Final report of the project

Root colonizing dark septate endophytic fungi of grasslands — diversity, taxonomy and secondary metabolites

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Background, state of the art at the start of the project, main aim

Fungal endophytes

Plants live together with a plethora of various microorganisms. Consequently, plants and their microbiome must be viewed as holobionts, where all interacting organisms contribute to the overall stability of the system (Vandenkoorhuyse et al. 2015; Hacquard 2016). Essential members of the plant microbiome are the fungal endophytes, which colonize plant tissues during some period of their life cycle, yet cause no symptoms of tissue damage to their hosts (Saikkonen et al. 1998; Schultz and Boyle 2005). Fungal endophytes can colonize different parts of the plant and are found in all biomes, where these fungi might play important roles in ecosystem functioning (Porras-Alfaro and Bayman 2011; Busby et al. 2016).

Root endophytes

Endophytic fungi are also present in healthy belowground tissues (Vandenkoornhuyse et al. 2002; Rodriguez et al. 2009), albeit knowledge of their general occurrence and their potential functions is lacking compared with what we know of mycorrhizal fungi. Apart from behaving as commensalistic symbionts, fungal endophytes also act as latent pathogens, latent saprotrophs, and mutualistic partners (Porras-Alfaro and Bayman 2011; Yakti et al. 2018). These form a group of root-colonizing endophytic fungi, generally called dark septate endophytes (DSEs), which refer to their mainly melanized and septate hyphae. These fungi dominate several biomes and climatic regions, including grasslands, yet their functions in relation to plants and the greater ecosystem are still elusive (Mandyam and Jumpponen 2005; Sieber and Grünig 2013). They might have an essential role as saprobes because comparative genomics, for example, of DSE fungi revealed an expansion of carbohydrate-active enzyme families (Knapp et al., 2018). Although their supposed important role in plant performance and survival (Giauque et al. 2019), information on root endophytic fungal communities, the dominant taxa, and their secondary metabolites are limited.

DSE community of grasslands

In arid, semiarid, and temperate grasslands of North America and Europe, DSE communities and non-mycorrhizal root-associated fungi have been thoroughly studied, and these fungi are relatively frequent in these ecosystems (e.g., Kovács and Szigetvári 2002; Mandyam and Jumpponen 2005; Porras-Alfaro et al. 2008; Sánchez-Márquez et al. 2008; Knapp et al. 2012). The results suggest that there are core members of those communities common to disparate regions, not only in North America (Khidir et al. 2010) but also worldwide (Knapp et al. 2012). In the past few years, only low number of studies has been published focusing on fungal root endophytes of Asian grasslands (but see e.g. Su et al. 2010; Li et al. 2018). However, information about DSEs from other sites and countries in the eastern part of the Steppe belt, including Mongolia and Kazakhstan, where the steppe represents a significant part of the area is not available.

DSE taxa of grasslands

A plethora of root endophytes belongs to the order Pleosporales, which is one of the most common ascomycetous orders comprising root-associated species in grassland ecosystems (Zhang et al. 2012; Jumpponen et al. 2017). Several common pleosporalean DSE fungi have been studied to date, including *Darksidea* species, *Flavomyces fulophazii* (Knapp et al. 2015), and the relatively well-studied *Periconia macrospinosa* (see Mandyam et al. 2010; Knapp et al. 2018). Pleosporales includes an increasing number of root endophytic species and genera; for example, in the last year only, several novel DSE lineages were investigated and formally described, such as *Laburnicola rhizohalophila* (Yuan et al. 2019), *L. zaaminensis* (Htet et al. 2021), and *Posidoniomyces atricolor* (Vohník et al. 2019). The other important order is Helotiales, where also some common and dominant DSE species belongs such as *Cadophora* sp. cf. *meredithiae* (Knapp et al. 2018) and *Polyphilus* species that can colonize nematodes and desert truffles, too (Ashrafi & Knapp et al. 2018). Despite the continuously increasing number of described DSE taxa, numerous lineages representing novel genera and species are waiting for formal description.

Secondary metabolites of DSE

There is a general assumption that endophytic fungi can generally produce a number of secondary metabolites (Elsebai et al. 2014; Tejesvi and Pirttilä 2018), among which, the presence of unidentified and biologically active metabolite may be significant (Schulz et al. 2002). Relatively few studies investigate the metabolites of endophytic fungi that colonize roots (but see Maciá-Vicente et al. 2018), however DSEs associated with microorganisms in the soil and in the plant itself presumably produce more bioactive by-products than fungi in plant organs due to this complex environment and specific interactions. To date, no study has been conducted on the metabolites and metabolic diversity of common DSE fungi in grasses found in dry and semi-arid grasslands, so metabolite profiling of these isolates and comparison of profiles may yield essential and many new results.

Aims

The main goal of the project was to gain information of the DSE communities occurring mainly in the semi-arid grasslands of the Great Hungarian Plain, Mongolia and Kazakhstan and the metabolite profiling of the DSE isolates. In our work, we aimed to (i) process the existing DSE strain collection and collect additional isolates from different grasses in semi-arid areas, (ii) carry out polyphasic taxonomy of the new lineages and description of the novel taxa found. Our further goal (iii) was to profile the metabolite of dominant DSE fungi belonging to different lineages and to identify the main compounds.

