

# PHYLOGENETIC ANALYSIS OF CORAL FISH FAMILIA *CHAETODONTIDAE* BASED ON *CHYTOCROME OXYDASE I* (COI) MARKINGS IN THE CORE ZONE AND LIMITED USE ZONE OF *TAMAN PULAU KECIL*, PADANG CITY, WEST SUMATRA IN THE CONTEXT OF ENVIRONMENTAL CONSERVATION

Rhavy Ferdyan<sup>1</sup>, Ramadhan Sumarmin<sup>2</sup>, \*Abdul Razak<sup>2</sup>

<sup>1</sup>Student Master (S2) Biology Education, Universitas Negeri Padang

<sup>2</sup>Departemen of Biology, Universitas Negeri Padang

\*E-mail: ar710322@gmail.com

Received: 01 Feb. 2021, Revised: 10 Jun. 2021, Accepted: 30 Jun. 2021

## ABSTRACT

This article aims to explain the coral reef fish relationship of the *Chaetodontidae* family based on the *Chytocrome* Oxydase I markers (COI). This research uses a qualitative descriptive approach. This qualitative descriptive study aims to determine the sequence of nucleotide bases offish *Chaetodontidae* in the COI area through the reading of sequences which are then used as material for making phylogenetic trees. Analyzes were performed using MEGA 10 software. The analysis was based on previous research, it was obtained the types of reef fish that inhabit the waters of the *Taman Pulau Kecil* of the city of Padang. The species are scattered in two zones, namely the core zone and the limited use zone in the *Taman Pulau Kecil* area of Padang city. From the results of the analysis using MEGA 10 software, it was obtained that the level of kinship between the species tested was very close, including the species *Chaetodon trifasciatus*, *Chaetodon vagabandus*, *Chaetodon triangulum*, and *Chaetodon lineolatus*. The genetic mean distance of all species was 0.02. In general, the species relatedness found is several species found in the same location with almost the same morphology and food. By obtaining the results of the phylogenetic analysis which directly describes the level of the kinship of the *Chaetodontidae* group, these results can be used as the material in the development of molecular ecology and a reference in environmental conservation.

**Keywords:** *Chaetodontidae* family, COI markers, environment, phylogenetics, phylogenetic analysis



This work is licensed under the Creative Commons Attribution-ShareAlike 4.0 International License

## INTRODUCTION

Pisces in Indonesian terms is known as "fish" which includes all types of fish, both those without jaws (including the superclass: *Agnatha*) and fish that have jaw (included in the superclass: *Gnathostomata*) which consists of *cartilaginous* fish (*chondrichthyes class*) and bony fish (*osteichthyes class*) (Pratomo & Rosadi, 2010). Fish are the most diverse group of vertebrates, with several species more than 27,000 species worldwide. The body structure of the fish is largely formed by its skeleton, bones the constituent of the body is cartilage, and there is true bone. Their gills and tail help them to move quickly in the water.

Fish are also a group of animals that have special habitats in waters. Fish can be found in almost any "puddle" of water which is either freshwater, brackish water, and saltwater with varying depths. In waters, fish can also inhabit places beneficial at the same time doing symbiosis mutualism in it and supporting for the breed, one of which is coral reefs. Coral reefs are community organisms that live in the bottom of the waters and are in the form of limestone (CaCO<sub>3</sub>) formations strong enough to withstand ocean wave forces.

Meanwhile, the dominant organisms that live here are coral animals that have a limestone skeleton, and a lot of algae among them also contain lime.

*Chaetodontidae* are organisms that interact directly with reefs abundance of coral, and their presence is influenced by the condition of the reef coral. The coral reef is an ecosystem mainly built by coral animals including the *Chaetodontidae* family (Giyanto *et al*, 2017). The relationship of coral reefs to rugosity the total abundance of fish is positive (Rafly & Karang, 2020). Another factor affecting is a close relationship between reef fish abundance and the percentage of cover coral (Riskiani *et al*, 2019). *Chaetodontidae* is a fish with wide distribution as well as a group of indicator fish in coral reef ecosystems. The exploitation of coral reef ecosystems has an influence on animal associations in the ecosystem Coral reefs include fish *Chaetodontidae* which are coral reef inhabitants who use coral polyps as food. (Nurjirana, 2017). Coral substrate for food very much as determining survival as the survival of the *Chaetodontidae* studied in Okinawa, Japan. Diverse substrates are important for maintaining the high diversity of butterflyfish species and substrate changes are likely to change spatial patterns and behavior looking for food (Nanami, 2020). The association of *Chaetodontidae* fish with the environment shows that temperature, salinity, pH, brightness, dissolved oxygen, and current velocity as well as live corals, algae, and abiotic components have a close association with data quality of 85.87% (Nurhasinta *et al*, 2019). However, other research states that the index fluctuation diversity, diversity, and dominance can be used as indicators of damage to reefs coral (Titaheluw *et al*, 2020).

The impact of changes in the environmental quality of coral reef ecosystems can be observed by looking at physical, chemical, and biological indicators. Changes in the coral reef ecosystem can be identified by the discovery of fish species in the family *Chaetodontidae* so that these can be classified into biological indicators. Coral fish which are resident species can also be used for indicators of the health, diversity, and productivity of coral reefs. Damage to coral reef ecosystems has a very direct impact on the condition of *Chaetodontidae* which are true inhabitants of coral reefs and are obligate coral feeders (100% of which are mainly coral).

