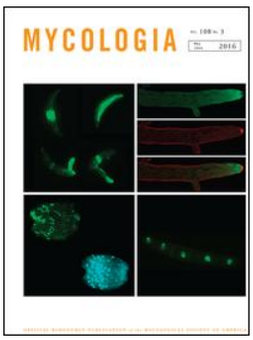

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Nine novel species of *Huntiella* from southern China with three distinct mating strategies and variable levels of pathogenicity

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ABSTRACT

The ascomycete genus *Huntiella* (Microascales) has a cosmopolitan distribution and occurs on a wide range of woody plants. Little is known regarding the identity, diversity, origin, or impact of these fungi in China. Recently, isolates of *Huntiella* spp. were collected from stumps of freshly felled trees or wounds on plantation-grown *Eucalyptus* in Guangdong, Guangxi, Fujian, and Hainan provinces of southern China. Additional isolates were obtained from stumps of *Acacia confusa* near *Eucalyptus* plantations in Hainan Province. The aim of this study was to identify these *Huntiella* species and to test their pathogenicity on *Eucalyptus* seedlings. Morphology and multigene phylogenies of the nuclear rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region and partial β-tubulin (*BT1*) and translation elongation factor 1α (*TEF1α*) genes revealed nine previously unknown *Huntiella* species, eight from *Eucalyptus* and one from *A. confusa*. The mating types of these species were determined, showing that seven are heterothallic, one is homothallic, and one is unisexual (*MAT1-2-1* gene). Pathogenicity tests showed that the nine *Huntiella* species can produce lesions on *Eucalyptus* seedlings, larger than wounds caused by controls on these plants. This study provides a basic understanding of the distribution, diversity, and pathogenicity of *Huntiella* species in southern China.

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Ceratocystidaceae; *Ceratocystis moniliformis* complex; phylogeny; plantation forestry; 9 new taxa

INTRODUCTION

The genus *Huntiella* belongs to the family Ceratocystidaceae (Microascales, Ascomycetes) as defined by De Beer et al. (2014). Other genera in the family include *Ambrosiella, Berkeleyomyces, Bretziella, Ceratocystis, Chalaropsis, Davidsoniella, Endoconidiophora, Meredithiella, Phialophoropsis*, and *Thielaviopsis* (De Beer et al. 2014, 2017; Mayers et al. 2015; Nel et al. 2017). The Ceratocystidaceae includes many important fungal pathogens of trees and agents of blue stain of timber globally (Wingfield et al. 1993; Roux and Wingfield 2009; De Beer et al. 2014). These fungi infect their hosts through wounds and are most commonly spread by insects, including bark beetles, nitidulid beetles, flies, and mites (Hayslett et al. 2008; Heath et al. 2009; Seifert et al. 2013; Mbenoun et al. 2016; Wingfield et al. 2017). Most species of Ceratocystidaceae share similar morphological characters, having dark, globoid ascomata and elongated necks that exude sticky ascospore masses at their tips. These characters reflect a general adaptation to insect dispersal (Upadhyay 1981; Wingfield et al. 1993; Seifert et al. 2013).

Until relatively recently, species of *Huntiella* were treated in *Ceratocystis* and commonly referred to as the *C. moniliformis* complex (Wingfield et al. 2013; De Beer et al. 2014). *Huntiella* species are distinguished from other genera in the Ceratocystidaceae based on their ecology, morphological characters, and phylogenetic relationships inferred from DNA sequence data (De Beer et al. 2014). Species of *Huntiella* are similar to those of *Ceratocystis*, having "hat-shaped" ascospores, but they differ in that *Huntiella* species have ascomata with "thick collar plates" connecting the ascomatal necks and bases. The ascomatal bases of *Huntiella* are rough-walled and ornamented with spines, whereas those of *Ceratocystis* are generally smooth-walled (Hedgcock 1906; De Beer et al. 2014). In addition, it is rare to find aleurioconidia in *Huntiella* species, whereas these are commonly produced by most *Ceratocystis* species (Hedgcock 1906; Seifert et al. 2013).

Huntiella includes at least 20 species on a broad range of hosts, with a cosmopolitan distribution (Van Wyk et al. 2006; De Beer et al. 2014; De Errasti et al. 2015; Mbenoun et al. 2016). For example, *H. moniliformis* is reported worldwide, including Africa (Luc 1952; Heath et al. 2009; Kamgan Nkuekam et al. 2012), Asia (Kitajima

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1936; Roldan 1962), Europe (Bakshi 1951; Kowalski and Butin 1989), North America (Hedgcock 1906; Davidson 1935), and South America (Cristobal and Hansen 1962; Van Wyk et al. 2011). *Huntiella* species are commonly encountered on tree wounds and generally regarded as nonpathogenic (Davidson 1935; Van Wyk et al. 2006; De Errasti et al. 2015; Mbenoun et al. 2016). Some species produce lesions in artificial inoculation experiments (Tarigan et al. 2010; Chen et al. 2013; De Errasti et al. 2015; Mbenoun et al. 2016), raising concern that under certain situations, they could be more important than has generally been assumed.

Species boundaries in *Huntiella* are not easily defined. Most have similar morphologies, and as new species are described it has become increasingly difficult to delimit them based on morphological characters alone (Van Wyk et al. 2006; Kamgan Nkuekam et al. 2008; Mbenoun et al. 2016). It is possible to distinguish between species based on DNA sequence comparisons and phylogenetic inference. Unfortunately, nuclear rDNA internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region, selected as the barcoding region for fungal species identification (Schoch et al. 2012), is of limited use in delineating *Huntiella* species (Van Wyk et al. 2004, 2006, 2011; Mbenoun et al. 2014). In contrast, partial β-tubulin (*BT1*) and translation elongation factor 1α (*TEF1α*) gene sequences provide better resolution among species, despite examples of incongruency between these regions (Kamgan Nkuekam et al. 2008; Mbenoun et al. 2014).

In surveys of potential fungal pathogens of plantation-grown forest tree species in southern China, several isolates resembling species of *Huntiella* were obtained from fresh stumps and wounds on *Eucalyptus* and *Acacia* trees. The aim of this study was to identify these isolates based on morphology and multigene phylogenies of the ITS, *BT1*, and *TEF1α* sequences.

MATERIALS AND METHODS

Fungal isolates.—Wood chips with structures resembling those of *Huntiella* species were collected from wounds of fallen trees and recently harvested stems (up to 1 mo old) of *Eucalyptus* species (FIG. 1A–B) and other tree species in the vicinity of *Eucalyptus* plantations. These samples were collected within the Guangdong, Guangxi, Fujian, and Hainan provinces of southern China between Sep 2013 and Apr 2014. Cultures were isolated by transferring ascospore masses from ascomata growing on the surfaces of the wood chips to 2% malt extract agar medium (MEA; 20 g/L malt extract [Biolab, Midrand, South Africa], 20 g/L agar [Difco, Maryland, USA]), and incubated at 25 C for 1 wk. Isolates were regularly inspected under a dissecting microscope and purified by isolating single hyphal tips onto 2% MEA.

Cultures of *Huntiella* isolates were deposited in the culture collection of the China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), Zhanjiang, China. Duplicate cultures have also been preserved in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Furthermore, representative isolates of novel species were deposited at the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands. Dried specimens were also deposited at the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA extraction, PCR, and sequencing.—Isolates obtained during this study were used for DNA sequence–based characterization. DNA was extracted from the mycelium of single hyphal tip isolates grown on 2% MEA for 2–3 wk at 25 C, using the protocol developed by Möller et al. (1992). Final DNA concentrations of ~100 ng/μL were prepared for polymerase chain reaction (PCR) amplification using a Thermo Scientific NanoDrop ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, Delaware).

Three markers were amplified for sequencing and phylogenetic analysis. The ITS was amplified with the primers ITS1 and ITS4 (White et al. 1990). A partial fragment of the *BT1* was amplified with the primers Bt1a and Bt1b (Glass and Donaldson 1995), and a partial fragment of *TEF1α* was amplified with the primers TEF1F and TEF2R (Jacobs et al. 2004).

Standard PCR reactions of 25 μL were conducted for each gene region. These reactions contained 50 ng of DNA template, 1 μM of each primer, 5 μL MyTaq PCR buffer (Bioline, London, UK), and 1 unit of MyTaq DNA polymerase (Bioline). The amplification reactions were conducted on a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, California). The thermocycling protocol for all three gene regions was as follows: an initial denaturation step at 95 C for 5 min, followed by 35 cycles of 30 s at 95 C, 45 s at 56 C, and 60 s at 72 C. The reaction was completed with a final extension at 72 C for 10 min.

PCR products were purified using ExoSap-IT PCR Product Clean-up Reagent (Thermo Fisher Scientific, Waltham, Massachusetts) to remove excess primers and dNTPs. Purified products were sequenced using the BigDye Terminator 3.1 cycle sequencing premix kit (Applied Biosystems), employing the same forward and reverse primers as used for PCR. The sequencing protocol consisted of 25 cycles of 10 s at 96 C, 5 s at

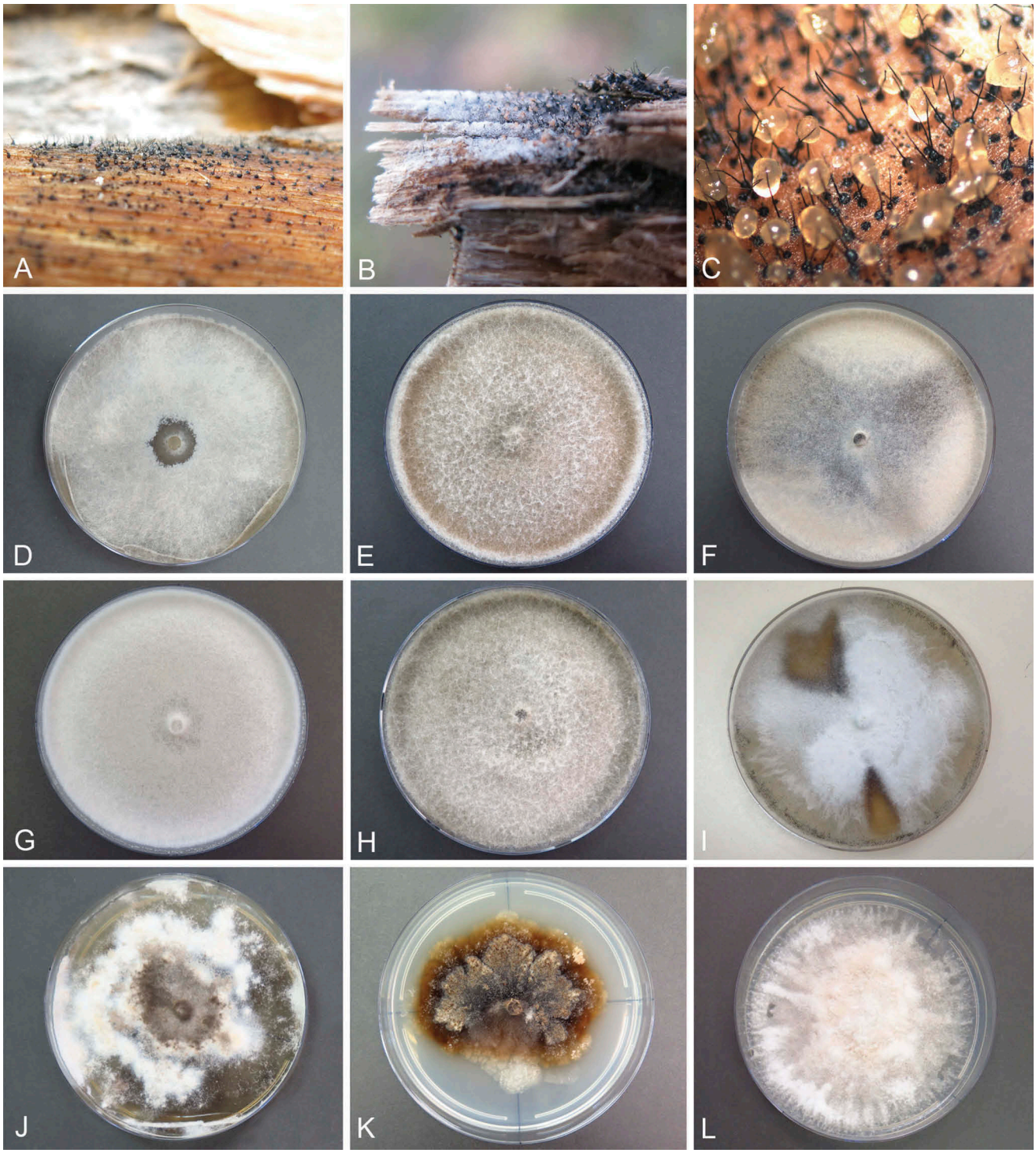

Figure 1. Structures of *Huntiella* on wood in the field and colony morphology on agar medium. A. Abundant *Huntiella* structures produced on a *Eucalyptus* stump. B. Ascomata on *Eucalyptus* stump producing ascospore masses in cold weather. C. *Huntiella fecunda* producing abundant ascomata and ascospores on the surface of 2% MEA. D–L. Colony morphologies of different *Huntiella* species on 90-mm Petri dishes containing 2% MEA after 2 wk. D. *H. ani*. E. *H. bellula*. F. *H. confusa*. G. *H. eucalypti*. H. *H. fabiensis*. I. *H. fecunda*. J. *H. glaber*. K. *H. inaequabilis*. L. *H. meiensis*.

56 C, and 4 min at 60 C. An ABI PRISM 3100 autosequencer (Applied Biosystems) was used for sequencing.

Phylogenetic analyses.—Sequence data for the representative type isolates of all published *Huntiella* species were downloaded from GenBank. Sequences

produced in this study and those from GenBank were checked manually in MEGA 7 and Geneious 7.0 was used to analyze the consensus sequences. All sequences were aligned using MAFFT 7 (http://align.bmr.kyushuu.ac.jp/mafft/on-line/server; Katoh et al. 2002) and then confirmed manually. Sequence alignments for all data sets were deposited in TreeBASE (study no. 22889), and sequences for the novel species were deposited in GenBank (TABLE 1).

Maximum parsimony (MP) phylogenetic analyses of single-gene data sets for the ITS, *BT1*, and *TEF1α* gene regions and the combined sequence data sets based on parsimony were carried out using PAUP 4.0b10 (Swofford 2003). The heuristic tree search algorithm was selected to generate phylograms, with the following options: sequence randomization (reps = 1000), tree bisection reconnection (TBR) branch swapping, and gaps treated as a fifth character. To determine the confidence intervals of the branch nodes, 1000 bootstrap replicates were conducted with the full heuristic search option. The parameters estimated for the most parsimonious trees included the tree length (TL), retention index (RI), consistency index (CI), and rescaled consistency index (RC). A partition homogeneity test (PHT; Swofford 2003) was run to verify that data for the three gene regions could be combined in the analyses.

Maximum likelihood (ML) analysis was carried out using PhyML 3.1 (Guindon and Gascuel 2003) on the data sets for the individual gene regions and the combined data set. Substitution models were selected for each data set with the Akaike information criterion (AIC) in jModeltest 2.1.5 (Posada 2008). Confidence levels for nodes were estimated using 1000 replicate bootstrap analyses. For both MP and ML analyses, *Ceratocystis cercfabiensis* (CMW 43029; Liu et al. 2015) was used as the outgroup taxon.

Morphological and growth studies.—For each of the putative new species identified in the phylogenetic analyses, growth and morphological studies were conducted. Cultures were grown on 2% MEA for 1–2 wk at 25 C. Descriptions of colony morphology and color (upper and reverse surfaces) were based on the mycological color charts of Rayner (1970). The morphology of fruiting structures was studied using 1–2-wk-old cultures on 2% MEA, which were incubated at 25 C. Microscope slides were prepared by placing each structure in 80% lactic acid, and these were examined using a Nikon H550L microscope (Nikon, Yokohama, Japan). Fifty measurements of characteristic morphological structures were made for each isolate. All measurements were computed and presented as (minimum–)(mean − SD)–(mean + SD)(–maximum).

