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Review Article

Comparison of the *Chaetodontidae* Mitochondrial Genome

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Abstract

The mitochondrial genome is useful for identification because the mutation rate of mtDNA is higher than nDNA. Previous studies have carried out many analyses on the mitochondrial genome of several species, the Chaetodontidae group. Fish in this group are biological indicator fish of coral reefs, so their molecular characteristics must be enriched to conserve these fish. This article aims to review several forms of the mitochondrial genome of the Chaetodontidae group of species, including: *Chaetodon speculum*, *Chaetodon octofasciatus*, *Chaetodon wiebeli*, and *Heniochus chrysostomus.* Data were collected qualitatively by reviewing previous research articles related to the Chaetodontidae mitochondrial genome. The review focused on the characteristics of each species' mitochondrial genome, including length, overall composition, tRNA, and non-coding region. Each species exhibits various features of the mitochondrial genome and is mapped in its phylogenetic analysis.

Keywords: Chaetodontidae, Phylogenetics, Mitochondrial Genome, Comparison

1. Introduction

Chaetodontidae is a type of fish group directly related to coral reefs in large numbers. Also, it functions as a fish group that is an indicator of the coral reef ecosystem. The area is relatively wide, but the condition of coral reefs also influences its existence. This family is an indicator of the condition of coral reefs because overall, these fish inhabit coral reefs so that if one day there is the degradation of the coral reef ecosystem, the appearance of these fish can be used as a guide to assess and monitor the condition of coral reefs.

Chaetodontidae is one of the organisms that interact directly with coral reefs in abundance, and the conditions of the coral reefs influence its existence. Coral reefs are ecosystems that coral animals build, including the Chaetodontidae family (Giyanto et al., 2017). The relationship of the rugosity of coral reefs to total fish abundance is positive (Rafly, 2020). Another factor that affects is the close relationship between coral fish abundance and the percentage of coral cover (Riskiani, Budimawan, & Bahar, 2019). *Chaetodontidae* is a fish with a broad distribution and indicates the fish group on coral reef ecosystems. The exploitation of coral reef ecosystems influences associated animal associations. In coral reef ecosystems, including fish *Chaetodontidae,* coral reef inhabitants use coral polyps to their food (Nurjirana, 2017).

There have been several studies that discuss some of these groups, *Chaetodontidae*. This generally aims to keep the species in the group *Chateodontidae* preserved and reproduced, given their central role in ecology. These studies were carried out regarding the complete composition of the mitochondrial genome of each *Chaetodontidae*. mtDNA is found in mitochondria, a cell organelle that produces energy for activity needs. Mitochondria contain 2-10 mtDNA. mtDNA is useful for identification because the mutation rate of mtDNA is higher than nDNA (high variation in the population) cells (Hidayat, 2017). The mitochondrial genome (mtDNA) is a circular double-stranded DNA molecule with thirteen protein-coding genes, two rRNA genes, and twenty-two tRNA genes (Yuriadi, Widayanti, & Artama, 2014). This study will provide valuable genetic information for the future phylogenetic and taxonomic classification of *Chaetodontidae*.

This article will review some of the research that has been done regarding the mitochondrial genome of *Chaetodontidae*. This study compares several species of *Chaetodontidae* that have been analyzed for mitochondrial genomes in previous studies, including the mitochondrial genome *Chaetodon speculum*, *Chaetodon octofasciatus*, *Chaetodon wiebeli*, and *Heniochus chrysostomus* (Yang et al., 2021; Zhu et al., 2018; Yukai et al., 2019; Yu et al., 2021). The complete mitochondrial genomes of several of these species have been analyzed and linked as materials for phylogenetic analyses between these species with several *Chaetodontidae* and other groups (*outgroups*) of reef fish. This review article aims to add information about the mitochondrial genome in Chaetodontidae fish and enrich scientific knowledge, especially in cell and molecular biology and biodiversity.

2. Material and Method

This article was written by examining several previous research articles that discuss the mitochondrial genome of the Chaetodontidae group. The search was carried out by searching for articles on Google Scholar, ScienceDirect, and ERIC. The data from the research results were compared and reviewed using a qualitative approach (Siswanto, 2010). Furthermore, it is supported by literature relevant to the review topic to enrich the discussion in this article.

