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Ceratocystis omanensis, a new species from diseased mango trees in Oman

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ABSTRACT

Mango (*Mangifera indica*) sudden decline is an important disease in Oman, which is closely associated with infections by *Ceratocystis fimbriata* and *Lasiodiplodia theobromae*. Another *Ceratocystis* species has also been found associated with symptoms on diseased trees. In this study, we identify that *Ceratocystis* based on morphology and DNA sequences. Morphological comparisons showed that the fungus from dying mango trees in Oman is similar to *C. moniliformis*. Both fungi have distinct hat-shaped ascospores, disc-shaped plates at the bases of the ascomatal necks and spines on the ascomatal bases. However, comparison of DNA sequences for ITS1-2, the 5.8S RNA gene, the β-tubulin gene, and Transcription Elongation Factor (EF1-α) gene, confirmed that the fungus from Oman is distinct from *C. moniliformis* and other related species. Phylogenetically, this fungus formed one of four strongly supported sub-clades. The other sub-clades included isolates of *C. bhutanensis*, *C. moniliformis* and *C. moniliformopsis*, respectively. Based on morphological characteristics and differences in DNA sequences for three gene regions, we conclude that the *Ceratocystis* sp. from wounds on mango in Oman is a new species, for which we provide the name *Ceratocystis omanensis* sp. nov.

Introduction

During 1999, a serious disease resulting in the decline of mango (*Mangifera indica*) appeared in Oman. This disease has affected up to 60 % of trees in parts of the Al Batinah region. Symptoms on trees include gum exudation from the trunks, wilting and eventual browning of leaves on single branches. Tree death occurs about six months after the first appearance of symptoms. The wood is stained dark brown, spreading from the point of infection. Damage caused by the ambrosia beetle *Cryphalus scabrecollis* (*Coleoptera: Scolytidae*; IIE no. 24214) is frequently associated with the disease (Al-Adawi 2002; Al-Adawi *et al.* 2003).

The cause of Mango decline in Oman has not yet been resolved, but various potential pathogens have been identified. These include a fungus tentatively identified as *Ceratocystis fimbriata* and *Lasiodiplodia theobromae* (Al-Adawi 2002). Isolates of another *Ceratocystis* sp. were commonly recovered from

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wounds on Mango trees suffering from decline. The aim of this study was to identify this fungus based on morphological characters, and comparisons of sequence data from the 5.8S rRNA operon including the ITS regions (ITS1,2), the β-tubulin gene, and Transcription Elongation Factor (EF1-α) gene.

Materials and methods

Sampling and fungal isolation

Stem cross-sections were made on 50 mango trees during April and May 2003 in the Al-Batinah region of Oman. These were incubated in a moist environment to promote fungal growth. Ascomata typical of *Ceratocystis* developed on these wood samples after one week, and isolations were made from these structures. Ascospore droplets at the apices of the ascomatal necks were transferred to 2 % Malt Extract Agar (MEA) (20 % w/v) (Biolab, Midrand, SA). Ascospore masses were transferred from primary isolation plates to 2 % MEA supplemented with Streptomycin sulphate (0.001 $gvol^{-1}$; Sigma, Steinheim, Germany) and thiamine (0.001 $gvol^{-1}$; Sigma) to obtain pure cultures and to encourage sporulation. All isolates used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht (Table 1). The holotype specimen as well as paratypes of the *Ceratocystis* from Oman, consisting of dried down cultures of isolates (CMW 11048, CMW 11046 and CMW 11050, respectively), on 2 % MEA, have been lodged with the National Collection of Fungi (PREM), Pretoria (Table 1).

Morphological characteristics

Three isolates (CMW 3777, CMW 11047 and CMW 11048) were randomly chosen from a larger collection to assess growth characteristics in culture. Single ascospore masses were transferred to 2 % MEA and incubated at 25 °C for one week. Mycelial discs were taken from the edges of vigorously growing cultures using a 5 mm cork borer and a single disc was transferred to the centres of 90 mm Petri dishes containing 2 % MEA. Five Petri dishes for each isolate were incubated at 4, 10, 15, 20, 25, 30 and 35 °C, respectively. Colony diameter was measured every 24 h for each culture by taking two diameter measurements at right angles to each other. Averages were computed for all growth measurements. The experiment was repeated once and differences in growth rate for the cultures were analysed statistically.

