Molecular Identification of Fire Worms in Indonesian Intertidal Waters: The First COI Gene-Based DNA Barcode of *Eurythoe complanata*

Vivian Rosyada, Nurliah Buhari*, Edwin Jefri

Program Studi Ilmu Kelautan, Fakultas Pertanian, Universitas Mataram
Gedung H, Jl. Pendidikan No.37, Mataram, Nusa Tenggara Barat, 83114
Email: nurliah.buhari@unram.ac.id

Abstract

Polychaetes, including fireworms, play crucial ecological roles such as nutrient recycling and forming a vital part of the marine food web. However, their accurate identification is challenging due to morphological complexity and the presence of cryptic species. DNA barcoding, particularly targeting the cytochrome oxidase subunit I (COI) gene, has emerged as a powerful tool for species delineation due to its high accuracy and reproducibility. This study aims to molecularly identify fireworm specimens collected from Indonesian intertidal zones using COI gene barcoding. Fireworm samples were collected from Lombok waters, followed by DNA extraction, PCR amplification, electrophoresis, and DNA sequencing. The DNA data were analyzed using MEGA-X software to compare the sequences with those in the database and identify the species. A phylogenetic tree was constructed to illustrate the relationships between the analyzed species. The results show that the fireworm sample from Lombok is classified as Eurythoe complanata, a member of the Amphinomidae family. COI genetic analysis revealed a 658 bp DNA fragment with 77.19% similarity to the same species from India. The phylogenetic tree indicated a close relationship with the Indian species, supported by a bootstrap value of 85. Genetic distances ranged from 0.02 to 0.48, and the dominance of A+T nucleotides suggests the presence of genetic variation.

Keyword: *Amphinomidae; Fireworms; DNA barcoding; Eurythoe complanata; Lombok*

INTRODUCTION

The intertidal zone of Indonesia's coastal waters constitutes a highly biodiverse ecosystem that plays a crucial role in maintaining marine ecological stability (Miloslavich *et al*., 2016; Jefri *et al*., 2025). Among the dominant organisms in this zone are polychaetes, which serve essential ecological functions such as nutrient recycling (Maximov *et al*., 2015)and forming a key part of the food web (Jumar *et al*., 2015). Despite their ecological significance, the accurate identification of polychaetes remains challenging due to their morphological complexity. Traditional taxonomic approaches based solely on morphology are often insufficient, particularly because of the presence of cryptic species—genetically distinct taxa that exhibit very similar external features (Purnamasari *et al*., 2016).

In response to these challenges, molecular techniques, especially DNA barcoding targeting the cytochrome oxidase subunit I (COI) gene, have become an essential tool for species delineation in modern taxonomy owing to their high accuracy and reproducibility (Hebert *et al*., 2003; Simbolon *et al*., 2021). Numerous studies have successfully employed this approach to identify polychaete species, particularly within the family Amphinomidae. For example, Jördens *et al*. (2004) characterized *Eurythoe complanata* from Germany, Leray *et al*. (2013) examined *Pherecardia striata* in the United States, Wang *et al*. (2019) investigated *Chloeia bimaculata* in China, and Calderon-Gutierrez *et al*. (2024) focused on *Hermodice carunculata* in North America. Collectively, these studies underscore the effectiveness of DNA barcoding in providing more precise species identification.

However, molecular data regarding Amphinomidae from Indonesian intertidal waters remain limited, highlighting a significant knowledge gap that hinders effective conservation and sustainable resource management in the region. This study aims to molecularly identify fireworm specimens collected from Indonesian intertidal zones using COI gene barcoding. By contributing novel genetic data, this research seeks to enrich the global genetic repository while enhancing understanding of the species' distribution and genetic diversity within the Indo-Pacific. Ultimately, these insights are

*) Corresponding author
www.ejournal2.undip.ac.id/index.php/jkt
Diterima/Received : 10-04-2025, Disetujui/Accepted : 30-05-2025
DOI: https://doi.org/10.14710/jkt.v28i2.26551

anticipated to facilitate more informed conservation strategies and promote sustainable management of marine biodiversity in the region.

MATERIALS AND METHODS

The specimen collection was conducted in September 2024 in the intertidal waters of Labuhan Pandan, East Lombok, as shown in (Figure 1), and the subsequent stages of the research were carried out from October to November 2024. Specimens were carefully collected by hand using protective gloves, and each specimen was photographed prior to further processing (Figure 2a). The preparation stage involved dissecting the head region of the fireworms, which were then preserved in tubes containing 96% ethanol to maintain sample integrity prior to analysis. This approach aligns with the study by Hadadi *et al*. (2023), which also utilized ethanol in concentrations ranging from 70% to 96% for sample preservation before use. Preservation with 96% ethanol is effective in maintaining sample quality over an extended period before further examination.

