Table 2 Name (year of sampling), localization (latitude and longitude in degrees, minutes and seconds), number of individuals ($N_{ind.}$), mean number of alleles per locus (N_{all}), observed heterozygosity (H_O), Nei's gene diversity (H_E) and $\hat{f}$-values for the 12 populations studied

Population	Localization	$N_{ind.}$	N_{all}	H_O	H_E	$\hat{f}$
Itasy (1999)	19 02 25/46 44 24	20	2.1	0.03	0.22	0.86
Nanarena (1999)	22 29 68/45 46 06	20	1.0	0	0.00	—
Man (1990)	07 26 12/07 55 06	18	1.1	0	0.01	1

—, monomorphic populations at all loci.

and one both a dinucleotide (5 repeats) and a tetranucleotide repeat (3 repeats). Nine microsatellite loci differing markedly in structure and number of repeats were retained for further analyses (Table 1). Screening was performed on individuals originating from two sites in Madagascar (Itasy and Nanarena) separated by about 500 kilometres, and from an African population (Man, Western Ivory Coast). The allele range was discontinuous at all loci (Table 1). Allelic diversity was rather high, with a mean of 3.3 alleles per locus, and correlated with the number of repeats of the largest stretch of pure repeats across loci (Spearman's coefficient of rank correlation, $P < 0.003$). It was possible to test for genotypic disequilibrium in only nine cases using exact tests implemented by GENEPOP version 3.1d (Raymond & Rousset 1995b). Applying Bonferroni sequential corrections, significant results at the 2% level were obtained for the pairs Bpf8/Bpf12 ($P < 0.011$), Bpf9/Bpf10 ($P < 0.018$) and Bpf10/Bpf12 ($P < 0.0086$) in Itasy. Gene diversity values ranged between 0 and 0.22 (Table 2). A striking result is that no heterozygotes were observed, except in Itasy (Table 2). Departures from Hardy–Weinberg equilibrium were tested within each population using exact tests. Significant heterozygote deficiencies were indeed observed, and $\hat{f}$-values, where $\hat{f}$ is the estimator of F_{IS}, ranged between 0.86 and 1. This indicated that the selfing rate ranged between 0.92 and 1 (for details about estimators, see Viard *et al*. 1996). This and the wide fluctuations of population size are likely causes of the limited variability observed (Viard *et al*. 1996). Exact tests performed over all loci using GENEPOP revealed that all pairs of population were significantly different ($P < 0.05$). F_{ST} were high in all pairwise comparisons, ranging between 0.84 and 0.99 and indicating that most genetic variance was distributed among populations. Substantial differentiation was also observed between the Ivory Coast and Malagasy populations, even if some alleles were shared at some microsatellite loci. Assessing more precisely the relationship between Malagasy and African populations on the basis of microsatellite variability would be a worthwhile task for the future, especially as *B. pfeifferi* has probably been recently introduced in Madagascar.

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Rapid isolation and characterization of microsatellites from the genome of Asian arowana (*Scleropages formosus, Osteoglossidae, Pisces*)

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Asian arowana (dragonfish; *Scleropages formosus*), is an ancient osteoglossid fish from south-east Asia (Kottelat *et al*. 1993) with extreme values on the ornamental and food market. Due to the high demand of the ornamental fish trade the species came close to extinction, and was classified by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as an Appendix I protected fish (Joseph *et al*. 1986). The green, white and gold strains of Asian arowana have lower commercial value than the red one (Kottelat *et al*. 1993).

Asian arowana is now bred regularly in Singapore and sold with CITES' permission, however, the genetic structure of the brood stock is unknown due to the lack of genetic markers. Microsatellites are the ideal DNA markers for studying fish populations because they are highly polymorphic and easy to analyse (O'Connell & Wright 1997). In this paper we describe the first microsatellites from the green strain of Asian arowana.

A $(CA)_n$-enriched plasmid library was constructed according Fischer & Bachmann (1998) with some modifications. Approximately 500 ng genomic DNA was digested with *Rsa*I, then an adaptor produced by hybridizing 5'-phosphorylated 25-mer and 21-mer oligonucleotides (and containing an *Mlu*I restriction

Table 1 Characterization of 21 microsatellites on 25 individuals of green Asian arowana

