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Diet Composition of the Wild Stump-Tailed Macaque (*Macaca arctoides*) in Perlis State Park, Peninsular Malaysia, Using a Chloroplast tRNL DNA Metabarcoding Approach: A Preliminary Study

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Simple Summary: This study investigated plant diet of wild *Macaca arctoides* in the Malaysia–Thailand border region using a chloroplast tRNL DNA metabarcoding approach. It is a comprehensive molecular technique to assess foods eaten by primates. We have chosen chloroplast tRNL because this region has been widely used for identifying plant species. Chloroplast tRNL DNA was amplified and sequenced using the Illumina MiniSeq platform. Sequences were analyzed using the CLC Genomic Workbench software version 12.0 to check for *M. arctoides* plant diet. Across these samples, we successfully identified 29 plant orders, 46 families, 124 genera, and 145 species. As the first report in Malaysia, the findings provide an important understanding on diet of wild *M. arctoides* that only reside in Perlis State Park, Malaysia.

Abstract: Understanding dietary diversity is a fundamental task in the study of stump-tailed macaque, *Macaca arctoides* in its natural habitat. However, direct feeding observation and morphological identification using fecal samples are not effective and nearly impossible to obtain in natural habitats because this species is sensitive to human presence. As ecological methods are challenging and time-consuming, DNA metabarcoding offers a more powerful assessment of the diet. We used a chloroplast tRNL DNA metabarcoding approach to identify the diversity of plants consumed by free-ranging *M. arctoides* in the Malaysia–Thailand border region located in Perlis State Park, Peninsular Malaysia. DNA was extracted from three fecal samples, and chloroplast tRNL DNA was amplified and sequenced using the Illumina MiniSeq platform. Sequences were analyzed using the CLC Genomic Workbench software. A total of 145 plant species from 46 families were successfully identified as being consumed by *M. arctoides*. The most abundant species were yellow saraca, *Saraca thaipingensis* (11.70%), common fig, *Ficus carica* (9.33%), aramata, *Clathrotropis brachypetala* (5.90%), sea fig, *Ficus superba* (5.44%), and envireira, *Malmea dielsiana* (1.70%). However, *Clathrotropis* and *Malmea* are not considered Malaysian trees because of limited data available from Malaysian plant DNA. Our study is the first to identify plant taxa up to the species level consumed by stump-tailed macaques based on a DNA

metabarcoding approach. This result provides an important understanding on diet of wild *M. arctoides* that only reside in Perlis State Park, Malaysia.

Keywords: Malaysian primates; *Macaca arctoides*; plant metabarcoding; tRNL

1. Introduction

Macaca arctoides also known as the stump-tailed macaque or bear macaque (Geoffroy 1831), is widely distributed throughout South Asia (India, Bangladesh), Southwestern China, and Southeast Asia (Myanmar, Bangladesh, Thailand, Laos, Cambodia, Vietnam, and Malaysia) [1]. In Malaysia, the stump-tailed macaque is locally known as beruk kentoï and only inhabits the Perlis State Park (PSP) in the northern region of Peninsular Malaysia [2,3]. There have been few studies determining how the macaque interacts and thrives in this habitat. PSP is located on the western border between Perlis State and Thailand and includes the Mata Ayer Forest Reserve and the Wang Mu Forest Reserve which encompasses a total area of 5015 ha [4]. It is also located within the Nakawan Range, the longest continuous range of limestone hills in Malaysia [5]. PSP shares a unique distribution of flora and fauna with the Thaleban National Park in southern Thailand signifying both as prominent transboundary international parks [6]. The population of wild *M. arctoides* in Malaysia is threatened due to illegal hunting in PSP [3].

The availability and distribution of food resources are important factors in determining the ecological variations of primate species [7]. In natural habitats, primates consume certain parts of over 100 plant species including buds, fruits, young leaves, bark, roots, and flowers [8,9]. However, the diet of *M. arctoides* remains largely unknown. Indeed, more foraging and dietary studies have been done on long-tailed macaque and pig-tailed macaque. For example, Sha and Hanya [10] determined the natural foods consumed by the long-tailed macaque (*Macaca fascicularis*) which included 17 genera of plants in Singapore. *Axonopus compressus* (grass), *Villebrunea rubescens* (daun jilat), and *Caryota mitis* (fishtail palm) were the three most frequent plant species consumed by *M. fascicularis* in Telaga Warna, Bogor, West Java Indonesia [11]. In addition, the five most frequent plant species consumed by *M. fascicularis* were oil palm, *Elaeis guineensis* (11%), fig, *Ficus variegata* (4%), senduduk, *Melastoma malabathricum* (3.1%), bamboo, *Schizostachyum jaculans* (3.1%), and brown salwood, *Acacia mangium* (2.7%) in a mixed landscape consisting of urban, agro-forested areas and forest fragments in Malaysia [12]. Another macaque group in Peninsular Malaysia, pig-tailed macaque (*Macaca nemestrina*), was observed to consume oil palm parts, including attached and fallen oil palm fruits, seeds, and flowers [13].

DNA metabarcoding is used to identify organisms from a sample containing DNA materials. Taberlet et al. [14] introduced the term 'DNA metabarcoding' for the identification of multiple species using total or often degraded DNA extracted from bulk samples of entire organisms or from an environmental sample. Taberlet et al. [15] designed the oligonucleotide primer used for the identification of shorter fragments of the chloroplast tRNL (UAA) intron. These regions are highly conserved, and the amplification system is very robust for plant species identification [16]. Metabarcoding using tRNL produced thousands of reads consistent with the known diet of the red-shanked douc langurs (*Pygathrix nemaeus*); however, the barcodes were too short to identify several plant species within a genus [17]. This technique revealed at least 53 plant species from 33 families in a dietary profile of an endangered population of the banded leaf monkey (*Presbytis femoralis*) [18]. In addition, tRNL yielded greater numbers of sequences with similar sequencing effort, and a higher resolution of taxonomic identification in dietary studies of wild white-faced capuchins (*Cebus capucinus*) [19].

A recent field observation study of the Malaysian stump-tailed macaques indicated that they were only found in the Wang Kelian forest areas of PSP [3]. All individuals were counted in two free-ranging groups and the natural habitat may significantly influence their diet. Because no information is available regarding the natural food consumed by wild *M. arctoides*, we investigated the plant diversity