Diseases of Cymbopogon citratus (Poaceae) in China:
Curvularia nanningensis sp. nov.

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Abstract
Five Curvularia strains isolated from diseased leaves of lemongrass (Cymbopogon citratus) in Guangxi Province, China, were examined. NCBI-Blast searches of ITS sequences suggested a high degree of similarity (99–100%) to Curvularia akaii, C. akaiiensis, C. bothriochloae, C. heteropogonis and C. sichuanensis. To accurately identify these strains, we further analysed their morphology and phylogenetic relationships based on combinations of ITS, GAPDH, and tef1 gene sequences. Morphological observations indicated that the key character differing from similar species was conidial size, whereas phylogenetic analyses indicated that the five strains represent one species that is also distinct from C. akaii, C. akaiiensis and C. bothriochloae by conidial size and conidiophore length. Thus, the strains examined are found to represent a new species described herein as Curvularia nanningensis. The pathogenicity test on the host and detached leaves confirmed the new species to be pathogenic on Cymbopogon citratus leaves. Standardised requirements for reliable identification of Curvularia pathogens are also proposed.

Keywords
Cymbopogon, phylogeny, plant disease, Pleosporaceae, taxonomy
Introduction

*Cymbopogon citratus* Stapf (lemongrass), believed to be a native of Malaysia, is now widely distributed in all continents and particularly in America, China, Guatemala and Southeast Asia. Essential oil from lemongrass is often used in aromatherapy (Williamson et al. 1996; Noel et al. 2002; Yang and Lei 2005; Shah et al. 2011). As a traditional Chinese medicine, lemongrass is known to provide relief from a variety of ailments including eczema, cold, headache and stomach-ache (Zhou et al. 2011). Guatemala is known to be the main exporter of lemongrass with about 250 tons per year. China produces 80 to 100 tons of lemongrass annually and the USA and Russia each imports about 70 tons per year (DAFF 2012). Depending on climatic conditions, lemongrass can be severely infected with a rust disease caused by *Puccinia nakanishikii* Dietel in Hawaii and California (Gardner 1985; Koike and Molinar 1999). In Brazil, a rust on lemongrass caused by another *Puccinia* species named *P. cymbopogonis* Massee has been reported (Vida et al. 2006). Joy et al. (2006) summarised the various disease symptoms and their causal agents of lemongrass.

*Curvularia* spp. infect many herbaceous plants including *Cymbopogon* Spreng. (Smith et al. 1989). *Helminthosporium cymbopogoni* C.W. Dodge (≡ *Curvularia cymbopogonis* (C.W. Dodge) J.W.Groves & Skolko) is responsible for a severe disease of lemongrass in the lowlands of Guatemala (Dodge 1942). Barua and Bordoloi (1983) discovered *C. verruciformis* causing disease on *Cymbopogon flexuosus* Stapf. *Curvularia andropogonis* (Zimm.) Boedijn led to foliage blight of *Cymbopogon nardus* (L.) Rendle in the Philippines (Sato and Ohkubo 1990). Thakur (1994) reported *C. lunata* (Wakker) Boedijn as the causal agent of a new blight disease of *Cymbopogon martini* (Roxb.) Wats. var. *motia* Burk. Chutia et al. (2006) discovered that a leaf blight of *Cymbopogon winterianus* Jowitt is caused by *Curvularia* spp., resulting in a dramatic change in oil yield and its constituents. Recently, Santos et al. (2018) characterised the morphological and molecular diversity of the isolates of *C. lunata*, associated with *Andropogon* Linn. seeds.

Starting in 2010, there have been outbreak reports of pathogenic *Curvularia* in Asian countries, especially India and Pakistan (Pandey et al. 2014; Avasthi et al. 2015; Majeed et al. 2015). As China is a neighbouring country, we felt obligated to evaluate the potential threat of *Curvularia* to our crops. A severe *Curvularia* leaf blight disease was observed in three farms of *Curcuma aromatica* Salisb. in Hainan Province during 2010 (Chen et al. 2013). Gao et al. (2012) reported a new rice black sheath spot disease caused by *C. fallax* Boedijn in Hunan Province. Our research group is also conducting a disease survey on the occurrence of *Curvularia* diseases in Southwest China since 2017. Two new pathogens (*C. asianensis* Manamgoda, L. Cai & K.D. Hyde and *C. microspora* Y. Liang, K.D. Hyde, J. Bhat & Yong Wang bis), which affected *Epipremnum pinnatum* (L.) Engl. and *Hippeastrum rutilum* Herb. (Liang et al. 2018; Wang et al. 2018), respectively, were found.

Meanwhile, a severe leaf blast disease on lemongrass was found in Guangxi Province, China, that first appeared on the tips of leaves. As the infection progressed, more than 30% of leaves showed different degrees of abnormalities, while in the later stages
more than 50% of the upper leaves appeared diseased and disease incidence reached 80% or above in the lower leaf blades. We provide a detailed morphological description and phylogenetic analyses of the pathogen confirming it as a new Curvularia species. Koch’s postulates (see later text) have been carried out to confirm its pathogenicity. Our study provides a further understanding of Curvularia disease on lemongrass in China.

**Materials and methods**

**Isolation**

Leaves of Cymbopogon citratus showing leaf blast symptoms were collected from Guangxi Medicinal Botanical Garden in Nanning, China, during 2017. Diseased leaf pieces were surface disinfected with 70% ethanol for 30 s, 1% NaClO for 1 min and repeatedly rinsed in sterile distilled water for 30 s. For isolation of Curvularia, conidia were removed from the diseased tissue surface using a sterilised needle and placed in a drop of sterilised water followed by microscopic examination. The spore suspension was drawn with a Pasteur pipette and transferred to a Petri dish with 2% water agar (WA) or 2% malt extract agar (MEA) and 100 mg/l streptomycin to inhibit the growth of bacteria. The plates were incubated for 24 h in an incubator (25°C) and examined for single spore germination under a dissecting microscope. Germinating conidia were transferred separately to new 2% MEA plates (Chomnunti et al. 2014).

