Diversity in *Scedosporium dehoogii* (Microascaceae): *S. deficiens* sp. nov.

J. Rainer[1] & J. Kaltseis[1]

[1] Institute of Microbiology, Leopold-Franzens-University Innsbruck, Austria.

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In the past few years a number of new *Pseudallescheria* (teleomorph) and *Scedosporium* (anamorph) species were described, among them *Scedosporium dehoogii*. This fungus was found frequently in the environment, whereas only a limited number of clinical cases have been reported. Some strains of *S. dehoogii* were recognised differing from the type strain in its ITS-sequences by clinicians during routine identification work. In an earlier work on the abundance of *Pseudallescheria* and *Scedosporium* in soil, a large number of *S. dehoogii* strains have been isolated. These strains grouped in two clades during sequence comparison of the ITS region as well as in the β-tubuline (BT2) gene. Differences were also found in the growth rates on polyvinyl alcohol agar supplemented with Diesel, rapeseed oil, and without supplement, in the colony appearance on oil-containing media and at 41 °C on PDA. Therefore the new species *Scedosporium deficiens* is proposed.

Keywords: Human pathogenic fungi, Microascaceae, *Scedosporium dehoogii*, *Pseudallescheria*, new species, oil.

The genus *Pseudallescheria* Negr. & I. Fisch. differs from other members of the Microascaceae Luttr. ex Malloch by its black to dark brown, non-ostiolate cleistothecia, produced by many strains in culture. Nevertheless, some members of recently described species are not able to produce ascomata and are therefore named by one of their anamorphic epithets in the genus *Scedosporium* Sacc. ex Castell & Chalm. The type species *P. boydii* (Shear) McGinnis, A. A. Padhye & Ajello, was long seen as a single species, variable in morphological characteristics and ecological features. Also a high diversity in clinical pictures caused by this opportunistic pathogen was recognised together with a certain variability in antimycotic drug resistance. In the early 1990ies, first molecular studies were carried out which revealed the existence of at least three distinct groups. This concept was worked over and expanded in recent years (Gilgado *et al.* 2005, Rainer & de Hoog 2006, Gilgado *et al.* 2008, Gilgado *et al.* 2010). Sequence comparisons resulted in five (clade 1-5 [Gilgado *et al.* 2005]) to seven (PbA – PbG [Rainer & de Hoog 2006]) distinct clades. Gilgado *et al.* (2005, 2008) proposed new species names for their clades 1 to 3 (*S. aurantiacum* Gilgado *et al.*, *P. minutispora* Gilgado *et al.*, *S. dehoogii* Gilgado *et al.*).

Furthermore, they assigned the described clade 4 to *P. apiosperma* Gilgado *et al.* with the anamorph *S. apiospermum* Sacc. ex Castell & Chalm. which has been treated as anamorph of *P. boydii* until recently. *Pseudallescheria boydii* (anamorph *S. boydii* Gilgado *et al.*) is left to denote a heterogenous group of strains containing controversially discussed species, which were renamed by McGinnis *et al.* (1982): *P. angusta* (Malloch & Cain) McGinnis *et al.*, *P. ellipsoidea* (Arx & Fassat.) McGinnis *et al.*, and *P. fusoidea* (Arx) McGinnis *et al.*

This work is focused on *S. dehoogii*, for which Gilgado *et al.* (2008) have already recognised differences in the TUB region of the beta-tubuline gene. These deviations resulted in two homogenous clusters within the otherwise well-delineated *S. dehoogii* branch. Physiological tests and experiments evaluating the impact of carbohydrates on populations in soil revealed also a bipartition of the isolated *S. dehoogii* strains concerning their growth rates and abundance. Recently, doubts on the correct identification of *S. dehoogii* based on ITS sequences occurred from clinicians, reflecting the impact of the grouping within the *S. dehoogii* clade.

In this work an accurate description of the morphology and physiologic particularities of the members of *S. dehoogii* clades is given, as well as an analysis of their molecular characteristics.

