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Evaluation of DNA polymorphism in the Red Sea *Epinephelus* species using 12s rRNA and inter simple sequence repeats

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The true phylogenetic relation among *Epinephelus* species is under debate. The present investigation was designed for evaluation of DNA polymorphism in some Red sea *Epinephelus* species using 12s rRNA and Inter Simple Sequence repeats. DNA polymorphism values were detected and evaluated within all estimated fishes. Both applied techniques revealed that *E. malabaricus* is closely related to *E. summana*. The distance value between *E. chlorostigma* and *E. areolatus* is lower than the distance value between *E. chlorostigma* and *E. radiatus*. The Serranidae evolutionary variations were evaluated comparatively with other ray-finned fishes belonging to four fish genera (Carangidae, Labridae, Mugilidae and Cichlidae) based on 12s rRNA sequence variations (obtained from NCBI). The developed DNA markers were reliably branched in the evaluated fishes. More molecular investigations using more spatial and temporal fish samples should be carried out in the future for exploring the true *Epinephelus* species biodiversity in the Red sea.

[**Keywords**: DNA polymorphism, *Epinephelus*, Genetics, ISSR, 12s rRNA]

Introduction

Up to date, about 100 *Epinephelus* species (belongs to family Serranidae) were recognized in the world. Most of these economic aquatic biological resources were distributed in the Asian sea areas[1]. In some cases, due to the limitation of morphometric visibilities of *Epinephelus* species, these fishes have low characterization power. The accurate classification of these fishes is still unclear. In addition, the true phylogenetic relationship among these fishes is under debate. Some of *Epinephelus* species are facing the risk of extinction due to its long-life and late maturation. In addition, overfishing is considered the main factor for losing *Epinephelus* species, especially in the Red sea.

Up to date, the analysis of DNA markers is increasingly applied to resolve various problems in animal evolution and conservation[2]. Developing more informative DNA markers is undoubtedly still the best choice for evaluating the true genetic variations in fish evolutionary studies. Developing informative molecular markers[3] for future investigations of *Epinephelus* speciation will be effective for understanding the evolution in these fishes[1]. Evaluation of DNA polymorphism in such fish species (for detecting the informative molecular markers) will be constituted the basic principle for designing *Epinephelus* conservation strategies. Identification and evaluation of the informative molecular markers will facilitate the utilization of such markers in the conservation of these fish resources.

Due to its higher mutation rates compared to nuclear genes, the mitochondrial DNA has proved to be widely used in fish molecular characterization and evolution studies[4-6].

The inference the true evolutionary variations among and within fish species should be revealed from nuclear and mitochondrial DNA polymorphism.

A lot of molecular techniques were applied for detecting biodiversity and inferring the true evolutionary variations among fish genera and/or species[3,5,6]. The molecular markers revealed from such techniques were classified into dominant and co-dominant genetic markers.

Analysis of mitochondrial genes sequence variations was recommended for re-constructing the phylogenetic relations among fish species. Analysis of partial sequences for mitochondrial genes (such as12s rRNA and 16s r-RNA) was effective as molecular markers for exploring the evolution and diversification in salmonid species[7].

Recently, the analysis of complete mitochondrial genome sequences was applied for reconstructing the

phylogenetic relationships among different fish species[1,8,9]. Concerning *Epinephelus*, the complete mitochondrial genome (16894 bp) of *E. Chlorostigma* (from the south China sea) was sequenced for evaluating the molecular variations in this fish species comparatively with another grouper (*Epinephelus* and *Cephalopholis*) fishes[1]. They found that *E. Chlorostigma* was closely related to *E. areolatus*).

Analysis of informative dominant markers such as ISSR and Co-dominant markers such as DNA sequencing is recommended by many researchers for re-reconstructing the phylogenetic relationships and studying the evolution in fish species[1,10].

The development of informative systems including different nuclear DNA, mt-DNA and morphological characters at intra and inter *Epinephelus* species level will improve the classical identification and classification of these fish biological resources.

The present investigation was designed for the evaluation of DNA polymorphism in some Red Sea *Epinephelus* species using 12s rRNA and Inter Simple Sequence repeats systems.

