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ORIGINAL ARTICLE

Pyrigemmula, a novel hyphomycete genus on grapevine and tree bark

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Abstract The anamorphic taxon *Pyrigemmula aurantiaca* gen. et sp. nov. is described and illustrated from specimens that were collected from the inner bark of living woody hosts (*Vitis vinifera*, *Pyrus communis*, *Mespilus germanica*, *Platanus hybrida*, *Elaeagnus angustifolia*) and plant debris in Hungary. The fungus is generically distinct in the nature of the pyriform, golden conidiogenous cell with a solitary terminal pore and the ellipsoidal, distoseptate, phragmoconidia that germinate from each end and that have a rarely noted internal hilum quite unlike the hilum of the conidiogenous cell. The new fungus is compared with the type species of a number of allied genera of hyphomycetes. Free spores of the fungus were trapped in air, honeydew sap and rainwater samples. Aerobiological studies showed that the spores are infrequent in the air, whereas their concentration increased with higher atmospheric pressure. *Pyrigemmula aurantiaca* lives in bark fissures and rarely becomes airborne and the spores are mainly dispersed by rain splash.

Keywords Bark · Corticolous · Dematiaceous hyphomycete · Dispersal · Honeydew sap

Taxonomic novelties *Pyrigemmula* D. Magyar & R. Shoemaker, gen. nov., *Pyrigemmula aurantiaca* D. Magyar & R. Shoemaker, sp. nov.

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Introduction

Stems and branches of woody hosts such as grapevines and fruit trees have in recent years been shown to share the same range of fungi, which are able to migrate between these different hosts (Mostert et al. 2005, 2006; Damm et al. 2007, 2008a, b, 2010; Essakhi et al. 2008). This movement of fungal organisms is usually enhanced by the fact that vineyards are frequently planted adjacent to fruit tree orchards. Upon closer examination, many of these fungi are associated with symptoms of brown wood discoloration, although several appear to simply be endophytic or saprobic (Van Niekerk et al. 2004; Mostert et al. 2006). These include several hyphomycetes such as *Phaeomoniella* (Petri disease in grapevines, and brown wood streaking in fruit trees; Mostert et al. 2006; Damm et al. 2008a), *Phaeoacremonium*/*Togninia* (brown wood streaking; Mostert et al. 2006; Essakhi et al. 2008), *Coniochaeta*/*Lecythophora* (endophytes on various substrates, but also pathogens of humans, and associated with food spoilage; Damm et al. 2010), *Collophora* (brown wood streaking; Damm et al. 2010), *Calosphaeria* and *Jattaea* species (endophytic in wood and bark; Damm et al. 2008a). Several coelomycetous species have also been found to share these hosts, namely species of *Paraconiothyrium* (endophytic, plant pathogenic; Damm et al. 2008b), and members of the *Botryosphaeriaceae* (endophytic, plant pathogenic; Slippers et al. 2007; Phillips et al. 2008), to name but a few. Some of these species have proven

sexual states or synanamorphs, which enable them to survive under different environmental conditions (Crous et al. 2006b), or to have different modes of dispersal, including insects (Mostert et al. 2006), air and rainwater (Magyar et al. 2009). Most of these genera are, however, typical inhabitants of wood and bark, occurring on a broad spectrum of trees and shrubs worldwide (Schoch et al. 2009; Zhang et al. 2009).

During a survey of alternative hosts of grapevine trunk disease pathogens, an unusual hyphomycetous fungus was found on bark and in honeydew samples of various woody hosts. Based on these findings, aerobiological studies were undertaken to understand its mode of dispersal. Furthermore, the fungus was isolated in pure culture, and is subsequently described here as a novel hyphomycete genus and species. It is contrasted to members of a number of other hyphomycete genera, from which we believe it to be distinct.

Materials and methods

Isolation

During investigations of bark-inhabiting fungi of living grapevines and a variety of trees, pieces of bark were collected in different locations in Hungary. The samples were incubated in moist chambers at room temperature in the laboratory and examined after 3–5 days. Monospore isolates were initiated from colonies found on the bark of *Elaeagnus angustifolia* and *Vitis vinifera.* The resulting colonies sporulated on synthetic nutrient-poor agar (SNA) at 25°C, but not on malt extract agar (MEA) (Crous et al. 2009b). All isolates were slow-growing. Small pieces of agar with the colonies of the fungus were placed on sterile filter paper and autoclaved bark of *Elaeagnus angustifolia* and *Vitis vinifera.* Dishes were incubated in a moist chamber for 2 weeks at 25°C to induce improved sporulation to facilitate morphological description. Digital photomicrographs were taken with an Olympus BX-51 microscope at ×800 magnification. Fungal structures were mounted on glass slides with clear lactic acid for microscopic examination. Thirty measurements per relevant microscopic structure were determined, with extremes given in parentheses. Colony colours were determined using the colour charts of Rayner (1970) after 7 days at 25°C on the bench. Reference strains are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW), Utrecht, the Netherlands. Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

