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Four new *Ceratocystis* spp. associated with wounds on *Eucalyptus, Schizolobium* and *Terminalia* trees in Ecuador

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Abstract Species of *Ceratocystis* commonly infect wounds on trees. In this study, artificially induced wounds were made on the stems of *Eucalyptus*, *Schizolobium* and *Terminalia* trees in Ecuadorian lowland forests, in an effort to determine the presence of *Ceratocystis* spp. in that environment. Species belonging to the *C. fimbriata sensu lato* (*s.l.*) complex and others in the *C. moniliformis s.l.* complex were collected. Phylogenetic analyses for both major groups in *Ceratocystis* using three gene regions (ITS, β-tubulin and EF1-α) revealed three distinct clades in the *C. fimbriata s.l.* complex and two in the *C. moniliformis s.l.* complex. Isolates in the three clades representing the *C. fimbriata s.l.* complex represent morphologically distinct species that are described here as *C. curvata* sp. nov., *C. ecuadoriana* sp. nov. and *C. diversiconidia* sp. nov. Isolates in one of the two clades in the *C. moniliformis* species complex represented *C. moniliformis sensu stricto* (*s.s.*) and the other was of a species with a distinct morphology that is described here as *C. sublaevis* sp. nov.

Keywords Fungal phylogenetics · Tree disease · Wounds

Introduction

Ceratocystis spp. are perithcial Ascomycetes that commonly infect wounds or the cut surfaces of recently felled trees (Kile 1993; Barnes et al. 2003; Van Wyk et al. 2009). They are transferred to these infection sites by sap-feeding insects such as nitidulid beetles (Juzwik 1999). In addition, a small number of *Ceratocystis* species are associates of conifer-infesting bark beetles (Wingfield et al. 1997).

Some species of *Ceratocystis* are important pathogens of fruit and forest trees or root crops (Kile 1993). In addition, many species are weakly pathogenic or saprophytes causing sap stain on timber. Most species that are weakly pathogenic or saprophytic reside in the *C. moniliformis* (Hedgc.) C. Moreau species complex (Hedgcock 1906; Van Wyk et al. 2006a) and some of the species related to *C. coerulescens* (Münch) B.K. Bakshi also cause sap stain particularly in conifers (Wingfield et al. 1997; Witthuhn et al. 1998). Species in the *C. fimbriata* Ellis & Halst. *s.l.* complex include important pathogens of trees causing vascular wilt and canker stain diseases (Roux et al. 1999; Engelbrecht et al. 2007; Roux and Wingfield 2009).

Early studies treated *Ceratocystis moniliformis* and *C. fimbriata* as single species although it was recognised that *C. fimbriata* included substantial variability (Webster and Butler 1967). In recent years, numerous cryptic species that would previously have been accommodated in *C. moniliformis* have been described (Yuan and Mohammed 2002; Van Wyk et al. 2004, 2006a). Likewise, *C. fimbriata s.l.* accommodates a relatively large number of cryptic taxa (Van Wyk et al. 2004; Engelbrecht and Harrington 2005; Johnson et al. 2005) with *C. fimbriata s.s.* defined by isolates phylogenetically related to those from sweet potato in the USA (Engelbrecht and Harrington 2005). It is likely that DNA sequence phylogenies and other emerging species concepts will reveal many other cryptic species in this group.

In Ecuador, plantation forestry is based largely on *Eucalyptus* spp. (~50%) and *Pinus* spp. (~40%) but various other trees are being tested for their potential as plantation species. These include the non-native *Terminalia ivorensis*

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A. Chev. and the native *Schizolobium parahyba* (Vell.) S.F. Blake. One of the key considerations in evaluating species for plantation development in Ecuador concerns their health and in this regard, various disease problems have been encountered (Geldenhuis et al. 2004; Lombard et al. 2008). In a previous study, *C. fimbriata s.l.* and *C. moniliformis* were isolated from machete wounds on diseased *S. parahyba* in Ecuador (Geldenhuis et al. 2004). Given the importance of members of the *C. fimbriata s.l.* complex as tree pathogens (Kile 1993; Roux et al. 2004; Heath et al. 2009; Roux and Wingfield 2009), there has been concern that these fungi could present constraints to forestry in Ecuador.

The tropical environment in areas of Ecuador where hardwood species are grown, results in a lush vegetation and considerable competition due to rapidly growing understory plants. These are typically cleared manually using machetes and physical wounds are common at the bases of trees. These wounds present ideal sites for infection by species of *Ceratocystis s.l.* Therefore, this study was conducted to determine which of these fungi might be present in the local environment. This was done by artificially wounding tree stems and collecting *Ceratocystis* spp. for identification.

Materials and methods

Isolates

Wounds were made on the stems of *Eucalyptus deglupta* Blume, *Terminalia ivorensis* and *Schizolobium parahyba* trees in Ecuador at three different sites and times. The first wounding trial was conducted on trees growing on the farms Rio Silanche and La Celica near Salinas in March 2004, where wounds were made on *E. deglupta* trees. A second trial was conducted at Rio Pitzara near Salinas during November 2005 and in this case, wounds were made on *E. deglupta* and *T. ivorensis* trees. A third trial at Rio Pitzara was conducted during February 2006 where wounds were induced on *S. parahyba* trees. Wounds were made on the stems of trees using a machete and were similar to those described by Barnes et al. (2003).

Pieces of wood were collected from the treated trees approximately 4 weeks after wounding and fungi were isolated directly from structures on the wood surface or through carrot baiting (Moller and De Vay 1968). Spore droplets were removed from ascomatal necks, transferred to 2% (*w*/*v*) malt extract agar (MEA) (Biolab, Midrand, South Africa) supplemented with Streptomycin Sulphate (0.001 g vol^{-1}, SIGMA, Steinheim, Germany) and incubated at 25°C until cultures sporulated. All isolates collected in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and representative isolates have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Material consisting of dried cultures on 2% MEA of representative isolates were lodged with the National Fungal Herbarium (PREM), Pretoria, South Africa.

DNA sequence comparisons

DNA was extracted from selected isolates as described by Van Wyk et al. (2006b). PCR conditions and reactions for three gene regions; the Internal Transcribed Spacer (ITS) regions 1 and 2 including the 5.8S rRNA operon, part of the Beta tubulin (βt) gene and part of the Transcription Elongation Factor 1-alpha (EF1-α), were as described by Van Wyk et al. (2006b). The primers utilized in these PCR reactions were ITS1 & ITS4 (White et al. 1990) for the ITS region, βt1a & βt1b (Glass and Donaldson 1995) for the βt gene region and EF1F & EF1R (Jacobs et al. 2004) for the EF1-α gene region. Purification of these amplified products was achieved with 6% Sephadex G-50 columns (Steinheim, Germany). The amplicons were sequenced in both directions using the ABI PRISM$_{TM}$ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California) as described in previous studies (Van Wyk et al. 2006b).

Sequence and phylogenetic analyses

The chromatographs were analysed using the freeware Cromas Lite 2.01 (www.technelysium.com.au). All isolates were subjected to ITS PCR and sequences were submitted to BLAST on NCBI (http://www.ncbi.nlm.nih.gov) for initial identification before they were selected for further sequencing. The sequences were subsequently divided into two groups based on the results of these analyses. They either grouped in the *C. fimbriata s.l.* complex or in the *C. moniliformis s.l.* complex. Sequence data for closely related species were taken from previous studies (Van Wyk et al. 2006a, 2007a, b, 2009) on these two groups of fungi. The sequences for the three gene regions for each of the two datasets (*C. moniliformis s.l.* and *C. fimbriata s.l.*) were aligned using MAFFT version 6.606 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) (Katoh et al. 2002), and the alignments were confirmed manually in MEGA4 (Tamura et al. 2007).

Phylogenetic analyses based on parsimony were done using PAUP version 4.0b10* (Swofford 2002). A Partition Homogeneity Test (PHT) was run to determine whether the sequences of three gene regions for each of the two datasets could be combined (Swofford 2002). Phylograms were

generated using a heuristic tree search algorithm. Sequences were randomised (reps=1,000), Mulpar was effective and a TBR branch swapping algorithm was used in the search. Gaps were treated as a fifth character. Confidence intervals of branch nodes using 1,000 bootstrap replicates with the full heuristic search option were calculated. *Ceratocystis virescens* (R.W. Davidson) C. Moreau was used as the outgroup taxon for both the datasets. All sequences derived from this study were deposited in GenBank (Table 1 and 2).

MrModeltest2 (Nylander 2004) was used to determine the most appropriate model for the analyses of each gene region. The suggested partition-specific models were then included in the Bayesian analyses. Phylogenetic trees based on Bayesian inference were generated using MrBayes version 3.1.1. (Ronquist and Huelsenbeck 2003). One million generations were run during which random trees were generated using the MCMC procedure. Four chains were applied and sampled every 100th generation. To avoid including trees that had been sampled before convergence, tree likelihood scores were assessed to determine the number of trees that were formed before stabilization. Trees outside the point of convergence were discarded by means of the burn-in procedure in MrBayes.

The three gene regions of the five groups of isolates from Ecuador (three in the *C. fimbriata s.l.* group and two in the *C. moniliformis s.l.* group) were analyzed separately for each gene region (ITS, βt and EF1-α). The PAUP settings were the same as above for the combined dataset excluding an outgroup. To determine the amount of variation between the isolates from Ecuador, Molecular Evolutionary Genetics Analysis (MEGA) 4 (Tamura et al. 2007) was used. Fixed alleles were sought in each gene region of the respective groups. Allele networks were drawn using the software TCS (Clement et al. 2000) in order to observe the shared alleles between the three groups in the *C. fimbriata s.l.* dataset and the two groups in the *C. moniliformis s.l.* dataset.

Culture characteristics and morphology

Culture morphology was assessed after 3 or 7 days of growth on 2% MEA (*w*/*v*) supplemented with Streptomycin Sulphate (100 mg per litre). Colony colour was assigned using the colour charts of Rayner (1970). Two distinctly different colony morphologies and colony colours were observed in the isolates from Ecuador when grown on 2% MEA. The two colony morphologies were representative of species in the *C. fimbriata s.l.* and *C. moniliformis s.l.* complexes.

It was not possible to distinguish between isolates representing the *C. fimbriata s.l.* complex based on colony morphology. Three clades in this group were, however, identified based on phylogenetic inference. Three isolates representing each of these phylogenetic clades in the *C. fimbriata s.l.* group were selected for further morphological comparisons.

The isolates representing species in the *C. moniliformis s.l.* complex had indistinguishable colony morphology. Two clades were identified in this group based on phylogenetic inference. One of these represented *C. moniliformis s.s.* and the other an undescribed taxon. Three isolates representing the latter group were thus selected for morphological characterisation.

Optimum growth temperatures were determined by placing 4 mm plugs taken from the margins of actively growing cultures, mycelium side down, at the centres of 90 mm 2% MEA plates. Five replicates were made for each culture at seven different temperatures between 5°C and 35°C at 5°C intervals. The growth was assessed 7 days after incubation for the isolates representative of species in the *C. fimbriata s.l.* complex. For isolates representing species in the *C. moniliformis s.l.* complex, growth was measured 3 days after incubation.

For morphological characterisation, fungal structures, taken from 10 d-old cultures grown on 2% MEA were mounted in lactic acid. Photographic images were captured for all taxonomically important structures with a Carl Zeiss microscope and a Zeiss Axio Vision camera system. Three isolates were selected for each of the discrete phylogenetic clades in the *C. fimbriata s.l.* and *C. moniliformis s.l.* complexes. Fifty measurements were taken for all taxonomically useful characteristics for one isolate in each group and an additional 10 measurements were made for the remaining two isolates. Minimum, maximum, average and standard deviation (stdv) were calculated for each structure and these are presented as (minimum–) stdv minus the mean – stdv plus the mean (–maximum).

