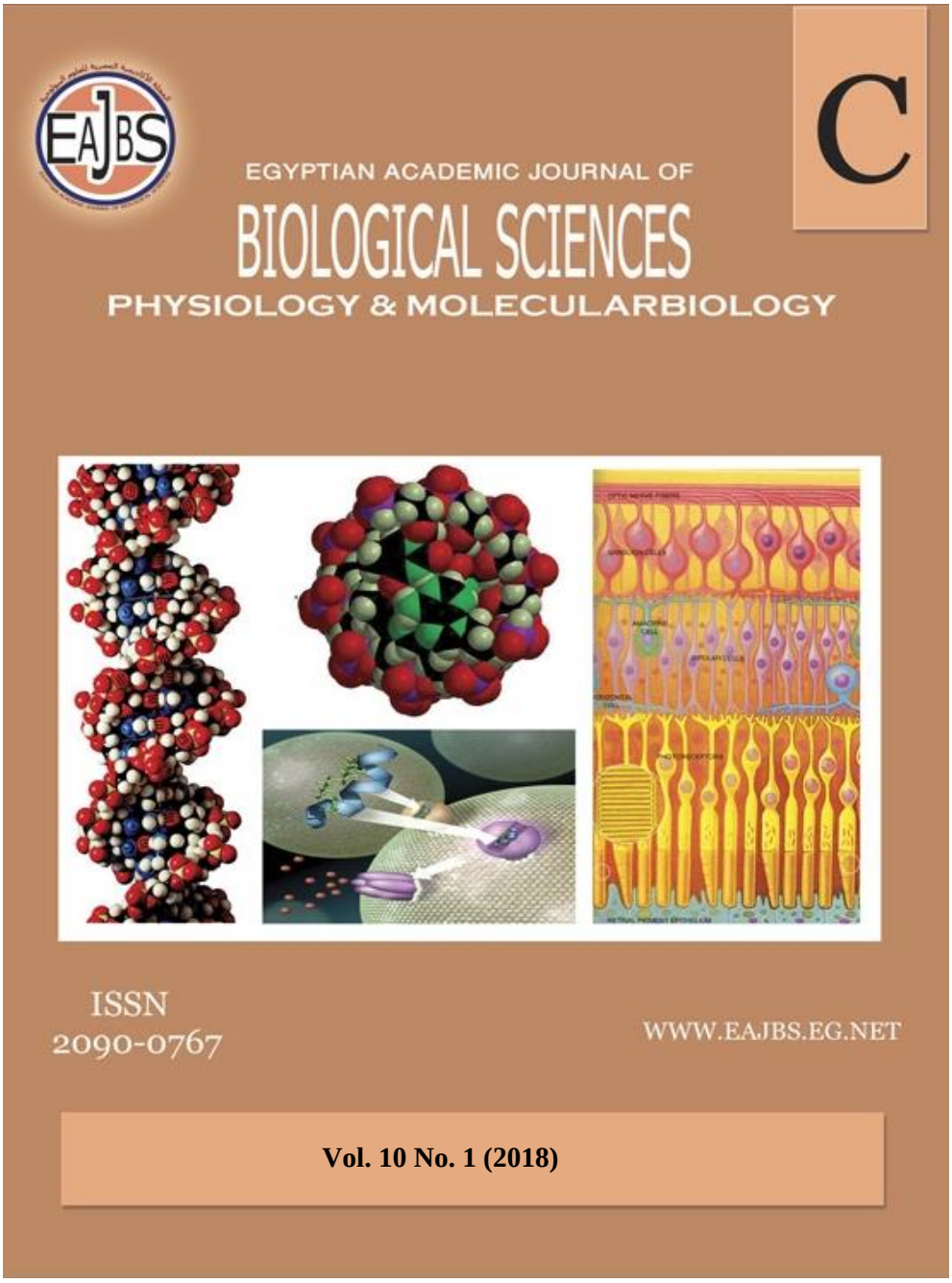

Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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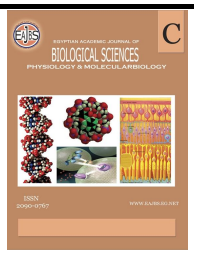
Egypt. Acad. J. Biolog. Sci., 10(1): 95- 113 (2018)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbs.eg.net



Molecular Phylogenetic Taxonomy of Some Parrotfish Species (Perciformes, Scaridae) From the Red Sea Using α-Actin Gene

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ARTICLE INFO
Article History
Received: 29/3/2018
Accepted: 19/4/2018

***Keywords*:**
α--actin gene, nucleotide sequence, phylogenetics and parrotfish

ABSTRACT

α--actin gene (ACTA 1) was studied as a potential phylogenetic marker for selected members of Subfamily Scarinea (Scaridae, Perciformes). The samples collected from the Red Sea. The nucleotide sequences of six parrotfish (*Scarus niger, Scarus fuscopurpureus, Scarus ferrugineus, Scarus psittacus, Chlorurus gibbus* and *Hipposcarus harid*) were analyzed with respect to their molecular evolution and phylogenetic relationships among themselves and other related percoid species depending on available sequence data. α-skeletal muscle actin gene segments isolated from the skeletal muscle of the six species that were sequenced and recorded in gene bank with the Accession number for the first time. The six-nucleotide sequences compared to fourteen other percoid sequences from Gene Bank/NCBI, altogether comprising 20 percoid sequences and 3 outgroup sequences (Order Scoraeniformes). The scores of p-distance and sequence divergence of the alpha-skeletal muscle actin gene among the tested species were calculated. Studied A+T of the six sequence rates were variant between 44.4 and 52.4 % for all species. The phylogenetic trees for 23 species (6 parrotfish and 14 sequences of other percoid families from GeneBank together with 3 fishes as outgroup) were developed using actin gene and 5 different analytical approaches: Neighbour Joining (NJ), Minimum Evolution (ME), Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference method (BI). The analysis revealed a monophyletic origin for the five tested species of the scarinea, which was the principal subfamily investigated (87, 92, 100, 88 and 100% support in our NJ, ME, MP, ML and BI analyses, respectively). While the sixth species *Scarus fuscopurpureus* of the tested fishes formed a complete separate clade that indicates this species more related to genus *labrus* than genus *scarus* .The phylogenetic implications of actin gene or other phylogenetic markers in the family Scaridae or even all families of Order Perciformes until now were shortly discussed.

INTRODUCTION

Teleosts have developed unique features in the structure and physiology of muscles during their evolutionary history, Muscle growth in teleosts is signified by property phenomenon, the increase in fiber number as well as increase in fiber size, which is not found in other vertebrates (Kiessling *et al.* 2006 and Johnston *et al*. 2011). Such adaptive processes have appeared in modifications of the genetic pathways modulating muscle growth and functions in fish (Mudalige *et al.* 2007). Furthermore, metabolic and contractile characteristics of teleost muscles also, represent significant flexibility concern to environmental conditions as temperature and food supplying (Johnston 2006 and Johnston *et al.* 2008) and reproductive status as gonad maturation (Mathana *et al*. 2012). Thus, identification of genetic determinants for muscle-specific genes from fish represented an important basis to study the evolutionary and diversification of the musculature in the fish's lineage.

Actin is one of the major components of muscle tissues. Actins play important roles in maintaining cytoskeletal structure, cellular mobility, cell division and differentiation, intracellular movement, and contractile processes, which are associated with a wide range of physiological aspects in fish and all vertebrates, thus actins an evolutionarily conserved protein (Perrin and Ervasti 2010 and Lee *et al.* 2017). The genome of vertebrate species usually contains six different actin genes, four of these genes code for muscle isoforms (α-skeletal muscle, □-cardiac muscle, α-vascular and α-enteric muscles) and two other genes code for (the gama- and beta-cytoplasmic) types (Vandekerckhove and Weber, 1979, Kusakabe *et al.*,1997). In spite of Muscle actin gene has three isoforms (α-skeletal, α-cardiac, α-vascular and α-enteric), these isoforms share remarkably high sequence identity one another, each isoform exhibits distinct regulation pattern for its spatial and temporal expression (Adriane *et al*., 2007, Perrin and Ervasti 2010, Glasauer and Neuhauss 2014, and Lee *et al.* 2017).

Order Perciformes, is comprises 156 recent families. Family Scaridae (parrotfish) is a distinctive group of order perciformes. The Scaridae is relatively small family, with 90 species in ten genera. It comprises small to large species with maximum adult sizes ranging from 110 to 1000 mm (Streelman *et al*., 2002). Scarid fishes are present in the Red Sea, tropical Atlantic and Indian and Pacific Oceans. Despite the economic importance of the family, the systematics of the Scaridae has been in a state of confusion for many years (David and Bellwood 1994). Scarus was established as the first genus of the family Scaridae following the International Commission on Zoological Nomenclature decision (Opinion 261) to invalidate names in Gronow's Zoophylacii Gronoviani (1763). (Forsklll 1775) reported sex new species of genus Scarus from the Red Sea. (Jordan and Gilbert 1882) designated *Scarus psittaeus* as the species of the genus *Scarus*. The Scaridae was first recognised as a distinct family by (Bleeker 1859b) who later, in 1862, provided detailed descriptions of the two families: the Scaroides (parrotfishes) and the Labroides. Prior to this classification, the two groups were both placed in a single family, the Labridae (Cuvier and Valenciennes, 1840; Kner, 1860; Gunther, 1862). However, the actual identity of this species was not resolved until 1978 (Randall and Ormond 1978). A number of recent studies have presented evidence which indicates that the Labridae, Odacidae and Scaridae

represent a monophyletic assemblage (Liem and Greenwood, 1981, Stiassny and Jensen 1987), and if the Scaridae represents a monophyletic group, then the immediate sister group of the Scaridae must be contained within the Labridae or Odacidae. In other studies, the genus *Scarus* appeared to be paraphyletic by (Bellwood 1986) and, subsequently, by (Bellwood and Choat 1990) and they were suggested that the genus *Scarus* was comprised of two distinct phyletic lineages. These two groups were identified as separate functional groups. There are numerous taxonomic problems associated with many groups of Perciformes (Nelson 2006).

