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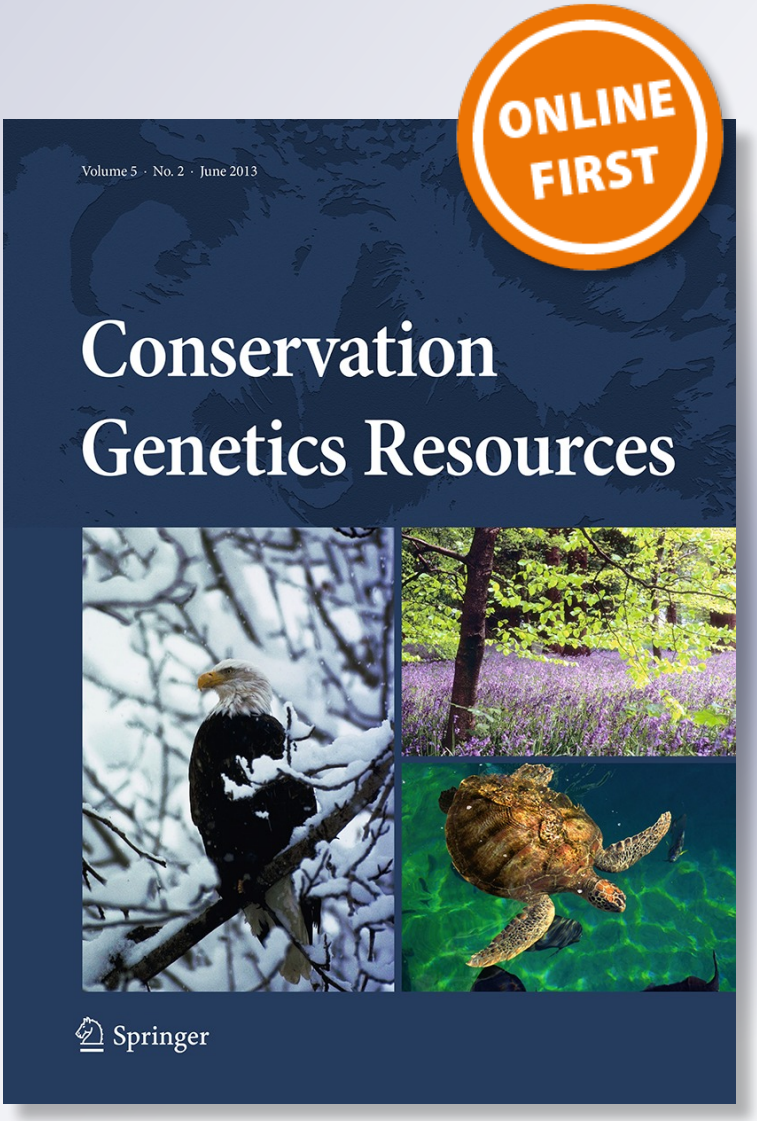

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MICROSATELLITE LETTERS

Development of 28 polymorphic microsatellite markers for the endemic Azorean spider *Sancus acoreensis* (Araneae, Tetragnathidae)

Aristeidis Parmakelis · Katerina Balanika · Sofia Terzopoulou · Francois Rigal · Rochelle R. Beasley · Kenneth L. Jones · Stacey L. Lance · Robert J. Whittaker · Kostas A. Triantis · Paulo A.V. Borges

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We isolated and characterized a total of 28 microsatellite loci from *Sancus acoreensis.* Loci were screened in 26 individuals originating from seven (Flores, Faial, Pico, São Jorge, Terceira, São Miguel, and Santa Maria) out of the nine islands of the Azores. The number of alleles per locus ranged from 2 to 14, observed heterozygosity ranged from 0.040 to 0.708, and the probability of identity values ranged from 0.02 to 0.97. *Sancus acoreensis* is a Laurel forest specialist species, endemic to the Azores, and is facing a great extinction risk due to the severe fragmentation of its habitat. The newly developed microsatellite loci will aid in detecting signs of population bottlenecks and pinpoint the island populations that are facing the greatest risk of extinction.

Sancus acoreensis (Araneae, Tetragnathidae) is an endemic Azorean species that inhabits the Laurel forest remnants within the Azores. It is facing a high risk of extinction due to the fragmentation and degradation of its habitat (Triantis et al. 2010). Using ecological indicators of risk, this species was found to be among the arthropod species mostly threatened. In order to assess the genetic diversity levels, and the population structure of this spider species throughout the Azores, we isolated and characterized 28 polymorphic microsatellites.

