PRIMER NOTE

Development and characterization of eight new microsatellite markers for the haremic sandperch, *Parapercis cylindrica* (family Pinguipedidae)

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Abstract

Eight di-, tri- and tetranucleotide microsatellite markers were developed for the haremic sandperch *Parapercis cylindrica* using a linker-ligated, magnetic bead enrichment protocol. Screening of at least 17 individuals showed these markers to be polymorphic with observed heterozygosity ranging from 0.381 to 1.000 (mean = 0.742) and the numbers of alleles ranging from three to 18. The average polymorphic information content for these eight loci was 0.723. These markers may be used for parentage studies aimed at exploring the complex mating strategies employed by this haremic coral reef fish and will be valuable for population genetic studies.

Keywords: enrichment, microsatellites, parentage, population genetics, reef fish

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Parapercis cylindrica is a monandric hermaphrodite that forms haremic groups on shallow reefs of the Great Barrier Reef. Males of this species defend harems of two to 10 females who are permanently site attached (Walker & McCormick 2004). When habitat supports a large population these harems border one another, leading to intense male–male competition. Small subordinate males may employ a range of sneak fertilization strategies to enhance reproductive success (S. Walker & M.I. McCormick, unpublished data), but little is known of the fitness consequences of sneak fertilization on fitness of the dominant harem master in haremic fishes. Here we report the development of eight microsatellite loci that can be used to quantitatively address hypotheses relating to fitness consequences of the haremic mating strategy in *P. cylindrica*.

Microsatellites were isolated using the following enrichment protocol. Twenty micrograms of genomic DNA was extracted from fin clips of a single *P. cylindrica* individual using a QIAGEN DNeasy kit (QIAGEN). Approximately 3 μg of genomic DNA was digested with combinations of *Hha*I plus *Nhe*I and *Hae*III or *Alu*I restriction enzymes (New England Biolabs) in separate reactions. Digested DNA was pooled and enriched in two groups for microsatellites using Streptavidin MagneSphere® Paramagnetic Particles (Promega) using the linker-ligated enrichment protocol described by Hamilton *et al*. (1999). The first group was enriched for two tri- (AAG, ATC) and two tetranucleotide (AATT, AAAC) microsatellite repeats. The second group was enriched for two di- (AC, AG), one tri- (AGC) and four tetranucleotide (ACAG, AACC, AAGG, AGCG) microsatellite repeats. Enriched single-stranded DNA was PCR amplified for 15 cycles at 95 °C for 1 min, 60 °C for 20 s, and 72 °C for 1 min 30 s, using the SNX forward primer (Hamilton *et al*. 1999). SNX forward primer was used at a final concentration of 0.6 μM in a reaction including 1 U of *Taq* DNA polymerase and 1× buffer (QIAGEN), 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 2.5 μg/μL bovine serum albumin and 2 μL of enriched single-stranded DNA. Double-stranded, microsatellite-enriched PCR products were *Nhe*I digested and mung bean nuclease treated (New England Biolabs) before being size selected for products over 200 base pairs using Sepharose CL6B resin (Amersham Pharmacia). Selected products were blunt-end cloned into a pMOS blue vector and transformed into JM109 cells (Amersham BioSciences).

Approximately 1300 colonies were picked and the inserts amplified using standard T7 and U19 primers (GeneWorks). Two microlitres of the amplified inserts was dot-blotted onto NytranN nylon membrane (Schleicher & Schuell BioScience) and fixed by heating to 50 °C for 15 min.

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Table 1 Primer sequences, motif characteristics for sequenced allele and amplification conditions of eight *Parapercis cylindrica* microsatellite loci. *15 µL reactions

Locus	Motif	Primer sequence 5′– 3′	Annealing temp. (°C)	$MgCl_2$ (mM)	dNTP (mM)	Primer (µM)	Template (ng)*	GenBank Accession no.
JCU_PC1-E9	$[GT]_6$	For — FAM CCAGTGAAAGCAGACTAGAGC Rev — CATGAGTAGAGTCAGCCACC	55	1.5	0.4	0.2	2	DQ398083
JCU_PC2-F10	$[GTTT]_9$	For — TET GGCTTTACTTTATATGTACTGAG Rev — ATTTCCAATGCCAGTTGTCTG	55	3.0	0.4	0.4	2	DQ398084
JCU_PC3-C1	$[AAG]_{18}$	For — FAM CGGAAGAGGAAACACTCTGGA Rev — GTAGTATTTACTGTACATGAGTC	55	1.5	0.4	0.8	5	DQ398085
JCU_PC3-D2	$[GAT]_8$	For — TET CTTTATAGCGACCTGAGCAG Rev — ACCTGACCAGTTCATTCACTC	55	1.5	0.4	0.8	2	DQ398086
JCU_PC3-E12	$[CA]_{10}$	For — FAM CTGAGACGCCAGGTTTGAGTC Rev — GTTTATGATGATTATCACAGTGTC	55	3.0	0.4	0.4	2	DQ398087
JCU_PC5-B10	$[CAT]_{14}$	For — TET AATCACCAACAGATAATAAACAG Rev — GGATAGATGAGGACAACAGTG	58	5.0	0.2	0.2	2	DQ398088
JCU_PC5-D12	$[CAT]_3$cct $[CAT]_6$	For — TET CTTCATCCTCACCTTCACCAC Rev — CACTGTGTATTGAGGTCGGTG	58	5.0	0.4	0.2	10	DQ398089
JCU_PC5-E9	$[GAT]_{13}$	For — FAM GAGAACAATCCAAAACACTCTG Rev — GTCACAGGAGGTTCCCAAAC	55	1.5	0.1	0.2	2	DQ398090

