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Revealing Cryptic Diversity: DNA Barcoding of Sea Cucumbers from Nusa Tenggara Barat and Nusa Tenggara Timur, Indonesia

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ABSTRACT

Sea cucumbers are ecologically and economically significant marine organisms, but accurately identifying their species remains a challenge due to morphological similarities and the presence of cryptic species. This issue is particularly pronounced in regions viz. West Nusa Tenggara (NTB) and East Nusa Tenggara (NTT), Indonesia, where high biodiversity and overexploitation pressures necessitate precise species identification for effective management and conservation. This study aimed to evaluate the effectiveness of DNA barcoding and phylogenetic analysis in identifying sea cucumber species in these regions. A total of ten sea cucumber samples were collected from various locations in NTB and NTT and were analyzed using the mitochondrial COI gene as a barcode marker. PCR amplification and sequencing were performed, followed by BLAST analysis and phylogenetic tree construction using the maximum likelihood method. The results indicated that eight samples were successfully identified at the species level, with percent identity ranging from 96.39 to 100%. This confirmed the high accuracy of DNA barcoding in distinguishing sea cucumber species. Notably, *Stichopus monotuberculatus* was identified with 100% identity, while *Holothuria flavomaculata* showed slightly lower identity values, suggesting potential intraspecific genetic variation or the presence of cryptic species. Phylogenetic analysis further supported these findings, revealing distinct clades among the identified species and indicating significant genetic divergence within the studied populations. However, limitations such as incomplete reference databases and suboptimal sequence quality hindered the identification of two samples. These findings emphasize the need to expand genetic reference data and improve sequence quality to enhance the reliability of DNA barcoding. Overall, this study demonstrates the utility of molecular approaches in addressing species identification challenges, contributing to the sustainable management and conservation of sea cucumber populations in NTB and NTT.

INTRODUCTION

Sea cucumbers are very important to marine ecosystems due to their bioturbation activities, which help in nutrient cycling as well as maintaining the health of the marine

environment **(Wulandari *et al.*, 2012)**. Sea cucumbers are economically significant, particularly in Asia, where they are valued as traditional commodities in agriculture and export due to their high nutritional and medicinal properties **(He *et al.*, 2024)**. However, identifying sea cucumber species is challenging due to morphological similarities and cryptic species, which complicates conservation efforts and compliance with international trade regulations **(Sulardiono *et al.*, 2022)**. This problem is exacerbated by the need for comprehensive genetic data, as only a limited number of complete mitochondrial genomes are available, hampering evolutionary and taxonomic studies **(He *et al.*, 2024)**. Accurate and efficient identification methods are critical to support these marine resources' conservation and sustainable use **(Guo *et al.*, 2022)**. Furthermore, the global trade of marine species such as *Humphead Wrasse* highlights the importance of regulatory frameworks to prevent overexploitation and ensure sustainable practices **(Chen & Justin, 2009)**. The discovery of new species and an understanding of their ecological roles, similar to studies conducted on other marine organisms, can offer valuable insights into biodiversity and evolutionary processes. This knowledge is essential for developing effective conservation strategies **(McGrath, 2020)**.

Identification of sea cucumber species through traditional morphological approaches is challenging due to significant intraspecific morphological variation and interspecies similarities, making cryptic species difficult to distinguish visually. This problem is particularly noticeable in areas such as West Nusa Tenggara (NTB) and East Nusa Tenggara (NTT) in Indonesia, known for their rich marine biodiversity. The lack of accurate local taxonomic data exacerbates the problem, leading to misidentification and inaccuracies in species reporting, negatively impacting fisheries management, conservation efforts, and trade policies. For example, studies on *Stichopus* cf. *horrens* emphasize the complexity of species boundaries and the presence of cryptic species, which can only be accurately resolved through genetic markers like mitochondrial COI and microsatellite markers, rather than relying solely on morphological traits **(Lizano *et al.*, 2024)**.

