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# Fungal endophytes of stems and roots of *Juncus trifidus* subsp. *trifidus* from Poland

## Introduction

Fungal and bacterial pathogens, mycorrhizal fungi, nematophagous fungi (Jansson, Lopez-Llorca, 2001), fungal root endophytes such as *Fusarium* spp. (Damicone, Manning, 1982; Blok, Bollen, 1995), *Oidiodendron maius* G.L. Barron (Rice, Currah, 2006) and dark septate endophyte (DSE) fungi (Jumpponen, Trappe, 1998) are a few of the microorganisms that colonise roots. One of the plants colonised by endophytes is the highland rush, *Juncus trifidus* L. subsp. *trifidus*. The taxonomical position and geographical distribution of the highland rush was shortly reported by Chlebicki (2009). He also noted some fungal endophytes from the stems and leaves of the plant. Suková and Chlebicki (2004) noted DSE fungi (possibly *Phialocephala* sp.) in the roots of *J. trifidus* from the Hruby Jesenik Mts. in the Eastern Sudetes. Chlebicki (2009) and Jacewski et al. (2019) cited available information about the occurrence of fungal species noted so far on the stems, leaves and inflorescences of this host plant.

DSE (dark septate endophytes) symbionts are root-inhabiting fungi that can act as parasites, mutualists or be neutral (Jumpponen, 2001). Intercellular and intracellular interfaces indicate mutualistic or parasitic symbioses (Smith, Smith, 1990). It is difficult to clearly define DSE, especially since many unknown fungi are included within this group (Jumpponen, 2001). Dark-pigmented fungi and DSE possess Dihydroxynaphthalene melanin (DHN) in their hyphae brown pigment (Fig. 1), which plays an important role in plant-fungus interactions (Butler, Day, 1998). Some DSE fungi belong to so-called "black yeasts." One of them (*Aureobasidium pullulans* (de Bary) G. Arnaud ex Cif., Ribaldi & Corte) was found in the stems of highland rush (Chlebicki, 2009). Some black yeasts were isolated as plant endophytes, i.e., *Exophiala salmonis* Carmich. and *Cyphellophora vermispora* Walz & de Hoog in grass roots (Feng et al., 2012). *Aureobasidium pullulans* was also noted in the bark and xylem of *Eucalyptus* (Bettucci et al., 1999) and leaf fern *Pteridium aquilinum* (L.) Kuhn (Fisher, 1996), the twigs, leaves, buds and roots of *Acer pseudoplatanus* L. and other trees (Pugh, Buckley, 1971), as well as in the leaves of *Juncus trifidus* (Chlebicki, 2009). DSE such as *Phialophora mustea* Neerg. and *Exophiala salmonis* were isolated from the roots of *Astragalus adsurgens* Pall. (Ban et al., 2012). *Exophiala mansonii* (Castell.) de Hoog, which causes meningitis in humans, was occasionally noted as an endophyte of *Dryas octopetala* L. (Robinson et al., 1998).

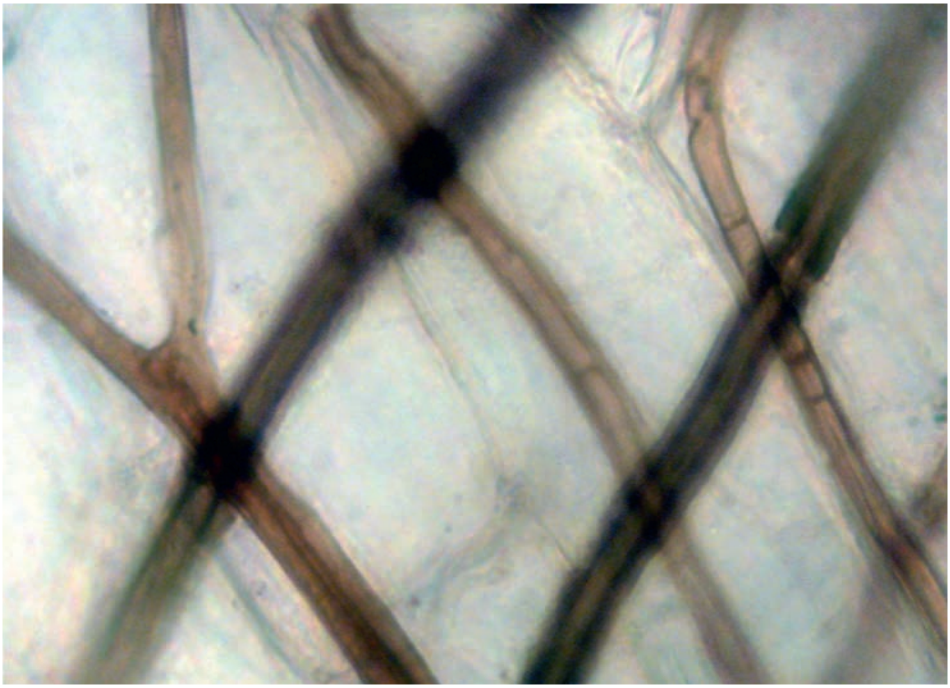

**Fig. 1.** DSE (dark septate endophytes) in roots of *Juncus trifidus* L. from Hruby Jesenik Mts. in Czech Republic (Photo. A. Chlebicki)