These events can be used as indicators as well as guidelines for assessing and monitoring the condition of coral reefs. Changes that occur in coral reefs will be indicated by the presence of *Chaetodon* fish, where these fish will move to healthier coral reefs if a location is considered no longer representative as a place to live.

Referring to the Governor's Decree No. 523.6-150-2017, Padang City as one of the areas included in the area that has a Regional Water Conservation Area (KKPD) has determined area management and conservation area called the *Taman Pulau Kecil* area of Padang City which is a reserved area for the conservation of the ecosystem. sea with an area of 2,274.96 Ha. (Reserve Decree Numb. 224/2011). *Taman Pulau Kecil* area is one of the areas inhabited by coral reefs and coral fish or *Chaetodontidae*.

Research conducted has obtained data regarding the family reef fish *Chaetodontidae* in the *Taman Pulau Kecil* of Padang City in the Core Zone area and Limited Use Zone (Amrullah & Rahmadani, 2020). The core zone is a zone in a *Taman Pulau Kecil* consisting of a water zone cold and Bidalang Island. In a limited use zone in *Taman Pulau Kecil*, Padang City is a water area that is used as a tourism area, both for tourism land and marine tourism. In this study, obtained data on which family reef fish the most common *Chaetodontides* found in the core zone are *Chaetodon trifasciatus* and *Chaetodon Triangulum*, with a total of 171 and 143 individuals per hectare, respectively. Meanwhile in the limited use zone are *Chaetodon trifasciatus*, *Chaetodon vagabundus*, *Chaetodon lineolatus*. However, this area is only mostly used for tourism, different infused as a

mining site. This is due to Marine Mining Activities resulting in fish trying to find new places to take shelter and find food (Andrian *et al*, 2020).

To enrich the scientific repertoire related to family reef fish *Chaetodontiae* this, as well as to describe the phylogenetic classification through kinship analysis, the authors modified the previous research by continuing with the analysis of inter-species kinship found in the core zone and the use zone in *Taman Pulau Kecil* in the city of Padang. The analysis is very important to do about the development of taxonomy, which up to now can classify based on genetic information (*cytotaxonomy*), more precisely by analyzing kinship based on studies of genetic resources so that kinship between species can be determined more specifically and clearly. Regarding natural resources, the wealth of genetic fish resources, Indonesia is the richest country in the world. The species richness in Indonesia is more than 4,644. Studies on fish genetic resources are also very much done.

The study of genetic structure is important because it can provide information on genetic exchange in different populations so that population status can be determined (Akbar *et al*, 2018). For this reason, it is necessary to conduct a study of the relationship through phylogenetic tree analysis reef fish *Chaetodontidae* in *Taman Pulau Kecil* uses the gene *Chytocrome Oxidase I* (COI). The proper way to analyze the phylogeny of species with other natural species is based COI gene (Meng *et al*, 2017). This is to find out the genetic diversity, kinship, lineage, *enrich germplasm* data, and support programs breeding *breed* upcoming.

## METHODS

This paper is made in the form of a modification of previous research by continuing to analyze the relationship between reef fish found in the *Taman Pulau Kecil* area. This research uses a qualitative descriptive approach. This qualitative descriptive study aims to determine the sequence of nucleotide bases offish *Chaetodontidae* in the COI area through the reading of sequences which are then used as material for making phylogenetic trees. Analyzes were performed using MEGA 10 software. *Molecular Evolutionary Genetics Analysis* (MEGA) is any form of software computer that has been discovered in 1993. Devices this is very useful as a statistical tool in carrying out molecular analysis. This device is often used to study the occurrence of evolution in the genetic material of an organism, which is studied in the field of Bioinformatics and Biosystematics (Nugroho & Rahayu, 2017). The analysis was carried out on the fish species of the *Chaetodontidae* family which were found mostly in the core zone and limited use zone in *Taman Pulau Kecil*, Padang city.

## RESULTS

The reef fish group is the largest group level of vertebrate animals that interact with coral reefs, even inhabiting coral reefs with the highest diversity. In general, the presence and abundance of coral fish species from the *Chaetodontidae* family in the waters can show a picture of the condition of local coral reefs. The diversity shown by the *Chaetodontidae* group opens up opportunities for inter-species phylogenetic or kinship analysis. This article describes the relationship analysis between *Chaetodon* reef fish found in the core zone and the limited use zone in the *Taman Pulau Kecil* of Padang city. The species to be tested are the species that are most commonly found based on previous

research, including *Chaetodon trifasciatus*, *Chaetodon triangulum*, *Chaetodon vagabundus*, and *Chaetodon lineolatus*. Fish kinship relationships need to be studied to determine the closeness between fish species in water and also provide scientific information in the field of taxonomy. Knowing the morphological characteristics and the number of fish in the water can describe the changes that occur in a fish species which are probably caused by environmental factors that undergo a change process (Tjitrosoepomo, 1993).

Genetic variation describes the diversity in one species. The diversity can be seen from the characteristics of the fish, both from within (genotype) and from outside (phenotype). When viewed genotypically, the genetic variations found in fish from crosses have different variations (Muharam *et al*, 2012). However, this *Chaetodontidae* reef fish is not the result of a cross, therefore it is necessary to carry out a relationship analysis related to the zone where the fish were found.

