

Calonectria metrosideri, a highly aggressive pathogen causing leaf blight, root rot, and wilt of *Metrosideros* spp. in Brazil

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Summary

The genus *Metrosideros* includes several tree, shrub and vine species, native to the Pacific Islands. Seedlings from 25 seed lots of *Metrosideros polymorpha* and two seed lots of *M. tremulooides* with symptoms of root rot, stem girdling, wilting and round, purple leaf spots were observed in the Forestry Nursery at the Universidade Federal de Viçosa, Brazil. In the original disease site, seedling mortality reached up to 71% in *M. polymorpha* and 34% in *M. tremulooides*. Single conidial cultures obtained from infected leaf, root and stem samples of *M. polymorpha* were used to identify the fungal species. Morphological characters and DNA sequences of four loci, containing partial sequences of β -tubulin (TUB2), histone H3 (HIS3), calmodulin (CAL) and the elongation factor (*tef-1x*) genes of three isolates, indicated that they belong to a new species, described here as *Calonectria metrosideri* sp. nov. Potting medium infestation and inoculation of seedlings of *M. polymorpha* with an inoculum suspension at 1×10^4 conidia ml⁻¹ induced typical symptoms of the disease (leaf spots, root rot and wilt), similar to those observed under natural conditions. *Calonectria metrosideri* was re-isolated, which fulfilled Koch's postulates, and confirmed its status as a pathogen.

1 Introduction

Metrosideros is a genus that includes several tree, shrub and vine species native to the Pacific Islands from the Philippines to New Zealand. *Metrosideros polymorpha* Gaudich, popularly known as ohia (Fig. 1) is the species dominant in Hawaiian ecosystems, occupying a wide variety of habitats (Cordell et al. 1998). This species and others of this genus can be used for medicinal purposes, wood production for energy, poles and several other uses (Friday and Herbert 2006).

In Hawaii, approximately 80% of native forests are composed of species of *Metrosideros*, especially *M. polymorpha* (Uchida et al. 2006). In April 2005, a rust fungus (*Puccinia psidii* Winter), a highly damaging pathogen in myrtaceous hosts in South America, was found on plants of *Metrosideros* spp. in Hawaii, and this rust pathogen is considered a threat to Hawaiian forest ecosystems (Uchida et al. 2006). Subsequently, half-sib families of Hawaiian ohia seeds were germinated and grown in Brazil to assess the genetic resistance to Brazilian strains of *P. psidii*. However, during a routine inspection at the nursery, seedlings of *M. polymorpha* showing symptoms of leaf spots, defoliation, young leaf wilt and seedling death were recorded (Fig. 2). In addition, stem necrosis and girdling with root rot were observed. Seedlings with the above disease symptoms kept in a moist chamber showed intense sporulation of a *Calonectria* sp. on the lesions. Thus, the objective of this study was to characterize the causal agent of this disease through a combination of morphological and molecular data and pathogenicity tests.

2 Material and methods

2.1 Sampling and fungal isolation

Samples of infected plants of *M. polymorpha*, containing round, purplish leaf spots, and stem cankers and root rot, were collected in the Forest Nursery at the Universidade Federal de Viçosa.

The samples were kept in a moist chamber at 26°C for 48 h. After incubation, single conidial cultures of the *Calonectria* sp. were obtained on malt extract agar (MEA) at 26°C for 10 days. Three selected isolates (LPF 101, LPF 103 and LPF 104) used in this study were deposited at CBS Fungal Biodiversity Institute in the Netherlands (CBS), and nomenclatural data were submitted to MycoBank (Crous et al. 2004b).

2.2 DNA extraction, amplification and purification

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 Corporation, WI, USA) kit. For PCR, the DreamTaq[™] Master Mix (MBI Fermentas, Vilnius, Lithuania) was used, following the manufacturer's protocol.

Four loci, including fragments of β -tubulin (TUB2), histone H3 (HIS3), elongation factor (*tef-1x*) and calmodulin (CAL) gene regions were amplified using the primers T1 (O'Donnell and Cigelnik 1997) and CYLTUB1R (Crous et al. 2004a) for

