Analysis of Genetic signature for some *Plectropomus* species based on some dominant DNA markers

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Abstract: Successful conservation of fish such as ***Plectropomus* species** depend on analysis of genetic signature and relations among fish species and subspecies. Knowledge about the genetic signature and structure of *Plectropomus* species needs to increase several times to enable us for conserve these economic fish in the future. The current study aims to develop and analysis of genetic signature for three *Plectropomus* species (*P.maculates, P.leopardus and P.areolatus*) based on some dominant DNA markers (RAPD and ISSR). A total of 9 ISSR and 12 RAPD primers were used to develop some DNA markers and their loci were estimated. Most studied DNA markers were polymorphic. The overall gene diversity (h) based on RAPD data was higher than based on ISSR data. Data analysis showed that, the *P.areolatus* was distantly related from both *P.maculates* and *P.leopardus*. RAPD and ISSR markers proved to be powerful methods to detect genetic signature for the applied *Plectropomus* species. The genetic markers which developed in this study will play a valuable role in monitoring the ***Plectropomus*** genetic resources in the future.

[Y. M. Saad; AbuZinadah, O. A. H.; El-Domyati, F. M. and Sabir, J. M. **Analysis of Genetic signature for some *Plectropomus* species based on some dominant DNA markers.** *Life Sci J* 2012;9(4):2370-2375] (ISSN:1097-8135). http://www.lifesciencesite.com. 351

Key words: *Plectropomus*, DNA polymorphism, RAPD and ISSR markers.

1. Introduction

Overfishing of *Plectropomus* species as high value marine food in the Red sea (Zhu *et al.,* 2005) makes them difficult to manage (FAO 2009).

Developing of genetic signature of such species will facilitate the analysis of fish genetic structure for management of such aquatic genetic resources in the future. In addition, the effective management of fisheries requires the ability to discriminate among fish stocks (Rico *et al.,* 1997) and/or species (Madhan *et al.,* 2011 and Rashed *et al.,* 2011).

Up to date, the application of DNA-based genetic analysis in *Plectropomus* species research, stock development and management is still not fully maximized. In addition, the level of speciation and evolutionary relationships between *Plectropomus* species remains unclear.

RAPD (Rashed *et al*., 2011), SSR (Rashed *et al*., 2009) and ISSR (Raina, *et al*. 2001) techniques should find major population genetics applications, notably in the field of genetic conservation, where molecular markers need to be developed at a reasonable cost.

The information revealed from the analysis of genetic polymorphism for fish species (Rashed *et al*., 2011) and subspecies (Rashed *et al.,* 2008 and Saad *et al.,* 2009) is widely used in the management of aquatic genetic resources because management of aquatic genetic resources should ideally involve a continuum of activities (Eknath, 1994). These activities are documentation of genetic resources and the variety of ecosystems; characterization to determine the genetic structure, evaluation to estimate economic potential and utilization in sustainable breeding schemes.

The current study aims to develop and analysis of genetic signature for some of *Plectropomus* species (*P.maculates, P.leopardus and P.areolatus*) based on some dominant DNA markers (RAPD and ISSR).

2. Material and Methods

Fish Material:

The fish samples (*Plectropomus maculates*, *Plectropomus leopardus* and *Plectropomus_areolatus*) were obtained from **MBSPR** project (funded from DSR, King Abdulaziz Univ., KSA during year of (2012). Jeddah province is the main source of these fish samples. A total of 20 individuals from each studied fish species were analyzed.

DNA extraction:

Small pieces of caudal fins (0.1g) were stored in 95% ethyl alcohol immediately. DNA was extracted and performed according to Hillis *et al.* (1996).

Genetic signature based on RAPD (Random Amplified Polymorphic DNA) markers:

Twelve RAPD primers (Operon Technologies) were tested for detection of applied fish genetic signature. The primer codes and sequences were presented in Table (1). PCR mixture, reaction conditions and product separation were carried out as described by Rashed *et al*. (2008) with some

modifications. Initial denaturation was for 4 min at 94°C; followed by 30 cycles of 40 s at 94°C, 40 s at 37°C, and 1 min at 72°C; with a final 15 min extension at 72°C.

Genetic signature based on ISSR (Inter Simple Sequence Repeat) markers:

A total of nine ISSR primers (Table 1) were originally tested and selected to develop genetic signature for the three applied fish genomes. PCR

mixture, reaction conditions and product separation were carried out as described by Raina, *et al*. (2001) with minor modifications. The amplification was carried out in a 10 µl reaction volume containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.1 mM each dNTP, 200 µM primer, 1 U Taq DNA polymerase, and 5 ng of genomic DNA. Initial denaturation was for 2 min at 94°C; followed by 30 cycles of 30 s at 94°C, 45 s at 44°C, and 2 min at 72°C; with a final 15 min extension at 72°C.

