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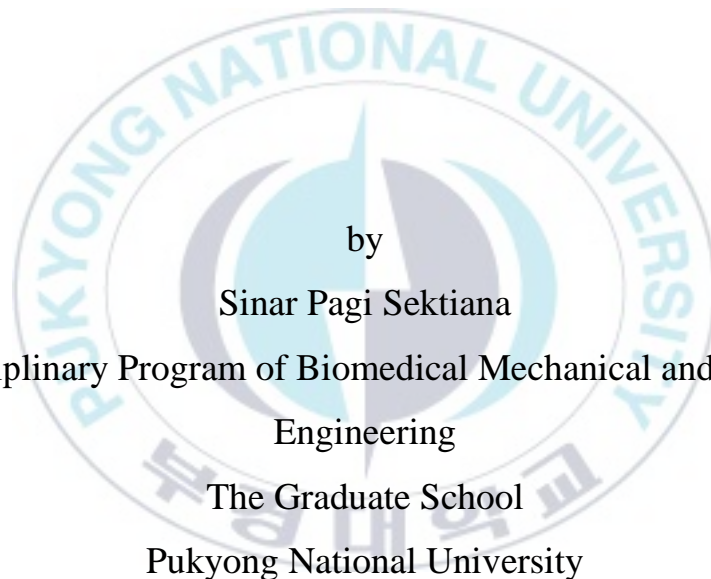
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Thesis for the Degree of Doctor of Philosophy

Fish biodiversity in the Coral Reef of Java  
Island, Indonesia by  
Genomic Analysis



by

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Interdisciplinary Program of Biomedical Mechanical and Electrical  
Engineering

The Graduate School

Pukyong National University

February 22, 2019

Fish biodiversity in the Coral Reef of Java  
Island, Indonesia by  
Genomic Analysis  
인도네시아 자바 섬의 산호초에서  
물고기의 생물 다양성  
게놈 분석

Advisor: Prof. Hyun Woo Kim

by  
Sinar Pagi Sektiana

A thesis submitted in partial fulfillment of the requirements  
for the degree of

Doctor of Philosophy

In Interdisciplinary Program of Biomedical Mechanical and Electrical  
Engineering, The Graduate School  
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February 22, 2019

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## **Fish biodiversity in the Coral Reef of Java Island, Indonesia by Genomic Analysis**

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### **Abstract**

Coral reefs are the most productive and rich biodiversity ecosystem. Their extent is relatively small, around 0.2 % of ocean surface were distributed around the world. However, almost 25 % of marine species and nearly 4,000 marine fish species are using coral reef as their habitat. Its ecosystem is now has been damaged by the global climatic changes, local pressure caused by the anthropogenic activity, destructive fishing practices, and increased sedimentation. It results in a significant impact on the abundance and diversity of coral reef fishes. Fish biodiversity has important parameters for marine ecosystem function. It is recognized to be global importance and becomes of increasing concern in the management of the marine ecosystem. Since the marine habitats continue to be under increasing pressure, its driving responsibility for conserving biodiversity and for managing development for the sustainable way. Hence, a biodiversity survey was applied to provide basic information for environmental management.

Thousand Islands and Tabuhan Island located in the Java Sea, it is a coastal area with coral reefs ecosystem. It has been reported as Indonesian archipelago local hotspot reef fish biodiversity. Furthermore, the biodiversity of both locations provided goods and service to the ecosystem and exploited since 1960 for fisheries product and ornamental fish. However, in the last decade, Thousand Island's coral reef experienced high degradation and reduction in the fish diversity.

During the period from 1984 to 2018, the biodiversity research in the Thousand Islands extensively employed underwater visual censuses (UVC) and fishes identified visually to the

level of species taxonomy based on its morphological characteristics as reef ecosystem are characteristically fragile and present special difficulties. The researchers recorded a total of 479 species of fish. In the year of 2016, another researcher identified 216 species of 29 families. Furthermore, according to the IUCN red list database, a total 442 species consisting ten species listed as data deficient (DD), 219 species as least concern (LC), 192 as not evaluated (NE) and eleven rare fish species were listed as near threatened (NT) and vulnerable (VUL) including three species from a subclass of Elasmobranchii. On the contrary, a few information of biodiversity research in Tabuhan Island was found, 34 fish species of 14 families and one near threatened species were reported in Bangsring coast, Banyuwangi as the closest location. Since fish species identified visually based on its morphological characteristics, 40 species were identified as unclear nomenclatures.

In a recent study, DNA sequencing and bioinformatics provide cheaper and simplified analysis and enable to identify cryptic species. Consequently, researchers can analyze the species biodiversity by sequencing of total mitochondrial DNA, DNA barcodes from specific species and another molecular information. Despite the long sequence of total mitochondrial DNA well determined the cryptic species, a short sequence of mitochondrial DNA has advantageous to genetic marker and biodiversity survey.

The COI sequence is a conserved protein-coding gene and was used as species DNA barcoding for the standard genetic marker and rapid single species identification. However, the COI sequence less effective to identify impurity, mixture, degraded, short, fragmented DNA and bulk sample from environmental DNA (eDNA). Furthermore, the Internal transcribed spacer (ITS) is a less conserved sequence which is situated between two conserved genes, 12S rRNA and 16S rRNA. It is commonly used for genetic population study and potentially effective to characterized species composition in bulk diversity sample or eDNA. Since COI sequence utilized as standard species DNA barcoding, a large number of COI sequence database was deposited in GenBank. However, a few ITS sequence database were deposited.

For this reason, we generate ITS database of fish collected from a coral reef in Java Sea, Indonesia and using COI region as an identity marker. The COI sequence ranged from 588-682 bp in length and the ITS sequence ranged from 583-1123 bp in length. Its sequence representing 120 species, 78 genera, 30 family and five orders, and we added a total of 87 new ITS sequence database and five new COI sequences to enrich the existing reference of DNA barcode. The ITS sequence contains two conserved genes (12S rRNA, and 16s rRNA), and tRNA<sup>Val</sup> showed hypervariable nucleotide were adequate to amplify the target gene and contain sufficient nucleotide variation to discriminate closely related congeneric fish species.

Furthermore, we designed the Chord primer by aligning 1,846 sequences from Chordata, targeting a fragment (344 bp in length) of ITS sequence and compare with MiFish primer targeting fragment (163 to 185 bp in length) of fish mitochondrial 12S rRNA gene sequence to obtain fish diversity in eDNA sample. As a result, MiFish primer obtained higher reads and OTUs number (105,860 reads and 146 OTUs) compared with the Chord primer (84,230 reads and 115 OTUs). Due to the higher numbers of sequences covering the MiFish primer regions in the database, a total 91.7 % merged reads (130 OTUs) were described as fish species. In contrast, only 58.3% merged reads (74 OTUs) were described as fish, and 39.7% merged reads (37 OTUs) were identified as unknown fish species by Chord primer. The taxonomic composition of fish species by MiFish primer exhibited 130 species consisting one subclass, eight orders, 31 families and 60 genera. while Chord primer covered 74 species comprising two subclasses, nine orders, 31 families and 60 genera. Different from MiFish primer set, three species of two family in Elasmobranchii were detected exclusively by Chord primer set. The short sequence of MiFish primer set showed a higher species number than the longer sequence of Chord primer set. However, both primer sets resulted in higher diversity than the previous study and revealed hidden fish diversity in the Thousand Islands. In conclusion, we developed a longer sequence of Chord primer set for eDNA metabarcoding. Although there is a lack of data in the ITS sequences database, this primer set exhibited higher resolution and enable to detect Elasmobranch group compare to the MiFish primer. It is potentially used as a tool to analyze

fish diversity and could serve as an alternative, non-invasive method for coral reef fish biodiversity surveys.

Keywords: DNA barcoding, DNA metabarcoding, Environmental DNA, Thousand Island, Highthroughput sequencing,



## Fish biodiversity in the Coral Reef of Java Island, Indonesia by Genomic Analysis

게놈 분석에 의한 인도네시아 자바 섬의 산호초에서의 생선 생물 다양성

Sinar Pagi Sektiana

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### 요약

산호초는 가장 생산적이고 풍부한 생물 다양성 생태계입니다. 그 범위는 비교적 작으며, 바다 표면의 약 0.2%가 전 세계에 분포되어 있다. 그러나 거의 25%의 해양 생물과 거의 4,000 종의 해양 생물이 산호초를 서식지로 사용하고 있습니다. 그 생태계는 전 지구 적 기후 변화, 지역 인위적 활동, 파괴적인 어업 활동 및 증가 된 퇴적물에 의해 손상되었습니다. 산호초 어류의 풍부 성과 다양성에 중요한 영향을 미친다. 중요한 생태계 기능을 위한 어류의 생물 다양성. 세계적인 중요성으로 인식되어 해양 생태계의 관리에 대한 관심이 증가하고 있습니다. 해양 습관은 계속해서 압력을 가중시키고 있으며, 생물 다양성 보전에 대한 책임과 지속 가능한 개발을 위한 관리를 책임지고 있습니다. 따라서 생물 다양성 조사는 환경 관리에 대한 기본 정보를 제공하기 위해 적용되었습니다.

서인도 제도과 타부 한 섬은 산호초 생태계 인 자바 해에 위치하고 있습니다. 그것은 인도네시아 군도 지방의 핫스팟 어류 생물 다양성으로 보고되었습니다. 또한 두 지역의 생물 다양성은 생태계에 대한 재화와 서비스를 제공하고 1960 년 이래 수산 식품과 관상용 어류에 이용되었습니다. 그러나 지난 10 년 동안 사우 전 섬의 산호초는 어류의 다양성이 크게 악화되고 감소했습니다.

1984 년부터 2018 년까지 UVC (Underwater Visual Census)와 물고기의 후원하에 생물 다양성 연구가 특징적으로 취약하고 현재 특별한 어려움이 있음이 확인되었습니다. 연구자들은 총 479 종의 물고기를 기록했다. 또 다른

연구원은 2016 년에 29 종의 216 종을 확인했습니다. 또한, IUCN 레드리스트 데이터베이스에 따르면 292 종은 최소 관심사로, 143 종은 평가되지 않음으로 표시되었습니다. 희귀 한 어류는 Elasmobranchii 의 하위 분류에서 7 종을 포함하여 거의 위협 받고 취약한 것으로 분류되었습니다. 반대로, 타 부한 (Tabuhan) 섬의 생물 다양성 연구에 관한 많은 정보가 발견되었는데, 14 군데의 어류 34 종과 Bangsring 해안에서 가장 가까운 곳인 바 누유이 (Banyuwangi)에서 가장 가까운 생물로 보고 된 종 중 하나. 확인 된 어종은 전형적으로 그 형태 학적 특성에 기반을 두고 있기 때문에 불명확 한 식별, 잘못 표기된 데이터베이스 및 데이터베이스에 정보가 없기 때문에 속 40 종을 확인했다.

최근의 연구에서 DNA 시퀀싱은 합리적인 생물 정보학 및 단순화 된 분석을 제공하고 비밀 종을 식별 할 수 있습니다. 결과적으로, 연구원은 총 미토콘드리아 DNA, 특정 종의 DNA 바코드 및 다른 분자 정보의 시퀀싱을 통해 생물 다양성 종을 분석 할 수 있습니다. 전체 미토콘드리아 DNA 가 잘 결정된 비밀스런 종의 긴 서열에도 불구하고 미토콘드리아 DNA 의 짧은 서열은 유전 적 마커 및 생물 다양성 조사에 유리하다.

COI 서열은 보존 된 단백질 암호 유전자이며, 표준 유전자 마커 및 신속한 단일 종 식별을위한 DNA 의 종으로 사용되었다. 그러나, COI 서열은 환경 DNA (eDNA)로부터의 불순물, 혼합물, 분해 된 단편 DNA 및 벌크 샘플을 확인하는데 덜 효과적이다. 또한 내부 전사 된 스페이서 (ITS)는 2 개의 보존 된 유전자 인 12SRNA 와 16sRNA 사이의 순차적 서열이다. 그것은 일반적으로 유전 연구에 사용되며 대량 다양성 샘플 또는 eDNA 에서 성분을 특성화하는 데 잠재적으로 효과적입니다. COI 서열이 표준 DNA 바코딩 종으로 이용 되었기 때문에 많은 수의 COI 서열 데이터베이스가 GenBank 에 기탁되었다. 그러나, 몇몇 ITS 서열 데이터베이스가 기탁되었다.

이러한 이유로 우리는 인도네시아 자바 해역의 산호초에서 수집 한 신원 확인 지표로 COI 영역을 생성했습니다. COI 서열의 길이는 588-682 bp 이며, ITS 서열은 583-1123 bp 이다. 그것의 순서는 120 종, 78 주, 30 가족 다섯 주문을 대표하고, 우리는 87 개의 새로운 ITS 데이터베이스 시퀀스와 기존의 DNA 바코드 참조를 풍부하게하는 5 개의 시퀀스를 가지고 있습니다. ITS 서열은 2 개의 보존 유전자 (12s rRNA 및 16s rRNA)를 포함하고 있으며,

tRNAval은 표적 유전자를 증폭시키기에 충분한 초 가변 뉴클레오타이드를 보여 주며, 밀접한 관련성이있는 동종 어류를 식별하기에 충분한 뉴클레오타이드 변이를 함유한다.

또한 Chordata의 1,846 염기 서열을 3484 bp의 ITS 서열을 표적으로하고 물고기 미토콘드리아 12S rRNA 유전자 서열의 MiFish 1 차 표적 단편 (163 ~ 185 bp 길이)과 비교하여 eDNA 샘플에서 물고기의 다양성. 결과적으로 1 차 MiFish는 기본 화음 (84,230 판독 및 115 OTU)과 비교하여 더 높은 판독 및 OTU 수 (105,860 판독 및 146 OTU)를 획득했습니다. 데이터베이스의 MiFish 주요 영역을 다루는 서열 수가 더 많기 때문에 합계 91.7 %의 병합 읽기 (130 OTUs)가 어종으로 묘사되었습니다. 대조적으로, 58.3 % 병합 읽기 (74 OTUs)는 물고기로 기술되었으며 39.7 % 합병 된 읽기 (37 OTU)는 기본 코드에 의해 알려지지 않은 물고기 종으로 확인되었습니다. MiFish 프라이머에 의한 어종의 분류 학적 구성은 하나의 서브 클래스, 8 개의 주문, 31 개의 계열 및 60 개의 장으로 구성된 130 종을 나타냈다. Chord Primary는 두 가지 하위 클래스, 9 개의 주문, 31 개의 가족과 60 개의 속을 포함하는 74 종을 대상으로했습니다. MiFish 프라이머 세트와 달리, Elasmobranchii의 3 종은 주로 Chord set에 의해 독점적으로 검출되었습니다. MiFish 프라이머 세트의 짧은 서열은보다 긴 프라이머 코드 서열보다 더 높은 종 다양성을 보였다. 그러나 두 가지 주요 세트는 이전의 연구보다 더 높은 다양성으로 생산되어 Thousand Islands의 숨겨진 물고기 다양성을 드러 냈습니다. 결론적으로, 우리는 eDNA 메타 코딩을 위해 설정된보다 긴 주 코드 줄을 개발했습니다. ITS 서열 데이터베이스에는 데이터가 부족하지만,이 프라이머 세트는 더 높은 해상도를 나타내고, MiFish 프라이머와 비교하여 Elasmobranchii 군을 검출 할 수있게한다. 잠재적으로 산호초 어류 생물 다양성 조사를위한 대체 어류, 비 침습적 방법을 분석하는 도구로 사용됩니다.

키워드: DNA 바코드, 환경 DNA, Thousand Island, 처리량 시퀀싱, DNA 대사 작용, 환경 DNA, 사우 전드 아일랜드, Hightroughput 시퀀싱.

## Chapter I. Introduction

Coral reefs are one of the most productive and vulnerable ecosystems (Roberts et al., 2002, Paulay et al., 2002). The size of the coral reef is relatively small than other ecosystems (approximately 250,000 km<sup>2</sup>), which collectively covers only 0.2 % of ocean surface in the world (Reaka-Kudla, 1997). As reported, it is a home for more than 25 % of marine species among which there are nearly 4,000 species of marine fishes (Paulay, 1997). The coral reef ecosystems are currently being damaged globally by various factors that include climatic change and other anthropogenic activities (Bellwood et al., 2004, Wilkinson and Souter, 2008), destructive fishing practices (Asoh et al., 2004, McManus et al., 1997) and increased sedimentation (Fabricius et al., 2003). Approximately, 75% of the total world coral reefs are now diagnosed as threatened (Burke et al., 2012), which also causes the loss of its associated fish species (Feary et al., 2007). Several studies have reported a change in the composition of the coral biodiversity (Schrandt and Lema, 2011, Pratchett et al., 2011b, Pratchett et al., 2011a, Musembi and Cowburn, 2014). The Indonesian archipelago, the largest coral reefs area in south-east Asia, is among the most threatened as the damaged area has increased up to 10 % between 1994 and 2004 (Tun and Chou, 2004). As reported in 2008, the area of coral reefs with good condition accounted for only 3 % (Tun et al., 2008). That has impact on the abundance and diversity of coral reef fishes significantly (Moore, 2006, Ndobe et al., 2012).

Biodiversity typically measures the variation in the genetics and life forms of populations, species, communities, and ecosystems (Hiddink et al., 2008, Paulay, 1997). It is recognized to be of global importance and has become an increasing concern in the management of marine ecosystem (Nevill, 2008, Hiddink et al., 2008) and thought to be important parameters for ecosystem function (Musembi and Cowburn, 2014). Since the marine species and their habitats are continuously exposed to the increased pressure, drives a public and corporate responsibility for its conservation biodiversity and managing its development in a sustainable way (Kok et al., 2014). For this reason, surveying and monitoring become important (Cairns and Pratt, 1993, Aronson et al., 1994).

Field survey is an initial step in the study of ecology and provides basic information for environmental management. Different variety of survey methods have been used to estimate abundance and diversity of species influencing both accuracy and precision of the data (Baker et al., 2016). Traditionally, a scientist focuses on the species level biodiversity by studying fishes captured in routine fisheries survey (Hiddink et al., 2008). It employs a fishery-independent and fishery-dependent approach to collect fish catch based data. The collected samples of fish are subsequently analysed morphologically for identification. This morphological methods are widely used for fisheries biodiversity and fisheries assessment (Fischer, 2014) since a large amount of fish morphological database is available (Keat-Chuan Ng et al., 2017). The coral

reef fish associated fisheries present special difficulties in the conventional approach of assessment and management (Houk et al., 2012) and tools were used during survey potentially harmed the coral reefs. Underwater Visual Census (UVC) is based on fishery independent survey. It is extensively used to quantify reef fish assemblages (Samoilys and Carlos, 2000, Caldwell et al., 2016). This method used to monitor reef fish communities are based on the data collected from surveys diving or deploying recording equipments in the environment. fishes were determined visually throughout morphological features (body shape, skin colour and pattern, marking and other physical features) by comprehensive photograph (Allen, 1991, Allen and Robertson, 1994). It provides a rapid estimate of relative abundance, biomass and length frequency distribution of reef fishes. However, UVC method is difficult to measure entire fish community (Jind, 2012), larger species may leave survey area and tendency to attract larger predator fishes, and unable to identify indistinct species (Prato et al., 2017). Further, advance knowledge for fish morphological identification are required to minimize taxonomic errors (Keat-Chuan Ng et al., 2017). Consequently, *in situ* training to identify target species by morphological methods is necessary for new observers (Halford and Thompson, 1994). More expensive tools such as sonar transect and underwater video technique were available to enhance data resolution (Patterson et al., 2007, Cappo et al., 2003). However, according to several study the morphological based identification potentially underestimate the data result

because of the presence of cryptic fishes, wide area of study, and poor visibility under water (Caldwell et al., 2016, Jind, 2012, Samoilyls and Carlos, 2000).

Recently, the molecular study is an advanced methods that successfully reveals limitation of previous methods. Species-level taxonomic resolution is important for defining the representative unit of biodiversity (Cook et al., 2008). The failure to recognize species can have a serious negative impact on biodiversity conservation and fisheries management (Piggott et al., 2011, Bickford et al., 2007). The cryptic species are the worst case of taxonomic incompleteness and a challenge for the biologists (Delić et al., 2017, Bickford et al., 2007). In addition to the morphological study, several molecular studies have successfully uncovered cryptic species throughout mitochondrial genome identification (Sektiana et al., 2017, Thomas Jr et al., 2014, Iannelli et al., 2007, Cai et al., 2013). Molecular technique includes Polymerase Chain Reaction (PCR) which can amplify a small amount of sample containing DNA fragment (with sufficient information to allow identification) in conjunction with certain DNA marker provide fast and reliable data information within a short period of time. It have now made possible to identify any species of any kind of organic substrate without undermining the ecosystem (Teletchea, 2009). Although in initial decade these method was expensive (Anderson et al., 1981), after the Next Generation Sequencing (NGS) technology has been introduced, the cost ratio are reduced along with high speed and high capacity analysis technology in large scale genome analysis project (Margulies et al., 2005).

One of the most widely used molecular marker is the DNA region of the mitochondrial DNA (mtDNA), is located in the mitochondrial matrix. The mtDNA is transmitted maternally and typically has a high mutation rate (Montooth and Rand, 2008, Taanman, 1999, Wallace and Chalkia, 2013). The accelerated evolutionary rate of mtDNA is useful for identification of species by the significant amount of sequence variation in its genome (Yang et al., 2014). In the vertebrates, the mtDNA is a highly conserved circular molecule (approximately 16 kb), which encodes 37 genes including 2 rRNAs, 22 tRNAs, and 13 protein (Ingman and Gyllensten, 2006b, Pesole et al., 2012). The most commonly used region is COI region, which is the partial sequence of Cytochrome C oxidase I (COX1) gene (approximately 670 bp in length). COI represents an informative source of phylogenetic data and species differentiation through DNA barcoding (Ladoukakis and Zouros, 2017, Hebert et al., 2003a, Arif et al., 2011, Yacoub et al., 2013).

DNA barcoding is a tool for rapid identification of species based on its DNA sequence (Kress and Erickson, 2008, Hebert et al., 2003a). It consist of standardized short sequences of DNA that easily generate and characterize species (Savolainen et al., 2005). Several mtDNA fragments were used for DNA barcoding such as nuclear ribosomal RNA (rRNAs), and protein coding genes (Cytochrome b, Cytochrome oxidase 1) (Zhang and Hanner, 2012, Kochzius et al., 2010). Since Cytochrome oxidase 1 (COI) is faster in evolution rate, its ability to amplify from the various animal groups is suitable for species-level

detection and attributed to a standard barcoding (Hebert et al., 2003b, Hebert et al., 2003a). However, it is less effective for impure, mixture, degraded and short fragmented DNA (Hebert et al., 2003a, Savolainen et al., 2005). Hereafter rRNA gene which is more conserved (Patwardhan et al., 2014) is potentially effective for DNA metabarcoding.

DNA metabarcoding is a rapid method of biodiversity assessment that combines two technologies like DNA barcoding and highthroughput DNA sequencing. It enables more direct evaluation of biodiversity by screening many species simultaneously as compared to screening one specimen at a time. This method contribute in the field of ecology that enables to characterize species composition in bulk diversity sample or environmental DNA (eDNA).

Since 1980, eDNA is described as particulate DNA, a genetic material detached by the species in a nonliving component of the environment (soil, sediment, or water) (Díaz-Ferguson and Moyer, 2014). It was first analyzed in sediment disclosing DNA from extinct and extant animals (Thomsen and Willerslev, 2015), and was focused primarily to detect a single species (Barnes and Turner, 2016, Deiner et al., 2017), specifically invasive (Adrian-Kalchhauser and Burkhardt-Holm, 2016, Scriver et al., 2015), rare and endangered species (Klymus et al., 2017, Jerde et al., 2011), more recently eDNA has started to provide new tools to assess biodiversity in marine ecosystems (Thomsen and Willerslev, 2015, Deiner et al., 2017).

In this study, a survey method is developed through eDNA metabarcoding for marine ornamental fish biodiversity. Since we attributed rRNA fragment for eDNA metabarcoding, partial 12S rRNA, tRNA<sup>val</sup> and partial 16s rRNA database of marine ornamental fish DNA barcoding are added. Additionally eDNA metabarcoding is applied to understand the fish biodiversity in Thousand Island National Park, Indonesia.



## **Chapter II. Fish diversity in Thousand Island and Tabuhan Island**

### **2.1 Introduction**

To understand the condition of marine ecosystem and for the sustainable use of its resources it is important to understand fish diversity. It comprises of genetic, taxonomic, phylogenetic and ecological components (Hiddink et al., 2008). The rise of threat of species extinction through ecosystem degradation, global change and pressure of human population has escalated these concerns (Chiarucci et al., 2011). Indonesia is an archipelagic country and rich in biodiversity with coral reefs reaching 75,000 km<sup>2</sup> (Hutomo and Moosa, 2005). Currently 2,057 reef fish species have been reported, which are spread over four endemic hotspots and 8 local hotspots including the Java Sea (Allen and Adrim, 2003).

The Java sea has a group of islands spread throughout the west and east. It has coral reef ecosystem that contain diverse fish which provides goods and service to ecosystem such as fisheries products like pelagic and ornamental fish (Durand and Petit, 1995) and tourism (Hutomo and Moosa, 2005, Wilkinson et al., 1995). A shallow water is located between Kalimantan, Java, Sumatra, and Sulawesi, within an area of 310,000 km<sup>2</sup>. The Java Sea contribute 31% of the

national marine fisheries production. Increase in fish consumption and rise in human population has resulted in increase in the demand for fishes thus stimulating the development of fishing in these area (Purwanto, 2003). However, the biodiversity in the Indonesian coral reef is facing a threat with global climatic changes, antropogenic activities, fisheries and sedimentation (Edinger et al., 1998). Furthermore, the Java Sea has experienced a large impact from these activities (Purwanto, 2003).

Thousand Island is located in the northwestern of Java sea in the north of Jakarta province, the capital city of Indonesia. They were exploited since 1960 (Idris et al., 2013) and designated as a protected area (strict nature reserve and National Park) in 1982 (Uneputty and Evans, 1997) . Its biodiversity was an important point of concern for researchers and policy makers (Alder et al., 1994). However, long term continuous research were rarely conducted (Setyawan and Yusri, 2009). In contrast, Tabuhan Island which is located in Banyuwangi Distric of East Java is a small island of interest for tourism activities, water sports and ornamental fish related fisheries (Damayanti, 2012) and has no previous study in fish biodiversity.

For the conservation of the biodiversity, information is needed about each specimen with data ranging from systematic position to molecular aspect. 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 problem of misidentification in conservation and management purposes so species determination becomes important.

We have summarized the information collected from the reseach on fish diversity and the purpose of this study is to describe the current status of fish biodiversity in the Thousand Island and Tabuhan Island.