Material & Methods

Strains

Strains used for studying morphological and physiological characteristics (Tab. 1) were isolated during field studies in Innsbruck (Kaltseis *et al.* 2009) except the type strain CBS 117406, which we obtained from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, NL. These isolates represent a collection of more than 72 ITS-sequenced *S. dehoogii* strains. These strains were isolated from urban soils and rural areas with intensive agricultures. For comparative sequence analysis sequences from Genbank and the curated working database of the CBS were used.

Sequence analysis

DNA extraction, PCR, and sequencing were done with the primer-pairs ITS4, ITS5 and BT2-F, BT2-R according to Kaltseis *et al.* (2009) and Gilgado *et al.* (2005). Alignments were done with the help of MUSCLE (Edgar 2004); maximum parsimony was calculated by means of MEGA (Tamura *et al.* 2007) software.

Physiological tests

Growth rates on potato dextrose agar (PDA) were measured at 25 °C, 37 °C, 39 °C, 41 °C, 43 °C, and 45 °C. Strains were inoculated

Tab. 1. – Strains and sequences used with source of isolation and Genbank numbers if available.

Species	Collection no.	Source of isolation	Genbank accession no.	
			ITS	BT2
P. apiosperma	CBS 330.93	Near-drowning patient, the Netherlands	AY863196	–
	CBS 117407	Keratitis, Brazil	AJ888416	AJ889584
	CBS 118233	Near-drowning patient, Graz, Austria	CBS[1]	–
	CBS 119712	Lung and brain tissues, Japan	EF151356	–
	JK 51	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 57	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 68	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 107	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 131	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 134	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 137	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 142	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 149	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 150	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 176	Soil, agricultural land, Inn valley, Austria	CBS1[1]	CBS[1]
	JK 181	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 199	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 203	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 223	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 249	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 254	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
P. boydii	CBS 101.22 T	Mycetoma, Galveston, Texas, USA	AJ888435	AJ889590
	CBS 117412	Pleural liquid, Madrid, Spain	AJ888392	AJ888960
	CBS 115829	Disseminated infection, Thessaloniki, Greece	CBS[1]	–
	CBS 418.73	Soil, Republic of Tajikistan	AJ888426	AJ889595
	JK 52	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 193	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 234	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	CBS 106.53 T	Goat dung, Aligarh, India	AJ888428	AJ889601
	CBS 254.72 T	Half-digested sewage tank, Ohio, USA	AJ888414	AJ889604
P. minutispora	CBS 116911 T	River sediment, Tordera river, Spain	AJ888384	AJ889592
	JK 194	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
S. aurantiacum	CBS 116910 T	Ulcer of ankle, Santiago de Compostela, Spain	AJ888440	AJ889597
	JK 53	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 229	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
S. dehoogii	CBS 117406 T	Garden soil, Barcelona, Spain	AJ888389	AJ888958
	CBS 101723	Mud, Eempolder, the Netherlands	AY878947	–
	FMR 8532	Cultivated soil, Montsia, Spain	AJ888407	AJ889858
	MUCL 8522	Humic soil, Baarn, the Netherlands	AJ888421	AJ889589
	FMR 8622	Foot skin, Barcelona, Spain	AJ888419	AJ889587
	MUCL 20263	Greenhouse soil, Herverlee, Belgium	AJ888423	AJ889852
	JK 16a	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 111a	Soil, agricultural land, Inn valley, Austria	CBS[1]	CBS[1]
	JK 207a	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 270a	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 277a	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 287a	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 46b	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 63b	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 200b	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 210b	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 235b	Soil, park and playground, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 260b	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 268b	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]
	JK 269b	Soil, industrial area, Innsbruck, Austria	CBS[1]	CBS[1]

[1] Curated sequence database of the CBS.

