

# Diversity of the Tabuhan Island coral reef fish revealed by DNA barcoding and implication on conservation strategy in Banyuwangi, Indonesia

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**Abstract.** *Sektiana SP, Abdillah AA, Alam JM, Isoni W, Dewi NM, Kim HW, Andriyono S. 2022. Diversity of the Tabuhan Island coral reef fish revealed by DNA barcoding and implication on conservation strategy in Banyuwangi, Indonesia. Biodiversitas 23: 4844-4851.* Tabuhan Island, Banyuwangi, Indonesia, is one of the mainstays of coastal tourism with the charm of coral reefs and has a fairly high potential for reef fish diversity. Coral reef ecosystems provide suitable habitats for reef fish to spawning ground, feeding ground, and nursery ground which provide suitable nurturing areas. Here, studies have been carried out on the diversity of reef fish by molecular approaches. The molecular identification approach provides accuracy in identification to the species level. In this study, samples of reef fish species from Tabuhan Island waters were identified molecularly in the mitochondrial DNA region of the cytochrome c oxidase subunit I (COI). The identification results showed that 53 specimens had been identified, and some of the themes were registered in the GenBank database to strengthen the genetic information of reef fish in the tropical region of Indonesia. A total of 53 specimens were identified, spread over 49 species, 3 orders, and 17 families dominated by reef fish groups from Labridae (20 species). The phylogenetic tree reconstruction shows that the family collects several species, but some species are classified as paraphyletic. The results of this molecular identification have also succeeded in registering 35 COI sequences in the Genbank database. The mtDNA sequence data is very important and becomes the basis for the genetic conservation resources in coral reef ecosystems.

**Keywords:** Conservation, coral reef ecosystem, diversity, marine fish, sustainable

## INTRODUCTION

The Java Sea, Indonesia, has a group of islands spread throughout the west and east of the Pacific Ocean. It has a coral reef ecosystem that contains diverse species of fishes that provide goods and services to the ecosystem, such as fisheries products like pelagic and ornamental fish (Durand 1997) and tourism. The Java Sea is included as shallow water between Kalimantan, Java, Sumatra, and Sulawesi, within 310,000 km<sup>2</sup>. The Java Sea contributes about 10.69% of the national marine fisheries production (Nainggolan et al. 2019). An increase in fish consumption and a rise in the human population have increased fish demand, thus stimulating the development of fishing in this area (Purwanto 2003). However, the biodiversity in the Indonesian coral reef is threatened by global climate changes, various anthropogenic activities, fisheries, and sedimentation. Furthermore, the biodiversity of the Java Sea has also experienced a tremendous impact from these activities (Purwanto 2003).

Along the waters of the Java Sea, there are several conservation areas in the form of archipelagic areas. For example, in Banten Province, Tunda Island is one of the

island's tourist areas (Prameswara and Suryawan 2019). Meanwhile, Central Java has a marine National Park area famous for its high diversity conditions, Karimunjawa National Park (Hafsaridewi et al. 2018). In East Java, apart from Bawean Island (Riskiani et al. 2019) in Gresik District, there is Tabuhan Island in Banyuwangi District (Luthfi et al. 2016).

Tabuhan Island is an empty island in the waters of Banyuwangi District, precisely included in the administrative area of Bangsring Village, Wongsorejo Sub-district. The island is about 20 km from the mainland of Bangsring Village in the Bali Strait, with an area of about 5 hectares. The uninhabited island is an attractive small island to become one of the marine tourism destinations in the form of tourism activities, air sports, and ornamental fisheries (Damayanti 2012). Research on reef fish has been carried out, but a molecular approach has never been done through a morphological approach (Azhar et al. 2019). In connection with the objectives of biodiversity conservation, information on each specimen is needed with data ranging from complete and accurate systematic positions, including the use of molecular approaches in collecting biodiversity information in this area. It is kept as a species

nomenclature, including conservation status (Shanmughavel 2007). The number of species in a community is called species richness. This is the most dominant measure of biodiversity because it can be easily monitored and recorded (Hillebrand et al. 2018).