Locus	Pepeat motif	Primer (5′–3′)	T_a (°C)	No. of alleles	Size range (bp)	H_O	H_E	Genbank Accession no.
D01 (–)	$(CA)_{10}$	F GAATGCTTAAAGTGGCAGTGAA R CTGGCCTTACGCCCTGTGTTAC	55	11	174–216	0.61	0.82	AF219951
D04 (F)	$(GT)_{41}$	F GCTTAAACCCATTACAGACAGG R TTTCTTCATGCAAAACCACTTT	55	19	171–229	0.84	0.94	AF219952
D11 (H)	$(GT)_{16}$	F TGGTTTCCACCTACAGTCCAAAGA R GTTACGAGTATCTGGCCCAATGG	55	4	154–166	0.60	0.74	AF219953
D13 (–)	$(GT)_{12}$ $(CT)_{23}$	F AGCTGCTGTGTCTGTGGTGGTCTA R CATGCCCATGGAGAGGGAGAG	55	14	94–136	0.44	0.91	AF219954
D14 (–)	$(CA)_{12}$	F AAGGGAGCAGCAGTTAGGTAGACG R CCGTGGTGAATTAACATTTCCTCT	55	14	196–246	0.28	0.92	AF219955
D15 (F)	$(GT)_{16}$	F GACTGGCGTCCCGTCCTG R TTAGTGTCAATGGGAAAGAGCATA	50	6	224–236	0.28	0.54	AF219956
D16 (F)	$(GT)_{20}$	F CTTGCGCCCTGTGTTGC R AAGGCCTTTTCTGCTGGTAA	55	10	127–163	0.72	0.74	AF219957
D27 (F)	$(CA)_{17}$	F GTGTCAGTATAGTGAATCTGTAG R TGACAATGGCAGCATAATGAGAT	55	11	97–127	0.52	0.84	AF219958
D31 (F)	$(GATA)_{15}$	F GTTTGTCCCTCCATGCACTGAGAG R GTGATTGCCACATGGTTTTGTTGG	50	15	154–223	0.84	0.88	AF219959
D32 (H)	$(CA)_{13}$	F AGCACCCTGTTACTGGAAGAGA R AGTGTGATGCTTTTGCTTTGAGAA	55	9	236–292	0.84	0.80	AF219960
D33 (H)	$(CA)_{12}AA$ $C(CA)_4$	F TATTACCATGCGCCCAGCACAC R TGGGTGAGCCAGAAGCAGGACT	55	5	130–138	0.40	0.32	AF219961
D35 (F)	$(GT)_{17}$	F GTTTCTTCTAGGTGCTCTGGTTTC R CTACTTACACTGGGTCACTCATCC	55	12	130–156	0.32	0.91	AF219962
D37 (F)	$(GT)_{51}$	F GCCTTACGCCCTGTGTTGC R TGGATATCTGTGAGTGGTGGTGAA	55	13	223–294	0.72	0.88	AF219963
D38 (F)	$(GT)_{24}$	F TTGGGGTCATGCCACTGG R CAATAAATACCAAACAGGGAACC	50	22	179–227	0.61	0.95	AF219964
D42 (F)	$(CA)_{19}$	F AGGAACATCACTGACAACACT R TGGACTAACTAGGAGCACAT	50	20	145–201	0.92	0.94	AF219965
D72 (–)	$(CA)_{14}$	F AGCAGGTTAATTTGGAGACT R CGACCCTGTATGGGACAAG	50	9	78–98	0.60	0.89	AF219966
D85 (H)	$(CA)_{10}$	F GTTCCACAGGGGCTGAGAAAAT R GAGGACGGAACAAAAGCATTGG	55	8	140–154	0.60	0.79	AF219967
D88 (H)	$(GT)_{11}$	F TTTCTTTCTGAGACTGAGG R CAACTCTTATCCACCATTT	50	13	128–164	0.64	0.90	AF219968
D92 (H)	$(GT)_{13}$	F AGTCGCACACCACCACCTCAG R TCAGCGATAACCCCACACCT	55	12	146–174	0.72	0.89	AF219969
D94 (H)	$(CA)_{16}$	F CAGCAGCAGTGACACGGGTTCG R TCGCAGGCTGATTAAAGGTGTG	55	8	195–217	0.64	0.77	AF219970
D95 (T)	$(CA)_9$	F CCTGCGGAAGAAGAAAAGACT R CATGGTGTTGGCTGTGAGGAG	55	10	165–205	0.48	0.79	AF219971

(H), F primer labelled with HEX; (F), F primer labelled with 6FAM; (T), F primer labelled with TET; (–), primers unlabelled.
T_a, optimal annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity.

