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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite markers from the marine isopods *Serolis paradoxa* and *Septemserolis septemcarinata* (Crustacea: Peracarida)

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Abstract

This study reports the successful isolation of highly informative microsatellite marker sets for two marine serolid isopod species. For *Serolis paradoxa* (Fabricius, 1775), 13, and for *Septemserolis septemcarinata* (Miers, 1875), eight polymorphic microsatellite markers were isolated using the reporter genome enrichment protocol. The number of alleles per locus (N_A) and the observed heterozygosity (H_O) encompass a wide range of variation within *S. paradoxa* (N_A 3–31, H_O 6–89%) and *S. septemcarinata* (N_A 2–18, H_O 9–94%). The suitability of the newly isolated markers for population genetic studies is evaluated.

Keywords: enrichment, microsatellites, population genetics, reporter genome protocol, Serolidae, Strait of Magellan, Tierra del Fuego

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Members of the marine isopod family Serolidae Dana, 1852, are predominately distributed on the continental shelves in the Southern Hemisphere (Brandt 1988; Wägele 1994). *Serolis paradoxa* (Fabricius, 1775) is restricted to the marine sublittoral around the Falkland Islands and the Magellan Strait region where it is locally abundant (Gappa & Sueiro 2007). In contrast, *Septemserolis septemcarinata* (Miers, 1875) is distributed in shallow waters of remote Antarctic islands (Brandt 1991; see Fig. 1). Both species brood their offspring and lack pelagic larval stages. The species' dispersal should thus be limited. However, the amount of gene flow between populations and the species' realized dispersal has never been estimated. This study reports two highly polymorphic microsatellite marker sets that allow to estimate population substructure and gene flow patterns.

Specimens of *S. paradoxa* were sampled at Bahia Laredo, near Punta Arenas, Chile (BL) and from the Atlantic opening of the Strait of Magellan (CE). Specimens of *S. septemcarinata* were sampled around South Georgia (SG) and Bouvetoya (BT, Fig. 1). Genomic DNA was isolated from muscle tissue using the QIAGEN DNeasy Mini Kit. A genomic library enriched for microsatellites was created for each species using the reporter genome protocol (Nolte *et al*. 2005) as described in Held & Leese (2007). Hybridization chips (Hybond N+, GE Healthcare) with DNA from *Mus musculus* and *Drosophila melanogaster* (Canton S) as reporter genomes were used for enrichment. As a modification to Held & Leese (2007), 0.03 U/μL Hotmaster *Taq* (Eppendorf) were used in the polymerase chain reaction (PCR). Also, nick repair and PCR were carried out in one reaction tube by incubating for 10 min at 65 °C prior to PCR (94 °C for 2 min, followed by 25 cycles of 30 s at 94 °C, 45 s at 52 °C, 80 s at 65 °C and 10 min final elongation at 65 °C). For elution, hybridization chips were transferred into 500 μL TE buffer (pH 8.0, 80 °C) for 5 min. DNA was precipitated using a standard isopropanol-sodiumacetate protocol.

The enriched fragments were PCR-amplified in 25 μL reactions and purified using the QIAGEN Qiaquick Kit. Purified fragments were cloned into pCR2.1-TOPO vector and transformed into competent TOP10F' *Escherichia coli* (Invitrogen).

For *S. paradoxa*, plasmid preparation of 167 colonies and shotgun sequencing using a standard M13-forward primer was conducted by GATC-Biotech (Konstanz, Germany). Analysis of electropherograms, vector clipping, assembly

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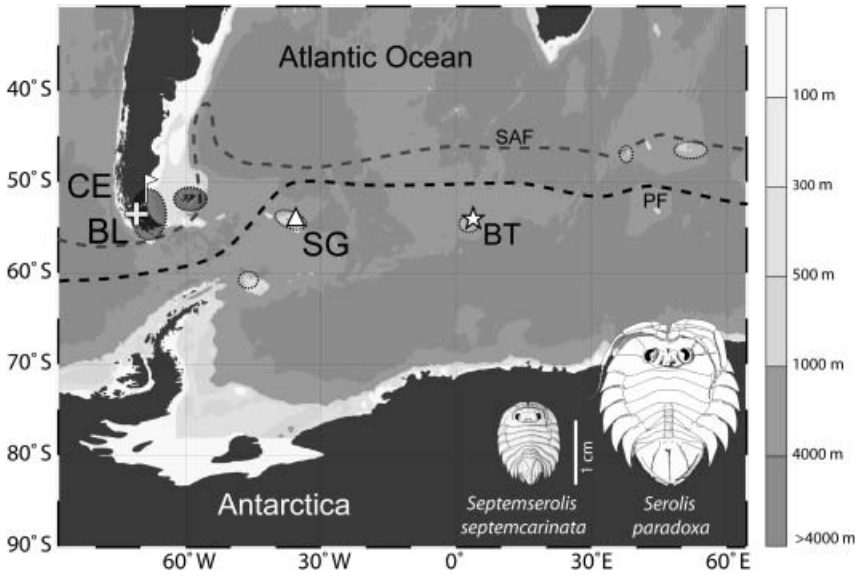

