC)_{13}$	F:[HEX]GCCCACTCAGCTGACACAC	60.48	21	199	191–197	12	0.29	0.27	1.00	−0.07
	STC13366		R:TGTGTTGTGCATCATAGTGTATGG	60.73			195					
Stor06	HG798929	$(AC)_{15}$	F:[6FAM]CTTGTTGCTCACCCTGTGG	60.29	21	156	145–159	14	0.71	0.65	0.80	−0.05
	STC15546		R:TAGAGGCAGCCAACCTTCTG	60.53			151, 153					
Stor07	HG798930	$(GT)_{15}$	F:[6FAM]CTGTCTGGGCATGAGAAGG	59.35	24	153	139–153	8	0.65	0.65	0.28	−0.03
	STC18373		R:TTTGCAGTCAGTCAGTACAAAGC	59.62			143, 153					
Stor08	HG798931	$(ATCC)_{18}$	F:[6-FAM]CACAGCTGCTCTGGGAATC	59.52	24	162	220–236	9	0.50	0.38	0.27	−0.14
	STC2321		R:ACAAGGGATGGAGGGACAG	59.90			224					
Stor09	HG798932	$(GT)_{13}$	F:[HEX]TCGGTGTCTGTTGGTATTGC	59.57	19	200	200–224	8	0.74	0.77	0.69	0.01
	STC2764		R:GCAGCTGCCTTTCTGTATGTC	60.04			202, 208					
Stor10	HG798933	$(TAA)_{13}$	F:[6FAM]TTGAAAGGTTACCCTGTTTGTG	59.04	23	210	195–221	26	0.89	0.85	0.46	−0.03
	STC3033		R:GGTACATTTCTGCTTTCAGATCC	59.15			207, 213					
Stor11	HG798934	$(GT)_{15}$	F:[HEX]AGAGTGGCAACTTGTTCTTGG	59.39	24	143	139–147	15	0.71	0.67	0.84	−0.04
	STC3205		R:TGTACCAGCTCTGCATCATTG	59.88			139, 143					
Stor12	HG798935	$(TGA)_{18}$	F:[HEX]GGCAGTGGCATCTGATTTG	60.22	24	217	193–239	23	0.35	0.29	0.56	−0.09
	STC3964		R:GGGAGATTCTCAACAGTGCAG	59.86			193					
Stor13	HG798936	$(TGA)_{19}$	F:[HEX]TGCTGACAAAGGGAGAAAGC	60.52	23	214	189–215	25	0.86	0.86	0.12	−0.01
	STC4021		R:CTGTAATCCGGCAGCACAC	60.28			193, 205					
Stor14	HG798937	$(GT)_{12}$	F:[6-FAM]CCATGTAAAGCACTTCCAAACC	60.75	22	141	138–144	8	0.75	0.75	0.42	−0.01
	STC4937		R:GGGAGAGGCAGGAACTGG	60.76			142					
Stor15	HG798938	$(CT)_{11}$	F:[6FAM]TCATTAAGGTTTGACTGTGTTGC	59.19	21	125	120–124	7	0.46	0.49	0.05	0.06
	STC7592		R:AAGGGCAAGATTTCCTGTTG	59.17			120, 124					
Stor16	HG798939	$(AC)_{15}$	F:[6FAM]AAACAGAACAAACCTCCATGTG	59.00	23	201	194–206	5	0.60	0.64	0.65	0.02
	STC11720		R:CTCCAGCCTATTGTATCATCACTATC	59.49			200					
Stor17	HG798940	$(CAAA)_{13}$	F:[HEX]AACAGAACTGCTGGCAACTG	59.09	22	232	221–237	5	0.70	0.76	0.15	0.02
	STC2521		R:TTGATGGTTTCAGCATGGTC	59.50			233					

