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Records of the rare and extremely osmophilic fungi *Penicillium eremophilum* and *Xerochrysium dermatitidis* on soy bars

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The strongly osmophilic and slowly growing fungi *Penicillium eremophilum* and *Xerochrysium dermatitidis* were found on soy protein bars. These fungi are worldwide rarely recorded and have been recorded in the Czech Republic for the first time. *Penicillium eremophilum* is exceptional in the genus *Penicillium* by the production of 2-spored asci and by the absence of a conidial state. *Xerochrysium dermatitidis* is predominantly known from food, but some affinity to clinical material has also been recorded. The identification of the strains was confirmed by DNA sequencing. Their macroscopic and microscopic descriptions with photographs completed with known literature data are given.

Key words: food-borne fungi, food spoilage, *Eurotiomycetes*, *Eurotiales*, *Aspergillaceae*, xerophiles.

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Kubátová A., Hubka V. (2021): Nálezy vzácných a extrémně osmofilních hub *Penicillium eremophilum* a *Xerochrysium dermatitidis* na sojových sucích. – Czech Mycol. 73(2): 185–198.

Extrémně osmofilní a pomalu rostoucí houby *Penicillium eremophilum* a *Xerochrysium dermatitidis* byly izolovány ze sojových suků. Obě houby jsou celosvětově zaznamenávány zřídka, v České republice poprvé. *Penicillium eremophilum* je výjimečné tvorbou vřecek se dvěma askosporami a absencí konidiového stádia, jinak běžného v rodě *Penicillium*. *Xerochrysium dermatitidis* je známo především z potravin, ale byla u něj zaznamenána i určitá afinita ke klinickému materiálu. Identifikace hub byla potrvrzena sekvenováním DNA. V článku je uveden makroskopický i mikroskopický popis doplněný fotografiemi a známými literárními údaji.

INTRODUCTION

Osmophilic fungi (often referred to as xerophilic) are capable of growing on substrates with very limited water availability (i.e. with low water activity), such

as dried foods or foods preserved by salting or sugaring. Osmophilic fungi can also be found in household dust, hypersaline water of salterns, leather and textile goods. They cause significant damage to specimens deposited in herbaria, and also to works of art, especially paintings, and deteriorate the quality of books in archives and libraries (Micheluz et al. 2015, Sklenář et al. 2017, Visagie et al. 2017, Chung et al. 2019). Osmophilic fungi include representatives of the orders *Eurotiales* (*Aspergillus* species of the sections *Aspergillus* and *Restricti*, *Penicillium eremophilum*, *Eremascus albus*, *Xeromyces bisporus*, *Xerochrysium* spp.), *Leotiales* (*Bettsia*, *Skoua*) but also of basidiomycete order *Wallemiales* (*Wallemia*). Osmophilic fungi are often overlooked in routine mycological analyses of foods, especially due to their slow growth on commonly used agar media. However, compared to other fungi, they mostly do not pose a significant health risk, as they usually do not produce any hazardous mycotoxins.

Soy bars are sweet products, sometimes with a chocolate coating, on which the presence of osmophilic microfungi can be expected. During the past years, a tiny whitish growth on the surface of soy bars has occasionally been observed. Two rare fungi, *P. eremophilum* and *Xerochrysium dermatitidis*, were found on them and identified using a combination of morphological, physiological and genetic traits. A brief description with photographs of these rarely found fungi is given and their ecology and known biological properties are summarised.

MATERIAL AND METHODS

Material studied

Locality: Prague, Czech Republic.

Substrate: *Penicillium eremophilum* was isolated from a mouldy soy bar in October 2011 (Fig. 1a), and *Xerochrysium dermatitidis* from chocolate glaze on a soy bar in December 2019 by A. Kubátová.

Specimens: Living isolates are maintained at the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague and the Westerdijk Fungal Biodiversity Institute, Utrecht (The Netherlands) under codes CCF 6323 and CBS 148469, respectively (*P. eremophilum*), and CCF 6436 and CBS 148470, respectively (*X. dermatitidis*). The herbarium specimens are stored in the Herbarium collections of Charles University (PRC) under codes PRC 4683 (*P. eremophilum*) and PRC 4684 (*X. dermatitidis*).

