

## First record of the fungus *Pringsheimia smilacis* E. Müller 1958 (Ascomycota: Dothioraceae) from Hungary

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**Abstract:** The 'black yeast' form of the fungus *Pringsheimia smilacis*, isolated from the exudate of a *Salix* sp ornamental tree, is reported as new for the mycoflora of Hungary. The species is illustrated with microphotographs and images of the colonies.

**Key words:** black yeast, new record, mycoflora, tree exudate, *Hormonema*, *Pringsheimia*.

**Összefoglalás:** A *Pringsheimia smilacis* E. Müller 1958 gombafaj (Ascomycota: Dothioraceae) első magyarországi adata. A *Pringsheimia smilacis* nevű gombafaj *Salix* sp. díszfa exudátumáról izolált 'fekete élesztő' alakját közöljük a magyarországi mikoflóra új adataként. A fajt mikroszkópos felvételek és a telepek képei illusztrálják.

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### Introduction

The complete than-known microfungal flora of Hungary was reviewed almost 3 decades ago (Bánhegyi et al. 1985) in a series of 3 books, but this work can be considered taxonomically outdated, especially since the new rules of the "International Code of Nomenclature for algae, fungi and plants" (McNeill et al. 2012) were adopted. In the last decades in Hungary, there have been a number of new records of fungal species (e.g. Révay 1998 and references therein). Completely new species (or even genera) are also regularly isolated and described from environmental substrates, plants or fermentation environments from the country (e.g. Péter et al. 2003; Sipiczki 2003; Tóth et al. 2010; Péter et al. 2012). But without doubt, there are still many unreported species of microfungi in the Hungarian mycoflora.

Here we report on the first known isolation of the species *Pringsheimia smilacis* from Hungary, found during a survey of tree sap fauna and flora in the city of Debrecen. The so-called black yeast stage of this species, traditionally referred as *Hormonema* sp [an anamorph of several teleomorphic genera in Dothideales (Yurlova et al. 1996)], was observed. Black yeasts (a diverse group of usually slow-growing, melanized filamentous fungi that

produce slimy, yeast-like conidia by multilateral or polar budding) are important factors of biodegradation and biodegeneration, frequently studied worldwide. Their teleomorphs are often plant-parasitic fungi. The yeast-like stage is generally restricted to special habitats and substrates (Bills et al. 2004) (Sterflinger 2006).

### Materials and Methods

**Sampling and strains** – Samples of tree sap were collected aseptically in the city of Debrecen from *Platanus x hybrida*, *Quercus* spp and *Salix* sp. The first two types of tree exudates yielded no yeast-like colonies, but from the sample from an ornamental *Salix* sp tree (inhabited by Ceratopogonidae midge larvae), two strains were isolated (date of isolation: 27.10.2010.; see photograph of isolation source: Fig. 1). Isolation of the strains was carried out on YPG-agar (1% yeast extract, 1% peptone, 2% glucose, 2% agar, all w/v). Strains are deposited in the strain collection of the Department of Genetics and Applied Microbiology, University of Debrecen under the identifiers 11-1083 and 11-1085 and also in the National Collection of Agricultural and Industrial Microorganisms (Faculty of Food Sciences, Corvinus University of Budapest).

**Media** – Strains were kept on YPG-agar and also studied on EMMA minimal medium (Mitchinson 1970) and in liquid YPG medium. Colony and cell morphology were studied after 5-7 days following inoculation and in the case of EMMA 1 month after inoculation. Incubation was at room temperature.

**Molecular identification of the strains** – Yeast cells were grown aerobically at 24°C for one day in liquid YPG. DNA was isolated and purified according to the method described in Hanna and Xiao (2006). The D1/D2 domains of the large-subunit (LSU) rRNA genes were amplified with primers NL-1 and NL-4 and sequenced with both primers as described by Sipiczki (2003). The BLAST network service of the NCBI database (<http://ncbi.nlm.nih.gov/blast>) was used for DNA sequence similarity searches with the strains, and the sequences of the amplified fragments were also compared to the sequence of the *Pringsheimia smilacis* strain CBS873.71 (obtained from the CBS database). As the type strain of the species, CBS375.59 lacks any sequences, it could not be compared to our strains.

Novel sequences are deposited in GenBank (accession numbers KF274497 and KF274498).

**Photography** – Colonies and isolation source were photographed with a DSLR camera, cells and spores with an Olympus BD40 microscope equipped with an Olympus 100x phase contrast lens and with an Olympus digital microscope camera. Photographs were recorded and measurements were made with the program DP-Controller (Olympus).

