Comparison of Metabarcoding Techniques for Dietary Assessment in Herbivores and Omnivores

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Abstract. Dietary assessment plays a crucial role in comprehending the
ecological dynamics and nutritional needs of herbivores and omnivores. The
metabarcoding technique has emerged as a potent tool for exploring the
dietary composition of these animals. However, various metabarcoding
techniques have been developed, each with its own advantages and
limitations. This study aims to compare the performance of different
metabarcoding techniques in herbivores and omnivores diet. We
systematically reviewed 159 published manuscripts in Scopus and Google
Scholar, and thematic analysis was conducted across several categories,
including the marker, platform, and database utilized. Preliminary findings
reveal significant variations among metabarcoding techniques across these
two animal groups. The trnL gene exhibited higher taxonomic resolution for
herbivorous species, whereas the combination of the 'trnL + 16s rRNA' gene
exhibited superior performance for omnivorous species. The Illumina
platform emerged as the most commonly used method for analyzing the diets
of both herbivores and omnivores, with the primary reference database being
the National Centre for Biotechnology Information (NCBI). This study
offers valuable insights into the strengths and limitations of different
metabarcoding techniques for dietary assessment in herbivores and
omnivores and optimizing metabarcoding protocols, facilitating more
precise and reliable diet analyses within these ecological groups.

1 Introduction

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DNA metabarcoding involves identifying a multitude of species within a single sample, which may consist of complete organisms or environmental specimens like water, soil, and degraded DNA [1]. This innovative approach is rapidly emerging as a practical substitute for traditional morphology-based species identification, particularly in large-scale investigations conducted in environments where logistical or financial constraints pose challenges [2]. Metabarcoding is a standard tool in numerous recent studies focusing on trophic interactions, including dietary analysis [3]. In the context of diet analysis, the diet is inferred from DNA traces found in fecal samples or the gut contents of consumers [4]. This molecular dietary research, including sequencing prey identification, can expose exact food webs throughout ecosystems using a reference library [5]. Forister *et al.* [6] proposed that diets frequently display imbalanced distributions, prioritizing a few primary resources while incorporating numerous rare ones.

Conventional methods for diet studies, such as direct observation, videotaping, or fecal microscopy, have various drawbacks and limitations [7]. Observing wild animals consuming unexpected foods that defy conventional knowledge is a common occurrence, as highlighted by Mehrkam [8]. While large mammalian herbivores possess the ability to consume a variety of food plants, they tend to exhibit preferences for specific options while avoiding others [9; 10]. Certain mammals exhibit dietary specialization, depending on a specific food source, whereas others are dietary generalists capable of consuming diverse foods [11]. Additionally, the food supplies that are accessible in a mammal's habitat have a significant impact on what it consumes [12]. Mammals have three main dietary groups: carnivores, omnivores and herbivores. Herbivores are mammals that own a primary diet based on plant material. They are further categorized into more specialized diets, such as folivores, frugivores, or granivores, based on their specific nutritional preferences and adaptations [10; 13]. In contrast, omnivores have a more diverse diet that encompasses both plant and animal matter, allowing them to adapt to fluctuations in food availability.

Symondson [14] is the first researcher who used DNA barcoding to assess the diets of wild animals, focusing on invertebrates. These investigations identified the predator's diet even after the food had been digested. Even though DNA metabarcoding has been chosen as the most practical tool for studying diet since it has enhanced the process of identifying foods, assessing dietary diversity, and measuring the relative abundance of taxa in the diets of wild animals [15, 16]. However, this method has occasionally come under scrutiny due to concerns about the accuracy of DNA identification, which affects both the choice of barcode markers and the technique used to analyze the results [17, 18]. Achieving precise taxonomic information at the species or genus level can be challenging through DNA metabarcoding, especially when reference databases are incomplete [19]. Additionally, implementing and conducting DNA metabarcoding experiments can incur substantial expenses and demand specialized equipment and expertise [20]. In this review, we compiled published research that employed metabarcoding techniques for the analysis of herbivores' and omnivores' diets to identify the barcode region, platforms and database utilized in previous studies. The strengths and weaknesses of various metabarcoding approaches for nutrition evaluation in herbivores and omnivores will be discussed to improve metabarcoding procedures, enabling more precise and trustworthy diet assessments in these ecological populations.

2 Methodology

Bibliographic searches were employed to acquire data from previous studies conducted on diet metabarcoding. Peer-reviewed articles were searched in both the Scopus database and Google Scholar, focusing on indexed titles, abstracts, keywords, and topics using the keyword "METABARCODING." Relevant studies, including theses and reports related to dietary analysis based on metabarcoding approaches, were included in the search. Figure 1