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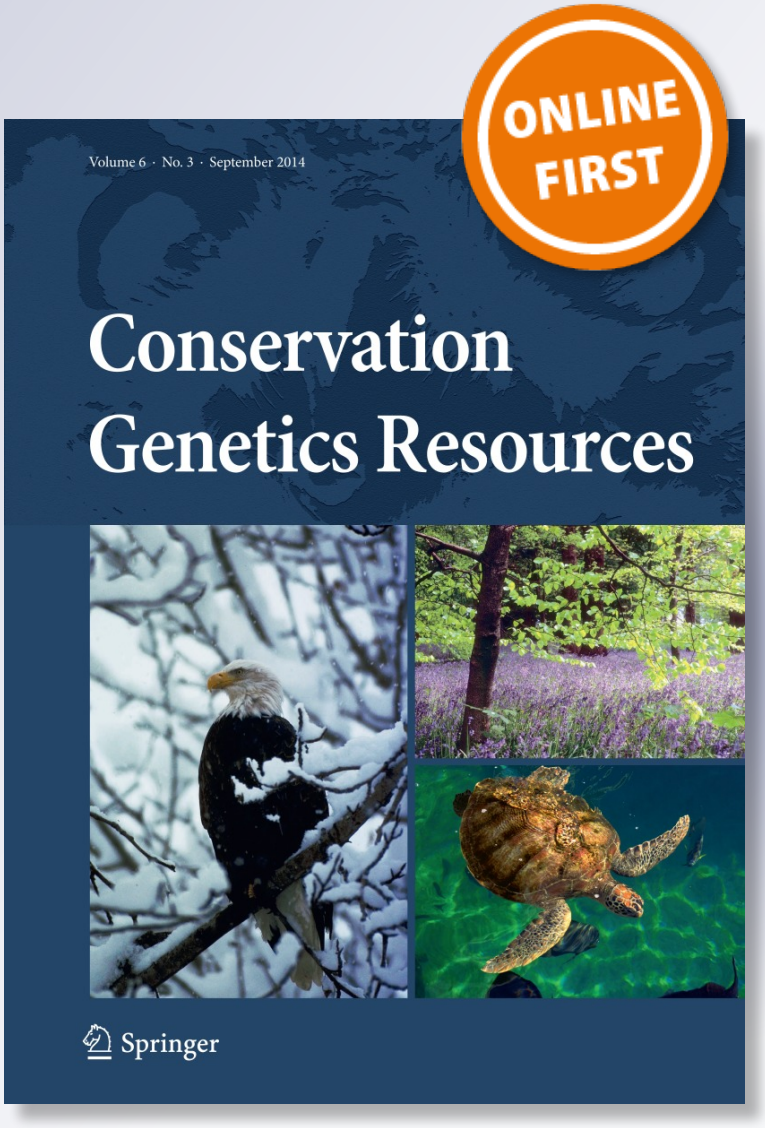
Conservation Genetics Resources

ISSN 1877-7252

Conservation Genet Resour
DOI 10.1007/s12686-014-0327-0



Springer

Conservation Genet Resour
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MICROSATELLITE LETTERS

Development and characterization of nine polymorphic microsatellite markers for the blue sheep (*Pseudois nayaur*)

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Received: 13 August 2014 / Accepted: 28 August 2014

Abstract *Pseudois nayaur* is a major component of Caprini and a keystone species in Qinghai-Tibet Plateau ecosystems. Due to the excessive hunting and habitat loss, its numbers are being reduced drastically. We developed and characterized the first set of microsatellite markers in this species to investigate its population genetic diversity and structure. Nine polymorphic loci displayed an average of 5.73 alleles (range between 2 and 14) and the levels of expected heterozygosity ranged from 0.422 to 0.816. Cross-amplification within dwarf blue sheep was successful for the nine loci. The loci characterized here will be useful for detecting fine-scale spatial structuring and resolving the taxonomic status of divergent *Pseudois* populations.

Keywords *Pseudois nayaur* · Microsatellite markers · Polymorphism · Population genetics

Min Yang and Quekun Peng have contributed equally to this work.

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Blue sheep (*Pseudois nayaur*), a Central Asian ungulate with restricted geographic distribution, exhibits extensive variation in morphology and phylogeographic structure (Zeng et al. 2008). The composition of species and subspecies in the genus *Pseudois* is controversial, particularly with respect to the taxonomic designation of geographically restricted populations (Tan et al. 2012). The genus *Pseudois* includes two morphologically variable taxa, blue sheep (*P. nayaur*) and dwarf blue sheep (*P. schaeferi*). Blue sheep have a wide distribution range across the entire Tibetan Plateau region, while dwarf blue sheep were only found in the gorges of the upper Yangtze River (Jinsha River) near Batang county, Sichuan province. As the body size of dwarf blue sheep is only about a half of blue sheep, its taxonomic status in the genus *Pseudois* has been argued for several decades since its discovery in 1937 (Schafer 1937). More genetic evidences from blue sheep and dwarf blue sheep are required to clarify this question.

