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Molecular Identification of *Uroteuthis chinensis* (Gray, 1849) (Myopsida: Loliginidae) from Selat Nasik, Belitung Island Using DNA Barcoding of the COX1 Gene

Robin[1]*, Eva Utami[2], Umroh[2], Fitri Sil Valen[1]

[1]Department of Aquaculture, Faculty of Agriculture, Fisheries and Marine, Universitas Bangka Belitung, Jl Kampus Terpadu UBB, Balunijuk 33127, Bangka Belitung, Indonesia.
[2]Department of Marine Science, Faculty of Agriculture, Fisheries and Marine, Universitas Bangka Belitung, Jl Kampus Terpadu UBB, Balunijuk 33127, Bangka Belitung, Indonesia.

***Corresponding Author: robin@ubb.ac.id**

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ABSTRACT

Uroteuthis chinensis is a commercially important squid species in Indonesia, particularly in the Bangka-Belitung waters, where it holds high economic and ecological significance. Due to its high export quality, it is a key commodity for the region's fisheries and marine sectors. Belonging to the Loliginidae family, which comprises approximately 50 species, *Uroteuthis chinensis* exhibits overlapping populations with closely related species in the Western Pacific, complicating morphological identification. DNA-based approaches, particularly mitochondrial Cytochrome C Oxidase Subunit I (COX1) gene analysis, offer a more accurate and efficient alternative for species identification. DNA was extracted from Bangka squid specimens using the gSYNCTM DNA Extraction Kit, and the COX1 gene was amplified using PCR with MyTaq HS Red Mix. Electrophoresis and BLASTn analysis confirmed the species identity with 99-100% similarity to *Uroteuthis chinensis*. Phylogenetic analysis, using the neighbor-joining method with 1000 bootstrap replicates, revealed that the Belitung Island population clusters within the same clade as *Uroteuthis chinensis*, with minimal genetic divergence (0.004). A total of 446 nucleotide base pairs were analyzed using the primers LCO1490 and HCO2198. These results confirm the evolutionary relationship of *Uroteuthis chinensis* and demonstrate the effectiveness of DNA barcoding for cephalopod species identification. This study contributes to biodiversity monitoring and provides a molecular reference for *Uroteuthis chinensis* in Indonesia. The sequence data has been deposited in the NCBI GenBank to support further research on marine biodiversity.

INTRODUCTION

Indonesia's marine biodiversity plays a crucial role in supporting the nation's fisheries economy **(Hasan & Islam, 2020; Amkieltiela *et al.*, 2022; Nurjirana *et al.*, 2022)**. Among the species contributing to this sector, *Uroteuthis chinensis*, also referred to as the Chinese squid, has emerged as a valuable resource due to its quality and global market appeal. The Bangka-Belitung region provides an optimal habitat for this species,

making it integral to the local fisheries. According to field studies and community converses, local squid traders and fishermen reported that Chinese squid, also known as Bangka squid, possesses a tender and flavorful meat texture, accompanied by a sweeter taste, making it more desirable in both local and export markets. The volume, processing products, and production value of Bangka squid significantly enhance the community's economy. The fisheries export statistics of the Bangka Belitung Islands Province indicate that squid commodities are the foremost superior commodity within the small pelagic resource category and the sixth superior export commodity on the provincial fisheries scale.

Chinese squid is a species from Loliginidae family **(Sin *et al.*, 2009)**, an Indo-Pacific species that can be found in waters ranging from the western Pacific to the Indian Ocean including the waters of Indonesia **(Zamroni *et al.*, 2024a)**. Loliginidae is a family of neritic squids including approximately 50 known species **(Sales *et al.*, 2013)**, some of them are from the genus *Uroteuthis*. Moreover, there are several species of *Uroteuthis* exist in Indonesia, namely *U. bartschi, U. edulis, U. duvaucelii, U. sibogae, U. s singhalensis,* and *U. pickfordi* **(Afiati *et al.*, 2022)**. However, some species exhibit overlapping populations in the Western Pacific **(Jin *et al.*, 2022)** and are additionally difficult to differentiate morphologically.

