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Reconsidering species boundaries in the *Ceratocystis paradoxa* complex, including a new species from oil palm and cacao in Cameroon

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Abstract: The *Ceratocystis paradoxa* complex accommodates a group of fungal pathogens that have become specialized to infect mostly monocotyledonous plants. Four species currently are recognized in this group, including *C. paradoxa*, which has a widespread distribution and broad host range. In this study, multigene phylogenetic analyses involving sequences of the ITS, β-tubulin and TEF-1α gene loci, in combination with phenotypic and mating studies, were used to characterize purported *C. paradoxa* isolates from Cameroon and to compare them with isolates from elsewhere, including protologs and type specimens of known species. We show that the *C. paradoxa* complex comprises substantially greater species diversity than previously recognized. One new species in this group is described from Cameroon as *Ceratocystis cerberus*, while *C. paradoxa* sensu stricto (s. str.) and four other species are redefined. Lectotypes are designated for *C. ethacetica* and *Endoconidium fragrans* (synonym of *C. ethacetica*), while epitypes are designated for *C. paradoxa* s. str., *C. ethacetica* and *C. musarum*. A neotype is designated for *Catenularia echinata* (synonym of *C. ethacetica*) and two species, previously treated in *Thielaviopsis*, are transferred to *Ceratocystis*.

Key words: Ceratocystidaceae, Microascales, phylogenetic species concept, *Thielaviopsis*

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Introduction

Ceratocystis paradoxa is a soilborne fungal plant pathogen, widely distributed across the globe and capable of infecting a broad range of hosts (Morgan-Jones 1967, Anonymous 1987, Garofalo and McMillan 2004). First discovered associated with black rot of pineapple fruit in France by de Seynes (1886), the impact of this fungus is best known from tropical, subtropical and arid regions where it represents a serious constraint to the cultivation of monocotyledonous crops (Kile 1993, Garofalo and McMillan 2004). Some of the well known diseases caused by *C. paradoxa* include rots of pineapple, sett rot of sugarcane and bud and trunk rots affecting almost all species of palm (Garafalo and McMillan 2004, Girard and Rott 2004).

In most previous reports identification of *C. paradoxa* was based on morphological characters of the fungus and relied primarily on two studies of approximately 100 y ago. In the first of these, Petch (1910) reviewed all earlier work and produced a comprehensive account of the asexual state of the fungus. Dade (1928) subsequently described the sexual state and provided the first insights into the mating system of *C. paradoxa*. The power of DNA sequencing technologies and molecular phylogenetics, which have deeply influenced fungal taxonomy during the past two decades, has not yet been fully explored in the recognition and delineation of *C. paradoxa* and related species.

Ceratocystis belongs to the family Ceratocystidaceae, order Microascales in the Sordariomycetes (Réblová et al. 2011, de Beer et al. 2013a) and is characterized by ascomata with bulbous bases and long necks that give rise to ascospores in sticky masses (Hunt 1956, Upadhyay 1981, Wingfield et al. 1993, Seifert et al. 2013). All species have tubular, phialidic conidiophores with diverse forms of endoconidia. In addition, some species also produce thick-walled, pigmented conidia. Under the dual nomenclature system, the asexual states of *Ceratocystis* species were treated in the genus *Chalara* (Nag Raj and Kendrick 1975) and were more recently consolidated in *Thielaviopsis* (Paulin-Mahady et al. 2002). Based on DNA sequence analyses, it is now recognized that there are distinct phylogenetic lineages in *Ceratocystis*, defining complexes in which species have similar morphological and ecological characteristics (Harrington et al. 1996, Harrington and Wingfield 1998,

Baker et al. 2003, Johnson et al. 2005, van Wyk et al. 2006). Indeed, it has been suggested that some of these lineages represent discrete genera (Wingfield et al. 2013). The species related to *C. paradoxa*, sometimes referred to as *C. paradoxa* sensu lato (s. lat.), most likely represent one of these generic entities within *Ceratocystis*.

Four species currently are recognized in the *C. paradoxa* complex, but results from phylogenetic studies using sequences from single loci suggest that this group includes at least two undescribed species (Harrington 2009, Álvarez et al. 2012). The previously described species include *C. paradoxa*, *C. radicicola* (Bliss 1941), *C. musarum* (Riedl 1962) and *Thielaviopsis euricoi* (Batista and Vital 1956, Paulin-Mahady et al. 2002). The first two species are well known as soilborne and pathogenic to monocotyledonous plants (Garofalo and McMillan 2004, Abdullah et al. 2009). While *C. paradoxa* has been recognized as a cosmopolitan fungus recorded on various hosts and from many parts of the world (Morgan-Jones 1967, Anonymous 1987), little is known regarding the incidence of the other three species. For example, there are no reports of *C. musarum* or *T. euricoi* other than from their first descriptions.

The species in the *C. paradoxa* complex, of which the sexual states are known, typically produce characteristic digitate ornamentations on their ascomatal bases (Dade 1928, Bliss 1941). Like many other *Ceratocystis* species, the asexual states of these species often produce a variety of conidial forms in culture. In his generic diagnosis of *Thielaviopsis*, Went (1893) recognized two major types of conidia, endoconidia (also referred to as phialoconidia, enteroblastic-phialidic ameroconidia), as opposed to thick-walled, pigmented conidia produced in chains at the tips of specialized hyphae. In most of the earlier studies the various forms of endoconidia were simply described as a range or continuum (as ''cylindrical to ellipsoid/doliiform, hyaline to brown''; Bliss 1941, Hunt 1956, Nag Raj and Kendrick 1975, Upadhyay 1981). However, in the taxonomic literature of *Ceratocystis* during the past decade, the endoconidia have been described consistently as of two forms. These have been referred to as cylindrical versus doliform endoconidia (Baker-Engelbrecht and Harrington 2005, Johnson et al. 2005) or by other authors as primary versus secondary conidia (van Wyk et al. 2006, 2007, 2009, 2011; Heath et al. 2009). For the purpose of the present study we define the endoconidia produced in phialides as primary conidia when they are aseptate, hyaline and cylindrical. In contrast, secondary conidia are cylindrical to oblong, often doliiform (barrel-shaped), initially hyaline, turning brown and thick-walled with age.

In addition to the two forms of endoconidia, species in the *C. paradoxa* complex also form what have been referred to as chlamydospores or aleurioconidia. Went (1893) described and illustrated pigmented conidia in short chains on terminal ends of hyphae of *Thielaviopsis*, while Peyronel (1918) established *Chalaropsis* for species with aleuriospores borne solitarily at the end of a branched conidiophore (Hennebert 1967). These structures often have been called chlamydospores (Nag Raj and Kendrick 1975; Upadhyay 1981; van Wyk et al. 2007, 2009, 2011; Kamgan et al. 2008; Heath et al. 2009), but the term ''aleurioconidia'' was reintroduced to taxonomic studies in *Ceratocystis* by Paulin-Mahady et al. (2002). Seifert et al. (2011) use the term aleurioconidia for ''solitary, thallic conidia that secede rhexolytically, usually with thickened and sometimes darkened walls'' and distinguishes it from chlamydospores, described as ''thick-walled resting spores, intercalary or terminal on hyphae … usually not readily liberated''. We concur with the definition of aleurioconidia by Paulin-Mahady et al. (2002) and Harrington (2009) as thick-walled, survival spores produced singly or in chains from the tips of specialized hyphae, which also corresponds to the definition by Seifert et al. (2011).

The aim of this study is to characterize recent isolates resembling *C. paradoxa* s. lat. from oil palm (*Elaeis guineensis*), cacao (*Theobroma cacao*) and pineapple (*Ananas comosus*) in Cameroon, with multigene DNA phylogenies, morphology and mating tests. Species boundaries and previously suggested synonymies of all species in the complex also are reconsidered. For this purpose, the Cameroonian isolates were compared with herbarium specimens and representative isolates of known species.

MATERIALS AND METHODS

Isolates.—Those forming the core material of this study were collected in Cameroon in 2009 and 2010, during field surveys for *Ceratocystis* species occurring on pineapple, oil palm and cacao. The surveys were conducted at various localities of the central, littoral and southwestern regions of the country. On cacao, fungi resembling *C. paradoxa* s. lat. were isolated mostly from discarded pod husks (FIG. 1), but a few isolates also were obtained from artificially induced wounds on the stems of cacao trees. Oil palm isolates were obtained from trunks and stumps of felled trees, cut basal ends of leaf fronds and rotting palm-nut bundles (FIG. 1). Pineapple isolates were obtained from rotting fruits, fruit peduncle sections and damaged leaves. Fungal structures, including mycelium and less commonly ascomata (only from cacao pod husks), generally were observed directly on plant tissue in the field. When they were not present, incubation of tissue samples in moist chambers for a few days was useful for inducing growth and sporulation.

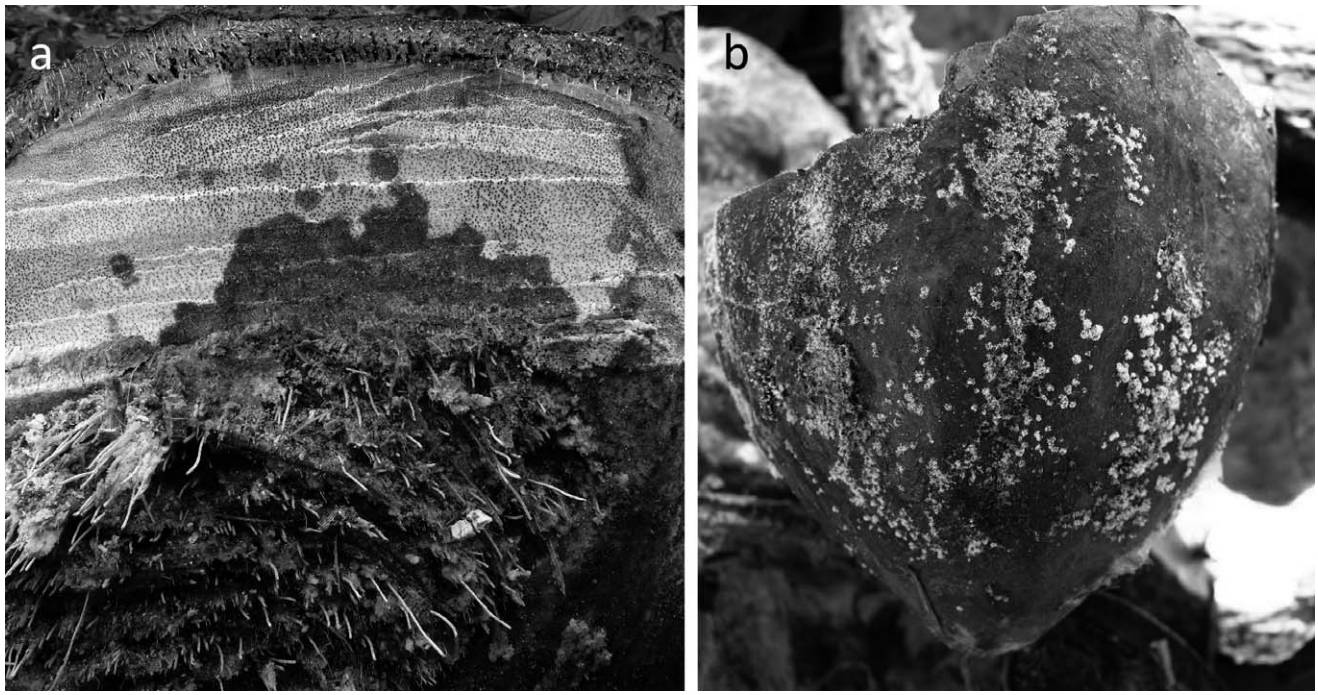

FIG. 1. Symptoms of infection by *Ceratocystis paradoxa* s. lat. a. Basal section of felled *Elaeis guineensis* tree. b. External surface of *Theobroma cacao* pod husk.

Isolations were made by aseptically lifting a few strands of aerial mycelium or single ascospore droplets from the surfaces of infected plant material with a sterile needle and transferring these to sterile 2% malt extract agar (MEA) (Biolab, Midrand, South Africa), amended with ~ 0.01 g/L streptomycin sulphate (Sigma, Steinheim, Germany). Isolates were purified further by subculturing from single hyphal tips, and they were maintained on MEA.

The *Ceratocystis* collections from Cameroon were supplemented with cultures obtained from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, and the Commonwealth Agricultural Bureau International Bioscience (CABI), UK. Additional isolates were sourced from international fungal culture collections and specifically chosen to represent different hosts and geographic regions. All fungal isolates included in this study are maintained in the culture collection (CMW) at FABI (TABLE I). Herbarium specimens of types of *Ceratocystis musarum*, *Ceratostomella paradoxa* and *Stilbochalara dimorpha* also were investigated (see TAXONOMY).

DNA extraction, PCR and sequencing.—DNA was extracted from 7 d old cultures maintained on MEA at 25 C. Mycelium was scraped from the surfaces of cultures and transferred to Eppendorf tubes for freeze-drying. Dried mycelium was crushed into a fine powder in a Retsch cell disrupter (Retsch Gmbh, Germany); thereafter total genomic DNA was isolated with the CTAB (cetyl trimethyl ammonium bromide) protocol described by Möller et al. (1992). Final DNA working concentrations were adjusted to ~ 75 ηg μL^{-1}, with a Thermo Scientific NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware).

