

Pilidiella tibouchinae sp. nov. associated with foliage blight of *Tibouchina granulosa* (quaresmeira) in Brazil

Bruno E.C. Miranda¹, Robert W. Barreto¹, Pedro W. Crous², and Johannes Z. Groenewald²

¹Universidade Federal de Viçosa, Departamento de Fitopatologia, 36570-000, Viçosa, MG, Brazil; corresponding author e-mail: Robert W. Barreto, rbarreto@ufv.br

²CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands

Abstract: *Tibouchina granulosa* (Melastomataceae), Brazilian glorytree (Brazilian common name – quaresmeira), a common tree of the Atlantic Forest of Brazil, is widely used as an ornamental for its violet or pink blossoms. Little is known about fungal diseases affecting this species, although these represent a known limitation for its cultivation in nurseries. Among these there is a foliage blight that occurs in combination with distortion of branch apices and die-back. A consistent association of a species of *Pilidiella* with the diseased tissues was observed. The fungus was isolated in pure culture and based on its morphology and DNA phylogeny, we conclude that it represents a new species, for which the name *Pilidiella tibouchinae* is introduced.

Key words:

Coniella
Diaporthales
 ITS
 LSU
Sordariomycetes
 systematics

Article info: Submitted: 9 January 2012; Accepted: 29 February 2012; Published: 5 April 2012.

INTRODUCTION

Tibouchina granulosa (Melastomataceae), the Brazilian glorytree, is a fast growing tree native that occurs in the Atlantic Forest of Brazil. It is a common and important component of the native flora, particularly in secondary forests. It is also a highly prized ornamental, which is widely used in gardens and parks because of its spectacular violet or pink blossoms that each year appear prior and during Easter. The period of lent (quaresma, in Portuguese) is an important period in the catholic tradition, from which the Brazilian common name for this tree, “quaresmeira” is derived (Lorenzi 2002). Usually there is a high demand for young *T. granulosa* plants in the garden nursery market in Brazil, particularly for the variety that produces pink flowers. Nevertheless, diseases are known to be a limiting factor in nursery production of *T. granulosa*. However, very little is known about the diseases affecting this tree. The record of *Chrysosporthe cubensis* (syn.: *Cryphonectria cubensis*) on *T. granulosa* in Brazil by Seixas *et al.* (2004) is the sole record of a fungal pathogen on this host in the Brazilian database (<http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fichahp.asp?id=1912>)—and there are only three records of fungi on this host in the USDA fungal database (Farr & Rossman 2010). This is somewhat surprising for such a common plant in the neotropics, and possibly reflects the limited existing knowledge about plant pathogenic fungi occurring on wild plants in this region. Here we clarify the identity of the fungus associated with a severe foliage blight (often evolving into a form of die-back) of quaresmeira. This is one of the most widespread

and damaging diseases affecting *T. granulosa* in the field, in gardens, and also in nurseries.

MATERIAL AND METHODS

Isolates

Samples of young abnormal branches of *Tibouchina granulosa* bearing diseased leaves were collected at two localities in Brazil (states of Rio de Janeiro and Minas Gerais), dried in a plant press and brought to the laboratory for further examination. Representative specimens of the fungus were deposited in the herbarium at the Universidade Federal de Viçosa (VIC). Pure cultures were obtained by transfer of conidia, using a sterile fine-pointed needle, from lesions onto plates containing VBA (vegetable broth-agar) as described in Pereira *et al.* (2003). Pure cultures are deposited in the fungal culture collection at the Universidade Federal de Viçosa and also at the CBS-KNAW Fungal Biodiversity Centre (CBS) in Utrecht, The Netherlands. Representative voucher specimens are deposited in VIC and at CBS.

DNA isolation, amplification and analyses

Genomic DNA was isolated from fungal mycelium grown on MEA, using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocols. The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA

© 2012 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).
Non-commercial: You may not use this work for commercial purposes.
No derivative works: You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

gene (SSU), the internal transcribed spacer 1, the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS) and the first 900 bases at the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al.* 1990) and LSU1Fd (Crous *et al.* 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment, and subsequent phylogenetic analysis followed the methods of Crous *et al.* (2006, 2009b). Additionally, partial translation elongation factor 1- α (TEF) sequences were determined as described by Bensch *et al.* (2010). Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search and alignments were constructed based on these results for ITS. For LSU, the novel sequence were added to an alignment modified from Lamprecht *et al.* 2011 (TreeBASE study S11805). Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous *et al.* 2004).