with a needle in the centre of the plates. Diameter of colonies was measured after 14 d incubation. Growth rates on solid media with Diesel and rapeseed oil [PVA agar modified after Janda-Ulfig *et al.* (2008): 5 g Bactopeptone (Difco), 5 g NaCl, 10 g rapeseed oil or diesel oil, 1.5 g polyvinyl alcohol (PVA), 0.8 g bromthymol blue, 15 g Bactoagar (Difco), 1000 mL deionised water] were measured after 14 d at 25 °C. The medium base was autoclaved at 121 °C, 1 bar; Diesel, rapeseed oil, and polyvinyl alcohol were supplemented after autoclaving. The utilisation of C-sources was carried out in liquid media using microplate technology, according to Gilgado *et al.* (2008). Deviating, only sucrose, D-glucosamine, L-Rhamnose, Lactose, Glycerol, Inositol, Melezitose, D-Gucitol (Sorbit), and Creatinine were tested, as for these C-sources variable results were observed previously (Gilgado *et al.* 2008). The liquid media were prepared as described in MycoBank (www.mycobank.org). The results for D-glucosamine were verified by assimilation tests in 15-mL tubes containing 4 mL assimilation medium and 10^5 mL^{-1} conidia.

Mating experiments

Mating experiments between strains of the clades *S. dehoogii* A and B (compare Fig. 1) were carried out. Strains belonging to either group were inoculated opposite on each plate on Silva-Hutner-Weizmann agar (Kane *et al.* 1997) with a needle. Production of ascomata in the contact zone was observed after 12 weeks.

Morphology

Material for microscopic examination (Nikon Optiphot 2, Nomarski interference contrast, 100x, 1000x oil immersion; mounted in 3 % KOH) obtained from 14 d cultures on PDA. Nine strains, including the type strain of *S. dehoogii* (CBS 117406) were compared.

Results

Sequence analysis

Two clades within *S. dehoogii* – clade A containing the type strain, and *S. dehoogii* clade B – were found during comparison of ITS sequences (bootstraps ≥ 82). Four polymorphic sites were detected which were responsible for the bipartition of the *S. dehoogii* branch in the most parsimonious tree (Fig. 1). In the BT2 sequence-based tree (Fig. 2), this bipartition was also found, and, in addition, two branches with the type strain and four other strains were separated (bootstraps ≥ 95). The polymorphic sites between the three clades are listed in Tables 2 and 3.

