Debunking *Korunomyces*

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Abstract

Korunomyces is a genus including fungi that produce stipitate, profusely branched, multicellular asexual reproductive structures (propagules) on leaves and in culture. Three species have been described in the genus: *Korunomyces terminaliae* – the type species, *K. prostratus* and *K. zapatensis*. No molecular studies have ever been conducted to elucidate the phylogenetic placement of *Korunomyces*. Recently, DNA sequences were obtained from pure cultures of *K. prostratus* and *K. terminaliae*, enabling an elucidation of their taxonomic placement. Isolates of *K. prostratus* obtained from diseased tissues of *Miconia calvescens* were observed for the first time to form pycnidial conidiomata in culture. A multi-gene phylogeny, including the large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS) region, polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*TEF1*), placed *K. prostratus* and *K. terminaliae* within *Coniella* (*Schizoparmaceae*). As *Korunomyces* is younger than *Coniella*, it is reduced to synonymy, and a new name and a new combination are proposed for these two species, namely: *Coniella ferreirense* nom. nov. and *Coniella prostrata* comb. nov. An emended description of *Coniella* to include the occasional formation of distinct and elaborate asexual propagules is also provided.

Introduction

The genus *Korunomyces* was proposed by Hodges & Ferreira (1981) for a fungus found producing stipitate, profusely branched, multicellular asexual reproductive structures (propagules) on infected leaves and in culture. The type species of the genus was described as *K. terminaliae*, causing a leaf spot disease on *Terminaliae ivorensis* in Brazil (Hodges & Ferreira, 1981). *Korunomyces zapatensis* was later described from dead leaves of *Nectandra coriaceae* in Cuba (Holubová-Jechová & Castaneda, 1986). The latest addition to the genus was *K. prostratus*, found causing foliage blight on *Miconia calvescens* in Brazil (Seixas et al., 2007).

Although originally described from *Terminalia ivorensis* in Brazil, *K. terminaliae* was also reported to cause leaf spots on members of the *Combretaceae* family, such as *Bouchenavia* sp., *T. catappa, T. ivorensis* and *T. myriocarpa* (Hodges & Ferreira, 1981; Farr & Rossman, 2021). Hodges and Ferreira (1981) compared the fungus on *T. ivorensis* with *Cristulariella*, *Papulaspora viridis* (= *Trichoderma matsushimae*) and *Aegerita candida* (= *Bulbillomyces farinosus*), all of which are known to produce non-conidial asexual propagules with more or less elaborate morphology. The differences in branching, hyphal width and colour, as well as the branching pattern of the propagules were regarded by Hodges and Ferreira (1981) as sufficient to distinguish each of these agonomycetous fungi from *Korunomyces*. One additional distinctive feature of *Korunomyces* separating it from *Aegerita candida* was the absence of clamp connections, which are present on the propagules and hyphae of the latter. *Papulaspora*, besides being restricted to a lignicolous (damp wood) habitat is a dematiaceous hyphomycete whereas *Korunomyces* mycelium and propagules were found to be hyaline. *Cristulariella depraedans*, similarly to *K. terminaliae* (and also *K protratus*) is a leaf parasite, but when growing in culture it produces phialoconidia and sclerotia (Redhead 1975). Based on such distinctions, Hodges and Ferreira (1981) decided to propose the new genus *Korunomyces* to accommodate the fungus on *T. ivorensis*.

Korunomyces zapatensis was the second species of *Korunomyces* included in the genus. Contrarily to the two other species, it was not associated with leaf spots, but was originally collected from leaf litter of *Nectandrae coriaceae* in Cuba. *Korunomyces zapatensis* produces asexual propagules which are morphologically similar to

those of *K. terminaliae*, but differences in the width of the propagule branches and sizes of the terminal cells and stalk were recognized as sufficient to propose it as a new species (Holubová-Jechová & Castaneda 1986).

The third species to be included in the genus was *Korunomyces prostratus.* It was proposed by Seixas et al. (2007) based on a fungus found in Brazil associated to leaf spots which coalesce to cause leaf blight on *M. calvescens*. Despite the significant similarities with *K. terminaliae*, the propagules of *K. prostratus* – as indicated by the name – are always prostrate, whereas in *K. terminaliae* these are always formed in an upright position. It was speculated that the propagules in *K. prostratus* function as infection pads, whereas in *K. terminaliae* these might serve as fungal analogues of the wind-dispersed pappus-bearing achenes produced by many plants of the *Asteraceae*.

No additions to the genus were made since 2007. The absence of a sexual morph or other morphological markers, such as clamp connections and the lack of molecular information for a phylogenetic study left the taxonomic placement of *Korunomyces* unresolved.

Here we report the results of a study involving the recollection of *K. prostratus* combined with the study of the ex-type culture of *K. terminaliae* aimed at resolving the taxonomy of this agonomycetous genus.

