Coniella vitis sp. nov. Is the Common Pathogen of White Rot in Chinese Vineyards

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Abstract

Grape white rot is a common disease and causes considerable yield losses in many grape-growing regions when environmental conditions are favorable. We surveyed grape white rot in five provinces in China and collected 27 isolates from diseased grape tissues. Multigene phylogenetic analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2), the 28S large subunit of nuclear ribosomal RNA (LSU), partial translation elongation factor 1- α gene (TEF 1- α), and partial histone 3 gene (HIS), coupled with genealogical concordance phylogenetic species recognition and

morphological observations, revealed that *Coniella vitis* sp. nov. and *C. diplodiella* are the causal agents of grape white rot in China. Koch's postulates were performed on *Vitis vinifera* cv. Summer Black in a greenhouse. These results confirmed the pathogenicity on grapes, as symptoms were reproduced, and also indicated significant variations in the virulence among *C. vitis* isolates. This work provides evidence that *C. vitis* is the main pathogen of grape white rot in China and also provides an optimized multi-gene backbone for resolving *Coniella* species.

Grape (*Vitis vinifera* L. and *Vitis* spp.), one of the most widely planted fruit crops, is mainly used as a fresh fruit and for wine production (Shao-Hua 2001). Due to its adaptability to diverse climatic conditions, grape cultivation has expanded around the world, reaching a total of 7.5 million hectares (OIV 2016). China is ranked second in the world for grape cultivation, with an area of 830,000 ha in 2015 (OIV 2016), whereas it is ranked first for grape production, with 11.5 million tonnes, producing 15% of the world's grapes (OIV 2014). Most of the grape production in China originates from *V. vinifera* cultivars, which are highly susceptible to fungal diseases (Li et al. 2008a; Magarey et al. 1993). In most Chinese grape-growing regions, the ripening period coincides with the heavy rainfall season, which is influenced by the East Asia monsoon climate (He 1999; Li et al. 2008a). The rainy season, together with warm summer temperatures, favors the spread of many diseases, resulting in a reduction in vine growth and grape yield and an increase in annual production costs (Úrbez-Torres et al. 2009).

Grape white rot has been reported as one of the main grape fungal diseases in China (He 1999; Li et al. 2008a) and results in an annual production loss of 16.3% (He 1999). However, larger losses can be expected when extreme climatic events such as hailstorms cause wounds that favor the infection (Li et al. 2008a). Hence, the disease is also known as "hail disease"; however, hailstorms are not essential for development of the disease (Cortesi 2015). Wounds caused by climatic events, together with wounds caused by insects or other fungal diseases such as powdery mildew, become the entry points for pathogens (Cortesi 2015). Grape white rot is mainly caused by the pathogenic fungus *Coniella diplodiella* (Speg.) Petr. & Syd. (Bisiach 1988; Crous and Carstens 2000; Crous et al. 2000; van Niekerk et al. 2004), which has also been reported in China (Changyuan et al. 1999). According to Crous and Carstens (2000), other *Coniella* Höhn. spp. such as *C. fragariae* (Oudem.) B. Sutton have also been reported from grapevines in South Africa. However, their ability to cause white rot has not been confirmed (Crous et al. 2000; Matthee and Thomas 1981; van Niekerk et al. 2004; Verbeek 1977). The genus *Coniella*, which was typified by *C. fragariae*, is cosmopolitan in distribution and consists of pathogens associated with foliar, fruit, stem, and root diseases in a wide

range of hosts (Alvarez et al. 2016; van Niekerk et al. 2004). In inoculation studies on grapevine, Tiedemann (1985) showed that *C. diplodiella* and *C. fragariae* cause white rot symptoms and that the latter exhibits the symptoms to a lesser degree. As mentioned by Cortesi (2015), pathogen normally overwinters as mycelium or pycnidia (containing 300 to 2000 conidia per gram of soil) in mummified berries, infected canes, and rachises that fall to the soil. This soil inoculum can remain viable for many years. Under wet or humid conditions, thousands of conidia are discharged from these pycnidia, and the soil-borne inoculum is distributed via rain, hailstorms, or farm equipment onto wound surfaces. Wounds act as entry points into berries, while on rachises and pedicels, infections occur via direct penetration (Cortesi 2015).

In the current study, we investigated *Coniella* spp. associated with white rot in China. Previous studies that identified *C. diplodiella* as the causal agent of grape white rot in China were based only on morphological identifications (Changyuan et al. 1999; Hu et al. 2010; Li et al. 2008b; Li 2012; Liu 2003). To date, no phylogenetic analyses have been combined with morphology to identify the white rot pathogens in China. Since species in this genus are morphologically similar, DNA-based approaches are required for their accurate identification, which is the basis for disease management.

Materials and Methods

Fungal isolates and morphological studies. Diseased grapevine samples were collected from the Beijing, Guangxi, Hebei, Henan, and Jilin provinces of China. Diseased tissue samples obtained from infected fruit, grape pedicels, rachises of clusters, and shoots, as well as from symptomatic leaves, were surface sterilized by washing with 1% sodium hypochlorite for 1 min, 70% ethanol for 1 min, and finally in sterilized water three times prior to culture on potato dextrose agar (PDA) at 28°C. If present, pycnidia were directly isolated from the surface of infected tissues. Single-spore isolation obtained pure cultures as described in Chomnunti et al. (2014). For long-term storage, cultures were transferred to sterilized filter paper at -20°C. Morphological observations were recorded as described in van Niekerk et al. (2004). Observations and photographs of the fungal structures were obtained using an Axio Imager Z2 photographic microscope (Carl Zeiss Microscopy, Oberkochen, Germany) and measurements were taken using ZEN PRO 2012 software (Carl Zeiss Microscopy). Forty conidial measurements were collected per isolate. As the species overlap in their conidial dimensions but differ in their conidial volume (Nag Raj 1993), the average conidial length:width ratios were calculated to further assist distinguishing the species. Growth rates and cultural characteristics were determined for all cultures after 5 days. The growth rate was calculated as the mean of two perpendicular measurements. Herbarium specimens

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were deposited in the Mae Fah Luang University (MFLU) herbarium, Thailand, and the Herbarium of Cryptogams (HKAS), Kunming Institute of Botany, China. All isolates were deposited in the culture collections at Mae Fah Luang University, Thailand (MFLUCC), the culture collection at Kunming Institute of Botany (KUMCC), China, and the Beijing Academy of Agricultural and Forestry Sciences (JZB), China. Following Jayasiri et al. (2015), Faces Of Fungi numbers were acquired.

DNA extraction, PCR amplification, and sequencing. Genomic DNA was extracted by modifying the protocol described in Guo et al. (2000). For the extraction, 500 mg of fresh fungal mycelium were scraped from fresh cultures grown on PDA plates at 28°C for 3 to 7 days. Gene sequences were obtained from four gene regions, including the internal transcribed spacer region (ITS1-5.8S-ITS2), the 28S large subunit of nuclear ribosomal RNA (LSU), partial

translation elongation factor 1- α gene (TEF 1- α), and partial histone 3 gene (HIS) using the specific primers shown in Table 1.