Table 2 Genetic diversity statistics of eight polymorphic microsatellite loci in a single population of *Parapercis cylindrica*, observed (H_O) and expected (H_E) heterozygosity, (PIC) polymorphic information content, (P_{HW}) probability of conformance to Hardy–Weinberg equilibrium (values in bold are significant after Bonferroni correction, alpha = 0.006)

Locus	No. of alleles	Sample size	Size range (bp)	H_O	H_E	PIC	P_{HW}
JCU_PC1-E9	9	19	257–276	0.895	0.858	0.816	0.015
JCU_PC2-F10	18	23	367–433	0.826	0.886	0.858	0.753
JCU_PC3-C1	10	18	181–235	0.889	0.862	0.819	0.030
JCU_PC3-D2	4	21	180–208	0.381	0.375	0.346	0.658
JCU_PC3-E12	18	17	178–266	0.706	0.945	0.911	0.024
JCU_PC5-B10	8	24	183–210	0.833	0.818	0.775	0.436
JCU_PC5-D12	3	22	102–118	0.409	0.498	0.471	**0.001**
JCU_PC5-E9	12	17	266–320	1.000	0.881	0.840	0.531

Membranes were probed for a subset of the microsatellite repeats using radiolabelled complementary oligonucleotides and standard screening conditions (Sambrook *et al*. 2001). Of the ~350 putative positive clones, 54 were sequenced with a standard T7-promoter primer. Thirty-nine of the 54 sequenced clones contained a microsatellite repeat and from these 18 primer sets were designed and tested using standard polymerase chain reaction (PCR) conditions (55 °C annealing, 1.5 mM $MgCl_2$). Fourteen of these primer sets worked well in initial trials of 10 *P. cyclindrica* samples from Orpheus Island (Australia) when screened using ethidium bromide staining on a Gel-Scan 2000 (Corbett Life Sciences). Twelve loci were polymorphic and 10 of the simplest of these microsatellite loci were chosen for subsequent screening with fluorescently labelled primers. Reagent concentrations for PCR with fluorescently labelled primers were optimized using a modified Taguchi method (Cobb & Clarkson 1994) and standard PCR cycling conditions at 95 °C for 3 min; 95 °C for 30 s, 55 or 58 °C for 45 s, 72 °C for 1 min (35 cycles); and at 72 °C for 10 min. Eight of the 10 primer sets specifically amplified the target locus in 15 µL reactions utilizing 0.5 U of *Taq* DNA polymerase and 1× buffer (QIAGEN) together with reagent concentrations and PCR conditions given in Table 1.

Genotype data were obtained from 17 to 23 *P. cylindrica* samples collected from the central Great Barrier Reef (Australia) and extracted using an SDS–silica plate method (Elphinestone *et al*. 2003). Fluorescent PCR products were ammonium acetate precipitated (Sambrook *et al*. 2001) and size separated on a MegaBACE 1000 automated sequencer (LG). Alleles for each locus were scored on the basis of size using the FRAGMENT PROFILER version 1.2 software (LG). Standard genetic diversity statistics were generated using CERVUS version 2.0 software (Marshall *et al*. 1998) and GENEPOP (http://wbiomed.curtin.edu.au/genepop/genepop_op1.html) (Table 2). The tetranucleotide locus

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JCU_PC5-B10 contained several alleles shifted by two base pairs from the expected size, suggesting that an insertion/deletion is present in the flanking DNA of some alleles. All other loci possessed alleles of the expected size based on simple changes in the number of repeat motifs. All loci conformed to Hardy–Weinberg expectations with the exception of the trinucleotide locus JCU_PC5-D12 (Table 2), which contained the fewest alleles (Table 1). No significant linkage disequilibrium was detected between pairs of loci after Bonferroni correction. These microsatellite loci will be valuable for parentage studies exploring mating strategies of the haremic sandperch, as well as for population genetic studies for this species.

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