

# Aquatic biodiversity in a pond on the airport landside areas through environmental DNA metabarcoding: Implementation for Aviation Security Management

DWI SENDI PRIYONO<sup>1,\*</sup>, AKBAR REZA<sup>1</sup>, RURY EPRILURAHMAN<sup>1</sup>, DONAN SATRIA YUDHA<sup>1</sup>,  
FARADINA MUFTI<sup>1</sup>, NOORMAN HENDRY FAUZY<sup>2</sup>, RADEN BAMBANG TRIYONO<sup>3</sup>,  
PANGGIH KURNIA ADHI<sup>3</sup>

<sup>1</sup>Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknik Selatan, Sleman 55281, Yogyakarta, Indonesia.  
Tel./fax.: +62-274-580839. \*email: dwisendipriyono@ugm.ac.id

<sup>2</sup>Department of Conservation of Forest Resources and Ecotourism, Faculty of Forestry and Environment, Institut Pertanian Bogor. Jl. Ulin Lingkar  
Akademik, Dramaga, Bogor 16680, West Java, Indonesia

<sup>3</sup>PT Angkasa Pura I, Yogyakarta International Airport. Palihan, Temon, Kulon Progo 55654, Yogyakarta, Indonesia

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**Abstract.** Priyono DS, Reza A, Eprilurahman R, Yudha DS, Mufti D, Fauzy NH, Triyono RB, Adhi PK. 2022. Aquatic biodiversity in a pond on the airport landside areas through environmental DNA metabarcoding: Implementation for Aviation Security Management. *Biodiversitas* 23: 3638-3645. Environmental DNA (eDNA) has become a widely used tool for aquatic biodiversity monitoring, as well as for formulating effective landscape management strategies. Yogyakarta International Airport (YIA), Indonesia, has a green belt landscape located on the Indian Ocean coast. YIA's landscape biodiversity analysis is still unexplored. Herein, we first used high throughput sequencing DNA metabarcoding to identify and investigate aquatic biodiversity in a pond on the airport landside areas. The metabarcoding eDNA sequencing analysis yielded 224,737 raw reads from the metagenomics utilizing Illumina MiSeq sequencing. We identified 588 eukaryote species from 205 families and 300 genera. Actinopteri is the most dominant class with a diversity of 160 taxa, followed by Mammalia (88), Amphibia (60), and Chlorophyceae (25). At the order level, Anura has the highest order diversity (50), followed by Rodentia (31), Chiroptera (17), Eulipotyphla (16), Squamata (15), and Cypriniformes (14). We found that *Plasmodium* was the genus with the highest relative abundance in this pond (18,030 reads). Furthermore, the large variety of fish and other taxa in this pond may attract waterbirds, increasing the risk of bird strike. The abundance of *Plasmodium* sp. in this airport area is an important issue, especially regarding airport malaria risk. Integration of biodiversity monitoring using eDNA with aviation security management provides valuable information for the airport's wildlife hazard management plan. To prevent the recurrence of bird strikes and prevent airport malaria, aviation security strategies utilizing habitat management approaches are recommended.

**Keywords:** Airport malaria, bird strike, biomonitoring, environmental DNA metabarcoding, wildlife hazard management plan

**Abbreviations:** eDNA: environmental DNA, YIA: Yogyakarta International Airport

## INTRODUCTION

Global biodiversity loss is a major issue for humanity and slowing or preventing it is a goal embraced by a broad international political consensus (Thomsen and Willerslev 2015). The lack of global information about biodiversity conditions and patterns is a fundamental impediment to reaching this goal (Geijzenendorffer et al. 2016). Traditionally, such monitoring has relied on morphological identification of species through visual surveys and individual counts. Limitations of taxonomic knowledge for morphology identifications and non-standard collection methodologies could make these attempts challenging (Corlett 2017). In recent years, molecular approaches have been used to address an important demand for reliable vast biodiversity assessment (Bohmann et al. 2014; Hunter et al. 2018). In this way, molecular approaches, more recently, metabarcoding, contribute to biodiversity assessment (Ji et al. 2013). Metabarcoding was proven to recover significant portions of existing biodiversity and reveal previously

unknown patterns of biodiversity in its first few years, and it has since been effectively applied to global-scale biodiversity surveys (Aylagas et al. 2014; Leray and Knowlton 2015; Taberlet et al. 2018).

