Trop. plant pathol.
DOI 10.1007/s40858-017-0143-7

ORIGINAL ARTICLE

Eucalypt powdery mildew caused by *Podosphaera pannosa* in Brazil

Natália R. Fonseca[1] · **Lúcio M. S. Guimarães**[1] · **Raul P. Pires**[1] · **Ned B. Klopfenstein**[2] · **Acelino C. Alfenas**[1]

Received: 29 September 2016 / Accepted: 9 February 2017

Abstract Eucalypt powdery mildew is an important disease in greenhouses and clonal hedges of *Eucalyptus* spp. in Brazil, which can cause leaf and shoot distortion, shoot discoloration, and growth reduction that results in production losses. Because reliable information regarding the causal agent of the disease is lacking, this study used ITS and 28S rDNA sequencing and morphological analyses to identify the powdery mildew pathogen that occurrs in eucalypt nurseries within different regions in Brazil. Based on the results of morphological characteristics and phylogenetic analyses, the pathogen isolates were identified as *Podosphaera pannosa*, also known as the rose powdery mildew pathogen. Cross inoculations with pathogen isolates from rose and eucalypt demonstrated that *P. pannosa* can infect both host species. The ITS sequence-based phylogeny showed that 42 sequences generated in this study were comprised within a single clade containing *P. pannosa*, which was supported by a posterior probability of 88%. Identical ITS sequences were obtained from all 42 pathogen isolates, which suggests a clonal population.

Keywords *Eucalyptus* · *Rosa* · Bayesian analysis · Cross inoculation · Erysiphales · ITS rDNA · 28S rDNA

Section Editor: Alan Wood

✉ Acelino C. Alfenas
aalfenas@ufv.br

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

[2] USDA Forest Service - Rocky Mountain Research Station, 1221 South Main Street, Moscow, ID 83843, USA

Introduction

The production system of eucalypt (*Eucalyptus* spp. L'Hér.) cuttings in Brazil has been evolving over recent decades with the introduction of improved clonal mini-cuttings and enhanced nursery facilities (Alfenas et al. 2009). The current environmental conditions in nurseries, such as nursery coverage and drip irrigation, are disadvantageous to most diseases except powdery mildew, which is favored under these conditions (Silva et al. 2003). Although powdery mildew rarely occurs in eucalypt plantations, it is commonly encountered in greenhouses and clonal hedges where it can cause severe leaf and shoot distortion, shoot discoloration, growth reduction, and production losses (Keane et al. 2000). As a result, powdery mildew has become one of the most important diseases of eucalypt clonal hedges in Brazil.

Powdery mildew diseases are caused by biotrophic fungi in the Erysiphales. These fungal pathogens are readily observed as conspicuous external mycelia, typically forming white patches that may cover the entire leaf surface (Fig. 1) (Braun and Cook 2012). Several eucalypt species are infected by powdery mildew pathogens (Old et al. 2003). Four species of powdery mildew pathogens that infect eucalypt in nurseries have been identified worldwide: 1) *Golovinomyces orontii* (Castagne) V. P. Heluta (≡*Erysiphe orontii* Castagne) in New Zealand (Boesewinkel 1981) previously referred to as *Erysiphe* (*Golovinomyces*) *cichoracearum* in United Kingdom, New Zealand, and USA (Stone 1962; Gardner and Yarwood 1974; Boesewinkel 1979; Matheron and Matejka 1992); 2) *Podosphaera aphanis* (Wallr.) U. Braun & S. Takam. [≡*Sphaerotheca aphanis* (Wallr.) U. Braun] in New Zealand, Australia, and Japan (Boesewinkel 1981; Cunnington et al. 2003; Tanda and Hirose 2003); 3) *Podosphaera macularis* (Wallr.) U. Braun & S. Takam. [≡*Sphaerotheca macularis* (Wallr.) Magnus 1899] in

Published online: 17 March 2017

Fig. 1 **(a)** *Eucalyptus* nursery in the state of Minas Gerais, Brazil, with plants showings powdery mildew signs. **(b)** Plants highly infected with powdery mildew pathogen. **(c)** Eucalypt leaf with mycelia and conidia of powdery mildew pathogen

Germany (Brandenburger 1961) though this record probably belongs to *Podosphaera pannosa* (Wallr.) de Bary; 4) *P. pannosa* [≡*Sphaerotheca pannosa* (Wallr.) Lév.] in Italy, United Kingdom, Denmark, Poland, Portugal, Argentina, New Zealand, Australia, South Africa, and Korea (Grasso 1948; Glasscock and Rosser 1958; Spaulding 1961; Gibson 1975; Boesewinkel 1981; Crous et al. 1989; Cunnington et al. 2003; Delhey et al. 2003; Cho et al. 2016). Powdery mildew has also been reported on field-grown *Corymbia citriodora* in Brazil, with leaf deformation and loss of apical dominance observed in young plants (Ferreira 1997).

In Brazil, powdery mildew on *Eucalyptus* was first reported in 1936 by Grillo, and subsequent reports determined that *Oidium* sp. or *Oidium eucalypti* Rostrup. was the causal agent, based on the anamorphic stage of the fungal pathogen (Mucci et al. 1980). Most powdery mildew pathogen anamorphs are poorly differentiated at the species level based on morphology (Braun and Cook 2012). Furthermore, accurate identification of the eucalypt powdery mildew pathogen in Brazil is hampered because sexual reproductive structures are lacking (Bedendo 2011). Previous artificial inoculation studies indicated that powdery mildew pathogen isolates from *Rosa* sp. L. and *Dahlia* sp. Cav., which were classified as *P. pannosa* and *G. cichoracearum* (DC.) V.P. Heluta, respectively, were also pathogenic to *E. pellita* (Silva et al. 2001). Based on morphological features of the anamorph, Silva et al. (2001) concluded that the eucalypt powdery mildew pathogen found in Brazil was similar to the rose powdery mildew pathogen.

Currently, the widely accepted concept of "one fungus, one name" proposes to end the dual nomenclature of pleomorphic fungi, and provides one species name that comprises the teleomorphic and anamorphic stages of the same fungus (Taylor 2011; Wingfield et al. 2012). With the advent of molecular techniques, substantial DNA sequencing data of Eysiphales have been generated (Mori et al. 2000; Limkaisang et al. 2006). DNA sequence comparisons allow Erysiphales anamorphs to be linked with their respective teleomorph, even when the teleomorph is not observed (Cunnington et al. 2003; Wingfield et al. 2012). Despite the available sequence data, the eucalypt powdery mildew pathogen in Brazil remains known solely by the anamorphic species *Oidium eucalypti*, which is not well characterized (Braun and Cook 2012), or *Oidium* sp., which can be attributed to several Erysiphales teleomorphs.

Studies that help develop technologies for managing powdery mildew are an urgent need. For disease management, it is essential to know precisely which powdery mildew pathogen species is/are causing disease on eucalypt in Brazil. Identification of powdery mildew pathogens is needed to 1) help breeding programs aimed at developing resistant plants, 2) contribute to more effective chemical and cultural control, which can be influenced by pathogen species or races, and 3) determine if the pathogen is native or introduced. Thus, the objective of this study was to identify the powdery mildew pathogen(s) infecting eucalypt in different regions of Brazil using phylogenetic analyses and morphological characteristics.

Material and methods

Sample sources

Eucalypt powdery mildew pathogen isolates were collected from March to September 2014 from mini-clonal hedges in greenhouses from several clonal *Eucalyptus* spp. nurseries in five states of Brazil (Table 1). Six isolates of the rose powdery mildew pathogen (*Podosphaera pannosa*) from nurseries near Viçosa, MG, were also included in this study. Specimens (mycelia and conidia) collected by scraping a leaf from one diseased plant or clone were considered as an isolate.

