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Jurnal : Egyptian Journal of Aquatic Biology and Fisheries

Penulis : Sapto Andriyono, Suciyono Suciyono

No.	Perihal	Tanggal	Keterangan
1	Bukti Submit dan Artikel yang di submit	13 April 2020	Page 2, Lampiran 1
2	Bukti reviewed minor revision	15 April 2020	Page 2, Lampiran 2
3	Bukti Accepted	5 Mei 2020	Page 3, Lampiran 3
4	Link Artikel terpublikasi	08 Mei 2022	Page 3

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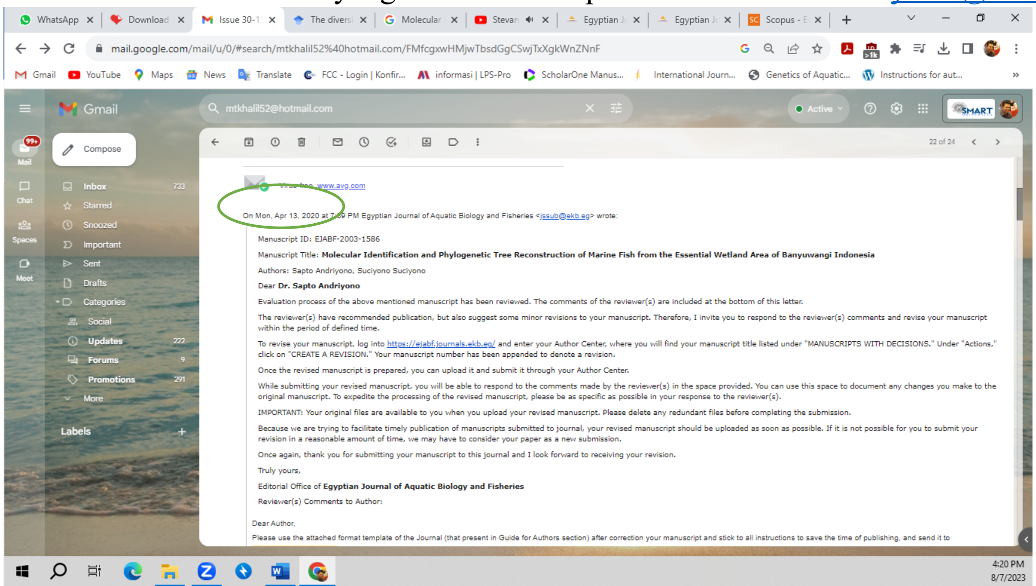

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Manuscript ID: EJABF-2003-1586

Manuscript Title: **Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi Indonesia**

Authors: Sapto Andriyono, Suciyono Suciyono

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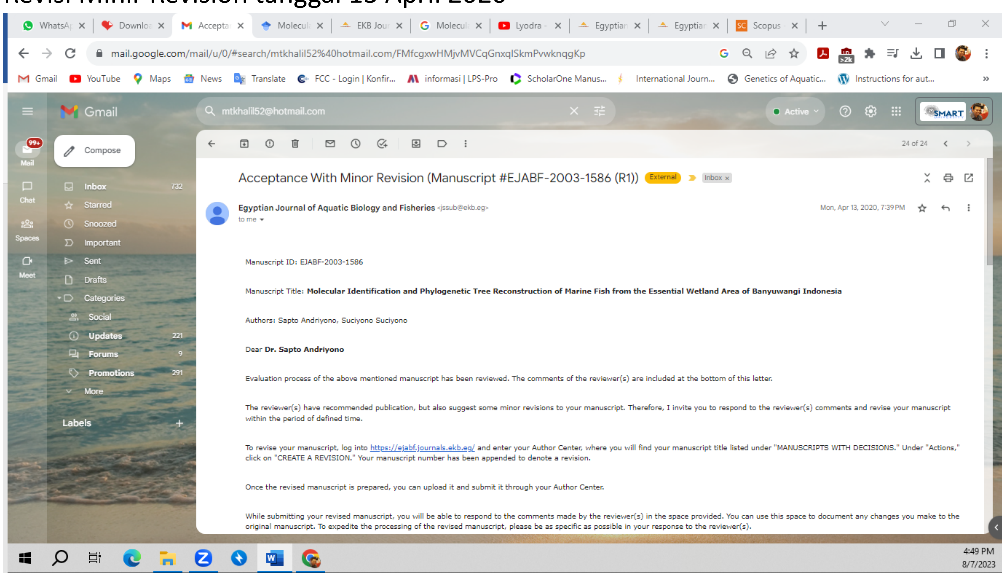

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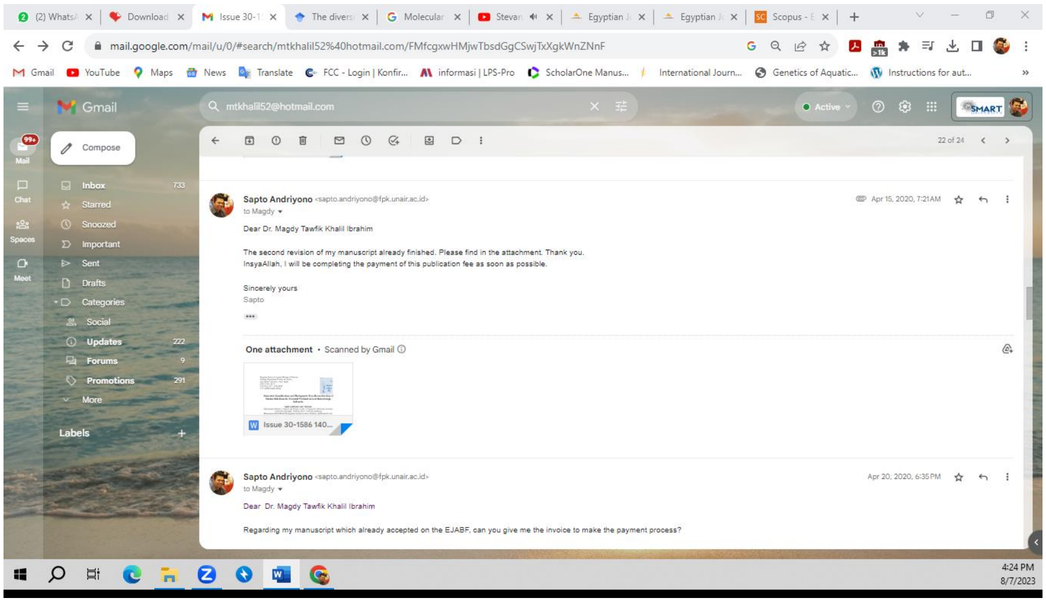

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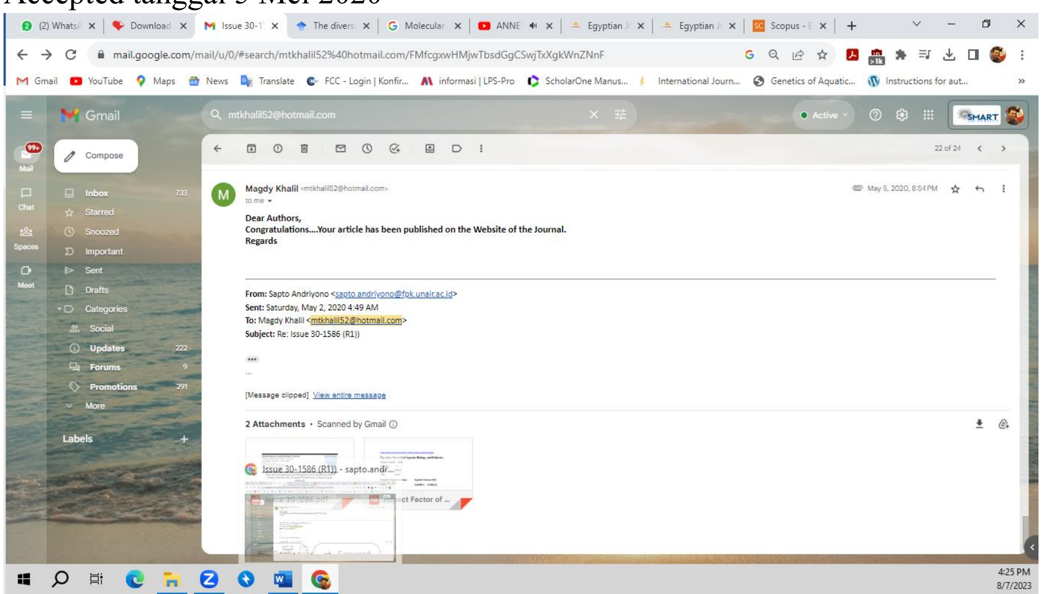

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Artikel 1, Lampiran 1

Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi Indonesia

Abstract

Pangpang Bay is necessary to get serious attention because it is one of the essential wetlands in Banyuwangi, Indonesia. This area able to generate income for traditional fishers and ecological supports the surrounding area because it is possible to be a spawning area and nursery ground for many estuary fish. The identification of fisheries resources is carried out using a molecular approach in the Cytochrome oxidase subunit I (COI) region that is commonly used in barcoding. Identification with this method is expected to minimize errors in determining the name of the species of species that exist in Pangpang Bay. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and 4 orders. The results of this study are the initials of molecular information on fisheries resources in the Pangpang Bay area.

Keywords: molecular, identification, estuary, Pangpang Bay, essential area, Indonesia

Introduction

Pangpang Bay is in the region of the Banyuwangi Regency, which is directly bordered by the waters of the Bali Strait and the Indian Ocean. There is a massive potential in this region, including the capture fisheries, fisheries processing, aquaculture, and the presence of mangrove ecosystems (Buwono et al. 2015, Kawamuna et al. 2017, Raharja et al. 2014). However, the pattern of resource utilization that has not been appropriately managed causes environmental problems such as a decrease in water quality due to the disposal of organic waste in the fisheries industry without processing (Anugrah 2013), overfishing status in the sardine fisheries, deforestation of mangrove forests for ponds as well as urban development activities, declining pond production, and coastal area abrasion.

Currently, the Indonesian government is conducting a study by setting several areas to become the development areas for fishery products as Minapolitan, through the Decree of the Minister of Maritime Affairs and Fisheries No.32/MEN/2010 (Suryawati and Purnomo 2017). One of the Minapolitan is the Muncar sub-district, which located in Banyuwangi. This area is made as one of the most potential minapolitan capture fisheries locations with consideration in several aspects. Muncar is showing potential fishery commodities, strategic geographical location, and having production chains (upstream-downstream) that are already running well. Another hand, Muncar also has adequate main supporting facilities such as transportation and communication facilities, as well as environmental feasibility and local government support. Before the Muncar was established as a minapolitan in 2010, the community had relied on fish resources that had developed since the mid-'60s (Wiratama 2012). This condition can be seen from a number of studies exploring the Muncar area relating to the use of traditional fishing technologies. In its development, purse-seines were introduced by the Marine Fisheries Research Institute (BPPL) in the 1972s and continues to be used today(Perkasa et al. 2016).

