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RESEARCH ARTICLE

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Calonectria spp. causing leaf spot, crown and root rot of ornamental plants in Tunisia

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Key words

Calonectria
crown and root rot
DNA phylogeny
leaf spot
pathogenicity
systematics

Abstract *Calonectria* spp. are important pathogens of ornamental plants in nurseries, especially in the Northern Hemisphere. They are commonly associated with a wide range of disease symptoms of roots, leaves and shoots. During a recent survey in Tunisia, a number of *Calonectria* spp. were isolated from tissues of ornamental plants showing symptoms of leaf spot, crown and root rot. The aim of this study was to identify these *Calonectria* spp. using morphological and DNA sequence comparisons. Two previously undescribed *Calonectria* spp., *C. pseudomexicana* sp. nov. and *C. tunisiana* sp. nov., were recognised. *Calonectria mexicana* and *C. polizzii* are newly reported for the African continent. Pathogenicity tests with all four *Calonectria* spp. showed that they are able to cause disease on seedlings of *Callistemon* spp., *Dodonaea viscosa*, *Metrosideros* spp. and *Myrtus communis*.

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INTRODUCTION

Species of *Calonectria* are common pathogens of a wide range of plant hosts in nurseries cultivated through seedings or vegetative propagation (Crous 2002, Lombard et al. 2010a). Nursery disease symptoms associated with these fungi include crown, collar and root rot, leaf spots and cutting rot (Crous 2002, Vitale & Polizzi 2008, Polizzi et al. 2009, Lombard et al. 2010a). *Calonectria* spp. have been reported worldwide from agricultural and forestry nurseries (Crous et al. 1991, Crous 2002, Lombard et al. 2009, 2010a, b, d), whereas in Europe, they have only been reported from commercial ornamental nurseries (Polizzi & Crous 1999, Polizzi 2000, Crous 2002, Henricot & Culham 2002, Polizzi et al. 2007a, b, Vitale & Polizzi 2008, Polizzi et al. 2009, Lombard et al. 2010a).

Past reports have shown that *C. morganii* and *C. pauciramosa* are the most common *Calonectria* spp. found in ornamental nurseries in the Northern Hemisphere (Polizzi & Crous 1999, Polizzi 2000, Polizzi & Catara 2001, Polizzi et al. 2006a, b, 2007a, b). Based on phylogenetic studies, *C. morganii* appears to be restricted to Brazil, Europe and the USA (Crous et al. 1993, Overmeyer et al. 1996, Schoch et al. 2000), whereas *C. pauciramosa* has a more global distribution and has been shown to better adapt to different environmental conditions (Crous 2002, Lombard et al. 2010b, Chen et al. 2011). *Calonectria pauciramosa* was also regarded as the dominant pathogen in nurseries in Australia and South Africa (Crous 2002, Schoch et al. 2001, Lombard et al. 2010b).

In November 2010, a survey was conducted in an ornamental nursery in Carthage, Tunis, Tunisia. Various plant species were collected showing symptoms of leaf spots, crown and root rot. Isolations consistently yielded *Calonectria* spp. and the aim of this study was to identify these species, and confirm their pathogenicity.

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MATERIALS AND METHODS

Disease survey and isolates

During November 2010, an ornamental nursery located in Carthage, Tunis, Tunisia was surveyed for diseased plants. Several samples of *Callistemon* spp., *Dodonaea viscosa*, *Myrtus communis* and *Metrosideros* spp. showing leaf spots, crown and root rot were randomly collected for analysis (Fig. 1, Table 1). Infected tissues collected from symptomatic plants were superficially disinfected with 1.0 % sodium hypochlorite for 2 min, rinsed with sterile water, placed on potato-dextrose agar (PDA, Oxoid) and incubated in the dark at 24 °C. Representative isolates of *Calonectria* from each ornamental species were obtained from single-spore colonies made from 14 d old cultures grown on PDA. Representative isolates have been deposited at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (Table 1).

DNA sequence comparisons

Total genomic DNA was extracted from single-conidial isolates grown on 2 % malt extract agar (MEA) for 7 d, using the UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc., California, USA) according to the manufacturer's protocol. Partial gene sequences were determined for β-tubulin (BT), histone H3 (HIS3) and translation elongation factor-1α (TEF-1α) using the primers and protocols described by Lombard et al. (2010c).

To ensure the integrity of the sequences, the amplicons were sequenced in both directions using the same primer pairs used for amplification. Sequence data from Lombard et al. (2010b, d) were used as reference data and subsequent alignments were generated using MAFFT v. 6 (Katoh & Toh 2010) and manually corrected where necessary.