To determine the optimal growth temperature for the putative new species, two or three isolates of each species were used for growth studies at temperatures ranging from 5 to 35 C, at 5-degree intervals. Five replicate plates were prepared for each isolate at each temperature, by transferring a 4-mm plug to the centers of 90-mm Petri dishes containing 2% MEA. Plates were incubated at the test temperatures for 4 d. Measurements were taken along two perpendicular axes, centered on the plugs and at right angles to each other for each colony, and the averages of diameter measurements at each temperature were computed.

Mating tests.—The mating type of each isolate of the new species was determined by positive amplification using specific mating type primers developed by Wilson et al. (2015). Primers Oman_111_F and Oman_111_R amplify a 335-bp fragment of the *MAT1-1-1* gene, and primers Om_Mo_121_F and Om_Mo_121_R amplify a 572-bp fragment of the *MAT1-2-1* gene. The same PCR protocol described above was used, with the exception that the annealing temperature was 53 C (Wilson et al. 2015).

Isolates of opposite mating type of the same species were crossed with each other in all possible combinations in an attempt to induce the production of ascomata. Mycelium-covered agar blocks were placed next to each other ca. 2 cm apart on a single plate. These pairings were done on 90-mm Petri dishes containing 2% MEA and incubated in the dark for 1–2 mo at 20 C. Crosses were inspected once per wk for the presence of ascomata.

Pathogenicity tests.—Seventeen isolates representing the nine *Huntiella* species were selected for pathogenicity trials to inoculate on *Eucalyptus* clone CEPT-11 (*Eucalyptus urophylla* × *E. grandis*) in a glasshouse. Isolates were grown on 2% MEA at 25 C for 7 d before inoculation. The seedlings were 2 m in height and had an average diameter at the root collar of 2 cm.

Inoculations were conducted using the same method described by Liu et al. (2018). Fifteen seedlings were inoculated for each of the 17 isolates, and 15 additional seedlings were inoculated with sterile MEA plugs to serve as negative controls. The inoculated seedlings were arranged in a randomized design in the glasshouse. The inoculated plants were evaluated after 6 wk by measuring lesion lengths in the

Table 1. List of *Huntiella* and *Ceratocystis* species included in this study.

Species[a]	CMW no.[b]	Other no.[b]	GenBank accession no.[c]			Host (or substrate)	Origin	Latitude and longitude	Reference
			ITS	*BT1*	*TEF1a*				
Ceratocystis cercfabiensis	CMW 43029	CERC 2170; CBS 139654	KP727592	KP727618	KP727643	*Eucalyptus* sp.	Hainan, China		Liu et al. 2015
Huntiella ani	**CMW 44684**[d,e]	**CERC 2827; CBS 143283**	**MH118602**	**MH118635**	**MH118668**	*Eucalyptus* sp.	Guangxi, China	23°26′34″N, 108°14′40″E	Present study
H. ani	**CMW 44686**[d,e]	**CERC 2829 CBS 143282**	**MH118603**	**MH118636**	**MH118669**	*Eucalyptus* sp.	Guangxi, China	23°26′34″N, 108°14′40″E	Present study
H. ani	**CMW 44687**	**CERC 2830**	**MH118604**	**MH118637**	**MH118670**	*Eucalyptus* sp.	Guangxi, China	23°26′34″N, 108°14′40″E	Present study
H. ani	**CMW 49315**[d]	**CERC 2871; CBS 143284**	**MH118611**	**MH118644**	**MH118677**	*Eucalyptus* sp.	Guangxi, China	24°28′2″N, 109°45′50″E	Present study
H. bellula	**CMW 49312**[d,e]	**CERC 2854; CBS 143286**	**MH118607**	**MH118640**	**MH118673**	*Eucalyptus* sp.	Guangxi, China	24°28′2″N, 109°45′50″E	Present study
H. bellula	**CMW 44702**	**CERC 2859**	**MH118608**	**MH118641**	**MH118674**	*Eucalyptus* sp.	Guangxi, China	24°28′2″N, 109°45′50″E	Present study
H. bellula	**CMW 49313**	**CERC 2861**	**MH118609**	**MH118642**	**MH118675**	*Eucalyptus* sp.	Guangxi, China	24°28′2″N, 109°45′50″E	Present study
H. bellula	**CMW 49314**[d,e]	**CERC 2862; CBS 143285**	**MH118610**	**MH118643**	**MH118676**	*Eucalyptus* sp.	Guangxi, China	24°28′2″N, 109°45′50″E	Present study
H. bellula	**CMW 49316**[d]	**CERC 2880; CBS 143287**	**MH118612**	**MH118645**	**MH118678**	*Eucalyptus* sp.	Guangxi, China	24°20′33″N, 110°4′59″E	Present study
H. bhutanensis	CMW 8242	CBS 112907	AY528951	AY528956	AY528961	*Picea spinulosa*	Jelekha, Bhutan		Van Wyk et al. 2004
H. bhutanensis	CMW 8217	CBS 114289	AY528957	AY528962	AY528952	*Picea spinulosa*	Jelekha, Bhutan		Van Wyk et al. 2004
H. ceramica	CMW 15245	CBS 122299	EU245022	EU244994	EU244926	*Eucalyptus grandis*	Malawi		Heath et al. 2009
H. ceramica	CMW 15248	CBS 122300	EU245024	EU244996	EU244928	*Eucalyptus grandis*	Malawi		Heath et al. 2009
H. chinaeucensis	CMW 24658	CBS 127185	JQ862729	JQ862717	JQ862741	*Eucalyptus* sp.	Guangdong, China		Chen et al. 2013
H. chinaeucensis	CMW 24661	CBS 127186	JQ862731	JQ862719	JQ862743	*Eucalyptus* sp.	Guangdong, China		Chen et al. 2013
H. chlamydoformis	CMW 36932	CBS 131674	KF769087	KF769109	KF769098	*Theobroma cacao*	Cameroon		Mbenoun et al. 2016
H. chlamydoformis	CMW 37102	CBS 131675	KF769088	KF769110	KF769099	*Terminalia superba*	Cameroon		Mbenoun et al. 2016
H. confusa	**CMW 49300**[d,e]	**CERC 2141; CBS 143289**	**MH118582**	**MH118615**	**MH118648**	*Acacia confusa*	Hainan, China	19°42′57″N, 109°37′3″E	Present study
H. confusa	**CMW 43452**[d]	**CERC 2158; CBS 143577**	**MH118583**	**MH118616**	**MH118649**	*Acacia confusa*	Hainan, China	19°42′57″N, 109°37′3″E	Present study
H. confusa	**CMW 43453**[d,e]	**CERC 2162; CBS 143288**	**MH118584**	**MH118617**	**MH118650**	*Acacia confusa*	Hainan, China	19°42′57″N, 109°37′3″E	Present study
H. cryptoformis	CMW 36826	CBS 131277	KC691462	KC691486	KC691510	*Terminalia sericea*	South Africa		Mbenoun et al. 2014
H. cryptoformis	CMW 36828	CBS 131279	KC691464	KC691488	KC691512	*Ziziphus mucronata*	South Africa		Mbenoun et al. 2014
H. decipiens	CMW 25918	CBS 129735	HQ203218	HQ203235	HQ236437	*Eucalyptus cloeziana*	South Africa		Kamgan Nkuekam et al. 2013
H. decipiens	CMW 25914	CBS 129737	HQ203219	HQ203236	HQ236438	*Eucalyptus maculata*	South Africa		Kamgan Nkuekam et al. 2013
H. eucalypti	**CMW 44692**[d,e]	**CERC 2840; CBS 143291**	**MH118605**	**MH118638**	**MH118671**	*Eucalyptus* sp.	Guangxi, China	24°40′38″N, 108°20′16″E	Present study
H. eucalypti	**CMW 44693**[d,e]	**CERC 2841; CBS 143290**	**MH118606**	**MH118639**	**MH118672**	*Eucalyptus* sp.	Guangxi, China	24°40′38″N, 108°20′16″E	Present study
H. fabiensis	**CMW 44370**[d,e]	**CERC 2736; CBS 143293**	**MH118589**	**MH118622**	**MH118655**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 49307**[d]	**CERC 2753; CBS 143294**	**MH118596**	**MH118629**	**MH118662**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 49308**	**CERC 2755**	**MH118597**	**MH118630**	**MH118663**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 44382**	**CERC 2762**	**MH118598**	**MH118631**	**MH118664**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 49309**[d,e]	**CERC 2763; CBS 143292**	**MH118599**	**MH118632**	**MH118665**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 49310**	**CERC 2764**	**MH118600**	**MH118633**	**MH118666**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fabiensis	**CMW 49311**	**CERC 2765**	**MH118601**	**MH118634**	**MH118667**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. fecunda	**CMW 49301**[d,e]	**CERC 2446; CBS 143304**	**MH118585**	**MH118618**	**MH118651**	*Eucalyptus* sp.	Fujian, China	24°44′36″N, 117°50′5″E	Present study

(*Continued*)

Table 1. (Continued).

Species[a]	CMW no.[b]	Other no.[b]	GenBank accession no.[c] ITS	*BT1*	*TEF1a*	Host (or substrate)	Origin	Latitude and longitude	Reference
H. fecunda	**CMW 49302**[d]	**CERC 2449; CBS 143296**	**MH118586**	**MH118619**	**MH118652**	*Eucalyptus* sp.	Fujian, China	24°44′36″N, 117°50′5″E	Present study
H. fecunda	**CMW 49303**[d,e]	**CERC 2451a; CBS 143295**	**MH118587**	**MH118620**	**MH118653**	*Eucalyptus* sp.	Fujian, China	24°44′36″N, 117°50′5″E	Present study
H. fecunda	**CMW 49304**	**CERC 2451f**	**MH118588**	**MH118621**	**MH118654**	*Eucalyptus* sp.	Fujian, China	24°44′36″N, 117°50′5″E	Present study
H. glaber	**CMW 43436**[d,e]	**CERC 2132; CBS 143298**	**MH118580**	**MH118613**	**MH118646**	*Eucalyptus exserta*	Hainan, China	19°2′14″N, 110°30′32″E	Present study
H. glaber	**CMW 49299**[d]	**CERC 2133; CBS 143297**	**MH118581**	**MH118614**	**MH118647**	*Eucalyptus exserta*	Hainan, China	19°2′14″N, 110°30′32″E	Present study
H. inaequabilis	**CMW 44372**[d,e]	**CERC 2740; CBS 143300**	**MH118590**	**MH118623**	**MH118656**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. inaequabilis	**CMW 49306**[d,e]	**CERC 2749; CBS 143299**	**MH118595**	**MH118628**	**MH118661**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. inquinana	CMW 21106		EU588587	EU588666	EU588674	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. inquinana	CMW 21107	CBS 124009	EU588588	EU588667	EU588675	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. meiensis	**CMW 44374**[d,e]	**CERC 2742; CBS 143302**	**MH118591**	**MH118624**	**MH118657**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. meiensis	**CMW 44375**	**CERC 2743**	**MH118592**	**MH118625**	**MH118658**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. meiensis	**CMW 49305**[d]	**CERC 2744; CBS 143303**	**MH118593**	**MH118626**	**MH118659**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. meiensis	**CMW 44376**[d,e]	**CERC 2746; CBS 143301**	**MH118594**	**MH118627**	**MH118660**	*Eucalyptus* sp.	Guangdong, China	24°44′3″N, 116°22′39″E	Present study
H. microbasis	CMW 21117	CBS 124013	EU588593	EU588672	EU588680	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. microbasis	CMW 21115	CBS 124015	EU588592	EU588671	EU588679	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. moniliformis	CMW 9590	CBS 116452	AY431101	AY528985	AY529006	*Eucalyptus grandis*	South Africa		Van Wyk et al. 2006
H. moniliformis	CMW 4114	CBS 118151	AY528997	AY528986	AY529007	*Shizolobium parahyba*	Ecuador		Van Wyk et al. 2006
H. moniliformopsis	CMW 9986	CBS 109441	AY528998	AY528987	AY529008	*Eucalyptus obliqua*	Australia		Yuan and Mohammed 2002
H. moniliformopsis	CMW 10214	CBS 115792	AY528999	AY528988	AY529009	*Eucalyptus sieberi*	Australia		Yuan and Mohammed 2002
H. oblonga	CMW 23803	CBS 122291	EU245019	EU244991	EU244951	*Acacia mearnsii*	South Africa		Heath et al. 2009
H. oblonga	CMW 23802		EU245020	EU244992	EU244952	*Acacia mearnsii*	South Africa		Heath et al. 2009
H. omanensis	CMW 11048	CBS 115787	DQ074742	DQ074732	DQ074737	*Mangifera indica*	Oman		Al-Subhi et al. 2006
H. omanensis	CMW 3800	CBS 117839	DQ074743	DQ074733	DQ074738	*Mangifera indica*	Oman		Al-Subhi et al. 2006
H. pycnanthi	CMW 36916	CBS 131672	KF769096	KF769118	KF769107	*Theobroma cacao*	Cameroon		Mbenoun et al. 2016
H. pycnanthi	CMW 36910		KF769095	KF769117	KF769106	*Theobroma cacao*	Cameroon		Mbenoun et al. 2016
H. salinaria	CMW 25911	CBS 129733	HQ203213	HQ203230	HQ236432	*Eucalyptus maculata*	South Africa		Kamgan Nkuekam et al. 2013
H. salinaria	CMW 30703	CBS 129734	HQ203214	HQ203231	HQ236433	*Eucalyptus saligna*	South Africa		Kamgan Nkuekam et al. 2013
H. savannae	CMW 17300	CBS 121151	EF408551	EF408565	EF408572	*Acacia nigrescens*	South Africa		Kamgan Nkuekam et al. 2008
H. savannae	CMW 17297		EF408552	EF408566	EF408573	*Combretum zeyheri*	South Africa		Kamgan Nkuekam et al. 2008
H. sublaevis	CMW 22449	CBS 122517	FJ151431	FJ151465	FJ151487	*Terminalia ivorensis*	Ecuador		Van Wyk et al. 2011
H. sublaevis	CMW 22444	CBS 122518	FJ151430	FJ151464	FJ151486	*Terminalia ivorensis*	Ecuador		Van Wyk et al. 2011
H. sumatrana	CMW 21109	CBS 124011	EU588589	EU588668	EU588676	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. sumatrana	CMW 21111	CBS 124012	EU588590	EU588669	EU588677	*Acacia mangium*	Indonesia		Tarigan et al. 2010
H. tribiliformis	CMW 13011	CBS 115867	AY528991	AY529001	AY529012	*Pinus merkusii*	Indonesia		Van Wyk et al. 2006
H. tribiliformis	CMW 13012	CBS 118242	AY528992	AY529002	AY529013	*Pinus merkusii*	Indonesia		Van Wyk et al. 2006
H. tyalla	CMW 28917		HM071899	HM071909	HQ236448	*Eucalyptus grandis*	Australia		Kamgan Nkuekam et al. 2012
H. tyalla	CMW 28920		HM071896	HM071910	HQ236449	*Eucalyptus grandis*	Australia		Kamgan Nkuekam et al. 2012

[a]Species indicated in bold are newly described in this study.
[b]CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CERC = culture collection of China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), Zhanjiang, Guangdong Province, China; CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.
[c]GenBank accession numbers indicated in bold were generated in this study.
[d]Isolates used for the growth study.
[e]Isolates used for the pathogenicity study.

cambium. The inoculated fungi were reisolated by cutting small pieces of wood from the edges of the lesions/wounds and cultivating them in 2% MEA at 25 C. Reisolations were made from five randomly selected seedlings per test isolate and all seedlings that served as negative controls. The data were analyzed using one-way analyses of variance (ANOVAs) using SAS 9.3 (SAS Institute Inc. 2011).

