



XIV Congresso Italiano
di Teriologia

Abstract Book

XIV Congresso Italiano di Teriologia
3 - 5 Giugno 2026, Bolzano

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research



Associazione
Teriologica
Italiana
E.T.S.

N. 118

Towards resolving species-level plant diets in Alpine wildlife through shotgun sequencing

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Accurate diet characterization is fundamental to understanding the ecology, habitat requirements, and health of wildlife in sensitive alpine ecosystems. While DNA metabarcoding of fecal samples has become a standard tool, it often lacks the taxonomic resolution required to distinguish between closely related plant species. In this study, we evaluate a methodological framework based on shotgun sequencing to achieve species-level resolution in plant diet analysis, providing a high-precision alternative for monitoring mountain-dwelling wildlife. Our approach is currently being developed and validated using fecal samples from the rock ptarmigan (*Lagopus muta*), a high-altitude specialist that serves as a model for testing dietary resolution in alpine flora. We evaluate the performance of shotgun sequencing together with the Kraken2 classifier across different reference database configurations, including full genomes, chloroplast markers, and Internal Transcribed Spacer (ITS) sequences. A key component of our work involves the enhancement of available reference databases through the integration of the latest sequences from NCBI-GenBank in a quality-controlled and curated manner, ensuring the highest possible taxonomic coverage today for alpine-specific taxa and beyond. Preliminary results highlight the critical role of database selection in taxonomic assignment and the ecological insights gained through co-occurrence network analysis. We demonstrate that shotgun metagenomics allows for the parallel identification of plant content and bacteria within the same fecal samples. Interestingly, our co-analysis suggests a functional link between diet and the gut microbiome. Specifically, we found that certain medicinal plants, such as *Lavandula angustifolia*, negatively correlate with potentially pathogenic bacteria, suggesting a possible role in modulating animal health. Additionally, the detection of specific bacteria such as *Pseudomonas putida* serves as an indicator of habitat quality and soil stress. Furthermore, the presence of plant pathogens like *Rhizobium tumorigenes* across habitats demonstrates the potential for the simultaneous monitoring of animal health and plant-ecosystem integrity. This shotgun-based approach offers a scalable and high-resolution alternative for studying the dietary ecology and potential health implications for wild animals. By refining these bioinformatic methods in a controlled alpine model, we provide a robust toolset that is highly transferable to the conservation and management of alpine mammals, such as the Alpine chamois or the mountain hare, where precise knowledge of host-diet-microbiome interactions is vital for ecosystem resilience.