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Genetic Structure and Phylogenetic Relationships of the Mitre Squid *Uroteuthis chinensis* (Gray, 1849) (Myopsida: Loliginidae) from Bangka-Belitung Islands

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ABSTRACT

This study examines the genetic structure and phylogenetic relationships of *Uroteuthis chinensis* (Gray, 1849) in Bangka Belitung waters, Indonesia, using mitochondrial cytochrome c oxidase subunit I (COI) gene sequences. Twenty-four sequences were analyzed, comprising four new field-collected samples and 20 GenBank references. Alignment with ClustalW in MEGA X produced a consensus length of 446 bp. BLAST analysis confirmed species identity, showing ≥99% similarity with *U. chinensis* references. Nucleotide composition revealed an AT-rich profile (61.6% AT content), consistent with typical mitochondrial DNA of marine invertebrates. Genetic diversity analysis identified eight haplotypes, with haplotype diversity (Hd) of 0.681 and nucleotide diversity (π) of 0.00219, indicating moderate diversity with low nucleotide differences. Eight polymorphic sites were found, including seven singleton variable sites and one parsimony-informative site. Pairwise genetic distances (K2P model) ranged from 0.002 to 0.009, suggesting low to moderate intraspecific variation. Neighbor-joining phylogeny with 1,000 bootstrap replicates revealed several clusters (bootstrap 28–50), indicating localized genetic diversification. These findings suggest *U. chinensis* populations in Bangka Belitung are genetically homogeneous but retain unique haplotypes relevant to population dynamics and local adaptation. Broader geographic sampling and additional genetic markers are recommended to clarify population structure and connectivity in Indonesian waters.

INTRODUCTION

Indonesia is recognized as one of the world's biggest biodiversity countries (**Hasan *et al.*, 2022**; **Robin *et al.*, 2023**), characterized by exceptionally high levels of marine biodiversity (**Handayani *et al.*, 2022**). The Indonesian waters, forming part of the global Coral Triangle, serve as habitats for thousands of fish species and other marine organisms (**Ceccarelli *et al.*, 2022**). This biodiversity encompasses various taxonomic groups, including cephalopods, which hold significant ecological and economic value (**Pratasik**

***et al.*, 2022)**. However, the majority of fishery resources in Indonesia are still exploited without sufficient baseline information on genetic structure and population dynamics—key elements for sustainable resource management (**Soeparna *et al.*, 2024**).

Cephalopods, particularly squids of the family Loliginidae, play a key role in tropical and subtropical marine ecosystems (**Guo *et al.*, 2023**). One of the most commercially valuable species in this group is *U. chinensis* (**Puspasari *et al.*, 2025**), commonly known as the Chinese squid. This species is widely distributed throughout the western Indo-Pacific region, including the South China Sea, northern Australia, and Indonesian waters (**Morgan *et al.*, 2024; Zamroni *et al.*, 2024b; Robin *et al.*, 2025b**). Due to its high economic value, mitre squid *Uroteuthis chinensis* is intensively targeted by artisanal and commercial fisheries across its range, including the Bangka-Belitung Islands (**Puspasari *et al.*, 2025**).

While several studies have investigated the population genetics of *U. chinensis* in parts of the Indo-Pacific, such as in Chinese, Vietnamese, and Taiwanese waters (**Zamroni *et al.*, 2024a**), genetic data from the Indonesian region remain scarce. This limits our understanding of regional population connectivity and potential genetic differentiation across biogeographic boundaries (**Mzingirwa *et al.*, 2019; Arranz *et al.*, 2024**). Mitochondrial DNA markers, particularly the cytochrome c oxidase subunit I (COI) gene, have proven effective in resolving population structure (**Arai *et al.*, 2023**), detecting cryptic diversity (**Costa *et al.*, 2023**), and inferring phylogenetic relationships in cephalopods and other marine taxa (**Kautsari *et al.*, 2024; Mahrus *et al.*, 2025**).

The Bangka-Belitung Islands, located at the confluence of the Indian Ocean and the South China Sea (**Xu *et al.*, 2021**), represent a biogeographically strategic zone that may influence gene flow patterns and population differentiation (**Huang *et al.*, 2023**). Given this context, studying the genetic structure of *U. chinensis* populations in this region offers valuable insights into the broader evolutionary and ecological dynamics of marine species in the Indo-Pacific.

This study aims to analyze the genetic structure and phylogenetic relationships of *U. chinensis* populations from the Bangka-Belitung Islands using mitochondrial COI gene sequences. The results are expected to contribute to the understanding of intraspecific genetic diversity and population connectivity (**Riera *et al.*, 2025**) and to provide a scientific foundation for conservation strategies and the sustainable management of cephalopod fisheries in Indonesian waters.

MATERIALS AND METHODS

1.Sampling site and Fish samples collection

A total of 16 individuals of *U. chinensis* were collected through targeted sampling during July–August 2025 from four coastal sites on the Bangka Belitung Islands, Indonesia. Specimens were obtained using active capture techniques (e.g., hand lines and squid jigs) in collaboration with local fishers. The sampling sites were determined based on variations in land use, GPS coordinates, and differences in environmental

characteristics. The first sampling site (Red Circle 1) is located along the coastal waters of Tuing and Deniang Villages, Bangka Regency. The second site (Red Circle 2) is in the coastal area of Selat Nasik Village, East Belitung Regency. The third site (Red Circle 3) encompasses the coastal waters of Sadai Village, South Bangka Regency. The fourth and final site (Red Circle 4) includes Kampak and Air Nyatoh Villages in West Bangka Regency.

Of the 16 specimens collected, four were preserved in 96% molecular-grade ethanol shortly after capture for subsequent mitochondrial DNA analysis (**Nazran *et al.*, 2025**). Eleven live specimens were transported to the Fisheries Hatchery of the University of Bangka Belitung to support further research on breeding, reproductive biology, domestication, and larval development. The remaining individual was fixed in 10% (**Valen *et al.*, 2025**) buffered formalin and deposited as a voucher specimen in the Ichthyological Collection of the Aquaculture Laboratory at the University of Bangka Belitung. All specimen handling procedures followed institutional ethical guidelines and were conducted under appropriate research permits.