RESULTS

Fungal isolates.—Collectively, 33 isolates with morphology typical of species in *Huntiella* were obtained (FIG. 1C–L). Of these, 30 were from *Eucalyptus* species and included 13 from Guangdong Province, 11 from Guangxi Province, 4 from Fujian Province, and two from Hainan Province (TABLE 1; FIG. 2). The remaining three were obtained from *Acacia confusa* in Hainan Province. All isolates were fast growing on MEA, produced dark ascomata with conical spines ornamenting their bases, had long necks, and produced hat-shaped ascospores. Additionally, all cultures also produced both sexual and asexual structures in culture, typically within 1 wk. However, the ability to produce these structures diminished over time. All of these characters are typical of species in *Huntiella* (Van Wyk et al. 2004; De Beer et al. 2014). Isolates obtained from this study have been preserved in the three culture collections described above (TABLE 1).

Sequencing and phylogenetic analyses.—PCR products and sequence data were generated for all 33 isolates, which were approximately 540 bp for ITS, 530 bp for *BT1*, and 830 bp for *TEF1α*. All sequences obtained for the 33 *Huntiella* isolates in this study were deposited in GenBank (TABLE 1).

Four data sets were used in the phylogenetic analyses, namely, the ITS (72 taxa, 612 characters), *BT1* (72 taxa, 564 characters), *TEF1α* (72 taxa, 844 characters), and a combined data set (72 taxa, 2020 characters). The aligned sequences for the data sets were deposited in TreeBASE (no. 22889). Statistical values for the parameters used in the phylogenetic trees for the MP analyses and the best fit substitution models of ML are provided in TABLE 2. Partition homogeneity tests (PHTs) with 1000 replicates for the three gene regions produced a $P = 0.045$, indicating that the data sets could be combined (Cunningham 1997).

Data for 19 previously described species of *Huntiella* were included in the analyses, which also included the 33 isolates from China. Topologies of the trees resulting from the MP and ML analyses were concordant, showing similar phylogenetic relationships among the species for all data sets (FIG. 3; SUPPLEMENTARY FIGS. 1, 2, and 3). Furthermore, the isolates from China formed nine well-resolved and unique phylogenetic groups based on three of the data sets, excluding ITS (SUPPLEMENTARY FIG. 1). Phylogenetic groups 1–6 were composed of isolates from China, were most closely related to *H. bhutanensis*, and formed subclade 1 of the Asian Clade (FIG. 3). Groups 7 and 8 were phylogenetically close to *H. chinaeucensis, H. inquinans, H. microbasis, H. omanensis*, and *H. sumatrana* in subclade 2 of the Asian Clade (FIG. 3). Phylogenetic group 9 was most closely related to *H. moniliformis, H. sublaevis*, and *H. tyalla* of the Indo-Pacific Clade (FIG. 3).

Morphological and growth studies.—The isolates representing the nine new phylogenetic groups all had morphological characteristics typical of *Huntiella* species. They produced hat-shaped ascospores and had short conical spines on their ascomatal bases. Colonies of isolates for the nine phylogenetic groups grew rapidly on MEA, covering the surfaces of 90-mm Petri dishes in 4–5 d (FIG. 1C–L) and had optimal growth at temperatures between 25 and 30 C. Mycelium was white when young and turned darker with age (FIG. 1C–L). Morphological differences were observed between isolates representing each phylogenetic group and their closest phylogenetic neighbor, especially in the sizes of ascomatal bases and conidia. Furthermore, there were also cases of growth rate differences.

Mating tests.—All isolates in phylogenetic group 8 (*H. glaber*) had both the *MAT1-1-1* and *MAT1-2-1* genes in a single isolate, were able to sexually reproduce in isolation, and are thus homothallic. Isolates in group 9 (*H. fecunda*) had only the *MAT1-2-1* gene but were able to sexually reproduce in isolation and are thus unisexual. Isolates in the seven other groups were heterothallic, having either a *MAT1-1-1* gene or a *MAT1-2-1* gene. In the case of these groups, isolates of opposite mating type were crossed and produced sexual structures in culture. This confirmed their heterothallic nature.

Pathogenicity tests.—All 17 *Huntiella* isolates tested for pathogenicity on the *Eucalyptus* seedlings produced localized lesions on the cambium after 6 wk. The negative controls produced only a wound response or callus around the inoculation sites (FIGS. 13 and 14). Results of ANOVA tests showed that the

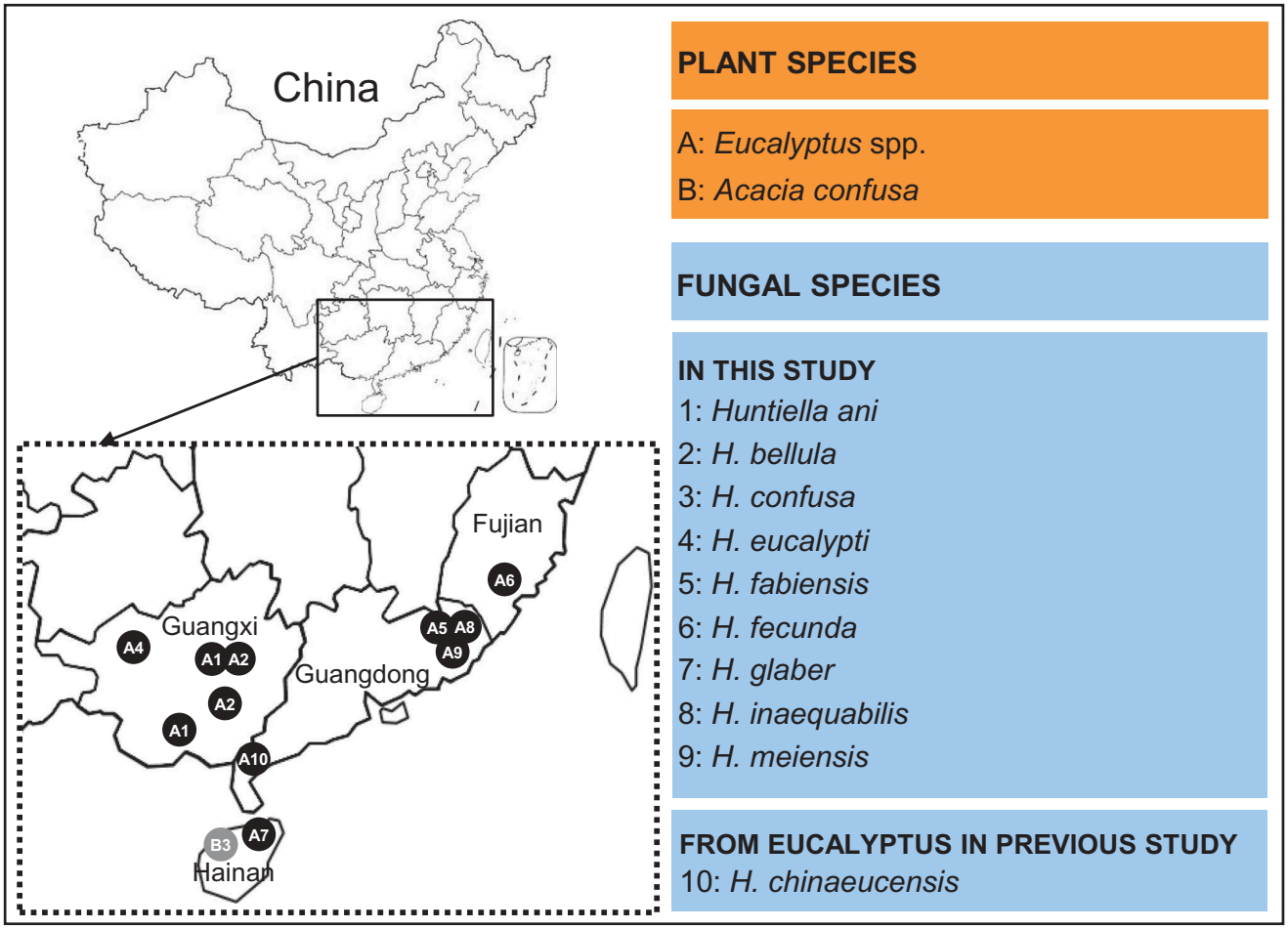

Figure 2. Map showing the nine species of *Huntiella* detected from different regions and plant hosts in China. The different *Huntiella* species are indicated as numbers 1 to 9, and the plant hosts are shown as letters A to B. A1, for example, indicates *H. ani* isolated from *Eucalyptus* spp. in Guangxi Province.

Table 2. Parameters used in phylogenetic analyses in this study.

Analysis	Parameter	ITS	*BT1*	*TEF1a*	Combined
MP	No. of taxa	72	72	72	72
	No. of bp	612	564	844	2020
	PIC	16	67	202	285
	Number trees	3000	42	3000	128
	Tree length	29	130	454	626
	CI	0.69	0.738	0.782	0.752
	RI	0.919	0.933	0.945	0.936
	HI	0.31	0.262	0.218	0.248
ML	Subst. model	TPM2uf+I	TrN+I+G	TPM2uf+G	TIM2+I+G
	NST	6	6	6	6
	P-inv	0.468	0.513	—	0.474
	Gamma	—	0.658	0.271	0.589

Note. bp = base pairs; PIC = number of parsimony informative characters; CI = consistency index; RI = retention index; HI = homoplasy index; Subst. model = best-fit substitution model; NST = number of substitution rate categories.

lesions produced by *H. bellula* (CMW 49312 and CMW 49314), *H. eucalypti* (CMW 44693), *H. fabiensis* (CMW 44370), *H. fecunda* (CMW 49301 and CMW 49303), *H. glaber* (CMW 43436), and *H. meiensis* (CMW 44376) were significantly longer than the wounds produced by negative controls ($P < 0.05$) (FIG. 13). Isolate CMW 44370 (*H. fabiensis*) was the most aggressive and produced significantly longer lesions. In contrast, isolates CMW 44684 and CMW 44686 (*H. ani*) had the lowest level of aggressiveness (FIG. 13). Significantly different lesion lengths were associated with different isolates of some species,

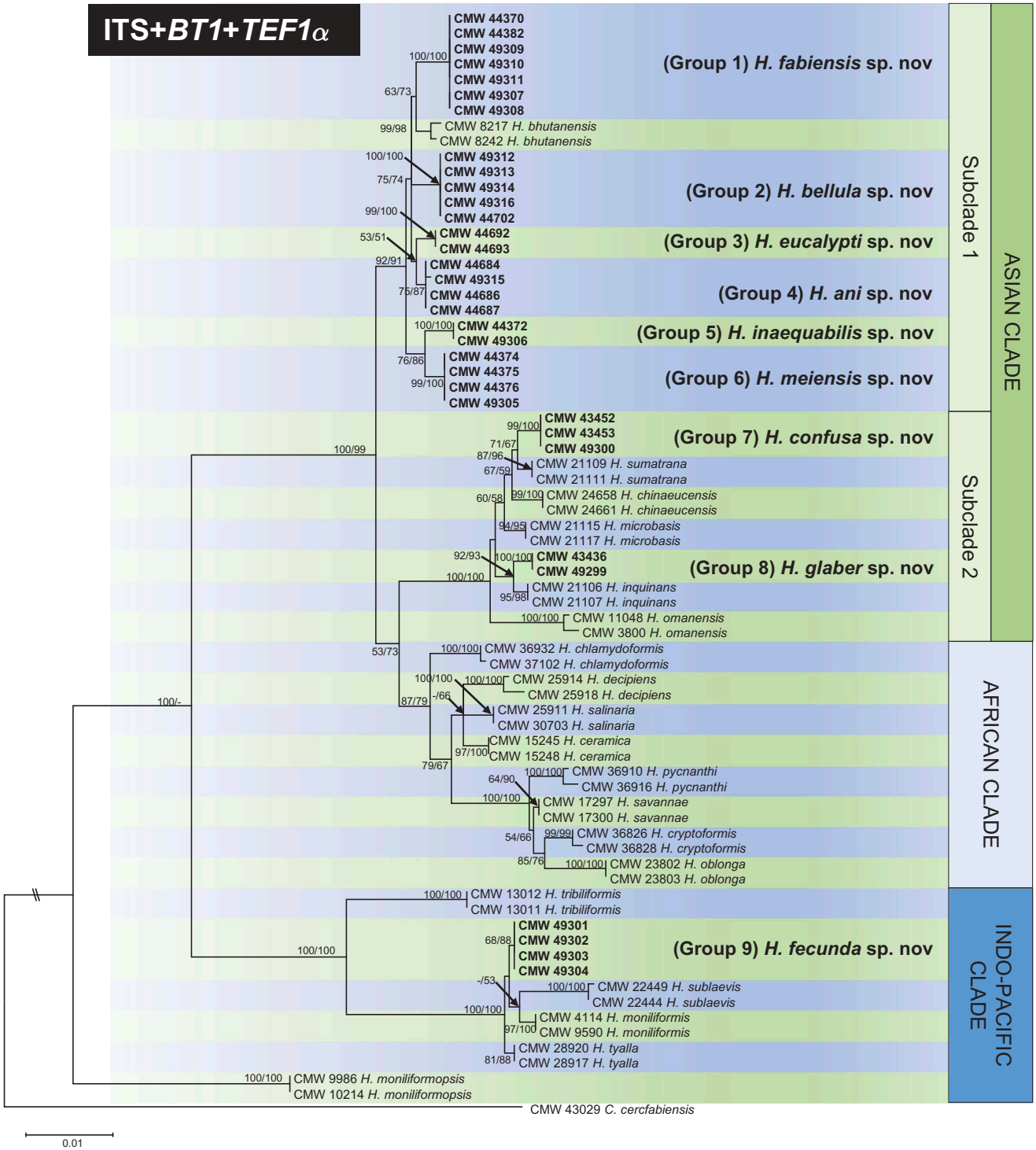

Figure 3. Phylogenetic trees based on maximum likelihood (ML) analysis of a combined data set of ITS, *BT1*, and *TEF1α* gene sequences for *Huntiella* species. Isolates in bold were obtained and sequenced in this study. Bootstrap values >50% for maximum parsimony (MP) and ML are presented above branches as MP/ML. Bootstrap values lower than 50% are marked with *. Nodes lacking bootstrap support are marked with -. *Ceratocystis cercfabiensis* (CMW 43029) represents the outgroup.

such as *H. fabiensis* and *H. meiensis* (FIG. 13). The inoculated fungi were easily reisolated from lesions on inoculated seedlings, but not from the negative controls, thus fulfilling Koch's postulates.