Biodiversity studies in coral reef areas generally examine macrobenthos (Quimpo et al. 2018), coral reef cover (Annas et al. 2017; Putra et al. 2018) and symbiotic fish species in this essential ecosystem area (Sahetapy et al. 2018). Furthermore, reef fish are important biota as an indicator of the health of coral reef ecosystems by identifying certain types of fish such as Chaetodontidae (Hamuna et al. 2019). In addition, the number of reef fish is also a target for traditional fishermen because they have a fairly high price, such as snapper (Arai et al. 2015) and grouper (Nanami 2021). In addition, the number of endemic fish and protected fish (Hobbs et al. 2013; Cowman et al. 2017) also makes coral reefs an important area for breeding, foraging, and raising children. Therefore, conservation activities for small islands in Indonesia will continue to be carried out by taking into account the important role of coral reefs.

One of the efforts in managing conservation areas is the availability of biodiversity data in the Tambuhan Island area. This important information can then be used as supporting data in making more appropriate management decisions. In the collection of biodiversity data, currently, many molecular approaches have been carried out. This is done to reduce errors and the accuracy of the resulting data. This report summarizes DNA barcodes and phylogenetic reconstructions of several reef fish from Tabuhan Island, Banyuwangi. This information will be very important for further research on the biology of reef fish and other research related to the genetics of coral reef fisheries in Indonesia.

## MATERIALS AND METHODS

### Sampling site

We have collected 53 fish specimens from the coral reefs in 2019 at Tabuhan Island of Banyuwangi, East Java, Indonesia (8°3'35.52"S, 114°27'42.08"E). Each specimen was kept in the freezer (at -20°C) in a 96% ethanol preservation solution. Parts of the body, including the muscles or dorsal fins, were used for further DNA sequence analysis.

### Genomic DNA extraction, amplification, and sequencing

The genomic DNA was extracted from muscles or fins of each fish sample using an Accuprep Genomic DNA Extraction Kit (Bioneer, Korea) after homogenization by TissueLyser II (Qiagen) according to the manufacturer's instructions. The purified genomic DNA is eluted in TE buffer, then quantified with Nanodrop (ThermoFisher Scientific D1000), and stored at -70°C for further analysis.

Fish Cytochrome oxidase I (COI) universal primer pairs BCL (TCA ACY AAT CAY AAA GAT ATY GGC AC) and BCH (ACT TCY GGG TGR CCR AAR AAT CA) (Baldwin et al. 2009) were used in PCR reaction to obtain

barcoding sequence for molecular identification (Hebert et al. 2003). The PCR reaction (20 µL) contained 11.2 µL ultrapure water, 1 µL of each primer (0.5 µM), 0.2 µL Extaq Hotstart version DNA polymerase (TAKARA, Japan), 2 µL 10x Extaq buffer, 2 µL dNTPs (1 µM, TAKARA, Japan), 0.6% total volume DMSO and 200 ng Genomic DNA as a template. Initial denaturation at the first stage of the PCR was carried out at 94°C for 3 minutes. Next, the primary PCR process includes denaturation (35 cycles at 94°C for 30 sec), annealing (50°C for 30 sec), and extension (72°C for 45 sec). The last step is the final extension at 72°C for 5 minutes. The PCR products were purified using a gel extraction kit (Bioneer, Daejeon, Korea) by following the manufacturer's standard protocol.

### DNA sequence analysis

The COI partial sequences obtained were assembled manually using Chromas ver 2.5.0. The low-quality sequences (QV < 20) were trimmed for further analysis. Species identification of each specimen was conducted by its DNA sequence identity to the GenBank database using the Basic Local Alignment Search Tool (BLAST) program (<http://www.ncbi.nlm.nih.gov/blast>). Sequences having both high query coverage (> 99%) and sequence identity (> 99%) to the GenBank database were considered as the same species. The morphological identification based on the comprehensive photograph method (Halford and Thompson 1994) was used to reconfirm species with a lower similarity and query coverage of the COI sequences (< 99%). All new sequences were submitted to the GenBank database to get accession numbers.

