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Dietary composition of the silvery gibbon (*Hylobates moloch*) in the Northwestern Dieng Mountains, Indonesia using fecal metabarcoding and field surveys

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Abstract. *Nuri TSK, Suratman, Susilowati A, Karyanto P. 2026. Dietary composition of the silvery gibbon (*Hylobates moloch*) in the Northwestern Dieng Mountains, Indonesia using fecal metabarcoding and field surveys. Biodiversitas 27 (4): d270443. https://doi.org/10.13057/biodiv/d270443.* Forest degradation has altered vegetation structure and composition, affecting the silvery gibbon's ability to adapt to fluctuating food availability. Understanding these adaptations provides essential baseline information for assessing population viability. We tested the hypothesis that *Hylobates moloch* exhibits dietary selectivity by comparing plant abundance in fecal metabarcoding data against vegetation metrics from field surveys, including species richness and the Importance Value Index (IVI). Additionally, we evaluated whether metabarcoding provides a broader dietary profile than traditional ground-based methods. We employed *rbcL*-based Next-Generation Sequencing (NGS) on five pooled fecal samples collected during the early dry season, complemented by the Point-Centered Quarter (PCQ) method and ethnobiological interviews. NGS analysis yielded 13,040 Amplicon Sequence Variants (ASVs), representing 28 orders, 47 families, 64 genera, and 29 species. Of these 47 families, 40.43% (19 families) were fully corroborated by both PCQ vegetation surveys and local interviews, underscoring the strong complementarity of these techniques. While the survey identified more than 40% tree species, the NGS data revealed a more specialized dietary niche. Field surveys effectively captured essential canopy food sources, whereas metabarcoding complemented these findings by identifying non-woody, rare, and understory taxa typically excluded from conventional tree plots. The three most abundant taxa based on Relative Read Abundance (RRA), *Ficus benjamina*, family Moraceae, and *Gnetum* spp., were identified as primary dietary components. Our findings yielded vital dietary data underscoring a practical conservation plan for Javan gibbons. We confirm that *H. moloch* selectively forages on specific taxa, with Moraceae serving as the predominant food source during the early dry season. Consequently, long-term conservation should prioritize restoring key fruit taxa and maintaining forest diversity to ensure their survival.

Keywords: Dietary ecology, fecal metabarcoding, *Hylobates moloch,* non-invasive sampling, *rbcL*

INTRODUCTION

The silvery gibbon (*Hylobates moloch* Audebert 1798) is an endemic arboreal primate that is primarily frugivorous and therefore, relies heavily on well-structured vegetation for food and locomotion (Kim et al. 2011; Widyastuti et al. 2023). Despite these facts, however, massive forest degradation across Java has affected its populations by reducing habitat connectivity and tree diversity (Chaves et al. 2023; Stead 2025). Driven by this excessive degradation, this species is experiencing a population decline and, therefore, is currently registered as Endangered on the IUCN Red List database (Nijman 2020). This decline tells us that the silvery gibbon may have limited behavioral flexibility to habitat changes, underscoring the urgency for a comprehensive study (Webber et al. 2022).

Alterations in vegetation structure significantly impact the foraging behavior of arboreal primates, including the silvery gibbon (Machado et al. 2023; Stewart et al. 2025). While certain primates exhibit dietary plasticity, others demonstrate an inability to adapt (Karyanto et al. 2022; Karyanto et al. 2025). As demonstrated by other resilient primates, the silvery gibbon should exhibit behavioral flexibility by modifying its foraging flexibility to exploit available food resources, aiming to maximize survival and reproductive success, thereby supporting population persistence in changing environments. These dietary shifts are most rigorously assessed using comprehensive dietary analysis. The empirical findings from such analyses provide robust data for conservation strategies. Determining the silvery gibbon's dietary pattern and its capacity to withstand habitat degradation yields critical insights into gibbon foraging ecology amidst habitat change, thereby facilitating the strategic prioritization of conservation interventions (Zhong et al. 2023; Richardson 2024).

Previous studies of the silvery gibbon diet still rely on visual observations, leading to an underestimation of dietary diversity in complex canopies and producing inappropriate data for a practical conservation plan (Kim et al. 2011; Rytkönen et al. 2019). Consequently, relying solely on incomplete observational data risks misinforming conservation strategies. A comprehensive understanding of its dietary requirements is urgently needed to effectively protect critical food resources and guide habitat restoration

in degraded landscapes. These limitations are effectively addressed by *rbcL* DNA metabarcoding using a non-invasive approach, a best practice recommended for endangered species. To our knowledge, this study represents the first molecular-based investigation of the silvery gibbon's diet, uniquely characterized within a degraded montane habitat. Unlike previous studies, we define our novelty through a dual validation framework, where high-resolution NGS results are cross-referenced with ground-truth data from the PCQ method and interviews to address biases. This study used the *rbcL* primer, known to yield high-resolution plant sequences, facilitating the accurate detection of plants consumed by silvery gibbons. By utilizing this primer, the metabarcoding approach facilitates the detection of a broad spectrum of its diet, including rare or highly degraded plant materials that typically evade conventional macro-contextual analysis. This molecular approach overcomes the limitations of continuous behavioral observation, providing enhanced taxonomic resolution necessary to identify cryptic or heavily processed plant taxa that fall below the detection thresholds of traditional methods (Bell et al. 2017; Schneider et al. 2023; Thiry et al. 2025).

Our research was carried out in the northwestern Dieng Mountains to represent a degraded habitat. Because NGS may yield misleading results due to biases, we cross-referenced the molecular results with a ground-truth survey using the PCQ method and interviews. To interpret the gibbons' foraging decisions, Optimal Foraging Theory was grounded to predict that individuals will maximize energy intake while minimizing foraging effort, particularly when high-quality resources become scarce. Under such conditions, animals may shift to fallback foods to meet their energy requirements. Based on this integrated approach, we tested two hypotheses: i) the silvery gibbon's dietary composition will be disproportionate to vegetation abundance, indicating selective foraging behavior and their adaptive capacity to live in the degraded habitats, and ii) NGS will capture a broader taxonomic dietary richness than what is typically captured by traditional surveys and local ecological knowledge. For the hypotheses, our analytical approach evaluated dietary selectivity by comparing the relative abundances of plant taxa in metabarcoding data with PCQ-derived values and interviews to determine the potential dietary preferences of the silvery gibbon. We evaluated differences in dietary breadth by comparing the taxa detected via NGS with those recorded during the ground check.

MATERIALS AND METHODS

Study area

We studied the diet of the silvery gibbon (Figure 2) in the secondary forest around Petungkriyono, Pekalongan, Central Java, Indonesia (Figure 1), located at 7°6'3.39"S, 109°40'20.13"E, encompasses 7,600-7,700 ha, and spans an elevation of 900 to 1,600 m asl. Although the landscape is primarily secondary forest, it retains the structural complexity of vegetation and biodiversity. The landscape is managed by the government as a mosaic of protected and production forests, resulting in varying degrees of disturbance and regeneration across the area. Anthropogenic disturbances in the forest are mainly associated with land-use changes for tourism development and pine and coffee plantations.

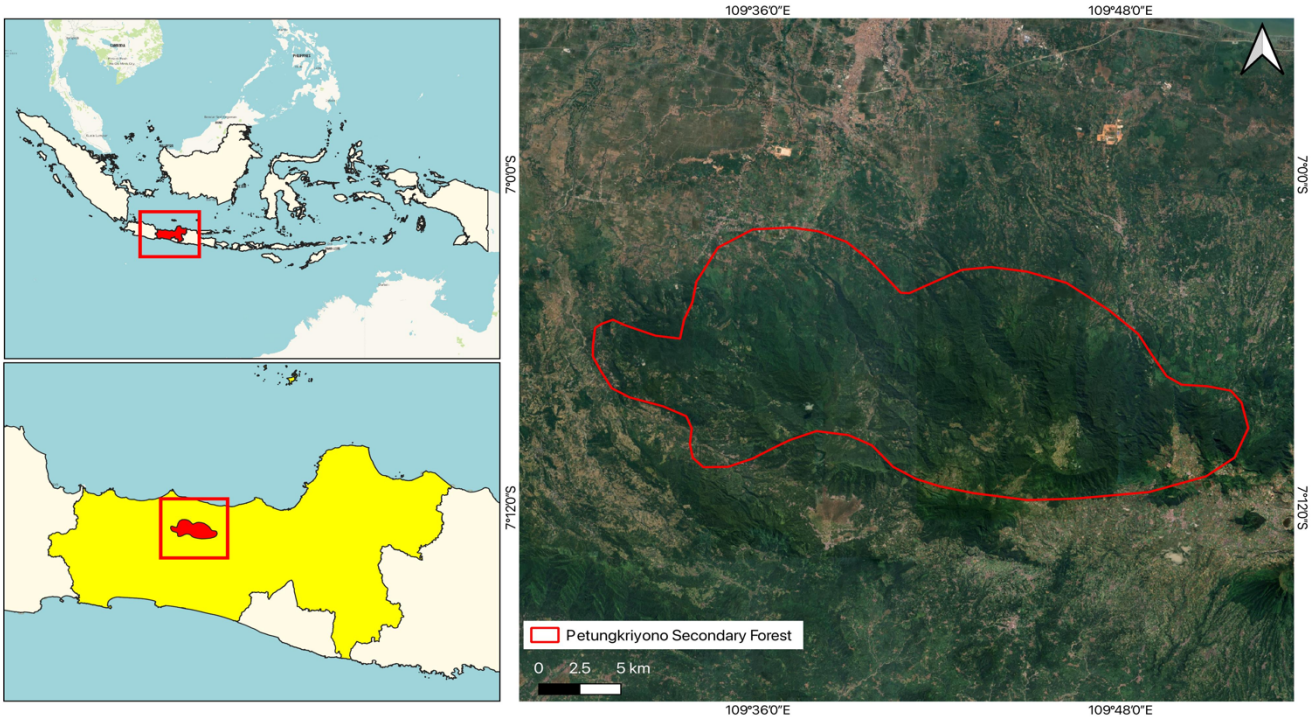

Figure 1. Location of study site in Petungkriyono secondary forest, Pekalongan, Central Java, Indonesia

Figure 2. The silvery gibbon *Hylobates moloch* at the study site is exhibiting typical brachiation behavior within the mid-to-upper canopy, photo taken by Puguh Karyanto

Samples collection was carried out during April-July, a period of the early dry season in Central Java, Indonesia. This period may be associated with lower rainfall and, consequently, influence plant phenology and, in turn, the availability of fruits, young leaves, and flowers relevant to dietary interpretation. In addition to vegetation complexity, qualitative visual observations along an 8.8 km segment of the main road crossing the forest (from 7°3'47.80"S, 109°42'58.16"E to 7°07'3.0"S, 109°44'24.1"E) indicated that the most conspicuous taxa were Moraceae (e.g., *Ficus* spp. and *Artocarpus*), Sapindales (e.g., *Dracontomelon dao*), and Malpighiales (*Pangium* sp.). At the time of the survey, several *Ficus* trees were observed to be actively fruiting, whereas the observed *Artocarpus* and *D. dao* were not bearing fruit. Additionally, several wild and cultivated fruit trees did not appear to be in active flowering or fruiting stages during this period.

