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Pidoplitchkoviella terricola – an interesting fungus from the Domica Cave (Slovakia)

Alena Nováková¹

Abstract:

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The microfungus *Pidoplitchkoviella terricola* – until now it was known only as Kirilenko's specimen isolated from *Quercus robur* rhizosphere in Ukraine - was found in earthworm casts in the Domica Cave, NP Slovak Karst, Slovakia. A description and microphotographs of this strain are provided in this article.

Keywords: Domica Cave, earthworm casts, *Pidoplitchkoviella terricola*

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INTRODUCTION

Considering the constant temperature and humidity, absence of light, and mostly the absence or limited amount of organic material in caves, caves are a very appropriate environment for living organisms. The microfungal community in caves is very specific, and caves can frequently be a source of very interesting species including relicts of fossilized microfungal spores (Rutherford & Huang, 1994). Long-term viable microfungal spores can be found in various cave substrates, such as cave air and sediments, but also in substrates accumulated by bats and other mammals visiting caves (marten, dormouse, etc.) or created by cave dwelling invertebrates (earthworm casts, arthropods faeces). The aim of our study was to estimate microfungal diversity in various cave substrates in several caves of the Slovak Karst National Park (Nováková, 2004, 2005).

Pidoplitchkoviella terricola (Ascomycetes, Xylariales) was isolated by Kirilenko from the rhizosphere of *Quercus robur* in Ukraine (CBS Fungi database) and until now it was known only from this one isolate. In 2002 this microfungal species was isolated by us from earthworm casts in the Domica Cave.

MATERIAL AND METHODS

A sample was collected from earthworm casts situated on speleothems (Fig. 1) in an adjoining corridor near the Japanese Teahouse in the Domica Cave (Slovak Karst National Park, Slovakia) on 24 April 2002.

The soil dilution method and three isolation media (Martin's agar with soil extract, Sabouraud's and beer

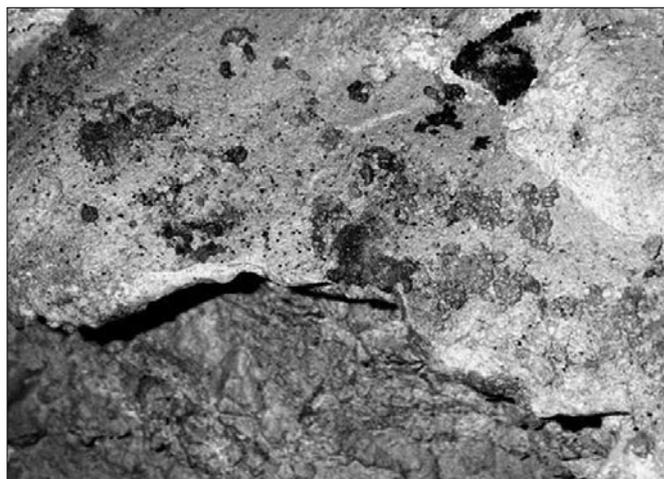


Fig. 1. A view on earthworm casts on the speleotheme, adjoining corridor near the Japanese Teahouse in the Domica Cave.

wort agars) with rose bengal, chloramphenicol and streptomycin were used for the isolation of microfungi (Garrett, 1981; Kreisel & Schauer, 1987). Three Petri dishes for each isolation medium were incubated in the dark at 25 °C for 7 days. Determination of isolated microfungi was carried out on malt extract agar (MEA), potato dextrose agar (PDA) and carrot agar (CA) (Kreisel & Schauer, 1987). A fungus that showed dark pigmented ascospores production on CA was identified as *Pidoplitchkoviella terricola* Kiril. following the criteria of predominantly non-ostiolate, dark brown ascospores and triangular ascospores.

Small agar blocks removed from ten days' colonies on PDA and MEA were fixed in a 2.5 % glutaraldehyde solution and a 0.2 M phosphate buffer followed by fixation in a 2% OsO₄ solution, dehydrated in a graded series of acetone solutions and by the critical point drying method (Kučerová – pers. comm.).

¹ Biology Centre, AS CR, v.v.i., Institute of Soil Biology, Na Sádkách 7, CZ-370 05 České Budějovice, Czech Republic, E-mail address: alena@upb.cas.cz

Observations were done on a JEOL 6300 scanning electron microscope.

DESCRIPTION OF THE ISOLATED STRAIN OF *PIDOPLITCHKOVIELLA TERRICOLA* KIRIL.

Colonies growing on CA attained 1.0-1.2 cm in diameter, and were sulcate, brown-black with dark pigmented reverse (Fig. 2A); colonies on PDA attained 0.5-0.6 cm in diameter, and were sulcate with radial wrinkles at the margins, natural white with a greyish to black centre, a yellowish pigmented reverse, and a light yellow pigment diffusing to the medium was observed; colonies on MEA attained 2-2.2 cm in diameter, white to cream, wrinkled, after 12-14 days with visible darker pigmented areas due to the production of ascomata; reverse beige to light ochre (Fig. 2B).

