Journal of Animal and Veterinary Advances 10 (5): 555-561, 2011
ISSN: 1680-5593

Genetic Variability in Cultured Stocks of *Scleropages formosus* in Mainland China Revealed by Microsatellite Markers

[1,2]X.D. Mu, [1]Y.C. Hu, [1]X.J. Wang, [1]H.M. Song, [1]Y.X. Yang and [1]J.R. Luo
[1]Pearl River Fisheries Research Institute,
Chinese Academy of Fishery Sciences, 510380 Guangzhou, Guangdong Province, P.R. China
[2]Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes,
Ministry of Agriculture, Freshwater Fisheries Research Center,
Chinese Academy of Fishery Science, 214081 Wuxi, Jiangsu Province, P.R. China

Abstract: The Asian arowana, *Scleropages formosus* (Osteoglossidae) is one of the most valuable species of the ornamental fish which has an important role on academic study and economic development. The present study assessed the genetic variation of three *S. formosus* stocks cultured in ponds using 39 polymorphic microsatellite primer combinations. The results showed the middle level of genetic diversity among all three stocks. The average values of observed Heterozygosity (Ho) expected Heterozygosity (He) for the red stocks are the highest which were 0.509 and 0.552, respectively. Genetic diversity of the green stocks was higher than the golden group. The inbreeding coeffcients within the subpopulation (Fis) and the differentiation index of population (Fst) analyses showed low genetic differentiation among three cultured stocks. The cluster analysis based on Nei's standard genetic distance showed that the golden and the green stocks clustered together and the red stock was in another clade. These results would have important implications for future breeding programs, conservation and production, suitable management guideline projects for *S. formosus* in mainland China.

Key words: *Scleropages formosus*, microsatellite, genetic variability, cluster analysis, primer combinations, China

INTRODUCTION

Scleropages formosus commonly known as Asian arowana, the Dragonfish, bony-tongue belongs to the order *Osteoglossiformes*, one of the ancestral teleost clade with extant representatives restricted to freshwater habitats in Southeast Asia. Three basic colour (green, golden and red) varieties were identified for *S. formosus* which were probably related to freshwater habitats during the Pleistocene glacial ages. Depending on lovely elegance shape and a long-life, *S. formosus* acquired a special status in some Asian countries, a very popular but extremely valuable ornamental fish. An adult red or golden arowana individual might cost over $20,000 at the ornamental fish market (Yue *et al*., 2004). Due to its popularity and great demand, *S. formosus* have been fiercely hunted in its native habitat, leading to its including species threatened with extinction fish in the wild listed by the Convention on International Trades in Endangered Species of Wild Fauna and Flora (CITES) as an endangered species since 1980. From then on, their commercial export, import and sales were normally prohibited in all members of CITES. On the other hand, *S. formosus* is a primitive fish retaining anatomical characteristics from the Jurassic era and of great value on scientific research to explore genetic evolution.

Given their great evolutionary and economic importance, more information should be required on genetic variation of the inter or intra-population which has important implication in fishery management and development of aquaculture technology. Previous studies have demonstrated that genetic diversity among *S. formosus* isolates in Southeast Asian countries including wild caught stocks (Shafiqur *et al*., 2008) and cultured stocks (Yue *et al*., 2000, 2006a; Mu *et al*., 2009) using several DNA-based molecular markers and techniques such as RAPD (Yue *et al*., 2002), AFLP (Yue *et al*., 2004), STS (Yue *et al*., 2003), mtDNA cytochrome b (cytb) gene (Hu *et al*., 2009), D-loop regions (Pan *et al*., 2005), ATP synthase gene (Tang *et al*., 2004) of mitochondrial (mt) DNA. Generally, the choice of an appropriate genetic marker contributed to a population genetics survey

Corresponding Author: Y.C. Hu, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, 1 Xingyu road, Liwan District, 510380 Guangzhou, Guangdong Province, The People's Republic of China

(Sunnucks, 2000). Above studies focused on approaching different questions such as identification of different stocks whether wild or cultured, conservation of different sampling locations using different mtDNA markers. In addition compared with RAPDs that results thought not to promise (Fernando, 1997), microsatellite is proved to be a powerful tool emerged as those with finest resolution for labeling of populations and individuals and have been widely used accurate genetic assessment of population differentiation of aquaculture fish species such as grass carp (Zhang *et al*., 2005; Liu *et al*., 2009), tilapia (Rutten *et al*., 2004) and Asian seabass (Zhu *et al*., 2006), due to their high variation, abundance, neutrality, co-dominance and unambiguously scoring of alleles (Tautz, 1989). Although, relatively few scientific studies using microsatellite have been published about different stocks in peer-reviewed, in order to avoid the loss of genetic variability posing a major stumbling block to aquaculture, the objectives of the present study was to examine genetic diversity for future commercial production within three stocks of *S. formosus* cultured in mainland China using microsatellite.

