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Partial and complete sequence of small and large subunit ribosomal RNA genes, tRNA-Val gene in some species of family Labridae

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ABSTRACT

Background: Mitochondrial genomes play a key role in molecular biology research by providing essential information about evolutionary links, population history, and genetic diversity.

Aim: The aim of this investigation was to produce a partial sequence of *12S rRNA* and *16S rRNA* genes, as well as a complete sequence of tRNA-Val gene in some species of family Labridae.

Methods: Five species of labrid fishes (*Oxycheilinus digramma, Cheilio inermis, Epibulus insidiator, Coris aygula,* and *Gomphosus caeruleus*) belonging to Family Labridae were collected from the Red Sea, thereafter, taken to a laboratory for morphological identification in accordance with. Using forward and reverse primers, genome DNA was amplified through polymerase chain reaction.

Results: The tRNA-Val gene's entire sequence, the *12S rRNA* gene's partial sequence, and the *16S rRNA* gene's partial sequence were all submitted to GenBank/NCBI with accession numbers (PP962382.1—PP962386.1). The sequences' outcomes showed that the average $A + T$ values were higher than the $C + G$ values.

Conclusion: The partial sequences of *12S RNA* and *16S RNA*, and the whole sequence of the tRNA-Val gene, were arranged so that, the *12S RNA* and *16S RNA* have been distinguished by the tRNA-Val gene.

Keywords: Mitochondrial *12S rRNA* gene, *16S rRNA* gene, tRNA-Val gene, Labridae.

Introduction

Found in nearly all eukaryotic species, mitochondria are fundamental components of cells because they control apoptosis, aging, energy metabolism, and several illnesses (Sergi *et al*., 2019). For systematic research, mitochondrial DNA is an important molecular marker. Its simple structure, quick rate of evolution, large number of copies, and simplicity of isolation make it commonly utilized. Because of these features, mtDNA is a useful and efficient tool for analyzing phylogenetic patterns and genetic links (Mishmar *et al*., 2019). In molecular biology research, mitochondrial genomes are crucial because they offer vital details regarding genetic variety, population history, and evolutionary links (Boore, 1999). They are widely used in the identification, categorization, and analysis of species, allowing for the discovery of the evolutionary links between species and assisting in the creation of a genus's evolutionary tree (Machado *et al*., 2016). Furthermore, the analysis of gene flow, patterns of migration, and genetic variation amongst species is made possible by mitochondrial genomes (Sun *et al*., 2021).

Studies on mitochondrial genomes have yielded extensive knowledge about patterns of population dynamics, molecular evolution, and adaptive mechanisms across a diverse range of animals (Zhong *et al*., 2022; Ding *et al*., 2023; Li *et al*., 2023; Palacios-Barreto *et al*., 2023; Plancarte and Solórzano, 2023; Zhou *et al*., 2023). Mitochondrial genomes' tiny size, high substitution rate, lack of recombination, and massive copy number have made them crucial for molecular evolution and worldwide genetic barcoding efforts for species identification (Brown *et al*., 1979; Wang *et al*., 2016; Zhang *et al*., 2021).

Due to processing-related constraints in morphological identification, molecular data is required (Muñoz-Colmenero *et al*., 2015; Pardo *et al*., 2016).

Fish mitochondrial DNA, just like in other vertebrates, is arranged as closed circular, extranuclear, double-stranded molecules made up of light (L) and heavy (H) strands (Xiao and Zhang, 2000; Satoh *et al*., 2016).

Fish mitochondrial DNA normally has a size of 15–18 kb and contains 13 PCGs (protein-coding genes), 22 tRNAs (transfer RNA), two rRNAs (ribosomal RNA), and one control region (D-loop) (Brown, 2008; Satoh *et al*., 2016). Since bioinformatics research and high-production DNA sequencing techniques have developed swiftly in recent years, fish mitochondrial genomes are becoming more and more successfully sequenced and identified (Zhang *et al*., 2023).

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Analysis of mitochondrial genomes frequently aids in our understanding of speciation and adaptive divergence (Crampton-Platt, 2016).

The Red Sea coasts represent one of the greatest levels of endemism and diversity among the coral reef fishes globally (Alwany and Stachowitsch, 2007).

