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Diplogelasinospora princeps (Sordariales), the first record in the Czech Republic

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Diplogelasinospora princeps, *Sordariales*, was isolated from the root of a sessile oak (*Quercus petraea*) in the Křivoklátsko region (Czech Republic) during a study of the endophytic mycoflora of roots. This is not only the first record in the Czech Republic, but probably the first record from Europe, too. Growth of the isolated strain under different conditions was tested.

Key words: roots, bark, *Diplogelasinospora princeps*, Křivoklátsko region, endophytes, *Quercus petraea*, oak

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Diplogelasinospora princeps, patřící do řádu *Sordariales*, byla izolována při studiu endofytické mykoflóry kořene dubu zimního (*Quercus petraea*) na Křivoklátsku v České republice. Tento nález je první nejen z České republiky, ale pravděpodobně i z Evropy. U nalezeného kmene byla testována růstová rychlost při různých teplotách a na různých agarových médiích.

INTRODUCTION

Diplogelasinospora princeps Cain 1961 was the first described species of *Diplogelasinospora* (Cain 1961). This genus is classified in the family *Sordariaceae*, order *Sordariales* (Hawksworth et al. 1995). So far three species of this genus are known: *D. grovesii*, *D. inaequalis*, *D. princeps* (Udagawa et al. 1973). Probably all strains of these species isolated and deposited by other mycologists were found in North America, Japan or South-east Asia. They were obtained from soil or flax seed (Cain 1961, Udagawa and Horie 1972, Udagawa et al. 1973, Anonymus 1996, Huang and Schmitt 1975).

MATERIALS AND METHODS

This species was isolated in October 1997 from peridermal bark of a 3 cm thick root of sessile oak (*Quercus petraea*) in an oak wood near the village of Nižbor in

the Křivoklátsko region, Central Bohemia, Czech Republic. Roots were studied in order to learn their endophytic mycoflora.

The roots were brushed under running water, their surface sterilised (96% ethanol 1 min., sodium hypochlorite (NaClO) 3 min., 96% ethanol 0.5 min.), cut and separated into wood, subperidermal bark and peridermal bark. Pieces of tissue were laid on 2% malt extract agar and incubated at room temperature for four weeks.

Growth of the isolated strain was tested on 2% malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oat-meal agar (OA). Incubation on MA2 was conducted at seven different temperatures (5, 15, 25, 30, 37, 42 and 45 °C). Mycelium of the tested strain was transferred to three Petri dishes per medium and temperature.

RESULTS AND DISCUSSION

Description of *Diplogelasinospora princeps* CCM 8255

Only a single strain of this fungus with the working designation DA/T/V2 was isolated. This strain was freeze-dried and also preserved under mineral oil. It was deposited as CCM 8255 in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, Czech Republic and as CCF 3188 in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic.

Macroscopic description

(Comparison of growth rates under different conditions are given in the tables 1 and 2)

MA2, 25 °C: colonies white-brown to brown, low, cottony, exudate light brown to brown, reverse dark grey-blue in the centre and dark brown on margins. Perithecia soon formed.

PDA, 25 °C: colonies brown, low, cottony, exudate absent, reverse dark grey-blue in centre and dark brown on margins. Perithecia formed sooner than on MA2, but later than on OA.

PCA, 25 °C: colonies green-brown, low, cottony, exudate absent, reverse green-brown. Perithecia formed sooner than on MA2, but later than on OA. Conidia abundant.

OA, 25 °C: colonies white-grey brown to brown, low, cottony, exudate clear, reverse dark grey-blue to brown. Perithecia appeared early, abundant.

MA2, 5 °C: colonies white to white brown, low, cottony, exudate absent, reverse green-brown. Perithecia were not observed during this study.