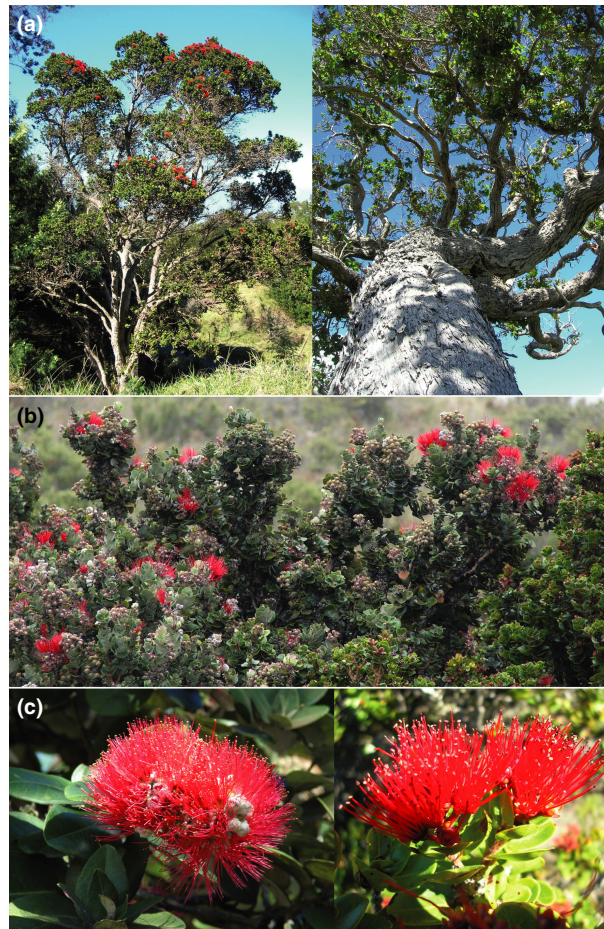


Fig. 1. *Meterosideros polymorpha* in natural stands: (a) Adult trees; (b) Flowering plants; (c) Typical red flowers. (Photos: Forest & Kim Starr, Starr Environmental, Bugwood.org).

TUB2, CYLH3F and CYLH3R (Crous et al. 2004a) for HIS3, EF1-728F (O'Donnell et al. 1998) and EF-2 (Carbone and Kohn 1999) for TEF-1 α and CAL-228F and CAL-737R (Carbone and Kohn 1999) for CAL. 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 initial 72°C for 1 min and 4 min final extension at 72°C. The PCR product was visualized on a 2% agarose gel, to determine fragment size and purity. PCR products were purified with an ExoSAP-IT[®] kit, according to the manufacturer's recommended protocol (2 μ l reagent per 5 μ l amplified DNA product) and incubated in a thermal cycler for 15 min at 37°C followed by an additional incubation for 15 min at 80°C.

2.3 Sequencing and phylogenetic analysis

Sequencing was performed at the Laboratory of Genomics in the Institute of Biotechnology Applied to Agriculture (BIO-AGRO) at the Universidade Federal de Viçosa, Brazil. Sequences quality was checked by means of Sequence Scanner software v. 1.0 (Applied Biosystems, Foster City, CA, USA) and edited using the software package SeqMan from DNASTar Inc. (Madison, WI, USA, www.DNASTAR.com) All sequences were manually corrected, and the arrangement of nucleotides in ambiguous positions was corrected using the sequences of primers in the forward and reverse directions. New sequences derived from this study were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>), and other sequences used in phylogenetic analysis were obtained from GenBank (Table 1).

Consensus regions were compared in the GenBank database using the Mega BLAST program. Based on the results of the BLAST, new sequences were added to the alignment of Lombard et al. (2011). All sequences were assembled in the MAFFT v. 6 online version (<http://mafft.cbrc.jp/alignment/server/>) (Katoh and Toh 2010), and aligned sequences were then manually corrected when necessary using MEGA v. 5 (Tempe, AZ, USA) (Tamura et al. 2011). Spaces (gaps) (insertions/deletions) were treated as absent.

PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Sunderland, MA, USA; Swofford 2002) was used to analyse the DNA sequence data sets. A partition homogeneity test (Farris et al. 1994) and a 70% reciprocal bootstrap method (Gueidan et al. 2007) were applied to determine whether the data sets were consistent and combinable. Phylogenetic relationships