## Results

Two strains were isolated and subsequently identified as the *Hormonema*-like form of *Pringsheimia smilacis* based on the comparison of the partial ribosomal D1/D2 domains of the isolates and the strain CBS 873.71, which was isolated from Italy from the twig of the plant *Smilax aspersa* [the type strain, which has no available sequences in the databases, was also isolated from this species in Uttar Pradesh, India (Müller 1958)]. Comparison of the sequences of the strain 11-1083 and CBS 873.71 showed an identity of 99.7% (586/588) with 0.2% gaps (1/588).

These values for the strain 11-1085 are 99.5% (585/588) and 0.2% (1/588), respectively.

The yeast-like stage of *P. smilacis* can be described as round, pale yellowish-pinkish colonies on rich medium after 5 days of incubation at room temperature. Cells are oval, irregular in shape (Fig. 2.; 3. a-c). The black-coloured stage of this fungus was observed after one month of incubation on EMMA minimal medium (Fig. 4). Small-scale filamentous growth was observed both on YPG-agar and EMMA media on the edges of the colonies.

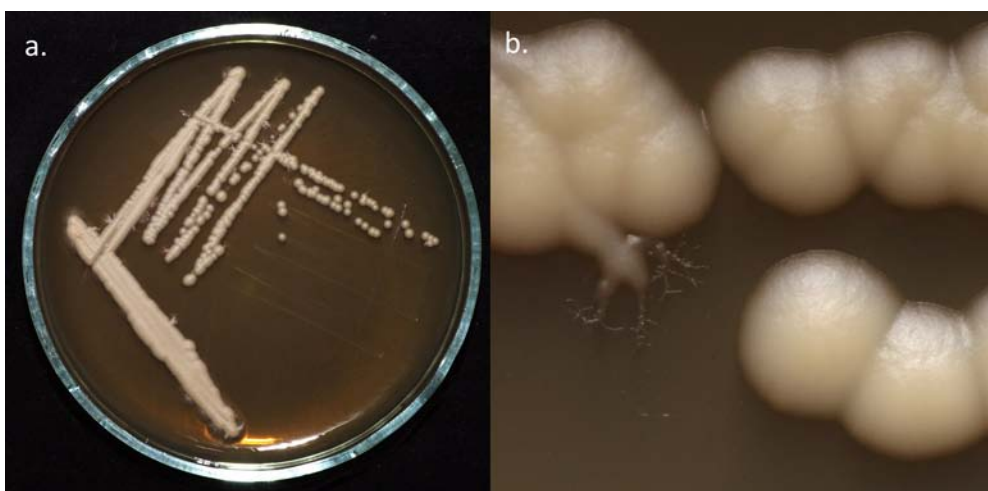
## Discussion

Molecular identification of fungal strains nowadays cannot be circumvented in the fields of taxonomy, agriculture, medicine or fungal ecology. The vast number of species that were historically treated under the group of imperfect fungi are nowadays more or less integrated into the monophyletic genera and higher taxonomic categories of the Fungal Kingdom. Many species names given to imperfect stages are to be sunken into synonymy with their perfect (sporulating) form (McNeill et al. 2012). Among these, the black yeasts are an interesting group of yeast-like forms of otherwise filamentous, usually plant-infecting fungi. The species isolated and observed by us and according to our knowledge, reported for the first time for Hungary, *Pringsheimia smilacis*, was originally described from the Himalayas (Müller 1958) from the plant *Smilax aspersa*, but as many *Pringsheimia* species, it also possesses a black, yeast-like hyphomycetous anamorph which was listed as a *Hormonema* species, but never given a species name (de Hoog & Yurlova 1994). Following the new rules of nomenclature, we used the species name of the teleomorph here (McNeill et al. 2012).

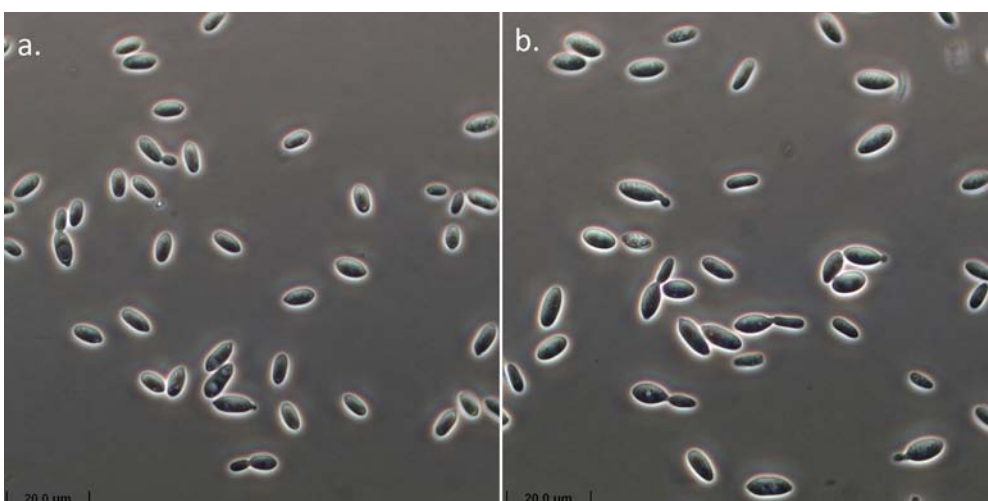
**Acknowledgements** – The authors thank dr. Gábor Péter for his valuable comments on the manuscript. This research was implemented in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program” subsidized by the European Union and Hungary.



