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Potato leaf infection caused by *Colletotrichum coccodes* and *C. nigrum*

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Abstract *Colletotrichum coccodes* is an important pathogen of potatoes. Recently, the closely related *C. nigrum* has been reported as an important pathogen of solanaceous crops but not as a pathogen of potato. This study confirms *C. coccodes* as the most prevalent *Colletotrichum* species infecting foliar and tuber tissue of potatoes in Australia and the USA. In addition, three isolates from the USA, previously identified as *C. coccodes,* were re-identified as *C. nigrum*. The gene loci *TUB2*, *ACT*, *GAPDH*, *CHS-1* or *HIS3* were all suitable for differentiating *C. nigrum* from *C. coccodes*. Australian and USA tuber and foliar isolates of *C. coccodes* and *C. nigrum* had similar pathogenicity on detached potato leaves. This is the first report of *C. nigrum* as a pathogen of potato. Moreover, the recently described *C. dianense* was revealed to be a synonym of *C. nigrum*.

Keywords *Colletotrichum dianense* · Pathogenicity · Phylogeny · Potato · *Solanum tuberosum*

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Black dot is a cosmopolitan disease of potato (*Solanum tuberosum L.*), caused by the fungal pathogen *Colletotrichum coccodes*. In 2013, *C. nigrum* was delineated from *C. coccodes* by a five-gene phylogenetic dataset, of which 13 isolates previously classified as *C. coccodes* were re-identified as *C. nigrum* (Liu et al., 2013). Recently, Zheng et al. (2022) reported a new species, *C. dianense*, closely related to *C. nigrum*, from the aquatic plant *Alternanthera philoxeroides* in the Yunnan Province, China. However, the identification of *C. dianense* was based on only one isolate, differences in the gene sequences of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene and the shape of its conidia. In addition, only the ex-type strains of *C. nigrum* and *C. coccodes* had been included in the phylogeny and not a broader distribution of isolates. Both *C. coccodes*

and *C. nigrum* have been reported as pathogens of a range of *Solanaceae* plant species producing lesions on leaves and fruit, and in the case of *C. coccodes* production of microsclerotia on potato tubers (black dot). *Colletotrichum nigrum* has not previously been reported as a pathogen of potato (Liu et al., 2013).

Although potato black dot has been reported on a global scale from potato tubers, foliar symptoms are not reported in some countries, including Australia. In contrast, in North America and Israel, black dot has also been shown to cause significant damage to potato leaves (Johnson, 1994; Tsror et al., 1999). These differences in pathogenicity in different countries may be due to different *Colletotrichum* species or pathotypes.

Virulence differences among *C. coccodes* isolates originating from different plant tissues and geographical origins have been reported in pathogenicity tests conducted on potato and tomato plants (Barkdoll & Davis, 1992; Daami-Remadi et al., 2010; Nitzan et al., 2002). Pathogenicity assays of nine *C. coccodes* isolates conducted by Barkdoll and Davis (1992) on potato leaves wounded by sandblasting with autoclaved sand prior to inoculation showed variable severity of leaf lesions between isolates. Nitzan et al. (2002) separated 110 *C. coccodes* isolates from potatoes from Israel, France and the Netherlands into four vegetative compatibility groups (VCGs) and found the VCGs to be associated with differences in virulence among the isolates inoculated onto potato plants. Ben-Daniel et al. (2010) separated 79 Australian *C. coccodes* isolates from potato into six VCGs and demonstrated significant differences in aggressiveness of isolates from different geographical locations on mature green tomato fruits.

Similarly, significant genetic variability has been reported within *C. coccodes* populations worldwide. Alananbeh et al. (2014) identified 855 *C. coccodes* isolates from potatoes in the USA, Israel, Europe, Australia and South Africa using the *C. coccodes*-specific primers designed by Cullen et al. (2002) that were based on the sequences of the internal transcribed spacers and the intervening 5.8S region (ITS). Genetic characterisation of these isolates using amplified fragment length polymorphism markers identified six VCGs. The group VCG/AFLP6/7, which clustered separately from the other groups, included isolates originating from the USA, South Africa and a single isolate from Europe. All the isolates originating from hosts other than potato clustered in this group, which may indicate that it represents a different *Colletotrichum* species. Due to lack of variation in the ITS sequences between *C. coccodes* and *C. nigrum*, any *C. nigrum* isolates would have been incorrectly identified as *C. coccodes.* The ITS sequences are often uninformative in differentiating closely related species, even though this has been widely used in previous molecular phylogenetic studies of *C. coccodes* (Alananbeh et al., 2014; Cullen et al., 2002).