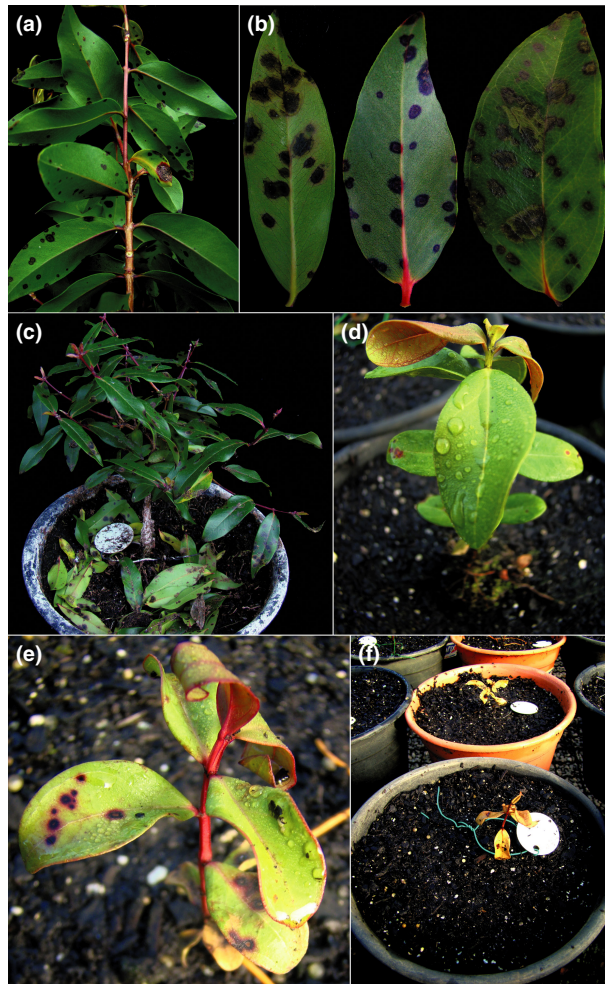


Fig. 2. Incidence of *Calonectria metrosideri* on seedlings of *Metrosideros polymorpha* in nursery. a–b: Leafspots; c–Defoliation; d–e: Wilting of young leaves on seedlings; f: Dead seedlings.

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 consistency index (RC). Bootstrap analyses (Hillis and Bull 1993) were based on 1 000 replications.

Analysis of Bayesian inference (BI) was performed using the algorithm of Markov chain Monte Carlo (MCMC), and the model of nucleotide substitution used was determined using the MrModeltest v. 2.3 (Posada and Crandall 1998). The models were estimated separately for each gene region. The likelihood values were calculated, and the model was selected according to Akaike information criterion (AIC). BI analysis was completed with MrBayes v. 3.1.1 (Ronquist and Heulsenbeck 2003) with 10 million random generations. Trees were sampled at every 1 000 generations, resulting in 10 000 trees. The first 2 500 trees were discarded from the analysis. The posterior likelihood values (Rannala and Yang 1996) were determined using the consensus tree. The convergence of the log likelihood was analysed using the software TRACER v. 1.4.1 (Auckland, New Zealand; Rambaut & Drummond 2009), and no indication of lack of convergence was detected. *Calonectria colombiensis* Crous and *Calonectria chinensis* (Crous) L. Lombard, M.J. Wingf. & Crous were used as outgroups in the analysis.

2.4 Morphological characterization

Single conidial cultures were grown on synthetic nutrient poor agar (SNA) (Nirenburg 1981) at 26°C, following the protocols set for *Calonectria* by Lombard et al. (2009, 2010b,c). After 7 days of incubation, the morphological characteristics were determined by mounting fungal structures in clear lactic acid, and 30 measurements at $\times 1\,000$ magnification were determined for each isolate using a Zeiss Axioscope 2 microscope (Jena, Germany) 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.

Table 1. Accession numbers, *Calonectria* species, gene regions sequenced of *Calonectria* spp. and host/substrate columns.