Thus, it is obvious that much molecular taxonomy and phylogenetic research on Scaridae and other percoid families remain to be conducted. In this study, we focus on the Subfamily Scarinae discuss 6 species and compared to 14 representatives of other families of percoid fishes by molecular phylogenetic and taxonomic considerations using α-skeletal muscle actin sequence data. Two aims were considered: 1) whether the Subfamily Scarinea is monophyletic, and 2) whether the nucleotide diversity at α-skeletal muscle actin gene supports the currently accepted intra-family, intra-subfamily and intra-genus divisions.

MATERIAL AND METHODS

a**. Samples**:

Six fish's species belonging to Subfamily Scarinea, Family Scaridae , Order Perciformes were collected from the Red Sea. First, the tested fish's species were identified morphologically according to (Randall, 1982) (Table 1). Skeletal muscles tissues were isolated from the collected fish samples and were preserved in -80ºC until used.

b. **DNA Isolation:**

Total genomic DNA was isolated from skeletal muscles of six species of Scarinea fishes using QIAamp DNA Mini kit (Qiagen, Hidden, Germany) following the manufacturer's protocol.

c. **Primer and PCR Conditions:**

To get the alpha actin gene sequence data of the six specimens. First, we used PCR to obtain alpha actin segments with the set of 2 primers (forward and reverse) Actin F1 (5'-GTA TTG TGC TGG ACT CTG GTG-3') Actin R1 (5'-GAA GCA CTT GCG GTG GAC GAT-3'). The forward and reverse primers were designed from the α-actin gene sequence described for the fish *Ictalurus punctatus* (Kim *et al.*, 2000). PCR reactions were carried out with 12.5 µL PCR master mix (Qiagen, Hidden, Germany), 0.5 µL (10 pmoles/ µL) of each primer and ~100 ng of genomic DNA in a final reaction volume of 25 µL, the two primers that were used in this study. PCR reaction was performed as following, an initial denaturation: 95 ºC for 2 minutes, followed by 34 cycl 30s at 95 ºC, annealing: 55ºC for 30s and extension: 1 min at 72 °C plus a final 5 min extension at 72 °C.

d. **Gel Electrophoresis:**

The PCR products were electrophoresed in 1% agarose gel, stained with ethidium bromide and visualised under UV light. Electrophoresis analysis induced a single segment with each species that indicate the alpha actin gene segment, the fragments of the gene amplified is approximately 900 bp (Fig. 1), which is similar with the fragments length of the primer expect.

Table 1: Classification of understudying parrotfish and related percoid species were retrieved from the Gen Bank/ NCBI and references.

Classification	Species	References
Class: Actinopterygii (Teleosti)	-	-
Order: Perciformes	-	-
Family: Scaridae (Parrotfishes)	-	-
Subfamily: Scarinea	-	-
Genus: Scarus	*Scarus niger*	Forsskal, 1775
-	*Scarus ferrugineus*	Forsskal, 1775
-	*Scarus psittacus*	Forsskal, 1775
-	*Scarus fuscopurpureus*	Kluninger, 1871
-	*Scarus iseri*	-
Genus: Chlorurus	*Chlorurus gibbus*	Ruppell, 1829
Genus: Hipposcarus	*Hipposcarus harid*	Forsskal, 1775
Family: Labridae	-	-
Genus: Labrus	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
-	*Labrus bergylta*	Ascanius, 1767
Genus: Thalassoma	*Thalassoma bifasciatum*	Bloch, 1791
Genus: Parajulis	*Parajulis poecilepterus*	Temminck and Schlegel, 1845
Family : Nototheniidae	-	
Genus: Notothenia	*Notothenia coriiceps*	Richardson, 1844
Genus: Trematomus	*Trematomus bernacchii*	Boulenger,190
Family: Serranidae	-	-
Genus: Epinephelus	*Epinephelus coioides*	Hamilton, 1822
Order: Scoraeniformes	-	-
Family: Scopaenidae	-	-
Genus: Sebastes	*Sebastes schlegelii*	Hilgendorf, 1880
-	*Sebastes inermis*	Cuvier and Valenciennes,
Genus: Sebastiscus	*Sebastiscus marmoratus*	1829

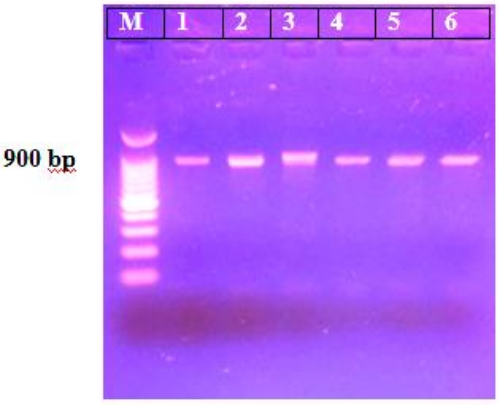


Fig. 1. Electrophoretic analysis of PCR products of the alpha skeletal muscle actin fragments. Lanes 1 - 6 PCR products for tested fish samples (*Hipposcarus harid,Scarus niger, S. fuscopurpureus, S. ferrugineus, S. psittacus* and *S. gibbus* respectively*)* and M, molecular weight marker

.

e. Sequencing of PCR Products:

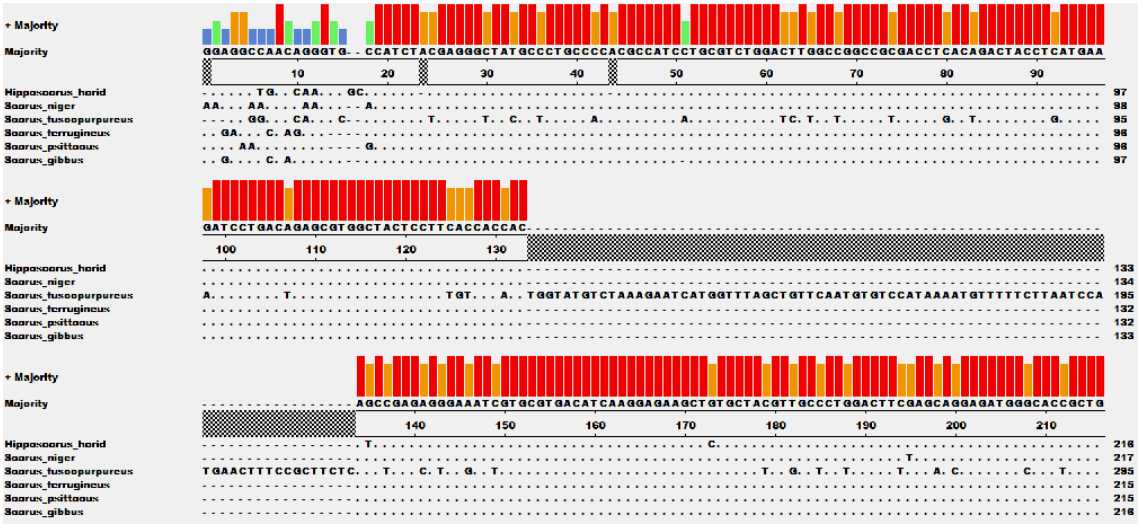
When good PCR products were obtained, each sample was purified before sequencing. The purified products then were sent to Macrogen Company to make standard sequencing, using both forward and reverse strands. Nucleotide sequences of the □-skeletal muscle actin gene (ACTA 1) segment of nuclear DNA were established for 23 fish.

Differing variants of the sequenced regions of the six parrotfish were deposited in the GenBank/NCBI for the first time under accession numbers MH203326, MH203328, MH203329,MH203327, MH203330 and MH203325 (Scarus niger, Scarus ferrugineus, Scarus psittacus, Scarus fuscopurpureus, Chlorurus gibbus and *Hipposcarus harid* respectively), The following actin gene sequences from the related percoid species were retrieved from the GenBank and used in the present study. the 14 related percoid species were retrieved from the Gen Bank/ NCBI and used in the present study under accession numbers (one species only of Family Scaridae that recorded previosly and available in Gen Bank *Scarus iseri* (HM 120258.1) ten species from Family Labridae eight of genus *Labrus, L. bergylta* (accession no. XM 020638243.1, XM 020638683.1, XM 020639600.1, XM 020643138.1), XM 020651307.1, XM 020656609.1, XM 020657440.1 and XM 02069999.1) and, one species of genus Thalassoma, *Thalassoma bifasciatum* (JQ 639047.1) and one species of genus Parajulis, *Parajulis poecilepterus* (DQ 073096.1), two species of Family Notothenіidae, genus Notothenia, *Notothenia coriiceps* (AF 503590.1) *and* one of Genus Trematomus, *Trematomus bernacchii (AF 503589.1),* one species of Family Serranidae, genus Epinephelus, *Epinephelus coioides* (AY 735013.1) together with three out group (Table 2). For comparative analysis, sequences from the GenBank were combined with the sequences of the present study and used to infer the phylogenetic relationship of scarinae species of the Red Sea. For the analysis, three representatives of order Scoraeniformes was used as the outgroup, *Sebastes schlegelii, Sebastes inermis and Sebastiscus marmoratus* with accession no. (JN 226152.1, JN 226153.1 and HQ 906886.1) respectively.

