CZECH MYCOL. 61(1): 117–124, 2009

Geographical distribution of *Scleroconidioma sphagnicola* in coniferous forests in Europe and Canada

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Koukol O. (2009): Geographical distribution of *Scleroconidioma sphagnicola* in coniferous forests in Europe and Canada. – Czech Mycol. 61(1): 117–124.

The distribution of the dematiaceous microfungus *Scleroconidioma sphagnicola* in coniferous litter in Europe and Canada was assessed using a culture-dependent approach. Needles of various coniferous species were sampled between May and November of 2008. Twenty needles from each sample were cultivated on 2° malt agar after surface sterilisation with 30 % hydrogen peroxide. The formed mycelia were identified based on their morphology. The identifications were further confirmed by analyses of ITS rDNA. *S. sphagnicola* was recorded at 8 of the 53 sites. The highest abundance of *S. sphagnicola* was recorded in mountain areas of the Czech Republic. Further records of *S. sphagnicola* were acquired from the literature and the GenBank database. *S. sphagnicola* does not seem to be a ubiquitous coloniser of coniferous litter. However, this study showed that this fungus is found in various regions and the data obtained may serve to make an evaluation of the potential spread of *S. sphagnicola*.

Key words: Ascomycota, *Larix decidua*, *Pinus* spp., *Picea* spp., litter needles.

Koukol O. (2009): Rozšíření druhu *Scleroconidioma sphagnicola* v jehličnatých lesích Evropy a Kanady. – Czech Mycol. 61(1): 117–124.

Rozšíření demaciové mikroskopické houby druhu *Scleroconidioma sphagnicola* v opadu jehličnatých lesů Evropy a Kanady bylo stanoveno pomocí izolací na agarové půdy. Jehlice různých druhů jehličnanů byly sbírány v květnu až listopadu 2008. Z každé lokality bylo odebráno 20 jehlic, povrchově sterilizováno ve 30 % peroxidu vodíku a kultivováno na 2° sladinovém agaru. Vyrůstající mycelia byla určena na základě morfologických znaků a určení bylo následně potvrzeno analýzou ITS rDNA. *S. sphagnicola* byla zaznamenána na 8 z celkem 53 lokalit. Nejvyšší četnost druhu *S. sphagnicola* byla zaznamenána v horských oblastech ČR. Další oblasti, kde byl druh *S. sphagnicola* zaznamenán, byly získány z literárních údajů a z databáze GenBank. Nezdá se, že by druh *S. sphagnicola* byl ubikvitní kolonizátor jehličnatého opadu, ale současná studie ukázala, že může být nalezen v různých oblastech. Získaná data mohou sloužit k odhalení případného šíření druhu *S. sphagnicola*.

INTRODUCTION

Scleroconidioma sphagnicola Tsuneda, Currah & Thormann is an anamorphic fungus (Ascomycota, Dothideomycetes) with dark melanised mycelium and pleomorphic conidiogenesis. *S. sphagnicola* was originally described as a necro-

trophic parasite of *Sphagnum fuscum* (Schimp.) Klinggr. in Canada (Tsuneda et al. 2000, 2001). In other regions where *S. sphagnicola* was isolated (Sweden and the Czech Republic) it saprotrophically colonised coniferous litter and wood (Vasiliauskas et al. 2005, Koukol and Kovářová 2007). A high incidence of *S. sphagnicola* was recorded in *Picea abies* litter in Šumava National Park (Southern Bohemia, Czech Republic) (Koukol and Kovářová 2007; Koukol, unpublished data). This made me believe that *S. sphagnicola* might also be present in coniferous forests in other regions. To confirm this hypothesis, I isolated microfungi from coniferous litter sampled in various parts of Europe and Canada, from where most of the records of *S. sphagnicola* originate. I identified isolated strains based on their morphology and ITS rDNA. I also hypothesised that the morphological similarity of *S. sphagnicola* to widespread soil and litter colonisers [*Aureobasidium pullulans* (de Bary) G. Arnaud and *Hormonema dematioides* Lagerb. & Melin] belonging to the same class (Dothideomycetes) might be responsible for misidentifications. To confirm this hypothesis, I checked twelve strains from the Culture Collection of Fungi (CCF, Prague) labelled as "*Aureobasidium*-like". Finally, I gathered relevant literature data from biodiversity studies on coniferous litter as well as the GenBank database.