In connection with this tremendous potential of water resources, the government (Ministry of Forestry) has determined the Pangpang bay area to be an essential area that has synergistic ecological and economic roles(Setyaningrum 2017). Balanced management and utilization efforts are expected to facilitate the community around the Pangpang Bay area, which is almost entirely dependent on the existence of these water resources. In this study, the initial information in management is the collection of a database of fisheries resources owned by this region. The morphological approach in identifying fish and the combination of molecular methods to reduce errors in fish identification in the Pangpang Bay area has been carried out. Previous studies have not found data that specifically inventory fish species in this area. Much research has been done on mangrove vegetation and aquatic fauna that are not specifically on fish species (Buwono 2017, Buwono et al. 2015). Information on the results of this study is essential for related research to the genetic and biological diversity of fisheries in economically important marine fish species obtained from this crucial region, as well as being the basis for formulating policies in sustainable management.

This molecular identification can be called DNA barcoding. This DNA barcoding method has been agreed globally to ensure the identification of both plant and animal species, both fresh samples and processed product samples(Giusti et al. 2017, Pepe et al. 2007).DNA barcoding efforts on aquatic biota have been carried out and are currently receiving a lot of attention, especially efforts to increase the database on GenBank. Compared to the morphological identification method, DNA barcoding has an accuracy that is close to 100% matched(Meyer and Paulay 2005). Single region of mitochondrial DNA Cytochrome C Subunit I gene (COI) that will be deposited in the Genbank database as central bioinformatics becomes the gene marker that is used as the basis for DNA barcoding. Scientists have proven their effectiveness in DNA barcoding in freshwater fish(Hubert et al. 2008), and marine fish (Lakra et al. 2011, Ward et al. 2005). Meanwhile, research on DNA barcoding in Indonesia has also begun to develop. However, it is still limited to several areas such as freshwater fish in Java and Bali(Dahruddin et al. 2017), freshwater fish Lake Laut Aceh (Ariyanti 2012, Muchlisin et al. 2013), the Pogar River Sulawesi Island (Arai et al. 1999), Lake Matano Sulawesi (Roy et al. 2004), and Malang (Andriyono et al. 2019b). Whereas DNA barcoding has done in Indonesia is still limited to certain species such as Shark (Prehadi et al. 2014, Sembiring et al. 2015) and Orange-Spotted grouper (Antoro et al. 2006).

Another researcher study about DNA barcoding of Grouper (Jefri et al. 2015), seahorse (Lourie and Vincent 2004), and goby fish species (Winterbottom et al. 2014). Several scientist concern on coral reefs fish species such as three-spot dascullus (Bernardi Giacomo et al. 2001), anemone-fishes (Madduppa et al. 2014), and Banggai cardinalfish (Bernardi G and Vagelli 2004). Due to the limited genetic information of marine fishes, this research is significant to report of Pangpang Bay fish resources through molecular approach.

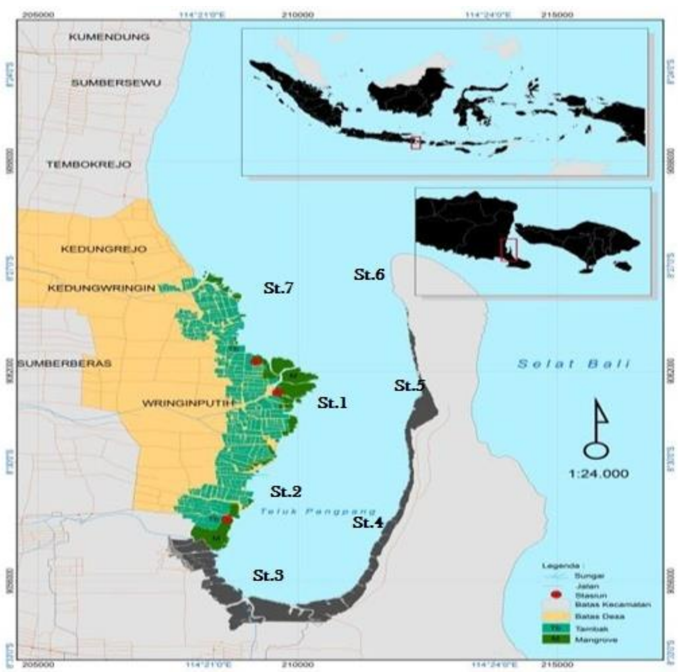

Figure 1. Map of sampling site distribution around Pangpang Bay, Banyuwangi

Materials and Methods

Sampling Site

Sampling sites for sample collection was performed in Pangpang Bay, Banyuwangi district of East Java Province (Figure 1). No specific permit was required for this study. All sample has been collected from the traditional fisherman in Wringinputih Village, Muncar, Banyuwangi. That specimen was dead upon purchasing, and none of the collected specimens were in the endangered category based on the IUCN Red List database. The digital camera has taken the individual sample

DNA extraction

Each sample has been compiled based on the morphological characters and after collection directly preserved in 90% ethanol. Genomic DNA was extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's guidelines. The anal fin (1 cm) was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA was performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70oC for further analysis.

PCR condition and Data Analysis

The universal fish primer, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), used to obtain the partial sequences of cytochrome c oxidase I (COI) region. The PCR mixture (20µL) contained 11.2 µL ultra-pure water, 1 µL primer forward and reversed (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan). This PCR reaction, we used 2 µL genomic DNA as a template. The PCR condition was carried out under the following setting: 95^{o}C for 5 min in initial denaturation. After that, denaturation performed at 95^{o}C for 30 s in 40 cycles, followed by 50^{o}C for 30 s in annealing and 72^{o}C for 45 s in extension step. The final step of PCR are final extension at 72^{o}C for 5 min. The PCR products of COI was purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

Data Analysis

All sequences were aligned and submitted to GenBank (Table 1). The pairwise evolutionary distance among the family was determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree was constructed, and 1000 bootstrap analysis was carried by Mega 7 (Kumar et al. 2016).

Results

Species Identification

In this study, 40 fish samples were identified in 37 marine fish species distributed in 25 families, and four orders (Table 1). The types of fish are the catch of traditional fishermen who use fishing gear in the form and a small portion using fishing rods and fishing nets. In addition to identification based on morphology, molecular identification is also carried out.

Table 1. List of online BLASTN results through NCBI database from marine fish specimens of Pangpang Bay, Banyuwangi

No.	Sampel ID	Order	Family	Species Name	Query Cover (%)	Identity (%)
1	bw32	Clupeiformes	Clupeidae	*Sardinella melanura*	100%	99%
2	bw38		Engraulidae	*Thryssa baelama*	96%	99%
3	bw42			*Thryssa baelama*	97%	99%
4	bw45	Perciformes	Ambassidae	*Ambassis sp.*	97%	99%
5	bw33		Apogonidae	*Jaydia novaeguineae*	98%	99%
6	bw44			*Archamia bleekeri*	99%	99%
7	bw18		Carangidae	*Scomberoides tala*	96%	100%
8	bw31			*Carangoides chrysophrys*	99%	100%
9	bw23		Eleotridae	*Butis amboinensis*	97%	99%
10	bw28		Gerreidae	*Gerres filamentosus*	100%	99%
11	bw29			*Gerres filamentosus*	100%	99%
12	bw43		Gobiidae	*Acentro gobiuscaninus*	95%	100%
13	bw21			*Oxyurichthys ophthalmonema*	98%	92%
14	bw9		Haemulidae	*Diagramma pictum*	97%	99%
15	bw12			*Plectorhinchus lineatus*	97%	100%
16	bw27			*Pomadasys kaakan*	98%	99%
17	bw10		Labridae	*Choerodonan chorago*	99%	99%
18	bw11			*Cheilinus fasciatus*	98%	99%
19	bw1		Latidae	*Psammoperca waigiensis*	95%	99%
20	bw26		Leiognathidae	*Leiognathus equulus*	100%	99%
21	bw15		Lethrinidae	*Lethrinus lentjan*	100%	99%
22	bw6		Lutjanidae	*Lutjanus fulviflamma*	100%	99%
23	bw7			*Lutjanus carponotatus*	97%	99%
24	bw16			*Lutjanus argentimaculatus*	100%	100%
25	bw25			*Lutjanus indicus*	99%	100%
26	bw17	Perciformes	Mugilidae	*Planiliza macrolepis*	98%	100%
27	bw5		Mullidae	*Parupeneus heptacanthus*	98%	100%
28	bw20			*Upeneus sulphureus*	94%	99%
29	bw8		Nemipteridae	*Pentapodus bifasciatus*	98%	100%
30	bw14		Scaridae	*Scarus vetula*	97%	99%
31	bw22		Scatophagidae	*Scatophagus argus*	100%	99%
32	bw4		Serranidae	*Cephalopholis miniata*	100%	100%

33	bw34			*Epinephelus coioides*	100%	100%
34	bw13		Siganidae	*Siganus sutor*	94%	99%
35	bw39			*Siganus vermiculatus*	98%	99%
36	bw36		Terapontidae	*Terapon jarbua*	100%	100%
37	bw37			*Terapon jarbua*	100%	100%
38	bw40		Trichiuridae	*Trichiurus lepturus*	99%	99%
39	bw41	Pleuronectiformes	Cynoglossidae	*Cynoglossus cf. cynoglossus*	96%	99%
40	bw19	Scorpaeniformes	Platycephalidae	*Platycephalus indicus*	92%	99%

