Monitoring mite diversity in European soils using (e)DNA metabarcoding

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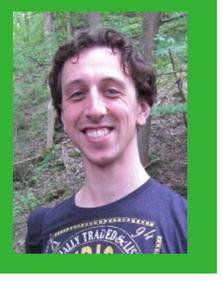

Background & Objectives

Within the EU FP7-project *EcoFINDERS*, various European partners collaborate to gain more insights in links between soil diversity and ecosystem services, across different soils, climate types and land uses. To allow rapid diversity screening of many soils throughout Europe, new tools are being developed for high-throughput species identification based on the DNA contained in soil extracts. Alterra participates in this metabarcoding project by developing a new approach for DNA metabarcoding of soil mites (Oribatida, Astigmata, Mesostigmata and Prostigmata).

Set-up

I. Soil samples were collected from agricultural and semi-natural grasslands around Europe. Mites were extracted for two purposes:

II. The development of a reference database with DNA barcodes of all potentially encountered taxa (~3 ecotypes / taxon). We use a newly designed 209bp minibarcode for soil microarthropods, located within the mitochondrial cytochrome c oxidase subunit 1 (CO1) fragment.

III. Samples from each site were split in two parts (*Figure 1*):
- Part 1: Subjected to DNA extraction and metabarcoding.
- Part 2: Identified morphologically to validate the method.

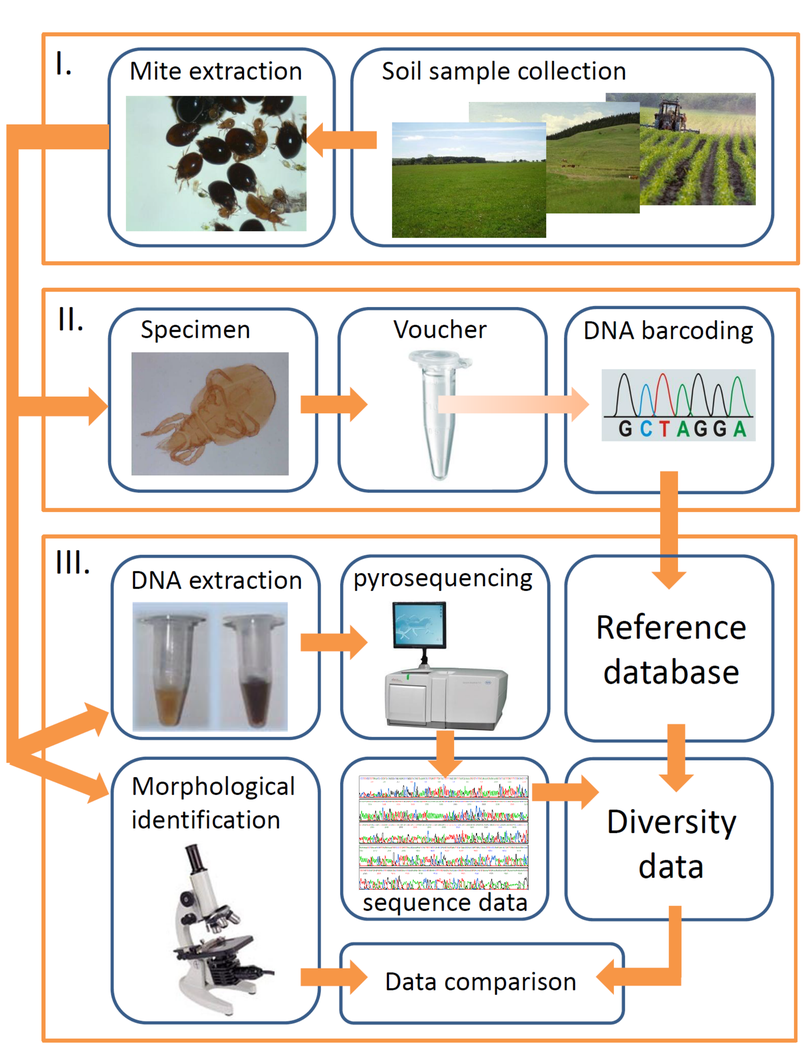

Figure 1. Schematic overview of the development and application of a new barcoding tool for rapid diversity screening based on soil samples. The numbers I-III indicate the workflow.

Towards direct (e)DNA extraction

In order to reduce the processing time, we currently explore different protocols for direct extraction of cellular and extracellular DNA from soil. Subsamples from identical localities will be subjected to various extraction methods, prior to DNA metabarcoding.