TAXONOMY

Based on phylogenetic analyses, growth, and morphological studies, the 33 *Huntiella* isolates from *Eucalyptus* and *Acacia* in China represent nine novel

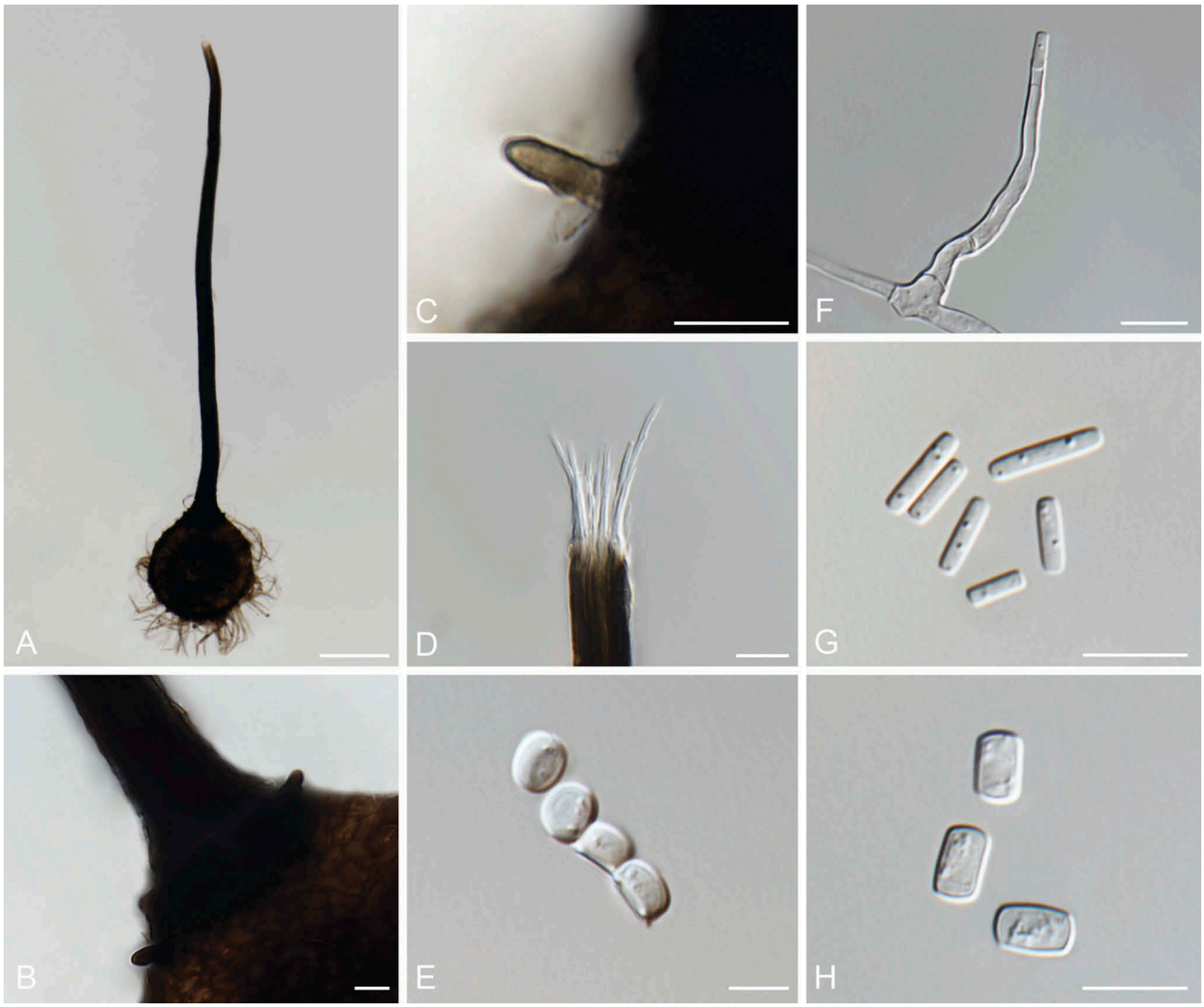

Figure 4. Morphological characters of *Huntiella ani*. A. Ascoma with obpyriform base and extended neck. B–C. Conical spines on the surface of ascomatal base. D. Tip of ascomatal neck with divergent ostiolar hyphae. E. Hat-shaped ascospore in top view and side view. F. Flask-shaped conidiophore. G. Various sizes of bacilliform conidia. H. Barrel-shaped conidia. Bars: A = 100 µm; B–D, F–H = 10 µm; E = 5 µm.

species. The following descriptions for these species are provided.

Huntiella ani F.F. Liu & S.F. Chen, sp. nov. FIG. 4
MycoBank MB826735

Typification: CHINA. GUANGXI PROVINCE: Nanning region, *Eucalyptus* plantation (23°26′34″N, 108°14′40″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu* & *G.Q. Li* (**holotype** PREM 62020). Ex-holotype culture: CMW 44686 = CBS 143282 = CERC 2829.

Etymology: The name refers to the Chinese name "An" for the host *Eucalyptus*, from which it was collected.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases dark brown, globose to obpyriform, (104.5–)124–167(–224) µm long and (117.5–)128.5–173(–232) µm wide, ornamented with conical, thick-walled, dark brown spines, (6–)8.5–15.5(–21) µm long. Ascomatal necks dark, erect, slender, (493–)520–667(–845) µm long, (9.5–)10.5–12.5(–14) µm wide at apex and (29.5–)33–44.5(–54) µm wide at base. Ostiolar hyphae present, hyaline, divergent, (14.5–)19.5–28.5(–33) µm long. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (4.5–)4.5–5.5(–6) µm long and (1.5–)2.5–3(–3.5) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (18.5–)27–45.5(–59) µm long, (1.5–)1.5–2.5(–3) µm wide at apex and (2–)3–4.5(–5.5) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)5–8.5(–12.5) µm long and (1.5–)1.5–2.5(–3) µm wide; and (ii) barrel-shaped conidia hyaline, aseptate, in chains, (4–)5–7.5(–9) µm long and (2.5–)3–4(–5) µm wide. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 C, no growth at 5, 30, and 35 C. After 4 d, colonies grew 7.1 mm at 10 C, 32.2 mm at 15 C, 51.7 mm at 20 C, and 66.4 mm at 25 C. Colonies round with even margins. Mycelium fluffy, smooth, dense on MEA, initially hyaline to white, turning to grayish sepia (15"") after 2–3 wk, reverse honey (19") turning chaetura drab (17""k) when older. Colony surfaces scattered with dark brown to black ascomata.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangxi Province, China.

Other specimens examined: CHINA. GUANGXI PROVINCE: Nanning region, *Eucalyptus* plantation (23°26′34″N, 108°14′40″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li*, PREM 62021, culture CMW 44684 = CBS 143283 = CERC 2827. GUANGXI PROVINCE: Liuzhou region, *Eucalyptus* plantation (24°28′2″N, 109°45′50″E). Isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li*, PREM 62022, culture CMW 49315 = CBS 143284 = CERC 2871.

Notes: Huntiella ani is closely related to *H. eucalypti*. The asexual structures of the two species are similar. *Huntiella ani* can be distinguished from *H. eucalypti* based on its optimal temperature for growth in culture. *Huntiella ani* did not grow at 5 C, but *H. eucalypti* grew 10.5 mm at 5 C after 4 d. It also differed from *H. eucalypti* in five bases in the *TEF1α* gene and one base in the *BT1* gene.

Huntiella bellula F.F. Liu & S.F. Chen, sp. nov. FIG. 5
MycoBank MB826738

Typification: CHINA. GUANGXI PROVINCE: Liuzhou region, *Eucalyptus* plantation (24°28′2″N, 109°45′50″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li* (**holotype** PREM 62023). Ex-holotype culture: CMW 49314 = CBS 143285 = CERC 2862.

Etymology: "*bellus*" (Latin) = beauty, referring to the beauty of the fungal structures of this species.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases black, globose to subglobose, (172.5–)179–228.5(–250.5) µm long and (184–)195–230.5(–244) µm wide, ornamented with conical, thick-walled, dark brown spines, (5–)7.5–16.0(–20) µm long. Ascomatal necks dark brown to black, erect, slender, forming an obvious bulbous collar at junction with ascomatal base, (395–)450–631(–707.5) µm long, (10.5–)11.5–13.5(–14.5) µm wide at apex and (42.5–)45–59.5(–69) µm wide at base. Ostiolar hyphae present, hyaline, divergent, (18–)20–29.5(–32.5) µm long. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (5–)5.5–6.5(–6.5) µm long and (2.5–)2.5–3(–4) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (21.5–)26.5–38.5(–50) µm long, (1–)1.5–2(–2.5) µm wide at apex and (2–)2.5–4(–6) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (5.5–)6.5–9(–12) µm long and (1.5–)1.5–2(–2) µm wide; and (ii) barrel-shaped conidia not observed. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 C, no growth at 35 C. After 4 d, colonies grew 5.3 mm at 5 C, 10.5 mm at 10 C, 33.6 mm at 15 C, 54.4 mm at 20 C, 75.5 mm at 25 C, and 5.7 mm at 30 C. Colonies were round and smooth with even margins. Aerial mycelium fluffy, extensive on MEA, initially white, turning to grayish sepia (15"") after 2–3 wk, reverse chamois (19"b) turning fuscous black (7""k) when older. Colony surfaces scattered with dark brown to black ascomata.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangxi Province, China.

Other specimens examined: CHINA. GUANGXI PROVINCE: Liuzhou region, *Eucalyptus* plantation (24°28′2″N, 109°45″50″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li*, PREM 62024, culture CMW 49312 = CBS 143286 = CERC 2854. GUANGXI PROVINCE: Laibin region, *Eucalyptus* plantation (24°20′33″N, 110°4′59″E). Isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li*, PREM 62025, culture CMW 49316 = CBS 143287 = CERC 2880.

Notes: Huntiella bellula is closely related to *H. fabiensis* and *H. bhutanensis* (Van Wyk et al. 2004). However, *H. bellula* can be distinguished from these

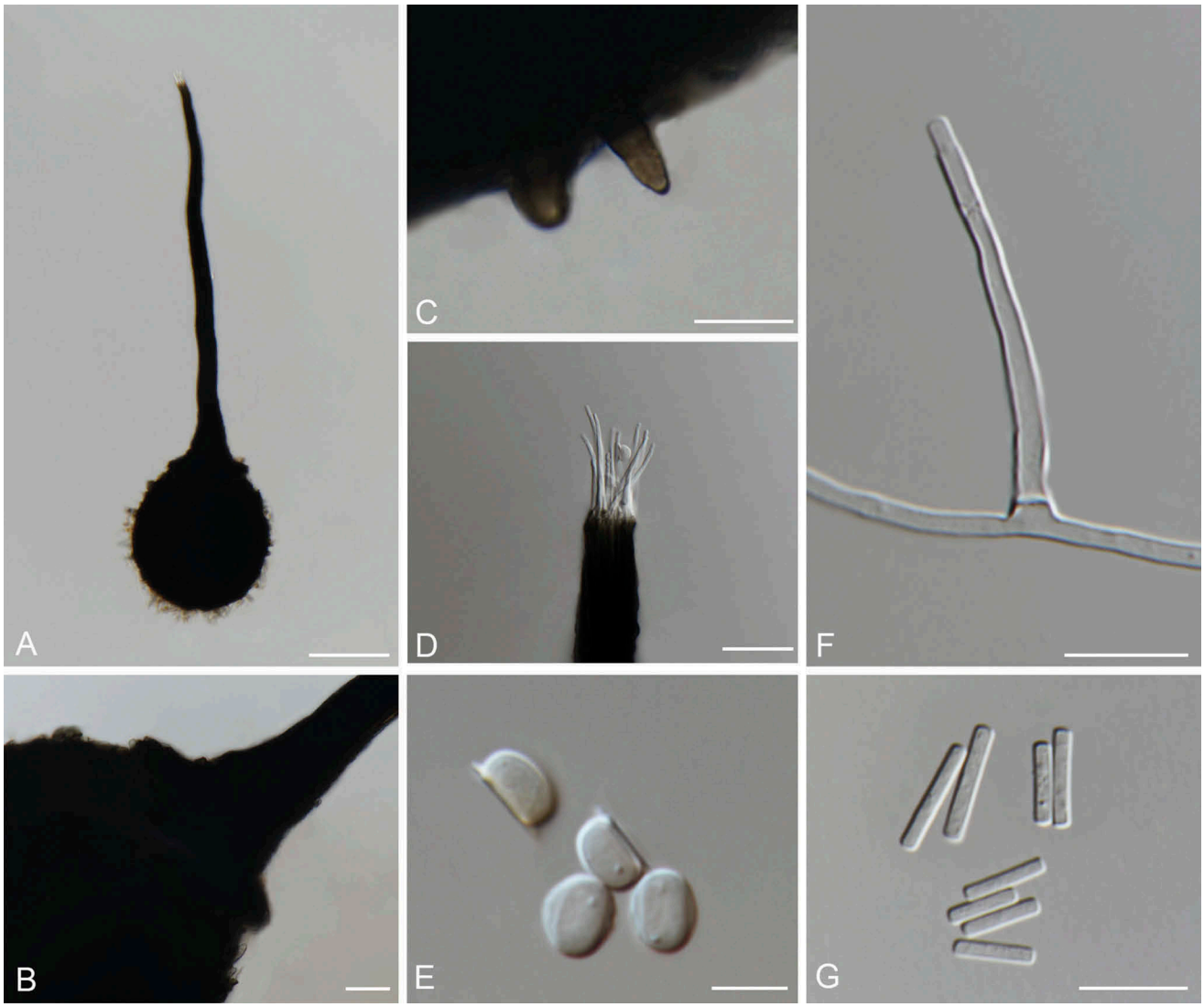

Figure 5. Morphological characters of *Huntiella bellula*. A. Ascoma with subglobose base and extended neck. B. Ascomatal base showing bulbous collar structure at neck base. C. Conical spines on the surface of ascomatal base. D. Tip of ascomatal neck with divergent ostiolar hyphae. E. Hat-shaped ascospore in top view and side view. F. Flask-shaped conidiophore. G. Various sizes of bacilliform conidia. Bars: A = 100 µm; B, D = 20 µm; C, F–G = 10 µm; E = 5 µm.

two species by the sizes of the ascomatal bases and necks, ascospores, and bacilliform conidia (TABLE 3). Ascomatal bases of *H. bellula* (average 203.5 × 212.5 µm) are larger than those of *H. bhutanensis* (average 158 × 158 µm). Ascomatal necks of *H. bellula* (average 540.5 µm) are longer than those of *H. bhutanensis* (average 486 µm) and *H. fabiensis* (average 373.5 µm). Ascospores of *H. bellula* (average 6 µm) are longer than those of *H. bhutanensis* (average 5 µm) and shorter than those of *H. fabiensis* (average 6.5 µm). Bacilliform conidia of *H. bellula* (average 7.5 × 1.5 µm) are smaller than those of *H. bhutanensis* (average 8 × 2 µm) and *H. fabiensis* (average 8 × 2 µm). Barrel-shaped conidia are present in *H. fabiensis* and *H. bhutanensis*, but not observed in *H. bellula*. Based on DNA sequence data, *H. bellula* differs from *H. fabiensis* in seven bases in the *TEF1α* gene and seven bases in the *BT1* gene, and from *H. bhutanensis* in five bases in the *TEF1α* gene, five bases in the *BT1* gene, and one base in the ITS region.

Huntiella confusa F.F. Liu & S.F. Chen, sp. nov. FIG. 6
MycoBank MB826740

Typification: CHINA. HAINAN PROVINCE: LinGao County, *Acacia confusa* tree (19°42′57″N, 109° 37′3″E), isolated from the fresh stump of a fallen tree, Sep 2013, *S.F. Chen, F.F. Liu, T. Huang & B. Liu* (**holotype** PREM 62026). Ex-holotype culture: CMW 43453 = CBS 143288 = CERC 2162.

Table 3. Morphological comparisons of closely related *Huntiella* species.

