Species Identification among Fish Samples taken from Mangrove Ecosystem in Lampung Coastal Bay through DNA Barcoding Technique

Yanti Ariyanti[1]*, Ika Agus Rini[1,2], Novriadi Novriadi[1], Fidya Septiana[1]

[1]*Biology Study Program, Institut Teknologi Sumatera*
Jl. Terusan Ryacudu, Lampung 35313, Indonesia
[2]*Department of Integrative Biotechnology, Sungkyunkwan University*
Gyeonggi-do, Suwon, Jangan-gu, Cheoncheon-dong, Seobu-ro 2066, South Korea
Email: yanti.ariyanti@bi.itera.ac.id

Abstract

Mangrove forests are essential for supporting the habitats of numerous fish species, some of which are vital for local economies. These forests serve as breeding and nurturing environments for fish, providing a safe haven for juvenile fish and protecting them from predators and environmental stressors. There are many different species of fish that use mangrove forests as a breeding and nursery ground. Although many studies on the biodiversity of mangrove organisms on Sumatra Island exist, data on fish species diversity in Lampung Bay is still lacking. Morphological identification is often inadequate to distinguish between potential fish species, even across different life stages such as adults, juveniles, or larvae. However, DNA barcoding has the potential to identify species at any developmental stages accurately. Thus, this study aimed to identify and confirm fish species originating from the mangrove ecosystem in Lampung Bay specifically the Sebalang Mangrove Ecopark (South Lampung) and Petengoran Mangrove Forest, Gebang Village (Pesawaran), through a molecular approach using DNA barcoding techniques. Twenty samples can be identified at the species/genus level, demonstrating that using short, standardized genetic region Cytochrome c oxidase I (COI) mitochondrial gene sequences can accurately and quickly classify fish samples. Furthermore, twenty mitochondrial DNA sequences of various fishes have been submitted to the massive genetic database, GenBank. By identifying species accurately and quickly, DNA barcoding can improve the knowledge about fish biodiversity especially samples taken from the mangrove ecosystem in Lampung Coastal Bay.

Keywords: *barcoding; biodiversity; CO1 gene; identification; Lampung Bay; mangrove*

Introduction

The mangrove ecosystem is a unique and brackish environment found in the intertidal zone. Due to the salinity and tidal fluctuations in water level, the forest floor is regularly flooded. The close interaction between marine, brackish, river, and land waters is essential in supporting this area with high species diversity (Mazda, 2013; Tri Martuti, 2014). The mangrove ecosystem provides ecological functions such as resisting intrusion, wind, abrasion, flooding, and serving as a habitat for various aquatic biotas (Lee *et al*., 2014). According to Faridah-Hanum *et al*. (2014), Sumatra has the second-largest region of mangrove forests after Papua. However, Sumatra has experienced a significant decline in mangrove forests, estimated at 60% due to the conversion of shrimp ponds and approximately 20% due to abrasion, fishing activities, and land clearing for agriculture, especially in Lampung Province (Onrizal 2010; Firdaus *et al*., 2021). The decline of mangrove forests not only threatens economic aspects but also ecologically, such as the diversity of the various aquatic biota that inhabits mangrove forests such as crabs, shrimps, mollusks and fish (Robertson 1991; Lee *et al*., 2014; Firdaus *et al*., 2021).

Mangrove trees provide shelter and protection from predators, as well as a source of food for the biotas that live among the roots. Many different species of fish use mangrove forests as a breeding and nursery ground, with specific species varying depending on the location. Identifying fish at the species level is critical for ecological monitoring, environmental impact assessment, fisheries compensation, resource management, anti-smuggling, and setting marine protected areas such as mangrove ecosystem (Valdez-Moreno *et al*., 2010). Although several morphological features, such as body shape, color pattern, meristic count, and measurements, can be used to identify each life stage of fish, they are sometimes insufficient to identify all species, especially rare and cryptic species (Matarese *et al*. 2011; Azmir *et al*. 2017). Morphometric measurements can also overlap between different species (Victor *et al*. 2009) or at any developmental stages of fish (Shao *et al*., 2002), leading to misidentification and inaccurate

*) Corresponding author

https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.28.2.173-180
Received : 03-10-2022
Accepted : 24-05-2023

estimation of fish diversity in certain areas such as mangrove ecosystems.

DNA barcoding offers scientists the key benefit of quickly and precisely recognizing species, even in the absence of physical specimens or when distinguishing between them proves challenging using traditional techniques like morphological analysis or physical identification (Sigwart and Garbett 2018; Insafitri *et al.,* 2023). This allows for the investigation of numerous uncertain species, species composition, and the discovery of cryptic species in the study areas (Packer *et al*., 2009; Ko *et al*., 2013), as well as the differentiation of species that appear morphologically similar.

