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Ophiostoma stenoceras and O. grandicarpum (Ophiostomatales), first records in the Czech Republic

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Two species of ophiostomatoid fungi were observed in oaks. *Ophiostoma stenoceras* was isolated during a study of endophytic mycobiota of the roots and seedlings of a sessile oak (*Quercus petraea*). *Ophiostoma grandicarpum* was recorded in the stem of a pedunculate oak (*Q. robur*). These fungi have not yet been reported from the Czech Republic. The knowledge on the occurrence of ophiostomatoid fungi in the Czech Republic is reviewed.

Key words: ophiostomatoid fungi, distribution, oak, roots, bark, *Ceratocystis*, *Quercus petraea*, *Quercus robur*

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Během studia mykobioty dubů byly pozorovány dva druhy ophiostomatálních hub. Druh *Ophiostoma stenoceras* byl izolován při studiu endofytické mykobioty kořenů dubů a mladých dubových semenáčků (*Quercus petraea*). Druh *Ophiostoma grandicarpum* byl nalezen na kmeni dubu letního (*Q. robur*). V případě obou druhů se jedná o první nálezy z České republiky. V článku je uveden přehled dosud zjištěných druhů ophiostomatálních hub z České republiky.

INTRODUCTION

Ophiostomatoid fungi are associated with different species of bark beetles or plants (especially trees). During the last 25 years, great attention has been paid to these fungi because they cause plant diseases (Upadhyay 1981, Wingfield et al. 1993, Jacobs and Wingfield 2001). This group includes nine genera, which are classified in the orders Microascales and Ophiostomatales (Kirk et al. 2001). Species of *Ceratocystis* and *Ophiostoma* are studied most frequently.

Ophiostomatoid fungi have been known in the Czech Republic since the nineteenth century. The species recorded in the Czech Republic are summarised in Table 1. Corda (1837) described *Graphium penicillioides* on wood of poplar near the city of Prague. Great attention has been paid to the occurrence of ophiostomatoid fungi in the Czech Republic since the 1930s, especially since 1970s, because of a great dieback of elms caused by *Ophiostoma ulmi* ("Dutch

Table 1. Survey of ophiostomatoid fungi recorded in the Czech Republic.

Species	Substrate
Ceratocystis autographa Bakshi	*Tomicus piniperda* (Kotýnková-Sychrová 1966)
Ceratocystis polonica (Siemaszko) C. Moreau	*Ips typographus* (Jankovský et al. 2001)
Ophiostoma bicolor Davidson et Wells	*Ips typographus* (Kotýnková-Sychrová 1966, Jankovský et al. 2001)
Ophiostoma canum (Münch) H. et P. Sydow	*Tomicus minor* (Kotýnková-Sychrová 1966)
Ophiostoma cuculatum H. Solheim	*Ips typographus* (Jankovský et al. 2001)
Ophiostoma minus (Hedgcock) H. et P. Sydow	*Myelophilus piniperda* (Kotýnková-Sychrová 1966)
Ophiostoma minutum Siemaszko	*Ips typographus* (Kotýnková-Sychrová 1966, Jankovský et al. 2001)
Ophiostoma penicillatum (Grosm.) Siemaszko	*Ips typographus* (Kotýnková-Sychrová 1966)
Ophiostoma piceae (Münch) H. et P. Sydow	bark beetles (Kotýnková-Sychrová 1966), cave (Hajdušková 2000), roots of *Quercus robur* and *Quercus petraea* (Novotný 2001), *Scolytus intricatus* (Kubátová et al. 2002), *Ips typographus* (Jankovský et al. 2001)
Ophiostoma piceaperdum (Rumbold) Arx	*Ips typographus*, *Pityogenes chalcographus*, *Xyloterus lineatus* (Kotýnková-Sychrová 1966), *Ips typographus* (Jankovský et al. 2001)
Ophiostoma piliferum (Fr.) H. et P. Sydow	*Pinus* sp., *Picea* sp. (Kotýnková-Sychrová 1966), wood (Páčová et al. 1999)
Ophiostoma serpens (Goidanich) von Arx	*Ips typographus*, *Pityogenes chalcographus* (Kotýnková-Sychrová 1966)
Ophiostoma tetropii Mathiesen	*Picea* sp. (Kotýnková-Sychrová 1966)
Graphium penicillioides Corda	*Populus* sp. (Corda 1837), soil of peat-bog (Kubátová 1998), *Populus nigra* cv. *Italica* (Okada et al. 2000)
Graphium pycnocephalum Grosm.	*Ips typographus* (Kotýnková-Sychrová 1966)
Leptographium lundebergii Lagerberg et Melin	*Hylurgops palliatus*, *Xyloterus lineatus* (Kotýnková-Sychrová 1966)