Polymerase chain reaction (PCR) was conducted in an Applied Biosystems Veriti Thermal Cycler according to White et al. (1990). The PCR conditions were as follows: initial denaturation for 3 min at 95°C, followed by 34 cycles of denaturation for 30 s at 95°C and 30 s of annealing and 1 min elongation at 72°C, and a final extension for 10 min at 72°C. The annealing temperatures used in the cycling program were as follows: 52°C for LSU and TEF 1- α , 55°C for ITS1-5.8S-ITS2, and 58°C for HIS. The PCR solution mixture was composed of 0.3 μ l of TaKaRa Ex-Taq DNA polymerase, 2.5 μ l of 10x Ex-Taq buffer, 3.0 μ l of dNTPs, 1 μ l of genomic DNA, 1 μ l of each primer, and 16.2 μ l of ddH₂O. Following the PCR amplification, products were visualized on 1% agarose gel under UV light using a Gel Doc XR+ Molecular

Table 1. Primers used in the study, with sequences and sources

Gene	Product name	Primer	Direction	Sequence (5'–3')	Reference
ITS1-5.8S-ITS2	Internal transcribed spacer	ITS 1	Forward	TCC GTA GGT GAA CCT GCG G	White et al. 1990
		ITS 4	Reverse	TCC TCC GCT TAT TGA TAT GC	
HIS	Histone 3	H3-1a	Forward	ACT AAG CAG ACC GCC CGC AGG	Glass and Donaldson 1995
		H3-1b	Reverse	GCG GGC GAG CTG GAT GTC CTT	
LSU	28S large sub unit of nuclear ribosomal RNA	LR 7	Forward	TAC TAC CAC CAA GAT CT	Vilgalys and Hester 1990
		LROR	Reverse	ACC CGC TGA ACT TAA GC	Rehner and Samuels 1994
TEF 1- α	Partial translation elongation factor 1- α	TEF1-728F	Forward	CAT CGA GAA GTT CGA GAA GG	Carbone and Kohn 1999
		TEF1-986R	Reverse	TAC TTG AAG GAA CCC TTA CC	

Table 2. Isolates and sequences of *Coniella* species used in the study

Species	Culture collection ^a	Host	Country	GenBank accession numbers ^b			
				ITS	Histone	LSU	TEF 1- α
<i>Coniella africana</i>	CBS 114133 ^c	<i>Eucalyptus nitens</i> leaf litter	South Africa	AY339344	AY339309	AY339293	AY339364
<i>Coniella crousii</i>	NFCCI 2213	<i>Terminalia chebula</i> fallen fruits	...	HQ264189	...	...	...
<i>Coniella diploidiella</i>	CBS 111858 ^c	<i>Vitis vinifera</i> stems	France	AY339323	AY339297	AY339284	AY339355
<i>Coniella diploidiella</i>	CBS 166.84	<i>V. berlandieri</i> $\times$ <i>V. riparia</i> twig	Germany	AY339325	AY339299	AY339285	AY339356
<i>Coniella diploidiella</i>	CBS 111857	<i>V. vinifera</i>	South Africa	AY339331	AY339305	AY339286	AY339357
<i>Coniella diploidiopsis</i>	CBS 590.84 ^c	<i>V. vinifera</i> canes	Italy	AY339334	AY339308	AY339288	AY339359
<i>Coniella diploidiopsis</i>	CBS 109.23	<i>V. vinifera</i>	Switzerland	AY339332	AY339306	AY339287	AY339358
<i>Coniella erumpens</i>	CBS 523.78 ^c	Rotten wood	Chile	KX833533	...	KX83361	KX833630
<i>Coniella eucalyptigena</i>	CBS 139893 ^c	<i>Eucalyptus brassiana</i> leaves	Malaysia	KR476725	...	KR476760	...
<i>Coniella eucalyptorum</i>	CBS 112640 ^c	<i>Eucalyptus grandis</i> $\times$ <i>E. tereticornis</i> hybrid leaves	Australia	AY339338	...	AY339290	KX833637
<i>Coniella eucalyptorum</i>	CBS 114134	<i>Eucalyptus camaldulensis</i> ssp. <i>simulata</i>	Vietnam	AY339339	...	AY339289	AY339361
<i>Coniella eucalyptorum</i>	CBS 111023	<i>Eucalyptus phyla</i>	Mexico	AY339337	...	KX833363	AY339360
<i>Coniella fragariae</i>	CBS 172.49 ^c	<i>Fragaria</i> sp. stem base	Belgium	AY339317	...	AY339282	AY339352
<i>Coniella fragariae</i>	CBS 167.84	<i>V. berlandieri</i> $\times$ <i>V. riparia</i> twig	Germany	AY339318	...	EU754149	KX833662
<i>Coniella fusiformis</i>	CBS 141596 ^c	<i>Eucalyptus</i> sp. leaves	Indonesia	KX833576	...	KX833397	KX833674
<i>Coniella fusiformis</i>	CBS 114850	<i>Eucalyptus pellita</i>	Australia	KX833574	...	KX833395	KX833672
<i>Coniella granati</i>	CBS 252.38	<i>V. vinifera</i>	Italy	AY339342	...	AY339291	AY339362
<i>Coniella granati</i>	CBS 814.71	<i>Punica granatum</i> fruit	Turkey	KX833582	...	AY408380	KX833682
<i>Coniella javanica</i>	CBS 455.68 ^c	<i>Hibiscus sabdariffa</i> leaf spot	Indonesia	KX833583	...	KX833403	KX833683
<i>Coniella koreana</i>	CBS 143.97 ^c	...	South Korea	KX833584	...	AF408378	KX833684
<i>Coniella lanneae</i>	CBS 141597 ^c	<i>Lannea</i> sp. leaves	Zambia	KX833585	...	KX833404	KX833685
<i>Coniella limoniformis</i>	CBS 111021 ^c	<i>Fragaria</i> sp.	South Africa	AY339346	AY339310	KX833405	KX833686
<i>Coniella macrospora</i>	CBS 524.73 ^c	<i>Terminalia ivoriensis</i> stem	Ivory coast	AY339343	...	AY339292	AY339363
<i>Coniella malaysiana</i>	CBS 141598 ^c	<i>Corymbia torrelliana</i> leaves	Malaysia	KX833588	...	KX833406	KX833688
<i>Coniella musaiaensis</i>	CBS 109757	<i>Hibiscus</i> sp.	Africa	KX833589	...	AF408337	KX833689
<i>Coniella nicotianae</i>	CBS 875.72 ^c	<i>Nicotiana tabacum</i>	Jamaica	KX833590	...	KX833407	KX833690
<i>Coniella nigra</i>	CBS 165.60 ^c	Soil	India	AY339319	...	KX833408	KX833691
<i>Coniella obovata</i>	CBS 111025	Leaf litter	South Africa	AY339313	...	KX833409	KX833692
<i>Coniella paracastaneicola</i>	CBS 141292 ^c	<i>Eucalyptus</i> sp. leaves	Australia	KX833591	...	KX833410	KX833693
<i>Coniella paracastaneicola</i>	CPC 25498	<i>Eucalyptus</i> sp.	Australia	KX833592	...	KX833411	KX833694

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^a CBS, CPC = Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; JZB = Beijing Academy of Agriculture and Forestry Sciences Culture Collection, China; NFCCI = National Fungal Culture Collection of India, India.

