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Cylindrocladiella species from *Cunninghamia lanceolata* plantation soils in southwestern China

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Abstract: Species of *Cylindrocladiella* are saprobic or plant pathogenic, and widely distributed in soil in tropical and sub-tropical regions of the world. Limited information is available about the species diversity and distribution of *Cylindrocladiella* in China. The aim of this study was to identify the *Cylindrocladiella* isolates from soils collected in a *Cunninghamia lanceolata* plantation in the Yunnan Province of southwestern China. Species identification was based on DNA phylogeny of *his3*, ITS, *tef1* and *tub2* regions, combined with morphological characteristics. Isolates obtained were identified as *Cylindrocladiella longistipitata* and a novel species, described here as *C. yunnanensis sp. nov*. Further studies are required, however, to elucidate the lifestyles of these taxa.

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INTRODUCTION

The genus *Cylindrocladiella* was proposed by Boesewinkel (1982) to accommodate several cylindrocladium-like species producing small conidia. This genus is distinguished from the *Cylindrocladium* (asexual morph of *Calonectria*) by its penicillate and/or subverticillate symmetrically branched conidiophores, small (< 20 µm in length), cylindrical, 1-septate conidia, aseptate stipe extensions, and *Nectricladiella* sexual morphs (Boesewinkel 1982, Crous & Wingfield 1993, 1994). The confirmation of the generic status of this genus was subsequently supported by phylogenetic inference of ITS and β-tubulin regions (Schoch *et al*. 2000).

Species of *Cylindrocladiella* are soil-borne fungi, and are distributed globally in temperate, sub-tropical and tropical regions (Crous 2002, Lombard *et al*. 2012, 2017, Pham *et al*. 2018). They are generally considered as plant pathogens or saprobes of a number of plant hosts. They have been associated with disease symptoms that include leaf spot, damping off, shoot die-back and root rot of agricultural, horticultural and forestry plants (Boesewinkel 1982, Crous *et al*. 1991, Crous & Wingfield 1993, Crous 2002, Erper *et al*. 2013, Armengol & Gramaje 2016, Carlucci *et al*. 2017, Aiello *et al*. 2020).

Relatively little knowledge is known on the species diversity and distribution of *Cylindrocladiella* species in China. At least 45 species have thus far been identified based on morphological characteristics and DNA sequence data (Crous 2002, Lombard *et al*. 2012, 2017, Crous *et al*. 2017, Pham *et al*. 2018, Tibpromma *et al*. 2018, Marin-Felix et al. 2019). The majority of these species were isolated from soil (Crous 2002, Lombard *et al*. 2012, 2017, Pham *et al*. 2018). In China, six species of *Cylindrocladiella* have thus far been reported. These include *C. camelliae*, *C. elegans*, *C. lageniformis*, *C. parva*, *C. peruviana* and *C. xishuangbannaensis* (Lu *et al*. 2000, Zhuang 2001, Crous 2002, Tibpromma *et al*. 2018, Ye *et al*. 2021). Of these, only *C. lageniformis* and *C. xishuangbannaensis* were identified based on molecular data (Tibpromma *et al*. 2018, Ye *et al*. 2021).

Recently, *Cylindrocladiella* isolates were obtained from soils collected from a *Cunninghamia lanceolata* plantation in the Yunnan Province in southwestern China. The aim of this study was to identify these isolates based on multigene sequence comparisons and morphological characteristics.

MATERIALS AND METHODS

Fungal isolations

Soil samples were collected from a *Cunninghamia lanceolata* plantation in the Puer Region, Yunnan Province in July 2021. The plantation was approximately 10 hectares. Trees in the plantation were 30-yr-old, and were planted near a lake at the top of a mountain (Fig. 1A). The leaf litter layer was removed before soil samples were collected from the upper 20 cm of the soil profile (Fig. 1B). Each soil sample was placed in a plastic bag and transferred to the laboratory for fungal isolation, molecular analyses and morphological comparisons.

Soil samples were baited with *Medicago sativa* (alfalfa) seeds as described by Crous (2002) and Liu & Chen (2023). White conidial masses with typical morphological characteristics

Fig. 1. Soil sampling in a plantation of *Cunninghamia lanceolata* in Yunnan Province, China. **A.** Thirty-year-old plantation near a lake. **B.** Leaf litter and upper 20 cm of the soil profile under *C. lanceolata* trees.

of *Cylindrocladiella* species on infected alfalfa tissue were distributed onto 2 % malt extract agar (MEA) (20 g malt extract powder and 20 g agar, 1 L H_2O). The single-conidium cultures were obtained from soil samples as described by Liu & Chen (2023). All obtained single conidium cultures are maintained in the Culture Collection (CSFF) at the Forest Pathogen Center (FPC), College of Forestry, Fujan Agricultural and Forestry University (FAFU) in Fuzhou, Fujian Province, China. Representative isolates were also deposited at the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. Dried specimens were deposited in the Mycological Fungarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS), Beijing, China.