According to **Rothamel *et al*. (2023)**, the economic and ecological significances of sea cucumbers, as well as their overexploitation, are well documented, with species such as *Holothuria fuscogilva* and *Holothuria nobilis* included in CITES Appendix II due to their threatened status, underscoring the need for accurate species identification to prevent illegal trade and ensure sustainable management.

Furthermore, the global trade in sea cucumbers, driven by their high commercial value and health benefits, requires a strong conservation strategy to combat overfishing and habitat loss, as seen in the case of India, where a general ban on harvesting was implemented to protect this species **(Harini *et al.*, 2024)**. The challenge of measuring sea cucumber body size due to its morphological plasticity further complicates fisheries management, as highlighted in a study that emphasized the need for standardized measurement techniques to obtain reliable data for management purposes **(Trenholm *et***

***al.*, 2024)**. In addition, understanding the ecological roles of sea cucumbers, such as their contribution to nutrient cycling and habitat preferences, can inform conservation strategies and support sustainable management of this vital marine resource **(Pangulimang *et al.*, 2023)**. The integration of genetic, ecological, and technological advances in species identification is essential for the sustainable utilization and protection of sea cucumber biodiversity in NTB and NTT, ensuring compliance with international trade regulations and the long-term viability of this valuable marine resource **(Azevedo *et al.*, 2024; Pérez-Lloréns & Mouritsen, 2024)**.

The study of sea cucumber diversity in the West Nusa Tenggara (NTB) and East Nusa Tenggara (NTT) regions of Indonesia is crucial due to the high potential of marine biodiversity and the threats posed by exploitation and habitat degradation. Despite its importance, molecular approaches such as DNA barcoding are underutilized in these regions, creating a significant gap in the literature. This gap is evident compared to other regions where molecular techniques have been widely applied. The use of DNA barcoding on other marine species in Indonesia has demonstrated the effectiveness of this method in revealing genetic diversity and aiding species identification, which is critical for sustainable management **(Joesidawati *et al.*, 2023; Wora *et al.*, 2024)**. The lack of genetic data for sea cucumbers in NTB and NTT hinders understanding their biodiversity and developing effective conservation strategies. Addressing this gap through molecular studies will improve species identification, especially for cryptic species, and support more effective marine biodiversity protection and management efforts. This is particularly important given sea cucumbers' economic and ecological significances and the pressures they face from overexploitation, as seen in other areas where fisheries have expanded due to declining resources **(Kovačević *et al.*, 2023)**. Therefore, integrating molecular approaches such as DNA barcoding into sea cucumber studies in NTB and NTT is essential to advance conservation efforts and ensure sustainable use of marine resources in this biodiverse yet vulnerable region.

MATERIALS AND METHODS

Research location and sampling

This study was conducted in two central locations, namely West Nusa Tenggara (NTB) and East Nusa Tenggara (NTT), Indonesia, known as areas with high sea cucumber diversity. Sampling was conducted at several points representing diverse sea cucumber habitats, including coastal areas and coral reefs on Lombok Island, Sumbawa Island, Flores Island, and Timor Island. Sampling sites were selected based on accessibility, significant sea cucumber populations, and input from local fishermen on crucial fishing grounds (Fig. 1a, b).

Sea cucumbers sampling technique

In this study, sea cucumber specimens were collected for DNA barcoding following rigorous, standardized protocols to ensure the reliability and reproducibility of genetic

data. Specimens were sampled across multiple geographically distinct locations within the target marine habitats to capture a representative range of genetic diversity within and between populations. Random stratified sampling minimizes environmental and population biases, ensuring proportional representation from various depth ranges and substrate types within each location.

Each sea cucumber was collected using hand-operated diving techniques to minimize physical damage, preserving the integrity of the specimens for both morphological and genetic analysis. Upon collection, a small tissue biopsy, typically a 1-2cm section of the body wall or tube foot, was excised from each specimen using sterilized surgical tools. Care was taken to ensure that biopsies were non-lethal, allowing for the potential return of live specimens to their natural environment when applicable, in compliance with ethical and conservation guidelines. Tissue samples were immediately preserved in 95% ethanol or frozen at –20°C to prevent DNA degradation and subsequently transported to the laboratory for further molecular analysis.

