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Two fungal species associated with canker disease of Jujube tree in China

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Abstract

Chinese Jujube (*Ziziphus jujuba*) is a common fruit tree used in landscaping, medicine and timber. However, Jujube trees are threatened by various pathogens in the process of planting and cultivation. In this study, destructive canker diseases of *Z. jujuba* were investigated in Beijing, China. Based on morphological comparison and DNA sequence analysis, the causal organisms of these diseases were identified as *Dothiorella acericola* and *Nothophoma spiraeae*. This is the first report of *D. acericola* and *N. spiraeae* on *Z. jujuba*. This study improves our understanding of fungal species causing canker or dieback disease on this economically important tree and provides insights on selecting the effective disease management strategies for *Z. jujuba* in China.

Keywords: Botryosphaeriaceae, Didymellaceae, fungal disease, phylogeny, taxonomy

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Introduction

Chinese Jujube (*Ziziphus jujuba* Mill.) is a common native fruit tree, which has high nutritional and nutraceutical values, health benefits, along with the wide adaptation to drought conditions (Liu and Zhao 2009). More than 700 cultivars of Jujube have been discovered in China (Gao et al. 2013). At present, China is the only country in the world that exports Jujube fruits, with an annual output of 400,000 tons of fresh Jujube fruits and an annual export of about 4,700 tons of dried Jujube fruits (Gao et al. 2013). *Ziziphus jujuba* is distributed in about 47 countries throughout Africa, America, Asia, Europe and Oceania (Liu 2006, Liu and Wang 2009). However, many Jujube trees are under serious threat of dieback or canker diseases caused by phytopathogenic fungi, resulting in a severe reduction in yield and quality of the final products.

Previous studies on diseases of Jujube focused mainly on fruits and roots. Zhang et al. (2011) reported *Alternaria alternata*, *Phoma destructiva* and *Fusicoccum* sp. to cause the Jujube fruit shrink disease. *Fusarium oxysporum* was isolated from discoloured stem vascular tissues of diseased Jujube trees with wilting symptoms (Gao et al. 2012), and *F. incarnatum* from the diseased fruits (Gao et al. 2015). Red rot disease caused by *Botryosphaeria dothidea* was reported by Ren et al. (2018), which affected the vales of grey Jujube fruits. Du et al. (2013) reported *Cytospora sacculus* from symptomatic rhizome samples in China, while Bai et al. (2016) observed brown spot of Jujube caused by *Nothophoma quercina* (= *Phoma fungicola*). Recently, Zhu et al. (2018) isolated *Aplosporella javeedii* that caused canker disease and dieback on *Z. jujuba*, which was the first record in China. Thus, further studies are required to improve our understanding of pathogens causing canker and dieback diseases of *Z. jujuba*.

In this study, a total of 12 specimens of *Z. jujuba* with obvious disease symptoms were collected from Beijing, China. In a systematic study based on morphology and DNA sequence analysis, the disease-causing agents were identified to be two fungal species. This is the first report on *Dothiorella acericola* (Botryosphaeriaceae) and *Nothophoma spiraeae* (Didymellaceae) causing diseases in *Z. jujuba* in China.

Materials and methods

Isolates

Symptomatic branches and twigs of *Ziziphus jujuba* were collected from Beijing, China in the year 2018, for pathogen isolation. Isolates were obtained directly from conidiomata, removing conidial masses to clean potato dextrose agar (PDA) medium in Petri dish, incubated at 25 °C for up to 24 h. Single germinating conidia were transferred to new PDA plates and incubated at 25 °C in the dark. Specimens were deposited at the Museum of Beijing Forestry University (BJFC) and at the working Collection of X. L. Fan (CF) housed at the Beijing Forestry University. Axenic cultures were deposited at the China Forestry Culture Collection Centre (CFCC).

DNA extraction, amplification and sequencing

Fungal isolates were grown on PDA medium for one week to produce mycelia for DNA extraction. Genomic DNA was extracted using the modified CTAB method (Doyle and Doyle 1990). DNA amplifications were performed by polymerase chain reaction (PCR) in a DNA Engine Peltier Thermal Cycler (PTC-200; Bio-Rad Laboratories, Hercules, CA, USA). The PCR was conducted as described in Fan et al. (2020). The primers and PCR conditions are listed in Table 1. The PCR amplification products were electrophoresed and visualized in gels. DNA sequences generated by each primer combination were used to obtain consensus sequences using Seqman v. 7.1.0 in the DNASTAR lasergene core suite software (DNASTAR Inc., Madison, WI, USA). Newly generated sequences were deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/).

Phylogenetic analyses

We constructed two separate phylogenetic trees, based on different gene regions, for two genera (*Dothiorella* and *Nothophoma*) isolated from *Ziziphus jujuba*. The first analysis was performed on the combined dataset of ITS and *tef1-α* sequences to compare *Dothiorella* species from the current study with other sequences obtained from GenBank (Table 2). Another analysis was performed using a combined multi-gene dataset of ITS, LSU, *rpb2* and *tub2* sequences to compare *Nothophoma* species (Table 3). For the phylogenetic study, sequences were aligned using MAFFT v. 6 (Katoh and Standley 2013) and adjusted using MEGA v. 6.0 (Tamura et al. 2013). The alignment was used to infer phylogenetic relationships based on Maximum Parsimony (MP) using PAUP v. 4.0b10 (Swofford 2003), Maximum Likelihood (ML) using PhyML v. 3.0 (Guindon et al. 2010) and Bayesian Inference (BI) analyses using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003).

MP analysis was performed using a heuristic search (1000 bootstraps) with random-addition sequences (Hillis and Bull 1993), and the tree bisection and reconnection (TBR) algorithm was selected (Swofford 2003). The branches of zero length were collapsed using the command minbrlen, and all equally most parsimonious trees were saved. Parsimony scores including tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated (Swofford 2003). ML analysis was performed with a GTR site substitution model including estimation of gamma-distributed rate heterogeneity and a proportion of invariant sites. The branch support from MP and ML analyses were evaluated with a bootstrapping (BS) method of 1000 replicates (Hillis and Bull 1993). MrModeltest v. 2.3 was used to estimate the best nucleotide substitution model settings for each gene following the Akaike Information Criterion (AIC) (Posada and Crandall 1998). BI analysis was performed with a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (BPP) (Rannala and Yang 1996). Two MCMC chains were run from random trees for 1,000,000 generations and trees were sampled for each 100^{th} generation. The first 25 % of trees were discarded as the burn-in phase of each analysis. BPP was calculated to assess the remaining trees (Rannala and Yang 1996). Phylogenetic trees were shown using Figtree v. 1.3.1 (Rambaut and Drummond 2010). Newly generated sequences from the current study were deposited in GenBank (Crous et al. 2004). The aligned matrices used for phylogenetic analyses were deposited in TreeBASE (www. treebase.org).

