

# Uncovering the biodiversity of root endophytic fungi in Europe

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*Extensive sampling of root endophytic fungi across Europe led to the discovery of two novel fungal genera, Endoradiciella and Extremopsis, and the description of six species new to science.*

Endophytic fungi are an ecological group of endosymbionts capable of colonizing the internal tissues of plants without causing any visible symptoms. They can be present in any part of the plant host such as roots, leaves and stems during their whole life cycles or for certain periods only. Plants acquire their endophytes through horizontal transmission via asexual propagules produced by the fungus and spread out across the plant population or by vertical transmission to the plant offspring via the host seeds. They contribute to the plant fitness and performance by increasing their tolerance and adaptation to several biotic and abiotic stressors such as plant pathogens, drought, high soil salinity or extreme temperatures, while producing bioactive compounds and metabolites with many potential applications [1]. Phylogenetically, fungal endophytes are a highly diverse, polyphyletic assemblage of taxa mainly belonging to the phylum Ascomycota, and this peculiar lifestyle is known to have evolved multiple times within distantly related lineages of the kingdom Fungi.

During extensive sampling events for root endophytic fungi across Europe, a large number of isolates were obtained from surface-sterilized root pieces of different plant hosts. They were grouped into operational taxonomic units (OTUs) after sequencing their nuclear ribosomal DNA internal transcribed spacer region (ITS) followed by comparison with reference databases. Poorly represented OTUs usually consisting of one or a few isolates or those taxonomically assigned to rare species were subjected to further blast searches in GenBank to confirm their initial identification. If their percent identity was low or below a certain threshold, they were selected for further investigation. Isolates were plated in different agar media to study their morphological and cultural features and to induce sporulation. None of them, however, sporulated in any of the media used and they were considered sterile. Besides their ITS, other gene markers used nowadays in fungal identification were sequenced for novelty confirmation and comparison with those available in GenBank. Strains were deposited at the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS) in The Netherlands and novel DNA sequences were deposited in GenBank.

As a result, two novel endophytic genera, *Endoradi-*

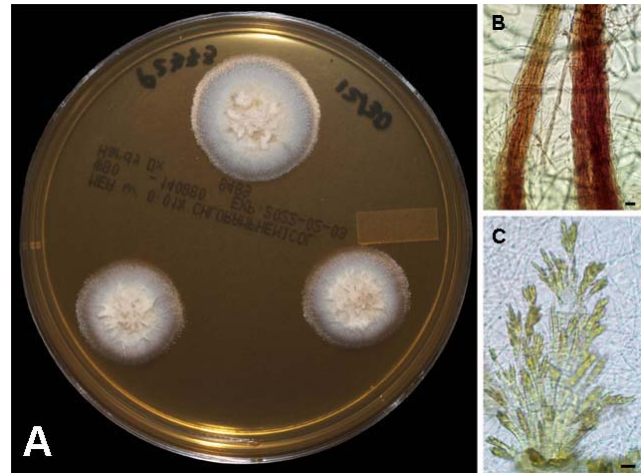


Figure 1: *Endoradiciella communis*. Colonies on MEA after 2 weeks at 25°C in surface view (A). Hyphal cords (B). Bundles of needle-shaped crystals (C). Scale bars = 10 µm. Source [2]

*ciella* and *Extremopsis*, and six species, *Cyphellophora endoradicis*, *Endoradiciella communis*, *Extremopsis radiculicola*, *Halocryptovalsa endophytica*, *Hymenoscyphus radialis* and *Tetraploa endophytica*, were described new to science from root samples obtained in Spain, Germany, France and Croatia.