Fig. 1 Distribution and sampling sites of *Serolis paradoxa* (dark grey circles, BL and CE) and *Septemserolis septemcarinata* (bright grey circles, SG and BT). Illustrations of *S. paradoxa* from Wägele (1994), of *S. septemcarinata* from Brandt (1991). SAF, Subantarctic Front; PF, Polar Front, according to Belkin & Gordon (1996) and Cortese & Gersonde (2007).

of contigs, redundancy filtering and primer design were performed using a newly developed, automated software pipeline based on the STADEN package (Staden 1996; Beszteri *et al*. in preparation) to which the microsatellite search tool PHOBOS 3.0 (Mayer, in preparation, www.rub.de/spezzoo/cm) and the primer design tool PRIMER 3 (Rozen & Skaletsky 2000) had been added. Among the 167 inserts sequenced, 124 (74%) were unique and contained at least one microsatellite. Only inserts containing microsatellites with a perfection of ≥ 95% were chosen using PHOBOS and considered for primer design. Insert sequences outside the microsatellites were additionally screened for the presence of duplications, inversions and higher-order repeat structures using DOTLET (Junier & Pagni 2000) and a self-written software to avoid problems in subsequent PCR amplification. Primer pairs for 22 adequate inserts were devised by PRIMER 3. The entire process was made highly automated due to the software pipeline.

For *S. septemcarinata*, plasmid preparation of 161 colonies was conducted using the Eppendorf Fast Plasmid Mini Kit. All 161 inserts were sequenced on an ABI 3130xl automated sequencer using M13-forward and reverse primers. A total of 103 inserts (64%) were unique with at least one microsatellite. Sequence analysis was conducted manually using the software LASERGENE (DNAStar). PHOBOS and DOTLET were used to select 16 adequate microsatellites from the 103 sequences. Primer pairs were designed using FASTPCR (Kalendar 2003).

For both species, the optimal annealing temperature for microsatellite PCR was determined on a gradient from 48 to 65 °C. The PCR protocol for 15 μL or 20 μL reactions was 2 min at 94 °C followed by 34 cycles of 20 s at 94 °C, 15 s at the annealing temperature (Table 1), 30 s at 65 °C, plus a final extension step of 5 min at 65 °C. PCR reagents consisted of 0.2 mM dNTPs, 0.5 μM primer (unlabelled), 0.5 M Betaine, 2.5 mM MgCl, 0.03 U/μL Hotmaster *Taq* (Eppendorf), 2–40 ng DNA.

For *S. paradoxa*, 21 of 22, and for *S. septemcarinata*, 14 of the 16 primer combinations yielded distinct PCR products. Microsatellite variability for *S. paradoxa* was evaluated using specimens from populations BL ($n = 35$) and CE ($n = 32$) and for *S. septemcarinata* using specimens from SG ($n = 23$) and BT ($n = 52$). PCRs were repeated, substituting one unlabelled primer with a 5′-fluorescently labelled primer (Table 1), reducing the number of cycles to 28–34 plus adding a final elongation step of 45 min at 65 °C. The denatured PCR products were analysed on an ABI 3130xl sequencer using ROX GS500 size standard (ABI). Genotyping was implemented using the software GENEMAPPER 4.0. The data sets were examined for genotyping errors, allelic dropout and null alleles using MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*. 2004). Tests for Hardy–Weinberg equilibrium (HWE) and genotypic disequilibrium were performed using GENEPOP 4.0.6 (Rousset 2007) and ARLEQUIN 3.11 (Excoffier *et al*. 2005). The unbiased probability of identity was calculated using GIMLET 1.3.3 (Valiere 2002).