DNA extraction, PCR amplification, and sequencing

All *Cylindrocladiella* isolates obtained in the current study were used for total genomic DNA extraction. Mycelia of each isolate were collected from 7-d-old cultures using a sterilized scalpel, and transferred into 2 mL Eppendorf tubes. The total genomic DNA of each isolate was extracted using the cetyltrimethylammonium bromide (CTAB) method described by Van Burik *et al*. (1998). The extracted DNA was dissolved by adding 30 µL TE buffer (1 M Tris-HCl and 0.5 M EDTA, pH 8.0), and 2.5 µL RNase (10 mg/mL) was further added to degrade the RNA. The mixture was incubated at 37 °C for 1 h. The DNA concentration of each isolate was measured using a NanoDrop Lite Plus spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All DNA samples were diluted to approximately 100 ng/uL using DNase/RNasefree ddH_2O (Sangon Biotech Co., Ltd., Shanghai, China) and stored at -20 °C for further use.

Based on previous research results, partial regions of histone H3 (*his3*), internal transcribed spacer regions and intervening 5.8S nrRNA (ITS), translation elongation factor 1-alpha (*tef1*) and β-tubulin (*tub2*) were considered as reliable DNA barcodes to distinguish species in *Cylindrocladiella* (Lombard *et al*. 2012, 2017, Pham *et al*. 2018). The primer pairs CYLH3F/CYLH3R (Crous *et al*. 2004a), ITS1F (Gardes & Bruns 1993)/ITS4 (White *et al*. 1990), EF1-728F (Carbone & Kohn 1999)/EF-2 (O'Donnell *et al*. 1998), and T1 (O'Donnell & Cigelnik 1997)/CYLTUB1R (Crous *et al*. 2004a) were used to amplify the fragments of *his3*, ITS, *tef1*, and *tub2* regions, respectively. The amplification reactions were performed as described by Liu *et al*. (2020).

Amplification products of all *Cylindrocladiella* isolates were sequenced in both the forward and reverse directions using the same primers used for PCR amplification. Sequence reactions were performed by Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China. The *his3*, ITS, *tef1*, and *tub2* regions were sequenced for all *Cylindrocladiella* morphology-like isolates obtained in this study. All obtained sequences were edited and assembled using MEGA v. 7.0 software (Kumar *et al*. 2016) and deposited in GenBank (https://www.ncbi.nlm.nih.gov).

Multigene phylogenetic analyses

The preliminary identities of all the *Cylindrocladiella* isolates were obtained by conducting a standard nucleotide BLAST search using the sequences of *his3*, ITS, *tef1*, and *tub2* regions of all sequenced isolates. Sequences of the ex-type strains of closely related *Cylindrocladiella* species were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov) and used for phylogenetic analyses.

Sequences generated in this study and sequences downloaded from the NCBI database were aligned using MAFFT online v. 7 (http://mafft.cbrc.jp/alignment/server) (Katoh & Standley 2013), with the iterative refinement method (FFT-NS-i setting). The alignments were further edited manually with MEGA v. 7.0 software (Kumar *et al*. 2016) when necessary. Sequences of each of the *his3*, ITS, *tef1*, and *tub2* regions, as well as the combination of these four regions were analysed.

The Maximum likelihood (ML) approach was used for the phylogenetic analyses for the four individual sequences of *his3*, ITS, *tef1*, and *tub2*, as well as for a concatenated dataset of all four regions. The ML analyses were conducted using PHYML v. 3.0 (Guindon & Gascuel 2003). The analyses were conducted with the default GTR substitution matrix. In PHYML, the maximum number of retained trees was set to 1 000, and nodal support was performed by non-parametric bootstrapping with 1 000 replicates. Sequence data of two isolates of *Calonectria pauciramosa* (CBS 138824 & CBS 137243) were used as outgroup