Morphology

Slides containing fungal structures were mounted in lactophenol or lactofuchsin, with 30 measurements determined per structure. Sections were prepared with the help of a freezing microtome (Microm HM 520). Observations of fungal structures and measurements, as well as preparation of photographs, were performed with an Olympus BX 51 light microscope fitted with an Olympus E330 camera. Colony characters and pigment production were noted after 6 d of growth on 2 % malt extract agar (MEA) and potato carrot agar (PCA) (Crous *et al.* 2009c) plates incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

RESULTS

Phylogeny

Approximately 1700 bases, spanning the ITS and LSU regions, were obtained from the sequenced culture.

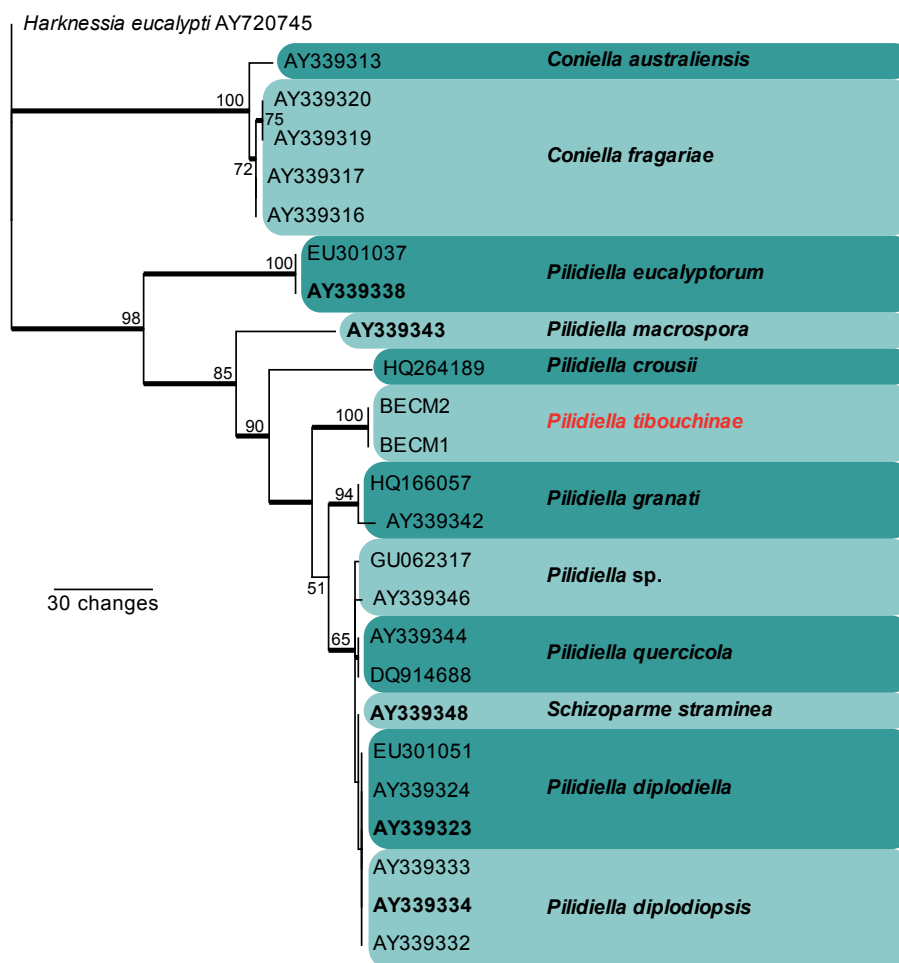


Fig. 1. The first of 24 equally most parsimonious trees obtained from a heuristic search with 50 random taxon additions of the ITS sequence alignment (Tree length = 229, CI = 0.834, RI = 0.904, RC = 0.754). The scale bar shows 30 changes, and bootstrap support values from 1000 replicates are shown at the nodes. Accession numbers of ex-type strains are shown in **bold** and the novel species in this study in red. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Harknessia eucalypti* (GenBank accession no. AY720745).

