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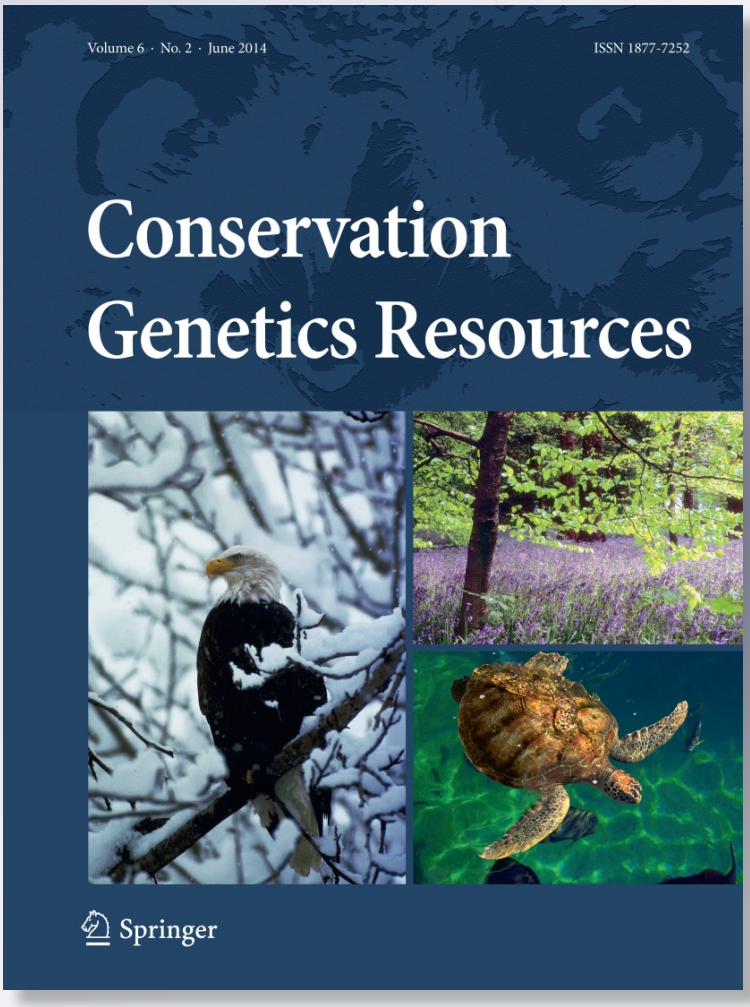

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TECHNICAL NOTE

Characterization of microsatellite loci markers for the critically endangered Montseny brook newt (*Calotriton arnoldi*)

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Abstract Fifteen novel tetranucleotide polymorphic microsatellite loci are described and characterized for the critically endangered Montseny brook newt *Calotriton arnoldi*. Until now little is known about genetic variability and dispersal of this species across its very limited distribution area, mainly represented by two main sectors (eastern and western from the Tordera river valley). The level of genetic diversity of the new loci was assessed in 23 individuals from the eastern sector. Number of alleles per locus ranged from 2 to 8 (mean = 4.7) and observed and expected heterozygosity ranged from 0.087 to 1.000 and 0.162 to 0.814, respectively. Almost all loci were found to be in Hardy–Weinberg equilibrium and were not linked. These polymorphic loci can be used in population structure, gene flow, and population differentiation. The value of such information should be high for successful management and conservation of this potentially highly threatened species.

Keywords *Calotriton arnoldi* · Montseny brook newt · Microsatellites · Conservation genetics · Captive breeding program

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The critically endangered Montseny brook newt (*Calotriton arnoldi*) is one of the most endangered amphibians in Europe (Carranza and Martínez-Solano 2009). The current global population size is estimated at less than 1,500 mature individuals, limited to an area of only 20 km^2 in the Montseny Mountains Natural Park, NE Iberian Peninsula. Their disconnected populations are found in seven closely located brooks, fragmented into two main sectors (eastern and western) on both sides of the Tordera river valley and separated by an unsuitable habitat. To date, one of the management measures for the conservation of this species is the development of a captive breeding program, which started in 2007 by the Catalonian Government. This breeding program maintained individuals from both sectors separately into two evolutionary significant units Previous genetic studies based on mitochondrial and nuclear sequence analysis suggested the isolation of eastern and western sectors confirming the need for the continuation of the maintenance of the two breeding stocks (Valbuena-Ureña et al. 2013). Furthermore, genetic studies applying high resolution genetic markers, such as microsatellite loci are needed to infer the degree of current gene flow among populations within sectors. The development of microsatellite markers in *C. arnoldi* should greatly enhance our understanding of this species in both an evolutionary and conservation oriented framework and should serve as an important tool for future studies aiming at: (1) assessing