Phylogenetic analysis refers primarily to biological evolution. Evolution is a complex process, an organism that transforms simple species into more complex ones through the accumulation of changes over several generations. Offspring will have some differentiation from their ancestors because of the progress of changes in evolution. In the study of genetic variation and differentiation between populations from one another, genetic distance can be calculated from the number of polymorphic base differences of a gene locus for each population based on DNA sequences. The diversity of this species starts from genetic diversity and is expressed to form phenotypes in the form of color, body size, fin shape, and various other variations. The diversity that is formed comes from a material likened to the genotype and phenotype characters of each organism. The differences in these characters are expressed in various genetic materials.

These variations can be grouped based on the similarity of characteristics both in the genotype and phenotype characters. The results of grouping will show the kinship relationship between one type and another, one individual to another in one type, and can find out their origin. The analysis carried out moved from the original research conducted where data were obtained from several types of *Chaetodontidae* fish found in *Taman Pulau Kecil* (Amrullah & Rahmadani, 2020).

In the core zone of *Taman Pulau Kecil*, Padang City, observation of coral fish from the *Chaetodontidae* family was carried out in the waters of Cold Water and Bindalang Island. In the waters of the "Cold Water" core zone, 4 fish species from the *Chaetodontidae* family were found, with 33 individuals, 20 for *Heniochus pleurodynia* and 6 for *H. singularis*. Meanwhile, in the core zone of P. Bindalang, there were 6 types of fish from the *Chaetodontidae* family with a total of 15 fish, where the fish species found were species *Chaetodon trifasciatus* and *Forcipiger flavissimus* with 4 each, and *Chaetodontidae triangulum* with 6 species. While the graph of the diversity of fish species from the *Chaetodontidae* family in the core zone of the *Taman Pulau Kecil*, for Cold Water waters, it was found *Heniochus pleurotaenia* 61% and *H. singularis* 18%. In the waters of P. Bindalang off found *Forcipiger flavissimus* were, 27%, 27% of *Chaetodon trifasciatus* and *Heniochus pleurotenia* 20%. Because the analysis taken was only in the *Chaetodontidae* genus, the authors took the types of *Chaetodontidae* fish that were analyzed which were the genus *Chaetodontidae* which were mostly found, namely *Chaetodontidae trifasciatus* and *Chaetodontidae triangulum*.

The limited use zone in *Taman Pulau Kecil*, Padang City is a water area that is utilized as a tourism area, both land tourism, and marine tourism. In the observation, the Utilization zone is divided into 3 locations, namely Pasumpahan Island in the East, Sikuai Island in the Southeast, and the West. In the waters of the eastern part of Pasumpahan Island, 27

individuals of coral fish of the *Chaetodontidae* family were found, 18 in the waters of P. Sikuai in the southeast and 13 in the West of P. sikuai with a total number of individual reef fish in the Utilization Zone of 58 individuals). Specifically for the limited use zone, the authors took the types of *Chaetodontidae vagabundus* and *Chaetodontidae lineolatus* (Amrullah & Rahmadani, 2020).

The various types of fish are grouped based on the same characteristics to obtain information about kinship and origin. In this analysis, the grouping is carried out based on the genetic material in the form of the nucleotide base sequence. The basis for thinking about the use of DNA sequences in phylogenetic studies is the change in nucleotide bases over time so that the rate of evolution that occurs can be predicted and the evolutionary relationship between one group of organisms can be re-examined.

Several reasons why DNA sequencing is used: 1) DNA is the basic piece of information that codes for organisms; 2) it is relatively easy to extract and combine information about the evolutionary process of a group of organisms so that it is easy to analyze; 3) evolutionary events are comparatively easy to model, and 4) produce many and varied information, so there will be a lot of evidence about the truth of a phylogenetic relationship. In this study, several *Chaetodontidae* reef fish sequences were found, including *Chaetodon trifasciatus*, *Chaetodon triangulum*, *Chaetodon vagabundus*, and *Chaetodon lineolatus*.

The selected COI sequence is then stored in the FASTA format and multiple sequence alignment is carried out in the MEGA 10 program. The alignment results are stored in the MEGA format. Biological sequence studies cannot always be avoided from alignment. The purpose of the juxtaposition process is to match homologous characters, namely characters who have the same ancestor (Kemena & Notredamie, 2009).

In addition, the main purpose of this stage is to determine whether one sequence of DNA or protein is homologous to another. Alignment involving two homologous sequences is called pairwise alignment, while one that involves multiple homologous sequences is called multiple alignments. The success of phylogenetic analysis depends on the accuracy of the alignment process. The author has carried out the analysis using MEGA X software and obtained phylogenetic tree data and analysis of genetic distances between species found. The phylogenetic tree is presented in Fig 1 below.

