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eDNA metabarcoding in mangrove ecosystems for fish conservation and stock assessment of *Sardinops sagax melanostictus* in the Philippines

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Abstract. *Balatero TP, Molina Z, Dalayap RM, Dechavez R, Manubag JJ, Sumaya NH, Peña JD, Tabugo SRM. 2025. eDNA metabarcoding in mangrove ecosystems for fish conservation and stock assessment of* Sardinops sagax melanostictus *in the Philippines. Biodiversitas 26: 345-355.* Mangrove forests are essential ecosystems that provide many benefits, including nursery grounds for various marine species; however, identifying juveniles is often challenging, as visual fish surveys primarily rely on the characteristics of adult specimens for accurate classification. These vital ecosystems are threatened by overfishing, habitat destruction, pollution, and climate change. Therefore, this study employs environmental DNA (eDNA) metabarcoding as a non-invasive tool to identify fish species in the mangrove ecosystems of Mindanao, Philippines. eDNA metabarcoding facilitates species detection by analyzing genetic material found in environmental samples, offering a cost-effective and sensitive method for identifying fish species. Seawater samples were collected from selected mangrove areas, and the extracted eDNA was analyzed using high-throughput next-generation sequencing (NGS). The sampling sites were South Cotabato, Surigao del Norte, Misamis Oriental, and Tawi-Tawi, which recorded 29 species across 20 families. Notable species included *Zenarchopterus dunckeri* (Zenarchopteridae), a rare fish in the aquarium hobby; *Hippocampus comes* (Syngnathidae), the tiger-tail seahorse, which is classified as Vulnerable by the IUCN; *Sardinops melanostictus* (Clupeidae), known for its high commercial value; *Siganus corallinus* (Siganidae), valued for both food and the aquarium trade; and *Gymnothorax flavimarginatus* (Muraenidae), an invasive species, that plays a vital role in the food chain as a natural predator of lionfish. Tawi-Tawi Island emerged as the most diverse site, recording species from 11 families. The results highlight the critical role of mangroves as nurseries in supporting diverse fish populations and underscore the effectiveness of eDNA metabarcoding as complementary to traditional species inventory techniques.

Keywords: eDNA, mangroves, metabarcoding, NGS, nurseries

INTRODUCTION

Mangrove forests display a variety of structures within their ecosystems, supporting rich and diverse forms of life. Coastal marine ecosystems offer numerous benefits (Costanza et al. 2017; Thomas et al. 2017; Zu et al. 2020); however, they have experienced significant levels of degradation (Smale et al. 2019) due to factors like overfishing (Duarte et al. 2020), habitat destruction, pollution, and shifts in climate (He and Silliman 2019). This deterioration is worsened by the continuous growth of human populations in coastal areas (Neumann et al. 2015).

Mangroves serve as nurseries for various marine species, including fish and shrimp, during their initial life stages (Nagelkerken et al. 2017; Romañach et al. 2018; Carrasquilla-Henao et al. 2019). They act as feeding areas, reduce the threat of predation for numerous fish species, and potentially increase the abundance of fish near coral reefs (Serafy et al. 2015). Studies have shown that the catch amount is directly linked to the extent of mangrove areas (Anneboina et al. 2017; Podda et al. 2021). The mangroves also provide plenty of food for developing juveniles contaminated with bacteria and detritus, and their dense roots help them hide from predators. As they grow, they leave the mangroves and migrate to nearby reefs. In this way, mangroves play a pivotal role in replenishing a portion of the ocean's fish population. Conducting fish inventories in these habitats poses significant challenges, particularly due to the difficulty of identifying juveniles. Traditional visual fish census methods rely heavily on adult morphological characteristics for accurate classification, making them less effective for juveniles. This limitation highlights the perils of relying solely on visual surveys and underscores the need for a complementary approach, such as eDNA metabarcoding. This advanced technique can overcome these challenges by identifying species at various life stages, offering a more comprehensive understanding of fish diversity and population dynamics within these ecosystems (Romañach et al. 2018).

Environmental DNA, often called eDNA, encompasses the genetic material in ecological samples like sediment, water, and air. This includes complete cells and extracellular DNA (Barnes and Turner 2016; Ruppert et al. 2019). The eDNA can be collected from environmental samples and subsequently preserved, extracted, amplified, sequenced, and classified according to its genetic sequence. The eDNA retrieved from water samples can be concentrated by employing filtration techniques. Specifically, utilizing a high-throughput Next-Generation Sequencing (NGS) platform enables the eDNA metabarcoding method to identify numerous species concurrently (Goldberg et al. 2016). Using PCR with broad-spectrum primers amplifies a short environmental DNA (eDNA) segment from target organisms, such as fish. Adapters and index sequences are added to the amplified fragments, enabling extensive parallel sequencing through a next-generation sequencing (NGS) platform. This process produces millions of amplicon sequences from different sampling locations. Bioinformatics analysis is then applied to process the data and identify species, resulting in a preliminary list of species for each site (Miya et al. 2015; Bautista et al. 2023).

Due to eDNA metabarcoding's low cost and excellent sensitivity for fish species detection, it has been used in a variety of aquatic research, including the lotic fish study (McDevitt et al. 2019; Bautista et al. 2023) in lentic freshwater settings (Sato et al. 2017; Fujii et al. 2019; Bautista et al. 2023), and coastal inlet environments (Zhang et al. 2020; Zou et al. 2020; Bautista et al. 2023). In most of this research, broad-spectrum metabarcoding primers like 16S Fish (Beng and Corlett, 2020; Berry et al. 2017; Bautista et al. 2023) and MiFish-U (Miya et al. 2015; Bautista et al. 2023) are used; they specifically target the fish mitochondrial genome's 12S sections. Furthermore, these studies demonstrated the ability to identify multiple fish species within each ecosystem. This study utilized environmental DNA (eDNA) metabarcoding as a non-invasive method and pioneering approach to identify fish species in remote mangrove areas as essential nursery habitats for fish in Mindanao, Philippines.

MATERIALS AND METHODS

Study area

After obtaining prior informed consent and permits from the Local Government Unit (LGU), the Bureau of Fisheries and Aquatic Resources (BFAR), and the Department of Environment and Natural Resources (DENR), researchers collected samples in mangroves and nearby areas located in South Cotabato (6.3358°N, 124.7741°E), Misamis Oriental (8.5046° N, 124.6220° E), Surigao del Norte (9.5148°N, 125.6970°E), and Tawi-Tawi (5.1338°N, 119.9509°E), Philippines (Figure 1).

Collection of water samples for eDNA

Seawater samples were collected from mangrove habitats and nearby areas. Water parameters, including temperature, pH, and salinity, were measured to assess the viability of environmental DNA (eDNA) in the ecosystem. 30 L of seawater per area (10 L per site) were collected. Samples were collected in sterile, properly labeled bottles, which were opened underwater and sealed after being filled.

After being collected, the seawater samples underwent on-site filtration using a sterile 60 mm Buchner funnel equipped with a 50 mm Polyether Sulfone (PES) membrane with a pore size of 0.22 µm. Following filtration, the membranes were carefully placed in sealed, sterile containers. During transportation, the membranes were stored in a portable cooler/icebox to prevent any degradation of eDNA. Filter membranes were immediately brought to the Molecular Systematics and Conservation Genomics Laboratory, Center for Biodiversity Studies and Conservation (CBSC), Premier Research Institute of Science and Mathematics (PRISM), MSU-IIT, for DNA extraction.

