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Short Communication

Characterization of 22 New Polymorphic Microsatellite Loci from the Endangered Buff-Throated Partridge (*Tetraophasis szechenyii*) by using Next-Generation Sequencing

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QL and JHR designed the research, performed the experiment and data analyses and drafted the article. FWL completed the part of experimental operation. NY collected the samples. XYZ and JHR critically revised the manuscript.

Key words
***Tetraophasis szechenyii*, Tetranucleotide microsatellite, DNA molecular markers, Polymorphism, Next-generation sequencing.**

ABSTRACT

The buff-throated partridge (*Tetraophasis szechenyii*) is an endemic species which is protected in first-grade state in China. Here, 22 polymorphic tetranucleotide microsatellite markers were isolated from *T. szechenyii* using a next-generation sequencing technology. The allele number of these loci ranged from two to six in genotyped 35 individuals. Polymorphism information content ranged from 0.2735 to 0.7717 with an average of 0.5149. Observed and expected heterozygosities at each locus ranged from 0 to 0.879 and 0.332 to 0.814, respectively. These markers could be used to better understand the breeding system and protection manage of this species.

The buff-throated partridge (*Tetraophasis szechenyii*) is an endemic species in China, which belongs to the family Phasianidae in the Galliformes (Johnsgard, 1988). The species occurs in a narrow zone of Southeast Tibet, South Qinghai, West Sichuan and Northwest Yunnan, and inhabits mainly in mixed coniferous forests, rhododendron shrubs, oak thickets, alpine meadows and rocky ravines at 3350-4600m ASL (Mackinnon *et al*., 2000). Due to poaching, the habitat destruction or fragmentation as a result of deforestation, the number of its stocks is rapidly decreasing, and it has been considered to be endangered in the Red Book of China and has been classified as a national first-grade protected animal in China. From 2006-2008, we found an interesting cooperative breeding in a wild population of *T. szechenyii* (Xu *et al*., 2011; Wang *et al*., 2017), which is rare in the Galliformes (Cockburn, 2006). However, the behavioral field data did not allow any conclusions regarding the reproductive contributions of differently ranked males. Therefore, investigations on the kinship and breeding system in buff-throated partridge using molecular data are necessary, and it would provide useful information for conservation management of this endangered species.

As co-dominant molecular marker, microsatellites were powerful tools for genetic identification, parentage and kinship analysis (Christiakov *et al*., 2006; Henry *et al*., 2013). Previously, dozens of microsatellite loci were identified and characterized by using cross-amplification and traditional enrichment method (Zhou *et al*., 2009; Wu *et al*., 2010; Yan *et al*., 2011). However, most of these loci were dinucleotide. Compared to tri- and tetra-nucleotide, dinucleotide has been generally to display a high level of stutter bands, which easily and frequently cause scoring errors if the two alleles are closely spaced (Perlin *et al*., 1995). More, our previous preliminary parentage analysis based on dinucleotide loci indicated that different dinucleotide loci may result in different results. Here, we first report 22 tetranucleotide microsatellite DNA markers isolated for *T. szechenyii* using the GS Junior (Roche) next-generation sequencer, it would be a great benefit to understand breeding system of this species.

Materials and methods

Blood samples of buff-throated partridge were

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Table I.- Primer sequences and characteristics of 22 tetranucleotide microsatellite loci isolated in *Tetraophasis szechenyii*.