The significant morphological variations between these two species include the mantle ratio, the length of the hectocotylus in males, and the quantity and configuration of the arm sucker ring teeth **(Zamroni *et al.*, 2024b)**. Notwithstanding the aforementioned differences, intraspecific changes in these physical traits are insufficiently pronounced to clearly differentiate these two species. Moreover, the hectocotylus is solely effective in differentiating mature males and is not applicable for distinguishing females or juvenile squid. Therefore, the teeth shape of the arm sucker ring appears to be the sole dependable morphological characteristic for differentiation. The failure to consistently differentiate these species may have resulted in inconsistencies in their documented geographical distributions.

Moreover, morphological analyses are exceedingly time-consuming and require numerous taxonomic specialists **(Zamroni *et al.*, 2024b)**. Furthermore, the existence of cryptic diversity may affect the outcomes of species analysis. Due to the complexity of *Uroteuthis* identification using existing morphological criteria, it is prudent to employ DNA systematics for species identification **(Valen *et al.*, 2024a; Zamroni *et al.*, 2024a)**. The discovery of DNA barcoding, a standardized technique for organism identification utilizing specific DNA sequences **(Robin *et al.*, 2022)**, along with the building of reference DNA barcode databases **(Valen *et al.*, 2023)**, has transformed biodiversity study **(Koblmüller *et al.*, 2024)**. The primary DNA barcode utilized for animals is the mitochondrial DNA Cytochrome C Oxidase subunit I gene (COX1) **(Robin *et al.*, 2023)**. It has demonstrated efficacy in differentiating closely related species and identifying cryptic taxa **(Anslan & Tedersoo, 2015)**. The COX1 gene has been extensively utilized

as a tool for species identification in marine organisms, including squid and other cephalopods **(Jin *et al.*, 2022)**. This study aimed to identify squid species present in Belitung waters based on the COX1 gene. Moreover, it aimed to provide preliminary data on confirmed species, supporting as a preliminary inquiry into the population structure and genetic diversity of squid inhabiting Belitung Island.

MATERIALS AND METHODS

1. Sampling site and fish samples collection

Bangka squid specimens were obtained from Nasik Strait, Belitung Islands, Indonesia on July 6, 2024. The squids were captured using a fish trap with a mesh size of 0.5-1cm. A total of 15 squids were collected and subsequently researched for their DNA characteristics. The remaining two specimens were preserved in a 96% ethanol solution **(Lutfiatunnisa *et al.*, 2020; Valen *et al.*, 2022)** for genetic examination at the Genetics Science Laboratory in Jakarta. The other 11 squids were transported to the Bangka Belitung University Fisheries Hatchery as a live specimens for the next stage of research (breeding, reproduction, domestication and larva rearing). The last one specimen was kept as a voucher using the 10% solution **(Valen *et al.*, 2024b; Syarif *et al.*, 2025)** of formalin and stored in the Aquaculture Laboratory (Ichthyological collecting) at the University of Bangka Belitung.

2. DNA purification and PCR processes

The DNA extraction and amplification occurred from August 5 to August 10, 2024. Genomic DNA extraction utilizing the gSYNCTM DNA Extraction Kit (Geneaid, GS300), comprising 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, denaturation at 96°C for 10 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 45 seconds. The steps of denaturation, annealing, and extension were performed over 35 cycles each. The final stage was held 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. 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.

3. Data analysis

Species identification was conducted using BLASTn (Basic Local Alignment Search Tool-nucleotide) on the NCBI GenBank platform (https://blast.ncbi.nlm.nih.gov). Prior to species identification, the quality of DNA sequences was assessed by visually inspecting nucleotide chromatograms of the DNA fragments using Sequence Scanner software. This preliminary step ensured the accuracy of the sequences. High-quality sequencing results were characterized by clear, well-separated peaks, whereas overlapping or merged peaks indicated suboptimal sequencing quality. To enhance the reliability of the analysis, low-quality regions were removed from the sequences. Subsequent to quality control, the sequences were aligned using the muscle algorithm, which facilitated accurate sequence alignment and enhanced the overall data quality **(Valen *et al.*, 2024c)**. Phylogenetic analysis was conducted using the neighbor-joining method to elucidate the evolutionary relationships among the species. The robustness of the tree topology was evaluated through bootstrap analysis with 1,000 replicates. Evolutionary distances, expressed as the average number of base substitutions per site, were calculated using the Maximum Composite Likelihood method. This approach provided an accurate estimation of genetic divergence among the species. All evolutionary analyses, including assessments of nucleotide composition, 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 lengthened posterior rays. The caudal fin is rounded, while the anal fin has elongated posterior rays. The pectoral fin is rounded, and the pelvic fin is elongated with a filamentous tip (Fig. 1).