elm disease" – in the Czech Republic in the periods 1932–1935 and 1972–1983; see Jančařík 1981, 1992) and because *Ophiostoma* species were considered to be the reason of the dieback of oaks ("oak decline") and other trees in this region (Jančařík 1992).

Ophiostoma piceae s. l., *Ophiostoma* spp., *Graphium* sp. and *Leptographium* sp. were isolated from several tree species (e.g. oaks, spruces, pines; see Kubátová and Prášil 1995; Novotný 2001, 2003).

A lot of ophiostomatoid fungi are associated with bark beetles. Kotýnková-Sychrová (1966) studied mycobiota of eight species of beetles (including *Ips typographus*) and recorded 13 species of these fungi. Jankovský et al. (2001) found eight taxa of these micromycetes in mycobiota associated with *Ips typographus* from the Šumava Mts. *Ophiostoma piceae* s.l. and *Ophiostoma* spp. were found on the surface of *Scolytus intricatus* and in its galleries (Kubátová et al. 2002).

O. piceae s.l. was detected by wiping off the surface of a floor of the limestone cave Ostrovské síně (near town Blansko – South Moravia) with cotton swab. The cave is used for speleotherapy (Hajdušková 2000).

Materials and methods

Strains of *Ophiostoma stenoceras* were isolated in November 1999 from peridermal and subperidermal bark of thick roots (2–5 cm) and from fine roots (0.1–0.3 cm) of sessile oak (*Quercus petraea*) from an oak stand locality near Dřevíč in the Křivoklát region in Central Bohemia, Czech Republic. This species was also detected in May 1999 in stems of oak seedlings (*Quercus petraea*) in the village Jíloviště-Strnady (Central Bohemia, near the city of Prague, Czech Republic).

The roots and seedlings were brushed under running water, their surface sterilised (96 % ethanol 1 min., sodium hypochlorite (NAClO) 3 min., 96 % ethanol 0.5 min.) and cut. The thick roots were separated into wood, subperidermal bark and peridermal bark. The seedlings were divided into leaves, stem and roots and were then cut. Pieces of tissues or organs were laid on 2 % malt extract agar and incubated at room temperature up to four weeks.

A strain of *Ophiostoma grandicarpum* was isolated in November 2000 from a branch of *Quercus robur* from the dam of pond Koclířov, near the town of Lomnice nad Lužnicí, Třeboň region, South Bohemia, Czech Republic. Samples of branches were cut into slices 0.5–2 cm thick, which were brushed under running water and then put in sterile, glass moist chambers with sterile cotton wool and sterile filter paper. They were incubated at room temperature for 4–7 weeks.

The isolated strains were deposited in two culture collections of fungi in the Czech Republic [Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, and the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague].

Strains were freeze-dried, preserved under mineral oil or saved agar slant. The strains of *O. stenoceras* were deposited as CCM 8317, CCM 8329, CCF 3261, and the strain of *O. grandicarpum* as CCM 8331.

Growth of the isolated strain was tested on 2 % malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oatmeal agar (OA). Mycelium of the tested strain was transferred to three Petri dishes per medium.

The identification was based on morphological and cultural features.

RESULTS AND DISCUSSION

Ophiostoma stenoceras (Robak) Melin et Nannf. 1934

= *Ophiostoma albidum* Math.-Käärik 1953. ≡ *Ceratocystis albida* (Math.-Käärik) Hunt 1956. = *Ceratocystis gossypina* Davidson 1971. ≡ *Ophiostoma gossypinum* (Davidson) J. Taylor 1976. = *Ceratocystis eucastaneae* Davidson 1971.

