

REVIEW OPEN ACCESS

Understanding the Current Use of Environmental DNA in Southeast Asia: Promoting Accessibility Through Networking and Capacity Building

Genetic Reference Gaps Limit eDNA Metabarcoding and Biodiversity Monitoring of Tropical Mangrove Ecosystems

Earl Kevin T. Cooper¹ | Jessah P. Trillo^{1,2}  | Erwin Jones A. Tucong^{1,3}  | Abner C. Evasco Jr.¹  | Christian Jay R. Nob¹  | Bernadeth Grace S. Pananganan^{4,5}  | Venus E. Leopardas^{1,3}  | Jerpe E. Honghong Jr.¹  | Phresnofi N. Don¹ | Allyn Duvin P. Hinoguin^{1,3} | Nonillon M. Aspe^{1,6}  | Ruby C. Gonzales^{1,3} | Roger G. Dolorosa⁷ | Mohammad Basyuni^{8,9} | Tadashi Kajita^{4,5}

¹Environmental DNA Research and Development Center, Mindanao State University at Naawan, Naawan, Misamis Oriental, Philippines | ²Department of Science and Technology-Science Education Institute, Taguig, Metro Manila, Philippines | ³College of Fisheries and Marine Sciences, Mindanao State University at Naawan, Naawan, Misamis Oriental, Philippines | ⁴United Graduate School of Agricultural Sciences, Kagoshima University, Kagoshima, Japan | ⁵Iriomote Station, Tropical Biosphere Research Center, University of the Ryukyus, Taketomi-cho, Yaeyama-gun, Okinawa, Japan | ⁶College of Environment and Life Sciences, Mindanao State University at Naawan, Naawan, Misamis Oriental, Philippines | ⁷College of Fisheries and Natural Sciences, Western Philippines University – Puerto Princesa Campus, Puerto Princesa City, Palawan, Philippines | ⁸Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan, Indonesia | ⁹Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia

Correspondence: Venus E. Leopardas (venus.leopardas@msunaawan.edu.ph) | Tadashi Kajita (tkaji@cs.u-ryukyu.ac.jp)

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ABSTRACT

Mangroves support high faunal diversity and provide essential ecological services. However, biodiversity assessments in these habitats remain constrained by their structural complexity, resulting in limited accessibility, low visibility, and persistent taxonomic gaps. Rapid advances in molecular techniques use environmental DNA (eDNA) metabarcoding as an emerging, powerful alternative to conventional surveys. However, its effectiveness in tropical systems, such as Philippine mangroves, is constrained by gaps in publicly available genetic reference data, particularly for invertebrates. To address this, we systematically reviewed 48 years (1977–2025) of literature records of Philippine mangrove-associated fauna and assessed their representation in public genetic databases to address three questions: (a) What is the current state of faunal biodiversity in Philippine mangroves? (b) How complete are public genetic reference libraries for eDNA applications? and (c) How can integrating eDNA with conventional surveys enhance ecological monitoring? We outlined a workflow for literature screening, taxonomic standardization, and programmatic retrieval and quality-filtering of GenBank accessions. Across 999 species spanning nine phyla, the ray-finned fishes (40.4%) dominated the records, followed by gastropods (21.7%) and bivalves (16.5%). Only 70.27% of species had at least one mitochondrial marker, dominated by COI (89.03%), followed by 16S (70.23%), 12S (60.97%), and Cytb (2.45%). Coverage was particularly low for invertebrates and some commercially important conservation-relevant fishes, limiting species-level detection in eDNA metabarcoding. Nevertheless, integrating eDNA with traditional methods increases the detection of cryptic, rare, juvenile, and hard-to-sample taxa, enabling more comprehensive biodiversity assessments. This combined approach enhances the capacity for spatial and temporal monitoring and supports more informed conservation strategies. Additionally, these data have the potential to enhance faunal assessments and strengthen biodiversity management practices. Overall, this review underscores the need to expand locally curated, voucher-linked reference databases through targeted sequencing and highlights how national and regional partnerships can improve the accuracy and conservation relevance of eDNA-based biodiversity monitoring.

Earl Kevin T. Cooper, Jessah P. Trillo and Erwin Jones A. Tucong contributed equally to this work and are designated as co-first authors.

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1 | Introduction

Mangrove forests provide essential ecosystem services, such as shoreline protection, carbon sequestration, and habitat and breeding ground for numerous species of flora and fauna and serve as critical breeding and nursery grounds for many marine organisms, such as invertebrates, fish, and birds, thereby supporting local and global biodiversity (Honda et al. 2013). The Philippines, with an extensive coastline and rich marine biodiversity, has one of the most diverse mangrove ecosystems in the world (Long and Giri 2011). However, mangrove ecosystems in the Philippines are undergoing rapid change. Deforestation, coastal development, aquaculture expansion, pollution, and climate change have contributed to habitat degradation and biodiversity loss in many regions of the country. The cumulative effects of these natural and man-made disturbances reduce stability and biodiversity in the mangrove ecosystem, which in turn disrupt the ecological, social, and economic functions (Dayal et al. 2022). Similar patterns of mangrove decline are observed globally, highlighting the vulnerability of these ecosystems under increasing environmental pressures. Despite their ecological and socio-economic importance, many components of mangrove biodiversity in the Philippines remain insufficiently documented, particularly at the genetic level. Although the country hosts one of the most extensive mangrove areas in Southeast Asia, associated mangrove faunal communities remain comparatively understudied due to logistical challenges in accessing mangrove habitats, taxonomic limitations for many invertebrate groups, a historical research bias toward coral reef ecosystems, and a long-standing emphasis on applied mangrove rehabilitation and management rather than baseline biodiversity assessments. As a result, many components of mangrove biodiversity in the Philippines remain insufficiently documented, particularly at the genetic level. The lack of comprehensive and accessible genetic reference data further limits the effectiveness of emerging molecular biomonitoring approaches, such as environmental DNA (eDNA) metabarcoding, which rely heavily on well-curated sequence databases for accurate species detection and biodiversity assessment.

Environmental DNA metabarcoding has emerged as a powerful and innovative tool for biodiversity monitoring, offering a non-invasive, efficient, and scalable approach to detect species from environmental samples such as water, soil, or sediment (Thomsen and Willerslev 2015; Wee et al. 2023). This technique involves extracting DNA fragments shed by organisms into the environment and amplifying specific genetic markers to identify the species present (Hebert et al. 2003; Ruppert et al. 2019). Compared with traditional survey methods that rely on physical sampling or direct observation, eDNA metabarcoding enables detection of both conspicuous and cryptic taxa—including rare, elusive, or seasonally occurring species—thereby overcoming many limitations of conventional field techniques. This makes it particularly valuable for assessing faunal communities in complex ecosystems like the mangroves, where dense vegetation, high species richness, and habitat heterogeneity often hinder effective sampling. The application of eDNA metabarcoding has been demonstrated to significantly enhance biodiversity inventories, reduce sampling effort, and improve species detection rates, especially in data-poor regions or in areas with limited taxonomic expertise (Antinero et al. 2024; Naputo et al. 2024).

However, the success of eDNA-based studies is heavily dependent on the availability and completeness of reference sequence databases (Valentini et al. 2016). Accurate taxonomic identification relies on matching the amplified DNA sequences to curated reference libraries such as GenBank or the Barcode of Life Data System (BOLD). In regions where such reference data are sparse or incomplete, particularly for understudied and endemic taxa, the ability to assign sequences to species or even genera becomes limited. This results in ambiguous identifications or complete failure to detect certain species, ultimately undermining the reliability and potential of eDNA as a biodiversity monitoring tool. Thus, while eDNA metabarcoding presents immense promise for faunal surveys in Philippine mangrove ecosystems, its effectiveness is directly constrained by the current state of genetic reference data for local fauna.

This challenge may be especially relevant in biodiversity-rich yet undersampled regions such as the Philippines. Although the country is recognized as a global hotspot for marine and coastal fauna, the extent to which mangrove-associated species are represented in major barcode repositories is unclear and requires systematic assessment. Preliminary observations suggest that existing genetic data may be unevenly distributed across taxa, with greater coverage for commercially important groups and comparatively limited representation for many ecologically significant but lesser-known species. If such patterns hold, the completeness of reference libraries could influence the accuracy of eDNA-based biodiversity assessments and potentially limit the detection of the full faunal diversity present in Philippine mangrove ecosystems.

Understanding the extent and patterns of these potential gaps in genetic data is essential not only for improving species detection accuracy but also for informing strategic priorities for barcoding and sequencing efforts. By determining which taxonomic groups are adequately represented and which remain undersampled or unsequenced, researchers and institutions can more effectively direct resources toward building comprehensive, high-quality, and locally relevant reference libraries. Enhancing these repositories is a critical step toward strengthening the reliability of eDNA metabarcoding applications, ultimately supporting more accurate biodiversity assessments and more informed conservation and ecological monitoring initiatives.

In this study, we assess the status of genetic reference data for Philippine mangrove-associated fauna across major barcode repositories, evaluating the extent of data availability and identifying critical gaps at both the taxonomic and ecosystem levels. Specifically, we seek to address the following key questions: (a) What is the current state of faunal biodiversity documented for Philippine mangrove ecosystems? (b) What is the current coverage of DNA barcode data (COI, 12S, 16S, Cytb) for Philippine mangrove-associated fauna across different taxonomic groups? (c) Which taxa or faunal groups in Philippine mangroves are underrepresented in public genetic databases, and how do these gaps affect the potential accuracy of eDNA metabarcoding? (d) How can the existing barcode repositories inform the selection of genetic markers and primers for effective biodiversity monitoring of mangrove ecosystems in the Philippines? By doing so, we highlight the importance of improving molecular reference resources to support robust, accurate, and science-based

conservation efforts for the country's mangrove ecosystems, which are among the world's most important tropical biodiversity hotspots.

2 | Methodology

2.1 | Literature Review and Taxonomic Standardization

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework to systematically review documented species and assess the extent of research on mangrove-associated fauna in the Philippines. The review process adhered to the workflow outlined in Figure 1.

Relevant online literature were retrieved from Google Scholar, PubMed, Scopus, and Web of Science, covering the period from 1955 to 2025, using targeted keyword searches across all databases following Boolean style: TITLE-ABS-KEY("mangrove*") AND TITLE-ABS-KEY(Philippines) AND TITLE-ABS-KEY(fauna OR animal* OR bivalve* OR mollusk* OR gastropod* OR cephalopod* OR crustacea* OR decapod* OR echinoderm* OR polychaete* OR sponge* OR tunicate* OR fish OR "ray-finned fish" OR amphibian* OR reptile* OR mammal* OR bird*). PubMed search results were retrieved and processed in R (version ≥ 4.0) using the *rentrez*, *tidyverse*, *xml2*, and *openxlsx* packages. Search results from Scopus, Web of Science, and Google Scholar were accessed and exported via institutional subscriptions and manual retrieval, using the same search strings. Titles, abstracts, and full texts of the identified records were screened for eligibility. The inclusion criteria for evaluating the articles that were related to the subject of interest were: (1) studies conducted in the Philippines; (2) explicit mention of mangrove-associated fauna; and (3) taxonomic identification to at least the species level. Articles that did not meet the eligibility criteria were excluded: (1) studies outside the Philippines; (2) absence of faunal data; and (3) purely abiotic focus. Inter-rater reliability between two reviewers was assessed using Cohen's kappa ($k = 0.874$), indicating strong agreement, following the interpretation benchmark of Landis and Koch (1977). More recent methodological literature, however, cautions that kappa should be interpreted in context, as factors such as prevalence bias or imbalanced ratings can influence its magnitude (e.g., Sim and Wright 2005; BMC Cancer 2023). Only studies that met all inclusion criteria based on full-text review were retained for the final analysis. All document types were considered, regardless of indexing status, including research articles, review papers, short communications, theses, technical reports, and conference proceedings. Although theses and technical reports are not consistently indexed in major databases, the ones used in this paper were retrieved through Google Scholar, which indexes institutional repositories and author-uploaded materials. Additional access points included Web of Science, which occasionally indexes citations to these documents, and ResearchGate, where authors have deposited publicly accessible copies. This inclusive approach aimed to maximize documentation of faunal diversity within Philippine mangrove ecosystems, thereby reflecting the current extent of species records available in the literature. The resulting data are intended to support efforts in biodiversity

assessment, conservation planning, and sustainable management of mangrove habitats.

Following the literature review, an inventory of the reported species was compiled. To ensure taxonomic accuracy, species names were verified using the World Register of Marine Species (WoRMS Editorial Board 2022) and cross-referenced with FishBase (<https://www.fishbase.se>) and the Catalog of Fishes (<https://www.calacademy.org/scientists/projects/catalog-of-fishes>). This step involved standardizing scientific names, checking for synonyms, and correcting typographical or spelling inconsistencies.