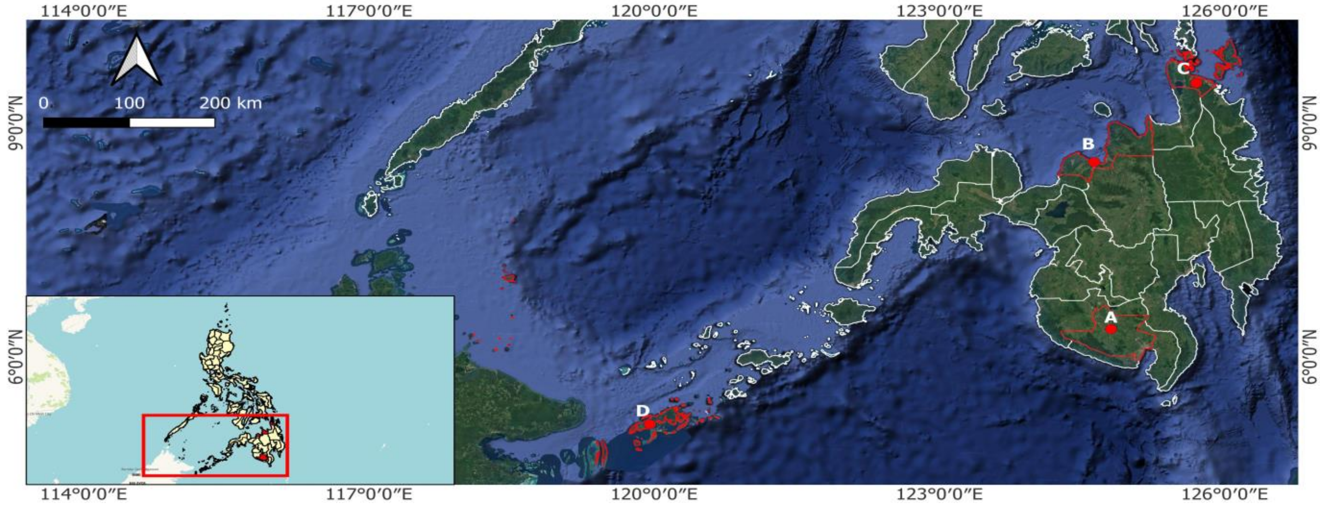

Figure 1. Sampling areas. A. South Cotabato; B. Misamis Oriental; C. Surigao del Norte; D. Tawi-Tawi Island, Philippines

DNA extraction, amplification, and MiSeq sequencing

The environmental DNA (eDNA) was extracted from the water samples using the HiPurA Water Purification Kit (HiMedia), following the manufacturer's instructions. The extracted eDNA was then evaluated through gel electrophoresis using Certified Molecular Biology Agarose gel (BIO-RAD) in a 1× TBE buffer with the Cleaver Scientific electrophoresis system (MSMINIONE). For visualization, the gels were stained with GelGreen (California, USA) 10,000× in water. The DNA samples were sent to Macrogen in Korea for Metagenome Custom Amplicon Sequencing.

After a thorough quality check, twelve amplicon libraries were produced (with triplicates for each area). However, some libraries did not successfully read fish DNA sequences and were therefore excluded from the data analysis. These libraries were produced using a custom primer set known as MiFish-U, which consists of forward primer (sequence: 5'-`GTCGGTAAAACTCGTGCCAGC`-3') and reverse primer (sequence: 5'-`CATAGTGGGGTATCTAATCCCAGTTG`-3'). This primer set was specifically designed to target various fish species' 12S mitochondrial DNA genes (as described by Miya et al. 2015; Bautista et al. 2023).

The polymerase chain reaction (PCR) followed this profile: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 98°C for 20 seconds, annealing at 65°C, extension at 72°C for 15 seconds, and a final extension at 72°C for 5 minutes. Sequencing was done on MiSeq 300bp PE (Bautista et al. 2023).

Data preprocessing and taxonomic assignment

The MitoFish pipeline version 3.89 (accessible at http://mitofish.aori.u- tokyo.ac.jp/mifish/), developed by Sato et al. (2018), was employed to preprocess and analyze the MiSeq raw reads. The initial step involved uploading the paired FASTQ files onto the pipeline. After that, these files underwent processing through FastQC (Brown et al. 2017; Bautista et al. 2023) to assess the quality of the sequences. Tail trimming was executed using SolexaQA. The paired-end reads underwent merging using the Fast Length Adjustment of Short Reads (FLASH) tool. Furthermore, any erroneous reads were subsequently eliminated from the dataset. TagCleaner (Chen et al. 2018) was implemented to remove primer sequences, allowing a maximum of 3 base mismatches. Taxonomic assignment procedures were carried out using the NCBI Basic Local Alignment Search Tool (BLAST). To consolidate the dataset, redundant sequences were merged while retaining count information. A strategy was employed to remap sequences with low read numbers (<10) onto sequences with higher read counts (>10), with a defined sequence-similarity threshold of 99%. Any sequences that did not remap were discarded (Sato et al. 2018; Bautista et al. 2023).

For sequence comparisons against a reference database, specifically the MitoFish database, Blastn searches were performed. The criteria for these searches involved identity cutoff values of 97% and an e-value threshold of 10^{-5}. The updated version (3.89, dated April 8, 2023) of the MitoFish database was utilized for accurate de novo annotations of fish mitogenomes. Subsequently, species names associated with the top-hit sequences were extracted. Molecular phylogenetic trees were constructed for each environmental sample. Multiple sequence alignments were performed using MAFFT (Katoh and Standley 2013), and neighbor-joining (NJ) phylogenetic trees were generated with Morphy. The results are compiled into an HTML report, which can also be used to calculate various ecological indices like alpha diversity, beta diversity, and correlation coefficients. This report includes links to critical databases such as FishBase, Barcode of Life, the Global Biodiversity Information Facility (GBIF), and MitoFish for further reference (Froese et al. 2012; Bautista et al. 2023).

Phylogenetic analysis

To validate the matched identities obtained from the eDNA data, a representative sequence was chosen to conduct phylogenetic inference. The multiple sequence alignment was generated using MAFFT, a tool developed by Katoh et al. (2019). To determine the most suitable evolutionary model, jModelTest 2 on XSEDE was employed; this model was selected based on criteria such as AIC, AICc, and BIC values. AIC (Akaike Information Criterion), AICc (Corrected Akaike Information Criterion), and BIC (Bayesian Information Criterion) are statistical measures used to select the best-fit model in evolutionary studies. They balance model accuracy with simplicity, penalizing overly complex models. Lower values for each criterion indicate a better-fitting model, with AICc adjusting for small sample sizes and BIC favoring simpler models more strongly.

Subsequently, Bayesian Inference was performed to infer phylogenetic relationships among the species using Mr.Bayes 3.2.2 on XSEDE Gateway v.3.3 Web Portal (www.phylo.org). The inference involved using Markov chains, with samples taken every 1000 generations. The analysis continued for a total of 100,000 generations. The resulting Markov Chain Monte Carlo (MCMC) samples were utilized to calculate Posterior Probabilities (PP) values, expressed as percentages, as a measure of confidence.

In the context of Bayesian Inference trees, Bayesian Posterior Probability values (BIPP) exceeding 0.95 were considered significant. These trees were rooted using *Clupea harengus* as the outgroup. The FigTree 1.4.0 software was utilized to visualize and make necessary edits to the obtained tree (Bautista et al. 2023).

RESULTS AND DISCUSSION

Detected important fish species based on eDNA

After post-quality control, there were a total of 76, 563 Amplicon Sequence Variants (ASVs) obtained from six successful amplicon libraries out of twelve (Table 1 and 2) and were made publicly available with SRA accession numbers SRR31745061-SRR31745066. The approach identified species belonging to 20 families comprising Zenarchopteridae, Syngnathidae, Ambassidae, Sillaginidae, Mugilidae, Bagridae, Eleotridae, Clupeidae, Cyprinidae, Pomacentridae, Siganidae, Muraenidae, Atherinidae, Cirrhitidae, Engraulidae, Apogonidae, Labridae, Lutjanidae, Balistidae, Tetraodontidae and identified 26 genera (Table 3).