DNA extraction

Total DNA was extracted from conidia and mycelia by the Chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). Conidia were added to 50 μL of 5% Chelex (Bio-

Table 1 Powdery mildew pathogen identity (ID), host, origin, and GenBank accession number

Isolate ID	Clone/variety	Host	Origin	GPS coordinates	GenBank accession number		Collector/reference
					ITS	28S	
LPF 610	6061	*Eucalyptus* sp.	Viçosa, MG	20°46′48.59″S/42°49′29.63″W	KX185528	KX185529	N. R. Fonseca
LPF 611	C219	*Eucalyptus* sp.	Viçosa, MG	20°46′48.59″S/42°49′29.63″W	KX185528	KX185529	N. R. Fonseca
LPF 612	VM3	*Eucalyptus* sp.	Viçosa, MG	20°46′48.59″S/42°49′29.63″W	KX185528	KX185529	N. R. Fonseca
LPF 613	10	*Eucalyptus* sp.	Belo Oriente, MG	19°18′48.59″S/42°23′30.09″W	KX185528	KX185529	A. G. B. Medeiros
LPF 614	37036	*Eucalyptus* sp.	Aracruz, ES	19°50′32.17″S/40°04′47.76″W	KX185528	KX185529	R. G. Mafia
LPF 615	1183	*Eucalyptus urophylla*	Viçosa, MG	20°46′48.59″S/42°49′29.63″W	KX185528	KX185529	L. M. S. Guimarães
LPF 616	1183	*Eucalyptus urophylla*	Viçosa, MG	20°46′27.7″S/42°52′35.75″W	KX185528	KX185529	N. R. Fonseca
LPF 617	BA 2004	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 618	BA 1922	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 619	BA 1922	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 620	144	*E. grandis* X *E. urophylla*	Brás Pires, MG	20°49′10.3″S/43°15′22.4″W	KX185528	KX185529	N. R. Fonseca
LPF 621	1528	*E. grandis* X *E. urophylla*	Brás Pires, MG	20°49′10.3″S/43°15′22.4″W	KX185528	KX185529	N. R. Fonseca
LPF 622	144	*E. grandis* X *E. urophylla*	Lima Duarte, MG	21°45′57.7″S/43°37′22.3″W	KX185528	KX355455	N. R. Fonseca
LPF 623	144	*E. grandis* X *E. urophylla*	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	KX185528	KX185529	N. Borges Junior
LPF 624	32864	*E. saligna*	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	–	KX185529	N. Borges Junior
LPF 625	BA2004	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 626	BA2004	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 627	3367	*Eucalyptus* sp.	Curvelo, MG	18°50′43.4″S/44°35′13.0″W	–	KX185529	N. R. Fonseca
LPF 628	3335	*Eucalyptus* sp.	Curvelo, MG	18°50′43.4″S/44°35′13.0″W	–	KX185529	N. R. Fonseca
LPF 629	2682	*Eucalyptus* sp.	Curvelo, MG	18°50′43.4″S/44°35′13.0″W	KX185528	KX185529	N. R. Fonseca
LPF 630	PEM 02598	*Eucalyptus* sp.	Paraopeba, MG	19°17′15.9″S/44°29′24.4″W	–	KX185529	N. R. Fonseca
LPF 631	VM3	*Eucalyptus* sp.	Paraopeba, MG	19°17′15.9″S/44°29′24.4″W	–	KX185529	N. R. Fonseca
LPF 632	PEM 04098	*Eucalyptus* sp.	Paraopeba, MG	19°17′15.9″S/44°29′24.4″W	KX185528	KX185529	N. R. Fonseca
LPF 633	BSCDT 47702	*Eucalyptus* sp.	Paraopeba, MG	19°17′15.9″S/44°29′24.4″W	KX185528	KX185529	N. R. Fonseca
LPF 634	A08 (3301)	*Eucalyptus* sp.	Jequitibá, MG	19°09′21.8″S/43°58′25.6″W	–	KX185529	N. R. Fonseca
LPF 635	144	*E. grandis* X *E. urophylla*	Jequitibá, MG	19°09′21.8″S/43°58′25.6″W	KX185528	KX185529	N. R. Fonseca
LPF 636	1528	*E. grandis* X *E. urophylla*	Jequitibá, MG	19°09′21.8″S/43°58′25.6″W	KX185528	KX185529	N. R. Fonseca
LPF 637	224	Hybrid of *E. urophylla*	Caetanópolis, MG	19°19′47.8″S/44°21′43.3″W	KX185528	KX185529	N. R. Fonseca
LPF 638	144	*E. grandis* X *E. urophylla*	Caetanópolis, MG	19°19′47.8″S/44°21′43.3″W	KX185528	KX185529	N. R. Fonseca
LPF 639	2034	(*E. camaldulensis* X *E. grandis*) X *E. urophylla*	Caetanópolis, MG	19°19′47.8″S/44°21′43.3″W	KX185528	KX185529	N. R. Fonseca
LPF 640	224	Hybrid of *E. urophylla*	Inimutaba, MG	18°41′30.0″S/44°15′07.2″W	KX185528	KX185529	N. R. Fonseca
LPF 641	1528	*E. grandis* X *E. urophylla*	Inimutaba, MG	18°41′30.0″S/44°15′07.2″W	KX185528	KX185529	N. R. Fonseca
LPF 642	144	*E. grandis* X *E. urophylla*	Inimutaba, MG	18°41′30.0″S/44°15′07.2″W	KX185528	KX185529	N. R. Fonseca
LPF 643	144	*E. grandis* X *E. urophylla*	Três Marias, MG	18°14′59.0″S/45°10′54.0″W	KX185528	KX185529	N. R. Fonseca

Table 1 (continued)

Isolate ID	Clone/variety	Host	Origin	GPS coordinates	GenBank accession number		Collector/reference
					ITS	28S	
LPF 644	1528	*E. grandis* X *E. urophylla*	Três Marias, MG	18°14′59.0″S/45°10′54.0″W	KX185528	KX185529	N. R. Fonseca
LPF 645	42864	*Eucalyptus* sp.	Viçosa, MG	20°46′27.7″S/42°52′35.75″W	KX185528	KX185529	N. R. Fonseca
LPF 646	D18	*E. dunnii*	Viçosa, MG	20°46′27.7″S/42°52′35.75″W	KX185528	KX185529	N. R. Fonseca
LPF 647	BA2004	*Eucalyptus* sp.	Itabatã, BA	18°02′34.17″S/39°55′25.77″W	KX185528	KX185529	E. A. V. Zauza
LPF 648	E13	*E. grandis* X *E. urophylla*	Três Lagoas, MS	20°59′30.61″S/51°47′39.78″W	KX185528	KX185529	J. F da Silva
LPF 649	E17	*Eucalyptus* sp.	Três Lagoas, MS	20°59′30.61″S/51°47′39.78″W	KX185528	KX185529	J. F da Silva
LPF 650	CNB 005	*E. grandis* X *E. urophylla*	Belo Oriente, MG	19°18′48.59″S/42°23′30.09″W	KX185528	KX185529	A. G. B. Medeiros
LPF 651	CNB 010	*E. grandis* X *E. urophylla*	Belo Oriente, MG	19°18′48.59″S/42°23′30.09″W	KX185528	KX185529	A. G. B. Medeiros
LPF 652	CNB 011	*E. grandis* X *E. urophylla*	Belo Oriente, MG	19°18′48.59″S/42°23′30.09″W	KX185528	KX185529	A. G. B. Medeiros
LPF 653	G26	*Eucalyptus* sp.	Viçosa, MG	20°46′27.7″S/42°52′35.75″W	KX185528	KX185529	N. R. Fonseca
LPF 654	57	*Eucalyptus* sp.	Viçosa, MG	20°46′27.7″S/42°52′35.75″W	KX185528	KX185529	N. R. Fonseca
LPF 655	D25	*E. dunnii*	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	KX185528	KX185529	N. Borges Junior
LPF 656	D18	*E. dunnii*	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	KX185528	KX185529	N. Borges Junior
LPF 657	BE 314	*E. benthamii*	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	–	KX185530	N. Borges Junior
LPF 658	37036	*Eucalyptus* sp.	Guaíba, RS	30°07′54.61″S/51°19′07.56″W	KX185528	KX185529	N. Borges Junior
LPF 659	Nórdia	*Rosa* sp. var. 'Nórdia'	Viçosa, MG	20°45′24.85″S/42°50′37.83″W	KX355453	KX355456	N. R. Fonseca
LPF 660	Greta	*Rosa* sp. var. 'Greta'	Viçosa, MG	20°45′24.85″S/42°50′37.83″W	KX355454	KX355456	N. R. Fonseca
LPF 661	Grand Gala	*Rosa* sp. var. 'Grand Gala'	Viçosa, MG	20°45′24.85″S/42°50′37.83″W	KX355454	KX355456	N. R. Fonseca
LPF 662	Karola	*Rosa* sp. var. 'Karola'	Viçosa, MG	20°45′24.85″S/42°50′37.83″W	KX355454	KX355456	N. R. Fonseca
LPF 663	Not identified 1	*Rosa* sp.	Viçosa, MG	20°45′24.85″S/42°50′37.83″W	KX355453	KX355456	N. R. Fonseca
LPF 664	Not identified 2	*Rosa* sp.	Teixeiras, MG	20°38′47.20″S/42°50′50.64″W	KX355454	KX355456	P. S. Hermenegildo
P. negeri	–	*Escalloniaceae - Escallonia rubra*	Argentina	–	AB525919	AB525919	Takamatsu et al. 2010
P. negeri	–	*Escalloniaceae - Escallonia rubra*	Argentina	–	AB525920	AB525920	Takamatsu et al. 2010
P. fugax	–	*Geraniaceae - Geranium thunbergii*	Japan	–	AB525922	AB525922	Takamatsu et al. 2010
P. fugax	–	*Geraniaceae - Geranium nepalense*	–	–	AB026134	–	Takamatsu et al. 2000
P. lini	–	*Linaceae - Linum usitatissimum*	Switzerland	–	AB525925	AB525925	Takamatsu et al. 2010
P. clandestina	–	*Rosaceae - Amelanchier laevis*	Germany	–	AB525927	AB525927	Takamatsu et al. 2010
P. clandestina	–	*Rosaceae - Crataegus oxyacantha*	Argentina	–	AB525931	AB525931	Takamatsu et al. 2010
P. clandestina	–	*Rosaceae - Crataegus* sp.	Argentina	–	AB525932	AB525932	Takamatsu et al. 2010
P. pannosa	–	*Rosaceae - Rosa rubiginosa*	Argentina	–	AB525937	AB525937	Takamatsu et al. 2010
P. pannosa	–	*Rosaceae - Rosa* sp.	–	–	AB022348	AB022347	Mori et al. 2000
P. pannosa	–	*Rosaceae - Rosa* sp.	Mexico	–	KM001666	–	Felix-Gastelum et al. 2014
P. pannosa	–	*Rosaceae - Rosa* sp.	Mexico	–	KM001669	–	Felix-Gastelum et al. 2014