Materials and Methods

Fungal isolates and specimens

We isolated fungi mainly from the roots of *Festuca vaginata*, *Stipa borysthenica* in the (semi)arid sandy grasslands of the Kiskunság, Hungary. We isolated root endophytes from *Stipa krylovii* from a natural steppe zone in the Nalaikh district, Mongolia. And roots were also collected, and isolates were gained from mainly gramineous species such as barley, wheat and *Stipa capillata* in agricultural and non-agricultural areas of the steppe zone of Akmola region Northern Kazakhstan. Altogether these samplings resulted ~750 new isolates to our strain collection (~150 Hungarian and ~600 Kazakh) and we also examined ~140 Mongolian and ~150 Hungarian isolates and for certain taxonomic studies ~100 DSE isolates from the United States, Spain, Germany, and other European Countries. During our field works on grasslands, several interesting ascomata and basidiomata were collected and these were also designated for polyphasic taxonomic works.

Molecular identification, phylogeny and taxonomy

For the molecular taxonomic identification after DNA extraction using mainly the CTAB method and NucleoSpin Plant II DNA Isolation Kit (MACHEREY-NAGEL, Germany) the DNA-barcode sequence of the fungi (Schoch et al. 2012) the nrDNA internal transcribed spacer (ITS) region of the isolates were amplified. The sequencing of the samples was carried out with the amplification primers from LGC GmbH (Berlin, Germany). Altogether, we sequenced the ITS region of ~1100 isolates. In case of the chosen lineages, further loci was amplified and sequenced such as the nrDNA 28S large subunit (LSU) region with the primers LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990), the nrDNA 18S small subunit (SSU) region with the primers NS1 and NS4 (White et al. 1990), the partial actin gene (ACT) using the primers ACT-512F (Carbone and Kohn 1999) and ACT-2Rd (Quaedvlieg et al. 2011), part of the translation elongation factor 1-α gene (TEF) using EF1-983F and EF1-2218R (Rehner and Buckley 2005) primers, part of the calmodulin gene (CAL) using the primers CAL-228F (Carbone and Kohn 1999) and CAL-2Rd (Groenewald et al. 2013) and ß-tubulin gene (TUB) with the primers CYLTUB1F (Groenewald et

al. 2013) and Bt-2b (Glass and Donaldson 1995). The RNA polymerase II largest subunit (rpb1) was amplified with the primer pair RPB1-Af and RPB1-Cr (Stiller and Hall 1997; Matheny et al. 2002). For RNA polymerase II second largest subunit (RPB2) RPB2-6F and RPB2-7R (Liu et al. 1999). The mitochondrial ATPase subunit 6 (atp6) were obtained using the primers atp6-2 and atp6-3 (Kretzer and Bruns 1999).

For molecular phylogenetic analyses, Bayesian analysis (BI) were performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and Maximum Likelihood (ML) phylogenetic analysis with the raxmlGUI v. 1.3 (Silvestro and Michalak 2012) implementation of RAxML (Stamatakis 2014).

The microscopic and morphological observations were carried out using light microscopes equipped with Nomarski differential interference contrast (DIC) optics, epi-fluorescent microscopy and scanning electron microscopy (SEM).

Metabolite profiling and identification of secondary metabolites

Isolates chosen for metabolite analyses were grown in three replicates on Potato dextrose agar medium at room temperature in dark for 30 days, then fungal mycelium and the medium were lyophilized and pulverized. After methanolic extraction, metabolic profiling of isolates was carried out by analytical HPLC hyphenated with UV and high-resolution Orbitrap mass spectrometry detections. A Dionex Ultimate 3000 UHPLC system (3000RS diode array detector (DAD), TCC-3000RS column thermostat, HPG-3400RS pump, SRD-3400 solvent rack degasser, WPS-3000TRS autosampler), hyphenated with a Orbitrap Q Exactive Focus Mass Spectrometer equipped with electrospray ionization (ESI) (Thermo Fischer Scientific, Waltham, MA, USA) was used for chromatographic separation and high-resolution mass spectral analysis. For metabolite isolation a Pharmacia LKB HPLC (Uppsala, Sweden) system (2248 pumps, VWM 2141 UV detector) connected to a preparative HPLC column was used. Nuclear magnetic resonance (NMR) spectroscopy spectra of the isolated compounds were recorded in methanol-d4 and chloroform-d and DMSO d6 on a Varian DDR spectrometer equipped with a dual 5 mm inverse detection gradient (IDPFG) probe-head. Standard pulse sequences and parameters were used.

Diversity of DSE fungi in different grasslands

DSE fungi from Mongolia

In this work, we investigated root-colonizing fungal endophytes of a common grass species of the steppes of Mongolia, which represent extended semiarid grasslands (**Knapp et al. 2019**). We gained information on DSE community of a common dominant gramineous plant species, *Stipa krylovii*. The majority of the 135 isolates examined in detail was found to belong to several orders

