ISSN 1479-2362
Chinese Journal of Agricultural Biotechnology **4**(1); 39–46
doi:10.1017/S1479236207001210

Genetic diversity and molecular markers of five snapper species

Liu Li and Liu Chu-Wu*
Fisheries College, Guangdong Ocean University, Zhanjiang 524025, China

Received 20 May 2005; Accepted 2 September 2005

First published in *Journal of Agricultural Biotechnology* 2006, 14(3): 349–355

Abstract

In order to protect and develop valuable snappers (*Lutjanus* spp.), genetic diversity and molecular markers of five species (*Lutjanus vitta*, *L. fulvus*, *L. fulviflamma*, *L. sebae* and *L. stellatus*) were detected and analysed using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) techniques. The polymorphic loci ratio (*P*) (86.00–92.11%), the mean intraspecies genetic distances (*D*) (0.1775–0.3431) and the intraspecies genetic diversity indexes (*Hi*) (0.1022–0.1634) were calculated using the RAPD technique. The genetic diversities of *L. fulviflamma* and *L. vitta* were richest in terms of *P*, and *D* and *Hi*, respectively. The results of SSR showed that low effective numbers of alleles (1.7893–3.6591), medium average heterozygosities (0.332–0.676) and medium polymorphism information contents (PIC) (0.302–0.641) occurred in five species of snappers, indicating comparatively rich genetic diversity among these fish. Nine molecular markers in the products amplified by primers OPA8 and OPP10, and six molecular markers in 11 microsatellite loci were found to be useful as specific markers to identify five species of snappers. Two neighbour-joining (NJ) dendrograms based on the results of RAPD and SSR suggested that *L. stellatus* and *L. sebae* are closely related and clustered in one branch, with *L. vitta*, *L. fulviflamma* and *L. fulvus* in the other.

Keywords: genetic diversity; *Lutjanus vitta*; *L. fulvus*; *L. fulviflamma*; *L. sebae*; *L. stellatus*; molecular marker; random amplified polymorphic DNA; simple sequence repeats

Introduction

Random amplified polymorphic DNA (RAPD) analysis has been widely used in genetic diversity studies, identification of fish species, genetic differentiation in intra- or interpopulation breeding, DNA detection and so on, as it is a quick, sensitive and easy technique (Bardakci and Skibinski, 1994; Ding *et al.*, 1998; Z.H. Liu *et al.*, 1999; Z.J. Liu *et al.*, 1999; Yoon and Kim, 2001). The use of simple sequence repeats (SSR) methods has attracted researchers' attention for their rich polymorphism, stability and reliability. The SSR technique has also been used to identify polygenetic relationships, to study the genetic intra- or interpopulation variations and to analyse biological species diversity (Nielsen *et al.*, 1994; Reilly *et al.*, 1999; Heist, 2000). *Lutjanus vitta*, *L. fulvus*, *L. fulviflamma*, *L. sebae* and *L. stellatus* (*Perciformes, Lutjanidae, Lutjanus*) are economically important marine fishes, colonizing tropical and subtropical seas (Meng *et al.*, 1995). They are highly appreciated for their beautiful scales, delicious muscle and high nutritional value. Many studies on *Lutjanus* fishes have been conducted at morphological, cytological and molecular level (Chow *et al.*, 1993; Chen, 1997; Allman and Lombardi-Carlson, 2000; Collins *et al.*, 2001; Li *et al.*, 2001; Allman and Churchill, 2002; Cao *et al.*, 2002; Yi and Liu, 2002; Liu *et al.*, 2003a, b).

So far, very little attention has been focused on *Lutjanus* fishes in the South China Sea. In 2004, Zhou *et al.* (2004a, b) compared the sequences of mitochondrial DNA (MtDNA), 16S rRNA and *Cyt*b genomic

* Corresponding author. E-mail: swyjs@gdou.edu.cn

Table 1. Genetic diversity of five species of snappers (*Lutjanus* spp.)

Species	Polymorphic loci ratio	Genetic similarity coefficient	Genetic distances	Genetic diversity indexes
L. vitta	89.30%	0.6569	0.3431	0.1634
L. fulvus	86.70%	0.7870	0.2130	0.1095
L. fulviflamma	92.11%	0.6879	0.3121	0.1353
L. sebae	86.47%	0.8174	0.1825	0.1022
L. stellatus	86.00%	0.8225	0.1775	0.1024

fragments of *L. argentimaculatus, L. stellatus, L. sebae, L. russelli* and *L. orythopterus*. Recently, Yi and Liu (2005) performed RAPD analysis on *L. russelli*, *L. johni*, *L. argentimaculatus* and *L. orythopterus*.

