($n = 15$), observed heterozygosity was low for three loci (SsCTAB6, SsCTB24 and SsCA58). A significant excess of homozygotes, and hence a positive inbreeding coefficient ($f = 0.30$), was found in the 15 individuals of the Bochum population using the AMOVA procedure implemented in Arlequin 3.1 (Schneider *et al*. 1997). This indicates that individuals of this hermaphroditic species exhibit selfing. However, due to the small sample size, it was not possible to test for the presence of null alleles (Van Treuren 1998). No significant linkage disequilibrium could be detected between any locus pair using the software Arlequin 3.1 ($P > 0.04$ in all six comparisons for the Bochum population, Bonferroni correction not applied). Ten individuals of the Bochum population (66%) exhibited three PCR fragments for the locus SsCA25, indicating the presence of a third allele, possibly due to gene duplication. Overall observed heterozygosity in the samples from the UK ranged between 0.88 and 0.62. A third gene copy at the locus SsCA25 was not observed in those worms. We also tested the identified primers in two other cestodes, *Diphyllobothrium latum* and *Taenia solium*. However, PCR amplification was not successful. The high polymorphism of our markers in *S. solidus* suggests that they will be useful for paternity assessment, the estimation of selfing rates and the study of gene flow and population structure.

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Polymerase chain reaction primers for polymorphic microsatellite loci in the invasive toad species *Bufo marinus*

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Originally from South America, the cane toad (*Bufo marinus*) was deliberately introduced to various Atlantic and Pacific islands, and to Australia, early this century as a biological control (reviewed in Easteal 1981). Because of its well known introduction history and likelihood of strong founder events and demographic flushes, the cane toad provides a good empirical model for studying the population genetics of nonequilibrium systems. Surveys of enzyme variation in the introduced populations in Australia enabled an assessment of some of the genetic consequences of colonization processes (Easteal 1988). However, low level of polymorphism observed from enzyme markers limited resolution. Thus, the development of microsatellite markers in *B. marinus* represents a significant progression in the study of this invasive species.

Two libraries enriched for microsatellites were constructed and screened for $(GT)_n$ repeats following the protocols of Armour *et al*. (1994) and Paetkau (1999). Following the former protocol, 8000 clones were probed, from which 21 positives were picked and sequenced. Eleven of those clones had flanking regions of at least 20 bp and a core sequence of at least 10 repeats, and hence selected for primer design. Following the latter protocol, 400 clones were obtained, 100 sequenced, and 18 selected for primer design. Of the 29 clones selected, preliminary genotyping tests indicated that 17 loci were not suitable as they produced dubious amplification patterns. Two *B. marinus* populations, British Guyana (source population) and Gordonvale (introduced population in Australia), were genotyped with the remaining 12 loci. The primer and core sequences of those loci are provided in Table 1.

Extractions of DNA from individuals (usually a toe section) were performed following Estoup *et al*. (1996). Polymerase chain reactions (PCRs) contained: 1–2.5 mM $MgCl_2$ (see Table 1), 0.17 mM of each dNTP, 1 × PCR buffer [100 mM Tris (pH 8.8)], 1% Triton X-100, 500 mM KCl, 160 μg/mL (BSA), 0.07–0.14 μM of fluorescent labelled primer (Table 1), 0.25 μM of unlabelled primer, and 0.2 units of DyNAzyme DNA polymerase (Finnzymes). PCRs were performed in a GeneAmp PCR System 9700 thermal cycler using the following programme: one cycle of 94 °C for 1 min 45 s; five 'touchdown' cycles: 94 °C for 15 s, 1 °C drop per cycle to a final annealing temperature of 57 °C or 52 °C (Table 1) for 20 s, 72 °C for 10 s; 27 cycles of 94 °C for 15 s, 57 °C or 52 °C for 20 s, 72 °C for 1 s; and a final hold of 72 °C for 2 min. Genotypes were scored using ABI PRISM Genotyper 2.0 software (Perkin Elmer).

