







Unveiling prey preferences of endangered wild Malayan tiger, *Panthera tigris jacksoni*, in Peninsular Malaysia through scat analysis via COI DNA metabarcoding

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Abstract

Understanding the prey preference of Malayan tiger (*Panthera tigris jacksoni*) in Malaysia is important to guide conservation planning initiatives. The utilisation of DNA metabarcoding provides valuable insights, particularly in the field of carnivora diet research. This technique has been proven to be effective for identifying various species within complex mixtures such as scat materials, where visual identification is challenging. The Cytochrome c oxidase subunit I (COI) locus has been selected as it is a widely used as an effective non-invasive approach for diet studies. Hence, given this advance approach, Malayan tiger scats were collected on the basis of existing records of their presence in two types of habitats, namely, protected areas (PA) and human–tiger conflict (HTC) areas. This study aimed to identify prey species in Peninsular Malaysia, based on Malayan tiger scat samples using DNA metabarcoding. Based on the partial mitochondrial COI region, DNA metabarcoding led to the taxonomic resolution of prey DNA remnants in scats and the identification of prey species consumed by Malayan tiger, which were predominately small-to-medium-sized prey, including livestock. The dominant DNA prey detected belongs to the family Canidae, followed by Bovidae, Vespertilionidae, Homonidae, Felidae, Phasianidae and Muridae. A significant difference ($p < 0.05$) was observed in alpha and beta diversity using the Shannon index and PERMANOVA with regard to prey richness and evenness in two different habitat groups, namely, PA and HTC. Our finding provides insights into Malayan tiger dietary requirements, which can be used to develop conservation plans and strategies for Malayan tiger, particularly for habitat priorities.



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Introduction

The Malayan tiger (*Panthera tigris jacksoni*) is a prominent apex predator, which has received considerable attention in Malaysia. Peninsular Malaysia constituted approximately 6% of the land areas in Malaysia and is designated as totally protected, which comprises national parks and wildlife reserves managed by the Department of Wildlife and National Parks (PERHILITAN), as well as state parks under the supervision of state governments (Kawanishi et al. 2010). According to Kawanishi et al. (2010), 51% of Malaysia's land cover was identified as a potential tiger habitat, which encompasses 29% confirmed tiger habitat, 9% expected tiger habitat and 13% possible tiger habitat. The potential and expected tiger habitats have decreased in recent times. Malayan tiger requires large prey and vast habitats to survive. However, continuous anthropogenic activities, such as illegal hunting, industrial and agricultural expansion and the establishment of human settlements, have resulted in habitat fragmentation and degradation, which have led to a significant decline in tiger population in Malaysia (Topani 1990; Elagupillay et al. 2001; Kawanishi et al. 2006; Shevade et al. 2017; Ten et al. 2021). At present, less than 200 Malayan tigers are left in the wild habitat (Halim et al. 2019). Other factors that led to the decline of Malayan tiger population include continuous depletion of prey species in the Malayan tiger habitat, which are considered as the current leading threats to tigers. The availability of prey species will determine the behaviour, health, social structure and survival of the Malayan tiger. Tigers can live in a diverse habitat and environment, in which they can consume prey that is of varying sizes and their hunting strategies will change depending on the habitat, prey type and prey size (Karanth 2003).

Predators play a crucial role in shaping the structure of food webs within ecosystems (Ritchie et al. 2012). The selection of prey can alter the composition and function of communities and the predator–prey relationship can affect the structure of habitats, behaviour of populations and their survival rates. Habitat loss and fragmentation also influence the relationship between predator and their prey (Haapakoski et al. 2013). In addition, habitat degradation greatly affects large predators that require a large habitat. The social structure of large predators, such as big cats, depends on the availability of prey biomass (Simcharoen et al. 2014) and the availability of the prey in the wild indicates the resources for their growth, survival and reproduction. The selection of their prey determines their eating habits, which, in turn, determines their life history strategies, including movement, habitat selection, social structure, geographical distribution and reproductive success (Sunquist and Sunquist 1989). However, information regarding real-time prey selection by the Malayan tiger in Peninsular Malaysia is lacking.

Determining the real-time prey selection by Malayan tiger is necessary to recognise the essential conservation needs of this endangered species in the future. Studies on prey selection of predators have been conducted in different ways, such as direct observation and scat analysis through undigested prey items remains in scats, such as hair, fur, bone and nails by using microscopic morphological analysis (Reddy et al. 2004; Ramesh et al. 2009; Kumar 2015; Upadhyaya et al. 2018; Matthews et al. 2020) and a molecular DNA approach (Xiong et al. 2017; Thuo et al. 2019; Hacker et al. 2021; Lu et al. 2021). Direct ob-