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RESEARCH ARTICLE / *ARTIGO*

A new species of *Calonectria* causing leaf blight and cutting rot of three forest tree species in Brazil

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ABSTRACT

Several species of *Calonectria* cause diseases on a wide range of forest tree species that are propagated either via seedlings or rooted cuttings. In nurseries these fungi cause damping-off, cutting and root rots, stem lesions, and leaf blights. Recently a *Calonectria* sp. was isolated from rooted cuttings of *Anadenanthera peregrina* (Fabaceae), *Piptadenia gonoacantha* (Fabaceae), and *Azadirachta indica* (Meliaceae) exhibiting leaf blight and cutting rot in a forest nursery at the Universidade Federal de Viçosa, Brazil. Morphological comparisons and DNA sequences of three loci containing partial gene sequences of β-tubulin (TUB2), calmodulin (CAL), and elongation factor (TEF-1α) indicated that these isolates represent an unnamed species of *Calonectria*, described here as *C. hodgesii* sp. nov. Spray-inoculated plants of all three hosts with a suspension at $1x10^4$ conidia mL^{-1} induced leaf lesions, cutting rot, and intense defoliation as observed under natural conditions. *Calonectria hodgesii* was re-isolated from infected tissue, which fulfilled Koch's postulates and confirmed its status as a pathogen with a wide host range.

Key words: forest pathology, Hypocreales, pathogenicity, phylogeny, taxonomy, tropical fungi.

INTRODUCTION

In recent years there has been an increasing demand for planting forest tree species in Brazil, in part due to the government's initiatives to restore degraded areas and mitigate global warming. *Anadenanthera peregrina* (L.) Speg. (Angico Vermelho) and *Piptadenia gonoacantha* (Mart.) J.F. Macbr. (Pau-Jacaré) are among the most commonly used native species (Carvalho, 1994; Araújo et al., 2006). There has also been an increase in plantations of *Azadirachta indica* A. Juss. (Neem), which is native to India. The increased planting of *A. indica* is largely due to its multiple applications in the pharmaceutical industry, use in agriculture as a natural insecticide, and more recently for biodiesel production (Mossini & Kemmelmeier, 2005). Nevertheless, propagation of these species either via cuttings or seedlings is generally still done in nurseries with low technology, where appropriate management practices for disease control are not employed (Mafia et al., 2007).

Numerous pathogenic fungal species, especially species of *Calonectria*, have been described from forest nurseries and have been reported as pathogens of a wide range of plant hosts cultivated via seedlings or vegetative propagation (Crous, 2002). Among the major nursery diseases, damping-off, cutting rot, stem girdling and leaf blight caused by *Calonectria* spp. are commonly encountered (Hodges & May, 1972; Lombard et al., 2010a). In recent years several new species of *Calonectria* have been newly described from hosts in forestry nurseries using a polyphasic approach incorporating morphological and molecular data (Lombard et al., 2010c; Alfenas et al., 2013).

Because of the importance of the genus *Calonectria* as plant pathogen in tropical and subtropical climates, we have for the past two years been collecting plant and soil samples from different hosts throughout Brazil to facilitate population biology studies of *Calonectria* spp.

During one these collecting in the Forest Nursery at the Universidade Federal de Viçosa, Brazil, in May 2011, we found rooted cuttings of *A. peregrina*, *A. indica* and *P. gonoacantha* exhibiting necrotic leaf blight, defoliation and cutting rot symptoms with brown and necrotic tissues at the stem base, covered by profuse white sporulation typical of *Calonectria* infection (Figure 1). The same symptoms were observed on all three hosts. The primary aim of this study was to identify the causal agent of this disease through a combination of morphological and molecular characterization, and pathogenicity tests.

MATERIAL AND METHODS

Isolates

Single conidial isolates of a *Calonectria* sp. were obtained from leaves and cuttings of infected plants of *A. peregrina*, *A. indica* and *P. gonoacantha*.

To obtain single conidial cultures, pathogen structures observed under a stereoscopic microscope (45