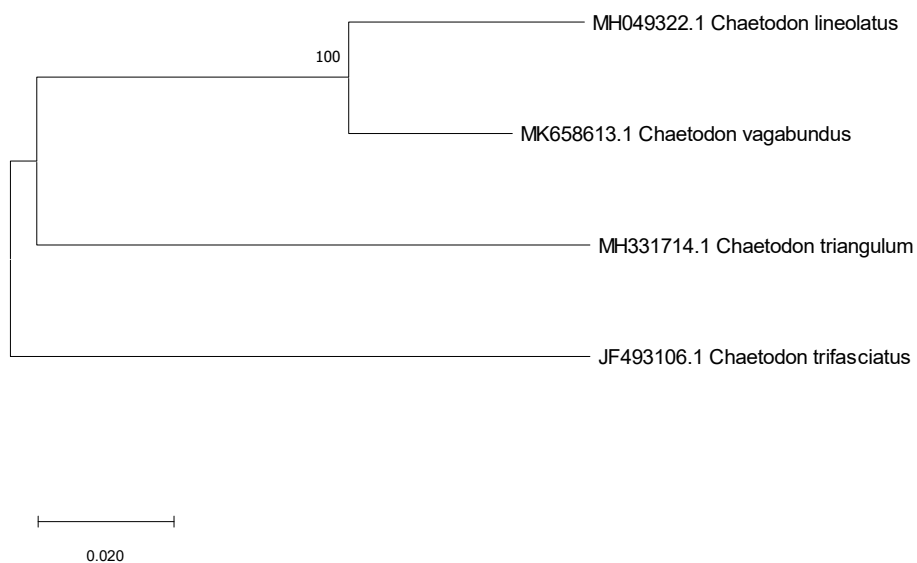


Fig. 1 Phylogenetic tree of *Chaetodontidae* in the waters of *Taman Pulau Kecil*, Padang City

Results of the phylogenetic analysis showed that the subgroup *Chaetodon lineolatus* very closely with *Chaetodon vagabundus* (100%), while the *Chaetodon triangulum* and *Chaetodon vagabundus* are also closer. For *Chaetodon trifasciatus* it is close to *Chaetodon triangulum* and slightly further from *Chaetodon lineolatus*. However, based on these data, it is estimated that each *Chaetodon* has a close kinship with other *Chaetodon* groups. In the phylogenetic tree, these reef fish species form very coherent groups, which is supported by a high bootstrap value (100%) and an average genetic distance of 0.02. The results above are more or less similar to the results of research that analyzed the kinship between the *Chaetodon wiebeli* species and other species in the waters of the South China Sea (Yukai *et al*, 2019). The results show that *Chaetodon wiebeli* has a close relationship with the *Chaetodon* found in the same place (100%).

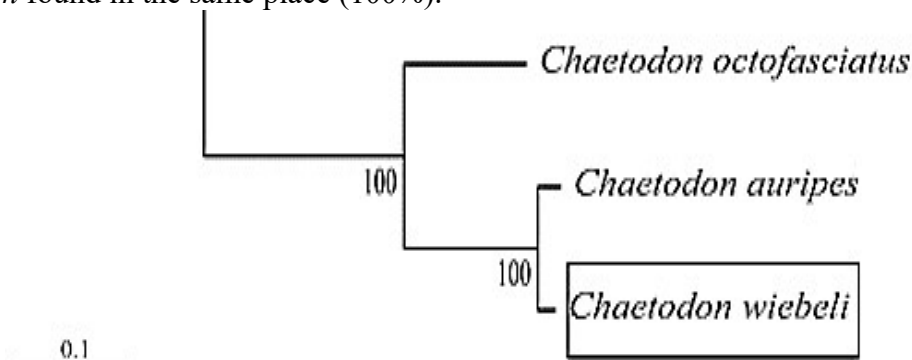


Fig. 2 Phylogenetic tree of *Chaetodon wiebeli*

When the nucleotide or protein sequences of two different organisms have a similarity, then they are thought to be derived from a common ancestor sequence. The alignment sequence will show where the position of the sequence is unchanged/conserved and where it is divergent/or evolves to be different from common ancestors (Zhu *et al*, 2019).

Phylogenetic tree yields using the sequence *Cytochrome Oxidase I* depicted fish samples *Chaetodontidae* corals share a common ancestor with the sequences in the NCBI species the same one. The phylogenetic tree forms a simple clade consisting of sequences *Chaetodon trifasciatus*, *Chaetodon triangulum*, *Chaetodon vagabundus*, and *Chaetodon lineolatus*. The phylogenetic tree formed is arranged based on the Neighbor-Joining method which has the principle taxa grouping based on the calculation of evolutionary distances where the evolutionary velocity is not the same in each branch. A phylogenetic tree based on the Neighbor-Joining Method was built to provide a connection within *Percoidea*, which can be a useful basis for the management of this species (Zhu *et al*, 2018).

In addition, the level of kinship is also determined by genetic inheritance. The inheritance of genetic material comes from the mother. In the fusion process of sperm and egg cells, only a small amount of genetic material from the sperm cell is fused, some of which do not enter and join infusing into the egg. When the sperm cells fertilize the ovum, only the head part fuses into the egg, while in the neck and locomotion the tail or flagellum does not fuse. This part only functions as a driving force for sperm cells to reach the ovum cells. The movement of the flagellum is a result of the energy produced by the mitochondria in the cell neck. Apart from being a producer of energy, mitochondria are also one of the cell organelles that contain genetic material. A large number of tail mitochondria and cell necks that do not fuse in the ovum is the reason for the inheritance of a trait that is dominated by the mother. Based on the phylogenetic tree that has been presented, it can be seen that the gene *Cytochrome Oxydase I* has a conservative area so that

the mutation in that area is very small. Several studies have found mutations in the nucleotide bases of the COI sequence but did not cause changes in amino acids. This fertilization is called a *silent mutation*. This mutation does not affect the work and function of the COI gene, which is called the synonym codon. Phylogenetic analysis of a species can be seen in the morphological characters and genes that reside inside and outside the body with *Mitochondrial* DNA sequences. The use of DNA sequences mitochondria clarifies the evolutionary relationships of species obscured by morphological variations (Awise, 1994). *Mitochondrial* DNA sequence shows a population's DNA variation, change breeding of an individual, and isolation of the population.