To provide more information for establishing relevant protective and developmental measures, and to ensure stock protection, timely conservation, reasonable exploitation and continual usage of resources, this article describes the genetic diversity and molecular markers of five *Lutjanus* fishes using RAPD analysis and the SSR technique.

Materials and methods

Materials

One hundred individuals of five *Lutjanus* fish species (20 samples each) were collected from the South China Sea from April 2001 to August 2003. Blood was collected intravenously from fish tails and stored in acid citrate dextrose (ACD) solution (81.7 mmol/l D-glucose, 22.8 mmol/l citric acid monohydrate, 44.9 mmol/l sodium citrate dihydrate) at −70 °C.

Apparatus and reagents

A polymerase chain reaction (PCR) Instrument (Hema 8000, Zhuhai, Guangdong, China) and a high speed refrigerated centrifuge (SIGMA3K30, B. Braun, Germany) were used. RAPD random primers were synthesized by SABC (Sino-American Biotechnology Company, Beijing, China). Microsatellite loci primers were synthesized by Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd (Shanghai, China). Reagents, such as PCR markers, deoxynucleoside triphosphates (dNTPs) and proteinase K, were from Promega (Madison, Wisconsin, USA) or SABC Biology Company.

Isolation of genomic DNA

The genomic DNA was extracted according to Sambrook *et al.* (1989) and Lu (1993), detected by UV spectrophotometry and tested quantitatively.

Reaction conditions of PCR and test of PCR products

The PCR reaction conditions and PCR products test for RAPD analyses were developed from the method by Liu *et al.* (2003a, b). The SSR analysis was carried out based on the protocols of Lin and Luo (2003).

Data analysis of RAPD

The polymorphic loci ratio (*P*), intraspecies genetic similarity coefficient (*S*), interspecies genetic distances (D_{xy}), genetic similarity coefficient (*I*) and Shannon information indexes (*Hi*) were calculated following Lynch (1990), Nei (1972) and Nei and Li (1979). A population molecular phylogenetic tree was constructed using MEGA software (Kumar *et al.*, 2001) with genetic distance as a parameter, based on the neighbour-joining (NJ) method.

Data analysis of SSR

The effective numbers of alleles (*Ne*), population heterozygosity (*H*), Nei standard genetic distance (*Ds*), genetic distance (*DA*) and polymorphic information content (*PIC*) were calculated by the method of Botsein *et al.* (1980). Two molecular phylogenetic trees were constructed according to the genetic distance.

Results

Intraspecies genetic diversity

The results of the amplification products of the 22 primers showed comparatively rich genetic diversity among the five fish species. The genetic diversity of *L. fulviflamma* was the richest in polymorphic loci ratio (*P*) and that of *L. vitta* in genetic distance and diversity index (Table 1).

Interspecies genetic diversity and genetic relationships

Table 2 showed that the genetic distance between *L. fulvus* and *L. sebae* was the longest (0.5032) and that

Table 2. Genetic distances (D_{xy}) among five species of snappers (*Lutjanus* spp.)

Species	*L. vitta*	*L. fulvus*	*L. fulviflamma*	*L. sebae*
L. vitta	–			
L. fulvus	0.3617	–		
L. fulviflamma	0.2878	0.3426	–	
L. sebae	0.4005	0.5032	0.3209	–
L. stellatus	0.4408	0.4926	0.3748	0.4014

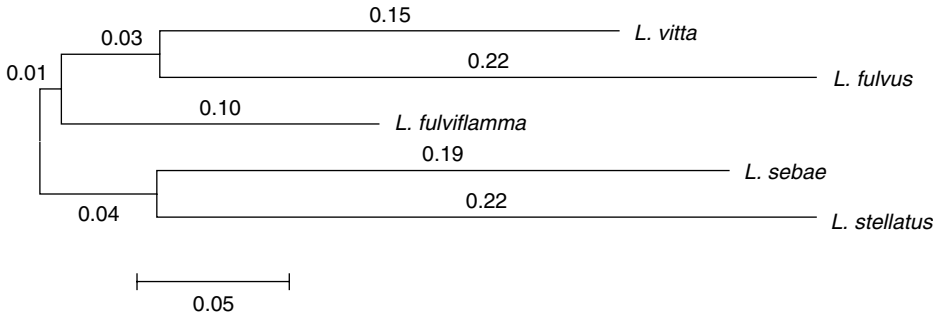

Fig. 1. Neighbour-joining (NJ) tree based on D_{xy}.