Materials and Methods

Sample collection, DNA extraction and amplification

A total of six *Epinephelus* species were sampled from the natural habitats, Yanbu (Red sea port at western KSA). The collected fish species were *E. areolatus* (n = 15), *E. malabaricus* (n = 5), *E. summana* (n = 5), *E. radiatus* (n = 7), *E. tauvina* (n = 10) and *E. chlorostigma* (n = 7). Fish samples were placed on ice in the field and small pieces of caudal fins were preserved in 95 % ethanol for DNA extraction. Total DNA was extracted from caudal fins tissues from each fish sample[11].

ISSR analysis

Six ISSR primers were selected to estimate the molecular variations in the evaluated fish species. The ISSR primer sequences were 5' [GA]7-RG, 5' [CT]8RC 3', 5' [CA]6AC 3', 5' [CA]6GG 3', 5' [AG]7YC 3' and 5' [CT]8TC 3'.

PCR reactions were prepared in a 10 µl contained a 50 ng of DNA, a 0.3 µM of primer, a 0.2 mM of dNTPs, a 25 mM of $MgCl_2$, a 0.5 unit of Taq polymerase and a 1 X buffer and water up to 10 µl. The PCR program consisted of one cycle for 3 min at 94 °C, 40 cycles for (30 sec at 94 °C, 45 sec at 45 °C & 1 min at 72 °C) and one cycle for 15 min at 72 °C. The separated PCR products (1.7 % agarose gels) were photographed. Data were analyzed and presented. Some DNA polymorphism parameters within each evaluated species were detected to monitor the genetic diversity among applied fish species[3]. The data were analyzed, and the phylogenetic relation was re-constructed (based on *Nei's* genetic distances) among evaluated *Epinephelus* species using POPGENE (version 1.32)[12].

12s rRNA analysis

The mitochondrial 12s rRNA regions were amplified, identified and sequenced in this study. The primer pairs (53F:cacaaaggcttggtcctgacttt and 613R:tcggttctagaacaggctcctctag)[13] were used.

The PCR reactions were achieved (Promega, Madison, WI 53711-5399, USA) in a reaction volume containing 10 µl of 5X Green Go Taq reaction buffer, 1 µl of dNTP Mix, 10 mM each, 1 µl forward primer (F), 1 µl reverse primer (R), 0.25 µl Go Taq DNA polymerase (5u/µl), 1µl of template DNA (0.5 µg/50 µl) and water up to 50 µl. PCR amplification was achieved with denaturation for 3 min at 95 °C; 40 cycles of 95 °C for 30s, 56 °C for 40s, 72 °C for 90s, and a final extension at 72 °C for 10 min.

Out of the 49 fish samples only 38 12s rRNA fragments (around 547 bp) from *E. areolatus* (n = 8), *E. summana* (n = 7), *E. malabaricus* (n = 5), *E. radiatus* (n = 7), *E. tauvina* (n = 6) and *E. chlorostigma* (n = 5) were estimated. The resulted 12s rRNA fragments were purified using QIAGEN PCR purification kit. 12s rRNA fragments were ligated to pGEM-T TA cloning kit (Promega). The samples were introduced for sequencing (Macrogen Inc, Republic of Korea).

Analysis of DNA sequences

Some of the 12s rRNA fragment sequences were submitted to the National Center for Biotechnology Information (NCBI). The accession numbers for *E. areolatus* (KU877246), *E. summana* (KU877249), *E. malabaricus* (KU877247), *E. radiatus* (KU877252), *E. tauvina* (KU877248) and *E. chlorostigma* (KU877253) were registered in NCBI. The DNA polymorphisms were calculated using DNA sp. (Ver.5.10.01)[14].

The evolutionary variations among evaluated Mugilidae fish species were inferred using the Maximum Likelihood (ML) methods. This analysis was carried out using MEGA6[(ref. 15)].

