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Submission date: 11-Jun-2023 06:39AM (UTC+0700)
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To cite this article: Amrullah Taqwa *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1083** 012029

Analysis of Morphometric and Gene of Bombay-duck (*Harpodon nehereus*) in Tarakan Waters

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Abstrak. Bombay-duck (*Harpadon nehereus*) was one of economically valuable fish at Tarakan waters. This fish was caught by trawl and jermal. In this study, samples were taken from two fishing area, that were Juata and Amal. 20 characters morphometric was measure carried out on 12 samples from each area. Molecular identification and phylogeny of this species used Cytochrome C Oxidase Subunit I (*COI*) gene. The phylogenetic tree was constructed used MEGA 5.0 program. There were 5 significantly different characters between sample from Juata and Amal, that were body weight ($P = 0.00$), distance between pelvic and anal fins ($P = 0.001$), caudal fin height ($P = 0.009$), pelvic fin base length ($P = 0.015$), and dorsal fin base length ($P = 0.031$). Molecular identification showed that samples were the same species. The genetic distance between samples from Juata and Amal was 0 (zero).

Keywords: ***Bombay-duck, morphometric, Cytochrome C Oxidase Subunit I .***

1. Introduction

Bombay-duck was widespread in the Western Indo-Pacific from India to the Solomon Islands [1]. This species was fishing in Tarakan waters have been carried out since decades ago and production continues to increase from year to year, and there is no management of fishing. In 2001 the amount of production was 58.8 tons, in 2007 it increased to 73.5 tons, in 2010 it increased to 84.9 tons, and then in 2014 it increased to 150 tons [2]. This species is benthopelagic and is oceanodromous, living in brackish water and seawater, which can be found in soft-water coastal and estuary waters [3]. Occupy deep waters off the coast for most of the year, but also gather at river mouths to find food during the rainy season [4]. These aggressive predators prey on small fish [5].

Many studies on Bombay duck in Tarakan waters have been carried out, such as its reproduction biology [5], size structure and length-weight relationship [6] and contamination status of pollutants, especially Polycyclic Aromatic Hydrocarbons (PAHs) [7]. This species fishing has been carried out in two locations in Tarakan waters, namely Juata and Amal. The fishing gear used in Juata is a mini trawl, while stow nets is used in Amal. This study was conducted to find out whether both populations are the same species or not. This study was conducted at Tarakan waters.

Previous research has proven that morphological and meristik characters in both location are *Harpodon nehereus* [8], but this has not been proven genetically. Genetics identification of fishes needs

to be done and this is important for fisheries management. Genetic barcodes are used to identify species in mall-scale fisheries in Myanmar and revealed that a huge range of species were caught and entered local market [9], an important step for future studies aimed at improving fisheries management. Applied of genetic markers consist of genetic sequence which detect differences between individual of the sa species, the markers used to determine the origin of an individual [10]. The research focused on the queen conch (*Lobatus gigas*), an important fishery throughout the Caribbean

2. Materials and Method

2.1. Sample Collection

Samples were taken from two fishing area, that were Juata (station 1) and Amal (station 2), these location can be seen on **Figure 1**. Molecular identification and phylogeny of this species used *COI* gene.

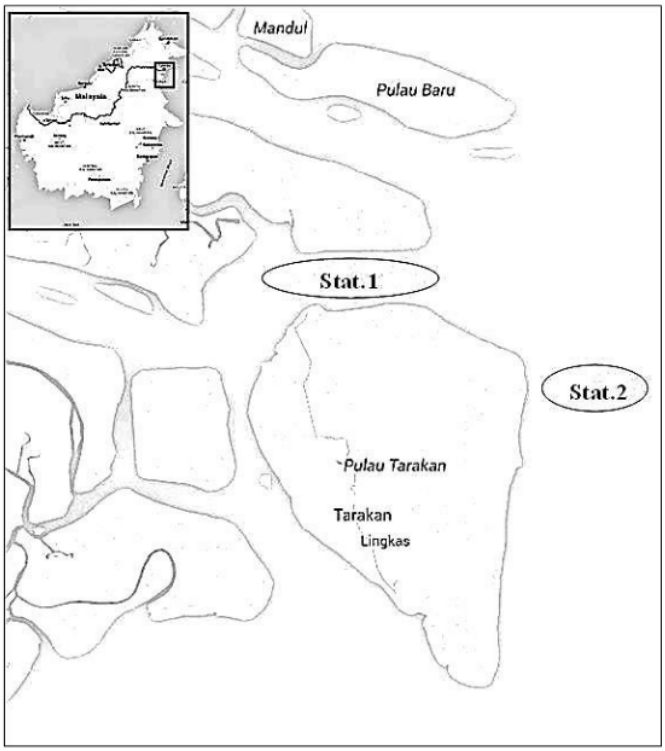

Figure 1. Bombay duck fishing area. Stat.1: Juata and Stat.2: Amal

2.2. Morphometric Measured

Morphometric characters (**Figure 2**) were measure carried out on 100 samples from each area. Morphometric measurement of 20 characters (figure 7) as follows: total length (X1), fork length (X2), standard length (X3), head length (X4), mouth length (X5), head height (X6), body height (X7), distance between the tip of the head and the base of the dorsal fin (X8), length of dorsal fin base (X9), dorsal fin height (X10), length of pectoral fin base (X11), pectoral fin height (X12), length of ventral fin base (X13), ventral fin height (X14), length of anal fin base (X15), anal fin height (X16), distance between the ventral fin and the anal fin (X17), height of caudal stem (X18), length of caudal fin (X19), caudal fin height (X20).