Table 3. Species-specific bands of five species of snappers (*Lutjanus* spp.)

Species	Molecular weight of species-specific markers
L. vitta	$OPA8^{-413\,bp}$
L. fulvus	$OPA8^{-140\,bp}$, $OPP10^{-418\,bp}$
L. fulviflamma	$OPA8^{-697\,bp}$, $OPP10^{-526\,bp}$
L. sebae	$OPA8^{-361\,bp}$, $OPP10^{-449\,bp}$
L. stellatus	$OPA8^{-311\,bp}$, $OPP10^{-599\,bp}$

between *L. vitta* and *L. fulviflamma* the shortest (0.2878). The population molecular phylogenetic tree was constructed with the NJ method (Fig. 1). The five species of snappers were separated into two groups (*L. stellatus* and *L. sebae* in one group, *L. vitta* and *L. fulvus* in the other group), then clustered with *L. fulviflamma*.

Specific intraspecies markers

Characteristic bands for one or two fish could be found in the amplification products of all 22 primers, but few of them could be used to identify the five species at a time, apart from primers OPA8 and OPP10 (Table 3, Fig. 2).

Selection of primers for SSR

Eleven of 20 primer pairs (Table 4), originally developed for the western Atlantic snappers, could produce clear and stable bands in samples from the five *Lutjanus* fishes (not shown).

Effective numbers of alleles (Ne)

The effective number of alleles of *L. stellatus* was the largest (3.6591) in the five species, followed by those of *L. fulvus* (3.3793), *L. fulviflamma* (3.2957), *L. vitta* (1.9610) and *L. sebae* (1.7893), by comparing the amplification products of the 11 primer pairs.

Mean genetic heterozygosity

Heterozygosity of the fish ranged from 0.3 to 0.8 with *L. stellatus* (0.676), *L. fulviflamma* (0.593), *L. fulvus* (0.462), *L. sebae* (0.367) and *L. vitta* (0.332).

Polymorphic information content

The polymorphic information content (PIC) is always used to assess the variability of microsatellite loci in SSR analysis. The mean PIC of the fish was as follows: *L. stellatus* (0.641), *L. fulviflamma* (0.554), *L. fulvus* (0.438), *L. sebae* (0.332) and *L. vitta* (0.302).

Genetic distance

The genetic distance (DA) parameter allows the interspecies estimation of genetic relationships. Tables 5 and 6 list the genetic standard distance and genetic distance among the five species of snappers. The maximum