Table 1 (continued)

Isolate ID	Clone/variety	Host	Origin	GPS coordinates	GenBank accession number		Collector/reference
					ITS	28S	
P. pannosa	–	*Rosaceae - Catharanthus roseus*	USA	–	KF703448	–	Romberg et al. 2014
P. pannosa	–	*Rosaceae - Prunus cerasus*	France	–	JN654341	–	Hubert et al. 2012
P. pannosa	–	*Rosaceae - Rosa* sp.	France	–	DQ139421	–	Leus et al. 2006
P. pannosa	–	*Rosaceae - Rosa* sp.	Germany	–	DQ139425	–	Linde and Debener 2003
P. pannosa	–	*Rosaceae - Rosa* sp.	Germany	–	DQ139427	–	Leus et al. 2006
P. pannosa	–	*Rosaceae - Prunus* sp.	Belgium	–	DQ139429	–	Leus et al. 2006
P. pannosa	–	*Myrtaceae - Eucalyptus* sp.	Australia	–	AF298543	–	Cunnington et al. 2003
P. pannosa	–	*Rosaceae - Rosa maltiflora*	Japan	–	AB525939	–	Takamatsu et al. 2010
P. spiraeae	–	*Rosaceae - Filipnedula purpurea* var. *purpurea*	–	–	AB022385	AB022384	Mori et al. 2000
P. xanthii	–	*Asteraceae - Calendula officinalis*	Argentina	–	AB525914	AB525914	Takamatsu et al. 2010
P. xanthii	–	*Asteraceae - Helianthus annuus*	Japan	–	AB040311	AB462774	Ito and Takamatsu 2010
P. xanthii	–	*Asteraceae - Lactuca raddeana* var. *elata*	Japan	–	AB040352	AB462776	Ito and Takamatsu 2010
P. xanthii	–	*Verbenaceae - Verbena hybrid*	Japan	–	AB040347	AB462780	Ito and Takamatsu 2010
P. xanthii	–	*Lamiaceae - Lycopus lucidus*	Japan	–	AB040343	AB462778	Ito and Takamatsu 2010
P. astericola	–	*Asteraceae - Zinnia elegans*	Japan	–	AB040335	AB462779	Ito and Takamatsu 2010
P. balsaminae	–	*Balsaminaceae - Impatiens balsamina*	Japan	–	AB462803	AB462788	Ito and Takamatsu 2010
P. balsaminae	–	*Balsaminaceae - Impatiens noli-tangere*	Japan	–	AB462805	AB462789	Ito and Takamatsu 2010
P. aphanis	–	*Rosaceae - Agrimonia pilosa*	–	–	AB000938	–	Takamatsu et al. 1998
P. aphanis	–	*Rosaceae - Agrimonia pilosa* var. *japonica*	–	–	AB026141	–	Takamatsu et al. 2000
P. aphanis	–	*Myrtaceae - Eucalyptus* sp.	Australia	–	AF073355	–	Cunnington et al. 2003
P. aphanis	–	*Rosaceae - Fragaria chiloensis*	Argentina	–	AB525933	–	Takamatsu et al. 2010
P. tridactyla	–	*Rosaceae - Prunus laurocerasus*	Switzerland	–	AY833654	–	Cunnington et al. 2005
P. tridactyla	–	*Rosaceae - Prunus persica*	Australia	–	AY833653	–	Cunnington et al. 2005
P. tridactyla	–	*Rosaceae - Prunus japonica*	–	–	–	AB022393	Mori et al. 2000
P. tridactyla	–	*Rosaceae - Prunus* sp.	–	–	AB000943	–	Takamatsu et al. 2000
P. longiseta	–	*Rosaceae - Prunus grayana*	–	–	AB000945	AB022423	Takamatsu et al. 2000
C. wrightii	–	*Fagaceae - Quercus glauca*	–	–	AB000932	AB022355	Takamatsu et al. 2000
C. lanestris	–	*Fagaceae - Quercus agrifolia*	–	–	AB000933	AB022353	Takamatsu et al. 2000

Rad) in a 1.5-mL microcentrifuge tube and incubated at 56 °C for 2 h. After mixing vigorously, the extract was incubated in boiling water for 8 min. The extract was mixed again and centrifuged at 15,000 *g* for 5 min. The supernatant was transferred to another tube and used as DNA template.

PCR amplification

The nuclear rDNA region including the ITS regions were amplified by nested-PCR using primers ITS5 (5′-GAA GTA AAA GTC GTA ACA AGG-3′) (White et al. 1990) and P3 (5′-GCC GCT TCA CTC GCC GTT AC-3′) (Kusaba and Tsuge 1995) for the first amplification. The first PCR product was used as a template for the second PCR using primers ITS5 and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al. 1990). For PCR amplification of the 28S rDNA gene, primers PM3 (5′-GKG CTY TMC GCG TAG T-3′) (Takamatsu and Kano 2001) and TW14 (5′-GCT ATC CTG AGG GAA ACT TC-3′) (Mori et al. 2000) were used. The reaction was performed in a final volume of 25 μL. The amplification program consisted of an initial step of denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. The PCR products were analyzed by electrophoresis in 1.4% (w/v) agarose gels, stained with ethidium bromide (1.0 μg/mL), and photodocumented using UV light.