On tropical reefs around the world, the Labridae family of fish, better known as wrasses, is one of the most abundant and noticeable fish families. Furthermore, the colors, shapes, and sizes of wrasses are remarkably diverse, and they frequently show notable differences, even within the same species (Parenti and Randall, 2011).

Members of the Labridae family exhibit a wide variety of trophic behaviors, they are important members of reef communities as herbivores, planktivores, piscivores, durophages, feeders of ectoparasites, and eaters of different invertebrates linked with the reef (Randall, 1983; Lieske and Myers, 1994; Floeter *et al*., 2007; Khalaf Allah, 2013; AL-Zahaby, 2015; Sampaio *et al*., 2016; Pradhan and Mahapatra, 2017).

The aim of this investigation was to produce a partial sequence of *12S rRNA* and 16S rRNA genes, as well as a complete sequence of tRNA-Val gene in some species of family Labridae. For forthcoming studies aimed at understanding the evolutionary history and geneic diversity of the family Labridae, this sequence data will be an important genomic resource.

Materials and Methods

Samples collection

Five species of labrid fishes (*Oxycheilinus digramma, Cheilio inermis, Epibulus insidiator, Coris aygula,* and *Gomphosus caeruleus*) belong to Family Labridae were collected from the Red Sea, thereafter, taken to a laboratory for morphological identification in accordance with (Randall, 1982). Individual muscle tissues were separated and kept at −20°C until genomic DNA was extracted.

DNA isolation

Following the manufacturer's instructions, each fish's genomic DNA was isolated from its muscular tissues using the DNA Mini kit (Qiagen, Germany).

PCR conditions

Genomic DNA amplification through the polymerase chain reaction (PCR) was carried out using forward and reverse primers according to (Wang *et al*., 2000). One µl of forward and reverse primers, genomic DNA, and 22 ml of PCR master mix were used in each PCR reaction, with a final reaction volume of 50 µl. A four-minute initial denaturation at 95°C was followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for thirty seconds, and extension at 72°C for 10 minutes in the PCR process The 1.3% agarose gel stained with ethidium bromide was used to visualize the PCR results.

PCR product sequencings and sequence alignments

After PCR amplification, each species produced a single band during the agarose gel electrophoresis. Macrogen (Seoul, South Korea) performed the DNA sequencing. The sequences of *12S rRNA* gene, tRNA-Val gene, and *16S rRNA* genes were uploaded to GenBank/NCBI to receive the accession numbers. MEGA version 7.0 18 (Kumar *et al*., 2016) was used to align the sequences.

Results

The tRNA-Val gene's entire sequence, the *12S rRNA* gene's partial sequence, and the *16S rRNA* gene's partial sequence were all submitted to GenBank/NCBI with accession numbers (PP962382.1—PP962386.1).

Sequence variation using partial sequence of small subunit ribosomal RNA gene

The nucleated sequence lengths using a partial sequence of small subunit ribosomal RNA gene in five species of labrid fishes (*Oxycheilinus digramma, Cheilio inermis, Epibulus insidiator, Coris aygula,* and *Gomphosus caeruleus*) were ranged from 724 bp and 965 bp. (Table 1). In all samples, the $A + T$ ratio of the *12S rRNA* is more than the $C + G$ (Fig. 1). There were 668, 297, and 103 conserved sites, variable sites, and parsimony informative sites, respectively, among the 1077 bp that made up the final alignments (Fig. 2).

Sequence variation using complete sequence of tRNA-Val gene

The lengths of a nucleated sequence of *tRNA-Val* gene in five species of labrid fishes (*Oxycheilinus digramma, Cheilio inermis, Epibulus insidiator, Coris aygula,* and *Gomphosus caeruleus*) were ranged from 72 bp and 92

Table 1. Nucleotide frequencies of partial sequence of *12S rRNA* gene in five species of labrid fishes.