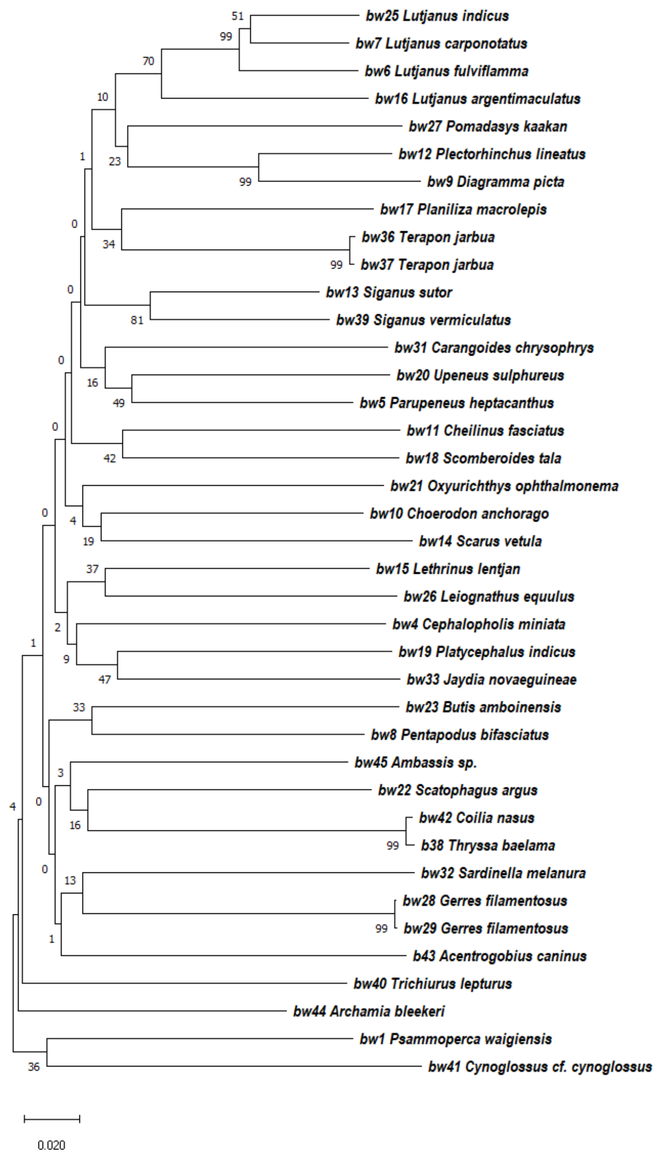

Figure 2. Phylogenetic reconstruction of COI gene sequences of marine fish from Pangpang Bay using Neighbor-Joining algorithm using Mega7

Phylogenetic Reconstruction

The results of the phylogenetic tree reconstruction showed that all Perciformes clustered in large clades, whereas in the Order Pleuronectiformes separated with *Psammoperca waigiensis*. This phylogenetic also shows that economically important Lutjanidae species form a clade. In this study, four species of *Lutjanus* (*L. indicus*, *L. carponotatus*, *L. fulviflamma*, dan *L. argentimaculatus*), were found, two species of *Siganus* (*S. sutor* dan *S. vermiculatus*), and one species of *Sardinella melanura*. Lutjanus genetic information has received the attention of many researchers, even information about the complete mitochondrial genome is known as *Lutjanus vitta* (Andriyono et al. 2018), *Lutjanus carponotatu*s (Kim et al. 2019), and *Lutjanus fulviflama* (Andriyono et al. 2019d). In this study, the *Sardinella melanura* species (Andriyono et al. 2019c) identified from Pangpang Bay waters, also supplement the information that not only *Sardinella lemuru* is commonly found in the Strait of Bali (Pradini et al. 2017, Wujdi et al. 2016). Whereas in the species Siganidae, *Siganus sutor* and *Siganus vermiculatus* are estuary fish groups that can be found throughout the year. This species is also reported to be found in Sulawesi waters, and even the *S. sutor* species is the first record found in Sulawesi waters (Burhanuddin et al. 2014). Information in the study also complements previous research that *S. sutor* was also found in Pangpang Bay waters.

Table 2. The measurement results of Physico-chemical parameters in Pangpang Bay waters.

No.	**Parameter**	**unit**	**Station**						
			1	**2**	**3**	**4**	**5**	**6**	**7**
Chemical									
1	DO	mg/L	6,2	12,2	8,4	10,7	6,2	8	6,7
2	Salinity	PSU	30	31	30	30	34	30	19
3	pH		6,5	7,5	7	7,5	7,5	7,5	7
4	Nitrate	mg/L	0,05	0,07	0,03	0,03	0,02	0,03	0,03
5	Nitrite	mg/L	0,12	0,2	0,2	0,11	0,11	0,09	0,02
6	Phosphate	mg/L	0,11	0,79	0,3	0,04	0,12	0,39	1,56

Physical									
7	Brightness	m	30	45	60	80	100	100	60
8	Depth	m	1	1,5	2	3	6	10	6
9	Temperature	^oC	32	31,9	32,6	31,8	31,6	31	31

Water quality parameters

The physical condition of the waters and the water's quality are vital components forming the habitat of a living thing. The results of measurements of some physic and chemical parameters of the seawaters carried out in situ included six chemical parameters and three physical parameters (Table 2).

Discussion

Indonesia has committed to protecting biodiversity by signing international treaties since 1992. The government also realized the commitment by enacting Law No. 5 of 1994 concerning the ratification of the Convention on Biological Diversity two years after ratifying the international treaty, as a continuation of the responsibilities undertaken in Brazil in 1992. The Indonesian government uses two approaches in protecting biodiversity, namely conservation of the area and preservation of species, including their genetic resources (Gunawan and Sugiarti 2015).

Slightly different from the conservation area, the Pangpang Bay, Banyuwangi Regency, has been designated by the Ministry of Forestry as an essential ecosystem. This determination was motivated by the Republic of Indonesia Presidential Instruction Number: 03 of 2010 concerning Equitable Development, namely the Millennium Development Goals (MDGs) program, the focus of its activities is the guarantee of Environmental Sustainability(Lundine et al. 2013). The uniqueness of Pangpang Bay, which is determined as an essential area, is a determination based on many things, one of which is excellent mangrove potential(Buwono 2017, Kawamuna et al. 2017, Raharja et al. 2014). Based on field visit results, fact-finding and group and plenary discussions from participants in the preparation of the Pangpang Bay Banyuwangi Bay Wetland/Mangrove Action Plan, there are 6 (six) main issues in the management of Pangpang Bay mangroves. These issues are environmental aspects, improving community welfare, regulation, and law enforcement, increasing capacity and human resources, improving infrastructure, and developing institutional management of the area. One of the environmental aspects that can be conveyed is the availability of accurate information about fisheries resources owned by Pangpang Bay, Banyuwangi, presented in this study.

Marine fish identification

Based on the type of fish caught on the static fishing gear (banjang) fishing gear, the size and species identified are quite diverse. The dominant fish caught were the Perciformes (89.2%), followed by the Cluperiformes (5.4%), Pleuronectiformes (2.7%), and Scorpaeniformes (2.7%). Although the Perciformes dominates from the results of identification, species from other orders also become a common catch of traditional fishermen. In the Perciformes, relevant economic groups include the Carangidae, Lutjanidae, and Serranidae families, have been identified in this report. Carangid fish species caught are species of *Scomberoides tala* and*Carangoideschrysophrys* (Table 1). Many research reports found this type in Pangpang Bay waters throughout the year with varying sizes. Apart from being the primary source of income for the traditional fishing community of Pangpang Bay, it is estimated that this area will become a hidden area that stores a high diversity of aquatic biota. Pangpang bay area is located near the Alas Purwo National Park area. The national park at the eastern end of the island of Java is a biodiversity reserve in the region. Previous studies mention that mangrove areas that live in the National Park are recorded to have a diversity of potential nekton (fish), including *Lutjanus fulviflamma* and *Ephinephelus gibbonus*, even though the Gobiidae (*Acentrogobius caninus*) dominates in that region (Ainullah et al. 2015).

Pangpang bay area is believed to be a nursery ground and also feeding zone, both at the juvenile stage and in the fingerling of several economic marine fish species. The catch shows that the fish are mostly young fish, which are likely feeding and growing in this area. With the condition of waters protected from waves (because they are bays), non-pelagic and small pelagic fish are commonly found (Table 1). In addition to these fish groups, due to the substrate waters that tend to be mud, demersal fish that were foraging in the bottom are also found, such as goby fish, *Acentrogobius caninus*, and *Trypauchen vagina* (Andriyono et al. 2019a).

In the type of Clupeiformes, *Sardinela melanura* was identified in this study although many reports mention that *Sardinella lemuru* is a species of Clupeidae that has habitat in this region (Pradini et al. 2017, Sartimbul et al. 2010). The identified *Sardinela melanura* is the first recorded of the complete mitochondrial genome isolated from fish in the Indonesian region (Andriyono et al. 2019c). In Indonesia, several *Sardinella* species have been reported to have habitats, such as *Sardinella fimbricita* (Rahardjo and Simanjuntak 2017), *Sardinella jussieu* (Sektiana et al. 2017), *Sardinella fijiensis* (Wang et al. 2019). Among some species of *Sardinella*, *S. albella*, *S. fimbricata* and *S. gibbosa* have received less attention in taxonomy (Stern et al. 2016). The economic value of sardine fish is sufficiently taken into account in the global fisheries market (Genisa 1999) in particular as a processed product and to obtain fish oil that contains essential fatty acids such as DHA (17.07%) and EPA (13.82%) (Andriyani et al. 2017). Fisheries processing companies in Indonesia that make processed sardine (fish cane) as a significant commodity are also entirely developed and become a mainstay in increasing export revenues.

Although Pangpang bay waters have high fishery potential, fishery activities around the area are also one of the biggest threats to the destruction of fisheries resources in the region. The use of fishing gear that is not environmentally friendly has the potential to damage fish populations, such as the condition of over-fishing in Pangpang Bay today(Ekawaty 2015). This situation can be seen with the use of trap net (traditional name) and chart trap (floating trap net), which are passive and not selective fishing gear by using tidal conditions (Cochrane 2002), which are widely installed on the coast and mangroves. An intake of fishes is carried out following the tidal system in this area, which can be done in the morning or evening. This region experiences tidal current around date 12-19 and 27-6 every month. Meanwhile, the fishermen do not take their catch when the water from stagnant happens around 20-27 and 5-10 each month. In these stagnant conditions, aquatic faunas are caught less, so they prefer to repair or clean the nets and wait when the seawater starts to tide. From the catches obtained, the length of fish caught varies in size from about 2 cm to around 30 cm in total length, and also, the number (capacity) of fish found has been decreased from year to year (Ekawaty 2015). With this method of fishing, it is expected to further reduce fish resources due to the pressure of exploitation that is increasingly high by the community(Andriyono et al. 2019a).

Physical-Chemical Parameters

The sedimentation process in the Pangang bay area is quite high, with some rivers entering the bay, including the Setail River and several other rivers from the west. With this high sedimentation, this area has become an ideal habitat for mangrove plants which were found to be quite dominant in the eastern edge of the Pangpang Bay area of 490 ha(Radiarta et al. 2017).