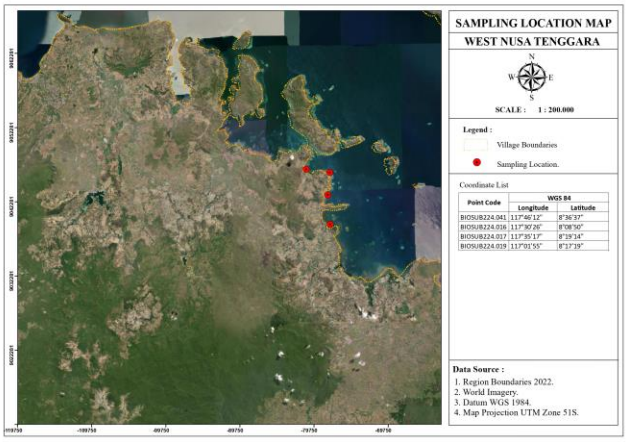

Fig. 1a. Sea cucumber sampling locations in West Nusa Tenggara

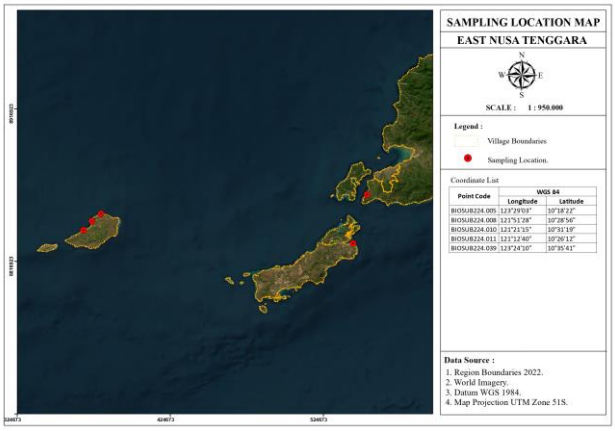

Fig. 1b. Sea cucumber sampling locations in East Nusa Tenggara

Amplification

Tissue samples were then taken (approximately 10g in size), and the extraction process was carried out to isolate DNA. The extraction method used follows the *Qiagen* protocol. The extraction results are then analyzed for the next stage, namely PCR (*Polymerase Chain Reaction*). The PCR process uses the BIONESIA laboratory protocol **(Kautsari *et al.*, 2024)**. The primer used in the amplification process for sea cucumber samples is COI ceF (5'-ACTGCCCACGCCCTAGTAATGATTTTTTATGGTNATGCC-3') reverse primer COI ceR (5'-TCGTGTGTCTACGTCCATTCCTACTGTRAA CATRTG -3') **(Hoareau & Boissin, 2010)**. The total PCR reaction was 26µL consisting of the mixture: 2µL of extracted DNA *template*, 1.25µL of each primer in 10 mM concentration, 9µL of ddH2O, and 12.5µL of *Ready mix*. The reaction mixture was amplified using an Applied Biosystems™ 2720 Thermal Cycler machine. The temperature and time profile of the PCR protocol used was as follows: Pre-denaturation:

95°C for 3 minutes, followed by denaturation stage: 94°C for 45 seconds, *annealing* 55-60°C for 1.0 minute 10 seconds, and *extension* stage 72°C for 1.0 minute 20 seconds. Denaturation to *extension* stage was carried out for 30 cycles, the last stage was final extension: 72°C for 5 minutes. PCR results were then visualized on 1.0% of agarose gel with *nucleic acid gel stain* (GelRed®). Positive samples (showing DNA bands) were then carried out to read DNA (*sequencing*) using the Sanger deoxy method at PT Genetika Science Jakarta.

Data analysis

The results of samples periously sequenced in the form of sequence data (Ab1 file) were then analyzed using a computer. The sequence data obtained were then edited and aligned using the ClustalW method in the MEGA 7 program. Each base arrangement was then manually checked, and all data used were ensured to be of good quality. Data that had poor sequence results were then subjected to PCR and re-sequencing.