Spore sampling

The characteristic conidia of the unknown fungus were previously observed during different surveys when data on the occurrence of free spores in air, rainwater and stem sap samples were collected. Air samples were obtained using two 7-day recording air samplers (Hirst 1952; VPPS 2000; Lanzoni, Bologna, Italy). The first trap operated to coincide with the blooming period of grape vines in Italy (May–June): 27 May to 13 June 1994; from 5 June to 3 July 1995; and from 13–24 June 1996, at 12 m height, in a vineyard (approximately 2,500,000 m^2, kept by the Luganotti Company) near the city of Brufa (Central Italy). The second sampler was located at 150 cm above ground level, in the experimental field of the Plant Protection Institute of the Hungarian Academy of Sciences in Nagykovácsi, where air sampling was conducted between 30 June 2007 and 16 October 2007. The spore trap worked continuously, aspirating air at a rate of 10 l/min. The airborne fungal spores impacted on a tape (MELINEX® strip) coated with a thin adhesive layer (silicone oil). The greased tape was mounted on a rotating drum within the trap, rotating 2 mm/h. The exposed tape was removed weekly and cut into 48 mm segments, thus representing 24 h periods. The segments were placed on microscope slides and stained with basic fuchsin in mounting medium (glycerine-jelly). In the air samples, 12 transverse traverses were scanned at ×400 magnification of an Olympus BX 51 microscope.

The technique to prepare honey-sap samples was: 10 g were taken from 500 g of previously homogenised honey, dissolved in 20 ml of distilled water at 40°C, centrifuged for 5 s at 2,500 *g* and allowed to settle. The sediment was recovered in 10 ml of distilled water and again centrifuged. The sediment was then collected with a Pasteur pipette and dried onto microscope slides at 40°C. It was then mounted in glycerine-gelatine and covered (Louveaux et al. 1978). The entire surface of each preparation was scanned under a microscope and fungal spores were identified. A total of 83 of these samples were examined from Croatia, Greece, Hungary, Italy, Mexico, New Zealand, Portugal, Slovakia, South Africa, Spain, and Tanzania.

Stem-flow rainwater samples were occasionally collected from living trees (24 samples in 2003 and 2004), and from a water-filled tree hollow on a maple tree (*Acer platanoides*; 65 samples collected between 18 July 2003 and 5 September 2007) in Budapest, Hungary. Depending on the intensity of rainfall, various quantities of water could be collected (2–10 ml) in centrifuge tubes. One ml of FAA (50% ethanol 5% glacial acetic acid, 10% formaldehyde) was added for each sample (Ingold 1975). Water samples were settled, then one drop of the sediment was mounted on a microscope slide and allowed to dry. Lactophenol with cotton blue was added to the dried sediment to prepare the sample for further studies.

Biometeorology

To clarify the connection between spore counts and meteorological variables, a weather station (Weather Station

WS-3600; Conrad Electronic, Hirschau, Germany) was placed in the vicinity of the sampling site in Hungary. The daily records of temperature (°C), dew point (°C), and relative humidity were measured with a hygrothermometer. Rainfall (mm) and the duration of precipitation (min) were recorded with a rain gauge. An anemometer and an electronic barometer were used to measure wind speed (m s^{-1}), wind direction, and atmospheric pressure (Pa), respectively. To study the effect of meteorological factors (temperature, rain, atmospheric pressure) on the airborne dispersal of the fungus, a logit regression model was used (Statistica, StatSoft). Spore counts were reduced to presence and absence data, because of the low airborne concentration of the spores.

Molecular phylogeny

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's protocols. Part of the nuclear rDNA operon spanning the 3' end of the 18 S nrRNA gene (SSU), the internal transcribed spacer 1, the 5.8 S nrRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28 S nrRNA gene (LSU) was amplified and sequenced as described in Frank et al. (2010). The primers ITS4 (White et al. 1990) and LR0R (Rehner and Samuels 1994) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search and results are discussed below in the species notes. The sequence alignment and subsequent phylogenetic analysis using PAUP v. 4.0b10 (Swofford 2003) followed the methods of Crous et al. (2006a, 2009a). Alignment gaps were treated as new character states for the parsimony analysis. Novel sequence data were deposited in NCBI's GenBank database (CBS 126743: GenBank HM241692, CBS 126744: GenBank HM241693; GenBank accessions span the ITS region and the first 900 bases at the 5' end of the 28 S nrRNA gene).