Specie[a]	Ascomatal base length[b]	Ascomatal base width	Ascomatal neck length	Ostiolar hyphae length	Ascospore height	Ascospore width	Bacilliform conidia length	Bacilliform conidia width	Barrel-shaped conidia length	Barrel-shaped conidia width
Huntiella ani	(104.5–)124–167(–224) (av. 145.5)	(117.5–)128.5–173 (–232) (av. 150.5)	(493–)520–667(–845) (av. 593.5)	(14.5–)19.5–28.5(–33) (av. 24)	(1.5–)2.5–3(–3.5) (av. 3)	(4.5–)4.5–5.5(–6) (av. 5)	(4–)5–8.5(–12.5) (av. 7)	(1.5–)1.5–2.5(–3) (av. 2)	(4–)5–7.5(–9) (av. 6)	(2.5–)3–4(–5) (av. 3.5)
H. bellula	(172.5–)179–228.5 (–250.5) (av. 203.5)	(184–)195–230.5(–244) (av. 212.5)	(395–)450–631(–707.5) (av. 540.5)	(18–)20–29.5(–32.5) (av. 25)	(2.5–)2.5–3(–4) (av. 3)	(5–)5.5–6.5(–6.5) (av. 6)	(5.5–)6.5–9(–12) (av. 7.5)	(1.5–)1.5–2(–2) (av. 1.5)	NA[c]	NA
H. bhutanensis	(112–)138–178(–206) (av. 158)	(112–)138–178(–206) (av. 158)	(450–)453–519 (av. 486)	(13–)18–26(–34) (av. 22)	2–5 (av. 3.5)	4–6 (av. 5)	(6–)7–9(–10) (av. 8)	1–3 (av. 2)	3–5 (av. 4)	(1.5–)2–3(–3.5) (av. 2.5)
H. confusa	NA	NA	NA	NA	NA	NA	(4.5–)5.5–7.5(–10) (av. 6.5)	(1.5–)1.5–2(–3) (av. 2)	NA	NA
H. chinaeucensis	(166–)212–271(–315) (av. 241.5)	(196–)232–304(–355) (av. 268)	(333–)410–551(–629) (av. 480.5)	(20–)24–37(–47) (av. 30.5)	3–4 (av. 3.5)	6–8(–9) (av. 7)	(5–)6–8(–9) (av. 7)	1–3 (av. 2)		
H. sumatrana	(148–)168–218(–293) (av. 193)	(158–)187–235(–296) (av. 211)	(323–)390–574(–687) (av. 482)	(21–)24–32(–35) (av. 28)	3–4 (av. 3.5)	5–7 (av. 6)	5–7(–8) (av. 6)	2–4(–5) (av. 3)	(4–)5–7(–8) (av. 6)	1–3 (av. 2)
H. eucalypti	NA	NA	NA	NA	NA	NA	(4–)6–8.5(–11) (av. 7)	(1.5–)1.5–2(–2.5) (av. 2)	NA	NA
H. fabiensis	(146.5–)163.5–219.5 (–244.5) (av. 191.5)	(158.5–)163–222.5 (–259.5) (av. 192.5)	(254.5–)316–431.5 (–470.5) (av. 373.5)	(12.5–)16.5–27.5(–28.5) (av. 22)	(2–)3–3.5(–4) (av. 3.5)	(5.5–)6–7(–8) (av. 6.5)	(4–)6–10(–13.5) (av. 8)	(1.5–)1.5–2(–2.5) (av. 2)	(3–)5–8(–10.5) (av. 6.5)	(2.5–)3–5(–7) (av. 4)
H. fecunda	(92.5–)106–173(–234) (av. 139.5)	(111–)118.5–184(–253) (av. 151.5)	(365.5–)521–828 (–1052.5) (av. 674.5)	(15–)18–23.5(–25.5) (av. 20.5)	(2–)2–2.5(–3) (av. 2.5)	(4.5–)5–5.5(–5.5) (av. 5.5)	(4–)5–7(–8.5) (av. 6)	(1.5–)1.5–2(–3) (av. 2)	NA	NA
H. tyalla	143.0–175.5(–195.5) (av. 159.25)	(116.5–)136.0–167.0 (–177.5) (av. 201.5)	(428.5–)466.5–607.5 (–772.5) (av. 537)	(14.5–)18.0–24.5(–28.5) (av. 21.25)	(2.0–)2.0–2.5(–3.0) (av. 2.25)	(3.5–)4.0–4.5(–5.0) (av. 4.45)	(8.5–)9.0–11.0 (–12.0) (av. 10)	(1.5–)2.0–2.5 (–3.5) (av. 2.25)	(5.0–)6.5–8.5(–9.5) (av. 7.5)	(1.5–)2.0–2.5(–3.0) (av. 2.25)
H. sublaevis	(98–)131–173(–187) (av. 152)	(102–)144–192(–231) (av. 168)	(522–)598–802(–990) (av. 700)	(15–)18–24(–25) (av. 21)	2–4 (av. 3)	4–6 (av. 5)	5–8 (av. 6.5)	1–3 (av. 2)	3–6 (av. 4.5)	2–3 (av. 2.5)
H. glaber	(133–)152.5–210.5(–237) (av. 181.5)	(128.5–)146.5–207.5 (–228) (av. 177)	(259.5–)328–548.5 (–722.5) (av. 438.5)	NA	(2–)2.5–3(–3.5) (av. 3)	(4.5–)5.5–6.5(–7) (av. 6)	(7–)8.5–12(–15.5) (av. 10)	(1.5–)2–3(–3) (av. 2.5)	NA	NA
H. microbasis	(65–)82–122(–162) (av. 101)	(82–)100–146(–185) (av. 246)	(185–)301–499(–574) (av. 400)	(9–)14–22(–25) (av. 18)	2–4 (av. 3)	5–7 (av. 6)	(3–)4–6(–11) (av. 5)	1–3 (av. 2)	NA	NA
H. inquinans	(116–)149–205(–236) (av. 177)	(130–)161–217(–270) (av. 189)	(347–)393–575(–687) (av. 484)	(20–)24–34(–38) (av. 29)	2–4 (av. 3)	5–7 (av. 6)	(5–)6–8(–11) (av. 7)	(2–)3–5(–7) (av. 4)	(4–)5–7(–8) (av. 6)	1–3 (av. 2)
H. omanensis	(154–)206–254(–279) (av. 230)	(154–)206–254(–279) (av. 230)	(385–)443–819(–1097) (av. 631)	(10–)18–36(–50) (av. 27)	2–4 (av. 3)	5–7 (av. 6)	6–8(–9) (av. 7)	2–3 (av. 2.5)	(5–)6–8(–10) (av. 7)	3–5
H. inaequabilis	NA	NA	NA	NA	NA	NA	(4–)4.5–9(–17.5) (av. 6.5)	(1.5–)1.5–2(–2) (av. 2)	(4.5–)5.5–7.5(–9.5) (av. 6.5)	(3.5–)3.5–5(–6) (av. 4)
H. meiensis	(119.5–)139–195(–219.5) (av. 167)	(129.5–)145–194.5 (–220) (av. 169.5)	(290–)299.5–347.5 (–416.5) (av. 323.5)	(9)12–18.5(–22.5) (av. 15.5)	(2–)2.5–3.5(–4) (av. 3)	(4.5–)5.5–6.5(–7) (av. 6)	(4–)5–7.5(–10.5) (av. 6.5)	(1.5–)1.5–2.5 (–3.5) (av. 2)	(5.5–)6–8.5(–11) (av. 7.5)	(3.5–)4–5.5(–6.5) (av. 5)

[a]Species indicated in bold are newly described in this study.
[b]Measurements are in micrometres.
[c]NA: not observed.

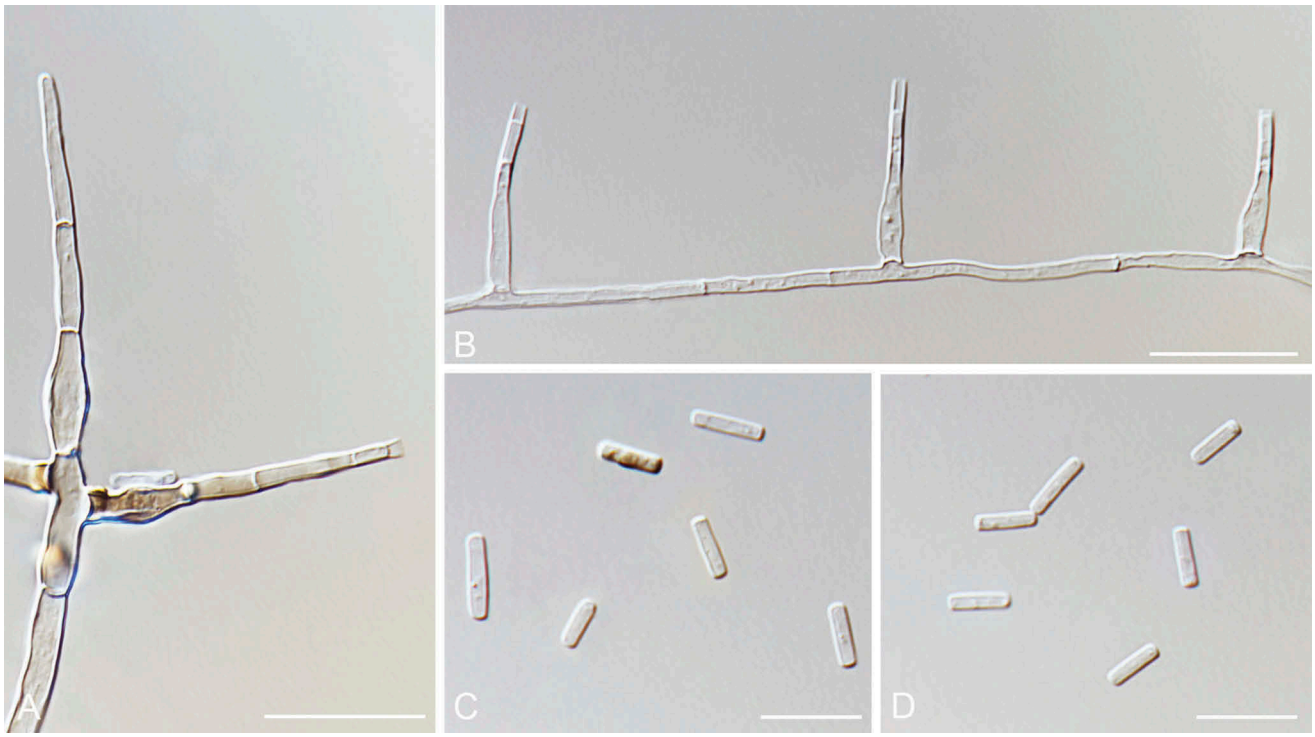

Figure 6. Morphological characters of *Huntiella confusa*. A–B. Flask-shaped conidiophore. C–D. Various sizes of bacilliform conidia. Bars: A–B = 20 µm; C–D = 10 µm.

Etymology: The name refers to the species epithet of the host *Acacia confusa*, from which it was collected.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Not observed.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (17–)20–33(–48) µm long, (1.5–)1.5–2(–3) µm wide at apex and (2–)2.5–4.5(–6.5) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4.5–)5.5–7.5(–10) µm long and (1.5–)1.5–2(–3) µm wide; and (ii) barrel-shaped conidia not observed. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 30 C, no growth at 5 C. After 4 d, colonies grew 2.3 mm at 10 C, 19.0 mm at 15 C, 40.5 mm at 20 C, 59.5 mm at 25 C, 69.3 mm at 30 C, and 30.4 mm at 35 C. Colonies round with even margins. Mycelium fluffy, dense on MEA, initially white, turning to dark brick (7''k) after 2–3 wk, reverse buff (19''f) to sepia (13''k) when older.

Habitat: Wounds on fallen *Acacia confusa* trees.

Distribution: Hainan Province, China.

Other specimens examined: CHINA. HAINAN PROVINCE: LinGao County, *Acacia confusa* tree (19°42′57″N, 109°37′3″E), isolated from stump of a fallen tree, Sep 2013, *S.F. Chen, F.F. Liu, T. Huang & B. Liu*, PREM 62027, culture CMW 49300 = CBS 143289 = CERC 2141; PREM 62028, culture CMW 43452 = CBS 143577 = CERC 2158.

Notes: Huntiella confusa is closely related to *H. chinaeucensis* (Chen et al. 2013) and *H. sumatrana* (Tarigan et al. 2010) but can be distinguished by the size of its bacilliform conidia (TABLE 3). Bacilliform conidia of *H. confusa* (average 6.5 × 2 µm) are longer than those of *H. sumatrana* (average 6 × 3 µm) and shorter than those of *H. chinaeucensis* (average 7 × 2 µm). In addition, barrel-shaped conidia are not observed in *H. confusa* and *H. chinaeucensis* but are present in *H. sumatrana*. *Huntiella confusa* differs from *H. chinaeucensis* in nine bases in the *TEF1α* gene and two bases in the *BT1* gene, and from *H. sumatrana* in three bases in the *TEF1α* gene and three bases in the *BT1* gene.

Huntiella eucalypti F.F. Liu & S.F. Chen, sp. nov. FIG. 7

MycoBank MB826741

Typification: CHINA. GUANGXI PROVINCE: Hechi region, *Eucalyptus* plantation (24°40′38″N, 108°20′16″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu & G.Q. Li* (**holotype** PREM 62029). Ex-holotype culture: CMW 44693 = CBS 143290 = CERC 2841.

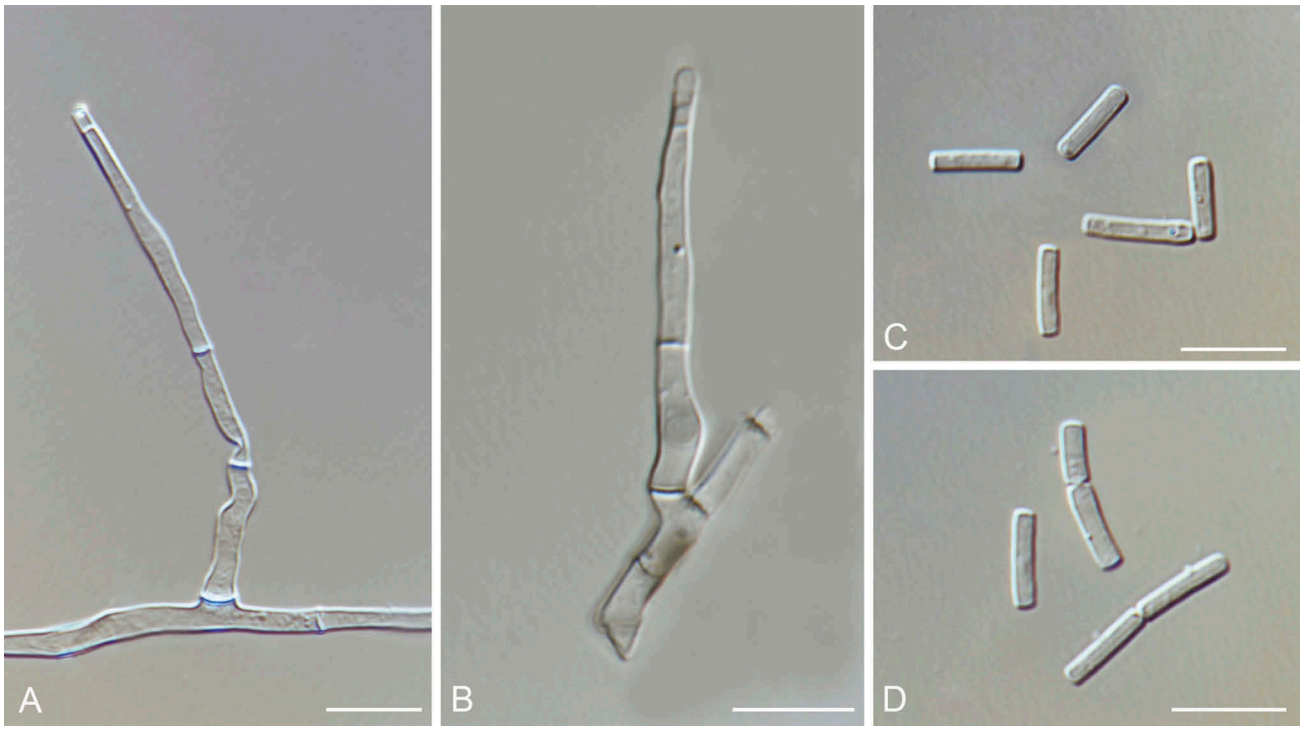

Figure 7. Morphological characters of *Huntiella eucalypti*. A–B. Flask-shaped conidiophore. C–D. Various sizes of bacilliform conidia Bars: A–D = 10 µm.

Etymology: The name refers to *Eucalyptus*, the host from which it was collected.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Not observed.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (24.5–)27–44.5(–56) µm long, (1.5–)1.5–2(–2) µm wide at apex and (2.5–)2.5–3.5(–4.5) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)6–8.5(–11) µm long and (1.5–)1.5–2(–2.5) µm wide; and (ii) barrel-shaped conidia not observed. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 C, no growth at 35 C. After 4 d, colonies grew 10.5 mm at 5 C, 14.4 mm at 10 C, 32.9 mm at 15 C, 53.2 mm at 20 C, 67.5 mm at 25 C, and 4.5 mm at 30 C. Colonies round with even margins. Aerial mycelium white, fluffy, extensive on MEA, reverse white to mikado brown (13"i) after 2–3 wk.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangxi Province, China.

Other specimens examined: CHINA. GUANGXI PROVINCE: Hechi region, *Eucalyptus* plantation (24° 40′38″N, 108°20′16″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, F.F. Liu* & *G.Q. Li*, PREM 62030, culture CMW 44692 = CBS 143291 = CERC 2840.

Huntiella fabiensis F.F. Liu & S.F. Chen, sp. nov. FIG. 8

MycoBank MB826743

Typification: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li* & *C. Chen* (**holotype** PREM 62031). Ex-holotype culture: CMW 49309 = CBS 143292 = CERC 2763.

