© Indian Academy of Sciences

ONLINE RESOURCES

New microsatellite markers for large yellow croaker (*Pseudosciaena crocea*) and cross-amplification in closely related species

LIHUA JIANG* and AIYI ZHU

National Engineering Research Center of Marine Facilities Aquaculture, College of Marine Sciences, Zhejiang Ocean University, Zhoushan 316022, People's Republic of China

[Jiang L. and Zhu A. 2014 New microsatellite markers for large yellow croaker (*Pseudosciaena crocea*) and cross-amplification in closely related species. *J. Genet.* **93**, e113–e117. Online only: http://www.ias.ac.in/jgenet/OnlineResources/93/e113.pdf]

Introduction

Microsatellites, are among the most frequently used molecular markers used in genotype identification, pedigree analysis and estimation of genetic distance (O'connell and Wright 1997). They have been widely used in the fields of biological sciences and medicine. In aquatic organism research, the microsatellites markers have been the powerful markers in analyses of genetic diversity and genetic mapping in the past decade (Liu and Cordes 2004). However, the development of simple sequence repeats (SSR) is costly and time-consuming. Therefore, researchers have expanded microsatellite searches to related species and have generated good results. There have been many reports on the study and application of related species PCR amplification and amplified polymorphic DNA analyses with SSR. Hai *et al.* (2009) reported that 17 pairs of primers designed for *Perca schrenki* amplified in *Perca fluviatilis* and *Perca flavescens*, of which 10 pairs could be used, and there were six pairs in *P. flavescens* and five in *P. flavescens* that resulted in high polymorphism (PIC > 0.5). Liu (2011) developed 216 microsatellite markers derived from the spotted halibut (*Verasper variegatus*) for cross-species amplification in three related species. Of these, 137 markers were successfully amplified in barfin flounder (*Verasper moseri*), and 82 of these loci were polymorphic. Additionally, 95 markers were successfully amplified in *Pleuronichthys cornutus* (Liu 2011). Lin and Luo (2003) employed 28 pairs of common carp primers designed for microsatellites containing CA motifs to amplify microsatellite loci in the genome of grass carp. Seven primer pairs (25%) amplified specific products successfully and four primer pairs (14. 3%) showed polymorphism (Lin and Luo 2003).

Sciaenids (croakers or drum fish, family Sciaenidae) are an important group of fish in temperate to tropical coastal waters and estuaries throughout the world (Jiang *et al*. 2014). They are particularly abundant at the mouths of large continental rivers (Trewavas 1962). In the past decades, Sciaenids in the West China Sea have been nearly exhausted due to overfishing and heavy marine pollution (Seikai National Fisheries Research Institute 2001). The objective of this study was to determine the genetic relationship and diversity among species related to the large yellow croaker (*P. crocea*) using known SSR markers, for saving the cost of developing additional SSR markers and to assess cross-species primer hybridization potential. In particular, very little information is available on cross-species SSR amplification in *Johnius belangerii, Nibea albiflora* and *Argyrosomus japonicas*. The information obtained can facilitate parental selection based on the genetic distance between Sciaenid varieties, species, genera and conservation needs for future breeding.

Materials and methods

Sample collection and DNA extraction

Nibea albiflora, A. japonicus and *J. belangerii* were captured from the wild and from aquaculture net cages using hand nets and specialized spears (tables 1 & 2). Initially, the fish were identified based on both their morphologic features and the mitochondrial gene *CO1* (cytochrome C oxidase 1). Tissue samples were preserved in 95% ethanol for molecular analysis. Whole genome DNA was extracted from fin clips of individual specimens using the phenol–chloroform method (Arai and Mukaino 1997). DNA concentration was quantified using a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Madison, USA) and DNA quality was assessed

*For correspondence. E-mail: florencejiang@126.com.

Keywords. simple sequence repeat; cross-amplification; related species.

Table 1. Identification and origin of fish samples.

Genus	Species	Collection site	Sample size
Larimichthys (Jordan and Starks 1904)	*Larimichthys crocea* (Richardson 1846)	Dongji island	30
Argyrosomus (De la Pylaie 1835)	*Argyrosomus japonicus* (Temminck and Schlegel 1843)	Xiushan island	30
Johnius (Bloch 1730)	*Johnius belangerii* (Cuvier 1830)	Liuheng island	31

on a 0.8% agarose gel. Samples were stored at –4°C for subsequent analysis.

