A new species of *Galactomyces* and first reports of four fungi on wheat roots in the United Kingdom

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A new species, *Galactomyces britannicum* (IMI395371, MycoBank 511261), is described from the roots of wheat in the UK. *Dendryphion penicillatum* var. *sclerotiale, Fusariella indica, Pseudogymnoascus appendiculatus* and *Volucrispora graminea* are reported for the first time from roots, rhizosphere or stem bases of wheat in the UK. A microconidiogenus synanamorph is described for *V. graminea* and the species is epitypified to reflect this amendment.

Keywords: *Dendryphion penicillatum* var. *sclerotiale, Fusariella indica, Galactomyces britannicum*, *Pseudogymnoascus appendiculatus,* taxonomy, *Volucrispora graminea*.

The introduction of synthetic low nutrient agar (SNA; Nirenberg 1976) to induce fungal sporulation in *Fusarium* has proved invaluable for the assessment of fungal diversity on or in the roots and stem bases of cereal plants (Bateman & Kwaśna 1999, Dawson & Bateman 2001a, b). The medium stimulates a fungal sporulation and allows the isolation of slow-growing species. Isolation studies on SNA have led to the recovery of new species of fungi and fungi previously unknown from cereal crops.

This paper describes one new species and reports four rare species isolated from the roots, rhizosphere, or stem bases at soil level, of wheat grown in the UK.

Material and methods

Isolation of fungi

Stem bases or upper parts of the roots of winter wheat (*Triticum aestivum* L. cv. Hereward) were collected from crops on Rothamsted Farm in 1998–2001 and pre-washed in running water to remove loose soil. Pieces (1 cm long) were serially washed 20 times for 3 min in

cold (4 °C), sterile water. The stem and root pieces, or water from the first washing, were placed on SNA (KH_2PO_4, 1 g L^{-1}; KNO_3, 1 g L^{-1}; $MgSO_4.7\ H_2O$, 0.5 g L^{-1}; KCl, 0.5 g L^{-1}; glucose, 0.2 g L^{-1}; sucrose, 0.2 g L^{-1}; agar, 20 g L^{-1}) and incubated for 2–4 weeks under natural day/night light conditions at 20–25 °C and for an additional 50 weeks at 4 °C to stimulate the sporulation of fungi. The fungi were transferred to potato dextrose agar (PDA; Difco), 2 % malt extract agar (MEA; Difco), malt extract yeast agar (MEYA; malt extract, 10 g L^{-1}; yeast extract, 4 g L^{-1}; glucose, 4 g L^{-1}; agar, 15 g L^{-1}) and SNA for identification by morphology. Growth rates were determined on PDA under a natural day/night cycle. Measurements of morphological structures were made from cultures grown on SNA and mounted in water. Mean values from 50 measurements are shown with extreme values in brackets.

Morphologic and physiologic characteristics

Morphologic and physiologic characteristics were examined according to Yarrow (1998) and Barnett *et al*. (2000). Morphology was examined by microscopy (Nikon). The utilization of various carbon sources and other physiological characteristics were determined with YT microplate (Biolog, Hayward, CA) and API 20C AUX (Biomérieux-Vitek, Hazelwood, MO) according to the manufacturers' instructions. The maximum growth temperature was determined in YM Broth (Difco™) using metal block baths (ISOCAL-6, Isotech, Southport, UK).

DNA extraction

For the molecular analyses, DNA was extracted from *D. penicillatum* var. *sclerotiale, G. britannicum*, *P. appendiculatus* and *V. graminea* grown in LB broth (tryptone, 10g L^{-1}; yeast extract, 5 g L^{-1}; sodium chloride, 10 g L^{-1}) at 25 °C for 10 d. The mycelium was separated by vacuum filtration, then freeze-dried in 2-ml microcentrifuge tubes and ground using a metal rod. DNA extraction was based on the method of Lee & Taylor (1990) as described previously by Ward & Gray (1992).

rDNA amplification

Consensus fungal primers ITS4 and ITS5 (White *et al*. 1990) were used to amplify the ITS1/2 rDNA. Each ITS4/ITS5 PCR mixture of 25 µL contained 25 pmol of each primer, 0.25 units of MBI *Taq* polymerase (MBI Fermentas, St. Leon-Rot, Germany), buffer (10 mM

Tris-HCl pH 8.8, 50 mM KCl, 0.08% Nonidet P-40, 0.1 mg mL^{-1} BSA, 1.5 mM $MgCl_2$), 0.2 mM deoxyribonucleoside triphosphates (dNTPs) and 100 ng fungal DNA. Cycling conditions were an initial denaturation step at 94 °C for 10 min, followed by 30 cycles of 94 °C for 30 s, 42 °C for 1 min and 72 °C for 2 min. This was followed by a final extension of 72 °C for 10 min.

PCR-RFLP

For restriction fragment length polymorphism analysis, aliquots of 8 µL of DNA from *V. graminea* (CBS114630 and CBS895.72) were digested with 10 U of restriction endonucleases: *Alu*I, *Cfo*I, *Dde*I, *Hae*III, *Hin*cII, *Hpa*II, *Sau*3A1 and *Taq*I at 37 °C, overnight. Separation of the restriction digests was done by electrophoresis in agarose gel (2.0% NuSieve GTG agarose supplemented with 1.0% FMC agarose) for 2 h at 5.5 V cm^{-1} in TBE buffer and detected by ethydium bromide gel staining. 1 kb ϕX-174 DNA digested with *Hae*III (1 µg) (Sigma, Saint Louis, Missouri, USA) and Low DNA Mass Ladder (1 µg) (Invitrogen, Paisley, UK) were used as molecular weight markers in the first two lines of the gel. The gel was photographed under UV light at 254 nm. The DNA was quantified by comparison on the gel with a known standard of similar size (bands from ϕX-DNA *Hae*III digest and Low DNA Mass Ladder).

Sequence analysis

Amplicons generated using ITS4 + ITS5 were purified using the MinElute PCR Purification Kit (Qiagen, Crawley, UK) according to the manufacturer's protocol. DNA sequences were determined using the ABI Prism Big Dye terminator cycle sequencing ready reaction kit (version 3.1, Applied Biosystems, Foster City, CA 94404, USA) with primers ITS4, ITS5 or ewfitsrev1 (5' TCC TCC GCT TAT TGA TAT GCT T; kindly provided by E. Ward). Reactions were run at the DNA Sequencing Facility, Oxford University, UK (http://polaris.bioch.ox.ac.uk/dnaseq/index.cfm).

DNA sequences from the fungi described and others used for comparison (Tab. 1) were assembled using the STADEN package (Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK). Sequence analyses were performed using the program BLAST. The DNA sequences with greatest similarity were obtained from EMBL/GenBank and were aligned with sequences of the fungi being studied using VECTOR NTI ADVANCE™10 (http://www.invitrogen.com/content.cfm?pageid=10129). An alignment was edited manually using GENEDOC (Nicholas & Nicholas 1997). Gaps gen-

erated in the alignment were treated as missing data. Phylogenetic analysis was carried out using MEGA 3.1 (Kumar *et al*. 2004) and PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8 (Swofford 2001). Trees were produced using both neighbor-joining (NJ) and maximum-parsimony (MP) analyses for the ITS sequence database. The Kimura two-parameter distance calculation was used for NJ analysis (Kimura 1980). For MP analysis, the heuristic search option with 1000 random addition sequences and TBR branch-swapping options were used. Stability of clades was assessed with 1000 bootstrap replications in a heuristic search. Other measures used were tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI).

Table 1. Fungi used for comparative purposes with isolate number, origin and depositor and EMBL Accession number. Numbers in bold were created for this study.