Cultivation. Malt extract agar with yeast extract and 40% sucrose (M40Y), a medium suitable for osmophilic fungi, was used for isolation. For *P. eremophilum*, colony diameters were measured after 14 days at 10, 15, 20, 25, 30 and 35 °C on malt extract agar with 20% sucrose (MA20S) and on malt extract agar with yeast extract and 50% glucose (MY50G). Growth was tested on malt extract agar (MEA), Czapek yeast extract agar (CYA), Czapek yeast extract agar with 5% NaCl (CYAS), dichloran 18% glycerol agar (DG18), and also creatine sucrose agar

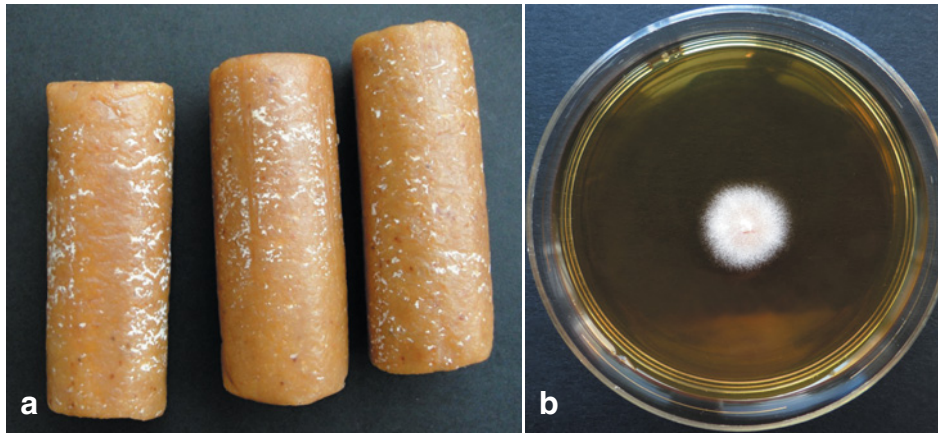


Fig. 1. *Penicillium eremophilum.* **a** – mouldy soy bars, **b** – strain CCF 6323, colony on MY50G (14 days, 25 °C, Petri dish 6 cm diam.). Photo A. Kubátová.

(CREA) (according to Frisvad et Samson 2004, Samson et al. 2010). The growth of *X. dermatitidis* was observed after 14 days at 25 °C on MY50G, M40Y, DG18, CYA, and MEA. Growth on MY50G at 30 °C and 37 °C was also tested.

Micromorphology. Microscopic characters were examined after 15–17 days from colonies on MY50G or M40Y and mounted on slides with lactic acid including cotton blue. An Olympus BX51 microscope with a DP72 camera was used for observation (maximum magnification 1600×). Photomicrographs and measurements (n = 50) were made with the QuickPHOTO MICRO 3.0 and Helicon Focus 5.0 software.

Molecular analyses. A Quick-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, Orange, USA) was used to isolate genomic DNA from 14-day-old colonies grown on the M40Y medium. The internal transcribed spacer region (ITS1-5.8S-ITS2 cluster) and partial large subunit (LSU) ribosomal DNA region were amplified and sequenced with the primers ITS1 (White et al. 1990), and NL4 (O'Donnell 1993), respectively. The obtained DNA sequences were compared with those available in the GenBank database and reference sequences were derived from ex-type strains (Houbraken et al. 2020) and deposited into the European Nucleotide Archive (ENA) database under accession numbers LR983937 (*P. eremophilum*) and LR983933 (*X. dermatitidis*). The phylogenetic trees based on the maximum likelihood method were constructed with IQ-TREE v. 1.4.4 (Nguyen et al. 2015). The support values at branches were obtained from 500 bootstrap replicates. The best-fitting model was determined using the Bayesian information criterion (BIC) in jModelTest 2.1.7 (Posada 2008).