Procedures

A non-invasive fecal metabarcoding approach using the *rbcL* primer on the Illumina platform was used to determine the diet of the silvery gibbon within the study area. To ascertain the robustness of the molecular results, we cross-verified the NGS data against a local floristic dataset generated using a vegetation sampling technique involving PCQ and interviews. The complete methodological workflow, from fecal sample collection to dietary inference, is illustrated in Figure 3.

Fecal sample collection and DNA extraction

We located our initial sampling sites based on research by Widyastuti et al. (2023) and our preliminary observations prior to fecal collection. Morning field explorations to collect fecal samples were then conducted from April to July 2022, during the early dry season, when fruits and young plant phenology may be limitedly available in the forest. Morning sampling is conducted to ensure that the genetic material in the fecal samples matches that of plants ingested a day before collection. Nine fresh fecal samples from the silvery gibbon were successfully collected, preserved in 96% alcohol, and stored in the refrigerator. All samples were encoded with the identifier 'Ho' and are detailed in Table 1. To ensure no misattribution to non-target species, feces were collected when an individual gibbon or a group was detected nearby. We also confirmed that the collected feces belong to the silvery gibbon by conducting species barcoding using the *Cytb* marker before running the NGS (the sequence has also been submitted to NCBI and received Accession Numbers).

To reduce spatial heterogeneity, effectively capture diet within the study site, and facilitate further analysis of potential differences between distinct forest physical structures, samples were composited into five composites. While this pooling strategy limits the statistical power to assess individual-level dietary variance, it minimizes the risk of false negatives in a single-sample extraction. It maximizes the detection of rare dietary taxa across the group. Samples Ho 1 and Ho 3 were composited to represent the forest characterized by dense, continuous canopy and high species richness. Samples Ho 6 and Ho 8 were grouped to represent a sparse forest formation. In contrast, Ho 7 and Ho 9 were composited to isolate the agroforestry edge effect, distinguishing them by their specific proximity to plantation activities. Finally, Ho 10 and Ho 11 were combined as replicates of the Highly Degraded zone. This way, treating each composite as a representative unit of its specific habitat, thereby allowing a more precise analysis from the forest to the anthropogenically disturbed patch. The composite samples were newly encoded as S089.1-S089.5 (Table 2).

The composition of the five composites was driven by the physical structure and ecological characteristics of the forest at each fecal encounter site. While composites S089.1 through S089.4 each consist of two pooled samples to maximize taxonomic detection within their respective zones, S089.5 was maintained as a single-sample unit representing sample Ho 12. This unequal structure was necessary because Ho 12 originated from a unique, fragmented, and isolated forest patch that did not align ecologically with the other sampling locations. Maintaining S089.5 as an independent unit ensures that the dietary profile of this specific high-richness but isolated habitat is not confounded by data from distinct forest formations, thereby prioritizing spatial accuracy over symmetrical sample distribution.

Quality control, sequencing, and downstream bioinformatics analysis

Next-generation sequencing with a short DNA barcode was utilized to identify the plant taxa eaten by the silvery gibbon, given its demonstrated effectiveness in analyzing the diet (Ingala et al. 2021). The chloroplast ribulose-1,5-bisphosphate carboxylase (*rbcL*) locus was amplified using the initiation sequence F: 5'-CTT ACC AGY CTT GAT CGT TAC AAA GG-3' and R: 5'-GTA AAA TCA AGT CCA CCR CG-3' following Kress and Erickson (2007). This primer pair has been widely adopted in plant DNA barcoding research due to its broad universality. The primers generate an amplicon of approximately 379 bp within the standard *rbcL* barcoding region. This enables broad amplification across terrestrial plants. It also allows for high-resolution

taxonomic assignment, typically to the family and frequently to the species level.

Quality control was maintained during DNA extraction, PCR, library preparation, and sequencing. The Quick-DNA Zymo Fecal/Soil Microbe DNA Miniprep Kit (D6010) was used to extract DNA from 150 mg of each fecal sample, following the manufacturer's instructions. We assessed the quantity and purity of the extracted DNA using a NanoDrop spectrophotometer and a Qubit 2.0 fluorometer (Thermo Fisher Scientific). To verify DNA integrity, we used 1% agarose gel electrophoresis. Using 2× EmeraldAmp MAX PCR Master Mix, forward and reverse primers, template DNA, and nuclease-free water, the *rbcL* gene sequence was amplified in 25 µL reactions. PCR was done on an ABI 2720 thermocycler. Initial denaturation at 95°C for 4 minutes; 35 cycles of 20 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C; and final extension for 5 minutes. The PCR products were purified with AMPure XP beads and used as templates for Nextera XT indexed library construction, followed by a final step of PCR and bead cleanup.

Paired-end (2×250 bp) sequencing was conducted on a NovaSeq™ 6000 v1.5 platform. Negative controls (PCR mix and primers without a DNA template) showed no visible amplification on the gel, though minor background reads were observed during the initial sequencing phase. To address this, we applied a strict zero-tolerance filtering threshold for potential contamination: any amplicon sequence variants (ASVs) detected in the negative control were entirely removed from the downstream dataset of the biological samples. Because these background reads were comprehensively filtered out during the DADA2 processing on the Galaxy platform, they did not influence the final dietary analysis.

Raw reads were processed using the DADA2 pipeline on the open-source web-based platform Galaxy (v1.34.0) at https://usegalaxy.org/. Following initial quality profiling, sequences were filtered and trimmed with a quality threshold (truncQ) of 2. We applied no truncation of read length or sequence removal from the start/end of the reads (truncLen: 0, trimLeft: 0, trimRight: 0). Error rates were estimated using a magnitude of 8 bases. Amplicon sequence variants (ASVs) were then inferred by processing samples independently. Forward and reverse reads were merged, requiring a minimum overlap of 12 bases and allowing 0 mismatches.

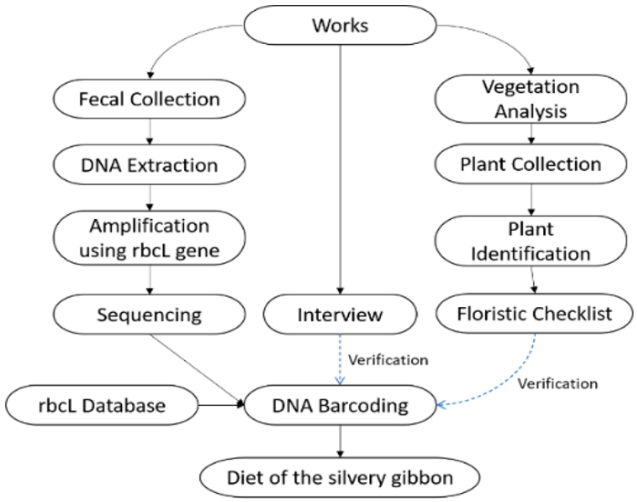

Figure 3. The methodological workflow from fecal sample collection to dietary inference. This tripartite approach combines high-sensitivity molecular profiling (DNA metabarcoding) with traditional botanical surveys and local ecological knowledge (interviews) to minimize the taxonomic biases inherent in single-method studies

Table 1. Fecal samples, coordinates, and forest profile description. NCBI will publicly release accession numbers upon publication

Sample code	Accession number (pending release)	Coordinates	Date of sampling	Forest physical structure description
Ho1	PX252238	S7° 4' 37.58" E109° 43' 25.43"	05-April-2022	Dense and interconnected canopy exhibiting high species richness with a dominance of Moraceous trees.
Ho3	PX252240	S7° 4' 20.52" E109° 43' 19.47"	14-May-2022	Dense and interconnected canopy exhibiting high species richness with a dominance of Moraceous trees.
Ho6	PX252243	S7° 5' 5.22" E109° 43' 26.36"	16-May-2022	Dense canopy, exhibiting lower species richness with a dominance of Moraceous trees.
Ho7	PX252244	S7° 4' 54.49" E109° 43' 28.29"	15-June-2022	Less dense canopy, exhibiting less species richness, located near a plantation, with dominance of Moraceous trees.
Ho8	PX252245	S7° 4' 9" E109° 43' 18.9"	17-June-2022	Dense canopy, exhibiting lower species richness with a dominance of Moraceous trees.
Ho9	PX252246	S7° 5' 42" E 109° 43' 18"	18-June-2022	Less dense canopy, exhibiting less species richness, located near a plantation, with dominance of Moraceous trees.
Ho10	PX252247	S7° 7' 1.89" E 109° 35' 19.74"	26-June-2022	Highly degraded and fragmented, with very low species richness.
Ho11	PX693698	S7° 7' 1.9" E 109° 35' 30.2"	26-June-2022	Highly degraded and fragmented, with very low species richness.
Ho12	PX693699	S7° 6' 11.16" E 109° 44' 10.09"	06-July-2022	Dense and interconnected canopy, exhibiting high species richness. Fragmented and isolated location.