Several studies have applied this technique to confirm fish species originating from the mangrove ecosystem in Sundarbans, Bangladesh (Habib *et al*., 2021), Merbok Estuary, Malaysia (Zainal Abidin *et al*., 2021), as well as freshwater fish species from Manna River (Bengkulu) and Semangka River (Lampung), and Lake Lebo Taliwang, West Nusa Tenggara (Wibowo *et al*., 2013; Arisuryanti *et al*., 2019). As a potential area with high diversity, mangrove forests are interesting for studying the diversity of biota, especially fish. This study aims to identify and confirm fish samples originating from the mangrove ecosystem in Lampung Bay through a molecular approach using DNA barcoding techniques.

Materials and Methods

Fish specimens were collected from Sebalang Mangrove Ecopark (South Lampung) and Petengoran Mangrove Forest, Gebang Village (Pesawaran) (Figure 1.). The sampling stations were defined using the purposive sampling method, with three stations for each site. Specimens were collected by using various fishing devices such as hand net, electrofishing (2A), and throwing net. We tried to collect at least three individuals for each morphotype/species, but for certain morphotype/species, a single individual was also collected.

Sample processing and morphological identification

A total of 212 samples (146 individuals from Sebalang and 66 individual from Dewi Mandapa) were initially identified on species level by using morphological traits as described in Kottelat and Whitten (1996) and Fishbase references (www.fishbase.org). The samples used in this study consisted of numerous morphotypes of fish in various life stages and were dominated by fish at the juvenile stage. There was 17 morphotypes of fish that included for barcoding analysis. Each specimen had been labeled containing field data and collector; then, documentation was carried out by the digital camera (Canon EOS1200). The collected specimens were fixed with 5% formalin for long-term storage.

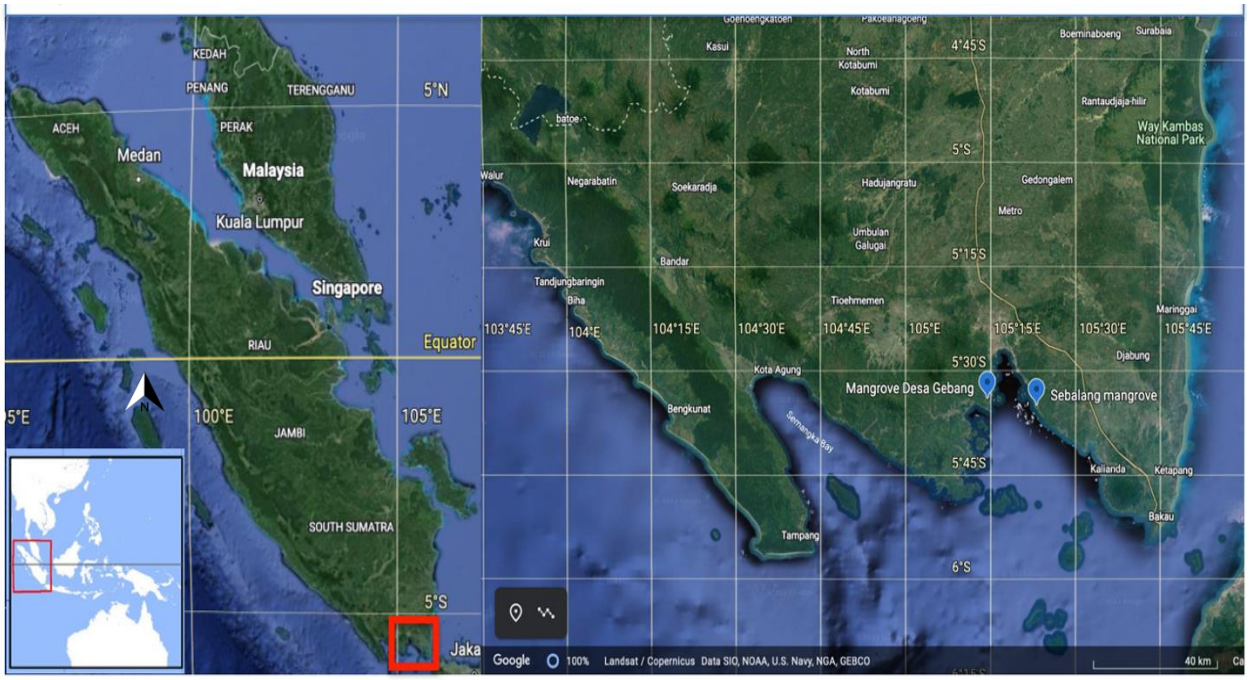

Figure 1. Location of Lampung Bay, blue point indicating the sampling sites of specimens in this study: point 1. Sebalang Mangrove Ecopark (5°35'15"S 105°23'04"E), point 2. Dewi Mandapa, Petengoran Mangrove Forest (5°34'18"S 105°14'29"E).