Results

Isolates

A total of 57 isolates representing *Ceratocystis* spp. were collected from the artificially induced wounds on trees. Of these, 42 were collected from *E. deglupta*, six were from *T. ivorensis* and nine were from wounds made on *S. parahyba*. Based on morphology, these isolates represented two discrete groups, one typical of species in the *C. moniliformis s.l.* complex and one in the *C. fimbriata s.l.* complex. Of these, nine in the *C. moniliformis* complex were from *S. parahyba* trees and two were from *T. ivorensis* trees. Forty two isolates in the *C. fimbriata s.l.* complex were from *E. deglupta* trees while four were from *T. ivorensis* trees.

Table 1 Isolates and species within the *C. fimbriata s.l.* species complex used in this study

Species	Isolate no.	GenBank accession no.	Host	Geographical origin
C. albifundus	CMW4068	DQ520638 EF070429 EF070400	*Acacia mearnsii*	RSA
C. albifundus	CMW5329 CBS119681	AF388947 DQ371649 EF070401	*Acacia mearnsii*	Uganda
C. atrox	CMW19383 CBS120517	EF070414 EF070430 EF070402	*Eucalyptus grandis*	Australia
C. atrox	CMW19385 CBS120518	EF070415 EF070431 EF070403	*Eucalyptus grandis*	Australia
C. cacaofunesta	CMW15051 CBS152.62	DQ520636 EF070427 EF070398	*Theobroma cacao*	Costa Rica
C. cacaofunesta	CMW14809 CBS115169	DQ520637 EF070428 EF070399	*Theobroma cacao*	Ecuador
C. caryae	CMW14793 CBS114716	EF070424 EF070439 EF070412	*Carya cordiformis*	USA
C. caryae	CMW14808 CBS115168	EF070423 EF070440 EF070411	*Carya ovata*	USA
C. colombiana	CMW9565 CBS121790	AY233864 AY233870 EU241487	Soil	Colombia
C. colombiana	CMW5751 CBS121792	AY177233 AY177225 EU241493	*Coffea arabica*	Colombia
C. colombiana	CMW9572	AY233863 AY233871 EU241488	Mandarin	Colombia
***C. curvata* (CF1)**	**CMW22432**	**FJ151439 FJ151451 FJ151473**	***Eucalyptus deglupta***	**Colombia**
***C. curvata* (CF1)**	**CMW22433 CBS122513**	**FJ151438 FJ151450 FJ151472**	***Eucalyptus deglupta***	**Colombia**
***C. curvata* (CF1)**	**CMW22435 CBS122604**	**FJ151437 FJ151449 FJ151471**	***Eucalyptus deglupta***	**Colombia**
***C. curvata* (CF1)**	**CMW22442 CBS122603**	**FJ151436 FJ151448 FJ151470**	***Eucalyptus deglupta***	**Colombia**
***C. diversiconidia* (CF3)**	**CMW22445 CBS123013**	**FJ151440 FJ151452 FJ151474**	***Terminalia ivorensis***	**Colombia**
***C. diversiconidia* (CF3)**	**CMW22446**	**FJ151443 FJ151455 FJ151477**	***Terminalia ivorensis***	**Colombia**
***C. diversiconidia* (CF3)**	**CMW22447 CBS122818**	**FJ151442 FJ151454 FJ151476**	***Terminalia ivorensis***	**Colombia**
***C. diversiconidia* (CF3)**	**CMW22448 CBS122605**	**FJ151441 FJ151453 FJ151475**	***Terminalia ivorensis***	**Colombia**
***C. ecuadoriana* (CF2)**	**CMW22092 CBS124020**	**FJ151432 FJ151444 FJ151466**	***Eucalyptus deglupta***	**Colombia**
***C. ecuadoriana* (CF2)**	**CMW22093 CBS124021**	**FJ151433 FJ151445 FJ151467**	***Eucalyptus deglupta***	**Colombia**
***C. ecuadoriana* (CF2)**	**CMW22097 CBS124022**	**FJ151434 FJ151446 FJ151468**	***Eucalyptus deglupta***	**Colombia**
***C. ecuadoriana* (CF2)**	**CMW22405**	**FJ151435 FJ151447 FJ151469**	***Eucalyptus deglupta***	**Colombia**
C. fimbriata s.s	CMW15049 CBS141.37	DQ520629 EF070442 EF070394	*Ipomoea batatas*	USA
C. fimbriata s.s.	CMW1547 CBS123010	AF264904 EF070443 EF070395	*Ipomoea batatas*	Papua New Guinea
C. fimbriatomima	CMW24174 CBS121786	EF190963 EF190951 EF190957	*Eucalyptus* sp.	Venezuela
C. fimbriatomima	CMW24176 CBS121787	EF190964 EF190952 EF190958	*Eucalyptus* sp.	Venezuela
C. larium	CMW25434 CBS122512	EU881906 EU881894 EU881900	*Styrax benzoin*	Indonesia
C. larium	CMW25435 CBS122606	EU881907 EU881895 EU881901	*Styrax benzoin*	Indonesia
C. manginecans	CMW13851 CBS121659	AY953383 EF433308 EF433317	*Mangifera indica*	Oman
C. manginecans	CMW13852 CBS121660	AY953384 EF433309 EF433318	*Mangifera indica* infected with *Hypocryphalus mangiferae*	Oman
C. neglecta	CMW17808 CBS121789	EF127990 EU881898 EU881904	*Eucalyptus* sp.	Colombia
C. neglecta	CMW18194 CBS121017	EF127991 EU881899 EU881905	*Eucalyptus* sp.	Colombia
C. obpyriformis	CMW23807 CBS122608	EU245004 EU244976 EU244936	*Acacia mearnsii*	South Africa
C. obpyriformis	CMW23808 CBS122511	EU245003 EU244975 EU244935	*Acacia mearnsii*	South Africa
C. papillata	CMW8857	AY233868 AY233878 EU241483	*Annona muricata*	Colombia
C. papillata	CMW8856 CBS121793	AY233867 AY233874 EU241484	*Citrus limon*	Colombia
C. papillata	CMW10844	AY177238 AY177229 EU241481	*Coffea arabica*	Colombia
C. pirilliformis	CMW6569	AF427104 DQ371652 AY528982	*Eucalyptus nitens*	Australia
C. pirilliformis	CMW6579 CBS118128	AF427105 DQ371653 AY528983	*Eucalyptus nitens*	Australia
C. platani	CMW14802 CBS115162	DQ520630 EF070425 EF070396	*Platanus occidentalis*	USA
C. platani	CMW23918	EF070426 EF070397 EU426554	*Platanus* sp.	Greece
C. polychroma	CMW11424 CBS115778	AY528970 AY528966 AY528978	*Syzygium aromaticum*	Indonesia
C. polychroma	CMW11436 CBS115777	AY528971 AY528967 AY528979	*Syzygium aromaticum*	Indonesia
C. polyconidia	CMW23809 CBS122289	EU245006 EU244978 EU244938	*Acacia mearnsii*	South Africa
C. polyconidia	CMW23818 CBS122290	EU245007 EU244979 EU244939	*Acacia mearnsii*	South Africa
C. populicola	CMW14789 CBS119.78	EF070418 EF070434 EF070406	*Populus* sp.	Poland
C. populicola	CMW14819 CBS114725	EF070419 EF070435 EF070407	*Populus* sp.	USA
C. smalleyi	CMW14800 CBS114724	EF070420 EF070436 EF070408	*Carya cordiformis*	USA
C. smalleyi	CMW26383 CBS114724	EU426553 EU426555 EU426556	*Carya cordiformis*	USA

Table 1 (continued)

Species	Isolate no.	GenBank accession no.	Host	Geographical origin
C. tanganyicensis	CMW15991 CBS122295	EU244997 EU244969 EU244929	*Acacia mearnsii*	Tanzania
C. tanganyicensis	CMW15999 CBS122294	EU244998, EU244970, EU244939	*Acacia mearnsii*	Tanzania
C. tsitsikammensis	CMW14276 CBS121018	EF408555 EF408569 EF408576	*Rapanea melanophloeos*	South Africa
C. tsitsikammensis	CMW14278 CBS121019	EF408556 EF408570 EF408577	*Rapanea melanophloeos*	South Africa
C. variospora	CMW20935 CBS114715	EF070421 EF070437 EF070409	*Quercus alba*	USA
C. variospora	CMW20936 CBS114714	EF070422 EF070438 EF070410	*Quercus robur*	USA
C. virescens	CMW11164 CBS123166	DQ520639 EF070441 EF070413	*Fagus americana*	USA
C. virescens	CMW3276 CBS123216	AY528984 AY528990 AY529011	*Quercus robur*	USA
C. zombamontana	CMW15235 CBS122297	EU245002 EU244974 EU244934	*Eucalyptus* sp.	Malawi
C. zombamontana	CMW15236 CBS122296	EU245000 EU244972 EU244932	*Eucalyptus* sp.	Malawi

Isolates in bold are the ones obtained with this study

Table 2 Isolates and species in the *C. moniliformis s.l.* species complex used in this study

Species	Isolate no.	GenBank accession no.	Host	Geographical origin
C. bhutanensis	CMW8217 CBS114289	AY528957 AY528962 AY528952	*Picea spinulosa*	Bhutan
C. bhutanensis	CMW8242 CBS112907	AY528956 AY528961 AY528951	*Picea spinulosa*	Bhutan
C. moniliformis	CMW4114 CBS118151	AY528997 AY528986 AY529007	*Shizolobium parahyba*	Ecuador
C. moniliformis	CMW8379	AY529005 AY528995 AY529016	*Cassia fistula*	Bhutan
C. moniliformis	CMW9590 CBS116452	AY431101 AY528985 AY529006	*Eucalyptus grandis*	South Africa
C. moniliformis	CMW9990 CBS155.62	FJ151423 FJ151457 FJ151479	*Theobroma cacao*	Costa Rica
C. moniliformis	CMW10134 CBS118127	FJ151422 FJ151456 FJ151478	*Eucalyptus grandis*	South Africa
C. moniliformis	**CMW22458**	**FJ151424 FJ151458 FJ151480**	***Shizolobium parahyba***	**Ecuador**
C. moniliformis	**CMW22459**	**FJ151425 FJ151459 FJ151481**	***Shizolobium parahyba***	**Ecuador**
C. moniliformis	**CMW22460**	**FJ151427 FJ151461 FJ151482**	***Shizolobium parahyba***	**Ecuador**
C. moniliformis	**CMW22462**	**FJ151426 FJ151460 FJ151483**	***Shizolobium parahyba***	**Ecuador**
C. moniliformopsis	CMW9986 CBS109441	AY528998 AY528987 AY529008	*Eucalyptus obliqua*	Australia
C. moniliformopsis	CMW10214 CBS115792	AY528999 AY528988 AY529009	*Eucalyptus sieberi*	Australia
C. oblonga	CMW23802 CBS122820	EU245020 EU244992 EU244952	*Acacia mearnsii*	South Africa
C. oblonga	CMW23803 CBS122291	EU245019 EU244991 EU244951	*Acacia mearnsii*	South Africa
C. omanensis	CMW3800 CBS117839	DQ074743 DQ074733 DQ074738	*Mangifera indica*	Oman
C. omanensis	CMW11048 CBS115787	DQ074742 DQ074732 DQ074737	*Mangifera indica*	Oman
C. savannae	CMW17278 CBS121019	EF408553 EF408567 EF408574	*Eucalyptus macarthurii*	South Africa
C. savannae	CMW17300 CBS121151	EF408551 EF408565 EF408572	*Acacia nigrescens*	South Africa
C. sublaevis	**CMW22415**	**FJ151428 FJ151462 FJ151484**	***Eucalyptus deglupta***	**Ecuador**
C. sublaevis	**CMW22422 CBS122516**	**FJ151429 FJ151463 FJ151485**	***Eucalyptus deglupta***	**Ecuador**
C. sublaevis	**CMW22444 CBS122518**	**FJ151430 FJ151464 FJ151486**	***Terminalia ivorensis***	**Ecuador**
C. sublaevis	**CMW22449 CBS122517**	**FJ151431 FJ151465 FJ151487**	***Terminalia ivorensis***	**Ecuador**
C. tribiliformis	CMW13011 CBS115867	AY528991 AY529001 AY529012	*Pinus merkusii*	Indonesia
C. tribiliformis	CMW13012 CBS118242	AY528992 AY529002 AY529013	*Pinus merkusii*	Indonesia
C. virescens	CMW11164 CBS123166	DQ520639 EF070441 EF070413	*Fagus americana*	USA
C. virescens	CMW3276 CBS123216	AY528984 AY528990 AY529011	*Quercus robur*	USA
Thielaviopsis ceramica	CMW15245 CBS122299	EU245022 EU244994 EU244926	*Eucalyptus grandis*	Malawi
T. ceramica	CMW15248 CBS122300	EU245024 EU244996 EU244928	*Eucalyptus grandis*	Malawi

Isolates in bold are the ones obtained with this study

Phylogeny

Two separate phylogenetic datasets were constructed. One of these was for isolates in the *C. fimbriata s.l.* complex and the other for isolates in the *C. moniliformis s.l.* complex.