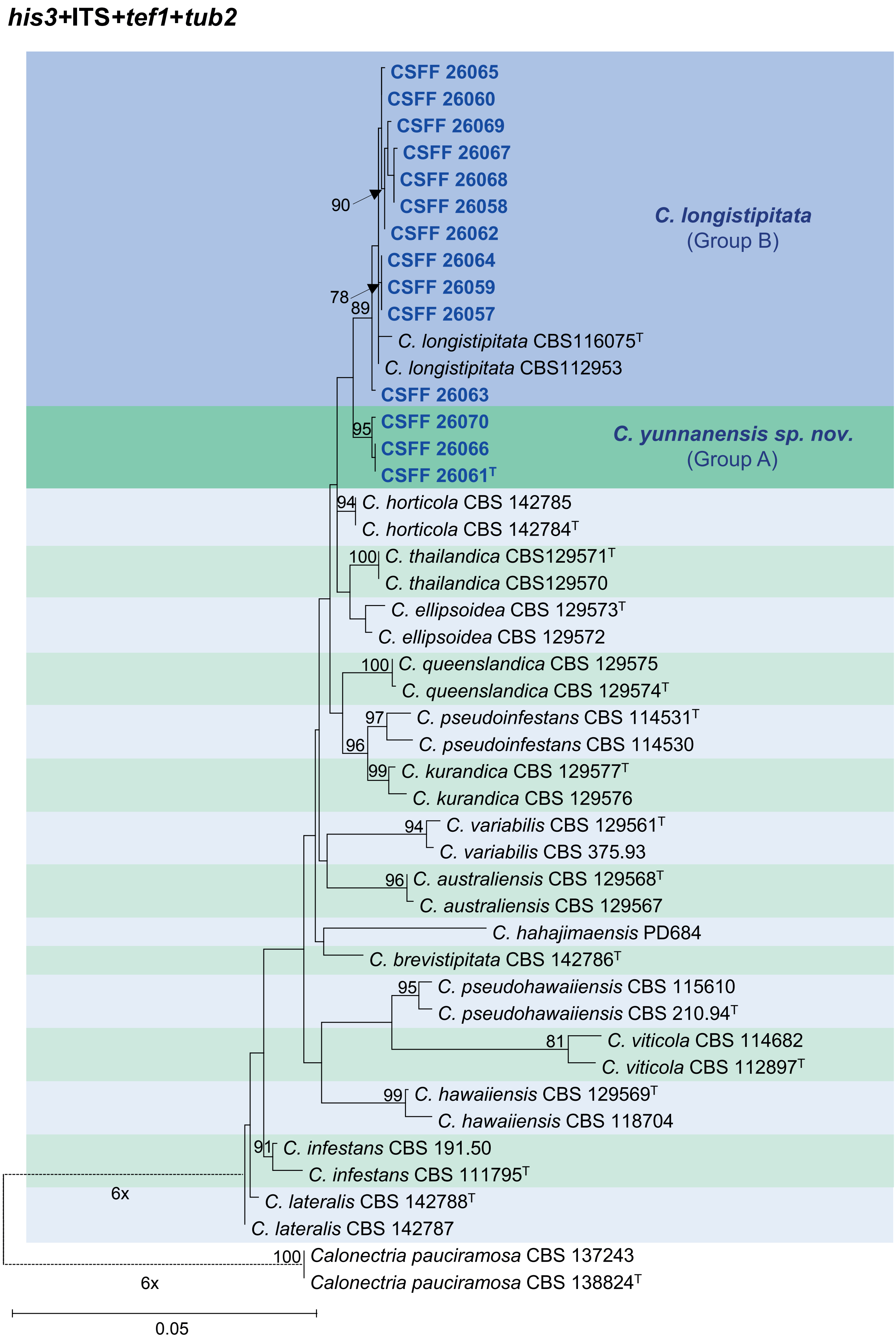

Fig. 2. Phylogenetic tree obtained from the maximum likelihood (ML) analysis of the combined dataset of *his3*, ITS, *tef1*, and *tub2*. Bootstrap values ≥ 75 % from the ML analysis are indicated at nodes. Isolates reported in this study are highlighted in bold and blue.