Morphology

Macroscopic and microscopic characters were recorded. A Leica stereomicroscope (M205) was used to capture the structure and size of conidiomata, the colour, shape and size of discs, the presence or absence of special structures such as number and diameter of ostioles per disc, and the arrangement of locules. Over 30 conidiomata were randomly sectioned and 50 conidia were selected randomly to measure their lengths and widths by a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Ri2 high-definition colour camera with differential interference contrast (DIC). Cultural characteristics of isolates incubated on PDA were

Table 1. Gene regions analysed in this study, with PCR primers, primer DNA sequence, optimal annealing temperature and corresponding references.

Locus	Definition	Primers	Primer DNA sequence (5′–3′)	Optimal annealing temp (°C)	Reference
ITS	internal transcribed spacer of ribosomal RNA	ITS1	TCCGTAGGTGAACCTGCGG	51	White et al. 1990
		ITS4	TCCTCCGCTTTTGATATGC		
LSU	large subunit of ribosomal RNA	LR0R	ACCCGCTGAACTTAAGC	55	Vilgalys and Hester 1990
		LR7	TACTACCACCAAGATCT		
act	actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	61	Carbone and Kohn 1999
		ACT-783R	TACGAGTCCTTCTGGCCCAT		
rpb2	RNA polymerase II second largest subunit	RPB2-5F	GA(T/C)GA(T/C)(A/C)G(A/T)GATCA (T/C)TT(T/C)GG	52	Liu et al. 1999
		RPB2-7cR	CCCAT(A/G)GCTTG(T/C)TT (A/G)CCCAT		
tef-1α	translation elongation factor 1-alpha	EF1-668F	CGGTCACTTGATCTACAAGTGC	55	Alves et al. 2008
		EF1-1251R	CCTCGAACTCACCAGTACCG		
tub2	beta-tubulin	Bt2a	GGTAACCAAATCGGTGCTGCTTTG	55	Glass and Donaldson 1995
		Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		

Table 2. Strains of *Dothiorella* used in the molecular analyses in this study.

Species	Strain[1]	Host	Origin	GenBank accession numbers		Reference
				ITS	*tef1-α*	
Dothiorella alpina	CGMCC 3.18001[T]	*Platycladus orientalis*	Yunnan, China	KX499645	KX499651	Zhang et al. 2016
D. acericola	KUMCC 18-0137[T]	*Acer palmatum*	Yunnan, China	MK359449	MK361182	Phookamsak et al. 2019
D. acericola	**CFCC 54007**	*Ziziphus jujuba*	Beijing, China	MW690189	MW701410	In this study
D. acericola	**CFCC 54008**	*Z. jujuba*	Beijing, China	MW690190	MW701411	In this study

D. acericola	**CFCC 54009**	*Z. jujuba*	Beijing, China	MW690191	MW701412	In this study
D. acericola	**CFCC 54010**	*Z. jujuba*	Beijing, China	MW690192	MW701413	In this study
D. acericola	**CFCC 54011**	*Z. jujuba*	Beijing, China	MW690193	MW701414	In this study
D. acericola	**CFCC 54012**	*Z. jujuba*	Beijing, China	MW690194	MW701415	In this study
D. acericola	**CFCC 54013**	*Z. jujuba*	Beijing, China	MW690195	MW701416	In this study
D. americana	CBS 128309^{T}	*Vitis vinifera*	USA	HQ288218	HQ288262	You et al. 2017
D. americana	CBS 128310	*V. vinifera*	USA	HQ288219	HQ288263	You et al. 2017
D. brevicollis	CMW 36463^{T}	*Acacia karroo*	South Africa	JQ239403	JQ239390	You et al. 2017
D. brevicollis	CMW 36464	*A. karroo*	South Africa	JQ239404	JQ239391	You et al. 2017
D. capri-amissi	CMW 25403^{T}	*A. erioloba*	South Africa	EU101323	EU101368	You et al. 2017
D. capri-amissi	CMW 25404	*A. erioloba*	South Africa	EU101324	EU101369	You et al. 2017
D. casuarini	CMW 4855^{T}	*Casuarina* sp.	Australia	DQ846773	DQ875331	You et al. 2017
D. casuarini	CMW 4857	*Casuarina* sp.	Australia	DQ846774	DQ875333	You et al. 2017
D. citricola	CBS 124728	*Corylus sinensis*	New Zealand	EU673322	EU673289	Abdollahzadeh et al. 2014
D. citricola	CBS 124729^{T}	*C. sinensis*	New Zealand	EU673323	EU673290	Abdollahzadeh et al. 2014
D. dulcispinae	CMW 36460^{T}	*Acacia karroo*	South Africa	JQ239400	JQ239387	You et al. 2017
D. dulcispinae	CMW 36461	*A. karroo*	South Africa	JQ239401	JQ239388	You et al. 2017
D. dulcispinae	CMW 36462	*A. karroo*	South Africa	JQ239402	JQ239389	You et al. 2017
D. dulcispinae	CBS 121764	*A. mellifera*	Namibia	EU101299	EU101344	Slippers et al. 2014
D. dulcispinae	CBS 121765	*A. mellifera*	South Africa	EU101300	EU101345	You et al. 2017
D. iberica	CBS 115041^{T}	*Quercus ilex*	Spain	AY573202	AY573222	You et al. 2017
D. iberica	CBS 113188	*Q. suber*	Spain	AY573198	EU673278	You et al. 2017
D. iranica	CBS 124722^{T}	*Olea europaea*	Iran	KC898231	KC898214	You et al. 2017
D. lampangensis	MFLU 18–2145^{T}	Fruit pericarp	Thailand	MK347758	MK340869	Jayasiri et al. 2019
D. longicollis	CMW 26165	*Lysiphyllum cunninghamii*	Australia	EU144053	EU144068	You et al. 2017

