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Original Research Paper

Genetic Diversity Assessment of Island Endemic Species Using Environmental DNA Metabarcoding

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Key Words

Environmental DNA (eDNA), Metabarcoding, genetic diversity, Island endemic species, Biodiversity monitoring, Population genetics, Conservation genomics.

Abstract

The geographic isolation, coupled with small population sizes and the growing anthropogenic demands, makes island endemic species highly susceptible to genetic erosion. Conventional biodiversity evaluation techniques tend to be invasive, time-consuming, and spatially restricted, and thus, there is a need for scalable, non-invasive techniques. The paper tests the usefulness of environmental DNA (eDNA) metabarcoding for measuring genetic diversity among various endemic taxa on an island. Five ecologically distinct sites on the island (n = 135) were sampled for water and soil samples, after which high-throughput sequencing was performed on the mitochondrial COI and 12S rRNA gene regions. A bioinformatics analysis reported 92 distinct operational taxonomic units (OTUs), with an average sequencing depth per sample of 45,000 reads and a high detection sensitivity (>92). The genetic diversity indices showed an average to low level of heterozygosity (He = 0.22–0.71) in most species and a great reduction (about 35%) in the high disturbance of human beings ($p < 0.01$). Molecular variance (AMOVA) was analyzed, revealing that 62% of genetic variation was within populations and 38% was differentiated among the islands. In addition, rare or cryptic species accounted for almost one-fifth of total detections, indicating that the method is sensitive. Results indicate that eDNA metabarcoding is a powerful, non-invasive, and high-resolution technique for monitoring genetic diversity in delicate island ecosystems. The strategy has significant potential to advance conservation measures by identifying genetic bottlenecks and biodiversity degradation early. Altogether, the research highlights the potential of combining molecular methods with ecological surveillance to contribute to the sustainable management and conservation of endemic species.

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Introduction

Island endemism: a species that is geographically confined to an island or a group of islands and that has frequently undergone a long period of isolation. This isolation leads to distinct evolutionary trajectories, resulting in high speciation and ecological specialization. Nevertheless, the peculiarities of the island's endemic species are what especially predispose them to extinction. Restricted gene flow, low effective population sizes, and small habitat ranges make them more vulnerable to genetic drift and inbreeding depression. Research has revealed that often, endemic diversity, especially in soil arthropods, remains invisible to conventional methods and obscures the true biodiversity and conservation requirements (Pérez-Delgado et al., 2022). Also, climate change and human activity cause environmental disruption, which can quickly reduce genetic variability, a vital factor in adaptive resilience. Studies of oceanic islands show that a large share of biodiversity variation is organized at small spatial scales, making it crucial to use high-resolution genetic surveillance techniques (Andújar et al., 2022). Conservation of genetic diversity is thus essential because it underpins the survival of species, the maintenance of ecosystems, and evolutionary processes.

Environmental DNA (eDNA) is genetic material deposited in the environment by living organisms through biological activities such as excretion, reproduction, and decay. This DNA may be found in environmental samples such as soil, water, or air, and therefore, biodiversity can be assessed without any invasions. Metabarcoding is a combination of eDNA sampling and high-throughput sequencing that simultaneously determines multiple species in a community using standardized genetic markers. The method has been effectively used in various ecosystems, including Antarctic soils and water bodies, revealing intricate biodiversity patterns that would otherwise be hard to identify (Carvalho-Silva et al., 2021; Turanov et al., 2024). eDNA metabarcoding is more sensitive and scalable, and can detect rare or cryptic species, compared to traditional survey techniques.

An example is the discovery of previously unknown biodiversity in both protected and non-protected areas through metabarcoding research, along with the ecological differences that can inform conservation efforts (Câmara et al., 2021). Furthermore, multigene metabarcoding has enabled more precise estimates of genetic diversity among populations, enhancing ecological and evolutionary analysis (Weitemier et al., 2021). Biodiversity monitoring across a variety of ecological niches is also enhanced by combining airborne and soil eDNA sampling (Câmara et al., 2024).