The diversity and spatial distribution of endophytic fungi in roots, as well as the interaction between them, are poorly investigated. Some of them can be transferred from the roots to the leaves. *Stagonospora nodorum* (Berk.) E. Castell. & Germano initially colonises roots, but later is transferred to the stems and leaves (Sieber et al., 1988). Similarly, the root endophytes of *Phragmites australis* (Cav.) Trin. ex Steud. are also transferred to the stems (Ernst et al., 2003). Aaltonen and Barrow (2003) noted the occurrence of DSE in the leaves of *Bouteloua eriopida* (Torr.) Torr. growing in dry conditions. Schulz and Boyle (2006) also reported the expansion of root endophytes from the roots to the stems in some cases. Menkis et al. (2004) noted the occurrence of DSE in roots, stems and debris, including in small pieces of wood. Hyphae of *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken were detected neither in the roots, stems or leaves (Waller et al., 2005). *Pezicula cinnamomea* (DC.) Sacc. and its anamorph *Cryptosporiopsis grisea* (Pers.) Petr., growing as endophyte in wood and bark, can also spread into the roots of dying trees (Kowalski, 1983). It is difficult to confirm that the *Phialocephala dimorphosphora* W.B. Kendr. strains isolated from living and declining trees (Kowalski, Kehr 1992; Holdenrieder, Sieber, 1992; Kehr, Wulf, 1993) are the same. These tissues could be colonised by different strains from the air or soil.

How it is possible for fungi to move from the roots to the stems? The radial and axial water that flows through the network of xylem vessels can probably enable the growth of fungal hyphae inside the hydraulic architecture of root and stem systems and possibly in intercellular cavities. However, rot hypodermis can protect the inner root tissues from fungi colonisation (Galmany et al., 1992). The stem/trunk base (the root-stem transition zone) is a very specific place where geotropism and organ function change. Is it a barrier for fungi? It is known that some endophytes, i.e., *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin, can reduce the arbuscular mycorrhizas after the death of the host, *Schedonorus phoenix* (Scop.) Holub in other plants (Antunes et al., 2008). We examined both roots and stems and tried to find the simultaneous occurrence of these fungi in organs of the same individuals of the host plant.

## Methods

Twenty-four samples of highland rushes *Juncus trifidus* subsp. *trifidus* were collected in the north-eastern slope of Mt. Małołączniak in the Tatra Mts, (49º 55'N, 19º 14'E) in September 2009, and 20 samples were collected in Mt. Diablak in the Babia Góra Range. A small part of tufts of the collected plants with roots was put in paper bags and then stored in the refrigerator at -20ºC for six months. Fresh plant material, was used to isolate endophytes. In the same places, airborne fungi were collected with an Eco Mass 100 sampler on 90 mm Petri dishes that contained pure agar and potato dextrose agar (PDA) and dichloran rose-bengal chloramphenicol DRBC media. Only viable and cultivable fungal spores were quantified. After sampling, the plates were placed in an incubator at 10°C in the dark. The colonies were then counted and identified.

### Isolation procedure

Three green culms and separated roots of each sample were used. Eight roots of each sample were put on a single plate and 360 pieces were examined. Culms were cut into two segments. Six to ten roots from each sample were used. All culm segments and roots were dipped for 1 minute (min) in 96% ethanol, then its surface was sterilised for 3 min in 3% chlorox (NaOCl), rinsed in ethanol 70% for 1 min and transferred to 90 mm Petri plates with a Ferency medium and then incubated in the incubator at 10°C. The Ferency medium consisted of 5 g of malt extract, 5 g of glucose, 1.25 g of peptone, 1.25 g of yeast extract,7.5 g of agar, 500 ml of $H_2O$ and 5 ml of oxytetracycline (100 mg in 50 ml acetone). Obtained strains were then transferred on PDA and malt extract agar MEA media and incubated at 10°C or in the case of the species of the genus *Penicillium* on MEA, incubated at room temperature (25°C). The colour of the colonies, their structures and size were investigated. For observation of the fungal morphological structures, Nikon SMZ 1500 and Nikon Eclipse 80i light microscopes were used. Photographs were taken with a Digital Sight DS-Ri1 Nikon camera.

### Molecular analysis

The mycelium was cultivated on MEA plates for two weeks at room temperature. The mycelium was scraped off the medium into Eppendorf-tubes with a CTAB buffer and glass bulbs. The probes were disrupted and later incubated for 10 min at 65°C. After the addition of a chloroform/phenol solution was centrifuged at 14000 rpm. The supernatant was treated with 2 vol. of ice-cold ethanol (70%). The samples were stored overnight at -20°C for DNA precipitation, then centrifuged for 5 min at 14000 rpm. The resulting pellet was washed in ethanol, then dried and dissolved in $ddH_2O$. The PCR reaction was performed in 25 µl volume containing 1x buffer, 200 µM dNTP, 0.25 µM ITS-1F and ITS-4 primers, 0.05 U Taq polymerase (Sigma Aldrich). A PCR reaction included an initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 seconds (s), annealing at 50°C for 30 s, extension at 72°C for 60 s, and a final extension at 72°C for 10 min. Sequences were aligned and adjusted using ClustalX2.1. Standard Nucleotide Blast programme from Gene Bank was used for identification of strains.

## Results

### Identification of isolated strains

Fourteen taxa of fungi in the stems and roots of *Juncus trifidus* subsp. *trifidus* were found (Tab. 1).

**Tab. 1.** Distribution of fungal endophytes on plant organs of *Juncus trifidus* L. subsp. *trifidus*

| Fungus | Stem | Root | Locality |
|---|---|---|---|
| *Aureobasidium pullulans* var. *pullulans* | + | + | Mt. Babia Góra |
| *Ascochyta* sp. | | + | Mt. Babia Góra |
| *Paraconiothyrium* sp. | +* | +* | Mt. Babia Góra |
| *Botrytis cinerea* | + | | Tatra Mts |
| *Cladosporium herbarum* | + | | Mt. Babia Góra, Tatra Mts |

| | | | |
|---|---|---|---|
| *Clonostachys rosea* | | + | Tatra Mts |
| *Epicoccum nigrum* | + | | Mt. Babia Góra |
| *Oidiodendron griseum* | | + | Tatra Mts |
| *Penicillium miczynskii* | | + | Tatra Mts |
| *Hyaloscypha finlandica* | | + | Tatra Mts |
| *Phoma* sp. | + | | Tatra Mts |
| *Trichoderma viridescens* | | + | Mt. Babia Góra |
| *Alternaria chartarum* | + | + | Tatra Mts |
| *Umbelopsis autotrophica* | | + | Mt. Babia Góra |