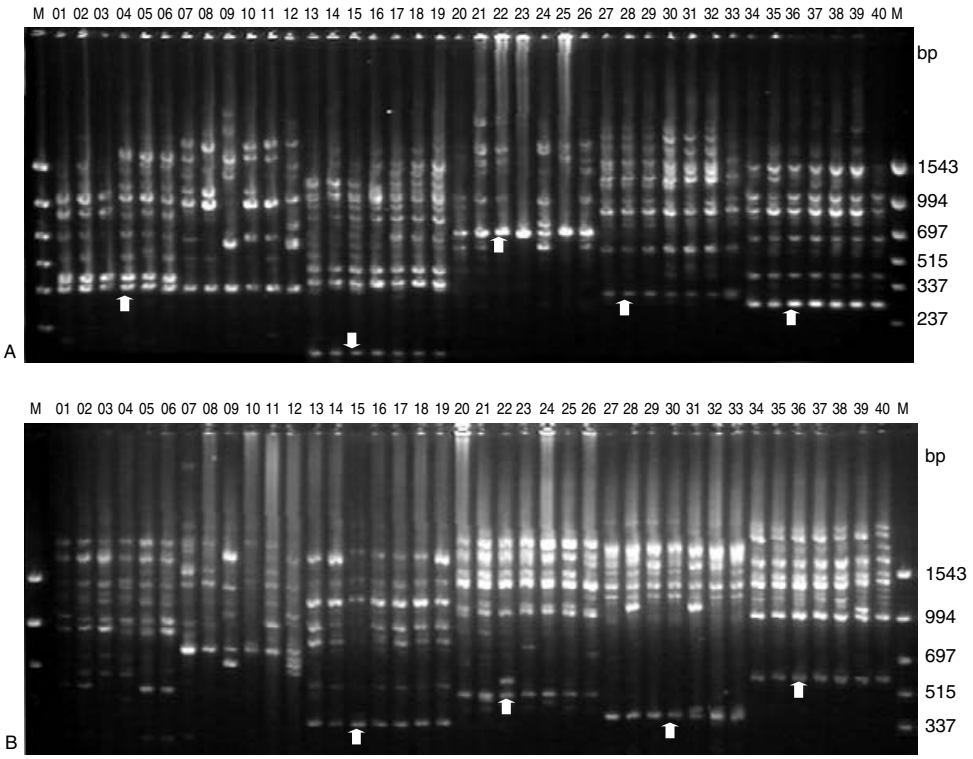

Fig. 2. RAPD bands generated by primers OPA8 (A) and OPP10 (B) in five species of snappers (*Lutjanus* spp.). Lane M, molecular size marker. Lanes: 01–12, *L. vitta*; 13–19, *L. fulvus*; 20–26, *L. fulviflamma*; 27–33, *L. sebae*; 34–40, *L. stellatus*. (A) Arrows, species-specific markers from left to right 413, 140, 697, 361 and 311 bp, respectively. (B) Arrows, species-specific markers from left to right, 418, 526, 449 and 599 bp, respectively.

Table 4. Sequence of 11 microsatellite marker primers

Microsatellite marker	Sequence of primers (5′–3′)	Microsatellite marker	Sequence of primer (5′–3′)
1 Lca20	CAA CCC TCT GGC TAG TGT CA ATC CTG AAG CCC TGG TTT AC	15 Prs240	CAA GAG GGT GAT GAA TGA AAT GAA TA CCC ACT GCT
2 Lca22	TCC ACA GGC TTT CAC TCT TTC AG TGC TCT TTT CTT TCC GTC ATT CC	17 Prs257	AAA GTT CTT GTG ATG TGT GAG AAA ATG TTG GAA TGA
4 Lca43	ACT GAA ATG CTG CTC TCC TT CAC TGT TTA CTT CTT CTG TT	18 Prs260	GGT AAA ATG CTC CCT TCC T GTG GTA GTG GGT GAA ATT CT
7 Lca91	GCA TCC ACC CTA AAC ATT TT GTT CAT CAG AGC AGC ATC CT	19 Prs275	CAC AGA TAC AAA CCC AGA CA AGT AGG TCT TTG GTC ATC A
11 Prs221	AGT TTG CTA ATG TCT GAG TCA CC CCA TTG TCT TCG CTT ACT T	21 Prs282	CAG AGG AGG CAG AAC AGA ACC ACA CTA ATG CAC ACA C
13 Prs229	CAC ATT GAA CCG TTT AAC CC GAA ATG ATG ACC CAG CAC AG		

Table 5. Genetic standard distance between the five species of snappers (*Lutjanus* spp.)

	L. vitta	*L. fulvus*	*L. fulviflamma*	*L. sebae*
L. vitta	–			
L. fulvus	0.3349	–		
L. fulviflamma	0.2859	0.1520	–	
L. sebae	0.3952	0.3574	0.3214	–
L. stellatus	0.2710	0.2305	0.2163	0.2430

Table 6. Genetic distance between the five species of snappers (*Lutjanus* spp.)

