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Phylogenetic Analysis and Genetic Diversity Among Some Red Sea Reef Fishes (Perciformes: Labridae) Based on Partial 16S Mitochondrial Gene Sequences

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ABSTRACT

This study investigated the sequence variation and phylogenetic relationships among 14 labrid fish species (Family: Labridae) from the coral reefs of Hurghada, the Red Sea, Egypt, using a fragment of the 16S mitochondrial gene (16S mt-rDNA). Comparison with similar species from the GenBank/NCBI published sequences was conducted. Sequencing analysis and phylogenetic tree construction employed maximum likelihood, neighbor-joining, and maximum parsimony methods. The results demonstrated the efficiency of 16S mt-rDNA in illustrating the genetic variation, indicating close genetic relations and shared ancestry among the studied genus and species, viz. *Epibulus* with *Cheilinus* and genus *Gomphosus* with *Thalassoma*. The phylogenetic hypotheses (ML, NJ and MP) produced similar topologies with slight differences in the bootstrap support values. Two main lineages, cheilines and julidines, each containing clades of genera, revealed a monophyletic group of labrid species. Distinct clades and clusters among genera highlighted evolutionary relationships within the Labridae family. The 16S gene effectively elucidates genetic diversity and phylogenetic patterns, underscoring its utility as a molecular marker for reef fish phylogenetic studies. The development of such molecular markers helps detect biodiversity and understand molecular phylogenetic relationships in this important aquatic biological resource.

INTRODUCTION

The Labridae family, commonly known as wrasses, stands out as one of the most widespread and conspicuous fish families on tropical reefs worldwide. Wrasses exhibit a remarkable diversity in colors, forms, and sizes, often displaying significant variations, even within a single species **(Parenti & Randall, 2011)**. Representing the third largest family within the Perciformes order, Labridae comprises over 600 species distributed across 82 genera, showcasing a wide array of morphological and ecological adaptations

in tropical and subtropical environments **(Sanderson, 1990; Parenti & Randall, 2000; Tatom-Naecker & Westneat, 2018; Ghezelayagh *et al*., 2022; Baldwin *et al*., 2023)**.

Members of the Labridae family exhibit a diverse range of trophic behaviors, playing prominent roles in reef communities as herbivores, planktivores, piscivores, durophages, ectoparasite feeders, and consumers of various reef-associated invertebrates **(Randall, 1983; Gomon & Randall, 1984; Lieske & Myers, 1994; Floeter *et al.*, 2007; Khalaf-Allah, 2013; AL-Zahaby, 2015; Sampaio *et al*., 2016; Pradhan & Mahapatra, 2017)**. This dietary variation is mirrored in the diverse functional morphology observed within the family **(Westneat, 1995; Burress & Wainwright, 2019; Evans *et al*., 2019)**.

The Red Sea coasts represent one of the highest degrees of endemism and diversity among the coral reef fishes globally **(Alwany & Stachowitsch, 2007)**. While the taxonomic knowledge of the Red Sea ichthyofauna is relatively well-developed compared to other tropical Indo-Pacific regions, research on the community structure of shore fishes remains less investigated **(Tatom-Naecker & Westneat, 2018; Ghezelayagh *et al*., 2022; Baldwin *et al*., 2023)**. Within the Red Sea reefs, the Labridae family emerges as the most species-rich family after the damselfishes and ranks among the top three most abundant families in the northern Red Sea **(Alwany & Stachowitsch, 2007)**, thus underscoring its significance in phylogenetic investigations. Previous studies have reported on the phylogenetic relationships of several subfamilies of the labrid fish **(Westneat, 1993; Bellwood, 1994; Gomon, 1997; Hanel *et al.*, 2002; Streelman *et al*., 2003; Clements *et al.*, 2004; Barber & Bellwood, 2005; Westneat & Alfaro, 2005; Phillips *et al*., 2016)**.

Genetic analysis offers opportunities to enhance data accuracy and accessibility of species characteristic information. With the limitations associated with morphological investigations, genotypic studies have emerged as a valuable alternative for exploring species-level relationships **(Syam & Syahputra, 2016)**. However, more precise, sensitive molecular identification techniques are required to elucidate the true evolutionary relationships among animal species including fish **(Ramadan, 2011; Saad *et al*., 2012)**. DNA barcoding techniques, in particular, provide simple and reliable approaches for species identification through standardized genomic regions, facilitating the detection of genetic variations among fish genera, species, and populations **(Hebert *et al*., 2004; Ward *et al*., 2005; Saad & Abd El-Sadek, 2017; Saad, 2019)**.

Mitochondrial DNA variants serve as valuable barcoding systems for studying fish speciation and other aquatic taxa, offering considerable potential in genetic population analysis and evolution studies **(Miya & Nishida, 2000; Saad *et al*., 2019)**. The interest in mitochondrial DNA stems from its ability to describe the maternal inheritance, rapid evolution, and unique recombinant DNA events, making it a valuable

tool for reconstructing phylogenetic relationships among fish species **(Craig *et al*., 2001; Ding *et al*., 2006; Ghorashi *et al*., 2008; Nematzadeh *et al*., 2013; Qi *et al*., 2013)**.

Molecular phylogenetics has significantly influenced the Labrids taxonomy, providing consistent resolutions of phylogenetic relationships through multi-locus data analysis **(Clements *et al*., 2004; Westneat & Alfaro 2005; Smith *et al*., 2008; Choat *et al*., 2012; Aiello *et al*., 2017; Hughes *et al*., 2023)**. Utilizing the 16S mitochondrial gene as a marker, previous studies have examined phylogenetic relationships within the Labridae family, such as those conducted by **Bernardi *et al.* (2004)** on the genus *Thalassoma*, **Westneat *et al.* (2005)** across major Labrid clades, and more recent work by **Baldwin *et al.* (2023)** describing new species and providing phylogenetic placements.