Materials And Methods

Sample collection processing and observation of fungus morphology

Samples of diseased foliage of *Miconia calvescens* were collected from the type locality (Angra dos Reis, state of Rio de Janeiro, Brazil). These were screened under a dissecting microscope and parts of the samples bearing sporulating colonies of the fungi were selected and dried in a plant press. Fungal structures were removed from the sample surface with a scalpel and mounted in lactophenol and lactofuchsin. Observations were made with an Olympus BX53 compound microscope adapted with differential contrast lighting and equipped with a digital camera (Olympus Q-Color 3 ™). Biometric data were obtained from at least 30 observations per representative fungal structure. A representative specimen was deposited in the fungarium at the Universidade Federal de Viçosa – state of Minas Gerais, Brazil (Herbarium VIC).

Isolations were performed by aseptic transfer of hyphal tips from the leaf surfaces onto 2 % potato dextrose-agar (PDA) plates with a sterile scalpel. Culture descriptions were based on the observation of 14-day-old (*K. prostratus*) colonies formed in plates containing either PDA, vegetable broth-agar (according to Pereira et al, 2002) or potato carrot-agar (PCA) (Crous *et al.* 2019), maintained at 25 °C under a 12-h day/night light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). The colour terminology followed Rayner (1970).

DNA isolation

Total genomic DNA was extracted from 7-day-old cultures on PDA by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described in Pinho et al. (2012).

PCR amplification

The large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS), polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*TEF1*) regions from each fungus included in the study were sequenced with the primers LSU1Fd (Crous et al. 2009) and LR5 (Vilgalys and Hester 1990) and IT5 + ITS4 (White et al. 1990), EF1Fd + EF2Fd (Groenewald et al. 2013) or EF1-728F + EF1- 986R (Carbone & Kohn 1999) or EF-2 (O'Donnell et al. 1998) and fRPB2-5F + fRPB2-7cR (Liu et al. 1999), respectively. PCR amplifications were performed in a total volume of 12.5 µL containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 µL DMSO (99.9 %), 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq DNA polymerase (Bioline GmbH Luckenwalde, Germany). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at 94 ºC followed by 35 cycles of 30 s at 94 ºC, 30 s at 48 ºC and 90 s at 72 ºC for nc LSU rDNA, ITS and 40 cycles of 30 s at 94 ºC, 30s at 52 ºC / 59 ºC and 45 s at 72 ºC for *TEF1* and a final elongation step of 7 min at 72 ºC. The partial *RPB2* gene was obtained by using a touchdown PCR protocol: start step of 5 min at 94 °C, followed by 5 cycles of 45 s at 94 °C, 45 s at 60 °C annealing temperature, and 2 min at 72 °C; 5 cycles of 45 s at 94 °C, 45 s at 58 °C annealing temperature, and 2 min at 72 °C; 30 cycles of 45 s at 94 °C, 45 s at 54 °C annealing temperature, and 2 min at 72 °C followed by a final step of 8 min at 72 °C. Amplicons were analysed on 0.8 % agarose electrophoresis gels stained with GelRed (InstantAgarose) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analysis

The nucleotide sequences were edited and contigs were generated with software SeqAssem v. 07/2008 (Hepperle 2004). The consensus sequences were compared with others deposited in the GenBank database using the MegaBLAST program. Sequences obtained from GenBank (www.ncbi.nlm. nih.gov) and the novel sequences generated during this study were aligned using MEGA v. 6 (Tamura et al. 2013) (Table 1).

Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo method were performed with all sequences, first with each locus separately and then with the concatenated sequences. Before launching the BI, the best nucleotide substitution models were determined for each gene with MrMODELTEST 2.3 (Posada and Buckley 2004). Once the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC). The SYM + I + G model of evolution was used for ITS region, GTR + I + G was used for LSU and *RPB2*, and SYM + I + G for *TEF1*. One concatenated tree with the four regions was generated with Mesquite v. 3.1 (Maddison and Maddison 2011) and estimated on the CIPRES web portal using MrBayes on XSEDE v. 3.2.6 (Miller et al. 2011). Phylogenetic trees were visualized with the program FigTree v. 1.3.1 (Rambaut 2009).

Additionally, a Maximum likelihood (ML) tree was generated using CIPRES web portal. The trees inferred by means of ML used the RAxML-HPC v. 8.2.12 (Stamatakis 2014). The bootstrap resampling was configured to perform 10,000 bootstraps, seeking to assess the stability of the inferred trees and define the best tree. The chain stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates.

The resulting tree topologies using the two methods (ML and BI) were then compared and the phylogram was edited with InkScape 0.91 (www.inkscape.org).

Sequences of *Melanconiella hyperopta* (CBS 131696) were used as the outgroups in the *Coniella* phylogeny. Sequences derived from this study were lodged in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). Taxonomic novelties were deposited in MycoBank (www.MycoBank.org).