Eight day-old cultures were used for morphological comparisons made using a Zeiss Axioplan 2 light microscope (Carl Zeiss, Heidenheim). A Zeiss Axio Vision camera system was used to photograph images. Colour descriptions were determined using the colour charts of Rayner (1970). Measurements were taken for each taxonomically relevant character; fifty random measurements were taken for isolate CMW 11048, and ten random measurements were taken for isolates CMW 11046, CMW 11050 and CMW 11056 to corroborate the measurements for the type specimen. Averages, ranges and standard deviations of the measurements were computed. The measurements are given in the format: (minimum-) mean minus standard deviation – mean plus standard deviation (-maximum).

DNA extraction and PCR amplification

Total DNA was extracted from five isolates (Table 1) of the *Ceratocystis*. A single ascospore mass for each isolate was transferred to 2 % MEA and incubated at 25 °C. After eight days, mycelium, including ascomata and spores, was scraped from the agar surface using a sterile scalpel blade and placed into 1.5 ml Eppendorf tubes. The tubes containing the mycelium were lyophilised. The lyophilised mycelium was placed in liquid nitrogen and ground to a powder using a glass rod. DNA was extracted using the method described by Barnes *et al.* (2001).

Primer pairs ITS1 and ITS4 (White *et al.* 1990) were used to amplify the entire ITS region (ITS1 and ITS2) including the 5.8S gene of the ribosomal DNA (rDNA) operon at an annealing temperature of 50 °C. The β-tubulin gene was partially amplified using the primers βt1a and βt1b at an annealing temperature of 52 °C (Glass & Donaldson 1995). Primers EF1-728F and EF1-986R were used to amplify the EF1-α gene of the rDNA operon at an annealing temperature of 56 °C (Carbone & Kohn 1999).

Polymerase chain reaction (PCR) was performed in 25 µl reaction volumes, containing 5–10 ηg 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), 1 × Expand HF Buffer containing 1.5 mM $MgCl_2$ (supplied with the enzyme) and reaction volumes were adjusted with sterile water. Amplifications were performed in Mastercycler gradient thermal cycler (Eppendorf, Perkin-Elmer, Germany) using the following parameters: a 2 min step at 96 °C, followed by 10 cycles of 20 s at 94 °C, 40 s at x °C (x = annealing temperature specified for each set of primers) 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 followed by an elongation step at 72 °C, and then a final 10 min at 72 °C. The resulting products were resolved by electrophoresis in a 2 % agarose gel (Roche Diagnostics), stained with ethidium bromide. The PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics).

DNA sequencing and analysis

DNA sequencing was performed on both strands using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, CA). The primers ITS1, ITS4, βt1a, βt1b, EF1-728F and EF1-986R used for DNA amplification were also used for sequencing. Sequences were determined using an ABI PRISM™ 3100 Autosequencer (Applied BioSystems) and sequence data were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems).

The sequences of the ITS region, β-tubulin gene and EF1-α gene for the *Ceratocystis* sp. from mango trees were compared with those of morphologically similar *Ceratocystis* species (Table 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 fifth character state. To

Table 1 – List of *Ceratocystis* isolates used in this study

Species	Isolate no.[d]	Alternative numbers[e]	GenBank accession no.	Year of isolation	Host	Geographical origin	Associated insect	Collector(s)
C. moniliformis	CMW 9590[a,c]	CBS 116452	AY528985[f] AY528996[g] AY529006[h]	2002	*Eucalyptus grandis*	Mpumalanga, South Africa	None	*J. Roux*
C. moniliformis	CMW 4114[a]	None	AY528986[f] AY528997[g] AY529007[h]	1997	*Schizolobium parahybum*	Ecuador, South America	None	*M. J. Wingfield*
C. moniliformopsis	CMW 9986[a]	CBS 109441	AY528987[f] AY528998[g] AY529008[h]	1999	*Eucalyptus obliqua*	Tazmania, Australia	None	*Z. Q. Yuan*
C. moniliformopsis	CMW 10214[a]	CBS 115792	AY528988[f] AY528999[g] AY529009[h]	1989	*Eucalyptus sieberi*	Victoria, Australia	None	*M. J. Dudzinski*
C. bhutanensis	CMW 8242[a]	CBS 112907 PREM 57809	AY528951[f] AY528956[g] AY528961[h]	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	*T. Kirisits & D. B. Chhetri*
C. bhutanensis	CMW 8217[a]	CBS 114289 PREM 57807	AY528952[f] AY528957[g] AY528962[h]	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	*T. Kirisits & D. B. Chhetri*
C. bhutanensis	CMW 8215[a]	CBS 114290 PREM 57805	AY528953[f] AY528958[g] AY528963[h]	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	*T. Kirisits & D. B. Chhetri*
C. bhutanensis	CMW 8399[a]	CBS 115772	AY528954[f] AY528959[g] AY528964[h]	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	*T. Kirisits & D. B. Chhetri*
C. bhutanensis	CMW 8396[a]	CBS 114286 PREM 57812	None	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	*T. Kirisits & D. B. Chhetri*
C. omanensis	CMW 11050[b]	PREM57816	None	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	*A. O. Al-Adawi*

