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Identification of *Botryosphaeriaceae* species in the South African National Collection of Fungi, including a novel species of *Oblongocollomyces*

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The *Botryosphaeriaceae* is a diverse group of fungi that includes several important plant pathogens. This study aimed to identify the 201 *Botryosphaeriaceae* isolates deposited at the South African National Collection of Fungi (NCF). The isolates were revived and phylogenetic analyses, based on multiple loci (ITS, *tef1-α, rpb2, tub2*), revealed several distinct clades, leading to the identification of 15 known species from *Botryosphaeria*, *Lasiodiplodia*, *Neofusicoccum* and a new species of *Oblongocollomyces*. This study expands the known diversity and host ranges of the *Botryosphaeriaceae* in South Africa and China, significantly contributing to our understanding of this family. The results highlight the importance of national collections as valuable resources for fungal biodiversity research. Further research is needed to explore the pathogenicity and distribution of these species in local agricultural ecosystems.

Keywords: Botryosphaeriales, latent pathogen, South Africa, China.

Introduction

The *Botryosphaeriaceae* Theiss. & Syd. was originally established to include three genera: *Botryosphaeria* Ces. & De Not., *Phaeobotryon* Theiss. & Syd. and *Dibotryon* Theiss. & Syd. (Theissen & Sydow 1918). Early classifications relied heavily on morphological characteristics, such as the production of large, ovoid to oblong, typically hyaline, aseptate ascospores that darken and become septate with age, contained within bitunicate asci in either unilocular or multilocular ascomata (Sivanesan 1984; Phillips et al. 2013). The asexual forms of the *Botryosphaeriaceae* exhibit a diverse range of conidial morphologies that are significant for taxonomic classification (Phillips et al. 2013). However, the taxonomy of the *Botryosphaeriaceae* has undergone substantial changes since the first molecular taxonomic study by Crous et al. in 2006. That study revealed ten lineages within the family, which were subsequently recognised as individual genera based on their sexual morphs. Over the years, this classification has been refined multiple times (Liu et al. 2012; Phillips et al. 2013; Yang et al. 2017; Zhang et al. 2021; Rathnayaka et al. 2023) and the family currently includes 23 genera and approximately 230 species. Notable genera within this family include *Neofusicoccum* Crous, Slippers & A.J.L. Phillips (45 species), *Dothiorella* Sacc. (44 species), *Lasiodiplodia* Ellis & Everh. (35 species) and *Diplodia* Fr. (30 species) (Bhunjun et al. 2024; Pem et al. 2024).

Botryosphaeriaceae species are cosmopolitan in distribution, existing as saprobes, endophytes or pathogens on a wide array of plant hosts in both human-altered (agricultural and urban) and natural ecosystems (Slippers & Wingfield 2007). Among the known species, three are the most common including *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. reported from 97 countries

(666 hosts), *Botryosphaeria dothidea* (Moug.) Ces. & De Not. reported from 66 countries (403 hosts) and *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips reported from 50 countries (223 hosts). Eleven countries exhibit the highest described diversity of *Botryosphaeriaceae* species, with China hosting the most species (72) and South Africa ranking second (62) (Batista et al. 2021). However, numerous new species have recently been described in China (Rathnayaka et al. 2022; Sun et al. 2022; Wu et al. 2024) and South Africa (Zhang et al. 2021), leading to an increased number of documented hosts and species from both these countries.

The South African National Collection of Fungi (NCF) is located in Pretoria at the Agricultural Research Council (ARC). This collection houses over 60 000 fungal specimens (Rong & Baxter 2006), representing a diverse array of plant pathogenic groups. Although the collection is extensive, many isolates have only been identified to the genus level based on morphological features. In recent years, however, increased funding has enabled the use of molecular methods to improve the identification of these isolates. This study focuses specifically on isolates deposited under the *Botryosphaeriaceae*, with the aim of advancing their taxonomic resolution through molecular techniques.

Materials and methods

Isolates

The isolates of this study were obtained from the National Collection of Fungi at the Agricultural Research Council, Plant Health and Protection, South Africa. The 201 isolates were collected from 42 different host species, including 69 isolates from South Africa, 130 isolates from China and 2 isolates from Benin (Supplementary material, Table S1). All isolates were revived on potato dextrose agar (PDA) and incubated at 25°C in the dark for seven days.

DNA sequence analyses

DNA was extracted using a Zymo Quick-DNA Fungal/Bacterial Miniprep (Zymo Research) kit from mycelium taken from four-day-old pure cultures. DNA sequences were generated for the internal transcribed spacer (ITS) region of the ribosomal RNA (rRNA) operon amplified with primers ITS-1 (Gardes & Bruns 1993) and ITS-4 (White et al. 1990), the translation elongation factor 1-α (*tef1-α*) gene amplified with primers EF1-728F and EF1-986R (Carbone & Kohn 1999), the β-tubulin (*tub2*) gene amplified with primers BT2a and BT2b (Glass & Donaldson 1995), and the RNA polymerase II second largest subunit (*rpb2*) using primers RPB2-6F and RPB2-7cR (Liu et al. 2012). Polymerase Chain Reaction (PCR) amplifications were performed on a MyCycler thermal cycler (Bio-Rad Laboratories, Inc). The PCR mixtures consisted of 1 µl genomic DNA, 10 µl OneTaq® 2X Master-Mix (New England, Biolabs) with Standard Buffer (Catalogue No. M0482S), 1 µl of each 10 nM primer and 7 µl nuclease free water in a total volume of 20 µl. To amplify the ITS region the following PCR protocol was used: initial denaturation (94°C, 5 min), 35 amplification cycles (94°C, 30 s; 50°C, 30 s; 68°C, 1 min), and a final extension (68°C, 10 min). The same PCR protocol was used for *tef1-α* and *rpb2* with the exception of the annealing temperature, which was increased to 53°C.