## **2.2 Research on Fish biodiversity**

Research on fish biodiversity in the Thousand islands has been carried out since 1979 with fish community and structure as the main subjects (Hutomo and Djamali, 1979). Short-term research has been conducted to collect brief information in certain areas of the Thousand Islands (Hutomo and Djamali, 1984, Iskandar and Mawardi, 1997, Mujiyanto et al., 2009, Riani and Affandi, 2016). However, it is not enough for long-term studies (Setyawan and Yusri, 2009, Setiawan et al., 2011). Contrarily, no research has been executed on the fish biodiversity in Tabuhan Island. However, there are several studies performed in Bangsring, Banyuwangi, a coastal area adjacent to the Tabuhan island (Kamaali et al., 2017, Asadi and Andrimida, 2017, Anggraini et al., 2017).

Data about the abundance and diversity of species were obtained by several methods. Furthermore, it is done extensively by underwater visual censuses

(UVC) as the fisheries independent survey method (English et al., 1997, Caldwell et al., 2016). This method was based on surveys diving with transect line method. It was applied as reef ecosystem are characteristicly fragile and present special difficulties (Houk et al., 2012).

According to UVC method, fishes are identified visually to the level of species taxonomy *in situ* based on its morphological characteristics (Halford and Thompson, 1994). In addition, a few research employed certain fishing gear to collect the target fish from non-reef ecosystem for different research purpose. Further, a more detailed process of species identification was carried out in the laboratory (Hutomo and Djamali, 1984, Riani and Affandi, 2016).

Since 'species' is an important taxonomic category of an organism and play a particular ecological role, so proper species determination is important for the study of biodiversity (NRC, 1992). Hereafter, morphological based identification is widely accepted as a standard for the species identification protocol (Patrick et al., 2014, Wilson and Green, 2009). Recently, a molecular based species identification was conducted for the study of biodiversity (Zhang and Hanner, 2012, Barman et al., 2018a).

Cryptic species is a major problem in morphological based identification (Piggott et al., 2011), species identification errors often occur in a study. The use of molecular approaches has managed to overcome this problem even if the fish is at the larvae stage (Rezaghlinejad et al., 2016, Hubert et al., 2012). In

addition, rare and invasive species in an aquatic system were described successfully in molecular base surveillance and monitoring through eDNA detection (Mahon and Jerde, 2016, Nevers et al., 2018). Further, molecular-based study in Thousand Island and Jakarta Bay remains low. Only limited number of studies employed the molecular-based detection methods to identify the marine fish larvae (Wibowo et al., 2018), sea cucumber (Madduppa et al., 2017) and sponges (Patantis et al., 2013).

### **2.3 Fish diversity in Thousand Island and Tabuhan Island**

In this study, we summarized the previous fish biodiversity studies in Thousand Islands (Pulau Seribu). From 1984 to 2018 several surveys targeting the specific fish taxa were conducted. Numbers of identified species varied from six to 216 species (Table 2.1). According to Madduppa et al. (2013) a total 216 fish species (29 families) were identified, which is the highest numbers in Thousand Islands among the currently known reports. They also found that species richness, diversity and abundance gradually increased from the coastal area to offshore water (Madduppa et al., 2013).

We counted the total numbers of fish species from 16 different studies (Table 2.1). From our counting, total 442 species were identified which covers 154 genera and 56 families (Table 2.1). According to World Register of Marine Species (WoRMs), 64 species names were changed (Table 2.2) and 40 unclear

nomenclatures were excluded from further analysis. Among family, Labridae and Pomacentridae were dominant in number followed by Apogonidae, Pomacanthidae, Scaridae, Serranidae Nemipteridae, Chaetodontidae, Ephippidae, Caesionidae, Haemullidae, Lutjanidae, and Siganidae (Figure 2.1). According to the IUCN red list database (<http://www.iucnredlist.org>), ten species were listed as Data Deficient (DD), 219 were listed as the Least Concern (LC) and 192 as Not Evaluated (NE). Seven species were listed as near threatened (NT) and four species were Vulnerable (Vul). Seven NT species were observed in the Elasmobranchii including *Carcharhinus leucas* (Carcharhinidae) and *Taeniura lymma* (Dasyatidae) and Actinopttherigii ie; *Scarus hypselopterus* (Labridae) *Epinephelus bleekeri* (Serranidae) *Epinephelus malabaricus* (Serranidae) *Plectropomus leopardus* (Serranidae) *Plectropomus leopardus* (Serranidae) and four vulnerable species were Elasmobranchii ie; *Glaucostegus granulatus* (Glaucostegidae) and Actinopttherigii ie ; *Amblyglyphidodon batunai* (Pomacentridae), *Bolbometopon muricatum* (Scaridae) *Cromileptes altivelis* (Serranidae).

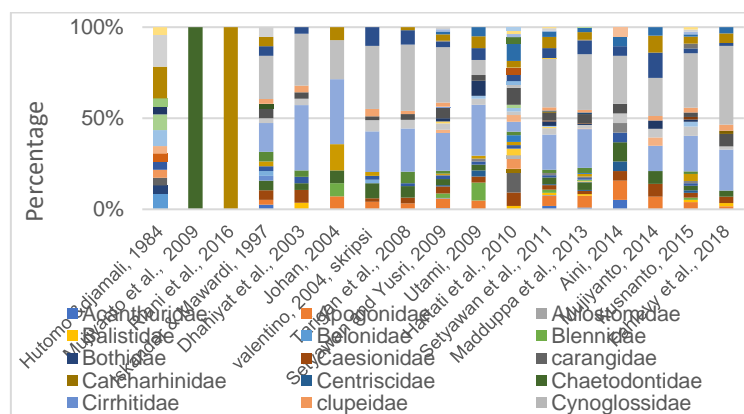


Figure 2.1 Composition of fish family were observed in Thousand Island

Compared with Thousand islands much fewer studies have been conducted about the fish surveys at Tabuhan Island, Banyuwangi. Total 34 species (14 families) were currently identified (Table 2.3) and the near threatened reef fish species from the family Chaetodontidae, *Chaetodon trifascialis* were discovered. (Anggraini et al., 2017).

## **2.4 Threat to Fish diversity in Thousand Island**

Thousand Islands are located in the front of Jakarta Bay and support local fisheries (Idris et al., 2013), aquaculture (Meina, 2013, Hudaya and Masri, 2015) and tourism industry (Lusianawati and Masful, 2015). Coral reefs in Thousand Island have been severely damaged in the last decades resulting in reduction of the biodiversity (Giyanto et al., 2017, Baum et al., 2015). Among them, Onrust Island, which is the closest island to the main land, experienced the highest degradation of the coral reef ecosystem and reduction of the fish diversity (Setyawan and Yusri, 2009).

Reef ecosystem degradation has a direct impact on coastal communities that depend on reef resources for their livelihood (Lagbas and Habito, 2016). The challenge of ecosystem conservation and management plans are connecting local habitats and the conflicting demand of various stakeholders on the resources (Sale et al., 2014). In general, there are several threats in the diversity of fish on the Thousand islands, in addition to the global threats such as climatic

change, sedimentation and anthropogenic activities (Hoegh-Guldberg et al., 2017). The Jakarta Bay, which is the estuary of three major rivers is influenced by sedimentation (Farhan and Lim, 2012). As the third largest metropolitan city in the world, Jakarta contributes to the local anthropogenic activities (van der Meij and Hoeksema, 2010). Both sedimentation and local anthropogenic activities have an impact on the change in the coral reef ecosystems and diversity (Adrianto et al., 2016). Further, destructive fishing activity such as poison fishing, blast fishing, coral mining and overfishing lead to coral reef deterioration and exhibited coral reef status as bad to medium condition (Arifin, 2004). It reflects a decline in the reefs ecosystem services (Heery et al., 2018).

Protection of marine biota in the future must be guaranteed with sufficient effort to conserve and manage the ecosystem. Further, basic data on the ecosystem and habitat including the fauna is necessary. Therefore, molecular based research on biodiversity has become an alternative to accelerate information of diversity.

Table 2. 1 List of fish species collected from Thousand Islands between 1984 and 2018

No	Family	Species	IUCN redlist	Reference
1	Acanthuridae	<i>Acanthurus albipectoralis</i>	LC	12
2		<i>A. blochii</i>	LC	12
3		<i>A. tristis</i>	LC	12
4		<i>A. triostegus</i>	LC	13
5		<i>Ctenochaetus striatus</i>	LC	8
6		<i>Zebrasoma scopas</i>	LC	16
7	Apogonidae	<i>Apogon semiornatus</i>	NE	13
8		<i>A. victoriae</i>	NE	5;14
9		<i>Archamia zosterophora</i>	NE	8;12
10		<i>A. fucata</i>	NE	13
11		<i>Cheilodipterus artus</i>	NE	8; 12; 13
12		<i>C. isostigmus</i>	NE	13
13		<i>C. quinquelineatus</i>	NE	8; 12; 15
14		<i>Nectamia bandanensis</i>	NE	12
15		<i>Ostorhinchus aureus</i>	NE	8; 13
16		<i>O. chrysotaenia</i>	NE	8
17		<i>O. fleurieu</i>	LC	8
18		<i>O. angustatus</i>	NE	13
19		<i>O. apogonoides</i>	NE	13
20		<i>O. cavitensis</i>	NE	13
21		<i>O. chrysopomus</i>	NE	6; 9; 12; 13
22		<i>O. compressus</i>	LC	2; 5; 6; 8; 9; 12; 13; 15; 16
23		<i>O. cookie</i>	NE	9
24		<i>O. cyanosoma</i>	NE	4
25		<i>O. novemfasciatus</i>	NE	13
26		<i>O. sealei</i>	NE	13; 16
27		<i>O. multilineatus</i>	NE	12
28		<i>Pristiapogon taeniopterus</i>	NE	14
29		<i>P. trimaculatus</i>	NE	14
30		<i>Zoramia fragilis</i>	NE	16
31	Aulostomidae	<i>Pristicon rhodopterus</i>	NE	15
32		<i>Sphaeramia nematoptera</i>	NE	8; 12; 13
33		<i>Aulostomus strigosus</i>	LC	12; 13
34	Balistidae	<i>Balistoides viridescens</i>	NE	10; 12
35		<i>Melichthys indicus</i>	NE	13
36		<i>Odonus niger</i>	NE	15
37		<i>Rhinecanthus acueatus</i>	NE	3

Table 2.1 Continued

No	Family	Species	IUCN redlist	Reference
38	Balistidae	<i>R. verrucosus</i>	NE	16
39		<i>Sufflamen fraenatum</i>	LC	12
40	Belonidae	<i>Tylosurus punctulatus</i>	NE	1
41	Bleniidae	<i>Cirripectes spingeri</i>	LC	9
42		<i>C. variolosus</i>	LC	9
43		<i>Ecsenius bathi</i>	LC	9
44		<i>Meiacanthus atrodorsalis</i>	LC	9
45		<i>Pseudobalistes flavimarginatus</i>	NE	9
46		<i>Aspidontus taeniatus</i>	LC	8; 9
47		<i>Atrosalarias fuscus</i>	LC	8
48		<i>Ecsenius cf bandanus</i>	LC	12
49		<i>Meiacanthus smithi</i>	LC	8; 12
50		<i>Petroscirtes springeri</i>	LC	4
51		<i>Salarias ceramensis</i>	LC	15
52		<i>S. fasciatus</i>	LC	8
53	Bothidae	<i>Bothus pantherinus</i>	LC	1
54	Caesionidae	<i>Caesio cuning</i>	LC	8; 9; 10; 12;
55		<i>C. lunaris</i>	LC	3; 14
56		<i>C. teres</i>	LC	6; 8; 9; 12; 13; 15; 16
57		<i>C. caeruleaurea</i>	LC	10
58		<i>Pterocaesio chrysozona</i>	LC	8; 10; 12; 13
59		<i>P. digramma</i>	LC	8
60		<i>P. tile</i>	LC	8; 10; 13
61		<i>P. pisang</i>	LC	8
62		<i>P. tessellata</i>	LC	8
63		<i>P. trilineata</i>	LC	12
		<i>Carangiodes</i>		
64	Carangidae	<i>coeruleopinnatus</i>	LC	10
65		<i>Caranx sexfasciatus</i>	LC	1
66		<i>C. tille</i>	LC	10
67		<i>Gnathanodon speciosus</i>	LC	10
68		<i>Selar crumenophthalmu</i>	LC	10
69		<i>selaroides leptolepis</i>	LC	10
70	Carcharhinidae	<i>Carcharhinus leucas</i>	NT	10
71	Centriscidae	<i>Aeoliscus strigatus</i>	DD	8; 9; 12; 13; 15 ; 16
72		<i>Centriscus scutatus</i>	LC	9
73	Chaetodontidae	<i>Chaetodon collare</i>	LC	13

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
74	Chaetodontidae	<i>C.kleinii</i>	LC	7; 14
75		<i>C. lunulatus</i>	LC	6
76		<i>C. melannotus</i>	LC	5; 13
77		<i>C. meyeri</i>	LC	13
78		<i>C. octofasciatus</i>	LC	4; 5; 6; 8; 9; 12; 13; 14; 15; 16; 16
79		<i>C. ornatissimus</i>	LC	14
80		<i>C. oxycephalus</i>	LC	7
81		<i>C. rafflesi</i> Anonymous	LC	8
82		<i>C. speculum</i>	LC	8
83		<i>Chelmon muelleri</i>	LC	2; 6
84		<i>C. rostratus</i>	LC	5; 8; 9; 12; 13; 15; 16
85		<i>Coradion chrysozonus</i>	LC	8; 12
96		<i>C. melanopus</i>	LC	7
87		<i>Heniochus acuminatus</i>	LC	12
88		<i>H. chrysostomus</i>	LC	6; 13
89		<i>H. diphreutes</i>	LC	7
90		<i>H. monoceros</i>	LC	7; 8
91		<i>H. pleurotaenia</i>	LC	8; 13
92		<i>H. varius</i>	LC	8; 13
93		<i>Parachaetodon ocellatus</i>	LC	8; 16
94	Cirrhitidae	<i>Cirrhitichthys falco</i>	LC	2
95	Clupeidae	<i>Amblygaster sirm</i>	LC	10
96		<i>Sardinella fimbriata</i>	NE	10
97	Clupeidae	<i>S. lemuru</i>	NE	10
98		<i>Spratelloides delicatulus</i>	LC	1
99	Cynoglossidae	<i>Cynoglossus arel</i>	NE	10
100	Dasyatidae	<i>Taeniura lymma</i>	NT	10; 13
101	Diodontidae	<i>Diodon hystrix</i>	LC	2; 5
102	Ephippidae	<i>Platax orbicularis</i>	NE	1; 8
103		<i>P. pinnatus</i>	NE	2; 5; 8; 13; 16
104		<i>P. teira</i>	NE	3; 6; 8; 9; 10; 12; 13; 15
105	Glaucostegidae	<i>Glaucostegus granulatus</i>	vul	1; 3
106	Gobidae	<i>Istigobius ornatus</i>	LC	16
107		<i>Amblyeleotris periphthalma</i>	NE	9
108		<i>Cryptocentrus cinctus</i>	NE	12
109		<i>C. leptcephalus</i>	NE	8
110		<i>Exyrias belissimus</i>	LC	12; 13

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
111	Gobidae	<i>Istigobius decorates</i>	NE	13; 15
112		<i>Valenciennesa strigata</i>	NE	12
113	Haemulidae	<i>diagramma punctatum</i>	NE	10
114		<i>Plectorhinchus chaetodonoides</i>	NE	8; 13
115		<i>P. chrysotaenia</i>	NE	13
116		<i>P. gaterinoides</i>	NE	5
117		<i>P. lineatus</i>	NE	15
118		<i>P. orientalis</i>	NE	4; 15
119		<i>P. picus</i>	NE	9
120		<i>P. vittatus</i>	NE	13
121		<i>P. chaetodonoides</i>	NE	4
122	Hemiramphidae	<i>Hemiramphus far</i>	NE	10; 13
123		<i>H. brasiliensi</i>	NE	10
124		<i>Hyporhamphus quoyi</i>	NE	1
125	Holocentridae	<i>Myripristis berndti</i>	LC	13
126		<i>M. botche</i>	LC	8
127		<i>M. hexagona</i>	LC	12
128	Holocentridae	<i>M. murdjan</i>	LC	6; 8; 12
129		<i>M. violacea</i>	LC	2
130		<i>M. vitata</i>	NE	6
131		<i>Sargocentron cornutum</i>	LC	15
132		<i>S. diadema</i>	LC	2; 8; 10; 13
133		<i>S. punctatissimum</i>	LC	15
134		<i>S. rubrum</i>	LC	6; 12; 13
135		<i>S. tiereoides</i>	LC	13
136	Labridae	<i>Anampses lineatus</i>	DD	8
137		<i>A. meleagrides</i>	LC	12
138		<i>Bodianus mesothorax</i>	LC	3; 8; 13; 14; 15; 16
139		<i>Cheilinus chlorourus</i>	LC	8; 12; 13; 16
140		<i>C. fasciatus</i>	LC	5; 6; 8; 9; 12; 13; 14; 15; 16
141		<i>C. trilobatus</i>	LC	8; 10; 13; 14
142		<i>Oxycheilinus arenatus</i>	LC	12
143		<i>O. bimaculatus</i>	LC	12
144		<i>O. celebicus</i>	LC	8
145		<i>O. diagramma</i>	LC	8; 12; 13
146		<i>O. oxyrhynchus</i>	LC	8; 12; 13

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
147	Labridae	<i>O. rhodochrous</i>	LC	12
148		<i>O. unifasciatus</i>	LC	13
149		<i>Choerodon anchorago</i>	LC	6; 8; 9; 10; 12; 13 15; 16
150		<i>C. fasciatus</i>	LC	13
151		<i>Cirrhilabrus cyanopleura</i>	DD	5; 8; 13; 15; 16
152		<i>Coris batuensis</i>	LC	8; 12
153		<i>Diproctacanthus xanthurus</i>	LC	4; 5; 6; 8; 9; 12; 13; 15
154		<i>Epibulus insidiator</i>	LC	8; 10; 12; 13
155		<i>Gomphosus caeruleus</i>	LC	12
156		<i>G. varius</i>	LC	12; 13
157		<i>Halichoeres argus</i>	LC	12
158		<i>H. binotopsis</i>	LC	8; 13
159		<i>H. biocellatus</i>	LC	13; 15
160		<i>H. chlorocephalus</i>	LC	9
161		<i>H. choropecterus</i>	LC	4; 5; 8; 9; 12; 13; 15; 16
162		<i>H. melanurus</i>	LC	8; 9; 12; 13; 15,5,6,16
163		<i>H. chrysus</i>	LC	12
164		<i>H. nigrescens</i>	LC	5; 13
165		<i>H. hortulanus</i>	LC	3; 4; 8; 12; 13; 14; 15; 16
166		<i>H. marginatus</i>	LC	13
167		<i>H. melanochir</i>	LC	13
168		<i>H. melasmapomus</i>	LC	5
169		<i>H. ornatissimus</i>	LC	8; 13; 14
170		<i>H. leucurus</i>	LC	3; 8; 12
171		<i>H. richmondi</i>	LC	8; 9; 12; 13; 15; 16
172		<i>H. scapularis</i>	LC	8; 12; 13
173		<i>H. papilionaceus</i>	LC	5
174		<i>H. vrolikii</i>	LC	3; 5; 8; 9; 13; 16
175		<i>Hemigymnus fasciatus</i>	LC	8; 12
176		<i>H. melapterus</i>	LC	5; 8; 9; 12; 13; 15
177		<i>Iniistius pentadactylus</i>	LC	9
178		<i>Labrichthys unilineatus</i>	LC	12
179		<i>Labroides bicolor</i>	LC	6
180		<i>L. dimidiatus</i>	LC	2; 3; 4; 5; 6; 8; 9; 12; 13; 15; 16
181		<i>L. pectoralis</i>	LC	6
182		<i>Leptojulius cyanopleura</i>	LC	8

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
183	Labridae	<i>L. chrysotaenia</i>		13
184		<i>Macropharyngodon negrosensis</i>	LC	13
185		<i>M. ornatus</i>	LC	12
186		<i>Oxycheilinus arenatus</i>	LC	8
187		<i>O. orientalis</i>	LC	8
188		<i>O. unifasciatus</i>	LC	12
189		<i>Pseudocheilinus hexataenia</i>	LC	13
190		<i>P. cerasinus</i>	DD	13
191		<i>Pteragogus amboinensis</i>	LC	13
192		<i>P. guttatus</i>	LC	12
193		<i>Scarus hypselopterus</i>	NT	12
194		<i>Stethojulis bandanensis</i>	LC	8
195		<i>S. interrupta</i>	LC	9
196		<i>S. trilineata</i>	LC	9; 13
197		<i>Thalassoma amblycephalum</i>	LC	3
198		<i>T. genivittatum</i>	LC	3
199		<i>T. hardwicke</i>	LC	3; 6; 8
200		<i>T. janseni</i>	LC	9
201		<i>T. lunare</i>	LC	2; 4; 5; 6; 8; 9; 12; 13; 15; 16
202		<i>T. lutescens</i>	LC	3; 6; 8; 12; 13
203		<i>T. purpureum</i>	LC	13
204		<i>T. quinquevittatum</i>	LC	13
205	Lethrinidae	<i>Lethrinus erythropterus</i>	LC	13
206		<i>L. harak</i>	LC	8
207		<i>L. lentjan</i>	LC	8; 14
208		<i>L. obsoletus</i>	LC	8; 14
209	Lutjanidae	<i>Lutjanus biguttatus</i>	LC	9; 6; 8; 13; 15; 16
210		<i>L. bohar</i>	LC	12
211		<i>L. carponotatus</i>	NE	5; 6; 12; 14
212		<i>L. decussatus</i>	LC	2; 5; 8; 9; 10; 12; 13; 14; 15
213		<i>L. fulviflamma</i>	LC	6; 8; 15
214	Lutjanidae	<i>L. fulvus</i>	LC	15
215		<i>L. monostigma</i>	LC	16
216		<i>L. nifolineatus</i>	LC	8
217		<i>L. quinquelineatus</i>	LC	8
218		<i>L. kasmira</i>	LC	3; 12; 13
219		<i>L. russellii</i>	LC	13
220		<i>L. sebae</i>	LC	5
221	Microdesmidae	<i>Ptereleotris evides</i>	LC	8; 12

Table 2.1 Continued

No	Family	Species	IUCN redlist	Reference
222	Microdesmidae	<i>P. microlepis</i>	NE	8
223	Monacanthidae	<i>Acreichthys tomentosus</i>	LC	9; 12; 15
224		<i>Monacanthus chinensis</i>	LC	1; 12
225		<i>Pseudomonacanthus macrurus</i>	NE	15
226		<i>Rudarius minutus</i>	LC	10
227	Mugillidae	<i>Ellochelon vaigiensis</i>	LC	10
228		<i>Parupeneus barberinus</i>	LC	8; 12; 13; 14
229		<i>P. bifasciatus</i>	NE	9; 14
230		<i>P. macronema</i>	NE	8; 9; 12
231		<i>P. multifasciatus</i>	LC	9
232		<i>Parupeneus pleurostigma</i>	LC	9
233		<i>P. trifasciatus</i>	NE	12
234		<i>Upeneus tragula</i>	LC	8; 9; 12
235	Muraenidae	<i>Gymnothorax javanicus</i>	NE	15
236	Nemipetridae	<i>Pentapodus bifasciatus</i>	NE	8; 9
237		<i>P. caninus</i>	LC	1; 8; 13
238		<i>P. porosus</i>	NE	9
239		<i>P. trivittatus</i>	LC	8; 12
240		<i>P. setosus</i>	NE	12
241		<i>P. vitta</i>	LC	13
242		<i>P. emeryii</i>	LC	10; 12
243		<i>Scolopsis ciliate</i>	LC	8; 13
244		<i>S. margaritifera</i>	NE	8; 12; 13; 15; 16
245		<i>S. bilineata</i>	LC	2; 3; 8; 15; 16; 5; 6; 13
246		<i>S. lineata</i>	LC	2; 8; 12; 16 6; 13
247		<i>S. monogramma</i>	LC	15
248		<i>S. temporalis</i>	LC	13
249		<i>S. trilineata</i>	LC	12; 16; 1; 10; 13
250	Ostraciidae	<i>Ostracion cubicus</i>	NE	12; 16
251	Pempheridae	<i>Pempheris adusta</i>	NE	12
252		<i>P. oualensis</i>	NE	13
253	Plesiopidae	<i>Callopleysiops altivelis</i>	NE	8
254	Pomacanthidae	<i>Centropyge eibli</i>	LC	8; 14; 16
255		<i>C. flavissima</i>	LC	8
256		<i>C. vrolikii</i>	LC	13; 16
257		<i>Chatodontoplus mesoleucus</i>	LC	2; 3; 5; 6; 8; 12; 13; 14

Table 2.1 Continued

No	Family	Species	IUCN redlist	Reference
258	Pomacanthidae	<i>Genicanthus lamarck</i>	LC	3
259		<i>Pomacanthus imperator</i>	LC	15
260		<i>P. sexstriatus</i>	LC	5; 8; 12; 13
261		<i>Pygoplites diacanthus</i>	LC	12
262		<i>Cheiloprion labiatus</i>	NE	13
263	Pomacentridae	<i>Abudefdu saxatilis</i>	LC	3
264		<i>A. bengalensis</i>	LC	5; 8; 12; 13; 16;
265		<i>A. lorenzi</i>	LC	5; 8
266		<i>A. septemfasciatus</i>	LC	6; 13; 15; 14
267		<i>A. sexfasciatus</i>	LC	4; 5; 6; 12; 13; 15; 16
268		<i>A. sordidus</i>	LC	13
269		<i>Pomacanthus sexstriatus</i>	LC	8
270	Pomacentridae	<i>A. vaigiensis</i>	LC	2; 5; 6; 8; 12; 13; 14; 15; 16
271		<i>A. whitleyi</i>	LC	3
272		<i>Acanthochromis polyacanthus</i>	LC	13
273		<i>Amblyglyphidodon aureus</i>	LC	6; 12; 13; 16
274		<i>A. batunai</i>	vul	12; 13; 15
275		<i>A. curacao</i>	LC	13; 14; 15; 16; 16
276		<i>A. leucogaster</i>	LC	5; 6; 8; 12; 13; 16
277		<i>Neoglyphidodon nigroris</i>	NN	13
278		<i>Amphiprion akallopisos</i>	LC	6; 8; 12; 13; 15
279		<i>A. akindynos</i>	LC	13
280		<i>A. clarkia</i>	NE	8; 12; 13
281		<i>A. frenatus</i>	LC	5
282		<i>A. ocellaris</i>	NE	2; 8; 12; 13; 16
283		<i>A. perideraion</i>	LC	13
284		<i>A. sandaracinos</i>	LC	13; 15
285		<i>Chromis amboinensis</i>	LC	6; 8; 12; 13; 16
286		<i>C. analis</i>	LC	2; 8; 14
287		<i>C. atripectoralis</i>	NE	13
288		<i>C. s atripes</i>	LC	6
299		<i>C. xanthura</i>	NE	8; 13
300		<i>Chrysiptera biocellata</i>	NE	5
301		<i>C. bleekeri</i>	NE	8
302		<i>C. cyanea</i>	NE	13
303		<i>C. hemicyanea</i>	NE	12; 13
304		<i>C. oxycephala</i>	NE	8; 12
305		<i>C. parasema</i>	NE	8; 12; 13