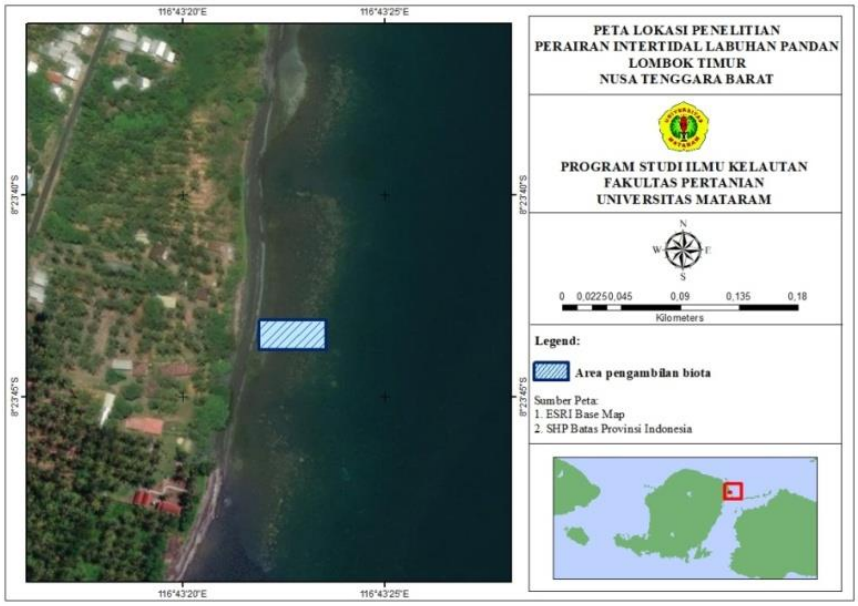

Figure 1. Study Area. The research location is indicated by the area shaded in blue

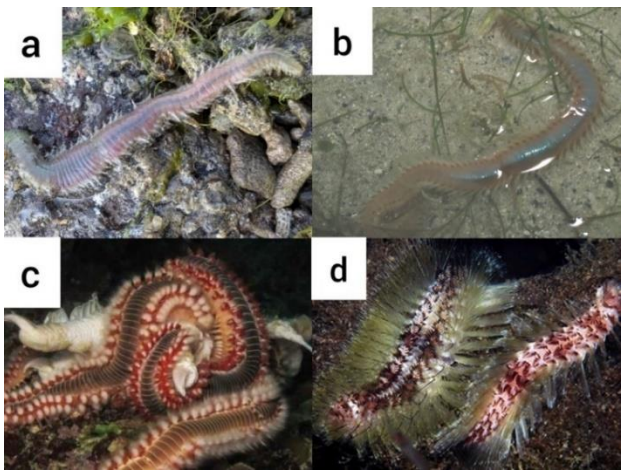

Figure 2. Fireworms from family Amphinomidae: (a) Specimen; (b) *E. complanata* (Nakamura *et al*., 2009); (c) *Hermodice carunculata* (Toso *et al*., 2022); (d) *Chloeia flava* (Salazar-Vallejo, 2023)

The analysis was conducted through several stages, including DNA extraction, Polymerase Chain Reaction (PCR), electrophoresis, and sequencing. According to Maduppa (2020), DNA extraction is performed to isolate DNA from the tissue of the research specimen. DNA extraction in this study was carried out using the Geneaid DNA Isolation Kit. This extraction process involved the homogenization of fireworm tissue samples after the working area was properly sterilized. The study employed primers LCO1490 and HCO2198, which are commonly used for the amplification of the Cytochrome Oxidase Subunit I (COI) gene, as they are designed based on highly conserved regions of the COI gene across a wide range of animal species (Sharma & Kobayashi, 2014).

DNA was then amplified using a Sensoquest PCR machine. A total of 12.5 µL of prepared DNA sample was mixed with 5 µL each of the primers HCO2198 and LCO1490. Subsequently, 25 µL of 2× MyTaq HS Red Mix was added per tube, followed by the addition of 2.5 µL of ddH_2O. The PCR tubes were then placed into the Sensoquest thermal cycler for amplification, which ran for approximately 1 hour and 49 minutes. The thermocycling program was set as follows: pre-denaturation at 94°C for 2 minutes, followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 49°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 3 minutes (Barroso *et al*., 2010). To confirm the presence of the target DNA band, electrophoresis was conducted using a 1.5% agarose gel, with a voltage of 90 V for 35 minutes. The electrophoresis results were visualized under a UV illuminator. Successfully amplified PCR products were sent to PT. Genetika Science Indonesia, located in Tangerang, Banten, for sequencing analysis.