The amplification products in both cases (RAPD and ISSR) were size-separated by standard horizontal electrophoresis in 1.5% agarose (Sigma) gels and stained with ethidium bromide.

Table (1): Codes and sequences of RAPD and ISSR primers used in the study.

RAPD code	Sequence	ISSR code	Sequence
A01	5' CAG GCC CTT C 3'	7	5' $[CT]_8$RG 3'
A02	5' TGC CGA GCT G 3'	814	5' $[CT]_8$TC 3'
A03	5' AGT CAG CCA C 3'	844	5' $[CT]_8$RC 3'
B01	5' GTT TCG CTC C 3'	898	5' $[CA]_6$RY 3'
B02	5' TGA TCC CTG G 3'	899	5' $[AG]_7$YC 3'
B03	5' CAT CCC CCT G3'	JOHN	5' $[AG]_7$YC 3'
C01	5' TTC GAG CCA G 3'	DAT	5' $[GA]_7$RG 3'
C02	5' GTG AGG CGT C 3'	TERRY	5' $[GTG]_4$RC 3'
C03	5' GGG GGT CTT T 3'	MAO	5' $[CTC]_5$RC 3'
D01	5' ACC GCG AAG G 3'		
D02	5' GGA CCC AAC C 3'		
D03	5' GTC GCC GTC A3'		

Data analysis:

All gels were analyzed using Total Lab program V2.01. These data were analyzed as diploid data for Dominant markers by standard POPGENE program (version 1.32), for population genetic analysis (Yeh and Boyle,1997).

3.Results

I-Analysis of genetic signature based on RAPD markers:

The number of detected fragments (ranged from 6 to 19 bands), number of polymorphic fragments (ranged from 6 to 19 bands) and ranges of fragment sizes generated by each primer were scored (Table 2).

1-Genetic variations within each *Plectropomus* species:

Most of detected RAPD bands were polymorphic. The percentages of polymorphic fragments were 61.01%, 37.74% and 11.95% in *P. maculates*, *P. leopardus* and *P. areolatus* respectively (Table 3).

Our results presented 23 species-specific RAPD markers. Four of them were detected in *Plectropomus* ***maculates*** using primers B02, (321bp and 85bp), C01 (442bp) and D01 (229bp). Regarding *Plectropomus leopardus*, five specific RAPD markers were identified at molecular sizes 410 bp (primer A02), 194 bp (primer B03), 996 bp & 296 bp (primer C01) and 221bp (primer D02). 14 RAPD markers were specific to *Plectropomus areolatus* at molecular sizes 610 bp, 404 bp, 200 bp (primer A01), 829 bp, 283 bp (primer A02), 430 bp, 232 bp (primer A03), 352 bp, 266 bp, 186 bp, 108bp (primer B01), 977bp, 106bp (primer B02) and 211bp (primer C01).

The mean of Actu**al** number of alleles (na), Effective number of alleles (ne), Nei's gene diversity (h) and Shannon's Information index (I) within each applied fish species were calculated (Table 3). *Plectropomus maculates* had the highest values for the previous estimates.

2-Genetic distance among applied *Plectropomus* species:

The genetic distance values among the applied fish species were calculated. The lengths among applied fish species, and nodes on the phylogenetic tree were presented in Table (4). The genetic distance values were 0.1685, 0.4265 and 0.399 between (*P. maculates* and *P. leopardus*), (*P. maculates* and *P. areolatus*) and (*P. leopardus* and *P. areolatus*) fish pairs, respectively (Table5). The dendrogram revealed genetic relationships among the applied fish species based on RAPD polymorphism was presented in Figure (1-a).

II- Analysis of genetic signature based on ISSR markers:

Generally, all studied loci were polymorphic. The molecular weight of each detected band was estimated. The ranges of these molecular sizes were presented in Table (Table 2).

All the nine studied loci, were informative in detecting the genetic signature of applied fish species (Table 2).

1-Genetic variations within applied fish species:

The actual number of alleles (na), effective number of alleles (ne) and Shannon's information index (I) were calculated within each estimated fish species and for all studied fish species for all primers. The mean of actual number of alleles (na), Effective number of alleles (ne), *N*ei's gene diversity (h) and Shannon's Information index (I) values were presented in Table (3). *P. areolatus* had the highest ne (1.146), h (0.087) and I (0.131) values relatively.

Table (2): Locus code, detected bands, polymorphic alleles (bands), and range of fragment generated by RAPD and ISSR markers.

