## Taxonomy and genetics

## eDNA metabarcoding of wild boar diet: a tool for local biodiversity assessment of understudied taxa of fungi and invertebrates

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Faeces from omnivorous terrestrial mammals are considered important "biodiversity capsules" and a complementary tool for improving local biodiversity assessments. Investigating the diet of opportunistic feeders such as wild boar (*Sus scrofa*) by using DNA metabarcoding provides important insights into local biodiversity, including cryptic species that are difficult to monitor using traditional methods. The opportunity to study biodiversity with DNA metabarcoding provides many advantages as follows: higher resolution of identification, higher detection probabilities of rare, elusive and quickly digested items, and the ability to sample in various environments. Moreover, in the case of studying wild boar diet, we also gain deeper insights into the ecological role of the species, trophic interactions, and the potential causes and consequences of rooting behaviour, which can all contribute to improving management decisions.

We used fresh faecal and/or gastrointestinal samples (n = 37) of wild boar, collected in central parts of Slovenia and Croatia, to investigate diet as well as local biodiversity of two of the most understudied taxonomic groups: invertebrates and fungi. We considered wild boar diet to serve as a potential indicator of local biodiversity for various reasons: (i) wild boar inhabit relatively large and diverse habitats; (ii) they have opportunistic feeding strategy, consuming a broad range of taxa available at specific periods and/or locations; (iii) there are known to root due to searching for several fungal and invertebrate taxa present in soil. We compared the influence of season and individual factors (sex and age) of individuals to variability in diet composition. The 157-bp long mitochondrial COI for barcoding invertebrates and 150-350-bp nuclear ITS1 universal primers for barcoding fungi were used. We detected 175 fungal genera (>270 species) and 114 invertebrate genera (>120 species) in faecal samples. Results showed seasonal variations in detected fungal and invertebrate genera in investigated faecal samples. Metabarcoding of wild boar faeces revealed great applicability especially for detecting microfungi, with the most frequently detected genera belonging to Candida, Pichia and Pilidium. We have also successfully detected several arthropods, especially ground beetles (e.g. Carabus, Abax, Harpalus, and Poecilus), which are considered as important food source for wild boar, but also many other insects (e.g. Lucilla, Drosophylla, Tipula, Culex) as well as some other taxa, important as food source for wild boar (e.g. flatworms, earthworms, and slugs). However, to get better insight into dietary preferences of wild boar in relation to season and location as well as the potential effect of sex/age on diet selection the dataset should be enlarged both considering geographical aspect and number of samples per area.