PCR amplification and genotyping

Our research group sequenced the draft genome of *L. crocea* in 2011 (http://news.sciencenet.cn/htmlnews/2011/2/243565.shtm). We extracted whole microsatellites from the genome, 60 primer pairs were randomly selected, synthesized and used to analyse *L. crocea* polymorphisms and 27 primer pairs worked (Lü *et al.* 2013). We used 27 primer pairs for cross-amplification in *N. albiflora*, *A. japonicus* and *J. belangerii* (table 3). PCR reactions were performed in a total volume of 25 μL, which contained ∼50 ng of template DNA. PCR was performed using the following programme: one cycle at 95°C for 5 min, 35 cycles at 94°C for 30 s, 52–62°C for 30 s and 72°C for 30 s, followed by a final extension step at 72°C for 5 min. PCR reaction was optimized by altering the annealing temperature from 52°C to 62°C for individual primer sets.

Locus characterization and genotypes were determined by PCR based on genomic DNA from 30 samples. PCR products were analysed using 8% denaturing polyacrylamide electrophoresis (19: 1, acrylamide: bis-acrylamide) followed by silver staining. Allele size was identified according to pBR322 DNA/*Msp*I molecular weight marker (Tiangen, Beijing, China).

Data analysis

For data codification, polymorphism parameters, expected (H_e) and observed (H_o) heterozygosity, number of alleles per locus (N), and Hardy–Weinberg equilibrium (HWE) were analysed using PopGene software (Yeh and Boyle 1997). Null alleles were calculated by Micro-Checker (Van *et al.* 2004). Polymorphism information content (PIC) was examined using the formula:

$$\text{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2.$$

Where p_i and p_j are frequencies of the ith and jth allele, respectively (Botstein *et al.* 1980). Genotypic linkage disequilibrium between these loci was calculated by Arlequin 3.11 software (Schneider *et al.* 2000). All test results were adjusted using Bonferroni correction (Rice 1989).

Results

Among the 27 polymorphic loci cross-amplified in *N. albiflora*, *A. japonicus* and *J. belangerii*, 24 (88%), 26 (96%), and 25 (92%) loci, respectively, amplified successfully (figure 1). The loci revealed polymorphism among the fishes, giving altogether 23, 23 and 25 polymorphic alleles, respectively. Each polymorphic primer pair created 6–14 alleles with an average of nine alleles. All polymorphic loci were isolated using primers developed in the large yellow croaker. The numbers of alleles found per primer pair for *N. albiflora*, *A. japonicus* and *J. belangerii* are summarized in table 3. The expected heterozygosity (H_e) of *N. albiflora*, *A. japonicus* and *J. belangerii* ranged from 0.617 to 0.900, from 0.656 to 0.903, and from 0.515 to 0.906, respectively. The observed heterozygosity (H_o) of *N. albiflora, A. japonicus* and *J. belangerii* ranged from 0.084 to 0.372, from 0.080 to 0.332, and from 0.078 to 0.476, respectively. Typical values for PIC of microsatellites in *N. albiflora, A. japonicus* and *J. belangerii* ranged from 0.575 to 0.891, from 0.605 to 0.896, and from 0.497 to 0.897, respectively. Most of the loci we analysed were highly polymorphic (PIC $>$ 0.5) after Bonferroni correction ($P < 0.0045$, adjusted value). For *N. albiflora*, there were 15 loci that significantly deviated from HWE in the sampled population. For *A. japonicus*, 18 loci deviated significantly from the HWE in the sampled population. For *J. belangerii*, there were eight loci that significantly deviated from HWE in the sampled population. Details of the polymorphic microsatellite loci in *N. albiflora*, *A. japonicus* and *J. belangerii* and their variability were measured across 30 samples, which are summarized in table 3. There were no stuttering errors and no evidence of allelic dropouts in any of the loci analysed by Micro-Checker following Bonferroni correction. No significant linkage disequilibrium was found between all pairs of these 27 loci after Bonferroni correction ($P < 0.0045$, adjusted value).