For primary identification of *Ceratocystis* isolates, the internal transcribed spacer regions, ITS1 and ITS2 and intervening 5.8S rDNA of the ribosomal RNA gene cluster (ITS) were amplified with the polymerase chain reaction (PCR) and sequenced for all isolates. Two additional gene regions, including part of the beta tubulin gene (β-tubulin) and part of the translation elongation factor 1-alpha gene (TEF-1α), were sequenced for selected isolates and used in phylogenetic reconstructions. The oligonucleotide primer pairs included in the reactions were respectively the ITS1 (5′–TCCGTAGGTGAACCTGCGG–3′) and ITS4 (5′–TCCTCCGCTTATTGATATGC–3′) for the ITS region (White et al. 1990), βt1a (5′–TTCCCCCGTCTCCACTTCTTCATG–3′) and βt1b (5′–GACGAGATCGTTCATGTTGAACTC–3′) for the β-tubulin region (Glass and Donaldson 1995), and EF1F (5′–TGCGGTGGTATCGACAAGCGT–3′) and EF2R (5′–AGCATGTTGTCGCCGTTGAAG–3′) for the TEF-1α region (Jacobs et al. 2004). PCR reaction mixtures contained 1 μL DNA template working solution, 0.5 μL each oligonucleotide primer (10 mM), 0.5 μL MyTaq™ DNA polymerase (Bioline) and 5 μL 5× MyTaq™ reaction buffer (supplied with the enzyme). The final reaction volumes were adjusted to 25 μL with sterile distilled water (SABAX water, Adcock Ingram Ltd, Bryanston, South Africa). PCR reactions were performed on a Biorad thermo-cycler (BIO-RAD, Hercules, California). The same cycling conditions were applied for both the ITS and β-tubulin regions, which comprised an initial denaturation step at 96 C for 2 min, followed by 35 cycles of 30 s at 94 C (denaturation), 60 s at 54 C (annealing), 90 s at 72 C

TABLE I. Isolates of *C. paradoxa* s. lat. included in this study

Species name	CMW No.	Other	GenBank accession Nos. ITS	β-tubulin	TEF-1α	Host	Origin	Collector (supplier)
C. cerberus sp. nov.	35021[b]	CBS 130763	JX518355	JX518387	JX518323	*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	35024		JX518356	JX518388	JX518324	*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36641		JX518345	JX518377	JX518313	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36653[b]	CBS 130764	JX518349	JX518381	JX518317	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36668[b]	CBS 130765	JX518348	JX518380	JX518316	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
C. ethacetica	14790	CBS 374.83	JX518329	JX518361	JX518297	*Phoenix canariensis*	Spain	(CBS)
	28533	CBS 601.70	JX518331	JX518363	JX518299	*Ananas comosus*	Brazil	M. Barreto Figueiredo (CBS)
	28534	CBS 453.66	JX518332	JX518364	JX518300	*Cocos nucifera*	Nigeria	O.A. Akinrefon (CBS)
	36644[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36662[a,b]		JX518353	JX518385	JX518321	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36671[a,b]	CBS 130757	JX518351	JX518383	JX518319	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36691[b]		JX518343	JX518375	JX518311	*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36725[a,b]	CBS 130758	JX518347	JX518379	JX518315	*Ananas comosus*	Cameroon	M. Mbenoun & J. Roux
	36735[a]					*Ananas comosus*	Cameroon	M. Mbenoun & J. Roux
	36741[a,b]	CBS 130759	JX518354	JX518386	JX518322	*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36743[a]					*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36745[a]					*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36747[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36771		JX518330	JX518362	JX518298	*Saccharum* sp.	South Africa	N. van Wyk (FABI)
	37450	CBS 128.32	JX518337	JX518369	JX518305	*Elaeis guineensis*	NA	(CBS)
	37775	IMI 50560	JX518341	JX518373	JX518309	*Ananas comosus*	Malaysia	(CABI)
	37777	IMI 344082	JX518339	JX518371	JX518307	*Cocos nucifera*	Tanzania	(CABI)
	37778	IMI 378943	JX518340	JX518372	JX518308	*Elaeis guineensis*	Papua New Guinea	(CABI)
C. euricoi	8788		JX518326	JX518358	JX518294	*Cocos nucifera*	Indonesia	M.J. Wingfield (FABI)
	8790		JX518327	JX518359	JX518295	*Cocos nucifera*	Indonesia	M.J. Wingfield (FABI)
	8799[b]		JX518328	JX518360	JX518296	*Cocos nucifera*	Indonesia	M.J. Wingfield (FABI)
	28537[b]	CBS 893.70	JX518335	JX518367	JX518303	NA	Brazil	E. de Matta (CBS)
	28538[b]	CBS 107.22	JX518336	JX518368	JX518304	*Cocos nucifera*	NA	(CBS)
C. paradoxa s. str.	36642[a,b]	CBS 130760	JX518346	JX518378	JX518314	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36650[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36654[a,b]		JX518350	JX518382	JX518318	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36655[a,b]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36674[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36683[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36686[a,b]	CBS 130762	JX518352	JX518384	JX518320	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	36689[a,b]	CBS 130761	JX518342	JX518374	JX518310	*Theobroma cacao*	Cameroon	M. Mbenoun & J. Roux
	36754[b]		JX518344	JX518376	JX518312	*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux
	37755[a]					*Elaeis guineensis*	Cameroon	M. Mbenoun & J. Roux

TABLE I. Continued

Species name	CMW No.	Other	GenBank accession Nos. ITS	β-tubulin	TEF-1α	Host	Origin	Collector (supplier)
C. paradoxa s. lat. 1	28535	CBS 101054	JX518333	JX518365	JX518301	*Rosa* sp.	Netherlands	J.W. Veenbaas- Rijks (CBS)
C. paradoxa s. lat. 2	28536	CBS 116770	JX518334	JX518366	JX518302	Palm species	Ecuador	M.C. Aime (CBS)
C. musarum	1546[a]		JX518325	JX518357	JX518293	*Musa* sp.	New Zealand	T.W. Canter-Visscher (FABI)
C. radicicola	1032	CBS 114.47	KF612023	KF612025	KF612024	*Phoenix dactylifera*	USA	D.E. Bliss
	26389	CBS 167.67	KF953932	KF953931	KF917202	*Lawsonia inermis*	Mauritania	G.L. Hennebert
	37776[b]	IM I 316225	JX518338	JX518370	JX518306	*Phoenix dactylifera*	Iraq	(CABI)

CABI: Commonwealth Agricultural Bureaux International Bioscience, formerly International Mycological Institute (IMI).
CBS: Centraalbureau voor Schimmelcultures.
CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.
[a] Isolates used in mating tests.
[b] Isolate used for microscopic and/or growth studies.
NA: Data not available.

(extension) and a final extension step at 72 C for 10 min to complete the reaction. For the TEF-1α gene region, the cycling conditions included 4 min initial denaturation at 96 C, 10 cycles of 40 s at 94 C, 40 s at 55 C (annealing step) and 45 s at 72 C (extension), followed by 30 additional cycles of the same sequence, with a 5 s increase in the annealing step per cycle. Reactions were completed by a final extension step at 72 C for 10 min. To confirm amplification of targeted gene regions, 4 μL aliquots reaction products were mixed with 1.5 μL GelRed™ (Biotium Inc., USA) dye and electrophoresed on 2% agarose gels with a DNA molecular weight marker (100 bp ladder) (Fermentas O'Gene Ruler™) and visualized under UV light.

PCR products were purified with 6% Sephadex G-50 (50–150 μm diam beads) columns (Sigma, Steinheim, Germany), following the manufacturer's instructions. Aliquots (4 μL) purified PCR products were used in the forward and reverse sequencing reactions, with the ABI PRISM™ BIG DYE Terminator Cycle Sequencing Ready Reaction kit (Applied BioSystems, 142 Foster City, California). In addition to the PCR products, sequencing mixtures contained 2.5 μL sequencing buffer, 0.5 μL Big Dye ready reaction mixture and 1 μL selected primer (10 mM). The final reaction volume was adjusted to 12 μL with sterile Sabax water. The sequencing PCR conditions comprised 25 cycles of a 10 s denaturation step at 96 C, 5 s annealing step at 52 C and a primer extension step at 60 C for 4 min. Sequencing products were cleaned with Sephadex G-50 columns and concentrated in an Eppendorf 5301 vacuum concentrator at 45 C. They were run on an ABI PRISM™ 3100 DNA Analyzer (Applied BioSystems, 142 Foster City, California). Bidirectional reads were assembled into consensus sequences and edited with CLC Main Workbench 6.1 software package (CLC Bio, Aarhus, Denmark).

Phylogenetic analyses.—As a means of primary identification and sorting of Cameroonian isolates, their ITS sequences were aligned with MUSCLE (Edgar 2004) as implemented in MEGA 5 (Tamura et al. 2011), and a neighbor-joining (NJ) phylogenetic tree was generated with MEGA. Representative sequences from each resulting group were submitted to BLASTN query in GenBank on NCBI (http://www.ncbi.nlm.nih.gov). The same BLASTN procedure was applied to the ITS sequences of all isolates obtained from the culture collections to confirm their identities. The NJ tree resolved the isolates from Cameroon into three groups, each showing 97–100% sequence similarity with *C. paradoxa* accessions in GenBank.

Five representatives of each NJ group, originating from different localities and hosts in Cameroon, were selected for phylogenetic analyses together with related CMW, CBS and CABI isolates (TABLE I). Three datasets, including sequences generated in this study and relevant GenBank accessions, were constructed for the three gene regions, as well as one dataset combining all gene regions. *Ceratocystis virescens*, isolate CMW 11164, (van Wyk et al. 2007) was used as the outgroup taxon in the analyses. Alignments were constructed with MAFFT 6 (http://www.align.bmr.kyushu-u.ac.jp/mafft/online/server/) (Katoh et al. 2005) and trimmed in

MEGA. The three gene regions were considered separately and in a three-partition combination, applying three different approaches of phylogenetic inference, that is Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP). Where applicable, models of nucleotide substitution were selected with jModelTest 2.2 (Posada 2008).

ML analyses were performed with the PhyML 3.0 online interface (Guindon et al. 2010 http://www.atgc-montpellier.fr/phyml/). Confidence support for branch nodes were estimated with 1000 bootstrap replications.

BI analyses based on Markov chain Monte Carlo (MCMC) algorithms were performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The MCMC procedure was initiated from a random tree topology and involved 1 000 000 random tree generations with four parallel chains and tree sampling every 100th generation. The software Tracer 1.5 (Rambaut and Drummond 2007) was used to examine the convergence of the chains from the distribution of posterior probabilities. The default burn-in of 10% of the generations (i.e. the first 1000 trees sampled), which fell beyond the point of convergence in all cases, was enforced during the construction of 50% majority rule consensus trees.

MP analyses were performed with PAUP 4.0b10* (Swofford 2002). Uninformative characters were excluded and gaps coded as a fifth character state. All characters were unordered and of equal weight. Trees were generated via a heuristic search, with random stepwise addition of 1000 replicates and tree bisection and reconstruction (TBR) branch-swapping algorithms. Branches of zero length were collapsed, and all multiple, equally parsimonious trees were saved. Statistical support for branch nodes in the most parsimonious trees (MPTs) was assessed with 1000 bootstrap replicates with the full heuristic search. Tree length (TL), consistency index (CI), retention index (RI), homoplasy index (HI) and rescaled consistency index (RC) also were calculated for MPTs. Finally, the genealogical concordance of the three gene loci was assessed with partition homogeneity tests (PHT) with 1000 heuristic search replicates (Swofford 2002).

Mating studies.—A trial was set to characterize the mating system of the putative *C. paradoxa* isolates from Cameroon and to produce possible sexual structures for morphological descriptions. This involved 10 randomly selected isolates from each discrete phylogenetic group, which produced only asexual structures. Single hyphal tip cultures were paired against themselves as well as against each other on 2% MEA and water agar (WA) plates in the presence of either sterile pineapple or sugarcane chips. This was done by plating the two isolates, opposite each other, approximately 5 cm apart, and separated by a line of plant material. Plates were incubated 2–4 wk in the dark at 25 C. The experiment was done in duplicate by preparing two plates for each pairing combination. Four isolates shown to represent opposite mating types from each group also were used in reciprocal crosses to assess the presence of reproductive barriers between isolates in different groups.

Morphology and taxonomy.—We studied growth and morphology of three isolates for each of the *Ceratocystis* species identified from Cameroon. For each isolate, five replicate plates were prepared by cutting out single agar plugs, with an 8 mm diam cork borer, from the margin of an actively growing culture on 2% MEA. These were transferred, mycelium side down, to the centers of 90 mm Petri dishes containing fresh, sterile 2% MEA. Plates were incubated 3 d in the dark at 10–35 C at 5 C intervals. Growth diameter measurements were taken for each colony on two axes at right angles, and averages were computed.

Morphological characteristics were determined for 10 d old cultures maintained on 2% MEA at their optimum growth temperature and herbarium specimens of type material when available. The color for fungal colonies and structures was determined with the mycological color charts of Rayner (1970). Microscope slides were prepared for fungal structures by mounting these in 85% lactic acid. Slides were examined under a Zeiss Axioskop microscope fitted with HRc Axiocam digital camera and Axiovision 3.1 software (Carl Zeiss Ltd, Germany) used for image capture and to determine sizes of structures. Where possible, 50 measurements were made for each taxonomically informative morphological character for isolates used as holotypes, with an additional 10 measurements for each isolate designated as a paratype. Mean and standard deviation values were computed for each character. These measurements are presented as the extremes in brackets and the range calculated as the mean of the overall measurements plus or minus the standard deviation.

Morphometric data and other phenotypic information for all previously known species related to *C. paradoxa* s. lat. were obtained from the literature. Additional information was generated through microscopic examination of isolates representing these species from isolates obtained from culture collections. A comparison of phenotypic features across all species in the *C. paradoxa* complex was made to identify possible diagnostic characters for each species considered in this study.

All isolates used as type cultures in morphological descriptions were deposited with the CBS culture collection. Dried herbarium specimens, including paired cultures of sexually compatible isolates, also have been deposited in the South African National Herbarium at the Plant Protection Research Institute (PREM), Pretoria, South Africa.

RESULTS

Isolates.—A total of 143 fungal isolates representing general growth and morphological characteristics of *C. paradoxa* s. lat. were collected in Cameroon. Of these, 22 were isolated from pineapple, 69 from oil palm and 52 from cacao (SUPPLEMENTARY TABLE I). Two additional isolates were collected from a fallen *Erytrophleum ivorense* tree alongside a cacao plantation after a windstorm. Based on NJ sequence comparisons of ITS sequence data, the Cameroonian isolates represented three distinct groups that we designated CMR1, CMR2 and CMR3. Group CMR1 included ~ 80% of isolates (SUPPLEMENTARY FIG. 1),

TABLE II. Summary of the phylogenetic information for the individual and combined nuclear regions used in this study

Dataset		ITS	β-tubulin	TEF-1α	Combined
Taxa (Nr)		49	36	41	35
Character (Nr)	Total	408	508	771	1684
	Variable	70	134	367	561
	constant	338	374	404	1123
MP	PIC (Nr)	40	88	269	382
	MPT (Nr)	1	2	10	70
	TL	60	164	770	911
	CI	0.817	0.787	0.666	0.720
	HI	0.183	0.213	0.334	0.280
	RI	0.959	0.959	0.902	0.917
	RC	0.783	0.754	0.601	0.660
BI/ML	Model	HKY+I	TIM+G	TVM+I+G	GTR+I+G
	Gamma shape	—	0.1670	0.9030	0.5730
	P-inv	0.7840	—	0.4090	0.4650
	ti/tv	2.4725	—	—	—

with all the isolates from pineapple, most (~ 90%) isolates from cacao as well as some (~ 57%) from oil palm. Group CMR2 was the second most prevalent and represented ~ 16% of the isolates, mostly from oil palm with a single isolate from cacao. Group CMR3 was the least prevalent of the three groups, representing less than 5% of the collections. It included isolates from oil palm and cacao, with cacao isolates originating from artificially induced trunk wounds in one orchard.

Phylogenetic analyses.—For the 32 putative *C. paradoxa* s. lat. isolates, DNA sequence data of approximately 500, 500 and 800 bp were generated respectively for the ITS, β-tubulin and TEF1-α loci (see TABLE I for GenBank accession numbers). A summary of the phylogenetic information for each of the three loci, as well as the combined dataset, is provided (TABLE II). There was a general concordance between the BI, ML and MP phylogenies in tree topology and phylogenetic relationship among taxa.