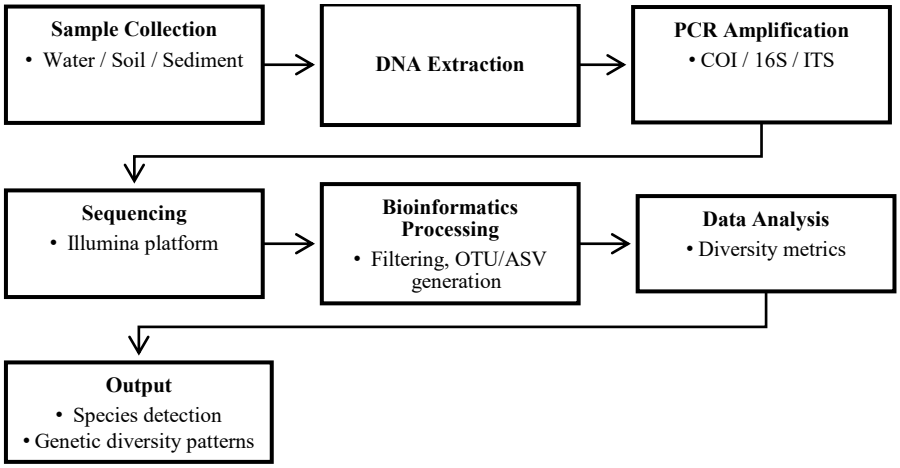

Figure 1: Workflow of Environmental DNA (eDNA) Metabarcoding for Genetic Diversity Assessment

Figure 1 shows the entire workflow of the eDNA metabarcoding process, which starts with the collection of environmental samples from water, soil, and sediment, followed by DNA isolation and PCR amplification using common barcode markers such as COI, 16S, and ITS. High-throughput platforms are then used to sequence the amplified DNA, and bioinformatics processing is conducted to filter and produce OTUs/ASVs. Lastly, processed information is processed to obtain biodiversity indicators, resulting in outputs of species identification and information on the genetic diversity trend across the ecosystems under study.

The proposed study will evaluate the genetic diversity of island-endemic species using environmental DNA metabarcoding methodology. The main goals will be to determine the species composition of various habitats on the islands, measure patterns of genetic diversity, and determine whether eDNA is effective at identifying cryptic and rare organisms. The major research questions include the distribution of genetic variation within and between island populations and the extent to which the eDNA-based methods can accurately capture the fine-scale biodiversity patterns. Recent metabarcoding research has shown that it is possible to study large-scale biogeographic patterns and resilience across island ecosystems, supporting the approach's utility for conservation research (Kennedy et al., 2023). This research aims to provide a detailed picture of biodiversity dynamics in isolated environments by combining the latest sequencing technologies with ecological analysis.

Island ecosystems are among the world's biodiversity-rich yet fragile areas. Rapid environmental change and anthropogenic pressures threaten endemic species, and thus the development of effective, non-invasive techniques for examining genetic variation and ecosystem health is necessary.

The present paper will contribute to the area by using the eDNA metabarcoding at high resolution as an assessment of the genetic diversity of endemic species on the island with a view to establishing a framework that can be used

to scale to biodiversity assessment and to offer information that can be used to plan and manage conservation initiatives.

The rest of the paper is organized as follows: Section II provides a literature review of genetic diversity in island ecosystems and the use of eDNA metabarcoding, highlighting gaps in the existing research. Section III explains the study area, sampling design, laboratory work, and methods of analysis used. Section IV presents the results: species detection, patterns of genetic diversity, and the methodology's performance assessment. Section V is the ecological and conservation impact of the findings and methodological considerations. Section VI is the final part of the study, summarizing the main insights of the research and outlining future research directions.