Species	Base pair length	Nucleotide number %			
		T	*C*	*A*	*G*
Oxycheilinus digramma	954	21.28	27.67	28.93	22.12
Cheilio inermis	956	21.23	25.52	31.28	21.97
Epibulus insidiator	965	21.55	26.84	29.02	22.59
Coris aygula	724	22.79	24.03	31.63	21.55
Gomphosus caeruleus	784	21.30	25.26	31.63	21.81

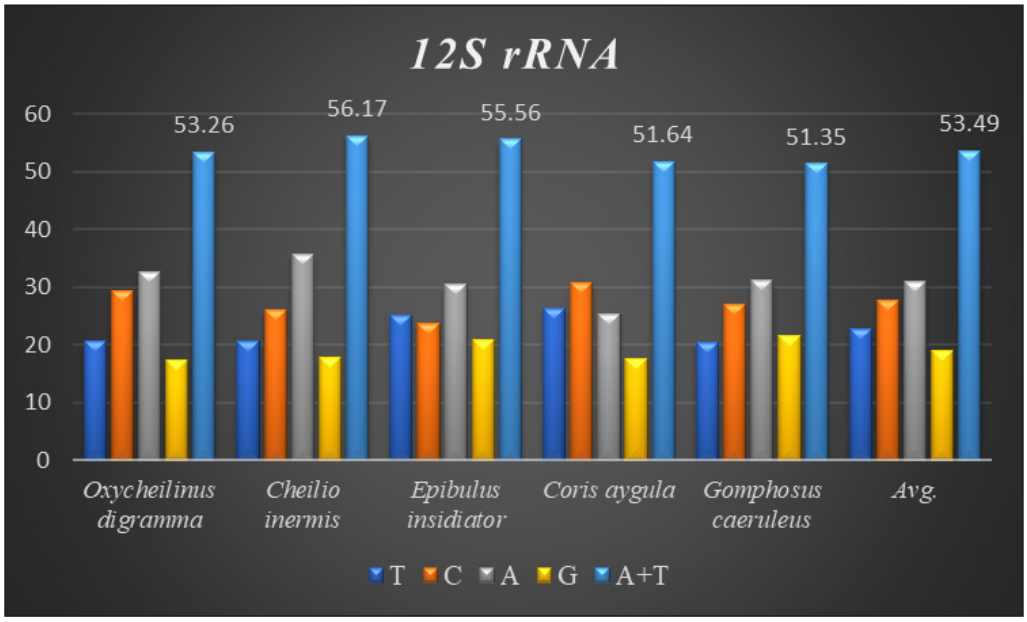

Fig. 1. The average partial sequence of the *12S rRNA* gene and nucleotide frequencies in five species of labrid fishes.

bp. (Table 2). In all samples, the *A* + *T* ratio of the *tRNA-Val* is more than the *C* + *G* (Fig. 3). There were 27, 56, and 10 conserved sites, variable sites, and parsimony informative sites, respectively, among the 103 bp that made up the final alignments (Fig. 4).

Sequence variation using partial sequence of large subunit ribosomal RNA gene

The lengths of the nucleated sequence using a partial sequence of *16S rRNA* gene in five species of labrid fishes (*Oxycheilinus digramma, Cheilio inermis, Epibulus insidiator, Coris aygula,* and *Gomphosus caeruleus*) were ranged from 114 bp and 240 bp (Table 3). In all samples, the *A* + *T* ratio of the *16S rRNA* is more than the *C* + *G* (Fig. 5). There were 112, 122, and 8 conserved sites, variable sites, and parsimony informative sites, respectively, among the 278 bp that made up the final alignments (Fig. 6).

Discussion

Due to its high copy number within the cell, ease of separation from the nuclear genome, short size, and quick rate of mutation accumulation, the mitochondrial genome has been extensively used in evolutionary and population genetics research (Moritz *et al*., 1987; Sotelo *et al*., 1993; Unseld *et al*., 1995). Mitochondrial DNA has many characteristics including the absence of introns, limited recombination, uniparental inheritance (mostly in animal phyla), and increased rate of evolution (Galtier *et al*., 2009; Tiwary *et al*., 2016).

The fundamental idea behind using molecular markers to investigate biodiversity in fishes is the analysis of nucleotide variations (Noikotr *et al*., 2013; Saad and Abd El-Sadek, 2017; Saad, 2019).

