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## SHORT RESEARCH NOTES

# DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman

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*Abstract.* A serious mango decline disease has recently been reported from the Sultanate of Oman. Based on morphological characteristics, the pathogen responsible for the disease was previously identified as *Ceratocystis fimbriata*. *C. fimbriata* is one of the most important pathogens of mango in Brazil and its appearance in Oman is of great concern. Recent phylogenetic studies have shown that *C. fimbriata* most likely represents a species complex. The aim of this study was to confirm the identity of the mango pathogen in Oman based on DNA sequences. Sequence data were obtained for the internal transcribed spacer 1 and 2 regions and the 5.8S rRNA gene regions and these were compared with sequence data of *C. fimbriata* from several hosts and geographic areas. The isolates from Oman where shown to represent *C. fimbriata sensu lato* and also to be most closely related to an isolate from mango in Brazil. This provides some evidence that the mango pathogen in Oman might have originated in Brazil.

*Ceratocystis fimbriata* is an important canker and wilt pathogen on a wide range of hosts. These include many fruit and plantation trees as well as several root crops (Kile 1993). *C. fimbriata* is a soil borne pathogen and it also produces a fruity odour facilitating dispersal by casual insects such as flies (Diptera) and picnic beetles (Coleoptera: Nitidulidae) (Himelick and Curl 1958; Upadhyay 1981; Kile 1993). The pathogen typically infects wounds on plants either through soil borne structures or via sexual or asexual spores carried to these wounds by insects.

Mango decline caused by *C. fimbriata* is one of the most serious diseases affecting production of *Mangifera indica* (mango trees) in Brazil (Batista 1960). In that country, *C. fimbriata* causes mango blight, also known as 'seca' or 'murcha' (Viegas 1960; De Toledo Piza 1966; Ribiero 1980). Symptoms are identical to those of Recife sickness, caused by *Diplodia recifensis* (Ploetz 2003). A Scolytid beetle, the *Hypocryphalus mangifera* (Coleoptera: Scolytidae) was identified by Ribiero (1980) to be the primary species responsible for the dissemination of *C. fimbriata* in Brazil. Until recently, mango disease caused by *C. fimbriata* was known only in Brazil.

*Ceratocystis fimbriata*, possibly in association with *Diplodia theobromae*, has recently been reported to cause mango decline in the Sultanate of Oman. In this situation, the disease is associated with the beetle *Cryphalus scabrecollis* (Coleoptera: Scolytidae) (Al-Adawi *et al*. 2003). Symptoms include dark staining of the wood that spreads from the points of infection, gum exudation from the trunks, wilting and browning of leaves on single branches, and eventually tree death. The disease severely threatens mango production in Oman and studies are currently underway to reduce its affect.

*Ceratocystis fimbriata* has a wide range of hosts and it has long been recognised as probably encompassing more than one taxonomic entity (Webster and Butler 1967). Contemporary DNA-based studies have reinforced this view, with some species initially described as *C. fimbriata* now

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clearly recognised as discrete taxa (Barnes *et al*. 2003; Van Wyk *et al*. 2004). The wilt pathogen of the forest plantation tree *Acacia mearnsii*, *Ceratocystis albifundus*, provides a relevant example (Wingfield *et al*. 1996). Contemporary DNA-based studies have shown that *C. fimbriata* represents discrete groups based on host specificity as well as geographical areas (Harrington 2000; Barnes *et al*. 2001; Baker *et al*. 2003).

Identification of *C. fimbriata* associated with mango decline in Oman was based solely on morphological characteristics (Al-Adawi *et al*. 2003). Although the morphological structures defining this species are reasonably well defined, these characteristics are insufficient to recognise emergent groupings within *C. fimbriata*. The aim of this study was to confirm the identity of *C. fimbriata* isolates from mango in Oman and to compare DNA sequences of the internal transcribed spacer (ITS) 1 and 2 regions and the 5.8S rRNA gene for these isolates with those from other hosts and geographic areas.

