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Original article

Assessment of genetic diversity and phylogenetic relationship among grouper species *Epinephelus* spp. from the Saudi waters of the Arabian Gulf

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ABSTRACT

Understanding of fish genetic characterization plays a vital role in the conservation and utilization of fish genetic resources of grouper species. The present study was carried out to assess the genetic diversity and phylogenetic relationships in five grouper species, *Epinephelus* spp. from eastern Saudi Arabian coast using two molecular marker systems, inter simple sequence repeat (ISSR) and microsatellite (SSR) markers. In total, 219 individuals grouper specimens (*Epinephelus tauvina, E. coioides, E. bleekeri, E. malabaricus, and E. areolatus*) were genotyped with 10 ISSR and 11 SSR selected primers. The ISSR produced 94 DNA fragments, of which 44 were polymorphic with an average of 2.13 fragment per primer. While SSR primers generated 107 alleles, all of them were polymorphic with an average 9.72 per primer. ISSR and SSR techniques demonstrated a high level of gene diversity and genetic distances illustrated by UPGMA dendrograms among the grouper species. The results proved that the SSR markers were highly informative and efficient in detecting genetic variability and relationships of the *Epinephelus* spp.

1. Introduction

Grouper is a member of the sub-family *Epinephelinae* (family, *Serranidae*). There are about 159 species of groupers in 15 genera (Koedprang, et al., 2007). They are found in the tropical and subtropical waters of all oceans (Tupper and Sheriff, 2008). Grouper is a commercially important marine fish species due to its desirable taste and high market demand in many parts of the world as well as in Saudi Arabia. Groupers are the best fish species for intensive aquaculture because of its fast growth; undertake environmental stress and efficient feed conversion (Craig and Hastings, 2007). Many grouper species *Epinephelus* spp. distributed in the Red Sea and Arabian Gulf (Priest, et al., 2016). The most important grouper species for both capture and aquaculture are orange-spotted grouper *Epinephelus coioides*, greasy grouper *E. tauvina*, king grouper or Malabar grouper *E. malabaricus*, giant grouper *E. lanceolatus*, potato grouper *E. tukula* and longfin grouper *E. quoyanus* (Wang, et al., 2011). The *E. tauvina* known in the Eastern Saudi Arabia as Arabian grouper. The lack of genetic diversity information about grouper species in the Arab Gulf especially, on the eastern Saudi coast magnifies the difficulty of selecting elite species in grouper breeding programs.

The grouper has problems such as determining the exact classification of the species of grouper where the morphological traits of the species overlap, so it is difficult to classify the species. For example, *E. coioides* is often confused with *E. tauvina,* and often mistaken with *E. malabaricus* (Rimmer and Glamuzina, 2019). In addition, the grouper is a protogynous hermaphrodite, which begins its sex life as a female and then change sex into male. Due to grouper sex traits in nature, catch of mature male is difficult and it is the most significant limitation to artificial larvae production of grouper (Oh, et al., 2013; An, et al., 2014). Furthermore, genetic diversity decreased among the solitary and non-social fish species, especially grouper, because of overfishing, marine pollution, and habitat destruction (Martinez, et al., 2018).

Knowledge of the population and subpopulation (stock) structure is important for conservation, fishery management, and fish

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breeding programs development (Grandcourt, et al., 2005). Molecular marker techniques are commonly used for fish stock characterization (Cuéllar-Pinzón, et al., 2016). The identification, genetic diversity and estimate of genetic variation among grouper species have been reported by many scientists who used PCR based methods, including microsatellite (Antoro, et al., 2006; Koedprang, et al., 2007; Wang, et al., 2011; Yang, et al., 2011; An, et al., 2014; Vaini, et al., 2019), mitochondrial DNA (Maggio, et al., 2005; Jackson, et al., 2014; JEFRI, et al., 2015; Ketchum, et al., 2016; Galal-Khallaf, et al., 2019), random amplified polymorphic DNA (RAPD) (Govindaraju and Jayasankar, 2004; Noikotr, et al., 2013; Roy, et al., 2014) and inter simple sequence repeat (ISSR) (Chiu, et al., 2012). Therefore, the current investigation aimed to determine genetic relationship and diversity among five *Epinephelus* spp. in the eastern Saudi Arabian coast using ISSR and SSR markers and to construct a molecular fingerprint database.

