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Short Communication

A core set of microsatellite markers for conservation genetics studies of Korean goral (*Naemorhedus caudatus*) and its cross-species amplification in Caprinae species

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In order to screen microsatellites for conservation genetics studies of the species, a total of 23 microsatellite loci from Korean goral (*Naemorhedus caudatus*), including 15 previously developed loci and 8 new loci in this study, were tested. Eleven microsatellites were screened and subjected to cross-species amplification using a test panel of four Caprinae species, Japanese serows (*Capricornis crispus*), Chinese gorals (*Naemorhedus goral*), Northern chamois (*Rupicapra rupicapra*) and domestic goats (*Capra hircus*). In addition, all eleven microsatellites (SY3A, SY12A, SY12B, SY48, SY58, SY71, SY76, SY84, SY84B, SY112, and SY129) satisfied the criteria to be a core set of microsatellites. This core set of microsatellites and cross-species amplification of Korean goral microsatellites were found to be helpful for high-resolution studies for conservation and management of Korean goral and other endangered Caprinae species.

Keywords: genetic diversity, Korean goral, microsatellites, *Naemorhedus caudatus*

The long-tailed goral (*Naemorhedus caudatus*) is the only mountain ungulate of the tribe Caprini found in Korea [4]. Since it is known that Korean goral populations have been reduced and fragmented by habitat reduction and road construction [11], understanding the current status of genetic diversity within and between populations is an essential component for their long-term survival. Few molecular studies measuring the genetic diversity of Korean goral have been carried out. Recently, 15 microsatellite loci were isolated and characterized for 20 Korean gorals [1]. Data from that study revealed that nine of 15 loci were in Hardy-Weinberg equilibrium (HWE) and only five of the 9 HWE loci exhibited more than 0.5 in polymorphic information content (PIC), an index of polymorphic level. Therefore, there is need for a sufficient number of polymorphic loci for genetic variability and population structure studies.

Recent studies have demonstrated that the cross-species amplification approach is advantageous as it allows population studies on species for which microsatellites have not yet been developed. Korean goral loci which had successful cross amplification in related endangered species such as Japanese serow (*Capricornis crispus*), Chinese goral (*Naemorhedus goral*), and Northern chamois (*Rupicapra rupicapra*), are thought to be helpful for the conservation genetics study of such species. Here, we report eight novel polymorphic microsatellite loci derived from a Korean goral genomic library and a core set of microsatellite markers that can be used across laboratories for future population genetics studies and conservation management of Korean goral. We also present cross-species amplification of Korean goral loci in other Caprinae species.

The enrichment protocols described by An *et al*. [1] were used to develop Korean goral specific microsatellites, with a slight modification by An *et al*. [1]. PCR amplification and genotyping were carried out as described by An *et al*. [1]. Genomic DNA from 38 Korean gorals, 10 Japanese serows, 7 Chinese gorals, 10 Northern chamois, and 5 domestic goats (*Capra hircus*) were tested in this study. Deviations from HWE and linkage disequilibrium between loci were tested by the Markov chain method implemented in GENEPOP version 4.0 [8]. Significant levels were adjusted using

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Table 1. Characteristics of the 8 newly developed and optimized loci for Korean goral (*Naemorhedus caudatus*)