3. Results and Discussion

Comparison of *Chaetodontidae* Mitochondrial Genomes

DNA (mtDNA) has a size of 16,569 base pairs and is located in the mitochondrial matrix, is circular, and has two kinds of double strands consisting of strands *heavy* (H) and *light* (L). MtDNA consists of *coding and non-coding regions* (Maksum & Rasdi, 2018). MtDNA consists of 37 genes coding for two rRNA, 22 tRNA, and 13 polypeptides subunits of the enzyme complex involved in oxidative phosphorylation. From the description above, it is clear that there are several aspects of the mitochondrial genome that we will compare between species in this review article.

First, this paper will review the mitochondrial genome of the species *Chaetodon speculum* previously studied. It is now essential to characterize the complete mitochondrial genome of this species, which can be used in research on the taxonomy of resolution, population genetic structure and phylogeography, and phylogenetic relationships. The study results (Yang et al., 2021) showed the complete mitochondrial genome of obtained C. speculum. The whole mitochondrial genome of *C. speculum is* 16,537 bp long. Its genome organization is identical for a typical vertebrate mitochondrial genome, including two rRNA genes, 13 protein-coding genes, 22 tRNA genes, the origin of light-strand replication (OL), and putative control region (CR). The overall baseline composition was 27.8% from A, 26.6% from T, 29.1% from C, and 16.5% from G, with a slight AT bias (54.4%) like other vertebrate mitochondrial genomes. All the compositions mentioned above correspond to the typical mitochondrial genome of the Chaetodontidae. *The C. speculum has two non-coding regions: the locus* of origin for the L strand replication (36 bp) located between tRNA-Asn and tRNA-Cys, and the control region (846 bp) within tRNA-Pro and tRNA-Phe. Except for eight tRNAs (tRNA-Ser, tRNA-Pro, tRNA-Glu, tRNA-Tyr, tRNA-Cys, tRNA-Asn, tRNA-Ala, and tRNA-Gln), the ND6 gene is encoded on the L-strand, the others are encoded on H-strand. This trait is similar to the mitochondrial genome of other fish. The complete mitochondrial genome sequence has 16 s RNA (1,664 bp) and 12 s RNA (847 bp), which lie between tRNA-Phe and tRNA-Leu and are separated by tRNA-Val. It is located the same as most other vertebrates (Yang, Meng, Wang, & Shi, 2017). The results also showed that *C. speculum* had a closer relationship with *Chaetodon autofasciatus*.

Second, the species to be reviewed is *Chaetodon octofasciatus* (Zhu et al., 2018). The peculiarities of the genome of the vertebrate mitochondria. The mitochondrial genome of *C. octofasciatus* is a closed double-stranded circular molecule with a length of 16,485 bp, containing 13 protein-coding genes, two ribosomal RNA genes, 22 tRNA genes, and two central non-coding regions (Zhu et al., 2018). The overall baseline composition was 28.3%, 27.4%, 28.2%, and 16.1% for A, T, C, and G, respectively, with a slight AT bias (55.7%). Most mitochondrial genes are encoded on the H-strand except for ND6 and the eight tRNA genes (Gln, Ala, Asn, Cys, Tyr, Ser, Glu, Pro), which are encoded on the L-strand. Most of them have TAA as a stop codon, whereas ND3 ends with TAG, and three protein-coding genes (COII, ND4, and Cytb) end with an incomplete T codon. 12S rRNA and 16S rRNA are 950 and 1767 bp, both of which lie in a typical position between tRNA-Phe and tRNA-Leu (UUA), separated by tRNA-Val. To explore phylogenetic position, a phylogenetic tree was constructed based on the NJ analysis of 12 PCG coded by heavy strands. The results of this study support that *C. octofasciatus* has the closest relationship with *C. auripes*, strongly supported by a bootstrap value of, we willlittle about the mitochondrial genome arrangement of *100*.

Third discuss a *Chaetodon wiebelii* (Yukai et al., 2019). The complete mitochondrial genome of *C. wiebeli is* 16,523 bp long. The genome organization is identical to a typical vertebrate mitochondrial genome, including two rRNA genes, 13 protein-coding genes, 22 tRNA genes, the origin of light strand replication (OL), and presumed control region (CR). The overall baseline composition was 27.9% from A, 26.4% from T, 29.1% from C, and 16.6% from G, with a slight AT bias (54.3%) like other vertebrate mitochondrial genomes. The features mentioned above correspond to the typical genome of Chaetodontidae fish mitochondrial. For the 13 protein-coding genes, 12 genes started with ATG while only COI started with GTG. Five genes share the TAA termination codons (COI, ATPase8, ND1, ND4L, and ND6), one with TAG (ND5), the rest have incomplete codon termination (COII, COIII, ND2, ND3, ND4, ATPase6, and Cytb). It is a common feature among vertebrate mitochondrial protein-coding genes. Chaetodon wiebeli has two non-coding regions, the origin of the L-strand replication (36 bp) located between the Asn and tRNA-Cys tRNAs, and the control region (860 bp) located within the tRNA-Pro and tRNA-Phe. Except for the eight tRNAs (tRNA-Ser, tRNA-Pro, tRNA-Glu, tRNA-Tyr, tRNA-Cys, tRNA-Asn, tRNA-Ala, and tRNA-Gln) and ND6 genes, which are encoded on the L-strand, which the other is encoded on the H-strand. This trait is similar to that of other fish mitochondrial genes. The complete mitochondrial genome sequence having 16s RNA (1693 bp) and 12s RNA (949 bp) lies between tRNA-Phe and tRNA-Leu and is separated by the tRNA-Val gene. The results also show that *C. wiebelii* has the closest relationship with *C. auripes.*