2. Materials and methods

2.1. Sampling

All investigations in this study were approved by the Institutional Animal Care and Use Committee of King Faisal University (KSA). All specimens were purchased from local fishermen, hence no specific licenses were required for the described field sampling as the fishermen were required to react with local laws regarding fishing. The species sampled are not protected or endangered. Two hundred and nineteen samples of multiple species of grouper *Epinephelus* spp. were collected from commercial landings in four locations along the eastern Saudi Arabia coast: Khafji (28.4257°N, 48.4887°E), Al-Jubail (26.9598°N, 49.5687°E), Al-Qatif (26.5765°N, 49.9982°E), and Salwa (24.76 10°N 50.7452°E) (Fig. 1). All the specimens were collected within three months to avoid errors due to seasonal variation. The samples were identified based on morphological characters following (Heemstra and Randall, 1993; Craig, et al., 2011; Allen and Erdmann, 2012) and were photographed (Fig. 2). Grouper species examined were *Epinephelus tauvina* (number = 46, and standard length = 276–334 mm), *E. coioides* (n = 67, and SL = 281–369 mm), *E. bleekeri* (n = 35, and SL = 355–371 mm), *E. malabaricus* (n = 38, and SL = 390–422 mm), and *E. areolatus* (n = 33, and SL = 263–349 mm).

2.2. DNA extraction and quantification

Fins were used for DNA extraction. A small piece of caudal fin samples was clipped (approximately 500–1000 mg), and preserved in 100% ethanol which was then stored at −20 °C. Genomic DNA was obtained through the DNeasy Tissue Kit (Qiagen, Hilden, Germany) using the manufactureŕs protocols. The DNA samples were checked for quantity and quality by spectrophotometer and 1% agarose gel electrophoresis, respectively. The DNA samples were diluted to 20 ng/μL then suspended in TE buffer (50 mM Tris-HCL; 10 mM EDTA) and then stored at 4 °C until used for PCR amplification.

2.3. ISSR electrophoresis and profile analysis

Out of the 30 ISSR primers (the University of British Columbia - UBC) screened, only ten primers giving most polymorphic, reproducible and clear fingerprints were selected for *Epinephelus* species analyses (Table 1). ISSR-PCR reactions were carried out in thermocycler GeneAmp® PCR System 9700. The amplification was per-

Fig. 1. Map of the eastern of Saudi Arabia coast showing the sample sites.

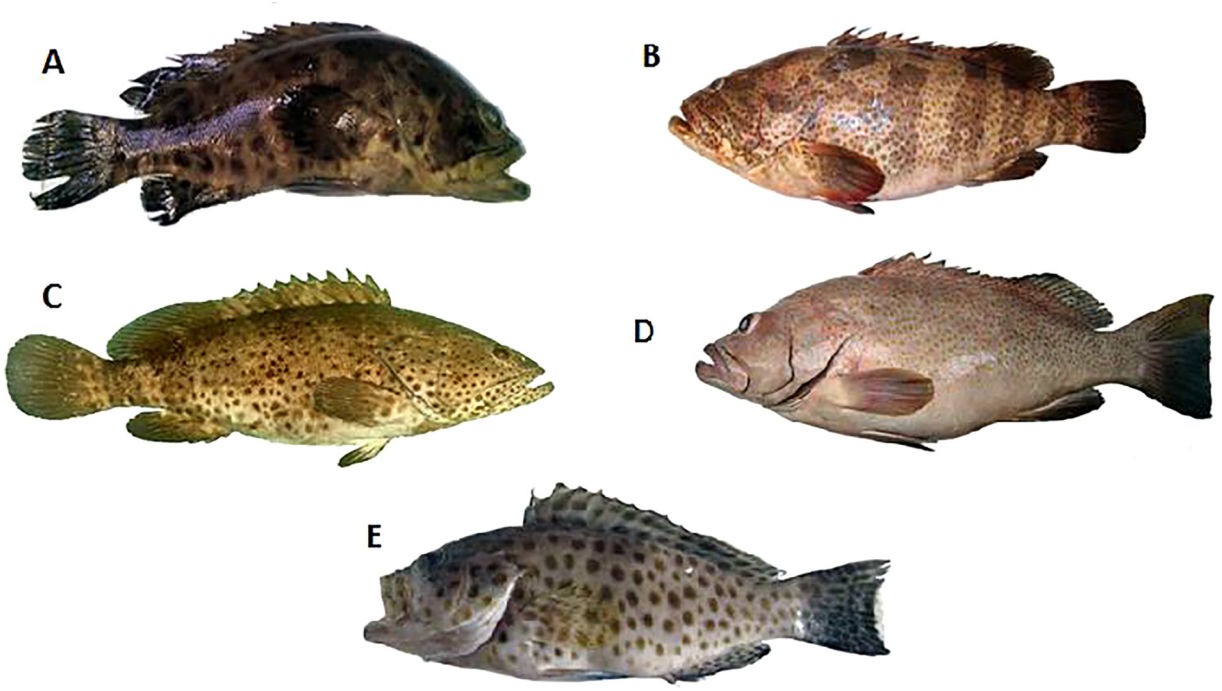

Fig. 2. The five grouper species collected from eastern of Saudi Arabia coast: (A) *E. tauvina*, (B) *E. coioides*, (C) *E. malabaricus*, (D) *E. bleekeri*, and (E) *E. areolatus*.