Results

Phylogenetic analysis

Approximately 1,500 bases, spanning the ITS and LSU regions, were obtained for the two isolates sequenced. The LSU region was used in the phylogenetic analysis for the generic placement (Fig. 1) and ITS to identify possible species-level relationships in NCBI's GenBank database.

The manually adjusted LSU alignment contained 41 taxa (including the outgroup sequence) and, of the 841 characters (including alignment gaps) used in the phylogenetic analysis, 254 were parsimony-informative, 61 were variable and parsimony-uninformative and 526 were constant. Five equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 1 (TL=10,78, CI=0.456, RI=0.680, RC=0.310). The obtained phylogenetic tree places the isolates in the *Chaetosphaeriaceae*, with the closest sister species being *Ellisembia brachypus* and *Lecythothecium duriligni*. The ITS sequences did not reveal any close hits during the blast search and the isolates are therefore described as a novel genus and species below.

Taxonomy

Pyrigemmula D. Magyar & R. Shoemaker, gen. nov.
MycoBank MB 517148

Etymology Pyrum, L. and *gemmula* L. referring to the pear-shaped conidiogenous cells.

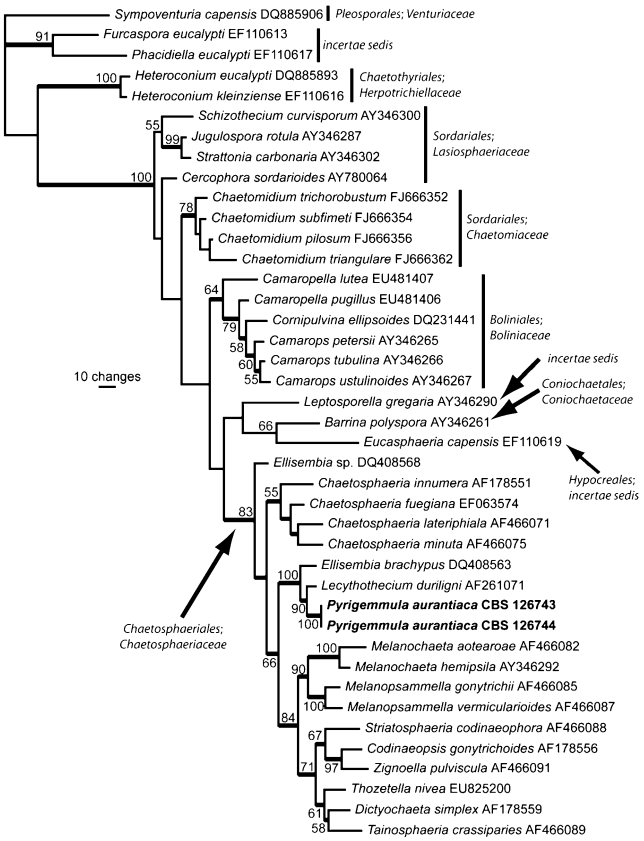

Fig. 1 The first of five equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The bootstrap support values from 1,000 replicates are shown at the nodes. Novel sequences generated for this study are shown in bold. Branches present in the strict consensus tree are thickened. The tree was rooted to a sequence of *Sympoventuria capensis* (GenBank accession DQ885906)

Mycelium hyalinum, tenuitunicatum, pauciseptatum, rarium ramosum rarium anastomosum. Conidiophora rara, hyalina, curvata, tenuitunicata, demum bifurcata. Cellula conidiogena sessilis in hyphis, rara in conidiophoris, terminalis, aurantiaca, ovoidea, pyriformis, rara sphaerica vel cylindrical; hilum terminale, solitarium, inclusum; abseque isthmo. Conidia solitaria, ellipsoidea, tenuitunicata, distoseptata, castanea, eguttulata; hilum inclusum. Hypha germinalis terminalis, hyalina, guttulata, recta, demum bifurcata; leniter crescens.