Congruency of the sequence datasets for the separate loci was determined using tree topologies of 70 % reciprocal Neighbour-Joining bootstrap trees with Maximum Likelihood distances that were compared visually to identify conflicts between partitions (Gueidan et al. 2007). Molecular evolution models for the separate gene regions were determined in Modeltest v. 3.7

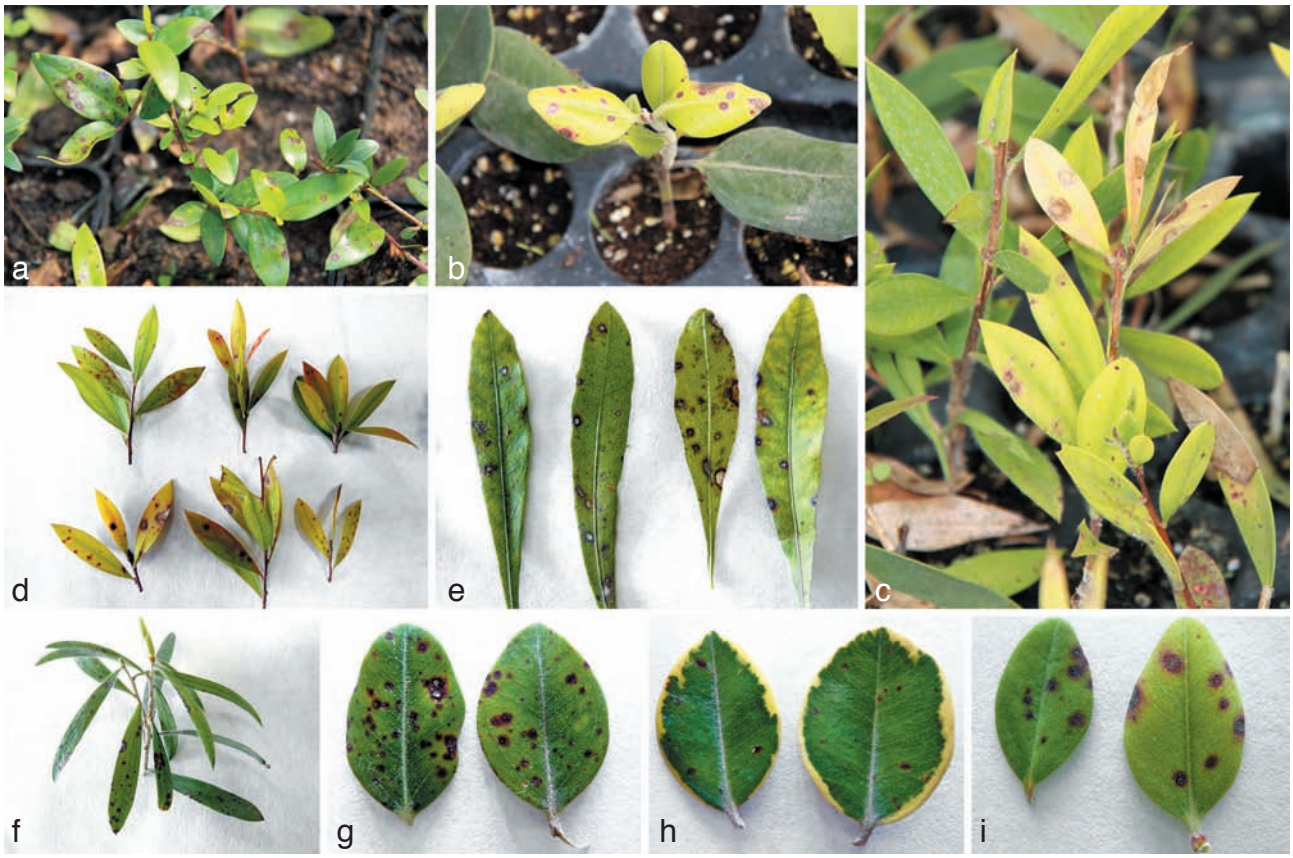

Fig. 1 Symptoms of leaf spot caused by *Calonectria* spp. on several ornamental plants. a. *Myrtus communis*; b. *Metrosideros thomasii*; c. *Callistemon* sp.; d. *Callistemon* sp.; e. *Dodonaea viscosa*; f. *Callistemon viminalis*; g. *Metrosideros excelsa*; h. *Metrosideros excelsa* cv. Aurea; i. *Metrosideros* sp.

(Posada & Crandall 1998) and bootstrap analyses were run for 10 000 replicates.

PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10, Swofford 2002) was used to analyse the DNA sequence datasets. Phylogenetic relationships were estimated by heuristic searches with 1 000 random addition sequences and tree bisection-reconnection was used, with the branch swapping option set on 'best trees' only. All characters were weighted 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 analysis (Hillis & Bull 1993) was based on 1 000 replications.