(continued on next page)

Table 1 – (continued)

Species	Isolate no.[d]	Alternative numbers[e]	GenBank accession no.	Year of isolation	Host	Geographical origin	Associated insect	Collector(s)
C. omanensis	CMW 11056[b]	None	None	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. omanensis	CMW 3777[a,c]	None	DQ074740[f] DQ074730[g] DQ074735[h]	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. omanensis	CMW 11047[a,c]	None	DQ074741[f] DQ074731[g] DQ074736[h]	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. omanensis	CMW 11048[a,b,c]	CBS 115780 PREM 57815	DQ074742[f] DQ074732[g] DQ074737[h]	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. omanensis	CMW 3800[a]	None	DQ074743[f] DQ074733[g] DQ074738[h]	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. omanensis	CMW 11046[a,b]	CBS 118112 PREM57814	DQ074739[f] DQ074729[g] DQ074734[h]	2003	*Mangifera indica*	Al Batinah, Oman	*Cryphalus scabrecollis*	A. O. Al-Adawi
C. virescens	CMW 3276[a]	None	AY528984[f] AY528990[g] AY528991[h]	1963	*Quercus* sp.	Warrenber, USA	None	T. Hinds

a Isolates sequenced.
b Isolates used for morphological descriptions.
c Isolates in the growth studies.
d CMW, the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.
e CBS, Centraalbureau voor Schimmelcultures, Utrecht; and PREM, National Fungal Herbarium (PREM), Pretoria.
f GenBank accession nos of the ITS sequences.
g GenBank accession nos of the β-tubulin sequences.
h GenBank accession nos of the elongation factor sequences.

determine the confidence intervals of branching points, 1000 bootstrap replicates were used. The tree was rooted using *C. virescens* as the out-group taxon (Table 1). A partition homogeneity test (Swofford 2002) was used to evaluate the possibility of combining the data for the three gene regions.

Results

Sampling, fungal isolation and morphological characteristics

In all, 22 morphologically similar isolates of a *Ceratocystis* sp. were collected from diseased mango trees. The isolates chosen for detailed examination (CMW 3777, CMW 11047 and CMW 11048) grew rapidly in culture. The optimal temperature range for growth was 25–30 °C. Minimal growth was observed at 10 °C with no growth occurring at 4 °C. At 30 °C, cultures reached an average diam of 80 mm within 3 d. At 35 °C cultures reached an average of 60 mm in 3 d. These growth parameters are very different to those obtained for *C. moniliformis*, *C. moniliformopsis* and *C. bhutanensis*. The optimum temperature ranges for growth of *C. moniliformis*, *C. moniliformopsis* and *C. bhutanensis* were 20–25 °C, 15–20 °C, and 20–25 °C respectively. *C. bhutanensis* was the only species that grew at 4 °C, while the *Ceratocystis* sp. from Oman was the only species able to grow at 35 °C.