2.2 | Gene Marker Selection Criteria

Only taxa identified to the species level were considered for downstream genetic marker analyses. Out of 22 gene markers considered (13 mitochondrial and nine nuclear), only four mitochondrial gene markers (12S rRNA, 16S rRNA, COI, and Cytb) were retained and selected based on the optimal performance in species-level detection and recent evaluations on their detection sensitivity, species-level resolution, amplification reliability, availability of reference sequences in GenBank, and suitability for eDNA applications (see Table 1).

2.3 | Data Analysis

For each species identified in the literature, we queried the National Center for Biotechnology Information (NCBI) GenBank using R (v4.3.2) with the *rentrez* package (Winter 2017). A standardized list of species names, compiled from the literature review, was provided as an excel file and used to perform exact species-level matching to minimize ambiguity in retrieval. To target gene markers relevant for eDNA metabarcoding, we applied a comprehensive set of marker-specific keywords, including synonyms and variants for cytochrome c oxidase subunit I (COI/COX1), 12S rRNA, 16S rRNA, cytochrome b (Cytb), as well as "mitochondrial genome" terms. These keywords were searched across multiple metadata fields in GenBank records (e.g., *definition*, *title*, *gene*, *product*).

Records annotated as "mitochondrial genome" in GenBank were not analyzed as whole mitochondrial genome sequences. Instead, these records were screened and retained only if the GenBank feature table contained clearly annotated gene regions corresponding to the target markers (COI, 12S rRNA, 16S rRNA, and Cytb). When present, the accession was counted under the respective marker category based on existing gene labels. We did not perform de novo sequence extraction or trimming from mitochondrial genomes. Marker assignment was based strictly on GenBank annotations to ensure transparency and reproducibility. Complete mitochondrial genome entries lacking clear gene annotations or species-level resolution were excluded from the dataset.

Sequence inclusion criteria required a minimum length of 300bp and species-level taxonomic identification. Exclusion criteria included nuclear or ambiguous markers, sequence-lacking

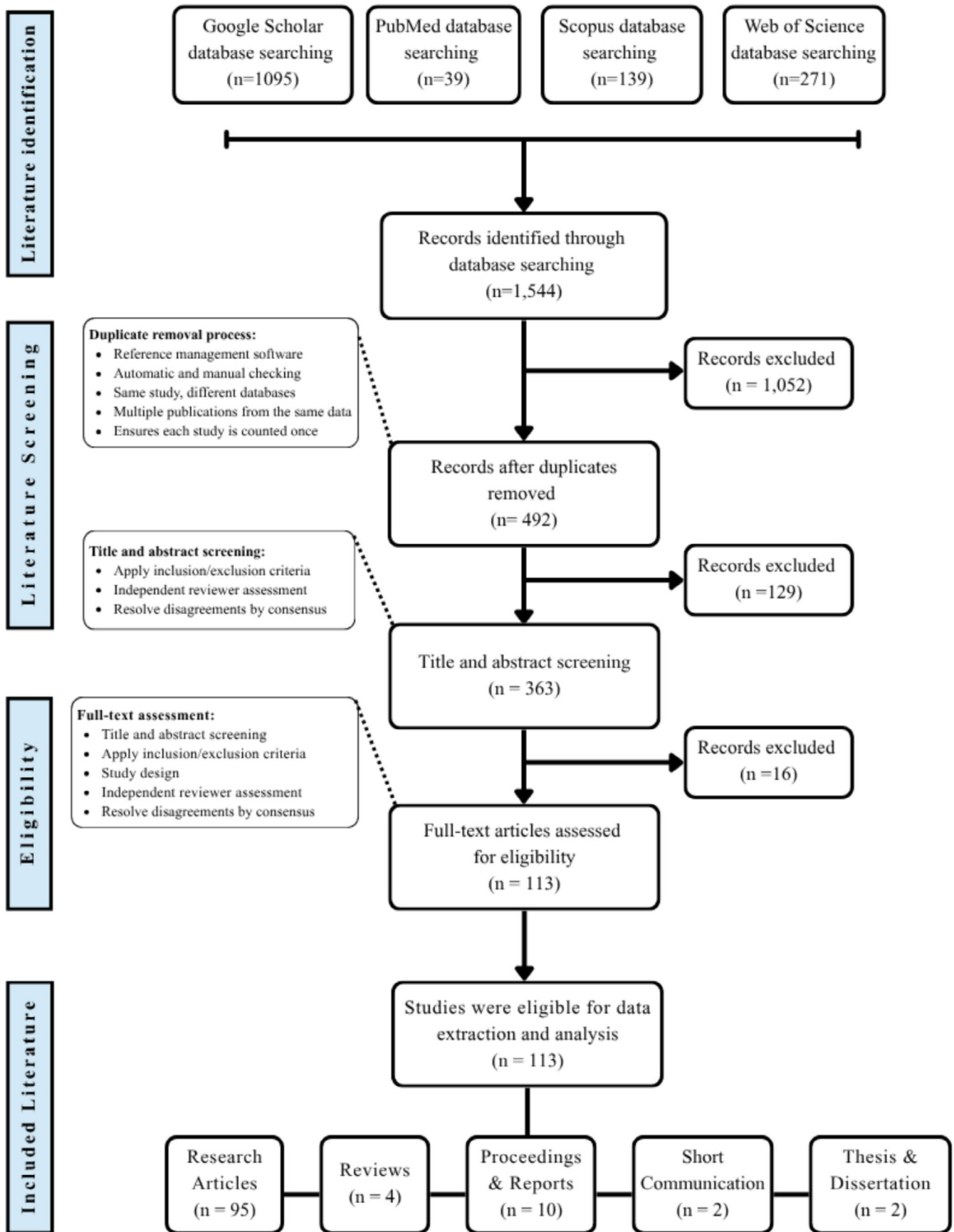


FIGURE 1 | PRISMA-based workflow illustrating the review process used in this study.

TABLE 1 | Comparative evaluation of genetic markers used in environmental DNA (eDNA) metabarcoding for biodiversity assessment.

Marker	Detection sensitivity	Species resolution	eDNA suitability	Justification	Inclusionstatus	References
12S	High	High	Excellent	Short conserved amplicons (e.g., MiFish primers) provide high amplification success for degraded DNA and strong detection sensitivity across vertebrates especially used in fishes eDNA studies.	Included	Miya et al. (2015); Collins et al. (2019); Kumar et al. (2022); Miya (2022); Wang et al. (2023)
COI	Moderate	High	Excellent	Global animal barcode with extensive reference databases (GenBank, BOLD). Broad taxonomic utility but lower sensitivity in degraded environmental samples compared to 12S.	Included	Elbrecht and Leese (2016); Deiner et al. (2017); Collins et al. (2019); Harper et al. (2019); Wang et al. (2023)
16S	Moderate	Moderate	Useful	Broad taxonomic applicability (especially invertebrates and microbes). Lower vertebrate resolution than 12S, but useful where 12S primers perform poorly	Included	Elbrecht et al. (2016); Harper et al. 2019; Kumar et al. (2022); Wang et al. (2023)
Cytb	Moderate	High	Useful	High species-level resolution across vertebrates and fishes; commonly applied in phylogenetics and increasingly in metabarcoding studies.	Included	Hanfling et al., (2016); Zhang et al. (2020); Jiang et al. (2022); Kumar et al. (2022)
ATP6/ATP8	Low	Moderate	Rarely used	Poor primer standardization and limited barcode reference libraries restrict the application in eDNA metabarcoding.	Excluded	Ratnasingham and Hebert (2007); Taberlet et al. (2012); Deiner et al. (2017); Machida et al. (2017); Zhang et al. (2020)
ND1–ND5 (NADH genes)	Low	Moderate	Rarely used	Primarily applied in mitogenomics; inconsistent amplification and lack of universal primers limit eDNA utility.	Excluded	Machida et al. (2017); Leray et al. (2022);
COII/COIII	Low	Moderate	Limited	Not standardized for metabarcoding; low amplification consistency and limited databases make them poor eDNA targets.	Excluded	Machida et al. (2017); Collins et al. (2019); Leray et al. (2022)
18S	High	Low	Caution	High copy number and broad taxonomic range suitable for protists and general community surveys, but insufficient species-level resolution for animals.	Excluded	Machida et al. (2017); Gunther et al., (2018); Zhou et al. (2025)

(Continues)

TABLE 1 | (Continued)

Marker	Detection sensitivity	Species resolution	eDNA suitability	Justification	Inclusionstatus	References
28S	Low	Low	Inappropriate	Highly conserved loci provide poor species-level discrimination for eDNA-based biodiversity surveys	Excluded	Deiner et al. (2017); Machida et al. (2017); Zhang et al. (2020)
ITS1/ITS2	Moderate	High	Taxon-specific	Excellent for fungi and useful in some invertebrates; unsuitable for vertebrate-focused eDNA surveys.	Excluded	Schoch et al. (2012); Nilsson et al. (2019); Krehenwinkel et al. (2018)
RAG1/RAG2; H3	Low	Moderate	Phylogenetics only	Single-copy nuclear genes are powerful for evolutionary reconstruction but yield low recovery from degraded environmental DNA.	Excluded	Colgan et al. (1999); Groth and Barrowclough (1999); Deiner et al. (2017); Zhang et al. (2020)
Microsatellite/SNPs	Very Low	Very High	Not viable	Require high-quality genomic DNA and targeted genotyping approaches; not compatible with highly degraded environmental samples.	Excluded	Thomsen and Willerslev (2015); Deiner et al. (2017); Ruppert et al. (2019)

taxonomic resolution, or incomplete metadata. Additional quality filters required associated information, such as specimen voucher, collection locality, or organelle annotation, where available. To ensure accuracy, GenBank hits were cross-checked against the Barcode of Life Data System (BOLD) when possible. Cross-validation using BOLD was performed exclusively for COI sequences, as BOLD primarily contains cytochrome c oxidase subunit I records. For non-COI mitochondrial markers (12S rRNA, 16S rRNA, and Cytb), validation was conducted through internal GenBank annotation consistency checks, including verification of gene names within the feature table, concordance between gene and product annotations, and confirmation of species-level taxonomic assignments in the NCBI records. When available, supporting metadata such as specimen vouchers, collection locality, and associated publication references were used to strengthen confidence in the reliability of the sequences. No external marker-specific re-annotation platforms (e.g., MitoAnnotator in MitoFish) were applied, as the study focused on the availability and representation of reference sequences in public repositories rather than on de novo re-annotation or sequence curation for the validation of gene regions. Data processing, analysis, and visualization were performed in RStudio (version 2024.04.2 + 764) using relevant R packages, while mapping the spatial distribution of mangrove-associated faunal studies across the Philippines was done in QGIS.

3 | Results and Discussion

3.1 | Trends and Characteristics of Mangrove-Associated Faunal Studies in The Philippines

In this review, literature searches covered the period 1955 to 2025 across major academic databases. However, research on mangroves and related studies in the Philippines dates back to the 17th century (Fortes and Salmo III 2017). After applying inclusion criteria requiring studies to document mangrove-associated fauna with taxonomic identification to at least the species level, only publications from 1977 to 2025 met the eligibility standards. Within this 48-year span, a total of 113 publications were included in this review. The majority were peer-reviewed research articles (84.1%, $n=95$); followed by proceedings and technical reports (8.8%, $n=10$), review articles (3.5%, $n=4$), short communications (1.8%, $n=2$), and theses and dissertations (1.8%, $n=2$). The temporal distribution of these studies (Figure 2a) exhibited a generally increasing trend in publication output since the early 2000s (≥ 10 publications per year). However, there may still be several studies conducted in many areas of the country that remain unpublished or inaccessible to the public. Nevertheless, this review considers all available documents, regardless of indexing status, to comprehensively examine historical trends and research interests in faunal biodiversity of Philippine mangrove ecosystems. While the bulk of research on the mangrove ecosystem in the country has focused on conservation and management, the ecology and biodiversity of these important habitats need to be studied more (Fortes and Salmo III 2017).