The data were then matched with the database in the data bank (Genbank NCBI) through the Basic *local alignment search tools (BLAST)* method on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Each dataset was then recorded for similarity and accuracy.

In addition to the BLAST method, the data were then analyzed using a *phylogenetic tree* to see the kinship relationship between samples while confirming the BLAST results in identifying up to the species level. The kinship tree was created using the neighbor-joining (NJ) method with 1000 bootstrap replications in MEGA 7 software. The genetic distance value was then analyzed using the *p-distance* method to compare one sample with another.

Table 1. Barcoding results using BLAST of samples using FISH FI / FISH R1 primers

Field ID	Lab ID	Species	bp	Gene	Accession Number	Query Cover (%)	Identity (%)
TBL 05	BIOSUB224.005	*Stichopus monotuberculatus*	722	*coi*	KC424500.1	92%	100%
SB 01	BIOSUB224.008	*Holothuria leucospilota*	721		MK562379.1	82%	100%
SB 03	BIOSUB224.010	*Holothuria hilla*	708		OP898065.1	99%	99,15%
SB 04	BIOSUB224.011	*Stichopus monotuberculatus*	709		KC424498.1	93%	99,85%
SB 08	BIOSUB224.015	-	-		-	-	-
1	BIOSUB224.016	*Holothuria flavomaculata*	705		JN207626.1	92%	96,93%
2	BIOSUB224.017	*Holothuria flavomaculata*	708		JN207626.1	91%	96,77%
4	BIOSUB224.019	*Stichopus monotuberculatus*	712		KC424500.1	93%	99,85%
PT 03	BIOSUB224.039	*Holothuria hilla*	708		MK477996.1	75%	98,51%
5	BIOSUB224.041	*Holothuria hilla*	709		MK562388.1	83%	100%

RESULTS

Of the ten samples analyzed, eight were successfully identified to the species level with high accuracy. The query cover rate ranged from 75 to 99%, while the percent

identity reached 96.39 to 100%. These results demonstrate the high success of the DNA barcoding technique in identifying sea cucumber species in the NTB and NTT regions. This technique plays an important role in revealing genetic diversity that may not be apparent through morphological identification alone.

The sample with Lab ID BIOSUB224.005 was identified as *Stichopus monotuberculatus* with a query cover of 92% and a percent identity reaching 100%. These results indicate that the DNA barcoding method used was highly accurate in identifying this species, confirming that the sample DNA sequences matched perfectly with the reference data in the database. In addition, these results reinforce the validity of the DNA barcoding technique in identifying species that are difficult to distinguish visually.

However, variation in results was found in the samples with Lab ID BIOSUB224.016 and BIOSUB224.017, which were identified as *Holothuria flavomaculata* with query cover of 92 and 91% and percent identity of 96.39 and 96.77%, respectively. Although the species identification was successful, the slightly lower identity rate compared to the previous sample suggests the possibility of genetic variation within the local population of *Holothuria flavomaculata*. This could indicate the presence of cryptic species or intra-specific variation not detected through traditional morphological identification. Such genetic differences need to be further explored through more in-depth studies using additional genetic markers to confirm the presence of cryptic speciation.

Two samples, SB 08 and Field ID 4, showed no specific identification results. This inability to identify these samples could stem from several factors, including suboptimal sequence quality, contamination, or mismatches between the sample DNA sequences and the reference database. The issue of database mismatches highlights a key challenge in DNA barcoding: the limited scope of reference data, which can hinder the identification of species that are not well documented in public databases.

The resulting phylogenetic tree demonstrated clear clustering among sea cucumber species, supporting the molecular identification results from DNA barcoding. *Stichopus monotuberculatus*, one of the identified species, formed a distinct clade with high bootstrap values, indicating significant genetic divergence from other species in the analyzed group. The strong bootstrap values (>90%) in this clade provide confidence in the phylogenetic tree's accuracy, suggesting that *Stichopus monotuberculatus* possesses a unique evolutionary history compared to other sea cucumber species in the same region.