Isolates	Species ¹	GenBank accession nr ²					Host/substrate
		β – tubulin (<i>TUB2</i>)	Histone 3 (<i>HIS3</i>)	Elongation factor (<i>tef-1α</i>)	Calmodulin (<i>CAL</i>)		
CBS 230.30	<i>C. brasiliensis</i>	GQ267241	GQ267259	GQ267328	GQ267421	<i>Eucalyptus</i> sp.	
CBS 114257	<i>C. brasiliensis</i>	GQ267242	GQ267260	GQ267329	GQ267422	Leaf litter	
CBS 123693	<i>C. cerciana</i>	FJ918510	FJ918528	FJ918559	GQ267369	<i>E. grandis</i> \times <i>urophylla</i>	
CBS 123695	<i>C. cerciana</i>	FJ918511	FJ918529	FJ918560	GQ267370	<i>E. grandis</i> \times <i>urophylla</i>	
CBS 112744	<i>C. chinensis</i>	AY725618	AY725660	AY725709	AY725746	Soil	
CBS 115127	<i>C. colombiana</i>	FJ972423	FJ972442	FJ972492	GQ267455	Soil	
CBS 115638	<i>C. colombiana</i>	FJ972422	FJ972441	FJ972491	GQ267456	Soil	
CBS 112220	<i>C. colombiensis</i>	GQ267207	AY725662	AY725711	AY725748	Soil	
CBS 111870	<i>C. hawksworthii</i>	AF333407	DQ190649	FJ918558	GQ267386	<i>Nelumbo nucifera</i>	
CBS 114558	<i>C. insularis</i>	AF210861	FJ918526	FJ918556	GQ267389	Soil	
CBS 114559	<i>C. insularis</i>	AF210862	FJ918525	FJ918555	GQ267390	Soil	
CBS 109166	<i>C. leucothoës</i>	FJ918508	FJ918523	FJ918553	GQ267392	<i>Leucothoë axillaris</i>	
CBS 110918	<i>C. mexicana</i>	AF210863	FJ972460	FJ972526	GQ267396	Soil	
CBS 1303533	<i>C. mexicana</i>	JN607280	JN607265	JN607295	–	<i>Dononaea viscosa</i>	
CBS 110666	<i>C. morganii</i>	FJ918509	FJ918527	FJ918557	GQ267423	<i>Ilex vomitoria</i>	
CBS 119669	<i>C. morganii</i>	DQ521599	DQ521601	GQ421796	–	<i>Pistacia lentiscus</i>	
CMW 5683	<i>C. pauciramosa</i>	FJ918514	FJ918531	FJ918565	GQ267405	<i>E. grandis</i>	
CPC 416	<i>C. pauciramosa</i>	FJ918515	FJ918532	FJ918566	GQ267404	<i>E. grandis</i>	
CBS 123402	<i>C. polizzii</i>	FJ972419	FJ972438	FJ972488	–	<i>Arbutus unedo</i>	
CBS 125270	<i>C. polizzii</i>	FJ972417	FJ972436	FJ972486	GQ267461	<i>Callistemon citrinus</i>	
CBS 1303513	<i>C. polizzii</i>	JN607270	JN607255	JN607285	–	<i>Myrtus communis</i>	
CBS 1303523	<i>C. polizzii</i>	JN607275	JN607260	JN607290	–	<i>Metrosideros thomasi</i>	
DISTEF-TMC2	<i>C. polizzii</i>	JN607269	JN607254	JN607284	–	<i>Myrtus communis</i>	
DISTEF-TMEA1	<i>C. polizzii</i>	JN607272	JN607257	JN607287	–	<i>Metrosideros excelsa</i> cv. Aurea	
DISTEF-TMN3	<i>C. polizzii</i>	JN607274	JN607259	JN607289	–	<i>Metrosideros</i> sp.	
CBS 1303543	<i>C. pseudomexicana</i>	JN607281	JN607266	JN607496	–	<i>Callistemon</i> sp. (rouge)	
CBS 1303553	<i>C. pseudomexicana</i>	JN607282	JN607267	JN607497	–	<i>Callistemon</i> sp. (rouge)	
DISTEF-TCROU4	<i>C. pseudomexicana</i>	JN607283	JN607268	JN607498	–	<i>Callistemon</i> sp. (rouge)	
CBS 125256	<i>C. pseudoscoparia</i>	GQ267228	GQ267277	GQ267348	GQ267440	<i>E. grandis</i>	
CBS 125257	<i>C. pseudoscoparia</i>	GQ267229	GQ267278	GQ267349	GQ267441	<i>E. grandis</i>	
CPC 1675	<i>C. scoparia</i>	FJ972426	FJ972476	FJ972525	GQ267367	<i>Eucalyptus</i> sp.	
CPC 1679	<i>C. scoparia</i>	GQ421779	GQ267246	GQ267298	GQ267368	<i>Eucalyptus</i> sp.	
CBS 133603	<i>C. metrosideri</i> sp. nov	KC294313	KC294307	KC294310	KC294304	<i>Metrosideros</i> <i>polymorpha</i> (leaf)	
CBS 133604	<i>C. metrosideri</i> sp. nov	KC294314	KC294308	KC294311	KC294305	<i>Metrosideros</i> <i>polymorpha</i> (leaf)	
CBS 133605	<i>C. metrosideri</i> sp. nov	KC294315	KC294309	KC294312	KC294306	<i>Metrosideros</i> <i>polymorpha</i> (root)	
CBS 112689	<i>C. spathulata</i>	AF308463	FJ918524	FJ918554	GQ267426	<i>E. viminalis</i>	
CBS 555.92	<i>C. spathulata</i>	GQ267215	GQ267261	GQ267331	GQ267427	<i>Araucaria angustifolia</i>	
CBS 125248	<i>C. sulawesiensis</i>	GQ267223	GQ267272	GQ267343	GQ267435	<i>Eucalyptus</i> sp.	
CBS 125253	<i>C. sulawesiensis</i>	GQ267220	GQ267269	GQ267340	GQ267432	<i>Eucalyptus</i> sp.	
CBS 1303563	<i>C. tunisiana</i>	JN607277	JN607262	JN607292	–	<i>Callistemon</i> sp. (rouge)	
CBS 1303573	<i>C. tunisiana</i>	JN607276	JN607261	JN607291	–	<i>Callistemon laevis</i>	
DISTEF-TCV1	<i>C. tunisiana</i>	JN607278	JN607263	JN607293	–	<i>Callistemon viminalis</i>	
DISTEF-TCROSA4	<i>C. tunisiana</i>	JN607279	JN607264	JN607294	–	<i>Callistemon</i> sp. (rosè)	
DISTEF-TME1	<i>C. tunisiana</i>	JN607271	JN607256	JN607286	–	<i>Metrosideros excelsa</i>	
DISTEF-TMN1	<i>C. tunisiana</i>	JN607273	JN607258	JN607288	–	<i>Metrosideros</i> sp.	
CBS 112691	<i>C. variabilis</i>	GQ267240	GQ267264	GQ267335	GQ267458	<i>Eucalyptus</i> sp.	
CBS 114677	<i>C. variabilis</i>	AF333424	GQ267263	GQ267334	GQ267457	<i>Eucalyptus</i> sp.	
CMW 9188	<i>C. zuluensis</i>	FJ972414	FJ972433	FJ972483	GQ267459	<i>Eucalyptus</i> sp.	
CMW 9896	<i>C. zuluensis</i>	FJ972415	FJ972434	FJ972484	GQ267460	<i>Eucalyptus</i> sp.	