Table 1 continued

Locus	EMBL accession number and clone name	Repeat motif	Primer sequence (5′–3′)	T_m (°C)	n	Exp. allele size (bp)	Obs. allele size range and genotype of AR02F¥ (bp)	N_A	H_E	H_O	$_PHWE$	Est. null allele freq.
Stor18*	HG798941	$(CTG)_{11}$	F:[HEX]GAGTCTCCAAGCTCTGCCTTC	60.66	24	155	145–170	7	0.69	0.71	**0.02**	0.02
	STC10500		R:TCCTTGAGGAGAGGGTTAAAGG	60.93			–					
Stor23*	HG798946	$(GGAT)_{16}$	F:[HEX]GCATCCTGAGACCATGTGTG	60.12	24	193	191–215	8	0.83	0.79	**0.01**	−0.03
	STC2689		R:CGTCCATACCCATGTCAGTG	59.83			195, 199					
Stor24*	HG798947	$(ATCC)_{22}$	F:[6FAM]AGAGCTGAGTCCTTCCCAAAG	60.00	23	240	238–256	5	0.48	0.90	**0.00**	0.30
	STC4625		R:CCTGGATCAGGCAAGTGG	60.20			–					
Stor26*	HG798949	$(ATCC)_{12}$	F:[HEX]AATTCTTCATCCCATTTCCATTATAG	59.86	23	152	137–159	7	0.79	0.76	**0.00**	0.11
	STC6257		R:GAGCTGGGACCACAAGATTC	59.66			–					

Locus	*S.t. axillaris* Kenya (24 tested)			*S.t. rubicola* Spain (30 tested)					*S. caprata* Indonesia (7 tested)		*S. dacotiae* Canary Islands (24 tested)				
	Exp. allele size (bp) ¥	n	Obs. allele size range and genotype of AR02F¥ (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$	n	Obs. allele size range (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$
(b)															
Stor01	194	24	181–209 193, 205	24	160–230	0.81	0.83	**0.03***	6	182–226	19	182–202	0.63	0.74	1.00
Stor02	198	23	194–202 198	22	186–206	0.48	0.46	**0.007***	7	190–204	21	192–198	0.18	0.14	0.14
Stor03	161	22	159–183 159, 171	26	155–177	0.73	0.73	0.21	6	159–179	19	155–177	0.82	0.90	0.90
Stor04	199	24	151–207 191, 197	21	133–201	0.88	0.86	0.92	6	133–191	20	137–187	0.87	0.65	**0.03***
Stor05	199	21	191–197 195	27	181–197	0.14	0.11	0.11	6	189–195	22	187–193	0.31	0.36	1.00
Stor06	156	21	145–159 151, 153	26	147–155	0.55	0.54	0.05	7	139–153	22	149–153	0.53	0.59	0.84
Stor07	153	24	139–153 143, 153	27	150–158	0.67	0.	0.14	7	144–174	22	148–150	0.30	0.36	1.00
Stor08	162	24	220–236 224	26	224	–	–	–	5	224	20	224	–	–	–
Stor09	200	19	200–224 202, 208	25	200–236	0.88	0.88	1.00	5	202–230	20	190–200	0.31	0.20	**0.01***
Stor10	210	23	195–221 207, 213	25	190–194	0.18	0.12	0.20	6	186–190	20	190	–	–	–

Table 1 continued

Locus	*S.t. axillaris* Kenya (24 tested)			*S.t. rubicola* Spain (30 tested)					*S. caprata* Indonesia (7 tested)		*S. dacotiae* Canary Islands (24 tested)				
	Exp. allele size (bp) ¥	n	Obs. allele size range and genotype of AR02F[¥] (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$	n	Obs. allele size range (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$
Stor11	143	24	139–147 139, 143	26	139–173	0.87	0.65	**0.005***	7	133–159	20	133–163	0.05	0.05	–
Stor12	217	24	193–239 193	27	181–211	0.87	0.81	0.37	7	177–205	22	181–191	0.09	0.09	1.00
Stor13	214	23	189–215 193, 205	24	184–214	0.88	0.75	**0.01***	6	166–224	20	190–198	0.23	0.25	1.00
Stor14	141	22	138–144 142	25	137–159	0.50	0.48	1.00	6	135–159	18	137–143	0.29	0.33	1.00
Stor15	125	21	120–124 120, 124	28	121–135	0.57	0.46	**0.001***	7	123–167	22	117–129	0.50	0.41	0.60
Stor16	201	23	194–206 200	27	194–202	0.58	0.52	0.08	7	182–204	21	194–196	0.05	0.05	–