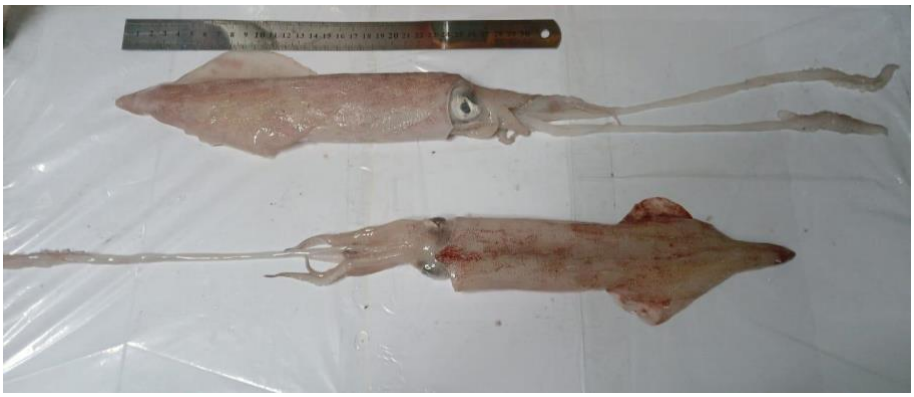

Fig. 1. Specimes of *Uroteuthis chinensis* from Selat Nasik of Belitung Island

2. DNA barcoding

The DNA barcoding of Bangka squid from Sleat Nasik, Belitung Island was accurately determined by sequencing the COX1 gene using the LCO1490 and HCO2198 primers **(Folmer *et al.*, 1994)** with a base-pair length of 446bp (Table 1).

Table 1. DNA barcoding of *Uroteuthis chinensis* based on COI gene

DNA barcoding of Bangka Squid from Selat Nasik Belitung Island, Indonesia
GGGGTTTCGGAAACTGATTAGTCCCATTAATATTAGGAGCTCCAGATATAGC CTTCCCCCGTATAAATAATATAAGATTCTGATTACTTCCACCTTCATTAACAC TACTATTAGCCTCATCCGCAGTTGAAAGAGGAGCCGGAACAGGGTGAACAGT ATACCCACCCTTATCCAGTAACCTTTCTCATGCAGGTCCTTCAGTTGACTTGG CTATTTTCTCTCTACACTTAGCTGGAATCTCATCCATCTTAGGTGCCATTAACT TTATCACAACTATTATAAATATACGCTGAGAAGGCTTATTAATAGAACGAAT ATCATTATTTGTATGATCTGTTTTCATTACAGCAATTCTATTGCTTCTTTCCCT CCCAGTATTAGCTGGAGCAATTACTATACTCTTAACTGACCGAAATTTTAACA CTACCTTTTTTGATCCGAGAGGG

3. Species identification based on COX1 gene

The 446 base-pair (bp) fragment of the mitochondrial cytochrome c oxidase subunit I (COX1) gene from *Uroteuthis chinensis* were collected from Selat nasik, Belitung Island, Indonesia. The COX1 gene sequence was analyzed and compared with existing data in the NCBI GenBank using the BLAST (Basic Local Alignment Search Tool) method. The results provided a molecular fingerprint for the species, confirming its identity and enhancing the understanding of cephalopod biodiversity in the region (Table 2).