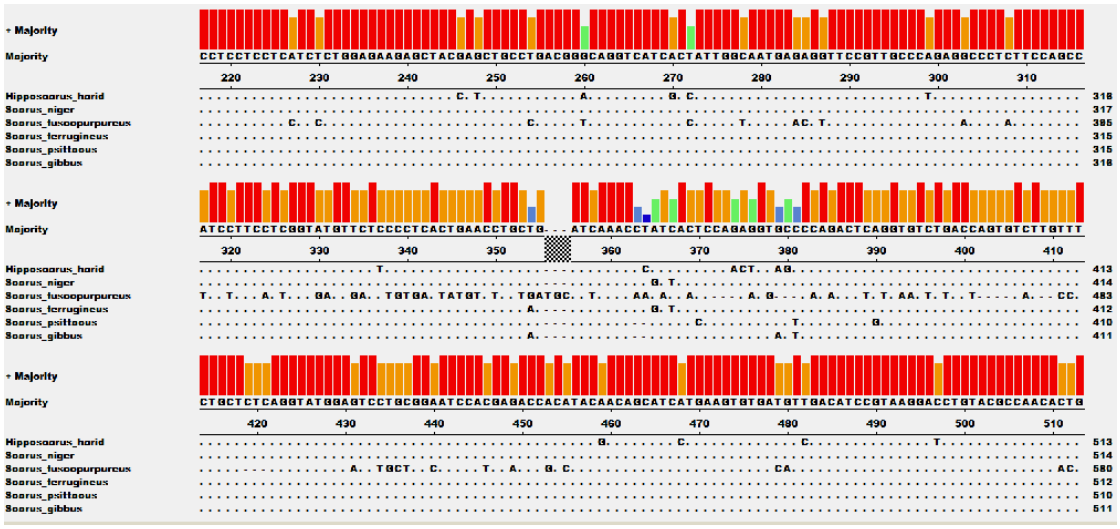
Table 2: Species and accession numbers of Actin gene sequences of understudying species (6 parrotfish species) and that obtained from gene bank (13 percoid species) together with outgroup species (3 of Order Scoraeniformes, Teleosti).

Species	Actin type	Accession number
Scarus niger	alpha-actin S. muscle	MH203326
Scarus ferrugineus	alpha- actin S. muscle	MH203328
Scarus psittacus	alpha- actin S. muscle	MH203329
Scarus fuscopurpureus	alpha- actin S. muscle	MH203327
Scarus iseri	beta-actin	HM120258.1
Chlorurus gibbus	alpha-actin S. muscle	MH203330
Hipposcarus harid	alpha- actin S. muscle	MH203325
Labrus bergylta	alpha-actin C. muscle	XM 020638243.1
Labrus bergylta	alpha-actin C. muscle	XM 020638683.1
Labrus bergylta	alpha- actin C. muscle	XM 020639600.1
Labrus bergylta	alpha- actin S. muscle	XM 020643138.1
Labrus bergylta	actin non –muscle 6.2	XM 020651307.1
Labrus bergylta	alpha-actin S. muscle	XM 020656609.1
Labrus bergylta	actin cytoplasmic 2	XM 020657440.1
Labrus bergylta	*beta-actin*	XM 02069999.1
Thalassoma bifasciatum	*beta-actin*	JQ 639047.1
Parajulis poecilepterus	*beta-actin*	DQ 073096.1
Notothenia coriiceps	alpha- actin	AF 503590.1
Trematomus bernacchii	alpha- actin	AF 503589.1
Epinephelus coioides	alpha- actin	AY 735013.1
Sebastes schlegelii	beta-actin	JN 226152.1
Sebastes inermis	beta-actin	JN 226153.1
Sebastiscus marmoratus	beta-actin	HQ 906886.1

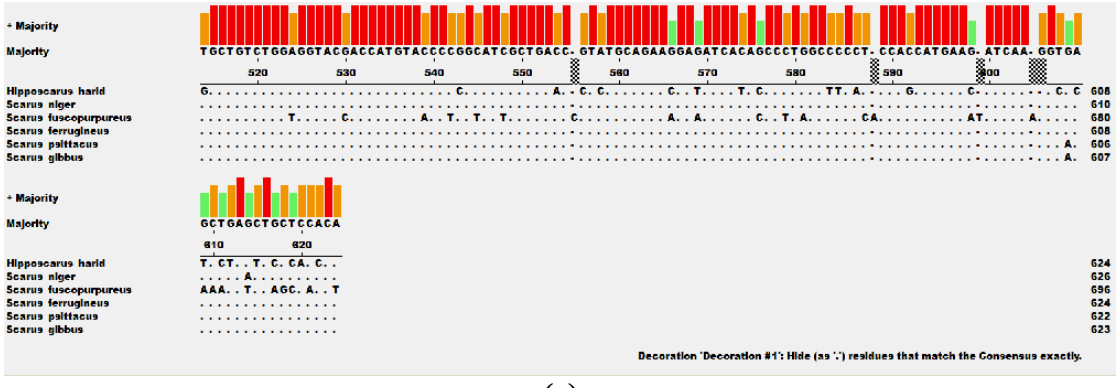
S. muscle = skeletal muscle C. muscle = Cardiac muscle



(a)



(b)



(c)

Fig. 2. Alignment report α-Actin Gene nucleotide sequences of the six parrotfish species, Photo. (a, b, and c). Dots indicate identical nucleotides and A,T,C and G indicate the difference nucleotides

f. Sequence Alignments:

All analyses, starting with new DNA extraction, will be repeated for this specimen.Nucleotide sequences from the six parrotfish specimens were compared and aligned among themselves (Fig. 2) and in respect to other sequences present in databases using the Clustal W computer software package. The sequences were aligned using the multiple-alignment algorithm in Megalign (DNASTAR, window version 3.12e) and MEGA version 7.0 18 (Kumar *et al*., 2016), then the alignments were refined manually and all gaps were then deleted manually. During alignment, several sequences were removed from further analysis because of their low proximity to the bulk of the data, most sequences removed were those from GenBank that did not fit the length limit of our samples. The multiple aligned dataset generations involved both pair wise and progressive alignments based on a guide tree. Five approaches were used for alpha actin tree building, Neighbour-joining (NJ) (Saitou and Nei 1987), Minimum Evolution (ME) (Rzhetsky 1982), Maximum Parsimony (MP) (Fitch 1971), Maximum Likelihood (ML) (Takahashi and Nei 2000) and Bayesian inference method (BI) (Huelsenbeck and Ronquist, 2001), trees using Kimura 2-parameter distances were created to provide a graphical representation of the pattern of divergence between species, using Verify the robustness of the internal nodes, bootstrap analysis was carried out using 1,000 replicates (Felsenstein, 1985).

RESULT

alpha-actin gene was studied as a potential phylogenetic marker for six species of parrotfish from family scaridae, subfamily scarinea include three genus (*Scarus*, *Chlorurus*, and *Hipposcarus*). Two primers; the forward and reverse primers were used to amplify the particular target region of all the species. Alpha skeletal muscle actin genes (ACTA 1) are amplified to give the general view about the molecular genetic relationships among the understudying species and other related species of Order Perciformes depending on available sequence data from gene bank/NCBI. The genomic DNA of parrotfish (*Hipposcarus harid*, *Scarus niger*, *Scarus fuscopurpureus*, *Scarus ferrugineus*, *Scarus psittacus* and *Chlorurus gibbus*) were generated a single segment (Fig. 1), that indicate all the species showed different alpha nucleotide sequences and hence successfully barcode the region, the nucleated sequence lengths were of approximately (889, 902, 696, 905, 889 and 880bp respectively). No insertion/deletion or stops codon was found, supporting the view that all of the amplified sequences constitute functional alpha actin gene sequences.

Genetic distance :

For the sequences of six parrotfish species, the average nucleotide frequencies of thymine (T), cytosine (C), adenine (A) and guanine (G) were 22.2 %, 30.3 %, 23.9 %, and 23.1 % respectively, and were varied between 20.9 and 31.9 %. The average content of G+C (53.4 %) was higher than that of A+T (46.6 %) (Table 3). Pair wise genetic distances among the twenty percoid species and the three outgroups were estimated by MEGA 7 (Kumar *et al*., 2016) using the Kimura two-parameter model (Kimura 1980) with gamma correction (Table 4), that assumes transition mutations should occur more often than transversion. Pairwise genetic distances among and within the sex-species and the outgroup, *Polyodon spathula,* were estimated by MEGA (Kumar *et al*., 2004) using the K2P method with gamma correction. The *P*-distance between the understudying fishes was lowest between *Scarus niger* and *Scarus fuscopurpureus* (79.9%) and highest between *Scarus psittacus* with

Chlorurus gibbus and also with *Hipposcarus harid* (99.8%). The results indicate also, the gene of *Scarus ferrugineus* genotype was the longest (905 bp), and the *Scarus fuscopurpureus* gene was the shortest (696 bp). The *Scarus fuscopurpureu*s fish was AT rich, with A+T contents of 52.4%, however, the A+T contents of the rest species were quite similar which were (46.8, 45,8, 44.7, 45.2, 44.4 and 46.6%) (Table 3).

Table 3. Average of base composition (A, T, C and G) percentage in 6 parrotfish species for alpha actin sequence.

Species	Base pair length	Nucleotide Number %				A+T Content (%)	G+C Content (%)
		A %	T %	C %	G %		
Hipposcarus harid	**889**	**25.6**	**21.2**	**32.3**	**20.9**	**46.8**	**53.2**
Scarus niger	**902**	**23.5**	**22.3**	**31.9**	**22.3**	**45.8**	**54.2**
Scarus fuscopurpureus	**696**	**25.7**	**26.7**	**25.4**	**22.1**	**52.4**	**47.6**
Scarus ferrugineus	**905**	**22.8**	**21.9**	**31**	**24.3**	**44.7**	**55.3**
Scarus psittacus	**889**	**22.7**	**22.5**	**30.3**	**24.5**	**45.2**	**54.8**
Chlorurus gibbus	**880**	**22.8**	**21.6**	**31**	**24.5**	**44.4**	**55.6**
Average *%*	**-**	**23.9**	**22.7**	**30.3**	**23.1**	**46.6**	**53.4**

Phylogenetic Analysis:

Sequence data reported from sequencer followed by the corrected raw sequence (Fig 2), before it recorded for the first time in GenBank with accession numbers (MH203325, MH203326, MH203327, MH203328, MH203329 and MH203330) for the six understudying species (*Hipposcarus harid, Scarus niger, Scarus fuscopurpureus, Scarus ferrugineus, Scarus psittacus* and *Chlorurus gibbus* respectively*).* For phylogenetic purposes, the six scarus alpha-actin gene sequences were subjected to an analysis together with 14 of other percoid sequences representing all the available and appropriate species of four families (one species *Scarus iseri* of family scaridae, ten of family Labridae, eight of *Labrus bergylta species, Thalassoma bifasciatum* and *Parajulis poecilepterus,* two of Nototheniidae *Notothenia coriiceps* and *Trematomus bernacchii* and one of Serranidae *Epinephelus coioides*), that were taken for the comparative purposes and retrieved from Gen Bank/NCBI, in addition to 3 outgroup sequences from order Scoraeniformes (Teleosti) (*Sebastes schlegelii Sebastes inermis Sebastiscus marmoratus*) (Table 1). All 23 nucleotide sequences (including outgroups), are aligned (Fig. 2). The genetic distances and genetic divergences among all sequences of the six tested species of parrotfish with the available species of the order perciformes together with three species as outgroup are calculated (Table 4).