A second phylogenetic analysis using a Markov Chain Monte Carlo (MCMC) algorithm was done to generate trees with Bayesian probabilities in MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). Nucleotide substitution models were determined using MrModeltest (Nylander 2004) for each gene region and included in the analyses. Two analyses of four MCMC chains were run from random trees for one million generations and sampled every 100 generations. All runs converged on the same likelihood score and tree topology and therefore the first 800 trees were discarded as the burn-in phase of each analysis and posterior probabilities determined from the remaining trees.

The phylogenetic analyses included 46 partial gene sequences for each gene region, representing 20 *Calonectria* spp. (Table 1). *Calonectria colombiensis* (CBS 112221) and *C. chinensis* (CBS 112744) were used as outgroup taxa in both analyses (Lombard et al. 2009). All novel sequences were deposited in GenBank and the alignments in TreeBASE (http://www.treebase.org).

Taxonomy

Morphological characterisation of the *Calonectria* isolates was done using single conidial cultures prepared on MEA and synthetic nutrient-poor agar (SNA; Nirenburg 1981, Lombard et al. 2009). Inoculated plates were incubated at room temperature and examined after 7 d. Gross morphological characteristics of the anamorph state were determined by mounting fungal structures in clear lactic acid and 30 measurements at ×1 000 magnification were made for each isolate using a Zeiss Axioscope 2 microscope with 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. Colony characteristics were noted after 7 d of growth on MEA at 24 °C and colony colours determined using the colour charts of Rayner (1970). Descriptions, nomenclature and illustrations were deposited in MycoBank (Crous et al. 2004).

Pathogenicity

In order to test the pathogenicity of the *Calonectria* spp. collected in this study, seven isolates representing different *Calonectria* species identified by morphology and DNA sequence comparisons were selected for inoculation trials (Table 1). A conidial suspension (1.0×10^5 conidia/mL) was prepared for each isolate by adding sterile water to plates of carnation leaf agar (CLA; Fisher et al. 1982) 7 d after inoculation and dislodging the conidia. The conidial suspension was sprayed onto the canopy (until run-off) of potted 2–6 mo old plants of *Callistemon citrinus* cv. Splendens, *C. laevis*, *C. viminalis*, *Dodonaea viscose*, *Metrosideros excelsa*, *M. excelsa* cv. Aurea, *M. thomasii*, *Myrtus communis*, *M. communis* subsp. *tarentina*. The conidial suspension of the isolate CBS 130351 was also applied to the crown of *M. communis* plants (10 mL/plant). All plants were subsequently covered with plastic bags for 48 h and maintained

Table 1 *Calonectria* isolates used in the phylogenetic analyses and pathogenicity trials.