Table 2. Sequence similarity of *Uroteuthis chinensis* Belitung Island

Specimen	Genbank similarity (Species outcome)	Gene	Accession Number	Query Cover (%)	Identity (%)
Uroteuthis chinensis of Selat Nasik, Belitung	*Uroteuthis chinensis*	COX1	EU349429.1	100	100
	Uroteuthis chinensis	COX1	LC552692.1	100	99
	Uroteuthis chinensis	COX1	MG192387.1	100	99
	Uroteuthis chinensis	COX1	LC552693.1	100	99

4. Classification of *Uroteuthis chinensis*

Uroteuthis chinensis belongs to the following hierarchical taxonomic classification:

- Kingdom : Animalia
- Phylum : Mollusca
- Class : Cephalapoda
- Subclass : Coleoidea
- Superorder : Decapodiformes
- Order : Myopsida

- Family : Loliginidae
- Genus : Uroteuthis
- Species : *Uroteuthis chinensis* Gray, 1849
- Synonim names : *Loligo chinensis* Gray, 1849

This classification places *Uroteuthis chinensis* within the order Teuthida, which encompasses all squid species. The genus *Uroteuthis* is a part of the family Loliginidae, commonly known as the market squids, characterized by their large eyes and long, slender bodies. The species *Uroteuthis chinensis* was first described by Gray in 1849 and is one of the most widely distributed squid species in the Indo-Pacific region, particularly around Southeast Asia.

5. Genetic distances

Utilizing the cytochrome c oxidase I (COX1) gene, the genetic divergence was calculated between these populations. The results indicate a low genetic distance of approximately 0.004, suggesting minimal genetic differentiation across populations. This study provides valuable insights into the genetic structure of *Uroteuthis chinensis* and contributes to understanding the population dynamics and evolutionary processes of this species.

Table 3. Genetic distance of *Uroteuthis chinensis* from Selat Nasik, Belitung Island, and other populations

		1	2	3	4	5
1	*Uroteuthis chinensis* Selat Nasik Belitung					
2	OR939423.1_*Uroteuthis chinensis*	0,004				
3	MG192453.1_*Uroteuthis edulis*	0,483	0,474			
4	KF413886.1_*Uroteuthis duvauceli*	0,597	0,587	0,491		
7	KF854081.1_*Uroteuthis sibogae*	0,544	0,534	0,496	0,380	

6. Molecular phylogeny

Phylogenetic analyses based on genetic data have shed light on the evolutionary relationships within the Loliginidae family. Studies suggest that *Uroteuthis chinensis* is closely related to other species within the genus *Uroteuthis*, such as *Uroteuthis duvauceli* and *Uroteuthis sibogae*. However, genetic divergences in the COX1 gene highlight the distinctiveness of *Uroteuthis chinensis*, particularly in its geographic distribution and reproductive biology. The evolutionary traits of *Uroteuthis chinensis*, such as its adaptability to various marine environments and its role in the food web, contribute to its phylogenetic uniqueness (Fig. 2).

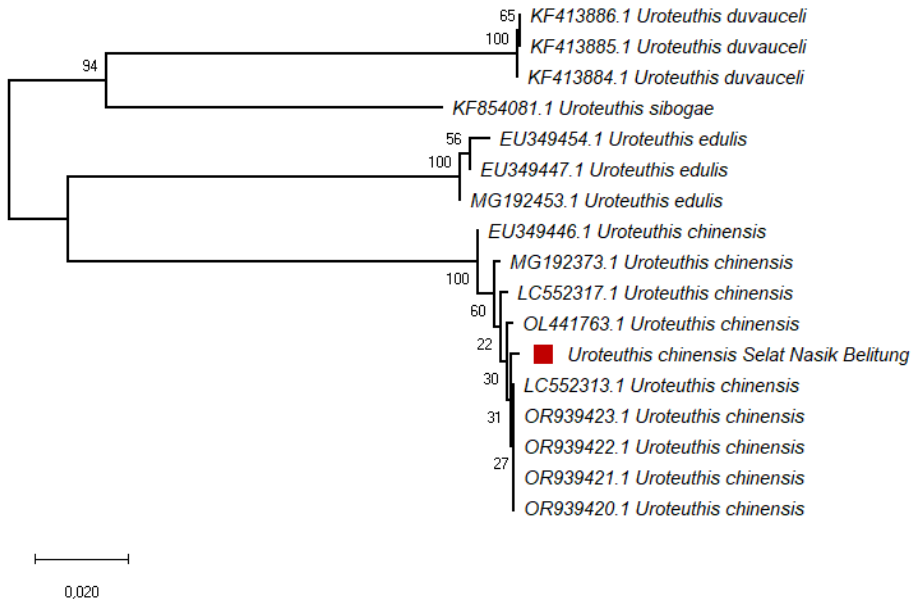

Fig. 2. Phylogenetic analysis of *Uroteuthis chinensis*: Insights into evolutionary relationships within the Loliginidae family