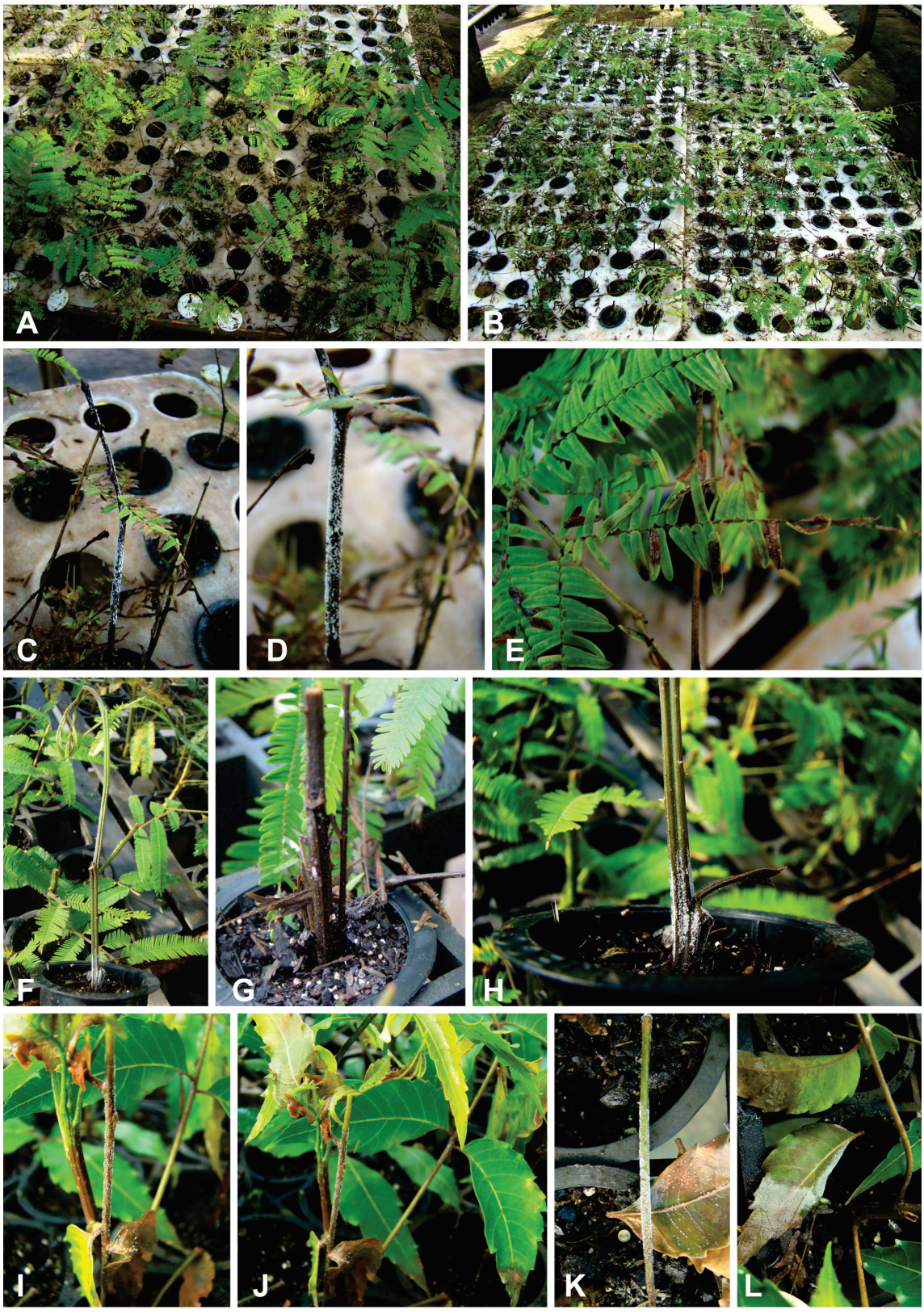

FIGURE 1 - Leaf blight and cutting rot caused by *Calonectria hodgesii* on rooted cuttings of forest species in a nursery. **A,B:** General view containing infected rooted cuttings of *Anadenanthera peregrina* and *Piptadenia gonoacantha*; **C-E:** Cutting rot and leaf blight in *A. peregrina*; **F-H:** Cutting rot in *P. gonoacantha*; **I-L:** Cutting rot and leaf blight with intense sporulation on seedlings of *Azadirachta indica*.

x) were deposited on Petri dishes containing water agar medium [WA, 1.5% (w/v) agar]. Subsequently 2 mL of sterile distilled water were added to each WA dish, and shaken manually. Excess water was removed by inverting the Petri dish, and under a stereomicroscope (45 x) a single conidium was transferred to a Petri dish containing malt extract agar (MEA). Plates were maintained at 26ºC for 5 days to promote fungal growth. One representative isolate from each host was selected for further studies. To maintain viable isolates strains were stored in a glycerol solution (10%) at -80ºC.

DNA extraction and amplification

Mycelia of the respective isolates were scraped from colonized MEA plates, and placed separately in 2 mL microtubes for genomic DNA extraction using the Wizard Genomic DNA Purification (Promega) kit. For PCR, the DreamTaq Master Mix (Fermentas) was used, following the manufacturer's protocol.

