Phytotaxa 630 (1): 040–050
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.630.1.3

Eucalyptus leaf spot disease caused by *Coniella eucalyptorum* in Sri Lanka

RASHIKA S. BRAHMANAGE[1,2,3,4,6], NALIN N. WIJAYAWARDENE[1,4,7], CHANDRIKA M. NANAYAKKARA[2,8], CHAMINDA K. MUTHUMALA[5,9], SIRIL WIJESUNDARA[3,10], DONG Q. DAI[1,11]* & KAHANDAWA G.S.U. ARIYAWANSA[2,12]*

[1]*Center for Yunnan Plateau Biological Resources Protection and Utilization, Qujing Normal University, Qujing, Yunnan Province, 655011 P.R. China.*
[2]*Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 00700, Sri Lanka.*
[3]*National Institute of Fundamental Studies, Hanthana, Kandy, 20000, Sri Lanka.*
[4]*Tropical Microbiology Research Foundation, 96/N/10, Meemanagoda Road, 10230 Pannipitiya, Sri Lanka.*
[5]*State Timber Corporation Sri Lanka, No.82, "Sampath Paya", Rajamalwatte Road, Sri Jayawardenepura Kotte, 10100 Sri Lanka.*
[6] *sajith2007@gmail.com; https://orcid.org/0000-0002-7846-5890*
[7] *nalinwijayawardene@yahoo.com; https://orcid.org/0000-0003-0522-5498*
[8] *chandi@pts.cmb.ac.lk; https://orcid.org/0000-0002-4683-9288*
[9] *ck_muthumala@yahoo.com; https://orcid.org/0000-0001-9358-7717*
[10] *siril.wi@nifs.ac.lk; https://orcid.org/0000-0002-6754-8201*
[11] *cicidaidongqin@gmail.com; https://orcid.org/0000-0001-8935-8807*
[12] *sameera@pts.cmb.ac.lk; https://orcid.org/0000-0002-5485-6053*
**Corresponding authors; Dong Qin, cicidaidongqin@gmail.com; Kahandawa G.S.U., sameera@pts.cmb.ac.lk*

Abstract

Coniella has a wide distribution as a plant pathogen in *Eucalyptus* species. Here we report a leaf spot disease caused by *Coniella eucalyptorum* on *Eucalyptus camaldulensis* in Sri Lanka. The pathogen was isolated from symptomatic leaf samples of *E. camaldulensis* plants from Polonnaruwa District in Sri Lanka. The isolated fungus was identified as *C. eucalyptorum* based on morphological characteristics and multi-gene phylogenetic analysis of combined sequences of the internal transcribed spacer region (ITS), the partial translation elongation factor 1-α gene (*tef1-α*), and large subunit of nuclear ribosomal RNA (LSU). The pathogenicity test was performed to confirm its pathogenicity to the host, which produced symptoms similar to those observed in the field. This is the first report of *C. eucalyptorum* associated with *Eucalyptus* plantations in Sri Lanka. This finding is important to expand the knowledge of plant pathogen diversity in *Eucalyptus* species and other economically important crops in Sri Lanka.

Key words: Foliar pathogen, new geographical record, *Pilidiella*, South Asia, timber trees

Introduction

Eucalyptus, a genus of over 900 species, is native to Australia and Tasmania (Bayle 2019). This evergreen perennial is now distributed worldwide in a wide range of geographical and environmental gradients (Sivananthawerl & Mitlöhner 2011). *Eucalyptus* species are commercially cultivated for food, beverages, cosmetics, pharmaceuticals, perfumery, timber, fuel, pulp, and paper industries. *Eucalyptus* species were first introduced to Sri Lanka in the late 18th century from Australia and the first plantation was established in 1890 (Sivananthawerl & Mitlöhner 2011). Currently, *Eucalyptus* plants play an essential role in filling the timber demand in Sri Lanka due to their fast growth, ability to adapt to harsh environmental conditions and impressive quantity and quality of the timber produced (Amarasinghe *et al*. 2021). In Sri Lanka, *Eucalyptus grandis*, *E. microcorys*, *E. Pilularis,* and *E. robusta* are commonly found in some parts of the upcountry, whereas *E. camaldulensis* and *E. tereticornis* are majorly established in dry and intermediate zones of the lowlands (Sivananthawerl & Mitlöhner 2011). In addition, *E. citriodora*, *E. torelliana*, *E. paniculata*, and *E. globulus* are found as scattered small plantations (Sivananthawerl & Mitlöhner 2011).

Eucalyptus species are susceptible to a wide range of diseases, including leaf diseases caused by fungi (Park *et al*. 2000, Crous *et al.* 2019). They can cause a significant reduction in the growth and productivity of *Eucalyptus* trees while making the trees more susceptible to other pests and diseases (Smith *et al*. 2017, Andjic *et al*. 2019). Some of the most common fungal diseases of *Eucalyptus* species are listed by Crous *et al*. (1998). *Eucalyptus* leaf spot diseases are characterized by the formation of small, brown spots on the leaves (Dick *&* Gadgil 1983, Greyling *et al*. 2016, Crous *et al*. 2019). These diseases are caused by several causal agents *viz*., *Colletotrichum, Coniella, Diaporthe, Pestalotiopsis,* and *Phaeomoniella* (Crous *et al.* 1998, 2019, Park *et al*. 2000). In particular, *Coniella* leaf spots caused by *C. tibouchinae, C. javanica, C. eucalyptorum*, and *C. granati* are responsible for significant damage to plantations, leading to reduced growth, yield, and quality of timber (Crous *et al*. 2016).

Eucalyptus plantations have become an important part of the Sri Lankan landscape since their introduction in the late 18th century. However, the common and impactful diseases of different *Eucalyptus* species are yet to be reported and described. Consequently, this study was carried out to identify and characterize the fungal diseases of *Eucalyptus* species in Sri Lanka, and it reports the first record of a leaf spot disease caused by *C. eucalyptorum* in *E. camaldulensis* plantations.