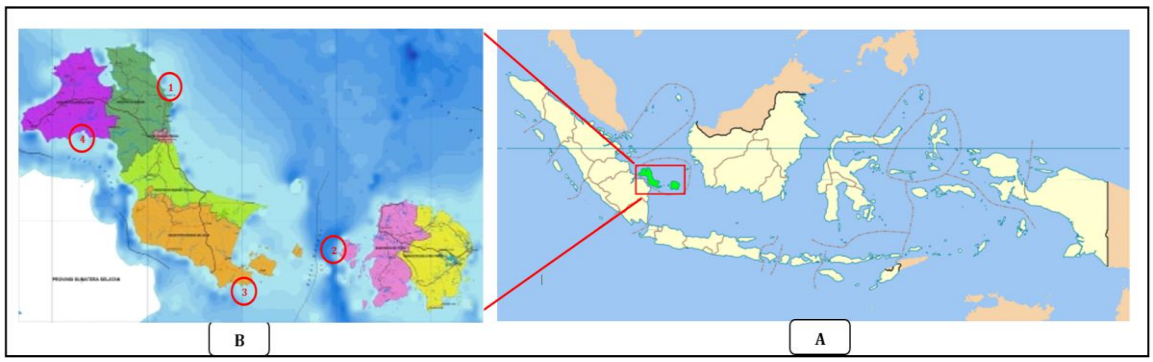

Fig. 1. Sampling locations of *U. chinensis* in natural habitats across the coastal waters of the Bangka Belitung Islands, Indonesia

2. Additional sequence data

In addition to newly obtained sequences, 20 mitochondrial DNA sequences of the cytochrome c oxidase subunit I (COI) gene from *U. chinensis* individuals in the Bangka Belitung region were included to support genetic and phylogenetic analyses. These sequences were downloaded from the GenBank database (accession numbers OR939404–OR939423) and aligned with the sequences generated in this study. Their inclusion was intended to enhance the resolution of population structure and phylogenetic relationships within a shared geographic context. All sequences underwent quality control checks prior to analysis.

3. DNA purification and PCR processes

The DNA extraction and amplification occurred from August 5 to August 10, 2025. Genomic DNA extraction utilizing the gSYNCTM DNA Extraction Kit (Geneaid, GS300) comprises four stages: lysis, binding, washing, and elution. Amplification PCR

utilizing MyTaq HS Red Mix (Bioline, BIO-25048) with a total volume of 25µl, containing 9.5µl of ddH2O, 12.5µl of MyTaq HS Red Mix, 10µM LCO1490 (5'-GGTCAACAAATCATAAAGATAATTGG-3'), 10µM HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (**Folmer *et al.*, 1994**), and DNA template. The reaction mixture was further increased utilizing the BioRad T100TM apparatus. The PCR cycle parameters consist of initial denaturation at 95°C for 3 minutes, and denaturation at 96°C for 10 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 45 seconds (**Robin *et al.*, 2025a**). Those steps were performed over 35 cycles each. The final stage was conducted at a temperature of 4°C for one cycle. The PCR results were subsequently examined in a 1% agarose gel by electrophoresis, utilizing nucleic acid gel dye (GelRed®) for staining (**Syarif *et al.*, 2025**). Agarose consists of 5ml of TAE buffer, 45ml of distilled water, and 8µl of red gel dye incorporated into the solution. Five microliters of the DNA sample was combined with one microliter of loading dye and introduced into the agarose gel well. The positive sample (luminous DNA band) was subsequently subjected to DNA sequencing via the Sanger dideoxy technique at PT. Genetics Science Jakarta.

4. Phylogenetic and genetic diversity analysis

Phylogenetic analysis was conducted to investigate the genetic relationships among *U. chinensis* individuals from the coastal waters of Bangka Belitung. A total of 24 COI gene sequences were used in the analysis, comprising four newly obtained sequences and 20 additional sequences retrieved from the GenBank database (accession numbers OR939404–OR939423). All sequences were analyzed using MEGA X software (**Kumar *et al.*, 2018**). The initial step involved sequence alignment using the ClustalW algorithm (**Daugelaite *et al.*, 2013**), followed by manual inspection to ensure the absence of misreads or significant gaps. The final aligned sequence length used for analysis was approximately 446 base pairs (bp). Prior to phylogenetic tree construction, the optimal nucleotide substitution model was identified using the "Find Best DNA/Protein Models (ML)" function in MEGA X (**Kumar *et al.*, 2018**), based on the Akaike Information Criterion (AIC). The Kimura 2-Parameter (K2P) model was selected as the best fit for the dataset (**Wennmann *et al.*, 2018**). A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (**Saitou *et al.*, 1987**) with 1,000 bootstrap replicates to assess the statistical support for each branch (**Russo *et al.*, 2018**). One sequence from an outgroup species, *Uroteuthis edulis*, was included to clarify the evolutionary position of *U. chinensis*. In addition, nucleotide composition analysis was performed in MEGA X to determine the proportions of the four nitrogenous bases (A, T, C, G) across the sequences. Genetic distances between sequences were calculated using the K2P model in MEGA X to quantify levels of genetic similarity or divergence among individuals. Further analyses of haplotype structure, nucleotide polymorphism, number of

polymorphic sites, and haplotype diversity (Hd) were performed using DnaSP v6 (**Rozas *et al.*, 2018**).

RESULTS

1.Aligment DNA

In total, 24 mitochondrial COI gene sequences of *U. chinensis* were included in this study. Four sequences were newly generated from specimens collected in the Bangka Belitung region and labeled as Babel 0 through Babel 3. An additional 20 reference sequences were obtained from the GenBank database (accession numbers OR939404–OR939423). All sequences were aligned using the ClustalW algorithm in MEGA X, and a final alignment of 446 base pairs in length was used for further genetic and phylogenetic analyses.