D. longicollis	CMW 26166^{T}	*L. cunninghamii*	Australia	EU144054	EU144069	You et al. 2017
D. magnoliae	CFCC 51563^{T}	*Magnolia grandiflora*	China	KY111247	KY213686	You et al. 2017
D. magnoliae	CFCC 51564	*M. grandiflora*	China	KY111248	KY213687	You et al. 2017
D. mangifericola	CBS 124726	*Mangifera indica*	Iran	KC898222	KC898205	Abdollahzadeh et al. 2014
D. mangifericola	CBS 124727^{T}	*M. indica*	Iran	KC898221	KC898204	Abdollahzadeh et al. 2014
D. moneti	MUCC 505^{T}	*Acacia rostellifera*	Australia	EF591920	EF591971	You et al. 2017
D. moneti	MUCC 506	*A. rostellifera*	Australia	EF591921	EF591972	You et al. 2017
D. neclivorem	DAR 80992^{T}	*Vitis vinifera*	Australia	KJ573643	KJ573640	You et al. 2017
D. oblonga	CMW 25407	*Acacia mellifera*	South Africa	EU101300	EU101345	You et al. 2017
D. oblonga	CMW 25408^{T}	*A. mellifera*	South Africa	EU101301	EU101346	You et al. 2017
D. ostryae	MFLU 18-0177^{T}	*Ostrya carpinifolia*	Italy	MN533805	MN537429	Hongsanan et al. 2020
D. parva	CBS 124720^{T}	*Corylus avellana*	Iran	KC898234	KC898217	You et al. 2017
D. parva	CBS 124721^{T}	*C. avellana*	Iran	KC898235	KC898218	You et al. 2017
D. plurivora	CBS 124724^{T}	*Citrus* sp.	Iran	KC898225	KC898208	Abdollahzadeh et al. 2014
D. plurivora	CBS 124724	*Prunus armeniaca*	Iran	KC898230	KC898213	Abdollahzadeh et al. 2014
D. plurivora	DAR 78869	*Vitis vinifera*	Australia	EU603287	HM800507	You et al. 2017
D. plurivora	DAR 78872	*V. vinifera*	Australia	EU603292	HM800510	You et al. 2017
D. pretoriensis	CMW 36480^{T}	*Acacia karroo*	South Africa	JQ239405	JQ239392	Jami et al. 2012
D. pretoriensis	CMW 36481	*A. karroo*	South Africa	JQ239406	JQ239393	Jami et al. 2012
D. prunicola	CBS124723^{T}	*Prunus dulcis*	Portugal	EU673313	EU673280	You et al. 2017
D. rosulata	CBS 121760^{T}	*Acacia karroo*	Windhoek, Namibia	EU101290	EU101335	Slippers et al. 2014
D. rosulata	CBS 121761	*A. mellifera*	South Africa	EU101293	EU101338	Slippers et al. 2014
D. santali	MUCC 508	*Santalum acuminatum*	Australia	EF591923	EF591974	You et al. 2017
D. santali	MUCC 509^{T}	*S. acuminatum*	Australia	EF591924	EF591975	You et al. 2017
D. sarmentorum	IMI 63581b^{T}	*Ulmus* sp.	England	AY573212	AY573235	You et al. 2017

D. sarmentorum	CBS 115038	*Malus pumila*	Netherlands	AY573206	AY573223	You et al. 2017
D. sempervirentis	CBS 124718^{T}	*Cupressus sempervirens*	Iran	KC898236	KC898220	You et al. 2017
D. sempervirentis	CBS 124719	*C. sempervirens*	Iran	KC898237	KC898219	You et al. 2017
D. striata	CBS 124730	*Citrus sinensis*	New Zealand	EU673320	EU673287	You et al. 2017
D. symphoricarposicola	MFULCC 13-0497^{T}	*Symphoricarpos* sp.	Italy	KJ742378	KJ742381	You et al. 2017
D. symphoricarposicola	MFULCC 13-0498	*Symphoricarpos* sp.	Italy	KJ742379	KJ742382	You et al. 2017
D. thailandica	CBS 133991^{T}	*Bambusa* sp.	Thailand	JX646796	JX646861	You et al. 2017
D. thripsita	BRIP 51876^{T}	*Acacia harpophylla*	Australia	KJ573642	KJ573639	You et al. 2017
D. uruguayensis	CBS 124908^{T}	*Hexachlamis edulis*	Uruguay	EU080923	EU863180	Pérez et al. 2010
D. vidmatera	DAR 78992^{T}	*Vitis vinifera*	Australia	EU768874	EU768881	You et al. 2017
D. vidmatera	DAR 78993	*V. vinifera*	Australia	EU768876	EU768882	You et al. 2017
D. vinea-gemmae	DAR 81012^{T}	*V. vinifera*	Australia	KJ573644	KJ573641	You et al. 2017
D. viticola	CBS 117009^{T}	*V. vinifera*	Spain	AY905554	AY905559	Phillips et al. 2008
D. viticola	CBS 117010	*V. vinifera*	Spain	AY905558	AY905561	Phillips et al. 2008
D. westrale	DAR 80529^{T}	*V. vinifera*	Australia	HM009376	HM800511	Pitt et al. 2015
D. westrale	DAR 80530	*V. vinifera*	Australia	HM009377	HM800512	Pitt et al. 2015
D. westrale	DAR 80531	*V. vinifera*	Australia	HM009378	HM800513	Pitt et al. 2015
D. yunnana	CGMCC 3.17999^{T}	*Camellia* sp.	China	KX499643	KX499649	Zhang et al. 2016
D. yunnana	CGMCC 3.17998	*Acer buergerianum*	China	KX499646	KX499652	Zhang et al. 2016
Neofusicoccum luteum	CBS 110299^{T}	*Vitis vinifera*	Portugal	AY259091	AY573217	You et al. 2017
N. luteum	CBS 110497	*V. vinifera*	Portugal	EU673311	EU673277	You et al. 2017

Notes: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Centre; CMW: Culture collection of Michael Wingfield, University of Pretoria, South Africa; ICMP: International Collection of Microorganisms from Plants, New Zealand. The new strains from the current study are in bold. Ex-type taxa are marked with a ^{T}.

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Table 3. Strains of *Nothophoma* used in the molecular analyses in this study.