Methodology

Sample collection and disease symptoms

Symptomatic leaf samples (n=15) with leaf spots ranging from light brown to dark brown and appearing with scattered black spots on the leaf surface, were randomly collected from an *E. camaldulensis* Dehnh. plantation (Forest Department of Sri Lanka) situated in the Polonnaruwa District (Latitude 7.7293889 N and Longitude 81.2109594 E, elevation 382 m) of Sri Lanka in 2022. The symptoms were recorded, and specimens were taken back to the Department of Plant Sciences, University of Colombo, in sterile zip-lock plastic bags within 24 hrs. Symptomatic leaves were observed using a stereo microscope (ACCU-SCOPE Inc., USA). Thereafter, the samples were incubated in plastic containers with moistened cotton wool at 28 °C for one week for sporulation, and then the fruiting bodies were examined using a stereo microscope (ACCU-SCOPE Inc., USA). Squash mounts and sections of the fruiting structures were mounted in water and stained with Melzer's reagent as necessary for microscopic studies and photomicrography.

Fungal isolation and morphological studies

The morphological characteristics of fungi were examined and photographed using a ZEISS Primos Star compound light microscope (Carl Zeiss Microscopy, USA) and a digital camera fitted to the microscope (Zeiss AxioCam ERc 5s). Measurements, such as the height and diameter of conidiomata, were made with the Image Framework program, and images used for figures were processed with Adobe Photoshop CC 2019 software (Adobe Systems Inc., US). At least 50 measurements were taken for each type of fungal structure for evaluation.

Single conidial isolation was performed to isolate the causal fungus onto 1.0% water agar medium containing 150 mg/L streptomycin according to the method described by Senanayake *et al*. (2020). The inoculated water agar plates were incubated at 28 °C for two days, and the germinating conidia were transferred onto potato dextrose agar (PDA) containing 150 mg/L streptomycin. The specimens and living cultures were deposited in the Herbarium of the National Institute of Fundamental Studies Material Collection (Herb. NIFSMC-RB-LP31), Hanthana, Sri Lanka, and in the Culture Collection of the University of Colombo (UOCCC-RB-LP31), Colombo, Sri Lanka, respectively.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from mycelial scrapes (50 mg) obtained from fungal cultures grown on PDA for five days at 28 °C according to the protocol described by Dissanayake *et al.* (2020). Mycelial scrapes were added to a 1.5 mL Eppendorf tube, homogenized in liquid nitrogen, and mixed with 500 µL of pre-heated extraction buffer (60 °C) containing SDS. The resulting DNA pellets were resuspended in 50 µL TE and stored at -20 °C. The quality of DNA samples was checked by loading 2−3 µL on a 1% agarose gel, and the concentration was determined using a NanoDrop 1000 Spectrophotometer. Using genomic DNA, the three loci, rDNA ITS (White *et al*. 1990), LSU (Vilgalys & Hester 1990), and *tef1-α* (Carbone & Kohn 1999), were amplified by polymerase chain reaction (PCR) in a T100™ Thermal

Cycler (Bio-Rad, USA) and primer pairs ITS1-F/ITS4, LR0R/LR5, and TEF1-728F/TEF1-1221R were used to amplify ITS, LSU and *tef1-α* loci, respectively. The PCR cycle conditions for all three amplifications consisted of an initial denaturation step (95 °C for 5 min), followed by 35 cycles of denaturation (95 °C for 30 s), annealing (52 °C for 1 min for ITS, 48 °C for 1 min for LSU and 52 °C for 1 min for *tef 1-α*) and extension (72 °C for 1 min) steps and a final extension at 72 °C for 10 min. Amplifications were performed in 25 µL PCR mixtures containing 5.5 µL of ddH_2O, 12.5 µL of PCR Master mix (GoTaq Green Master Mix Promega, Madison, WI, USA), 2 µL of genomic DNA (2 ng/µL), and 2.5 µL of each primer (10 µM). The PCR products were visualized on 1% agarose gels stained with ethidium bromide using the Gel Doc Imager (BIO-RAD, USA). Purification and sequencing of PCR products were carried out at Torrington Genomics, Colombo, Sri Lanka. DNA sequences generated in this study were deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

Molecular phylogenetic analyses

Similarity searches were performed for ITS, *tef1-α*, and LSU sequences to determine the closely related taxa using NCBI-BLAST (http://blast.ddbj. nig.ac.jp/top-e.html, accessed on 10th May 2023). The reference nucleotide sequences for ITS, *tef1-α*, and LSU sequences for multiple sequence alignment and phylogenetic analyses were retrieved from recently published data (Alvarez *et al*. 2016) and the GenBank (Table 1). Multiple sequence alignment was performed with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html; Katoh *et al.* 2019) and the sequences were further edited and improved using BioEdit v.7 (Hall 1999) and MEGA 5.0 (Tamura *et al*. 2013).

Phylogenetic analyses were performed using ITS, *tef1-α*, and LSU combined sequences. Maximum likelihood (ML) and Bayesian inference (BI) were used for the multi-locus analyses to construct phylogenetic trees. Optimal evolutionary models for each locus were selected using MrModeltest v. 2.2 under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b1 for BI and ML analyses (Nylander 2004) under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b10. For all gene regions in both BI and ML analyses, the GTR+G model was selected as the best model. The ML analyses were performed using RAxML GUI version 1.3 (Silvestro & Michalak 2012). The optimal ML tree search was done with 1000 bootstrap (BS) replications using the default algorithm of the program with a random starting tree for each run. The final tree amongst suboptimal trees was selected from each run by comparing the likelihood scores under the GTR+G substitution model, and the tree with the best score was selected. Bayesian analysis was performed for the combined data set using MrBayes v. 3.2.6 (GTR+G model) (Ronquist *et al*. 2012). Six simultaneous Markov Chain Monte Carlo analyses were run for 2,000,000 generations, and trees were sampled at every 100th generation, resulting in 20,001 trees. The first 25% of trees were discarded as the burn-in phase, while the remaining 15,001 trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. The resulting trees were incorporated into ML trees, visualized with Tree View v. 1.6.6 (Rambaut 2012), and rearranged with Microsoft PowerPoint (2010).