levels of genetic diversity and population structure; (2) checking the genetic connectivity between populations within sectors and the degree of isolation; (3) determining which units are of particular importance for maintaining species genetic diversity; and (4) addressing these results to the conservation strategies by studying the effect of the captive breeding program on the genetic diversity of the species. Here, we describe new primer pairs for the successful amplification of 15 unlinked polymorphic loci for this species.

Genomic DNA was extracted from tail-tips of *C. arnoldi*. Tissue sampling and export were carried out under the authority permits. Microsatellite loci were isolated by Ecogenics GmbH (Switzerland) on the basis of fifteen individual DNA samples from the complete range of the species. Size selected fragments from genomic DNA were enriched for simple sequence repeat (SSR) content by using magnetic streptavidin beads and biotin- labeled GTAT, GATA, AAC, AAG, TAC and ATC repeat oligonucleotides. The SSR-enriched library was analyzed on a Roche 454 platform using the GS FLX titanium reagents. In total 76804 sequence reads were obtained with an average length of 354 bp. Of these, 1,087 contained a tetra- or tri nucleotide microsatellite motif of at least 6 repeat units or either a dinucleotide motif of at least 10 repeat units. Suitable primers could be designed for 501 potential loci, of which 72 were tested for amplification and polymorphism. Total genomic DNA from the 15 individuals was extracted using DNeasy Blood and Tissue Kit (Qiagen). Reactions of 10 μl containing 1 × buffer, 200 μM of

Table 1 Characterization and level of variability at 15 microsatellite loci in A1 population ($n = 23$) of *Calotriton arnoldi*. Observed heterozygosity (H_O), expected heterozigosity (H_E), probability of Hardy–Weinberg equilibrium (P_{HW})

Primer ID	Sequences 5′-3′		Repeat motif	Allele range (bp)	No. of alleles	H_O	H_E	P_{HW}
Calarn_02248	F:	CACAACAACAGGCGAATGAC	$(TATC)_{18}$	185–209	6	0.739	0.642	0.1161
	R:	ACTTTAGGTCTTGCGTTGGC						
Calarn_29994	F:	ACCAGCTGCACTCTGCTATC	$(TATC)_8$	169–173	2	0.087	0.162	0.1318
	R:	GTGCTGCTCATCAAATAGTCAAC						
Calarn_37825	F:	CATCCTTGTAGCAGGCCTTTG	$(AGAT)_{18}$	227–255	8	0.870	0.814	0.5412
	R:	CTACCAGGGGTTGATCTCAGG						
Calarn_14961	F:	TTGAGAAATGCAAGGTCGCC	$(TATC)_{11}$	201–221	4	0.609	0.649	0.2238
	R:	GTCAGGATGACGCGTTTCG						
Calarn_15906	F:	TCAATCAAGGGCAAGATGATGAC	$(CTAT)_{14}$	113–125	4	0.913	0.702	0.1373
	R:	ACCAATGACCTATCACAGCC						
Calarn_12022	F:	CTCTCACGGAAAAGCTCAGG	$(TCTA)_{14}$	224–248	5	0.391	0.430	0.0286
	R:	GCGTGGCCCAATACATATTCC						
Calarn_06881	F:	AGCGCATTGCTGCCTGTG	$(AGAT)_{15}$	162–174	4	0.391	0.490	0.1454
	R:	TACAGAGGGAGTGGGAGGAG						
Calarn_50748	F:	ATTGGGGTATATTGGGGCTC	$(AGAT)_{13}$	201–213	4	0.565	0.579	1.0000
	R:	GGCATCCATCACCGATTATCTATC						
Calarn_36791	F:	TTGGAGGTGTCATCAGTGGG	$(TCTA)_{18}$	128–148	5	0.826	0.729	0.8491
	R:	AACCACAGAAATTCACCAGTC						
	F:	GTAGGTTTGGTGCGAAGTGG	$(AGAT)_{15}$	224–236	4	0.478	0.410	1.0000
	R:	GTACGAGATCTTCTCAGTGGC						
Calarn_52354	F:	AAACTGTGGCATCTTGTGGC	$(ATCT)_{12}$	220–240	4	0.565	0.467	0.8995
	R:	AGACAGCATCTGTGTCCTCTG						
Calarn_30143	F:	AGGTTAGGTTTAGGTTTACTGCAC	$(TCTG)_7$	190–230	5	0.609	0.690	0.5285
	R:	AGCTTCGTCATTCTTGTACCC						
Calarn_31321	F:	GCTTACATCCATCCTTCTCGTC	$(ATCT)_{19}$	173–193	6	0.652	0.757	0.3864
	R:	AGGCAGATGTTTTGATGGGTG						
Calarn_15136	F:	GCTAGTTTGCTTTGGCAGTTC	$(TGTA)_{15}$	165–177	4	0.478	0.506	0.4615
	R:	CTGCCTTTTGGCTAGGTTCG						
Calarn_37884	F:	GGGGCGCAAGTTACAGTTAG	$(ATAG)_{10}$	248–267	5	1.000	0.710	0.0013*
	R:	ATGTAGTGTGGCAGGTGAGG						
M13		TGTAAAACGACGGCCAGT						