Species	Strain[1]	Host	Origin	GenBank accession numbers				Reference
				ITS	LSU	*rpb2*	*tub*	
Nothophoma acaciae	CBS 143404^T	*Acacia melanoxylon*	Australia	MG386056	MG386109	MG386144	MG386167	Hou et al. 2020
N. anigozanthi	CBS 381.91^T	*Anigozanthus maugleisii*	Netherlands	GU237852	GU238039	KT389655	GU237580	Chen et al. 2015
N. arachidis-hypogaeae	CBS 125.93	*Arachis hypogaea*	India	GU237771	GU238043	KT389656	GU237583	Chen et al. 2015
N. brennandiae	CBS 145912^T	Garden soil	Netherlands	MN823579	MN823430	MN824604	MN824753	Hou et al. 2020
N. brennandiae	CBS 140540	House dust	Canada	MN973558	MN943761	MT018202	MT005601	Hou et al. 2020
N. eucalyptigena	CBS 142535^T	*Eucalyptus* sp.	Australia	KY979771	KY979826	KY979852	KY979935	Hou et al. 2020
N. infossa	CBS 123395^T	*Fraxinus pennsylvanica*	Argentina	FJ427025	GU238089	KT389659	FJ427135	Chen et al. 2015
N. infuscata	CBS 121931^T	*Acacia longifolia*	New Zealand	MN973559	MN943766	MT018203	MT005662	Hou et al. 2020
N. gossypiicola	CBS 377.67	*Gossypium* sp.	USA	GU237845	GU238079	KT389658	GU237611	Chen et al. 2015
N. macrospora	CBS 140674^T	Human respiratory tract	USA	LN880536	LN880537	NA	LN880539	Crous et al. 2016
N. multilocularis	AUMC 12003^T	*Rhazya stricta*	Saudi Arabia	NA	KY996744	NA	NA	Abdel-Wahab et al. 2017
N. nullicana	CPC 32330^T	*Acacia falciformis*	Australia	NR156665	MG386107	MG386143	MG386165	Hou et al. 2020
N. prosopidis	CPC 21699^T	*Prosopis* sp.	South Africa	KF777149	KF777204	NA	NA	Hou et al. 2020
N. prosopidis	CPC 21701	*Prosopis* sp.	South Africa	KF777150	KF777205	NA	NA	Hou et al. 2020
N. pruni	MFLUCC 18-1600^T	*Prunus avium*	Beijing, China	MH827007	MH827028	MH853664	MH853671	Chethana et al. 2019
N. pruni	JZB 380015	*P. avium*	Beijing, China	MH827004	MH827025	MH853661	MH853668	Chethana et al. 2019
N. pruni	MFLUCC 18-1601	*P. avium*	Beijing, China	MH827005	MH827026	MH853662	MH853669	Chethana et al. 2019
N. pruni	JZB 380017	*P. avium*	Beijing, China	MH827006	MH827027	MH853663	MH853670	Chethana et al. 2019
N. quercina	CBS 633.92	*Microsphaera alphitoides*	Ukraine	GU237900	EU754127	KT389657	GU237609	Chen et al. 2015
N. raii	MCC 1082^T	Soil	India	MF664467	NA	NA	MF664468	Crous et al. 2017
N. spiraeae	CFCC 53928^T	*Spiraea salicifolia*	Beijing, China	MN737833	MN737828	MN879292	MN879295	Zhang et al. 2019

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N. spiraeae	CFCC 53929	*S. salicifolia*	Beijing, China	MN737834	MN737829	MN879293	MN879296	Zhang et al. 2019
N. spiraeae	CFCC 53930	*S. salicifolia*	Beijing, China	MN737832	MN737830	MN879294	MN879297	Zhang et al. 2019
N. spiraeae	**CFCC 54002**	*Ziziphus jujuba*	Beijing, China	MW690196	MW690203	MW701400	MW701405	In this study
N. spiraeae	**CFCC 54003**	*Z. jujuba*	Beijing, China	MW690197	MW690204	MW701401	MW701406	In this study
N. spiraeae	**CFCC 54004**	*Z. jujuba*	Beijing, China	MW690198	MW690205	MW701402	MW701407	In this study
N. spiraeae	**CFCC 54005**	*Z. jujuba*	Beijing, China	MW690199	MW690206	MW701403	MW701408	In this study
N. spiraeae	**CFCC 54006**	*Z. jujuba*	Beijing, China	MW690200	MW690207	MW701404	MW701409	In this study
N. variabilis	CBS 142457^{T}	Human respiratory tract	USA	LT592939	LN907428	LT593078	LT593008	Valenzuela-Lopez et al. 2018
Phoma herbarum	CBS 615.75	*Rosa multiflora* cv. *Cathayensis*	Netherlands	FJ427022	EU754186	KP330420	KF252703	Valenzuela-Lopez et al. 2018
Vacuiphoma bulgarica	CBS 357.84	*Trachystemon orientale*	Bulgaria	GU237837	GU238050	LT623256	GU237589	Valenzuela-Lopez et al. 2018

Notes: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; JZB: Beijing Academy of Agriculture and Forestry Sciences culture collection, Beijing, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA. The new strains from the current study are in bold. Ex-type taxa are marked with a ^{T}

recorded after 3 and 30 days according to the colour charts of Rayner (1970). Adobe Bridge CS v. 6 and Adobe Photoshop CS v. 5 were used for manual editing.

Results

For *Dothiorella*, the combined matrix of ITS and *tef1-α* included 72 ingroup and two outgroup taxa, comprising 845 characters including gaps, of which 558 characters were constant, 41 variable characters were parsimony-uninformative, and 246 characters were variable and parsimony-informative. A heuristic MP search generated 200 parsimonious trees from which one was selected and shown in Fig. 1 (TL= 699, CI= 0.869, RI= 0.900, RC= 0.404). All trees from the ML and Bayesian analyses were identical to that of the MP tree shown (Data not shown). The phylogenetic tree with high support value (MP/ML/BI= 98/98/1) and the morphological characteristics revealed a known species *Dothiorella acericola*.

For *Nothophoma*, five isolates from *Z. jujuba* were sequenced for ITS, LSU, *rpb2* and *tub2* gene regions, which contained 29 *Nothophoma* ingroup and two outgroup sequences with a total of 2,280 characters including gaps. Of the 1,957 characters used in the phylogenetic analysis, 100 variable characters were parsimony-uninformative, and 223 characters were variable and parsimony-informative. MP analysis generated 16 parsimonious trees, one of which is presented in Fig. 2 (TL = 630, CI = 0.608, RI = 0.719, RC = 0.392). ML and Bayesian analyses were similar to the MP tree (Data not shown). *Nothophoma spiraeae* represented a monophyletic clade with a high support value (MP/ML/BI= 96/89/1).