Sequencing reactions were performed using an ABI 3500XL Genetic Analyzer at Inqaba Biotec (Pretoria, South Africa). Sequences were edited using the CLC-Bio Workbench V9.0 (Qiagen, Hilden, Germany). DNA sequences for relevant *Botryosphaeriaceae* species previously published were retrieved from GenBank (https://www.ncbi.nlm.nih.gov). The resulting data matrices were aligned online using MAFFT (https://mafft.cbrc.jp/alignment/server) version 6 (Katoh et al. 2019) and checked manually for alignment errors. Phylogenetic analyses for all datasets were performed using Maximum Likelihood (ML) using PhyML 3.0 online (http://www.atgc-montpellier.fr/phyml; Guindon et al. 2010). The confidence levels for ML were determined with 1 000 bootstrap replications.

Morphology

Cultures of a possible novel species were incubated on half-strength PDA to induce spore production for morphological characterisation. The fungal structures were mounted in water on microscope slides and characteristic morphological structures were observed using a Zeiss compound microscope (Zeiss, Germany). Images were captured with a Zeiss Axiocam mounted on the microscope and measurements were made using the imaging software Zeiss Zen 3.9. Up to 20 measurements were made for spores and other morphologically characteristic structures, where these were found.

Results

Molecular identification

Blast N searches in GenBank revealed the presence of four genera of *Botryosphaeriaceae* among the 201 isolates sequenced in this study. Four sets of phylogenetic trees were generated from the derived DNA sequence data individually for *Lasiodiplodia* species (Figure 1), *Neofusicoccum* species (Figure 2), *Botryosphaeria* species (Figure 3) and *Oblongocollomyces* Tao Yang & Crous species (Figure 4). The topologies of the trees emerging