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
289	Pomacentridae	<i>C.caudalis</i>	LC	3
290		<i>C. dimidiata</i>	LC	16
291		<i>C. flavipectoralis</i>	LC	12
292		<i>C. fumea</i>	LC	2; 8; 13
293		<i>C. lepidolepis</i>	NE	16
294		<i>C. margaritifer</i>	NE	12
295		<i>C. nitida</i>	NE	13
296		<i>C. scotochilopterus</i>	NE	13
297		<i>C. ternatensis</i>	NE	6; 8; 13; 15
298		<i>C. viridis</i>	NE	4; 6; 8; 12; 13; 16
306		<i>C. unimaculata</i>	LC	16
307		<i>Cheiloprion labiatus</i>	NE	13
308		<i>Dascyllus melanurus</i>	NE	13
309		<i>D. reticulatus</i>	NE	2; 13
310		<i>D.s trimaculatus</i>	NE	8; 12; 13; 16
311		<i>Dischistodus chrysopoecilus</i>	NE	12
312		<i>D. melanotus</i>	NE	13
313		<i>D. perspicillatus</i>	NE	8; 13; 14; 15; 16
314		<i>D. prosopotaenia</i>	NE	5; 6; 8; 12; 13 ; 15; 16
315		<i>Hemiglyphidodon plagiometopon</i>	NE	3; 8; 12; 13; 14
316		<i>Lepidozygus tapienosoma</i>	NE	8
316		<i>Neoglyphidodon bonang</i>	NE	6; 13
318		<i>N. crossi</i>	NE	6; 13; 15
319		<i>N. leucogaster</i>	LC	13
320		<i>N. melas</i>	NE	6; 8; 13; 15; 16
321		<i>N. nigroris</i>	NE	13; 15
322		<i>N. oxyodon</i>	NE	8; 15; 16
323		<i>N. thoracotaeniatus</i>	NE	13; 16
324		<i>Neopomacentrus anabatoides</i>	NE	8; 12
325		<i>N. azysron</i>	NE	4; 8; 12
326		<i>N. bankieri</i>	LC	13
327		<i>N. cyanomos</i>	NE	8; 12; 13
328		<i>N. filamentosus</i>	NE	3; 5; 8; 13

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference	
329	Pomacentridae	<i>Plectroglyphidodon lacrymatus</i>	NE	2; 8	
330		<i>Pomacentrus amboinensis</i>	NE	5; 13; 15	
331		<i>P. adelus</i>	NE	8	
332		<i>P. alexanderae</i>	NE	3; 5; 12; 13; 16	
333		<i>P. auriventris</i>	NE	9	
334		<i>P. azuremaculatus</i>	NE	8	
335		<i>P. bankanensis</i>	NE	8	
336		<i>P. brachialis</i>	NE	2; 6; 13; 16	
337		<i>P. burroughi</i>	NE	6; 8; 9; 12; 13; 15; 16	
338		<i>P. chrysurus</i>	NE	12	
339		<i>P. coelestis</i>	NE	8; 12; 13; 15	
340		<i>P. cuneatus</i>	NE	13	
341		<i>P. javanicus</i>	DD	9; 13	
342		<i>P. lepidogenys</i>	NE	6; 12; 15; 16	
343		<i>P. littoralis</i>	NE	12; 13	
344		<i>P. milleri</i>	LC	13	
345		<i>P. moluccensis</i>	NE	3; 5; 6; 8; 9; 12; 13; 14; 16; 16	
346		<i>P. nagasakiensis</i>	NE	8; 16	
347		<i>P. nigromarginatus</i>	NE	3	
348		<i>P. pavo</i>	NE	2	
349		<i>P. philippinus</i>	NE	6; 8; 16	
350		<i>P. polyspinus</i>	NE	6	
351		<i>P. reidi</i>	NE	8	
352		<i>P. saksoni</i>	NE	9; 12	
353		<i>P. simsiang</i>	NE	8; 13; 16	
354		<i>P. smithi</i>	LC	6; 8; 12; 13; 16	
355		<i>P. taeniometopon</i>	NE	8	
356		<i>P. vaiuli</i>	NE	8	
357		<i>P. xanthosternus</i>	NE	13	
358		<i>Premnas biaculeatus</i>	NE	8; 12; 13; 15	
359		<i>Pristotis obtusirostris</i>	NE	13	
360		<i>stegastes apicalis</i>	NE	5	
361		<i>S. leucostictus</i>	LC	8	
362		Psetteodidae	<i>Psettodes erumei</i>	NE	10
363		Pseudochromidae	<i>Congrogadus subducens</i>	NE	12
364	Scaridae	<i>Bulbometopon muricatum</i>	vul	12; 14	
365		<i>Cetoscarus bicolor</i>	LC	8; 12	

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
366	Scaridae	<i>Chlorurus bleekeri</i>	LC	12; 13; 15
367		<i>C. capistratoides</i>	LC	16
368		<i>C. microrhinos</i>	LC	13
369		<i>C. sordidus</i>	LC	13; 6; 8; 12; 13; 14
370		<i>Hipposcarus harid</i>	LC	6
371		<i>H. longiceps</i>	LC	6
372		<i>Scarus arcuatus</i>	LC	14
373		<i>S. atropectoralis</i>	LC	14
374		<i>S. bleekeri</i>	LC	5
375		<i>S. chameleon</i>	LC	5; 13
376		<i>S. dimidiatus</i>	LC	9; 13
377		<i>S. flavipectoralis</i>	LC	9; 13; 14
378		<i>S. forsteni</i>	LC	5
379		<i>S. frenatus</i>	LC	13
380		<i>S. ghobban</i>	LC	2; 8; 10; 12;
381		<i>S. globiceps</i>	LC	5; 13
382		<i>S. niger</i>	LC	6; 8; 13
383		<i>S. psittacus</i>	LC	5
384		<i>S. quoyi</i>	LC	13; 15; 16
385		<i>S. rivulatus</i>	LC	8; 9; 10 ; 13;
386		<i>S. tricolor</i>	LC	3; 9
387		<i>S. xanthopleura</i>	LC	12; 13
388	Scombridae	<i>Rastrelliger brachysoma</i>	DD	10
389		<i>R. kanagurta</i>	DD	10
390	Scorpaenidae	<i>Pterois volitans</i>	LC	8; 13
391		<i>P. antennata</i>	LC	15
392	serranidae	<i>Anyperodon leucogrammicus</i>	LC	11
393		<i>Cephalopholis micripriion</i>	LC	11
394		<i>Cephalopholis sexmaculata</i>	LC	11
395		<i>C. argus</i>	LC	13
396	Serranidae	<i>C. boenak</i>	LC	6; 8; 9; 11; 13; 14; 16
397		<i>C. cyanostigma</i>	LC	11
398		<i>C. microprion</i>	LC	8; 12; 13; 15; 16
399		<i>C. miniata</i>	LC	8; 12; 16
400		<i>C. urodeta</i>	LC	12
401		<i>Chelodon patoca</i>	LC	1
402		<i>Cromileptes altivelis</i>	vul	4

Table 2. 1 Continued

No	Family	Species	IUCN redlist	Reference
403	Serranidae	<i>Diploprion bifasciatum</i>	LC	8; 13
404		<i>Epinephelus areolatus</i>	LC	9; 12
405		<i>E. bleekeri</i>	NT	9
406		<i>E. bontoides</i>	DD	11
407		<i>E. coeruleopunctatus</i>	LC	1
408		<i>E. epistictus</i>	No	14
409		<i>E. fasciatus</i>	LC	2; 8; 11; 12; 13
410		<i>E. hexagonatus</i>	LC	2; 14
411		<i>E. longispinis</i>	LC	11
412		<i>E. malabaricus</i>	NT	11
413		<i>E. merra</i>	LC	11
414		<i>E. ongus</i>	LC	11; 9; 15
415		<i>E. quoyanus</i>	LC	9; 10; 11; 12
416		<i>E. rivulatus</i>	LC	13
416		<i>E. sexfasciatus</i>	DD	13
418		<i>Plectropomus leopardus</i>	NT	8; 14
419		<i>P. maculatus</i>	LC	10; 11; 12
420		<i>P. oligacanthus</i>	NT	11
421	Siganidae	<i>Siganus argenteus</i>	LC	8; 13
422		<i>S. canaliculatus</i>	LC	10; 12; 13
423		<i>S. corallinus</i>	LC	12
424		<i>S. guttatus</i>	LC	2; 8; 10; 13; 16
425		<i>S. puellus</i>	LC	1; 15; 16
426		<i>S. punctatus</i>	LC	9; 10; 14
427		<i>S. vermiculatus</i>	LC	10
428		<i>S. virgatus</i>	NE	8; 9; 10; 13; 14; 16
429		<i>S. vulpinus</i>	LC	9; 13
430	Siluridae	<i>Plotosus lineatus</i>	NE	2; 10
431	Sphyraenidae	<i>Sphyraena barracuda</i>	LC	8; 10; 15
432		<i>S. flavicauda</i>	NE	12
433		<i>S. forsteri</i>	LC	8
434	Synodontidae	<i>Plectorhinchus gibbosus</i>	LC	1
435		<i>Saurida gracilis</i>	LC	1
436		<i>Synodus dermatogenys</i>	LC	12; 15
437		<i>S. jaculum</i>	LC	2; 8; 12

Table 2.1 Continued

No	Family	Species	IUCN redlist	Reference)*
438	Synodontidae	<i>S. variegatus</i>	LC	8
438	Tetraodontidae	<i>Arothron mappa</i>	LC	15
440		<i>A. reticularis</i>	LC	1
441		<i>A. nigropunctatus</i>	LC	12
442	Triakidae	<i>Mustelus manazo</i>	DD	10
442	Zanclidae	<i>Zanclus cornutus</i>	LC	13

- )\* 1 : (Hutomo and Djamali, 1984) 9 : (Utami, 2010)  
 2 : (Iskandar and Mawardi, 1997) 10 : (Hartati et al., 2017)  
 3 : (Dhahiyat et al., 2017) 11 : (Riani and Affandi, 2016)  
 4 : (johan, 2004) 12 : (Setiawan et al., 2011)  
 5 : (Valentino, 2004) 13 : (Madduppa et al., 2013)  
 6 : (Tarigan et al., 2008) 14 : (Mujiyanto, 2014)  
 7 : (Mujiyanto et al., 2009) 15 : (Kusnanto et al., 2015)  
 8 : (Setyawan and Yusri, 2009) 16 : (Fahlevy et al., 2018)

Table 2. 2 List of updated species nomenclature according to WoRMS database

No	Family	species name	WoRMS Database
1	Apogonidae	<i>Archamea fucata</i>	<i>Archamia fucata</i>
2		<i>Apogon bandanensis</i>	<i>Nectamia bandanensis</i>
3		<i>A. angustatus</i>	<i>Ostorhinchus angustatus</i>
4		<i>A. apogonides</i>	<i>O. apogonoides</i>
5		<i>A. aureus</i>	<i>O. aureus</i>
6		<i>A. cavitiensis</i>	<i>Ostorhinchus cavitiensis</i>
7		<i>A. chrysopomus</i>	<i>O. chrysopomus</i>
8		<i>A. chrysotaenia</i>	<i>O. chrysotaenia</i>
9		<i>A. compressus</i>	<i>O. compressus</i>
10		<i>A. cookii</i>	<i>O. cookii</i>
11		<i>A. cyanosoma</i>	<i>O. cyanosoma</i>
12		<i>A. fleurieu</i>	<i>O. fleurieu</i>
13		<i>A. novemfasciatus</i>	<i>O. novemfasciatus</i>
14		<i>A. sealei</i>	<i>O. sealei</i>
15		<i>A. taeniopterus</i>	<i>Pristiapogon taeniopterus</i>
16		<i>A. trimaculatus</i>	<i>Pristicon trimaculatus</i>
17		<i>A. fragilis</i>	<i>Zoramia fragilis</i>
18	Aulostomidae	<i>Aulostomus chinensis</i>	<i>Aulostomus strigosus</i>
19	Balistidae	<i>Sufflamen fraenatus</i>	<i>Sufflamen fraenatum</i>
20	Caesionidae	<i>Caesio erythrogaster</i>	<i>Caesio cuning</i>
21		<i>C. chrysozona</i>	<i>Pterocaesio chrysozona</i>
22	Carangidae	<i>Carangiodes uii</i>	<i>Carangoides coeruleopinnatus</i>
23		<i>Selar crumenophthalmus</i>	<i>Selar crumenophthalmus</i>
24	Chaetodontidae	<i>Chaetodon rafflesii</i>	<i>Chaetodon rafflesii</i> Anonymous

Table 2.2 Continued

No	Family	species name	Worms Database
25	Chaetodontidae	<i>Heniochus diohreutes</i>	<i>Heniochus diphreutes</i>
26	Glaucostegidae	<i>Rhinobatus granulatus</i>	<i>Glaucostegus granulatus</i>
27	Gobiidae	<i>Istigobius decoratus</i>	<i>Istigobius decorates</i>
28	Hemirhamphidae	<i>hemirhamphus melanurus</i>	<i>Hyporhamphus quoyi</i>
29	Labridae	<i>hortulanus purpurescens</i>	<i>Halichoeres leucurus</i>
30		<i>Halichoeres chrysotaenia</i>	<i>H. melanurus</i>
31		<i>H. dussumieri</i>	<i>H. nigrescens</i>
32		<i>H. scwartzii</i>	<i>H. papilionaceus</i>
33		<i>T. genvittatum</i>	<i>Thalassoma genivittatum</i>
34	Lutjanidae	<i>Lutjanus nifolineatus</i>	<i>Lutjanus rufolineatus</i>
35	Monacanthidae	<i>monacanthus mylii</i>	<i>Monacanthus chinensis</i>
36	Mugillidae	<i>Liza valgiensis</i>	<i>Ellochelon vaigiensis</i>
37	Nemipteridae	<i>Scolopsis caninus</i>	<i>Pentapodus caninus</i>
38		<i>S. lineatus</i>	<i>Scolopsis lineata</i>
39		<i>S. bilineatus</i>	<i>S. bilineata</i>
40		<i>S. margaritifera</i>	<i>S. margaritifera</i>
41		<i>S. ciliatus</i>	<i>S. ciliata</i>
42		<i>S. trilineatus</i>	<i>S. trilineata</i>
43	Pomacanthidae	<i>Centropyge flarissima</i>	<i>Centropyge flavissima</i>
44	Pomacentridae	<i>Abudefduf curacao</i>	<i>Amblyglyphidodon curacao</i>
45		<i>Chromis dimidiata</i>	<i>Chromis dimidiata</i>
46		<i>Paraglyphidodon melas</i>	<i>Neoglyphidodon melas</i>
47		<i>P. oxyodon</i>	<i>N. oxyodon</i>
48		<i>Pomacentrus azmeomaculatus</i>	<i>Pomacentrus azuremaculatus</i>
49		<i>P. caudalis</i>	<i>Stegastes leucostictus</i>
50	Scaridae	<i>Scarus bleekeri</i>	<i>Chlorurus bleekeri</i>
51		<i>Cheilinus arenatus</i>	<i>Oxycheilinus arenatus</i>
52		<i>C. bimaculatus</i>	<i>Oxycheilinus bimaculatus</i>
53		<i>C. celebicus</i>	<i>Oxycheilinus celebicus</i>
54		<i>C. oxyrhynchus</i>	<i>Oxycheilinus celebicus</i>
55		<i>C. diagramma</i>	<i>Oxycheilinus digramma</i>
56		<i>C. rhodochrous</i>	<i>Oxycheilinus orientalis</i>
57	Scaridae	<i>C. unifasciatus</i>	<i>Oxycheilinus unifasciatus</i>
58		<i>Oxycheilinus digramma</i>	<i>Oxycheilinus diagramma</i>
59		<i>Scarus arcuatus</i>	<i>Scarus rivulatus</i>
60		<i>S. atropectoralis</i>	<i>S. xanthopleura</i>
61	Serranidae	<i>Cephalopholis boenack</i>	<i>Cephalopholis boenack</i>
62		<i>C. micripriion</i>	<i>Cephalopholis micropriion</i>
63		<i>Chelodon patoca</i>	<i>Chelonodon patoca</i>
64	Synodontidae	<i>gaterin niger</i>	<i>Plectorhinchus gibbosus</i>

Table 2. 3 List of fish species collected in Bangsring, Banyuwangi (Anggraini et al., 2017)

No	Family	Species	IUCN redlist
1	Acanthuridae	<i>Acanthurus nigricans</i>	LC
2		<i>Acanthurus nigrofuscus</i>	LC
3		<i>Zebrasoma scopas</i>	LC
4	Antennariidae	<i>Antennarius maculatus</i>	NE
5	Bleniidae	<i>Ecsenius bicolor</i>	LC
6		<i>Atrosalarias fuscus</i>	LC
7	Caesionidae	<i>Pterois antennata</i>	LC
8	Callionymidae	<i>Synchiropus stellatus</i>	NE
9	Chaetodontidae	<i>Chaetodon trifascialis</i>	NT
10		<i>Coradion chrysozonus</i>	LC
11		<i>Forcipiger flavissimus</i>	LC
12	Cirrhitidae	<i>Oxycirrhites typus</i>	LC
13	Labridae	<i>Halichoeres chrysus</i>	LC
14		<i>Labroides dimidiatus</i>	LC
15		<i>Macropharyngodon ornatus</i>	LC
16		<i>Pseudocheilinus hexataenia</i>	LC
17		<i>Stethojulis bandanensis</i>	LC
18		<i>Thalassoma lutescens</i>	LC
19	Microdesmidae	<i>Nemateleotris decora</i>	LC
20	Ostraciidae	<i>Ostracion meleagris</i>	NE
21	Pomacanthidae	<i>Centropyge argi</i>	LC
22		<i>Centropyge bicolor</i>	LC
23		<i>Centropyge eibli</i>	LC
24		<i>Centropyge tibicen</i>	LC
25		<i>Centropyge vrolikii</i>	LC
26		<i>Genicanthus lamarck</i>	LC
27		<i>Paracentropyge multifasciata</i>	LC
28	Pomacentridae	<i>Amphiprion bicinctus</i>	LC
29		<i>Amphiprion ocellaris</i>	NE
30		<i>Pomacentrus auriventris</i>	NE
31		<i>Pseudanthias dispar</i>	LC
32	Pseudochromidae	<i>Pictichromis coralensis</i>	NE
33	Tetraodontidae	<i>Canthigaster valentini</i>	LC
34		<i>Arothron nigropunctatus</i>	LC

Full form of abbreviations used in above table :

DD: Data deficient

NE: Not Evaluated

LC: Least concern

NT: Near threatened

Vul: Vulnerable

# **Chapter III. Mitochondrial COI and ITS region DNA barcoding of Java Sea Coral reef fish, Indonesia**

## **3.1 Introduction**

The DNA based approach to determine species diversity has been initiated for more than two decades (Wilson, 1995, Kurtzman, 1994). This is simply based on sequence differences in a single gene by comparing the sample's sequence against a reference library (Lakra et al., 2011). Although its accuracy may have been debatable (Will et al., 2005, Collins and Cruickshank, 2013), molecular identification has been widely used due to several reasons including rapidity, conveniency, and low ambiguity among the relative species (Li et al., 2016, Zhang and Hanner, 2012).

The marker DNA used in vertebrates were preferably extracted from the mitochondrial genome because its recombination is rare and haploid (Hebert et al., 2003a), well conserved (Gissi et al., 2008), evolves rapidly, lacks intron and has high copy number (Luo et al., 2011). The mitochondrial DNA encodes 37 genes that consist of 2 rRNAs, 22 tRNAs, and 13 protein coding gene (Anderson et al., 1981, Taanman, 1999, Pereira, 2000) and provides a resource genetic population (Ingman and Gyllensten, 2006a). The mitochondrial DNA also has been widely used to study biodiversity, evolution, and phylogeny (Nagpure et

al., 2015). Additionally several mt-DNA genes are used as a tool for species identification through DNA barcoding (Zhang and Hanner, 2012, Kochzius et al., 2010, Hebert et al., 2003a, Ward et al., 2005).

A part of the mitochondrial genome, the cytochrome c oxidase 1 (COI) gene has been used to determine fish species as a complementary for morphological identification (Landi et al., 2014). The COI region is suitable to determine an unknown fish tissue sample and useful for the authentication of processed fish product (Shokralla et al., 2015). It has become a standardized DNA barcoding marker and are broadly used for species identification since it has the ability to amplify from the various group, has faster evolution rate and determine differences within species (Hebert et al., 2003b, Hebert et al., 2003a). Recently, COI region is used as a part of the protocol of species identification (Handy et al., 2011). Since high throughput sequencing (HTS) technique were developed molecular based identification for biodiversity continues to increase. HTS technique generally generate a large amount of sequence data with the substantially low cost. However, their maximal read length ranged between 100 bp and 400 bp according to the system, which is relatively shorter than traditional Sanger's method (Kircher and Kelso, 2010). This made it difficult to adopt HTS system to use the most widely used marker, COI region, which is approximately 670 bp (Hebert et al., 2003b). From the reason, a shorter and reliable DNA marker was needed to adopt HTS technique for the massive barcoding analysis.

An Intergenic transcriber spacer (ITS) region is a noncoding region located between Small Subunit (SSU) and Large Subunit (LSU) ribosomal RNA. It has faster evolution rate and potentially magnifies the differences between the species (Chow et al., 2009). It is extensively sequenced for fungi (Kumar and Shukla, 2005, Fujita et al., 2001, Henry et al., 2000) and several higher taxa frequently uses ITS as a marker for phylogenetic and population analysis (Stothard et al., 1996, Vogler and DeSalle, 1994). In recent, several study discovered a short fragment range from SSU to LSU were enable to differentiated environmental DNA (eDNA) samples into species level (Miya et al., 2015, Yamamoto et al., 2017, Deiner et al., 2017, Olds et al., 2016, Evans et al., 2016). However, ITS region database for higher taxa was insufficient, therefore querying the database with unknown sources often fails to identify (Chow et al., 2009). In this study, we aimed to generate ITS database of fish collected from a coral reef in Java sea, Indonesia using our design primer and the COI region were used as an identity marker.

## 3.2 Materials and Methods

### 3.2.1 Sample Collection

We collected 163 fish specimens from the coral reefs in 2017. Two sample stations are located in the Thousand Islands (Th) National Park, Jakarta ( $5^{\circ}43'48.68''\text{S}$ ,  $106^{\circ}33'50.87''\text{E}$ ) and Tabuhan Island (Ti) of Banyuwangi, West Java ( $8^{\circ}3'35.52''\text{S}$ ,  $114^{\circ}27'42.08''\text{E}$ ), Indonesia (Fig 3. 1). 130 and 43 specimens were collected from Thousand Islands and Tabuhan Island, respectively. Each specimen was stored in the freezer at  $-20^{\circ}\text{C}$  stored in 96 % ethanol (Duksan pure chemical co.ltd, South Korea), which is located in Jakarta Fisheries University, Jakarta Indonesia. Parts of body including the muscles or dorsal fins were used for the further DNA sequence analysis.

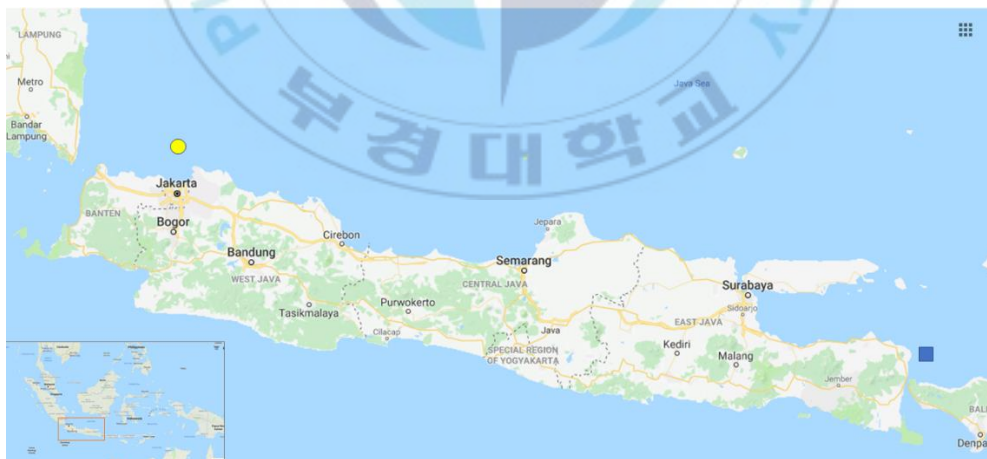


Figure 3. 1 Two sample stations in Thousand Island (yellow circle) and Tabuhan Island (Blue square)

### 3.2.2 Genomic DNA extraction and molecular identification

Genomic DNA was extracted from the muscles or fins using the Accuprep Genomic DNA Extraction Kit (Bioneer, Korea) according to the manufacturer's instructions. Purified genomic DNA was quantified with Nanodrop (ThermoFisher Scientific D1000) and stored at -70 °C for further analysis.

Identification of the specimen was by analyzing COI region, which was designed previously (Baldwin et al., 2009). The fish-specific COI universal primer pairs were BCL (5'-TCAACYAATCAYAAAGATATYGGCAC-3') and BCH (5'-ACTTCYGGGTGRCCRAARAATCA-3'). In order to supplement the ITS region, fish-specific degenerated ITS primers were designed based on the 1846 Chordate mitogenome sequences in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>). Two primers were Fish\_F2 (5'-CCMYCTAGAGGAGCCTGTYCTRDA-3') and Fish\_R1 (5'-CATGATGCAAAGGTAC-3'). The quality of all the primers in this study was analyzed by the OligoAnalyzer 3.1 (<http://s.idtdna.com/calc/analyzer>) and commercially synthesized by Bioneer Co. (South Korea). Each PCR reaction (20 µL) contained 11.2 µL ultrapure water, 1 µL of each primer (0.5µM), 0.2 µL Extaq Hotstart version DNA polymerase (TAKARA, Japan), 2 µL 10x Extaq buffer, 2 µL dNTPs (1µM, TAKARA, Japan), 0.6 % total volume DMSO and 200 ng Genomic DNA as a template. PCR was carried out with 3 step standard PCR protocol under the following conditions: initial denaturation at

94°C for 3 min, followed by 35 cycle of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 sec (1 min/kb) the process was completed with a final extension at 72 °C for 5 min. The PCR products (COI and ITS) were purified using a gel extraction kit (Bioneer, Daejeon, South Korea) by following the manufacturer's manual protocol.

The COI partial sequences and ITS sequences obtained from direct sequencing were assembled manually using Chromas ver 2.5.0. Sequences with low quality (QV < 20) were trimmed for the further analysis. Species identification of each specimen was conducted by its DNA sequence identity to GenBank database using Basic Local Alignment Search Tool (BLAST) program (<http://www.ncbi.nlm.nih.gov/blast>). Sequences both with a high query coverage (> 99 %) and a sequence identity (> 99 %) were considered as the same species. Furthermore, a lower query coverage value of the ITS sequence was assigned as new ITS sequence database and was named based on the COI sequence BLAST result. Species with a lower similarity and query coverage of the COI sequences (< 99 %) were identified morphologically by comprehensive photograph method (Halford and Thompson, 1994, Allen and Adrim, 2003) and assigned as putative species and submitted to GenBank to get accession number.