Results

Phylogeny

Phylogenetic analysis using the ITS, nc LSU rDNA, *RPB2* and *TEF1* regions were based on 51 *Coniella* strains, one isolate of *K. terminaliae*, two isolates of *K. prostratus* and one outgroup sequence (Fig. 1). The combined alignment was comprised of 3576 characters with gaps (777 for ITS, 1314 for nc LSU rDNA, 768 for *RPB2* and 717 for *TEF1*). The phylogenetic analyses generated by Maximum likelihood (ML), and Bayesian inference (BI) indicate that *K. terminaliae* and *K. prostratus* grouped within the genus *Coniella* and formed a monotypic well-supported clade (100%/1.00, ML/BI supports, respectively). Additionally, *K. prostratus* formed a distinct lineage and was a sister to a strain of *K. terminaliae*.

Taxonomy

Coniella Höhn., Ber. dt. bot. Ges. 36 (7): 316 (1918), emend.

Synomyms: *Schizoparme* Shear, Mycologia 15: 120. 1923.

Baeumleria Petr. & Syd., Beih. Reprium nov. Spec. Regni veg. 42: 268. 1927.

Pilidiella Petr. & Syd., Beih. Reprium nov. Spec. Regni veg. 42: 462. 1927.

Anthasthoopa Subram. & K. Ramakr., Proc. Indian Acad. Sci., Sect. B 43: 173. 1956.

Cyclodomella Mathur *et al.*, Sydowia 13: 144. 1959.

Embolidium Bat., Brotéria, N.S. 33(3–4): 194. 1964 non Sacc. 1978.

Korunomyces Hodges & F.A. Ferreira, Mycologia 73: 335, 1981.

Pathogens, saprobes. *Ascomata* brown to black, collapsed collabent, erumpent, becoming superficial, globose, papillate, with central periphysate ostiole. *Asci* clavate to subcylindrical, with distinct apical ring, floating free at maturity. *Paraphyses* lacking. *Ascospores* ellipsoid, aseptate, hyaline, at times becoming pale brown at maturity, smooth, with or without mucoid caps. *Conidiomata* pycnidial, immersed to semi-immersed, unilocular, glabrous, ostiolate. Ostiole central, circular or oval, often situated in a conical or rostrate neck. *Conidiomata wall* brown to dark brown or black wall of thin, pale brown *textura angularis* on exterior, and hyaline, thin-walled, *textura prismatica* in the inner layers except at base, which has a convex, pulvinate tissue of hyaline *textura angularis* giving rise to conidiophores or conidiogenous cells. *Conidiophores* mostly reduced to conidiogenous cells, occasionally septate and branched at base, invested in mucus. *Conidiogenous cells* discrete, cylindrical, subcylindrical, obclavate or lageniform, hyaline, smooth-walled, proliferating percurrently, or with visible periclinal thickening. *Conidia* ellipsoid, globose, napiform, fusiform or naviculate with a truncate base and an obtuse to apiculate apex, unicellular, thin- or thick-walled, smooth, olivaceous brown to brown, sometimes with a

longitudinal germ-slit, with or without a mucoid appendage extending from apex to base on one side; basal hilum with or without short tubular basal appendage. Spermatophores formed in same conidioma, hyaline, smooth, 1-septate with several apical conidiogenous cells, or reduced to conidiogenous cells. *Spermatogenous cells* hyaline, smooth, lageniform to subcylindrical, with visible apical periclinal thickening. *Spermatia* hyaline, smooth, red-shaped with rounded ends. Synasexual morph, when present, agonomicetous composed of complex, multicellular, repeatedly dichotomous/dendrictily branched, chandelier-like propagules ended in digitate projections, formed on a simple cylindrical stalk and erect or directly from hyphae and prostrate.

Typus: *Coniella* fragariae (Oudem.) B. Sutton 1977 (syn. *Coniella* pulchella Höhn. 1918).

Coniella ferreirense **B.W. Ferreira & R.W. Barreto, nom. nov.**

MycoBank: MB840982

Etymology: Named after the forest pathologist and mycologist Francisco Alves Ferreira (1950–2018) (Chico Fungo) who first collected and described the fungus on *T. ivorensis*, and proposed the genus *Korunomyces*.

≡ *Korunomyces terminaliae* Hodges & F.A. Ferreira, Mycologia 73(2): 335 (1981)

Typus: Brazil: Pará: Belém, on *Miconia calvescens*, 22 Sep 1979, F. A. Ferreira, USDA United States National Fungus Collections (BPI 71913 – culture ATCC 42410 and CBS 224.80).

Notes: Although the type culture (CBS 224.80) was recovered from culture and found to have remained viable, only undifferentiated mycelium and sterile pycnidium-like structures were formed. A recollection and novel examination of material from the type locality would be of interest for a more detailed examination of *C. ferreirense* in fresh cultures.

Coniella prostrata **(Seixas & R. W. Barreto) B.W. Ferreira & R.W. Barreto, comb. nov. and emend.** (Fig. 2).