MATERIALS AND METHODS

Fungal isolation. Strains of *S. sphagnicola* were isolated from litter needles sampled at 53 sites from May to November 2008. The sampling sites were distributed over various areas of Europe and Canada. Natural coniferous stands were prioritised, although samples also originated from managed monocultures. A list of the sites, incl. coordinates, elevations, and dates of sampling are given in Tab. 1.

Small bunches of needles were sampled into clean zip-lock polyethylene bags. In the laboratory, twenty spruce (fir) needles or twenty 2-cm segments of pine needles (cut from the central part of the needles) were randomly selected. Needles or their segments were surface sterilised for 90 s with hydrogen peroxide (30 %). Next, they were incubated at 23–26 °C in Petri dishes containing malt agar (2° MA; 2 % w/v sucrose content; 18 g agar) with ten needles in each Petri dish. Formed mycelia of *S. sphagnicola* were transferred to Petri dishes with 2° MA, and the number of needles colonised by *S. sphagnicola* was recorded. Also isolated fungi that resembled *S. sphagnicola* (e.g. *H. dematioides*) were recorded. Representative strains from various geographical regions are preserved in the author's fungal collection or in the Culture Collection of Fungi (CCF, Prague).

Morphological identification. Strains formed from the isolates and the CCF collection were cultivated on 2° MA. The strains were also cultivated on cornmeal agar with dextrose (CMAD) to induce sporulation and reduce vegetative mycelium (Tsuneda et al. 2000). Microscope slides were mounted in Melzer's reagent and observed using a light microscope (Olympus BX–51).

DNA analysis. DNA was isolated from pure cultures growing on 2° MA with the Ultra Clean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California) using the manufacturer's procedure. The first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second internal transcribed spacer (ITS2), and the 5' end of the 28S rRNA gene (LSU) were amplified with the primers ITS1 and ITS4 (White et al. 1990). PCR fragments were sequenced with the above-mentioned primers (Macrogen, Korea).

Tab. 1. Records of *Scleroconidioma sphagnicola* from litter needles in coniferous forests in selected areas of Europe and Canada.
[1] code of one representative strain kept in the CCF collection
– no strain preserved because of contamination
The values in italics are approximate (not measured in the field).

Area	Country	GPS N	GPS E	GPS W	Elevation (m a.s.l.)	Date of collection	Num. of needles colonised	Collection code 1
Niederösterreich	AUS	47° 45' 49"	15° 57' 01"		537	2 July 2008		
Šumava NP	CZ	48° 46' 53"	13° 50' 27"		1235	17 May 2008	20	CCF3875
CHKO Lužické hory	CZ	50° 46' 48"	14° 32' 52"		480	4 May 2008		
Central Bohemia	CZ	49° 52' 09"	14° 31' 36"		310	14 May 2008		
Bohemian Switzerland NP	CZ	50° 55' 57"	14° 25' 57"		340	24 May 2008	1	CCF3886
Beskydy Mts.	CZ	49° 23' 30"	18° 16' 37"		550	8 July 2008		
Králický Sněžník Mts.	CZ	50° 12' 37"	16° 53' 15"		935	3 Oct 2008	20	CCF3890
Králický Sněžník Mts.	CZ	50° 11' 56"	16° 50' 08"		922	3 Oct 2008		
CHKO Český kras	CZ	49° 56' 13"	14° 03' 09"		230	8 May 2008		
CHKO Lužické hory	CZ	50° 46' 46"	14° 34' 08"		390	4 May 2008	5	CCF3885
CHKO Český ráj	CZ	50° 35' 40"	15° 15' 42"		740	16 July 2008		
Karula NP	EST	57° 43' 48"	26° 30' 50"		80	3 Aug 2008		
Vidumae	EST	58° 17' 33"	22° 05' 42"		35	6 Aug 2008		
Kuopio	FIN	62° 54' 06"	27° 40' 29"		100	18 Aug 2008		
Helsinki	FIN	60° 10' 50"	24° 52' 40"		60	18 Aug 2008		
Pitlorchy	GB	*56° 44'*		*3° 42'*	*300*	1 July 2008		
Tintern	GB	51° 41' 48"	2° 40' 45"		245	26 Nov 2008		
Glenmore Forest NP	GB	*57° 10'*		*3° 42'*	*330*	2 July 2008		
Loch Sheilah	GB	*57° 46'*		*4° 13'*	*210*	6 July 2008		
Glen Affric, Chrisholm Bridge	GB	57° 19' 29"	4° 47' 32"		350	7 July 2008		
Gauja NP	LAT	57° 16' 43"	25° 07' 37"		50	2 Aug 2008		
Kemeru NP	LAT	56° 54' 30"	23° 27' 32"		18	1 Aug 2008		
Aukshtaitia NP	LIT	55° 20' 02"	26° 06' 28"		135	31 July 2008		
Białowieża NP	POL	52° 47' 53"	23° 49' 27"		150	28 July 2008		
Warszawa	POL	52° 32' 34"	21° 26' 32"		91	12 July 2008		
Kursk	RU	55° 17' 43"	20° 59' 08"		56	7 Aug 2008		
Ryazan Oblast	RU	55° 11' 23"	40° 09' 38"		118	20 July 2008	1	CCF3887
Bashkortostan	RU	55° 18' 12"	40° 09' 17"		100	29 July 2008		
CHKO Slovenské rudohorie	SK	48° 50' 06"	20° 18' 13"		1220	1 May 2008		
CHKO Slovenské rudohorie	SK	48° 47' 07"	20° 12' 59"		1470	2 May 2008		
CHKO Slovenské rudohorie	SK	48° 48' 04"	20° 10' 28"		1409	2 May 2008		
CHKO Slovenské rudohorie	SK	48° 51' 56"	20° 19' 36"		700	1 May 2008		
CHKO Muráňská planina	SK	48° 48' 22"	20° 14' 31"		1390	1 May 2008		
Velká Fatra Mts.	SK	*48° 57'*	*19° 05'*		*1200*	1 July 2008		
Veľká Fatra Mts.	SK	*48° 58'*	*19° 11'*		*1300*	4 July 2008		
Strážske	SK	48° 52' 16"	21° 47' 38"		179	16 Aug 2008		

