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Isolation and characterization of polymorphic microsatellite loci in black sea bream (*Acanthopagrus schlegeli*) by cross-species amplification with six species of the Sparidae family

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Abstract – We tested cross-species amplification of 68 existing microsatellite loci in 6 species of the Sparidae family: *Acanthopagrus butcheri*, *Sparus aurata, Pagrus auratus, Chrysophrys major, Pagellus bogaraveo, Pagellus erythrinus* and one species of Bothidae, *Paralichthys olivaceus*. Of the 68 loci screened, sixteen were found to be polymorphic when tested in 20 individual black sea bream, *Acanthopagrus schlegeli*. The number of alleles per locus ranged from 2 to 9, and the observed and expected heterozygosity ranged from 0.55 to 0.95, and from 0.58 to 0.87, respectively. Our results show that cross-species amplification of known microsatellite loci in closely related species is a highly promising source of microsatellite markers for *A. schlegeli*.

Key words: Microsatellites / Cross-species amplification / *Acanthopagrus schlegeli*

Résumé – Isolation et caractérisation de locus de microsatellites polymorphiques chez *Acanthopagrus schlegeli* par amplification interspécifique avec six espèces de Sparidés. Nous testons l'amplification interspécifique de 68 locus de microsatellites existants de six espèces de Sparidés : *Acanthopagrus butcheri*, *Sparus aurata*, *Pagrus auratus*, *Chrysophrys major*, *Pagellus bogaraveo*, *Pagellus erythrinus* et une espèce de Bothidé, *Paralichthys olivaceus*. Des 68 locus étudiés, seize sont polymorphes lorsqu'ils sont testés chez 20 individus de *Acanthopagrus schlegeli*. Le nombre d'allèles par locus varie de 2 à 9, et l'hétérozygosité (la diversité génétique) s'étend de 0,55 à 0,95, et de 0,58 à 0,87, respectivement. Nos résultats montrent que l'amplification interspécifique de locus de microsatellites chez des espèces proches est une source prometteuse de marqueurs de microsatellites pour *A. schlegeli.*

1 Introduction

Black sea bream, *Acanthopagrus schlegeli*, a protandrous marine hermaphrodite, is of particular interest for commercial aquaculture and a major target for breeding programmes (Chang and Yueh 1990). This species is widely distributed along the West Pacific coasts from Japan and Korea to the East China Sea and Taiwan. Only a limited few reports are currently available that concern the molecular phylogeny and population structure of this species using different molecular marker techniques (Jean et al. 1996; Jean et al. 1998).

Microsatellites are simple DNA sequences, repeated in tandem, and widely dispersed throughout the genomes of eukaryotic and prokaryotic organisms (Tautz et al. 1986). Microsatellites are highly variable and most are thought to be selectively neutral, making them amenable to population genetic theory. However, only a limited number of microsatellite markers are available for *A. schlegeli*. A current lack of sufficient and polymorphic microsatellite markers is severely limiting the development of population structure, molecular phylogeny and molecular-derived breeding data in this important marine fish species. Thus, screening for polymorphic microsatellite markers in *A. schlegeli* is very important for analyzing genome organization and evolution and for developing breeding techniques. However, ascertaining microsatellite markers that are sufficiently polymorphic in species of interest is challenging. The identification of novel microsatellite markers requires some technical expertise and, on average, several months work (Zane et al. 2002). Microsatellites are usually discovered by sequencing fragments of genomic DNA.