MycoBank: MB840983

≡ *Korunomyces prostratus* Seixas & R.W. Barreto, Mycologia 99 (1): 105 (2007)

Leaf spots necrotic, initially circular, greyish brown centrally with a brown periphery, becoming irregular with age with concentric dark brown peripheral rings often resulting in a scale-like pattern, with a yellowish halo, coalescing and leading to extensive leaf blight; older parts of lesions often cracking and falling out to leave irregular holes in the leaf lamina. External mycelium amphigenous, branched, septate, initially hyaline becoming yellow or orange-brown later. Internal mycelium indistinct. Propagulophores either absent or difficult to distinguish from ordinary hyphae, cylindrical, simple, length indeterminate, individual cells 11–27 µm long, 3–4 µm diam at the base, increasing to about 5–8 µm diam immediately below the propagule, hyaline, smooth, point of rupture indistinct or absent. Propagules complex, repeatedly branched, chandelier-like, subglobose to irregular outline at maturity, formed on prostrate hyphae or occasionally on erect propagulophores, multicellular, composed of primary branches with an initial dichotomous branching pattern, becoming dendritic at maturity, 69–273 × 64–272 µm, branch elements 4–10 µm diam, terminal elements digitate, 4–5 × 7–13 µm, initially hyaline becoming orange when mature, smooth.. Additional synasexual morph formed in pure culture (on VBA): Conidiomata pycnidial, globose to slightly depressed globose, 100–260 × 100–370 µm, wall composed of 1–3

cell-thick layers dark brown *textura angularis*, 7–12 µm diam; dehiscence ostiolate, central. Conidiophores formed on a dense, basal, cushion-like aggregation of hyaline cells, mostly reduced to conidiogenous cells, subcylindrical, branched next to base, 7–13 × 3–4 µm, smooth, hyaline, 1–2-septate. Conidiogenous cells enteroblastic, phialidic with apical periclinal thickening, 7–12 × 2–3 µm, smooth, hyaline, with minute collarette. Conidia mostly broadly ellipsoidal, often somewhat flattened on one side, oblong, subreniform, ovoid to subovoid, 9–12 × 3–5 µm, apex rounded to subtruncate, hilum sometimes slightly protuberant, aseptate, hyaline when immature, becoming chestnut-brown at maturity, smooth, guttulate.

In culture: on PDA and PCA, fast-growing (7–7.4 cm diam in 7 days), colonies with cottony-woolly aerial mycelium, orange centrally and becoming white at the margin, diurnal zonation distinct; dark-orange to umber or ochreous to orange reverse on PDA; on PCA colonies with flattened aerial mycelium surrounded by isolated areas of sparse aerial mycelium and strongly irregular superficial growth; sporulation (pycnidiospores) abundant on both media (but appearing only after 14 d).

Material examined: Holotype: Brazil: Rio de Janeiro: Angra dos Reis, Ilha Grande, 04 Jan 2000, VIC 22213. **Paratype**: Brazil: Rio de Janeiro: Angra dos Reis, Ilha Grande, road from Vila Abrahão to Dois Rios, 13 Jan 2002, VIC 22218. **Epitype:** Brazil: Rio de Janeiro: Angra dos Reis, Praia Brava, on *Miconia calvescens*, 28 Jul 2018, R. W. Barreto, Herbarium Universidade Federal de Viçosa (VIC 47147 – epitype designated here, MBT 10002681, ex-epitype culture COAD 2597).

Additional material: Brazil: Rio de Janeiro: Estrada de Guapiaçu, Cachoeiras do Macacú, on *Miconia calvescens*, 8 Jan 2021, R. W. Barreto VIC 47491, culture COAD 3306).

Notes: The ex-type culture was no longer viable. Hence, a new isolate obtained from the same region from where the type material originated was collected to serve as epitype, as indicated above. An ex-epitype culture was obtained, deposited in the culture collection and used, together with a supplementary specimen obtained during the study.

Discussion

In the present study multigene phylogenetic analyses revealed two of the three *Korunomyces* spp. known from culture, *K. terminaliae* and *K. prostratus*, to form a well-supported clade within the genus *Coniella*, resolving *Korunomyces* as an agonomycetous synasexual morph of *Coniella* (*Schizoparmaceae*). Since *Coniella* has nomenclatural priority over *Korunomyces*, *Korunomyces* is reduced herein to a synonym for *Coniella* and a new name and combination are proposed here for *K. terminaliae* and *K. prostratus*: *Coniella ferreirense* and *Coniella prostrata*. Unfortunately the taxonomic affinity of *K. zapatensis* will remain unclear until this fungus is recollected and epitypified.

Coniella was introduced by von Höhnel (1918) and typified by *Coniella pulchella* (= *Coniella fragariae*). Many species of *Coniella* are known as plant pathogens, causing leaf, fruit, stem, and root diseases of a wide range of hosts, including economically important species, and have received considerable attention in phytopathological literature (van Niekerk et al. 2004; Alvarez et al. 2016; Chethana et al. 2017). Other species in this genus have a saprobic lifestyle, occurring in litter, decaying bark and in soil, whereas others occur as endophytes or as

secondary invaders of plant tissues infected by other organisms or injured by other causes (Alvarez et al. 2016; Ferreira et al. 1997).