This study aimed to investigate sequence variations and molecular phylogenetic relationships among some species of the coral reef fishes from the Labridae family inhabiting the Red Sea coasts in Egypt, utilizing partial gene sequencing of the 16S mitochondrial DNA.

MATERIALS AND METHODS

Sample collection and identification

A total of 14 specimens of the reef fish belonging to the family Labridae (*Epibulus insidiator, Cheilinus lunulatus, Cheilinus fasciatus, Cheilinus cholourus, Oxycheilinus unifasciatus, Oxycheilinus digramma, Hemigymnus fasciatus, Hemigymnus melapterus, Cheillio inermis, Thalassoma rueppellii, Gomphosus caeruleus, Coris aygula, Stethojulus bandanensis*, and *Novaculichthys taeniourus*) were collected from the Red Sea near Hurghada City, Egypt. Morphological identification of each fish was conducted according to **Randall (1983)**. Muscle tissues were isolated from each specimen and preserved at -80 °C until further processing.

DNA extraction and PCR amplification

Genomic DNA extraction from the preserved samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany) following the manufacturer's instructions. A partial sequence of the mitochondrial 16S mt-DNA gene was amplified using the primers 16S_L (CGCCTGTTTATCAAAAACAT) and 16S_H (CCGGTCTGAACTCAGATCACG) **(Palumbi, 1996)** (100pmol/ µl, Macrogen Inc., Seoul, Korea).

Polymerase chain reaction (PCR) was carried out using a thermocycler (PeQLab, Primus 25) in a total volume of 50µl, comprising 25µL Taq PCR Master Mix Kit (Qiagen, Germany), 1µL of each forward and reverse primer, 1µL of genomic DNA template, and 22µL Nuclease-Free Water. The PCR amplification conditions included an initial denaturation at 94°C for 4min, followed by 35 cycles of denaturation at 94°C for

1min, annealing at 56°C for 1min, and extension at 72°C for 1min, with a final extension at 72 °C for 10min.

Five microliters of each PCR product were mixed with 2µl of 5X gel loading dye (Qiagen) and loaded into a 1.5% agarose gel stained with ethidium bromide, alongside 5µL of 100bp DNA ladder (Qiagen). Gel electrophoresis was conducted using a documentation system (MicroDoc Cleaver Scientific Ltd, United Kingdom) to confirm the presence of PCR product bands.

Sequencing analysis

The amplified DNA was purified using a QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's protocol. Sequencing reactions were performed in an MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the manufacturer's protocols with the same primers used for PCR amplification. All sequencing procedures were carried out by Macrogen Inc., Seoul, Korea.

Data analysis

The newly generated 16S mt-DNA sequences were submitted to the GeneBank, National Center for Biotechnology Information (NCBI) (Table 1). These sequences were aligned with previously published sequences of the most similar Labridae taxa obtained from a Blast search on the GenBank NCBI database (Table 1). Alignments were performed using the Clustal W tool, and conserved region analysis was conducted using BioEdit software version 7.2.5 **(Hall, 1999)**, as illustrated in Fig. (1).

The resulting alignments were manually refined, and sequences from the studied gene were trimmed to the size of the smallest fragment to minimize the amount of introduced missing data. Pairwise distances were calculated using MEGA X software version 10.2.2 **(Kumar *et al.*, 2018)**, as shown in Table (2).

A phylogenetic tree was constructed using three different methods implemented in MEGA X software version 10.2.2 **(Kumar *et al.*, 2018)**. Methods applied were: Neighbor-joining (NJ) **(Saitou & Nei, 1987)**, maximum likelihood (ML) **(Tamura & Nei, 1993)**, and maximum parsimony (MP) **(Nei & Kumar, 2000)**. Moreover, thebranch relative support was assessed using the bootstrap test with 1000 replicates **(Felsenstein, 1985)**, all methods are depicted in Figs. (2, 3). Sequence divergences were calculated using Kimura's two-parameter distances **(Kimura, 1980)**, and the majority-rule consensus tree from the parsimony analysis was presented.

Table 1. The 14 studied labrid fish species of the current study and 17 species from the most similar published sequences of family Labridae taxa with their (GeneBank/ NCBI) submitted accession numbers. *Epinephelus polyphekadion* is used as an outgroup species.

No.	Species	Accession number	Reference
1	*Epibulus insidiator*	MW332305	Present study
2	*Epibulus insidiator*	JF457451	GeneBank
3	*Epibulus brevis*	KY815393	GeneBank
4	*Hemigymnus fasciatus*	MW332308	Present study
5	*Hemigymnus melapterus*	MW332309	Present study
6	*Hemigymnus fasciatus*	JF457499	GeneBank
7	*Hemigymnus melapterus*	DQ076711	GeneBank
8	*Cheilio inermis*	MW332310	Present study
9	*Cheilio inermis*	JF457361	GeneBank
10	*Thalassoma rueppellii*	MW332311	Present study
11	*Thalassoma genivittatum*	JF457670	GeneBank
12	*Thalassoma lutescens*	KY815461	GeneBank
13	*Cheilinus lunulatus*	MW332313	Present study
14	*Cheilinus fasciatus*	MW332314	Present study
15	*Cheilinus chlorourus*	MW332318	Present study
16	*Cheilinus fasciatus*	JF457349	GeneBank
17	*Cheilinus trilobatus*	JF457358	GeneBank
18	*Cheilinus abudjubbe*	KY815371	GeneBank
19	*Cheilinus lunulatus*	KY815373	GeneBank
20	*Coris aygula*	MW332315	Present study
21	*Coris aygula*	AY279692	GeneBank
22	*Oxycheilinus digramma*	MW332317	Present study
23	*Oxycheilinus unifasciatus*	MW332323	Present study
24	*Oxycheilinus digramma*	JF457549	GeneBank
25	*Oxycheilinus unifasciatus*	JF457554.1	GeneBank
26	*Novaculichthys taeniourus*	MW332322	Present study
27	*Novaculichthys taeniourus*	JF457546	GeneBank
28	*Gomphosus caeruleus*	MW332312	Present study
29	*Gomphosus varius*	AY279700	GeneBank
30	*Gomphosus caeruleus*	KY815396	GeneBank
31	*Stethojulis bandanensis*	MW332321	Present study
32	*Epinephelus polyphekadion*	AY947569	GeneBank