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Several screening strategies for microsatellite isolation have been described so far: partial genomic libraries, primer extension for the production of libraries enriched in microsatellite loci, selective hybridization protocols and the sequencing of expressed sequence tags (EST) (Zane et al. 2002). Unfortunately, all of these procedures are currently too expensive and too time consuming, particularly for small-scale laboratories with limited resources. Thus, the strategy of cross-species amplification, using loci already developed in a related species, may provide a cost-effective alternative to microsatellite isolation and development in *A. schlegeli*. This approach offers significant potential for the low cost development of SSR markers for species with very little or no sequence information, by simply screening primers from different sources. Microsatellites have now been isolated from a wide range of species from the Sparidae family, including *Acanthopagrus butcheri* (Yap et al. 2000), *Sparus aurata* (Brown et al. 2005; Launey et al. 2003), *Pagrus auratus* (Takagi et al. 1997; Adcock et al. 2000), *Chrysophrys major* (Chen et al. 2005), *Pagellus bogaraveo* (Stockley et al. 2000), *Pagellus erythrinus* (RamSak et al. 2003). In addition, five microsatellite loci have been identified in another marine fish species of the Bothidae family, *Paralichthys olivaceus* (Liu et al. 2006). In the present study, our aim was to employ cross-species primers from the abovementioned species to amplify *A. schlegeli* DNA in order to develop polymorphic microsatellites.

2 Materials and methods

2.1 Fish material and DNA isolation

A. schlegeli were obtained from the coast side of Qingdao, China. A total of 20 *A. schlegeli* were used in the present study. DNA extraction was performed as described by Liu et al. (2005a). Blood samples (100 μl) were collected using a 1 ml syringe and immediately expelled into a tube containing 500 μl DNA extraction buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8, 25 mM EDTA, 0.5% SDS, and freshly added 0.1 mg ml^{-1} proteinase K). Blood wasexpelled into lysis buffer quickly to disperse the blood cells. The lysates were incubated at 55 °C overnight. DNA was then extracted twice with phenol and once with chloroform. DNA was precipitated by adding half the original blood volume of 7.5 M ammonium acetate and two volumes of ethanol. DNA was collected by brief centrifugation and washed twice with 70% ethanol, airdried, and resuspended in TE buffer. The concentration was measured with a GENEQUANT Pro (Pharmacia Biotech Ltd) RNA/DNA spectrophotometer at 260 nm.

2.2 Microsatellite amplification

A total of 68 microsatellites previously identified in *Acanthopagrus butcheri*, *Sparus aurata, Pagrus auratus, Chrysophrys major, Pagellus bogaraveo, Pagellus erythrinus* and, *Paralichthys olivaceus* were used in the study (Table 1).

The polymerase chain reaction (PCR) was performed in a 25-μl reaction mixture that included 10 pmol of each primer set, 100 μM of dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1 unit of Taq polymerase (TaKaRa), and approximately 100 ng of template DNA. Amplification was performed on a Peltier Thermal Cycler (PTC-200) (Gene Corp.). PCR cycles were as follows: 5 min pre-amplification denaturation at 94 °C, 35 cycles of 45 s at 94 °C, 40 s at a primer-specific annealing temperature, and 1 min at 72 °C. As a final step, products were extended for 5 min at 72 °C. The specific annealing temperature of each primer set is given in Table 2.

2.3 Polymorphism identification of microsatellite markers

Microsatellite polymorphism was screened with 20 *A. schlegeli* individuals, collected from coastal Waters in Qingdao, China, using an ECP3000 DNA sequencer system (Liuyi Corp.). Alleles were designated according to the PCR product size relative to a 10-bp SSR DNA ladder (Invitrogen Corp.). The PCR products were separated by electrophoresis on an 8% denaturing polyacrylamide gel. For the separation of microsatellite markers, we used a gel with uniform 0.4-mm thickness using a comb with "sharkteeth wells". Analysis was carried out by silver staining as described by Liu et al. (2004, 2005b).

3 Results

We attempted to amplify 68 microsatellite markers (Table 1) previously characterized in 6 Sparidae species: *Acanthopagrus butcheri*, *Sparus aurata, Pagrus auratus, Chrysophrys major, Pagellus bogaraveo, Pagellus erythrinus* and one Bothidae species, *Paralichthys olivaceus*. We found that amplification success (the detection of monomorphic or polymorphic amplification loci) in species belonging to the Sparidae family as the target species was high, in the range of 57.1% (*Pagellus erythrinus*) to 80.0% (*Acanthopagrus butcheri*), whereas amplification success in *Paralichthys olivaceus* was lower (20.0% of tested loci amplified, Table 1).

