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PRIMER NOTE

Isolation and characterization of microsatellite markers in the African catfish *Clarias gariepinus* (Burchell, 1822)

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The African catfish *Clarias gariepinus* is an economically important species, apart from being a model organism in research (Volckaert *et al.* 1994). Its natural distribution extends all over Africa and Asia Minor; it is cultured intensively and extensively in Africa, Europe and Asia (Huisman & Richter 1987). Little is known about the genetic background of the natural populations and the cultured stocks. Specific highly polymorphic markers are required for example to assess the inbreeding level (Bentzen *et al.* 1991) associated with the aquaculture of this species (Van Der Bank *et al.* 1992).

Due to the relatively high polymorphism microsatellite loci may be used as markers in studies of parentage, quantitative genetics and population genetics (Tautz 1989). $(GT)_n$-repeats, in particular, prove to be highly abundant (Queller *et al.* 1993). We developed specific microsatellite primers for *Clarias gariepinus* in order to perform paternity tests and to characterize wild and domesticated populations. We report the isolation and characterization of seven $(GT)_n$ markers and their potential use in other catfish species.

The microsatellite primers were isolated through the production and screening of a library of short fragments of genomic DNA of *Clarias gariepinus* (Queller *et al.* 1993). After extraction of high-molecular-weight DNA, 50 µg DNA of a single individual was restricted with three different restriction enzymes (*Hae*III, *Rsa*I and *Hin*cIII). The fragments were separated on a 1% low melting point agarose gel and the 300–800-bp fragments isolated. These fragments were ligated into a pUC18 vector. The ligation products were transformed in DH5α-competent cells (*E. coli*; GIBCO BRL) which were plated on to agar plates. The resulting colonies were blotted on Hybond-N membranes which were hybridized with a synthetic $(GT)_{15}$ probe end-labelled with [γ-^{32}P]ATP. The colonies that hybridized were isolated and cultured in a liquid medium. The colonies were sequenced and primer sets were developed for the specific flanking regions of the microsatellite repeats. Fifty-three positive clones were sequenced (Sanger's dideoxy method; T7 DNA-polymerase kit from USB) of which 33 contained GT-repeats (39 arrays in total). A total of 10 different primer sets have been developed specific for *Clarias gariepinus* (Cga01–10).

Genomic DNA for genotyping was prepared using the phenol–chloroform extraction method or by boiling for 2 h in a 10% Chelex solution (Chelex 100 resin from BIORAD). Initially random labelling with ^{35}S was used to visualize the PCR-products (Weber & May 1989). The final concentrations of the reagents in a volume of 12.5 µL were as follows: 10–100 ng of genomic DNA, 1 × PCR-buffer (75 mM Tris-HCl pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 1 mM $MgCl_2$, 15 pmol of each primer, 75 µM of dNTPs (-dATP), 0.25 units of *Taq* polymerase (Goldstar, Eurogentec S.A.) and 50 nmol [α-^{35}S]dATP (ICN). A drop of mineral oil was added before PCR to prevent evaporation and 9 µL of Stop-solution after PCR. The initial denaturation took 4 min at 94 °C followed by 30 cycles (94 °C for 45 s, 30 s at a specific annealing temperature as specified in Table 1 and 72 °C for 30 s) and 10 min at 72 °C in a thermocycler (Triblock, Eurogentec S.A.).

After 5 min of denaturation at 93 °C, 4–5 µL of the PCR-products were separated on a 6% denaturing polyacrylamide-gel at 30–50 mA during 2.5 h of vertical electrophoresis. After fixation and drying, the gel was exposed to a X-ray film during 48 h. The alleles were scored by using a labelled marker (M13 sequencing reaction) whose size is known up to the base pair. We also used high-resolution 4% agarose (NuSieve GTG from FMC) gels in TBE buffer to separate PCR products (alleles) differing at least eight basepairs.