MATERIALS AND METHODS

Fish samples and DNA extraction: Three stocks of *S. formosus* were collected from Guangzhou tiny-lake aquatic organism technology co., ltd, including green (17), golden (21), red (15) arowana individuals. Fin clip of each fish was collected and kept in absolute ethanol. DNA was extracted according to the instruction of kit (Tianwei Co. Ltd, China) and dissolved in deionized distilled water. The integrity and purity of the DNA samples were examined on a 0.8% agarose gel by electrophoresis. All DNA samples were stored at -20°C until use.

Microsatellite analysis: A total of forty four microsatellite primer combinations described by previous studies (Yue *et al*., 2000, 2006a; Tang *et al*., 2004) were used. Detail information of all microsatellite primers were shown in Table 1. PCR reactions were carried out in a volume of 20 μL containing 2 mM of each $MgCl_2$, 0.2 mM of each dNTPs, 0.5 mM primer, 2.0 μL 10×rTaq buffer, 0.15 U Taq DNA polymerase (Takara) and 1 μL DNA in a thermocycler (Bio-Rad) under the following conditions:

Table 1: Microsatellites DNA primer

Primer	Repeat motif	Primer sequences (5'-3')	Genebank	Tm	Allele number
D01	$(CA)_{10}$	F:GAATGCTTAAAGTGGCAGTGAA			
		R: TAACACAGGGCGTAAGGCCAG	AF219951	55°C	2
D04	$(GT)_{41}$	F:GCTTAAACCCATTACAGACAGG			
		R:AAAGTGGTTTTGCATGAAGAAA	AF219952	55°C	3
D11	$(GT)_{16}$	F:GTTTCTTCTAGGTGCTCTGGTTTC			
		R:GGATGAGTGACCCAGTGTAAGTAG	AF219953	55°C	3
D13	$(GT)_{12}$ $(CT)_{23}$	F AGCTGCTGTGTCTGTGGTGGTCTA			
		R CATGCCCATGGAGAGGGAGAG	AF219954	53°C	3
D14	$(CA)_{12}$	F:AAGGGAGCAGCAGTTAGGTAGCAG			
		R:AGAGGAAATGTTAATTCACCACGG	AF219955	55°C	5
D15	$(GT)_{16}$	F:GACTGGCGTCCCGTCCTG			
		R:TATGCTCTTTCCCATTGACACTAA	AF219956	57°C	2
D16	$(GT)_{20}$	F:CTTGCGCCCTGTGTTGC			
		R:TTACCAGCAGAAAAGGCCTT	AF219957	57°C	1
D27	$(CA)_{17}$	F:GTGTCAGTATAGTGAATCTGTAG			
		R:ATCTCATTATGCTGCCATTGTCA	AF219958	57°C	5
D31	$(GATA)_{15}$	F:GTTTGTCCCTCCATGCACTGAGAG			
		R:CCAACAAAACCATGTGGCAATCAC	AF219959	52°C	1
D32	$(CA)_{13}(CA)_{12}AA$	F:AGCACCCTGTTACTGGAAGAGA			
		R:TTCTCAAAGCAAAAGCATCACACT	AF219960	55°C	1
D33	$C(CA)_4$	F:GTTCTTCTAGGTGCCGCGTTTC			
		R:CTACTTACACTGGGTCACTCATCC	AF219961	50°C	5
D35	$(GT)_{17}$	F:GTTTCTTCTAGGTGCTCTGGTTTC			
		R: CTACTTACACTGGGTCACTCATCC	AF219962	52°C	2
D37	$(GT)_{51}$	F: GCCTTACGCCCTGTGTTGC			
		R:TGGATATCTGTGAGTGGTGGTGAA	AF219963	45°C	7
D38	$(GT)_{24}$	F:TTGGGGTCATGCCACTGG			
		R:CAATAAATACCAAACAGGGAACC	AF219964	53°C	5
D42	$(CA)_{19}$	F:AGGAACATCACTGACAACACT			
		R:TGGACTAACTAGGAGCACAT	AF219965	50°C	2
D72	$(CA)_{14}$	F:AGCAGGTTAATTTGGAGACT			
		R:CGACCCTGTATGGGACAAG	AF219966	55°C	4
D85	$(CA)_{10}$	F:GTTCCACAGGGGCTGAGAAAAT			
		R:GAGGACGGAACAAAAGCATTGG	AF219967	50°C	2
D88	$(GT)_{11}$	F:TTTCTTTCTGAGACTGAGG			
		R:CAACTCTTATCCACCATTT	AF219968	50°C	5
D92	$(GT)_{13}$	F:AGTCGCACACCACCACCTCAG			
		R:TCAGCGATAACCCCACACCT	AF219969	50°C	2
D94	$(CA)_{16}$	F:CAGCAGCAGTGACACGGGTTCG			
		R:TCGCAGGCTGATTAAAGGTGTG	AF219970	53°C	4