The $(AC)_n$ microsatellite-enriched libraries were constructed based on the protocol described by Peng et al. (2011). The primers amplifying the microsatellites were designed using the software PRIMER5. Finally, 9 primer pairs were characterized using PCRs (Table 1). The primers that were characterized successfully using PCR were labeled with fluorescence and PCR products were analyzed using an ABI PRISM377 squencer and GeneMapper version 3.2. GENETIX 4.0.5 was used to check allelic richness and expected and observed heterozygosity. Hardy–Weinberg equilibrium and Linkage disequilibrium was tested by GENEPOP v.4.1.4 and the frequency of null alleles was estimated using MICRO-CHECKER.

DNA samples from 25 blue sheep were examined for the 9 loci. Cross-amplification within dwarf blue sheep was successful for the nine loci. The observed and expected heterozygosites (H_O and H_E) ranged from 0.167 to 0.792

Published online: 06 September 2014

Table 1 Primer sequences and characteristics of nine microsatellite loci isolated in *P. nayaur*

Locus	Primer sequence(5′–3′)	Repeat motif	Labeled dye	T_a (°C)	Size range (bp)	A	H_O	H_E	PIC	GenBank accession no.
PND01	F: CTACTGGACTGAGGCAAGATTTACC[a]	$(AC)_{19}AT(AC)_6$	HEX	61	144–174	4	0.792	0.562	0.487	KM272976
	R: ACTATTCGATGTCATAACTAGGGGAG									
PND02	F: CTAATCAGTCCCTTTGCTCC[a]	$(AC)_{22}$	FAM	55	190–228	9	0.542	0.715	0.666	KM272977
	R: CATCTCAGGTGGGGTGTCTT									
PND03	F: TCCAAAGAGTCAGATACAAT[a]	$(AC)_6$ $AT(AC)_5$ $AT(AC)_9$	TAMAR	55	102–116	4	0.542	0.587	0.483	KM272978
	R: TAACAGGACAACCACAGC									
PND04	F: CGACTGAAATGAAGGAAACGA[a]	$(CT)_{25}(CTCCCT)_3$	HEX	55	220–244	14	0.750	0.816	0.780	KM272979
	R: ACAACAAGGGGGATGGTGAA	$(CA)_{20}$								
PND05	F: CAAAGAGTTGGGTACAACTGGGTGA[a]	$(AC)_{17}$	FAM	60	166–174	5	0.167	0.479	0.439	KM272980
	R: GTTCCAATGTCGGAAGCATCTATTA									
PND06	F: GCCCAGGGAGGATAAGTAAC[a]	$(GT)_4ATCT(GT)_7CT$	HEX	59	183–189	3	0.625	0.536	0.424	KM272981
	R: TCCGTGGCAAAGAGTCAGC	$(GT)_{15}$								
PND07	F: GTTCCCGCAGATGGCATTATT[a]	$(GT)_{18}$	FAM	57	180–186	4	0.167	0.451	0.413	KM272982
	R: GAGAAGCCCAAGTAGCAACAAAG									
PND08	F: GTGTGAAATCATTACTGTCGGA[a]	$(GT)_7GAACAT(GT)_7$	TAMAR	55	93–121	2	0.333	0.422	0.328	KM272983
	R: CCACCTCTTAAATCAAGCAAAA									
PND09	F: ATAGTCAAGACGAGCAAAGAAAG[a]	$(AC)_{29}$	TAMAR	56	208–256	4	0.533	0.486	0.497	KM272983
	R: CTGCCTCCATAATCTGAACTG									

T_a Annealing temperature of primer pair, *A* number of alleles, *H_o* observed heterozygosity, *H_E* expected heterozygosity, *PIC* polymorphism information content

[a] Primers labeled with the fluorocchrome

and 0.422 to 0.816 respectively. The average of number of alleles per locus (A), polymorphism information content, and observed and expected heterozygosity (H_O and H_E) in the blue sheep were 5.73, 0.489, 0.574, and 0.505, respectively. No significant deviations from Hardy–Weinberg equilibrium and linkage association was found among all loci. Therefore, these 9 microsatellite loci were highly polymorphic, suggesting that these markers will be valuable for studies on the genetic structure of blue sheep populations.

Acknowledgments We are grateful to Ningxia Helan Mountain National Nature Reserve Administration for their assistance in this study. This research was supported by the Research Project of Chinese Ministry of Education (No. 113054A), and Sichuan Provincial Training Foundation for Academic and technical Leaders (2012DTP Y008), and National Natural Science Foundation of China (31401970).

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