C. fimbriata s.l. species complex

A *P*-value of 0.01 was obtained from the PHT for the dataset that included the three gene regions considered. Although this value was low, it indicated that the datasets could be combined (Sullivan 1996; Cunningham 1997). A total of 1,996 characters represented this dataset of which 1,087 were constant, 44 were parsimony uninformative and 865 characters were parsimony informative. Four most parsimonious trees were obtained after a heuristic search, one of which was selected for presentation (Fig. 1). The tree length and goodness of character fit indices were as follows: tree length=1,956 steps, consistency index (CI)= 0.7, retention index (RI)=0.9 and a rescaled consistency index (RC) of 0.6.

The HKY+G model was determined to be appropriate for the ITS gene region with the software MrModeltest2. The GTR+G model was best suited for the βt gene region, while the HKY+G model was found to be best suited for the EF1-α gene region. These model settings were included in the Bayesian analyses with 4,000 trees discarded because they were outside the point of convergence. The posterior probabilities of the branch nodes supported the bootstrap values of the selected tree obtained in PAUP (Fig. 1).

Parsimony analysis placed isolates from Ecuador in three separate clades with 100, 96 and 100% support respectively (Fig. 1). The clades were designated as CF1, CF2, and CF3 on the phylogram and are, henceforth, referred to using this notation. All isolates in Groups CF1 and CF2 were from *E. deglupta* with isolates CMW22432, CMW22433, CMW22435, CMW22442 residing in CF1 and isolates CMW22092, CMW22093, CMW22097, CMW22405 residing in Group CF2 (Fig. 1). Isolates in Group CF1 were phylogenetically distinct from all species in the *C. fimbriata s.l.* species complex with 100% bootstrap and Bayesian support. The isolates in Group CF1 were sister to *C. colombiana* M. van Wyk & MJ. Wingf., a pathogen of various tree crops in Colombia (Van Wyk et al. 2010a) (Fig. 1). Isolates residing in Group CF2 were phylogenetically distinct (Bootstrap 96%, Bayesian 98%) from all other species in the *C. fimbriata s.l.* species complex and formed a sister group to *C. neglecta* M. van Wyk, Jol. Roux & Rodas, a pathogen of *Eucalyptus* trees in Colombia (Rodas et al. 2008). Isolates in Group CF3 included those that were obtained from *T. ivorensis* trees (Fig. 1). This group was phylogenetically distinct from all species in this complex with 100% bootstrap and Bayesian support, with no sister group present.

The tree topologies for each of the datasets of the seperate gene regions appeared similar when compared to the tree topology of the dataset for the combined gene regions (Fig. 2). The three seperate groups could still be identified, however, support for the branches was not as high as in the combined dataset. All three gene regions had fixed polymorphisms for all three groups within the *C. fimbriata s.l.* dataset. In the ITS region, there were 33 differences between CF and CF2, 52 differences between CF2 and CF3 and 64 differences between CF1 and CF3. In the BT region there were four differences between CF1 and CF2, nine differences between CF2 and CF3 and seven differences between CF1 and CF3. In the EF region, there were six differences between CF1 and CF2, 9 differences between CF2 and CF3 and nine differences between CF1 and CF3. The allele networks obtained showed that the three groups observed in the phylogenetic trees were also seen in the allele network as the single dataset of the three gene regions produced three separate allele networks (Fig. 3).

C. moniliformis s.l. species complex

The PHT for the isolates in the *C. moniliformis* complex gave a *P*-value of 0.01, and the datasets were thus combined (Sullivan 1996; Cunningham 1997). This dataset consisted of 1,824 characters of which 1,389 were constant, 11 parsimony-uninformative and 424 characters were parsimony informative. One of six trees obtained was selected for presentation (Fig. 4) and it had a length of 610 steps, a CI of 0.8, a RI of 0.9 and a RC of 0.8.

With MrModeltest2, the HKY+I model was selected for the ITS dataset while the GTR+G model was selected for the βt gene region and the SYM+G model was best suited for the EF1-α gene region. These models were incorporated into the Bayesian analyses in MrBayes. Three thousand trees were discarded (burn-in) as they were outside the point of convergence. The posterior probabilities for the nodes were supportive of the bootstrap values obtained in PAUP (Fig. 4).

Two well resolved clades emerged from the phylogenetic analyses. In one of these, referred to as CM1 (CMW22458, CMW22459, CMW22462, CMW22460) isolates from Ecuador grouped with *C. moniliformis s.s*. (61% bootstrap and 100% Bayesian) and they were all from *S. parahyba* (Fig. 4). Isolates (CMW22415, CMW22422, CMW22444, CMW22449) in the second clade (CM2) were phylogenetically distinct from all other isolates (97% bootstrap, 100% Bayesian) in the *C. moniliformis s.l.* species complex (Fig. 4). The latter isolates originated from both *T. ivorensis* and *E. deglupta* trees.

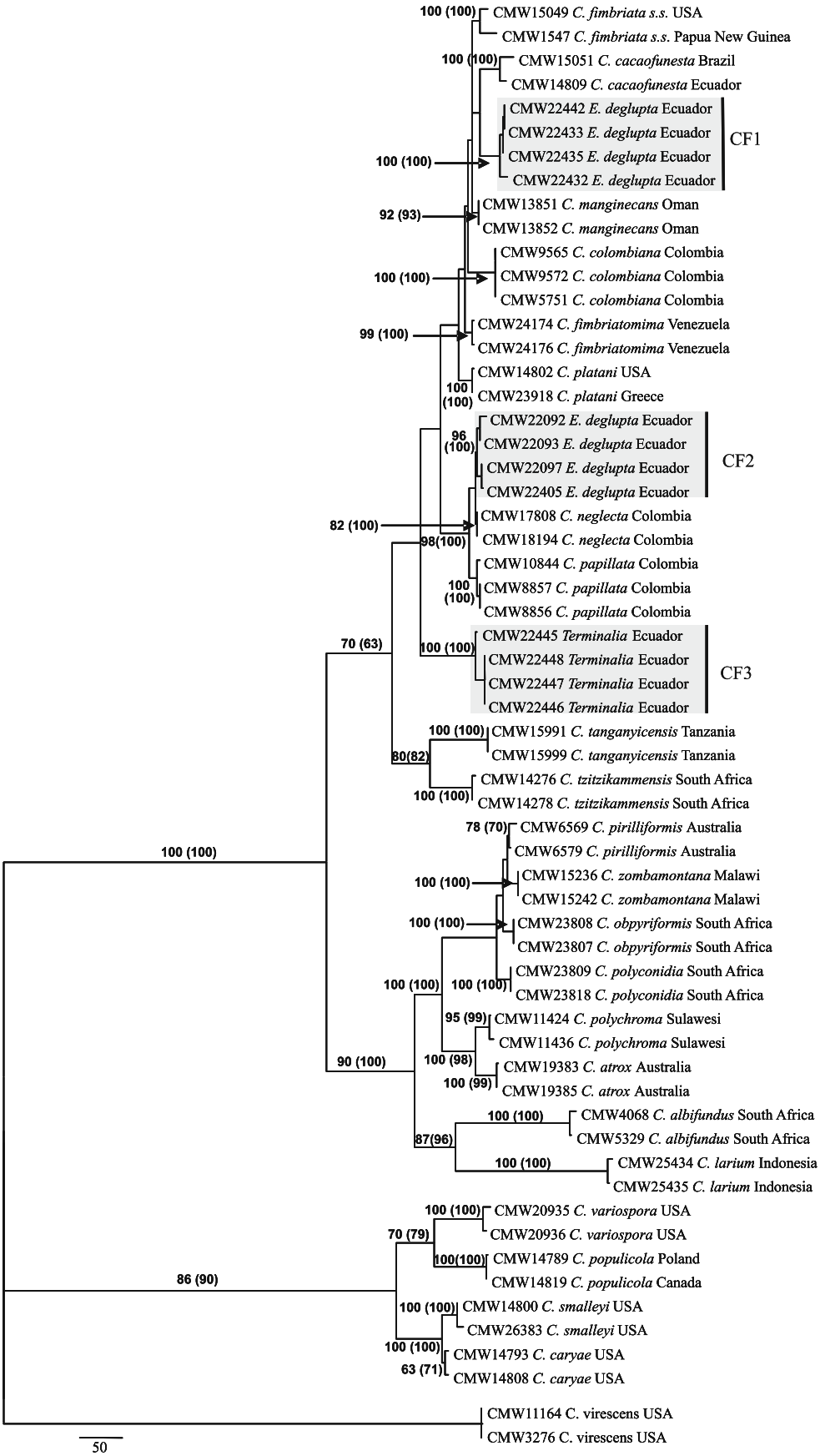

Fig. 1 Isolates of Ecuador obtained from *E. deglupta* and *T. ivorensis* trees in a phylogenetic tree based on the combined regions of the ITS, βt and EF1-α for species in the *C. fimbriata s.l.* species complex. *Ceratocystis virescens* represents the out-group taxon. Bootstrap values and Bayesian posterior probabilities (in brackets) are indicated at the branch nodes

