Cryptosporiopsis radicicola and Pezicula eucrita – neglected species of microscopic fungi in the Czech Republic

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Cryptosporiopsis radicicola was frequently found during a study of endophytic mycobiota of oak roots in the Czech Republic from 1996 to 1999. This is the first record from this region. Pezicula eucrita was isolated from the bark of spruce stem in South Moravia. This species was frequently recorded during the revision of collections of Pezicula from conifers deposited in herbaria in the Czech Republic. A lot of strains of the related species Pezicula cinnamomea were obtained in pure culture. Till now, this fungus had only been known in the Czech Republic from herbarium specimens.

Key words: endophytes, first record in the Czech Republic, roots, bark, Picea abies, Quercus, Pezicula cinnamomea, Prunus, Pezicula livida

Novotný D. (2003): Cryptosporiopsis radicicola a Pezicula eucrita – přehlížené druhy mikroskopických hub v České republice. – Czech Mycol. 55: 57–72

V letech 1996–1999 byl při studiu endofytické mykoflóry kořenů dubů hojně pozorován druh Cryptosporiopsis radicicola. Jedná se o první záznam tohoto druhu z území České republiky. Druh Pezicula eucrita, který byl do této doby vzácně zaznamenáván na území České republiky, byl izolován z kůry smrku. Při revizi herbářových položek Pezicula z jehličnanů byl zmiňovaný druh zjištěn z více lokalit v České republice. Příbuzný druh Pezicula cinnamomea byl mnohokrát získán v čisté kultuře. Do této doby byla tato houba v České republice uchovávána pouze ve formě herbářových položek.

Introduction

Cryptosporiopsis and Pezicula species are frequently recorded during the study of endophytic mycobiota of different species of plants (Bissegger and Sieber 1994, Collado et al. 1996, Fisher et al. 1995, Petrini 1984, Kowalski and Kehr 1992, Sieber et al. 1991).

The genus *Pezicula* is classified in the family *Dermateaceae*, order Helotiales (Kirk et al. 2001). Twenty-six species of this genus are known at present. Most of them are associated with *Cryptosporiopsis* anamorphs.

So far, seven species of Cryptosporiopsis with unknown teleomorphic states were described. C. radicicola and C. melanigena occurring in oak roots belong to

this group (Verkley 1999). C. radicicola has been recorded in roots of oak, beech, pine and spruce up to now. C. melanigena has been found in roots of oak only (Ahlich and Sieber 1996, Kowalski and Bartnik 1995, Kowalski et al. 1998).

Pezicula eucrita was described by Karsten, but most authors treat it as synonym of P. livida. Cultivation on agar media and genetic studies showed the differences between these species. P. eucrita differs morphologically from P. cinnamomea (syn. P. livida) and P. sporulosa in the presence of 4 ascospores per ascus. Eight spores develop in asci of P. cinnamomea and P. sporulosa. P. eucrita occurs predominately on conifers (Abies, Larix, Picea, Pinus, Pseudotsuga) and was also recorded on Acer platanoides and Carpinus betulus (Anonymus 2001, Verkley 1999).

P. eucrita has been recorded very sparsely in the Czech Republic, because it has not been distinguished from P. livida. To date, no cultures of Pezicula nor Cryptosporiopsis species have been reported from the Czech Republic.

MATERIALS AND METHODS

Strains of Cryptosporiopsis radicicola were frequently isolated in the years 1996–1999 from bark and wood of roots of pedunculate oak (Quercus robur) in an oak stand near the village Dešov near Moravské Budějovice in Southwest Moravia and of sessile oak (Quercus petraea) in four oak stands (Dřevíč, Nižbor, Křivoklát and Vlastec) in the Křivoklát region in Central Bohemia (Fig. 1). Some strains from the Křivoklát region were deposited in culture collections of fungi.

The strain of *Pezicula eucrita* was isolated by L. Jankovský from bark of a stem of spruce (*Picea abies*) from a spruce stand near the village Jinošov near Náměšť nad Oslavou in South Moravia in October 1999.

The roots and the sample of the stem 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 in Petri dishes and incubated for four weeks at room temperature. The sample of the spruce stem was washed in sterile water, its surface sterilised (96 % ethanol 1 min., sterile water, dipping in ethanol), placed on 2 % malt extract agar and incubated at room temperature.