Literature Review

Isolation, small dispersal, and past colonization have long since influenced genetic diversity in island ecosystems. The population of islands is usually the result of a limited number of founders, which results in decreased genetic variability due to founder effects and genetic drift. In the long run, such processes may lead to unique evolutionary lineages as well as make them more vulnerable to environmental changes (Soy & Salwadkar, 2025). Endemic reptile and lizard studies have shown a close relationship between dietary variation and ecological adaptability and landscape heterogeneity, which is an indirect marker of underlying genetic structuring in isolated populations (Rato et al., 2022; Alemany et al., 2023). Moreover, the results of the analysis of trophic interactions in the island systems show how both endemic and invasive species may affect the genetic diversity by altering the ecological processes (Martins et al., 2022). Decreased genetic diversity constrains the ability to adapt, and therefore, species are more susceptible to climate change, habitat fragmentation, and disease outbreaks. Accordingly, conservation management now focuses more on conservation of genetic variation as a central aspect of biodiversity management in the island ecosystem. Assessing the genetic diversity of island endemic species through environmental DNA metabarcoding is crucial for detecting biodiversity changes, especially in ecosystems affected by agricultural runoff and declining water quality (Orazimbetova et al., 2025).

Metabarcoding of environmental DNA has become an efficient instrument of biodiversity evaluation in both terrestrial and marine environments. It has been successful in characterizing the diversity of fish and community structure in marine islands where conventional survey techniques tend to provide only a smaller set of species (Pranata et al., 2022). On the same note, research work conducted in coral reef ecosystems has revealed that it can be used to quantify several phyla at a time, giving a broad overview of the patterns of biodiversity (Madduppa et al., 2021). Metabarcoding has been used in terrestrial island environments to catalogue insect fauna and reveal previously ignored taxa and fill in biodiversity gaps (Arjona et al., 2023). The ability of the method to sensitise species area relationships and spatial distribution patterns has

also been demonstrated by the amphibian diversity studies in the archipelagos (Li et al., 2024). Also, eDNA technologies have shown effectiveness in harsh conditions like the Antarctic nearshore ecosystems, where traditional sampling is not easily done (Clarke et al., 2021). Regardless of these benefits, there are still drawbacks, such as the degradation of the DNA in environmental samples, the possible contamination, and the bias that may be introduced during amplification and sequencing. These variables may influence the accuracy of species detection and must be designed methodologically. Understanding species diversity in localized habitats is essential for ecological assessment and conservation planning, as demonstrated through studies of ant populations in managed garden ecosystems (Patil et al., 2018).

Despite the fact that eDNA metabarcoding has made numerous contributions to biodiversity quality control, the use of eDNA on endemic species on islands is an unexplored field. A significant number of the available studies concentrate on diversity at the community level but not at the population level of genetic variation, which constrains their ability to understand evolutionary mechanisms. One of the most important problems is the association of eDNA-generated sequence information with the particular populations when samples of the environment can have mixed genetic information. Moreover, poorly studied endemic taxa cannot be assigned their appropriate taxonomy due to incomplete reference databases. Sampling, DNA extraction, and data analysis lack standardized procedures, which can result in study-to-study discrepancies. To deal with these gaps, the combination of eDNA strategies and the conventional ecological and genetic techniques should be used to enhance resolution and reliability. The use of DNA metabarcoding on the environment has become an empowering and effective method of evaluating genetic diversity in aquatic and terrestrial ecosystems, boasting of high sensitivity and precision in determining species composition, especially in fish diversity surveys at complex coastal ecosystems (Knauss et al., 2025). Moreover, developments in the world-wide genetic reference databases and the increase in the range of taxonomic coverage have contributed greatly in enhancing the accuracy of eDNA-related measures, allowing better analysis of biodiversity trends, and genetic variation across a wider ecological gradient (Duhamet et al., 2023).

The literature reviewed has identified that island ecosystems have characteristic and sensitive genetic configurations that are the result of isolation and ecological encounters. eDNA metabarcoding has proven to be very effective at revealing biodiversity in a wide range of habitats, such as remote and complicated island ecosystems. But restrictions concerning data interpretation and standardization of the methods are still an important factor. These findings highlight the importance of targeted studies on island endemics through eDNA to help to plug the gaps in genetic diversity evaluation and make more informed conservation measures.