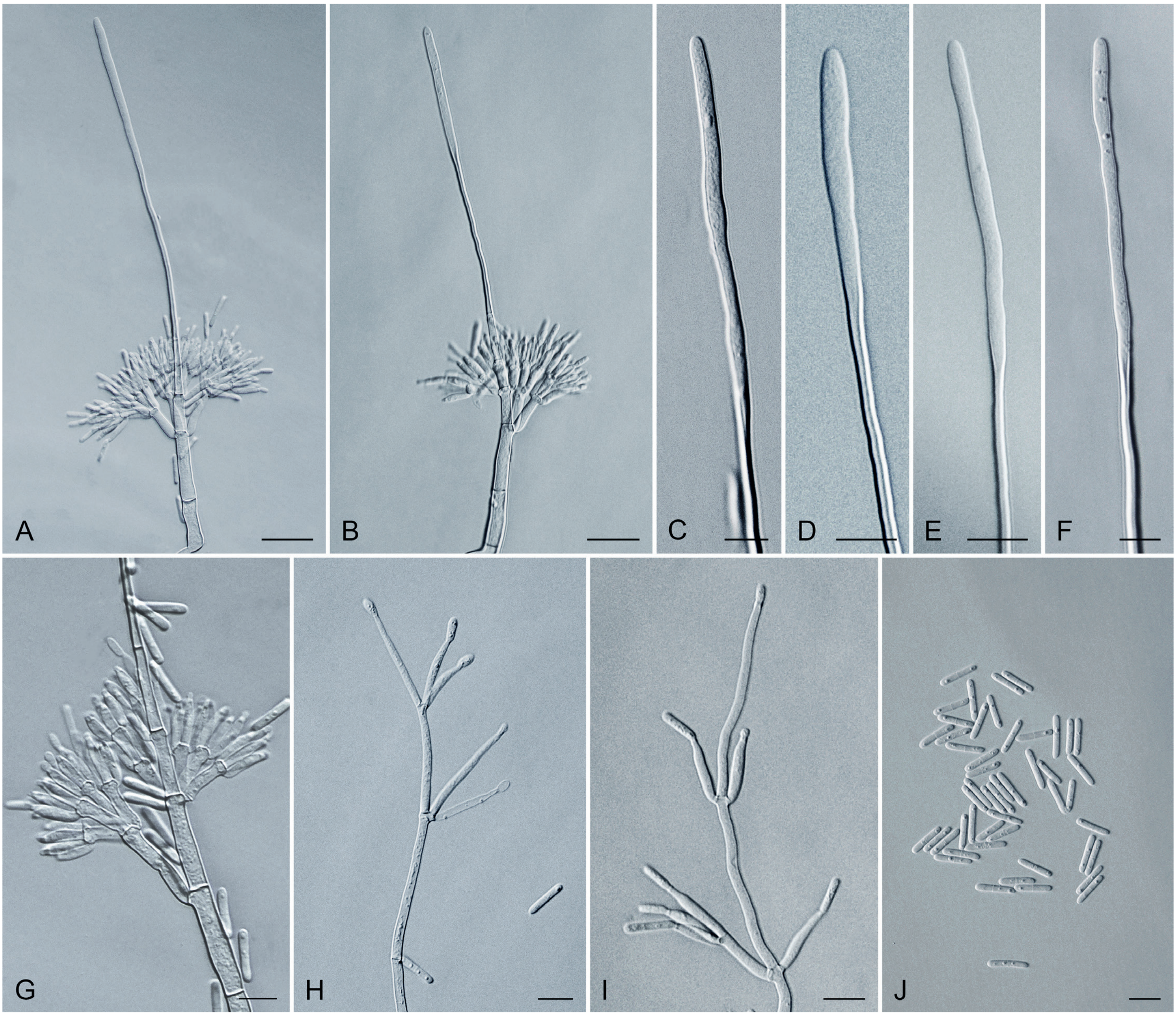

Fig. 3. *Cylindrocladiella yunnanensis* (ex-type CSFF 26061). **A, B.** Penicillate conidiophores. **C–F.** Cylindrical vesicles. **G.** Penicillate conidiogenous apparatus. **H, I.** Subverticillate conidiophores. **J.** Conidia. Scale bars: A, B = 20 µm; C–J = 10 µm.

taxa (Liu *et al*. 2020). Phylogenetic trees were viewed using MEGA v. 7.0 (Kumar *et al*. 2016). The final alignments were deposited in TreeBASE (http://treebase.org).

Morphology

For the novel species identified based on DNA sequence comparisons, representative isolates were used for morphological study. To induce sporulation, mycelium plugs from pure single conidium cultures were transferred onto Synthetic Nutrient-poor Agar (SNA; Nirenburg 1981), and incubated at 25 °C for 4–7 d. Pieces of autoclaved carnation leaf were also added to the SNA plates to induce sporulation. Fungal structures were mounted in 85 % lactic acid and examined with a Zeiss Axio Imager.A2 compound microscope (Carl Zeiss Ltd., Germany). To induce sexual structures, isolates of each species were crossed with each other in all possible combinations on Minimum Salt Agar (MN; Guerber & Correll 2001), with sterile toothpicks placed on the surface of the media (Guerber & Correll 2001). The crosses were incubated at 25 °C for 2–8 wk. Isolates were also crossed with themselves as control. Crosses were considered as successful when isolate combinations produced perithecia extruding viable ascospores.

Fifty measurements were made for each taxonomically informative structure (such as the conidial length and width). Minimum, maximum and average (mean) values are presented as (minimum–) (average – standard deviation) – (average + standard deviation) (–maximum). Only extremes are presented for other fungal structures.