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RESULTS

The sequence data obtained from the partial 16S mitochondrial gene with accession numbers (Table 1) exhibited remarkable resolution among genera and species within the Labridae family. The trimmed sequences had a length of 526bp, containing 162 polymorphic sites and 126 parsimony informative sites, with 14 gap sites excluded. Analysis using the BioEdit program revealed the presence of six conserved regions, each with varying segment lengths (Fig. 1). Pairwise distance analysis (Table 2) highlighted notable differences between species. The highest pairwise distances were observed between the sequences of *Gomphosus varius* (AY279700) with *Oxycheilinus unifasciatus* (MW332323) (0.198), and with both species *Cheilinus chlorourus* (MW332318) and *Cheilinus abudjubbe* (KY815371) (0.197). Conversely, different sequences exhibited no pairwise distance (0.000), indicating a genetic similarity between these species.

In this study, multiple methods of phylogenetic analysis produced similar topologies of relationships among the species, with some differences in the support values (Figs. 2, 3). Such phylogenetic analyses are particularly useful in species-rich genera like the labrid fishes, which have few distinctive morphological characteristics.

The different constructed phylogenetic trees of the present study illustrated various major clades and clusters. The first major group includes the cheilines lineage genera, with distinct clusters for each species. For instance, *Epibulus insidiator* clustered with high support values (NJ = 100, ML = 97, MP = 98) and exhibited similarity to *Cheilinus fasciatus* (NJ = 78, ML = 70, MP = 74). Meanwhile, *Oxycheilinus* species formed a separate clade, indicating their evolutionary divergence from other cheilines. The species *Stethojulis bandanensis* (MW332321) of the current study is represented in a separated clade in the different constructed trees methods.

Another major group consisted of julidines lineage genera, with distinct clusters for each species. For example, the *Hemigymnus* species formed a distinct clade, as did *Gomphosus* and the *Thalassoma* species. Among julidines lineage genera the genus *Coris* form a paraphyletic clade with the two genus of the current study *Coris aygula* (MW332315) and *Novaculichthys taeniourus* (MW332322) which was clustered together with high support values (NJ, ML =100, MP =96), meanwhile *Novaculichthys taeniourus* (JF457546) was clustered to *Cheilio inermis* (MW332310.1) and *Cheilio inermis* (JF457361) with support values (NJ =81,ML=80, MP =56).

Table 2. Pairwise distance of the partial 16S mitochondrial rDNA nucleotide sequences of the labrids fish species of the current study and GenBank/NCBI similar sequences.