RESULTS

Penicillium eremophilum (A.D. Hocking et Pitt) Houbraken, Leong et Vinnere Pettersson, Stud. Mycol. 86: 47, 2017

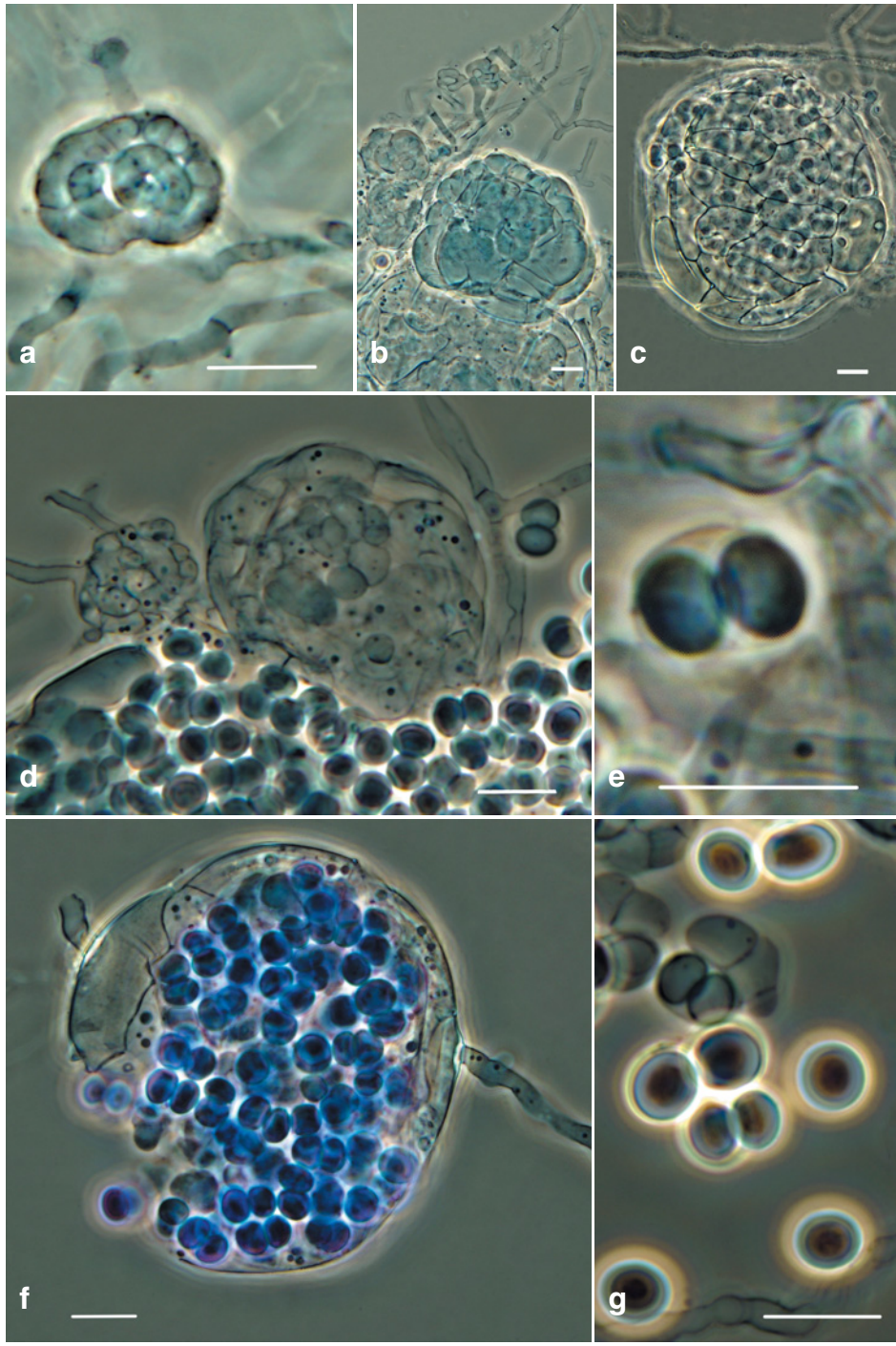
Basionym: *Monascus eremophilus* A.D. Hocking et Pitt, Mycologia 80(1): 84, 1988

Colonies (isolate CCF 6323; Fig. 1b). Colonies on MY50G after 14 days growing very slowly: 2 mm at 10 °C, 5 mm at 15 °C, 12 mm at 20 °C, 15 mm at 25 °C, and 12–13 mm at 30 °C. At 35 °C, only germination was observed. No growth was observed on MEA, CYA, CYAS, DG18 or CREA after 14 days at 25 °C. Colonies after 14 days at 25 °C white, margins fimbriate, without sulci, exudate or soluble pigment. Reverse brownish. Optimal growth temperature 25 °C, good growth was observed in the temperature range 20–30 °C. Hocking et Pitt (1988) found somewhat smaller colonies on MY50G after 14 days at 25 °C (6–9 mm). On MA20S, no growth was observed.