Environmental DNA (eDNA) and DNA metabarcoding are transforming biodiversity monitoring at all scales because they reduce the limitations of morphological identification and allow for the efficient observation of many taxa that are challenging to collect and identify using conventional methods (Taberlet et al. 2018; Ruppert et al. 2019). eDNA is DNA obtained from environmental materials without the isolation of any target organism. This strategy has recently been broadened to include eukaryotic species by taking use of the concept that all species produce environmental DNA (eDNA) remnants of their genetic material in the environment through releasing and accumulating waste products (Valentini et al. 2016). Environmental DNA has a wide range of potential applications, including detecting endangered or unwanted organisms without having to catch them, characterizing

communities in difficult-to-sample areas like large rivers, and reducing the impact and increasing the power of biological surveys (Goldberg et al. 2016). This eDNA method provides a lot of biodiversity data, which has the potential to provide essential and effective recommendations for landscape management plans. For example, civil transportation landscapes such as airports, use a relatively wide landscape and require an appropriate biodiversity analysis.

Yogyakarta International Airport (YIA) is a unique airport with a green belt area that is located near the Indian Ocean's coast. The character YIA's wide, flat, and open area may be appealing to some species looking for a place for resting, loafing, foraging, or hiding from predators. However, biodiversity studies such as wildlife population in the YIA landscape are still scarce. On the other hand, civil aviation's ability to satisfy the requirements of global business is dependent on safety and revenue. Aviation accidents with birds and other wildlife (wildlife strikes) constitute a growing safety and cost risk to the global civil aviation industry (Dolbeer et al. 2021). A metabarcoding analysis was performed at Perth Airport to reliably identify prey species of birds that are at high hazard of bird-strike (Coghlan et al. 2013). Dietary information generated from DNA has the ability to reveal important information about feeding ecosystems inside and around the airport.

Habitat conditions that need to be an issue in relation to wildlife are the presence of several relatively large natural ponds inside the YIA area. Thus, it is important to invest in information on habitat management studies, especially in this pond, as a Wildlife Hazard Management Plan (WHMP) to support flight safety at Yogyakarta International Airport. In this study, we first used high throughput DNA metabarcoding to detect and explore aquatic biodiversity in pond on the airport landside area. A biodiversity viewpoint is crucial in airport business development and safety management because it can influence aviation safety (Ntampakis 2013; Smith et al. 2020). Our approach provides a useful opportunity to incorporate biodiversity monitoring through DNA metabarcoding into airport planning.

## MATERIALS AND METHODS

### eDNA water sample collection

Sampling was conducted in a pond on the YIA landside areas (7°53'26.44"S 110° 3'39.06"E) (Figure 1A). This pond is characterized by herbaceous and shrubby, shallow (about 1.5 m deep) (Figure 1B). Two liters of water from pond were obtained. Water sample was collected at a depth of 0.5m. Due to the sensitivity of eDNA samples, the sample bottles were carefully packaged and stored in the icebox. The eDNA water samples were stored in a 20°C for 3-4 days until total eDNA extraction was completed.