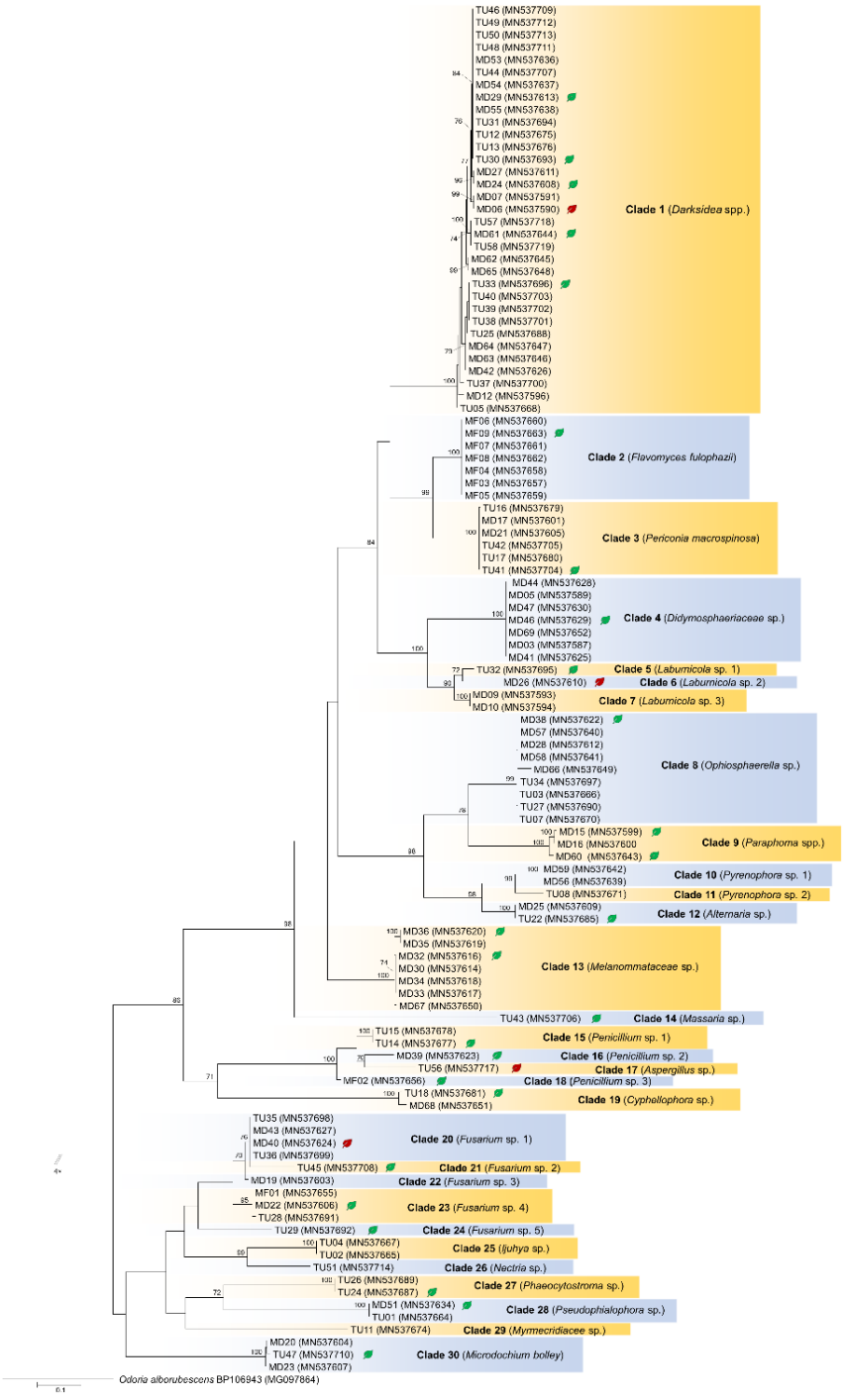

Fig. 1 Maximum likelihood (RAxML) phylogenetic tree of ITS sequences of isolates belonging to Ascomycota. ML bootstrap support values (>70) are shown at the branches. After the isolate names, GenBank accession numbers are shown in brackets. Leaves indicate the representative isolates tested by inoculation of leek; isolates with negative effect are labeled with red, and green leaves indicate no visible symptoms caused by the isolate. The scale bar indicates 0.1 expected changes per site per branch (Knapp et al. 2019).

of Ascomycota (110 isolates, **Fig. 1**) and some to Basidiomycota (25 isolates). We established *in vitro* resynthesis systems with leek to test the endophytic nature of isolates. A significant number of the isolates collected represented presumably novel taxa, and dominant similarities of the lineages have been found with relatively frequent and known grass root endophytes of semi-arid areas in other geographic regions. These endophytes included *Periconia macrospinosa*, *Microdochium bolley*, and *Darksidea*, the genus of which comprised one fourth of the isolates. We found numerous lineages, which have been detected not only from Asian steppe ecosystems, but also from prairies in North America and sandy grasslands in Europe. Therefore, our results strengthen the hypothesized worldwide presence of a common and dominant core group of a DSE community in arid and semiarid grasslands reinforcing our previous hypotheses on that core fungal community of those areas (Knapp et al., 2012).

DSE fungi from Kazakhstan

In this part of the project, we aimed to gain information on the DSE community of different plant species of agricultural and non-agricultural areas in the steppe zone of Northern Kazakhstan (**Akhmetova et al. 2022** *before submission*). Altogether, 587 isolates were collected from roots of different agricultural and non-agricultural plants in Northern Kazakhstan. Most of the isolates represented various fungal genera in mainly Pezizomycotina (Ascomycota), but basidiomycetes and mucoromycetes were also found. The most common and widespread genus was *Fusarium* represented by half of the isolates (290) comprising both known species such as *F. oxysporum*, *F. tricinctum* and *F. sambucinum* and also novel lineages within this genus. Besides the isolates of the complex *Fusarium* genus, 66 different lineages were represented by the isolates gained. We also found here (like in case of the Hungarian and Mongolian grasslands) the dominant DSE fungi characteristic to grasslands, such as *Periconia macrospinosa*, *Darksidea species*, *Microdochium bolley*, and several novel DSE lineages were identified (*see below at the taxonomy section*).

