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TECHNICAL NOTE

Isolation of 12 microsatellite markers for geelbeck (*Atractoscion aequidens* (Cuvier, 1860), Sciaenidae), an overexploited marine fish

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Abstract Geelbeck (*Atractoscion aequidens*) represents a valuable fishery resource in the Benguela Current region in southwestern Africa. Due to overexploitation the species is considered depleted in South Africa, and little information is available for the rest of its distribution in the region. Genetic studies of the species are essential to inform management plans for sustainable harvesting and conservation. To facilitate such studies 12 polymorphic microsatellite DNA loci were isolated from an enriched genomic library. Genotyping of 41 individuals revealed an average of 17.75 alleles per locus (range 2–37), and observed/expected heterozygosity per locus of 0.024–0.951 and 0.024–0.962, respectively. These loci constitute the first microsatellite markers isolated for geelbeck and are applicable to multiple areas of research on this species.

Keywords Enriched library · Fishery resource · Benguela cold current · Management plan · Stock · Conservation

Geelbeck (*Atractoscion aequidens* (Cuvier 1860)) is a benthopelagic sciaenid fish, occurring off southern Africa and eastern Australia. Geelbeck is considered an overexploited fishery resource in the Benguela Cold Current region off southwestern Africa, where it is targeted both by commercial and recreational fisheries (Hutton et al. 2001; Heemstra and Heemstra 2004). In South Africa it has been among the most important handline fish resources since the line-fishery started in the 19th century (Hutton et al. 2001), but continuous exploitation led to depletion of the stock by 2002 (FAO 2005). In Namibia and Angola little is known about the ecology and population dynamics of the species. Determining the species genetic population structure is fundamental to establish an effective management plan and achieve sustainable exploitation of the resource. To facilitate this goal 12 species-specific microsatellite markers were developed.

Microsatellites were isolated from an enriched partial genomic library created by methods outlined by Glenn and Schable (2005) and McKeown and Shaw (2008). Genomic DNA extracted from fin tissue was digested with RsaI restriction enzyme (New England Biolabs), ligated to matching linkers and amplified by polymerase chain reaction (PCR) using the linkers as PCR primers. Enrichment was performed by selective hybridisation of biotin-labelled repeat motif oligonucleotide probes $(TG)_{12}$, $(GA)_{12}$, $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$ and $(AGAT)_8$ to the PCR products. Hybridised complexes were captured using streptavidin-coated magnetic beads (DYNAL). Microsatellite enriched eluates were PCR amplified and cloned using the TOPO-TA cloning kit (Invitrogen). Recombinant colonies were identified by inactivation of the B-galactosidase gene, individually transferred into 50 µl of 10 mM Tris–HCL (pH 8.5) and incubated at 95°C for 10 min to promote plasmid DNA release. One µl of each plasmid extract was submitted to PCR involving M13 forward and reverse primers. The amplification reaction contained 1× buffer, 1.5 mM $MgCL_2$, 0.2 mM dNTPs, 0.2 U of *Taq* DNA polymerase (Bioline, UK), 10 pmol of each primer and was performed through 30 cycles of 30 s at

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95°C, 30 s at 52°C and 30 s at 72°C. PCR products were cleaned using ExoSap and sequenced using the internal T7 vector primer. Sequences were analysed using the TANDEM REPEATS FINDER (Benson 1999) and, where appropriate, primer pairs designed using Primer 3 (Rozen and Skaletsky 2000).

From 32 primer pairs tested 12 polymorphic loci (Table 1) were selected for screening of genetic variation in 41 geelbeck individuals collected at Namibe, Angola. For each locus the respective forward primer was labelled with a fluorescent dye at the 5′-end (Applied Biosystems). Each locus was individually amplified in a 10 µl reaction mixture containing 100–200 ng of DNA, 1× buffer, 2.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 pmol of each primer, 0.8 U of *Taq* DNA polymerase (Bioline, UK). PCR thermoprofiles included an initial denaturation step of 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, optimized annealing temperature (T_a—see Table 1) for each primer pair for 30 s, and 72°C for 1 min. PCR products were separated using an AB3500 (Applied Biosystems), and allelic calls performed using the GENEMAPPER software (version 4.1, Applied Biosystems).

