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Unveiling the First DNA Barcoding of *Betta* cf. *uberis* Fish (Anabantiformes: Osphronemidae) from Belitung Island, Indonesia

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ABSTRACT

Betta uberis, a member of the *Betta coccina* group, was first discovered on the Island of Borneo in 2006. A recent expedition to Belitung Island, in Dukong Village, a Swamp Forest Stream in East Belitung Regency, identified a species with strong morphological similarities to *Betta uberis*. To confirm the relationship between the species, a genetic analysis was conducted using DNA barcoding with the cytochrome oxidase subunit 1 (COI) gene. Molecular analysis revealed that the specimens from Belitung Island exhibit approximately 94.41% similarity to *Betta uberis* from Kalimantan. According to genetic guidelines, the specimens are considered non-identical if discrepancies exceed 3%, which is supported by the significant genetic divergence between them—a genetic distance of 0.057, corresponding to 33 DNA base variations out of 626. Phylogenetic analysis indicates that both the Belitung Island specimens and *Betta uberis* from Kalimantan belong to the same clade and share the closest genetic relationship among species in the *Betta* genus. Therefore, we have designated this species as *Betta cf. uberis*. Additionally, the DNA barcode for *Betta cf. uberis* has been recorded in the GenBank database under the accession identifier PQ675811.1.

INTRODUCTION

Indonesia possesses an incredible variety of fish, with at least 26% of the world's fish species inhabiting its waters, including 1,272 freshwater species exclusive to Indonesia **(Valen *et al.*, 2020; Hasan *et al.*, 2023a; Robin *et al.*, 2023)**. Furthermore,

Indonesia, an archipelagic nation, has a significant amount of endemicity; at least 141 endemic species of freshwater fish are spread in this country. However, 1/3 of freshwater fishes are threatened with extinction due to the destruction of their natural habitat and the presence of invasive fish **(Hasan *et al.*, 2020; Serdiati *et al.*, 2021)**. *Betta Bleeker* is known for being the most diverse genus in the Osphronemidae family **(Valen *et al.*, 2023a)**. Approximately, 65 species of Betta are located in Southeast Asia. These species are classified into distinct categories **(Tan & Ng, 2005)**. At least 52 species of them are found in Indonesia **(Nur *et al.*, 2022)**.

Betta uberis is a member of the *Betta coccina* group and was initially documented on the island of Borneo in 2006 **(Tan & Ng, 2006)**. During our recent expedition to Belitung Island, we discovered a species that closely resembles *Betta uberis* on the basis of its morphological characteristics. We employed a genetic methodology to do additional identification in order to ascertain the proximity of the two species. We employed the DNA barcoding technique utilizing the cytochrome oxidase subunit 1 gene found in mitochondrial DNA. The COI gene has been used as a standard instrument for species identification by scientists **(Bingpeng *et al.*, 2018)**. It provides a dependable basis for differentiating among various animal species **(Panprommin *et al.*, 2019)**. Utilizing the COI gene as a species identification method has proven to be effective in identifying freshwater fish in Indonesia **(Syarif *et al.*, 2023a)**.

In addition to validating the species, this study aimed to expand the knowledge base of freshwater fish species through molecular DNA analysis. Furthermore, we will ensure the registration of the DNA barcode in the NCBI GenBank, which will facilitate species identification using molecular techniques. This research seeked to provide valuable insights into biodiversity, genetic diversity, and the life history of the species. Ultimately, it aimed to promote the development and implementation of conservation policies.

MATERIALS AND METHODS

1. Sampling site and fish samples collection

The research was carried out in the Swamp Forest Stream of Dukong Village, located in the East Belitung Regency of Belitung Island, Indonesia, from July 8th to July 14th, 2024. The collection of samples was conducted utilizing environmentally sustainable fishing equipment, specifically a scoop net. Fifteen specimens of *Betta* cf. *uberis* were captured throughout the sampling process. All captured specimens were collected and delivered to the laboratory for the next stage of research. As part of the study of the habitat of *Betta* cf. *uberis*, we also performed on-site examinations of water quality, which involved measuring temperature, pH levels, and dissolved oxygen.

