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Root endophytic *Chaetomiaceae (Sordariales)* from *Musa acuminata (Musaceae)* in Brazil, including *Intraradix grisea gen. et sp. nov.*

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Abstract: Banana is one of the main agronomic crops produced worldwide and has a great economic importance. Endophytic fungi can play an important role in the resistance to plant pathogens. During a survey that aimed to identify endophytic fungi from healthy banana roots in an area with a high incidence of Fusarium wilt, fungal isolates belonging to *Chaetomiaceae* were obtained. Polyphasic approach was applied for the identification of the isolates. Phylogenetic analyses were performed using Internal Transcribed Spacer (ITS), 28S large subunit rDNA (LSU), RNA polymerase second-largest subunit (*RPB2*), and beta-tubulin (*TUB2*) sequences. An endophytic isolate from cassava roots was added to the phylogenetic analysis of this study, after a preliminary megablast search of NCBI's GenBank nucleotide database. A new genus within *Chaetomiaceae* from banana and cassava roots, *Intraradix grisea gen. et sp. nov.*, was identified and described. In the phylogenetic analysis, *I. grisea* was grouped next to the genera *Thermothielavioides* and *Floropilus*. In addition, a new species belonging to the genus *Dichotomopilus*, namely *Dichotomopilus endophyticus sp. nov.*, is introduced. Futhermore, *Dichotomopilus variostiolatus* and *Chaetomium globosum* were first reported as part of the root endophytic community associated with banana roots.

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INTRODUCTION

Banana is a crop of huge economic interest, with associated fungal diversity that can be influenced by plant variety, the type of tissue evaluated, climatic conditions, and other factors (Souza Junior *et al.* 2018, Savani *et al.* 2021). Banana fruit has nutritional and medicinal properties, and can be consumed fresh, fried, cooked, or processed (Ranjha *et al.* 2020). Most edible bananas consumed result from crosses between diploids belonging to the species *Musa acuminata*, which generates triploids, such as the Cavendish subgroup (Christelová *et al.* 2017). Although there are other banana varieties, Cavendish is the most commercialized representing approximately 50 % of global production. However, the occurrence of fungal diseases has been limiting worldwide (Cordeiro *et al.* 2016).

Fusarium wilt of banana, also known as Panama disease is the most destructive disease in banana crops, causing great economic losses (Buddenhagen 2009). This disease is caused by soil-borne *Fusarium* spp. (Heck *et al.* 2021, Maryani *et al.* 2019a, b, Ujat *et al.* 2021). Studies report that different *Fusarium* species such as *F. odoratissimum*, *F. hexaseptatum*, *F. tardichlamydosporum*, *F. duoseptatum*, *F. grosmichelii*, *F. phialophorum*, and *F. purpurascens* within the *Fusarium oxysporum* species complex (FOSC) and *F. sacchari* in the *Fusarium fujikuroi* species complex (FFSC) can cause Fusarium wilt on banana (Maryani *et al.* 2019a, b, Ujat *et al.* 2021). These plant pathogens produce toxic substances that lead to cell death and development of disease symptoms (Ploetz 2015, Sanchez-Espinosa *et al.* 2020). The control of Fusarium wilt is still a challenge, as chemical methods do not achieve satisfactory results, whereas genetic breeding may require considerable time and market acceptance (Ordonez *et al.* 2015). Therefore, the use of endophytic fungi as biological control agents can be a promising alternative for the management of this vascular wilt.

Different endophytic fungal genera, including isolates from bananas, have been studied as potential biocontrol agents (Silva *et al.* 2019). For example, the root endophytic fungus *Serendipita indica* from *Musa* spp. may improve the resistance of banana plants to *Fusarium oxysporum* f. sp. *cubense* tropical race 4, which is the major pathogen in this crop (Cheng *et al.* 2020). Other fungi, such as *Trichoderma asperellum*, *Penicillium simplicissimum*, and *Leptosphaeria* sp., have also demonstrated the potential to control phytopathogens, such as Fusarium wilt on gerbera (Brandler *et al.* 2017) and Verticillium wilt on cotton seedlings (Yuan *et al.* 2017).

During a survey of endophytic fungi from asymptomatic roots of *Musa acuminata* located in a farm with a high incidence of Fusarium wilt in Brazil, fungi with morphological characteristics belonging to the genus *Chaetomium* were identified. Some *Chaetomium* species have been reported to act as biocontrol agents against plant pathogens by suppressing the development of soil-borne fungi, including *Fusarium* spp. (Dhingra *et al.* 2003, Yue *et al.* 2018, Soytong *et al.* 2021). As an example, *Chaetomium globosum* produces substances known as Chaetoviridis and Chaetomugilins, which can inhibit some pathogens, such as *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium graminearum*, *Phytophthora capsici*, and *Fusarium moniliforme* (Yan *et al.* 2018, Omar *et al.* 2022). *Chaetomium* belongs to the family *Chaetomiaceae*, which plays a significant role in the medical, ecological, and biotechnological fields because it produces substances of commercial interest (Yan *et al.* 2018, Tian & Li 2022).

Chaetomiaceae comprises 53 genera and approximately 300 species and was historically defined by its sexual morph, producing superficial ostiolate ascoma with an outer wall covered by appendages, asci that develop in basal fascicles with an evanescent wall, brownish ascospores, and mostly lacking an asexual morph (Winter 1885, Wang *et al.* 2016a, 2022, Sastoque *et al.* 2025). With the advanced use of molecular characters in phylogenetic analysis, the taxonomy of this group of fungi has undergone significant changes, and most members of *Chaetomiaceae* have been redefined within the family (Wang *et al.* 2016a, 2022). Studies have proposed the definition of new genera within the family to accommodate species with morphological characteristics similar to those of *Chaetomium*, known as chaetomium-like (Wang *et al.* 2016a, b, 2022).

Members of this family have a wide geographic distribution and can be found in different habitats, including plant endophytes (Wang *et al.* 2016a, 2022). The endophytic habitat appears to harbour a great diversity of unknown *Chaetomiaceae* species. Therefore, species belonging to the genera *Achaetomium*, *Arcopilus*, and *Chaetomium* have been described and reported as endophytes of various plant families (Crous *et al.* 2017, Sousa *et al.* 2020, Yang *et al.* 2024). Moreover, the genera *Allocanariomyces* and *Parachaetomium* were described as seed endophytes of *Triticum boeoticum* and *Aegilops triuncialis*, respectively (Mehrabi *et al.* 2020).

Previous studies have reported that the fungi *Chaetomium* sp., *C. globosum, Dichotomopilus erectus*, and *D. funicola* are associated with the genus *Musa* in Papua New Guinea, the Philippines, and Thailand (Farr & Rossman 2024). However, to the best of our knowledge, they have not been isolated as endophytes, and there are not any reports of *Chaetomiaceae* in banana plants in Brazil. Thus, the present study aimed to identify and describe endophytic *Chaetomiaceae* species in banana roots using morphological and phylogenetic analysis.

MATERIAL AND METHODS

Sample collection and isolation

The collections take place in a farm with a high incidence of Fusarium wilt, in the municipality of Itinga do Maranhão, Maranhão, Brazil (Fig. 1). Healthy root samples were collected from vigorous banana plants (*Musa acuminata*) of two varieties, Dwarf Cavendish and Prata type, in December 2021. The collected plants were grown together with the diseased or withered plants. Root samples were placed in paper bags, stored at 4 °C and carefully transported to the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas, housed at the Departamento de Fitopatologia of the Universidade Federal de Viçosa, Minas Gerais.

Fungi were isolated by the indirect isolation method based on the methodology described by Pereira & Zambolim (2012). The samples were washed in running water, fragmented, and disinfested in 70 % alcohol (1 min) and 2 % sodium hypochlorite (3 min) and washed in sterile distilled water three times (1 min each). After disinfestation, the samples were dried on filter paper and transferred to Petri dishes containing Potato Dextrose Agar (PDA) culture medium. The plates were incubated at 25 °C until fungal growth was observed and transferred to new Petri dishes containing PDA. Pure cultures were obtained by transferring hyphal tips grown in water agar to PDA plates (Dhingra & Sinclair 1995).

The isolates were stored in sterile distilled water, in anhydrous silica gel, at 5 °C, and in 2 mL microtubes containing 10 % glycerol solution at -20 °C (Castellani 1939, Dhingra & Sinclair 1995). The isolates were deposited in the fungal culture collection "Coleção Octávio Almeida Drummond" (COAD). Dry cultures were deposited in the VIC Herbarium. Both collections are housed at the Universidade Federal de Viçosa. Nomenclatural novelties and descriptions were deposited in the MycoBank (www.mycobank.org).

In addition, one isolate deposited in the culture collection of "Micoteca URM" at Universidade Federal de Pernambuco was added to this study. The strain URM 9211 was isolated as a root endophyte of *Manihot esculenta* and had the same characteristics of one of the isolates obtained from banana roots.