Name	Isolate number	Origin and depositor	EMBL no.
Alternaria japonica Yoshii	ATCC13618[a]	*Pryor B.M., Gilbertson R.L.,* 2000	AF229474
Dendryphiella sp.	Pf 96[b]	From opium poppy, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2004	AY376645
Dendryphiella sp.	Colombia 1[b]	From opium poppy, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376649
Dendryphiella sp.	414296[b]	From opium poppy, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376652
Dendryphion penicillatum (Corda) Fr.	Cf[c]	*Farr D.F., O'Neill N.R., vanBerkum P.,* 1998	AF102889
D. penicillatum	EGS37-134[d]	Switzerland, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376641
D. penicillatum	1841[b]	Austria, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376647
D. penicillatum	381488-1[b]	Iran, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376656
D. penicillatum	414349[b]	Afghanistan, *Inderbitzin P., Shoemaker R.A., O'Neill N.R., Berbee M.L.*, 2003	AY376660
D. penicillatum var. *sclerotiale* Meffert	CBS208.50	From *Papaver somniferum* seed, Germany, *Meffert M.E.,* 1950	AY376662
D. penicillatum var. *sclerotiale*	CBS117147[e]	From *Triticum aestivum* stem base, Harpenden, UK, *Kwaśna H,* 2005	**AJ876894**[n]

Table 1. – continued

Name	Isolate number	Origin and depositor	EMBL no.
Dipodascus aggregatus Francke-Grosmann	CBS152.57	From *Ips pini* frass in roots of *Pinus resinosa,* USA, *Batra L R.*, 1957	AY788292
D. aggregatus	CBS175.53	From pupal galleries of *Ips acuminatus* in *Pinus sylvestris,* Germany, *Francke-Grosmann H.*, 1953	AY788294
D. albidus de Lagerheim	CBS766.85	From exudates of angiosperm tree *Quercus serrata*, Japan, *Nakase T.*, 1985	AY788342
D. armillariae W. Gams	CBS624.82	From gills of *Armillaria mellea*, Ardennes, Fond d'Auffe, Belgium, *Gams W.*, 1982	AY788332
D. australiensis Arx & J.S.F. Barker	CBS372.83	From *Euphorbia ingens*, Pretoria, South Africa, *van der Walt J. P.,* 1983	AY788314
D. geniculatus de Hoog, M.T. Smith & Guého	CBS184.80	From pulp of *Psidium guajava*, Maharashtra, India, *Bhide V. P.*, 1980	AY788301
D. macrosporus Madelin & Feest	CBS260.82	From slime trail plasmodium of *Badhamia utricularis,* U.K., *Madelin M. F.*, 1982	AY788311
Galactomyces britannicum Kwaśna & G.L. Bateman	CBS117695 IMI 393237[f]	From roots of *Triticum aestivum*, Harpenden, UK, *Kwaśna H.,* 2000	**AJ938163**[n]
Galactomyces citri-aurantii E.E. Butler	CBS175.89	From soil of orange orchard, Salisbury, Zimbabwe, *Butler E.*, 1989	AY788295
G. geotrichum (E.E. Butler & L.J. Petersen) Redhead & Malloch	CBS773.71	From soil, Puerto Rico, *Butler E.E.*, 1971	AY788343
G. geotrichum	CBS774.71	*Butler E.E.*, 1971	AY788344
Galactomyces geotrichum	CBS775.71	From soil, Puerto Rico, *Butler E.E.*, 1971	AY788345
G. geotrichum	CBS866.68	From wheat field soil, Germany, Kiel-Kitzeberg,	AY788351
G. reessii (Van der Walt) Redhead & Malloch	CBS179.60	From cold-water retting of *Hibiscus cannabinus,* Indonesia, Java, *van der Walt J.P.*, 1960	AY788299
G. asperulatus (Sigler & Carmichael) van Oorschot	CBS124.77	Forest soil, Massachusetts, USA, *Carmichael J.W.,* 1977	AJ390390
G. asperulatus	UAMH 9032[g]	From decayed spruce, Canada, *Lumley T.C.*	DQ117449
G. pannorum (Link) Sigler & (Carmichael)	KCTC6060[h]	*Shin Y.K.*, 1997	AF015789

Table 1. – continued

Name	Isolate number	Origin and depositor	EMBL no.
G. pannorum	S6C2[i]	From soil, U K, *Barratt S.R.*	AJ509866
G. pannorum	S33A1/B1	From soil, U K, *Barratt S.R.*	AJ509867
G. pannorum	S9A4	From soil, U K, *Barratt S.R.*	AJ509868
G. pannorum	S9A3/A2[i]	From soil, U K, *Barratt S.R.*	AJ509869
G. pannorum	S6A4	From soil, U K, *Barratt S.R.*	AJ509870
G. pannorum	S6A3	From soil, U K, *Barratt S.R.*	AJ509871
G. pannorum	T1.1[i]	From soil, U K, *Barratt S.R.*	AJ549922
G. pannorum	UAMH 1030	From cold storage food, USA, *Kuehn H.H.*	DQ117436
G. pannorum	ASIGP1[j]	From soil, *Cosgrove L., McGeechan L., Robson G.D., Handley P.S.,* 2006	DQ779788
G. vinaceus Dal Vesco	GFI 21[i]	From plasticised polyvinylchloride, Bulgaria, *Sabev H.,* 2003	AJ608972
Geotrichum fermentans (Diddens & Lodder) Arx	CBS409.34	From woodpulp mill, Sweden, Värmland, Sunne, *Melin E.,* 1934	AY788315
G. fragrans (Berkhout) Morenz	CBS152.25	*Smit J.,* 1925	AY788291
G. klebahnii (Stautz) Morenz	CBS179.30	From sime flux *Taxus baccata, Stautz W.*, 1930	AY788298
G. restrictum de Hoog & M.T. Sm.	CBS111234	From *Picea abies,* Sweden, *Vasiliauskas R.*	EF126738
Gymnostellatospora alpina (E. Müll. & Arx) Udagawa	UAMH 9430	From *Erica carnea* rhizosphere, Switzerland, *Müller E.*	DQ117459
G. canadensis T.C. Lumley, Sigler & Currah	UAMH 8899	From decayed spruce, Canada, *Lumley T.C.*	DQ117448
G. canadensis	UAMH 9238	From decayed spruce, Canada, *Lumley T.C.*	DQ117453
G. frigida Uchiy., Kamiya & Udagawa	UAMH 9304	From alpine, forest soil, Japan *Udagawa S.*	DQ117457
G. japonica Udagawa, Uchiy. & Kamiya	UAMH 9240	From decayed spruce, Canada, *Lumley T.C.*	AF062818
Gymnostellatospora japonica	UAMH 9239	From decayed spruce, Canada, *Lumley T.C.*	DQ117454
G. subnuda Sigler, T.C. Lumley & Currah	UAMH 9242	From decayed spruce, Canada, *Lumley T.C.*	DQ117456
Magnusiomyces capitatus (de Hoog, M.T. Sm. & E. Guého) de Hoog & M.T. Sm.	CBS162.80	From bovine mastitic milk, Weybridge, UK, *Pepin, G.A.*, 1980	AY788293