It is important to revisit genetic and virulence variability of *Colletotrichum* isolates associated with potato crops in the light of recent advances in *Colletotrichum* taxonomy and the separation of *C. coccodes* from *C. nigrum*. Identification of *Colletotrichum* species causing tuber and leaf lesions of potato as different species may have important implications for disease management as different species may require different approaches to integrated disease control, especially if each species infects different tissues of a plant or responds differently to resistant cultivars. The aims of this study were therefore to 1) assign and validate the correct taxonomy to new and previously identified isolates of *C. coccodes* collected from potatoes in Australia and the USA; and 2) determine their pathogenicity to potato leaves.

A total of 101 isolates were collected from asymptomatic petiole tissue and tubers with black dot symptoms in South-Eastern Australia. Four isolates were obtained from the culture collection of the AgriBio Centre for AgriBiosciences, Victoria, and five from the collection of the South Australian Research and Development Institute. In addition, 30 *C. coccodes* isolates were received from the USA, Department of Plant Pathology, North Dakota State University, which were isolated from potatoes (tissue unknown) in 13 different states of the United States. All isolates from the USA had previously been identified as *C. coccodes* by PCR with a *C. coccodes*-specific marker based on ITS sequences (Alananbeh et al., 2014).

A phylogenetic tree based on *GAPDH* sequences identified the isolates from Australia and the USA as *C. coccodes,* except for three isolates from the USA (USANY, USAMN3, USAWA4), which clustered in the *C. nigrum* clade (Supplementary Table 1, Supplementary Fig. 1). Twenty Australian isolates and five isolates from the USA were then selected for a combined six-gene phylogenetic analysis (Wang et al., 2024), which included sequences of six *C. coccodes*

and five *C. nigrum* isolates downloaded from GenBank. In addition, sequences of the ex-type strain of *C. dianense,* CGMCC 3.18943, were included that had been re-sequenced in this study. Maximum likelihood and Bayesian analysis clearly showed all Australian isolates to cluster with *C. coccodes,* along with four isolates from the USA, while isolate USANY clustered in the *C. nigrum* clade (Fig. 1, Supplementary Table 1). Within the Australian isolates there was no clustering of isolates based on geographical location or host tissues from which isolates were obtained. Sequences of the beta tubulin (*TUB2*), actin (*ACT*), chitin synthase (*CHS-1*), *GAPDH* and histone (*HIS3*) genes differentiated the two closely related species *C. coccodes* and *C. nigrum*.

The newly generated sequences of all six loci of CGMCC 3.18943, originally described as *C. dianense* were identical to the ex-type strain of *C. nigrum.* However, the *GAPDH* and *CHS-1* sequences differed from the sequences of the same strain originally published by Zheng et al. (2022) by 1 bp each at the 3' and 5' ends, respectively, which may be a result of sequencing errors. The study by Zheng et al. (2022) was based on sequences of ITS, *ACT, GAPDH, CHS-1* and *TUB2* but there had been no *TUB2* sequences generated for *C. dianense*. A difference in spore size between *C. dianense* and *C. nigrum* as reported by Zheng et al. (2022) is not a sufficient morphological character to distinguish a new species (Cai et al., 2009). Given that the sequences of all six loci of the ex-type strains of *C. dianense* and *C. nigrum* are identical, and the phylogenetic analyses showed that the ex-type strain of *C. dianense* resided within the *C. nigrum* clade, *C. dianense* is regarded as a synonym

