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ARTICLE

Parascedosporium and its relatives: phylogeny and ecological trends

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Abstract: The genus *Scedosporium* and its relatives comprising microascalean anamorphs with slimy conidia were studied. *Graphium* and *Parascedosporium* also belong to this complex, while teleomorphs are found in *Pseudallescheria*, *Petriella*, *Petriellopsis,* and *Lophotrichus*. Species complexes were clearly resolved by rDNA ITS sequencing. Significantly different ecological trends were observed between resolved species aggregates. The *Pseudallescheria* and *Scedosporium prolificans* clades were the only lineages with a marked opportunistic potential to mammals, while *Petriella* species were associated primarily with soil enriched by, e.g. dung. A consistent association with bark beetles was observed in the *Graphium* clade. The ex-type strain of *Rhinocladium lesnei*, CBS 108.10 was incorrectly implicated by Vuillemin (1910) in a case of human mycetoma; its sequence was identical to that of the ex-type strain of *Parascedosporium tectonae*, CBS 127.84.

Key words:
Parascedosporium tectonae
Rhinocladium lesnei
Graphium putredinis
Doratomyces putredinis
Scedosporium
Pseudallescheria
Microascales, ecology

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INTRODUCTION

This study aims to provide a phylogenetic overview of the relatives of the human-opportunistic genus *Pseudallescheria*. Members of this genus, producing *Scedosporium* and eventually *Graphium* (syn)anamorphs, are found in industrially or agriculturally enriched environments. They are frequently observed in subcutaneous and systemic infections. Among the infections caused is a unique clinical entity, the near-drowning cerebral abscess (Guarro *et al.* 2006). The species are also encountered as persistent colonizers of the respiratory tract of patients with cystic fibrosis (Lu *et al*. 2011). In contrast, monomorphic *Graphium* species have been reported from galleries of bark beetles in conifer wood (Jacobs *et al.* 2003). This study was undertaken to establish, whether these widely different ecologies show any phylogenetic consistency.

Gilgado *et al.* (2007) introduced the polymorphic generic name *Parascedosporium* Gilgado in the framework of a taxonomic revision of *Scedosporium*, its synanamorph *Graphium* and its teleomorphs *Pseudallescheria, Petriella,* and *Petriellopsis*. *Parascedosporium* was segregated from *Scedosporium* and its synnematous synanamorph *Graphium* on the basis of sympodial conidiogenesis of the *Scedosporium* morph, conidia being borne on large, blunt denticles, and annellidic in the *Graphium* morph. *Scedosporium,* consistently present in members of the teleomorph genera mentioned above, has percurrent conidiogenesis resulting in somewhat slimy conidia. The description of *Parascedosporium* provided by Gilgado *et al.* (2007) was based on an authentic strain of *Graphium tectonae* (Booth 1964), CBS 127.84, which they renamed *Parascedosporium tectonae*. The strain had been isolated from *Tectonia grandis* seeds in Jamaica and its relation to the plant-inhabiting species *Graphium putredinis* needed to be established. We uncovered the ex-type strain of *Rhinocladium lesnei* which appeared to be molecularly similar to *P. tectonae*, but, according to its original description, it was associated with a case of human mycetoma. These widely different origins of supposedly interrelated strains required a thorough re-evaluation of the material.

Table 1. Strains used for phylogenetic tree construction. Syntypes are marked with (ST), epitypes with (ET), and ex-type strains with (T).