DNA extraction, PCR and sequencing

The isolates were grown on PDA culture medium for 7 d at 25 °C in the dark. The fungal structures were removed and transferred to 2.0 mL microtubes containing 600 uL of Nuclei Lysis Solution – Wizard Genomic DNA Extraction Kit (Promega), 100 mg of Polyvinylpyrrolidone (PVP; Sigma-Aldrich), and four spheres of steel. Subsequently, the samples were mixed and crushed on the L-BEADER 6 cell and tissue disruptor (Loccus Biotecnologia) for 60 s at 4000 rpm. After maceration, the extraction was conducted according to the methodology described by Pinho *et al.* (2013).

The internal transcriber spacer (ITS), nuclear 28S rDNA region (LSU), RNA polymerase II second largest subunit (*RPB2)*, and beta-tubulin (*TUB2)* gene regions were amplified. The combination of primers used were: ITS1 / ITS4 (White *et al.* 1990), ITS1 / LR6 (Vilgalys & Hester 1990), fRPB2-5F2 (Sung *et al.* 2007) and fRPB2-7cR (Liu *et al.* 1999), T1 (O'Donnell & Cigelnik 1997) / TUB4Rd (Groenewald *et al.* 2013) for ITS, LSU, *RPB2* and *TUB2*, respectively. The gene regions were amplified by Polymerase Chain Reaction (PCR), and each reaction was prepared using 18 µL of Platinum® PCR SuperMix, 0.4 µL of each 10 µM forward and reverse primer synthesized by Invitrogen (Carlsbad, USA), 1.2 µL of genomic DNA (25 ng/µL). The reactions were incubated using a thermocycler, with an initial denaturation temperature of

Fig. 1. Banana plants in the field. **A.** Banana field in which roots were collected. **B.** Plants with vigorous growth next to dead plant with Fusarium wilt symptoms**. C.** Root samples, the white arrow indicates the healthy root.

94 °C/2 min and 35 cycles of denaturation at 94 °C / 30 s, annealing (ITS and LSU 49 °C, *RPB2*, and *TUB2* 56 °C) / 30 s, extension at 72 °C / 90 s. The PCR products were purified and sequenced by Macrogen Inc., South Korea.

Phylogenetic analysis

The BioEdit v. 7.0.9 (Hall 2012) was used to visualize and form the consensus sequence of the obtained sequences. The consensus sequences were compared against the GenBank database, using their megaBLAST program for the first identification of fungal genera. The sequences generated in the present study presented high similarity with the sequences of the *Chaetomiaceae*, highlighting the genera *Chaetomium*, *Dichotomopilus*, *Floropilus*, and *Thermothielavioides*. Additional sequences were obtained from the GenBank to create databases of the entire *Chaetomiaceae* and the genus *Dichotomopilus* (Table 1). The sequences retrieved were aligned with those generated in this study, using the MUSCLE® algorithm [Multiple Sequence Comparison by Log -Expectation (Edgar 2004)], carried out in the MEGA X program (Kumar *et al.* 2018), using the default parameters. The alignments were verified, and manual adjustments were carried out where necessary. The resulting alignment was deposited at figshare.com (doi: 10.6084/m9.figshare.28339313). The best nucleotide substitution model was determined for each gene by MrModeltest v. 2.3 (Posada & Buckley 2004), according to the Akaike Information Criterion (AIC). Phylogenetic analyses were performed individually and concatenated for the ITS, LSU, *RPB2*, and *TUB2* genes (see supplementary material, Figs S1–S8).

Phylogenetic analysis by Bayesian Inference were performed on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2015), using MrBayes v. 3.2.6 (Ronquist & Heulsenbeck 2003). In MrBayes, the data were partitioned by locus, and the parameters of the nucleotide substitution models for each partition were defined as described above. Four MCMC chains were run concurrently in each run, starting from random trees for 10 M generations. The trees were sampled every 1000 generations, totalling 20002 trees. The first 2500 trees were discarded in the burning phase of each analysis. Further probabilities (Rannala & Yang 1996) were determined from a majority-rule consensus tree generated from the remaining trees. Maximum Likelihood analysis with 10000 bootstrapping replicates (Hoang *et al.* 2018) were conducted in IQ-TREE v. 2.2.0 (Minh *et al.* 2020), and ModelFinder (Kalyaanamoorthy *et al.* 2017) as implemented in IQ-TREE was used to calculate the best nucleotide substitution model for each region. The trees generated were visualized in the application FigTree v. 1.4.4 (Rambaut 2018) and later edited in graphics programs. The trees were rooted with *Pseudoechria longicollis* CBS 610.97^{T} and *Pseudoechria prolifica* CBS 250.71^{T}, for phylogenetic analysis of the family, and *Chaetomium elatum* CBS 142034^{neoT} for phylogenetic analysis of the genus *Dichotomopilus*.

Morphology

Morphological characteristics of *Chaetomiaceae* isolates were described following the methodology suggested by Wang *et al.* (2022). Colony characteristics were recorded in four different culture media, for 7 d, at 25 °C (or 37 and 42 °C), in the dark: cornmeal agar (CMA), malt extract agar (MEA), oatmeal agar (OA), and potato carrot agar (PCA) (Crous *et al.* 2009, Samson *et al.* 2010). Colour names mentioned in the descriptions adhere to Rayner (1970). After 7–14 d of incubation, the fungal structures were transferred to slides containing a drop of lactoglycerol or Shear's solution and later added and covered with a coverslip. Photographs were taken with an Olympus BX53 microscope equipped with a digital camera, Olympus Q-Color5™. The relevant morphological characteristics such as the length and width of the ascoma, the diameter of the ascomatal appendix, and the length and width of the asci and ascospores, were measured (n = 30) using the Olympus cellSens Dimension v. 1.9 software system.

RESULTS

Root endophytic isolates

A total of 79 endophytic isolates were obtained from banana roots of which six belonging to the family *Chaetomiaceae*. The isolates COAD 3645, COAD 3647, and COAD 3648 were obtained from Dwarf Cavendish bananas, whereas COAD 3646, COAD 3649, and COAD 3910 were obtained from Prata type bananas. Morphological and phylogenetic analysis revealed that four isolates (COAD 3645, COAD 3646, COAD 3647, and COAD 3648) belonged to the genus *Dichotomopilus* and one isolate (COAD 3649) belonged to the genus *Chaetomium*. One isolate (COAD 3910) could not be assigned to any known genus belonging to *Chaetomiaceae*. In addition, the ITS sequence of the strain URM 9211, obtained as root endophyte of cassava plants (*Manihot esculenta*), presented high similarity with COAD 3910 and was included in the present study to better comprehend the relation of these isolates in the *Chaetomiaceae*.

Phylogenetic analyses

Phylogenetic analyses were performed for each genus separately and for the *Chaetomiaceae*. The best substitution models for BI and ML analysis chosen for each DNA region are presented in Table 2.

Sequences from the regions of *TUB2* and *RPB2* were sufficient to identify almost all the isolates in the phylogenetic analysis, with ITS and LSU being more informative to separate the genus within the family. In the concatenated phylogenetic analysis by Bayesian Inference and Maximum Likelihood, the isolate COAD 3649 was identified as *Chaetomium globosum* (Fig. 2). Isolate COAD 3648 grouped with *Dichotomopilus variostiolatus* CBS 179.84^{T} in a single well-supported clade (Fig. 3).

Three isolates (COAD 3645, COAD 3646, and COAD 3647) formed a single well-supported clade distinct from every known species of *Dichotomopilus* and represent a new species (Fig. 3). This new species is phylogenetically related to *D. erectus*, *D. pseudoerectus*, and *D. ramosissimus*, in the individual and combined phylogenetic analysis. The isolates COAD 3910 and URM 9211 formed a distinct clade closest to the genera *Floropilus* and *Thermothielavioides*, constituting a new genus within *Chaetomiaceae* (Fig. 2).

Table 1. *Chaetomiaceae* species and sequences used in the phylogenetic analysis.