Seixas et al. (2007) when differentiating *C. prostrata* from *C. ferreirense*, speculated that the propagules of *C. prostrata* would not be functional as dispersion units due to the prostrate condition of the structure and were likely to function, instead, as infection pads. The authors suggested that the dispersion in *C. prostrata* would probably depend on some spore stage that had not been observed until then. Several attempts to induce sporulation of *C. prostrata* were made by the authors, but without success. In this study we observed for the first time the formation of sporulating pycnidia in *C. prostrata* in culture, and found it to produce a typical *Coniella* asexual morph. The *Schizoparme* sexual morph is yet to be observed, but it is likely that the pycnidial phase may be formed on infected leaves of *M. calvescens* at an advanced stage of the necrosis and will represent the dispersal stage of this fungus, as suggested earlier by Seixas et al. (2007).

In the phylogenetic tree *C. ferreirense* and the two isolates of *C. prostrata* formed distinct lineages within a clade separated from the other species of *Coniella*.

Other species of *Coniella* have been described from *Terminalia* spp., namely: *C. crousii* on *T. chebula* from India (Alvarez et al. 2016), *C. macrospora* on *T. ivorensis* from the Ivory Coast (Alvarez et al. 2016), *C. pseudogranati* on *T. stuhlmannii* from Zambia (Alvarez et al. 2016; Chethana et al. 2017), *C. fragariae* on *T. chebula* and *T. paniculata* from India (Rajeshkumar et al. 2011), *C. terminaliae* on *T. tomentosa* from India (Rajeshkumar et al. 2011; Alvarez et al. 2016) and *C. terminaliicola* on *T. superba* from Ecuador (Alvarez et al. 2016). *Coniella crousii*, *C. macrospora*, *C. pseudogranati*, and *C. fragariae* are phylogenetically distant from *C. prostrata* and *C. ferreirense*. There are no sequences available on GenBank for *C. terminaliae* and *C. terminaliicola*. Pycnidial formation was not described for *C. terminaliicola* and *C. ivorensis*, making morphological comparison impossible. *Coniella terminaliae* has globose to subglobose spores, 2–8 × 2–3.5 µm, whereas *C. prostrata* has ellipsoidal conidia, 9–12 × 3–5 µm. Although the size, shape and colour of the conidia are overlapping characteristics in some species, the formation of propagules and propagulophores are unique for *C. ferreirense* and *C. prostrata*.

Seixas et al. (2007) and Hodges & Ferreira (1981) performed pathogenicity tests with *C. ferreirense* and *C. prostrata*. The results of these inoculations showed that *C. prostrata* was capable of causing necrosis on leaves of *M. calvescens*, *T. ivorensis* and *E. grandis*, but not on *T. catappa*, whereas *C. ferreirense* was able to infect three species of *Terminalia*, but not *Eucalyptus grandis* (Hodges and Ferreira 1981). Despite the partial overlap of the host range, there are differences between the two species. Based on the DNA phylogeny and morphology presented here and host differences reported by Seixas et al. (2007) and Hodges & Ferreira (1981), the two species are considered as distinct.

Unfortunately, a phylogenetic study of *K. zapatensis* was not possible, because the species is only represented by herbarium material available only in Cuba and there are no ex-type cultures or DNA sequences available for this species (Holubová-Jechová & Castaneda, 1986). However, the morphological similarity with the other species previously belonging to *Korunomyces*, indicates that this is yet another agonomycetous synasexual morph of *Coniella*. For now, this particular species should be treated as *incertae sedis*.

Declarations

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Contributions

BWF conducted the isolation of strains, DNA extractions, PCR amplifications, phylogenetic analyses and wrote the manuscript. JLA and PWC prepared the morphological characterization and participated in writing of the manuscript. RWB is the research leader. He corrected the text and guided throughout the development of the study.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability

The datasets generated and analysed during the current study are available either in GenBank at NCBI (National Center for Biotechnology Information), as indicated in the text, or available from the corresponding author.

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Tables

Table 1. Taxa and collections used for multi-gene phylogenetic analyses in this study.

Species name	Strain accession number[1]	GenBank accession number[2]				References
		ITS	LSU	*RPB2*	*TEF1*	
Coniella africana	CBS 114133^{T} = CPC 405	AY339344	AY339293	KX833421	KX833600	Van Niekerk et al. (2004); Alvarez et al. (2016)
C. crousii		HQ264189	–	–	–	Rajeshkumar et al. (2011)
C. diplodiella	CBS 111858^{ET} = CPC 3708	AY339323	KX833335	KX833423	KX833603	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 111857 = CPC 3735	AY339325	AY339285	KX833422	KX833602	Alvarez et al. (2016)
	CBS 112333 = CPC 3775	AY339329	KX833336	KX833424	KX833604	Alvarez et al. (2016)
C. diplodiopsis	CBS 590.84^{T} = CPC 3940	AY339334	AY339288	–	–	Alvarez et al. (2016)
	CBS 10923 = CPC 3933	AY339332	AY339287	KX833440	KX833624	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 112637 = CPC 4228	KX833530	KX833355	KX833441	KX833625	Alvarez et al. (2016)
	CBS 112702 = CPC 3866	KX833531	KX833356	KX833442	KX833626	Alvarez et al. (2016)
C. duckerae	VPRI 13689 = CBS 142045^{T}	KY924929	–	–	–	Marin-Felix et al. (2017)
C. erumpens	CBS 52378^{T}	KX833535	KX833361	KX833446	KX833630	Alvarez et al. (2016)
C. eucalyptigena	CBS 139893^{T}	KR476725	–	–	–	Crous et al. (2015a, b)
C. eucalyptorum	CBS 112640^{T} = CPC3904	AY339338	AY339290	KX833452	KX833637	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 110674 =	KX833536	KX833362	KX833447	KX833631	Alvarez et al. (2016)