Table 1. – continued

Name	Isolate number	Origin and depositor	EMBL no.
M. ovetensis (Peláez & C. Ramírez) de Hoog & M.T. Sm.	CBS192.55	From tannin concentrate, Spain, *Ramírez C.,* 1955	AY788303
M. magnusii (F. Ludw.) Redhead & Malloch	CBS108.12	*Lindner P.,* 1912	AY788289
M. spicifer (de Hoog, M.T. Sm. & E. Guého) de Hoog & M.T. Sm.	CBS244.85	From cactus rot, Arizona, USA, *Lachance M.A.,* 1985	AY788308
M. starmeri (Phaff, Blue, Hagler & Kurtzman) de Hoog & M.T. Sm.	CBS780.96	From rotting *Carnegiae gigantean,* Arizona, USA, *Phaff H.J.,* 1996	AY788346
M. tetrasperma (Macy & M.W. Mill.) de Hoog & M.T. Sm.	CBS765.70	From wet conveyor, at a prune dehydration plant, California, USA, *Miller M.W.,* 1970	AY788340
Myxotrichum chartarum Kunze	UAMH 1997	From soil, Japan	AF062813
Phlebiopsis gigantea (Fr.) Jülich	B-P160[k]	*Vainio E.J,; Hantula J.,*1998	AF087484
Pleospora papaveracea (De Not.) Sacc.	Pf[c]	From *Papaver somniferum, Farr D.F., O'Neill N.R., vanBerkum P.,* 1998	AF102888
P. papaveracea	wb383[l]	*Buzina W.,* 2001	AF455453
P. papaveracea	wb275	*Buzina W.,* 2001	AF455497
Pseudogymnoascus appendiculatus Rice and Currah	RR135[m]	From roots of *Triticum aestivum,* UK, Harpenden, *Bateman G.L.,* 1999	**AJ938164**[n]
P. appendiculatus	UAMH 10510	From brown-rotted *Picea mariana* wood under *Sphagnum fuscum* peat, Canada, Alberta, *Rice V.,* 2002	DQ117437
P. appendiculatus	UAMH 10511	From brown-rotted *Picea mariana* wood under *Sphagnum fuscum* peat, Alberta, Canada, *Rice V.,* 2002	DQ117438
P. appendiculatus	UAMH 10512	From brown-rotted *Picea mariana* wood under *Sphagnum fuscum* peat, Alberta, Canada, *Rice V.,* 2002	DQ117439
P. roseus Raiłło	UAMH 1658	From forest soil, *Ghillini C.A.*	DQ117443
P. roseus	UAMH 2879	From alpine soil, Canada, *Widden P.*	DQ117445
P. roseus	UAMH 9163	From ectomycorrhizal root tip, Canada, *Fernando A.A.*	DQ117451
Pseudogymnoascus roseus	UAMH 9222	From decayed spruce, Canada, *Lumley T.C.*	DQ117452

Table 1. – continued

Name	Isolate number	Origin and depositor	EMBL no.
P. verrucosus Rice and Currah	UAMH 10579	From brown-rotted *Picea mariana* wood under *Sphagnum fuscum* peat, Alberta, Canada, *Rice V.,* 2002	DQ117440
P. verrucosus	UAMH 10580	From brown-rotted *Picea mariana* wood under *Sphagnum fuscum* peat, Alberta, Canada, *Rice V.,* 2002	DQ117441
Saprochaete clavata (de Hoog, M.T. Sm. & E. Guého) de Hoog & M.T. Sm.	CBS425.71	From lung tissue of a man, Baltimore, USA, *Ahearn* D.G., 1971	AY788317
S. gigas (Smit & L. Meyer) de Hoog & M.T. Sm.	CBS126.76	From oily debris, Japan, *Goto S.,* 1976	AY838940
S. ingens (Van der Walt & Kerken) de Hoog & M.T. Sm.	CBS517.90	From wine cellar, South Africa, *van der Walt J.P.*	AY788321
Volucrispora graminea Ingold, P.J. Mc Doughall & Dann	CBS895.72	From *Holcus lanatus,* Hollingstedt, Germany, *Schlösser U.G.*, 1972	
V. graminea	CBS114630 IMI391620	From rhizosphere of *Triticum aestivum,* UK, Harpenden, *Kwaśna H.,* 2001	**AJ748690**[n]

[a] American Type Culture Collection, 10801 University Boulevard, Manassas (VA), 20110-2209, USA;
[b] Department of Botany, University of British Columbia, 3529-6270 University Blvd., Vancouver, British Columbia V6T1Z4, Canada;
[c] SARL, USDA ARS, 10300 Baltimore Blvd, Beltsville, MD 20705, USA;
[d] Emory G. Simmons Culture Collection, 717 Thornwood Road, Crawfordsville, Indiana, USA;
[e] Centraalbureau voor Schimmelcultures, P. O. Box 85167, 3508 AD Utrecht, The Netherlands;
[f] CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK;
[g] University of Alberta, Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, T6G 2E1 Canada;
[h] Korean Collection for Type Cultures, Korean Research Institute of Bioscience and Biotechnology, Yusong, Korea;
[i] Immunology, Microbiology and Development, University of Manchester, 1.800 Stopford Building, Oxford Road, Manchester, M13 9PT, UK;
[j] School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK;
[k] Finnish Forest Research Institute, PO Box 18, Vantaa 01301, Finland;
[l] Laboratory for Mycology and Molecular Biology, ENT-University Hospital, Auenbruggerplatz 26-28, Graz A 8036, Austria;
[m] Rothamsted Research, Harpenden, AL5 2JQ, UK;
[n] deposited by authors.

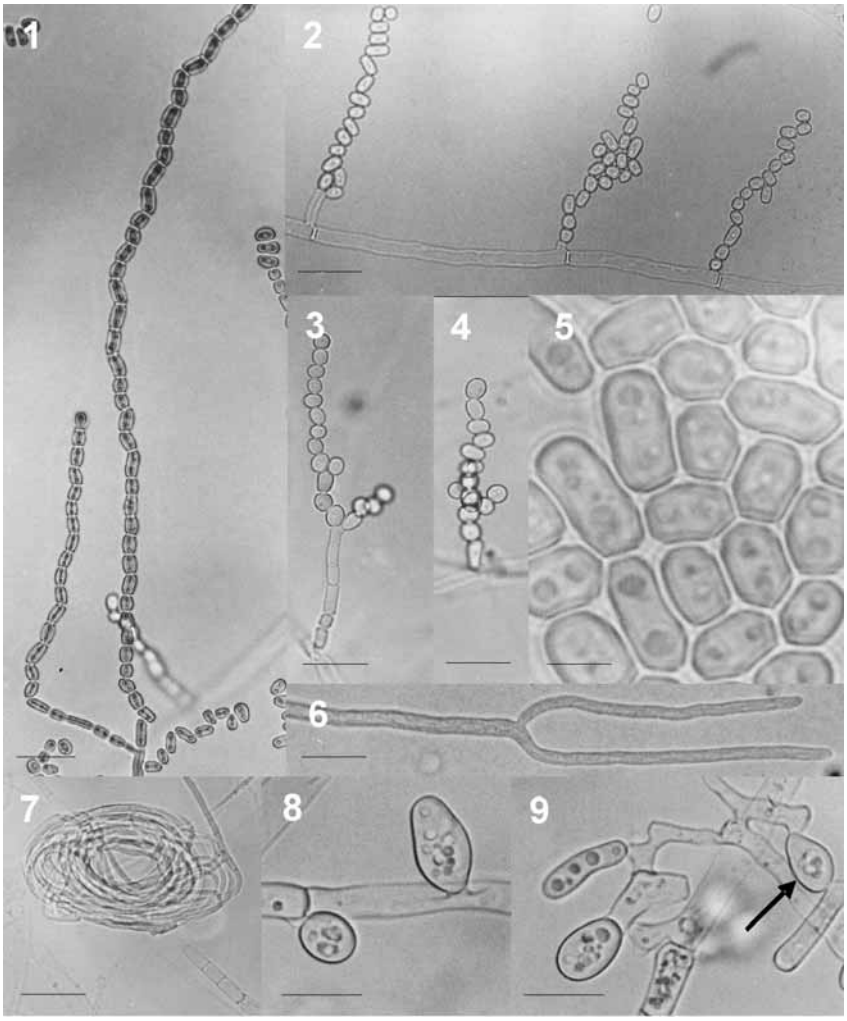
Results and Discussion

One new and four rare species of fungi were isolated from roots, rhizosphere and stem base of wheat. They are the first records of these fungi in the United Kingdom, confirming that not everything is yet known about the true scale of fungal diversity in/on cereal roots, which have been relatively well studied (Roughgarden & Diamond 1986, Rayner & Boddy 1988, Christensen 1989, Bateman & Kwaśna 1999). The indication of fungal species found previously only in warmer climate points to the possibility of changes in the fungal communities resulting from climatic warming.