Results and Discussion

Genetic polymorphism in the evaluated Red sea *Epinephelus* species using ISSR variations

A group of parameters was used to assess the genetic variations within each evaluated *Epinephelus* species. The percentage (65.22) of polymorphic loci, the actual number of alleles (1.65), the effective

Table 1 — The mean and standard deviation of observed number of alleles (n_a), effective number of alleles (n_e), gene diversity (*h*), Shannon's Information index (I), number of polymorphic loci (NP) and the percentage of polymorphic loci (% NP) of the evaluated Red Sea *Epinephelus* species

	na ± SD	ne ± SD	*h* ± SD	I ± SD	NP	% NP
eta_1	1.33 ± 0.4	1.15 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	23	33.3
era_2	1.34 ± 0.4	1.24 ± 0.3	0.13 ± 0.2	0.2 ± 0.2	24	34.7
ear_3	1.33 ± 0.4	1.23± 0.3	0.13 ± 0.19	0.19± 0.28	23	33.33
ech_4	1.31 ± 0.4	1.17 ± 0.33	0.10 ± 0.17	0.15 ± 0.25	22	31.88
ema_5	1.2 ± 0.4	1.15 ± 0.32	0.08 ± 0.17	0.12 ± 0.25	14	20.29
esu_6	1.05 ± 0.2	1.03 ± 0.16	0.02± 0.09	0.03 ± 0.13	4	5.8
Total	1.65 ± 04	1.34 ± 0.3	0.196± 0.20	0.29 ± 0.28	45	65.22

ear = Epinephelus areolatus, *esu = Epinephelus summana*, *ema = Epinephelus malabaricus*, *era =Epinephelus radiatus*, *eta = Epinephelus tauvina* and *ech = Epinephelus chlorostigma*

Table 2 — Genetic distance values among estimated *Epinephelus* species based on ISSR (above diagonal) and 12s rRNA consensus sequence (under diagonal) variations

species	eta	era	ear	ech	ema	esu
eta		0.1278	0.0980	0.0703	0.2702	0.2695
era	0.070		0.0534	0.1096	0.1806	0.1600
ear	0.085	0.108		0.0610	0.2026	0.1523
ech	0.082	0.102	0.082		0.2147	0.2229
ema	0.065	0.088	0.112	0.117		0.1199
esu	0.065	0.082	0.118	0.111	0.007	

ear = *E. areolatus*, *esu = E. summana*, *ema = E. malabaricus*, *era = E. radiatus*, *eta = E. tauvina* and *ech = E. chlorostigma*

number of alleles (1.34), *Nei's* gene diversity (0.196) and Shannon's information index (0.29) were averaged across all the evaluated *Epinephelus* species.

The numbers of bands (each band is considered as a dominant allele for the locus defined and designated by this allele) were variable among the estimated fish species. Results of the analyzed data showed that the percentage of polymorphic loci were 33.3, 34.7, 33.33, 31.88, 20.29 and 5.8 for *E. tauvina* (eta), *E. radiatus* (era), *E. areolatus* (ear), *E. chlorostigma* (ech), *E. malabaricus* (ema) and *E. summana* (esu) respectively (Table 1). The gene diversity (h) values were 0.1, 0.13, 0.13, 0.1, 0.08 and 0.02 in *E. tauvina* (eta), *E. radiatus* (era), *E. areolatus* (ear), *E. chlorostigma* (ech), *E. malabaricus* (ema) and *E. summana* (esu) fish species, respectively. The highest Shannon's information index value (0.2) was detected for both *E. tauvina* (eta) and *E. radiatus* (era). On the other hand, the lowest Shannon's information index value was calculated for (esu) fish species. The same number of polymorphic bands (23) was calculated for both *E. tauvina* (eta) and *E. areolatus* (ear). The highest percentage of polymorphic bands (34.7 %) was detected in *E. radiatus* (era) fish species. The number of polymorphic bands (NP) in *E. malabaricus* (ema) was higher than (NP) in *E. summana* (esu).