The 29 recorded species inhabit various environments, ranging from marine and brackish waters to freshwater ecosystems. The species that inhabit marine ecosystems include *Doboatherina magnidentata,* first recorded in the Philippines by Bautista et al. (2023), *Hippocampus comes* (Tiger-tail seahorse), *Abudefduf sordidus* (Blackspot sergeant), *Dascyllus albisella* (Hawaiian dascyllus), *Chromis xanthochira* (Yellow axil chromis), *Amblyglyphidodon aureus* (Golden damselfish), *Chromis viridis* (Blue-green damselfish), *Siganus corallinus* (Blue-spotted spine foot), *Gymnothorax flavimarginatus* (Yellow-edged moray), *Echidna nebulosa* (Starry moray), *Cirrhitus pinnulatus* (Stocky hawkfish), *Stethojulis interrupta* (Cutribbon wrasse), *Arothron mappa* (Map puffer), *Balistapus undulatus* (Orange-lined triggerfish), and *Sillaginops macrolepis* (Large-scale sillago). On the other hand, species like *Zenarchopterus dunckeri* (Duncker's river garfish), *Ambassis urotaenia* (Banded-tail glassy perchlet), *Moolgarda engeli* (Long-finned mullet), *Lutjanus ehrenbergii* (Blackspot snapper), *Lutjanus fulvus* (Blacktail snapper), and *Ophiocara porocephala* (Northern mud gudgeon) are primarily found in marine environments. Still, they can also inhabit freshwater and brackish ecosystems. These species demonstrate high adaptability, enabling them to thrive in various habitats.

Table 1. Number of Total raw reads per amplicon library

Library	Total raw reads
Misamis Oriental (MO1)	15,309 sequences
Surigao del Norte (S1)	15,312 sequences
Surigao del Norte (S2)	15,314 sequences
South Cotabato (SC1)	15,315 sequences
Tawi-Tawi (T1)	15,315 sequences
Tawi-Tawi (T2)	15,313 sequences

Table 2. Number of total reads per fish species after quality check

Sample name/Library	Species	Total reads
South Cotabato eDNA (SC1)	*Zenarchopterus dunckeri* Mohr 1926	128
	Hippocampus comes Cantor 1849	38
	Pseudobagrus koreanus Uchida 1990	30
	Moolgarda engeli Bleeker 1858	24
	Sillaginops macrolepis Bleeker 1858	18
	Ambassis urotaenia Bleeker 1852	12
Misamis Oriental eDNA (MO1)	*Ophiocara porocephala* Valenciennes 1837	30
	Zenarchopterus dunckeri Mohr 1926	16
Surigao del Norte eDNA (S1, S2)	*Hippocampus comes* Cantor 1849	38
	Nipponocypris koreanus Uchida 1990	20
	Hippocampus comes Cantor 1849	123
	Sardinops melanostictus Temminck and Schlegel 1846	18
	Hippocampus comes Cantor 1849	31
	Phoxinus oxycephalus subsp. *jouyi* Jordan and Snyder 1901	10
Tawi-tawi eDNA (T1, T2)	*Halichoeres nebulosus* Valenciennes 1839	3943
	Cirrhitus pinnulatus Forster 1801	75
	utjanus ehrenbergii Peters 1869	81
	Engraulis encrasicolus Linnaeus 1758	46
	Abudefduf sordidus Forsskål 1775	39
	Dascyllus albisella Gill 1862	34
	Chromis xanthochira Bleeker 1851	32
	Hipposcarus longiceps Valenciennes 1840	29
	Balistapus undulatus Park 1797	24
	Amblyglyphidodon aureus Cuvier 1830	18
	Lutjanus fulvus Forster 1801	20
	Gymnothorax flavimarginatus Rüppell 1830	22
	Echidna nebulosa Ahl 1789	14
	Siganus corallinus Valenciennes 1835	22
	Carangidae sp.	12
	Ostorhinchus taeniophorus Regan 1908	12
	Abudefduf sexfasciatus Lacepède 1801	12
	Doboatherina magnidentata Sasaki, Kimura, Satapoomin and Nguyen 2019	34
	Arothron mappa Lesson 1831	24
	Chromis viridis Cuvier 1830	18
	Stethojulis interrupta Bleeker 1851	18

Table 3. The list of fish detected in Mindanao, Philippines, using Next-Generation Sequencing platform (NGS), as referenced to the MitoFish Database and GBIF

Family	Scientific name	Common name	Habitat	Threat to humans	IUCN red list status	Importance in fisheries
Zenarchopteridae	*Zenarchopterus dunckeri* Mohr 1926	Duncker's river garfish	Marine, brackish, and freshwater	Harmless	Not Evaluated	Minor commercial
Syngnathidae	*Hippocampus comes* Cantor 1849	Tiger-tail seahorse	Marine	Harmless	Vulnerable	Commercial
Cyprinidae	*Phoxinus oxycephalus* subsp. *jouyi* Jordan and Snyder 1901	Japanese minnow	Freshwater, brackish	Harmless	Not Available	Minor commercial
Ambassidae	*Ambassis urotaenia* Bleeker 1852	Banded-tail glassy perchlet	Marine, brackish, freshwater	Harmless	Least Concern	Minor commercial
Sillaginidae	*Sillaginops macrolepis* Bleeker 1858	Large-scale sillago; large-scale whiting	Marine, brackish, freshwater	Harmless	Not Evaluated	Minor commercial
Mugilidae	*Moolgarda engeli* Bleeker 1858	Long-finned mullet	Marine, brackish, freshwater	Harmless	Not Evaluated	Minor commercial
Bagridae	*Pseudobagrus koreanus* Uchida, 1990	Korean bullhead	Freshwater	Harmless	Not Evaluated	Minor commercial
Eleotridae	*Ophiocara porocephala* Valenciennes 1837	Northern mud gudgeon	Marine, brackish, freshwater	Harmless	Least Concern	Of no interest
Clupeidae	*Sardinops melanostictus* Temminck and Schlegel 1846	Japanese sardine	Marine	Harmless	Not Available	High commercial value
Pomacentridae	*Abudefduf sordidus* Forsskål, 1775	Blackspot sergeant	Marine	Harmless	Least Concern	Minor commercial
	Dascyllus albisella Gill 1862	Hawaiian dascyllus	Marine	Harmless	Not Evaluated	Minor commercial
	Chromis xanthochira Bleeker 1851	Yellow axil chromis	Marine	Harmless	Not Evaluated	Minor commercial
	Amblyglyphidodon aureus Cuvier 1830	Golden damselfish	Marine	Harmless	Least Concern	Minor commercial
	Abudefduf sexfasciatus Lacepède 1801	Scissortail sergeant	Marine	Harmless	Least Concern	Minor commercial
	Chromis viridis Cuvier 1830	Blue-green damselfish	Marine	Harmless	Not Evaluated	Minor commercial
Siganidae	*Siganus corallinus* Valenciennes 1835	Blue-spotted spine foot	Marine	Venomous	Least Concern	Minor commercial
Muraenidae	*Gymnothorax flavimarginatus* Rüppell 1830	Yellow-edged moray	Marine	Reports of ciguatera poisoning	Least Concern	Commercial
	Echidna nebulosa Ahl 1789	Starry moray	Marine	Harmless	Least Concern	Minor commercial
Atherinidae	*Doboatherina magnidentata* Sasaki, Kimura, Satapoomin and Nguyen 2019	Tropical silverside	Marine	Harmless	Not Evaluated	Minor commercial
Cirrhitidae	*Cirrhitus pinnulatus* Forster 1801	Stocky hawkfish	Marine	Harmless	Least Concern	Commercial
Engraulidae	*Engraulis encrasicolus* Linnaeus 1758	European anchovy	Marine	Harmless	Least Concern	Highly commercial
Apogonidae	*Ostorhinchus taeniophorus* Regan 1908	Reef-flat cardinalfish	Marine	Harmless	Not Evaluated	Minor commercial
Labridae	*Halichoeres nebulosus* Valenciennes 1839	Nebulous wrasse	Marine	Harmless	Least Concern	Minor commercial
	Stethojulis interrupta Bleeker 1851	Cutribbon wrasse	Marine	Harmless	Least Concern	Minor commercial
Scaridae	*Hipposcarus longiceps* Valenciennes 1840	Pacific longnose parrotfish	Marine	Harmless	Least Concern	Commercial
Lutjanidae	*Lutjanus ehrenbergii* Peters 1869	Blackspot snapper	Marine, brackish, freshwater	Harmless	Least Concern	Commercial
	Lutjanus fulvus Forster 1801	Blacktail snapper	Marine, brackish, freshwater	Reports of ciguatera poisoning	Least Concern	Commercial
Balistidae	*Balistapus undulatus* Park 1797	Orange-lined triggerfish	Marine	Traumatogenic	Not Evaluated	Commercial
Tetraodontidae	*Arothron mappa* Lesson 1831	Map puffer	Marine	Poisonous to eat	Least Concern	Minor commercial