For *S. paradoxa*, 15 loci could be reliably genotyped of which 13 were polymorphic with three to 31 alleles per locus. The observed heterozygosities ranged from 0.059 to 0.89. Loci Spa04 and Spa41 (BL), Spa08, Spa10, Spa30 and Spa39 (BL and CE), and Spa34 (CE) were characterized by a significant global heterozygosity deficiency ($P < 0.01$). Although this might be an indication of null alleles, it needs to be considered whether it could be a consequence of local inbreeding, a Wahlund effect, a sampling bias or of recent population expansion in the Strait of Magellan

Table 1 Microsatellite loci for *Serolis paradoxa* and *Septemserolis septemcarinata*: Locus name, primer sequences, 5′-fluorescent dyes, repetitive sequence, number of alleles identified (N_A), product size range of the identified alleles, annealing temperature (T_a), observed (H_O) and expected (H_E) heterozygosity for the populations from Bahia Laredo (BL) and the Opening of the Strait of Magellan to the Atlantic Ocean (CE) for *S. paradoxa*, and from South Georgia (SG) and Bouvetoya (BT) for *S. septemcarinata*, unbiased probability of identity (PI) and GenBank accession numbers.

Species	Locus name	Primer sequence (5′–3′)	Dye	Repeat motif	N_A	Size range (bp)	T_a (°C)	H_O/H_E for populations	PI (unbiased)	Accession no.
S. paradoxa	Spa04	F: GAGCTTACGAACAAAACTGC R: CGTCTCCAACCTTACTTCAG	HEX	$(CA)_9$	5	126–140	62	BL: 0.47/0.68† CE: 0.47/0.44	1.594×10^{-1}	EU127468
S. paradoxa	Spa07	F: TGTCTGTCTGTTGGTCGATA R: AAGCAAACAGGCAGTCTAAC	6FAM	$(TGTC)_5$	3	109–117	62	BL: 0.059/0.058 CE: monomorphic	9.368×10^{-1}	EU127457
S. paradoxa	Spa08	F: AAGATAATCCAGAAGGCGTA R: GCAGTGCTTCTTTCTCTGTT	HEX	$(AGTG)_{15}$	16	264–332	55	BL: 0.32/0.90† CE: 0.13/0.92†	1.700×10^{-2}	EU127458
S.paradoxa	Spa10	F: TGTTTTGGTGATACTGACGA R: AGTGTAGGAGTGACGAAAGC	NED	$(AC)_{23}$	24	256–318	55	BL: 0.71/0.95† CE: 0.78/0.95†	3.496×10^{-3}	EU127459
S. paradoxa	Spa12	F: CAAATCCAAAAAGGAATCTG R: TTCCTTCTGTTCGTTCATTT	HEX	$(AC)_{18}$	6	188–202	55	BL: 0.26/0.30 CE: 0.13/0.12	2.885×10^{-1}	EU127460
S. paradoxa	Spa13	F: TCCTCAAAGAATTTCACGTT R: GCATTTTTCTTCAAGTGTCC	6FAM	$(CA)_{25}$	6	153–181	60	BL: 0.50/0.71 CE: 0.42/0.54	2.172×10^{-1}	EU180576
S. paradoxa	Spa30	F: AGGTAGCCCCACTCATTTAC R: AGTGTTGTTCAATGCACGTA	HEX	$(AC)_{23}AA(AC)_3$	31	155–198	62	BL: 0.80/0.94* CE: 0.63/0.95†	2.192×10^{-3}	EU127461
S. paradoxa	Spa34	F: CTCCCAAAAAGTAGCACATC R: AGAAAGGGATCAGCGAATA	NED	$(AC)_{23}$	20	145–191	60	BL: 0.88/0.92 CE: 0.66/0.74*	1.373×10^{-2}	EU127462
S. paradoxa	Spa35	F: TATTTGCCTGTGCATGTTTA R: ATGATCTGAGTGTGCGTGT	HEX	$(CA)_8$	10	227–255	62	BL: 0.68/0.67 CE: 0.63/0.73	6.910×10^{-2}	EU127463
S. paradoxa	Spa39	F: TGTCTCGAACGAGAAACTCT R: GTGTGCAAGTGTATCGATGT	NED	$(ACAG)_{20}$	19	172–256	62	BL: 0.63/0.92† CE: 0.69/0.89†	7.899×10^{-3}	EU127464
S. paradoxa	Spa41	F: AGTGTAGGAGTGACGAAAGC R: ACCACATACAACACAAGCAA	6FAM	$(GT)_{22}$	28	120–280	62	BL: 0.73/0.95† CE: 0.87 0.95	3.292×10^{-3}	EU127465
S. paradoxa	Spa42	F: TATGCGTTTCTTTTCACCTT R: CACACATAGGGTAACACCAA	NED	$(GT)_{22}$ $AGG(GT)_6/(TG)_4CG(TG)_5$	20	160–208	55	BL: 0.89/0.91 CE: 0.78/0.89	1.205×10^{-2}	EU127466
S. paradoxa	Spa43	F: GAGGGAAGGAAAGAATGAAT R: GTTTAGGTCCTCCTCTGGTC	HEX	$(GAAT)_3/(AGA)_3/(TGAA)_4$	4	174–182	59	BL: 0.40/0.39 CE: 0.38/0.48	2.136×10^{-1}	EU127467
S. septemcarinata	Sse04	F: TATTTGTGTCGGCGTGTG R: TCCACGTGCAAGTAGGCGGT	6FAM	$(AT)_7$	12	230–258	65	SG: 0.48/0.56 BT: 0.73/0.80	4.106×10^{-2}	EU056269
S. septemcarinata	Sse05	F: AGCACAAGCGCTTAGAGGGTCCAG R: AGTACGTCTAGAGCTAGCAAGTGTG	6FAM	$(CT)_9$	2	215–217	63	SG: 0.09/0.09 BT: 0.25/0.25	6.497×10^{-1}	EU056270
S. septemcarinata	Sse07	F: ACGCGTGATTCACTGGCAGAGTTC R: AGATTCGGCCAAGCGGCTGTTC	HEX	$(ATT)_6$	4	210–223	65	SG: 0.39/0.37 BT: 0.42/0.43	3.557×10^{-1}	EU056272
S.septemcarinata	Sse08	F: TCGAAAGTCGAATTGCGTGTG R: AGAAACCGCCCAGAGTGG	HEX	$(AG)_{42}$	16	215–254	65	SG: 0.70/0.68 BT: 0.83/0.86	2.873×10^{-2}	EU056273
S. septemcarinata	Sse10	F: GCCCCAACACAATATGGAGGCTGTG R: AGAAGGCCGTGACATCGGTTAGGG	HEX	$(GT)_4AT(GT)_{21}$	18	155–203	60	SG: 0.70/0.88 BT: 0.94/0.93	5.388×10^{-3}	EU056273
S. septemcarinata	Sse13	F: TCTTGACAGGGTGGAGCGCAAACC R: GGCAGCGAGCCTAGTGCCTCGATTC	NED	$(AG)_{26}AT(AG)_4$	11	188–212	65	SG: 0.87/0.85 BT: 0.85/0.83	2.774×10^{-2}	EU056276
S. septemcarinata	Sse14	F: GGTCTAAGGGTAGATGACTCGACCG R: GGCGATTCTACTGGTGCCGCCATCA	NED	$(AC)_8CT(AC)_{39}$	13	263–288	65	SG: 0.22/0.73† BT: 0.83/0.85	2.323×10^{-2}	EU056277
S. septemcarinata	Sse15	F: TGGCGGCACCAGTAGAGTCGCCATG R: ACGGTGACGCAGTGGGGCTTCGAG	NED	$(AG)_{11}$	15	126–182	60	SG: 0.78/0.80 BT: 0.83/0.86	1.396×10^{-2}	EU056277