### Water filtration and DNA extraction

A 1,000 mL water sample was filtered via new sterile 0.45 m pore size Nitrocellulose membrane (Whatman™, USA) and membrane filter holder apparatus. The

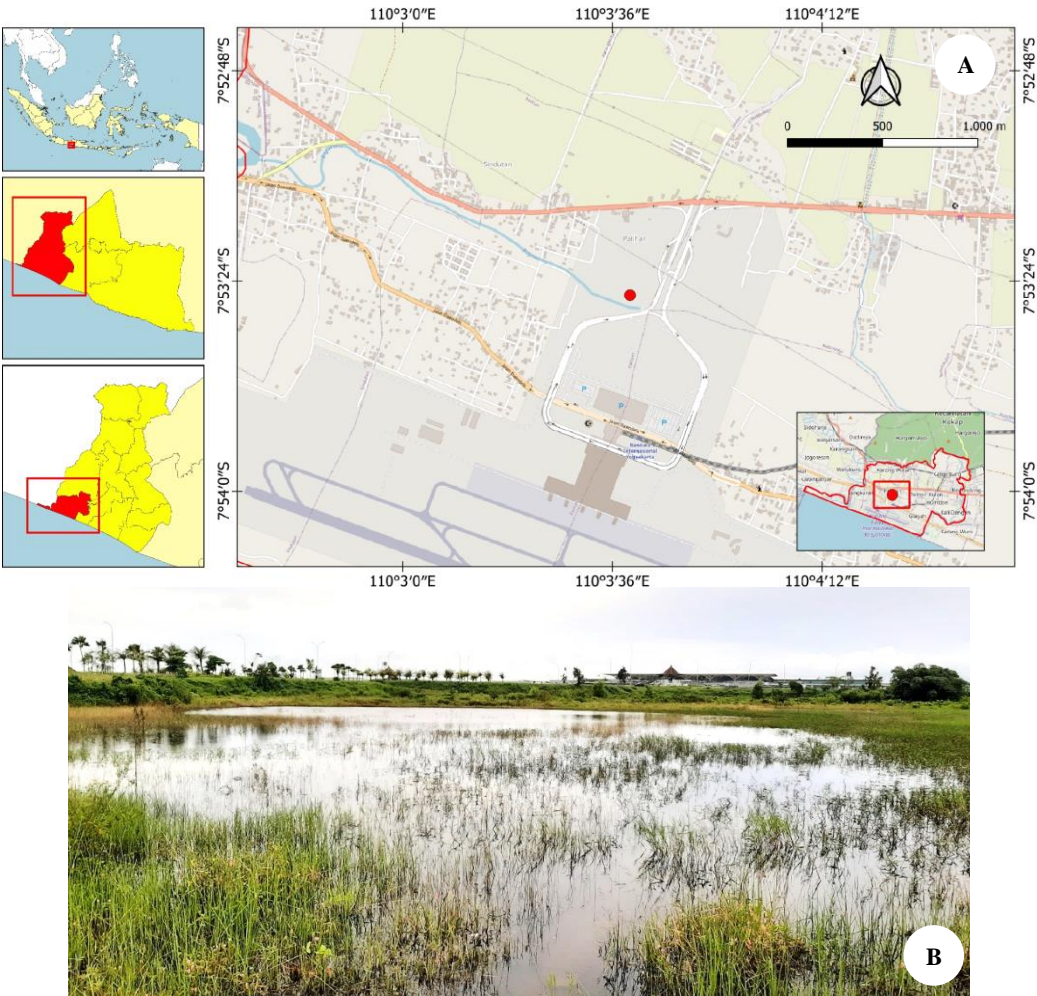
membrane was removed aseptically. The ZymoBIOMICS™ DNA Mini was used to isolate DNA genome from the filter membrane (Qiagen, Hilden, Germany). To ensure that the eDNA extraction processes were free of cross-contamination, eDNA was isolated from distilled water as a negative control. The quality of the extracted DNA was then checked using Qubit fluorometric quantitation. After the DNA extract was checked using fluorometric, eDNA extraction from the negative control showed no DNA content, so it can be confirmed that there was no cross-contamination in the eDNA extraction process.