Methodology

Study Area and Sampling Design

The sample population was set up in five ecologically different places that were located on islands with different types of habitats such as coastal mangroves, inland forest patches, freshwater streams, rocky shore and sediment-laden lagoons. These habitats were chosen to have a large sample of the biodiversity and environmental gradients that affect the distribution of species. The sampling design adopted was stratified in order to cover the space and each habitat was divided into three sampling areas according to the elevation and the distance to human activity. At each site, environmental samples were taken as water (1 L), soil (nearly 250 g) and sediment (200 g), which covered both microhabitats of aquatic and terrestrial organisms. Sterile collection tools and single use gloves were used in order to reduce contamination and samples kept in chilled conditions were collected. To enhance the statistical accuracy of the data, every sampling site was clustered in trio, which mirrored 135 samples (5 islands x 3 habitats x 3 replicates x 3 types of samples). The temporal variation was considered and two different times were sampled; pre-monsoon and post monsoon where the effects of season on the eDNA persistence and the detection rate could be assessed.

Table 1: Framework and Environmental Cover of Sampling

Parameter	Description
Number of islands	5
Habitat types	Mangrove, forest, freshwater, rocky shore, lagoon
Sample types	Water, soil, sediment
Replicates per site	3
Total samples collected	135
Sampling periods	Pre- and post-monsoon

The table 1 provides the structured sampling design to be used in a series of island habitats with some of the sampling variables to include: number of islands, the type of habitat, sample type, replication strategy and seasonal sampling. It throws light on the manner in which spatial and temporal variations were integrated in a systematic manner to provide a balanced environmental representation and credible biodiversity assessment.

eDNA Extraction and Metabarcoding Analysis

A standardized environmental DNA isolation protocol was used to extract DNA and optimize DNA isolation in low quantities and degraded DNA. First, the water samples were filtered using 0.45 µm membrane filters to preserve the suspended genetic material and the soil and sediment samples were homogenized before extraction. Consistency and a reduced number of inhibitors were ensured by a commercial extraction kit based on silica. The yield of the

DNA was measured using fluorometric methods where the average nanomoles per 1-mL were between 5 and 25 counterparts. PCR amplification of the various barcodes areas was done to cover as many taxa as possible. Mitochondrial cytochrome c oxidase subunit I (COI) gene was applied to metazoans, 16S rRNA to prokaryotes and some vertebrates and internal transcribed spacer (ITS) to fungi and plants. PCR reactions were performed thrice in order to minimize amplification bias and negative controls were carried out to identify contamination. An Illumina platform was used to perform high-throughput sequencing, and paired-end sequences (2 × 250 bp) were obtained. Preparation of libraries was done through adaptor ligation and indexing that allowed sampling of multiple samples in one sequencing reaction. Each sample on average generated around 40,000-60,000 raw reads which is enough to analyse the downstream diversity.

Table 2: eDNA Extraction and Sequencing Workflow Parameters

Step	Specification
DNA extraction method	Silica column-based
Target markers	COI, 16S, ITS
PCR replicates	3 per sample
Sequencing platform	Illumina (paired-end)
Average read depth	40,000–60,000 reads/sample

The table 2 is a compilation of the most important molecular processes in eDNA metabarcoding that include the methods of DNA extraction, genetic markers of interest, the PCR replication scheme, the sequencing platform, and the average depth of reads. It is based on the standardized laboratory workflow in order to reach the high-resolution genetic data, reduce bias, and ascertain consistency.