The multiple alignment of the sequences were conducted using Multiple Sequence Comparison by Log Expectation (MUSCLE) program (Edgar, 2004).

Nucleotide composition, transition and transversion bias estimation, overall pairwise distance and Minimum Evolution (ME) tree reconstruction were calculated using Kimura two-parameter (K2P) distance model using MEGA6 program (Tamura et al., 2013). The ME tree was created with 1000 bootstrap replications to provide a graphical representation of the divergence pattern.

### **3.3 Result**

#### **3.3.1 Molecular identification of specimen**

As the result of DNA sequence analysis with 163 specimens, we were able to identify 120 species, which covered 78 genera, 30 families, and 5 orders (Table 3.1). Among them, both COI and ITS sequence information of 33 species were already deposited in the GenBank database (Table. 3.1). Only COI sequences for 79 species were previously known and ITS sequences corresponding to each was deposited on the GenBank database (Table 3.1). GenBank Accession numbers for each ITS sequences were shown in Table 3.1. Eight putative species showed lower sequence identity to the database (85% - 98%) and the morphological analysis of those species were conducted (Table 3.2). Both COI and ITS sequences for the eight putative species were submitted to GenBank and their accession number were received (Fig 3.2 and Table 3.2).

A Total 30 families were identified from 120 species and Labridae and Pomacentridae were among the two major families, in which 51 and 25 species were identified, respectively (Table 3.1). Four to seven species in each family Pomacanthidae, Acanthuridae, Chaetodontidae, Apogonidae, Gobiidae, Blennidae and Scaridae were identified.

A total of 51 Labridae divided into 31 species of 15 genera have been collected. Among all the species, 19 species were located in Jakarta and 16 species in Banyuwangi. *Thalassoma lunare*, *Macropharyngodon ornatus*, *Anampses meleagrides*, and *Halichoeres melanurus* were discovered in both the locations. According to COI and ITS sequences of BLAST search result, three species *Anampses caeruleopunctatus*, *Macropharyngodon negrosensis* and *Anampses meleagrides* were discriminated. Additionally, 27 species were identified by the COI sequences, whose ITS sequences result is similar to COI sequences result yet has low query coverage, and ITS sequences with different result from COI sequences. Furthermore, we deposited ITS sequences in the GenBank as a species name of COI sequence result to obtain its accession number (Table 3.1). However, one sequence from Banyuwangi has a low COI sequence identity (85%) and was closely related to *Labaricus quadrilineatus* (MF123934). In accordance with morphological identification, it is referred as *Diproctacanthus xanthurus*. Its putative species were deposited its COI and ITS sequences in the GenBank to obtain the accession number.

A total of 25 species reflecting 15 genera were grouped as Pomacentridae. 25 species were successfully identified by COI sequence and five species reinforced with the ITS sequence. Later on, according to COI sequence result, ITS sequences of 20 species were deposited in the GenBank database (Accession number were presented in the Table 3.1). An ambiguous species from Jakarta (dkiart22) and Banyuwangi (bwicor55) has 98% sequence identity which is closely related to *Chrysiptera rolandi* voucher from Lizard Island, Australia (KP194302) were reconfirmed by its morphological identification. It was subsequently registered to the Genbank and accession number MH049092, MH049141 was received for COI and ITS sequences respectively.

In our result, Acanthuridae family were gathered comprised of three genera as Acanthurus, Paracanthurus, and Naso. The COI and ITS sequences of *Acanthurus lineatus* (COI and ITS accession number EU273284) and *Paracanthurus hepatus* (COI and ITS accession number HM180752 and KT826539 respectively) were identified. Identification for the rest of specimens were accomplished by COI sequences and their ITS sequences were deposited in the GenBank.

Total five species of the family Scaridae were obtained. According to COI sequences BLAST search result four species were successfully distinguished (Table 3.1). Furthermore, Since its ITS sequence database were not available, the sequences to the GenBank database was proposed later on. One indistinct

species (95% COI sequence identity) closely related to *Chlorurus gibbus* (accession number MF123809) and morphologically correspond to *C. blekeeri*.

In our study, six species of Apogonidae were collected. Among them five species successfully confirmed by COI sequence (Table 3.1). Meanwhile, one species related to *Taeniamia kagoshimasus* (accession number AB889678). has low sequence identity (85% COI sequence identity) and morphological identification was referred to *T. macroptera* (Fig 3.2 Table 3.2). Afterwards, COI and ITS sequence were submitted to the GenBank database. Since complete mitochondrial DNA of *Pterapogon kaudermi* were established (accession number AP005997), the ITS sequence was determined perfectly. However, the other members of Apogonidae do not have ITS database yet, this new sequences were added to the GenBank database.

Table 3. 1 Summary of coral reef fishes collected from Jakarta (DKI) and Banyuwangi (BWI)

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	Species	ITS BLAST result			Submitted ITS sequence accession number
				Genbank Accession number	QC	ID			QC	ID	Genbank Accession number	
<b>Perciformes</b>												
<b>Labridae</b>												
dki12	DKI		<i>Anampses caeruleopunctatus</i>	AY850746	100	100	The Indo West Pasific	<i>Anampses caeruleopunctatus</i>	99	99	JN935296	-
bwicor42	BWI		<i>Macropharyngodon negrosensis</i>	KP013102	100	100	-NA-	<i>Macropharyngodon negrosensis</i>	99	99	KP013102	-
bwicor7, dkiart2, dkisp23, dki23c	BWI, DKI, DKI, DKI		<i>Anampses meleagrides</i>	DQ164157, JF434744	99	99	Pohnpei Micronesia, Madagascar	<i>Anampses meleagrides</i>	100	99	JN935312	-
bwicor28	BWI		<i>Halichoeres chrysus</i>	AY850791	100	100	The Indo West Pasific	<i>Halichoeres chrysus</i>	93	100	AY850853	MH049120
bwicor43	BWI		<i>Thalassoma amblycephalum</i>	KJ968295	100	100	French Polynesia : Society Islands	<i>Thalassoma quinguevittatum</i>	38	100	KX354995	MH049131
dki40	DKI		<i>Coris cuvieri</i>	JF434944	100	100	Australia	<i>Coris gaimard</i>	90	99	AY8500826	MH049028
bwicor56	BWI		<i>Coris pictoides</i>	AY850778	100	100	The Indo Pasific	<i>Coris pictoides</i>	86	99	AY850840	MH049142
dkiA1	DKI		<i>Gomphosus caeruleus</i>	JF434977	100	100	Australia	<i>Thalassoma quinguevittatum</i>	99	92	KX354995	MH049082
dki86	DKI		<i>Labroides dimidiatus</i>	FJ583596	100	100	Philippines	<i>Labrichthys unilineatus</i>	93	91	AY850812	MH049067
bwicor14, bwicor18	BWI, BWI		<i>Bodianus mesothorax</i>	KM224695	100	100	Indonesia, West Nusa Tenggara	<i>Bodianus mesothorax</i>	91	90	AY850503	MH049107 MH049109
dki37c, dkiA3	DKI, DKI		<i>Hemigymnus melapterus</i>	FJ237784	100	100	The South China Sea	<i>Halichoeres trimaculatus</i>	99	89	EU087704	MH049026 MH049084
bwicor44	BWI		<i>Pseudocheilinus hexataenia</i>	FJ583969	100	100	Vietnam, Ho Chi Minh	<i>Lutjanus erythropterus</i>	99	83	KP939271	MH049132

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	Species	ITS BLAST result			Submitted ITS sequence accession number
				Genbank Accession number	QC	ID			QC	ID	Genbank Accession number	
<b>Perciformes</b>												
<b>Labridae</b>												
dki10	DKI		<i>Thalassoma hardwicke</i>	JQ432194	99	100	French Polynesia : Society Islands	<i>Thalassoma quinguevittatum</i>	100	95	KX354995	MH049007
dki48, dki7, dki47	DKI DKI DKI		<i>Halichoeres chloropterus</i>	FJ583490	98	100	Indonesia, Jakarta	<i>Halichoeres chloropterus</i>	92	99	AY850861	MH049035 MH049060 MH049034
dkisp28	DKI		<i>Cirrhilabrus cyanopleura</i>	FJ583216	100	99	Indonesia, Jakarta	<i>Cirrhilabrus cyanopleura</i>	51	100	KY815256	MH049161
bwicor1	BWI		<i>Pseudocheilinus evanidus</i>	KP194506	100	99	Australia, Lizard Island, Queensland	<i>Pseudocheilinus evanidus</i>	48	100	KY815327	MH049111
bwicor13	BWI		<i>Coris gaimard</i>	AY850764	100	99	Australia	<i>Coris gaimard</i>	93	99	AY850826	MH049106
dki85, dki60, dki86b	DKI DKI DKI		<i>Halichoeres scapularis</i>	AY850762	100	99	The Indo Pasific	<i>Halichoeres scapularis</i>	93	99	AY850824	MH049065 MH049045 MH049066
bwicor40	BWI		<i>Halichoeres prosopion</i>	AY850776	100	99	The Indo Pasific	<i>Halichoeres prosopion</i>	92	99	AY850838	MH049128
bwicor53	BWI		<i>Hologymnosus doliatus</i>	AY850771	100	99	The Indo Pasific	<i>Hologymnosus annulatus</i>	90	99	AY850834	MH049140
dki30, dki31, bwicor26	DKI DKI BWI		<i>Thalassoma lunare</i>	KF809426	100	99	Philippines	<i>Thalassoma lunare</i>	52	99	AY850799	MH049020 MH049021 MH049118
dkiar24	DKI		<i>Cirrhilabrus lubbocki</i>	FJ583234	100	99	Indonesia, Jakarta	<i>Cirrhilabrus lubbocki</i>	49	99	AY279587	MH049093
dkisp29, bwicor22, dki29b	DKI, BWI, DKI		<i>Halichoeres melanurus</i>	FJ83514, JQ839492	100	99	Indonesia, Jakarta Indonesia,Bali	<i>Halichoeres melanurus</i>	99	97	AP006018	MH049018 MH049114 MH049018

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
				Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Perciformes</b>												
<b>Labridae</b>												
dki24, dkisp24, bwicor29	DKI, DKI, BWI		<i>Macropharyngodon ornatus</i>	FJ583646	100	99	Sri Lanka	<i>Macropharyngodon ocyanoguttatus</i>	96	97	JN935323	MH049015 MH049160 MH049121
dki68, dki36	DKI DKI		<i>Cheilinus chlorourus</i>	KF714912	100	99	Philippines	<i>Cheilinus undulatus</i>	100	90	KM461717	MH049051 MH049025
dki9, dki62	DKI DKI		<i>Cheilinus fasciatus</i>	KF809396	100	99	Philippines	<i>Cheilinus undulatus</i>	100	90	KM461717	MH049009 MH049046
dkisp22, dki25	DKI DKI		<i>Epibulus insidiator</i>	JF434968	100	99	Madagascar	<i>Cheilinus undulatus</i>	99	90	KM461717	MH049158 MH049016
dki38	DKI		<i>Choerodon anchorago</i>	KF714916	100	99	Philippines	<i>Choerodon schoenleinii</i>	100	89	KM487697	MH049027
bwicor24	BWI		<i>Bodianus dictynna</i>	KC684980	100	99	Indonesia, Bali	<i>Bodianus rufus</i>	90	88	AY850503	MH049116
dki46, dki32b, dki29	DKI DKI DKI		<i>Halichoeres hortulanus</i>	FJ583504	98	99	Indonesia, Jakarta	<i>Halichoeres hortulanus</i>	86	99	AY850823	MH049033 MH049022 MH049019
<b>Pomacentridae</b>												
dkiart8, bwicor16	DKI BWI		<i>Amphiprion clarkii</i>	KJ174498	100	100	China, Paracel Island	<i>Amphiprion clarkii</i>	100	99	KJ174498	-
dki88	DKI		<i>Amphiprion ocellaris</i>	AP006017	100	99	-NA-	<i>Amphiprion ocellaris</i>	100	99	AB980197	-
dki104	DKI		<i>Amphiprion perideraion</i>	KJ833753	100	99	China, fish market	<i>Amphiprion perideraion</i>	100	99	KJ833753	-
dkiA10	DKI		<i>Pomacentrus moluccensis</i>	KP194142	100	99	Australia, Lizard Island, Queensland	<i>Pomacentrus moluccensis</i>	100	99	KJ833754	-

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
				Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Perciformes</b>												
<b>Pomacentridae</b>												
dkiart10, dki32c, dkiA9	DKI DKI DKI		<i>Chrysiptera parasema</i>	FJ583190	100	99	Philippines	<i>Amphiprion clarkii</i>	98	92	AB979449	MH049089 MH049088 MH049023
dkiart5, dki75, dkiart9	DKI DKI DKI		<i>Premnas biaculeatus</i>	LC089001	100	99	Thailand	<i>Premnas biaculeatus</i>	100	99	KJ833754	-
dki5	DKI		<i>Neoglyphidodon melas</i>	FJ583726	100	100	Philippines	<i>Amphiprion frenatus</i>	100	91	LC089039	MH049044
dki91, dki72	DKI DKI		<i>Pomacentrus coelestis</i>	FJ583901	100	100	Philippines	<i>Premnas biaculeatus</i>	100	91	KJ833754	MH049073 MH049055
dki101	DKI		<i>Chromis atripectoralis</i>	KP194775	100	100	Australia, Lizard Island, Queensland	<i>Abudefduf hoefleri</i>	99	90	KT374291	MH049003
dki82, bwicor19	DKI, BWI		<i>Dascyllus aruanus</i>	MF123849	100	100	Israel	<i>Abudefduf vaigiensis</i>	99	87	KU363024	MH049062 MH049110
bwicor49	BWI		<i>Dascyllus trimaculatus</i>	FJ583337	100	100	Philippines	<i>Abudefduf vaigiensis</i>	99	87	KU363024	MH049135
dki94, dkiart3, bwicor30	DKI, DKI BWI		<i>Dascyllus reticulatus</i>	KP194023	100	100	Australia, Lizard Island, Queensland	<i>Abudefduf vaigiensis</i>	97	87	KU363024	MH049076 MH049097 MH049123
dki28	DKI		<i>Abudefduf septemfasciatus</i>	KR090613	98	100	India	<i>abudefduf hoefleri</i>	100	96	KT374291	MH049017
dkiart11	DKI		<i>Dascyllus melanurus</i>	FJ583330	100	99	Philippines	<i>Abudefduf hoefleri</i>	99	97	KT374291	MH049090
dki92, dki8	DKI DKI		<i>Hemiglyphidodon plagiometopon</i>	KP194554	100	99	Australia, Lizard Island, Queensland	<i>Amphiprion bicinctus</i>	99	93	JQ030887	MH049074 MH049071
dki57	DKI		<i>Dischistodus prosopotaenia</i>	FJ583365	100	99	Philippines	<i>Amphiprion sebae</i>	100	92	AB979696	MH049042

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
				Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Perciformes</b>												
<b>Pomacentridae</b>												
dki74	DKI		<i>Neoglyphidodon nigroris</i>	KP194471	100	99	Australia, Lizard Island, Queensland	<i>Amphiprion akallopisos</i>	98	92	AB979273	MH049057
dki93	DKI		<i>Chrysiptera cyanea</i>	FJ583168	100	99	Indonesia, Jakarta	<i>Amphiprion sebae</i>	98	91	AB979696	MH049075
dki98	DKI		<i>Chrysiptera talboti</i>	KP194622	100	99	Australia, Lizard Island, Queensland	<i>Amphiprion clarkii</i>	99	90	AB979449	MH049079
dkiA5	DKI		<i>Neopomacentrus azysron</i>	JF435054	100	99	Madagascar	<i>Amphiprion bicinctus</i>	99	88	JQ030887	MH049086
dki83	DKI		<i>Neoglyphidodon oxyodon</i>	FJ583735	99	99	Philippines	<i>Amphiprion frenatus</i>	99	91	LC089039	MH049063
dki45	DKI		<i>Amblyglyphidodon curacao</i>	KF929588	97	99	Fiji Island	<i>Amphiprion clarkii</i>	100	92	KJ174498	MH049032
dkiart6, dkiart7	DKI		<i>Amphiprion sebae</i>	KU987434	96	99	India	<i>Amphiprion polymnus</i>	98	99	KJ101554	MH049101
bwicor51	BWI		<i>Chromis retrofasciata</i>	KU944320	87	99	Taiwan	<i>Chromis retrofasciata</i>	49	100	FJ606323	MH049138
<b>Acanthuridae</b>												
dkisp13	DKI		<i>Acanthurus lineatus</i>	EU273284	100	100	India	<i>Acanthurus lineatus</i>	99	100	EU273284	-
bwicor3	BWI		<i>Paracanthurus hepatus</i>	HM180752	100	100	South Korea	<i>Paracanthurus hepatus</i>	100	99	KT826539	-
dkisp14	DKI		<i>Acanthurus leucosternon</i>	EF649259	98	100	Africa, Republic of Seychelles	<i>Acanthurus leucosternon</i>	100	99	EU136032	-
dkisp16	DKI		<i>Naso elegans</i>	KJ658922	100	100	UEA	<i>Naso lopezi</i>	100	95	AP009163	MH049155
bwicor38	BWi		<i>Acanthurus nigricans</i>	EF648269	100	99	Indian Ocean, Keeling island	<i>Acanthurus leucosternon</i>	100	99	EU136032	MH049125
dkisp19	DKI		<i>Acanthurus auranticavus</i>	KC623655	100	99	USA, fish market	<i>Acanthurus monroviae</i>	99	96	KT283582	MH049156
bwicor4	BWI		<i>Acanthurus olivaceus</i>	KJ967832	99	100	French Polynesia : Society Islands	<i>Ctenochaetus striatus</i>	100	95	KU244260	MH049136

Table 3.1 Continued

Sample code.	Sample Loc.	Order Family	Species	COI			Locations	Species	ITS		Genbank Accession number	Submitted ITS sequence accession number
				Genbank Accession number	QC	ID			QC	ID		
<b>Perciformes</b>												
<b>Pomacanthidae</b>												
bwicor61	BWI		<i>Centropyge vrolikii</i>	NC036949	100	100	Philippines	<i>Centropyge vrolikii</i>	99	100	MF001440	-
bwicor41	BWI		<i>Centropyge nox</i>	KR870993	100	100	Indonesia, Bali	<i>Centropyge nox</i>	100	99	KR870993	-
dki103	DKI		<i>Chaetodontoplus mesoleucus</i>	KP218262	100	99	Philippines	<i>Chaetodontoplus mesoleucus</i>	100	100	KP218262	-
bwicor45	BWI		<i>Centropyge acanthops</i>	KR870990	100	99	Africa, Kenya	<i>Centropyge acanthops</i>	99	99	KR870990	-
bwicor11	BWI		<i>Centropyge bicolor</i>	FJ582945	100	100	Philippines	<i>Centropyge deborae</i>	99	95	KX499480	MH049105
dki80	DKI		<i>Pomacanthus sexstriatus</i>	KJ542548	100	99	Taiwan	<i>Pomacanthus xanthometopon</i>	98	97	KP218258	MH049061
<b>Scaridae</b>												
dkiA4	DKI		<i>Cetoscarus bicolor</i>	JQ349874	100	100	Madagascar	<i>Bolbometopon muricantum</i>	100	90	KY235362	MH049085
dki32	DKI		<i>Leptoscarus vaigiensis</i>	FJ237788	100	100	China, The South China Sea	<i>Scarus forsteni</i>	100	97	FJ619271	MH049024
dkiA13	DKI		<i>Scarus vetula</i>	<u>FJ584083</u>	100	99	Vietnam, Ho Chi Minh	<i>Thalassoma quinguevittatum</i>	100	91	KX354995	MH049081
dki4	DKI		<i>Chlorurus japanensis</i>	KU944735	87	100	Taiwan	<i>Chlorurus sordidus</i>	99	97	AP006567	MH049037

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	COI BLAST Result				Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
			Species	Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Perciformes</b>												
<b>Blennidae</b>												
dkiart4	DKI		<i>Salarias fasciatus</i>	NC004412	100	99	-NA-	<i>Salarias fasciatus</i>	100	99	NC004412	
bwicor23	BWI		<i>Ecsenius bicolor</i>	FJ583381	100	99	Sri Lanka	<i>Ecsenius bicolor</i>	99	93	KP013109	MH049115
dki67	DKI		<i>Meiacanthus grammistes</i>	KX230183	100	99	-NA-	<i>Petrosciartes breviceps</i>	95	89	AP004450	MH049050
bwicor35, bwicor46	BWI BWI		<i>Plagiotremus rhinorhynchus</i>	KP194373	100	100	Australia, Lizard Island, Queensland	<i>Petrosciartes breviceps</i>	98	85	AP004450	MH049124 MH049134
<b>Gobiidae</b>												
dki69	DKI		<i>Amblygobius phalaena</i>	FJ582724	100	99	Philippines	<i>Amblygobius phalaena</i>	99	99		-
dki50	DKI		<i>Valenciennea longipinnis</i>	HQ536648	99	99	Papua New Guinea	<i>Valenciennea longipinnis</i>	99	99	KF415487	-
dki95, dki59	DKI DKI		<i>Valenciennea muralis</i>	FJ584203	100	100	Indonesia, Jakarta	<i>Valenciennea longipinnis</i>	98	94	KF415487	MH049077 MH049043
bwicor52	BWI		<i>Valenciennea strigata</i>	FJ584225	100	100	Vietnam, Ho Chi Minh	<i>Valenciennea strigata</i>	93	99	KF415488	MH049139
<b>Apogonidae</b>												
dki102	DKI		<i>Pterapogon kauderni</i>	AP005997	100	99	Indonesia	<i>Pterapogon kauderni</i>	100	99	AP005997	-
dki43	DKI		<i>Apogon coccineus</i>	JQ349718	100	99	Madagascar	<i>Zoramia leptacantha</i>	99	96	AB206136	MH049031
dki41	DKI		<i>Cheilodipterus quinquelineatus</i>	JQ349887	97	99	Madagascar	<i>Cheilodipterus quinquelineatus</i>	100	92	AB889639	MH049029
dki42	DKI		<i>Ostorhinchus sealei</i>	FJ582864	97	100	Vietnam, Ho Chi Minh	<i>Ostorhinchus sealei</i>	100	97	AB889659	MH049030
dki71	DKI		<i>Sphaeramia orbicularis</i>	AB890107	100	99	Japan, Aquarium shop	<i>Apogon hyalosoma</i>	100	99	AB889674	MH049054

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			ITS BLAST result					
				Genbank Accession number	QC	ID	Sample Loc.	Species	QC	ID	Genbank Accession number	Submitted ITS sequence accession number
<b>Perciformes</b>												
<b>Chaetodontidae</b>												
dkiA6, dkisp5	DKI		<i>Chelmon rostratus</i>	KM978959	100	99	The South China Sea	<i>Chelmon rostratus</i>	100	100	KM978959	-
dkisp3, dkisp10, bwicor21	DKI , BWI		<i>Chaetodon kleinii</i>	HQ561505	100	100	Mozambique	<i>Chaetodon auripes</i>	99	91	AP006004	MH049163 MH049151 MH049113 MH049157
dkisp1	DKI		<i>Chaetodon lineolatus</i>	KF489525	100	99	South Africa	<i>Chaetodon auripes</i>	98	97	AP006004	MH049157
<b>Callionymidae</b>												
dkiart25	DKI		<i>Dactylopus dactylopus</i>	<u>FJ583316</u>	100	100	Philippines	<i>Synchiropus ocellatus</i>	100	92	AP012315	MH049094
dkipsm84	DKI		<i>Synchiropus splendidus</i>	AP012317	100	99	-NA-	<i>Synchiropus splendidus</i>	100	99	AP012317	-
<b>Cirrhitidae</b>												
bwicor50	BWI		<i>Oxycirrhites typus</i>	FJ583793	100	100	Philippines	<i>Cirrhitichthys aprinus</i>	75	89	AP006011	MH049137
<b>Echeneidae</b>												
dki79	DKI		<i>Echeneis naucrates</i>	KF021242	100	99	Thailand	<i>Echeneis naucrates</i>	100	99	KF021242	-
<b>Ephippidae</b>												
dki70	DKI		<i>Platax orbicularis</i>	AP006825	100	99	-NA-	<i>Platax orbicularis</i>	99	99	AP006825	-
<b>Haemulidae</b>												
dki87, dkiart26, bwicor10	DKI DKI DKI		<i>Plectorhinchus chaetodonoides</i>	FJ583863	100	100	Philippines	<i>Plectorhinchus lineatus</i>	100	96	KM099284	MH049068 MH049104 MH049095

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	Species	ITS BLAST result			Submitted ITS sequence accession number
				Genbank Accession number	QC	ID			QC	ID	Genbank Accession number	
<b>Perciformes</b>												
<b>Kuhliidae</b>												
dki90	DKI		<i>Kuhlia mugil</i>	AP011065	100	99	-NA-	<i>Kuhlia mugil</i>	99	99	AP011065	-
<b>Microdesmidae</b>												
bwicos25	BWI		<i>Ptereleotris heteroptera</i>	KM357753	100	100	Indonesia, Raja Ampat	<i>Ptereleotris carinata</i>	99	90	KF415456	MH049117
<b>Mullidae</b>												
bwicos20	BWI		<i>Parupeneus multifasciatus</i>	KF009641	100	99	Philippines	<i>Parupeneus multifasciatus</i>	100	99	AP012314	-
dki54	DKI		<i>Upeneus tragula</i>	AB355920	100	99	-NA-	<i>Upeneus tragula</i>	100	99	AB355920	-
<b>Nemipteridae</b>												
dki56	DKI		<i>Scolopsis margaritifera</i>	KY362951	100	100	Indonesia, Bali	<i>scolopsis vosmeri</i>	100	89	KT692978	MH049041
dki20	DKI		<i>Pentapodus trivittatus</i>	KY362927	100	100	Philippines	<i>Nemipterus virgatus</i>	98	86	KU933270	MH049010
<b>Pseudochromidae</b>												
dki66	DKI		<i>Pseudochromis fuscus</i>	FJ583981	100	100	Indonesia, Jakarta	<i>Labracinus cyclophthalmus</i>	71	85	AP009125	MH049049
dki96, dkiart12, bwicos2	DKI, DKI, BWI		<i>Pictichromis paccagnellae</i>	KT826540	100	99	Philippines	<i>Pictichromis paccagnellae</i>	99	99	KT826540	-

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
				Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Perciformes</b>												
<b>Serranidae</b>												
bwior6	BWI		<i>Pseudanthias squamipinnis</i>	FJ583941	100	100	Philippines	<i>Pseudanthias squamipinnis</i>	39	94	EF120810	MH049149
bwior39	BWI		<i>Belonoperca chabanaudi</i>	KF929657	100	99	Pacific Ocean, Tonga Island	<i>Macrorhamphosodes uradoi</i>	99	85	AP009171	MH049126
<b>Siganidae</b>												
dkiA2	DKI		<i>Siganus virgatus</i>	KF715023	100	100	Philippines	<i>Siganus guttatus</i>	99	99	KJ420577	MH049083
<b>Beryformes</b>												
<b>Holocentridae</b>												
dki22	DKI		<i>Sargocentron rubrum</i>	EU600150	100	99	The South China Sea	<i>Sargocentron rubrum</i>	100	100	AP004432	-
<b>Scorpaeniformes</b>												
<b>Scorpaenidae</b>												
dki73	DKI		<i>Pterois miles</i>	LK022697	100	100	Israel, The Red Sea	<i>Pterois miles</i>	100	100	LK022697	-
bwior63	BWI		<i>Pterois volitans</i>	KM488633	100	100	Mexico	<i>Pterois volitans</i>	99	99	KM488633	-
<b>Syngnathiformes</b>												
<b>Centriscidae</b>												
dki49	DKI		<i>Aeoliscus strigatus</i>	AP009198	100	99	-NA-	<i>Aeoliscus strigatus</i>	100	99	AP009198	-

Table 3.1 Continued

Sample code	Sample Loc.	Order Family	Species	COI BLAST Result			Sample Loc.	ITS BLAST result			Submitted ITS sequence accession number	
				Genbank Accession number	QC	ID		Species	QC	ID		Genbank Accession number
<b>Tetraodontiformes</b>												
<b>Balistidae</b>												
dkisp12	DKI		<i>Abalistes stellaris</i>	AP009202	100	99	-NA-	<i>Abalistes stellaris</i>	100	100	AP009202	-
<b>Monacanthidae</b>												
dkis53	DKI		<i>Acreichthys tomentosus</i>	AP009213	99	99	-NA-	<i>Acreichthys tomentosus</i>	100	100	AP009213	-
<b>Ostraciidae</b>												
bwicor60, dki89	BWI, DKI		<i>Ostracion cubicus</i>	JQ861019	100	100	USA, fish market	<i>Ostracion immaculatus</i>	100	99	AP009176	MH049146
<b>Tetraodontidae</b>												
bwicor27	BWI		<i>Canthigaster valentini</i>	AP011912	100	99	Japan	<i>Canthigaster valentini</i>	100	99	AP011912	-

Two species of Blennidae presented identical with COI sequence BLAST search result as *Salarias fasciatus* (Accession number NC004412) and *Ecsenius bicolor* from Sri Lanka (KP013109) respectively (Table 3.1). The other COI sequence were delineated as *Meiacanthus grammistes* (accession number KX230183) and *Plagiotremus rhinorhynchos* (accession number KP194373) (Table 3.1). Since the ITS sequence of *E. bicolor* exhibited lower identity (93%), we submitted the sequences to Genbank database in conjunction with *M. grammistes* and *P. rhinorhynchos* and received their accession number (Table 3.1).