To determine the optimal temperature for growth, mycelial plugs of representative isolates were taken from the actively growing margins of cultures and plated onto fresh MEA plates. These were incubated at temperatures ranging from 5 °C to 35 °C at 5 °C intervals, and five replicate plates per temperature per isolate. After 7 d, the colony diameters were measured. Colony morphology and colour were described based on the colour charts of Rayner (1970) using 7-d-old cultures on MEA incubated at 25 °C. All fungal descriptions and associated metadata were deposited in MycoBank (Crous *et al*. 2004b).

RESULTS

Fungal isolations

Fifty soil samples were collected from the plantation. The *Cylindrocladiella* spp. were observed on the baited alfalfa seeds in 11 soil samples. One to four single conidium isolates were obtained from each sample with white conidiophore masses with typical morphological characteristics of *Cylindrocladiella* species. Fourteen *Cylindrocladiella* isolates were retained for study (Table 1).

Multigene phylogenetic analyses

The amplicons generated for the *his3*, ITS, *tef1*, and *tub2* regions of fungi isolated in this study were approximately 510, 560, 490, and 570 bp, respectively. Sequences of ex-type specimen strains and additional strains of 16 *Cylindrocladiella* species that are closely related to our 14 isolates, were downloaded from GenBank for sequence comparisons and phylogenetic analyses (Table 2).

The phylogenetic analyses of the *his3*, *tef1*, and *tub2* and the combined four gene datasets (TreeBASE No. 31339) showed the 14 isolates in this study grouped in two clades (Fig. 3). Isolates CSFF 26061, CSFF 26066, and CSFF 26070 (Group A) formed an independent clade in each of *his3*, *tef1*, and *tub2* and combined dataset trees (Figs 3, S1, S3 and S4), which were supported with a high bootstrap value in the *his3* tree (100 %, Fig. S1) and the combined dataset tree (96 %, Fig. 3). The other 11 isolates (Group B) grouped close to the ex-type isolate CBS 116075 of *C. longistipitata* (Figs 3, S1, S3, S4). The phylogenetic analyses indicated that the *Cylindrocladiella* species were not well distinguished based on the ITS dataset (Fig. S2), but CSFF 26061, CSFF 26066, and CSFF 26070 grouped separately from the ex-type isolates of *C. longistipitata* (Fig. S2). Based on the phylogenetic analyses of the *his3*, ITS, *tef1*, *tub2* and combined datasets, the three isolates (CSFF 26061, CSFF 26066, and CSFF 26070) in Group A were identified as a novel species. Isolates in Group B were identified as *C. longistipitata*.

Morphology

The crossing tests using three isolates (CSFF 26061, CSFF 26066, and CSFF 26070) representing a novel species on MSA did not produce sexual structures. Asexual structures were common in these isolates on the SNA medium. The novel species is described as follows:

Taxonomy

Cylindrocladiella yunnanensis Y. Liu & S.F. Chen ***sp. nov.*** MycoBank MB 853678. Fig. 3.

Etymology: Name refers to Yunnan Province, where this fungus was isolated.

Diagnosis: Similar to *C. longistipitata* (conidia av. = 15 × 3 µm) but differs in its smaller conidial dimensions (av. = 11.5 × 2 µm).

Description: *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 41–91.5 × 4–6.5 µm (av. = 57.5 × 5 µm); stipe extension aseptate, straight, 75.5–117 µm (av. = 95.5 µm) long, thick-walled with one basal septum, terminating in thin-walled, cylindrical vesicles, 2.5–4.5 µm (av. = 3.5 µm) wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 10.5–19.5 × 2.5–4 µm (av. = 15.5 × 3 µm), secondary branches aseptate, 7.5–13 × 2–3.5 µm (av. = 10 × 2.5 µm), each terminal branch producing 2–4 phialides; phialides cylindrical to cymbiform, hyaline, aseptate, 7.5–11 × 1.5–2.5 µm (av. = 9 × 2.5 µm), apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* in moderate numbers, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 14.5–41 × 2–4.5 µm (av. = 23.5 × 3 µm); phialides cymbiform to cylindrical, hyaline, aseptate, 12–30.5 × 2–3 µm (av. = 17.5 × 2.5 µm), apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (9–)10.5–12.5(–14) × (1.5–)2(–3) µm (av. = 11.5 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characters: Colonies white to buff on the surface and pale luteous to salmon in reverse on MEA after 7 d; smooth margins; extensive aerial mycelium in the middle and the margins; chlamydospores not observed. Optimal growth temperature at 25 °C, no growth at 35 °C; after 7 d, colonies at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 9.1 mm, 30.6 mm, 56.4 mm, 73.9 mm, 79.0 mm and 38.6 mm, respectively.