All specimens were cataloged and deposited at Zoology Laboratory, Institut Teknologi Sumatera. Among them, a total of 48 individuals were selected for DNA barcoding analyses. An epaxial tissue muscle, fin, or whole body (for larval or juveniles) from each fresh specimen was taken and stored in 96% ethanol for further molecular analysis.

DNA isolation and sequencing

Mitochondrial DNA (mtDNA) was extracted using a Genomic DNA Mini Kit Geneaid GT300 following the animal tissue DNA extraction protocol. The purity and concentration of the isolated DNA were measured using a NanoDrop 2000/c spectro-photometer (Thermofisher) and stored at − 18 °C until further analysis. The target region of the COI gene was amplified using the combination of the following primers previously designed by Ward *et al*. (2005): FishF1-5'TCAACCAACCACAAAGACATTGGCAC-3' and FishR1-5'-TAGACTTCTGGGTGGCCAAAGAATCA-3', and expected to gain an approximately 650 bp fragment.

Each mtDNA sample was amplified in a final volume of 25 µL, containing 12.5 µL of Gotaq® Green Master Mix (Promega), 1 µL of each primer, 1 µL of genomic DNA, and adequate distilled water to complete the final reaction volume. Each amplification set was performed with the thermal cycling conditions as follows: initial denaturation at 95°C for 2 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 1 min; then a final extension at 72°C for 10 min. The PCR products were run in 1% agarose gel electrophoresis to check for successful amplification. All intense amplifications were sent for bidirectional sequencing on BigDye® Terminator v. 3.1 platforms provided by 1st BASE Laboratories (Singapore).

Data analysis

Each generated chromatogram was manually checked using the sequence editor BioEdit v. 7.0.9.0 prior to DNA alignment in MEGA X (Kumar *et al*., 2018). The sequences were proofread, independently aligned, and then thoroughly checked for deletions, insertions, and stop codons using the same software. A similarity search of the generated sequences was performed using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/) and The Barcode of Life Data System (BOLD) databases (www.boldsystem.org). The sequences were then aligned using Clustal W in MEGA X and compared by the Neighbor-Joining (NJ) and maximum Likelihood (ML) method with the correction model of p-distance method. The NJ and ML tree clade confidence was estimated with a bootstrap analysis of 1000 replications. The COI sequence of *Himantura* sp. (EU398861.1) was obtained from GenBank for an outgroup.

Result and Discussion

Out of 48 specimens, 20 were successfully sequenced for the partial CO1 gene region. All twenty barcodes generated had sequence lengths greater than 600 bp and no indels or stop codons were detected. Based on the partial COI gene sequences, nucleotide BLAST and BOLD analysis results verified twenty species (Table 1). All specimens from Sebalang ecopark were indicated by KT code, while specimens from Dewi Mandapa and Petengoran mangrove forest were indicated by DM code. Those sequences have been submitted to the GenBank with the accession number as cited at the Table 1 (OP491861- OP491880).

Sample KT3_3 was confirmed as *Trichogster tricoptherus* (KC789556.1), KT2_4 as *Channa striata* (KJ937421.1), KT4_13 as *Anabas testudineus* (OM674614.1), KT4_2 as *Oreochromis niloticus* (MT079202.1), KT4_8 as *Gerres filamentosus* (KU692497.1), KT1_22 as *Apogon coccineus* (JQ349718.1), DM_10 and DM_11 as *Fibramia lateralis* (AB890040.1), DM_18 *Siganus fuscescens* or *Siganus canaliculatus* (OL851675.1), KT5_9 confirmed as *Barbodes binonatus* (MT483452.1), KT2_6 and KT2_11 as *Megalops cyprinoides* (MG923390.1), DM_17 as *Leiognathus robustus*, DM_20, and DM_23 as *Zenarcoptherus* sp. (MK777142.1) and DM_24 confirmed as *Cynoglossus* cf. *cynoglossus* (HQ564331.1). Sample numbers KT2_8 and KT2_10 are clustered into one group; however, there were some different names for these species. Nevertheless, based on the BOLD databases, KT2_8 samples can only be identified to the genus level, *Ambassis* sp. (JN021210.1), with a 100% similarity score. KT4_15 and DM_07 were clustered with three different names: *Crenimugil buchanani* (MT884995.1) and *Crenimugil seheli* (MN795451.1). In fact, those names are a synonym for an accepted scientific classification of *Crenimugil buchanani* (Froese and Pauly 2018).