In accordance with COI sequences BLAST search result, the Gobiidae were two species of *Amblygobius* and three species of genera *Valenciennea* (Table. 3.1). One species of *Amblygobius* has a low sequence identity (88%) corresponding to *Amblygobius decussatus* (accession number FJ582723) thus its morphological identification resemble as *Amblygobius stethophthalmus* (Fig 3.2 and Table 3.2). The ITS sequence database of *Ablygobius phalaena*, *Valenciennea longipinnis* and *Valenciennea strigata* matched with the resulting COI sequences (accession number HQ536648 and FJ584225 respectively) (Table 3.1). However, *V.strigata* has fewer query coverage, and *Valenciennea muralis* generated different result (Table 3.1). furthermore, its ITS sequences were submitted to the GenBank and accession number was received (Table 3.1).

In this study, we suggested a putative species, *Heniochus pleurotaenia* by its morphological characteristic (Fig 3.2) since COI sequence revealed no database, whose BLAST search resulted 96% sequence identity related to *Heniochus dipherutes* (accession number AP006005) (Table 3.2). The other Chaetodontidae were validated as *Chelmon rostratus* by COI and ITS sequences (accession number KM978959). Meanwhile, *Chaetodon kleinii* and *Chaetodon lineolatus* were differentiated by COI sequences (accession number HQ561505 and KF489525, respectively). Hereafter, COI and ITS sequences of putative species, *H. pleurotaenia* added in the Genbank jointly with *C. kleinii* and *C. lineolatus* ITS sequences (Table 3.2 and Table 3.1, respectively).

There are 20 other fish families with less than 3 members species. Within the family, Echineidae, Ehippidae, Mullidae, Holocentridae, Scorpaenidae, Centriscidae, Balistidae, Monacanthidae, and Tetraodontidae have been verified using COI and ITS sequences. The ten remaining families are identified by using the COI region, while the ITS sequences are not listed in the GenBank database (Table 3.1).

### **3.3.2 Morphological Identification**

We observed nine specimens in which the sequence BLAST search resulted COI sequences identity was less than 99% (Table 3.2). We reconfirmed it by using

morphological identification and three species were proposed as a similar species (*Chrysiptera rollandi*, *Cirrhitichthys oxycephalus*, *Caesio xanthonota*), with the sequences reference, five specimens were identified as different species in which four species were clustered in the same genera (*Heniochus pleurotaenia*, *Taeniamia macroptera*, *Chlorurus bleekeri*, *Amblygobius stethophthalmus*) and one species was in the same family (*Diproctacanthus xanthurus*) (Figure 3.2).

#### **Sequences concensus characteristic**

DNA sequences were generated from 120 coral reef fish species. For forward direction sequencing the length of COI sequences were 588-682 nucleotide base pairs. Its alignment produced 571 bp in length exhibited average nucleotide frequencies as 29.8 (T), 29.1 (C), 23.7 (A), and 17.3 (G) %. The nucleotide concensus were conserved without insertions, deletions or stop codon (Fig 3.3a). The GC content was 46.5 % and the average ratio of transition pairs and transversion pairs was 1.89, The average transition pairs (si= 65.44) were higher than an average transversional pair (sv = 34.56).

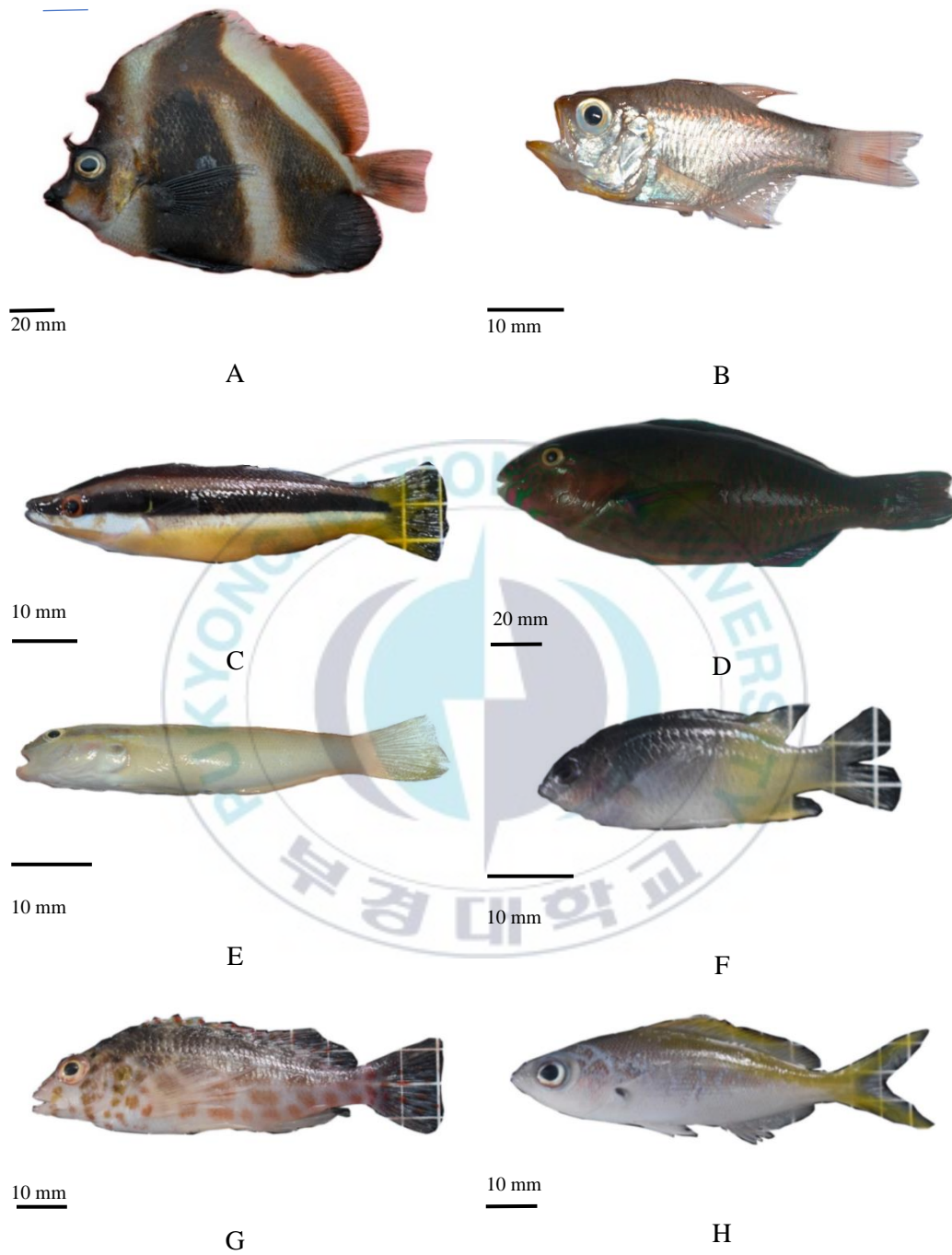


Figure 3. 2 Morphological features of fishes with low sequences identity in COI sequences fathomed as *Heniochus pleurotaenia* (A), *Taeniamia macroptera* (B), *Diproctacanthus xanthurus* (C), *Chlorurus bleekeri* (D), *Amblygobius stethophthalmus* (E), *Chrysiptera rollandi* (F), *Cirrhitichthys oxycephalus* (G), *Caesio xanthonota* (H)

Table 3. 2 List of morphologically identified 8 fishes specimens with low sequence identity to GenBank database

code	Location	Molecular identification			Morphological Identification				
		GenBank Accession	Qv	Id	Species name	Morphological description	Putative species name	Reference	Submitted COI sequence Accession number
dki100	DKI	AP006005	100	96	<i>Heniochus dipherutes</i>	Body bands merging only in upper part of the body, leaving a white triangular space in a midventral portion of the body (Fig 2.a)	<i>Heniochus pleurotaenia</i>	(Pyle, 2001), www.fishbase.org	MH049002
dki63	DKI	AB889678	98	95	<i>Taeniamia kagoshimasus</i>	Basilingual teeth absent, Posterior preopercular edge completely serrate, ventral edge serrate on posterior half. Scaly sheath along anal-fin base poorly developed or absent (Fig 2.b)	<i>Taeniamia macroptera</i>	(Gon and Randall, 2003)	MH049047
bwicor5	BWI	MF123934	100	85	<i>Larabicus quadrilineatus</i>	The three-part broad black stripes from head to caudal fin where the stripes merge; the Head scales small. Lips thick and fleshy, forming a short tube when the mouth is closed. Caudal fin rounded to truncate; pelvic fins rounded (Fig 2.c)	<i>Diproctacanthus xanthurus</i>	(Debelius and Baensch, 1994)	MH049145
dki21c	DKI	MF123809	100	95	<i>Chlorurus gibbus</i>	Caudal fin truncate in both phases. Lips do not cover dental plates. Adults with 1-2 canines posteriorly on side of the upper dental plate. The initial phase is reddish-brown with a diffuse yellowish patch in the center of the caudal peduncle and markings on the lips similar to those of the terminal male (Ref. 1602). Males identified by the white patch on cheek and females strongly barred (Fig 2.d )	<i>Chlorurus bleekeri</i>	(RANDALL and CHOAT, 1980) www.fishbase.org	MH049011
dki69	DKI	FJ582723	100	88	<i>Amblygobius decussatus</i>	Characterized by white-edged brown stripe from the snout, through the eye, to the anterior side below first dorsal fin, similar stripe on the cheek to pectoral fin base; dark freckling on snout; the presence of short bars on back; upper caudal fin base with a dark spot (Fig 2.e )	<i>Amblygobius stethophthalmus</i>	(Allen and Erdmann, 2012) www.fishbase.org	MH049052
bwicor55, dkiart22	BWI, DKI	KP194302	100	98	<i>Chrysiptera rollandi</i>	Dorsal part of the head and anterior portion of the back dark brown with blue streaks, creamy white below (Fig 2.f )	<i>Chrysiptera rollandi</i>	(Allen, 1991) www.fishbase.org	MH049092 MH049141
bwicor50	BWI	JF493204	100	98	<i>Cirrhitichthys oxycephalus</i>	Variable in color from pale with grey to black blotches to pink with bright red blotches. Pale with horizontal rows of subquadrate dark brown spots (Fig 2.g).	<i>Cirrhitichthys oxycephalus</i>	(Debelius and Baensch, 1994)	MH049143
bwicor59	BWI	HQ561536	98	98	<i>Caesio xanthonota</i>	Upper 1/3 of body and caudal fin bright yellow, middle third blue, lower white. Forked caudal fin (Fig 2.h)	<i>Caesio xanthonota</i>	(Carpenter, 1987)	MH049144

The ITS sequence of 120 species were generated with sequences alignment of 1,056 nucleotide in a length ranging from 583 to 1123 Nucleotide base pairs. The consensus showed hypervariable nucleotide including insertion, deletion, and substitution with several conserved places. Start from the initial (0-380bp), middle (650-720bp) and end of the sequences (950-1056 bp) (Fig 3.3).

The nucleotide average frequencies was 20 (T), 24.6 (C), 34.4 (A), and 21.1 (G) %. The GC content was 45.7%. and the average ratio of transition pairs and transversion pairs is 2.35. The average transition pairs (si = 70.16) were higher than an average transversional pair (sv = 29.84).

### **3.3.3 Phylogenetic analysis**

Each phylogenetic tree of 120 fish species was constructed with COI and ITS sequences, respectively (Figure 3.4, Figure 3.5). Generally, COI sequences phylogenetic tree were clustered each species in their family as expected (Figure 3.4). A similar result were showed for ITS sequences phylogenetic tree. Further, the Scaridae and Labridae were closely related family (Figure 3.5).

We used The COI phylogenetic tree at the family level for species confirmation by aligning the collection of sequences with the references obtained from the Genbank database. Nine families with more than 3 species were separated into different phylogenetic tree, whereas the families with less than three species were grouped into another family phylogenetic tree.

## Labridae

The COI and ITS sequences phylogenetic tree reconstruction with minimum evolution algorithm for 31 species of Labridae clustered each species into their genera with the exception *Coris pictoides* (accession number AY85077) which is clustered in *Halichoeres* genera (Fig. 3.6 and Fig. 3.7, respectively). According to the COI phylogenetic tree, a total of 19 species were haplotype with the reference (Figure 3.6). In addition, the COI sequences of *Anampses meleagrides* which is collected from Th(dkiart2, dkisp23, dki23c) inline with Madagascar voucher (accession number JF434744) were indicated haplotype with Ti specimens (bwicor7) which is identical to *A. meleagrides* from Micronesia (accession number DQ1641570) (Fig 3.6). Surprisingly, the distance value of species collected from Th (dkisp29 and dki291) which is inline with *H. melanurus* voucher from Th (accession number FJ83514) and species obtained from Ti (bwicor 22) which is related to *H. melanurus* from Bali (JG839492) exhibited more than 3% (d: 0.07) (Fig 3.6). In contrast, the distance value of *Coris cuvieri* (Th collection, accession number JF434944) and *Coris gaimard* (Th collection, accession number AY850764) were exposed less than 3% (d:0.013) (Fig 3.6). Furthermore, the phylogenetic tree indicated putative species, *Diproctacanthurus xanthurus* which is the COI sequence identity were closely related to *Labaricus quadrilineatus* (MF123934) were sister species



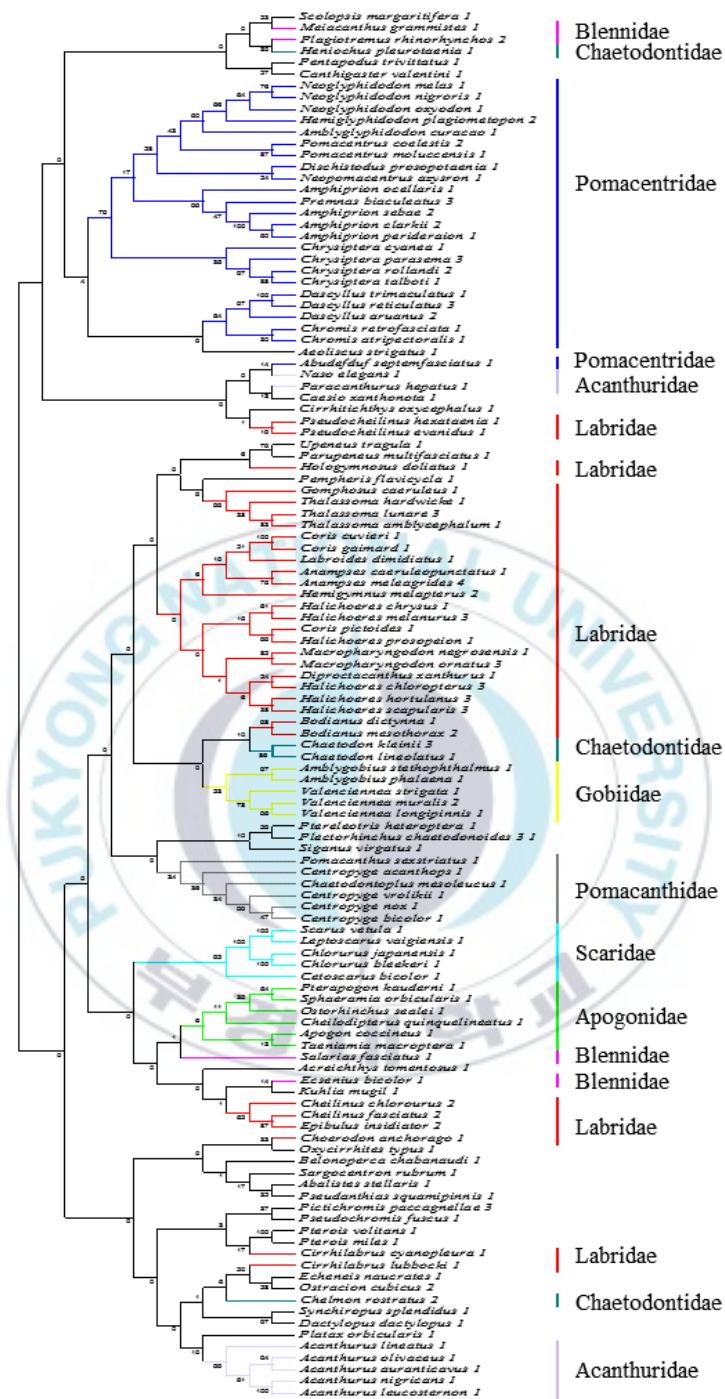


Figure 3. 4 Summary of the Phylogenetic tree of COI sequence derived from 120 fish species. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

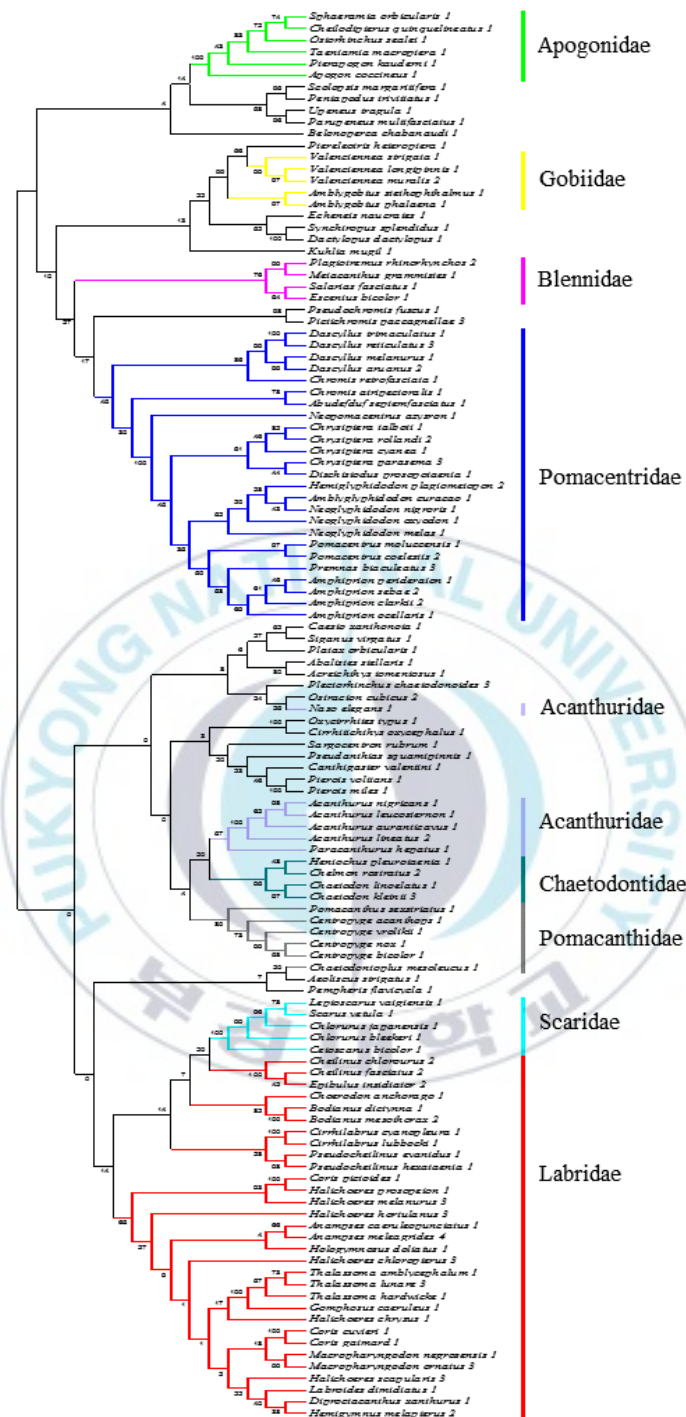


Figure 3. 5 Phylogenetic tree of ITS sequence derived from 120 fishes species. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

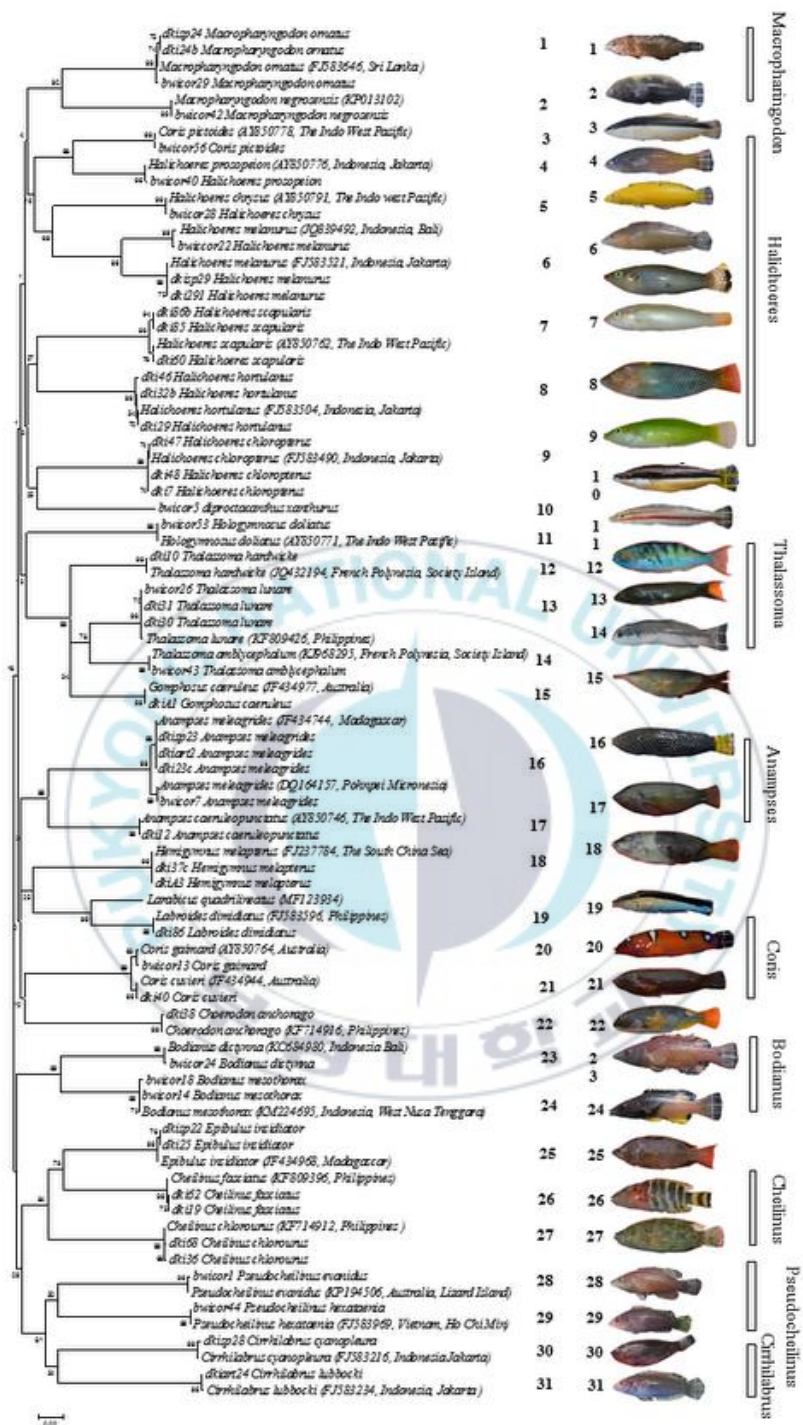


Figure 3. 6 Phylogenetic tree of COI sequence derived from 31 species of family Labridae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

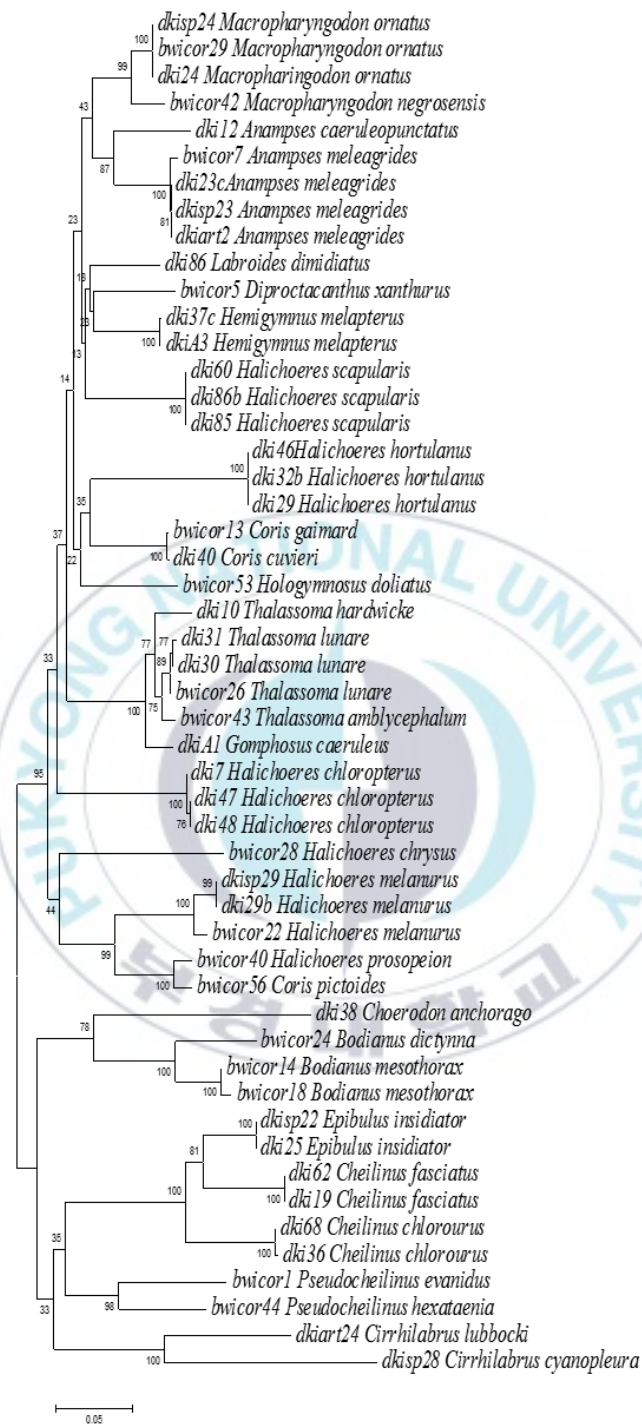


Figure 3. 7 Phylogenetic tree of ITS sequence derived from 31 species of family Labridae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method.