Finally, we will also composition the mitochondrial genome to *discuss the Heniochus chrysostomus.* This fish is still classified into the Chaetodontidae group even though it is not in the Chaetodon genus. The complete mitochondrial genome of *H. chrysostomus* is circular and 16,650 bp long with a G content of 16.24%, showing a clear anti-guanine bias in general. The overall elemental composition was 28.64% A, 25.47% T, 29.65% C, and 16.24% G with AT bias (54.11%). The mitochondrial genome consists of 13 protein-coding genes, 22 tRNA genes, two rRNA genes (12S rRNA and 16S rRNA), and two presumed non-coding regions (control region and L-strand replication origin). All protein-coding genes found in other vertebrates are also found in *H. chrysostomus*. The mitochondrial genome contains 22 tRNA genes interspersed between rRNA and protein-coding genes. Fourteen tRNA genes are transcribed on the H-strand, while the other eight tRNA genes are L-strand oriented (Cheng, Ma, Song, & Gao, 2012; Li, Zhang, Zhao, & Gao, 2013). The tRNA gene varies in size from 65 bp to 75 bp. The phylogenetic tree shows that *H. chrysostomus* has a closer relationship with *Heniochus diphreutes*.

Table. 1. Comparison of Mitochondrial Genome of several Chaetodontidae species

No	Aspects Compared	*C. speculum*	*C. octofasciatus*	*C. wiebelii*	*H. chrysostomus*
1	Length	16.537 bp	16.485 bp	16.523 bp	16.650 bp
2	Composition Basic	4 Non-regions coding 27.8% of A, 26.6% of T, 29.1% of C, and 16.5% of G with a slight bias AT (54.4%)	2 in the non-region coding 28.3%, 27.4%, 28.2% and 16.1% respectively for the A, T, C, and G, with a slight bias AT (55.7%)	2 non-regions coding Thebasic composition overallwas 27.9% of A, 26.4% of T, 29.1% of C, and 16.6% of G with slight AT bias (54.3%)	28.64% A, 25.47% T, 29.65% C and 16.24% G with AT bias (54.11%)
3	tRNA on H and L strands	L strand replication origin region (36 bp) located between tRNA Asn and tRNA Cys, and the control region (846 bp) in the tRNA Pro and tRNA Phe	Most of the mitochondrial gene is encoded on untai- H except for ND6 and the eight tRNA genes (Gln, Ala, Asn, Cys, Tyr, Ser, Glu, Pro), which are encoded on the L-strand	The origin replication of the L-strand (36 bp) lies between the Asn-tRNA and the Cys tRNA, and theregion control (860 bp) lies within the tRNA-Pro and thetRNA Phe	The tRNA genes totaling 14 were transcribed on the H-strand,H-strand. while the other 8 tRNA genes were oriented to the L strand
4	Non areas coding	2 non-coding areas	2 non-coding areas	2 non-coding areas	2 non-coding areas

From the research results obtained, we can see and compare the length of the mitochondrial genome, elemental composition, tRNA in the H and L strands, and non-coding regions. The length of the mitochondrial genome *Chaetodon speculum* was 16,537 bp, *Chaetodon octofasciatus* 16,485 bp, *Chaetodon wiebelii* 16,523 bp, and *Heniochus chrysostomus* 16,650 bp. We can see that Heniochus chrysostomus has the longest mitochondrial genome of the four species above. The size of the mitochondrial DNA genome in each organism varies greatly. This can be seen in comparing several species in the Chaetodontidae above. Even though the differences are only slight, it already has diversity. This difference in length has become a benchmark for studies of interspecific genetic diversity that can produce phylogenic reconstructions of several species that are close to each other.

