Study of gut microbiome of wild sloth bear using non-invasive techniques to understand the role of microorganisms in sloth bear nutrition and health

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Rational:

Most mammal-associated microbes reside in their host's gastrointestinal tract, comprising the gut microbiome (GMB) (Ley et al., 2008). The GMB facilitates critical processes essential for host health and survival such as energy uptake, vitamin synthesis, development of immunity as well as immune response (Turnbaugh et al., 2006; Hooper et al., 2012). Many facets of host health are impacted by both early colonization and continuous cultivation of a diverse GMB community. Furthermore, the GMB may serve the host as a buffer against environmental perturbations and shifts in resource availability by promoting nutritional efficiency (Amato et al., 2015) and modulating fat storage (Martin et al., 2019). As such, a study by Stumpf et al. (2015) suggests the GMB plays an important factor in host resiliency as global change continues to impact ecosystems.

Several field studies revealed that GMB communities in wild animals is sensitive to habitat disturbance, often resulting in reduced microbial diversity. An imbalance, or dysbiosis, of the GMB, which is typically associated with reduced GMB diversity, can have major consequences to host health. For example, dysbiosis has been linked to intestinal disorders, cardiovascular disease, asthma, obesity, and host mortality (Trujillo et al., 2022). However, before we can understand the impact of microbiome dysbiosis in wildlife, we must first understand and explore the extent of among individual GMB variation within a species. Further, greater GMB diversity is not inherently "good", in that more diverse communities are not always more beneficial to the host (Huston, 2000). Therefore, identifying factors that drive observed GMB variation in specific species may be important and imperative in providing a contextualized framework for using GMB diversity as an indicator of the health of an animal.

The study of the gut microbiota of wild species is a promising field on conservation research, because it provides an important source of information related with the health status of individuals, and may serve for the monitoring and surveillance of entire populations (Stumpf et al., 2016). Further, it can reflect the diet designed for the bears under captivity where the low diversity of food resource can act as a stressor leading to disease development (Borbon et al, 2017). Despite of being an omnivore and feeding on large variety of plant foods, the bears have a simple digestive tract unlike the other herbivores hence for fibre degradation, they must have established a symbiosis with cellulolytic microorganisms in their gestor-intestinal (GI) tract in order to fulfil their nutritional needs (Borbon et al., 2017). However, the gut microbial diversity of bears and the role of gut microorganism in digestion of a variety of

food is poorly studied; most of the studies are carried out on brown bears and other European and American bear species; however, no such work has been carried out on the Asian bears.

Sloth bear (*Melursus ursinus*) are considered among one of the largest omnivores in the ecosystems of Indian subcontinent. The species is listed as vulnerable (VU) according to IUCN Redlist (Dharaiya et al., 2016) and schedule I in the Indian wildlife protection act (1972). The myrmecophagous feeding habit i.e., feeding on termite and ants has been studied by several researchers across its geographic range and found a significant spatio-temporal variation in their diet composition and the preference for the food. The studies also revealed that the food preference of sloth bear is largely depending on the habitat condition, availability of fruiting species and termite and ants in the different seasons (Joshi et al., 1997; Mevada and Dharaiya 2010; Bargali et al., 2004). Microbiome biology is still a poorly explored field in conservation biology in general and specifically in sloth bears.

We are developing a study on the gut microbial diversity of a sloth bear in which we will study the gut microflora from the scats of the wild sloth bears. Though the gut microbiota is a dynamic community, time andfield conditions can drive changes in the gut microbiota and sometime deviate related-conclusions (Hale et al., 2016), hence we have planned to collect the fresh scat samples from the field and maintain the conditions such as the exposure time, storage of the sample and its analysis. This will be probably a pioneer study on the wild sloth bears and we hope that this study will help to understand many aspects of sloth bear biology including host-microbiome interaction, health status of a wild sloth bear, habitat condition and its impacts on sloth bear food preference and digestion abilities and the role of GMB in the sloth bear nutrition and myrmecophagy.

As this is the first study of this kind on sloth bear, the proposed study is designed to understand the gut microbial diversity of free ranging sloth bears in the dry deciduous habitats of Gujarat with the following objectives:

1. To study the gut microbial diversity of wild sloth bears
2. To understand the seasonal variation in gut microbiota according to food uptake
3. To study the role of microorganisms in toxin degradation

Study Area:

The proposed study will be conducted in Gujarat state, western India which is a westernmost limit of sloth bear distribution. The state of Gujarat encompasses an area of approximately 196,024 km^2, according to a report submitted by the Forest Survey of India in 2017, approximately 14,757 km^2 is covered by forest. The sloth bear landscape in Gujarat is divided into north, north-east, central and south Gujarat where the sloth bears are patchily distributed in fragmented habitats. Though the sloth bears are protected in five wildlife sanctuaries, a significant number of bears are also found in the non-protected areas of the state. According to Champion and Seth (1968), the state has a wide variety of forest types such as dry deciduous, tropical moist, mixed and scrub forests.

Methodology:

Sample collection:

Different forest patches of Gujarat with high sloth bear density will be identified through the recent sloth bear population estimation data and will be surveyed for the scat sample collection. The fresh scats of the sloth bear will be identified and collected in a sterile bottle containing normal saline solution and stored at 4 ^{0}C temperature for the microbial analysis. During the scat collection, the collection details such as date, time, location and food content in the scats will be recorded.

Laboratory analysis:

The fresh scats samples will be isolated by conventional microbiological methods like isolation on growth medium as well as identifying their Gram characteristics. Further identification will be carried out using Vitek-2 compact system which is an automated rapid identification system used for bacteria, yeast and bacillus identification. Genotypic analysis will be carried out using 16s rRNA analysis through Sanger sequencing which is considered as "Gold" standard followed by data analysis using BLASTN.

Potential outcomes and significance of study:

Microbiome biology is still a poorly explored field in conservation biology and has an enormous potential for elucidating the effects of habitat degradation, lower resource availability, population isolation, and captivity maintenance conditions on host health. Being a first study of its kind, the findings of the study will generate the baseline information about the gut microflora of wild sloth bears and help to understand the host-microbiome interaction and how the microorganisms play a role in the digestion of food and nutrition of the sloth bear. As the sloth bear is myrmecophagous, this study will also help to find out the important microbes which help in degrading the toxins produced by termites like naphthalene, formic acid etc. Also, it can reflect the effect of diets designed for captive individuals, where the low diversity of resources can act as a stressor, leading the individual to dysbiosis and further disease development.

Microbiome analyses could be a powerful tool for forest department and policy makers to improve the current management plans for such a threatened and understudied wild species like sloth bear, as very little is known about the current health status of populations.

Estimated budget (USD):

No	Particulars	Estimated budget	Support received*	Requesting for financial support
1	Basic instruments for lab	4000	--	4000
2	Consumables and kits	1800	800	1000
3	Advanced molecular analysis through outsourcing	2000	--	2000
4	Field work and travel	1500	700	800
5	Contingency	500	500	00
6	Publication	250		250
	Total	10050	2000	8050

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