Three loci, including fragments of the β-tubulin (TUB2), calmodulin (CAL), and elongation factor (TEF-1α) gene regions were amplified using the primers T1 (O'Donnell & Cigelnik, 1997) and CYLTUB1R (Crous et al., 2004) for TUB2, CAL-228F and CAL-737R (Carbone & Kohn, 1999) for CAL, and EF1-728F (O'Donnell et al., 1998) and EF-2 (Carbone & Kohn, 1999) for TEF-1α. Amplification was performed with an initial denaturing at 96°C for 5 min followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min and a final 4 min extension at 72°C. The PCR product was visualized on a 2% agarose gel to determine fragment size and purity. PCR products were prepared for sequencing with the ExoSAP-IT kit (Affymetrix) according to the manufacturer's protocol.

Sequencing and phylogenetic analyses

Sequencing was performed at the Laboratory of Genomics of the Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO) at the Universidade Federal de Viçosa, Brazil. Sequence quality was checked via Sequence Scanner Software v. 1.0 (Applied Biosystems), and edited using the software package Seqman from DNAStar Inc. Consensus regions of edited sequences were compared in the NCBI GenBank nucleotide database (www.ncbi.nlm.nih.gov) using the nucleotide collection (nr/nt) optimised for highly similar sequences (megablast). *Calonectria* sequences generated in this study were deposited in GenBank (Table 1). All sequences were assembled in MAFFT v. 6 (Katoh & Toh, 2010), using the FFT-NS-i (Slow; iterative refinement method) alignment strategy with the 200PAM/ K=2 scoring matrix and a gap opening penalty of 1.53 with an offset value of 0.0. Aligned sequences were then manually corrected when necessary using MEGA v. 5 (Tamura et al., 2011).

PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford, 2002) was used to analyse the DNA sequence datasets. A partition homogeneity test (Farris et al., 1994) was applied to determine whether the data sets were consistent and combinable. Phylogenetic relationships were estimated by heuristic searches based on 1,000 random addition sequences and tree bisection-reconnection, with the branch swapping option set on 'best trees' only. All characters were weighed equally and alignment gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull, 1993) were based on 1,000 replications.

Analysis of Bayesian Inference (BI) was performed with MrBayes v. 3.1.1 (Ronquist & Heulsenbeck, 2003) using the algorithm of Markov Chain Monte Carlo (MCMC) with two sets of four chains (one cold and three heated) with 10 million random generations. The sample frequency was set to 1,000 and the first 25% of trees were removed as burnin. Likelihood values were calculated and the best model of nucleotide substitution for each gene was selected according to the Akaike Information Criterion (AIC) using MrModeltest v. 2.3 (Posada & Buckley, 2004).

The convergence of the log likelihood was analysed using TRACER v. 1.5 (Rambaut & Drummond, 2009) and no indication of lack of convergence was detected. *Calonectria chinensis* (Crous) L. Lombard, M. J. Wingf. & Crous was used as outgroup in the analysis.

Morphological characterization

For morphological characterization single conidial cultures were grown in synthetic nutrient-poor agar (SNA) at 26°C for 7 days. Fungal structures were mounted in clear lactic acid for morphological examination, and 30 measurements of each structure determined at 1,000 × magnification using a Zeiss Axioscope-2 microscope with differential interference contrast (DIC) illumination. The 95% confidence levels were determined and extremes of conidial measurements are given in parentheses. For other structures, only extremes are presented.

Pathogenicity test

Single conidial cultures were transferred aseptically to Petri dishes (90 mm diam) containing Malt Extract Agar (MEA), and subsequently incubated at 26°C for 10 days for pathogenicity studies. Healthy rooted cuttings (five per species) of *A. peregrina*, *A. indica* and *P. gonoacantha* were spray-inoculated with a conidial suspension of 1×10^4 conidia mL^{-1} of each isolate, as described by Graça et al. (2009). Five plants of each host species were treated with distilled water to serve as controls. The inoculated plants were maintained in a greenhouse under controlled conditions (25°C±3°C) and the development of symptoms was monitored daily for 10 days, after which time the fungus was re-isolated from the lesion margins.