The ITS constituted the largest dataset but had the least resolution (SUPPLEMENTARY FIG. 2). Cameroonian isolates separated into three clades. Isolates in groups CMR1 and CMR2 formed two separate but monophyletic clades, forming a cluster with high statistical support, and each was composed of several GenBank accessions as well as isolates obtained from culture collections. Clade CMR1 had the greatest diversity in terms of hosts and geographic origin, with taxa originating from Cameroon and other countries in Africa, Asia, Europe, North and South America and Oceania, collected from banana (*Musa* sp.), *Butia* sp., cacao, date palm (*Phoenix* sp.), coconut palm, pineapple, oil palm and sugarcane (*Saccharum* sp.). Clade CMR2 included, apart from the isolates from Cameroon, the ex-type (CMW 28537 = CBS 893.70) of *Thielaviopsis euricoi*, which originally was collected from air in Brazil, isolate CMW 28535 = CBS 101054 from *Rosa* sp. in the Netherlands, five isolates collected from coconut palm (*Cocos nucifera*) in Brazil and Indonesia and one GenBank accession from Jamaica, also associated with coconut palm. Isolates in group CMR3 formed a phylogenetic clade, separate from CMR1 and CMR2 isolates, supported by strong bootstrap and posterior probability values. In addition to the isolates from Cameroon, this clade included one GenBank accession representing an isolate from a palm species in Colombia.

Apart from the three clades containing the Cameroon isolates, three other well supported clades were present in the ITS tree. One of these had a basal position and included an ex-type isolate (CMW 1032 = CBS 114.47) of *C. radicicola* and two additional taxa from date palm in three countries (Iraq, Oman, USA). The second clade was sister to group CMR3 isolates from Cameroon, including an isolate (CMW 28536 = CBS 116770) from a palm species in Ecuador and one GenBank accession. The third clade was monophyletic with group CMR1 and included one taxon (CMW 1546) from banana in New Zealand and one from date palm in Saudi Arabia.

Sequence analyses of the β-tubulin and TEF-1α loci had better resolution than those for the ITS region. All the clades delineated in the ITS tree were recovered in the β-tubulin (SUPPLEMENTARY FIG. 3) and TEF-1α (SUPPLEMENTARY FIG. 4) trees, with stronger statistical support. Furthermore, clade CMR2 including *T. euricoi* was separated into two well supported subclades (CMR2, *T. euricoi*) and one nonaligned isolate, CMW28535 = CBS 101054.

Polymorphisms also were observed within clade CMR1 in the TEF-1α tree where at least three subclades were identified.

There was some conflict among the individual gene trees in the deep branches, especially regarding the relationship among the three CMR groups. While the CMR1 and CMR2 clades in the ITS tree were monophyletic with strong statistical support, the CMR1 clade in the TEF-1α tree was monophyletic with the CMR3 clade, also with strong statistical support. In the β-tubulin tree, a common ancestry was shared between the CMR2 and CMR3 clades, although this was less well supported. This incongruence was reflected in the PHT, which resulted in a *P* value of 0.001. Overall, only two well supported deep branches were identified in the combined species tree (FIG. 2). The one branch linked *C. radicicola* and group CMR3 as well as isolate CMW 28536 = CBS 116770, each representing a distinct taxon. The second branch also linked three taxa, *T. euricoi*, group CMR2 and isolate CMW 28535 = CBS 101054. A third deep branch connected *C. musarum* and group CMR1. However, this link was supported statistically only in the MP tree.

Mating studies.—Isolates in the CMR3 group from Cameroon produced ascomata in single-spore or hyphal-tip cultures, and this appears to be a homothallic taxon. In contrast, none of the single-spore or hyphal-tip cultures representing groups CMR1 and CMR2 produced ascomata. Ten randomly selected isolates representing each of these two groups were used in mating trials. Of the 55 paring combinations used for each group, we obtained 12 and six fertile combinations respectively for CMR1 and CMR2, with ascomata forming along the lines of interaction between the two colonies. The pattern of fertile combinations clearly indicated the existence of two mating types in each group and thus a heterothallic mating system (FIG. 3). However, reproductive compatibility between pairs of isolates was variable. This was illustrated in the relative abundance of ascomata produced by the various pairs and the fact that some isolates did not produce ascomata in any combination. These infertile isolates could not be assigned a mating type (FIG. 3). Mating was never observed between isolates representing the two different phylogenetic groups.

TAXONOMY

Phylogenetic analyses and careful comparisons of material collected in Cameroon with protologs, type specimens and isolates from elsewhere made it possible to delineate *C. paradoxa* s. str. and other species in the *C. paradoxa* complex. All described species included in the complex at present are listed below, and issues related to typification, synonymies, nomenclature and morphology are discussed for each species. Morphological differences among the species are summarized (TABLE III).

Ceratocystis paradoxa (de Seynes) C. Moreau, Rev. Mycol. (Paris) Suppl. Col. 17:22. 1952. FIGS. 4–6
MycoBank MB294224

= *Sporoschisma paradoxum* de Seynes, Recherches pour Servir à l'Histoire Naturelle des Végétaux Inférieurs 3:30. 1886. (basionym)
= *Chalara paradoxa* (de Seynes) Sacc., Syll. Fung. 10:595. 1892.
= *Thielaviopsis paradoxa* (de Seynes) Höhn., Hedwigia 43:295. 1904.
= *Ceratostomella paradoxa* (de Seynes) Dade, Trans. Br. Mycol. Soc. 13:191. 1928.
= *Ophiostoma paradoxum* (de Seynes) Nannf., In Melin & Nannf., Svenska SkogsvFör. Tidskr. 32:408. 1934.
= *Endoconidiophora paradoxa* (de Seynes) R.W. Davidson, J. Agric. Res. 50:802. 1935.
= *Stilbochalara dimorpha* Ferd. & Winge, Bot. Tidsskr. 30:220. 1910.

Ascomata perithecial, forming on sugarcane chips on WA and on agar in paired cultures of compatible strains, absent in single colonies, but observed in nature on endosperma of cacao pod husks. Ascomatal bases fully or partially submerged in substrata, mostly globose, (201–)237–317(–392) µm high × (249–)279–348(–382) µm wide, straw (21f), partially or completely covered by aleurioconidia and ascomatal appendages. Ascomatal appendages digitate, (29–)31–45(–51) × (10–)11–25(–26), mostly on exposed areas of ascomatal bases. Ascomatal necks umber (9), erect, (961–)1063–1367(–1538) µm long, (20–)26–37(–45) µm wide at apices; bases of the necks occasionally swollen, forming collar-like structures, (53–)72–96(–110) µm wide. Ostiolar hyphae hyaline, divergent, (62–)102–150(–158) µm long. Asci not observed. Ascospores invested in mucous sheaths, hyaline, aseptate, ellipsoidal, (9–)9–11(–12) × (3–)3–4(–4) µm, accumulating in mucilaginous, buff (19''f) droplets at tips of ascomatal necks. Conidiophores hyaline to grayish sepia (17'''i), 1–4 septa, phialidic, lageniform, (73–)93–179(–301) µm long, (4–)5–8(–9) µm wide at bases and (4–)4–6(–6) µm wide at apices, mononematous with enteroblastic conidium ontogeny, commonly solitary, but occasionally aggregated in synnemata, variable in size, (406–)488–1312(–1640) µm long × (43–)47–65(–69) µm wide. Primary conidia hyaline, aseptate, cylindrical, (8.1–)10–14(–20) × (4–)4–5(–6) µm. Secondary conidia

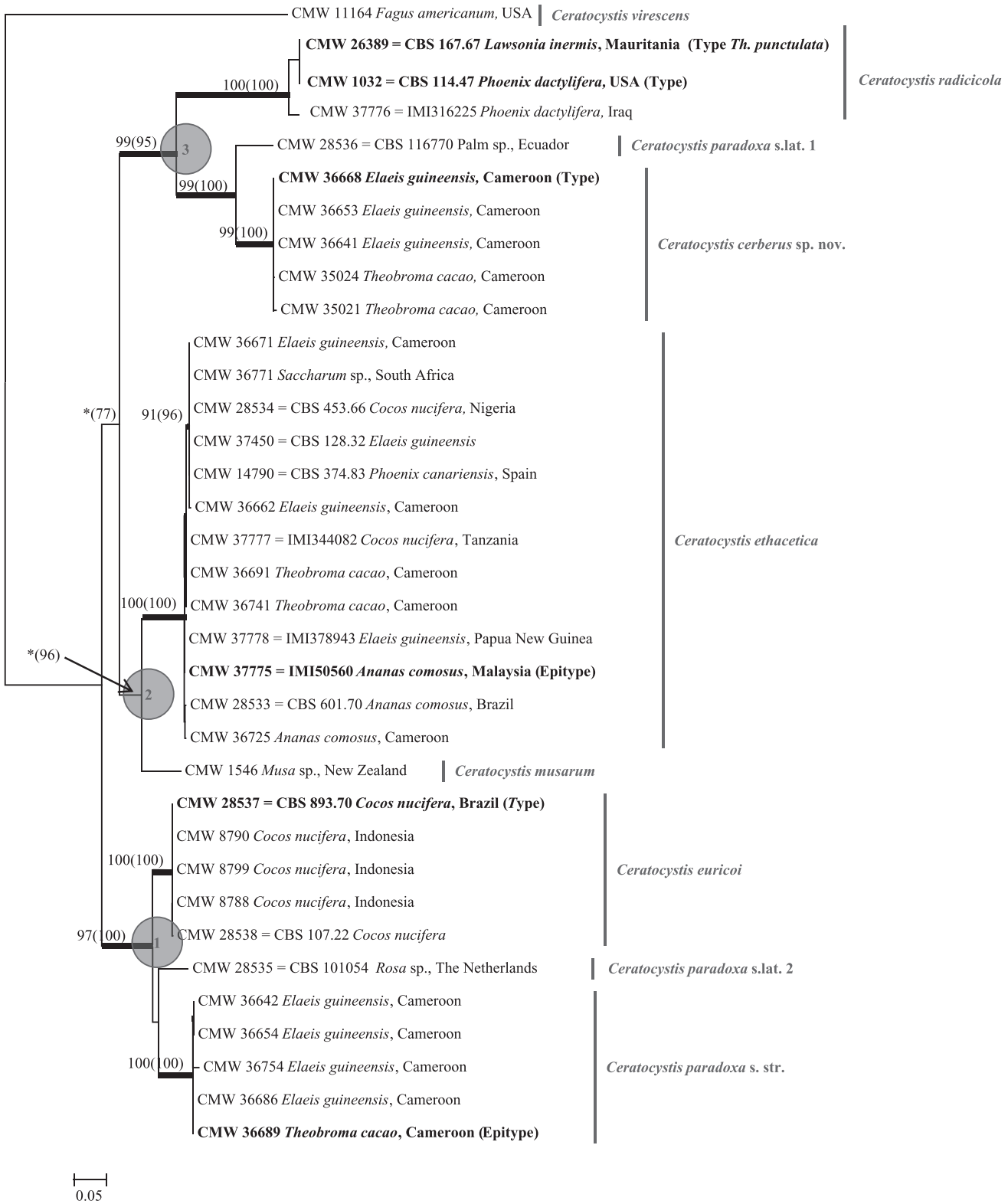

FIG. 2. Species tree of *Ceratocystis paradoxa* s. lat. derived from ML analysis of combined ITS, β-tubulin and TEF1-α sequences Bootstrap support values ≥ 70% from 1000 heuristic search replicates are indicated next to branch nodes as ML (MP). Thick branches are those supported by ≥ 95% posterior probability in Bayesian analyses. The phylogeny is subdivided in three major clades highlighted by blue circles.

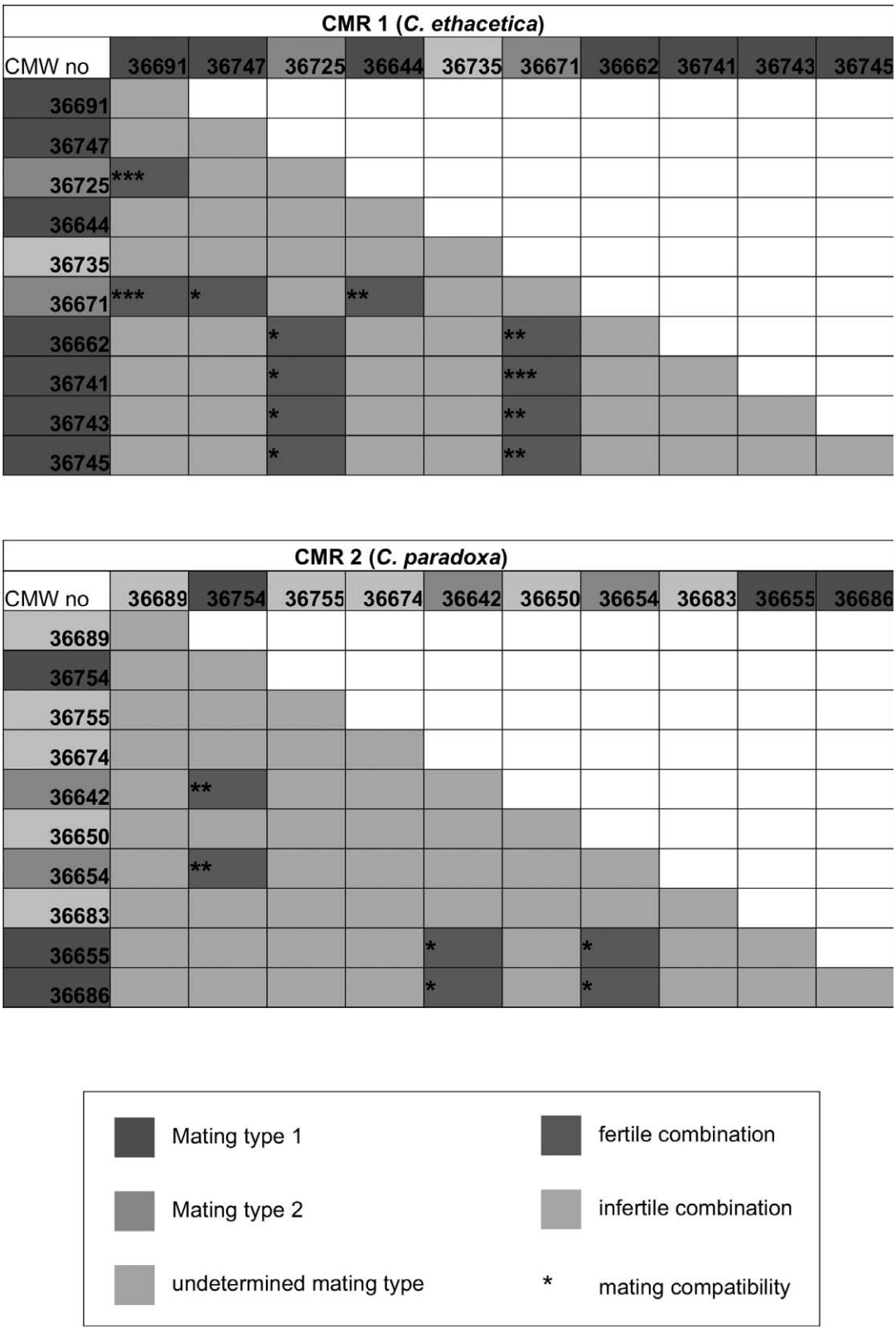

FIG. 3. Summary of mating tests realized with 10 isolates each of group CMR1 (*Ceratocystis ethacetica*) and group CMR2 (*Ceratocystis paradoxa* s. str.) groups. The number of stars is indicative of the relative abundance of ascomata obtained with the various mating combinations.