The species recorded also present varying levels of threat to humans based on their physical characteristics or consumption risks. Most species, such as *H. comes* (Tiger-tail seahorse), *A. sordidus* (Blackspot sergeant), *D. albisella* (Hawaiian dascyllus), *C. xanthochira* (Yellow axil chromis), *A. aureus* (Golden damselfish), *C. viridis* (Blue-green damselfish), and *C. pinnulatus* (Stocky hawkfish), are classified as harmless, meaning they pose no direct threat to humans through contact or consumption. In contrast, species like *S. corallinus* (Blue-spotted spinefoot) are classified as venomous, possessing spines capable of delivering painful stings if mishandled (Bellwood et al. 2016). *G. flavimarginatus* (Yellow-edged moray) can cause ciguatera poisoning if consumed. This foodborne illness occurs from eating certain reef fish contaminated with ciguatoxins, which are toxins produced by marine dinoflagellates such as *Gambierdiscus toxicus.* Though *G. flavimarginatus* is generally non-aggressive unless provoked (Dao et al. 2020). Similarly, *A. mappa* (Map puffer) is poisonous, as it contains tetrodotoxin, a dangerous neurotoxin that can be fatal if ingested (Park et al. 2021). *B. undulatus* (Orange-lined triggerfish) poses a potential risk as it has been labeled tumorigenic, meaning its consumption could result in health complications (Wolfe et al. 2021) but more research is likely needed to confirm a definitive link between *B. undulatus* consumption and health complications.

Furthermore, under IUCN Conservation Status, species such as *A. urotaenia* (Banded-tail glassy perchlet), *O. porocephala* (Northern mud gudgeon), *Abudefduf sordidus* (Blackspot sergeant), *A. sexfasciatus* (scissortail sergeant), *Amblyglyphidodon aureus* (Golden damselfish), *S. corallinus* (blue-spotted spinefoot), *G. flavimarginatus* (Yellow-edged moray), *E. nebulosa* (Starry moray), *C. pinnulatus* (Stocky hawkfish), *Engraulis encrasicolus* (European anchovy), *Halichoeres nebulosus* (Nebulous wrasse), *S. interrupta* (cutribbon wrasse), *Hipposcarus longiceps* (pacific longnose parrotfish), *L. ehrenbergii* (Blackspot snapper), *L. fulvus* (Blacktail snapper), *A. mappa* (Map puffer) were listed as Least Concern (LC), suggesting that these species are relatively stable in the wild (Šlechtová et al. 2021). However, vulnerable species like *H. comes* (Tiger-tail seahorse), an essential species in commercial fisheries due to its use in traditional medicine and the aquarium trade (Hou et al. 2018; Foster et al. 2019; Reis et al. 2019), highlight potential conservation concerns, especially given the pressures of habitat degradation and overfishing in Southeast Asia (Pollom et al. 2021). In light of the seahorse's declining populations, their survival will depend on the region's improved biomonitoring and conservation efforts (Nester et al. 2020; Bautista et al. 2023).

Unfortunately, species such as *Z. dunckeri* and *S. macrolepis* have yet to be evaluated for conservation status, suggesting a gap in our understanding of these species' population trends and ecological roles (Zapata-Hernández et al. 2021). Several of the identified species are commercially important, contributing to local fisheries either as a major or minor component. Species like the *E. encrasicolus* (European anchovy) that is native to the Atlantic Ocean (Mutalipassi et al. 2024) and *S. melanostictus* (Japanese sardine) are categorized as highly commercial, forming a vital part of marine resource-based livelihoods (Southwick et al. 2018; De Carvalho et al. 2020). Minor commercial species, such as the *A. sordidus* (Blackspot sergeant) and *A. sexfasciatus* (Scissortail sergeant), etc. play a less significant but still notable role in local fisheries (Vanderklift et al. 2021). Data shows that commercially important species are often listed as Least Concern (LC) by the IUCN, indicating that current exploitation levels may be sustainable. However, continuous monitoring is crucial to ensure that overfishing or environmental changes do not negatively impact their populations. The ecological roles of these species are varied.

According to Hirota et al. (2015), *Z. dunckeri* (Duncker's river garfish) has a highly developed lateral line system, which enhances its ability to feed at the water's surface. This adaptation underscores the species' specialized ecological role in its habitat, allowing it to detect prey in shallow waters and strengthening its role as a critical insect predator in estuarine ecosystems. The *A. sexfasciatus* (Scissortail sergeant) contributes to the stability of coral reef ecosystems by grazing on algae, helping to control algal populations and promote healthy coral growth (Kano et al. 2011). *H. comes* (Tiger-tail seahorse) serves as a predator of small crustaceans, helping maintain ecological balance in reef habitats (Lourie et al. 2016). Also, herbivorous species like the *S. corallinus* (Blue-spotted spinefoot) and *H. longiceps* (Pacific longnose parrotfish) play a crucial role in controlling algal growth on coral reefs (Cruz-Torres and Reyes-Bonilla 2020; Munday and Jones 2020), while predatory species such as the *B. undulatus* (Orange-lined triggerfish) and *Lutjanus fulvus* (Blacktail snapper) help maintain the balance of marine food webs (Friedlander and Parrish 2020) *H. nebulosus* (Nebulous wrasse) plays a significant role in coral reef cleaning by consuming parasites and dead tissues, which helps maintain reef health (Eschmeyer et al. 2017). The structural complexity of mangroves, combined with their proximity to shallow and brackish waters, provides an ideal refuge for various marine organisms (Sun et al. 2016; Enochs and Glynn 2017). This unique environmental setting fosters higher species diversity, as reflected in biodiversity indices like Shannon, Simpson, and Chao1 (Figure 2), which indicates a rich and diverse fish community in these mangrove areas. Tables 2 and 3 show the number of raw reads from successful eDNA libraries generated and the number of clean reads for each fish species after the quality check.

Moreover, the seawater samples collected revealed a diverse range of fish species, with Tawi-Tawi showing the highest relative abundance (Figure 2.A). This pattern is consistent with species diversity indices (Shannon, Simpson, Chao1) (Figures 2.B, 2.C, and 2.D), which indicate that Tawi-Tawi hosts the largest fish assemblage in the region. This may be attributed to the site's specific location, as Tawi-Tawi has a rich diversity of fish species in its mangrove ecosystems, which can be linked to the abundant shelter and food resources these habitats provide. These resources are essential for the survival and growth of both juvenile and adult fish, playing a vital role in sustaining the region's overall biodiversity (Babcock et al. 2019; Serag et al. 2024).

In addition, as seen in Figure 3, *H. nebulosus* and *A. mappa* show high relative abundance (represented by the red nodes), indicating that these species are more dominant in the Tawi-Tawi samples. This could be related to their ecological adaptability and the favorable conditions of Tawi-Tawi's mangrove habitats. Other species, such as *Lutjanus ehrenbergii* and *C. viridis*, which have green nodes, indicate lower relative abundance (<1%). These species might be present in fewer numbers due to specific habitat preferences or resource competition. The phylogenetic tree corroborates the species richness and diversity suggested by biodiversity indices like Shannon, Simpson, and Chao1 (Figure 2). The clustering of species shows clear distinctions in abundance, reflecting a well-structured ecosystem with both dominant and rare species coexisting. Furthermore, this data highlights the rich biodiversity of the Mindanao, Philippines' mangroves and reef ecosystems. It also underscores the need for a balanced conservation and fisheries management approach to preserve biodiversity and local communities' livelihoods.