C. omanensis is homothallic, as all the isolates produced perithecia. The ascomatal bases were globose, black and covered with short spines (Fig 1a,d). The ostiolar hyphae were

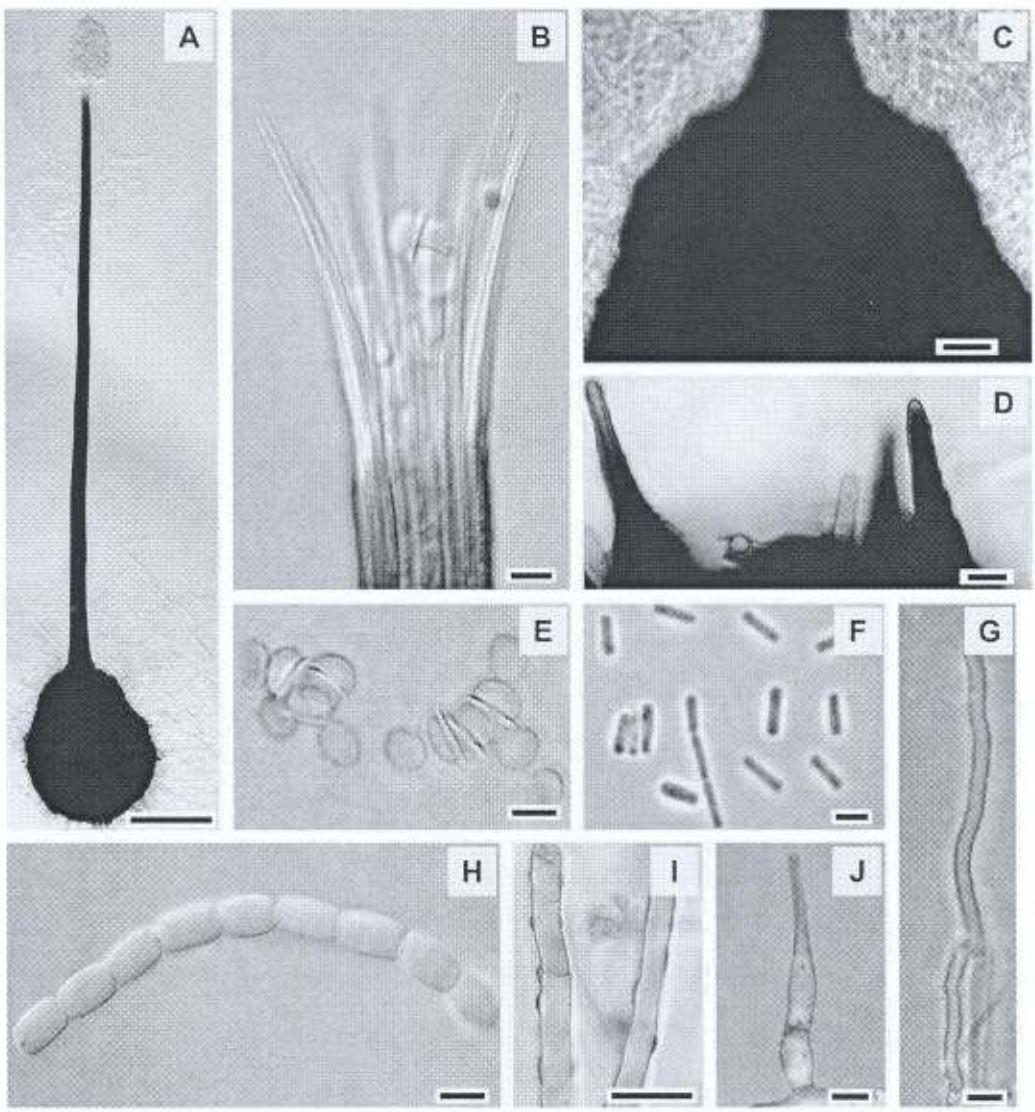

Fig 1 – *Ceratocystis omanensis* (CMW 11048). (A) Ascomata; (B) ostiolar hyphae; (C) ascoma neck base; (D) Conical spines on the bases of ascomata; (E) hat-shaped ascospores in side view; (F) cylindrical conidia; (G) smooth-edged hyphae with septa; (H) barrel-shaped conidia in a chain; (I) rough edged hyphae; and (J) phialide. Bars: A = 100 μm; C = 20 μm; G & I = 10 μm; and B, D F & J = 5 μm.

divergent (Fig 1b) and the bases of the necks disc-shaped, similar to those of *C. moniliformis* (Fig 1c). The ascospores were the characteristically hat-shaped (Fig 1e). Conidiophores (Fig 1j) were typical of *Thielaviopsis* anamorphs of *Ceratocystis* with cylindrical conidia (Fig 1f). Barrel-shaped conidia were also present (Fig 1h). Two types of hyphae were present, one with smooth walls (Fig 1g) and another with a granular appearance (Fig 1i).