Species	Culture accession	Substrate/Host	Gen Bank accession number				Reference
			ITS	LSU	*TUB2*	*RPB2*	
Achaetomiella gracilis	CBS 146.60^T	Soil	KX976648	KX976743	KX976990	KX976842	Wang *et al.* (2022)
A. virescens	CBS 148.68^T	Agricultural soil	KX976654	KX976749	KX976996	KX976848	Wang *et al.* (2022)
Achaetomium globosum	CBS 332.67^T	Rhizosphere	KX976570	KX976695	KX976911	KX976793	Wang *et al.* (2016a)
Ac. lentum	CBS 618.68	Rhizosphere	KX976570	KX976695	KX976911	KX976793	Wang *et al.* (2022)
Ac. macrosporum	CBS 102436	Garden soil	MZ334718	AJ312099	MZ343007	MZ342966	Wang *et al.* (2016a)
Ac. strumarium	CBS 333.67^T	Soil	AY681204	AY681170	AY681238	KC503254	Wang *et al.* (2016a)
Acrophialophora nainiana	CBS 100.60^T	Farm soil	MK926793	MK926793	MK926893	MK876755	Wang *et al.* (2019a)
Acr. teleoafricana	CBS 281.79^T	Soil	MK926795	MK926795	MK926895	MK876757	Wang *et al.* (2019a)
Allobotryotrichum blastosporum	CGMCC 3.19343^T	Roots of *Saccharum officinarum*	MN215716	MN215554	MN329887	MN255397	Raza *et al.* (2019)
Allocanariomyces americanus	CBS 147185^T	Human right hip subcutaneous tissue	MT902181	MT902391	MT904876	MT904877	Wang *et al.* (2022)
Al. tritici	IRAN 3450C^T	Seed endophyte of *Triticum boeoticum*	MT568839	MT568842	MT568850	MT568845	Mehrabi *et al.* (2020)
Amesia atrobrunnea	CBS 379.66^T	Mouldy mattress	JX280771	JX280666	KX976916	KX976798	Wang *et al.* (2016a)
Am. cymbiformis	CBS 176.84	Tent rope	KX976577	KX976702	KX976919	KX976801	Wang *et al.* (2016a)
Aporothielavia leptoderma	CBS 538.74^T	Soil	NR_16421	NG_067253	MZ343025	MZ342986	Wang *et al.* (2022)
Arcopilus aureus	CBS 153.52	Unknown	KX976582	KX976707	KX976924	KX976806	Wang *et al.* (2016a)
	CBS 560.80	Dung of moose	KX976584	KX976709	KX976926	KX976808	Wang *et al.* (2016a)
Ar. macrostiolatus	CBS 102435^T	Forest soil	MZ334722	MZ351418	MZ343006	MZ342965	Wang *et al.* (2022)
Ar. turgidopilosus	CBS 169.52^T	Top of storage tent	KX976588	KX976713	KX976930	KX976812	Wang *et al.* (2016a)
Arxotrichum repens	CBS 233.82^T	Soil	MK919282	MK919282	MK919396	MK919338	Wang *et al.* (2022)
Arx. wyomingense	CCF 5691^T	Soil	LT968153	LT968143	LT971393	—	Crous *et al.* (2018)
Batnamyces globulariicola	CBS 144474^T	Roots of *Globularia alypum*	MT075917	MT075917	MT075919	MT075918	Noumeur *et al.* (2020)
Bommerella trigonospora	CBS 324.69^T	Soil	—	MZ351419	MZ343022	MZ342984	Wang *et al.* (2022)
Botryoderma lateritium	CBS 586.66^T	Soil mixed with leaf litter	MK919287	MK919287	MK919401	MK919343	Wang *et al.* (2022)
B. rostratum	CBS 184.68^T	Sandy soil	MK919288	MK919288	MK919402	MK919344	Wang *et al.* (2022)
Botryotrichum murorum	CBS 163.52	Unknown	KX976591	KX976716	KX976933	KX976815	Wang *et al.* (2016a)
Bo. piluliferum	CBS 654.79^T	Pastry	KX976597	KX976722	KX976939	KX976821	Wang *et al.* (2016a)
Brachychaeta variospora	CBS 414.73^T	Soil	MK926797	MK926797	MK926897	MK876759	Wang *et al.* (2019a)
Canariomyces arenarius	CBS 507.74^T	Desert soil	MK926798	MK926798	MK926898	KM655438	Wang *et al.* (2019a)
C. notabilis	CBS 548.83^T	Litter of *Phoenix canariensis*	MK926802	MK926802	MK926902	MK876763	Wang *et al.* (2019a)
Carteria arctostaphyli	CBS 229.82^T	*Arctostaphylos uva-ursi*	MK926807	MK926807	MK926907	MK876767	Wang *et al.* (2019a)
Chaetomium elatum	CBS 142034^T	Cardboard	KX976612	KX976733	KX976954	KX976832	Wang *et al.* (2016a)
Ch. globosum	CBS 160.62^T	Compost	KT214565	KT214596	KT214742	KT214666	Wang *et al.* (2016b)
	CBS 105.40	Mouldy book	KT214566	KT214597	KT214743	KT214667	Wang *et al.* (2016b)
	COAD 3649	**Roots of *Musa acuminata***	**OR626086**	—	**OR614380**	**OR611985**	**This study**
Ch. meridionalense	COAD 3451^T	Leaf litter from a cave	ON989660	ON979679	ON988187	OP131292	Condé *et al.* (2023)
	COAD 3122	Leaf litter from a cave	ON989661	ON979680	ON988188	OP131291	Condé *et al.* (2023)
Ch. neoglobosporum	CBS 108.83^T	Green leaf of *Triticum aestivum*	KC109750	KC109750	KC109768	KF001825	Wang *et al.* (2016b)
Ch. subglobosum	CBS 149.60^T	Dead herbaceous stem	JN209930	JN209930	JN256200	KF001808	Wang *et al.* (2016b)
Ch. tenue	CBS 139.38^T	Unknown	KT214568	KT214599	KT214745	KT214669	Wang *et al.* (2016b)
Ch. umbonatum	CBS 293.83^T	Soil	KT214575	KT214606	KT214752	KT214676	Wang *et al.* (2016b)
Ch. unguicola	CBS 128446^T	Nail of *Homo sapiens*	KT214567	KT214598	KT214744	KT214668	Wang *et al.* (2016b)
Chrysanthotrichum allolentum	CBS 644.83^T	Soil	MK926808	MK926808	MK926908	MK876768	Wang *et al.* (2019a)
Chr. lentum	CBS 339.67^T	Soil	MK926809	MK926809	MK926909	MK876769	Wang *et al.* (2019a)
Chr. leptolentum	CBS 126.85^T	Dung of elephant	MK926810	MK926810	MK926910	MK876770	Wang *et al.* (2019a)
Chr. peruvianum	CBS 732.68^T	High mountain tundra soil	MK926812	MK926812	MK926912	MK876772	Wang *et al.* (2019a)
Chrysocorona lucknowensis	CBS 727.71^T	Dung of deer	MK926813	MK926813	MK926913	MK876773	Wang *et al.* (2019a)

Table 1. (Continued)

Collariella bostrychodes	CBS 163.73	Dung of antelope	KX976641	KX976738	KX976983	KX976837	Wang *et al*. (2016a)
Col. carteri	CBS 128.85^{T}	Air	KX976647	KX976742	KX976989	KX976841	Wang *et al*. (2016a)
Condenascus tortuosus	CBS 610.97^{T}	Soil	MK926817	MK926817	MK926917	MK876777	Wang *et al*. (2019a)
Corynascella humicola	CBS 337.72^{T}	Soil	KX976656	KX976751	KX976998	KX976850	Wang *et al*. (2016a)
Corynascus sepedonium	CBS 111.69^{T}	Soil	HQ871751	KX976777	KX977027	KX976892	Wang *et al*. (2016a)
Cor. sexualis	CBS 827.96^{T}	Soil	MK919295	MK919295	MK919409	MK919352	Wang *et al*. (2022)
Dichotomopilus dolichotrichus	CBS 162.48T	Unknown	HM449049	JF772462	HM449063	KX976852	Wang *et al*. (2016a)
D. dolichotrichus	CGMCC 3.14189	Discarded cloth	HM449048	JF772455	HM449062	KX976853	Wang *et al*. (2016a)
D. endophyticus	**COAD 3645**	**Roots of *Musa acuminata***	—	—	**OR614381**	**OR611982**	**This study**
	COAD 3646	**Roots of *Musa acuminata***	**OR626088**	**PV014878**	**OR614382**	**OR611983**	**This study**
	COAD 3647^{T}	**Roots of *Musa acuminata***	**OR626089**	**PV014879**	**OR614383**	**OR611981**	**This study**
D. erectus	CBS 140.56^{T}	*Petroselinum sativum*	HM449044	JF772458	HM449058	KX976854	Wang *et al*. (2016a)
	CGMCC 3.12900	Soil	KC109760	KC109778	KC109760	KX976855	Wang *et al*. (2016a)
D. finlandicus	SZMC 26529^{T}	Inlet air filter, public building	MW541926	MZ665529	—	MZ665531	Kedves *et al*. (2021)
D. funicola	CBS 159.52^{T}	Unknown	GU563369	JF772461	GU563354	KX976856	Wang *et al*. (2016a)
	CBS 136.38	Unknown	HM449046	JF772457	HM449060	KX976857	Wang *et al*. (2016a)
D. fusus	CBS 372.66^{T}	Leaf litter	KX976660	KX977002	KX976754	KX976859	Wang *et al*. (2016a)
	CBS 114.83	*Tectona grandis* or calyx	KX976661	KX977003	KX976755	KX976860	Wang *et al*. (2016a)
D. indicus	CGMCC 3.14184^{T}	Rhizosphere of *Panax notoginseng*	GU563367	JF772453	GU563360	KX976861	Wang *et al*. (2016a)
	CGMCC 3.14182	Rhizosphere of *Panax notoginseng*	GU563366	JF772451	GU563358	KX976862	Wang *et al*. (2016a)
D. pratensis	CBS 133396^{T}	Soil	GU563372	JF772450	GU563357	KX976866	Wang *et al*. (2016a)
	CBS 804.83	Wood of celar	KX976665	KX977007	KX976759	KX976867	Wang *et al*. (2016a)
D. pseudoerectus	CBS 252.75^{T}	Air	KX97666	KX977009	KX976761	KX976869	Wang *et al*. (2016a)
D. pseudofunicola	CBS 142033^{T}	Dust	KX976668	KX977010	KX976762	KX976870	Wang *et al*. (2016a)
D. ramosissimus	CGMCC 3.14183^{T}	Rhizosphere of *Panax notoginseng*	GU563371	JF772452	GU563361	KX976871	Wang *et al*. (2016a)
	CGMCC 3.12930	Soil	HM449045	JF772449	HM449059	KX976872	Wang *et al*. (2016a)
D. reflexus	CBS 157.49^{T}	Germinating seed	HM449051	JF772460	HM449055	KX976873	Wang *et al*. (2016a)
	CBS 141.56	Seed	KX976669	KX977011	KX976763	KX976874	Wang *et al*. (2016a)
D. subfunicola	CGMCC 3.12892^{T}	Soil	JX867125	JX867122	JX867125	KX976875	Wang *et al*. (2016a)
	CGMCC 3. 9466	Rhizosphere of *Panax notoginseng*	GU563368	JF772446	GU563353	KX976876	Wang *et al*. (2016a)
D. variostiolatus	CBS 179.84^{T}	Tarpaulin	KX976672	KX977014	KX976766	KX976879	Wang *et al*. (2016a)
	DTO 319-A2	Dust	KX976673	KX976767	KX977015	KX976880	Wang *et al*. (2016a)
	COAD 3648	**Roots of *Musa acuminata***	**OR626087**	—	**OR614384**	**OR611984**	**This study**
Floropilus chiversii	CBS 558.80^{T}	Dung of moose	MK926818	MK926818	MK926918	MK876778	Wang et al. (2019a)
Humicola fuscoatra	CBS 118.14^{T}	Soil	LT993579	LT993579	LT993660	LT993498	Wang *et al*. (2019b)
H. olivacea	CBS 142031^{T}	Dust	LT993589	LT993589	LT993670	LT993508	Wang *et al*. (2019b)
H. quadrangulata	CBS 111771^{T}	Soil	LT993593	LT993593	LT993674	LT993512	Wang *et al*. (2019b)
Hyalosphaerella fragilis	CBS 456.73^{T}	Rhizosphere of *Pennisetum typhoideum*	KX976693	KX976791	KX977042	MK876779	Wang *et al*. (2019a)
Intraradix grisea	**COAD 3910^{T}**	**Roots of *Musa acuminata***	**PQ898842**	**PQ898841**	**PQ898405**	**PQ898404**	**This study**
	URM 9211	**Roots of *Manihot esculenta***	**PQ898843**	—	**PQ898406**	**PQ898403**	**This study**
Madurella mycetomatis	CBS 109801^{T}	Foot mycetoma of a woman	MK926820	MK926820	MK926920	MK876781	Wang *et al*. (2019a)
M. pseudomycetomatis	CBS 129177^{T}	Mycetoma of a man's lower jaw	MK926821	MK926821	MK926921	MK876782	Wang *et al*. (2019a)
Melanocarpus albomyces	CBS 638.94^{T}	Chicken nest straw	KX976679	KX976773	KX977021	KX976886	Wang *et al*. (2019a)
Microthielavia ovispora	CBS 165.75^{T}	Root of *Avena sativa*	MK926826	MK926826	MK926926	MK876787	Wang *et al*. (2019a)

