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Molecular Genetic Variations in Mitochondrial Control Region Among Some Species of Family Labridae

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ABSTRACT

The current study aimed to amplify and analyze the sequences of the tRNA-Thr (trnT) gene, tRNA-Pro (trnP) gene, and the control region in five species of the family Labridae: *Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Hemigymnus fasciatus*, *Hemigymnus melapterus*, and *Thalassoma rueppellii*. The resulting sequences from these five labrid species were submitted to GenBank/NCBI under accession numbers PP723722–PP723726. The average nucleotide frequencies of adenine (A), thymine (T), cytosine (C), and guanine (G) in the partial sequence of the trnT gene were 16.35%, 30.77%, 33.65%, and 19.23%, respectively. In the complete sequence of the trnP gene, the nucleotide frequencies were 33.33%, 27.35%, 25.64%, and 13.68%, respectively. For the complete sequence of the control region, the frequencies were 32.27%, 28.72%, 23.81%, and 15.2%, respectively.

INTRODUCTION

Coral reefs are among the most successful and diverse ecosystems on Earth; nevertheless, they are also among the most endangered. The structure and function of coral reef ecosystems worldwide are significantly affected by overfishing, pollution, sedimentation, eutrophication, habitat modification, and disease—all of which are further exacerbated by climate change **(Jackson *et al.*, 2001; Pandolfi *et al.*, 2003; Bellwood *et al.*, 2004)**.

The morphology and feeding strategies of labrids, which are among the most prevalent and functionally important inhabitants of coral reef ecosystems, exhibit remarkable variation **(Wainwright *et al.*, 2004; Bellwood *et al.*, 2006; Price *et al.*, 2011)**. The Labridae family, commonly referred to as wrasses, is one of the most widespread and visible fish groups on tropical reefs. Even within a single species, wrasses show an impressive range of colors, shapes, and sizes, often displaying notable variation **(Parenti & Randall, 2011)**.

Members of the Labridae family engage in a wide range of trophic behaviors and occupy key ecological roles on reefs as planktivores, herbivores, durophages, piscivores,

ectoparasite feeders, and consumers of other reef-associated invertebrates **(Randall, 1983; Lieske & Myers, 1994; Floeter *et al.*, 2007; Khalaf-Allah, 2013; AL-Zahaby, 2015; Sampaio *et al.*, 2016; Pradhan & Mahapatra, 2017)**.

Nearly all eukaryotic cells contain mitochondria—essential and energy-producing organelles with their own genetic material **(Margulis, 1970; Sato & Sato, 2013)**. The mitochondrial (mt) genome of Metazoa serves as an excellent model system for evolutionary genomic studies **(Gissi *et al.*, 2008)**. Its small size allows for the systematic investigation of genomic features such as gene order, gene structure, genome size, and gene count **(Wu *et al.*, 2014)**. In vertebrates, the mitochondrial genome is a circular, compact, double-stranded molecule (16–17 kb), usually containing 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and two non-coding regions—the control region (CR) and the origin of L-strand replication (OL) **(Satoh *et al.*, 2016)**.

The mitogenome contains abundant genetic information and offers advantages such as rapid evolution due to haploid and matrilineal inheritance, a relatively low recombination rate, and efficient amplification. These features make it especially useful in resolving previously unresolvable phylogenetic relationships, particularly in cases of rapid radiation **(Moore, 1995; Breton *et al.*, 2007; Dong *et al.*, 2018; Liu *et al.*, 2023)**.

The control region (CR), a non-coding portion of the mitochondrial genome, evolves two to five times faster than coding regions **(Meyer, 1993)**. Due to its high mutation rate, the CR has proven valuable in addressing intraspecific evolutionary questions **(Brown *et al.*, 1986; Palumbi, 1996)**. The D-loop, the most variable part of mtDNA, exhibits substantial genetic diversity even among individuals of the same species. Haplotype analysis of the D-loop region is a useful method for identifying genetic diversity, which is critical for species conservation **(Najjar Lashgari *et al.*, 2017)**.