¹CBS, Culture collection of the Centraal bureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, Netherlands; CPC, Cultures of Pedro Crous housed at CBS; LPF, Laboratory of Forest Pathology; DFT-UFV, Viçosa Minas Gerais Brazil; isolate number in **bold** were sequenced in this study.

²GenBank Accession Number. – No sequences in GenBank.

2.5 Pathogenicity

Because the undescribed *Calonectria* species was isolated from infected *M. polymorpha*, this plant was selected to confirm pathogenicity. For this test, 10 seedlings were spray-inoculated with a conidial suspension at $1 \times 10^4 \text{ ml}^{-1}$ of each isolate, as described by Graça et al. (2009). Potting medium (Mec Plant® substrate, Telêmaco Borba, Paraná, Brazil) supporting 10 healthy seedlings was also infested by adding 30 ml of the same conidial suspension in each of the four holes made around each plant. Five plants treated with distilled water served as control. The development of symptoms was monitored daily for 10 days.

2.6 Source of inoculum

To determine the inoculum source of inoculum, samples of irrigation water and samples of unused/ used substrate were tested for the presence of the pathogen using the castor bean leaf bio-baiting method (Gonçalves et al. 2001).

2.7 Disease progress

Disease progress was evaluated on plants growing at the original nursery where the disease was discovered by counting the number of wilted or dead plants at biweekly intervals from April to July, 2010.

3 Results

3.1 Phylogenetic analysis

Amplicons of approximately 450 bases for HIS3 and 500 bases each for TUB2, TEF-1 α and CAL were generated. Based on preliminary *tef-1 α* sequence analyses with 49 taxa including outgroups (Fig. 3), the multigene analysis was performed with closely related species, which belong to the *Calonectria scoparia* complex.

The combined sequence analysis was performed with 18 taxa, including outgroups. Comparing the tree topologies of the 70% reciprocal bootstrap trees indicated no conflicts. Subsequently, the data sets were combined, and this resulted in a data set consisting of 1 899 characters including gaps. Of these, 1 634 were constant and parsimony uninformative, and

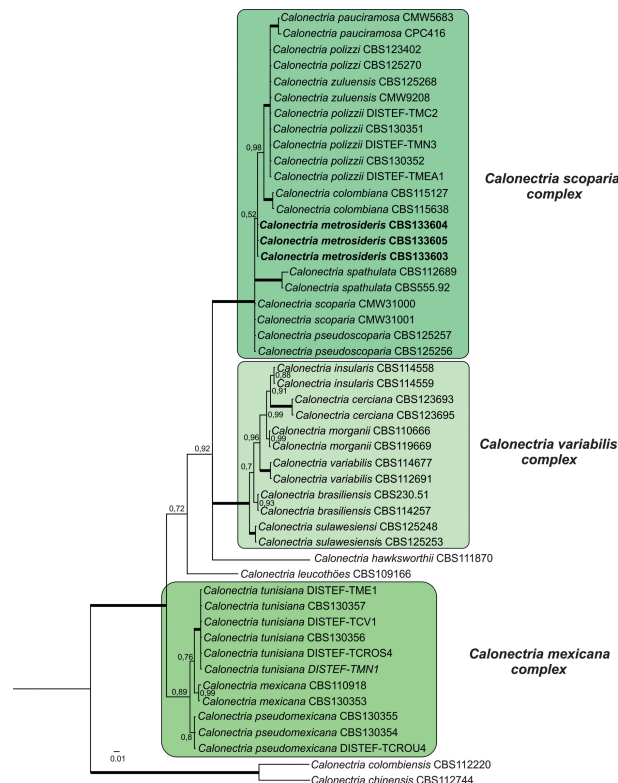


Fig. 3. Phylogenetic tree obtained by Bayesian inference using sequences of translation elongation factor. 1a sequence alignments of the *Calonectria* isolates. The bold lines indicate posterior probability values of 1.00. The tree was rooted to *C. chinensis* (CBS 112744) and *C. colombiensis* (CBS 112220). Isolates in bold were obtained during the survey.

