Assessing Methodological Variability in Gut Microbiome Studies: Lessons from Southeast Asian for Effective Conservation Strategies

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> Abstract. Gut microbiome studies have gained significant attention in recent years due to their potential in unveiling the role of microbial communities in animals' health and ecological processes. However, the lack of standardized protocols in sample handling and processing across studies introduces variability, impeding the comparability of findings. This study addresses this issue by examining methodological variations in gut microbiome research on wildlife and domesticated animals in Southeast Asia. A comprehensive search of 91 relevant studies on the SCOPUS database yielded 54 suitable publications for review, encompassing diverse taxa such as invertebrates (20), fishes (7), reptiles (3), birds (5), and mammals (19). Notably, various methodological approaches were employed to characterize microbial communities, including the source of isolation, various culture-based approaches, sequencing methods, and the targeted markers. Based on the information provided in this study, future studies should strive to develop guidelines and best practices specific to gut microbiome studies. This would enhance comparability and facilitate the integration of findings. Such efforts will also advance our understanding of the microbial diversity associated with wildlife, and its potential implications for their health and conservation.

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1 Introduction

Wildlife gut microbiome studies have gained substantial attention within ecological and conservation research, providing deep insights into the complex relationship between microorganisms residing in the gastrointestinal tract of various animal species. To date, the majority of microbiome studies have primarily focused on humans and domesticated animals, and for wildlife, efforts are now becoming more widespread to address this research gap [1]. This coincides with the advancement of technologies such as high-throughput sequencing which has enabled faster and cost-effective identification of microbial taxa inhabiting the gastrointestinal tract system, including their genetic material [4]. Understanding the diversity, composition, and functional roles of these microbial communities is critical for solving the complexities of ecosystems and their influence on the fitness, health, and behaviour of wildlife [5-7].

Various methodological approaches, practices, new challenges, and opportunities are emerging in the rapidly advancing field of wildlife gut microbiome research. Currently, there is no standardized set of best practices guiding the gut microbiome among wildlife. This necessitates a critical analysis of methodological and technical variability [1, 2]. Furthermore, current evidence suggests that the impacts of the gut microbiome on host species and environments differ across various aspects. These include behavior, such as social patterns and stress [8-11] and health aspects like the digestive system, hormone metabolites, and the immune system [6, 12-14]. Anthropogenic disturbances can significantly impact the gut microbiomes of wildlife, potentially rendering them more susceptible to diseases. This vulnerability arises from the fact that gut bacteria play a crucial role in the development of the mucosal innate immune system [4]. They achieve this through direct interactions with intestinal epithelial cells, acting as our first line of defense against pathogens and toxins. Hence, subsequent investigations in this area could contribute to a more effective conservation and management plan for the wildlife species, especially in cases where understanding can lead to the development of strategies and protocols to mitigate the risk of extinction for endangered species [2, 6].

However, much remains to be further explored on the wildlife species in Southeast Asian countries since the study is very scarce and limited. Despite being recognized as a global hotspot for biodiversity and endemism, this region is notably one of the most biotically threatened areas with regards to its biodiversity [15]. Therefore, this study aims to conduct a comprehensive assessment of methodological differences in gut microbiome studies, specifically focusing on studies conducted in Southeast Asian countries. Understanding this variability is crucial for accurately interpreting research findings in this region and to help provide set of protocols that minimize bias in this field, enabling better-informed conservation and management strategies tailored to the unique characteristics of our Southeast Asian wildlife.

2 Methodology

Bibliographic searches were utilized to gather data on the methodological variability in gut microbiome studies. This review consists of a few stages, which are (1) identifying the search strategy, (2) sorting the exported research articles, (3) identifying the relevant studies, and (4) summarizing and tabulating the results.