	CPC 610					
	CBS 111023 = CPC 3843	KX833537	KX833363	KX833448	KX833632	Alvarez et al. (2016)
C. ferreirense	CBS 224.80^{T}	MH861257	MH873026	–	–	Vu et al. (2019)
C. fragariae	CBS 17249^{NT} = CPC 3930	AY339317	AY339282	KX833472	KX833663	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 45468	KX833571	KX833393	KX833477	KX833670	Alvarez et al. (2016)
C. fusiformis	CBS 141596^{T} = CPC 19722	KX833576	KX833397	KX833481	KX833674	Alvarez et al. (2016)
	CBS 114850	KX833574	KX833395	KX833479	KX833672	Alvarez et al. (2016)
	CBS 114851	KX833575	KX833396	KX833480	KX833673	Alvarez et al. (2016)
C. granati	CBS 132860	KX833577	KX833400	KX833484	KX833677	Alvarez et al. (2016)
	CBS 130974 = CPC 19625	JN815312	KX833398	KX833482	KX833675	Alvarez et al. (2016)
	CBS 130975 = CPC 19626	JN815313	KX833399	KX833483	KX833676	Alvarez et al. (2016)
C. hibisci	CBS 109757^{ET}	KX833589	–	–	KX833689	Marin-Felix et al. (2017)
C. javanica	CBS 45568^{T}	KX833583	KX833403	KX833489	KX833683	Alvarez et al. (2016)
C. koreana	CBS 14397	KX833584	AF408378	KX833490	KX833684	Alvarez et al. (2016)
C. lanneae	CBS 141597^{T} = CPC 22200	KX833585	KX833404	KX833491	KX833685	Alvarez et al. (2016)
C. limoniformis	CBS 111021^{T} = PPRI 3870	KX833586	KX833405	KX833492	KX833686	Alvarez et al. (2016)
C. lustricola	DAOMC 251731^{T}	MF631778	MF631799	MF651900	MF651899	Jayawardena et al. (2019)
C. macrospora	CBS 52473^{T} =	KX833587	AY339292	KX833493	KX833687	Alvarez et al. (2016)

	CPC 3935					
C. malaysiana	CBS 141598^{T} = CPC 16659	KX833588	KX833406	KX833494	KX833688	Alvarez et al. (2016)
C. musaiaensis	CBS 109757 = AR 3534	KX833589	AF408337	–	KX833689	Alvarez et al. (2016)
C. nicotianae	CBS 87572^{T} = PD 72/793	KX833590	KX833407	KX833495	KX833690	Alvarez et al. (2016)
C. nigra	CBS 16560^{T} = IMI 181519	AY339319	KX833408	KX833496	KX833691	Van Niekerk et al. (2004); Alvarez et al. (2016)
C. obovata	CBS 111025 = CPC4196	AY339313	KX833409	KX833497	KX833692	Van Niekerk et al. (2004); Alvarez et al. (2016)
C. paracastaneicola	CBS 141292^{T} = CPC 20146	KX833591	KX833410	KX833498	KX833693	Alvarez et al. (2016)
	CPC 25498	KX833592	KX833411	–	KX833694	Alvarez et al. (2016)
C. peruensis	CBS 110394^{T} = RMF 7401	KJ710463	KJ710441	KX833499	KX833695	Crous et al. (2015a, b)
C. prostrata	COAD 3306	MZ727003	MZ726999	MZ772857	MZ772859	This study
	COAD2597^{T}	MZ727004	MZ727000	MZ772858	MZ772860	This study
C. pseudogranati	CBS 137980^{T}	KJ869132	–	–	–	Crous et al. (2014)
C. pseudostraminea	CBS 112624^{T} = IMI 233050	KX833593	KX833412	KX833500	KX833696	Alvarez et al. (2016)
C. quercicola	CBS 90469^{NT}	KX833595	KX833414	KX833502	KX833698	Alvarez et al. (2016)
	CBS 283.76	KX833594	KX833413	KX833501	KX833697	Alvarez et al. (2016)
	CPC 12133	KX833596	–	KX833503	KX833699	Alvarez et al. (2016)
C. solicola	CBS 76671^{T}	KX833597	KX833416	KX833505	KX833701	Alvarez et al. (2016)
	CBS 114007 =	AY339320	KX833415	KX833504	KX833700	Alvarez et al. (2016)