Several studies on fish mitochondrial genomes have found 22 tRNAs, of which eight (Gln, Ala, Asn, Cys, Tyr, Ser, Glu, and Pro) are encoded on the light strand, while the remaining 14 are encoded on the heavy strand. These tRNAs typically exhibit a cloverleaf secondary structure, except for tRNA-Ser, which lacks the entire dihydrouridine (D) stem **(Qi *et al.*, 2013; Zhang *et al.*, 2021; Patil *et al.*, 2023; Zhou *et al.*, 2024)**.

The present study aimed to amplify and evaluate the sequences of the tRNA-Thr (trnT) gene, tRNA-Pro (trnP) gene, and the control region in selected species of the Labridae family. These sequence data will serve as a valuable genomic resource for future studies on molecular genetic variation in Labridae.

MATERIALS AND METHODS

Samples collection

Following their collection from the Red Sea, five species of the family Labridae (*Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Hemigymnus fasciatus*, *Hemigymnus melapterus*, and *Thalassoma rueppellii*) were morphologically identified **(Randall, 1982;**

Akel & Karachle, 2017). Prior to genomic DNA extraction, individual muscle tissues were isolated and stored at −20 °C.

DNA isolation and PCR amplification

Genomic DNA was extracted from the stored muscle tissues using a Biospin extraction kit, following the manufacturer's instructions. PCR amplification of the target regions was performed using the primers:

- **Forward (F):** AGCACCGGTCTTGTAAACCG
- **Reverse (R):** GGGCTCATCTTAACATCTTC, as described by **Cheng *et al.* (2012)**.

Each PCR reaction was carried out in a 50µL volume consisting of 1.0µL of genomic DNA, 1.0µL of each primer (forward and reverse), and 25µL of PCR master mix. The thermal cycling protocol included:

- Initial denaturation at 94°C for 5 minutes
- 30 cycles of:
    - Denaturation at 94°C for 1 minute
    - Annealing at 54°C for 1 minute
    - Extension at 72°C for 1 minute
- Final extension at 72°C for 5 minutes.

PCR products were visualized using 1.5% agarose gel electrophoresis stained with ethidium bromide.

PCR product sequencing and sequence alignment

Each species produced a single distinct band on the agarose gel following PCR amplification. DNA sequencing was conducted by Macrogen (Seoul, South Korea). The sequences of the tRNA-Thr (trnT) gene, tRNA-Pro (trnP) gene, and control region were subsequently submitted to GenBank/NCBI to obtain accession numbers.

Multiple sequence alignment was performed using MUSCLE **(Edgar, 2004)** under default parameters, implemented in MEGA version 11.0.11 **(Tamura *et al.*, 2021)**.

RESULTS

The *tRNA-Thr* (*trnT*) gene, *tRNA-Pro* (*trnP*) gene and control region sequences in five species of labrid fishes were all inserted into the GenBank/NCBI with accession numbers (PP723722 - PP723726).

Sequence variability utilizing the *tRNA-Thr* (*trnT*) gene's partial sequence

The lengths of partial sequence of *tRNA-Thr* (*trnT*) gene in the five labrid species (*Cheilinus lunulatus, Cheilinus abudjubbe, Hemigymnus fasciatus, Hemigymnus melapterus* and *Thalassoma rueppellii*) ranged from 12 and 30bp. The average frequencies of the nucleotides were 16.35, 30.77, 33.65, and 19.23% for adenine (A), thymine (T), cytosine (C), and guanine (G), respectively (Table 1). Among the 30bp that

comprised the final alignments, there were 3, 7, and 21 conserved, parsimony informative and variable sites, respectively (Fig. 1).

Table 1. Nucleotide frequencies of the *tRNA-Thr* partial sequence (*trnT*) in five labrid fish species

	Base pair length	A	T	C	G	A + T
Cheilinus lunulatus	24	20.83	33.33	25	20.84	54.16
Cheilinus abudjubbe	12	0	33.33	58.33	8.34	33.33
Hemigymnus fasciatus	30	20	26.67	26.67	26.66	46.67
Hemigymnus melapterus	24	20.83	29.17	29.17	20.83	50
Thalassoma rueppellii	14	7.14	35.71	50	7.15	42.85
Avg.	-	16.35	30.77	33.65	19.23	47.12