Table 1
List of ISSR primers and number of polymorphic/number of bands generated per primer within five *Epinephelus* species.

Primer	Nucleotide sequence	AT	*E. tauvina*	*E. coioides*	*E. bleekeri*	*E. Malabaricus*	*E. areolatus*	Overall species
			Number of polymorphic/number of bands					
UBC807	AGAGAGAGAGAGAGAGT	48 °C	2/9	2/8	2/9	4/10	0/9	4/11
UBC812	GAGAGAGAGAGAGAGAA	51 °C	2/8	2/8	2/8	3/8	2/8	4/9
UBC815	CTCTCTCTCTCTCTCTG	53 °C	4/10	3/11	3/9	3/8	3/9	5/10
UBC816	CACACACACACACACAT	48 °C	0/8	2/9	2/8	2/8	2/8	3/8
UBC822	TCTCTCTCTCTCTCTCA	48 °C	3/9	3/10	3/9	3/9	3/9	4/10
UBC826	ACACACACACACACACC	53 °C	3/7	2/7	2/7	2/8	2/7	4/8
UBC828	TGTGTGTGTGTGTGTGA	50 °C	5/11	4/10	2/9	4/9	2/9	6/12
UBC835	AGAGAGAGAGAGAGAGYC	51 °C	3/7	3/7	3/7	3/7	2/7	5/8
UBC874	CCCTCC CTCCCTCCCT	50 °C	2/5	1/4	2/7	3/5	3/7	4/8
UBC880	GGAGAGGAGAGGAGA	55 °C	4/8	4/9	3/9	3/8	3/9	5/10
	Total		28/82	26/83	24/82	30/80	22/82	44/94
	% of polymorphism		34.15	31.32	29.26	37.50	26.82	46.8
Average number of polymorphic bands per primer			2.92	3.19	3.41	2.66	3.72	2.13

AT, Annealing temperature.

Table 2
Estimates of ISSR variation in *Epinephelus* species.

	E. tauvina	*E. coioides*	*E. bleekeri*	*E. malabaricus*	*E. areolatus*	Across species	P-values
Na ± SD	1.341 ± 0.053	1.317 ± 0.059	1.293 ± 0.047	1.378 ± 0.051	1.268 ± 0.041	1.420 ± 0.038	*
Ne ± SD	1.267 ± 0.042	1.234 ± 0.039	1.215 ± 0.028	1.285 ± 0.036	1.193 ± 0.042	1.239 ± 0.018	*
I ± SD	0.213 ± 0.033	0.194 ± 0.034	0.178 ± 0.037	0.233 ± 0.049	0.162 ± 0.035	0.216 ± 0.017	**
He ± SD	0.148 ± 0.023	0.133 ± 0.018	0.123 ± 0.026	0.161 ± 0.023	0.111 ± 0.027	0.143 ± 0.010	**
Co-efficient of genetic differentiation (GST)						0.407	***
Gene flow (Nm)						0.911	

Observed number of alleles (Na), effective number of alleles (Ne), Shannon's Index (I), heterozygosity (He) and standard deviation.
* $P < 0.05$.
** $P < 0.001$.
*** $P < 0.0001$.

formed in 25 μl, containing 2.5 μl template DNA (50 ng); 1X PCR buffer with Mgcl2; 0.5 μl (200 mM each) dNTP mix; 0.5 μl (5unit/μl) Taq polymerase (Promega); 1 μl (20 pM) primer; and Millipore water to volume. ISSR-PCR was carried out in an initial denaturation for 3 min at 94° C, then 35 cycles: 45 s at 94 °C, 45 s at 50–56 °C depended on the primers base composition, 45 s at 72 °C followed by final extension at 72 °C for 7 min. PCR products were electrophoresed by running on 1.5% agarose gel stained with ethidium bromide (0.5-μg mL^{-1}). PCR band size was estimated by 100 base pairs (bp) molecular size ladder (Promega, USA). Then

gels were photographed by the Gel Doc (TM) XY System (Bio-Rad, Hercules, USA).