Typus *Pyrigemmula aurantiaca*

Hyphae hyaline, thin-walled, straight, rarely branched at acute or right angles, sometimes with anastomoses. *Conidiogenous cell* usually arising directly from hyphae or rarely from delicate conidiophores. *Conidiophores* (when present) slightly curved as acute-angled projection from hypha, hyaline and thin-walled. *Conidiogenous cell* terminal, brown, narrow ovoid, pyriform, rarely spherical or short-filamentous; pore terminal, solitary; hilum flush, not thickened, not exerted, without isthmus. *Conidia* solitary, short to long ellipsoidal, thin-walled, distoseptate, reddish brown, eguttulate; hilum inconspicuous, internal, not flush or exserted (atrium type of Alcorn 1983, p. 50, figs 43, 45–48). Germination axial from each pole with one hyaline, guttulate, straight hypha that later branches dichotomously. Germination evident after 2 days; germ tube growth slow, 90–150 μm in 6 days. Surface of the colony a golden, powdery mass of conidia and conidiogenous cells. To the naked eye, the thin hyaline surface hyphae are not evident.

Pyrigemmula aurantiaca D. Magyar & R. Shoemaker, sp. nov. Figs. 2, 3 and 4

MycoBank MB 517031

Etymology: aurantiaca L. referring to the golden or orange colour of the conidiogenous cells.

Hyphae pauci septatae (15–20 μm). Conidiophora 3–5(–10)×2–3 μm diam. Cellula conidiogena 6–14×4–6 μm; hilum terminale ca. 2 μm diam. Conidia 18–30×(5–)8–9 μm, 0–5(–7) septata. Hypha germinales leniter crescens, 90–150 μm in 6 deis.

Hyphae septate at 15–20 μm. *Conidiophores* ca. 3–5×2–3 μm, later extending to ca. 10×3 μm with a branch budding from the outside of the curvature, finally branching bifurcately to produce 4 or 5 cells. *Conidiogenous cells* (9.6–)11(–14.4)×(4.8–)5.7(–7.2) μm, with one solitary terminal pore 2 μm diam. *Conidia* (17.6–)22.4–24(–27.2)×6.5–8 μm, 0–5(–7) septate.

Germination axial from each pole with one hyaline, guttulate, straight hypha that later branches dichotomously. Germination slow, after 2 days; germ tube growth slow, 90–150 μm in 6 days. Surface of the colony a golden, powdery mass of conidia and conidiogenous cells. The thin, hyaline surface hyphae are hardly visible. Colonies on MEA greyish brown, slimy, pulvinate, consisting of a cortical layer, at the margins lobed, slow growing, attaining 5 mm diam in 7 days (Figs. 2, 3 and 4). The colony brownish in reverse. On MEA, the fungus does not sporulate, the isolate