The multiple alignments of sequences were conducted using the MUSCLE program (Edgar 2004). Nucleotide composition, transition and transversion bias estimation, overall pairwise distance, and Minimum Evolution (ME) tree reconstruction were calculated using the Kimura two-parameter (K2P) distance model using the MEGA 6.0 program (Tamura et al. 2013). The Neighbour Joining (NJ) algorithm tree was created with 1000 bootstrap replications to provide a graphical representation of the divergence pattern.

## RESULTS AND DISCUSSION

### Results

In this research, molecular identification has been carried out to complete the morphological identification that has been done so far. A total of 53 fish samples showed similarities with the reference BLASTN results with a database on GenBank with a value of 99-100%. Of the 53 samples, only 16 specimens have not yet received the GenBank accession numbers, because the registration process has not been completed (still in process). However, all sequences, included in the resulting phylogenetic tree are grouped into three broad groups, namely Labridae (the most dominant family), Pomacentridae and Pomochantidae, and a small number of other families of Teleostei (small groups of families).

### Phylogenetic tree reconstruction of different families

#### Labridae

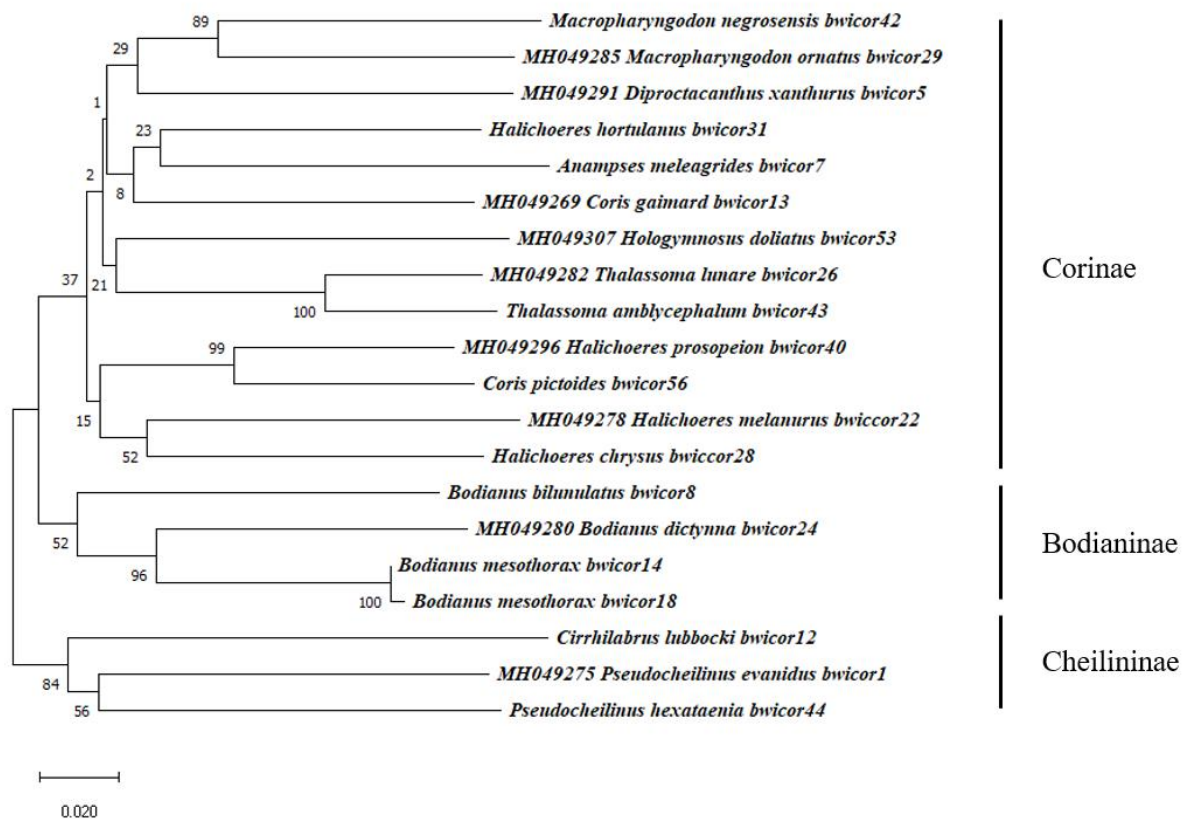
A total of 20 Labridae species were identified, but only nine species received GenBank accession numbers. Registration of other sequences is still in the process of recording on the NCBI database through the online system (<https://www.ncbi.nlm.nih.gov/>), which is expected to be verified shortly. The Labridae family group is a major fish group in coral reef ecosystems (Dhahiyat et al. 2017; Putra and Akbar 2017). From the phylogenetic tree (Figure 1), we can see the family Labridae belonging to the order, Cheilinae made a separate clade, while the other clades consist of Bodianinae and Corinae orders.