Table 1. Analysis of genetic distance between species

		1	2	3	4
1	MK658613.1 <i>Chaetodon vagabundus</i>		0.0187	0.0183	0.0105
2	MH331714.1 <i>Chaetodon triangulum</i>	0.1512		0.0196	0.0191
3	JF493106.1 <i>Chaetodon trifasciatus</i>	0.1578	0.1698		0.0195
4	MH049322.1 <i>Chawtodon lineolatus</i>	0.0585	0.1606	0.1698	

The result of genetic distance analysis showed that the closest distance was 1.05% between *Chaetodon vagabandus* and *Chaetodon lineolatus*. Meanwhile, the farthest distance was 16.98% between *Chaetodon lineolatus* and *Chaetodon trifasciatus*. It is well known that the genetic distance is the distance that separates the two genes on the same chromosome. The distance between the two genes is written in map units. Now, that term used is "centimorgan" (cM) as a form of appreciation for Morgan find genetic distances. From the observations, the distance was inconspicuous between *Chaetodon vagabundus* with other *Chaetodon* groups, where *Chaetodon vagabundus* is 1.87% with *Chaetodon triangulum*, 1.83% with *Chaetodon trifasciatus*, and 1.05% with *Chaetodon lineolatus*. Meanwhile, there is a very striking difference between *Chaetodon lineolatus* with *Chaetodon trifasciatus* with a distance of 16.98% and with *Chaetodon triangulum* with a distance of 16.06%. The smaller the value of the genetic distance between two organisms illustrates the closeness of the kinship relationship, meaning that the smaller the distance, the closer the kinship is between the two (Tallei *et al*, 2017). The *E-value* is equal to 0.0 indicating a significant alignment of the sequences, meaning that the specimen sequences searched through the NCBI website in this study come from the identical genus (Tindi *et al*, 2017). Meanwhile, the variation of nuclei is found at the 7th site which experiences differences between *Chaetodon trifasciatus* and others.

Table 2. Nucleotida Variations of Species

JF493106.1 <i>Chaetodon trifasciatus</i>	-1	0	0	0	A	A	A	C	T	T	A	A	C	A	C	A	C	T	T	T	C	T	T	G	A	C	C	T	G	C	A
MH049322.1 <i>Chaetodon lineolatus</i>	-1	0	0	0	.	.	.	.	C	.	G	.	.	T	.	.	.	.	C	.	T	.	.	.	T	.	C	.	.	.	
MH331714.1 <i>Chaetodon triangulum</i>	-1	0	0	0	G	.	.	.	C	.	G	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	
MK658613.1 <i>Chaetodon vagabundus</i>	-1	0	0	0	.	.	.	.	C	.	C	.	.	T	.	.	.	.	C	.	T	.	C	.	.	.	.	C	.	.	

Meanwhile, the variation of nuclei is found at the 7th site which experiences differences between *Chaetodon trifasciatus* and others. The subtle differences are located at sites 6, 10, 19, and 21. This shows that several species have the same nitrogen base and only one distinguishing species with a different nitrogen base. For example at site 6, *Chaetodon trifasciatus* has a nitrogen base *thymine* (T), while other *chaetodons* have a nitrogen base cytosine (C). The difference in each species being compared indicates that the use of COI gene sequences is good enough to be used as an indicator of phylogenetic analysis of the *Chaetodontidae* group in the waters of *Taman Pulau Kecil*, Padang City.

The genetic distance of this *Chaetodontidae* fish is also influenced by a stable environment. A stable environment will find less allele variation than an environment with unstable conditions because the mutation rate and environmental selection are relatively low. The same thing happened in previous research conducted on freshwater fish, namely *Kelabau* fish in West Kalimantan. The results showed that the low diversity of genes in *Kelabau* fish in a West Kalimantan river. Based on the average value of *heterozygosity* and *polymorphism* percentage of *Kelabau* fish, it can be seen that the genetic diversity of *Kelabau* fish in West Kalimantan is very low compared to other freshwater fish species (Iriana, 2011).