Discussion

Previous research has shown that microsatellite sequences within species found in the same genus or closely related species are often conserved, which makes it possible to use those sequences for microsatellite analysis on related species (McConnell *et al*. 1995; Morris *et al*. 1996; Rico *et al.* 1996; Presa and Guyomard 1996). There have been many

Table 2. Primer sequence and characteristics of 27 microsatellite loci isolated from *P. crocea.*

Loci	Primers sequence (5′–3′)	Size (bp)	T_a (°C)	Repeat motif	N_a	H_o	H_e	PHWE	PIC	GenBank accession number
26 FR	F: AGCTGACCTTTCACCTGGAG R: AGGTGGACGAGACTGAAGGA	178–230	61	$(GT)_{15}$	10	0.452	0.812	0.001	0.767	KC773844
34 FR	F:CGATCAATGAGATCATAGTCAG ACTTC R: GCCCTGCACTGTAGGTCTTTG	176–200	62	$(GT)_{10}$	6	0.594	0.832	0.117	0.742	KC773845
41 FR	F: GGGTGACAACATCTGTGTGG R: AAGAGCAGCACCATCTGTGA	130–174	60	$(CA)_{10}$	8	0.7	0.88	0.505	0.758	KC773846
48 FR	F: ATCCGGTGCTCTCAGTCCT R: ATTTGGACGCGTGTAGACAG	204–224	62	$(AC)_8$	5	0.412	0.769	0.235	0.433	KC773847
57 FR	F: CAACGCATGTCAGATTGGAG R: CGCAACTCAGCACAGGATTA	150–184	58	$(CA)_6TA(CA)_6$	5	0.5	0.778	0.449	0.64	KC773848
77 FR	F: AGACCGGTTCTCAAGCAGCT R: TAGCTCACGGGAAGGAGAGA	172–196	62	$(AC)_{12}$	6	0.455	0.792	0.078	0.625	KC773849
79 FR	F: TCTGAATGGATGCAATCCA R: GGCATTTCCCCTTTTCTTTC	158–198	61	$(CA)_{11}$	6	0.258	0.822	0.002[a]	0.701	KC773850
82 FR	F: GTTTGGCCTGTTTCACTGTACT R: GTGGCAGCTTCCTGACAGAT	274–326	62	$(GT)_9$	8	0.781	0.901	0.464	0.814	KC773851
86 FR	F: ACTCCAAGGCAGAGCTGACT R: CATACCAGGATGCCGATCAT	252–330	62	$(TG)_{13}$	8	0.633	0.71	0.512	0.837	KC773852
90 FR	F: TCTGTGTGCCTGTGTGAACAG R: TCCTCCTCACTACCGCATTC	192–218	58	$(CA)_8$	7	0.483	0.827	0.011	0.762	KC773853
92 FR	F: GCATATCAACCAAAGCGGGAG R: AGAGTGGGCCTTGGAGTGTG	186–226	62	$(AC)_{14}$	5	0.742	0.813	0.736	0.772	KC773854