#### Pomacentridae and Pomacanthidae

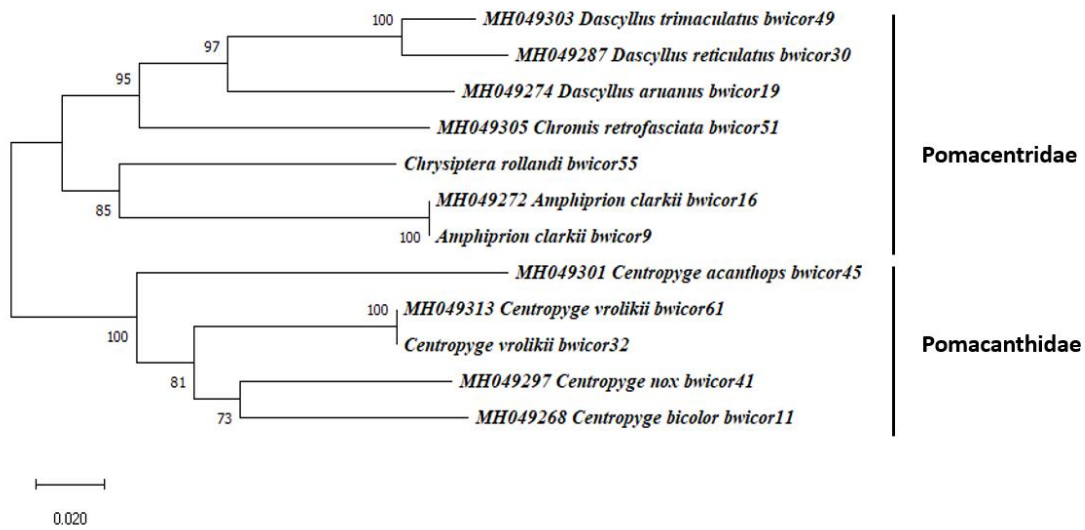
The Pomacentridae and Pomacanthidae families are still included in the major fish groups that make up the coral reef ecosystem. The previous studies in the Trenggalek waters found a large number of fish species under the Pomacentridae family (21 species), while only six species were from the Pomacanthidae family (Wibowo and Adrim 2014). Although the number of species found in this study is not as much as studies conducted in other regions. The analysis of phylogenetic tree reconstruction shows that the Pomacentridae and Pomacanthidae separate families form a distinct clade on the phylogenetic tree produced (Figure 2).