Tab. 1. Continuing

Area	Country	GPS N	GPS E	GPS W	Elevation (m a.s.l.)	Date of collection	Num. of needles colonised	Collection code 1
Malá Fatra NP	SK	*49° 04'*	*18° 35'*		*460*	26 Sep 2008		
Saxon Switzerland NP	SRN	50° 54' 25"	14° 15' 17"		460	28 Aug 2008		
Saxon Switzerland NP	SRN	50° 53' 25"	14° 14' 50"		320	28 Aug 2008		
Saxon Switzerland NP	SRN	50° 54' 02"	14° 14' 20"		250	28 Aug 2008	9	CCF3889
Saxon Switzerland NP	SRN	50° 54' 31"	14° 14' 59"		440	28 Aug 2008	6	–
Kiev	UKR	50° 13' 03"	30° 46' 32"		200	19 July 2008		
Berdychiv	UKR	50° 26' 33"	29° 42' 18"		178	13 Aug 2008	8	CCF3888
Kiev	UKR	*50° 27'*	*30° 31'*		*200*	19 July 2008		
Calgary	CAN	51° 05'		114° 03'	1050	15 Aug 2008		
Banff NP	CAN	51° 24'		116° 13'	1640	6 May 2008		
Banff NP	CAN	51° 32'		114° 28'	1200	11 May 2008		
Banff NP	CAN	51° 32'		114° 28'	1200	14 May 2008		
Banff NP	CAN	52° 22'		114° 52'	1860	17 May 2008		
Banff NP	CAN	53° 17'		114° 58'	1580	21 May 2008		
Banff NP	CAN	51° 10'		115° 25'	1560	8 May 2008		
Banff NP	CAN	51° 25'		116° 14'	1750	12 May 2008		
Jasper NP	CAN	52° 38'		117° 30'	1600	7 Aug 2008		

RESULTS AND DISCUSSION

Isolations from litter needles

S. sphagnicola was isolated from 8 of the 53 sites where litter needles were sampled (Tab. 1). The abundance of *S. sphagnicola* varied from 1 to 20 needles colonised. Colonies of *S. sphagnicola* usually appeared after several days of cultivation of the needles. Since it is relatively fast growing, *S. sphagnicola* was not affected by other fungi. Moreover, the sterilisation technique was sufficient to eliminate fast-growing soil-borne fungi, and only a few Petri dishes were completely overgrown by *Trichoderma* spp.