Genetic distances for each sequence comparing with reference sequences retrieved from GenBank ranged from 0.00-0.03. Lower genetic distances (0.00) were observed between KT4_15 with *Crenimugil buchanani* (MT884995.1), DM_07 with *Moolgarda seheli* (MN795451.1), KT3_3 with *Trichogster tricoptherus* (KC795556.1), KT2_4 with *Channa striata* (KJ937421.1), KT4_13 with *Anabas testudineus* (OM674614.1), KT4_2 with *Oreochromis niloticus* (MT079202.1), DM_18 with *Siganus fuscescens* (OL851675.1), KT1_22 with *Apogon coccineus* (JQ349718.1), DM_10 and DM_11 with *Fibramia lateralis* (MN733538.1), DM_24 with

Cynoglossus cf. *cynoglossus* (HQ564331.1), KT2_8 with *Ambassis* sp. (JN021210.1), KT5_9 with *Barbodes binonatus* (MT483452.1), KT2_6 and KT2_11 with *Megalops cyprinoides* (MG923390.1). The genetic distance between KT4_8 with *Gerres filamentosus* (KU692497.1), DM17 with MN511936.1, DM24 with HQ564331.1 were 0.01, while genetic distance between DM23 and DM 20 with MK777142.1 were 0.03.

Phylogenetic tree was constructed for all studied species and sequences retrieved from the Genbank. The Neighbor-Joining method was used to infer the evolutionary history (Saitou and Nei, 1987), and the optimal tree with the sum of branch length = 2.10033743 is shown (Figure 2.). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of amino acid differences per site. This analysis involved 38 nucleotide sequences, and all ambiguous positions were removed for each sequence pair (pairwise deletion option). There were total of 596 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018; Stecher *et al*., 2020). The tree has the same topology as the ML tree (Figure 3.). The initial ML tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Petengoran Mangrove Forest is located in Gebang village, Teluk Pandan district, Pesawaran regency, Lampung province. This location is close to

Table 1 Identification result based on GenBank (BLAST analysis) and BOLD databases

No sample	GenBank Accession (present study)	Origin	Blast result	GenBank Accession (references)
KT2_6	OP491861	Sebalang Ecopark	*Megalops cyprinoides*	MG923390.1
KT2_11	OP491864		*Megalops cyprinoides*	MG923390.1
KT5_9	OP491862		*Barbodes binotatus*	MT483452.1
KT3_3	OP491863		*Trichogaster trichopterus*	KC789556.1
KT4_8	OP491865		*Gerres filamentosus*	KU692497.1
KT4_13	OP491866		*Anabas testudineus*	OM674614.1
KT4_2	OP491867		*Oreochromis niloticus*	MT079202.1
KT2_4	OP491868		*Channa striata*	KJ937421.1
KT2_8	OP491869		*Ambassis* sp	JN021210.1
			Ambassis macrachantus	MW498508.1
KT2_10	OP491870		*Ambassis gymnocephalus*	KX621134.1
			Ambassis sp.	MN243472.1
KT4_15	OP491871		*Crenimugil buchanani*	MT884995.1
KT1_22	OP491872		*Apogon coccineus*	JQ349718.1
DM_07	OP491873	Petengoran Mangrove	*Moolgardal seheli*	MN795451.1
DM_24	OP491874		*Cynoglossus* cf. *cynoglossus*	HQ564331.1
DM_10	OP491875		*Ostorhinchus lateralis*	AB890040.1
			Fibramia lateralis	MN733538.1
DM_11	OP491876		*Ostorhinchus lateralis*	AB890040.1
			Fibramia lateralis	MN733538.1
DM_23	OP491877		*Zenarchopterus* sp	MK777142.1
DM_18	OP491878		*Siganus canaliculatus*	OL851675.1
DM_20	OP491879		*Zenarchopterus* sp	MK777142.1
DM_17	OP491880		*Leiognathus robustus*	MN511936.1