Generally, the same pattern is observed in review of mangrove restoration studies in Southeast Asia, in which the number of studies on mangrove-associated fauna increased over time. At

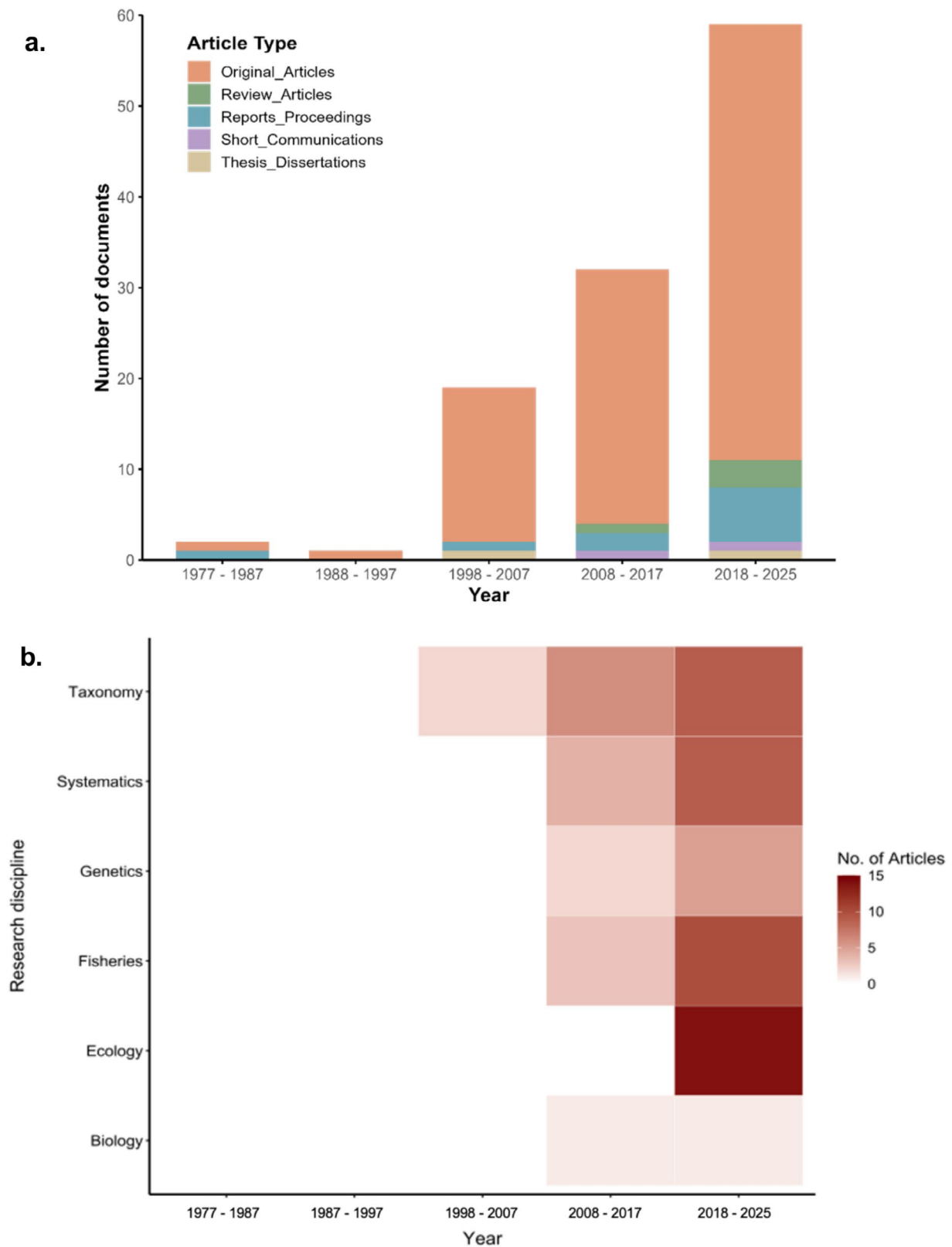


FIGURE 2 | Trends in published studies on associated fauna in Philippine mangrove ecosystems. (a) Temporal distribution of faunal studies, highlighting a notable increase in research output from the 2000s onward (≥ 10 articles). (b) Decadal distribution of research disciplines. Ecological studies comprise the highest proportion of publications in the most recent period (2018–225), indicating a recent shift toward ecological research relative to other disciplines.

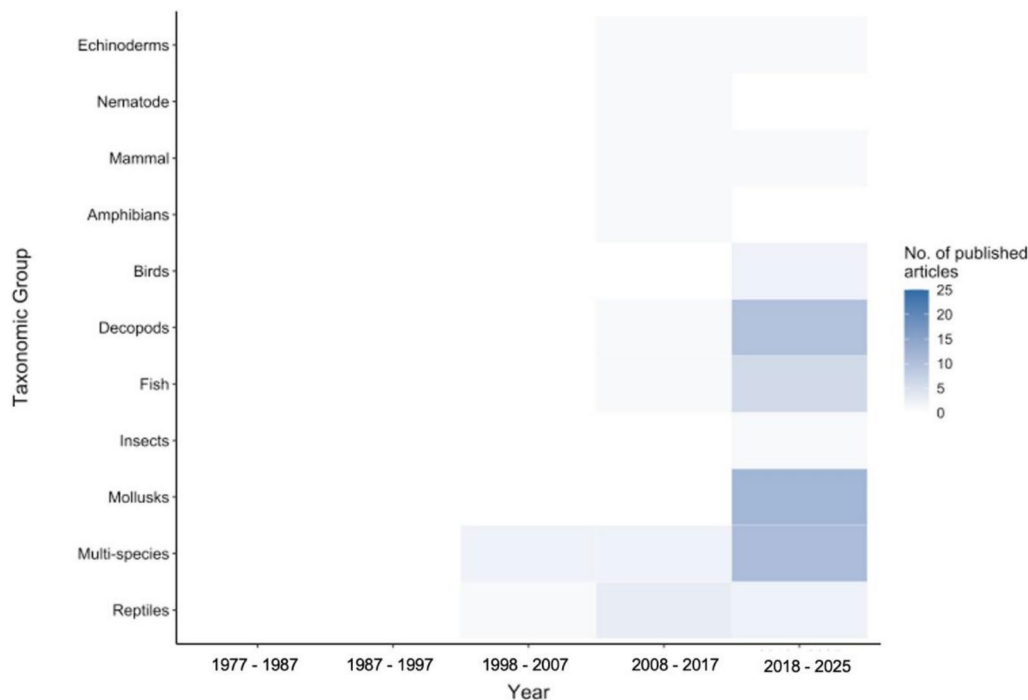


FIGURE 3 | Temporal trend in research studies on mangrove-associated fauna in the Philippines.

present, there is greater emphasis on studying the ecology of mangrove-associated fauna than on other research disciplines (Figure 2b). These studies will eventually enhance understanding of conservation efforts by elucidating species relationships and ecosystem functions, which are important for biodiversity conservation and management strategies. Meanwhile, fisheries, systematics, and taxonomic studies have begun to be explored, serving as a valuable complement to ecological studies by refining species identification, updating taxonomic records, clarifying species distributions, and delving into the fisheries aspects associated with each faunal species. However, biological-related studies have received less attention over the years and need to be considered, as biological aspects are critical, such as describing the developmental status of mangrove-associated fauna in relation to their resilience to sudden or gradual ecosystem change.

3.2 | Geographical Distribution and Taxonomic Focus of Mangrove Faunal Research in The Philippines

The earliest records of faunal studies in the mangrove ecosystem of the Philippines address the broader ecology of these faunal associates. Among these pioneering works, Motoh and Solis (1977) made a significant report on decapods from Batan Bay, Panay Island, documenting 15 species belonging to three families. In the early 2000s, researchers focused on conducting species-specific studies, such as a collection of the highly commercial mangrove clams (*Anodontia edentula*) in Panay and Guimaras Islands, to monitor recruitment and yields of the three species of mud crab (*Scylla* spp.) in the mangroves of Ibaay, Aklan (Lebata et al. 2007). Conversely, aside from being species-specific, several studies have been conducted to investigate diversity and species composition, such as those on fish assemblages in Panay (Ikejima et al. 2006) and mollusk diversity

in Catanduanes (Masagca et al. 2010) and the Lingayen Gulf in Pangasinan (Salmo and Duke 2010). Newly recorded species were also reported in some areas of the country, like the two Dolichopodidae (long-legged flies) species in the Province of Bohol (Ramos et al. 2018), and the two decapod species *Thalassina spinosa* (Bedi and Primavera 2018) and *T. kelanang* (Albarico et al. 2020) in Ibaay, Aklan, and Sagay City, Negros Occidental, respectively.

Over the past two decades, there has been a noticeable increase in research on mangrove-associated fauna in the Philippines, with the highest number of publications observed beginning in 2006 and rising over the years. The majority of early studies focused on mollusks, decapods, and fish (Figure 3). However, during the early 2000s, research shifted from species-specific studies to examining faunal diversity across multiple species. Thus, significant focus has been given to mollusks (particularly on bivalves and gastropods) over the past ten years compared to other faunal groups. This shift indicates broader, more expanded studies, reflecting a more comprehensive understanding of various faunal groups and mangrove-associated faunal species.

All major islands of the country were represented. Still, the majority of scientific outputs came from the Visayas islands in central Philippines, as indicated by the number of available literature for each region (Figure 4; Table S1). The faunal documentations show a wide spatial distribution of short-term studies; however, the majority of the mangrove areas, including those with dense mangrove cover, have noticeable gaps on faunal research, specifically in the areas of northern and southern Luzon; Masbate and most of the eastern parts of Visayas, which include Leyte and Samar; and Surigao del Norte, Zamboanga, and the majority of the southern parts in Mindanao. With the study trends across time and localities, there is still much to be done in terms of studying the associated fauna in the mangrove

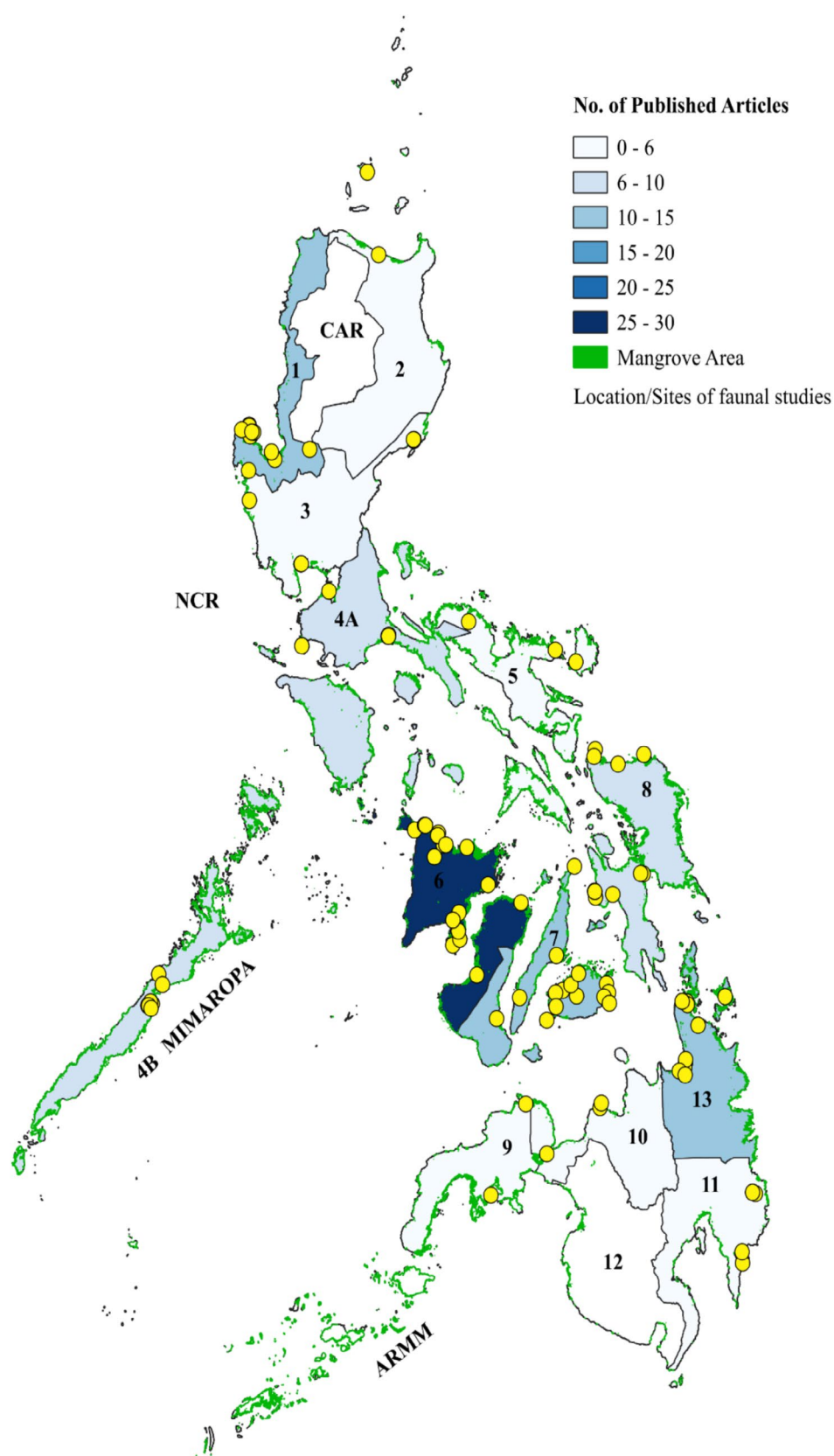


FIGURE 4 | Geographic distribution of studies related to mangrove-associated fauna in the Philippines. The regions illustrated on the map are as follows: 1—Ilocos region; CAR—Cordillera Administrative Region; 2—Cagayan Valley; 3—Central Luzon; NCR—National Capital Region; 4A—CALABARZON (Cavite, Laguna, Batangas, Rizal, Quezon); 4B—MIMAROPA (Mindoro, Marinduque, Romblon, Palawan); 5—Bicol Region; 6—Western Visayas; 7—Central Visayas; 8—Eastern Visayas; 9—Zamboanga Peninsula; 10—Northern Mindanao; 11—Davao Region; 12—SOCCSKSARGEN (South Cotabato, Sultan Kudarat, Sarangani, General Santos); 13—Caraga Region; BARMM—Autonomous Region of Muslim Mindanao.

ecosystem of the country. The same observation is apparent in other regions of the world, such as in other parts of Asia (Rajpar and Zakaria 2014) and Australia (Hutchings and Recher 1981). Region 6 (Western Visayas), which includes the islands of Panay and Negros Occidental, has been widely studied, as evidenced by the number of available published studies. Most of the studies in this area have focused on decapods and mollusks. Similarly, in many other regions of the country, research has predominantly centered on mollusks.