In terms of morphology, habitat, and food, each *Chaetodon* has similarities. *Chaetodon trifasciatus* has a less conspicuous dorsal patch under the dorsal fin and the anal fins are mostly dark, while the latter have caudal and fin fins rectum is black. Usually, live in the sea which is rich in coral and marine coral reefs semi-shielded. Meanwhile, the *Chaetodon Triangulum* also has less conspicuous spots under the fins dorsal and anal fins are mostly dark in color but differ only in numbers anal and dorsal fins. *Chaetodon vagabundus* is found in reef plains, lagoons, and ocean reefs. Body pattern with narrow stripes on the side; black band across the posterior body which does not cover the entire back of the dorsal fin. Reef fish show diversity which is high in measures of chromatic acuity and degree of color patterning on both the fine and coarse scales (Alfaro *et al*, 2019). Color patterns develop rapidly and often are the only morphological feature separating closely related species (McMillan *et al*, 2019). For food, more than half of *Chaetodontidae* reef fish feed on substrate coral (Konow *et al*, 2017), although other studies have also existed mentioning that no correlation shows phylogenetic distance with food similarity (Nunes *et al*, 2020). However, phylogenetics is closely related to diet (Floeter *et al*, 2018). Another point also explains the phylogenetic effect against bite speed (Liedke *et al*, 2018).

*Chaetodontidae* mostly lives on coral reefs or rocky seabed, usually live in tropical and warm climates at a depth of less than 20 m. Several species are more numerous in deep water. Some species that live in the Indo-Pacific region penetrate brackish water. *Chaetodontidae* are usually solitary or in pairs, (juveniles are mostly solitary), although a few species in the Indo-Pacific form a large group. *Chaetodontidae* feed on coral polyps, anemones colonial seas (*zoantharians*), as well as other invertebrates and algae. Many show color patterns at night, usually dark and sometimes with sparkling light. Because its size relatively small (no species in the western Atlantic exceed 15 cm), *Chaetodontidae* is not suitable as forage fish. *Chaetodontidae* does have commercial value as aquarium fish because almost every species appears in the aquarium field trade. Then among the marine ornamental fish popular in local and international markets including in the *Chaetodontidae* family (Ihya *et al*, 2020). When viewed from the tree and the genetic distance analysis, the *Chaetodon* Genus Fish shows close kinship based on the location where it was found, where Species *Chaetodon trifasciatus* and *Chaetodon Triangulum* were found in the same zone in the islet park to be precise in the core zone. The two fish were proven in the phylogenetic tree analysis to have a strong relationship. The same thing was found in research by Twindiko (2017) regarding genus fish *Pichticromis* which have the same level of kinship in one location where found. Fish *Pichticromis rutilus* Banda 65m is included in the *Pichticromis litus* found in place in the waters of Raja Ampat (Twindiko *et al*, 2013).

Other studies have also mentioned that some of the species discussed in this article are frequently coexisting with other genus *Chaetodontidae*. For example *Chaetodon trifasciatus*. From research conducted by Riansyah *et al*, (2018), found that the abundance of *Kepe* fish species - *Kepe* at the station obtained as many as 136 individuals identified from 9 species, namely *Chaetodontidae melannotus*, *Chaetodontidae vagabundus*,



*Chaetodontidae trifasciatus*, *Chaetodontidae collare*, *Chaetodontidae kleinii*, *Chaetodontidae auriga*, *Chaetodontidae ephipium*, *Chaetodontidae meyeri*, *Chaetodontidae lunulatus*. *C. triphasiatus* is a frequent species found at every station. Based on research by Adrim & Hutomo (1989) species *C. trifasciatus*, *C. vagabundus*, *C. kleinii*, and *C. Baronsesa* are often found in waters that have coral reefs' high brightness levels. For an anatomical study, we can see the typical eye structure of reef fish *Chaetodontidae*. The cone cells in reef fish, like other fish cone cells, are patterned like mosaics. The arrangement of the mosaics is in the form of lines or patterns of single or double squares. In most types of fish, the double cone cells are identical to the twin, whereas the single cone cells only have one type. The double cone cells usually contain the same visual pigment but can also contain different pigments (Razak, 2017).

Research on phylogenetic analysis of *Chaetodontidae* reef fish using COI markers adds to the sequence data from local Indonesian fish. Indonesia with its mega biodiversity can be a strategic place as land to study all forms of organisms. And can add value in the field of local fish development in Indonesia so that its sustainability is maintained. Some Previous research has been carried out regarding the relationship between marine fish in the same place as a *skipjack* tuna in North Maluku. Overall the results of the analysis of genetic distances between populations of *skipjack* tuna based on primary and secondary data show a close genetic distance between population and strong genetic flow (Akbar, 2020). *Julung* fish in Indonesian waters too already researched. The close relationship between the *Julung-Julung* fish samples obtained in the waters of Indonesia is shown by the low value of genetic distance in the analysis of phylogenetic trees, namely 0,000-0,005. Indonesian samples of *Julung-Julung* fish have high diversity in comparison with rolled fish in another area (Achmad *et al*, 2019). Internal factors include aspects of genetics, age and size, and behavior (behavior). Genetic differences lead to differences in morphology, physiological responses, and adaptability towards the environment (Razak, 2017).