Growth of the isolated strains was tested on 2 % malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oatmeal agar (OA). Incubation on MA2 was conducted at five different temperatures (5, 15, 25, 30, 36 °C). Tested strains were cultivated on three Petri dishes per for each medium and each temperature.

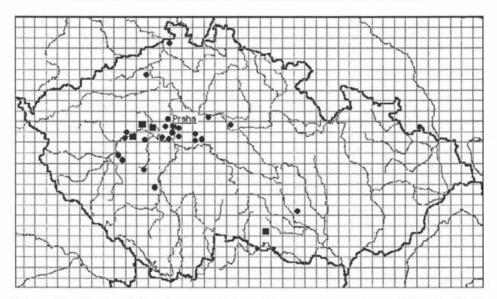


Fig. 1. Distribution of Pezicula eucrita (●) and Cryptosporiopsis radicicola (■) in the Czech republic.

Some of the isolated strains were deposited in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, Czech Republic or in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic.

The examined specimens were loaned from two herbaria in the Czech Republic (PRM and BRNM). Sixty specimens of *Pezicula* from conifers were revised.

RESULTS AND DISCUSSION

Cryptosporiopsis radicicola Kowalski et Bartnik 1995

Many strains of this species were isolated. Six of them were regarded representative:

CCM 8294: root of Quercus petraea, Nižbor, Křivoklát region, Czech Republic, No. DA/L/1, X. 1997

CCM 8295: peridermal bark of root of Quercus petraea, Dřevíč, Křivoklát region, Czech Republic, No. CL/T/V1, VIII. 1997

CCM 8299: root of Quercus petraea, Dřevíč, Křivoklát region, Czech Republic, No. FC/L/10, X. 1999

CCF 3232: root of Quercus petraea, Dřevíč, Křivoklát region, Czech Republic, No. ER/L/7, X. 1997

CCF 3233: peridermal bark of root of Quercus petraea, Křivoklát, Křivoklát region, Czech Republic, No. EK/N/V3, VII. 1997

CCF 3234: root of Quercus petraea, Nižbor, Křivoklát region, Czech Republic, No. DC/L/10, X. 1997

Macroscopic charaters

(A comparison of growth rates on different cultivation media is given in Table 1) MA2, 25 °C (Fig. 2a): Colonies whitish brown to brown, fasciculate with prominent elevated central circle, exudate absent or clear to light brown, reverse brown in the centre and whitish on margins, soluble pigment absent or brown. Conidia and sporodochia more readily formed than on PDA, but later than on PCA and OA.

PCA, 25 °C: Colonies white or whitish to brown, fasciculate with prominent elevated central circle, on the margin adpressed, low, exudate absent or clear to light brown, reverse whitish to green-brown or brown, soluble pigment absent. Conidia and sporodochia appeared early (after 17–20 days).

OA, 25 °C: Colonies whitish or brown with whitish margin, low, with prominent elevated central circle, exudate absent or clear to light brown, reverse dark grey-blue to brown, soluble pigment absent. Conidia and sporodochia appeared early (after 20–24 days), but later than on PCA.

PDA, 25 °C (Fig. 2b): Colonies whitish brown to brown, fasciculate with elevated central circle, on the margin adpressed, compact, sometimes sulcate, exudate absent or clear to light brown, reverse maroon or dark brown in the centre and whitish on margins, soluble pigment absent. Conidia and sporodochia were formed lately.

The studied strains grow most quickly on PDA medium. The slowest growth was on MA2. Among the studied strains differences in growth rates were observed. The sporodochia and conidia arise most readily on PCA medium.