3.3 | Documented Fauna in Philippine Mangrove Ecosystems

A total of 999 faunal species belonging to nine distinct phyla, 24 classes, 275 families, and 565 genera from marine, brackish water, terrestrial, and freshwater environments were documented in this review (See Table S1). Of the nine identified phyla, Phylum Chordata, specifically the ray-finned fishes (40.4%, $n=404$), had the most extensive species list. This could be because many studies on finned fish were geared toward broader assessments rather than species-specific studies. Of the faunal groups listed, ray-finned fishes also account for the highest number of recorded species followed by gastropods (21.7%, $n=217$) and bivalves (16.5%, $n=165$), then decapods (7.4%), birds (6.3%), echinoderm (2.2%), reptiles (1.4%), mammals (1.0%), amphibians (0.8%), tunicates (0.3%), chiton (0.2%) and brachiopods, cephalopods, crustaceans, nematodes, peanut worm, polychaetes, sea jellies and sponges with (0.1%) each (Figure 5). In comparison with neighboring countries in Southeast Asia, reported mangrove-associated

marine fauna vary considerably. For example, there are ~730 marine faunal species that have been documented in Malaysia, composed of 138 fish species, 384 gastropod species (e.g., Baharuddin et al. 2018, 2019) and 206 bivalve species (Hamli et al. 2021); while there are >4130 species in Vietnam composed of >2000 fish species (e.g., Van Nguyen and Mai 2020), ~1500 gastropod species (e.g., Nhat et al. 2025) and 814 bivalve species (Zvonareva et al. 2019; Lutaenko 2016); >4700 species in Thailand composed of >2100 fish species (e.g., Derrick et al. 2017; Eiamsa-Ard and Amornchairojkul 1997), <2000 gastropod species and ~600 bivalve species (Sanpanich 2011; Sanpanich and Duangdee 2013; Wells et al. 2021); and >4300 species in Indonesia composed of ~3700 fish species (e.g., Allen and Adrim 2003; Peristiwady 2021) and >665 marine mollusk species (Hutomo and Moosa 2005; Kartika and Mu 2014). This reflects the extensive national inventories across multiple coastal habitats. However, such differences should be interpreted cautiously, as species counts are influenced by variation in spatial coverage, taxonomic resolution, habitat inclusion criteria, and research intensity rather than representing direct biodiversity contrasts. These regional comparisons highlight a shared challenge across Southeast Asia. At the same time, the region is globally recognized as a marine biodiversity hotspot, and mangrove-associated fauna remain unevenly documented, with research often concentrated on commercially valuable groups such as fishes, gastropods, and bivalves. Similar patterns are evident in the Philippines, where commercially important taxa receive disproportionate attention relative to less conspicuous invertebrate and non-commercial groups. Finfish and some mollusks, such as bivalves and gastropods, have gained considerable attention because they generate quite high market values

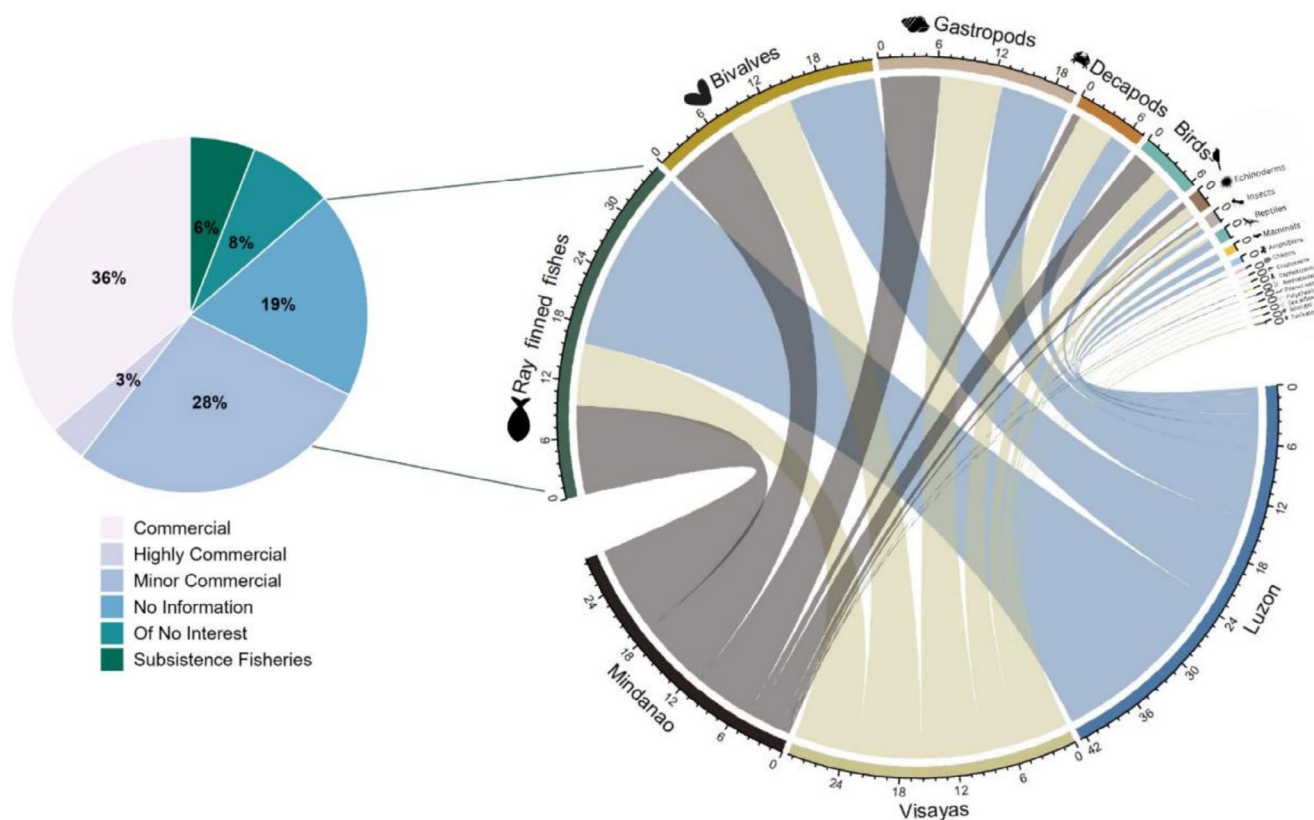


FIGURE 5 | Circular plot showing the composition of faunal groups documented in mangrove ecosystems of the Philippines. Emphasis is given on the economic significance of ray-finned fishes (Class Actinopterygii), which represent a major component of the reported fauna.

(Hutchison et al. 2014) and are among the primary foods for the majority of individuals, especially in coastal areas. The economic importance of fisheries further underscores the significance of understanding fish and macroinvertebrate communities. Other faunal groups, on the other hand, received comparatively less attention, primarily due to their perceived lack of direct importance to consumers. Unlike fish, bivalves, and gastropods, these other groups might not have the same immediate economic impact or widespread consumption, resulting in reduced emphasis in research. Importantly, while regional species inventories provide insight into faunal richness patterns across Southeast Asia, the utility of these biodiversity records for contemporary monitoring depends increasingly on the availability of corresponding genetic reference data. Disparities in molecular representation within public databases therefore mirror differences in species documentation across countries. In this context, evaluating the genetic coverage of documented Philippine mangrove fauna becomes essential, as incomplete reference libraries directly constrain the effectiveness of environmental DNA (eDNA) metabarcoding for biodiversity assessment and cross-regional comparability.

Among the 404 species documented within the Class Actinopterygii, only 14 were classified as Highly Commercial, accounting for only 3.47% (Figure 5; Table S1). These highly commercial species include *Channa striata* (Striped snakehead), *Chanos chanos* (Milkfish), *Epinephelus malabaricus* (Malabar grouper), *Hilsa kelee* (Kelee shad), *Hippocampus kuda* (Spotted seahorse), *Hypophthalmichthys nobilis* (Bighead carp), *Lates calcarifer* (Barramundi), *Lethrinus lentjan* (Pink ear emperor), *Lutjanus lutjanus* (Bigeye snapper), *Muraenesox cinereus* (Daggertooth pike conger), *Oreochromis niloticus* (Nile tilapia), and *Sardinops melanostictus* (Pacific sardine). Species classified as Commercial and Minor Commercial made up the majority, with 146 (36.14%) and 113 (27.97%) species, respectively. Although a significant portion of the documented fishes has commercial importance, at least 31 species (7.67%) were reported to have no commercial value (or of no interest). Additionally, 24 species, comprising 5.94%, were identified for subsistence fisheries, while 76 (18.81%) species have no data about their human uses (Figure 5). For ray-finned fishes, commercially valued species were mostly documented, supporting the mangrove ecosystem's role in fisheries and food security (Pantallano et al. 2018).

The literature search shows that many mangrove-associated taxa are poorly documented in terms of the employment of molecular data or are sparsely reported in the literature, despite spanning 48 years (1977–2025). Many faunal groups were represented by fewer than five species across all published studies combined, including brachiopods, cephalopods, chitons, crustaceans, nematodes, peanut worms, polychaetes, sea jellies, sponges, and tunicates. At the same time, the following groups have species with minimal documentation relative to expected biodiversity levels: insects, amphibians, and reptiles.

Globally, mangrove ecosystems harbor exceptionally diverse and unique faunal assemblages (Nagelkerken et al. 2008; Lee et al. 2014). The relatively low species counts documented in the Philippines, despite its status as a megadiverse country, suggest that large components of mangrove biodiversity remain overlooked. Understudied groups often include cryptic, small-bodied, or soft-bodied taxa that are difficult to survey

morphologically but can be detected efficiently using eDNA approaches, provided reference sequences exist (Stat et al. 2017). The lack of molecular data, therefore, represents a major bottleneck in closing the knowledge gap for Philippine mangrove fauna. These small counts suggest either a true low mangrove association or substantial under-sampling, both biologically and genetically. Many of these taxa inhabit microhabitats (e.g., sediment and cryptic niches) that are less frequently sampled and difficult to access, contributing to their underrepresentation in biodiversity inventories and genetic repositories. Sequencing, however, is not inherently limiting once representative samples are obtained.

3.4 | Genetic Data on Documented Mangrove Fauna in The Philippines: Applicability to eDNA Metabarcoding

Molecular data revealed that of the 999 documented mangrove-associated fauna, 70.27% ($n=702$) of species had at least one available gene marker, indicating that a substantial portion of the fauna has been partially characterized in global barcode repositories such as NCBI and BOLD. Whereas 29.73% ($n=297$) of species lack molecular data (Figure 6a; Table 1). This pattern indicates that although a considerable portion of the Philippine mangrove fauna has been genetically characterized, nearly one-third remains unrepresented in the barcode database. Such gaps are consistent with global assessments showing that reference libraries for biodiversity monitoring continue to suffer from substantial taxonomic and geographic biases, particularly in species-rich tropical ecosystems such as mangrove forests, which are often undersampled relative to their diversity (Weigand et al. 2019; Porter and Hajibabaei 2020). Additionally, this reflects long-standing global patterns in molecular repositories, where molecular data are concentrated in well-studied groups and economically valuable species. At the same time, many ecologically important taxa receive little attention (Stat et al. 2017).