### Polymerase Chain Reaction (PCR) amplification, library preparation, and sequencing

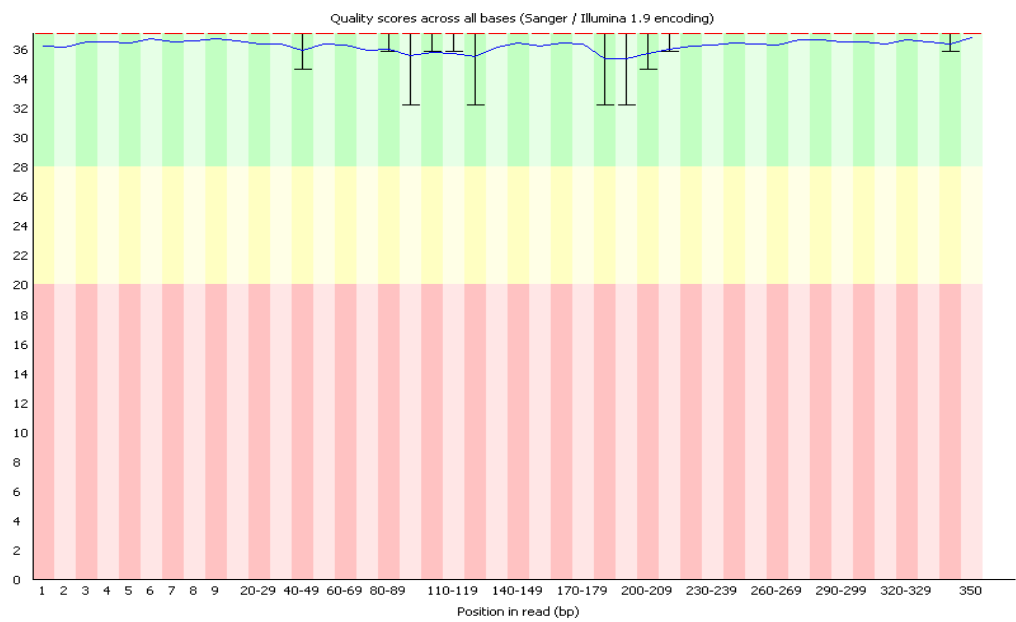
Subsequently, for PCR amplification, universal primer pair targeting the cytochrome oxidase subunit 1(COI) gene was chosen. The COI gene primer sequences were: mICOIntF 5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'; and mICOIntR 5'-GGRGGRTASACSGTTCASCCSGTSCC-3' (Leray et al. 2013). The PCR was carried out in triplicate, and all of the PCR amplicons were pooled together in the same volume. eDNA samples, blanks, and negative controls were included in each set of replicates. The total volume of the three reactions was 25 µL, which included 5 µL 5x buffer, 2 µL dNTP (2.5 mM), 1 µL (10 µM) forward primer mICOIntF, 1 µL (10 µM) reverse primer mICOIntR, 1.2 µL DNA template, 0.25 µL DNA polymerase, molecular grade water added to 25 µL. Thermal conditions for the PCR step were initial denaturation at 95°C for 2 min, 35 cycles of denaturation at 95°C for 30s, annealing at 65°C for 20 s, elongation at 72°C for 30s, and final elongation at 72°C for 10 min. The 1.5% agarose gel electrophoresis was used to detect, and gel cut for PCR product purification. Furthermore, libraries were sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) to use a MiSeq Reagent v3 Kit according to the manufacturer's instructions.

### Bioinformatics and data analysis

The sequence reads quality of the original high-throughput sequencing data was evaluated. The index and barcode information were separated from the sequences, and the barcode sequences were removed. FASTQ format was used to save the sequencing data (Cock et al. 2010). The FastQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) was used to check the quality of the generated Illumina raw read. Sequencing adapters and reads with less than 100 bp were deleted. Bases with an average quality of less than Q25 were trimmed. Then, the filtered forward and reverse reads obtained were merged by overlapping paired end reads in order to increase the quality of data analyses. To denoise and delete chimeric sequences, and to identify amplicon sequence variants (ASVs), the DADA2 plugin in QIIME2 (Callahan et al. 2016) has been used in single-read mode. DADA2 quality control generated a dereplicated sequence, which was classified as an amplicon sequence variant (ASV). With 100 percent similarity clustering, ASV is equal to OUT (McDonald et al. 2012).