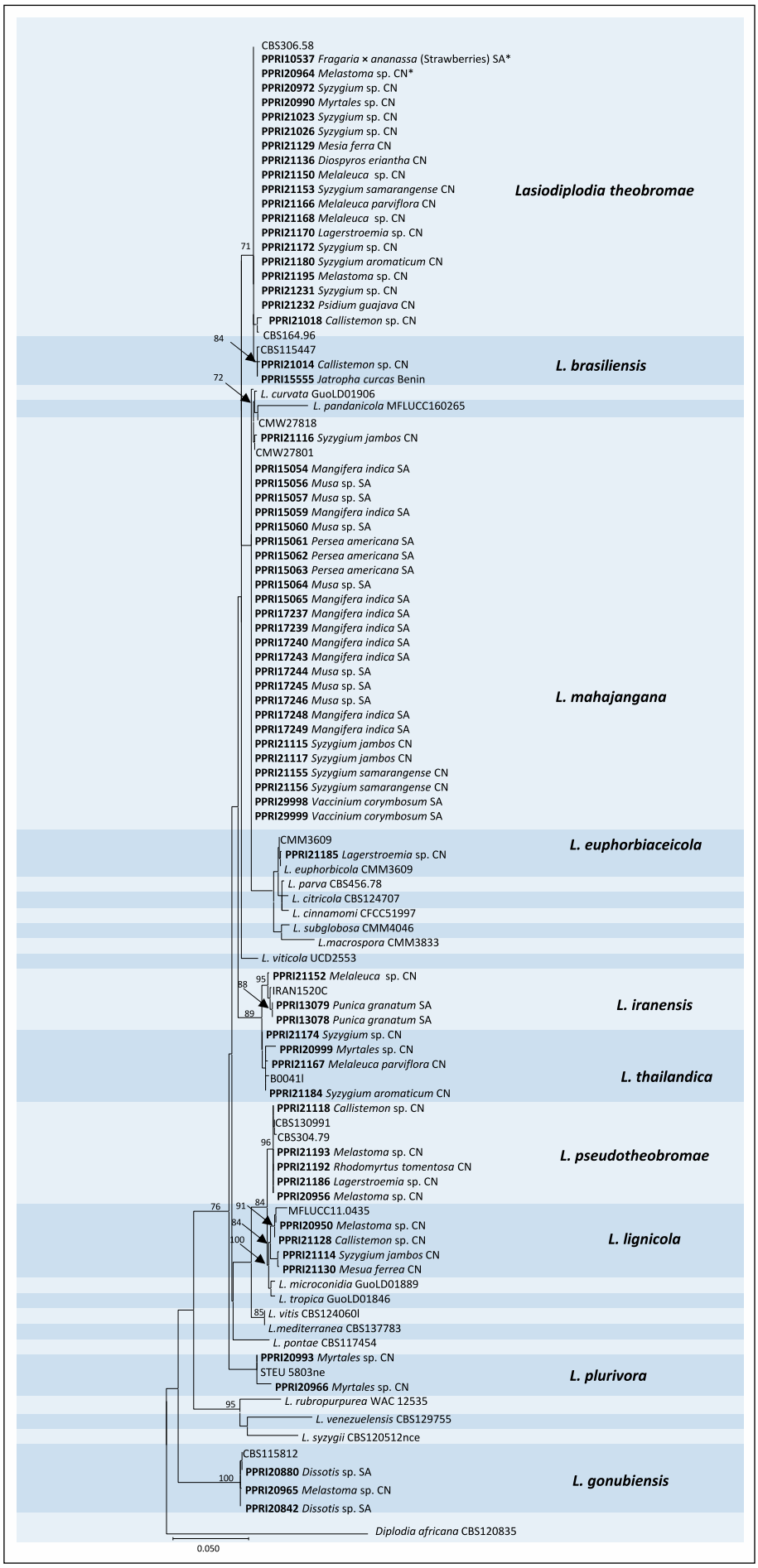

Figure 1. Maximum likelihood (ML) tree. The combined data set of ITS, *tef1-α* and *rpb2* loci sequences of *Lasiodiplodia*. The tree is rooted to *Diplodia africana* (CBS120835). Bootstrap values above 75% are given at the nodes. Isolates of this study are indicated in bold. *SA = South Africa, CN = China.

from the ML analyses were congruent in individual gene regions, as well as in the combined dataset for the clades representing the species isolated in this study.

The sequence dataset for *Lasiodiplodia* contained 531 characters for ITS, 357 characters for *tef-1α* and 623 characters for the *rpb2* dataset, and the combined dataset consisted of 1 511 characters. Ten clades were identified from analyses, and these resolved as *Lasiodiplodia brasiliensis* M.S.B. Netto, M.W. Marques & A.J.L. Phillips, *L. euphorbiaceicola* A.R. Machado & O.L. Pereira, *L. gonubiensis* Pavlic, Slippers & M.J. Wingf.,

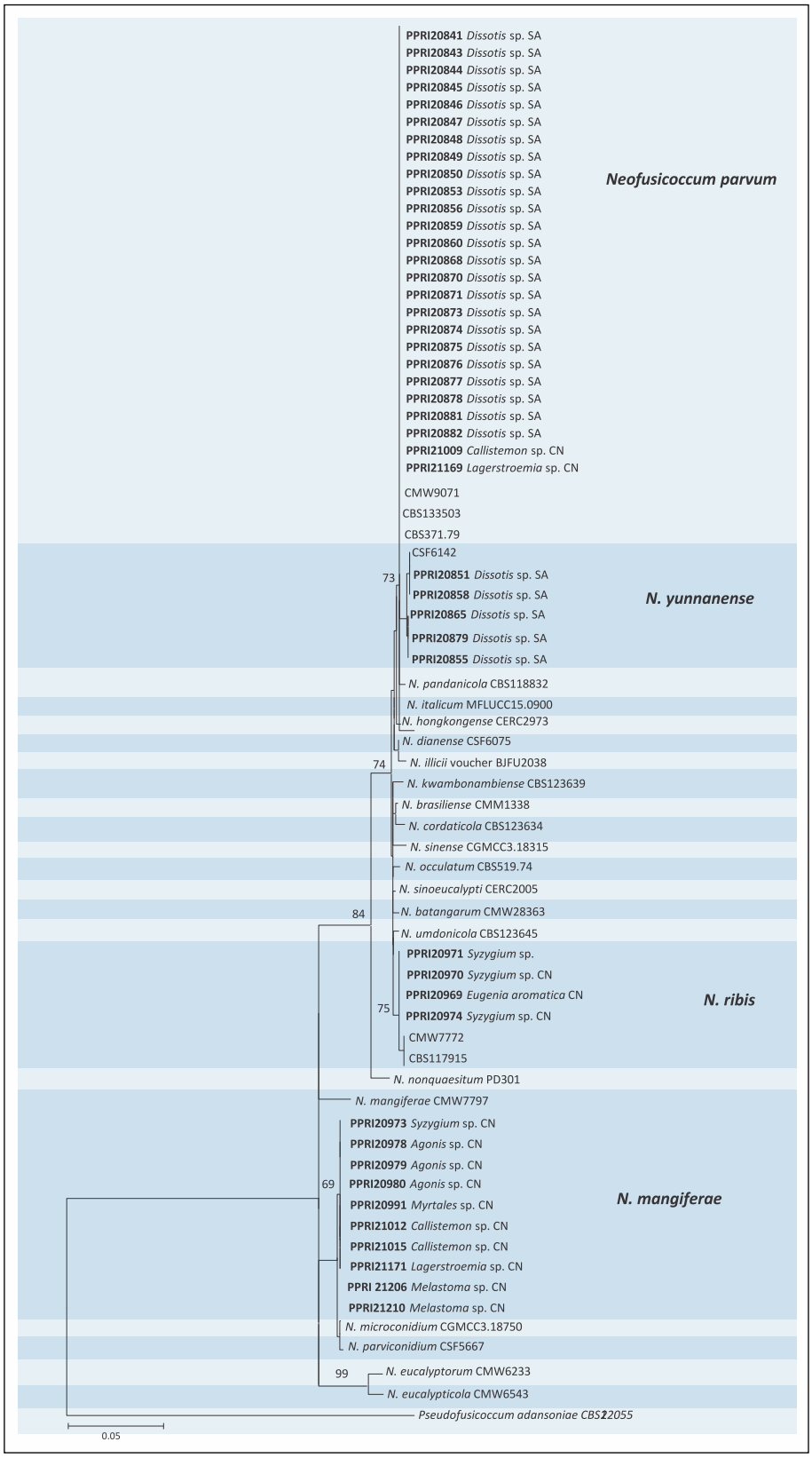

Figure 2. The combined data set of ITS, *tef1-α* and *rpb2* loci sequences of *Neofusicoccum*. The tree is rooted to *Pseudofusicoccum adansoniae* (CBS122055). Bootstrap values above 75% are given at the nodes. Isolates of this study are indicated in bold. *SA = South Africa, CN = China.