Locus	GenBank accession No.	Primer sequence	Repeat motif	Dye	T (C)	*K*	Size range	Ho	He	PIC	*p*-value	±SE
SY14	DQ355011	F:5′-GGA ACC CTA CCA ATG CTC TG-3′ R:5′-CAA AGT GAA TCG CCC GTC-3′	$(CA)_{19}$	6FAM	56	6	98～126	0.211	0.558	0.521	0.0000*	0.0000
SY22	GQ120509	F:5′-GAG GAG AGG GTG TTT ATT TTG C-3′ R:5′-GAG AAT GCT GGA GTG GGT TG-3′	$(GT)_{17}$	HEX	60	5	230～242	0.123	0.590	0.534	0.0000*	0.0000
SY71	DQ355013	F:5′- TGG AGT TTA GGG GCA GGA-3′ R:5′- CAC AGT GAG TAT TGT TTT GCT TAT TA-3′	$(GT)_2(GT)_{23}$	6FAM	54	9	93～113	0.711	0.756	0.710	0.0563	0.0045
SY76	DQ355014	F:5′-AGG GTT TGC TTT TCA GGA C-3′ R:5′-CAT CCA TTA CAG GAA GAC TGC-3′	$(GT)_{14}$	6FAM	54	13	105～171	0.684	0.836	0.805	0.0013*	0.0006
SY112	DQ355015	F:5′-TCA ATA ATC AGG GCA GGC TC-3′ R:5′-GTC CTT GTG TAG TCT GTG TGG G-3′	$(CA)_6CNCT(CA)_{10}C(CA)_5$	HEX	54	5	192～202	0.579	0.715	0.650	0.1057	0.0031
SY128	GQ120510	F:5′- TGA CCC TTT GCT GTA TCC TG-3′ R:5′- GGT GAG CCC AGA GAA TCT TC-3′	$(GT)_3GC(GT)_3GC(GT)_3GC(GT)_{17}$	HEX	54	2	140～142	0.000	0.417	0.327	0.0000*	0.0000
SY129	DQ355016	F:5′-GAA AAA GAA GCA CAC ACA CG-3′ R:5′-AAG GTT TGT CCC CAC ATT C-3′	$(CA)_{13}$	6FAM	56	5	134～142	0.500	0.675	0.605	0.0029	0.0005
SY141	DQ355017	F:5′-CAT AGC CTT GAC TAA ACG GAC C-3′ R:5′-CAC CTG CCA CAT TCG GG-3′	$(CA)_{19}$	HEX	56	7	252～268	0.684	0.802	0.761	0.0020	0.0005

*Significant deviation from Hardy-Weinberg equilibrium at $\alpha = 0.05$. T: annealing temperature, *K*: number of allele, size range: size range of PCR product, Ho and He: observed and expected heterozygosities, PIC : polymorphic information content.

Table 2. Summary of loci characteristics for inclusion in the core set of *Naemorhedus* (*N.*) *caudatus* microsatellites and cross-species amplification of the *N. caudatus* loci in four caprine species

Locus	Polymorphism†	Null alleles‡		Selective neutrality§		Linkage between loci‖	Applicability of cross-species amplification			
		Presence	Brookfield	Presence	Slatkin's exact *p* value		Japanese serow (n = 10)	Chinese goral (n = 7)	Domestic goat (n = 5)	Northern chamois (n = 10)
SY3A*	Moderate	No	0.1422	Yes	0.719	−	+	+	+	+
SY3B	Moderate	Yes	0.2574	No	0.008	−	+	+	ND	ND
SY12A*	Moderate	No	0.054	Yes	0.645	SY17, SY449	+	+	−	+
SY12B*	Moderate	No	0	Yes	0.279	−	+	+	+	+
SY14	Moderate	Yes	0.2191	Yes	0.405	−	+	+	ND	ND
SY17	Moderate	No	0.0932	Yes	0.207	SY12A, SY449	+	+	+	ND
SY22	Moderate	Yes	0.2847	Yes	0.262	SY129	ND	+	ND	ND
SY48*	Moderate	No	0.1331	Yes	0.378	−	+	+	+	−
SY50	Moderate	No	0.1156	No	0.047	−	+	+	+	ND
SY58*	Moderate	No	0.0236	Yes	0.581	−	+	+	+	+
SY71*	Moderate	No	0.0206	Yes	0.419	−	+	+	+	ND
SY76*	High	No	0.0774	Yes	0.603	SY93	+	+	+	ND
SY84*	High	No	0.0309	Yes	0.165	−	+	+	+	+
SY84B*	Moderate	No	0.0699	Yes	0.182	−	+	+	+	+
SY93	Moderate	Yes	0.1733	Yes	0.229	SY141	+	+	+	+
SY112*	Moderate	No	0.0743	Yes	0.218	−	+	+	+	ND
SY128	Moderate	Yes	0.2915	Yes	0.19	SY434	+	+	+	ND
SY129*	Moderate	No	0.0999	Yes	0.291	SY22, SY449	+	+	+	ND
SY141	High	No	0.0601	No	0.044	SY93	+	+	+	ND
SY242	Moderate	No	0	No	0.005	−	+	+	+	ND
SY259	High	No	0.1224	No	0.015	SY17, SY449	+	+	+	+
SY434	Moderate	Yes	0.2347	No	0.008	SY128	+	+	+	+
SY449	Moderate	Yes	0.3327	No	0.028	SY12A, SY17, SY128, SY129, SY259	+	+	+	ND