Meanwhile, two species of the *Holothuria* genus, *Holothuria hilla*, and *Holothuria flavomaculata*, belong to a closer clade, indicating a close relationship between them. This finding is consistent with the hypothesis of the existence of cryptic species in the genus *Holothuria*, where morphologically similar species have low genetic divergence while showing some observable genetic variation through molecular analysis. This is important to note, as cryptic species are often difficult to identify morphologically and

may lead to errors in biodiversity assessment if not further scrutinized using molecular approaches.

Some specimens, including the one identified as *Holothuria leucospilota*, displayed slightly different phylogenetic positions compared to the reference sequences available in GenBank. These differences likely reflect intraspecific genetic variation, potentially arising from geographic, ecological, or adaptive evolutionary factors. Another possibility is the presence of subspecies that have not yet been genetically documented in public databases. Such genetic variation often indicates localized population divergence, which is crucial for understanding the dynamics of fisheries resource conservation and management. Recognizing these variations can aid develop more targeted conservation strategies to ensure the sustainability of sea cucumber populations in their respective habitats.

Statistical support and phylogenetic validation

Bootstrapping, used to assess the reliability of branches on a phylogenetic tree, showed good support for most of the identified clades, with some clades achieving bootstrap values above 90%. This indicates a high confidence level in most of the phylogenetic relationships. The robust clustering of species identified by DNA barcoding further validates this phylogenetic approach, reinforcing the conclusion that DNA barcoding methods and *maximum likelihood* phylogenetic analysis are complementary in uncovering evolutionary relationships between species.

In addition, this phylogenetic tree clarifies the kinship structure between species, which provides a better understanding of the evolutionary dynamics and biodiversity of sea cucumbers in the NTB and NTT regions. For example, the significant genetic divergence in *Stichopus monotuberculatus* species and genetic proximity among *Holothuria* species provide essential insights into the different evolutionary pathways within species living in the same geographical area.