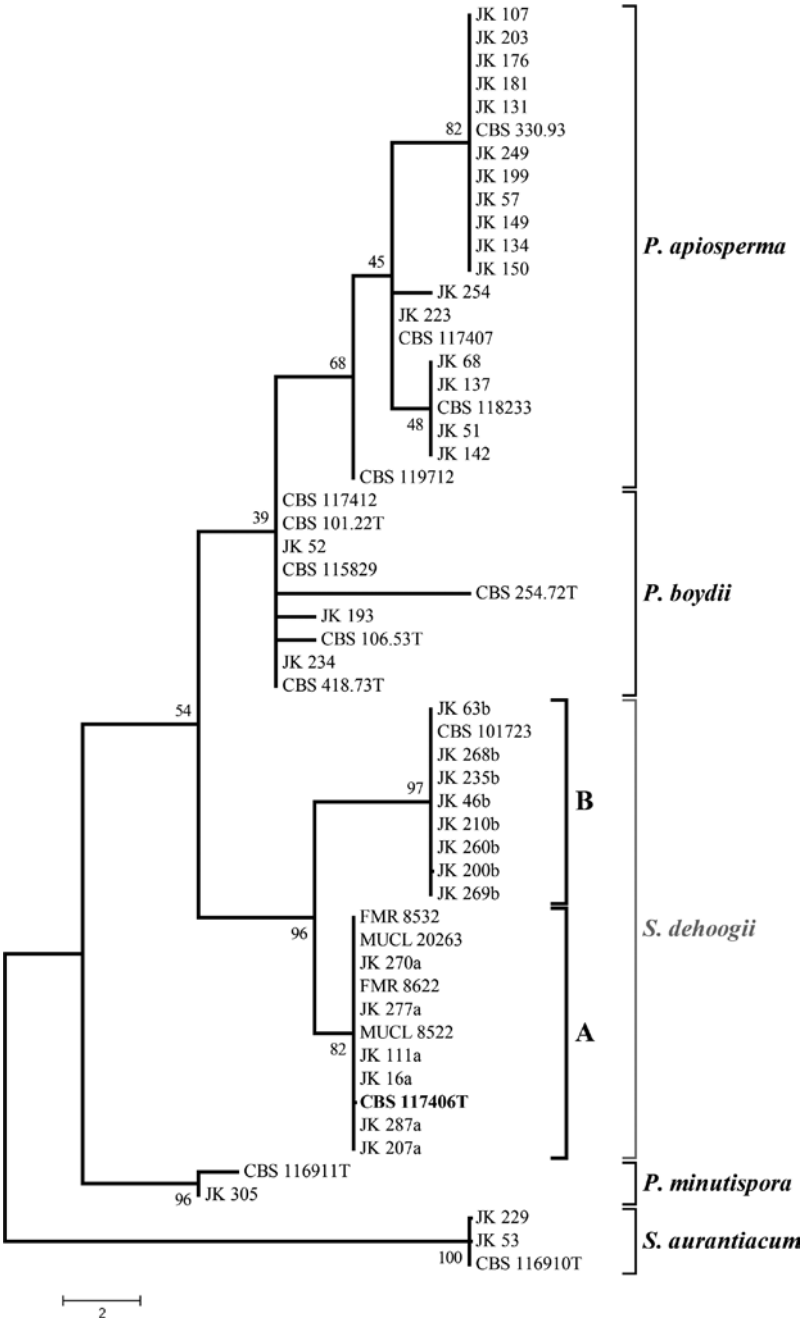

Fig. 1. – Phylogenetic tree, based on ITS sequences: Most parsimonious tree (n = 847), consistency index = 0.815789, bootstrap test with 200 replicates. Positions containing gaps and missing data were eliminated from the dataset. 29 of 517 positions were parsimony informative.