	L. vitta	*L. fulvus*	*L. fulviflamma*	*L. sebae*
L. vitta	–			
L. fulvus	0.3612	–		
L. fulviflamma	0.347	0.292	–	
L. sebae	0.4167	0.4022	0.4147	–
L. stellatus	0.3516	0.3535	0.3748	0.3527

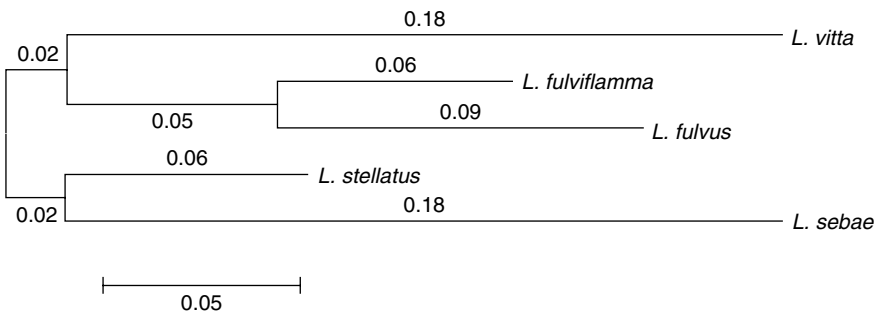

Fig. 3. Neighbour-joining (NJ) tree based on genetic standard distance (*DS*).

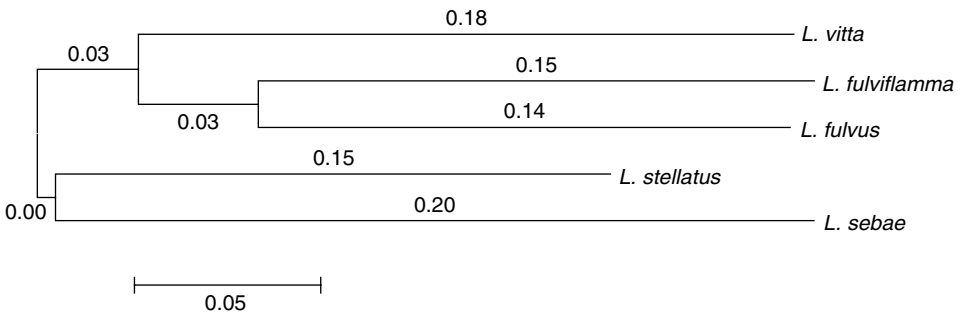

Fig. 4. Neighbour-joining (NJ) tree based on genetic distance (*DA*).

genetic distance was found between *L. vitta* and *L. sebae*, and the minimum between *L. fulvus* and *L. fulviflamma*.

Dendrogram analysis

The construction of population molecular phylogenetic trees of the five fish were performed by NJ method with genetic distance and standard genetic distance as parameters. The NJ trees were in accordance with two separate groups: *L. stellatus* and *L. sebae* were closely related in one group, and *L. fulviflamma* and *L. fulvus* in the other group, joined later by *L. vitta* (Figs 3 and 4).

Specific interspecies markers

Six specific markers in four species were found from the amplified products of the 11 microsatellite primer pairs: 13 $Prs229^{-115\,bp}$, 4 $Lca43^{-212\,bp}$, 4 $Lca43^{-240\,bp}$, 13 $Prs229^{-288\,bp}$, 19 $Prs275^{-156\,bp}$ and 7 $Lca91^{-118\,bp}$ (Table 7, Fig. 5), but none was found in *L. vitta*.

Discussion

Genetic diversity analysis

In RAPD analysis, polymorphic loci ratio (*P*) and heredity diversity index (*Hi*) are important parameters for estimation of intraspecies heredity diversity levels. The heredity diversity level of the five species under study, scored with *P* and *Hi*, was similar. According to *P, L. fulviflamma* presented the richest heredity diversity (92.11%), followed by *L. vitta* (89.30%), *L. fulvus* (86.70%), *L. sebae* (86.47%) and *L. stellatus* (86.00%). However, taking *Hi* into account, *L. vitta* displayed the richest diversity (0.1634), followed by *L. fulviflamma* (0.1353), *L. fulvus*