Dewi Mandapa, one of the famous beach in Lampung, while Sebalang Ecopark is located in Katibung Village, Tarahan District, South Lampung Regency. The existing mangrove forest at the Sebalang Ecopark is about five hectares, and its location is close to the Steam Power Plant (PLTU) Tarahan, which was built in 2004. The Sebalang Ecopark's mangrove forest boasts unique characteristics that set it apart from others. The area is secluded by roads and infrastructure, which

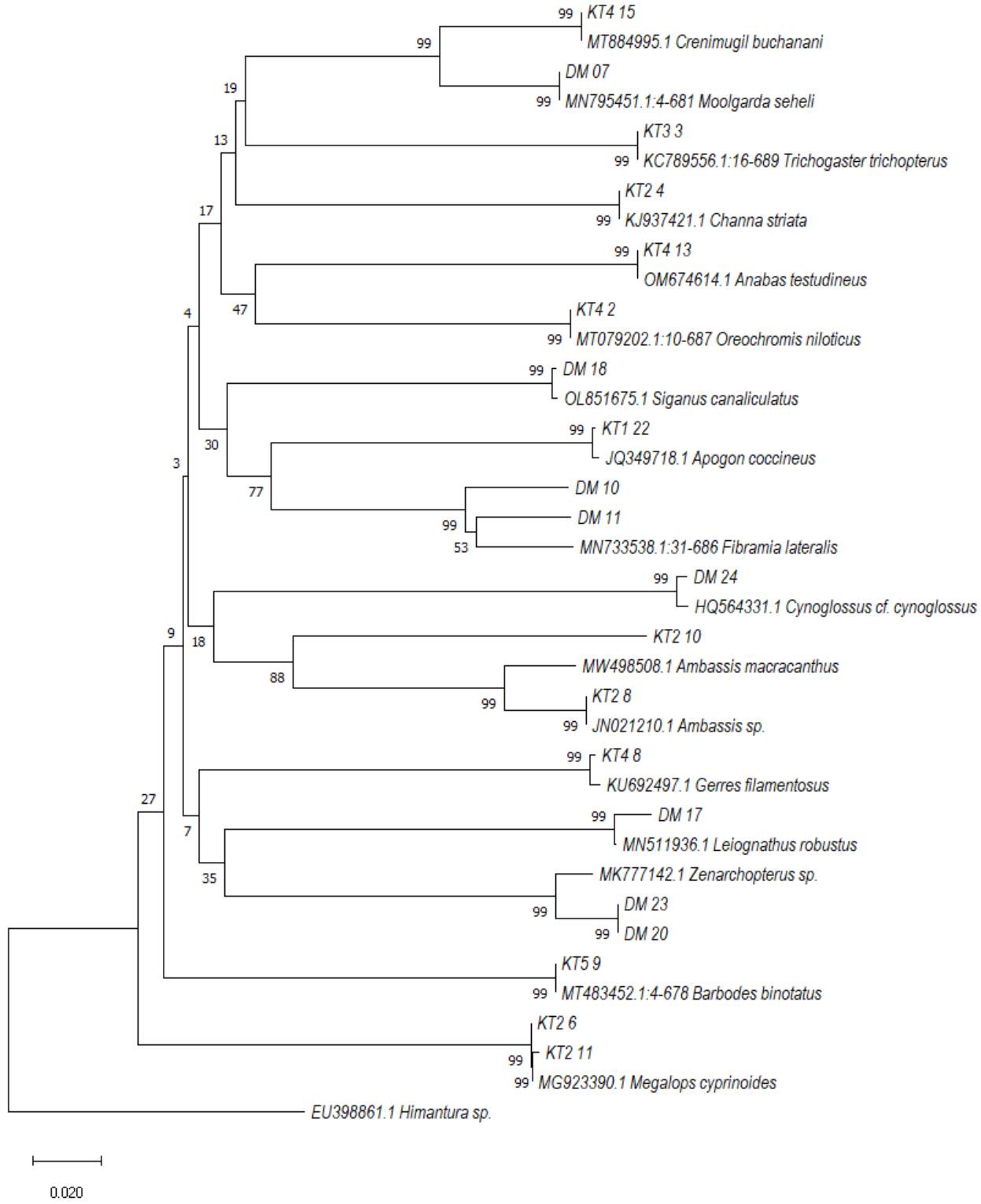

Figure 2. Neighbor-Joining (NJ) tree constructed based on COI sequences with a bootstrap analysis of 1000 replications

prevents direct exposure to sea tides, unlike most mangrove ecosystems. Over a decade ago, the mangrove forest of Sebalang was a popular spot for fishing enthusiasts seeking different types of fish. However, with the change in function of the forest, the community began to complain about the disappearance of several fish species that were once common in the mangrove ecosystem and regularly caught by locals. As a result, the community took action by planting fish seeds in the isolated mangrove forest area, suspecting that these may have been non-native or introduced.