Taxonomic works of DSE and other fungi of grasslands

During the project, we carried out polyphasic taxonomic works using both classical morphological, molecular and further special techniques. Altogether 15 taxa have been formally described comprising 4 novel genera and 11 novel species of fungi (see the shaded **textbox**).

Alfoldia D.G. Knapp, Imrefi & Kovács, **gen. nov**.
A. vorosii D.G. Knapp, Imrefi & Kovács, **sp. nov.**
Babosia D.G. Knapp, Zagyva, Trappe & Kovács, **gen. nov.**
B. variospora D.G. Knapp, Zagyva, Trappe & Kovács, **sp. nov.**
Fusarium campestre Akhmetova, D. G. Knapp, Kovács, & Molnár **nom. prov.**
Fusarium kazakhstanicum Akhmetova, Özer, D.G. Knapp, Kovács, & Molnár **nom. prov.**
Fusarium rhizicola Akhmetova, Knapp, D. G. Kovács, & Molnár **nom. prov.**
Fusarium steppicola Akhmetova, D.G. Knapp, Kovács, & Molnár **nom. prov.**
Fuscosphaeria D.G. Knapp & Pintye **gen. nov.**
F. hungarica D.G. Knapp & Pintye **sp. nov.**
Geastrum dolomiticum Finy, Dima & V. Papp **sp. nov.**
Kiskunsagia D.G. Knapp, Imrefi & Kovács, **gen. nov.**
K. ubrizsyi D.G. Knapp, Imrefi & Kovács, **sp. nov.**
Murispora kazachstanica Akhmetova, Kovács & D.G. Knapp, **sp. nov.**
Stouffera gilkeyae D.G. Knapp, Zagyva, Trappe & Kovács, **sp. nov.**

Novel taxa from Hungary

Using isolates from our culture-collection and recently isolated strains, three new DSE species have been described representing three novel genera. Namely, ***Kiskunsagia ubrizsyi*** (the genus name is referring to the sandy collection site within the Kiskunság National Park, and the species name was given in honour of the outstanding Hungarian mycologist Gábor Ubrizsy (1919–1973)) (**Crous et al. 2019**), ***Alfoldia vorosii*** (the genus name is referring to the sampling site, the Great Hungarian Plain, which is called 'Alföld' in Hungarian and the species name was given in honour of another outstanding Hungarian mycologist József Vörös (1929–1991)) (**Crous et al. 2019**), and ***Fuscosphaeria hungarica*** (the genus name was given by its dark (fuscus) round (spharion) structures (**Fig. 2**), and the species was named "hungarica" because its occurrence solely in one semiarid Hungarian grassland) (**Pintye and Knapp 2021**).

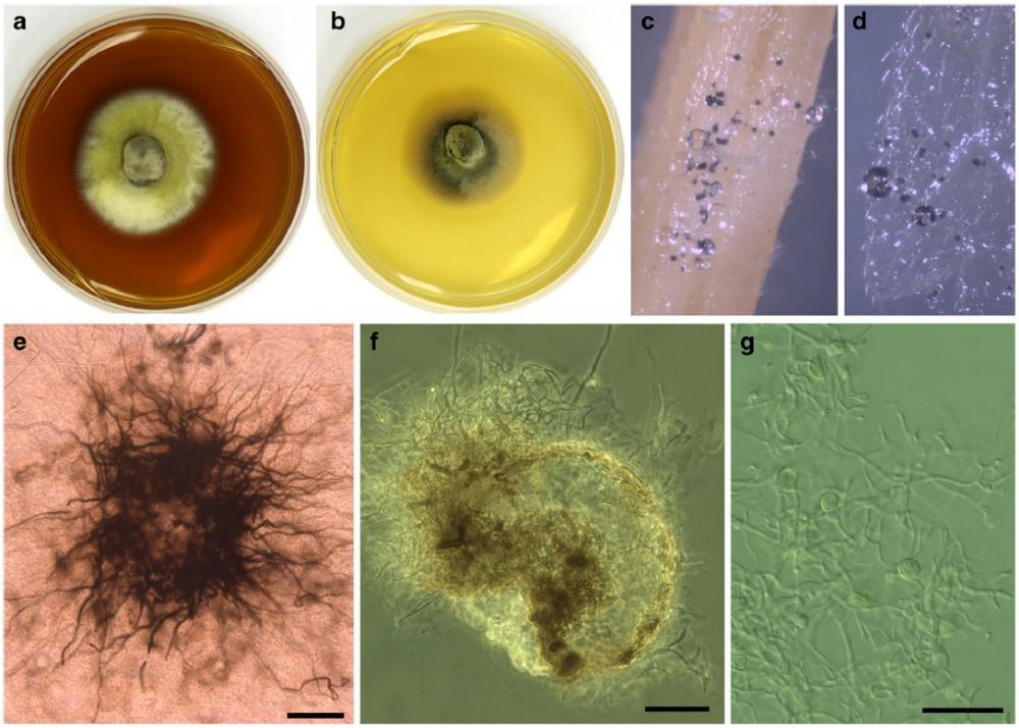

Fig. 2 *Fuscosphaeria hungarica* (ex-holotype, CBS 147250). **a** Colony on MEA. **b** Colony on PDA. **c**, **d** Sporocarp-like structure produced on the surface of stinging nettle. **e** Developing sporocarp-like structure. **f** Cross section of a sporocarp-like structure formed submerged in WA media supplemented with pine needles. **g** Terminal chlamydospores produced on PDA. Scale bars: 50 µm (Pintye and Knapp, 2021).