Etymology: The name refers to the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria in South Africa, where this work was conducted.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases black, globose, (146.5–)163.5–219.5(–244.5) µm long and (158.5–)163–222.5(–259.5) µm wide, ornamented with conical, thick-walled, dark brown spines, (7–)8.0–15.5(–18) µm long. Ascomatal necks dark brown to black, erect, slender, (254.5–)316–431.5(–470.5) µm long, (10.5–)11.5–15(–17.5) µm wide at apex and (34–)

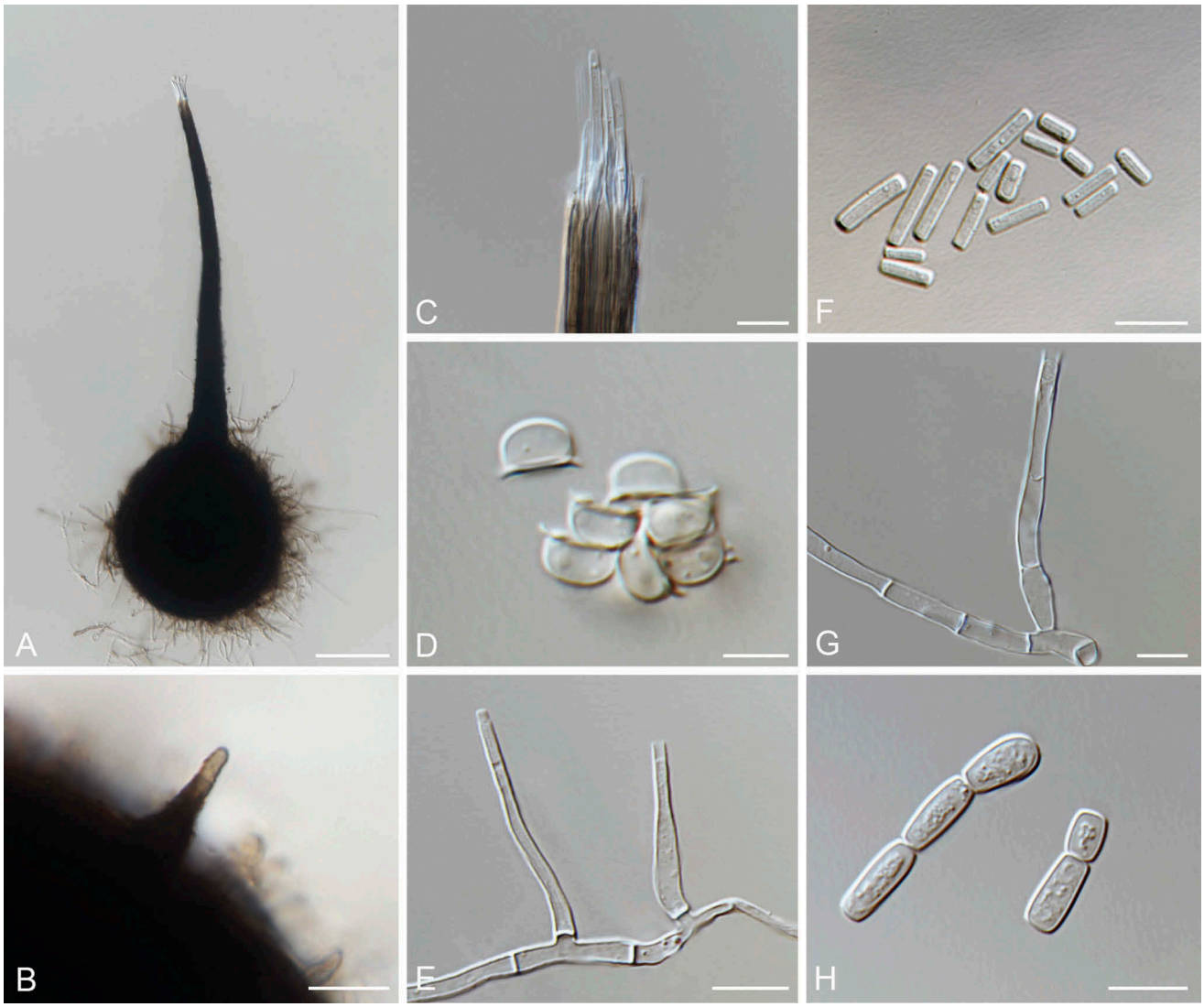

Figure 8. Morphological characters of *Huntiella fabiensis*. A. Ascoma with globose base and extended neck. B. Conical spines on the surface of ascomatal base. C. Tip of ascomatal neck with convergent ostiolar hyphae. D. Hat-shaped ascospore in side view. E. Flask-shaped conidiophores. F. Various sizes of bacilliform conidia. G. Secondary conidiophores with emerging barrel-shaped conidia. H. Barrel-shaped conidia in chains. Bars: A = 100 µm; B–C, E–H = 10 µm; D = 5 µm.

37.5–57.5(–71) µm wide at base. Ostiolar hyphae present, hyaline, convergent, (12.5–)16.5–27.5(–28.5) µm long. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (5.5–)6–7(–8) µm long and (2–)3–3.5(–4) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (19–)24.5–52(–75.5) µm long, (1.5–)1.5–3(–4) µm wide at apex and (2.5–)3–4(–5.5) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)6–10(–13.5) µm long and (1.5–)1.5–2(–2.5) µm wide; and (ii) barrel-shaped conidia hyaline, aseptate, in chains, (3–)5–8(–10.5) µm long and (2.5–)3–5(–7) µm wide. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 C, no growth at 35 C. After 4 d, colonies grew 7.7 mm at 5 C, 16.7 mm at 10 C, 35.5 mm at 15 C, 59.6 mm at 20 C, 70.9 mm at 25 C, and 3.4 mm at 30 C. Colonies round with even margins. Mycelium fluffy, smooth on MEA, initially hyaline to white, turning to smoke gray (21’’’’f) after 2–3 wk, reverse buff (19’’f) turning fuscous black (3’’’’k) when older. Colony surfaces scattered with dark brown to black ascomata.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangdong Province, China.

Other specimens examined: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li & C. Chen*, PREM 62032, culture CMW 44370 = CBS 143293 = CERC 2736; PREM 62033, culture CMW 49307 = CBS 143294 = CERC 2753.

Notes: Huntiella fabiensis is closely related to *H. bhutanensis* (Van Wyk et al. 2004). However, *H. fabiensis* can be distinguished from *H. bhutanensis* by the sizes of the ascomatal bases, ascospores, and barrel-shaped conidia (TABLE 3). Ascomatal bases of *H. fabiensis* (average 191.5 × 192.5 µm) are larger than those of *H. bhutanensis* (average 158 × 158 µm). Ascospores of *H. fabiensis* (average 3.5 × 6.5 µm) are wider than those of *H. bhutanensis* (average 3.5 × 5 µm). Barrel-shaped conidia of *H. fabiensis* (average 6.5 × 4 µm) are larger than those of *H. bhutanensis* (average 4 × 2.5 µm). The optimal growth temperature for *H. fabiensis* is 25 C, but that of *H. bhutanensis* is 20 to 25 C (Van Wyk et al. 2004). Based on DNA sequence data, *H. fabiensis* differs from *H. bhutanensis* by four bases in the *TEF1α* gene, six bases in the *BT1* gene, and one base in the ITS region.

Huntiella fecunda F.F. Liu & S.F. Chen, sp. nov. FIG. 9
MycoBank MB826744

Typification: CHINA. FUJIAN PROVINCE: Zhangzhou region, *Eucalyptus* plantation (24°44′36″N, 117°50′5″E), isolated from recently harvested tree

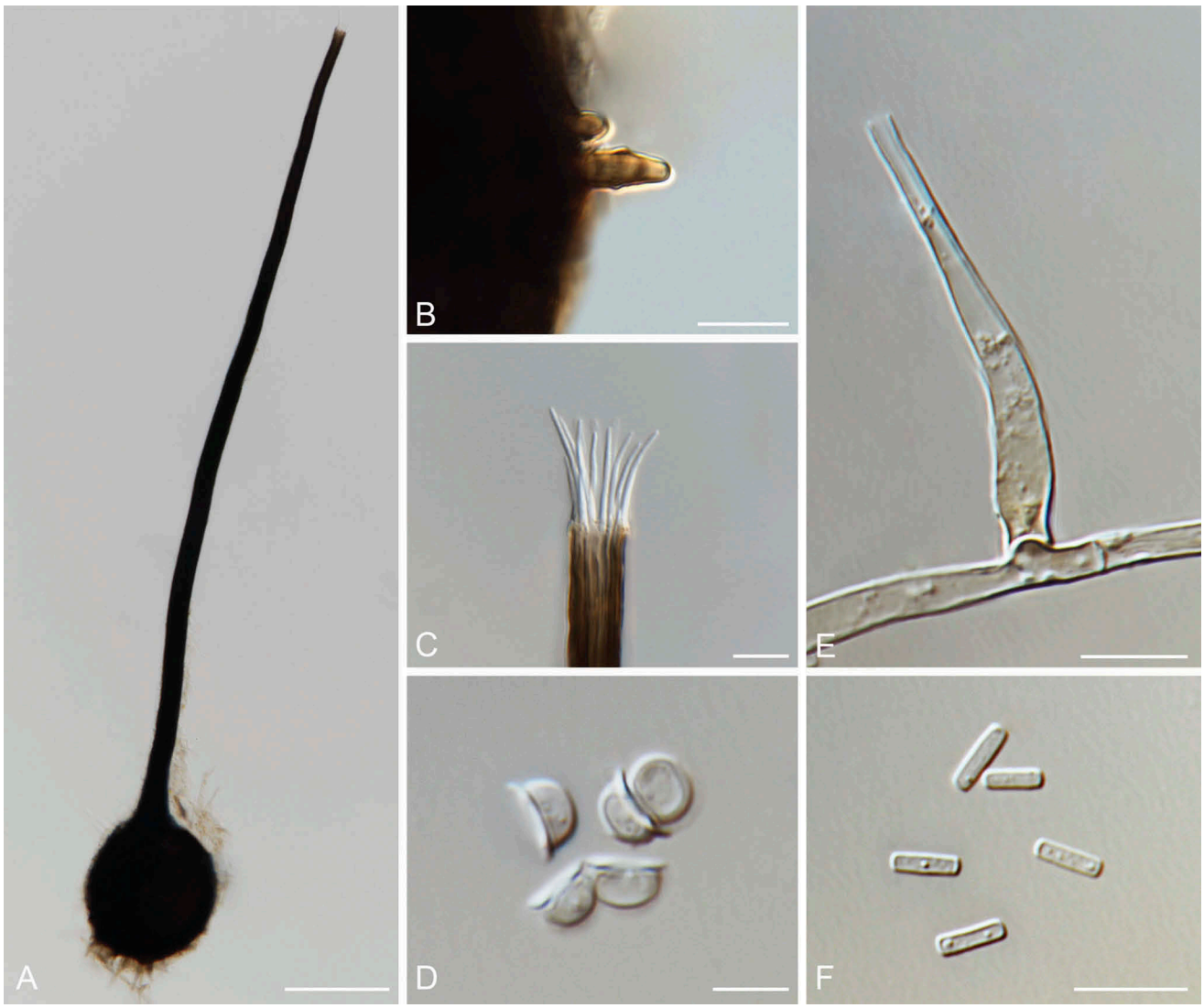

Figure 9. Morphological characters of *Huntiella fecunda*. A. Ascoma with subglobose base and extended neck. B. Conical spines on the surface of ascomatal base. C. Tip of ascomatal neck with divergent ostiolar hyphae. D. Hat-shaped ascospore in top view and side view. E. Flask-shaped conidiophore. F. Various sizes of bacilliform conidia. Bars: A = 100 µm; B–C, E–F = 10 µm; D = 5 µm.

stump, Oct 2013, *S.F. Chen, F.F. Liu & G.Q, Li* (**holotype** PREM 62034). Ex-holotype culture: CMW 49303 = CBS 143295 = CERC 2451a.

Etymology: "*fecundus*" (Latin) = fecund, referring to the prolific production of ascomata in culture.

Mating strategy: Unisexual, sexually reproducing isolates possess only the *MAT1-2-1* gene.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases dark brown to black, globose or obpyriform, (92.5–)106–173(–234) µm long and (111–)118.5–184(–253) µm wide, ornamented with conical, thick-walled, dark brown spines, (5–)7.0–18.5(–23) µm long. Ascomatal necks dark brown to black, erect, slender, forming a bulbous collar at junction with ascomatal base, (365.5–)521–828(–1052.5) µm long, (9.5–)10–12(–13.5) µm wide at apex and (23.5–)29.5–47.5(–66.5) µm wide at base. Ostiolar hyphae present, hyaline, divergent, (15–)18–23.5(–25.5) µm long. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (4.5–)5–5.5(–5.5) µm long and (2–)2–2.5(–3) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (16–)18.5–32.5(–53) µm long, (1.5–)1.5–2(–2.5) µm wide at apex and (2–)2.5–3.5(–5.5) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)5–7(–8.5) µm long and (1.5–)1.5–2(–3) µm wide; and (ii) barrel-shaped conidia not observed. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 to 30 C, no growth at 5 and 35 C. After 4 d, colonies grew 4.8 mm at 10 C, 29.2 mm at 15 C, 45.6 mm at 20 C, 61.0 mm at 25 C, and 63.7 mm at 30 C. Colonies round with even margins. Aerial mycelium fluffy, smooth, extensive on MEA, initially white, turning to dark brown after 7–10 d, especially under areas where ascomata produced, reverse white turning fuscous black (7""k) when older. Colony surfaces scattered with abundant dark brown to black ascomata.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Fujian Province, China.

Other specimens examined: CHINA. FUJIAN PROVINCE: Zhangzhou region, *Eucalyptus* plantation (24°44′36″N, 117°50′5″E), isolated from recently harvested tree stump, Oct 2013, *S.F. Chen, F.F. Liu & G.Q. Li*, PREM 62035, culture CMW 49301 = CBS 143304 = CERC 2446; PREM 62036, culture CMW 49302 = CBS 143296 = CERC 2449.

Notes: Huntiella fecunda is closely related to *H. moniliformis* (Van Wyk et al. 2006), *H. sublaevis* (Van Wyk et al. 2011), and *H. tyalla* (Kamgan Nkuekam et al. 2012). *Huntiella fecunda* and *H. moniliformis* can be distinguished based on growth characters, with *H. moniliformis* rarely growing below 20 C (Van Wyk et al. 2006), whereas *H. fecunda* grows well at 15 C and has reduced growth at 10 C. *Huntiella fecunda* can be distinguished from *H. sublaevis* and *H. tyalla* by the sizes of the ascomatal bases and bacilliform conidia (TABLE 3). Ascomatal bases of *H. fecunda* (average 139.5 × 151.5 µm) are smaller than those of *H. sublaevis* (average 152 × 168 µm) and *H. tyalla* (average 159.25 × 201.5 µm). Bacilliform conidia of *H. fecunda* (average 6 × 2 µm) are shorter than those of *H. sublaevis* (average 6.5 × 2 µm) and *H. tyalla* (average 10 × 2.25 µm). Barrel-shaped conidia are present in *H. sublaevis* and *H. tyalla* but were not observed in *H. fecunda*. *Huntiella fecunda* can also be distinguished from *H. sublaevis* and *H. tyalla* based on growth at different temperatures. *Huntiella sublaevis* does not grow below 15 C, but *H. fecunda* grows at this temperature. *Huntiella fecunda* does not grow at 35 C, but *H. tyalla* grows 34 mm at 35 C in 3 d on MEA.

Based on DNA sequence data, *H. fecunda* differs from *H. moniliformis* in three bases in the *TEF1α* gene and one base in the *BT1* gene. *Huntiella fecunda* differs from *H. sublaevis* in 15 bases in the *TEF1α* gene and two bases in the *BT1* gene, and from *H. tyalla* in one base in the *TEF1α* gene, two bases in the *BT1* gene, and one base in the ITS region.