Pathogenicity assay

The pathogenicity of our isolate was confirmed by applying Koch's postulates, as demonstrated by Bhunjun *et al*. (2021). Asymptomatic detached branches (5–6 leaves per branch) were collected from the *E. camaldulensis* plantation at Aralaganwila, Polonnaruwa District, and the pathogenicity test was conducted within three hours. The leaves were surface sterilized by dipping them in 2% (v/v) sodium hypochlorite for 1 min (Chen *et al*. 2006), then washing them three times with sterile distilled water, followed by air drying under a laminar flow for 15 min. Five replicates of randomly selected leaves were considered for wounded and non-wounded assays using both conidial spore suspensions (50 µl of 10^8 spores per mL) and mycelial plugs. Mycelial plugs (5 mm in diameter) were obtained from the colony margins of 3-day-old cultures grown on PDA. Wounded leaves were obtained by inducing symmetrical injuries at four distinct spots on each leaf, uniformly affecting the upper epidermal layer within a 4 mm diameter circle using a sterile needle. Another set of five leaves remained non-wounded. Control inoculations were performed using sterile water and sterile PDA plugs for wounded and non-wounded leaves. While still attached to the stem, leaf samples were incubated in a moist chamber at 28 °C with 95% relative humidity under a 12-hour light period until symptoms manifested. Lesion lengths were recorded three days post-inoculation. Koch's postulates were confirmed by re-isolating the inoculated fungus from infected leaves. The re-isolated fungus was identified based on cultural and morphological characteristics.

Results

Phylogenetic analyses

The ITS, LSU, and *tef1-α* regions of the isolate RB-LP31 were amplified, and sequences were deposited in the GenBank. Phylogenetic tree was constructed based on combined ITS, LSU, and *tef1-α* sequence data of RB-LP31 isolate and respective sequences of fifty-seven strains of *Coniella* with *Melanconiella* sp. (CBS110385) as the outgroup taxon (Fig. 1). Fifty-eight taxa were included in the combined alignment which comprised 1678 characters (ITS = 593, LSU = 789, and *tef1-α* = 296). Both ML and BI analyses obtained similar tree topologies. The best RaxML tree with a final likelihood value of -11577.557573 is presented in Figure 1. The matrix had 583 distinct alignment patterns, with 8.44% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.250384, C = 0.238986, G = 0.259540, T = 0.251091; substitution rates AC = 1.352072, AG = 2.236271, AT = 1.430103, CG = 0.922002, CT = 4.834449, GT = 1.0; gamma distribution shape parameter α = 0.162079. The monophyletic clade of *Coniella eucalyptorum* with high BS (100%) included the type species CBS 112640. The average standard deviation of split frequencies was 0.009 after 2,000,000 generations. According to phylogenetic analyses, our strain (NIFSMC-RB-LP31) formed a monophyletic clade of *Coniella eucalyptorum* with 100% ML and 1.00 PP bootstrap support (Fig. 1).

Taxonomy

Coniella eucalyptorum (Crous & M.J. Wingf.) L.V. Alvarez & Crous, *Stud. Mycol.* **85**: 15 (2016)
Index Fungorum Registration Identifier 817817, Fig. 2

Basionym: *Pilidiella eucalyptorum* Crous & M.J. Wingf., in Van Niekerk, Groenewald, Verkley, Fourie, Wingfield & Crous, (2004).

Pathogenic on leaves of *E. camaldulensis*. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 80−140 µm long, 150−200 µm in diameter (n= 15), solitary or aggregated, globose, brown, and dark brown to black from the top. *Conidioma wall* consisting of 2–3 layers of hyaline *textura prismatica* and 4−5 layers of brown *textura angularis* cells. *Conidiogenous cells* 12–15 × 1–2 µm ($\bar{x}$ = 13 × 2 µm, n = 20), annellidic, narrowing at the tip, smooth and hyaline. *Conidia* 10−13 × 3−6 µm ($\bar{x}$ = 12 × 5 µm, n = 30), hyaline to pale brown, becoming dark brown at maturity, smooth, broadly ellipsoidal, both sides gradually tapering, smooth-walled, and multi-guttulate with one or two prominent guttules.

Culture characteristics: Colony on PDA reaching 25 mm diameter after seven days at 25 °C, colony circular, margin wavy, flat, velvety appearance, colony from above: pale pink to brown; reverse: light brown to dark brown.

Material examined: Sri Lanka, North Central Province, Polonnaruwa District, Aralaganwila, (Latitude 7.293889 Longitude 81.2109594) on leaves of *E. camaldulensis*, 5th of May 2022, Rashika S. Brahmanage, RB-LP31, (NIFSCC-RB-LP31, UOCCC-RB-LP31)

GenBank accession numbers: ITS = OR141147, LSU = OR143784, *tef1-α* = OR461287

Notes: Based on the morphological characteristics, our strain (NIFSMC-RB-LP31) is similar to the holotype of *Coniella eucalyptorum* (CBS 112640) collected from the leaves of *Eucalyptus grandis* × *E. tereticornis* hybrid (Alvarez *et al*. 2016). According to multi-gene phylogeny (ITS, LSU, and *tef1-α*), our strain (NIFSMC-RB-LP31) clustered with other strains of *C. eucalyptorum* (CBS 111023, CBS 112640, CBS 114134, and MFLU 17-0675) with 100% ML and 1.00 PP bootstrap support (Fig. 1). When comparing the ITS, LSU, and *tef1-α* gene regions, no base pair (0%) differences were observed between our strain (NIFSMC-RB-LP31) and the holotype of *C. eucalyptorum* (CBS 112640). *Coniella eucalyptorum* was previously recorded in Australia, Brazil, Chile, Indonesia, Malaysia, Mexico, Thailand, Venezuela, and Vietnam (Hyde *et al*. 2020). Therefore, we identified our fungal collection as the first geographical record from Sri Lanka.