Micromorphology (Fig. 2). Only sexual reproduction was observed. Cleistothecial initials similar to those of other representatives of the *Eurotiales*. Cleistothecia globose or subglobose, ca 30–90 µm (mean ± SD: 52 ± 7 µm) in diameter. Cleistothecial wall transparent, later yellowish, consisting of relatively large cells. Asci ellipsoid, with evanescent wall, 7–8 × 5.5–6 µm, containing two ascospores. Mature ascospores broadly ellipsoidal to subglobose, 5–7 × 4–5.8 µm (mean ± SD: 6.1 ± 0.4 × 4.8 ± 0.4 µm), smooth, hyaline, yellowish in mass. These data are in accordance with those by Hocking et Pitt (1988), with one exception: the cleistothecial wall is described by these authors as brownish orange at maturity.

Molecular studies. Using the BLAST similarity search, the ITS rDNA region of isolate CCF 6323 was almost identical to *Penicillium eremophilum* ex-type strain FRR 3338 (= IMI 313774 = CBS 123361 = ATCC 62925) (GenBank accession numbers: GU733341, MH863291). The sequence only differed by a single substitution in the ITS2 region. Other *Penicillium* species showed a similarity of 90% or lower. The partial LSU rDNA region was identical with that of the ex-type strain (GenBank JF922042). The maximum likelihood phylogenetic tree showing the relationship of isolate CCF 6323 to the ex-type strain of *P. eremophilum* (section *Eremophila*) and species from the related section *Charlesia* is shown in Fig. 3.

Fig. 2. *Penicillium eremophilum* CCF 6323. **a** – cleistothecial initials, **b–d** – developing cleistothecia, **e** – ascus with two ascospores, **f** – mature cleistothecium containing asci with ascospores, **g** – asci and ascospores. Scale bars = 10 µm. Phase contrast, photo A. Kubátová. ▶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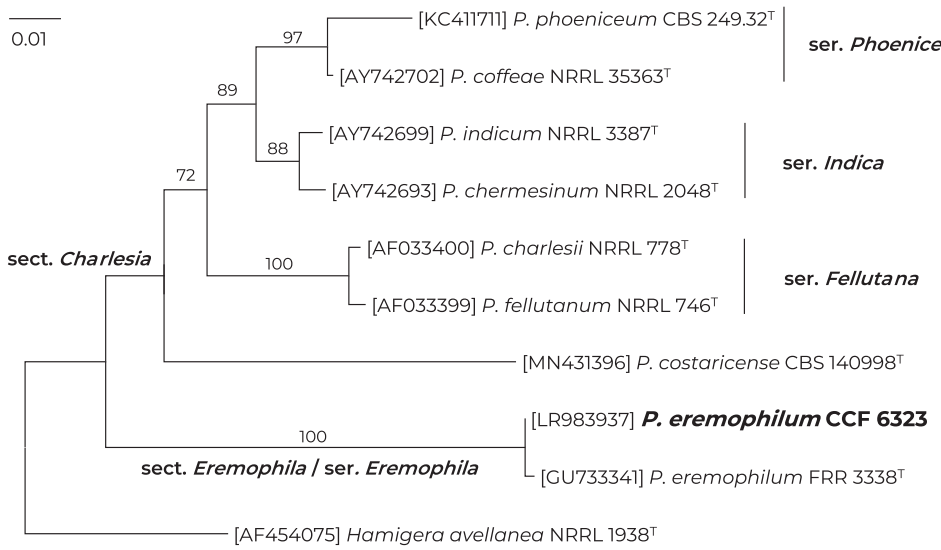

Fig. 3. Best scoring maximum likelihood tree based on the ITS and partial LSU rDNA regions showing the relationships of *Penicillium eremophilum* isolate CCF 6323 to species from the most closely related section *Charlesia* and corresponding series sensu Houbraken et al. (2020). The dataset contained 10 taxa and a total of 800 characters of which 134 were variable and 87 parsimony-informative. Substitution model TIM2+F+G4 and 500 bootstrap replicates were used for analysis. Only bootstrap support values ≥ 70% are shown. Ex-type strains are indicated by superscript ^{T}. The tree is rooted with *Hamigera avellanea*.