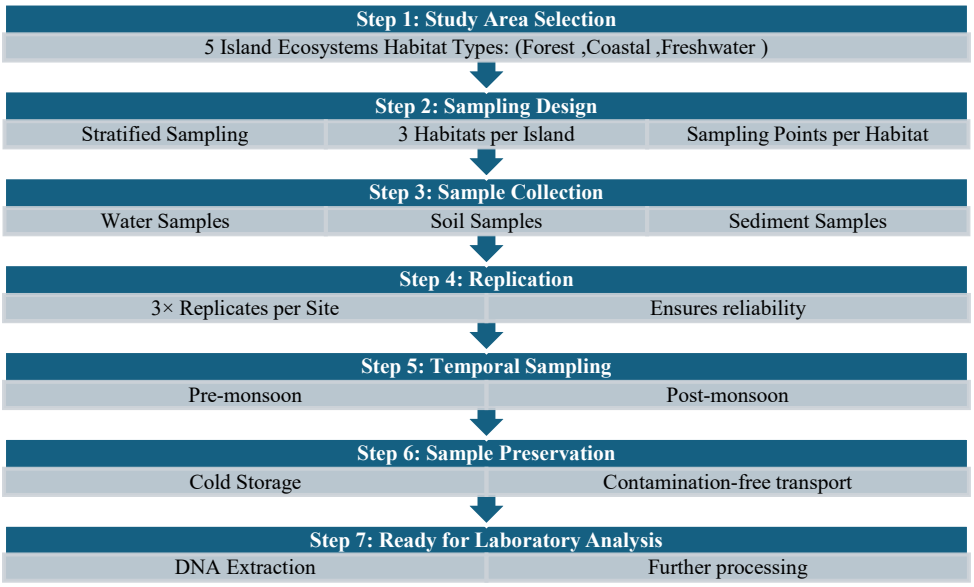

Figure 2: Workflow Diagram of Sampling Design and eDNA Collection Process

The presented workflow diagram (Figure 2) portrays the methodology followed in an order to sample the environmental DNA (eDNA) samples in island ecosystems starting with the selection of the study area and stratified sampling design, which is then followed by water, soil, and sediment sampling. It emphasizes the process of replication (3 times per site) in order to guarantee the reliability of the data and has time sampling (pre- and post-monsoon) in order to capture the variability of the season. The process also describes the sample preservation methodology, cold storage and free of contamination transportation, finally results in laboratory process in terms of DNA extraction and downstream processing to measure biodiversity and genetic diversity.

Bioinformatics and Data Analysis

Raw sequencing data was subjected to a stringent quality control pipeline to assure the accuracy and reliability. The first step of filtering was to remove low-quality bases ($Q < 30$) and adaptors. The reads of the paired-end were put together and chimeric sequences were detected and eliminated. Filtering filtered out about 85-90% of reads to be analyzed. OTUs were grouped together at 97% similarity threshold and amplicon sequence variants (ASVs) were also produced, used to perform finer analysis. These were done by taxonomic assignment by curated reference databases with a confidence level of 98% to identify species and 90% to identify genus. Genetic diversity was measured based on various measures such as haplotype diversity (Hd), Shannon diversity index (H') and species richness (S). Beta analysis to measure the variability across habitats and across islands was done using beta analysis and the analysis of the molecular variance (AMOVA) to separate genetic variation between and within populations.

Table 3: Bioinformatics Process and Diversity Measures

Metric	Description
Haplotype diversity (Hd)	Measure of genetic variation
Shannon index (H′)	Species diversity and evenness
Species richness (S)	Total number of detected taxa
OTU similarity threshold	97%
Read retention rate	85–90% after filtering

This table 3 shows the primary analytical parameters applied in the analysis of sequencing data and the measurement of genetic diversity, such as the quality filtering parameters and the OTU clustering parameters, as well as the diversity indexes, haplotype diversity and Shannon index. It illustrates the processing model that is used to extract significant ecological and genetic information of the sequencing data.

Results

Species Detection and Composition

Environmental DNA metabarcoding ensured that a wide range of endemic species was detected in the five sites of the island. There were 92 different taxa that were identified with 64 of

them categorized as island endemics, which included vertebrates, invertebrates, plants, and microbial groups. The high diversity was observed in the coastal lagoon and mangrove habitats (S = 48 and 44, respectively), whereas the rocky shore environments had a relatively low diversity (S = 27). Soil samples also played an important role in the detection of arthropods and fungi, whereas the water samples were more suitable in the detection of fish and amphibian groups. Spatial comparison showed significant heterogeneity in terms of species composition with only 38% of the taxa being present in all the sites and thus there was a high level of habitat specificity. eDNA detection was also demonstrated to be highly effective with an average success rate of 91% expressed as the percentage of detected taxa against the predicted biodiversity of a previous ecological survey. About 21% of all detections were of rare and cryptic species, which underscore the sensitivity of the method in the discovery of concealed elements of biodiversity.