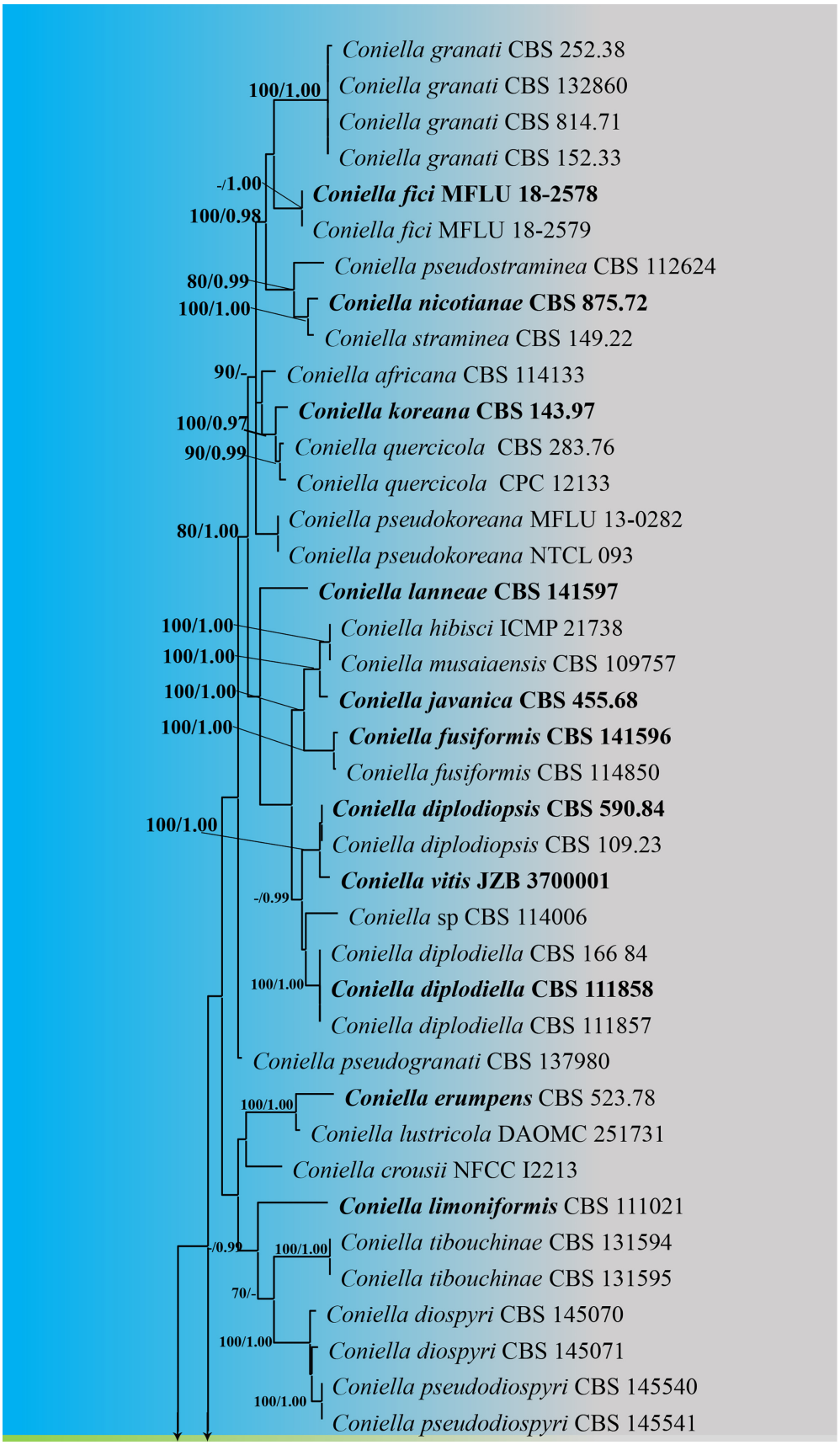

FIGURE 1. Phylogram generated for *Coniella* species from ML and BI analyses based on a combined dataset of ITS, LSU, and *tef1-α* sequences. The tree is rooted with *Melanconiella* sp. (CBS110385). Bootstrap support values for ML ≥ 65 % and Bayesian posterior probabilities (PP) ≥ 0.95 are shown at the nodes. The strain from the current study is in red. All ex-type strains are in bold black. Some branches were shortened to fit them to the pages, and these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines.

