

# Identification of the spaghetti eels (*Moringua Sp*) at Pelabuhan Ratu, Indonesia using mtDNA control region sequences

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**Abstract.** Eels are economic species of fish. The diversity of eel in the world is essential knowledge that we have to know to manage fisheries in the future. One of the types of eels is *Moringua Sp*. There are around 11 species of *Moringua sp*. in the world. In Indonesia, the diversity of *Moringua sp*. was no information yet. The aim of this study is to the identification of *Moringua sp* in Indonesia. The method used in this study is a sanger sequence method using the mtDNA control region. Base on this study, we found 726 and 745 base pairs alignment of *Moringua* by sequencing. The result of BLAST showed identically 87% to *Moringua edwardsi* and 89% as a *Moringua microchir*. The low percentage of BLAST yields shows that the sample used for this study is not strong enough to be declared as one of the *Moringua* species obtained from the results of BLAST analysis. Suspected of being another *Moringua* species. Thus, to get robust result, further research related to this species identification using COI or Cyt-b.

## 1 Introduction

Eels are economic species of fish. The diversity of eel in the world is essential knowledge that we have to know to manage fisheries in the future. The spaghetti eels or worm eels are a small family of Moringuidae, and they share the last name with other families of eels [1]. Moringuidae eels live in shallow tropical waters worldwide; the length of the body was around 15 to 40 cm with body shape was very narrow and cylindrical [1]. The Moringuidae contains two different looking genera [2]: *Moringua* Gray, 1831, and *Neoconger* Girard, 1858 [3].

Globally, Indonesian freshwater fish biodiversity is ranked second, after Brazil 129 [4, 5], while in terms of Asian endemic freshwater 130 fishes, Indonesia ranks third, behind India and China [6]. However, the distribution and systematics of many freshwater fishes are poorly understood 132 [7, 6, 8, 9]. Meanwhile, freshwater fishes new to 135 species (including cryptic species) and new species records for Indonesia are still being 136 discovered [10, 11].

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Pelabuhan Ratu is one of the Southern coastal waters of Java Island, Indonesia, which has great potential in providing glass eel. As the waters have a relationship with the Indian Ocean, it is suspected that this area is visited by many types of eels in waters of Indian Ocean. However, research on the kinds of eels that landed in this area, both morphologically and molecularly, has not been carried out, especially for *Moringua* sp. There are eleven marine species of the genus *Moringua* Gray 1831, currently [12, 13]. The diversity of *Moringua* sp was no information yet in Indonesia. So, the aim of this study is to the identification of *Moringua* sp. at Pelabuhan Ratu, Indonesia.

## 2 Methods

### 2.1 Samples collection and DNA extraction

Two samples of *Moringua* sp were collected in July 2018 from Cimandiri River in Pelabuhan Ratu, Sukabumi, Indonesia (7°01'14.75" S 106°34'06.81" E) by using a fyke net. Eels larvae called glass eels were then preserved in absolute ethanol and stored at -4°C until required. The samples were processed and analyzed in Research Institute for Inland Fishery and Extension, Palembang, South Sumatera, Indonesia. Approximately 25mg of caudal fin tissue was taken from each specimen using a sterile procedure to avoid contamination of samples. Total genomic DNA was extracted using Zymo Research Extraction Kit®, following the manufacturer's protocol.

### 2.2 Morphological identification

Morphological characters (morphometric and meristic) have long been used in fisheries biology to measure the distance and kinship in the taxonomy category variations. In this study, the identification for the glass eels stages is based on morphometric characteristics. Morphometric characteristic is related to the size of the body or the body part fish. That can be used as a taxonomic characteristic when identifying fish. Types of equipment used are a stereoscope ("stereomicroscope") and caliper graded to 0.1 mm.

### 2.3 DNA amplification and electrophoresis

Polymerase Chain Reaction (PCR) was done in a Model Mastercycler X50s (Eppendorf®), and reactions were carried out with 50µl reaction volume containing 20µl sterile distilled water, 25µl QuickTaq™ HS Dyemix (Toyobo), 1 µl of each primer and 3 µl of total DNA. The sequence of the forward primer was (5'-ACA TGA ATT GGA GGA ATA CCA GT-3') while the reverse primer was (5'-CCT GAA ATA GGA ACC AAA TG-3') [14]. Amplification parameters were 35 cycles, and the thermal cycler profile was as follows; predenaturation at 94°C for 2 minutes, denaturation at 94°C for the 30s, annealing at 50°C for 30s, extension at 68°C for 1 minute, and final extension at 94°C for 30s. The PCR products were electrophoresed on 1% agarose gel and stained with ethidium bromide for band characterization via ultraviolet transillumination within 90V for 40 minutes. The PCR products with the clear band were sent to Genetica Science Ltd for sequencing.

## 2.4 Sequences alignment

Two sequencing samples with specimen vouchers 1807PR027 and 1807PR039 were aligned using Molecular Evolutionary Genetics Analysis (MEGA) 6 software. The resultant fragments were approximately 726 – 745 base pairs (bp). The samples were identified using BLAST procedure in NCBI.