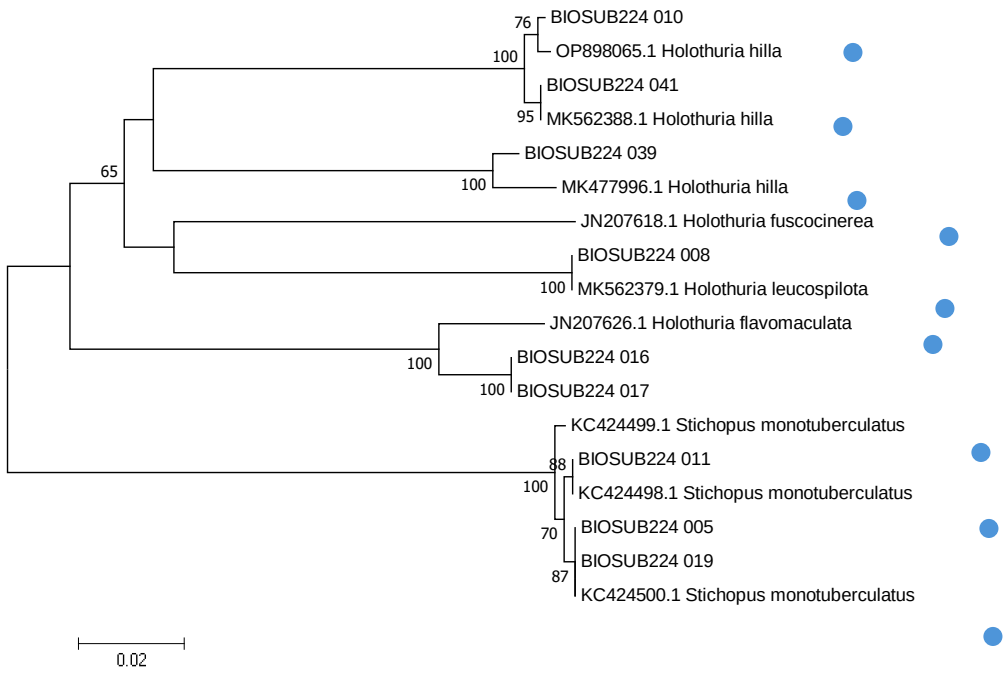

Fig. 2. Phylogenetic results of samples using CeF/ CeR primers

DISCUSSION

Identification accuracy with DNA barcoding

Eight of the ten samples analyzed were successfully identified at the species level with high query cover (75- 99%) and a percent identity that reached 96.39 to 100%. This indicates that DNA barcoding is an effective tool for identifying sea cucumber species. According to **Hebert *et al.* (2013)**, DNA barcoding can overcome complex species identification problems by providing a more objective and standardized genetic-based method than traditional morphological approaches. These findings align with existing literature that confirms that genetic analysis can reveal species diversity not visible at the morphological level, including intra-specific variation that may be relevant in a conservation context **(Thompson & Newmaster, 2014)**.

Kinship relationships and genetic variation

Phylogenetic analysis showed that *Stichopus monotuberculatus* forms separate clades with high bootstrap support. This suggests the presence of significant genetic divergence, which can be interpreted through speciation theory that explains how individuals within populations can develop genetic variation in response to environmental selection pressures **(Ricklefs, 2007)**. The apparent clustering between species within the genus *Holothuria* also confirms the *hypothesis* of cryptic species, supporting the notion

that similar-looking species can have different genetic backgrounds. This phenomenon has been widely discussed in the context of marine biodiversity **(Uthicke *et al*., 1998)**.

The genetic variation observed in *Holothuria flavomaculata* samples and the different phylogenetic positions of *Holothuria leucospilota* compared to the GenBank reference may reflect evolutionary dynamics influenced by geographical or ecological factors. Local adaptation theory suggests that populations can adapt to specific environmental conditions, leading to significant genetic variation **(Kerr *et al*., 2005)**. Therefore, these findings indicate the need for further research to understand the factors influencing genetic diversity and speciation within the *Holothuria* genus.

Conservation implications

This study has significant implications for sea cucumber conservation strategies in NTB and NTT. The presence of cryptic species and intra-specific genetic variation may influence resource management policies, especially in the context of sustainable utilization. Given that sea cucumbers have an essential role in marine ecosystems as decomposers and filters, loss of biodiversity in this group could negatively impact ecosystem health **(Purcell *et al*., 2016)**. Therefore, it is essential to integrate the results of this study into broader conservation policies, which consider not only the apparent presence of species but also the genetic variability that may exist within local populations.

Limitations and recommendations for future research

While the results demonstrate the high potential of DNA barcoding and phylogenetic analysis, several limitations must be acknowledged. Some samples could not be specifically identified, likely due to suboptimal sequence quality or mismatches with existing reference databases. This highlights the challenges faced in DNA barcoding, particularly in regions with high biodiversity but insufficient genetic reference data **(Keskin & Atar, 2013)**. Therefore, future research should focus on collecting additional sequence data from local populations to enhance database coverage. Additionally, employing more comprehensive analyses using additional genetic markers could provide deeper insights into species identification and biodiversity in these regions.

CONCLUSION

Eight of the ten samples analyzed were successfully identified at the species level with high accuracy, showing DNA identity match rates of 96.39 to 100%. The species *Stichopus monotuberculatus* was successfully identified with a 100% identity match rate.

The findings also indicate the potential for genetic variation within the local population of *Holothuria flavomaculata*, indicated by the slightly lower percentage identity. This indicates the possibility of cryptic species or intraspecific variation that is not detected morphologically. Further phylogenetic analysis supported these molecular

identification results, showing clear clusters among the identified sea cucumber species. *Stichopus monotuberculatus* formed a separate clade with high bootstrap values, showing significant genetic differences from other species.

However, this study still has limitations, mainly related to the insufficient coverage of genetic reference data and the suboptimal quality of DNA sequences from some samples. Two samples could not be specifically identified, possibly due to low sequence quality or mismatches with reference data in the database. This suggests that expanding the coverage of reference data and improving the quality of DNA sequences are necessary to increase the effectiveness of DNA barcoding.