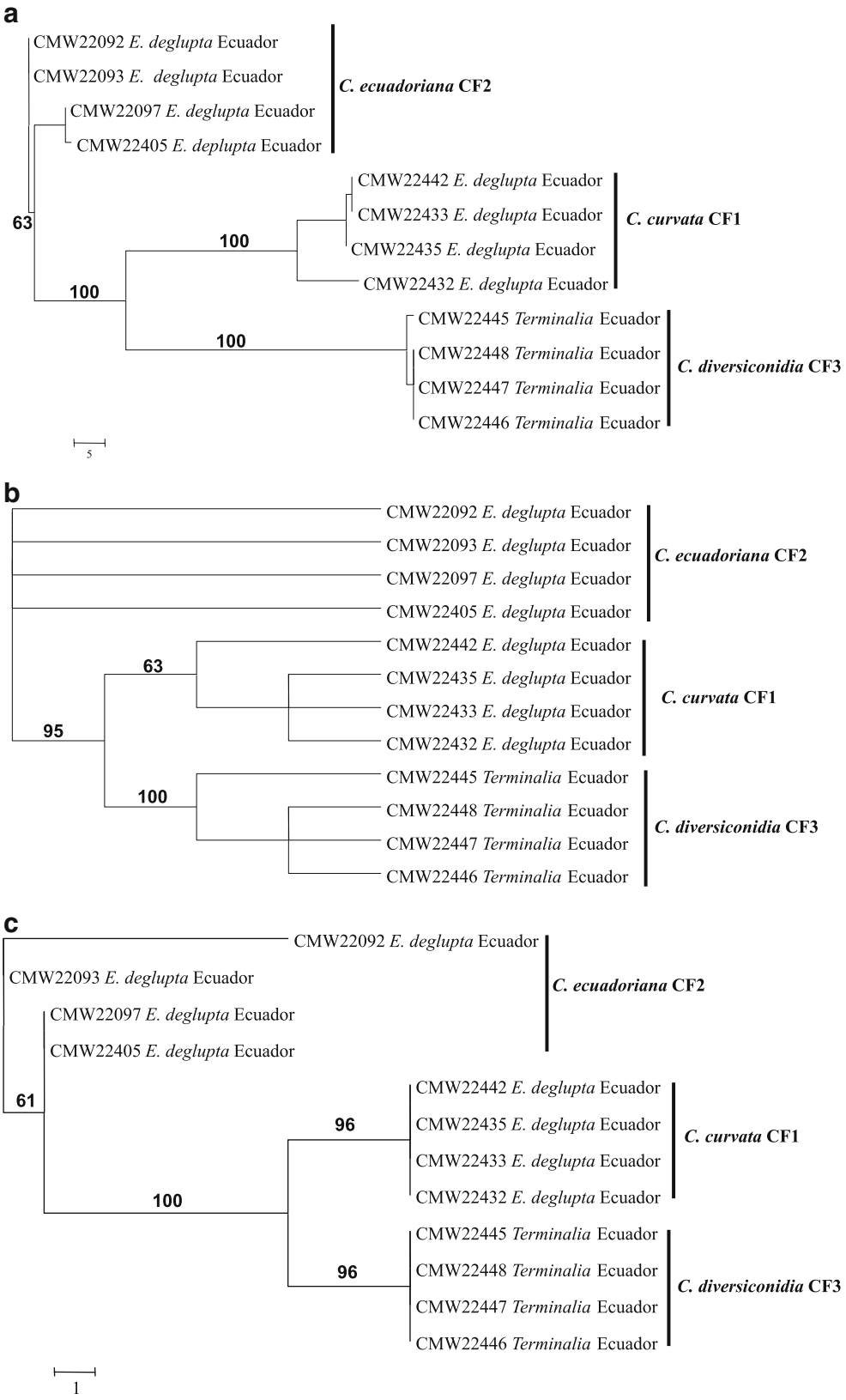

Fig. 2 Separate unrooted phylogenetic trees of the three groups in the *C. fimbriata s.l.* dataset representing the three gene regions. **a** ITS. **b** βt. **c** EF1-α. Bootstrap values are indicated on the branches

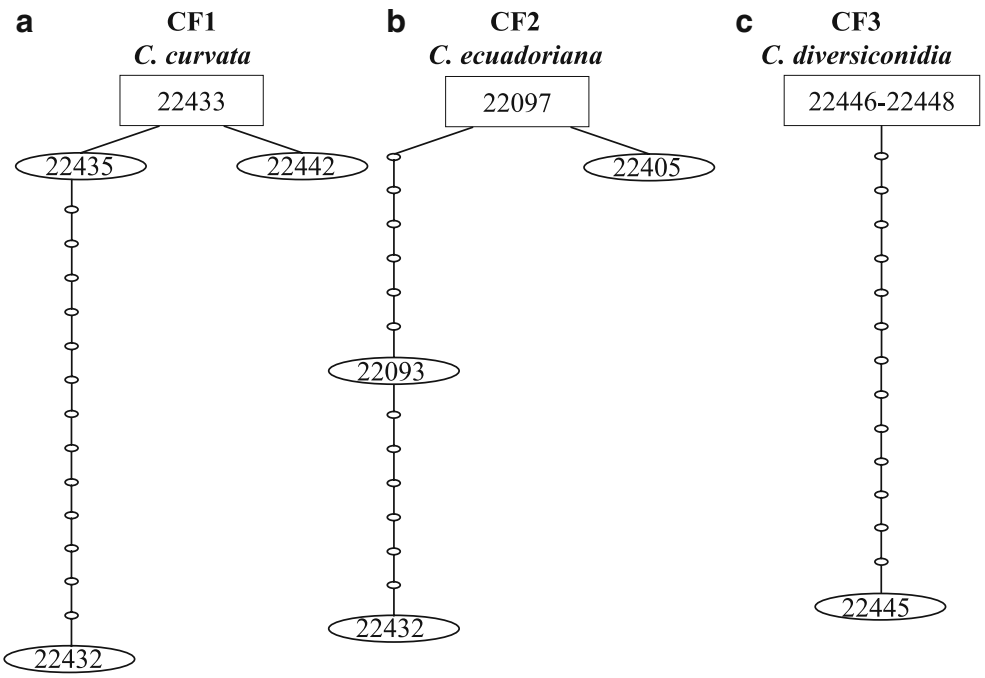

Fig. 3 Three separate allele networks obtained from one dataset, within the *C. fimbriata s.l.* complex, representing three gene regions (ITS, βt and EF1-α) of the isolates obtained from Ecuador on *E. deglupta* and *Terminalia* trees. The numbers represent CMW numbers as listed in Table 1

Analyses of the datasetes for the two groups in the *C. moniliformis s.l.* complex, based on single gene regions, gave results diferent to those emerging from the combination of the three gene regions (Fig. 5). For the ITS gene tree, there was no support for either of the two groups. For the βt gene region, all the isolates from Ecuador grouped in one clade. In the EF1-α gene tree, the two groups were well-defined with high bootsrap support and the tree had a topology similar to that for the combined gene sequences.

Fixed polymorphisms were observed in two of the three gene regions considered. The βt gene region had one base pair difference while the EF1-α gene region had 14 differences between *C. moniliformis* and the isolates representing an apparently different species from Ecuador. There were no differences between isolates based on sequence comparisions for the ITS gene region (Van Wyk et al. 2006a, 2010b). The allele network obtained for the combined genes for the isolates in the *C. moniliformis s.l.* complex from Ecuador together with the *C. moniliformis s.s.* isolates is presented in Fig. 6. All isolates representing *C. moniliformis s.s.* grouped together in one cluster while the isolates representing the CM2 clade grouped seperately from them.

Culture characteristics and morphology

The isolates from Ecuador could be separated into two groups based on their culture morphology. The first group included isolates that grew slowly and had a greenish colour. The second group grew faster and were white to light brown with abundant aerial mycelium or little to no mycelium but abundant ascomata that covered the plates. The first group of isolates were morphologically most similar to species in the *C. fimbriata s.l.* complex and the remaining group was morphologically most similar to species in the *C. moniliformis s.l.* species complex. These two morphological groups were also consistent with the two groups emerging from DNA sequence comparisons.

C. fimbriata s.l. species complex

Based on phylogenetic analyses, three distinct groups (CF1, CF2, CF3) were identified in the *C. fimbriata s.l.* complex. However, only one group could be identified based on culture morphology. All isolates that represented *C. fimbriata s.l.* had greenish to brown colonies and a banana odour. They, however, differed from each other with regard to growth in culture. After 7 days of incubation, isolates representing Group CF1 (CMW22432, CMW22435 and CMW22442) had an optimum growth at 25°C (36 mm). No growth was observed at 5°C, 10°C or 35°C, limited growth (14 mm) occurred at 15°C and intermediate growth was observed at 20°C (28 mm) and 30°C (32 mm). Isolates representing Group CF2 (CMW22092, CMW22093 and CMW22097) had an optimum growth at 25°C (47 mm). No growth was observed at 5°C, 10°C and 35°C, limited growth was observed at 15°C (20 mm) and 30°C (24 mm) and intermediate growth was observed at 20°C (36 mm). Isolates representing Group CF3 (CMW22445, CMW22446 and CMW22447) had an optimum growth at

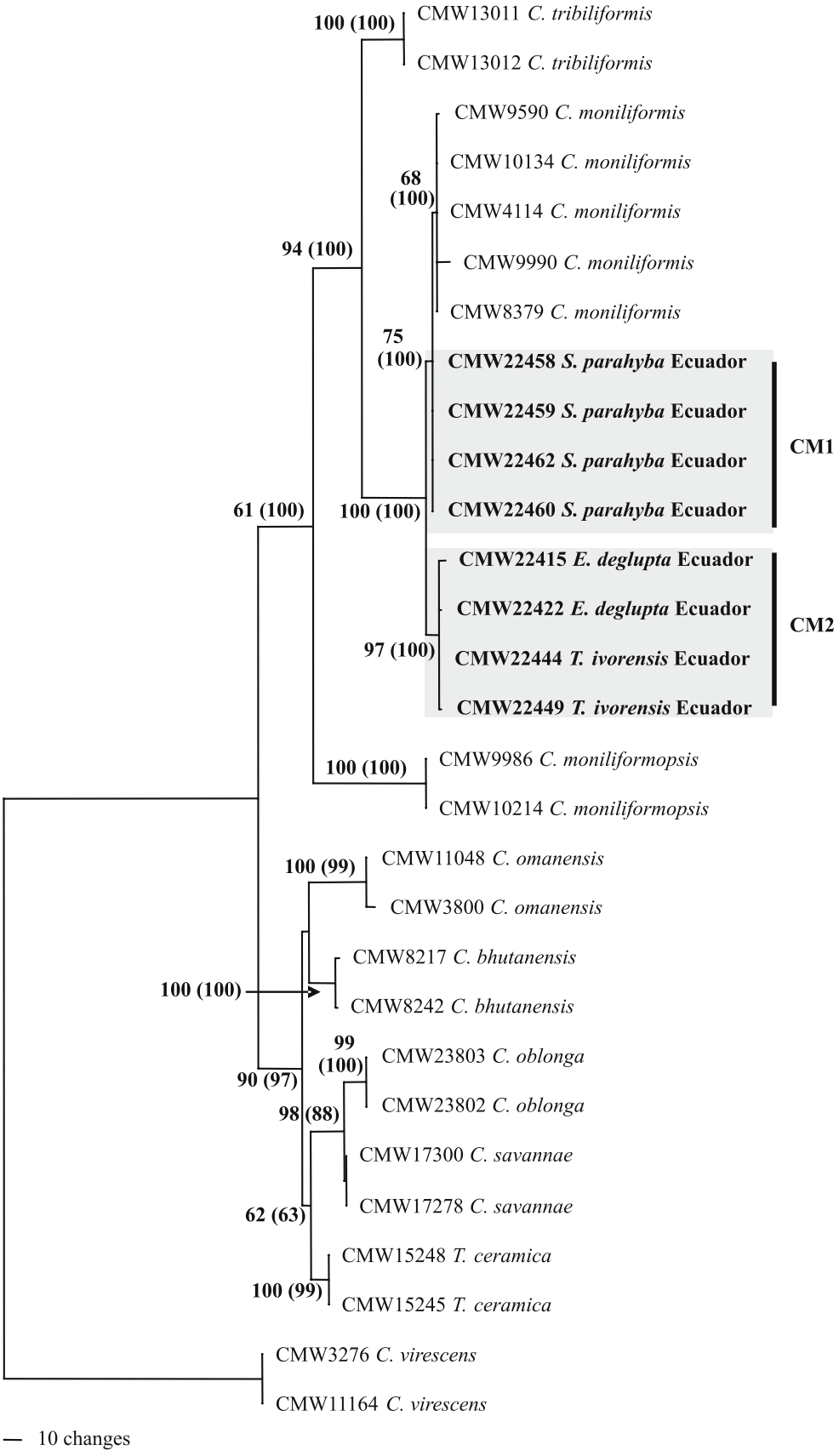

Fig. 4 Isolates of Ecuador obtained from *S. parahyba*, *E. deglupta* and *T. ivorensis* trees in a phylogenetic tree based on the combined regions of the ITS, βt and EF1-α for species in the *C. moniliformis s.l.* species complex. *Ceratocystis virescens* represents the out-group taxon. Bootstrap values and Bayesian posterior probabilities (*in brackets*) are indicated at the branch nodes