2. Preserve fish and morphological analysis

Fifteen individuals of *Betta* cf. *uberis* were captured using a scoop net over a period of one week of trapping. To facilitate the breeding and domestication of this species, the twelve individuals were stored as livestock at the Fish Reproduction Laboratory,

Aquaculture Department, University of Bangka Belitung, Indonesia. Furthermore, the pectoral fin on the right side of one fish was surgically removed in order to collect DNA sample. The fin was then stored in a solution containing 70% alcohol for 8 days before the molecular analysis. The other side of the specimen was utilized to the gather the morphomeristic analysis, which is often evident in photographs. Subsequently, the specimen was preserved in a 10% formalin solution and was archived in the ichthyological collection at the Aquaculture Laboratory of the University of Bangka Belitung. Additionally, the specimen was analyzed for morphological traits following the methods outlined by **Tan and Ng (2006)**.

3. DNA purification and PCR processes

The DNA purification and PCR procedure was conducted from the 20th to the 22nd of July, 2024 at the Laboratory of Biology, University of Bangka Belitung. The NexproTM DNA kit, FISH-F, and FISH-R primers (**Ward *et al.*, 2025)**, NexproTM Master mix, 10% DNA template and ddH2O, were used to process the DNA purification and PCR analysis. The PCR analysis was conducted using the BioRad type T100TM.

The PCR protocol begins with a denaturation state at 95 degrees Celsius for 15 seconds, subsequently annealing at 54°C, and extension at 72 degrees Celsius for 10 seconds, repeated for thirty-five cycles, closing with a final extension at 72 degrees Celsius for 10 minutes **(Syarif *et al.*, 2023)**. The quality of the PCR analysis results was assessed using electrophoresis **(Robin *et al.*, 2022)** with agarose, TAE buffer, distilled water, red gel dye, PCR DNA sample, and loading dye. The mixture was then introduced into the agarose gel wells. Positive samples with distinct DNA bands were considered to represent high-quality PCR outcomes. High-quality amplification results were further analyzed using the Sanger dideoxy DNA sequencing method.

4. Data analysis

Species identification was completed on NCBI GenBank (https://blast.ncbi.nlm.nih.gov), using BLASTn (Basic Local Alignment Search Tool-nucleotide) analysis. Previously, the quality of the DNA sequences of the species was evaluated by visually examining the nucleotide chromatograms of the DNA fragments using Sequence Scanner software. Graphs displaying sequencing results with distinct separation and prominent peaks demonstrated exceptional quality. Graphs exhibiting merged or overlapping peaks, conversely, indicated poor sequencing results. In order to establish a reliable foundation, we eliminated the low-quality segments of the sequence. The Muscle algorithm was used to align the sequences, ensuring their exceptional quality. The evolutionary history was deduced utilizing the Neighbor-Joining approach. A bootstrap analysis was conducted with 1000 replicates to evaluate the stability of the tree structure. Evolutionary distances, defined as the average number of base substitutions per site, were computed using the Maximum Composite Likelihood method. The

evolutionary studies, including assessments of nucleotide composition and polymorphism sites, were performed using MEGA X software **(Kumar *et al.*, 2018)**.

RESULTS

1. Identification of morphological features

Physical Characteristics: The head is rounded, while the body is thin, long, and has a rounded cross-section. The dorsal fin is positioned toward the rear, with a pointed shape and elongated posterior rays. The caudal fin is rounded, and the anal fin has elongated posterior rays. The pectoral fin is rounded, and the pelvic fin is elongated with a filamentous tip.

Chromatic Expression: The body is reddish-brown with a subtle iridescent green hue. The opercle displays twin soft gold bars. The eye features a vibrant blue patch near the lower part of the iris. The dorsal fin is red with a thin white border at the tip, and there is an iridescent green patch at the base of the fin, with iridescent green streaks between the fin rays. However, these streaks do not extend to the edge of the fin. The anal fin is red with iridescent green streaks on the interradial membrane, originating from the base of the fin, but not reaching its edge. The pelvic fin is red with a white tip.