Chimeras (bimeras) were removed from the inferred ASV sequence table using the dada2: remove Bimera Denovo function. For this step, we applied the "consensus" approach. This approach examines each sample independently and assigns a consensus chimera/non-chimera decision for each sequence variant. Finally, taxonomy was assigned using the *rbcL* reference library for seed plants provided by Bell et al. (2017). We tracked read attrition throughout the pipeline using the DADA2 sequence counts tool. This tracking included raw, filtered, denoised, merged, and non-chimeric read counts per sample. These detailed counts are presented in Table 3. We utilized a minimum bootstrap confidence of 50 for this assignment, which is the default setting in DADA2. While we acknowledge that this threshold is relatively permissive and may occasionally yield improbable species-level matches, it was intentionally chosen to maximize the initial recovery of dietary taxa, given the historically incomplete nature of DNA barcode reference databases for tropical Indonesian flora. To strictly mitigate this limitation and resolve any improbable matches, all NGS-derived taxonomic assignments were subsequently cross-referenced and validated against our robust field-based vegetation survey (PCQ) data. Following the DADA2 pipeline, downstream analysis was conducted in R v4.2.0, with visualizations generated using Krona Tools. The overall bioinformatics workflow is summarized in Figure 4.

PCQ vegetation analysis and interview

Vegetation analysis using the PCQ technique was conducted to refine the interpretation of NGS metabarcoding data by providing independent information on plant availability, thereby helping evaluate potential biases and gaps in the molecular dietary profile. PCQ points were purposively placed around sites of fecal encounters, during direct observations, and in broader areas to account for the population's daily movement radius of 1.18 km, as reported by Kim et al. (2011). The PCQ approach can quickly determine that the vegetation data represent the floristic composition of the actual habitat. The PCQ approach can quickly determine whether vegetation data represent the floristic composition of the actual habitat. This technique was applied at 63 sampling points (Figure 5) and 252 measured quarters, focusing on tree strata with diameter at breast height (DBH) that represent the tree layers essential for gibbon locomotion and feeding. In each quarter, the nearest tree was determined, the straight distance from the point to the tree was measured, species were recorded, and DBH exceeding 10 cm was measured at 1.3 m (Terra et al. 2018; Araújo and Shideler 2019). For each tree, diagnostic features were photographed and recorded to identify the taxa using The Mountain Flora of Java (Steenis 2006), the Global Biodiversity Information Facility (https://www.gbif.org), and Plants of the World Online (https://powo.science.kew.org). From the PCQ data, the Important Value Index (IVI) for each species was calculated as the sum of Relative Density, Relative Frequency, and Relative Dominance. The diversity profile of the tree community in the study area was summarized using the Shannon-Wiener index (H′) and Simpson's dominance index (C).

Table 2. Five composite samples and codes, and their qualitative habitat descriptions. The composition of the five composites was driven by the physical structure and ecological characteristics of the forest at each fecal encounter site

Composited samples	Code	Habitat description
(Ho 1 + Ho 3)	S089.1	Dense, continuous canopy with high species richness
(Ho 6 + Ho 8)	S089.2	Dense canopy with lower species richness
(Ho 7 + Ho 9)	S089.3	Agroforestry edge effect (less dense canopy, near the plantation)
(Ho 10 + Ho 11)	S089.4	Highly degraded and fragmented zone with very low species richness
(Ho 12)	S089.5	Dense canopy and high species richness, but in a highly fragmented and isolated patch

Table 3. Number of reads retained at each step of the DADA2 processing pipeline. Raw Reads indicates the initial number of sequenced paired-end reads. Reads were subsequently quality-filtered, denoised to remove sequencing errors, merged, and cleared of chimeric sequences (Bimera) to produce the final amplicon sequence variants (ASVs)

Samples	Filttrim reads.in	Filttrim reads.out	Dada forward	Dada reverse	MakePairs	SeqTab	Bimera	ASVs	Taxonomic assignment
S089.1	132795	132782	131588	131538	128592	128592	108138	2608	
S089.2	135082	135061	133779	133832	131574	131574	117820	2608	
S089.3	143596	143585	141564	141872	137991	137991	126442	2608	101 ASVs
S089.4	231184	231158	228341	228652	221318	221318	178293	2608	
S089.5	117702	117696	114678	115126	110229	110229	96148	2608	
								13040	

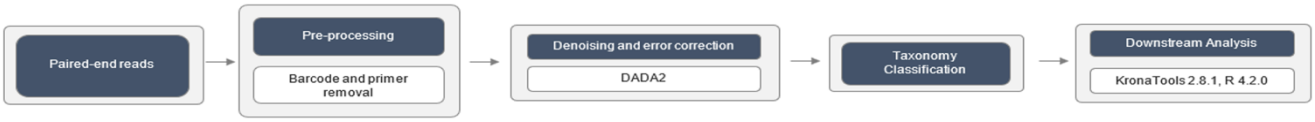

Figure 4. Bioinformatics procedures from paired-end reads to Krona visualization. This workflow utilizes the DADA2 algorithm for stringent denoising and error correction to resolve Amplicon Sequence Variants (ASVs)

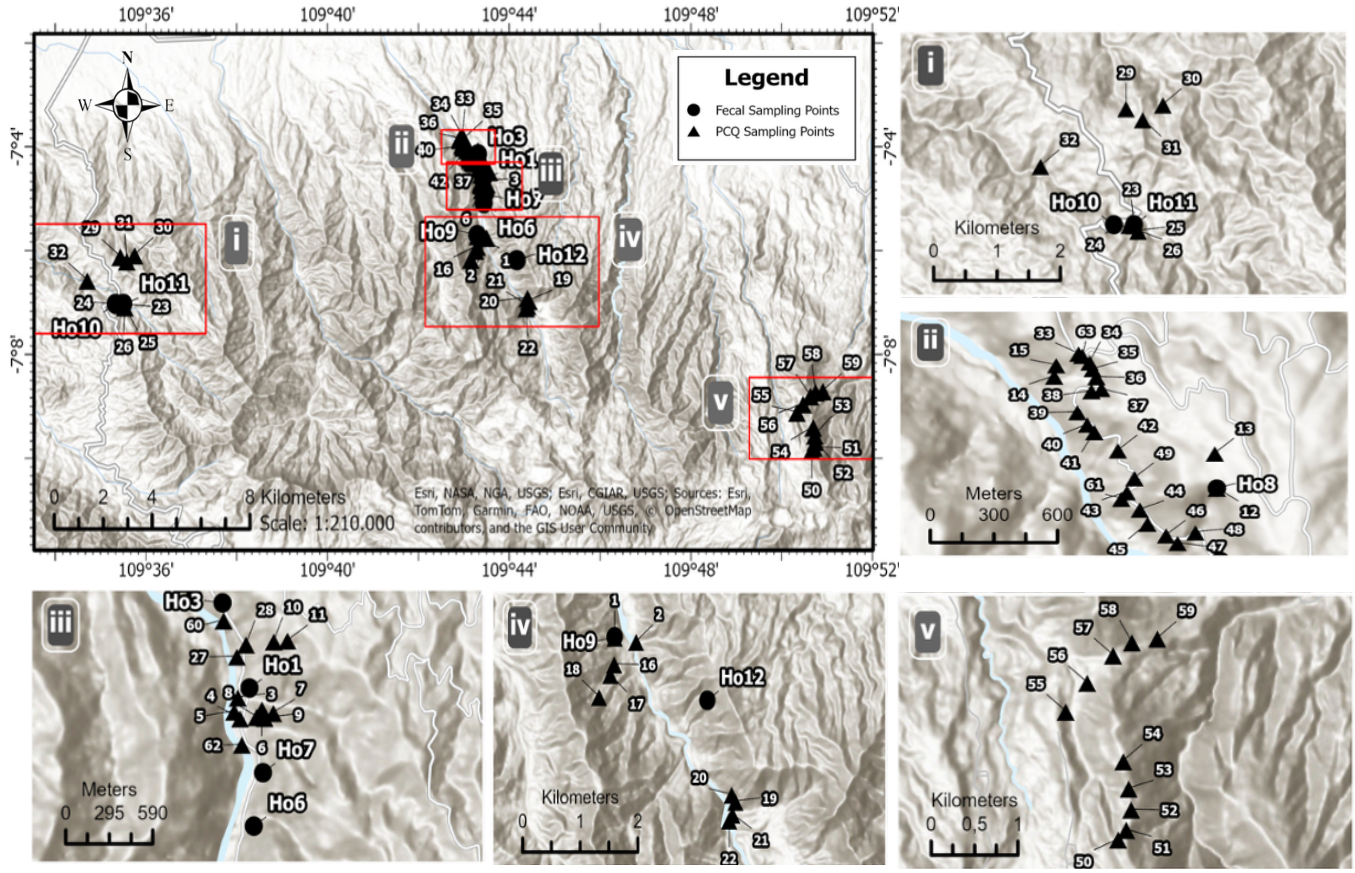

Figure 5. Map of sampling sites for fecal collection and vegetation surveys (PCQ). Topographic overview showing the strategic clustering of sampling blocks (i-v) to capture varying elevations and microhabitat types inhabited by the silvery gibbon. Detailed spatial arrangement of fecal collection points (circles) in relation to PCQ vegetation plots (triangles), designed to facilitate a direct analytical comparison between local resource availability and individual dietary selection

We note a limitation regarding our vegetation sampling design: the placement of the Point-Centered Quarter (PCQ) points was purposive rather than strictly randomized. This purposive approach was intentionally designed to target areas with observed silvery gibbon activity, ensuring that the surveyed vegetation closely reflected the immediate food resources available to the specific groups being studied. However, we explicitly acknowledge that this non-randomized placement introduces a potential sampling bias. Consequently, the resulting PCQ data characterizes the specific habitat-use areas of the targeted gibbon groups and should not be interpreted as a generalized or unbiased floristic representation of the entire Petungkriyono forest landscape.