Most of them are influenced by the environment, such as several studies conducted, including the lake fish in West Sumatra, namely *Bilih*. The study states that the chemical composition of the *Bilih* Fisheye lens from Lake Toba is influenced by aquatic environmental factors (Razak, 2018). So phylogenetic studies will provide useful genetic information for the future phylogenetic and taxonomic classification of *Chaetodontidae* (Yukai *et al*, 2019). One example that we can find in *Chaetodontidae* with the morphology and physiological responses described above. Thus, the phylogenetic analysis of *Chaetodontidae* reef fish in the waters of *Taman Pulau Kecil*, Padang City supports the main objectives of conservation. Conservation can be initiated with molecular genetic approaches and tools, including phylogenetic analysis such as that carried out in the *Chaetodontidae* group in Pulau Kecil Park. Its contributions to conservation are defining species and subspecies (via taxonomic boundaries and hybridization) and determining population viability (through determining genetic diversity within and between populations, population, and race-specific markers, identification of population structures, and identification of unidentified individuals). Genetic variation is the main key for populations and species in surviving environmental changes. Populations with higher genetic diversity are more likely to have several individuals who can withstand environmental changes and thus pass the genes to the next generation. From the genetic variations described in the *Chaetodontidae* reef fish in this article, it can be seen that species diversity is not too high. Therefore, the results of this analysis can be the first step in environmental conservation, especially for the *Chaetodontidae* fish group in the waters of *Taman Pulau Kecil*, Padang City, West Sumatra.

## CONCLUSION

Based on the results of the kinship analysis of *Chaetodontidae* reef fish found in the core zone and the limited use zone, it was found that the *Chaetodontidae* genus fish in that zone were closely related with the level of nitrogen base suitability reaching 100% in the same clade. This can be seen after going through the analysis process using MEGA 10 software with the neighbor-joining method to form a phylogenetic tree. When viewed from the phylogenetic tree, the *Chaetodon* Genus Fish shows a close relationship based on the location where it was found, where the *Chaetodontidae* fish in the core zone have close kinship with genetic distance 0.02. Thus, the results of the analysis that have described genetic diversity play a very important role in the context of environmental conservation, especially in the waters of Taman Pulau Kecil, Padang City. Phylogenetic analysis is the first step in environmental conservation so that existing species do not become extinct.

## REFERENCES

- Achmad, M. J., Djamhur, M., Fabanyo, M. A., & Akbar, N. (2019). Aplikasi DNA barcoding ikan *JulungJulung* (*Hemirhamphus* sp.) di Perairan Laut Maluku Utara. *Jurnal Iktiologi Indonesia*, 19(3), 463-473.
- Akbar, N., Aris, M., Irfan, M., Tahir, I., Baksir, A., Surahman, H. H., ... Kotta, R. (2018). Filogenetik ikan tuna (*Thunnus* spp.) di Perairan Maluku Utara, Indonesia. *J. Iktiologi Indonesia*, 18(1), 1-11. <https://doi.org/http://doi.org/10.32491/jii.v18i1.37>
- Alfaro, M. E., Karan, E. A., Schwartz, S. T., & Shultz, A. J. (2019). The evolution of color pattern in butterflyfishes (*Chaetodontidae*). *Integrative and Comparative Biology*, 59(3), 604-615.
- Amrullah, M. Y., & Rahmadani, W. (2020). Kondisi Ikan Karang Famili *Chaetodontidae* di Kawasan Zona Inti dan Zona Pemanfaatan Terbatas Taman Pulau Kecil Kota Padang. *SEMAH: Journal Pengelolaan Sumberdaya Perairan*, 4(1), 1-8.
- Andrian, E., Adibrata, S., & Sari, S. P. (2020). Analisis Kelimpahan Ikan Karang di Fish Shelter dan Terumbu Karang Alami Perairan Karang Melantut Pantai Rebo Kecamatan Sungailiat, Kabupaten Bangka. *Journal of Tropical Marine Science*, 3(1), 35-46.
- Floeter, S. R., Bender, M. G., Siqueira, A. C., & Cowman, P. F. (2018). Phylogenetic perspectives on reef fish functional traits. *Biological Reviews*, 93(1), 131-151.
- Giyanto, C., Abrar, M., Hadi, T., Budiyanoto, A., Hafizt, M., Salatalohy, A., & Iswar, M. (2017). *Status Terumbu Karang Indonesia*. Jakarta: Puslit Oseanografi.
- Ihya, S. S., Nuryanto, A., Prabowo, R. E., Bhagawati, D., & Kusbiyanto, K. (2020). Phylogenetic Relationships of Ornamental *Chaetodontidae* in The South Coastal of West Java, Indonesia. *Jurnal Biodjati*, 5(1), 82-89.
- Iriana, I. (2011). Karakterisasi Genetik Ikan Kelabau (*Osteochilus kelabau*) Dari Berb Agailokasidi Kalimantan Barat Menggunakan Metode Rpd (Random Amplified Polymorphism DNA). *Berita Biologi*, 10(4), 449-454.
- Kemena, C., & Notredamie, C. (2009). Upcoming challenges for multiple sequence