All 12 loci were polymorphic with an observed number of alleles per population ranging between one and 10, and heterozygosities between zero and 0.909 (Table 1). Exact tests performed using GENEPOP 3.1 (Raymond & Rousset 1995) with a correction for multiple comparisons (sequential Bonferroni procedure, Rice 1989), detected significant deviation from Hardy–Weinberg equilibrium and linkage disequilibrium for the loci BM102 and BM128. As the sex of the individuals was known for the Gordonvale (Australia) sample, we were able to detect that both these loci were sex linked. This finding

Table 1 Primer sequences, PCR conditions and polymorphism statistics for 12 microsatellite loci identified in *Bufo marinus*. G (30 individuals) and BG (22 individuals) refer to the Gordonvale (Australia) and British Guyana populations, respectively. H_O and H_E are observed and expected (i.e. 'gene diversity'; Nei 1987) heterozygosities, respectively. The first primer listed for each locus was flourescently labelled. The repeat motif is listed 5′ to 3′ with respect to the flourescent primer, and the final concentration in a PCR also refers to this primer. T_a indicates the lower annealing temperature in a PCR. Sex linked loci are marked with an asterisk. Sequences from which primers were designed have GenBank accession nos AF273090–AF273101

Locus	Primer sequences (5′ to 3′)	Repeat motif in library	T_a (°C)	$MgCl_2$ (mM)	Primer (μM)	Size range (bp)	No. alleles		H_O		H_E	
							G	BG	G	BG	G	BG
BM102*	GATCGGAGACATCTGGCA GATCCAACTCATACATACGTACA	$(GT)_{10}$	57	2	0.07	100–124	4	5	0.800	0.455	0.693	0.515
BM121	GACCCCATTTGTGCTGAG AACCAGCATTGCTGAAGTATC	$(GT)_{21}$	57	1.5	0.07	144–169	5	8	0.600	0.909	0.761	0.821
BM128*	GCCATATTTCGTGACTGTAGC CTGGGTTATCTAATATATAAAGCTGAG	$(AC)_{23}AT(AC)_2$	57	1	0.11	115–145	3	8	0.800	0.818	0.595	0.777
BM217	AACATGACAACCCAGCCAT GCGGTGCAGATTCCTTTAGT	$(TA)_{10}$ … $(GT)_{10}$	57	1.5	0.07	149–164	1	5	0.000	0.364	0.000	0.329
BM218	GGTATGCAACTGCATGAGC GGTTGCTACTTAGTAAGTTCGC	$(CA)_{12}$	57	1.5	0.07	161–173	2	3	0.267	0.636	0.282	0.566
BM224	GGGATCTGTGCAGATGGG GCTGATCTTGCACAATCTTTG	$(TG)_5TA(TG)_5TA(TG)_2$ … $(AG)_{15}$	57	1.5	0.07	142–154	5	5	0.467	0.818	0.631	0.710
BM229	ACTAAATTTATCATGTGCGCC ACACTGTAGCCATGCTGCA	$(TG)_{14}$	52	2	0.14	84–94	2	4	0.467	0.591	0.488	0.721
BM231	GTACCTTAATGGGGAAAGATC AACAGTGCCAGTCAATAGAAG	$(AC)_3TC(AC)_6GC(AC)_2GC(AC)_{11}$	57	1.5	0.07	170–174	2	3	0.333	0.136	0.325	0.449
BM235	GAAAATGAATTGACAGGTCCTC GTGACATCACTAGTATAGAAAGAGGC	$(TA)_8(CA)_{14}$	52	2.5	0.14	228–246	3	7	0.433	0.318	0.518	0.783
BM239	AAGTAGAGTTTTGCCGCAC AGCTACGTTCCCTTCAAC	$(TG)_4CG(TG)_{16}(CG)_4$	57	1.5	0.07	100–123	4	10	0.700	0.864	0.747	0.873
BM279	GGAGAAGTTTGTATTGCGAAC ATGGAGCATATCTGATTTGTGTAG	$(TG)_{12}$	57	1.5	0.07	247–257	3	4	0.400	0.773	0.392	0.735
BM322	AATCCACTCTTTACAAGTCCG ATTGATGCCCTATCCTGAG	$(GT)_{15}$	52	2.5	0.14	228–251	2	7	0.433	0.636	0.463	0.814

Table 2 Results of cross-species amplification for each microsatellite locus using the PCR conditions standard for *Bufo marinus*. Size of products is given in bp, and number of individuals screened is indicated within parentheses. A hyphen indicates the absence of a visible fluorescent PCR amplimer