TABLE 1 - Details pertaining to *Calonectria* spp. studied

Species	Isolates	GenBank accession no.			Host/substrate	Country	Reference
		β - tubulin (*TUB2*)	Elongation factor (*TEF1α*)	Calmodulin (*CAL*)			
C. brasiliensis	CBS 230.51	GQ267241	GQ267328	GQ267421	*Eucalyptus* sp.	Brazil	Lombard et al. (2010c)
C. brasiliensis	CBS 114257	GQ267242	GQ267329	GQ267422	*Eucalyptus* sp.	Brazil	Lombard et al. (2010c)
C. canadiana	CBS 110817	AF348212	GQ267297	AY725743	*Picea* sp.	Canada	Kang et al. (2001)
C. cerciana	CBS 123693	FJ918510	FJ918559	GQ267369	Hybrid "urograndis"	China	Lombard et al. (2010d)
C. cerciana	CBS 123695	FJ918511	FJ918560	GQ267370	Hybrid "urograndis"	China	Lombard et al. (2011)
C. chinensis	CBS 112744	AY725618	AY725709	AY725746	Soil	China	Crous et al. (2004)
C. citri	CBS 186.36	AF333393	GQ267299	GQ267371	*Citrus sinensis*	U.S.A	Lombard et al. (2010c)
C. colombiana	CBS 115127	FJ972423	FJ972492	GQ267455	Soil	Colombia	Lombard et al. (2010c)
C. colombiana	CBS 115638	FJ972422	FJ972491	GQ267456	Soil	Colombia	Lombard et al. (2010c)
C. colombiensis	CBS 112221	AY725620	AY725712	AY725749	Soil	Colombia	Crous et al. (2004)
C. densa	CMW 31182	GQ267232	GQ267352	GQ267444	Soil	Ecuador	Lombard et al. (2010c)
C. densa	CMW 31184	GQ267230	GQ267350	GQ267442	Soil	Ecuador	Lombard et al. (2010c)
C. hodgesii	CBS 133608	KC491227	KC491224	KC491221	*Piptadenia gonoacantha*	Brazil	This study
C. hodgesi	CBS 133609	KC491228	KC491225	KC491222	*Anadenanthera peregrina*	Brazil	This study
C. hodgesii	CBS 133610	KC491229	KC491226	KC491223	*Azadirachta indica*	Brazil	This study
C. humicola	CMW 31183	GQ267233	GQ267353	GQ267445	Soil	Ecuador	Lombard et al. (2010c)
C. humicola	CMW 31187	GQ267235	GQ267355	GQ267447	Soil	Ecuador	Lombard et al. (2010c)
C. insularis	CBS 114558	AF210861	FJ918556	GQ267389	Soil	Madagascar	Lombard et al. (2010d)
C. insularis	CBS 114559	AF210862	FJ918555	GQ267390	Soil	Madagascar	Lombard et al. (2011)
C. kyotensis	CBS 170.77	GQ267209	GQ267308	GQ267380	*Robina pseudoacacia*	Japan	Crous (2002)
C. kyotensis	CBS 413.67	GQ267208	GQ267307	GQ267379	*Paphiopedilum callosum*	Germany	Crous (2002)
C. leucothoës	CBS 109166	FJ918508	FJ918553	GQ267392	*Leucothoë axillaris*	U.S.A	Lombard et al. (2010c)
C. morganii	CBS 110666	FJ918509	FJ918557	GQ267423	*Rosa* sp.	U.S.A	Lombard et al. (2010c)
C. pauciramosa	CMW 5683	FJ918514	FJ918565	GQ267405	*Eucalyptus* sp.	Brazil	Lombard et al. (2010b)
C. pauciramosa	CMW 30823	FJ918515	FJ918566	GQ267404	*Eucalyptus grandis*	South Africa	Lombard et al. (2010b)
C. polizzii	CBS 125270	FJ972417	FJ972486	GQ267461	*Callistemon citrinus*	Italy	Lombard et al. (2010b)
C. polizzii	CBS 125271	FJ972418	FJ972487	GQ267462	*Callistemon citrinus*	Italy	Lombard et al. (2010b)
C. pseudospathiphylli	CBS 109165	FJ918513	FJ918562	GQ267412	Soil	Ecuador	Lombard et al. (2010d)
C. scoparia	CMW 31000	FJ972426	FJ972525	GQ267367	*Eucalyptus*	Brazil	Lombard et al. (2010b)
C. scoparia	CMW 31001	GQ421779	GQ267298	GQ267368	*Eucalyptus*	Brazil	Lombard et al. (2010b)
C. spathiphylli	CBS 114540	AF348214	GQ267330	GQ267424	*Spathiphyllum* sp.	U.S.A	Kang et al. (2001)
C. spathiphylli	CBS 116168	FJ918512	FJ918561	GQ267425	*Spathiphyllum* sp.	U.S.A	Lombard et al. (2010d)
C. spathulata	CBS 112689	AF308463	FJ918554	GQ267426	*Eucalyptus viminalis*	Brazil	Lombard et al. (2010c)
C. spathulata	CBS 555.92	GQ267215	GQ267331	GQ267427	*Araucaraia angustifolia*	Brazil	Lombard et al. (2010c)
C. sulawesiensis	CBS 125248	GQ267223	GQ267343	GQ267435	*Eucalyptus* sp.	Indonesia	Lombard et al. (2010c)
C. sulawesiensis	CBS 125253	GQ267220	GQ267340	GQ267432	*Eucalyptus* sp.	Indonesia	Lombard et al. (2010c)
C. variabilis	CBS 112691	GQ267240	GQ267335	GQ267458	*Theobroma grandiflorum*	Brazil	Crous (2002)
C. variabilis	CBS 114677	AF333424	GQ267334	GQ267457	*Schefflera morotoni*	Brazil	Crous (2002)
C. zuluensis	CBS 125268	FJ972414	FJ972483	GQ267459	*Eucalyptus grandis*	South Africa	Lombard et al. (2010b)
C. zuluensis	CMW 9896	FJ972415	FJ972484	GQ267460	*Eucalyptus grandis*	South Africa	Lombard et al. (2010b)