The records of new or rare fungi contribute to knowledge on plant-microbial interactions. Representatives of Ascomycota, including the fungi reported here, are usually neutral inhabitants or mutualists deterring herbivores and enhancing host physiology and increasing resistance to pathogens. Expression of host-fungus association varies depending on the plant and environmental conditions affecting growth and activity of both the plant and the fungus. The host-fungus associations may have a long-term ecological effects measured in terms of persistence and total productivity (Malinowski & Belesky 2006).

Galactomyces britannicum Kwaśna & G. L. Bateman **sp. nov.** – Figs. 1–10.

Coloniae in MEYA celeriter crescens, 30 mm diam post 4 dies ad 25 °C, albae, butyraceae, modice madidae; mycelio aerio tenui floccoso obtectae, planae, leves, margine regulari circumdatae; reversum album ad cremeum. Odor fructuous. Hyphae rectae, bilaterale ramosae, hyalinae, leves, primum tenuitunicatae, deine saepe inspissatae, saepe intercalariter inflatae et partim parietibus inspissatis, 4–7.5 µm crassis, apicibus rotundatibus. Hyphis marginalis saepe dichotome ramosis. Conidiophora plerumque simplicis, ramosa ad angulos 45–90°, quoque septo 1 (–2) ramos laterales ferente, 5–55 × 2.5–4 µm. Hyphis marginalis et ramis perpendicularibus in arthroconidia fragmentatia. Arthroconidia unicellularia, primo cylíndrica vel rectangularia, 3–8 (10) × 2.5–4 (4.5) µm, deine inflata et globosa, 4–10 µm diam vel ellipsoidea, 6.5–13 × 3.5–6 (8) µm, plerumque biguttulatae. Typici asci absentes. Partes terminales hypharum nonnumquam inflatae et crassitunicatae, ad 1-2 cellulas terminales, ellipsoideas vel clavatas, hyalinas, 10–13 × 5 µm, plasma granulosum continentes. Chlamydosporae globoseae, hyalinae, singulariae vel 2–3 catenulatae 7.5–10 µm diam. Fermentatio nulla. Assimilantur D-glucosum, D-galactosum, L-sorbosum, D-xylosum, glycerolum, D-glucitolum, D-galacturonatum, DL-acidium lacticum, acidium succinicum, ethanolum, propane-1,2-diolum, butano-2,3-diolum. Non assimilantur D-glucosaminum, D-ribosum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, trehalosum, cellobiosum, salicinum, arbutinum, lactosum, raffinosum, amylum solubile, erythritolum, ribitolum, D-mannitolum, D-gluconatum, D-glucuronatum, acidium citricum, methanolum. Assimilantur ethylaminum, L-lysinum, cadaverinum et glucosaminum. Non assimilantur kali nitratum, sodii nitritum, creatinum, creatininum. Vitaminum externum ad crescentiam non est necessarium. Augmentum non fiunt in temperatura 35 °C. Crescit in medio 10 µg mL^{-1} cycloheximido addito.



Figs. 1–9. *Galactomyces britannicum* (CBS117695, IMI393237). **1–4**. Conidiophores with arthroconidia. **5.** Arthroconidia. **6.** Marginal hyphae dichotomously branched. **7.** Coil formed in young, aerial mycelium. **8, 9**. Cells resembling immature asci. Chlamydospore indicated by an arrow. Bars **1–4, 6, 7** = 20 µm; **5** = 5 µm; **8, 9** = 10 µm.

Holotypus. IMI395371 (IMI Herbarium, CABI Bioscience) – cultura exsiccata ex radix, *Triticum aestivum* L., Jul 2000, H. Kwaśna, Harpenden, UK (0° 30' W, 51° 45' N).

Isotypus. CBS117695 (Centraalbureau voor Schimmelcultures), IMI393237 (CABI Bioscience), DNA406 (Rothamsted Research, Harpenden, UK), KFL406 (Agricultural University, Poznan, Poland). MycoBank 511261.

Colonies on MEYA 30 mm diam after 4 d at 25 °C, whitish, butyrous, slightly moist, with thinly floccose aerial mycelium, flat,

smooth, with a regular and sharp margin; reverse whitish to cream-colored. Odour fruity. Hyphae straight, bilaterally branched, hyaline, smooth, thin-walled, later often thick-walled, in older colonies with intercalary swellings and with locally thickened walls, 4–7.5 µm wide, with rounded apices. Aerial mycelium occasionally forms coils. Marginal hyphae often dichotomously branched. Conidiophores usually simple, branched at angles of about 45–90°, each septum with 1 (–2) lateral branches, 5–55 × 2.5–4 µm. Marginal hyphae and branches soon disarticulating into arthroconidia. Arthroconidia 1-celled, at first cylindrical and rectangular, 3–8 (10) × 2.5–4 (4.5) µm, inflated and globose, 4–10 µm diam to ellipsoidal, 6.5–13 × 3.5-6 (8) µm after liberation, with two or many oil drops in the younger and older conidia, respectively. Arthroconidia collected in huge assemblies. Typical asci absent. Terminal parts of hyphae often swell and become thick-walled, forming one to two terminal, broadly ellipsoidal to clavate cells, 10–13 × 5 µm, with granular contents. These cells resemble immature asci. Chlamydospores globose, hyaline, single or in chains of 2–3, 7.5–10 µm diam.

Physiology – *Galactomyces britannicum* is non-fermentative. Carbon assimilation; able to assimilate D-glucose, D-galactose, L-sorbose, D-xylose, glycerol, D-glucitol, D-galacturonate, DL-lactate, succinate, ethanol, propane 1,2-diol, butane 2,3-diol, unable to assimilate D-glucosamine, D-ribose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α-trehalose, cellobiose, salicin, arbutin, lactose, raffinose, soluble starch, erythritol, ribitol, xylitol, D-mannitol, D-gluconate, D-glucuronate, citrate, methanol. Nitrogen assimilation; able to assimilate ethylamine, L-lysine, cadaverine, unable to assimilate nitrate, creatine, creatinine. Vitamins requirements; growth without vitamins. Growth temperature; no growth at 35 °C (Tab. 2).

Table 2. Physiological characteristics of *Galactomyces britannicum* compared with *G. geotrichum* and *G. reessii* (de Hoog *et al*. 1986).

Characteristic	***G. britannicum***	***G. geotrichum***	***G. reessii***
Fermentation			
D-glucose	–	Possible, weak	–
Carbon assimilation			
D-Glucose	+	+	+
D-Galactose	+	+	+
D-Sorbose	+	+	+
D-Glucosamine	–	–	–
D-Ribose	–	Possible, weak	–
D-Xylose	+	+	+
L-Arabinose	–	–	–

Table 2. – continued

Characteristic	*G. britannicum*	*G. geotrichum*	*G. reessii*
D-Arabinose	–	–	–
L-Rhamnose	–	–	–
Sucrose	–	–	–
Maltose	–	–	–
A-Trehalose	–	–	–
Cellobiose	–	–	–
Salicin	–	–	–
Arbutin	–	–	–
Lactose	–	–	–
Raffinose	–	–	–
Soluble starch	–	–	–
Glycerol	+	+	+
Erythritol	–	–	–
Ribitol	–	Possible	–
Xylitol	–	–	–
D-Glucitol	+	+	+
D-Mannitol	–	+	–
D-Galacturonate	+	–	–
D-Gluconate	–	Possible	–
D-Glucuronate	–		
DL-Lactate	+	+	+
Succinate	+	+	+
Citrate	–	Possible	+
Methanol	–		
Ethanol	+	+	+
Propane 1,2-diol	+		
Butane 2,3-diol	+		
Nitrogen assimilation			
Ethylamine	+	+	+
Nitrate	–	–	–
L-lysine	+		
Cadaverine	+		
Creatine	–		
Creatinine	–		
Vitamin requirements			
w/o witamins	+	+	–
Growth temperature			
at 35 °C	–	+	–
at 37 °C	–	Possible	–
Growth on			
50% D-glucose	Weak		
10% NaCl	–		

Galactomyces britannicum is the fourth species in the genus *Galactomyces* Redhead & Malloch to be described. The others are *G. citri-aurantii* E. E. Butler, *G. geotrichum* (E. E. Butler & L. J. Peterson) Redhead & Malloch and *G. reessii* (van der Walt) Redhead & Malloch (de Hoog & Smith 2004).