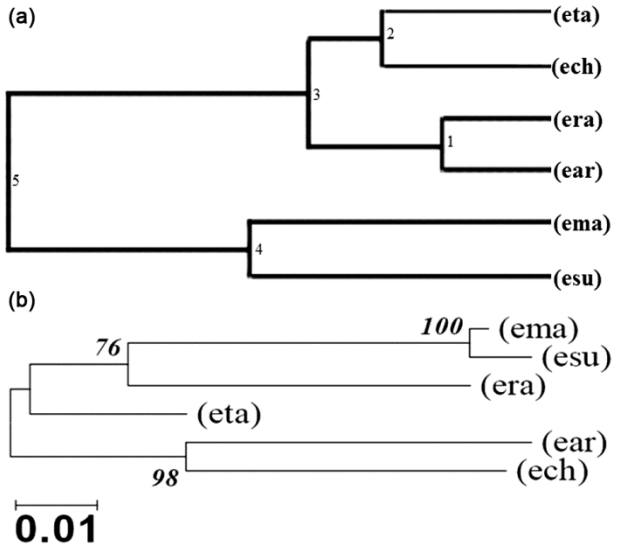

Fig. 1 — The phylogenetic relationships among the evaluated Red sea *Epinephelus* species based on ISSR: (a) and 12s rRNA consensus sequence, (b) variations ear = *E. areolatus*, esu = *E. summana*, ema = *E. malabaricus*, *era* = *E. radiatus*, eta = *E. tauvina* and ech = *E. chlorostigma*. In (a), the lengths between (5 and 3), (3 and 2), (2 and eta), (2 and ech), (3 and 1), (1 and era), (1 and ear), (5 and 4), (4 and ema), (4 and esu) were 5.49, 1.43, 3.51, 3.51, 2.28, 2.66, 2.66, 4.45, 5.99 and 5.99, respectively. Concerning (b), the values represented the bootstrapping values

The phylogenetic relations among the evaluated Red Sea *Epinephelus* species based on ISSR variations

Nei's genetic distance values among the evaluated Red Sea *Epinephelus* species were presented in Table 2. High genetic distance values were calculated between *E. tauvina* (eta) and both *E. malabaricus* (ema) and *E. summana* (esu) fishes. On the other hand, low genetic distance values were recorded between *E. tauvina* (eta) and both *E. areolatus* (ear) and *E. chlorostigma* (ech) fishes. The lowest distance value was recorded between *E. areolatus* (ear) and *E. radiatus* (era) fish species. All the previous observations were reflected by the dendrogram (Fig. 1a). The *E. chlorostigma* (ech) is

distantly related to both *E. malabaricus* (ema) and *E. summana* (esu). The distance between *E. summana* (esu) and *E. areolatus* (ear) is higher than the distance between *E. summana* (esu) and *E. malabaricus* (ema). This dendrogram represents the inferred phylogenetic relationships among the estimated Red sea *Epinephelus* species.

Genetic polymorphism in the evaluated Red sea *Epinephelus* species using 12s rRNA sequence variations

The total number of analyzed sites (412), the averages of nucleotide compositions (T = 20.5, C = 25.6, A = 31.7 and G = 22.2), the sequence conservation value (Sc = 0.820), conservation threshold (CT = 0.8), the average values of GC (0.477), GC2 (0.471), GC3 (0.558), single nucleotide polymorphism (SNPs = 71), Parsimony informative sites (PIS = 59), estimates of the haplotype diversity (hd = 0.859), Nucleotide diversity (Pi = 0.067), theta from polymorphic sites (Θ = 0.046), the average number of nucleotide differences (k = 27.582) were calculated for all evaluated 12s rRNA sequences. Some fragment sequences were submitted to the NCBI (based on haplotype diversity).

The phylogenetic relations among the evaluated *Epinephelus* species based on 12s rRNA sequence variations

The phylogenetic relations among the evaluated fish species was reconstructed using the Maximum Likelihood (Fig. 1b). All identified Red sea fish species can be differentiated by 12s rRNA gene sequence analysis. The relationships among evaluated fishes on the phylogenetic trees are reflected by the distance values presented in Table 2.

The lowest distance value was detected between *E. malabaricus* (ema) and (esu). Both of *E. malabaricus* (ema) and *E. summana* (esu) are distantly related to both of *E. areolatus* (ear) and (ech). The distance value between *E. tauvina* (eta) and *E. chlorostigma* (ech) is higher than distance value between (eta) and the most evaluated *Epinephelus* species (ema, esu and era). The distance between *E. tauvina* (eta) and *E. areolatus* (ear) is similar to distance between *E. tauvina* (eta) and *E. chlorostigma* (ech).

Genetic polymorphism in the Serranidae using 12s rRNA sequence variations compared with some other fish families

A total of fifty two 12s rRNA fragments from 52 fish species belonging to 38 fish genera for the five families (Carangidae, Labridae, Serranidae, Mugilidae and Cichlidae) were analyzed for all evaluated DNA sequences. The total number of analyzed sites (425), the sequence conservation value (SC = 0.564), conservation threshold (CT = 0.8), the average values of GC (0.480), GC_2 (0.516), GC_3 (0.523), single nucleotide polymorphism (SNPs = 150), estimates of the haplotype diversity (hd = 1), Nucleotide diversity (Pi = 0.114), theta from polymorphic sites (Θ = 0.111), the average number of nucleotide differences (k = 43.60) were calculated for all the evaluated 12s rRNA sequences.