*strains noted on stems and roots of single plant specimen

***Paraconiothyrium*** sp. (PbgEn-19) colonies, measuring 25 mm in diameter, after seven days at room temperature, covered all surface of the Petri plate after 30 days. The margin was slightly fimbriate and the surface was velvety (Fig. 2) and a pale to dark grey. The underside was a pale orange-yellow to dark grey, and the colonies were dark with a brown mass of conidia, conidia globose, covered in small warts: 4.6–5.3(6,2) × 3.3–4 µm (Fig. 3). This strain was in 100% similar to the *Paraconiothyrium* sp. isolated from the roots of *Poa flabellata* (Lam.) Hook from breeding colonies of albatrosses in Kidney Island in the Falkland Islands archipelago (Hargreaves et al. 2018), accession number MN28307.

Locality: Poland, the Western Carpathians, Babia Góra Massif, endophyte of the roots of *Juncus trifidus* from Mt. Diablak, collected (coll.) by A. Chlebicki, strain PBgEn-19 from the root and stem, PBgEn-18 from the root.

***Aureobasidium pullulans*** (de Barry) G. Arnaud var. ***pullulans***

Locality: Poland, the Western Carpathians, Babia Góra Massif, endophyte of the roots of *Juncus trifidus* from Mt. Diablak, coll. by A. Chlebicki, strain PBgEn-11.

Comments

> The ITS1-5ITS2 sequence of the strains was investigated in detail by Owczarek-Kościelniak, Chlebicki and Sterflinger (2016). The fungus was present in three roots (of the 360 investigated roots) from sample PBgEn-11 (Fig. 2) together with other fungi such as *Umbelopsis autotrophica* (E.H. Evans) W. Gams and non-sporulated mycelium with dark chlamydospores.

***Hyaloscypha finlandica*** (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová, root endophyte, strain PTEn-19, PTEn-11

Basionym: *Phialophora finlandica* C.J.K. Wang & H.E. Wilcox [as 'finlandia'], Mycologia 77: 953. 1985. Synonyms: *Cadophora finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew [as 'finlandia'C], Mycotaxon 87: 147. 2003.

The colonies were lobate, olive to dark brown (Fig. 2/7, 2/8), convex, with a velvety surface and a feathery margin (Fig. 4). Their reverse-side was black and the aerial hyphae

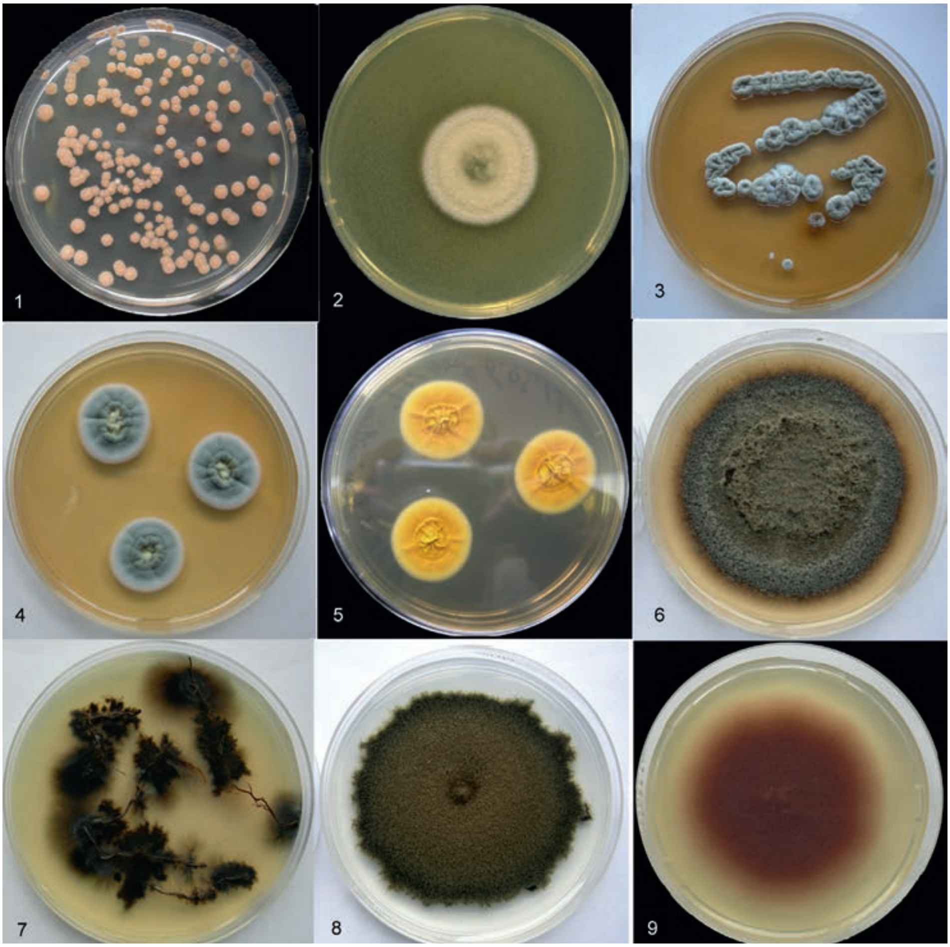

**Fig. 2.** Some root endophytes isolated from highland rush (*Juncus trifidus* L.). 1 – *Aureobasidium pullulans* var. *pullulans* (after 7 days); 2 – *Paraconiothyrium* sp. (after 7 days); 3 – *Oidiodendron griseum* Robak (after 1 month); 4 – *Penicillium miczynskii* K.W. Zaleski (after 10 days, MEA); 5 – *Penicillium miczynskii* (revers); 6 – *Alternaria chartarum* Preuss (after 3 months); 7 – root endophyte, *Hyaloscypha finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová (PTEn-11); 8 – root endophyte, *Hyaloscypha finlandica* (PTEn-11) after 3 months; 9 – *Umbelopsis autotrophica* (E.H. Evans) W. Gams (after 1 month) (Photo. A. Chlebicki)