RESULTS

Phylogenetic analysis

Amplicons of approximately 500 bases each for TUB2, TEF-1α, and CAL were generated. The 70% reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions separately, and therefore they were combined in a dataset consisting of 1,531 characters including gaps. Of these 1,100 were constant and parsimony uninformative and 431 were parsimony informative. Analysis of the 431 parsimony informative characters yielded three equally most parsimonious trees (TL = 1033 CI = 0.691, RI = 0.871, RC = 0.602). Evolution models HKY + G for TUB2, TEF, and CAL were selected and incorporated into the Bayesian analysis. The consensus tree obtained for the Bayesian analyses confirmed the tree topology obtained with parsimony. The isolates of *Calonectria* from *A. peregrina*, *A. indica* and *P. gonoacantha* formed a distinct, well-supported clade (PP=1,00) (Figure 2).

Taxonomy

Based on the DNA sequence data and morphological features, we conclude that the *Calonectria* isolates from *A. peregrina, A. indica* and *P. gonoacantha* represent a novel species, which is described below:

Calonectria hodgesii R.F. Alfenas, O.L. Pereira, Crous & Alfenas, sp. nov. MycoBank MB 803943 (Figure 3);

Etymology: Named after Dr. Charles S. Hodges, in honor of his contribution to forest pathology in the tropics;

Hosts: *Anadenanthera peregrina*, *Azadirachta indica* and *Piptadenia gonoacantha*;

Specimen examined: Brazil, Minas Gerais state, Viçosa, on *Anadenanthera peregrina*, May 2011, Rafael F. Alfenas (Holotype CBS H-21147, Culture ex-type CBS 133609).

Conidiophores containing a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 40-82 × 5-7 µm; stipe extensions septate, straight to flexuous 136-196 µm long, 2-4 µm wide at the apical septum, terminating in pyriform to ellipsoidal or ovoid to sphaeropedunculate vesicles, 6-11 µm diam. Conidiogenous apparatus 45-65 µm long, 61-72 µm wide at apex; primary branches aseptate, 18-27 × 4-5 µm; secondary branches aseptate, 12-24 × 3-4 µm, and tertiary branches aseptate, 9-18 × 3-5 µm, each terminal branch producing 2-6 phialides; phialides doliiform to reniform, hyaline, 5-10 × 2-4 µm; apex with minute periclinal thickening and inconspicuous collarette. Macroconidia cylindrical, rounded at both ends, straight, (44-)49-51(-55) × 3-5 µm (av. = 50 × 4.5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Mega- and microconidia not seen.

Notes: *Calonectria hodgesii* is phylogenetically closely related to *C. brasiliensis* and *C. sulawesiensis*, but *C. hodgesii* can easily be distinguished from these species based on the size of its macroconidia, vesicle shape, number of macroconidiophore branches, and DNA sequence data.

Culture characteristics: Colonies sienna to umber on the surface and sepia to brown-vinaceous in reverse, with moderate aerial mycelium; chlamydospores moderate to extensive, occurring throughout the colony, forming microsclerotia; extensive sporulation on the aerial mycelium; moderate to rapid growth (50–65 mm) diam after 7 days at 25ºC on MEA.

Pathogenicity test

After 10 days spray-inoculated plants showed necrotic leaf blight, cutting rot with brown and necrotic tissues of the basal stem, and intense defoliation as observed under natural conditions in the nursery. Profuse sporulation was also observed on necrotic lesions of inoculated organs of all three host species.