Table 1: Continued

Primer	Repeat motif	Primer sequences (5'-3')	Genebank	Tm	Allele number
D95	$(CA)_9$	F:CCTGCGGAAGAAGAAAAGACT			
		R: CATGGTGTTGGCTGTGAGGAG	AF219971	53°C	\
D101	$(CA)_{16}$	F:CTGAATTTGATGGAAGTCTGTGT			
		R:AGTTGGTTTAAAGTGGCTGGAG	AY894662	53°C	4
D102	$(AC)_{27}(CT)_{17}ACC$	F:GTTATCAGCCCTCACACCT			
		R:TTCCTCCCACAGTCCAAAGA	AY894663	53°C	2
D104	$A(TC)_{12}$	F:AGGCCACACCTCCTCCCTCAGAC			
		R:CAGCCTCCTCCTGGGAACACTGTC	AY894664	50°C	3
D105	$(CA)_{18}$	F:GATGCCGGTGTTATCAGTGAC			
		R:TTGGATGAGGGAGAAACAAATAAT	AY894665	50°C	6
D106	$(GT)_{16}$	F:ATATTGCGCTTTGATTCATAAACCG			
		R:GCAGGGCGTGCTCTCCACT	AY894666	50°C	4
D107	$(AC)_{10}$	F:CTGCTGCTGCTGGGAGCGTAGC			
		R:AGCTGTGGAAAGAGCTGGCCTTCA	AY894667	50°C	3
D108	$(AC)_9$	F:ATCTGCAAAAGAAGTGCCACAGC			
		R:CACTAAACCGGTGTCCAATCCTG	AY894668	50°C	7
D109	$(CA)_{19}$	F:ACCTCCGCGCTTTACTTTTTGAGC			
		R:CGGAGCGCCCTGCCTGCTA	AY894669	50°C	4
D111	$(TG)_{22}$	F:TTCCTCCCACACTCCAAAGAC			
		R:TATCATTCCACAAACCTCATTCAC	AY894670	50°C	2
D114	$(GT)_{17}(AT)_2(GT)_{15}$	F:CCATGACCCCGCTTGGAAC			
		R:AGACCACCGGATGTAGAGAAGTGC	AY894671	50°C	7
D115	$(GT)_{21}$	F:GTCGCTCCAGCTCTGCTACTTT			
		R:GTGAGGCTGGGTTCGTCCTG	AY894672	53°C	5
D116	$(AC)_{19}$	F:CAGCCTCCCTCCCACTCTCCTT			
		R:TTCCCCTGCCCCTATCAACATCT	AY894673	50°C	3
D117	$(CA)_{28}$	F:CGATCCCTGCTCAGTCTGTGT			
		R:AAAGGGTCACTCAGCCATACATC	AY894674	53°C	2
D118	$(GT)_7$	F:ACTATGATCCGCATCGGGAGAT			
		R:AGGGGAAAAGGGTCTAAGATGATG	AY894675	50°C	2
D119	$(CA)_{28}$	F:TGGGGGCTTTCGTTGCAATAACAC			
		R:TCCCCGGTCAGGATGGACGAT	AY894676	50°C	7
K10	$(CA)_{20}$	F:GCACCTAACTGAAGAGCATT			
		R:AAAATTACCTGCTTGTGTGC	AY173130	50°C	3
K13	$(CA)_5CG(CA)_3$	F: GCACTGTTAAGTTCTGGTGTC			
		R GATACGCATGACATTCTGTG	AY173131	50°C	2
K16	$(TG)_5$	F:CAGTGGTTGCACACTTACAG			
		R:AAAGTCGGCATGATGAAATA	AY173136	50°C	/
K17	$(CA)_6$	F:ATATTTCATCATGCCGACTT			
		R:TGGTATTTTCTCGTGCATTA	AY173137	50°C	4
K20	$(CA)_8$	F:AGCTGACACTTTGAAGCACT			
		R:GTGCTAATTCAGCGACTCTT	AY173134	50°C	2
K27	$(CA)_{16}$	F:CCATTAACCCCTTGTCCTCA			
		R:AAGGATGCAGGAGAGCAAAA	AY173135	50°C	2
K29	$(TG)_5$	F:CCCAGTGGTTGCACACTTAC			
		R:TGAAAGGAATTTTCAAGGGTTT	AY173133	50°C	4
K37	$(CA)_4$	F: CCATTAGCAAACCCATGCTT			
		R:TGGAAATGTGTCATCCTTCAG	AY173132	50°C	2

F: Forward primer; R: Reverse primer

after an initial denaturation at 94°C for 4 min, then 94°C for 30 sec (denaturation); 45°C for 57 sec (Table 1) for 30 sec (annealing); 72°C for 30 sec (extension) for 30 cycles, followed by a final extension at 72°C for 7 min. Amplification products were separated by electrophoresis on 8% nondenaturing polyacrylamide gels, visualized by silver staining and photographed (Huo *et al*., 2005). Size of amplification products were estimated using DNA size marker DL2000.