The molecular phylogenetic trees were constructed based on the alpha actin gene sequences of six parrotfish species and the fourteen available percoid species from Gene Bank/NCBI together with the out group species to determine the root of the trees. The dataset was analyzed with Neighbour-joining (NJ), Minimum evolution (ME) these analysis were performed by the MEGA version 7.0.18 software, the, Maximum-parsimony (MP) and Maximum-likelihood (ML) these analysis were performed by the MEGA version 7.0.18 software and PAUP (version 4.0a150), ML analysis was based on the Akaike Information Criteria (AIC) test in

MrModel test 2.3. Bayesian inference (BI) algorithms, phylogenetic analysis by BI utilising Monte Carlo Markov Chain (MCMC) analysis in MrModeltest 2.3. (Fig. 3-5). The robustness of the tree was corroborated with bootstrap analyses (Bootstrap value=1.000). The results showed, all phylogenetic trees obtained are widely based on the same topology with Scarus, Labrus and other percoid species. Bootstrap support ranges from > 50 to 100% under all tree-building methods. In tested scarinae species, the maximum genetic divergences have occurred between the *Scarus niger* and *Scarus fuscopurpureus* (22.5%) whereas the maximum *p*-distance was observed between the *Scarus ferrugineus* and the two species, *Scarus psittacus and Scarus gibbus* (99.8%) (Table 4). To establish the relationship between sex tested fish and other available perciforme species, the alpha actine gene sequences of scarid sprecies were aligned with the previously published sequences of 14 species of Order Perciformes, one species of family scaridae, *S. iseri* (accession no. HM 120258.1) ten species from family Labridae eight of genus Labrus, *L. bergylta* (accession no. XM 020638243.1, XM 020638683.1, XM 020639600.1, XM 020643138.1), XM 020651307.1, XM 020656609.1, XM 020657440.1 and XM 02069999.1) and, one species of genus Thalassoma, *Thalassoma bifasciatum* (JQ 639047.1) and one species of genus Parajulis, *Parajulis poecilepterus* (DQ 073096.1), two species of family Notothenіidae, genus Notothenia, *Notothenia coriiceps* (AF 503590.1) *and* one of Genus Trematomus, *Trematomus bernacchii (AF 503589.1),* one species of family Serranidae, genus Epinephelus, *Epinephelus coioides* (AY 735013.1) obtained from GenBank. Five trees Neighbour-joining (NJ), Minimum Evolution (ME), Maximum Parsimony (MP), Maximum leikhood (ML) and Bayesian inference method (BI) were constructed by using three outgroup of order Scoraeniformes (Fig. 3-5), the three outgroup belong to Family Scopaenidae, two of genus Sebastes, *Sebastes inermis* (JN 226152.1) and *Sebastes schlegelii* (JN 226153.1) and one of genus Sebastiscus, *Sebastiscus marmoratus* (HQ 906886.1).

Phylogenetic analysis of our dataset resulted in, four tested samples forming a monophyletic clade (*Scarus niger, Scarus ferrugineus, Scarus psittacus* and *Chlorurus gibbus*) (Figs 3, a (NJ) and b (ME) and fig. 4, b (ML), with strong support (NJ= 87, ME= 93 and ML=88) to the exclusion of outgroup taxa. While *Hipposcarus harid* in the three trees (NJ, ME and ML) forming the basal clade to scarus species. On the other hand *Scarus fuscopurpureus* completely separated from scarus samples where it was found closer to the labrus sample according to the results of the all trees, with variant support, weakly with (NJ=66 and MP=60), while it formed a sister clade with *Labrus bergylta* in (ME and BI) With strong support (BI=95), that indicate *Scarus fuscopurpureus* is close to Labrus species than scarus species. On the other hand, four scarid species *(Scarus niger, Scarus ferrugineus, Scarus psittacus* and *Chlorurus gibbus)* together with *Scarus iseri* formed a monophyletic group in the tree (MP) with strong support (bootstrap value, 100%) to the exclusion of outgroup taxa. Whereas, *Hipposcarus harid* formed the basal clade to genus *Scarus* in (ME) tree but it was fused with Scarus genus to form one clade in (BI) tree. While, *Scarus fuscopurpureus* formed a separate basal clade with other percoid species clade with somewhat a weakly bootstrap value of 60% (MP). BI tree (Fig. 5) exhibit Polyphyletic group of the tested scaridae species (*Scarus niger* and *Chlorurus gibbus* formed the sister clade, whereas *Scarus psittacus* formed the basal clade to the Scarid species, *Hipposcarus harid* formed the

basal clade for the species (*Scarus niger* and *Chlorurus gibbus* and *Scarus ferrugineus*). *Scarus fuscopurpureus* formed a separate basal clade together *Labrus bergylta* clade with strong support 95%. The non- members of the family Scaridae as family Labridae (members of the genera *labrus, Thalassoma and Parajulis*), family Nototheniidae (members of genera Notothenia and Trematomus) and family Serranidae (genus Epinephelus) in Figs 3 (a and b) exhibit a polytomy (9-way polytomy) while and Fig. 4 (a and b) and (Fig 5) formed 10 polytomy. These polytomy contain some resolved relationships of acceptable to strong support values as follwing: *Thalassoma/ Parajulis* (NJ=69, ME= 51, Mp=100, ML= 68 and BI=94), genus *Notothenia coriiceps/ Trematomus bernacchii* with strong support (NJ=99,ME=99, MP=100, ML=97 and BI=94) and *Epinephelus coioides*/ *Labrus bergylta* with somewhat strong support (NJ=65, ME=71 and MP=100 and ML=70) and in BI tree *Epinephelus coioides* is separate and formed a basal clade to other percoid species. Weak support values which observed in the some clades may represent an indicator that the relation among or within these clades either not fully resolved or more information is needed.

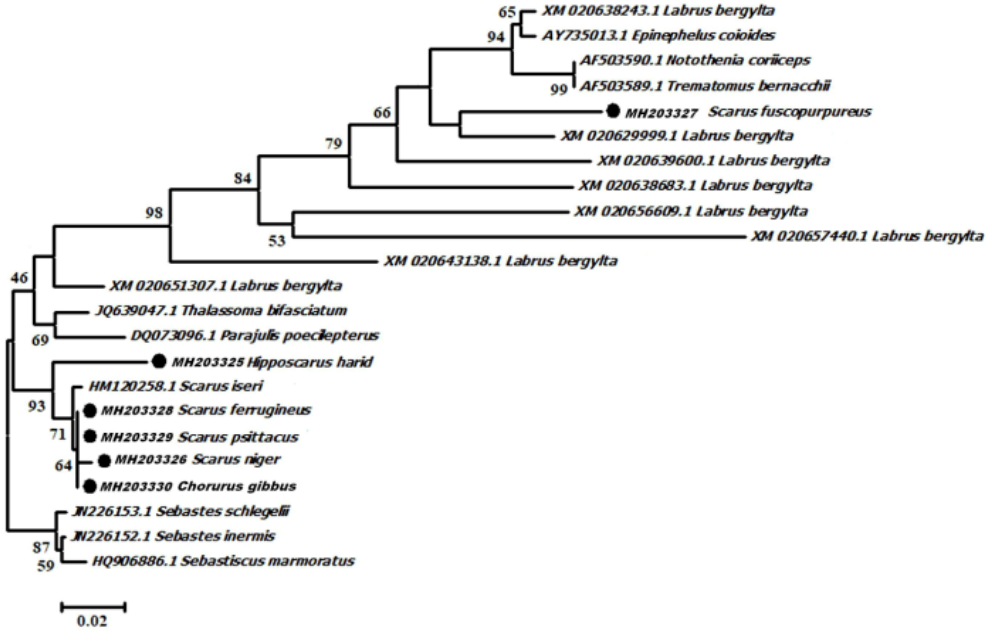
With simple exclusion to these data, it can be observed that tested species of family Scaridae form one distinct clade, *Scarus fuscopurpureus* is presente in all trees in a distinct separated clade with *Labrus bergylta* with weak support except strong support in (BI=95), without any interrelationship with scarus clade, it has a strong support with labrus species in BI tree and weak support with the rest trees that indicate it not fully resolved. According to the resulting phylogenetic trees, it is observed that both families Labridae and Nototheniidae and Serranidae are very close to each other. In all trees family Scaridae forming the basal clade to other families of order perciformes.