**Figure 1.** Sampling location for eDNA analysis. A. map of sampling locations inside YIA, B. the landscape character of the pond sampling location



**Figure 2.** Per base sequence quality of sample as generated by FASTQC. The X-axis indicates the position of the nucleotide in read and the Y-axis indicates the sequence quality score (Q). The mean quality scores are shown by the blue line. The 10% and 90% points are represented by the upper and lower whiskers, respectively

We combined ASV sequences with ASV tables once we finished denoising all libraries, and we deleted singleton ASVs. The size distributions of high-quality sequences have been calculated. Annotation screening was carried out using the QIIME2 software. UCLUST was applied to assemble the readings prior to taxonomy assignment. Identity >95% and E-value:  $10^{-5}$  were adopted as comparison criteria (Edgar 2010). Downloaded databases from Barcode of Life Data (BoLD) system (<https://www.boldsystems.org>) were used to make taxonomic annotations for COI. Finally, using the sequence frequency within the same species, the relative abundance was estimated. The hierarchical visualization and taxonomic compositions of species were classified according to Krona (Ondov et al. 2011).

## RESULTS AND DISCUSSION

### Sequence reads

The water sample yielded a total of 224,737 raw reads from the metabarcoding eDNA sequencing analysis in this study, with >98 percent of reads retained, and the average length is 311 bp after quality processing. The total number of readings obtained after the quality-control process was 151,763 reads. The distribution of per base sequence quality of the effective tags can be seen in Figure 2, The per base sequence quality stays within the green area (>Q28).

### Taxonomic assignments

An assignment analysis in the metabarcoding pipeline was performed on environmental DNA water samples to determine the composition, taxonomy diversity, and relative abundance in a pond inside the airport. Eukaryotes were the most commonly assigned domain, accounting for 98% of all assignments, followed by Bacteria (0.8%) and unidentified (1.2%). The Krona, a web browser metagenomic visualization tool, was used to visualize the diversity of the assignment at various taxonomic levels for eukaryote (Figure 3A). Any assignment with an ambiguous or undefined status was eliminated from the relative abundance chart value in any taxonomic unit. In this study area, 588 eukaryotes species from 205 families and 300 genera were identified.

Figure 3B displays hierarchical data at various taxonomic levels. Taxonomy diversity was 46.6% at the species level, 23.8% at the genus level, 16.3% at the family level, 8.6% at the order level, 3.2% at the class level, and 1.6% at the phylum level from the total assignment. A total of 20 phyla with five dominant phyla sequences were chordates (385), Chlorophyta (33), arthropods (8), oomycota (8), and mollusks (5). The number of taxonomic diversity at the class level from the assignment is 40 classes. Actinopteri is the most dominant class with a diversity of 160 taxa, followed by Mammalia (88), Amphibia (60), Chlorophyceae (25), and Ascidiacea (24). At the order level, Anura has the highest order diversity (50), followed by Rodentia (31), Chiroptera (17), eulipotyphla (16), Squamata (15), Cypriniformes (14),

Sphaeropleales (14), Perciformes (13), and Siluriformes (13). Soricidae (15) was the frequently assigned family in eukaryotes, followed by Gobiidae (11), Characidae (9), and Vespertilionidae (9). At the level of genera, diversity is nearly uniform, including *Didemnum* (7), *Johnius* (7), *Molgula* (5), *Sorex* (5), *Thoracosphaera* (5), *Crocidura* (5), *Rana* (4), *Ascidia* (4), *Astyanax* (4), *Episoriculus* (4), *Pandorina* (4), *Myotis* (4), *Phelsuma* (4), *Phyllomys* (4), and *Theلودerma* (4).