	IMI 253210					
	CPC 17308	KX833598	KX833417	–	KX833702	Alvarez et al. (2016)
C. straminea	CBS 14922 = CPC 3932	AY339348	AY339296	KX833506	KX833704	Van Niekerk et al. (2004); Alvarez et al. (2016)
C. tibouchinae	CBS 131595^{T} = CPC 18512	JQ281774	KX833418	KX833507	JQ281778	Miranda et al. (2012); Alvarez et al. (2016)
	CBS 131594^{T} = CPC 18511	JQ281774	KX833418	KX833507	JQ281778	Alvarez et al. (2016)
C. vitis	MFLUCC 16–1399^{T}	KX890008	KX890083	–	KX890058	Jayawardena et al. (2019)
C. wangiensis	CBS 132530^{T} = CPC 19397	JX069873	JX069857	KX833509	KX833705	Crous et al. (2012); Alvarez et al. (2016)
Melanconiella hyperopta	CBS 131696	JQ926281	JQ926281	KX833510	KX833706	Miranda et al. (2012); Alvarez et al. (2016)

1 ET: ex-epitype culture; NT: ex-neotype culture; T: ex-type culture.

2 ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S nrDNA; *RPB2*: DNA-directed RNA polymerase II second largest subunit; *TEF1*: translation elongation factor 1-alpha.

Figures

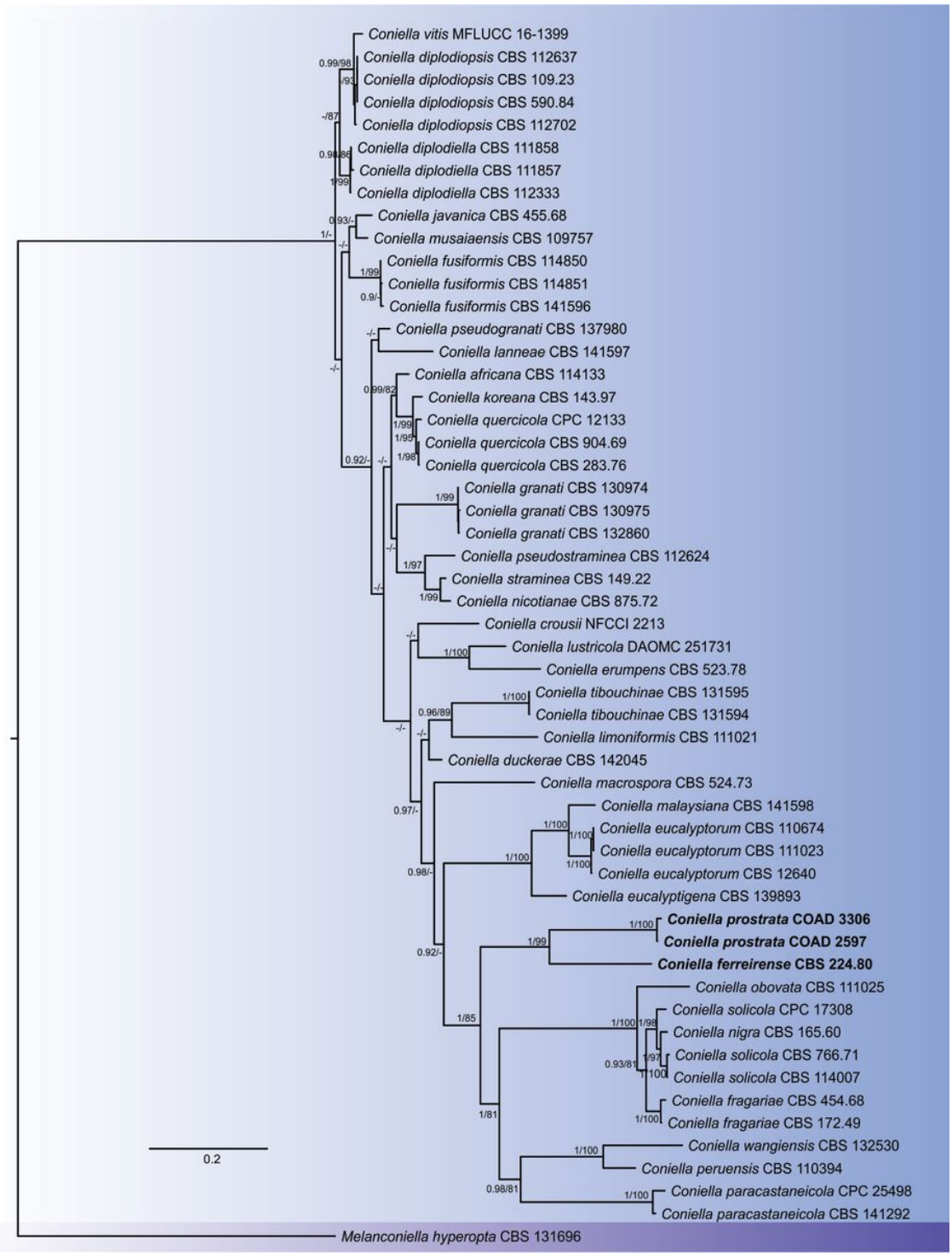

Figure 1

Maximum Likelihood (ML) tree based on combined nc LSU rDNA, ITS, RPB2 and TEF1 showing the relationship of Coniella ferreirense and C. prostrata with other closely related species within Coniella. Bootstrap support values or Bayesian posterior probabilities higher than 70 % or 0.90 are indicated above or below thickened branches (– indicates lack of support). Isolates from this study are indicated by bold text.