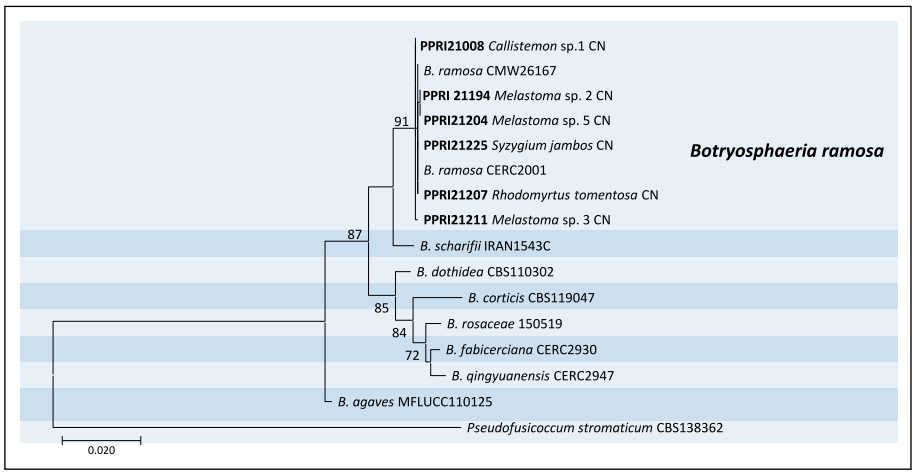

Figure 3. The combined data set of ITS, *tef1-α* and *rpb2* loci sequences of *Botryosphaeria*. The tree is rooted to *Pseudofusicoccum stromaticum* (CBS138362). Bootstrap values above 75% are given at the nodes. Isolates of this study are indicated in bold. *SA = South Africa, CN = China.

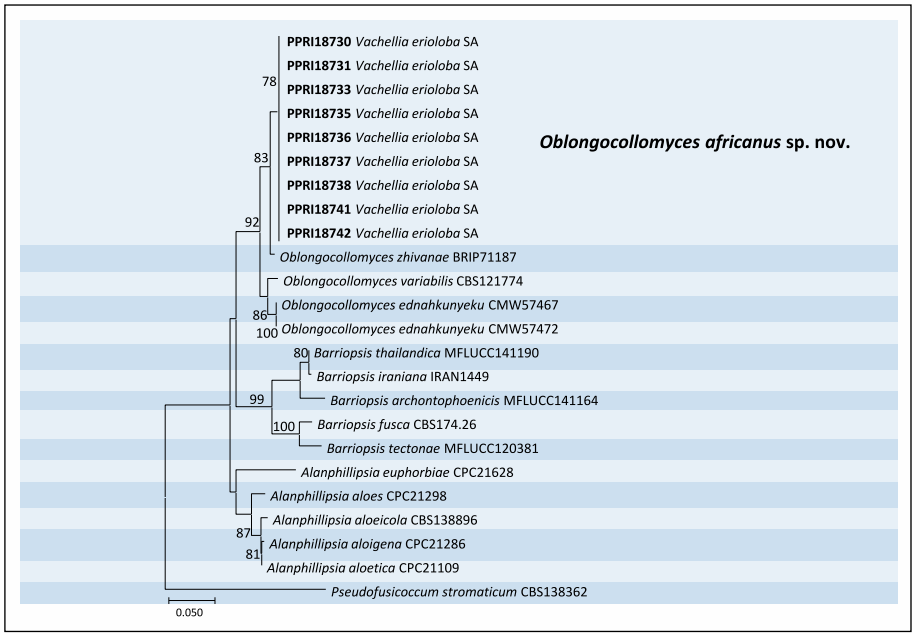

Figure 4. The combined data set of ITS, *tef1-α*, *tub2* and *rpb2* loci sequences of *Oblongocollomyces*. The tree is rooted to *Pseudofusicoccum stromaticum* (CBS138362). Bootstrap values above 75% are given at the nodes. Isolates of this study are indicated in bold. *SA = South Africa, CN = China.

L. iraniensis Abdollahz., Zare & A.J.L. Phillips*, L. lignicola* (H.A. Ariy., Jian K. Liu & K.D. Hyde) A.J.L. Phillips, A. Alves & Abdollahz.*, L. mahajangana* Begoude, Jol. Roux & Slippers*, L. plurivora* Damm & Crous*, L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous*, L. thailandica* Trakun., L. Lombard & Crous and *L. theobromae* (Pat.) Griffon & Maubl. The sequence dataset for *Neofusicoccum* contained 477 characters for ITS, 369 characters for *tef-1α* and 639 characters for the *rpb2* dataset, and the combined dataset consisted of 1 485 characters. Four clades were identified from the analyses, and these resolved as *Neofusicoccum mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips*, N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips*, N. ribis* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *N. yunnanense* G.Q. Li & S.F. Chen. The sequence dataset for *Botryosphaeria* contained 525 characters for ITS, 329 characters for *tef-1α* and 684 characters for the *rpb2* dataset, and the combined dataset consisted of 1 538 characters. One clade was identified from analyses, and this resolved as *Botryosphaeria ramosa* (Pavlic, T.I. Burgess & M.J. Wingfield) A.J.L. Phillips & A. Alves. The sequence dataset for *Oblongocollomyces* contained 535 characters for ITS, 394 characters for *tef-1α,* 592 characters for the *rpb2* dataset, and 452 characters for *tub2*, and the combined dataset consisted of 1 973 characters. One new clade was identified from the analyses, and it was described as a new species. Among all these species, five species overlapped between China and South Africa (Figure 5).

