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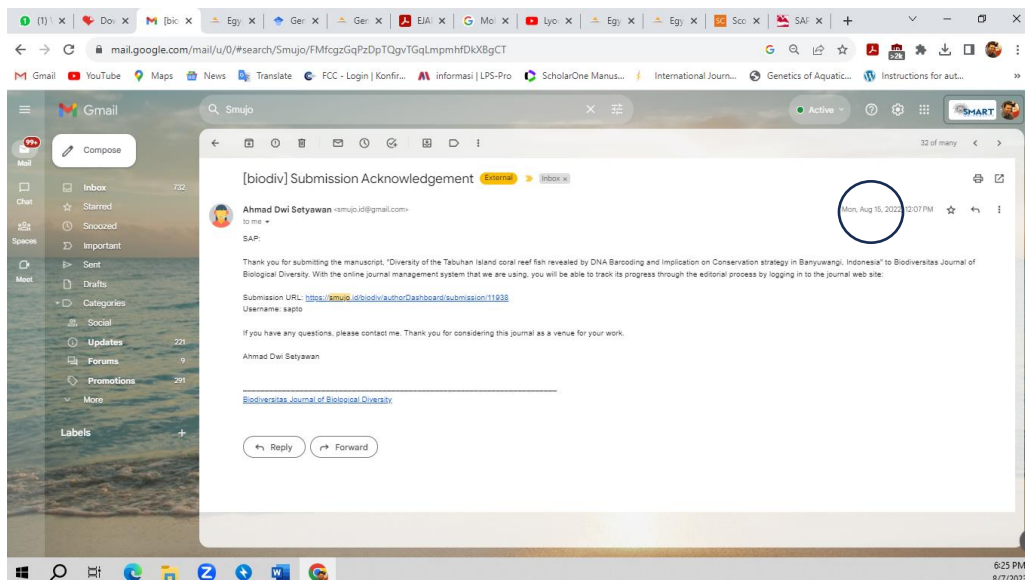
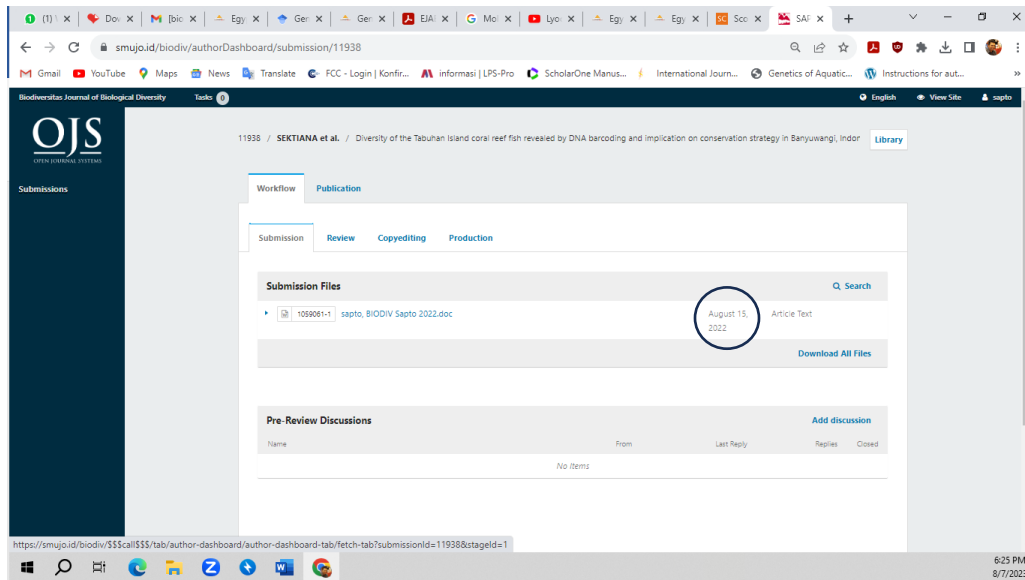
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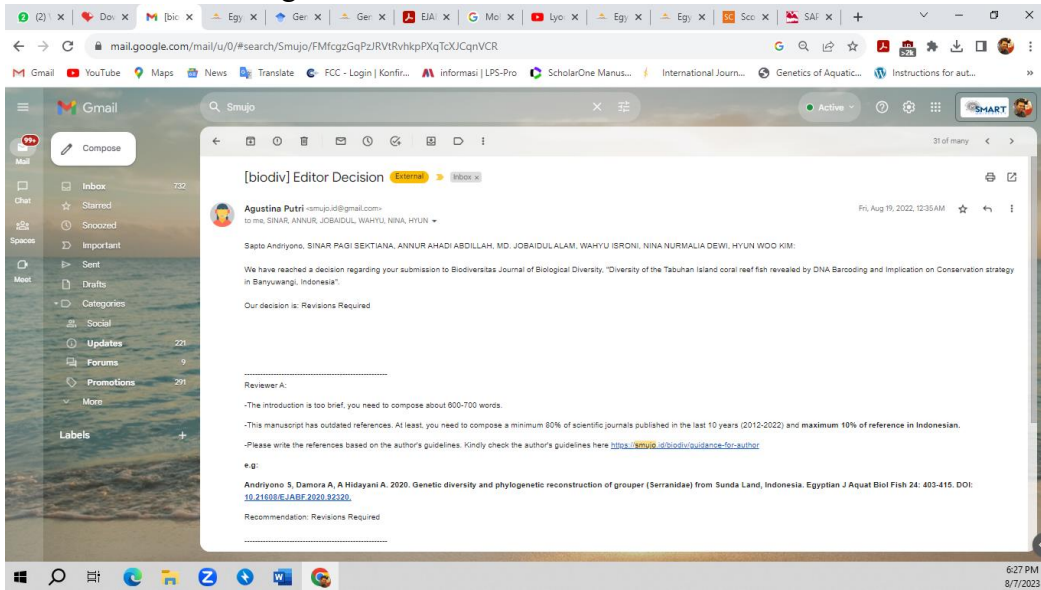
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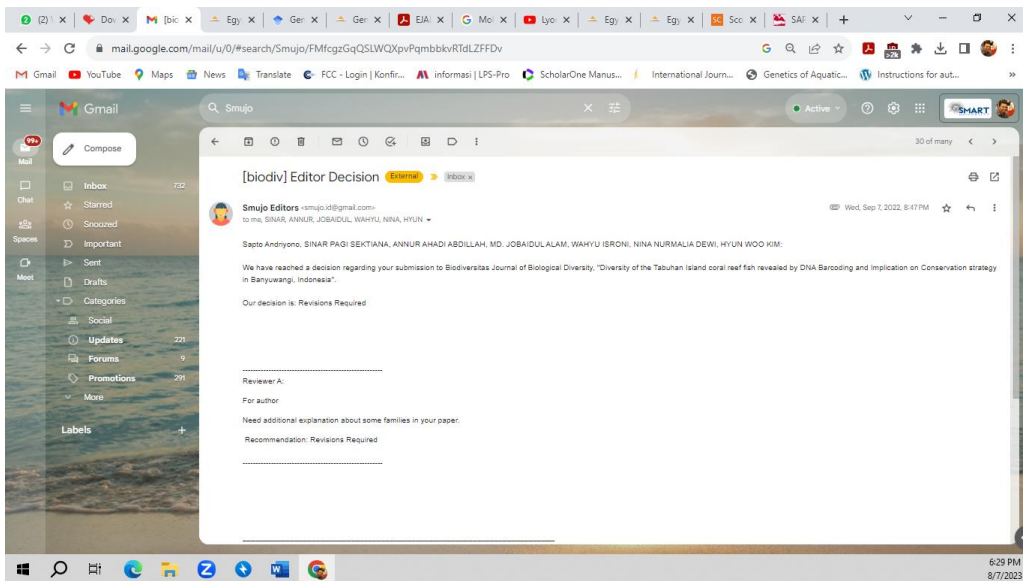
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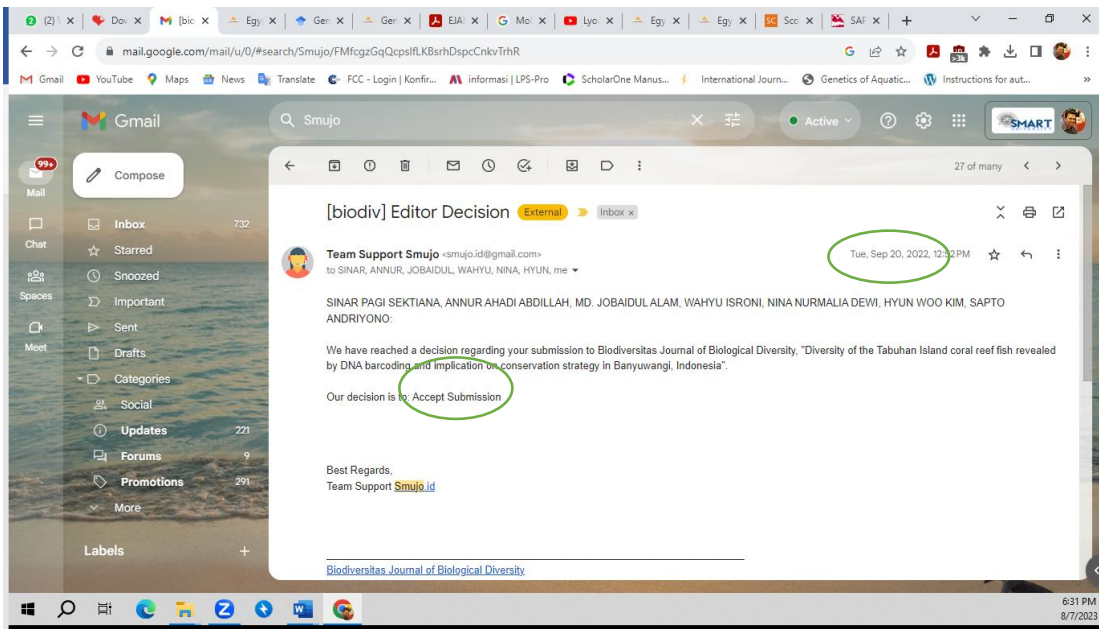
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Diversity of the Tabuhan Island coral reef fish revealed by DNA Barcoding and Implication on Conservation strategy in Banyuwangi, Indonesia

Author(s) name:

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SAPTO ANDRIYONO

Diversity of the Tabuhan Island coral reef fish revealed by DNA Barcoding and Implication on Conservation strategy in Banyuwangi, Indonesia

SINAR PAGI SEKTIANA¹, ANNUR AHADI ABDILLAH², MD. JOB Aidul ALAM³ WAHYU ISRONI⁴, NINA NURMALIA DEWI⁴, HYUN WOO KIM^{2,5} AND SAPTO ANDRIYONO^{2*}

¹Aquaculture Technology Study Program. Jakarta Technical University of Fisheries, Jl. AUP Pasar Minggu Jakarta Selatan 12520 Jakarta, Indonesia.

²Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga C Campus

Jl. Mulyorejo Surabaya East Java, 60115, Indonesia

³Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh. Email: jobaidul_dof@yahoo.com

⁴Department of Aquaculture, Fisheries and Marine Faculty, Universitas Airlangga, Surabaya, East Java, Indonesia.

⁵Department of Marine Biology, School of Fisheries Sciences, Pukyong National University, Busan, South Korea.

*email: sapto.andriyono@fpk.unair.ac.id

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Abstract. Tabuhan Island, one of the mainstays of coastal tourism with the charm of coral reefs, 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 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 reconstruction of the phylogenetic tree shows that several species are collected by family, 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: diversity, coral reef ecosystem, marine fish, conservation, sustainable

Running title: Sektiana et al. Diversity of the Tabuhan Island

INTRODUCTION

The Java Sea 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 and Petit 1995) and tourism (Hutomo and Moosa 2005, Wilkinson et al. 1995). The Java Sea is included as shallow water located between Kalimantan, Java, Sumatra, and Sulawesi, within an area of 310,000 km². 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 has increased the demand for fishes thus stimulating the development of fishing in this area (Purwanto 2003). However, the biodiversity in the Indonesian coral reef is facing a threat with global climate changes, various anthropogenic activities, fisheries, and sedimentation (Edinger et al. 1998). Furthermore, the biodiversity of the Java Sea has also experienced a tremendous impact from these activities (Purwanto 2003).