A consistent result was shown in the ITS phylogenetic tree of Labridae (Figure 3.7). It described that *A. meleagrides* were haplotype species between Th collection and Ti collection. Subsequently, both of *H. melanurus* (collected from Th and collected from Ti) was separated with the distance value 0.03 and *Coris gaimard* were inline with *Coris cuvieri* (Fig 3.7). and The putative species, *Diproctacanthurus xanthurus* was clustered with *Labroides dimidiatus* and *Hemigymnus melapterus* as closely related species (Figure 3.7).

#### Pomacentridae

The CO1 Pomacentridae phylogenetic trees reconstruction with minimum evolution algorithm successfully clustered Anemone fish group (*Premnas* and *Amphiprion* genera) and damselfish group (*Chrysiptera*, *Dischistodus*, *Hemiglyphidodon*, *Pomacentrus*, *Neopomacentrus*, *Amblyglyphidodon*, *Neoglyphidodon*, *Dascyllus*, *Chromis*, and *Abudefduf* genera) (Figure 3.8). Among 25 species of Pomacentridae, no species references were generated from Indonesia, with the exception *Chrysiptera cyanea* which was collected from Jakarta, Indonesia (accession number FJ583168). Further, 16 species were haplotype with sequence reference (*Premnas biaculatus*, *Amphiprion sebae*, *A. clarkii*, *Chrysiptera rollandi*, *C. talboti*, *C. parasema*, *C. cyanea*, *Dischistodus prosopotaenia*, *Pomacentrus coelestis*, *P. moluccensis*, *Neopomacentrus azysron*, *Amblygophidodon curacao*, *Neoglyphidodon oxyodon*, *N. nigroris*, *Dascyllus aruanus* and *Chromis retrofasciata*). In addition, *A. clarkii* collected from Th (dkiart8) which was in line with reference voucher from China

(Accession number KJ 174499). were haplotype with *A. clarkii* collected from Ti (bwicor 16) .

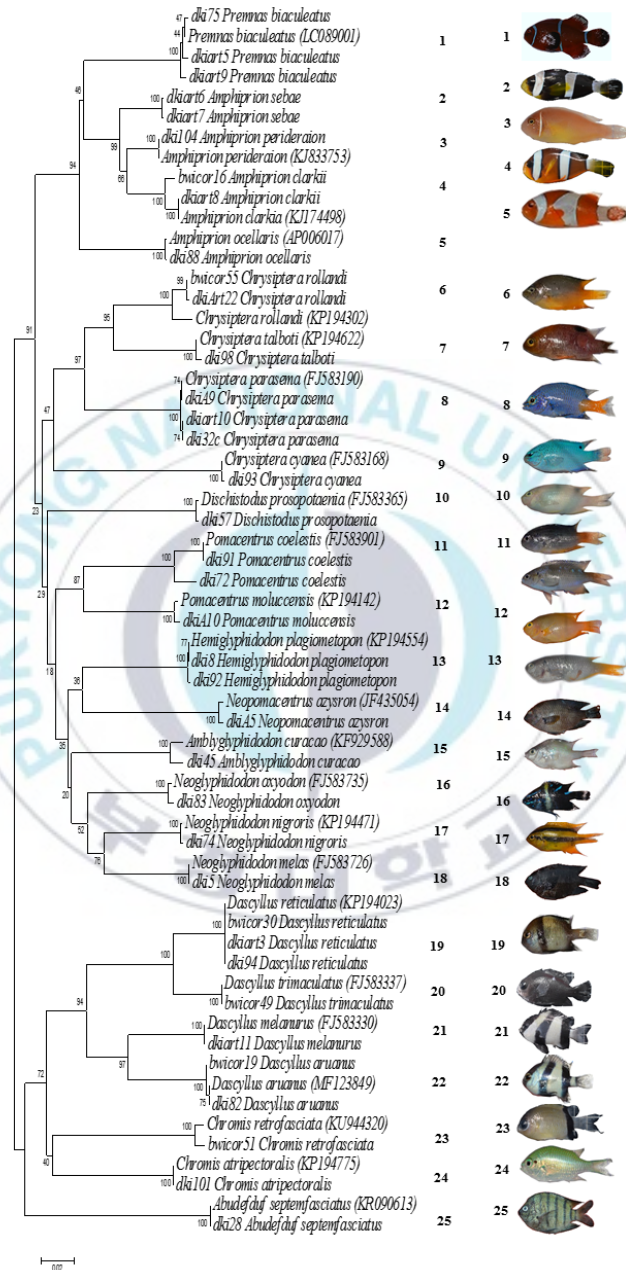


Figure 3. 8 Phylogenetic tree of COI sequences derived from 25 species of family Pomacentridae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

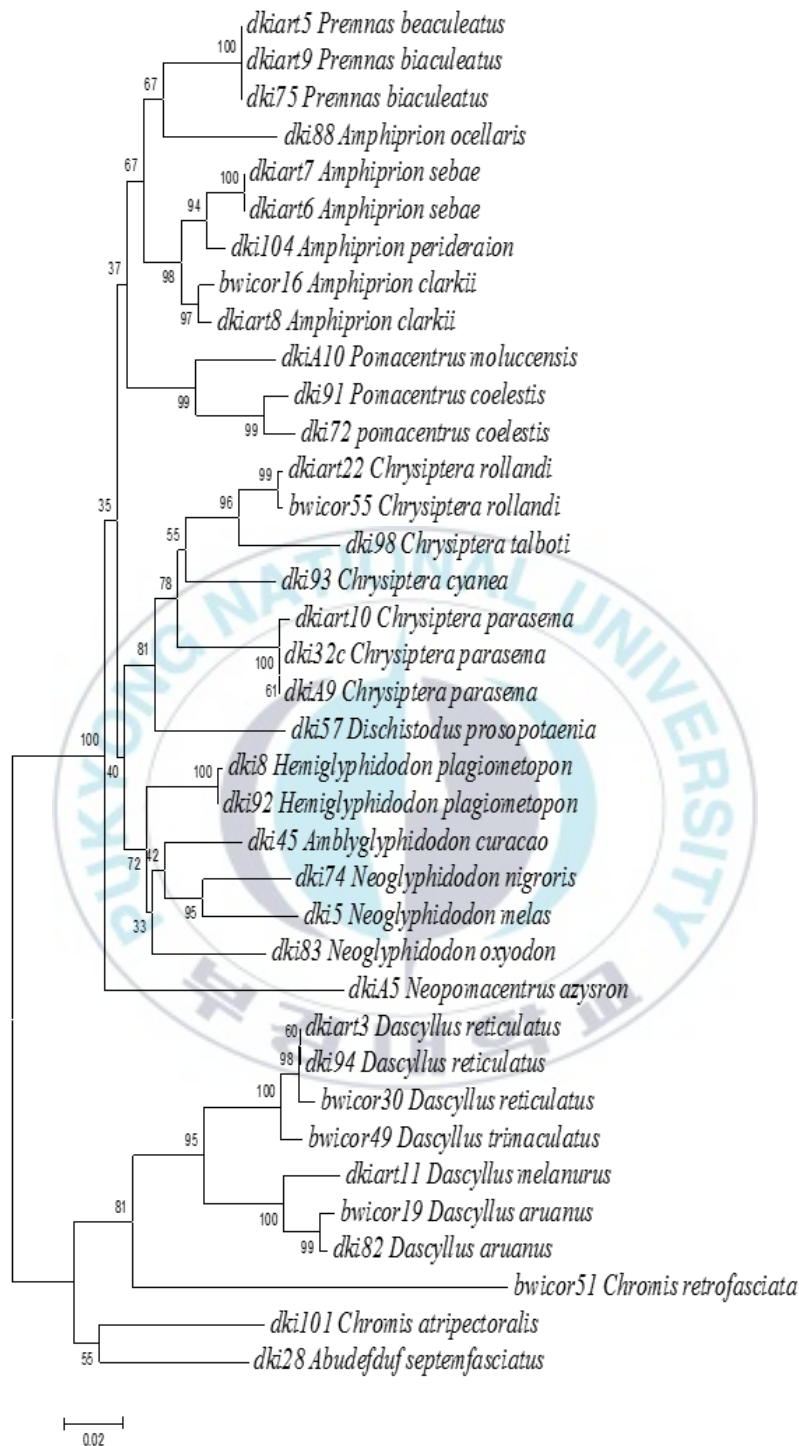


Figure 3. 9 Summary of the Phylogenetic tree of ITS sequence derived from 25 species of Pomacentridae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

The phylogenetic tree of ITS sequence specified four haplotype species (*A. clarkii*, *C. rolandi*, *C. parasema*, *D. reticulatus*, *D. aruanus*) between Th and Ti collection (Figure 3.9).

#### Acanthuridae

We constructed the phylogenetic trees with COI and ITS sequences of seven species in the family Acanthuridae. It was similar to each other (Fig. 3.10). Each species were clustered into tree genera (*Acanthurus*, *Naso* and *Paracanthurus*, respectively). We observed that *Acanthurus nigricans* (*bwicor38*) were haplotype with the COI sequence reference from Keeling Island (accession number EF648269), and *Acanthurus auranticavus* (*dkisp19*) were haplotype with COI sequence reference obtained from fish exported for ornamental fish market in USA (Accession number KC623655). Further, we realized that The COI phylogenetic tree of *Acanthurus nigricans* collected from Ti (*bwicor38*) and *Acanthurus leucosternon* collected from Th (*dkisp14*) has a short distance value in phylogenetic tree (d: 0.013) (Fig 3.10).

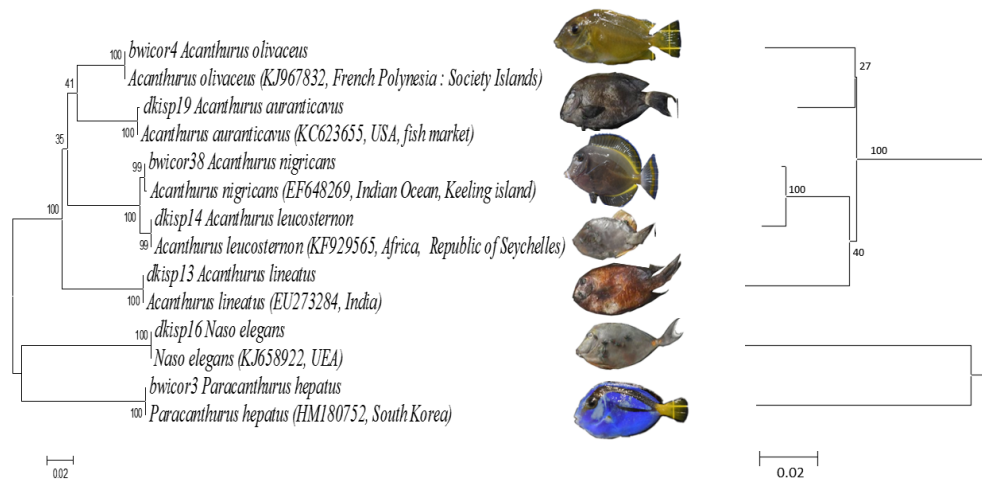


Figure 3. 10 Phylogenetic tree of COI sequences (left) and ITS sequences (right) derived from seven species of Acanthuridae family. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis (MEGA6.0) program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

#### Pomacanthidae

In the family of Pomacanthidae, total of six species of both COI and ITS were clustered with minimum evolution algorithm (Figure 3.11). Its phylogenetic tree produced the similar topology.(Figure 3.11). We inspected the voucher sequences of *Centropyge acantops* from Kenya (Accession number KR 870990), *Pomacanthus sextriatus* from Taiwan and *Chaetodontoplus mesolencus* from Philippines (KP218262) were haplotype with our collection.

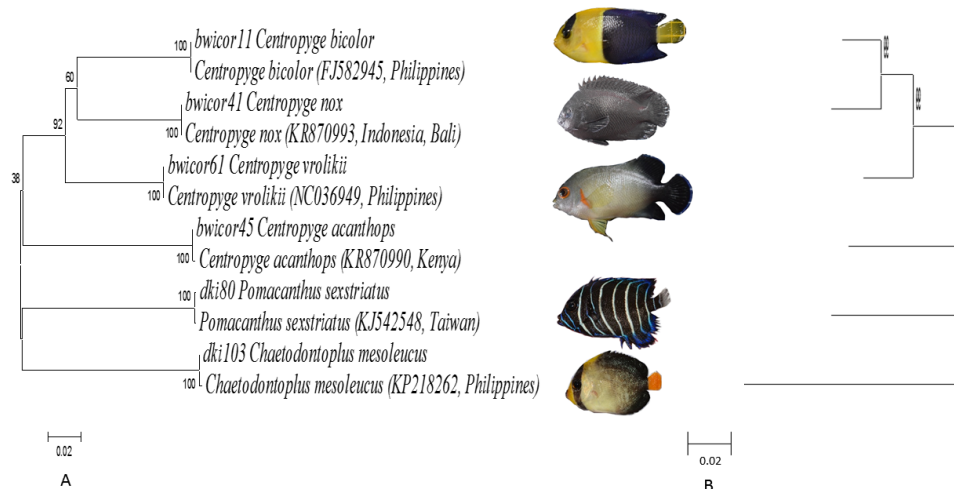


Figure 3. 11 Summary of the Phylogenetic tree of COI sequences (left) and ITS sequences (right) derived from six species of pomacanthidae family. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

## Scaridae

The phylogenetic tree with minimum evolution algorithm for Scaridae was constructed from COI and ITS sequences of five species (Figure 3.12). It was characterized a single species of family Scaridae, *Scarus vetula* (dkiA13) as haplotype along with reference from Vietnam (FJ584083) (Figure 3.12). We also defined *C. blekeeri* (dki21c) which has low COI sequence identity were clustered together as a sister species with *C. gibbus* (Fig. 3.12).



Figure 3. 12 Summary of the Phylogenetic tree of COI sequences (left) and ITS sequences (right) derived from five species of Scaridae family. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

#### Blennidae

The species dataset of family Blennidae were constructed for COI and ITS phylogenetic tree (Fig. 3.13). It was displayed *M. grammistes*, *P. rhinorhynchos*, *S. fasciatus* and *E. bicolor* (Figure 3.13).

In our result, the COI phylogenetic tree of *M. grammistes*, *S. fasciatus* and *E. bicolor* were haplotype with the voucher reference originated from USA (KX230183 ), Japan (NC004412 ) and Sri Lanka (FJ583381) respectively and *P. rhinorhynchos* which collected from Ti were considered as similar species with voucher reference from Australia (KP194373). However, haplotype were appeared between *P. rhinorhynchos* in the ITS phylogenetic tree (Fig. 3.13).

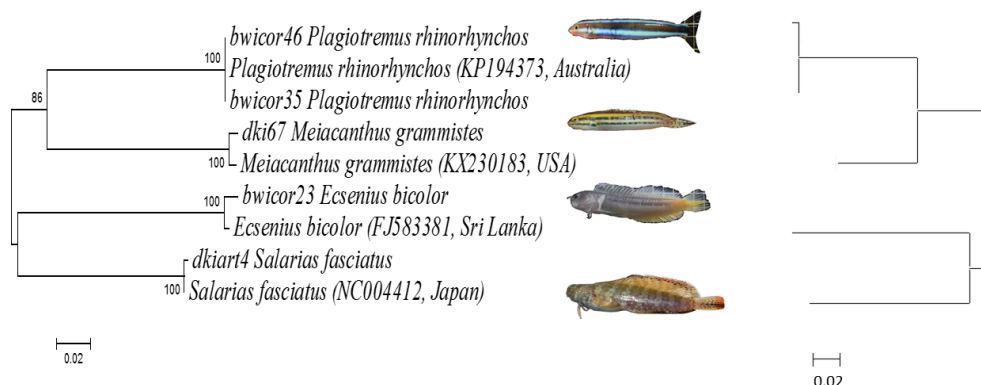


Figure 3.13 Summary of the Phylogenetic tree of COI sequence (left) and ITS sequences (right) derived from four species of Blennidae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

#### Gobiidae

The Gobiidae dataset consists of five species belonging to genera Valencienea and Amblygobius. It presented similar pattern between COI and ITS phylogenetic tree (Fig 3.14). The COI sequences clustering resulted that *V. longipinnis* were haplotype with reference collected from Papua New Guinea (PNG) (accession number HQ536648) and *V. muralis* which collected from Th were haplotype species with the voucher reference collected from Jakarta, Indonesia (Accession number FJ584203) (Figure 3.14). In addition, the COI and ITS phylogenetic trees suggested the putative species, *Amblygobius stethophthalmus* as a sister species of *Amblygobius phalaena* (Fig 3.14).



Figure 3.14 Summary of the Phylogenetic tree of COI sequences (left) and ITS sequences (right) derived from 5 species of Gobiidae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

#### Apogonidae

Six species of family Apogonidae were examined for phylogenetic tree reconstruction with the minimum evolution algorithm. The phylogenetic tree exhibited different position of species among COI and ITS sequences. in which COI phylogenetic tree composed of *O. sealei* and *S. orbicularis* as sister species, and *P. kaudermi*, *C. quinquelineatus*, *A. coccineus*, and putative species *T. macroptera* were sister species (Figure 3.15).

Haplotype were found for all species with the references except *O. sealei* which is similar to voucher from Vietnam (accession number FJ582864). The COI sequence of phylogenetic tree confirmed that *T. macroptera* was parallel with *T. kagoshimasus* and can be suggested as sister species. furthermore, the ITS phylogenetic tree revealed *T. macroptera* was closely related species to *O. sealei* and swiched *S. orbicularis* (Figure 3.16).

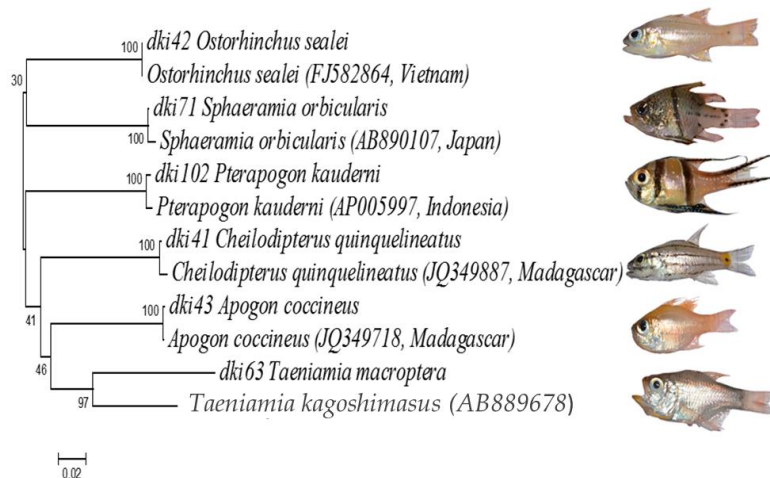


Figure 3. 15 Summary of the Phylogenetic tree of COI sequences derived from six species of Apogonidae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

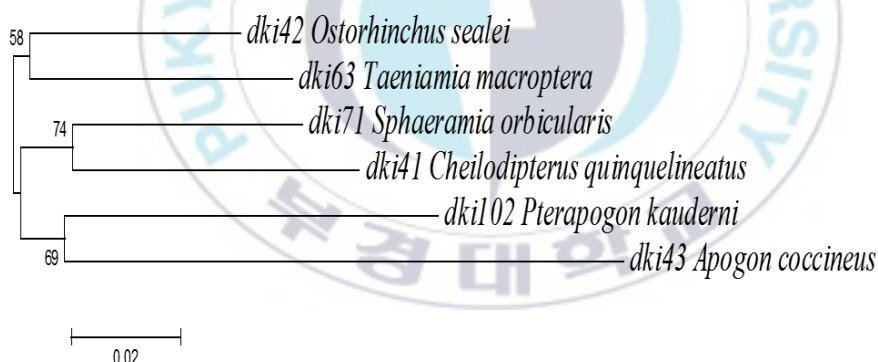


Figure 3. 16 Summary of the Phylogenetic tree of ITS sequences derived from six species of Apogonidae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

### Chaetodontidae

Five species of family Chaetodontidae have been analyzed and the Chaetodon genera appears to be in one branch of the phylogenetic tree, whereas Chelmon

and *Heniochus* were in another (Figure 3.17). Based on COI phylogenetic tree, all the species of family Chaetodontidae are haplotypes to the voucher reference (Fig 3.17). On the other hand, ITS phylogenetic tree illustrated *Chelmon rostratum* collected from Th (dkisp10, dkisp3) and Ti (bwicor21) were haplotype although the COI does not show any sequence difference. We aligned ambiguity species, *Heniochus pleurotaenia* that have less COI sequence identity (95% sequence identity) and have shown the close relationship with the genus *Heniochus* as expected (Fig. 3.17).

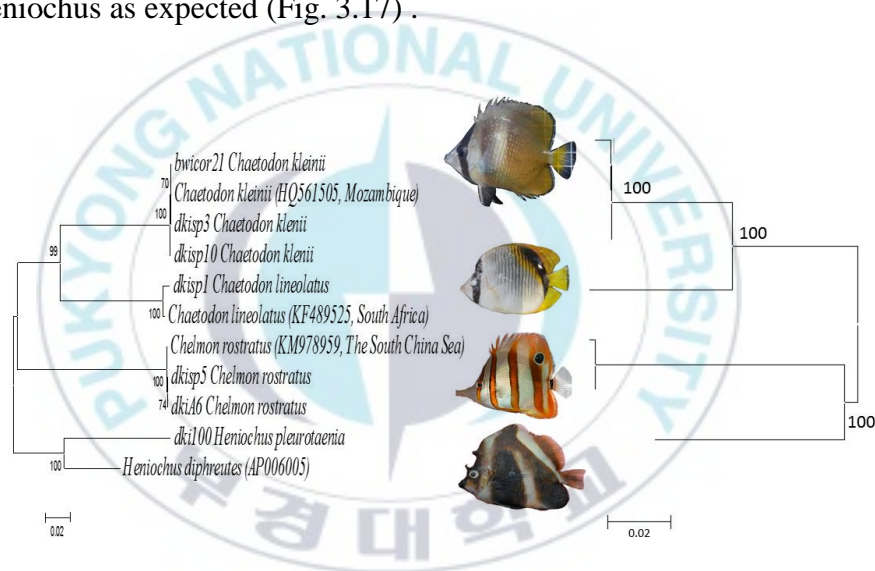


Figure 3. 17 Summary of the Phylogenetic tree of COI sequences (left) and ITS sequence (right) derived from five species of Chaetodontidae. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

#### Other family

A total 21 fish families with the collection less than three species were congested in a single COI and ITS phylogenetic tree (Fig 3.18 and Fig. 3.19, respectively).

We defined that more than 50% (15 species) were haplotype (Fig 3.18). The

haplotype pattern was included for *Caesio xanthonota* (bwicor59) which has 98% COI sequence identity and it confirmed as a correct species.