Locus	*S. gutturalis* Timor-Leste (6 tested)		*S. insignis* Nepal (1 tested)	*S. maura* Kazakhstan (4 tested)		*S. rubetra* Spain (15 tested)					*S. sibilla* Madagascar (6 tested)		*S. tectes* Réunion (1 tested)
	n	Obs. allele size range (bp)	Obs. allele size range (bp)	n	Obs. allele size range (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$	n	Obs. allele size range (bp)	Obs. allele size range (bp)
(b)													
Stor01	3	172–226	–	1	198–226	4	160–226	0.86	0.75	0.64	4	190–218	206–214
Stor02	5	190–204	194–196	2	192–196	8	190–202	0.77	0.50	0.05	6	198–202	200–202
Stor03	6	147–175	155–163	2	163	11	147–187	0.87	0.55	**0.03***	6	163–219	183–191
Stor04	3	147–207	137–145	2	149–161	12	131–159	0.88	0.75	**0.005***	6	151–217	193
Stor05	6	187–195	191–193	3	189–191	14	177–203	0.70	0.71	0.40	6	195–199	195
Stor06	5	143–159	–	3	149–151	13	139–153	0.89	0.85	0.68	6	145–155	151–153
Stor07	6	128–158	150	3	146–156	12	144–204	0.91	0.67	**0.002***	6	144–166	150–152
Stor08	4	224	224	2	224	11	224	–	–	–	6	224	224
Stor09	4	196–218	–	2	190–202	11	196–220	0.84	0.46	**0.0003***	5	200–214	204–214
Stor10	5	178–192	186–190	2	190–194	11	172–196	0.75	0.72	1.00	4	190–210	190–196
Stor11	3	133–163	133–163	4	143–163	14	133–167	0.73	0.71	0.72	6	137–159	141–145
Stor12	6	181–207	181–187	3	181–191	14	177–209	0.87	0.71	**0.03***	6	191–235	205–209
Stor13	5	192–208	182–198	2	194–208	9	176–230	0.92	0.89	0.63	3	188–220	194–196

Table 1 continued

Locus	*S. gutturalis* Timor-Leste (6 tested)		*S. insignis* Nepal (1 tested)	*S. maura* Kazakhstan (4 tested)		*S. rubetra* Spain (15 tested)				*S. sibilla* Madagascar (6 tested)		*S. tectes* Réunion (1 tested)
	n	Obs. allele size range (bp)	Obs. allele size range (bp)	n	Obs. allele size range (bp)	n	Obs. allele size range (bp)	H_E	H_O	$_PHWE$	n	Obs. allele size range (bp)	Obs. allele size range (bp)
Stor14	4	153	137–143	2	155–163	10	137–159	0.92	0.90	0.77	6	137–141	137
Stor15	5	129–159	117–129	3	121–137	12	119–177	0.97	0.95	1.00	6	119–121	121
Stor16	6	182–228	196–198	4	198–210	13	176–209	0.89	0.81	1.00	5	196–204	196–200

S. t. axillaris Saxicola torquata axillaris, *S. t. rubicola Saxicola torquata rubicola*, ¥ based on the sequenced *Saxicola torquata axillaris* individual from which primers were designed (AR02F)

Tm melting temperature (°C) of primer, *bp* base pairs, *Exp.* expected, *Obs.* observed allele size, *NA* number of alleles observed, *n* number of individuals genotyped, sex based on adult plumage, H_O observed heterozygosity, H_E expected heterozygosity, $_PHWE$ probability of deviation from Hardy–Weinberg equilibrium, *Est.* estimated null allelic frequency

* Loci (shown in bold) and taxa, which deviated from Hardy–Weinberg equilibrium ($p < 0.05$):

Stor18, *Stor23*, *Stor24*, and *Stor26* or *Saxicola torquata axillaris*

Stor01, *Stor02*, *Stor10*, *Stor13* and *Stor15* for *Saxicola torquata rubicola*

Stor04 and *Stor09* for *Saxicola dacotiae*

Stor03, *Stor04*, *Stor07*, *Stor09* and *Stor12* for *Saxicola rubetra*

adult *S. t. axillaris* individuals sampled at Mount Meru, Arusha National Park, Tanzania. Genomic DNA was extracted using an ammonium acetate precipitation method. Primer sets were amplified individually in all 24 individuals. Each 2-µl PCR contained approximately 10 ng of air-dried genomic DNA, 0.2 µM of each primer and 1 µl QIAGEN PCR mix (QIAGEN Inc.). PCR amplification was performed using a DNA Engine Tetrad ®Thermal Cycler (MJ Research, Bio-Rad, Hemel Hempstead, Herts, UK) with the following program: 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 90 s, 72 °C for 60 s, 45 cycles of 60 °C for 30 min. Locus-specific products were loaded separately on an ABI 3730 48-well capillary DNA Analyzer (Applied Biosystems, California, USA) and allele sizes assigned using GENEMAPPER v4.1 (Applied Biosystems). The numbers of alleles and heterozygosities were calculated for each locus using CERVUS v3.0.6. Tests for deviation from Hardy–Weinberg equilibrium and linkage disequilibrium were conducted using GENEPOP web version 4.2. To identify sex-linked loci, 318 (76 females, 242 males) *S. t. axillaris* individuals were assessed for heterozygosity (sex based on adult plumage and the Z-002A and Z-002D sex-typing markers; Dawson 2007) and the chromosomal location of each locus was assigned in the zebra finch (*Taeniopygia guttata*) assembled genome by performing a BLAST search for sequence similarity (via http://www.ensembl.org/index.html), following Dawson et al. (2006; homologous sequences possessed E-values less than E−05) and a figure created using MAPCHART v2.2.