Tabuhan Island, located in the Banyuwangi District of East Java, is a small island of interest for tourism activities, water sports, and ornamental fisheries (Damayanti 2012) and there is no previous study about the fish biodiversity on Tabuhan Island. For the conservation of biodiversity, information is necessary about each specimen with data ranging from systematic position to molecular aspects. It is stored as species nomenclature, including conservation status (Shanmughavel 2007). The number of species within a community is called species richness. It is the most dominant measure of biodiversity as it can be easily monitored and recorded (Hillebrand et al. 2018).

Furthermore, decision-makers face the problem of misidentification for conservation and management purposes, so species determination becomes essential. In this report, we have summarized the DNA barcoding and phylogenetic reconstruction of several coral reef fish from Tabuhan Island, Banyuwangi. This information will be crucial for further studies on coral reef fish biology and other research related to the field of Genetic of coral reef fisheries in Indonesia.

49 Sampling site

50 We have collected 53 fish specimens from the coral reefs in 2019 at Tabuhan Island of Banyuwangi, West Java (8°
51 3'35.52"S, 114°27'42.08"E), Indonesia (Fig 3. 1). Each specimen was kept in the freezer (at -20°C) in a 96 % ethanol
52 preservation solution. Parts of the body, including the muscles or dorsal fins, were used for further DNA sequence
53 analysis.

54 Genomic DNA extraction, amplification, and Sequencing

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

59 Fish Cytochrome oxidase I (COI) universal primer pairs BCL (TCA ACY AAT CAY AAA GAT ATY GGC AC) and
60 BCH (ACT TCY GGG TGR CCR AAR AAT CA) (Baldwin et al., 2009) were used in PCR reaction to obtain barcoding
61 sequence for molecular identification (Hebert et al. 2003). The PCR reaction (20 µL) contained 11.2 µL ultrapure water, 1
62 µL of each primer (0.5µM), 0.2 µL Extaq Hotstart version DNA polymerase (TAKARA, Japan), 2 µL 10x Extaq buffer, 2
63 µL dNTPs (1µM, TAKARA, Japan), 0.6 % total volume DMSO and 200 ng Genomic DNA as a template. Initial
64 denaturation at the first stage of the PCR was carried out at 94°C for 3 minutes. Next, the primary PCR process includes
65 denaturation (35 cycles of at 94°C for 30 sec), annealing (50°C for 30 sec), and extension (72°C for 45 sec). The last step is
66 the final extension at 72°C for 5 minutes. The PCR products were purified using a gel extraction kit (Bioneer, Daejeon,
67 Korea) by following the manufacturer's standard protocol.

68 DNA Sequence analysis

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

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

RESULTS AND DISCUSSION

85 Results

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

92 Phylogenetic tree reconstruction of different families:**93 Labridae**

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

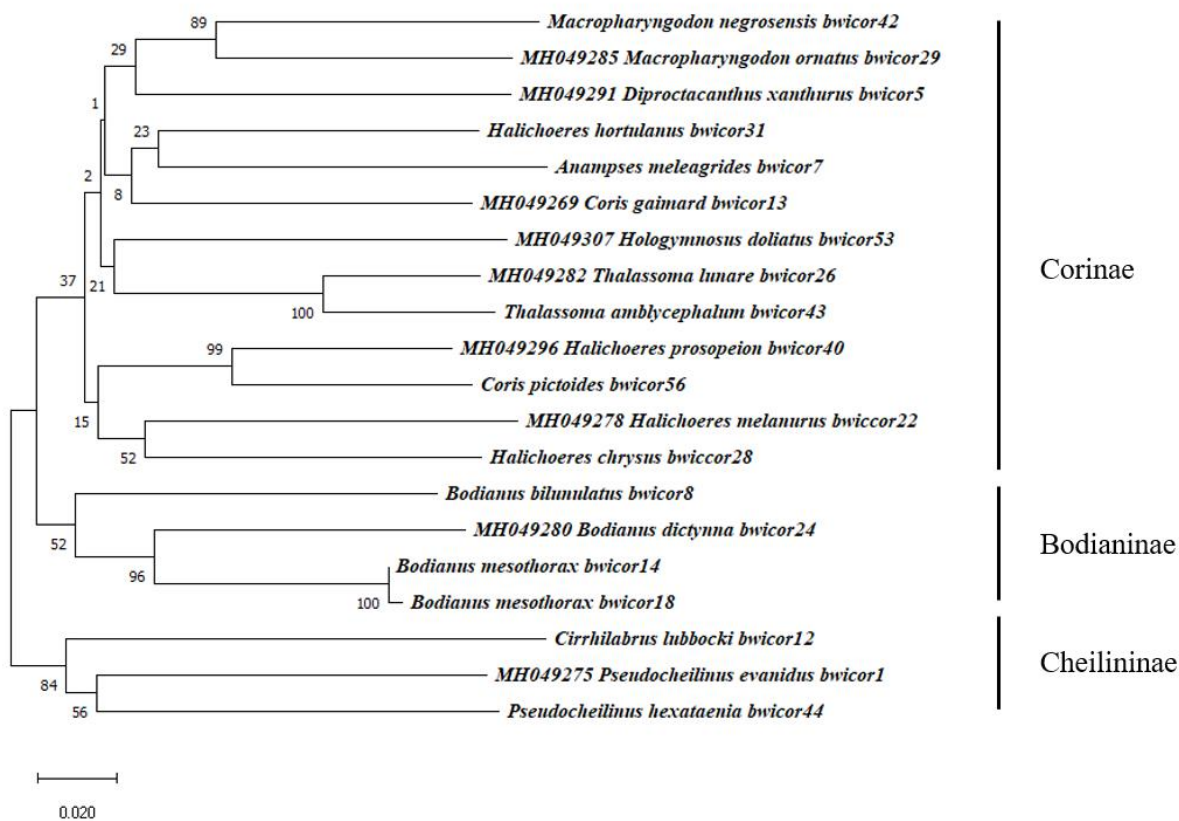


Figure 1. Phylogenetic reconstruction of Labridae from Tabuhan Island using Neighbour Joining algorithm

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).

Others

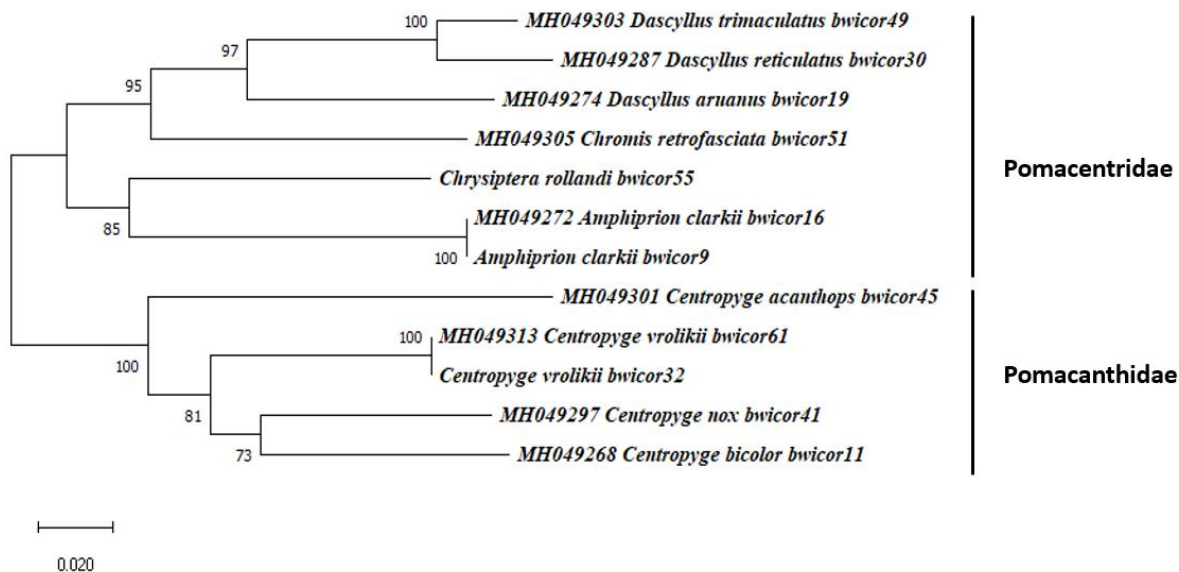


Figure 2. Phylogenetic reconstruction of Pomacentridae and Pomacanthidae from Tabuhan Island using Neighbour Joining algorithm

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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. 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 that 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.

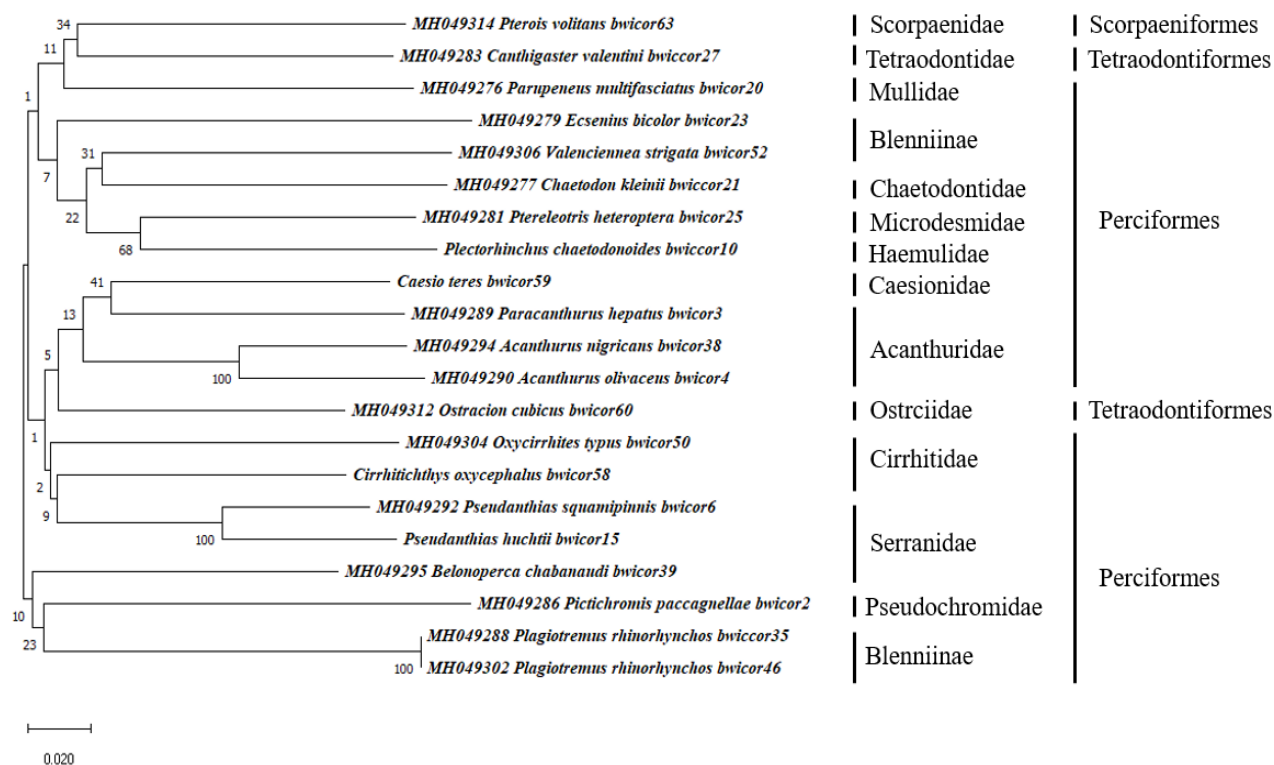


Figure 2. Phylogenetic reconstruction of a small number of families from Tabuhan Island using Neighbour Joining algorithm

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Discussion

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 the exploitation of 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 study related to conservation biology in the formulation of 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. Some researchers have carried out studies in several areas and carried out molecular identification, such as coral fish on Bali's Nusa Penida Island (Twindiko 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.

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, and Mullidae fishes. In this study, the Serranidae family was represented by *Pseudanthias squamipinnis*, *Pseudanthias huchtii*, and *Belonoperca chabanaudi*. Two species of *Pseudanthias* are identified as seawater ornamental fish because they have an attractive color.