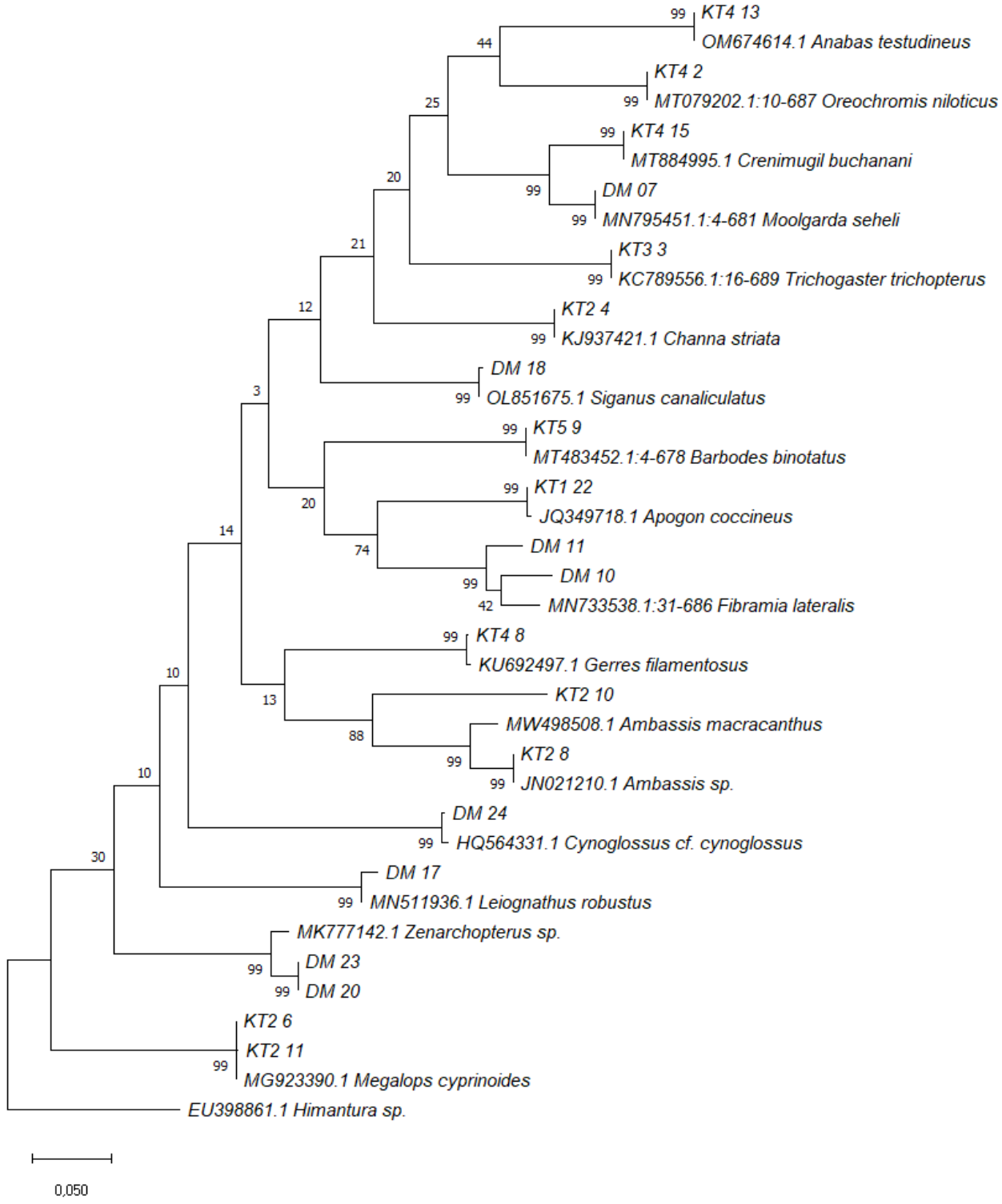

Figure 3. Maximum Likelihood (ML) tree constructed based on COI sequences with a bootstrap analysis of 1000 replications

This present work provides information about species diversity and potential threats of non-native fishes in the Lampung Bay mangrove ecosystem. For instance, we found several non-native fish species, such as *Oreochromis niloticus*, *Channa striata*, and *T. trichopterus*, to be more frequently occurring in Sebalang Mangrove Ecopark. These species typically inhabit calm waters with low oxygen levels, such as swamps. *Trichogaster trichopterus* belongs to the suborder Anabantoidei, which has a labyrinth organ in its gills that enables it to breathe oxygen directly from the air above the water's surface (Murjani, 2009). This species can tolerate brackish waters due to its ability to tolerate a wide range of water hardness, pH, temperature, salinity, and dissolved oxygen conditions (Geheber *et al*., 2010). The presence of non-native fish in the isolated Sebalang Mangrove Ecopark may have the potential to displace native species, impact the community structure and diversity of native mangrove forest organisms (Alidoost Salimi *et al*., 2021), and have economic implications.