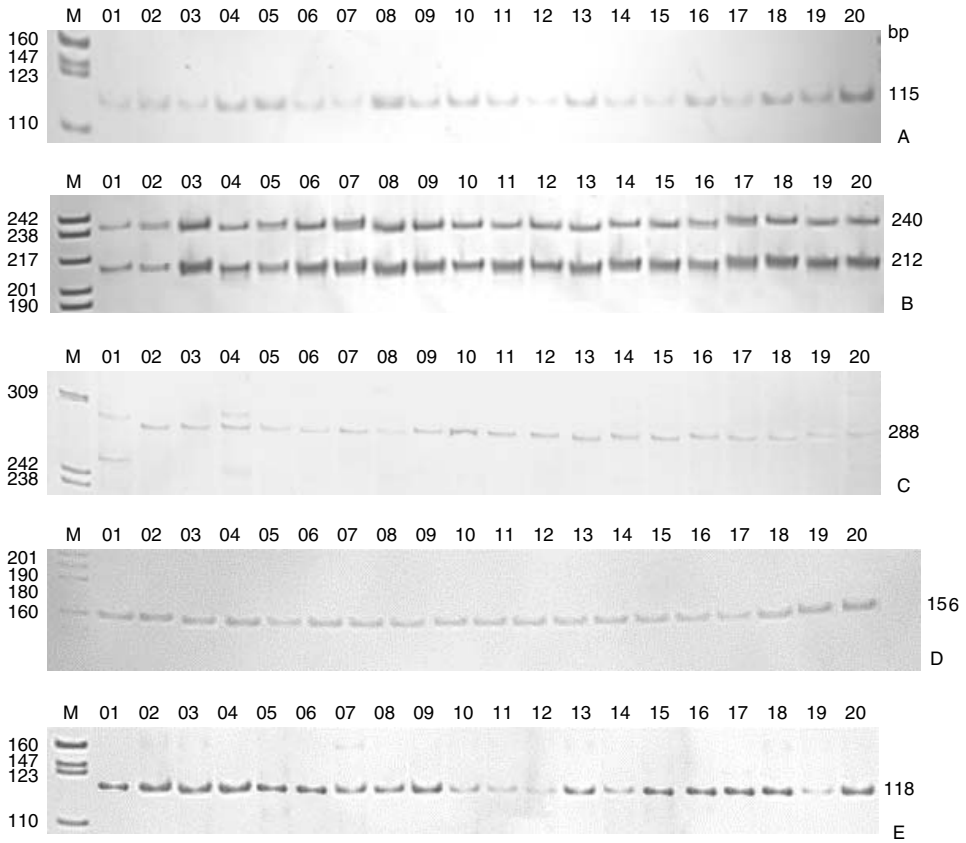

Fig. 5. Electrophoretic patterns of PCR products amplified by different primers in *Lutjanus*. (A) Primer 13, *L. fulvus*; (B) Primer 4, *L. fulviflamma*; (C) Primer 13, *L. sebae*; (D) Primer 19, *L. stellatus*; (E) Primer 7, *L. stellatus*. Lanes: M, molecular size markers; 01–20, 20 samples.

Table 7. Species-specific bands of five species of snappers (*Lutjanus* spp.)

Species	Molecular weight of species-specific markers
L. vitta	
L. fulvus	13 $Prs229^{-115\,bp}$
L. fulviflamma	4 $Lca43^{-212\,bp}$, 4 $Lca43^{-240\,bp}$
L. sebae	13 $Prs229^{-288\,bp}$
L. stellatus	19 $Prs275^{-156\,bp}$, 7 $Lca91^{-118\,bp}$

(0.1095) and *L. sebae* (0.1022) and *L. stellatus* (0.1024). All the five species examined showed rich genetic diversity when comparing the RAPD results with those of other fish. As important fish in the South China Sea, this resource is still at a favourable level and no evident degeneration has appeared in their germ plasm at present.

Heterozygosity (*H*) and polymorphic information content (*PIC*) are important parameters for analysis of the polymorphism of microsatellite loci using the SSR technique. Heterozygosity generally ranges from 0.3 to 0.8, when calculated through microsatellite markers. Population mean heterozygosity is a suitable parameter to estimate species variation and it can indicate the genetic consistency in a population regarding a given molecular marker. Lower population heterozygosity means fewer population variations, lower population genetic diversity and higher genetic consistency of the population. This study showed that the heterozygosity of the five species of snappers ranged from 0.3 to 0.8, that of *L. stellatus*, *L. fulviflamma* and *L. fulvus* was higher with 0.676, 0.593 and 0.462. A lower heterozygosity was observed in *L. sebae* and *L. vitta* with 0.367 and 0.332, respectively. Polymorphic information content is the possibility for an offspring to get a same allele from its parents. $PIC > 0.5$ indicates a high polymorphism, whereas $0.25 < PIC < 0.5$ refers to the mid-range and $PIC < 0.25$ to lower polymorphism. In this study, 11 microsatellite loci have been used to estimate the polymorphism of the five *Lutjanus* fishes from the South China Sea. The results suggest that the mean polymorphic information content of *L. stellatus* and *L. fulviflamma* exceeded 0.5 and that of *L. fulvus*, *L. sebae* and *L. vitta* ranged from 0.25 to 0.5. It showed high polymorphism

in *L. stellatus* and *L. fulviflamma* and relatively high polymorphism in the other three species.