^b Sequence data of internal transcribed spacer region (ITS1-5.8S-ITS2), histone gene (HIS), elongation factor 1- α (TEF1- α), and large subunit (LSU).

^c Ex-type, neo-type, and epitype cultures.

^d Taxonomic novelties.

Imager (BIO-RAD, USA) after ethidium bromide staining. DNA sequencing was done at the Sunbiotech Company, Beijing, China. The DNA sequences generated in this study were deposited in GenBank and their accession numbers are given in Table 2.

Phylogenetic analyses. Consensus sequences were generated using DNASTar v.5.1 and were analyzed using the National Center for Biotechnology Information (NCBI) search engine GenBank BLASTn. Fungal members closely associated with the new taxa, belonging to the same and different genera, were included in the analyses. Reference sequences used in previous studies were obtained from GenBank (Alvarez et al. 2016; Crous et al. 2012, 2014, 2015; Miranda et al. 2012; Rajeshkumar et al. 2011; van Niekerk et al. 2004) and listed in Table 2. Individual sequence datasets of ITS1-5.8S-ITS2, LSU, HIS, and TEF 1- α genes were aligned using the default settings of MAFFT v.7 (Kato and Toh 2008; <http://mafft.cbrc.jp/alignment/server>) and improved manually where necessary using BioEdit (Hall 1999). Then, the aligned sequence datasets were concatenated manually.

Maximum parsimony (MP) analysis was performed in PAUP (phylogenetic analysis using parsimony) v.4.0b10 (Swofford 2002) using the heuristic search option with tree bisection-reconnection (TBR) branch swapping and 1000 random sequence additions. Ambiguous regions in the alignment were excluded, and gaps were treated as missing data. Stability of the trees was evaluated by 1000 bootstrap replications (Hillis and Bull 1993). Branches of zero length were collapsed, and all multiple parsimonious trees were saved. Descriptive statistics including tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were calculated.

Differences between the trees inferred under different optimality criteria were evaluated using Kishino-Hasegawa tests (KHT) (Kishino and Hasegawa 1989).

The best model of evolution was determined by MrModeltest 2.2 for each gene (Nylander 2004). Maximum likelihood (ML) analysis was performed in raxmlGUIv.0.9b2 (Silvestro and Michalak 2010) with 1000 nonparametric bootstrapping iterations, using the general time reversible model (GTR) with a discrete gamma distribution. A tree with the final likelihood value of -12360.77 was selected as the best-scoring tree and the replicates were plotted relative to it.

Bayesian analyses were performed in MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003). The following models were applied: a symmetrical model with a discrete gamma distribution (SYM + G) for the ITS1-5.8S-ITS2 gene region, the Kimura 2-parameter model coupled with a proportion of an invariant site (K80 + I) for the LSU gene region, a general time-reversible model with a proportion of an invariant site (GTR + I) for the HIS gene region, and a general time-reversible model with a discrete gamma distribution coupled with a proportion of an invariant site (GTR + I + G) for the TEF 1- α gene region. The Markov Chain Monte Carlo sampling (BMCMC) analysis was conducted with four simultaneous Markov chains. They were run for 1,000,000 generations; sampling the trees at every 100th generation. From the 10,000 trees obtained, first 2,000 representing the burn-in phase were discarded. Remaining 8000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Crous et al. 2006). Resulting phylogenetic trees were drawn using Treeview v.1.6.6 (Page

Table 2. (continued from previous page)

Species	Culture collection ^a	Host	Country	GenBank accession numbers ^b			
				ITS	Histone	LSU	TEF 1- α
<i>Coniella peruensis</i>	CBS 110394 ^c	Soil of rain forest	Peru	KJ710463	...	KJ710441	KX833695
<i>Coniella pseudogranati</i>	CBS 137980 ^c	<i>Terminalia stuhlmannii</i>	Zambia	KJ869132	...	KJ869189	...
<i>Coniella pseudostraminea</i>	CBS 112624 ^c	<i>Fragaria</i> sp.	South Africa	KX833593	...	KX833412	KX833696
<i>Coniella quercicola</i>	CBS 904.69 ^c	<i>Quercus robur</i> leaf litter	Netherland	KX833595	...	KX833414	KX833698
<i>Coniella solicola</i>	CBS 766.71 ^c	Soil	South Africa	KX833597	...	KX833416	KX833701
<i>Coniella</i> sp.	CBS 114006	<i>V. vinifera</i>	India	AY339347	AY339311	AY339295	KX833703
<i>Coniella straminea</i>	CBS 149.22	<i>Fragaria</i> sp.	USA	AY339348	AY339312	AY339296	AY339366
<i>Coniella tibouchinae</i>	CBS 131594 ^c	<i>Tibouchina granulosa</i> leaves	Brazil	JQ281774	...	JQ281776	JQ281778
<i>Coniella tibouchinae</i>	CBS 131595	<i>Tibouchina granulosa</i> leaves	Brazil	JQ281775	...	JQ281777	JQ281779
<i>Coniella vitis</i> ^d	JZB3700001 ^c	<i>V. vinifera</i>	China	KX890008	KX890033	KX890083	KX890058
<i>Coniella vitis</i> ^d	JZB3700002	<i>V. vinifera</i>	China	KX889992	KX890017	KX890067	KX890042
<i>Coniella vitis</i> ^d	JZB3700003	<i>V. vinifera</i>	China	KX889993	KX890018	KX890068	KX890043
<i>Coniella vitis</i> ^d	JZB3700004	<i>V. vinifera</i>	China	KX889994	KX890019	KX890069	KX890044
<i>Coniella vitis</i> ^d	JZB3700005	<i>V. vinifera</i>	China	KX889995	KX890020	KX890070	KX890045
<i>Coniella vitis</i> ^d	JZB3700006	<i>V. vinifera</i>	China	KX889996	KX890021	KX890071	KX890046
<i>Coniella vitis</i> ^d	JZB3700007	<i>V. vinifera</i>	China	KX889997	KX890022	KX890072	KX890047
<i>Coniella vitis</i> ^d	JZB3700008	<i>V. vinifera</i>	China	KX889998	KX890023	KX890073	KX890048
<i>Coniella vitis</i> ^d	JZB3700009	<i>V. vinifera</i>	China	KX889999	KX890024	KX890074	KX890049
<i>Coniella vitis</i> ^d	JZB3700010	<i>V. vinifera</i>	China	KX890000	KX890025	KX890075	KX890050
<i>Coniella vitis</i> ^d	JZB3700011	<i>V. vinifera</i>	China	KX890001	KX890026	KX890076	KX890051
<i>Coniella vitis</i> ^d	JZB3700012	<i>V. vinifera</i>	China	KX890002	KX890027	KX890077	KX890052
<i>Coniella vitis</i> ^d	JZB3700013	<i>V. vinifera</i>	China	KX890003	KX890028	KX890078	KX890053
<i>Coniella vitis</i> ^d	JZB3700014	<i>V. vinifera</i>	China	KX890006	KX890031	KX890081	KX890056
<i>Coniella vitis</i> ^d	JZB3700015	<i>V. vinifera</i>	China	KX890005	KX890030	KX890080	KX890055
<i>Coniella vitis</i> ^d	JZB3700016	<i>V. vinifera</i>	China	KX890004	KX890029	KX890079	KX890054
<i>Coniella vitis</i> ^d	JZB3700018	<i>V. vinifera</i>	China	KX890009	KX890034	KX890084	KX890059
<i>Coniella vitis</i> ^d	JZB3700019	<i>V. vinifera</i>	China	KX890012	KX890037	KX890087	KX890062
<i>Coniella vitis</i> ^d	JZB3700020	<i>V. vinifera</i>	China	KX890013	KX890038	KX890088	KX890063
<i>Coniella vitis</i> ^d	JZB3700021	<i>V. vinifera</i>	China	KX890015	KX890040	KX890090	KX890065
<i>Coniella vitis</i> ^d	JZB3700022	<i>V. vinifera</i>	China	KX890016	KX890041	KX890091	KX890066
<i>Coniella vitis</i> ^d	JZB3700023	<i>V. vinifera</i>	China	KX890007	KX890032	KX890082	KX890057
<i>Coniella vitis</i> ^d	JZB3700024	<i>V. vinifera</i>	China	KX890010	KX890035	KX890085	KX890060
<i>Coniella vitis</i> ^d	JZB3700025	<i>V. vinifera</i>	China	KX890011	KX890036	KX890086	KX890061
<i>Coniella vitis</i> ^d	JZB3700026	<i>V. vinifera</i>	China	KX890014	KX890039	KX890089	KX890064
<i>Coniella wangiensis</i>	CBS 132530 ^c	<i>Eucalyptus</i> sp.	Australia	NR_111764	...	NG_042686	KX833705
<i>Melanconella</i> sp.	CBS 110385	Soil rain forest	Peru	KX833599	...	KX833420	KX833707

