BIODIVERSITAS
Volume 26, Number 5, May 2025
Pages: 2508-2518

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260546

Dietary diversity of the vulnerable leaf-eating monkey *Presbytis fredericae* in the degraded forest of Mount Merbabu, Indonesia

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Manuscript received: 9 March 2025. Revision accepted: 15 May 2025.

Abstract. *Karyanto P, Tazkia D, Nayasilana IN, Susilowati A, Sunarto, Wahyudi J, Atmoko SSU, Nor SM. 2025. Dietary diversity of the vulnerable leaf-eating monkey* Presbytis fredericae *in the degraded forest of Mount Merbabu, Indonesia. Biodiversitas 26: 2508-2518.* Extensive forest degradation and fragmentation on Java Island, Indonesia, have resulted in significant habitat loss and a sharp decline in tree diversity. The remnant forests, characterized by declining plant diversity and composition, could have a major impact on the diversity and food preferences of a vulnerable leaf-eating monkey (*Presbytis fredericae*). In response to changes in a new floristic structure, *P. fredericae* may develop new foraging adaptations, allowing them to diversify their diet by consuming a variety of plant species. However, such behavior may not be performed, as the monkey's food choices are evidence of an evolutionary strategy to maximize nutrient and energy intake for survival. Therefore, dietary diversity can serve as a good proxy to examine the extent to which folivore monkeys' foraging behavior adapts to food resource change. This study investigated the food diversity of plants selected by *P. fredericae* in a degraded montane forest on Mount Merbabu, Indonesia. We employed a molecular-based Next-Generation Sequencing (NGS) technique to analyze 13 fecal samples. Our downstream analysis in RStudio identified 132 Operational Taxonomic Units (OTUs). By organizing the OTUs by their abundance in Microsoft Excel and comparing our results to an existing floristic database from previous vegetation surveys, we identified eight orders, nine families, and nine species in the tree stratum, and 11 orders, 12 families, and 14 species in the forest floor community. These findings indicate that *P. fredericae* selectively consumed only 34% of the total tree species and 14% of the available forest floor plant species. This food selection suggests that *P. fredericae* exhibits selective feeding and optimal foraging behavior, primarily by consuming immature foliage from a wide range of plant taxa. This behavior helps them meet their nutritional needs while minimizing the risk of toxin accumulation.

Keywords: Dietary diversity, forest degradation and fragmentation, next-generation sequencing, plant selection, *Presbytis fredericae*

INTRODUCTION

Forest degradation and fragmentation are the primary drivers for primate population decline and extinction (Estrada et al. 2020). Most primates persist in degraded forests, surrounded by an anthropogenically created matrix that negatively impacts their distribution, dispersal, behavior, gene flow, and population viability (Dhawale et al. 2020; Gould et al. 2020; Pinto et al. 2023; Ramsay et al. 2023; Shanee et al. 2023). Conservation biologists have intensified research on the impacts of habitat degradation on primate populations, providing valuable ecological insights to support conservation actions (de Figueiredo et al. 2023). This growing body of research has significantly advanced our understanding of the complex relationships between habitat degradation and primate population dynamics.

Primate responses to forest degradation and fragmentation varied from extinction (Estrada and Garber 2022) to exhibiting adaptive behavioral changes (Widyastuti et al. 2022). Food selection is one of the primates' adaptive behaviors to this habitat change. Food selection is a reliable proxy for the adaptation of primates to habitat degradation and fragmentation. This foraging behavior is an important response that primates must adapt to survive in newly formed food resources due to human interference (Stewart et al. 2023). The process of food selection influences several aspects of primate life, including their distribution, demography, and habitat preferences (Wang et al. 2022), habitat selection (Aristizabal et al. 2019), reproduction (Windley et al. 2022), and population persistence (Ozgul et al. 2023). Food selection is closely related to dietary diversity, as the choices made regarding food consumption directly determine the range of nutrients and food groups that make up an individual's diet. Understanding food diversity is, therefore, critical to assessing primates' ability to adapt to changes in food availability and environmental conditions (Almeida-Warren et al. 2022).

Presbytis fredericae (Sody, 1930) (Figure 3) may have an adaptive hindgut structure, allowing them to respond to changes in plant composition (Liu et al. 2022). However, the necessity to meet nutritional requirements drives *P. fredericae's* food selection process, which is influenced by ecological complexity (Felton et al. 2009). Therefore, forest degradation may have a detrimental effect on *P. fredericae's* food sources and selection despite its adaptive capabilities. Therefore, in order to provide scientific evidence for their conservation initiatives, it is imperative to investigate the nutritional diversity of *P. fredericae*.

Existing data on their diet are limited to visual observations. Since the Next-Generation Sequencing (NGS) techniques provide more accurate identification of dietary diversity (Quéméré et al. 2013). This study used a non-invasive DNA metabarcoding approach using the NGS technology to accurately identify the dietary diversity of *P. fredericae* (Tsuji et al. 2019). Our study aims to investigate *P. fredericae*'s dietary diversity in degraded habitats, providing valuable insights for conservation efforts.

Presbytis fredericae may have an adaptive hindgut structure, allowing them to respond to changes in plant composition (Liu et al. 2022). However, food selection depends on ecological complexity and is driven by the need to meet nutritional needs (Felton et al. 2009). Despite this adaptive ability, forest degradation may negatively impact *P. fredericae*'s food resources. Therefore, understanding the dietary diversity of *P. fredericae* is crucial for providing scientific data to support their conservation programs. Existing data on their diet are limited to visual observations. Since the NGS techniques provide more accurate identification of dietary diversity (Quéméré et al. 2013). This study used a non-invasive DNA metabarcoding approach using the NGS technology to accurately identify the dietary diversity of *P. fredericae* (Tsuji et al. 2019). Our study aims to investigate *P. fredericae*'s dietary diversity in degraded habitats, providing valuable insights for conservation efforts.