Ascomata superficial, spherical, without ascomatal hairs, predominantly non-ostiolate, ostiolate in some cases (Fig. 3A, B, 4A), dark brown, 50-120 μm in diameter, ascomatal wall pale, with *textura epidermoidea*, asci cylindric-clavate, fasciculate, thin-walled, 8-spored, evanescent, 28-36 x 7-9 μm (Fig. 3C); ascospores 1-celled, aseptate, triangular in lateral view, pale brown, 7-9 x 2,8-3,5 μm , without germ pores (Fig. 3D, 4B). No anamorph was observed.

Examined material: strain CMF 1376 (Collection of Microscopic Fungi ISB)(Nováková, 2007), earthworm casts, the Domica Cave, Slovak Karst National Park, Slovakia, coll. A. Nováková, 24 April 2002.

DISCUSSION

Unfortunately only one strain of *P. terricola* could be studied in the present study, although several samples of earthworm casts and also samples of cave sediments from various sites of the Domica Cave were collected in the same time and repeatedly in the following years, this fungus did not succeed in being isolated again. Morphological features of our isolate corresponded with Kirilenko's original diagnosis (predominantly created non-ostiolate, but occasionally ostiolate ascomata). However, false statements about *P. terricola* resulting probably from incorrect translations

of original paper were published in former times, e.g., only non-ostiolate ascomata (von Arx et al., 1986) and the isolation from the rhizosphere of *Quercus rubra* (Suh & Blackwell, 1999). This fungus also has some similarities with some species of the family *Chaetomiaceae* (Ascomycetes, Sordariales) such as *Chaetomidium triangulare* Stchigel & Guarro (Stchigel et al., 2004), *Chaetomium trigonosporum* (Marchal) Chivers and *C. microascooides* Guarro and with other ascomycete species with triangular ascospores, such as *Microascus trigonosporus* C. W. Emmons & B. O. Dodge var. *trigonosporus* and *M. trigonosporus* var. *macrosporus* G. F. Orr. *Chaetomidium triangulare* has also non-ostiolate ascomata without hairs, but differs from *P. terricola* by having triangular ascospores with a visible germ pore and in the *textura angularis* of the peridial wall. *Chaetomium microascooides* has ostiolate and beaked ascomata with straight or reflexed hairs, but *C. trigonosporum* differs from *P. terricola* mainly by having seta-like hairs and *Scopulariopsis* anamorph (von Arx et al., 1986). The species of the genus *Microascus* differs by ostiolate ascocarps (Domsch et al., 2007), obovate to spherical asci, straw coloured to reddish brown ascospores, and by the presence of *Scopulariopsis* or *Wardomyces* anamorphs (von Arx et al., 1988; Stchigel et al., 2004).

Kirilenko's original diagnosis shows affinities of the genus *Pidoplitchkoviella* to the genera *Microascus* Zukai, *Pithoascus* Arx, (Microascaceae) *Melanospora* Corda (Ceratostomataceae), and *Tripospora* Sacc. (Coryneliaceae). On the contrary, von Arx et al. (1988) placed this genus as a member of the family Pithoascaceae (Sordariaceae Ascomycetes; Sphaeriales) together with the genera *Pithoascus*, *Faurelina*, and *Leuconeurospora*, whereas ascospores without germ pores are typical of this family. Currently *Pidoplitchkoviella* is included to the family Xylariaceae (Xylariales, Xylariomycetidae, Sordariomycetes) (Lumbsch & Huhndorf, 2007). This family includes mainly stromatic ascomycetes with perithecial ascomata with hairs and dark brown pigmented ascospores (Hawksworth et al., 1996). Although *P. terricola* is non-stromatic species with

