

First record of *Ceratocystis laricicola* (Ascomycota, *Ceratocystidaceae*) in the Czech Republic

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During the study of large larch bark beetle (*Ips cembrae*) mycobiota, the species *Ceratocystis laricicola* was isolated. It was detected in galleries and on the surface of the beetle body. This fungus is a well-known larch pathogen and this observation is the first report of its occurrence from the Czech Republic.

Key words: Ascomycota, ophiostomatoid fungi, *Larix decidua*, distribution, *Ips cembra*.

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Při studiu mykobioty lýkožrouta modřínového (*Ips cembrae*) byl z chodbiček a povrchu těla tohoto brouka izolován druh *Ceratocystis laricicola*, který je znám jako patogen modřínů. Jedná se o první nálezy z České republiky.

INTRODUCTION

Ophiostomatoid fungi are *Ophiostoma*-like genera usually associated with bark beetles. The genus *Ceratocystis* (*Ceratocystidaceae*, *Microascales*, *Sordariomycetes*, *Ascomycota*) belongs to the best known taxa in this group. *Ophiostoma* and *Ceratocystis* are not closely related; they belong to the orders *Ophiostomatales* and *Microascales*, respectively. They differ in anamorphs, cycloheximide sensitivity, composition of cell wall and molecular-genetic features (Kirk et al. 2001, Zipfel et al. 2006).

Ceratocystis species live more frequently in tropic regions than in the temperate zone. *Ceratocystis fagacearum* (cause of oak wilt in North America), *C. fimbriata*, *C. coerulescens*, and *C. polonica* are the best known species living in the temperate zone (Kile 1993, Marin et al. 2005).

Ceratocystis laricicola is associated with large larch bark beetle (*Ips cembrae*) living on larch. It is very similar and related to *C. polonica* (living in connection with bark beetles on spruce) and *C. fujiensis*, living in connection with *Ips subelongatus* on *Larix kaempferi* in Japan. *Ceratocystis laricicola* and *C. polonica* are known pathogens of larch and spruce, respectively (Harrington et al. 2002, Marin et al. 2005).

To the present day, two *Ceratocystis* species (*C. autographa* and *C. polonica*) have been recorded in the Czech Republic. *Ceratocystis autographa* was observed by Kotýnková-Sychrová (1966) in connection with the bark beetle *Tomicus piniperda*. *Ceratocystis polonica* was recorded repeatedly in the mycobiota of *Ips typographus* (Jankovský and Mrkva 1997, Novotný and Jankovský 2005).

Until recently, *Ceratocystis laricicola* had not been recorded in the Czech Republic. This is the first report. The aim of this paper is to provide information on the occurrence of this fungal species in the Czech Republic.

MATERIALS AND METHODS

During 2006, fungi living on adult beetles of *Ips cembrae* (*Curculionidae*, Coleoptera) and in their galleries in *Larix decidua* were investigated on Hudečka hill near Stříbrná Skalice in the Sázava region in Central Bohemia, Czech Republic. Twenty beetles and samples of detritus from their galleries were separately inoculated on Petri dishes with 2 % malt extract agar. After 14–21 days of incubation at room temperature the fungi were isolated and identified.

The isolated strain of *Ceratocystis laricicola* was preserved under mineral oil and deposited as CPPF 309 in the Collections of phytopathogenic microorganisms (Crop Research Institute, Division of Plant Medicine, Department of Mycology, Prague).

Growth of the isolated strain was tested on 2 % malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA), oatmeal agar (OA) and 0.1 % malt extract agar with a small piece of larch branch (MA01+LAR) at 20 °C (four replicates in all media). The strain was cultivated at 32 °C, too.

The identification was based on morphological, physiological and ecological features according to Redfern et al. (1987), Yamaoka et al. (1998), and Marin et al. (2005).

The strain *Ceratocystis polonica* (Siemaszko) C. Moreau CPPF 252 was used for comparison. It was obtained from a gallery of the bark beetle *Ips typographus* in Southwest Moravia near Mohelno, Czech Republic.

