Novel endophytic nematode antagonistic fungi – potential for nematode biocontrol

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Overview

Cereal cyst nematodes (CCNs) can lead to significant yield reductions of grains. The use of nematicidal chemicals is banned in many countries due to their generally high non-target toxicity. Antagonistic microorganisms controlling nematodes are an important alternative.

Microscopic observations of cyst samples of the CCN *Heterodera filipjevi* obtained from wheat fields in Turkey regularly revealed nematode cysts containing fungal colonised eggs (Fig. 1). The aim of this study was to:

- isolate and classify fungi isolated from nematode eggs, and fulfil Koch's postulates,
- study the nematode-fungus interaction microscopically,
- identify secondary metabolites produced by these fungi.

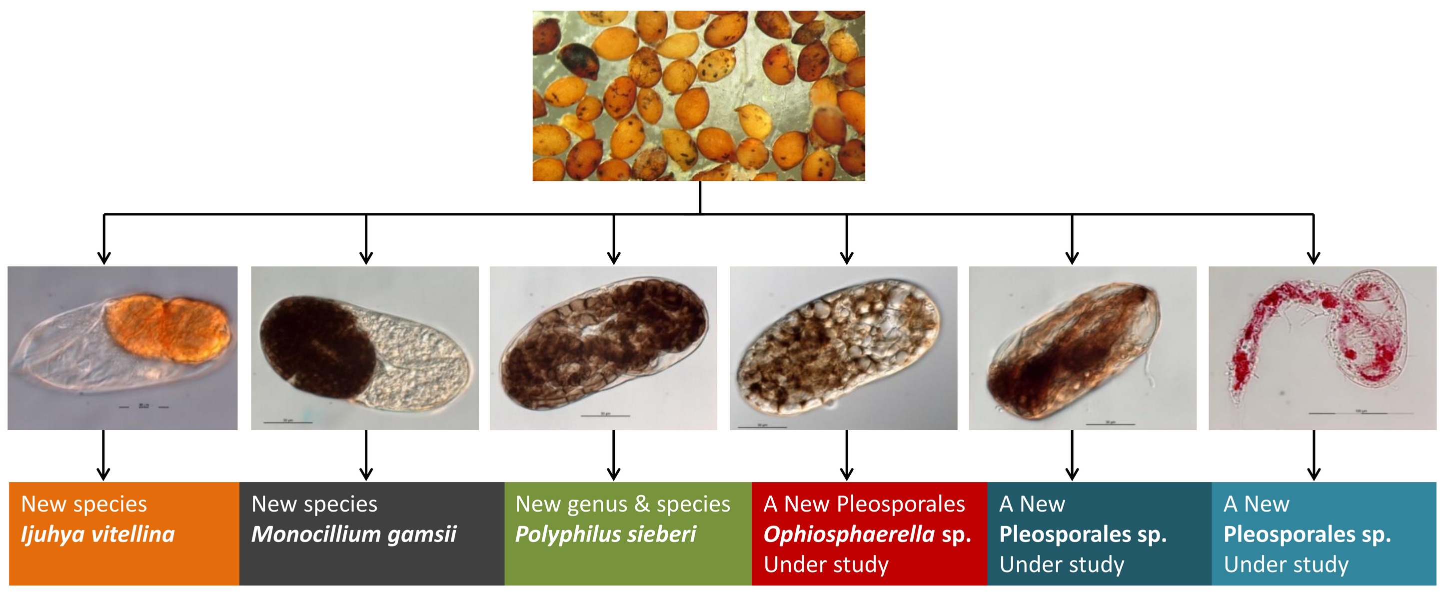

Fig. 1. Symptomatic eggs of *H. filipjevi* colonised with different fungal species. Several novel fungal species producing new natural compounds were found during this study.

Methodology

A single-egg based technique was developed to isolate fungi from symptomatic nematode eggs (Fig. 2A):

- Collection of diseased eggs from each symptomatic cyst
- Individually plating of single eggs on agar plates
- Fungal identification and taxonomy
- *In vitro* examination of isolated fungi against nematode eggs

Metabolite profiling of the discovered fungi (Fig. 2B):

- Small and medium-scale fermentation of fungal cultures
- Application of EtOAc and HPLC-based techniques for extraction, purification and structural elucidation of secondary metabolites
- Nematode bioassays and cytotoxicity of obtained compounds