DISCUSSION

In the present study we describe a new species of *Calonectria* associated with necrotic leaf blight and cutting rot of *A. peregrina*, *A. indica* and *P. gonoacantha* in Brazil based on morphological and molecular data. *Calonectria hodgesii* formed a distinct and well-supported phylogenetic clade, closely related to *C. brasiliensis* and *C. sulawesiensis*, which belong to the *C. morganii* species complex. This complex, characterised by having uniseptate macroconidia and vesicles varying from pyriform to obpyriform or ovoid to ellipsoidal, includes *C. cerciana* L. Lombard, M.J. Wingf. & Crous, *C. insularis* C.L. Schoch & Crous, *C. morganii*, *C. sulawesiensis*, *C. hawksworthii* (Peerally) L. Lombard, M.J. Wingf. & Crous, *C. leucothöes* (El-Gholl, Leahy & T.S. Schub.) L. Lombard, M.J. Wingf. & Crous, *C. variabilis* Crous, B.J.H. Janse, D. Victor, G.F. Marias & Alfenas and *C. brasiliensis* (Peerally) L. Lombard, M.J. Wingf. & Crous (Schoch et al., 2001; Lombard et al., 2010c).

Calonectria hodgesii is characterised by having macroconidia larger than those of *C. brasiliensis*, *C. morganii* and *C. sulawesiensis*, but smaller than *C. variabilis* (Table 2). Superficially *C. hodgesii* resembles *C. variabilis* in having vesicles that vary in shape, and it is quite probable that many isolates previously identified as *C. variabilis*, were in fact representative of *C. hodgesii.* The two species can be distinguished, however, in that *C. hodgesii* has 1-septate conidia, while *C. variabilis* has (1-)3(-4)-septate conidia. Although the vesicle shape of *C. hodgesii* is also quite variable, it is mainly obpyriform to ellipsoidal, while those of *C. variabilis* vary from clavate to ellipsoidal. Interestingly, the new species shares morphological characteristics with phylogenetically distant species, such as *Calonectria citri* (H.S. Fawc. & Klotz) L. Lombard, M.J. Wingf. & Crous and *Calonectria canadiana* L. Lombard, M.J. Wingf. & Crous.