No.	Acc. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	MW332305																															
2	JF457451	0.000																														
3	KY815393	0.016	0.016																													
4	MW332308	0.160	0.160	0.157																												
5	MW332309	0.157	0.157	0.160	0.019																											
6	JF457499	0.165	0.165	0.162	0.016	0.019																										
7	DQ076711	0.157	0.157	0.160	0.019	0.000	0.019																									
8	MW332310	0.172	0.172	0.174	0.126	0.121	0.131	0.121																								
9	JF457361	0.172	0.172	0.174	0.128	0.123	0.133	0.123	0.002																							
10	MW332311	0.164	0.164	0.166	0.081	0.074	0.076	0.074	0.140	0.142																						
11	JF457670	0.166	0.166	0.169	0.083	0.076	0.078	0.076	0.140	0.142	0.004																					
12	KY815461	0.166	0.166	0.169	0.083	0.076	0.078	0.076	0.140	0.142	0.004	0.000																				
13	MW332313	0.068	0.068	0.076	0.176	0.170	0.180	0.170	0.178	0.178	0.181	0.184	0.184																			
14	MW332314	0.040	0.040	0.044	0.149	0.149	0.156	0.149	0.161	0.161	0.155	0.158	0.158	0.070																		
15	MW332318	0.070	0.070	0.074	0.173	0.168	0.178	0.168	0.178	0.178	0.182	0.184	0.184	0.027	0.081																	
16	JF457349	0.044	0.044	0.044	0.146	0.151	0.153	0.151	0.161	0.161	0.158	0.160	0.160	0.074	0.004	0.085																
17	JF457358	0.068	0.068	0.076	0.176	0.170	0.180	0.170	0.178	0.178	0.181	0.184	0.184	0.000	0.070	0.027	0.074															
18	KY815371	0.070	0.070	0.074	0.173	0.168	0.178	0.168	0.178	0.178	0.182	0.184	0.184	0.027	0.081	0.000	0.085	0.027														
19	KY815373	0.068	0.068	0.076	0.176	0.170	0.180	0.170	0.178	0.178	0.181	0.184	0.184	0.000	0.070	0.027	0.074	0.000	0.027													
20	MW332315	0.169	0.169	0.173	0.073	0.069	0.078	0.069	0.131	0.134	0.074	0.076	0.076	0.183	0.152	0.181	0.155	0.183	0.181	0.183												
21	AY279692	0.169	0.169	0.173	0.073	0.069	0.078	0.069	0.131	0.134	0.074	0.076	0.076	0.183	0.152	0.181	0.155	0.183	0.181	0.183	0.000											
22	MW332317	0.092	0.092	0.083	0.160	0.165	0.170	0.165	0.172	0.172	0.179	0.181	0.181	0.094	0.095	0.087	0.090	0.094	0.087	0.094	0.185	0.185										
23	MW332323	0.128	0.128	0.127	0.170	0.181	0.178	0.181	0.207	0.207	0.187	0.189	0.189	0.122	0.124	0.134	0.119	0.122	0.134	0.122	0.196	0.196	0.083									
24	JF457549	0.092	0.092	0.083	0.160	0.165	0.170	0.165	0.172	0.172	0.179	0.181	0.181	0.094	0.095	0.087	0.090	0.094	0.087	0.094	0.185	0.185	0.000	0.083								
25	JF457554	0.100	0.100	0.087	0.160	0.167	0.170	0.167	0.182	0.182	0.187	0.189	0.189	0.090	0.097	0.100	0.092	0.090	0.100	0.090	0.190	0.190	0.022	0.083	0.022							
26	MW332322	0.174	0.174	0.178	0.077	0.073	0.082	0.073	0.136	0.138	0.078	0.080	0.080	0.188	0.157	0.186	0.160	0.188	0.186	0.188	0.004	0.004	0.190	0.196	0.190	0.195						
27	JF457546	0.172	0.172	0.177	0.143	0.148	0.153	0.148	0.126	0.126	0.153	0.153	0.153	0.178	0.175	0.178	0.175	0.178	0.178	0.178	0.148	0.148	0.181	0.212	0.181	0.184	0.144					
28	MW332312	0.174	0.174	0.176	0.083	0.080	0.081	0.080	0.139	0.142	0.018	0.022	0.022	0.192	0.163	0.192	0.165	0.192	0.192	0.192	0.067	0.067	0.178	0.193	0.178	0.186	0.071	0.157				
29	AY279700	0.177	0.177	0.179	0.085	0.083	0.083	0.083	0.142	0.144	0.022	0.026	0.026	0.192	0.165	0.197	0.168	0.192	0.197	0.192	0.071	0.071	0.180	0.198	0.180	0.189	0.076	0.157	0.004			
30	KY815396	0.174	0.174	0.176	0.083	0.080	0.081	0.080	0.139	0.142	0.018	0.022	0.022	0.192	0.163	0.192	0.165	0.192	0.192	0.192	0.067	0.067	0.178	0.193	0.178	0.186	0.071	0.157	0.000	0.004		
31	MW332321	0.163	0.163	0.163	0.167	0.172	0.182	0.172	0.165	0.168	0.176	0.178	0.178	0.176	0.153	0.168	0.150	0.176	0.168	0.176	0.179	0.179	0.165	0.193	0.165	0.178	0.179	0.182	0.181	0.183	0.181	