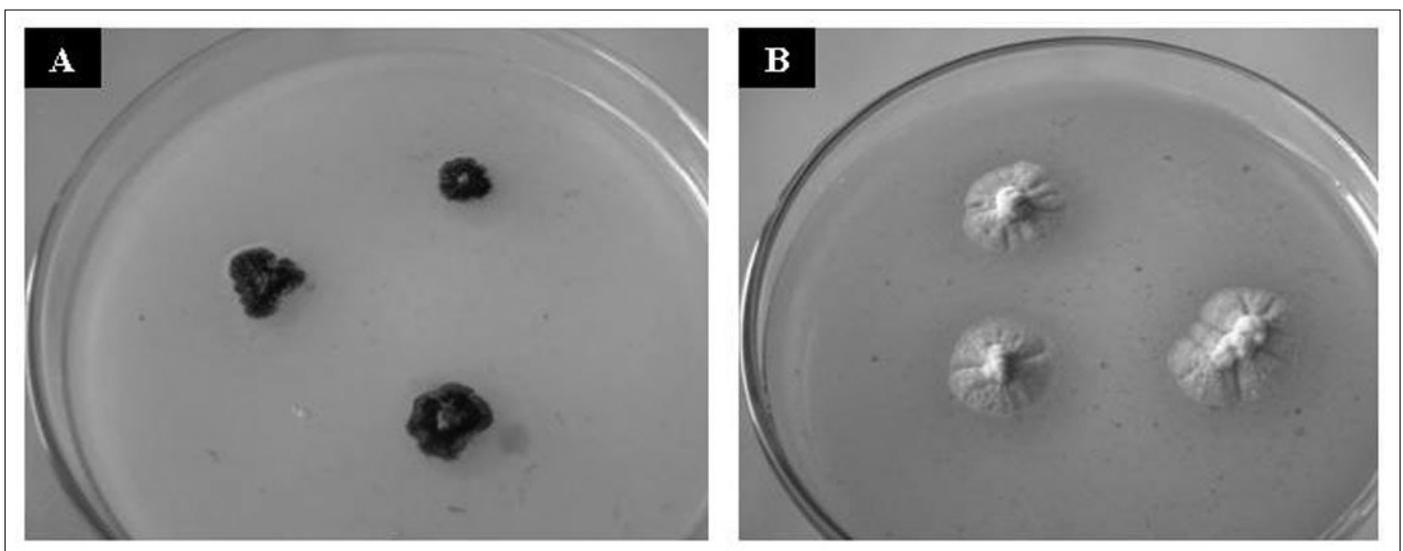


Fig. 2. Colonies of *P. terricola* on carrot (A) and potato dextrose (B) agars, 10 days at 25°C.