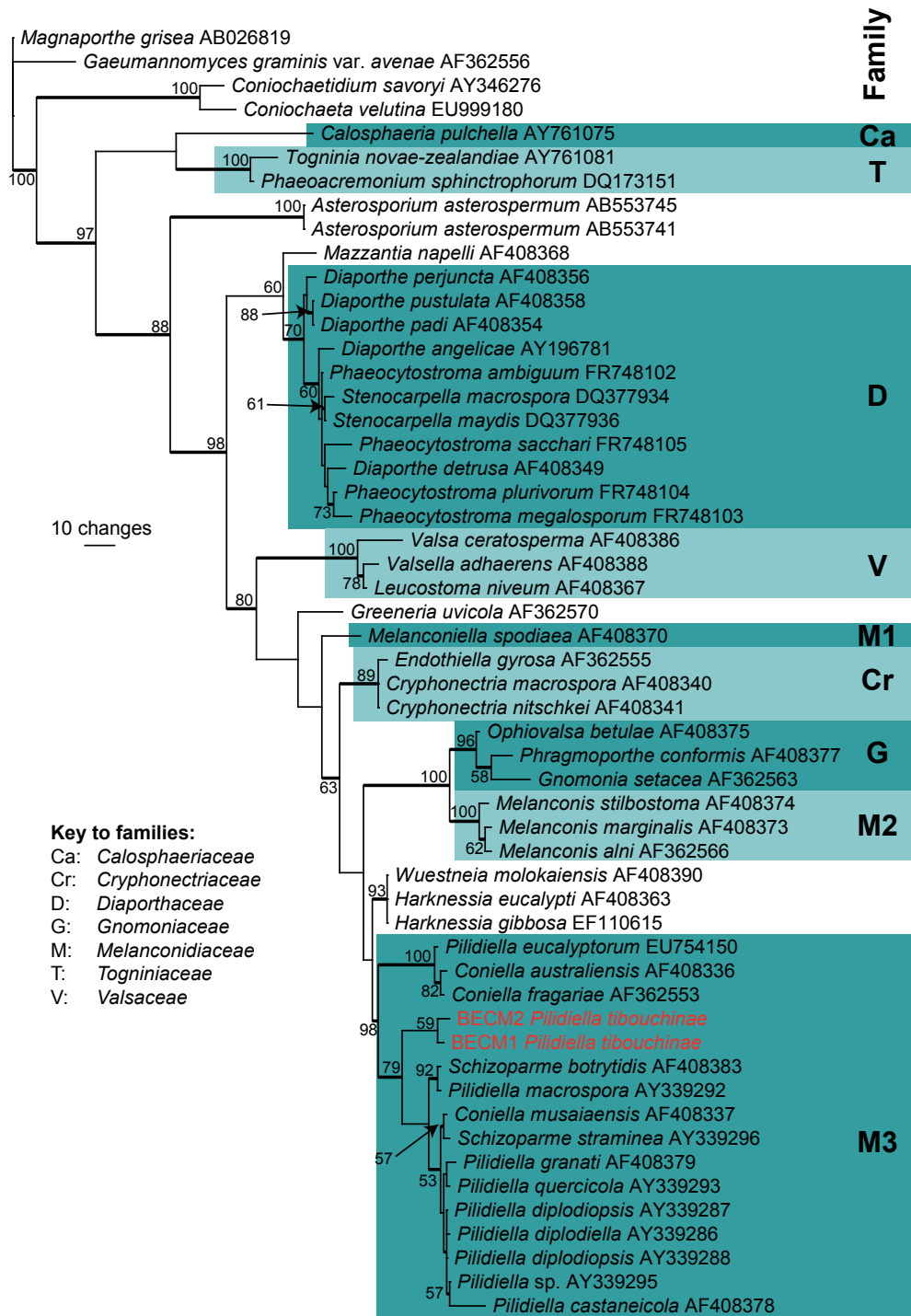


Fig. 2. The first of 180 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment (Tree length = 594, CI = 0.515, RI = 0.817, RC = 0.421). The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. The novel species in this study is indicated in red and families are indicated to the right of the tree according to the key on the figure. Branches present in the strict consensus tree are thickened and the tree was rooted to sequences of *Magnaporthe grisea* and *Gaeumannomyces graminis* var. *avenae* (GenBank accession nos AB026819 and AF362556, respectively).