Morphologically, the Betta specimen from Belitung Island, Indonesia, closely resembles Betta uberis **(Tan & Ng, 2006)** (Fig. 1).

Fig. 1. Life specimen of *Betta* cf. *uberis* from Belitung Island (Female)

2. DNA barcoding

The DNA Barcoding of *Betta* cf. *uberis* from Belitung Island was accurately determined by sequencing the COI gene using the Fish_F2 and Fish_R2 primers with a base-pair length of 626 bp (Table 1).

Table 1. DNA barcoding of *Betta* cf. *uberis* based on COI gene

DNA barcoding of *Betta* cf. *uberis*
CCGGAATGGTTGGTACCGCCCTGAGTCTGCTCATTCGAGCCGAGCTGAGCCAACCGGGGG CCCTTCTTGGGGATGACCAGATCTACAATGTCATTGTTACAGCGCACGCTTTTGTAATAA TCTTCTTTATGGTAATACCTGTAATAATCGGGGGTTTCGGGAACTGACTTGTCCCCCTCA TGATCGGGGCGCCAGACATGGCCTTTCCTCGAATGAATAATATGAGTTTCTGACTCCTAC CACCCTCTTTTTTACTGCTATTAACATCTTCTGGGGTAGAAGCTGGTGCTGGTACTGGTT GAACCGTGTACCCCCCACTAGCCAGCAACTTAGCTCATGCGGGCGCATCTGTAGATTTAA CAATTTTTTCACTTCACCTAGCAGGTGTATCATCTATCTTGGGGGCCATTAACTTTATTA CCACAATCATTAACATGAAACCACCTGCAATTTCCCAATATCAAACACCTCTGTTTGTAT GGGCCGTATTAATCACGGCTGTACTACTCCTTCTATCACTTCCCGTCTTAGCTGCCGGAA TCACAATGCTTTTAACAGACCGAAATCTAAACACAACCTTTTTTGACCCCGCAGGGGGTG GTGACCCTATCTTATACCAACACTTA

3. Species identification based on COI gene

The COI gene of the species discovered on Belitung Island was studied and compared to the NCBI GenBank using the BLAST (Basic Local Alignment Search Tool-nucleotide) method (https://blast.ncbi.nlm.nih.gov). The findings of the two analyses are presented in Table (2).

Table 2. Sequence similarity of *Betta* cf. *uberis* Belitung Island

Specimen	Genbank similarity (Species outcome)	Gene	Accession Number	Query Cover (%)	Identity (%)
Betta cf. uberis Belitung Island	*Betta uberis*	COI	GQ911983.1	100	94.41
	Betta burdigala	COI	OR167622.1	96	93.57
	Betta livida	COI	KM485460.1	100	88.98
	Betta tussyae	COI	KM485462.1	99	86.08

4. Genetic distances

Genetic distance measures the gene variation level, calculated by evaluating differences among species or groups. Based on genetic distance (Table 3), the species

Betta cf. *uberis* Belitung and *Betta uberis* Kalimantan were the most genetically similar (0.057 COI distance).

Tabel 3. Genetic distances of *Betta coccina* group

		1	2	3	4	5	6	7
1	*Betta cf. uberis Belitung*							
2	*Betta uberis Kalimantan*	0,057						
3	*Betta burdigala Bangka*	0,067	0,027					
4	*Betta tussyae*	0,162	0,176	0,183				
5	*Betta coccina*	0,122	0,109	0,122	0,160			
6	*Betta persephone*	0,161	0,158	0,168	0,203	0,179		
7	*Betta livida*	0,119	0,109	0,124	0,156	0,028	0,186	

5. Molecular phylogeny

We reconstructed the phylogenetic connection between *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan based on the mitochondrial COI gene to discover both species' evolutionary connection (Fig. 2).