Genomic DNA was extracted using a kit (NucleoSpin Tissue kit, Macherey–Nagel) from a single individual. An Illumina paired-end shotgun library was prepared by shearing 1 μg of DNA using a Covaris S220 and following the standard protocol of the Illumina TruSeq DNA Library Kit and using a multiplex identifier adaptor index. Illumina sequencing was conducted on the HiSeq with 100 bp paired-end reads. Five million of the resulting reads were analyzed with the program *PAL_FINDER_v0.02.03* (Castoe et al. 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexa-nucleotide microsatellites. Once

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A. Parmakelis (✉) · K. Balanika · S. Terzopoulou · K. A. Triantis
Department of Ecology and Taxonomy, Faculty of Biology, University of Athens, Panepistimioupoli Zografou, 15784 Athens, Greece
e-mail: aparmakel@biol.uoa.gr

A. Parmakelis · S. Terzopoulou · F. Rigal · K. A. Triantis · P. A.V.Borges
Departamento de Ciências. AgráriasAzorean Biodiversity Group (CITA-A) and Platform for Ecological and Environmental Research (PEER), Universidade dos Açores, Rua Capitão João d'ávila, São Pedro, 9700-042 Angra do Heroismo, Terceira, Portugal

R. R. Beasley · S. L. Lance
Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29802, USA

K. L. Jones
Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO 80045, USA

R. J. Whittaker · K. A. Triantis
Conservation Biogeography and Macroecology Programme, Oxford University Centre for the Environment, South Parks Road, Oxford OX1 3QY, UK

R. J. Whittaker
Center for Macroecology Evolution and Climate, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

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Table 1 The 28 polymorphic microsatellite loci developed for *S. acoreensis*

Locus	Repeat motif	Size (bp)	N	*K*	H_o	H_e	PI	TD
Saac1	ATT	262–268	25	3	0.200	0.274	0.55	TD65
Saac 2	ATT	132–159	26	8	0.538[a]	0.752	0.10	TD65
Saac 3	ATT	176–182	25	2	0.040	0.039	0.92	TD65
Saac 4	ATT	186–231	25	12	0.560[a]	0.890	0.02	TD65
Saac 5	ATAC	211–271	25	12	0.680	0.827	0.04	TD65
Saac 6	AC	226–246	25	7	0.280[a]	0.710	0.12	TD65
Saac 7	TTGG	191–219	26	7	0.654	0.794	0.07	TD65
Saac 8	AT	103–117	26	7	0.538	0.695	0.14	TD65
Saac 12	ACC	352–370	25	8	0.360[a]	0.814	0.06	TD65
Saac 13	TTCC	214–242	25	5	0.280[a]	0.702	0.13	TD65
Saac 14	AAC	184–199	26	6	0.346[a]	0.675	0.16	TD65
Saac 15	ATT	282–288	23	3	0.261	0.264	0.57	TD65
Saac 17	AAC	288–309	23	7	0.174[a]	0.656	0.15	TD65
Saac 18	AAAC	217–289	25	11	0.320[a]	0.870	0.03	TD65
Saac 19	ATT	178–187	24	4	0.333	0.355	0.44	TD65
Saac 20	ATT	134–149	26	6	0.346[a]	0.712	0.12	TD65
Saac 22	ATT	223–244	25	8	0.360[a]	0.779	0.07	TD65
Saac 26	ATT	143–146	23	2	0.391	0.500	0.37	TD65
Saac 27	ATT	236–364	25	4	0.120[a]	0.382	0.43	TD65
Saac 29	AAAC	162–202	24	10	0.333[a]	0.834	0.04	TD65
Saac 30	AAC	137–154	25	6	0.680	0.761	0.09	TD65
Saac 31	ATT	161–185	26	9	0.423[a]	0.857	0.03	TD65
Saac 37	ATT	274–286	26	5	0.385[a]	0.579	0.23	TD65
Saac 38	TTCC	190–226	24	10	0.500	0.766	0.08	TD65
Saac 39	ATT	174–195	24	8	0.667	0.733	0.11	TD65
Saac 42	AAAC	254–319	24	14	0.708	0.884	0.02	TD65
Saac 44	ATT	137–173	25	8	0.600	0.694	0.14	TD65
Saac 45	TTGG	119–155	25	7	0.560[a]	0.806	0.06	TD65

The size of the observed alleles in base pairs including the length of the CAG tag is provided; N is the number of individuals genotyped; *K* is number of alleles observed; H_o is observed and H_e is expected heterozygosity; PI is the probability of identity for each locus, TD refers to the touchdown protocol used for PCR. The sequences of the primers appear in Table S1

[a] Significant deviations from Hardy–Weinberg expectations after Bonferroni corrections

positive reads were identified in *PAL_FINDER_v0.02.03* they were batched to a local installation of the program Primer3 (version 2.0.0) for primer design. To avoid issues with copy number of the primer sequence in the genome, loci for which the primer sequences only occurred one or two times in the 5 million reads were selected. Forty-eight loci of the 1,134 that met this criterion were chosen. One primer from each pair was modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTC ATCA-3′) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled. The sequence GTTT was added to primers without the universal CAG tag addition.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals. Primers were screened as described in O'Bryhim et al. (2013). Twenty-eight of the tested primer pairs amplified high quality PCR products that exhibited polymorphism.

We assessed the variability of the 28 loci in 26 specimens. Conditions and characteristics of the loci are provided in Table 1. We estimated the number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e), and probability of identity (PI) using GenAlEx v6.4 (Peakall and Smouse 2006). Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction for multiple comparisons 15 loci showed significant deviations from expectations under HWE and linkage disequilibrium was detected for 5 of the 378 paired loci comparisons (7–45, 20–31, 15–31, 8–17, and 1–15). The deviations from the Hardy–Weinberg equilibrium were anticipated since the specimens scored did not originate from a single population but from neighboring islands of the Azores. These new loci will allow us to evaluate the extinction risk of the species populations throughout the Azores.

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