In this study, the used primers (Wang *et al*., 2000) generated PCR fragments containing *12S rRNA*, $tRNA^{VAL}$, and *16S rRNA*. This gene order is common throughout all vertebrate's mitochondrial genomes with only minor variations in length (Wang *et al*., 2000). As well as the mitochondrial genome of *Halichoeres nigrescens* was 17,252 bp long and comprised two rRNA genes, thirteen protein-coding genes, twenty-two tRNA genes, and one large non-coding region. *Halichoeres nigrescens* shares the same arrangement of mitochondria genes as other common fishes (Shi *et al*., 2018). Like that, the complete mitochondrial of the fish *Thalassoma lunare* was 17,073 bp in length. The complete mitochondrial sequence had *12S RNA* and *16S RNA*, which were separated by tRNA-Val gene and situated between tRNA-Phe and tRNA-Leu (Yukai *et al*., 2019). Also, the complete mitochondrial genome of *Iniistius trivittatus* was inserted into the NCBI database (MG976729) with 16,820 bp in length. The *12S rRNA* gene was situated between the $tRNA^{Phe}$ and $tRNA^{Val}$ genes, and the *16S rRNA* gene was situated between the $tRNA^{Val}$ and $tRNA^{Leu}$ genes (Liu *et al*., 2020). Likewise, the gene order of the *Pseudocheilinus hexataenia* mitochondrion (17,111 bp, GenBank accession no. MZ357706) was identical to those of all known wrasse mitogenomes (Nam *et al*., 2022).

Our results revealed that the *12S rRNA*, tRNA-Val, and *16S rRNA* genes were encoded on the H-strand, this was similar to that observed for other Labridae studies. Similar to, Qi *et al*. (2013) who observed that the *12S rRNA*, $tRNA^{Val}$, and *the 16S rRNA* genes of *Cheilinus undulatus* were encoded on the H-strand. Likewise, Liu *et al*. (2020) studied the mitochondrial genome