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REFERENCES

Azevedo e Silva, F.; Brito, A. C.; Simões, T.; Pombo, A.; Sousa, J.; Venâncio, E.; and Félix, P. M. (2024). Estimating age and growth parameters for three commercial NE-Atlantic sea cucumbers, Holothuria mammata, H. forskali and H. arguinensis, in a marine protected area. *Frontiers in Marine Science*, *11*(February), 1–10. https://doi.org/10.3389/fmars.2024.1295196

Chen, J. N. S., and Justin, S. R. (2009). Regulating the Humphead Wrasse (Cheilinus undulatus) Trade in Sabah, Malaysia. *Ambio*, *38*(2), 123–125. http://www.jstor.org/stable/25515818

Guo, M., Yuan, C.; Tao, L.; Cai, Y.; and Zhang, W. (2022). Life barcoded by DNA barcodes. *Conservation Genetics Resources*, *14*(4), 351–365. https://doi.org/10.1007/s12686-022-01291-2

Harini, R.; Natarajan, V.; and Sunil, C. K. (2024). Sea cucumber significance: Drying techniques and India's comprehensive status. *Journal of Food Science*, *89*(7), 3995–4018. https://doi.org/https://doi.org/10.1111/1750-3841.17153

He, Y.; Zhao, H.; Wang, Y.; Qu, C.; Gao, X. and Miao, J. (2024). A novel deep-benthic sea cucumber species of Benthodytes (Holothuroidea, Elasipodida, Psychropotidae) and its comprehensive mitochondrial genome sequencing and evolutionary analysis. *BMC Genomics*, *25*(1), 689. https://doi.org/10.1186/s12864-024-10607-5

Hebert, P. D. N.; DeWaard, J. R.; Zakharov, E. V,; Prosser, S. W. J. and John L.S

(2013). A DNA "Barcode Blitz": Rapid digitization and sequencing of a natural history collection. In *PloS one*. journals.plos.org. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0068535

Hoareau, T. B., and Boissin, E. (2010). Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. Molecular Ecology Resources, 10(6), 960–967. https://doi.org/10.1111/j.1755-0998.2010.02848.x

Joesidawati, M. I.; Nursalim, N.; Kholilah, N.; Kurniasih, E. M.; Cahyani, N. K. D. and Ambariyanto, A. (2023). Utilizing DNA barcoding approach to study the diversity of the blue swimming crab from Tuban District, East Java, Indonesia. *Biodiversitas*, *24*(9), 4731–4737. https://doi.org/10.13057/biodiv/d240913

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

Kerr, A. M.; Janies, D. A.; Clouse, R. M.; Samyn, Y.; Kuszak, J.; and Kim, J. (2005). Molecular phylogeny of coral-reef sea cucumbers (Holothuriidae: Aspidochirotida) based on 16S mitochondrial ribosomal DNA sequence. *Marine Biotechnology (New York, N.Y.)*, *7*(1), 53–60. https://doi.org/10.1007/s10126-004-0019-y

Keskin, E. and Atar, H. H. (2013). DNA barcoding commercially important aquatic invertebrates of Turkey. *Mitochondrial DNA*, *24*(4), 440–450. https://doi.org/10.3109/19401736.2012.762576

Lizano, A. M. D.; Kim, K. M.; Juinio-Meñez, M. A. and Ravago-Gotanco, R. (2024). Pseudocryptic diversity and species boundaries in the sea cucumber Stichopus cf. horrens (Echinodermata: Stichopodidae) revealed by mitochondrial and microsatellite markers. *Scientific Reports*, *14*(1), 4886. https://doi.org/10.1038/s41598-024-54987-w

McGrath, C. (2020). Highlight: A Search for New Species on the High Seas. *Genome Biology and Evolution*, *12*(9), 1679–1680. https://doi.org/10.1093/gbe/evaa156

Pangulimang, J.; Mege, R. A.; Manampiring, N. and Ogi, N. L. I. M. (2023). Morphological Identification Of Sea Cucumber And Proximate Analysis Of The Habitat Substrate Of Mudy Sea Cucumber On Molosing Island And Biau Beach Bolaang Mongondow Regency. *Indonesian Biodiversity Journal*, *4*(3 SE-), 12–20. https://doi.org/10.53682/ibj.v4i3.7618