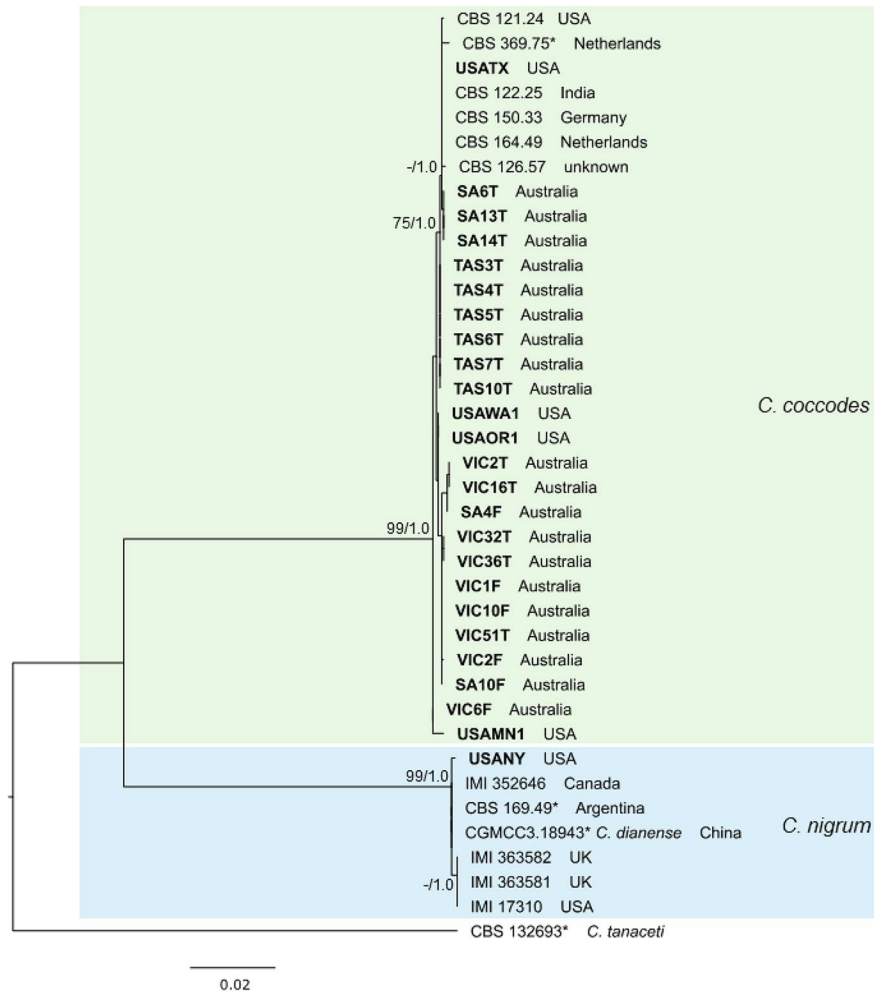

Fig. 1 Phylogenetic tree based on combined sequences of six loci (ITS, *TUB2*, *ACT*, *GAPDH*, *CHS-1*, *HIS3*) of *Colletotrichum coccodes* and *C. nigrum* isolates using Maximum Likelihood (ML) and Bayesian Inference (BI) with 1000 bootstrap replicates. ML bootstrap values above 70% and BI Posterior Probabilities above 0.9 are provided at the nodes (ML/BI). Node labels include isolate accession numbers and country of collection. Isolates sequenced in this study are indicted in bold. Ex-type strains are indicated by an asterisk. The tree was rooted with *C. tanaceti* (CBS 132693). The scale bar represents nucleotide substitutions per site

of *C. nigrum* based on the chronological order of publication.

Colletotrichum nigrum Ellis & Halst., New Jers. Agric. Exp. Sta. Bull.: 297 (1895).
= *Colletotrichum dianense* Z.F. Yu & H. Zheng, Journal of Fungi 8(1, no. 87): 11 (2022).

The three isolates from the USA (10%) that had been classified as *C. coccodes* by Alananbeth et al. (2014) and were identified as *C. nigrum* in this study belonged to VCG 6/7 (Gumstead, personal communication), while the other 27 isolates from the USA that belonged to other VCGs were verified as *C. coccodes* in this study, indicating a strong correlation between VCGs and the two species. In fact, 42 of the 465 isolates from the USA (9%) studied by Alananbeh et al. (2014) were classified as VCG 6/7. Similarly, 60% of the South African isolates were included in VCG 6/7. These isolates can probably all be regarded as *C. nigrum*.

One way analysis of variance of a pathogenicity bioassay based on inoculating non-wounded, detached leaves of potato cultivar Russet Burbank according to the method of Chang (2016) indicated significant differences between individual isolates in lesion sizes 6 days after inoculation (Table 1). There was no apparent grouping of isolates originating from asymptomatic leaf petiole tissue or infected tubers, or between geographic origins. The one *C. nigrum* isolate (USANY) produced relatively small lesions, but was still within the range of the *C. coccodes* isolates.