B23FR	F: ACCAGTGGCCTTAATCACATG R:GTGATAGGGTCAATCAATCAGGA	177–212	55	$(CA)_{13}$	7	0.515	0.814	0.651	0.769	KC773855
B24 FR	F: CTTGACTGTCTGGCCTGACCT R: GCTGACACACACTCAATGCAA	180–207	58	$(GT)_{12}$	8	0.781	0.833	0.464	0.803	KC773856
B42 FR	F: CACCCTGAATTCACGGAGA R: TGAGGGGGTGGAAACTAATG	245–278	55	$(CAA)_7$	7	0.546	0.825	0.14	0.605	KC773857
B43 FR	F: GGAGGTGGCTTCAGTGGATA R: GCCCGTCTCTTCGAGTCAA	142–168	57	$(TG)_{10}$	7	0.375	0.813	0.376	0.383	KC773858
B45 FR	F: GAGCGATCCCCACAGGAT R: CTGTGAGTCAACATGGATGGAG	142–168	60	$(GT)_8AT(GT)_{10}$	6	0.367	0.814	0.225	0.412	KC773859
B46 FR	F: ACTCACAGAGAGACAGCGGTG R: GGACTGAAAGCAGCAAGAGC	222–272	54	$(TG)_{17}$	6	0.4	0.788	0.151	0.714	KC773860
B48 FR	F: TGGTCATAAAAGCCCAGTCTG R:GAGCAACAAAGTCAAACACACC	178–216	60	$(CA)_{13}$	5	0.6	0.861	0.484	0.49	KC773861
B69 FR	F: ACGGTCCTCAATAGTTTTCTGC R: TGGAGAAGACGGAGCAAGAT	158–194	58	$(TG)_{12}$	6	0.778	0.876	0.652	0.658	KC773862
B85 FR	F:AGAGGAAAAGTGTGGCACATAAG R:CGGGTTTACCCTCCACTGTA	160–180	62	$(AC)_{11}$	5	0.515	0.773	0.133	0.575	KC773863
B86 FR	F: GTGCATGTGTATCATGGCCT R: TCCTCTCATCTGGGCTTGTC	180–212	60	$(ATGA)_5$	5	0.677	0.798	0.358	0.693	KC773864
B90 FR	F: AGAGGCAGCCTCCATTCC R: CCCACATGGTGTGTGCTTAG	180–214	60	$(AC)_9$	8	0.727	0.862	0.139	0.717	KC773865
B93 FR	F: CGTCAAGCACTCTGCTATCAG R: ATGCACGCACGTATTCAAAC	170–220	55	$(TG)_{11}$	8	0.844	0.896	0.49	0.806	KC773866
B95 FR	F: CCACATCCCAGTGCCAGTAAA R: CTCGGCGCTCATAGGTAAAG	150–184	60	$(CACT)_7$	6	0.394	0.797	0.016[a]	0.483	KC773867
B96 FR	F: CGTTGGCCTACTTAAACAGCA R: TCGGAAAGTGACATCACAGC	190–238	58	$(TG)_9$	7	0.588	0.879	0.010[a]	0.599	KC773868
B98 FR	F: TACTGTTGAGGGCCAAGTGA R: CCCTGGAAGTCCAACTTCAT	146–180	62	$(TG)_8$	6	0.663	0.856	0.221	0.719	KC773869
97 FR	F: GAATACATCCCTCTGAGACAG R: TGAGCAAAGAGTTGATGAGA	178–240	60	$(AC)_{15}$	11	0.833	0.893	0.000[a]	0.866	KC773870