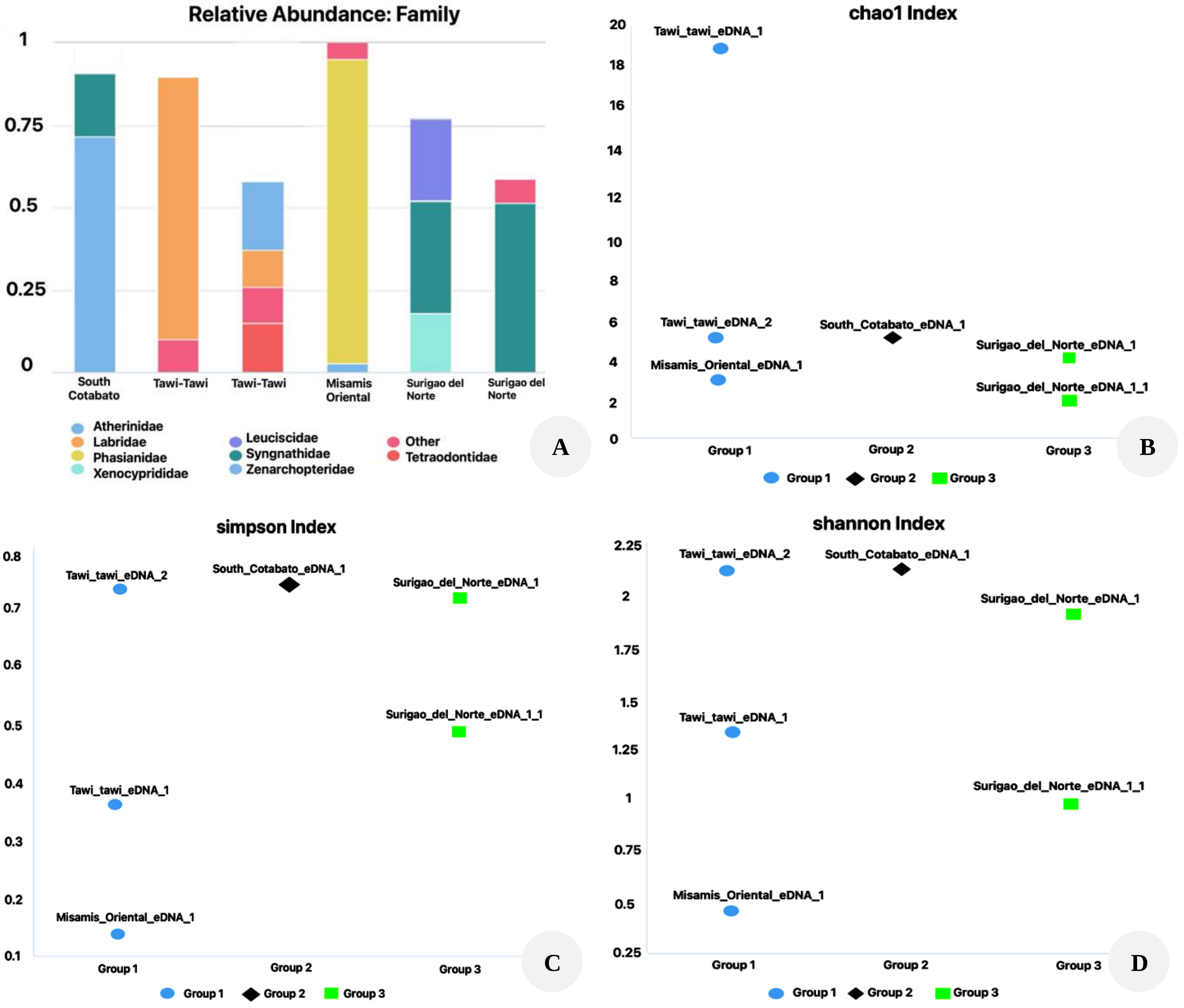

Figure 2. Relative abundance and Biodiversity indices of fish eDNA from the water samples of Mindanao, Philippines. A. Relative abundance; B. Chao1 index; C. Simpson index; D. Shannon Index. All indices were analyzed and visualized from the MiFish pipeline (http://mitofish.aori.u-tokyo.ac.jp/mifish/)

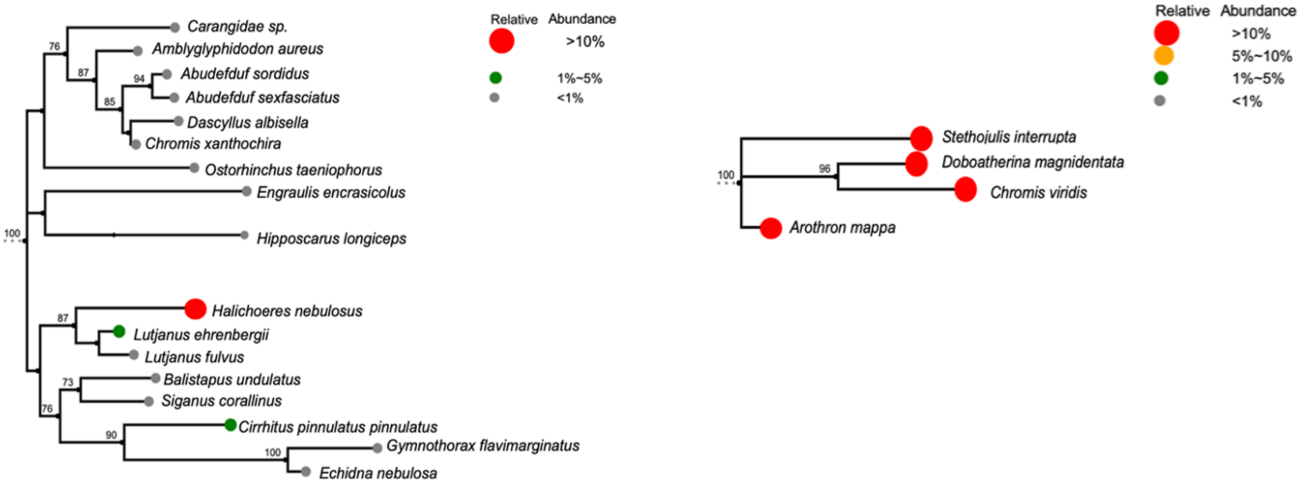

Figure 3. Neighbor-joining (NJ) phylogenetic tree constructed after multiple sequence alignment with MAFFT for Tawi-Tawi eDNA samples, analyzed and visualized from the MiFish pipeline (http://mitofish.aori.u-tokyo.ac.jp/mifish/)

Furthermore, based on eDNA signatures, *S. melanostictus* (Japanese sardine) was detected, marking what may be the first record of this species in the Philippines, but it needs further confirmation. This discovery helps clarify uncertainties regarding its distribution and taxonomy. According to the World Register of Marine Species (WORMS), this species belongs to the family Alosidae of the order Clupeiformes. Initially classified as *Clupea sagax*, its accepted name became *Sardinops sagax* Jenys 1842. *S. melanostictus* was considered a synonym and later recognized as a subspecies; however, in the Global Biodiversity Information Facility (GBIF) database, it is placed in the family Clupeidae.

The Japanese sardine is distinguished by its elongated, cylindrical body, ventral operculum with evident bony striae, and characteristic blackish-blue to greenish hues. It plays an essential ecological role in marine ecosystems, primarily in the waters of East Asia, including the Pacific Ocean, the Sea of Japan, and the East China Sea. Sardine catches have fluctuated over the last 50 years, peaking in the 1980s before collapsing in the early 1990s. These fluctuations have sparked significant research into their migration, biology, and fisheries, particularly in the Sea of Japan and the northern Pacific Ocean. Over the years, various scientific publications have focused on specific research topics related to the Japanese sardine; however, finding recent comprehensive studies that consolidate all current knowledge about this species in the scientific literature remains challenging, emphasizing the need for updated research on these fish. Their short lifespan and low position in the food web make gaining knowledge on reproductive strategy pertinent because sardines can produce large quantities of eggs during an extended spawning season. They frequent the Pacific waters and are distributed in the northwestern Pacific Ocean, central Pacific, and especially in the Bering Sea, where they are in high abundance. They have been recorded in the coastal waters of Japan, Chinese waters, and Korea’s Japanese and Yellow Sea coasts.

In Russia, they were recorded in the Gulf of Tartary, Sakhalin, and in and near the eastern shore of the Kamchatka Peninsula Kronotzki Cape (Sarr et al. 2021). Data mining from the Global Biodiversity Information Facility (GBIF) reveals that based on fish collections from the National Museum of Nature and Science, most tagged locations for *S. melanostictus* come from Japan. The earliest recorded specimens date back to 1825 from New Zealand and August 1896 from Japan. The 1825 fish specimens from New Zealand are with the fish collection (IC) of the Muséum National d' Histoire Naturelle (MNHN-Paris). Meanwhile, the 1896 fish specimens are housed in the Department of Zoology at the National Museum of Nature and Science in Japan.