A first example of a root endophytic novelty discovered in Europe is *Endoradiciella*. The fungus is represented by its type species, *E. communis*, which was isolated from roots of *Microthlaspi perfoliatum* and *M. erraticum* (Brassicaceae) in France and Germany, respectively [2]. Mycelium is composed of hyphae often aggregated in tightly packed, reddish brown hyphal cords and produces greenish yellow bundles of needle-shaped or thin rectangular-bladed crystals on malt extract agar (MEA) after two months of incubation (Figure 1). Curiously, multigene phylogenetic analyses revealed that the three recovered strains of *E. communis* formed a well-supported lineage with fifteen other isolates available in GenBank and annotated as *Helotia* sp. or *Helotiales* sp. They all share a similar root endophytic lifestyle and their ITS sequences are identical or almost identical to those of *Endoradiciella*, suggesting they might be conspecific. Most of them were isolated from several locations all over Europe including Bosnia & Herzegovina, Croatia, France or the

UK and the reason of the specific epithet of the fungus ‘*communis*’ or widespread. They clustered within the family Porodiplodiaceae belonging to the order Helotiales of the class Leotiomycetes.

Another example of a novel root endophyte is *Extremopsis*, based on *Ex. radiculicola* as the type species, and isolated from surface-sterilized, asymptomatic roots of an *Arabidopsis thaliana* (Brassicaceae) plant inoculated with soil originated from wet heathlands in Spain [3]. The three recovered strains of the fungus did not sporulate in any of the different culture media used including MEA, Potato Dextrose Agar, Modified Cellulose Agar or Water Agar supplemented with wooden toothpicks, but they produced abundant chlamydospores in all media (Figure 2). Phylogenetically, they grouped together in a strongly supported monophyletic lineage within the family Extremaceae belonging to the order Mycosphaerellales of the class Dothideomycetes. Similar to *Endoradiciella*, two other strains represented in GenBank by their ITS sequences and obtained from soil samples in Austria and Germany also clustered within the *Extremopsis* clade in multigene phylogenetic analyses. This suggests that they are conspecific with *Ex. radiculicola* and the known distribution of the genus is therefore expanded from southern Spain to other localities across Europe.

A last example is the novel species *Halocryptovalsa endophytica*, isolated from surface-sterilized, asymptomatic roots of a wild plant of *Salicornia patula* (Amaranthaceae) in Spain [4]. This is a halophyte host which is well adapted to conditions of high salinity and common in saline ecosystems of the Mediterranean region. The two previously described species of *Halocryptovalsa* were also found associated with this type of host in marine environments but *H. endophytica* is the first member of the genus with an endophytic lifestyle. The two recovered strains of the fungus grouped together in a strongly supported monophyletic clade with a third isolate identified as ‘*Libertella* sp. F6’ and represented in GenBank by an unpublished ITS sequence. This other isolate was originally obtained in Iran from another halophyte, *Juncus acutus*, and therefore it could be considered conspecific with *H. endophytica* based on molecular and ecological evidence. Multigene phylogenetic analyses placed the fungus within the family Diatrypaceae belonging to the order Xylariales of the

class Sordariomycetes.

The examples outlined above show how fungal endophytes, despite being extensively studied nowadays, still represent a largely untapped reservoir of biodiversity awaiting to be discovered and it is our goal to continue describing them in the near future.

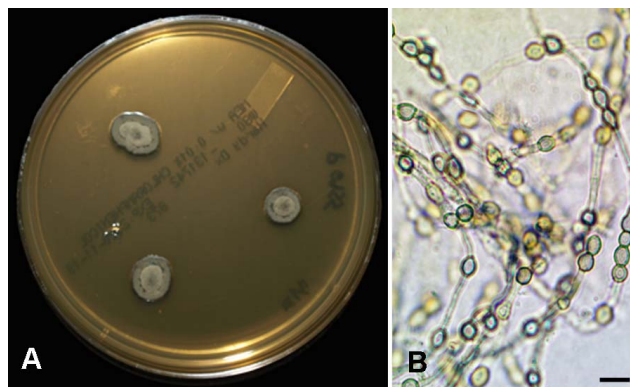


Figure 2: *Extremopsis radiculicola*. Colonies on MEA after 3 weeks at 25°C in surface view (A). Chlamydospores (B). Scale bar = 10 µm. Source [3]

## References

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