The DNA polymorphism values within each evaluated fish family was calculated and presented in Table 3. The highest DNA polymorphism parameter values (SNPs, Pi, Θ, K and h) were detected in the Serranidae family (Table 3). Besides, the lowest sequence conservation (SC) value was detected in the same family. The SNP, Θ, K and Pi values were varied among all evaluated fish families. The lowest SNP and the highest SC values were detected in the Carangidae family. The average of distance values within each of Carangidae, Mugilidae and Cichlidae is similar. The haplotype diversity value within each fish family was equal (1). The consensus sequence for each evaluated fish family was detected. The variable sites among the evaluated fish families revealed from consensus sequences were estimated (Fig. 2).

The phylogenetic relations among the evaluated fish families based on 12s rRNA consensus sequence variations

The phylogenetic relations among evaluated fishes were reflected by Figure 3. The distance values among evaluated fish families were calculated based on the consensus sequences revealed from each analyzed 12s rRNA sequence within each evaluated family (Fig. 4). The divergence value within each evaluated fish family was averaged and presented in Table 3. Serranidae is distantly related to all the evaluated fish genera. Cichlidae and Mugilidae were the closest taxa.

The distance between Labridae and Mugilidae is higher than thedistance between Labridae and Cichlidae. Mugilidae is distantly related to Carangidae comparatively with Cichlidae. The same distance value was calculated between Serranidae and each of Cichlidae and Labridae. The distance between Labridae and Carangidae is higher than the distance between the Labridae and Cichlidae.

The fish morphological characterization is not parallel with the molecular identification in some cases. This observation was confirmed in grey mullets (Family Mugilidae), in *Plectropomus* (Family Serranidae) and Parrotfishes (Family Labridae) using different DNA markers[3,16,17]. The lack of parallel variations between morphological characterization and

Table 3 — DNA polymorphism, sequence conservation and genetic distance values in each evaluated fish family based on 12s rRNA sequence variations

Family	Carangidae	Labridae	Serranidae	mugilidae	Cichlidae	TOTAL
Parameters						
Genera	5	5	7	9	12	38
Fragments	6	6	15	12	13	52
Species	6	6	15	12	13	52
SNP	59	75	130	84	81	150
GC	0.455	0.483	0.470	0.485	0.496	0.480
GC_2	0.486	0.463	0.456	0.561	0.474	0.516
GC_3	0.474	0.459	0.508	0.520	0.552	0.523
Pi	0.074	0.088	0.118	0.073	0.075	0.114
Ɵ	0.0708	0.093	0.125	0.079	0.075	0.111
K	30.4	35.86	47.36	29.43	29.97	43.6
h	6	6	15	12	13	52
hd	1	1	1	1	1	1
SC	0.856	0.803	0.667	0.773	0.778	0.564
CT	0.8	0.9	0.8	0.8	0.8	0.8
PIS	37	30	92	53	55	133
Dist.	0.081	0.096	0.134	0.079	0.081	0.128

Single nucleotide polymorphism = (SNP), averages of T, C, A, G contents, nucleotide diversity = (Pi), theta from site = (Ɵ), average number of nucleotide differences = (K), haplotype diversity = (hd), sequence conservation = (SC), Conservation threshold = (CT), Parsimony informative sites = (PIS) and distance value within each evaluated fish family (D)