Data analysis: Amplified products were manually scored as 1 for the presence and 0 for the absence of a band as binary data using each of the primer. The effective number of alleles each primer (Ae) observed (Ho) and expected (He) Heterozygosities and Hardy-Weinberg Equilibrium (HWE) were analyzed using program POPGENE (Version 1.31) (Yeh and Boyle, 1997). Nei's genetic distances (d) among stocks were calculated using MEGA4.0 (Tamura *et al*., 2007) and used to draw phylogenetic trees by an Unweighted Pair-Group Method using an Arithmetic mean method (UPGMA). The reliability of the dendrogram was evaluated with 1,000 bootstraps.

RESULTS AND DISCUSSION

The current study analyzed DNA polymorphism for forty-four microsatellite primer and assessed the genetic variability of three cultured stocks of *S. formosus* in mainland China. Although, each primer was used to amplify in all stocks, the 39 polymorphic primers except for

Table 2: Genetic diversity of three stocks of *Scleropages formosus*

	Golden arowana						Red arowana						Green arowana					
Primers	Ae	Ho	He	PIC	Fis	Day	Ae	Ho	He	PIC	Fis	Day	Ae	Ho	He	PIC	Fis	Day
D01	1.68	0.190	0.418	0.325	0.533	-0.545	1.63	0.105	0.398	0.314	0.728	-0.736	1.60	0.214	0.388	0.305	0.428	-0.448
D04	2.97	0.238	0.679	0.608	0.641	-0.649	2.87	0.105	0.670	0.593	0.838	-0.843	2.94	0.214	0.685	0.619	0.675	-0.688
D11	1.66	0.523	0.407	0.397	-0.316	0.285	1.92	0.631	0.493	0.464	-0.314	0.280	1.50	0.428	0.349	0.280	-0.272	0.226
D13	2.13	0.190	0.543	0.527	0.641	-0.650	3.03	0.105	0.688	0.651	0.843	-0.847	1.98	0.214	0.515	0.488	0.569	-0.584
D14	3.13	0.809	0.698	0.652	-0.188	0.159	3.28	0.789	0.714	0.666	-0.135	0.105	3.13	0.928	0.706	0.638	-0.363	0.314
D15	1.96	0.857	0.501	0.370	-0.750	0.711	1.76	0.631	0.443	0.339	-0.461	0.424	1.96	0.857	0.507	0.370	-0.750	0.690
D27	3.69	0.666	0.746	0.698	0.085	-0.107	4.34	0.684	0.790	0.752	0.110	-0.134	4.00	0.785	0.777	0.728	-0.047	0.010
D33	3.03	0.571	0.686	0.666	0.147	-0.168	2.62	0.578	0.635	0.572	0.064	-0.090	3.66	0.642	0.754	0.721	0.115	-0.149
D35	1.09	0.095	0.092	0.087	-0.050	0.033	1.11	0.105	0.102	0.096	-0.055	0.029	1.15	0.142	0.137	0.124	-0.076	0.036
D37	4.52	0.809	0.797	0.767	-0.039	0.015	4.10	0.789	0.776	0.783	-0.044	0.017	3.76	0.857	0.761	0.714	-0.166	0.126
D38	3.35	0.857	0.718	0.685	-0.221	0.194	3.32	0.789	0.718	0.697	-0.128	0.099	3.43	0.857	0.735	0.694	-0.208	0.166
D42	1.89	0.761	0.483	0.360	-0.615	0.576	1.91	0.789	0.490	0.364	-0.652	0.610	1.84	0.714	0.476	0.354	-0.555	0.500
D72	2.52	1.000	0.619	0.527	-0.654	0.616	2.59	1.000	0.631	0.548	-0.626	0.585	2.63	1.000	0.642	0.551	-0.613	0.558
D85	1.99	0.952	0.511	0.374	-0.909	0.863	1.91	0.789	0.490	0.364	-0.652	0.610	1.98	0.928	0.515	0.374	-0.866	0.802
D88	4.10	0.476	0.774	0.732	0.370	-0.385	3.74	0.526	0.752	0.710	0.281	-0.301	3.66	0.357	0.754	0.691	0.508	-0.527