were brown to olive-brown and often covered by wart-like, distinctly paler blisters. In the peripheral part of the colony, numerous clusters of darker, thick-walled cells (Fig. 4), which are similar to intracellular structures named 'microsclerotia' (Haselwandter, Read, 1980) or 'sclerotial bodies' (Wilcox, Wang, 1987), were formed. The strains were difficult to precisely determine because of the lack of conidia and conidiogenous cells. Some anamorphs of Leotiomycetes O.E. Erikss. & Winka have been suggested to be root endophytes but without possessing any clear teleomorph connections (Sutton, Hennebert, 1994). According to Day et al. (2011), *Mollisia* species with smaller

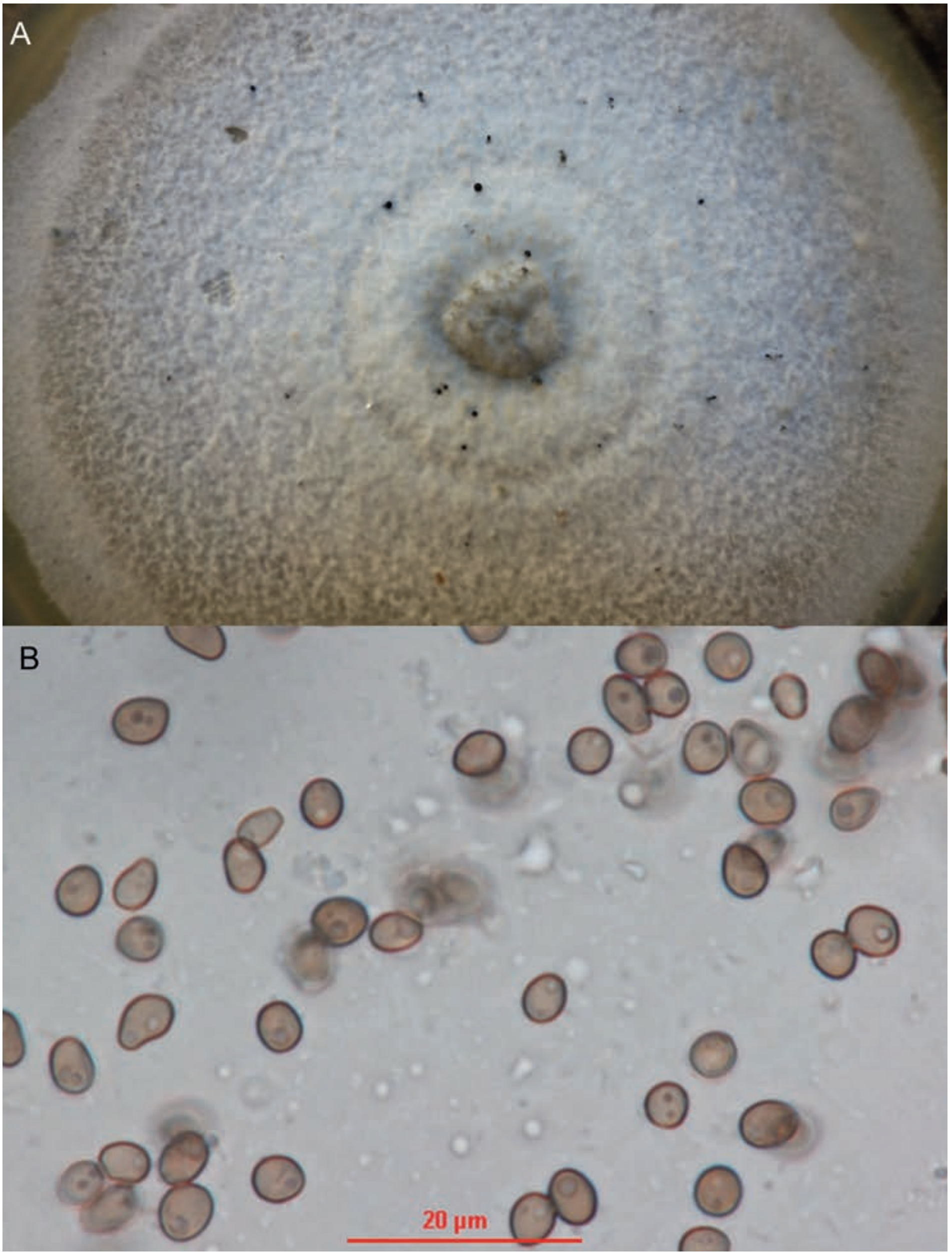

**Fig. 3.** *Pycnothyria* sp. – A, and conidia of *Paraconiothyrium* sp. – B (Photo. A. Chlebicki)

asci would be expected to have *Phialocephala* anamorphs. However, the next molecular investigation of PTEn-19 (Wilczek, n.a.) indicated a 99% similarity with the *Phialophora verrucosa* Medlar strain WRCF-AB6. Identification of the *Phialophora* species using sequence data (Ko Ko et al., 2011) is difficult. Some of them, such as *P. verrucosa*, are