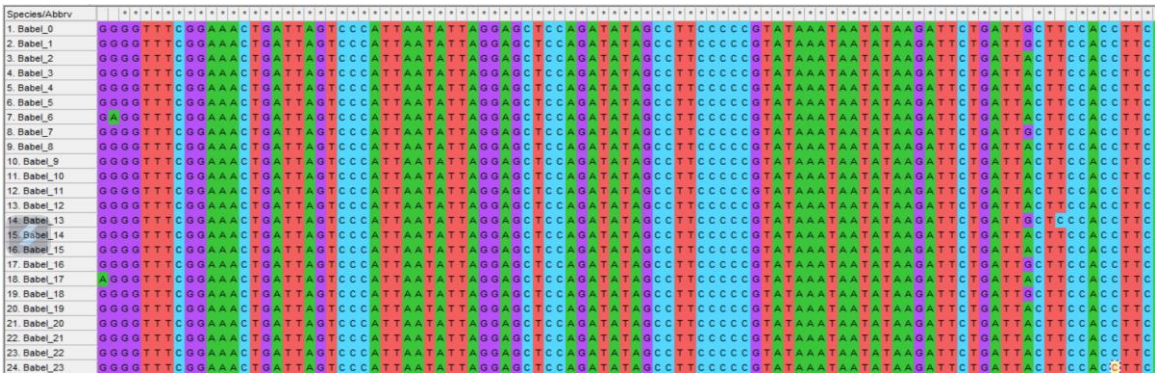

Fig. 1. Aligment DNA

2. BLAST DNA

Four *U. chinensis* samples collected from the coastal waters of Bangka Belitung (designated Babel 0 to Babel 3) were analyzed using the Basic Local Alignment Search Tool (BLAST) against the GenBank database. BLAST results indicated that all samples shared very high sequence similarity (≥99%) with reference sequences of *U. chinensis* stored in GenBank (Table 1).

Table 1. Identification of *U. chinensis* DNA sequences from Bangka Belitung based on nucleotide BLAST (BLASTn) analysis against the GenBank database

Sample Code	Sequence Identity (%)	Query Coverage (%)	Genbang Accession	Refrences Species
Babel_0	100%	100%	LC552681.1	*U. chinensis*
Babel_1	100%	100%	LC552681.1	*U. chinensis*
Babel_2	99.5%	100%	MK984358.1	*U. chinensis*
Babel_3	99.3%	100%	MZ938994.1	*U. chinensis*

Note: “Babel” indicates sampling locations within Bangka Belitung Province, Indonesia.

Based on the BLAST output, the alignment length for nearly all matches was 446 base pairs, with 100% query coverage. An E-value of 0.0 indicated that the sequence alignments were statistically highly significant. The percentage of sequence identity ranged from 99.27 to 100%, confirming consistent species-level identification (**Valen *et al.*, 2024a**). All top matches corresponded to *U. chinensis*, thereby verifying that the field-collected samples belong to the same species.

3. Nucleutide composition

Nucleotide composition analysis of the COI (cytochrome c oxidase subunit I) gene was performed on eight specimens of *U. chinensis* collected from the Bangka Belitung Islands, each yielding a sequence length of 446 base pairs. The analysis revealed a strong bias toward adenine (A) and thymine (T), which together accounted for an average of 61.6% of the total nucleotide composition (A: 28.3%, T: 33.3%), consistent with the AT-rich nature of mitochondrial DNA in marine invertebrates (Table 2). The proportions of cytosine (C) and guanine (G) were lower, averaging 22.7 and 15.7%, respectively. Minor variations were observed across the eight haplotypes, but the overall compositional pattern remained conserved.

Table 2. Nucleotide composition of *U. chinensis* from the Bangka Belitung Islands

Haplotype	T(U) (%)	C (%)	A (%)	G (%)	Length (bp)
1	33.4	22.6	28.0	15.9	446
2	33.2	22.9	28.3	15.7	446
3	33.2	22.9	28.3	15.7	446
4	33.4	22.6	28.3	15.7	446
5	33.4	22.6	28.5	15.5	446
6	33.2	22.9	28.0	15.9	446
7	33.4	22.6	28.5	15.5	446
8	33.4	22.4	28.7	15.5	446
Mean	**33.3**	**22.7**	**28.3**	**15.7**	**446**

4. Haplotype identification

Based on COI (cytochrome c oxidase subunit I) gene sequence analysis of 24 *U. chinensis* samples from the coastal waters of Bangka Belitung using DnaSP v6 software, the following results were obtained: the total number of haplotypes was eight, with eight variable sites. The haplotype diversity (Hd) value was 0.6812. Haplotype distribution analysis revealed that haplotype 4 (Hap_4) was the most dominant, occurring in 13 individuals, while five haplotypes were singletons, each represented by only one individual (Table 3).

Table 3. Haplotype distribution of *U. chinensis* from the coastal waters of Bangka Belitung based on COI gene sequences

Haplotype ID	Number of Individuals	Sample Code(s)
Hap_1	5	Babel_0, Babel_1, Babel_7, Babel_16, Babel_18
Hap_2	1	Babel_2
Hap_3	1	Babel_3
Hap_4	13	Babel_4, Babel_5, Babel_8, Babel_9, Babel_10, Babel_11, Babel_12, Babel_14, Babel_19, Babel_20, Babel_21, Babel_22, Babel_23
Hap_5	1	Babel_6
Hap_6	1	Babel_13
Hap_7	1	Babel_15
Hap_8	1	Babel_17

Note: "Babel" indicates sampling locations within Bangka Belitung Province, Indonesia.