Huntiella glaber F.F. Liu & S.F. Chen, sp. nov. FIG. 10
MycoBank MB826745

Typification: CHINA. HAINAN PROVINCE: Chengmai County, *Eucalyptus exserta* plantation (19° 2′14″N, 110°30′32″E), isolated from exposed wood of fallen tree, Sep 2013, *S.F. Chen, F.F. Liu, T. Huang & B. Liu* (**holotype** PREM 62037). Ex-holotype culture: CMW 49299 = CBS 143297 = CERC 2133.

Etymology: "*glaber*" (Latin) = hairless or bald, referring to the absence of ostiolar hyphae at the apices of the ascomata.

Mating strategy: Homothallic, with sexually competent isolates having both the *MAT1-1-1* and *MAT1-2-1* genes.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases black, globose, (133–)152.5–210.5(–237) µm long and (128.5–)146.5–207.5(–228) µm wide, ornamented with conical, thick-walled, dark brown spines, (8.0–)8.0–17.5(–26) µm long. Ascomatal necks dark brown to black, erect, slender, forming a bulbous collar at junction with ascomatal base, (259.5–)328–548.5(–722.5) µm long, (8.5–)10–

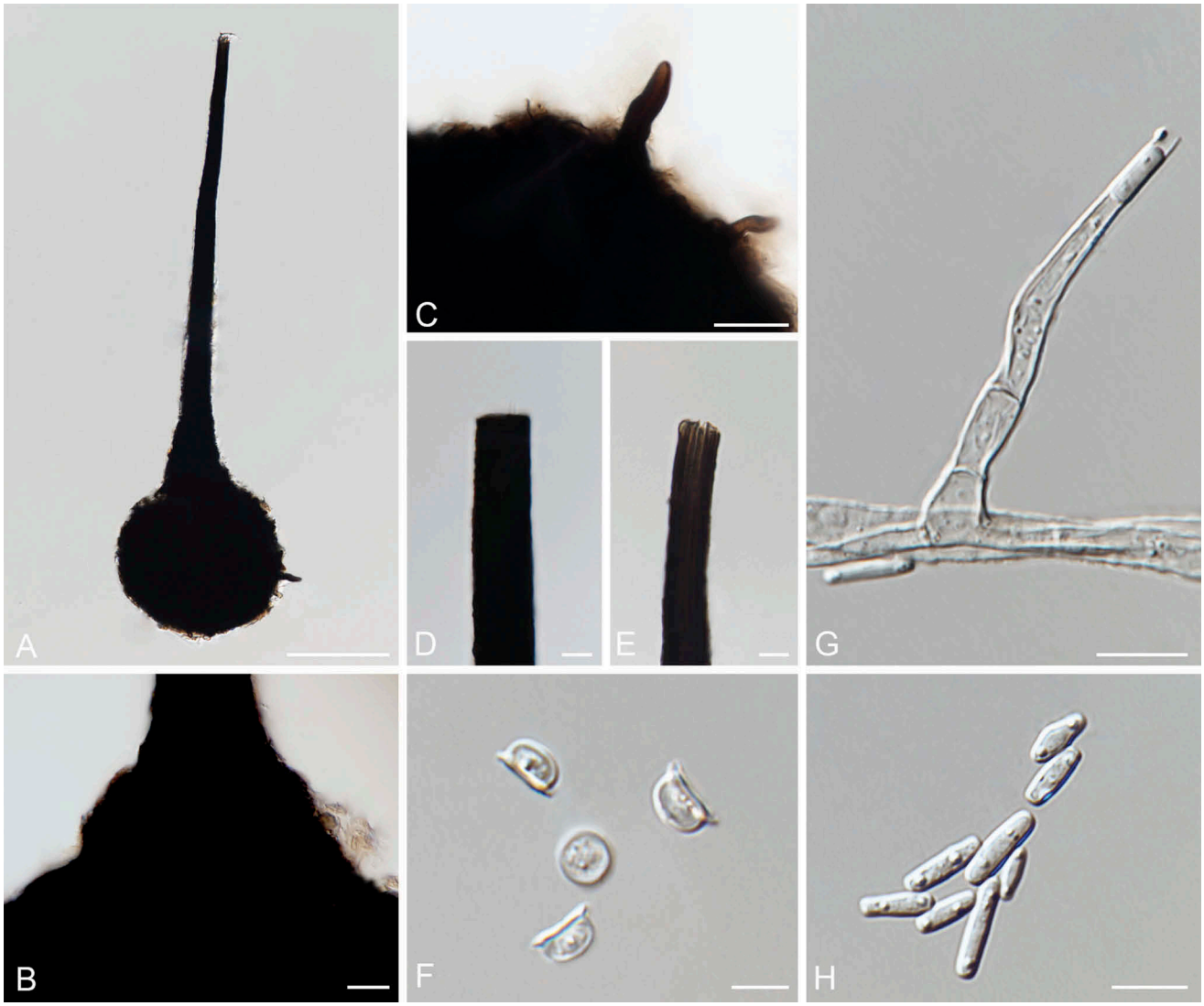

Figure 10. Morphological characters of *Huntiella glaber*. A. Ascoma with globose base and extended neck. B. Ascomatal base showing bulbous collar structure at neck base. C. Conical spines on the surface of ascomatal base. D–E. Tip of ascomatal neck without ostiolar hyphae. F. Hat-shaped ascospore in top view and side view. G. Flask-shaped conidiophore. H. Various sizes of bacilliform conidia. Bars: A = 100 µm; B–C = 20 µm; D–E, G–H = 10 µm; F = 5 µm.

15(–23) µm wide at apex and (42–)49–67.5(–78.5) µm wide at base. Ostiolar hyphae very few or absent. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (4.5–)5.5–6.5(–7) µm long and (2–)2.5–3(–3.5) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (19–)25–45.5(–79) µm long, (1.5–)2–2.5(–3) µm wide at apex and (2.5–)3.5–4.5(–6) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (7–)8.5–12(–15.5) µm long and (1.5–)2–3(–3) µm wide; and (ii) barrel-shaped conidia not observed. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 30 C, no growth at 5 C. After 4 d, colonies grew 5.7 mm at 10 C, 26.4 mm at 15 C, 56.0 mm at 20 C, 79.6 mm at 25 C, 86.0 mm at 30 C, and 42.3 mm at 35 C. Colonies round with even margins. Aerial mycelium fluffy, extensive on MEA, initially white, turning to grayish sepia (15"") after 2–3 wk, reverse white turning brown vinaceous (5"m) when older. Colony surfaces scattered with dark brown to black ascomata.

Habitat: Exposed wood of fallen *Eucalyptus exserta* trees.

Distribution: Hainan Province, China.

Other specimens examined: CHINA. HAINAN PROVINCE: Chengmai County, *Eucalyptus exserta* plantation (19°2′14″N, 110°30′32″E), isolated from exposed wood of fallen tree, Sep 2013, *S.F. Chen, F.F. Liu, T. Huang & B. Liu*, PREM 62038, culture CMW 43436 = CBS 143298 = CERC 2132.

Notes: Huntiella glaber is closely related to *H. inquinans* (Tarigan et al. 2010), *H. microbasis* (Tarigan et al. 2010), and *H. omanensis* (Al-Subhi et al. 2006). *Huntiella glaber* can be distinguished by the sizes of the bacilliform conidia and number of ostiolar hyphae (TABLE 3). Bacilliform conidia of *H. glaber* (average 10 × 2.5 µm) are longer than those of *H. inquinans* (average 7 × 4 µm), *H. microbasis* (average 5 × 2 µm), and *H. omanensis* (average 7 × 2.5 µm). In addition, ostiolar hyphae are very few or absent in *H. glaber*, but present in *H. inquinans* (Tarigan et al. 2010), *H. microbasis* (Tarigan et al. 2010), and *H. omanensis* (Al-Subhi et al. 2006). Based on DNA sequence data, *H. glaber* differs from *H. inquinans* in seven bases in the *TEF1α* gene and one base in the *BT1* gene; *H. glaber* differs from *H. microbasis* in five bases in the *TEF1α* gene and seven bases in the *BT1* gene; and from *H. omanensis* in 16 bases in the *TEF1α* gene, five bases in the *BT1* gene, and three bases in the ITS region.

Huntiella inaequabilis F.F. Liu & S.F. Chen, sp. nov. FIG. 11

MycoBank MB826746

Typification: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li & C. Chen* (**holotype** PREM 62039). Ex-holotype culture: CMW 49306 = CBS 143299 = CERC 2749.

Figure 11. Morphological characters of *Huntiella inaequabilis*. A–B. Flask-shaped conidiophores. C–D. Secondary conidiophores with emerging barrel-shaped conidia. E–F. Bacilliform conidia. G–H. Barrel-shaped conidia in chains. Bars: A–H = 10 µm.

Etymology: "*inaequabilis*" (Latin) = unequal and irregular, referring to the shape of the culture colony, distinguishing *H. inaequabilis* from other *Huntiella* species.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Not observed.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (27.5–)35–56(–72) µm long, (1.5–)1.5–2(–2.5) µm wide at apex and (2.5–)2.5–4(–7) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)4.5–9(–17.5) µm long and (1.5–)1.5–2(–2) µm wide; and (ii) barrel-shaped conidia hyaline, aseptate, in chains, (4.5–)5.5–7.5(–9.5) µm long and (3.5–)3.5–5(–6) µm wide. Chlamydospores not observed.

Culture characters: Colonies on MEA slow growing, optimal temperature for growth 25 C, no growth at 35 C. After 4 d, colonies grew 4.3 mm at 5 C, 9.1 mm at 10 C, 19.1 mm at 15 C, 26.5 mm at 20 C, 31.6 mm at 25 C, and 14.6 mm at 30 C. Colony margins unequal and irregular. Mycelium cottony, dense on MEA, initially white, turning to chaetura drab (17""k) after 7–10 d, reverse buff (19"f) to mars brown (13'm) when getting older.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangdong Province, China.

Other specimens examined: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li* & *C. Chen*, PREM 62040, culture CMW 44372 = CBS 143300 = CERC 2740.

Notes: Huntiella inaequabilis is closely related to *H. meiensis*. Because sexual structures for *H. inaequabilis* could not be induced in vitro, it can only be distinguished from other *Huntiella* species based on the morphology of the asexual state. The barrel-shaped conidia of *H. inaequabilis* (average 6.5 × 4 µm) are smaller than those of *H. meiensis* (average 7.5 × 5 µm). In addition, the colonies of *H. inaequabilis* on MEA at 25 C are small and irregular in outline, whereas those of *H. meiensis* are have smooth round margins. Growth of *H. inaequabilis* at 25 C is slower (average 31.6 mm) than for *H. meiensis* (average 71.5 mm). Based on DNA sequence data, *H. inaequabilis* differs from *H. meiensis* by six bases in the *TEF1α* gene and four bases in the *BT1* gene.

Huntiella meiensis F.F. Liu & S.F. Chen, sp. nov. FIG. 12

MycoBank MB826747

Typification: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li* & *C. Chen* (**holotype** PREM 62041). Ex-holotype culture: CMW 44376 = CBS 143301= CERC 2746.

Etymology: The name refers to the Meizhou, Guandong Province of China, where this fungus was collected.

Mating strategy: Heterothallic with isolates having either a *MAT1-1-1* gene or a *MAT1-2-1* gene.

Sexual state: Ascomata superficial, scattered near center of colony. Ascomatal bases black, globose to oval, (119.5–)139–195(–219.5) µm long and (129.5–)145–194.5(–220) µm wide, ornamented with many conical, thick-walled, dark brown spines, (6–)8.0–17.0(–28) µm long. Ascomatal necks dark brown to black, erect, slender, (290–)299.5–347.5(–416.5) µm long, (10–)11–13(–14.5) µm wide at apex and (33–)40–59(–77.5) µm wide at base. Ostiolar hyphae present, hyaline, convergent, (9)12–18.5(–22.5) µm long. Asci not observed. Ascospores hat-shaped, invested in sheath, aseptate, (4.5–)5.5–6.5(–7) µm long and (2–)2.5–3.5(–4) µm wide with sheath in side view. Ascospores accumulating in creamy to yellow droplets at tip of ascomatal neck.

Asexual state: Conidiophores arising laterally from vegetative hyphae, scattered or arising in clusters, multiseptate, hyaline, consisting of 2–3 cylindrical cells terminating in a phialide. Conidiogenous cells phialidic, cylindrical, (28–)31.5–52(–73) µm long, (1.5–)1.5–2.5(–2.5) µm wide at apex and (3–)3.5–4.5(–6) µm wide at base. Conidia of two forms: (i) bacilliform conidia hyaline, aseptate, cylindrical, (4–)5–7.5(–10.5) µm long and (1.5–)1.5–2.5(–3.5) µm wide; and (ii) barrel-shaped conidia hyaline, aseptate, in chains, (5.5–)6–8.5(–11) µm long and (3.5–)4–5.5(–6.5) µm wide. Chlamydospores not observed.

Culture characters: Colonies on MEA fast growing, optimal temperature for growth 25 C, no growth at 5 and 35 C. After 4 d colonies grew 12.7 mm at 10 C, 38.4 mm at 15 C, 60.2 mm at 20 C, 71.5 mm at 25 C, and 5.8 mm at 30 C. Colonies round with even margins. Mycelium flocky, dense on MEA, initially white, turning to mouse gray (13"") after 2–3 wk, reverse white turning dark brick (7"k) when older. Colony surfaces scattered with dark brown to black ascomata.

Habitat: Stumps of recently cut *Eucalyptus* trees.

Distribution: Guangdong Province, China.

Other specimens examined: CHINA. GUANGDONG PROVINCE: Meizhou region, *Eucalyptus* plantation (24°44′3″N, 116°22′39″E), isolated from recently harvested tree stump, Jan 2014, *S.F. Chen, J.N. Li* & *C. Chen*, PREM 62042, culture CMW 44374 = CBS 143302 = CERC 2742; PREM 62043, culture CMW 49305 = CBS 143303 = CERC 2744.

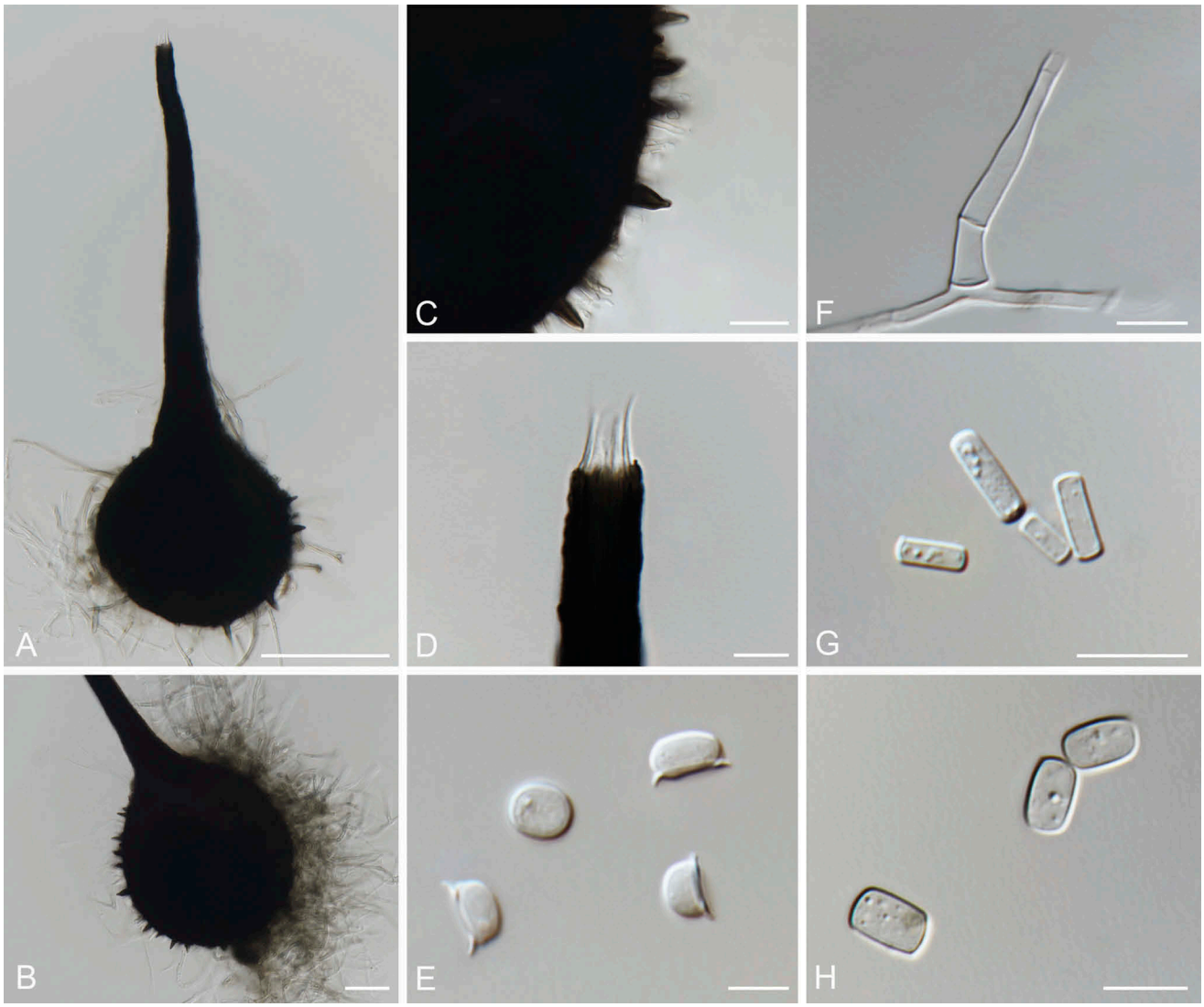

Figure 12. Morphological characters of *Huntiella meiensis*. A. Ascoma with globose base and extended neck. B–C. Conical spines on the surface of ascomatal base. D. Tip of ascomatal neck with convergent ostiolar hyphae. E. Hat-shaped ascospore in top view and side view. F. Flask-shaped conidiophore. G. Bacilliform conidia. H. Barrel-shaped conidia. Bars: A = 100 µm; B = 50 µm; C = 20 µm; D, F–H = 10 µm; E = 5 µm.