1996). Sequences derived in this study were deposited in GenBank; alignments and trees were deposited in TreeBase (S20261).

Genealogical concordance phylogenetic species recognition analysis. The Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model with a pairwise homoplasy index (PHI) test was used to analyze new species, their limits, and their most closely related neighbors as described by Quaedvlieg et al. (2014). The recombination level within phylogenetically closely related species was determined with the PHI test performed using SplitsTree4 (Huson 1998; Huson and Bryant 2006). The concatenated dataset (ITS1-5.8S-ITS2, LSU, HIS, and TEF 1- α) was used for *C. vitis* and its sister taxa, *C. diplodiella* and *C. diplodiopsis* (Crous & van Niekerk) L.V. Alvarez & Crous. The relationships between these three species were visualized in splits graphs with both the Log-Det transformation and splits decomposition options. A pairwise homoplasy index below a 0.05 threshold ($\Phi_w < 0.05$) indicated the presence of significant recombination in the dataset.

Pathogenicity test. Detached leaf inoculation. Young, healthy detached leaves of *Vitis vinifera* cv. Summer Black were collected from plants in a greenhouse and used for the pathogenicity tests. Six of the newly isolated species strains (JZB3700001, JZB3700006, JZB3700009, JZB3700012, JZB3700014, and JZB3700023) were selected. Surface sterilization was performed on leaves as follows: washed in 75% ethanol for 1 min, then 10% sodium hypochlorite for 1 min, followed by washing with distilled water three times. The experiment was conducted using five leaves per isolate (including one control), inoculated using two methods: nonwound and wound inoculation. The latter was performed on symmetrical halves of each leaf, by injuring the upper epidermal layer of the leaf, using a sterile needle. PDA plugs (5 mm diameter) with fungal mycelia (from 3-day-old culture) were inoculated on both the wounded and nonwounded sites of each of the leaves. Sterile agar plugs (5 mm diameter) were used as the control. All leaves were incubated in a moist chamber at 28°C with a 95% relative humidity, until symptoms appeared. Lesion lengths were recorded three days after inoculation. Once the symptoms appeared, Koch's postulates were confirmed by reisolation of the inoculated fungus and the identification was based on cultural and morphological characters.

Detached fruit inoculation. Pathogenicity tests were conducted on healthy young berries of uniform size in detached clusters, collected from *V. vinifera* cv. Summer Black using the same isolates previously mentioned. Berries were collected at different growth stages, including pea size, green, and well-separated, and berries in closed bunches at veraison. These clusters were broken down into small clusters with their rachis intact. Berries were surface sterilized as mentioned before. The experiment was conducted using five small clusters per isolate (including one control), inoculated using two methods. For the wound inoculation, the epidermis was superficially wounded using a sterilized needle, but for the clusters at their formation stage, wounds were made on the rachis as the berries were of smaller size (diameter 4 to 7 mm). One hundred μ l of conidial suspension (10^6 spores/ml) from each of the isolates were microinjected using a sterile inoculator on to the wounds of the berries. For the nonwound inoculation, conidial suspensions were placed on the berries without making any wounds. Sterile water was used as the control. Each inoculated berry cluster was placed in a 12-cm-diameter petri dish. During the incubation period at 28°C, wetted cotton swabs were placed in petri dishes to maintain humidity and plates were placed inside the incubator at 80% RH. Initial symptoms appeared 5 days after the inoculation. Finally, Koch's postulates were confirmed by reisolation of the inoculated fungus and the identification was carried out based on cultural and morphological characters. All inoculated leaves and berry clusters were autoclaved before being disposed.

Direct plant inoculation. Direct plant inoculations were conducted to determine whether the new species can cause the disease in a live plant, rather than detached plant parts. For this experiment, five young plants of *V. vinifera* cv. Summer Black from the greenhouse were selected for inoculation and another five plants were used as controls. Only the holotype culture (JZB3700001) was selected for the pathogenicity assay. From each plant three new young leaves and three green shoots were wounded using a sterilized needle. The wound on the leaf

was made by injuring the upper epidermal layer and the wound on the green shoot was made at the distal end using a sterilized needle. Wounded shoots and leaves were inoculated with 100 μ l of spore suspension (10^6 spores/ml). For the control we used 100 μ l of sterilized water. These plants were maintained at 70 to 80% RH and a temperature at 28°C.

Data Analysis. One-way analysis of variance (ANOVA) was used to analyze data obtained from the pathogenicity tests at 5% significance level. The analysis was implemented in the MINITAB statistical program v.16.0 (Minitab Inc., State College, PA). Normality tests of Shapiro-Wilk and Jarque-Bera were conducted for all datasets. The means of the different isolates were compared using Fisher's test at the 5% significance level.

Results

Isolation of fungi. *Coniella* isolates were obtained from various white rot-affected tissues, including infected fruit (directly from conidiomata), pedicels, rachises, shoots, and leaves. Twenty-five isolates were obtained from 20 diseased samples of *V. vinifera* cvs. Summer Black, and Centennial seedless grape, collected from Beijing, Guangxi, Hebei, Henan, and Jilin provinces.