site) was ligated onto the blunt ends (Edwards *et al*. 1996). The ligation product was polymerase chain recation (PCR)-amplified in 25 μL containing 10 mM Tris-HCl (pH 8.8), 150 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol of the 21-mer oligonucleotide as primer, 250 ng template and 2.0 U DyNAzyme II DNA-polymerase (Finnzymes). The PCR conditions were: 2 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 2 min. The PCR products were then hybridized to a biotinylated $(CA)_{10}$ probe in 6× SSC at 55 °C for 20 min. The products from this hybridization were captured by using streptavidin-coated magnetic beads (350 μg MagnaBind™ from Pierce in 10 μL 6× SSC). The unhybridized genomic DNA and $(CA)_{10}$ probe were washed away and the captured fragments were eluted according to Fischer & Bachmann (1998). The eluate (1–2 μL) was then used as template for PCR amplification (see above for conditions). The amplification products were digested with *Mlu*I and ligated into pPCR-Script Cam vector (Stratagene) linearized with *BssH*II. The ligation products were transformed into XL-gold Kan supercompetent cells (Stratagene) and plated upon LB-agar containing 30 mg/L choramphenicol, 60 mg/L IPTG and 40 mg/L X-gal. More than 99% of the 10 500 clones obtained were white. The insert length was determined by colony PCR using M13–20 and M13 reverse

primers. Approximately 85% (244/288) of the tested clones contained inserts between 250 and 1000 bp. They were purified using GeneClean II Kit (Bio 101), then sequenced using M13–20 or M13 reverse primers using the ABIPrism Big Dye terminator cycle sequencing kit (Applied Biosystems). Extension products were separated on an automated ABI 377 sequencer (Applied Biosystems).

Out of 45 sequenced clones 35 (77.8%) contained CA repeats proving the efficiency of our approach. Twenty-eight out of 35 (80.0%) of these CA clones showed sufficient flanking sequences for primer design (PrimerSelect, DNASTAR).

PCR amplification of microsatellites was performed on a PTC-100 thermal cycler (MJ Research) using 30 ng genomic DNA in 25 μL volume. The concentration of components in the PCR reaction were as above, except for 0.2 μM of the primers. PCR conditions were: 94 °C for 2 min followed by 33 cycles at 94 °C for 30 s, annealing temperature (50 or 55 °C) for 30 s and 72 °C for 30 s with a final extension for 5 min at 72 °C. Unlabelled PCR products were separated either on 8% denaturing polyacrylamide or 4% Metaphor Agarose (FMC BioProducts) gels, then visualized by using silver staining or ethidium bromide staining, respectively. For fluorescent detection on the ABI 377 sequencer, one primer per pair was labelled with either 6FAM, TET or HEX dyes (Geneset). The length of the fluorescently labelled PCR products was determined using GENESCAN and GENOTYPER software programs (Applied Biosystems). Allele sizes of unlabelled PCR products were calculated against a 20-bp ladder. Twenty-eight microsatellite primer pairs were tested in 25 green Asian arowana individuals obtained from farms in Singapore and Indonesia (pedigrees unknown). Twenty-one out of 28 microsatellites showed specific products and polymorphism (Table 1). The average number of alleles was 11.7, while the average heterozygosity was 0.58.

Our microsatellite isolation method requires approximately one tenth of DNA (500 ng) than the original protocol of Fischer & Bachmann (1998). This made sample collection from live individuals less invasive, which is important for the study of an endangered species. Sequencing reactions were performed directly on the glassmilk-purified product of colony PCR, eliminating the need for minipreps.

Experiments are in progress to investigate the applicability of these markers to the gold and red strains, as well as to develop multiplex-PCRs for studies on genetic diversity of local Asian arowana brood stocks.

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Microsatellite variation in the freshwater schistosome-transmitting snail *Biomphalaria glabrata*

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Biomphalaria glabrata is a tropical freshwater snail and the main vector of schistosomiasis in South America and the Caribbean. This snail is a common inhabitant of slow flowing streams, drainage ditches and ponds from Brazil to Haiti. Reduced genetic variation has been previously reported in local populations of this species on the basis of allozyme studies (Mulvey & Vrijenhoek 1982), which may be related to large variation in population size. The mating system may also reduce variability because this species is a hermaphrodite that can self-fertilize. Microsatellite loci previously proved to be useful for producing variability in such situations (see Viard *et al*. 1997).

We characterized microsatellite loci in *B. glabrata*. DNA was extracted from foot tissue using the QIAmp Tissue kit (Qiagen). A partial genomic library (hereafter the classical library) and a GATA/GACA-rich library (hereafter the enriched library) were constructed following the protocols described by Estoup *et al*. (1993) and Billotte *et al*. (1999), respectively. Screening was performed following Waldbieser (1995). Positive colonies (1.4%, 23 and 19%, 19) were detected with $(GATA)_6$ and $(GACA)_5$ probes in the classical and enriched libraries, respectively (Table 1). Thus, the enrichment protocol yielded 13 times more tetranucleotide loci than the classical one. The classical library also yielded 2.2% (36) positive colonies for $(CT)_{10}$ and $(GT)_{10}$ probes. Only positive colonies with large inserts, as determined by polymerase chain reaction (PCR) with forward and reverse M13 primers, were sequenced. Ninety–100% of inserts sequenced contained at least one microsatellite (Table 1).