DNA sequencing and data analysis

Both amplicon strands were sequenced using ITS5/ITS4 primers for ITS rDNA sequencing, and primers PM3/TW14 for 28S rDNA sequencing. PCR-amplified regions were sequenced using an ABI PRISM 3100 sequencer (Applied Biosystems). Sequences were edited and used in similarity searches in GenBank database using BLASTn (Altschul et al. 1990) at NCBI (http://www.ncbi.nlm.nih.gov) to confirm them as Erysiphales. The sequences were aligned with homologous sequences available in GenBank. A total of 37 ITS sequences of *Podosphaera* spp. were aligned and used for analyses. For 28S rDNA-sequence alignment, 20 sequences of *Podosphaera* spp. were used, which are available in GenBank (Table 1). Sequences of *Cystotheca wrightii* Berk. & M.A. Curtis and *Cystotheca lanestris* (Harkn.) Miyabe were used as outgroups, based on Mori et al. (2000). Analyses were performed with the MUSCLE algorithm (Edgar 2004) implemented in MEGA 4 (Tamura et al. 2007), followed by manual adjustments. The Bayesian inference method was used to construct phylogenetic trees using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). The substitution model was chosen based on the Akaike Information Criterion (AIC). The posterior probability in the distribution of the trees was calculated using the MCMC algorithm (Metropolis-coupled Markov chain Monte Carlo), with two chains from a random tree and 1×10^6 generations executed, discarding the first 25% of the trees. Phylogenetic trees were viewed and edited with FigTree v. 1.3.1. (http://tree.bio.ed.ac.uk/software/). Ambiguous bases were coded using IUPAC (International Union of Pure and Applied Chemistry) codes.

Morphological study

Eucalypt powdery mildew pathogen isolate LPF 615 was selected for morphological studies. In addition, rose powdery mildew pathogen isolates LPF 659 and LPF 660 were also analyzed. Mycelia, conidiophores, and conidia were scraped from the infected surface of a fresh leaf and placed into a drop of lactic acid on a glass slide for light microscopy. Alternatively, mycelia, conidiophores and conidia were stripped off the leaf surfaces with clear adhesive tape, mounted on a microscope slide and examined in water using a light microscope at 40x magnification. Germination tests were performed by lightly tapping an infected leaf upon a glass slide. The slide was placed in a plastic germination box (germbox) containing a wet paper towel, and the box was closed to constitute a moist chamber. The slide was maintained in the germbox for 24 h. After that period, the slide was examined in water using light microscope at 40x magnification. Morphological characters, including size and shape of conidia, presence or absence of fibrosin bodies in fresh materials, characteristics of the conidiophore, nature of conidiogenesis, hyphal morphology, position of conidial germ tubes, and shape of germ tube-derived appressoria were observed and recorded.

Cross inoculations

To evaluate the capability of eucalypt powdery mildew pathogens to infect rose plants, 10 healthy rose cuttings (*Rosa* sp. var. 'Ambiance') were kept in a growth chamber at 19 ± 2 °C with a 12-h photoperiod and light intensity of 40 μmol/s/m^2, which was free of powdery mildew inoculum. Cuttings were monitored for possible latent infections for a period of 10 days. Eucalypt powdery mildew pathogen isolate LPF 615, collected in Viçosa, MG, and maintained on eucalypt cuttings in a growth chamber free of other inoculum sources, was used for inoculation. Inoculations were performed with a small soft brush by dusting conidia from an infected eucalypt leaf onto young leaves of rose. The inoculated rose plants were then watered, covered separately with a plastic bag, and closed on the bottom with a rubber band to constitute a moist chamber. The inoculated plants were covered for 24 h. After that period, they were uncovered and placed interspersed among five *Eucalyptus* plants with powdery mildew. Rose plants were evaluated daily for powdery mildew. The same procedure was used to test whether eucalypt cuttings could be infected by powdery mildew pathogens from rose. Ten healthy *E. urophylla* cuttings were kept in a growth chamber at 19 °C

with a 12-h photoperiod and light intensity of 40 μmol/s/m^2, free of powdery mildew inoculum. Rose plants with powdery mildew were used for inoculation as described previously. Eucalypt plants were evaluated daily for powdery mildew signs and symptoms. To reconfirm the identity of the pathogen on inoculated eucalypt and rose plants, ITS rDNA sequencing was performed using ITS5/ITS4 primers and both amplicon strands were sequenced.

Results

DNA extraction, PCR amplification and DNA sequencing

Of 82 samples of powdery mildew pathogens collected from eucalypt plants, 49 samples yielded sufficient DNA for PCR, which resulted in 42 sequences of ITS rDNA and 49 sequences of 28S rDNA. The sequences were 417 bases in length for ITS rDNA and 768 bases for 28S rDNA. All 42 ITS sequences were identical without insertions, deletions, or substitutions. For this reason, only one sequence was deposited in GenBank (accession number KX185528), and the same number was assigned to ITS sequences of all eucalypt powdery mildew pathogen isolates. Among the ITS sequences of rose powdery mildew pathogens, the isolates LPF 659 and LPF 663 differed from other rose isolates by one base, forming a subclade within the *P. pannosa* clade. The rose isolates LPF 660, LPF 661, LPF 662, and LPF 664 were identical to ITS sequences of the eucalypt powdery mildew pathogen isolates. For the 28S rDNA sequence analysis, the partial sequence of the 28S rDNA gene including the D1/D2 region was determined. The 28S rDNA sequence alignment showed that sequences of eucalypt isolates LPF622 and LPF657 differed from other isolates by two and one base(s), respectively. ITS sequencing of isolate LPF657 was unsuccessful. The 28S rDNA sequences of rose isolates did not differ and were identical to the 28S sequences of eucalypt isolates. The 28S rDNA sequences were also deposited in GenBank (Table 1).

Searches on BLASTn revealed that all sequences were very similar to species within the genus *Podosphaera* for both ITS and 28S rDNA regions. The best evolutionary model selected by MrModeltest 2.3 was the model GTR + I + G, with parameters I = 0.5848 and G = 1.3165. The general time reversible (GTR) model takes into account the rate of substitution for each pair of nucleotides and considers the frequencies of the four nucleotides. According to the ITS-based phylogenetic tree, all sequences generated in this study were comprised within a single clade that contains *Podosphaera pannosa*, supported by 88% posterior probability (Fig. 2).

For 28S rDNA sequence data, the best evolutionary model was GTR + I, with I = 0.8710. The resulting 28S rDNA-based phylogenetic tree did not result in a well-defined separation among species of *Podosphaera* (Fig. 3), in contrast to the ITS-based tree. The 49 sequences of eucalypt powdery mildew pathogens and six rose powdery mildew pathogens generated in this study were contained within a polytomy comprising *P. pannosa*, *P. clandestina*, *P. fugax*, *P. lini*, *P. spiraeae*, and *P. negeri* in a clade supported by 100% posterior probability. An additional subclade comprising other *Podosphaera* species is also evident.

Morphological study

The isolates studied presented morphological characters typical of *Podosphaera*, having conidia formed in chains and presence of fibrosin bodies. Hyphae were septate, branched, and hyaline with nipple-shaped appressoria; conidiophores were epiphytic, septate, hyaline; foot-cells were cylindrical, 42 − 68 × 7.3 − 9.5 μm; conidia were produced in chains at the apex of the conidiophores, ellipsoid-ovoid to doliiform, 21.6 − 32.5 × 10.9 − 19 μm, aseptate, hyaline with germ tubes terminal to lateral *Fibroidium* type, orthotubus subtype (Fig. 4). Although *P. aphanis* [previously included in *Sphaerotheca* (*Podosphaera*) *macularis* s. lat.] has been reported on *Eucalyptus* (Braun and Cook 2012), this species differs in having much longer conidiophore foot-cells (Braun 1987), which were not observed on infected eucalypt and during morphological analyses.