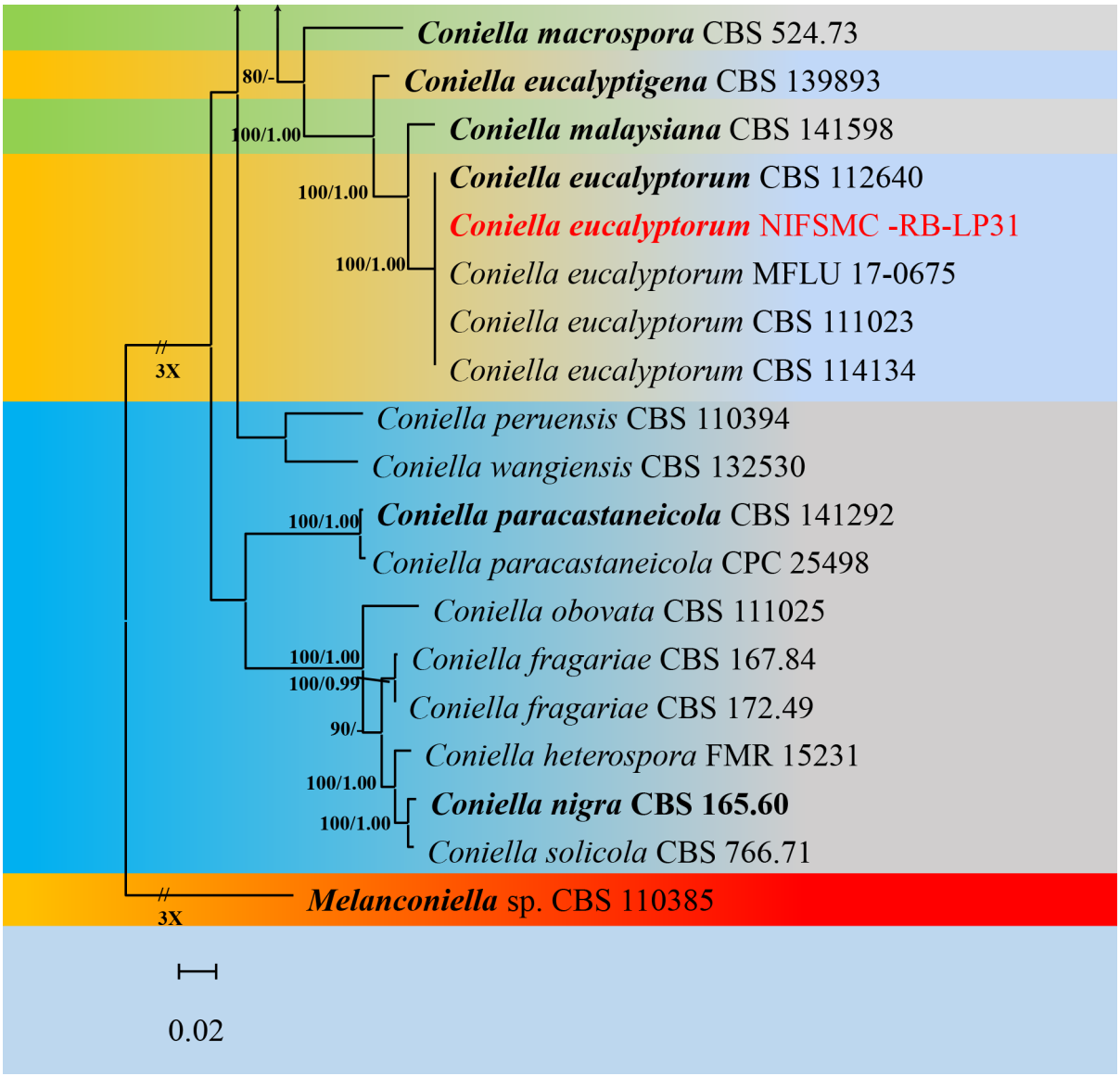

FIGURE 1. (Continued)

Pathogenicity assay

Results of the pathogenicity test showed that the RB-LP31 strain (identified as *C. eucalyptorum*) can cause disease on only wounded host leaves. The initial symptoms were seen on wounded leaves after two to three days of inoculations with mycelial plugs (Fig. 3, e1–e4). Small light-brown to brown lesions emerged at the inoculation site of the leaves. Subsequently, these lesions rapidly enlarged (diameter 2–5.5 cm) and transformed into brown to dark brown lesions after five days of inoculation. Symptoms continuously spread, and sparse white mycelia appeared on the lesions three days post-inoculation and subsequently spread throughout the host leaf. Initial symptoms were seen on wounded leaves treated with spore suspensions of *C. eucalyptorum* after 10–12 days of inoculation (Fig. 3, a1–a4), and 1–3 cm lesions appeared after 16 days of inoculation. Non-wounded leaves inoculated with both spore suspensions and mycelial plugs showed no symptoms even after 16 days of inoculation (Fig. 3, b and f), similar to the negative control treatments (Fig. 3, c, d, g, and h). Based on the morphological characteristics, we identified the re-isolated fungus as *C. eucalyptorum*.

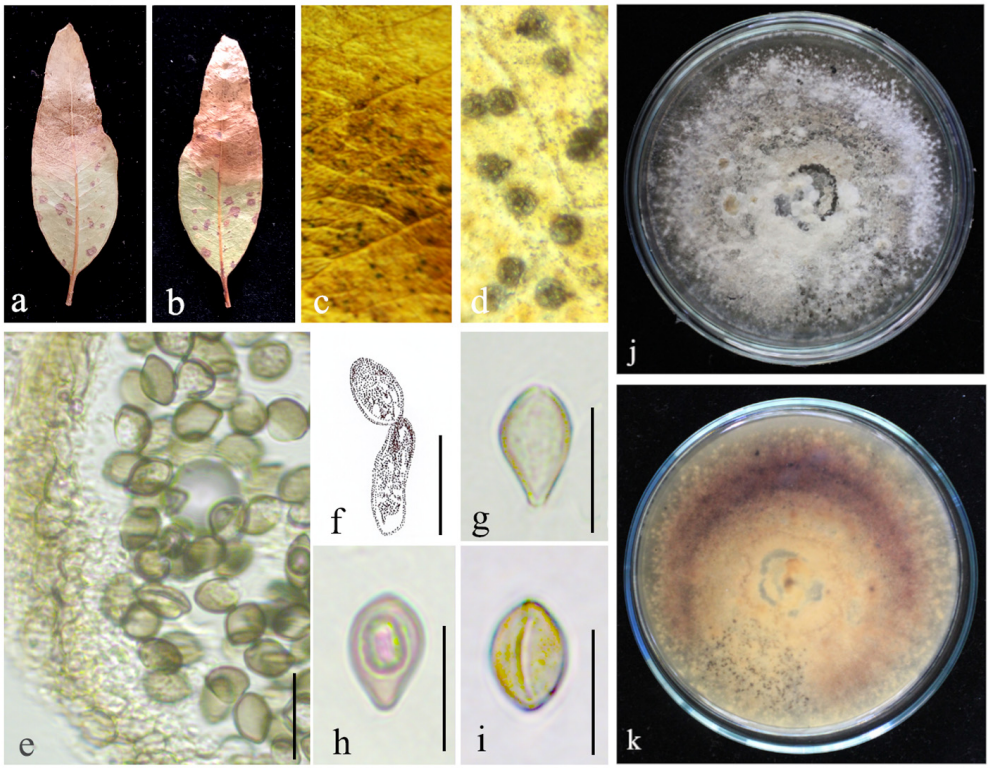

FIGURE 2. *Coniella eucalyptorum* (NIFSMC-RB-LP31) **a**–**b** Disease symptoms on the leaves of *Eucalyptus camaldulensis.* **c**–**d** Appearance of Conidiomata on *E. camaldulensis.* **e** Wall of conidiomata. **f** Conidiogenous cell. **g**–**i** Conidia. **j** Above view of the culture on PDA **k** Reverse view of the culture **e** = 50 µm, **f**–**i** = 20 µm.

Discussion

The present study collected symptomatic leaves with leaf spots from an *E. camaldulensis* plantation in Polonnaruwa District, Sri Lanka. We isolated a *Coniella* species through single conidial isolation. Based on the morphological examination and multi-gene (ITS, LSU, and *tef1-α*) phylogenetic analyses, we identified the causative agent of *E. camaldulensis* leaf spot disease as *C. eucalyptorum*.