2.4. Microsatellite amplification and genotyping

Out of the fifteen microsatellite markers tested, only eleven primers gave good PCR results. Microsatellite markers were selected from the GenBank database (http//:www.ncbi.nlm.nih.gov/) for genetic analyses. The sequence of eleven pairs of microsatellite primers listed in Table 3. PCR reactions were carried out in thermocycler GeneAmp® PCR System 9700. The amplification was carried out in 25 μl volume with 1 × PCR buffer (Promega, Madison, WI, USA), 250 mM dNTPs, 250 nM each primer, 2.25mMMgcl2, 50 ng DNA and 0.25U Taq polymerase (Promega, USA). The following PCR conditions were used pre-denaturation for 3 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 55–56 °C, 60 s at 72 °C, and final extension step at 72 °C for 7 min. The PCR product (3 μl) was loaded onto a 7% polyacrylamide gel electrophoresis (PAGE). The gels were visualized using silver nitrate staining (Promega Kit) of the polyacrylamide gel.

2.5. Statistical analyses for ISSR/SSR markers

From gel photographs, DNA bands produced by ISSR profile was scored for presence (1) or absence (0) of a band (binary data). Each primer of the ISSR marker was referred to as an allele and ISSR matrix used for analysis. For the SSR data, The size of each allele per locus was estimated by the program Quantity with the standard DNA ladder marker pBR322 DNA/MspI (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China) was loaded. The measurement of genetic diversity parameters (percentage of polymorphism (%P), observed number of alleles (Na), effective number of alleles (Ne), Shannon's Index (I), and heterozygosity (He)) in the five grouper species were estimated employing the software POPGENE version 1.31 (Yeh, et al., 1999). The degree of genetic differentiation for ISSR data was figured by a coefficient of genetic differentiation (G_{ST}). POPGENE version 1.31 (Yeh, et al., 1999), was employed for analysis of microsatellites data: allele frequencies of all loci, number of alleles, observed and expected heterozygosity, and the Hardy–Weinberg equilibrium. Also, F-Statistics, the genetic differentiation index between grouper species (genetic distance, F_{ST}), inbreeding coefficient of an individual relative to the grouper species (within inbreeding, FIS), and inbreeding coefficient of an individual relative to the total species (total inbreeding, F_{IT}) were estimated using GenAlEx Version 6.5 (Peakall and Smouse, 2012). Two genetic distance matrices for ISSR and SSR markers were established between grouper species based on Nei's coefficients (Nei, 1978), in GenAlEx 6.4 (Peakall and Smouse, 2012) and thereafter used to create dendrograms based on UPGMA clustering algorithm implemented in NTSYSpc 2.2 (Rohlf, 2000). The cophenetic correlation test was carried out for calculating the correlation between each of the distance matrices and its corresponding dendrogram by Mantel's test (Mantel, 1967) with 1000 replications using NTSYSpc 2.2 (Rohlf, 2000). Correlation coefficients between ISSR and SSR systems were also calculated using Mantel's test with the same software.

3. Results

3.1. ISSR analysis

The total number of fragments that were detected consistently for the overall five *Epinephelus* species based on ten decamer primers were 94, out of which 44 fragments were found to be polymorphic (46.8%). The product size ranged from 500 to 3000 bp. (Table 1). The number of bands generated per primer varied from 8 to 11. With these ten primers, each primer in *Epinephelus areolatus, E. bleekeri, E. coioides, E. tauvina, and E. malabaricus,* generated on average 3.72, 3.41, 3.19, 2.92 and 3.66 polymorphic bands respectively. For overall species, the average number of polymorphic band per

primer was 2.13. The proportion of polymorphism detected was lower for *E. areolatus* (26.82%) compared with that of *E. malabaricus* (37.50%). A total of 82, 83, and 82 amplified bands were produced from *E. tauvina*, *E. coioides* and *E. bleekeri* species of which 28, 26, and 24 bands were polymorphic (34.15%, 31.32%, and 29.26% respectively) (Table 1). The heterozygosity (He) ranged between 0.111 ± 0.213 (*E. areolatus*) to 0.148 ± 0.119 (*E. tauvina*). The *E. coioides, E. bleekeri and E. malabaricus* showed a heterozygosity of 0.133, 0.123 and 0.161 respectively. For overall species, effective number of alleles (Ne), Shannon index (I) and the heterozygosity (He) were 1.239 ± 0.289, 0.216 ± 0.190 and 0.143 ± 0.171 respec-

Table 3
Sequence of eleven pairs of microsatellite primers, number of alleles and fixation indices (F_{ST}, F_{IS}, and F_{IT}) at each locus over five grouper species.