151 *Pseudanthias squamipinnis* fish distribution in the Western Indian Ocean reefs to the Red Sea and Christmas in South
152 Africa (Heemstra and Akhilesh 2012). Also reported, these fish inhabit the waters of northern Japan, southern Australia.
153 Whereas the *Pseudanthias huchtii*, fish has an attractive green color. It has the potential to become a seawater ornamental
154 commodity that has habitat distribution in the Western Central Pacific covering Sulawesi and the Philippines to Vanuatu,
155 to the southern Great Barrier Reef and Palau regions in the Micronesian islands (Myers 1999).

156 The proportion of major fish compared to target fish in this study was 90%. This value also occurs in almost all studies
157 of reef fish that have a higher composition of major fish than the target fish or indicator fish. Research conducted in Palu
158 Bay waters found a composition of major fish by 54% and only 40% as a target fish. Whereas the study that is currently
159 conducted, only takes ornamental fish samples so that the proportion of major fish is very dominant compared to the target
160 fish, which is only 7.5%. In this study, the Pomacentridae and Pomacathidae groups were identified as many as 7 and 5
161 species, respectively. This type of fish is fish that has characteristics of maintaining the territory of its habitat so that this
162 group of fish is a permanent resident (resident species) in the coral reef ecosystem.

163 Whereas Chaetodontidae as indicators of coral reef (Reese 1995), this study found only one species in the
164 Chaetodontidae, namely *Chaetodon kleinii*. Species indicators show that the waters of the Tabuan Island still have the
165 coral cover that allows reef fish to live in this region. The identification of these fish also needs to be carried out further
166 research on the condition of the coral cover of Tabuan Island. Research on coral reefs of the Tabuhan Island shows that the
167 conditions are quite weak, with values below 24.9% (Suprayogi 2017). However, this condition still allows some reef fish
168 to live in this area with conservation activities carried out independently by the community accompanied by several
169 academic institutions and local non-governmental organizations in Banyuwangi, Indonesia (Erwanto and Masluha 2019a).

170 In the group of fish that have venom, *Pterois volitans* are identified in the Tabuhan Island and become one of the
171 traded species. This lionfish species is a common species traded along with other lionfish species *P. miles* (Lyons et al.
172 2017). However, several references indicate that *P. volitans* has the potential to be invasive. The researches have
173 demonstrated that *P. volitans* invading the North America region (Whitfield et al. 2002), Florida (Freshwater et al. 2009),
174 and other regions in the Indo-pacific region such as the Atlantic coast of mainland USA, the Western North Atlantic, and
175 the Caribbean Sea (Morris et al. 2011, Schofield 2009). As a native fish in the Indo-Pacific region, The red lionfish (*P.*
176 *volitans*) plays a role in controlling other reef fish species because of their carnivorous nature (Morris and Akins 2009,
177 Morris et al. 2011). Although, it is mentioned that this species of lionfish is abundant in the Indo-Pacific region (Green and
178 Côté 2009), fishers in Banyuwangi do not exploit this species much because these fish have venom which is quite
179 dangerous to humans (Church and Hodgson 2002).

180 181 **Conservation strategy**

182 The increase in tourism activities in Banyuwangi also has an impact on increasing tourist visits to Tabuhan Island
183 (Erwanto and Masluha 2019b). This tourist visit can hurt efforts to conserve coral reef ecosystems in this area. Most
184 domestic tourists are not equipped with adequate conservation knowledge so it can have an impact on damage and bring in
185 plastic waste in this area (Barlinti 2020, Mirsalila 2020). Therefore, it is necessary to limit tourism activities in this area as
186 well as to monitor and educate the importance of protecting fishery and marine resources in general. This restriction is
187 adjusted to the ability of the Region to receive visits. The concept of this restriction has been applied in several tourist
188 areas that pay attention to the carrying capacity of the area, such as in the Duyung Island Archipelago, Riau Archipelago
189 (Mukhlis et al. 2022), Sebesi Island, Lampung (Johan Yar 2016), Karimun Jawa Archipelago, Jawa Tengah (SULISYATI
190 2016), Dodola Island of Morotai Archipelago, Maluku Utara (Kismanto Koroy and Mustafa 2018).

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

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

204 A number of fish associated with coral reefs become the target of fishing catches such as grouper, snapper and
205 napoleon fish. Several studies have shown that grouper species are also very diverse inhabiting coral reef ecosystems
206 (Andriyono et al. 2020b, Jefri et al. 2015). Meanwhile, napoleon fish species even have a very fantastic selling price even
207 though they are currently in a protected status (Miñarro et al. 2016). The knowledge and understanding of the community
208 need to be improved so that the concept of sustainable fisheries can be applied properly. The concept of community-based
209 conservation is deemed more appropriate and can have a significant impact on the sustainability of marine biota in addition
210 to the application of protected areas in the form of National Parks or Marine Protected Areas. The concept of community

211 based has been applied in a number of regions of Indonesia (Damastuti et al. 2022, Gurney et al. 2016) and is expected to
212 preserve Indonesia's marine water resources for the future.

213 CONCLUSIONS

214
215 The diversity of reef fish resources has a great potential in the Tabuhan Island can be maintained, even though the
216 Banyuwangi is currently developing for tourism industry. In this study, 53 specimens were identified, including 49 species
217 of 3 orders and 17 families. Among the identified families, the most family is Labridae, with 20 species identified. The
218 Labridae that are currently identified consist of three subfamilies namely Corinae, Bodianinae, and Cheilinae; each
219 subfamily has formed a separate clade in phylogenetic tree reconstruction. The Pomacentridae and Pomacanthidae are
220 separated to develop their respective clades. The Tetraodontiformes were identified as being scattered between
221 *Canthigaster valentine* and *Ostracion cubicus*, which may show polyphyletic properties. We have not got yet the Genbank
222 accession numbers for 18 sequences, on the other hand, a total of 35 COI sequences were successfully deposited in the
223 GenBank database and it became important information for the study of biodiversity and genetic of coral reef fish in
224 Indonesian waters.
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233

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381 **Table 1.** Summary of coral reef fishes identified from Tabuhan Island, Banyuwangi, Indonesia

No.	Sample ID	Order*	Family*	Species name	Habitat distribution **	GenBank accession No.***
1	bwicor1	Perciformes	Labridae	<i>Pseudocheilinus evanidus</i>	Indo-Pacific: the Red Sea to South Africa	MH049275
2	bwicor44	Perciformes	Labridae	<i>Pseudocheilinus hexataenia</i>	Indo-Pacific	nr
3	bwicor13	Perciformes	Labridae	<i>Coris gaimard</i>	Pacific Ocean	MH049269
4	bwicor56	Perciformes	Labridae	<i>Coris pictoides</i>	Western Pacific	nr
5	bwicor40	Perciformes	Labridae	<i>Halichoeres prosopeion</i>	Western Pacific	MH049296
6	bwicor31	Perciformes	Labridae	<i>Halichoeres hortulanus</i>	Indo-Pacific	nr
7	bwicor28	Perciformes	Labridae	<i>Halichoeres chrysus</i>	Eastern Indian Ocean	nr
8	bwicor53	Perciformes	Labridae	<i>Hologymnosus doliatus</i>	Indo-Pacific, South Africa	MH049307
9	bwicor26	Perciformes	Labridae	<i>Thalassoma lunare</i>	Indo-Pacific	MH049282
10	bwicor43	Perciformes	Labridae	<i>Thalassoma amblycephalum</i>	Indo-Pacific	nr
11	bwicor22	Perciformes	Labridae	<i>Halichoeres melanurus</i>	Indo-Pacific: Bali, Indonesia, Australia	MH049278
12	bwicor29	Perciformes	Labridae	<i>Macropharyngodon ornatus</i>	Indo-Pacific	MH049285
13	bwicor42	Perciformes	Labridae	<i>Macropharyngodon negrosensis</i>	Eastern Indian Ocean	nr
14	bwicor24	Perciformes	Labridae	<i>Bodianus dictynna</i>	Western Pacific	MH049280
15	bwicor14	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
16	bwicor18	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
17	bwicor8	Perciformes	Labridae	<i>Bodianus bilunulatus</i>	Indo-West Pacific	nr
18	bwicor5	Perciformes	Labridae	<i>Diproctacanthus xanthurus</i>	Western Central Pacific	MH049291
19	bwicor12	Perciformes	Labridae	<i>Cirrhilabrus lubbocki</i>	Western Central Pacific	nr
20	bwicor7	Perciformes	Labridae	<i>Anampses meleagrides</i>	Indo-Pacific	nr
21	bwicor16	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	MH049272
22	bwicor9	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	nr
23	bwicor19	Perciformes	Pomacentridae	<i>Dascyllus aruanus</i>	Pacific Ocean	MH049274
24	bwicor49	Perciformes	Pomacentridae	<i>Dascyllus trimaculatus</i>	Indo-Pacific	MH049303
25	bwicor30	Perciformes	Pomacentridae	<i>Dascyllus reticulatus</i>	Eastern Central Indian Ocean to Western Pacific	MH049287
26	bwicor51	Perciformes	Pomacentridae	<i>Chromis retrofasciata</i>	Western Pacific	MH049305
27	bwicor55	Perciformes	Pomacentridae	<i>Chrysiptera rollandi</i>	Eastern Indian Ocean	nr
28	bwicor3	Perciformes	Acanthuridae	<i>Paracanthurus hepatus</i>	Indo-Pacific	MH049289
29	bwicor38	Perciformes	Acanthuridae	<i>Acanthurus nigricans</i>	Eastern Indian Ocean	MH049294
30	bwicor4	Perciformes	Acanthuridae	<i>Acanthurus olivaceus</i>	Pacific Ocean	MH049290
31	bwicor61	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	MH049313
32	bwicor32	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	nr
33	bwicor41	Perciformes	Pomacanthidae	<i>Centropyge nox</i>	Western Pacific	MH049297
34	bwicor45	Perciformes	Pomacanthidae	<i>Centropyge acanthops</i>	Western Indian Ocean	MH049301
35	bwicor11	Perciformes	Pomacanthidae	<i>Centropyge bicolor</i>	Indo-Pacific	MH049268
36	bwicor23	Perciformes	Blennidae	<i>Ecsenius bicolor</i>	Indo-Pacific	MH049279
37	bwicor35	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchus</i>	Indo-Pacific	MH049288
38	bwicor46	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchus</i>	Indo-Pacific	MH049302
39	bwicor52	Perciformes	Blennidae	<i>Valenciennesa strigata</i>	Indo-Pacific	MH049306
40	bwicor21	Perciformes	Chaetodontidae	<i>Chaetodon kleinii</i>	Indo-Pacific, Eastern Pacific	MH049277
41	bwicor50	Perciformes	Cirrhitidae	<i>Oxyrrhites typus</i>	Indo-Pacific	MH049304
42	bwicor58	Perciformes	Cirrhitidae	<i>Cirrhitichthys oxycephalus</i>	Indo-Pacific	nr

43	bwicor25	Perciformes	Microdesmidae	<i>Ptereleotris heteroptera</i>	Indo-Pacific	MH049281
44	bwicor20	Perciformes	Mullidae	<i>Parupeneus multifasciatus</i>	Pacific Ocean	MH049276
45	bwicor2	Perciformes	Pseudochromidae	<i>Pictichromis paccagnellae</i>	Western Pacific, Palau	MH049286
46	bwicor6	Perciformes	Serranidae	<i>Pseudanthias squamipinnis</i>	Indo-West Pacific	MH049292
47	bwicor15	Perciformes	Serranidae	<i>Pseudanthias huchtii</i>	Western Central Pacific	nr
48	bwicor39	Perciformes	Serranidae	<i>Belonoperca chabanaudi</i>	Indo-Pacific	MH049295
49	bwicor10	Perciformes	Haemulidae	<i>Plectorhinchus chaetodonoides</i>	Indo-West Pacific	nr
50	bwicor59	Perciformes	Caesionidae	<i>Caesio teres</i>	Indo-West Pacific	nr
51	bwicor63	Scorpaeniformes	Scorpaenidae	<i>Pterois volitans</i>	Pacific Ocean	MH049314
52	bwicor60	Tetraodontiformes	Ostraciidae	<i>Ostracion cubicus</i>	Indo-Pacific	MH049312
53	bwicor27	Tetraodontiformes	Tetraodontidae	<i>Canthigaster valentini</i>	Indo-Pacific	MH049283

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383 * WoRMS : <http://www.marinespecies.org/>

384 ** Fishbase database : <https://www.fishbase.se/>

385 *** NCBI database : <https://www.ncbi.nlm.nih.gov/>

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Diversity of the Tabuhan Island coral reef fish revealed by DNA Barcoding and Implication on Conservation strategy in Banyuwangi, Indonesia

Author(s) name:

SINAR PAGI SEKTIANA, ANNUR AHADI ABDILLAH, MD. JOB Aidul Alam, WAHYU ISRONI, NINA NURMALIA DEWI, HYUN WOO KIM, SAPTO ANDRIYONO

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SAPTO ANDRIYONO

Diversity of the Tabuhan Island coral reef fish revealed by DNA Barcoding and Implication on Conservation strategy in Banyuwangi, Indonesia

SINAR PAGI SEKTIANA¹, ANNUR AHADI ABDILLAH², MD. JOB Aidul Alam³ WAHYU ISRONI⁴, NINA NURMALIA DEWI⁴, HYUN WOO KIM^{2,5} AND SAPTO ANDRIYONO^{2*}

¹Aquaculture Technology Study Program, Jakarta Technical University of Fisheries, Jl. AUP Pasar Minggu Jakarta Selatan 12520 Jakarta, Indonesia.