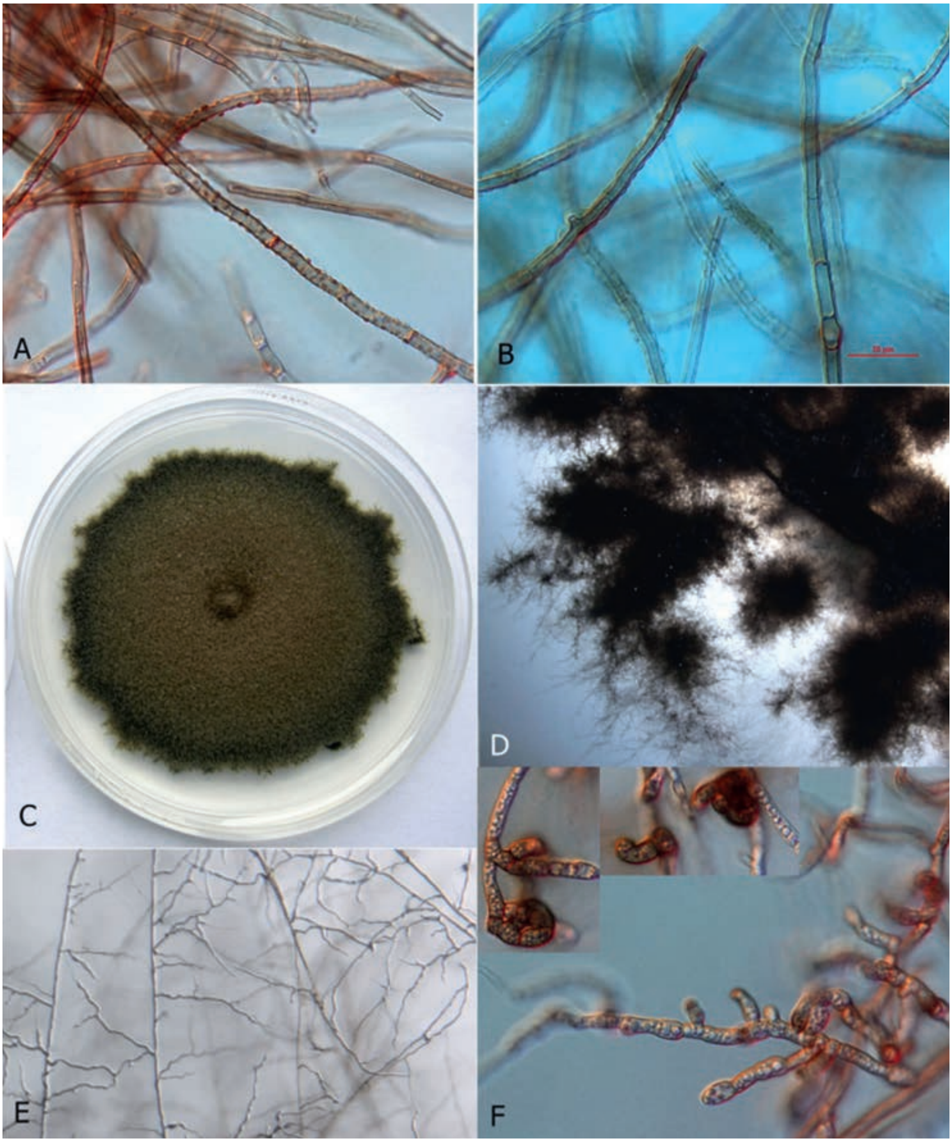

**Fig. 4.** Root endophyte *Hyaloscypha finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová strain PTEn-19; hyphae covered by wart-like blisters – A, B; 3-month-old colony on PDA – C; hyphae growing from a rush root on Petri plate – D, E; 'microsclerotia' – F (Photo. A. Chlebicki)

known to commonly occur as plant roots endophytes. This taxon has been recognised to be part of a complex group, *P. verrucosa*/*P. americana* (Nannf.) S. Hughes (Yan et al., 1995). De Hoog et al. (1999) described two new *Phialophora* species from the *Phialophora verrucosa* complex*: P. reptans* de Hoog *and P. sessilis* de Hoog. Untereiner et al. (2008) also separated *P. reptans*, *P. sessilis* and *P. europea* de Hoog, Mayser & Haase

from the *P. verrucosa* complex. Moreover, they separated *P. verrucosa* from *P. americana*. According to Su et al. (2010), most endophytic *Phialophora* strains have not yet been identified at a species level. My recent try of identification of this strain indicates 100% similarity with *Cadophora finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew sequence ID KJ817298. Recently Fehrer et al. (2019) synonymised *Phialophora finlandica* (*Cadophora finlandica*) with the genus *Hyaloscypha* and species *H. finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Reblova. The species is known as endophyte of *Picea*, *Pinus* (Fehrer et al. 2019) and *Vaccinium vitis-idaea* L. sequence ID KJ817298. Fungal mycelium of these strains PTEn-11 and PTEn-19 covered all parts of the roots. It is a very competitive strain.
Locality: The Tatra Mts., Mt. Małołączniak, in the roots of *Juncus trifidus*, (T-11, T-17b, T-18, T-19, T-24). coll. by A. Chlebicki, photograph: strain PTEn-19.