H. dematioides, another member of Dothideomycetes, was isolated from 11 litter samples (3 of them together with *S. sphagnicola*). These two species colonise the same habitat and may occur simultaneously. On the other hand, *A. pullulans* was completely absent from the present study. This dothidealean species is frequently found in coniferous litter and soil (Domsch et al. 2007) and was isolated from *P. abies* litter in such a habitat in Šumava National Park at frequencies of 12–16% (Kubátová et al. 1998). This finding suggests that either the isolation technique affects the spectra of fungi obtained or *S. sphagnicola* may re-

place related species colonizing litter. The effect of cultivating media on the fungal spectrum obtained is a well-known phenomenon (Mueller et al. 2004). However, less is known about the sterilisation technique. The surface sterilisation with hydrogen peroxide might have favoured *S. sphagnicola* and *H. dematioides* over *A. pullulans*. The latter hypothesis may be confirmed by competition tests with the above-mentioned species. Previous findings suggest that *S. sphagnicola* is able to withstand competition by other fungal species and adverse abiotic conditions (Koukol et al. 2006, Hambleton et al. 2003). It may thus potentially replace other fungi sharing the same habitat if conditions become stressful.

Distribution

The highest number of needles colonised by *S. sphagnicola* occurred in mountain areas with *Picea abies*, although the fungus was isolated also at lower altitudes (170–440 m a.s.l.). This study, a previous one (Koukol and Kovářová 2007) and ongoing research in the mountain areas of Šumava National Park have revealed an almost ubiquitous presence of *S. sphagnicola* in *P. abies* litter. *S. sphagnicola* reached a high frequency of 84 % from all fungal species in litter needles in permanent plots in this area. On the other hand, *S. sphagnicola* was not isolated from living spruce needles and green needles still attached to fallen trees. This suggests that *S. sphagnicola* is not present as an endophyte in living needles and does not represent a latent pathogen of coniferous trees (Koukol, unpublished data). Regular microfungal isolations will have to reveal changes in the abundance of *S. sphagnicola* during succession in spruce litter.

The sites where needles were colonised by *S. sphagnicola* varied in management, age and vegetation. *S. sphagnicola* was isolated from highly protected, unmanaged forests as well as young plantations. The type of understory vegetation had apparently no effect on the presence of *S. sphagnicola*. However, this conclusion cannot be statistically tested because of the low number of sites.

I did not isolate *S. sphagnicola* from needles in Canada, Great Britain or Slovakia, although I sampled at more than five sites in each country. I also obtained negative results from Finland, Estonia, Latvia, Lithuania, and Austria. However, these results may be caused by a limited number of samples (only one site in each country).

Identification

S. sphagnicola may be identified according to its colony morphology. It has several characteristic features, including a greenish black colour, a white brindled margin and numerous microsclerotia (Tsuneda et al. 2000, Koukol and Kovářová 2007). However, before the microsclerotia are formed, the mycelium of *S. sphagnicola* may be confused with other fungi that produce greenish young colonies (e.g.

Chalara spp., common in spruce litter) or with *H. dematioides*, producing shiny reddish black colonies with a dense substrate mycelium. The aerial mycelium of *S. sphagnicola* is characterised by continuous growth, individual tufts or tufts with spherical shapes that may resemble haired pycnides [similar to *Allantophomopsis lycopodina* (Höhn.) Carris, also known from coniferous litter].

Conidiogenous cells on the surface of the microsclerotia represent a characteristic feature of *S. sphagnicola*. However, it was frequently absent. So far, I recorded only one strain producing conidiogenous cells on the surface of its microsclerotia. The strain (CCF 3875) was isolated from *Picea abies* litter needles in Šumava National Park. Surprisingly, numerous other strains were isolated at the same locality but did not produce conidiogenous cells. The production of conidiogenous cells probably depends on cultivation conditions. I did not observe this morphological character in the ex-type strain UAMH 9731 cultivated in my laboratory on various media, including CMAD.

Reliable identification of *S. sphagnicola* may be accomplished by aligning its ITS rDNA region with sequences in the GenBank database. The ITS region was amplified from the DNA of 8 representative strains and compared with the sequence of the type strain (UAMH 9731). All of the strains had identical sequences when compared to the type strain. Based on numerous previous alignments of *S. sphagnicola* I have completed, this region seems to have no intraspecific variability. Therefore, it is useful in the identification of *S. sphagnicola* from environmental DNA.