### The small number of families

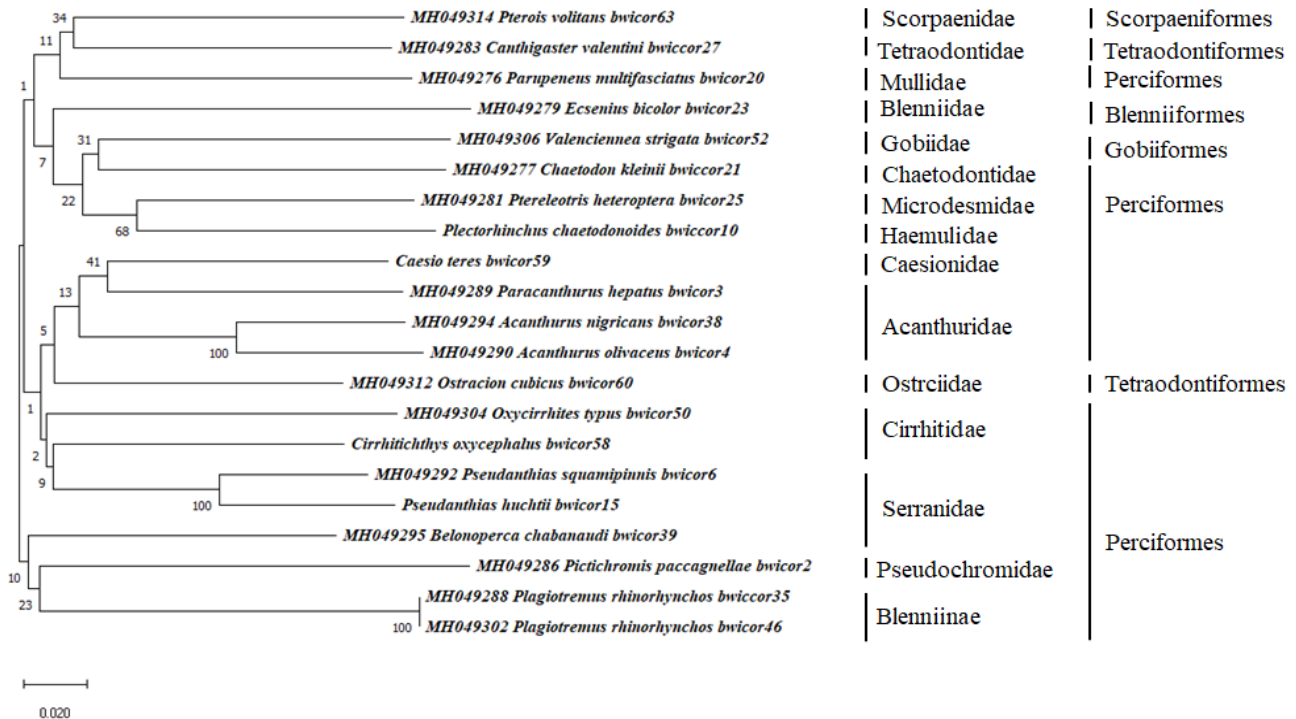
Besides the three major families (Labridae, Pomacentridae, and Pomacanthidae), many target fish species those fall into this category are economically essential fishes, such as Caesionidae, Serranidae, and Mullidae (Figure 3). A study of the fish stock of these three groups in the Karimun National Park in Java shows that the Order Serranidae has been exploited beyond its sustainability limit, while the other two families (Caesionidae and Mullidae) are still below their sustainability limit (Yuliana et al. 2016). While from another family, Scorpaeniformes, is a group of seawater ornamental fish species that is quite important and this fish has a poisonous gland that is quite dangerous. Although it has poison glands, some ornamental fish traders make this decorative fish commodity to be quite exclusive. Besides, this group also found an indicator of fish which is the family Chaetodontidae, which is an indicator of coral health. This fish is also found in Trenggalek waters and is an indicator of the coral reef ecosystem in this region, which is also still awake (Wibowo and Adrim 2014). The existence of indicator fish is fundamental because it also reflects the condition of the waters and ecosystems of coral reefs that are still in good condition.



**Figure 1.** Phylogenetic reconstruction of Labridae from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm



**Figure 2.** Phylogenetic reconstruction of Pomacentridae and Pomacanthidae from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm



**Figure 3.** Phylogenetic reconstruction of a small number of families from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm

## Discussion

### Diversity

The waters of Tabuhan Island are an uninhabited island that is currently an area for nature tourism, besides that, this area is also an area for catching reef fish as an essential ornamental fish commodity in Indonesia. Banyuwangi area, which is adjacent to Bali (a famous spot for tourists), becomes a strength in exploiting coral, which is quite large.

The ornamental fish market in Bali is attractive enough for traditional fishers to make decorative fish as an alternative income source for local and international tourists besides consuming fish that has become a common catch.

The diversity of reef fish requires accurate identification, although there have been many studies of reef fish species, most of the identification is based on morphological information alone. In this study, we

identified molecularly as well as listed the sequences produced as sequences from the tropical waters of Tabuhan Island, Banyuwangi, Indonesia. This information is crucial for the study of molecular biology and other studies related to conservation biology in formulating policies for the conservation of coastal ecosystems, including coral reef ecosystems.