MATERIALS AND METHODS

Study area

The Indonesian Research Institute and Mount Merbabu National Park, Indonesia, reported the presence of *P. fredericae* in 2010 in a small area of fragmented and degraded secondary forest on Mount Merbabu, with a very small population size (Sawitri et al. 2010). We were interested in this newly recorded information and collected 13 samples from elevations ranging from 1,997 to 2,290 masl in the mountain's early and late successional secondary forests (7º28'20" S and 110º27'20" E). Our samples were collected in these forests (Figure 1), as these two types of forests are the only habitats occupied by *P. fredericae* and exhibit a very low vegetation diversity index (Nurmiyati et al. 2023; Saputra et al. 2023). The monkeys may have responded to these low H' values by exhibiting specific dietary behaviors, as they need to demonstrate a level of foraging flexibility that matches the vegetation resources available in their habitat (Tsuji et al. 2019).

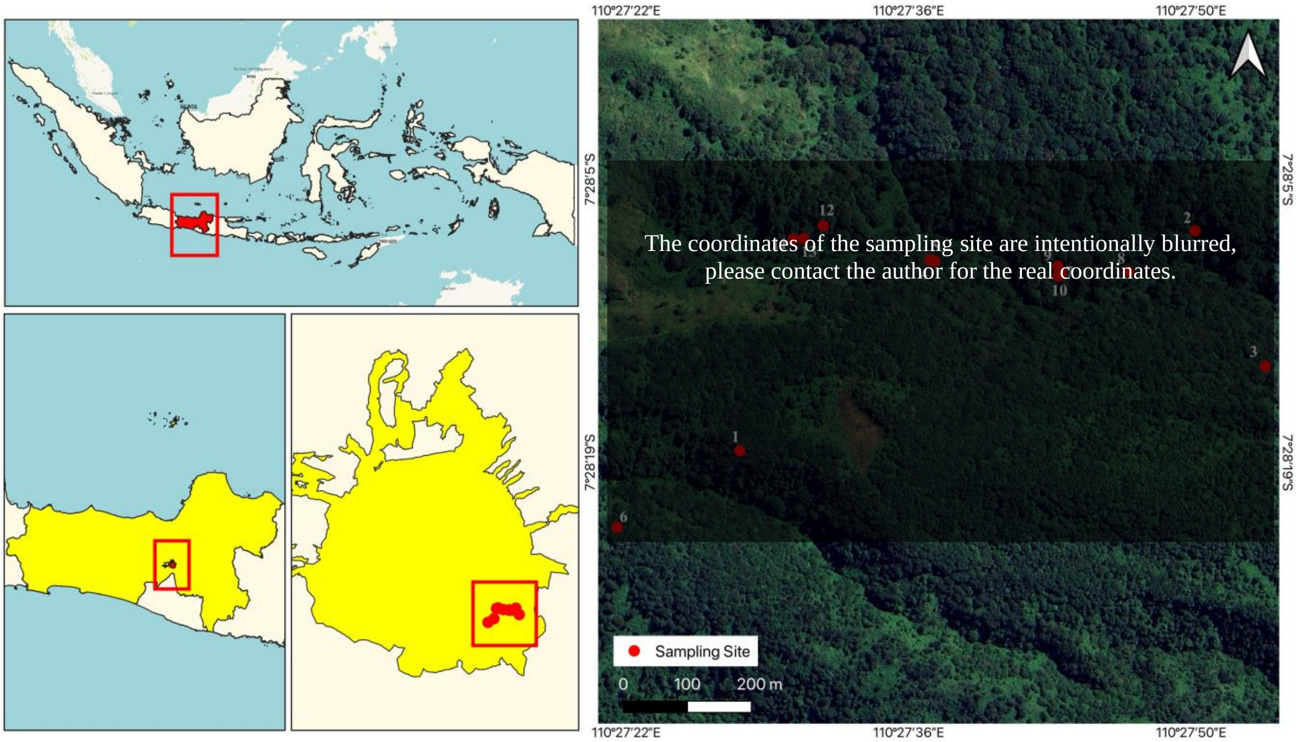
The coordinates of the sampling site are intentionally blurred, please contact the author for the real coordinates.

Figure 1. The study site in Mount Merbabu National Park, Indonesia is located at 7º28'20" S and 110º27'20" E. Points 1 to 13 are sites where the feces of *Presbytis fredericae* were collected

Procedures

General outline

Figure 2 outlines the overall steps of this study, from feces collection to dietary diversity analysis using a genetic approach. We performed the NGS method using plant mitochondrial-DNA mini barcodes to ensure the identification of plant food diversity consumed by *P. fredericae*, because this sequence-based method has been proven effective and efficient in revealing animal diets (Satam et al. 2023). However, NGS analysis only produces tiny DNA sequences, potentially leading to bias in providing actual dietary information (Gerwing et al. 2016). Small DNA fragments from fecal material are also rapidly degraded, resulting in low-quality bands during Quality Control (QC) (Zhang et al. 2019). Therefore, we confirmed the output by comparing the Operational Taxonomic Units (OTUs) generated from NGS with the floristic checklist of Mount Merbabu, according to Karyanto et al. (2022).

Fecal sampling and NGS validation using the local vegetation database

We collected *P. fredericae*'s feces in the dry seasons from June to August 2020 and 2021 and in September 2022, considering accessibility in Mount Merbabu, as there was no significant difference in tree composition and phenology between the two definitive seasons (dry and wet seasons). Considering that the DNA in the feces may be degraded soon after collection, our thirty fecal samples were fresh and immediately collected after defecation during the day or the afternoon, ensuring that the plant DNA in all fecal samples was a reliable proxy for the plant materials the monkeys ingested several hours before the sampling (de Flamingh et al. 2023). We collected each sample using a glass rod and put it in a marked 15 mL centrifuge tube. We preserved all of our fecal collection in 96% ethanol following the procedure conducted by Yang et al. (2020) and stored the entire sample in a freezer before the extraction. Our samples were coded as PK 1 through PK 13.