Species	Isolate number[1]	β-tubulin[2]	Histone H3[2]	TEF-1α[2]	Host/substrate
Calonectria brasiliensis	CBS 230.30[4]	GQ267241	GQ267259	GQ267328	*Eucalyptus* sp.
	CBS 114257	GQ267242	GQ267260	GQ267329	Leaf litter
C. cerciana	CBS 123693[4]	FJ918510	FJ918528	FJ918559	*E. grandis* × *urophylla*
	CBS 123695	FJ918511	FJ918529	FJ918560	*E. grandis* × *urophylla*
C. chinensis	CBS 112744	AY725618	AY725660	AY725709	Soil
C. colombiana	CBS 115127[4]	FJ972423	FJ972442	FJ972492	Soil
	CBS 115638	FJ972422	FJ972441	FJ972491	Soil
C. colombiensis	CBS 112220[4]	GQ267207	AY725662	AY725711	Soil
C. hawksworthii	CBS 111870[4]	AF333407	DQ190649	FJ918558	*Nelumbo nucifera*
C. insularis	CBS 114558[4]	AF210861	FJ918526	FJ918556	Soil
	CBS 114559	AF210862	FJ918525	FJ918555	Soil
C. leucothoës	CBS 109166	FJ918508	FJ918523	FJ918553	*Leucothoë axillaris*
C. mexicana	CBS 110918[4]	AF210863	FJ972460	FJ972526	Soil
	CBS 130353[3]	JN607280	JN607265	JN607295	*Dodonaea viscosa*
C. morganii	CBS 110666	FJ918509	FJ918527	FJ918557	*Ilex vomitoria*
	CBS 119669	DQ521599	DQ521601	GQ421796	*Pistacia lentiscus*
C. pauciramosa	CPC 971	FJ918514	FJ918531	FJ918565	*E. grandis*
	CPC 416	FJ918515	FJ918532	FJ918566	*E. grandis*
C. polizzii	CBS 123402[4]	FJ972419	FJ972438	FJ972488	*Arbutus unedo*
	CBS 125270	FJ972417	FJ972436	FJ972486	*Callistemon citrinus*
	CBS 130351[3]	JN607270	JN607255	JN607285	*Myrtus communis*
	CBS 130352[3]	JN607275	JN607260	JN607290	*Metrosideros thomasii*
	DISTEF-TMC2	JN607269	JN607254	JN607284	*Myrtus communis*
	DISTEF-TMEA1	JN607272	JN607257	JN607287	*Metrosideros excelsa* cv. Aurea
	DISTEF-TMN3	JN607274	JN607259	JN607289	*Metrosideros* sp.
C. pseudomexicana	**CBS 130354**[3,4]	JN607281	JN607266	JN607496	*Callistemon* sp. (rouge)
	CBS 130355[3]	JN607282	JN607267	JN607497	*Callistemon* sp. (rouge)
	DISTEF-TCROU4	JN607283	JN607268	JN607498	*Callistemon* sp. (rouge)
C. pseudoscoparia	CBS 125256	GQ267228	GQ267277	GQ267348	*E. grandis*
	CBS 125257[4]	GQ267229	GQ267278	GQ267349	*E. grandis*
C. scoparia	CPC 1675	FJ972426	FJ972476	FJ972525	*Eucalyptus* sp.
	CPC 1679	FJ972427	GQ267246	GQ267298	*Eucalyptus* sp.
C. spathulata	CBS 112689	AF308463	FJ918524	FJ918554	*E. viminalis*
	CBS 555.92[4]	GQ267215	GQ267261	GQ267331	*Araucaria angustifolia*
C. sulawesiensis	CBS 125248	GQ267223	GQ267272	GQ267343	*Eucalyptus* sp.
	CBS 125253	GQ267220	GQ267269	GQ267340	*Eucalyptus* sp.
C. tunisiana	**CBS 130356**[3]	JN607277	JN607262	JN607292	*Callistemon* sp. (rouge)
	CBS 130357[3,4]	JN607276	JN607261	JN607291	*Callistemon laevis*
	DISTEF-TCV1	JN607278	JN607263	JN607293	*Callistemon viminalis*
	DISTEF-TCROS4	JN607279	JN607264	JN607294	*Callistemon* sp. (rosè)
	DISTEF-TME1	JN607271	JN607256	JN607286	*Metrosideros excelsa*
	DISTEF-TMN1	JN607273	JN607258	JN607288	*Metrosideros* sp.
C. variabilis	CBS 112691	GQ267240	GQ267264	GQ267335	*Eucalyptus* sp.
	CBS 114677	AF333424	GQ267263	GQ267334	*Eucalyptus* sp.
C. zuluensis	CBS 125268[4]	FJ972414	FJ972433	FJ972483	*Eucalyptus* sp.
	CBS 125272	FJ972415	FJ972434	FJ972484	*Eucalyptus* sp.

[1] CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Pedro Crous working collection housed at CBS; DISTEF: Dipartimento di Scienze e Tecnologie Fitosanitarie, Catania, Italy.
[2] GenBank accession numbers.
[3] Isolates used for the pathogenicity trials.
[4] Ex-type cultures; Isolates in **bold** obtained during survey.

in a growth chamber at 25 ± 1 °C for 14 d. Five plants for each isolate and host were used and the same number of control plants were treated using sterile water. Pathogenicity tests were evaluated 5, 10 and 25 d after inoculation.

RESULTS

Disease survey and isolates

During the survey, a total of 46 *Calonectria* isolates were collected from ornamental hosts sampled. Majority of the isolates (41) were associated with leaf spots or leaf blight of *Callistemon* spp. (18), *D. viscosa* (1), *Metrosideros* spp. (17) and *Myrtus communis* (5), and the remaining (5) with crown and root rot of *M. communis*. Leaves showed minute brown spots, which often enlarged, forming a necrotic centre surrounded by a dark purple halo (Fig. 1). Young, non-lignified terminal shoots often exhibited dieback or lesions similar to those on the leaves. Severe defoliation was observed on *M. communis* and *M. excelsa* cv. Aurea. Several seedlings of *M. communis* had crown and root rot, and fungal sporulation occurred on the lower part of the crown. Initial symptoms were brown lesions that expanded rapidly to girdle the stem at the seedling crown, above and below the soil line, resulting in plant death.

DNA sequence comparisons

Amplicons of approximately 450 bases for HIS3 and 500 bases each for BT and TEF-1α were generated. The 70 % reciprocal bootstrap trees showed no conflict in tree topologies for the three gene regions and therefore they were combined in a dataset consisting of 1 532 characters including gaps. Of these characters, 1 187 were constant and parsimony uninformative. Analysis of the 345 parsimony informative characters yielded 16 equally most parsimonious trees (TL = 814, CI = 0.721, RI = 0.923, RC = 0.666), of which the first tree is presented (Fig. 2). A HKY+I model for BT, a GTR+I+G model for HIS3 and a GTR+G model for TEF-1α was selected for Bayesian analysis. The Bayesian consensus tree confirmed the tree topology obtained with maximum parsimony including bootstrap support.