The elemental composition of the mitochondrial genome discussed in several species above can be seen as the difference in the AT bias. *Chaetodon speculum* has an AT bias of 54.4%, *Chaetodon octofasciatus* 55.7%, *Chaetodon wiebelii* 54.3%, and *Heniochus chrysostomus* 54.11%. Judging from the data above, the Chaetodontidae group has an AT bias of around 54- 55%. *Chaetodon octofasciatus* has a higher AT preference than the others. This bias is related to the rate of evolution in the mitochondrial genome. This bias in AT makes the mitochondrial genome usually have a faster evolutionary rate than the nuclear genome. Comparison of tRNA on the H and L strands of several species above shows that *Chaetodon speculum* and *Chaetodon wiebelii* Most of the genomes are encoded on the L strand. Meanwhile, *Chaetodon octofasciatus* and *Heniochus chrysostomus are* on the H strand. Origin and control region in each mitochondrial genome.

Furthermore, the similarities between species that we can take from the review of the mitochondrial genome are the non-coding region. All of them have two coding regions. This is based on the similarity in the mitochondrial genome arrangement in fish and other vertebrates. The part that does not code for mtDNA has a size of 1122 bp, starting with nucleotides 16024 to 576, and is located between the tRNA pro and tRNA phe genes. This area contains an area with a height variation called a displacement loop (D-loop). D-loop is a three-stranded region *(triple-stranded) that* is increasingly recognized as the third strand of DNA 7S.

Phylogenetic analysis of several species.

Phylogenetic analysis is an approach that is generally used for the protection and preservation of germplasm and is useful for amplifying phylogenetic relationships and uncovering the reproductive history of populations (Putra, Razak, & Sumarmin, 2021). This section will discuss the relationships of some species that we differ from other species. The results of previous research recapitulated can be seen in the following table.

Table 2. Summary of Phylogenetic Analysis of Several Chaetodontidae

No	Species discussed	Species that are closely related based on phylogenetic analysis
1	*Chaetodon speculum*	*Chaetodon octofasciatus*
2	*Chaetodon octofasciatus*	*Chaetodon auripes*
3	*Chaetodon wiebelii*	*Chaetodon auripes*
4	*Heniochus chrysostomus*	*Heniochus diphreutes*

Chaetodon speculum is closely related to the *Chaetodon octofasciatus* where we compared these two species by finding differences in their mitochondrial genomes. The hallmark of *Chaetodon octofasciatus* is its beautiful color and is located on coral reefs worldwide. *The Chaetodon speculum* is also marked with attractive colors in Indonesia, Japan, and Papua New Guinea. The areas where these two species are likely found are the same.

Chaetodon octofasciatus and *Chaetodon wiebelii* are closely related to *Chaetodon auripes*. *Chaetodon auripes* is a fish with a local term in Indonesia with butterfly fish which is very much found in East Asia. *Chaetodon octofasciatus* and *Chaetodon wiebelii* share the same living area as *Chaetodon auripes.*

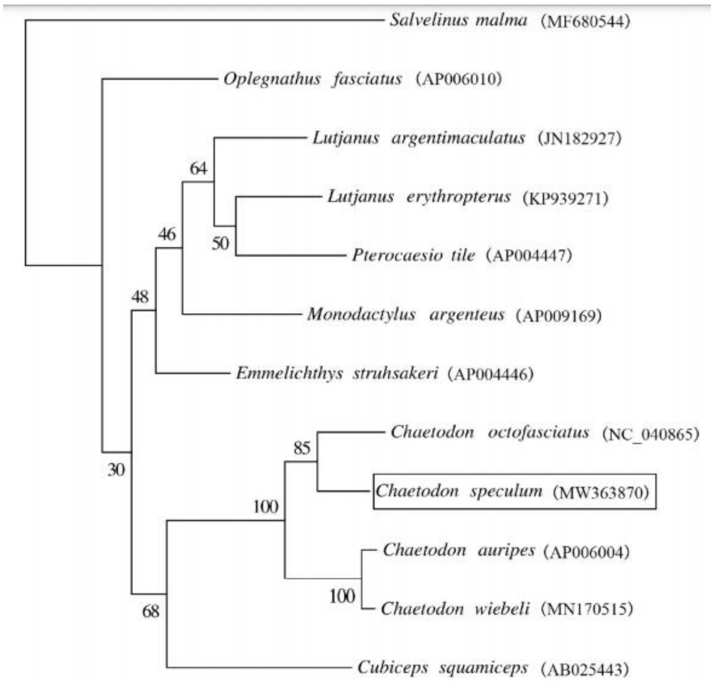

Figure 1. Phylogenetic tree Based on Mitochondrial Genome which indicates *C. speculum is* closely related to *C. octofasciatus* (Yang et al., 2021)