Xerochrysium dermatitidis (A. Agostini) Pitt, IMA Fungus 4: 23, 2013

Basionym: *Glenosporella dermatitidis* A. Agostini, Atti Ist. Bot. Univ. Pavia 2: 93, 1930
Synonym: *Chrysosporium inops* J.W. Carmich., Can. J. Bot. 40: 1156, 1962

Colonies (isolate CCF 6436; Fig. 4). Colonies grow very slowly. After 14 days on MY50G at 25 °C: 14–15 mm, MY50G at 30 °C: 16–17 mm, M40Y at 25 °C: 11–12 mm, DG18 at 25 °C: 9–10 mm, MEA at 25 °C: 2 mm, CYA at 25 °C: 22 mm. On MY50G after 14 days at 25 °C white, more or less flat, compact, without sulci, exudate or soluble pigment. Reverse almost uncoloured. Colonies on M40Y white, umbonate, with a few sulci. Colonies on DG18 also white and umbonate; reverse light brown. Fungus growing with difficulties on CYA and MEA. On MY50G at 30 °C, growth good and colonies similar to those at 25 °C. No growth observed at 37 °C. Pitt et al. (2013) reported analogous data.

Micromorphology (Fig. 5). Only the asexual state was observed. Mycelium white. In the course of two weeks, conspicuous thick-walled smooth conidia (chlamydoconidia according to Pitt et al. 2013) formed terminally and

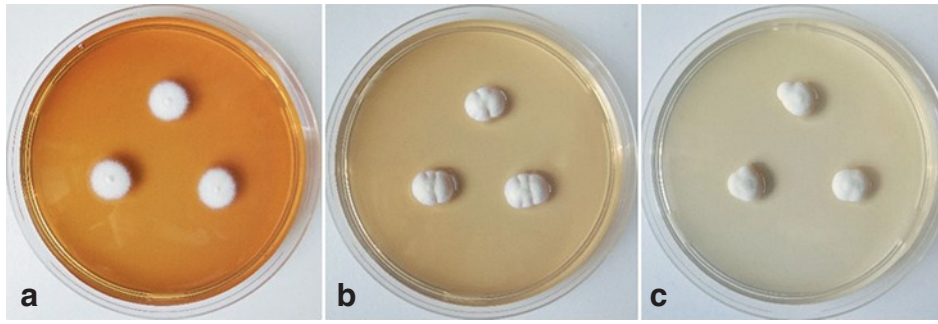

Fig. 4. *Xerochrysium dermatitidis* CCF 6436. Colonies after 14 days at 25 °C. **a** – MY50G, **b** – M40Y, **c** – DG18. Photo A. Kubátová.