295 were parsimony informative. Analysis of the 295 parsimony informative characters yielded four equally most parsimonious trees (TL = 561, CI = 0.904, RI = 0.911, RC = 0.823). Evolution models HKY+I for TUB2 and CAL, and GTR+G for HIS3 and *tef-1 α* were selected and incorporated into the Bayesian analysis.

The preliminary tree performed with *tef-1 α* can distinguish *Calonectria scoparia* complex from the other *Calonectria* complexes (*C. variabilis* and *C. mexicana*); however, it is not useful for separating *C. metrosideri* from other species within the *C. scoparia* complex (Fig. 3). The newly described *C. metrosideri* can be distinguished from other *Calonectria* spp. within the *C. scoparia* complex using an additional three loci (HIS3, TUB2 and CAL). The multigene analysis formed a distinct and well-supported clade close to but distinct from *C. pseudoscoparia* and *C. scoparia* (Fig. 4).

3.2 Taxonomy

Based on the DNA sequence data and morphological features of the anamorph, we conclude that the *Calonectria* isolates from *M. polymorpha* represent an undescribed new species, described below as follows:

Calonectria metrosideri R.F. Alfenas, O.L. Pereira, P.W. Crous & A.C. Alfenas, sp. nov. MycoBank MB 802511 (Fig. 5).

Etymology: In reference to the genus *Metrosideros*, from which the fungus was isolated.

Hosts: *Metrosideros polymorpha*.

Distribution: Brazil.

Conidiophores containing a stipe bearing penicillate suites of fertile branches, stipe extension and terminal vesicle; stipe septate, hyaline, smooth, 40–105 \times 4–7 μ m; stipe extensions septate, straight to flexuous, 90–170 μ m long, 2–4 μ m wide at the apical septum, terminating in spatulate to obpyriform vesicles, 5–9 μ m diam (abnormal bifurcate vesicles frequently observed). *Conidiogenous apparatus* 40–65 μ m long, 60–75 μ m wide; primary branches aseptate, 18–30 \times 4–5 μ m; secondary branches aseptate, 18–22 \times 3–4 μ m; tertiary and additional branches (–4), aseptate, 8–15 \times 3–4 μ m, each terminal branch producing 2–6 phialides; phialides elongate doliform to reniform, hyaline, aseptate, 8–11 \times 3–4 μ m; apex with minute periclinal thickening and inconspicuous collarette. *Macroconidia* cylindrical, rounded at both ends, straight, (40–)44–46 (–51) \times 3–5 μ m (av. = 45 \times 4 μ m), 1-septate, lacking a visible abscission scar, held in parallel cylindrical clusters by colourless slime. *Mega-* and *microconidia* were not seen observed.

Notes: *Calonectria metrosideri* (conidia av. 45 \times 4 μ m) can be distinguished from *C. scoparia* (conidia av. 60 \times 4.5 μ m) and *C. pseudoscoparia* (conidia av. 48 \times 4 μ m) based on smaller macroconidia and on being phylogenetically distinct. Mating tests resulted in no successful matings, suggesting that the fungus either is heterothallic, with no compatible tester strains found, or has lost the ability for sexual mating.

Culture characteristics: Rapid growth 50–55 mm diam after 10 days at 25°C on Malt Extract Agar (MEA), aerial mycelial and sporulation sparse; chlamydospores forming brown, thick-walled microsclerotia.

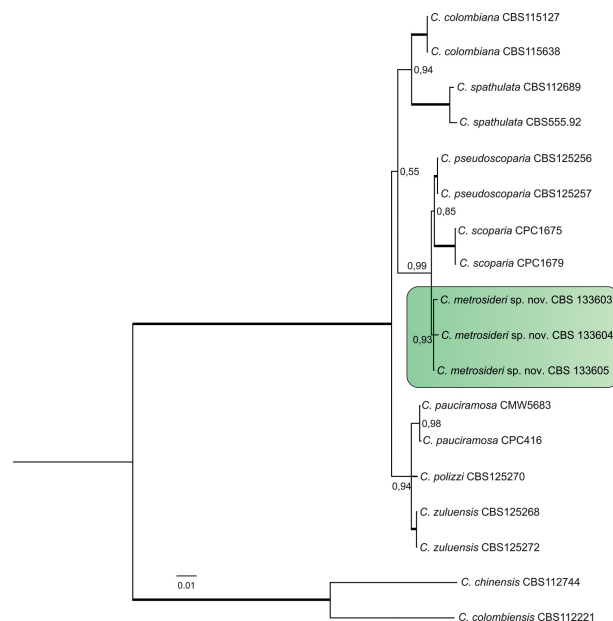


Fig. 4. Phylogenetic tree obtained by Bayesian inference using combined sequences of p-tubulin, histone H3, translation elongation factor 1a and calmodulin sequence alignments of *Calonectria* isolates. The bold lines indicate posterior probability values of 1.00. The tree was rooted to *C. chinensis* (CBS 112744) and *C. colombiensis* (CBS 112220). Isolates in bold were obtained during the survey.