In order to avoid radioactivity but to maintain a high resolution, we currently apply 5'-fluorescently labelled primers (Fluos-Phosphororamidite, Eurogentec S.A.) to visualize PCR-products on a 7% acrylamide-gel (HydroLink) in an A.L.F. DNA Sequencer (Pharmacia Biotech). The gels can be reused once.

PCR conditions for seven of the 10 sets (Table 1) could be optimized to yield clear bands, whose sizes were in accordance with the sizes predicted by the sequence

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Table 1 Characterization of seven *Clarias gariepinus* $(GT)_n$-microsatellite primer sets, including locus name, repeat array, primer sequences, Genbank Accession number, specific annealing temperature, size-range of PCR-products and observed number of alleles (n = 38 individuals)

Locus	Repeat array	Primer sequences (5′→3′)	Genbank Accession number	$[MgCl_2]$ in mM	Annealing temp. (°C)	Size range of PCR product (bp)	No. of alleles
Cga01	$(GT)_{15}$	GGCTAAAAGAACCCTGTCTG TACAGCGTCGATAAGCCAGG	U30862	1	59	92–104	5
Cga02	$(GT)_{10}N_2(GT)_8$	GCTAGTGTGAACGCAAGGC ACCTCTGAGATAAAACACAGC	U30863	1	58	102–110	5
Cga03	$(GT)_{21}$	CACTTCTTACATTTGTGCCC ACCTGTATTGATTTCTTGCC	U30864	1	56	142–168	13
Cga05	$(GT)_{11}N_2(GT)_2$	TCCACATTAAGGACAACCACCG TTTGCAGTTCACGACTGCCG	U30866	1.5	60	204–212	5
Cga06	$(GT)_5N_2(GT)_9$	CAGCTCGTGTTTAATTTGGC TTGTACGAGAACCGTGCCAGG	U30867	1.5	60	134–142	5
Cga09	$(GA)_3N_3(GT)_{11}N$ $(GT)_6N_2(GT)_4$	CGTCCACTTCCCCTAGAGCG CCAGCTGCATTACCATACATGG	U30871	1	65	180–196	7
Cga10	$(GT)_2N_2(GT)_{15}$	GCTGTAGCAAAAATGCAGATGC TCTCCAGAGATCTAGGCTGTCC	U30870	1	60	102–138	14

Table 2 Number of alleles detected in three African catfish species after PCR amplification with seven microsatellite primer sets developed for *Clarias gariepinus*

		Locus						
Species	Sample size	Cga01	Cga02	Cg03	Cga05	Cga06	Cga09	Cga10
Clarias anguillaris	32	9	6	8	8	5	10	9
Clarias alluaudi	1	2	2	2	1	1	2	2
Heterobranchus longifilis	20	5	6	2	4	1	1	1

information. All of these primer sets produced PCR bands that were inherited in a Mendelian fashion (unpublished data).

The seven markers were used to analyse samples originating from Kenya (Lake Victoria), revealing a fairly high amount of allelic polymorphism (Table 1). The number of alleles per locus ranged from 5 to 14 and the heterozygosity from 43 to 89% (mean 61%) for 38 samples analysed. The number of heterozygotes was as expected at Hardy–Weinberg equilibrium for most of the markers as calculated by the GENEPOP program (Raymond & Rousset 1995). A significant deficit ($P < 0.05$) in heterozygotes at loci Cga05 and Cga09 might be, amongst others, due to substructuring of the sample (Wahlund effect) or the presence of null alleles.

In addition, the *Clarias gariepinus* primers amplified microsatellite loci in other species. A mean of eight alleles was amplified by all primers in *C. anguillaris* (Linnaeus) DNA (32 samples). All primer sets also amplified DNA, yielding PCR products of similar size, of *Heterobranchus longifilis* Valenciennes, 1840, (20 samples) and *C. alluaudi* (one single sample) (Table 2). However, some loci (Cga06, 09 and 10) appeared to be monomorphic in *Heterobranchus longifilis*. The possibility to use primer sets interspecifically has been demonstrated in other teleosts such as cyprinids (Zheng *et al.* 1995).

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