Table 1. Growth of Cryptosporiopsis radicicola (strains ER/L/7, EK/N/V3, FC/L/10, CL/T/V1, DA/L/1) on different media at 25 °C

Medium	Colony diam.		
	7 days (mm)	10 days (mm)	14 days (mm)
MA2	19–28	23-42	30-50
PCA	21-30	27-43	33-51
0A	17-34	26-48	33-65
PDA	30-37	41-58	54-74

Microscopic characters

Hyphae white to light brown, 2–6 μ m wide, smooth. Conidiomata are sporodochiae, orange-brown, 240–300 μ m in diam. Depending on the medium used, the first sporodochia appear after 17 days of incubation. Macroconidiophores (Figs. 2c,d; 3a) smooth, hyaline or light brown, branched, with hyaline, smooth,

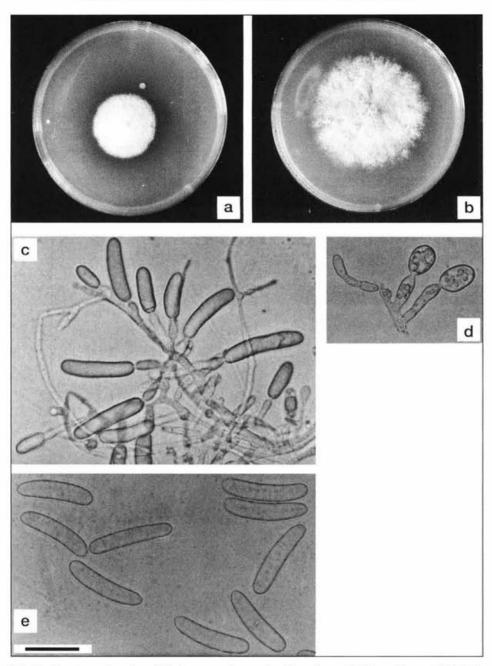


Fig. 2. Cryptosporiopsis radicicola – a: colony after 2 weeks on MA2 medium at 25° C; b: colony after 2 weeks on PDA medium at 25° C; c, d: macroconidiophores with phialides and macroconidia; e: macroconidia. Scale bar for a-b = 25 mm, for c-e = 20 μ m.

cylindrical phialides 12–20 × 4–5 μ m. Macroconidia (Figs. 2c,e, 3b) straight to slightly curved, hyaline, smooth, 23–30 × 5–7 μ m. Young macroconidia 1-celled, old macroconidia with 1–3 septa, arising first solitarily, than in sporodochia. Microconidiophores (Fig. 3c) smooth, hyaline or light brown, with smooth hyaline phialides, 8–10 × 2.5–3.5 μ m. Microconidia (Fig. 3d) obovate, hyaline, 4–6 × 1–2 μ m. Dark setae in sporodochia were not observed.

Occurrence

This species is similar to *Cryptosporiopsis melanigena*, which was described from oak roots, too. They differ in growth rates (most different at 25 °C), in culture pigmentation, size of macroconidia and shape of setae (Kowalski et al. 1998). Kowalski & Bartnik (1995) described sporodochia of this species with dark setae, but the author of the present study observed no setae.

Cryptosporiopsis radicicola was described from oak roots (Quercus robur) in Poland (Kowalski and Bartnik 1995). So far, this species has been recorded in roots of Q. robur in Poland (Bartnik 1996), Q. petraea in Austria (Kowalski et al. 1998), beech (Fagus sylvatica; Switzerland, Germany), fir (Abies alba; Switzerland), spruce (Picea abies; Switzerland, Germany) and pine (Pinus sylvestris; Switzerland, Germany) (Ahlich and Sieber 1996).

In the Czech Republic the author has frequently isolated this species from roots of *Quercus robur* and *Q. petraea*. This report is the first record from the Czech Republic. The species belongs to the most frequently recorded species from oak roots in Poland (Bartnik 1996) and the Czech Republic (Novotný 2002). It was not observed in branches, stems or leaves of plants.

The similar species *Cryptosporiopsis melanigena* was not recorded during the studies of the author. This species was described in Austria (Kowalski et al. 1998) and it probably occurs in the Czech Republic, too.

Pezicula eucrita (P. Karst.) P. Karst.

A single strain of this fungus (as JSM15/3) was isolated. It was deposited as CCM 8297 in the Czech Collection of Microorganisms. It is preserved under mineral oil. Twenty-four specimens of *P. eucrita* were recorded during the revision of thirty herbarium collections of *Pezicula* from conifers (Fig. 1).

Specimens examined

Central Bohemia, Roblín, *Picea abies*, leg. J. Velenovský, V. 1925, det. J. Velenovský as *Dermatea livida*, rev. D. Novotný (PRM 148974). – Central Bohemia, Mnichovice, *Picea abies*, leg J. Velenovský, VII. 1925, det. J. Velenovský as *D. livida*, rev. D. Novotný (PRM 150686). – Central Bohemia, Mnichovice-Záduší, bark of *Pinus*, leg. J. Velenovský, 2. VII. 1926, det.