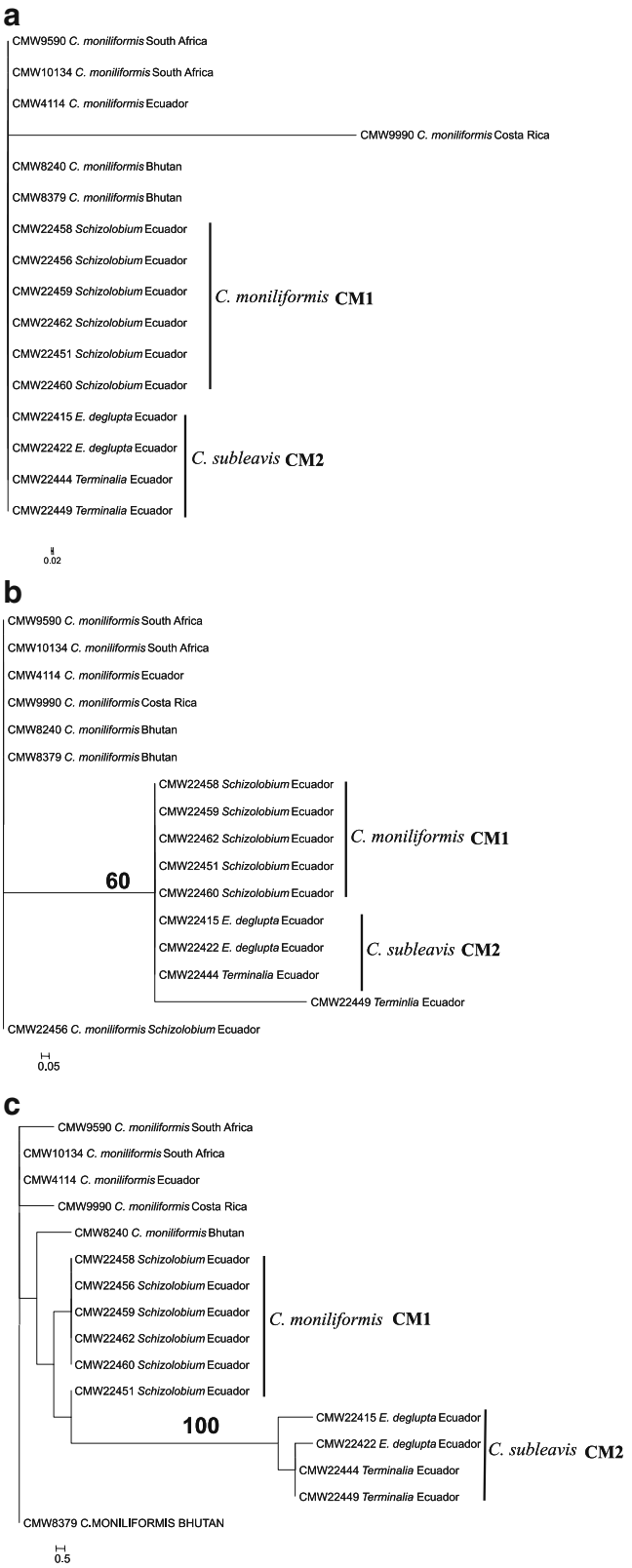

Fig. 5 Three separate unrooted phylogenetic trees of the two groups in the *C. moniliformis s.l.* dataset representing three gene regions. **a** ITS. **b** βt. **c** EF1-α. Bootstrap values are indicated on the branches. Where there are no bootstrap values this indicates that the bootstrap was less than 60%

25°C (21 mm). No growth was observed at 5°C, 10°C or 35°C, limited growth was observed at 15°C (8 mm) and intermediate growth was observed at 20°C (16 mm) and 30°C (14 mm).

C. moniliformis s.l. species complex

Based on phylogenetic analyses, two groups (CM1, CM2) of isolates were identified in the *C. moniliformis s.l.* species complex. However, no clear distinction could be made between them based on colony morphology. Both groups displayed very rapid growth, had white to light brown colonies with abundant aerial mycelium and a banana odour. Isolates representing Group CM1 (CMW22451, CMW22456, CMW22458, CMW22459, CMW22460, CMW22462) were identified as *C. moniliformis s.s.* based on the phylogenetic analysis and no further morphological characterisation was made for them. Isolates representing

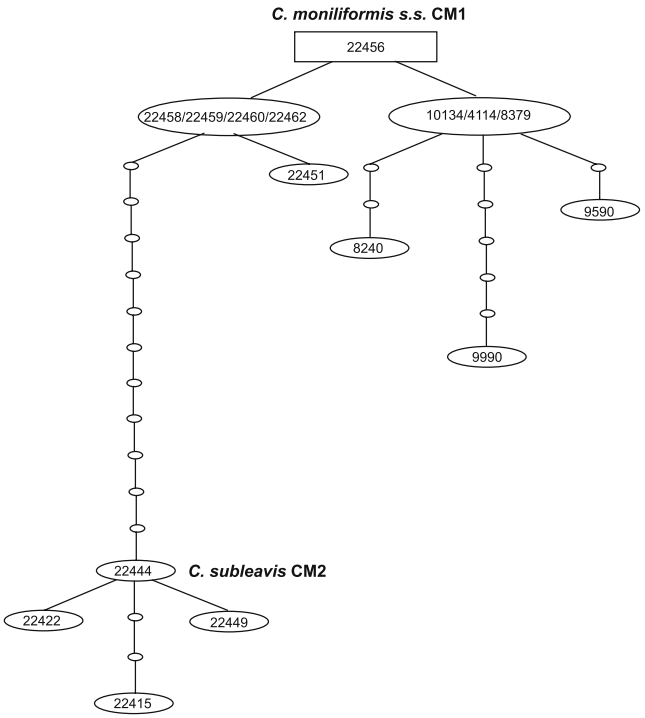

Fig. 6 Allele network for representative isolates in the *C. moniliformis s.l.* complex. Isolates representing *C. moniliformis s.s.* and isolates obtained in this study from Ecuador were selected for this dataset. The ITS, βt and EF1-α gene regions were combined and a single allele network was obtained. The numbers in the network represent isolates in the CMW collection (Table 2)

Group CM2 (CMW22444, CMW22449, CMW22410 and CMW22415) had an optimum growth after 3 days at 25°C (56 mm). No growth was observed at 5°C, 10°C or 35°C, limited growth was observed at 15°C (19 mm) and intermediate growth was observed at 20°C (41 mm) and 30°C (40 mm).

Taxonomy

The *Ceratocystis* isolates from Ecuador considered in this study clearly represent four previously unknown taxa, three in the *C. fimbriata s.l.* species complex and one in the *C. moniliformis s.l.* species complex. These differ from each other based on minor morphological characteristics and they are primarily distinguished based on DNA sequence comparisons for three gene regions. They are thus described as follows:

C. curvata M. van Wyk & M.J. Wingf. **sp. nov.** (Fig. 7a–i)

MB 512846

Etymology: The name refers to the curved ascomatal necks in this species.

Bases ascomatum nigrae globosae vel obpyriformes (162–) 185–239 (–280) μm longae, (170–) 192–240 (–280) μm latae. *Colla ascomatum* atrobrunnea vel nigra, apicibus undulates. *Hyphae ostiolares* divergentes, (34–) 39–49 (–59) μm longae. Conidiophora biformia in mycelio singuli disposita, *primaria* phialidica hyalina basi tumescentia, apicem versus contracta (44–) 55–95 (–139) μm longa; *secondaria* tubularia, expansa hyalina, (34–) 42–66 (–90) μm longa. *Chlamydosporae* atro-brunneae, parietibus crassis, globosae vel subglobosae 11–17×9–12 μm.

Colony brownish olive (19″m) on malt extract agar. *Mycelium* submerged and aerial. *Optimal temperature* for growth 25°C, no growth below 15°C and above 35°C. *Hyphae* smooth, not constricted at septa. *Ascomatal bases* black, globose to obpyriform, no ornamentation, bases (162–) 185–239 (–280) μm long, (170–) 192–240 (–280) μm wide, (166–) 188–240 (–280) μm in diam. *Ascomatal necks* dark brown to black, undulating at apices, (419–) 498–644 (–714) μm long, (12–) 15–21 (–25) μm wide at apices, (19–) 23–35 (–56) μm wide at bases. *Ostiolar hyphae* divergent, hyaline, (34–) 39–49 (–59) μm long. *Asci* not observed. *Ascospores* hat-shaped in side view, aseptate, hyaline, invested in a sheath, 3–5×4–6 μm without sheath, 3–5×5–8 μm with sheath. Ascospores accumulating in buff-yellow (19 d) mucilaginous masses at the apices of ascomatal necks.

Thielaviopsis anamorph: *Conidiophores* of two types occurring singly on mycelium, *primary conidiophores*, phialidic, hyaline, swollen at the base, tapering towards the apices, (44–) 55–95 (–139) μm long, 4–6 μm wide at bases, 4–7 μm wide at widest point in middle, 3–4 μm wide at apices. *Secondary conidiophore*, phialidic, flaring, hyaline, (34–) 42–66 (–80) μm long, 3–6 μm wide at bases, 4–6 μm wide at apices. *Conidia* of two types: *Primary conidia*, hyaline, aseptate, cylindrical, (10–) 13–21 (–31)× 3–6 μm. *Secondary conidia*, hyaline, aseptate, barrel-shaped 7–11×4–8 μm. *Chlamydospores* dark brown, thick walled, globose to perprolate, 11–17×(7–) 9–11 (–12) μm.

Habitat: Freshly wounded *Eucalyptus deglupta* wood.

Known distribution: Ecuador.

Material examined: Ecuador, near Salinas isolated from wood of *E. deglupta*, 2004, M.J. Wingfield (PREM60151 – holotype), live strain: CMW22442, CBS122603.

Additional specimens examined (paratypes): same data (PREM60154), live strain CMW22432; same data (PREM60153), live strain CMW22433; same data (PREM60152), live strain CMW22435, CBS122604.

Notes: *Ceratocystis curvata* is distinct from all other species within the *C. fimbriata s.l.* complex in that it generally has ascomatal necks that are curved at the apices. Several other structures differ in size when compared to other species in the *C. fimbriata s.l.* complex (Table 3).

C. ecuadoriana M. van Wyk & M.J. Wingf. **sp. nov.** (Fig. 8a–h)

MB 512847

Etymology: Name reflects the country where the fungus was first collected.

Bases ascomatum nigrae globosae, (180–) 215–265 (–290) μm longae, (184–) 208–250 (–279) μm latae. *Colla ascomatum* atrobrunnea vel nigra, (515–) 615–851 (–1,021) μm longa. Conidiophora biformia in mycelio singuli disposita, *primaria* phialidica hyalina basi tumescentia, apicem versus contracta (60–) 72–94 (–98) μm longa; *secondaria* phialidica expansa hyalina, (52–) 61–85 (–98) μm longa. *Conidia* biformia: *primaria* hyalina non septata cylindrica (9–) 14–20 (–24)×3–5 μm.

Colony brownish olive (19″m) on malt extract agar. *Mycelium* submerged and aerial. *Optimal temperature* for growth 25°C, no growth below 15°C and above 35°C. *Hyphae* smooth, not constricted at septa. *Ascomatal bases* black, globose, no ornamentation, bases (180–) 215–265 (–290) μm long, (184–) 208–250 (–279) μm wide, (182–) 212–258 (–285) μm in diam. *Ascomatal necks* dark brown to black, (515–) 615–851 (–1,021) μm long, (14–) 17–23 (–28) μm wide at apices, (24–) 28–38 (–46) μm wide at bases. *Ostiolar hyphae* divergent, hyaline, (29–) 32–42 (–50) μm long. *Asci* not observed. *Ascospores* hat-shaped in side view, aseptate, hyaline, invested in sheath, 3–4×3–6 μm without sheath, 3–4×5–8 μm with sheath. Ascospores accumulating in buff-yellow (19 d) mucilaginous masses on the apices of ascomatal necks.