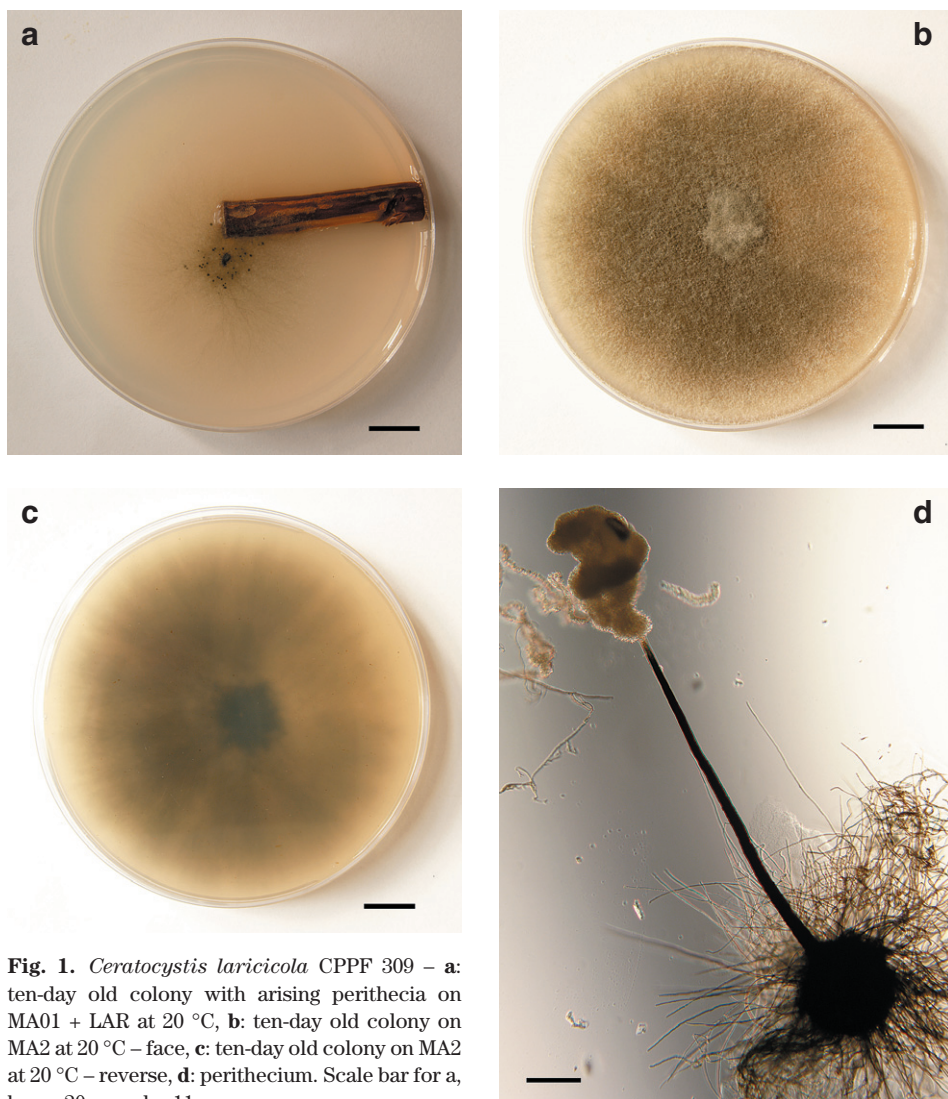


Fig. 1. *Ceratocystis laricicola* CPPF 309 – **a:** ten-day old colony with arising perithecia on MA01 + LAR at 20 °C, **b:** ten-day old colony on MA2 at 20 °C – face, **c:** ten-day old colony on MA2 at 20 °C – reverse, **d:** perithecium. Scale bar for a, b, c = 20 mm, d = 11 μ m.