During our field works on grasslands, several interesting ascomata and basidiomata were collected and these were also designated for polyphasic taxonomic works. Several *Geastrum* species was found that resembled to *G. granulosum* but based on microscopic and molecular features it differed from all known European species (**Finy et al. 2021**). These samples originated from calcareous open rocky grasslands on dolomite bedrock. This species was described as ***Geastrum dolomiticum*** (The epithet refers to the habitat of the species, in open rocky grasslands on dolomite bedrock) (**Fig. 3**). Truffles with distinct morphological and anatomical features were collected from semiarid sandy grasslands of the Great Hungarian Plain in Hungary (**Knapp et al. 2020**). The truffles were found to represent two novel lineages that grouped with the *Marcelleina-Peziza gerardii* clade of the Pezizaceae (**Fig 4**). One belonged to a lineage clustered with the rarely collected

Fig. 3 *Basidiomata of Geastrum dolomiticum* (Finv et al. 2021).

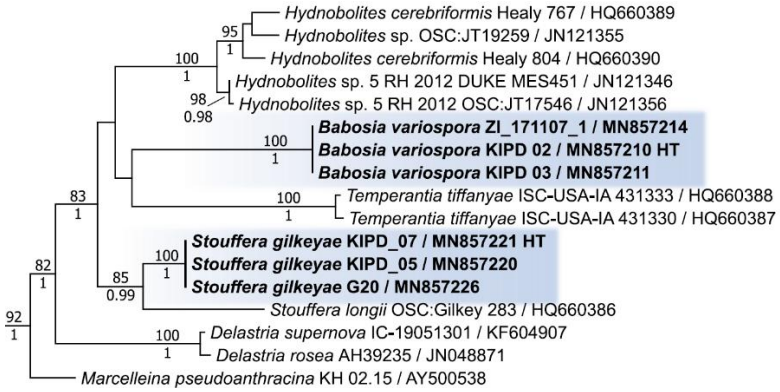

Fig. 4 Part of the ML phylogenetic tree of 28S sequences of the two novel truffle species and representative taxa of Pezizaceae (Knapp et al. 2020).

American truffle *Stouffera longii* and share with it similar spore ornamentation and habitat features. However, our material differs from *S. longii* by geographic origin, the quick and strong coloration of the ascomata to dark gray at cut surface or bruised area, varying spore number in

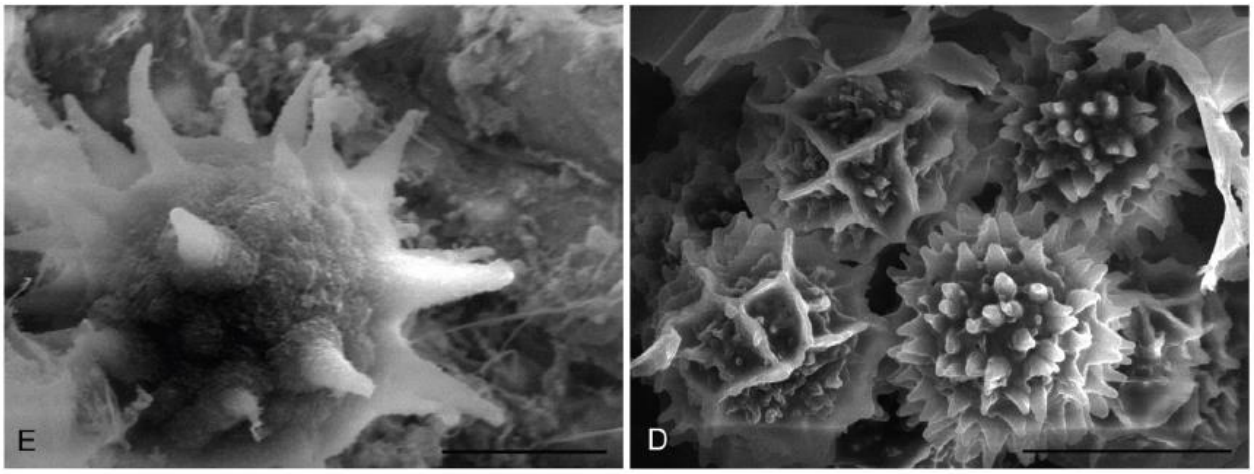

Fig. 5 Micromorphology of *Babosia variospora*. E. Spiny ascospore in the gleba, D. Scanning electron micrographs of diverse spores in asci of *B. variospora*. (Knapp et al. 2020).

asci, and smaller spore size; thus, we describe it as a new species, ***Stouffera gilkeyae*** (in honor of the outstanding mycologist and botanist Helen M. Gilkey (1886–1972)). The other specimens formed a distinct lineage, for which we propose a new genus ***Babosia*** (in honor and memory of the outstanding Hungarian mycologist Margit Babos (1931–2009)) with a new truffle species ***B. variospora*** (in reference to the variability of spore ornamentation varying even within one ascus, **Fig 5**). Taxonomic description of at least two other novel DSE species belonging to the Lentitheciaceae and Didymosphaeriaceae families and several *Tulostoma* species of Hungarian grasslands are in preparation.

Novel taxa from Mongolia, Kazakhstan, and the United States

From Mongolia, Kazakhstan and also from Hungary several isolates found to represent novel lineages within the genus *Laburnicola* (**Knapp et al. 2022a** *in prep*). These represent three different species within the genus, and one of these isolates grouped together with isolates obtained from cysts and eggs of the nematode *Heterodera filipjevi* (**Fig. 6**). This is not the first time that novel root endophytes are found within nematodes (see Ashrafi & Knapp et al. 2018). The taxonomic works and formal description of the three species are in preparation.