Thielaviopsis anamorph: *Conidiophores* of two types occurring singly on mycelium, *primary conidiophore*, phialidic, hyaline, swollen at the base, tapering towards the apices, (60–) 72–94 (–98) μm long, 4–6 μm wide at bases, 5–7 μm

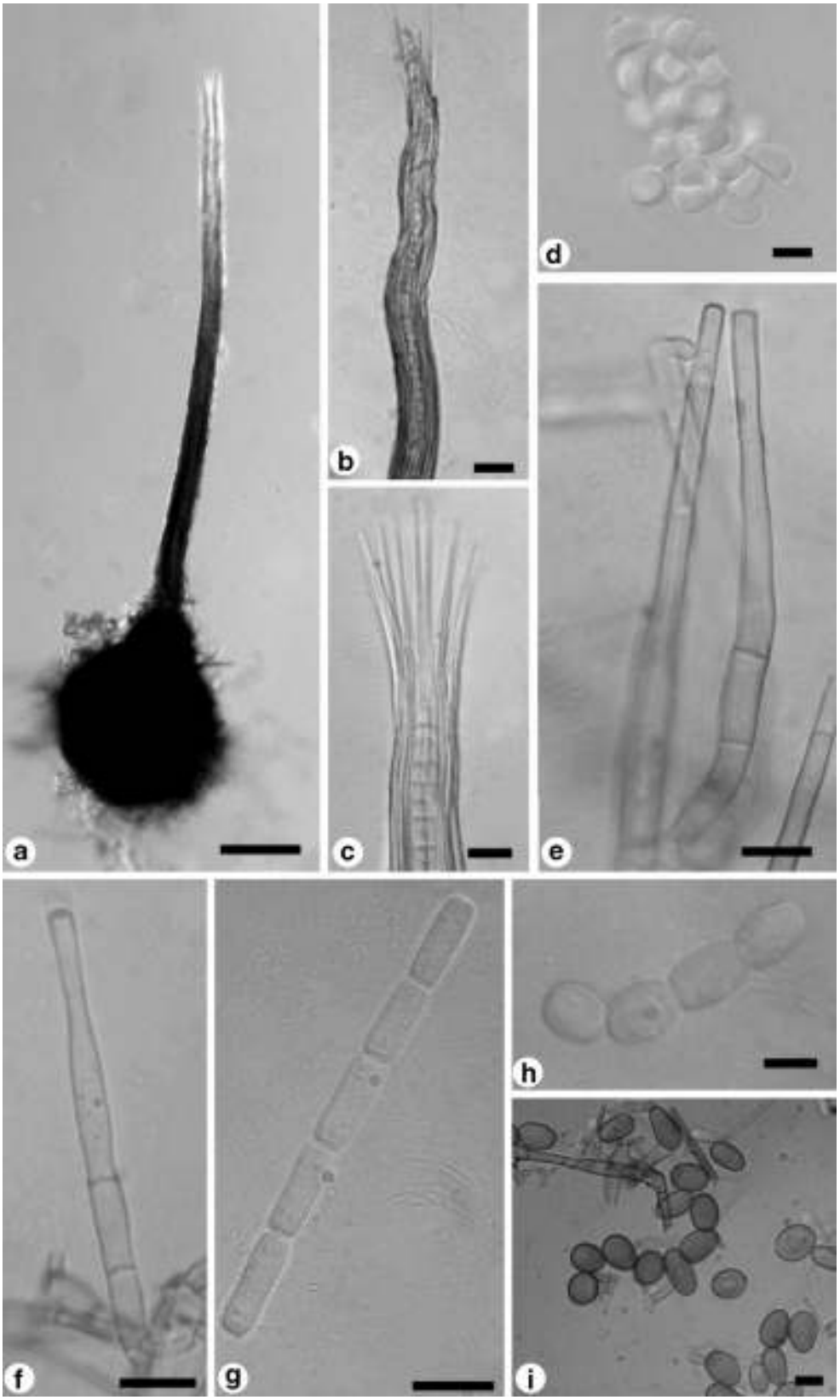

Fig. 7 Morphological characteristics of *Ceratocystis curvata*. **a** Ascoma with globose to obpyriform base. **b** Ascomatal neck undulating at apex. **c** Divergent ostiolar hyphae. **d** Hat-shaped ascospores. **e** Primary conidiophore, flask-shaped phialides. **f** Secondary conidiophore, flask-shaped but flaring at apex. **g** Chain of cylindrical conidia. **h** Chain of barrel-shaped conidia. **i** Dark, globose to perprolate chlamydospores Bars; **a** = 100 μm. **b, d, h** = 5 μm. **c, e, f, g, i** = 10 μm

Table 3 Morphological features of *C. curvata*, *C. ecuadoriana* and *C. diversiconidia* compared with each other and related species in the *C. fimbriata sensu lato* species complex

Species	*C. curvata*	*C. cacaofunesta*	*C. ecuadoriana*	*C. neglecta*	*C. diversiconidia*	*C. fimbriata s.s.*
Characteristics						
Ascomatal bases						
Shape	Globose to obpyriform	Globose	Globose	Globose	Globose	Globose
Length	(162–)185–239(–280)	100–275	(180–)215–265(–290)	(173–)202–244(–281)	(97–)115–155(–196)	110–250
Width	(170–)192–240(–280)	95–305	(184–)208–250(–279)	(153–)178–228(–250)	(97–)122–170(–223)	120–250
Ascomatal necks	Undulating at apices	Straight	Straight	Straight	Straight	Straight
Length	(419–)498–644(–714)	310–1,010	(515–)615–851 (–1,021)	(691–)745–840(–889)	(245–)368–520(–599)	440–770
Width (bases)	(19–)23–35(–56)	20–45	(24–)28–38(–46)	(27–)31–39(–46)	(19–)23–29(–34)	28–40
Width (apices)	(12–)15–21(–25)	12–25	(14–)17–23(–28)	(14–)16–20(–22)	(12–)15–21(–25)	16–24
Ostiolar hyphae						
Shape	Divergent	Divergent	Divergent	Divergent	Divergent	Divergent
Length	(34–)39–49(–59)	30–125	(29–)32–42(–50)	(35–)41–49(–54)	(40–)45–61(–72)	20–120
Ascospores						
Length	3–5	3–4	3–4	3–6	3–5	3–4.5
Width (excluding sheath)	4–6	4.5–6.5	3–6	4–7	4–7	3.5–5
Width (including sheath)	5–8	3.5–5.5	5–8	5–8	6–8	5–7.5
Primary phialides						
Length	(44–)55–95(–139)	12–85	(60–)72–94(–98)	(75–)80–114(–152)	(58–)82–132(–162)	27–60
Width (bases)	4–6	N.A.	4–6	(4–)5–7(–8)	4–7	N.A.
Width (broadest point)	4–7	2–9	5–7	5–9	4–9	4–8.5
Width (apices)	3–4	2–6.5	3–5	(3–)4–6(–7)	3–6	3–6
Secondary phialides						
Length	(34–)42–66(–80)	Present (sizes N.A.)	(52–)61–85(–98)	(38–)48–76(–89)	(40–)49–65(–68)	Absent
Width (bases)	3–6	Present (sizes N.A.)	3–6	(3–)5–7(–8)	4–6	Absent
Width (apices)	4–6	Present (sizes N.A.)	4–6	(3–)5–7(–8)	5–7	Absent
Primary conidia						
Length	(10–)13–21(–31)	8–40	(9–)14–20(–24)	(11–)15–27(–30)	(10–)13–21(–30)	9–33
Width	3–6	2.5–5	3–5	(3–)5–6	4–6	3.5–5
Secondary conidia						
Length	7–11	Present (sizes N.A.)	7–9(–11)	(6–)10–11	(5–)8–10(–11)	Absent
Width	4–8	Present (sizes N.A.)	4–7	(4–)5–7(–9)	4–8	Absent
Chlamydospores						
Shape	Globose to perprolate	Globose to pyriform	Globose to subglobose	Globose	Pyriform to obpyriform	Globose to pyriform
Length	(7–)9–11(–12)	10–20(–37)	(9–)11–16(–16)	(8–)10–12(–13)	(12–)14–18(–19)	11–16
Width	11–17	3.5–11.5	(7–)9–11(–12)	(9–)10–14(–16)	(9–)10–12(–15)	6.5–12
Reference	This study	Engelbrecht and Harrington 2005	This study	Rodas et al. 2008	This study	Engelbrecht and Harrington 2005

All measurements are in μm. (Measurements not available are denoted as N.A.)

wide at widest point in middle, 3–5 μm wide at apices. *Secondary conidiophore*, tubular, flaring, hyaline, (52–) 61–85 (–98) μm long, 3–6 μm wide at bases, 4–6 μm wide at apices. *Conidia* of two types: *Primary conidia*, hyaline, aseptate, cylindrical, (9–) 14–20 (–24)×3–5 μm. *Secondary conidia*, hyaline, aseptate, barrel-shaped 7–9 (–11)×4–7 μm. *Chlamydospores* dark brown, thick walled, globose to sub–globose, (9–) 11–15 (–16)(7–) 9–11 (–12) μm.

Habitat: Freshly wounded wood of *Eucalyptus deglupta*.

Known distribution: Ecuador.

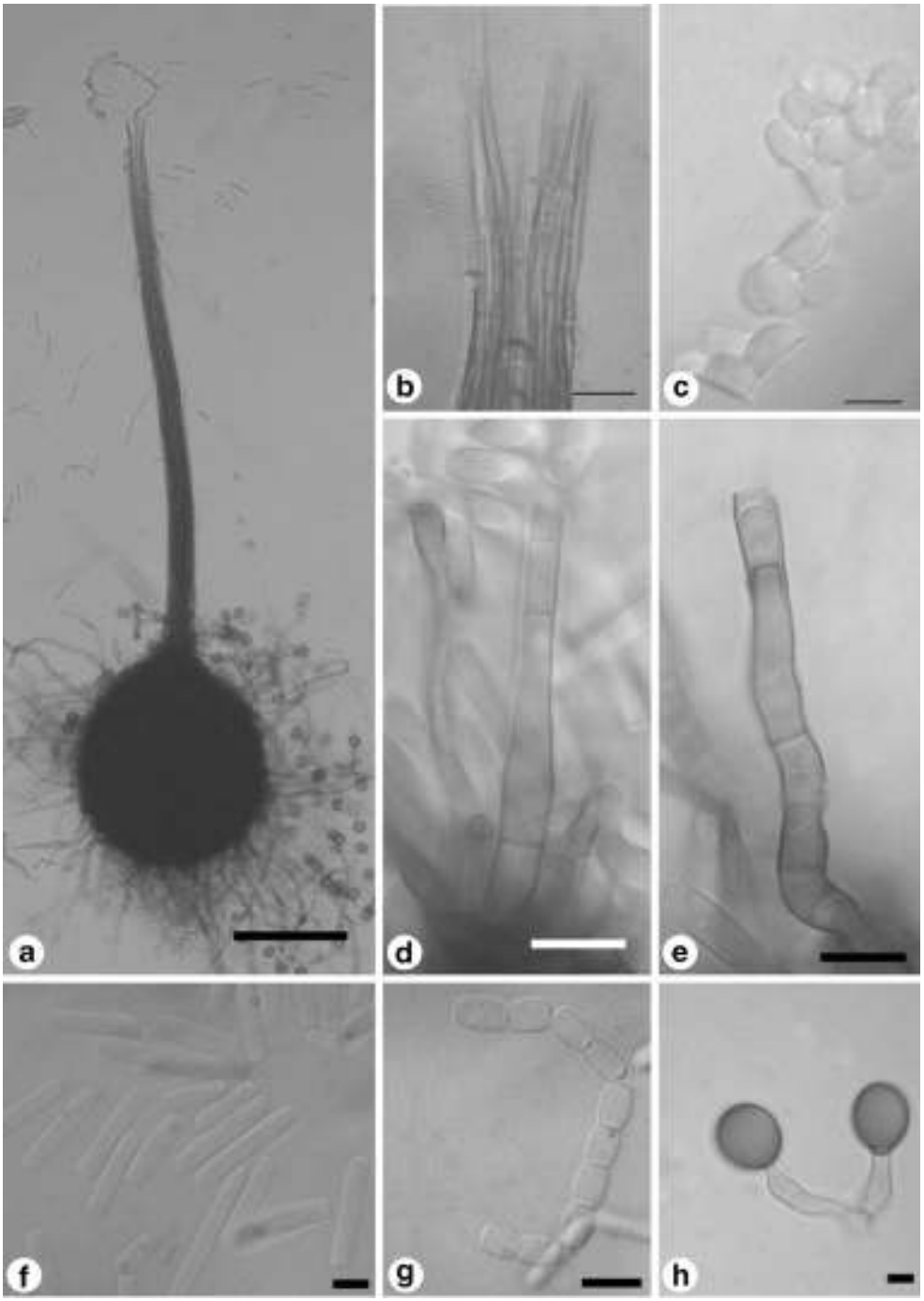

Fig. 8 Morphological characteristics of *Ceratocystis ecuadoriana.* **a** Ascoma with globose base. **b** Divergent ostiolar hyphae. **c** Hat-shaped ascospores. **d** Primary conidiophore, flask-shaped phialides. **e** Secondary conidiophore, tubular flaring at apex. **f** Cylindrical conidia. **g** Chain of barrel-shaped conidia. **h** Dark, globose to sub-globose chlamydospores Bars; **a** = 100 μm. **c, f, h** = 5 μm. **b, d, e, g** = 10 μm

Material examined: Ecuador, near Salinas isolated from wood of *E. deglupta*, 2004, M.J. Wingfield (PREM60155 – holotype), live strain: CMW22092, CBS124020.