Taxonomy

An undescribed species of *Oblongocollomyces* emerged from the phylogenetic analyses and it is described as follows:

Oblongocollomyces africanus *Jami, Roux & Pavlic* sp. nov. Figure 6. MycoBank: MB857578.

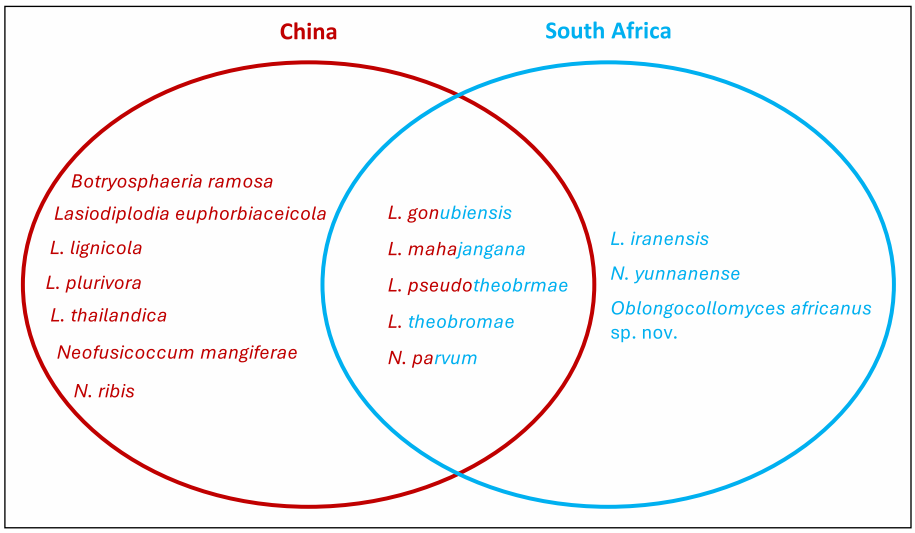

Figure 5. The pattern of overlapping *Botryosphaeriaceae* species between South Africa and China.

Conidiomata stromatic, formed on half strength PDA in vicinity of edges of plates after 21 days, often covered with aerial hyphae, producing black spore masses in single or multiple locules. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* borne on the inner most layer of compressed hyaline cells, cylindrical to lageniform, enteroblastic, 5–11 (avg. 6.5–9.5 μm) × 2–8 μm (avg. 3.5–6.5 μm). *Conidia* hyaline, becoming brown when mature, 0–1 septate, septation occurring mostly when pigmented, fusiform to ellipsoidal, bases truncated, apex round, slightly tapering towards base, 14–24 (avg. 15.5–21.5 μm) × 5–9 μm (avg. 6.5–8.5 μm).

Etymology

The species name refers to Africa, the continent from which this species was collected.

Specimens examined

SOUTH AFRICA, **Limpopo**: twigs of *Vachellia erioloba* (E.Mey.) P.J.H.Hurter., Nov. 2018, J. *Roux and C. Ncedana*, holotype, cultures ex-holotype PPRI18730, PPRI18731, PPRI18733, PPRI18735, PPRI18736, PPRI18737, PPRI18738, PPRI18741, PPRI18742.