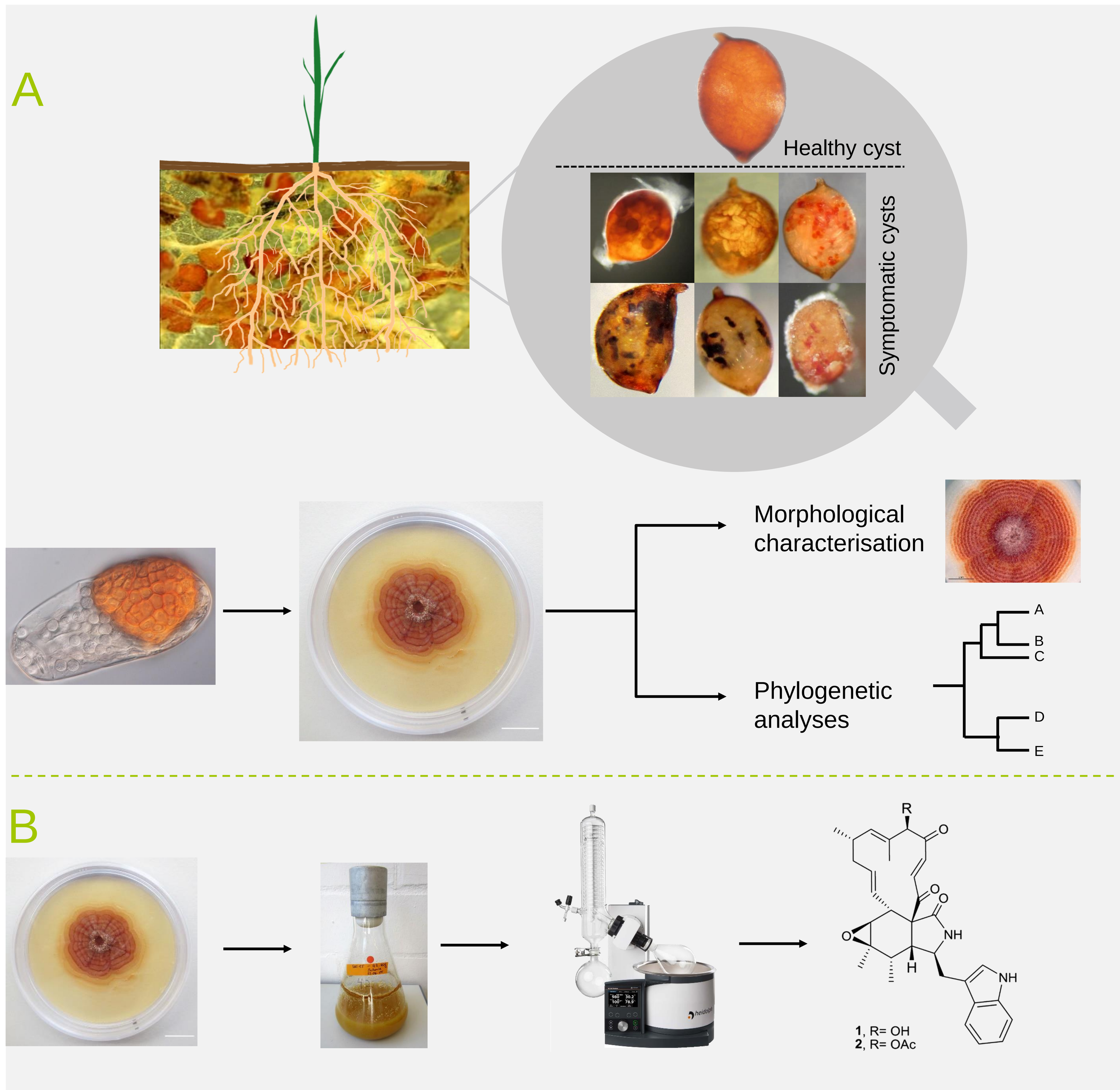

Fig. 2. A general overview of the methods applied in this study. **A)** methodes applied in cultural studies. **B)** methods applied for secondary metabolite profiling

Results

The approach applied in this study resulted in finding of eight new fungal species, all isolated from symptomatic nematode eggs.The newly described *Ijuhya vitellina* and *Monocillium gamsii* belong to the Bionectriaceae and Niessliaceae, respectively. The new fungal genus *Polyphilus* was proposed to accommodate the new species *P. sieberi* representing a dark septate endophyte (DSE). Two more species were preliminarily characterized as DSEs belonging to the Pleosporales. These are the first DSEs found to parasitize nematode eggs. All newly-found species could be successfully re-isolated from artificially infected nematodes and Koch's postulates were thus fulfilled (Fig. 3A-C).