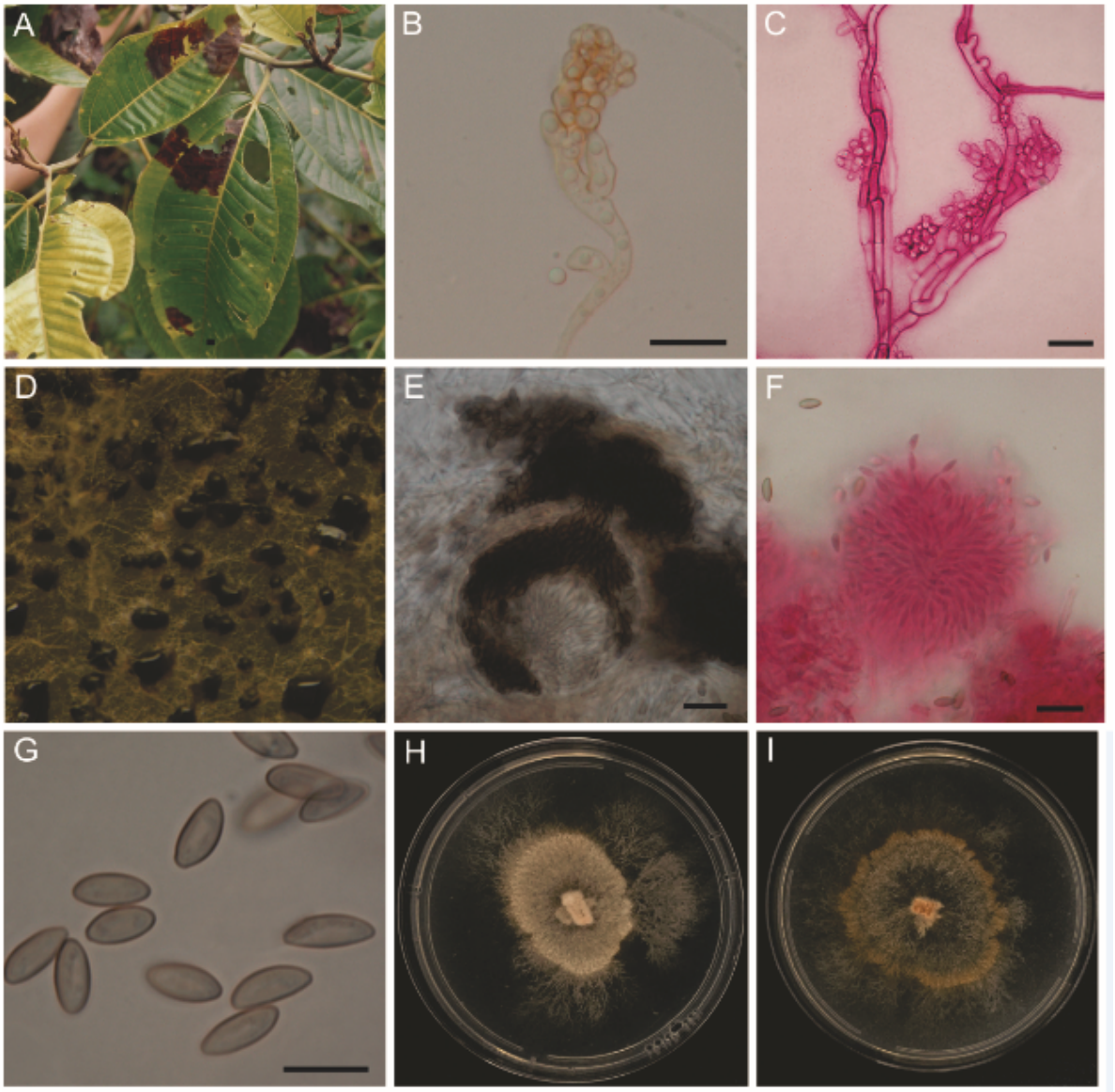

Figure 2

Coniella prostrata comb. nov. (VIC 47147). A Miconia calvescens individual bearing typical leaf blight symptoms resulting from attack by C. prostrata at type locality. B–C Young propagules and propagulophores. D Mature conidiomata on PDA. E Ostiolate mature conidiomata. F Conidiogenous cells. G Conidia H Colony on PDA after 14 days (incubation at 25 °C in 12 h light/dark cycle). I Colony on PCA after 14 days (incubation at 25 °C in 12 h light/dark cycle). Scale bars: a, b, c, e and f = 20 µm; g = 10 µm.