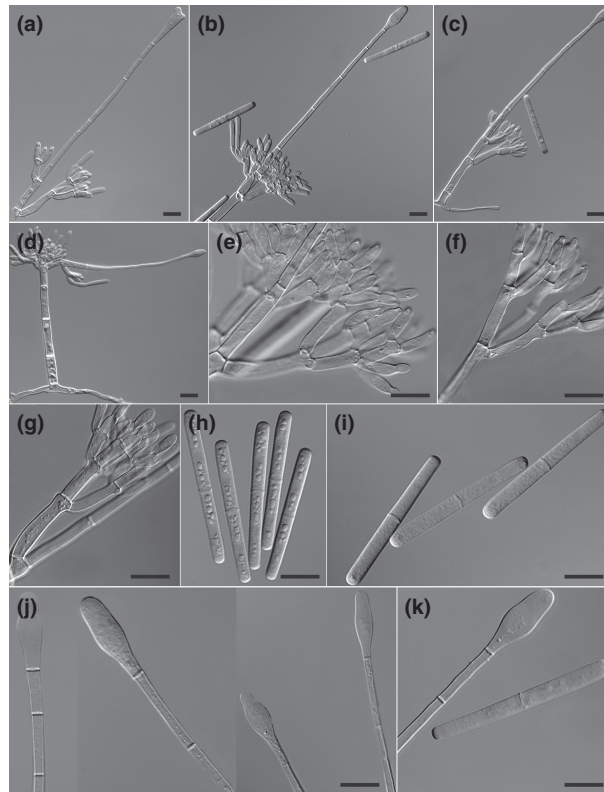


Fig. 5. Morphological characteristics of *Calonectria metrosideri*. a: Macroconidiophores containing an abnormal bifurcate vesicles; b–d: Macroconidiophores containing typical vesicles; e–g: Three branched conidiophores; h–i: 1-septate macroconidia and j–k: Spathulate to obpyriform vesicles. Scale bars = 10 μ m.

3.3 Pathogenicity

As observed in the nursery under natural infection, spray-inoculated plants showed leaf spots, and seedlings grown in pathogen-infested substrate exhibited root rot and wilt symptoms and eventually died. Intense defoliation was also found with spray-inoculated plants.

3.4 Source of inoculum

Irrigation water and potting medium were *Calonectria* free. However, used substrate produced pathogen colonization in 6.3% of castor bean leaf baits. These results indicate that neither the irrigation water nor the substrate was the primary inoculum source of the fungus.

3.5 Disease progress

The number of infected plants of *M. polymorpha* and *M. tremuloides* increased significantly over time, reaching up 71 and 34% of diseased seedlings, respectively, in about four months. Higher disease levels occurred on *Metrosideros polymorpha* compared with *M. tremuloides* (Fig. 6).

4 Discussion

To characterize the causal agent of the *Metrosideros* disease, isolates of a *Calonectria* sp. obtained from infected plants were identified as a phylogenetically undescribed species. This is described here as *Calonectria metrosideri* sp. nov., which is closely related to the *C. scoparia* complex (Schoch et al. 1999, 2001). This complex includes *C. pauciramosa* C.L. Schoch & Crous, *C. scoparia* Peerally, *C. mexicana* CL Schoch & Crous, *C. spathulata* El-Gholl, Kimbr, E.L. Barnard, Alfieri & Schoult and *C. insularis* Schoch & Crous (Schoch et al. 1999). More recently, Lombard et al. (2010b, 2011) added a further five species to this complex, namely *C. zuluensis* Lombard, Crous & MJ Wingf., *C. polizzi* Lombard, Crous & MJ Wingf., *C. colombiana* L. Lombard, Crous & MJ Wingf., *C. pseudomexicana* L. Lombard, G. Polizzi & Crous and *C. tunisiana* L. Lombard, G. Polizzi & Crous. Species of *C. scoparia sensu lato* are characterized by having obpyriform (= as spathulate) to ellipsoidal vesicles, as well as uniseptate conidia (Schoch et al. 1999).