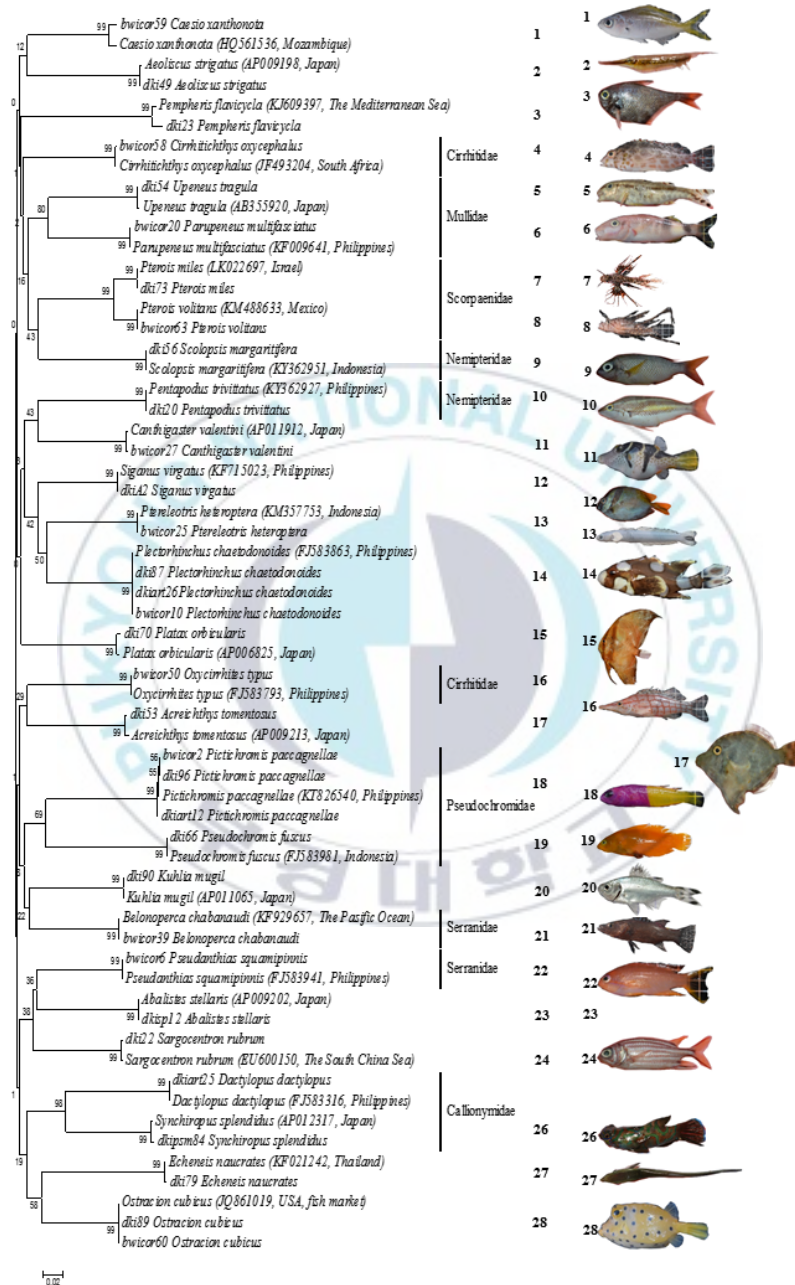


Figure 3. 18 Summary of the Phylogenetic tree of COI sequence derived from 21 species of Other species. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

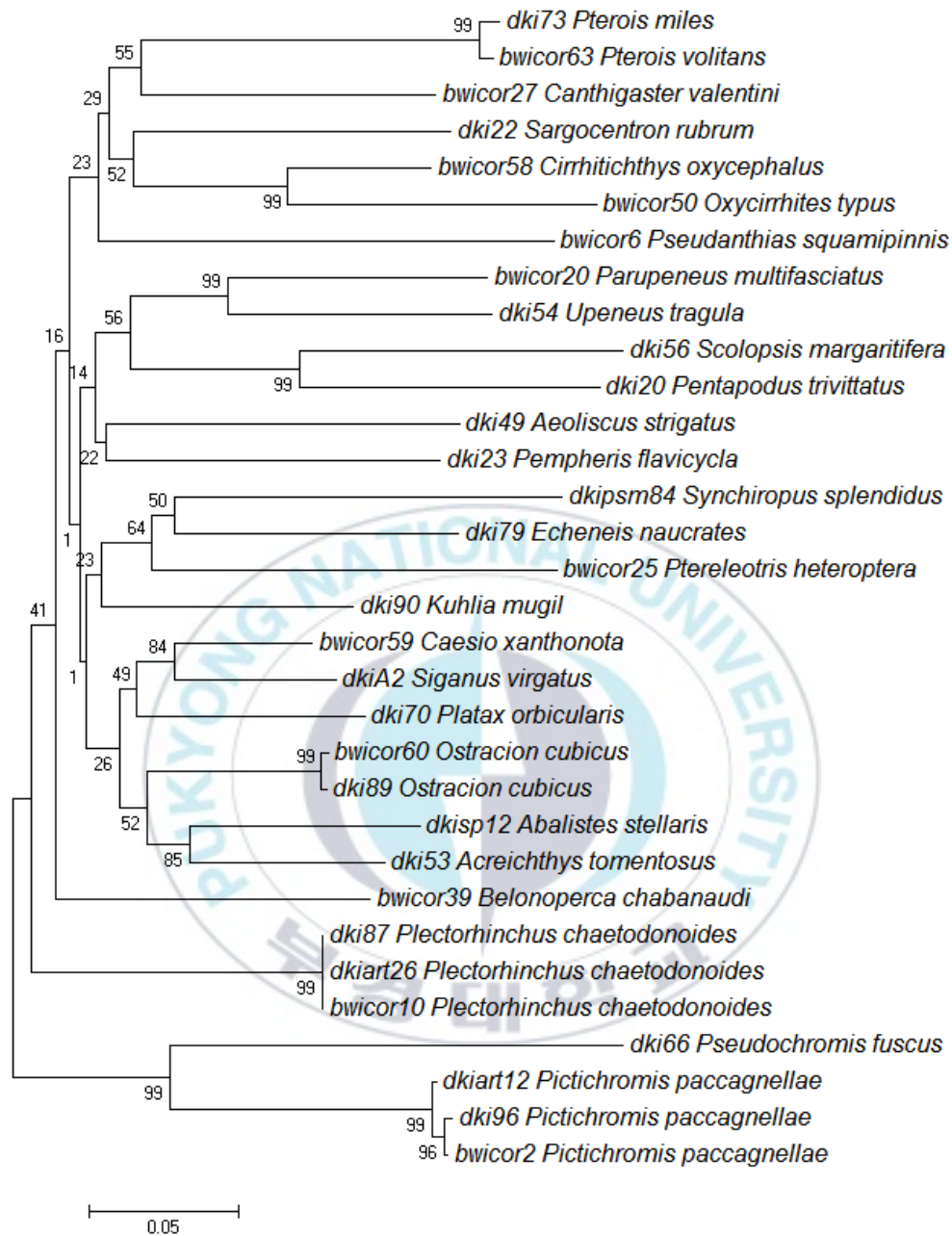


Figure 3. 19 Summary of the Phylogenetic tree of ITS sequence derived from 21 species of the Other family. The phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis ver. 6.0 (MEGA 6, MEGA Inc. Englewood, NJ), program with the Minimum Evolution algorithm. The evolutionary distance were computed using Kimura 2-Parameter Method

### 3.4 Discussion

Our analysis indicate that the sequences were successfully amplified by COI and ITS primer pairs. It was potent to identify and determine 120 fish species in Th and Ti which describes 5 orders (Perciformes, Scorpaeniformes, Tetraodontiformes, Beryciformes, Syngnathiformes) and 30 families. The specimen were dominated by coral reef fish family (Asadi and Andrimida, 2017, Tarigan et al., 2009) and were generally collected for ornamental fish trade by local fisherman (Asiseh and Andriyono, 2015). According to our data, 112 species were successfully identified by COI sequences. In addition, 33 species exhibited similar result between COI and ITS sequences. Subsequently, we discovered that eight species has less than 99% COI sequence identity. Since DNA barcoding works rely on a reference library (Hartvig et al., 2015), an insufficient database was the problem to detect an unknown species. For this reason, the morphological characteristic was employed for identification of eight coral reef fish in which five putative species were added in a new COI sequence database. Further, we added 87 species in a new ITS sequences database.

Species determination is often defined as a basic requirement to understand ecology and biodiversity (Randler, 2008, Steele and Pires, 2011, Barman et al., 2018b). Successful assessing of the real number of species provided a representative ecological picture and helps in fisheries management (Ferris and

Tuomisto, 2013). In contrast, the lack of information and failure to recognize a species potentially lead to wrong diagnosis and undesired fisheries management (Fischer, 2014, Keat-Chuan Ng et al., 2017).

Recent decade molecular approach was broadly utilized as an alternative for fish identification (Zhang and Hanner, 2012). This method were conducted by creating a barcode from a particular DNA with a standardized short fragment with recorded based on a morphological determination as a reference (Hubert and Hanner, 2015). The external and internal features of the morphology of fish historically have been used as the primary source by a taxonomist to distinguish species (Strauss and Bond, 1990). In addition, reef fish survey extensively employed external feature such as general body shape, lateral line, fins, pigmentation and color pattern (Strauss and Bond, 1990, Halford and Thompson, 1994, Caldwell et al., 2016).

The COI nucleotide consensus with 571bp in length exhibited the conserved region from the beginning to the end of the sequence (Fig 3.3a), whereas the ITS nucleotide consensus with 583-1123 bp in length displayed both conserved and variable region (Figure 3.3b). In accordance to the DNA barcoding consensus (Hubert and Hanner, 2015, Patwardhan et al., 2014), several properties should be fulfilled for the genes as a marker such as; universal primers should amplify the target genes (Isenbarger et al., 2008), rapid evolution rate was needed to differentiate the sequences (Palumbi et al., 2002), and easy to align the sequences for the phylogenetic tree analysis purpose. For this

reason, the COI region was proposed as the standard barcode (Ratnasingham and Hebert, 2007). Despite there was another potential region as a marker (Dentinger et al., 2011, Ardura et al., 2013). Although the COI sequence is recognized as a standard barcode, its sequences of at least 500 bp in length was suggested for better resolution (Ward et al., 2009).

In our result, the ITS region provided two benefits. First, the primer pairs were adequate to amplify the target genes. Since our ITS primer pairs targeted ITS region that lies between the mitochondrial rRNA contained conserved region (12S rRNA, 16s rRNA) (Patwardhan et al., 2014, Wang et al., 2000). It can be amplified by universal primer and extensively used in molecular taxonomy and phylogeny study (Jogayya et al., 2013, Yang et al., 2014). In addition, the tRNA-valine were included in the sequence to recognize the target sequence and minimize pseudogenes (Wang et al., 2000). Second, the previous study separately employed 12S rRNA and 16s rRNA as a marker and concluded that this marker can provide genetic information at the species level, but its conserved genes do not contain sufficient nucleotide variation to discriminate closely related congeneric fish species (Cawthorn et al., 2012). However, our sequence enclosed both the 12S rRNA and 16s rRNA regions comprising ITS region located among them which is more variable and suggested contain species-specific (Dugosz and Wiñiewski, 2006). A previous study suggested that the ITS region is widely used in fungal taxonomy and phylogeny (Chen et al., 2016, Badotti et al., 2017, Groenewald et al., 2011, Schoch et al., 2012) and

useful to describe congeneric species (Hao et al., 2010). In addition, the variable region leads that the specific species is sufficient to discriminate the species (Kocher et al., 1989, Vawter and Brown, 1986) even in short sequence (Xie et al., 2015).

In contrast to the COI sequence, the indel (insertion, deletion) and substitution base occurred in the ITS sequences alignment (fig 3.3b). It created a sequence gap and problematic situation for phylogenetic tree reconstruction (Simmons and Ochoterena, 2000, Blair and Murphy, 2010). However, since base indel were common for rRNA sequences (Chu et al., 2006, Kitahara et al., 2010) it was ignored for phylogenetic tree analysis (Qi et al., 2004).

Our phylogenetic reconstruction provides an analysis for the evolutionary relationship of 120 coral reef fishes species from Th, Jakarta, and Ti, Banyuwangi, East Java. In general, our result indicates that both phylogenetic trees have the ability to assign into a species level, and classified into each genera. However, the ITS region described Labridae and Scaridae as the group in the most recent common ancestor and revealed as sister species (Ashlock, 1971). It was reasonable since the Scaridae is a descent derived from Labridae (Schultz, 1958) and morphologically both family have been grouped into Labridae (Westneat and Alfaro, 2005). However, Bellwood (1994) and Parenti and Randall (2011) updated Scaridae is as a sister family of Labridae.

We have recognized the haplotype species in every family group, which is the result of variation and mutation of nucleotides (Salisbury et al., 2003). In addition, the geographical distribution contributes variation within individual of the population (Ward et al., 2008, Delrieu-Trottin et al., 2014). It suggested that collected specimen of haplotype species were Th population and Ti population. However, our specimen collection was deficient and genetic population study was needed for confirmation.

We noticed two species were potentially misclassified. Although BLAST search produced similar species, *Halichoeres melanurus* specimen from Th (dkisp29, dki291) and specimen from Ti (bwiccor22) has the distance value of phylogenetic tree more than 3% (fig 3.6), which make it as a different species (Hebert et al., 2003a). But according to its morphological features, the specimen from Ti is similar to *Halichoeres leucurus* (fishbase.org). Contrary, *Coris gaimard*, and *Coris cuvieri* were likely to be the sub species or even same species since the distance is less than 3%. (Ahti et al., 2016). Similar pattern uncovers in the Acanthuridae family for *Acanthurus nigricans* and *Acanthurus leucosternon*. It confirmed that natural hybrid occurred in Acanthuridae genera and probably mixed the gene flow (Marie et al., 2007). It suggested that DNA Barcoding discovered misclassification, species delineation, and occurrence of hybridization (Zhang and Hanner, 2012, Hubert and Hanner, 2015).

### 3.5 Conclusion

The COI and ITS sequences exhibited ability to distinguish fish species and suggested eight COI sequences and 87 ITS sequence to the database of coral reef fish in the Java Sea, Indonesia. These sequences enriched the existing reference barcode in public database and these would be useful for the study of fish biodiversity in the coral reef ecosystem.



# **Chapter IV. Primer set Comparison for environmental DNA (eDNA) metabarcoding: Fish Biodiversity in the Coral Reef of Java Island, Indonesia**

## **4.1 Introduction**

The abundance and biodiversity fish species in an aquatic ecosystem are considered to be significantly affected by the anthropogenic activities (Argillier et al., 2013). From the reason, many surveys are conducted to obtain information about fish composition and abundance with the different methodologies (Li et al., 2018). The traditional methods can be further classified into the fishery-dependent and fishery-independent approach according to the data collection methods (Pennino et al., 2016). However, both methods still make an adverse effect on the coral reefs ecosystem threatening them.

Alternatively, the aquatic organism can be detected by extracting and analyzing the DNA present in the water bodies using PCR technique. The origin of DNA is the body excretion such as feces, urine, sperm, and egg (Schultz and Lance, 2015). This DNA, called environment DNA (eDNA) are used to test the presence of target species in the monitored water body and widely used for the conservation study to detect invasive and endangered species (Schultz and Lance, 2015, Huver et al., 2015, Brozio et al., 2017, Mahon and Jerde, 2016).

eDNA surveys methods provide a non invasive genetic method to assess biodiversity in marine ecosystems (Thomsen and Willerslev, 2015, Deiner et al., 2017) and has great potential for application in ecology and biodiversity management (Taberlet et al., 2018).

Universal PCR primer pairs (MiFish primer) were recently developed for eDNA metabarcoding of fish species targeting short sequence from a hypervariable region of 12S rRNA (Miya et al., 2015). It was confirmed by detecting 232 fish species (70 families and 152 genera) through multiplex PCR strategy. This approach presented an alternative method for the survey replacing the traditional surveys. However, there are several challenges to replace the traditional fish surveys including potential contaminations, resolution of Mifish region to discriminate species, and low taxon coverage, for example, Elasmobranchii group (sharks and rays).

The subclass Elasmobranchii is an important predatory and scavenging species within aquatic ecosystem (Sims, 2015) and plays an important element within marine foodwebs (Bornatowski et al., 2014). The Elasmobranchii were reported to be at higher risk of extinction than most of the other vertebrates (Dulvy et al., 2014) and threatened due to overfishing (Gemaque et al., 2017). Its conservation has become a world concern since its population has decreased tremendously and have low productivity in relation to teleost fishes (Stevens et al., 2000).

MiFish primers set have short fragments ranging from 163 to 185 bp (Miya et al., 2015). Since the species level detection are important in the study of biodiversity, the short sequences may have low resolution to discriminate the closely related species (Hajibabaei et al., 2006). Therefore increasing the sequence length may be required for those closely related fish species (Schloss et al., 2016).

In order to obtain longer region to identify fish species, we designed Chord primer set targeting ITS (internal transcribed spacer) fragment ranging from 12S rRNA to 16s rRNA genes (288-310bp) which is denoted as Chord region for further research purposes. and compared with MiFish primer sets targeting 12S rRNA genes (163-185 bp) by eDNA metabarcoding in Thousand Island National Park in December 2017. These data would provide an useful information in fish species diversity analysis through eDNA metabarcoding.

## **4.2 Material and Methods**

### **4.2.1 Samples collection**

Water samples were collected from two stations in the Thousand Island national Park in December 2017 (Fig 4.1). All the equipment was sterilized by 10% bleach before use to avoid contamination. One liter water sample was collected in every 100 m by roving the sampling point to obtain twenty liters in total.

Five-liter water samples were subsequently used for subsampling and each litre sample was filtered using 0.45 mm GN-6 membrane (Pall Life sciences, Mexico) as five replications. Membranes were put in the 1 x lysis buffer (Biosesang, South Korea) immediately after filtration and stored on ice. All filtration the sample was frozen at -20°C before extraction.



Figure 4. 1 Sampling point in the Thousand Island National Park (red square)

#### 4.2.2 Genomic DNA extraction

Total genomic DNA was extracted from the filtering membrane using the DNeasy mini kit (Qiagen, Germany) according to the manufacture's instructions. Extracted genomic DNA was quantified using Nanodrop spectrophotometer ND1000 (Thermo Scientific, Waltham, MA, USA) and stored at -80°C for further analysis.

### 4.2.3 Library preparation and Next Generation Sequencing (NGS) analysis

The Nextera XT index kit (Illumina, USA) was used to construct the library for NGS analysis. The first PCR of MiFish primer (MiFish F-R) and Chordata primer (Chord F-R) (Table 4.1) was performed to connect the adapters. An exception to the nested strategy which is performed PCR primer set (Chord 1<sup>st</sup> F-R) reaction to isolated 420 bp target sequence before connecting the adapter with Chord F-R primer set (table 4.1). The adapter primers were forward adapter sequences (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse adapter sequences (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') respectively. The final PCR used N7xx and S5xx primer including Illumina Nextera XT indexing primers. Libraries finally were constructed on Miseq 600-cycle Reagent Kit v3 (Illumina, USA).

Table 4. 1 Primer list were used in this study

Primer	Sequences (5' to 3')	Target region	Reference
MiFish F	GTCGGTAAACTCGTGCCAGC	12S rRNA	(Miya et al., 2015)
MiFish R	GTTTGACCCTAATCTATGGGGTGATAC		
Chord F	AAGTCGTAACAYGGTAAG	Chord region	In this study
Chord R	CATGATGCAAAAGGTACRRG		

#### 4.2.4 Bioinformatics analysis of NGS data

The paired-end sequences were filtered from the obtained raw read. Low-quality read ( $QV < 20$ ) and short read length were truncated at CLC Genomic workbench v 8.0 (CLC Bio, USA). Mothur software was employed to assemble paired-end mergeds and using cut off based on size selection (MiFish167- 180bp, and Chord primer 300bp). Overlapping sequences of more than 6 bp, and omitting mismatches with the  $pdiffs = 0$  options set (Schloss et al., 2009). All sequences with 99% similarity were clustered into Operational Taxonomic Unit (OTUs) and chimeras were detected using UCHIME software v8.1 (Edgar et al., 2011). The processed sequences were subjected to BLAST Searches database (BLASTN version 2.2.30+) under the condition if sequence identity between queries and the top BLAST hit were  $\geq 97\%$ , the assembled sequence was assigned to the top hit species (species level). If the sequence identity was  $\geq 90\%$  to 97 %, the assembled sequence was assigned to the top hit genus or taxon (genus level), and sequences with less than 90% identity were as “unknown”. The result from the BLAST searches was automatically tabulated with scientific names, higher level taxon, the total number of the reads and representative sequences. Moreover, biological information for each detected species was obtained from WoRMS (<http://www.marinespecies.org/>) and FishBase (<http://www.fishbase.org>).

## 4.3 Result

### 4.3.1 Environmental DNA universal primer comparison

We compared NGS data between MiFish primer and newly designed Chordata primer (Chord primer) (Table 4.2). After trimming the raw reads, total 105,860 and 84,230 merged reads were obtained by MiFish primer and by Chord primer, respectively (Table XXX). 146 genotypes were obtained by MiFish primer, whereas 115 genotypes were obtained by Chord primer. It also represent higher fish sequence reads proportion of MiFish primer (91.7 %), than Chord primer (58.3 %). In contrast, for an unknown fish species (<90% similarity) reads proportion of Chord primer (39.7%) was higher than MiFish primer (7.8 %), and a small portion (less than 5%) were assigned as nonfish species (table 4.2).

The NGS sequences BLAST search detected 115 OTUs were fish at species level ( $\geq 97\%$  similarity) for MiFish primer and 44 for Chord primer. Furthermore, the OTUs with lower similarity ( $\geq 90\%$  and  $< 97\%$  similarity) was assigned as genus level were higher for Chord primer (30 species) than MiFish primer (15 species) (Table 4.2). According to the Fishbase ([www.fishbase.org](http://www.fishbase.org)), it was dominated by coral reef-associated fish (50% - 77.86%), and a small portion were noncoral reef associated fish (8.4%-14.86%). Higher proportion of the coral reef- associated fish by MiFish primer 77.86 % may have come from the limited numbers of Chord sequence in the database, which suggests the supplement of its sequence data (Fig 4.2).

Table 4. 2 A summary of the NGS contig reads for the primers DNA metabarcoding

Sequence reads	MiFish primer			Chord primer		
	Merged	Proportion (%)	genotypes	Merged	Proportion (%)	genotypes
Total Reads	105,860		146	84,230		115
Fish species :						
≥ 97% identity	92,397	87.3	115	29,858	35.4	44
≥ 90% and <97% identity	4,656	4.4	15	19,249	22.9	30
Unknown (<90% identity )	8,222	7.8	12	33,404	39.7	37
Nonfish species	585	0.6	4	1,719	2.0	4

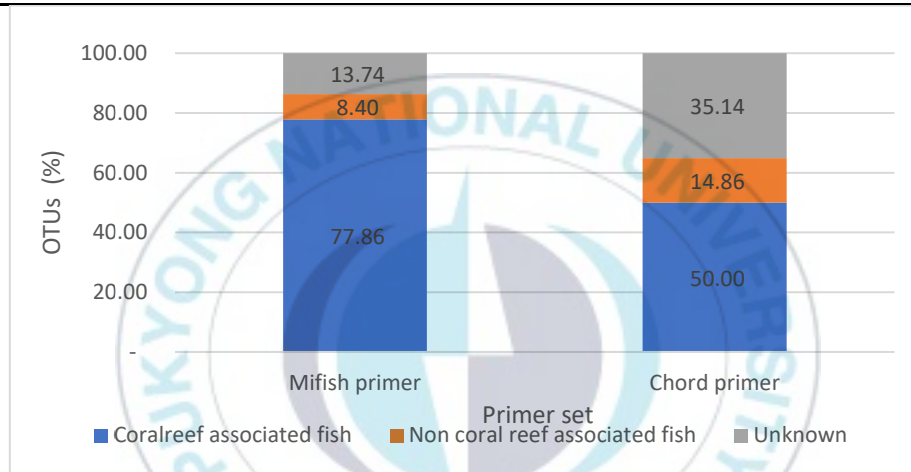


Figure 4. 2 Fish species composition based on the habitat (according to [www.fishbase.org](http://www.fishbase.org) )

#### 4.3.2 The taxonomic composition

The MiFish primer presented one subclass, eight orders, 34 families, and 85 genera while Chord primer amplified two subclasses, nine orders, 31 families, 60 genera. Among 130 species identified in Thousand Island by the MiFish primer in this study, 72 species have already been reported by the previous surveys. Due to the limited numbers of database, only 30 species were identified as the previously identified species in the result by Chord primer set (Table 4.3).

the remaining identified species have not been reported in Thousand Island. We classified the species in each family and the family with highest proportion was Pomacentridae (21.37%) followed by Labridae (11.45%), and Gobiidae (9.16%) by MiFish primer, whereas the Gobiidae (13.51%), Labridae (8.11%), and Pomacentridae, Apogonidae, Carangidae, Serranidae (6.76%), were among the largest families for Chord primer (Fig 4.3). We also investigated that though two primers have different length and target sequence both the primers shared 34 species and 24 families. Further the unique species and family of MiFish primer were higher than Chord primer (fig 4.4).

Although MiFish primer exhibited various taxonomic compositions, and higher in total number of family and species. We realized that Elasmobranchii was not amplified, while three species of Elasmobranchii from Dasyatidae and Myliobatidae were detected by Chord primers. In addition, we figured out the Bluespotted fantail ray (*Taeniura lymma*) from the family of Dasyatidae was near threatened species according the IUCN Red List ([www.iucnredlist.org](http://www.iucnredlist.org)).

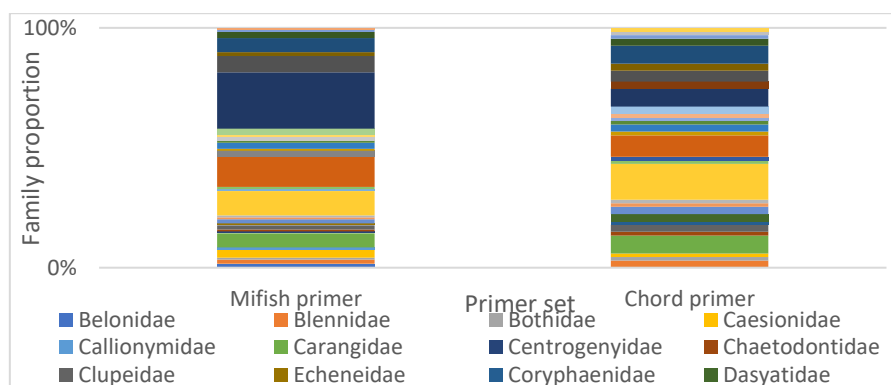
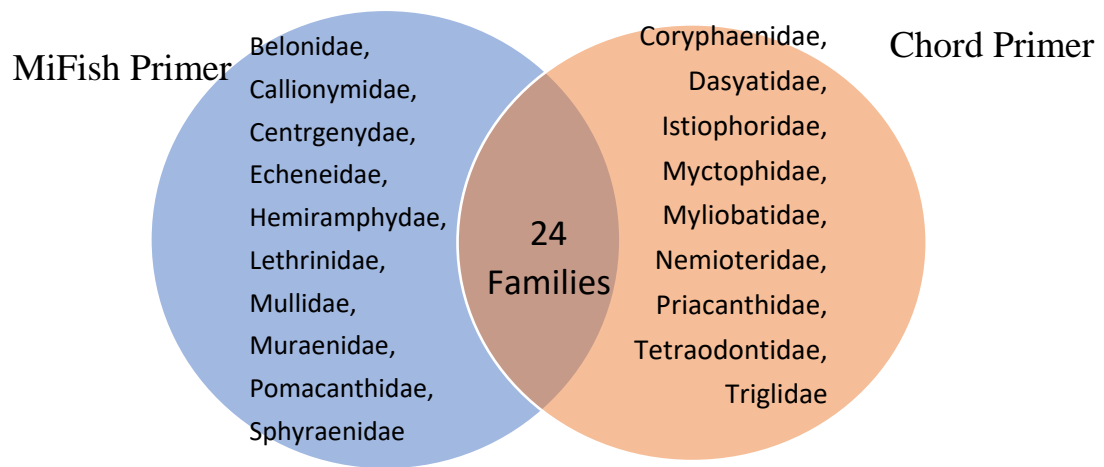
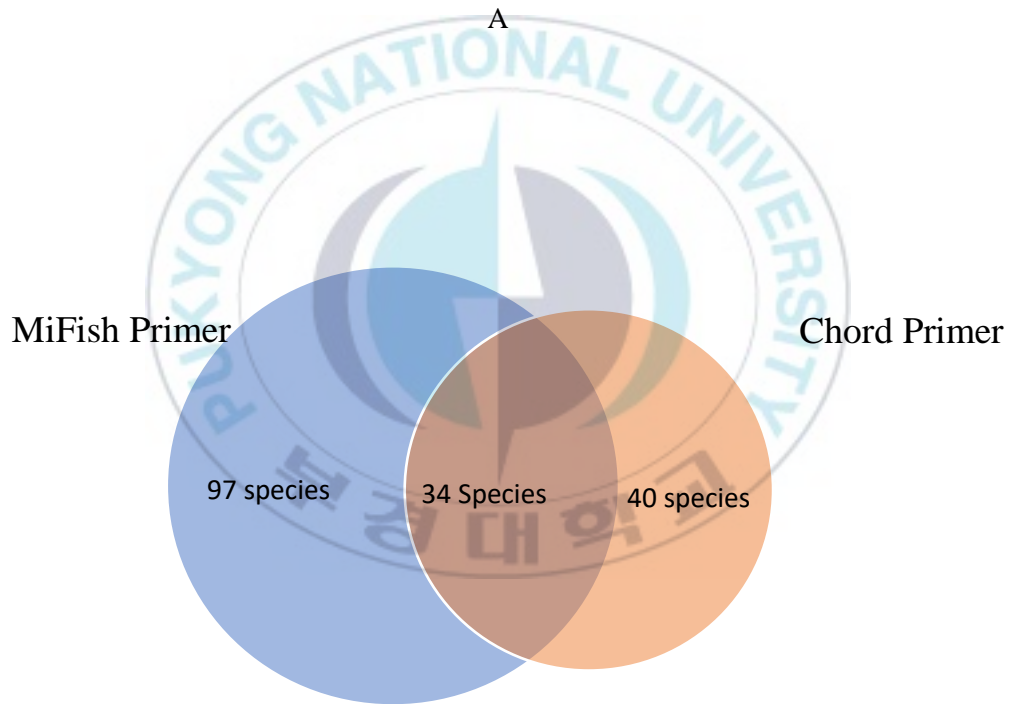