Table 1. (Continued)

Myceliophthora lutea	CBS 145.77[T]	Hay	HQ871775	KM655351	KX977026	KX976891	Wang *et al.* (2019a)
Mycothermus thermophiloides	CBS 183.81[T]	Soil	LT993603	LT993603	LT993684	LT993522	Wang *et al.* (2019b)
My. thermophilus	CBS 625.91[T]	Chicken nest straw	LT993604	LT993604	LT993685	LT993523	Wang *et al.* (2019b)
Ovatospora brasiliensis	CBS 130174	Soil	KX976682	KX976780	KX977030	KX976895	Wang *et al.* (2019a)
O. medusarum	CBS 148.67[T]	Soil	KX976684	KX976782	KX977032	KX976897	Wang *et al.* (2019a)
Parachaetomium biporatum	CBS 244.86[T]	Soil	MK919303	MK919303	MK919417	MK919360	Wang *et al.* (2022)
P. perlucidum	CBS 141.58[T]	Dead herbaceous stem	MK919308	MK919308	MK919422	MK919365	Wang *et al.* (2022)
Parahumicola guana	COAD 3110[T]	Bat guano from a cave	ON989659	OP108407	OP131290	ON995381	Condé *et al.* (2023)
Parathielavia hyrcaniae	CBS 353.62[T]	Sand dune soil	KM655329	KM655368	KX977043	KM655401	Wang *et al.* (2019a)
Par. kuwaitensis	CBS 945.72[T]	Desert soil	KM655332	KM655371	KX977044	KM655404	Wang *et al.* (2019a)
Parvomelanocarpus tardus	CBS 541.76[T]	Cotton jacket	KX976681	KX976775	KX977023	KX976888	Wang *et al.* (2019a)
Parv. thermophilus	CBS 886.97	Soil	KM655350	MH874288	MZ343037	KM655434	Wang *et al.* (2019a)
Pseudoechria longicollis	CBS 368.52[T]	Deteriorating material	MK926847	MK926847	MK926947	MK876809	Wang *et al.* (2019a)
Ps. prolifica	CBS 250.71[T]	Dung of *Cobus defassa*	MK926848	MK926848	MK926948	MK876810	Wang *et al.* (2019a)
Pseudohumicola semispiralis	CBS 723.97[T]	Paper	LT993597	LT993597	LT993678	LT993516	Wang *et al.* (2019b)
Pse. subspiralis	CBS 148.58	Leaf fragments in soil	LT993599	LT993599	LT993680	LT993518	Wang *et al.* (2019b)
Pseudothielavia subhyaloderma	CBS 473.86[T]	Forest soil	MK926833	MK926833	MK926933	MK876794	Wang *et al.* (2019a)
Pseu. terricola	CBS 165.88[T]	Barren soil	KX976694	KX976792	KX977045	MK876795	Wang *et al.* (2019a)
Remersonia tenuis	CBS 784.85[T]	Dung of horse	LT993609	LT993609	LT993690	LT993528	Wang *et al.* (2019b)
R. thermophila	CBS 643.91	Compost	LT993610	LT993610	LT993691	LT993529	Wang *et al.* (2019b)
Staphylotrichum boninense	CBS 112059	Twig	LT993616	LT993616	LT993697	LT993535	Wang *et al.* (2019b)
S. coccosporum	CBS 364.58[T]	Soil	LT993620	LT993620	LT993701	LT993539	Wang *et al.* (2019b)
Stellatospora terricola	CBS 811.95[T]	Paddy soil	MK926835	MK926835	MK926935	MK876797	Wang *et al.* (2019a)
Stolonocarpus gigasporus	CBS 112062[T]	Dung of *Camelus dromedarius*	MK926836	MK926836	MK926936	MK876798	Wang *et al.* (2019a)
Subramaniula flavipila	CBS 446.66[T]	Dead leaves	KP862600	KP970647	KP900706	KP900669	Wang *et al.* (2019b)
Su. thielavioides	CBS 122.78[T]	Dung of nilgai	KP862597	KP970654	KP900708	KP900670	Wang *et al.* (2019b)
Tengochaeta nigropilosa	CBS 639.83[T]	Soil from a *Pinus* forest	MZ334730	—	MZ343029	MZ342990	Wang *et al.* (2022)
Thermocarpiscus australiensis	CBS 493.74[T]	Nesting material of incubator bird	KM655339	KM655378	MZ343024	KM655419	Wang *et al.* (2022)
Thermochaetoides dissita	CBS 180.67[T]	Straw of *Typha*	MK919319	MK919319	MK919433	MK919375	Wang *et al.* (2022)
T. thermophila	CBS 144.50[T]	Decaying wheat straw	MK919314	MK919314	MK919428	KM655436	Wang *et al.* (2022)
Thermothelomyces fergusii	CBS 406.69[T]	Mushroom compost	HQ871794	KX976776	KX977024	MK919378	Wang *et al.* (2022)
Th. termophilus	CBS 117.65[T]	Dry pasture soil	MK919331	MK919331	MK919445	MK919387	Wang *et al.* (2022)
Thermothielavioides terrestris	CBS 117535[T]	Soil	MK926837	MK926837	MK926937	MK876799	Wang *et al.* (2022)
	CBS 492.74	Soil	MK926838	MK926838	MK926938	MK876800	Wang *et al.* (2019a)
Trichocladium acropullum	CBS 114580[T]	Soil	LT993626	LT993626	LT993707	LT993545	Wang *et al.* (2019b)
Tr. asperum	CBS 903.85[T]	Acidic soil	LT993632	LT993632	LT993713	LT993551	Wang *et al.* (2019b)
Xanthiomyces spinosus	CBS 789.71	Culture of algae	MH860357	MZ351429	MZ343034	MZ342995	Wang *et al.* (2022)

"T" = ex-type strains.

Table 2. Alignment length and the best nucleotide substitution model for Bayesian inference (BI) and maximum likelihood (ML) analysis used in this study.