Pathogen identification

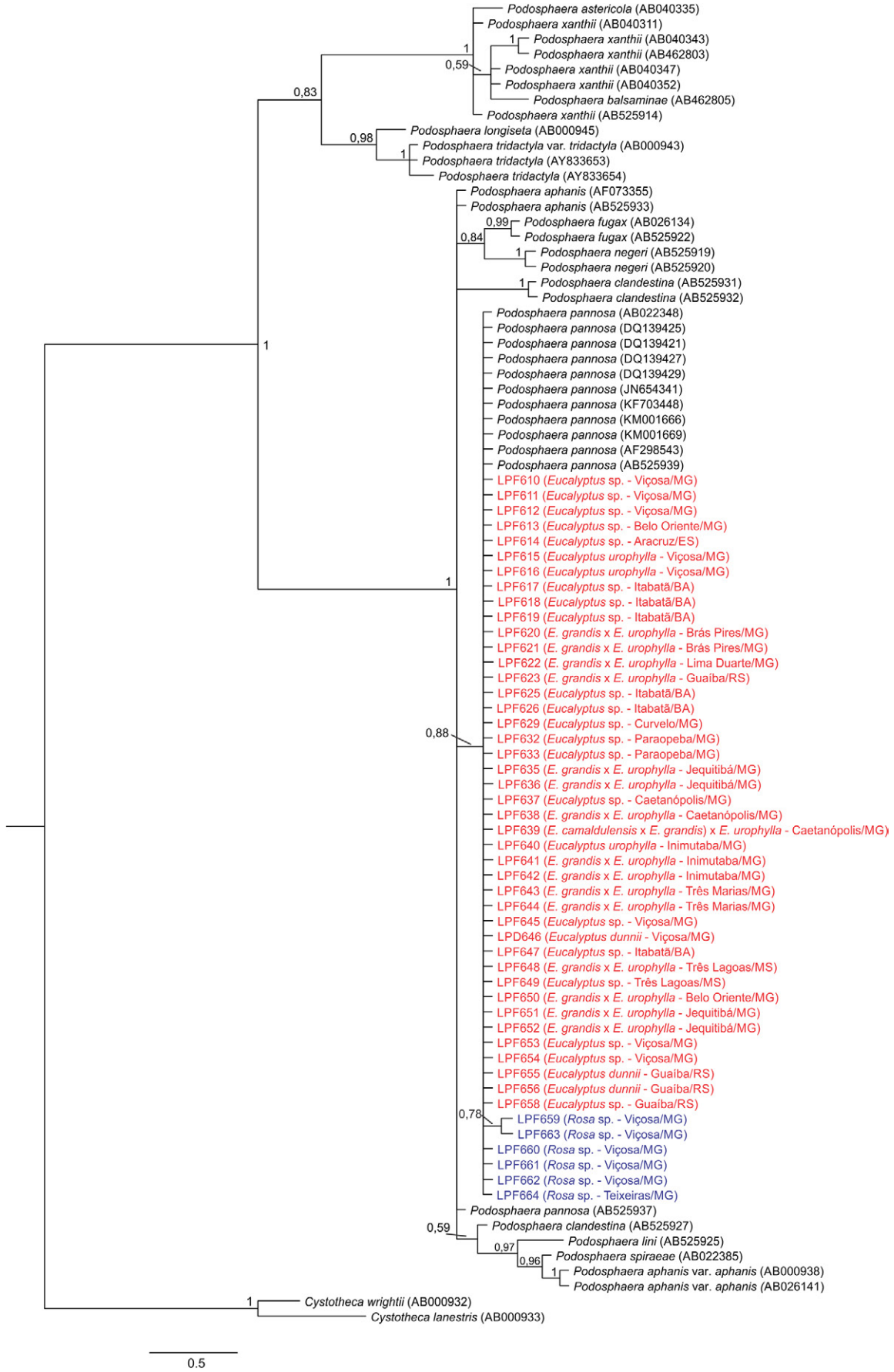
Based on comparisons of DNA sequences and morphological analyses, it is concluded that all eucalypt powdery mildew pathogen isolates collected from different regions of Brazil belong to the same species, *Podosphaera pannosa.*

Cross inoculations

After rose plants were inoculated with eucalypt powdery mildew pathogen isolate LPF 615, mycelial signs of powdery mildew were first observed at 10 days post-inoculation (Fig. 4). Thus, the eucalypt powdery mildew pathogen is capable of infecting roses. For eucalypt inoculation with rose powdery mildew pathogens, the first signs of powdery mildew were observed at 7 days post-inoculation (Fig. 4). Thus, the rose powdery mildew pathogen is capable of infecting eucalypt. ITS sequencing reconfirmed the identity of powdery mildew pathogen isolates collected from eucalypt and rose plants. Generated sequences were aligned with previous ITS sequences of this study and resulted in 100% identity amongst them.

Discussion

Based on comparisons of DNA sequences and anamorph morphology, all isolates of eucalypt powdery mildew pathogen


Podosphaera astericola (AB040335)
Podosphaera xanthii (AB040311)
Podosphaera xanthii (AB040343)
Podosphaera xanthii (AB462803)
Podosphaera xanthii (AB040347)
Podosphaera xanthii (AB040352)
Podosphaera balsaminae (AB462805)
Podosphaera xanthii (AB525914)
Podosphaera longiseta (AB000945)
Podosphaera tridactyla var. tridactyla (AB000943)
Podosphaera tridactyla (AY833653)
Podosphaera tridactyla (AY833654)
Podosphaera aphanis (AF073355)
Podosphaera aphanis (AB525933)
Podosphaera fugax (AB026134)
Podosphaera fugax (AB525922)
Podosphaera negeri (AB525919)
Podosphaera negeri (AB525920)
Podosphaera clandestina (AB525931)
Podosphaera clandestina (AB525932)
Podosphaera pannosa (AB022348)
Podosphaera pannosa (DQ139425)
Podosphaera pannosa (DQ139421)
Podosphaera pannosa (DQ139427)
Podosphaera pannosa (DQ139429)
Podosphaera pannosa (JN654341)
Podosphaera pannosa (KF703448)
Podosphaera pannosa (KM001666)
Podosphaera pannosa (KM001669)
Podosphaera pannosa (AF298543)
Podosphaera pannosa (AB525939)
LPF610 (Eucalyptus sp. - Viçosa/MG)
LPF611 (Eucalyptus sp. - Viçosa/MG)
LPF612 (Eucalyptus sp. - Viçosa/MG)
LPF613 (Eucalyptus sp. - Belo Oriente/MG)
LPF614 (Eucalyptus sp. - Aracruz/ES)
LPF615 (Eucalyptus urophylla - Viçosa/MG)
LPF616 (Eucalyptus urophylla - Viçosa/MG)
LPF617 (Eucalyptus sp. - Itabatã/BA)
LPF618 (Eucalyptus sp. - Itabatã/BA)
LPF619 (Eucalyptus sp. - Itabatã/BA)
LPF620 (E. grandis x E. urophylla - Brás Pires/MG)
LPF621 (E. grandis x E. urophylla - Brás Pires/MG)
LPF622 (E. grandis x E. urophylla - Lima Duarte/MG)
LPF623 (E. grandis x E. urophylla - Guaíba/RS)
LPF625 (Eucalyptus sp. - Itabatã/BA)
LPF626 (Eucalyptus sp. - Itabatã/BA)
LPF629 (Eucalyptus sp. - Curvelo/MG)
LPF632 (Eucalyptus sp. - Paraopeba/MG)
LPF633 (Eucalyptus sp. - Paraopeba/MG)
LPF635 (E. grandis x E. urophylla - Jequitibá/MG)
LPF636 (E. grandis x E. urophylla - Jequitibá/MG)
LPF637 (Eucalyptus sp. - Caetanópolis/MG)
LPF638 (E. grandis x E. urophylla - Caetanópolis/MG)
LPF639 (E. camaldulensis x E. grandis) x E. urophylla - Caetanópolis/MG)
LPF640 (Eucalyptus urophylla - Inimutaba/MG)
LPF641 (E. grandis x E. urophylla - Inimutaba/MG)
LPF642 (E. grandis x E. urophylla - Inimutaba/MG)
LPF643 (E. grandis x E. urophylla - Três Marias/MG)
LPF644 (E. grandis x E. urophylla - Três Marias/MG)
LPF645 (Eucalyptus sp. - Viçosa/MG)
LPD646 (Eucalyptus dunnii - Viçosa/MG)
LPF647 (Eucalyptus sp. - Itabatã/BA)
LPF648 (E. grandis x E. urophylla - Três Lagoas/MS)
LPF649 (Eucalyptus sp. - Três Lagoas/MS)
LPF650 (E. grandis x E. urophylla - Belo Oriente/MG)
LPF651 (E. grandis x E. urophylla - Jequitibá/MG)
LPF652 (E. grandis x E. urophylla - Jequitibá/MG)
LPF653 (Eucalyptus sp. - Viçosa/MG)
LPF654 (Eucalyptus sp. - Viçosa/MG)
LPF655 (Eucalyptus dunnii - Guaíba/RS)
LPF656 (Eucalyptus dunnii - Guaíba/RS)
LPF658 (Eucalyptus sp. - Guaíba/RS)
LPF659 (Rosa sp. - Viçosa/MG)
LPF663 (Rosa sp. - Viçosa/MG)
LPF660 (Rosa sp. - Viçosa/MG)
LPF661 (Rosa sp. - Viçosa/MG)
LPF662 (Rosa sp. - Viçosa/MG)
LPF664 (Rosa sp. - Teixeiras/MG)
Podosphaera pannosa (AB525937)
Podosphaera clandestina (AB525927)
Podosphaera lini (AB525925)
Podosphaera spiraeae (AB022385)
Podosphaera aphanis var. aphanis (AB000938)
Podosphaera aphanis var. aphanis (AB026141)
Cystotheca wrightii (AB000932)
Cystotheca lanestris (AB000933)
0,83
0,98
0,59
0,99
0,84
0,88
0,78
0,97
0,96
0.5