Locus/ Accession No.	Primer sequence (5'-3')	Repeat motif	Tm (°C)	N	NA	Size (bp)	H_o	H_e	PIC	PHWE
TSZ-4/ KU236435	F: GGAACTGGTGGATGCAGTTG R: ACCACACTTAGGGTAACTGGA	(TTAT)9	59.0	34	3	285-293	0.3824	0.5263	0.4642	0.019
TSZ-5/ KU236436	F: GACAGACTGGGCAGAGATGT R: GGAGCCACGAAGAAAAGCAA	(GGAT)8	61.5	33	5	197-217	0.8788	0.7422	0.6905	0.030
TSZ-6/ KU236437	F: CTGTCCTGGTTCTGTCCCAT R: TGATGCTCATTTTGACTTTGGGA	(GTTT)8	62.0	33	5	174-214	0.3939	0.6541	0.5820	0.011
TSZ-7/ KU236438	F: AGCCTAGCCTGTTCAACCAT R: TGATCATCTTCCAGCCAGCA	(CAAA)8	61.5	34	2	260-264	0.0000	0.3319	0.2735	0 (<0.001)
TSZ-8/ KU236439	F: AGTGACCTGGTGATGGATGG R: GGAGTTGTGACACTGAGGGA	(CATC)8	61.5	34	6	245-265	0.8529	0.8139	0.7717	0.329
TSZ-9/ KU236440	F: GCTGGCTTTTACGTCACTGG R: GCTGGAGGAGAGGCATATGT	(CTAT)8	59.0	34	5	172-216	0.2941	0.4175	0.3817	0.089
TSZ-10/ KU236441	F: ACAGCAATTCCAGCCTGTTG R: CGCTTATGAGACCAGTTGCC	(AAAC)10	64.0	34	5	228-260	0.4412	0.6870	0.6264	0.025
TSZ-11/ KU236442	F: CTGTCCTGGTTCTGTCCCAT R: GACTTGGAAGAGGGAAATGCA	(GTTT)8	59.0	34	3	337-343	0.2647	0.4614	0.4068	0.010
TSZ-14/ KU236443	F: ACAGGGCAGCTATTGTGTTG R: CACTCTCACAAATGCTGCCA	(ATCT)10	60.5	33	5	282-298	0.6667	0.7110	0.6611	0.104
TSZ-17/ KU236444	F: GTCCAAGCTCTCCCACTGAT R: TCTGGGTCGGTAATGCTGTT	(CAAA)8	64.0	34	3	150-162	0.7941	0.5088	0.3895	0.0002 (<0.001)
TSZ-19/ KU236445	F: AAACCTTCCCTGTCTCCCTC R: AGCATGTCCTCTAGTGGCAA	(CAAA)9	62.0	32	6	231-253	0.4688	0.6141	0.5730	0.002
TSZ-20/ KU236446	F: GCCATTGGTTAGGCTTCAGG R: TCTGGGTCGGTAATGCTGTT	(CAAA)8	64.0	34	2	266-270	0.6765	0.4965	0.3695	0.033
TSZ-22/ KU236447	F: GCTGTGAGTGTGAACTGTGG R: TCAGTTCAGCACATAACTCTGT	(AAGG)17	60.5	33	6	245-279	0.4242	0.7828	0.7392	0 (<0.001)
SCZ-2/ KU236448	F: TTCGTGATTGCCTCCTATCC R: CTCTGTGCCATTCATGGAAAT	(AAAT)12	62.5	34	6	200-224	0.7353	0.7243	0.6772	0.268
SCZ-4/ KU236449	F: TGTTCTACAAATCCCTCTGTACCA R: ATAAGCCCGCCTCCTAAAAA	(AAAT)11	62.7	34	3	224-232	0.4412	0.5404	0.4760	0.037
SCZ-6/ KU236450	F: CAGGGCAGCTATTGTGTTGA R: CTCAGGTATGGGCTTAACAGG	(TATC)10	63.0	34	5	182-198	0.7059	0.7221	0.6738	0.291
SCZ-9/ KU236451	F: CCCAGGGACTGTTTCTTCAG R: TGACAACAAACCACACTTAGGG	(TTAT)8	63.7	34	4	224-236	0.4706	0.5588	0.4965	0.036
SCZ-10/ KU236452	F: ATTCAGAGCCAGGTTTGCTG R: CTTTGGAACTGAAACCCAGAA	(TTAT)8	62.0	34	6	200-224	0.5000	0.6062	0.5320	0.201
SCZ-13/ KU236453	F: TGAGTGGAAACAGGAAAACAGA R: CCAGTTTGGCAAGCAATAGG	(TTTA)9	65.7	34	3	250-258	0.5000	0.4649	0.4136	0.194
SCZ-18/ KU236454	F: TTGAAATCTTTACCCTCCCTGA R: GTGGCAGAAAGGTTGGAAAG	(AAAC)6	63.5	34	3	145-161	0.3235	0.3376	0.2862	0.409
SCZ-22/ KU236455	F: TCCTCTGCCCTGTTTTGCTA R: TTGACCAGGATGACTTTGGA	(AATA)7	64.9	34	2	214-218	0.4118	0.3652	0.2951	0.411
SCZ-24/ KU236456	F: GCACAACGTGGACTTTCTCA R: GCATCTGCCTTGCACTGAAT	(ATTT)7	65.5	34	5	266-286	0.6176	0.5944	0.5471	0.648