*, †refer to markers that depart from HWE at $P < 0.05$ and $P < 0.01$, respectively.

instead. A significant genotypic disequilibrium was reported for the locus Spa10/Spa41 ($P < 0.01$). The probability of identity (excluding locus Spa10) was high, PI = 2.214×10^{-17}. Two microsatellites were monomorphic for the populations investigated: Spa01 (EU127455) and Spa16 (EU127456).

For *S. septemcarinata*, 13 loci could be reliably genotyped of which eight were polymorphic with two to 18 alleles per locus. The observed heterozygosities ranged from 0.09 to 0.94. Population SG displayed a highly significant homozygosity excess for locus Sse14 that is likely to be due to null alleles. None of the other loci deviated from HWE. Significant genotypic disequilibrium was reported for loci Sse14/Sse15 ($P = 0.025$), which are located on the same insert, 131 bp apart. The probability of identity (excluding locus Sse14) was high, PI = 5.689×10^{-10}. Five additional microsatellites were monomorphic for the populations investigated: Sse01, Sse02, Sse06, Sse12, Sse16 (EU056267, EU056268, EU056271, EU056275, EU056279).

The novel marker sets reported in this study are appropriate for studying microevolutionary processes, especially gene flow, to quantify the species' dispersal capabilities in the context of their unique biology and habitat characteristics. In addition, the markers can provide insight into the poorly understood reproductive strategies of these two benthic key species.

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