Main clade	Current ID	Obsolete name	Strain numbers	Status	Cross reference numbers	Associated insect	Host/Source	Country	GenBank number
Graphium	*G. basitruncatum*	*Graphium basitruncatum*	CBS 320.72	(T)	JCM 9300; MFC 2997		Forest soil	Solomon Islands	AB038427
Graphium	*G. basitruncatum*	*Graphium basitruncatum*	JCM 8083				Soil	Japan	AB038425
Graphium	*G. penicillioides*	*Graphium penicillioides*	CBS 102630		JCM 10496		*Populus nigra*, wood core	Czech Republic	AB038430
Graphium	*G. penicillioides*	*Graphium penicillioides*	CBS 102632	(ET)	JCM 10498; PRM 842988		*Populus nigra*, wood core	Czech Republic	AB038432
Graphium	*G. penicillioides*	*Graphium penicillioides*	JCM 10499				*Populus nigra*, wood core	Czech Republic	AB038433
Graphium	*G.laricis*	*Graphium laricis*	CMW 5598		DAOM 229754	*Ips cembrae*	*Larix decidua*	Scotland	AY148181
Graphium	*G.laricis*	*Graphium laricis*	CMW 5601	(T)	DAOM 229757	*Ips cembrae*	*Larix decidua*	Austria	AY148183
Graphium	*G.laricis*	*Graphium laricis*	CMW 5603		DAOM 229759	*Ips cembrae*	*Larix decidua*	Austria	AY148182
Graphium	*G.laricis*	*Graphium laricis*	CBS 116195		CMW 5602; DAOM 229758	*Ips cembrae*	*Larix decidua*	Austria	AY148184
Graphium	*G. pseudomiticum*	*Graphium pseudormiticum*	AsaP. 18			*Ips typographus*	Unknown	Sweden	FJ824623
Graphium	*G. pseudomiticum*	*Graphium pseudormiticum*	CMW 503	(T)		*Orthotomicus erosus*	*Pinus* sp.	South Africa	AY148186
Graphium	*G. pseudomiticum*	*Graphium pseudormiticum*	CMW 5611			*Tomicus minor*	*Pinus sylvestris*	Austria	AY148185
Graphium	*G. pseudomiticum*	*Graphium pseudormiticum*	CMW 12285				*Tsuga dumosa*	China	FJ434981
Graphium	*P. putredinis*	*Rhinocladium lesnei*	CBS 108.10	(T)	dH 14860; MUCL 11709; MUCL 15754		Human, foot mycetoma	France	HQ185347
Graphium	*R. fimbriasporum*	*Graphium fimbriisporum*	CBS 421.94		CMW 3353; CMW 5607	*Ips typographus*	*Picea abies*	Austria	AY148179
Graphium	*R. fimbriasporum*	*Graphium fimbriisporum*	CBS 422.94		CMW 3352; CMW 5606	*Ips typographus*	*Picea abies*	Austria	AY148178; AY148180
Graphium	*R. fimbriasporum*	*Graphium fimbriisporum*	CMW 5605	(T)		*Ips typographus*	*Picea abies*	France	AY148177
Lophotrichus	*L. fimeti*	*Pseudallescheria fimeti*	CBS 129.78	(T)	dH 14898		Dung of goat	India	AY879799
Microascus	*Microascus trigonosporus var. trigonosporus*	*Microascus trigonosporus var. trigonosporus*	CBS 665.71		NRRL A-8019		Soil	USA	AM774156
Parascedosporium	*P. putredinis*	*Graphium calicioides*	CBS 102085		JCM 9765		decayed wood	Japan	AB007686
Parascedosporium	*P. putredinis*	*Graphium pudretinis*	CBS 102083		JCM 8082		*Chrysalidocarpus lutescens*	Japan	HQ185348
Parascedosporium	*P. putredinis*	*Graphium putredinis*	HSAUP052348				Unknown		FJ914685
Parascedosporium	*P. putredinis*	*Graphium tectonae*	CBS 100392		dH 11150		Bathroom flask	Netherlands	GQ 476983
Parascedosporium	*P. putredinis*	*Parascedosporium tectonae*	CBS 118694				*Actinidia deliciosa*, leaf lesions	New Zealand	AM749735; AM749149

Table 1. (Continued).

Main clade	Current ID	Obsolete name	Strain numbers	Status	Cross reference numbers	Associated insect	Host/Source	Country	GenBank number
Parascedosporium	*P. tectonae*	*Parascedosporium tectonae*	CBS 127.84	(T)	dH14863; JCM 9753		*Tectona grandis*, seed	Jamaica	AY228113
Petriella	*P. guttulata*	*Petriella guttulata*	CBS 362.61	(ST)	MUCL 9886; TRTC 33049; UAMH 3996		Dung of partridge	Germany	AY879800
Petriella	*P. setifera*	*Petriella setifera*	CBS 265.64				Unknown		AY882349
Petriella	*P. setifera*	*Petriella setifera*	CBS 347.64		MUCL 8138		Compost	Belgium	AY882346
Petriella	*P. setifera*	*Petriella setifera*	CBS 391.75				*Tursiops truncatus*, skin lesion	The Nether-lands	AY882344
Petriella	*P. setifera*	*Petriella setifera*	CBS 395.69				Maize-field soil	Canada	AY882348
Petriella	*P. setifera*	*Petriella setifera*	CBS 710.96		FMR 5550		Soil	Singapore	AY882347
Petriella	*P. sordida*	*Melanospora asymmetrica*	CBS 297.58				Compost soil	Germany	AY882359
Petriella	*P. sordida*	*Petriella guttulata*	CBS 520.72				Unknown	Germany	AY882355
Petriella	*P. sordida*	*Petriella setifera*	ATCC 26490				Unknown		AF043596
Petriella	*P. sordida*	*Petriella setifera*	CBS 385.87				Human, nail	Finland	AY882345
Petriella	*P. sordida*	*Petriella sordida*	CBS 124169		dH 19097		Bathroom flask	Netherlands	GQ426957
Petriella	*P. sordida*	*Petriella sordida*	CBS 184.73	(T)			Wood	Sweden	AY882360
Petriellidium	*P. desertorum*	*Petriellidium desertorum*	CBS 489.72	(T)	UAMH 3993		Salt-marsh soil	Kuwait	AY879798
Pseudallescheria	*P. africana*	*Pseudallescheria africana*	CBS 311.72	(T)	dH 14874		Brown, sandy soil	Namibia	AY879797; AY228115; AJ888425
Pseudallescheria	*P. angusta*	*Pseudallescheria angusta*	CBS 254.72	(T)			Unknown		AY228114; AJ888414
Pseudallescheria	*P. apiosperma*	*Graphium eumorphum*	CBS 987.73		JCM 9748		Human, otitis externa	Czecho-slovakia	AY877352
Pseudallescheria	*P. apiosperma*	*Pseudallescheria apio-sperma*	CBS 117407	(T)	FMR 8619; dH 14354		Kereatitis	Brazil	AJ888416; AJ889584
Pseudallescheria	*P. boydii*	*Pseudallescheria boydii*	CBS 119707	(T)			Unknown		HQ185312
Pseudallescheria	*P. minutispora*	*Scedosporium minutispora*	CBS 116911	(T)			Leukemia patient, subcuta-neous mycosis of the leg	Hungary	HQ185354
Pseudallescheria	*S. aurantiacum*	*Scedosporium aurantiacum*	CBS 116910	(T)	FMR 8630; dH 14360		Human, ulcer of ankle	Spain	AJ888440; AJ889597; AJ890133; AJ890219
Pseudallescheria	*S. dehoogii*	*Scedosporium dehoogii*	CBS 117406	(T)	FMR 6921; dH 14338		Garden soil	Spain	AJ888389; HQ185341