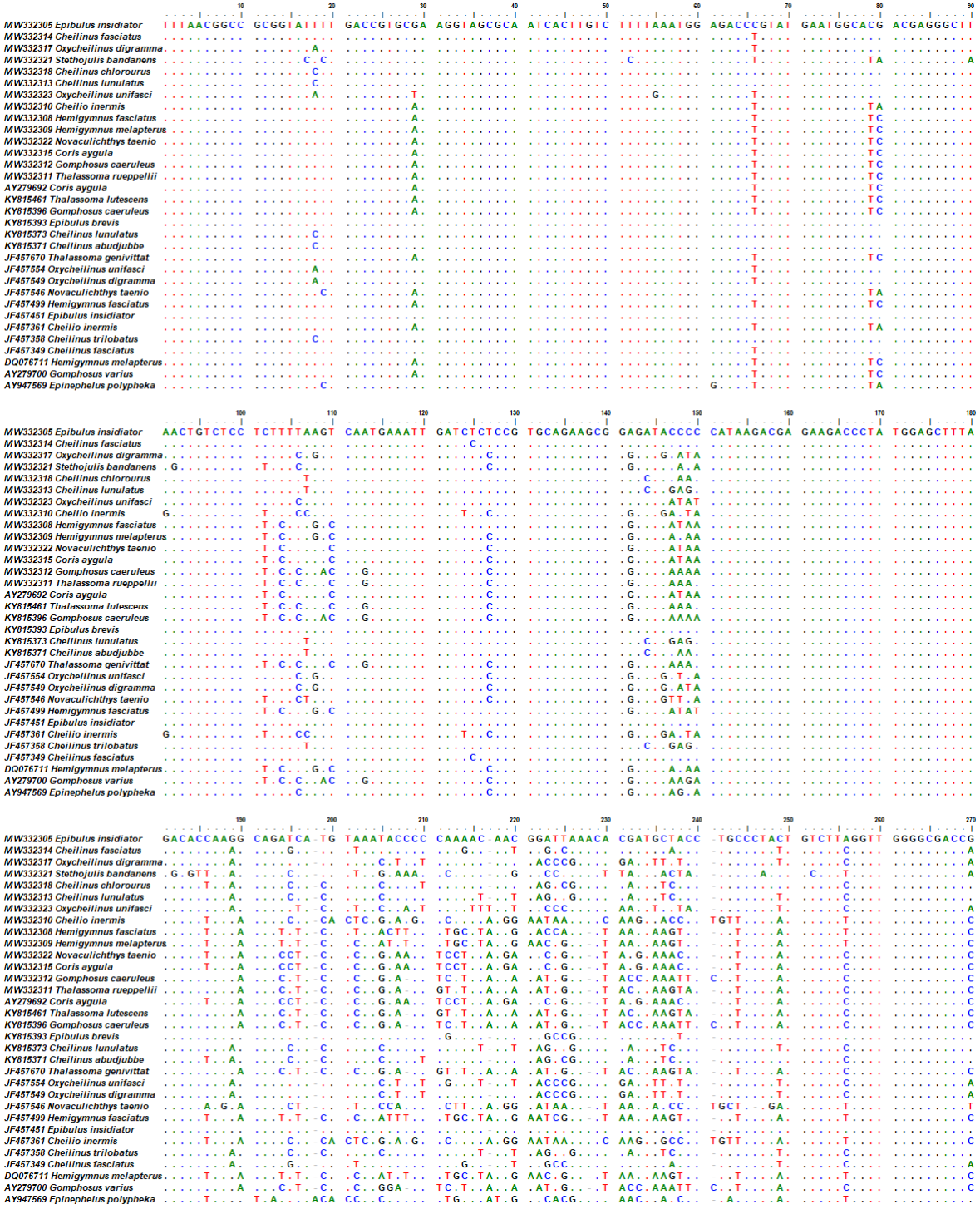

Fig. 1. Multiple sequence alignment of the partial 16S mitochondrial rDNA nucleotide sequences of the labrids fish species of the current study and GenBank/NCBI similar sequences after trimming the ends, a dot indicates identity with the top sequence. The alignment is from 1- 270 bp.

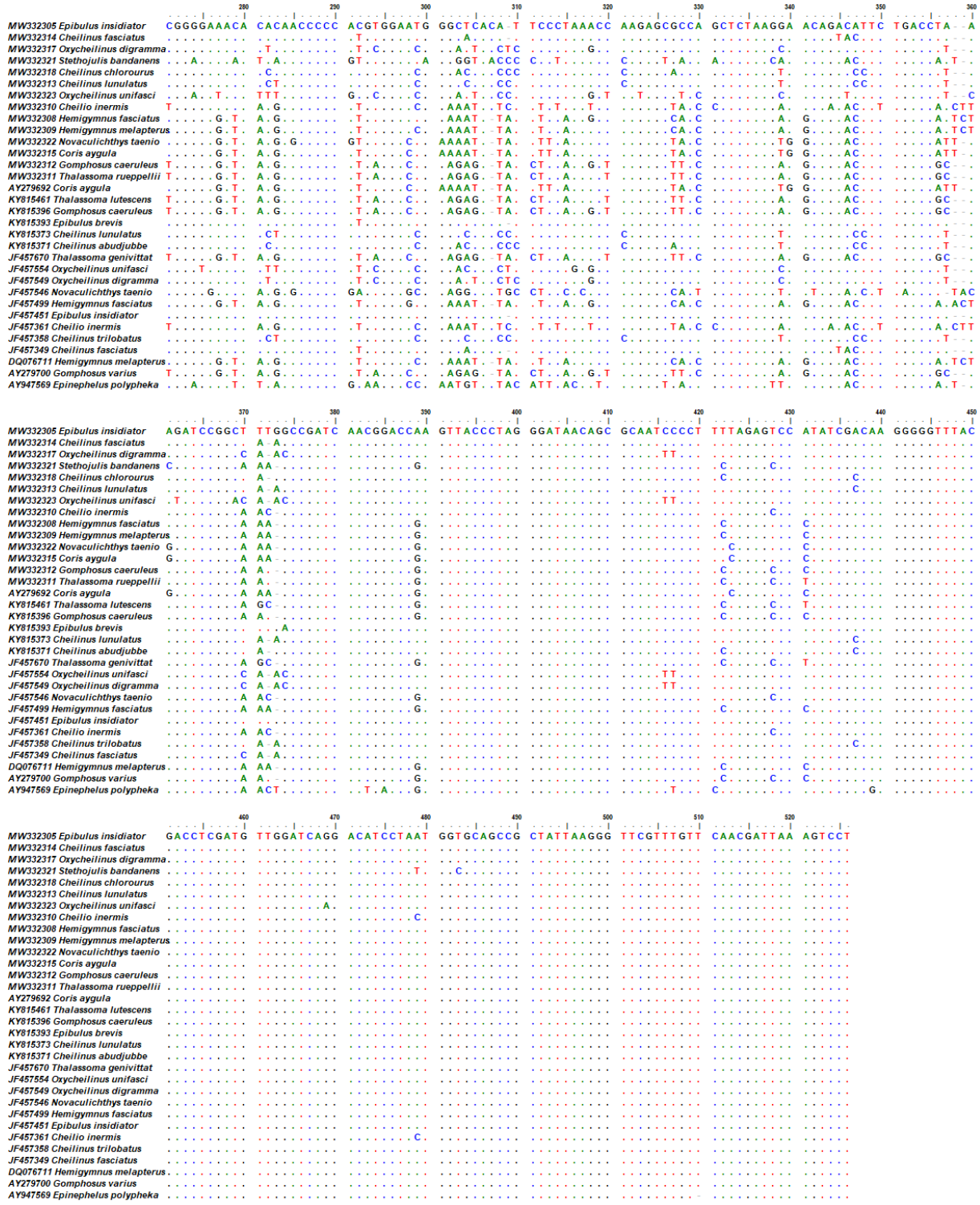

Fig. 1. Multiple sequence alignment of the partial 16S mitochondrial rDNA nucleotide sequences of the labrids fish species of the current study and GenBank/NCBI similar sequences after trimming the ends, a dot indicates identity with the top sequence. Alignment is from 271- 526 bp.