Perithecial necks in the Oman fungus were consistently shorter (434–596 μm) than those described by Hunt (1956) for *C. moniliformis* (900 μm), and *C. moniliformopsis* (480–780 μm) (Yuan & Mohammed 2002). Neck lengths were within the same range as those of *C. bhutanensis* (453–519 μm) (Van Wyk *et al.* 2004), and another description of *C. moniliformis* by Upadhyay (1981) (550–1000 μm).

Based on characteristics in culture, the species from Oman was distinct from *C. moniliformis*, *C. moniliformopsis*, and *C. bhutanensis*. *C. moniliformis* has a white to grey/black (Hedgcock 1906) cultural appearance, while *C. moniliformopsis* has white to grey centres, which become green (Yuan & Mohammed 2002), *C. bhutanensis* has cream-buff to dark olive to black cultures (Van Wyk *et al.* 2004), while the *Ceratocystis* sp. from mango in Oman has white to wood-brown cultures.

DNA extraction PCR amplification and analysis of sequence data

Successful amplification of the three gene regions for the *Ceratocystis* sp. from Oman resulted in amplicons of ~500 bp, ~500 bp and ~300 bp for the ITS, β-tubulin and EF1-α genes, respectfully.

The partition homogeneity tests for three sequence data sets (ITS, β-tubulin and EF1- α) gave a value ($P = 0.46$) greater than the minimum required to combine the data. The three sequence datasets were thus combined. The total length of the sequence for the three gene regions was 1398 characters, including gaps. The tree length was 667, and the dataset contained 866 constant characters, 369 uninformative characters and 163 informative characters. The tree statistics were: CI = 0.9353, HI = 0.0647, RI = 0.8822, and RC = 0.8251.

A heuristic search resulted in four well-resolved trees, one of which (Fig 2) was chosen for presentation. Four clades were obvious in the phylogenetic tree that included *Ceratocystis* spp. with similar morphological features. The five isolates of the *Ceratocystis* sp. from diseased mango trees in Oman resided in one of these subclades, with strong (100 %) bootstrap support. The other subclades included isolates of *C. bhutanensis*, *C. moniliformis* and *C. moniliformopsis*, respectively (Fig 2). These data strongly support the morphological observations suggesting that the Oman fungus represents an undescribed species. Thus, both phylogenetic comparisons and comparisons based on morphology support the view that this fungus represents a new species which we describe here.

Taxonomy

Ceratocystis omanensis Al-Subhi, M. J. Wingf., M. Van Wyk & Deadman, **sp. nov.** (Fig 1)

Etym.: '*omanensis*' refers to the country in which the fungus was collected.

Coloniae juvenes albae, seniores lignum brunneum contaminantes. *Mycelium* in medium immersum, mycelium aerium album vel lignicolor adest. *Crescit* optime ad 30–35 °C, ad 4 °C non crescit. *Hyphae* leves vel granulatae, in septis non constrictae, (2–)3–5(–7) μm latae. *Bases ascomatarum* atrobrunneae vel nigrae, globosae, spinis hyphisque ornatis, spinis atrobrunneis vel nigris, (4–)9–19(–26) μm, bases (154–)206–254(–279) μm diametro. *Colla ascomatarum* basi atrobrunnea vel nigra, apicem versus laete brunnescentia, (385–)443–819(–1097) μm longa, basi (30–)43–57(–64) μm, apice (14–)16–22(–26) μm lata, basi discoideo. *Hyphae* ostiolares divergentes, hyalinae, (10–)18–36(–50) μm longae. *Ascos* non vidi. *Ascosporae* lateraliter visae culculatae, aseptatae, hyalinae, vagina investitae, cum vagina 2–4 × 5–7 μm, sine illa 2–4 × 4–6 μm. Ascosporae in apicibus collorum ascomatarum in massis mucosis fulvoluteis convenientes. *Anamorpha Thielaviopsis*: coniciophorae singuli in mycelio crescentes, hyalinae, basi tumidae, apicem versus angustatae, (19–)22–36(–56) μm longae, basi (1–)2–4(–5) μm, apicibus 1–3 μm latae. Evolutio *conidii* phialidici per parietes annulares faciendas, *conidia* in catenas biformibus facta: conidia primaria hyalina, aseptata, cylindrica 6–8(–9) × 2–3 μm; conidia secondaria hyalina, aseptata, doliiformia, (5–)6–8(–10) × 3–5 μm.