Table 4. Kimura 2-parameter pairwise distances based on Alpha actin sequence data for 20 percoid in addition to three outgroup**.**

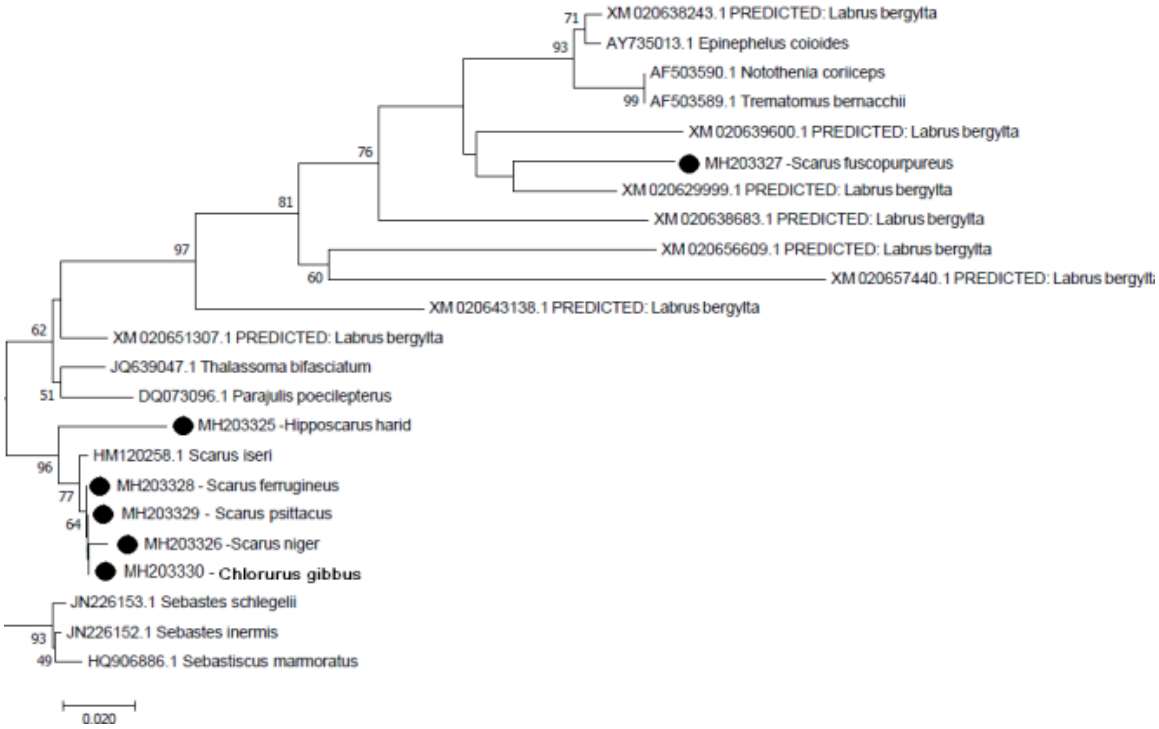
Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1		94.0	79.9	94.4	94.2	94.2	82.1	91.5	91.8	91.8	91.5	91.3	85.1	81.7	83.5	81.3	80.9	81.5	81.1	80.7	80.1	79.9	44.1	1	Hipposcarus_harid
2	5.9		83.5	99.6	99.6	99.4	84.7	95.4	95.0	95.4	95.2	95.0	88.3	85.5	86.1	84.7	84.7	84.3	84.5	85.3	84.3	83.1	44.3	2	Scarus_niger
3	22.5	18.2		83.9	83.7	83.7	72.2	85.9	84.7	83.7	85.5	84.1	84.3	88.7	82.9	88.5	88.9	88.7	88.7	91.3	86.7	80.5	38.0	3	Scarus_fuscopurpureus
4	5.5	0.4	17.7		99.8	99.8	85.1	95.8	95.4	95.8	95.6	95.4	88.7	85.9	86.5	85.1	85.1	84.7	84.9	85.7	84.7	83.5	44.5	4	Scarus_ferrugineus
5	5.7	0.4	17.9	0.2		99.6	84.9	95.6	95.2	95.6	95.4	95.2	88.5	85.7	86.3	84.9	84.9	84.5	84.7	85.5	84.5	83.3	44.5	5	Scarus_psittacus
6	5.5	0.4	17.7	0.0	0.2		84.9	95.6	95.2	95.6	95.4	95.2	88.5	85.7	86.3	84.9	84.9	84.5	84.7	85.5	84.5	83.3	44.7	6	**Chlorurus_gibbus**
7	3.7	1.0	16.8	0.5	0.7	0.5		81.9	81.5	82.1	81.7	82.1	75.3	72.4	73.6	71.6	71.8	72.2	71.4	73.0	72.0	70.8	58.8	7	HM120258.1_Scarus_iseri
8	8.6	4.8	15.1	4.4	4.6	4.4	4.4		95.8	94.8	97.4	94.8	88.1	86.5	86.7	86.1	86.1	87.1	86.3	86.3	85.1	84.3	43.9	8	JQ639047.1_Thalassoma_bifasciatum
9	8.4	5.3	16.7	4.8	5.1	4.8	4.9	4.4		95.0	96.0	95.0	88.5	87.5	86.5	87.1	87.5	85.7	86.9	86.5	85.5	84.7	44.9	9	XM_020651307.1_Labrus_bergylta
10	8.4	4.8	17.9	4.4	4.6	4.4	4.2	5.5	5.3		95.0	99.6	88.3	85.1	85.7	84.7	84.7	84.9	84.9	85.5	85.1	83.7	45.1	10	JN226152.1_Sebastes_inermis
11	8.6	5.0	15.6	4.6	4.8	4.6	4.7	2.7	4.2	5.3		95.0	87.7	86.3	87.7	85.5	85.9	86.3	85.7	85.3	85.7	84.3	43.7	11	DQ073096.1_Parajulis_poecilepterus
12	8.8	5.3	17.4	4.8	5.1	4.8	4.2	5.5	5.3	0.4	5.3		88.5	84.7	85.5	84.3	84.7	84.9	84.5	85.9	85.3	83.5	45.9	12	JN226153.1_Sebastes_schlegelii
13	16.3	12.8	17.0	12.4	12.6	12.4	13.3	13.1	12.6	12.8	13.6	12.6		83.9	84.5	83.5	83.5	83.7	83.3	84.3	83.3	81.7	41.4	13	XM_020643138.1_Labrus_bergylta
14	20.8	16.3	11.7	15.8	16.0	15.8	17.4	15.1	13.8	16.8	15.3	17.4	18.4		85.1	97.4	97.6	88.7	97.2	90.5	87.3	84.7	39.6	14	XM_020638243.1_Labrus_bergylta
15	18.3	15.5	18.9	15.0	15.2	15.0	15.5	14.8	15.0	16.0	13.5	16.3	17.7	16.8		84.7	84.5	84.1	84.9	82.5	83.9	84.5	39.6	15	XM_020656609.1_Labrus_bergylta
16	21.3	17.3	11.9	16.8	17.1	16.8	18.6	15.6	14.3	17.3	16.4	17.9	18.9	2.7	17.3		97.0	89.5	99.4	91.1	87.3	84.3	39.0	16	AF503590.1_Notothenia_coriiceps
17	21.9	17.4	11.5	16.9	17.1	16.9	18.3	15.6	13.8	17.4	15.9	17.4	19.0	2.5	17.6	3.1		89.7	96.4	91.5	87.5	84.7	39.8	17	AY735013.1_Epinephelus_coioides
18	21.1	17.9	11.7	17.3	17.6	17.4	17.7	14.3	16.1	17.1	15.3	17.1	18.6	12.4	18.0	11.5	11.2		89.3	90.5	88.9	85.3	39.0	18	XM_020639600.1_Labrus_bergylta
19	21.6	17.6	11.7	17.1	17.3	17.1	18.9	15.3	14.5	17.1	16.1	17.6	19.2	2.9	17.0	0.6	3.7	11.7		90.9	87.7	84.5	39.0	19	AF503589.1_Trematomus_bernacchii
20	22.2	16.6	8.6	16.1	16.3	16.1	16.5	15.3	15.1	16.3	16.6	15.8	17.8	10.2	20.2	9.5	9.1	10.3	9.8		88.1	84.3	39.6	20	XM_020629999.1_Labrus_bergylta
21	23.0	17.8	14.1	17.3	17.6	17.4	17.9	16.9	16.3	16.8	16.0	16.5	19.1	14.2	18.3	14.2	13.9	12.2	13.7	13.2		84.7	39.4	21	XM_020638683.1_Labrus_bergylta
22	23.2	19.3	22.0	18.8	19.0	18.8	19.7	17.7	17.2	18.5	17.8	18.8	21.2	17.3	17.5	17.8	17.3	16.5	17.5	17.7	17.3		38.0	22	XM_020657440.1_Labrus_bergylta
23	6.4	5.0	19.6	4.6	4.6	4.6	5.1	6.0	3.6	0.9	6.5	1.3	12.0	16.8	16.6	18.4	16.2	18.3	18.4	16.7	17.2	21.1		23	HQ906886.1_Sebastiscus_marmoratus
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		

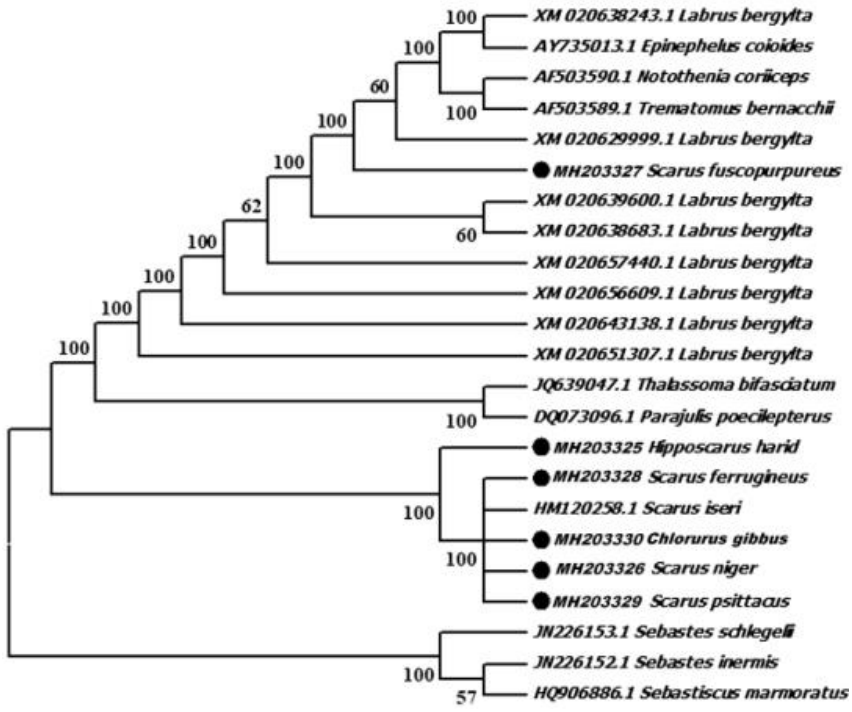


(a, NJ)