The phylogenetic tree illustrates a number of well-supported clades containing the *Calonectria* isolates obtained during the survey. Some of the isolates clustered in a clade representing *C. polizzii* with a bootstrap value (BP) of 97 and a Bayesian posterior probability (PP) value of 1.00. Several isolates also grouped with and close to *C. mexicana* in two separate well-supported clades (BP = 68, PP = 0.95 and BP = 78, PP = 0.98, respectively), which could represent novel phylogenetic species.

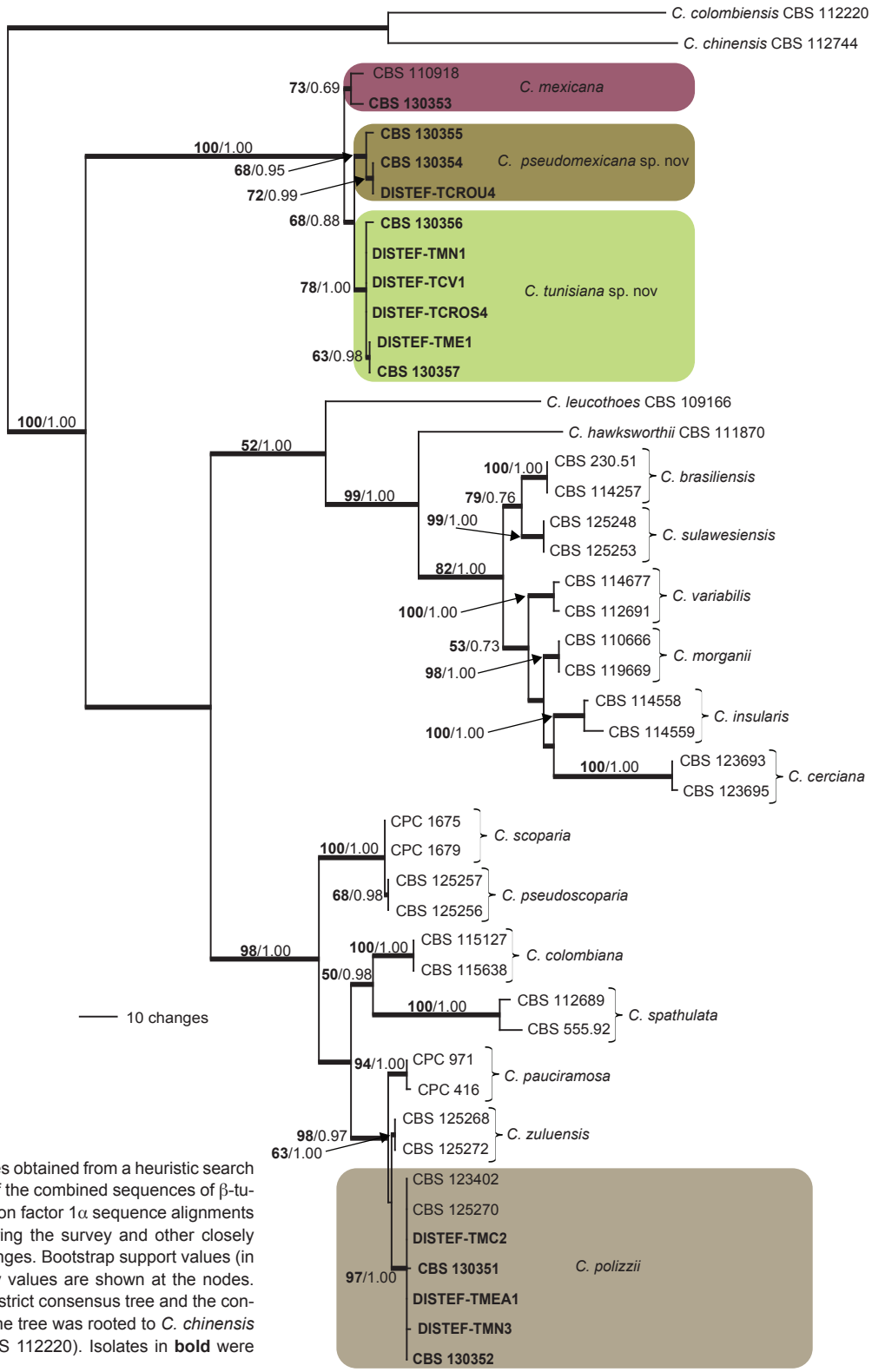

Fig. 2 One of 16 most parsimonious trees obtained from a heuristic search with 1 000 random addition sequences of the combined sequences of β-tubulin, histone H3 and translation elongation factor 1α sequence alignments of the *Calonectria* isolates obtained during the survey and other closely related species. Scale bar shows 10 changes. Bootstrap support values (in **bold**) and Bayesian posterior probability values are shown at the nodes. Thickened lines indicate branches in the strict consensus tree and the consensus tree of the Bayesian analyses. The tree was rooted to *C. chinensis* (CBS 112744) and *C. colombiensis* (CBS 112220). Isolates in **bold** were obtained during the survey.