N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PHWE, Hardy–Weinberg equilibrium P values; T_a, annealing temperature; PIC, polymorphic information content. [a]Represents significant deviation from HWE ($P < 0.05$, after sequential Bonferroni adjustment) (Lü *et al.* 2013).

Table 3. Characteristics of the 27 polymorphic SSR markers used for three Sciaenidae species.

	N. albiflora				*A. japonicus*				*J. belangerii*				F_{st} among
Loci	A	H_o	H_e	PIC	A	H_o	H_e	PIC	A	H_o	H_e	PIC	three populations
26 FR	14	0.105	0.880	0.868	7	0.171	0.814	0.790	14	0.079	0.904	0.898	0.082
34 FR	8	0.258	0.730	0.699	10	0.139	0.846	0.828	13	0.080	0.904	0.896	0.113
41 FR	12	0.089	0.894	0.884	7	0.194	0.790	0.702	7	0.476	0.515	0.497	0.122
48 FR	13	0.126	0.859	0.848	13	0.080	0.903	0.896	9	0.198	0.788	0.764	0.080
57 FR	14	0.125	0.859	0.844	13	0.084	0.900	0.891	12	0.168	0.818	0.802	0.067
77 FR	7	0.179	0.807	0.78	6	0.299	0.688	0.635	11	0.157	0.827	0.809	0.065
82 FR	8	0.212	0.774	0.745	6	0.332	0.656	0.605	–	–	–	–	0.063
86 FR	10	0.112	0.872	0.860	11	0.105	0.879	0.867	14	0.078	0.905	0.897	0.063
90 FR	–	–	–	–	12	0.093	0.891	0.881	5	0.236	0.751	0.711	–
92 FR	10	0.121	0.863	0.849	9	0.139	0.846	0.827	9	0.123	0.862	0.846	0.027
97 FR	8	0.149	0.836	0.816	15	0.083	0.901	0.892	9	0.138	0.847	0.829	0.029
B23FR	8	0.182	0.803	0.778	8	0.213	0.773	0.739	10	0.123	0.861	0.846	0.115
B24 FR	6	0.372	0.617	0.575	6	0.182	0.803	0.774	7	0.209	0.777	0.748	0.049
B42 FR	11	0.131	0.854	0.838	8	0.174	0.812	0.789	13	0.078	0.906	0.899	0.067
B43 FR	11	0.118	0.866	0.853	14	0.119	0.865	0.852	13	0.089	0.895	0.886	0.029
B45 FR	6	0.258	0.728	0.696	10	0.136	0.849	0.832	14	0.094	0.891	0.881	0.093
B46 FR	9	0.170	0.816	0.794	10	0.128	0.857	0.840	11	0.139	0.847	0.829	0.091
B48 FR	8	0.157	0.828	0.806	4	0.283	0.703	0.645	11	0.144	0.841	0.824	0.141
B85 FR	8	0.201	0.785	0.762	13	0.110	0.874	0.863	10	0.135	0.850	0.834	0.085
B86 FR	8	0.182	0.803	0.777	9	0.165	0.821	0.797	12	0.174	0.812	0.794	0.085
B90 FR	11	0.149	0.836	0.816	11	0.107	0.877	0.865	11	0.115	0.869	0.856	0.037
B93 FR	8	0.206	0.780	0.752	8	0.229	0.757	0.735	11	0.141	0.845	0.82	0.117
B95 FR	9	0.126	0.858	0.843	9	0.114	0.870	0.856	7	0.198	0.788	0.762	0.064
B96 FR	13	0.084	0.900	0.891	8	0.153	0.832	0.812	8	0.252	0.735	0.697	0.069
B98 FR	–	–	–	–	10	0.126	0.858	0.843	12	0.111	0.873	0.861	–

–, unsuccessful PCR amplification; A, number of alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, the value of polymorphic information constant; F_{st}, fixation index.

reports on microsatellite primers that are shared among different aquatic animal species and genera. Kim *et al.* (1996) used salmon microsatellite loci to amplify across species and nearly all microsatellite primers produced amplification products in multiple species. Zhu *et al.* (1999) used four microsatellite loci found in lake sturgeon (*Acipenser fulvescens*) to amplify loci in the Chinese sturgeon (*Acipenser sinensis*) and all loci were amplified successfully. Dubut (2010) assayed 41 cyprinid-specific polymorphic microsatellite loci (including 10 novel loci isolated from *Chondrostoma nasus*, *Chondrostoma toxostoma* and *Leuciscus leuciscus*) for 503 individual samples from 15 European cyprinid species and they successfully cross-amplified.

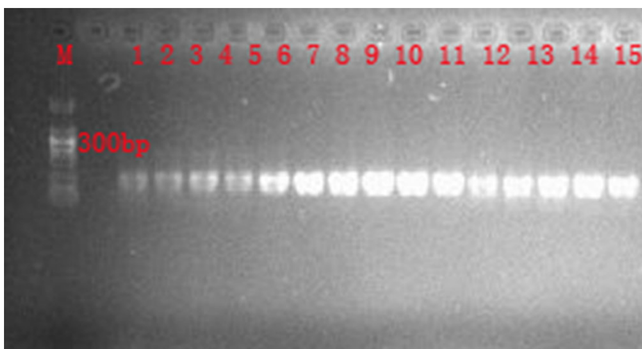

Figure 1. The PCR products amplified with prime 48. M, marker ladders; lanes 1–5, PCR products of *A. japonicus*; lanes 6–10, PCR products of *N. albiflora*; lanes 11–15, PCR products of *J. belangerii*.

Our results show that most of the large yellow croaker microsatellite primers studied are versatile and can be used in *N. albiflora*, *A. japonicus* and *J. belangerii*, with the exception of locus 90.98B. These microsatellite loci showed high diversity and heterozygosity in the populations and were useful in studying genetic diversity and systematic evolution concurrently. These loci can be used as common molecular markers within the family *Sciaenidae*. As there has been one hybridization study report between *Sciaenidae* species (Ma *et al.* 2002), we could conceivably use these microsatellites for identification of the hybrid offspring and their parental lineages through the observed allele polymorphism.

Acknowledgement

This study was supported by the National High-Technology Research and Development Programme of China (2012AA10A413-5).