In the Philippines, sardines are one of the most commercially important species, making up a substantial proportion of the fish catch, and are considered an accessible source of animal protein for most Filipinos. In 2005, the combined production of two sardine species, the fimbriated sardine (*Sardinella fimbriata*) and the Bali sardine (*Sardinella lemuru*), was recorded at approximately 331,298 metric tons. This production was valued at around USD 146,300,000 (PHP 8.06 billion) based on the exchange rates at the time, according to data from the Bureau of Agricultural Statistics (BAS) (Willette et al. 2011). Published literature on the exact number of sardine species recorded in the country must be precise. Herre (1953) listed nine species of sardines (*Sardinella aurita, Sardinella gibbosa, Sardinella longiceps, Sardinella melanura, Sardinella samarensis, Sardinella sindensis, Sardinella brachysoma, Sardinella fimbriata,* and *Sardinella sirm*). Whitehead (1985) also reported nine species, with another five in adjacent water bodies, such as Sulawesi and South China. Meanwhile, Conlu (1986) reported seven species: *S. brachysoma, S. fimbriata, S. longiceps, S. melanura, S. samarensis, S. sinensis,* and *S. sagax* (Peruvian pacific sardine). Only one species (*S. fimbriata*) is corroborated across the three reports, whereas other inclusions do not have ranges that extend to the Philippines or are found exclusively in different oceans. Sardines have many local names including lao-lao, manamsi, tamban, tunsoy, turay, and tabagak (Willette et al. 2011). The International Union for Conservation of Nature (IUCN) has assessed and listed the Japanese sardine (*Sardinops melanostictus*) as Not Evaluated (NE); however, several studies have affirmed that this fish stock is slightly overfished (Sarr et al. 2021).

To confirm the eDNA identification, a Bayesian phylogenetic analysis was conducted using the TIM2+G model, as determined by jModelTest2. The resulting phylogenetic tree, with high posterior probabilities, verified the identity of *S. melanostictus* and its very high similarity to *S. sagax* (Figure 4). The length of the branches in the tree can indicate the extent of evolutionary change over time, but it does not necessarily reflect genetic distance directly. This supports findings that state that S. *melanostictus* is a subspecies of *S. sagax*. It is noted that *Sardinops*, in the Clupeidae family, is widely distributed in the Indo-Pacific and East Pacific Oceans and is known by various common names depending on the subspecies, such as the Australian pilchard (*Sardinops sagax* subsp. *neopilchardus*), Californian pilchard (*Sardinops sagax* subsp. *caeruleus*), Peruvian Pacific sardine (*Sardinops sagax* subsp. *sagax*), South American pilchard, Chilean sardine (*Sardinops sagax* subsp. *sagax*), Japanese pilchard (*Sardinops sagax* subsp. *melanostictus*), and Southern African pilchard (*Sardinops sagax* subsp. *ocellatus*). This confirms several studies stating that *Sardinops* species range from southern Africa to the eastern Pacific in the Indo-Pacific. Through cluster and parsimony analyses of haplotypic divergences, three distinct lineages have been confirmed: the south African (*S. sagax* subsp. *ocellatus*) and Australian lineages (*S. sagax* subsp. *neopilchardus*); the Chilean (*S. sagax* subsp. *sagax*) and Californian lineages (*S. sagax* subsp. *caeruleus*); and the Japanese lineage (*S. sagax* subsp. *melanostictus*) (Sarr et al. 2021; Froese and Pauly 2024). In general, this information contributes to the knowledge of *Sardinop's* taxonomy. These findings are essential for informing conservation efforts and ensuring sustainable management.

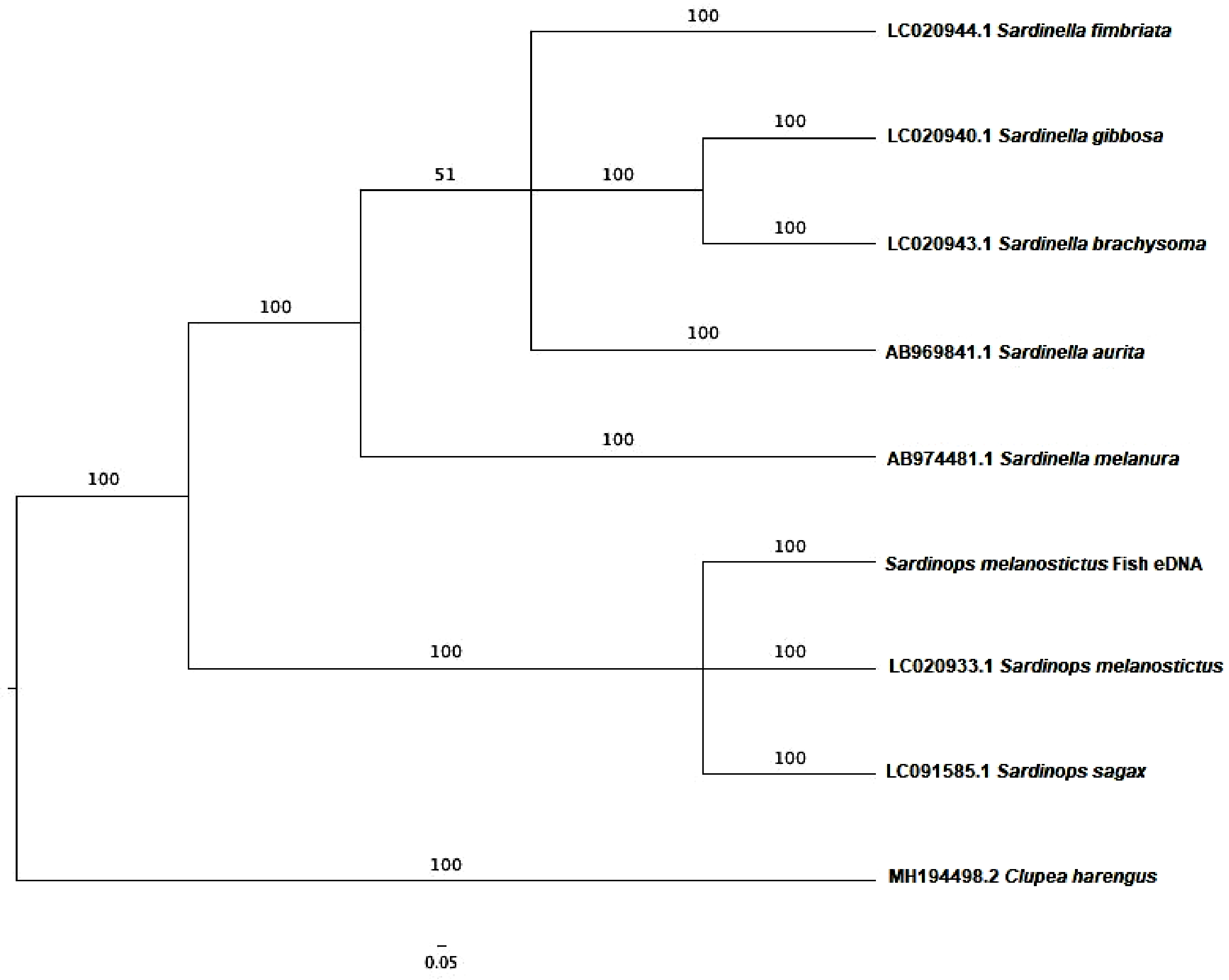

Figure 4. Phylogenetic consensus tree based on Bayesian inference analysis for the *S. melanostictus* fish species detected from eDNA (Fish_eDNA) on Hinatuan Island, Surigao del Norte, Philippines; Bayesian posterior probabilities are expressed as percent probabilities in nodes; *Clupea harengus* (outgroup)

In conclusion, this study demonstrates eDNA technology as a promising approach to complement traditional methods for species detection for future studies. It successfully identified 29 species belonging to 20 families within the mangrove ecosystems serving as nursery grounds of Mindanao, Philippines, demonstrating the ecological richness of these environments. Notable species were *Z. dunckeri* (Zenarchopteridae), a rare fish in the aquarium hobby; *H. comes* (Syngnathidae), the tiger-tail seahorse with conservation status of vulnerable by IUCN; *S. melanostictus* (Clupeidae), being of high commercial value; *S. corallinus* (Siganidae), noted for food and aquarium trade and *G. flavimarginatus* (Muraenidae), which plays a vital role in the food chain as a natural predator to the lionfish (an invasive species). Tawi-Tawi Island was considered the most diverse site, recording species from 11 families. The results highlight the critical role of mangrove nurseries in supporting diverse fish populations and underscore the effectiveness of eDNA metabarcoding in providing an inventory of species.