Of the 68 loci screened as microsatellite markers for *A. schlegeli*, 52 were discarded for one or more of the following reasons: non-amplification, poor amplification, or detection of only one allele within the test set (Table 1). Sixteen loci amplified well, provided reproducible genotypes, and revealed multiple alleles in the test set of individuals. All of the 16 loci found to be polymorphic were from species of the Sparidae family. These loci were then used to genotype a total of 20 *A. schlegeli* individuals.

We used the POPGENE software package (Yeh et al. 1999) and ARLEQUIN (Schneider et al. 2000) to calculate observed (Ho) and expected (He) heterozygosity, and linkage disequilibrium (LD), respectively. Significance values for all multiple tests were corrected following the Sequential Bonferroni procedure (Rice 1989). The number of alleles observed per locus ranged from two (PbMS 4) to 9 (CM001742), with an average of 4.75 (Table 2). The alleles observed at each locus fell within a limited size range, as is typically observed. For two of the sixteen loci, the observed range of allele sizes in *A. schlegeli* differed from the predicted minimum allele size

Table 1. Microsatellite markers and amplification results tested in *A. schlegeli* from *Acanthopagrus butcheri, Sparus aurata, Pagrus auratus* (Sparidae) *Chrysophrys major, Pagellus bogaraveo, Pagellus erythrinus* and one species of Bothidae, *Paralichthys olivaceus*.

Locus	Species	Repeat motif	GenBank accession no.	References	Amplification Amplification results
pAb1H1	*Acanthopagrus butcheri*	(TG)1	AF284351	Yap et al. (2000)	+
pAb2B7		(TG)24	AF284352		++
pAb4D5		(TG)60	AF284353		–
pAb2A5		(TG)19	AF284354		++
pAb2D11		(TG)15	AF284355		+
SaI10	*Sparus aurata*	(GT)37	AY322107	Brown et al. (2005)	++
SaI12		(GT)30	AY322108		+
SaI14		(GT)27	AY322109		–
SaI15		(GT)26	AY322110		+
SaI19		(GT)25	AY322111		++
SaI21		(GT)41	AY322112		–
SauA25INRA	*Sparus aurata*	(TG)n	AY173031	Launey et al. (2003)	+
SauANINRA		(TG)18	AY173032		+
SauD69INRA		(TG)19T(TG)7	AY173033		++
SauD182INRA		(CA)9+ (CA)8	AY173034		+
SauE82INRA		(CA)12AA(CA)7	AY173035		–
SauE97INRA		(CA)30	AY173036		–
SauG46INRA		(GT)6(GA)6GGAA (GA)8(GT)19	AY173037		++
SauH94INRA		(CA)12N19(TG)7	AY173038		–
SauH98INRA		(CA)17+ (TG)6	AY173039		–
SauI41INRA		(CA)9CG(CA)4 CG(CA)13	AY173040		+
SauI47INRA		(TG)19	AY173041		+
SauK140INRA		(CA)23	AY173042		+
Pma1	*Pagrus auratus*	(GT)21	AB042989	Takagi et al. (1997)	+
Pma2		(GT)24	AB042990		+
Pma3		(GT)20	AB042991		–
Pma5		(GT)22	AB042993		–
GA1A	*Pagrus auratus*	(TC)11(N)12(TC)	AF202880	Adcock et al. (2000)	+
GA1B		(GAA)4(GGA)4	AF202880		–
GA2A		(AG)16	AF202885		++
GA2B		(AC)17	AF202885		++
GT2		(GT)16	AF202884		+
GT3		(CA)10N(CA)15	AF202883		–
GT4		(GT)16	AF202881		–
GT6		(TG)11C (GT)5	AF202882		+
CM000278	*Chrysophrys major*	(TG)16	AY696574	Chen et al. (2005)	++
CM000925		(TAT)11	AY696585		++
CM000953		(GT)11GC(GT)3(GC) (GT)(GC)4(GT)2	AY696586		–
CM001034		(GT)7A(TG)6AT (TA)2 (TG)6	AY696587		++
CM001143		(CTGT)7	AY696589		++
CM001742		(TG)12	AY696598		++
CM002101		(C A)16	AY696603		–