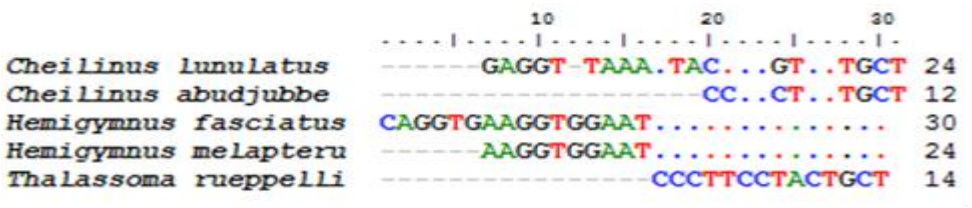

Fig. 1. The partial sequence alignment of *tRNA-Thr (trnT)* gene in in five labrid fish species

Sequence variability utilizing the *tRNA-Pro* (*trnP*) gene's complete sequence

The lengths of complete sequence of *tRNA-Pro* (*trnP*) gene in the five labrid species (*Cheilinus lunulatus, Cheilinus abudjubbe, Hemigymnus fasciatus, Hemigymnus melapterus*, and *Thalassoma rueppellii*) ranged from 69, and 71bp. The average frequencies of the nucleotides were 33.33, 27.35, 25.64, and 13.68% for adenine (A), thymine (T), cytosine (C), and guanine (G), respectively. In all samples the A+T ratio is greater than the C+G ratio (Table 2). Among the 71bp that comprised the final alignments, there were 13, 17, and 54 parsimony informative, variable, and conserved sites, respectively (Fig. 2).

Table 2. Nucleotide frequencies of the *tRNA-Pro* complete sequence (*trnP*) in five labrid fish species

	Base pair length	A	T	C	G	A + T
Cheilinus lunulatus	71	32.39	22.54	28.17	16.9	54.93
Cheilinus abudjubbe	71	30.99	22.54	29.57	16.9	53.53
Hemigymnus fasciatus	70	35.71	31.43	22.86	10	67.14
Hemigymnus melapterus	70	35.71	31.43	22.86	10	67.14
Thalassoma rueppellii	69	31.88	28.99	24.64	14.49	60.87
Avg.	-	33.33	27.35	25.64	13.68	60.68

Fig. 2. The complete sequence alignment of *tRNA-Pro* (*trnP*) gene in in five labrid fish species

Sequence variability utilizing the control region gene's complete sequence

The lengths of complete sequence of control region in the five labrid species (*Cheilinus lunulatus, Cheilinus abudjubbe, Hemigymnus fasciatus, Hemigymnus melapterus*, and *Thalassoma rueppellii*) ranged from 837, and 957 bp. The average frequencies of the nucleotides were 32.27, 28.72, 23.81, and 15.2% for adenine (A), thymine (T), cytosine (C), and guanine (G), respectively. In All samples the A+T attribution is great than the C+G attribution (Table 3). Among the 989bp. that comprised the final alignments, there were 261, 471, and 512 parsimony informative, variable, and conserved sites, respectively (Fig. 3).

Table 3. Nucleotide frequencies of the control region sequence in five labrid fish species

	Base pair length	A	T	C	G	A + T
Cheilinus lunulatus	957	33.02	28.42	22.57	15.99	61.44
Cheilinus abudjubbe	957	33.34	27.69	22.15	16.82	61.03
Hemigymnus fasciatus	848	31.96	28.42	25.47	14.15	60.38
Hemigymnus melapterus	848	31.96	28.42	25.47	14.15	60.38
Thalassoma rueppellii	837	30.82	30.82	23.78	14.58	61.64
Avg.	-	32.27	28.72	23.81	15.2	60.99

DISCUSSION

Mitochondrial genomes have been widely used in evolutionary and population genetics studies due to their high copy number within cells, ease of extraction compared to nuclear DNA, small genome size, and rapid rate of mutation accumulation **(Moritz *et al.*, 1987; Sotelo *et al.*, 1993; Unseld *et al.*, 1995)**. Several distinctive features of mitochondrial DNA—such as the absence of introns, limited recombination, uniparental inheritance (primarily maternal in animals), and accelerated evolutionary rate—further enhance its utility **(Galtier *et al.*, 2009; Tiwary *et al.*, 2016)**. The analysis of nucleotide variation forms the basis for employing genetic markers in studies of fish biodiversity **(Noikotr *et al.*, 2013; Saad & Abd El-Sadek, 2017; Saad, 2019)**.