Table 1. (Continued).

Main clade	Current ID	Obsolete name	Strain numbers	Status	Cross reference numbers	Associated insect	Host/Source	Country	GenBank number
Scedosproium prolificans	*S. prolificans*	*Scedosporium prolificans*	CBS 100391				Disseminated infection, AIDS patient with Burkitt lymphoma	Germany	AY882368
Scedosproium prolificans	*S. prolificans*	*Scedosporium prolificans*	CBS 116906		dH 14616; IHEM 14076		Sputum of cystic fibrosis patient	France	HQ185323
Scedosproium prolificans	*S. prolificans*	*Scedosporium prolificans*	CBS 452.89	(T)			Human, blood	France	HQ185322

MATERIALS AND METHODS

Strains and sequences

Strains and sequences included in this study, as well as isolation data and GenBank accession numbers, are listed in Table 1. Sequences compromised those downloaded from GenBank (Jacobs *et al.* 2003, Okada *et al.* 1998, Paciura *et al.* 2010) supplemented with sequences generated from material in the CBS collection of fungus cultures and other sequences from CBS not yet incorporated into the public collection.

DNA extraction and sequencing

Strains were cultured for 10–14 d on malt extract agar (MEA); genomic DNA was extracted according to Möller *et al.* (1992). ITS sequence data spanned the entire internal transcribed spacer region and the partial 28S ribosomal gene (D1/D2), generated with primers V9G and LS266 (de Hoog & Gerrits van den Ende 1998) and sequenced with primers its5 and its4. PCR reaction mixtures (total volume 25 µL) contained 0.4 µM forward and backward primers, 0.185 mM of each deoxynucleoside triphosphate (GC Biotech, Alphen aan de Rijn, The Netherlands), 10-fold concentrated NH_4 BioTaq Reaction buffer (GC Biotech), and 20 ng DNA. For performing the PCR reactions a Thermal cycler 2720 (Applied Biosystems, Foster City, U.S.A.) was used. PCR conditions were as follows: a) initial cycle 94 °C for 5 min, b) 35 cycles of 94 °C for 60 s, 53 °C 60 s, 72 °C for 120 s and c) final cycle of 10 min at 72 °C for 7 min. Strains were sequenced with an abi 3700xl instrument using BigDye Terminator Sequencing Kits (Applied Biosystems). Electropherograms were edited, using the Lasergene software package (DNAstar, www.dnastar.com).

Phylogenetic tree construction

Phylogenetic trees were calculated on the basis of 47 ITS sequences. Alignment was made automatically and adjusted manually using the Muscle package (www.ebi.ac.uk/Tools/muscle/index.html). A maximum likelihood ITS tree was calculated using Mega5 (www.megasoftware.net). The calculation parameters were 1000 bootstrap replicates with substitution model GTR + GI and heuristic search set to close-neighbour-interchange. *Microascus trigonosporus* was used as outgroup to root the tree.

Morphological characteristics

Morphological characteristics of strains CBS 192.61, CBS 102082, CBS 102083, CBS 18694, and CBS 108.10 were recorded. Strains were grown at room temperature under daylight on potato dextrose agar (PDA). Slide cultures were made of cultures in the early stages of conidiation, transferring a 5 mm-sized piece of the colony with agar to a fresh PDA plate, covering the transferred piece with a cover slip and incubating it at room temperature until growth on the cover slip could be observed. Slides were stained with Cotton blue. Microscopic features were photographed using a Nikon Eclipse 80*i* microscope with a Nikon digital sight DS-Fi1 camera.