Typus: **Oman**: Sohar, isolated from stem of *Mangifera indica* affected with decline disease, in association with the insect *Cryphalus scabrecollis*, April 2003, *A. O. Al-Adawi & M. L. Deadman* (PREM 57815 – **holotypus**; cultura viva ex tipo – CMW 11048, CBS 115787).

Colonies white when young, turning wood brown (17‴) when older (Rayner 1970). *Mycelium* submerged in medium, white to wood brown (17‴) aerial mycelium present. *Optimal temperature* range for growth 30–35 °C, with no growth at 4 °C. *Hyphae* smooth or granulated, not constricted at septa, (2–)3–5(–7) μm wide. *Ascomatal bases* dark brown to black, globose, ornamented with spines and hyphae, spines dark brown to black, (4–)9–19(–26) μm long, bases (154–)206–254(–279) μm in diam. *Ascomatal necks* dark brown to black at base, becoming light brown towards the apex, (385–)443–819(–1097) μm long, (30–)43–57(–64) μm wide at the base, (14–)16–22(–26) μm wide at the apex, with a disc-like base. *Ostiolar hyphae* divergent, hyaline, (10–)18–36(–50) μm long. *Asci* not observed. *Ascospores* cucullate in side view, aseptate, hyaline, invested in sheath, 2–4 × 5–7 μm with sheath, 2–4 × 4–6 μm without sheath. *Ascospores* accumulating in buff-yellow (19 d) mucilaginous masses on the apices of ascomatal necks. *Thielaviopsis anamorph* conidiophores occurring singly on mycelium, hyaline swollen at the base, tapering towards the apex, (19–)22–36(–56) μm long, (1–)2–4(–5) μm wide at base, 1–3 μm wide at the apices. *Conidium development* phialidic through ring wall building, *conidia* formed in chains of two types: cylindrical conidia hyaline, aseptate, 6–8(–9) × 2–3 μm, barrel-shaped conidia hyaline, aseptate, (5–)6–8(–10) × 3–5 μm.

Additional specimens examined: **Oman**: Sohar, isolated from stem of *Mangifera indica* affected with decline disease in association with *Cryphalus scabrecollis*, April 2003, *A. O. Al-Adawi & M. L. Deadman* (PREM 57814; culture CMW 11046, CBS 118112; PREM 57816; culture CMW 11050).