RAPD				ISSR			
Code	DB	PB	R FS (Bb)	Code	DB	PB	R FS (Bp)
A01	17	16	924-122	7	32	32	1315-99
A02	15	15	859-114	814	23	23	1038-79
A03	14	14	775-114	844	17	17	570-120
B01	16	16	1005-108	JOHN	40	40	909-75
B02	9	9	977-106	899	12	12	785-111
B03	10	10	989-850	DAT	7	7	761-208
C01	12	12	1012-211	MANNY	40	40	874-67
C02	6	6	302-99	TERRY	27	27	524-99
C03	10	10	1100-234	MAO	25	25	855-109
D01	17	17	1356-135				
D02	19	19	656-155				
D03	14	14	1353-108				

DB= number of detected bands, PB= polymorphic bands and R FS= range of fragment size.

Table (3): Mean ±SE of Actual number of alleles (na), Effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I) and percentage of polymorphic loci (%PL) for each studied fish species.

	RAPD				ISSR			
	P.m	P.l	P.a	Total	P.m	P.l	P.a	Total
na	1.61 ±0.48	1.37 ±0.486	1.119 ±0.322	1.981 ±0.136	1.313 ±0.46	1.336 ±0.47	1.251 ±0.4346	1.995 ± 0.067
ne	1.35 ±0.40	1.23 ±0.370	1.085 ± 0.25	1.46 ± 0.33	1.127 ±0.26	1.128 ±0.25	1.146 ±0.293	1.302 ± 0.318
h	0.20 ±0.20	0.133 ± 0.197	0.047 ±0.135	0.28 ±0.164	0.07 ±0.14	0.08 ±0.14	0.087 ±0.16	0.19 ±0.16
I	0.302 ±0.29	0.198 ±0.28	0.0691 ±0.193	0.433 ±0.216	0.124 ±0.217	0.129 ±0.214	0.131 ±0.237	0.31 ±0.219
%PL	61.01 %	37.74 %	11.95 %	98,11%	31.39 %	33.63 %	25.11 %	99.55

P.m=*P.maculates*,.P.l=*P.leopardus.and*.P.a=*P.areolatus*

2-Genetic distance among applied fish species:

The genetic distance values among the applied fish species were calculated. The lengths among applied fish species, and nodes on the phylogenetic tree were presented in Table (4).

The genetic distance values were 0.1461, 0.3007 and 0.3729 between (*P. maculates* and *P. leopardus*), (*P. maculates* and *P. areolatus*) and (*P. leopardus* and *P. areolatus*) fish pairs, respectively (Table 5). The dendrogram revealed genetic relationships among the applied fish species based on ISSR polymorphism was presented in Figure (1-b).

Table (4): The lengths among applied fish species and nodes on the phylogenetic tree (based on RAPD and ISSR)

Based on RAPD			Based on ISSR		
Between	And	Length	Between	And	Length
2	1	12.31	1	1	12.21
1	*P. maculates*	7.94	*P. maculates*	*P. maculates*	8.42
1	*P. leopardus*	7.94	*P. leopardus*	*P. leopardus*	8.42
2	*P. areolatus*	20.25	*P. areolatus*	*P. areolatus*	20.62

Table (5): Genetic distance based on ISSR (lower half) and RAPD markers (upper half) among the three *Plectropomus* species

	P.maculates	***P.leopardus***	***P.areolatus***
P.maculates		0.1685	0.4265
P.leopardus	0.1461		0.3990
P.areolatus	0.3007	0.3729	

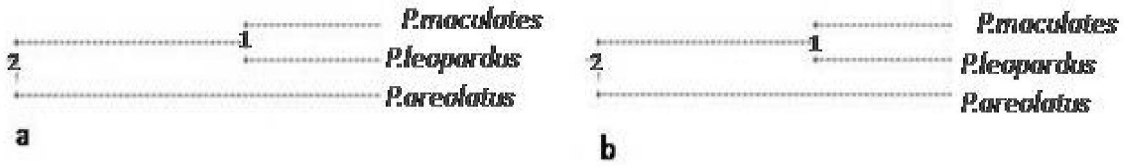

Figure (1): Reconstruction of phylogenetic relationships among the applied fish species based on RAPD (a) and ISSR (b) polymorphism.

4.Discussion

The capacity to conserve and to use natural resources wisely requires identifying taxa and variation at both the individual and/or population levels (Rashed *et al.,* 2008 and Saad *et al*., 2011).

Conservation or good management of aquatic genetic resources should ideally involve a continuum of activities. These activities are documentation, characterization, evaluation and utilization of aquatic genetic resources (Eknath, 1994).