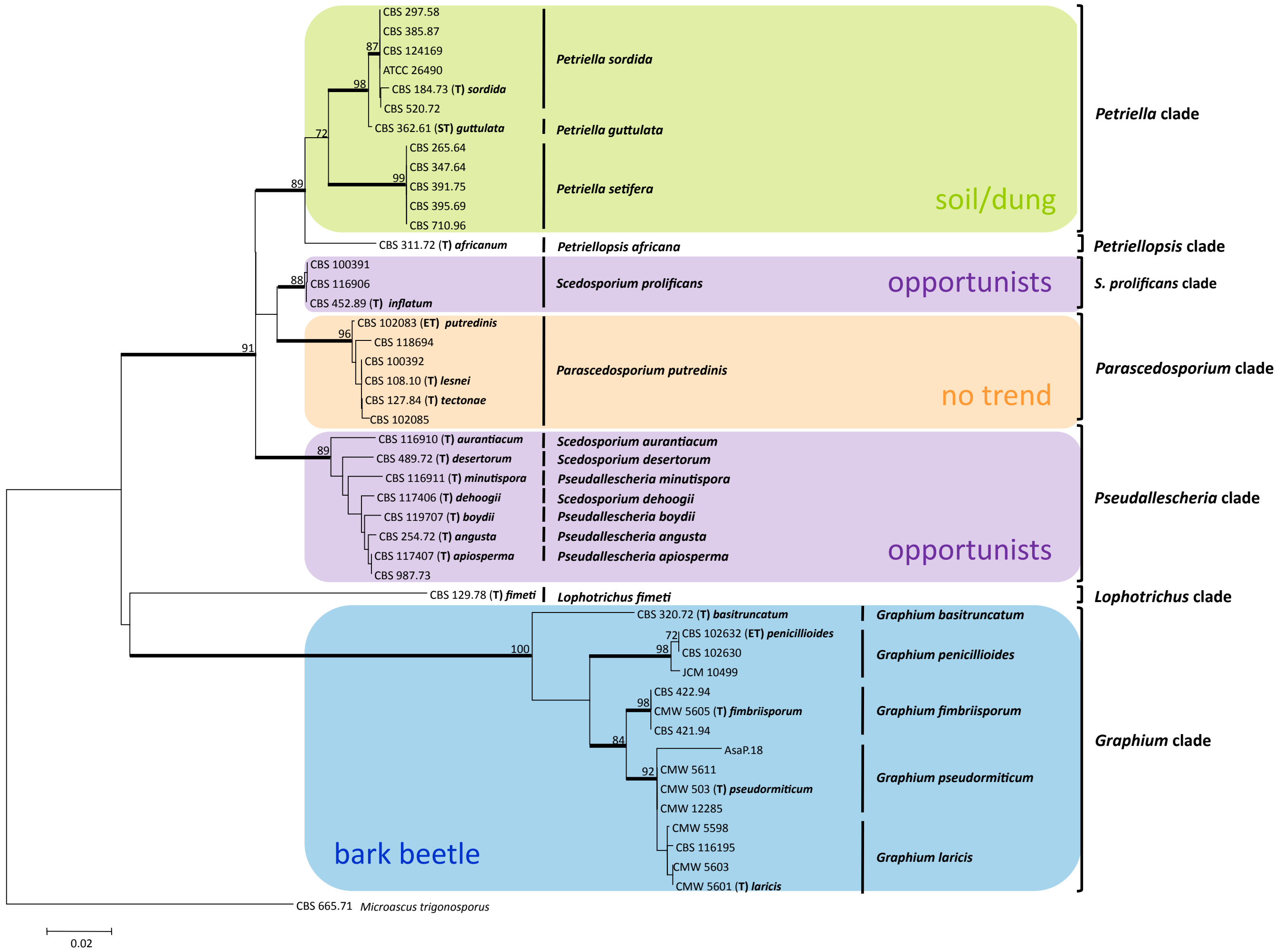

Fig. 1. Overview of treated members of *Microascales* including strains of *Graphium, Pseudallescheria, Petriella*, *Lophotrichus, Petriellopsis*, and *Parascedosporium*. The maximum likelihood ITS tree was calculated with 200 bootstrap replicates. Strains shaded in green represent strains isolated from dung, strains in shades of purple represent those with medical relevance and/or associated with hydrocarbon-polluted soil, and those shaded in blue are associated with trees and/or bark beetles, while strains shaded in orange do not have any apparent ecological trend. Bootstrap-supported branches (>80 %) are in bold and bootstrap values are indicated. Ex-type strains are marked with a bold (**T**), epitypes are marked with bold (**ET**), and syntypes with a bold (**ST**) after the strain number. The name used in the first description is given in italic bold type.

RESULTS

Sequences of strains of the following groups were included in the phylogenetic analyses: (1) *Petriella* clade (*P. sordida, P. guttulata*, and *P. setifera*), (2) *Petriellopsis africana*, (3) *Scedosporium prolificans*, (4) *Pseudallescheria* clade (*S. aurantiacum, P. desertorum, P. minutispora, S. dehoogii, P. boydii, P. angusta,* and *P. apiosperma*), (5) *Lophotrichus fimeti*, and (6) *Graphium* clade (*G. penicilliodes, G. fimbriisporum, G. pseudormiticum,* and *G. laricis*). We were unable to locate ex-type strains of (a) *G. bulbicola*, (b) *G. cuneiferum*, (c) *Nematographium stilboideum* (previously known as *G. stilboideum*), and (d) *G. fructicola*.