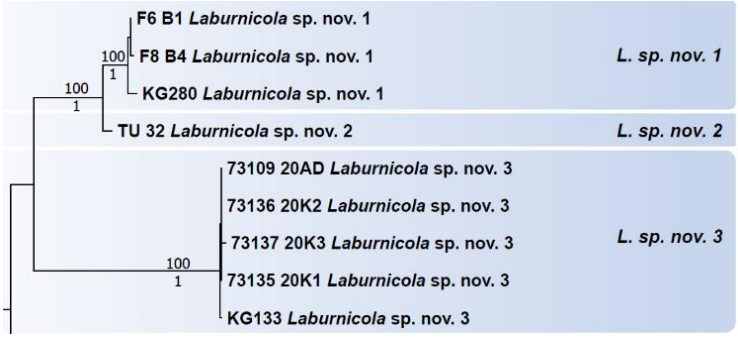

Fig. 6 Part of the ML phylogenetic tree of ITS, LSU, SSU, and TEF sequences of the three novel *Laburnicola* species and representative taxa of the genus (Knapp et al. 2022 *in prep*).

From different sites in Kazakhstan, several clades including novel lineages of the widespread genus *Fusarium* were found in the roots of different grass species. Four new species (***F. steppicola*** nom. prov., ***F. campestre*** nom. prov., ***F. rhizicola*** nom. prov., and ***F. kazakhstanicum*** nom. prov.) are being described (see the shaded **texbox**) among which *Fusarium steppicola* (nom. prov) represents a novel monotypic lineage/species complex (**Akhmetova et al. 2022** *before submission*). The epithets refer to their original ecosystem, country of origin or to their root-

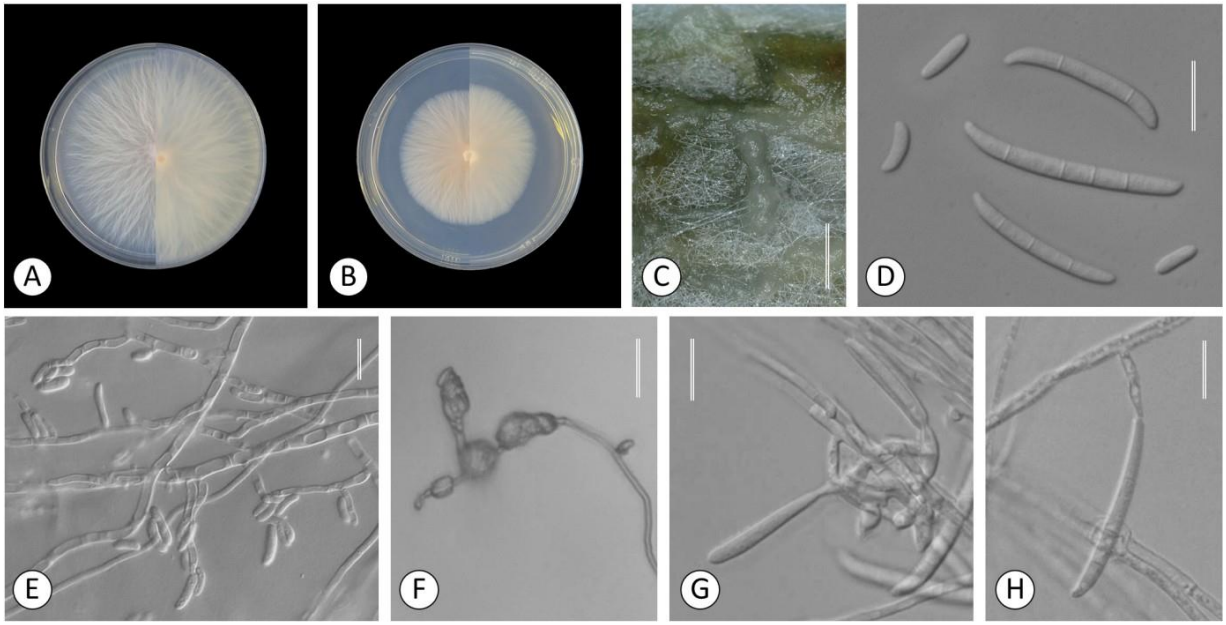

Fig. 7 *Fusarium rhizicola* A-B. Colony morphology after 8 d growth on PDA using a 12/12 h photoperiod. Colony surface is shown on left half of each plate and colony undersurface on right half. A. KG327. B. KG483. C. Sporodochia of KG327 formed on carnation leaf. D-H. KG483. D. O- and multi-septate aerial conidia. E-F. Microconidia in false heads on monophialides. G-H. Aerial conidia developed on monophialides. Bars: C = 1 mm; D-H =20 µm. (Akhmetova et al.2022 *in prep, before submission*)

endophytic nature. Besides the multilocus analyses of seven DNA loci, comprehensive microscopic works were carried out to find correct and robust micromorphological markers for species delimitation that is crucial in case of this complex and widespread genus (**Fig. 7**)

From roots of different grass species of Kazakhstan, a novel root colonizing dark septate endophyte species has been described that belonged to the *Murispora* genus (Amniculicolaceae family) (**Crous et al. 2021**) (**Fig. 8**). This species, ***Murispora kazakhstanica*** (the epithet refers to the to the origin country of the species, Kazakhstan).