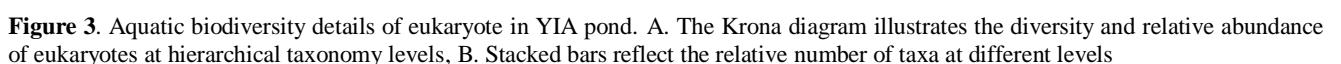
The clustered chart in Figure 4 shows various dominant classes based on the relative abundance of sequences read generated. The top ten classes with the most dominant abundance frequencies were actinopteri (45,616), Mammalia (25,503), aconoidasida (18,572), Amphibia (10,819), Phaeophyceae (5,875), Dinophyceae (5,435), Hexapoda (4,473), Ascidiacea (4,153), Chlorophyceae (3,362), and Discosea (2,822). Interestingly, we found that plasmodium was the genus with the highest relative abundance (18,030), followed by *Rasbora* (7,219), *Rana* (4,613), *Ostorhinchus* (4,593), *Homidia* (4,473), *Pseudorhombus* (4,451), *Desmarestia* (4,083), *Ostreopsis* (3,923), and *Glossophaga* (3,915).

### Discussion

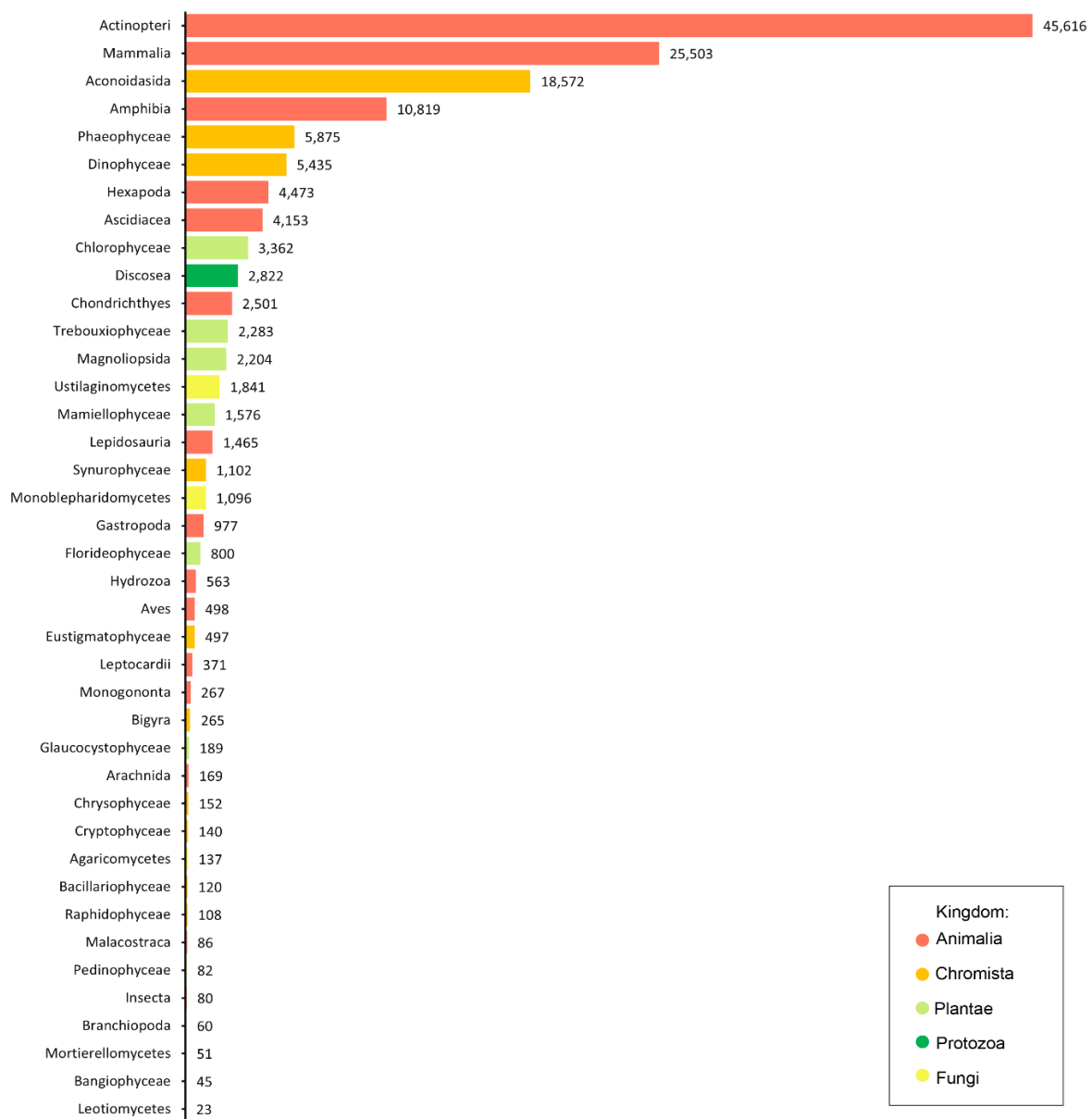
Biodiversity monitoring using molecular tools has been proven to effectively and accurately identify various species. The prospect of biodiversity monitoring is incredibly optimistic because eDNA improves species detectability, requires less effort, doesn't harm ecosystems, and may be utilized in areas where conventional surveys are unfeasible (Valentini et al. 2016). This is the first study to uncover aquatic biodiversity in an unexplored pond on the landside YIA area. As a result of the identification utilizing eDNA through water samples, a wide range of aquatic biodiversity has been revealed. We identified 588 eukaryotic species in this pond, expanding the range of aquatic organisms that can be investigated with minimal volumes of water.