Isolates of *C. fimbriata* were obtained from wood taken from infected mango trees in the Al-Batinah region of Oman. Perithecia from primary isolations were induced by incubating infected tissue in moist chambers or by baiting through placing tissue between two slices of carrot (Moller and De Vay 1968). Pure cultures were obtained by lifting ascospore masses from the apices of perithecia developing on infected wood after incubation in moist chambers and transferring these to 2% malt extract agar (20 g/L malt, 20 g/L agar) (Biolab, Midrand, South Africa) and maintained at 25°C. Cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

DNA was extracted using a modified version of the technique described by Raeder and Broda (1985). Polymerase chain reaction (PCR) amplifications were conducted with the primer pairs, ITS1 and ITS4 (White *et al*. 1990) for the gene region ITS1, 5.8S, ITS2. The final reaction volumes of the PCR were adjusted to 25 µL with sterile water. The PCR mixture consisted of 5–10 ng of genomic DNA, 0.2 mM of dNTP, 0.2 µM of each primer, 1.75 U Expand High Fidelity PCR System enzyme mix (Roche Diagnostics, Mannheim, Germany) and 1× Expand HF Buffer containing 1.5 mM $MgCl_2$ (supplied with the enzyme). Amplifications were performed in a Mastercycler gradient thermal cycler (Eppendorf, Germany) using the following parameters: a 2-min step at 96°C, followed by ten cycles of 20 s at 94°C, 40 s at 55°C and 45 s at 72°C. The last three temperature intervals were repeated for another 30 cycles with a 5-s increase per cycle for the annealing step at 55°C, and then a final elongation step for 10 min at 72°C, and then a final 10 min at 72°C. Products were resolved by electrophoresis in a 2% agarose gel (Roche Diagnostics, Mannheim, Germany), stained with ethidium bromide. The PCR products were purified using 6% Sephadex columns, 1 g in 15 mL sterile water (Sigma, Steinheim, Germany).

For sequencing, the same primers were used as for the generation of the PCR products. Sequences were determined using an ABI PRISM 3100 Autosequencer (Applied BioSystems, Foster City, California, USA) and sequence data were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California). The sequences of the ITS region for the *Ceratocystis* spp. from mango trees were compared with those of morphologically similar *Ceratocystis* spp. obtained from GenBank (Fig. 1). Sequences were aligned manually and analysed using PAUP version 4.0b10* (Swofford 2002). The heuristic search was performed with 100 random addition sequence replications. Gaps were treated as a fifth character 'newstate'. Confidence intervals of branching points were determined using 1000 bootstrap replicates. The tree was rooted using *C. albifundis* as the out-group taxon.

The DNA sequence data for the ITS region provided support for the identification of *C. fimbriata* isolated from diseased mango trees in Oman. This is important as the fungus could easily be mistaken for morphologically similar sibling species such as *C. polychroma* (Van Wyk *et al*. 2004) or *C. pirilliformis* (Barnes *et al*. 2003). The three isolates from Oman (CMW 13851/AY953383, CMW 13852/AY953384 and CMW 13854/AY953385) grouped together in a clade with a bootstrap support of 91%.

The *C. fimbriata* isolates from diseased mango trees in Oman grouped most closely (bootstrap support, 55%) to an isolate from Brazil (CMW 14797/AY953382) that was also isolated from mango trees. Although a comparison with a greater number of isolates from mango would be desired, this does suggest that the fungus on mango in Oman and Brazil represent the same form of *C. fimbriata*. It is possible that the fungus was introduced into the Sultanate of Oman from Brazil by a route that has yet to be determined. More detailed studies of larger numbers of isolates using microsatellite markers are currently underway to consider this hypothesis.

Isolate CMW 15052 (AY157964), obtained from mango in Brazil grouped separately from the other isolates from mango used in this study. This isolate clustered together with isolate CMW 14812 (AY953386) from cacao in Brazil (90% bootstrap support). With the exception of the clade containing isolates CMW 15052 and CMW 14812, all other clades in the phylogenetic tree of *C. fimbriata* reflected either host specificity of isolates or geographical area of isolation, or both (Fig. 1). This supports the view that *C. fimbriata* is both host and geographically restricted (Harrington 2000). Isolates from coffee in Colombia (CMW 10844/AY177238, CMW 5746/AY953388 and CMW 5747/AY9533898, CMW 9555/AY177232) resided in two distinct clades as previously shown by Marin *et al*. (2003) and Barnes *et al*. (2002). These groups of isolates are not necessarily strictly host related, implying strong host specificity in some cases and not in