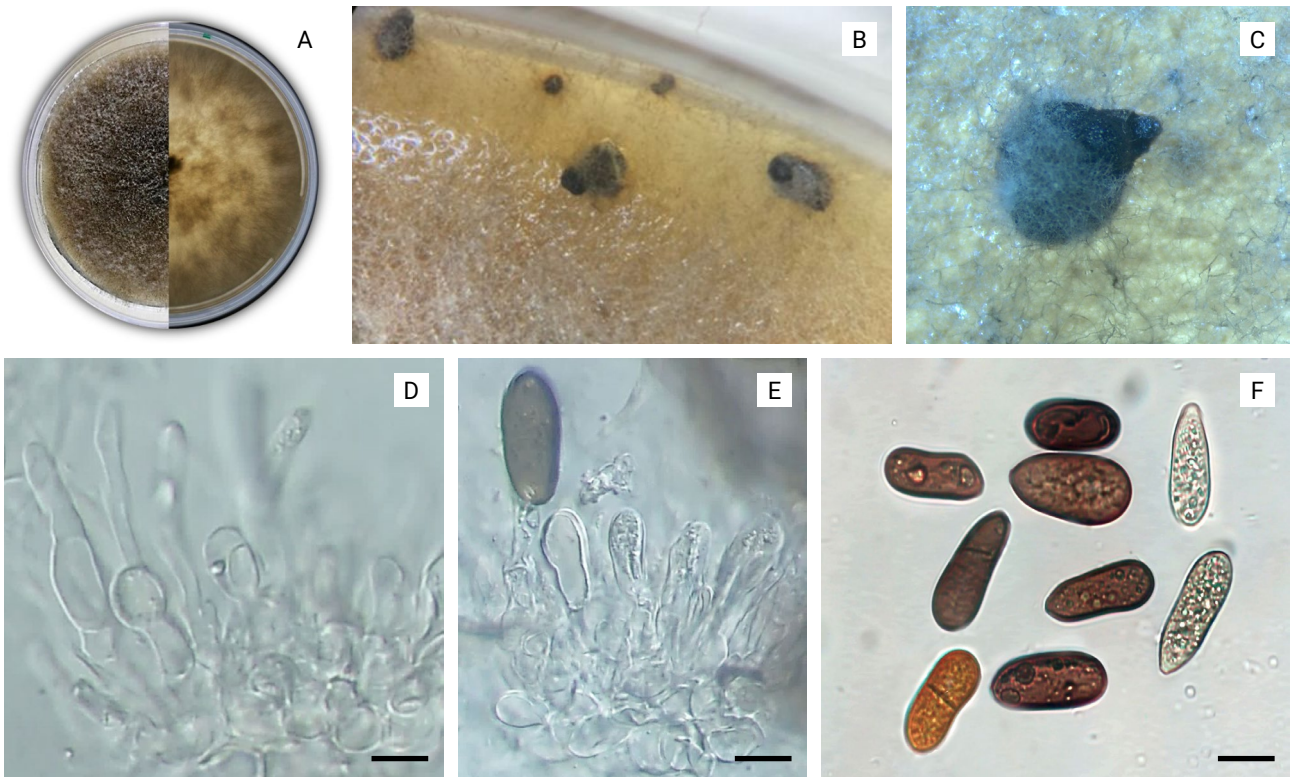

Figure 6. Morphological characteristic of *Oblongocollomyces africanus* sp. nov., A, Colony on PDA at 25°C after 5 days; B & C, Emerging pycnidia after 21 days; D, Conidiogenous cells, developing conidia and paraphyses; E, Immature conidia and mature conidium; F, Mature conidia. Scale bars = 10 μm.

Note

Oblongocollomyces africanus differs from its closest known phylogenetic neighbour, *O. zhivanae* (BRIP71187) by 3 bp in the ITS region and 4 bp in the *tub2* locus.

Discussion

The results of this study enhance our understanding of the taxonomic diversity and host ranges within the *Botryosphaeriaceae* family, with a particular focus on isolates preserved in the NCF. Using multilocus phylogenetic analyses (ITS*, tef1-α, rpb2, tub2*), 15 known species were identified, including *Botryosphaeria ramosa*, *L. brasiliensis, L. euphorbiaceicola, L. gonubiensis, L. iraniensis, L. lignicola, L. mahajangana, L. plurivora, L. pseudotheobromae, L. thailandica,* and *L. theobromae, N. mangiferae*, *N. parvum*, *N. ribis* and *N. yunnanense*, as well as a novel species within the genus *Oblongocollomyces*. This new species, along with two other species were found exclusively in South Africa. Five species were shared between South Africa and China, while seven species were found exclusively in China. These results contribute to the broader understanding of *Botryosphaeriaceae* diversity in both South Africa and China.

In this study *L. theobromae* was the most prevalent species, isolated from 26 different hosts. This dominance is not surprising, as *L. theobromae* is the most widespread species in the genus, having been reported from over 600 hosts worldwide (Batista et al. 2021). *Lasiodiplodia pseudotheobromae* was the second most prevalent species, isolated from 16 hosts. This species has been reported from 124 hosts across 44 countries (Batista et al. 2021). This study expands the hosts range of these two species, including the first reports of *L. theobromae* from *Dissotis* sp., *Diospyros eriantha* Champ. ex Benth*, Rhodomyrtus tomentosa* (Aiton) Hassk and *L. pseudotheobromae* from *Syzygium jambos* (L.) Alston.

Lasiodiplodia mahajangana was the third most prevalent species, identified on seven hosts. This species was originally described from *Terminalia catappa* L. in Madagascar (Begoude et al. 2010). In 2021, Zhang et al. synonymised seven *Lasiodiplodia* species, including *L. americana* S.F. Chen, G.Q. Li & Michailides (Chen et al. 2015), *L. caatinguensis* I.B.L. Cout., F.C. Freire, C.S. Lima & J.E. Cardoso (Coutinho et al. 2017), *L. curvata* Y. Zhang ter & S. Lin (Wang et al. 2019), *L. exigua* Linald., Deidda & A.J.L. Phillips (Linaldeddu et al. 2015), *L. irregularis* Y. Zhang ter & S. Lin (Wang et al. 2019), *L. macroconidia* Y. Zhang ter & S. Lin (Wang et al. 2019) and *L. pandanicola* Tibpromma & K.D. Hyde (Tibpromma et al. 2018) under *L. mahajangana*. To date, this species has been reported from 33 different hosts across 19 countries (https://fungi.ars.usda.gov).