In the present study, three *Plectropomus* species (*P.maculates, P.leopardus* and *P.areolatus*) were identified using two molecular techniques (RAPD and ISSR) to detect genetic signature of these economic fish. RAPD and ISSR were chosen because they are cheaper, simple, and fast techniques for detecting genetic polymorphism at a molecular level. In addition, just only one primer could obtain the different profiles for genomic analysis (Rina *et al.,* 2001 and Antunes, *et al.,* 2010).

In the present study, most detected DNA markers were polymorphic reflecting a rich allelic diversity in the applied fish species. So the primers of these loci are recommended to detect the genetic polymorphism and inferring the genetic signature for the applied *Plectropomus* species.

A comparison of RAPD and ISSR patterns in *Plectropomus* species samples, ISSR primers generate higher polymorphism (99.55%) than those generated by RAPD primers (98.11%). It could provide simple and convenient method to discriminate genetic variation of *Plectropomus* species.

It is necessary to estimate intra and inter population variations and phylogenetic relationships among fish genomes (Saad *et al*., 2011) to help the breeder in designing suitable breeding programs for fish improving and/or conservation. In addition, this will be useful in detecting any genetic contamination in these fish genomes.

In the present study the *P.areolatus* was distantly related from both *P.maculates, P.leopardus.* The genetic distance values among the applied fish species were calculated for determining genetic dissimilarity among them. Determining true genetic dissimilarity between fish individuals based on molecular marker analysis is a decisive point to cluster and analyzing diversity within and among fish species because different dissimilarity indices may yield conflicting outcomes.

Our results presented some species-specific RAPD and ISSR markers. These markers could be used in different two ways. In the first way these

markers will be used, as a species genetic signature. Regarding the second way, these markers are useful as marker assisted selection (Rashed *et al*., 2009) in breeding (to develop local fish breeds) and restocking programs.

The species-specific PCR method could be potentially used by regulatory agencies as routine control assay for the commercial fish production.

PCR-based methods commonly used for fish species identification include PCR-sequencing, random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR). Those are simple, specific and sensate methods for genetic characterization (Asensio *et al.,* 2009).

Restocking and stock enhancement programs are now recognized as an important tool for the management of fishery resources. It is important, however, to have an adequate knowledge on the genetic population structure of both the released stock and the wild population before carrying out such programs (Pereira *et al.,* 2010). So, the genetic markers should be conducted to provide the information needed for a sound management of this an economic aquatic resources in wild fish stocks and/or farms (Saad *et al*., 2011). In addition, development of local *Plectropomus* species breeds is important because many of them will be resilient to climatic stress and represent a unique source of genes for improving *Plectropomus* species production and conservation in the future.

Detection and analysis of genetic signature for *Plectropomus* species is an important for studying the fish genetic fragmentation. Fish fragmentation into subpopulations revealing groups that is genetically different from main source.

To date, very few studies have reported and/or used Nei's gene diversity and Shannon's based on RAPD and ISSR markers in *Plectropomus* species in the Red sea. The N*ei'*s gene diversity (h) and Shannon's information index (I) estimates were calculated in the present study to detect genetic diversity within applied fish species. They were slightly different. Based on RAPD results, both of them were higher than in the case of results revealed from ISSR. Shannon's information index has general applications in ecology and is relatively insensitive to the skewing effects caused by the inability to detect heterozygous loci (Dawson *et al*., 1995). To avoid the different approaches for its calculation we adopted the approach employed by Yeh and Boyle (1997).

Our results will be useful in studying the *Plectropomus* sub-species genetic structure because the genetic structure of *Plectropomus* sub-species and populations are not fully maximized and unclear. Generally, genetic diversity which is required for populations to be more adaptive with the environmental changes can be measured using an array of molecular methods (Rashed *et al.,* 2008). The present study showed that, both RAPD and ISSR primers were informative in detecting species specific DNA markers.

Conclusion:

In our study, RAPD and ISSR markers proved to be powerful methods for the detection of genetic signature for *Plectropomus* species. The future of *Plectropomus* species conservation will rely on development of sound management techniques such as RAPD and ISSR to characterize and monitor *Plectropomus* genetic resources. This will increase our knowledge of the genetic characteristics of each fish genetic resource and to examine the effects of management practices on the gene pools of these fish species.

The detected of genetic signature using RAPD and ISSR techniques will assist in the definition of appropriate units for fish conservation thus providing a restricted focus for good management of *Plectropomus* genetic resources.

Acknowledgment

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, KSA, under grant no. (509/ 130/ 1432H). The authors, therefore, acknowledge with thanks (DSR) technical and financial support.

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10/11/2012