Table 1 Primer sequences and characteristics of 10 variable (n = 408) and invariant (n = 12) microsatellite loci in *Cristatella mucedo*. GenBank Accession nos for these sequenced clones are AF085418–AF085427

Locus	Primer sequence (5'— 3')	Repeat *	Size (bp)*	No. of alleles	H_O	H_E
1.1	F:CTGCCTTTCTGCTTCTACATAC R:TTGGTGTTGATGTGGTGAATAC	$(AG)_{32}$	217	13	0.1446	0.8509
1.3	F:TTGGTGTTGATGTGGTGAATAC R:ATCCTTCACGGCTTGTTCAG	$(AG)_{28}$	154	2	0	0.4638
2.2	F:GCAATTCTCACTTTCCATGTGCTC R:CTCGCGTCTGCCTGTCATTACA	$(CTGT)_7(CTGC)_3$ $(CTGT)_5(CT)_{51}$	251	17	0.4216	0.8764
2.9	F:TGTTGATAGCCTACAGGAATAC R:GCGTTGTTAGAATAGCCTGT	$(TG)_{48}$	164	1	0	
5.9	F:TTGTCAAAGAGTTCAGCCGAATA R:GCGTGTTGTATCTTAGTGCTTGG	$(AG)_{53}$	235	25	0.0245	0.9135
6.4	F:GTGTGTGACCTGTTATATCAACC R:ACCAATAGTACCAAGCTACAGC	$(CG)_6CCC(GT)_{11}$	196	1	0	
6.7	F:TACGACCCTACTGAATTACTCATGG R:TTCCCTCGTGTCATTCACAG	$(AG)_{13}$	248	14	0.1299	0.5891
7.2	F:CACCTGCTCACATAATACACTCC R:TCTGAGGGCGCTTTATCAAG	$(CT)_{20}$	190	1	0	
8.1	F:AAAGGTACAACTGAGTGTCATCTGC R:TACCGGGATCCGTTCTTTTC	$(AG)_{36}$	170	1	0	
9.4	F:ACAACAAACCCTTCAGCCAATG R:ACAGTGGAGTTCAGCCAGTAATG	$(GT)_{15}$	174	8	0.0074	0.7282

H_O, observed heterozygosity; H_E, expected heterozygosity.
*Sequenced allele.

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Isolation and characterization of microsatellite loci from the three-spined stickleback (*Gasterosteus aculeatus* L.)

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The three-spined stickleback, *Gasterosteus aculeatus*, is frequently used as a model organism for questions concerning evolution, ecology, and behaviour (Bell & Foster 1994). This small, territorial fish species has exclusive male parental care and conspicuous male nuptial colouration and courtship. The species is well known for 'sneaking' behaviour, an alternative reproductive tactic in which a rival male tries to steal fertilizations in another male's nest (Goldschmidt *et al.* 1992). It has a wide geographical distribution and can be studied relatively easily both in nature and in the laboratory. It is furthermore remarkably variable for a wide array of features, and is actually a large complex of differentiated allopatric

populations and biological species (Bell & Foster 1994). Microsatellite loci would allow the possibility of tackling various behaviour–ecological and population–genetical issues in this species. Here we report the developement of primers for PCR amplification of 10 new microsatellite loci of the three-spined stickleback.

Stickleback microsatellite loci were cloned as described in Estoup *et al.* (1993) and in detailed protocols by A. Estoup and J. Turgeon available at the internet site http://www.inapg.inra.fr/dsa/microsat/microsat.htm. The genomic library was constructed with approximately 10 μg of DNA isolated from muscle tissue of a single stickleback from a population near Roche/Montreux, Switzerland (46°26′N, 6°55′E). Approximately 1600 colonies were screened for microsatellites using a mixture of six probes $(TC)_{10}$, $(TG)_{10}$, $(CAC)_5CA$, $CT(CCT)_5$, $CT(ATCT)_6$, and $(TGTA)_6TG$ yielding 113 positively hybridizing clones. Plasmid DNA of positive clones was purified with a QIAprep Spin Miniprep Kit (Qiagen). Both strands of the stickleback DNA inserts were sequenced using a Thermo sequenase cycle sequencing kit (Amersham) and M13 forward and reverse primer, which were endlabelled with fluorescent dye (IRD800; Li-Cor). Miniprep preparation and sequencing reactions were carried out according to the recommendations of the manufacturers and sequence reaction products were resolved on an automated DNA sequencer (model 4200; Li-Cor).

