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TECHNICAL NOTE

Isolation and characterization of novel microsatellite markers in Water monitor (*Varanus salvator*)

Meilan Fu · Dongmei Yu · Jianjun Peng · Ying Wang · Saifei Gao · Lili Wang · Xiqing Liu · Shijia Hu · Lingfu Gao

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Abstract The Water monitor (*Varanus salvator*) is the second largest of all the monitor lizards of the world, after the Komodo dragon. It was listed in CITES Appendix II as an endangered species. Investigation of genetic variation for *Varanus salvator* can help to conserve and improve this endangered monitor salvator. Here, sixteen microsatellite loci were developed and twelve polymorphic loci were used to investigate the genetic variation on 30 wild individuals. The number of alleles per locus ranged from 2 to 15, with the average of 6,000. The observed and expected heterozygosity values varied from 0.2333 to 0.9000 and 0.2096 to 0.9203, respectively. Only one locus (YBJX28) significantly deviated from Hardy–Weinberg equilibrium after Bonferroni correction. No significant linkage disequilibrium was detected. These polymorphic markers should be useful tool for assessing population genetics of *Varanus salvator*.

Keywords *Varanus salvator* · Microsatellite · Genetic variation

The Water monitor (*Varanus salvator*) can attain a length of three meters and is a large species of monitor lizard (Taylor 1963). As one of the most common monitor lizards, Water monitors are widely distributed through Asia. They inhabit in tropical rain forest, mangrove forest and freshwater swamp areas (Erdelen 1991). However, its wild population has declined rapidly because of excess hunting and habitat destruction (Komsorn and Kumthorn 2001). Their skins and meat are used for dietary protein, medicine, and leather goods (Gaulke 1992). It is both the largest lizard in the leather trade, and the most heavily exploited species (Shine et al. 1996). In order to conserve and improve this endangered monitor salvator, it is urgent for researchers to study its genetic structure. This study reports twelve polymorphic microsatellite loci isolated from *V. salvator*, which should be useful tool for further study.

A microsatellite-enriched partial genomic library was constructed following the procedure described by Yu et al. (2010). The genomic DNA was extracted from a small tail cut piece of one individual, using the DNeasy Tissue Kits (Qiagen). Extracted DNA was digested with restriction enzyme *Sau* 3AI (TaKaRa) for 3–4 h, and the fragments of 400–900 bp were excised from agarose gel and purified using DNA purification kit (Takara). The selected fragments were ligated to a blunt-end adapter (SAULA: GC GGTACCCGGGAAGCTTGG, SAULB: GATCCCAAG CTTCCCGGGTACCGC) with T4 DNA ligase (Takara). Ligation mixture was incubated at 16°C for 16 h, and the ligation products were amplified by polymerase chain reaction using the adapter SAULA as primers. Amplified fragments were denatured and hybridized with biotin-labeled probes dinucleotide repeats $(CA)_{12}$ GCTTGA-Biotin and $(GA)_{12}$ GCTTGA-Biotin. The hybridization mixture was incubated with VECTREX Avidin D (Vector Laboratories) at 37°C for 30 min and washed with TBS (100 mMTris, 150 mMNaCl, pH 7.5) for four times at different temperatures (50, 50, 68, 68°C) to remove

M. Fu · D. Yu (✉) · J. Peng (✉) · Y. Wang · S. Gao · L. Wang · X. Liu · S. Hu · L. Gao
Guangdong Provincial Public Laboratory on Wild Animal Conservation and Management, South China Institute of Endangered Animals, No. 105 Xin Gang Xi Road, Guangzhou 510260, China
e-mail: yudongmei50@163.com

J. Peng
e-mail: jjpeng74@163.com

Table 1 Isolation and characterization of polymorphic microsatellite loci from the Water monitor (*Varanus salvator*)