The taxonomic status of *Galactomyces candidus* de Hoog & M. Th. Smith and *G. pseudocandidus* de Hoog & M. Th. Smith proposed by Hoog & Smith (2004) are currently uncertain. Sequences of both species, i.e. AY788300 (CBS182.33), AY788304 (CBS194.35), AY788297 (CBS178.71), AY788327 (CBS557.83), AY788334 (CBS626.83) and AY788288 (CBS100812) have been deposited in EMBL GenBank as *G. geotrichum* or *Geotrichum fragrans*.

Galactomyces britannicum differs morphologically from the other species in producing enormous numbers of arthroconidia, which densely cover the entire surface of the colony after a few days of incubation. Shortly after liberation, the conidia inflate to become globose to ellipsoidal, while conidia of other *Galactomyces* species show no or little inflation. Typical asci and ascospores have not been observed. The fungus produced, however, round to ellipsoid cells filled with a coarse granulation which resembled immature asci formed by the self-fertilizing *G. geotrichum* cultures (de Hoog *et al*. 1986). *Galactomyces britannicum* produced chlamydospores and characteristic coils of thin hyphae in young aerial mycelium, which have not been reported previously in *Galactomyces* species. The physiological characters of *G. britannicum* are similar to other species of *Galactomyces*. *Galactomyces britannicum* can be distinguished from *G. geotrichum* and from *G. reessii* by its inability to assimilate D-mannitol and citrate, growth with no vitamins and no growth at 35 °C .

The ITS1/2 rDNA region of *G. britannicum* was remarkably short, as is typical among species of *Geotrichum* and its teleomorphs (de Hoog & Smith 2004). The 5.8S rDNA gene was 156 bp and the ITS1 and ITS2 regions were 64 and 60 bp, respectively. The ITS sequences of 26 strains of *Dipodascus, Galactomyces, Geotrichum, Magnusiomyces* and *Saprochaete*, mostly retrieved from GenBank, were included in the phylogenetic analysis. The sequences included had the greatest similarity to the described species in the Blast search. The maximal identity was 97–92%. The alignment of sequences included 215 nucleotide positions. Of these, 93 characters were constant and 122 were informative. The 28 equally parsimonious trees generated from the heuristic search exhibited low levels of homoplasy as indicated by CI = 0.898, RI = 0.885, RC = 0.805 and HI = 0.157. The topology of the trees differed from one another only in the positions of isolates within terminal groupings. Tree topologies resulting from neighbor-joining and maximum parsimony analyses

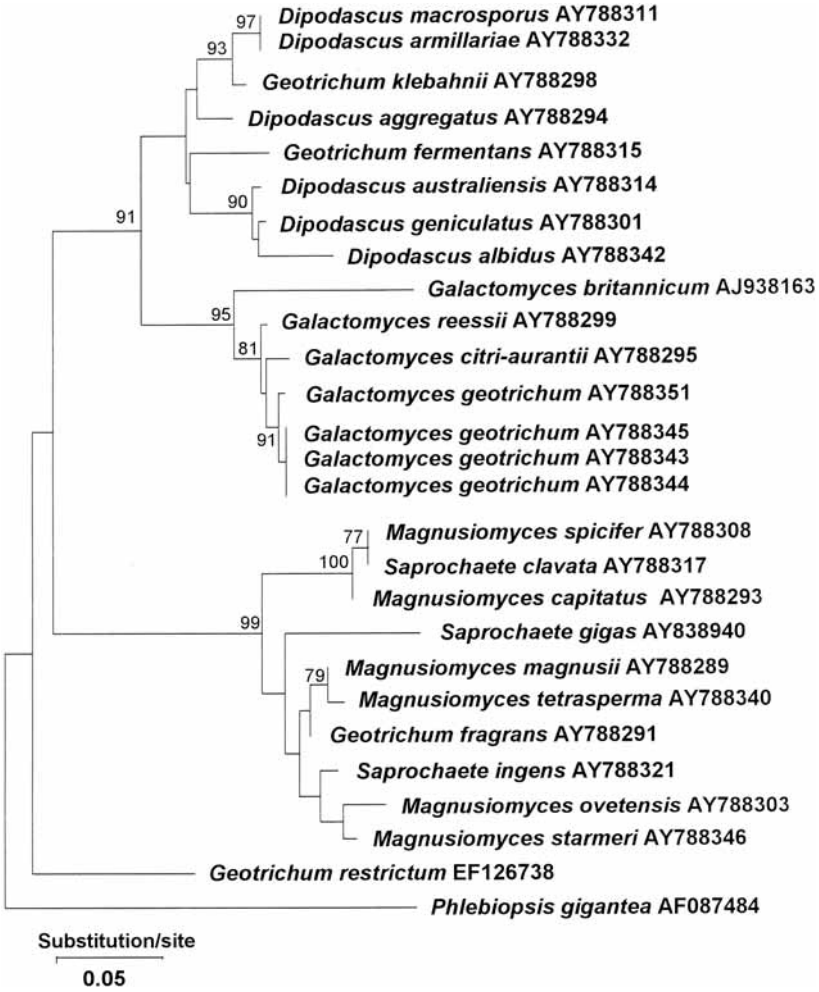


Fig. 10. Phylogenetic position of *Galactomyces britannicum*. Neighbor-joining tree based on nucleotide sequences of the ITS1/2 rDNA. Branch lengths are proportional to distances. Bootstrap values above 75% are indicated above the internodes. *Phlebiopsis gigantea* was used as an outgroup.

were similar and only the former is shown (Fig. 10). Phylogenetic analysis of the ITS1/2 rDNA distinguished *G. britannicum* from other *Galactomyces* species.

Dendryphion penicillatum* var. *sclerotiale Meffert – Figs. 11–15.

Materiale examined. *Dendryphion penicillatum* var. *sclerotiale* Meffert: United Kingdom, Harpenden, Rothamsted Research, on stem base of *Triticum aestivum* L., 12 Jul 1998, *leg.* W.A.J.M. Dawson (CBS117147, IMI392920, RR248).

The sequenced ITS1/2 rDNA of the fungus, comprising small fragments of the 18S and 26S genes (455 bp) was 100% similar to the type strain of *D. penicillatum* var. *sclerotiale* (CBS208.50, AY376662) and *D. penicillatum* (AF102889, AY376641, AY376647, AY376656, AY376660). The ITS sequences of 12 strains of *Dendryphiella*, *Dendryphion* and *Pleospora* which had the greatest similarity to our fungus, retrieved from GenBank were included in the phylogenetic analysis. The maximal identity was 100–92%. The 10 equally parsimonious trees generated from the heuristic search exhibited low level of homoplasy indicated by CI = 0.909, RI = 0.881, RC = 0.815 and HI = 0.171. Due to the similarity of topology of trees resulting from neighbor-joining and maximum parsimony analyses only the former is shown (Fig. 11).