J. Velenovský as D. livida, rev. D. Novotný (PRM 150654). – Central Bohemia, Hrusice, Pinus sylvestris, leg. J. Velenovský, VII. 1926, det. J. Velenovský as D. livida, rev. D. Novotný (PRM 148283). - Central Bohemia, Ondřejov, Pinus, leg. J. Velenovský, 12. XII. 1928, det. J. Velenovský as D. livida, rev. D. Novotný (PRM 149973). – Central Bohemia, Hřebečníky (distr. Rakovník), rotten branch of Picea abies, leg. J. Herink, 29. XI. 1940, det J. Herink as D. livida, rev. D. Novotný (PRM 669681). - Central Bohemia, Hracholusky (distr. Rakovník), rotten branch of Picea abies, leg. J. A. Herink, 6. XII. 1940, det. M. Svrček as D. livida, rev. D. Novotný (PRM 669689). - Central Bohemia, near the city of Prague, Hájek near Jeneč, abundantly on bark of rotten stem of Picea abies, leg. J. A. Herink, 17. VIII. 1941, det. M. Svrček as D. livida, rev. D. Novotný (PRM 669685). – Prague-Modřany, on bark of Pinus, leg. V. Vacek, 12. X. 1941, det. V. Vacek as D. livida, rev. D. Novotný (PRM 669682). - Prague-Modřany, on bark of Pinus and Picea abies, leg. V. Vacek, 23. XI. 1944, det. V. Vacek as D. livida, rev. D. Novotný (PRM 669686). - Central Bohemia, Vodopády near the village of Srbsko, bark of fallen stem, leg. M. Svrček, IX. 1949, det. M. Svrček as P. livida, rev. D. Novotný (PRM 895532). Prague-Krč, Picea abies, leg. O. Dvořák, 5. VII. 1958, det. M. Svrček as P. livida, rev. D. Novotný (PRM 614550). - Northern Bohemia, České středohoří mountains, Velemín, elevation point 391 m a.s.l., Picea abies, leg. M. Svrček, 18. XI. 1960, det. D. Novotný (PRM 895539). – West Bohemia, Svojkovice near Rokycany, Picea abies, leg. K. Cejp, 12. VIII. 1961, det. D. Novotný (PRM 780236). – West Bohemia, in the wood Zdar near Rokycany, Picca abies, 24. VIII. 1961, leg. K. Cejp, det. D. Novotný (PRM 780228). – Central Bohemia, Tuchoměřice near Prague, Picea abies, leg. A. Příhoda, 4. IX. 1961, det. A. Příhoda as D. livida, rev. D. Novotný (PRM 669698). – Central Bohemia, Brdy mountains, Nesvačily, Picea, leg. E. Wichanský, 8. IX. 1963, det. M. Svrček as P. livida, rev. D. Novotný (PRM 624044). - South Bohemia, Vrábsko near the wood of Čimelice, Kovářka, branch of Pinus sylvestris, leg. M. Svrček, 1. VIII. 1966, det. D. Novotný (PRM 626158). – North Bohemia, village Mezná near Hřensko, in wooded gorge under elevation point 346 m a.s.l., Pinus sylvestris, leg. M. Svrček, 11. VII. 1970, det. D. Novotný (PRM 712371). - Central Bohemia: Zdice, Holý vrch hill, on the bark of many Pinus sylvestris trees, leg. M. Svrček, 3. X. 1971, det. D. Novotný (PRM 895540). - Prague-Velká Chuchle, in wooded gorge Slivenecká rokle, Pinus sylvestris, leg. and det. M. Svrček as P. livida, 12. IX. 1972, rev. D. Novotný (PRM 895533). – Prague-Butovice, wood declining into Prokopské údolí valley, Pinus nigra, leg. M. Svrček, 2. VII. 1977, det. D. Novotný (PRM 895541). – Central Bohemia, Přerov nad Labem near Lysá nad Labem, Pinus sylvestris, leg. and det. D. W. Minter as P. livida, X. 1979, rev. D. Novotný (PRM 821678). – Central Bohemia, Veltruby near Kolín, Pinus sylvestris, leg. and det. D. W. Minter as P. livida, 9. X. 1979, rev. D. Novotný (PRM 821691).