All loci generated high quality products with allele sizes differing by expected multiples of their repeated motifs. Standard diversity indices for each locus, calculated in FSTAT (Goudet 1995), are presented in Table 1 along with primer sequences and allele size ranges. Tests for linkage disequilibrium (LD) and deviations of genotype proportions from expectations of Hardy–Weinberg equilibrium (HWE) were performed using default parameters in GENEPOP 4.0 (Raymond and Rousset 1995). No significant LD was detected between any locus pair. Genotype proportions conformed to HWE expectations for 10 of the 12 screened loci, with significant departures to HWE found in loci *Geelb28* and *Geelb31* due to an excess of homozygotes (Table 1).

The markers described here represent the first microsatellites isolated for geelbeck and will provide insights

Table 1 Primer sequences (GenBank accession numbers JF927900-JF927911) and characteristics of 12 microsatellite loci developed for geelbeck (*Atractoscion aequidens*): optimal annealing temperature (T_a), allele numbers (N_a) and size range, observed (H_O)/expected (H_E) heterozygosity and *P*-values for tests of Hardy–Weinberg equilibrium (P_{HW})

Locus	Primer sequences (5′–3′)	Repeat motif in cloned allele	T_a (°C)	N_a	Size range (bp)	H_O	H_E	P_{HW}
Geelb5	F: GCAAGGGTGGGCTTTATT (VIC) R: GCACACAGGTGTGAGCAT	$(GA)_{14}$	56	18	142–196	0.872	0.907	0.244
Geelb7	F: TTGTCTTCTCCATCGCTGA (6-FAM) R: CCTCTGCAAAATGTTTGTGTT	$(CA)_8AA(CA)_4$	54	2	86–90	0.024	0.024	–
Geelb13	F: AACACTGCAGCTTCTGTCAA (PET) R: AGGGCTGACCGAGCTAAC	$(CTAA)_9$	56	8	101–129	0.610	0.643	0.485
Geelb16	F: CGCCGTCACGTAAGTCTG (NED) R: CAGCAGACGCACCTTGTT	$(CTAT)_{17}$	56	19	116–192	0.951	0.921	0.547
Geelb21	F: GCCATGAGCCTCACACAA (6-FAM) R: CCGGATGGGACAGACAC	$(CA)_{25}$	56	20	117–167	0.878	0.864	0.246
Geelb25	F: AATGTGCTTTGGCAATGG (VIC) R: GGAAGAGATGTCTCTGAAGGAA	$(GATA)_{30}$	56	19	146–234	0.925	0.914	0.786
Geelb27	F: TGGCCACCAGACTTTGTT (NED) R: GTTGGAGCTCTCTTTTTCCT	$(CTAT)_{27}$	54	19	175–255	0.902	0.926	0.540
Geelb28	F: CCTAATTTCCCTTGGGGTA (PET) R: GCACGTAAATGAAAATGATGG	$(CTAT)_{14}$	50	19	110–190	0.525	0.899	**0.000**
Geelb29	F: TGTGATGGAAATAGGCTGAA (NED) R: TGACGATTGCATGTTCTTG	$(CTAT)_{15}$	56	19	106–182	0.854	0.911	0.203
Geelb30	F: GGTAAACATGTCCCTGCCTA (6-FAM) R: TTGGCAACAAGACTTTCCA	$(GATA)_{17}$	54	17	174–258	0.927	0.911	0.127
Geelb31	F: GCTGTTACATAAACATAATATAGTGAA (VIC) R: TGCTGCTACTGGATCTTTG	$(GATA)_{25}$	54	16	120–216	0.732	0.907	**0.000**
Geelb32	F: GGGGCTGAAGATGACCA (PET) R: TGGGCTCCTTTTTGTTGTT	$(GATA)_{24}(GATA)_{25}(GATA)_9$	54	37	179–313	0.875	0.962	0.029

P-value in bold were significant following Bonferroni correction (Rice 1989)

into species genetic diversity levels, population structure and migration patterns. Thus, these loci will provide information vital to the design of sustainable fisheries management plans and ultimately ensuring the maintenance of the species long-term evolutionary potential.

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