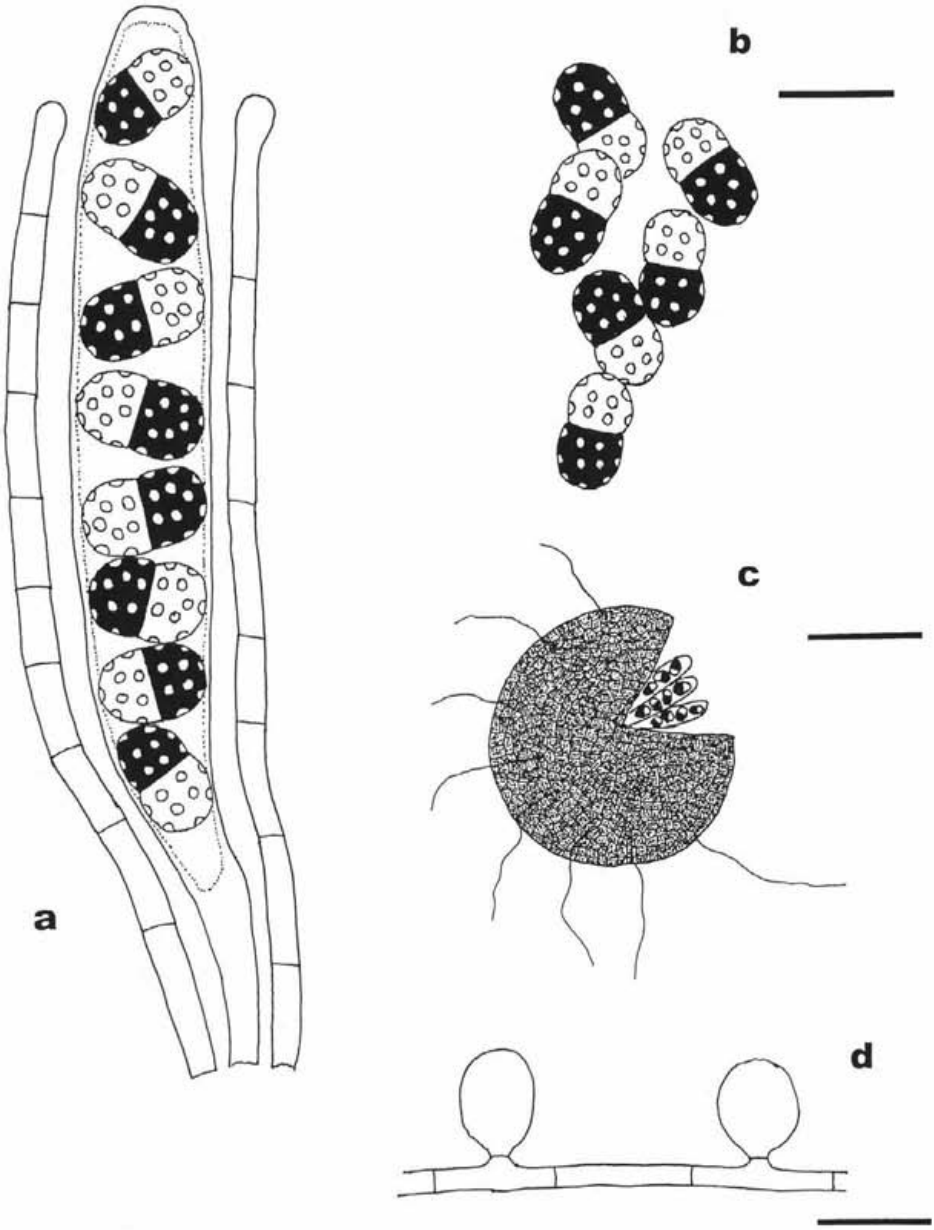

Fig. 1. *Diplogelasinospora princeps* – A: ascus with ascospores and paraphyses. B: two-celled ascospores. C: ascoma. D: conidia. Scale bar for A, B = 20 μm, for C = 150 μm, for D = 10 μm.