Since the small fragment of DNA sequence extracted from the feces might result in ambiguity during the NCBI BLAST (Zhong et al. 2019), we confirmed the results of the NGS using tree and lower-stratum species catalog of Mount Merbabu published by Karyanto et al. (2022). The list of floristic composition of two vegetation strata at the *P. fredericae*'s habitat yielded 98 species in 87 genera for the understory community and 45 families and 25 species in 23 genera for the tree community (Tables 1 and 2).

DNA extraction

To extract the DNA from the fecal sample, we used ZYMO RESEARCH's ZymoBIOMICS DNA Microprep Kit (D4300). We added 750 µL of ZymoBIOMICS lysis solution to each collected sample in a ZR Bashing bead lysis tube (0.1 and 0.5 mm). We followed the manufacturer's methodology till the filtered DNA was acquired to enter further downstream applications. Since the ribulose-bisphosphate carboxylase gene (*rbcL*) primer has been widely recognized as a standard primer in *rbcL* investigations, we utilized a little mini-barcode for the *rbcL* gene during the PCR to amplify the plant DNA (Botha et al. 2023). Following Erickson et al. (2017), We used the forward primer sequence 5'-CTTACCAGYCTTGATC GTTACAAAGG-3' and the reverse primer sequence 5'-GTAAAATCAAGTCCACCRCG-3' due to their ability to consistently produce a 379 base pair product across the alignment of the 500 *rbcL*. DNA extraction, PCR, library preparation, and sequencing are examples of laboratory procedures that can impact the quality of the data and the outcomes of the analysis (Haendiges et al. 2021). Therefore, we employ Quality Control (QC) methods for the DNA samples, including the use of Nanodrop to assess DNA purity, agarose gel electrophoresis to verify DNA integrity and potential contamination, and a Qubit 2.0 fluorometer to quantify DNA concentration. This allows us to verify the reliability of the data by performing QC at every stage of the process (Kekre and Pascoe 2023).

Next, to achieve higher taxonomic resolution and more precise plant identification, PCR was performed using the *rbcL* primers (Letsiou et al. 2024). During the dry laboratory steps, libraries were prepared on a paired-end Illumina platform to have 250bp paired-end reads. We entrusted the reads according to their unique barcode and cut by trimming the barcode and primer sequence (Schwartz et al. 2011). The paired-end readings were combined using FLASH (Chen et al. 2022). Therefore, to obtain high-quality and clean tags, quality filtering was performed on the raw tags under conventional filtering circumstances.

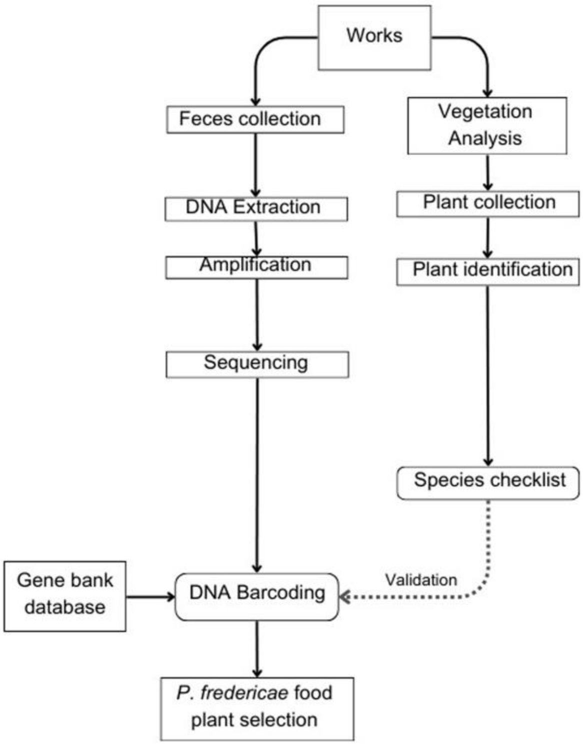

Figure 2. The general outlines of this study

Table 1. List of understory communities in the primary and secondary forests in Mount Merbabu, Indonesia