Abbreviations: F, forward primer; R, reverse primer; Tm, annealing temperature of primer pair; N, sample size; K, number of alleles; Ho, observed heterzygosity; He, expected heterzygosity; PIC, polymorphism information content; PHWE, probability of deviation from the Hardy-Weinberg equilibrium.

collected from 35 individuals in a wild population at Pualing Mountain, Yajiang County, Western China, and genomic DNA was extracted using E.Z.N.A. Tissue DNA Kits (Omega, USA). We constructed a shotgun genomic library using ~5µg of genomic from a single individual, which was sequenced using 454 Life Sciences Genome Sequencer FLX Titanium instrument (Roche Applied Science) at Shanghai Majorbio Biopharm Technology Co.,

Ltd. Over 185,734 unique reads with an average length of 447.7 bp were generated after quality filtering. We screened the high-throughput sequencing data to locate tetra-nucleotide microsatellite loci with at least seven perfect repeats by software MSDB2.4.2 (Du *et al*., 2013) and the primers were designed using the online software PRIMER. The PCR reaction mixture had a final volume of 25 µL, which contained 1 uL DNA (50ng/µL), 2.5 µL 10*PCR buffer (plus Mg^{2+}), 1 µL dNTPs(10 mmol/L each), 0.5 µL for each primer (10 µLmol/L), 0.5U rTaq DNA polymerase (Takara, Japan), and 18.7 µL ddH_2O. The amplification profiles include an initial denaturation at 95°C for 5 min, followed by 35 cycles 30s at 94°C, 45s at 59-65.7°C, 30s at 72°C, and a final extension for 10 min at 72°C (Table I). The PCR product size were measured using the ABI PRISM 377 Genetic Analyzer (Applied Biosystems) according each forward primer labeled with fluorescent dyes (FAM, TAMRA or HEX). Polymorphism information, observed and expected heterozygosities were calculated using CERVUS 3.0.3 software (Kalinowski *et al*., 2007).

Results and discussion

The 22 loci were subsequently used to screen all of 35 individuals. Using CERVUS 3.0.3 software to analysis, the number of allele in each locus ranged from two to six. The observed and expected heterozygosities (Ho and He) varied from 0 to 0.879 and 0.332 to 0.814, respectively; the PIC values ranged from 0.2735 to 0.7717 with an average of 0.5149 (Table I). Deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using GENEPOP 3.3 (Raymond and Rousset, 1995) and the presence of null alleles was assessed at a 95% confidence interval using MICRO-CHECKER 2.2.3 (Oosterbout *et al*., 2004). Three of the loci (TSZ-7, TSZ-17 and TSZ-22) showed significant deviation from HWE ($P<0.001$), suggesting the possibility of null alleles, non-random mating or Wahlund effect. There was no evidence of significant LD for all pairs of loci ($P<0.005$). The combined first-parent non-exclusion probability for the 22 markers was 0.00701993, and the second-parent non-exclusion probability was 0.00006956, as calculated by CERVUS 3.0.3 (Kalinowski *et al*., 2007).

The microsatellite markers described here will be useful for conservation genetic studies of the buff-throat partridge, such as evaluating the genetic diversity, exploring population structure, and understanding the breeding system of the species.

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Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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