The characteristics of the bay waters can be observed from the tidal system, which is included in the mixed type with two ebb and two tides. Tidal conditions are aquatic dynamics that significantly affect the oceanographic process in waters. The characteristics of sea level elevation data are obtained from the Meneng station. The Meneng Station is a station owned by Deshidros of Indonesia Navy Station, at latitude 08011'00'S; longitude 1140 54 '00' 'T. The data used is the average water level elevation per hour for one year in 2013. The tidal type at the Meneng station is a mixture leaning to a Mixed Daily semi-diurnal tidal pattern (Buwono et al. 2015). Besides, the dynamics of water parameters in the Pangpang bay area are observed to be quite diverse (Table 1) and overall are still in moderate criteria for marine aquaculture activities if developed in this area (Radiarta et al. 2017). Nonetheless, waste discharges from several fishing industries in Muncar (Setiyono and Yudo 2018)allow the accumulation of organic material and other wastes that increase the burden of waters, especially for the living needs of aquatic biota. From previous studies, the contaminants of Fe, Pb, Cr, and Zn have been measured in the Pangpang Bay region with an average concentration of 0.02 mg/L, 0.09 mg/L, 0.03 mg/L, and 0.8 mg/L, respectively (Radiarta et al. 2017).

Conclusion

The potential of marine fish diversity in the Pangpag Bay area needs to be considered, as well as getting a severe threat with the rise of fishing activities that are less environmentally friendly. The use of "*banjang*" which produces non-selected catches of various sizes, is the leading cause of the decline in the biodiversity value of this region. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and four orders. Based on this identification, we believe there are still many other species of marine fish that have not been identified in this study due to the limitations of fishing gear and the scope of the research conducted. Environmental DNA metabarcoding can be applied by performing a combination of traditional surveys with various fishing tools (passive and active) to obtain complete and useful information in the formulation of conservation policies in this region.

Acknowledgments

We are grateful for the Ministry of Education and Culture, the Republic of Indonesia. Besides, We also want to thank the graduate students of Molecular Physiology Laboratory, Pukyong National University, Busan, Korea for their helpful suggestions during these experiments.

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Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia

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ABSTRACT

Pangpang Bay is necessary to get serious attention because it is one of the essential wetlands in Banyuwangi, Indonesia. This area able to generate income for traditional fishers and ecological supports the surrounding area because it is possible to be a spawning area and nursery ground for many estuary fish. The identification of fisheries resources is carried out using a molecular approach in the Cytochrome oxidase subunit I (COI) region that is commonly used in barcoding. Identification with this method is expected to minimize errors in determining the name of the species of species that exist in Pangpang Bay. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and 4 orders. The results of this study are the initials of molecular information on fisheries resources in the Pangpang Bay area.

INTRODUCTION

Pangpang Bay is in the region of the Banyuwangi Regency, which is directly bordered by the waters of the Bali Strait and the Indian Ocean. There is a massive potential in this region, including the capture fisheries, fisheries processing, aquaculture, and the presence of mangrove ecosystems (**Buwono et al., 2015, Kawamuna *et al*., 2017, Raharja *et al*., 2014**). However, the pattern of resource utilization that has not been appropriately managed causes environmental problems such as a decrease in water quality due to the disposal of organic waste in the fisheries industry without processing (**Anugrah 2013**), overfishing status in the sardine fisheries, deforestation of mangrove forests for ponds as well as urban development activities, declining pond production, and coastal area abrasion.

Currently, the Indonesian government is conducting a study by setting several areas to become the development areas for fishery products as Minapolitan, through the Decree of the Minister of Maritime Affairs and Fisheries No.32/MEN/2010 **(Suryawati**

and Purnomo 2017). One of the Minapolitan is the Muncar sub-district, which located in Banyuwangi. This area is made as one of the most potential minapolitan capture fisheries locations with consideration in several aspects. Muncar is showing potential fishery commodities, strategic geographical location, and having production chains (upstream-downstream) that are already running well. Another hand, Muncar also has adequate main supporting facilities such as transportation and communication facilities, as well as environmental feasibility and local government support. Before the Muncar was established as a minapolitan in 2010, the community had relied on fish resources that had developed since the mid-'60s (**Wiratama 2012**). This condition can be seen from a number of studies exploring the Muncar area relating to the use of traditional fishing technologies. In its development, purse-seines were introduced by the Marine Fisheries Research Institute (BPPL) in the 1972s and continues to be used today **(Perkasa *et al*. 2016**).

In connection with this tremendous potential of water resources, the government (Ministry of Forestry) has determined the Pangpang bay area to be an essential area that has synergistic ecological and economic roles (**Setyaningrum 2017**). Balanced management and utilization efforts are expected to facilitate the community around the Pangpang Bay area, which is almost entirely dependent on the existence of these water resources. In this study, the initial information in management is the collection of a database of fisheries resources owned by this region. The morphological approach in identifying fish and the combination of molecular methods to reduce errors in fish identification in the Pangpang Bay area has been carried out. Previous studies have not found data that specifically inventory fish species in this area. Much research has been done on mangrove vegetation and aquatic fauna that are not specifically on fish species **(Buwono 2017, Buwono *et al*., 2015**). Information on the results of this study is essential for related research to the genetic and biological diversity of fisheries in economically important marine fish species obtained from this crucial region, as well as being the basis for formulating policies in sustainable management.

The molecular identification can be called DNA barcoding. This DNA barcoding method has been agreed globally to ensure the identification of both plant and animal species, either fresh samples or processed product samples **(Giusti *et al*., 2017, Pepe *et al*. 2007**). DNA barcoding efforts on aquatic biota have been carried out and are currently receiving a lot of attention, especially efforts to increase the database on GenBank. Compared to the morphological identification method, DNA barcoding has an accuracy that is close to 100% matched (**Meyer and Paulay 2005**). A single region of mitochondrial DNA Cytochrome C Subunit I gene (COI) that will be deposited in the Genbank database as central bioinformatics becomes the gene marker that is used as the basis for DNA barcoding. Scientists have proven their effectiveness in DNA barcoding in freshwater fish(Hubert et al. 2008), and marine fish (**Lakra *et al*., 2011, Ward *et al*., 2005**). Meanwhile, research on DNA barcoding in Indonesia has also begun to develop. However, it is still limited to several areas such as freshwater fish in Java and Bali (**Dahruddin *et al*., 2017**), freshwater fish Lake Laut Aceh (**Ariyanti 2012, Muchlisin *et al*., 2013**), the Pogar River Sulawesi Island (**Arai *et al*., 1999**), Lake Matano Sulawesi (**Roy *et al*. 2004**), and Malang (**Andriyono *et al*. 2019b**). Whereas DNA barcoding has done in Indonesia is still limited to certain species such as Shark **(Prehadi *et al*., 2014, Sembiring *et al*., 2015**) and Orange-Spotted grouper (**Antoro *et al*., 2006**). Other researchers studied DNA barcoding of a grouper (**Jefri *et al*., 2015**), seahorse **(Lourie** and **Vincent 2004**), and goby fish species (**Winterbottom *et al*., 2014**). Several scientists concerned on coral reef fish species such as three-spot dascullus (Bernardi Giacomo et al. 2001), anemone-fishes (**Madduppa *et al*., 2014**), and Banggai cardinalfish **(Bernardi G and Vagelli 2004**). Due to the limited genetic information of marine fishes, this research is significant to report of Pangpang Bay fish resources through molecular approach.

MATERIALS AND METHODS

Sampling Site

Sampling sites for sample collection was performed in Pangpang Bay, Banyuwangi district of East Java Province **(Figure. 1)**. No specific permit was required for this study. All samples had been collected from the traditional fishermen in Wringinputih Village, Muncar, Banyuwangi. That specimens were dead upon purchasing, and none of the collected specimens were in the endangered category based on the IUCN Red List database. The digital camera has taken for each individual sample

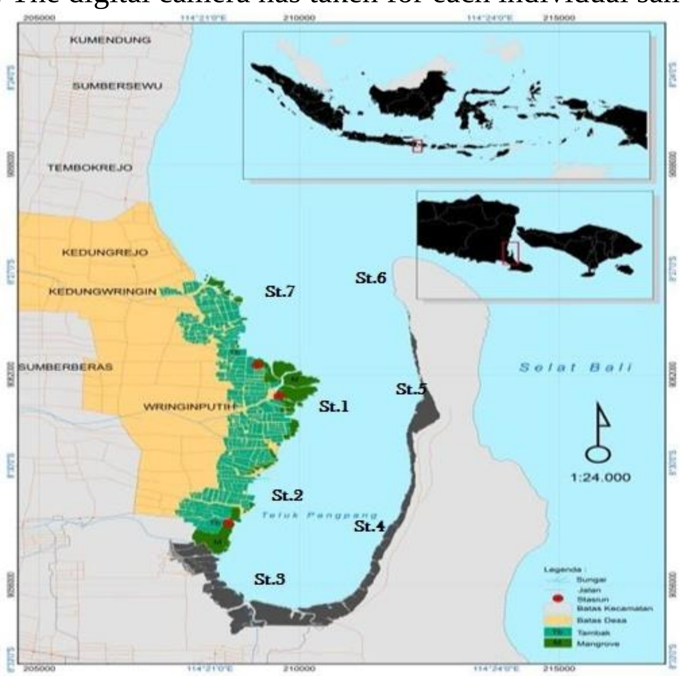

Fig 1. Map of sampling site distribution around Pangpang Bay, Banyuwangi

DNA extraction

Each sample has been compiled based on the morphological characters. After collection, it directly preserved in 90% ethanol. Genomic DNA was extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's guidelines. The anal fin (1 cm) was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA was performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

PCR condition and Data Analysis

The universal fish primer, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), used to obtain the partial sequences of cytochrome c oxidase I (COI) region. The PCR mixture (20µL) contained 11.2 µL ultra-pure water, 1 µL primer forward and reversed (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan). This PCR reaction, we used 2 µL genomic DNA as a template. The PCR condition was carried out under the following setting: 95°C for 5 min in initial denaturation. After that, denaturation performed at 95°C for 30 s in 40 cycles, followed by 50°C for 30 s in annealing and 72°C for 45 s in extension step. The final step of PCR are final extension at 72°C for 5 min. The PCR products of COI was purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

Data Analysis

The pairwise evolutionary distance among the family was determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree was constructed, and 1000 bootstrap analysis was carried by Mega 7 **(Kumar *et al.*, 2016**).

RESULTS

Species Identification

In this study, 40 fish samples were identified in 37 marine fish species distributed in 25 families, and four orders **(Table 1)**. The types of fish are the catch of traditional fishermen who use fishing gear in the form and a small portion using fishing rods and fishing nets. In addition to identification based on morphology, molecular identification is also carried out.

Phylogenetic Reconstruction

The results of the phylogenetic tree reconstruction **(Figure. 2)** showed that all Perciformes clustered in large clades, whereas in the Order Pleuronectiformes separated with *Psammoperca waigiensis*. This phylogenetic also shows that economically important Lutjanidae species form a clade. In this study, four species of *Lutjanus* (*L. indicus, L. carponotatus, L. fulviflamma, dan L. argentimaculatus*), were found, two species of *Siganus* (*S. sutor dan S. vermiculatus*), and one species of *Sardinella melanura*. *Lutjanus* genetic information has received the attention of many researchers, even information about the complete mitochondrial genome is known as *Lutjanus vitta* (**Andriyono *et al*., 2018**), *Lutjanus carponotatus* (**Kim *et al*., 2019**), and *Lutjanus fulviflama* (**Andriyono *et al*., 2019d**).