Order	**Family**		**Species**
Alismatales	Araceae	2 genera, 3 species	*Scindapsus* sp., *Arisaema triphyllum, Arisaema tortuosum*
Apiales	Apiaceae	1 genus, 1 species	*Centella asiatica*
	Araliaceae	2 genera, 2 species	*Aralia laevis, Hydrocotyle ranunculoides*
Arales	Araceae		
Asparagales	Orchidaceae	1 genus, 1 species	*Oberonia similis*
Asterales	Asteraceae	13 genera, 13 species	*Ambrosia acanthicarpa, Ageratina riparia, Erigeron sumatransis, Crassocephalum crepidiodes, Anaphalis javanica, Galinsoga parviflora, Emilia sonchifolia, Gamochaeta claviceps, Sonchus palustris, Ageratum conyzoides, Gnaphalium* sp.*, Chromolaena odorata, Acmella uliginosa*
Caryophyllales	Amaranthaceae	2 genera, 2 species	*Achyranthes aspera, Alternanthera* sp.
	Phytolaccaeae	1 genus, 1 species	*Phytolacca icosandra*
Cucurbitales	Cucurbitaceae	1 genus, 1 species	*Gynostemma pentaphylum*
Cyatheales	Cyaheaceae	1 genus, 1 species	*Cyathea* sp.
Cyperales	Cyperaceae	2 genera, 3 species	*Cyperus odoratus, Cyperus rotundus, Kyllinga monocephala*
Equisetales	Equisetaceae	1 genus, 1 species	*Equisetum hyemale*
Fabales	Fabaceae	2 genera, 2 species	*Desmodium repandum, Parochetus communis*
Gentianales	Rubiaceae	2 genera, 2 species	*Richardia brasiliensis, Rubia cordifolia*
Geraniales	-	-	*Unidentified Geraniales*
Gleicheniales	Gleicheniaceae	1 genus, 1 species	*Dicranopteris linearis*
Lamiales	Lamiaceae	4 genera, 4 species	*Buddleja davidii, Clinopodium vulgare, Scutellaria serrata, Solenostemon scutellarioides*
Liliales	Colcicaceae	1 genus, 1 species	*Disporum cantoniense*
	Smilacaceae	1 genus, 1 species	*Smilax anceps*
Lycopodiales	Lycopodiaceae	1 genus, 1 species	*Huperzia selago*
Malphigiales	Violaceae	1 genus, 1 species	*Viola reichenbachiana*
Myrtales	Melatomataceae	2 genera, 3 species	*Clidemia hirta, Melastoma* sp., *Melastoma malabatricum,*
Myricales	Myricaceae	1 genus, 1 species	*Morella cerifera*
Piperales	Piperaceae	1 genus, 1 species	*Peperomia rotundifolia*
Plantaginales	Plantaginaceae	1 genus, 1 species	*Plantago major*
Poales	Poaceae	14 genera, 15 species	*Arthraxon hispidus, Brachypodium sylvaticum, Briza minor, Digitaria sanguinalis, Imperata cylindrica, Isachne globosa, Microlena stipoides, Oplismenus hirtellus, Panicum effusum, Pennisetum purpureum, Poa annua, Setaria pumila, Setaria parviflora, Themeda quadrivalvis, Zoysia matrella*
	Cyperaceae	1 genus, 1 species	*Carex baccans*
Polygonales	Polygonaceae	3 genera, 4 species	*Persicaria chinensis, Polygonum nepalense, Polygonum chinensis, Rumex obtusifolius*
Primulales	Myrsinaceae	1 genus, 1 species	*Lysimachia* sp.
Polypodiales	Aspleniceae	1 genus, 1 species	*Pteridium aquilium*
	Dennstaedtiaceae	1 genus, 1 species	*Polystichum braunii, Polystichum semifertile*
	Dryopteridaceae	1 genus, 2 species	*Asplenium pruemorium*
	Pteridaceae	1 genus, 1 species	*Adiantum caudatum*
	Lindsaeaceae	1 genus, 1 species	*Odontosoria chinensis*
	Polypodiaceae	2 genera, 2 species	*Lepisorus sublinearis, Leptocilus decurrens*
Ranunculales	Ranunculaceae	3 genera, 4 species	*Actaea rubra, Clematis cirrhosa*
	Berberidaceae	1 genus, 1 species	*Ranunculus lanuginosus, Ranuculus repens*
Rosales	Rhamnaceae	1 genus, 1 species	*Rhamnus alaternus*
	Rosaceae	4 genera. 8 species	*Agrimonia procera, Rubus rosaefolium, Parietaria Judaica, Potentilla tabernaemontanii, Rubus nifeus, Rubus moluccanus, Rubus fraxinifolius, Rubus lineatus*
	Urticaceae	1 genus, 1 species	*Debregeasia longifolia*
Sapindales	Rutaceae	1 genus, 1 species	*Tripasia trifolia*
Selaginellales	Selaginellaceae	1 genus, 1 species	*Selaginella saginata*
Solanales	Convolvulaceae	1 genus, 1 species	*Ipomea violacea*
	Solanaceae	1 genus, 1 species	*Solanum nigrum*
Vitales	Vitaceae	1 genus, 1 species	*Cayratia japonica*
32 orders	43 families	86 genera	96 species

The fastq-filter command from USEARCH was used to trim the primer sequence data and denoise the readings according to the expected error value (Müller and Nebel 2021). After producing 250 bp paired-end raw reads using an Illumina paired-end platform, the amplicon was assembled and pre-treated to get clean tags. Next, to obtain the final tags, the chimeric sequences on the clean tags were chosen and separated (Prodan et al. 2020). Data from previous high-throughput sequencing analyses were transformed into sequenced reads through base calling. Raw data were saved in a FASTQ format consisting of reads with sequencing quality information following Ansorge et al. (2021). Low-quality reads with an expected error >1 were excluded. Figure 4 summarizes the overall steps of the OTUs clustering process. We used the unique reads with a 97% criterion to produce the final OTUs, excluding low-quality reads with an expected error >1.

Data analysis

We identified the OTUs using BLAST analysis against the GenBank database at https://www.ncbi.nlm.nih.gov/. Subsequently, we evaluated the sampling efforts of the OTU results using the rarefaction curve of the alpha diversity in RStudio to ensure data saturation (Willis 2019). When multiple OTUs represented the same taxa, we manually examined the OTU lineage table generated by the NGS, followed by a grouping analysis based on taxonomic similarity. Using the sort and filter functions in Microsoft Excel, we sorted the taxa and their abundance data from the highest to the lowest. We removed the zero values from each sample to ensure the results reflect only plant DNA in the monkeys' diet. Finally, we confirmed the OTU identities by comparing the NGS results against the vegetation database of Mount Merbabu, as also performed by Karyanto et al. (2022). The ultimate results were visualized using Microsoft Excel to display the plant taxa selected by *P. fredericae*.

Figure 3. The Javan fuscous leaf monkey, *Presbytis fredericae*. Photo credit to Jarot Wahyudi, forest officer at the Mount Merbabu National Park, Central Java, Indonesia

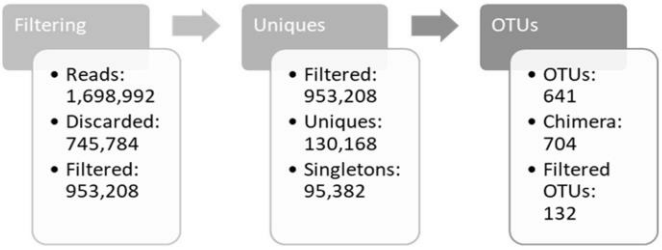

Figure 4. The OTUs clustering

Table 2. List of tree communities in the primary and secondary forests in Mount Merbabu, Indonesia