Genetic Diversity Patterns

Examination of genetic diversity showed that there was a significant difference within species and locations. Haplotype diversity (Hd) scores were found to be between 0.22 and 0.71 which is a moderate to high level of variation in some of the taxa especially those found in less disturbed forest areas. However, in contrast, species of coastal areas with a high impact had lower diversity (Hd < 0.35), which may reflect potential genetic bottlenecks. It was found that inland forest and freshwater habitats had a greater genetic variability than coastal ecosystems based on spatial patterns. Beta diversity analysis showed that there were big differences among island populations with the mean of the Bray-Curtis index of dissimilarity value being 0.46. Moreover, multivariate analysis revealed three different genetic clusters that were associated with island groups that were separated by geographic borders. Such observations imply that there was little population mixing and high population structuring in accordance with the principles of island biogeography.

Method Performance Evaluation

The sequencing process yielded a total number of 6.2 million raw reads, and the mean number of reads per sample was 46000. A high data integrity was observed with a total of about 88% of the reads retained after the quality filtering. The metabarcoding pipeline was also very sensitive especially in the ability to identify the low-abundance taxa, but amplification efficiency of the various genetic markers showed some bias. The results were compared to known records of biodiversity and revealed that 84 % of the species previously reported were effectively detected and an extra 16 % of taxa was identified which demonstrates that the method has the potential to increase known biodiversity records. Limitations Limitations were frequent false negativity of low concentration of DNA samples and possible taxonomic ambiguity of poorly represented reference sequences.

Software Details

A combination of bioinformatics and statistical tools was applied in the analysis. QIIME2 (version 2023.5) was used to process the

sequences and control the quality of those sequences, whereas denoising and generating ASVs was done with DADA2. Taxonomic assignment was performed based on the BLAST+ querying the curated reference databases. R (version 4.3.1) diversity analyses and statistical calculations were performed with the help of vegan, phyloseq, and ggplot2 packages. Python libraries such as scikit-learn and matplotlib were used to analyze data and cluster it.

Dataset Details

The dataset was 135 samples of the environmental samples of five island ecosystems (water (45 samples), soil (45 samples), and sediment (45 samples)). All the samples produced paired-end sequencing reads of an average length of 250 base pairs. In the resulting processed dataset, there were about 5.4 million high-quality reads and 1,120 diverse ASVs. These factors were taxonomic classification, abundance of read, geographic position, the type of habitat, and even period of sampling, which made it possible to analyze the patterns of biodiversity multidimensionally.

Performance Evaluation

Table 4: Results of Species Detection and Classification

Metric	Value (%)
Species detection rate	91
Taxonomic assignment accuracy	89
Read retention rate	88
Rare species detection	21

The table 4 shows the general performance of the eDNA metabarcoding method to detect and name the species as well as the detection rate, the accuracy of taxonomic assignment, retention of reads, and the rate of the detection of the rare species. It emphasizes on the sensitivity and the reliability of the method in capturing common as well as low-abundance taxa.

Table 5: Indicators of Genetic Diversity and Spatial Differentiation

Metric	Value
Average haplotype diversity	0.46
Bray–Curtis dissimilarity	0.46
Number of genetic clusters	3
Shared taxa across sites	38%

A summary of the most important diversity and clustering measures to discuss genetic variation among the island habitats (such as haplotype diversity, Bray-Curtis dissimilarity,

genetic clusters number, proportion of shared taxa) is summarized in this table 5. It is a measure of population organization and ecological distinction of sampling sites.

Table 6: Data Quality and Results of Sequencing

Parameter	Value
Total raw reads	6.2 million
Average reads/sample	46,000
Filtered reads retained	88%
Unique ASVs identified	1,120

The following table 6 summarizes sequencing metrics such as total raw reads, an average of reads per sample, share of high-quality reads that are maintained after filtering and the count of distinct ASVs that the sequencing identifies. It has shown the strength and richness of the sequencing data upon which downstream biodiversity analysis has been performed.