Taxonomy

Dothiorella acericola Phookamsak, D.S. Tennakoon & K.D. Hyde, Fungal Diversity 95:78 (2019) **Fig. 3**

Asexual morph: *Conidiomata* pycnidial, stromatic, globose to conical, immersed to erumpent from bark surface, separate or aggregated into botryose clusters. *Locule* undivided, globose to subglobose, 195–455(–675) µm ($\bar{x}$ = 350 µm, n = 30) in diam. *Ectostromatic disc* hazel to dark, circular to ovoid. *Ostioles* hazel to fuscous black at the same level as the disc, surrounded below disc by lighter entostroma, inconspicuous. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, thin-walled, holoblastic, cylindrical to subcylindrical, (6.5–)8.0–11.5(–15.5) × 2.0–5.0 µm ($\bar{x}$ = 9.8 × 2.4 µm, n = 30). *Conidia* initially hyaline, oblong to subcylindrical with rounded ends, thin-walled, smooth, unicellular, becoming dark brown and 1-septate when mature, moderately thick-walled, (15.5–)18.0–19.0(–21.0) × 8.0–10.0 µm ($\bar{x}$ = 18.6 × 9.2 µm, n = 50). **Sexual morph:** not observed.

***Culture characteristics*:** Colonies on PDA medium was initially white and growing fast, entirely covering the 9 cm Petri dish after 3 d. Aerial mycelium present, colonies cottony, dark olive to greyish, become fully darkened after 12 d. Colonies moderately dense with sparse aerial mycelium, conidiomata sparse and distributed irregularly on the medium surface.

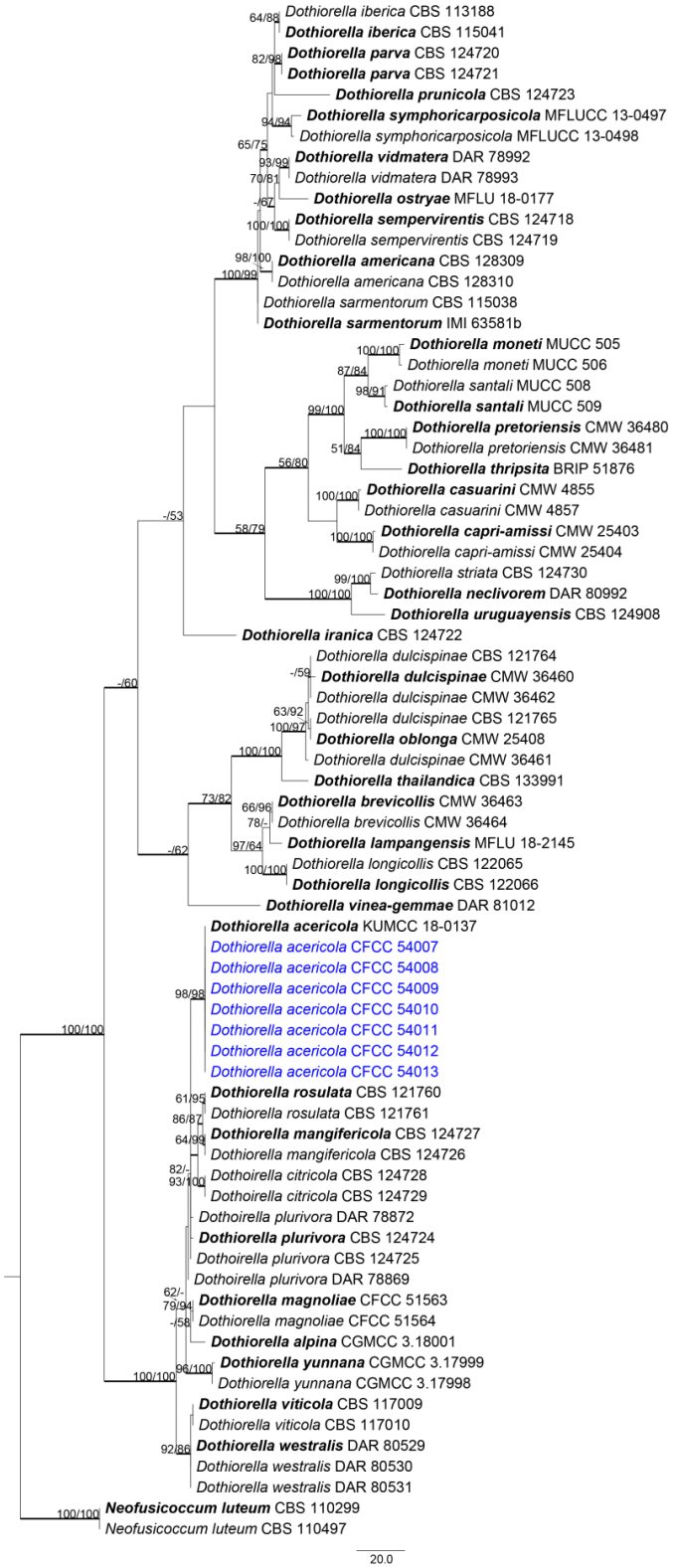

Fig. 1 Phylogram of *Dothiorella* based on combined ITS and *tef1-α* gene regions. MP and ML bootstrap support values above 50 % are shown at the first and second positions. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains from this study are in blue.

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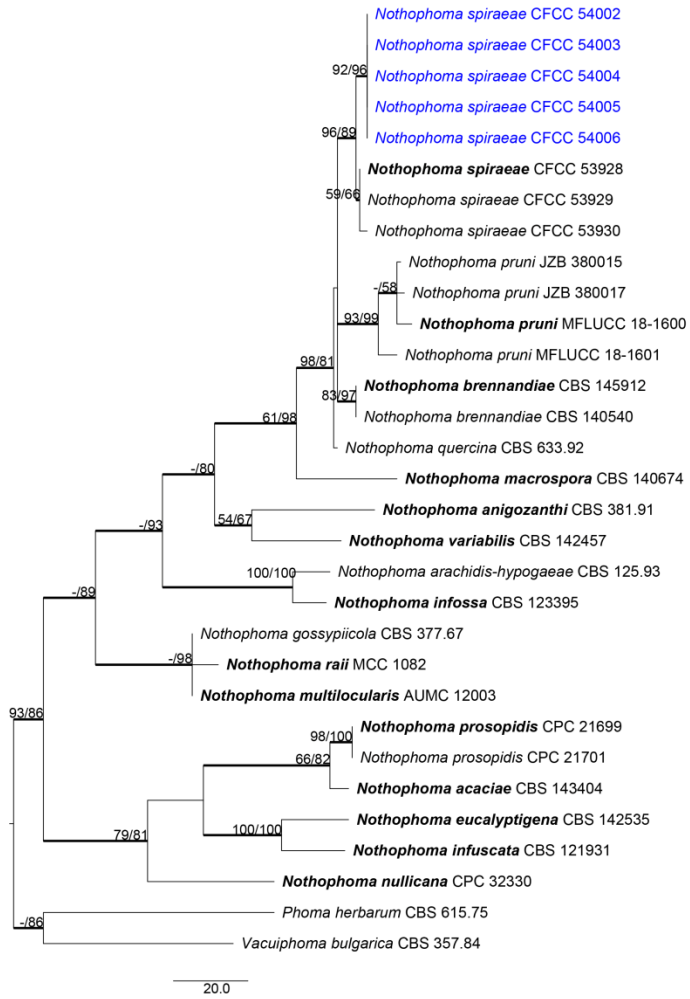