Order	Family	Species	
Apiales	Araliaceae	1 genus, 1 species	*Macropanax dispermus*
	Pittosporaceae	1 genus, 1 species	*Pittosporum moluccanum*
Casuarinales	Casuarinaceae	1 genus, 1 species	*Casuarina junghuhniana*
Ericales	Ericaceae	1 genus, 1 species	*Rhododendron javanicum*
	Theaceae	1 genus, 2 species	*Schiima norhoe, Schiima wallichii*
Euphorbiales	Phyllanthaceae	1 genus, 1 species	*Glachidion kollmannianum*
Fabales	Fabaceae	2 genera, 2 species	*Acacia decurens, Paraserianthes lophanta*
Fagales	Juglandaceae	1 genus, 1 species	*Engelhardia spicata*
	Fagaceae	1 genus, 1 species	*Littocarpus* sp.
Gentianales	Rubiaceae	1 genus, 1 species	*Cinchona* sp.
Laurales	Laurceae	1 genus, 2 species	*Cinnamomum parthenoxylon, Cinnamomum verum*
Malpighiales	Euphorbiaceae	1 genus, 1 species	*Homalanthus giganteus*
	Pandaceae	1 genus, 1 species	*Galearia filiformis*
Malvales	Thymelaeaceae	1 genus, 1 species	*Daphnopsis americana*
Oxalidales	Elaeocarpaceae	1 genus, 1 species	*Elaeocarpus stipularis*
Pinales	Cupressaceae	2 genera, 3 species	*Cupressus arizonica, Cupressus lusitanica, Pinus merkusii*
Sapindales	Meliaceae	2 genera, 2 species	*Aglaia odoratissima, Chisocheton pentandrus*
	Sapindaceae	1 genus, 1 species	*Dodonaea viscosa*
Theales	Actinidiaceae	1 genus, 1 species	*Saurauia bracteosa*
Urticales	Moraceae	1 genus, 1 species	*Ficus fistulosa*
15 orders	20 families	23 genera	26 species

RESULTS AND DISCUSSION

Result

The forward 5’-CTTACCAGYCTTGATCGTTACAA AGG-3' and reverse 5’-GTAAAATCAAGTCCACCRCG-3' successfully resulted in 132 filtered OTUs. The rarefaction curve in the alpha diversity analysis (Figure 5) indicates that the lines approach an asymptote, suggesting that further sampling efforts would not alter the cumulative richness and composition of OTUs. We finally identified eight orders, nine families, nine species belonging to the tree category, and 11 orders, 12 families, and 14 species of understory communities consumed by *P. fredericae*. All the plants selected by *P. fredericae* are presented in Table 3 and Figure 6.

Discussions

Applicability of the vegetation survey to support the NGS for dietary diversity analysis of Presbytis fredericae

This research is the first molecular-based study to reveal the dietary diversity of the Javan leaf monkeys *P. fredericae*. Several previous visual-based surveys on plant food selected by this leaf monkey resulted in inaccurate data due to observers' limitations in following their full-day foraging activities. Indeed, by relying on the time budget data of the monkeys' foraging activities, such eye-based observations faced challenges related to the observers' limitations in determining sufficient time allocation. Previous visual observations resulted in only three plant species that the monkeys mostly selected: *Engelhardtia spicata, Acacia decurrens*, and *Paraserianthes lophanta*. In contrast, we identified 9 plant taxa in the tree stratum and 14 plant taxa in the understory community. Our molecular-based observation using *rbcL* primer identified more plant taxa consumed by the monkeys and is therefore considered more effective than the previously conducted visual surveys. Our results proved that this DNA metabarcoding approach offered several benefits compared to direct observation of foraging behavior. First, it is time- and cost-effective, as it allows researchers to ascertain the dietary diversity of many individuals simultaneously. Second, the metabarcoding approach yields more accurate results when assessing the actual diet of the monkeys. This approach, of course, can be employed to overcome the limitations faced by eye-aided observation methods. Indeed, the metabarcoding technique can assess all plant materials consumed by the monkeys throughout the day, whereas direct observation cannot track their overall foraging activities. Based on these benefits, we concluded that the metabarcoding technique could be employed to conduct a comprehensive, large-scale analysis to shape the pattern of foraging and dietary diversity of *P. fredericae* at the landscape scale.

Table 3. List of plants selected by *Presbytis fredericae* at Mount Merbabu, Indonesia. The list presents the original OTUs ID, their abundance, and the results of verified plant food taxa consumed by the monkey

Examples of OTUs ID	Order	Families	Species	Local name	OTU abundance
Tree					
OTU 2, 27, 93	Fabales	Fabaceae	*Acacia decurrens*	Akasia	20314
OTU 8, 89	Apiales	Araliaceae	*Macropanax dispermus*	Pampung	9592
OTU 5, 16, 18	Fagales	Juglandaceae	*Engelhardia spicata*	Kesowo	9393
OTU 7, 22	Ericales	Theaceae	*Schima wallichii*	Puspa	6387
OTU 14, 80	Pinales	Pinaceae	*Pinus merkusii*	Pinus	4070
OTU 13, 44, 83	Sapindales	Sapindaceae	*Dodonea viscosa*	Tesek	3257
OTU 15, 28, 43, 67, 75, 77, 79, 95, 97, 107	Malphigiales	Phylantaceae	*Glochidion kollmannianum*	Dempul	2320
OTU 19, 120	Fagales	Casuarinaceae	*Cupressus lusitanica*	Kipres	1062
OTU 33, 46, 60, 85	Ericales	Actinidiaceae	*Saurauia bracteosa*	Lotrok	246
31 OTUs	7 Order	9 Families	9 Species		
Lower Crop					
OTU 1	Vitales	Vitaceae	*Cayratia japonica*	-	14053
OTU 3	Caryophylales	Polygonaceae	*Persicaria chinensis*	Aseman	10681
OTU 4, 82	Asterales	Asteraceae	*Chromolaena odorata*	Kirinyu	9031
OTU 56, 68, 71	Rosales	Urticaceae	*Debregeasia longifolia*	Urang urang	5816
OTU 6, 24, 52, 101	Rubiales	Rubiaceae	*Wendlandia paniculata*	Lotrok	5524
OTU 10, 34, 81, 124	Ranunculales	Berberidaceae	*Berberis julianae*	Jerukan	4364
OTU 12, 100	Poales	Poaceae	*Themeda quadrivalvis*	Bulu kucing	4237
OTU 56, 68, 71	Cucurbitales	Cucurbitaceae	*Gynostemma pentaphyllum*	Jiaogulan	1316
OTU 21, 50, 70, 84	Rosales	Rosaceae	*Rubus* sp.	Rubus	1267
OTU 17, 30, 104, 109, 111, 119	Poales	Cyperaceae	*Carex baccans*	Kerisan	1132
OTU 32	Solanales	Solanaceae	*Solanum nigrum*	Lencak	304
OTU 35, 53	Myrtales	Melastomaceae	*Melastoma* sp.	Senggani	183
OTU 37	Poales	Poaceae	*Omplismenus hirtellus*	-	119
OTU 91	Lycopodiales	Lycopodiaceae	*Huperzia selago*	-	2
33 OTUs	11 Order	13 Families	14 Species		