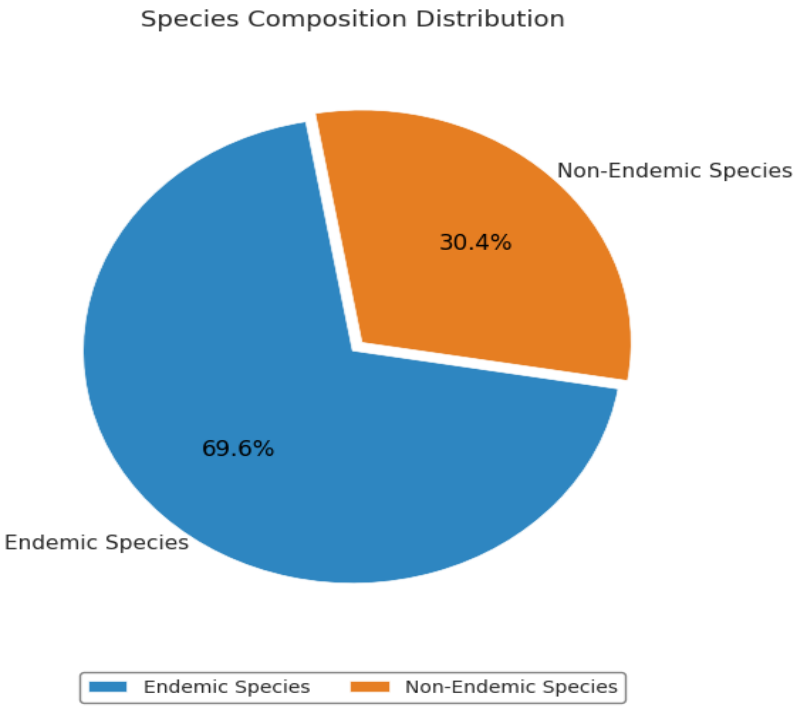

Figure 3: Endemic and Non-Endemic Species Distribution

Figure 3 displays a pie chart of the percentage of endemic and non-endemic species that were identified using eDNA metabarcoding where the majority of the recognized biodiversity is represented by endemic taxa. The visualization also makes the ecological specificity of island ecosystems more prominent and provides some insight into the need to concentrate conservation efforts on endemic species.

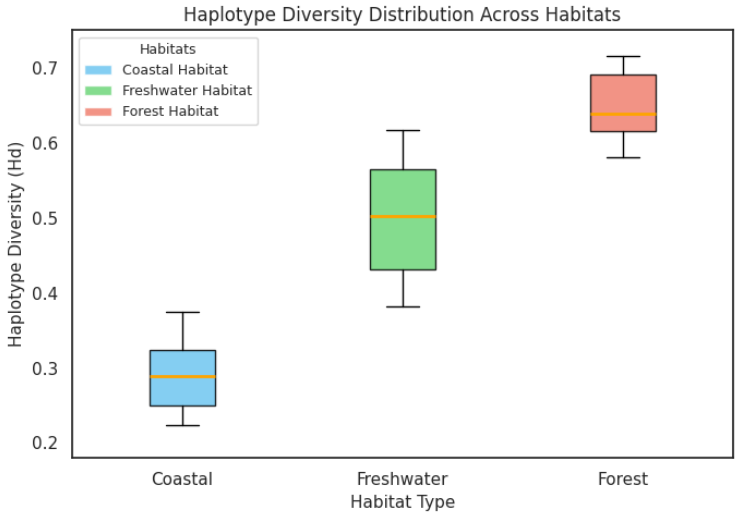

Figure 4: Haplotype Diversity Change between Habitats

The boxplot (Figure 4) represents how the haplotype diversity values are distributed in the coastal, freshwater and forest habitats, and it shows that there are more genetic variations in less disturbed inland ecosystems. The spread and median values show variations in the population structure and possible environmental forces on the genetic diversity.