In this study, the *Sardinella melanura* species (**Andriyono *et al*., 2019c**) identified from Pangpang Bay waters, also supplement the information that not only *Sardinella lemuru* is commonly found in the Strait of Bali **(Pradini *et al.*, 2017, Wujdi *et al*., 2016**). Whereas in the species Siganidae, *Siganus sutor* and *Siganus vermiculatus* are estuary fish groups that can be found throughout the year. This species is also reported to be found in Sulawesi waters, and even the *S. sutor* species is the first record found in Sulawesi waters (**Burhanuddin *et al*., 2014**). Information in the study also complements previous research that *S. sutor* was also found in Pangpang Bay waters.

Table 1. List of online BLASTN results through NCBI database from marine fish specimens of Pangpang Bay, Banyuwangi

No.	Sampel ID	Order	Family	Species Name	Query Cover (%)	Identity (%)
1	bw32	Clupeiformes	Clupeidae	*Sardinella melanura*	100%	99%
2	bw38		Engraulidae	*Thryssa baelama*	96%	99%
3	bw42			*Thryssa baelama*	97%	99%
4	bw45	Perciformes	Ambassidae	*Ambassis sp.*	97%	99%
5	bw33		Apogonidae	*Jaydia novaeguineae*	98%	99%
6	bw44			*Archamia bleekeri*	99%	99%
7	bw18		Carangidae	*Scomberoides tala*	96%	100%
8	bw31			*Carangoides chrysophrys*	99%	100%
9	bw23		Eleotridae	*Butis amboinensis*	97%	99%
10	bw28		Gerreidae	*Gerres filamentosus*	100%	99%
11	bw29			*Gerres filamentosus*	100%	99%
12	bw43		Gobiidae	*Acentro gobiuscaninus*	95%	100%
13	bw21			*Oxyurichthys ophthalmonema*	98%	92%
14	bw9		Haemulidae	*Diagramma pictum*	97%	99%
15	bw12			*Plectorhinchus lineatus*	97%	100%
16	bw27			*Pomadasys kaakan*	98%	99%
17	bw10		Labridae	*Choerodonan chorago*	99%	99%
18	bw11			*Cheilinus fasciatus*	98%	99%
19	bw1		Latidae	*Psammoperca waigiensis*	95%	99%
20	bw26		Leiognathidae	*Leiognathus equulus*	100%	99%
21	bw15		Lethrinidae	*Lethrinus lentjan*	100%	99%
22	bw6		Lutjanidae	*Lutjanus fulviflamma*	100%	99%
23	bw7			*Lutjanus carponotatus*	97%	99%
24	bw16			*Lutjanus argentimaculatus*	100%	100%
25	bw25			*Lutjanus indicus*	99%	100%
26	bw17	Perciformes	Mugilidae	*Planiliza macrolepis*	98%	100%
27	bw5		Mullidae	*Parupeneus heptacanthus*	98%	100%
28	bw20			*Upeneus sulphureus*	94%	99%
29	bw8		Nemipteridae	*Pentapodus bifasciatus*	98%	100%
30	bw14		Scaridae	*Scarus vetula*	97%	99%
31	bw22		Scatophagidae	*Scatophagus argus*	100%	99%
32	bw4		Serranidae	*Cephalopholis miniata*	100%	100%
33	bw34			*Epinephelus coioides*	100%	100%
34	bw13		Siganidae	*Siganus sutor*	94%	99%
35	bw39			*Siganus vermiculatus*	98%	99%
36	bw36		Terapontidae	*Terapon jarbua*	100%	100%
37	bw37			*Terapon jarbua*	100%	100%
38	bw40		Trichiuridae	*Trichiurus lepturus*	99%	99%
39	bw41	Pleuronectiformes	Cynoglossidae	*Cynoglossus cf. cynoglossus*	96%	99%
40	bw19	Scorpaeniformes	Platycephalidae	*Platycephalus indicus*	92%	99%

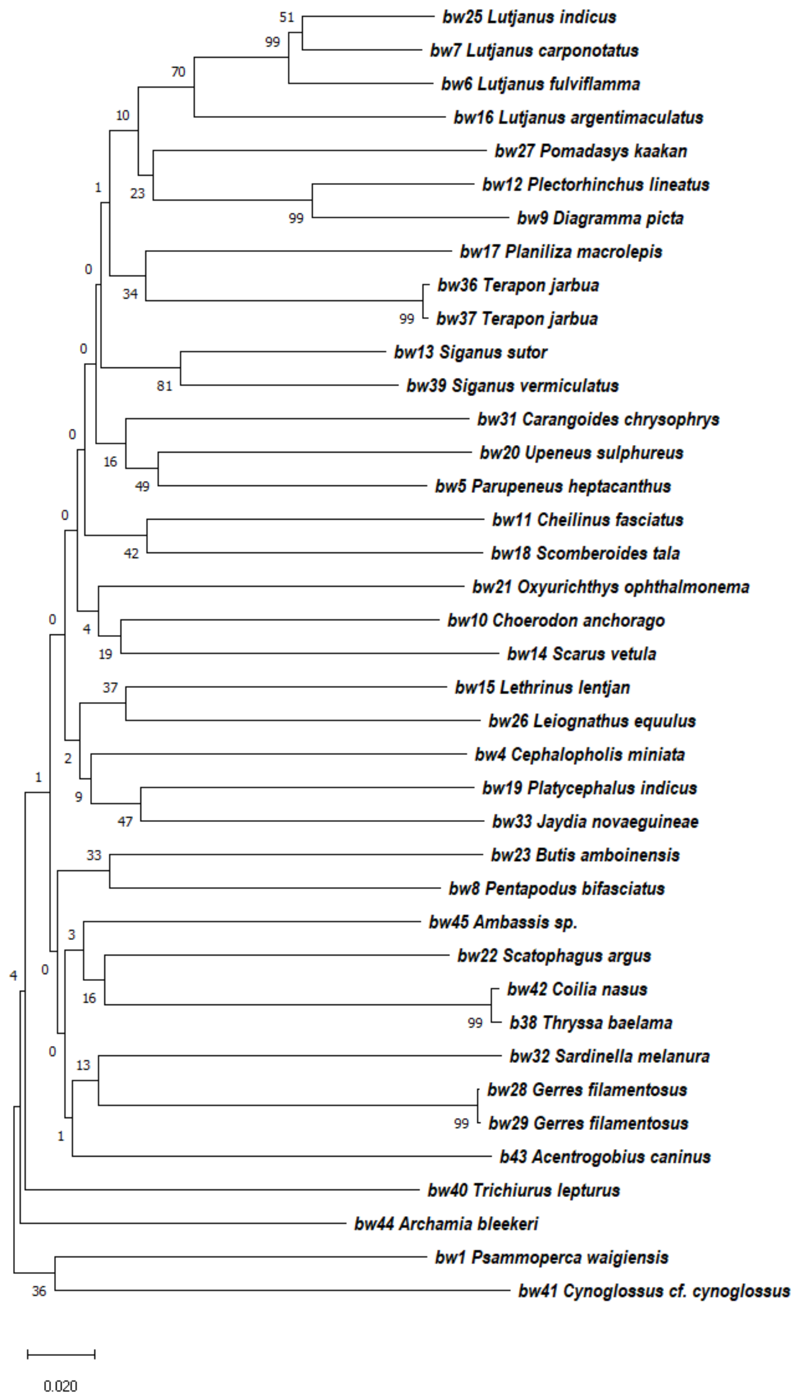

Fig 2. Phylogenetic reconstruction of COI gene sequences of marine fish from Pangpang Bay using Neighbor-Joining algorithm using Mega7

Water quality parameters

The physical condition of the waters and the water's quality are vital components forming the habitat of a living thing. The results of measurements of some physical and chemical parameters of the seawaters carried out in situ included six chemical parameters and three physical parameters **(Table 2)**.

Table 2. The measurements of physico-chemical parameters in Pangpang Bay waters.

No.	Parameter	unit	Station						
			1	2	3	4	5	6	7
Chemical									
1	DO	mg/L	6,2	12,2	8,4	10,7	6,2	8	6,7
2	Salinity	PSU	30	31	30	30	34	30	19
3	pH		6,5	7,5	7	7,5	7,5	7,5	7
4	Nitrate	mg/L	0,05	0,07	0,03	0,03	0,02	0,03	0,03
5	Nitrite	mg/L	0,12	0,2	0,2	0,11	0,11	0,09	0,02
6	Phosphate	mg/L	0,11	0,79	0,3	0,04	0,12	0,39	1,56
Physical									
7	Brightness	m	30	45	60	80	100	100	60
8	Depth	m	1	1,5	2	3	6	10	6
9	Temperature	^{o}C	32	31,9	32,6	31,8	31,6	31	31

DISCUSSION

Indonesia has committed to protecting biodiversity by signing international treaties since 1992. The government also realized the commitment by enacting Law No. 5 of 1994 concerning the ratification of the Convention on Biological Diversity two years after ratifying the international treaty, as a continuation of the responsibilities undertaken in Brazil in 1992. The Indonesian government uses two approaches in protecting biodiversity, namely conservation of the area and preservation of species, including their genetic resources (**Gunawan and Sugiarti 2015**).

Slightly different from the conservation area, the Pangpang Bay, Banyuwangi Regency, has been designated by the Ministry of Forestry as an essential ecosystem. This determination was motivated by the Republic of Indonesia Presidential Instruction Number: 03 of 2010 concerning Equitable Development, namely the Millennium Development Goals (MDGs) program, the focus of its activities is the guarantee of Environmental Sustainability (**Lundine *et al*., 2013**). The uniqueness of Pangpang Bay, which is determined as an essential area, is a determination based on many things, one of which is excellent mangrove potential (**Buwono 2017, Kawamuna *et al.*, 2017, Raharja *et al*., 2014**). Based on field visit results, fact-finding and group and plenary discussions from participants in the preparation of the Pangpang Bay Banyuwangi Bay Wetland/Mangrove Action Plan, there are 6 (six) main issues in the management of Pangpang Bay mangroves. These issues are environmental aspects, improving community welfare, regulation, and law enforcement, increasing capacity and human resources, improving infrastructure, and developing institutional management of the area. One of the environmental aspects that can be conveyed is the availability of accurate information about fisheries resources owned by Pangpang Bay, Banyuwangi, presented in this study.