Additional specimens examined (paratypes): same data (PREM60156), live strain CMW22093, CBS124021; same data (PREM60158), live strain CMW22097, CBS124022; same data (PREM60157), live strain CMW22405.

Notes: There is no single morphological characteristic that distinguishes this species from other species within the *C. fimbriata s.l.* complex. It does, however, have very long ascomatal necks when compared to other species. Differences in the sizes and shapes of various other structures are also seen when compared to the other species within the *C. fimbriata s.l.* complex (Table 3).

C. diversiconidia M. van Wyk & M.J. Wingf. **sp. nov.** (Fig. 9a–i)

MB 512848

Etymology: Name reflects the wide range of variation in size of both the cylindrical and barrel shaped conidia.

Bases ascomatum nigrae globosae, (97–) 115–155 (–196) μm longae, (97–) 122–170 (–223) μm latae. *Colla ascomatum* atrobrunnea vel nigra, (245–) 368–520 (–599) μm longa, basi

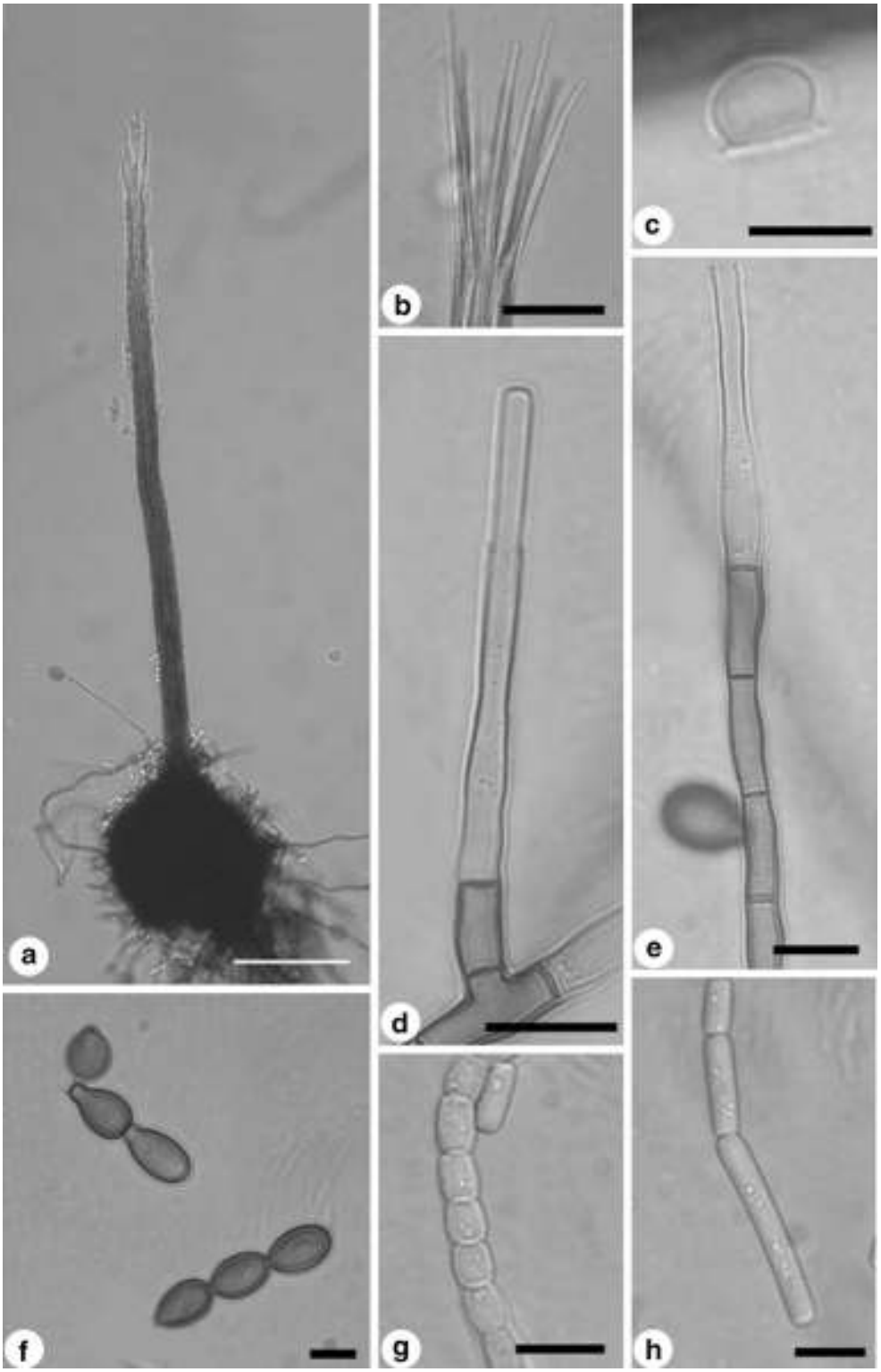

Fig. 9 Morphological characteristics of *Ceratocystis diversiconidia.* **a** Ascoma with globose base. **b** Divergent ostiolar hyphae. **c** Hat-shaped ascospore. **d** Primary conidiophore, flask-shaped phialides. **e** Secondary conidiophore, flask-shaped but flaring at apex. **f** Dark, pyriform to obpyriform chlamydospores. **g** Chain of barrel-shaped conidia with different sizes. **h** Primary conidia, cylindrical to oblong with truncated ends. Bars; **a** = 100 μm. **b** = 20 μm. **c** = 5 μm. **d–h** = 10 μm

(19–) 23–29 (–34) μm lata. *Hyphae ostiolares* divergentes, hyalinae, (40–) 45–61 (–72) μm longae. Conidiophora biformia in mycelio singuli disposita, *primaria* phialidica hyalina basi tumescentia, apicem versus contracta (58–) 82–132 (–162) μm longa; *secondaria* phialidica expansa hyalina, (40–) 49–65 (–68) μm longa.

Colony brownish olive (19″m) on malt extract agar. *Mycelium* submerged and aerial. *Optimal temperature* for growth 25°C, no growth below 15°C and above 35°C. *Hyphae* smooth, not constricted at septa. *Ascomatal bases* black, globose no ornamentation, bases (97–) 115–155 (–196) μm long, (97–) 122–170 (–223) μm wide, (97–)

119–163 (–210) μm in diam. *Ascomatal necks* dark brown to black, (245–) 368–520 (–599) μm long, (12–) 15–21 (–25) μm wide at apices, (19–) 23–29 (–34) μm wide at bases. *Ostiolar hyphae* divergent, hyaline, (40–) 45–61 (–72) μm long. *Asci* not observed. *Ascospores* hat-shaped in side view, aseptate, hyaline, invested in sheath, 3–5×4–7 μm without sheath, 3–5×6–8 μm with sheath. Ascospores accumulating in buff-yellow (19 d) mucilaginous masses on the apices of ascomatal necks.

Thielaviopsis anamorph: *Conidiophores* of two types occurring singly on mycelium, *primary conidiophores*, phialidic, hyaline, swollen at the base, tapering towards the apices, (58–) 82–132 (–162) μm long, 4–7 μm wide at bases, 4–9 μm wide at widest point in middle, 3–6 μm wide at apices. *Secondary conidiophores*, phialidic, flaring, hyaline, (40–) 49–65 (–68) μm long, 4–6 μm wide at bases, 5–7 μm wide at apices. *Conidia* of two types: *Primary conidia*, hyaline, aseptate, cylindrical to oblong, apices truncate, (10–) 13–21 (–30)×4–6 μm. *Secondary conidia*, hyaline, aseptate, barrel-shaped (5–) 8–10 (–11)×4–8 μm. *Chlamydospores* dark brown, thick walled, pyriform to obpyriform, (12–) 14–18 (–19)×(9–) 10–12 (–15) μm.

Habitat: Freshly wounded wood of *Terminalia ivorensis*.

Known distribution: Ecuador.

Material examined: Ecuador, near Salinas, isolated from wood of *T. ivorensis*, 2004, M.J. Wingfield (PREM60160 – holotype), live strain: CMW22445, CBS123013.

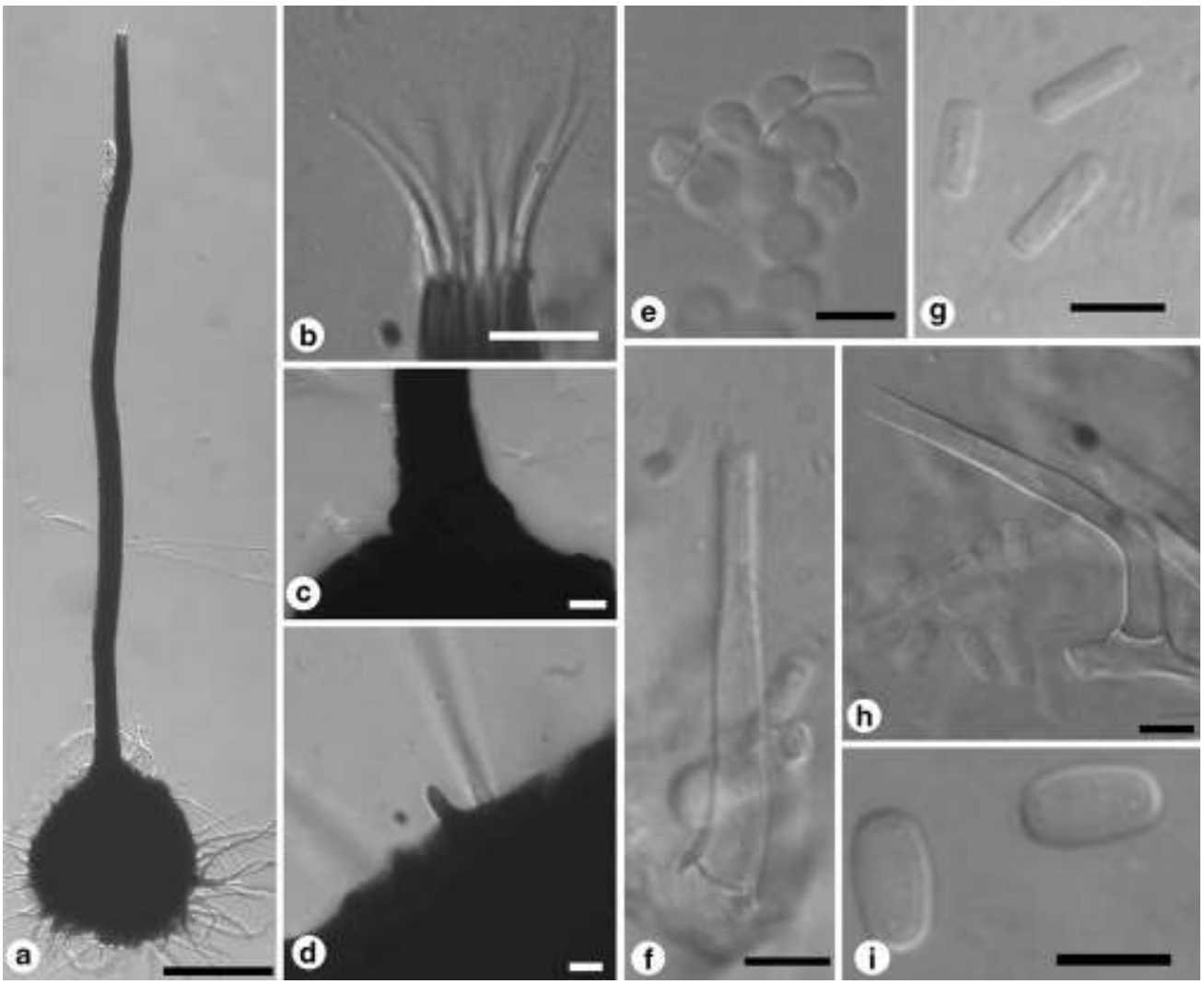

Fig. 10 Morphological characteristics of *Ceratocystis sublaevis*. **a** Ascoma with globose base with limited amount of conical spines. **b** Divergent ostiolar hyphae. **c** Ascomatal neck disk-shaped at base. **d** One of a limited number of conical spines on ascomatal base. **e** Hat-shaped ascospores. **f** Primary conidiophore, flask-shaped phialides. **g** Primary conidia, cylindrical with truncated ends. **h** Secondary conidiophore, tubular flaring at apex. **i** Barrel-shaped conidia. Bars; **a** = 100 μm. **b** = 10 μm. **c–i** = 5 μm

Additional specimens examined (paratypes): same data (PREM60162), live strain CMW22448, CBS122605; same data (PREM60161), live strain CMW22446; same data (PREM60159), live strain CMW22447, CBS122818.