Locus/accession no.	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	*A*	H_o	H_e	HWE *P* value
Varsa01/HQ896216	GATACATTACAGGGAGGCA CCAACCCATCATACACCA	$(AC)_7$	62	5	0.4333	0.4452	0.5848
Varsa02/HQ896217	GTCAGAACTCAGCAGCAT ACTTCCTCCTCACAATCA	$(AG)_5$	58	2	0.4333	0.3452	0.1883
Varsa06/HQ896218	CCTGCCTCATTTCCTTCA CCCAATCCCTGGCTCTTA	$(CA)_4GA(AC)_{13}$	64	1	–	–	–
Varsa07/HQ896219	CTATCGCCAATCTCAAAC ACACTTGGGATACTCTGC	$(GT)_{10}$	64	3	0.4000	0.5898	0.9989
Varsa08/HQ896220	GAGCGTCCTGACCTACTTGA GAGACAGTGCGGGTTTCC	$(AG)_8N(AG)_{13}$	66	1	–	–	–
Varsa09/HQ896221	AGAGGTGGCACGAGAACT CACCAAACAGCAGCAAAA	$(TG)_7$	62	4	0.4000	0.6254	0.9930
Varsa10/HQ896222	CACCAGCCTTGTGAAGAA TAGCCTCCAGGTAAACCA	$(AG)_{24}$	64	8	0.7000	0.7994	0.8119
Varsa11/HQ896223	TGTGGAGGTGGTGAGGAG ACTGAGATGAGCGAGTGC	$(AG)_{17}$	64	1	–	–	–
Varsa12/HQ896224	ACTGTGGAGGTGGTGAGG CCAAATGACGGAGGTGTT	$(TG)_5$	62	2	0.2333	0.2096	0.6733
Varsa15/HQ896227	CCCAGACCATCTCCATCA GGCAAGTTTGTTCCCTTT	$(GT)_{14}$	62	1	–	–	–
Varsa21/HQ896230	TCGATGCGACTCTTGTTT GTCCTGGTCTGACTGTTGG	$(GA)_{27}$	66	9	0.2333	0.8266	1.0000
Varsa22/HQ896231	CAATCCACAAGAAGCGAAGA GGCAGCAGCAATGACAGG	$(TC)_{13}(TG)_9$	66	15	0.7667	0.9203	0.9843
Varsa24/HQ896233	ACAAGAGTTCCCACTACCT AACACCCACCTTCTGCTA	$(GT)_{16}(GA)_{29}$	64	8	0.3000	0.8034	1.0000
Varsa27/HQ896235	ACACGACTTCGCAACTAACA GGCTCCACCTCTTCTCCT	$(AAC)_6$	62	3	0.2333	0.2153	0.6817
Varsa28/HQ896236	CAGGGAAGGAGTCCAGAA TTTCAACATGAGGGCAGA	$(CA)_{10}$	62	6	0.9000	0.6090	0.0000
Varsa33/HQ896237	ACAGCCATTCTGCTCCAA AGGGCAAGTTCTCAAGCT	$(GT)_6CT(GT)_5$	64	7	0.3667	0.7407	0.9959

Ta annealing temperature, *A* no. of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *HWE* Hardy–Weinberg equilibrium

unbound fragments. The bound fragments were eluted with 80 μl ddH_2O at 95°C for 10 min, and amplified by PCR. Fragments ranging from 400 to 900 bp were excised from agarose gel and recovered, and then were ligated with pMD19-T vector (Takara) and transformed to *E. coli* DH5*a* competent cells (Takara). Recombinant clones were detected using blue/white screening. White clones were screened by PCR, PCR products were checked on 1.5% agarose gels, and clones that generated two or more bands were selected.

Sixty positive clones were identified from 120 recombinant colonies via PCR with linker primers and M13 universal primers (Takara), 50 sequences contained microsatellite repeats, of which 40 possess sufficient flanking sequence appropriate for primer design. Thirty pairs of primers were designed using Primer 5.0 program (PREMIER Biosoft International, Silicon Valley, USA). All the microsatellite loci developed here were evaluated on other 30 wild samples for subsequent evaluation.

All 30 specimens of *V. salvator* were collected from Guangzhou Forest Police Bureau in Guangzhou, China. Following DNA extracted with the Dneasy Tissue Kit (Qiagen), polymerase chain reaction (PCR) was carried out in 10 μl reactions containing 1 μl of DNA, 0.2 μl of each 10 μM primer, 5 μl of the *Ex* premix *Taq* polymerase (Takara) and 3.6 μl of water for a total volume of 10 μl. The PCR conditions for amplification were as follows: denaturation at 95°C for 5 min, followed by 35 cycles of

94°C for 30 s, a primer-specific annealing temperature for 30 s and 72°C for 40 s, with a final extension at 72°C for 10 min. The PCR products were checked by electrophoresis on 8% non-denaturing polyacrylamide gel and visualized with silver staining. In the study, 16 microsatellite loci amplified clear and specific products consistently.

Allele size was determined with software Gel-Pro Analyzer 4.5. Number of alleles, heterozygosity, test of Hardy–Weinberg expectations (HWE) of genotype frequencies and linkage disequilibrium (LD) between loci were analyzed using GENEPOP 3.4 (Raymond and Rousset 1995). Conditions and characteristics of the 16 loci were shown by Table 1. Sequences of these loci have been deposited with GenBank under accession nos. HQ896216–HQ896237.

Four of the 16 loci that amplified were monomorphic; 12 loci were polymorphic among them. The number of alleles per polymorphic locus varied from 2 to15, with the average of 6.000. The observed and expected heterozygosity values ranged from 0.2333 to 0.9000 and 0.2096 to 0.9203, respectively. Only 1 of these 12 loci (YBJX28) significantly deviated from Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.01$). In contrast, no significant linkage disequilibrium was detected.

These 12 polymorphic microsatellite loci reported here will be useful in population genetics, conservation and other relevant studies of *V. salvator*.

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