The ITS region was used in the phylogenetic analysis to determine species-rank relationships (Fig. 1) and the LSU region for the generic placement (Fig. 2). The manually adjusted ITS alignment contained 25 taxa including the outgroup sequence and, of the 501 characters used in the phylogenetic analysis, 86 were parsimony-informative, 49 were variable and parsimony-uninformative, and 366 were

constant. Twenty-four equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 1. The phylogenetic tree of the ITS region (Fig. 1) shows that the obtained sequences cluster between *Pilidiella crousii* and *Pilidiella granati*. The manually adjusted LSU alignment contained 54 taxa including the two outgroup sequences and, of the 840 characters used in

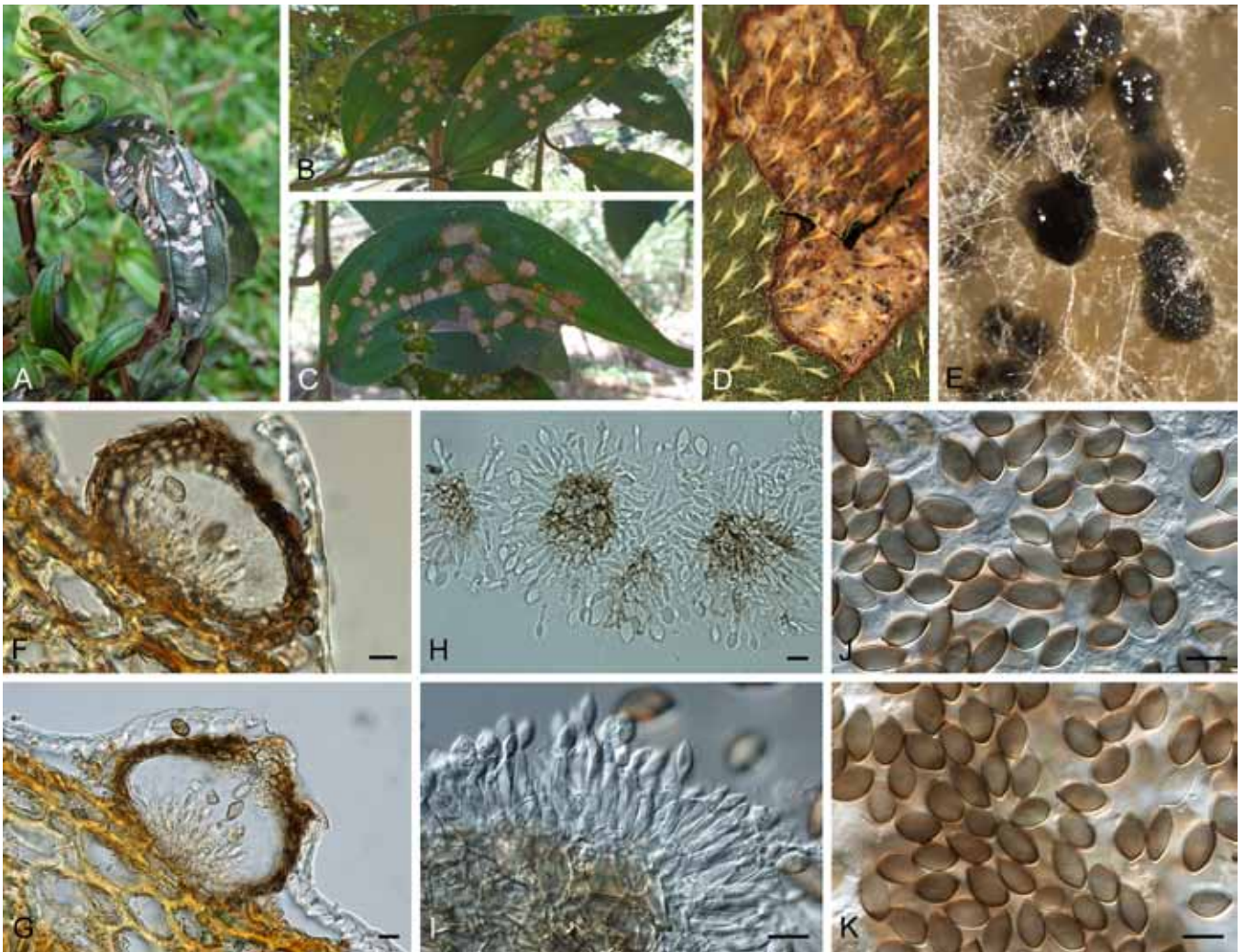


Fig. 3. *Pilidiella tibouchinae*. **A–D.** Leaf spots and curling on *Tibouchina granulosa*. **E.** Colony on oatmeal agar. **F, G.** Vertical section through pycnidia. **H, I.** Conidiogenous cells. **J, K.** Conidia. Bars = 10 μ m.

the phylogenetic analysis, 190 were parsimony-informative, 54 were variable and parsimony-uninformative, and 596 were constant. From this heuristic search, 180 equally most parsimonious trees were retained, the first of which is shown in Fig. 2. Phylogenetic analysis of the LSU region (Fig. 1) confirms the placement of the novel sequences in *Pilidiella*. The partial TEF sequences did not have any high identity to those sequences available in GenBank (data not shown).

Taxonomy

A pycnidial coelomycete was regularly associated with diseased tissues on the samples collected at the two separate localities. Its morphology conformed to that of species in the genus *Pilidiella* (Nag Raj 1993, van Niekerk et al. 2004), although it appeared to represent a distinct taxon. Accordingly, a new species name is introduced below to accommodate the fungus occurring on *T. granulosa*.

Pilidiella tibouchinae B.E.C. Miranda, R.W. Barreto & Crous, **sp. nov.**
Mycobank MB563992
(Fig. 3)

Etymology: Named after the host genus on which it occurs, *Tibouchina*.

Diagnosis: Similar to *Pilidiella eucalyptorum* but lacking conidial germ slits, and similar to *P. petrakioidea* but lacking mucoid appendages on conidia.