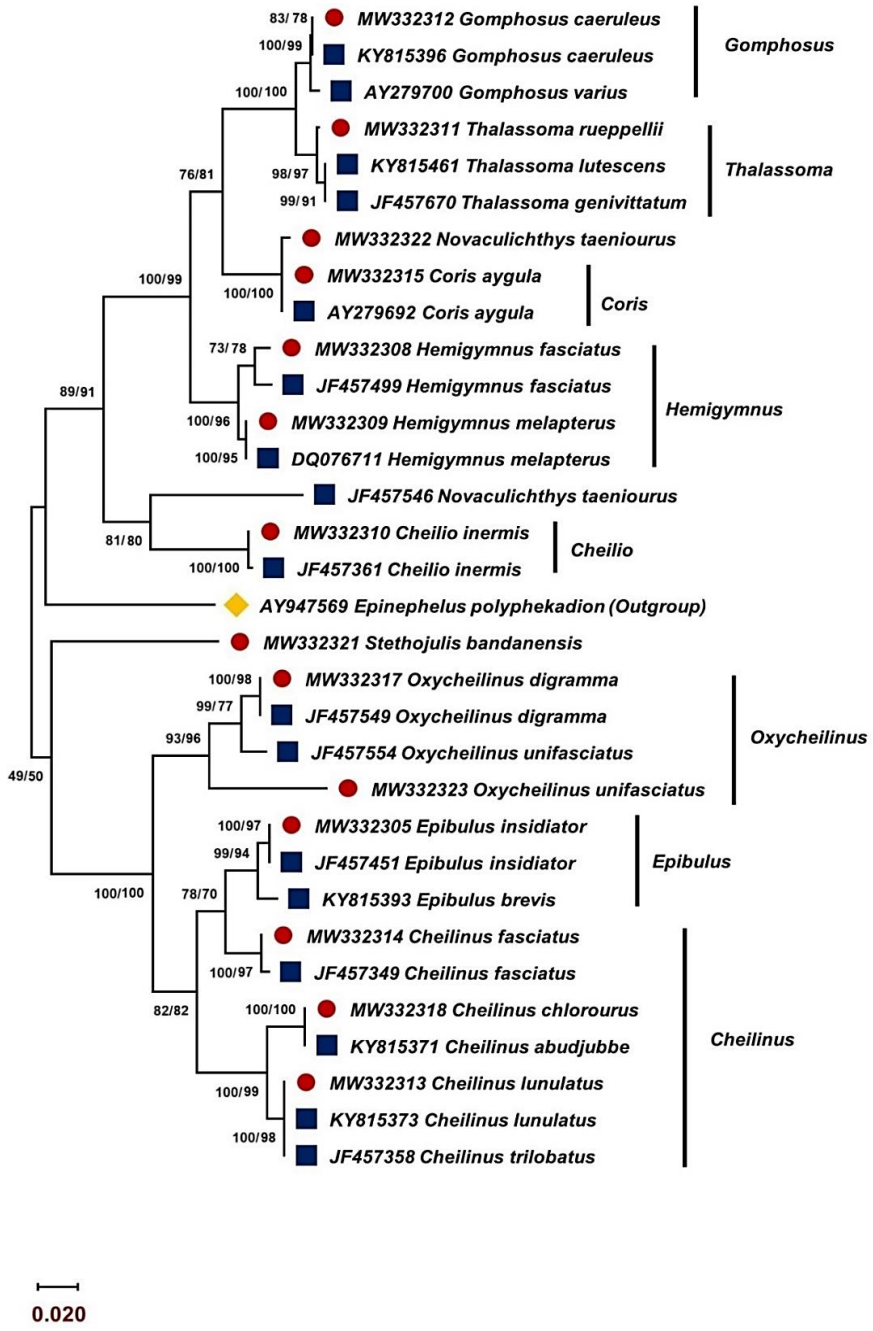

Fig. 2. Neighbor-joining (NJ) and Maximum likelihood (ML) trees constructed using the 16S mitochondrial rDNA fragment sequences of the family Labridae species, sequences of this study labrids fishes are labeled with the symbol ●, the most identical published GenBank/NCBI sequences from Blast search of labrids species marked with symbol ■, the sequences marked with the symbol ◆ rooted the tree as an out-group. The numbers above the tree branches indicate the bootstrap confidence values of the (ML) and (NJ) hypotheses, and branch lengths are proportional to genetic distance.