Reference database

- Our database of CO1 barcodes for soil mites from Dutch and French agricultural and (semi-)natural grasslands currently contains 245 sequences of 91 taxa, comprising 67 genera from 47 families.
- 89 % of the species and 84% of the genera in the database formed its own well-supported monophyletic clade (*Figure 2*).
- 72% of the species showed a proper "barcoding gap".

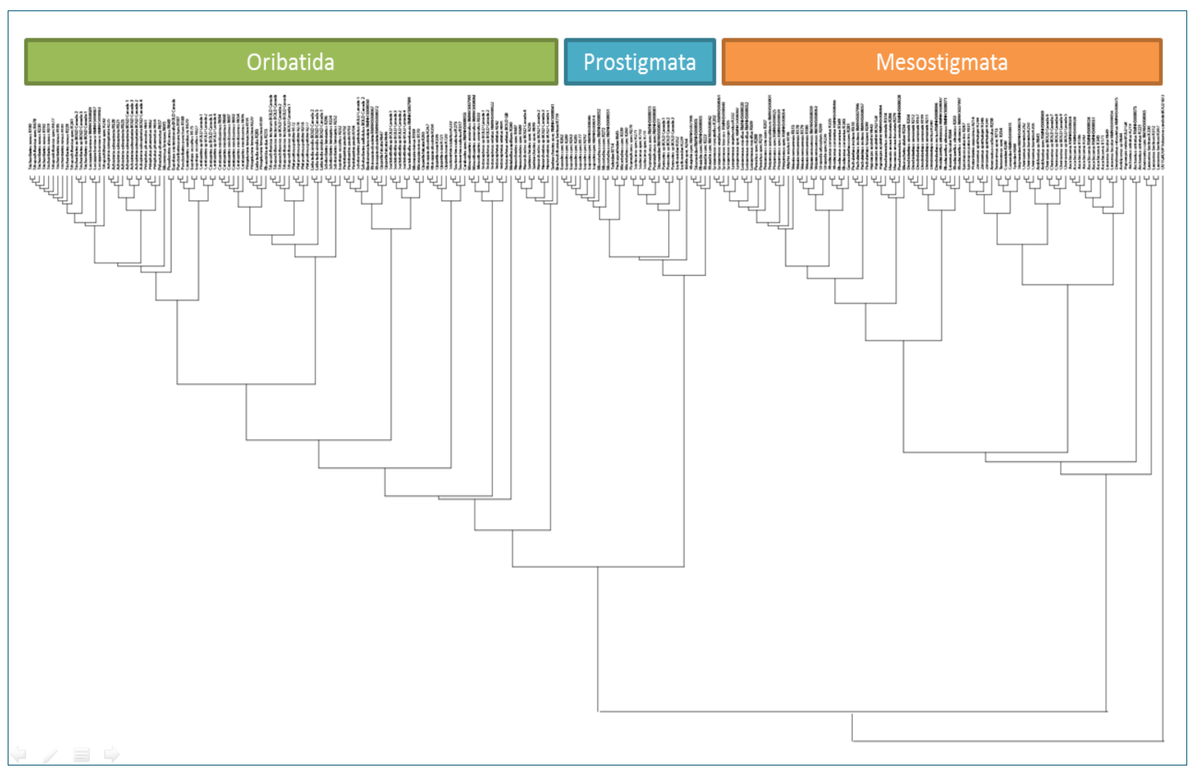

Figure 2. Neighbour-joining tree for all 245 CO1 barcodes in the current database.

DNA metabarcoding

- Soil samples (N=9) were taken in semi-natural grasslands (Veluwe, the Netherlands) and agricultural lands (Lusignan, France) with contrasting land and soil management regimes.
- Microarthropod communities were extracted from each soil sample and subjected to DNA extraction.
- CO1 barcodes were amplified and amplicons were sequenced on a Roche 454 GS FLX platform, resulting in a total of ~110.000 reads.
- Following bioinformatic analysis (currently in progress), we will compare species composition data gained molecular and morpho-logical methods, in order to validate potential for taxon identification, retrieval of relative abundances and evaluation of land use types.

A general metabarcoding framework for soil fauna

Within *EcoFINDERS* [3], integrated pipelines have been developed for simultaneous diversity screening of microarthropods (mites and springtails), lumbricids (earthworms), enchytraeids (pot worms), nematodes (round worms) and protists (single-celled eukaryotes). Alterra has coordinated these efforts and is currently evaluating the first data gained using these pipelines.

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