Type: **Brazil:** *Minas Gerais:* Viçosa, campus of the Universidade Federal de Viçosa, on leaves of *Tibouchina granulosa*, 8 March 2010, B. C. Miranda (VIC 31443 – holotype; CBS H-20827 – isotype; cultures ex-holotype CPC 18511, CPC 18512 = CBS 131595).

(GenBank accession numbers for VIC 31443 and VIC 31444: ITS = JQ281774, JQ281775; LSU = JQ281776, JQ281777; TEF = JQ281778, JQ281779)

Other specimen examined: **Brazil:** *Minas Gerais:* Viçosa, campus of the Universidade Federal de Viçosa, on leaves of *T. granulosa*, 17 May 2010, B. C. Miranda (VIC 31444).

Description: Lesions on living leaves and young stems, firstly as adaxial straw-coloured necrotic spots, mostly appearing near the leaf veins, becoming yellowish to greyish with a dark brown to dark purple border, irregularly shaped, coalescing and leading to necrosis and distortion of large parts of the leaf lamina; loss of necrotic leaf parts usually creating the impression of insect damage. In conjunction to these symptoms, a shortening of branch internodes, leaf distortion, bud death, necrosis and die-back of young stems are also observed. Stunting and decline of severely affected plants are observed even for adult plants. *Conidiomata* pycnidial, adaxial, subcuticular, solitary, globose to depressed globose, 42.5–75 × 75–112.5 µm, wall composed of dark greyish brown *textura angularis* of 1–3 cell layers, 7–12 µm thick, dark brown; dehiscence ostiolate, central; *conidiophores* formed on a dense, basal, cushion-like aggregation of hyaline cells, mostly reduced to conidiogenous cells, subcylindrical, branched below, 8–15 × 3–4 µm, smooth, hyaline, 1–2-septate. *Conidiogenous cells* enteroblastic, phialidic with apical periclinal thickening, 5–10 × 2–3 µm, smooth, hyaline, with minute collarette, and covered in mucilage. *Conidia* mostly broadly ellipsoidal, often somewhat flattened on one side, oblong, subreniform, ovoid to subovoid, 10–13 × 6–8 µm (l:b = 1.7), apex rounded, subtruncate at base, hilum sometimes slightly protuberant, aseptate, hyaline when immature, becoming smoky-brown at maturity, smooth, guttulate (usually with one large guttule but sometimes biguttulate or eguttulate).