Studied strains (three strains of this species were isolated):

CCM 8317: peridermal bark of thick root of *Quercus petraea*, Dřevíč, Křivoklátsko region, Czech Republic, isol. and det. D. Novotný as no. FF/T/V3, XI. 1999
CCM 8329: oak seedling (*Quercus petraea*), Jíloviště-Strnady (Central Bohemia, near the city of Prague), Czech Republic, isol. and det. D. Novotný as no. D2K3/2TN, V. 1999
CCF 3261: fine root of *Quercus petraea*, Dřevíč, Křivoklátsko region, Czech Republic, isol. and det. D. Novotný as no. FB/L/11, XI. 1999

Macroscopic description

MA2, 28 days, 25 °C: colonies white to luteous, low, exudate absent, reverse pale luteous, pigment absent.

PDA, 28 days, 25 °C: colonies luteous, flat or elevated, exudate absent, reverse pale luteous, pigment absent.

PCA, 28 days, 25 °C: colonies white, flat, low, exudate absent, reverse pale luteous, pigment absent.

OA, 28 days, 25 °C: colonies white, flat, low, exudate absent, reverse white to pale luteous, pigment absent.

The studied strains grow most quickly on OA. The slowest growth was on PDA. Daylight induced formation of perithecia. Perithecia developed earliest and most abundant on OA and PCA medium.

Table 2. Growth of *Ophiostoma stenoceras* on different media at 25 °C

Medium	Colony diam. (mm)		
	7 days	14 days	28 days
MA2	5–7	9.5–11	29–30
PCA	8–9	13–14.5	35–36
OA	12–13	18–22	39–41
PDA	6–9	11–12	18–21