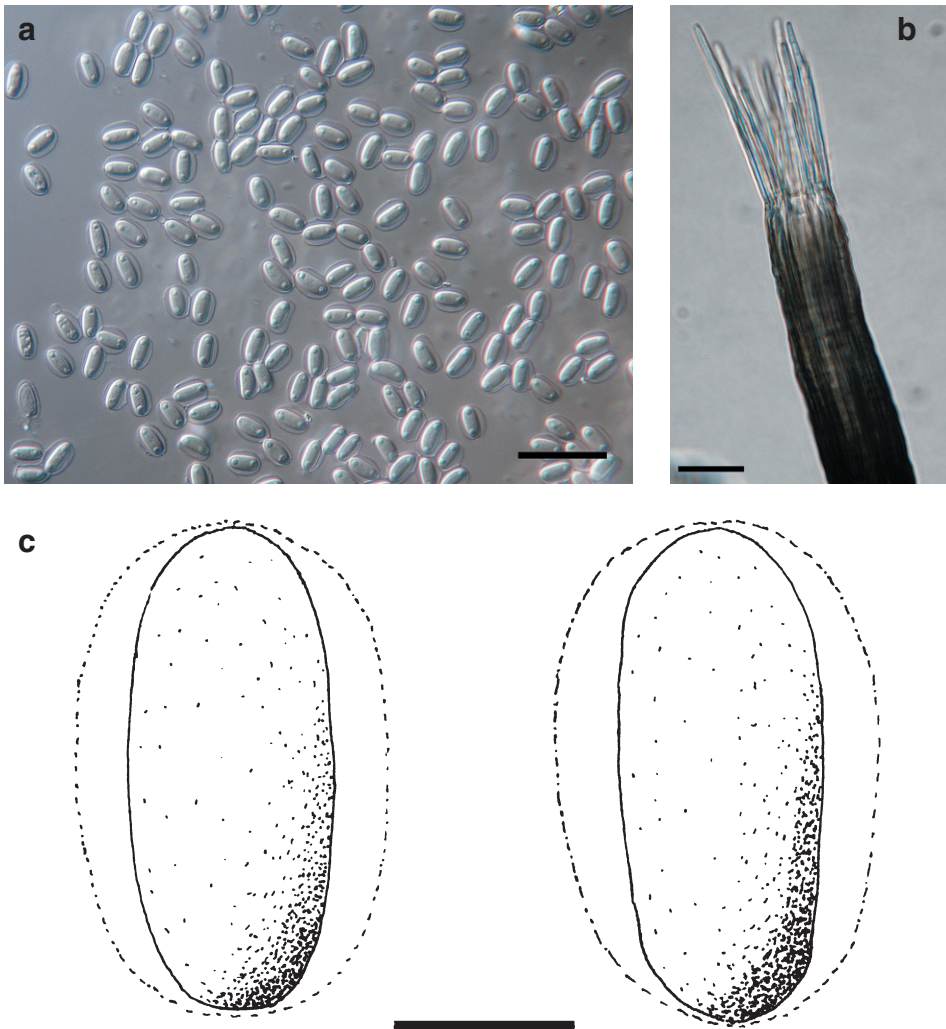


Fig. 2. *Ceratocystis laricicola* CPPF 309 – **a:** ascospores, **b:** apex of neck with ostiolar hyphae, **c:** ascospores. Scale bar for a = 11 μm , b = 12 μm , c = 2 μm .

RESULTS

Ceratocystis laricicola Redfern et Minter 1987

The fungus occurred in 50 % of investigated beetle bodies and 55 % of the beetle's galleries.

The description is based on a single representative strain: CPPF 309: gallery of bark beetle *Ips cembrae*, branch of *Larix decidua*, slope of Hudečka hill near Stříbrná Skalice, Sázava region, Central Bohemia, Czech Republic, isol. and det. D. Novotný as no. IC15L, VI. 2006.

Only one strain was deposited and investigated, because the species was recorded to be associated with *Ips cembrae* on one tree at one locality only.

Colony characteristics (growth rates see in Tab. 1)

MA2, 10 days, 20 °C: colonies grey-white, flat, exudate absent, reverse grey brown-green, soluble pigment absent.

OA, 10 days, 20 °C: colonies grey-white, flat, low, exudate absent, reverse pale to greyish, soluble pigment absent.

PDA, 10 days, 20 °C: colonies grey-white, flat, exudate absent, reverse fuscous, soluble pigment absent.

PCA, 10 days, 20 °C: colonies grey, flat, exudate absent, reverse grey-green, soluble pigment absent.