D92	1.26	0.238	0.214	0.188	-0.135	0.112	1.11	0.105	0.102	0.095	-0.055	0.029	1.32	0.285	0.254	0.215	-0.166	0.122
D94	2.05	0.714	0.526	0.455	-0.390	0.357	2.05	0.736	0.527	0.446	-0.434	0.397	1.95	0.642	0.505	0.444	-0.319	0.271
D101	2.51	0.285	0.616	0.559	0.525	-0.537	2.23	0.315	0.567	0.493	0.428	-0.444	2.52	0.285	0.627	0.563	0.527	-0.545
D102	1.68	0.285	0.418	0.325	0.300	-0.318	1.99	0.210	0.512	0.375	0.577	-0.590	1.68	0.285	0.423	0.325	0.300	-0.326
D104	2.96	0.285	0.679	0.610	0.569	-0.580	2.88	0.210	0.671	0.604	0.678	-0.687	2.70	0.285	0.653	0.562	0.546	-0.564
D105	3.93	0.666	0.764	0.723	0.106	-0.128	3.12	0.789	0.698	0.642	-0.160	0.130	4.26	0.642	0.793	0.752	0.160	-0.190
D106	2.55	0.571	0.623	0.572	0.061	-0.083	2.87	0.578	0.670	0.608	0.112	-0.137	2.56	0.642	0.632	0.576	0.160	0.016
D107	2.25	0.333	0.570	0.494	0.401	-0.416	2.16	0.473	0.553	0.470	0.120	-0.145	1.89	0.214	0.489	0.410	0.545	-0.562
D108	4.74	0.809	0.808	0.849	-0.026	0.001	4.40	0.894	0.793	0.758	-0.157	0.127	4.72	0.785	0.817	0.775	0.003	-0.039
D109	3.03	0.714	0.686	0.627	-0.066	0.041	3.26	0.684	0.712	0.661	0.014	-0.039	2.78	0.642	0.664	0.583	-0.004	-0.033
D111	1.32	0.285	0.250	0.215	-0.167	0.140	1.23	0.210	0.193	0.171	-0.117	0.080	1.32	0.285	0.254	0.215	-0.166	0.122
D114	5.06	0.714	0.822	0.912	0.110	-0.131	5.55	0.736	0.842	0.873	0.101	-0.126	4.96	0.785	0.828	0.901	0.016	-0.052
D115	3.75	0.857	0.751	0.722	-0.167	0.141	3.70	0.789	0.749	0.708	-0.081	0.053	3.73	0.857	0.759	0.721	-0.170	0.129
D116	2.88	0.333	0.669	0.620	0.450	-0.502	2.46	0.315	0.610	0.555	0.468	-0.484	2.90	0.285	0.679	0.620	0.564	-0.580
D117	1.56	0.095	0.371	0.297	0.738	-0.744	1.69	0.052	0.422	0.327	0.872	-0.877	1.60	0.071	0.388	0.305	0.809	-0.817
D118	1.38	0.142	0.284	0.239	0.486	-0.500	1.29	0.157	0.234	0.202	0.309	-0.329	1.50	0.142	0.349	0.280	0.575	-0.593
D119	5.37	0.857	0.833	0.892	-0.053	0.029	3.88	0.684	0.762	0.841	0.078	-0.102	5.60	0.928	0.851	0.881	-0.130	0.090
K10	2.71	0.381	0.646	0.567	0.397	-0.410	2.35	0.421	0.591	0.517	0.269	-0.288	2.64	0.428	0.645	0.559	0.311	-0.336
K13	1.26	0.238	0.214	0.188	-0.135	0.112	1.00	0.000	0.000	0.000	0.000	/	1.15	0.142	0.137	0.124	-0.076	0.036
K17	2.46	0.523	0.608	0.544	0.118	-0.140	2.46	0.684	0.610	0.523	-0.151	0.121	2.31	0.571	0.589	0.526	-0.004	-0.031
K20	1.38	0.333	0.284	0.239	-0.200	0.173	1.36	0.315	0.273	0.231	-0.187	0.154	1.23	0.214	0.198	0.173	-0.120	0.081
K27	1.80	0.666	0.455	0.346	-0.500	0.464	1.76	0.631	0.443	0.339	-0.461	0.424	1.77	0.642	0.452	0.342	-0.473	0.420
K29	2.51	0.381	0.616	0.525	0.367	-0.381	2.62	0.578	0.635	0.555	0.064	-0.090	2.70	0.571	0.653	0.573	0.093	-0.126
K37	1.15	0.142	0.135	0.124	-0.077	0.052	1.23	0.210	0.193	0.171	-0.117	0.088	1.07	0.071	0.071	0.066	-0.037	0.000
Mean	2.60	0.509	0.552	0.503	-	-	2.53	0.493	0.543	0.489	-	-	2.57	0.509	0.549	0.491	-	-