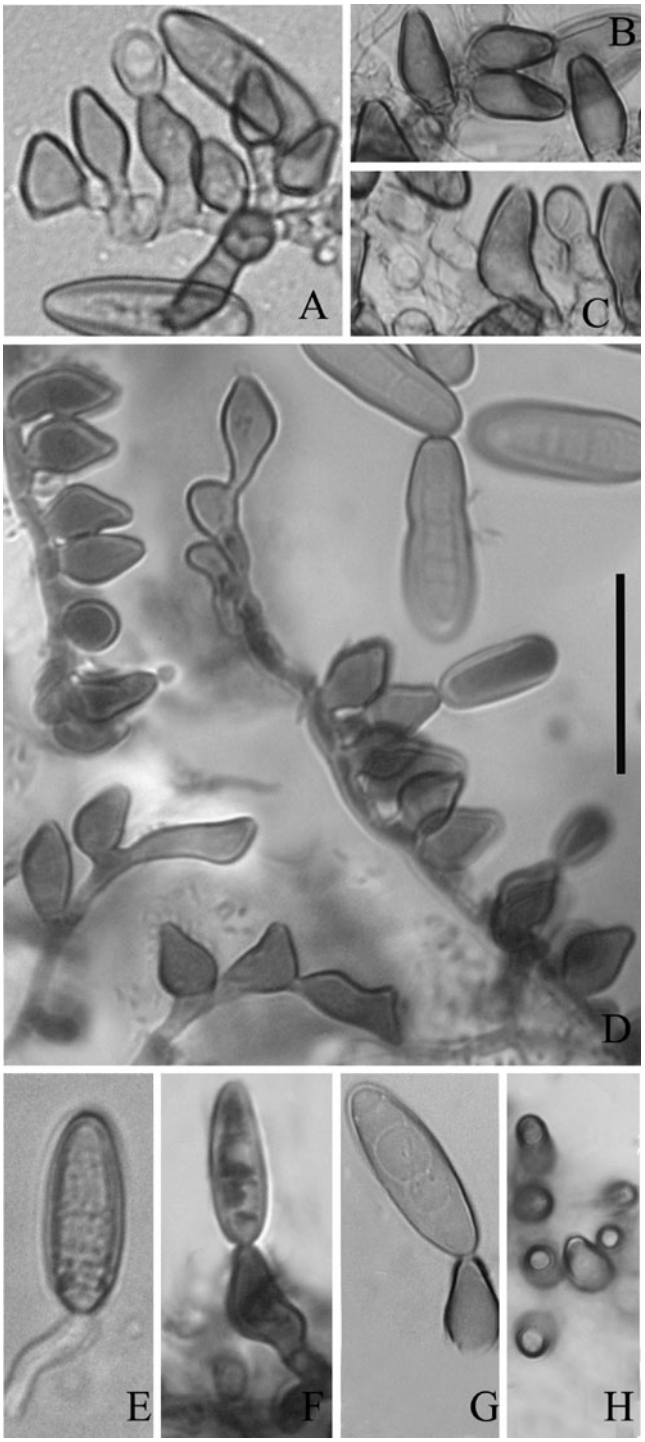

Fig. 2 *Pyrigemmula aurantiaca*. **a–d** Solitary and clustered conidiogenous cells. **e** Conidium germinated after 6 days on SNA. **f,g** Conidia prior to detachment. **h** Conidiogenous cell hilum. *Scale bar* 20 μm

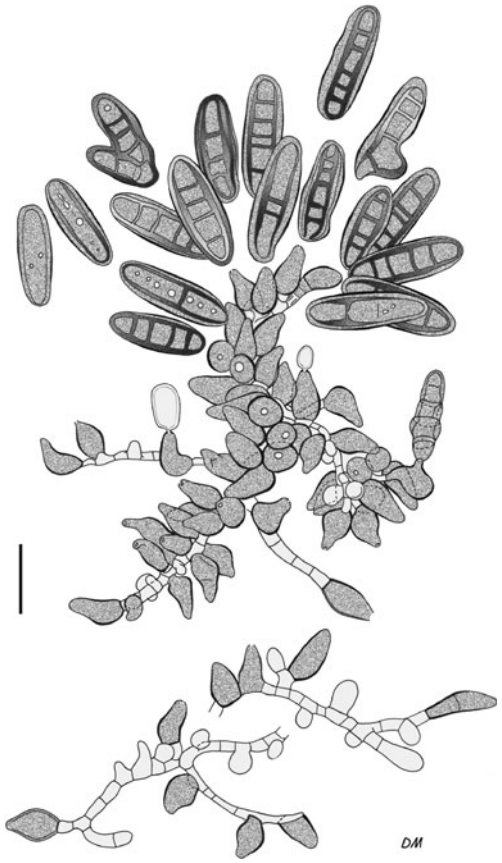

Fig. 3 *Pyrigemmula aurantiaca* conidiogenous cells and conidia. *Scale bar* 20 μm

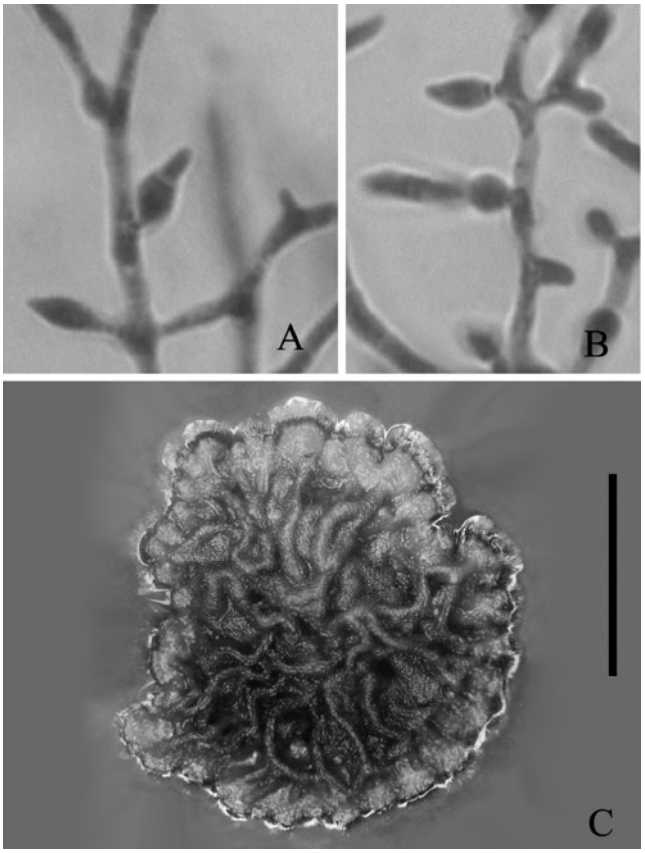

Fig. 4 *Pyrigemmula aurantiaca* on MEA. **a,b** Sterile hyphae. **c** Colony after 40 days of incubation. *Scale bars* (**a,b**) 20 μm, (**c**) 15 mm

sporulated when transferred to filter paper and sterilised bark (isolates from *Vitis* were transferred to *Elaeagnus* bark and *vice versa*). Filter paper, SNA and bark cultures do not differ in morphological characters from those of herbarium material.