Bootstrap-supported branches (≥ 80) are indicated in bold in the ITS-tree (Fig. 1 and Table 1). Within the *Petriella* clade three branches were bootstrap-supported: (a) a branch of CBS 362.61 (ex-syntype of *P. guttulata*), (b) a branch of CBS 184.73 (ex-type *Petriella sordida*) including the strains identified in the GenBank and/or the CBS database as *P. setifera* (ATCC 26490, CBS 385.87), *Melanospora asymmetrica* (CBS 297.58), *P. sordida* (CBS 124169), and *P. guttulata* (CBS 520.72), (c) a third branch referred to as *P. setifera*. The latter branch did not contain any ex-type strain, but all strains attributed to this group (CBS 395.69, CBS 391.75, CBS 265.64, CBS 347.64, and CBS 710.96) were identified by GenBank and/or CBS database as *P. setifera*. *Petriellopsis africana* was represented only by its ex-type strain CBS 311.72. Sequences of strains of *Scedosporium prolificans*, CBS 452.89 (ex-type), CBS 116906, and CBS 100391 clustered in an isolated, bootstrap-supported clade which was the nearest neighbour of *Parascedosporium*.

The *Parascedosporium* clade (96 % bootstrap support) compromised CBS 102085, CBS 118694, and CBS 102083 of *Graphium putredinis* (according to Okada *et al.* 1998), the ex-type of *Rhinocladium lesnei* (CBS 108.10), and the ex-type plus one additional sequence of *Parascedosporium*

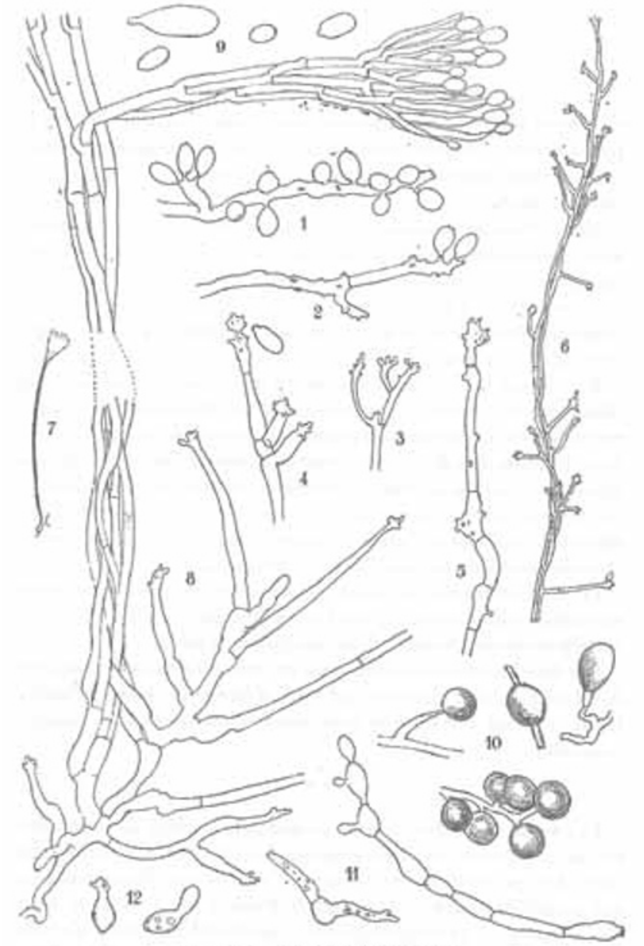

Fig. 2. Reproduction of the original drawings of *Rhinocladium lesnei* (Vuillemin 1910).

tectonae (CBS 127.84 and CBS 100392). Within the clade differences of maximally two bases were noted, but branches were statistically unsupported.

The bootstrap-supported *Pseudallescheria* clade was represented by ex-type strains of all currently recognized sibling species, including *Scedosporium aurantiacum* (CBS 116910), *Petriellidium desertorum* (CBS 489.72; according to latest taxonomic changes *Pseudallescheria desertorum*), *Pseudallescheria minutispora* (CBS 116911), *Scedosporium dehoogii* (CBS 117406), *P. angusta* (CBS 254.72), *P. boydii* (CBS 119707), and *P. apiosperma* (CBS 117407). Strain CBS 987.73 had been reported as causative agent of otitis externa. It was previously identified as *Graphium eumorphum* (Frágner & Hejzlar 1973), but the ITS sequence was 100 % identical to that of *P. apiosperma,* ex-type strain. *Lophotrichus fimeti* was represented exclusively by its ex-type strain (CBS 129.78).

The *Graphium* clade with 100 % bootstrap support included four bootstrap-supported branches. The first branch included the epitype of *G. penicillioides* (CBS 102632) and sequences of the *G. penicillioides* strains CBS 102630 and JCM 10499. The second branch contained a sequence of the ex-type of *Stilbum basitruncatum* (CBS 320.72; currently known as *G. basitruncatum*). The third branch was formed by strains of *G. fimbriisporum* (CMW 6505 ex-type strain, CBS 422.94, and CBS 421.94). The fourth bootstrap-supported subclade within the *Graphium* main clade included the ex-type strain of *G. pseudormiticum* (CMW 503) and the ex-type strain of *G. laricis* (CMW 5601), varying in ITS from each other by a single nucleotide change. Moreover, the sequences of the following strains were found in the same cluster: *G. pseudormiticum* (CMW 12285, CMW 5611, and AsaP. 18) and *G. laricis* (CBS 116195, CBS 5598, and CMW 5603). The two species *G. pseudormiticum* and *G. laricis* described by Jacobs *et al*. (2003) differed only by a single ITS nucleotide, which was similar to the *G. laricis* intra-species variation. Recently Cruywagen *et al*. (2010) distinguished the two species with *TEF1* sequences.