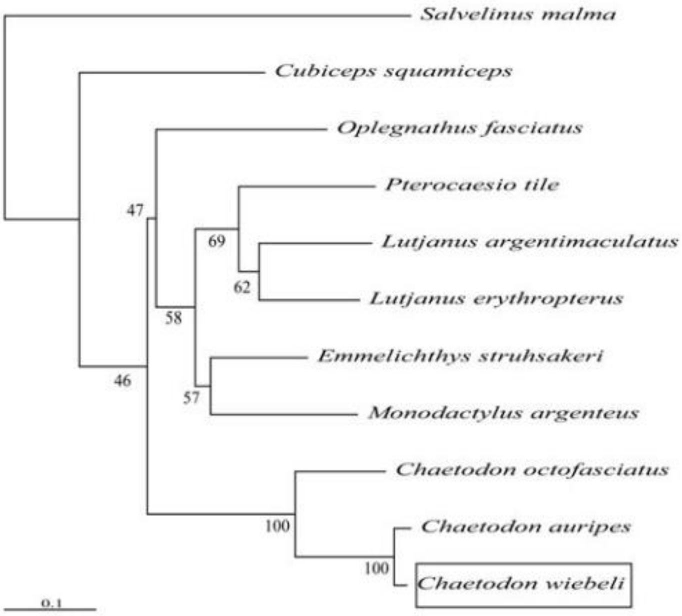

Figure 2. Phylogenetic tree Based on Mitochondrial Genome which *indicates C. wiebelii is* closely related to *C. auripes* (Yukai et al., 2019)

Meanwhile, *Heniochus chrysostomus* is closely related to a species of one genus, namely *Heniochus diphreutes.* A white base color characterizes it with two broad black stripes. Dorsal and caudal fins are a soft yellow. While *Heniochus chrysostomus* has similarities, the area where it was found is also the same, namely in the Indo-Pacific region.

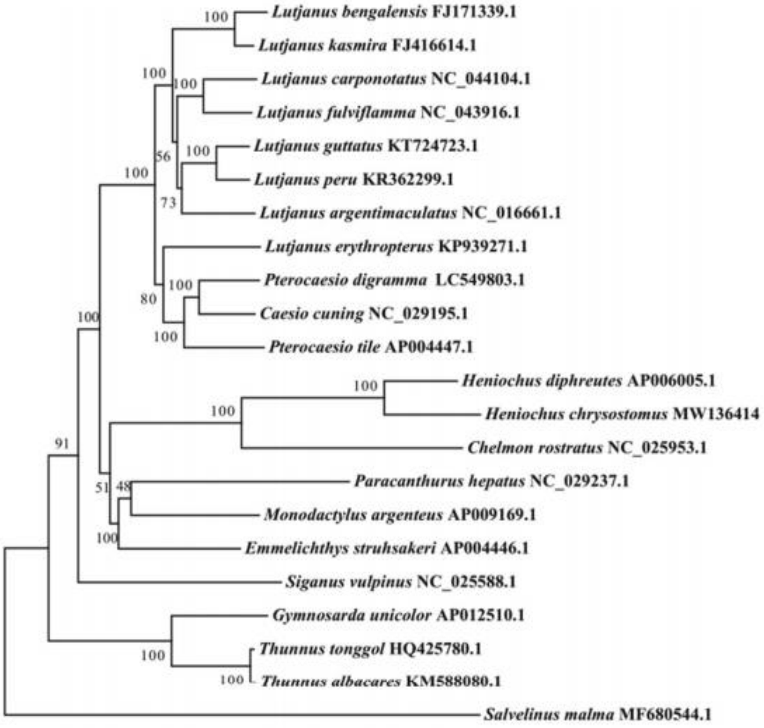

Figure 3. Phylogenetic tree Based on Mitochondrial Genome, which indicates that *H. chrysostomus is* closely related to *H. diphreutus* (Yu et al., 2021)

Conclusion

This review concludes that the mitochondrial genome of several Chaetodontidae species can be used as information for taxonomy preparation and conservation. Each species analyzed has different mitochondrial genome conditions ranging from genome length, genome composition, tRNA, and non-coding regions that exist in the genome. The mitochondrial genome present in the species has been used to carry out phylogenetic analysis in previous studies and determine which species Chaetodon is closely related.

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