Dietary analysis and validation based on vegetation structure data

Semi-quantitative data of amplicon sequence variants (ASVs) were processed using the sort and filter functions in Microsoft Excel, following Karyanto et al. (2025) to organize the identified plant taxa alongside their semi-quantitative values (reads/frequencies) to calculate the Relative Read Abundance (RRA) in descending order to clarify the composition and relative abundance of plant species in the silvery gibbon's diet. The RRA was calculated according to this formula:

$$RRA_i = \left(\frac{R_i}{R_{total}}\right) \times 100$$

Where, *RRAi*: The relative read abundance of a specific taxon; R_i: The frequencies of taxa assigned to that specific taxon; R_{total}: The total number of frequencies for each taxon in each composite.

To cross-validate taxa identified through NGS analysis and the PCQ survey, an ethnobiological approach was applied. To confirm these species, which occur locally, we employed three experienced Swaraowa Foundation surveyors to cross-check the NGS findings. We identified shared taxa to confirm the NGS results. This process allowed us to distinguish between verified taxa and those detected solely through metabarcoding. To evaluate sampling adequacy, rarefaction curves were generated in PAST v2.17 with a confidence level of 0.95. These curves plotted the cumulative number of detected plant taxa against sequencing depth, confirming that the dataset provided sufficient coverage of the silvery gibbon's diet.

RESULTS AND DISCUSSION

Genomic DNA extraction assessment

Analysis of the extracted gDNA from all fecal composites demonstrated consistent quality, providing reliable templates for downstream PCR and metabarcoding. All composite samples yielded sufficient gDNA concentrations for library preparation (17.3 to 40.5 ng/µL) and exhibited high purity with A260/280 ratios between 1.80 and 1.95 (Table 4).

Sequencing of the *rbcL* marker yielded an initial 13,040 raw ASVs. To isolate biologically meaningful dietary components, we applied a strict bioinformatic hierarchy using our DADA2 Galaxy workflow. To ensure rigorous quality control, our criteria ensure that any ASVs present in the negative control were completely subtracted and excluded from all biological samples during the pre-processing stage. Although the intermediate ASV counts and minor background reads were not retained in the final exported sequence table following these strict filtering steps, subsequent taxonomic filtration successfully retained 101 distinct plant ASVs. Because individual ASVs can overrepresent true diversity due to multi-copy genes or intra-specific variation, we then collapsed these 101 plant ASVs into biologically meaningful dietary taxa, resulting in 28 orders, 47 families, and 64 genera. The final dietary profile included these taxonomically retained taxa, provided they were present in at least one of the five composites.

The ASVs identified in this study match different plant taxa that the silvery gibbons eat. Figure 6 shows a Krona plot that breaks down these taxa. To address the limitations of the Krona plot, a supplementary pie chart (Figure 7) was provided to clearly distinguish between confirmed, implausible, and unmapped taxa, providing the necessary cross-validation for the taxonomic interpretation. Figure 8 illustrates the rarefaction curve for the *rbcL* ASVs, which rises rapidly before distinctly leveling off. Analytically, this plateau demonstrates that sample saturation was successfully attained, confirming that our methodology of using five composite samples was highly adequate for this dietary profiling. The asymptote indicates that the current sequencing depth captured the vast majority of the plant diversity in the silvery gibbon's diet, and that increasing the composite count or sequencing deeper would be unlikely to reveal a significant number of additional new plant taxa.

Table 5 details the relative read abundance (RRA) of the silvery gibbon's diet, expanding on the taxonomic breakdown from the Krona plot. Following quality filtering and taxonomic assignment, we recovered 101 distinct ASVs. We then collapsed these ASVs, which may result from multi-copy genes or intra-specific variation, into 28 orders, 47 families, and 64 genera. The 64 species presented in Table 5 represent a refined list of taxa. This selection was achieved by excluding all 'NA' (unidentified) entries and consolidating fully identified taxa, specifically those marked with single (*) and double asterisks (**).

Taxonomic assignment success predictably declined at lower ranks. While we successfully assigned 100% of ASVs to an Order and 98.0% (99 of 101) to a Family, genus-level assignments fell to 79.2% (80 of 101). Notably, 21 ASVs lacked genus-level resolution, including a large Moraceae cluster representing 48,949 reads. Species-level assignments had the lowest resolution, with only 29 ASVs (28.7%) confidently identified.

While the *rbcL* marker provided extensive taxonomic profiling of the diet, we explicitly exercise caution regarding certain species-level assignments. Several taxa identified in our dataset (e.g., *Artabotrys hongkongensis*, *Castanea sativa*, *Euscaphis japonica*, and *Pinus massoniana*) are biogeographically improbable for Java. Because the *rbcL* region often lacks the mutational resolution to reliably discriminate between regional congeners, and considering the potential geographical bias in global reference databases, these taxonomic assignments should not be treated as definitive species occurrences. Instead, we interpret these results as 'nearest-reference matches. They likely represent local congeneric species or closely related regional flora consumed by the silvery gibbons, whose specific reference sequences are currently unrepresented in the database.

Table 4. Nanodrop spectrophotometry results detailing genomic DNA concentration and A260/280 purity ratios for composite and fecal samples

Code	Volume (µL)	Nanodrop reading	
		gDNA concentration (ng/µL)	A260/280
S089.1	35	29.6	1.89
S089.2	35	17.3	1.95
S089.3	35	34.1	1.95
S089.4	35	18.3	1.88
S089.5	35	40.5	1.80

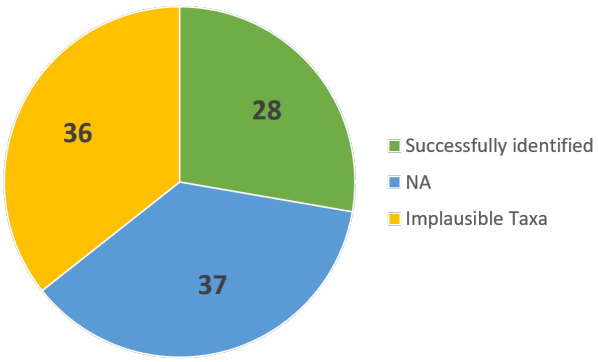

Figure 7. Classification reliability of detected taxa, categorizing results into identified, implausible, and non-applicable (NA) groups

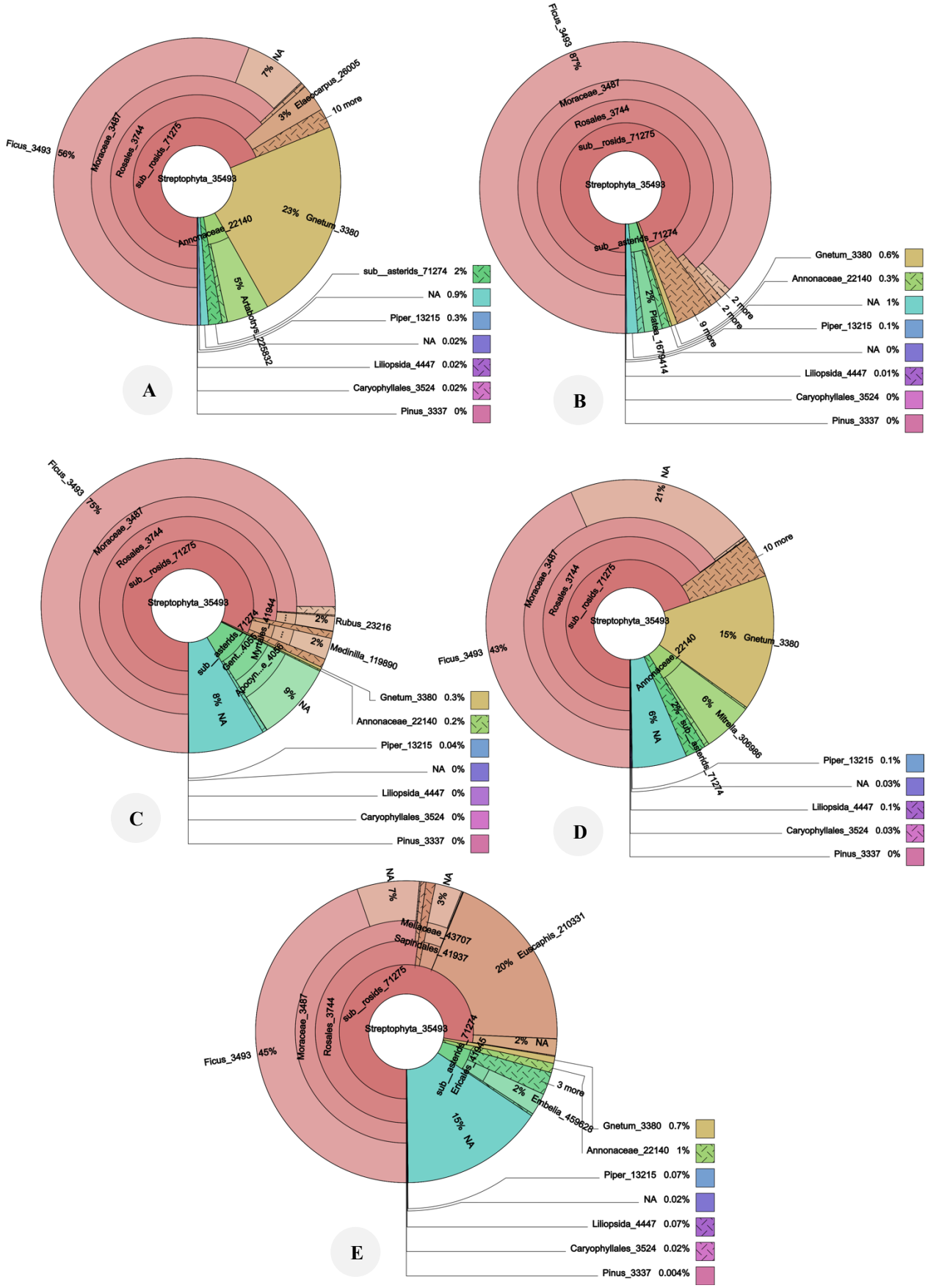

Figure 6. Krona visualization of the primary and secondary plant taxa consumed by the silvery gibbon. *Ficus* emerges as the most dominant genus across all five composites representing different habitat conditions. A. S089.1 (dense canopy, high richness), B. S089.2 (dense canopy, lower richness), C. S089.3 (agroforestry edge), D. S089.4 (highly degraded zone), E. S089.5 (fragmented/isolated patch)