Holotype Hungary, Noszvaj, on cortex of *Vitis vinifera* L., 22 Nov. 2009, D. Magyar, BP 101176, culture ex-type CBS 126743.

Additional specimens examined. Bark samples were collected in different sites in Hungary from the marginal surface of the bark of living *Acer saccarinum* L. (1 sample), *Betula pendula* Roht. (2 samples), *Elaeagnus angustifolia* L. (6 samples), *Mespilus germanica* L. (1 sample), *Quercus* sp. (1 sample), *Platanus hybrida* Brot. (6 samples), *Pyrus communis* L. (1 sample), *Vitis vinifera* L. (2 samples) and on litter (1 sample). Near the stream Szén-patak, Mountain Börzsöny, on litter (possibly *Fagus sylvatica* L.), 14 Feb. 1981, Á. Révay and J. Gönczöl (BP 11/23 and BP 11/24 as slides); Budapest, in Városliget Park, 47°30′44.83”N19°04′ 58.73”E, on *Quercus* sp., 20 Nov. 2006, D. Magyar (T09/ 10 as slide); Szokolya, 47°51′54.67″N,19°00′21.87″E, on *M. germanica*, 20 Jan. 2007, D. Magyar (T09/5 as slide); Budapest, Nagyvárad square, 47°28′42.33″N, 19°05′24.95″ E, on *A. saccharinum*, 12 Nov. 2009, D. Magyar (T62/3 and T63/1 as slide, Tk0911/1 as bark sample); Budapest, Nagyvárad square, on *B. pendula*, 11 Dec. 2009, D. Magyar (CBS 126744 as MEA culture, GenBank accession number HM241693, T63/1 and T62/5 as slide, T160 as MEA culture); Budapest, Korong str., on *B. pendula*, 26 Nov. 2009, D. Magyar (T62/5, T63/2 and T63/4 as slide); Budapest, in the park of the Plant Protection Institute, 47° 30′50.75″N, 19°00′39.28″E, on *E. angustifolia*, 09 May 2007, D. Magyar (BP 99816 as slide, T09/1 as bark sample, DAOM 239578 as dried SNA culture); Pákozd, Isle Szúnyog-sziget, 47°12′37.03″N, 18°34′30.39″E, on *E. angustifolia*, 04 July 2007, D. Magyar (T09/2 as slide); Belsőbáránd, by the side of the watercourse Dinnyés-Kajtori-csatorna, 47°05′56.04″N, 18°30′48.60″E, on *E. angustifolia*, 07 Aug. 2007, D. Magyar (T09/3 as slide); between Tinnye and Perbál, on *E. angustifolia*, 26 May 2008, É. Szita (T09/11 as slide); Kecskemét, Új-Városföld, 46°52′11.85″N, 19°42′11.37″E, on *E. angustifolia,* 19 Sept. 2008, D. Magyar (T09/12 as slide); Visegrád, on *E. angustifolia*, 12 May 2010, D. Magyar (Tk1005/1 as bark sample); Budapest, in Városliget Park, 47°31′00.15″N, 19° 05′08.48″E, on *P. hybrida*, 11 July 2009, D. Magyar (T09/ 21a as slide); Budapest, in Városliget Park, on *P. hybrida*,