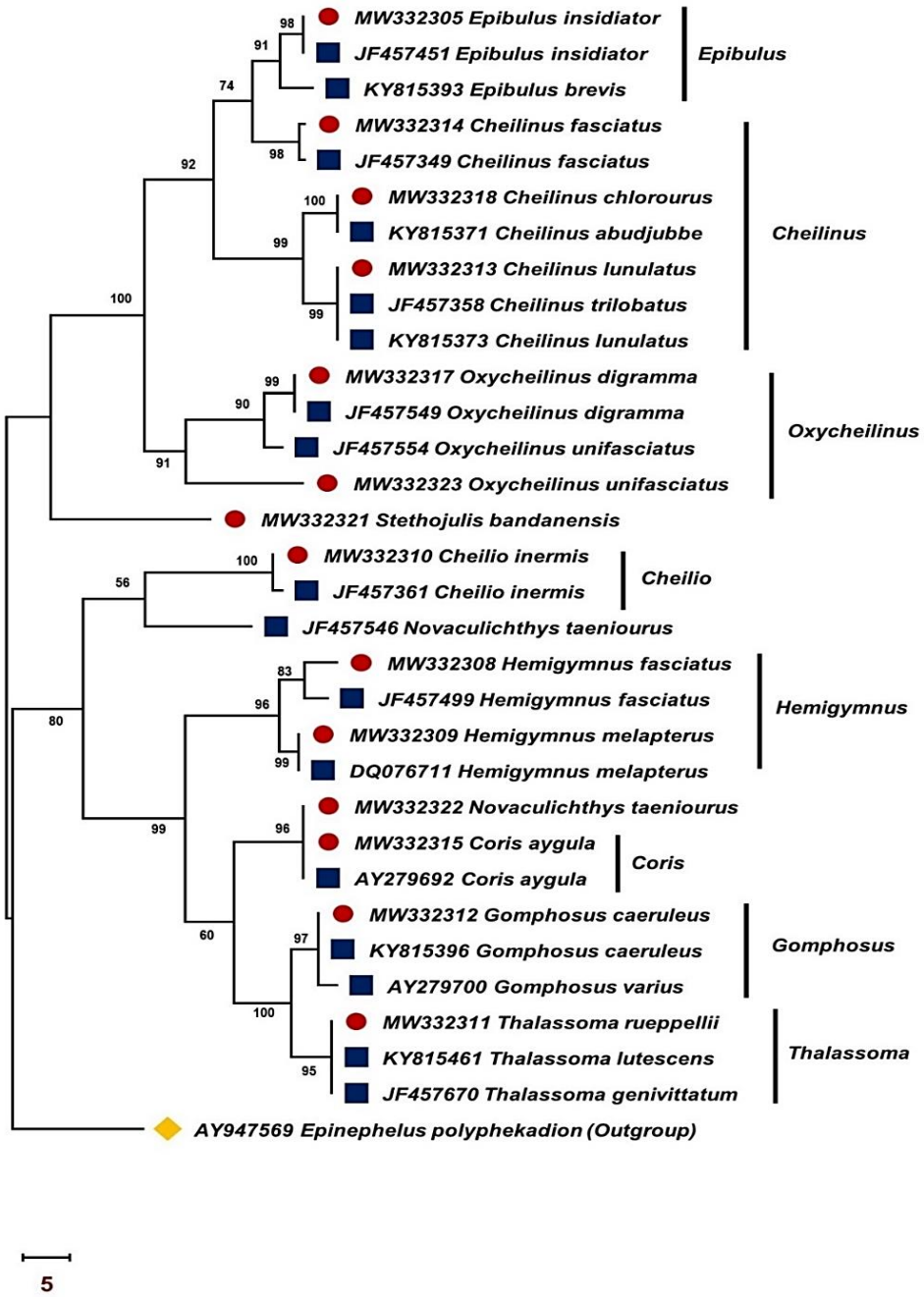

Fig. 3. Maximum parsimony (MP) tree constructed using the 16S mitochondrial rDNA fragment sequences of the family Labridae species, sequences of this study labrids fishes are labeled with the symbol ● the most identical published GenBank/NCBI sequences from Blast search of labrids species marked with the symbol ■ , the sequences marked with the symbol ◆, rooted the tree as an out-group. The numbers above the tree branches indicate the bootstrap confidence value and branch lengths are proportional to genetic distance.

DISCUSSION

It is important to note that marker selection is a complex decision influenced by multiple factors, and no single marker is universally applicable. Researchers determine which molecular markers to use in their phylogenetic analyses based on several factors, including the specific research question, the evolutionary timescale of interest, the taxonomic group under study, and the characteristics of the markers themselves **(Kocher *et al.*, 1989; Baker & DeSalle, 1997; Slowinski & Page, 1999; Wiens, 2004; Avise, 2009; Toonen & Grosberg, 2011; Taberlet *et al.*, 2012; McCormack *et al.*, 2013; Edwards *et al.*, 2016; Lemmon *et al.*, 2019)**.

Sequencing of the 16S mitochondrial rDNA gene is a common choice for phylogenetic analyses due to several key considerations. Researchers often opt for this marker based on its evolutionary rate and variation, which are suitable for the taxonomic group and timescale of interest. The 16S rDNA gene typically evolves at a moderate pace, making it appropriate for resolving both deep and recent phylogenetic relationships within a taxonomic group **(Baker & DeSalle, 1997; Toonen & Grosberg, 2011)**.

In terms of informativeness and phylogenetic signal, the 16S rDNA gene possesses sufficient variability to resolve relationships of interest. Researchers assess its utility by evaluating its variability across taxa and its ability to capture informative genetic changes, such as substitutions within the gene **(Slowinski & Page, 1999; Wiens, 2004)**. Another advantage of using mitochondrial 16S rDNA sequencing is its widespread availability and practicality. Well-established protocols, existing reference databases, and primers for marker amplification facilitate its use in phylogenetic studies. Researchers often benefit from the wealth of resources accumulated from previous studies utilizing this marker **(Kocher *et al.*, 1989; Taberlet *et al.*, 2012)**.

When considering marker selection, researchers also assess the coherence and compatibility of mitochondrial 16S rDNA with other markers, especially when combining multiple markers. Compatibility ensures consistency in inheritance patterns and evolutionary dynamics, minimizing the potential for conflicting results **(Edwards *et al.*, 2016; Lemmon *et al.*, 2019)**. Comparative analyses further support the use of mitochondrial 16S rDNA by highlighting its performance in resolving evolutionary relationships within a taxonomic group. Studies on closely related taxa or within the same taxonomic group provide valuable insights into the marker's suitability and efficacy **(Avise, 2009; McCormack *et al.*, 2013)**.