Nucleotide sequence editing and alignment were performed using MEGA-X, which was also used to analyze DNA sequences and identify the species sampled. A phylogenetic tree was constructed using the Neighbor-Joining method with the Maximum Composite Likelihood model (Tamura *et al*., 2004). A consensus tree was generated with 1000 bootstrap replicates to assess the taxonomy and genetic relationships of the samples (Felsenstein, 1985). The tree was reconstructed using MEGA-X version 11 and ClustalW to analyze nucleotide diversity and determine the genetic distance among species (Tamura *et al*., 2021). Nucleotide sequences were identified by aligning sequencing results with available data from the National Center for Biotechnology Information (NCBI) using the BLAST (Basic Local Alignment Search Tool) method.

RESULTS AND DISCUSSION

The electrophoresis results showed that the amplified DNA band was located at approximately ±700 bp (base pairs). This is consistent with the findings of Sharma & Kobayashi (2014), who reported that primers LCO1490 and HCO2198 amplified DNA fragments ranging from 681 to 710 bp in their study. The electrophoresis results are presented in (Figure 3). In Figure 3, the presence of DNA bands indicates successful amplification of purified DNA, with fragment lengths determined using a standard-sized DNA marker (Novitasari *et al*., 2014). The clearly visible bands reflect good quality and are suitable for the sequencing stage. According to Buchori *et al*. (2023), sharp and distinct bands indicate high DNA concentration and successful isolation, while faint or absent bands may suggest contamination or DNA degradation. The editing and sequencing results using MEGA X.11 software produced a fragment of 658 bp in length. This is consistent with the findings of Folmer *et al*. (1994), which indicated that COI gene sequencing using LCO1490 and HCO2198 primers in invertebrates typically ranges between 651–710 bp. The results of the DNA sequencing analysis using NCBI are presented in (Table 1).

The sequencing results showed a higher similarity with the genetic database of *Eurythoe complanata*. This is supported by the highest values of query cover and total score, high identity percentage, and a very low e-value. Based on the analysis (Table 1), the BLAST results yielded an e-value of 8.00e-20, a total score of 89.7, an identity percentage of 77.19%, and a query cover of 16%.

The obtained e-value of 8.00e-20 indicates a strong similarity with sequences in the database and is unlikely to be due to chance (Sabbathini *et al*., 2017). The e-value represents the statistical significance and reliability of the database match. Narita *et al*. (2012) further emphasized that the

closer the value is to zero, the more accurate the result. The BLAST results also showed a total score of 89.7, indicating a good alignment between the analyzed sequence and those available in the database (Gaffar & Sumarlin, 2020).

The identity percentage value of 77.19% indicates that nearly 80% of the base pairs in the analyzed sequence are identical to those in the database. This suggests that the species may be related but not identical (Tobe *et al*., 2010). According to Hebert *et al*. (2003), if the genetic identity difference between species exceeds 3%, then a similarity below 80% may indicate that the sequence originates from a different species, despite sharing a portion of identical base pairs. The identity value also reflects the match with the query cover, which denotes the relationship between the sample's nucleotide length and the sequences in the GenBank database (Gaffar & Sumarlin, 2020). Therefore, this result suggests a degree of nucleotide base pair similarity between the sample and the reference sequences analyzed.

The BLAST results revealed that the query cover value of the fireworm sample was relatively low, at only 16%. This low value is attributed to suboptimal sequencing quality and the detection of bacterial sequences. The identification of these bacteria suggests a potential symbiotic relationship between the fireworm and certain bacterial species. The detection of bacteria was likely due to the use of universal primers LCO1490 and HCO2198. This finding is consistent with the study by Gaffar & Sumarlin (2020), which also reported low query cover values as a result of poor sequencing quality. Therefore, further analysis such as the construction of a phylogenetic tree is necessary to gain a deeper understanding of the taxonomic and evolutionary relationships of the sample species.