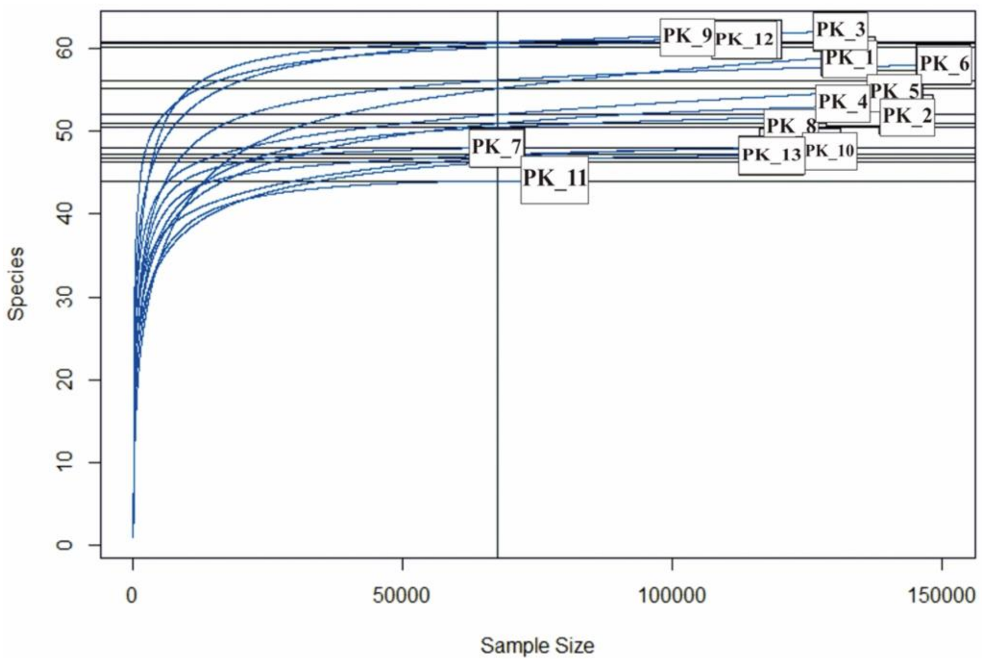

Figure 5. The rarefaction curve of the Alpha diversity. The figure shows sufficient sampling efforts as the rarefaction curve has consistently plateaued