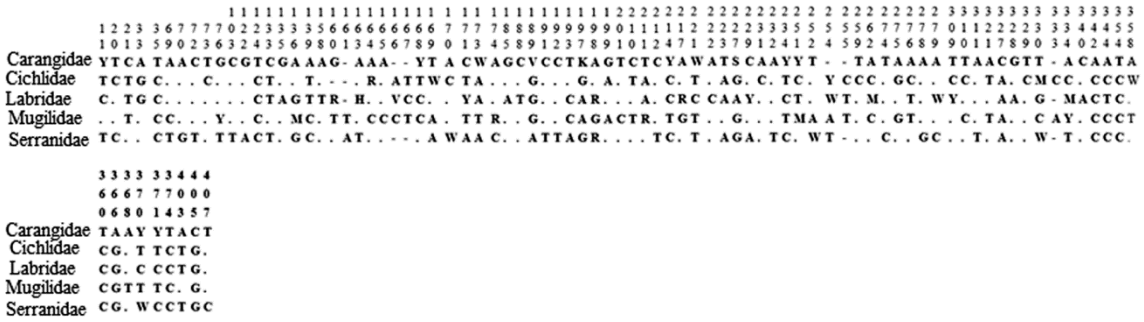

Fig. 2 — Variable 12s rRNA sequence sites among the evaluated fish families

some informative DNA markers may be explained by alterations in the selective constraints between the two characters[16,18].

Two different molecular techniques, ISSR and partial sequencing of the mitochondrial 12s rRNA were applied in the present study for evaluating the genetic polymorphism among the six Red sea *Epinephelus* species (*E. areolatus*, *E. malabaricus*, *E. summana*, *E. radiatus*, *E. tauvina* and *E. chlorostigma*).

ISSR technique (Ng & Tan[10]) was applied in the present study due to its advantages in fish characterization[3,10,17]. The ISSR as dominant markers demands fewer experimental steps and incurred costs are relatively low[19]. The divergence within each evaluated fish species was reflected by calculated the observed number of alleles, the effective number of alleles, gene diversity, Shannon's Information index and the percentage of polymorphic loci. Also, the calculated genetic distance values reflect the divergence levels among these *Epinephelus* species. All of these values were affected by the number of polymorphic ISSR bands within and among the estimated fish samples[3].

The numbers of amplified ISSR bands were varied within and among evaluated fish species. The percentages of polymorphic bands across all the evaluated fish samples was considered sufficient for confirming the technique sensitivity in species identification and the future population genetic studies[20].

Concerning the second technique (12s rRNA sequence analysis), all revealed mitochondrial 12s rRNA fragment sequences were analyzed for detecting molecular variability and inference the evolutionary variations among the evaluated fish