Fig. 8 Part of the ML phylogenetic tree of ITS, LSU, SSU, and TEF sequences of *Murispora kazakhstanica* and representative *Murispora* species, and the colony of the type culture (Crous et al. 2021).

From various grasslands of the United States, several *Darksidea* isolates have been gained representing a novel lineage within the genus (**Romero-Jiménez et al. 2022**). The new species, ***Darksidea phi*** was named referring to the letter of the Greek alphabet that represents the golden ratio following the line of naming *Darksidea* species (see Knapp et al. 2015). *D. phi* represented a sister clade with *D. beta* and *D. gamma*, and the isolates showed variable colony morphology that is characteristic to the genus (**Fig. 9**). Formal description of at least six more *Darksidea* species is in preparation (**Knapp et al. 2022b** *in prep*).

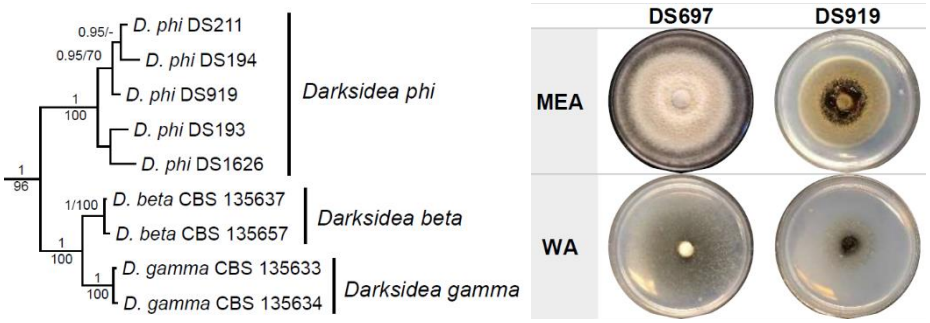

Fig. 9 Part of the ML phylogenetic tree of ITS, LSU, SSU, TEF, ACT, TUB, CAL sequences of *Darksidea phi* and representative *Darksidea* species, and the colony of two isolates culture (Romero-Jiménez et al.

Secondary metabolites of DSE fungi

In this project, more than 600 cultures of DSE fungi have been chosen for metabolite profiling including the dominant genera such as *Darksidea*, *Flavomyces*, *Periconia* originating from different grasslands. Secondary metabolites of DSE fungi belonging to the order Pleosporales have been studied during our investigations. We have information about compounds of several pleosporalean fungi (e.g. Zhang et al. 2015; Kellogg and Raja 2017; Maciá-Vicente et al. 2018) however, metabolite production of numerous pleosporalean fungi is unknown.

When the species *Flavomyces fulophazii* was formally described, the "flavo" referred to the remarkable yellow pigments of this DSE, secreted into the culture media (Knapp et al., 2015; **Fig. 10**). Later we isolated further strains from Hungarian and Mongolian grasslands, producing also yellow pigments (Knapp et al., 2015, 2019). During our studies, different isolates of *F. fulophazii* were analyzed (**Berek-Nagy et al. 2021**). The culture extracts contained a main compound vermelhotin (**Fig. 10**). To date, few fungal isolates have been reported to produce vermelhotin. This tetramic acid compound of *F. fulophazii* is important in high yield vermelhotin production, because it has antiproliferative activity against cancer cell lines. Altogether 11 previously undescribed compounds, including four tetramic acids (dihydroxyvermelhotin, hydroxyvermelhotin, methoxyvermelhotin, oxovermelhotin), and 7 chlorinated azaphilones (flavochlorines A–G), together with the known vermelhotin, were identified (**Fig. 11**).