Ecology and distribution: *Cylindrocladiella yunnanensis* isolated from soil in a forest of 30-yr-old *Cunninghamia lanceolata* trees in Jinggu County, Puer Region, Yunnan Province, China.

Typus: **China**, Yunnan Province, Puer Region, Jinggu County, Yongping Town, 23°22’58.1916”N, 100°9’5.436”E, from soil in a *Cunninghamia lanceolata* plantation, Jul. 2021, *S.F. Chen, Y. Liu, X.Y. Liang, B.Y. Chen & L.Q. Lu* (**holotype** HMAS 352737, fungarium specimen preserved as dried culture in metabolically inactive state, culture ex-type CSFF 26061 = CGMCC 3.27213). GenBank accession number PP763877 (*his3*), PP741549 (ITS), PP763905 (*tef1*), PP763891 (*tub2*).

Additional material examined: **China**, Yunnan Province, Puer Region, Jinggu County, Yongping Town, 23°22’58.1916”N, 100°9’5.436”E, from soil in a *C. lanceolata* plantation, Jul. 2021, *S.F. Chen*, *Y. Liu*, *X.Y. Liang*, *B.Y. Chen* & *L.Q. Lu* (HMAS 352738, fungarium specimen of dried culture, culture CSFF 26066 = CGMCC 3.27214). GenBank accession number.: PP763878 (*his3*), PP741550 (ITS), PP763906 (*tef1*), PP763892 (*tub2*).

Notes: *Cylindrocladiella yunnanensis* is phylogenetically closely related to *C. longistipitata*. However, the vesicles of *C. yunnanensis* (2.5–4.5 µm) are narrower than that of *C. longistipitata* (5–7 µm), and conidia of *C. yunnanensis* (av. = 11.5 × 2 µm) are smaller than those of *C. longistipitata* (av. = 15 × 3 µm). Furthermore, the ex-type isolate of *C. longistipitata* (CBS 116075), and isolates of *C. yunnanensis* (CSFF 26061, CSFF 26066) have unique fixed nucleotides for four loci: *his3* position 31 (T), 36 (-), 63 (C), 77 (A), 89 (C), 103 (C), 122 (C), 133 (T), 289 (G), 295 (T), 398 (C); ITS position 109 (T), 110 (T), 463 (C); *tef1* position 29 (-), 247 (T), 487 (-); *tub2* position 434 (T), 441 (-), 442 (-).

Table 1. Isolates sequenced and used for phylogenetic analyses in this study.

Species	Isolate No.[a]	Host	Locality	GPS information	Collector	GenBank Accession number.[b]			
						his3	ITS	*tef1*	*tub2*
C. longistipitata	CSFF 26057	Soil under *Cunninghamia lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100°9’5.436”E	S.F. Chen *et al.*	PP763875	PP741547	PP763903	PP763889
	CSFF 26058	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763880	PP741552	PP763908	PP763894
	CSFF 26059	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100°9’5.436”E	S.F. Chen *et al.*	PP763876	PP741548	PP763904	PP763890
	CSFF 26060	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100°9’5.436”E	S.F. Chen *et al.*	PP763879	PP741551	PP763907	PP763893
	CSFF 26062	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763884	PP741556	PP763912	PP763898
	CSFF 26063	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763886	PP741558	PP763914	PP763900
	CSFF 26064	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763887	PP741559	PP763915	PP763901
	CSFF 26065	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100°9’5.436”E	S.F. Chen *et al.*	PP763881	PP741553	PP763909	PP763895
	CSFF 26067	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763885	PP741557	PP763913	PP763899
	CSFF 26068	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763882	PP741554	PP763910	PP763896
	CSFF 26069	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763888	PP741560	PP763916	PP763902
C. yunnanensis sp. nov.	CSFF 26061 = CGMCC 3.27213[T]	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763877	PP741549	PP763905	PP763891
	CSFF 26066 = CGMCC 3.27214	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100º9’5.436”E	S.F. Chen *et al.*	PP763878	PP741550	PP763906	PP763892
	CSFF 26070	Soil under *C. lanceolata* trees	Yunnan Province, China	23°22’58.1916”N, 100°9’5.436”E	S.F. Chen *et al.*	PP763883	PP741555	PP763911	PP763897

[a] CSFF: Culture Collection located at Forest Pathogen Center (FPC), College of Forestry, Fujan Agricultural and Forestry University, Fuzhou, Fujian Province, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; [T]: Ex-type cultures.

[b] *his3*: histone H3; ITS: internal transcribed spacer regions and intervening 5.8S nrRNA gene; *tef1*: translation elongation factor 1-alpha; *tub2*: β-tubulin.