TABLE III. Morphological comparisons between species of the *Ceratocystis paradoxa* complex

	C. cerberus	*C. ethacetica*	*C. euricoi*	*C. musarum*[a]	*C. paradoxa* s. str.	*C. radicicola*[b]
Mating system	Homothallic	Heterothallic	Sexual state not observed	Undetermined	Heterothallic	Heterothallic
Ascomatal bases						
Rooting	Superficial	Submerged		Submerged	Submerged	Submerged
Shape	Globose	Globose		Spherical–ellipsoid	Globose	Spherical
Ornamentations	Digitate–stellar	Digitate		Absent	Digitate	Digitate
Length	(137–)260–340(–370)	(107–)154–215(–260)		300	(201–)237–317(–392)	180–320
Width	(148–)268–348(–368)	(125–)156–216(–251)		350	(249–)279–348(–382)	NA
Acomatal necks						
Shape	Erect/curled	Erect		NA	Erect	NA
Length	(280–)650–984(–1244)	(451–)672–862(–983)		1100–1200	(961–)1063–1367(–1538)	440–980
Width (apice)	(24–)29–43(–56)	(20–)22–29(–35)		17–18	(20.2–)26–37(–45)	24
Width (base)	(43–)61–83(–101)	(33–)44–64(–79)		50	(53–)72–96(–110)	71
Ostiolar hyphae						
Shape	Divergent	Divergent		Convergent	Divergent	Fimbriate
Length	(47–)70–108(–142)	(71–)92–112(–121)		~100	(62–)102–150(–158)	56–112
Ascospores						
Shape	Ellipsoidal	Ellipsoidal		Cylindrical/ellipsoidal	Ellipsoidal	Ellipsoidal
Length	(6–)7–9(–13)	(7–)7–9(–10)		6–11	(9–)9–11(–12)	8–15
Width	(3–)3–4(–6)	(2–)3–4(–4)		2–3.5	(3–)3–4(–4)	3–4
Phialides						
Shape	Lageniform	Lageniform	Lageniform	Lageniform	Lageniform	Lageniform
Length	(63–)92–150(–215)	(75–)87–148(–257)	(77–)101–144(–171)	(174–)206–252(–295)	(73–)93–179(–301)	(108–)141–207(–252)
Width (apice)	(3–)4–5(–6)	(3–)4–5(–6)	(3–)4–6(–7)	(4–)4–5(–6)	(4–)4–6(–6)	(4–)4–5(–6)
Width (base)	(5–)6–7(–8)	(4–)6–8(–10)	(6–)7–10(–12)	(5–)7–9(–10)	(4–)5–8(–9)	(5–)7–8(–10)
Synnemata	Absent	Absent	Present	Absent	Present	Absent
Primary conidia						
Shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Length	(7–)7–12(–19)	(5–)7–8(–10)	(8–)9–11(–12)	(9–)10–12(–13)	(8–)10–14(–20)	(6–)8–14(–16)
Width	(3–)4–5(–6)	(2–)4–5(–6)	(3–)4–5(–5)	(3–)4–5(–5)	(4–)4–5(–6)	(3–)4–5(–5)
Second. conidia	Not observed					
Shape		Cylindrical/oblong	Cylindrical/oblong	Cylindrical/oblong	Cylindrical/oblong	Cylindrical/oblong
Length		(7–)8–12(–16)	(10–)11–16(–25)	(9–)10–13(–15)	(4–)6–7(–8)	(3–)4–6(–6)
Width		(4–)6–7(–7)	(5–)6–7(–8)	(5–)6–7(–8)	(8–)9–13(–16)	(6–)10–14(–16)

TABLE III. Continued

	C. cerbenus	*C. ethacetica*	*C. euricoi*	*C. musarum*[a]	*C. paradoxa* s. str.	*C. radicicola*[b]
Aleurioconidia						
Aggregation	Singly/in chain	Singly/in chain	Singly/in chain	Singly/in chain	Singly/in chain	Singly
Shape	Obovoid-subglobose	Obovoid-subglobose	Obovoid-subglobose	Obovoid-subglobose	Obovoid-subglobose	Subglobose
Length	(7–)9–12(–16)	(9–)14–18(–19)	(9–)11–14(–17)	(10–)12–16(–18)	(8–)10–16(–20)	(10–)13–16(–18)
Width	(4–)6–8(–10)	(6–)8–11(–12)	(7–)8–11(–13)	(4–)6–9(–11)	(7–)8–12(–19)	(9–)10–12(–13)

[a] Sexual state data from Riedl (1961).
[b] Sexual state data from Bliss (1941).

aseptate, initially hyaline, turning grayish sepia (17'''i) to umber (9), thick-walled when mature, cylindrical to oblong, (8–)9–13(–16) × (4–)6–7(–8) μm. Aleurioconidia produced holoblastically, singly or in short chains, dark mouse umber (9), granulated, thick-walled, mostly oblong to subglobose, (8–)10–16(–20) × (7–)8–12(–19) μm. Colonies on MEA initially hyaline to white, becoming green-olivaceous (23'''i) or gray-olivaceous (23''''i) after 10 d, reverse gray-olivaceous (23''''i). Mycelium aerial, submerged, hyphae hyaline, smooth, often terminating as conidiophores, septate, no constriction at septa. Optimal temperature for growth 25–30 C, fast growing, 60–80 mm diam after 36 h at 30 C, marginal growth at 35 C, no growth at 10 C after 10 d.

Mating system: Heterothallic.

Types: Lectotype of *Sporoschisma paradoxum* (designated by Nag Raj and Kendrick [1975], p. 129, MBT 178353): FRANCE, exact origin unknown, on fruit of *Ananas comosus*, 1886, coll. *J. de Seynes*, represented by illustrations (Plate I, Figs. 22–24) from de Seynes (1886).

Previously considered lectotype for *Ceratostomella paradoxa* (designated by Hunt 1956 p 19, but see argument below): GHANA, Anyinam, on discarded *Theobroma cacao* pod husks, 1927, coll. *H.A. Dade*, IMI 41297 = CB 449.

Epitype of *Ceratocystis paradoxa* (designated here, MBT 178352): CAMEROON, southwest region, Kumba (N4 35.271 E9 28.112), on endosperm of *Theobroma cacao* pod husk, 12 Oct 2010, coll. *M. Mbenoun & J. Roux*, dried culture PREM 60766, living ex-epitype culture CMW 36689 = CBS 130761.

Holotype of *Stilbochalara dimorpha* (MBT 178354): VENEZUELA, Las Trincheras, on rotten *Theobroma cacao* pod husks, 25 Dec. 1891, coll. *H. Lassen*, unnumbered specimen from Museum Botanicum Hauniense.

Additional material examined (MBT 178354): CAMEROON, Littoral Region, Dibamba (N3 54.510 E9 50.177), on cut end of *Elaeis guineensis* leaf, 15 Oct 2010, coll. *M. Mbenoun & J. Roux*, living culture CMW 36642 = CBS 130760, herbarium specimen of dried culture PREM 60765; South West Region, Kumba (N4 35.271 E9 28.112), on cut end of *Elaeis guineensis* leaf, 12 Oct. 2010, coll. *M. Mbenoun & J. Roux*, living culture CMW 36686 = CBS 130762, herbarium specimen of dried culture PREM 60767.

Sexual state: CAMEROON, herbarium specimens with sexual structures obtained from crosses, PREM 60777 (CMW 36642 × CMW 36655), PREM 60775 (CMW 36654 × CMW 36686), PREM 60776 (CMW 36654 × CMW 36655).

Notes: The asexual state of *Ceratocystis paradoxa* first was described as *Sporoschisma paradoxum* de Seynes from pineapple in France, although the origin of the pineapple collection remains unknown (de Seynes 1886). De Seynes (1886) did not designate a type

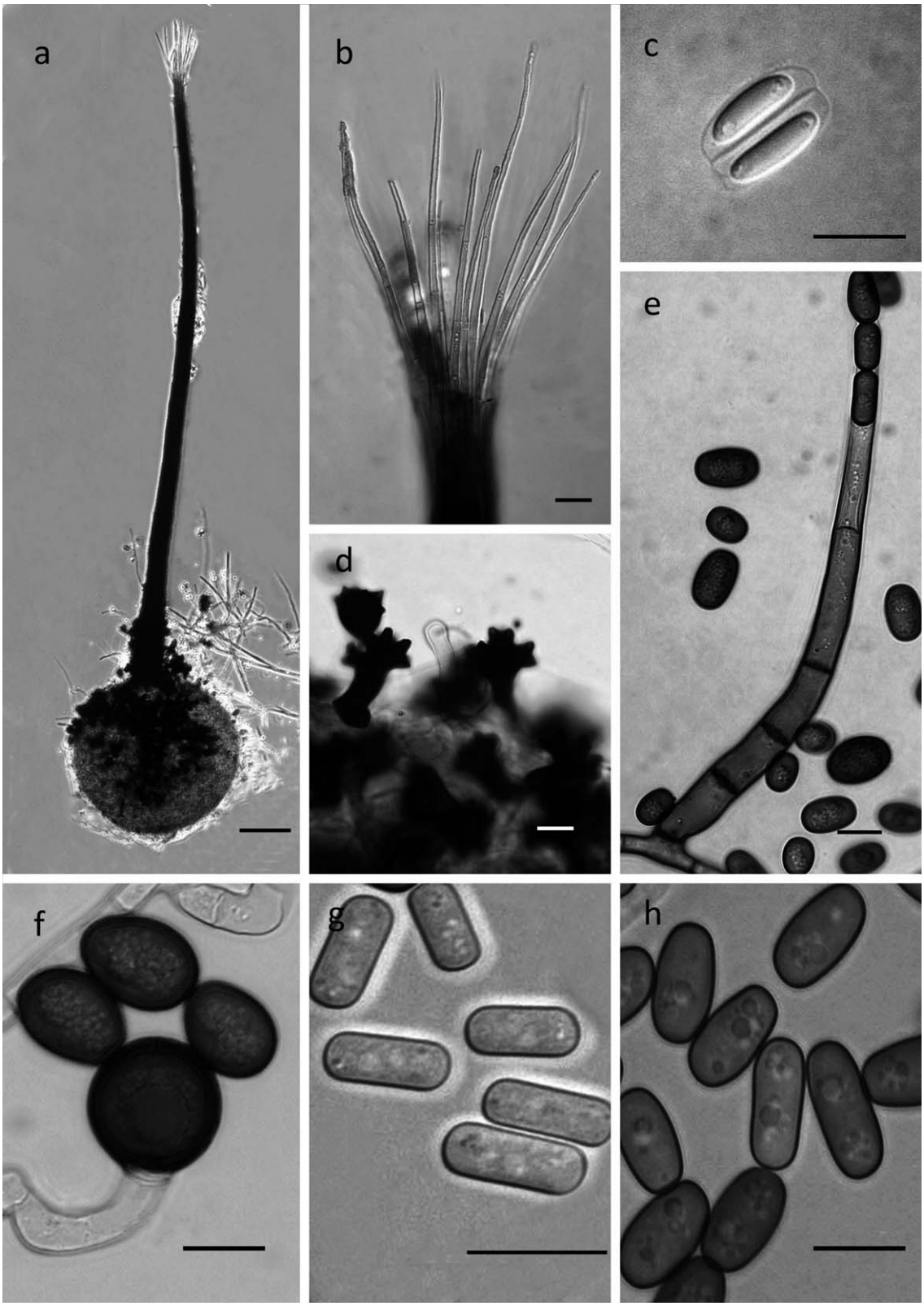

FIG. 4. Sexual and asexual structures from fresh cultures of *Ceratocystis paradoxa* s. str. (isolates CMW 36642, CMW 36686 and CMW 36689). a. Ascomata with globose base and extended neck. b. Divergent ostiolar hyphae. c. Ellipsoidal ascospores in mucous sheaths. d. Digitate ascomatal ornamentations. e. Flasked-shaped phialidic conidiophore. f. Thick-walled aleurioconidia. g. Cylindrical primary conidia. h. Obovoid secondary conidia. Bars: a = 100 μm, b–h = 10 μm.

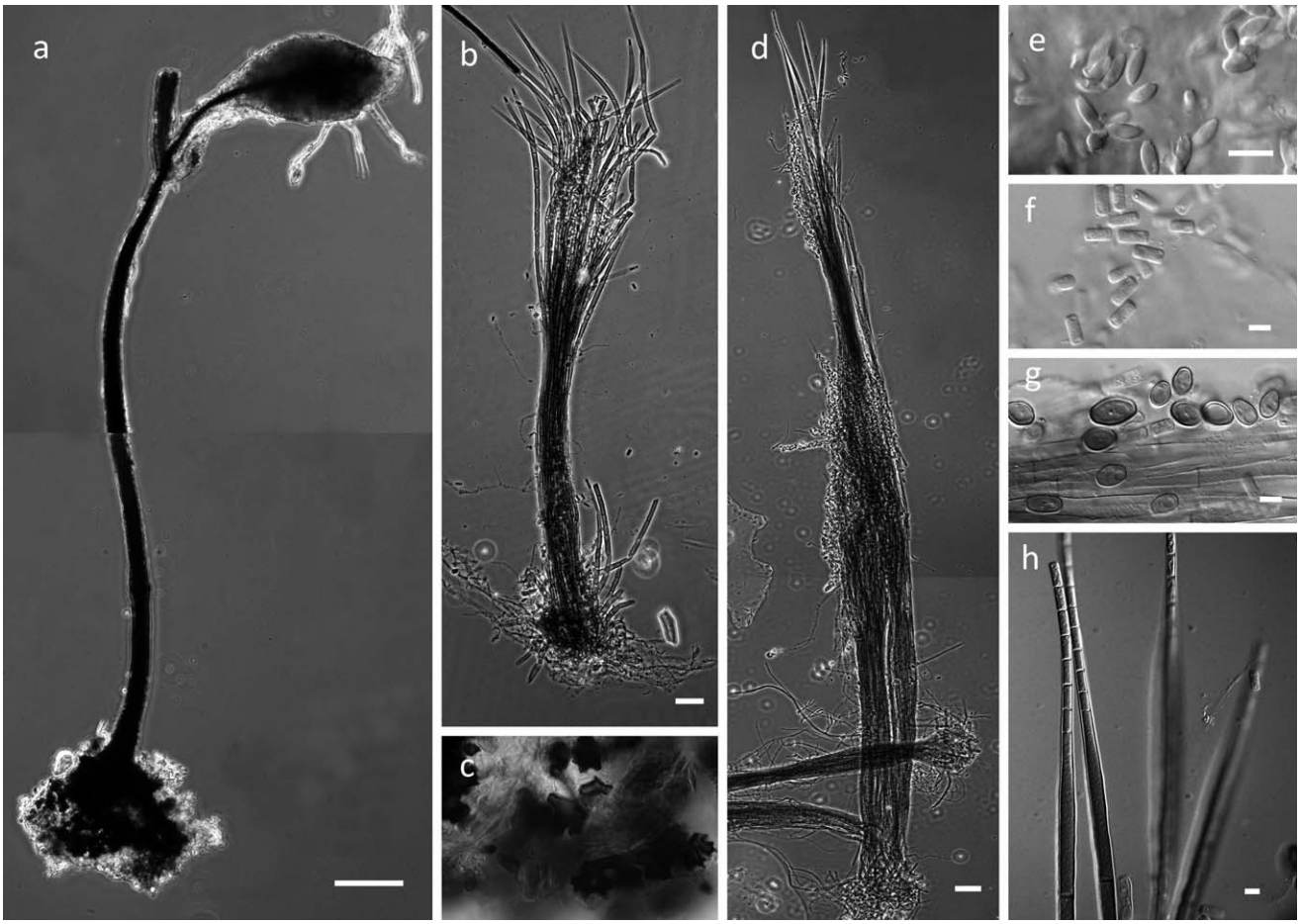

FIG. 5. Sexual and asexual structures from the herbarium specimen (IMI 41297) previously treated as lectotype of *Ceratostomella paradoxa*. a. Perithecium. b, d. Synnemata. c. Ascomatal appendages. e. Ascospores. f. Primary conidia. g. Secondary condia. h. Phialides extruding chains of primary conidia. Bars: a = 100 μm, b–h = 10 μm.