◀ **Fig. 2** ITS sequence-based Bayesian phylogenetic analysis of powdery mildew pathogens of *Eucalyptus* spp. (*red*) and *Rosa* sp. (*blue*). Posterior probability support percentages are indicated at the branch nodes

collected from different regions of Brazil in this study were identified as *P. pannosa.* These results confirm the hypothesis raised by Silva et al. (2001), who previously suggested that the eucalypt powdery mildew pathogen in Brazil was similar to the rose powdery mildew pathogen, *P. pannosa*, on the basis of morphological analyses of the anamorph and cross-inoculation studies. The results generated in this study also corroborate the results of Cho et al. (2016), who reported *P. pannosa* as the causal agent of powdery mildew on *Corymbia citriodora* in Korea. This is a significant finding because it now confirms *P. pannosa* as the causal agent of a very important disease on eucalypt in Brazil. It cannot be excluded that *O. eucalypti* represents a younger heterotypic synonym of *P. pannosa*, but this question remains open and unresolved due to the lack of type material, not allowing a re-examination and reassessment of this name, above all since

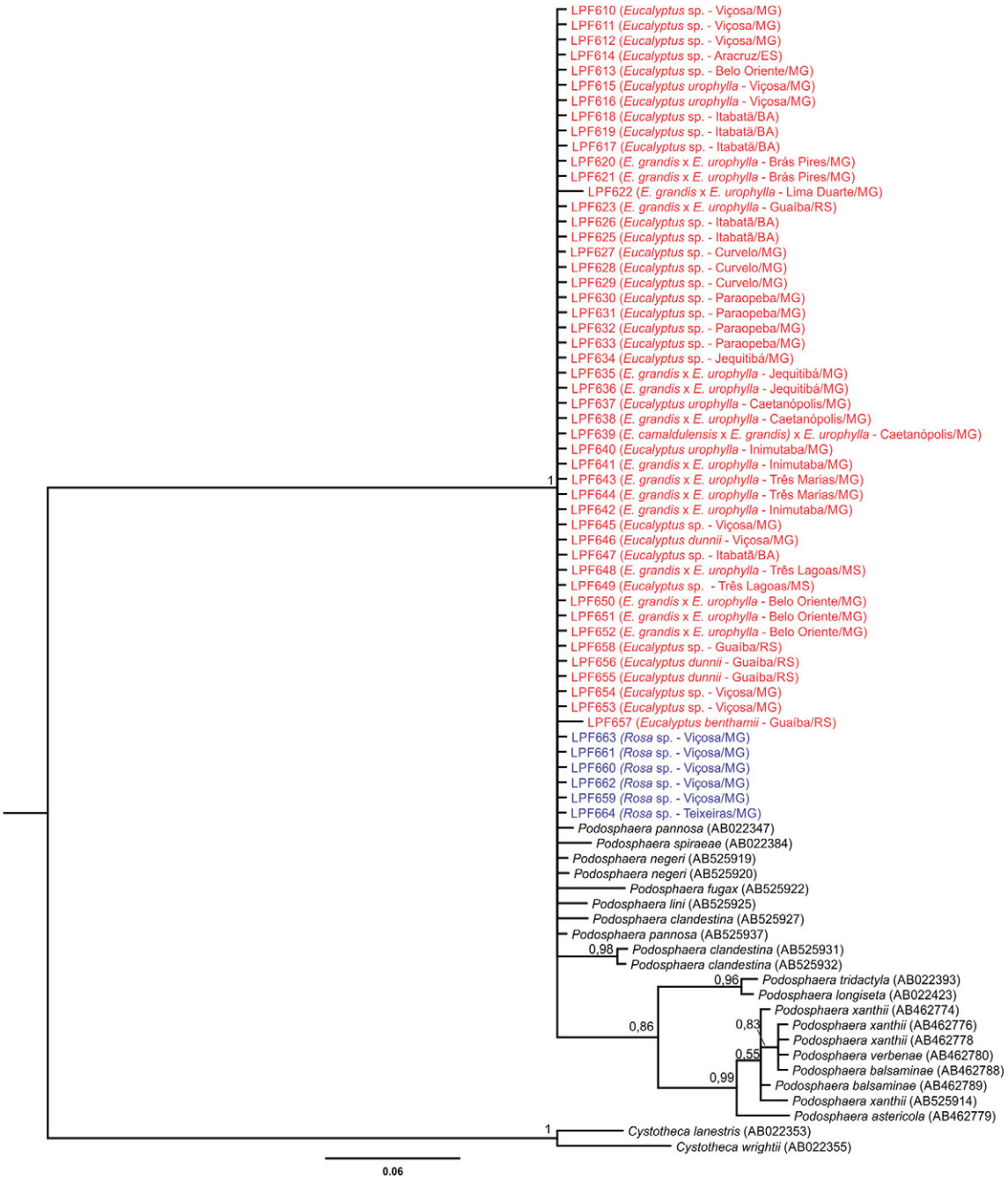


Fig. 3 28S rDNA sequence-based Bayesian phylogenetic analysis of powdery mildew pathogens of *Eucalyptus* spp. (*red*) and *Rosa* sp. (*blue*). Posterior probability support percentages are indicated at the branch nodes