Prompt and precise identification of plant pathogenic fungal species is critical for providing information on their biology, host range, distribution, pathogenicity, and impacts on food security (Jayawardena *et al*. 2021, Chen *et al*. 2022). Recently, misidentification of plant pathogenic fungi has occurred due to the use of morphology alone (Jayawardena *et al*. 2021). Results from the pathogenicity test revealed that *C. eucalyptorum* can cause disease in the host from which it was initially isolated and developed similar symptoms under laboratory conditions. Additionally, it showed that *C. eucalyptorum* can only infect wounded leaves. Thus, we can conclude that *C. eucalyptorum* requires wounds to enter and infect the host.

It has been contested whether the asexually typified genera *Coniella* (Höhn 1918) and *Pilidiella* (Petr & Syd 1927), along with their sexual morphs in *Schizoparme* (Shear 1923), are congeneric. Alvarez *et al*. (2016) phylogenetically proved that all three genera are congeneric. Therefore, *Pilidiella* and *Schizoparme* were considered synonyms of *Coniella*, the older name. The members of *Coniella* are reported as saprobes, pathogens, and endophytes on a diverse host range, including *Eucalyptus*, *Fragaria*, *Hibiscus*, *Psidium*, *Punica*, *Terminalia*, and *Vitis* (Alvarez *et al*. 2016, Crous *et al*. 2019). Additionally, they have been found on leaf litter, rotting barks, and soil (Alvarez *et al.* 2016), and are mostly isolated from leaf litter rather than diseased trees (Crous *et al*. 2019). *Coniella eucalyptorum* has been abundantly recorded from *Eucalyptus* species such as *E. grandis, E. camaldulensis, E. microcorys, E. pellita, E. urophylla,* and *E. torelliana* (Alvarez *et al*. 2016, Hyde *et al*. 2020) and is considered a major foliar pathogen in certain *Eucalyptus* species, causing a variety of leaf disease symptoms (Crous *et al*. 2019). Carnegie (2007) mentioned that following periods of high rainfall in sub-tropical Australia, *C. eucalyptorum* can inflict substantial damage on *E. dunnii* plantations.

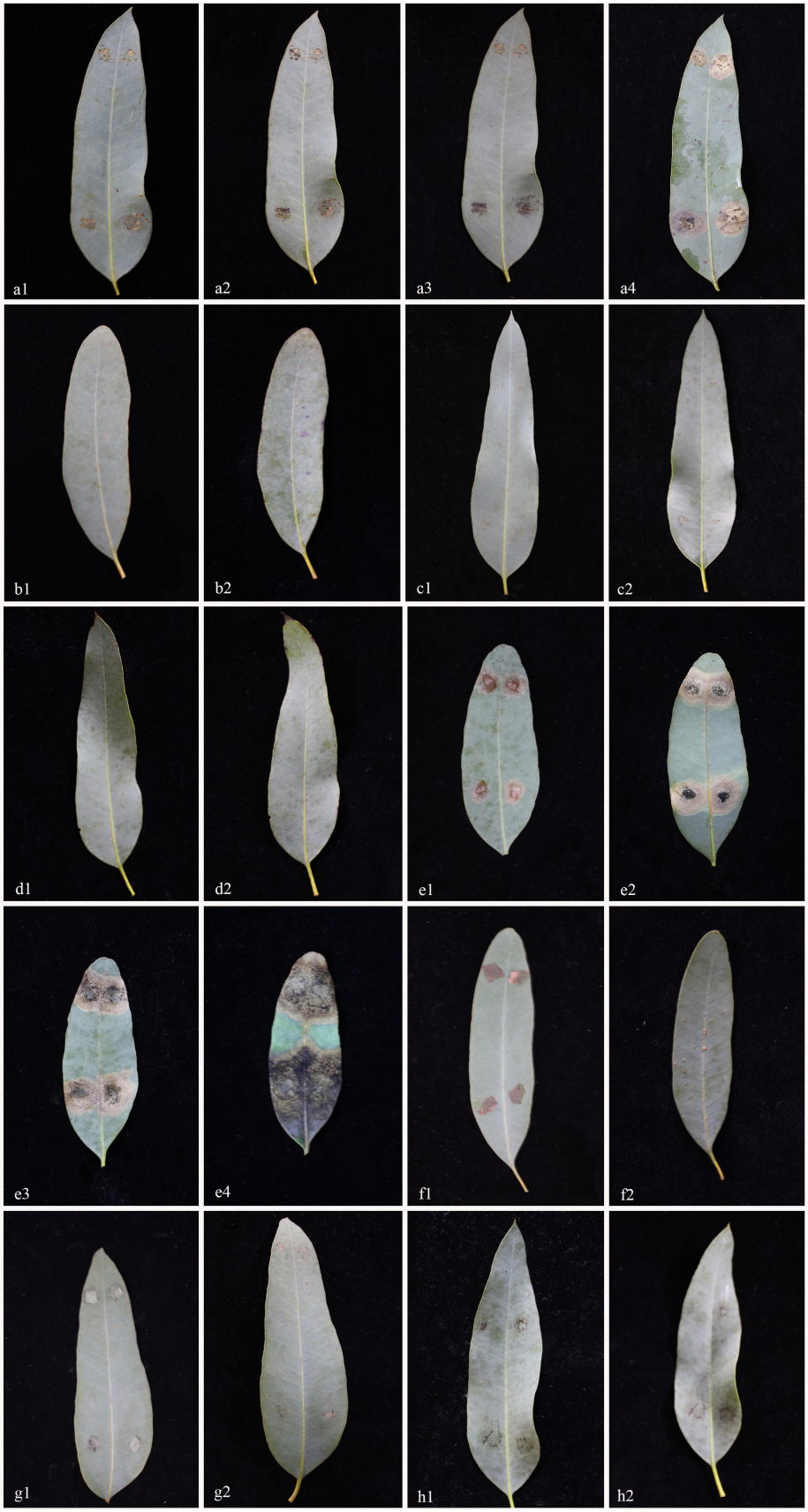

FIGURE 3. Pathogenicity test results for *C. eucalyptorum* on *E. camaldulensis* leaves. **a1–a4** wounded leaves inoculated with *C. eucalyptorum* spore suspensions. **b1–b2** non-wounded leaves inoculated with *C. eucalyptorum* spore suspensions. **c1–c2** non-wounded leaves inoculated with sterile water (control). **d1–d2** wounded leaves inoculated with sterile water (control). **e1–e4** wounded leaves inoculated with *C. eucalyptorum* mycelial plugs. **f1–f2** non-wounded leaves inoculated with *C. eucalyptorum* mycelial plugs. **g1–g2** wounded leaves inoculated with sterile PDA plugs (control). **h1–h2** non-wounded leaves inoculated with sterile PDA plugs (control).