Taxonomy

DNA sequence and morphological comparisons of the *Calonectria* isolates obtained during the survey show that these isolates belong to *C. mexicana* and *C. polizzii* and also constitute two previously undescribed taxa. Based on morphological comparisons, isolate CBS 130353 agrees with *C. mexicana* (Schoch et al. 1999) and isolates DISTEF-TMC2, CBS 130351, DISTEF-TMEA1, DISTEF-TMN3 and CBS 130352 represent *C. polizzii* (Lombard et al. 2010b). The remaining isolates are newly described as follows:

Calonectria pseudomexicana L. Lombard, G. Polizzi & Crous, *sp. nov*. — MycoBank MB563138; Fig. 3

Teleomorph unknown.

Calonectria mexicana morphologice similes sed minus ramis conidiophorae.

Etymology. Name reflects the fact that this species closely resembles *C. mexicana*.

Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 38–69 × 5–9 µm; stipe extensions septate, straight to flexuous, 175–251 µm long, 3–6 µm wide at the apical septum, terminating in a fusiform to broadly ellipsoidal vesicle

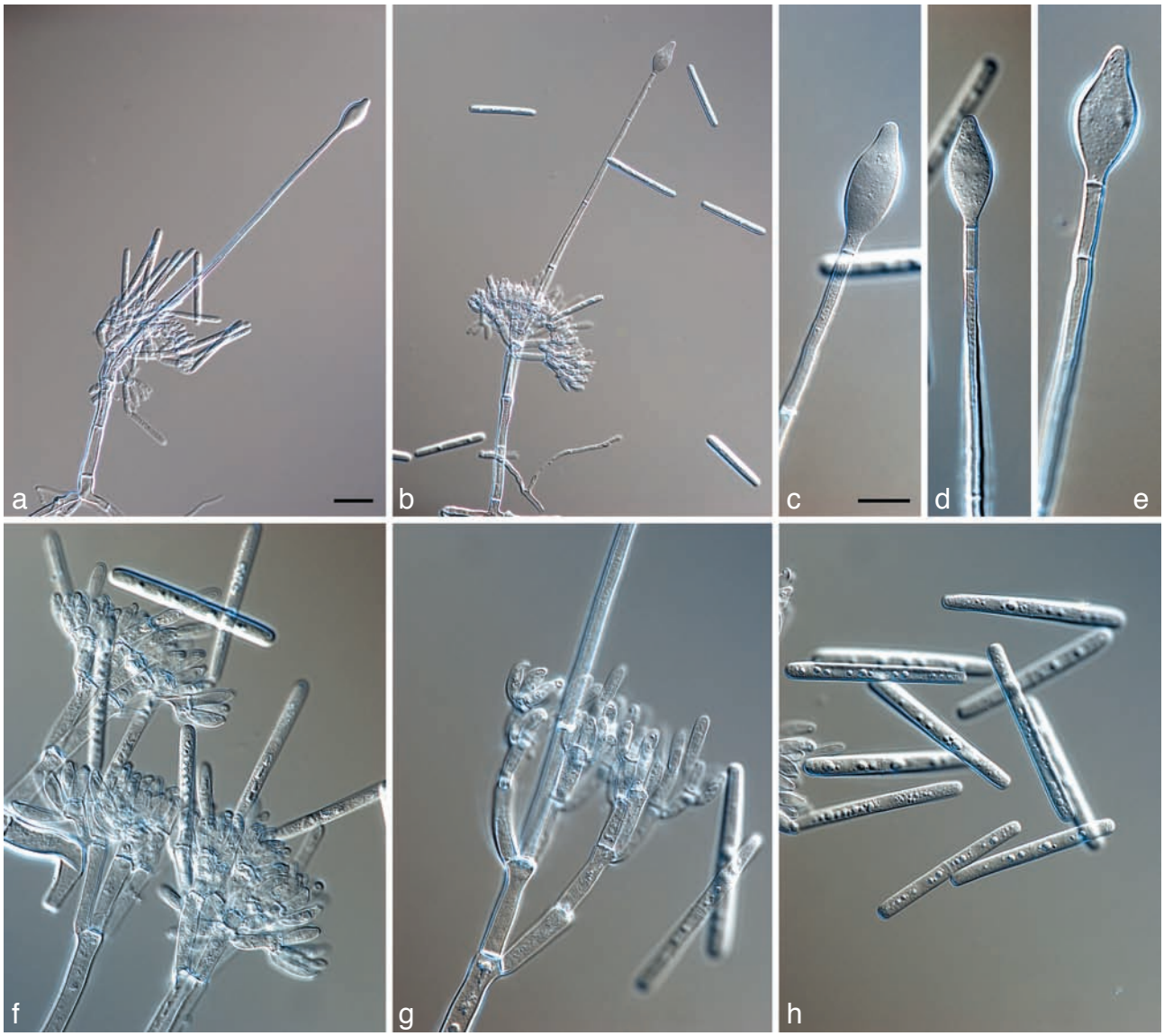

Fig. 3 *Calonectria pseudomexicana*. a, b. Macroconidiophores; c–e. fusiform to broadly ellipsoidal vesicles with papillate apices; f, g. conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides; h. 1-septate macroconidia. — Scale bars = 10 µm.

9–14 µm diam with papillate apex. *Conidiogenous apparatus* 38–68 µm long, 32–64 µm wide; primary branches aseptate or 1-septate, 21–43 × 4–7 µm; secondary branches aseptate, 13–26 × 4–7 µm; tertiary branches and additional branches (–4), aseptate, 10–18 × 2–6 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 6–14 × 2–6 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight, (40–)43–48(–49) × (4–)5–6 µm (av. = 45 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Megaconidia and microconidia not seen.

Culture characteristics — Colonies fast growing at 24 ºC on MEA, sienna to bay on surface, reverse sienna after 7 d; moderate white aerial mycelium with sparse to moderate sporulation; chlamydospores extensive throughout medium.

Specimens examined. Tunisia, Carthage, Tunis, from *Callistemon* sp., Nov. 2010, *G. Polizzi*, (CBS H-20685, holotype of *C. pseudomexicana*, culture ex-type CBS 130354 = DISTEF-TCROU1); Carthage, Tunis, from *Callistemon* sp., Nov. 2010, *G. Polizzi*, culture CBS 130355 = DISTEF-TCROU3; Carthage, Tunis, from *Callistemon* sp., Nov. 2010, *G. Polizzi*, culture DISTEF-TCROU4.