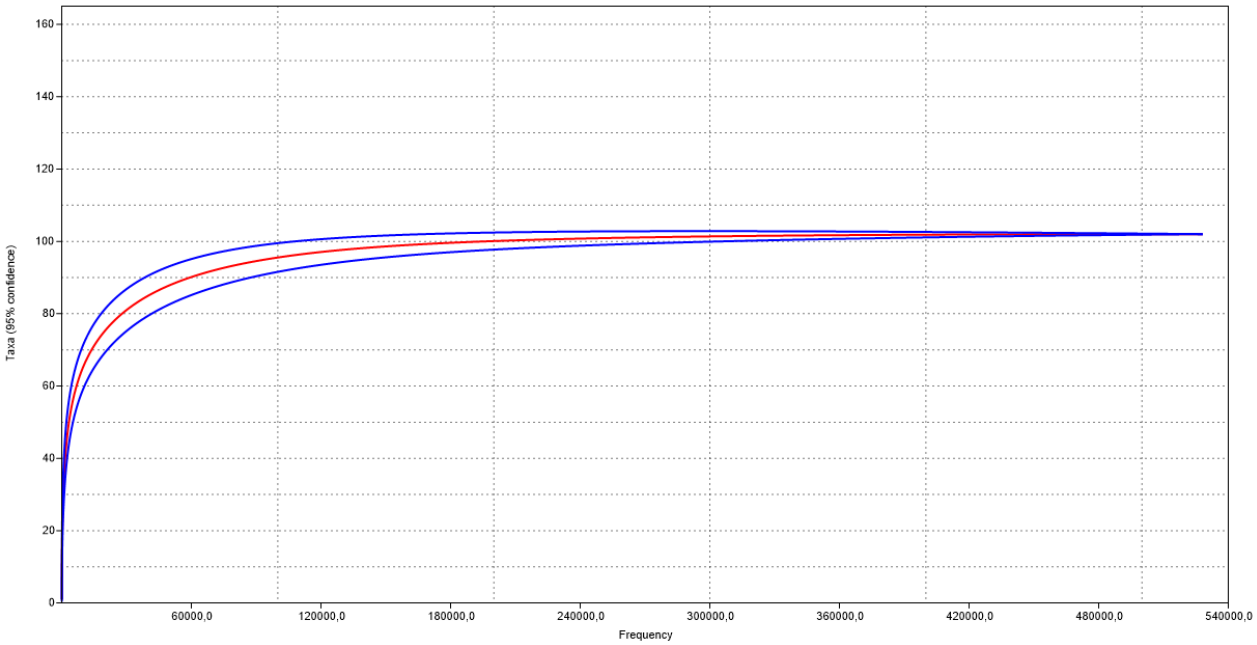

Figure 8. Sequencing depth adequacy based on *rbcL* ASV rarefaction. The plateauing of the *rbcL* Amplicon Sequence Variant (ASV) accumulation curve indicates that the sequencing depth (exceeding 300,000 reads per frequency unit) was adequate to capture the vast majority of the plant DNA diversity present in the fecal samples

Table 5. Top 10 representative dietary taxa of the silvery gibbon identified via NGS during the early dry season

ASVs	Order	Family	Genus	Species	RRA S089.1	RRA S089.2	RRA S089.3	RRA S089.4	RRA S089.5
341558	Rosales	Moraceae	*Ficus*	*Ficus benjamina*	0.55745	0.87303	0.75118	0.42558	0.44453
48949	Rosales	Moraceae	NA	NA	0.06995	0.01242	0.00873	0.21361	0.06691
28056	Gnetales	Gnetaceae	*Gnetum*	*Gnetum latifolium*	0.21930	0.00650	0.00299	0.02545	0.00493
21755	Gnetales	Gnetaceae	*Gnetum*	*Gnetum montanum**	0.01083	0.00000	0.00000	0.12717	0.00235
17303	Crossosomatales	Staphyleaceae	*Euscaphis**	*Euscaphis japonica**	0.00061	0.01104	0.00048	0.00818	0.19545
11300	Gentianales	Apocynaceae	NA	NA	0.00525	0.00693	0.08631	0.00033	0.00190
10334	Magnoliales	Annonaceae	*Mitrella*	*Mitrella kentii*	0.00509	0.00018	0.00000	0.05650	0.00932
5665	Magnoliales	Annonaceae	*Artabotrys*	*Artabotrys hongkongensis**	0.04762	0.00219	0.00167	0.00211	0.00000
4573	Oxalidales	Elaeocarpaceae	*Elaeocarpus*	*Elaeocarpus glabripetalus**	0.03028	0.00386	0.00352	0.00394	0.00000
4570	Ericales	Primulaceae	*Embelia*	*Embelia cotinoides**	0.00199	0.00369	0.00000	0.01362	0.02354

Note: This curated subset highlights core nutritional resources, excluding environmental DNA. Asterisks denote ecological status: (*) identifies potentially rare or novel regional flora detected by molecular sensitivity, while (**) indicates nearest-neighbor database matches for unsequenced local congeners. The dominance of these taxa reflects the gibbon's selective foraging strategy toward high-value fallback resources (e.g., Moraceae)

Metabarcoding analysis using the *rbcL* primer successfully amplified dietary plant DNA from the fecal samples. The family Moraceae, specifically *F. benjamina*, recorded the highest Relative Read Abundance (RRA) across all five samples, ranging from 0.425 to 0.873. Another notable Moraceae signal with the second-highest RRA was also detected. Field observations during the sampling period confirmed that *Ficus* spp. were in optimum fruiting condition. Silvery gibbons were observed foraging in these trees, and *Ficus* fruit remnants and seeds were visibly dominant in the collected fecal samples (Figure 9). The NGS data revealed inter-sample variation in secondary dietary taxa. For example, *Gnetum latifolium* reached an RRA of 0.219 in sample S089.1, and *Gnetum* sp. reached 0.127 in sample S089.4. Composite sample S089.5 contained an abundance of an unidentified species in the Staphyleaceae family (RRA 0.195), a taxon absents from the other composites. Trace taxa were also detected, including *Rubus* (<0.017), *Piper* spp. (<0.003), and *Sandoricum koetjape* (<0.004). Although *Artocarpus elasticus* trees were frequently occupied by gibbons during field observations, they were absent from the NGS dietary profile during this dry-season sampling.

Community structure, vegetation diversity, and NGS verification

The PCQ analysis yielded 89 taxa, with 69 successfully identified to the species level. The species list and structural dominance are ranked by the Important Value Index (IVI) and presented in Table 6. The Point-Centered Quarter (PCQ) sampling technique identified *A. elasticus* as the structurally dominant species in the forest (Important Value Index [IVI] Rank 1, IVI: 23.230), followed by *Schleichera oleosa* (IVI Rank 2, IVI: 19.051) and *Mallotus paniculatus* (IVI Rank 3, IVI: 12.169). In contrast, *F. benjamina* held an IVI rank of 23 (IVI: 4.313), with other *Ficus* species scattered across ranks 20, 35, 40, 41, and 56. Plant community structure under habitat modification is presented in Table 6.

Table 7 exhibited high species richness (Dmg: 15.915) and low dominance (C: 0.022), alongside moderate diversity (H: 1.796) and evenness (E: 0.400). Collectively, these metrics characterize a species-rich assemblage where dominance is distributed across multiple taxa, though relative abundances remain moderately uneven. The Point-Centered Quarter (PCQ) results and the calculated Importance Value Index (IVI), combined with verification from ground-truthed interviews and the final verification outcomes, are presented in Table 8.

Of the 47 plant families detected in the silvery gibbon's diet via NGS, only 40.43% (19 families) were fully corroborated by both PCQ vegetation surveys and local interviews. Interestingly, interviews proved to be a robust supplementary verification tool, confirming the absence of 19.15% (9 families) of the NGS taxa from the PCQ data. In contrast, the PCQ surveys verified only 8.51% (4 families) on their own. Comparison at the species level showed that 17 out of 29 NGS-detected species (58.6%) were also recorded in the PCQ vegetation plots.

The remaining 12 species detected by NGS were identified as non-tree taxa (e.g., lianas and shrubs) or small-diameter trees that did not meet the PCQ inclusion criteria (DBH>10 cm), demonstrating the high sensitivity of metabarcoding in detecting diverse growth forms. Notably, nearly a third of the NGS-detected families (31.91%, or 15 families) remained unconfirmed by either traditional method. A comparison between the NGS diet profile and PCQ data revealed that the silvery gibbons consumed 17 of the 89 recorded tree species (19%). Comparison at the species level showed that 17 out of 29 NGS-detected species (58.6%) were also recorded in the PCQ vegetation plots. The remaining 12 species detected by NGS were identified as non-tree taxa (e.g., lianas and shrubs) or small-diameter trees that did not meet the PCQ inclusion criteria (DBH>10 cm), demonstrating the high sensitivity of metabarcoding in detecting diverse growth forms. Several species detected with high frequency by NGS (specifically climbers such as *M. kentii*, *Artabotrys* sp., and *Embelia* sp.) were not recorded in the PCQ survey. Furthermore, the NGS data provided taxonomic resolution at the family or order level (e.g., Caryophyllales, Sapindaceae) where visual morphological identification failed. Cross-verification of the plant taxa highlighted distinct disparities between the methods. While 21 core species (predominantly large trees such as *F. benjamina*, *Cananga odorata*, and *D. dao*) were confirmed across methods, local interviews identified an additional 21 taxa not detected by the PCQ survey, including *Artocarpus altilis* and *Samanea saman*.