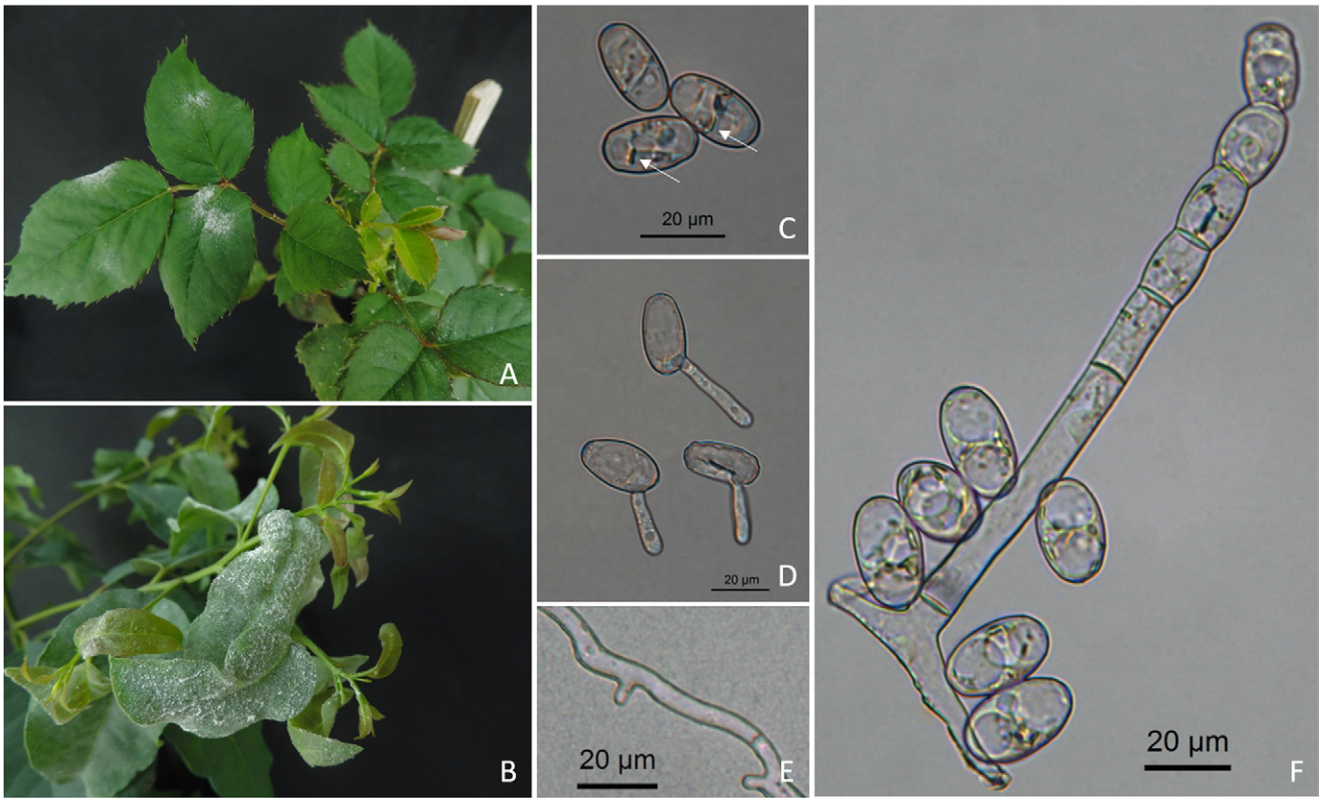


Fig. 4 Infected leaves exhibiting powdery mildew signs of *Podosphaera pannosa* on (**a**) rose (*Rosa* sp.) and (**b**) eucalypt (*Eucalyptus urophylla*) after cross inoculation. (**c**) Conidia with fibrosin bodies (*arrows*). (**d**) Germinating conidia. (**e**) hyphal appressorium. (**f**) conidiophore and conidia

two powdery mildew species with catenescent conidia are known, *Podosphaera pannosa* and the plurivorous *Golovinomyces orontii*. In addition, we determined that all isolates collected in this study belong to a single species with identical ITS sequences among the isolates. Because the identity of eucalypt powdery mildew pathogen species in Brazil has now been confirmed, disease management activities can focus on the appropriate causal pathogen.

Until the 1990s, taxonomy within Erysiphales was fundamentally based on fungal morphology and biology, with a focus on the sexual structures. In many tropical regions where chasmothecia (= cleistothecia) are rarely formed, studies on disease etiology were often compromised (Bedendo 2011). With the introduction of a new generic concept by Braun et al. (2002), which includes morphological, biological, molecular information of the organism, and the use of phylogenetic analysis based on ITS and 28S rDNA sequences, it is now possible to connect a majority of anamorphic species with their teleomorphic species, even when only the anamorph is found (Cunnington et al. 2003). This study further corroborates the utility of these tools to better classify species within Erysiphales.

The ITS sequences provided a more robust phylogenetic tree than the 28S rDNA sequences, reflecting the phylogeny on generic and higher levels. Although some authors discourage the use of the ITS region for phylogenetic analyses in fungi (e.g., Harrington et al. 2014), the ITS and 28S rDNA regions were selected in our study because they are the most employed and well elucidated genetic regions for Erysiphales (Glawe 2008; Braun and Cook 2012), while also providing separation among taxa. In a preliminary study, beta-tubulin and translation elongation factor primers were tested to assess diversity among isolates, but amplifications were unsuccessful (*data not shown*). Perhaps low-quantity DNA contributed to reduced amplification, or perhaps more precise sequence information for these genetic regions is needed to develop better primers. In addition, the lack of available information about these genetic regions in species of Erysiphales limits reliable classification based on comparisons of these sequences.

Podosphaera pannosa is a cosmopolitan species occurring on several species of different families, such as *Rosa* spp. and *Prunus* spp. (Rosaceae), *Cotinus coggygria* (Anacardiaceae), *Forsythia* spp. (Oleaceae), *Eucalyptus* spp. and *Corymbia citriodora* (Myrtaceae) (Braun and Cook 2012; Cho et al. 2016). Studies revealed a close evolutionary relationship between *Podosphaera* spp. and Rosaceae, suggesting the Rosaceae may have been the first host for *Podosphaera*, and host jumps from the Rosaceae to other plant families may have occurred spontaneously during the evolution of *Podosphaera* (Takamatsu et al. 2010). Our cross-inoculation

studies support the hypothesis that eucalypt powdery mildew may be the result of a host jump from *Rosa* sp.. However, more focused population genetic studies are needed to confirm this hypothesis.

The identical ITS sequences of eucalypt powdery mildew pathogen isolates obtained in our study could indicate a clonal population structure attributable to the lack of sexual reproduction in tropical regions, perhaps due to the absence of cold environmental conditions or maybe due to the presence of only one mating-type in the population. This powdery mildew pathogen was reported earlier from Brazil (Grillo 1936). Thus, it can be surmised that this pathogen has been in Brazil for several decades. Because sexual reproduction is lacking, several decades is perhaps too short of a time period to allow the evolution of significant genetic differences, which could also explain the low sequence diversity found in powdery mildew pathogen isolates from different regions of Brazil.

Improvement of disease management practices, such as resistance breeding/screening programs and cultural practices, depend on a precise understanding of the pathogens that cause disease. The identification of *P. pannosa* as the causal agent of eucalypt powdery mildew allows us to transfer management techniques used for other hosts, such as roses and *Prunus* spp., and test them with eucalypt. Recently, studies using eco-friendly disease control measures have been effective for rose powdery mildew. A silicon treatment reduced powdery mildew development by inducing host defense responses (Shetty et al. 2012), and ultraviolet irradiance exposure suppressed powdery mildew via reduction of spore germination, disease severity, and sporulation of surviving colonies (Suthaparan et al. 2012). In addition, correct pathogen identification can enhance chemical control methods, because different species may respond differently to various fungicides with specific modes of action.

This is the first unequivocal report of *P. pannosa* on *Eucalyptus* spp. in Brazil, based on detailed analyses of morphology, DNA sequence data, and pathogenicity tests. The identification of *P. pannosa* as the cause of eucalypt powdery mildew provides a baseline for continued studies to better understand this pathosystem for which information is largely lacking.

Acknowledgements The authors thank CNPq, CAPES, and FAPEMIG for financial support. The authors also are very grateful to the eucalypt nurseries that provided materials for this study.