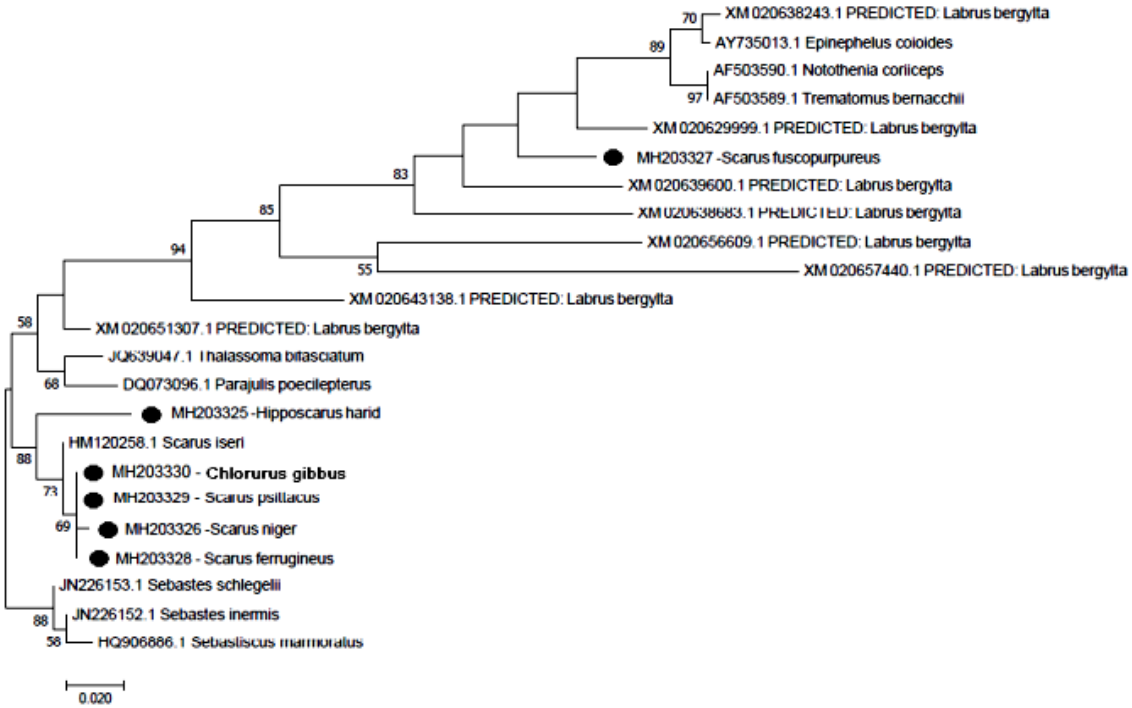


(b, ME)

Fig. 3. Phylogenetic trees showing the genetic relationships among members of the Scaridae sequences of the α-actin gene (ACTA 1) and with other 14 percoid species (a) Neighbor-Joining (NJ) and (b) Minimum Evolution (ME). Trees were drawn with amino acid sequences of selected α-actin using MEGA7 software (ver. 7.0.18). including three species of order Scoraeniformes as outgroups. Each node was tested by bootstrap method (1000 replicates), and only bootstrap values of 50% or above were shown here.



(a, MP)



(b, ML)

Fig. 4. Phylogenetic trees showing the genetic relationships among members of the Scaridae sequences of the α-actin gene (ACTA 1) and with other 14 percoid species (a) Maximum Parsimony (MP) and (b) Maximum-Likelihood (ML) method. Trees was drawn with amino acid sequences of selected α-actin isoforms using MEGA7 software (ver. 7.0.18). including three species of order Scoraeniformes as outgroups. ach node was tested by bootstrap method (1000 replicates), and only bootstrap values of 50% or above were shown here.

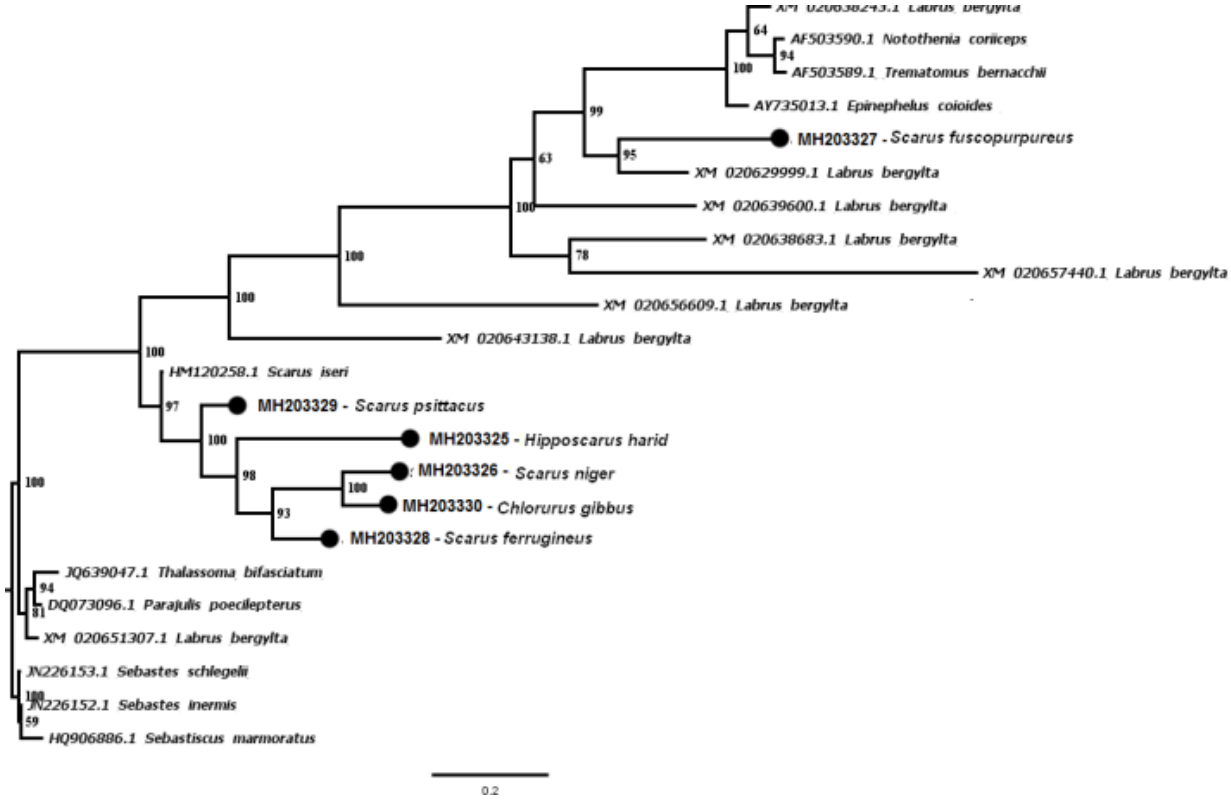


Fig. 5. Phylogenetic relationships among members of the Scaridae inferred from actin gene (ACTA 1) following analysis using Bayesian Inference(BI) method. The numbers above or below branches indicate bootstrapping values (1000 Replications). The percentage of replicate trees are indicated for all nodes.

The present paper will help in support new paths to the future of systematic Ichthyology, and probably in resolving evolutionary relationships. Our study for alpha-actin gene was provided several quite long sequences for the gene alpha actin of the tested samples, which allowed us to make a molecular taxonomic and phylogenetic evaluation for tested fishes on this basis. However, taking into consideration the large diversity in *Scarus fuscopurpureus* species. Five different tree building method (NJ, ME and MP, ML and BI) constructed using Kimura 2-parameter distance shows *Hipposcarus harid* species being having the basic and longest branch length that it consideres the most ancestral of all species considered, while the *Scarus fuscopurpureus* forms completely separate clade indicating this species has highly diverged from the rest of the scarus species (Fig 3-5) and may share advanced character with Labrus species and may be primitive characters with Scarus species.

Our own data show that the investigated representatives of the family Scaridae formed a monophyletic clade. The BI tree showed the difference compared to the NJ, ME, MP and ML trees mostly in somewhat there is deference in the position of *Scarus psittacus* and *Hipposcarus harid* in the Scarus clade. Nevertheless, all trees showed 4 major properties: (1) a separate cluster for outgroup species, (2) *Scarus* clade forming basal clade for the rest families of perciformes. 3) *Scarus fuscopurpureus* formed a separate clade with *Labrus bergylta* far from *scarus* species that indicat *Scarus fuscopurpureus* more close to genus Labrus than genus Scarus. 4) *Epinephelus coioides* also, form a clade with *Labrus bergylta* that indicate it close to genus *Labrus*. 5) *Notothenia coriiceps/*

Trematomus bernacchii form one clad in the all trees that indicate they belong to a separate family that close to family Labridae than family Scaridae. To our knowledge, the monophyletic origin of most families in the order Perciformes has not yet been investigated thoroughly.

DISCUSSION

The present study compared six parrotfish samples obtained from the Red Sea in Egypt, both within the samples and with other previous studies by means of a sequence analysis of the alpha actin gene. Approximately 900 base pair (bp) fragments of the nuclear DNA alpha skeletal muscle acten gene were amplified and then sequenced from the tested samples, and 14 samples of other family of Order Perciformes and three outgroup from Order Scoraeniformes (Teleosti) were obtained from Gene Bake/NCBI to use in this study. The current study revealed that the A+T nucleotide ratio for the six species *Hipposcarus harid, Scarus niger, Scarus fuscopurpureus, Scarus ferrugineus, Scarus psittacus* and *Chlorurus gibbus* are (46.8, 45.8, 52.4, 44.7, 45.2, 44.4 and 46.6) respectively. *Scarus fuscopurpureus* that formed a complete separate clade far from the rest of scarus species showed a high A+T bias that may be confirms a common phenomenon in fish, a high in an A+T nucleotide bias, when present this tends to accumulate in hyper variable sites (Simon, 1991 and Çiftci 2013).