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REFERENCES

Anneboina LR, Kumar KK. 2017. Economic analysis of mangrove and marine fishery linkages in India. Ecosyst Serv 24: 114-123. DOI: 10.1016/j.ecoser.2017.02.004.

Babcock RC, Bustamante RH, Fulton EA, Fulton DJ, Haywood MD, Hobday AJ, Vanderklift MA. 2019. Severe continental-scale impacts of climate change are happening now: Extreme climate events impact marine habitats, forming communities along 45% of Australia's coast. Front Mar Sci 6: 466674. DOI: 10.3389/fmars.2019.00411.

Barnes MA, Turner CR. 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv Genet 17 (1): 1-17. DOI: 10.1007/s10592-015-0775-4.

Bautista JA, Manubag JJ, Sumaya NH, Martinez JG, Tabugo SR. 2023. Environmental DNA (eDNA) metabarcoding and fish visual census reveals the first record of *Doboatherina magnidentata* in the Philippines. Biodiversitas 24: 3063-3072. DOI: 10.13057/biodiv/d240562.

Bellwood DR, Goatley CH, Khan JA, Tebbett SB. 2016. Site fidelity and homing in juvenile rabbitfishes (Siganidae). Coral Reefs 35: 1151-1155. DOI: 10.1007/s00338-016-1466-4.

Beng KC, Corlett RT. 2020. Environmental DNA (eDNA) applications in ecology and conservation: Opportunities, challenges, and prospects. Biodivers Conserv 29 (7): 2089-2121. DOI: 10.1007/s10531-020-01980-0.

Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, Stat M, Bejder L, Bunce M. 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). Ecol Evol 7 (14): 5435-5453. DOI: 10.1002/ece3.3123.

Brown J, Pirrung M, McCue LA. 2017. FQC Dashboard: Integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinform 33 (19): 3137-3139. DOI: 10.1093/bioinformatics/btx373.

Carrasquilla-Henao M, Ban N, Rueda M, Juanes F. 2019. The mangrove-fishery relationship: A local ecological knowledge perspective. Mar Pol 108: 103656. DOI: 10.1016/j.marpol.2019.103656.

Chen Y, Chen Y, Shi C, Huang Z, Zhang Y, Li S, Chen Q. 2018. SOAPnuke: A map reduce acceleration-supported software for integrated quality control and preprocessing of high-throughput sequencing data. Gigascience 7 (1): gix120. DOI: 10.1093/gigascience/gix120.

Conlu PV. 1986. Guide to Philippine flora and fauna. IX. Manila, Philippines: National Resources Management Center, Ministry of Natural Resources, and University of the Philippines.

Costanza R, De Groot R, Braat L, Kubiszewski I, Fioramonti L, Sutton P, Grasso M. 2017. Twenty years of ecosystem services: How far have we come, and how far do we still need to go?. Ecosyst Serv 28: 1-16. DOI: 10.1016/j.ecoser.2017.09.008.

Cruz-Torres J, Reyes-Bonilla H. 2020. Herbivory by reef fishes and its importance in maintaining coral reef ecosystems: A review. Mar Ecol Prog Ser 644: 151-164. DOI: 10.3354/meps13277.

Dao HV, Pham KX, Hoang BX, Tanioka M, Watanabe R, Suzuki T. 2020. Occurrence of tetrodotoxin in three *Nassarius* gastropod species in Khanh Hoa Province, Vietnam. Fish Sci 86: 181-186. DOI: 10.1007/s12562-019-01375-3.

De Carvalho BM, Ferreira Junior AL, Fávaro LF, Artoni RF, Vitule J. 2020. Human-facilitated dispersal of the Gulf toadfish *Opsanus beta* (Goode & Bean, 1880) in Guaratuba Bay, south-eastern Brazil. J Fish Biol 97 (3): 686-690. DOI: 10.1111/jfb.14421.

Duarte CM, Agusti S, Barbier E, Britten GL, Castilla JC, Gattuso JP, Worm B. 2020. We are rebuilding marine life. Nature 580 (7801): 39-51. DOI: 10.1038/s41586-020-2146-7.

Enochs IC, Glynn PW. 2017. Trophodynamics of eastern Pacific coral reefs. In: Glynn P, Manzello D, Enochs I (eds). Coral Reefs of The Eastern Tropical Pacific: Persistence And Loss in A Dynamic Environment. Springer, Dordrecht. DOI: 10.1007/978-94-017-7499-4_9.

Eschmeyer WN, Fricke R, Van der Laan R. 2017. Catalogue of Fishes: Genera, Species, References. Zootaxa, New Zealand. DOI: 10.11646/zootaxa.3882.1.1.

Foster SJ, Kuo TC, Wan AKY, Vincent AC. 2019. Global seahorse trade defies export bans under CITES action and national legislation. Mar Pol 103: 33-41. DOI: 10.1016/j.marpol.2019.01.014.

Friedlander AM, Parrish JD. 2020. The role of predation in reef fish assemblages: Implications for fisheries management. Ecol Appl 30 (5): e02138. DOI: 10.1002/eap.2138.

Froese R, Pauly D. 2024. FishBase. *Sardinops sagax* (Jenyns, 1842). World Register of Marine Species. https://www.marinespecies.org/aphia.php?p=taxdetails&id=217452 on 2024-10-03

Froese R, Zeller D, Kleisner K, Pauly D. 2012. What catch data can tell us about the status of global fisheries. Mar Biol 159: 1283-1292. DOI: 10.1007/s00227-012-1909-6.

Fujii K, Doi H, Matsuoka S, Nagano M, Sato H, Yamanaka H. 2019. Environmental DNA metabarcoding for fish community analysis in backwater lakes: A comparison of capture methods. PLoS One 14 (1): e0210357. DOI: 10.1371/journal.pone.0210357.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Taberlet P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7 (11): 1299-1307. DOI: 10.1111/2041-210X.12595.

He Q, Silliman BR. 2019. Climate change, human impacts, and coastal ecosystems in the Anthropocene. Curr Biol 29 (19): R1021-R1035. DOI: 10.1016/j.cub.2019.08.042.

Herre AW. 1953. Check list of Philippine fishes. 20. US Government Printing Office.

Hirota K, Asaoka R, Nakae M, Sasaki K. 2015. The lateral line system and its innervation in *Zenarchopterus dunckeri* (Beloniformes: Exocoetoidei: Zenarchopteridae). Ichtiol Res 62: 286-292. DOI: 10.1007/s10228-014-0442-7.

Hou F, Wen L, Peng C, Guo J. 2018. Identification of marine traditional Chinese medicine dried seahorses in the traditional Chinese medicine market using DNA barcoding. Mitochondrial DNA A: DNA Mapping Seq Anal 29 (1): 107-112. DOI: 10.1080/24701394.2016.1248430.

Kano Y, Ohnishi K, Tomida Y, Ikeda N, Iwawaki N, Miyagawa M, Watanabe K. 2011. Fluctuation and variation in stream-fish assemblages after a catastrophic flood in the Miyagawa River, Japan. Environ Biol Fish 92: 447-460. DOI: 10.1007/s10641-011-9861-8.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: Multiple sequence alignment, interactive sequence choice, and visualization. Brief Bioinform 20 (4): 1160-1166. DOI: 10.1093/bib/bbx108.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol 30 (4): 772-780. DOI: 10.1093/molbev/mst010.