Phylogenetic analyses. *Single-gene phylogenetic analyses, optimized multigene phylogeny and species recognition.* Sequences from 25 isolates from the current study and those generated in previous studies for the ITS, LSU, TEF 1- α , and HIS gene regions (Alvarez et al. 2016; Crous et al. 2012, 2014, 2015; Miranda et al. 2012; Rajeshkumar et al. 2011; van Niekerk et al. 2004) were used to construct single-gene phylogenetic trees. These phylogenetic analyses consisted of 66 *Coniella* isolates obtained from various sources and different geographical locations. All the isolates from the current study clustered in a subclade within the *C. diplodiella*/*C. diplodiopsis* clade (see Figure 1). It was observed that ITS phylogeny separated single species from species complexes in the genus *Coniella*, and LSU phylogeny provided a higher taxonomic order for separation of the *Coniella* isolates. The TEF1- α alignment provided better separation between *C. diplodiella*, *C. diplodiopsis*, *C. eucalyptorum* (Crous & M.J. Winf.) L.V. Alvarez & Crous, *C. wangiensis* (Crous & Summerell) L.V. Alvarez & Crous, *C. crousii* (Raheshk. et al.) L.V. Alvarez & Crous, *C. obovata* L.V. Alvarez & Crous, and *C. fragariae* isolates. However, in all of these alignments, *C. diplodiella*, *C. diplodiopsis*, and the novel species introduced in this study clustered together. These isolates clustered into different clades when aligned using the HIS gene due to its high variability, caused probably by a high ratio of parsimony uninformative variable sites in the histone gene region (87 variable, parsimony uninformative positions) compared with phylogenetically informative changes (64 positions).

In addition to the single-gene trees constructed in the current study, most of the possible gene combinations were tested to obtain a better resolution for the identification of *Coniella* species. Among all of the alignment combinations, the best resolution with the highest bootstrap values was obtained when all loci were used for the analysis in the following order: ITS, LSU, HIS, and TEF 1- α . The combined dataset contained 66 sequences representing 30 taxa with *Melanconiella* sp. (CBS 110385) as the outgroup (Fig. 2). Similar multigene phylogenetic trees resulted from all the analyses, including MP, ML, and Bayesian. The parsimony analysis comprised 2877 total characters (585 in the ITS, 1189 in the LSU, 608 in the TEF 1- α , and 485 in the histone gene regions), including gaps. The concatenated alignment contained 558 parsimony informative characters, 238 variable and parsimony uninformative characters, and 2081 constant characters. The first of 1000 equally parsimonious trees is shown in Figure 2, which enabled the identification of the isolates to species level, with a better resolution than the single-gene analyses (TL = 2643, CI = 0.514, RI = 0.739, RC = 0.380, HI = 0.486).

In the combined analysis, the 66 isolates grouped into different clades representing different taxa. In contrast to the single-gene analyses, the combined analysis revealed distinct separate clades for the *C. diplodiella*/*C. diplodiopsis* complex. The phylogenetic analyses provided good evidence that all of the isolates from the current study belonged to a new species, named *C. vitis*, which formed a clade that

was well separated from *C. diplodiopsis*, with high bootstrap value (MP = 100%, ML = 100%) and Bayesian posterior probability (1.00).

Genealogical concordance phylogenetic species recognition analysis. In order to determine evolutionary independence, the GCPSR concept was applied to the *C. vitis* dataset and its neighboring taxa. A

pairwise homoplasy index (PHI or Φ_w) below 0.05 provide evidence for the presence of significant recombination within a dataset. According to the GCPSR analysis, our dataset showed PHI of 1.0, indicating no significant genetic recombination between *C. vitis* and its sister taxa, *C. diplodiella* and *C. diplodiopsis* (Fig. 3). Hence,

Fig. 1. Maximum likelihood analyses based on sequence data of single gene regions of *Coniella* species. **A**, 28S large subunit (LSU). **B**, Translation elongation factor 1- α (TEF1- α). **C**, Internal transcribed spacer region (ITS1-5.8S-ITS2). **D**, Histone gene. Bootstrap support values greater than 50% are indicated near the nodes. The ex-typa are in bold. Scale bars indicate 10 changes for LSU and TEF1- α genes, 1 change for ITS1-5.8S-ITS2, and 0.1 change for histone gene. The trees are rooted with *Melanconella* sp. (CBS 110385) for all the genes, except for histone gene, which is rooted with *Fusarium proliferatum* (NRRL 31071).

it is concluded that these three taxa were significantly different from each other. As we observed a high phylogenetic and pathogenic diversity among the newly introduced species, we applied the GCPSR concept to a dataset, consisting of only *C. vitis* isolates. The analysis resulted in a PHI below 0.05 ($\Phi_w = 0.0001207$) for all the *C. vitis* isolates, indicating a significant recombination among the *C. vitis*

isolates. This confirms that all of these isolates belong to the same species.

Taxonomy. Isolates associated with white rot of *V. vinifera* clustered in a distinct clade in the multigene phylogeny (Fig. 2). The new species described here, *Coniella vitis* (Fig. 4), is closely related to *C. diplodiopsis*, which was originally described as another pathogen

Fig. 2. Maximum likelihood analysis phylogram based on combined ITS, LSU, histone, and TEF1- α sequence data of *Coniella* species. Maximum likelihood and parsimony bootstrap support values greater than 50% are indicated near the nodes, and branches with Bayesian posterior probabilities greater than 0.95 are given in bold. The ex-types are in bold. The scale bar indicates 0.1 changes. The tree is rooted with *Melanconiella* sp. (CBS 110385).

associated with *V. vinifera* in Italy and Switzerland (van Niekerk et al. 2004).

Disease symptoms identified in the field. During this study, symptomatic vines were observed in several vineyards in different provinces. Initially, infected immature berries change color from pale green to pinkish and then dark brown. When the observations were made at the end of summer, in almost all of the vineyards the disease has passed its initial stage. However, only five to six observations of immature dark brown berries were made in several diseased clusters. All the observed diseased vines were carrying completely or partially desiccated clusters. During this process, berries lost their turgidity, shriveled, and were covered by gray to black pycnidia. Pycnidia on the berry skin cause the cuticle to detach from the epidermis, resulting in white berries. At maturity, these pycnidia becomes greyish white. More than 20 partially desiccated diseased clusters were observed during the survey. In most of these clusters, more than half of the berries have been mummified. Infected rachises and pedicels initially appear as brown pin-point spots, which later become pale brown with elongated depressions. These symptoms were observed on all the diseased vines. Under high relative humidity, these infections spread from pedicels into uninjured berries. Within few days, whole clusters become desiccated. Cankers were observed on non-lignified shoots. The infections on green shoots often localized around the nodes. These colonized tissues become necrotic, exhibiting elongated, brownish, depressed areas. Irregular lesions with brown centers were observed on the leaf blades near the injured areas, which became lighter toward the diffused edges. In all of the diseased vines, leaf symptoms were visible clearly (Fig. 5).

Morphological characterization. Isolates of the newly identified *Coniella* species had identical sequences and produced pycnidial conidiomata in culture that contained narrowly ellipsoidal, hyaline

conidia. All the isolates demonstrated identical cultural characteristics. Colonies on PDA medium had crenulated edges with concentric rings and were fast growing up to 8 cm in diameter after 5 days of incubation at 28°C. Mycelia were mostly immersed, and were rarely visible due to sparse aerial mycelia. The upper surface of colonies was mostly flat and white with many pycnidia, and the bottom surface was buff (Fig. 4). Conidial masses secreted from conidiomata were white. The following taxonomic description provides the detailed morphological characters of this new *Coniella* species.