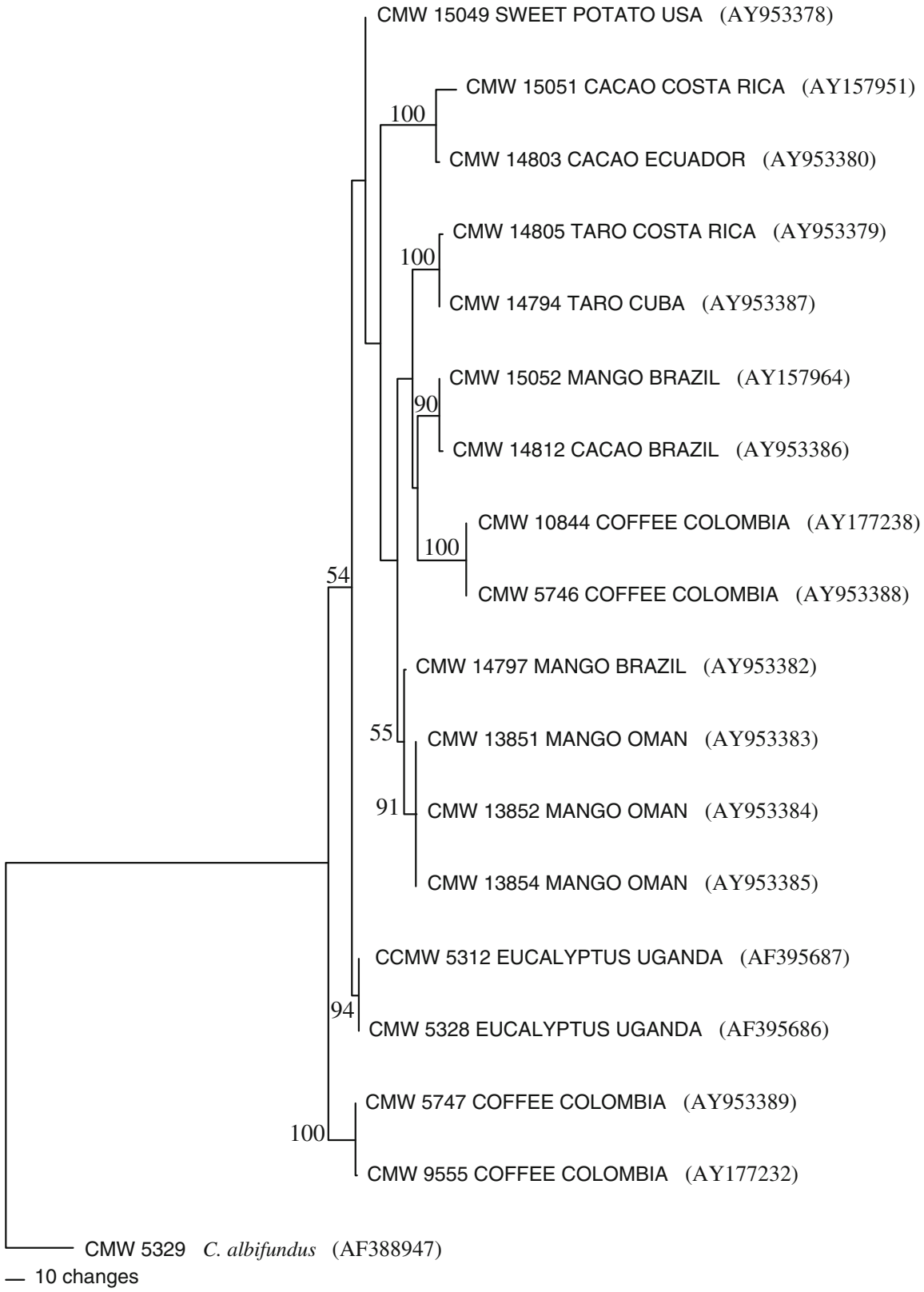


**Fig. 1.** Phylogenetic tree based on parsimony analysis of the ITS1–5.8S rRNA gene-ITS2 region of *Ceratosystis fimbriata*. Bootstrap values are indicated above the branches. *Ceratocystis albifundus* is used as the out-group taxon. GenBank accession numbers are indicated in brackets. The scale bar indicates 10 nucleotide changes.

others. This might also explain why one mango isolate from Brazil in this study was more closely related to an isolate from cacao than to others from mango.

The disease of mango in Oman that is apparently caused by *C. fimbriata* is extremely severe and has a very significant impact on mango production in the country. This study provides some evidence that the pathogen has been introduced into the country from Brazil, where it is also a serious pathogen. In Brazil, infections by *C. fimbriata* occur in association with the insect *Hypocryphalus mangifera*. In Oman, the disease is also closely associated with the insect *C. scabrecollis*. These insects appear to have a similar biology and their association with *C. fimbriata* has clearly given rise to serious disease problems.

Management of the mango disease associated with *C. fimbriata* in Oman is receiving attention. The best possible option to reduce the affect of the disease will be through planting disease resistant tress. In this regard, an understanding of the population biology of the pathogen in Oman will be important and research on the origin and population biology of *C. fimbriata* in this country is currently underway.

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