Differences of the results from the two methods

Comparison of the results of RAPD and SSR analyses of the five *Lutjanus* species showed that the genetic diversity of *L. vitta*, *L. fulvus* and *L. fulviflamma* was richer than that of *L. sebae* and *L. stellatus* with the RAPD analysis. But, with SSR analysis, the diversity of *L. stellatus*, *L. fulvus*, *L. fulviflamma* was more outstanding than that of *L. sebae* and *L. vitta*. In the NJ tree constructed from RAPD analysis, *L. fulvus* clustered with *L. vitta* in a group. In the SSR analysis, however, *L. fulvus* and *L. fulviflamma* were grouped together. *L. sebae* and *L. stellatus* were closely related in one group in the trees constructed with both methods. As to the genetic diversity of *L. vitta* and *L. stellatus*, the result from RAPD was not in accordance with that from SSR.

Two possible reasons could explain this result. First, the differences in research methodology could induce different results. As a matter of fact, investigating *Gossypium hirsutum* L. and cucumis, respectively, Zhu *et al.* (2003) and Chen *et al.* (2003) drew the same conclusion. Zhu observed that the RAPD result from dendrogram analysis was more consistent with the family tree.

The second reason is probably related to the problems existing in the morphological classification of *L. vitta*: any fish with two vertical brown or yellow bands on both sides of the body (especially in living fish) was identified as *L. vitta*. However, in this study, 10 samples with brown avertical bands and 10 others with yellow avertical bands were collected. Brown fishes were remarkably different from the yellow ones at the molecular level, but the differences were probably not totally consistent when analysing the five *Lutjanus* species with both RAPD and SSR methods, and maybe this is one of the reasons for inconsistent analytical results.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC) (No. 30671610), the important scientific project of Guangdong Provincial Department of Science and Technology (No. A3050202) and the target-oriented key project of Zhanjiang Bureau of Science and Technology (No. 200017).

References

Allman RJ and Churchill BG (2002) Temporal and spatial dynamics of spawning, and growth of gray snapper (*Lutjanus griseus*) from the West Florida shelf as determined from otolith microstructures. *Fishery Bulletin* 100(3): 391–403.

Allman RJ and Lombardi-Carlson LA (2000) Age structure of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico by fishing mode and region. *Proceedings of the Gulf and Caribbean Fisheries Institute* 53: 482–495.

Bardakci F and Skibinski DOF (1994) Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity* 73: 117–123.

Botsein D, White RL, Skolnick M, *et al.* (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32: 314–331.

Cao FJ, Li CL and Liu CW (2002) Studies on the karyotypes of *Lutjanus erythopterus, Lutjanus argetimaculatus* and *Lutjanus bohar*. *Marine Sciences* 26(11): 43–46 (in Chinese with English abstract).

Chen G (1997) Studies on age, growth and life-history pattern of *Lutjanus russelli Bleeker*. *Journal of Fisheries of China* 21(1): 6–12 (in Chinese with English abstract).

Chen JF, Zhuang FY, Lu MH, Qian CT and Ren G (2003) Phylogenetic relationships in cucumis (Cucurbitaceae) revealed by SSR and RAPD analyses. *Acta Phytotaxonomica Sinica* 41(5): 427–435 (in Chinese with English abstract).

Chow S, Clarke ME and Walsh PJ (1993) PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): A simple method for species and stock identification. *Fishery Bulletin US* 91: 619–627.

Collins LA, Fitzhugh GR, Mourand L, *et al.* (2001) Preliminary results from a continued study of spawning and fecundity in the red snapper (*Lutjanidae: Lutjanus campechanus*) from the Gulf of Mexico, 1998–1999. *Proceedings of the Gulf and Caribbean Fisheries Institute* 52: 34–47.

Ding SX, Wang J, Quan CG and Su YQ (1998) Gentic diversity in population of cultured *Nibea miichthioides*. *Bulletin of Science* 43(21): 2294–2299 (in Chinese).