specimen, and no herbarium specimens or cultures from his study are available. Nag Raj and Kendrick (1975) designated the illustrations from the original publication as lectotype, which is legitimate under the current code (Articles 8.1, 40.4; McNeill et al. 2012). Saccardo (1892) treated the species in *Chalara* and Höhnel (1904) placed it in *Thielaviopsis*. Dade (1928) described the sexual state of this fungus as *Ceratostomella paradoxa* Dade, mentioning several specimens but not designating one as type. Hunt (1956) designated one of Dade's original specimens as lectotype (IMI 41301), but Nag Raj and Kendrick (1975) listed IMI 41297 as holotype. The latter specimen also came from the original collection of Dade and is the only specimen presently available in IMI (CABI). The specimen (IMI 41297) currently is labeled as lectotype and clearly corresponds in all respects with the description of Dade (1928). However, considering the abolishment of the dual nomenclature system (Hawksworth 2011, Hawksworth et al. 2011), and based on the suggestions by Hawksworth et al. (2013), the sexual state of *C. paradoxa*, described subsequently to the asexual state, should not be treated as a new species (i.e. *Ceratostomella paradoxa* Dade), but as a formal error for a new combination (i.e. *Ceratostomella paradoxa* [de Seynes] Dade). We thus replaced Dade with de Seynes in the authors of the homotypic synonyms *Ceratocystis paradoxa*, *Ophiostoma paradoxum* and *Endoconidiophora paradoxa*. Following this interpretation, the type of the sexual morph (IMI 41297) no longer has nomenclatural status because the type for the species is that of the basionym (i.e. the illustrations from de Seynes 1886). Although the option was available to designate IMI 41297 (a herbarium specimen) from cacao husks in Ghana as epitype, we chose to designate as epitype a dried specimen of a living culture from which DNA could be obtained, originating also from cacao husks but from Cameroon.

Ainsworth and Bisby (1943) listed *Stilbochalara* as a synonym of *Thielaviopsis*, based on study of the

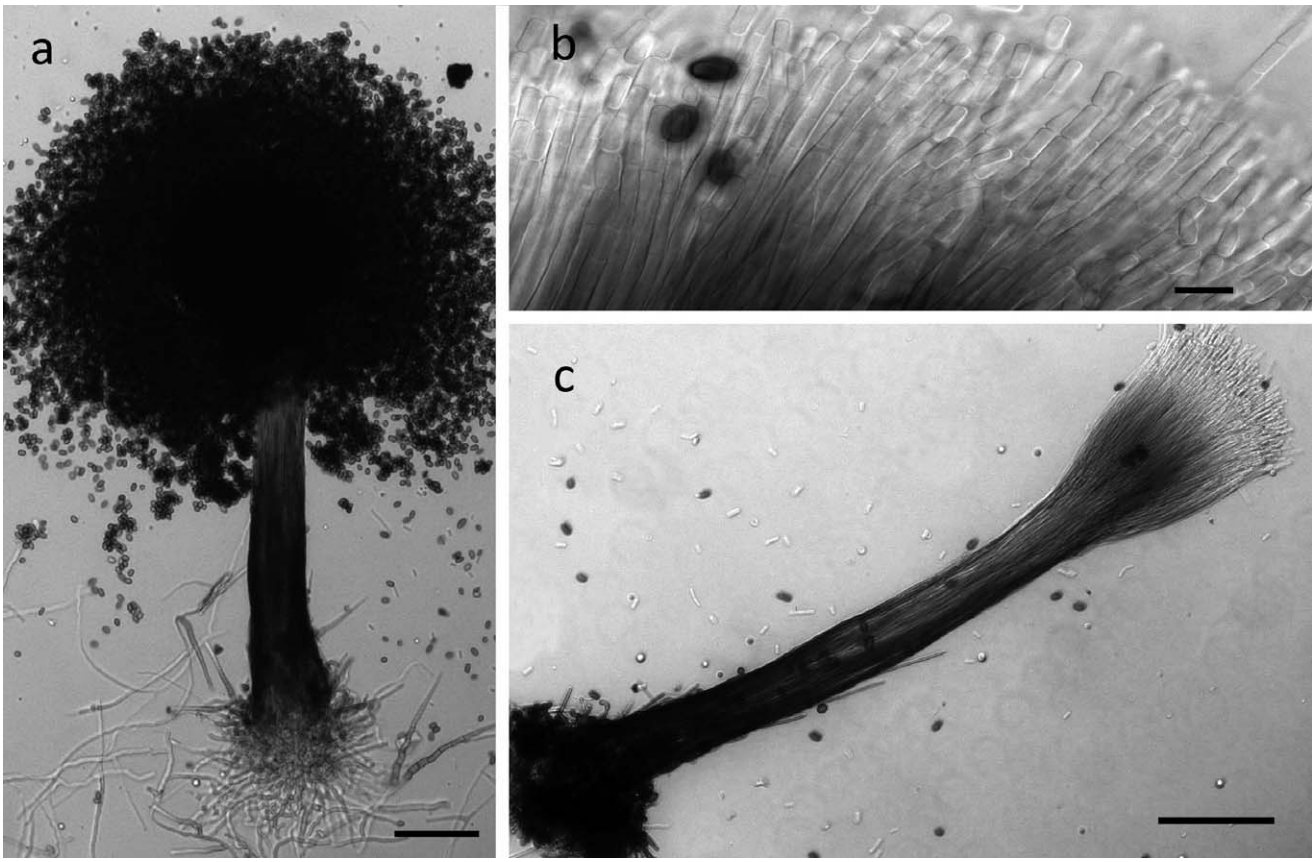

FIG. 6. Synnemata from a fresh culture of *Ceratocystis paradoxa* s. str. (isolate CMW 36642). a. Mature synnema with round, dark head. b, c. Young synnema producing hyaline conidia. Bars: a, c = 100 μm; b = 10 μm.

holotype of its type species, *Stilbochalara dimorpha* (Ferdinandsen and Winge 1910). This specimen, previously stored in Museum Botanicum Hauniense, was obtained for the present study from the Natural History Museum of Denmark. It also was studied by Nag Raj and Kendrick (1975) who listed the species as a synonym of *C. paradoxa*, as did Paulin-Mahady et al. (2002).

In the original account of *Sporochisma paradoxum*, de Seynes (1886) did not mention synnemata. However, shortly thereafter he expanded his description of the same material and described synnemata as fructifications reminiscent of those of species in the genera *Isaria*, *Stysanus* or *Sporocybe* (de Seynes 1888). These also were mentioned by several other early authors (Höhnel 1904, 1909; Petch 1910; Dade 1928) and were observed in this study on IMI 41297, previously treated as lectotype of *Ceratostomella paradoxa* (FIG. 4). Confusion emerged when Höhnel (1904) reduced *T. ethacetica* to synonymy with *Sporochisma paradoxum* and treated the latter species in *Thielaviopsis*. *Thielaviopsis ethacetica* was described earlier without synnemata from sugarcane in Java (Went 1893). Although Nag Raj and Kendrick (1975) did not mention synnemata in their diagnosis of the species (p 128), they stated on p 55 that ''*Chalara paradoxa* … is also known to form occasional lax coremia'' (= synnemata). Based on the descriptions of synnemata in *Sporochisma paradoxum* by de Seynes (1888) and the obvious synnemata observed in the original material of *Ceratostomella paradoxa* in this study, we conclude that *Sporochisma paradoxum* and *Ceratostomella paradoxa* represent the same taxon and we define *C. paradoxa* s. str. as a species forming synnemata. The shape and size of these structures are highly variable even within the same culture.

In the present study isolates of *C. paradoxa* s. str. produced synnemata under the same conditions as, and alongside ascomata, in paired cultures on water agar supplemented with chips of sugarcane. They consisted of bundles of erect phialides, held together but individually extruding slimy conidia at their apices (FIG. 5). Conidia are agglutinated in round spore drops at the tips of synnemata. Initially slimy and cream-colored, the spore drops turned black when old, consisting essentially of matured, dry

secondary conidia (FIG. 5). We thus designated a dried specimen of a fresh, synnema-producing culture as epitype based on its similarity to IMI 41297. The similar structures observed on the holotype of *Stilbochalara dimorpha*, although fragmented (SUPPLEMENTARY FIG. 6), confirmed that this species represents the same fungus and is best treated as synonym of *C. paradoxa* s. str. The forms of *C. paradoxa* that do not produce synnemata are discussed under *C. ethacetica*.

Ceratocystis ethacetica (Went) Mbenoun & Z.W. de Beer, comb. nov. FIG. 7

MycoBank MB805506

≡ *Thielaviopsis ethacetica* Went, Meded. Proefstn W. Java 'Kagok' 5:4. 1893. [as '*ethaceticus*'] (basionym)

= *Endoconidium fragrans* Delacr., Bull. Soc. Mycol. Fr. 9:184. 1893.

= *Catenularia echinata* Wakker in Wakker & Went, de Ziekten van het Suikerriet op Java, EJ Brill, Leiden p 196. 1898.

Ascomata perithecial, formed in patches along the lines of interaction between mating-compatible colonies in paired cultures on agar (MEA and WA) and on plant chips (sugarcane and pineapple), absent in single colonies but observed in nature on endosperma of cacao pod husks. Ascomatal bases fully or partially submerged in substrata, mostly globose, (107–)154–215(–260) µm high × (125–)156–216 (–251) µm wide, originally straw (21f), appearing dark in old cultures when surrounded with aleurioconidia and ascomatal appendages. Ascomatal appendages stellate or digitate, (22–)22–41(–51) × (12–) 14–23(–27), mostly restricted to aerial parts of partially submerged ascomatal bases. Ascomatal necks dark mouse gray (14''''k), erect, (451–)672–862(–983) µm long, (20–)22–29(–35) µm wide at apices, (33–)44–64(–79) µm wide at bases. Ostiolar hyphae hyaline, divergent, (71–)92–112(–121) µm long. Asci not observed. Ascospores in sheaths, hyaline, aseptate, ellipsoidal, (7–)7–9(–10) × (2–)3–4(–4) µm, accumulating in mucilaginous droplets, buff (19''f) at tips of ascomatal necks with strong adherence to ostiola hyphae when old and dry. Conidiophores mostly hyaline, phialidic, lageniform, (75–)87–148(–257) µm long, (4–)6–8(–10) µm wide at bases and (3–)4–5 (–6) µm wide at apices, mononematous with enteroblastic conidium ontogeny, solitary. Primary conidia hyaline, aseptate, cylindrical, (5–)7–8(–10) × (2–)4– 5 (–6) µm. Secondary conidia aseptate, initially hyaline, turning grayish sepia (17'''i), thick-walled at maturity, cylindrical to oblong, (7–)8–11(–16) × (4–)6–7(–7) µm. Aleurioconidia produced holoblastically, singly or in chains of 2–10 units, grayish sepia (17'''i) to umber (9), granulated, thick-walled, subglobose, oblong or ovoid, (9–)14–18(–19) × (6–)8–11(–12) µm. Colonies on MEA initially hyaline to white, progressively darkening, turning citrine-green (25''b), green-olivaceous (23'''i) or gray-olivaceous (23''''i) after 10 d, reverse gray-olivaceous (23''''i). Mycelium aerial and submerged, hyphae hyaline, smooth, often terminating as conidiophores, septate, no constriction at septa. Optimal temperature around 30 C, fast growing, reaching ~ 75 mm in 36 h at 30 C, marginal growth at 35 C, no growth at 10 C after 10 d.

Mating system: Heterothallic.

Types: Lectotype of *Thielaviopsis ethacetica* (designated here, MBT 178355): INDONESIA, Java, Tegal, on stems, fruit, and leaves of *Saccharum* sp., 1893, coll. *F.A.F.C. Went*, represented by line drawings (Plate III, Figs. 1–6) from Went (1893).

Epitype of *Thielaviopsis ethacetica* (designated here, MBT 178356): MALAYSIA, Western Malaysia, on fruit of *Ananas comosus*, 8 Jul 1952, coll. *A. Johnson*, dried culture PREM 60961, living ex-epitype culture IMI 50560 (CABI) = MUCL 2170 = CMW 37775.

Lectotype of *Endoconidium fragrans* (designated here, MBT 178357): FRANCE, Paris, in fermented pineapple juice, 1893, coll. *G. Delacroix*, represented by line drawings (Plate XI, Fig. IIa, b) from Delacroix (1893).

Neotype of *Catenularia echinata* (designated here, MBT 178358): SOUTH AFRICA, KwaZulu-Natal Province, *Saccharum* sp., 2010, coll. N. van Wyk, dried culture PREM 60963, living ex-neotype culture CMW 36771.

Additional specimens examined: CAMEROON, southwest region, near Tiko (N4 14.488 E9 21.698), on stump of felled *Elaeis guineensis* tree, 13 Oct 2010, coll. *M. Mbenoun & J. Roux*, culture CMW 36671 = CBS 130757, herbarium specimen PREM 60762; littoral region, Njombe (N4 34.032 E9 37.204), on damaged leaf of *Ananas comosus*, 14 Oct 2010, coll. *M. Mbenoun & J. Roux*, culture CMW 36725 = CBS 130758, herbarium specimen PREM 60763; center region, Ngomedzap (N316.107 E11 14.498), on endosperm of *Theobroma cacao* pod husk, Dec 2010, coll. *M. Mbenoun*, culture CMW 36741 = CBS 130759, herbarium specimen PREM 60764; herbarium specimens with sexual structures obtained from crosses, PREM 60774 (CMW 36671 × CMW 36691), PREM 60771 (CMW 36671 × CMW 36741), PREM 60772 (CMW 36671 × CMW 36644), PREM 60773 (CMW 36671 × CMW 36745).