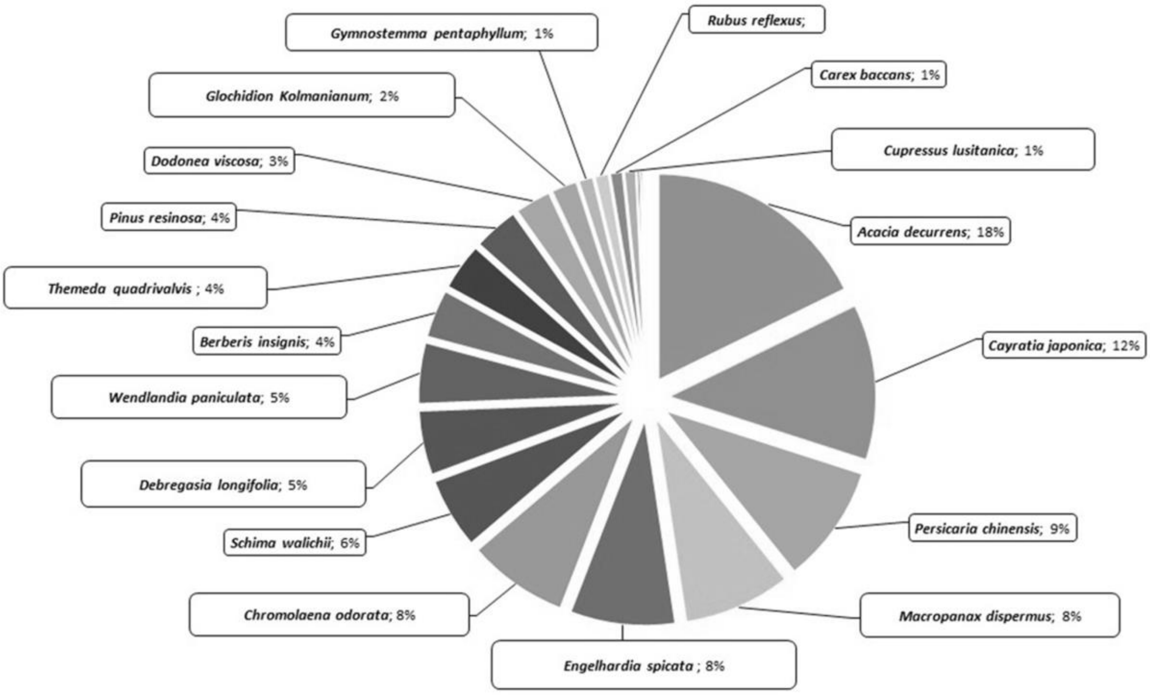

Figure 6. Dietary diversity of *Presbytis fredericae*

Utilizing metabarcoding techniques in the dietary analysis of *P. fredericae* offers several benefits. However, using universal primers, such as *rbcL*, to sequence tiny fragments of plant DNA may produce inaccurate results during the BLAST. The NGS result indicates that despite the highly conserved gene in the *rbcL* sequence (Pere et al. 2023), the primer targeting this sequence still resulted in a limited ability to differentiate taxa in the order and lower taxonomic categories. For example, in OTU 8 and 89 (Table 3), the barcode primer yields low discrimination results across differing families within the Apiales. According to the Krona analysis, the NGS identifies such a taxon only at the order rank. In contrast, our vegetation survey reported two families in the Apiales: Araliaceae (*Macropanax dispermus*) and Pittosporaceae (*Pittosporum moluccanum*). The possibility of misidentification underlines the need for caution when using *rbcL* primer in dietary analysis. To specifically identify the plant taxa consumed by *P. fredericae,* we cross-validated the results derived from our single primer in the NGS analysis with a plant catalog obtained through our Point-Centered Quarter (PCQ) and plot technique of vegetation survey, along with the fecal collection. Through this approach, we validated our NGS data with a cost-effective technique to supplement the use of the *rbcL* primer.

The vegetation survey, conducted using the PCQ and plot technique, identified 15 orders, 20 families, 23 genera, and 26 species in the tree stratum. Species with the best 5 ecological importance values were the native montane tropical trees *E. spicata* (47.86), *Dodonaea viscosa* (35.92), *Chisocheton pentandrus* (23.81), and *M. dispermus* (22.51), the non-invasive tree *Cupressus arizonica* (24.10), and 31.24 for the invasive tree species *A. decurrens* (Saputra et al. 2023). The community of the lower stratum consisted of 32 orders, 43 families, 86 genera, and 96 species, and the 4 importance values were the invasive species *Ageratina riparia* (77.70) and *Chromolaena odorata* (16.97) and the non-invasive *Brachypodium sylvaticum* (25.13) and 18.51 for *Selaginella saginata* (Nurmiyati et al. 2023). The local floristics list resulting from the vegetation survey effectively confirms all the OTUs identified through the NGS analysis, making this validation technique one of the most cost-effective methods for validating data from metabarcoding techniques. In addition to this, accurate plant taxonomic identification is crucial for the effective examination of the plant food consumed by *P. fredericae*, as plants identified in the survey serve as the primary taxonomic reference against which all the OTUs are confirmed. We concluded that a vegetation survey could become one of the cheaper and more effective techniques to complement metabarcoding-based dietary analysis and enhance the accuracy of this molecular-based approach to identification and analysis.

Food selection and dietary diversity of Presbytis fredericae in degraded montane forest

Presbytis fredericae are folivorous monkeys that consume mostly leaves that may be evenly and abundantly distributed in the forest. In the undegraded forest, they need less effort to forage. However, anthropogenic activities such as deforestation and habitat fragmentation can create an environment that is unsuitable for their survival, potentially having a negative impact. As with some other colobine primates, their existence in a given habitat is profoundly impacted by their foraging success in coping with the challenge of food plant availability. Habitat degradation can cause plant food resources to be less diversified and insufficiently available in the forest. *P. fredericae* requires foraging adaptations to survive in changing food resources. An adaptation to the altered floristic structure is highly possible for them. Their semi-ruminant ventriculus with three-partite lobes and a longer intestinal tract allows them, as a folivorous primate, to consume a wide range of food plants with high fiber content and maximize energy gain from the plant materials (Hoshino et al. 2021). Their anatomical uniqueness enables them to select and diversify their food plant options in the forest habitat. Therefore, conducting a dietary diversity analysis is a suitable approach to examine the foraging adaptations they perform in degraded forests by determining their food plant choice and variability.

Aside from their ability to digest high cellulose materials, *P. fredericae* at the study site demonstrates extraordinary adaptability by selecting specific plant phenology and species for their diet. Table 3 displays a case where *P. fredericae* diversified their diet at our research location by consuming 9 plant taxa from a tree stratum and 14 from the ground cover. However, according to our local vegetation database (Tables 1 and 2), monkeys consume only about 34% of the trees and 14% of the plants in lower-crop communities (Figure 7). It shows that although *P. fredericae* is hypothetically a generalist folivore that can consume various plant species, the monkey still performs a foraging selection toward particular plant species, considering that the selected food plant is provided abundantly in *P. fredericae* habitat. This food plant selection is likely related to the monkey's optimum foraging behavior to meet their need for protein, fiber, and secondary metabolites, with optimum foraging efforts (Liu et al. 2022). Additionally, our experience with the bitter taste of the three most eaten food plants by *P. fredericae* shows that the monkey selects a particular plant with a higher tannin content. This selection is likely a form of the ultimate strategy employed by *P. fredericae*, as its sympatric species, the Javan langur (*Trachypithecus auratus*), exhibits similar foraging choices. These two sympatric langurs choose such food selection to make a niche for themselves that foliage foragers will not disrupt (Nishi et al. 2018).

In this study site, *P. fredericae* exhibits a discernible food selection strategy during the dry period. Through our visual observation, we ascertained that *P. fredericae* prefers immature leaves over mature ones. This preference enables the monkey to perform an effortless consumption event by masticating and digesting the plant food, extracting higher energy and protein with lower fiber content. Although leaves serve as the major source of protein, fiber, and secondary metabolites essentially needed by the leaf-monkey, high levels of these organic compounds prevalent in mature leaves can be indigestible, unpalatable, or even toxic for them (Ganzhorn et al. 2017; Matsuda et al. 2019; Windley et al. 2022). The selection of *P. fredericae* towards the immature foliage is also indicated by its runny feces, as depicted in Figure 8. The watery consistency of the monkey's feces shows their selection toward the immature foliage that has a relatively lower fiber content and a mature cell wall (Nijboer et al. 2006).