Lourie SA, Pollom RA, Foster SJ. 2016. A global revision of the seahorse's *Hippocampus* Rafinesque 1810 (Actinopterygii: Syngnathiformes): Taxonomy and biogeography with recommendations for further research. Zootaxa 4146 (1): 1-66. DOI: 10.11646/zootaxa.4146.1.1.

McDevitt AD, Sales NG, Browett SS, Sparnenn AO, Mariani S, Wangensteen OS, Benvenuto C. 2019. Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals. J Fish Biol 9 5(2): 679-682. DOI: 10.1111/jfb.14053.

Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Iwasaki W. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. R Soc Open Sci 2 (7): 150088. DOI: 10.1098/rsos.150088.

Munday PL, Jones GP. 2020. Parrotfish: The key to coral reef health. Trends Ecol Evol 35 (7): 610-622. DOI: 10.1016/j.tree.2020.02.013.

Mutalipassi M, D'Anza E, Pugliano M, Firmamento R, Murano C, Ruocco N. 2024. Casting light on the European anchovy: From biology to conservation and industry. Front Ecol Evol 12: 1352055. DOI: 10.3389/fevo.2024.1352055.

Nagelkerken I, Huebert KB, Serafy JE, Grol MGG, Dorenbosch M, Bradshaw CJA. 2017. Highly localized replenishment of coral reef fish populations near nursery habitats. Mar Ecol Prog Ser 568: 137-150. DOI: 10.3354/meps12062.

Nester GM, De Brauwer M, Koziol A, West KM, DiBattista JD, White NE, Power M, Heydenrych MJ, Harvey E, Bunce M. 2020. Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (Family: Syngnathidae). Environ DNA 2 (4): 614-626. DOI: 10.1002/edn3.93.

Neumann B, Vafeidis AT, Zimmermann J, Nicholls RJ. 2015. Future coastal population growth and exposure to sea-level rise and coastal flooding: A global assessment. PLoS One 10 (3): e0118571. DOI: 10.1371/journal.pone.0118571.

Park BK, Jang WJ, Park KH, Lee HS, Lee WO, Choi KS, Lee J. 2021. Tetrodotoxin and its analogues: A review of analysis methods and levels in pufferfish. J Food Hyg Saf 36 (2): 105-117. DOI: 10.13103/JFHS.2021.36.2.105.

Podda C, Palmas F, Pusceddu A, Sabatini A. 2021. Hard times for catadromous fish: The case of the European eel (*Anguilla anguilla*, L. 1758). Adv Oceanogr Limnol 12: 47-64. DOI: 10.4081/aiol.2021.9997.

Pollom RA, Ralph GM, Pollock CM, Vincent AC. 2021. Global extinction risk for seahorses, pipefishes, and their near relatives (Syngnathiformes). Oryx 55 (4): 497-506. DOI: 10.1017/S0030605320000782.

Reis V, Hermoso V, Hamilton SK, Bunn SE, Fluet-Chouinard E, Venables B, Linke S. 2019. Characterizing seasonal dynamics of Amazonian wetlands for conservation and decision making. Aquat Conserv Mar Freshw Ecosyst 29: 1073-1082. DOI: 10.1002/aqc.3051.

Romañach SS, DeAngelis DL, Koh HL, Li Y, Barizan RSR, Zhai L. 2018. Conservation and restoration of mangroves: Global status, perspectives, and prognosis. Ocean Coast Manag 154: 72-82. DOI: 10.1016/j.ocecoaman.2018.01.009.

Ruppert KM, Kline RJ, Rahman MS. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Glob Ecol Conserv 17: e00547. DOI: 10.1016/j.gecco.2019.e00547.

Sato H, Sogo Y, Doi H, Yamanaka H. 2017. Usefulness and limitations of sample pooling for environmental DNA metabarcoding of freshwater fish communities. Sci Rep 7: 14860. DOI: 10.1038/s41598-017-14978-6.

Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W. 2018. MitoFish and MiFish pipeline: A mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. Mol Biol Evol 35 (6): 1553-1555. DOI: 10.1093/molbev/msy074.

Sarr O, Kindong R, Tian, S. 2021. Knowledge on the biological and fisheries aspects of the Japanese sardine, *Sardinops melanostictus* (Schlegel, 1846). J Mar Sci Eng 9: 1403. DOI: 10.3390/jmse9121403.

Serafy JE, Shideler GS, Araújo RJ, Nagelkerken I. 2015. Mangroves enhance reef fish abundance at the Caribbean regional scale. PLoS One 10 (11): e0142022. DOI: 10.1371/journal.pone.0142022.

Serag KJ, Elloran C, Serag K. 2024. Tawi Tawi, Reef Fishes, Philippines. Version 1.21. ASEAN Centre for Biodiversity (ACB). Occurrence dataset. https://www.gbif.org/dataset/7f30b6c4-bdd7-4dc0-b32e-699a8d96dc47/project. DOI: 10.15468/nvy0u9.

Šlechtová V, Musilova Z, Tan HH, Kottelat M, Bohlen J. 2021. One northward, one southward: Contrasting biogeographical history in two benthic freshwater fish genera across Southeast Asia (Teleostei: Cobitoidea: *Nemacheilus*, *Pangio*). Mol Phylogenet Evol 161: 107139. DOI: 10.1016/j.ympev.2021.107139.

Smale DA, Wernberg T, Oliver EC, Thomsen M, Harvey BP, Straub SC, Moore PJ. 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Chang 9 (4): 306-312. DOI: 10.1038/s41558-019-0412-1.

Southwick R, Holdsworth JC, Rea T, Bragg L, Allen T. 2018. Estimating marine recreational fishing's economic contributions in New Zealand. Fish Res 208: 116-123. DOI: 10.1016/j.fishres.2018.06.016.

Sun D, Cheney KL, Werminghausen J, McClure EC, Meekan MG, McCormick MI, Grutter AS. 2016. Cleaner wrasse influences habitat selection of young damselfish. Coral Reefs 35: 427-436. DOI: 10.1007/s00338-015-1391-y.

Thomas N, Lucas R, Bunting P, Hardy A, Rosenqvist A, Simard M. 2017. Distribution and drivers of global mangrove forest change, 1996-2010. PLoS One 12: e0179302. DOI: 10.1371/journal.pone.0179302.

Vanderklift MA, Pillans RD, Hutton M, De Wever L, Kendrick GA, Zavala-Perez A, Howard A. 2021. High herbivory rates in remote northwest Australian seagrass meadows by rabbitfish and green turtles. Mar Ecol Prog Ser 665: 63-73. DOI: 10.3354/meps13657.

Whitehead PJ. 1985. FAO species catalogue. 7. Clupeoid fishes of the world (Suborder Clupeoidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies, and wolf-herrings. Part 1. Chirocentridae, Clupeidae & Pristigasteridae.

Willette DA, Bognot ED, Mutia M, Santos MD. 2011. Biology and ecology of sardines in the Philippines: A review. BFAR Tech Pap Ser 13: 1-20.

Wolfe K, Kenyon TM, Mumby PJ. 2021. The biology and ecology of coral rubble and implications for the future of coral reefs. Coral Reefs 40 (6): 1769-1806. DOI: 10.1007/s00338-021-02185-9.

Zapata-Hernández G, Sellanes J, Letourneur Y, Harrod C, Morales NA, Plaza P, Gaymer CF. 2021. Tracing trophic pathways through the marine ecosystem of Rapa Nui (Easter Island). Aquat Conserv Mar Freshw Ecosyst 31 (2): 304-323. DOI: 10.1002/aqc.3500.

Zhang Y, Pavlovska M, Stoica E, Prekrasna I, Yang J, Slobodnik J, Dykyi E. 2020. Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to marine mammals. Environ Intl 135: 105307. DOI: 10.1016/j.envint.2019.105307.

Zou K, Chen J, Ruan H, Li Z, Guo W, Li M, Liu L. 2020. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. Sci Total Environ 702: 134704. DOI: 10.1016/j.scitotenv.2019.134704.

Zu EPS, Mukherjee N, Worthington TA et al. 2020. Fishers who rely on mangroves: Modelling and mapping the global intensity of mangrove-associated fisheries. Estuar Coast Shelf Sci 248: 107159. DOI: 10.1016/j.ecss.2020.106975.