2.3. *DNA extraction and amplification*

DNA extraction according to a standard phenol-chloroform protocol [10]. The DNA dilution was carried out to obtain a concentration of 100 ng/ml. PCR was perfomed in 25 ml reaction volume, which contained approximately 100 ng genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 mM of each primer and 1 U of *Taq* DNA polymerase. The *COI* barcode region of approximately 100 bp.

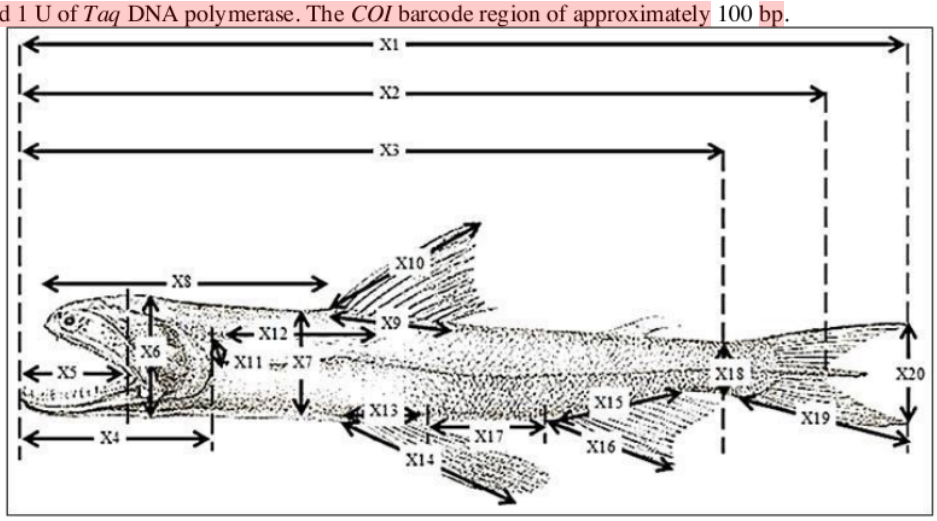

Figure 2. Morphometric measured

3. Result and Discussion

3.1. *Morphometric*

The discriminant analysis of morphometric characters shows that there were 5 characters have a significantly different (**Table 1**).

Table 1. Significant characters between samples from Station1 and Station 2.

Characters	Ratio characters to total length		Sig.
	Station 1	Station 2	
body height (X7)	0.16 ± 0.010	0.15 ± 0.010	0.000
length of dorsal fin base (X9)	0.13 ± 0.023	0.12 ± 0.007	0.031
length of ventral fin base (X13)	0.04 ± 0.004	0.04 ± 0.003	0.015
distance between the ventral fin and the anal fin (X17)	0.25 ± 0.019	0.24 ± 0.023	0.001
height of caudal stem (X18)	0.05 ± 0.003	0.05 ± 0.004	0.009

These differences to be caused by the difference in the total length of the samples from the both stations, total length (mm) from station 1 and station 2 respectively was 213 ± 34.11 and 206 ± 34.5 ($P = 0.15$). The sizes of fish in Amal are generally smaller than Juata once [6].

3.2. *DNA*

Partial *COI* sequences with an aligned length of 100 bp were obtained from individuals of sample from Station 1 and Station 2 (**Table 2**). No insertions or deletions were observed in any of the sequences. Figure 3 shows *COI* gene band from Bombay-duck genome amplification. Figure 4 shows Phylogenetic tree of samples A and B inferred from DNA sequences of gene *COI*.

Table 2. Partial *COI* sequences of samples from Station1 (sample A) and Station 2 (sample B).

