

# Barcoding Slovakia as a tool for nature conservation and protection

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## Abstract

In 2015 Slovak National Museum-Natural History Museum in Bratislava, Slovakia, obtained financial support of 1.7 mil. EUR from EU Commission Operational Program of Research and Development and co-financed with the European Fund for Regional Development (EFRD) for building a DNA lab which will serve for the barcoding of Slovak flora and fauna in 2016 – 2023. This lab has been built and project of Barcoding Slovakia has been launched. The main activity in term of research objective by 2023 is gathering, identifying and sequencing of selected species of bacteria, fungi, plants and animals from the area of Slovakia. The aim of the research plan till the year 2023 is at least 1,000 species to be sequenced, voucher specimens will be obtained by field research from different areas of Slovakia, from soil samples from environmental research and from collections of SNM-NHM. Within the range of project activities aimed towards the conservation and protection of threatened species and habitats has already started.

## Building a barcode library of native orchid species and populations, threatened by illegal harvesting for commercial purposes.

Since 2013 several cases of illegal harvesting of protected orchid species in protected sites were observed in Slovakia. Hundreds of specimens mainly *Dactylorhiza coriophora* and *Orchis ustulata* were removed (dug out) at each locality. Repeated poaching has become the subject of the criminal investigation and a committee composed of experts such as botanists, environmentalists, nature conservationists and molecular biologists was established in 2016 to help find some solutions. One of the proposals how to fight illegal trade with threatened species was the use of barcoding in revealing their use in various commercial products.

As the first step, in 2017, building of the national barcode library of native species and population has begun at the Slovak National Museum-Natural History Museum in Bratislava. The aim of the project is to create a database of voucher DNA sequences of most of the orchid species growing in Slovakia. The database will serve as a tool for identifying these species in commercial products such as remedies, herbal mixes, teas or ice creams (Salep) and others.

## Environmental sampling on mine wastes and contaminated soils, for further analysis.

Technogenic soils (listed as technosols in WRB 2006) are formed during the reclamation of overburdens, tailings, mining and industrial wastes having technic material with an artefact content of more than 50% by volume (Levyk et al. 2007). Mining activities, disposal of metals or metal containing materials inevitably cause contamination of the surrounding ecosystem and changes in the size, structure and activity of soil microbial communities (Camargo et al. 2005; Ge & Zhang 2011).

Studied technosols from Zemianske Kostolany (Slovakia) represent a unique system of 50 years old environmental burden. Long-term and significant contamination of soils with As is mainly due to dam failure of one of the coal-ash ponds in 1965 (Jurkovič et al. 2011), when 3 million m<sup>3</sup> of As-rich ash slurry were released into the surrounding environment, and thus contaminating a large area of agricultural soils. The released ashes rich in arsenic with a thickness of 1–2 m were covered by 40 cm thick layer of soil (Fig. 1). Long-term exposure and selection pressure of elevated concentrations of arsenic (a range of 93–634 µg/g) induced the formation of the specific adapted autochthonous microorganisms.

Based on the phylogenetic analysis, isolated bacterial strains (Fig. 2) were composed of 4 phyla and represented by common strains in soils: Proteobacteria (60.9%), Firmicutes (21.7%), Actinobacteria (8.7%) and Bacteroidetes (8.7%). The phylum Proteobacteria was represented by the species *P. baetica*, *P. fluorescens*, *P. chlororaphis*, *P. korensis*, *P. putida*, *P. reinekei* and *Pseudomonas* sp. The phylum Firmicutes was represented by species *Bacillus cereus* and *Bacillus pumilus*. There were further recorded two isolates of the genus *Chryseobacterium* (Bacteroidetes). The genus *Streptomyces* and *Rhodococcus* (Actinobacteria) were represented by only one species.

## Fungal taxonomy as a tool for better understanding inter specific relations within the habitats.

Species rich semi-natural grasslands are rapidly decreasing in Europe. According to NATURA 2000 reports (Calaciura & Spinelli) grassland habitats are steadily decreasing, mainly due to abandonment or change in landuse. According to FAO the area of grasslands in the EU declined by 12.8% from 1990 to 2003; only a few Member States managed to buck this trend. Pressure caused by land use changes on grassland habitats is steadily increasing. More than 75% of the grasslands habitats in EU are in an unfavourable conservation status, according to draft data provided by Member States under Article 17 of the Habitats Directive.

Since 1999 systematic research of biodiversity of grassland fungi is going on in Slovakia. Fungi of the families Hygrophoraceae, Clavariaceae, or Geoglossaceae are often treated as valuable indicators of well preserved, diversity rich, traditionally managed meadows and pastures and are given high scores in several evaluating systems of such habitats. The research is focused on taxonomy, distribution, ecological demands and their role in grassland habitats as well as occurrence in Slovakia. For understanding of ecology and interspecific relationships, precise identification of the species is of primary importance. But it is often almost impossible without use of molecular taxonomy and barcoding database.(Kautmanova et al. 2012)

## Barcoding of reptiles and amphibians – threatened and invasive species

In Slovakia there is a deficient research of amphibians and reptiles in comparison with other animal taxonomic groups. Both of these taxonomic groups are considered as highly vulnerable in a global scale. In Slovakia, the situation became serious in correlations with decreasing habitat quality, overused chemical substances in agriculture and invasive pathogens. Combination of these factors weakens the populations of numerous species. The distribution of all amphibian and reptile species in Slovakia will be monitored within this project, while the survey focused on genetic variability will be simultaneously performed (Fig. 1). The outcome of the research will be the comprehensive publication comprising the distribution of amphibians and reptiles in Slovakia. The results will be used for the protection, conservation and reintroduction of the species. Simultaneously, results will be used for the increasing the quality of the environmental and habitat management. The impact of the invasive animal species on the autochthonous populations will be also evaluated within the project. The results will help with applied solutions in environmental management in cooperation with state authorities and agricultural and economical field.