The ex-type strain CBS 108.10 of *Rhinocladium lesnei* was originally reported from a human mycetoma (Vuillemin 1910). His original illustration is reproduced in Fig. 2. The drawings show a fungus with elongate cells producing one-celled sympodial conidia on hyphae, in addition to hyphae aggregating in large synnemata and producing a drop of mucous sympodial conidia at the apex (Fig. 2). The characteristic features of the mononematous anamorph are still exhibited in the authentic strain, CBS 108.10 (Fig. 3) and match with the descriptions and illustrations provided by Gilgado *et al*. (2007) for that of *P. tectonae* CBS 102083. We investigated the morphological characteristics of the strains CBS 192.61 (= *G. putredinis*), CBS 102083 (= *G. putredinis*), and CBS 118694 (= *Parascedosporium tectonae*). All isolates displayed characteristic solitary scedosporium-like conidiophores bearing lateral, cylindrical conidiogenous cells with 2–5 cylindrical denticles. Strains CBS 102082, CBS 118694, and CBS 102083 formed additional synnemata with an annellidic type of conidiation. The synnematous anamorph of *R. lesnei* was no longer produced, but Vuillemin (1910) illustrated it as being sympodial (Fig. 2). Hence, we cannot be certain of its identity and therefore treat *R. lesnei* as being doubtful.

Graphium putredinis has previously been attributed to *Doratomyces* (Morton & Smith, 1963, Matsushima 1975), a microascalean genus with strongly hydrophobic conidia. This interpretation had been adopted in major databases such as *Index Fungorum* and MycoBank. However, Okada *et al.* (1998), following Hughes (1958), assigned it to the *Pseudallescheria* relationship comprising species with slimy conidia. The genus *Doratomyces* was introduced by Corda (1829), with a clearly recognizable description and illustration, rendering confusion with a mucous gaphium-like species unlikely. *Gaphium cuneiferum* is often listed as a synonym, although Hughes (1958), in the absence of authentic material, regarded this synonymy as doubtful. Hence we conclude that the nomenclature of the species at hand is as follows:

Parascedosporium putredinis (Corda) Lackner & de Hoog, **comb. nov.**
MyoBank MB519652
(Fig. 3)

Fig. 3. Macro- and micromorphological characteristics of *Parascedosporium putredinis*. **A.** Four day old culture on malt-extract agar (CBS 108.10). **B.** *Graphium* stage, with basal ring structure and conidia (CBS 320.72). **C.** Conidiogenous cells of the *Graphium* synnemata (CBS 102082). **D.** (CBS 118694). **E.** (CBS 118694). **F.** (CBS 102083): solitary conidiophores with lateral, cylindrical conidiogenous cells. Bar = 10 µm.

Basionym: *Stysanus putredinis* Corda, – *Icon. Fung,* **3**: 12 (1829).
Synonyms: *Graphium putredinis* (Corda) S. Hughes, – *Can. J. Bot.* **36**: 772 (1958).
Doratomyces putredinis (Corda) F.J. Morton & G. Sm., *Mycol. Pap.* **86**: 83 (1963); *non sensu* Matsushima (1975: 63).

Type: **Czech Republic:** Prague, on rotten stem of *Echium* sp., 1838, *A.C. Corda* (PR 155673 – holotype, slide ex-type DAOM 40745); **Japan** (Ogasawara, Chichijima, Komagari)**:** isolated by G. Okada from *Chrysalidocarpus lutescens in* 1999, (CBS 102083 –***epitypus hic designatus*;** JCM 8082 – isoepitypus).

? *Stilbum cuneiferum* Berk. & Broome, *Ann. Mag. nat. Hist., ser 4* **15**: 33 (1875).
Synonyms: *Sporocybe cuneifera* (Berk. & Broome) Sacc., *Syll. Fung*. **4**: 606 (1886).
Cephalotrichum cuneiferum (Berk. & Broome) Kuntze, *Rev. Gen. Pl*. **3**: 453 (1898).
Graphium cuneiferum (Berk. & Broome) E.W. Mason & M.B. Ellis, *Mycol. Pap*. **56**: 41 (1953).

Type: **United Kingdom:** on rotten stem of cabbage, (K(M) – holotype n.v.).

Rhinocladium lesnei Vuill., *Bull. Séanc. Soc. Sci. Nancy* **11**: 143 (1910).
Sporotrichum lesnei (Vuill.) Castell. & Chalm., *Man. Trop. Med.,* edn 3 : 1121 (1919).
Graphium lesnei (Vuill.) E.W. Mason, *Mycol. Pap.* **4**: 94 (1937).