Notes: Thielaviopsis ethacetica was described originally as the type species for a new genus, *Thielaviopsis*, from sugarcane in Java (Went 1893). In his description of the species, Went (1893) did not mention or illustrate synnemata or any similar structures. Höhnel (1904) found a similar fungus on coconut in Vienna, Austria, but it produced synnemata in addition to other conidiogenous structures. He thought his

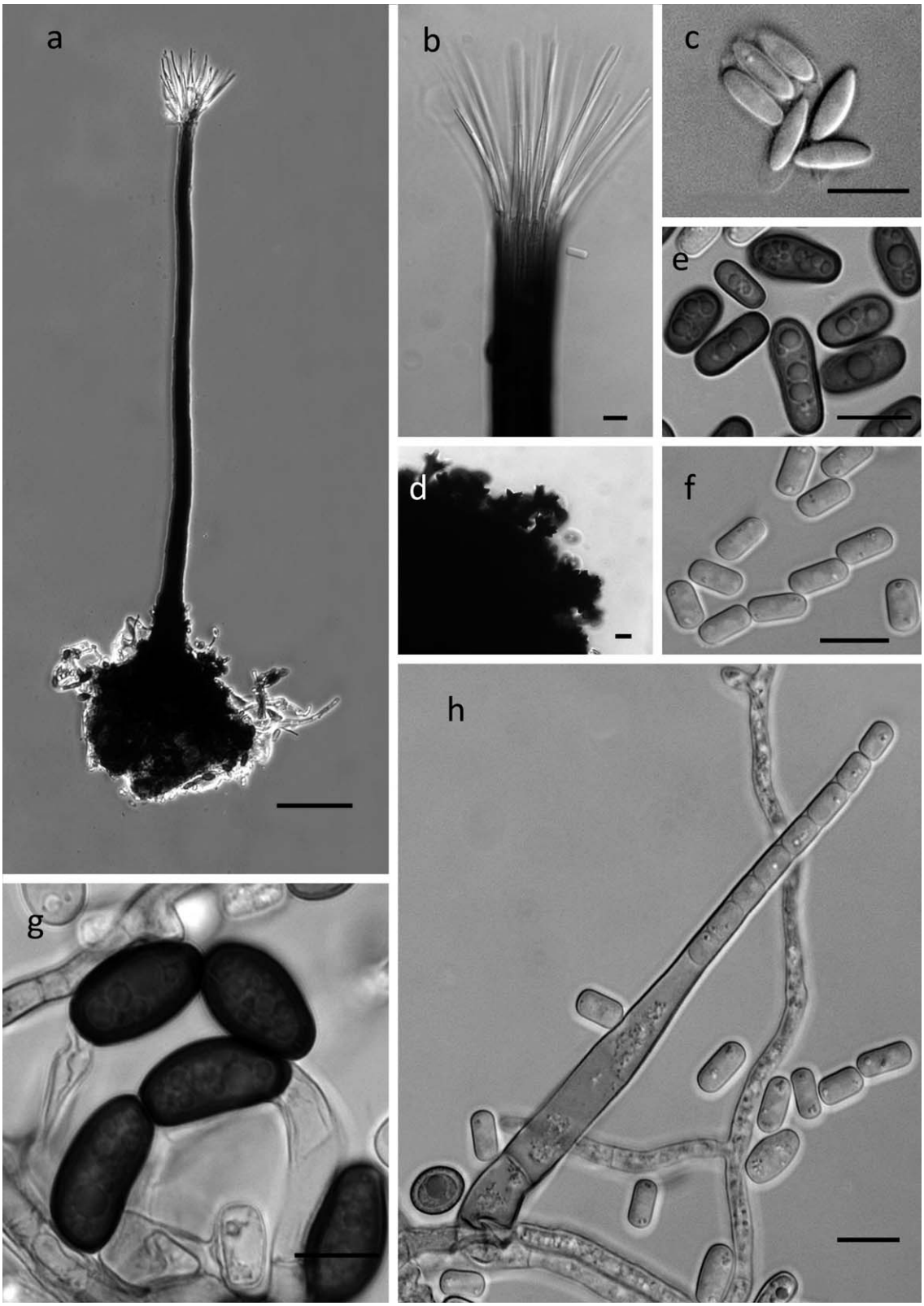

FIG. 7. Sexual and asexual structures from fresh cultures of *Ceratocystis ethacetica* (isolates CMW 36671, CMW 36725, CMW 36741, CMW 37775). a. Perithecium with globose base. b. Divergent ostiolar hyphae. c. Ellipsoidal ascospores invested in mucous sheaths. d. Digitate ornamentations on perithecial base. e. Oblong secondary conidia. f. Cylindrical primary conidia. g. Thick-walled aleurioconidia in short chains. h. Flask-shaped phialidic conidiophore. Bars: a = 100 μm, b–e = 10 μm.

material represented *Sporoschisma paradoxum* but sent it to Went for confirmation. Went suggested that the fungus from Vienna was conspecific with *T. ethacetica* (Höhnel 1904), despite the presence of synnemata. Höhnel (1904) transferred *Sporoschisma paradoxum* to *Thielaviopsis*, which he considered a more appropriate genus, and treated *T. ethacetica* as synonym of *T. paradoxa*, based on the older epithet. Höhnel's (1904) synonymy was accepted in all major treatments of the species (Petch 1910, Dade 1928, Davidson 1935, Hunt 1956, Nag Raj and Kendrick 1975, Upadhyay 1981, Paulin-Mahady et al. 2002). Of note, in the majority of descriptions of *C. paradoxa* following Dade (1928) no mention was made of synnemata (Davidson 1935, Hunt 1965, Morgan-Jones 1967, Upadhyay 1981, Paulin-Mahady et al. 2002). As explained under *C. paradoxa*, results of this study show that *C. paradoxa* s. str. is characterized by synnemata and that isolates with and without synnemata, but otherwise morphologically similar, are phylogenetically distinct. The name *T. ethacetica* therefore is reinstated for species without synnemata. In view of the lack of type material for *T. ethacetica* from sugarcane in Indonesia, the illustrations from Went (1893) are designated as lectotype. To ensure stability, a dried specimen of a morphologically similar living culture from pineapple in Malaysia, which lacks synnemata and clustered separately from *C. paradoxa* s. str. in phylogenetic analyses, is designated as an epitype for this species.

Delacroix (1893) described and illustrated a fungus with hyaline conidia and without synnemata from fermented pineapple juice in France as *Endoconidium fragrans*. Höhnel (1909) thought the species represented an immature stage of *T. paradoxa* and treated it as a synonym of the latter species. The synonymy was accepted in most subsequent taxonomic treatments (Saccardo 1913, Nag Raj and Kendrick 1975, Paulin-Mahady et al. 2002). Nag Raj and Kendrick (1975) reported that the original specimen of *Endoconidium fragrans* had been lost. Because no synnemata were illustrated or mentioned in the cryptic description by Delacroix (1893), we suggest that the name should be treated as a synonym of *C. ethacetica*. However, to ensure taxonomic stability, we designated the illustrations of Delacroix (1893) as lectotype for *Endoconidium fragrans*.

In an extensive treatment of diseases of sugarcane in Java (Wakker and Went 1898), Wakker described *Catenularia echinata* with pigmented macroconidia and hyaline, enteroblastic conidia. In the same paper *T. ethacetica* was treated as distinct, but the descriptions of the two species largely overlap and no explanation was provided as to why these fungi should be treated separately. Based on their similarities, Höhnel (1909) suggested *Catenularia echinata* as a possible synonym of *T. paradoxa*. Apart from Saccardo (1899), who initially listed it as a distinct species but later followed Höhnel's synonymy (Saccardo 1913), the species was ignored in all subsequent treatments of the species complex. In the absence of synnemata and based on its similarities with *C. ethacetica*, *Catenularia echinata* is best treated as a synonym of the latter species. With no material or illustrations available for *Catenularia echinata*, lectotypification is not possible, but we designated a neotype for the name from a morphologically similar isolate from sugarcane in South Africa.

Ceratocystis euricoi (Bat. & A.F. Vital) Mbenoun, Z.W. de Beer, comb. nov. FIG. 8a–d, FIG. 9
MycoBank MB805507

≡ *Hughesiella euricoi* Bat. & A.F. Vital, Anais Soc. Biol. Pernambuco 14:142. 1956. (basionym)

≡ *Thielaviopsis euricoi* (Bat. & A.F. Vital) A.E. Paulin, T.C. Harr. & McNew, in Paulin-Mahady et al., Mycologia 94:70. 2002.

Ascomata not observed. Conidiophores hyaline, 1–4 septa, phialidic, lageniform, (77–)101–144(–171) µm long, (6–)7–10(–12) µm wide at bases and (3–)4–6(–7) µm wide at apices, mononematous with enteroblastic conidium ontogeny, commonly solitary, occasionally aggregated in synnemata, variable in size, (853–)946–1295(–1380) µm long × (54–)56–82(–87) µm wide. Primary conidia hyaline, aseptate, cylindrical, (8–)9–11(–12) × (3–)4–5(–5) µm. Secondary conidia aseptate, initially hyaline, turning mouse gray (15''''') and thick-walled at maturity, mostly oblong, (10–)11–16(–25) × (5–)6–7(–8) µm. Aleurioconidia produced holoblastically, singly or in short chains, dark mouse gray (15'''''k), granulated, thick-walled, mostly subglobose to globose, (9–)11–14(–17) × (7–)8–11(–13) µm. Colonies on MEA initially hyaline to white, becoming gray-olivaceous (23''''i) with age.

Mating system: Undetermined.

Type: Holotype of *Hughesiella euricoi* (MBT 178359): BRAZIL, Bahia, Salvador, atmospheric air, 1956, coll. *E.A.F. da Matta*, holotype (not seen) URM 640, isotype (not seen) DAOM 75211, culture exholotype CBS 893.70 = CMW 28537 = MUCL 1887 = UAMH 1382.

Additional specimens examined: INDONESIA, Sulawesi, on trunk of *Cocos nucifera*, 29 Nov. 2002, *M.J. Wingfield*, CMW 8799. UNKNOWN ORIGIN, on endosperm of *Cocos nucifera*, Mar 1922, coll. H.G. Derx, CBS 107.22 = CMW 28538 = MUCL 8358.

Notes: Hughesiella euricoi was described originally from an air sample in Brazil as type species of the genus *Hughesiella* (Batista and Vital 1956). Nag Raj and Kendrick (1975) considered the species a synonym of *Chalara paradoxa* based on morpholog-

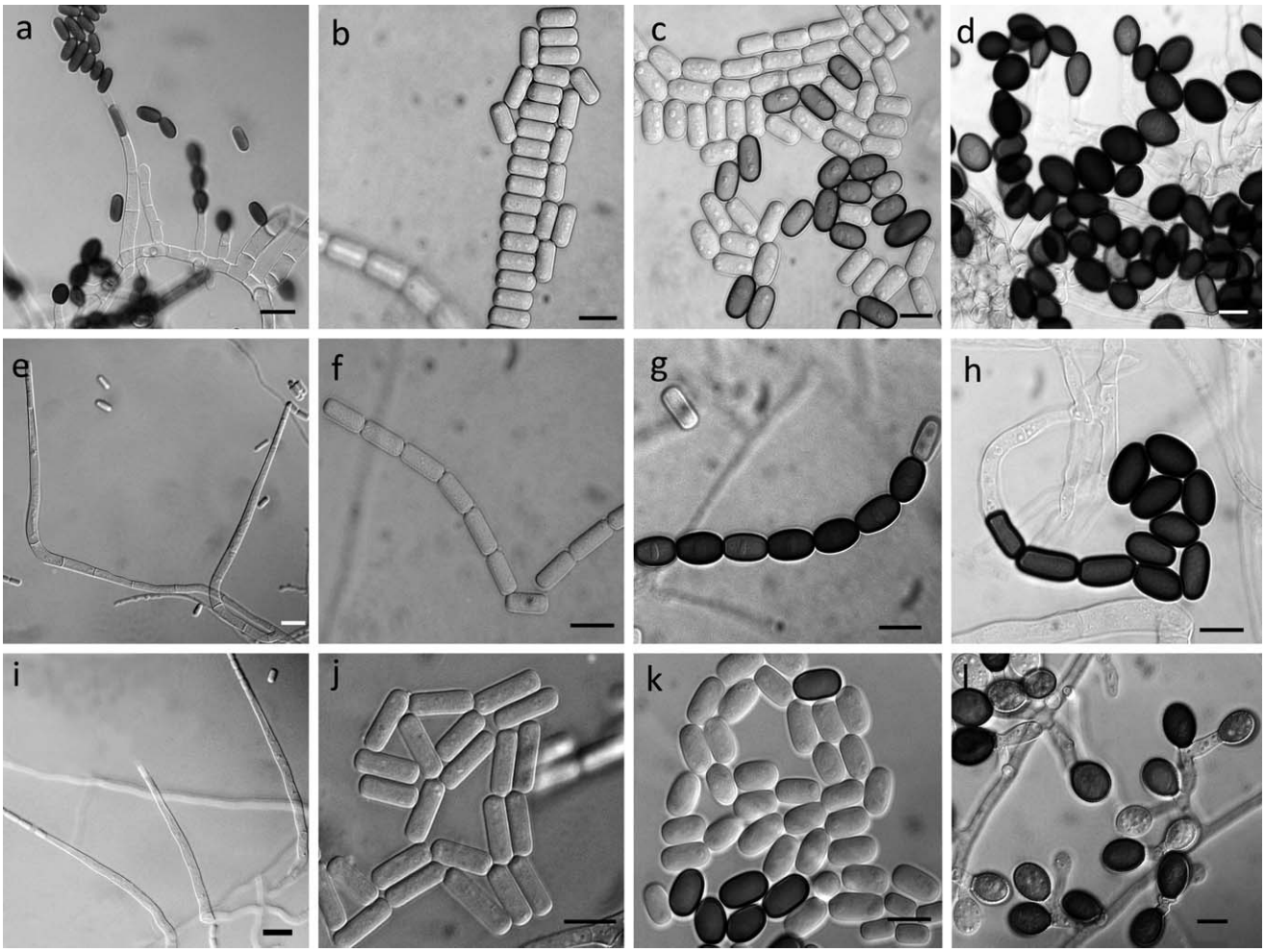

FIG. 8. Asexual structures from fresh cultures of *Ceratocystis euricoi* (ex holotype CMW 28537, CMW 28538, CMW 8799). a. Phialide. b. Primary conidia. c. Secondary conidia. d. Aleurioconidia. *Ceratocystis musarum* (isolate CMW 1546). e. Phialides. f. Primary conidia. g. Secondary conidia. h. Aleurioconidia. *Ceratocystis radicicola* (isolate CMW 37776). i. Phialides. j. Primary conidia. k. Secondary conidia. l. Aleurioconidia. Bars: a, e, i = 20 µm; b, c, d, f, g, h, j, k, l = 10 µm.