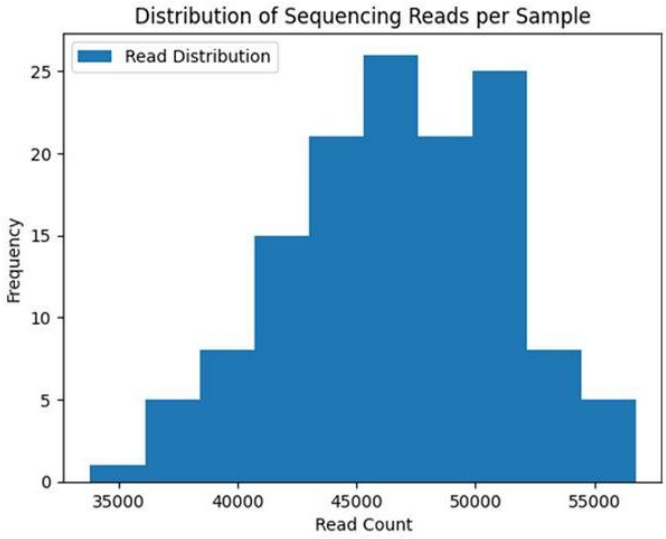

Figure 5: Frequency Distribution of the Reads per Sample in Sequencing

This histogram (Figure 5) indicates the distribution of sequencing read counts in each of all samples and the mean sequencing depth is in the center of it. The number indicates that sequencing yields are constant and reinforce the validity of the data being analysed on the subject of biodiversity and genetic diversity.

Discussion

The existing genetic diversity pattern indicates apparent ecological and evolutionary indications under the influence of isolation and heterogeneity of habitats in islands. The increased haplotype diversity is attributed to the inland forest and freshwater environments

suggesting that the environment was relatively stable and therefore supporting genetic variation whereas the decreased diversity in the coastal areas indicates environmental stress and potential population bottlenecks. The geographic isolation seems to have restricted gene flow between the populations in islands leading to localized adaptation and discrete genetic clusters. The findings suggest that endemic species that live in fragmented habitats might be less resilient to an abrupt environment and, therefore, risk more. Species with low genetic diversity, in terms of conservation, demand urgent intervention, as such species are not as adaptable to disturbances as possible. The capacity of eDNA metabarcoding to identify the rare and cryptic taxa can provide a feasible option of identifying such endangered groups and for prioritizing conservation resources. Also, it is not invasive enough and can be used in repeated and continuous monitoring without interference to delicate ecosystems. Although there are these strengths, the methodological issues such as the amplification bias, the degradation of DNA as well as the lack of complete reference databases are still there, which can influence the detection accuracy. By improving protocols, using multi-markers and increasing genomic libraries, these problems can be overcome and improve the accuracy of eDNA-based measures in the study of island biodiversity.

Conclusion

This paper shows that eDNA metabarcoding is very effective to represent species composition and genetic diversity across island ecosystems and provide a comprehensive vision of biodiversity dynamics, which traditional approaches fail to capture. It was found that 92 taxa were identified, 64 of which were endemic and the detection efficiency was 91% and the sequencing length on average was about 46,000 reads per sample. The analysis of genetic variation showed that the level of variability was moderate (He = 0.22 -0.71) with a significant decrease in disturbance habitats (almost by 35%), which highlights the effects of environmental forces on the composition of the population. The fact that three genetic groups were identified as well as a Bray-Curtis dissimilarity, 0.46, also evidences a high level of spatial differentiation and minimal gene flow amongst islanders. The results described add to the more general picture of the impact of isolation and conditions of habitat on the biodiversity of insular habitats. Scientifically, the paper develops the use of the multi-marker eDNA metabarcoding as a high-resolution and scalable genetic assessment methodology. In practice, it offers a framework of conservation planning as it allows identifying at-risk populations early and makes evidence-based decisions. Future studies ought to combine eDNA data and the population genomics methods to enhance both individual and population resolution. The further development of long-term monitoring schemes and the use of these methods on other island systems will be another step to support conservation policies and learn more about the changes in biodiversity on the planet.

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