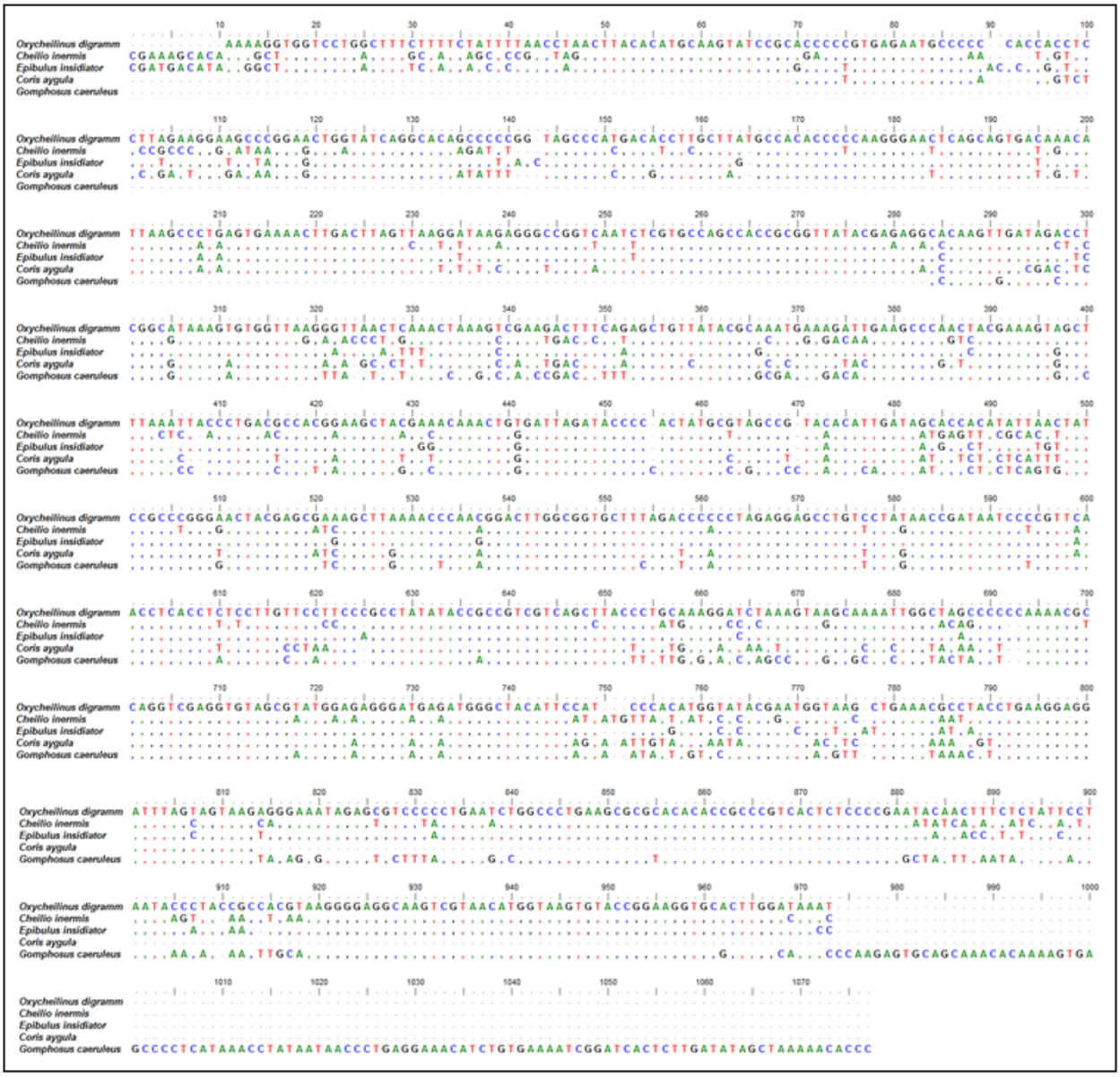

Fig. 2. Multiple sequence alignment of the partial sequence of *12S rRNA* gene in five species of labrid fishes.

Table 2. Nucleotide frequencies of complete sequence of *tRNA-Val* gene in five species of labrid fishes.

Species	Base pair length	Nucleotide number %			
		T	*C*	*A*	*G*
Oxycheilinus digramma	92	20.65	29.35	32.61	17.39
Cheilio inermis	73	20.55	26.03	35.62	17.81
Epibulus insidiator	72	25.00	23.61	30.56	20.83
Coris aygula	91	26.37	30.77	25.27	17.58
Gomphosus caeruleus	74	20.27	27.03	31.08	21.62

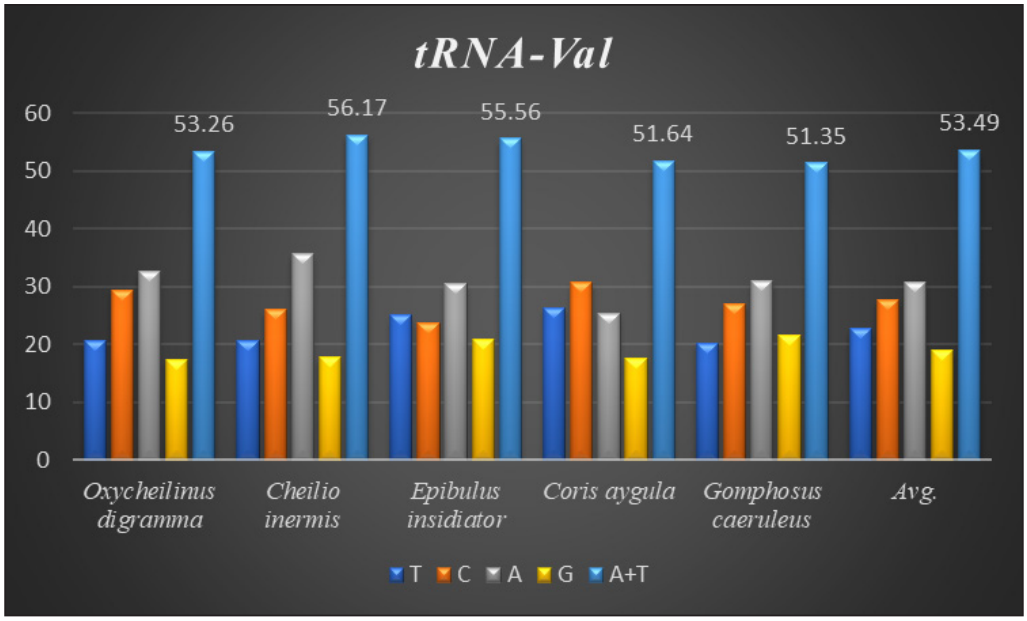

Fig. 3. The average partial sequence of the *tRNA-Val* gene and nucleotide frequencies in five species of labrid fishes.