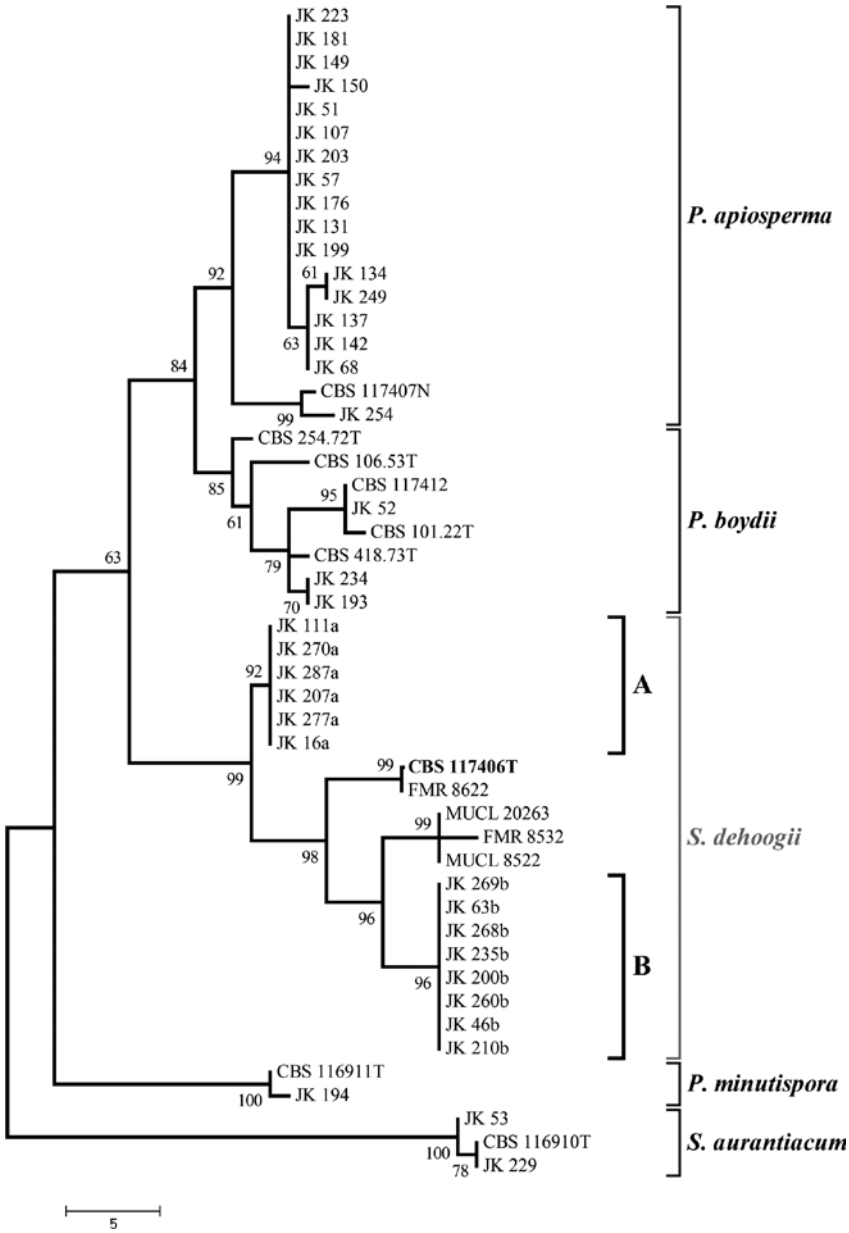

Fig. 2. – Phylogenetic tree, based on BT2 sequences: most parsimonious tree (n = 510), consistency index 0.876289, bootstrap test with 200 replicates. Positions containing gaps and missing data were eliminated from the dataset. 74 of 371 were parsimony informative.

Tab. 2. – Polymorphic sites in the BT2 region of the tubuline gene. Numbers indicate the position forward from the primer site.

Strain/clade	Nucleotide at polymorphic site at indicated position																			
	11	**30**	**53**	**108**	**120**	**137**	**145**	**184**	**250**	**286**	**287**	**291**	**294**	**299**	**301**	**317**	**322**	**326**	**328**	**434**
CBS 117406T	T	A	G	T	G	C	A	T	C	A	A	C	G	A	C	T	A	C	A	T
Clade A	A	.	C	C	A	A	–	C	.	.	.	.	.	G	T	C	T	.	G	.
Clade B	.	.	.	.	A	–	.	.	A	C	C	T	C	G	T	.	.	T	G	C

Tab. 3. – Polymorphic sites in the ITS region of the ribosomal genes. Numbers indicate the position forward from the beginning of the ITS region.

Strain/clade	Nucleotide at polymorphic site at indicated position						
	76	**77**	**78**	**79**	**80**	**81**	**82**
CBS 117406T	C	C	C	G	A	A	A
Clade A	.	.	.	.	.	.	.
Clade B	T	.	.	C	C	.	C

Taxonomy

Scedosporium deficiens Rainer & Kaltseis, **sp. nov.** – Figs. 3a–c.
MycoBank: MB 518386

Coloniae dilute griseae usque ad fuscae nonnumquam facile roseae sunt. Conidiophora solitaria plerumque non-ramosa. Conidia sessilis subhyalina vel dilute grisea. Conidia producid sessilis vel in distinctis annelidibus vel in synnematis. Caules synnematae coloratae nigrae. Diameter coloniae in potato dextrose agar post 14 dies ad 25 °C, 42.5–47.0 mm; Polyvinyl alcohol (PVA) agar cum diesel oleo, 26.5–34.5 mm; PVA agar cum brassica oleifera, 40.0–45.0 mm; PVA agar sine oleo, 38.0–41.0 mm. Coloniae similes cerae in PDA ad 41 °C, si denique coloniae aducent. Coloniae habent fines claros in PVA agar. Pars aversa coloniarum cana usque nigra est in PVA agar cum et sine oleo (nullus flavus color). Teleomorphosis ignota. D-glucosaminae non assimilatae.