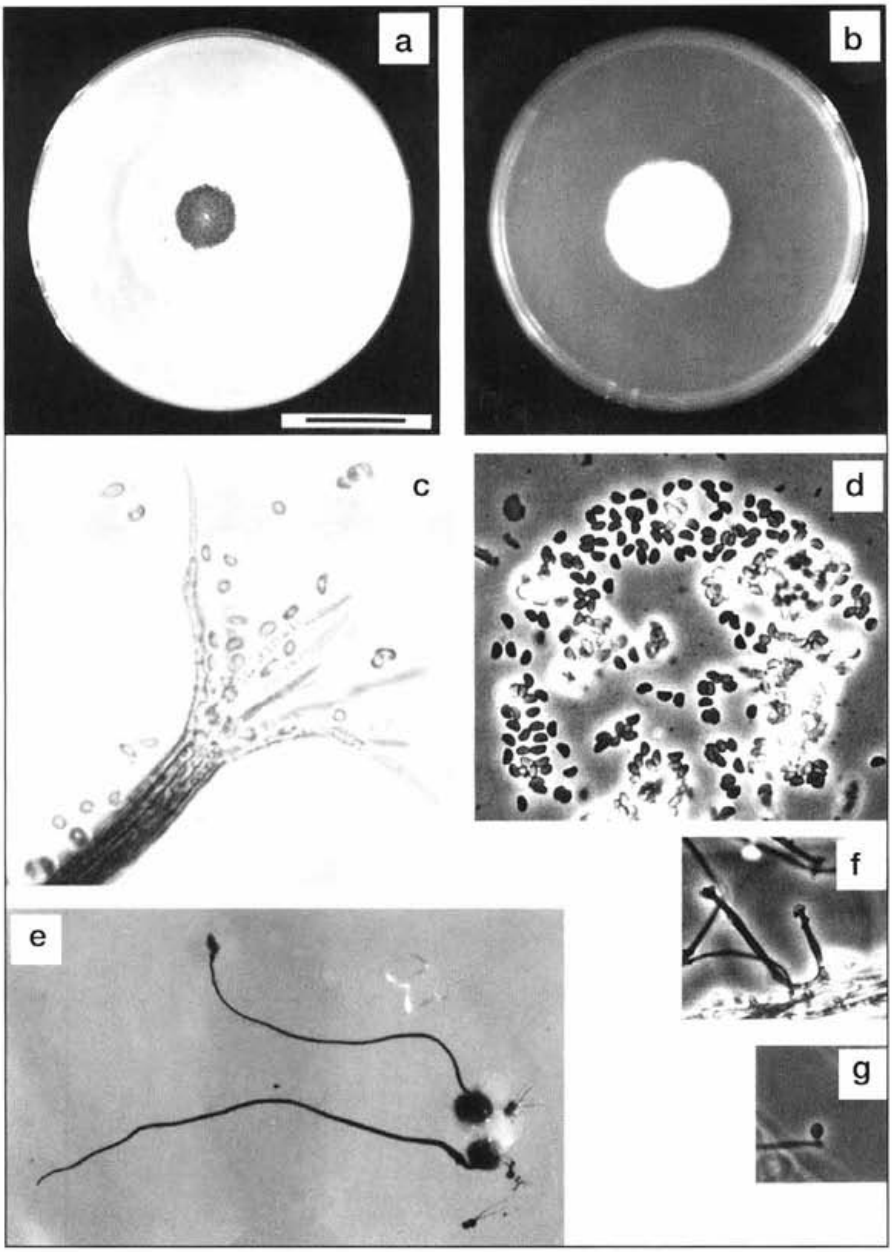

Fig. 1. *Ophiostoma stenoceras* – **a:** four week old colony on OA. **b:** four week old colony on MA2. **c:** apex of neck with ostiolar hyphae. **d:** ascospores. **e:** polyblastic conidiogenous cells. **f:** conidia. **g:** perithecia of *O. grandicarpum* (larger bases with very long necks) and *O. stenoceras* (small perithecia with necks at the bases of *O. grandicarpum*). Scale bar for a, b = 23 mm, c-e = 20 μm, g = 1500 μm.