## Other fungi noted in roots

***Clonostachys rosea*** (Link) Schroers, Samuels, Seifert & W. Gams, Mycologia 91(2): 369, 1999.
Locality: Poland, the Tatra Mts., Mt. Małołączniak, in the roots of *Juncus trifidus*, September 2009, coll. by A. Chlebicki, strain PTEn-12.
***Epicoccum nigrum*** Link, in Mag. Gesell. naturf. Freunde. Berlin 7: 32, 1816.
Locality: Poland, the Western Carpathians, Babia Góra Massif, in the roots of *Juncus trifidus* from Mt. Diablak, coll. by A. Chlebicki.
***Oidiodendron griseum*** Robak, Saertryck ur Svensk. Skogsvardsforeningens Tidsk. 3, 4: 440, 1934.
Locality: Poland, the Tatra Mts., Mt. Małołączniak, in the roots of *Juncus trifidus*, September 2009, coll. by A. Chlebicki, strain PTEn-1.
***Penicillium miczyńskii*** K.M. Zaleski, Bulletin Acad. Polonaise Sci., Math. Et Nat., Ser. B: 482, 1927.
Locality: Poland, the Tatra Mts., Mt. Małołączniak, in the roots of *Juncus trifidus*, September 2009, coll. by A. Chlebicki, strain PTEn-12P.
***Phoma* sp.** Poland, the Tatra Mts, Mt. Małołączniak, in the roots of *J. trifidus*, September 2009, coll. by A. Chlebicki.
***Trichoderma viridescens*** (A.S. Horne & H.S. Williamson) Jaklitsch & Samuels, Stud. Mycol. 56:156. 2006.
Locality: Poland, the Western Carpathians, Babia Góra Massif, in the roots of *Juncus trifidus* from Mt. Diablak, coll. by A. Chlebicki, strain PBgEn-12.
***Alternaria chartarum*** Preuss, Botanische Zeitung 6: 412. 1848
Locality: Poland, the Tatra Mts., Mt. Małołączniak, in the roots of *Juncus trifidus*, September 2009, coll. by A. Chlebicki, strain PTEn-17.
***Umbelopsis autotrophica*** (E.H. Evans) W. Gams, Mycol. Res. 107(3): 349, 2003.

Syn. *Mortierella ramanniana* var. *autotrophica* E.H. Evans Tr. Br. Mycol. Soc. 56: 214, 1971.
Locality: Poland, the Western Carpathians, Babia Góra Massif, in the roots of *Juncus trifidus* from Mt. Diablak, coll. by A. Chlebicki, strain PBgEn-20.

### Distribution of endophytes inside plant organs

I noted 10 taxa of fungal endophytes in roots (Tab. 1), whereas 31 taxa of microfungi (Chlebicki, 2009; Suková, Chlebicki, 2004) and 7 species of endophytes were noted in stems. *Botrytis cinerea* Pers., *Cladosporium herbarum* (Pers.) Link and *Epicoccum nigrum* Link were also noted on the stem surfaces and isolated as endophytes from the stems. Only some taxa of DSE were noted in roots, such as *Aureobasidium pullulans*, strain (*Hyaloscypha finlandica*) PTEn-19 and *Alternaria chartarum*. Moreover, *Paraconiothyrium* sp. was noted in the roots and stems of the same plant specimens (strains PBgEn-18, PBgEn-19).

## Discussion

### Notes on the species

Similar fungus to *Clonostachys rosea* was noted by Philipson (1991) inside the stems of *Lolium perenne* L. and by Petrini and Dreifuss (1981) in the roots of unidentified orchids from French Guyana. According to Schroers et al. (1999), this anamorph is related to *Bionectria ochroleuca* (Schwein.) Schroers & Samuels. The fungus is a famous antifungal agent that produces compounds against *Sclerotinia sclerotiorum* (Lib.) de Bary, *Fusarium* spp., *Botrytis cinerea* and *Phytophtora cactorum* J. Schröt (Sutton et al., 2008; Huang et al., 2009; Smith et al., 1990). *C. rosea* was frequently noted in Poland as *Gliocladium catenulatum* J.C. Gilman & E.V. Abbott (Kozak, Kozłowska, 2008). *Epicoccum nigrum* is a ubiquitous fungus that displays an endophytic lifestyle (Schulz, Boyle, 2005; Martini et al., 2009). The strain isolated from the roots of *Ornithidium rigidum* (Barbr. Rodr.) can control pathogenic yeasts as *Candida krusei* (Castell.) Berkhout (Vaz et al 2009). The species is known as a sugarcane endophyte that can also induce root growth (Fávaro et al., 2012). The strain of *Oidiodendron griseum* from the Tatra Mts. differ from the type collection has longer conidiophores that reach 200 **μm**. Also, specimens cited by Barron (1962) have longer conidiophores. The species was isolated from the soil, rhizosphere and roots of trees and grasses in Poland (Truszkowska, 1976; Kowalski, 1980; Kwaśna, 2002).

Dalpé (1991) found that *Vaccinium angustifolium* Benth. forms mycorrhiza with *Oidiodendron griseum*. Rice and Currah (2006) found a similar species, *Oidiodendron maius*, in ericaceous roots that was identified as a mycorrhizal endophyte. Moreover, *O. maius* was often isolated to form the members of Ericaceae in arctic and alpine meadows. It was also an inhabitant of bacterial biofilm in cold mines in Złoty Stok. *Penicillium miczyńskii* belongs to the subgenus *Furcatum* Pitt series *Citrina* (Houbraken et al. 2011), which includes taxa with two biverticillate structures of conidiophores and phialides shorter than the supporting metulae. Members of the subgenus *Furcatum* are mostly soil fungi or root endophytes of various plants (Samson, Frisvad, 2004; Houbraken et al., 2010). *P. miczynskii* sensu str. was isolated from north temperate conifers or heathland soils (Christensen et al., 1999). This species was found by Zaleski (1927) in the soil near Morskie Oko Lake in the Tatra Mts. Various fungi that form the genus *Penicillium* were noted as root endophytes in the following plants: *P. nigricans* in *Picea abies* (L.) H. Karst., *Penicillium griseofulvum* Dierckx R.P. on *Dendrobium nobile* Lindl. and *Penicillium* spp. in roots of *Holcus lanatus* L. (Sanchez Marquez et al., 2010), *Stipa grandis* P.A. Smirn. (Su et al., 2010) and *Lepanthes* sp. (Bayman et al., 1997).