Notes — *Calonectria pseudomexicana* is morphologically similar to *C. mexicana*. *Calonectria pseudomexicana* has four or less conidiophore branches while *C. mexicana* has five as reported by Schoch et al. (1999).

Calonectria tunisiana L. Lombard, G. Polizzi & Crous, *sp. nov*. — MycoBank MB563139; Fig. 4

Teleomorph unknown.

Calonectria mexicana morphologice similes sed minus conidiophorae ramis et fructibus breviores sunt stipe augue.

Etymology. Name refers to the country Tunisia, where the fungus was collected.

Conidiophores with a stipe bearing penicillate suites of fertile branches, stipe extensions, and terminal vesicles; stipe septate, hyaline, smooth, 42–95 × 7–11 µm; stipe extensions septate, straight to flexuous, 147–199 µm long, 4–5 µm wide at the apical septum, terminating in a fusiform to broadly ellipsoidal vesicle 8–14 µm diam with papillate apex. *Conidiogenous apparatus* 40–68 µm long, 30–66 µm wide; primary branches aseptate or 1-septate, 17–41 × 5–7 µm; secondary branches aseptate, 10–22 × 4–7 µm; tertiary branches aseptate, 9–18 × 4–5 µm, each terminal branch producing 2–6 phialides; phialides doliiform to reniform, hyaline, aseptate, 8–13 × 3–5 µm; apex with minute periclinal thickening and inconspicuous collarette. *Conidia* cylindrical, rounded at both ends, straight,

Fig. 4 *Calonectria tunisiana*. a, b. Macroconidiophores; c–e. fusiform to broadly ellipsoidal vesicles with papillate apices; f, g. conidiogenous apparatus with conidiophore branches and doliiform to reniform phialides; h. 1-septate macroconidia. — Scale bars = 10 µm.

(43–)47–51(–53) × 4–6 µm (av. = 49 × 5 µm), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. Megaconidia and microconidia not seen.

Culture characteristics — Colonies fast growing at 24 °C on MEA, sienna to bay on surface, and reverse sienna after 7 d; sparse white aerial mycelium with sparse sporulation; chlamydospores extensive throughout the medium.

Specimens examined. Tunisia, Carthage, Tunis, from *Callistemon laevis*, Nov. 2010, *G. Polizzi*, (CBS H-20684, holotype of *C. tunisiana*, culture ex-type CBS 130357 = DISTEF-TCL1); Carthage, Tunis, from *Callistemon* sp., Nov. 2010, *G. Polizzi*, culture CBS 130356 = DISTEF-TCROU2; Carthage, Tunis, from *Metrosideros excelsus*, Nov. 2010, *G. Polizzi*, culture DISTEF-TME1.