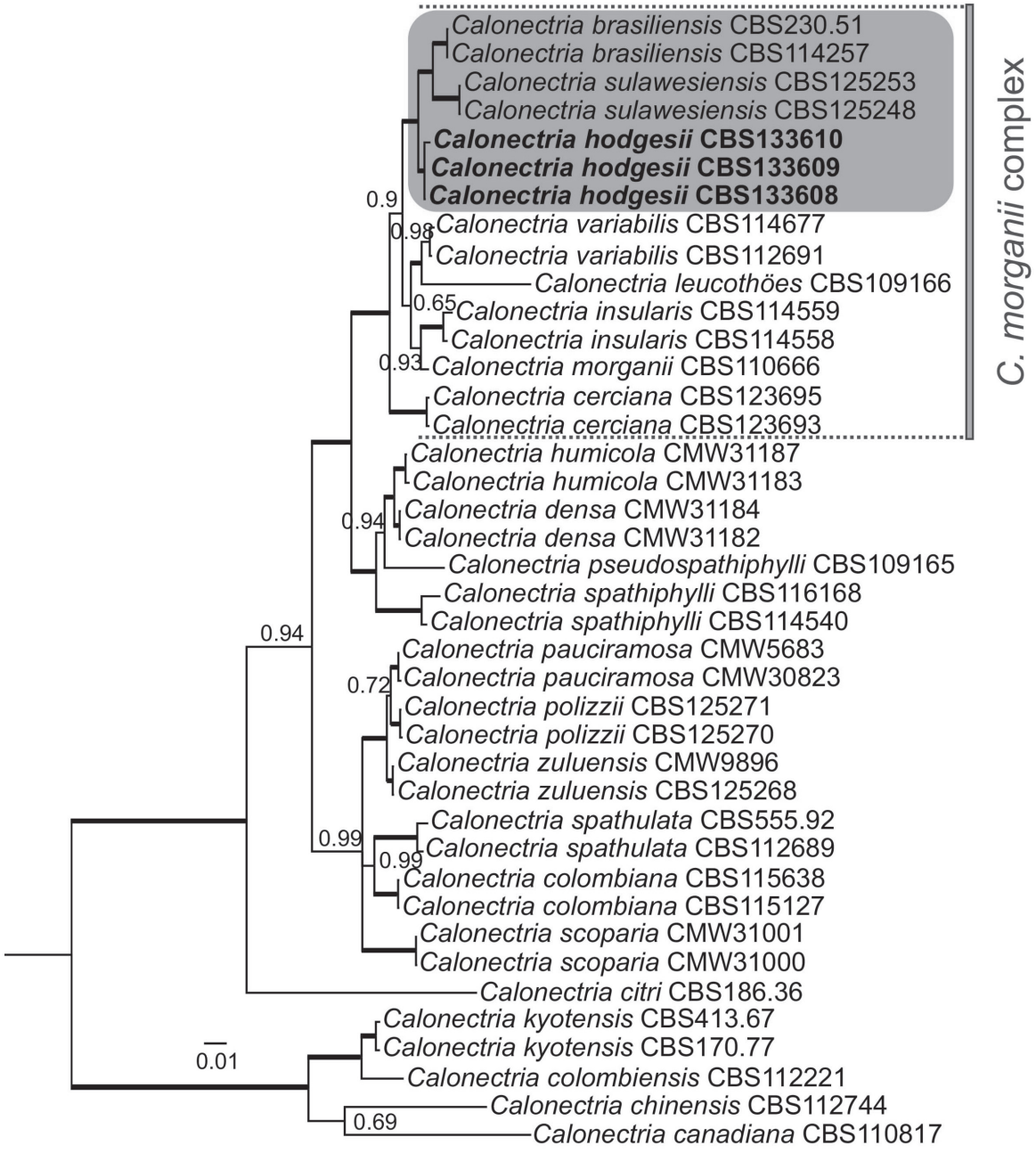

FIGURE 2 - Phylogenetic tree obtained from Bayesian inference using combined sequences of the β-tubulin, translation elongation factor-1α and calmodulin genes of *Calonectria* isolates. The bold lines indicate posterior probability values of 1.00. The tree was rooted to *C. chinensis* (CBS 112744). Isolates in bold were obtained during the survey.

Species of the *C. morganii* complex are well-known pathogens of various hosts worldwide (Crous, 2002), and some, like *C. brasiliensis*, are known to be highly aggressive to *Eucalyptus* seedlings (Batista, 1951). Originally, *C. brasiliensis* was described as a variety of *C. morganii* Crous, Alfenas & M.J. Wingf. (as *Cylindrocladium scoparium* var. *brasiliensis* Batista & Ciferri) based on having macroconidia smaller than those of *Calonectria morganii* (Batista, 1951; Peerally, 1974). Based on morphological characteristics and total protein banding patterns however, Crous et al. (1993a) reduced *C. brasiliensis* to synonymy under *C. morganii*. By employing multigene DNA sequence data, Lombard et al. (2010b) recently showed that the ex-type culture of *C. brasiliensis* (CBS 230.51) is phylogenetically and morphologically distinct from *C. morganii*, and therefore reinstated it to species level.