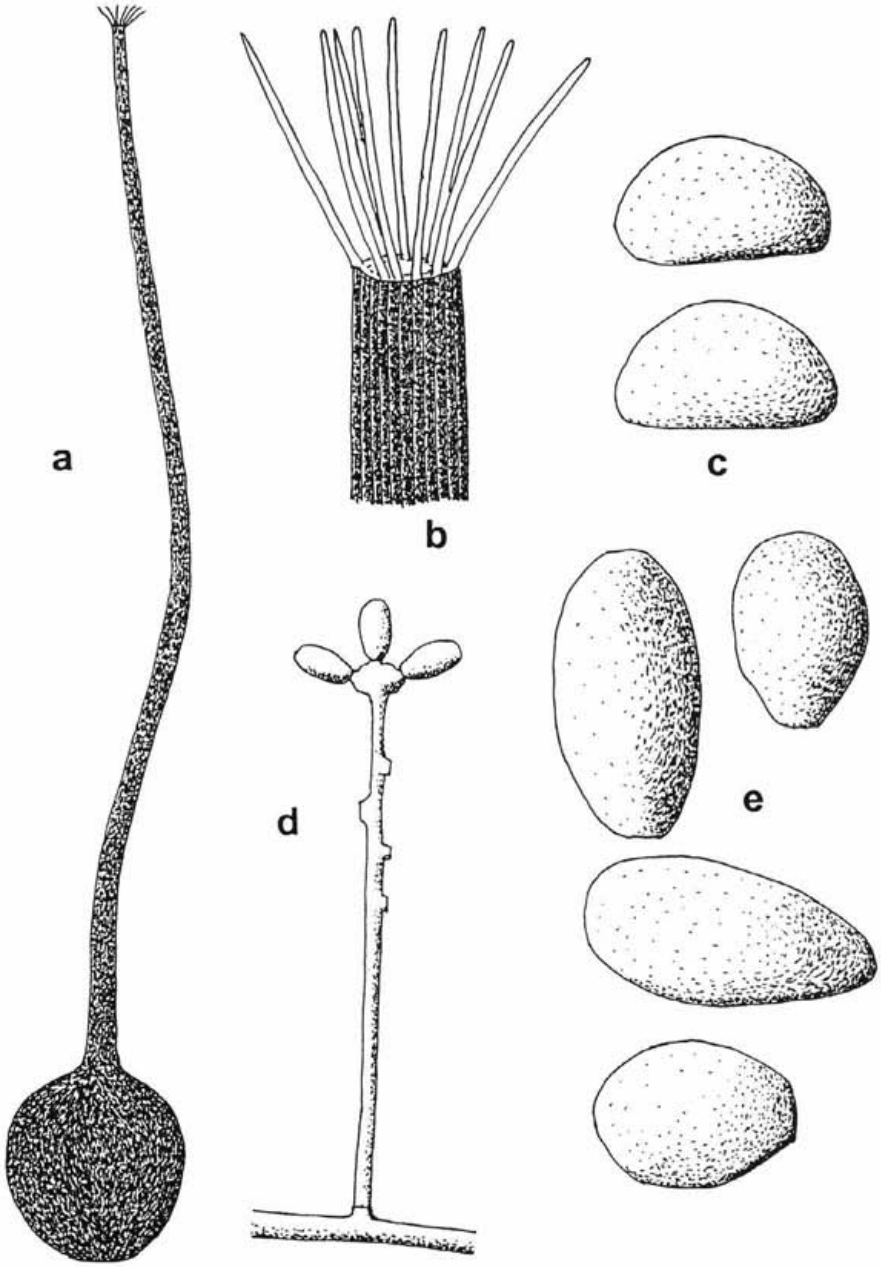

Fig. 2. *Ophiostoma stenoceras* – **a**: perithecium. **b**: apex of neck. **c**: ascospores. **d**: conidiophores. **e**: conidia. Scale bar for a = 80 μm, b = 15 μm, c = 2 μm, d = 10 μm, e = 2 μm. Del. D. Novotný

Microscopic features

Hyphae hyaline, septate, 1–1.5 μm wide, smooth. In culture, perithecia develop superficially on the substrate within 3–4 weeks. Bases globose, 102–132 μm in diameter, black or dark brown. Necks straight or curved, brown to black in colour, 419–756 μm in length, 20–30 μm wide at base, 9–10 μm wide at the tip immediately below the apex. Ostiolar hyphae present, hyaline, septate, divergent, 20–30 μm long and 1.5–2 μm wide at the base, tapering to apex. Ascospores hyaline, one-celled, orange-section shaped in side view, ellipsoid in face view and globose in the end view, 3 × 1.7–2 μm, sheath absent, emerging from the ostiole and forming a spore ball at tip.

Anamorph: *Sporothrix* sp. Conidiophores mononematous or semi-macronematous, hyaline, 1–1.5 μm wide. Conidiogenous cells polyblastic, integrated or discrete, terminal to intercalary, sympodial, denticulate 15–45 μm. Conidia produced sympodially upon the denticles, hyaline, one-celled, obovoid, ellipsoid to globose, tapering towards the base, 3–6 × 2–2.5 μm, solitary or aggregated in a head.

Discussion

So far, this species has been isolated from oaks, pines, chestnut, fir, soil, seawater, *Erica gracilis* and man (Anonymus 2001, Kowalski and Butin 1989, Przybyl 1991, Upadhyay 1981). It has been recorded in oaks in Poland, Austria and Germany. It was found in discoloured or necrotic spots of bark or wood of oaks with oak decline symptoms (Balder 1991, Cech 1991, Kowalski and Butin 1989, Przybyl 1991). In the present study, Novotný isolated this fungus from two healthy roots (without any discoloured or necrotic spots) of two oak trees (*Quercus petraea*) without or with a low degree of oak decline and in a healthy oak seedling. This report is the first one from the Czech Republic.

There were differences observed in growth rates between the strains from the Czech Republic and the strains studied by Kowalski and Butin (1989) and Upadhyay (1981). The strains from the Czech Republic grow slower than those recorded by other mycologists (Kowalski and Butin 1989, Upadhyay 1981).

The ascospores of strains from the Czech Republic (the present study) and Poland (Kowalski and Butin 1989) are broader than those recorded by Upadhyay (1981). The anamorphic state of this species is similar to that of *Sporothrix schencki* Hektoen et Perkins. The conidia of both species are ellipsoid or subglobose (Summerbell et al. 1993).

In the present study, the identification of *O. stenoceras* was based on differences in ascospore, anamorph and perithecium morphology (length of necks and presence of ostiolar hyphae). Five *Ophiostoma* species [*O. epigloeum* (Guerrero) de Hoog, *O.*

grandicarpum (Kowalski et Butin) Rulamort, *O. introcitrinum* (Olchow. et J. Reid) Georg Hausner, J. Reid et Klassen, *O. megalobrunneum* (Davidson et Toole) de Hoog et Scheffer and *O. stenoceras*] are similar in having orange-section shaped ascospores without a gelatinous sheath (Kowalski and Butin 1989, Upadhyay 1981).