It has been demonstrated that whole-eukaryotic community assessments utilizing eDNA metabarcoding advance efficient biomonitoring techniques and get us further to a comprehensive inventory of biodiversity. For instance, the Caribbean Sea's eDNA metabarcoding, which generates a high level of eukaryotic MOTU (molecular operational taxonomic unit) richness (12,769 MOTUs) (Bakker et al. 2019). Ratcliffe et al. (2021) compared the method of sampling fish larvae using nets with the eDNA metabarcoding method, resulting in equivalent estimates of species richness and diversity, with 75% agreement. Used eDNA metabarcoding for monitoring fish communities in coral reef lagoons in Okinawa, Japan. The eDNA metabarcoding results detected various species as well as clear differences in fish communities between reef edges and shore-side seagrass beds even in small lagoons, indicating habitat separation. These instances advance eDNA observation as a robust noninvasive sampling technique for practical use with increased reliability in a variety of systems and throughout the branches of the tree of life.

autochthonous (locally acquired) malaria is known as "airport malaria," since cases are primarily localized around international airports (Siala et al. 2015; Alenou and Etang 2021). The travels of *Anopheles* mosquito vectors and humans infected with *Plasmodium* spp. parasites are strongly linked to the epidemiological patterns of imported malaria (Alenou and Etang 2021; Van Bortel et al. 2021). Furthermore, airport safety management measures are necessary to minimize and prevent the risk of malaria, considering that this disease can rapidly become fatal if not diagnosed and treated promptly.







**Figure 4.** The sequence read abundance of various classes from the YIA pond eDNA water sample

Even though the pond emerged naturally inside YIA, we found a high level of species diversity. Actinopteri is the class with the highest level of taxonomy diversity, indicating habitat suitability for freshwater fishes in this pond. Several variables affect the diversity of freshwater fish assemblages, including physical, chemical, and landscape parameters (López-Delgado et al. 2020). While numerous factors can go into establishing a suitable habitat for fish, one of the most important appears to be the abundance of various types of fish diet available in this pond, such as diverse species of algae, insects, and mollusks.