Partition	*Chaetomiaceae*			*Dichotomopilus*		
	Length	Best model		Length	Best model	
		BI	ML		BI	ML
ITS	734	GTR+I+G	GTR+F+I+G4	584	GTR+I+G	TN+F+R2
LSU	570	GTR+I+G	TIM3e+I+R3	561	GTR+I	K2P+I
RPB2	534	GTR+I+G	TIM+F+R5	525	GTR+I+G	TN+F+G4
TUB2	1036	GTR+I+G	K3Pu+F+I+R5	716	HKY+I+G	K2P+G4
Concatenated	2874	—	—	2386	—	—

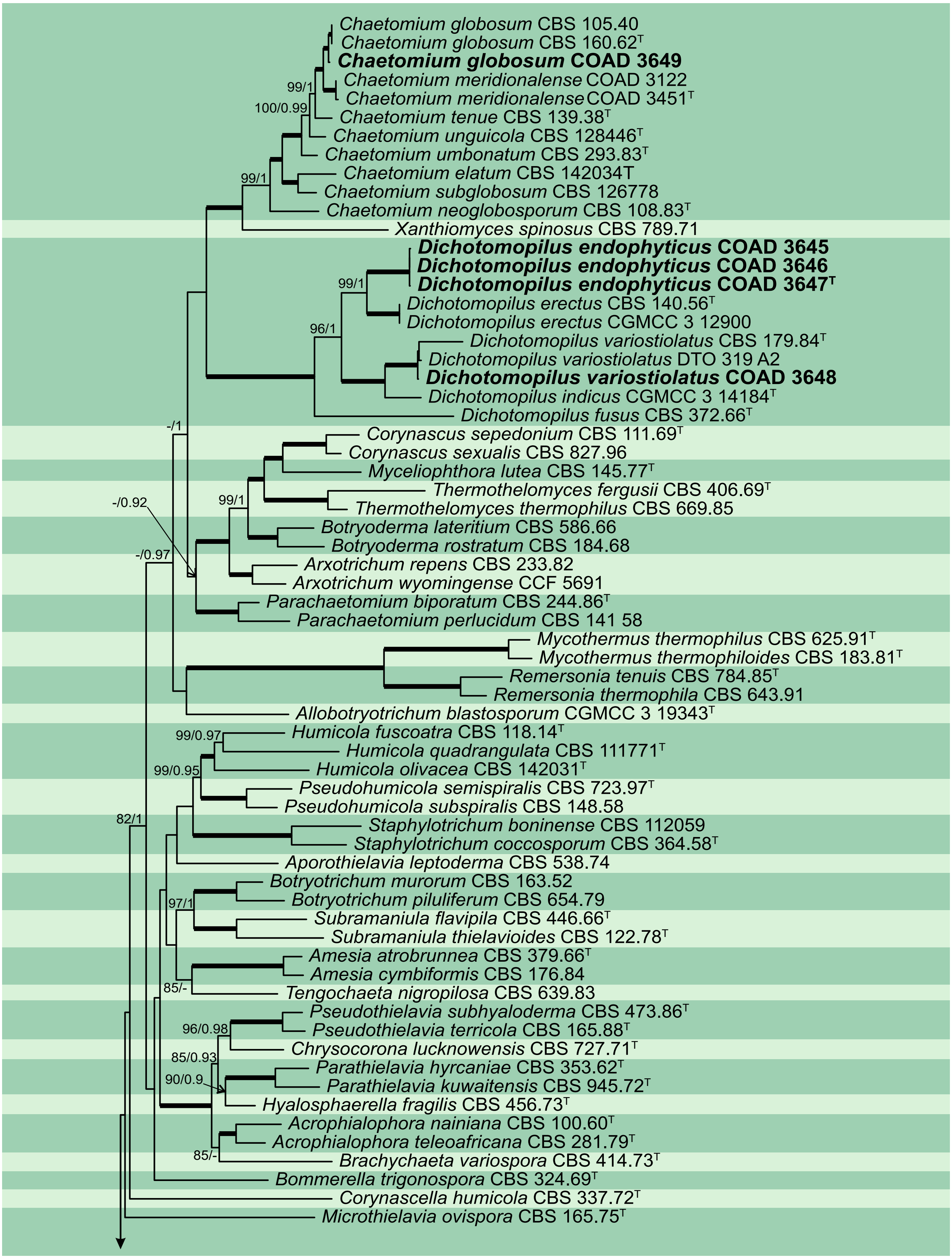

Fig. 2. Maximum-likelihood tree of *Chaetomiaceae* based on concatenated dataset of ITS, LSU, *TUB2*, and *RPB2* sequences. Isolates found in this study are shown in **bold**. Ex-type isolates are marked with ''T''. Only bootstrap support (bs) values ≥ 80 % and posterior probabilities (pp) ≥ 0.90 are shown at branches (''–'' means no statistical support). The branches that presented full statistical support (bs = 100 % and pp = 1) are thickened. The tree is rooted with *Pseudoechria longicollis* CBS 368.52 and *Pseudoechria prolifica* CBS 250.71 (*Schizotheciaceae*).

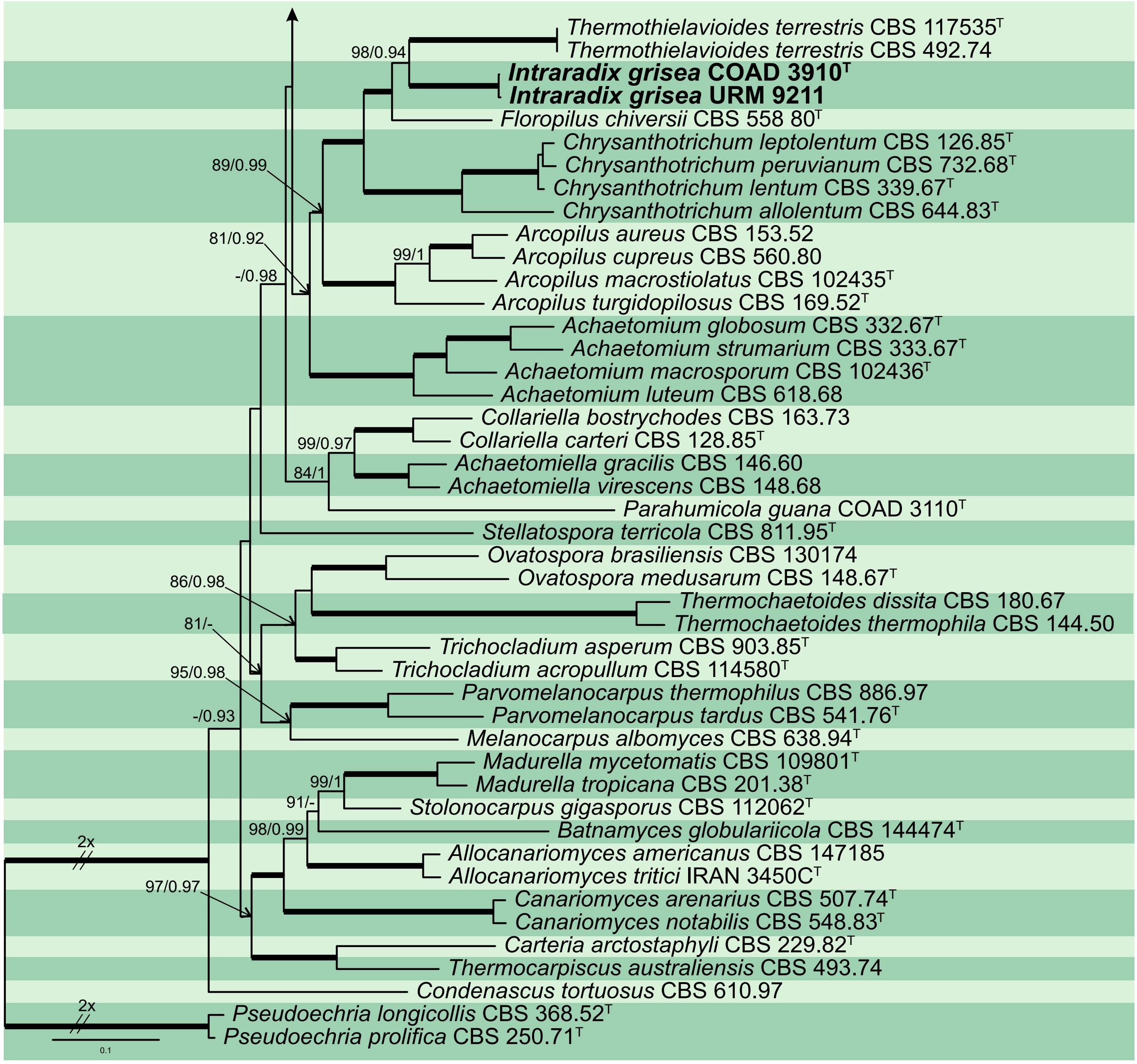

Figure 2. (Continued)

Taxonomy

Chaetomium Kunze, *Mykol. Hefte* **1**: 16. 1817.

Chaetomium globosum Kunze, *Mykol. Hefte* **1**: 16. 1817.

Description and illustration: Wang *et al.* (2016b).

Material examined: **Brazil**, Maranhão state, Itinga do Maranhão municipality, isolated from roots of *Musa acuminata*, Dec. 2021, *F.A. Custódio* (living culture COAD 3649).