- South Moravia, Jinošov near Náměšť nad Oslavou, bark of stem of spruce (Picea abies) from a spruce stand, sample of bark collected by L. Jankovský, isol. and det. D. Novotný, X. 1999.

Macroscopic characters

Comparisons of growth rates under different conditions are given in the Tables 2 and 3.

MA2, 25 °C (Fig. 4d): Colonies grey or brown, cottony, fasciculate, sulcate, elevated, exudate minute, clear to light brown, reverse umber to red-brown, pigment yellow-brown. Orange apothecia more readily formed than on PCA, but later than on OA. No sporodochia were observed.

PDA, 25 °C (Fig. 4b): Colonies whitish to yellowish in the centre and whitish on margins, fasciculate, cottony, elevated to high, exudate absent, reverse brown to maroon in centre, whitish on margins, between them black-brown, pigment yellow-brown. Sporodochia producing microconidia appeared, but no apothecia were recorded.

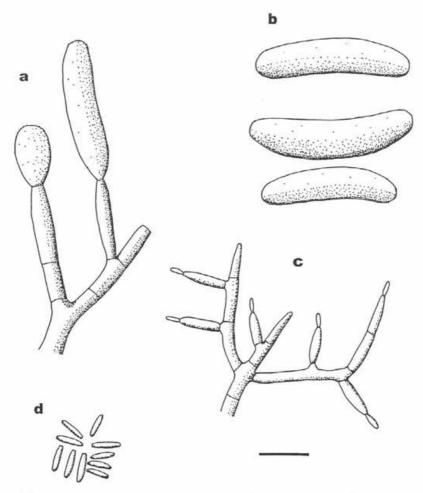


Fig. 3. Cryptosporiopsis radicicola – a: macroconidiophores; b: macroconidia; c: microconidiophores; d: microconidia. Scale bar for a-d = $10 \mu m$.

PCA, 25 °C (Fig. 4a): Colonies whitish, finely cottony, depressed with elevated centre, margin compact, exudate absent, reverse maroon in centre, whitish on margins, between them black brown, pigment yellow brown. Orange apothecia and sporodochia producing microconidia appeared.

OA, 25 °C (Fig. 4c): Colonies white-grey, cottony, fasciculate, elevated to high, margins lobed, exudate absent, reverse greenish in the centre and green on margins. Sporodochia appeared early (after 21–24 days). Orange apothecia appeared earliest of all tested media, abundant. No sporodochia were observed.

MA2, 5 °C: Colonies white, inconspicuous.

MA2, 10 °C: Colonies white to white-brown, fasciculate, elevated, exudate absent, reverse cream, in the centre orange-brown.

MA2, 15 °C: Colonies cream to brown, fasciculate, elevated, exudate absent or clear, pigment brown, reverse maroon.

MA2, 30 °C: Colonies fasciculate, brown, exudate dark, reverse dark grey-blue. MA2, 36 °C: no growth.

Table 2. Growth of Pezicula eucrita on different media at 25 °C

Medium	Colony diameter			
	7 days (mm)	10 days (mm)	14 days (mm)	
MA2	6–7	10-13	15-18	
OA	10-11	15-21	25-32	
PDA	11-14	16-20	22-26	
PCA	4.5-8	7–11	10-14.5	

Table 3. Growth of Pezicula eucrita on MA2 at different temperatures

Temperature (°C)	Colony diameter			
	7 days (mm)	10 days (mm)	14 days (mm)	
5	0–1	1-2	1-2.5	
10	1-2	2.5-4	3-6	
15	3.5-5.5	6-9	8-10	
25	6–7	10-13	15-18	
30	5-6	5-6	5-6	
36	0	0	0	

The studied strain grows most quickly on OA medium. No growth was recorded at a temperature of 36 °C. Apothecia arise earliest and most abundantly on OA medium. Sporodochia producing microconidia develop on PCA and PDA media. Daylight induces formation of apothecia and sporodochia.