5. Polymorphic sites

Analysis of 24 COI (cytochrome c oxidase subunit I) gene sequences from *U. chinensis* collected in the Bangka Belitung region revealed that, out of a total of 446 nucleotide positions analyzed, eight were polymorphic (variable sites) and 438 were monomorphic (invariable), with no gaps or missing data. Among the eight polymorphic sites, seven were singleton variable sites—nucleotide positions exhibiting mutations in only a single individual—located at positions 1, 2, 89, 245, 290, 359, and 416 (Table 4). The remaining site, position 86, was identified as a parsimony-informative site with two nucleotide variants, which can be used for phylogenetic inference. No sites were found with more than two variants (i.e., tri-allelic or tetra-allelic sites), indicating that the observed genetic variation among sequences was simple and consisted predominantly of single nucleotide polymorphisms (SNPs).

Table 4. Polymorphic sites in COI gene sequences of *U. chinensis* from the Bangka Belitung region

Parameter	Value/Position	Description
Total nucleotide positions	446 bp	Sequence length analyzed
Polymorphic sites (variable sites)	8	Total positions exhibiting mutations
Monomorphic sites (invariable sites)	438	Positions showing no variation
Gaps / missing data	None	Complete alignment with no missing data
Singleton variable sites	7 (Positions: 1, 2, 89, 245, 290, 359, 416)	Mutations occurring in only one individual
Parsimony-informative site	1 (Position: 86)	Site with two variants, useful for

		phylogenetic inference
Sites with three or four variants	None	No complex mutations detected, supporting predominance of simple SNPs
Dominant mutation type	SNP (single nucleotide polymorphism)	Genetic variation among individuals is simple and limited

6. Haplotype diversity

Analysis of 24 COI (cytochrome c oxidase subunit I) gene sequences from *U. chinensis* in the Bangka Belitung region revealed eight unique haplotypes, with a haplotype diversity (Hd) of 0.681 accompanied by a standard deviation of 0.092 and a variance of 0.00845. This value indicates that, although the majority of individuals share the same haplotype—particularly haplotype 4—there remains a moderate level of genetic diversity within the population. In addition, the nucleotide diversity (π) was recorded at 0.00219, suggesting that the average pairwise nucleotide differences within the population are relatively low. The Theta (θ) per site was estimated at 0.00480 based on η (the total number of mutations) and at 0.00486 based on π (Table 5).

Table 5. Genetic diversity indices for *U. chinensis* from the Bangka Belitung region based on COI gene sequences

Parameter	Value	Description
Number of nucleotide sites	446 bp	COI region analyzed
Polymorphic sites (S)	8	Number of variable sites
Number of mutations (Eta)	8	Total nucleotide changes
Number of haplotypes (h)	8	Total unique haplotypes
Haplotype diversity (Hd)	0.681	Genetic diversity based on haplotypes
Variance of haplotype diversity	0.00845	—
Standard deviation of haplotype diversity	0.092	—
Nucleotide diversity (π)	0.00219	Average nucleotide differences between individuals
Theta (per site) from Eta	0.00480	Estimated genetic variation based on mutations
Theta (per site) from S (Theta-Watterson)	0.00480	Estimated variation based on segregating sites
Theta (per site) from π	0.00486	Estimated variation based on nucleotide differences

7. Analysis of local sample sequences

A summary of the four COI (Cytochrome C Oxidase Subunit I) gene sequences obtained from field collections in the Bangka Belitung region is presented Table (6).

Table 6. Summary of COI gene sequences from field-collected *U. chinensis* samples in the Bangka Belitung region

Sample Code	Haplotype Name	Sequence Length (bp)	Notes
Babel_0	Haplotype 1	446	Identical to Babel_1
Babel_1	Haplotype 1	446	-
Babel_2	Haplotype 2	446	1–2 polymorphic sites
Babel_3	Haplotype 3	446	Difference at position 250 (G → C)

Note: "Babel" indicates sampling locations within Bangka Belitung Province, Indonesia.

8. Genetic distances

Genetic distances among haplotypes were calculated using the Kimura 2-Parameter (K2P) model. The results, presented in Table (7), indicate that genetic distances among samples ranged from 0.002 to 0.009, suggesting low to moderate levels of intraspecific genetic diversity.

Tabel 7. Pairwise genetic distances among *U. chinensis* haplotypes from the Bangka Belitung region based on the Kimura 2-Parameter (K2P) model

Haplotype	1	2	3	4	5	6	7	8
1								
2	0.005							
3	0.005	0.005						
4	0.002	0.002	0.002					
5	0.004	0.005	0.005	0.002				
6	0.002	0.007	0.007	0.005	0.007			
7	0.004	0.005	0.005	0.002	0.004	0.007		
8	0.007	0.007	0.007	0.005	0.007	0.009	0.007	

9. Molecular phylogeny

A phylogenetic tree was constructed using the neighbor-joining (NJ) method with 1,000 bootstrap replicates. The Kimura 2-Parameter (K2P) model was applied to estimate evolutionary distances. *U. edulis* was included as an outgroup. The analysis revealed the formation of several genetic clusters, with moderate to high bootstrap support values (28–50), indicating the presence of local genetic diversification within the Bangka Belitung population (Fig. 2).