Furthermore, these deficiencies highlight the need to expand and sustain molecular documentation efforts to improve reference databases, as current barcode repositories still capture only a fraction of species diversity, limiting the accuracy and completeness of downstream applications such as eDNA metabarcoding and conservation assessments (Hickerson et al. 2006; Leray and Knowlton 2015; Berba and Matias 2022; Schmid et al. 2025). Because eDNA metabarcoding relies on accurate, taxonomically validated reference sequences, the lack of molecular data for nearly 300 mangrove-associated species poses a substantial barrier to biodiversity detection. Incomplete barcode libraries in eDNA-based monitoring lead to false negatives, ambiguous taxonomic assignments, or restricted detection at higher taxonomic levels (Deiner et al. 2017; Zinger et al. 2019; Weigand et al. 2019; Ruppert et al. 2019; Porter and Hajibabaei 2020).

Figure 6a also shows that marker availability varied across the four targeted mitochondrial genes used in eDNA metabarcoding (12S, 16S, COI & Cytb), with considerable variability in representation across taxonomic groups. Among species with available molecular data, COI emerged as the most represented marker (625 species; 89.03%), followed by 16S (493 species;

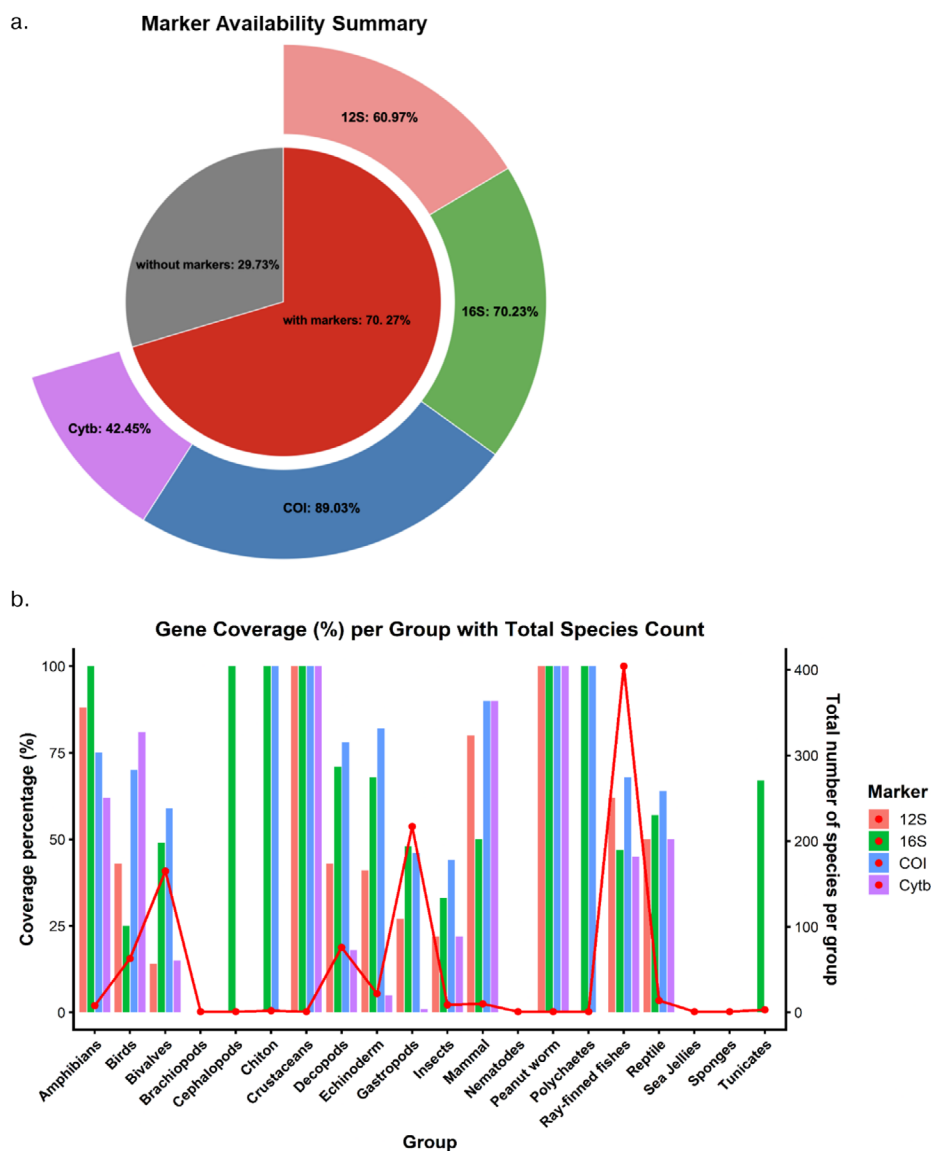


FIGURE 6 | (a) Clustered pie chart summarizing mitochondrial marker availability. The inner circle represents the proportion of ‘with’ and ‘without available’ markers, while the outer circle shows the relative coverage of each of the four mitochondrial markers (12S, 16S, COI, and Cytb) among marker-positive samples. (b) Bar and line plot showing mitochondrial gene coverage (%) across major animal groups with corresponding total species counts. The bar plot represents the coverage of four mitochondrial markers, while the red line indicates the total number of species per group.

70.23%) and 12S (428 species; 60.97%), which exhibited moderate representation, while Cytb had the least representation (298 species; 42.45%). The higher coverage of COI is consistent, given its long-standing role in global DNA barcoding initiatives and its extensive inclusion in barcode databases, where COI constitutes the backbone of animal barcoding datasets (Hebert et al. 2003; Ratnasingham and Hebert 2007; Porter and Hajibabaei 2020). However, despite this dominance, recent studies have raised concerns about taxonomic biases and incomplete COI reference libraries, particularly for understudied or ecologically important taxa (Weigand et al. 2019). The wide availability of COI underscores its utility in molecular biodiversity studies and its importance in reference databases that power DNA barcoding and eDNA metabarcoding workflows, but its prevalence also reflects historical and economic priorities in sequencing (Keck et al. 2023). The 16S gene, frequently used in vertebrate and invertebrate metabarcoding surveys, showed consistent

results, underscoring the growing reliance on this marker for eDNA assessment in marine and freshwater systems (Deiner et al. 2017). Similarly, the 12S gene, a commonly preferred marker for vertebrate eDNA studies due to its short amplicon size and reliable taxonomic resolution, continues to be validated in recent research. Previous studies comparing primer sets have highlighted the advantages of the 12S primer, which often outperforms other markers in detecting fish diversity and in other marine vertebrates, particularly in fragmented habitats such as mangrove forests (Zhang et al. 2020). Moreover, benchmark studies on detecting marine fishes using eDNA show trade-offs among 12S, 16S, and COI markers in terms of taxonomic resolution and amplification efficiency, highlighting the need for careful marker selection (Fontes et al. 2024). However, methodological challenges remain; for instance, variation in primer performance and DNA polymerase can significantly affect 12S-based eDNA detection (Min et al. 2023).

Meanwhile, *Cytb*, which is typically employed in more specific phylogenetic studies, showed the lowest availability represented in public databases. This pattern has been reaffirmed in recent barcode gap analyses (Berba and Matias 2022). The limited coverage of *Cytb* reflects its secondary use compared to 12S, 16S, and COI in genetic barcoding initiatives, as well as its more specialized role in biodiversity research (Hoban et al. 2022; Min et al. 2023; Fontes et al. 2024). The underrepresentation of *Cytb* may reduce its utility and detection sensitivity for some taxa as well as limit marker selection flexibility for Philippine mangrove in metabarcoding approaches, particularly for high-resolution species identification in certain taxonomic groups, which is why there is a need for targeted sequencing efforts to fill this gap (Berba and Matias 2022; Hoban et al. 2022). These patterns displayed an unbalanced distribution of molecular information across gene regions, with a strong dominance of COI and relatively limited availability of short-fragment ribosomal markers (12S and 16S), which are most relevant to contemporary eDNA metabarcoding analysis. The underrepresentation of 12S and *Cytb*, in particular, could reduce taxonomic resolutions for some groups and limit detection sensitivity in eDNA-based surveys. This is crucial, as ribosomal RNA genes such as 12S and 16S are often preferred for detecting a wide array of taxa in environmental DNA monitoring due to their broad universality and robust amplification from degraded DNA samples (Deiner et al. 2017).

Subsequently, the distribution of molecular reference coverage among major taxonomic groups (Figure 6b; Table 1) reveals substantial differences in genetic representation among the Philippine mangrove-associated fauna. Across the 87,489 retrieved reference sequences of the selected gene markers representing 702 species with available molecular data, patterns are highly uneven, aligning with strong taxonomic biases observed in global barcode repositories and highlighting both well-represented groups and those that remain severely understudied. These inequities mirror well-documented international assessments showing that barcode libraries disproportionately favor commercially valuable, easily accessible, or historically well-studied taxa, while many ecologically critical marine invertebrates remain severely underrepresented (Leray and Knowlton 2015; Bucklin et al. 2021).

Ray-finned fishes constitute the largest molecularly represented group (285 species; 35,486 sequences), with COI representing more than half of all sequences (57.8%), followed by *Cytb* (30.2%), while 16S (8.4%) and 12S (3.6%). This pattern reflects long-standing momentum from the Fish Barcode of Life Initiative (FISH-BOL) and extensive COI sequencing efforts integrated into BOLD and GenBank, which have prioritized barcoding for fisheries monitoring and biodiversity assessments globally. The strong representation of ray-finned fishes shows comparatively high readiness for eDNA-based detection using COI, yet the limited 12S and 16S representation remains a bottleneck for modern vertebrate metabarcoding assays, which rely heavily on these genetic markers for broad vertebrate detection (Miya et al. 2015; Weigand et al. 2019; Porter and Hajibabaei 2020; Polanco et al. 2021). Thus, even within well-studied taxa, marker-specific gaps can limit the detection sensitivity of high-throughput eDNA workflows.

The groups of Bivalves (114 species; 20,259 sequences) followed the Ray-finned-fishes in the next most represented groups, though marker distribution varies considerably. In Bivalves, marker distribution is strongly skewed toward 16S (66.37%) and COI (31.74%). Coverage of *Cytb* (1.63%) and 12S (0.26%) remains minimal. These patterns mirror global challenges in molluscan barcoding, particularly for taxa with cryptic species boundaries, high intraspecific divergence, and complex life histories toward COI sequencing factors that have limited the development of balanced reference libraries for alternative markers (Sun et al. 2016; Van Der Loos and Nijland 2021; Buršić et al. 2021). Gastropods followed with 113 species (9802 sequences), with very strong COI representation (82.77%) relative to 16S (14.92%). However, as with bivalves, the markers 12S (2.29%) and *Cytb* (0.03%) remain poorly represented. This COI-dominant profile reflects the historical focus of mollusk barcoding initiatives on COIs, leaving substantial gaps in other markers commonly used in eDNA metabarcoding.

Decapods (59 species; 7268 sequences) and Echinoderms (18 species; 7079 sequences) exhibit similar COI biased profiles. For Decapods, COI accounts for 71.97% of all sequences, followed by 16S (22.47%), 12S (2.88%), and *Cytb* (2.68%). In Echinoderms, COI (80.29%) has the highest number of available reference sequences, followed by 16S (18.03%), 12S (1.67%), and *Cytb* (0.01%). Such patterns reflect global trends in which crustaceans and echinoderms are taxonomically complex, morphologically variable, and undersampled for non-COI markers (Collin et al. 2020; Hestetun et al. 2020). The scarcity of 12S and *Cytb* sequences in both groups limits their detectability by vertebrate- and broad-metazoan metabarcoding assays commonly used in tropical coastal ecosystems.

The remaining vertebrates and select invertebrate groups show progressively weaker molecular representation. Birds with 52 species comprising (3090 sequences), having moderate COI with (43.14%), followed by *Cytb* (31.52%), then 16S (22.27%), and 12S with (3.07%). Amphibians have 8 species (1219 sequences), comprising COI (37.65%), 16S (34.13%), 12S (20.67%), and *Cytb* (7.55%). Next to it is the Mammal group, with nine species (915 sequences), subdivided by genetic markers: *Cytb* (70.38%), COI (23.39%), 12S (3.93%), and 16S (2.30%). At the same time, Reptiles have 11 species (771 sequences), with COI (49.03%), *Cytb* (28.27%), 16S (15.43%), and 12S (7.26%). All exhibit moderate COI and *Cytb* coverage but limited availability of high-performing eDNA markers, such as 12S or 16S libraries, an issue repeatedly highlighted in the vertebrate eDNA monitoring literature (Ruppert et al. 2019; Wilting et al. 2021). This mismatch is particularly important for mangrove ecosystems, where vertebrates, especially birds and reptiles, serve as indicators of ecosystem integrity but are challenging to detect using eDNA surveys.