Table 3: Chi-square test for Hardy-Weinberg equilibrium of three stocks of *Scleropages formosus*

Primer	Gold arowana	Red arowana	Green arowana	Primer	Gold arowana	Red arowana	Green arowana	Primer	Gold arowana	Red arowana	Green arowana
D01	0.0096**	0.0008**	0.0731	D85	0.0000**	0.0062**	0.001**	D114	0.0160*	0.0001**	0.208
D04	0.0000**	0.0000**	0.0015**	D88	0.0069**	0.0000**	0.016*	D115	0.1000	0.1260	0.194
D11	0.5000	0.3010	0.3580	D92	0.5840	0.8650	0.594	D116	0.0070**	0.0010**	0.013*
D13	0.0000**	0.0000**	0.0000**	D94	0.4250	0.4310	0.841	D117	0.0003**	0.0000**	0.001**
D14	0.0000**	0.0000**	0.0230*	D101	0.0072**	0.0038**	0.016*	D118	0.0150*	0.1150	0.017*
D15	0.0008**	0.0558	0.0075**	D102	0.1320	0.0083**	0.198	D119	0.1350	0.0570	0.172
D27	0.2560	0.0018**	0.3870	D104	0.0005**	0.0000**	0.004**	K10	0.0004**	0.0000**	0.000**
D33	0.0620	0.0031**	0.0187*	D105	0.0190*	0.0210*	0.162	K13	0.5840	-	0.841
D35	0.8720	0.8650	0.8410	D106	0.0011**	0.0000**	0.014*	K17	0.6240	0.1380	0.814
D37	0.1440	0.0750	0.4880	D107	0.0150*	0.2300	0.013*	K20	0.4010	0.4600	0.718
D38	0.0010**	0.0140*	0.0313*	D108	0.0330*	0.0260*	0.070	K27	0.0280*	0.0550	0.098
D42	0.0066**	0.0062**	0.0505	D109	0.1720	0.0340*	0.056	K29	0.0450*	0.2780	0.321
D72	0.0068**	0.0019**	0.0795	D111	0.4900	0.6610	0.594	K37	0.7760	0.6600	1.000

*significant deviation from equilibaium;**terribly significant deviation from equilibaium; there is only one allele at the primer

D16, D31, D32, D95, K16 were included in the analyses. A total of 144 alleles were detected across polymorphic primer ranging from 2-7, corresponding to an average of 3.7 alleles per primer. The genentic diversity at the population level was determined using the effective number of alleles (Ae), observed (Ho) and expected (He) heterozygosities. Value of Ho ranged from 0.493 (Red) to 0.509 (Green and Golden) whereas He ranged from 0.552 (Golden) to 0.549 (Green) with the number of effective alleles ranging from 2.53 (Red) to 2.60 (Golden) (Table 2) showing middle diversity across each stocks. Probability of deviation from Hardy-Weinberg equilibrium per primer combination was shown in Table 3. The F_{ST} estimating within stocks ranged from 0.0026-0.01 with an average of

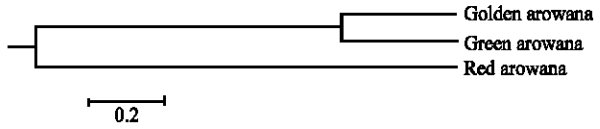

Fig. 1: Cluster analysis of three *Seleropages formosus* based on Ds using UPGMA method (Mu *et al*., 2009)

Table 4: Genetic Distances (Ds)\genetic similarity Indices (I) (above diagonal), gene flow (Nm)\genetic differentiation coefficient (F_{ST}) (below diagonal) among three stocks of *Scleropages formosus*

Stock	Gold arowana	Red arowana	Green arowana
Gold arowana	-	0.0197\0.981	0.0060\0.994
Red arowana	29.19\0.0085	-	0.0245\0.976
Green arowana	94.49\0.0026	24.83\0.010	-

Table 5: Heterozygosity of different stocks of *Scleropages formosus*

Stock	Sample	Ho	He
Gold arowana (Cultured)	Singapore	0.650	0.710
Gold arowana (Cultured)	Malaysian	0.522	0.407
Gold arowana (Cultured)	Malaysian	0.509	0.552
Red arowana (Cultured)	Singapore	0.720	0.740
Green arowana (Wild)	Indonesia	0.780	0.780
Red arowana (Cultured)	Malaysian	0.493	0.543
Green arowana (Cultured)	Singapore	0.650	0.750
Green arowana (Wild)	Singapore/Indonesia	0.580	0.580
Green arowana (Wild)	Indonesia	0.301	0.368
Green arowana (Wild)	Indonesia	0.304	0.405
Green arowana (Cultured)	Malaysian	0.509	0.549

0.0097 ($p<0.01$), (Table 4) showing significant genetic differences between different stocks. Low level of genetic distances were observed between the golden and green arowana ranging from 0.006-0.0245, suggested that *S. formosus* perhaps did not evolve to the level of specie. However, a little high level of distances were found between red and green arowana. Genetic relationships of three stocks were calculated using Nei's genetic distances to infer phylogenies by the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) (Fig. 1). The phylogenetic relationships among three stocks showed that golden and green arowana clustered together, sistered to the red stock.