Fig. 2 Phylogram of *Nothophoma* based on ITS, LSU, *rpb2* and *tub2* gene regions. MP and ML bootstrap support values above 50 % are shown at the first and second positions. Thickened branches represent posterior probabilities above 0.95 from BI. Ex-type strains are in bold. Strains from this study are in blue.

Materials examined: **China, Beijing City, Huairou District,** Labagoumen natural scenic spot, 116°25'48" E, 40°53'9" N, from branches of *Ziziphus jujuba*, 26 June 2018, C. X. Shang & X. L. Fan, deposited by C. X. Shang, BJFC CF20191106, living culture CFCC 54007; *ibid.* BJFC CF20191107, living culture CFCC 54008; *ibid.* BJFC CF20191108, living culture CFCC 54009; **China, Beijing City, Huairou District,** Labagoumen natural scenic spot, 116°24'36" E, 40°55'12" N, from branches of *Ziziphus jujuba*, 27 June 2018, C. X. Shang & X. L. Fan, deposited by C. X. Shang, BJFC CF20191109, living culture CFCC 54010; *ibid*. BJFC CF20191110, living culture CFCC 54011; **China, Beijing City, Huairou District,** Labagoumen natural scenic spot, 116°25'02" E, 41°15'42" N, from branches of *Ziziphus jujuba*, 27 June 2018, C. X. Shang & X. L. Fan, deposited by C. X. Shang, BJFC CF20191111, living culture CFCC 54012; *ibid*. BJFC CF20191112, living culture CFCC 54013.

***Notes*:** Phylogenetic analysis of combined ITS and *tef1-α* loci showed that our five isolates clustered with the type strain of *D. acericola* (KUMCC 18-0137) with high statistical values (MP/ML/BI = 98/98/1). *Dothiorella acericola* was first associated with the canker disease of *Acer palmatum* in Yunnan Province, China. In this study, we indicated *D. acericola* as the common pathogen of *Ziziphus jujuba*, which expanded its geographical distribution range and host range. This fungus can be identified by its globose to conical conidiomata with uni- to biloculate locule, producing initial hyaline, aseptate, becoming brown, 1-septate conidia (17.0–22.0 × 7–10 µm) when mature (Phookamsak et al. 2019).

Nothophoma spiraeae L.X. Zhang & X.L. Fan Phytotaxa 430:147–156 (2019) **Fig. 4**
Asexual morph: *Conidiomata* pycnidial, immersed in bark, erumpent through the surface of bark when mature, scattered, discoid to conical. *Locule* multiple, circular to ovoid, (290–)420–540 µm ($\bar{x}$ = 460 µm, n = 30) in diam. *Ectostromatic disc* purplish grey to fawn, circular to ovoid. *Ostioles* black, inconspicuous, at the same level as the disc surface. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth, ampulliform to dolliform, hyaline, thin-walled, 5.0–8.5 × 3.0–5.0 µm ($\bar{x}$ = 7.0 × 4.5 µm, n = 30). *Conidia* hyaline, incidentally olivaceous buff, ovoid, oblong to ellipsoidal, smooth, thin-walled, aseptate, 5–6.5(–7) × 3.5–4.5 µm ($\bar{x}$ = 6.0 × 4.0 µm, n = 50). **Sexual morph:** not observed.

***Culture characteristics*:** Colonies on PDA medium initially pale olivaceous grey and growing to 4 mm after 3 d, in some sectors covered by a low mat of floccose white to grey aerial mycelium. Colonies darkened after 14 d, more felted and denser, conidiomata sparse and distributed irregularly on the medium surface.

Materials examined: **China, Beijing City, Huairou District,** Labagoumen natural scenic spot, 116°25'48" E, 40°53'9" N, from branches of *Ziziphus jujuba*, 26 June 2018, C. X. Shang & X. L. Fan, deposited by C. X. Shang, BJFC CF20191101, living culture CFCC 54002; *ibid*. BJFC CF20191102, living culture CFCC 54003; **China, Beijing City, Huairou District,** natural scenic spot, 116°26'27" E, 41°54'23" N, from branches of *Ziziphus jujuba*, 26 June 2018, C. X. Shang & X. L. Fan, deposited by C. X. Shang, BJFC CF20191103, living culture CFCC 54004; *ibid*. BJFC CF20191104, living culture CFCC 54005; *ibid*. BJFC CF20191105, living culture CFCC 54006.

***Notes*:** *Nothophoma spiraeae* has previously been recorded on *Spiraea salicifolia* from China (Zhang et al. 2019). Our isolates are closely related to *N. spiraeae*, distinguished from them based on *rpb2* and *tub2* loci (nucleotide differences in concatenated alignment: three in *rpb2* and one in *tub2*). Morphologically, our isolates are also similar to *N. spiraeae* in having smooth-walled, aseptate and hyaline conidia (5–6.5 × 3.5–4.5 vs. 5–6.5 × 3.5–4 µm) (Zhang et al. 2019). Thus, the isolates obtained in this study were identified as *N. spiraeae*, representing a new record from symptomatic branches of *Z. jujuba*.