7. Nucleutide composition

This research focused on the nucleotide composition of *Uroteuthis chinensis* from Selat Nasik, Belitung Island, based on the mitochondrial Cytochrome C Oxidase Subunit I (COX1) gene. The findings reveal that the nucleotide composition of *Uroteuthis chinensis* includes thymine (T) 33%, cytosine (C) 23%, adenine (A) 28%, and guanine (G) 16%, from a total of 446 DNA bases (Table 4).

Table 4. Nucleotide composition of *Uroteuthis chinensis* from Selat Nasik, Belitung Island

Sequences	T(U)	C	A	G	Total
Uroteuthis chinensis Selat Nasik Belitung	33	23	28	16	446
OR939423.1 Uroteuthis chinensis	33	23	28	16	446
MG192453.1 *Uroteuthis edulis*	36	20	28	16	446
KF413884.1 Uroteuthis duvauceli	36	19	29	16	446
KF854081.1 *Uroteuthis sibogae*	34	21	29	16	446

DISCUSSION

The Chinese squid (*Uroteuthis chinensis*), belonging to the Loliginidae family, possesses distinctive features that are vital for identifying the species **(Paiva *et al.*, 2024)**. It has four pairs of arms, each with two rows of suckers, and its tentacles have four rows, with the inner ones being larger and more pronounced. The fins are diamond-shaped and cover a large portion of the mantle's posterior end, with their length comprising 55-70% of the total mantle length, which aids in efficient swimming. The mantle can grow up to

300mm in length and tapers toward the back. Its radula has seven transverse rows of teeth, common to cephalopods **(Norman & Lu, 1997)**. The mantle is rich in chromatophores, enabling quick color changes for camouflage and communication. Additionally, the squid has two gills with many filaments for effective gas exchange, and statocysts help maintain balance and spatial orientation during movement **(Clarke, 1978)**. These traits, along with molecular and ecological analyses, are essential for distinguishing *Uroteuthis chinensis* from other species in the same family.

Morphologically, *Uroteuthis chinensis* exhibits traits typical of the Loliginidae family, such as a slender, elongated body and the presence of a well-developed fin structure along the sides of the mantle. The squid also possesses eight arms and two tentacles, each equipped with suckers, which are key to its feeding strategy. Notably, its tentacular clubs are an important diagnostic feature, often used in distinguishing *Uroteuthis chinensis* from other squids in the Loliginidae family. The species also displays a chromatophore system, allowing it to change color for camouflage and communication. However, squid members are a diverse group of cephalopods that play vital roles in marine ecosystems. Accurate species identification is crucial for understanding their ecological roles, evolutionary history, and implementing conservation measures. Morphological methods, although historically significant, face substantial challenges such as intraspecific variability, cryptic diversity, and environmental influences on physical traits. Recent research advancements, particularly molecular analysis present a more reliable alternative **(Valen, *et al.*, 2024)**.

Molecular techniques have played an increasingly important role in clarifying the taxonomy of marine species **(Valen *et al.*, 2024)**. In the case of *Uroteuthis chinensis*, the mitochondrial cytochrome c oxidase subunit I (COX1) gene is frequently used for species identification due to its relatively fast rate of mutation and applicability across diverse taxa **(Paiva *et al.*, 2024)**. DNA barcoding using the COX1 gene has confirmed the species identity of *Uroteuthis chinensis*, distinguishing it from other closely related species within the *Uroteuthis* genus and the broader Loliginidae family. The comparison of genetic sequences with other members of the Loliginidae family has provided further evidence for the phylogenetic position of *Uroteuthis chinensis* **(Xu *et al.*, 2020)**.