for the original species (data not shown). The average observed heterozygosity was 0.75, with a range of 0.55–0.95. The distribution of alleles at CM001742 was found to differ from Hardy-Weinberg expectations ($p < 0.002$), and the observed heterozygosity exceeded the expected heterozygosity, while all other loci conformed to expectations. This departure and the variations in heterozygosity levels may be explained by the facts that individuals from a structured population were sampled, and a conservative approach was used to score the homozygous loci. There was no evidence for the presence of null alleles at this or any other of the sixteen loci, as judged by allele frequencies. Within the population characterized, this set of markers is powerful enough to be used for the genetic analysis of *A. schlegeli*. Out of 120 possible pairwise comparisons

Table 1. continued.

Locus	Species	Repeat motif	GenBank accession no.	References	Amplification results
CM002332	*Chrysophrys major*	(TG)12(AG)3(TG)3AG (CG)3CA(TG)3(CG)2	AY696607	Chen et al. (2005)	++
CM002640		(GCCA)5	AY696612		–
CM003195		(TG)13	AY696625		–
CMcon212		(TTA)17	AY696651		+
PbMS 1	*Pagellus bogaraveo*	(CA)34	AF209084	Stockley et al. (2000)	–
PbMS 2		(CTT)6CTC(CTT)3	AF209085		+
PbMS 4		(CA)4T(CA)22	AF209086		++
PbMS 6		(CA)43	AF209087		+
PbMS 15		(CA)24GAG(TC)3	AF209088		–
PbMS 16		(CA)33	AF209089		+
PbMS 17		(GA)5	AF209090		–
PbMS 18		(CA)10	AF209091		++
PbMS 19		(CAG)10	AF209092		–
PbMS 20		(GTGC)3(GT)11	AF209093		+
PAGEERY MS1	*Pagellus erythrinus*	(TG)10	AY188339	RamSak et al. (2003)	–
PAGEERY MS2		(ATGG)2ATTG (ATGG)12(GATA)26	AY188340		+
PAGEERY MS3		(CA)9	AY188341		+
PAGEERY MS4		(CA)21	AY188342		+
PAGEERY MS5		(CA)11	AY188343		–
PAGEERY MS6		(TG)10N13(TG)8	AY188344		–
PAGEERY MS8		(GT)18CT(GT)6	AY188346		+
OF101	*Paralichthys olivaceus*	(TCC)5	DQ207959	Liu et al. (2006)	–
OF102		(TCC)5	DQ207960		–
OF103		(TC)2TG(TC)7 TG(TC)2	DQ207961		–
OF104		(GA)9GGGG(GA)2	DQ207962		+
OF105		(TC)2TG(TC)7TG(TC)2	DQ207963		–

between the 16 loci applied to *A. schlegeli*, none showed significant LD ($p > 0.002$).

4 Discussion

Despite the relative ease of isolating microsatellites, their development still requires substantial time, financial and technical resources. The development and use of a more efficient method to provide microsatellite sequences for *A. schlegeli* is, therefore, highly desirable. Cross-species amplification is a very practical method to extend the utilization of microsatellite methods. For this reason there is considerable interest in using existing microsatellites on species from which markers have yet to be cloned. Microsatellite loci generally show considerable evolutionary conservation, suggesting that microsatellite primers developed for any one locus may often be useful across a wide range of taxa. In the present study, of the 68 loci tested, sixteen (23.5%) fulfilled the required criteria: good amplification, providing reproducible genotypes, and exhibiting levels of polymorphism adequate to distinguish individuals. Overall, these results suggest that time and money invested in evaluating the cross-species amplification of microsatellite loci in target species may be well spent. However, these cross-species amplification data also indicate that allelic diversity was low in *A. schlegeli* compared with the original species. This result might have been improved upon had greater attention been given to primer optimization or redesign. However, time and financial considerations suggested that the better strategy was to screen more primers rapidly, rather than devote significant resources to a few primers that did not give an early indication of utility.