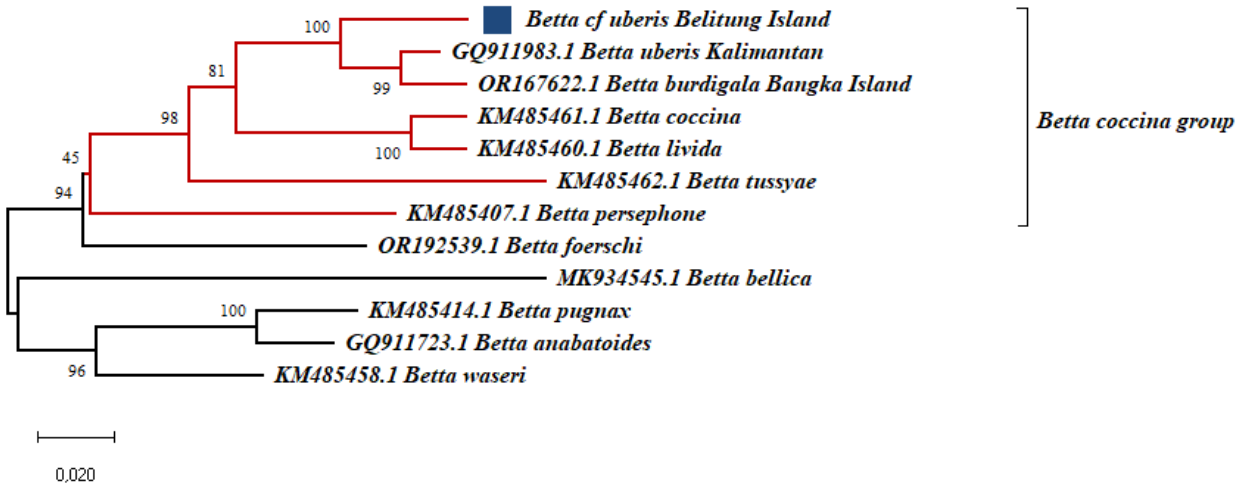

Fig. 2. Evolutionary tree of *Betta* cf. *uberis* (Red branches is *Betta coccina* group)

6. Genetic polymorpisms and genetic mutation

There are 33 nucleotide bases out of a total of 626 nucleotide bases that differ between *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan (Table 4).

Table 4. Analysis of polymorphic sites between *Betta* cf. *uberis* from Belitung and *Betta uberis* from Kalimantan

	17	20	23	32	62	65	68	92	101	146	149
Betta cf. uberis Belitung	C	C	G	C	C	T	T	C	A	A	C
Betta uberis Kalimantan	T	T	A	T	T	C	C	A	G	G	T
	167	179	185	191	209	212	242	257	266	311	318
Betta cf. uberis Belitung	A	C	C	G	T	A	A	G	A	C	C
Betta uberis Kalimantan	G	T	T	A	C	G	C	A	G	T	T
	335	359	386	407	428	431	452	471	482	492	590
Betta cf. uberis Belitung	T	A	T	C	C	T	T	C	G	A	C
Betta uberis Kalimantan	C	G	C	T	T	C	C	T	A	G	T

DISCUSSION

We successfully identified *Betta cf. uberis* from Belitung Island using gene sequences from the Cytochrome C Oxidase Subunit 1 (COI) gene, located within mitochondrial DNA. Previous studies have confirmed that the COI gene is an effective tool for identifying various species in freshwater environments **(Robin *et al.*, 2023b; Syarif *et al.*, 2023b; Valen *et al.*, 2024)**. We compared the COI gene sequence of the Belitung Island specimen (*Betta cf. uberis*) with sequences in the NCBI GenBank using the BLASTn method to assess sequence similarity. Based on the BLAST analysis, the genetic sequence of *Betta cf. uberis* from Belitung Island shows approximately 94.41% similarity to *Betta uberis* from Kalimantan. According to **Hebert *et al.* (2003)**, sequences with 97-100% similarity are typically considered identical, while differences of 3% or more indicate distinct species. *Betta cf. uberis* was discovered on Belitung Island, whereas *Betta uberis* is native to Kalimantan (Fig. 3).