Here we report 10 microsatellite loci (Table 1) for which we have so far successfully designed primer pairs. The genomic DNA for genotyping was prepared either using a phenol–ethanol extraction method or a rapid Chelex resin extraction protocol described by Estoup *et al.* (1996). PCR amplifications were carried out in 10 μL volumes using a PTC100-machine (MJ Research). Each reaction contained approximately 5–20 ng of genomic DNA, 2 pmol of each primer, one of which was endlabelled with an infrared fluorescent dye (IRD800; Li-Cor), $MgCl_2$ (concentration in Table 1), 20 μg/mL BSA, 60 μM of each dNTP, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, and 0.25 U *Taq* polymerase (Promega). Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, five cycles consisting of 30 s at 95 °C, 30 s at the annealing temperature (see Table 1), and 75 s at 72 °C, 25 cycles (except locus Gac7033PBBE: 21 cycles) consisting of 30 s at 94 °C, 30 s at the annealing temperature, and 75 s at 72 °C, followed by a final 5 min extension at 72 °C. PCR products were analysed on an automated DNA sequencer (model 4200; Li-Cor) and ampli-

Table 1 Characterization of 10 microsatellite loci in the three-spined stickleback based on a population sample of 50 individuals

Locus	Repeat array	Primer sequences (5'- > 3')	T (°C)	$MgCl_2$ (mM)	n	Size range (bp)†	H_O	H_E	GenBank Accession no.
Gac1097PBBE	$(CA)_{30}$	*AGGAACTCTCTTCTTCTCTG CCCGGGTTAGTCACT	56	1.0	6	100–130 (130)	0.50	0.54	AJ010352
Gac1116PBBE	$(CA)_{40}$	*GGTGTCATGTGGGGGCGAGCAG CCCGAAGCATTGTGGCATCATC	64	0.8	12	124–184 (166)	0.72	0.81	AJ010353
Gac1125PBBE	$(CA)_{24}$	*CATCACACCCAGCCTCTC CCTCCCTCCAACTCTTATCA	58	1.2	5	159–185 (179)	0.30	0.27	AJ010354
Gac2111PBBE	$(AC)_3AG(AC)_{18}$	*GTAGAGCACTTGAACTTGAACTG GACGTAGATTGTGGATGTAGAGG	54	1.2	5	225–235 (235)	0.74	0.71	AJ010355
Gac3133PBBE	$(CA)_{21}$	*CGCCCAGTTCCTGAACTTAG CATGGTGGGCTGACTGAC	64	0.8	6	138–150 (144)	0.46	0.52	AJ010356
Gac4170PBBE	$(CA)_{20}$	*GCCGAGCCACATAGAGA CCAATATAACAGCCGAGCAG	61	0.7	5	120–132 (124)	0.64	0.62	AJ010357
Gac4174PBBE	$(CA)_{24}CG(CA)_7$	*CCGCGATGATGAGAGTG GTGAAATGCGACAGATGATG	54	1.2	5	204–214 (214)	0.72	0.72	AJ010358
Gac5196PBBE	$(AC)_{19}$	*ACTTCTCCCCTCATTATGCT GGGGTCTGATGGATACAAA	56	0.8	5	157–169 (159)	0.58	0.63	AJ010359
Gac7033PBBE	$(CA)_{23}$	*AGGTGGATTGGTTTTCTG GGACGCTCGCTCTTTC	55	1.3	4	209–223 (217)	0.64	0.63	AJ010360
Gac7188PBBE	$(CA)_{45}$	*CCCCTCACACATAGTTACAC TTCAATTGGGAGAGAAGC	54	1.2	9	143–203 (199)	0.44	0.53	AJ010361

n, No. of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.
*Primer used for end-labelling.
†Cloned insert size in parentheses.

fied fragments of cloned alleles were used for size determination at the respective loci.