**Figure 1.** Isolation substrate of *P. smilacis*: *Salix* sp. tree exudate in Debrecen, Egyetem sugárút (N47.546848, E21.621547).



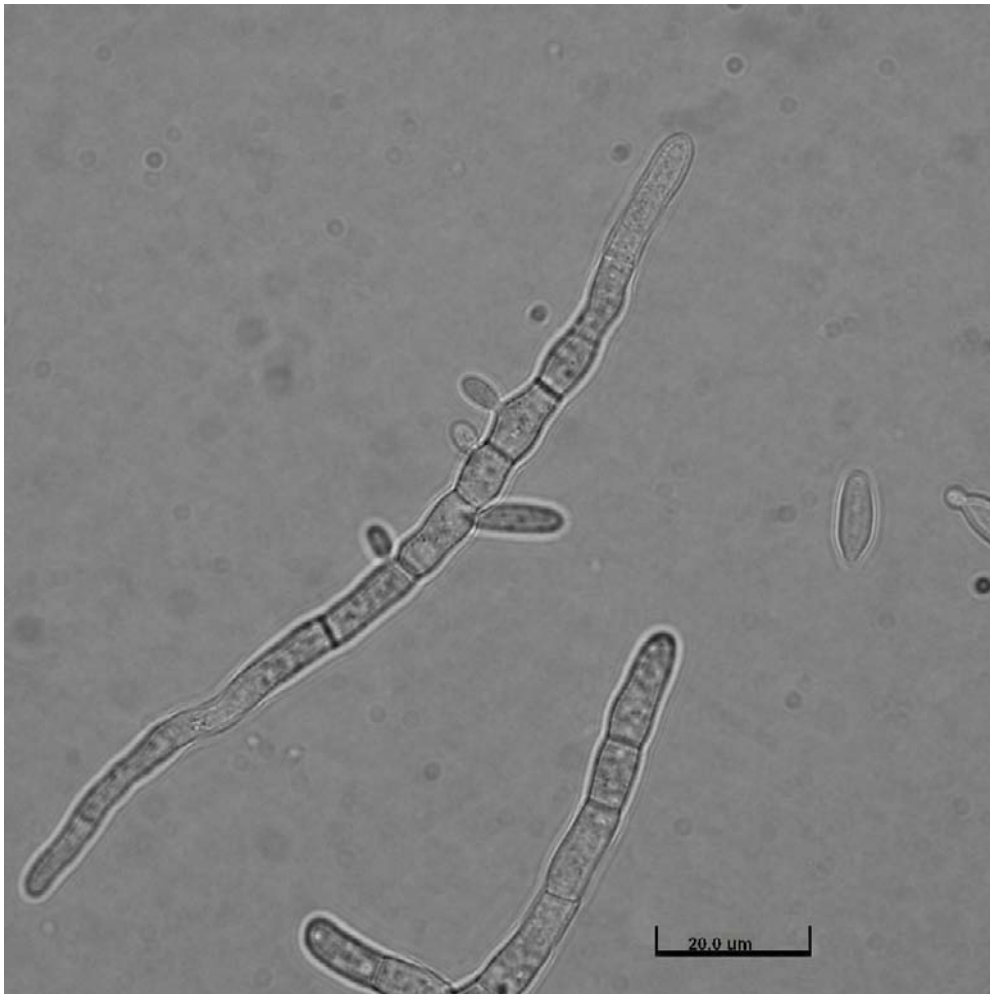
**Figure 2a-b.** Colonies of 11-1083 on YPG-agar after 5 days at room temperature.



**Figure 3.** Cells of 11-1085 on YPG-agar after 5 days at room temperature.  
Scale bar: 20  $\mu\text{m}$ .



**Figure 4.** Colonies of 11-1083 on EMMA after 1 month of incubation at room temperature.



**Figure 5.** Filamentous growth of 11-1083 in YPG liquid medium after 1 day at room temperature. Scale bar: 20 μm.

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