Colonies coloured light grey to brownish grey, sometimes with an old rose touch. Colony reverse grey to dark grey, sometimes with a greenish note. Colony diameters after 14 d (Tab. 4) at 25 °C on PDA 42.5–47 mm, on PVA- agar with Diesel 26.0–34.5 mm, on PVA- agar with rapeseed oil 40.0–45.0 mm, and PVA agar without oil 38.0–41.0 mm (Tab. 4). Colonies on PDA at 41 °C wax-like with colonies ≤ 6.0 mm, if growth is present. Colonies on PVA at 25.0 °C with sharp margins and radial wrinkles. Colony reverse on PVA-agar, with and without Diesel or rapeseed oil, dark grey to dark brown, without yellow indicator switch. Conidia subhyaline to light grey, 6.3–9.7 × 3.9–5.6 µm (Tab. 5), produced sessile or on distinct annellides (5–50 × 0.8–2.6 µm). Solitary conidia usually non-ramose. Synnemata (Graphium) with dark coloured stipes producing conidia in a slimy matrix. Synnemata sometimes only microscopic, composed of few hyphae. No teleomorphs were observed. D-glucosamine was not assimilated (Tab. 6).

Etymology. – Refers to the fact that ecophysiological abilities are constantly "inferior" compared to *S. dehoogii*.

Distinguishing characters. – *S. deficiens* can be distinguished from *S. dehoogii* by its growth rates on oil containing media (PVA and PVA with Diesel or rapeseed oil) as well as by the velvety to cottony, non-flower like colony appearance and sharp margins on PVA with and without oil. On PVA no indicator switch occurred, no yellow colony reverse was observed. At 41 °C, if growth is present, *S. dehoogii* forms an aerial mycelium, whereas *S. deficiens* exhibits a waxy colony surface.