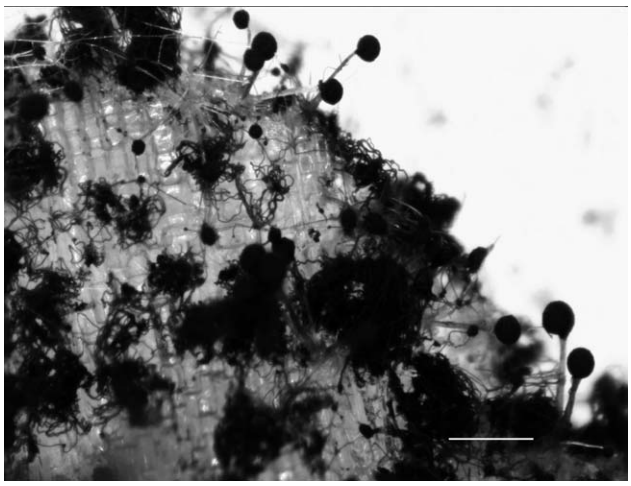

FIG. 9. Synnemata produced by *Ceratocystis euricoi* ex holotype (CMW 28537). Bars = 1000 µm.

ical similarities and thus listed the genus *Hughesiella* as synonym of *Chalara*. Paulin-Mahady et al. (2002) did not include any material of the species in their study but treated *T. euricoi* as distinct from *T. paradoxa* and listed *Hughesiella* as synonym of *Thielaviopsis*. In the present study, the ex-type culture (CMW 28537 = CBS 893.70) of the fungus studied by Batista and Vital (1956) appeared to have similar asexual morphology as *C. paradoxa* s. str., including the formation of synnemata (FIG. 11), not mentioned in the original description. However, DNA sequence analyses confirmed that *T. euricoi* belongs in the *C. paradoxa* complex but that this species is distinct from *C. paradoxa* s. str. and other species in the complex. Several isolates from coconut palm in Indonesia, as well as one (CMW 28538 = CBS 107.22) isolated by a Dutch mycologist in the 1920s from the same host but of an unknown origin, previously thought to be *C.*

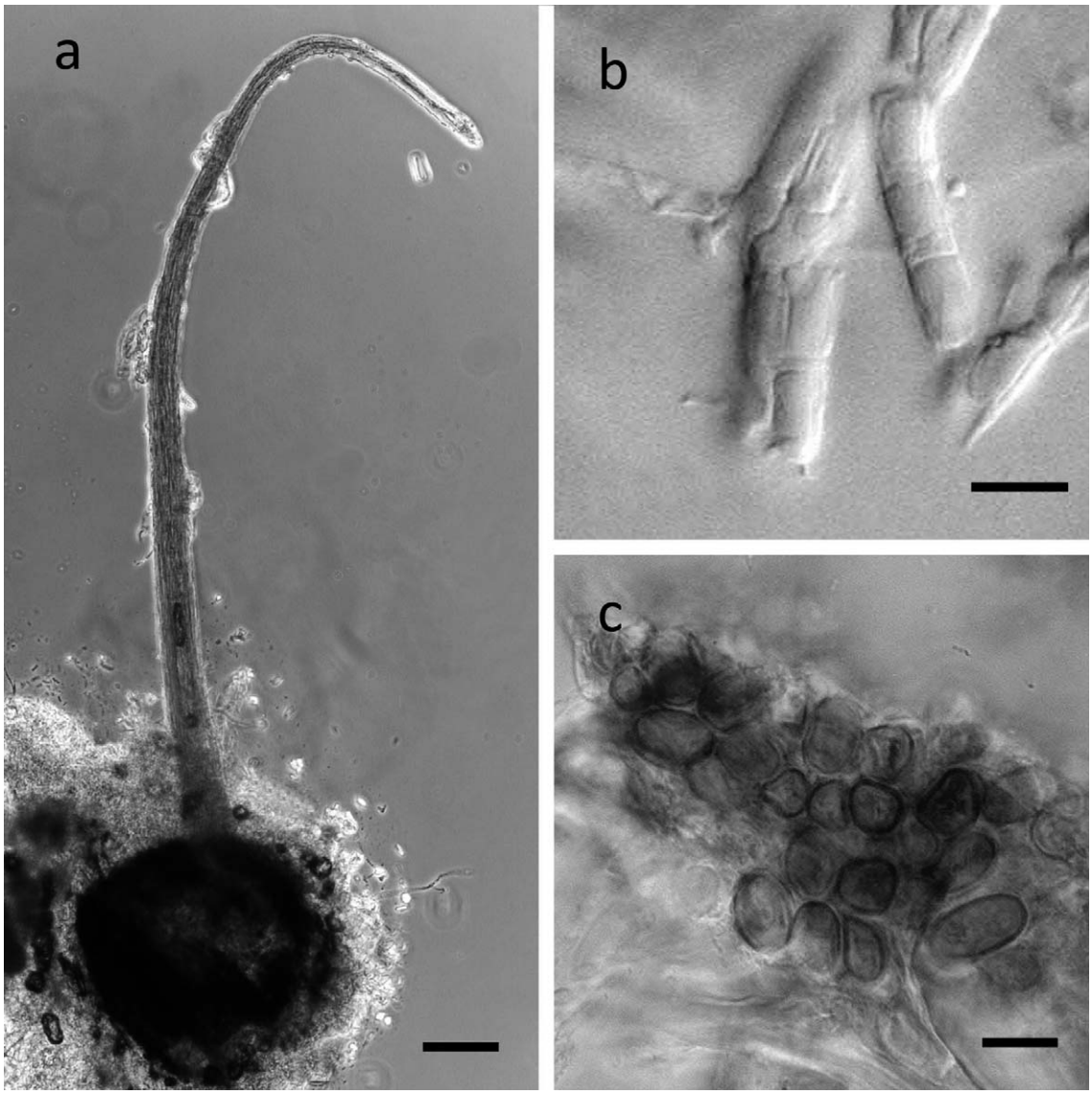

FIG. 10. Sexual and asexual structures from the holotype of *Ceratocystis musarum*. a. Ascomata. b. Primary conidia. c. Secondary conidia. Bars: a = 100 µm; b, c = 10 µm.

paradoxa, were shown to be conspecific with the Brazilian isolate. Although no sexual state has been observed for this species, the one fungus one name principles adopted in the Melbourne Code require that the species be treated in *Ceratocystis* (Hawksworth 2011, Hawksworth et al. 2011).

Ceratocystis musarum Riedl, Sydowia 15:248. 1962. FIGS. 8e–h, 10

MycoBank MB327636

= *Thielaviopsis musarum* (R.S. Mitchell) Riedl, Sydowia 15:249. 1962.

[= *Thielaviopsis paradoxa var. musarum* R.S. Mitchell, J. Coun. Sci. Ind. Res. Australia, 10:130. 1937. *nom. inval.*, Art. 39.1]

''Ascomata perithecial. Ascomatal bases partially submerged or on the surface of substrate, sienna (14i), globose to subglobose, 300 µm high ×350 µm wide, no ornamentations observed. Ascomatal necks sienna (14i) to orange (13), 1100–1200 µm long, 17–18 µm wide at apices and 50 µm wide at bases. Ostiolar hyphae hyaline, convergent, ~ 100 µm long. Ascospores hyaline, cylindrical, 6–11 µm long, 2–3.5 µm thick'' (Riedl 1962). Conidiophores hyaline, 1–5 septa, phialidic, lageniform, (174–)206–252(–295) µm long, (5–)7–9(–10) µm wide at bases and (4–)4–5(–6) µm wide at apices, mononematous with enteroblastic conidium ontogeny, solitary, emerging laterally or terminal on hyphae. Primary conidia hyaline, aseptate, cylindrical, (9–)10–12(–13) × (3–)

4–5(–5) µm. Secondary conidia aseptate, initially hyaline, turning umber (9) and thick-walled at maturity, mostly oblong, (9–)10–13(–15) × (5–)6–7(–8) µm wide. Aleurioconidia produced holoblastically, singly or in short chains, dark mouse gray (14''''k), granulated, thick-walled, mostly ovoid to subglobose, (10–)12–16(–18) × (4–)6–9(–11) µm wide. Colonies on MEA initially hyaline to white, becoming smoke gray (21''''f) with age.

Mating system: Undetermined.

Types: Holotype of *Ceratocystis musarum* (MBT 178360): AUSTRIA, Vienna, peduncles of *Musa speciosa* (= *M. ornata*), 20 Feb 1962, coll. *H. Riedl*, W 28259 (Naturhistorisches Museum Wien).

Epitype of *Ceratocystis musarum* (designated here, MBT 178360): NEW ZEALAND, on *Musa* sp., coll. *T. W. Canter-Visscher*, dried culture PREM 60962, living ex-epitype culture CMW 1546 = C 907.

Notes: Mitchell (1937) described a new variety of *T. paradoxa* associated with stem-end rot of banana in Australia. This description was invalid however in that it lacked a Latin diagnosis. Riedl (1962) isolated a similar fungus from banana stems in Vienna, although the source of the bananas was probably not Vienna, and described it as a new species, distinct from *T. paradoxa*. Following the practice of dual nomenclature, he named the sexual and asexual states of the fungus separately, altering the rank of the asexual state described by Mitchell (1937) from variety to species level. Both de Hoog (1974) and Nag Raj and Kendrick (1975) accepted the species from banana as distinct from *C. paradoxa*, while Upadhyay (1981) listed *C. musarum* as synonym of *C. paradoxa* based on a single specimen originating from banana in Canada. The material of Mitchell and that of Riedl was not included in the latter three studies. Paulin-Mahady et al. (2002) did not mention *T. musarum* in their treatment of *Thielaviopsis*. In a subsequent study (Harrington 2009) the sequence of an isolate (C 1480) presumably from banana, but of unknown origin, grouped separately from *C. paradoxa* s. str. and was labeled *C. musarum* in a phylogenetic tree. Based on this sequence, de Beer et al. (2013b) treated *C. musarum* as a distinct taxon.

In this study we compared the holotype of *C. musarum* and the original description of its asexual state (Riedl 1962) with an isolate (CMW 1546) from banana in New Zealand and found that they correspond morphologically. The dried specimen of the latter isolate is designated here as epitype for *C. musarum*. The ITS sequence for this isolate corresponded with the ITS sequence produced for the same isolate by Witthuhn et al. (1996 unpubl). The two sequences differed by three bp from a sequence produced in another study by Witthuhn et al. (1999) and an unpublished sequence of an isolate from date palm in Saudi Arabia (FIG. 3). The β-tubulin and TEF-1α sequences of the epitype were clearly distinct from all other species in the complex (SUPPLEMENTARY FIGS. 3–4), with the TEF-1α sequence corresponding with the one from banana in Harrington (2009).

Ceratocystis radicicola (Bliss) C. Moreau, Rev. Mycol. (Paris) Suppl. Col. 17:22. 1952. FIG. 8i–l
MycoBank MB294235

≡ *Ceratostomella radicicola* Bliss, Mycologia 33:468. 1941. (basionym)

≡ *Ophiostoma radicicolum* (Bliss) Arx, Antonie van Leeuwenhoek 18:211. 1952.

= *Chalaropsis punctulata* Hennebert, Antonie van Leeuwenhoek 33:334. 1967.

≡ *Thielaviopsis punctulata* (Hennebert) A.E. Paulin, T.C. Harr. & McNew, Mycologia 94:70. 2002.

''Ascomata perithecial. Ascomatal bases partially or completely submerged, faintly colored, nearly spherical, 180–320 µm diam. Ascomatal appendages variously branched, 35–90 µm long. Ascomatal necks dark, becoming hyaline at the apices, 440–980 µm long, 24–71 µm diam. Ostiolar hyphae hyaline and fimbriate. Asci deliquescent, not observed. Ascospores hyaline, ellipsoidal, sides unequally convex, continuous, 8–15 × 3–4 µm, covered with a mucous sheath'' (Bliss 1941). Conidiophores hyaline, phialidic, lageniform, (108–)141–207(–252) µm long, (5–)7–8(–10) µm wide at bases and (4–)4–5(–6) µm wide at apices, mononematous with enteroblastic conidium ontogeny, solitary, emerging laterally or terminal on hyphae. Primary conidia hyaline, aseptate, cylindrical or rectangular, (6–)8–14(–16) × (3–)4–5(–5) µm. Secondary conidia aseptate, initially hyaline, turning umber (9) and thick-walled at maturity, mostly oblong, (6–)10–14(–16) × (3–)4–6(–6) µm. Aleurioconidia produced holoblastically, produced singly, initially hyaline, dark mouse gray (14''''k) when mature, granulated, thick-walled, ovoid to subglobose with a flattened base, (10–)(11–)13–16(–18) × (9–)10–12(–13) µm wide. Colonies on MEA initially hyaline to white, becoming dark gray (21''''f) with age.

Mating system: Heterothallic (Bliss 1941, El-Ani et al. 1957).

Types: Holotype of *Ceratostomella radicicola* (MBT 378362): USA, California, Indio, root and trunk of *Phoenix dactylifera*, coll. *D.E. Bliss*, holotype (not seen) BPI 596268, isotype (not seen) IMI 036479, culture ex-holotype CBS 114.47 = CMW 1032 = MUCL 9526.

Holotype of *Chalaropsis punctulata* (MBT 2699): MAURITANIA, Atar, in roots of *Lawsonia inermis*, Apr 1966, coll. *J. Brun*, holotype not seen (CBS),

culture ex-holotype CBS 167.67 = ATCC 18454 = MUCL 8674 = CMW 26389 = IFAC H-A1.

Additional culture examined: IRAQ, on *Phoenix dactylifera*, 1993, A. Bahadli, CMW 37776 = IMI 316225.

Notes: Ceratocystis radicicola was isolated first and described from the roots of dying *Phoenix dactylifera* in California (Bliss 1941). The species was treated subsequently in *Ophiostoma* (von Arx 1952) and until present in *Ceratocystis* (Moreau 1952, Hunt 1956, de Hoog 1974, Nag Raj and Kendrick 1975, Upadhyay 1981). When Hennebert (1967) described *Chalaropsis punctulata*, he recognized that it was morphologically similar to the asexual state of *C. radicicola* but distinguished the two species based on conidial sizes. Nag Raj and Kendrick (1975) suggested that the conidial sizes overlapped and that the two species might be synonyms. Paulin-Mahady et al. (2002) showed that ITS sequences of the two species were identical and that the ex-type isolate of *Chalaropsis punctulata* could mate with an isolate of *C. radicicola*. In a phylogenetic tree based on concatenated sequence data for ITS, β-tubulin and TEF-1α produced by van Wyk et al. (2009), the ex-type of *Chalaropsis punctulata* (CMW 26389 = CBS 167.67) grouped not with *C. radicicola* but closest to *C. fagacearum*. When considered separately the TEF-1α blasted to *C. adiposa* (HM569644, Harrington 2009) and was in conflict with the other two gene regions. The ITS and β-tubulin sequences were identical to those obtained in this study for the ex-type of *C. radicicola*, CMW 1032 = CBS 114.47 and isolate CMW 37776 = IMI 316225. Based on these discrepancies, we have resequenced the three gene regions for isolate CMW 26389 = CBS 167.67 (ITS: KF953932, β-tubulin: KF953931, TEF-1α: KF917202). As found previously, the ITS and BT sequences for these isolates were identical. The TEF1-α sequence differed in one bp between CMW 26389 and CMW 1032 = CBS 114.47 and eight bp from CMW 37776 = IMI 316225, and grouped clearly within the *C. radicicola* clade (FIG. 2). Based on these results, we endorse the synonymy between *T. punctulata* and *C. radicicola* suggested by Paulin-Mahady et al. (2002).

Ceratocystis cerberus Mbenoun, M.J. Wingf. & Jol. Roux, sp. nov. FIG. 11

MycoBank MB805508

Etymology: Epithet refers to the multi-necked ascomata observed in the ex-type isolate of this species.