Notes: *Chaetomium globosum* is the type species of the genus *Chaetomium,* characterized by the production of superficial, ostiolate ascomata, greenish olivaceous or slightly dark olivaceous, ascomatal hairs, ascospores globose, ellipsoid, limoniform, ovate or obovate, olivaceous brown when mature. *Chaetomium globosum* was originally isolated from stems of *Dianthus carthusianorum,* in Leipzig, Germany, but the species has been obtained from different substrates, such as air, carpet, dust, indoor environment, plants, and others (Wang *et al.* 2016a, b). The isolate COAD 3649 is morphologically similar to the ex-type *C. globosum* CBS 160.62, presenting the same colony colour, shape, and length of ascomata, asci, and ascospores (Wang *et al.* 2016b). To the best of our knowledge, this is the first report of this species as endophyte of *Musa* spp.

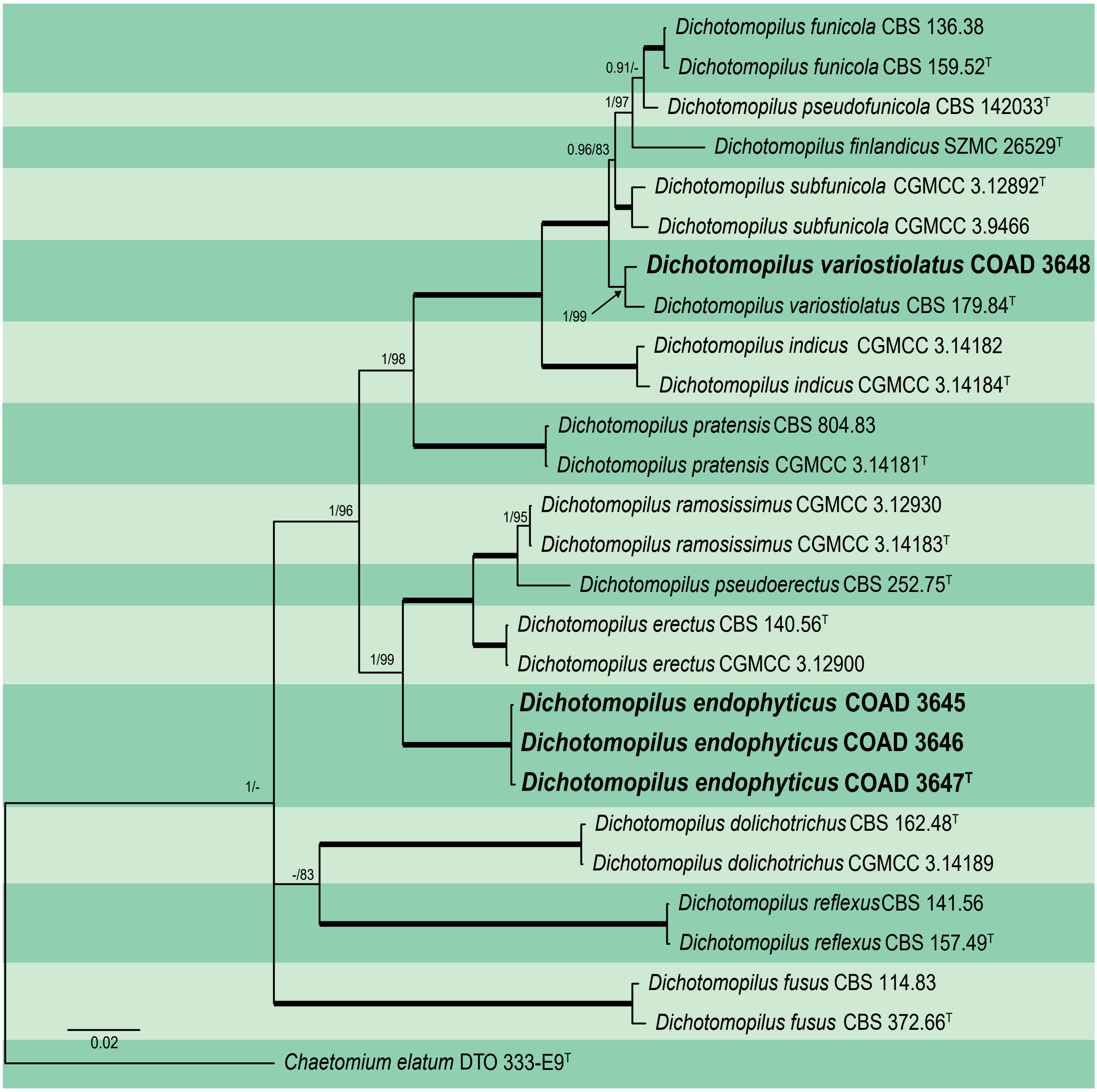

Fig. 3. Bayesian phylogenetic tree of *Dichotomopilus* based on concatenated dataset of ITS, LSU, *TUB2*, and *RPB2* sequences. Isolates found in this study are shown in **bold**. Ex-type isolates are marked with ''T''. Only bootstrap support (bs) values ≥ 80 % and posterior probabilities (pp) ≥ 0.90 are shown at branches (''–'' means no statistical support). The branches that presented full statistical support (bs = 100 % and pp = 1) are thickened. The tree is rooted with *Chaetomium elatum* DTO 333-E9.

Dichotomopilus X. Wei Wang *et al.*, *Stud. Mycol*. **84**: 179. 2016.

Description and illustration: Wang *et al.* (2016a).

Dichotomopilus endophyticus J.S. Santana, F.A. Custódio, D.O. Ramos & O.L. Pereira, ***sp. nov.*** MB 850545. Fig. 4.

Etymology: The name refers to the endophytic lifestyle of the ex-type isolate of the species, found endophytically in the roots of *Musa acuminata*.

Typus: **Brazil**, Maranhão state, Itinga do Maranhão municipality, isolated from roots of *Musa acuminata*, 15 Dec. 2021, *F.A. Custódio* (**holotype** VIC 49473 preserved as dried down culture, ex-type culture COAD 3647).

Description: *Ascomata* superficial, occasionally immersed in the medium, ostiolate, chestnut to olivaceous, globose to subglobose, (71.5–)78.5–130.5 µm in length and (71.5–)75–121(–126) µm diam. *Ascomata wall* brown composed of angular and irregular cells, textura angularis. *Terminal hairs* numerous, dark brown, verrucose, dichotomously branched at acute angles at the upper part, with a diameter close to

Fig. 4. *Dichotomopilus endophyticus* COAD 3647. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 d at 25 °C in the dark. **B.** Top view of ascomata on OA. **C, D.** Ascomata in side view. **E–G.** Ascomata. **H.** Surface of ascomatal wall. **I.** Terminal hairs. **J.** Swollen cells on the terminal ascomatal hairs. **K.** Asci. **L.** Ascospores. Scale bars: E–G = 100 µm; H–K = 20 µm; L = 10 µm.

the base, 3–6(–6.5) µm, sometimes with swollen cells similar to chlamydospores at apical end. *Lateral hairs* short and seta-like. *Asci* fasciculate, evanescent, with 8 ascospores in irregular arrangement, clavate, spore-bearing portion, 15–24 × 8.8–15.5 µm, with stalks being 4–6 µm long. *Ascospores* dark brown when mature, ovate to limoniform, bilaterally flattened, (5.5–)6–6.5(–7) × (3.7–)4.5–5(–5.5) µm, with one apical germ pore at the more attenuated end. *Asexual morph* unknown.

Culture characteristics (7 d at 25 °C in the dark): Colonies on OA attaining 47–50 mm diam.; edge entire; white aerial mycelium sparse to absent, flat, circular shape; intense to moderate production of ascomata; reverse buff; soluble pigment absent. Colonies on CMA attaining 45–49 mm diam.; edge undulate, aerial mycelium sparce to absent, white, flat; intense production of superficial and submerse ascomata; reverse buff; soluble pigment absent. Colonies on MEA attaining 48–49 mm diam., edge entire, white aerial mycelium sparse to absent, flat, circular shape; few ascomata at the centre of colonies; rancid odour; reverse buff; soluble pigment absent. Colonies on PCA attaining 47–48 mm diam., edge fimbriate, aerial mycelium moderate, olivaceous, flat, circular shape; moderate production of ascomata; reverse pale luteus; soluble pigment absent.

Additional material examined: **Brazil**, Maranhão state, Itinga do Maranhão municipality, isolated from roots of *Musa acuminata*, Dec. 2021, *F.A. Custódio* (**paratypes** VIC 49471 and VIC 49472, living cultures COAD 3645 and COAD 3646).

Notes: *Dichotomopilus endophyticus* is phylogenetically related to *D*. *erectus*, *D*. *pseudoerectus,* and *D*. *ramosissimus*. This species has dark brown ascospores, with terminal hair dichotomously branched at the ends, while *D. pseduoerectus* has olivaceous ascospores and terminal hairs branched near the base to the apical end. *Dichotomopilus endophyticus* has shorter and less ascomatal hairs, swollen cells in the terminal hair, and no pigmentation when grown on CMA and MEA, compared to the related species. Moreover, it produces ascomata immersed in the medium when cultured on CMA, whereas most *Dichotomopilus* species produce superficial ascomata.

Dichotomopilus variostiolatus (A. Carter) X. Wei Wang & Samson, *Stud. Mycol*. **84**: 203. 2016.