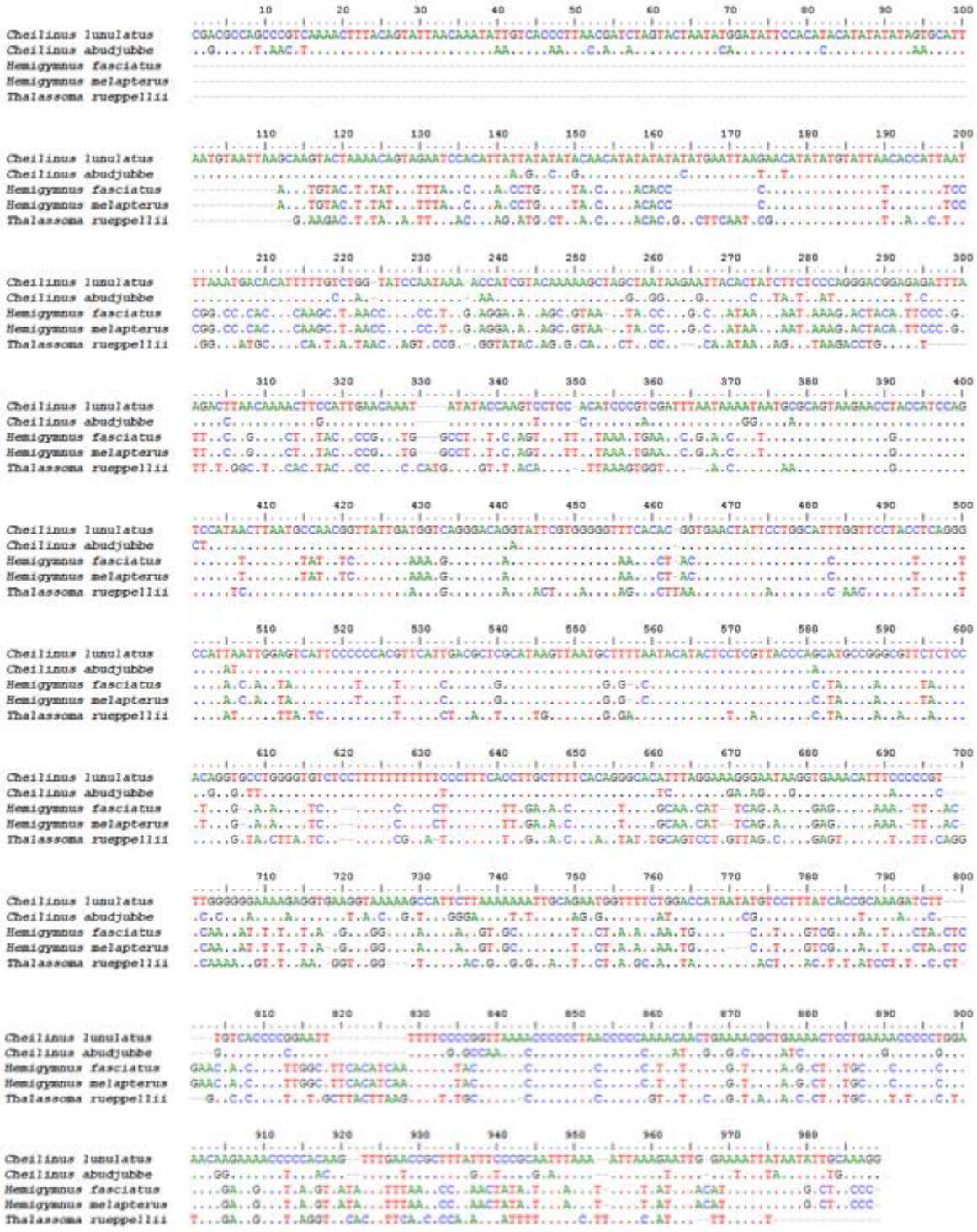

Fig. 3. The complete sequence alignment of control region gene in in five labrid fish species