Lasiodiplodia gonubiensis was identified from *Dissotis* sp. in South Africa and *Melastoma* sp. in China. Previously, this species was reported from *Syzygium cordatum, Pinus* sp. (Pavlic et al. 2004), *Sclerocarya birrea* subsp. *afra* (Sond.) Kokwaro (Jami et al. 2017) and *Ceriops tagal* (Perr.) C.B.Rob. (Osorio et al. 2017) in South Africa. It has also been reported from *Acmena smithii* (Poir.) Merr. & L.M.Perry, *Camellia sinensis* (L.) Kuntze and *Lenwebbia lasioclada* (F.Muell.) N.Snow & Guymer in Australia (Burgess et al. 2019; Tan et al. 2019), and *Phyllanthus emblica* L. in Thailand (Trakunyingcharoen et al. 2015). *Lasiodiplodia iraniensis,* has been reported from 27 hosts across 18 countries (https://fungi.ars.usda.gov), including *Adansonia digitata* L. and *Sclerocarya birrea* subsp. *afra* in South Africa (Cruywagen et al. 2017; Jami et al. 2017), *Bougainvillea spectabilis* Willd. and *Caryota mitis* Lour. in China (Li et al. 2015; Zhu et al. 2015). In this study *L. iraniensis*was identified on *Melaleuca* sp. in China and *Punica granatum* L. in South Africa.

Lasiodiplodia thailandica and *L. lignicola* were identified from six different hosts in China. *Lasiodiplodia thailandica* was found on a Myrtales species, *Melaleuca parviflora* and *Syzygium aromaticum*, while *L. lignicola* was identified from *Callistemon* sp., *Melastoma* sp., *Mesua ferrea* L. and *Syzygium jambos*. *Lasiodiplodia thailandica* was previously reported from *Acacia confusa* Merr., *Albizia chinensis* (Osbeck) Merr. and *Podocarpus macrophyllus* (Thunb.) Sweet. in China (Dou 2017; Li et al. 2018), as well as from *Mangifera indica* L., *Pandanus* sp. and *Phyllanthus acidus* (L.) Skeels in Thailand (Hyde et al. 2018). *Lasiodiplodia lignicola* has been reported from *Aquilaria crassna* Pierre. ex Lecomte in Laos (Wang et al. 2019), *Sterculia oblonga* Mast. in Germany (Yang et al. 2017) and *Canarium parvum* Leenh., *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg, *Rhodomyrtus tomentosa*, *Sterculia lychnophora* Hance and *Vaccinium uliginosum* L. in China (Dou 2017; Dou et al. 2017).

Some *Lasiodiplodia* species were identified exclusively from a single host in China. For instance, *L. euphorbiaceicola* and *L. plurivora* were identified on *Lagerstroemia* sp. and a Myrtales species, respectively, both of which are new hosts for these species. *Lasiodiplodia euphorbiaceicola* was initially described from *Jatropha curcas* L. (Machado et al. 2014) in Brazil and has since been reported from various hosts, including *Anacardium* sp. (Netto et al. 2017), *Cocos nucifera* L. (Coutinho et al. 2017), *Carica papaya* L. (Netto et al. 2014), *Vitis vinifera* L. (Correia et al. 2016) and *Nopalea cochenillifera* (L.) Salm-Dyck (Conforto et al. 2019) in Brazil. It has also been reported from *Adansonia digitata* in Botswana, Cameroon, Madagascar, Senegal and Zimbabwe (Cruywagen et al. 2017). *Lasiodiplodia plurivora*

was previously reported from *Prunus salicina* Lindl. and *Vitis* sp. in Australia and South Africa (Jami et al. 2017; Burgess et al. 2019). These findings significantly broaden the known host ranges of *Lasiodiplodia* species and emphasise the ongoing need to monitor their spread across diverse regions and plant species.

Neofusicoccum is among the 100 most cited fungal genera (Bhunjun et al. 2024). In this study, four species were identified including *N. mangiferae*, *N. parvum*, *N. ribis* and *N. yunnanense*. *Neofusicoccum parvum* was the most common species isolated from three different hosts in China and South Africa. *Neofusicoccum mangiferae* was identified exclusively in China, across six different hosts. This species has been reported from 13 countries from 25 hosts (https://fungi.ars.usda.gov) including China (Dissanayake et al. 2015; Lin et al. 2019; Wan et al. 2020) and South Africa (Pavlic et al. 2007; Jami et al. 2017). However, all six hosts, *Syzygium* sp., *Agonis* sp., a Myrtales species, *Callistemon* sp., *Lagerstroemia* sp., *Melastoma* sp., represent new host records. *Neofusicoccum yunnanense* was identified on *Dissotis* sp. in South Africa and was previously known only from *Eucalyptus* species in China (Li et al. 2020) and apples in Serbia (Vučković et al. 2022).

Neofusicoccum parvum and *N. ribis* are phylogenetically closely related and had been considered a species complex. The taxonomy of the *N. parvum*/*N. ribis* complex has been re-evaluated multiple times over the past two decades, leading to the introduction of six species, including *N. batangarum* Begoude, Jol. Roux & Slippers, *N. cordaticola* Pavlic, Slippers & M.J. Wingf., *N. kwambonambiense* Pavlic, Slippers & M.J. Wingf., *N. occulatum* Sakalidis & T.I. Burgess, *N. umdonicola* Pavlic, Slippers & M.J. Wingf. and *Neofusicoccum* sp. (Slippers et al. 2004; Pavlic et al. 2009; Sakalidis, et al. 2011a). However, a recent taxonomic revision by Zhang et al. (2021) synonymised *N. batangarum* and *N. umdonicola* under *N. ribis*. To date, *N. ribis* has been reported from 285 hosts across 47 countries (https://fungi.ars.usda.gov), including various hosts in South Africa (Pavlic et al. 2007) and China (Chen et al. 2011).