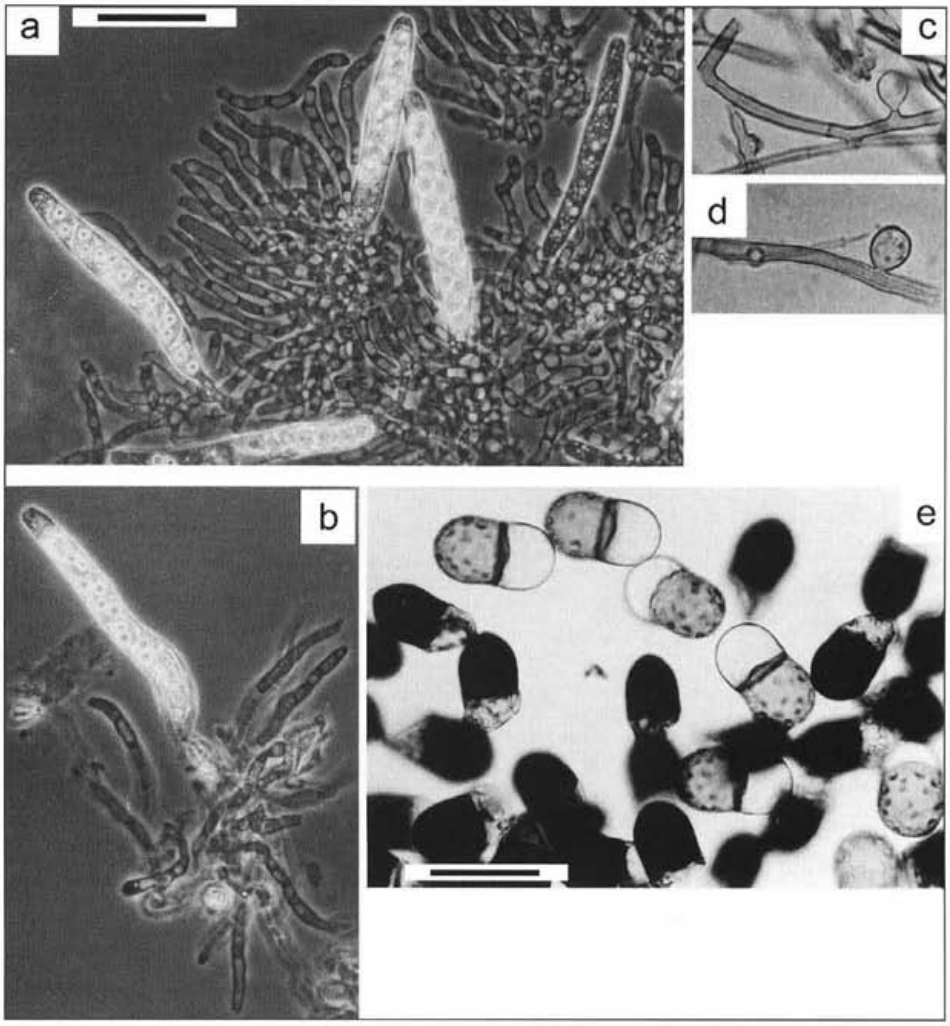

Fig. 2. *Diplogelasinospora princeps* – A, B: young asci with ascospores. C, D: conidia, E: two-celled ascospores. Scale bar for A, B = 50 μm, for C-E = 20 μm.

MA2, 15 °C: colonies white to white-brown, low, cottony, exudate absent, reverse dark grey-blue in the centre and green-brown on the margins. Perithecia were not observed during this study.

MA2, 30 °C: colonies grey-brown to brown, cottony with elevated central circle, exudate absent, reverse dark grey-blue in the centre and dark brown on the margins. Perithecia few.

MA2, 37 °C: colonies white-brown to brown, cottony with elevated central circle, exudate pale to yellow, reverse dark grey-blue in the centre and dark brown on the margins. Perithecia scarce.

MA2, 42 °C: colonies light brown, cottony with elevated central circle, exudate absent, reverse light brown, dark on the margins. Perithecia were not observed during the study.

MA2, 45 °C: growth nil.

Table 1. – Growth on different media at 25 °C.

Medium	Colony diameter		
	7 days (mm)	10 days (mm)	14 days (mm)
MA2	15–21	26–31	42–47
OA	20–24	32–34	55–57
PDA	26–28	43–46	69–72
PCA	34–37	57–61	70–74

The studied strain grows fastest on PCA medium at 25 °C and on MA2 at a temperature 30 °C. No growth was recorded at a temperature of 45 °C. Perithecia develop earliest and most abundant on OA medium.

Table 2. – Growth on MA2 at different temperatures.