Primers	GenBank no.	Primer sequence (5′-3′)	Tann (∘C)	Size range (bp)	Alleles Number	F_{ST}	F_{IS}	F_{IT}
Ec_3	GQ267993	F: GTTTCAGGGTCTCTTCAGCC R: CCCACAGTGAGGGATTAGAT	56	164–188	8	0.252	0.072	0.021
Ec_121	GQ267996	F: GACTGAATTTGCAACTACTG R: CATCTCTTGCCTTCAACACAC	56	136–196	10	0.211	0.081	0.030
Ec_122	GQ267997	F: CATTCCTTAAAGTATTCTGTG R: CCACAGCCAGTCTAGGTATTC	55	158–210	10	0.343	0.168	0.040
Ec_124	GQ267999	F: GCTCTGACTAAGTCAGGTGAG R: GTTTAATTTGATTTGAGTAGAGC	56	122–148	12	0.189	0.152	0.100
Ec_125	GQ268000	F: CTGCTATATAAATAAAAACAATGC R: CAGTGATCTGAGCTGAGTTAG	56	174–240	9	0.201	0.059	0.090
Ec_131	GQ268001	F: CACTTGCGATCGTCATTACAG R: CCCGTCCCACACACACAC	56	122–186	7	0.221	0.138	0.110
Ec_154	GQ429007	F: AGCTGCTCAACAGGTTGTGTT R: CAAGTTCCATATGTGCTCTGACA	56	189–235	11	0.301	0.024	0.032
Ec_157	GQ429008	F: TGGAACAAGTTGGCATGGTA R: CAAATACAACACCCTAGATTTT	56	242–270	13	0.428	0.192	−0.002
Ec_158	GQ429009	F: TGAGAGACAGTGGAGCACAAA R: CGTGGTTACATTCTACCCCCTA	56	148–170	10	0.352	0.142	0.119
An-06	JN185627	F: GCTCGAAGATGAGCTGGAAG R: AAGGTGCTGCTCCTGCTTT	60	192–210	9	0.209	0.182	0.020
An-15	JN185636	F: CCTGTGTGTGAGCTGGAGAA R: GGTGGAGGAGTACGAAACCA	58	186–242	8	0.196	0.191	0.140
Mean	–	–	–	–	9.72	0.263	0.127	0.063

tively (Table 2). Co-efficient of genetic differentiation (G_{ST}) estimated based on the ISSR data was 0.407. The gene flow (Nm) value among grouper species *Epinephelus* spp. was 0.911. ISSR analysis revealed that the mean genetic distance (D) for the five species ranged from 0.101 between *E. bleekeri* and *E. areolatus* populations to 0.240 between *E. tauvina* and *E. areolatus* populations (Table 5). The cluster analysis of the ISSR data (Fig. 3) categorized the five *Epinephelus* spp. into three groups: Group one included the *E. tauvina* and *E. coioides*; the second was *E. bleekeri* and *E. areolatus* and the third was the *E. malabaricus.*

3.2. SSR analysis

Microsatellites diversity indices among and within grouper species studied are summarized in Table 3. and Table 4. A total number of 107 alleles were obtained using eleven microsatellites in two hundred and nineteen individuals, with all of them being polymorphic. Ec_157 was the most variable locus with 13 alleles, and Ec_131 (7 alleles) was the lowest variable locus (Table 3). The number of alleles ranged from 5 to 13 (average 8.09), 6 to 13 (average 8.36), 4 to 11 (average 7.36), 5 to 12 (average 8.00) and 6 to 10 (average 7.90) for *E. tauvina, E. coioides, E. bleekeri, E. malabaricus,* and *E. areolatus*, respectively (Table 4). The mean effective number of alleles per locus varied from 4.73 for Ec_3 to10.06 for Ec_157. The average observed and expected heterozygosity had values of 0.635 to 0.782 and 0.755 to 0.860, respectively. All grouper populations showed high expected heterozygosity within population with overall mean 0.819 (±0.032) which showed high levels of genetic diversity in grouper populations studied. No private alleles were detected for any grouper species studied using eleven SSR markers.

All populations deviated significantly from HWE at most of the microsatellite loci. F-statistics were determined in a fixation index as genetic differentiation (F_{ST}) per locus varied from 0.189 (Ec_124) to 0.428 (Ec_157), and the mean F_{ST} of 11 loci was 0.263, the global heterozygote deficit among five *Epinephelus* spp. (F_{IT}) value ranging from −0.002 to 0.140 with average 0.063 and the heterozygote deficit within the species/among species (F_{IS}) among the 11 SSR markers value ranged between 0.024 and 0.192 with average 0.127 (Table 3). Average F_{ST} value indicated that about 26.3% of total genetic variation corresponded to differences between populations, while the remaining 73.7% corresponded to differences among individuals within populations. The mean genetic distance based on SSR analysis, ranged from 0.125 between *E. bleekeri* and *E. malabaricus* populations to 0.220 between *E. areolatus* and *E. tauvina* populations (Table 5). Using the genetic distance parameter, the UPGMA dendrogram indicated that grouper species under study could be classified into three clearly distinct groups (Fig. 4). The first category included *E. tauvina* and *E. coioides*; the other two categories included *bleekeri* and the *E. malabaricus*, with *E. areolatus* being the third population.