Marine fish identification

Based on the type of fish caught on the static fishing gear (banjang) fishing gear, the size and species identified are quite diverse. The dominant fish caught were the

Perciformes (89.2%), followed by the Cluperiformes (5.4%), Pleuronectiformes (2.7%), and Scorpaeniformes (2.7%). Although the Perciformes dominates from the results of identification, species from other orders also become a common catch of traditional fishermen. In the Perciformes, relevant economic groups include the Carangidae, Lutjanidae, and Serranidae families, have been identified in this report. Carangid fish species caught are species of *Scomberoides tala* and *Carangoides chrysophrys* **(Table 1)**. Many research reports found this type in Pangpang Bay waters throughout the year with varying sizes. Apart from being the primary source of income for the traditional fishing community of Pangpang Bay, it is estimated that this area will become a hidden area that stores a high diversity of aquatic biota. Pangpang bay area is located near the Alas Purwo National Park area. The national park at the eastern end of the island of Java is a biodiversity reserve in the region. Previous studies mention that mangrove areas that live in the National Park are recorded to have a diversity of potential nekton (fish), including *Lutjanus fulviflamma* and *Ephinephelus gibbonus*, even though the Gobiidae (*Acentrogobius caninus*) dominates in that region (**Ainullah *et al*., 2015**).

Pangpang Bay area is believed to be a nursery ground and also feeding zone, both at the juvenile stage and in the fingerling of several economic marine fish species. The catch shows that the fish are mostly young fish, which are likely feeding and growing in this area. With the condition of waters protected from waves (because they are bays), non-pelagic and small pelagic fish are commonly found **(Table 1)**. In addition to these fish groups, due to the substrate waters that tend to be mud, demersal fish that were foraging in the bottom are also found, such as goby fish, *Acentrogobius caninus*, and *Trypauchen vagina* (**Andriyono *et al*., 2019a**).

In the type of Clupeiformes, *Sardinela melanura* was identified in this study although many reports mention that *Sardinella lemuru* is a species of Clupeidae that has habitat in this region (**Pradini *et al*., 2017, Sartimbul *et al*., 2010**). The identified *Sardinela melanura* is the first recorded of the complete mitochondrial genome isolated from fish in the Indonesian region (**Andriyono *et al*., 2019c**). In Indonesia, several *Sardinella* species have been reported to have habitats, such as *Sardinella fimbricita* **(Rahardjo and Simanjuntak 2017**), *Sardinella jussieu* (**Sektiana *et al*., 2017**), *Sardinella fijiensis* (**Wang et al. 2019**). Among some species of *Sardinella*, *S. albella*, *S. fimbricata* and *S. gibbosa* have received less attention in taxonomy (**Stern *et al*., 2016**). The economic value of sardine fish is sufficiently taken into account in the global fisheries market (**Genisa 1999**) in particular as a processed product and to obtain fish oil that contains essential fatty acids such as DHA (17.07%) and EPA (13.82%) (**Andriyani *et al*., 2017**). Fisheries processing companies in Indonesia that make processed sardine (fish cane) as a significant commodity are also entirely developed and become a mainstay in increasing export revenues.

Although Pangpang bay waters have high fishery potential, fishery activities around the area are also one of the biggest threats to the destruction of fisheries resources in the region. The use of fishing gear that is not environmentally friendly has the potential to damage fish populations, such as the condition of over-fishing in Pangpang Bay (**Ekawaty 2015**). This situation can be seen with the use of trap net (traditional name) and chart trap (floating trap net), which are passive and not selective fishing gear by using tidal conditions (**Cochrane 2002**), which are widely installed on the coast and mangroves. An intake of fishes is carried out following the tidal system in this area, which can be done in the morning or evening. This region experiences tidal current around date 12-19 and 27-6 every month. Meanwhile, the fishermen do not take their catch when the water from stagnant happens around 20-27 and 5-10 each month. In these stagnant conditions, aquatic faunas are caught less, so they prefer to repair or clean the nets and wait when the seawater starts to tide. From the catches obtained, the length of fish caught varies in size from about 2 cm to around 30 cm in total length, and also, the number (capacity) of fish found has been decreased from year to year (**Ekawaty 2015**). With this method of fishing, it is expected to further reduce fish resources due to the pressure of exploitation that is increasingly high by the community (**Andriyono *et al*., 2019a**).

Physical-Chemical Parameters

The sedimentation process in the Pangang bay area is quite high, with some rivers entering the bay, including the Setail River and several other rivers from the west. With this high sedimentation, this area has become an ideal habitat for mangrove plants which were found to be quite dominant in the eastern edge of the Pangpang Bay area of 490 ha (**Radiarta *et al*., 2017**).

The characteristics of the bay waters can be observed from the tidal system, which is included in the mixed type with two ebb and two tides. Tidal conditions are aquatic dynamics that significantly affect the oceanographic process in waters. The characteristics of sea level elevation data are obtained from the Meneng station. The Meneng Station is a station owned by Deshidros of Indonesia Navy Station, at latitude 08011'00'S; longitude 1140 54 '00' 'T. The data used is the average water level elevation per hour for one year in 2013. The tidal type at the Meneng station is a mixture leaning to a Mixed Daily semi-diurnal tidal pattern (**Buwono *et al*., 2015**). Besides, the dynamics of water parameters in the Pangpang bay area are observed to be quite diverse **(Table. 2)** and overall are still in moderate criteria for marine aquaculture activities if developed in this area (**Radiarta *et al*., 2017**). Nonetheless, waste discharges from several fishing industries in Muncar (**Setiyono and Yudo 2018**) allow the accumulation of organic material and other wastes that increase the burden of waters, especially for the living needs of aquatic biota. From previous studies, the contaminants of Fe, Pb, Cr, and Zn have been measured in the Pangpang Bay region with an average concentration of 0.02 mg/L, 0.09 mg/L, 0.03 mg/L, and 0.8 mg/L, respectively (**Radiarta *et al*., 2017**).

CONCLUSION

The potential of marine fish diversity in the Pangpag Bay area needs to be considered, as well as getting a severe threat with the rise of fishing activities that are less environmentally friendly. The use of "*banjang*" which produces non-selected catches of various sizes, is the leading cause of the decline in the biodiversity value of this region. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and four orders. Based on this identification, we believe that there are still many other species of marine fish that have not been identified in this study due to the limitations of fishing gear and the scope of the research conducted. Environmental DNA metabarcoding can be applied by performing a combination of traditional surveys with various fishing tools (passive and active) to obtain complete and useful information in the formulation of conservation policies in this region.

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Molecular Identification and Phylogenetic Tree Reconstruction of Marine Fish from the Essential Wetland Area of Banyuwangi, Indonesia

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ABSTRACT

Pangpang Bay is necessary to get serious attention because it is one of the essential wetlands in Banyuwangi, Indonesia. This area able to generate income for traditional fishers and ecological supports the surrounding area because it is possible to be a spawning area and nursery ground for many estuary fish. The identification of fisheries resources is carried out using a molecular approach in the Cytochrome oxidase subunit I (COI) region that is commonly used in barcoding. Identification with this method is expected to minimize errors in determining the name of the species of species that exist in Pangpang Bay. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and 4 orders. The results of this study are the initials of molecular information on fisheries resources in the Pangpang Bay area.

INTRODUCTION

Pangpang Bay is in the region of the Banyuwangi Regency, which is directly bordered by the waters of the Bali Strait and the Indian Ocean. There is a massive potential in this region, including the capture fisheries, fisheries processing, aquaculture, and the presence of mangrove ecosystems (**Buwono et al., 2015**; **Kawamuna *et al*., 2017** and **Raharja *et al*., 2014**). However, the pattern of resource utilization that has not been appropriately managed causes environmental problems such as a decrease in water quality due to the disposal of organic waste in the fisheries industry without processing (**Anugrah, 2013**), overfishing status in the sardine fisheries, deforestation of mangrove forests for ponds as well as urban development activities, declining pond production, and coastal area abrasion.

Currently, the Indonesian government is conducting a study by setting several areas to become the development areas for fishery products as Minapolitan, through the

Decree of the Minister of Maritime Affairs and Fisheries No.32/MEN/2010 **(Suryawati and Purnomo, 2017**). One of the Minapolitan is the Muncar sub-district, which located in Banyuwangi. This area is made as one of the most potential minapolitan capture fisheries locations with consideration in several aspects. Muncar is showing potential fishery commodities, strategic geographical location, and having production chains (upstream-downstream) that are already running well. Another hand, Muncar also has adequate main supporting facilities such as transportation and communication facilities, as well as environmental feasibility and local government support. Before the Muncar was established as a minapolitan in 2010, the community had relied on fish resources that had developed since the mid-'60s (**Wiratama, 2012**). This condition can be seen from a number of studies exploring the Muncar area relating to the use of traditional fishing technologies. In its development, purse-seines were introduced by the Marine Fisheries Research Institute (BPPL) in the 1972s and continues to be used today **(Perkasa *et al*., 2016**).

In connection with this tremendous potential of water resources, the government (Ministry of Forestry) has determined the Pangpang bay area to be an essential area that has synergistic ecological and economic roles (**Setyaningrum, 2017**). Balanced management and utilization efforts are expected to facilitate the community around the Pangpang Bay area, which is almost entirely dependent on the existence of these water resources. In this study, the initial information in management is the collection of a database of fisheries resources owned by this region. The morphological approach in identifying fish and the combination of molecular methods to reduce errors in fish identification in the Pangpang Bay area has been carried out. Previous studies have not found data that specifically inventory fish species in this area. Much research has been done on mangrove vegetation and aquatic fauna that are not specifically on fish species **(Buwono *et al*., 2015** and **Buwono, 2017**). Information on the results of this study is essential for related research to the genetic and biological diversity of fisheries in economically important marine fish species obtained from this crucial region, as well as being the basis for formulating policies in sustainable management.