Description and illustration: Wang *et al.* (2016a).

Material examined: **Brazil**, Maranhão state, Itinga do Maranhão municipality, isolated from roots of *Musa acuminata*, Dec. 2021, *F.A. Custódio* (living culture COAD 3648).

Notes: Colonies of isolate COAD 3648 differed from the colonies of the ex-type of *D. variostiolatus* (CBS 179.84^{T}) by the absence of coloured exudates diffused in OA, and by the size of colonies on MEA (28–30 vs 34–40). Micromorphology showed a greater production of terminal hairs dichotomously branched (type 1) than the seta-like (type 2). Isolate COAD 3648 grouped with the reference isolate (ex-type) of *D. variostiolatus* CBS 179.84, in phylogenetic analysis of the three regions (ITS, *TUB2*, and *RPB2*) and the individual phylogeny of *TUB2*. However, in the individual phylogenetic analysis of the ITS and *RPB2* regions, isolate COAD 3648 clustered with other species within the complex (Figs S5, S8). This may have occurred due to the inclusion of *D. variostiolatus* in the *D. funicula* species complex (Wang *et al.* 2016a). This species is reported from the USA, Thailand and New Guinea on dust, tarpaulin and from a human nose (Wang *et al.* 2016a, Dolatabadi *et al.* 2022). To the best of our knowledge, this is the first report of this species in Brazil and as an endophyte in *Musa* spp.

Intraradix J.S. Santana, D.O. Ramos, F.A. Custódio & O.L. Pereira, ***gen. nov.*** MB 857367.

Etymology: Refers to the fungus habitat inside the roots, since it has an endophytic lifestyle.

Type species: *Intraradix grisea* J.S. Santana, D.O. Ramos, F.A. Custódio & O.L. Pereira

Description: *Ascomata* superficial, pale greenish grey due to ascomatal hairs in reflected light, globose to subglobose, ostiolate. *Ascomatal wall* brown, textura angularis. *Terminal hairs* erect in the lower part, apically coiled or undulate, brown, septate, verrucose. *Lateral hairs* straight or slightly flexuous. *Asci* fasciculate, clavate or fusoid, containing eight biseriate or irregularly arranged ascospores, evanescent. *Ascospores* aseptate, ellipsoid or fusoid, olivaceous buff to greenish olivaceous when mature, with one apical germ pore. *Asexual morph* not observed.

Notes: The phylogenetic analysis grouped this novel genus as sister to *Thermothielavioides* and related to *Floropilus* (Fig. 2). *Intraradix* can be distinguished from *Thermothielavioides* by the absence of an asexual morph, presence of ostiolate ascomata with terminal hairs undulate or coiled, asci clavate or fusoid and ascospores that are greenish olivaceous. In addition, *Floropilus* is mesophilic while *Intraradix* is thermotolerant with an optimal growth temperature at 37 °C.

Intraradix grisea J.S. Santana, D.O. Ramos, F.A. Custódio & O.L. Pereira, ***sp. nov.*** MB 857368. Fig. 5.

Etymology: Refers to the grey colour of the ascomatal hair in reflected light.

Typus: **Brazil**, Maranhão state, Itinga do Maranhão municipality, isolated from roots of *Musa acuminata*, 15 Dec. 2021, *F.A. Custódio* (**holotype** VIC 49596 preserved as dried down culture, ex-type culture COAD 3910).

Description: *Ascomata* superficial, pale greenish grey due to ascomatal hairs in reflected light, globose to subglobose, ostiolate, 74–163.5 × 72–118 µm. *Ascomatal wall* brown, textura angularis. *Terminal hairs* erect in the lower part, undulate to loosely coiled in the upper part, brown, septate, verrucose, 3–5 µm diam. near base. *Lateral hairs* straight or flexuous. *Asci* fasciculate, clavate or fusoid, spore bearing part 20.5–35 × 6–9 µm, with stalks being 4.5–7.5 µm long, containing eight biseriate or irregularly arranged

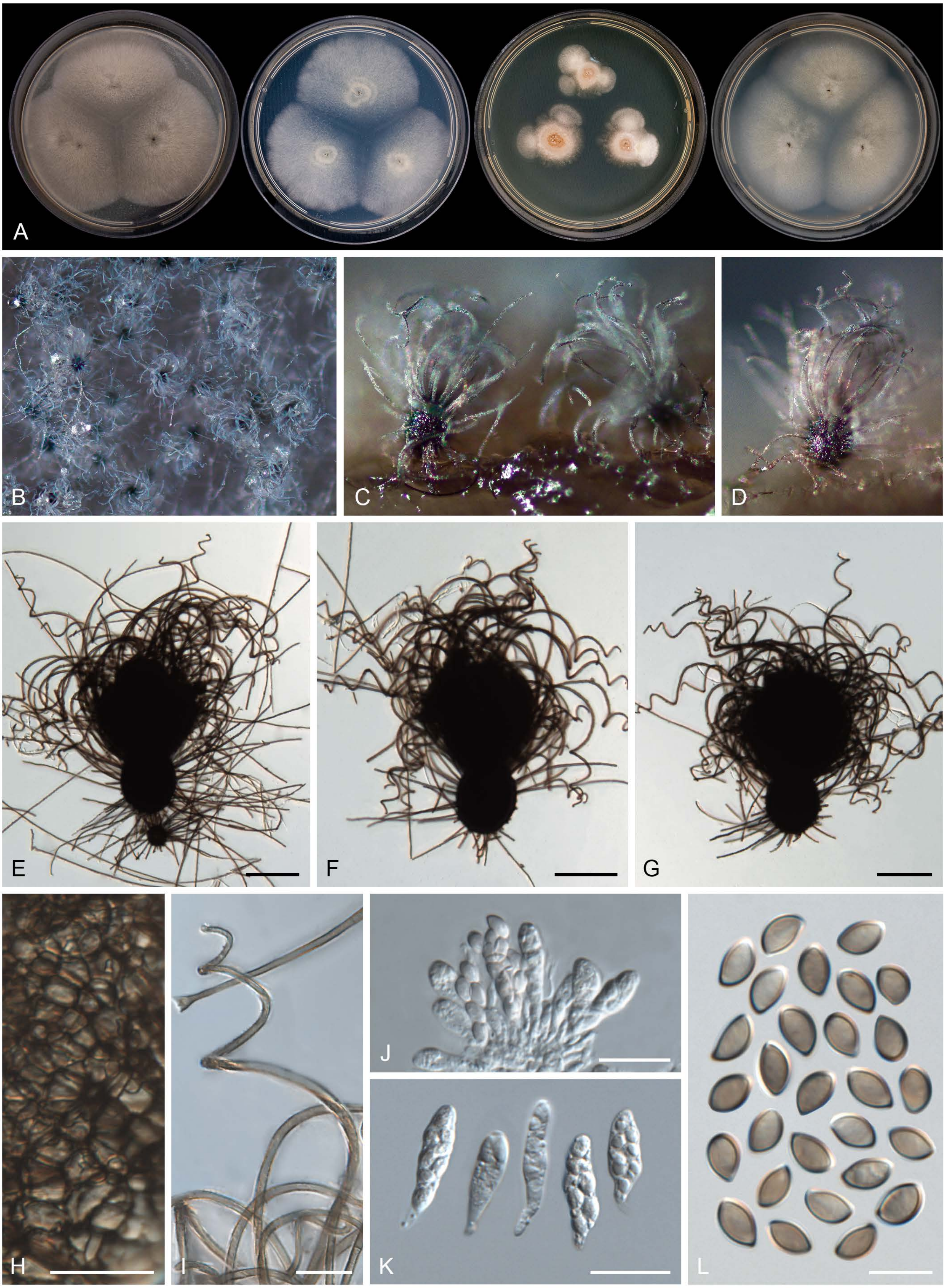

Fig. 5. *Intraradix grisea* COAD 3910. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 d at 37 °C in the dark. **B.** Top view of ascomata on OA. **C, D.** Ascomata in side view. **E–G.** Ascomata. **H.** Surface of ascomatal wall. **I.** Terminal ascomatal hairs. **J, K.** Asci. **L.** Ascospores. Scale bars: E–G = 100 µm; H–K = 20 µm; L = 10 µm.

ascospores, evanescent. *Ascospores* aseptate, ellipsoid or fusoid, olivaceous buff to greenish olivaceous when mature, smooth, (6–)7.5–8(–9) × (3.5–)4.5–5(–6) µm, with one apical germ pore. *Asexual morph* not observed.

Culture characteristics (7 d at 37 °C in the dark): Colonies on OA attaining 45–57 mm diam.; edge entire; white aerial mycelium sparse to absent, flat, circular shape; few immature ascomata in centre of colonies; reverse buff; soluble pigment absent. Colonies on CMA attaining 46–50 mm diam.; edge undulate, aerial mycelium sparce to absent, white, flat; no production of ascomata; reverse white; soluble pigment absent. Colonies on MEA attaining 20–30 mm diam., edge lobate, white aerial mycelium sparse to absent, flat, irregular shape; no sporulation observed; reverse rosy buff to vinaceous due to the presence of soluble pigment. Colonies on PCA attaining 50–60 mm diam., entire edge, white aerial mycelium sparse to absent, flat, circular shape; no production of ascomata; reverse rosy buff; soluble pigment absent.