References

Alfenas AC, Zauza EAV, Mafia RG, Assis TF (2009) Clonagem e Doenças do Eucalipto. Editora UFV, Viçosa

Altschul SF, Gish W, Miller W, Myers E, Lipmann DJ (1990) Basic local alignment search tool. J Mol Biol 219:403

Bedendo IP (2011) Oídios. In: Bergamin Filho A, Kimati H, Amorim L (eds) Manual de Fitopatologia, vol 1, 4th ed. Ceres, São Paulo, pp 866–871

Boesewinkel HJ (1979) Erysiphaceae of New Zealand. Sydowia 32:13–56

Boesewinkel HJ (1981) A first recording of rose mildew, *Sphaerotheca pannosa*, on three species of *Eucalyptus*. Nova Hedwigia 34:721–730

Brandenburger W (1961) Beobachtungen über das Auftreten einer Sphaerotheca-Art an *Eucalyptus*. Sydowia 15:194–196

Braun U (1987) A monograph of the *Erysiphales* (powdery mildews). Beihefte zur. Nova Hedwigia 89

Braun U, Cook RTA (2012) Taxonomic manual of the *Erysiphales* (powdery mildews). CBS biodiversity series 11. CBS, Utrecht

Braun U, Cook RTA, Inman AJ, Shin HD (2002) The taxonomy of the powdery mildew fungi. In: Belanger R, Dik AJ, Bushnell WR (eds) The powdery mildews: a comprehensive treatise. APS Press, St. Paul, pp 13–54

Cho E, Park JH, Lee SH, Lee CK, Shin HD (2016) Occurrence of powdery mildew caused by *Podosphaera pannosa* on lemon eucalyptus. For Pathol 46:264–266

Crous PW, Knox-Davies PS, Wingfield MJ (1989) A summary of fungal leaf pathogens of *Eucalyptus* and the diseases they cause in South Africa. South Afr For J 149:9–16

Cunnington JH, Takamatsu S, Lawrie AC, Pascoe I (2003) Molecular identification of anamorphic powdery mildews (*Erysiphales*). Australas Plant Pathol 32:421–428

Cunnington JH, Lawrie AC, Pascoe IG (2005) Genetic variation within *Podosphaera tridactyla* reveals a paraphyletic species complex with biological specialization towards specific *Prunus* subgenera. Mycol Res 109:357–362

Delhey R, Braun U, Kiehr M (2003) Some new records of powdery mildew fungi from Argentina (2). Schlechtendalia 10:79–90

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

Felix-Gastelum R, Herrera-Rodriguez G, Martinez-Valenzuela C, Maldonado-Mendoza IE, Quiroz-Figueroa FR, Brito-Vega H, Espinosa-Matias S (2014) First report of powdery mildew (*Podosphaera pannosa*) of roses in Sinaloa, Mexico. Plant Dis 98: 442

Ferreira FA (1997) A cultura do eucalipto II. Enfermidades do eucalipto no Brasil. Doenças em viveiros de eucalipto. Inf Agrop 18:5–19

Gardner MW, Yarwood CE (1974) A list of powdery mildews of California. University of California Agriculture Experimental Station Extension Service leaflet. UCAES, Sacramento

Gibson IAS (1975) Diseases of forest trees widely planted as exotics in the tropics and southern hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, Verbenaceae and Meliaceae. Commonw Mycol Inst: Kew, UK and Commonw Forest Inst: Oxford, UK

Glasscock HH, Rosser WR (1958) Powdery mildew on *Eucalyptus*. Plant Pathol 7:152

Glawe DA (2008) The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. Annu Rev Phytopathol 46:27–51

Grasso V (1948) L'oidio dell'eucalipto [Oidium of Eucalyptus]. Nuovo G Bot Ital 55:581–584

Grillo HVS (1936) Lista preliminar dos fungos assinalados em plantas do Brasil. Rodriguesia 2:39–96

Harrington TC, Kazmi MR, Al-Sadi AM, Ismail SI (2014) Intraspecific and intragenomic variability of ITS rDNA sequences reveals taxonomic problems in *Ceratocystis fimbriata* sensu stricto. Mycologia 106:224–242

Hirata T, Takamatsu S (1996) Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37:283–288

Hubert J, Fourrier C, Payen C, Fournie JL, Ioos R (2012) First report of powdery mildew caused by *Podosphaera pannosa* on *Prunus cerasus* in France. Plant Dis 96:1375

Ito M, Takamatsu S (2010) Molecular phylogeny and evolution of subsection *Magnicellulatae* (Erysiphaceae: *Podosphaera*) with special reference to host plants. Mycoscience 51:34–43

Keane PJ, Kile GA, Podger FD, Brown BN (eds) (2000) Diseases and pathogens of *Eucalyptus*. CSIRO Publishing, Collingwood

Kusaba M, Tsuge T (1995) Phylogeny of *Alternaria* fungi known to produce host-specific toxins on the basis of variation in internal transcribed spacers of ribosomal DNA. Curr Genet 28:491–498

Leus L, Dewitte A, Van Huylenbroeck J, Vanhoutte N, Van Bockstaele E, Höfte M (2006) *Podosphaera pannosa* (syn. *Sphaerotheca pannosa*) on *Rosa* and *Prunus* spp.: characterization of pathotypes by differential plant reactions and ITS sequences. J Phytopathol 154: 23–28

Limkaisang S, Cunnington JH, Liew KW, Salleh B, Sato Y, Divarangkoon R, Fangfuk W, To-Anun C, Takamatsu S (2006) Molecular phylogenetic analyses reveal a close relationship between powdery mildew fungi on some tropical trees and *Erysiphe alphitoides*, an oak powdery mildew. Mycoscience 47:327–335

Linde M, Debener TH (2003) Isolation and identification of eight races of powdery mildew of roses (*Podosphaera pannosa*) (Wallr.: Fr.) de Bary and the genetic analysis of the resistance gene *Rpp1*. Theor Appl Genet 107:256–262

Matheron ME, Matejka JC (1992) Powdery mildew caused by *Erysiphe cichoracearum* on five new *Eucalyptus* hosts in Arizona. Plant Dis 76:1077

Mori Y, Sato Y, Takamatsu S (2000) Evolutionary analysis of the powdery mildew fungi (*Erysiphales*) using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92:74–93

Mucci ESF, Pitta GPB, Yokomizo NKS (1980) O oídio em mudas de eucalipto. Bol Téc Inst Flor 34:27–38

Old KM, Wingfield MJ, Yuan ZQ (2003) A manual of diseases of eucalypts in South-East Asia. Center for International Forestry Research (CIFOR), Bogor

Romberg MK, Kennedy AH, Ko M (2014) First report of the powdery mildews *Leveillula taurica* and *Podosphaera pannosa* on rose periwinkle in the United States. Plant Dis 98:848

Ronquist F, Huelsenbeck JP (2003) MrBayes version 3.1.2: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574

Shetty R, Jensena B, Shettya NP, Hansena M, Hansenb CW, Starkeyb KR, Jorgensen HJL (2012) Silicon induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. Plant Pathol 61:120–131

Silva MDD, Alfenas AC, Maffia LA, Zauza EAV (2001) Etiologia do oídio do eucalipto. Fitopatol Bras 26:201–205

Silva MDD, Alfenas AC, Maffia LA, Zauza EAV (2003) Germinação de conídios de *Sphaerotheca pannosa* obtidos de eucalipto. Fitopatol Bras 28:674–677

Spaulding P (1961) Foreign diseases of forest trees of the world - an annotated list. USDA Agriculture Handbook 197. USDA, Washington, DC

Stone OM (1962) Alternate hosts of cucumber powdery mildew. Ann Appl Biol 50:203–210

Suthaparan A, Stensvand A, Solhaug KA, Torre S, Mortensen LM, Gadoury DM, Seem RC, Gislerod HR (2012) Suppression of powdery mildew (*Podosphaera pannosa*) in greenhouse roses by brief exposure to supplemental UV-B radiation. Plant Dis 96:1653–1660

Takamatsu S, Kano Y (2001) PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. Mycoscience 42:135–139

Takamatsu S, Hirata T, Sato Y (1998) Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (*Erysiphaceae*). Mycoscience 39:441–453

Takamatsu S, Hirata T, Sato Y (2000) A parasitic transition from trees to herbs occurred at least twice in tribe *Cystotheceae* (*Erysiphaceae*): evidence from nuclear ribosomal DNA. Mycol Res 104:1304–1311

Takamatsu S, Niinomi S, Harada M, Havrylenko M (2010) Molecular phylogenetic analyses reveal a close evolutionary relationship between *Podosphaera* (Erysiphales: Erysiphaceae) and its rosaceous hosts. Persoonia 24:38–48

Tamura K, Dudley J, Nei ME, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tanda S, Hirose T (2003) Powdery mildews and their causal fungi on some spice and medicinal plants. J Agric Sci 47:274–282

Taylor JW (2011) One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2:113–120

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: Innis MA, Gelfrand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous PW (2012) One fungus, one name promotes progressive plant pathology. Mol Plant Pathol 13:604–613