* P value < 0.01

dNTPs, 0.04 μM M13 tailed locus specific forward primer, 0.16 μM locus specific reverse primer, 0.16 μM universal M13 primer 5′ -end labeled with FAM (Metabion), 0.5 units of HotStarTaq (Qiagen) were used to amplify each locus via the nested PCR procedure described by Schuelke (2000). The PCR profile was 95 °C for 15 min, 30 cycles of 30 s at 95 °C, 45 s at 56 °C (annealing temperature), 45 s at 72 °C, followed by 8 cycles of 30 s at 95 °C, 45 s at 53 °C and 45 s at 72 °C; and a final elongation phase of 30 min at 72 °C. PCR products were sized on an ABI3730 Genetic Analyzer (Applied Biosystems) using GENEMAPPER V4 (Applied Biosystems) to assign genotypes. The 15 loci were screened for polymorphism in 23 individuals of *C. arnoldi* from A1 population originating from the eastern sector. Standard population genetics parameters were estimated using GENEPOP V4.1.4 (Rousset 2008) and details of the 15 polymorphic microsatellite loci are shown in Table 1. We detected 2–8 alleles per locus (mean = 4.7) and observed (Ho) and expected (He) heterozygosity values ranged from 0.087 to 1.000 and 0.162 to 0.814, respectively. The loci generally conformed to Hardy–Weinberg (HW) equilibrium expectations, although locus Calarn 37884 exhibited a heterozygote excess ($P < 0.01$), and no loci showed significant deviation from linkage disequilibrium. There was neither evidence for scoring error due to stuttering or due to large allele dropout and nor evidence for the presence of null alleles according to MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004).

The new fifteen polymorphic loci described here together with ten loci, which have been previously developed for *C. asper* and successfully cross-amplified in *C. arnoldi* (Drechsler et al. 2013) provide a powerful tool to challenge urgently needed conservation genetics studies in this critically endangered European amphibian. In turn, these new markers are also likely to be applicable for its closely related sister species, the Pyrenean brook newt *C. asper*.

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References

Carranza S, Martínez-Solano I (2009) *Calotriton arnoldi*. IUCN red list of threatened species. Version 2012.1 Accessed 04 July 2012

Drechsler A, Geller D, Freund K, Schmeller DS, Künzel S, Rupp O, Loyau A, Denoël M, Valbuena-Ureña E, Steinfartz S (2013) What remains from a 454 run: estimation of success rates of microsatellite loci development in selected newt species (*Calotriton asper*, *Lissotriton helveticus*, and *Triturus cristatus*) and comparison with Illumina-based approaches. Ecol Evol 3(11):3947–3957. doi:10.1002/ece3.764

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8(1):103–106. doi:10.1111/j.1471-8286.2007.01931.x

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat biotechnol 18(2):233–234

Valbuena-Ureña E, Amat F, Carranza S (2013) Integrative Phylogeography of *Calotriton* Newts (Amphibia, Salamandridae), with Special Remarks on the Conservation of the Endangered Montseny Brook Newt (*Calotriton arnoldi*). PLoS ONE 8(6):e62542. doi:10.1371/journal.pone.0062542

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol EcolSS Notes 4(3):535–538. doi:10.1111/j.1471-8286.2004.00684.x