Ultimately, the consensus between PCQ and interviews accounted for 40.43% of the families, while local knowledge from interviews verified 19.15% of the unique species unrecorded by the ecological survey. Although Table 8 indicates that 15 out of the 47 molecularly identified plant families (31.91%) detected via NGS remained unconfirmed by either PCQ surveys or local interviews, this pattern should not be interpreted as a direct discrepancy. Rather, it highlights the inherent differences and the complementary nature of these assessment methods. The lack of direct correspondence across datasets is primarily driven by limitations in taxonomic resolution, as numerous NGS sequences could only be resolved to higher taxonomic ranks (e.g., order or family) and remained unassigned ('NA') at the genus or species levels (Table 5). This broad resolution complicates direct matching with specific local plant names obtained through interviews. Furthermore, the PCQ sampling method exhibits a methodological bias toward prominent tree species, potentially overlooking non-tree taxa, such as lianas, epiphytes, and rare herbaceous flora, that are actively consumed by the silvery gibbons and are well captured by the high sensitivity of NGS. Consequently, rather than contradicting one another, these datasets collectively overcome individual methodological biases to provide a more holistic and robust overview of the silvery gibbon's diet.

Figure 9. Fresh silvery gibbon feces containing visible remnants of *Ficus* fruit skin and seeds. The presence of undigested *Ficus* fruit skin and seeds provides critical ground-truthing that corroborates the high Relative Read Abundance (RRA) of Moraceae detected in our NGS pipeline

Table 6. Floristic list (PCQ method) sorted by IVI rank; unidentified taxa are classified as local morphotypes (local names appended) rank

Species	Family	RDs (%)	RDm (%)	FR (%)	IVI	IVI rank
Artocarpus elasticus	Moraceae	6.746	9.442	7.042	23.230	1
Schleichera oleosa	Sapindaceae	5.952	8.403	4.695	19.051	2
Mallotus paniculatus	Euphorbiaceae	4.762	3.181	4.225	12.169	3
Castanopsis acuminatissima	Fagaceae	2.381	4.469	2.817	9.667	4
Pangium edule	Achariaceae	3.175	3.346	2.817	9.338	5
Schima wallichii	Theaceae	3.175	2.578	2.347	8.100	6
Lithocarpus sundaicus	Fagaceae	2.381	3.343	2.347	8.072	7
Nauclea orientalis	Rubiaceae	2.381	1.657	2.817	6.855	8
Pinus merkusii	Pinaceae	2.381	1.780	2.347	6.508	9
Maesopsis eminii	Rhamnaceae	2.381	1.489	2.347	6.217	10
Radermachera glandulosa	Bignoniaceae	2.778	1.513	1.878	6.168	11
Symplocos fasciculata	Symplocaceae	1.984	1.687	2.347	6.018	12
Morphotype 1 (Local: Picis)*	-	1.984	1.867	1.878	5.729	13
Dalrympelea sphaerocarpa	Staphyleaceae	1.190	2.983	1.408	5.582	14
Hypobathrum microcarpum	Rubiaceae	1.587	1.906	1.878	5.371	15
Sterculia lanceolata	Malvaceae	1.587	1.531	1.878	4.996	16
Dysoxylum sp. 1 (Morphotype)	Meliaceae	1.984	1.579	1.408	4.971	17
Castanopsis argentea	Fagaceae	1.587	1.777	1.408	4.772	18
Macropanax dispermus	Araliaceae	1.984	0.879	1.878	4.741	19
Ficus annulata	Moraceae	1.587	2.041	0.939	4.567	20
Callicarpa pentandra	Lamiaceae	1.587	1.564	1.408	4.559	21
Nephelium sp.	Sapindaceae	1.587	1.522	1.408	4.517	22
Ficus benjamina	Moraceae	1.190	1.714	1.408	4.313	23
Toona sinensis	Meliaceae	1.190	1.600	1.408	4.199	24
Platea latifolia	Metteniusaceae	1.587	1.089	1.408	4.085	25
Morphotype 2 (Local: Lembayungan)*	-	1.587	0.618	1.878	4.083	26
Alstonia scholaris	Apocynaceae	1.190	1.194	1.408	3.793	27
Lauraceae sp. 1 (Morphotype)*	-	1.190	0.984	1.408	3.583	28
Cinchona pubescens	Rubiaceae	1.190	0.678	1.408	3.277	29
Dillenia obovata	Dilleniaceae	1.190	1.038	0.939	3.168	30
Dysoxylum sp. 2 (Morphotype)	Meliaceae	1.190	1.026	0.939	3.156	31
Bischofia javanica	Phyllanthaceae	0.794	1.321	0.939	3.053	32
Engelhardia serrata	Juglandaceae	0.794	1.245	0.939	2.978	33
Sandoricum koetjape	Meliaceae	0.794	1.155	0.939	2.888	34
Ficus nervosa	Moraceae	0.397	1.918	0.469	2.784	35
Albizia procera	Fabaceae	0.794	0.990	0.939	2.723	36
Mangifera odorata	Anacardiaceae	0.794	0.948	0.939	2.681	37
Elaeocarpus angustifolius	Elaeocarpaceae	0.794	0.858	0.939	2.591	38
Cleistanthus monoicus	Phyllanthaceae	0.794	0.804	0.939	2.537	39
Ficus variegata	Moraceae	0.794	0.765	0.939	2.498	40
Ficus fistulosa	Moraceae	0.794	0.648	0.939	2.381	41
Morphotype 3 (Local: Kinjeng)*	-	0.794	0.639	0.939	2.372	42
Morphotype 4 (Local: Kemlekson)*	-	0.794	0.597	0.939	2.330	43
Albizia chinensis	Fabaceae	0.794	0.492	0.939	2.225	44
Morphotype 5 (Local: Kanjilan)*	-	0.794	0.444	0.939	2.177	45
Archidendron pauciflorum	Fabaceae	0.794	0.417	0.939	2.150	46
Heptapleurum polybotryum	Araliaceae	0.794	0.363	0.939	2.096	47
Trema orientale	Cannabaceae	0.794	0.735	0.469	1.998	48
Spondias pinnata	Anacardiaceae	0.397	1.044	0.469	1.911	49
Neolamarckia cadamba	Rubiaceae	0.794	0.609	0.469	1.872	50
Tectona grandis	Lamiaceae	0.794	0.561	0.469	1.824	51
Morphotype 6 (Local: Miri Sepet)*	-	0.794	0.528	0.469	1.791	52
Crypteronia paniculata	Crypteroniaceae	0.794	0.444	0.469	1.707	53
Planchonia valida	Lecythidaceae	0.397	0.780	0.469	1.647	54
Lauraceae sp. 2 (Morphotype)*	-	0.397	0.720	0.469	1.587	55
Ficus sp. 1 (Morphotype) (1)	Moraceae	0.397	0.678	0.469	1.545	56
Ficus sp. 2 (Morphotype) (2)	Moraceae	0.794	0.270	0.469	1.533	57
Ficus sp. 3 (Morphotype) (3)	Moraceae	0.397	0.645	0.469	1.512	58
Styrax benzoin	Styracaceae	0.397	0.639	0.469	1.506	59
Dracontomelon dao	Anacardiaceae	0.397	0.540	0.469	1.407	60
Lithocarpus elegans	Fagaceae	0.397	0.540	0.469	1.407	61
Ficus recurva	Moraceae	0.397	0.516	0.469	1.383	62

Dysoxylum sp. 2 (Morphotype)	Meliaceae	0.397	0.429	0.469	1.295	63
Morphotype 7 (Local: Kala Pacung)*	-	0.397	0.420	0.469	1.286	64
Symplocos fasciculata	Symplocaceae	0.397	0.390	0.469	1.256	65
Lauraceae sp. 3 (Morphotype)*	-	0.397	0.390	0.469	1.256	66
Melicope elleryana	Rutaceae	0.397	0.360	0.469	1.226	67
Lauraceae sp. 4 (Morphotype)*	-	0.397	0.360	0.469	1.226	68
Schoutenia kunstleri	Malvaceae	0.397	0.351	0.469	1.217	69
Morphotype 8 (Local: Kayu Semut)*	-	0.397	0.336	0.469	1.202	70
Morphotype 9 (Local: Kemejing)*	-	0.397	0.288	0.469	1.154	71
Vitex pinnata	Lamiaceae	0.397	0.276	0.469	1.142	72
Prasoxylon alliaceum	Meliaceae	0.397	0.255	0.469	1.121	73
Commersonia bartramia	Malvaceae	0.397	0.240	0.469	1.106	74
Syzygium samarangense	Myrtaceae	0.397	0.210	0.469	1.076	75
Homalanthus populneus	Euphorbiaceae	0.397	0.210	0.469	1.076	76
Artocarpus heterophyllus	Moraceae	0.397	0.210	0.469	1.076	77
Durio zibethinus	Malvaceae	0.397	0.204	0.469	1.070	78
Gnetum gnemon	Gnetaceae	0.397	0.201	0.469	1.067	79
Macaranga sp.	Euphorbiaceae	0.397	0.201	0.469	1.067	80
Terminalia bellirica	Combretaceae	0.397	0.186	0.469	1.052	81
Pittosporum moluccanum	Pittosporaceae	0.397	0.186	0.469	1.052	82
Hevea brasiliensis	Euphorbiaceae	0.397	0.159	0.469	1.025	83
Cananga odorata	Annonaceae	0.397	0.150	0.469	1.016	84
Acalypha macrostachya	Euphorbiaceae	0.397	0.150	0.469	1.016	85
Dendrocnide stimulans	Urticaceae	0.397	0.147	0.469	1.013	86
Hibiscus macrophyllus	Malvaceae	0.397	0.135	0.469	1.001	87
Parartocarpus bracteatus	Moraceae	0.397	0.120	0.469	0.986	88
Morphotype 10 (Local: pari)*	-	0.397	0.096	0.469	0.962	89