Table 1. BLAST Results of Fireworm Samples Obtained

Taxa	*Accession Number*	*E-Value*	*Ident.*	*Query Cover*	*Total Score*	*Base pare*
Eurythoe complanata	MG251652	8.00e-20	77.19%	16%	89.7	658
Bathychloeia cf. sibogae	ON903196	2.00e-10	79.38%	15%	71.8	682
Chloeia flava	JN852944	8.00e-06	78.75%	12%	56.4	590
Hermodice carantulata	MG251653	1.00e-04	77.38%	13%	69.8	658
Chloeia parve	MK696602	0.023	77.94%	10%	44.9	680
Linopherus sp.	OQ323218	0.023	74.49%	15%	44.9	658

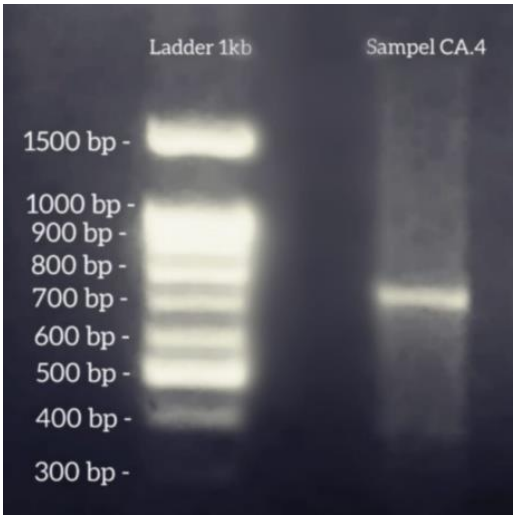

Figure 3. Electrophoresis result of the amplified target DNA band

Following the BLAST results, which specifically identified the species as *E. complanata*, five sequence data entries were selected for phylogenetic tree reconstruction. The sequence data used for the analysis are presented in Table 2. The use of only five *E. complanata* sequences from GenBank was due to the limited availability of COI-based identifications for this species in the NCBI BLAST database. The phylogenetic tree was constructed using these five *E. complanata* sequences, one fireworm sequence from the intertidal waters of Lombok (sample CA4 Seq5246781), and *Archinome rosacea* as the outgroup. Including an outgroup enhances the reliability of the phylogenetic analysis by minimizing grouping bias and providing a more accurate representation of evolutionary relationships (Hidayat & Pancoro, 2019). The resulting phylogenetic tree is shown in Figure 4.

The phylogenetic tree generated shows that the sample CA4 Seq5246781 clusters within the same clade as Eurythoe complanata from India. As illustrated in Figure 4, the bootstrap value obtained is high, at 85. This elevated value indicates that the interspecies relationships presented are robust and reliable, reflecting true evolutionary patterns (Saleky *et al*., 2020). Bootstrap values above

Table 2. Sequence Data Used in Phylogenetic Analysis from GenBank

Taxa	*Location*	*Accession Number*	*E-Value*	*Ident.*	*Query Cover*	*Total Score*	*Base pare*	*References*
Eurythoe complanata	India	MG251652	8.00E-20	77.19%	16%	89.7	658	Bharathidasan *et al*., 2017
Eurythoe complanata	USA	KP255001	9.00E-13	74.04%	15%	67.1	658	Leray dan Knowlton, 2015
Eurythoe complanata	Brazil	KY972418	3.00E-12	73.15%	15%	65.3	1548	Bernardino *et al*., 2017
Eurythoe complanata	China	OQ749906	8.00E-14	74.07%	15%	69.8	1548	He *et al*., 2023
Eurythoe complanata	USA	JN086548	2.00E-14	75.00%	15%	71.6	680	Borda *et al*., 2012

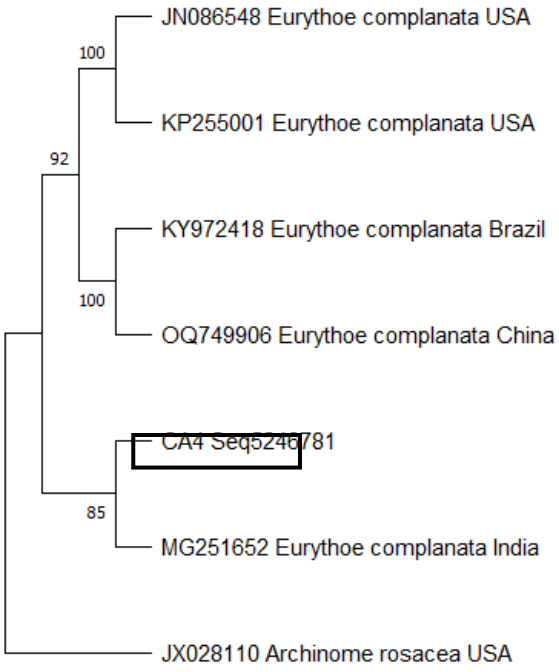

Figure 4. Phylogenetic tree of *E. complanata* from the waters of Lombok

70 generally denote strong branching, whereas values below this threshold are considered less reliable (Anafarida & Badruzsaufari, 2020). Lestari *et al*. (2018) further emphasized that higher bootstrap values correspond to stronger phylogenetic trees. These findings suggest considerable genetic sequence similarity in the region.