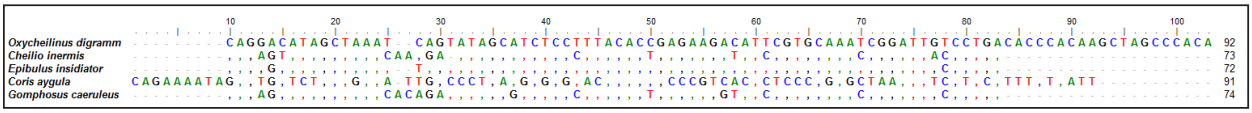

Fig. 4. Multiple sequence alignment of the partial sequence of *tRNA-Val* gene in five species of labrid fishes.

Table 3. Nucleotide frequencies of partial sequence of *16S rRNA* gene in five species of labrid fishes.

Species	Base pair length	Nucleotide number %			
		T	*C*	*A*	*G*
Oxycheilinus digramma	114	21.93	28.95	39.47	9.65
Cheilio inermis	132	16.67	28.79	42.42	12.12
Epibulus insidiator	130	20.00	27.69	41.54	10.77
Coris aygula	240	20.00	25.42	36.25	18.33
Gomphosus caeruleus	227	20.26	27.31	39.21	13.22

of *Iniistius trivittatus* and mentioned that the *12S rRNA*, $tRNA^{Val}$, and *the 16S rRNA* genes were encoded on the H-strand. Also, Wang *et al*. (2023) reported that the mitochondrial sequence of *Cheilinus trilobatus* was 17,292 bp in length, and *12S rRNA*, $tRNA^{Val,}$ and *16S rRNA* genes were encoded on the H-strand.

In all understudied species, our analysis of the *12S rRNA* gene indicated a higher *A* + *T* composition than the *C* + *G*. This is consistent with several studies. Norazila and Patimah (2002) applied the 12S rRNA/tRNA-Val gene on three varieties (normal, green, and yellow) of the tiger barb (*Puntius tetrazona*). Sivaraman *et al*. (2009) characterized the 12S rRNA gene in four Cyprinid species. Widayanti *et al*. (2021) examined the genetic diversity and phylogenetic reconstruction of the Indonesian catfish (baung fish) using the *12S rRNA* gene. Similarly, Mahrous and Allam (2022) found similar findings during their study on eleven catfish species using the 12S rRNA gene. Likewise, Aziz *et al*. (2024) observed similar results during their study on some species of the family Apogonidae using *12S rRNA* gene sequencing.