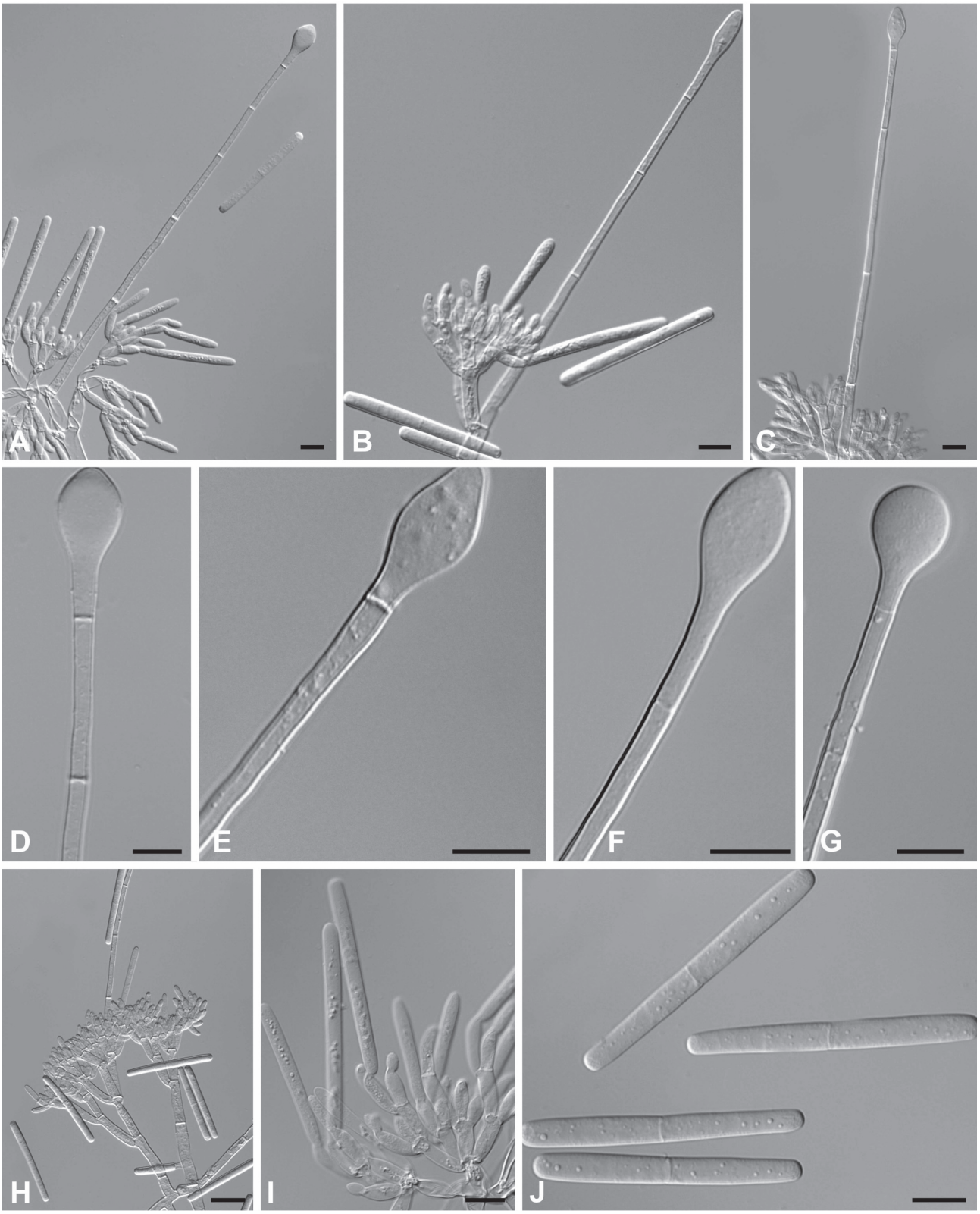

FIGURE 3 - Morphological characteristics of *Calonectria hodgesii*. **A-C:** Macroconidiophores containing obpyriform to ellipsoidal or sphaeropedunculate vesicles; **D-G:** Variation in vesicle shape; **H, I:** Macroconidiophores; **J:** Uniseptate macroconidia. Scale bars = 10 µm; H = 20 µm.

TABLE 2 - Distinctive morphological characters of *Calonectria hodgesii* and related species

Species	Vesicle shape	Vesicle diameter	Macroconidial size	Macroconidial septation
C. brasiliensis[a]	ellipsoidal to obpyriform	7–11 µM	(35–)38(–41) × 3–5 µM	1-septate
C. canadiana[b]	pyriform to sphaeropedunculate	6–10 µM	(38–)50(–65) × 4–5 µM	1-septate
C. citri[c]	obovoid to spathulate	6.5–10 µM	(50–)57.5(–65) × 3–4 µM	(1–)3-septate
C. hodgesii	ellipsoidal to pyriform, or ovoid to sphaeropedunculate	6–11 µM	(44–)50(–55) × 3– 5 µM	1-septate
C. morganii[d]	clavate, ellipsoid to pyriform	6–8 µM	(40–)45(–66) × 3–5 µM	1-septate
C. sulawesiensis[e]	broadly clavate to ellipsoidal	5–7 µM	(41–)48(–54) × 3–6 µM	1-septate
C. variabilis[d]	sphaeropedunculate to ovoid, or ellipsoid to clavate	6–11 µM	(48–)73(–85) × 4–6 µM	(1–)3(–4)-septate

[a]Lombard et al., 2010b; [b]Crous et al., 1993b and Kang et al., 2001; [c]Boedjin & Reitsma, 1950; [d]Crous et al., 1993a; [e]Lombard et al., 2010c

Calonectria sulawesiensis Lombard et al. (2010c), described from *Eucalyptus* sp. in Indonesia, is another phylogenetically closely related species, but it is morphologically distinct, and presently nothing is yet known regarding its pathogenicity and host range.

In our studies all three selected isolates (CBS133608, CBS133609, and CBS133610) of *C. hodgesii* tested were pathogenic, and induced leaf blight, defoliation and cutting rot in *A. peregrina*, *A. indica* and *P. gonoacantha* similar to that observed in the nursery under natural conditions.

The occurrence of *C. hodgesii* sp. nov. causing leaf blight and cutting rot on these hosts represents an alert for nurseries that propagate these forest species either from seedlings or rooted cuttings.

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