*Phoma fimeti* Brunaud was observed in the roots of *Vulpia ciliata* Dumort (Newsham, 1994). *Trichoderma viridescens* was isolated from many substrates, also as an endophyte in the trunks of *Fagus sylvatica* L. from North and South temperate regions (Jaklitsch et al., 2006; Samuels et al., 2008). *T. viridescens* belongs to the *viride* clade, which includes *T. viride* Pers., *T. atroviride* P. Karst., *T. koningii* Oudem., *T. asperellum* Samuels, Lieckf. & Nirenberg and *T. hamatum* (Bonord.) Bainier. According to Samuels (pers. inf.), *T. viridescens* is a species complex with well-supported internal branches. However, its correlation with biological characters is problematic. *Trichoderma* spp. can control *Phytophtora cactorum*'s roots and crown rot its apple (Smith et al., 1990). *Alternaria chartarum* was noted as an endophyte in *Hyoscyanus muticus* L. (El-Zayat et al., 2008). The species was also noted on grasses, wood and soil (Ellis, 1976). In the past, three species under name of *Alternaria* were isolated from various species of *Juncus*: *A. alternata* (Fr.) Keissl., *A. junci* McAlpine and *A. junci-glauci* Joly. The last species is a member of the genus *Stemphylium* Wallr. *A. junci* is not distinguishable as unique among the small spore species of *Alternaria* (Simmons, 2007). Similar fungus, such as *A. alternata*, was noted in the epidermal cells (substomatal cavity) of *J. bufonius* L., *J. effusus* L., *J. patens* E. Mey. and *J. bolanderi* Engelm. (Cabral et al., 1993) and also colonised the dead leaves of *Juncus maritimus* Lam. (Fell, Hunter, 1979). *Alternaria* sp. was noted as a rot endophyte of *Cypripedium calceolus* L. (Bayman, Otero, 2006).

Both *Umbelopsis* and *Mortierella* are very common soil fungi (Meyer, Gams, 2003). It is noteworthy that the fungi from the genus *Umbelopsis* prefer to grow in low temperatures. These fungi are common associates of woody roots. Many strains belonging to the genus *Umbelopsis* were noted in the roots of Douglas-firs (Hoff et al., 2004). The ecological role of *Umbelopsis* in forest ecosystems is poorly known. Unanswered questions include how *Umbelopsis* interacts with other fungi. A similar species, *Mortierella elongate* Linnem., was recognised as one of the endophytic species that completely suppresses diseases caused by *Plasmodiophora brassicae* Woronin in the roots of *Brassica* *campestris* L. (Narisawa et al., 1998; Usuki et al., 2002). Our observation indicates that *U. autotrophica* coexists with *Aureobasidium pullulans* in the same root (Fig. 5). Root endophytes of both localities were different. No common species were found in the roots. Also, the species noted in the air (*Pleospora helvetica* Niessl) were not found in the roots. These endophytes are generalist. No exclusive species that were restricted to the host plant were found.

**Fig. 5.** Endophytes isolated from the stem and roots of a single specimen of *Juncus trifidus* L. from the Tatra Mts: roots with a very limited colony of root endophyte – A; stems with *Cladosporium herbarum* (Pers.) Link and *Alternaria chartarum* Preuss – B (Photo. A. Chlebicki)

The root endophyte (*Hyaloscypha finlandica*) and *Trichoderma viridescens* distinctly dominated the host plant roots (Fig. 2/7). *Phialophora* complex is known as a plant and human pathogen, as well as an endophyte (Yan et al., 1995). Dark-pigmented fungi and DSE possess melanin in their hyphae. Four taxa isolated from roots were darkly pigmented (*Aureobasidium pullulans*, *Hyaloscypha finlandica* (PTEn-19), *Alternaria chartarum* and *Paraconiothyrium* (PBgEn-18, PBgEn-19). Also, taxa such as *Aureobasidium pullulans* var. *pullulans*, *Epicoccum nigrum*, *Cladosporium herbarum*, *Alternaria chartarum* and *Paraconiothyrium* were noted on the stems. Melanin protects fungal mycelia from physical and biological stress (Butler et al., 2001). Domination of the root system by a single genotype of root endophyte (*Hyaloscypha finlandica*) indicates their greater competitiveness. Such strains were noted only in the roots. The next fungus, *A. pullulans* var. *pullulans*, was noted in the roots but also occurred in the stems of other plant specimens (Chlebicki, 2009). *Alternaria chartarum* was noted both in the roots and stems but never in the same host plant shoots.

We suggest that the host plant was colonised from air and soil by different strains of fungi. Menkis et al. (2004) also noted that various plant tissue could be colonised by different strains of the same species. We did not find any hard confirmation of the transfer of DSE fungi from the roots to the stems. Only *Paraconiothyrium* with pale brown conidia was present both in the stems and roots of the same plant specimens. Verkley et al. (2004) erected a new genus *Paraconiothyrium*, consisting of four species: *P. brasiliense* Verkley, *P. estuarinum* Verkley, *P. cyclothyrioides* Verkley, and *P. fungicola* Verkley & Wicklow. One of them, *Paraconiothyrium brasiliense* was isolated from Chinese Maple leaves in Korea (Narayan, Hyand, 2014). Moreover, a new species of this genus was isolated from *Huperzia selago* (L.) Bernh. ex Schrank et Mart. in Babia Góra Massif (Budziszewska et al., 2011). None of them were the species from the *Juncus trifidus* roots. The strain from *Juncus trifidus* was in 100% similar to the *Paraconiothyrium* sp. noted by Hargreaves et al. (2018).

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## Conflict of interest

The author declare no conflict of interest related to this article.