Microscopic features

Mycelium hyaline to light brown, hyphae smooth, 3–7 μ m wide. Ascomata (apothecia) single or up to 4, subsessile, yellow, orange to red-brown apothecia, 0.3–1.2 mm diam. Depending on medium, the first ascomata appear after 6 weeks of incubation in light, but most frequently after 7–8 weeks. Asci (Figs. 5a, b; 6a)

NOVOTNÝ D.: CRYPTOSPORIOPSIS RADICICOLA AND PEZICULA EUCRITA

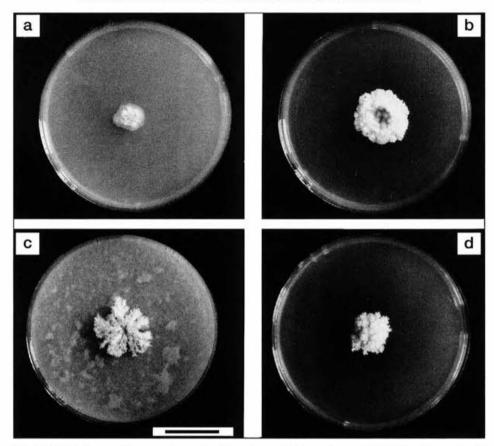


Fig. 4. Pezicula eucrita – a: colony after 2 weeks on PCA medium at 25°C; b: colony after 2 weeks on PDA medium at 25°C; c: colony after 2 weeks on OA medium at 25°C; d: colony after 2 weeks on MA2 medium at 25°C. Scale bar for a-d = 25 mm.

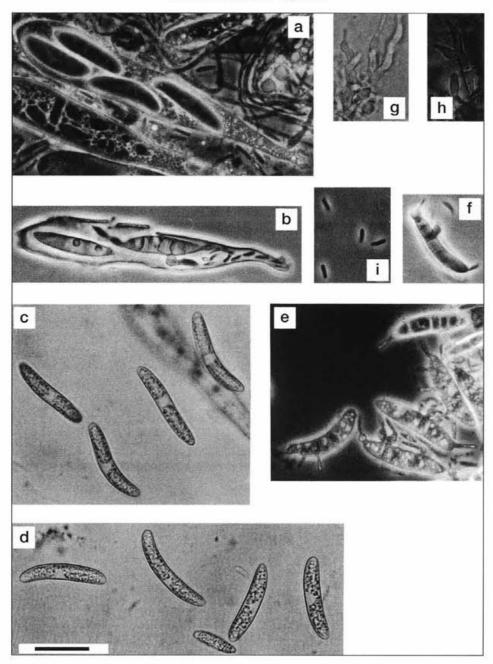


Fig. 5. Pezicula eucrita – a: ascus with four ascospores; b: ascus with ascospores and conidia developed from ascospores; c, d: ascospores; e, f: ascospores with conidia arising from them; g, h: anamorphic state – conidiophores with conidiogenous cells; i: conidia. Scale bar for a-i = 20 μ m.

clavate, 75–120 \times 12–19 μ m, with 4 as cospores, occasionally with 2–4 aborted as cospores, in Mėlzer's reagent negative. As cospores (Figs. 5c, d; 6b, c) smooth, hyaline, cylindrical, straight or curved, occasionally S-shaped, 1-celled, later with 4–7 septa, 20–35 \times 6–8 μ m. Length/width ratio (3.6-)4–5.2. Paraphyses filiform (Fig. 6a), hyaline, 80–100 μ m long, the top up to 2 μ m wide (strain CCM 8297) or swollen up to 7 μ m (herbarium specimens).

Conidiomata (sporodochia) superficial, at first spherical, then irregularly shaped, yellow-brown, 390–500 $\mu \rm m$ diam. Conidiophores (Figs. 5g, h; 6d) hyaline, septate, 20–30 × 2–4 $\mu \rm m$. Phialides hyaline, cylindrical, 6–11 × 2–3 $\mu \rm m$. Conidia (Figs. 5i, 6e) smooth, hyaline, cylindrical, 1-celled, 5–7 × 1–1.5 $\mu \rm m$. Macroconidophores and macroconidia not observed. Conidia develop from released ascospores or from ascospores closed in asci, too. They arise from small openings and from short cylindrical or bottle shaped phialides, width 2–5.5 × 2–3.5 $\mu \rm m$ (Figs. 5e, f; 6c). Conidia smooth, hyalinne, 1-celled, 5–7 × 1–1.5 $\mu \rm m$.