²Department of Marine, Fisheries and Marine Faculty, Universitas Airlangga C Campus Jl. Mulyorejo Surabaya East Java, 60115, Indonesia

³Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh

⁴Department of Aquaculture, Fisheries and Marine Faculty, Universitas Airlangga, Surabaya, East Java, Indonesia.

⁵Department of Marine Biology, School of Fisheries Sciences, Pukyong National University, Busan, South Korea.

*email: sapto.andriyono@fpk.unair.ac.id

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Abstract. Tabuhan Island, one of the mainstays of coastal tourism with the charm of coral reefs, 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 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 reconstruction of the phylogenetic tree 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: diversity, coral reef ecosystem, marine fish, conservation, sustainable

Running title: Sektiana et al. Diversity of the Tabuhan Island

INTRODUCTION

The Java Sea 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 and Petit 1995) and tourism. The Java Sea is included as shallow water between Kalimantan, Java, Sumatra, and Sulawesi, within 310,000 km². 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 has increased the demand for fishes 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. In Banten Province, there is Tunda Island which 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, Karimun Jawa National Park (Hafsaridewi et al. 2018, Yuliana et al. 2020). In East Java, apart from Bawean Island (Riskiani et al. 2019) in Gresik Regency, there is Tabuhan Island in Banyuwangi Regency (Luthfi et al. 2016).

Tabuhan Island is an empty island in the waters of Banyuwangi Regency, precisely included in the administrative area of Bangsring Village, Wongsorejo District. The island is about 20 km from the mainland of Bangsing 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 still through a morphological approach (Azhar et al. 2019), a molecular approach has never been done. 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

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

51 Biodiversity studies in coral reef areas generally examine macrobenthos (Quimpo et al. 2018), coral reef cover (Annas
52 et al. 2017, Putra Risandi Dwirama et al. 2018) and symbiotic fish species in this essential ecosystem area (Sahetapy et al.
53 2018). Reef fish are important biota as an indicator of the health of coral reef ecosystems by identifying certain types of
54 fish such as Chaetodontidae (Hamuna et al. 2019). In addition, the number of reef fish is also a target for traditional
55 fishermen because they have a fairly high price such as snapper (Arai et al. 2015) and grouper (Nanami 2021). In addition,
56 the number of endemic fish and protected fish (Cowman et al. 2017, Hobbs et al. 2013) also makes coral reefs an
57 important area for breeding, foraging and raising children. By taking into account the important role of coral reefs,
58 conservation activities for small islands in Indonesia will continue to be carried out.

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

66

MATERIALS AND METHODS

67 **Sampling site**

68 We have collected 53 fish specimens from the coral reefs in 2019 at Tabuhan Island of Banyuwangi, West Java (8°
69 3'35.52"S, 114°27'42.08"E), Indonesia (Fig 3. 1). Each specimen was kept in the freezer (at -20°C) in a 96 % ethanol
70 preservation solution. Parts of the body, including the muscles or dorsal fins, were used for further DNA sequence
71 analysis.

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73 **Genomic DNA extraction, amplification, and Sequencing**

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

78 Fish Cytochrome oxidase I (COI) universal primer pairs BCL (TCA ACY AAT CAY AAA GAT ATY GGC AC) and
79 BCH (ACT TCY GGG TGR CCR AAR AAT CA) (Baldwin et al., 2009) were used in PCR reaction to obtain barcoding
80 sequence for molecular identification (Hebert et al. 2003). The PCR reaction (20 µL) contained 11.2 µL ultrapure water, 1
81 µL of each primer (0.5µM), 0.2 µL Extaq Hotstart version DNA polymerase (TAKARA, Japan), 2 µL 10x Extaq buffer, 2
82 µL dNTPs (1µM, TAKARA, Japan), 0.6 % total volume DMSO and 200 ng Genomic DNA as a template. Initial
83 denaturation at the first stage of the PCR was carried out at 94°C for 3 minutes. Next, the primary PCR process includes
84 denaturation (35 cycles of at 94°C for 30 sec), annealing (50°C for 30 sec), and extension (72°C for 45 sec). The last step is
85 the final extension at 72°C for 5 minutes. The PCR products were purified using a gel extraction kit (Bioneer, Daejeon,
86 Korea) by following the manufacturer's standard protocol.

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88 **DNA Sequence analysis**

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

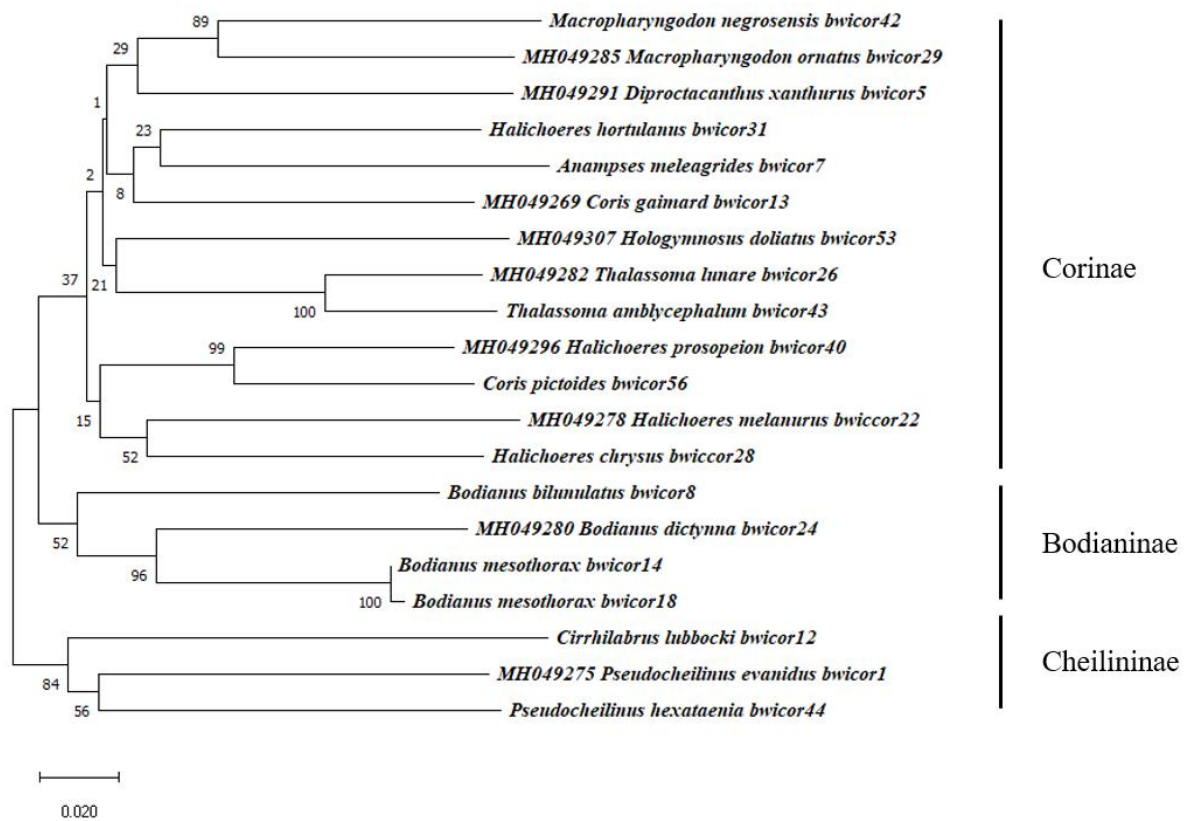
97 The multiple alignments of sequences were conducted using the MUSCLE program (Edgar 2004). Nucleotide
98 composition, transition and transversion bias estimation, overall pairwise distance, and Minimum Evolution (ME) tree
99 reconstruction were calculated using the Kimura two-parameter (K2P) distance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

103 **Results**

104 In this research, molecular identification has been carried out to complete the morphological identification that has
 105 been done so far. A total of 53 fish samples showed similarities with the reference BLASTN results with a database on
 106 GenBank with a value of 99-100%. Of the 53 samples, only 16 specimens have not yet received the GenBank accession
 107 numbers, because the registration process has not been completed (still in process). However, all sequences, included in
 108 the resulting phylogenetic tree are grouped into three broad groups, namely Labridae (the most dominant family),
 109 Pomacentridae and Pomacanthidae, and a small number of other families of Teleostei (small groups of families).
 110

111 **Phylogenetic tree reconstruction of different families:**112 **Labridae**

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



119 **Figure 1.** Phylogenetic reconstruction of Labridae from Tabuhan Island using Neighbour Joining algorithm
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122 **Pomacentridae and Pomacanthidae**

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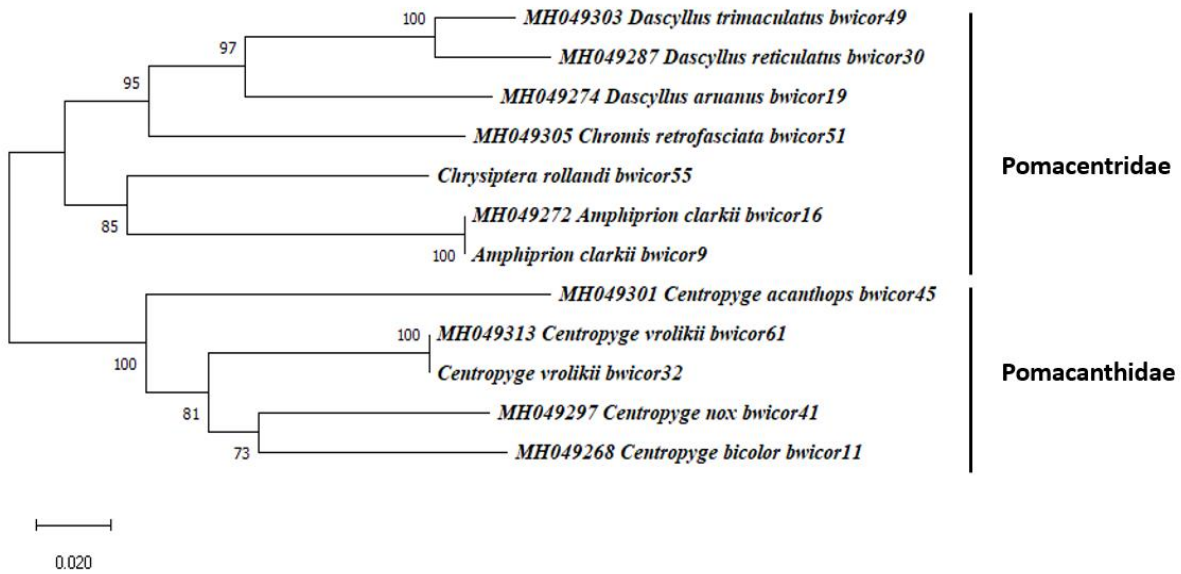


Figure 2. Phylogenetic reconstruction of Pomacentridae and Pomacanthidae from Tabuhan Island using Neighbour Joining algorithm

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. 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 that 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.