Dendryphion penicillatum has been described as the anamorph of *Pleospora papaveracea* (De Not.) Sacc. – a common pathogen of opium poppy (*Papaver somniferum* L.) (Ellis 1971, Sivanesan & Holliday 1982). However, morphological observation and AFLP analysis of the ITS1/2 rDNA by Farr *et al*. (2000) showed that the anamorph of *P. papaveracea* is an unnamed *Dendryphiella* sp., not *D. penicillatum*. Our analysis of the ITS 1/2 rDNA sequences supported the findings of Farr *et al*. (2000), with our isolate grouping with *D. penicillatum* and distinct from *P. papaveracea* and isolates of *Dendryphiella* sp.

Dendryphion penicillatum var. *sclerotiale* was originally isolated from *P. somniferum* (Meffert 1950), on which it is pathogenic, albeit less virulent than *P. papaveracea* (Farr *et al*. 2000, O'Neill *et al*. 2000). The two pathogens often co-occur on stems and leaves of *P. somniferum*. *Dendryphion penicillatum* var. *sclerotiale* can also grow on wheat culms (Farr *et al*. 2000).

The fungus produced, *in vitro*, micronematous conidiophores, conidia that were pale olivaceous, cylindrical, rounded at the ends or obclavate, minutely granulose, catenulate, 1–3 (–4) transversally septate, (8) 10–20 (30) × 5–6.5 µm, and microsclerotia consisting of few to several hyaline or dark thick-walled cells. Macronematous conidiophores characteristic of *D. penicillatum* var. *sclerotiale* from opium poppy were not produced even on filter paper placed on water agar with 0.1% yeast extract; a treatment that stimulated formation of macronematous conidiophores in *Dendryphion comosum* Wallr. (Reisinger 1968).

The fungus also produced, *in vitro*, conidia that were less septate and smaller than those of the isolates from opium poppy, which are usually 3-septate and (17) 23 (28) × 5–9 µm *in vitro* and up to 8-septate and up to 60 µm long *in situ* (Ellis 1971, Farr *et al*. 2000). The fungus also tended to produce smaller structures on inoculated wheat (Farr *et al*. 2000) (Figs. 12–15).

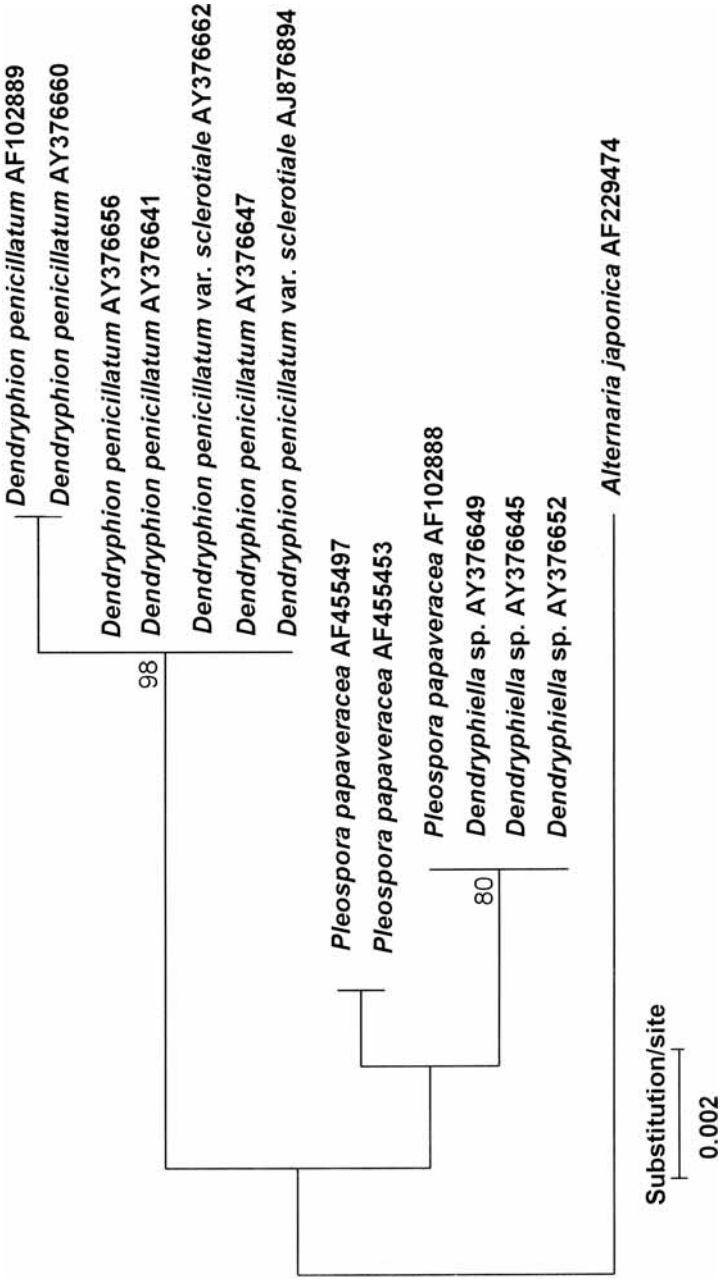
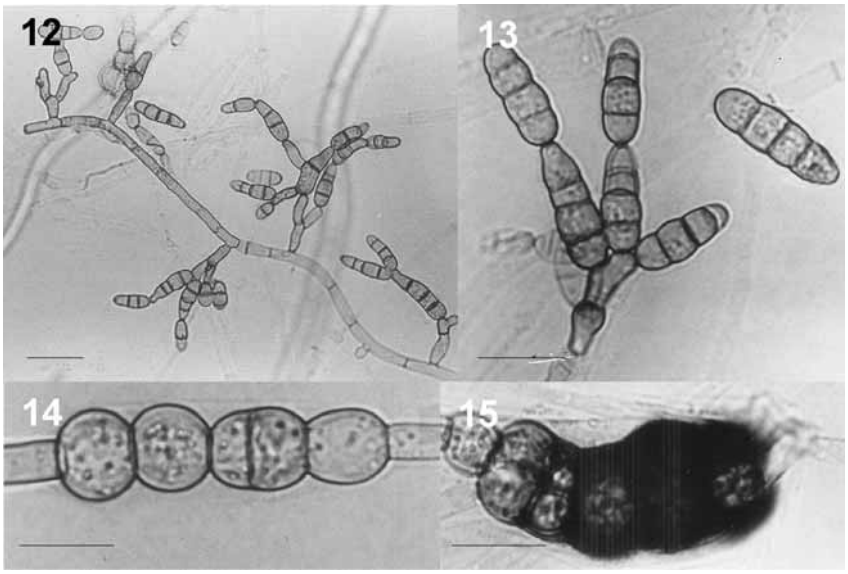


Fig. 11. Phylogenetic position of *Dendryphion penicillatum* var. *sclerotiale*. Neighbor-joining tree based on nucleotide sequences of the ITS1/2 rDNA. Branch lengths are proportional to distances. Bootstrap values above 75% are indicated above the internodes. *Alternaria japonica* was used as outgroup.



Figs. 12–15. *Dendryphion penicillatum* var. *sclerotiale* (CBS117147, IMI392920). **12–13.** Conidiophores with conidia. **14–15**. Sclerotia. Bars **12, 13** = 20 µm; **14, 15** = 10 µm.