Ascomata perithecial, formed superficially on surface of substratum or suspended in aerial mycelium. Ascomatal bases mostly globose, (137–)260–340 (–370) μm high × (148–)268–348(–368) μm wide, initially straw (21f), unornamented, dark, covered with stellar or digitate appendages at maturity, (26–)27–37(–39) × (17–)19–27(–27). Ascomatal necks dark brown, commonly erect or curled and branched as observed in the holotype, (280–)650–984(–1244) μm long, (24–)29–43(–56) μm wide at apices, (43–)61–83(–101) μm wide at bases. Ostiolar hyphae hyaline, divergent, (47–)70–108(–142) μm long. Asci not observed. Ascospores in sheaths, hyaline, aseptate, ellipsoidal (6–)7–9(–13) × (3–)3–4(–6), accumulating in easily discharged, mucilaginous, buff (19''f) droplets at apex of ascomatal necks. Conidiophores hyaline, phialidic, lageniform, tapering toward apices, (63–)92–150(–215) μm long, (5–)6–7(–8) μm wide at bases and (3–)4–5(–6) μm wide at apices, mononematous with enteroblastic conidium ontogeny, solitary. Primary conidia hyaline, aseptate, cylindrical, (7–)7–12(–19) × (3–)4–5(–6) μm. Secondary conidia not observed. Aleurioconidia produced holoblastically, singly or in short chains of 2–3 units, umber (9), granulated, thick-walled, subglobose, obovoid (7–)9–12(–16) × (4–)6–8(–10) μm. Colonies on MEA gray-olivaceous (21''''b) or olivaceous-gray (21'''''b), reverse gray-olivaceous (21''''b). Mycelium mostly aerial, hyphae smooth and septate, not constricted at septa. Optimal temperature around 30 C, fast growing, ~ 70 mm on average in 36 h, no growth at 10 C or 35 C after 10 d.

Mating system: Homothallic.

Types: Holotype of *Ceratocystis cerberus*: CAMEROON, southwestern region, near Tiko (N4 10.377 E9 25.419), on stump of felled *Elaeis guineensis* tree, 13 Oct 2010, coll. *M. Mbenoun & J. Roux*, holotype PREM 60770 (PREM), culture ex-holotype CBS 130765 = CMW 36668.

Paratypes of Ceratocystis cerberus: CAMEROON, littoral region, Dibamba (N3 54.510 E9 50.177) on cut end of *Elaeis guineensis* leaf, 15 Oct 2010, coll. *M. Mbenoun & J. Roux*, paratype PREM 60769 (PREM), culture ex-paratype CBS 130764 = CMW 36653; central region, Bokito (N4 30.279 E11 04.748), on wound on *Theobroma cacao*, Dec 2009, coll. *M. Mbenoun & J. Roux*, paratype PREM 60768 (PREM), culture ex-paratype CBS 130763 = CMW 35021.

Additional cultures examined: CAMEROON, central region, Bokito (N4 30.279 E11 04.748), from trunk wound on *Theobroma cacao*, Dec 2009, coll. *M. Mbenoun & J. Roux*, CMW 35024; littoral region, Dibamba (N3 54.510 E9 50.177) on cut end of *Elaeis guineensis* leaf, 15 Oct 2010, coll. *M. Mbenoun & J. Roux*, CMW 36641.

Notes: Ceratocystis cerberus is the only known homothallic species in the *C. paradoxa* complex. It is also the only species that did not produce distinctive secondary conidia under the conditions used in the present study (TABLE III). Under certain

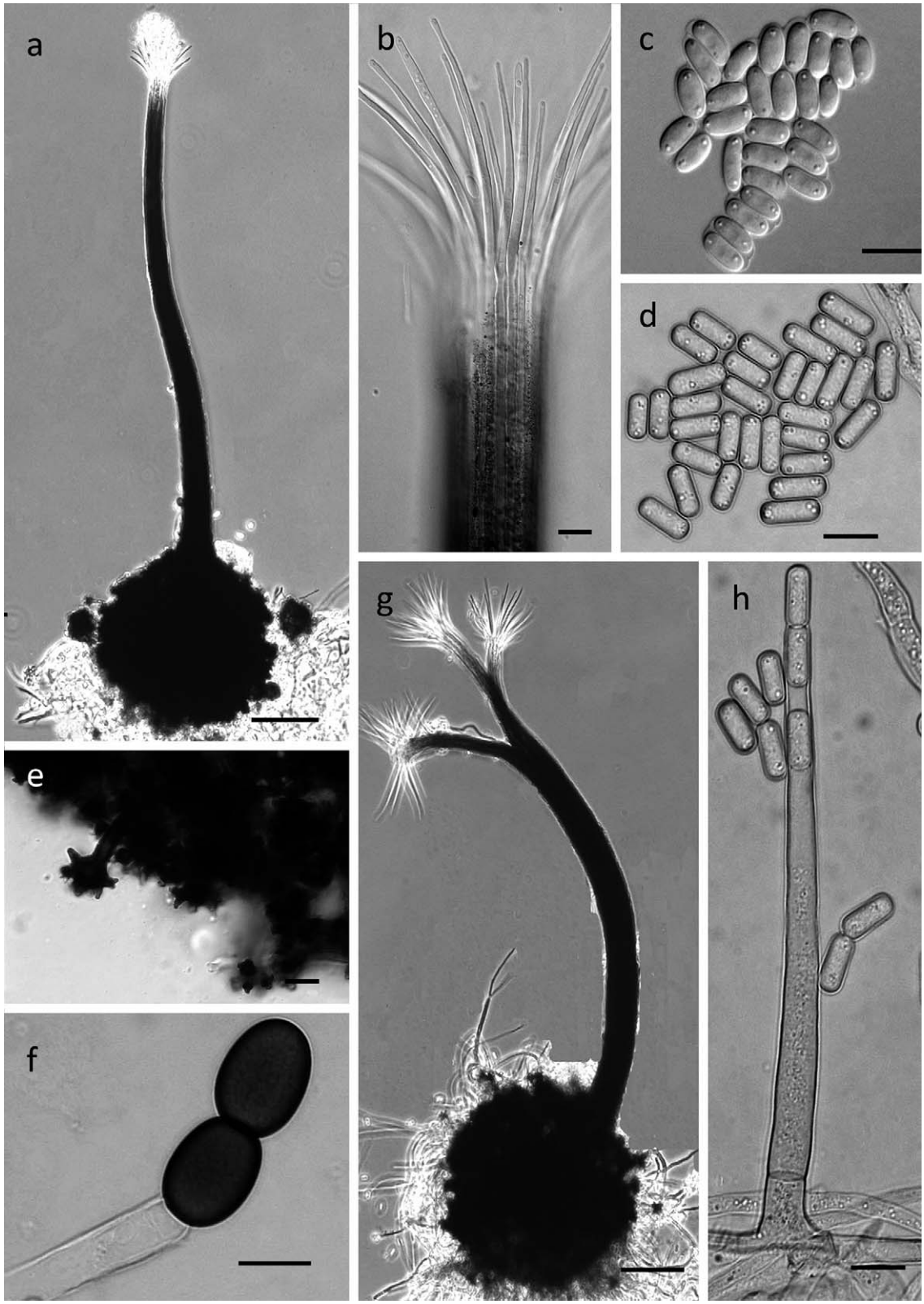

FIG. 11. Sexual and asexual structures from fresh cultures of *Ceratocystis cerberus* (isolates CMW 36668, CMW 36653, CMW 35021). a. Perithecium with globose base. b. Divergent ostiolar hyphae. c. Ellipsoidal ascospores invested in sheaths. d. Cylindrical primary conidia. e. Digitate ascomatal ornamentation. f. Thick-walled aleurioconidia in short chains. g. Perithecium with multiple dichotomous necks. h. Flasked-shaped phialidic conidiophore. Bars: a, g = 100 μm; b–e, g–h = 10 μm.

conditions *C. cerberus* forms ascomata with multiple dichotomous necks. In *Ceratocystis* this feature has been observed only in *C. mangivora* M. van Wyk & M.J. Wingf. (van Wyk et al. 2011).

DISCUSSION

Before this study, only four species were recognized in the *Ceratocystis paradoxa* complex, including *C. musarum*, *C. paradoxa*, *C. radicicola* and *Thielaviopsis euricoi* (Harrington 2009, Wingfield et al. 2013). Multigene DNA phylogenies in combination with mating studies and careful morphological examination of type material of all species and their synonyms revealed that the complex includes more species than previously recognized. Five species, including *C. paradoxa* s. str., are redefined and their descriptions amended. Lectotypes are designated for *C. ethacetica* and *Endoconidium fragrans* (synonym of *C. ethacetica*), epitypes were designated for *C. paradoxa* s. str., *C. ethacetica* and *C. musarum*, and a neotype was designated for *Catenularia echinata* (synonym of *C. ethacetica*). Two of the species, previously treated in *Thielaviopsis*, are transferred to *Ceratocystis* following the one fungus one name principles. A sixth species from Cameroon is described as new.

Few morphological differences could be used as a diagnostic tool to distinguish species within the *C. paradoxa* complex. Sexual structures are not known for all species and are not commonly observed in nature or in culture because of the predominantly heterothallic mating system in this group. Furthermore, the characteristics of these structures, where they have been observed, overlap among species (Dade 1928, Bliss 1941; see also TABLE III). Likewise, the shape and size of asexual structures mostly overlap. The only species with a potential diagnostic character is *C. radicicola*, which can be discriminated from all other known species based on its aleurioconidia that are borne singly. DNA sequences are therefore critically important for accurate identification of species in this complex. Of the three gene regions used in this study, the TEF1-α emerged as the marker providing the best resolution in the group while ITS showed the least resolution. In particular, ITS could not discriminate *C. paradoxa* s. str. and *C. euricoi*. The use of this marker as a default barcode system in the *C. paradoxa* complex therefore should be avoided, similar to what has been shown for members of the *C. moniliformis* sensu lato species complex (van Wyk et al. 2006; Kamgan Nkeukam et al. 2008, 2013).

Based on the dual nomenclature system for fungi, the sexual and asexual states of *Ceratocystis* species were placed in different genera. Following the rules of the Melbourne Code, these genera are now considered synonyms of *Ceratocystis* (de Beer et al. 2013b). Three of these genera that previously were considered anamorph-form genera are typified by currently recognized members of the *C. paradoxa* complex. The type species of *Thielaviopsis* is *T. ethacetica* (= *C. ethacetica*). *Stilbochalara dimorpha* (= *C. paradoxa* s. str.) is the type species of *Stilbochalara*, and *Hughesiella euricoi* (= *C. euricoi*) is the type species of *Hughesiella*. Following the Melbourne Code (Hawksworth 2011, McNeill et al. 2012) and the suggestions for a pragmatic approach to the naming of plant pathogens (Wingfield et al. 2012), these genus names are taxonomically convergent with *Ceratocystis* (de Beer et al. 2013b). However, if the genus *Ceratocystis* is split into smaller genera as proposed by Wingfield et al. (2013), *Thielaviopsis* could be reinstated based on priority to accommodate species in the *C. paradoxa* complex.

Based on combined ITS, β-tubulin and TEF1-α gene phylogenies, the genealogical structure of the *C. paradoxa* complex includes three major clades. Clade 1 occupies a basal position and includes *C. paradoxa* s. str., *C. euricoi* and an unnamed species represented by the isolate CMW 28535 = CBS 101054. The three species are conjointly characterized by the production of synnemata, a feature observed in the original description of de Seynes (1888) and subsequently reported only by Petch (1910). The latter author suggested that the production of synnemata is triggered by harsh conditions (e.g. impoverished and dry substrates). In the present study, only some of the isolates of *C. paradoxa* s. str. and *C. euricoi* produced synnemata. This occurred in paired cultures on WA supplemented with sugarcane chips originally set up to test for sexual compatibility. Of note, in the unassigned isolate CMW 28535 = CBS 101054, synnemata formed readily and abundantly on MEA. The formation of synnemata might represent a distinctive homologous character to members of Clade 1. Because it is variably expressed among and between species, the possibility that this feature is more common across the *C. paradoxa* s. lat. lineage and only requires adequate conditions to express, however, cannot be overruled a priori.

Clade 2 of *C. paradoxa* s. lat. is composed of *C. ethacetica* and *C. musarum*. These two species have similar morphological characteristics in their asexual states, shared by other members of the *C. paradoxa* complex. However, their sexual states differ markedly in morphology. For example, from the description of Riedl (1962), the sexual state of *C. musarum* includes the absence of ornamentation on ascomatal bases, convergent ostiolar hyphae and cylindrical ascospores without sheaths. These characteristics do not fit with the general descriptions of other members of *C.*

paradoxa s. lat. Based on examination of the holotype specimen of *C. musarum* we concluded that the fungus was at an early stage of development when it was preserved and, hence, ascomata were immature. We did not recover any ascospores from this specimen despite the abundant presence of ascomata. This also supports the view that Riedl (1962) could have confused primary conidia for ascospores. Vovlas et al. (1994) reported ''*C. paradoxa*'' in association with the nematode *Helicotylenchus multicinctus* (Cobb), causing necrotic lesions on roots and rhizomes of declining bananas in Sao Tome and Principe. Although the identity of the fungus cannot be ascertained, their description of its sexual structures conforms with those expected for *C. paradoxa* s. lat. and suggests a heterothallic mating system.

Although statistically well supported, Clade 3 of *C. paradoxa* s. lat. is heterogeneous in terms of morphological and biological characteristics of the fungi in this group. It could be separated into two subclades respectively represented by *C. radicicola* and *C. cerberus*. *Ceratocystis radicicola* is heterothallic (Bliss 1941) and the only species in which aleurioconidia are borne singly. On the other hand, *C. cerberus* forms aleurioconia in short chains and is the only species producing only one endoconidial form, in that no secondary conidia were observed in any of the isolates examined in this study. Moreover, *C. cerberus* is homothallic and produces large numbers of ascomata in isolates derived from single spores or hyphal tips on artificial media. This characteristic has not been observed in other species of *C. paradoxa* s. lat. with known sexual states. This suggests that homothallism is most likely a derived character in *C. cerberus*. Of note, Harrington (2009) mentioned a homothallic strain (C1753) of ''*C. paradoxa*'', which in this study was closely related with but distinct from *C. cerberus* based on TEF-1α. The putatively undescribed species represented by CMW 28536 = CBS 116770 also falls in the same clade. However, this strain did not produce perithecia in our tests. This does not preclude the possibility that it is homothallic and might have lost its ability to produce ascomata in culture.

The taxonomic reevaluation of *C. paradoxa* s. lat. prompts a reconsideration of the host range and geographic distribution of species in this complex. *Ceratocystis paradoxa* s. lat. has been considered cosmopolitan. The results of this limited study suggest that *C. paradoxa* s. str. is far less common than previously considered. It most commonly has been confused with *C. ethacetica*, which better fits the description of widespread distribution and broad host range generally associated with *C. paradoxa* s. lat. (Morgan-Jones 1967, Anonymous 1987). The host range of *Ceratocystis paradoxa* s. lat. includes economically important crops such as banana, cacao, coconut palm, date palm, oil palm, pineapple and sugarcane. These plants have spread around the world, expanding the geographic range of their associated and possibly coevolved pathogens, and providing them with new opportunities for host expansion. Clarifying the taxonomy of species in the *C. paradoxa* complex will assist in more accurate identification of these pathogens. It also should provide a solid foundation for research to determine their centers of origin, pathways of movement and the management of the diseases that they cause.

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