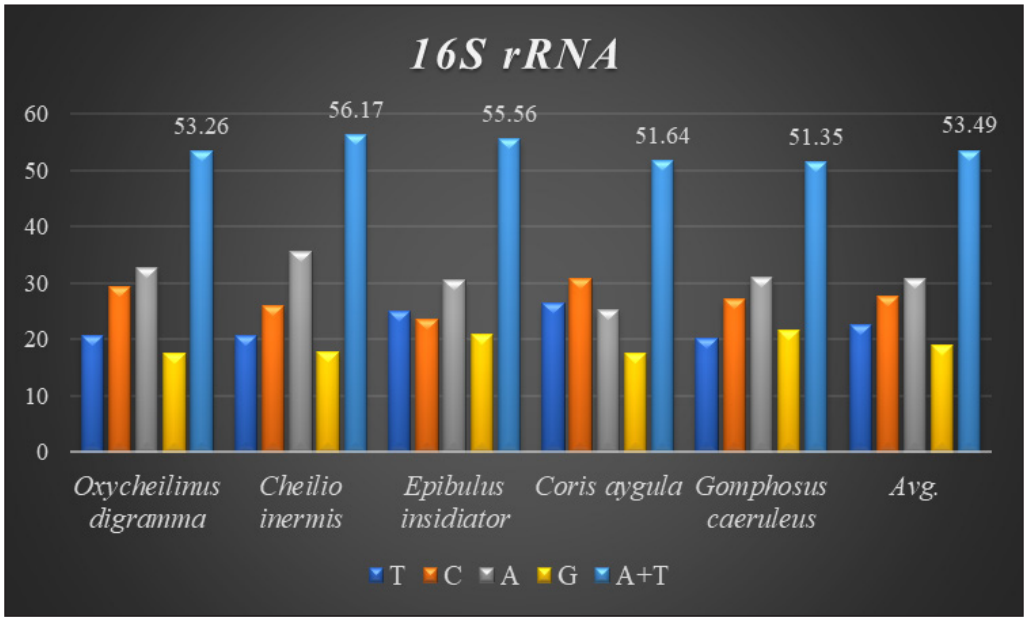

Fig. 5. The average partial sequence of the *16S rRNA* gene and nucleotide frequencies in five species of labrid fishes.

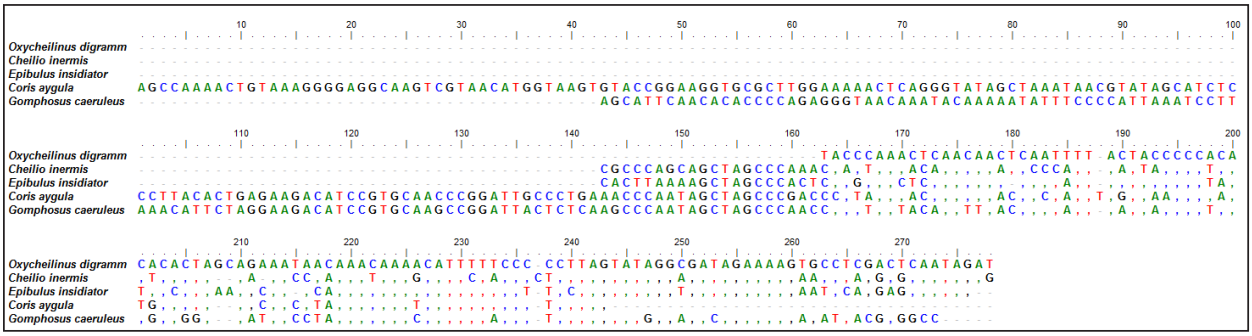

Fig. 6. Multiple sequence alignment of the partial sequence of *16S rRNA* gene in five species of labrid fishes.

In many fish's studies, the barcoding and identification of fishes have traditionally used the *16S rRNA* gene system since it is simpler to amplify and sequence (Miglietta *et al*., 2009; Moura *et al*., 2011; Rosas *et al*., 2018; Saad, 2019). When compared to *C* + *G*, the whole 16S rRNA gene exhibits *A* + *T* abundance (Bo *et al*., 2013). In All our samples the *A* + *T* ratio of the *16S rRNA* is more than the *C* + *G*, this was consistent with research on fish by Lakra *et al*. (2009) and Singh *et al*. (2015), which discovered high *A* + *T* levels in their study on fishes. As well as Basheer *et al*. (2015) during the study on Rastrelliger species found the *C* + *G* content of 16S rRNA was shorter than the *A* + *T*. Also, Mar'ie and Allam (2019) found a high *A* + *T* proportion compared to *C* + *G* in two puffer fish.

Conclusion

In this study, we used forward and reverse primers (Wang *et al*., 2000) to amplify tRNA-Val gene's entire sequence, the *12S rRNA* gene's partial sequence, and the *16S rRNA* gene's partial sequence. The sequences' outcomes showed that the average *A* + *T* values were higher than the *C* + *G* values. These sequences were arranged so that, the *12S RNA* and *16S RNA* have been distinguished by the tRNA-Val gene.

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Conflict of interest

The author declares that there is no conflict of interest.

Funding

Not applicable.

Authors' contributions

There is one author in this manuscript.

Data availability

All data are provided in the manuscript.

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