The average allele number in the study was 3.7 per primer, lower than that of the *S. formosus* previously reported by Yue *et al.* (2000, 2006a) and Tang e*t al.* (2004). The difference in allele number could be affected by sample size of the individuals used for the characterization of primers. Heterozygosity, gene diversity was an important measurement of population diversity at genetic level and has drawn much attention from ecologists and aquaculturists (Xu *et al*., 2001).

Heterozygosity of three stocks ranged from 0.543-0.552 which was similar to the statistical data of approximately 40000 individuals, 524 microsatellite primers of 78 species (DeWoody and Avise, 2000) suggested that the genetic diversity of three *S. formosus* stocks was relatively rich. However, compared with those of other wild or cultured *S. formosus* stocks, the results were higher than that between wild green and captive golden arowana stocks (Shafiqur *et al*., 2008) but far below to that between wild green and wild red arowana stocks (Yue *et al*., 2000, 2006a) (Table 5). The possible explaination could be the inheritance of single or multiple genes affected by factors in the environment. Intra-specific relationship relating to *S. formosus* among different geographical populations isolates in Southeast Asian countries has different results using different types of nuclear genome markers and techniques in earlier studies (Kumazawa and Mutsumi, 2000; Tang *et al*., 2004). The study showed that golden arowana and green arowana clearly fall into different clades from red arowana, other than the former studies (Tang *et al*., 2004; Yue *et al*., 2006b) which was relevant to different geographical population and size.

Traditionally, cluster analysis maybe solve the problem of compositor of classifications in one collectivity. Yue *et al.* (2004) monitoring phylogenetic relationship of three Asian arowana stocks by AFLP showed that red and green varieties clustered. It is the same with the results that of Tang *et al.* (2004) supported red arowana was closely related with Malaysian red-tail gold arowana. Something else, Hu *et al.* (2009) anaylzing mt cytb gene showed that red arowana clustered with golden arowana after clustering with green arowana.

This study render different results that gold arowana and green arowana clearly fall into different clades from red arowana but Tang *et al.* (2004) estimated the time of divergence between the green and red arowana (1.5-2.6 MYA) close to the probable time of the fluctuation of sea level during the late Pliocene to early Pleistocene era by mt ATPase subunit 6 and 8 assuming that the sequence divergence rate for ATPase determined for fish is 1.3% per million years (Bermingham *et al*., 1997). In view of the present case to be a primitive fish possessing of academic and economical value, it is necessary to make more researches to clarify the evolution and breeding of different color stocks for a fuller understanding using more molecular markers.

CONCLUSION

The present study assessed genetic diversity among different stocks of *S. formosus* cultured in mainland China using microsatellite markers. The results have important implication for studying genetic structure of *S. formosus* as well as for the effective breeding management and development.

ACKNOWLEDGEMENTS

Project support was provided by the People's Republic of China, Ministry of Agriculture Affairs State (2009-Z13) to YC Hu, Science and Technology Developing Fisheries Program of liwan (20082109029) to YC Hu and the Open Fund of Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture (BZ2009-10) to XD Mu.

REFERENCES

Bermingham, E., S. McCafferty and A. Martin, 1997. Fish Biogeography and Molecular Clocks: Perspectives from the *Panamian Isthmus*. In: Molecular Systematics of Fishes, Kocher, T. and C. Stepien (Eds.). Academic Press, New York, pp: 112-126.

DeWoody, J.A. and J.C. Avise, 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish Biol., 56: 461-473.

Fernando, A.A., 1997. DNA fingerprinting: Application to conservation of the CITES-listed dragon fish, *Scleropages formosus* (Osteoglossidae). Aquarium Sci. Conserv., 1: 91-104.

Hu, Y.C., X.D. Mu, X.J. Wang, C. Liu, P.X. Wang and J.R. Luo, 2009. Preliminary study on mitochondrial DNA cytochrome b sequences and genetic relationship of three Asian arowana *Scleropages formosus*. Int. J. Biol., 2: 28-32.