Culture characteristics: (MEA or PCA either under a 12 h light regime or in the dark): Colonies fast-growing (up to 86 mm diam after 6 d); flat, occasionally slightly raised centrally; mostly composed of immersed mycelium, aerial mycelium mostly sparse (but very dense cottony to woolly aerial mycelium on MEA in the dark); cottony to woolly to spider web-like white to grey olivaceous, sometimes with some small cinnamon areas centrally, occasionally becoming powdery towards the periphery, abundant olivaceous black fruit bodies crowded in zone rings on MEA/light. On PCA greyish black to greenish black centrally, with saffron margin in reverse; black fruit bodies less abundant, and in more distinct rings on PCA/light.

DISCUSSION

The fungus on *Tibouchina granulosa* clearly belongs to the *Coniella/Pilidiella*-complex that has *Schizoparme* teleomorphs (*Schizoparmaceae*, *Diaporthales*; Rossman *et al.* 2007). Fungi in *Schizoparmaceae* include several species associated with foliar diseases, sometimes occurring as secondary invaders of plant tissues infected by other organisms or injured by other causes (Ferreira *et al.* 1997). There is no record of any teleomorphic species of *Schizoparmaceae* in association with members of the genus *Tibouchina*, and a single doubtful record of a *Coniella* on another member of the *Melastomataceae*, *Miconia serrulata*

(Farr & Rossman 2010). Several members of *Myrtales*, which according to Bremer *et al.* (2003) includes up to 14 families, are known hosts of *Schizoparmaceae* (Farr & Rossman 2010). For instance, several species are known from *Myrtaceae* (*Acca*, *Blepharocalyx*, *Eucalyptus*, *Eugenia*, *Heteropyxis*, *Myrcia*, *Syzygium*), *Lythraceae* (*Lythrum*, *Punica*), and *Combretaceae* (*Anogeissus*, *Anogeissus*, *Terminalia*) (van Niekerk *et al.* 2004, Farr & Rossman 2010).

Sutton (1980) and Nag Raj (1993) treated *Pilidiella* as a synonym of *Coniella*. However, based on analyses of large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequences, Castlebury *et al.* (2002) concluded that *Pilidiella* is distinct from *Coniella*. *Pilidiella* has two main morphological criteria separating it from *Coniella*: the presence of conidia that are hyaline when young becoming pale brown with age (consistently brown in *Coniella*) (Castlebury *et al.* 2002) and having a length to breadth ratio larger than 1.5 (equal to or smaller than 1.5 for *Coniella*) (van Niekerk *et al.* 2004). Pigmentation alone is difficult to interpret (Table 1), although *P. tibouchinae* has hyaline conidia that become smoky brown at maturity and a l:b ratio of 1.7. Additionally the results of the phylogenetic analysis place *P. tibouchinae* in the *Pilidiella* clade, distinct from *Coniella* (Figs 1–2). Considering the combination of morphological and molecular data, we prefer to place this fungus in the genus *Pilidiella*. Nevertheless, several species in the group still need to be re-examined, as is evident from Fig. 2, where *Pilidiella eucalyptorum* clusters in the *Coniella* clade, and *C. musariensis* clusters in the *Pilidiella* clade.