Additional material examined: **Brazil**, Pernambuco state, Araripina municipality, isolated from healthy roots of *Manihot esculenta*, Apr. 2022, *A.R. Machado* (living culture URM 9211).

Notes: *Intraradix grisea* exhibited a higher growth rate at 37 °C, although it could also slowly grow at 25 °C. The isolate COAD 3910, from banana, produced a vinaceous pigment on MEA, whereas the isolate URM 9211 did not produce any soluble pigment on the same medium. However, a pale luteous pigment was observed only in URM 9211 when grown on CMA. Although the phylogeny grouped *I. grisea* as sister to *T. terrestris,* morphologically, it is quite similar to *F. chiversii*, but differs from it by producing smaller ascospores [(6–)7.5–8(–9) × (3.5–)4.5–5(–6) µm vs (9.5–)10–11.5(–12) × 6–7 µm)], absence of luteus exudates, and by the optimum growth temperature at 37 °C. Moreover, *I. grisea* differs from *T. terrestris* by producing ostiolate ascomata, asci clavate, larger ascospores [(6–)7.5–8(–9) × (3.5–)4.5–5(–6) µm vs 4–6.5(–7) × (3–)3.5–4.5(–5)] with olivaceous buff to greenish olivaceous colour, and by the absence of an asexual morph.

DISCUSSION

The *Chaetomiaceae* family was described by Winter (1885) to accommodate *C. globosum* and other fungi that produce ascomata with a membranaceous wall, ascomatal hairs, evanescent asci, and aseptate, pigmented ascospores. This family includes species with different lifestyles that are found in a wide range of substrates and environments, such as air, caves, plant endophytes, plant debris, animal dung, sandy soil, and volcanic soil (Melo *et al.* 2019, Sousa *et al.* 2020, Wang *et al.* 2022, Condé *et al.* 2023, Sastoque *et. al* 2025). In the present study, we identified root endophytic *Chaetomiaceae* from Brazil. Four different species in three genera were identified by morphological and phylogenetic analyses using a polyphasic approach, including *Chaetomium globosum* and *Dichotomopilus variostiolatus*. Three isolates did not belong to any known species of *Dichotomopilus* and were introduced as *D. endophyticus*. Moreover, a new genus and species from *Chaetomiaceae* was introduced here as *Intraradix grisea*.

The genus *Intraradix* was isolated as a root endophyte from banana and cassava roots. The strain COAD 3910 was obtained from banana roots located in an area in the Amazon biome, which has a tropical rainforest equatorial climate, and the average annual temperature that varies between 25–27 °C. On the other hand, the strain URM 9211 was isolated from cassava roots in the Caatinga biome, where the climate is semi-arid with a high average annual temperature ranging between 25–30 °C (AC 2025). The presence of isolates from the same genus or species in different climatic zones clarifies how this fungus is distributed in different biomes (Saremi & Saremi 2013). This highlights the possibility that *I. grisea* is widely distributed in Brazil under different climatic conditions and has an endophytic lifestyle in different crops.

Intraradix grisea showed greater growth at 37 °C and could grow at 42 °C, and is therefore considered as a thermotolerant species. *Chaetomiaceae* also includes thermotolerant species in the genera *Corynascus*, *Melanocarpus*, *Myceliophthora Parvomelanocarpus* and *Pseudohumicola* (Wang *et al.* 2019a, 2022, Sastoque *et. al* 2025). The new genus *Intraradix* is phylogenetically related to *Floropilus* and *Thermothielavioides*. The genera *Floropilus* and *Thermothielavioides* were introduced by Wang *et al.* (2019a), with *Floropilus chiversii* and *Thermothielavioides terrestris* as the type species, respectively. *Floropilus chiversii* is a mesophilic species that produces chaetomium-like ascomata and has been isolated from moose dung (Wang *et al.* 2019a). In contrast, *T. terrestris* is a thermophilic fungus with an optimal growth temperature of 45 °C, isolated from soil, cellulose in soil from palm oil estate, sun-heated soil, and sun-heated dung of rabbit (Wang *et al.* 2019a).

The species *I. grisea* described in the present study is thermotolerant and was obtained from regions with a tropical climate, characteristics that distinguish it from the mesophilic species *Floropilus chiversii*, which is related to other climates, such as isolated from Burt Lake, Ontario, Canada where the climate is harsh with cold winters (Wang *et al.* 2019a, OASDI 2025). *Thermothielavioides terrestris* is a thermophilic species reported in different environments and climates, such as the United Kingdom, which has a maritime, moist, and temperate climate with a moderate annual temperature range, or Hiroshima with temperate humid, mild winters and muggy, rainy summers (Wang *et al.* 2019a, WBCCKP 2021, CT 2020). These characteristics can contribute to the understanding of the evolutionary process leading to the emergence of a new genus, since the individual phylogeny of the gene regions of ITS and *TUB2* clustered *I. grisea* next to *F. chiversii*, whereas the concatenated phylogeny clustered *Intraradix* closest to *T. terrestris* (Figs S1, S3).

The *Chaetomiaceae* species *C. globosum*, *I. grisea*, *D. endophyticus*, and *D. variostiolatus* were reported here as root endophytic fungi from banana plants. Previous studies reported *C. globosum* as endophyte of several economically important plant crops, including maize (*Zea mays*) (Elshahawy & Khattab 2022), rice (*Oryza sativa*) (Naik *et al.* 2009), and grapevine (*Vitis vinifera*) (Longoni *et al.* 2012). However, to date, no reports have been provided of *C. globosum* being associated with banana tissues (*Musa acuminata*). In this study, *D. variostiolatus* was also reported for the first time as a root endophyte of banana plants. Furthermore, this is the first report of *D. variostiolatus* in Brazil, expanding its geographical distribution, as it has previously been reported from the USA, New Guinea, and Thailand (Wang *et al.* 2016a).

The genus *Dichotomopilus* includes species isolated from different substrates, but mainly from dust and soil (Wang *et al.* 2016a, 2022). Among them, *D. funicola* is the only species previously known to occur endophytically, having been isolated from the leaves of *Cajanus cajan* and *Mangifera indica* in China (Gu *et al.* 2018, Yang *et al.* 2023). In Brazil, *Dichotomopilus* species have only been reported on palm trees, *D. funicola* and *D. indicum*, which were reported in association with plant material of *Syagrus coronata,* a native palm tree in the Brazilian tropical dry forest (Caatinga) (Fortes & Vitória 2022). Recently, *Dichotomopilus* sp. was isolated as a root endophyte from *Acrocomia aculeata*, another palm native to Brazil (Oliveira *et al.* 2024). Therefore, for the first time, the genus *Dichotomopilus* has been reported from plants that do not belong to the *Arecaceae* family in Brazil.

The present study provides important insights into the distribution, ecology, and taxonomy of *Chaetomiaceae*. This is the first report of root endophytic *Chaetomiaceae* associated with banana plants and of the new species *D. endophyticus*. In addition, the new genus *Intraradix* was described occurring in different Brazilian biomes and important agricultural crops. The information obtained here will be potentially useful for future studies on the biological control of Fusarium wilt and growth promotion of banana plants, since the *Chaetomiaceae* species were obtained from healthy banana plants with vigorous growth in an area with a high incidence of vascular wilt.

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Supplementary Material: http://fuse-journal.org/

Fig. S1. Bayesian phylogenetic tree of *Chaetomiaceae* based on dataset of ITS sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Pseudoechria longicollis* CBS 368.52^{T} and *Pseudoechria prolifica* CBS 250.71^{T}.

Fig. S2. Bayesian phylogenetic tree of *Chaetomiaceae* based on dataset of LSU sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Pseudoechria longicollis* CBS 368.52^{T} and *Pseudoechria prolifica* CBS 250.71^{T}.

Fig. S3. Bayesian phylogenetic tree of *Chaetomiaceae* based on dataset of *TUB2* sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Pseudoechria longicollis* CBS 368.52^{T} and *Pseudoechria prolifica* CBS 250.71^{T}.

Fig. S4. Bayesian phylogenetic tree of *Chaetomiaceae* based on dataset of *RPB2* sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Pseudoechria longicollis* CBS 368.52^{T} and *Pseudoechria prolifica* CBS 250.71^{T}.

Fig. S5. Bayesian phylogenetic tree of *Dichotomopilus* based on dataset of ITS sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Chaetomium elatum* DTO 333-E9^{neoT}.

Fig. S6. Bayesian phylogenetic tree of *Dichotomopilus* based on dataset of LSU sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Chaetomium elatum* DTO 333-E9^{neoT}.

Fig. S7. Bayesian phylogenetic tree of *Dichotomopilus* based on dataset of *TUB2* sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Chaetomium elatum* DTO 333-E9^{neoT}.

Fig. S8. Bayesian phylogenetic tree of *Dichotomopilus* based on dataset of *RPB2* sequences. The isolates obtained in this study are highlighted in **bold**. Ex-type isolates are marked with ''T''. Posterior probabilities (pp) ≥ 0.70 are shown at branches. The tree is rooted with *Chaetomium elatum* DTO 333-E9^{neoT}.