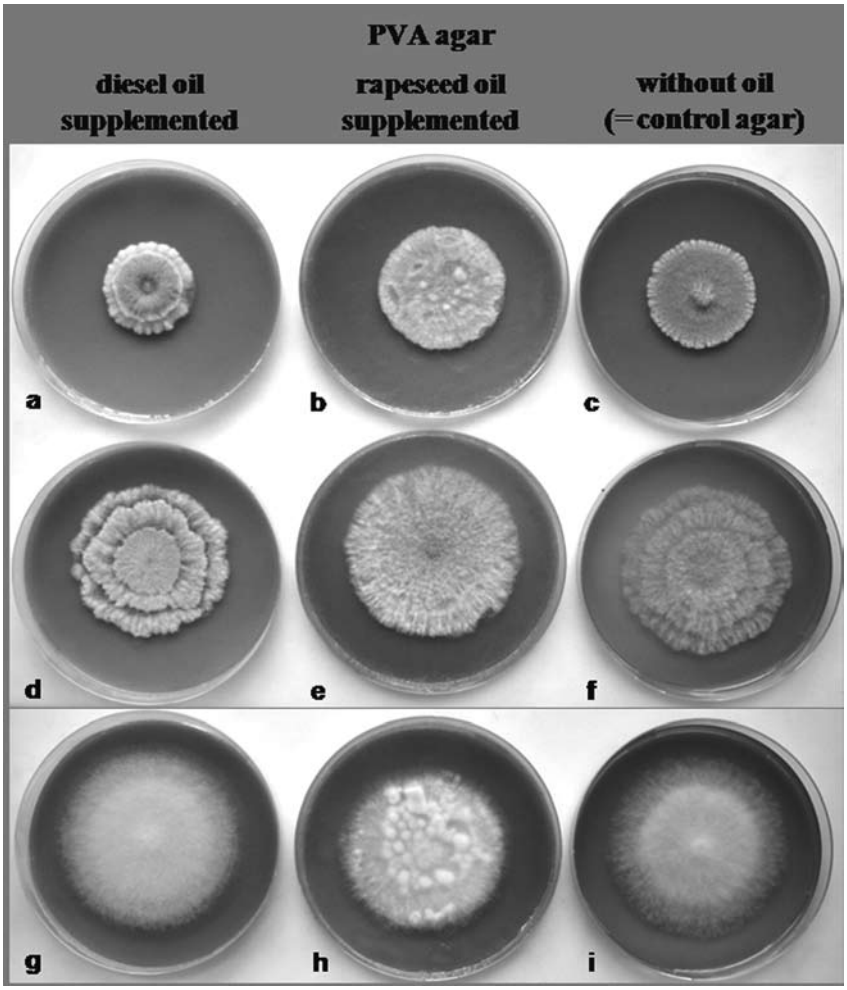

Fig. 3. – Colonies of *S. deficiens* (3a–c), *S. dehoogii* (3d–f), and the type strain of *S. dehoogii* (3g–i) on PVA at 25 °C with and without oil supplements.

Discussion

The sequence comparison of the ITS region revealed two distinct clades within *S. dehoogii*. One of these clades (A) contained the type strain of *S. dehoogii,* for the other clade, *S. deficiens* sp. nov. is described here. Constant phenotypic and molecular differences in the BT2 region between *S. dehoogii* strains in clade A and the *S. dehoogii* type strain were recognised but are in contrast to their identical ITS sequences. The two clusters (clade A with *S. dehoogii* and clade B with *S. deficiens* sp. no *S. deficiens* sp. nov.) were found also in the BT2 tree. This finding correlates with both morphological characteristics and growth rates on nutrition media containing Diesel or rapeseed oil. Slight differences in growth rates were found at 41 °C, at which strains of *S. deficiens* were growing slower and lacked aerial mycelia. In previous studies on *Pseudallescheria* and *Scedosporium* populations in soil supplemented with Diesel, it was reported that members of *S. deficiens* were not found at concentrations of 10 g Diesel/kg (Eggertsberger 2010). In contrast, *S. dehoogii* made up a large proportion of the *Scedosporium* and *Pseudallescheria* strains at higher Diesel concentrations in soil. Consequently, we presume that the different Diesel sensitivity might have a phylogenetic background.

Tab. 4. – Growth rates on PDA at different temperatures and on PVA at 25 °C with Diesel and rapeseed oil.

14 d, PDA	**Growth rates [mm]**		
	CBS 117406T	*S. dehoogii*	*S. deficiens*
25 °C	72.5	42.5 – 46.5	42.5 – 47.0
37 °C	60.0 – 65.0	36.0 – 40.0	37.0 – 49.0
39 °C	19.0 – 21.0	12.0 – 19.0	4.0 – 11.0
41 °C	5.0 – 6.0	0.0 – 7.0	0.0 – 6.0
43 °C	No growth	No growth	No growth
45 °C	No growth	No growth	No growth
14 d, 25 °C, PVA agar			
Diesel oil supplemented	68.0	37.0 – 60.0	26.0 – 34.5
Rapeseed oil suppl.	60.0 – 63.0	48.0 – 62.5	40.0 – 45.0
Without oil (= control agar)	63.0 – 66.0	45.5 – 60.5	38.0 – 41.0

Tab. 5. – Measures (µm) of the conidiogenous cells and conidia of representatives of *S. dehoogii, S. deficiens,* and the type strain of *S. dehoogii.*

Strain/clade	**Annellides**	**Conidia**		
		length	**width**	**l/w ratio**
CBS 117406T	1.4 (1.2 – 1.5)	7.7 (6.2 – 8.9)	4.6 (3.9 – 5.6)	1.67
S. dehoogii	1.8 (1.3 – 2.4)	7.2 (6.2 – 9.0)	5.1 (4.3 – 6.2)	1.40
S. deficiens	1.7 (0.9 – 2.6)	7.4 (6.3 – 9.6)	4.9 (3.9 – 5.6)	1.51