The vast diversity of fishes and other taxa, on the other hand, may attract a variety of waterbirds to forage in this pond. We frequently observe some waterbirds in the pond.

The availability of Nankeen kestrel (*Falco cenchroides*) diet at the airport ground has become one of the main causes of bird strike incidents at Brisbane Airport (Leach 2013). The presence of a water bird colony inside the airport will potentially increase the risk of a bird strike by aircraft. YIA is geographically located near the coast of the Indian Ocean. Airports located near the coast have also reported that waterbirds are priority management issue for aviation security to minimize the bird strike risk (Brown et al. 2001; DeVault et al. 2016; Fu et al. 2016). Aviation security could be jeopardized by bird strikes. Bird strikes have been more common in recent decades, causing not only significant financial losses for airports but also posing a risk to aviation security (Weber 2021). (Brown et al. 2001) suggested that habitat modifications and improving

the ability of the bird control unit to diminish bird flocks at the airport via nonlethal bird dispersal methods were ineffective in reducing bird strikes at the Jamaica Bay laughing gull populations. The other strategy is the use of trained raptors. When the frequency of a particular bird species existing at the airport is calculated, (Kitowski et al. 2011) observed that using trained raptors from European starlings and northern wings to disperse birds offers effective results. These aviation security management strategies need to be implemented to minimize the risk of birdstrike events.

However, we were unable to detect these waterbirds with eDNA. We assume that several limitations of eDNA affect the overall taxon identification results, including:

- (i) Inadequate sample collection. Imperfect identification is an inherent component of most abundance data: even in traditional ecological field research, individuals and species present at one site are not always identified all the moment and failing to account for imperfect detection can lead to biased conclusions (Lahoz-Monfort et al. 2014). According to (Ficetola et al. 2015), the optimal level of replication and the collection of multiple samples from a single sample can be altered to reduce the probability of false detections. As a result, adding sampling locations based on the study areas would improve the chances of identifying organisms.
- (ii) eDNA is not well preserved. The amount of eDNA secreted by an organism is proportionate to its size; However, distinct species release varying amounts and rates of DNA. (Russo et al. 2021). Furthermore, eDNA preservation is essential due to the fact that long transport after sample collection, duration, and other factors all have an impact on eDNA stability (Yudha et al. 2021). As a result, sample preservation should be carefully considered, and proper filtration upon sampling would reduce the level of eDNA degradation.
- (iii) Lack of local reference database. The misclassification of species that have not been detected in Yogyakarta, Indonesia, is the result of inadequacies in taxonomic records in databases, which led to the assignment of multiple taxa to the most similarly related species. The effectiveness of eDNA is dependent on the accuracy of the classification criteria and the reference sequence repository (Gold et al. 2021). We will explore building a local database in future investigations and studies.
- (iv) Inappropriate of COI primer to waterbirds. The primers we utilized may not be appropriate for identifying waterbirds in pond, which could explain the low number of successful identifications. Other primers, such as the mitochondrial 12S subunit of RNA primer for waterbird amplification, have been employed in many studies (Ushio et al. 2018; Neice and McRae 2021). Different primers could be produced in future tests to see if they are more suitable for YIA pond waterbirds.

As technology progresses and practices become more established, eDNA metabarcoding will likely continue as a novel technique that is still under improvement for a considerable amount of time. Although the use of eDNA is

growing rapidly on a worldwide level, there are still considerable gaps in understanding, particularly in terms of methodology and applications. The results of this study were successful in detecting numerous aquatic species in a transient and naturally formed pond inside the airport. The detection of plasmodium relative abundance has the potential to increase the risk of airport malaria. Furthermore, the existence of a water bird population inside the airport may increase the risk of an aviation bird strike. To reduce the probability of bird strike incidents, several aviation security management strategies must be adopted.

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