Table 2. Isolates from other studies used in the phylogenetic analyses.

Species	Isolate No.[a]	Substrate	Country	Collector	GenBank Accession No.[b]				References or Source of Data
					his3	**ITS**	***tef1***	***tub2***	
Cylindrocladiella australiensis	CBS 129567 = CPC 17507[T]	Soil	Australia	P.W. Crous	JN098932	JN100624	JN099060	JN098747	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129568 = CPC 17562	Soil	Australia	P.W. Crous	JN098931	JN100623	JN099059	JN098748	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. brevistipitata	CBS 142786[T]	Soil	Thailand	P.W. Crous	N/A[c]	N/A	MF444940	MF444926	Lombard *et al*. (2017)
C. ellipsoidea	CBS 129572 = CPC 17558	Soil	Australia	P.W. Crous	JN098943	JN100636	JN099073	JN098756	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129573 = CPC 17560[T]	Soil	Australia	P.W. Crous	JN098857	JN099094	JN098973	JN098757	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. hahajimaensis	PD684	Soil	Japan	T. Watanabe	N/A	JN687561	N/A	JN687562	Lombard *et al*. (2017)
C. hawaiiensis	CBS 118704	Soil	Hawaii	Y. Degawa	JN098878	JN099115	JN098996	JN098760	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129569 = CPC 12272[T]	Soil	Hawaii	Y. Degawa	JN098929	JN100621	JN099057	JN098761	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. horticola	CBS 142784[T]	Soil	Thailand	P.W. Crous	N/A	MF444911	MF444938	MF444924	Lombard *et al*. (2017)
	CBS 142785	Soil	Thailand	P.W. Crous	N/A	MF444912	MF444939	MF444925	Lombard *et al*. (2017)
C. infestans	CBS 111795 = ATCC 44816[T]	*Pinus pinea*	New Zealand	H.J. Boesewinkel	AY793513	AF220955	JN099037	AF320190	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 191.50 = IMI 299376	*Arenga pinnata*	Indonesia	K.B. Boedijn & J. Reitsma	AY793514	AF220956	JN099036	AY793475	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. kurandica	CBS 129576 = CPC 17547	Soil	Australia	P.W. Crous	JN098941	JN100634	JN099071	JN098764	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129577 = CPC 17551[T]	Soil	Australia	P.W. Crous	JN098953	JN100646	JN099083	JN098765	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. lateralis	CBS 142787	Soil	Thailand	P.W. Crous	N/A	MF444913	MF444941	MF444927	Lombard *et al*. (2017)
	CBS 142788[T]	Soil	Thailand	P.W. Crous	N/A	MF444914	MF444942	MF444928	Lombard *et al*. (2017)
C. longistipitata	CBS 112953 = CPC 4720	*Opisthiolepsis heterophylla*	Australia	C. Pearce & B. Paulus	N/A	N/A	N/A	JN098792	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 116075 = CPC 708[T]	Soil	China	M.J. Wingfield	AY793546	AF220958	JN098993	AY793506	Lombard *et al*. (2012), Lombard *et al*. (2017)

Table 2. (Continued).

Species	Isolate No.[a]	Substrate	Country	Collector	GenBank Accession No.[b]				References or Source of Data
					his3	ITS	*tef1*	*tub2*	
C. pseudohawaiiensis	CBS 210.94 = PPRI 4450[T]	*Eucalyptus* sp.	Brazil	A.C. Alfenas	JN098890	JN099128	JN099012	JN098819	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 115610 = CPC 909	Unknown	Madagascar	P.W. Crous	JN098901	JN100594	JN099024	JN098820	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. pseudoinfestans	CBS 114530 = CPC 2320	Soil	Madagascar	J.E. Taylor	JN098888	JN099126	JN099010	JN098821	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 114531 = CPC 2319[T]	Soil	Madagascar	J.E. Taylor	AY793548	AF220957	JN099004	AY793508	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. queenslandica	CBS 129574 = CPC 17568[T]	Soil	Australia	P.W. Crous	JN098861	JN099098	JN098977	JN098826	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129575 = CPC 17569	Soil	Australia	P.W. Crous	JN098860	JN099097	JN098976	JN098827	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. thailandica	CBS 129570 = CPC 18834	Soil	Thailand	P.W. Crous	JN098847	JN100581	JN098962	JN098833	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129571 = CPC 18835[T]	Soil	Thailand	P.W. Crous	JN098848	JN100582	JN098963	JN098834	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. variabilis	CBS 375.93 = IMI 317057	*Mangifera indica*	India	P.N. Chowdhry	JN098881	JN099119	JN099000	JN098836	Lombard *et al*. (2012), Lombard *et al*. (2017)
	CBS 129561 = CPC 17505[T]	soil	Australia	P.W. Crous	JN098950	JN100643	JN099080	JN098719	Lombard *et al*. (2012), Lombard *et al*. (2017)
C. viticola	CBS 112897 = CPC 5606[T]	*Vitis vinifera*	South Africa	G.J. van Coller	AY793544	AY793468	JN099064	AY793504	Coller *et al*. (2005), Lombard *et al*. (2012)
	CBS 114682 = IMI 297470	*Amorphophallus* sp.	Thailand	R. Stevenson	JN098919	JN100612	JN099047	JN098723	Lombard *et al*. (2012)
Calonectria pauciramosa	CBS 138824 = CMW 5683 = CPC 971[T]	Soil	South Africa	P.W. Crous	MT335565	MT359786	MT412856	MT413068	Schoch *et al*. (1999), Liu *et al*. (2020)
	CBS 137243 = CMW 36327	*Eucalyptus grandis* × *E. camaldulensis*	Mozambique	J. Roux & S. Maússe-Sitoe	MT335563	MT359784	MT412854	MT413066	Crous *et al*. (2013), Liu *et al*. (2020)