MA01+LAR, 10 days, 20 °C: colonies dark brown-green, sparse, flat, very low, exudate absent, reverse dark green in the middle, without colour at the margin, soluble pigment absent.

MA2, 8 days, 32 °C: colonies grey-white, 29–32 mm in size, flat, brown, exudate absent, reverse grey to brown-green.

The studied strain grows most quickly on PDA (see Tab. 1). The slowest growth was recorded on MA2. Perithecia developed earliest and most abundant on MA01+LAR medium.

Tab. 1. Growth of *Ceratocystis laricicola* CPPF 309 on five different cultivation media at 20 °C.

Medium	Colony diameter (mm)		
	5 days	7 days	10 days
MA2	16–23	20–35	43–64
PDA	23–32	49–54	78–85
OA	19–34	38–50	70–82
PCA	19–25	33–43	62–74
MA01+LAR	17–23	20–34	50–67

Microscopic features

Hyphae hyaline, light brown to dark brown, septate, (2–)2.5–3.0(–3.5) μm wide, smooth. In culture, perithecia develop superficially within 10 days. Bases globose, 160–240 μm in diameter, dark brown or black. Necks straight or slightly curved, brown to black, 580–890 μm in length, 25–43 μm wide at base, 14–23 μm wide at the tip immediately below the apex. Ostiolar hyphae present or absent in early stages, hyaline, septate, convergent or slightly divergent, 18–50 μm long and 2 μm wide at base, tapering to apex. Asci not observed. Ascospores hyaline, one-celled, oval, surrounded by mucous sheath, 5–6 \times 2–2.5(–3) μm , emerging from the ostiole and forming a whitish to yellowish droplet at the tip.

Anamorph: *Thielaviopsis* was not observed on the isolated strain.

DISCUSSION

Ceratocystis laricicola occurs in connection with *Ips cembrae* in Europe (Redfern et al. 1987, Marin et al. 2005, Jankowiak et al. 2007). So far, *Ceratocystis laricicola* has been isolated from larch in Poland (Jankowiak et al. 2007), Austria (Marin et al. 2005) and United Kingdom (Redfern et al. 1987). It probably occurs in other European countries where *Ips cembrae* lives. The present article gives the first report of the occurrence of this fungus from the Czech Republic. In Japan *C. fujiensis* occurs, which is very similar to this species. The description of *C. laricicola* given by Yamaoka et al. (1998) is in reality a description of *C. fujiensis*, as concluded by Marin et al. (2005), who revised some strains of *C. laricicola* described by Yamaoka et al. (1998) and identified them as *C. fujiensis*. Marin et al. (2005) recorded only *C. fujiensis* in Japan. They did not obtain any strain of *C. laricicola* from Japan.

C. laricicola, *C. polonica*, and *C. fujiensis* are very closely related but differ in growth rate at 32 °C, sequences of β -tubulin and MAT-2 HMG box and ecological niche. On 2 % malt extract agar after 8 days at 32 °C, colonies of *C. laricicola* are 11–29 mm in diameter, whereas colonies of *C. polonica* under the same conditions do not exceed a diameter of 0–3 mm. *C. fujiensis* occurs in connection with *Ips cembrae* living on *Larix kaempferi* only. *C. laricicola* lives in relationship with *I. cembrae* on *Larix decidua* in Europe and *C. polonica* is associated with *I. typographus*, *I. amitimus* and *I. duplicatus* living on *Picea abies* (Marin et al. 2005). In the present study the same significant differences in growth rate at 32 °C and ecological niche between *C. laricicola* and *C. polonica* were found, too. The Czech strain of *C. laricicola* formed 29–33 mm large colonies on 2 % malt extract agar after 8 days at 32 °C, whereas colonies of the Czech strain of *C. polonica* reached only a diameter of 4–10 mm under the same conditions. The strains of *C.*

laricicola and *C. polonica* were obtained from *L. decidua* and *P. abies*, respectively. The sequences of β -tubulin and MAT-2 HMG box of the Czech strain of *C. laricicola* were not studied.

The identification of the Czech strain was based on its growth at a temperature of 32 °C (29–33 mm colonies after 8 days) and specific ecological niche (gallery of *I. cembrae* from *L. decidua*).

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