Of the 28 markers tested, 21 were polymorphic with 2–26 alleles, two were monomorphic, and five amplified multiple non-specific products despite testing at various annealing temperatures (Table 1 and Online Resources 1 and 2). Loci *Stor20* and *Stor21* were monomorphic with the same allele sizes in three other populations (data not shown). None of the 21 polymorphic loci were sex-linked based on the presence of female heterozygotes and sequence similarity to zebra finch autosomes (Fig. 1). Locus *Stor26* displayed a high estimated null allele frequency (over 10 %). No groups of loci exhibited evidence of linkage disequilibrium.

Sixteen selected markers (*Stor01–Stor16*) were assessed in nine other *Saxicola* species, including taxa classified as near threatened (*S. dacotiae* and *S. gutturalis*) or vulnerable (*S. insignis*; Table 1 and Online Resource 1). Between 11 and 16 loci were variable per species (1–24 individuals tested per species). The avian genus *Saxicola* is distributed throughout Africa, Asia, Europe and various islands across Oceania (Illera et al. 2008). Many species within this complex are listed as endangered. Thus, these microsatellites will be useful to assess genetic diversity for conservation efforts.

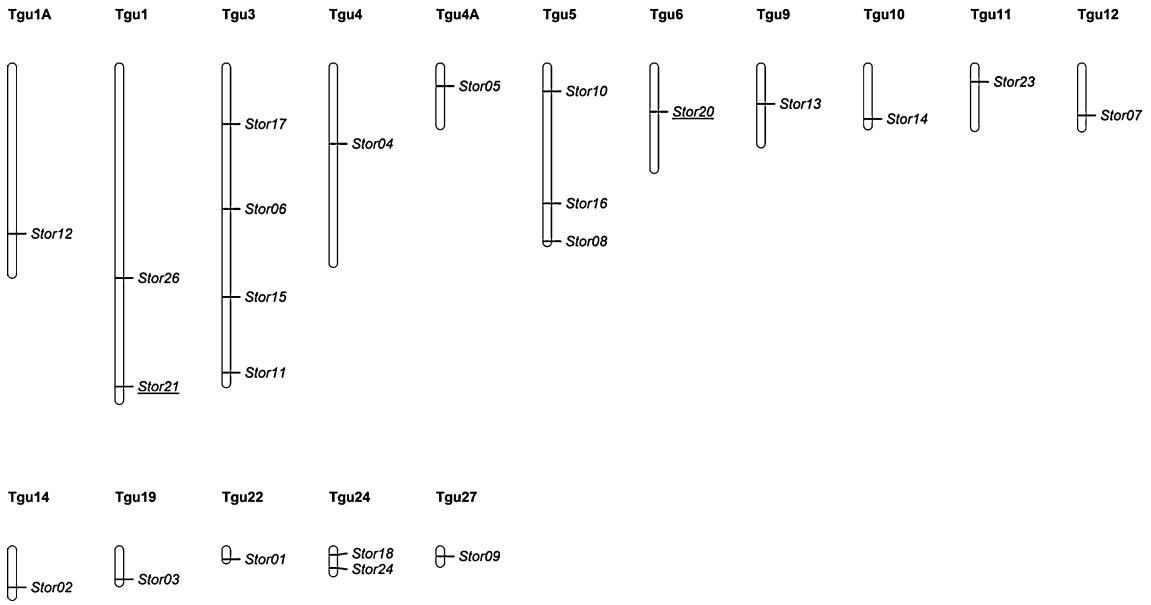

Fig. 1 Chromosomal locations of 23 African stonechat (*S. torquata*) loci in the zebra finch *T. guttata* (Tgu) genome. The two monomorphic loci are underlined. Chromosome locations are not assigned for the five loci that amplified multiple non-specific products

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