Temperature	Colony diameter		
(°C)	7 days (mm)	10 days (mm)	14 days (mm)
5	0–2	1–4	4–8
15	12–13	25–27	42–45
25	15–21	26–31	42–47
30	73–78	>90	>90
37	48–49	72–80	>90
42	3–4	6–9	14–18
45	0	0	0

Microscopic features

Hyphae pale brown, 2–4 μm wide, smooth, with cells 13–35 μm long. Ascomata (Fig. 1c) dark brown, globose or subglobose, 250–385 μm in diam. Ostiolum not observed. Peridium dark brown, with angular cells 4–10 × 4–10 μm. Depending on medium used, the first ascomata appear after 14 days of incubation, but most frequently after 4–6 weeks.

Asci (Fig. 1a, 2a, b) cylindrical, 150–170 × 10–17 μm, 8-spored, non-amyloid, broadest in the upper part, soon evanescent. Paraphyses hyaline, cylindrical, 120–150 × 3.5–5 μm, swollen up to 7 μm. Ascospores (Fig. 1b, 2e) at first

hyaline, ovate, one-celled, later becoming two-celled. Mature ascospores pitted (pits 1–1.5 μm in diam.), 19–23 × 11–13 μm, one cell is dark, semiglobose to semisubglobose, 11–15 × 11–14 μm, the other cell is hyaline, semiglobose to semisubglobose, 7–10 × 11–14 μm.

Only aleurioconidia (sessile blastoconidia) (Fig. 1d, 2c,d) were observed. These are elliptical, hyaline to pale brown and measure 8–11 × 4–5 μm. They arise singly, terminal or lateral on hyphae. Arthroconidia were not found.

Occurrence and discussion

Udagawa and Horie (1972) observed luxuriant growth in their isolate on PCA at 37 °C. They did not study the growth at different temperatures. In the present study the fastest growth of the isolated strain was recorded at 30 °C.

All records of this species known to the present author are hitherto from soil or plant plant (Anonymus 1996, Huang and Schmitt 1975). The studied strain was found in peridermal bark of an oak (*Quercus petraea*) root. It is part of the inner (endophytic) mycoflora of a plant, but it is also close to rhizosphere and to soil. During the author's study it was isolated only once. It is not a typical endophyte, but is probably capable of endophytic life.

This fungus was recorded in North America and Japan (Cain 1961, Udagawa and Horie 1972, Anonymus 1996, Huang and Schmitt 1975). Besides, *Diplogelasinospora grovesii* was found in Japan (Udagawa and Horie 1972), and *D. inaequalis* in New Guinea (Udagawa et al. 1973). The present author has not found any record of the occurrence of *Diplogelasinospora princeps* in the Czech Republic nor Europe.

There were observed differences in presence and size of some morphological structures between the strain from the Czech Republic and the strains found in Canada and in Japan (Table 3). The ascomata of the strain from the Czech Republic are similar in size to the ascomata from Canada (Cain 1961) and from Japan (Udagawa and Horie 1972). However, the asci of the strain from the Czech Republic are shorter and narrower than the asci of the strains from Canada (Cain 1961) and from Japan (Udagawa and Horie 1972). The ascospores are shorter than in non-European strains. Udagawa and Horie (1972) observed two types of conidia (aleurioconidia and arthroconidia). Cain (1961) did not observe any of them. In the present study aleurioconidia were observed. They are similar in shape and in size to the conidia of the Japanese strain. Sigler and Carmichael (1983) did not observe arthroconidia during their study of the strain NHL 2504 from Japan. The author of the present study did not observe this type of conidia either.

Table 3. – Comparison of the size of some morphological structures in three strains of *Diplogelasinospora princeps* (in μm)

Morphological structure	strain from Canada Cain (1961)	strain from Japan Udagawa and Horie (1972)	strain from the Czech Republic (present study)
Size of perithecia	250–400	325–400	248–387
Length of asci	180–220	160–200	150–170
Width of asci	18–21	16–20	10–17
Length of ascospores	19–25	20–27	19–23
Width of ascospores	10–14	10–15	11–13
Length of aleurioconidia	*	8–12	8–11
Width of aleurioconidia	*	3,6–5	4–6
Length of arthroconidia	*	7–14	absent

* – not recorded

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