Sample	Nucleotide sequence	Species	Identity
A	TTTCAACCAAACCCAAAGACATTGGCACCC TCTACCTCGTATTTGGTGCATGAGCTGGGA TAGTGGGAACCGCCCTGAGCCTTTTGATCC GTGCTGAGCTGAGCCGCCGGGGGCCCTGCT CGGTGACGATCAAATTTATAACGTAATCGT TACTGCCCACGCCTTCGTAATAATTTTCTTT ATAGTAATGCCAATTATGATCGGGGGCTTT GGAAATTGACTCATTCCCCTGATGATCGGT GCCCCCGATATGGCGTTTCCCCGAATGAAT AACATAAGCTTTTGACTCCTCCCACCCTCTT TCCTTCTTCTCTTGGCATCATCGGGAGTCGA AGCAGGGGCTGGAACCGGCTGAACAGTCT ATCCTCCGTTAGCGGGAAACCTTGCTCACG CTGGGGCCTCTGTAGATCTAACCATCTTCTC GCTACACTTGGCTGGGATTTCCTCTATTTTG GGAGCCATTAATTTTATTACGACAATTATC AATATAAAACCTCCCGCCATTTCACAATAC CAGACACCCCTCTTTGTTTGGGCTGTACTG ATTACGGCTGTCCTTCTCCTCCTCTCCTTAC CCGTTCTTGCAGCCGGAATCACAATGCTCT TAACTGATCGAAATCTTAATACCACCTTCTT TGACCCTGCAGGGGGCGGCGATCCCATCCT CTATCAGCACTTATTCTGATTCTTTGGCCAC	*Harpadon nehereus*	99%
B	TTTTCAACCCAACCCAAAAGACATTGGCAC CCTCTACCTCGTATTTGGTGCATGAGCTGG GATAGTGGGAACCGCCCTGAGCCTTTTGAT CCGTGCTGAGCTGAGCCAGCCGGGGGCCCT GCTCGGTGACGATCAAATTTATAACGTAAT CGTTACTGCCCACGCCTTCGTAATAATTTTC TTTATAGTAATGCCAATTATGATCGGGGGC TTTGGAAATTGACTCATTCCCCTGATGATC GGTGCCCCCGATATGGCGTTTCCCCGAATG AATAACATAAGCTTTTGACTCCTCCCACCC TCTTTCCTTCTTCTCTTGGCATCATCGGGAG TCGAAGCAGGGGCTGGAACCGGCTGAACA GTCTATCCTCCGTTAGCGGGAAACCTTGCT CACGCTGGGGCCTCTGTAGATCTAACCATC TTCTCGCTACACTTGGCTGGGATTTCCTCTA TTTTGGGAGCCATTAATTTTATTACGACAAT TATCAATATAAAACCTCCCGCCATTTCACA ATACCAGACACCCCTCTTTGTTTGGGCTGT ACTGATTACGGCTGTCCTTCTCCTCCTCTCC RTACCCGTTCTTGCAGCCGGAATCACAATG CTCTTAACTGATCGAAATCTTAATACCACC TTCTTTGACCCTGCAGGGGGCGGCGATCCC ATCCTCTATCAGCACTTATTCTGATTCTTTG GCC	*Harpadon nehereus*	99%

Figure 3. *COI* gene band from Bombay-duck genome amplification. Sample from station 1 (A) and station 2 (B)

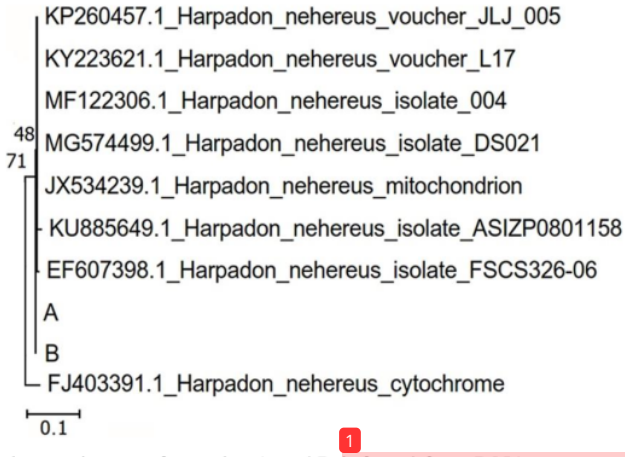

Figure 4. Phylogenetic tree of samples A and B inferred from DNA sequences of gene *COI*

Table 3. Genetic distances of Bombay duck from Tarakan and other places based on *COI* sequences.

	Tarakan	East Java	India	Vietnam
East Java	0,000			
India	0,036	0,036		
Vietnam	0,002	0,002	0,038	
China	0,002	0,002	0,038	0,000

A genetic distance more than 3% are cryptic species [12], Bombay duck from India was cryptic species for this species in Tarakan, East Java, Vietnam and China. A condition of habitat affected the genetic material of individual within a species [13]. A genetic variation of a species can be caused by

random mating, very large population size, migration, mutation, recombination and natural selection [14], [15], [16], [17], [18].

The accurate and rapid identification of closely related fish species is important for scientific research. DNA barcoding offers an opportunity for a standar system of species identification based on the analysis of small fragment of DNA [19]. Bombay duck was found in several location, their identification based on morphological characteristic is very ambiguous. A genetic distance between species in Tarakan was null, this indicated that these species were same species.

4. Conclusion

The results showed that genetically Bombay duck found at station 1 and station2 were the same species, but differ physically in some morphological characters which might be due to differences in fish size.

Acknowledgements

Thanks to Biotechnology Laboratory of Bogor Agricultural Insitute for analysed samples. This research was carried out with the help from the Institute for Research and Community Service. University of Borneo Tarakan.

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