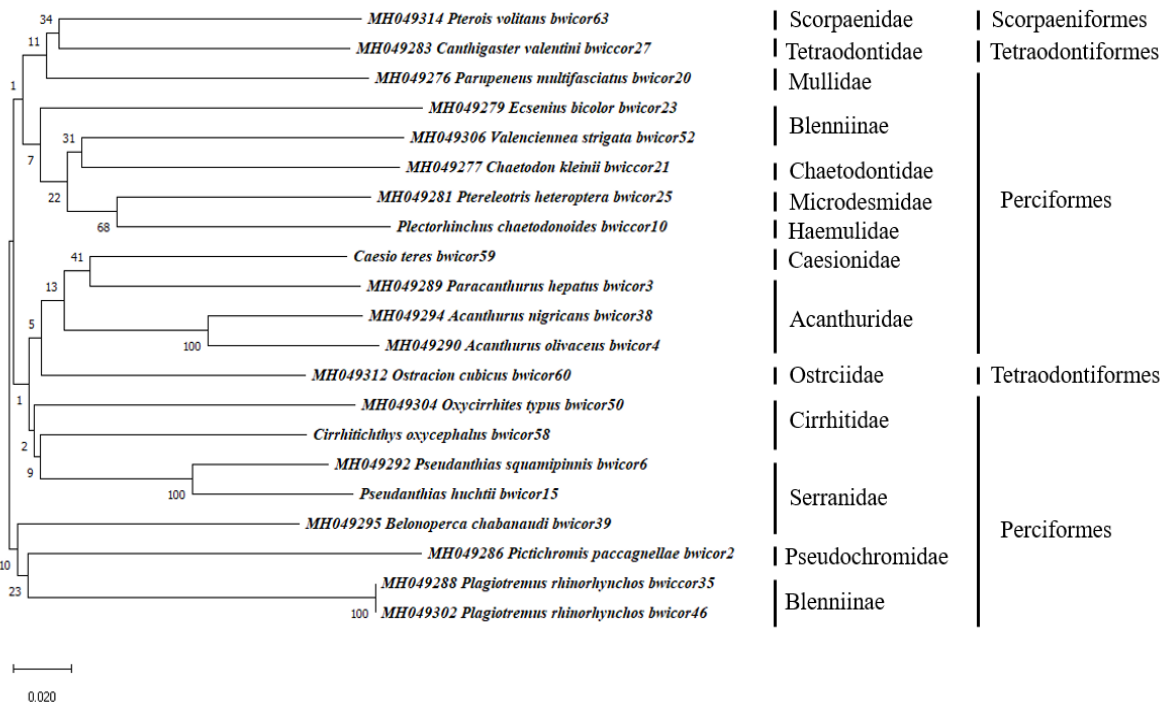


Figure 2. Phylogenetic reconstruction of a small number of families from Tabuhan Island using Neighbour Joining algorithm

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Discussion

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 the exploitation of 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 study related to conservation biology in the formulation of 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. Some researchers have carried out studies in several areas and carried out molecular identification, such as coral fish on Bali's Nusa Penida Island (Twindiko 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.

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, and Mullidae fishes. In this study, the Serranidae family was represented by *Pseudanthias squamipinnis*, *Pseudanthias huchtii*, and *Belonoperca chabanaudi*. Two species of *Pseudanthias* are identified as seawater ornamental fish because they have an attractive color. *Pseudanthias squamipinnis* fish 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, southern Australia. Whereas the *Pseudanthias 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.

The proportion of major fish compared to target fish in this study was 90%. This value also occurs in almost all studies of reef fish that have a higher composition of major fish than the target fish or indicator fish. Research conducted in Palu Bay waters found a composition of major fish by 54% and only 40% as a target fish. Whereas the study that is currently conducted, only takes ornamental fish samples so that the proportion of major fish is very 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 reef, this study found only one species in the Chaetodontidae, namely *Chaetodon kleinii*. Species indicators show that the waters of the 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 the 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 2019b).

In the group of fish that have venom, *Pterois volitans* are identified in the 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* invading 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 (Morris et al. 2011, Schofield 2009). As a native fish in the Indo-Pacific region, The red lionfish (*P. volitans*) plays a role in controlling other reef fish species because of their carnivorous nature (Morris et al. 2011). Although, it is mentioned that this species of lionfish is abundant in the Indo-Pacific region (Green and Côté 2009), fishers in Banyuwangi do not exploit this species much because these fish have venom which is quite dangerous to humans.

Conservation strategy

The increase in tourism activities in Banyuwangi also has an impact on increasing tourist visits to Tabuhan Island (Erwanto and Masluha 2019a). This tourist visit can hurt efforts to conserve coral reef ecosystems in this area. Most domestic tourists are not equipped with adequate conservation knowledge so it can have an impact on 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 Yar 2016), Karimun Jawa Archipelago, Jawa Tengah (Sulisiyati 2016), Dodola Island of Morotai Arcipelago, Maluku Utara (Kismanto Koroy and Mustafa 2018).

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

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

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

230 CONCLUSIONS

231
232 The diversity of reef fish resources has a great potential in the Tabuhan Island can be maintained, even though the
233 Banyuwangi is currently developing for tourism industry. In this study, 53 specimens were identified, including 49 species
234 of 3 orders and 17 families. Among the identified families, the most family is Labridae, with 20 species identified. The
235 Labridae that are currently identified consist of three subfamilies namely Corinae, Bodianinae, and Cheilinae; each
236 subfamily has formed a separate clade in phylogenetic tree reconstruction. The Pomacentridae and Pomacanthidae are
237 separated to develop their respective clades. The Tetraodontiformes were identified as being scattered between
238 *Canthigaster valentine* and *Ostracion cubicus*, which may show polyphyletic properties. We have not got yet the Genbank
239 accession numbers for 18 sequences, on the other hand, a total of 35 COI sequences were successfully deposited in the
240 GenBank database and it became important information for the study of biodiversity and genetic of coral reef fish in
241 Indonesian waters.

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248

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401 **Table 1.** Summary of coral reef fishes identified from Tabuhan Island, Banyuwangi, Indonesia

No.	Sample ID	Order*	Family*	Species name	Habitat distribution **	GenBank accession No.***
1	bwicor1	Perciformes	Labridae	<i>Pseudocheilinus evanidus</i>	Indo-Pacific: the Red Sea to South Africa	MH049275
2	bwicor44	Perciformes	Labridae	<i>Pseudocheilinus hexataenia</i>	Indo-Pacific	nr
3	bwicor13	Perciformes	Labridae	<i>Coris gaimard</i>	Pacific Ocean	MH049269
4	bwicor56	Perciformes	Labridae	<i>Coris pictoides</i>	Western Pacific	nr
5	bwicor40	Perciformes	Labridae	<i>Halichoeres prosopeion</i>	Western Pacific	MH049296
6	bwicor31	Perciformes	Labridae	<i>Halichoeres hortulanus</i>	Indo-Pacific	nr
7	bwicor28	Perciformes	Labridae	<i>Halichoeres chrysus</i>	Eastern Indian Ocean	nr
8	bwicor53	Perciformes	Labridae	<i>Hologymnosus doliatus</i>	Indo-Pacific, South Africa	MH049307
9	bwicor26	Perciformes	Labridae	<i>Thalassoma lunare</i>	Indo-Pacific	MH049282
10	bwicor43	Perciformes	Labridae	<i>Thalassoma amblycephalum</i>	Indo-Pacific	nr
11	bwicor22	Perciformes	Labridae	<i>Halichoeres melanurus</i>	Indo-Pacific: Bali, Indonesia, Australia	MH049278
12	bwicor29	Perciformes	Labridae	<i>Macropharyngodon ornatus</i>	Indo-Pacific	MH049285
13	bwicor42	Perciformes	Labridae	<i>Macropharyngodon negrosensis</i>	Eastern Indian Ocean	nr
14	bwicor24	Perciformes	Labridae	<i>Bodianus dictynna</i>	Western Pacific	MH049280
15	bwicor14	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
16	bwicor18	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
17	bwicor8	Perciformes	Labridae	<i>Bodianus bilunulatus</i>	Indo-West Pacific	nr
18	bwicor5	Perciformes	Labridae	<i>Diproctacanthus xanthurus</i>	Western Central Pacific	MH049291
19	bwicor12	Perciformes	Labridae	<i>Cirrhitilabrus lubbocki</i>	Western Central Pacific	nr
20	bwicor7	Perciformes	Labridae	<i>Anampses meleagrides</i>	Indo-Pacific	nr
21	bwicor16	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	MH049272
22	bwicor9	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	nr
23	bwicor19	Perciformes	Pomacentridae	<i>Dascyllus aruanus</i>	Pacific Ocean	MH049274
24	bwicor49	Perciformes	Pomacentridae	<i>Dascyllus trimaculatus</i>	Indo-Pacific	MH049303
25	bwicor30	Perciformes	Pomacentridae	<i>Dascyllus reticulatus</i>	Eastern Central Indian Ocean to Western Pacific	MH049287
26	bwicor51	Perciformes	Pomacentridae	<i>Chromis retrofasciata</i>	Western Pacific	MH049305
27	bwicor55	Perciformes	Pomacentridae	<i>Chrysiptera rollandi</i>	Eastern Indian Ocean	nr
28	bwicor3	Perciformes	Acanthuridae	<i>Paracanthurus hepatus</i>	Indo-Pacific	MH049289
29	bwicor38	Perciformes	Acanthuridae	<i>Acanthurus nigricans</i>	Eastern Indian Ocean	MH049294
30	bwicor4	Perciformes	Acanthuridae	<i>Acanthurus olivaceus</i>	Pacific Ocean	MH049290
31	bwicor61	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	MH049313
32	bwicor32	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	nr
33	bwicor41	Perciformes	Pomacanthidae	<i>Centropyge nox</i>	Western Pacific	MH049297
34	bwicor45	Perciformes	Pomacanthidae	<i>Centropyge acanthops</i>	Western Indian Ocean	MH049301
35	bwicor11	Perciformes	Pomacanthidae	<i>Centropyge bicolor</i>	Indo-Pacific	MH049268
36	bwicor23	Perciformes	Blennidae	<i>Ecsenius bicolor</i>	Indo-Pacific	MH049279
37	bwicor35	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchos</i>	Indo-Pacific	MH049288
38	bwicor46	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchos</i>	Indo-Pacific	MH049302
39	bwicor52	Perciformes	Blennidae	<i>Valenciennesa strigata</i>	Indo-Pacific	MH049306
40	bwicor21	Perciformes	Chaetodontidae	<i>Chaetodon kleinii</i>	Indo-Pacific, Eastern Pacific	MH049277
41	bwicor50	Perciformes	Cirrhitidae	<i>Oxycirrhites typus</i>	Indo-Pacific	MH049304
42	bwicor58	Perciformes	Cirrhitidae	<i>Cirrhitichthys oxycephalus</i>	Indo-Pacific	nr

43	bwicor25	Perciformes	Microdesmidae	<i>Ptereleotris heteroptera</i>	Indo-Pacific	MH049281
44	bwicor20	Perciformes	Mullidae	<i>Parupeneus multifasciatus</i>	Pacific Ocean	MH049276
45	bwicor2	Perciformes	Pseudochromidae	<i>Pictichromis paccagnellae</i>	Western Pacific, Palau	MH049286
46	bwicor6	Perciformes	Serranidae	<i>Pseudanthias squamipinnis</i>	Indo-West Pacific	MH049292
47	bwicor15	Perciformes	Serranidae	<i>Pseudanthias huchtii</i>	Western Central Pacific	nr
48	bwicor39	Perciformes	Serranidae	<i>Belonoperca chabanaudi</i>	Indo-Pacific	MH049295
49	bwicor10	Perciformes	Haemulidae	<i>Plectorhinchus chaetodonoides</i>	Indo-West Pacific	nr
50	bwicor59	Perciformes	Caesionidae	<i>Caesio teres</i>	Indo-West Pacific	nr
51	bwicor63	Scorpaeniformes	Scorpaenidae	<i>Pterois volitans</i>	Pacific Ocean	MH049314
52	bwicor60	Tetraodontiformes	Ostraciidae	<i>Ostracion cubicus</i>	Indo-Pacific	MH049312
53	bwicor27	Tetraodontiformes	Tetraodontidae	<i>Canthigaster valentini</i>	Indo-Pacific	MH049283

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* WoRMS : <http://www.marinespecies.org/>

** Fishbase database : <https://www.fishbase.se/>

*** NCBI database : <https://www.ncbi.nlm.nih.gov/>

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Diversity of the Tabuhan Island coral reef fish revealed by DNA Barcoding and Implication on Conservation strategy in Banyuwangi, Indonesia

Abstract. Tabuhan Island, one of the mainstays of coastal tourism with the charm of coral reefs, 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 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 reconstruction of the phylogenetic tree 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: diversity, coral reef ecosystem, marine fish, conservation, sustainable

Running title: Sektiana et al. Diversity of the Tabuhan Island

INTRODUCTION

The Java Sea 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 and Petit 1995) and tourism. The Java Sea is included as shallow water between Kalimantan, Java, Sumatra, and Sulawesi, within 310,000 km². 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 has increased the demand for fishes 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. In Banten Province, there is Tunda Island which 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, Karimun Jawa National Park (Hafsaridewi et al. 2018, Yuliana et al. 2020). In East Java, apart from Bawean Island (Riskiani et al. 2019) in Gresik Regency, there is Tabuhan Island in Banyuwangi Regency (Luthfi et al. 2016).