On the other hand, cross-species amplification is only effective if primer sequences are conserved between species. Generally the number of loci capable of being amplified tends to decrease with increasing divergence between species (Moore et al. 1991; Peakall et al. 1998), although extreme conservation of loci has also been reported (Rico et al. 1996; Scott et al. 2003).We found that amplification success in species belonging to the same family (Sparidae), especially the same genus, was high, whereas amplification of other fish was lower. Although cross-species amplification is convenient, it may lead to low or incomplete amplification. This may be due to as little as a single dinucleotide mismatch between the primer and the target DNA sequences. The consequence of this may be non-specific amplification of a second locus. If this occurs, individuals may exhibit three or more "alleles" with a single primer set because more than one locus is inadvertently being amplified. This problem can often be resolved by employing less stringent polymerase chain reaction (PCR) conditions such as lowering the annealing temperature or increasing the magnesium concentration in the reaction.

It is increasingly apparent that cross-species amplification using common microsatellite markers may pose a greater

Table 2. Microsatellite markers screened for *A. schlegeli* with primer sequences, PCR conditions, observed heterozygosity (Ho) and expected heterozygosity (He) in 20 individuals. Exact tests of Hardy-Weinberg equilibrium showed significant heterozygote departure after a sequential Bonferroni correction (*$p < 0.002$).

Locus Name	Repeat motif	Primer sequences (5'-3')	T (°C)	N (size range, bp)	Ho	He	P
pAb2B7	$(TG)_{24}$	F: GGTGCGTGCATTGTTAATGTGT R: GATCTGCTTTCCTTTGACTCAGC	63	3(90–110)	0.70	0.73	0.552
pAb2A5	$(TG)_{19}$	F: ACCTCTTCATCTGCGTGACATCT R: GACAACACCCTCACTCAGCTGA	62	3(110–120)	0.55	0.59	0.491
SaI10	$(GT)_{37}$	F: TCACGGGGGACCAAGACTG R: CTCACACTGCCTAATTAGCACAGA	61	6 (200–240)	0.85	0.82	0.950
SaI19	$(GT)_{25}$	F: ATTCTTCACAGGCCCAACACAAA R: GAAAACACCGGCCCAGTACGA	59	7(220–270)	0.80	0.75	0.756
GA2A	$(AG)_{16}$	F: ACGGACAGAGAGGGAGTGG R: CATCATCATCAGTCAGAGCTG	55	5 (80–110)	0.75	0.81	0. 015
GA2B	$(AC)_{17}$	F: CAGATACAGGCAGAGGAGC R: CAATTAGAGGAGGGAGAACG	55	4 (160–190)	0.65	0.68	0. 537
CM000278	$(TG)_{16}$	F: GTGTGCGATCATCTTTGTGA R: TTAGCGGCTGTAAGACCAT	52	7 (150–190)	0.65	0.70	0. 073
CM000925	$(TAT)_{11}$	F: CATTCTGTTCCTGGCAGTG R: TCACCCACACCGTCACAA	54	5 (520-580)	0.75	0.69	0. 715
CM001034	$(GT)_7A(TG)_6AT(TA)_2$ $(TG)_6$	F: ATTTGTCACCTGCAAACGCCAAGA R: TTCGGGCGCAGTGTCCAAAGAG	55	5 (160-240)	0.85	0.80	0.957
CM001143	$(CTGT)_7$	F: GTCCGACTCCACTCCATTCCTCT R: GTGCTCGATCCCTTGTGCTGATA	57	6 (110-240)	0.65	0.58	0.096
CM001742	$(TG)_{12}$	F: GGCCAAGCCGTAAGAATCAT R: AACACGTTAACCCGGCCA	53	9 (100-180)	0.90	0.73	0.001*
CM002332	$(TG)_{12}(AG)_3(TG)_3AG$ $(CG)_3CA(TG)_3(CG)_2$	F: CGCTACCCACCTTACACAG R: AAGCTCCACTATCTGCGC	54	4 (150-230)	0.70	0.74	0. 517
PbMS 4	$(CA)_4T(CA)_{22}$	F: CAACCTGAAGTGAACCGCAGTC R: TCCAGCCCTCTATCACATCAGC	62	2(170-190)	0.80	0.78	0.815
PbMS 18	$(CA)_{10}$	F: ACCTCTGCGTTAGCAACAGG R: GAGACTGAACACTTCCGTAGG	56	3(160-200)	0.80	0.84	0.624
SauD69INRA	$(TG)_{19}T(TG)_7$	F: CGTTGATCCCTGAGAAGC R: AATACACGGAGAGCCACTG	58	3(140–170)	0.60	0.65	0. 085
SauG46INRA	$(GT)_6(GA)_6GGAA(GA)_8$ $(GT)_{19}$	F: GTGAACACCTGCCAGACG R: GCATCGAGGTCAAGTACCTG	60	4 (150-190)	0.95	0.87	0.033

