I. INTRODUCTION

1.1.Background

Indonesia is well known as a mega biodiversity country for both marine and terrestrial animals. One of the most unique animals is Mudskipper fish, an amphibious fish known amongst local communities as ‘ikan blodo cina’, ‘ikan blodok’, ‘lunjah’, ‘glodok’, ‘layar’, ‘tembakul’, ‘tempakul’ and ‘ikan glodok’ (Saanin, 1968). Mudskippers are found along the Indo-West Pacific region, from East Africa to the Samoa and Tonga Islands, and along the Atlantic coast of Africa (Murdy, 1989). This species mostly inhabit estuaries, muds and mangrove swamps and shows their unique characteristics through skipping out of the water, climbing on mangrove trees, and sticking on rocks. The base of its pectoral fins is strongly muscular, so it can be bent and function like an arm for creeping and crawling. They have distinct flat faces with protruding eyes in the dorsal part of the head (Mukharomah, 2016).

Mudskipper fish species are cryptic species from the family Gobiidae (Thacker, 2003) which shows similar morphological features but is genetically different among the species and are often confused as one another due to their similarity of morphological characters, making their identification and differentiation a complex and time-consuming task (Arisuryanti et. al., 2018). Traditionally, fish species identification is often done based on their morphometrics and meristic characteristics, including

body shape, size and colours of scale or body, and number and type of fin (Strauss & Bond, 1990). However, in certain instances, solely relying on morphological characteristics for identification may be insufficient or unreliable due to several reasons. Some species of fish may exhibit similar characteristics or small differences between species (Teletchea, 2009). In other cases, morphological characteristics have been eliminated or altered before and during sample processing and it may become infeasible to rely on morphological characteristics alone (Strauss & Bond, 1990). For instance, a study from Callejas & Ochando (2001) showed the difficulty of identifying Barbus species of the Iberian Peninsula based on only morphological features because of the common interspecific variation. Arisuryanti et al (2018) also reported the case of identification difficulties in Mudskipper fish and even referred to it as "almost impossible task" to identify only based on morphological traits.

Several studies of Mudskipper identification using morphological traits in Indonesia have been done including from Mukharomah, et al (2016) who identified two species of Mudskipper (*Periophtalmus gracilis* and *Periopthalmus variabilis*) from Makarti Jaya and Sungsang estuaries through morphometric and meristic and descriptive methods. The result shows the two species of mudskipper have very similar morphological characteristics of body shape and scale type. The only characteristic differentiating the two species is the eye diameter of *Periophtalmus gracilis* being bigger and more prominent compared to *Periophthalmus*

variabilis. Another study from Baderan et al (2023) done in the mangrove ecosystem of coastal Bay of Tomini, Boalemo, Gorontalo Province. 561 individuals collected were identified using morphometric, meristic and comparing the results with identification keys. The result revealed five species found with high similarity in morphological features, namely *Periophthalmus argentilineatus*, *Periophthalmus kalolo*, *Periophthalmus malaccensis*, *Periophthalmus minutus*, and *Periophthalmus variabilis*.

The high similarity level of morphological features of Mudskipper emerging a problem in assigning proper names to mudskipper species, which in turn can impact conservation initiatives aimed at protecting these fish within their natural habitat. Therefore, molecular approaches offer a solution for this problem. Molecular approaches are the way forward especially in the form of DNA barcodes. The development of various molecular techniques that generate molecular markers has made it possible to accurately identify animals accurately with less time consuming. These techniques utilise differences at the level of the deoxyribonucleic acids (DNA) of the protein encoded by it. One of the widely used molecular approaches that can be used to identify the fish species quickly and accurately is DNA barcoding using the Cytochrome Oxidase Subunit I (COI) mitochondrial gene (Hogg & Hebert 2004). The COI mitochondrial gene has been accepted as a universal barcode to identify animals (Amin, 2013), and now considered highly preferable for the precise identification of fish

Despite the high rate of biodiversity amongst freshwater fish and high abundance of Mudskipper in Indonesia, studies investigating the DNA barcoding Mudskipper fish using COI gene marker in Indonesia is very limited and considered new as it just started less than 10 years ago by Dahruddin, et. al (2016). This study was conducted from the concern about the limited and uncertainty of Indonesian DNA barcodes library and database, especially for ichthyofauna. They noted that at that time Indonesia had very limited, unorganised, and non-inclusive databases, while the presence of proper, accurate, and inclusive databases are important in the development of taxonomic and phylogenetic studies in the future. Despite facing challenging difficulties, 1046 sequences belong to 159 species, 107 genera and 50 families of freshwater fishes from several locations along Java and Bali were successfully identified and deposited to GenBank, and 30 sequences of them includes 4 species of Mudskipper namely *Periophthalmus argentilineatus*, *Periohthalmus kalolo*, *Periophthalmus novemradiatus*, and *Boleophthalmus boddarti*. These sequences are notably being the first sequences of Mudskipper fish COI region from Indonesia deposited in GenBank, and helped initiate the development of DNA barcoding, taxonomic and phylogenetic studies of Mudskipper in Indonesia. However, difficulties persisted due to the limited availability of locations available for conducting phylogenetic studies

Until years later, Arisuryanti, et al. (2018) conducted a study on mudskipper fish collected from Bogowonto Lagoon and identified 2 species of Mudskipper from the location, namely *Periophthalmus argentilineatus* and *Periophthalmus kalolo*. Further phylogenetic analysis revealed that *P. argentilineatus* from Bogowonto Lagoon is closely related to *P. argentilineatus* from Pandeglang, and *P. kalolo* is closely related from *P. kalolo* from Cilacap. Phylogenetic studies of Mudskipper fish in Indonesia continue revealing a lot of new findings, as one from Arisuryanti, et. al. (2021) investigated Mudskipperfish from Tekolok Estuary, West Nusa Tenggara and suspected the presence of cryptic species in *P. argentilineatus* from the separation of two distinct clades with a relatively huge genetic distance that showed a distinct geographicpatterns between eastern and western area of Indonesia. However, as the database kept increasing, Arisuryanti et. al (2023) investigated *P. argentilineatus, P. kalolo* and *P. novemradiatus* from Baros Beach and Pasir Mendit Beach, Jogjakarta and found other evidence that did not support the initial theory of separation by geographical region happening in the population structure of *P. kalolo* and *P. argentilineatus*.

Despite the growing interest in DNA barcoding and phylogenetic studies of Mudskipper fish in Indonesia, it still can be considered very limited and there are still gaps to be understood and fill, such as further analysis for reconfirmation of suspected cryptic species existed in

Periophthalmus argentilineatus, and greater coverage of sampling areas need to be done to give more understanding about the probability of geographic patterns of Mudskipper fish in Indonesia. Most importantly, greater coverage of sampling areas and greater total of individuals identified can help develop a proper and better database of Mudskipper in Indonesia that can help provide an advanced understanding of the species, evolutionary relationships, and conservation plan in the future.

1.2. Problem Formulation

1.2.1. How is the process of identifying mudskippers using DNA barcoding technique to ensure accurate species identification?

1.2.2. How is the molecular diversity of mudskipper species based on the conducted phylogenetic analysis?

1.3. Objective

1.3.1. To implement the process of identifying mudskipper species using DNA barcoding techniques.

1.3.2. To analyze the molecular diversity of mudskipper species in Indonesia using a phylogenetic tree approach.