Tabuhan Island is an empty island in the waters of Banyuwangi Regency, precisely included in the administrative area of Bangsring Village, Wongsorejo 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 still through a morphological approach (Azhar et al. 2019), a molecular approach has never been done. 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

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

51 Biodiversity studies in coral reef areas generally examine macrobenthos (Quimpo et al. 2018), coral reef cover (Annas
52 et al. 2017, Putra Risandi Dwirama et al. 2018) and symbiotic fish species in this essential ecosystem area (Sahetapy et al.
53 2018). Reef fish are important biota as an indicator of the health of coral reef ecosystems by identifying certain types of
54 fish such as Chaetodontidae (Hamuna et al. 2019). In addition, the number of reef fish is also a target for traditional
55 fishermen because they have a fairly high price such as snapper (Arai et al. 2015) and grouper (Nanami 2021). In addition,
56 the number of endemic fish and protected fish (Cowman et al. 2017, Hobbs et al. 2013) also makes coral reefs an
57 important area for breeding, foraging and raising children. By taking into account the important role of coral reefs,
58 conservation activities for small islands in Indonesia will continue to be carried out.

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

66 MATERIALS AND METHODS

67 Sampling site

68 We have collected 53 fish specimens from the coral reefs in 2019 at Tabuhan Island of Banyuwangi, West Java (8°
69 3'35.52"S, 114°27'42.08"E), Indonesia (Fig 3. 1). Each specimen was kept in the freezer (at -20°C) in a 96 % ethanol
70 preservation solution. Parts of the body, including the muscles or dorsal fins, were used for further DNA sequence
71 analysis.

72 Genomic DNA extraction, amplification, and Sequencing

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

77 Fish Cytochrome oxidase I (COI) universal primer pairs BCL (TCA ACY AAT CAY AAA GAT ATY GGC AC) and
78 BCH (ACT TCY GGG TGR CCR AAR AAT CA) (Baldwin et al., 2009) were used in PCR reaction to obtain barcoding
79 sequence for molecular identification (Hebert et al. 2003). The PCR reaction (20 µL) contained 11.2 µL ultrapure water, 1
80 µL of each primer (0.5µM), 0.2 µL Extaq Hotstart version DNA polymerase (TAKARA, Japan), 2 µL 10x Extaq buffer, 2
81 µL dNTPs (1µM, TAKARA, Japan), 0.6 % total volume DMSO and 200 ng Genomic DNA as a template. Initial
82 denaturation at the first stage of the PCR was carried out at 94°C for 3 minutes. Next, the primary PCR process includes
83 denaturation (35 cycles of at 94°C for 30 sec), annealing (50°C for 30 sec), and extension (72°C for 45 sec). The last step is
84 the final extension at 72°C for 5 minutes. The PCR products were purified using a gel extraction kit (Bioneer, Daejeon,
85 Korea) by following the manufacturer's standard protocol.

86 DNA Sequence analysis

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

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

100

101

102

RESULTS AND DISCUSSION

103 Results

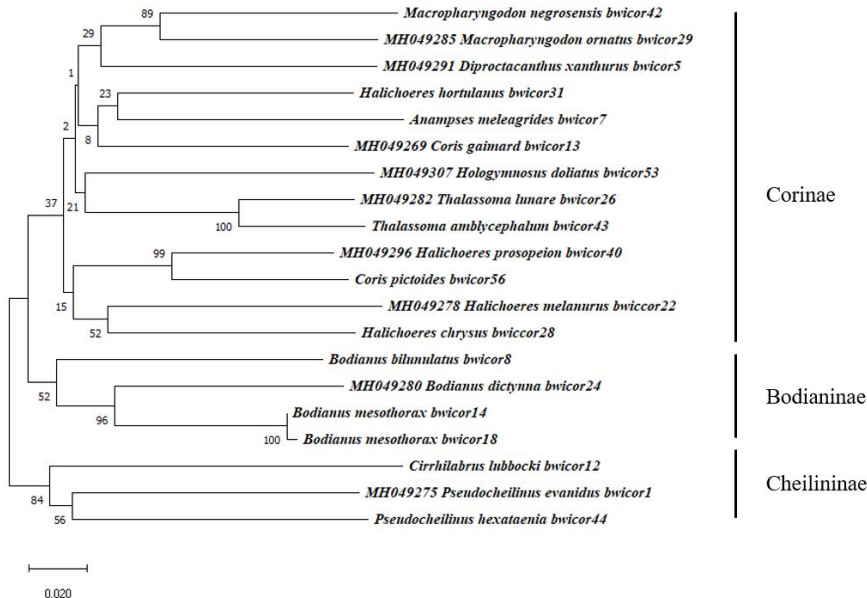
104 In this research, molecular identification has been carried out to complete the morphological identification that has
 105 been done so far. A total of 53 fish samples showed similarities with the reference BLASTN results with a database on
 106 GenBank with a value of 99-100%. Of the 53 samples,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

110 Phylogenetic tree reconstruction of different families

111 Labridae

112 A total of 20 Labridae species were identified, but only nine species received GenBank accession numbers.
 113 Registration of other sequences is still in the process of recording on the NCBI database through the online system
 114 (<https://www.ncbi.nlm.nih.gov/>), which is expected to be verified shortly. The Labridae family group is a major fish group
 115 in coral reef ecosystems (Dhahiyat et al. 2017, Putra Aswad Eka and Akbar 2017). From the phylogenetic tree (Figure 1),
 116 we can see the family Labridae belonging to the order, Cheilinae made a separate clade, while the other clades consist of
 117 Bodianinae and Corinae orders.
 118

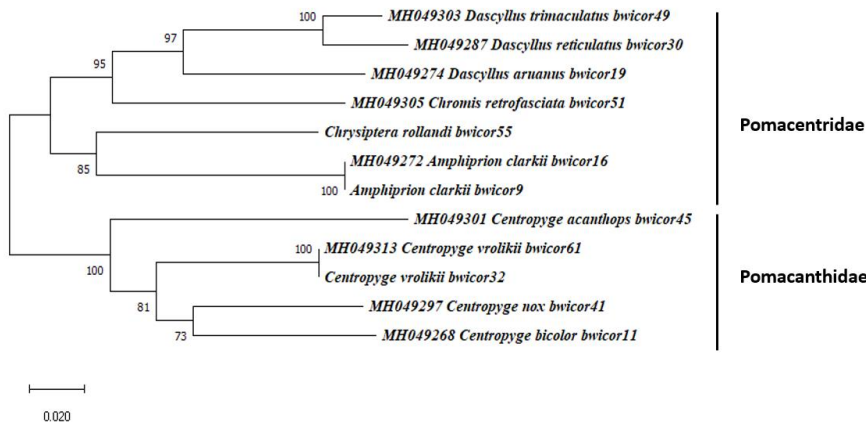
Commented [W11]: There is no explanation in the discussion



119 **Figure 1.** Phylogenetic reconstruction of Labridae from Tabuhan Island using Neighbour Joining algorithm
 120
 121

122 Pomacentridae and Pomacanthidae

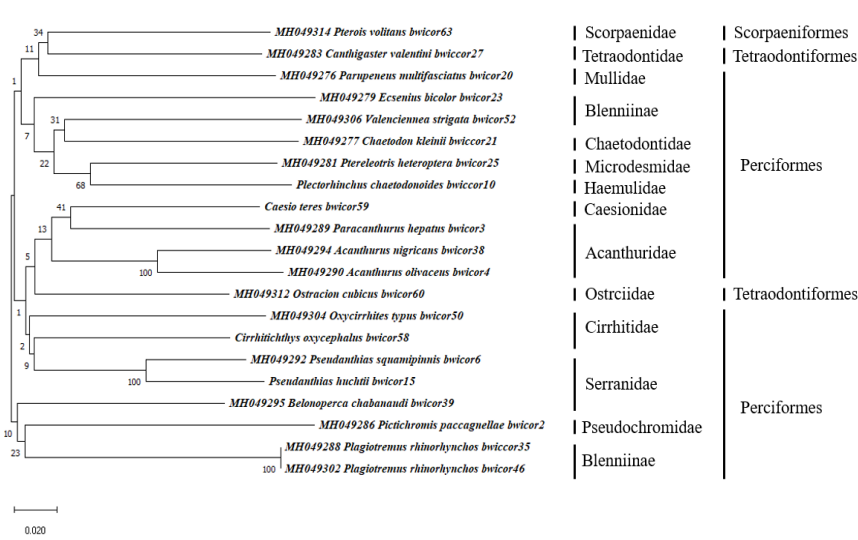
123 The Pomacentridae and Pomacanthidae families are still included in the major fish groups that make up the coral reef
 124 ecosystem. The previous studies in the Trenggalek waters found a large number of fish species under the Pomacentridae
 125 family (21 species), while only six species were from the Pomacanthidae family (Wibowo and Adrim 2014). Although the
 126 number of species found in this study is not as much as studies conducted in other regions. The analysis of phylogenetic
 127 tree reconstruction shows that the Pomacentridae and Pomacanthidae separate families form a distinct clade on the
 128 phylogenetic tree produced (Figure 2).
 129



130
131 **Figure 2.** Phylogenetic reconstruction of Pomacentridae and Pomacanthidae from Tabuhan Island using Neighbour Joining algorithm

132 **The small number of families**

133 Besides the three major families (Labridae, Pomacentridae, and Pomacanthidae), many target fish species those fall
134 into this category are economically essential fishes, such as Caesionidae, Serranidae, and Mullidae. A study of the fish
135 stock of these three groups in the Karimun National Park in Java shows that the Order Serranidae has been exploited
136 beyond its sustainability limit, while the other two families (Caesionidae and Mullidae) are still below their sustainability
137 limit (Yuliana et al. 2016). While from another family, Scorpaeniformes, is a group of seawater ornamental fish species
138 that is quite important and this fish has a poisonous gland that is quite dangerous. Although it has poison glands, some
139 ornamental fish traders make this decorative fish commodity to be quite exclusive. Besides, this group also found an
140 indicator of fish that is the family Chaetodontidae, which is an indicator of coral health. This fish is also found in
141 Trenggalek waters and is an indicator of the coral reef ecosystem in this region, which is also still awake (Wibowo and
142 Adrim 2014). The existence of indicator fish is fundamental because it also reflects the condition of the waters and
143 ecosystems of coral reefs that are still in good condition.
144



145
146 **Figure 2.** Phylogenetic reconstruction of a small number of families from Tabuhan Island using Neighbour Joining algorithm

Commented [W12]: Blenniinae or Blenniidae?

148 **Discussion**149 *Diversity*

150 The waters of Tabuhan Island are an uninhabited island that is currently an area for nature tourism, besides that this
 151 area is also an area for catching reef fish as an essential ornamental fish commodity in Indonesia. Banyuwangi area, which
 152 is adjacent to Bali (a famous spot for tourists), becomes a strength in the exploitation of coral, which is quite large. The
 153 ornamental fish market in Bali is attractive enough for traditional fishers to make decorative fish as an alternative income
 154 source for local and international tourists besides consuming fish that has become a common catch.