Notes — Morphologically, *C. tunisiana* is similar to *C. mexicana* and *C. pseudomexicana*, but can be distinguished from both taxa by its shorter stipe extensions. The conidiophores of *C. tunisiana* (–3) also form fewer fertile branches than *C. mexicana* (–5) and *C. pseudomexicana* (–4) (Schoch et al. 1999).

Pathogenicity

All plants inoculated with the *Calonectria* spp. in this study developed leaf spot, leaf blight or crown and root rot symptoms. The first symptoms of leaf spot and leaf blight were observed 5 d after inoculation on all test plants inoculated with the *Calonectria* spp., resembling the symptoms observed during the survey. Isolates of *C. pseudomexicana* (CBS 130354, 130355), *C. tunisiana* (CBS 130356, 130357) as well as the single isolate of *C. mexicana* (CBS 130353) produced the most severe symptoms. Isolates of *C. polizzii* (CBS 130351, 130352) also caused leaf spot and leaf blight on all inoculated plants, but less severe than the other three *Calonectria* spp. tested. Ten days after inoculation, severe or moderate defoliation of *M. communis* and *M. excelsa* cv. Aurea plants was observed.

All inoculated plants of *M. communis* developed crown rot, basal stem rot and root rot 25 d after inoculation with the isolate representing *C. polizzii* (CBS 130351). All un-inoculated control plants remained healthy and re-isolations from the test plants consistently yielded the test fungi.

DISCUSSION

During a survey of diseased plants at an ornamental nursery in Tunis, Tunisia, a number of *Calonectria* spp. were isolated from plants exhibiting crown, root rot and leaf spots. DNA sequence and morphological comparisons allowed the identification of two of these isolates as *C. mexicana* and *C. polizzii* as well as the description of two new species, *C. pseudomexicana* and *C. tunisiana*, both in the *C. scoparia* complex (Schoch et al. 1999).

Calonectria mexicana resides in the *C. scoparia* complex (Schoch et al. 1999) and can be distinguished from the other seven *Calonectria* spp. in the complex based on their unique papillate vesicles (Schoch et al. 1999, Lombard et al. 2010b, c, Chen et al. 2011). Until now, *C. mexicana* has only been reported from soil samples collected in Mexico, and its pathogenicity was unknown (Schoch et al. 1999, Crous 2002). This study represents the first report of this fungus outside Mexico, and also demonstrates its pathogenicity on some plant hosts.

Calonectria polizzii has previously been reported from ornamental plants collected at a nursery in Sicily, Italy (Schoch et al. 2001, Lombard et al. 2010b), although its pathogenicity was not confirmed. This study represents the first confirmation of the pathogenicity of *C. polizzii* and widens its distribution to Tunisia. *Calonectria polizzii* is a member of the *C. scoparia* complex and can be distinguished from the other members by its smaller macroconidial dimensions (Lombard et al. 2010b).

The description of *C. pseudomexicana* and *C. tunisiana* adds two more species to the *C. scoparia* complex. This complex is characterised by 1-septate macroconidia and the formation of ellipsoidal to obpyriform terminal vesicles on the stipe extensions (Schoch et al. 1999, Crous 2002, Lombard et al. 2010b). Based on phylogenetic inference, both these newly described species are closely related to *C. mexicana*, which they also resemble in morphology. They can be distinguished from *C. mexicana* and each other by the number of fertile branches produced on the conidiophores. *Calonectria tunisiana* (av. = 49 × 5 µm) has slightly larger macroconidia than both *C. mexicana* (av. = 45 × 4 µm; Schoch et al. 1999) and *C. pseudomexicana* (av. = 45 × 5 µm).

The pathogenicity tests with isolates of *C. mexicana*, *C. polizzii*, *C. pseudomexicana* and *C. tunisiana* clearly showed that they are able to cause symptoms similar to those observed during the survey. *Calonectria polizzii* was less virulent than the other three species, but should still be regarded as an important nursery pathogen. This supports the view that most *Calonectria* spp. can induce leaf spots if the environmental conditions are favourable (Crous 2002). All four species caused similar disease symptoms on the nine inoculated plant species, suggesting that little is known about the host specificity and mechanisms of infection of this group of plant pathogens.

This study stresses the importance of *Calonectria* spp. as nursery pathogens. Their soil-borne nature has contributed to their ease of movement globally and little is known about their origins. Furthermore, it is not known if these fungal pathogens originated from Tunisia or were introduced, and more isolates are needed for a study of their population dynamics.

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