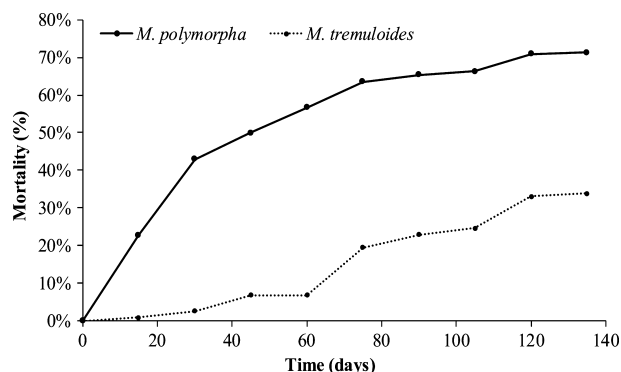


Fig. 6. Seedling mortality of *Meterosideros polymorpha* and *M. tremuloides* caused by *Calonectria metrosideri* in a forest nursery with temperatures of 25°C–30°C and irrigation daily.

Although *C. metrosideri* is phylogenetically and morphologically close to *C. scoparia* and *C. pseudoscoparia*, it grouped in a well-supported, distinct clade. Furthermore, it also has smaller conidia than the latter two species. Currently, identification of species based on phylogenetic inference has shown that many species of plant pathogens represent a species complex (Crous and Groenewald 2005; Hyde et al. 2010). The problem is that sometimes the phylogenetic species concept is not correlated with morphology, and the boundaries of separation between taxa remain unclear. In some cases, the separation of two or more groups of isolates as distinct taxa may occur, but in fact, they could belong to the same species (Summerell et al. 2010). Therefore, before assigning isolates to a new species, it is necessary to find robust differences by employing additional techniques (Summerell et al. 2010), as done in the present work.

Recent studies describing novel species of *Calonectria* have employed a combination of the phylogenetic and morphological species concepts (Lombard et al. 2010a,c). The difficulty of only adopting the biological species concept in *Calonectria* is that some isolates of different phylogenetically related species (*C. hawksworthii*, *C. insulare* and *C. scoparium*) can interbreed and produce fertile progeny.

Recently, Lombard et al. (2011) also described two new species of *Calonectria* from *Meterosideros* sp. (*C. pseudomexicana* and *C. tunisiana*) and underlined the importance of phytosanitary and quarantine measures, to prevent the introduction of these species into Hawaii. *Calonectria metrosideri* differs phylogenetically and morphologically from *C. pseudomexicana* and *C. tunisiana*, which also have wider conidia and broadly ellipsoidal vesicles.

Although the present description is based on characteristics of the anamorph (*Cylindrocladium*), the new species from ohia is named in the genus *Calonectria*, because all species of *Cylindrocladium* are phylogenetically connected to *Calonectria*. Moreover, the oldest name prevails (Crous 2002; Crous et al. 2004a, 2006; Schoch et al. 1999) and the use of *Calonectria* is being adopted in proposals of new species, even when the sexual state is not observed (Lombard et al. 2010a, 2011; Wingfield et al. 2012).

All three isolates (CBS133603, CBS133604 and CBS133605) of *C. metrosideri* tested were pathogenic and induced disease symptoms in seedlings of *M. polymorpha* similar to those observed in the nursery under natural infection. However, wilted and dead plants were only observed when the potting medium was infested with inoculum of the pathogen. In this case, the fungus infects the root system and induces seedling wilt. Species of *Calonectria* are soil-borne pathogens (Crous 2002). In a eucalypt cutting nursery, *Calonectria* spp. and other pathogens are spread and infect healthy plants mainly from inoculum in contaminated water (Mafia et al. 2008), infested substrate, tubes and scissors, as well as infected shoots used to make cuttings (Alfenas et al. 2009). In this study, we confirmed that the irrigation water and the potting medium used for growing ohia plants were pathogen inoculum free at the time of testing. Therefore, contaminated pots were probably the primary inoculum source of *C. metrosideri* on ohia. This conclusion is based on the fact that once pots were subjected to a hot water treatment (80°C min⁻¹) (Alfenas et al. 2009), the disease was successfully controlled.

As observed among and within species of *Eucalyptus* susceptible to leaf blight caused by *Calonectria pteridis* (Alfenas et al. 2009; Zarpelon et al. 2011), indications of differences in resistance to *C. metrosideri* infection were also observed between *M. polymorpha* and *M. tremuloides*. However, in nurseries, cultural practices aiming to eradicate the sources of inocula and to reduce the environmental conditions favourable to infection are the most important forms of disease control, whereas breeding for resistance is more applicable to the establishment of plantations or replacement of forest trees.

The description of *C. metrosideri* sp. nov. represents a novel species for Brazil. The rapid progress of this disease indicates the high aggressiveness of this pathogen and the urgent need for control methods, especially cultural practices, to minimize losses from the disease in forest nurseries.

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