Previous research on DNA barcoding in reef fish has been done in Indonesia but is very limited. However, some researchers have carried out studies in several areas and carried out molecular identification, such as coral fish on Bali's Nusa Penida Island (Twindikio et al. 2013), Fish around Pondok Dadap Harbor, Malang (Andriyono et al. 2019), and several areas in Java and Bali (Andriyono et al. 2020a). With the limited molecular information on reef fish, research on coral fish in Indonesia has become essential.

The Labridae family is the most dominant group of marine ornamental fish. The results of this study obtained 20 samples identified in this family. Previous research found the Labridae Family as the group most often found in coral reef ecosystems (Suliswati et al. 2016). This group of fish also has the habit of schooling and grazing together throughout their life cycle. In groups, Labridae fish are generally found on branching coral species (Edrus and Hadi 2020). In this study, several essential species were successfully identified molecularly. In target fishes, the Perciformes order is the dominant fish that becomes the target fish, such as the Serranidae, Caesionidae, Acanthuridae and Mullidae fishes. In this study, the Serranidae family was represented by *Pseudanthias squamipinnis* (Peters, 1855), *Pseudanthias huchtii* (Bleeker, 1857), and *Belonoperca chabanaudi* (Fowler & Bean, 1930). Two species of *Pseudanthias* are identified as seawater ornamental fish because they have an attractive color. The *P. squamipinnis* fish is distribution in the Western Indian Ocean reefs to the Red Sea and Christmas in South Africa (Heemstra and Akhilesh 2012). Also reported, these fish inhabit the waters of Northern Japan and Southern Australia. In comparison, the *P. huchtii* fish has an attractive green color. It has the potential to become a seawater ornamental commodity that has habitat distribution in the Western Central Pacific, covering Sulawesi and the Philippines to Vanuatu, to the southern Great Barrier Reef and Palau regions in the Micronesian islands. Besides, *B. chabanaudi* has been reported to inhabit the Indo-Pacific region, including Japan (Randall and Schraml 2010). Very limited reports regarding species *B. chabanaudi* as commercial ornamental fish, but he has nice color and size suitable and potential as ornamental fish.

The proportion of major fish compared to target fish in this study was 90%, including Blennidae and Labridae. This value also occurs in almost all studies of reef fish with a higher composition of major fish than the target fish or indicator fish. For example, research conducted in Palu Bay waters found a composition of major fish of 54% and only 40% as target fish. The study currently conducted only takes ornamental fish samples, so the proportion of major fish is dominant compared to the target fish, which is only

7.5%. In this study, the Pomacentridae and Pomacathidae groups were identified as many as 7 and 5 species, respectively. This type of fish is fish that has characteristics of maintaining the territory of its habitat so that this group of fish is a permanent resident (resident species) in the coral reef ecosystem.

Whereas Chaetodontidae as indicators of coral reefs, this study found only one species in the Chaetodontidae, namely *Chaetodon kleinii* (Bloch, 1790). Species indicators show that the waters of Tabuan Island still have the coral cover that allows reef fish to live in this region. The identification of these fish also needs to be carried out further research on the condition of the coral cover of Tabuan Island. Research on coral reefs of Tabuhan Island shows that the conditions are quite weak, with values below 24.9% (Suprayogi 2017). However, this condition still allows some reef fish to live in this area, with conservation activities carried out independently by the community accompanied by several academic institutions and local non-governmental organizations in Banyuwangi, Indonesia (Erwanto and Masluha 2019).

In the group of fish that have venom, *Pterois volitans* (Linnaeus, 1758) (Scorpaenidae) are identified in Tabuhan Island and become one of the traded species. This lionfish species is a common species traded along with other lionfish species, *P. miles* (Lyons et al. 2017). However, several references indicate that *P. volitans* has the potential to be invasive. The researches have demonstrated that *P. volitans* invaded the North America region, Florida (Freshwater et al. 2009), and other regions in the Indo-pacific region such as the Atlantic coast of mainland USA, the Western North Atlantic, and the Caribbean Sea (Schofield 2009; Morris et al. 2011). As a native fish in the Indo-Pacific region, The red lionfish (*P. volitans*) controls other reef fish species because of their carnivorous nature (Morris et al. 2011). Although, it is mentioned that this lionfish species is abundant in the Indo-Pacific region (Green and Côté 2009), fishers in Banyuwangi do not exploit this species much because they have venom, which is quite dangerous to humans.