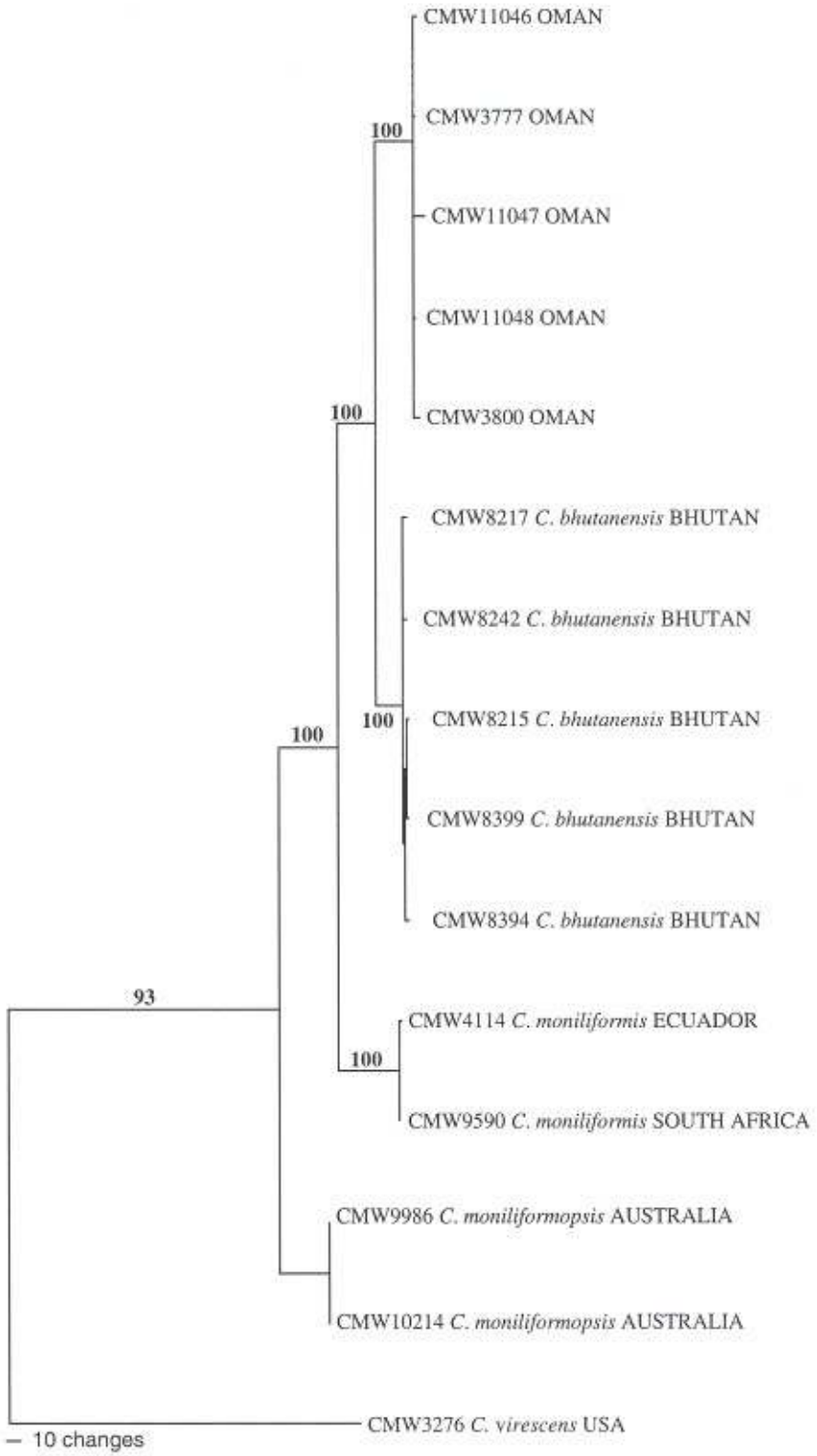

Fig 2 – Phylogenetic tree constructed by heuristic search for the combined sequence data of the ITS region, β-tubulin gene and EF-1α gene. (TL = 1399 bp, CI = 0.9353, HI =0.0647, RI = 0.8822 and RC = 0.8251). The values of bootstrap are indicated above the branches. *Ceratocystis virescens* was used as the out-group.

Discussion

In this study we describe the new species *Ceratocystis omanensis* from declining mango trees in Oman. Recognition of the fungus as a new taxon is based on both morphological and cultural characteristics, as well as differences in DNA sequence data. To the best of our knowledge, this is the first new *Ceratocystis* sp. to be discovered in Oman.

C. omanensis is morphologically most similar to *C. moniliformis*, *C. moniliformopsis*, and *C. bhutanensis*. It can, however, be distinguished from these species based on a number of phenotypic characters. There are two types of hyphae present in *C. omanensis*, with granular and smooth walls, as seen in *C. bhutanensis* (Van Wyk *et al.* 2004), but absent in both *C. moniliformis* and *C. moniliformopsis*. The average length of the perithecial necks of *C. bhutanensis* and *C. omanensis* is within the same size range but they are markedly shorter than those of *C. moniliformis* (Hedgcock 1906) and *C. moniliformopsis* (Yuan & Mohammed 2002).