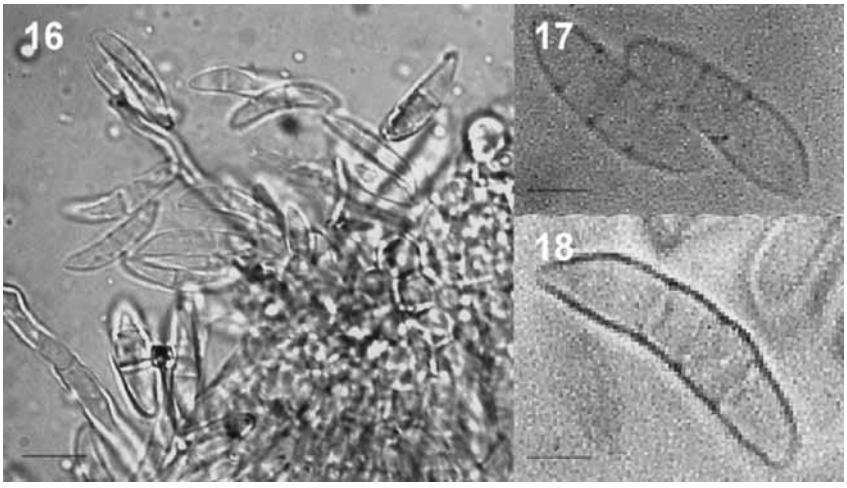
Fusariella indica Roy & B. Rai. – Figs. 16–18.

Material examined. *Fusariella indica* Roy & B. Rai.: UNITED KINGDOM, Harpenden, Rothamsted Research, on roots of *Triticum aestivum* L., 15 Jul 2001, *leg*. H. Kwaśna (RR34).

The fungus conforms closely to the description of *F. indica* by Ellis (1971). After storage at 4 °C for 1 year it produced a compact greenish-black colony with small, round, brownish-black, conidial aggregations resembling sporodochia. Conidia developed in basipetal succession on smooth, brownish, strongly branched, 70–90 × 2–3 µm conidiophores with subhyaline straight to curved phialides borne mostly apically. Three kinds of conidia, 8–16 × 4–6µm, were formed either separately or simultaneously in a single chain-like aggregation: (i) long obclavate, brown, with 1–3 transverse septa, (ii) short obclavate, brown, with one transverse septum, (iii) fusiform, brown or hyaline, with 1–3 transverse septa (Figs. 16–18).

Fusariella species are known as saprophytes on plant material. *Fusariella indica* is the only *Fusariella* species that produces three kinds of conidia, i.e. long and short obclavate, and fusiform, all of which were produced by our isolate. Previous reports of the fungus have been from warmer climates, notably from dead leaves of *Saccharum munja* Roxb. in India (Roy & Rai 1968). This is the first

record of *F. indica* from wheat and the first from the temperate region. The only species of *Fusariella* that has been recorded previously on *T. aestivum* is *F. obstipa*, which has colourless and smooth conidiophores, and conidia of one shape: fusiform, tapered to an acute apex, usually slightly curved, always in slipped chains, in mass black or blackish green, 14–20 (16) × 4.5–7 μm (Ellis 1971, Sharma & Munjal 1982).



Figs. 16–18. Conidia of *Fusariella indica* (RR34). Bars **17** = 10 μm; **16, 18** = 5 μm.

Pseudogymnoascus appendiculatus Rice & Currah – Figs. 19–21.

Material examined. *Pseudogymnoascus appendiculatus* Rice & Currah: United Kingdom, Harpenden, Rothamsted Research, on roots of *Triticum aestivum* L., 20 Jun 1999, *leg.* G. L. Bateman (CBS117696, IMI 393238, RR135).

The sequenced ITS1/2 rDNA, comprising small fragments of the 18S and 26S genes (460 bp) of the fungus (CBS11769), was 98% similar to that of three isolates of *P. appendiculatus* from a bog containing *Picea mariana* (P. Mill.) B.S.P. and *Sphagnum fuscum* (Schimp.) Klinggr. in Alberta, Canada, (DQ117437, DQ117438, DQ117439, UAMH 10510, UAMH 10511, UAMH 10512) (Rice & Currah 2006).

The ITS sequences of 29 strains of *Geomyces Gymnoastellatospora* and *Pseudogymnoascus* which had the greatest similarity to our isolate of *P. appendiculatus* in the Blast search, were included in the phylogenetic analysis. The 12 most parsimonious trees generated using a heuristic search exhibited low level of homoplasy (CI = 0.809, RI = 0.835, RC = 0.815, HI = 0.161). Tree topologies resulting

from neighbor-joining and maximum parsimony analyses were similar and only the former is shown (Fig. 19).

The fungus produced a white-gray-green colony with distinct sulphur-coloured pigmentation in the reverse and in the agar. It produced tree-like branched conidiophores with short chains of arthroconidia (Figs. 20–21). It did not produce intercalary arthroconidia, which were present in isolates described by Rice & Currah (2006). When incubated on PDA and SNA for 24 months at 4 °C it produced only a single, immature, orange ascocarp with smooth, peridial hyphae, lacking branched appendages, asci or ascospores. This is the second record of the fungus worldwide.

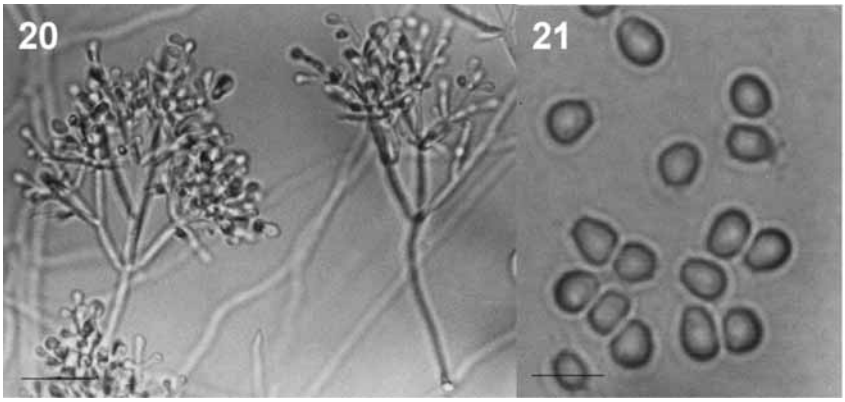
Fig. 19. Phylogenetic position of *Pseudogymnoascus appendiculatus*. Neighbor-joining tree based on nucleotide sequences of the ITS1/2 rDNA. Branch lengths are proportional to distances. Bootstrap values above 75% are indicated above the internodes. *Myxotrichum chartarum* was used as an outgroup.

Volucrispora Haskins

The genus and species descriptions have been revised and emended with a new concept of synanamorph.

Colonies (2% MA) white, in some isolates cream-colored or brownish after long cultivation, reverse pale, isabelline or brown. Some strains become pale pink when submerged in the medium and exposed to daylight. Conidiophores micro- to semi-macronematous, mononematous, mostly lateral, 0- to few-septate.

Conidiogenous cells terminal or lateral, polyblastic, discrete or integrated, sometimes concurrent with conidia, proliferation sympodial. Macroconidia terminal, fasciculate, compound, with an axis and one or rarely two, paired or alternate, laterals; elements subulate, slightly arcuate, 0- to few-septate, apex acute; axis often with a percurrent or eccentric basal extension; branching pleurogenous with insertions strongly and unequally constricted. Microconidia oval, fusiform to cylindrical, in heads. Conidial secession schizolytic. Teleomorph unknown.



Figs. 20–21. *Pseudogymnoascus appendiculatus* (CBS117696, IMI393238). **20.** Conidiophore with aleuroconidia. **21.** Conidia. Bars **20** = 10 µm; **21** = 5 µm.

Volucrispora graminea Ingold, P.J. Mc Doughall & Dann – Figs. 22–25.

Material examined. *Volucrispora graminea* Ingold, P.J. Mc Doughall & Dann: United Kingdom, Harpenden, Rothamsted Research, in rhizosphere of *Triticum aestivum* L., 21 Nov 2001, *leg.* H. Kwaśna (CBS114630, IMI391620, RR165).

Conidiophores lateral, solitary or branched, 0–2 septate, ampulliform, cylindrical to irregular, 4–7 (–12) × 3–3.5 µm. Conidiogenous cells obovate or irregular, polyblastic, sympodial, discretely denticulate, 2.5–5 (7) × 1.5–2 µm.