Madduppa *et al*. (2017) explained that genetic sequence similarity can be influenced by factors such as habitat connectivity, genetic exchange, and environmental similarity. Genetic exchange between the biota of Indonesia and India may be driven by geological and oceanographic factors. Geologically, Indonesia and India have experienced changes in landforms and sea levels that influenced marine organism dispersal routes. Hanebuth *et al*. (2000) noted that during glacial periods sea levels dropped, causing some Indonesian islands (Sunda Shelf) to connect, facilitating migration and genetic exchange among biota. When sea levels rose again and landmasses were separated, organisms that had dispersed retained genetic similarities.

Marine species like *E. complanata* can widely disperse across the Indo-Pacific region since their larvae can be carried by ocean currents (Adams *et al*., 2014). One principal current pattern in the area is the water movement from India to Indonesia, although the pattern and characteristics vary. According to La Ode *et al*. (2020), the Indonesian Throughflow (Arlindo) transports water from the Pacific Ocean to the Indian Ocean through Indonesian straits such as Makassar Strait, Lombok Strait, Ombai Strait, and Timor Sea. This current facilitates genetic exchange between marine biota from India and Indonesia.

The evolutionary pattern analysis of the phylogenetic tree is further supported by genetic distance values (pairwise distance). Halisah *et al*. (2024) clarified that genetic distance is calculated based on the differences in nucleotide base sequences between two DNA sequences and is used to measure the degree of relatedness among species or individuals within a species (Table 3).

Based on the results obtained, the genetic distance between CA4 Seq5246781 and *Eurythoe complanata* from India (MG251652) was 0.41, indicating a degree of genetic divergence. The closest genetic distance was observed between *E. complanata* from China (OQ749906) and Brazil (KY972418), with a value of 0.02. According to Halisah *et al*. (2024), genetic distance is calculated based on nucleotide sequence differences and is used to assess the degree of relatedness between species or individuals within the same species. A value of 0 indicates a very close relationship, while a value of 1 indicates the greatest genetic divergence (Elfianis *et al*., 2021). In this study, the genetic distance ranged from 0.02 to 0.48, demonstrating genetic variation within *E. complanata*. These findings are consistent with Rahayu & Handayani (2010), who reported genetic distances ranging from 0.267 to 0.957, indicating broad genetic variation within the studied species.

Genetic variation essentially occurs due to differences in the sequence of nucleotide bases such as adenine, thymine, guanine, and cytosine in DNA (Putra *et al*., 2021). The nucleotide composition of the analyzed sample showed significant differences between species. The results of the nucleotide composition in this study are presented in (Table 4).

Table 3. Genetic Distance of Fireworms

Taxa	1	2	3	4	5	6	7
CA4 Seq5246781							
MG251652 Eurythoe complanata India	0.41						
JN086548 Eurythoe complanata USA	0.46	0.18					
KY972418 Eurythoe complanata Brazil	0.48	0.19	0.19				
OQ749906 Eurythoe complanata China	0.47	0.19	0.19	0.02			
KP255001 Eurythoe complanata USA	0.46	0.18	0.02	0.19	0.19		
JX028110 Archinome rosacea USA	0.46	0.23	0.21	0.23	0.22	0.22	

Table 4. Nucleotide Composition of the Fireworm

Taxa	T/U (%)	C (%)	A (%)	G (%)	A+T (%)	C+G (%)
CA4 Seq5246781	30.5	24.5	20.1	24.9	50.6	49.4
E. complanata India	28.6	25.7	28.6	17.2	57.2	42.9
E. complanata USA	28.5	28.1	26.4	17.0	54.9	45.1
E. complanata Brazil	31.1	23.8	26.1	19.0	57.2	42.8
E. complanata China	30.9	24.0	26.1	19.0	57.0	43.0
E. complanata USA	29.1	27.4	26.1	17.4	55.2	44.8
Archinome rosacea USA	30.0	27.7	25.8	16.5	55.8	44.2

Table 4 shows that the percentage of A+T content is higher than that of C+G. According to Hapsari (2015), species with lower C+G content are considered more primitive, as they retain more similarities with their ancestors and have undergone fewer evolutionary changes. Niu *et al*. (2017) further explain that genomes with higher C+G content tend to exhibit a greater mutation rate. The mutation rate of DNA directly affects the genetic variation of an organism. Mutations can occur due to errors during DNA replication or external environmental factors such as exposure to foreign substances (Dailami *et al*., 2018). Nucleotide base substitutions may result in base variations in the form of transitions (changes between A and G or C and T) or transversions (changes between purine and pyrimidine bases) (Fauziyyah & Suhadi, 2021). In addition, biogeography influences genetic variation, where environmental factors such as climate and topography can limit the dispersal of organisms. Hasibuan *et al.* (2017) also noted that these mutations are a major cause of nucleotide base variation, particularly in the COI gene.