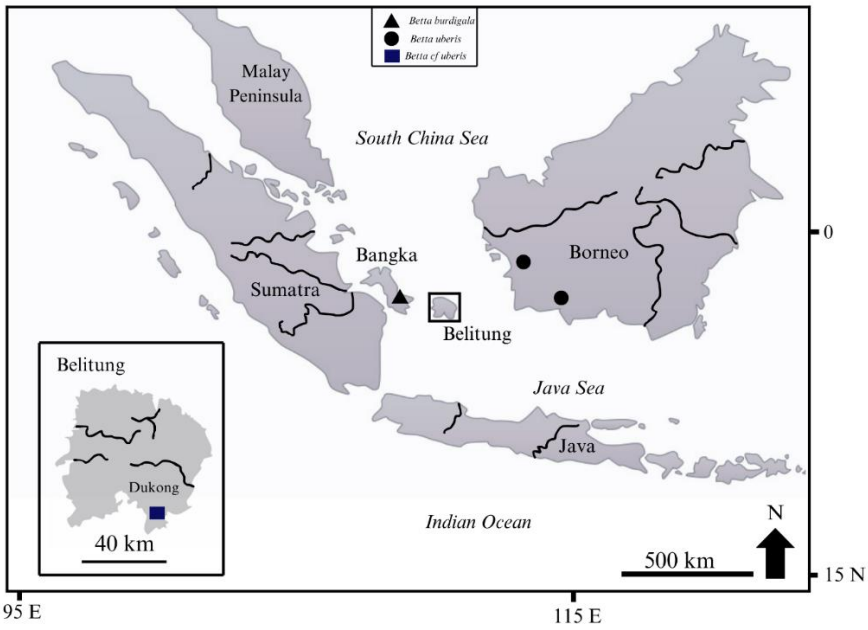

Fig. 3. Map of the location found of *Betta* cf. *uberis* (black square); *Betta uberis* (Black circle), *Betta burdigala* (black triangles) are based on **Ng and Tan (2006)** and **Valen *et al.* (2023a)**

There is evidence in historical texts that the islands of Kalimantan and Belitung were physically connected to the ancient mainland known as Sundaland, which existed thousands of years ago. The geographical location of Belitung Island can be found in the Greater Sunda Islands region of Indonesia. The Sundaland region spans over 1,800,000 square kilometers and comprises the Western of Indonesia (Java, Borneo and Sumatera including Bangka Belitung Island), and the land of Malay Peninsula. In addition, Belitung Island is situated approximately 500 kilometers to the southwest of the nearest community in Kalimantan **(Hasan *et al.*, 2023b)**. It is also known that Belitung Island is rich in biodiversity **(Kusumah *et al.*, 2023)**.

Genetic distance is a measure of the level of variation in a gene, calculated by evaluating differences among species or groups. The species *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan have the closest reported proximity, measuring roughly 0.057 units (Table 3). This indicates that among a collection of 1000 base pairs of nuclutides, there are 57 differences of nucleotide basis. **Nei (1972)** defines a distance of genetic of 0.010 to 0.099 as low, a genetic distance of 0.1 to 0.99 as medium, and a genetic distance of 1.00 to 2.00 as being large.

After determining the genetic variation within the two organisms, the phylogenetic connection was reconstructed between *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan in order to discover the history of the evolution of the two organisms (Fig. 3). A phylogenetic tree was constructed to illustrate the evolutionary relationship between *Betta cf. uberis* from Belitung and *Betta uberis* from Kalimantan. The bootstrap value of 1000 on this tree showed that the branching pattern was very accurate and consistent. A

branch of a phylogenetic tree that has a support value of greater than 70% is considered to have a high level of confidence, with a 95% confidence interval **(Valen *et al.*, 2023b)**. The phylogenetic tree consists of two branches and two clades. *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan belong to the same clade. This indicates a high degree of proximity in terms of their familial lineage and evolutionary past. This confirms our hypothesis that these species share comparable physical characteristics and are classified together as a single group, known as the coccina group. However, additional data are needed to fully understand the hereditary structure and evolutionary history of *Betta* cf. *uberis* Belitung and *Betta uberis* Kalimantan. A comprehensive genomic analysis is recommended to determine the degree of similarity between these two species, providing a more thorough understanding. Additionally, a full morphological comparison should be conducted to support these findings. To further explore the differences between these species, a genetic polymorphism analysis is suggested.