Huo, J.L., R. Zeng, W.R. Pan, J. Zhang and G.Y. Luo *et al*., 2005. Analysis and study of influence factors about microsatellite PCR-polyacrylamide gel-silver staining. J. Yunnan Agric. Univ., 20: 11-15.

Kumazawa, Y. and N. Mutsumi, 2000. Molecular phylogeny of Osteoglossoids: A new model for Gondwanian origin and plate tectonic transportation of the Asian arowana. Mol. Biol. Evol., 17: 1869-1878.

Liu, F., J.H. Xia, Z.Y. Bai, J.J. Fu, J.L. Li and G.H. Yue, 2009. High genetic diversity and substantial population differentiation in grass carp (*Ctenopharyngodon idella*) revealed by microsatellite analysis. Aquaculture, 297: 51-56.

Mu, X.D., Y.C. Hu, X.J. Wang, X.H. Li, H.M. Song and J.R. Luo, 2009. Genetic analysis of scleropages formosus golden Asian arowana using microsatellite DNA. J. Agric. Sci., 1: 53-58.

Pan, Y., J.J. Bai, X. Ye, X.J. Wang, H.H. Lao and J.R. Luo, 2005. Analysis on the D-loop structure of two stock of *Scleropages formosus*. J. Zhanjiang Ocean. Univ., 25: 5-8.

Rutten, M.J.M., H. Komen, R.M. Deerenberg, M. Siwek and H. Bovenhuis, 2004. Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers. Anim. Genet., 35: 93-97.

Shafiqur, R., Z. Mohd, Y.T. Pek and M. Sekaran, 2008. Microsatellite analysis of wild and captive population of Asian arowana (*Scleropages formosus*) in Peninsular Malaysis. J. Boil. Sci., 1: 1-9.

Sunnucks, P., 2000. Efficient genetic markers for population biology. Trends Ecol. Evol., 15: 199-203.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Tang, P.Y., J. Sivananthan, S.O. Pillay and S. Muniandy, 2004. Genetic structure and biogeography of Asian arowana (*Scleropages formosus*) determined by microsatellite and mitochondrial DNA analysis. Asian Fish. Sci., 17: 81-92.

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic markers. Nucleic. Acids Res., 17: 6463-6471.

Xu, Z.K., J.H. Primavera, L.D. de la Pena, P. Pettit, J. Belak and A. Alcivar-Warren, 2001. Genetic diversity of wild and cultured Black Tiger Shrimp (Penaeus monodon) in the Philippines using microsatellites. Aquaculture, 199: 13-40.

Yeh, F.C. and T.J.B. Boyle, 1997. Population genetic analysis of codominant and dominant markers and quantitative traits. Belgium J. Botany, 129: 157-157.

Yue, G., Y. Li, F. Chen, S. Cho, L.C. Lim, and L. Orban, 2002. Comparison of three DNA markers systems for assessing genetic diversity in Asian arowana (*Scleropages formosus*). Electrophoresis, 23: 1025-1032.

Yue, G.H., D. Ong and C.C. Wong, L.C. Lim and L. Orban, 2003. A strain-specific and a sex-associated STS marker for Asian arowana (*Scleropages formosus*, Osteoglossidae). Aquacult. Res., 34: 951-957.

Yue, G.H., F. Chen and L. Orban, 2000. Rapid isolation and characterization of microsatellites from the genome of Asian arowana (*Scleropages formosus*, *Osteoglossidae*, *Pisces*). Mol. Ecol., 9: 1007-1009.

Yue, G.H., W.C. Liew and L. Orban, 2006b. The complete mitochondrial genome of a basal teleost, the Asian arowana (*Scleropages formosus*, *Osteoglossidae*). BMC Genomics, 7: 242-242.

Yue, G.H., Y. Li, L.L. Lim and L. Orban, 2004. Monitoring the genetic diversity of three Asian arowana (*Scleropages formosus*) captive stocks using AFLP and microsatellites. Aquaculture, 237: 89-102.

Yue, G.H., Z.Y. Zhu, G. Lin, L.C. Lo and F. Feng, 2006a. Novel polymorphic microsatellites for studying genetic diversity of red Asian arowanas. Conserv. Genet., 7: 627-629.

Zhang, Z.W., Z.M. Cao, H.Yang, J.L. Wang, J.L. C, Y.P. Han and T.T. Wu, 2005. Microsatellites analysis on genetic variation between wild and cultured populations of *Ctenopharyngodon idella*. Zool. Res., 27: 189-196.

Zhu, Z.Y., G. Lin, L.C. Lo, Y.X. Xu, F. Feng, R. Chou and G.H. Yue, 2006. Genetic analyses of Asian seabass stocks using novel polymorphic microsatellites. Aquaculture, 256: 167-173.