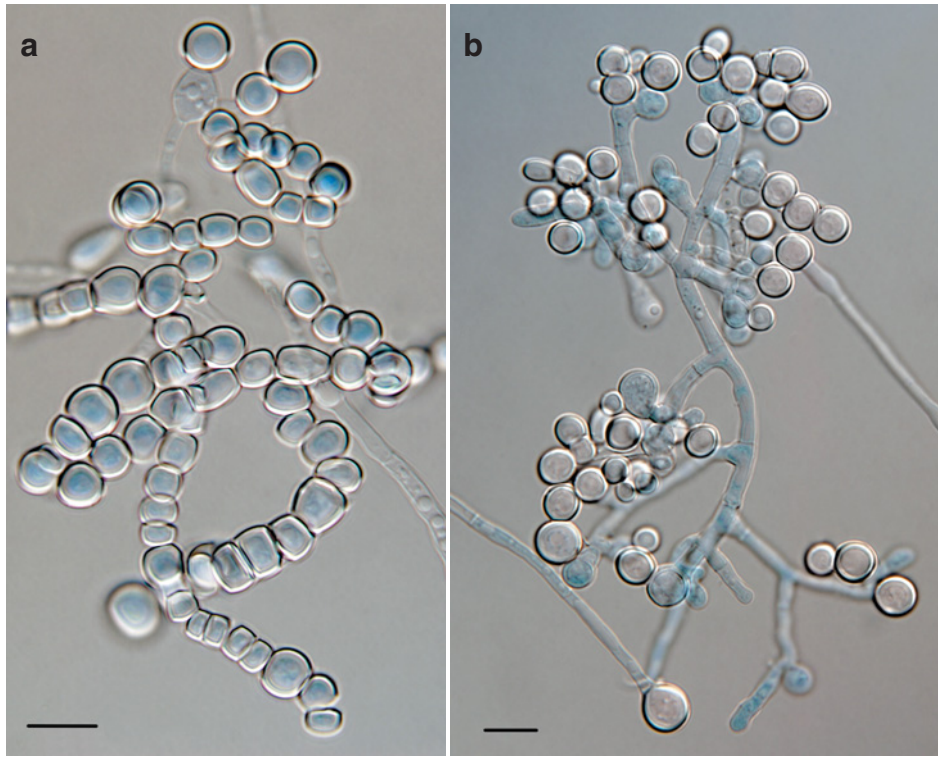

Fig. 5. *Xerochrysium dermatitidis*. **a** – conidia from chocolate glaze on a soy bar, **b** – strain CCF 6436, conidiophore with terminal conidia on M40Y. Scale bars = 10 µm. Photo A. Kubátová.

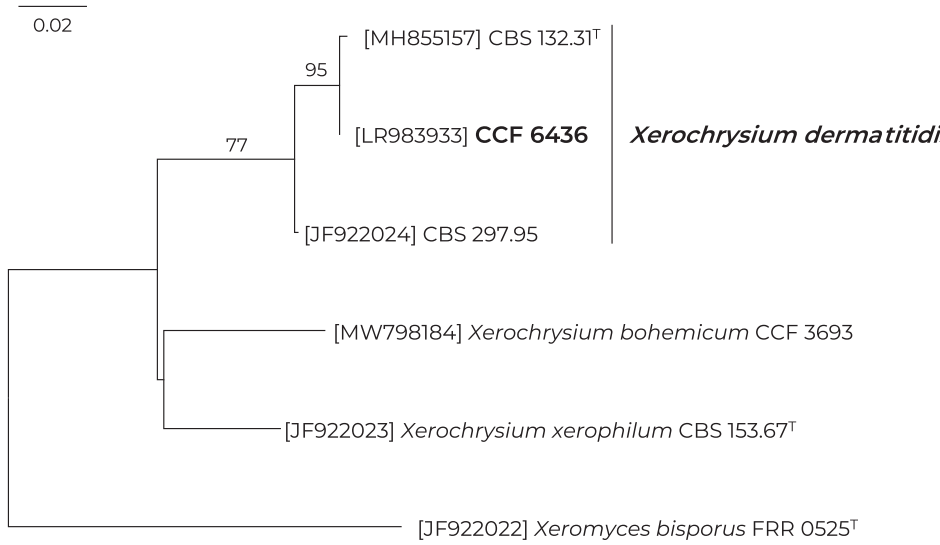

Fig. 6. Best scoring maximum likelihood tree based on the ITS rDNA (containing ITS1, 5.8S and ITS2 regions) showing the relationships of *Xerochrysium dermatitidis* CCF 6436 to other *Xerochrysium* species. The dataset contained 10 taxa and a total of 542 characters of which 71 were variable and 13 parsimony-informative. Substitution model K3P+I and 500 bootstrap replicates were used for the analysis. Only bootstrap support values ≥ 70% are shown. Ex-type strains are indicated by superscript ^{T}. The tree is rooted with *Xeromyces bisporus*.

intercalarly on the hyphae, later often forming chains. Intercalary conidia mostly barrel-shaped, 3–7 × 3–6.7 µm (mean ± SD: 5.0 ± 0.8 × 4.6 ± 0.8 µm), later rounded. Terminal conidia spherical, of variable size, 4.5–12 µm (mean ± SD: 7.5 ± 1.1 µm) in diameter. These data are consistent with those reported by Pitt et Hocking (2009) and Pitt et al. (2013).

Similar species. The remaining two members of the genus, *Xerochrysium xerophilum* and *X. bohemicum*, are very similar. However, *X. xerophilum* differs in somewhat faster growth on MY50G. The colony reverse of some isolates may be coloured light yellow, light orange to khaki. Moreover, *X. xerophilum* is able to grow at 37 °C compared to the other two species. *Xerochrysium bohemicum* differs by a faster growth on MY50G (see Tab. 1). Two other similar species, *Bettsia alvei* and *B. fastidia*, form aleurioconidia, grow faster on MY50G than both *X. dermatitidis* and *X. xerophilum*, and the colony reverse may also be coloured (see Pitt et al. 2013).