## 3 Results

This paper is the first paper for the *Moringua* sp. (Figure 1) at Pelabuhan Ratu, West Java, Indonesia. Thus, this paper can fill in information that has not been recorded regarding the distribution of this species in the Pelabuhan Ratu area and contribute to providing a better understanding of the ichthyofauna in the area. The samples of this study were identified by morphology and genetic analysis. The result of each identified is below

### 3.1 Morphology

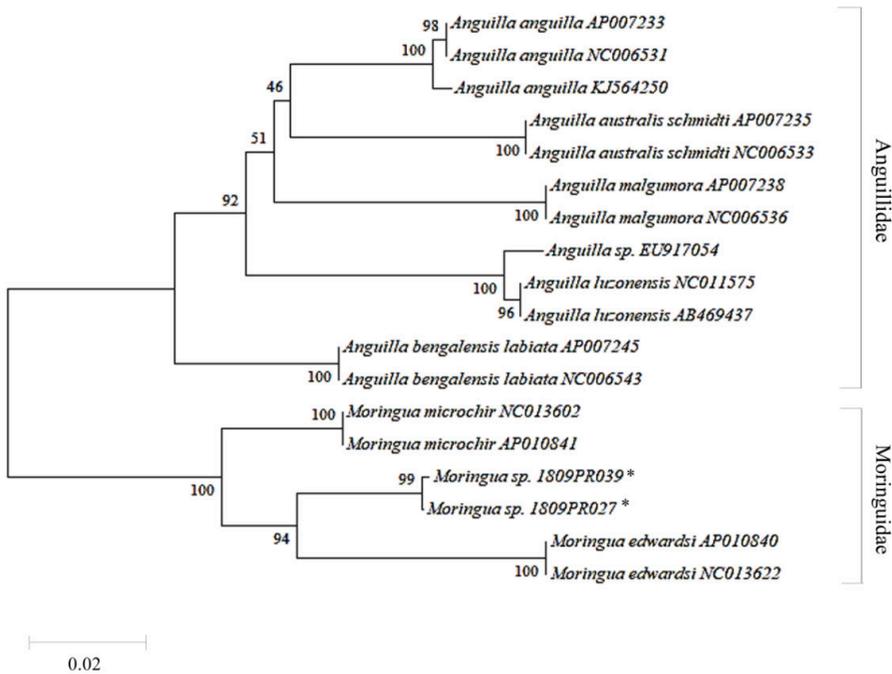
Description of two specimens that we found is the total length (mm) 515.9-521.6; head length (mm) 41.2-44.5; predorsal fin 320.4-326.2; preanal fin 348.3-353.3, lower jaw projects beyond upper jaw; glass eels specimen; have some spots in lateral line. Besides, there are limited references related to Moringuidae identification guide by morphological character and fewer data about *Moringua* sp., especially the glass eel stage.



**Fig. 1.** *Moringua* sp. voucher 1807PR039 (521.6 mm).

### 3.2 Genetic

The marker that we used in this genetic study is the mtDNA control region shows that the species identified as *Moringua* sp. It resulted from a 726–745 bp of sequences. BLAST analysis tool resulted in the identity to *Moringua edwardsi* 87% and *Moringua microchir* 89%. Based on the phylogenetic tree, derived from 16 sequences in GenBank database and two sequences of samples from this study using Neighbor-Joining (NJ) tree with bootstrap analysis (1000 replicates) indicated two significant groups of the order Anguilliformes (Figure 2).



**Fig. 2.** Phylogenetic tree using neighbor-joining methods with bootstrap analysis 1000 replication (\*samples of study).

## 4 Discussion

Indonesia is known as a mega biodiversity country, where Indonesia has more than 17.000 islands, so that high diversity of fish species [15]. Pelabuhan Ratu is one of the coastal waters on the island of Java in Indonesia which has excellent potential in supplying glass eel, presumably because these waters are connected to the Indian Ocean. Hence, this area is suspected to be visited by the types of eel from the Indian Ocean waters, so that these waters become the main eel fishing grounds in Indonesia [16, 17,18].

Nowadays, information related to the diversity of eel species on the Moringuidae family in the Pelabuhan Ratu area has not been found. This condition makes it difficult for the authors to identify the sample fish morphologically. Besides, the size of the eel sample fish still in the glass eel phase also supports the impossibility of determining the morphologically sampled eel fish. Phase glass eels are metamorphic stage between larva *Leptocephalus* end of leaf-shaped translucent and pigmented elver complete. Therefore, indicates of the progress metamorphosis is shown by the extent of skin pigmentation [14]. The best way to answer this problem is to identify the sample fish to overcome this condition genetically.

Then the phylogeny tree analysis was carried out on the results of the research samples' sequencing and the sequencing results of two types of *Moringua* found in Genbank, namely *Moringua microcher*; and *Moringua edwardsii*. The samples of this study are from the Moringuidae family. The Moringuidae family is one of the most challenging families of eels to classify [19]. According to [3], the Moringuidae family consists of two genera, namely *Moringua* Gray, 1981 and *Neoconger* Girard, 1858.

The analysis results show that the results of the sequencing of the research samples form *Moringua* groups. With BLAST analysis, 87% as a *Moringua edwardsi* and 89% as a

*Moringua microchir*. Both percentage analyses that less than 90% show that the sample used for this study is not strong enough to be declared as one of the *Moringua* species obtained from the results of BLAST analysis, suspected of being another *Moringua* species.

*Moringua* sp is a member family of Moringuidae that live in tropical and subtropical waters. At least there are eleven types of eel species of *Moringua*, namely: *M. abbreviate*, *M. arundinacea*, *M. bicolor*, *M. edwardsi*, *M. ferruginea*, *M. javanica*, *M. macrocephalus*, *M. macrochir*, *M. microchir*, *M. penni*, and *M. raitaborua* [20].

## 5 Conclusion

The mitochondrial DNA control region of the species from this study was identified as *Moringua* sp. as it resulted in a 726–745 bp of sequences. The low percentage of BLAST yields and the phylogeny tree analysis show the results of the sequencing of research samples that do not blend with any of the *Moringua* species found in Genbank. It is shown that the sample used for this study is not strong enough to be declared as one of the *Moringua* species obtained from the results of BLAST analysis, suspected of being another *Moringua* species. There is still a need for further research related to species identification using COI or *Cyt-b*.

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