The occurrence of *C. eucalyptorum* on *E. camaldulensis* has not been reported in Sri Lanka thus far. Therefore, this finding becomes the first record of *C. eucalyptorum* on *E. camaldulensis* in Sri Lanka. A comprehensive study is necessary to assess the impact of this pathogen on *Eucalyptus* plantations and other crops in Sri Lanka. Wijayawardene *et al.* (2022b) stressed the timely need to maintain species of quarantine importance in a centralized, national institute rather than in personal collections. Hence, Wijayawardene *et al.* (2022b) suggested establishing a material collection unit at the National Institute of Fundamental Studies of Sri Lanka (NIFSMC).

The findings from this study can be useful for pathologists to understand the plant pathogen diversity associated with *Eucalyptus* species in Sri Lanka. Further, this information can be applied for disease management and quarantine purposes. As suggested by Wijayawardene *et al.* (2022a), broadening studies on plant pathogens in underexplored regions is important to reveal the true fungal diversity. Therefore, further studies on *Coniella* species in the Dry Zone are crucial. Moreover, research into the identification of *Coniella* species associated with *Eucalyptus* broadens our understanding of its diversity, potentially leading to the discovery of new *Coniella* species.

Acknowledgments

Rashika S. Brahmanage acknowledges the Tropical Microbiology Research Foundation and the Green Movement of Sri Lanka for funding. The authors thank Achala Rathnayaka, Danushka S.Tennakoon, H.A. Dimutu, H. Dangalla, K.M. Wimalasena, J Rajendran and T. Rassool, for the assistance provided during the lab work. We would like to extend our appreciation to all the staff members of the Department of Plant Sciences, Faculty of Science, University of Colombo, Sri Lanka. The authors are grateful to Deepani Alawathugoda, Department of Forest Conservation, Sri Lanka. This work is partially funded by the Asian Development Bank grant (Grant number R3RJ2) provided by the Science and Technology Human Resource Development Project, Ministry of Higher Education, Sri Lanka. Dong-Qin Dai and Nalin Wijayawardene acknowledge the Yunnan Revitalization Talents Support Plan ("Young Talents" Program and "High-End Foreign Experts" Program).

References

Alvarez, L.V., Groenewald, J.Z. & Crous, P.W. (2016) Revising the Schizoparmaceae: *Coniella* and its synonyms *Pilidiella* and *Schizoparme*. *Studies in Mycology* 85: 1–34. https://doi.org/10.1016/j.simyco.2016.09.001

Amarasinghe, W.T.D., Terada, T. & Yamamoto, H. (2021) Enhancing the carbon sequestration of high-elevation *Eucalyptus* plantations in Sri Lanka for future carbon market activities. *Journal of Forest Research* 26: 351–357. https://doi.org/10.1080/13416979.2021.1927501

Andjic, V., Carnegie, A.J., Pegg, G.S., Hardy, G.E.S.J., Maxwell, A., Crous, P.W., Pérez, C., Wingfield, M.J. & Burgess, T.I. (2019) 23 years of research on *Teratosphaeria* leaf blight of *Eucalyptus*. *Forest Ecology and Management* 443: 19–27. https://doi.org/10.1016/j.foreco.2019.04.013

Bayle, G. (2019) Ecological and social impacts of *Eucalyptus* tree plantation on the environment. *Journal of Biodiversity Conservation and Bioresource Management,* 5: 93–104. https://doi.org/10.3329/jbcbm.v5i1.42189

Bhunjun, C.S., Phillips, A.J., Jayawardena, R.S., Promputtha, I. & Hyde, K.D. (2021) Importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on Koch's Postulates. *Pathogens* 10: 1096. https://doi.org/10.3390/pathogens10091096

Carnegie, A.J. (2007) Forest health condition in New South Wales, Australia, 1996–2005. II. Fungal damage recorded in eucalypt plantations during forest health surveys and their management. *Australasian Plant Pathology* 36: 225–239. https://doi.org/10.1071/AP07021

Chen, Y., Fu, D., Wang, W., Gleason, M.L., Zhang, R., Liang, X. & Sun, G. (2022) Diversity of *Colletotrichum* species causing apple bitter rot and *Glomerella* leaf spot in China. *Journal of Fungi* 8: 740. https://doi.org/10.3390/jof8070740

Chen, Z.Z., Ho, C.K., Ahn, I.S. & Chiang, V.L. (2006) *Eucalyptus,* Agrobacterium Protocols. *Methods in Molecular Biology* 2: 344. https://doi.org/10.1385/1-59745-131-2:125

Crous, PW (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* 21:

1–170.
https://doi.org/10.2307/3761362

Crous, P.W., Wingfield, M.J. & Burgess, T.I. (2016) Fungal Planet description sheets: 469–557. *Persoonia* 37: 218–403.
https://doi.org/10.3767/003158516X694499

Crous, P.W., Michael, J., Wingfield, R., Cheewangkoon, A.J., Carnegie, T.I., Burgess, B.A., Summerell, J., Edwards, Taylor, P.W.J. & Johannes, Z.G. (2019) Foliar pathogens of *Eucalypts*. *Studies in Mycology* 94: 125–298.
https://doi.org/10.1016/j.simyco.2019.08.001

Greyling, I., Wingfield, M.J., Coetzee, M.P., Marincowitz, S. & Roux, J. (2016) The *Eucalyptus* shoot and leaf pathogen *Teratosphaeria destructans* recorded in South Africa. *Southern Forests: a Journal of Forest Science* 78 (2): 123–129.
https://doi.org/10.2989/20702620.2015.1136504