DISCUSSION

Huntiella moniliformis, the type species of the genus, was first isolated from sweet-gum wood (*Liquidambar styraciflua*) in Texas (Von Schrenk 1903). Subsequently, the taxonomy of the species related to *H. moniliformis* presented considerable challenges, especially before the advent of DNA sequence data to resolve species boundaries. *Huntiella moniliformis* was first described as *Ceratostomella moniliformis* by Hedgcock (1906) and later was reduced to synonymy with the genus *Ceratocystis* (Moreau 1952). Several descriptions of *H. moniliformis* were subsequently presented (Davidson 1935; Kitajima 1936; Bakshi 1951; Luc 1952; Hunt 1956; Roldan 1962; Upadhyay 1981), although it became increasingly clear (Wingfield et al. 2013) that these fungi were not closely related to the morphological similar *Ceratocystis* spp. De Beer et al. (2014) presented the first conclusive evidence that *Huntiella* represents a discrete genus, clearly separated from *Ceratocystis*, using robust DNA sequence-based phylogenetic inference, morphology, and ecological characters.

Subsequent to the discovery of *H. moniliformis* in the USA more than 100 years ago (Von Schrenk 1903), species in this genus have been reported from many different parts of the world, including Africa, Australasia, and South America (Van Wyk et al. 2006; Heath et al. 2009; Kamgan Nkuekam et al. 2012; De Beer et al. 2014). As is true for other genera in Ceratocystidaceae (De Beer et al. 2014), many *Huntiella* species rely on insects for their dispersal

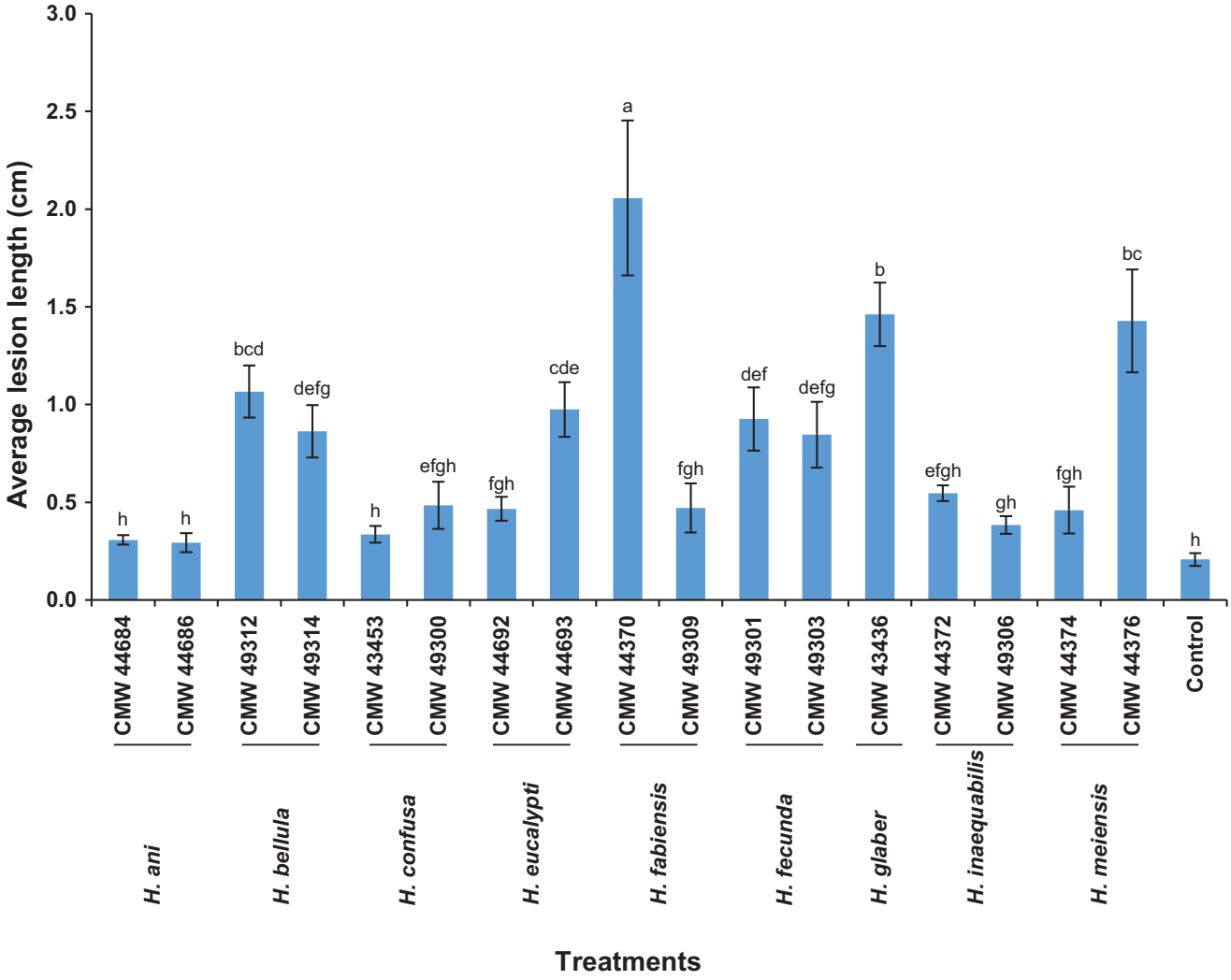

Figure 13. Histogram indicating the average lesion length (in cm) resulting from inoculation trials of *Eucalyptus* seedlings inoculated with 17 isolates of nine *Huntiella* species and the negative controls. Vertical bars represent standard error of means. Different letters above the bars indicate treatments that were statistically different ($P = 0.05$).

and fertilization (Kamgan Nkuekam et al. 2012; De Errasti et al. 2015). This has probably led to their being introduced accidentally into new areas, although very little is known regarding their pathways of spread.

In this study of *Huntiella* in southern China, a surprising number of new species were discovered, although only two host genera were examined. All of the newly described species, except for *H. confusa*, were obtained from freshly wounded *Eucalyptus* tissues in plantations across four provinces. *Huntiella confusa* was from an *Acacia confusa* tree in Hainan Province. All species were identified based on comparisons of ITS, *BT1*, and *TEF1α* sequence data and supported by morphological characters.

Different clades reflecting geographic origins were characterized within *Huntiella* (Mbenoun et al. 2014) based on analyses of the combined gene regions. Our isolates grouped in the two larger clades referred to as the Indo-Pacific Clade and Asian Clade. Only *H. fecunda* belonged to the Indo-Pacific Clade, the only species collected from Fujian Province, our northernmost sampling site. The remaining eight species grouped in two subclades (subclades 1 and 2) within the Asian Clade. *Huntiella confusa* and *H. glaber* grouped in subclade 2, including the previously described species *H. chinaeucensis, H. inquinans, H. microbasis*, and *H. sumatrana*. These two newly described species can be distinguished from their closest relatives by their morphological characters and phylogenetic inference. *Huntiella chinaeucensis* was described from *Eucalyptus* in Guangdong Province of China (Chen et al. 2013), whereas *H. sumatrana, H. microbasis*, and *H. inquinans* were

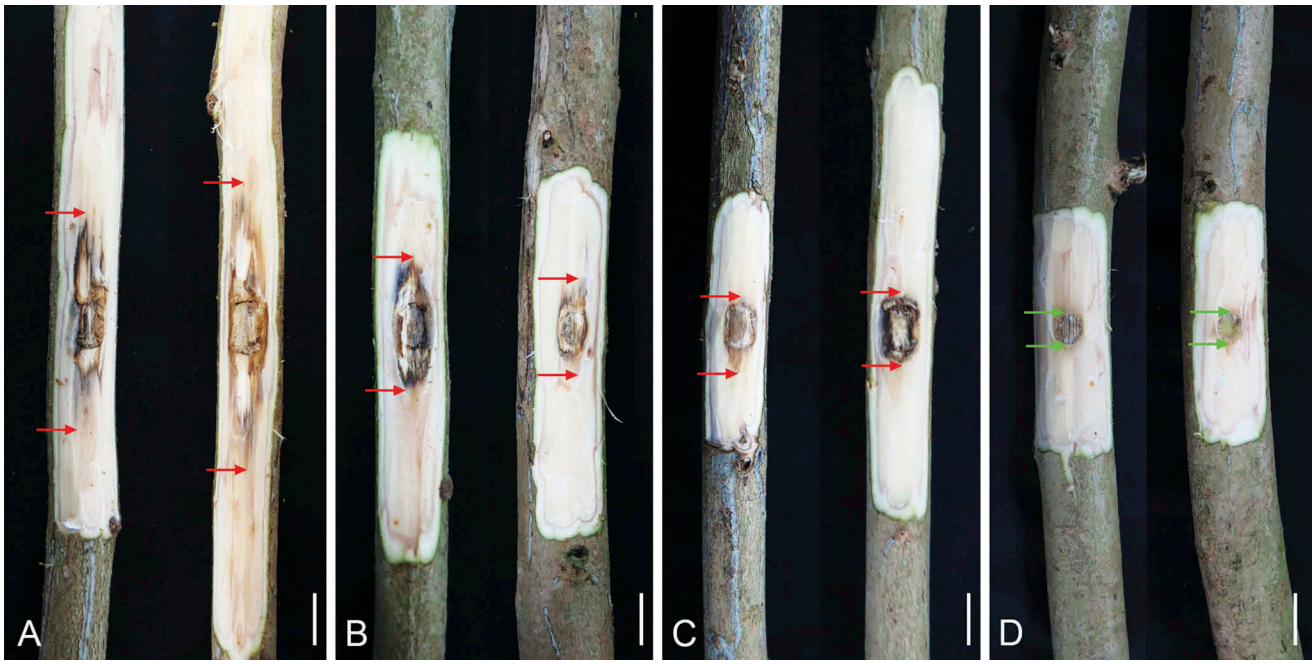

Figure 14. Lesions resulting from inoculations of *Huntiella* species onto *Eucalyptus* seedlings and wound responses on the negative controls. A–C. Lesions produced by *H. fabiensis* (CMW 44370), *H. fecunda* (CMW 49303), and *H. ani* (CMW 44684), respectively. D. Negative controls showing absence of lesion but only wound development; arrows indicate the terminal ends of the lesions (red arrows) and wounds (green arrows). Scale bars: A–D = 10 mm.

from *Acacia mangium* in Indonesia (Tarigan et al. 2010). All are known only from wounds on their hosts and have not been associated with diseases of these trees. *Huntiella confusa* and *H. glaber* were both collected only from Hainan Province, which is the southernmost province of China.

Six of the newly described *Huntiella* species (*H. ani, H. bellula, H. eucalypti, H. fabiensis, H. inaequabilis*, and *H. meiensis*), together with the previously described *H. bhutanensis*, formed subclade 1 of the Asian Clade of *Huntiella*. These six species could be recognized with confidence based on phylogenetic analyses of the *BT1* and *TEF1α* gene sequences. In addition, morphological and growth differences in culture can also be used to distinguish among them. The six species were collected from fresh wounds on recently cut *Eucalyptus* trees in Guangdong and Guangxi provinces, which are adjacent provinces with similar environments. It is relevant that all species obtained in these two provinces grouped in the same species complex, suggesting that the environmental conditions may play an important role in species adaptation.

Huntiella species are well known to lose their ability to produce sexual structures with successive transfers on artificial media (Van Wyk et al. 2004; De Beer et al. 2014). This was also true for the species described here. This prompted an effort to define the mating strategies of the species and to attempt to induce sporulation by pairing cultures of opposite mating type in culture. The majority of the newly described species were heterothallic, based on the genetic analysis of their mating genes. It was thus possible to induce the production of sexual structures in pairing tests with four of the seven species. Our results showed that mating strategies of *Huntiella* species differ in each of two subclades of the larger Asian Clade.

It was interesting that *H. fecunda* undergoes unisexual reproduction. This makes it one of only six species known to exhibit this sexual strategy (Glass and Smith 1994; Lin et al. 2005; Alby et al. 2009; Wilson et al. 2015; Schuerg et al. 2017). Unisexuality is a unique form of homothallism, recently recognized in *H. moniliformis* by Wilson et al. (2015). In this situation, only the *MAT1-2-1* gene is present in sexually reproducing cultures (Wilson et al. 2015).

Species of *Huntiella* are generally considered saprobes (Van Wyk et al. 2006; De Beer et al. 2014) that infect freshly cut wounds on trees. Several reports show that some species can cause lesions on the stems of artificially inoculated trees (Tarigan et al. 2010; Chen et al. 2013; De Errasti et al. 2015; Mbenoun et al. 2016). This is consistent with our pathogenicity tests, where all of the *Huntiella* species tested showed the capacity to

cause distinct lesions in sapwood when inoculated on healthy seedlings. Although we do not consider these fungi to be primary pathogens, they may contribute to tree mortality (De Errasti et al. 2015).

Prior to this study, only one *Huntiella* species, *H. chinaeucensis*, was known from China, where it was isolated from *Eucalyptus* trees in Guangdong Province (Chen et al. 2013). Application of multigene phylogenetic analysis has made it possible to identify nine novel species of *Huntiella* from China, bringing the total number of known *Huntiella* species to 30. The relatively large number of new species found in this study conducted in a fairly limited area suggests that many more novel species of *Huntiella* await discovery in China.

Until recently, the Ceratocystidaceae have been relatively poorly known in China. Although *Huntiella* spp. are not considered important agents of plant disease, numerous *Ceratocystis* have been described from China in contemporary studies. Other than *C. fimbriata* sensu stricto, which was isolated from *Ipomoea batatas* (Sy 1956), these include *C. cercfabiensis* from recently harvested *Eucalyptus* stumps in South China (Liu et al. 2015), *C. changhui*, the causal agent of black rot on *Colocasia esculenta* (Liu et al. 2018), *C. collisensis* from *Cunninghamia lanceolata* in Fujian Province (Liu et al. 2015), and *C. manginecans*, also from stumps of *Eucalyptus* in Guangdong Province (Chen et al. 2013). Our results, and recent reports of new species of *Ceratocystis*, suggest that a relatively high diversity of Ceratocystidaceae occur in this geographic region. Their ecological importance, especially as plant pathogens and agents of wood degradation, deserves further study.

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