*The core set of microsatellites of *N. caudatus*. +indicates the successful cross species amplification for 11 core set of microsatellites. −implies no amplification. ND: not determined yet (size range is available upon requested). †Based on the expected heterozygosity (He); $0 <$ low (L) < 0.4, $0.4 \leq$ moderate (M) < 0.8, $0.8 \leq$ high (H) < 0.9. ‡Presence of null allele based on MICRO-CHECKER analysis. §Yes denotes the null hypothesis of selective neutrality against the presence of selection was not rejected for that locus at $p = 0.05$, and No denotes the null hypothesis of neutrality was rejected for that locus. ‖ Linkage disequilibrium were present at $p = 0.05$ after Bonferroni correction for multiple testing.

Bonferroni correction for multiple testing. Expected (He) and observed (Ho) heterozygosities, and PIC were calculated using the CERVUS version 3.0 [5].

A core set of microsatellites for population genetics studies of Korean goral was screened by following criteria described by Kim *et al*. [6]. We investigated 1) readability of each marker, 2) level of polymorphisms, 3) occurrence of null alleles, 4) level of selective neutrality [2], and 5) linkage equilibrium between loci.

A total of 8 microsatellite loci were successfully optimized for 38 Korean gorals (Table 1). The observed number of alleles per locus ranged from 2 to 13 averaging 6.5 per locus. Expected (He) and observed (Ho) heterozygosity were in the range of 0.417 ~ 0.836 (mean = 0.669) and 0 ~ 0.711 (mean = 0.437) respectively. Genetic diversity, as indicated by mean He estimates in Korean goral, was not significantly higher than certain ungulates (0.842 in musk deer [10], 0.76 in Chital deer [3]).

After correction for multiple comparisons by applying a sequential Bonferroni correction ($\alpha = 0.05$) [7], Fisher's exact tests revealed four loci that were deviated from HWE. Although these deviations could be caused by null alleles, these results are more likely an artifact of biased sampling from captive specimens exhibiting a deficiency of heterozygotes (i.e. Wahlund's effect) [9].

A total of 23 loci, 8 new loci from this study and 15 loci developed by An *et al*. [1], were tested to determine whether they meet the criteria to be defined core sets of microsatellite markers as suggested by Kim *et al*. [6]. Eleven microsatellites satisfied all criteria (i.e. moderate to high polymorphism, no evidence of null alleles, apparent selective neutrality, and no linkage with other loci) and are recommended for future population genetics studies of Korean goral (Table 2). Of the core set of 11 loci tested, all loci were successfully amplified for Japanese serow and Chinese goral, whereas SY12A for domestic goat and SY48 for Northern chamois showed no amplification, and SY71, SY76, SY112, and SY129 have not been investigated for Northern chamois (Table 2).

This core set of microsatellites can be applied to better understand the genetic diversity and population structure of Korean gorals as well as other endangered Caprinae species. In addition, the applicability of cross-species amplification of Korean goral microsatellites could facilitate high-resolution studies for the conservation and management of other Caprinae species. This could lead to better decision-making in regards to the improvement of conservation management plans.

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