Cultures from CCF

I checked the morphology of 12 strains that were isolated during the last decade and preserved in the CCF collection as "*Aureobasidium*-like." One strain (AK156/99) resembled *S. sphagnicola* when grown on CMAD. An analysis of its ITS rDNA confirmed the identification. This strain was isolated from soil in a spruce (*Picea abies*) forest at Mt. Plechý (1378 m a.s.l.) in Šumava National Park by Alena Kubátová in July 1999. At approximately the same time, *S. sphagnicola* was isolated in Canadian peat bogs and described as a new species (Tsuneda et al. 2000).

Literature and GenBank records

Several studies have recently addressed the diversity of microfungi on coniferous litter. Lindahl and Boberg (2008) described a high incidence of *S. sphagnicola* in *Pinus sylvestris* litter needles in Sweden. *S. sphagnicola* was found in early stages of decomposition and persisted in the litter for up to two years. Surprisingly, *S. sphagnicola* was not retrieved from *Picea abies* litter needles in Finland and Poland in studies by Korkama-Rajala et al. (2008) and Przybył et al. (2008), respectively. In the former study, microfungi colonising litter needles were studied using a culture-dependent approach as well as isolation of DNA. It is very improb-

able that *S. sphagnicola* would be missed combining these approaches. In the latter study, fungi were solely isolated on malt extract agar after surface sterilisation of the needles (in ethanol and 3 % cupric chloride). Isolated microfungi were identified only morphologically. *A. pullulans* and *H. dematioides* were isolated with frequencies reaching 4.5 % and 8 %, respectively. Considering the similarity of these two species to *S. sphagnicola*, misidentification cannot be excluded. Unfortunately, the strains obtained during the latter study are not available.

Alignment with sequences from GenBank showed that *S. sphagnicola* was isolated from leaf litter of *Gaultheria shallon* Pursh ("*Scleroconidioma* sp.", Osono et al. 2008) and soil under *Pinus contorta* (Dougl.) ex Loud. in Canada ("uncultured Ascomycota clone"; Hartmann et al., unpublished). The sequences with accession numbers AB366648 and FJ553470 showed 100 % similarity to the type sequence. The former record shows that *S. sphagnicola* may be found in the litter of non-coniferous plants. Additionally, not all conifer species may represent suitable substrates for *S. sphagnicola*. In the present study, I did not isolate *S. sphagnicola* from *Larix decidua* (Mill.) litter in the Bohemian Switzerland (Czech Republic) and Saxon Switzerland (Germany) National Parks, where *S. sphagnicola* was regularly present in the litter of pine and spruce (Tab. 1). An interesting record with a sequence similarity of 99.2 % (517/521 bp) to *S. sphagnicola* was an unknown fungal species isolated from hair roots of *Rhododendron lochiae* F. Muell. in an Australian tropical cloud forest (Bougoure and Cairney 2005). This strain may represent another species of the genus *Scleroconidioma* distributed in the Southern Hemisphere. Unfortunately, the strain is not available.

Conclusions

The presented method for *S. sphagnicola* isolation and identification may be helpful for the study of fungal diversity in coniferous forests and should prevent future confusion of *S. sphagnicola* with other fungi. Previous literature and the data gathered in this study show that *S. sphagnicola* may be found in various parts of Europe and Canada, although not ubiquitously. The data on the current distribution of *S. sphagnicola* may be valuable for further studies assessing the diversity and role of saprotrophic microfungi in coniferous litter. Above all, the possible spread of *S. sphagnicola* and its effect on microfungal communities in coniferous litter and potential competitive exclusion of other fungal species (e.g. *A. pullulans*) deserves further study. At present, the role of *S. sphagnicola* as a parasite of coniferous needles or terrestric bryophytes in coniferous forests does not seem probable. *S. sphagnicola* was not isolated from living coniferous needles and selected strains failed to colonise *Sphagnum* plants in artificial conditions (Koukol and Kovářová 2007).

ACKNOWLEDGEMENTS

I am grateful to the numerous students and colleagues who eagerly sampled coniferous litter needles during their journeys abroad (namely Miloš Duchoslav, Celie Korittová, and David Svoboda). This study was supported by the Grant Agency of the Academy of Sciences of the Czech Republic, project Nr. KJB601110718 and the Grant Agency of the Czech Republic, project Nr. 206/09/P295.

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