Genetic polymorphisms refer to inheritable variations in the DNA sequence, often caused by mutations. These variations contribute to the genetic diversity within a population. Polymorphic site analysis can detect differences in nucleotide bases within a species. This study aimed to identify the sites exhibiting differences between *Betta cf. uberis* from Belitung and *Betta uberis* from Kalimantan. Out of a total of 626 nucleotide bases, 33 show differences between the two species.

The genetic mutations primarily consist of transition mutations, with 31 instances of G↔A and T↔C substitutions, and 2 transversion mutations, specifically C↔A. Transition mutations involve the substitution of purine bases (G, A) with other purines, or pyrimidine bases (T, C) with other pyrimidines. In contrast, transversion mutations involve the substitution of a pyrimidine with a purine or vice versa. While there are four possible transitions (G↔A, T↔C), there are eight possible transversions (C↔A, T↔A, C↔G, T↔G). Transversions generally have a higher likelihood of causing protein changes due to their more significant impact on amino acid generation **(Insani *et al.*, 2022)**.

Furthermore, *Betta cf. uberis* from Belitung inhabits peat swamps and adjacent blackwater streams in the natural primary forests of Dukong Village, Belitung Island, Indonesia (Fig. 4).

Fig. 4. Sampling site of *Betta* cf. *uberis* in Belitung Island, Indonesia

This habitat is characterized by the presence of humic acids and other compounds produced from the decomposition of organic materials. These substances typically give the water a dark color and result in low levels of dissolved minerals. The water's pH is 5.4, with a dissolved oxygen concentration of 3.0ppm, and the water temperature ranges from 27-28.9°C. The dense canopy above and the heavy growth of riparian plants reduce the amount of light that reaches the water's surface, resulting in limited light penetration into the habitat. The substrate is often obscured by submerged branches, decomposed foliage, and tree roots that extend to the bottom. During certain seasons, the fish may endure several weeks in damp leaf litter due to the absence of permanent water sources.

While studying the *Betta cf. uberis* habitat, we observed numerous other *Betta* species coexisting in the same environment, exhibiting a high tolerance to low pH levels **(Tan & Ng, 2005)**, including *Betta edithae* and *Betta* sp. *Belitung* **(Syarif *et al.*, 2023c)**. In addition to *Betta* species, we identified several other local species capable of adapting to acidic pH conditions, including *Rasbora einthovenii*, *Channa bankanensis*, *Luciocephalus pulcher*, *Nandus nebulosus*, and *Barbodes sellifer*.

However, the population of *Betta cf. uberis* in this habitat is relatively low. Limited ecological tolerance, a restricted habitat, and susceptibility to open tin mining prevent the species from dispersing to other areas within the waterways. To date, no conservation efforts have been made for *Betta cf. uberis* from Belitung. A major contributing factor is the lack of recognition of this fish, and no expert has yet focused on identifying its species. To ensure the accuracy of our research, preliminary investigations, especially focusing on morphological and genetic identification, are essential. While we have successfully identified the fish to the genus level, it is crucial to conduct a thorough

examination to accurately determine the species when introducing new fish. Therefore, we recommend further research, including the analysis of the full mitochondrial genome, complemented by morphological identification, with an emphasis on variations in meristic traits.]

CONCLUSION

The specimen *Betta cf. uberis* was identified through both morphological and molecular studies in the present research. Morphologically, *Betta cf. uberis* is closely related to *Betta uberis*; however, DNA barcoding provided a different insight. The COI gene analysis revealed that the genetic sequence of *Betta cf. uberis* from Belitung Island shares approximately 94.41% similarity with *Betta uberis* from Borneo. Additionally, sequences with a variance of 3% or greater are generally classified as distinct species. The evolutionary divergence between *Betta cf. uberis* and *Betta uberis* is significant, with a genetic distance of 0.057. The two species exhibit 33 nucleotide base differences out of an average of 626 base pairs.