The COX1 gene sequencing resulted in a 446bp fragment from the collected squid specimen. BLAST comparison of the sequences revealed a high degree of identity with *Uroteuthis chinensis*, confirming the species' identity. The sequence exhibited characteristic molecular markers typical of *Uroteuthis* species, including specific variations in the COX1 gene that distinguish it from other closely related species within the Loliginidae family. The use of the COX1 gene for DNA barcoding in this study was effective in providing a precise molecular identification of *Uroteuthis chinensis* from Sleat Nasik, Belitung Island. The successful amplification of a 446bp fragment highlights the applicability of COX1 as a genetic marker for cephalopod species identification in the region. The results also emphasize the utility of DNA barcoding for overcoming the

limitations of traditional morphological methods, which can be unreliable due to environmental and developmental variations. This research also contributes to expanding the molecular reference library for Indonesian cephalopods and offers a valuable tool for future biodiversity monitoring efforts. The accurate identification of species is essential for sustainable marine resource management, particularly in regions facing pressures from overfishing and habitat degradation **(Xu *et al.*, 2020; Paiva *et al.*, 2024)**.

The phylogenetic analysis showed a clear clustering of the Belitung Island population with other populations of *Uroteuthis chinensis*, suggesting a stable genetic lineage. The results further confirmed that the 446bp COX1 gene fragment is a reliable molecular marker for the species, providing a robust method for future studies on cephalopod biodiversity. The molecular phylogenetic analysis provided valuable insights into the evolutionary history of *Uroteuthis chinensis*. The clear genetic distinction between *Uroteuthis chinensis* and other species in the Loliginidae family suggests that this species underwent a separate evolutionary path, potentially driven by geographic isolation and ecological factors in the Indo-Pacific region **(Wang *et al.*, 2021)**.

However, the relatively low genetic variation observed across different populations of *Uroteuthis chinensis* suggests a high degree of genetic homogeneity in this species. This information is useful for understanding the population structure and potential migration patterns of the species in the region **(Sin *et al.*, 2009)**. The divergence observed between *Uroteuthis chinensis* and closely related species such as *Uroteuthis duvauceli* supports the hypothesis of speciation events in response to environmental pressures and habitat differentiation. Additionally, the phylogenetic positioning of *Uroteuthis chinensis* relative to other Loliginidae species highlights its role in the broader evolutionary context of squid diversification.

The genetic diversity within and among populations of marine species is crucial for understanding their evolutionary history, population structure, and adaptability to changing environmental conditions **(Morgan *et al.*, 2024)**. The genetic distance between the *Uroteuthis chinensis* population from Selat Nasik and other populations of the species was found to be approximately 0.004. This low genetic divergence suggests a high level of genetic similarity among the populations, indicating minimal differentiation across geographically separated groups. The COX1 gene sequences from Selat Nasik were closely related to those from other regions within the species' distribution range, supporting the hypothesis of gene flow between populations and the absence of significant genetic barriers. The observed low genetic distance of 0.004 between *Uroteuthis chinensis* from Selat Nasik, Belitung Island, and other populations suggests that gene flow is occurring between these populations, maintaining a relatively homogeneous genetic structure across the species' range. This genetic similarity is consistent with the hypothesis that *Uroteuthis chinensis* is a highly mobile species with the ability to disperse across vast marine environments. The lack of significant genetic differentiation may also reflect the species' adaptation to a wide range of ecological

niches in the Indo-Pacific region. However, further studies using additional genetic markers and larger sample sizes are needed to gain a deeper understanding of the population structure and potential local adaptation of *Uroteuthis chinensis* **(Xu *et al.*, 2020)**.

Moreover, the nucleotide composition of *Uroteuthis chinensis* from Selat Nasik, Belitung Island, based on the mitochondrial cytochrome c oxidase subunit I (COX1) gene were thymine (T) 33%, cytosine (C) 23%, adenine (A) 28%, and guanine (G) 16%, from a total of 446 DNA bases. Nucleotide composition, reflecting the proportional distribution of adenine, thymine, cytosine, and guanine in DNA, serves as a foundational parameter in genetic and evolutionary studies **(Insani *et al.*, 2022)**. In the *Uroteuthis* genus, the mitochondrial COX1 gene is a widely used genetic marker for species identification and phylogenetic analysis. The findings indicate that *Uroteuthis chinensis* exhibits a unique nucleotide composition compared to closely related species. The slightly elevated thymine percentage (33%) and reduced adenine percentage (28%) may suggest localized genetic adaptations to the Selat Nasik region. In contrast, cytosine (23%) and guanine (16%) levels remain consistent across species, reflecting conserved genetic elements within the genus.