- alignment methods in the highthroughput era. *Bioinformatics*, 25(1), 2455 – 2465.
- Konow, N., Price, S., Abom, R., Bellwood, D., & Wainwright, P. (2017). Decoupled diversification dynamics of feeding morphology following a major functional innovation in marine butterflyfishes. *Proceedings of the Royal Society B: Biological Sciences*, 284(1680), 112–121.
- L, Y., Meng, F., Wang, R., & Shi, G. (2017). Complete mitochondrial genome of the *Salvelinus malma* sp. (Salmoniformes, Salmonidae) with phylogenetic consideration. *Mitochondrial DNA Part B*, 2(1), 889–890.
- Liedke, A. M., Bonaldo, R. M., Segal, B., Ferreira, C. E., Nunes, L. T., & Burigo, A. P. (2018). Resource partitioning by two syntopic sister species of butterflyfish (Chaetodontidae). *Journal of the Marine Biological Association of the United Kingdom*, 98(7), 1767–1773.
- McMillan, W. O., Weigt, L. A., & Palumbi, S. R. (2019). Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution*, 53(1), 247–260.
- Muharam, E. G., Buwono, I. D., & Mulyani, Y. (2012). Analisis Kekerabatan Ikan Mas Koi (*Cyprinus carpio* Koi) Dan Ikan Mas Majalaya (*Cyprinus carpio* Carpio) Menggunakan Metode Rapd. *Jurnal Perikanan Kelautan*, 3(3), 23–34.
- Nanami, A. (2020). Spatial distribution and feeding substrate of butterflyfishes (family Chaetodontidae) on an Okinawan coral reef. *PeerJ*, 8(1), 96.
- Nugroho, E. D., & Rahayu, D. . (2017). *Pengantar Bioteknologi (Teori & Aplikasi)*. Jakarta: Deepublish.
- Nunes, L. T., Siqueira, A. C., Cord, I., Ford, B. M., Liedke, A. M., Ferreira, C. E., & Floeter, S. R. (2020). The influence of species abundance, diet and phylogenetic affinity on the co-occurrence of butterflyfishes. *Marine Biology*, 167(8), 1–11.
- Nurhasinta, N., Umroh, U., & Syari, I. A. (2019). Kelimpahan Ikan Chaetodontidae Dan Pomacentridae Di Ekosistem Terumbu Karang Pulau Ketawai Dan Pulau Gusung Asam Kabupaten Bangka Tengah. *Maspari Journal: Marine Science Research*, 11(2), 97–114.
- Nurjirana, A. I. B. (2017). Kelimpahan dan Keragaman Jenis Ikan Famili Chaetodontidae Berdasarkan Kondisi Tutupan Karang Hidup di Kepulauan Spermonde Sulawesi Selatan. *Spermonde*, 2(3), 34–42.
- Pratomo, H., & Rosadi, B. (2010). *Identifikasi Pisces*. Jakarta: Universitas Terbuka.
- Rafly, N. M., & Karang, I. W. G. A., Widiastuti, W. (2020). Hubungan Rugositas Terumbu Karang terhadap Struktur Komunitas Ikan Corallivor dan Herbivor di Perairan Pemuteran, Bali. *Journal of Marine Research and Technology*, 3(1), 6–11.
- Razak, A. (2017). *Fotoreseptor Mata Ikan Laut*. Jakarta: Taushia.
- Riskiani, I., Budimawan, B., & Bahar, A. (2019). The Analysis of Coral Reef Fishes Abundance Based on Coral Reef Condition in Marine Tourism Park of the Kapoposang Islands, South Sulawesi, Indonesia. *International Journal of Environment, Agriculture and Biotechnology*, 4(4), 41–50.
- Tallei, T. E., Rembet, R. E., Pelealu, J. J., & Kolondam, B. . (2017). Sequence variation and phylogenetic analysis of *Sansevieria trifasciata* (Asparagaceae). *Bioscience Research*, 13(1), 1–7.

- Tindi, T., Monalisa, N., Gustaf, F., Mamangkey, M., & Stenly, W. (2017). DNA barcode dan analisis filogenetik molekuler beberapa jenis bivalvia asal perairan Sulawesi Utara berdasarkan gen COI. *Jurnal Pesisir Dan Laut Tropis*, 1(2), 2–10.
- Titaheluw, S. S., Andriani, R., Naim, A., & Kotta, R. (2020). Condition of the Coral Reef of Maitara Island Based on Chaetodontidae Fish for Coral Reef Improvement in North Maluku Province. *5th International Conference on Food, Agriculture and Natural Resources (FANRes 2019)*. Atlantis Press., 1(1), 370–376.
- Tjitrosoepomo G. (1993). *Taksonomi Umum*. Yogyakarta: Gadjah Mada University Press.
- Twindik, A., Wijayanti, I. P., & Ambariyanto, A. (2013). Studi Filogenetik Ikan Karang Genus *Pseudochromis* dan *Pictichromis* di Perairan Indo-Pasifik. *Buletin Oseanografi Marina*, 2(1), 28–36.
- Yukai, Y., Xiaolin, H., Heizhao, L., Tao, L., Wei, Y., & Zhong, H. (2019). The complete mitochondrial genome of *Chaetodon wiebeli* (Chaetodontiformes, Chaetodontidae). *Mitochondrial DNA Part B*, 4(2), 3145–3146.
- Zhu, K., Gao, Y., Yuan, P., Cao, P., Ying, X., Tao, H., & Liu, B. (2019). The complete mitochondrial genome of *ostorhinchus fleurieu* (kurtiformes: Apogonidae) and phylogenetic studies of apogoninae. *Mitochondrial DNA Part B*, 4(2), 3691–3692.
- Zhu, K., Gong, L., Lü, Z., Liu, L., Jiang, L., & Liu, B. (2018). The complete mitochondrial genome of *Chaetodon octofasciatus* (Perciformes: Chaetodontidae) and phylogenetic studies of Percoidea. *Mitochondrial DNA Part B*, 3(2), 531–532.