The *trnT* gene was located on the heavy strand, while the *trnP* gene was located on the light strand **(Qi *et al.*, 2013; Singh *et al.*, 2023; Wang *et al.*, 2023; Zhang *et al.*, 2023)**. The lengths of the complete *trnP* gene sequence in the five labrid species

(*Cheilinus lunulatus*, *Cheilinus abudjubbe*, *Hemigymnus fasciatus*, *Hemigymnus melapterus*, and *Thalassoma rueppellii*) ranged from 69 to 71bp. This agrees with the findings of **Qi *et al.* (2013)**, who reported that the 22 tRNA genes of *Cheilinus undulatus* range in length from 68 to 77bp. In all samples, the A+T content of the *trnP* gene was higher than the C+G content, consistent with the findings of **Zhang *et al.* (2023)**, who reported that the A+T proportion in the 22 tRNA genes was 55.6%.

In vertebrates, the heavy and light strands of mitochondrial DNA each contain regulatory regions that form stable stem-loop structures **(Pereira, 2000)**. The D-loop region is the primary non-coding segment of mitochondrial DNA responsible for transcription and replication of the heavy strand **(Sbisà *et al.*, 1997; Shadel & Clayton, 1997)**. According to **Bourlat *et al.* (2009)**, the D-loop region in vertebrates is typically situated between the *trnP* and *trnF* genes. In their study of *Cheilinus undulatus*, **Qi *et al.* (2013)** found that the arrangement and gene order of the mitochondrial genome matched that of other teleost fish. They reported a 903bp control region (D-loop) located between *tRNA-Pro* and *tRNA-Phe*, accounting for 5.44% of the total mitochondrial genome.

The control region (CR) shows notable variation in fragment size between species and individuals, marked divergence in primary nucleotide sequences, and a high rate of nucleotide substitution **(Zhang & Hewitt, 1997)**. This is consistent with our results, which show that the control region lengths of the five Labridae species ranged from 837 to 957bp.

The control region—often referred to as the A+T-rich region—plays a critical role in the initiation of mitochondrial genome transcription and replication **(Wolstenholme, 1992; Zhang & Hewitt, 1997)**. In all samples examined in this study, A+T content exceeded C+G, consistent with findings from multiple other studies. **Singh *et al.* (2023)** reported that the D-loop region had an A+T content of 62.96%, while **Zhang *et al.* (2023)** observed up to 66.99% A+T content in the control region, making it the most A+T-rich part of the mitochondrial genome—characteristic of animal mitochondrial DNA **(Zhang & Hewitt, 1997; Satoh *et al.*, 2016)**. Similarly, **Aziz *et al.* (2025)** found that certain catfish species from the Nile River in Egypt also exhibited higher A+T than C+G content in their mitochondrial D-loop.

Numerous studies have examined the mitochondrial genomes of Labridae species to understand their evolutionary relationships. **Westneat and Alfaro (2005)** conducted a phylogenetic analysis of various labrid fishes using nuclear markers (RAG2 and Tmo4C4) and mitochondrial genes (12S rRNA and 16S rRNA). **Arnal *et al.* (2006)** constructed a phylogeny using partial 12S rRNA sequences from wrasse species collected from the Atlantic, Mediterranean, and Indo-Pacific regions. Additional studies have employed complete mitochondrial genomes in various labrid species, including *Cheilinus undulatus* **(Qi *et al.*, 2013)**, *Halichoeres nigrescens* **(Shi *et al.*, 2018)**, *Thalassoma lunare* **(Yukai *et al.*, 2019)**, *Pseudocheilinus hexataenia* **(Nam *et al.*, 2022)**, and *Cheilinus trilobatus* **(Wang *et al.*, 2023)**.

CONCLUSION

In this study, primers were used to successfully amplify the *trnT* gene, *trnP* gene, and control region in five Labridae species. The gene arrangement followed a conserved order: *trnT*, *trnP*, and then the control region. The resulting sequence data provide a valuable genomic resource for future research on molecular genetic variation, phylogenetic relationships, and evolutionary patterns within the Labridae family.

ETHICS STATEMENT

The entirety of the animal experimentation process complied with the South Valley University Faculty of Science's Ethics of Animal Experiments Committee (Permit No.: 005/03/2023).

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