All loci were analysed for a population sample of 50 individuals of sticklebacks from the Roche population. Departures from Hardy–Weinberg expectations for all loci and linkage equilibrium between all pairs of loci were assessed with the GENEPOP (version 3.0) program package (Raymond & Rousset 1995). Number of alleles per locus and observed heterozygosity are listed in Table 1. All loci were polymorphic, the number of alleles per locus ranging between four and 12 and the observed heterozygosity between 0.44 and 0.72. One locus (Gac5196PBBE) deviated significantly from Hardy–Weinberg equilibrium ($P = 0.026$) and among a total of 45 comparisons two pairs of loci (Gac2111PBBE×Gac4174PBBE and Gac4170PBBE×Gac3133PBBE; *P*-values: 0.033 and 0.027, respectively) showed significant devations from linkage equilibrium. Such a number of significant deviations is to be expected by chance given the number of tests made. In accordance with this, global tests across all loci or pairs of loci (Fisher's method or sequential Bonferroni correction) did not show significant departures from the two equilibria.

Together with the loci published by Rico *et al.* (1993) and by Taylor (1998), there is now a selection of 18 microsatellite loci for three-spined sticklebacks to choose from, considerably increasing the probability of obtaining a sufficient number of loci with an appropriate degree of polymorphism for various kinds of studies and populations.

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Identification and characterization of microsatellite loci in coconut (*Cocos nucifera* L.) and the analysis of coconut populations in Sri Lanka

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The coconut palm, *Cocos nucifera* L. is a major plantation crop and the most important palm of the wet tropics. It is the most extensively grown nut in the world and was originally disseminated from its centre of origin by sea currents and then further by human agency (Harries 1978; Ohler 1984). As a result of this, the genetic structure of coconut populations is probably influenced by genetic drift and founder effect (N'cho *et al.* 1993). According to the Coconut Genetic Resources Database (Version 2.2/IPGRI), over 300 coconut ecotypes have been documented throughout the world to date and the evaluation and characterization of coconut palm is mainly carried out on morphological and reproductive traits (Fernando *et al.* 1995). In Sri Lanka, coconut has been classified into three varieties: typica (tall type), nana (dwarf type) and aurantiaca (intermediate between tall and dwarf but shown previously to be more similar to dwarfs [Perera *et al.* 1998]), and 15 forms within these varieties based on their breeding habit, morphology and fruit characteristics (Liyanage 1958; Wickramaratne & Rathnasiri 1986; Perera *et al.* 1996). The high rate of current genetic erosion of coconut, mainly due to the fragmentation of land for industrial and/or urban development, has led to the gradual replacement of existing natural biodiversity with a reduced number of hybrids exhibiting a narrow genetic base. It is therefore important to assess the levels and patterns of genetic diversity in coconut populations in order to formulate appropriate conservation strategies as well as to identify ecotypes that represent a 'core gene pool' for *ex situ* maintenance. Simple sequence repeats (SSRs) or microsatellites (Powell *et al.* 1996a) provide an ideal tool for such studies due to their high information content, ease of genotyping through PCR, codominant and multiallelic nature and high discrimination power (Morgante & Olivieri 1993; Powell *et al.* 1996a,b; Russell *et al.* 1997). Microsatellites have been used in both agricultural and breeding studies as well as in the analysis of natural plant populations (see Powell *et al.* 1996a and references therein).

Eight coconut microsetellites were identified using a precloning enrichment procedure described by White & Powell (1997). The genomic DNA was enriched for $(AC)_n$ repeats using biotinylated oligomer $(AC)_{13}$ and the enriched fraction was then cloned into λ-Zap phage vector. Positive recombinant clones were identified by hybridization with an endlabelled $(AC)_{13}$ oligomer and sequenced on an ABI 377