Macroconidia hyaline, 1–7 septate, branched; main axis 25–40 µm long, 1.5–2 µm wide, slightly curved, lateral branch 16–20 µm long, 1.5 µm wide, 0-1 septate, arising near the middle, but closer to the base than to the apex, from the convex side of the main axis. Microconidia hyaline, smooth-walled, oval, fusiform to cylindrical (1.5) 2–4 (5.5) × (1) 1.5 (2) µm, with an inconspicuous basal hilum. Teleomorph unknown.

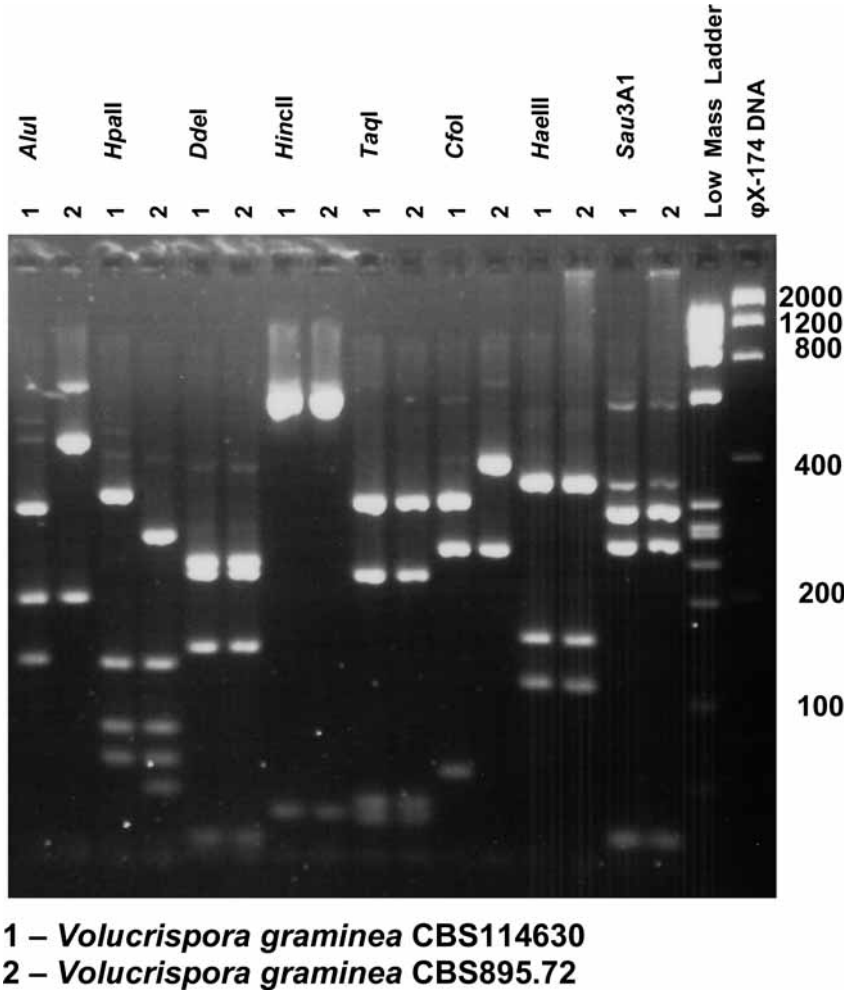


Fig. 22. RFLP analysis of the ITS rDNA region of *Volucrispora graminea* with eight restriction enzymes.

Morphological and molecular comparisons of the fungus from the wheat rhizosphere (CBS114630) with *V. graminea* from *Holcus lanatus* (CBS895.72) demonstrate that both isolates are at least congeneric.

Eight restriction enzymes generated informative RFLP patterns. The two isolates gave identical patterns for the ITS1/2 rDNA digested with *Dde*I, *Hae*III, *Hin*cII, *Sau*3A1 and *Taq*I. The patterns of the rDNA ITS1/2 with *Alu*I, *Cfo*I and *Hpa*II consisted of similar numbers of bands but had different locations for the peripheral bands (Fig. 22, Tab. 3). The positions of the intermediate sized fragments indicated, however, a close phylogenetic relationship between the isolates.

A microconidiogenus synanamorph is described for *V. graminea* and the species is epitypified to reflect this amendment. The microconidiogenus synanamorph in *V. graminea* (syn. *Ypsilina graminea*) was observed for the first time by Marvanová & Bärlocher (2001) in two out of 12 isolates studied. They emended the description of *V. graminea* with microconidia formed on lageniform phialides with distinct, up to 2 um deep or obscure collarettes. We found ampulliform to cylindrical conidiophores, with polyblastic, denticulate conidiogenous cells with few to several microconidia attached, unlike any reported by Marvanová & Bärlocher (2001) (Figs. 23–25). We observed that these conidiogenous cells were more distinct in young cultures. In older cultures, which stopped producing microconidia, the apices of the conidiogenous cells degenerated and resembled those of the collarette of the phialide.

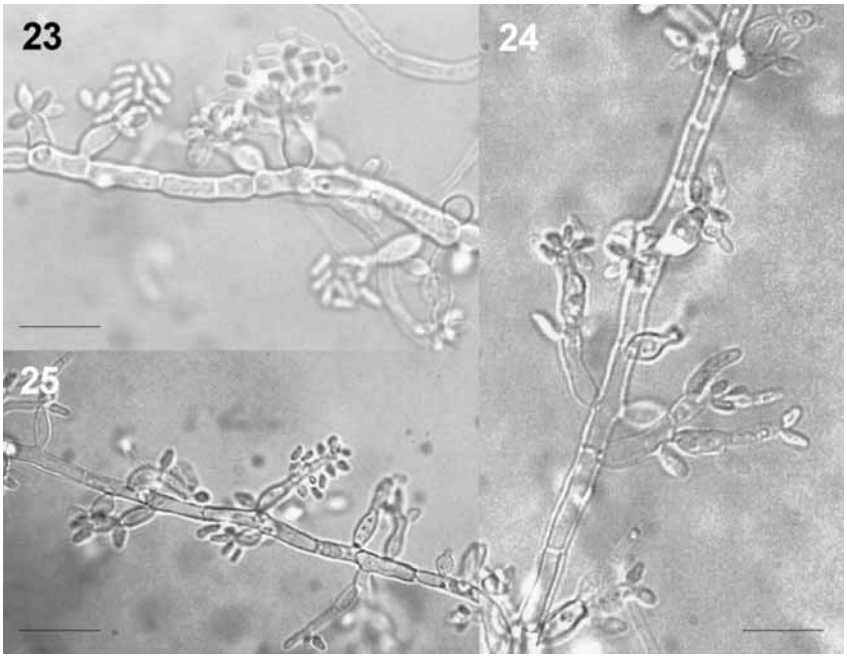


Fig. 23–25. *Volucrispora graminea* (CBS114630, IMI391620). Coniodophores with microconidia. Bar 10 µm.

The isolate CBS114630 provides the new type strain of *V. graminea*. Its sequences was deposited at EMBL with the accession number AJ748690.

Table 3. RFLP analysis of the ITS rDNA region with restriction enzymes (DNA fragment sizes shown as number of base-pairs).

Enzyme	*V. graminea* CBS114630	*V. graminea* CBS895.72
*Alu*I	529, 458, 304, 195, 142, 47	689, 458,195, 47
*Cfo*I	575, 304, 243, 72, 43	651, 383, 243, 42
*Dde*I	383, 235, 221, 148, 50	383, 235, 221, 148, 50
*Hae*III	336, 151, 119, 42	336,151, 119, 42
*Hin*cII	575, 57	575, 57
*Hpa*II	469, 422, 325, 138, 97, 81, 47	422, 325, 266, 138, 97, 81, 76, 47
*Sau*3A1	581, 535, 325, 283, 243, 47	581, 535, 325, 283, 243, 47
*Taq*I	310, 221, 60, 55	310, 221, 60, 55

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