Soil protozoa: Abundant, diverse, but largely unknown organisms

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Protozoa occupy key positions in soil food webs; they are the major consumers of bacterial production in soil due to their small size, high abundance and fast turnover rates. Despite their important functions for nutrient cycling at the base of the heterotrophic eukaryotic food web in soil we only have a vague idea on the identity of the dominant taxa. This lack of knowledge is caused by widespread methodological difficulties in cultivation and quantification in the opaque soil environment as well as a lack of taxonomic expertise. Especially naked amoebae have received little attention in the past, due to difficulties both, in isolation and identification. However, recent developments in molecular techniques now promise to reduce this methodological gap on this largely unknown trophic link in the soil food web.

We combined morphological and molecular methods to determine the normal operating range on the occurrence of soil naked amoebae within the EU-project EcoFINDERS.

In a trans-European selection of sites from long term observatories in the Netherlands, and Sardinia as well as from high altitude soils in Tibet we setup parallel cultures in intensive cultivation effort. Clonal cultures of individual amoebae were then grouped into morphotypes and sequenced.

Approximately 100 clonal cultures were isolated from each soil exhibiting a huge diversity of naked amoebae. The isolated taxa were highly diverse in morphology, revealing basically all known morphotypes at each site. In addition, several clones at each site possessed new morphological features which were supported by phylogenetic information as sequence identities could not be assigned to any described organism, suggesting a significant amount of undetected taxonomic diversity in soils. This interesting finding let us to focus part of our work on taxonomy in order to increase the reference data for future studies.

We are describing seven new species in the class Heterolobosea which is placed in the supergroup Excavata. We are describing six of them as new species within the genus *Allovahlkampfia* allowing us to revise the systematics of this and related genera, such as *Solumitrus palustris* that we rename to *A. palustris* based on morphological and phylogenetic information (Figure 1a,b). Other clones were identified as already described species in distinct genera such as *Naegleria*, *Tetramitus* and *Paravahlkampfia*. A remaining isolate of our cultures varied strongly in morphology and phylogeny from all currently described taxa. Focused phylogenetic analyses reveal that it branches together with unknown environmental sequences. Consequently, we are describing it as a new genus (Figure 1c).

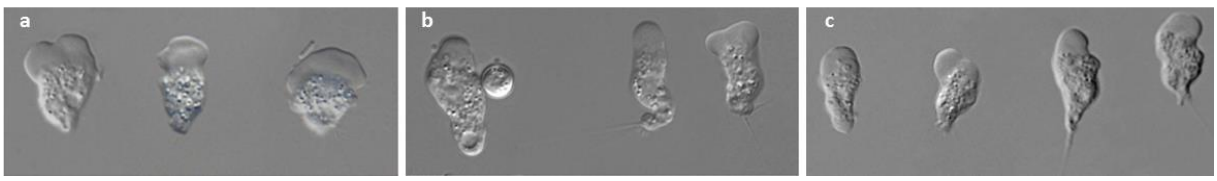

Figure 1: Light microscopic images of newly described Heterolobosea with differential interference contrast; new species of *Allovahlkampfia* (a,b) and new genus *Pagea* (c)

The supergroup Amoebozoa contains most other naked amoebae varying strongly in morphology and phylogeny. Our intense cultivation approach has supported this idea as we found not already described species within different genera, but also found new species. Currently we are describing 16 new species in all three classes of Amoebozoa; two in the class Tubulinea, three in the class Discosea and eleven in Variosea. The most innovative aspects about these species are the following; with the exception of three species, all species are placed in different genera demonstrating a large lack of knowledge on amoeba diversity. For example, eleven new species described in the class Variosea are placed in seven genera, five of them new to science. Further, we describe a new extremely small but abundant nanoamoeba species in the genus *Hartmannella* with probably cosmopolitan distribution, which has been overlooked in all environmental studies on soil protozoa (Figure 2).

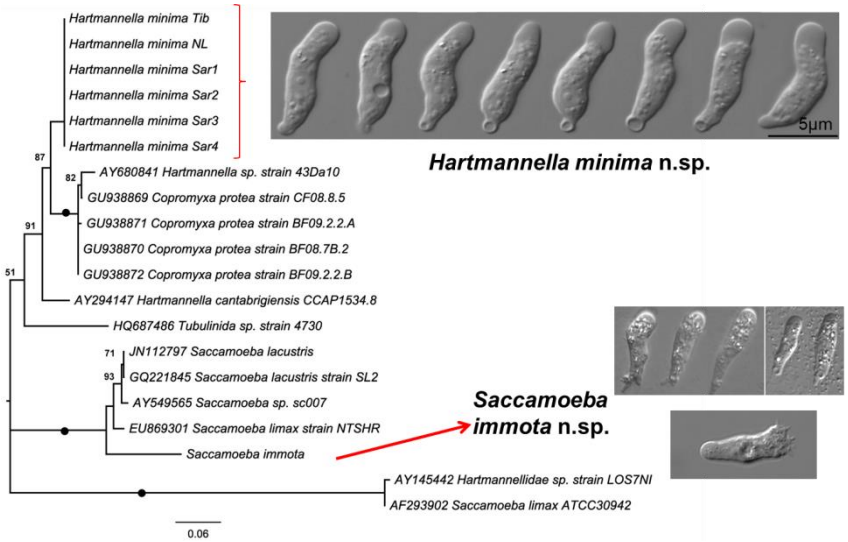

Figure 2: RAxML tree based on the SSU-rDNA focusing on the family Hartmannellidae showing the placement of two new species, *Saccamoeba immota* isolated from the Netherlands and *Hartmannella minima* cultivated independently from Sardinia, Tibet and the Netherlands. Support values at each node presented for RAxML with bootstrap values equal to 100% represented by a black dot. All branches are drawn to scale.

Besides the known species and above-mentioned new species we are describing, our immense cultivation resulted in several other clonal cultures, all placed in a wide range of genera with at least one new genus awaiting formal description.

Taken together, with our intensive cultivation programme and detailed morphological and molecular taxonomic work, we have been able to cultivate and identify a plethora of novel taxa of naked amoebae from soils investigated in EcoFINDERS. For the first time, our studies are describing the true diversity of Amoebozoa in European soils, the normal operation ranges of species abundances, as well as genetic diversity. Our study provides a significant contribution to foster the existing knowledge on this neglected, but abundant and ecologically important group of soil eukaryotes. Future studies will undoubtedly reveal more new species and genera, which are essential to gain a deeper understanding on the evolution and ecological functions of eukaryotes.