CONCLUSION

This study demonstrates the efficacy of DNA barcoding using the COX1 gene to accurately identify the Bangka squid from Sleat Nasik, Belitung Island. The molecular identification of *Uroteuthis chinensis* through a 446bp sequence contributes to the growing molecular database of marine species in Indonesia. It highlights the importance of molecular tools in modern biodiversity conservation strategies. The 446bp COX1 fragment was sequenced and compared with the NCBI GenBank database using the BLAST tool, confirming the species identity and contributing to the molecular reference library for cephalopods. This study highlights the importance of molecular techniques in advancing species identification and monitoring marine biodiversity in Indonesia. The phylogenetic relationships of *Uroteuthis chinensis* within the Loliginidae family using the COX1 gene as a molecular marker. The results confirm the distinctiveness of *Uroteuthis chinensis* and its separation from other closely related species, reinforcing its classification as a separate species within the genus *Uroteuthis*. Further research using additional genetic markers and broader sampling across the genus *Uroteuthis* is recommended to gain deeper insights into the evolutionary processes shaping the diversification of this important cephalopod group. The genetic distance of 0.004 observed between the *Uroteuthis chinensis* population from Selat Nasik, Belitung Island, and other populations indicates a high level of genetic similarity, with minimal differentiation across geographically distinct populations. These findings suggest that gene flow is extensive across populations of *Uroteuthis chinensis* in the Indo-Pacific. Further research on the population structure and genetic diversity of this species will

provide valuable information for conservation and management efforts, especially in the face of environmental change and anthropogenic pressures.

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REFERENCES

Amkieltiela; Handayani, C.N.; Andradi-Brown, D.A.; Estradivari; Ford, A.K.; Beger, M.; Hakim, A.; Muenzel, D.K.; Carter, E.; Agung, F.; Veverka, L.; Iqbal, M.; Lazuardi, M.E.; Fauzi, M.N.; Tranter, S.N. and Ahmadia, G.N. (2022). The rapid expansion of Indonesia's marine protected area requires improvement in management effectiveness. Marine Policy., 146: 105257.

Anslan, S. and Tedersoo, L. (2015). Performance of cytochrome c oxidase subunit I (COI), ribosomal DNA Large Subunit (LSU) and Internal Transcribed Spacer 2 (ITS2) in DNA barcoding of Collembola. European Journal of Soil Biology., 69: 1–7.

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. N

Clarke, M.R. (1978). The cephalopod statolith: Structure, development, and function. Symposia of the Zoological Society of London., 38: 277-289.

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. Mol Mar Biol Biotechnol., 1994 3(5): 294-9.

Hasan, V. and Islam, I. (2020). A new inland record of the bull shark *Carcharhinus leucas* (müller & henle 1839) from peninsular malaysia. Ecologica Montenegrina., 38: 12-17.

Insani, L.; Jatayu, D.; Valen, F.S; Widodo, M. and Hasan, V. (2022). Comparing genetic *Mystacoleucus marginatus* and *Mystacoleucus padangensis*

(Cypriniformes: Cyprinidae) based on Cythochrome C Oxidase subunit I (COI) gene. J. Ichthyol., 9(4): 195–203.

Jin, Y.; Wang, C.; Li, N. and Fang, Z. (2022). Species identification of two Loliginidae cryptic species in China Seas with morphological and molecular methods. Regional Studies in Marine Science., 55: 102549.

Koblmüller, S.; Resl, P.; Klar, N.; Bauer, H.; Zangl, L. and Hahn, C. (2024). DNA Barcoding for Species Identification of Moss-Dwelling Invertebrates: Performance of Nanopore Sequencing and Coverage in Reference Database. Diversity., 16(4): 196.

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.

Lutfiatunnisa; Widodo, M.S.; Faqih, A.R.; Sari, L.D. Valen, F.S. (2020). Molecular approach for identification of Asian seabass (*Lates calcarifer* Bloch 1790) based on COI gene sequence from Java, Indonesia. AACL Bioflux., 13(5): 2828–2834.

Morgan, J.A.T.; 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.