T, annealing temperature (°C); N, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity.

challenge than was previously believed (Primmer and Merila 2002; Smith et al. 2000). A key difficulty is that while cross species amplification may be possible for a sizable percentage of the markers tested, differences in allele numbers and frequencies in the new population of interest mean that only a few of these cross-amplified markers contribute as much statistical power to the analysis of the new species as they did to the first (Morin et al. 1998). This emphasizes the importance of conducting pilot studies to establish the efficiency of the planned genotyping system before large-scale projects are initiated.

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References

Adcock G.J., Bernal Ramirez J.H., Hauser L., Smith P., Carvalho G.R., 2000, Screening of DNA polymorphisms in samples of archived scales from New Zealand snapper. J. Fish Biol. 56, 1283-1287.

Brown R.C., Tsalavouta M., Terzoglou V., Magoulas A., McAndrew B.J., 2005, Additional microsatellites for *Sparus aurata* and cross-species amplification within the Sparidae family. Mol. Ecol. Notes 5, 605-607.

Chang C.F., Yueh W.S., 1990, Annual cycle of gonadal histology and steroid proWles in the juvenile males and adult females of the protandrous black porgy, *Acanthopagrus schlegeli*. Aquaculture 91, 179-196.

Chen S.L., Liu Y.G., Xu M.Y., Li J., 2005, Isolation and characterization of polymorphic microsatellite loci from an EST-library of red sea bream (*Chrysophrys major*) and cross-species amplification. Mol. Ecol. Notes 5, 215-217.

D'Amato M.E., Lunt D.H., Carvalho G.R., 1999, Microsatellite markers for the hake Macruronus magellanicus amplify other gadoid fish. Mol. Ecol. 8, 1086-1087.

Jean C.T., Lee S.C., Hui C.F., Chen C.T., 1996, Genetic variation of black porgy, *Acanthopagrus schlegeli* (Perciformes: Sparidae) in the coastal waters of Taiwan. Biochem. Syst. Ecol. 24, 211-219.

Jean C.T., Lee S.C., Hui C.F., Chen C.T., 1998, Variation in Mitochondrial DNA Sequences of Black Porgy, *Acanthopagrus schlegeli*, in the Coastal Waters of Taiwan. Zool. Stud. 37, 22-30.

Launey S., Krieg F., Haffray P., Bruant J.S., Vannier A., Guyomard R., 2003, Twelve new microsatellite markers for gilted seabream (*Sparus aurata* L.): characterization, polymorphism and linkage. Mol. Ecol. Notes 3, 457-459.