Note: *We have 14 unidentified species due to limited specimens' phenological data and labeled according to local names, RDs: Relative Density, RDm: Relative Dominance, FR: Relative Frequency, IVI: Important Value Index

Table 7. Vegetation diversity and dominance indices as indicators of food plant availability in the gibbon habitat based on the Point-Centered Quarter (PCQ) method

Diversity index (H')	Dominance index (C)	Margalef's species richness index (Dmg)	Species evenness index (E)
1.796 (Moderate)	0.022 (Low)	15.914 (High)	0.400 (Moderate)

Table 8. Verification status of plant families detected in the silvery gibbon diet via NGS. Cross-validation of 47 molecularly identified plant families using Point-Centered Quarter (PCQ) surveys and local interviews (categorized as confirmed by both, one, or neither)

Category	Description	Count	Total NGS taxa (%)
Fully Confirmed	Verified by both PCQ and Interview	19	40.43%
Interview Only	Verified by Interview (not reported by PCQ)	9	19.15%
PCQ Only	Verified by PCQ (not reported by Interviews)	4	8.51%
Unconfirmed	Found in NGS, but not reported by either	15	31.91%
Total		47	100%

Discussion

Diet composition inferred from NGS and field observation

To fully understand the foraging ecology of the silvery gibbon, it is essential to contextualize the dietary outcomes derived from fecal DNA against the available vegetation diversity. Our vegetation surveys indicated that the forest canopy is structurally dominated by species such as *A. elasticus* and *S. oleosa*. However, despite this availability, the DNA barcoding results revealed a heavily skewed dietary profile disproportionately dominated by Moraceae (specifically *Ficus*). The robust and consistent dominance of *F. benjamina* across all five composites firmly establishes its ecological significance as a primary staple resource for the silvery gibbon in this habitat. This molecular evidence aligns with its recognized role as a keystone species in tropical forest ecosystems, providing a reliable food source. According to the lens of Resource Selection Theory, our research reveals a strong dietary selectivity in Javan gibbons, underscored by the stark contrast between resource availability and actual consumption. Based on the PCQ vegetation analysis, the habitat is structurally dominated by *A. elasticus*. However, NGS diet profiling demonstrates a disproportionate preference for *Ficus* taxa. This quantitative disparity confirms that Javan gibbons actively select *Ficus* species, likely due to their year-round availability and high nutritional value as keystone resources, rather than foraging opportunistically based on the highest floral abundance. Such pronounced selectivity highlights the critical role of targeted conservation for *Ficus* species to sustain gibbon populations, even in forests dominated by other large canopy trees.

This strong reliance on *Ficus* is also reported in two previous studies by Zhang et al. (2022) and Zhong et al. (2023). Their study reported that gibbons select ripe figs when they are readily available in their habitat. This food selection of figs in the gibbon species probably indicates a primary food preference, a behavior commonly passed on

by adult gibbons to their offspring (Yi et al. 2020). Ecologically, *F. benjamina* and other *Ficus* species serve as essential food resources for silvery gibbons during the dry season, consistent with literature noting their year-round availability. Lower *Ficus* abundance likely increases survival pressure during seasons of food scarcity, particularly when the fruiting of *A. elasticus* (rank IVI 1) is unavailable in the habitat. Although *A. elasticus* trees were frequently observed and occupied by gibbons, they were absent from the diet during our dry-season sampling. Based on its high structural dominance in the habitat, we hypothesize that this species might serve as a primary or fallback food source during the rainy season when fruiting is abundant. However, multi-season dietary data are required to confirm this hypothesis.

Despite the observed high reliance on *Ficus,* a consistent *Ficus* signal in the NGS data also revealed significant inter sample heterogeneity, suggesting opportunistic foraging and spatial variation in response to site-specific food availability. For example, while Gnetaceae reads were generally low, *G. latifolium* reached an RRA of 0.219 in sample S089.1 (dense canopy with high species richness), and *Gnetum* sp. reached 0.127 in sample S089.4 (highly degraded zone). Since *Gnetum* spp. are fruiting all year-round and are known to be rich sources of protein (Krishnatreya et al. 2021), selection on these plant species is likely to provide the essential proteins needed to support gibbons' physiological processes. Similarly, composite sample S089.5 was uniquely characterized by a high abundance of an unidentified species in the Staphyleaceae family, a taxon completely absents from the other composites. Furthermore, identification of trace taxa such as *Rubus*, *Piper* spp., and *S. koetjape* demonstrated high NGS sensitivity in identifying rare or low-biomass food items that are typically not captured by ground observation. Tree selection shaped the evolution of gibbon foraging, highlighting the value of molecular dietary studies in understanding primate ecology (Lim et al. 2021).

In addition, although NGS provides a high-resolution taxonomic profile of the silvery gibbon's diet, we noted that fecal metabarcoding has methodological limitations. The great sensitivity of NGS allows it to detect unwanted DNA from non-target plants. Thus, gibbons may have accidentally eaten plant fragments while hunting fruits or insects, which may have affected their taxonomic richness, especially taxa with very low Relative Read Abundance (RRA). Thus, while our data shows a wide dietary spectrum, rare ASVs should be evaluated with caution to distinguish between purposely targeted food sources and inadvertent ambient exposure.

Vegetation structure assessed using the PCQ and interview

A comparison of amplicon sequence variants (ASVs) generated by next-generation sequencing with ground-truthing data revealed clear differences between local interviews and the Point-Centered Quarter (PCQ) method. All three approaches detected 21 core species, primarily large and easily recognizable trees such as *F. benjamina*, *C. odorata*, and *D. dao*. Local interviews proved essential for supplementing the dataset by identifying 21 taxa not detected by the PCQ technique, including notable species such as *A. altilis* (breadfruit) and *S. saman*. Although the PCQ method is highly efficient for rapid vegetation surveys, these findings underscore its limitations when used in isolation. The PCQ, as a plotless distance method, records only the nearest tree per quadrant and therefore statistically favors widespread, evenly spaced canopy species. Consequently, this method often overlooks rare, clumped, or non-tree plants such as lianas, epiphytes, and understory shrubs, which are frequently important components of the silvery gibbon's diet. Our results underscore that traditional vegetation surveys like PCQ may underestimate the actual dietary breadth of the Silvery gibbon. As evidenced by the 12 species detected exclusively through NGS, including various lianas and understory plants, metabarcoding provides a more holistic view of the diet that includes items often missed in tree-centric plots. This is particularly important for detecting fallback foods or cryptic dietary items during the early dry season.

At approximately 31.91% of the tree families identified through NGS were not corroborated by either the Point-Centered Quarter (PCQ) method or local interviews. Concordance between the PCQ method and interviews was observed for 40.43% of the families. The taxa that remained unconfirmed may include cryptic, rare, or non-woody species that traditional and visual surveys are not equipped to detect. For instance, NGS identified six distinct entries for *Gnetum* and several *Piper* species, none of which were present in the PCQ results. Furthermore, NGS data provided resolution at the family or order level (e.g., Caryophyllales, Sapindaceae) where morphological identification was limited. These unconfirmed records likely represent rare species present in the habitat, but outside the sampling point's spatial range, as well as potential environmental DNA (eDNA) traces from the broader ecosystem. This situation highlights the superior sensitivity of molecular methods in capturing the complete biological diversity of the site. Indeed, while NGS can uncover broader and more cryptic layers of biodiversity, these methods are most effective when supplemented by ground checks and local knowledge. Interviews offer a distinct advantage, as local knowledge identified 19.15% of unique species not recorded by the ecological survey, demonstrating that it is a cost-effective approach for biodiversity surveys.

Consistent with Optimal Foraging Theory, the difference between the structural dominance of trees (IVI) and the feeding behaviors of silvery gibbons shows that these primates use a selective foraging strategy rather than simply eating plants based on their availability, aiming to maximize nutritional intake relative to foraging effort. The examination of NGS and PCQ data revealed that only 17 of 89 tree species (19%) comprised their diet. This case means that the canopy trees they could consume were not significantly different from each other. Also, the fact that the tree species with the highest IVI scores are not the same as those they consume the most shows that the species that are most common in their area do not always determine what they eat. These ten plants presumably provide the silvery gibbon with the food necessary to meet its nutritional requirements, including new leaves, flowers, and both ripe

and unripe fruits. The gibbons probably only eat certain foods because of the trees' phenological rhythms in their forest habitat and their own dietary needs (Jang et al. 2021). Moreover, several species are frequently identified by NGS, in addition to specific PCQ identifications. This case indicates that gibbons utilize plant resources, especially lianas and non-tree vegetation, which are either underrepresented or not captured by tree-focused PCQ sampling.

The molecular traits of the Moraceae are closely tied to the physical makeup of the forest. Our initial field observation indicated that this family was the predominant tree species in most areas, with all *Ficus* species exhibiting fruiting. This study demonstrates that the elevated Relative Read Abundance (RRA) of *F. benjamina* accurately reflects resource utilization rather than being a byproduct of PCR amplification bias. The presence of various secondary dietary species at each site, such as *Gnetum* sp. and Staphyleaceae, further demonstrates that the environments are diverse. In landscapes with thick, broken-up, and edge microhabitats, gibbons optimize their foraging efficiency by balancing targeted high-value resources with easily accessible alternatives. They rely heavily on the abundant *Ficus,* which likely serves as a fallback food source to meet their energy requirements when preferred seasonal foods are scarce during our dry-season sampling period. As the canopy starts to break down, they strategically use resources available only in certain places to reach other fruits, reinforcing their behavioral flexibility in a suboptimal habitat. Indeed, the metabarcoding results demonstrate that silvery gibbons have many food choices to meet their nutritional needs, even though they depend on a few key families (Lauer et al. 2025).