C. omanensis is able to grow well at 35 °C, which is higher than is true for the other related species. The optimum temperature for most species of *Ceratocystis* is in the range of 23–27 °C and growth is usually inhibited at 35 °C (Upadhyay 1981; Yuan & Mohammed 2002; Van Wyk *et al.* 2004). *C. bhutanensis*, *C. moniliformis*, and *C. moniliformopis*, are characterised by disc-shaped plates at the bases of the ascomatal necks, and conical spines on the bases of the ascomata (Davidson 1935; Nag Raj & Kendrick 1975; Upadhyay 1981; Yuan & Mohammed 2002; Van Wyk *et al.* 2004). *C. omanensis* shares these characteristics. *C. omanensis* also produces a fruity aroma in culture but this turns to a fermented aroma when cultures age (2 wk). In contrast, *C. moniliformis* and *C. moniliformopsis* produce a fruity banana-oil odour, and *C. bhutanensis* produces a putrid odour that appears not to change in the same way with time (Yuan & Mohammed 2002; Van Wyk *et al.* 2004).

The various overlapping morphological characteristics in *C. omanensis* and other similar species of *Ceratocystis* illustrate the taxonomic limitations of morphological characterisation. For example, when *C. omanensis* is grown on 2 % MEA, it has globose bases ranging from 206–254 µm diam, within the range reported for *C. fimbriata*, *C. albifundus* and *C. moniliformis* (Wingfield *et al.* 1996; Grylls & Seifert 1993). Luc (1952) described four different forms of *C. moniliformis* based on small morphological differences. It would be interesting to know whether the isolates considered by Luc represent variation within *C. moniliformis* or whether they really reflect cryptic species. Apart from *C. omanensis*, hat-shaped ascospores occur in eight species of *Ceratocystis*. These include *C. fimbriata* (Upadhyay 1981), *C. acericola* (Grylls & Seifert 1993), *C. moniliformis* (Davidson 1935), *C. albifundus* (Wingfield *et al.* 1996), *C. moniliformopsis* (Yuan & Mohammed 2002), *C. pirilliformis* (Barnes *et al.* 2003) and *C. bhutanensis* (Van Wyk *et al.* 2004). Thus, although there are some morphological differences between *C. omanensis* and its close relatives, it would be difficult to rely on these for definitive identification.

While morphological differences were difficult to define for *C. omanensis*, this fungus is clearly distinct based on DNA sequence comparisons. The phylogenetic analyses using sequences of the three gene regions showed clearly that this fungus is distinct from *C. moniliformis*, *C. moniliformopsis* and *C. bhutanensis*. In terms of *Ceratocystis* as a whole, the genus can be separated into two main groups, the *C. coerulescens* group and the *C. fimbriata* group (Witthuhn *et al.* 1999; Paulin-Mahady *et al.* 2002). Phylogenetically, *C. omanensis* is most closely related to species in the *C. coerulescens* group, jointly with *C. bhutanensis*, *C. moniliformis* and *C. moniliformopsis*.

C. omanensis is the second species to be recorded from mango trees. The other, *C. fimbriata* was recently reported associated with Mango decline disease in Oman (Al-Adawi 2002; Al-Adawi *et al.* 2003). This fungus is a pathogen of mango trees in Brazil (Ribeiro *et al.* 1986; Ploetz & Prakash 1997) and it is probable that it contributes to mango decline in Oman. Isolates used in the present study did not include the isolate linked to the report of *C. fimbriata* killing mango trees in Oman. Clearly, pathogenicity tests with both *C. omanensis* and *C. fimbriata*, as well as with *Lasiodiplodia theobromae* are required to determine their respective roles in mango death in Oman.

C. omanensis is similar to other species of *Ceratocystis* in producing slimy droplets of spores from the apices of long beaked ascomata and in producing fruity aromas. Both of these factors are important in facilitating dispersal by insects (Hanssen 1993; Christen *et al.* 1997; Soares *et al.* 2000). Trees usually require wounds for the initiation of infection by these fungi (De Vay *et al.* 1963; Teviotdale & Harper 1991; Roux *et al.* 2000) and these wounds are usually visited by insects that transmit the fungal spores (Crone & Bachelder 1961; Hinds 1972). The insect identified as a species of *Cryphalus* (*Coleoptera*: *Scolytidae*) associated with infections on mango in Oman, is closely associated with mango decline disease in Oman (Al-Adawi 2002; Al-Adawi *et al.* 2003). The role of this insect in transmitting the agent of Mango decline is not known, but it has been shown to carry hat shaped ascospores similar to those of *C. omanensis* and *C. fimbriata* (Al-Adawi *et al.* 2003). The relationship between these insects and *C. omanensis* and the two fungi requires further investigation.

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