Type: **France:** isolated in 1910 by P. Vuillemin from pus of a mycetoma of human foot (CBS 108.10 – ex-holotype strain).

Graphium tectonae C. Booth, *Mycol. Pap.* **94**: 5 (1964).
Parascedosporium tectonae (C. Booth) Gilgado *et al*., *Int. J. Syst. Evol. Microbiol.* **57**: 2176 (2007).

Type: **Jamaica:** isolated from epicarp of *Tectona grandis* seed, July 1962, isol. *C. Booth* (IMI 95673d – holotype, n.v.; CBS 127.84 – ex-holotype strain).

The following description is of CBS 102083 on PDA after 14 d at 22 °C:
Colonies attaining about 50 mm diam, velvety, radially folded, grey, with numerous synnemata at the centre; reverse dark grey to black. *Conidiophores* when solitary, simple, undifferentiated, with lateral conidiogenous cells; *conidiogenous cells* cylindrical, 6–20 × 1.5–2.5 µm, hyaline, thin-walled, bearing 2–5 cylindrical denticles of up to 1 µm long; *conidia* obovoidal, 5–6 × 3–4 µm, smooth- and rather thick-walled. *Synnemata* erect, to 450 µm tall; stipe to 70 µm wide, olivaceous grey, apically splaying out and producing conidia in a slime droplet; *conidiogenous cells* percurrent, cylindrical, 10–37 × 1.5–2.5 µm; *conidia* (sub)cylindrical, 5.5–7.5 × 2.5–3.5 µm. Additional sessile conidia present in low frequency on undifferentiated hyphae. Maximum growth temperature was 37 °C.

Members of the *Pseudallescheria* clade, especially *Scedosporium dehoogii* including *S. deficiens* (Rainer & Kaltseis 2010), are frequently found in hydrocarbon-contaminated soils (Kaltseis *et al.* 2009). In temperate climates *P. apiosperma, S. aurantiacum,* and *P. minutispora* can be isolated from soils with increased nitrogen concentrations and lowered pH (Kaltseis *et al.* 2009). In arid areas, such as Australia and Spain, the member of the *Pseudallescheria* clade, most frequently isolated from environmental and clinical samples is *S. aurantiacum* (Rodriguez-Tudela *et al.* 2009). In contrast, *P. boydii* is the most frequently encountered agent in human infection (Kaltseis *et al.* 2009). In general, members of the *Pseudallescheria* clade tend to inhabit environments with increased human impact (agricultural soils, urban playgrounds, industrial areas, hydrocarbon-contaminated soils) and are also able to cause infections. In immunocompetent patients these infections are either traumatic or cerebral in almost drowned victims (Buzina *et al.* 2006), whereas in immunocompromised patients (e.g. transplant recipients) disseminated infections occur (Guarro et al. 2006). Strains of *Scedosporium prolificans* are frequently found in human infections and are regularly isolated from soil in arid areas of Australia (Heath *et al.* 2009), the USA (Spielberger *et al.* 1995)*,* and Spain (Rodriguez-Tudela *et al.* 2009). Members of this species are known to carry multiple resistances against all available antifungal drugs (Guarro *et al*. 2006).

Petriellopsis africana was isolated only once from brown sandy soil; its ecology and virulence are unknown. *Petriella* strains are rarely associated with vertebrate infections. A cutaneous infection was described by Poelma *et al.* (1974) in a bottlenosed dolphin *Tursiops truncatus*; the causative agent was *Petriella setifera* (CBS 391.75). The type of *P. guttulata* (CBS 362.61) was obtained from dung of a partridge, and *P. setifera* (CBS 385.87) came from a human nail. All other *Petriella* strains were isolated from soil, dung, compost, and once from a bathroom jar (Table 1).

In contrast, all strains in the ITS *Graphium* clade were isolated from wood or forest soil. A consistent association with wood infested by bark insects seems likely. The three strains of *G. fimbriisporum* from France and Austria were associated with the *Picea abies* and the bark beetle *Ips typographus*. Jacobs *et al*. (2003) described *G. laricis* as exhibiting a specific host/insect association (*Larix deciduas / Ips cembrae*), verified in four strains (CBS 116195, CMW 5598, CMW 5601, and CMW 5603). The remaining strains in the same cluster (CMW 503, CMW 5611, and CMW 12285) were derived from trees and/or bark beetles, but did not share an exclusive association with a particular insect (*Tomicus minor, Ips typographus, Orthomicus erosus*) on a specific host tree (*Pinus* sp., *Pinus sylvestris*, *Tsuga dumosa*). *Graphium penicillioides* was isolated from a wood core of *Populus nigra* in the Czech Republic. In contrast, *G. basitruncatum* seems to be atypical as it was isolated twice from soil (Solomon Islands and Japan) and once from a leukemic patient (Kumar *et al.* 2007).