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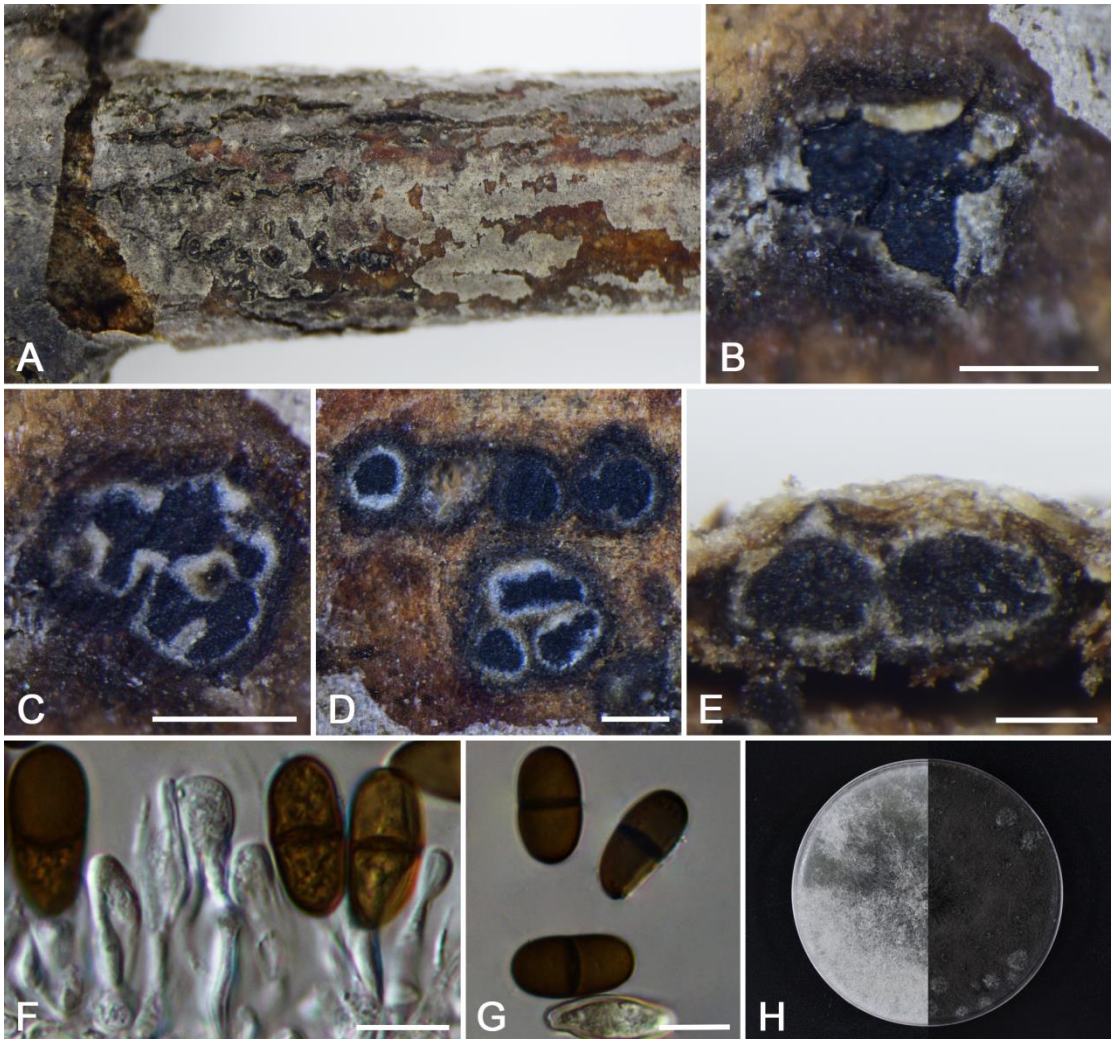

Fig. 3 Morphology of *Dothiorella acericola* from *Ziziphus jujuba* Mill. (CFCC 54007). A, B: Habitat of conidiomata on twig. C, D: Transverse sections of conidiomata. E: Longitudinal sections through conidiomata. F: Conidiophores and conidiogenous cells. G: Conidia. H: Colony on PDA at 3 days (left) and 30 days (right). Scale bars: B–E = 500 μm; F–G = 10 μm.

Discussion

In this study, species identification was supported by morphological characters and multigene phylogeny (ITS, LSU, *rpb2*, *tef1-α* and *tub2*). We report here that *Dothiorella acericola* and *Nothophoma spiraeae* were associated with canker disease on symptomatic twigs and branches of *Ziziphus jujuba* in China. *Nothophoma spiraeae* has been reported previously from *Spiraea salicifolia*, but without detailed morphological data of conidiomata on twigs (Zhang et al. 2019). We identified *Dothiorella acericola*, which formed a distinct clade with high support values (MP/ML/BI=98/98/1). This study further explored the species of plant pathogens with morphology and multi-locus phylogenies in Beijing, China (Zhu et al. 2018, 2019; Pan et al. 2019).

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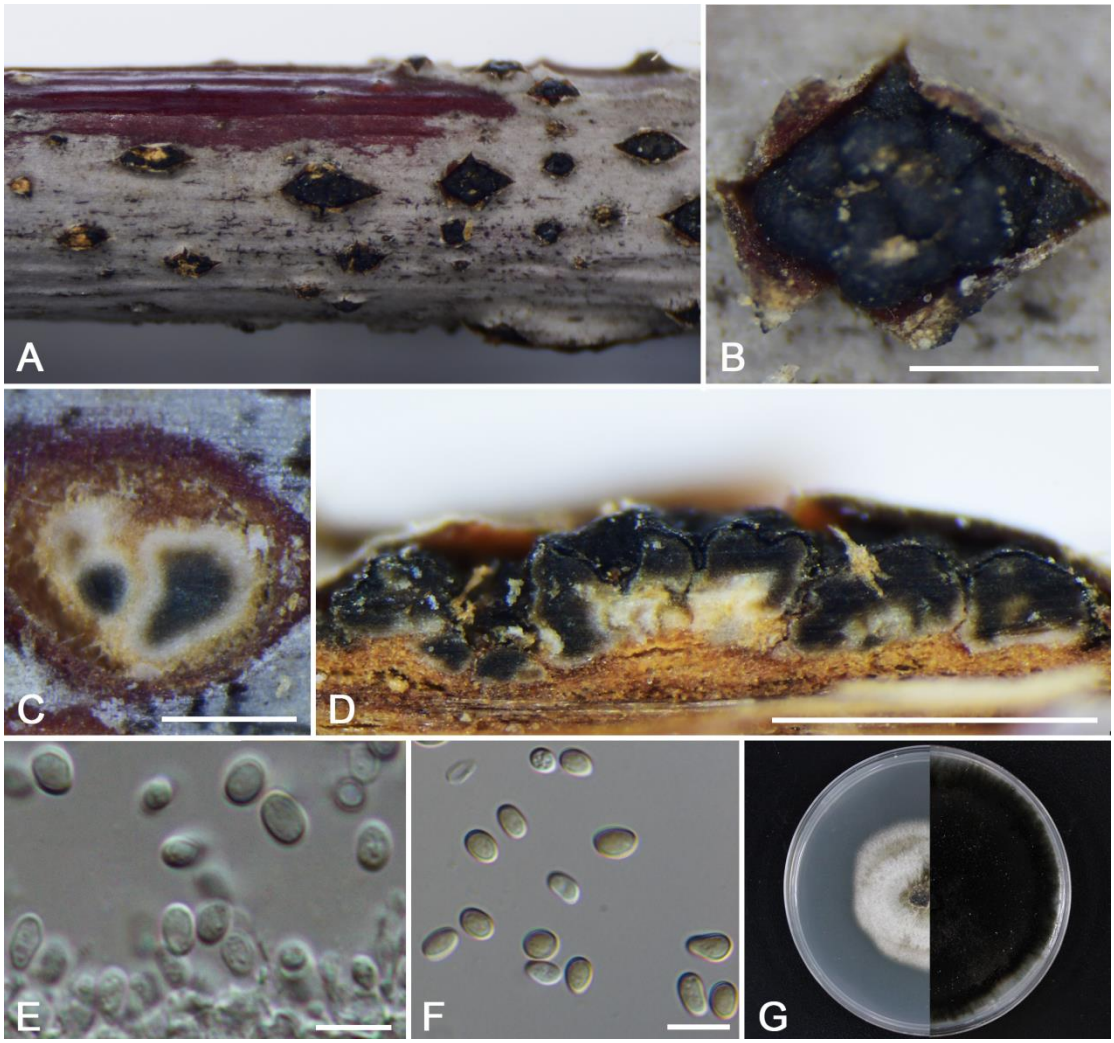

