

First genetic record and phylogenetic relationships *Cheilopogon spilonotus* (Bleker, 1865) Originates from the Makassar Strait, Indonesia

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ABSTRACT

Flying fish, including pelagic fish that live on the surface of the high seas, can jump out of the air and glide for great distances. The identification of fish species in Indonesia is still limited as morphology. It is therefore very important to validate using short DNA sequences which have proven to be fast, effective, and accurate. Fish specimens were collected from the Makassar Strait, Indonesia. The CO1 gene is amplified and sequenced. The results showed that the individuals obtained were the same as those from the North Pacific Ocean with a strong bootstrap value (100%) in the monophyletic clade. These findings can be sequential order references and basic information for the conservation of this species.

Key words : CO1, Flyingfish, Southeast Asia, Fish distribution

Introduction

Flying fish including pelagic fish, which live on the open ocean's surface, can jump out of the water and glide for great distances. The high diversity causes difficulties in the identification process of each species. Generally, flying fishes are members of the family *Exocoetidae* (Carpenter and Niem, 2001; 1996. Parin, NV 1996), composed of 71 valid species (Wu *et al.*, 2017), 18 of which were found in Indonesia (Carpenter and Niem, 2001; Fricke and Wantiez, 2011; Parin, 1999; Riede, 2004; Robins and Ray 1986; Shen, 1993; Sommer and Poutiers, 1996); (Febyanty

and Syahailatua, 2008); (Ferdiansyah and Syahailatua, 2011).

However, several studies that have been carried out in the Makassar Strait waters on the types of flying fish which have been collected, have not been determined morphologically and meristically. The individual fish of the same species at the age stage different species is often differ from each other apart from individuals of different species at the same age stage (Jayakumar, 2019). Based on this, we use molecular markers as one way to identify the types of flying fish caught in the Makassar Strait. These findings will be practically used for species validation

and identification references in the future. The absence of a flying fish gene Exocoetidae from the genus *Cheilopogon* originating from Indonesia in Gen Bank provides an opportunity for this study to produce the first record.

Materials and Method

In this study, Flying fish specimens were collected from the local fishermen in Majene Regency, West Sulawesi Province, Indonesia. The location of random sampling is based on the fishing position of gillnet fishermen in the Makassar Strait.

Thirty (30) individuals were collected from the Makassar Strait fishing area, each of them with morphological characteristics for the flying fish Exocoetidae. Five good fish specimens were randomly taken for DNA isolation. Then the whole body of specimen was stored in 96% ethanol until it reached the laboratory (Hasan and Tamam, 2019). All taken samples were stored under -20°C temperature in the Ichthyology Laboratory of Brawijaya University. Molecular confirmation of the species identification was carried out by using partial mtDNA cytochrome oxidase sub-unit I gene (COI).

The sequence data were translated into amino acids to confirm the absence of premature stop codons. The forward and reverse sequences were edited by using the Chroma 2.6.6 program, the consensus was drawn up by using the Ugene 1.32 pro-

gram, and the comparison sequences were applied by using the Mesquite program. DNA sequences developed in the present study and already available sequences for the species and related species in NCBI were aligned and edited by using MEGA version 10 (Ardiansyah *et al.*, 2021). Phylogenetic and molecular evolutionary were analyzed by using Kimura 2-parameter method (Kimura, 1980) and were conducted by using MEGA version 7.0 (Kumar *et al.*, 2016). The sequence of *Decapterus ruselli* was used as outgroup for phylogenetic analysis.

Results

We found that the results of molecular identification of flying fish originating from the Makassar Strait showed that the samples collected were 98.10% similar to the *Cheilopogon spilonotopterus* species originating from the South China Sea. The results of the alignment analysis showed that the amplicon sample sequence was similar to the *Cheilopogon nigricans* CO1 gene sequence, namely 100% (Accessi no. MH638792.1). Based on these similarity figures, it can be concluded that it is true that the sample used in this study is the CO1 oxidase gene from flying fish (*Cheilopogon spilonotopterus*).

Discussion

The flying fish in the waters of the Makassar Strait in this study proved to be fish from the Exocoetidae family. The value of the genetic distance between species with data from GeneBank is still very low,

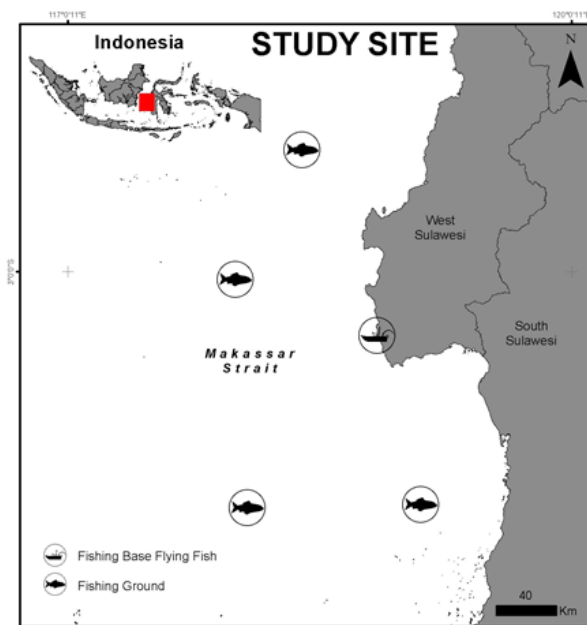


Fig. 1. Location of sampling

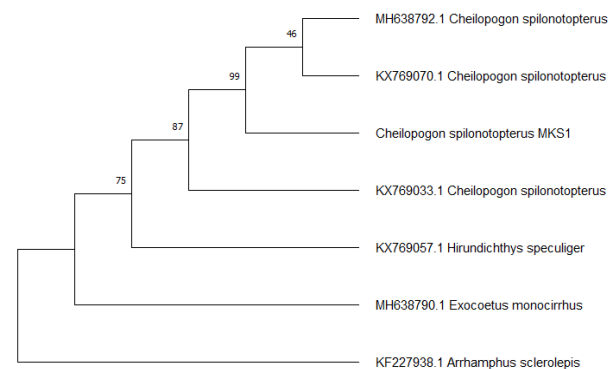


Fig. 2. Maximum likelihood phylogenetic tree with 1000 bootstrap replicates. Specimens from the Makassar Strait were grouped together with a strong bootstrap value (100%)

so it can be concluded that the used samples of isolate flying fish still have a very close relationship with *Cheilopogon spilonotopterus* species from the South China Sea. According to Tallei *et al.* (2016), the less the value of the genetic distance between two organisms, the closer the relationship between them.

The phylogeny tree analysis results show that the Indonesian Makassar Strait specimens are in the same group with *Cheilopogon spilonotopterus* from the Northwestern Pacific Ocean while *Cheilopogon spilonotopterus* from the South China Sea are in a different group. The difference in genetic distance between the two sample groups is presumed because geographically, there are boundaries that allow it to occur. Separation of groups from one another for example, straits or islands (Kurniawan *et al.*, 2021) or perhaps migration due to environmental suitability and food availability. Flying fish obtained in the Makassar Strait showed low genetic distance values in the phylogenetic tree analysis, namely 0.000-0.005. The genetic variation of flying fish (*Cheilopogon spilonotopterus*) in the Indonesian Makassar Strait waters consists of the same population group as the *Cheilopogon spilonotopterus* specimens from the Northwestern Pacific Ocean.

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