[a] ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC: Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; PPRI: Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa; [T]: Ex-type cultures.

[b] *his3*: histone H3; ITS: internal transcribed spacer regions and intervening 5.8S nrRNA gene; *tef1*: translation elongation factor 1-alpha; *tub2*: β-tubulin.

[c] N/A: information not available.

DISCUSSION

This study expanded our understanding of species diversity and distribution of *Cylindrocladiella* species in China. We identified two *Cylindrocladiella* species from soil in a plantation of *C. lanceolata* in southwestern China, namely *C. yunnanensis sp. nov*., and *C. longistipitata.* The identity of *C. yunnanensis* as a new species was supported by phylogenetic inference of multiple gene sequences and morphological characteristics.

Multigene phylogenetic inference based on DNA sequence comparisons promotes the identification and understanding of the diversity of *Cylindrocladiella* species (Lombard *et al*. 2012, 2017, Pham *et al*. 2018). Of the four regions used for phylogenetic analyses in this study, *his3*, *tef1* and *tub2* provided good resolution for *Cylindrocladiella* species delineation, with *his3* proving to be the best barcode. On the contrary, the ITS region failed to adequately distinguish species of *Cylindrocladiella*.

The relative limited number of reports of *Cylindrocladiella* species from China is probably because the genus is primarily soil-borne (Crous 2002, Lombard *et al*. 2012, 2017, Crous *et al*. 2017, Pham *et al*. 2018, Tibpromma *et al*. 2018), and these fungi tend to be overlooked. Combined with the results obtained in this study, *C. lageniformis*, *C. longistipitata*, *C. xishuangbannaensis*, and *C. yunnanensis* have now been confirmed as occurring in China (Tibpromma *et al*. 2018, Ye *et al*. 2021). With the exception of *C. lageniformis*, which was isolated from symptomatic trunk and root stock of *Vitis vinifera*, the other three species were all isolated from soil. Although inoculation results indicated that *C. lageniformis* was pathogenic to the tested shoots of *V. vinifera* (Ye *et al*. 2021), it remains to be determined if these other *Cylindrocladiella* species isolated in China are pathogenic.

This study resulted in the description of *C. yunnanensis* as a new species, and the first report of *C. longistipitata* from China. A more systematic soil collection from other plantations and regions is now required to provide a more comprehensive understanding *Cylindrocladiella* species diversity in China.

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Fig. S1. Phylogenetic tree obtained from the maximum likelihood (ML) analysis of the dataset of *his3* gene region. Bootstrap values ≥ 75 % from the ML analysis are indicated at nodes. Isolates reported in this study are highlighted in bold and blue.

Fig. S2. Phylogenetic tree obtained from the maximum likelihood (ML) analysis of the dataset of ITS region. Bootstrap values ≥ 75 % from the ML analysis are indicated at nodes. Isolates reported in this study are highlighted in bold and blue.

Fig. S3. Phylogenetic tree obtained from the maximum likelihood (ML) analysis of the dataset of *tef1* gene region. Bootstrap values ≥ 75 % from the ML analysis are indicated at nodes. Isolates reported in this study are highlighted in bold and blue.

Fig. S4. Phylogenetic tree obtained from the maximum likelihood (ML) analysis of the dataset of *tub2* gene region. Bootstrap values ≥ 75 % from the ML analysis are indicated at nodes. Isolates reported in this study are highlighted in bold and blue.