The NGS dietary profile, PCQ vegetation structure, and local interviews all have different detection ranges. This disparity shows how limited it is to use just one survey approach. The PCQ approach, a plotless distance methodology, limits sampling to trees that are nearest to each other. This limitation biases the data toward species that are both common and evenly distributed. As a result, NGS's sampling range naturally excludes non-tree species, including lianas (*M. kentii*, *Embelia*, etc.) and understory shrubs (*Piper* spp., *Rubus*), even though they are important parts of the diet. NGS found 31.91% of families that field surveys did not confirm. These families likely include cryptic species that are not evenly distributed, as well as environmental DNA (eDNA) from the larger ecosystem. This percentage shows how sensitive metabarcoding is to finding unusual or low-biomass food products that visual surveys overlook.

Local interviews, on the other hand, were very helpful because they showed that over 20% of the unique species the PCQ did not record were actually present. Local Ecological Knowledge (LEK) gathered through interviews reflects long-term, cumulative observations of the forest over broader spatial and temporal scales. Local communities possess intimate knowledge of cryptic and non-timber forest products, enabling them to identify canopy-dwelling and low-density taxa that visual ecological surveys typically miss. This emphasizes the ecological necessity of integrating traditional local knowledge to comprehensively validate genomic dietary data, particularly for canopy-foraging primates. This technique shows that local ecological knowledge is a useful and supplementary technique for qualitative biodiversity evaluation, in addition to the advantage of using NGS. This technique still bears a weakness. Due to the inherent limits of short-read barcode resolution and incomplete reference databases for tropical flora, some NGS assignments at the species level may be taxonomically uncertain and should be interpreted as genus-level or closely related molecular operational taxonomic units (MOTUs).

Silvery gibbons diet and its implication for conservation priority

According to the Optimal Foraging Theory by MacArthur and Pianka (1966, as cited in Krebs 2001; Chaves et al. 2023), the silvery gibbons use a diet-breadth foraging strategy to meet their energy needs while getting sufficient nutrients. During periods of seasonal food scarcity, such as the dry period, *Ficus* and other Moraceae are likely to function as a fallback food for the silvery gibbon. However, these foods provide insufficient nutritional value for the gibbons, and thus, they are forced to forage across a diverse range of tree species within their habitat. As a fallback tree species, *Ficus* and other Moraceae are critical to raise the possibility of gibbons' survival in their habitat due to their ability to provide sufficient carbohydrates for energy. By eating fallback foods and diversifying their diet, the silvery gibbons at our study site demonstrate optimal foraging, selecting food using their innate sensitivity or environmentally shaped foraging behavior (Jang et al. 2021) to maximize the surplus nutrients per unit foraging time. The availability of fruiting Moraceae, especially in the genus *Ficus* and probably *Arthocarpus*, is critical for the silvery gibbon's home range for foraging (Lim et al. 2021; Zhang et al. 2022). Due to the year-round availability of moraceous fruit and their asynchrony, Moraceae consumption may be associated with narrow home ranges performed by the silvery gibbon. Moraceae, notably *Ficus*, are a vital dry-season food, as well as a keystone taxon for forest gibbons. This taxon's prominence in conservation priorities will assure gibbon conservation success.

The silvery gibbon is entirely arboreal, usually brachiating among tree canopy branches and rarely descending to the ground during foraging. As reported by Borah et al. (2018) for Hoolock Gibbon, the silvery gibbon in our study sites tends to forage in the canopy. All gibbon-eaten plant families are tall trees or climbers that the gibbon can reach from the canopy. As arboreal primates, gibbons need forest canopy connectivity to move smoothly. Gibbons use the most profitable feeding strategy based on energy costs and rewards, and are protected from predators and humans when foraging in big trees with dense canopy connections (King and Marshall 2022). The phenological asynchrony of the key food taxa is critical. The abundance of *Ficus* as a reliable dry-season resource, mostly mentioned as a year-round fallback food in literature, combined with the phenological timing of *Artocarpus* (this species was found on non-fruiting phenology during the observation), *Gnetum*, and other primary foods, creates a temporal food calendar

that sustains the gibbon population. Habitat restoration programs should specifically prioritize planting confirmed Moraceae species (e.g., various *Ficus* and *Artocarpus* species) and other verified canopy fruit taxa to ensure a continuous phenological food supply and minimize seasonal shortages.

While Moraceae is the dominant genus, recurrent identification of secondary taxa reveals significant variation among samples. The presence of taxa such as *Gnetum* spp., noted for their year-round fruit production and high protein content (Krishnatreya et al. 2021), and Staphyleaceae indicates the spatial heterogeneity of the environment. When they reside in a mosaic of microhabitats, silvery gibbons seem to be the best at finding food. They usually eat *Ficus*, which is easy to find, but they also eat foods found only in their area, especially when the canopy is damaged, and they cannot reach other fruits. These results show that gibbons' foraging tactics are complex ways to obtain a variety of foods, which have evolved to meet important nutritional needs (Lim et al. 2021; Lauer et al. 2025).

Despite its high sensitivity, molecular dietary analysis necessitates critical interpretation due to inherent methodological biases. The elevated Relative Read Abundance (RRA) of *Ficus* aligns with our field observations of its intense consumption. However, RRA cannot be strictly interpreted as a direct quantitative measure of consumed biomass or volume. This limitation arises from multiple factors, including differential DNA degradation during digestion, varying chloroplast copy numbers among plant taxa, and PCR amplification biases that may preferentially amplify certain sequences over others. Furthermore, our study relied on the *rbcL* marker. While *rbcL* is highly universal across plant lineages and excellent for family- or genus-level dietary profiling, its slow mutation rate fundamentally limits taxonomic resolution at the species level. Unlike the Internal Transcribed Spacer 2 (ITS2) marker, which offers superior species-level discrimination but is prone to amplification failures in degraded fecal DNA, *rbcL* often struggles to resolve closely related congenerics. Consequently, some species-level assignments in our dataset should be interpreted cautiously as molecular operational taxonomic units (MOTUs).

Future multi-locus studies incorporating both *rbcL* and ITS2 would be highly beneficial to refine species-level dietary interactions. Pooled composite sampling presents an additional limitation. At the same time, although cost-effective for assessing group-level ecological strategies, it obscures individual dietary variation driven by age, sex, or social rank and may artificially inflate the group's perceived dietary breadth. Furthermore, while metabarcoding offers unparalleled sensitivity in detecting cryptic dietary items, it cannot capture the behavioral nuances of foraging, such as specific plant-part handling, social feeding interactions, or time-budget allocations, that are only accessible through direct field observation. Therefore, future longitudinal studies should prioritize the integration of high-resolution molecular data with systematic behavioral observations. Such a multi-proxy approach will provide an even more holistic understanding of *H. moloch* ecological dynamics, linking precise nutrient intake with the complex foraging behaviors that drive primate survival in fragmented landscapes.

However, we note two limitations, our dietary interpretations are restricted to the dry-season sampling period, requiring multi-season studies to confirm year-round fallback strategies, and some species-level NGS assignments should be interpreted with taxonomic caution due to barcode resolution limits. Ultimately, while this species shows remarkable adaptation to deteriorated habitats as a selective forager, the silvery gibbon will only survive in the long run if its habitat is restored by focusing on increasing plant diversity, restoring key fruit sources, and protecting continuous forest canopies essential for their mobility. Our findings provide a vital dietary baseline for optimizing the nutritional management of captive Javan gibbons, ensuring their health and readiness for successful reintroduction into the wild. It is important to note, however, that these findings may be specific to the unique floristic and anthropogenic conditions of the Dieng Mountains. Given that *H. moloch* inhabits diverse forest types across Java, ranging from lowland rainforests to sub-montane environments, their dietary plasticity likely leads to regional variations in resource utilization. Therefore, caution should be exercised when generalizing these results to all populations.

Our study underscores the necessity of localized ecological research to capture these site-specific differences, which are vital for tailoring conservation management plans to the particular needs of different regional populations. Additionally, our study design implies that sampling was limited to the early dry season. In this phase, fleshy fruits were scarcer, forcing the gibbon to eat more fallback foods. Thus, its strong reliance on *Ficus* is an effective foraging strategy during seasonal resource limitation, not its full annual diet. Extrapolating dry-season findings to the whole year may inflate gibbons' reliance on specific backup supplies and underestimate their nutritional diversification, which presumably increases during the wet season. To fully understand the silvery gibbon's nutritional flexibility and year-round foraging tactics, longitudinal research across multiple seasons is needed.

In conclusion, our study demonstrates that the dietary ecology of the silvery gibbon (*H. moloch*) in the Dieng Mountains is characterized by high foraging selectivity rather than opportunistic feeding. By integrating DNA metabarcoding with traditional surveys, we identified a broad dietary breadth comprising 47 families and 64 genera, with 31.9% of these families detected exclusively through NGS, including cryptic lianas and understory plants missed by visual methods. The stark disparity between habitat dominance, where *A. elasticus* held the highest IVI, and actual consumption, where Moraceae (specifically *Ficus*) dominated with a combined Relative Read Abundance (RRA) exceeding 45%, confirms that *Ficus* serves as a critical keystone resource and primary fallback food during the early dry season. Our findings explicitly support both hypotheses, the NGS data confirmed that molecular profiling reveals a broader and more sensitive dietary composition than traditional visual surveys by identifying numerous trace and non-tree taxa (e.g., cryptic lianas, *Piper* spp., and *Gnetum*) that were entirely missed by the PCQ

method. The stark quantitative disparity between the structural dominance of *A. elasticus* in the habitat and the gibbons' disproportionate dietary preference for *Ficus* taxa confirms that they exhibit targeted selective foraging driven by specific nutritional needs, rather than opportunistic feeding based on general tree abundance.

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