Taxonomic description. *Coniella vitis* Chethana, Yan, Li & K.D. Hyde, sp. nov. Fig. 4. MycoBank no. MB 819365. Faces Of Fungi no. FoF 02722.

The newly identified species is pathogenic on berries of *Vitis vinifera*. Asexual morph is coelomycetous. Conidiomata were 150 to 260 µm ($\bar{x}$ = 203 µm, n = 20) in diameter, pycnidial, solitary, submerged in PDA, globose to slightly depressed globose, with verruculose wall, initially hyaline and turned dark brown to black at maturity, with a central ostiole. Conidiophores were 5 to 8 × 1 to 3 µm ($\bar{x}$ = 6.8 × 2.3 µm, n = 10), formed on a dense, cushion-like aggregation of hyaline cells, subcylindrical, hyaline, smooth, simple or branched below, mostly reduced to conidiogenous cells. Conidiogenous cells were 8 to 13 × 1 to 3 µm ($\bar{x}$ = 10.7 × 2.5 µm, n = 10), hyaline, simple, slender, smooth with a prominent periclinal thickening. Conidia were 7.9 to 12.8 × 3.3 to 6.5 µm ($\bar{x}$ = 10.41 × 5.07 µm, n = 40), length: width ratio 2.0, inequilateral, hyaline when immature becoming pale brown, aseptate, straight to slightly curved, narrowly ellipsoidal, often somewhat flattened on one side, both sides gradually tapering towards the subobtusely rounded apex, subtruncate base, smooth-walled, and multi-guttulate with one or two prominent guttules. Sexual morph is undetermined.

Etymology. Named after the host genus on which it occurs, *Vitis*.

Fig. 3. The results of the pairwise homoplasy index (PHI) test for closely related species of *Coniella vitis* using both LogDet transformation and splits decomposition. PHI test results (Φ_w) < 0.05 indicate significant recombination within the dataset.

Material examined. CHINA. Yanqing, Beijing, on white rot-infected berries of *Vitis vinifera* (Vitaceae), 13 May 2015, XingHong Li JZB3700001 (MFLU 16-2677, holotype, dried sporulating culture on PDA), ex-type living culture, MFLUCC 16-1399; Isotype JZB3700019 (HKAS96352, dried sporulating culture on PDA), ex-type living culture, MFLUCC 16-1400).

Additional material examined. **Paratypes** CHINA. Guangxi province, leaf symptoms of *V. vinifera*, 3 May 2015, XingHong Li, JZB3700012 (living culture, MFLUCC 16-1401); CHINA. Henan

province, on infected shoots of *V. vinifera*, 5 May 2014, XingHong Li, JZB3700002 (living culture, MFLUCC 16-1402); CHINA. JiLin province, infected shoot of *V. vinifera*, 3 May 2015, XingHong Li, JZB3700014 (living culture, MFLUCC 16-1403); CHINA. Hebei province, infected berry of *V. vinifera*, 3 May 2015, XingHong Li, JZB3700016 (living culture, MFLUCC 16-1404); CHINA. Yanqing, Beijing, rachis of an infected berry cluster of *V. vinifera*, 3 May 2015, XingHong Li, JZB3700021 (living culture, MFLUCC 16-1405).

Fig. 4. Morphological characteristics of *Coniella vitis* (JZB3700001). **A**, Black pycnidia on heavily infected host tissue. **B**, Immature white pycnidia on the PDA. **C**, Mature pycnidia with whitish spore mass. **D**, Conidiogenous cells. **E**, Periclinal thickening at the apex of the conidiogenous cells. **F to L**, Different shapes of conidia. **M**, Upper view showing pycnidia on the PDA and, **N**, the reverse view of the colony on PDA. Scale bars: **B**, **C**, 100 μ m; **D**, **E**, 10 μ m; **F to L**, 5 μ m.

Pathogenicity test. Detached leaf inoculation. Lesions similar to those observed in the field were recorded on leaves after three days. Irregular necrotic lesions formed on the wounded leaves on the third day. After five days, these lesions expanded and the wounded areas became covered with brown to black pycnidia, which turned to gray-white at maturity after a week. No symptoms were observed on non-wounded inoculated leaves or on wounded and nonwounded leaves maintained as controls. Reisolation from lesions and from the pycnidia confirmed the inoculated fungus based on cultural and morphological characters such as white colonies with sparse, crenulated edges and concentric rings; and hyaline, inequilateral, straight to slightly curved and narrowly ellipsoidal conidia with a length:width ratio of 2.0. All four replicates yielded the same results (Fig. 6). Different isolates of *C. vitis* recorded in this study showed significantly different virulence on grape leaves ($F_5 = 68.86$, $P = 0.000$) as shown in Figure 6I. Among all isolates, JZB3700012, isolated from symptomatic leaves from Guangxi province, was the most virulent.

Detached fruit inoculation. The fruit inoculation assay was performed at three berry stages (Fig. 7 and 8). Among the three stages, clusters at the formation stage with pea sized berries were easily infected. This stage was the first to exhibit symptoms for all the isolates. Five days post inoculation, rachises of these clusters showed irregular necrotic lesions and pycnidia on the surface. However, clusters at other berry ripening stages did not show any symptoms. A week after the inoculation, black pycnidia appeared on these rachises and the infection spread to the berries (Fig. 7 and 8). Most of the berries in formation-stage clusters (pea-sized berries) turned from green to brown. In some isolates (JZB3700001 and JZB3700014), the berries and the rachises were completely covered with pycnidia at seven days post inoculation. Green and red berry clusters at their ripening stages lost their turgidity and started changing from green to pinkish. Few of the berries at these two stages were covered with pycnidia at seven days post inoculation. At two weeks post inoculation, all the pea-sized berry clusters were covered with pycnidia and on some berries pycnidia turned white (especially for JZB3700001 and JZB3700014). Water-soaked regions formed around the wound of wound-inoculated, red, young berries seven days after inoculation (Fig. 8). Subsequently, the skin around the water-soaked areas became discolored. White mycelia appeared on the water-soaked areas, and cracks appeared on the berry skin. Two weeks post inoculation, most of the berry surface was covered with light brown to black pycnidia, turgidity was lost, and some berries were shrunk. Berry clusters maintained as the control and the berry clusters with nonwound inoculation remained asymptomatic. The taxa reisolated from diseased fruits (from water-soaked tissues and mycelia and pycnidia that formed at the infection site) were identical to the original isolates. Identification of the reisolated fungus was confirmed based on cultural and morphological characters such as white colonies with sparse, crenulated edges and concentric rings, and hyaline, inequilateral, straight to slightly curved, and narrowly ellipsoidal conidia with a length:width ratio of 2.0. All four replicates yielded the same results, but the virulence was highest in JZB3700001. In addition, *C. vitis* isolates JZB3700006 and JZB3700014 also exhibited high levels of virulence compared with other isolates. These three isolates infected the clusters more rapidly compared with other isolates (Fig. 7 and 8).