Overall, these results suggest that the DNA barcoding technique using partial COI gene sequences is a reliable method for identifying and classifying fish samples in the mangrove ecosystem. This study has also contributed to enriching the sequence data submitted to the genetic database, GenBank. Further research could expand the sample size and geographic range to provide a more comprehensive understanding of the diversity and relationships among fish species in the mangrove ecosystem.

Conclusion

This study identified twenty fish samples from the Lampung Bay mangrove ecosystem using the partial COI mitochondrial gene sequences. Twenty samples were identified at the species level and at least at the genus level based on the GenBank and BOLD database. The use of short, standardized genetic region COI mitochondrial gene sequences as DNA barcode markers proved to be a fast and accurate method for identifying and classifying fish samples. This study has also contributed to enriching the sequence data submitted to the genetic database, GenBank.

Acknowledgement

This research was fully supported by the Smart ITERA Research Grant 2019 (Grant No. B/304/IT9.C1/PT.01.03/2019) from Institut Teknologi Sumatera. We would like to express our gratitude to the research student team who helped us collect the samples. The authors declare no conflicts of interest, and all co-authors have seen and approved the manuscript's contents without any financial interest to report.

References

Alidoost Salimi, P., Creed, J.C., Esch, M.M., Fenner, D., Jaafar, Z., Levesque, J.C., Montgomery, A.D., Alidoost Salimi, M., Edward, J.P., Raj, K.D. & Sweet, M. 2021. A review of the diversity and impact of invasive non-native species in tropical marine ecosystems. *Mar. Biodiv. Rec.*, 14(1): p.11. https://doi.org/10.1186/s41200-021-00206-8.

Arisuryanti, T., Hasan, R.L., Ayu, K.L., Ratman, N. & Hakim, L., 2019. Genetic Identification of Freshwater Fish Species Through DNA Barcoding from Lake Lebo Taliwang, West Nusa Tenggara. *J. Trop. Biodiv. Biotechnol.,* 4(3): 107-112. https://doi.org/ 10.22146/jtbb.46256.

Azmir, I.A., Esa, Y., Amin, S.M.N., Md Yasin, I.S. & Md Yusof, F.Z., 2017. Identification of larval fish in mangrove areas of Peninsular Malaysia using morphology and DNA barcoding methods. *J. App. Ichthyol.*, 33(5): 998-1006. https://doi.org/10.1111/jai.13425.

Faridah-Hanum, I., Latiff, A., Hakeem, K.R. & Ozturk, M. eds., 2013. Mangrove ecosystems of Asia: status, challenges and management strategies. Springer Science & Business Media. pp.1–471. https://doi.org/10.1007/978-1-4614-8582-7.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evol.,* 39: 783-791.

Firdaus, M., Hatanaka, K. & Saville, R. 2021. Mangrove forest restoration by fisheries communities in lampung bay: A study based on perceptions, willingness to pay, and management strategy. *For. Soc.*, 5(2): 224–244. https://doi.org/10.24259/fs.v5i2.12008.

Froese, R. & Pauly, D. 2018. World Wide Web electronic publication. www.fishbase.org.

Geheber, A.D., McMahan, C.D. & Piller, K.R., 2010. First record of the non-native three spot gourami, *Trichogaster trichopterus* (Pallas 1770) (Teleostei: Osphronemidae) in Jamaica. *Aquatic Invasions*, 5(1): S13-S16.

Habib, K.A., Neogi, A.K., Rahman, M., Oh, J., Lee, Y.H. & Kim, C.G., 2021. DNA barcoding of brackish and marine water fishes and shellfishes of Sundarbans, the world's largest mangrove ecosystem. *Plos One*, 16:1–17. https://doi.org/10.1371/journal.pone.0255110.