Pycnidia in *P. tibouchinae* are small when compared to the species of *Schizoparmaceae* treated by Sutton (1980), Nag Raj (1993), and van Niekerk *et al.* (2004). Morphologically, conidia of *P. tibouchinae* show some similarity to that of *P. eucalyptorum* and *P. petrakioidea*. However, conidia of *P. tibouchinae* lack conidial germ slits (present in *P. eucalyptorum*) and mucoid appendages (present in *P. petrakioidea*). It also has thinner pycnidial walls (7–12 µm), than those in *P. eucalyptorum* (to 25 µm thick), and has hyaline to pale smoky-brown conidia, whereas those of *P. eucalyptorum* are medium to dark reddish brown. Furthermore, *P. tibouchinae* also differs from *P. petrakioidea* in conidial morphology (narrowly ellipsoidal with acutely rounded apices in *P. petrakioidea*) and a l:b ratio larger than 1.9. Five species of *Coniella* have been described in association with members of the *Myrtaceae*: *C. australiensis*, *C. castaneicola*, *C. costae*, *C. fragariae*, and *C. minima*. Considering the close morphological similarity of *Pilidiella* and *Coniella*, the conidial morphology of these species is also provided here for comparison with that of *P. tibouchinae* (Table 1).

Pilidiella tibouchinae is the first species of the genus to be described on a host belonging to *Melastomataceae*, on which it appears to be associated with a rather serious foliar and dieback disease. Further investigations aimed at clarifying the pathological status of the fungus on *Tibouchina granulosa*, and evaluating potential disease control measures are now urgently required, and will be reported elsewhere.

Table 1. Conidial morphology of selected *Coniella* and *Pilidiella* species recorded from members of *Myrtales*.

Species	Size	l:b rate	Shape	Appendage	Germ slit	Reference
<i>Coniella australiensis</i>	(9–)10–11(–14) × (6–)7–8(–10) µm	1.4	Broadly ellipsoidal	+	-	van Niekerc <i>et al.</i> (2004)
<i>C. castanaeicola</i>	13–29 × 2.5–3.5 µm	7.3	Fusoid to falcate	+	-	Nag Raj (1993)
<i>C. costae</i>	19–28 × 7–7.5 µm	3.2	Fusoid to ellipsoid	-	-	Dianese <i>et al.</i> (1993)
<i>C. delicata</i>	7–9 × 2.5–3 µm	2.9	Ellipsoid	-	-	Sutton (1980)
<i>C. fragariae</i>	(8–)9–10(–12.5) × (5–)6–7(–8) µm	1.5	Ellipsoid	+	+	van Niekerc <i>et al.</i> (2004)
					in older conidia	
<i>C. macrospora</i>	(18.3–)25–29(–32.5) × (13–)16–20(–21.5) µm	1.5	Ovoid, ellipsoid, pyriform, globose	+	-	van der Aa (1983)
<i>C. minima</i>	6.5–7.5 × 3.5–4.5 µm	1.5	Globose to subglobose	-	-	Sutton (1969)
<i>C. terminaliae</i>	2–8 × 2–3.5 µm	2:01	Globose to subglobose	-	-	Firdousi <i>et al.</i> (1994)
<i>Pilidiella crousii</i>	(6–)7–12(–13.5) × (2.5–)3–5 µm	2.2	Narrowly ellipsoid to ellipsoid	-	-	Rajeshkumar <i>et al.</i> (2011)
<i>P. dipodiella</i>	(10–)12–15(–19) × (4–)5–6 µm	2.3	Narrowly ellipsoid	+	-	van Niekerc <i>et al.</i> (2004)
<i>Peucalyptorum</i>	(9–)10–12(–14) × (6–)7–8 µm	1.6	Broadly ellipsoid or limoniform	uncommon	+	van Niekerc <i>et al.</i> (2004)
<i>P. granati</i>	9–16 × 3–4.5 µm	2.8	Ellipsoid	+	-	Nag Raj (1993)
<i>P. jambolana</i>	19–22 × 3.5–4 µm	5.7	Elongate-fusoid	-	-	Ahmad (1967)
<i>P. petraktoidea</i>	12–14.5 × 6.5–8 µm	1.9	Narrowly ellipsoid	+	-	Nag Raj (1993)
<i>P. tibouchinae</i>	10–13 × 6–8 µm	1.7	Broadly ellipsoid	-	-	This publication

ACKNOWLEDGEMENTS

We thank the technical staff of CBS, Arien van Iperen (cultures), Marjan Vermaas (photographic plates), and Mieke Starink-Willemsse (DNA isolation, amplification and sequencing) for their invaluable assistance.