Occurrence

Pezicula eucrita was recorded from dead bark and cone scales of different species of Abies, Larix, Picea and Pinus in different countries of Europe and North America. It was isolated at the study of the endophytic mycobiota of branches of Carpinus betulus and from a branch of Acer platanoides (Verkley 1999). To date, this species was observed on Pinus sylvestris, P. nigra and Picea abies in the Czech Republic, especially in Central Bohemia and near the city of Prague. Many mycologists who collected this taxon live (or lived) in or near Prague. They probably preferentially studied the mycobiota of these parts of the Czech Republic.

Paraphyses of this taxon are branched, swollen up to 10 μm at the top (Verkley 1999). In the examined herbarium specimens, swollen paraphyses up to 7 μm were observed, but paraphyses of the studied strain CCM 8297 were filiform or very slightly swollen up to 2 μm . Branching of paraphyses was not observed during the present study.

There were observed differences in culture growing on oatmeal agar between the strain from the Czech Republic and strains studied by Verkley (1999). The Czech strain formed colonies 25–32 mm diam. after 14 days at 25 °C. Verkley (1999), who incubated strains at 18 °C, recorded 50–60 mm and 50–80 mm colonies after 14 days in strains from Europe and North America, respectively. During the present study the strain was cultivated at 25 °C, because it was not possible to set the temperature of any incubator in the Czech Collection of Microorganisms to 18 °C.

Notes on occurrence of Pezicula cinnamomea

Pezicula cinnamomea (DC.) Sacc. is a species related to P. eucrita. It has been frequently observed in the Czech Republic, but no cultures have been obtained.

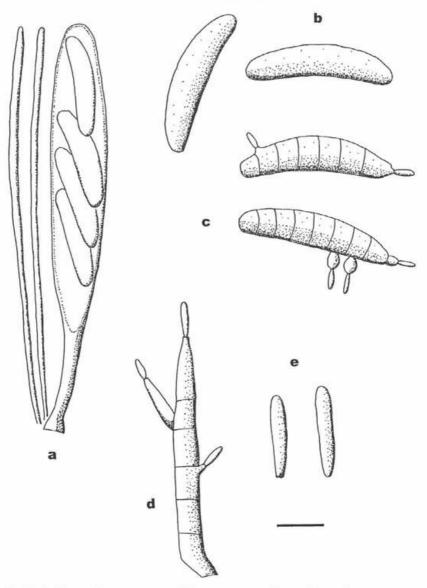


Fig. 6. Pezicula eucrita – a: ascus with ascospores and paraphyses; b: young one-celled ascospores; c: many-celled ascospores producing microconidia; d: conidiogenous cells and microconidia; e: microconidia. Scale bar for a = $20 \mu m$, for b-c = $10 \mu m$, for d = $6 \mu m$, e = $3 \mu m$.

The author has isolated many strains from oak branches in different places in the Czech Republic, one strain from branch of cherry and one from a branch of lime. The following ones were deposited in culture collections:

- CCM 8285: bark of branch of Quercus petraea, Dřevíč, Křivoklát region, Czech Republic, No. V2/SV5, V. 1998
- CCM 8302: bark of branch of Quercus robur, Jablonec nad Nisou Mšeno, Czech Republic, as No. K3K2, IV. 2000
- CCM 8301: bark of branch of Quercus robur, Bitouchov near Semily, Czech Republic, No. ZLB 4,5,6/V2, X. 1998
- CCM 8303: bark of branch of Prunus cerasus, near the village Holovousy, near Hořice, Czech Republic, No. TR3, VI. 1999
- CCM 8306: bark of branch of Tilia, cemetery, Jablonec nad Nisou, Czech Republic, No. L1K2, 1V. 2000
- CCF 3102: bark of branch of Quercus robur, Bitouchov near Semily, Czech Republic, No. ZLA H/H T V3, X. 1998
- CCF 3103: bark of branch of Quercus robur, Bitouchov near Semily, Czech Republic, No. ZLA 10/SK, X. 1998
- CCF 3235: bark of branch of Quercus robur, Jablonec nad Nisou Mšeno, Czech Republic, No. K1K2, IV. 2000

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