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## Appendix 1

**Tab. 2.** Fungi noted on stems and roots of *Juncus trifidus* L.; microfungi were noted on the stems (S) and roots (R) of *J. trifidus* according to the data of Scheuer (1988; 1996; 1999), Chlebicki (1990; 2002; 2009), Scheuer, Chlebicki (1997), Sukova (2004), Sukova, Chlebicki (2004), Owczarek-Kościelniak, Chlebicki, Sterfingler (2016), Jacewski et al. (2019) and this article (in the Alps, Carpathians, Sudetes and Urals)

| Name of species | Part of plant | Occurrence |
|---|---|---|
| *Alternaria chartarum* Preuss | R + S | endophyte |
| *Apiospora montagnei* Sacc. syn.: *Arthrinium arundinis* | S | |
| *Arthrinium cuspidatum* (Cooke & Harkn.) Tranzschel | S | |
| *Ascochyta junci* (Oudem.) Melnik | S | |
| *Ascochyta caricis-arenariae* Melnik, tel. *Didymella* | S | |
| *Ascochyta* sp. | R | endophyte |
| *Aureobasidium pullulans* var. *pullulans** | S + R | endophyte |
| *Botrytis cinerea* Pers. | S | endophyte |
| *Cladosporium herbarum* (Pers.) Link | S | endophyte |
| *Clonostachys rosea* (Link) Schroers, Samuels, Seifer & W. Gams | R | endophyte |
| *Coronellaria caricinella* P. Karst. | S | |
| *Cistella fugiens* (W. Philips) Matheis | S | |
| *Diplonaevia emergens* (P. Karst.) B. Hein | S | |
| *Epicoccum nigrum* Link | S | endophyte |
| *Hyaloscypha finlandica* (C.J.K. Wang & H.E. Wilcox) Vohník, Fehrer & Réblová | R | endophyte |
| *Hysteronaevia minutissima* (Rehm) Nannf. | S | |
| *Hysteropezizella diminuens* (P. Karst.) Nannf. | S | |
| *Lachnum calycioides* (Rehm) Rehm syn. *Brunnipila calycioides* | S | |
| *Lachnum diminutum* (Roberge ex Desm.) Rehm | S | |
| *Lachnum roseum* (Rehm) Rehm | S | |
| *Leptosphaeria sepalorum* (Vleugel) Lind syn. *Bricookea sepalorum* | S (inflorescence) | |
| *Lophodermium juncinum* (Jaap) Terrier | S | |
| *Mycosphaerella perexigua* (P. Karst.) Johanson | S | |
| *Naeviella paradoxa* (Rehm) Clem. | S | |
| *Nimbomollisia eriophori* (L.A. Kirchn.) Nannf., syn.: *Niptera eriophori* | S | |
| *Oidiodendron griseum* Robak | R | endophyte |
| *Paraconiothyrium* sp. | R + S | endophyte |
| *Penicillium expansum* Link. | S | |
| *Penicillium miczynskii* K.M. Zaleski | R | endophyte |
| *Periconia atra* Corda | S | |
| *Phaeosphaeria juncicola* (Rehm ex G. Winter) L. Holm | S | |
| *Phaeosphaeria vagans* (Niessl) O.E. Erikss. | S | |
| *Phoma* sp. | S | endophyte |
| *Phomatospora dinemasporium* J. Webster, syn.: *Dinemasporium strigosum* | S | |

| | | |
|---|---|---|
| *Pseudoseptoria* sp. | S | |
| *Pycnothyrium junci* Grove | S | |
| *Septoria* spp. | S | |
| *Septoria chanousiana* Ferraris | S | |
| *Stagonospora* sp. | S | |
| *Stagonospora junciseda* (Sacc.) Mussat. | S | |
| *Trichoderma viridescens* (A.S. Horne & H.S. Will.) Jaklitsch & Samuels | R | endophyte |
| *Umbelopsis autotrophica* (E.H. Evans) W. Gams | R | endophyte |
| *Unguicularia costata* (Boud.) Dennis | S | |
| *Xylodon detriticus* (Bourdot & Galzin) Tura, Zmitr., Wasser & Spirin, syn.: *Lagarobasidium detriticum* (Bourdot & Galzin) Jülich | S | |

* in different host plants

## Abstract

*Paraconiothyrium*, *Aureobasidium pullulans*, *Clonostachys rosea*, *Oidiodendron griseum*, *Penicillium miczynskii*, *Alternaria chartarum*, *Trichoderma viridescens*, *Umbelopsis autotrophica* and *Hyaloscypha finlandica* have been isolated from the roots of the highland rush (*Juncus trifidus* L.) from Babia Gora Massif and the Tatra Mts. in the Western Carpathians. Only the *Paraconiothyrium* sp. was found both in roots and stems and thus can possibly be transferred from the roots to the leaves (or in opposite direction).

**Key words:** *Juncus trifidus* distribution of endophytes, DSE

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## Endofity grzybowe łodyg i korzeni *Juncus trifidus* subsp. *trifidus* z Polski

## Streszczenie

*Paraconiothyrium*, *Aureobasidium pullulans*, *Clonostachys rosea*, *Oidiodendron griseum*, *Penicillium miczynskii*, *Alternaria chartarum*, *Trichoderma viridescens*, *Umbelopsis autotrophica* i *Hyaloscypha finlandica* zostały wyizolowane z korzeni situ skucina (*Juncus trifidus* L.) z masywu Babiej Góry i Tatr. w Karpatach Zachodnich. Spośród w/w taksonów, tylko rodzaj *Paraconiothyrium* sp. został stwierdzony w próbach wyizolowanych zarówno z korzeni jak i pędów, dlatego prawdopodobnie może zostać przeniesiony z korzeni na liście (lub w przeciwnym kierunku).

**Słowa kluczowe:** rozmieszczenie endofitów *Juncus trifidus*, DSE

## Information on the author

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His research to date has focused on fungi found on Arctic-Alpine plants. In addition, in recent years he has dealt with extremophiles found on rocks, mainly black yeast, and the influence of the electromagnetic field on the development of fungal colonies.