Tab. 1. Comparison of physiological characters of *Xerochrysium* species.

Species	Colony diameter on MY50G*, 14 days, 25 °C (mm)	Growth at 37 °C	Reference
X. dermatitidis	12–20	–	Pitt et al. 2013
X. xerophilum	20–38	+	Pitt et al. 2013
X. bohemicum	38–42	–	Crous et al. 2021

* MY50G – malt extract agar with yeast extract and 50% glucose

Molecular studies. BLAST analyses with the ITS region of isolate CCF 6436 showed 99% similarity (475/479 bp, 3 indels, 1 substitution) with the ex-type of *X. dermatitidis* CBS 132.31 (GenBank accession number: MH855157), 94% with *X. bohemicum* and ~89% with *X. xerophilum* isolates. The partial sequence of the LSU rDNA region was identical with *X. dermatitidis* strain CBS 297.95 (JF922024) and showed 98% similarity with *X. xerophilum* and *X. bohemicum*. The maximum likelihood phylogenetic tree showing the relationship of isolate CCF 6436 to presently accepted *Xerochrysium* species is shown in Fig. 6.

DISCUSSION

Taxonomy

The name *Penicillium* evokes an image of a brush-like conidiophores with most mycologists and microbiologists, while others associate it with the antibiotic penicillin. However, there are species of the genus *Penicillium* that do not correspond to this common idea. One of them is, for example, *Penicillium paradoxum* (older name *Aspergillus paradoxus*), whose conidiophores have a shape typical of the genus *Aspergillus* (see Kubátová et Hubka 2018). Another peculiar species is *Penicillium eremophilum* (formerly *Monascus eremophilus*), an extremely osmophilic fungus, which does not form conidiophores at all and reproduces only sexually. However, based on molecular phylogenetic studies, this fungus has also been, somewhat surprisingly, included in the genus *Penicillium* (Barbosa et al. 2017). Hocking et Pitt (1988), who described this species, provisionally included it in the genus *Monascus* (due to similar ascocarps) as the only xerophilic representative of this genus. They found that it is a strict xerophile and it does not grow on conventional media with 2% of the sugars commonly used in microfungi cultivation. Thus, they chose the species name 'eremophilus' derived from Greek, which means 'loving desert'. However, Park et al. (2004), who studied the *Monascus* phylogeny, found that *M. eremophilus* does not belong to the same clade as the other *Monascus* species (*Monascaceae*, *Eurotiales*). Barbosa

et al. (2017), based on an extensive phylogenetic study, then made a combination of this species into the genus *Penicillium* (*Aspergillaceae*, *Eurotiales*). Later, the separate section *Eremophila* of the genus *Penicillium* was created to accommodate this species (Houbraken et al. 2020).

Xerochrysium is a rather recently established genus (Pitt et al. 2013) comprising only three species, *X. dermatitidis*, *X. xerophilum* and *X. bohemicum*. The first two species have for a long time been included in the genus *Chrysosporium* Corda [*Onygenales*, see the classic works by Carmichael (1962) and van Oorschot (1980)]. Pitt et al. (2013), following the work by Vidal et al. (2000), found in their phylogenetic study based on the LSU region that the hitherto widely understood genus *Chrysosporium* includes representatives of three different orders: keratinophilic representatives of the order *Onygenales* (*Chrysosporium*), and xerophilic species of the orders *Leotiales* (*Bettsia*) and *Eurotiales* (*Xerochrysium*).

Occurrence

According to the Global Catalogue of Microorganisms (http://gcm.wdcm.org), only two isolates of *Penicillium eremophilum* are present in the world's collections: ex-type isolate FRR 3338, found in 1986 on dried mouldy plums in Australia (Hocking et Pitt 1988), and strain NBRC 111180, isolated from chocolate in Japan around 2015. The latest record of the occurrence of this fungus comes from Bovo et al. (2018), who studied environmental DNA in honey bee (*Apis mellifera*). *Penicillium eremophilum* was found in the flower honey of an orange tree native to Sicily. No reports of further finds could be found. Consequently, *P. eremophilum* is therefore either an extremely rare or overlooked fungus, which may have a worldwide distribution. We consider our isolate to be the first find in the Czech Republic.