However, further research is needed to fully understand the genetic makeup and evolutionary background of *Betta cf. uberis* from Belitung and *Betta uberis* from Kalimantan. To achieve a more comprehensive understanding, it is recommended to conduct additional studies on these populations, including data collection from other genes (especially mitochondrial genes) or even a full genome analysis. This will be crucial in determining whether *Betta cf. uberis* is a new species or merely a population of an already described one. Future studies should also incorporate additional haplotypes from the analyzed populations to observe potential interspecific variations. Furthermore, a thorough morphological comparison is essential to gather more data on these species, as well as documenting and understanding their morphological variations.

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REFERENCES

Bingpeng, X.; Heshan, L.; Zhilan, Z.; Chunguang, W.; Yanguo, W. and Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One., 13(6): e0198109.

Hasan, V.; Andraini, N.E.; Isroni, W.; Sari, L.A.; Nafisyah, A.L.; Dewi, N.N.; Putri, D.N.A.; Prasasti, T.A.B.; Ramadhani, A.A.; Daniel, K.; South, J.; Vieira, L.O. Ottoni, F.P.; Maftuch; Faqih, A.R.; Wirabuana, P.Y.A.P.; Tamam, M.B. and Valen, F.S. (2023a). Fish diversity of the Bengawan Solo River estuary, East Java, Indonesia. Biodiversitas., 24(4): 2207–2216.

Hasan, V.; Vieira, L.O.; South, J. and Ottoni, F.P. (2023b). First record of Mastacembelus notophthalmus (Actinopterygii: Synbranchiformes: Mastacembelidae) for Belitung Island, Indonesia. Acta Ichthyologica et Piscatoria., 53: 123–127.

Hasan, V.; Widodo, M.S.; Islamy, R.A. and Pebriani, D.A.A. (2020). New records of alligator gar, *atractosteus spatula* (Actinopterygii: Lepisosteiformes: Lepisosteidae) from Bali and Java, Indonesia. Acta Ichthyologica et Piscatoria., 50(2), 233-236.

Hebert, P.D.N.; Cywinska, A.; Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London., 270: 313–321.

Insani, L.; Jatayu, D.; Valen, F.S.; Sri Widodo, M. and Hasan, V. (2022). Comparing genetic *Mystacoleucus marginatus* and *Mystacoleucus padangensis* (Cypriniformes: Cyprinidae) based on Cythochrome C Oxidase sub unit I (COI) gene. Iranian Journal of Ichthyology., 9(4): 195–203.

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution., 35: 1547–1549.

Kusumah, W.; Hasan, V. and Samitra, D. (2023). Rediscovery of the Billiton Caecilian, *Ichthyophis billitonensis* Taylor, 1965, on Belitung Island, Indonesia, after more than five decades. Herpetology Notes., 16, 95-97.

Nei, M. (1972). Genetic Distance between Populations. The American Naturalist., 106(949), 283–292.

Nur, F.M.; Batubara, A.S.; Fadli, N.; Rizal, S.; Siti-Azizah, M.N. & Muchlisin, Z.A. (2022). Diversity, distribution, and conservation status of Betta fish (Teleostei:

Osphronemidae) in Aceh waters, Indonesia. The European Zoological Journal., 89(1): 142–151.

Panprommin, D.; Soontornprasit, K.; Tuncharoen, S.; Pithakpol, S.; and Keereelang, J. (2019). DNA barcodes for the identification of species diversity in fish from Kwan Phayao, Thailand. Journal of Asia-Pacific Biodiversity, 12(3): 382–389.

Robin, R.; Insani, L.; Swarlanda, S.; Prananda, M. and Valen, F. S. (2022). Range extension of Spanner barbs, *Barbodes lateristriga* (Valenciennes, 1842) (Cypriniformes: Cyprinidae) to Bangka Island, Indonesia. Iranian Journal of Ichthyology., 9(3): 149–157.

Robin, R.; Valen, F.S.; Nomleni, A.; Turnip, G.; Luhulima, M. Y. and Insani, L. (2023a). Presence of non-native freshwater fish in Indonesia: A review - Risk and ecological impacts. AACL Bioflux., 16(1): 66–79.

Robin, R.; Valen, F.S.; Ramadhanu, D.; Nomleni, A.; Turnip, G. & Insani, L. (2023b). A new distributional record of flying barb, *Esomus metallicus* (Actinopterygii: Cyprinidae), from Kapalo Banda River, West Sumatra, Indonesia. International Journal of Aquatic Biology., 11(1): 59–68.