Figure 4. 3 Fish structure at family level between MiFish and Chord primer in Thousand Island



A



B

Figure 4. 4 Venn diagram illustrating (A) the list of shared and unique family and (B) number of shared a unique species obtained by MiFish and Chord primer

Table 4. 3 Taxonomic comparison of eDNA fish biodiversity from Thousand Island National Park with different primer sets

Higher classification	Species	Primer set		Previous study in Thousand Island
		MiFish	Chordata	
Class Elasmobranchii				
Order Myliobatiformes				
Family Dasyatidae	<i>Pastinachus atrus</i>	-n/a-	12	-n/a-
	<i>Taeniura lymma</i>	-n/a-	129	Madduppa et al., 2013
Family Myliobatidae	<i>Aetobatus sp.</i>	-n/a-	15	-
Class Actinopterygii				
Order Anguilliformes				
Family Muraenidae	<i>Gymnothorax richardsonii</i>	321		-n/a-
Order Atheriniformes				
Family Atherinidae	<i>Atherinomorus lacunosus</i>	104		-n/a-
	<i>Atherinomorus regina</i>	3854		-n/a-
	<i>Hypoatherina celebesensis</i>	178		-n/a-
	<i>Hypoatherina temminckii</i>	557		-n/a-
	<i>Hypoatherina sp.</i>		50	-n/a-
Order Beloniformes				
Family Belonidae	<i>Strongylura incisa</i>	538		-n/a-
	<i>Tylosurus crocodilus</i>	256		-n/a-
Family Hemiramphidae	<i>Hemiramphus far</i>	99		Hartati et al., 2010
Order Beryciformes				
Family Holocentridae	<i>Myripristis sp.</i>		28	Dhahiyat et al., 2003
	<i>Myripristis murdjan</i>	232		tarigan et al., 2008
Order Clupeiformes				
Family Clupeidae	<i>Sardinella sp.</i>	10		-n/a-
	<i>Spratelloides gracilis</i>	241	758	-n/a-
	<i>Sardinella jussieu</i>		13036	-n/a-
	<i>Encrasicholina pseudoheteroloba</i>	103		-n/a-
Family Engraulidae	<i>Encrasicholina punctifer</i>	4761	21	-n/a-
	<i>Engraulis japonicus</i>		2494	-n/a-
Order Myctophiformes				
Family Myctophidae	<i>Benthoosema sp.</i>		4587	-n/a-
Order Perciformes				
Family Apogonidae	<i>Cheilodipterus sp.</i>		80	Madduppa et al., 2013
	<i>Fowleria isostigma</i>	49	83	-n/a-
	<i>Nectamia sp.</i>	1083	297	
	<i>Ostorhinchus compressus</i>	236	30	Iskandar & Mawardi, 1997
	<i>Pristicon rhodopterus</i>	426		Kusnanto, 2015
	<i>Sphaeramia nematoptera</i>	239		Setyawan & Wijoyo, 2009
	<i>Taeniamia kagoshimasus</i>		21	-n/a-
	<i>Taeniamia zosterophora</i>	406		-n/a-
	<i>Zoramia leptacantha</i>	1349		-n/a-

Table 4.3. (Continued)

Higher classification	Species	Primer set		Previous study in Thousand Island
		MiFish	Chordata	
Order Perciformes				
Family Blennidae	<i>Atrosalarias holomelas</i>	250		-n/a-
	<i>Salarias fasciatus</i>	2614	221	Setyawan & Wijoyo, 2009
	<i>Salarias sp.</i>		18	
Family Caesionidae	<i>Caesio cuning</i>	1690	255	Iskandar & Mawardi, 1997
	<i>Caesio lunaris</i>	22		Mujiyanto, 2014
	<i>Caesio sp.</i>	12		
	<i>Pterocaesio digramma</i>	615		Madduppa et al., 2013
Family Callionymidae	<i>Callionymus enneactis</i>	126		-n/a-
Family Carangidae	<i>Alepes djedaba</i>	1140	36	-n/a-
	<i>Alepes vari</i>	241		-n/a-
	<i>Caranx tille</i>		51	Hartati et al., 2010
	<i>Decapterus russelli</i>	58		-n/a-
	<i>Gnathanodon speciosus</i>	128		Hartati et al., 2010
	<i>Megalaspis cordyla</i>	101	2455	-n/a-
	<i>Selar crumenophthalmus</i>	240	197	Hartati et al., 2010
	<i>Selaroides leptolepis</i>	229	36	Hartati et al., 2010
Family Centrogenyidae	<i>Centrogenys vaigiensis</i>	232		-n/a-
Family Chaetodontidae	<i>Chaetodon octofasciatus</i>	871		Johan, 2004
	<i>Chaetodon sp.</i>		239	
Family Coryphaenidae	<i>Coryphaena hippurus</i>		950	-n/a-
Family Echeineidae	<i>Echeneis naucrates</i>	31		
				Hutomo & djamali, 1982
Family Ehippidae	<i>Platax orbicularis</i>	109		
	<i>Platax sp.</i>		18	-n/a-
Family Gerreidae	<i>Gerres oyena</i>	1633	236	-n/a-
Family Gobiidae	<i>Acentrogobius sp.</i>		237	-n/a-
	<i>Amblygobius sp.</i>	1379	80	-n/a-
	<i>Asterropteryx semipunctata</i>	933		-n/a-
	<i>Asterropteryx sp.</i>	16	38	-n/a-
	<i>Atherinomorus sp.</i>		114	
	<i>Cryptocentrus caeruleomaculatus</i>	349		-n/a-
	<i>Cryptocentrus sp.</i>		11	-n/a-
	<i>Eviota sp.</i>	221		
	<i>Exyrias belissimus</i>	91		Setyawan et al., 2011
	<i>Exyrias sp.</i>		38	-n/a-
	<i>Glossogobius giuris</i>	105	18	-n/a-
	<i>Gnatholepis sp.</i>	655	42	-n/a-
	<i>Istigobius decoratus</i>	87		Aini, 2014
Family Gobiidae	<i>Valenciennesa longipinnis</i>	290	87	Setyawan et al., 2011
	<i>Valenciennesa sp.</i>	271	12	-n/a-
	<i>Valenciennesa strigata</i>	121		Setyawan et al., 2011
Family Istiophoridae	<i>Istiompax indica</i>		18	-n/a-

Table 4.3. (Continued)

Higher classification	Species	Primer set		Previous study in Thousand Island
		MiFish	Chordata	
Order Perciformes				
Family Labridae	<i>Anampses meleagrides</i>	279		Setyawan et al., 2011
	<i>Cheilinus fasciatus</i>	28		Valentino, 2004
	<i>Choerodon anchorago</i>	2682		Setyawan & Wijoyo, 2009
	<i>Choerodon sp.</i>		206	Iskandar & Mawardi, 1997
	<i>Cirrhilabrus cyanopleura</i>	229		Kusnanto, 2015
	<i>Coris pictoides</i>	164		-n/a-
	<i>Epibulus insidiator</i>	51		Setyawan et al., 2011
	<i>Halichoeres argus</i>	8037		Setyawan et al., 2011
	<i>Halichoeres chloropterus</i>		36	Setyawan & Wijoyo, 2009
	<i>Halichoeres leucurus</i>	33		Madduppa et al., 2013
	<i>Halichoeres miniatus</i>		65	-n/a-
	<i>Halichoeres nebulosus</i>	124		-n/a-
	<i>Halichoeres sp.</i>	408	873	Madduppa et al., 2013
	<i>Hemigymnus melapterus</i>	720		Setyawan & Wijoyo, 2009
	<i>Hologymnosus annulatus</i>		10	-n/a-
	<i>Hologymnosus doliatus</i>	462		-n/a-
	<i>Labroides dimidiatus</i>	278		Dhahiyat et al., 2003
	<i>Labropsis manabei</i>	139		-n/a-
	<i>Stethojulis trilineata</i>		13	-n/a-
	<i>Thalassoma bifasciatum</i>	712		-n/a-
Family Lethrinidae	<i>Lethrinus harak</i>	1025		Setyawan & Wijoyo, 2009
	<i>Lethrinus lentjan</i>	280		Setyawan & Wijoyo, 2009
	<i>Lethrinus obsoletus</i>	624		Setyawan & Wijoyo, 2009
Family Lutjanidae	<i>Lutjanus fulviflamma</i>	245		Setyawan & Wijoyo, 2009
	<i>Lutjanus sp.</i>		1468	Setyawan & Wijoyo, 2009
Family Mugilidae	<i>Mugil cephalus</i>	299	183	-n/a-
Family Mullidae	<i>Parupeneus heptacanthus</i>	76		-n/a-
	<i>Upeneus tragula</i>	220		Setyawan & Wijoyo, 2009
Family Nemipteridae	<i>Nemipterus sp.</i>		47	Hartati et al., 2010
	<i>Pentapodus sp.</i>		219	-n/a-
Family Pomacanthidae	<i>Centropyge bicolor</i>	414		Anggraini et al., 2018
	<i>Centropyge fisheri</i>	121		-n/a-
	<i>Centropyge vrolikii</i>	655		Anggraini et al., 2018
Family Pomacentridae	<i>Abudefduf saxatilis</i>	223		Dhahiyat et al., 2003
	<i>Abudefduf sp.</i>	24	152	-n/a-
	<i>Abudefduf vaigiensis</i>	4159	3267	Aini, 2014
	<i>Acanthochromis sp.</i>	15		
	<i>Amblyglyphidodon curacao</i>	794		tarigan et al., 2008
	<i>Amblyglyphidodon ternatensis</i>	2779		Madduppa et al., 2013

Table 4.3. (Continued)

Higher classification	Species	Primer set merged reads		Previous study in Thousand Island
		MiFish	Chordata	
Order Perciformes				
Family Pomacentridae	<i>Amphiprion clarkii</i>		47	Setyawan et al., 2011
	<i>Amphiprion ephippium</i>	10		-n/a-
	<i>Amphiprion sp.</i>		7460	
	<i>Cheiloprion labiatus</i>	440		Madduppa et al., 2013
	<i>Chrysiptera rollandi</i>	21		
	<i>Chrysiptera sp.</i>	109		Setyawan et al., 2011
	<i>Chrysiptera unimaculata</i>	1385		-n/a-
	<i>Dascyllus trimaculatus</i>	41		Setyawan et al., 2011
	<i>Dischistodus chrysopoecilus</i>	1261		Setyawan et al., 2011
	<i>Dischistodus perspicillatus</i>	1031		Madduppa et al., 2013
	<i>Hemiglyphidodon plagiometopon</i>	10001		Madduppa et al., 2013
	<i>Hemiglyphidodon sp.</i>	39		Setyawan & Wijoyo, 2009
	<i>Neopomacentrus azysron</i>	1188		Johan, 2004
	<i>Neopomacentrus sp.</i>	17		
	<i>Pomacentrus albicaudatus</i>	1286		-n/a-
	<i>Pomacentrus alexanderae</i>	1589		Madduppa et al., 2013
	<i>Pomacentrus bankanensis</i>	247		Setyawan & Wijoyo, 2009
	<i>Pomacentrus brachialis</i>	12		Iskandar & Mawardi, 1997
	<i>Pomacentrus burroughi</i>	295		tarigan et al., 2008
	<i>Pomacentrus chrysurus</i>	191		-n/a-
	<i>Pomacentrus grammorhynchus</i>	14		-n/a-
	<i>Pomacentrus moluccensis</i>	200		Setyawan & Wijoyo, 2009
	<i>Pomacentrus tripunctatus</i>	158		-n/a-
	<i>Pomacentrus vaiuli</i>	222		Setyawan & Wijoyo, 2009
	<i>Premnas sp.</i>		65	
Family Priacanthidae	<i>Priacanthus arenatus</i>		1844	-n/a-
	<i>Priacanthus sp.</i>	92	102	-n/a-
Family Scaridae	<i>Chlorurus oedema</i>	47		-n/a-
	<i>Hipposcarus longiceps</i>	4761		Tarigan et al., 2008
	<i>Scarus forsteni</i>	144	15	Valentino, 2004, skripsi
	<i>Scarus ghobban</i>	42	159	Iskandar & Mawardi, 1997
	<i>Scarus niger</i>	69		Setyawan & Wijoyo, 2009
	<i>Scarus quoyi</i>	1042		Madduppa et al., 2013
Family Scaridae	<i>Scarus rivulatus</i>	86		Hartati et al., 2010
	<i>Scarus schlegeli</i>			-n/a-
	<i>Scarus sp.</i>	161	2519	Madduppa et al., 2013

Table 4.3. (Continued)

Higher classification	Species	Primer set		Previous study in Thousand Island
		MiFish	Chordata	
Order Perciformes				
Family Scombroidae	<i>Rastrelliger brachysoma</i>	4893	24	Hartati et al., 2010
	<i>Rastrelliger kanagurta</i>		119	Hartati et al., 2010
Family Serranidae	<i>Diploprion bifasciatum</i>	227	330	-n/a-
	<i>Cephalopholis boenak</i>	834		Utami, 2009
	<i>Cephalopholis cyanostigma</i>	834		Jimmi et al., 2011
	<i>Cephalopholis sp.</i>	607	84	Madduppa et al., 2013
	<i>Epinephelus fuscoguttatus</i>	3602	73	Setyawan & Wijoyo, 2009
	<i>Epinephelus ongus</i>	1061		-n/a-
	<i>Epinephelus quoyanus</i>	12	758	Jimmi et al., 2011
	<i>Epinephelus sp.</i>	122	348	Utami, 2009
Family Siganidae	<i>Pseudanthias sp.</i>			-n/a-
	<i>Siganus canaliculatus</i>	2560	342	Hartati et al., 2010
	<i>Siganus fuscescens</i>			-n/a-
	<i>Siganus guttatus</i>	33	120	Iskandar & Mawardi, 1997
	<i>Siganus vermiculatus</i>	3242		Hartati et al., 2010
	<i>Siganus virgatus</i>	200		Utami, 2009
Family Sphyraenidae	<i>Sphyraena jello</i>	320		-n/a-
Order Pleuronectiformes				
Family Bothidae	<i>Bothus pantherinus</i>		385	-n/a-
	<i>Tosarhombus sp.</i>	250		-n/a-
Family Monacanthidae	<i>Paramonacanthus choirocephalus</i>		49	
Family Soleidae	<i>Pardachirus pavoninus</i>	25	90	-n/a-
Order Scorpaeniformes				
Family Triglidae	<i>Chelidonichthys kumu</i>		205	-n/a-
Order Tetraodontiformes				
Family Balistidae	<i>Pseudobalistes flavimarginatus</i>	597	13	Utami, 2009
	Family Monacanthidae	<i>Acreichthys hajam</i>	289	
	<i>Aluterus scriptus</i>	139		-n/a-
	<i>Cantherhines sp.</i>		338	-n/a-
	<i>Monacanthus chinensis</i>	376		Setyawan et al., 2011
Family Tetraodontidae	<i>Arothron hispidus</i>		31	-n/a-
Unidentified species	<i>Unknown . Abalistes stellaris</i>		25	
	<i>U. Ablennes hians</i>		116	
	<i>U. Abudedefduf hoefleri</i>		1239	
	<i>U. Acentrogobius qhy-</i>	764		
	<i>U. Amoya chusanensis</i>		44	

Table 4.3. (Continued)

Higher classification	Species	Primer set		Previous study in Thousand Island
		MiFish	Chordata	
Unidentified species	Unidentified <i>Amphiprion clarkii</i>		51	
	<i>U. Anodontostoma chacunda</i>		1757	
	<i>U. Asterropteryx semipunctata</i>		606	
	<i>U. Atherinomorus lacunosus</i>		57	
	<i>U. Atrosalarias holomelas</i>	505		
	<i>U. Balistes vetula</i>		19	
	<i>U. Bodianus axillaris</i>	246		
	<i>U. Bollmannia macropoma</i>		1067	
	<i>U. Branchiostegus albus</i>		33	
	<i>U. Callionymus beniteguri</i>	378		
	<i>U. Cheilinus undulatus</i>		646	
	<i>U. Coelorinchus scaphopsis</i>		11	
	<i>U. Culaea inconstans</i>		462	
	<i>U. Dermogenys pusilla</i>		334	
	<i>U. Eviota saipanensis</i>	53		
	<i>U. Gadus morhua</i>		31	
	<i>U. Gobiosoma ginsburgi</i>		10	
	<i>U. Gymnothorax formosus</i>	391		
	<i>U. Gymnothorax maderensis</i>	330		
	<i>U. Halichoeres trimaculatus</i>		50	
	<i>U. Herichthys cyanoguttatus</i>		49	
	<i>U. Hypoatherina woodwardi</i>	1039		
	<i>U. Johnius belangerii</i>	183		
	<i>U. Lactoria diaphana</i>		33	
	<i>U. Lateolabrax latus</i>		42	
	<i>U. Maurolicus muelleri</i>		372	
	<i>U. Microgobius thalassinus</i>	151	20	
	<i>U. Myersina macrostoma</i>	301		
	<i>U. Opistognathus jacksoniensis</i>		178	
	<i>U. Ostorhinchus flagelliferus</i>		18	
	<i>U. Parexocoetus brachypterus</i>		85	
	<i>U. Perca flavescens</i>		69	
	<i>U. Pictilabrus laticlavus</i>		16	
	<i>U. Ptereleotris carinata</i>		191	
<i>U. Salaria fasciatus</i>		115		
<i>U. Scolopsis vosmeri</i>		333		
<i>U. Scorpaenopsis cirrosa</i>		178		
<i>U. Siniperca undulata</i>		155		
<i>U. Spratelloides gracilis</i>	3881			
<i>U. Synagrops japonicus</i>		24829		
<i>U. Synodus variegatus</i>		30		
<i>U. Toxotes chatareus</i>		26		
<i>U. Valenciennes longipinnis</i>		107		

Table 4. 4 Improvement of Chord region database in species identification

Low similarity species		Chord region local BLAST result	
Species name	Similarity (%)	Species name	Similarity (%)
<i>Unknown_Cheilinus undulatus</i>	0.88	<i>Cheilinus sp</i>	0.93
<i>Unknown_Scolopsis vosmeri</i>	0.89	<i>Scolopsis ciliata</i>	0.99
<i>Pentapodus sp.</i>	0.94	<i>Pentapodus trivittatus</i>	100
<i>Choerodon sp.</i>	0.91	<i>Choerodon anchorago</i>	0.99
<i>Unknown_Ptereleotris carinata</i>	0.87	<i>Ptereleotris heteroptera</i>	100
<i>Siganus guttatus</i>	0.98	<i>Siganus virgatus</i>	100
<i>Cheilodipterus sp.</i>	0.9	<i>Cheilodipterus quinquelineatus</i>	0.99
<i>Unknown_Halichoeres trimaculatus</i>	0.89	<i>Hemigymnus melapterus</i>	100
<i>Unknown_Herichthys cyanoguttatus</i>	0.89	<i>Dascyllus trimaculatus</i>	100
<i>Unknown_Amphiprion clarkii</i>	0.89	<i>Pomacentrus sp</i>	0.94
<i>Valenciennea sp.</i>	0.93	<i>Valenciennea muralis</i>	100

From Chord primer result, we identified that 38 were unknown OTUs species (table 4.4). In order to increase sequence detection, we employed updated Chord region database from previous chapter and performing Local BLAST. The result revealed new nine species at species level and two species at genus level (Table 4.4).

#### 4.3.3 Species diversity

The diversity represented by Shannon index were analysed to see the overall biodiversity of the samples, and the Pielou's evenness measured that the species are evenly distributed within a samples. Both Shannon index and Pielou's evenness index for MiFish primer were higher than Chord primer (Table 4.5).

Table 4. 5 Shannon diversity Index and Pieleu's Evenness were measured

	MiFish Primer	Chord Primer
Shannon index	3.83	2.75
Pieleu's Evenness	0.79	0.64

#### 4.4 Discussion

The fish biodiversity is important for future sustainability of marine resources since it affects the capacity of living system to respond the changes in the ecosystem. Previously, fishery survey through morphological analysis was managed at species-level biodiversity for supporting data fisheries management (Baker et al., 2016, English et al., 1997). However, molecular approach through eDNA survey enabled reserchers to analyze the biodiversity with the convenient, rapid and noninvasive ways (Stat et al., 2017). Near future, it is estimated that eDNA-based fish biodiversity survey would provide an overview of the biodiversity of fish in the ecosystem.

In this study, we compared two primer sets (MiFish & Chord) for the analysis of fish eDNA, which were collected from Thousand island National Park, Jakarta, Indonesia. Both primer pairs successfully presented the high degree of fish taxa from eDNA samples. Read length by MiFish primer (XXX bp) is shorter than Chord primer (XXX bP). Short sequence targeting primer has advantage to amplify low quality and degraded DNA (Deagle et al., 2006, Epp et al., 2012) suggesting the low amount of water samples such as one-liter of water would be enough as shown in the previous studies (Laramie et al., 2015).

Since our developed primer has longer sequence, it has the limitation to amplify a low quality of DNA sample (Ponce and Micol, 1992). Afterwards, it succeed to amplify and increase resolution for species detection, reduce the error rate and reveale cryptic species (Schloss et al., 2016). Net sampling strategy offered similar result in environmental genetic monitoring (Keller et al., 2017). It potentially collected more eDNA. Additionally, fish egg and fish larva trapped in the net were essential for study of biodiversity and fisheries management (Rabbaniha et al., 2015, Moser et al., 1974). Therefore, it is suggested that Chord primer is suitable for the detection of eDNA of collected net sample.

The world's concern is increasing over the exploitation of Elasmobranchii and Indonesia has the highest landing in the world which makes them vulnerable (Blaber et al., 2009, Wijayanti et al., 2018). At least 130 Elasmobranchii occurred in Indonesian coastal area (Aditya and Al-Fatih, 2017) and more than 25 species were rare and endemic (Fahmi and Dharmadi, 2012). Lack of data information on the status of the population and migration patterns is a challenge for the conservation and management of Elasmobranchii (Dulvy et al., 2014, Suryagalih, 2016). Several survey and monitoring were conducted to obtain its biodiversity and conservation status (Wijayanti et al., 2018, Subrata et al., 2017). However, the identification often experiences difficulties because some fish landed have dry fins, cartilages, skins and gills (Wehantouw et al., 2017, Mopay et al., 2017, Kinakesti and Wahyudewantoro, 2017). In the present study, the Chord primer set successfully amplified three species of

Elasmobranchii including the near threatened blue-spotted fantail ray (*Taeniura lymma*), from family Dasyatidae, Elasmobranchii (Compagno et al., 2005) which was failed in the MiFish primer. Chord primer set are expected to provide a breakthrough in fisheries survey methods including Elasmobranchii through DNA metabarcoding.

The DNA barcoding and metabarcoding works were relied on reference database (Rydberg, 2010). According to database in the GenBank (72507 sequences database using search filters: 12S rRNA, tRNA-Val, 16S rRNA and Chordata) compared with MiFish primer database in the GenBank (145.386 with 12S rRNA and Chordata search filters) Chord primer set targeting Chord region has insufficient database (Figure 4.2). Further, 95 additional ITS sequence database (see on chapter 3) performed better species identification (table 4.4). The Chord region consist of the end of 12S rRNA, tRNA-Val and initial of 16 srRNA whereas MiFish region consist of the initial of 12S rRNA (Miya et al., 2015) and both sequences fragment have a gap of at least 1 kb. Since new method succeeded to amplify long reads sequencing with average result of 10kb (Suzuki et al., 2018). An interesting strategy to perform and increase database was creating a bridge sequence between two primer pairs (MiFish primer set- Chord primer set) and its database would increase species detection (Hubert and Hanner, 2015).

The short sequence of MiFish primer set reveal a higher species number than the Chord primer set (Table 4.2, Fig 4.2, Table 4.3). However, the total species

in this study were almost similar and both primers pairs exhibited higher fish diversity than previous study (Table 4.5) (Madduppa et al., 2013). Further, new species identified by molecular strategy through eDNA metabarcoding revealed hidden fish diversity in Thousand Island.

#### **4.5 Conclusion**

We have demonstrated that although short sequences resulted from MiFish primer pairs provide larger number of taxonomic result which consist of one subclass, eight order, 34 family and 85 genera, as compared to longer sequences resulted from Cord primer pairs which is comprise of two subclass, nine order, 31 families and 60 genera, both primer pairs revealed species diversity of coral reef associated fish in Th, Jakarta, Indonesia using eDNA metabarcoding. Therefore, eDNA metabarcoding strategy could serve as an alternative non-invasive method for coral reef fish surveys. Since Chord primerset succeeded to amplify subclass Elasmobranchii it could be useful in its conservation and fishery management surveys. However, increasing Chord region sequences database were required.

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## ACKNOWLEDGEMENTS

This dissertation becomes a reality with the kind support and help of many individuals. I would like to extend my sincere thanks to all of them.

The research was successfully carried at the Interdisciplinary Program of Biomedical Mechanical and Electrical Engineering, Pukyong National University. A series of simulation and analysis had been finished from September 2015 to December 2018, under the successful supervision of Professor Hyun Woo Kim.

The study and research were permitted by Jakarta fisheries University, Ministry of marine affairs and Fisheries Republic of Indonesia and financially supported by Brain Korea 21 Program for Leading Universities and Students (BK21 PLUS), a Korean governmental research project supervised by National Research Foundation, and the support is gratefully acknowledged.

I am grateful to Professor Young Mog Kim for his help and precious discussions related to the novelty of my research. Then, I address gratitude to the reviewers of the dissertation, Professor Won Gyu Park, Dr Kyoung Dong Park and Professor Hak Jun Kim for their excellent comment and recommendation. I wish my best thanks to Professor Hong for his inspiring insight.

Special thanks and my great gratitude to my parents, brothers, my lovely wife Nurmaya Shinta and My sons Aman and Faza for their invaluable supports which driving me until this extent. Also because of them, I have chance walk on excellent and unforgettable experiences as both student and researcher throughout my study and life.

My success in completing this dissertation cannot be separated from discussion with outstanding colleagues across the globe for various aspects in both research objective and analysis technique. I give the deepest acknowledgement for Dr. Hye Eun Kang, Sapto Andriyono, Moch Jobaidul Alam, Nack Geun, Tae Hoo, Soo Rin, Ah Ran, Ji Hyun, Dong Eui, Eun Bi and Najia for their best interaction, their kindness and friendliness.

Busan, December 2018