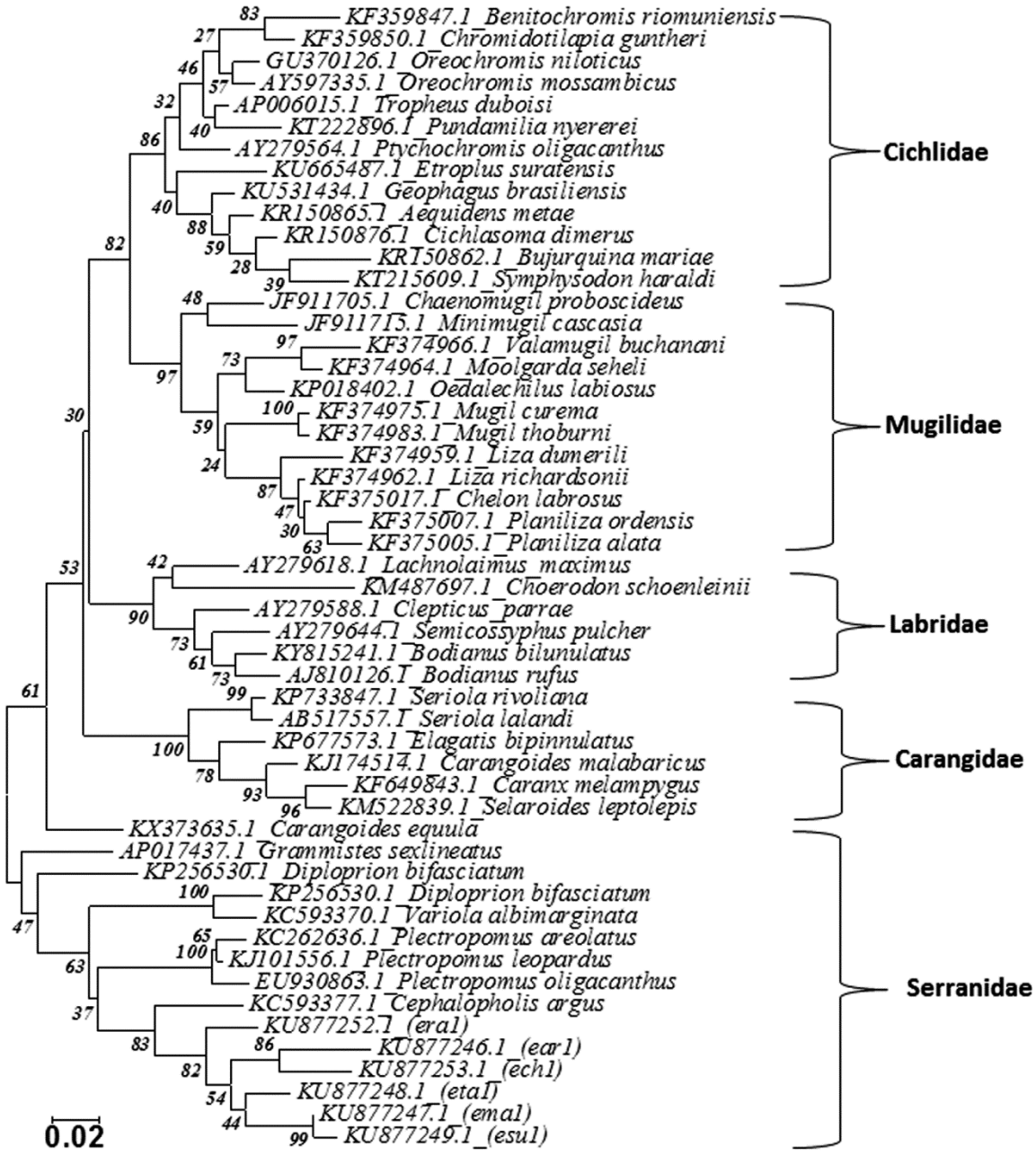

Fig. 3 — Evolutionary variations based on 12s rRNA gene fragment sequence differences among the evaluated fish species using the Maximum Likelihood method (MLM) method. The *ear1 = Epinephelus areolatus*, *esu1 = Epinephelus summana*, *ema1 = Epinephelus malabaricus*, *era1 = Epinephelus radiatus*, *eta1 = Epinephelus tauvina* and *ech1 = Epinephelus chlorostigma*

species. No individual variations were detected within each evaluated fish species (except one sample within *E. summana*). Therefore, not all identified fragment sequences were submitted to the NCBI.

The sequence conservation values (SC) among the evaluated 12s rRNA sequences were varied. The highest (0.856) and lowest (0.667) values were detected in Caranidae and Serranidae families, respectively. The sequence conservation values were calculated to reflect the evolutionary rate of the analyzed gene fragment sequences in differently evaluated fishes[16]. In addition, the calculated GC, GC_2 and GC_3 values explored the molecular variations among the evaluated fish families. The importance of such parameters in exploring the evolutionary variations among differently animal taxa was confirmed in different studies including aquatic animal taxa[2,21]. These differences may be due to mutation or selection pressure[16,22].

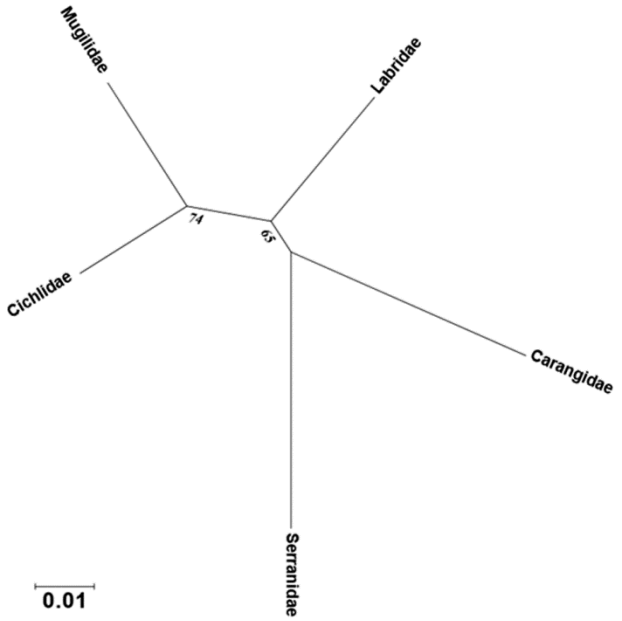

Fig. 4 — Evolutionary variations based on 12s rRNA consensus sequence differences among the evaluated fish families using the Maximum Likelihood method (ML) method. The tree with the highest log likelihood (-820.2770) is shown

Each technique was applied separately for exploring the evolutionary variations among the evaluated *Epinephelus* species. The relative relatedness among evaluated fishes was detected by each applied technique.