Notes: *Ceratocystis diversiconidia* is distinct from most species in the *C. fimbriata s.l.* complex due to its diverse conidial and chlamydospore sizes. There are also differences in sizes of various other structures when compared to other species in the *C. fimbriata s.l.* complex. (Table 3).

C. sublaevis M. van Wyk & M.J. Wingf., **sp. nov.** (Fig. 10a–h)

MB 512849.

Etymology: The name refers to the limited number of conical spines on the ascomatal bases that are somewhat smooth in comparison to other species in this group.

Colonia in MEA alba vel laete brunnea. *Mycelium* abundans, plerumque aerium. *Bases ascomatum* nigrae globosae sparse spinis nigris et hyphis ornatae, spinae (2–) 4–10 (–13) μm, bases (98–) 131–173 (–187) μm longae, (102–) 144–192 (–231) μm latae. *Colla ascomatum* atrobrunnea vel nigra (100–) 137–183 (–209) μm longa, discoidea vel papillata. *Hyphae ostiolares* divergentes, hyalinae, (15–) 18–24 (–25) μm longae. Conidiophora biformia in mycelio singuli disposita, *primaria* phialidica hyalina basi tumescentia, apicem versus contracta (15–) 23–37 (–50) μm longa; *secondaria* tubularia apicem versus non contracta, hyalina rara (19–) 25–34 (–36) μm longa. *Conidia* biformia: *primaria* hyalina non septata cylindrica 5–8×1–3 μm; *secondaria* hyalina non septata doliiformia rara 3–6×2–3 μm.

Colony white to light brown (19″f) on malt extract agar. *Mycelium* abundant mostly aerial. *Optimal temperature* range for growth 20–30°C, no growth below 15°C and above 35°C. *Hyphae* smooth, not constricted at septa. *Ascomatal bases* black, globose, ornamented sparsely with spines and hyphae, spines black, (2–) 4–10 (–13) μm long, bases (98–) 131–173 (–187) μm long, (102–) 144–192 (–231) μm wide, (100–) 137–183 (–209) μm in diam. *Ascomatal necks* dark brown to black, undulating, (522–) 598–802 (–990) μm long, (9–) 12–16 (–19) μm wide at apices, (24–) 31–45 (–55) μm wide at bases, with a disk to papillate base. *Ostiolar hyphae* divergent, hyaline, (15–) 18–24 (–25) μm long. *Asci* not observed. *Ascospores* hat-shaped in side view, aseptate, hyaline, invested in sheath, 2–4×3–5 μm without sheath, 2–4×4–6 μm with sheath. Ascospores accumulating in buff-yellow (19 d) mucilaginous masses on the apices of ascomatal necks.

Thielaviopsis anamorph: *Conidiophores* of two types occurring singly on mycelium, *primary conidiophores*, phialidic, hyaline, swollen at the base, tapering towards the apices, (15–) 23–37 (–50) μm long, 2–5 μm wide at bases, 2–5 μm wide at widest point in middle, 1–3 μm wide at apices. *Secondary conidiophores*, tubular, not-tapering towards apices, hyaline, scarce, (19–) 25–34 (–36) μm long, 2–4 μm wide at bases, 2–4 μm wide at apices. *Conidia* of two types: *Primary conidia*, hyaline, aseptate, cylindrical, 5–8×1–3 μm. *Secondary conidia*, hyaline, aseptate, scarce, barrel-shaped 3–6×2–3 μm.

Habitat: Freshly wounded wood of *Eucalyptus deglupta* and *Terminalia ivorensis*.

Known distribution: Ecuador.

Material examined: Ecuador, Salinas area, isolated from freshly wounded wood of *T. ivorensis*, 2004, M.J. Wingfield (PREM60164 – holotype), live strain: CMW22444, CBS122518.

Additional specimens examined (paratypes): Ecuador, isolated from wood of *T. ivorensis*, 2004, M.J. Wingfield (PREM60163), live strain CMW22449, CBS122517, Ecuador, isolated from wood of *E. deglupta*, 2004, M.J. Wingfield (PREM60165), live strain CMW22415; Ecuador, isolated from wood of *E. deglupta*, 2004, M.J. Wingfield (PREM60166), live strain CMW22422, CBS122603.

Notes: *Ceratocystis sublaevis* is morphologically distinct from all other species in the *C. moniliformis s.l.* complex. The most obvious distinguishing characteristic is found in its reatively small number of conical spines on the ascomatal bases. The very distinct disk-shaped structures at the bases of the ascomatal necks, typical of this group of fungi, is also very small in comparison to those seen in other species (Table 4).

Discussion

Five *Ceratocystis* spp. were isolated from freshly made wounds on the stems of plantation-grown trees in the lowland forests of Ecuador. Two of these species, *C. sublaevis* and *C. moniliformis s.s.* reside in the *C. moniliformis s.l.* species complex. The three remaining species reside in the *C. fimbriata s.l.* complex and they have been provided with the names *C. curvata, C. ecuadoriana* and *C. diversiconidia*. Both *C. ecuadoriana* and *C. curvata* strains were isolated from *Eucalyptus* trees, while *C. diversiconidia* was isolated from *Terminalia* trees. The *C. moniliformis s.s.* isolates were isolated from *Schizolobium* while *C. sublaevis* was found on both *Eucalyptus* and *Terminalia* trees.

The three new Ecuadorian *Ceratocystis* spp. in the *C. fimbriata s.l.* complex reside in three distinct phylogenetic groups. *Ceratocystis curvata* in clade CF1 is most closely related to *C. colombiana*. The latter species is a pathogen of various tree crops (Van Wyk et al. 2010a) in Colombia that is geographically close to Ecuador where *C. curvata* was found. *Ceratocystis ecuadoriana* in clade CF2 was sister to *C. neglecta* that is a pathogen of *Eucalyptus* trees also in Colombia (Rodas et al. 2008). In contrast, *C. diversiconidia* in clade CF3 was distant from other species in the *C. fimbriata s.l.* complex without

Table 4 Comparison of morphological characteristics for *C. moniliformis s.s.*, *C. sublaevis* and *C. tribiliformis*

Species	*C. moniliformis s.s.*	*C. sublaevis*	*C. tribiliformis*
Characteristics			
Ascomatal bases			
Shape	Globose	Globose	Globose to obpyriform
Length	N.A.	(98–)131–173(–187)	N.A.
Width	N.A.	(102–)144–192(–231)	N.A
Diam	90–180	(100–)137–183(–209)	(196–)203–249(–264)
Conical spines - length	12–16	(2–)4–10(–13)	(4–)6–10(–12)
Ascomatal necks	Straight	Undulating	Straight
Length	N.A	(522–)598–802(–990)	(741–)615–851(–1,047)
Width (bases)	N.A	(24–)31–45(–55)	(43–)44–50(–53)
Width (apices)	N.A.	(9–)12–16(–19)	(13–)14–18(–20)
Ostiolar hyphae			
Shape	Divergent	Divergent	Divergent
Length	12–18	(15–)18–24(–25)	(22–)25–31(–32)
Ascospores			
Length	3–4	2–4	2–3
Width (excluding sheath)	4–5	3–4	4–5
Width (including sheath)	N.A.	4–6	5–6
Primary phialides			
Length	N.A.	(15–)23–37(–50)	(21–)72–94(–46)
Width (bases)		2–5	3–4
Width (broadest point)		2–5	N.A.
Width (apices)		1–3	1–3
Secondary phialides	Absent		
Length		(19–)25–34(–36)	N.A.
Width (bases)		2–4	N.A.
Width (apices)		2–4	N.A.
Primary conidia			
Length	6–8	5–8	7–9
Width	1.8–2.2	1–3	2
Secondary conidia	Absent		
Length		3–6	7–9
Width		2–3	3–4
Chlamydospores	Absent	Absent	Absent
Shape			
Length			
Width			
Reference	Hedgcock 1906	This study	Van Wyk et al. 2006a

All measurements are in μm. (Measuring data not available denoted as N.A.)

clearly defined phylogenetic neighbours. Based on phylogenetic inference, it should not be difficult to recognise these three new species from other taxa in the *C. fimbriata s.l.* complex that have been defined in previous studies (Johnson et al. 2005; Van Wyk et al. 2004, 2007b).

It was not unusual to isolate *C. moniliformis* from wounds on trees in Ecuador as this fungus has previously been found in a similar habitat in the country (Geldenhuis et al. 2004). The new species, *C. sublaevis,* residing in the *C. moniliformis s.l.* complex was phylogenetically distinct from all species in this group. It was, however, closest related to *C. moniliformis s.s.* No differences were observed in the sequences of the ITS gene region when comparing isolates representing *C. moniliformis s.s.* and the isolates representing *C. sublaevis.* This was expected as it is known that, in contrast to isolates in the *C. fimbriata s.l.* complex, isolates in the *C. moniliformis s.l.* complex have no differentiation in the ITS gene region, while the EF1-α gene region has the highest degree of resolution (Van Wyk et al. 2006a, 2010b).

The morphological characteristics of species in the *C. fimbriata s.l.* complex are very similar and new species

can be recognised with confidence only through the application of phylogenetic inference. Yet, *C. curvata* has very distinct undulating ascomatal necks, *C. diversiconidia* has conidia and chlamydospores that differ significantly in size from other species in the group and *C. ecuadoriana* has unusually long ascomatal necks, which should assist in their identification.

Ceratocystis sublaevis can be distinguished from other members of the *C. moniliformis s.l.* complex morphologically. The most distinct differences observed are the limited occurrences of conical spines on the ascomatal bases as well as its unusually small basal plateau giving rise to the ascomatal necks.

Many *Ceratocystis* species are important plant pathogens (Kile 1993; Roux and Wingfield 2009). In this regard, nothing is known regarding the pathogenicity of the new *Ceratocystis* spp. described in this study. Given that there were no disease symptoms found on the wounded trees, it is possible that they all represent wound-infecting saprophytes. It would be worthwhile to consider their pathogenicity on the hosts from which they were isolated. In contrast, species in the *C. moniliformis s.l.* complex are typically non-pathogenic colonists of freshly wounded wood (Van Wyk et al. 2006a; Heath et al. 2009) and this is probably true for *C. moniliformis s.s.* and *C. sublaevis* found in this study.

The discovery of a relatively large number of new taxa in a well-known group of fungi, reflects strongly the fact that these fungi have been poorly studied in Ecuador. It is likely that similar studies in that country will yield additional new species in this group and some of these are potentially important pathogens. Their discovery will enhance the understanding of fungal diversity in Ecuador and their description would augment a growing understanding of an intriguing and ecologically important group.

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