Although Manova et al. (2022) identified one isolate of *C. nigrum* from potato stolon tissue in a multigene phylogenetic analysis, only the ITS sequences were published hence the identification of the species cannot be reevaluated, and besides, the pathogenicity of that isolate was not assessed. Since foliar symptoms of potato black dot have not been reported in Australia, further studies are needed to confirm whether the pathogen which causes foliar damage in the USA and Israel is *C. coccodes* or *C. nigrum* (Johnson, 1994; Tsror et al., 1999). *Colletotrichum nigrum* was not detected among the 101 Australian isolates screened. This result was supported by the study of Alananbeh et al. (2014), in which none of the 86 isolates from Australia belonged to VCG 6/7.

In conclusion, the DNA sequences and pathogenicity on detached potato leaves of *C. coccodes* isolates from Australia and the USA were similar. The results further suggest that 9% of the isolates from potatoes in the USA, that had been identified as *C. coccodes* by Alananbeh et al. (2014) were in fact *C. nigrum*. *Colletotrichum coccodes* and *C. nigrum* can neither be identified based on morphological characteristics nor on ITS sequences. In future surveys, instead of using the ITS region, any of the other loci used in this study (*TUB2*, *ACT*, *GAPDH*, *CHS-1* or *HIS3*) may be used to develop species-specific diagnostic probes to quickly distinguish *C. coccodes* from *C. nigrum*. There was no difference in pathogenicity among the Australian *C. coccodes* isolates from tubers or from leaves, or between *C. coccodes* isolates from Australia and the USA. Foliar infection of plants grown in the field by *C. coccodes* may have developed due to specific environmental conditions predisposing plants to infection, combined with high inoculum pressure.

Table 1 Pathogenicity of 19 *C. coccodes* and the *C. nigrum* isolates in a detached potato leaf bioassay

Isolate	Tissue	Country of origin	Mean lesion diameter (mm)*
VIC10F	Foliar	Australia	1.87^{a}
TAS7T	Tuber	Australia	1.80^{a}
VIC51T	Tuber	Australia	1.77^{ab}
TAS3T	Tuber	Australia	1.74^{abc}
USAMN1	Unknown	USA	1.72^{abc}
VIC1F	Foliar	Australia	1.72^{abc}
VIC32T	Tuber	Australia	1.66^{abc}
USATX	Unknown	USA	1.50^{abcd}
VIC36T	Tuber	Australia	1.35^{bcde}
TAS10T	Tuber	Australia	1.35^{bcde}
TAS6T	Tuber	Australia	1.30^{cde}
VIC2T	Tuber	Australia	1.22^{de}
USAWA1	Unknown	USA	1.22^{de}
USAOR1	Unknown	USA	1.09^{de}
VIC6F	Foliar	Australia	1.07^{de}
VIC2F	Foliar	Australia	1.05^{e}
TAS4T	Tuber	Australia	1.04^{e}
USANY (*C. nigrum*)	Unknown	USA	0.98^{e}
VIC16T	Tuber	Australia	0.97^{e}
TAS5T	Tuber	Australia	0.95^{e}

*Each mean has a 95% confidence interval of ± 0.30 and the Least Significant Difference (p = 0.05) between any pair of means is 0.43 mm. Different letters indicate treatment means that are significantly different

Colletotrichum nigrum was shown to be a potential pathogen of potato leaves because it was able to infect and cause lesions on detached potato leaves. Further research is needed to assess, if *C. nigrum* is present in Australia, and if this is the case, then to assess the effect this species might have on yield and marketability of Australian potato varieties. The identification of two *Colletotrichum* species causing disease in potatoes has implications for determining genetic composition of populations for implementing disease control measures.

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Authors' contribution The study conception and design were made by Jiang Chang, Pedro Crous, Peter Ades and Paul Taylor. Material preparation, data collection and analysis were performed by Jiang Chang, Pedro Crous, Peter Ades, Weixia Wang, Niloofar Vaghefi, Fang Liu, Ulrike Damm and Paul Taylor. The first draft of the manuscript was written by Jiang Chang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The sequences of isolates used in the phylogenetic analysis are available in GenBank. Further data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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