Fig. 3A. *Ijuhya vitellina.* **A, B)** Cysts and eggs of *H. filipjevi* naturally infested with *I. vitellina*. Infested eggs accommodate one, occasionally two globose to subglobose microsclerotia. **C)** Transformation of hyphae into microsclerotium. **D)** *In vitro* colonisation of a nematode egg by hyphae developed into microsclerotium **[1]**.

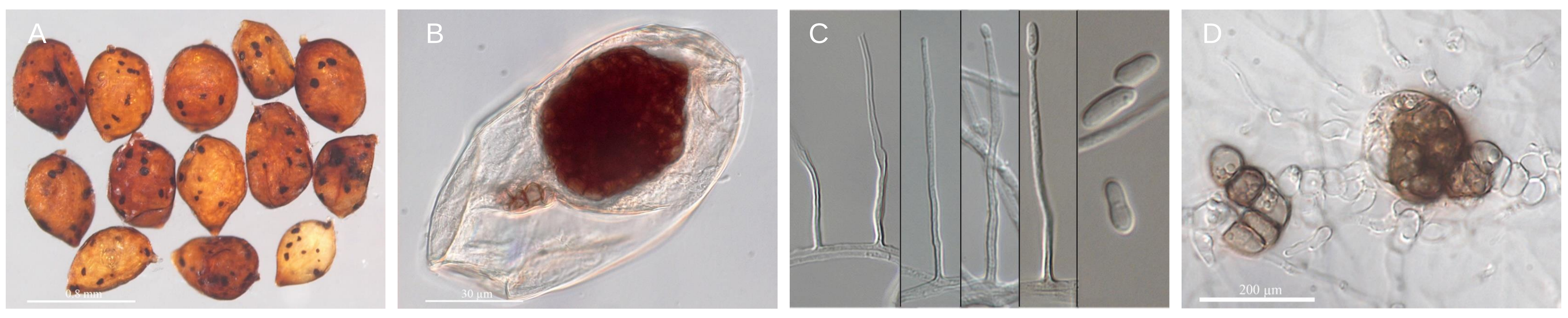

Fig. 3B. *Monocillium gamsii.* **A, B)** Naturally infested cysts and eggs of *H. filipjevi* naturally infested with *M. gamsii*. Infested eggs contain one or two microsclerotia. **C)** phialides and conidia. **D)** Formation of microsclerotia by development of dictyochlamydospore structures **[2]**.

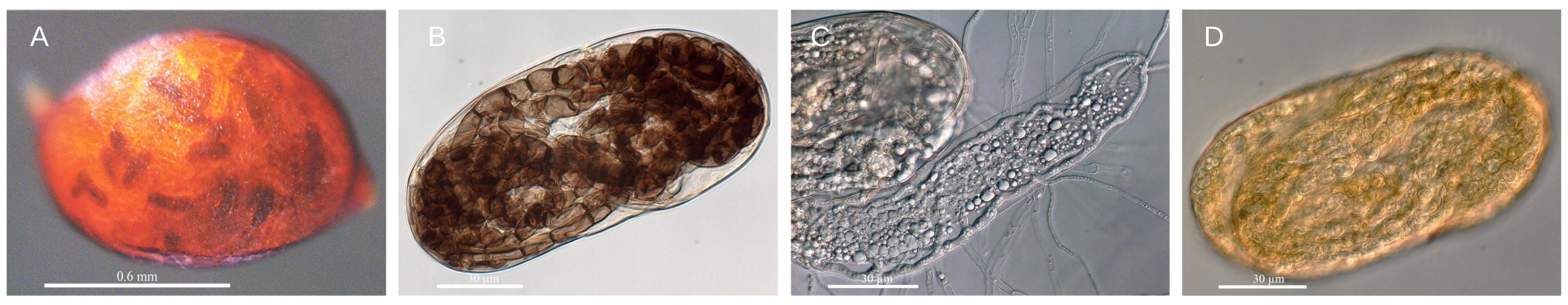

Fig. 3C. *Polyphilus sieberi.* **A, B)** Cysts and eggs of *H. filipjevi* naturally infested with *P. sieberi*. Infested eggs were colonised with moniliform and strongly melanised hyphae. **C, D)** fungal colonisation of a nematode juvenile and nematode egg *in vitro* **[3]**.

