

ORIGINAL ARTICLE

High-Throughput DNA Metabarcoding for the Gut Microbiome Assessment of Captive White-Handed Gibbon and Siamang

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ABSTRACT

Background: The gut microbiota plays a vital role in primates' overall health and well-being, including small apes (Hylobatidae). The symbiotic relationships between bacteria and the gut aid food digestion, maintain host health, and help them adapt to their environment, including captive conditions. Despite being listed as endangered in the International Union for Conservation of Nature (IUCN) red list category, molecular studies on the small ape's gut microbiome are limited compared to other primates. This study aimed to characterize the gut microbiota of captive small apes at Zoo Taiping and Night Safari, Peninsular Malaysia, by evaluating their microbial communities.

Methods: Seven fecal samples from Hylobatidae (white-handed gibbon and siamang) were collected, and the bacteria therein were successfully isolated and subjected to high-throughput sequencing of the 16S rRNA gene.

Results: The acquired amplicon sequence variants (ASVs) were successfully classified into 17 phyla, 82 families, 164 genera, and 43 species of microbes. Each small ape exhibited a unique gut microbiota profile. The phyla Bacteroidota and Firmicutes were dominant in each individual. Environmental conditions and host genetics are among the factors that influence the small ape's gut microbiome composition.

Conclusions: These findings provide valuable insights into the gut microbiota composition of small apes at Zoo Taiping and Night Safari, thus contributing to the health management and welfare efforts of small apes in captivity.

1 | Introduction

Small apes, including gibbons and siamang, are endemic to the South and Southeast Asia region [1]. They are notable for their beautiful morning calls and brachiating locomotion [1], and each gibbon and siamang has a distinct song [2]. Most of these species

live in tropical forests, moving bipedally on the ground and in trees [2]. In the natural habitat of small apes, coexistence is possible between *Hylobates lar* and *Symphalangus syndactylus* because of their different diet preferences [3]. These small apes are common nonhuman primates that are housed in zoos or captive centers [4], including Zoo Taiping, Peninsular Malaysia. Most

captive gibbons and siamang have been rescued from being kept illegally as pets [5]. Threats such as illegal hunting or being kept as pets have brought both gibbons and siamang to near extinction [6]. Gibbons and siamang under the family Hylobatidae in Malaysia are listed as endangered species in the IUCN Red List [7], including *H. lar* and *S. syndactylus*. Therefore, conservation translocation and reintroduction have been identified as key conservation tools to repopulate and reinforce extirpated or reduced populations, such as gibbons in Malaysia [8]. One of the major considerations before translocating and reintroducing these captive animals into the wild is assessing the condition of their gut health. Therefore, to be able to adapt themselves to the new surroundings after being released back into the wild, the composition of their gut microbiota needs to be nearly identical to that of their wild counterparts to reduce the probability of disease infection [9].

Gut microbiota refers to the collection of bacteria found in the gastrointestinal tract [10]. The symbiotic relationships of microbes with the intestinal tract play a significant role in many functions necessary for host physiology, such as food digestion, immunity, and pathogen resistance [11]. Each individual has a unique gut microbiota profile that is influenced by various factors, such as species, diet, and the environment [12]. The gut microbiota composition can also be influenced by its habitat type, whether in captivity or the wild [13]. The role of gut microbiota in host health has been extensively studied in humans and is often associated with various diseases, such as obesity, diabetes, and intestinal diseases [14]. Thus, the study of gut microbiota is important to understand the various biological aspects and promote the conservation management of individuals in captivity [12]. Currently, an increasing number of studies have been conducted on primate species to investigate how gut microbiota composition affects their health [15–18]. However, research specifically focusing on the gut microbiota of small apes in Peninsular Malaysia, such as *H. lar*, *H. agilis*, and *S. syndactylus*, remains limited [16, 18].

With the advancement of next-generation sequencing technology, such as DNA metabarcoding, it has become faster compared to conventional approaches [13] to determine the composition of gut microbiota, which can be inferred using 16S rRNA gene amplicon sequencing. The V3 and V4 regions of the 16S rRNA

gene alone are sufficient to provide bacterial diversity information [11]. DNA metabarcoding techniques have also been widely applied in other fields, including diet metabarcoding, such as diet metabarcoding in Asian elephants [19, 20] and primate Cercopithecidae [21, 22]. Several other microbiome DNA metabarcoding studies in mammals have recently been performed, such as for the Malayan tiger [12] and island flying fox [11]. In addition, several gut microbiome studies on Hylobatidae are available, which can be used as a good comparison. These microbiome studies on captive small apes in China include *H. lar*, *S. syndactylus* [23], *Nomascus leucogenys* [24], and *Hoolock tianxing* [25]. Therefore, this study is crucial to elucidate their adaptation to the surrounding environment through the characterization of gut microbiota diversity. The main objective of this study is to determine the gut microbiota diversity of Hylobatidae primates at Zoo Taiping and Night Safari using the 16S rRNA DNA metabarcoding method. Investigating the gut microbiota in small apes is important for the conservation management of captive primates at Zoo Taiping and Night Safari, Peninsular Malaysia.

2 | Materials and Methods

Seven Hylobatidae fecal samples were collected from Zoo Taiping and Night Safari (4.8550° N, 100.7510° E) (Table 1). This zoo was established in 1961 and is the oldest in Malaysia as well as the only zoo in the northern region of Peninsular Malaysia. The Hylobatidae samples were collected from seven different enclosures, where one of the samples was from *S. syndactylus* and the remaining six were from *Hylobates lar*. Noninvasive sampling methods were employed, and fecal samples were collected from *H. lar* and *S. syndactylus* [6]. All samples were stored in separate vials containing 99% absolute ethanol. Subsequently, the samples were stored at –20°C for long-term preservation [21].

The InnuPREP Stool DNA kit (Analytik Jena, Germany) was utilized for bacterial genomic DNA extraction according to the manufacturer's protocol [21]. The bacterial 16S rRNA gene was amplified using PCR and primers targeting the V3 and V4 regions F515 5'-TCGTCCGCGAGTCAGATGTGTATAAGAGA CAGGTGCCAGCMGCCGCGGTAA-3' and R806 5'-GTCTCG TGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVG

TABLE 1 | Shannon diversity (H'), Simpson diversity (D), and richness (Chao1) indices of the gut microbiome of each sample.

No.	Species	Sample ID	Nonchimeric sequence	ASV	Shannon (H')	Simpson (D)	Richness (Chao1)
1	<i>H. lar</i>	BMHLA 910	86 500	370	4.908	0.985	370.0
2		BMHLA 911	80 704	305	4.410	0.960	305.5
3		BMHLA 912	92 380	274	4.410	0.977	274.0
4		BMHLA 913	93 770	352	4.800	0.984	355.0
5		BMHLA 914	100 798	337	4.558	0.978	338.5
6		BMHLA 915	99 100	368	4.901	0.986	369.0
7	<i>S. syndactylus</i>	BMSSC 916	80 091	269	4.138	0.967	270.3
Total			633 343	1273	—	—	—

Note: Mann–Whitney U test p -value > 0.05.