Furthermore, a few phyla display extremely low representation despite documented occurrence in Philippine mangroves, such as Peanut Worms, with only one species (539 sequences) having *Cytb* (45.08%), COI (41.56%), 16S (13.17%), and 12S (0.19%). Similarly, the Insect group is represented by only five species (344 sequences), with sequences predominantly from COI (79.94%), followed by 16S (9.88%), 12S (5.81%), and *Cytb* (4.36%). The Crustacean group, excluding the Decapods, was

represented by one species (285 sequences), with marker distribution dominated by COI (94.39%), followed by 12S (4.21%), 16S (1.05%), and Cytb (0.35%). Then the Polychaete group with only one species (282 sequences), having both COI (50.71%) and 16S (49.29%). This is followed by the Cephalopod group, which also has one species (76 sequences), and is represented by the 16S gene marker (100%). Additionally, the groups of Chiton and Tunicates have two species, each having (65 total sequences) and (nine sequences) respectively, with Chiton having gene markers COI (89.23%) and 16S (10.77%). On the other hand, Tunicates are represented exclusively by the 16S genetic marker (100%), indicating extremely limited marker availability for this group. This restricted representation highlights broader challenges in generating comprehensive reference libraries for certain marine invertebrates. Similar patterns have been reported globally, particularly for taxonomically complex or understudied taxa that remain poorly represented in molecular repositories (Zinger et al. 2019). As a result, their minimal genetic coverage severely constrains eDNA-based biodiversity detection because taxa lacking robust reference sequences are effectively invisible to metabarcoding assays, regardless of their ecological presence.

On the contrary, among 29.73% ($n = 297$) species with no genetic data, including the groups of Brachiopods, Nematodes, Sea Jellies, and Sponges, with only one (0.34%) species being documented for each. These taxonomic groups remain largely unrepresented in genetic repositories. This gap highlights the need for extensive genetic sampling in mangrove ecosystems, particularly of groups crucial to understanding their biodiversity, to determine the steps for biomonitoring and which fauna require urgent protection. On the other hand, even within species-rich groups, some species lack available reference sequences for the selected genetic markers. Ray-finned fishes account for the largest deficit, with 119 species (40.07%) lacking molecular data, of which 66 are commercially important taxa. These include high-value species such as *Muraenox cinereus* (Forsskål, 1775) and conservation concern species such as *Hippocampus comes* (Cantor, 1850; Vulnerable). Additionally, two species were Data Deficient: *Acanthopagrus datnia* (Hamilton, 1822) and *Stolephorus waitei* (Jordan & Seale, 1926). At the same time, some species are Not Evaluated: *Sillago maculata* (Quoy & Gaimard, 1824) and *Strongylura strongylura* (van Hasselt, 1823).

Furthermore, 31 species have no information in terms of their economic value. Still, about their IUCN status, four were Data Deficient: *Arcygobius baliurus* (Valenciennes, 1837), *Fibramia amboinensis* (Bleeker, 1854), *Gobiopertus stellatus* (Herre, 1927), and *Neostethus amaricola* (Villadolid & Manacop, 1934), four were Not Evaluated: *Chelonodon reticulatus*, *Microgobius stellatus*, *Uropterygius hades*, and *Yongeichthys criniger* (Valenciennes, 1837), and the rest are categorized as Least Concern. Knowledge gaps of this magnitude are especially problematic because high-value and threatened species are often primary targets for monitoring but cannot be detected using eDNA without reference sequences (Gold et al. 2022). On the other hand, gastropods have 84 species (28.28%), followed by Bivalves and Decapods, with 51 (17.17%) and 15 (5.05%) species, respectively. The Echinoderms and Insects both have four species (1.35% each). Reptiles have three species (1.01%), and both Mammals and Tunicates have one species (0.34%), with no genetic data available for the selected genetic markers.

When compared with regional efforts in Southeast Asia, the Philippine barcode coverage gaps reveal both parallels and contrasts. For instance, a DNA-based taxonomic survey of fishes in the Merbok Estuary in Peninsular Malaysia uncovered 139 Molecular Operational Taxonomic Units (MOTUs) from 350 specimens, exceeding the 134 species initially identified by morphology (Zainal Abidin et al. 2021). This cryptic diversity underscores underappreciated taxonomic complexity in Southeast Asian mangrove fish communities, a complexity that may also be underdetected in the Philippines due to missing reference sequences. Moreover, similar barcode library construction efforts in Southern China's Greater Bay Area have produced verified COI libraries for >100 species of mangrove-associated crabs and gastropods (Bravo et al. 2021), demonstrating that more balanced regional barcoding is feasible and thereby highlighting the relative underdevelopment of molecular coverage in the Philippine mangrove context (Bravo et al. 2021). Conversely, evaluations of barcode completeness in the Western and Central Pacific Ocean, including regions of high mangrove biodiversity, reveal significant gaps: COI coverage may reach only moderate levels in some phyla, while sequence quality and taxonomic annotation remain problematic (Zhou et al. 2025). These comparative insights emphasize that although barcode gaps are widespread, the particularly low representation of invertebrate lineages in Philippine mangroves may be more severe than in some neighboring regions, reinforcing an urgent need for regionally coordinated barcoding initiatives.

A set of clear priorities emerges from this review for strengthening eDNA-based biodiversity assessment in Philippine mangroves. First, a targeted national barcoding initiative is essential, with priority given to sequencing the 297 species currently lacking markers and to improving coverage for underrepresented phyla such as polychaetes, sponges, tunicates, and nematodes. Coordinated sampling across bioregions and seasons, paired with the strategic use of museum specimens and ethically sourced market specimens, would greatly accelerate reference library development (Bemis et al. 2023; Schmid et al. 2025). Second, adopting a multi-marker metabarcoding framework that combines short 12S/16S assays for vertebrates, COI panels for invertebrates, and Cytb for selected taxa will improve detection breadth, provided that marker performance is validated both in silico and experimentally (Xiong et al. 2022; Wang et al. 2023). Marker choice should always be guided by the local completeness of reference databases. Third, implementing a voucher-driven workflow that synchronizes field surveys with morphological identification and multilocus sequencing will reduce taxonomic errors and strengthen confidence in species assignments (Deiner et al. 2017; Bemis et al. 2023). Strengthening regional coordination with neighboring Southeast Asian programs, including Malaysia and southern China, can further expand barcode coverage for shared mangrove taxa and harmonize methodologies (Bravo et al. 2021; Zainal Abidin et al. 2021). At the policy level, prioritizing barcode generation for commercially important and threatened species will immediately enhance fisheries monitoring, conservation planning, and IUCN assessments by embedding molecular data generation into national biodiversity frameworks.

Methodologically, detectability remains a critical consideration in eDNA workflows. Although COI is the most abundant

marker in public repositories, its typical amplicon length makes it less suitable for degraded environmental DNA, whereas short-amplicon 12S and 16S markers substantially improve vertebrate detectability but depend heavily on adequate reference coverage (Wilting et al. 2021; Wang et al. 2023). Given current marker gaps in the Philippines, an effective short-term strategy is to deploy parallel assays pairing short 12S/16S vertebrate primers with COI mini-barcodes for invertebrates and to interpret outputs using a context-aware assignment pipeline that reports confidence levels and flags ambiguous sequences for voucher-based follow-up (Weigand et al. 2019; Xiong et al. 2022). Together, these recommendations provide a practical roadmap for enhancing the accuracy, resolution, and conservation relevance of eDNA metabarcoding in Philippine mangrove ecosystems.

3.5 | Integrating eDNA Metabarcoding With Traditional Biodiversity Surveys to Enhance Monitoring and Conservation in Philippine Mangroves

The integration of environmental DNA metabarcoding with traditional biodiversity assessment offers a powerful, complementary framework for monitoring Philippine mangrove ecosystems. Traditional surveys such as netting, trapping, visual censuses, and expert-based species identification remain foundational for validating species presence, documenting morphology, and providing voucher specimens. However, many mangrove-associated fauna in the Philippines inhabit cryptic, sediment-rich, or structurally complex habitats, making conventional sampling difficult, labor-intensive, and often incomplete. This is especially true for small-bodied fishes, nocturnal or burrowing invertebrates, and soft-bodied taxa such as tunicates and sponges. eDNA metabarcoding, which detects DNA shed into the environment, has the potential to overcome these limitations by capturing a broader range of taxa with minimal disturbance (Thomsen and Willerslev 2015; Deiner et al. 2017).

In a megadiverse but understudied setting like the Philippines, where 999 mangrove-associated faunal species were identified in this review, many groups still remain severely underdocumented, and the eDNA tools can substantially expand detection capacity. For instance, cryptic or low-abundance taxa that are rarely encountered in field surveys can be detected from small-volume water samples, reducing sampling bias (Stat et al. 2017). Additionally, the eDNA tool enables temporal replication at lower cost, allowing monitoring programs to detect seasonal shifts, recruitment events, or population declines in real time, a capacity rarely feasible through traditional methods alone (Bohmann et al. 2014). However, the effectiveness of eDNA metabarcoding is constrained by the completeness and accuracy of reference databases. In the Philippines, important gaps persist: 297 mangrove-associated species lack any genetic marker, while others are represented only by COI, which is often used for short-read eDNA assays. This mismatch between eDNA markers (12S & 16S) and available reference sequences can lead to false negatives, incomplete community profiles, or ambiguous taxonomic assignments (Goldberg et al. 2015; Weigand et al. 2019). Consequently, species may be present in the environment but remain undetected due to insufficient barcode

coverage, a limitation particularly evident for mollusks, echinoderms, decapods, and other understudied phyla.

Primer choice further affects detection breadth. While primers such as MiFish-U and MiDeca-U have demonstrated strong amplification performance for fishes and decapods (Polanco et al. 2021; Madduppa et al. 2022), their effectiveness is ultimately dependent on the representation of target taxa in reference databases (Antinero et al. 2024). When relevant sequences are absent or sparse, even well-designed primers cannot reliably achieve species-level resolution. This limitation highlights a major methodological constraint: primer specificity and database completeness are deeply intertwined, and overlooking this relationship can lead to biased inferences about biodiversity (Zainal Abidin et al. 2022). Therefore, a comprehensive assessment of publicly available genetic resources such as NCBI GenBank, BOLD, and regional barcoding initiatives is critical prior to primer selection. Despite these limitations, the synergy between eDNA and traditional surveys offers substantial benefits. Field-based sampling provides morphological confirmation, ecological context, abundance estimates, and vouchered specimens, all of which are required to build accurate, region-specific reference libraries (Ficetola et al. 2008). In turn, eDNA expands detection sensitivity, enhances temporal and spatial coverage, and identifies species that evade physical capture. When combined, these approaches provide a more complete and reliable characterization of mangrove biodiversity, enabling improved conservation assessments, early detection of declining or invasive taxa, and more informed management actions.

With the Philippine mangrove ecosystems facing rapid habitat loss, fisheries pressure, and climate-driven changes, this integrative approach is especially timely. Strengthening reference databases, improving primer selection, and aligning molecular data with traditional ecological surveys will significantly enhance the ability of national agencies and researchers to monitor biodiversity, detect ecological shifts, and safeguard the ecological and economic functions of mangrove ecosystems. Overall, eDNA metabarcoding does not replace traditional biodiversity assessment; rather, it amplifies its effectiveness. When the two approaches are combined, they create a monitoring system capable of detecting more species, across more habitats, with greater accuracy and frequency than either could achieve alone.

4 | Future Directions

The significant gap in sequence references for documented mangrove faunal species in the Philippines carries implications for eDNA metabarcoding studies. The absence of a comprehensive sequence reference may limit the accuracy and sensitivity of eDNA analyses, impacting the effectiveness of biodiversity assessments in mangrove habitats. These limitations also imply that current eDNA biomonitoring surveys may underestimate true species richness, overlook cryptic species, and produce community profiles that do not fully reflect the ecological structure of mangrove habitats. This has direct consequences, for instance, for management because undetected species, such as threatened, commercially important, or habitat-indicator taxa, may not be incorporated into conservation planning or policy decisions. Moreover, the ability to detect population changes,

assess habitat conditions, and respond to emerging threats may be constrained by the incompleteness of existing molecular databases. Addressing this gap becomes crucial for enhancing the reliability of eDNA metabarcoding, enabling more informed conservation measures, and promoting a thorough understanding of genetic diversity within these ecosystems.