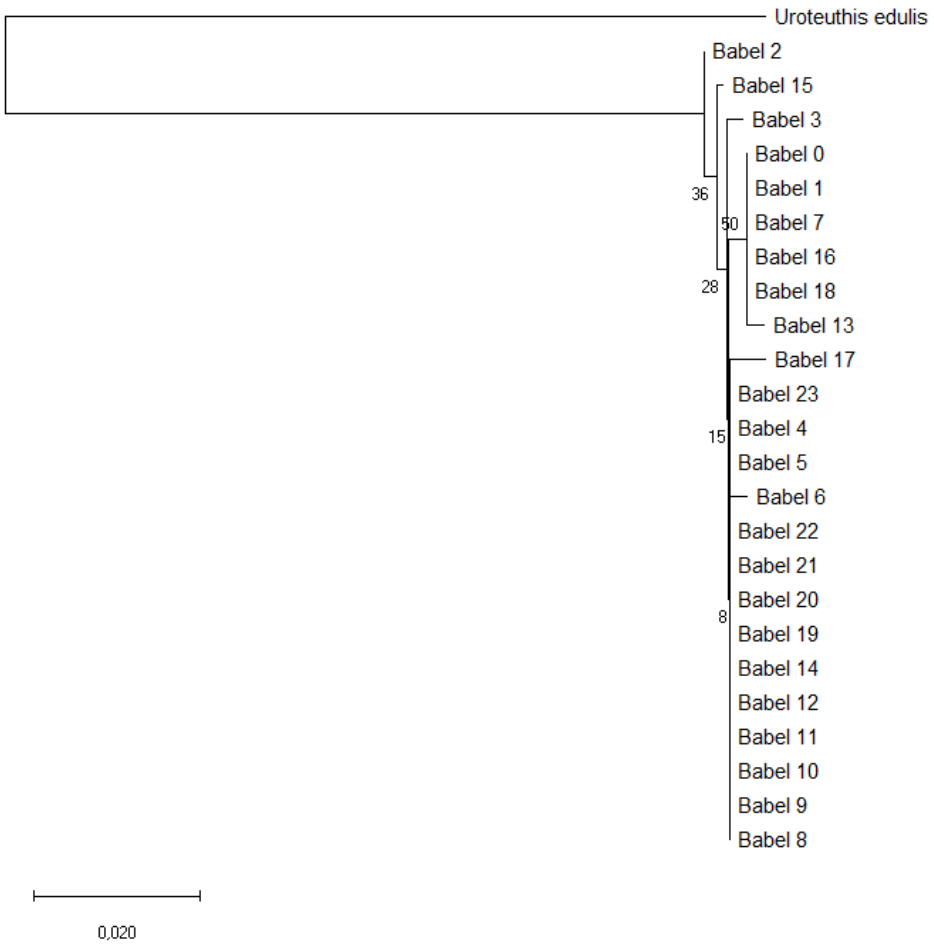

Fig. 2. Phylogenetic relationships among *U. chinensis* haplotypes from the Bangka Belitung region based on COI gene sequences (Note: "Babel" indicates sampling locations within Bangka Belitung Province, Indonesia)

The tree was constructed using the neighbor-joining (NJ) method with 1,000 bootstrap replicates, applying the Kimura 2-Parameter (K2P) model to estimate evolutionary distances. *U. edulis* was included as an outgroup. Bootstrap values (28–50) are shown at the corresponding nodes. The analysis reveals several genetic clusters, suggesting localized genetic diversification within the Bangka Belitung population.

DISCUSSION

BLAST analysis of four *U. chinensis* samples collected from the Bangka Belitung waters revealed very high nucleotide sequence similarity (≥99%) with reference sequences available in the GenBank database. Two of the four samples (Babel_0 and Babel_1) exhibited 100% identity with sequence LC552681.1, while the other two samples (Babel_2 and Babel_3) showed 99.5 and 99.3% identity with MK984358.1 and MZ938994.1, respectively. All BLAST results yielded an E-value of 0.0 and 100% query coverage, indicating that the matches were statistically highly significant and not due to random similarity (**Robin *et al.*, 2022; Bolaji *et al.*, 2023; Syarif *et al.*, 2023; Valen *et al.*, 2023; Yalla *et al.*, 2023**).

These findings provide strong evidence that the four field-collected samples belong to *U. chinensis*. The very high sequence identity between the local samples and reference sequences demonstrates that the COI molecular marker is effective for species-level identification (**Valen *et al.*, 2022; Robin *et al.*, 2025a**). Minor differences in sequence identity (<1%) likely reflect intraspecific variation or genetic divergence among populations from different geographic locations.

All mitochondrial COI sequences analyzed in this study had a final aligned length of 446 base pairs after alignment using the ClustalW algorithm. No large gaps or mutations disrupting the reading frame were detected, indicating that the COI segment lies within a conserved and functionally important region, as is commonly observed in mitochondrial sequences of marine invertebrates.

Meanwhile, validation through BLAST analysis and high-quality DNA sequence alignment offers a reliable basis for assessing genetic diversity and population structure in *U. chinensis*. Although all four samples from Bangka Belitung were confirmed as the same species, slight nucleotide variations detected among them may serve as indicators of population genetic differentiation or potential geographic isolation (**Li *et al.*, 2022**).

Analysis of COI sequences from 24 *U. chinensis* samples using DnaSP v6 identified eight distinct haplotypes with eight variable sites. The haplotype diversity (Hd) value of 0.6812 indicates a moderate level of genetic variation in the analyzed population. Haplotype distribution revealed dominance of Hap_4, present in 13 of the 24 individuals (54.2%), while five haplotypes were singletons, each represented by a single individual. This pattern suggests a non-homogeneous population structure, where most individuals belong to one dominant genetic group while others harbor unique genetic variants potentially important for local adaptation or genetic isolation (**Insani *et al.*, 2022**).

Analysis of the 446 nucleotide positions in the COI gene showed eight polymorphic sites, with the remaining 438 positions monomorphic. Seven of these polymorphic sites were singleton variable sites—mutations occurring in only one individual—while one site (position 86) was parsimony-informative, having two nucleotide variants useful for phylogenetic inference. No tri-allelic or tetra-allelic sites were found, indicating that genetic variation in this population is dominated by simple single-nucleotide polymorphisms (SNPs), as is common in mitochondrial DNA markers (**Castañeda *et al.*,**

2022). Although the overall variation was low, the presence of an informative site such as position 86 still provides genetic signals that can be used for phylogenetic tree construction, mapping individual relationships, and testing hypotheses on the origin and divergence of the population (**Zheng *et al.*, 2023; Kise *et al.*, 2024**).

The analysis of 24 COI gene sequences from *U. chinensis* individuals in the Bangka Belitung region yielded eight unique haplotypes, reflecting haplotype diversity (Hd) of 0.681, with a standard deviation of 0.092 and variance of 0.00845. These values suggest that, although most individuals share the dominant haplotype (Hap_4), the population retains a moderate level of genetic variation (**Li *et al.*, 2023**).