Pérez-Lloréns, J. L. and Mouritsen, O. G. (2024). Sea cucumber: A scavenger overexploited, traded and turned into food (even a gastronomic delicacy). *International Journal of Gastronomy and Food Science*, *37*, 100996. https://doi.org/https://doi.org/10.1016/j.ijgfs.2024.100996

Purcell, S.; Conand, C.; Uthicke, S. and Byrne, M. (2016). Ecological Roles of Exploited Sea Cucumbers. *Oceanography and Marine Biology*, *54*, 367–386. https://doi.org/10.1201/9781315368597-8

Ricklefs, R. E. (2007). History and Diversity: Explorations at the Intersection of Ecology and Evolution. *The American Naturalist*, *170*(S2), S56–S70. https://doi.org/10.1086/519402

Rothamel, E.; Borgerson, C.; Rajaona, D.; Razafindrapaoly, B. N.; Rasolofoniaina, B. J. R. and Feehan, C. (2023). Wild sea cucumber trade in rural Madagascar: Consequences for conservation and human welfare. *Conservation Science and Practice*, *5*(12), e13033. https://doi.org/https://doi.org/10.1111/csp2.13033

Sertić Kovačević, M.; Baričević, A.; Kružić, P.; Maurić Maljković, M. and Hamer, B. (2023). Barcoding (COI) Sea Cucumber Holothuria mammata Distribution Analysis: Adriatic Rare or Common Species? In *Genes* (Vol. 14, Issue 11). https://doi.org/10.3390/genes14112059

Sulardiono, B.; Hartoko, A.; Aini, A. N.; Wulandari, D. and Budiharjo, A. (2022). Genetic diversity of commercial sea cucumbers Stichopus (Echinoderm: Stichopodidae) based on DNA Barcoding in Karimunjawa, Indonesia. *Biodiversitas*, *23*(2), 922–927. https://doi.org/10.13057/biodiv/d230234

Thompson, K., and Newmaster, S. (2014). Molecular taxonomic tools provide more accurate estimates of species richness at less cost than traditional morphology-based taxonomic practices in a vegetation survey. *Biodiversity and Conservation*. https://doi.org/10.1007/s10531-014-0672-z

Trenholm, R. G.; Montgomery, E. M., Hamel, J.-F.; Rowe, S.; Gianasi, B. L. and Mercier, A. (2024). *Chapter 33 - Exploring body-size metrics in sea cucumbers through a literature review and case study of the commercial dendrochirotid Cucumaria frondosa* (A. Mercier, J.-F. Hamel, A. D. Suhrbier, & C. M. B. T.-T. W. of S. C. Pearce (eds.); pp. 521–546). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-95377-1.00040-0

Uthicke, S.; Benzie, J. A. H. and Ballment, E. (1998). Genetic structure of fissiparous populations of Holothuria (Halodeima) atra on the Great Barrier Reef. *Marine Biology*, *132*(1), 141–151. https://doi.org/10.1007/s002270050380

Wora, U. D.; Wijayanti, D. P.; Widowati, I.; Ginzel, F. I.; Nursalim, N.; Kholilah, N., and Bachtiar, M. (2024). Unveiling the DNA Barcoding of Threadfin Breams (Nemipteridae) at Oeba Fish Landing Site and Oesapa Fish Market in Kupang, East Nusa Tenggara. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences; Vol 29, No 2 (2024): Ilmu KelautanDO - 10.14710/Ik.Ijms.29.2.170-180* . https://ejournal.undip.ac.id/index.php/ijms/article/view/60388

Wulandari, N.; Majiana Krisanti, and Dewi Elfidasari. (2012). Keragaman Teripang asal Pulau Pramuka, Kepulauan Seribu Teluk Jakarta. *Unnes Life Of Science*, *1*(2), 133–139. http://journal.unnes.ac.id/sju/index.php/UnnesJLifeSci