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
https://doi.org/10.1021/bk-1999-0734.ch008

Hyde, K.D., de Silva, N.I., Jeewon, R., Bhat, D.J., Phookamsak, R.,Doilom, M., Boonmee, S., Jayawardena, R.S., Maharachchikumbura, S.S.N.,Senanayake, I.C., Manawasinghe, I.S., Liu, N., Abeywickrama, P.D., Chaiwan, N., Karunarathna, A., Pem, D., Lin, C.G., Sysouphanthong, P., Luo, Z.L., Wei, D.P., Wanasinghe, D.N., Norphanphoun, C., Tennakoon, D.S., Samarakoon, M.C., Jayasiri, S.C., Jiang, H.B., Zeng, X.Y., Li, J.F., Wijesinghe, S.N., Devadatha, B., Goonasekara, I.D., Brahmanage, R.S., Yang, E.F., Aluthmuhandiram, J.V.S., Dayarathne, M.C., Marasinghe, D.S., Li, W.J., Dissanayake, L.S., Dong, W., Huanraluek, N., Lumyong, S., Liu, J.K., Karunarathna, S.C., Jones, E.B.G., Al-Sadi, A.M.,Xu, J.C., Harishchandra, D., Sarma, V.V. & Bulgakov, T.S. (2020) AJOM new records and collections of fungi: 1–100. *Asian Journal of Mycology* 3: 22–294.
https://doi.org/10.5943/ajom/3/1/3

Index Fungorum (2022) *Index Fungorum.* Available from: http://www.indexfungorum.org/names/Names.asp (accessed: 10 June 2023).

Jayawardena, R.S., Hyde, K.D., de Farias, A.R.G., Bhunjun, C.S., Ferdinandez, H.S., Manamgoda, D.S., Udayanga, D., Herath, I.S., Thambugala, K.M., Manawasinghe, I.S., Gajanayake, A.J. Samarakoon, B.C., Bundhun, D., Gomdola, D., Huanraluek, N., Sun, Y., Tang, X., Promputtha, I. & Thines, M. (2021) What is a species in fungal plant pathogens? *Fungal Diversity* 109: 239–266.
https://doi.org/10.1007/s13225-021-00484-8

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.
https://doi.org/10.1093/bib/bbx108

Nylander, J.A.A. (2004) *MrModeltest* 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, pp. 2–4.

Park, R.F., Keane, P.J., Wingfield, M.J. & Crous, P.W. (2000) Fungal diseases of Eucalypt foliage. *Diseases and pathogens of Eucalypts* 153–239.

Rambaut, A. (2012) *Fig.Tree.* v. 1.4.0. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed: 1 May 2023).

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61: 539–542.
https://doi.org/10.1093/sysbio/sys029

Senanayake, I.C., Rathnayaka, A.R., Marasinghe, D.S., Calabon, M.S., Gentekaki, E., Lee, H.B., Hurdeal, V.G., Pem, D., Dissanayake, L.S., Wijesinghe, S.N., Bundhun, D., Nguyen, T.T., Goonasekara, I.D., Abeywickrama, P.D., Bhunjun, C.S., Jayawardena, R.S., Wanasinghe, D.N., Jeewon, R., Bhat, D.J. & Xiang, M.M. (2020) Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. *Mycosphere* 11: 2678–2754.
https://doi.org/10.5943/mycosphere/11/1/20

Shear, C.L. (1923) Life histories and undescribed genera and species of fungi. *Mycologia* 15: 120–131.
https://doi.org/10.1080/00275514.1923.12020405

Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337.
https://doi.org/10.1007/s13127-011-0056-0

Sivananthawerl, T. & Mitlöhner, R. (2011) *Eucalyptus grandis* and other important *Eucalyptus* species: A case study from Sri Lanka. *Silviculture in the Tropics* 463–472.
https://doi.org/10.1007/978-3-642-19986-8_29

Smith, A.H., Wardlaw, T.J., Pinkard, E.A., Ratkowsky, D. & Mohammed, C.L. (2017) Impacts of *Teratosphaeria* leaf disease on plantation *Eucalyptus globulus* productivity. *Forest Pathology* 47 (2): e12310.
https://doi.org/10.1111/efp.12310

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.

https://doi.org/10.1093/molbev/mst197

Von Höhnel, F. (1918) Dritte vorlaufige Mitteilung mycologischer Ergebnisse (Nr.201–304). *Berichte der Deutschen Botanischen Gesellschaft* 36: 309–317.

White, T.J., Bruns, T., Lee, S.J.W.T. & Taylor, J.L. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18: 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wijayawardene, N.N., Phillips, A.J.L., Pereira, D.S., Dai, D.Q., Aptroot, A., Monteiro, J.S., Druzhinina, I.S., Cai, F., Fan, X., Selbmann, L., Coleine, C., Castañeda-Ruiz, R.F., Kukwa, M., Flakus, A., Fiuza, P.O., Kirk, P.M., Kumar, K.C.R., Arachchi, I.S.l, Suwannarach, N., Tang, L.Z., Boekhout, T., Tan, C.S., Jayasinghe, R.P.P.K. & Thines, M. (2022a) Forecasting the number of species of asexually reproducing fungi (Ascomycota and Basidiomycota). *Fungal Diversity* 114 (1): 463–490.
https://doi.org/10.1007/s13225-022-00500-5

Wijayawardene, N.N., Rajakaruna, S, Dai, D.Q., Jayasekara, S., Warnakula, L., Ariyawansa, K.G.S.U., Fernando, E.Y., Jayasekera, P., Karunarathna, S.C., Singhalage, D., Ukuwela, K., Jayalal, R.G.U., Jayasinghe, R.P.P.K., Muthumala, C.K., Madawala, S., Hewajulige, I.G.N., Rajawardana, D.U., Ediriweera, A., Ediriweera, S., Alawathugoda, D., Rajawatta, K.M.W., Jin, X.C., Chandana, E.P.S., Nanayakkara, C. & Wijesundara, S. (2022b) Necessity of a National Fungarium and a Culture Collection for Fungi in Sri Lanka. *Chiang Mai Journal of Science* 49 (2): 248–271.
https://doi.org/10.12982/CMJS.2022.027