Phylogenetic Analysis:

Our results In agreement to the known phylogenetic positions of perciformes, the phylogenetic relationships of the species in this order are still controversial (Kaufman and Liem, 1982, Bellwood 1986, Stiassny and Jensen 1987, Smith *et al*., 2008 and Çiftci 2013). The phylogenetic relationships among six species of parrotfish (subfamily Scarinae) and available related fourteen species of Order Perciformes are resolved with a combination of alpha actin gene. All resulting trees (Figs. 3-5) clarify that *Chlorurus gibbus* is fused with genus *Scarus (Scarus niger Scarus ferrugineus, Scarus psittacus* and *Scarus iseri*) to form one clade that indicate as monophyletic clade with strong support values (NJ=87, ME=93, MP=100, ML=88 and BI=100) to the exclusion of outgroup taxa (three species of order Scopaenidae, Actinopterygii). *Chlorurus gibbus* previously classified and putted in genus Scarus, this results in agreement with The previous attempt at a cladistic analysis of scarid taxa also by (Bellwood 1986) who examined the phylogeny of genera in the subfamily Scarinae and sugesste that the two genera *Chlorurus* and *Scarus* placed in *Scarus*. Our results in this study agrees closely with all previous studies and that of (Bellwood, 1994) who reported the genera *Chlorurus* and *Scarus* as being two distinct monophyletic lineages. (Smith et al., 2008) suggested there is relatedness among species of the two genus *Chlorurus* and *Scarus* that have been based on color pattern and distribution information. Our result revealed also, *Hipposcarus harid* formed a basal clade with five scarinae species (*Scarus niger, Scarus ferrugineus, Scarus psittacus, Chlorurus gibbus* in addition to *Scarus iseri* from gene bank) with strong support (NJ=93, ME=96 and MP=100, ML=88 and BI=98) this results agrees closely with that of (Streelman *et al.* 2002) who regard that *gen*us *Hipposcarus* froms the ancestral splits of the scarinae genera and formed the basal clad of the parrotfish. On the other, (David and Bellwood 1994) reported *gen*us *Hipposcarus* being the immediate sister group of *Scarus*.

On the other hand, In BI tree only *Scarus psittacus* formed a basal clade with *Hipposcarus harid* clad with strong support (BI=100) this finding in agreement with (Smith et al., 2008) who reported *Scarus psittacus* is the sister to the rest of the *Scarus* clade in tree, and it

is one of the most broadly distributed species among all parrotfish, occurring from the the Red Sea to South Africa, all samples of our study were collected from the Red Sea. Furthermore, all our trees pattern revealed *Scarus fuscopurpureus* is fused with *Labrus bergylta* In a separate clade far from Scarus species, that indicate *Scarus fuscopurpureus* is very close to family Labridae than family Scaridae, this result in support that reported by (Kaufman & Liem, 1982 and Stiassny & Jensen, 1987) those authors reported, it is widely accepted that the Labridae and Scaridae represent a monophyletic assemblage. Some workers followed (Kaufman and Liem 1982) included the Scaridae in the Labridae, eg, (Stiassny and Jensen 1987). Others questioned this decision. On the other hand, (Richards and Leis 1984) cautioned against the fusion of the two families, based on observations on the early life history characters of the two families. (Bruce and Randall 1985) retained the Scaridae arguing that they are unique. Other studies suggested, the genus *Scarus* appeared to be paraphyletic, and it was suggested that the genus was comprised of two distinct phyletic lineages. These two groups were identified as separate functional groups by (Bellwood, 1986) and, subsequently, by (Bellwood and Choat 1990), this distinction also appeared to have a phylogenetic basis (Stiassny and Jensen 1987). To our knowledge, the monophyletic origin of most families in the order Perciformes has not yet been investigated thoroughly, there is a need for further examination of this question (Smith and Craig 2007).

Our results support the previous studies that suggested that The high degree of similarity among the nucleotide sequence and amino acid residues of the actin gene of scaride species and those of other percoid species is, unsurprisingly, not only a consequence of the narrow evolutionary divergence between several species but is also due to the origin of the actin genes. It seems that actin isoforms are encoded by a set of structurally related genes that developed as a consequence of gene duplication followed by functional divergence (Hightower and Meagher, 1986), resulting in highly conserved proteins.

Conclusion:

The present study strongly supports the monophyly of the Scaridae and indicate that the traditional characters are inadequate to recent classification. To our knowledge, the monophyletic origin of most families in the order Perciformes has not yet been investigated thoroughly. The actin gene proved to be a valuable phylogenetic marker, thus the investigation of more genes e.g. actin gene is required for the further development of a natural classification of the perciformes. In five resulting trees (Figs 3-5) Hipposcarus harid clade is a distinct clade which basal to all other subfamily scarinea and they are resolved as a monophyletic clade. Scarid clad is represented in all trees as a distance separated clad without interrelationships with other clads and have a strong support. *Chlorurus gibbus* is fused with scarus species in one clade of all trees that indicate it close to Scarus species and we prefer the name is *Scarus gibbus* than *Chlorurus gibbus* as named previously. The species *Scarus fuscopurpureus* are closely related to Labrid species and well separated from the Scarid species, this gives an indicator that *Scarus fuscopurpureus* share Labrid species in more characters than Scarus species.

REFERENCES

Adriane P. W., Fábio E., Severino, Flávia T., Presti, Andréia B., Poletto and Martins C., (2007). Partial molecular characterization of the Nile tilapia (*Oreochromis niloticus*) a-cardiac muscle actin gene and its relationship to actin isoforms of other fish species. Genet. Mol. Biol. vol.30 no.4.

Bellwood DR. and Choat JH. (1990). A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. Environmental Biology of Fishes 28: 189-214.

Bellwood DR (1994). A Phylogenetic Study of the Parrotfishes Family Scaridae (Pisces: Labroidei), with a Revision of Genera. Records of the Australian Museum Supplement 20.

Bellwood DR. (1986). The functional morphology, systematics and behavioural ecology of parrotfishes (family Scaridae). PhD dissertation, James Cook University of North Queensland, Townsville, 489 pp.

Bleeker P. (1859b). Enumeratio specierum piscum hucusque in Archipelago Indico observatarum, habitationibus citationibusque, ubi descriptiones earum recentiores reperiuntur, nec non speciebus Musei Bleekeriani Bengaleensibus, Japonicus, Capensibus Tasmanicisque. Acta Societatis Scientiarum Indo-Neerlandicae 6, xxxvi + 276.

Bleeker P. (1862). Atlas Ichthyologique des Indes Orientales Neerlandaises. I: Scaroides et Labroides 1, 168 pp, 48 pIs. Frederec Muller Amsterdam.

Bruce RW. & J.B. Randall (1985). Revision of the Indo-Pacific Parrotfish Genera *Calotomus* and *Leptosearus.* Indo-Pacific Fishes Number 5, 32 pp.

Çiftci Y, Eroğlu O and Firidin Şirin (2013). Mitochondrial Cytochrome b Sequence Variation in Three Sturgeon Species (*A. stellatus* Pallas, 1771, *A. gueldenstaedtii* Brandt, 1833, *H. huso* Linnaeus, 1758) from the Black Sea Coasts of Turkey. Turkish Journal of Fisheries and Aquatic Sciences 13: 291-303.

Cuvier, G. and Valenciennes A. (1840). Histoire Naturelle des Poissons, 14, pp. 1-464, pIs 389-420. Pitois-Levrault. Paris.

David R and Bellwood (1994). A Phylogenetic Study of the Parrotfishes Family Scaridae (Pisces: Labroidei), with a Revision of Genera. Records of the Australian Museum. 1-86.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Forssktll, P. (1775). Descriptiones Animalium Avium, Amphibiorum, Piscium, Insectorum, Vermium; quae in Itinere Orientali observavit. 164 pp., 43 pIs, Molleri Havniae.

Fitch WM. (1971). Towards defining the course of evolution: Minimum change for a specific tree topology. Systematic zoology, 20: 406-416.

Giinther A (1862). Catalogue of the Acanthopterygii, Pharyngognathi and Acanthini in the Collection of the British Museum. Vo!. 4. Catalogue of Fishes in the British Museum, xxii + 534 pp., British Museum (Natural History), London.

Glasauer SM and Neuhauss SC. (2014). Whole-genome duplication in

teleost fishes and its evolutionary consequences. Mol Genet Genomics. 289(6):1045-60.

Gronow, LT. (1763). Zoophylacii Gronoviani fasciculus primus, exhibens animalia quadrupeda, amphibia atque pisces, quae in museo suo adservat, rite examinavit, systematice disposuit, descripsis atque iconibus illustravit L.T. Gronovius, lU.D . Leiden: Haak & Luchtmans, 136 pp.

Giinther A. (1862). Catalogue of the Acanthopterygii, Pharyngognathi and Acanthini in the Collection of the British Museum. Vo!. 4. Catalogue of Fishes in the British Museum, xxii + 534 pp., British Museum (Natural History), London.

Hightower RC and Meagher RB (1986) The molecular evolution of actin. Genetics 114:315-332.

Huelsenbeck JP and Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17 (8): 754-755.

Johnston IA. (2006). Environment and plasticity of myogenesis in teleost fish. J Exp Biol. 209:2249–64.

Johnston IA, Bower NI and Macqueen DJ. (2011). Growth and the regulation of myotomal muscle mass in teleost fish. J Exp Biol. 214:1617–28.

Johnston IA, Macqueen DJ and Watabe S. (2008). Molecular biotechnology of development and growth in fish muscle. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard TD, Kaiser MJ, editors. Fisheries for global welfare and environment, 5th

world fisheries congress: TERRAPUB; p. 241–62.

Jordan DS. and Gilbert CH. (1882). A synopsis of the fishes of North America. Bulletin of the United States National Museum 16: i-lvi, 1-1018.

Kaufman L.S. and Liem KF. (1982). Fishes of the suborder Labroidei (Pisces perciformes): phylogeny, ecology, and evolutionary significance. Breviora 472: 1-19.

Kiessling A, Ruohonen K and Bjørnevik M. (2006). Muscle fibre growth and quality in fish. Arch Tierz Dummerstorf. 49: 137–46.