The presence of five singleton haplotypes—each found in only one individual—indicates low-frequency genetic variants in the population. Such unique haplotypes may represent recent mutations, local adaptations, or limited gene flow among subpopulations (**Miller *et al.*, 2012**). This distribution pattern is common in marine invertebrates with high fecundity and planktonic larval stages, where selection and dispersal play key roles in shaping population genetic structure (Hellberg, 2009).

The recorded nucleotide diversity (π) of 0.00219 reinforces the interpretation that the level of genetic variation at the nucleotide level in this population is low. This value represents the average number of nucleotide differences per random pair of individuals and is consistent with the high conservation of mitochondrial genes, such as COI, which have essential roles in cellular respiration **(Chen *et al.*, 2019)**.

Estimates of Theta (θ) per site, based on both the total number of mutations (η) and the number of segregating sites (S), yielded nearly identical values (0.00480), indicating consistent mutation rates across the analyzed gene segment. The agreement with the Theta value based on π (0.00486) also suggests that the population is close to a mutation–drift equilibrium, reflecting a stable population dynamic over its evolutionary history (**Angst *et al.*, 2022**).

The genetic diversity parameters indicate that *U. chinensis* populations in Bangka Belitung have a stable genetic structure with moderate diversity. This is supported by genetic distance analysis among haplotypes, which ranged from 0.002 to 0.009, indicating low to moderate levels of intraspecific variation (**Valen *et al.*, 2023; Sumana *et al.*, 2024**). The lowest genetic distance (0.002) was found between haplotypes 1 and 4, as well as between haplotype 4 and haplotypes 5 and 7, suggesting minimal nucleotide differences and possible derivation from the same genetic ancestor or recent divergence. Conversely, the highest distance (0.009) occurred between haplotypes 6 and 8, indicating greater genetic differences while still remaining within the intraspecific range.

In general, the observed genetic distance range suggests that, despite haplotype differentiation, all individuals belong to the same species with no evidence of deep phylogenetic divergence or cryptic speciation. However, genetic distances approaching 0.009 indicate that some individuals harbor higher genetic variation, potentially reflecting

micro-population structuring, local geographic isolation, or spatially driven selective pressures within the Bangka Belitung region (**Séré *et al.*, 2017**; **Robin *et al.*, 2025a, b**).

These genetic distance data provide important insights into the degree of genetic relatedness among individuals in the population and support phylogenetic analyses in detecting potential genetic clustering within the species (**Nousias *et al.*, 2021**; **Dong *et al.*, 2024**). The phylogenetic results also support earlier findings that *U. chinensis* in Bangka Belitung is relatively homogeneous yet contains hidden genetic variation that could develop into more complex population structures over time. The inclusion of *U. edulis* as an outgroup reinforced species-level phylogenetic boundaries and confirmed that all analyzed haplotypes belong to the same monophyletic clade of *U. chinensis*.

Overall, the phylogenetic analysis strengthens the hypothesis of local-level population differentiation that is not yet fully captured by conventional genetic parameters but is detectable through evolutionary analysis. The use of additional molecular markers, such as 16S, cyt b, or nuclear markers, would help to resolve a more detailed phylogenetic structure in future studies (**Xiong *et al.*, 2022**).

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REFERENCES

Ahmed, M.S.; Datta, S.K.; Saha, T. and Hossain, Z. (2021). Molecular characterization of marine and coastal fishes of Bangladesh through DNA barcodes. Ecology and Evolution., 11(9): 3696-3709.

Angst P, Ameline C, Haag CR, Ben-Ami F, Ebert D, Fields PD. (2022). Genetic Drift Shapes the Evolution of a Highly Dynamic Metapopulation. Molecular Biology and Evolution., 39(12): msac264.

Arai, T.; Taha, H.; Alidon, N., Jumat, J., Azmey, S., Zan, N. D., ... & Habib, A. (2023). Mitochondrial cytochrome c oxidase subunit I gene analysis of the yellowfin snapper, *Lutjanus xanthopinnis* in the Indo-Pacific region and a note on *Lutjanus lutjanus* population structure. Heliyon., 9(9): e19348

Arranz, V.; Fewster, R.M. and Lavery, S.D. (2024). Concordance and drivers of marine spatial structure determined using genogeographic clustering. Journal of Biogeography., 51(10): 2034-2046.

Bolaji, D.A.; Lawal-Are, A.O. and Kuton, M.P. (2023). DNA barcoding and misidentification of some marine fish species in Nigerian industrial trawl fishery. Scientific African., 20: e01662.

Castañeda V, Haro-Vinueza A, Salinas I, Caicedo A, Méndez MÁ. (2022). The MitoAging Project: Single nucleotide polymorphisms (SNPs) in mitochondrial genes and their association to longevity. Mitochondrion., 66:13-26

Ceccarelli, D.M.; Lestari, A.P. and White, A.T. (2022). Emerging marine protected areas of eastern Indonesia: Coral reef trends and priorities for management. Marine policy., 141: 105091.

Chen, L.; Huang, J.R.; Dai, J.; Guo, Y.F.; Sun, J.T. and Hong, X.Y. (2019). Intraspecific mitochondrial genome comparison identified CYTB as a high-resolution population marker in a new pest *Athetis lepigone*. Genomics., 111(4): 744-752.

Costa, A.C.P.; Garcia, T.M.; Tavares, T.C.L.; dos Santos Rocha, R.; Maggioni, R. and Soares, M.O. (2023). DNA barcoding reveals cryptic diversity of fish eggs in Equatorial Southwestern Atlantic. Regional Studies in Marine Science, 65, 103069.

Daugelaite, J.; O′ Driscoll, A. and Sleator, R.D. (2013). An overview of multiple sequence alignments and cloud computing in bioinformatics. International Scholarly Research Notices., 2013(1): 615630.