Concerning 12s rRNA results, we assessed the degree of support by the calculated bootstrap values for relations recovered by ML and NJ methods. The obtained bootstrap values were relatively high and sustain the reliability of our results. The application of ML and NJ methods generated similar results. This observation suggested that the data were free of positive-misleading systematic biases. In addition, this high level of similarity increased our confidence in the resulted topology of both trees[16,23].

Both applied techniques revealed that *E. malabaricus* (ema) is closely related to *E. summana* (esu). In addition, the distance between *E. chlorostigma* (ech) and *E. areolatus* (ear) is lower than distance between (ech) and *E. radiatus* (era). Our investigation supports these close relationships. The close relation between *E. chlorostigma* (ech) and *E. areolatus* (ear) is recently confirmed[1]. The complete mitochondrial genome of *E. chlorostigma* was identified and comparatively analyzed with other Serranidae fishes[1]. The relatedness among these Serranidae fishes (*Epinephelus* species and *Cephalopholis* species) was re-constructed *via* analysis of some protein coding genes sequences. The *E. chlorostigma* was the most closely related to *E. areolatus*.

The distance values revealed from analyzed ISSR variations were higher than distance values that revealed from 12s rRNA sequences analysis. The sensitivity of ISSR technique for detecting the genetic variability within and among aquatic organisms including fishes and shrimp was confirmed in many evolutionary studies[3,17]. The application of this technique for fish identification and invasions was also recommended[20,24].

The ranges of divergence values detected among the evaluated *Epinephelus* species based on ISSR and 12s rRNA were (from 0.053 to 0.270) and (from 0.007 to 0.118), respectively. These results are in general similar to another study (using RFLP) in some fish species especially in Mugilidae fishes based on the analysis of mt-DNA (12s rRNA, 16s rRNA) variations[16,25].

The highest degree of genetic distance value (0.134) has been detected within Serranidae comparatively with the other estimated fishes. This could be the result of the identified SNPs within this family relatively.

Generally, species characterization via mitochondrial rRNA molecular analysis was evaluated for designing general animal identification methods. A combination of this technique with suitable statistical analysis was recommended to provide a reliable way for animal classification[26].

The 12S rRNA gene as a promising tool for exploring genetic variations among different biological animal taxa was suitable for tracing the evolutionary variations among the evaluated Red Sea *Epinephelus* species[7]. On the other hand, this technique was not suitable for detecting the genetic variations within each estimated fish species. For estimating the genetic polymorphism within fish species and/or population studies, the ISSR technique was recommended[3,20].

Some traditional methods such as SDS-PAGE for protein electrophoresis, isoelectric focusing and immunological methods were not suitable for accurate fish species identification and inference the true phylogenetic relations comparatively with molecular methods[26].

Due to the fluctuations in the efficiencies of different molecular systems in animal taxa identification including fishes, only some of these systems were recommended for studying fish characterization and evolutions[2,27]. A fair evaluation of all of these techniques should concentrate on their discriminatory force and reproducibilities[2,26].

For the reconstruction of the true phylogenetic relations among fish taxa, development and analysis of informative nuclear and mitochondrial DNA markers should be applied[5]. ISSR and 12S rRNA approaches, when combined with bioinformatics, run a dependable design for the fish taxonomic and evolutionary studies.

Conclusion

Analysis of the 12s rRNA gene sequence variations revealed different haplotypes among the evaluated fishes. ISSR technique was succeeded in exploring the genetic variations within and among evaluated fish species. Therefore, there are still different opinions concerning species-level relationships. Based on the present results and the recent relevant data, we recommend a revision of the taxonomic status of the evaluated fish genera including their all integral species. More molecular investigations using more spatial and temporal fish samples should be carried out in the future for exploring the true *Epinephelus* species biodiversity in the Red sea.

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Conflict of Interest

The Authors declared no conflict of interest.

Author Contributions

AMS-O contributed in the conceptualization of problem statement; YMS performed formal analysis, investigation, software analysis, drafted the original manuscript, supervision and review of the manuscript; ZMA-H provided resources and edited the manuscript.

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