REFERENCES

- Aa HA van der (1983) A new species of *Coniella*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, C* **86**: 121–125.
- Ahmad S (1967) Contributions to the fungi of West Pakistan–VI. *Biologica* **13**: 15–42.
- Bensch K, Groenewald JZ, Dijksterhuis J, Starink-Willemsse M, Andersen B, Summerell BA, Shin H-D, Dugan FM, Schroers H-J, Braun U, Crous PW (2010) Species and ecological diversity within the *Cladosporium cladosporioides* complex (*Davidiellaceae, Capnodiales*). *Studies in Mycology* **67**: 1–94.
- Bremer B, Bremer K, Chase MW, Reveal JL, Soltis DE, *et al.* (2003) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141**: 399–436.
- Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G (2004) MycoBank: an online initiative to launch mycology into the 21st century. *Studies in Mycology* **50**: 19–22.
- Crous PW, Groenewald JZ, Risède J-M, Simoneau P, Hyde KD (2006) *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* **55**: 213–226.
- Crous PW, Groenewald JZ, Summerell BA, Wingfield BD, Wingfield MJ (2009b) Co-occurring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**: 38–48.
- Crous PW, Schoch CL, Hyde KD, Wood AR, Gueidan C, Hoog GS De, Groenewald JZ (2009a) Phylogenetic lineages in the *Capnodiales*. *Studies in Mycology* **64**: 17–47.
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009c) *Fungal Biodiversity*. [CBS Laboratory Manual Series 1.] Centraalbureau voor Schimmelcultures, Utrecht.
- Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the *Diaportheales* based on large subunit nuclear ribosomal DNA sequences. *Mycologia* **94**: 1017–1031.
- Dianese JC, Medeiros RB, Santos LTP, Sutton BC (1993) *Coniella costae* sp. nov. on leaves of *Myrcia tomentosa* from Brazilian cerrado. *Mycological Research* **97**: 1234–1236.
- FAO (1996) *International Standard for Phytosanitary Measures*. Rome: Secretariat of the International Plant Protection Convention.
- Farr DF, Rossman AY (2010) Fungal databases. Beltsville, MD: Systematic Mycology and Microbiology Laboratory, ARS, USDA; <http://nt.ars-grin.gov/fungalatabases/>.
- Ferreira FA, Alfenas AC, Coelho L (1997) Portas-de-entrada para *Coniella fragariae* em folhas de eucalipto. *Revista Árvore* **21**: 307–311.
- Firdousi SA, Sharma CD, Vyas KM (1994) A new species of *Coniella* from India. *Acta Botanica Indica* **22**: 134–135.

- Hoog GS de, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. *Mycoses* **41**: 183–189.
- Lamprecht SC, Crous PW, Groenewald JZ, Tewoldemedhin YT, Marasas WFO (2011) *Diaporthaceae* associated with root and crown rot of maize. *IMA Fungus* **2**: 13–24.
- Lorenzi H (2002) *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil*. Vol. 1. 4a edição. Nova Odessa, SP: Instituto Plantarum.
- Nag Raj TR (1993) *Coelomycetous Anamorphs with Appendage-bearing Conidia*. Waterloo: Mycologue Publications.
- Niekerk JM van, Groenewald JZ, Verkley GJM, Fourie PH, Wingfield MJ, Crous PW (2004) Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. *Mycological Research* **108**: 283–303.
- Rayner RW (1970) *A Mycological Colour Chart*. Kew: Commonwealth Mycological Institute.
- Rossmann AY, Farr DF, Castlebury LA (2007) A review of the phylogeny and biology of the *Diaporthales*. *Mycoscience* **48**: 135–144.
- Sutton BC (1969) Type studies of *Coniella*, *Anthasthoopa*, and *Cyclodomella*. *Canadian Journal of Botany* **47**: 603–608.
- Sutton BC (1980) *The Coelomycetes: fungi imperfecti with pycnidia, acervuli and stromata*. Kew: Commonwealth Mycological Institute.
- USDA/Fungal Database. Available at <http://nt.ars-grin.gov/fungalatabases/index.cfm>. (accessed 20 March 2010).
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
- White TJ, Bruns T, Lee J, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a guide to methods and applications*: 315–322. San Diego: Academic Press.