155 The diversity of reef fish requires accurate identification, although there have been many studies of reef fish species,
 156 most of the identification is based on morphological information alone. In this study, we identified molecularly as well as
 157 listed the sequences produced as sequences from the tropical waters of Tabuhan Island, Banyuwangi Indonesia. This
 158 information is crucial for the study of molecular biology and other study related to conservation biology in the formulation
 159 of policies for the conservation of coastal ecosystems, including coral reef ecosystems.

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

165 In this study, several essential species were successfully identified molecularly. In target fishes, the Perciformes order
 166 is the dominant fish that becomes the target fish such as the Serranidae, Caesionidae, and Mullidae fishes. In this study, the
 167 Serranidae family was represented by *Pseudanthias squamipinnis*, *Pseudanthias huchtii*, and *Belonoperca chabanaudi*.
 168 Two species of *Pseudanthias* are identified as seawater ornamental fish because they have an attractive color. *Pseudanthias*
 169 *squamipinnis* fish distribution in the Western Indian Ocean reefs to the Red Sea and Christmas in South Africa (Heemstra
 170 and Akhilesh 2012). Also reported, these fish inhabit the waters of northern Japan, southern Australia. Whereas the
 171 *Pseudanthias huchtii*, fish has an attractive green color. It has the potential to become a seawater ornamental commodity
 172 that has habitat distribution in the Western Central Pacific covering Sulawesi and the Philippines to Vanuatu, to the
 173 southern Great Barrier Reef and Palau regions in the Micronesian islands.

174 The proportion of major fish compared to target fish in this study was 90%. This value also occurs in almost all studies
 175 of reef fish that have a higher composition of major fish than the target fish or indicator fish. Research conducted in Palu
 176 Bay waters found a composition of major fish by 54% and only 40% as a target fish. Whereas the study that is currently
 177 conducted, only takes ornamental fish samples so that the proportion of major fish is very dominant compared to the target
 178 fish, which is only 7.5%. In this study, the Pomacentridae and Pomacathidae groups were identified as many as 7 and 5
 179 species, respectively. This type of fish is fish that has characteristics of maintaining the territory of its habitat so that this
 180 group of fish is a permanent resident (resident species) in the coral reef ecosystem.

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

188 In the group of fish that have venom, *Pterois volitans* are identified in the Tabuhan Island and become one of the
 189 traded species. This lionfish species is a common species traded along with other lionfish species *P. miles* (Lyons et al.
 190 2017). However, several references indicate that *P. volitans* has the potential to be invasive. The researches have
 191 demonstrated that *P. volitans* invading the North America region, Florida (Freshwater et al. 2009), and other regions in the
 192 Indo-pacific region such as the Atlantic coast of mainland USA, the Western North Atlantic, and the Caribbean Sea
 193 (Morris et al. 2011, Schofield 2009). As a native fish in the Indo-Pacific region, The red lionfish (*P. volitans*) plays a role
 194 in controlling other reef fish species because of their carnivorous nature (Morris et al. 2011). Although, it is mentioned that
 195 this species of lionfish is abundant in the Indo-Pacific region (Green and Côté 2009), fishers in Banyuwangi do not exploit
 196 this species much because these fish have venom which is quite dangerous to humans.

197 *Conservation strategy*

198 The increase in tourism activities in Banyuwangi also has an impact on increasing tourist visits to Tabuhan Island
 199 (Erwanto and Masluha 2019a). This tourist visit can hurt efforts to conserve coral reef ecosystems in this area. Most
 200 domestic tourists are not equipped with adequate conservation knowledge so it can have an impact on damage and bring in
 201 plastic waste in this area (Barlinti 2020, Mirsalila 2020). Therefore, it is necessary to limit tourism activities in this area as
 202 well as to monitor and educate the importance of protecting fishery and marine resources in general. This restriction is
 203 adjusted to the ability of the Region to receive visits. The concept of this restriction has been applied in several tourist

Commented [W13]: In this study, target fish from the Acanthuridae family were also found, although in small numbers

Commented [W14]: How about *Belonoperca chabanaudi*? Does it include ornamental fish? Any reports of its spread?

Commented [W15]: Blennidae and Labridae are also included as major fish

Commented [W16]: belonging to the family Scorpaenidae.

204 areas that pay attention to the carrying capacity of the area, such as in the Duyung Island Arcipelago, Riau Archipelago
205 (Mukhlis et al. 2022), Sebesi Island, Lampung (Johan Yar 2016), Karimun Jawa Archipelago, Jawa Tengah (Suliswati
206 2016), Dodola Island of Morotai Arcipelago, Maluku Utara (Kismanto Koroy and Mustafa 2018).

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

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

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

229 In conclusion, the diversity of reef fish resources has a great potential in the Tabuhan Island can be maintained, even
230 though the Banyuwangi is currently developing for tourism industry. In this study, 53 specimens were identified, including
231 49 species of 3 orders and 17 families. Among the identified families, the most family is Labridae, with 20 species
232 identified. The Labridae that are currently identified consist of three subfamilies namely Corinae, Bodianinae, and
233 Cheilinae; each subfamily has formed a separate clade in phylogenetic tree reconstruction. The Pomacentridae and
234 Pomacanthidae are separated to develop their respective clades. The Tetraodontiformes were identified as being scattered
235 between *Canthigaster valentine* and *Ostracion cubicus*, which may show polyphyletic properties. We have not got yet the
236 Genbank accession numbers for 18 sequences, on the other hand, a total of 35 COI sequences were successfully deposited
237 in the GenBank database and it became important information for the study of biodiversity and genetic of coral reef fish in
238 Indonesian waters.

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244 marine water ecosystem. The authors have no conflict of interest to declare.

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Table 1. Summary of coral reef fishes identified from Tabuhan Island, Banyuwangi, Indonesia

No.	Sample ID	Order*	Family*	Species name	Habitat distribution **	GenBank accession No.***
1	bwicor1	Perciformes	Labridae	<i>Pseudocheilinus evanidus</i>	Indo-Pacific: the Red Sea to South Africa	MH049275
2	bwicor44	Perciformes	Labridae	<i>Pseudocheilinus hexataenia</i>	Indo-Pacific	nr
3	bwicor13	Perciformes	Labridae	<i>Coris gaimard</i>	Pacific Ocean	MH049269
4	bwicor56	Perciformes	Labridae	<i>Coris pictoides</i>	Western Pacific	nr
5	bwicor40	Perciformes	Labridae	<i>Halichoeres prosopeion</i>	Western Pacific	MH049296
6	bwicor31	Perciformes	Labridae	<i>Halichoeres hortulanus</i>	Indo-Pacific	nr
7	bwicor28	Perciformes	Labridae	<i>Halichoeres chrysus</i>	Eastern Indian Ocean	nr
8	bwicor53	Perciformes	Labridae	<i>Hologymnosus doliatus</i>	Indo-Pacific, South Africa	MH049307
9	bwicor26	Perciformes	Labridae	<i>Thalassoma lunare</i>	Indo-Pacific	MH049282
10	bwicor43	Perciformes	Labridae	<i>Thalassoma amblycephalum</i>	Indo-Pacific	nr
11	bwicor22	Perciformes	Labridae	<i>Halichoeres melanurus</i>	Indo-Pacific: Bali, Indonesia, Australia	MH049278
12	bwicor29	Perciformes	Labridae	<i>Macropharyngodon ornatus</i>	Indo-Pacific	MH049285
13	bwicor42	Perciformes	Labridae	<i>Macropharyngodon negrosensis</i>	Eastern Indian Ocean	nr
14	bwicor24	Perciformes	Labridae	<i>Bodianus dictynna</i>	Western Pacific	MH049280
15	bwicor14	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
16	bwicor18	Perciformes	Labridae	<i>Bodianus mesothorax</i>	Western Pacific	nr
17	bwicor8	Perciformes	Labridae	<i>Bodianus bilunulatus</i>	Indo-West Pacific	nr
18	bwicor5	Perciformes	Labridae	<i>Diproctacanthus xanthurus</i>	Western Central Pacific	MH049291
19	bwicor12	Perciformes	Labridae	<i>Cirrhitilabrus lubbocki</i>	Western Central Pacific	nr
20	bwicor7	Perciformes	Labridae	<i>Anampses meleagrides</i>	Indo-Pacific	nr
21	bwicor16	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	MH049272
22	bwicor9	Perciformes	Pomacentridae	<i>Amphiprion clarkii</i>	Indo-West Pacific	nr
23	bwicor19	Perciformes	Pomacentridae	<i>Dascyllus aruanus</i>	Pacific Ocean	MH049274
24	bwicor49	Perciformes	Pomacentridae	<i>Dascyllus trimaculatus</i>	Indo-Pacific	MH049303
25	bwicor30	Perciformes	Pomacentridae	<i>Dascyllus reticulatus</i>	Eastern Central Indian Ocean to Western Pacific	MH049287
26	bwicor51	Perciformes	Pomacentridae	<i>Chromis retrofasciata</i>	Western Pacific	MH049305
27	bwicor55	Perciformes	Pomacentridae	<i>Chrysiptera rollandi</i>	Eastern Indian Ocean	nr
28	bwicor3	Perciformes	Acanthuridae	<i>Paracanthurus hepatus</i>	Indo-Pacific	MH049289
29	bwicor38	Perciformes	Acanthuridae	<i>Acanthurus nigricans</i>	Eastern Indian Ocean	MH049294
30	bwicor4	Perciformes	Acanthuridae	<i>Acanthurus olivaceus</i>	Pacific Ocean	MH049290
31	bwicor61	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	MH049313
32	bwicor32	Perciformes	Pomacanthidae	<i>Centropyge vrolikii</i>	Western Pacific	nr
33	bwicor41	Perciformes	Pomacanthidae	<i>Centropyge nox</i>	Western Pacific	MH049297
34	bwicor45	Perciformes	Pomacanthidae	<i>Centropyge acanthops</i>	Western Indian Ocean	MH049301
35	bwicor11	Perciformes	Pomacanthidae	<i>Centropyge bicolor</i>	Indo-Pacific	MH049268
36	bwicor23	Perciformes	Blennidae	<i>Ecsenius bicolor</i>	Indo-Pacific	MH049279
37	bwicor35	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchos</i>	Indo-Pacific	MH049288
38	bwicor46	Perciformes	Blennidae	<i>Plagiotremus rhinorhynchos</i>	Indo-Pacific	MH049302
39	bwicor52	Perciformes	Blennidae	<i>Valenciennesa strigata</i>	Indo-Pacific	MH049306
40	bwicor21	Perciformes	Chaetodontidae	<i>Chaetodon kleinii</i>	Indo-Pacific, Eastern Pacific	MH049277
41	bwicor50	Perciformes	Cirrhitidae	<i>Oxycirrhites typus</i>	Indo-Pacific	MH049304