On the other hand, there is an emerging concern about erroneous taxonomic IDs in sequences available in public databases, which must be taken into account. As such, there is a need for verification and further validation on the integrity of matching sequences from public databases. Thus, the collection and repository of voucher specimens that are properly curated must be considered when assessing the credibility of a particular sequence, especially since many sequences are shared among other species. Improving taxonomic accuracy and establishing, for example, Philippine-specific reference collections would not only enhance the reliability of eDNA-based technology but also strengthen long-term biodiversity monitoring programs, thereby enabling more ecological baselines and supporting evidence-based management strategies for mangrove conservation. Meanwhile, with the available genetic information for most faunal species, one could consider developing primers specifically for finfish and other macroinvertebrates, such as gastropods and bivalves. The expanse of genetic information available on these macroinvertebrates can serve as a tool for improving species identification by designing primers using publicly available genetic databases. This will then provide robust identification specifically for the application of eDNA in biodiversity monitoring of the country's mangrove areas.

Author Contributions

E.K.T.C., E.J.A.T., J.P.T., and V.E.L. contributed to the conception and design of the study. E.K.T.C., E.J.A.T., J.P.T., V.E.L., C.J.R.N., and A.C.E. were involved in data acquisition. E.K.T.C., E.J.A.T., J.P.T., V.E.L., and A.C.E. were involved in the data analysis and interpretation. E.K.T.C., E.J.A.T., and J.P.T. prepared the original draft of the manuscript, and all authors contributed to writing, reviewing, and editing the manuscript. V.E.L. and T.K. were responsible for funding acquisition, conceptualization, resources, supervision, and project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that all data supporting the findings of this study are provided in a separate [Supporting Information](#).

References

- Albarico, F. P. J., R. Gacutan, and A. M. Bedi. 2020. "First Record of *Thalassina kelanang* (Crustacea: Decapoda: Thalassinidae) From The Philippines." *Palawan Scientist* 12: 61–67.
- Allen, G. R., and M. Adrim. 2003. "Coral Reef Fishes of Indonesia." *Zoological Studies-Taipei* 42, no. 1: 1–72.
- Antinero, A., M. Balaba, V. Leopargas, N. M. Aspe, and T. Kajita. 2024. "Is It Really There? Addressing Inadequate Sampling and False Detection in Environmental DNA Metabarcoding." *Journal of Environmental Science and Management* 27, no. 2: 61–76.
- Baharuddin, N., N. H. M. Basir, and S. N. H. Zainuddin. 2019. "Tropical Intertidal Gastropods: Insights on Diversity, Abundance, Distribution and Shell Morphometrics of Pulau Bidong, Malaysia." *Aquaculture, Aquarium, Conservation & Legislation* 12, no. 4: 1375–1387.
- Baharuddin, N., N. B. Basri, and N. H. Syawal. 2018. "Marine Gastropods (Gastropoda; Mollusca) Diversity and Distribution on Intertidal Rocky Shores of Terengganu, Peninsular Malaysia." *Aquaculture, Aquarium, Conservation & Legislation* 11, no. 4: 1144–1154.
- Bedi, A. M. B., and J. H. Primavera. 2018. "New Record of *Thalassina spinosa* (Crustacea: Decapoda: Gebiidea: Thalassinidae) From The Philippines." *Philippine Journal of Science* 147, no. 3: 357–361.
- Bemis, K. E., M. G. Girard, M. D. Santos, et al. 2023. "Biodiversity of Philippine Marine Fishes: A DNA Barcode Reference Library Based on Voucher Specimens." *Scientific Data* 10, no. 1: 411.
- Berba, C. M. P., and A. M. A. Matias. 2022. "State of Biodiversity Documentation in The Philippines: Metadata Gaps, Taxonomic Biases, and Spatial Biases in the DNA Barcode Data of Animal and Plant Taxa in the Context of Species Occurrence Data." *PeerJ* 10: e13146.
- BMC Cancer. 2023. "Kappa Statistic Considerations in Evaluating Inter-Rater Reliability." *BMC Cancer* 23: 799.
- Bohmann, K., A. Evans, M. T. P. Gilbert, et al. 2014. "Environmental DNA for Wildlife Biology and Biodiversity Monitoring." *Trends in Ecology & Evolution* 29, no. 6: 358–367.
- Bravo, H., C. L. Y. Cheng, A. Iannucci, et al. 2021. "A DNA Barcode Library for Mangrove Gastropods and Crabs of Hong Kong and the Greater Bay Area Reveals an Unexpected Faunal Diversity Associated With the Intertidal Forests of Southern China." *BMC Ecology and Evolution* 21, no. 1: 180.
- Bucklin, A., K. T. C. A. Peijnenburg, K. N. Kosobokova, et al. 2021. "Toward a Global Reference Database of COI Barcodes for Marine Zooplankton." *Marine Biology* 168, no. 6: 78.
- Buršić, M., L. Iveša, A. Jaklin, et al. 2021. "DNA Barcoding of Marine Mollusks Associated With *Corallina officinalis* Turfs in Southern Istria (Adriatic Sea)." *Diversity* 13, no. 5: 196.
- Colgan, D. J., A. McLauchlan, G. D. Wilson, et al. 1999. "Histone H3 and U2 snRNA DNA Sequences and Arthropod Molecular Evolution." *Australian Journal of Zoology* 46, no. 5: 419–437.
- Collin, R., D. E. Venera-Pontón, A. C. Driskell, et al. 2020. "DNA Barcoding of Echinopluteus Larvae Uncovers Cryptic Diversity in Neotropical Echinoids." *Invertebrate Biology* 139, no. 2: e12292.

- Collins, R. A., J. Bakker, O. S. Wangenstein, et al. 2019. "Non-Specific Amplification Compromises Environmental DNA Metabarcoding With COI." *Methods in Ecology and Evolution* 10, no. 11: 1985–2001.
- Dayal, S. R., K. Ravuiwasa, and J. A. Bhat. 2022. "Deforestation and Degradation in the Mangrove Ecosystem: Implication on Environment and Livelihoods." In *Land Degradation Neutrality: Achieving SDG 15 by Forest Management*, 99–116. Springer Nature Singapore.
- Deiner, K., H. M. Bik, E. Mächler, et al. 2017. "Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities." *Molecular Ecology* 26, no. 21: 5872–5895.
- Derrick, B., P. Noranarttragoon, D. Zeller, L. C. Teh, and D. Pauly. 2017. "Thailand's Missing Marine Fisheries Catch (1950–2014)." *Frontiers in Marine Science* 4: 402.
- Eiamsa-Ard, M., and S. Amornchairojkul. 1997. "The Marine Fisheries of Thailand, With Emphasis on the Gulf of Thailand Trawl Fishery." In *Status and management of tropical coastal fisheries in Asia*. ICLARM Conference Proceedings (53, 208, pp. 85–95).
- Elbrecht, V., and F. Leese. 2016. "Development and Validation of DNA Metabarcoding COI Primers for Aquatic Invertebrates Using the R Package PrimerMiner." *PeerJ Preprints* 4: e2044v1.
- Elbrecht, V., P. Taberlet, T. Dejean, et al. 2016. "Testing the Potential of a Ribosomal 16S Marker for DNA Metabarcoding of Insects." *PeerJ* 4: e1966.
- Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. "Species Detection Using Environmental DNA From Water Samples." *Biology Letters* 4, no. 4: 423–425.
- Fontes, J. T., K. Katoh, R. Pires, P. Soares, and F. O. Costa. 2024. "Benchmarking the Discrimination Power of Commonly Used Markers and Amplicons in Marine Fish (e) DNA (Meta) Barcoding." *ARPHA Preprints* 5: e128777.
- Fortes, M. D., and S. Salmo III. 2017. "Status of Mangrove Research and Management in The Philippines: Challenges and Opportunities." in *Proceedings State of the Mangrove Summit*: 50–60.
- Gold, Z., A. R. Wall, T. M. Schweizer, et al. 2022. "A Manager's Guide to Using eDNA Metabarcoding in Marine Ecosystems." *PeerJ* 10: e14071.
- Goldberg, C. S., K. M. Strickler, and D. S. Pilliod. 2015. "Moving Environmental DNA Methods From Concept to Practice for Monitoring Aquatic Macroorganisms." *Biological Conservation* 183: 1–3.
- Groth, J. G., and G. F. Barrowclough. 1999. "Basal Divergences in Birds and the Phylogenetic Utility of the Nuclear RAG-1 Gene." *Molecular Phylogenetics and Evolution* 12, no. 2: 115–123.
- Hamli, H., A. H. M. Kamal, M. H. Idris, G. J. Gerusu, J. Ismail, and M. H. Abualreesh. 2021. "Bivalves (Mollusca: Bivalvia) in Malaysian Borneo: Status and Threats." *Journal of Threatened Taxa* 13, no. 11: 19553–19565.
- Harper, L. R., A. S. Buxton, H. C. Rees, et al. 2019. "Prospects and Challenges of Environmental DNA (eDNA) Monitoring in Freshwater Ponds." *Hydrobiologia* 826, no. 1: 25–41.
- Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003. "Biological Identifications Through DNA Barcodes." *Proceedings of the Royal Society B: Biological Sciences* 270, no. 1512: 313–321.
- Hestetun, J. T., E. Bye-Ingebrigtsen, R. H. Nilsson, A. G. Glover, P. O. Johansen, and T. G. Dahlgren. 2020. "Significant Taxon Sampling Gaps in DNA Databases Limit the Operational Use of Marine Macrofauna Metabarcoding." *Marine Biodiversity* 50, no. 5: 70.
- Hickerson, M. J., C. P. Meyer, and C. Moritz. 2006. "DNA Barcoding Will Often Fail to Discover New Animal Species Over Broad Parameter Space." *Systematic Biology* 55, no. 5: 729–739.
- Hoban, M. L., J. Whitney, A. G. Collins, et al. 2022. "Skimming for Barcodes: Rapid Production of Mitochondrial Genome and Nuclear Ribosomal Repeat Reference Markers Through Shallow Shotgun Sequencing." *PeerJ* 10: e13790.
- Honda, K., Y. Nakamura, M. Nakaoka, W. H. Uy, and M. D. Fortes. 2013. "Habitat Use by Fishes in Coral Reefs, Seagrass Beds and Mangrove Habitats in The Philippines." *PLoS One* 8, no. 8: e65735.
- Hutchings, P. A., and H. F. Recher. 1981. "The Fauna of Australian Mangroves." In *Proceedings of the Linnean Society of New South Wales* 106: 83–121.
- Hutchison, J., M. Spalding, and P. Zu Ermgassen. 2014. "The Role of Mangroves in Fisheries Enhancement." *Nature Conservancy and Wetlands International* 54: 434.
- Hutomo, M., and M. K. Moosa. 2005. "Indonesian Marine and Coastal Biodiversity: Present Status." *Indian Journal of Marine Sciences* 34, no. 1: 88–97.
- Ikejima, K., J. D. Ronquillo, V. L. Corre, and V. A. Dureza. 2006. "Fish Assemblages in Abandoned Ponds and Waterways Surrounding Brackish Water Aquaculture Ponds in Panay Island, the Philippines." *Asian Fisheries Science* 19, no. 3: 293.
- Jiang, R., J. L. Lusana, and Y. Chen. 2022. "High-Throughput DNA Metabarcoding as an Approach for Ichthyoplankton Survey in Oujiang River Estuary." *China. Diversity* 14, no. 12: 1111.
- Kartika, S., and Y. Mu. 2014. "A Study on Indonesian Mollusk Fishery and Its Prospect for Economy." *International Journal of Marine Science* 4: 61–66.
- Keck, F., M. Couton, and F. Altermatt. 2023. "Navigating the Seven Challenges of Taxonomic Reference Databases in Metabarcoding Analyses." *Molecular Ecology Resources* 23, no. 4: 742–755.
- Krehenwinkel, H., M. Fong, S. Kennedy, et al. 2018. "The Effect of DNA Degradation Bias in Passive Sampling Devices on Metabarcoding Studies of Arthropod Communities and Their Associated Microbiota." *PLoS One* 13, no. 1: e0189188.
- Kumar, G., A. M. Reaume, E. Farrell, and M. R. Gaither. 2022. "Comparing eDNA Metabarcoding Primers for Assessing Fish Communities in a Biodiverse Estuary." *PLoS One* 17, no. 6: e0266720.
- Landis, J. R., and G. G. Koch. 1977. "The Measurement of Observer Agreement for Categorical Data." *Biometrics* 33, no. 1: 159–174.
- Lebata, M. J. H. L., L. L. Vay, J. H. Primavera, M. E. Walton, and J. B. Biñas. 2007. "Baseline Assessment of Fisheries for Three Species of Mud Crabs (*Scylla* spp.) in the Mangroves of Ibayay, Aklan, Philippines." *Bulletin of Marine Science* 80, no. 3: 891–904.
- Lee, S. Y., J. H. Primavera, F. Dahdouh-Guebas, et al. 2014. "Ecological Role and Services of Tropical Mangrove Ecosystems: A Reassessment." *Global Ecology and Biogeography* 23, no. 7: 726–743.
- Leray, M., and N. Knowlton. 2015. "DNA Barcoding and Metabarcoding of Standardized Samples Reveal Patterns of Marine Benthic Diversity." *Proceedings of the National Academy of Sciences* 112, no. 7: 2076–2081.
- Leray, M., N. Knowlton, and R. J. Machida. 2022. "MIDORI2: A Collection of Quality Controlled, Preformatted, and Regularly Updated Reference Databases for Taxonomic Assignment of Eukaryotic Mitochondrial Sequences." *Environmental DNA* 4, no. 4: 894–907.
- Long, J. B., and C. Giri. 2011. "Mapping The Philippines' Mangrove Forests Using Landsat Imagery." *Sensors* 11, no. 3: 2972–2981.
- Lutaenko, K. A. 2016. "Biodiversity of Bivalve Mollusks in the Western South China Sea: An Overview." In *Biodiversity of the Western Part of the South China Sea*, 315–384. Dalnauka.
- Machida, R. J., M. Leray, S. L. Ho, and N. Knowlton. 2017. "Metazoan Mitochondrial Gene Sequence Reference Datasets for Taxonomic Assignment of Environmental Samples." *Scientific Data* 4, no. 1: 1–7.