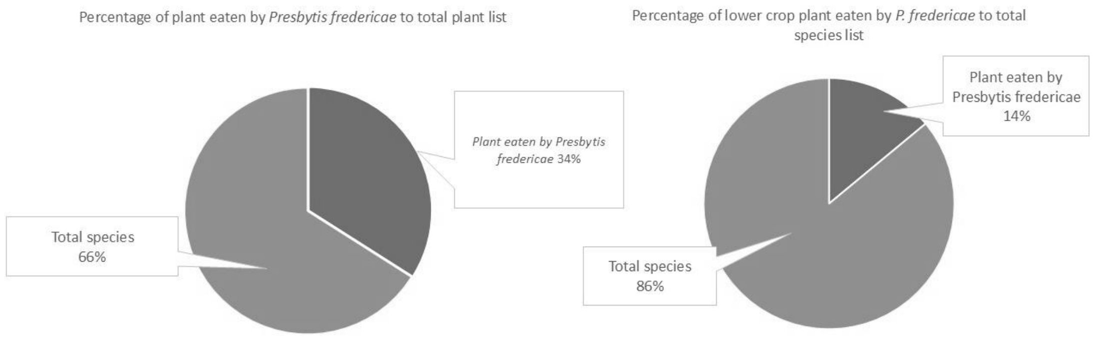

Figure 7. Percentage of plant species eaten by *Presbytis fredericae* compared to the total species of plant and lower crop species

Figure 8. The runny feces of *Presbytis fredericae*

In this research location, the dry season, characterized by minimal rainfall, can result in a scarcity of immature leaves. This seasonal condition can force *P. fredericae* to exhibit adaptive foraging behavior to locate an adequate supply of immature leaves in their habitat. Considering that the OTU abundance reflects semi-quantitative data of plant DNA the monkey ate, the OTU results revealed that the monkey mostly selects the three best plant foods in the tree stratum, i.e., *A. decurrens*, *M. dispermus*, and *E. spicata* (Table 3). These three species are the best sixth plant species with high ecological importance values and are the most abundant tree species in the monkey's habitat. These three tree species sufficiently support the dietary preference of *P. fredericae* by providing an adequate amount of immature leaves for the monkey, even during the dry period. *Macropanax dispermus* and *E. spicata* are plant foods mostly selected by *P. Fredericae*. Visual surveys conducted by Mount Merbabu National Park recorded that the monkey allocates their time budget to forage within the habitat where these two native plant foods are abundantly available. The invasive green wattle *A. deccurens* is an incredibly adaptive species that can grow in all seasons, even in dry and hot conditions. These three species serve as a new preferred plant food for the monkey (Afrianto et al. 2017). This fast-growing invasive species is closely related to the native *P. lophanta*, also known as the crested wattle, a preferred natural food source for monkeys that is rarely available due to almost periodic forest fires. The young herbaceous leaves of the native montane species *M. dispermus* are available all year round and provide sufficient watery leaves for *P. fredericae* (Karyanto et al. 2022). Additionally, the Great Malay Beam *E. spicata*, with its unique sprouting pattern outside the rainy season, ensures an abundance of immature leaves after flowering and fruiting from November to April (Upadhaya et al. 2018).

These tree species serve as new preferred plant foods for *P. fredericae* (Afrianto et al. 2017). When these species are abundant in the forest, the fallback food phenomenon, as reported in some colobines, does not occur, as the young phenology of leaves that are sufficiently available throughout the year (Hanya and Bernard 2012). The selection of these tree species by *P. fredericae* can also indicate a case of the optimal foraging theory, as shown by their closely related taxon, *Colobus angolensis* (Arseneau-Robar et al. 2021). Selecting the abundant species within the occupied habitat suggests that they optimize their foraging strategy by adjusting their movement to minimize travel costs and risks. Through this foraging behavior, they can access sufficient preferred food while avoiding predation and traveling in a disoriented state.

We reported the dietary diversity of the leaf-eating monkey *P. fredericae* based on the composition of the 132 OTUs resulting from the NGS. This analytical approach allowed us to examine the dietary diversity to identify the most frequently consumed food plants by the monkeys (Ando et al. 2013). The Shannon diversity indices generated a beta diversity range from 1.5 to 4.25. The species rarefaction curves for each sample, depicted in Figure 5, illustrate that each curve has reached an asymptote. The plateaued curves suggest that our sequencing efforts have appropriately represented all OTUs, reducing the likelihood of newly identified OTUs emerging (Schloss 2024). The obtained diversity indexes demonstrate that *P. fredericae* diversifies its consumption pattern by selecting various plant species in its dietary composition. By incorporating more varied food plant sources into their diets, *P. fredericae* at the sampling location is likely to exhibit a diet-breadth trade-off foraging strategy (Torregrossa et al. 2011). Such a behavioral mechanism is shown by the greater diversity of the young phenology of food plants in *P. fredericae* diets to reduce the amount of toxins consumed while still maximizing the nutrients and energy they require (Ganzhorn et al. 2017).

In conclusion, based on 132 OTUs from NGS, our study demonstrated that P. fredericae in degraded forests exhibited dietary breadth by selecting 8 orders, 9 families, and 9 species in the tree strata, and 11 orders, 12 families, and 14 species in the lower crop community. The monkeys also performed dietary selection and an optimal foraging strategy by consuming only 34% of trees from the total tree species and 14% of lower crops from the total lower plant species available in their habitat.

ACKNOWLEDGEMENTS

We are grateful for the support from Universitas Sebelas Maret (UNS), Indonesia, which enabled us to conduct this study. Additionally, we are grateful to Saeful Hidayat (Mount Merbabu National Park, Indonesia), Johan Setiawan, Nurpana Sulaksono, and Junita Parjanti for their invaluable assistance. I appreciate all of my students for the data collection.

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