Fig. 1. Sampling of threatened amphibians for DNA Barcoding.

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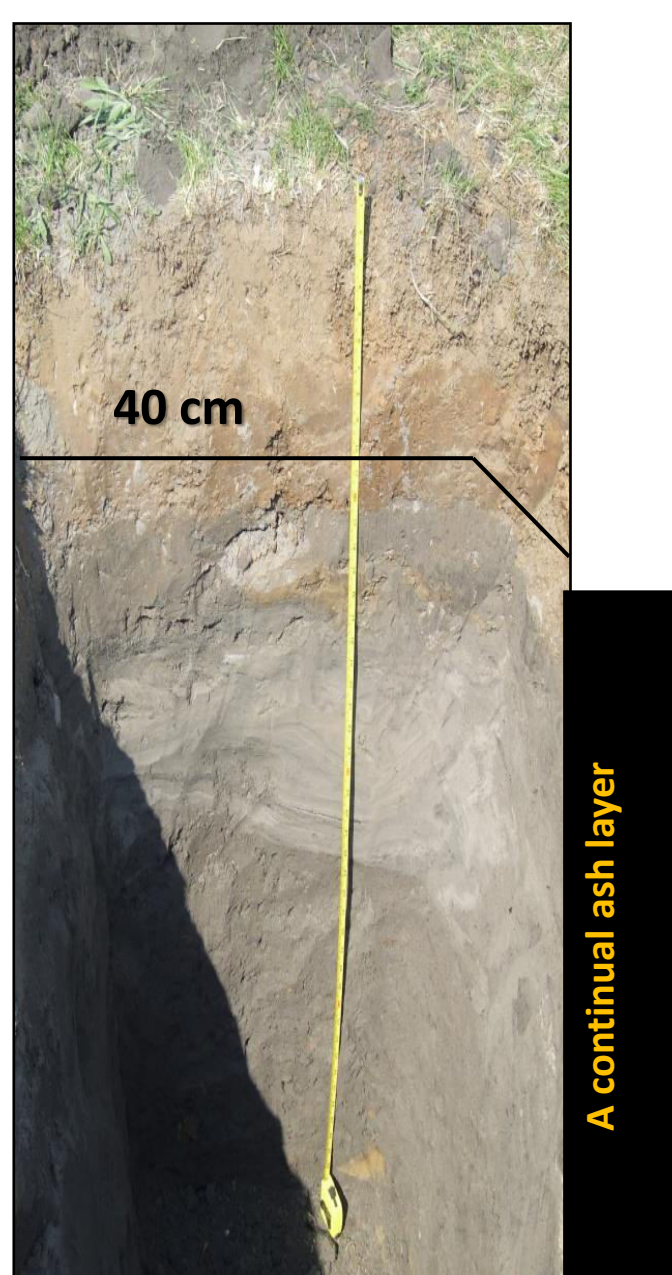


Fig. 1. Soil profile after covered of ash layer.

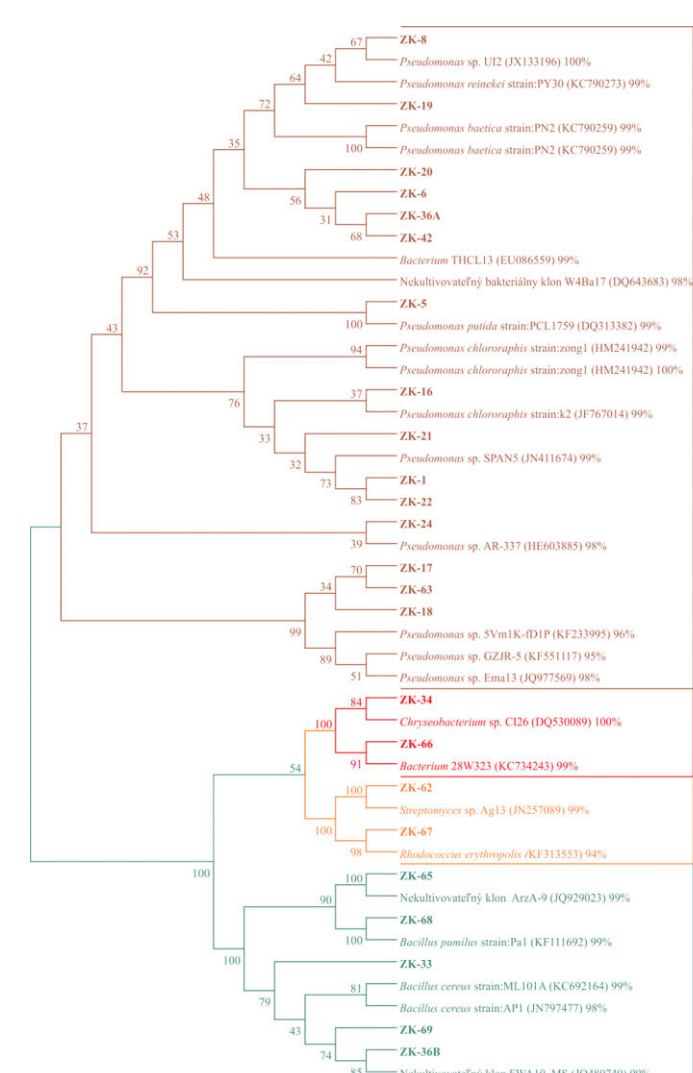


Fig.2. The phylogenetic tree of bacteria isolated from anthropogenic sediments and the affinity of their 16S rRNA sequences (percent similarity) to species in the GenBank database.