#### Conservation strategy

The increase in tourism activities in Banyuwangi also impacts increasing tourist visits to Tabuhan Island (Erwanto and Masluha 2019). This tourist visit can hurt efforts to conserve coral reef ecosystems in this area. In addition, most domestic tourists are not equipped with adequate conservation knowledge, which can impact damage and bring in plastic waste in this area (Barlinti 2020; Mirsalila 2020). Therefore, it is necessary to limit tourism activities in this area as well as to monitor and educate the importance of protecting fishery and marine resources in general. This restriction is adjusted to the ability of the region to receive visits. The concept of this restriction has been applied in several tourist areas that pay attention to the carrying capacity of the area, such as in the Duyung Island Arcipelago, Riau Archipelago (Mukhlis et al. 2022), Sebesi Island, Lampung (Johan 2016), Karimunjawa Archipelago, Jawa Tengah (Suliswati 2016), Dodola

Island of Morotai Arcipelago, Maluku Utara (Koroy et al. 2018).

Several strategies that need to be implemented with high fish biodiversity include catching environmentally friendly ornamental fish with non-destructive fishing gear, for example set net (Salim et al. 2019). It is also necessary to pay attention to the number and types of fish caught (Marwadi and Anggoro 2013). Some ornamental fish are not included in the protected category; however, their population continues to decline as the coral reef ecosystem is damaged (Setiawan et al. 2013; Ulfah et al. 2018). Thus, coral reef fish conservation strategies must also be accompanied by good management of coral reef ecosystems.

The condition of coral reef cover on Tabuhan Island needs to be considered. Activities to increase coral reef cover artificially can be done by transplanting corals (Erwanto and Masluha 2019). This activity has been successful in several places such as Bali (Nurcahyani 2018), Jakarta (Johan et al. 2016), Makassar (Kasmi et al. 2021), Bintan (Bukhari and Kurniawan 2021) and Papua (Harianto et al. 2013). With the increasing condition of coral reef cover, it is likely to be followed by an increasing number of reef fish living in this area. It has been proven that coral reefs provide a place for nurturing young fish, spawning, and foraging. Good environmental support will also impact people who depend on coral reef ecosystems for their lives.

Several fish associated with coral reefs become the target of fishing catches, such as grouper, snapper, and napoleon fish. Several studies have shown that grouper species are also very diverse inhabiting coral reef ecosystems (Jefri et al. 2015; Andriyono et al. 2020b). Meanwhile, napoleon fish species even have a very fantastic selling price even though they are currently in a protected status (Miñarro et al. 2016). The knowledge and understanding of the community need to be improved so that the concept of sustainable fisheries can be applied properly. The concept of community-based conservation is deemed more appropriate and can have a significant impact on the sustainability of marine biota in addition to the application of protected areas in the form of National Parks or Marine Protected Areas. The concept of a community-based has been applied in several regions of Indonesia (Gurney et al. 2016; Damastuti et al. 2022) and is expected to preserve Indonesia's marine water resources for the future.

In conclusion, the diversity of reef fish resources has great potential in Tabuhan Island can be maintained, even though the Banyuwangi is currently developing for the tourism industry. This study, 53 specimens were identified, including 49 species of 3 orders and 17 families. Among the identified families, the most family is Labridae, with 20 species identified. The Labridae currently identified consist of three subfamilies, namely Corinae, Bodianinae, and Cheilinae; each subfamily has formed a separate clade in phylogenetic tree reconstruction. The Pomacentridae and Pomacanthidae are separated to develop their respective clades. The Tetracteroidei were scattered between *Canthigaster valentini* (Bleeker, 1853) and *Ostracion*

*cubicus* (Linnaeus, 1758), which may show polyphyletic properties. We have not yet received the Genbank accession numbers for 18 sequences. On the other hand, a total of 35 COI sequences were successfully deposited in the GenBank database and became important information for studying the biodiversity and genetics of coral reef fish in Indonesian waters.

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