Fig. 10 Colony of *Flavomyces fulophazii*

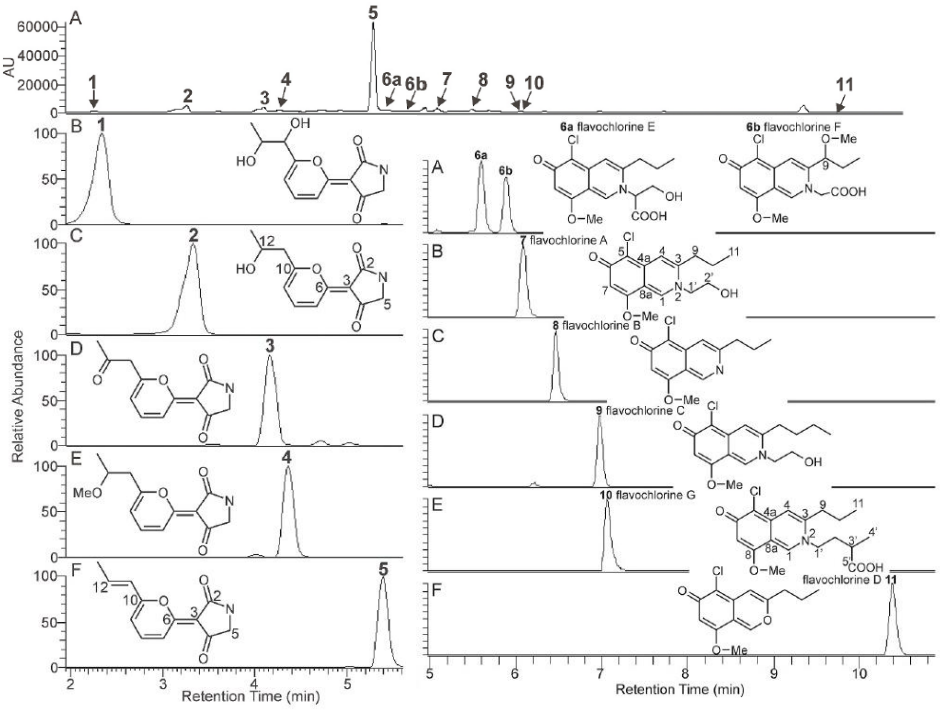

Fig. 11 A HPLC separation of the extract prepared from *Flavomyces fulophazii* culture sample HF-3A [full chromatogram A was recorded using UV detection (λ = 280 nm), and trace chromatograms (on the left, B–F) were obtained by MS detection of tetramic acids 1 (dihydroxy-vermelhotin), 2 (hydroxy-vermelhotin), 3 (oxo-vermelhotin), 4 (methoxy-vermelhotin) and 5 (vermelhotin), respectively, and of azaphilones (on the right, A–F) 6a (flavochlorine E), 7 (flavochlorine A), 8 (flavochlorine B), 9 (flavochlorine C), 10 (flavochlorine G) and 11 (flavochlorine D) (Berek-Nagy et al. 2021)

In case of the worldwide common and dominant DSE genus, *Darksidea*, 107 isolates from different grasslands of several countries of three continents such as United States, Spain, Germany, Hungary have been studied (**Knapp et al. 2022b** *in prep*). *Darksidea* represents a genetically diverse genus, and our recent analyses showed that based on metabolite production some of the lineages can be differentiated. Several main metabolites have been found such as petasol, isopetasol, neopetasol, ascomycone B, 5-deoxybostrycoidin and phomopsidin, which are mainly phytotoxic eremophilane sesquiterpenes, polyketides, terpenoids (**Fig. 12**). Majority of isolates belonging to *D. alpha* can produce brown-orange-red pigments into the media, which colour is due to the dominant presence of petasol

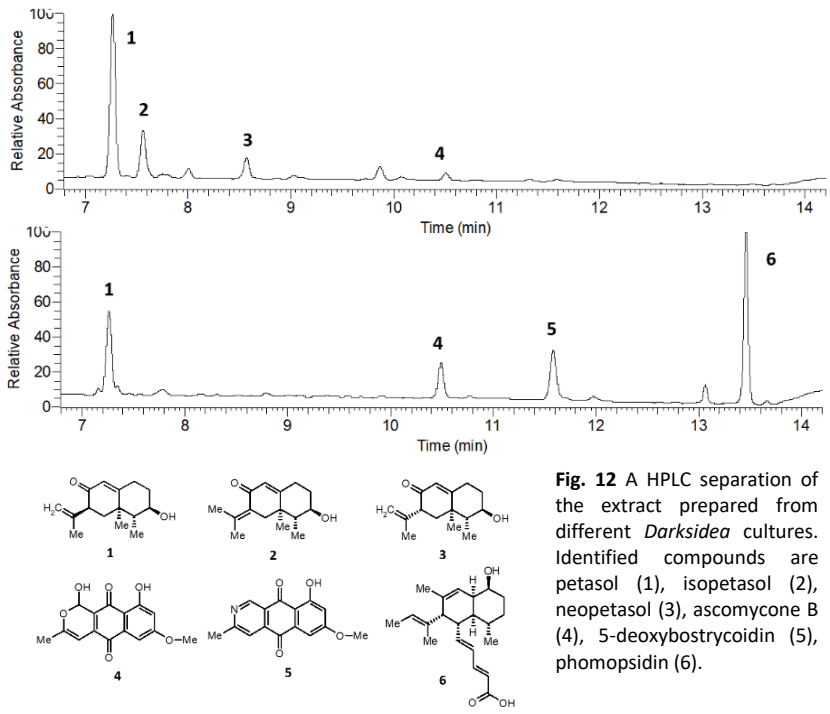

Fig. 12 A HPLC separation of the extract prepared from different *Darksidea* cultures. Identified compounds are petasol (1), isopetasol (2), neopetasol (3), ascomycone B (4), 5-deoxybostrycoidin (5), phomopsidin (6).

Fig. 13 Colony of *Darksidea alpha*

(**Berek-Nagy et al. 2022** *in prep*) (**Fig. 13**). Based on a four-loci phylogenetic analyses of the 107 isolates, at least 6 lineages represent potentially novel *Darksidea* species. Majority of these isolates shows characteristic metabolic profiles within the clades, however in some subgroups of the genus, totally different profiles and dominant compounds are present. Despite this problematic phenomenon, it seems that in case of majority of the *Darksidea* species and clades, the metabolic profiling can help in the taxonomic questions, hence it is truly important tool in case of this genetically diverse and taxonomically problematic genus.

Publications

Altogether **8 papers** have been published from the works reported above; 7 of them were published in scimago Q1 journals (3 out of them are D1) (summa **IF: 37.455**, google scholar **citations: 86**).

Knapp DG*, Imrefi I*, Boldpurev E, Csíkos S, Berek-Nagy PJ, Akhmetova G, Otgonsuren B, Kovács GM. 2019. Root colonizing endophytic fungi of the dominant grass *Stipa krylovii* from a Mongolian steppe grassland. *Frontiers in Microbiology* 10:2565. **equally contributed* [Q1, IF: 4,235]

Crous PW et al. (…, Imrefi I, Knapp DG, Kovács GM, …) *[multi-authored]* 2019. Fungal Planet Description Sheets 868–950. *Persoonia - molecular phylogeny and evolution of fungi* 42: 291–473. [D1, IF: 8,227]

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