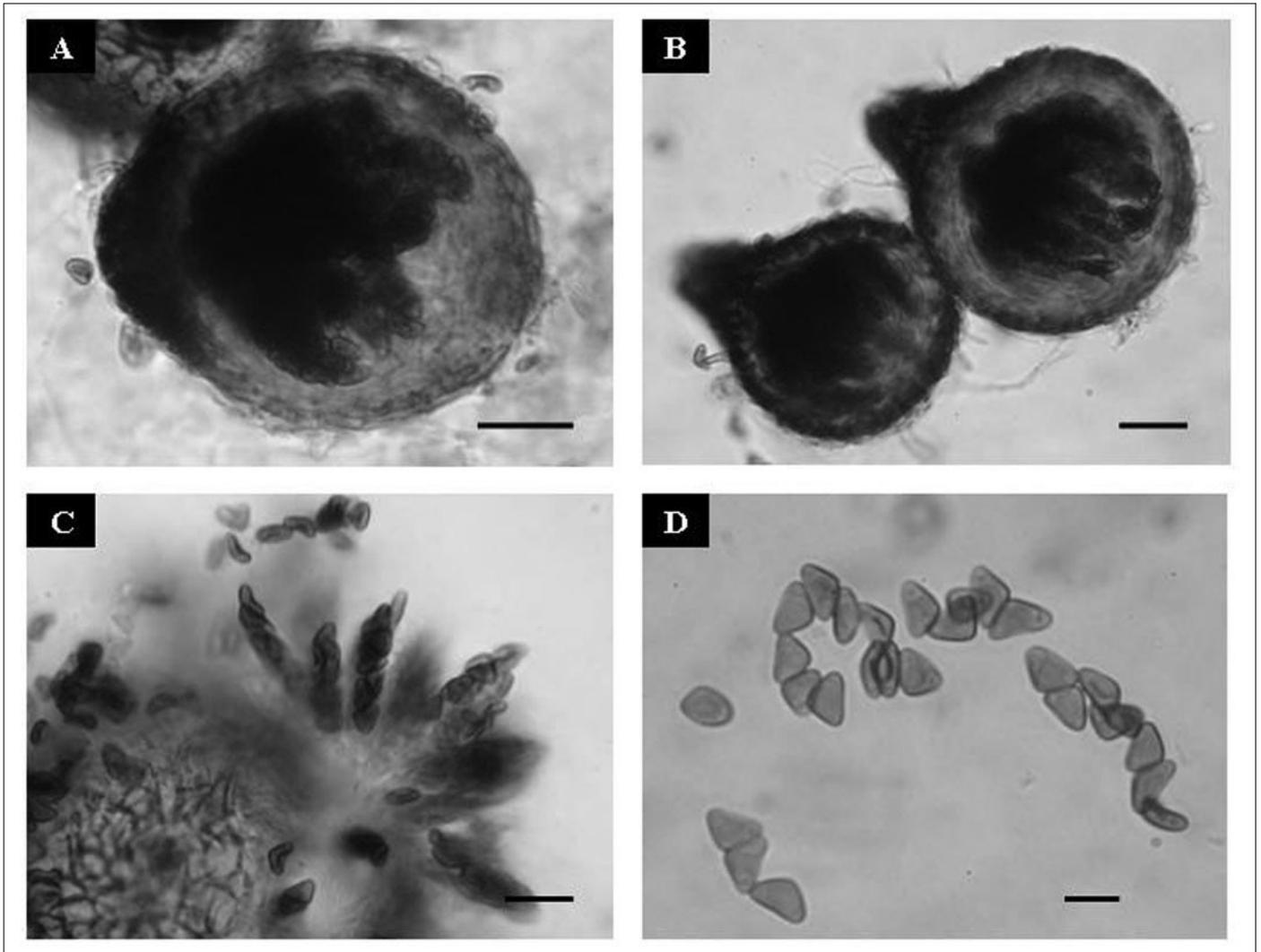


Fig. 3. A – Non-ostiolate ascocarp, B – ostiolate ascocarps, C – elongate asci with ascospores D – triangular ascospores (bar = 10 μ m).

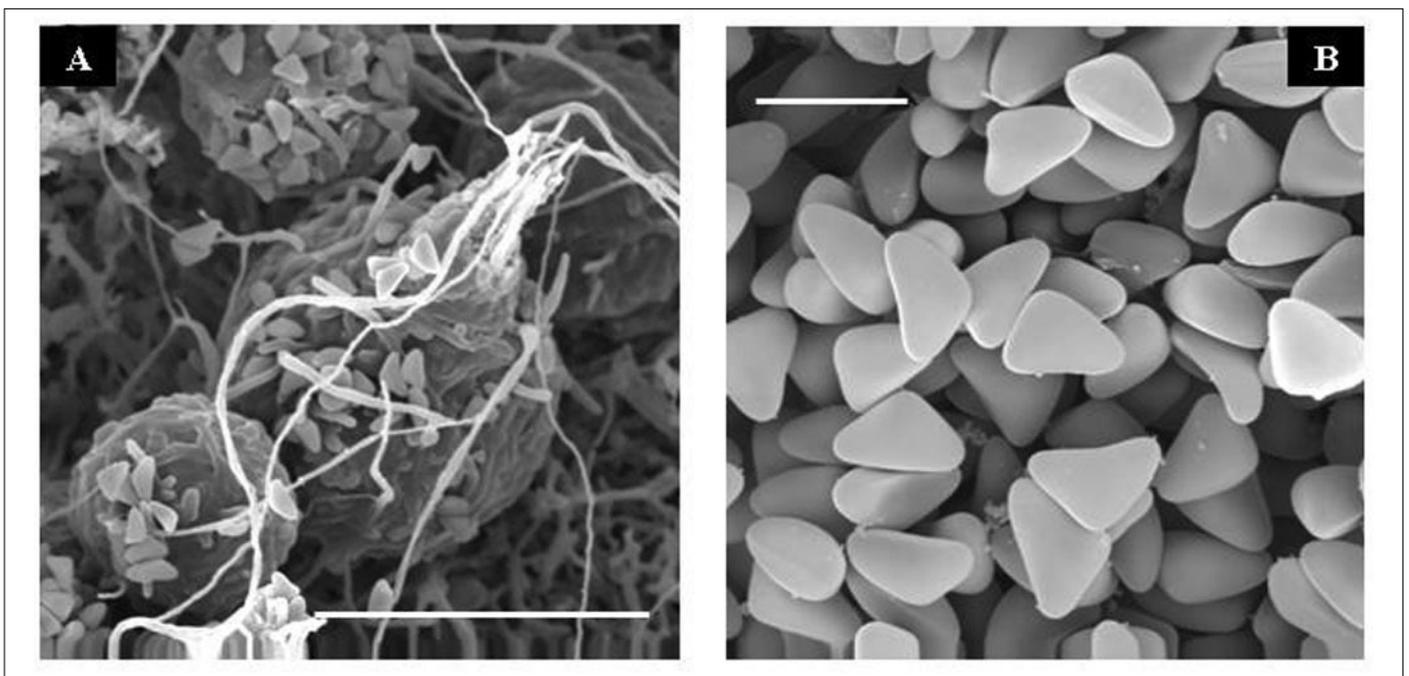


Fig. 4. SEM micrographs of *P. terricola*. A – ascocarps (bar = 50 μ m), B – triangular ascospores (bar = 10 μ m).

light pigmented ascospores, and with predominantly globose or subglobose cleistothecial, non-ostiolate ascomata without hairs, considering the SSU rDNA analyses, *P. terricola* was placed finally in the clades of Xylariales (Suh & Blackwell, 1999).

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