In the length of perithecial necks, *Ophiostoma stenoceras* (400–1400 μm) resembles *Ophiostoma megalobrunneum* (950–1500 μm), *O. introcitrinum* (337–510 μm) and *O. epigloeum* (170–700 μm), but differs in other perithecial characters (size of perithecia, presence of ostiolar hyphae, colony colour). Perithecia of *O. megalobrunneum* are 327–500 μm in diameter, necks have ostiolar hyphae and colonies are buff to dark brown or black (Upadhyay 1981). The necks of *O. introcitrinum* and *O. epigloeum* lack ostiolar hyphae and their perithecial bases are 117–225 μm and 115–170 μm in diameter, respectively. Colonies of *O. stenoceras* are white to dull grey, necks have ostiolar hyphae and perithecial bases are 65–200 μm in diameter (Upadhyay 1981). The necks of *O. grandicarpum* have no ostiolar hyphae and are several times longer than the necks (with ostiolar hyphae) of *O. stenoceras* (Kowalski and Butin 1989).

Ophiostoma stenoceras, *O. epigloeum* and *O. megalobrunneum* are associated with *Sporothrix* anamorphs, but the anamorphic states of *O. grandicarpum* and *O. introcitrinum* belong to the genera *Hyalorhinocladiella, Hyalodendron* and *Pesotum* (Upadhyay 1981, as *Hyalopesotum*), respectively. Further, *Ophiostoma megalobrunneum* is associated with a yeast anamorph (Kowalski and Butin 1989, Upadhyay 1981). Conidiogenous cells of the *Sporothrix* anamorph of *O. epigloeum* differ in shape from conidiogenous cells of the *Sporothrix* of *O. stenoceras* (Upadhyay 1981).

Ophiostoma grandicarpum (Kowalski et Butin) Rulamort 1990

≡ *Ceratocystis grandicarpa* Kowalski et Butin 1989

Representative strain (a single strain of this fungus was isolated only):

CCM 8331: branch of *Quercus robur*, dam of pond Koclířov – near the town of Lomnice nad Lužnicí, Třeboň region, South Bohemia, Czech Republic, isol. P. Šrůtka as no. Oph 24, XI. 2000, det. D. Novotný

Macroscopic description:

MA2, 28 days, 25 °C: colonies cream, low, exudate absent, reverse cream, pigment absent.

PDA, 28 days, 25 °C: colonies luteous, low, exudate absent, reverse pale luteous, pigment absent.