Direct plant inoculation. Living plants exhibited typical white rot symptoms on shoots and on leaves. Five weeks post inoculation brown necrotic areas were formed on the shoots. On the infected shoots, leaves exhibited light brown lesions, and some turned completely brown. One month after the inoculation, most of the leaves turned completely light brown and the necrotic areas on the shoots collapsed into necrotic shoots. All the five plants showed the typical symptoms, whereas control plants did not show any type of symptom (Fig. 6).

Discussion

Grape white rot is found in viticulture regions with warm, humid summers in Africa, Asia, Australasia, Europe, North and South America, and Oceania (Cho and Shin 2004; Crous et al. 2000; Mathur 1979; Plantwise Knowledge Bank 2012; van Niekerk et al. 2004; Zhuang

2001). It has also been reported in China, a country with one of the most extensive grape cultivation areas in the world. According to surveys conducted on grape white rot disease in China during 2015 and 2016, severe incidence of the disease was found on the fruit in major grapevine-cultivating provinces. The disease has been previously reported from many Chinese vineyards as a minor disease. According to grape growers, the disease was minimized by proper agricultural practices including maintaining trellis structures as high as possible (70 to 80 cm) above the soil to increase the height of the fruit and shoot position. In addition, shoot thinning, leaf removal, pruning, cluster thinning, and shoot positioning have also been conducted by growers to reduce humidity and increase air and light circulation in the vineyards. In 2016, most of the vine-growing provinces experienced extremely conducive environmental conditions including heavy rainfall, storms, and hailstorms. These conditions, especially hailstorms and heavy rainfall, facilitated the infections by creating wounds on berries, which contributed to the build up of inoculum. During the fruit formation period, berries and rachis were infected by the pathogen, leading to severe conditions during the fruit ripening stage. After these extremely conducive environmental conditions, we observed an increment on the incidence of white rot disease even in those areas where proper management agricultural practices were followed.

The white rot pathogen belongs to genus *Coniella*, a genus mainly composed of many pathogens and some secondary invaders of plant

Fig. 5. Disease symptoms of grape white rot on *Vitis vinifera* cv. Summer Black in the field. **A**, Immature berries change color from pale green to brown. **B**, Shrunk berries covered with pycnidia, infected pedicels, and rachis leading to desiccation and death of clusters. **C**, Cracks on long depressed brownish, necrotic areas on shoots. **D**, Leaf symptoms: Irregular lesions with brown center and amphigenous gray-white pycnidia on brown lesions.

tissues (Alvarez et al. 2016). *Coniella*, together with the asexual genus *Pilidiella* Petr. & Syd., typified by *P. quercicola* (Oudem.) Petr., and *Schizoparme* Shear, typified by *S. straminea* Shear, belong to the family Schizoparmaceae Rossman, D.F. Farr & Castl. (Maharachchikumbura et al. 2015, 2016; Rossman et al. 2007). Links between the genus *Schizoparme* and its asexual genera, *Coniella* and *Pilidiella*, were first recognized by Samuels et al. (1993), and several studies have confirmed this relationship (Alvarez et al. 2016; Miranda et al. 2012; Rossman et al. 2007). Initially, *Coniella* was positioned in Melanconidaceae, but later, Castlebury et al. (2002) showed their distinct lineage in Diaporthales and referred to them as the *Schizoparme* complex. *Coniella* and *Pilidiella* were treated as separate genera by van der Aa (von Arx 1973) and von Arx (1981), considering the pigmentations in their conidia. Nag Raj (1993) and Sutton (1980)

rejected this theory and synonymized *Pilidiella* under *Coniella*, considering their identical fruiting bodies, conidiogenesis, and conidiophore orientations. Following Castlebury et al. (2002), many studies confirmed that *Pilidiella* is distinct from *Coniella* based on their phylogenetic analyses (van Niekerk et al. 2004; Wijayawardene et al. 2016). In accordance to one name for one fungus based on the International Code of Nomenclature for algae, fungi and plants (McNeill et al. 2012), Alvarez et al. (2016) synonymized *Pilidiella* and *Schizoparme* under their older name *Coniella*.

Here, we defined a novel species, *Coniella vitis*, by considering its morpho-cultural characteristics and its placement in the phylogenetic trees inferred from ITS, LSU, HIS, and TEF 1- α gene regions. Single-gene and multigene analyses were conducted to delineate the pathogenic species of white rot with high resolution. Most of

Fig. 6. Detached-leaf pathogenicity assay and whole-plant inoculation results of *Coniella vitis* (JZB3700001) on *V. vinifera* cv. Summer Black. **A to E**, Symptoms on leaves inoculated with mycelial plugs of *C. vitis*: right, wound inoculation; left, nonwound inoculation. **A**, Grape leaves inoculated with PDA plug (control). **B, C, D**, Necrotic areas of leaves three days post inoculation by JZB3700001, JZB3700006, and JZB3700012 strains, respectively. **E**, Necrotic areas and pycnidia five days post inoculation by JZB3700012. **F to H, J, K**, Symptoms resulted from whole-plant inoculation on shoots and leaves of infected plants. **I**, Variations in the virulence of lesion area of *Coniella vitis* isolates on grape leaves resulted from the pathogenicity test three days post inoculation. JZB3700012 showed the highest virulence of all isolates. Red characters indicate the significant differences for lesion area. Isolates that do not share same letter are significantly different.

Fig. 7. Detached fruit inoculation assays on *V. vinifera* cv. Summer Black berry clusters. Control treatment with sterile water on, **A**, pea-sized berries and, **G**, young hard, green berries at the ripening stage. **B to D**, Symptoms on pea-sized berry clusters seven days after wound inoculation by *C. vitis*. **B**, Pycnidia were visible on most of the berries and rachises infected by highly virulent *C. vitis* isolate JZB3700001. Compared with *C. vitis* isolate JZB3700001, clusters inoculated by **C**, JZB3700014 and **D**, JZB3700006 showed less infection with only few berries and rachises covered pycnidia. **E, F**, Symptoms on pea-sized berry clusters 14 days after wound inoculation by *C. vitis* isolates JZB3700001 and JZB3700014. **E**, Fourteen days post inoculation, cluster infected by isolate JZB3700001 completely desiccated with pycnidia on berries and rachis, and the disease spread into berries in cluster infected by JZB3700006 with pycnidia on berries and rachis. **H to J**, Symptoms on young, hard, green berry clusters seven days after wound inoculation by *C. vitis* isolates JZB3700001, JZB3700014, and JZB3700006 respectively. Few berries showed pycnidia on the berry surface, and only the clusters infected by, **H**, JZB3700001 and, **J**, JZB3700006 showed pycnidia on the rachis. **K to M**, Symptoms on young, hard, green berry clusters 14 days after wound inoculation by *C. vitis* isolates JZB3700001, JZB3700006, and JZB3700014, respectively. Almost all the berries and rachis were covered with pycnidia in the cluster infected by, **K**, isolate JZB3700001. Only a few of the berries were completely covered with pycnidia, while the other berries has changed color in clusters infected by, **L**, isolates JZB3700006 and, **M**, JZB3700014. In these clusters rachises were completely infected with visible pycnidia on the surface.