Botryosphaeria ramosa is a relatively uncommon species, first described as *Fusicoccum ramosum* Pavlic, T.I. Burgess & M.J. Wingf. from asymptomatic branches of *Eucalyptus camaldulensis* Dehnh. in Australia (Pavlic et al. 2008). Later, Phillips et al. (2013) reclassified it under the correct name *Botryosphaeria ramosa*. This species was subsequently reported from *Acacia* sp., *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson and a Myrtaceae species in China (Zhou et al. 2017). *Botryosphaeria pseudoramosa* G.Q. Li & S.F. Chen was initially reported from *Eucalyptus* sp. and *Melastoma* in China (Li et al. 2018), but Zhang et al. (2021) later synonymised it under *B. ramosa*. In this study, *B. ramosa* was identified from *Callistemon* sp., *Syzygium jambos*, *Rhodomyrtus tomentosa*, and three *Melastoma* species, all of which represent new host records for this species. Interestingly, all known hosts of *B. ramosa* to date are in the same order, the Myrtales.

Oblongocollomyces is a rare genus within the *Botryosphaeriaceae* family, previously reported only from southern Africa and Australia. Described by Yang et al. (2017), its type species, *Oblongocollomyces variabilis* (F.J.J. Van der Walt, Slippers & G.J. Marais) Tao Yang & Crous, was originally identified as *Sphaeropsis variabilis* F.J.J. Van der Walt, Slippers & G.J. Marais and found on *Vachellia hebeclada* (DC.) Kyal. & Boatwr., *V. karroo* Hayne) Banfi & Galasso, and *Senegalia mellifera* (Vahl) Seigler & Ebinger in Namibia and South Africa (Slippers et al. 2014). Subsequently, *O. ednahkunjekuae* Slippers, Ramabulana & Coetzee was discovered on *Sclerocarya birrea* in South Africa (Slippers et al. 2024), and *O. zhivanae* on *Vachellia farnesiana* in Australia (Limbongan et al. 2023). In this study, we describe a novel species, *Oblongocollomyces africanus* isolated from *Vachellia erioloba* in South Africa. These findings highlight the expanding diversity and host range of *Oblongocollomyces*, suggesting that additional undescribed species may exist in unexplored regions and host plants.

The diverse isolate origins in this study have revealed distinct distributions of *Botryosphaeriaceae* species, including several first-time reports for each area, including *N. yunnanense* and *Botryosphaeria ramosa* for South Africa, *L. mahajangana*, *L. gonubiensis*, *L. euphorbiaceicola* and *L. plurivora* for China and *L. brasiliensis* for Benin. Additionally, multiple species were found on new hosts in both countries including *L. gonubiensis*, *L. iraniensis*, *L. mahajangana*, *L. pseudotheobromae, L. theobromae* and *N. parvum*. These findings significantly enhance our understanding of the geographical distribution of *Botryosphaeriaceae* species across China and South Africa. The identification of these fungi on various hosts in diverse regions underscores their extensive presence and ecological importance in both countries.

Botryosphaeriaceae species are known as latent pathogens that can cause disease when their hosts are under stress (Slippers & Wingfield 2007). Some of the identified species of this study are well-known pathogens associated with canker and branch dieback, including *L. pseudotheobromae, L. theobromae, N. mangiferae* and *N. parvum* (Sakalidis et al. 2011b; Mehl et al. 2013; Mehl et al. 2017). *Lasiodiplodia mahajangana* has been reported in association with dieback and canker on various hosts (Burgess et al. 2019) and shoot blight on pistachio (Chen et al. 2015). In this study, *L. mahajangana* was identified from black spots on mango, banana and avocado fruits, indicating its potential as a postharvest pathogen. *Neofusicoccum yunnanense* is another example that has been previously reported causing dieback and cankers on *Eucalyptus* (Li et al. 2020) and as

postharvest pathogen of apples in Serbia (Vučković et al. 2022). In this study, *N. yunnanense* was found on *Dissotis* sp., as an endophyte suggesting a broader host range than previously documented. Further studies are needed to assess its pathogenicity and potential impact on newly identified hosts.

Conclusion

This study highlights the critical role of fungal collections, such as the NCF, in addressing global gaps in fungal biodiversity. The identification of novel species and the overlap of species between South Africa and China highlights the importance of continued research and global collaboration to better understand the ecological roles and potential impacts of these fungi as pathogens across different regions. These findings emphasise the importance of continued exploration of fungal collections and ecosystems in underexplored regions. Such efforts will enhance fungal taxonomy, also inform plant health management strategies, and contribute to broaden the biodiversity conservation initiative.

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Authors' contributions

FJ conducted laboratory experiments, analysed fungal species, conceived and supervised the study, coordinated manuscript writing and editing. MT and DP contributed to the sample collection and isolations. SC and JR contributed to the sample collection.

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List of supplementary materials

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Table S1. Hosts and locations for *Botryosphaeriaceae* isolates of this study including GenBank accession numbers for the four gene regions ITS, β-tubulin (*tub2*), translation elongation factor 1α (*tef1-α*) and RNA polymerase II second largest subunit (*rpb2*) sequenced.

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