The fact that *Xerochrysium dermatitidis* is found relatively rarely is probably due to the use of inappropriate isolation methods. The first record of this fungus by A. Agostini is relatively unique. He isolated it from clinical material in Italy (skin lesion). Carmichael (1962) mentions a find from an orange concentrate in California in 1959. Kinderlerer (1987, 1997) found it on table fruit jelly and various types of chocolate in Great Britain. Pitt et Hocking (2009), who specialised in osmophilic fungi and purposefully used suitable isolating media, found it quite often in various spices, chopped dates, gelatine confectionery and starch. Finds on other substrates (under the older name *Chrysosporium inops*) also appear in the literature. These have, however, not been verified with molecular methods. For example, Abdel-Hafez et al. (1990) reported *C. inops* from airborne dust particles in Egypt, Erdogan et al. (2001) from various types of Turkish cheese, Lugauskas et al. (2004, 2006) from air of an organic waste recycling facility, raspberries and

black currants in Lithuania, Qiu et al. (2005) from a clinical specimen collected from corneal infection in China, Papini et al. (2008) from rat fur in Italy, and Pantoja et al. (2009) from the surface of ant bodies in a Brazilian hospital. *Chrysosporium inops* was also isolated from soil in India using various keratin baits (Kotwal et Sumbali 2016). Our find can be considered the first verified and documented record of *Xerochrysium dermatitidis* in the Czech Republic.

Ecology and physiology

Leong et al. (2010) studied extreme physiological abilities of *Penicillium eremophilum* and other osmophilic fungi and found that the optimal conditions for growth are a water activity (aw) = 0.88 and a temperature of 25 °C, in agreement with our observation. They stated that the extreme xerophile *Xeromyces bisporus*, found on dried prunes, shares similar conditions for growth, but is able to grow faster and therefore possesses more competitive abilities.

Carmichael (1962), who worked with an ex-type isolate of *Xerochrysium dermatitidis* from human skin, stated that this species does not attack hair (keratin) and has no cellulolytic abilities. Similarly, van Oorschot (1980) did not observe any keratinolytic properties in this fungus. Regarding temperature requirements, *Xerochrysium dermatitidis* does not grow at 37 °C (Carmichael 1962) but is able to grow well at 30 °C (van Oorschot 1980), which was confirmed in our work. Therefore, further studies are needed to confirm the pathogenic properties of *X. dermatitidis*.

Exometabolites

Many species of the genus *Monascus*, in which *P. eremophilum* was previously classified, produce red pigments applied in industry and mycotoxins (see e.g. Patakova 2013, Barbosa et al. 2017). No similar pigments are produced in *P. eremophilum*, and nothing is known about the production of secondary metabolites in general.

Similarly, nothing is known about mycotoxin production by *X. dermatitidis*. However, during our study, older Petri dishes were accidentally contaminated with *Cladosporium* species, which showed that *X. dermatitidis* is able to produce metabolites with antifungal properties manifesting as inhibition zones (Fig. 7).

CONCLUSION

Penicillium eremophilum is characterised by several interesting features. Being an extremely osmophilic species, it does not grow on common mycological media with a sugar content of 2% and grows very slowly even on media with low

Fig. 7. *Xerochrysium dermatitidis* CCF 6436. Colonies after 14 days on M40Y contaminated by *Cladosporium* spp. An inhibitory zone is visible. Photo A. Kubátová.

water activity. It represents a rarity in the genus *Penicillium* by reproducing only sexually and by producing 2-spored asci. It is considered to be an extremely rare and thus understudied fungus. Consequently, little is known about its biological properties and metabolites.

Xerochrysium dermatitidis is reported worldwide at a relatively low frequency, occurring mainly on foods with a low content of available water. It is therefore necessary to use agar media for osmophilic fungi to detect it. The formation of mycotoxins, bioactive or antimicrobial substances is not known, but our accidental observation of inhibition zones may indicate the production of certain antifungal substances.

This study adds to our knowledge on the ecology of these poorly explored fungi which are probably underreported as they are relatively inconspicuous and need special media to isolate them. We consider our finds to be the first documented records in the Czech Republic.

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