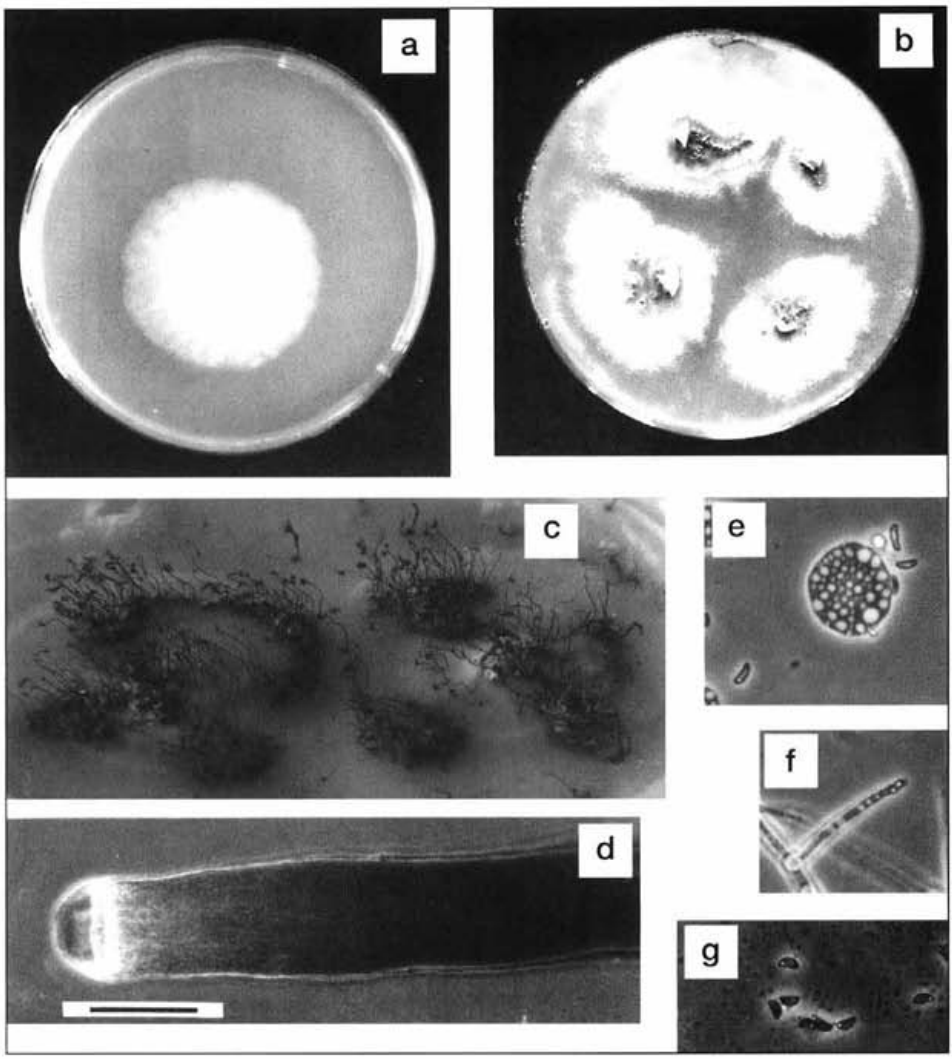

Fig. 3. *Ophiostoma grandicarpum* – **a:** four week old colony on PCA. **b:** three month old colonies on MA2. **c:** perithecia of *O. grandicarpum* in six month old colony on MA2. **d:** apex of neck. **e:** ellipsoid slightly curved conidia. **f:** conidiogenous cells. **g:** ascospores. Scale bar for a, b = 23 mm, c = 12 mm, d = 50 µm, e-g = 20 µm.

