

Publication

A powerful long metabarcoding method for the determination of complex diets from faecal analysis of the European pond turtle (*Emys orbicularis*, L. 1758)

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High-throughput sequencing has become an accurate method for the identification of species present in soil, water, faeces, gut or stomach contents. However, information at the species level is limited due to the choice of short barcodes and based on the idea that DNA is too degraded to allow longer sequences to be amplified. We have therefore developed a long DNA metabarcoding method based on the sequencing of short reads followed by de novo assembly, which can precisely identify the taxonomic groups of organisms associated with complex diets, such as omnivorous individuals. The procedure includes 11 different primer pairs targeting the COI gene, the large subunit of the ribulose-1,5-bisphosphate carboxylase gene, the maturase K gene, the 28S rRNA and the trnL-trnF chloroplastic region. We validated this approach using 32 faeces samples from an omnivorous reptile, the European pond turtle (*Emys orbicularis*, L. 1758). This metabarcoding approach was assessed using controlled experiments including mock communities and faecal samples from captive feeding trials. The method allowed us to accurately identify prey DNA present in the diet of the European pond turtles to the species level in most of the cases (82.4%), based on the amplicon lengths of multiple markers (168-1,379 bp, average 546 bp), and produced by de novo assembly. The proposed approach can be adapted to analyse various diets, in numerous conservation and ecological applications. It is consequently appropriate for detecting fine dietary variations among individuals, populations and species as well as for the identification of rare food items.

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