Fig. 4 Morphology of *Nothophoma spiraeae* from *Ziziphus jujuba* Mill. (CFCC 54002). A, B: Habit of conidiomata on twig. C: Transverse sections of conidiomata. D: Longitudinal section through conidiomata. E: Conidiophores and conidiogenous cells. F: Conidia. G: Colony on PDA at 3 days (left) and 30 days (right). Scale bars: B–E = 250 μm; F–G = 10 μm

Nothophoma Q. Chen & L. Cai was established by Chen et al. (2015) as a Phoma-like genus which clustered as a monophyletic clade in *Didymellaceae*. *Phoma* was a confused group with a long history (Sutton 1980). Boerema et al. (2004) subdivided *Phoma* into nine sections based on morphological characters. Later, de Gruyter et al. (2009) established *Didymellaceae* to accommodate several related Phoma-like genera and suggested five sections (*Macrospora*, *Peyronellaea*, *Phoma*, *Phyllostictoides* and *Sclerophomella*) clustered in *Didymellaceae*. Aveskamp et al. (2010) reported *Phoma* species in six distinct clades within the *Pleosporales*, which appeared to reside in different families.

The generic delimitation of *Didymellaceae* was clarified by multi-locus phylogenetic analyses and morphological observations (Chen et al. 2015), thus *Nothophoma* was first introduced and

described in detail, residing in *Didymellaceae*. *Nothophoma* accommodated five species (*N. anigozanthi*, *N. arachidis-hypogaeae*, *N. gossypiicola*, *N. infossa* and *N. quercina*) having *N. infossa* as the type (Chen et al. 2015). Subsequently, *N. macrospora*, *N. multilocularis*, *N. pruni*, *N. raii*, *N. variabilis* and *N. spiraeae* were introduced (Jami et al. 2012, Abdollahzadeh et al. 2014, Slippers et al. 2014, Abdel-Wahab et al. 2017, Hou et al. 2020). Currently, a total of 21 *Nothophoma* species have been registered in Index Fungorum (2021). Based on the previous studies, four loci (ITS, LSU, *rpb2* and *tub2*) were combined for phylogenetic reconstruction in this study. The topology of *rpb2* gene, which showed better resolution at the species level than others, is similar to the combined four loci tree (Chen et al. 2015). By contrast, the LSU locus has no distinct interspecific difference. Thus, we suggest it could be removed in future phylogenetic analysis.

In the past, identification of *Dothiorella* and *Spencermartinsia* was difficult due to the paucity of reliable morphotaxonomic features. However, Phillips et al. (2005) resurrected *Dothiorella* to harbour *Diplodia*-like species having brown and 1-septate conidia before dehiscence from conidiogenous cells, and thereafter, introduced *Spencermartinsia*, with the basionym *Dothiorella viticola* A.J.L. Phillips & J. Luque (Pitt et al. 2015). Phillips et al. (2008) revealed that *Spencermartinsia* differs from *Dothiorella* in having brown, 1-septate ascospores with an apiculus at either end of the ascospores. Subsequently, *Dothiorella* and *Spencermartinsia* were identified as two distinct genera in the Botryosphaeriaceae by multigene phylogenetic analysis (Liu et al. 2012; Phillips et al. 2013). However, *Spencermartinsia pretoriensis* and *S. uruguayensis* were found to reside in *Dothiorella* and they were re-combined in the genus (Phillips et al. 2013). With the increase in number of species, Yang et al. (2016) treated these two genera as one, because the phylogenetic separation between *Dothiorella* and *Spencermartinsia* has become less distinct. In this paper, we also synonymized *Spencermartinsia* species into *Dothiorella*. At present, although over 400 species records in *Dothiorella* have been registered in Index Fungorum (2021), the current study revealed that molecular data is available for less than 10 % of *Dothiorella* species (Phillips et al. 2013; Chen et al. 2015; Li et al. 2016; Yang et al. 2016; Wanasinghe et al. 2018; Hongsanan et al. 2020). It is worth mentioning that *Dothiorella westrale* is closely related to *D. viticola* in the phylogenetic analysis based on combined ITS and *tef1-α* dataset. Morphologically, *D. westrale* and *D. viticola* have similar-sized conidia (18.8–19.7 × 9.8–10.3 vs. 20.2–20.6 × 9.2–9.4) (Luque et al. 2005; Pitt et al. 2015). Thus, we suggest that these two species could be reduced into one. However, the specific results need to be further verified by detailed morphological and phylogenetic analyses.

In conclusion, our study implies that many additional species associating with Jujube trees from China are still undiscovered. In addition, Jujube is regarded as an endemic and commercial species in China, and an extensive study of phytopathogenic fungal species from fresh materials should be collected to help clarify the species concepts of taxa isolated from *Z. jujuba*. This can provide insights on effective disease management of *Z. jujuba* in China.

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Conflicts of Interest
The authors declare no conflict of interest.

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