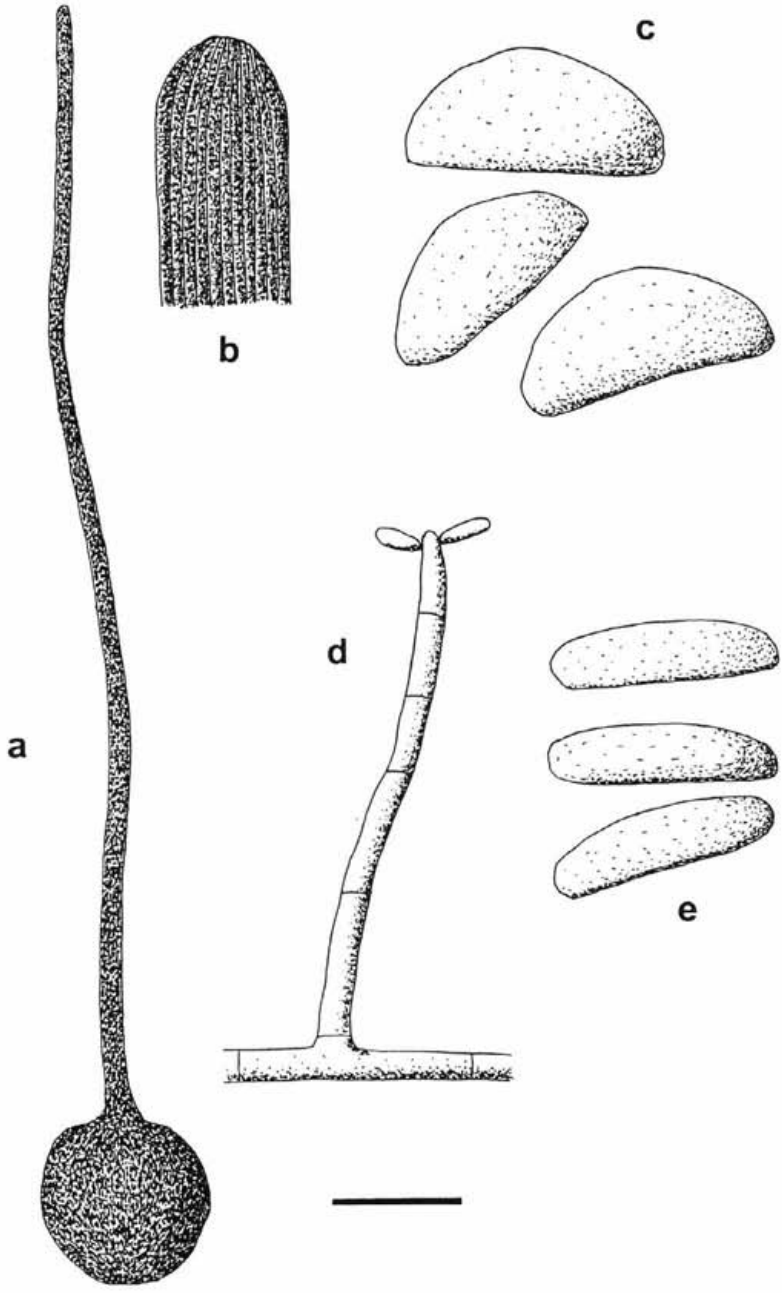

Fig. 4. *Ophiostoma grandicarpum* – **a**: perithecium. **b**: apex of neck. **c**: ascospores. **d**: conidiogenous cells. **e**: conidia. – Scale bar for a = 500 μm, b = 50 μm, c = 2 μm, d = 10 μm, e = 2 μm. Del. D. Novotný

PCA, 28 days, 25 °C: colonies white, exudate absent, reverse white to cream, pigment absent.

OA, 28 days, 25 °C: colonies white, flat, exudate absent, reverse white to cream, pigment absent.

Table 3. Growth of *Ophiostoma grandicarpum* on different media at 25 °C

Medium	Colony diam. (mm)		
	7 days	14 days	28 days
MA2	9–12	13–16.5	24–30
PCA	9–10	12–13	39–42
OA	8–10	19–20	40–44
PDA	8–9	12–14	22–24

The studied strain grows most quickly on OA and PCA. The slowest growth was on PDA. During the present study, perithecia developed on the MA2 medium, but they did not arise in the dark and on OA, PDA and PCA media. The formation of perithecia was induced by daylight.

Microscopic characters

Hyphae hyaline, 1–2 μm wide, smooth. In culture, perithecia develop superficially in the agar substrate within 4–5 months. Bases globose, 496–734 μm in diameter, black. Necks straight or curved, black in colour, 3470–7300 μm in length, 117.3–146.4 μm wide at base, 43.9–58.6 μm wide at the tip immediately below the apex. Ostiolar hyphae absent. Apex of necks of the 6–8 month old perithecia with brown to black hyphae. Asci globose, broadly ellipsoid, hyaline, 6–9 × 7–10 μm. Ascospores hyaline, one-celled, orange-section shaped in side view and ellipsoid in plane view, 3.5–4 × 1.5–2 μm, sheath absent, emerging from the ostiole. The forming of a spore ball at the tip was not observed.

Anamorph: *Hyalorhinocladiella* state. Conidiophores mononematous, hyaline, terminal, 13–39 × 1.5–2 μm. Conidia in heads, hyaline, one-celled, ellipsoid, the ends obtuse, slightly curved, 3.5–5 × 0.9–1.4 μm.

Discussion

The second author observed this species several times during his study of fungi associated with oak branches by using the moist chamber method, but

no other Czech mycologist recorded *Ophiostoma* species with such extremely long perithecial necks. This species was so far detected in oaks in Poland and Germany (Kowalski and Butin 1989, Balder 1991 etc.). Kowalski and Butin (1989) observed 1.6–1.8 μm wide conidia and two anamorph states: *Hyalorhinocladiella* and *Hyalodendron*.

In the present study, the *Hyalorhinocladiella* state producing 0.9–1.4 μm wide conidia appeared. The *Hyalodendron* state was not detected. The strain from the Czech Republic grows slower than strains obtained by Kowalski and Butin (1989).

Four *Ophiostoma* species with necks longer than 5000 μm are known (Grylls and Seifert 1993). *Ophiostoma novae-zelandiae* (Hutchinson et Reid) Rulamort has ostiolar hyphae at the apex of the neck and its ascospores are narrower than those of *O. grandicarpum* (Hutchinson and Reid 1988). Ascospores of *Ophiostoma nothofagi* (Butin) Rulamort are broader (2.5 μm, Butin and Aquilar 1984) than those of *O. grandicarpum*. The anamorphic state of *O. nothofagi* is *Sporothrix* (Butin and Aquilar 1984), but anamorphs of *O. grandicarpum* are *Hyalorhinocladiella* and *Hyalodendron*. The neck of *Ophiostoma multiannulatum* has divergent ostiolar hyphae, and ascospores are reniform or broadly elliptical in side view (Upadhyay 1981). Ascospores of *O. grandicarpum* are orange-section shaped in side view and the neck lacks ostiolar hyphae (Kowalski and Butin 1989). The strain from the Czech Republic has no ostiolar hyphae and the ascospores are orange-section shaped in side view.

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