DISCUSSION

The opportunistic, multi-drug resistant species *Scedosporium prolificans* represents the closest relative of *Parascedosporium putredinis* as a sister clade (Fig. 1). In contrast to *S. prolificans, P. putredinis* was only once reported to be involved in a human infection (Vuillemin 1910, as *Rhinocladium lesnei*). This strain, CBS 108.10 originated from pus exuded from a fistula of a human foot mycetoma (Vuillemin 1910). No histopathology was provided, and no fungal grains were biopsied and cultured. Since 1910, *P. putredinis* has never been found in human infections, and the infection described by Vuillemin (1910) lacks histopathological proof; we therefore doubt the credibility of this being the causal agent of the clinical condition. Even though *Scedosporium prolificans* represents the nearest neighbour of *P. putredinis*, we found no evidence of *P. putredinis* being virulent to humans. Analyzing the origins and sources of isolation of strains attributed to *P. putredinis* (Table 1), no clear ecological trend is evident; some strains were isolated from living plant material, while others were isolated from soil, dung, or plant debris.

Other closely related fungi are those in the *Petriella* clade (i.e. *Petriella setifera, P. guttulata,* and *P. sordida*) and *Petriellopsis africana.* These strains were mainly isolated from dung and soil, with two exceptions: CBS 391.75 which caused a skin lesion in *Tursiops truncatus* kept in a Dolphinarium in The Netherlands (Table 1), and CBS 385.87 from a human nail, the latter possibly representing a contaminant. There is consequently not much evidence of any *Petriella* bearing intrinsic pathogenicity to vertebrates.

The *Pseudallescheria* clade encompasses the largest number of clinical strains, and is implemented in a wide spectrum of diseases (Guarro *et al.* 2006). Environmental isolates of this cluster are mostly found in soil and water enriched by agricultural or industrial pollution (Kaltseis *et al*. 2009). Recently, enhancement of isolation by using biodiesel fuel was noted for *S. dehoogii* (Eggertsberger *et al.,* unpubl.) and for *S. deficiens* (Rainer & Kaltseis 2010); the latter species was described for one of the clusters within *S. dehoogii*. *Scedosporium aurantiacum* is generally more common in hot and arid areas such as Spain (Rodriguez-Tudela *et al.* 2009) and Australia (Heath *et al.* 2009), while the remaining species of the clade are prevalent in temperate climates.

Several clinical and animal cases attributed to *Graphium* species probably concerned misidentified strains. *Graphium eumorphum*, of which no type material is known to exist, was reported from a case of otitis externa (Frágner & Hejzlar 1973). The ITS sequence of the strain from this case, CBS 987.73 was found to be identical to the ex-type strain of *P. apiosperma*, CBS 117407. Käufer & Weber (1977) described a systemic infection with cerebral involvement in a dog caused by *G. fructicola* (Marchal & Marchal1921) – but neither the original fungal material nor the veterinary specimen are known as preserved. The brain abscesses including histopathology reported by Käufer & Weber (1977) were similar to those caused by *P. apiosperma* and *P. boydii* in human infections, so a species of the *Pseudallescheria* clade may have been involved.

Kumar *et al*. (2007) reported a fungemia in a patient with acute leukemia caused by *Graphium basitruncatum*, which is the only example of a clinical strain in the clade with monomorphic *Graphium* species otherwise showing association with bark beetle communities (Fig. 1) (Romón *et al.* 2007). This specialized type of ecology is well known for members of the order *Ophiostomatales* (Carlier *et al*. 2006). Such fungal associations are often specific to a particular host insect. The beetles transport fungal cells in their mycangia and use fungal gardens as food source. Jacobs *et al*. (2003) and Paciura *et al.* (2010) reported on *Graphium* species as associates of bark beetles (Table 1); the species concerned all belong to a single clade (Fig. 1). In the study of Paciura *et al*. (2010), *G. pseudormiticum* was directly isolated from bark beetle galleries, providing strong evidence that the fungus is cultured by the beetles. Other authors recognized that strains of *Graphium* cause brown to black wood staining (Stauffer *et al.* 2001, Geldenhuis *et al.* 2004). With ITS sequencing (Fig. 1), *G. pseudormiticum* and *G. laricis* were nearly indistinguishable. Okada *et al.* (1998) erected *G. laricis* on the basis of its ecological niche rather than on sequence differences, because in their data (rDNA SSU) *G. laricis* was identical to *G. pseudormiticum.* Kirschner (1998) reported *G. pseudormiticum* to be associated with bark beetles of the genera *Crypturgus, Dryocoetes, Hylurgops, Polygraphus, Trypodendron, Pityogenes,* and *Ips* species on spruce, and *Ips* and *Orthotomicus* species on pine trees, suggesting a low degree of host-specialization. Nevertheless, association with bark beetles is consistent in the *Graphium* clade.

In conclusion, according to current knowledge, we find only evidence of vertebrate-pathogenicity for strains of the *S. prolificans* clade and the *Pseudallescheria* clade (mainly strains of *P. apiosperma, P. boydii,* and *S. aurantiacum*). In contrast, strains affiliated to the *Graphium* clade, in particular *G. fimbriisporum, G. laricis,* and *G. pseudormiticum,* inhabit niches in association with different kinds of bark beetles, while the majority of *Petriella* and *Petriellopsis* strains show an increased affinity towards soil, dung and compost. *Parascedosporium putredinis* does not exhibit any ecological specialization.

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