CONCLUTION

Based on the results obtained, the fireworm sample from Lombok is classified as *E. complanata*, a member of the Amphinomidae family. COI genetic analysis revealed a 658 bp DNA fragment with 77.19% similarity to the same species from India. The phylogenetic tree indicated a close relationship with the Indian species, supported by a bootstrap value of 85. Genetic distances ranged from 0.02 to 0.48, and the dominance of A+T nucleotides indicates the presence of genetic variation.

ACKNOWLEDGEMENTS

The author would like to thank all colleagues who have assisted in various aspects and throughout the research process. Sincere appreciation is also extended to all parties who have provided support during the course of this study, enabling its successful completion.

REFERENCES

Adams, T.P., Aleynik, D., & Burrows, M.T. (2014). Larval dispersal of intertidal organisms and the influence of coastline geography. *Ecography, 37*(7), 698-710. doi: 10.48550/arXiv.1304.6319.

Anafarida, O., & Badruzsaufari, B. (2020). Phylogenetic analysis of mango (Mangifera spp.) based on the 5.8S rRNA gene. *Ziraa'ah: Agricultural Scientific Journal*, *45*(2), 120–126. doi: 10.31602/zmip.v45i2.3001

Barroso, R., Klautau, M., Solé-Cava, A.M., & Paiva, P.C. (2010). *Eurythoe Complanata* (Polychaeta: Amphinomidae), The 'Cosmopolitan'fireworm, Consists of At Least Three Cryptic Species. *Marine Biology*, *157*(1), 69-80. doi: 10.1007/s00227-009-1296-9.

Beesley, P.L., Ross, G.J.B., & Glasby, C.J. (2000). *Polychaetes & Allies The Southern Synthesis*. Environment Australia, Canberra.

Buchori, A., Firmansah, H., Anika, M., Ratnawati, S., Ulfa, U.T., & Zendrato, Y. (2023). Comparison of DNA extraction methods using rice leaves. *Agriculture and Biological Technology*, *1*(1), 40-50. doi: 10.61761/agiotech.1.1.40-50.

Calderón-Gutiérrez, F., Labonté, J.M., Gonzalez, B.C., Iliffe, T.M., Mejía-Ortíz, L.M., & Borda, E. (2024). Cryptic diversity patterns of subterranean estuaries. *Proceedings B, 291*, 20241483.

Dailami, M., Santi, D., Abubakar, H., & Toha, A.H. (2018). Genetic analysis of the cytochrome oxidase subunit 1 (COI) gene fragment from *Cirrhilabrus cf. ryukyuensis* Ishikawa 1904 (Labridae) from Cenderawasih Bay and Raja Ampat. *Jurnal Iktiologi Indonesia*, *18*(3), 209–222. doi: 10.32491/jii.v18i3.347

Elfianis, R., Warino, J., Rosmaina, R., Suherman, S., & Zulfahmi, Z. (2021). Genetic relationship analysis of rice plants (*Oryza sativa* L.) in Kampar Regency using Random Amplified Polymorphic DNA (RAPD) markers. *Jurnal Agroteknologi*, 11(2), 75–84. doi: 10.24014/ja.v11i2.10013

Fauziyyah, I., & Suhadi, S. (2021). Sequence variation and phylogenetic analysis of *Leptocorisa oratorius* (Fabricius) in East Java based on the COX2 gene. *Jurnal Ilmu Hayat*, *5*(2), 71–79.

Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using The Bootstrap. *Evolution*, *39*(4), 783-791. doi: 10.1111/j.1558-5646.1985.tb00420.x.

Flomer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA Primers For Amplification of Mitochondrial Cytochrome C Oxidase Subunit I From Diverse Metazoan Invertebrates. *Molecular Marine Biology Biotechnology*, *3*(5), 294-299.

Gaffar, S., & Sumarlin, S. (2020). COI mtDNA sequence analysis of blue-spotted stingray (Neotrygon kuhlii) landed at the Fish Landing Site in Tarakan City. *Jurnal Harpodon Borneo*, *13*(2), 80–89. doi: 10.35334/harpodon.v13i2.1835.

La Ode, M., Atmadipoera, A.S., Naulita, Y., & Nugroho, D. (2020). Struktur Vertikal Dan Variabilitas Arlindo Yang Masuk Ke Tepi Barat Laut Banda. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *12*(2), 455-469.