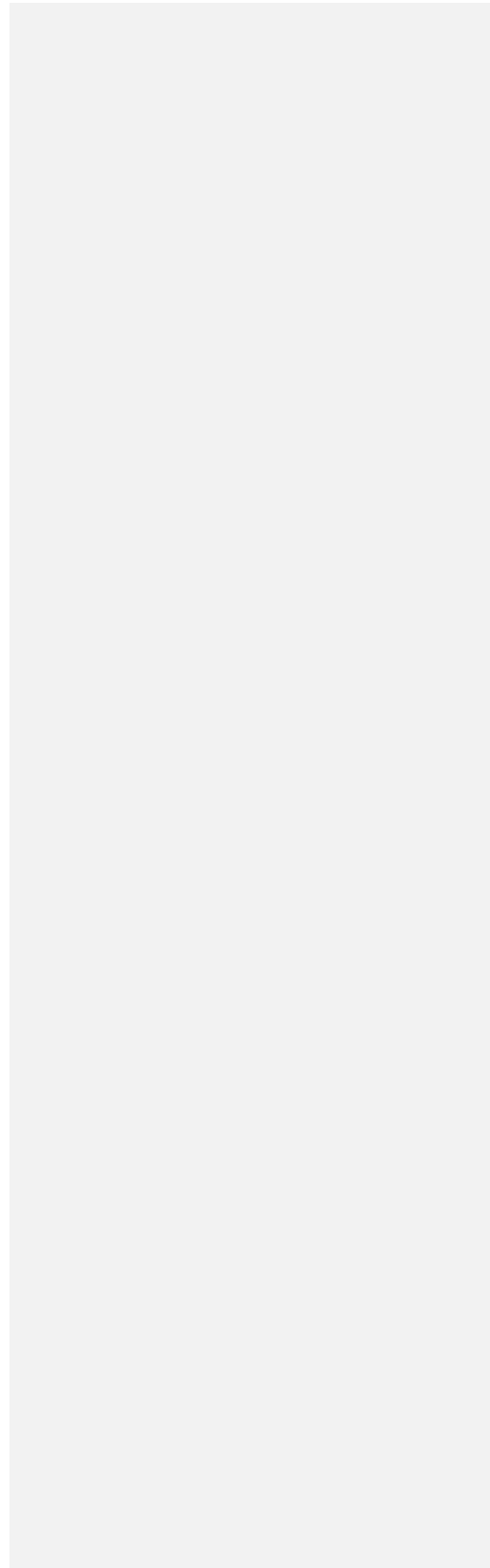
42	bwicor58	Perciformes	Cirrhiridae	<i>Cirrhitichthys oxycephalus</i>	Indo-Pacific	nr
43	bwicor25	Perciformes	Microdesmidae	<i>Ptereleotris heteroptera</i>	Indo-Pacific	MH049281
44	bwicor20	Perciformes	Mullidae	<i>Parupeneus multifasciatus</i>	Pacific Ocean	MH049276
45	bwicor2	Perciformes	Pseudochromidae	<i>Pictichromis paccagnellae</i>	Western Pacific, Palau	MH049286
46	bwicor6	Perciformes	Serranidae	<i>Pseudanthias squamipinnis</i>	Indo-West Pacific	MH049292
47	bwicor15	Perciformes	Serranidae	<i>Pseudanthias huchtii</i>	Western Central Pacific	nr
48	bwicor39	Perciformes	Serranidae	<i>Belonoperca chabanaudi</i>	Indo-Pacific	MH049295
49	bwicor10	Perciformes	Haemulidae	<i>Plectorhinchus chaetodonoides</i>	Indo-West Pacific	nr
50	bwicor59	Perciformes	Caesionidae	<i>Caesio teres</i>	Indo-West Pacific	nr
51	bwicor63	Scorpaeniformes	Scorpaenidae	<i>Pterois volitans</i>	Pacific Ocean	MH049314
52	bwicor60	Tetraodontiformes	Ostraciidae	<i>Ostracion cubicus</i>	Indo-Pacific	MH049312
53	bwicor27	Tetraodontiformes	Tetraodontidae	<i>Canthigaster valentini</i>	Indo-Pacific	MH049283

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* WoRMS : <http://www.marinespecies.org/>

** Fishbase database : <https://www.fishbase.se/>

*** NCBI database : <https://www.ncbi.nlm.nih.gov/>



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

SINAR PAGI SEKTIANA¹, ANNUR AHADI ABDILLAH², MD. JOB AIDUL ALAM³, WAHYU ISRONI⁴, NINA NURMALIA DEWI⁴, HYUN WOO KIM^{2,5}, SAPTO ANDRIYONO^{2,*}

¹Aquaculture Technology Program, Politeknik Ahli Usaha Perikanan. Jl. AUP Pasar Minggu, Jakarta Selatan 12520, Jakarta, Indonesia

²Department of Marine, Faculty of Fisheries and Marine Science, Universitas Airlangga. Jl. Mulyorejo, Kampus C, Surabaya 60115, East Java, Indonesia. Tel.: +62-31-5911451, *email: sapto.andriyono@fpk.unair.ac.id

³Department of Fisheries, Ministry of Fisheries and Livestock. Dhaka, Bangladesh

⁴Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Airlangga. Jl. Mulyorejo, Kampus C, Surabaya 60115, East Java, Indonesia

⁵Department of Marine Biology, School of Fisheries Sciences, Pukyong National University. Busan, South Korea

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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 km². 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, Cheiliniinae 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.

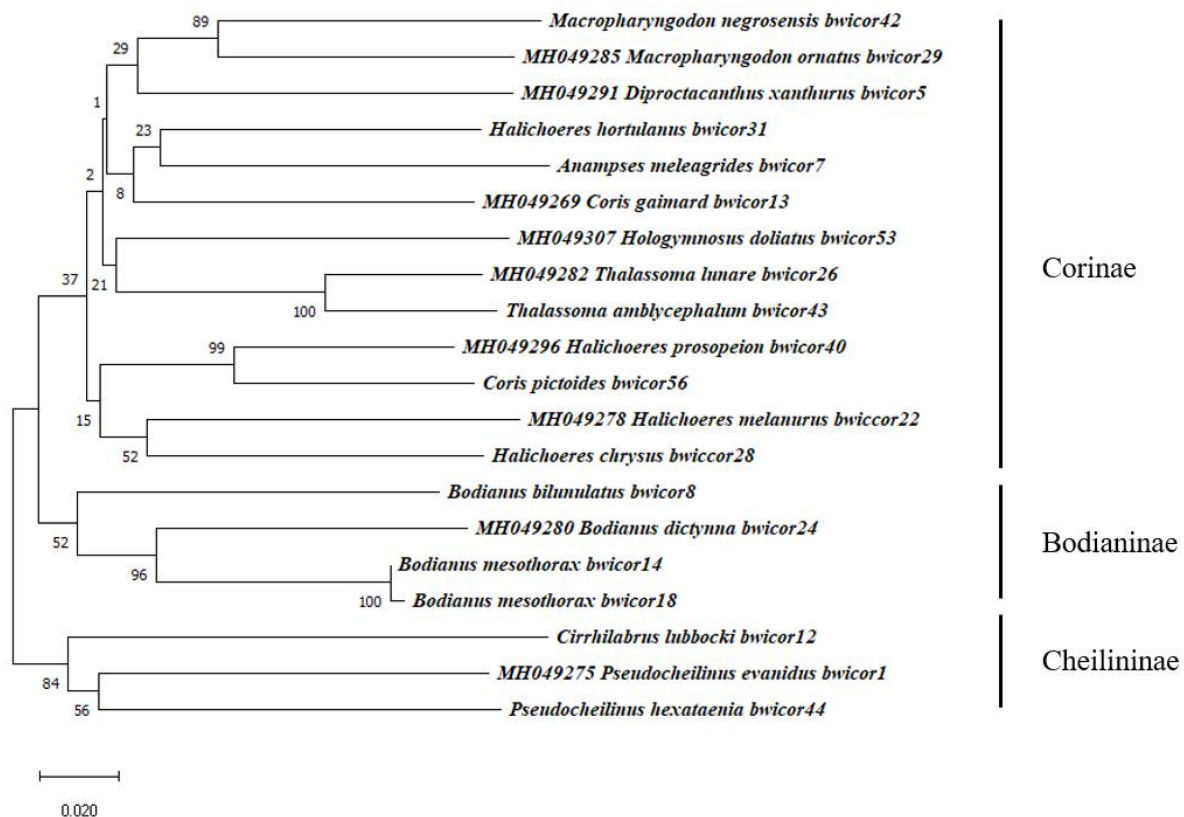


Figure 1. Phylogenetic reconstruction of Labridae from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm

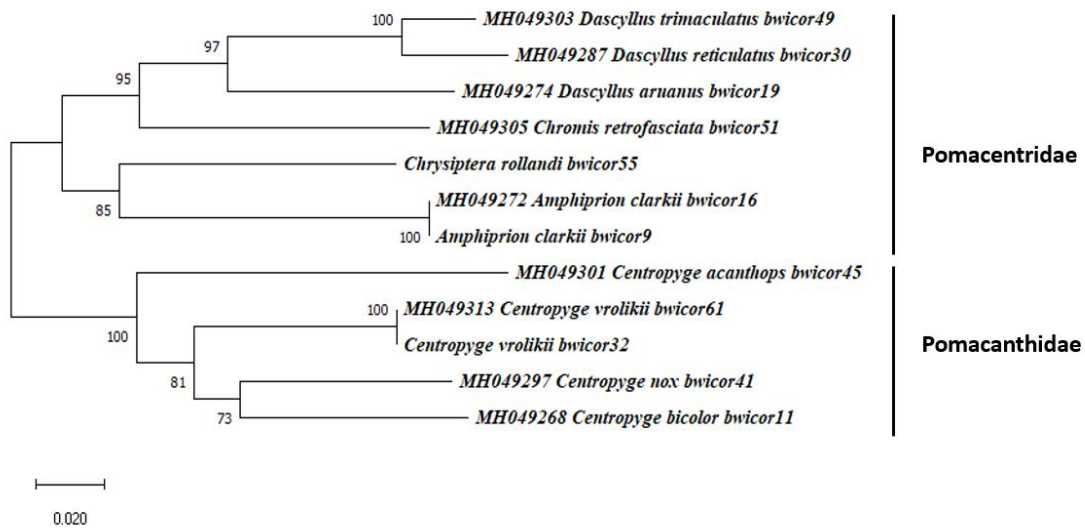


Figure 2. Phylogenetic reconstruction of Pomacentridae and Pomacanthidae from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm

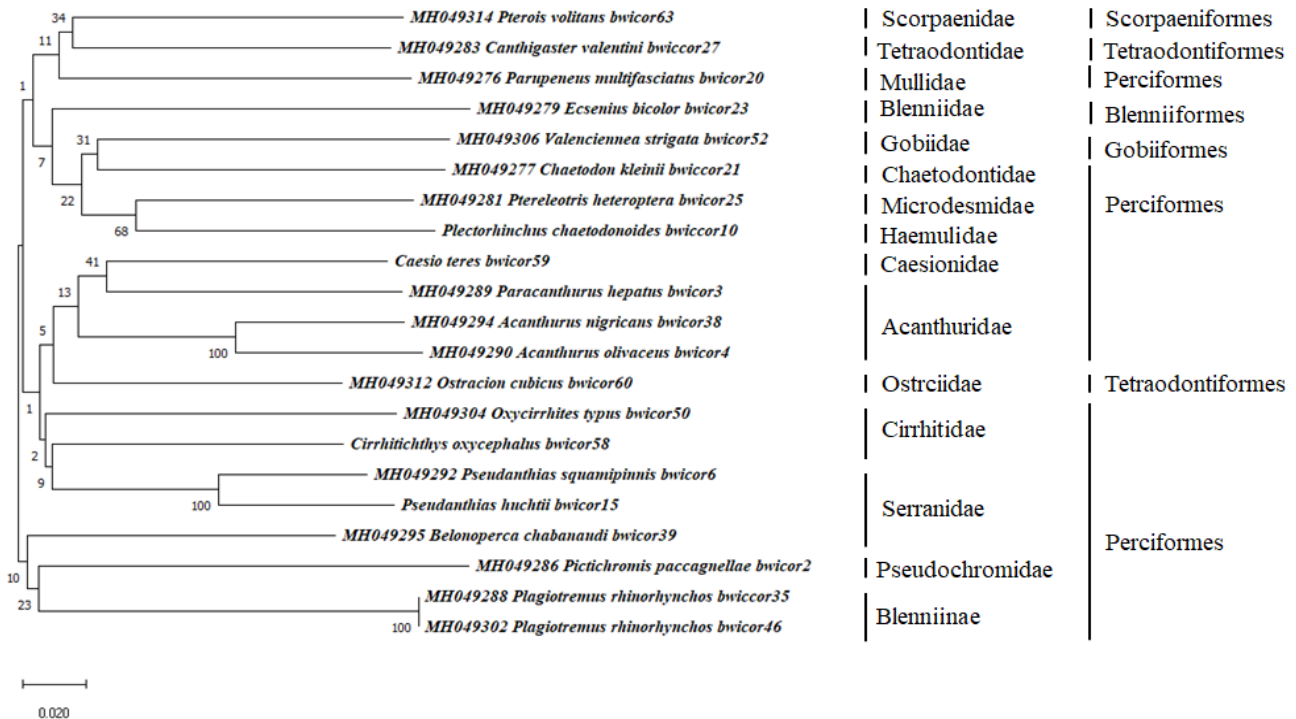


Figure 3. Phylogenetic reconstruction of a small number of families from Tabuhan Island, Banyuwangi District, Indonesia, using Neighbour Joining algorithm

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 Tetraodontiformes 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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