References

Arai K. and Mukaino M. 1997 Clonal nature of gynogenetically induced progeny of triploid (diploid tetraploid) loach, *Misgurnus anguillicaudatus* (*Pisces: Cobitididae*). *J. Exp. Zool.* **278**, 412–421.

Botstein D., White R. L., Skalnick M. H. and Davies R. W. 1980 Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet.* **32**, 314–331.

Dubut V. 2010 Cross-species amplification of 41 microsatellites in European cyprinids: a tool for evolutionary, population genetics and hybridization studies. *BMC Res. Notes* **3** 1, 135–139.

Hai S., Li J. L. and Liu F. 2009 Isolation of microsatellite loci from *Perca schrenkii* and its universal in related species. *Chin. J. Zoo.* **44**, 17–23.

Jiang L. H., Su Y. Q., Wu C. W., Chen Y. J., Zhu A. Y. and Gu C. P. 2014 Phylogenetic estimation of Sciaenidae in the East China Sea inferred from nuclear EPIC DNA sequence variation. *Biochem. Syst. Ecol.* **53**, 69–75.

Kim T. S., Judy R. G. and Raymond L. F. 1996 Isolation and characterization of novel salmon microsatellite loci: crossspecies amplification and population genetic applications. *Can. J. Fish. Aquat. Sci.* **53**, 833–841.

Liu Z. J. and Cordes J. F. 2004 DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* **238**, 1–37.

Liu Z. P. 2011 Research of in ducing triploid half-smooth tongue sole (*Cynoglossus semilaevis*) and the cross-species SSR study in flatfishes. Masters dissertation, pp. 47–53. Ocean University of China, Qingdao, China.

Lin K. D. and Luo C. 2003 Preliminary study on applicability of microsatellite primers developed from common carp for genomic analysis of grass carp. *Acta Laser Biol. Sin.* **12**, 121–127.

Lü Z. M., Li H. M., Liu L. Q., Cui W. T., Hu X. Y. and Wang C. F. 2013 Rapid development of microsatellite markers from the large yellow croaker (*Pseudosciaena crocea*) using next generation DNA sequencing technology. *Biochem. Syst. Ecol.* **51**, 314–319.

Ma L., Wang J. and Chen W. G. 2002 Study on embryonic development of two drum fish crossbreeding filial generation I. *J. Xiamen Uni. (Nat. Sci.)* **41**, 378–381.

McConnell S. K., Hamilton L. and Morris D. 1995 Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. *Aquaculture* **137**, 19–30.

Morris D. B., Richard K. R. and Wright J. M. 1996 Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic studies of salmonids. *Can. J. Fish. Aquat. Sci.* **53**, 120–126.

Rico C., Rico I. and Hewitt G. 1996 470 million years of conservation of microsatellite loci among fish species. *Proc. R. Soc. Lond.* **263**, 549–557.

Presa P. and Guyomard R. 1996 Conservation of microsatellites in three species of salmonids. *J. Fish. Biol.* **49**, 1326–1329.

O'Connell M. and Wright J. M. 1997 Microsatellite DNA in fishes. *Rev. Fish. Biol. Fish.* **7**, 331–363.

Rice W. E. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Schneider S., Roessli D. and Excoffier L. 2000 ARLEQUIN, version 2.000: a software for population genetics date analysis. Genetics and Biometry Laboratory. Department of Anthropology, University of Geneva, Switzerland.

Trewavas E. 1962 A basis for classifying sciaenid fishes of tropical west Africa. *Ann. Magazine Nat. Hist.* **5**, 167–176.

Van O. C., Hutchinson W. F., Wills D. P. M. and Shipley P. 2004 MICRO-CHECKER: software for identifying and correcting genotyping errors in mirosatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Yeh F. C. and Boyle T. J. B. 1997 Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg. J. Bot.* **129**, 157.

Zhu B., Chang J. B. and Tan X. C. 1999 Applicability of microsatellite DNA primers of lake sturgeon for the parentage analyzing of Chinese sturgeon. *Acta Hydrobiol. Sin.* **23**, 547–554.

Received 24 January 2014, accepted 9 June 2014
Unedited version published online: 26 June 2014
Final version published online: 13 November 2014