Hadadi, N., Madduppa, H., Azaraly, I.S., & Anggraini, N.P. (2023). DNA barcoding, morphological identification, and population density of *Tridacna* genus in the waters of North Maluku. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *15*(3), 349–368. doi: 10.29244/jitkt.v15i3.47110

Halisah, K.A.Z., Fitri, A., Irianto, S.N., Aulya, M., Sakti, B., Sanrina, S., Harmadi, P., Maha, N.S.S., & Cahyani, N.K.D. (2024). Identification of marine fish species using DNA barcoding technique. *Bioma: Berkala Ilmiah Biologi*, 26(1), 43–51. doi: 10.14710/bioma.2024.62478.

Hanebuth, T.J.J., Stattegger, K., & Grootes, P.M. (2000). Rapid Flooding of the Sunda Shelf: A Late-Glacial Sea-Level Record. *Science, 288*(5468), 1033-1035.

Hapsari, L. (2015). Genetic diversity and relationship of banana (*Musa* L.) in East Java based on internal transcribed spacer (ITS) sequence. [Master's thesis, Universitas Brawijaya]. Malang, Indonesia.

Hasibuan, F.E.B., Mantiri, F.R., & Rumende, R.R. (2017). Intraspecies sequence variation and phylogenetic study of Sulawesi black macaque (*Macaca nigra*) using COI gene. *Jurnal Ilmiah Sains*, 17(1), 59–67. doi: 10.35799/jis.17.1.2017.15558

Hebert, P.D.N., Ratnasingham, S., & de Waard, J.R. (2003). Barcoding Animal Life: Cytochrome C Oxidase Subunit 1 Divergences Among Closely Related Species. *Proceedings of the Royal Society B, 270*(1), 96–99.

Hidayat, T., & Pancoro, A. (2019). A review of molecular phylogenetic studies and their role in providing basic information to improve the quality of orchid genetic resources. *Jurnal AgroBiogen*, 4(1), 35–40.

Jefri, E., Nurliah, N., Damayanti, A.A., Astriana, B.H., & Lestariningsih, W.A. (2025). Biodiversity and Distribution of Gastropods in Relation to Shrimp Pond Effluents in the Intertidal Zone of Sambelia, East Lombok. *Jurnal Biologi Tropis, 25*(1), 341-348.

Jördens, J., Struck, T., & Purschke, G. (2004). Phylogenetic Inference Regarding Parergodrilidae and Hrabeiella Periglandulata ('Polychaeta', Annelida) Based on 18S Rdna, 28S Rdna and COI Sequences. *Journal of Zoological Systematics and Evolutionary Research, 42*(4), 270-280. doi: 10.1111/j.1439-0469.2004.00265.x.

Jumars, P.A., Dorgan, K.M., & Lindsay, S.M. (2015). Diet of worms emended: an update of polychaete feeding guilds. *Annual review of marine science, 7*(1), 497-520.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., Machida, R.J. (2013). A New Versatile Primer Set Targeting A Short Fragment of The Mitochondrial COI Region

For Metabarcoding Metazoan Diversity: Application For Characterizing Coral Reef Fish Gut Contents. *Frontiers in zoology, 10*, 1-14. doi: 10.1186/1742-9994-10-34.

Lestari, D. A., Azrianingsih, R., & Hendrian, H. (2018). Phylogenetics of Annonaceae species from East Java in the Purwodadi Botanic Garden collection based on coding and non-coding DNA sequences. *Journal of Tropical Biodiversity and Biotechnology*, *3*(1), 1–7. doi: 10.22146/jtbb.28308

Madduppa, H., Subhan, B., Anggraini, N.P., Fadillah, R., & Tarman, K. (2017). DNA barcoding reveals vulnerable and not evaluated species of sea cucumbers (Holothuroidea and Stichopodidae) from the Seribu Islands reefs, Indonesia. *Biodiversitas Journal of Biological Diversity*, *18*(3), 893–898. doi: 10.13057/biodiv/d180305

Maximov, A., Bonsdorff, E., Eremina, T., Kauppi, L., Norkko, A., & Norkko, J. (2015). Context-dependent consequences of *Marenzelleria* spp.(Spionidae: Polychaeta) invasion for nutrient cycling in the Northern Baltic Sea. *Oceanologia, 57*(4), 342-348.

Miloslavich, P., Cruz-Motta, J.J., Hernández, A., Herrera, C., Klein, E., Barros, F., Bigatti, G., Cárdenas, M., Carranza, A., Flores, A. & Gil-Kodaka, P. (2016). Benthic assemblages in South American intertidal rocky shores: biodiversity, services, and threats. *Marine benthos: biology, ecosystems, functions and environmental impact*, 83-137.