the previous phylogenetic studies conducted on *Coniella* species were based on single-gene phylogeny (Castlebury et al. 2002; Miranda et al. 2012; van Niekerk et al. 2004), except for Alvarez et al. (2016). Castlebury et al. (2002) and Miranda et al. (2012) used LSU and ITS phylogeny separately to identify the species in these taxa. In the study conducted by Alvarez et al. (2016), multigene phylogeny (ITS, LSU, RPB2, and TEF 1- α) was successfully used to resolve species within the genus. However, some *Coniella* species were not included in their multigene phylogenetic analyses (*C. croussii*, *Coniella eucalyptigena* (Crous & M.J. Winf.) L.V. Alvarez & Crous, *Coniella musaiaensis* B. Sutton, and *Coniella pseudogranati* (Crous) L.V. Alvarez & Crous). Similar to our study, van Niekerk et al. (2004) performed single-gene (ITS, LSU, histone, and TEF

1- α genes) phylogenetic analyses. Each of these gene regions addressed different aspects during the phylogenetic analyses, and they were combined using a partition homogeneity test. Therefore, in our study, we combined the gene regions, which provided the best topology compared with the single gene topologies. The combined analysis in our study allowed all of these aspects to be combined in one tree rather than having to generate several trees to represent each aspect. In addition, GCPSR analyses also confirmed *C. vitis* as a new species.

The multigene phylogenetic analyses showed 25 isolates belonging to *C. vitis* but with various genotypes. *Coniella vitis* isolates exhibited high phylogenetic divergence. According to the phylogenetic analyses, all of the isolates belonging to *C. vitis* formed a clade

Fig. 8. Detached fruit inoculation assays on *V. vinifera* cv. Summer Black berry clusters with young, hard, red berries. **A**, Control treatment with sterile water on young, red berries. **B to D**, Symptoms on young, red berry clusters seven days after wound inoculation by *C. vitis* isolates JZB3700001, JZB3700014, and JZB3700006 respectively. Only a few berries were covered with black pycnidia and water-soaked lesions were seen on few berries. **E to G**, Symptoms on young, red berry clusters 14 days after wound inoculation by *C. vitis* isolates **E**, JZB3700001, **G**, JZB3700014, and **F**, JZB3700006. Infections spread through rachis and berries; many berries and all the rachises were covered with black pycnidia. **H**, Susceptibility to infection by *C. vitis* on three different stages of berry clusters seven days post inoculation. Among the three stages, clusters at the formation stage with pea-sized berries were easily infected.

that was well separated from *C. diplodiopsis*. Morphologically, *C. vitis* is similar to *C. diplodiella* and *C. diplodiopsis* but differs from *C. diplodiopsis* (length:width ratio 1.7) by having longer conidia with subobtusely rounded apices and a higher length:width ratio (2.0) (van Niekerk et al. 2004). They differ from *C. diplodiella* (length:width ratio 2.3) by having smaller conidiophores and a lower length:width ratio (2.0) (van Niekerk et al. 2004). In accordance with the phylogenetic divergence, pathogenicity assays also demonstrated a high level of variability among the *C. vitis* isolates. Virulence toward the Summer Black cultivar differed significantly among the isolates. According to the pathogenicity results, berries exhibited high susceptibility to infection during fruit formation compared with the initial period of fruit formation. Especially during the fruit formation stage with pea-sized berries, *C. vitis* infections spread easily. Considering all of the molecular, morphological, and pathogenicity data, *C. vitis* is introduced as a new pathogenic species causing grape white rot in China. *Coniella vitis* exhibited certain divergence in the phylogenetic analyses as well as in the pathogenicity assays. However, according to the GPCSR analysis, it was confirmed that all of our *C. vitis* isolates belong to the same species. Hence, we suggest *C. vitis* as a species with a cryptic lineage.

To our knowledge, this is the first study that has identified and characterized the grape white rot pathogen in China by both morphological and molecular techniques. Pathogenicity tests also confirmed *C. vitis* as another causal agent of grape white rot in China. Identification of this new pathogen is important for disease management, which is based on identifying the disease symptoms at initial stage and taking actions during weak stages in the disease cycle. These management practices were based on the knowledge of a specific disease, life cycles of the pathogen, time and the method of infection, affected plant parts, pathogen distribution methods, and other agronomic and economic considerations (Mahmood and Kerr 2001). Even though early detection and monitoring are important for any disease management program, the most critical step is the accurate identification of the pathogen. Correct identification of the pathogen, *C. vitis*, becomes an important issue in the management of grape white rot, especially when pathogenicity varies with the species within a genus. Since there are many *Coniella* species isolated from grapevines that do not exhibit any pathogenicity, identification of *C. vitis* and its virulence will allow the improvement of management strategies.

In previous studies, *C. diplodiella* was the only causal agent of grape white rot in Chinese vineyards (Bisiach 1988; Cortesi 2015; Crous and Carstens 2000; Crous et al. 2000; van Niekerk et al. 2004). However, now a new pathogen, *C. vitis*, has been identified from China. The identification of *C. vitis* as a new pathotype in the field may illustrate the possibility of pathogenic taxa evolving in response to several environmental and human factors. The new pathogen evolved in the current context under the existing chemical control methods, and thus if the traditional fungicides used to control *C. diplodiella* are applied, there is a possibility that it would not work against *C. vitis*. Therefore, studies must be conducted to ensure the efficacy of traditional protective fungicides such as phthalimides, dichlofluanid, and chlorothalonil (Cortesi 2015) against the white rot caused by *C. vitis*. Representative isolates used in the current study for pathogenicity tests (JZB3700001, JZB3700006, JZB3700009, JZB3700012, and JZB3700014) can be utilized for screening high efficiency fungicides against grape white rot. However, proper agricultural practices such as maintaining trellis structures as high as possible will prevent creating wounds on berries. Since these wounds act as main entry points for *C. vitis*, preventing them will prevent its infection. High humidity conditions along with warm temperatures facilitate the spread of infections by favoring the growth of this pathogen. Therefore, cultural practices such as leaf removal, pruning, cluster thinning, and shoot positioning will help to reduce humidity and increase air circulation among the vines, thereby creating unfavorable conditions for *C. vitis*. Since most of *V. vinifera* cultivars are highly susceptible to white rot (Li et al. 2008a), planting resistant cultivars is important in preventing the disease. Li et al. (2008a) classified several Chinese wild *Vitis* species including *V. amurensis*, *V. piazekii*, *V. davidii* var. *cyanocarpa*, *V. liubanensis*, *V. qinlingensis*, *V. bashanica*, *V. yeshanensis*, and *V. hankockii*

as resistant to grape white rot. In order to overcome the pathogen adaptation and evolution against the management practices, disease management approaches need diversification. For this, further understanding on the impact and the epidemiology of grape white rot in China is necessary. In the current study, we only investigated several main grape-growing provinces. In future studies, disease-sample collection area should be further expanded to all the provinces in China. If possible, *Coniella diplodiella* cultures previously identified in China based only on morphological characters should be reinvestigated. In the current study, we have exhibited the variations in the pathogenicity of this grape white-rot pathogen. Research can now focus on species population dynamics and disease epidemiology to design more effective disease management strategies against this pathogen.

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