Dong, F.; Cheng, P.; Sha, H.; Yue, H.; Wan, C.; Zhang, Y.; ... & Wei, Q. (2024). Genetic diversity and population structure analysis of blunt snout bream (*Megalobrama amblycephala*) in the Yangtze River Basin: Implications for conservation and utilization. Aquaculture Reports., 35: 101925.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology., 3(5): 294-299.

Guo, R.; Li, N.; Fang, Z. (2023). The Fecundity Characteristics and Spawning Strategy of Uroteuthis edulis in the East China Sea. Animals., 13: 2786.

Handayani, C.N.; Andradi-Brown, D.A.; Ford, A.K.; Beger, M.; Hakim, A.; Muenzel, D.K.; ... and Ahmadia, G.N. (2022). The rapid expansion of Indonesia's marine protected area requires improvement in management effectiveness. Marine Policy., 146: 105257.

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. (2023). Fish diversity of the Bengawan Solo River estuary, East Java, Indonesia. Biodiversitas., 24(4): 2207-2216.

Hellberg, M.E. (2009). Gene flow and isolation among populations of marine animals. Annual Review of Ecology, Evolution and Systematics., 40: 291.

Huang, W.; Chen, Y.; Wu, Q.; Feng, Y.; Wang, Y.; Lu, Z.; ... & Yu, K. (2023). Reduced genetic diversity and restricted gene flow of broadcast-spawning coral *Galaxea fascicularis* in the South China Sea reveals potential degradation under environmental change. Marine Pollution Bulletin., 193: 115147.

Insani, L.; Jatayu, D.; Valen, F.S.; Widodo, M.S. 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.

Kautsari, N.; Suleman.; Djonu, A. and Valen, F.S. (2024). DNA barcoding and phylogenetic of lollyfish, *Holothuria atra* (Jaeger, 1833) (Actinopoda: Holothuriidae), from Tablolong Beach, Kupang, East Nusa Tenggara, Indonesia. AACL Bioflux., 17 (1): 1 – 12

Kise, H.; Saito, N.; Gibu, K.; Asato, M.; Kita, T.; Matsuzaki, K. and Iguchi, A. (2024). Population structure and genetic connectivity of the big blue octopus *Octopus cyanea* in the Ryukyu Archipelago, Japan. Aquatic Conservation: Marine and Freshwater Ecosystems., 34(5): e4153.

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(6): 1547-1549.

Li, M.; Huang, W.; Wu, Q.; Feng, Y.; Chen, Y.; Yu, K. ... and Wang, X. (2022). High genetic differentiation and moderate genetic diversity of the degenerative branching coral Pocillopora verrucosa in the tropical South China Sea. Science of the Total Environment., 819: 153076.

Li, S.; Lyu, Y.; Zhang, C. and Zheng, X. (2023). Morphological and Genetic Differentiation of Loliolus (Nipponololigo) beka (Cephalopoda: Loliginidae) in Coastal China. Diversity., 15(1): 41.

Mahrus, H.M.; Karnan, K. and Sedijani, P. (2025). Molecular and phylogenetic analysis of Sardines at the fish landing center-Tanjung Luar-east Lombok using DNA sequences of the CO1 gene. Fisheries and Aquatic Sciences., 28(3): 152-162.

Miller, M.R.; Brunelli, J.P.; Wheeler, P.A.; Liu, S.; Rexroad, C.E.; Palti, Y.; Doe, C.Q. and Thorgaard, G.H.A. (2012). conserved haplotype controls parallel adaptation in geographically distant salmonid populations. Molecular Ecology., 21(2):237-49.

Morgan, J.A.; Dunning, M.C.; Ovenden, J.R.; Broderick, D.; Street, R.; Davies, J. and Hall, K.C. (2024). Molecular delimitation of cryptic Australian squid species of the genus Uroteuthis Rehder, 1945 (Cephalopoda: Loliginidae), provides a baseline of diversity to resolve classification challenges throughout the Indo-Pacific. Journal of Molluscan Studies., 90(3): eyae028.

Mzingirwa, F.A.; Stomeo, F.; Kaunda-Arara, B.; Nyunja, J. and Mujibi, F.D. (2019). Genetic connectivity of the Sky Emperor, Lethrinus mahsena populations across a gradient of exploitation rates in coastal Kenya. Frontiers in Genetics., 10: 1003.

Nazran.; Syofriani.; Ulfauza.; Valen, F.S.; Oktaviandi, D.; Prayoga, A.; Ilmia, M.; Czech, M.; Kamarudin, A.S. and Hasan, V. (2025). DNA Barcoding of *Rasbora sp.* from Bangka Island: A Genetic Identification Basis and Its Potential Application in Aquaculture. Egyptian Journal of Aquatic Biology and Fisheries., 29(3): 213-226.

Nousias, O.; Tzokas, K.; Papaharisis, L.; Ekonomaki, K.; Chatziplis, D.; Batargias, C. and Tsigenopoulos, C.S. (2021). Genetic Variability, Population Structure,

and Relatedness Analysis of Meagre Stocks as an Informative Basis for New Breeding Schemes. Fishes., 6(4): 78.

Pratasik, S.B.; Lalamentik, L.T.H.X.; Manoppo, L. and Budiman, J. (2022). Deep sea squid in Sulawesi Sea, North Sulawesi Province, Indonesia. Biodiversitas., 23: 1774-1779.

Puspasari, R.; Rahman, A.; Sugianti, Y.; Ambalika, I.; Putri, M.R.A.; Amri, K. and Pamungkas, A. (2025). Projecting potential spawning area and habitat characterization of the Mitre Squid (*Uroteuthis chinensis* Gray, 1849) using socio-ecological methods. Egyptian Journal of Aquatic Research., 51(3): 322-331.