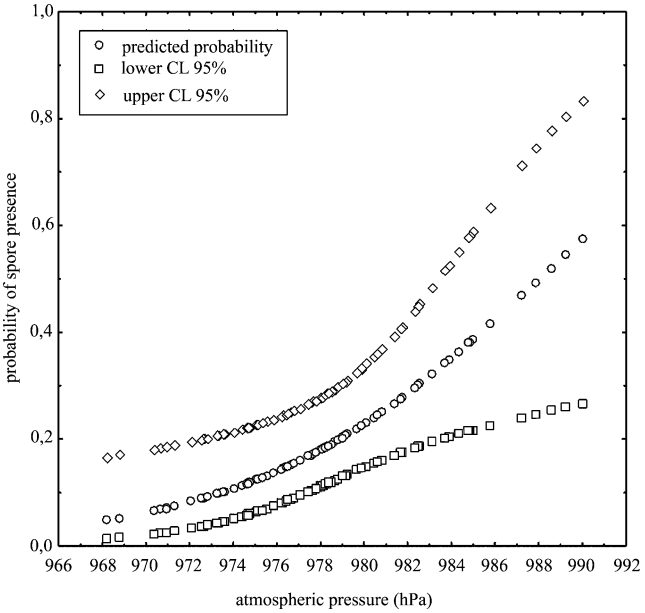

Fig. 5 Prediction model showing the increasing probability of the presence of *Pyrigemma aurantiaca* spores in air samples caused by an increase in atmospheric pressure.

26 Oct. 2009, D. Magyar (T09/21b as bark sample); Göd, 47°42′58.46″N, 19°08′03.18″E, on *P. hybrida*, 27 Oct. 2009, D. Magyar (BP 100757 as bark sample); Budapest, in the park of the National Center for Epidemiology, on *P. hybrida*, 09 Feb. 2009, D. Magyar (T09/23 as slide); Budapest, in the courtyard of the Hungarian Natural History Museum, on *P. hybrida*, 10 Feb. 2009, Á. Révay and J. Gönczöl (BP 100758 as bark sample); Budapest, near Zugló train station, on *P. hybrida*, 01 Jun. 2010, D. Magyar (T81/1 as slide); Budapest, Lőrinc, on *P. communis*, 08 Oct. 2008, J. Gönczöl (BP 100337 as bark sample); Budapest, in the park of the Plant Protection Institute, 47°30′50.51″N, 19°00′43.08″E, on *V. vinifera*, 07 Nov. 2007, D. Magyar (BP 99817 and T09/4 as slides); Noszvaj, 47°56′36.51″N, 20°28′19.51″E, on *V. vinifera*, 22 Nov. 2009, D. Magyar (BP 101176 as bark sample, CBS 126743 as MEA culture, GenBank accession number HM241692, T161 as MEA culture, T62/4 as slide, Tk0911/2 as bark sample).

Notes.*Pyrigemmula aurantiaca* clustered close to sequences of *Ellisembia brachypus* and *Lecythothecium duriligni*. *Ellisembia brachypus* is distinct by having solitary, septate conidiophores and rostrate conidia, while the anamorph of *L. duriligni* also has solitary, septate conidiophores (Réblová and Winka 2001), thus being morphologically distinct. *Pyrigemmula* was further contrasted with the type species of the following genera of hyphomycetes. *Murogenella*, typified by *M. terrophila*, has hypha-like conidiogenous cells, and the conidia have a broad truncate basal cell. *Bactrodesmiastrum*, typified by *B. obscurum,* has flask-shaped conidiogenous cells with a truncate apex, and the conidia are obovoid, versicoloured, and have a truncate exserted hilum. *Janetia*, typified by *J. euphorbiae,* has conidiogenous cells with one to several truncate openings, and the conidia have a truncate base. The monograph of *Janetia* by Goh and Hyde (1996) treated 17 species, and emended the description of the genus. However, the 17 species treated all had denticulate conidiogenous cells and the conidia exhibited a conspicuous, exerted truncate base quite unlike the comparable structures of *P. aurantiaca*. *Phragmospathula*, typified by *P. phoenicis,* has obovoid conidiogenous cells that proliferate and bear conidia that have a spathulate basal cell. *Heteroconium,* typified by *H. citharexyli,* has more elaborate filamentous conidiophores and conidia with eusepta and a truncate base. The recently introduced genus *Houjia* is morphologically similar to *Pyrigemmula,* in having conidiophores reduced to conidiogenous cells, and solitary, brown, scolecosporous conidia. However, it has euseptate conidia, and is a member of the *Capnodiales* (Yang et al. 2010), whereas *Pyrigemmula* is a member of the *Chaetothyriales*.

Pyrigemmula aurantiaca spores were found in stemflow samples draining from *Alnus glutinosa* (23 Feb. 2004), *Carpinus betulus* (12 Jan. 2004), *Celtis occidentalis*, *Crataegus monogyna* (29 July 2003), and *Cercis siliquastrum* (30 July 2003). In water-filled maple tree hollows, spores were found in low concentrations, but occurred throughout the year during the 5-year long observation period (samples v25, v20, v3, v5, respectively; preserved on slides).