Kim S, Karsi A, Dunham RA and Liu Z (2000). The skeletal muscle □-actin gene of channel catfish (*Ictalurus punctatus*) and its association with piscine specific SINE elements. Gene 252:173-181.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.Mol. Evol., 6, 111–120.

Kner R (1860). Zur Charakteristik und Systematik der Labroiden. Osterreichische Akademie de Wissenschaften. Sitzungsberichte Vienna 40 (7): 41-57, 2 pIs.Kumar S, Stecher G and Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. Jul, 33(7):1870-4.

Kusakabe T, Araki I, Satoh N and Jeffrey WR (1997). Evolution of chordate actin genes: Evidence from genomic organization and amino acid sequences. J Mol Evol 44: 289-298.

Lee SY, Lee J and Nam YK (2017). Molecular characterization of three muscle alpha actin genes in mud loach (Misgurnus mizolepis; Cypriniformes). Fisheries and Aquatic Sciences 20: 27. 1-11.Liem, KF. and

Greenwood PH. (1981). A functional approach to the phylogeny of the pharyngognath teleosts. American Zoologist 21: 83-101.

Mathana P, Raj ST, Nair RK and Selvamohan T. (2012). Seasonal changes in the biochemical composition of four different tissues of red spotted emperor Lethrinus lentjan (Family: Lethrinidae). Anal Biol Res. 3: 5078–82.

Mudalige W, Jackman DM, Waddleton DM and Heeley DH. (2007). A vertebrate slow skeletal muscle actin isoform. FEBS. 274:3452–61.

Nelson JS (2006). Fishes of the World. 4th edition. John Willey and Sons Inc., New York, 601 pp.

Perrin BJ and Ervasti JM. (2010). The actin gene family: function follows isoform. Cytoskeleton. 67: 630–4.

Randall JE and Ormond RF. (1978). On the Red Sea parrotfishes of Forsskill, Searus psittaeus and S. ferrugineus. Zoological Journal of the Linnean Society 63 (3): 239-248.

Randall JE. (1982). The diver's guide to Red Sea reef fishes biblios pub distribution service.

Richards WL. (1984). Kinds and abundances of fish larvae in the Caribbean Sea and adjacent areas. NOAA Tech. Rep. NMFS SSRF-776: 1-54 Robertson, D. R. (1991).

Rzhetsky A and Nei M (1982). A Simple Method for Estimating and Testing Minimum-Evolution Trees Mol. Biol. Evol., 9, 945–967.

Saitou N. and Nei M.(1987). Mol. Biol. Evol., 4, 406-425.

Simon, C. (1991). Molecular systematics at the species boundary: exploiting conserved and variable regions of the mitochondrial genome of animals via direct sequencing from amplified DNA. In: G.M. Hewitt, A.W.B. Johnston, J.P.W. Young, (Eds.), Molecular Techniques in Taxonomy.

Smith PJ, Mcveagh SM, and Steinke D. (2008). DNA barcoding for the identification of smoked fish products. Journal of Fish Biology. 72, 464–471.

WL. and Craig MT (2007). Casting the Percomorph Net Widely: The Importance of Broad Taxonomic Sampling in the Search for the Placement of Serranid and Percid Fishes Copeia (1):35-55.

Stiassny, MLJ. and Jensen JS. (1987). Labroid interrelationships revisited: morphological complexity, key innovations, and the study of comparative diversity. Bulletin of the Museum of Comparative Zoology 151: 269-319.

Streelman JT., Alfaro M., Westneat MW., Bellwood DR and Karl SA. (2002): Evolutionary history of the parrotfishes: biogeography, ecomorphology, and comparative diversity. Evolution, 56(5): 961-971.

Takahashi K and Nei M. (2000). Efficiencies of fast algorithms of phylogenetics inference under the criteria of maximum parsimony, minimum evolution and maximum likelihood when a large number of sequences are used. Molecular biology and evolution, 17 (8): 1251-1258.

Vandekerckhove J and Weber K (1979). The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle, and rabbit slow skeletal muscle: A protein-chemical analysis of muscle actin differentiation. Differentiation 14:123-133.

ARABIC SUMMARY

التقسيم العرقى الوراثى الجزيئى لبعض أنواع اسماك الببغاء
(بيرسيفورمس، اسكاريدى) من البحر الأحمر باستخدام جين ألفا أكتين

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تنتمى أسماك الببغاء الى عائلة سكاريدى (Scaridae) التى هى واحدة من عائلات رتبة بيرسيفورمس (Perciformes) من طائفة الأسماك العظمية (Teleosti) وتتميز اسماك الببغاء بالوانها الزاهية وأسنانها التى تجعل فمها يشبه فم الببغاء بالإضافة إلى أهميتها الاقتصادية. وهي اسماك بحرية تعيش في المياه الدافئة بين الشعاب المرجانية في المحيطات والبحار.

ومن الناحية التصنيفية فان التصنيف الحديث يسجل أن رتبة بيرسيفورمس تضم ١٥٦ عائلة، منها عائلة سكاريدى التى تعتبر من العائلات الصغيرة نسبيا وتضم حوالى تسعون نوعا صنفت فى عشرة أجناس. وقد كان جنس سكاريوس (*Scarus*) اول جنس تم تصنفه فى عائلة سكاريدى. وحتى الآن توجد مشاكل عديدة فى تصنيف الأنواع والعائلات فى رتبة بيرسيفورمس باستخدام التصنيف التقليدى بالرجوع إلى الشكل الظاهرى والتركيب التشريحى، فهى اسماك لها القدرة على التغيير فى الوانها و حتى فى اجناسها.

لذا كان من الواضح ان التصنيف الجزيئى والابحاث التى توضح التقسيم العرقى الوراثى للأنواع ضروريا استخدامه فى عائلات رتبة بيرسيفورمس. وقد كان الهدف من الدراسة الحالية القاء الضوء على التصنيف الجزيئى والتقسيم العرقى الوراثى لستة انواع من طويئفة سكارينى تنتمى الى ثلاثة اجناس هم (*Scarus, Chlorurus and Hipposcarus*) وذلك باستخدام تفاعل سلسلة البلمرة لجزىء جين الفا اكتين من العضلات الهيكلية للدنا النووية للستة أنواع *(Scarus niger, Scarus fuscopurpureus, Scarus ferrugineus, Scarus psittacus, Chlorurus gibbus* and *Hipposcarus harid*) وذلك باستخدام بادئين امامى وعكسى بطول ٢١ نيوكليوتيدة من جين الفا اكتين لمقارنة التباين الوراثى والمسافة الوراثية بين الانواع الستة وقد عمل البادئان بنجاح فى تفاعل سلسة البلمرة وتم الحصول على حزمة واحدة لكلا من الستة انواع تشير الى انها جزيء جين الفا اكتين. وقد تم تحليل تسلسل النيوكليوتيدات لجين الفا اكتين لكلا من العيينات الستة قيد الدراسة وتم تسجيلها فى بنك الجينات لاول مرة. ثم تم مقارنة تسلسل النيكليوتيدات فى الجين باستخدام برنامج ميجا ٧ لدراسة التباين الوراثى والمسافة الوراثية بين الانواع الستة وعلاقتها بالأربعة عشر نوعا من اسماك بيرسيفورمس الأخرى (واحدة فقط من عائلة سكاريدى قد تم تسجيل تسلسل النيكليوتيدات لجين الاكتين لها مسبقا فى بنك الجينات والثلاثة عشر الاخرى من ثلاث عائلات من رتبة بيرسيفورمس (عشرة من عائلة لابيريدى Labridae واثنان من عائلة نوتوثينيدى Nototheniidae واثنان من عائلة Serranidae) قد تم تسجيلهم مسبقا فى بنك الجينات بالاضافة إلى ثلاثة اسماك كمجموعة من الخارج من رتبة Scoraeniformes وذلك لتحديد التباين الوراثى بين الستة انواع من اسماك الببغاء (عائلة اسكاريدى) وعلاقتها بالانواع من العائلات القريبة من نفس الرتبة. ولتحقيق هذا الهدف تم رسم خمسة انواع من الشجرات التطورية باحصائيات مختلفة للحصول على نتائج دقيقة.

وقد أشار التحليل الإحصائى الدقيق للمسافات الوراثية والشجرة الوراثية للأصول أن خمسة انواع من مجموعة الاسماك التصنيفية Scarus التى تضم Scarus niger, Scarus ferrugineus, Scarus psittacus, Chlorurus gibbus and Scarus iseri وحيدة الاصل monophylytic . كما أن النوع Hipposcarus harid هو الجد السلفى للأنواع الأخرى تحت الدراسة من جنس سكاريوس Scarus . اما بالنسبة للنوع Scarus ferrugineus فقد اظهرت النتائج انها شقيقة لجنس لابريوس Labrus وانها ليست لها اى علاقة بجنس سكاريوس وذلك فى الخمسة اشجار التطورية. وجدير بالذكر ايضا ان النوع Chlorurus gibbus اندمجت تماما مع جنس سكاريوس فى الخمسة اشجار التطورية مما يوضح ان هذه المجموعة من الاسماك هى وحيدة الاصل وقد نشأت من اصل واحد لذا يفضل ان يستخدم اسمها Scarus gibbus وهذا الاسم قد سميت به سابقا حين نسبت الى جنس سكاريوس. وعلى حد علمى ان النوعان سكاريوس و كلوريورس قد دمجا معا فى بعض الدراسات وانفصلا لجنسين مستقلين فى دراسات اخرى وتكررذلك عدة مرات.

وقد اسفرت التحليلات المختلفه عن وجود درجات متفاوته من القرابه والاختلاف بين تلك الانواع وأن ما تم الحصول عليه من نتائج فى هذا البحث عن الأنواع تحت الدراسة تؤكد مدى أهمية أستخدام تقنية دلالات التكبير لجزيئات الحمض النووى DNA بما فى ذلك مؤشر جزىء جين الاكتين فى التصنيف والتعرف على الأنواع والسلالات التى قد تنشأ من الأنواع الموجودة وتحديد علاقات القرابة وتطور الانواع فيما بينها كدعم للطرق التقليدية فى علم التصنيف.