- Madduppa, H., L. M. I. Sani, K. C. Nugroho, et al. 2022. "eDNA Metabarcoding of Decapod Crustaceans Across Indonesian Seas Has Implications for Biodiversity Conservation and Fisheries Sustainability." *Frontiers in Marine Science* 9: 918295.
- Masagca, J. T., A. V. Mendoza, and E. T. Tribiana. 2010. "The Status of Mollusk Diversity and Physical Setting of the Mangrove Zones in Catanduanes Island, Luzon, Philippines." *Biotropia* 17, no. 2: 62.
- Min, X., F. Li, X. Zhang, F. Guo, F. Zhang, and Y. Zhang. 2023. "Choice of Primer Pairs and PCR Polymerase Affect the Detection of Fish eDNA." *Environmental Sciences Europe* 35, no. 1: 103.
- Miya, M. 2022. "Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities." *Annual Review of Marine Science* 14: 161–185.
- Miya, M., Y. Sato, T. Fukunaga, et al. 2015. "MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA From Fishes: Detection of More Than 230 Subtropical Marine Species." *Royal Society Open Science* 2, no. 7: 150088.
- Motoh, H., and N. B. Solis. 1977. "A Preliminary Report on the Fauna of Decapod Crustacea in the Mangrove and Estuarine Areas of Batan Bay, Panay Island, Philippines." Paper Presented at the International Workshop on Mangrove and Estuarine Area Development for the Indo-Pacific Region. Manila, Philippines. November 1977.
- Nagelkerken, I. S. J. M., S. J. M. Blaber, S. Bouillon, et al. 2008. "The Habitat Function of Mangroves for Terrestrial and Marine Fauna: A Review." *Aquatic Botany* 89, no. 2: 155–185.
- Naputo, C. F. P., Y. Isowa, M. E. Gerona-Daga, M. D. Artigas, T. Kajita, and S. G. Salmo III. 2024. "Application of eDNA Metabarcoding in the Assessment of Fish Biodiversity in Philippine Mangroves: Challenges and Opportunities." *Regional Studies in Marine Science* 77: 103642.
- Nhat, M. N. H., H. T. Cong, H. N. Thi, et al. 2025. "Species Composition and Distribution Characteristics of Gastropod Mollusks (Mollusca: Gastropoda) in Central Vietnam." *Veterinary Integrative Sciences* 24, no. 2: 1–17.
- Nilsson, R. H., K. H. Larsson, A. F. S. Taylor, et al. 2019. "The UNITE Database for Molecular Identification of Fungi: Handling Dark Taxa and Parallel Taxonomic Classifications." *Nucleic Acids Research* 47, no. D1: D259–D264.
- Pantallano, A. D. S., R. U. Bobiles, and Y. Nakamura. 2018. "Dependence of Fish on Subtropical Riverine Mangroves as Habitat in the Ryukyu Islands, Japan." *Fisheries Science* 84: 613–625.
- Peristiwady, T. 2021. "Ichthyological Research and Biodiversity of Marine Fishes in Indonesia." *IOP Conference Series: Earth and Environmental Science* 789, no. 1: 12009 IOP Publishing.
- Polanco, F. A., E. Richards, B. Flück, et al. 2021. "Comparing the Performance of 12S Mitochondrial Primers for Fish Environmental DNA Across Ecosystems." *Environmental DNA* 3, no. 6: 1113–1127.
- Rajpar, M. N., and M. Zakaria. 2014. "Mangrove Ecosystems of Asia: Status, Challenges and Management Strategies." In *Mangrove Fauna of Asia*, 153–197. Springer.
- Ramos, K., R. Meier, O. Nuneza, and P. Grootaert. 2018. "Two New Species of Mangrove Dolichopodidae From Bohol Island in The Philippines (Insecta: Diptera) and a Checklist of the Dolichopodidae of The Philippines." *Raffles Bulletin of Zoology* 66: 268–276.
- Ratnasingham, S., and P. D. Hebert. 2007. "BOLD: The Barcode of Life Data System." *Molecular Ecology Notes* 7, no. 3: 355–364.
- Ruppert, K. M., R. J. Kline, and M. S. Rahman. 2019. "Past, Present, and Future Perspectives of Environmental DNA (eDNA) Metabarcoding: A Systematic Review in Methods, Monitoring, and Applications of Global eDNA." *Global Ecology and Conservation* 17: e00547.
- Salmo, S. G., and N. C. Duke. 2010. "Establishing Mollusk Colonization and Assemblage Patterns in Planted Mangrove Stands of Different Ages in Lingayen Gulf, Philippines." *Wetlands Ecology and Management* 18: 745–754.
- Sanpanich, K. 2011. "Marine Bivalves Occurring on the East Coast of the Gulf of Thailand." *Science Asia* 37: 195–204.
- Sanpanich, K., and T. Duangdee. 2013. "The Biodiversity of Marine Gastropods of Thailand in the Late Decade." *Malaysian Journal of Science* 32: 47–64.
- Schmid, S., N. Straube, C. Albouy, et al. 2025. "Unlocking Natural History Collections to Improve eDNA Reference Databases and Biodiversity Monitoring." *Bioscience* 75: 1083–1095.
- Schoch, C. L., K. A. Seifert, S. Huhndorf, et al. 2012. "Nuclear Ribosomal Internal Transcribed Spacer (ITS) Region as a Universal DNA Barcode Marker for Fungi." *Proceedings of the National Academy of Sciences* 109, no. 16: 6241–6246.
- Sim, J., and C. C. Wright. 2005. "The Kappa Statistic in Reliability Studies: Use, Interpretation, and Sample Size Requirements." *Physical Therapy* 85, no. 3: 257–268.
- Stat, M., M. J. Huggett, R. Bernasconi, et al. 2017. "Ecosystem Biomonitoring With eDNA: Metabarcoding Across the Tree of Life in a Tropical Marine Environment." *Scientific Reports* 7, no. 1: 12240.
- Sun, S., Q. Li, L. Kong, et al. 2016. "DNA Barcoding Reveal Patterns of Species Diversity Among Northwestern Pacific Molluscs." *Scientific Reports* 6, no. 1: 33367.
- Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. "Towards Next-Generation Biodiversity Assessment Using DNA Metabarcoding." *Molecular Ecology* 21, no. 8: 2045–2050.
- Thomsen, P. F., and E. Willerslev. 2015. "Environmental DNA—an Emerging Tool in Conservation for Monitoring Past and Present Biodiversity." *Biological Conservation* 183: 4–18.
- Valentini, A., P. Taberlet, C. Miaud, et al. 2016. "Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding." *Molecular Ecology* 25, no. 4: 929–942.
- Van Der Loos, L. M., and R. Nijland. 2021. "Biases in Bulk: DNA Metabarcoding of Marine Communities and the Methodology Involved." *Molecular Ecology* 30, no. 13: 3270–3288.
- Van Nguyen, L., and D. X. Mai. 2020. "Reef Fish Fauna in the Coastal Waters of Vietnam." *Marine Biodiversity* 50, no. 6: 100.
- Wang, Z., X. Liu, D. Liang, Q. Wang, L. Zhang, and P. Zhang. 2023. "VertU: Universal Multilocus Primer Sets for eDNA Metabarcoding of Vertebrate Diversity, Evaluated by Both Artificial and Natural Cases." *Frontiers in Ecology and Evolution* 11: 1164206.
- Wee, A. K., S. G. Salmo Iii, K. Sivakumar, et al. 2023. "Prospects and Challenges of Environmental DNA (eDNA) Metabarcoding in Mangrove Restoration in Southeast Asia." *Frontiers in Marine Science* 10: 1033258.
- Weigand, H., A. J. Beermann, F. Čiampor, et al. 2019. "DNA Barcode Reference Libraries for Monitoring Aquatic Biota in Europe: Gap-Analysis and Recommendations for Future Work." *Science of the Total Environment* 678: 499. <https://pubmed.ncbi.nlm.nih.gov/31077928/>.
- Wells, F. E., K. Sanpanich, S. K. Tan, and T. Duangdee. 2021. *The Marine and Estuarine Molluscs of Thailand*, 195. Lee Kong Chian Natural History Museum, National University of Singapore.
- Wilting, A., T. V. Nguyen, J. Axtner, et al. 2021. "Creating Genetic Reference Datasets: Indirect Sampling of Target Species Using Terrestrial Leeches as Sample Collectors." *Environmental DNA* 4, no. 2: 311–325.
- Winter, D. J. 2017. "Rentrez: An R Package for the NCBI eUtils API." *R Journal* 9: 520–526.
- WoRMS Editorial Board. 2022. "World Register of Marine Species." <https://www.marinespecies.org>.

- Xiong, F., L. Shu, H. Zeng, X. Gan, S. He, and Z. Peng. 2022. "Methodology for Fish Biodiversity Monitoring With Environmental DNA Metabarcoding: The Primers, Databases and Bioinformatic Pipelines." *Water Biology and Security* 1, no. 1: 100007.
- Zainal Abidin, D. H., S. A. M. Nor, S. Lavoué, M. A. Rahim, N. A. Jamaludin, and N. A. Mohammed Akib. 2021. "DNA-Based Taxonomy of a Mangrove-Associated Community of Fishes in Southeast Asia." *Scientific Reports* 11, no. 1: 17800.
- Zainal Abidin, D. H., S. A. M. Nor, S. Lavoué, M. A. Rahim, N. A. Jamaludin, and N. A. Mohammed Akib. 2022. "Assessing a Megadiverse but Poorly Known Community of Fishes in a Tropical Mangrove Estuary Through Environmental DNA (eDNA) Metabarcoding." *Scientific Reports* 12, no. 1: 16346.
- Zhang, S., J. Zhao, and M. Yao. 2020. "A Comprehensive and Comparative Evaluation of Primers for Metabarcoding eDNA From Fish." *Methods in Ecology and Evolution* 11, no. 12: 1609–1625.
- Zhou, Y., A. Trujillo-González, S. Nicol, R. Huerlimann, S. D. Sarre, and D. Gleeson. 2025. "Evaluation of DNA Barcoding Reference Databases for Marine Species in the Western and Central Pacific Ocean." *PeerJ* 13: e19674.
- Zinger, L., A. Bonin, I. G. Alsos, et al. 2019. "DNA Metabarcoding—Need for Robust Experimental Designs to Draw Sound Ecological Conclusions." *Molecular Ecology* 28, no. 8: 1857–1862.
- Zvonareva, S. S., E. S. Mekhova, V. T. Hà, and Y. I. Kantor. 2019. "Checklist of Bivalve Molluscs in Mangroves of Khánh Hòa Province Vietnam." *Molluscan Research* 39, no. 4: 296–312.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Faunal species recorded in Philippine mangrove ecosystems with taxonomic groups and genetic markers suitable for eDNA metabarcoding.