Afiati, N.; Subagiyo.; Handayani, C.R.; Hartati, R.; and Kholilah, N. (2022). DNA Barcoding on Indian Ocean Squid, *Uroteuthis duvaucelii* (D'Orbigny, 1835) (Cephalopoda: Loliginidae) from the Java Sea, Indonesia. Jurnal Ilmiah Perikanan Dan Kelautan., 14(2): 231–245.

Norman, M.D. and Lu, C.C. (1997). Redescription of Uroteuthis chinensis (Gray, 1849) with notes on the loliginid squid fauna of Hong Kong. Asian Marine Biology., 14: 97-110.

Nurjirana; Burhanuddin, A.I.; Keith, P.; Haris, A.; Moore, A.M.; Afrisal, M.; Gani, A.; Hasan, V.; Wuniarto, E.; Bakri, A.A. and Adriany, D.T. (2022). Additional records of *Sicyopus discordipinnis* (Watson, 1995) (Oxudercidae: Sicydiinae) in Central Sulawesi, Indonesia. Cybium., 46(1): 41–43.

Paiva, B.L.; Rodrigues, A.E.S.; Almeida, I.O.deF.; Silva, K.deF.; Haimovici, M.; Markaida, U.; Charvet, P.; Faria, V.V.; Batista, B.B.; Tomás, A.R.G.; Rodrigues-Filho, L.F.daS.; Ready, J.S. and Sales, J.B.deL. (2024). Molecular

identification of whole squids and calamari at fairs and markets in regions of Latin America. Scientific Reports., 14(1): 30007.

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; 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.

Sales, J.B; de L.; Shaw, P.W.; Haimovici, M.; Markaida, U.; Cunha, D.B.; Ready, J.; Figueiredo-Ready, W.M.B.; Schneider, H. and Sampaio, I. (2013). New molecular phylogeny of the squids of the family Loliginidae with emphasis on the genus Doryteuthis Naef, 1912: Mitochondrial and nuclear sequences indicate the presence of cryptic species in the southern Atlantic Ocean. Molecular Phylogenetics and Evolution., 68(2): 293–299.

Sin, Y.W.; Yau, C. and Chu, K.H. (2009). Morphological and genetic differentiation of two loliginid squids, *Uroteuthis (Photololigo) chinensis* and *Uroteuthis (Photololigo) edulis* (Cephalopoda: Loliginidae), in Asia. Journal of Experimental Marine Biology and Ecology, 369(1): 22–30.

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.

Valen, F.S.; Alfian, R.A.; Prananda, M.; Hasmadi, M.; Gani, A. and Hasan, V. (2024a). DNA Barcoding of the Endemic Freshwater Catfish *Encheloclarias tapeinopterus* (Bleeker, 1853) (Siluriformes:Clariidae) from Bangka Island, Indonesia. IOP Conference Series: Earth and Environmental Science., 1392(1): 012034.

Valen, F.S.; Syaputra, D.; Mohd Hasmadi, I.; Swarlanda.; Kamarudin, A.S.; Hasan, V. and Itaya, I. (2024b). Unveiling the Enigmatic Dwarf Horseface Loach *Acanthopsoides molobrion*: A Groundbreaking Discovery in Indonesia. Biosaintifika: Journal of Biology & Biology Education.,16(2): 265-272.

Valen, F.S,; Mamat, N.B,; South, J,; Ottoni, F.P,; Vieira, L.O,; Kamarudin, A.S,; Afandi, A.Y. and Hasan V. (2024c). Unveiling the occurrence of vulnerable Sisorid catfish (Teleostei: Sisoridae) in Bangka based on morphological and molecular evidence. Biodiversitas., 25: 4543-4550.

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.; 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).

Wang, D.; Yao, L.; Yu, J. and Chen, P. (2021). The Role of Environmental Factors on the Fishery Catch of the Squid *Uroteuthis chinensis* in the Pearl River Estuary, China. Journal of Marine Science and Engineering, 9(2): 131.

Xu, L.; Wang, X. and Du, F. (2020). The complete mitochondrial genome of loliginid squid (*Uroteuthis chinensis*) from Minnan–Taiwan bank fishing ground. Mitochondrial DNA Part B., 5(1): 428–429.

Zamroni, A.; Boer, M.; Butet, N.A.; Zairion, Z. Wudianto. (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.; Zairion, Z. and Wudianto. (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.