Sajjad, A.; Jabeen, F.; Ali, M.; and Zafar, S. (2023). DNA barcoding and phylogenetics of Wallago attu using mitochondrial COI gene from the River Indus. Journal of King Saud University – Science., 35(6): 102725.

Serdiati, N.; Insani, L.; Safir, M.; Rukka, A. H.; Mangitung, S. F.; Valen, F. S.; Tamam, M. B. & Hasan, V. (2021). Range expansion of the invasive Nile tilapia *Oreochromis niloticus* (Perciformes: Cichlidae) in Sulawesi Sea and first record for Sangihe Island, Tahuna, North Sulawesi, Indonesia. Ecology, Environment and Conservation., 27(1): 168-171.

Syarif, A.F.; Valen, F.S.; Herjayanto, M. and Aisyah, S. (2023a). First genetic record and phylogenetic relationship of *Rasbora einthovenii* (Bleeker, 1851) (Cyprinidae : Danioninae) from Bangka Island, Indonesia. IOP Conf. Series: Earth and Environmental Science., 1260(1): 0120002.

Syarif, A.; Kurniawan, A.; Kurniawan, A.; Valen, F.S. and Robin, R. (2023b). First record and phylogenetic relationship of the endangered species *Betta foerschi* (Vierke, 1979) (Anabantiformes : Osphronemidae) from Belitung Island, Indonesia. IOP Conference Series: Earth and Environmental Science., 1289(1): 012010.

Syarif, A.F.; Valen, F.S. and Herjayanto, M. (2023c). First DNA barcoding and phylogenetics of wild *Betta edithae* (Anabantiformes: Osphronemidae) from Belitung Island, Indonesia. AACL Bioflux., 16(5): 2626–2636.

Tan, H.H. and Ng, P.K.L. (2005). The fighting fishes (Teleostei: Osphronemidae: Genus Betta) of Singapore, Malaysia, and Brunei. Raffles Bulletin of Zoology Supplement., 13:43–99.

Tan, H.H. and Ng, P.K.L. (2006). Six new species of fighting fish (Teleostei: Osphronemidae: Betta) from Borneo. Ichthyological Exploration of Freshwaters., 17(2): 97–114.

Valen, F.S.; Bidayani, E.; Alfian, R.A.; Prananda, M.; Notonegoro, H.; Susilo, N. B.; Ardi, W. D.; Swarlanda, S.; Aziz, M. A.; Puryoso, P.; Widodo, M. S.; Faqih, A. R. & Hasan, V. (2023a). Unveiling the close relationship between *Betta burdigala* and *Betta uberis* through DNA barcoding based on COI Gene. IOP Conference Series: Earth and Environmental Science., 1267(1): 012066.

Valen, F.S.; Notonegoro, H.; Pamungkas, A.; Swarlanda, S. and Hasan, V. (2023b). Revolutionary Breakthrough: Unveiling the first DNA Barcoding of the Endemic wild *Betta burdigala* (Kottelat and Ng 1994) (Anabantiformes: Osphronemidae): A Critically Endangered Wild Betta from Bangka Island, Indonesia. International Journal of Agriculture and Biology., 30(4):269–275.

Valen, F.S.; Syaputra, D.; Hasmadi, M.; Swarlanda, S.; Kamarudin, A.S.; Hasan, V. and Itaya I. (2024). Unveiling the Enigmatic Dwarf Horseface Loach *Acanthopsoides molobrion*: A Groundbreaking Discovery in Indonesia. Biosaintifika., 16(2): 265–272.

Valen, F.S.; Soemarno, S; Widodo, M.S.; Wiadnya, D.G.R.; and Hasan, V. (2020). Contemporary distribution records of yellow finned barb *Mystacoleucus marginatus* (Valenciennes, 1842) in Brantas Basin, Indonesia. Ecology, Environment and Conservation., 26, S40-S43.

Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R. and Hebert, P.D.N. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences., 360(1462): 1847–1857.