The results of this study underscore the effectiveness of partial 16S mitochondrial gene sequences in elucidating phylogenetic relationships and genetic diversity among the Labridae species. These findings align with previous molecular studies, which have also demonstrated the utility of 16S rDNA as a barcoding system for revealing genetic variation among fish species and validating its suitability for resolving genetic

relationships within this fish family **(Craig *et al*., 2001; Pondella *et al*., 2003; Mitani *et al*., 2009; Quraishia *et al.*, 2015; Singh *et al*., 2015; Saad, 2019; Wang, *et al*., 2023 ;Baldwin *et al.*, 2023)**.

The phylogenetic analyses conducted in this study yielded consistent topologies among species, albeit with some variations in support values. Such analyses are particularly valuable in species-rich genera like the labrid fish, where few distinctive morphological characteristics exist **(Bernardi *et al*., 2004; Rocha, 2004; Barber & Bellwood, 2005; Rocha *et al.*, 2005; Westneat & Alfaro, 2005)**. Establishing robust hypotheses for phylogenetic relationships within and among major groups of coastal marine and coral reef fish enhances our understanding of their evolutionary biology **(Westneat *et al*., 2005)**.

The constructed phylogenetic trees revealed distinct clades and clusters, highlighting evolutionary relationships among genera. For example, the cheilines lineage exhibited a clade comprising *Epibulus insidiator* and *Cheilinus fasciatus*, with strong support values. Meanwhile, the *Oxycheilinus* species formed a separate clade, indicating their evolutionary divergence. These results corroborate the results of **Westneat *et al*. (1995)** and the previous chromosomal study of **Almeida *et al*. (2017)**, supporting the hypothesis of a monophyletic group comprising *Cheilinus* and *Epibulus.* Regarding to the separated clade of *Oxycheilinus,* **Westneat *et al*. (1993)** in a phylogenetic analysis of the Cheilini group according to morphological characters supported the hypothesis that *Cheilinus* and *Oxycheilinus* form a monophyletic group with *Epibulus* outside that group.

In contrast, the julidines lineage genera formed distinct clusters, with notable phylogenetic relationships observed among species such as the clustering of *Gomphosus* with *Thalassoma* genus was supported by previous molecular studies, further emphasizing the importance of molecular markers in resolving phylogenetic relationships **(Westneat & Alfaro, 2005; Aiello *et al*., 2017; Hughes *et al*., 2023)**. The monophyly of the genus *Thalassoma* agrees with the previous results of the mitochondrial DNA study of **Mikami and Machida (1999)**, and the results of the phylogenetic relationships obtained by the study of **Bernardi *et al*. (2004)**.

The clustring of the genus *Stethojulis bandanensis* in a separated clade with different hypothesis, agreed with the results of the study of **Westneat *et al*. (2005)**, who concluded that the species *Stethojulis bandanensis* show no close relation with the other labrids in his study. In our results, the clade for the *Stethojulis bandanensis* exhibits no similar relation to the genus *Hemigymnus* clade, meanwhile molecular phylogenetic studies based on partial mitochondrial sequences and nuclear genes by **Westneat and Alfaro (2005)** and **Yi *et al*. (2019)** revealed that *Hemigymnus melapterus* has a close evolutionary relationship with the *Stethojulis strigiventer*, and they are grouped together.

In the current study results, the paraphyly of the clade of the genus *Coris* with the species *Novaculichthys taeniourus* nested within is supported with the result of the study

of **Westneat *et al*. (2005)**, who reported that several genera in the Labridae are paraphyly and the positions of *Cheilio inermis* as monotypic genera are uncertain in most analyses as *Cheilio* placed as the sister-species to the razorfish including the *Novaculichthys* genus (*Cheilio inermis* nested as a sister species to *Novaculichthys taeniourus* with strong parsimony support). In addition, molecular studies indicated that the monophyly of some julidines genera including the genus *Coris* is challenged **(Westneat & Alfaro, 2005; Aiello *et. al.*, 2017)**.

The present study contributes to our understanding of the Labridae species' genetic relationships and evolutionary history. Using the 16S mitochondrial rDNA gene as an informative marker facilitates the elucidation of genetic diversity among reef fishes, offering insights into their shared ancestry and evolutionary dynamics. However, further research incorporating complete mitogenome information and additional molecular markers is necessary to fully uncover the true phylogeny of the Labridae family **(Saad, 2019; Yi *et al.*, 2019)**.

CONCLUSION

The results of the discussed manuscript emphasize the effectiveness of partial 16S mitochondrial gene sequences in elucidating phylogenetic relationships and genetic diversity among the Labridae species. The phylogenetic analyses conducted in the study yielded consistent topologies among species and provided valuable insights into the evolutionary relationships within the Labridae. Distinct clades and clusters were observed, highlighting evolutionary relationships among genera. The findings corroborate previous studies and support hypotheses regarding the monophyletic nature of certain groups within the Labridae. While this study contributes to our understanding of the genetic relationships and evolutionary history of the Labridae species, further research incorporating complete mitogenome information and additional molecular markers is necessary for a comprehensive understanding of the true phylogeny of the Labridae family. In summary, this work demonstrates the utility of the 16S mitochondrial rDNA gene as an informative marker for studying genetic diversity and phylogenetic relationships among the reef fish that is important to conserve these valuable biological resources. The findings underscore the importance of molecular markers in elucidating shared ancestry and evolutionary dynamics and pave the way for future research in this field.

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Ethics approval

The experimental protocol was approved by the Research Ethics Committee of the Faculty of Science, South Valley University (REC-FSCI-SVU, Approval Number: 004/05/24).

Conflict of interest

The authors declare that there is no conflict of interest.

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