The cophenetic correlation of ISSR and SSR were r = 0.842 and 0.815, respectively. There was no significant correlation between the distance and cophenetic matrices of the two marker systems, while the highly significant correlation coefficient of the two genetic matrices from the two marker systems was 0.869 ($p < 0.01$).

4. Discussion

Understanding of genetic diversity for grouper species is highly important, for fisheries and aquaculture improvement. Morphological traits are a simple method to study biodiversity. The various genera and species cataloging rely on morphological traits. However, the strategy of conservation and management of genetic resources of grouper species based on morphological traits alone is extremely limited because these traits are influenced by age and various environmental factors (Becker, et al., 2015; Hulley, et al., 2018). Thus, it is essential to estimate the genetic diversity of such grouper species by molecular marker techniques so that it can be improved and preserved (Cuéllar-Pinzón, et al., 2016). Molecular markers are modern genetic tools, giving polymorphism at the DNA level, which are used to improve knowledge on the population structure of fish resources (Vignal, et al., 2002; Liu and Cordes, 2004).

Number of alleles per population (A), effective number of alleles (Ae), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (Fis), and probability estimates regarding deviation from Hardy-Weinberg equilibrium (p).

This study gave the first detailed analysis of the level of genetic variation and genetic relationship among the genotypes of grouper species *Epinephelus* spp. on the Arabian Gulf coast of Saudi Arabia revealed by ISSR (dominant) and SSR (co-dominant) molecular markers that have been proven to be valuable for the determination of genetic diversity and taxonomic discrimination of the species (Kubota, et al., 2014; Bernard, et al., 2016; Hassanien and Al-Rashada, 2019; Oliveira, et al., 2019).

The ISSR-PCR technique was applied to identify genetic similarity and diversity in grouper species *Epinephelus* spp. using ten polymorphic primers. The percentage of polymorphism at intra-population in grouper species was relatively low (ranged between 26.82% and 37.50%), but was high (46.8%) in the overall population, compared to giant grouper *Epinephelus lanceolatus*. In the same sense, a total of 166 bands produced from 17 ISSR primers included 58 were polymorphic bands (34.9%) in the giant grouper (*E. lanceolatus*) (Chiu, et al., 2012). *E. malabaricus* had the highest percentage observed in polymorphism, recommending this technique to be used in breeding programs. In the present study, a relatively high inter-population genetic diversity was observed in *Epinephelus* spp. (G_{ST} = 0.407), reported that 40.7% of the total genetic variation was among grouper species and 39.3% was within the populations. The Shannon information index across species was 0.216, indicating that 21.6% of effective alleles were on average identified per primer. Average gene flow (Nm) during the current investigation was determined as 0.911 showing limited gene flow across grouper species. These data could be attributed to the continual seasonal migration of grouper species influenced by thermal variation of water temperature in the Arabian Gulf during different seasons. According to Muneer, et al., (2009) the species with limited gene flow have a common trend to form well-defined population. ISSR is a dominant marker that has high reproducibility, compared with RAPD marker in the present study. The observed data showed that ISSR analyses were found more informative than RAPD technique (Chiu, et al., 2012).

Microsatellite analysis has been proofed to offer the best estimation of genetic diversity (by producing a greater amount of polymorphism) and a better understanding of intra- and inter-specific variability among and within these *Epinephelus* spp. (Koedprang, et al., 2007; An, et al., 2014; Kubota, et al., 2014). In the current investigation, the average number of alleles (A = 9.72) was lower than that found in *E. coioides* populations using microsatellite markers (Ec_122, Ec_154, and Ec_158) (Wang, et al., 2011). There is a high level of genetic diversity in all grouper species studied based on the average expected heterozygosity value (0.819) while the genetic variation was relatively low for *E. coioides* populations which collected from Thailand and Indonesia revealed by microsatellites (Antoro, et al., 2006). In addition, heterozygote deficiency was noted, as shown by the lower average value (0.719) compared to expected heterozygosity, as well as the positive value of F_{IS} at almost all the loci (Table 4). These results were disagreeing with the estimation of genetic dif-

Table 4
Microsatellites diversity indices for grouper species.