	South America		North America	South Africa		Europe
Locus	*B. paracnemis* (4)	*B. granulosis* (2)	*B. americanus* (2)	*B. gutturalis* (3)	*B. rangeri* (2)	*B. bufo* (2)
BM102	110, 112, 114	—	—	—	—	—
BM121	143, 145	—	163	125	148	—
BM128	113, 115, 119, 121	—	—	—	—	—
BM217	149, 151	—	135, 137	—	—	—
BM218	159, 169, 171, 173, 175	—	—	—	—	—
BM224	142	154, 156	146, 148, 152	140, 144, 148, 150	140, 142	136
BM229	82, 84, 88	88, 94	—	—	—	—
BM231	—	—	—	—	—	—
BM235	—	—	—	—	—	—
BM239	89, 95	89, 95, 97, 99	—	—	—	—
BM279	251, 253, 255	—	—	—	—	—
BM322	229, 231, 233	—	—	—	—	—

was confirmed with additional tests using other populations (unpublished data).

Using PCR conditions for *B. marinus*, the 12 loci were tested for interspecies priming for an additional six *Bufo* species (Table 2). BM224 was the only locus that provided an easily interpretable pattern for all species, and showed polymorphism within most species. The closely related species *B. paracnemis* (Slade & Moritz 1998) amplified successfully with nine of the 12 microsatellites and showed polymorphism at most loci. Other species could not be amplified for most loci which is not unexpected considering their more distant relationship to *B. marinus* and the deep evolutionary history of the genus *Bufo* (Goebel *et al*. 1999). These results are in agreement with the low cross-species priming found at eight microsatellites cloned from *B. calamita* (Rowe *et al*. 1997).

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Microsatellite markers for coral trout (*Plectropomus laevis*) and red throat emperor (*Lethrinus miniatus*) and their utility in other species of reef fish

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There is concern about sustainable management of stocks of reef fish, particularly as we do not fully understand population dynamics and response to harvest of many species (Mapstone *et al*. 1997). Evidence to date suggests that most harvested species have restricted movement ranges (Davies 1995). Genetic diversity may be reduced if particular populations are over-harvested. To effectively manage fishing in the Great Barrier Reef (GBR), managers require information on the stock structure and gene flow among populations of harvested species along the GBR. This will allow managers to balance conservation of biodiversity with sustainable exploitation. Genetic markers are useful in determining stock structure of fish and assessing gene flow among populations (e.g. Wright & Benzen 1994). The lack of suitable genetic markers is an impediment to our understanding of the population structure of tropical reef fish.

Microsatellite markers are powerful tools for detecting intra- and interpopulation genetic diversity. Here we report the development of microsatellite markers to assess genetic diversity for populations of *Plectropomus laevis* and *Lethrinus miniatus* on the GBR. These species represent two major families of harvested reef fish. Tests against several other taxa were undertaken to ascertain the utility of these markers to differentiate between populations in a wide range of reef fish.

Partial genomic libraries were constructed using genomic DNA extracted from liver/muscle tissue of *P. laevis* and *L. miniatus*, by standard methods (Sambrook *et al*. 1989). DNA was digested with *Alu*I and *Hae*III, fragments were separated by gel electrophoresis and fragments of 400–1000 nucleotides were excised (Rassman *et al*. 1991), purified and ligated into puc18/*Sma*I BAP (Invitrogen).

Nearly 3000 clones were screened using a T4 polynucleotide kinase — [γ^{33}P]-ATP end-labelled cocktail of di-, tri- and tetranucleotide oligonucleotides [$(GT)_{15}$, $(GA)_{15}$, $(GAA)_{10}$, $(CCA)_{10}$, $(CGG)_{10}$, $(CACG)_7$, $(CCTT)_7$ and $(GATA)_7$] as described by Glenn (1996). Fifty coral trout and 112 red throat emperor clones were sequenced (DyeDeoxy terminator cycle sequencing kits, Perkin Elmer/ABI). Vector sequences were eliminated and primer pairs manually designed and optimized for 22 markers of each species according to principles described by Ehrlich (1989) (Table 1). Eight of the 22 red throat emperor markers,