



Research Article

High-throughput DNA metabarcoding for determining the gut microbiome of captive critically endangered Malayan tiger (*Panthera tigris jacksoni*) during fasting

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Abstract

The Malayan tiger (*Panthera tigris jacksoni*) is a critically endangered species native to the Malaysian Peninsula. To imitate wild conditions where tigers do not hunt every day, numerous wildlife sanctuaries do not feed their tigers daily. However, the effects of fasting on the gut microbiota of captive Malayan tigers remains unknown. This study aimed to characterise the gut microbiota of captive Malayan tigers by comparing their microbial communities during fasting versus normal feeding conditions. This study was conducted at the Melaka Zoo, Malaysian Peninsula and involved Malayan tigers fasted every Monday. In total, ten faecal samples of Malayan tiger, two of Bengal tiger (outgroup) and four of lion (outgroup) were collected and analysed for metabarcoding targeting the 16S rRNA V3–V4 region. In total, we determined 14 phyla, 87 families, 167 genera and 53 species of gut microbiome across Malayan tiger samples. The potentially harmful bacterial genera found in this study included *Fusobacterium*, *Bacteroides*, *Clostridium* sensu stricto 1,

Solobacterium, *Echerichia shigella*, *Ignatzschineria* and *Negativibacillus*. The microbiome in the fasting phase had a higher composition and was more diverse than in the feeding phase. The present findings indicate a balanced ratio in the dominant phyla, reflecting a resetting of the imbalanced gut microbiota due to fasting. These findings can help authorities in how to best maintain and improve the husbandry and health of Malayan tigers in captivity and be used for monitoring in ex-situ veterinary care unit.

Keywords

Panthera tigris jacksoni, carnivora, next generation sequencing, 16S rRNA, metabarcoding

Introduction

Tigers (*Panthera tigris*) are the largest felid species and a widely recognised symbol of worldwide wildlife conservation. Six tiger subspecies exist in the wild, including the Malayan tiger (*Panthera tigris jacksoni*) (Luo et al. 2004, O'Brien et al. 2005), a charismatic iconic flagship species native to the Malaysian Peninsula. The Malayan tiger is currently classified as critically endangered (Kawanishi 2015) and totally protected in Malaysia under the Second Schedule of the Wildlife Conservation Act of 2010. The 1st National Tiger Survey (1st NTS) 2016–2018 found a Malayan tiger population of < 200 individuals. The Malayan tiger is threatened by anthropogenic disturbances, such poaching, forest clearance for agriculture, commercial and illegal logging and human settlements (Ten et al. 2021).

In the Malaysian Peninsula, the Department of Wildlife and National Parks (PERHILITAN) Malaysian Peninsula is the main authority responsible for managing and conserving the Malayan tiger. For instance, a project started in 2015 by the PERHILITAN under the 10th Malaysian National Tiger Conservation Action Plan NTCAP aimed to potentiate the conservation management of the Malayan tiger (PERHILITAN 2008). The National Wildlife Rescue Center (NWRC) and Malayan Tiger Conservation Center (MTCC) are two of the *ex-situ* tiger conservation centres. The NWRC in Sungkai Wildlife Reserve offered facilities for breeding and rescuing wildlife; meanwhile, the MTCC in Krau Wildlife Reserve is building facilities for rewilding Malayan tigers (Malaysian Palm Oil Industry 2020). In addition, zoological parks also contribute to the conservation of Malayan tigers as well as providing educational and tourism benefits (Boyd et al. 2014).

Ex-situ platforms are indispensable to conserve the biodiversity of large breeding groups of mammals, such as tigers and their prey species (Ten et al. 2021). Numerous efforts have been aimed at improving the husbandry and welfare of Malayan tigers in captivity, including their health development through veterinary care, health screening and diet regime. Zoos in the Malaysian Peninsula provide them with different types of meat, such as chicken, beef and lamb and/or even live prey animals for hunting enrichment. In addition, most food regimes applied by zoos involve either daily feeding or one day fasting per week. The latter aims to imitate the conditions of a wild habitat where tigers do not hunt every day (De