Locus		*E. tauvina*	*E. coioides*	*E. bleekeri*	*E. malabaricus*	*E. areolatus*	Mean
Ec_3	A	5	6	4	5	6	5.20
	Ae	4.96	5.00	4.00	4.40	5.30	4.73
	Ho	0.700	0.680	0.619	0.654	0.541	0.638
	He	0.863	0.845	0.659	0.777	0.634	0.755
	Fis	0.169	0.189	−0.131	0.126	−0.033	–
	P	0.001	0.000	0.314	0.005	0.002	–
Ec_121	A	8	7	9	8	8	8.00
	Ae	7.33	6.21	8.00	7.56	7.11	7.242
	Ho	0.706	0.780	0.763	0.717	0.690	0.731
	He	0.850	0.901	0.843	0.858	0.767	0.843
	Fis	0.121	0.010	0.132	0.049	−0.027	–
	P	0.021	0.053	0.006	0.010	0.035	–
Ec_122	A	8	9	7	9	10	8.6
	Ae	7.80	8.10	6.40	8.22	9.22	7.94
	Ho	0.789	0.813	0.743	0.711	0.811	0.773
	He	0.811	0.900	0.859	0.659	0.800	0.805
	Fis	0.129	0.118	0.212	0.184	0.234	–
	P	0.000	0.231	0.318	0.008	0.183	–
Ec_124	A	8	10	9	9	8	8.80
	Ae	7.67	9.34	8.24	8.56	7.00	8.16
	Ho	0.690	0.743	0.690	0.709	0.648	0.696
	He	0.759	0.871	0.801	0.810	0.790	0.806
	Fis	−0.030	−0.061	0.070	0.110	0.018	–
	P	0.020	0.013	0.018	0.009	0.043	–
Ec_125	A	9	9	7	9	8	8.40
	Ae	8.33	8.25	6.66	8.30	7.63	7.83
	Ho	0.732	0.771	0.720	0.751	0.729	0.740
	He	0.839	0.835	0.828	0.849	0.856	0.841
	Fis	0.232	0.178	0.290	0.229	0.210	–
	P	0.041	0.006	0.120	0.027	0.005	–
Ec_131	A	5	7	4	5	6	5.40
	Ae	4.28	6.47	3.21	4.50	5.34	4.76
	Ho	0.734	0.783	0.682	0.711	0.737	0.729
	He	0.863	0.885	0.739	0.833	0.848	0.833
	Fis	0.211	0.193	0.242	0.230	0.227	–
	P	0.038	0.145	0.091	0.004	0.087	–
Ec_154	A	9	8	11	11	10	9.8
	Ae	7.67	6.30	8.96	9.30	9.33	8.31
	Ho	0.750	0.831	0.795	0.694	0.841	0.782
	He	0.803	0.904	0.807	0.718	0.900	0.826
	Fis	0.149	0.134	0.174	0.160	0.195	–
	P	0.019	0.094	0.000	0.008	0.010	–
Ec_157	A	13	13	10	12	9	11.4
	Ae	11.22	11.67	8.33	10.44	8.67	10.06
	Ho	0.700	0.741	0.684	0.781	0.688	0.718
	He	0.893	0.844	0.823	0.910	0.757	0.845
	Fis	0.186	0.177	0.227	0.167	0.291	–
	P	0.211	0.164	0.000	0.000	0.110	–
Ec_158	A	7	8	6	8	8	7.4
	Ae	6.22	6.47	5.55	7.67	6.69	6.52
	Ho	0.750	0.642	0.769	0.714	0.680	0.711
	He	0.889	0.798	0.801	0.849	0.756	0.818
	Fis	0.144	0.161	0.221	0.176	0.210	–
	P	0.171	0.000	0.514	0.088	0.000	–
An-06	A	9	7	9	6	7	7.60
	Ae	8.00	6.39	8.22	5.37	6.14	6.82
	Ho	0.731	0.799	0.737	0.783	0.765	0.763
	He	0.856	0.874	0.890	0.809	0.874	0.860
	Fis	−0.023	0.069	0.170	0.125	−0.061	–
	P	0.004	0.086	0.156	0.128	0.067	–
An-15	A	8	8	5	6	7	6.80
	Ae	7.26	7.50	5.00	5.29	6.53	6.31
	Ho	0.683	0.629	0.597	0.603	0.667	0.635
	He	0.798	0.758	0.719	0.817	0.794	0.777
	Fis	0.211	0.291	0.077	0.191	0.068	–
	P	0.003	0.105	0.000	0.089	0.045	–
Mean							
	A	8.09	8.36	7.36	8.00	7.90	7.94
	Ae	7.34	7.42	6.59	7.23	7.17	7.15
	Ho	0.724	0.746	0.709	0.711	0.708	0.719
	±SE	±0.024	±0.019	±0.031	±0.017	±0.021	±0.022
	He	0.838	0.855	0.797	0.808	0.797	0.819
	±SE	±0.031	±0.011	±0.054	±0.037	±0.031	±0.032

Table 5
Nei's (1978) genetic distance between five *Epinephelus* species revealed by ten ISSR (above diagonal) and eleven microsatellite loci (below diagonal).