Heist EJ (2000) DNA microsatellite loci and genetic structure of red snapper in the Gulf of Mexico. *Transactions of the American Fisheries Society* 129(2): 469–475.

Kumar S, Tamura K, Jacobsen IB and Nei M (2001) MEGAZ: molecular evolutionary genetics analysis software. *Bioinformatics* 17: 1244–1245.

Li CL, Cao FJ, Liu CW and Chen G (2001) Microstructure of peripheral blood cells in *Lutjanus russelli*. *Journal of Zhanjing Ocean University* 21(3): 5–9 (in Chinese with English abstract).

Lin KD and Luo C (2003) Preliminary study on applicability of microsatellite primers developed from common carp for genomic analysis of grass carp. *Acta Laser Biology Sinica* 12(2): 121–127 (in Chinese with English abstract).

Liu L, Liu CW and Xu Q (2003a) Population genetics analysis on three species of snappers by random amplified polymorphic DNA (RAPD). *Journal of Zhanjing Ocean University* 23(3): 16–21 (in Chinese with English abstract).

Liu L, Liu CW and Xu Q (2003b) Population genetics analysis on two species of snappers by random amplified polymorphic DNA (RAPD). *Marine Sciences* 27(10): 59–62 (in Chinese with English abstract).

Liu ZH, Shi T, Liu XZ, Chen C and Kong J (1999) Identification of three populations (Chinese *Fugu rubripes*, Japanese *Fugu rubripes* and *Fugu pseudommus*) using RAPD markers. *Transactions of Oceanology and Limnology* 4: 38–41 (in Chinese).

Liu ZJ, Li P, Argue BJ and Dunham RA (1999) Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture* 174: 59–68.

Lu SD (1993) *Current Protocols for Molecular Biology*. Beijing: China Higher Education Press, pp. 95–1045 (in Chinese).

Lynch M (1990) The similarity index and DNA fingerprinting. *Molecular Biology and Evolution* 7: 478–484 (in Chinese with English abstract).

Meng QW, Su JX and Miao XZ (1995) *Systematics of Fishes*. Beijing: China Agriculture Press, pp. 686–687 (in Chinese).

Nei M (1972) Genetic distance between populations. *American Naturalist* 106: 283–292.

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proceedings of the National Academy of Sciences of the USA* 76(10): 5269–5273.

Nielsen JL, Gan CA, Wright JM, Morris DB and Thomas WK (1994) Biogeographic distributions of mitochondrial and nuclear markers for southern steelhead. *Molecular Marine Biology and Biotechnology* 3: 281–293.

Reilly A, Elliott NG, Grewe PM, Clabby C, Powell R and Ward RD (1999) Genetic differentiation between Tasmanian cultured Atlantic salmon (*Salmo salar* L.) and their ancestral Canadian population: comparison of microsatellite DNA and allozyme and mitochondrial DNA variation. *Aquaculture* 173: 459–469.

Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Yi LF and Liu CW (2005) RAPD analysis of four snapper species. *Fisheries Science* 24(1): 9–12 (in Chinese with English abstract).

Yi LF, Liu CW and Lu LQ (2002) RAPD analysis on genetic diversity of *Lutjanus johni*. *Journal of Fishery Sciences of China* 9(4): 379–381 (in Chinese with English abstract).

Yoon JM and Kim GW (2001) Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*. *Journal of Biosciences* 26(5): 641–647 (in Chinese with English abstract).

Zhou Fl, Jiang SG, Su TF and Lv JL (2004a) Comparative study of mtDNA 16S rRNA gene fragments among six *Lutjanus* fishes. *Journal of Fishery Sciences of China* 11(2): 99–103 (in Chinese with English abstract).

Zhou Fl, Jiang SG, Su TF and Lv JL (2004b) Comparison of mitochondrial cytochrome b gene fragments among six species of *Lutjanus*. *Journal of Tropical Oceanography* 23(4): 87–92 (in Chinese with English abstract).

Zhu LF, Zhang XL and Nie YC (2003) Analysis of genetic diversity in upland cotton (*Gossypium hirsutum* L.) cultivars from foreign countries by RAPDs and SSRs. *Journal of Agricultural Biotechnology* 11(5): 450–455 (in Chinese with English abstract).