Tab. 6. – Assimilation of C-sources by different strains belonging to *S. dehoogii* (Sdh) and *S. deficiens* (Sde). (+ growth, – no growth, (–) growth very weak)

Strains	C-Source											
	Negative control	Neg. con. + sucrose	Positive control	Sucrose	D-glucosamine	L-rhamnose	Lactose	Glycerol	Inositol	Melezitose	D-glucitol	Creatinine
Sdh CBS 117406T	–	–	–	+	+	+	–	+	–	–	–	–
Sdh JK 16a	–	–	–	+	+	+	–	–	–	–	–	–
Sdh JK 111a	–	–	–	+	+	+	–	–	–	–	–	–
Sdh JK 207a	–	–	–	+	+	-	–	–	–	–	–	–
Sdh JK 270a	–	–	–	+	+	+	–	–	–	–	–	–
Sdh JK 287a	–	–	–	+	+	+	–	+	–	–	–	–
Sde JK 46b	–	–	–	+	(–)	+	–	–	–	–	–	–
Sde JK 63b	–	–	–	+	(–)	+	–	–	–	–	–	–
Sde JK 200b	–	–	–	+	(–)	+	–	–	–	–	–	–
Sde JK 210b	–	–	–	+	–	+	–	–	–	–	–	–
Sde JK 235b	–	–	–	+	–	+	–	+	–	–	–	–
Sde JK 268b	–	–	–	+	–	+	–	–	–	–	–	–
Sde JK 269b	–	–	–	+	–	+	–	–	–	–	–	–

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References

Edgar R. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Eggertsberger M. (2010) Temperature dependence and environmental conditions enhancing the distribution of the opportunistic pathogens *Scedosporium* and *Pseudallescheria*. Master Thesis, University of Innsbruck, Austria, 93 pp.

Gilgado F., Cano J., Gene J., Sutton D. A., Guarro J. (2008) Molecular and phenotypic data supporting distinct species statuses for *Scedosporium apiospermum* and *Pseudallescheria boydii* and the proposed new species *Scedosporium dehoogii*. *Journal of Clinical Microbiology* **46**: 766–771.

Gilgado F., Cano J. F., Guarro J. (2005) Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *Journal of Clinical Microbiology* **43**: 4930–4942.

Gilgado F., Gene J, Cano J., Guarro J. (2010) Heterothallism in *Scedosporium apiospermum* and description of its teleomorph *Pseudallescheria apiosperma* sp. nov. *Medical Mycology* **48**: 122–128.

MycoBank http://www.mycobank.org/yeast/defaultpage.aspx 12 May 2010.

Janda-Ulfig K., Ulfig K., Cano J., Guarro J. (2008) A study of the growth of *Pseudallescheria boydii* isolates from sewage sludge and clinical sources on tributyrin, rapeseed oil, biodiesel oil and Diesel oil. *Annals of Agricultural and Environmental Medicine* **15**: 45–49.

Kaltseis J., Rainer J., de Hoog G. S. (2009) Ecology of *Pseudallescheria* and *Scedosporium* species in human dominated and natural environments and their distribution in clinical samples. *Medical Mycology* **47**: 398–405.

Kane J., Summerbell R.C., Sigler L., Krajden S., Land G. (1997) *Laboratory handbook of dermatophytes: A clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails*, Star Publishing Co. Belmont, California, 331 pp.

McGinnis M. R., Padhye A. A., Ajello L. (1982) *Pseudallescheria* Negroni et Fischer, 1943 and its later synonym *Petriellidium* Malloch, 1970. *Mycotaxon* **14**: 94–102.

Rainer J., de Hoog G. S. (2006) Molecular taxonomy and ecology of *Pseudallescheria, Petriella* and *Scedosporium prolificans* (Microascaceae) containing opportunistic agents on humans. *Mycological Research* **110**: 151–160.

Tamura K., Dudley J., Nei M., Kumar S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.

Von Arx J.A.(1973). The genera *Petriellidium* and *Pithoascus* (Microascaceae). *Persoonia* **7**: 367–375.

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