The molecular identification can be called DNA barcoding. This DNA barcoding method has been agreed globally to ensure the identification of both plant and animal species, either fresh samples or processed product samples **(Pepe *et al*., 2007** and **Giusti *et al*., 2017**). DNA barcoding efforts on aquatic biota have been carried out and are currently receiving a lot of attention, especially efforts to increase the database on GenBank. Compared to the morphological identification method, DNA barcoding has an accuracy that is close to 100% matched (**Meyer and Paulay, 2005**). A single region of mitochondrial DNA Cytochrome C Subunit I gene (COI) that will be deposited in the Genbank database as central bioinformatics becomes the gene marker that is used as the basis for DNA barcoding. Scientists have proven their effectiveness in DNA barcoding in freshwater fish(Hubert et al. 2008), and marine fish (**Ward *et al*., 2005** and **Lakra *et al*., 2011**). Meanwhile, research on DNA barcoding in Indonesia has also begun to develop. However, it is still limited to several areas such as freshwater fish in Java and Bali (**Dahruddin *et al*., 2017**), freshwater fish Lake Laut Aceh (**Ariyanti, 2012** and **Muchlisin *et al*., 2013**), the Pogar River Sulawesi Island (**Arai *et al*., 1999**), Lake Matano Sulawesi (**Roy *et al*., 2004**), and Malang (**Andriyono *et al*., 2019b**). Whereas DNA barcoding has done in Indonesia is still limited to certain species such as Shark

(Prehadi *et al*., 2014 and **Sembiring *et al*., 2015**) and Orange-Spotted grouper (**Antoro *et al*., 2006**). Other researchers studied DNA barcoding of a grouper (**Jefri e*t al*., 2015**), seahorse **(Lourie and Vincent, 2004**), and goby fish species (**Winterbottom *et al*., 2014**). Several scientists concerned on coral reef fish species such as three-spot dascullus (Bernardi Giacomo et al. 2001), anemone-fishes (**Madduppa *et al*., 2014**), and Banggai cardinalfish **(Bernardi and Vagelli, 2004**). Due to the limited genetic information of marine fishes, this research is significant to report of Pangpang Bay fish resources through molecular approach.

MATERIALS AND METHODS

Sampling Site

Sampling sites for sample collection was performed in Pangpang Bay, Banyuwangi district of East Java Province **(Figure, 1)**. No specific permit was required for this study. All samples had been collected from the traditional fishermen in Wringinputih Village, Muncar, Banyuwangi. That specimens were dead upon purchasing, and none of the collected specimens were in the endangered category based on the IUCN Red List database. The digital camera has taken for each individual sample

Fig 1. Map of sampling site distribution around Pangpang Bay, Banyuwangi

DNA extraction

Each sample has been compiled based on the morphological characters. After collection, it directly preserved in 90% ethanol. Genomic DNA was extracted using an Accuprep® Genomic DNA Extraction Kit (Bioneer) according to the manufacturer's guidelines. The anal fin (1 cm) was dissected and mix with 6X lysis buffer, which was further homogenized by the TissueLyser II (Qiagen). Quantification of purified genomic DNA was performed by nanoDrop (Thermofisher Scientific D1000), aliquoted and stored at the -70^{o}C for further analysis.

PCR condition and Data Analysis

The universal fish primer, BCL-BCH (Baldwin et al. 2009, Handy et al. 2011), used to obtain the partial sequences of cytochrome c oxidase I (COI) region. The PCR mixture (20µL) contained 11.2 µL ultra-pure water, 1 µL primer forward and reversed (0.5 µM), 0.2 µL Ex Taq DNA polymerase (TaKaRa, Japan), 2 µL 10X ExTag Buffer, 2 µL dNTPs (1 µM, TaKaRa, Japan). This PCR reaction, we used 2 µL genomic DNA as a template. The PCR condition was carried out under the following setting: 95°C for 5 min in initial denaturation. After that, denaturation performed at 95°C for 30 s in 40 cycles, followed by 50°C for 30 s in annealing and 72°C for 45 s in extension step. The final step of PCR are final extension at 72°C for 5 min. The PCR products of COI was purified with the AccuPrep®Gel purification kit (Bioneer, Korea).

Data Analysis

The pairwise evolutionary distance among the family was determined by the Kimura 2-Parameter method. The Neighbor-joining (NJ) tree was constructed, and 1000 bootstrap analysis was carried by Mega 7 **(Kumar *et al.*, 2016**).

RESULTS

Species Identification

In this study, 40 fish samples were identified in 37 marine fish species distributed in 25 families, and four orders **(Table 1)**. The types of fish are the catch of traditional fishermen who use fishing gear in the form and a small portion using fishing rods and fishing nets. In addition to identification based on morphology, molecular identification is also carried out.

Phylogenetic Reconstruction

The results of the phylogenetic tree reconstruction **(Figure, 2)** showed that all Perciformes clustered in large clades, whereas in the Order Pleuronectiformes separated with *Psammoperca waigiensis*. This phylogenetic also shows that economically important Lutjanidae species form a clade. In this study, four species of *Lutjanus* (*L. indicus, L. carponotatus, L. fulviflamma, dan L. argentimaculatus*), were found, two species of *Siganus* (*S. sutor dan S. vermiculatus*), and one species of *Sardinella melanura*. *Lutjanus* genetic information has received the attention of many researchers, even information about the complete mitochondrial genome is known as *Lutjanus vitta* (**Andriyono *et al.*, 2018**), *Lutjanus carponotatus* (**Kim *et al.*, 2019**), and *Lutjanus fulviflama* (**Andriyono *et al.*, 2019d**).

In this study, the *Sardinella melanura* species (**Andriyono *et al.*, 2019c**) identified from Pangpang Bay waters, also supplement the information that not only *Sardinella lemuru* is commonly found in the Strait of Bali **(Pradini *et al.*, 2017** and **Wujdi *et al.*, 2016**). Whereas in the species Siganidae, *Siganus sutor* and *Siganus vermiculatus* are estuary fish groups that can be found throughout the year. This species is also reported to be found in Sulawesi waters, and even the *S. sutor* species is the first record found in Sulawesi waters (**Burhanuddin *et al.*, 2014**). Information in the study also complements previous research that *S. sutor* was also found in Pangpang Bay waters.

Table 1. List of online BLASTN results through NCBI database from marine fish specimens of Pangpang Bay, Banyuwangi

No.	Sampel ID	Order	Family	Species Name	Query Cover (%)	Identity (%)
1	bw32	Clupeiformes	Clupeidae	*Sardinella melanura*	100%	99%
2	bw38		Engraulidae	*Thryssa baelama*	96%	99%
3	bw42			*Thryssa baelama*	97%	99%
4	bw45	Perciformes	Ambassidae	*Ambassis sp.*	97%	99%
5	bw33		Apogonidae	*Jaydia novaeguineae*	98%	99%
6	bw44			*Archamia bleekeri*	99%	99%
7	bw18		Carangidae	*Scomberoides tala*	96%	100%
8	bw31			*Carangoides chrysophrys*	99%	100%
9	bw23		Eleotridae	*Butis amboinensis*	97%	99%
10	bw28		Gerreidae	*Gerres filamentosus*	100%	99%
11	bw29			*Gerres filamentosus*	100%	99%
12	bw43		Gobiidae	*Acentro gobiuscaninus*	95%	100%
13	bw21			*Oxyurichthys ophthalmonema*	98%	92%
14	bw9		Haemulidae	*Diagramma pictum*	97%	99%
15	bw12			*Plectorhinchus lineatus*	97%	100%
16	bw27			*Pomadasys kaakan*	98%	99%
17	bw10		Labridae	*Choerodonan chorago*	99%	99%
18	bw11			*Cheilinus fasciatus*	98%	99%
19	bw1		Latidae	*Psammoperca waigiensis*	95%	99%
20	bw26		Leiognathidae	*Leiognathus equulus*	100%	99%
21	bw15		Lethrinidae	*Lethrinus lentjan*	100%	99%
22	bw6		Lutjanidae	*Lutjanus fulviflamma*	100%	99%
23	bw7			*Lutjanus carponotatus*	97%	99%
24	bw16			*Lutjanus argentimaculatus*	100%	100%
25	bw25			*Lutjanus indicus*	99%	100%
26	bw17	Perciformes	Mugilidae	*Planiliza macrolepis*	98%	100%
27	bw5		Mullidae	*Parupeneus heptacanthus*	98%	100%
28	bw20			*Upeneus sulphureus*	94%	99%
29	bw8		Nemipteridae	*Pentapodus bifasciatus*	98%	100%
30	bw14		Scaridae	*Scarus vetula*	97%	99%
31	bw22		Scatophagidae	*Scatophagus argus*	100%	99%
32	bw4		Serranidae	*Cephalopholis miniata*	100%	100%
33	bw34			*Epinephelus coioides*	100%	100%
34	bw13		Siganidae	*Siganus sutor*	94%	99%
35	bw39			*Siganus vermiculatus*	98%	99%
36	bw36		Terapontidae	*Terapon jarbua*	100%	100%
37	bw37			*Terapon jarbua*	100%	100%
38	bw40		Trichiuridae	*Trichiurus lepturus*	99%	99%
39	bw41	Pleuronectiformes	Cynoglossidae	*Cynoglossus cf. cynoglossus*	96%	99%
40	bw19	Scorpaeniformes	Platycephalidae	*Platycephalus indicus*	92%	99%

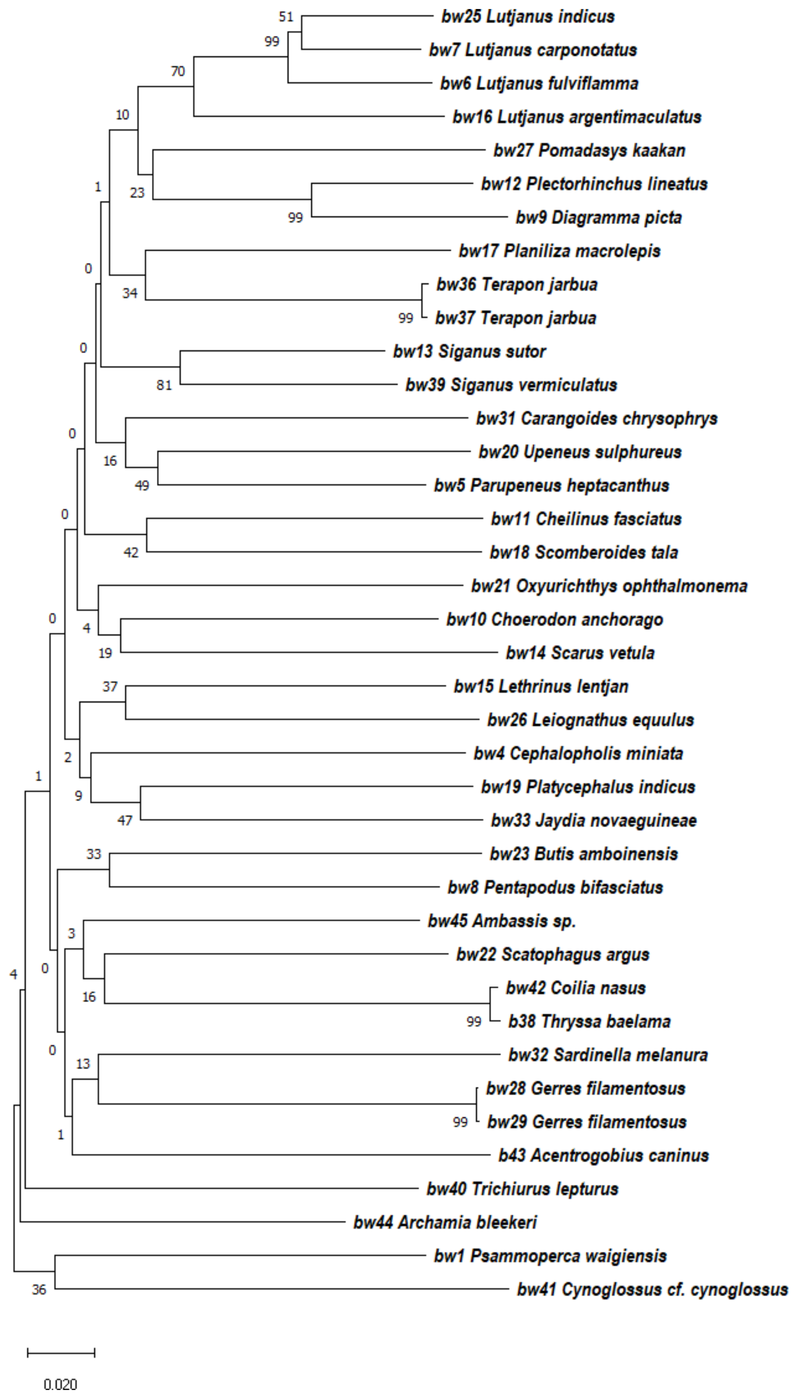

Fig 2. Phylogenetic reconstruction of COI gene sequences of marine fish from Pangpang Bay using Neighbor-Joining algorithm using Mega7