	Tauvina	*coioides*	*Bleekeri*	*Malabaricus*	*Areolatus*
Tauvina	0.00	0.117	0.226	0.119	0.240
Coioides	0.126	0.00	0.184	0.134	0.196
Bleekeri	0.156	0.128	0.00	0.209	0.101
Malabaricus	0.184	0.136	0.125	0.00	0.225
Areolatus	0.220	0.175	0.142	0.153	0.00

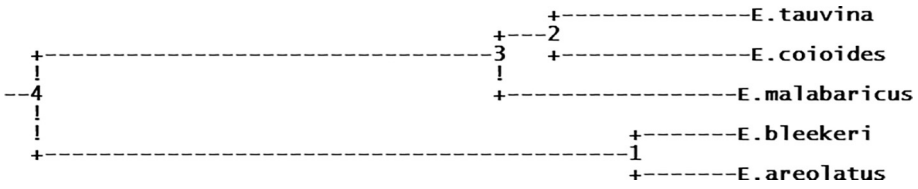

Fig. 3. UPGMA dendrogram of the five *Epinephelus* species based on genetic distances derived from ISSR data.

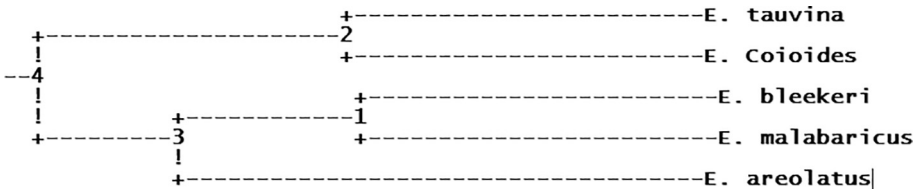

Fig. 4. UPGMA dendrogram of the five *Epinephelus* species based on genetic distances derived from SSR data.

ferentiation for eight grouper species based on microsatellites (Koedprang, et al., 2007). All microsatellite loci had significant differences ($P \leq 0.05$) from Hardy–Weinberg Equilibrium (HWE). The results indicate that the microsatellite technique produced a greater average estimation of alleles per locus compared to ISSR, suggesting a greater diversity of alleles in grouper species. The obtained data confirm that microsatellite markers are co-dominant in nature and help in identification of heterozygotes in the populations. No diagnostic alleles observed with eleven loci to genetic differentiation between five grouper species. The results confirm the importance of using another microsatellite or other molecular markers more accurately such as mitochondrial/nuclear markers. The estimation of genetic diversity and taxonomic status of grouper species in the United Arab Emirates revealed by mitochondrial DNA, indicated that there were three species of grouper (*Epinephelus coioides, E areolatus* and *E bleekeri*) that were thought to be one species (Ketchum, et al., 2016). In addition, the identification of Red Sea grouper species (*Serranidae*) in Egypt, proved that the data for mitochondrial DNA (COI) and nuclear gene (12srRNA) were an accurate tool for Egyptian Red Sea grouper species discrimination (Galal-Khallaf, et al., 2019).

The Mantel test was conducted on two marker systems to obtain reliable assessments of genetic distance among the grouper species investigated with their cophenetic correlation values. The cophenetic correlation for ISSR and SSR were r = 0.842 and 0.815 respectively, which indicated a good fit of the data for genetic diversity analysis. No significant correlation was found between the distance and cophenetic matrices of the two markers. The highly significant correlation coefficient estimated by the Mantel test of the two genetic matrices from ISSR and SSR systems was 0.869 ($p < 0.01$).

5. Conclusions

The present study identified the high profile of genetic diversity and genetic relationship of grouper species *Epinephelus* spp. such as *E. tauvina, E. coioides, E. bleekeri, E. malabaricus* and *E. areolatus* in the eastern Saudi Arabia coast. These results can have significant implications for the grouper in terms of fisheries conservation and genetic improvement programs. ISSR and SSR markers were useful tools for investigating genetic diversity and genetic structure of grouper species. The results indicated that the SSR molecular markers were better than ISSR molecular markers for the determination of the genetic diversity and genetic relationship between grouper species. More research is recommend for identification of grouper species based on more accurate molecular techniques like COI or other mitochondrial/nuclear markers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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