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Development of Polymorphic Microsatellite Markers for the Okinawa Pit Viper, *Ovophis okinavensis*

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Abstract: We isolated and characterized 11 microsatellite markers for *Ovophis* okinavensis, a viperid species endemic to the Ryukyu Archipelago. These markers showed polymorphism among 25 individuals from Okinawajima Island. The number of alleles per locus was 3–11, and observed and expected heterozygosity were 0.240–0.960 and 0.218–0.853, respectively. Cross-amplification confirmed that at least seven out of the 11 microsatellite markers were applicable to a putative relative, *Trimeresurus gracilis*. The markers provided here are expected to be valuable for population- and/or individual-level genetic studies of not only *O. okinavenesis*, but also its relatives.

Key words: *Ovophis okinavensis*; Primer sequence; Short tandem repeat; *Trimeresurus gracilis*; Viperidae

INTRODUCTION

Ovophis okinavensis is a short, stoutbodied viperid snake that inhabits forest areas, especially near streams, ponds, and marshes, on many subtropical islands of the Okinawa and Amami groups, Ryukyu Archipelago, Japan. This snake is a typical ambush forager and exhibits a foraging strategy that is adjusted to spatial and temporal fluctuations of the emergence of two frog species with different breeding periods (Kadota, 2011). The specialized foraging behavior together with its broad geographic range suggests that this snake has a heterogeneous population structure. However, there are currently no useful genetic markers (including those of the relatives) to obtain fine-scale genetic information on this species. Here, we report newly developed 11 microsatellite primers of O. okinavensis and examine the suitability of these markers for Trimeresurus gracilis, which is the putative sister taxon to O. okinavensis (Castoe and Parkinson. 2006: Malhotra et al., 2010).

MATERIALS AND METHODS

We isolated microsatellite loci from ventral

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TABLE 1. CI	laracteristics of 11 microsatellite loci develo	ped for Ovophis	okinave	<i>usis</i> and the resu	Its of (CLOSS-8	amplifica	tion in <i>Trimeres</i>	urus	gracil	IS.
Locus		Donot motif	Ę	Ovophis okin	rvensis	(n=2)	()	Trimeresurus	graci	lis (n=	4)
(Accession no.)	(c-c) equivalent termination of the second secon	керсан шош	л а	Size range (bp)	А	$H_{\rm O}$	H_{E}	Size range (bp)	А	H_0	$H_{\rm E}$
OvoP1	F: GCCCATCGACTTTGTTTTGC	$(AC)_{23}$	64	168–192	10	0.96	0.85	168	1	0.00	0.00
(LC414977)	R: GGTTTTCTTGCCTTCAGCAC										
OvoP7	F: GGTGCATACAACCATCAACAGG	(AC) ₁₂	64	243–249	4	0.68	0.56	240–252	б	0.50	0.41
(LC414978)	R: GAACAACGGAGGCAAAGAAG										
OvoP11	F: TCTGAGACATAGTGGAGGATGG	(GT) ₁₃	62	198–204	4	0.24	0.22	191 - 204	4	0.75	0.72
(LC414979)	R: GTGCCACAGGCAATGTTTTC										
OvoP12	F: AAAGAGGCAGTGGAGCATGT	(TG) ₁₂	64	248–264	4	0.64	0.7	256–268	2	1.00	0.78
(LC414980)	R: GATGAGAGACTGAGAGGATAGGG										
OvoP17	F: GTGGAAAGAAAGGTAAGCATTG	(GT) ₈	62	305-321	5	0.52	0.43	318-328	4	0.25	0.72
(LC414981)	R: ATGAAAACCCATGGAAGGTG										
OvoP24	F: AAGGGACATGTGTGTGTGTG	(TG) ₁₃	62	208-212	ŝ	0.56	0.65	190 - 208	ŝ	0.25	0.59
(LC414982)	R: CGAGTGCGGTATCTTCCC										
OvoP29	F: CACATACACACGCACAGG	(AC) ₉	64	200–210	5	0.56	0.71	191-217	4	1.00	0.69
(LC414983)	R: TGGGGCAAAGCTGACTTAAC										
OvoP30	F: GGTTGTCCAATTCCTTCTGG	$(GT)_{18}$	64	258–266	5	0.76	0.66			I	I
(LC414984)	R: GTTTGGCCCACAAATAGCC										
OvoP38	F: GTTACACATTGCTCGCTTGC	$(CA)_{22}$	62	265–383	11	0.88	0.83				
(LC414985)	R: CTTCCTGGTGGTCACGTTTT										
OvoP40	F: CCTCTTCACTCCACCCAGTC	(TG) ₁₆	62	306–320	~	0.56	0.66	311–312	0	*	*
(LC414986)	R: CGCTTAGCGACCAAAGTTAC										
OvoP45	F: AAAACAGTCGGGTTTGTGG	(TG) ₁₂	62	248–266	4	0.36	0.33	250-260	2	0.5	0.75
(LC414987)	R: CCAGTCTCCCTTTATCTCTTTGA										
Ta, annealin amplyfied.	g temperature (°C); A , number of alleles	; H_0 , observed 1	heterozy;	gosity; $H_{ m E}$, expe	cted h	letero	rygosity;	*only one indiv	vidua	succe	ssfully

scale tissue samples from Okinawajima Island snakes using the method slightly modified from Glenn and Schable (2005). The detailed methods for library preparation and primer design are described in Kurita et al. (2013). For amplification trials, 25 individuals of O. okinavensis from the northern part of Okinawajima Island were used (DNA identification numbers by Y. Kadota: 4, 100-102, 106, 107, 111, 114, 117, 128, 129, 133, 135, 138, 139, 145, 149, 151, 152, 154, 158, 164, 170, 175, 177). In addition, we tested cross-species amplification of developed markers on four individuals of T. gracilis from Taiwan (temporal specimen numbers by Ming-Chung Tu: 79804-805 and 79807-808). PCR amplification was conducted using fluorescently (FAM, HEX, NED) labeled M13(-21) universal primers (Schuelke, 2000) with the TaKaRa Ex Tag kit (Takara Bio, Otsu, Japan). Fragment sizes were measured with GeneScan 400HD ROX size standard (Applied Biosystems, Foster City, CA, USA) on ABI 3130xl Genetic Analyzer (Applied Biosystems) and analyzed by the Peak Scanner software (Applied Biosystems). Number of alleles (A), and observed (H_0) and expected heterozygosities $(H_{\rm F})$ were calculated using GenAlEx 6.503 (Peakall and Smouse, 2006, 2012). Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium between loci were tested using GENEPOP 4.7.0 (Rousset, 2008). Null allele frequency was estimated using CERVUS 3.0.7 (Kalinowski et al., 2007).

RESULTS AND DISCUSSION

The characteristics of the 11 isolated microsatellite loci are summarized in Table 1. These loci exhibited high or moderate allelic polymorphism in *O. okinavensis*. The number of alleles per locus ranged from 3 to 11. The observed and expected heterozygosity ranged from 0.240 to 0.960 and from 0.218 to 0.853, respectively. There was no evidence of deviations from the Hardy-Weinberg equilibrium at any locus and significant linkage disequilibrium at any pair of loci after the sequential Bonferroni correction (Rice, 1989). The estimated frequency of null alleles ranged from -0.13 to 0.12. In *T. gracilis*, eight of 11 loci were successfully amplified for all individuals examined, and seven loci showed polymorphism. As such, the microsatellite markers developed here will be useful for populationand/or individual-level genetic studies of *O. okinavensis* and its relatives, in the fields of ecology and population genetics (e.g. population structure, genetic variation, demography, and relatedness analyses including paternity).

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