Water quality parameters

The physical condition of the waters and the water's quality are vital components forming the habitat of a living thing. The results of measurements of some physical and chemical parameters of the seawaters carried out in situ included six chemical parameters and three physical parameters **(Table 2)**.

Table 2. The measurements of physico-chemical parameters in Pangpang Bay waters.

No.	Parameter	unit	Station						
			1	2	3	4	5	6	7
Chemical									
1	DO	mg/L	6,2	12,2	8,4	10,7	6,2	8	6,7
2	Salinity	PSU	30	31	30	30	34	30	19
3	pH		6,5	7,5	7	7,5	7,5	7,5	7
4	Nitrate	mg/L	0,05	0,07	0,03	0,03	0,02	0,03	0,03
5	Nitrite	mg/L	0,12	0,2	0,2	0,11	0,11	0,09	0,02
6	Phosphate	mg/L	0,11	0,79	0,3	0,04	0,12	0,39	1,56
Physical									
7	Brightness	m	30	45	60	80	100	100	60
8	Depth	m	1	1,5	2	3	6	10	6
9	Temperature	^oC	32	31,9	32,6	31,8	31,6	31	31

DISCUSSION

Indonesia has committed to protecting biodiversity by signing international treaties since 1992. The government also realized the commitment by enacting Law No. 5 of 1994 concerning the ratification of the Convention on Biological Diversity two years after ratifying the international treaty, as a continuation of the responsibilities undertaken in Brazil in 1992. The Indonesian government uses two approaches in protecting biodiversity, namely conservation of the area and preservation of species, including their genetic resources (**Gunawan and Sugiarti 2015**).

Slightly different from the conservation area, the Pangpang Bay, Banyuwangi Regency, has been designated by the Ministry of Forestry as an essential ecosystem. This determination was motivated by the Republic of Indonesia Presidential Instruction Number: 03 of 2010 concerning Equitable Development, namely the Millennium Development Goals (MDGs) program, the focus of its activities is the guarantee of Environmental Sustainability (**Lundine *et al*., 2013**). The uniqueness of Pangpang Bay, which is determined as an essential area, is a determination based on many things, one of which is excellent mangrove potential (**Raharja *et al*., 2014; Buwono, 2017** and **Kawamuna *et al.*, 2017;**). Based on field visit results, fact-finding and group and plenary discussions from participants in the preparation of the Pangpang Bay Banyuwangi Bay Wetland/Mangrove Action Plan, there are 6 (six) main issues in the management of Pangpang Bay mangroves. These issues are environmental aspects, improving community welfare, regulation, and law enforcement, increasing capacity and human resources, improving infrastructure, and developing institutional management of the area. One of the environmental aspects that can be conveyed is the availability of accurate information about fisheries resources owned by Pangpang Bay, Banyuwangi, presented in this study.

Marine fish identification

Based on the type of fish caught on the static fishing gear (banjang) fishing gear, the size and species identified are quite diverse. The dominant fish caught were the Perciformes (89.2%), followed by the Cluperiformes (5.4%), Pleuronectiformes (2.7%), and Scorpaeniformes (2.7%). Although the Perciformes dominates from the results of identification, species from other orders also become a common catch of traditional fishermen. In the Perciformes, relevant economic groups include the Carangidae, Lutjanidae, and Serranidae families, have been identified in this report. Carangid fish species caught are species of *Scomberoides tala* and *Carangoides chrysophrys* **(Table 1)**. Many research reports found this type in Pangpang Bay waters throughout the year with varying sizes. Apart from being the primary source of income for the traditional fishing community of Pangpang Bay, it is estimated that this area will become a hidden area that stores a high diversity of aquatic biota. Pangpang bay area is located near the Alas Purwo National Park area. The national park at the eastern end of the island of Java is a biodiversity reserve in the region. Previous studies mention that mangrove areas that live in the National Park are recorded to have a diversity of potential nekton (fish), including *Lutjanus fulviflamma* and *Ephinephelus gibbonus*, even though the Gobiidae (*Acentrogobius caninus*) dominates in that region (**Ainullah *et al*., 2015**).

Pangpang Bay area is believed to be a nursery ground and also feeding zone, both at the juvenile stage and in the fingerling of several economic marine fish species. The catch shows that the fish are mostly young fish, which are likely feeding and growing in this area. With the condition of waters protected from waves (because they are bays), non-pelagic and small pelagic fish are commonly found **(Table 1)**. In addition to these fish groups, due to the substrate waters that tend to be mud, demersal fish that were foraging in the bottom are also found, such as goby fish, *Acentrogobius caninus*, and *Trypauchen vagina* (**Andriyono *et al*., 2019a**).

In the type of Clupeiformes, *Sardinela melanura* was identified in this study although many reports mention that *Sardinella lemuru* is a species of Clupeidae that has habitat in this region (**Sartimbul *et al*., 2010** and **Pradini *et al*., 2017**). The identified *Sardinela melanura* is the first recorded of the complete mitochondrial genome isolated from fish in the Indonesian region (**Andriyono *et al*., 2019c**). In Indonesia, several *Sardinella* species have been reported to have habitats, such as *Sardinella fimbricita* **(Rahardjo and Simanjuntak 2017**), *Sardinella jussieu* (**Sektiana *et al*., 2017**), *Sardinella fijiensis* (**Wang *et al*., 2019**). Among some species of *Sardinella*, *S. albella*, *S. fimbricata* and *S. gibbosa* have received less attention in taxonomy (**Stern *et al*., 2016**). The economic value of sardine fish is sufficiently taken into account in the global fisheries market (**Genisa 1999**) in particular as a processed product and to obtain fish oil that contains essential fatty acids such as DHA (17.07%) and EPA (13.82%) (**Andriyani *et al*., 2017**). Fisheries processing companies in Indonesia that make processed sardine (fish cane) as a significant commodity are also entirely developed and become a mainstay in increasing export revenues.

Although Pangpang bay waters have high fishery potential, fishery activities around the area are also one of the biggest threats to the destruction of fisheries resources in the region. The use of fishing gear that is not environmentally friendly has the potential to damage fish populations, such as the condition of over-fishing in Pangpang Bay (**Ekawaty, 2015**). This situation can be seen with the use of trap net (traditional name) and chart trap (floating trap net), which are passive and not selective fishing gear by using tidal conditions (**Cochrane, 2002**), which are widely installed on the coast and mangroves. An intake of fishes is carried out following the tidal system in this area, which can be done in the morning or evening. This region experiences tidal current around date 12-19 and 27-6 every month. Meanwhile, the fishermen do not take their catch when the water from stagnant happens around 20-27 and 5-10 each month. In these stagnant conditions, aquatic faunas are caught less, so they prefer to repair or clean the nets and wait when the seawater starts to tide. From the catches obtained, the length of fish caught varies in size from about 2 cm to around 30 cm in total length, and also, the number (capacity) of fish found has been decreased from year to year (**Ekawaty, 2015**). With this method of fishing, it is expected to further reduce fish resources due to the pressure of exploitation that is increasingly high by the community (**Andriyono *et al*., 2019a**).

Physical-Chemical Parameters

The sedimentation process in the Pangang bay area is quite high, with some rivers entering the bay, including the Setail River and several other rivers from the west. With this high sedimentation, this area has become an ideal habitat for mangrove plants which were found to be quite dominant in the eastern edge of the Pangpang Bay area of 490 ha (**Radiarta *et al*., 2017**).

The characteristics of the bay waters can be observed from the tidal system, which is included in the mixed type with two ebb and two tides. Tidal conditions are aquatic dynamics that significantly affect the oceanographic process in waters. The characteristics of sea level elevation data are obtained from the Meneng station. The Meneng Station is a station owned by Deshidros of Indonesia Navy Station, at latitude 08011'00'S; longitude 1140 54 '00' 'T. The data used is the average water level elevation per hour for one year in 2013. The tidal type at the Meneng station is a mixture leaning to a Mixed Daily semi-diurnal tidal pattern (**Buwono *et al*., 2015**). Besides, the dynamics of water parameters in the Pangpang bay area are observed to be quite diverse **(Table 2)** and overall are still in moderate criteria for marine aquaculture activities if developed in this area (**Radiarta *et al*., 2017**). Nonetheless, waste discharges from several fishing industries in Muncar (**Setiyono and Yudo 2018**) allow the accumulation of organic material and other wastes that increase the burden of waters, especially for the living needs of aquatic biota. From previous studies, the contaminants of Fe, Pb, Cr, and Zn have been measured in the Pangpang Bay region with an average concentration of 0.02 mg/L, 0.09 mg/L, 0.03 mg/L, and 0.8 mg/L, respectively (**Radiarta *et al*., 2017**).

CONCLUSION

The potential of marine fish diversity in the Pangpag Bay area needs to be considered, as well as getting a severe threat with the rise of fishing activities that are less environmentally friendly. The use of "*banjang*" which produces non-selected catches of various sizes, is the leading cause of the decline in the biodiversity value of this region. Based on molecular identification information in the COI region, 37 marine fish species were distributed in 25 families, and four orders. Based on this identification, we believe that there are still many other species of marine fish that have not been identified in this study due to the limitations of fishing gear and the scope of the research conducted.

Environmental DNA metabarcoding can be applied by performing a combination of traditional surveys with various fishing tools (passive and active) to obtain complete and useful information in the formulation of conservation policies in this region.

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