Narita, V., Arum, A.L., Isnaeni, S., & Fawzya, N.Y. (2012). Web-based bioinformatics analysis for chitosanase enzyme exploration based on sequence similarity. *Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi*, *1*(4), 197–203. doi: 10.36722/sst.v1i4.84

Nakamura, K., Tachikawa, Y., & Uemura, D. (2009). Complanine, an inflammatory substance of marine fireworm: a synthetic study. *Beilstein Journal of Organic Chemistry*, *5*(1), 12. doi: 10.3762/bjoc.5.12.

Niu, Z., Xue, Q., Wang, H., Xie, X., Zhu, S., Liu, W., & Ding, X. (2017). Mutational Biases and GC-Biased Gene Conversion Affect GC Content in the Plastomes of Dendrobium Genus. *International Journal of Molecular Sciences, 18*(11), 2307.

Novitasari, D.A., Elvyra, R., & Roslim, D.I. (2014). Total DNA isolation and electrophoresis techniques in *Kryptopterus apogon* (Bleeker, 1851) from the Kampar Kiri and Tapung Hilir Rivers, Kampar Regency, Riau Province. *Online Journal of the Faculty of Mathematics and Natural Sciences*, *1*(2), 258–261.

Purnamasari, L., Farajallah, A., & Wowor, D. (2016). Application of DNA barcoding for species identification of freshwater shrimp from Jambi Province. *Jurnal Bioconcetta*, *2*(1), 50–59. doi: 10.22202/bc.2016.v2i1.1299

Putra, A.W., Sahara, F., Ritonga, I.R., Ramadhani, S., Wardhani, T.E., & Achyar, A. (2021). Analysis of genetic variation of the outer membrane protein (omp) gene sequence in *Salmonella enterica* subsp. *enterica* using in silico RFLP. In *Proceedings of the National Biology Seminar*, 289–295. doi: 10.24036/prosemnasbio/vol1/38

Rahayu, S. E., & Handayani, S. (2010). Genetic diversity of *Pandanus* from West Java based on inter-simple sequence repeat (ISSR) markers. *Makara Journal of Science*, *14*(2), 158–162.

Sabbathini, G.C., Pujiyanto, S., Wijanarka, W., & Lisdiyanti, P. (2017). Isolation and identification of *Sphingomonas* genus bacteria from rice leaves (*Oryza sativa*) in the Cibinong paddy field area. *Jurnal Akademika Biologi*, 6(1), 59–64.

Salazar-Vallejo, S.I. (2023). Revision of Chloeia Savigny in Lamarck, 1818 (Annelida, Amphinomidae). *Zootaxa*, *5238*(1), 1-134. doi: 10.11646/zootaxa.5238.1.1.

Saleky, D., Leatemia, S.P., Pattiasina, T.F., Isma, I., Pangaribuan, R.D., Welliken, M.A., Melmambessy, E.H. & Dailami, M.(2020). Analisis Pola Pertumbuhan dan Pendekatan DNA Barcoding untuk Identifikasi Turbo stenogyrus P. Fischer, 1873 (Mollusca: Gastropoda). *Biotropika: Journal of Tropical Biology,* 8(2), 79-86.

Sharma, P., & Kobayashi, T. (2014). Are "Universal" DNA Primers Really Universal? *Journal of Applied Genetics*, *55*(4), 485-496. doi: 10.1007/s13353-014-0218-9.

Simbolon, A.R., Masteria, Y.P., & Ismiliana, W. (2021). Species Identification Using DNA Barcoding to Support Sea Cucumber Aquaculture and Conservation in the Waters of Lampung. *Jurnal Riset Akuakultur*, *16*(1), 31–37. doi: 10.15578/jra.16.1.2021.31-37.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, *101*(1), 11030-11035.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027. doi: 10.1093/molbev/msab120.

Tobe, S.S., Kitchener, A.C., & Linacre, A.M. (2010). Reconstructing Mammalian Phylogenies: A Detailed Comparison of The Cytochrome B and Cytochrome Oxidase Subunit I Mitochondrial Genes. *PloS one*, *5*(11), e14156. doi: 10.1371/journal.pone.0014156.

Toso, A., Furfaro, G., Fai, S., Giangrande, A., & Piraino, S. (2022). A sea of fireworms? New insights on ecology and seasonal density of *Hermodice carunculata* (Pallas, 1766) (Annelida) in the Ionian Sea (SE Italy). *The European Zoological Journal*, *89*(1), 1104–1114. doi: 10.1080/24750263.2022.2113156

Wang, Z., Zhang, Y., Xie, Y.J., & Qiu, J.W. (2019). Two Species of Fireworms (Annelida: Amphinomidae: Chloeia) from Hong Kong. *Zoological Studies*, *58*(22), 1-12. doi :10.6620/ZS.2019.58-22.