Riera, J.; Tudurí, A.; Guijarro, B.; Ordines, F.; Picornell, A. and Ramírez-Amaro, S. (2025). Genetic Diversity, Connectivity and Demographic History of the Small Red Scorpionfish Scorpaena notata at a Small Scale in the Balearic Islands (Western Mediterranean). Diversity., 17: 405.

Robin, R.; Anjani, T.P. and Simanjuntak, S.W. (2025a). Molecular Evidence and Phylogenetic Analysis of the Mitochondrial COI Gene for Species Confirmation of Mitre Squid *Uroteuthis chinensis* (Gray, 1849) from Bangka Island. Egyptian Journal of Aquatic Biology & Fisheries, 29(5): 963 – 977

Robin, R.; Utami, E.; Umroh.; Valen, F.S. (2025b) Molecular Identification of *Uroteuthis chinensis* (Gray, 1849) (Myopsida: Loliginidae) from Selat Nasik, Belitung Island Using DNA Barcoding of the COX1 Gene. Egyptian Journal of Aquatic Biology & Fisheries, 29(1): 2325 – 2338

Robin, R.; Insani, L.; Swarlanda.; 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.; Ramadhanu, D.; Nomleni, A.; Turnip, G. and Insani, L. (2023). 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

Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E. and Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution, 34(12): 3299-3302.

Russo, C.A.D.M. and & Selvatti, A. P. (2018). Bootstrap and rogue identification tests for phylogenetic analyses. Molecular Biology and Evolution, 35(9): 2327-2333.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4(4): 406-25.

Séré, M.; Thévenon, S.; Belem AMG, De Meeûs T. (2017). Comparison of different genetic distances to test isolation by distance between populations. Heredity, 119(2):55-63.

Soeparna, I.I. and Taofiqurohman, A. (2024). Transversal policy between the protection of marine fishery resources and fisheries subsidies to address overfishing in Indonesia. Marine Policy, 163: 106112.

Sumana, S.L.; Wang, P.; Zhang, C.; Jing, X.; Zhu, J.; Tang, Y.; ... & Liao, Y. (2024). Genetic diversity of the common carp black strain population based on mtDNA (D-loop and cytb). Heliyon, 10(10): e30307

Syarif, A.F.; Valen, F.S.; Kurniawan, A.; Rudiansyah.; Herjayanto, M.; Mamat, N.B.; Ottoni, F.P.; Andriyono, S. and Hasan, V. (2025). Unveiling the First DNA Barcoding of *Betta cf. uberis* Fish (Anabantiformes: Osphronemidae) from Belitung Island, Indonesia. Egyptian Journal of Aquatic Biology & Fisheries, 29(1): 2023 – 2036.

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

Valen, F.S.; Widodo, M.S.; Islamy, R.A.; Wicaksono, K.P.; Soemarno.; Insani, L. and Hasan, V. (2022). Molecular phylogenetic of silver barb *barbonymus gonionotus* (bleeker, 1849) (cypriniformes: Cyprinidae) in Java, Indonesia. IOP Conference Series: Earth and Environmental Science, 1036(1): 012011.

Valen, F.S.; Bidayani, E.; Alfian, R.A.; Prananda, M.; Notonegoro, H.; Susilo, N.B.; Ardi, W.D.; Swarlanda.; Aziz, M.A.; Puryoso.; Widodo, M.S.; Faqih, A.R. and Hasan, V. (2023). 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. and Hasan, V. (2023). 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.; Mamat, N.B.; South, J.; Ottoni, F.P.; Vieira, L.O.; Kamarudin, A.S.; Afandi, A.Y. and Hasan, V. (2024). Unveiling the occurrence of vulnerable Sisorid catfish (Teleostei: Sisoridae) in Bangka based on morphological and molecular evidence. Biodiversitas, 25(11): 4543-4550.

Valen, F.S.; Syaputra, D.; Mohd Hasmadi, I.; Swarlanda.; 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.; Salmi, S. And Syarif, A.F. (2025). DNA Barcoding Reveals the Genetic Identity of Licorice Gourami *Parosphromenus deissneri*, an Endangered Species Threatened by Tin Mining in Bangka. Egyptian Journal of Aquatic Biology & Fisheries., 29(4): 573 – 589

Wennmann, J.T.; Keilwagen, J. and Jehle, J.A. (2018). Baculovirus Kimura two-parameter species demarcation criterion is confirmed by the distances of 38 core gene nucleotide sequences. Journal of General Virology, 99(9): 1307-1320.

Xiong, F.; Shu, L.; Zeng, H.; Gan, X.; He, S. and Peng, Z. (2022). Methodology for fish biodiversity monitoring with environmental DNA metabarcoding: The primers, databases and bioinformatic pipelines. Water Biology and Security., 1(1): 100007.

Xu, T.F.; Wei, Z.X.; Susanto, R.D.; Li, S.J.; Wang, Y.G.; Wang, Y., ... & Fang, G. H. (2021). Observed water exchange between the south China Sea and Java Sea through karimata strait. Journal of Geophysical Research: Oceans., 126(2): e2020JC016608.

Yalla, S.K. and Mohanraju, R. (2019). DNA barcoding of commercially important cephalopods from Andaman Islands, India. Regional Studies in Marine Science, 25: 100479.

Zamroni, A.; Boer, M.; Butet, N.A. and Zairion, Z. (2024a). Dominant species of squid in the waters of Western Kalimantan and Northern Java identified by DNA barcoding method. Egyptian Journal of Aquatic Research, 50(1): 121-126.

Zamroni, A.; Boer, M.; Butet, N.A. and Zairion, Z. (2024b). Kinship relationship of *Uroteuthis chinensis* in the waters of western Kalimantan and northern Java based on morphometric characters and genetic structure. Regional Studies in Marine Science, 72: 103442.

Zheng, J.; Tang, Y.; Xu, R.; Zhang, X. and Zheng, X. (2023). Molecular phylogenetics and population demographic history of Amphioctopus fangsiao, inferred from mitochondrial and microsatellite DNA markers. Acta Oceanologica Sinica, 42(6): 39-48.