Liu Y.G., Zheng M.G., Liu L.X., Lin H., Wang D.F., Li B.F., Sun X.Q., 2006, Five new microsatellite loci for Oliver flounder (*Paralichthys olivaceus*) from an EST-library and cross-species amplification. Mol. Ecol. Notes 6, 371-373.

Liu Y.G., Chen S.L., Li B.F., 2005a, Assessing the genetic structure of three Japanese flounder (*Paralichthys olivaceus*) stocks by microsatellite markers. Aquaculture 243, 103-111.

Liu Y.G., Chen S.L., Li B.F., Wang Z.J., Liu Z.J., 2005b, Analysis of genetic variation in selected stocks of hatchery flounder, *Paralichthys olivaceus*, using AFLP markers. Biochem. System. Ecol. 33, 993-1005.

Liu Y.G., Wang X.Y., Liu L.X., 2004, Analysis of genetic variation in surviving apple shoots following cryopreservation by vitrification. Plant Sci. 166, 677-685.

Moore S.S., Sargeant L.L., King T.J., Mattick J.S., Georges M., Hetzel D.J.S., 1991, The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10, 654-660.

Morin P.A., Mahboubi P., Wedel S., Rogers J., 1998, Rapid screening and comparison of human microsatellite markers in baboons: allele size is conserved, but allele number is not. Genomics 53,12-20.

Peakall R., Gilmore S., Keys W., Morgante M., Rafalski A., 1998, Cross-species amplification of soybean (*Glycine max*) simple sequence repeats within the genus and other legume genera: Implications for the transferability of SSRs in plants. Mol. Biol. Evol. 15, 1275-1287.

Primmer C.R., Merila J., 2002, A low rate of crossspecies microsatellite amplification success in Ranid frogs. Conserv. Genet. 3, 445-449.

RamSak A., Garoia F., Guarniero I., Mannini P., Tinti F., 2003, Novel polymorphic microsatellite markers for the common pandora (*Pagellus erythrinus*) Mol. Ecol. Notes 3, 553-555.

Rice W.R., 1989, Analyzing tables of statistical tests. Evolution 43, 223-225.

Rico C., Rico I., Hewitt G., 1996, 470 million years of conservation of microsatellite loci among fish species. Proc. R. Soc. London, Ser. B 263, 549-557.

Schneider S., Roessli D., Excoffier L., 2000, ARLEQUIN: A software for population genetics data analysis. Genetics and Biometry Laboratory. University of Geneva, Geneva.

Scott L.J., Shepherd M., Henry R.J., 2003, Characterization of highly conserved microsatellite loci in *Araucaria cunninghamii* and related species. Plant System. Evol. 236, 115-123.

Smith K.L., Alberts S.C., Bayes M.K., Bruford M.W., Altmann J., Ober C., 2000, Crossspecies amplification, non-invasive genotyping, and non-Mendelian inheritance of human STRPs in Savannah baboons. Am. J. Primatol. 51, 219-227.

Stockley B.M., Rogers A.D., Iyengar A., Menezes G., Santos R., Long A., 2000, Ten microsatellite loci isolated and developed for the blackspot seabream, *Pagellus bogaraveo* (Brunnich 1768). Mol. Ecol. 9, 999-1000.

Takagi M., Taniguchi N., Cook D., Doyle R.W., 1997, Isolation and characterization of microsatellite loci from red sea bream Pagrus major and detection in closely related species. Fish. Sci. 63, 199-204.

Tautz D., Trick M., Dover G., 1986, Cryptic simplicity in DNA is a major source of genetic variation. Nature 322, 652-656.

Yap E.S., Spencer P.B., Chaplin J.A., Potter I.C., 2000, The estuarine teleost, *Acanthopagrus butcheri* (Sparidae), shows low levels of polymorphism at five microsatellite loci. Mol. Ecol. 9, 2225-2226.

Zane L., Bargelloni L., Patarnello T., 2002, Strategies for microsatellite isolation: a review. Mol. Ecol. 11, 1-16.