Four novel compounds with nematicidal activity were isolated from a hitherto undescribed pleosporalean strain (DSM106825) belonging to the family of Phaeosphaeriaceae (Fig. 4A-E) that parasitises eggs of *H. filipjevi* **[4]**.

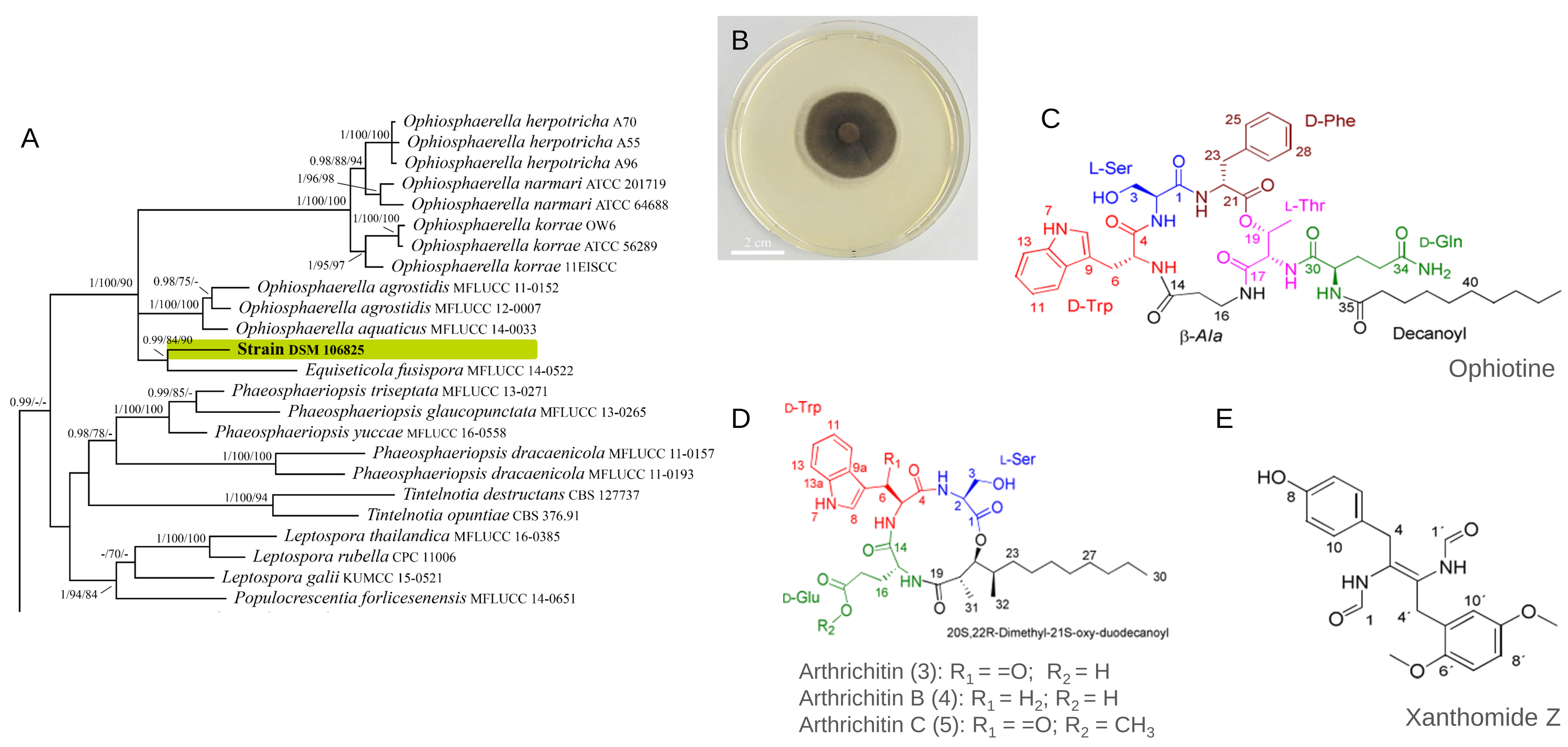

Fig. 4. A new Phaeosphaeriaceous fungus isolated from eggs of *H. filipjevi*, and the source of the here presented new comppounds. **A)** BI of the strain DSM106825 based on mutli-gene (ITS-LSU-TEF1) phylogenetic analyses. **B)** A pure culture of the fungus. **C)** The new compounds isolated from the fungus.

References

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