Free spores of *P. aurantiaca* were also observed in saphoney samples (1–2 spores/10 g), but only in those of honeydew origin (often called as ‘forest honey’) or *Castanea* honeys, which were contaminated with honeydew (m20326, from *Abies* sp., Italy; m21884, unspecified forest honey, Lazio, Italy; m21888, unspecified forest honey, Friuli Venezia Giulia, Italy; m21892, honeydew honey from *Abies alba* Mill., Italy; m20406 and m20409, honeys from *Castanea sativa* Mill., Italy; m21935, unspecified forest honey, Toscana, Italy; m21639 and m30093, unspecified forest honeys, Liguria, Italy; m00025, unspecified forest honey, Ózd, Hungary; m00026, unspecified forest honey, Croatia; m00028, unspecified forest honey, Slovakia). Spores were absent, however, from floral honeys.

Air samplers caught these spores only sparsely both in Italy and Hungary. The airborne concentration of the fungus fluctuated between 0–3 spores/m^3, Logit regression analysis performed on airborne spore data showed that atmospheric pressure had significant effect (p=0.009). Temperature and rain, having no significant effects, were excluded from the model. The prediction of the probability of the presence of *P. aurantiaca* spores in air samples is

depicted in Fig. 5. The amount of increase of such probability corresponds with the calculated odds ratio (1.162, lower CL 95% 1.038, upper CL 95% 1.302), which implies that by 1 hPa increase of the atmospheric pressure the presence of spores is 1.162 times more likely.

Discussion

The finding that *P. aurantiaca* could occur in the inner bark or bark fissures of grapevines and a variety of other woody hosts, is not that surprising. This phenomenon could be more common among ascomycetes than previously accepted, as several phytopathogenic species of the *Botryosphaeriaceae*, *Calosphaeriaceae, Togniniaceae*, etc. have been shown to migrate from branches and stems of grapevines and fruit trees to other woody hosts in the immediate vicinity (Crous et al. 2006b; Damm et al. 2007). According to Kubátová et al. (2004), some species of *Phaeoacremonium* could be dispersed between woody hosts by bark beetles, as they have also been isolated from these vectors. Based on the results obtained in this study, *P. aurantiaca* appears to lack host specificity, and currently there is also no indication what ecological role it plays, nor if it could be pathogenic to any of these hosts.

The low frequency and concentration (0–3 spores/m^3) of *P. aurantiaca* spores in the air samples suggests that this fungus is rarely dispersed by wind. The conditions for wind dispersal for this fungus living in the inner marginal surface of the bark are poor, because the air may be still inside these fissures (Gregory 1961). Deposits of free spores were often observed inside the bark fissures and probably were carried there by stem-flow rainwater (Magyar 2008). It is hypothesised that stem-flow may play an important role providing microscale dispersal between fissures. Spores carried by stem-flow are trapped and accumulated in the fissures, thus the fungus could colonise new fissures in the bark.

Furthermore, these data suggests that the spores of *P. aurantiaca* are frequent in South and Central Europe in forest- and honeydew honeys, as well as in *Castanea* honeys. Honeydew, a product of piercing insects feeding on the trees are harvested and transported by honeybees to the hives and processed into honeydew honey. Honeys from silver-fir, oak-trees, etc. are marketed worldwide and often called “forest honey”. Therefore, these honeys were in contact with tree bark, and essentially act as a conidial trap (Magyar *et al*. 2005).

Pyrigemmula represents yet another novel genus of hyphomycetes from the inner bark of woody hosts like *Phaeomoniella*, *Phaeoacremonium* and *Collophora* (Mostert et al. 2006; Damm et al. 2010). The fact that so many novel genera are currently being recorded from unusual substrates such as fruit surfaces (Batzer et al. 2008; Frank et al. 2010; Yang et al. 2010), stem-sap and bark, leaf trichomes (Dornelo-Silva and Dianese 2004), extremotolerant environments (Selbmann et al. 2008) rocks (Gueidan et al. 2008; Ruibal et al. 2008, 2009), endophytes (Strobel and Daisy 2003), intestinal tracks of insects (Suh et al. 2005), suggests that mycologists have just been scratching the surface and sampling obvious habitats when looking at fungal diversity. New DNA sequencing technologies that are able to detect obscure and frequently non-cultivatable taxa, will further highlight the inability of presently employed techniques to collect and assist in describing the fungal biodiversity currently expected to exist (Hibbett et al. 2009). This fully underlines the need for a novel approach to dealing with the magnitude of undescribed biodiversity in Kingdom Fungi.

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