Insafitri, I., Nursalim, N., Kholilah, N., Kurniasih, E.M., Cahyani, N.K.D., Nugraha, W.A. & Ambariyanto, A., 2023. DNA barcode of seven species coral

from Sepulu, Madura Island, Indonesia. *Biodiversitas*, 24(1):317-323. https://doi.org/10.13057/biodiv/d240138

Ko, H.L., Wang, Y.T., Chiu, T.S., Lee, M.A., Leu, M.Y., Chang, K.Z., Chen, W.Y. & Shao, K.T., 2013. Evaluating the Accuracy of Morphological Identification of Larval Fishes by Applying DNA Barcoding. *Plos One,* 8(1): e53451.

Kottelat, M. & Whitten, A.J. 1996. Freshwater Fishes of Western Indonesia and Sulawesi : Additions and Corrections. (p. 8). Hong Kong: Periplus editions.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.,* 35: 1547-1549.

Lee, S.Y., Primavera, J.H., Dahdouh-Guebas, F., McKee, K., Bosire, J.O., Cannicci, S., Diele, K., Fromard, F., Koedam, N., Marchand, C. & Mendelssohn, I., 2014. Ecological role and services of tropical mangrove ecosystems: A reassessment. *Glob. Ecol. Biogeogr.,* 23(7): 726-743. https://doi.org/10.1111/geb.12155.

Matarese, A.C., Spies, I.B., Busby, M.S. & Orr, J.W. 2011. Early larvae of *Zesticelus profundorum* (family: Cottidae) identified using DNA barcoding. *Ichthyol. Res.,* 58: 170-174.

Mazda, Y. 2013. The Mangrove Ecosystem Utilizes Physical Processes. *Glob. Environ. Res.,* 17:165–172.

Murjani, A. 2009. Budidaya Ikan Sepat Rawa (*Trichogaster trichopterus*) dengan Pemberian Pakan Komersil. Laporan Penelitian Mandiri. Fakultas Perikanan Universitas Lambung Mangkurat.

Nei, M. & Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Onrizal. 2010. di Pantai Timur Sumatera Utara Periode 1977-2006. *J. Biologi Indonesia*, 6(2): 163–172.

Packer, L., Gibbs, J., Sheffield, C., & Hanner, R. 2009. DNA barcoding and the mediocrity of morphology. *Mol. Ecol. Res.,* 9: 42–50.

Robertson, A.I. 1991. Plant-animal interactions and the structure and function of mangrove forest ecosystems. *Austral. J. Ecol.*, 16(4): 433–443. https://doi.org/10.1111/j.1442-9993.1991.tb01073.x.

Saitou, N. & Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biolog. Evol.,* 4: 406-425.

Shao, K.T., Chen, K.C. & Wu, J.H., 2002. Identification of marine fish eggs in Taiwan using light microscope, scanning electron microscope and mtDNA sequencing. *Mar. Freshw. Res.,* 53: 355–365.

Sigwart, J.D. & Garbett, A. 2018. Biodiversity assessment, DNA barcoding, and the minority majority. *Integr. Comp. Biol.*, 58(6): 1146–1156. https://doi.org/10.1093/icb/icy076

Stecher G., Tamura K. & Kumar S. 2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Mol. Biol. Evol.,* 37(4): 1237-1239. https://doi.org/10.1093/molbev/msz312).

Tri Martuti, N.K. 2014. Keanekaragam Mangrove Di Wilayah Tapak, Tugurejo, Semarang. *J. MIPA Unnes*, 36(2): 113503.

Valdez-Moreno, M., Vásquez-Yeomans, L., Elías-Gutiérrez, M., Ivanova, N.V. & Hebert, P.D. 2010. Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. *Mar. Freshw. Res.*, 61(6): 655-671.

Victor, B.C., Hanner, R., Shivji, M., Hyde, J. & Caldow, C., 2009. Identification of the larval and juvenile stages of the cubera snapper, *Lutjanus cyanopterus*, using DNA barcoding. *Zootaxa* 2215(2): 24–36.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D., 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.,* 360(1462): 1847-1857. https://doi.org/10.10 98/rstb.2005.1716.

Wibowo, A., Farajalah, A. & Husnah, H. 2013. Dna Barcoding of Freshwater Fish Species of Manna River (Bengkulu) and Semanka River (Lampung). *Indones. Fish. Res. J.,* 19(1): p.9. https://doi.org/10.15578/ifrj.19.1.2013.9-17.

Zainal Abidin, D.H., Mohd. Nor, S.A., Lavoue, S., A. Rahim, M., Jamaludin, N.A. & Mohammed Akib, N.A. 2021. DNA-based taxonomy of a mangrove-associated community of fishes in Southeast Asia. *Sci. Rep.*, 11(1): 1–15. https://doi.org/10.1038/s41598-021-97324-1.