**Morphological studies**

Single germinated spores were transferred to PDA or MEA and incubated at 28°C in a light incubator with 12 h light/12 h darkness. Ten days later, the colony and morphological characters were recorded according to Manamgoda et al. (2011, 2012). Colony diameters on PDA and MEA were measured at 1, 3, 5 and 7 days post-inoculation and average growth rates were calculated. Conidia and conidiophores were examined using a compound microscope fitted with a digital camera (Olympus BX53). The holotype specimen is deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). An ex-type culture is deposited in the Culture Collection of the Department of Plant Pathology, Agriculture College, Guizhou University, China (GUCC) and Mae Fah Luang University Culture Collection (MFLUCC) in Thailand (Table 1).

**DNA Extraction and Sequencing**

Fungal cultures were grown on PDA at 28°C until the entire Petri dish (90 mm) was colonised. Fresh fungal mycelia were scraped off the surface of the PDA using a sterilised scalpel. A BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416,
**Table 1.** Sequences used for phylogenetic analysis.

| Species name                  | Strain number | ITS GenBank Accession numbers | GAPDH GenBank Accession numbers | tef GenBank Accession numbers |
|-------------------------------|---------------|------------------------------|---------------------------------|-----------------------------|
| *Curvularia aeria*            | CBS 294.61†   | HE861850                     | HF565450                         | –                           |
| *C. affinis*                  | CBS 154.34†   | KJ909780                     | KM230401                         | KM196566                    |
| *C. abrusenzis*               | CBS 144673†   | KX139029                     | MG428693                         | MG428686                    |
| *C. akati*                    | CBS 317.86    | KJ909782                     | KM230402                         | KM196569                    |
| *C. akatiensis*               | BRIP 16080    | KJ415539                     | KJ415407                         | KJ415453                    |
| *C. alcornii*                 | MFLUCC 10-0703† | JX256420      | JX276433                         | JX266589                    |
| *C. americana*                | UTHSC 08-3414 | HE861833                     | HF565488                         | –                           |
| *C. asiatica*                 | MFLUCC 10-0711† | JX256424      | JX276436                         | JX266593                    |
| *C. australiensis*            | BRIP 12044†   | KJ415540                     | KJ415406                         | KJ415452                    |
| *C. australis*                | BRIP 12521†   | KJ415541                     | KJ415405                         | KJ415451                    |
| *C. bannonii*                 | BRIP 10972†   | MH444892                     | MH433638                         | MH433654                    |
| *C. bealayi*                  | BRIP 12942†   | MH444894                     | MH433634                         | MH433657                    |
| *C. boreriae*                 | CBS 859.73    | HE861848                     | HF565455                         | –                           |
| *C. bothriochloae*            | MFLUCC 11-0422 | KF400638       | KP419987                         | KM196571                    |
| *C. brachyspora*              | CBS 186.50    | KJ922372                     | KM061784                         | KM230405                    |
| *C. buchloes*                 | CBS 246.49†   | KJ909765                     | KM061789                         | KM196588                    |
| *C. carica-papayae*           | CBS 135941    | HG779021                     | HG779146                         | –                           |
| *C. chiangmaiensis*           | CPC 28829     | MF490814                     | MF490836                         | MF490857                    |
| *C. chlamydospora*            | UTHSC 07-2764 | HG779021                     | HG779151                         | –                           |
| *C. clavata*                  | BRIP 61680b   | KU552205                     | KU552167                         | KU552159                    |
| *C. coatesiae*                | BRIP 24261†   | MH444897                     | MH433636                         | MH433659                    |
| *C. colbranii*                | CBS 192.29    | JN192373                     | JN600962                         | JN601006                    |
| *C. dactyloctenii*            | CBS 135941†   | HG778984                     | HG779146                         | –                           |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. deightonii*               | BRIP 13524†   | KJ415544                     | KJ415402                         | KJ415448                    |
| *C. dactyloctenii*            | CBS 135941†   | HG778984                     | HG779146                         | –                           |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenii*            | CBS 135941†   | HG778984                     | HG779146                         | –                           |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 13524†   | KJ415544                     | KJ415402                         | KJ415448                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| *C. dactyloctenicola*          | CPC 28810     | MF490815                     | MF490837                         | MF490858                    |
| *C. dactyloctenii*            | BRIP 12846†   | KJ415545                     | KJ415401                         | KJ415447                    |
| Species name                  | Strain number  | ITS       | GAPDH     | tef        |
|------------------------------|----------------|-----------|-----------|------------|
| *Curvularia nanningensis* sp. nov. | BRIP 12919<sup>T</sup> | KJ415550  | KJ415397  | KJ415443   |
| *C. neergaardii*              | GUCC 11000     | MH885316  | MH980000  | MH980006   |
| *C. nanningensis* sp. nov.    | GUCC 11001     | MH885317  | MH980001  | MH980007   |
|                              | GUCC 11002     | MH885318  | MH980002  | MH980008   |
|                              | GUCC 11003     | MH885319  | MH980003  | MH980009   |
|                              | GUCC 11005<sup>T</sup> | MH885321  | MH980005  | MH980011   |
| *C. neovindica*               | BRIP 17439     | AF081449  | AF081406  | –          |
| *C. nicotiae*                 | CBS 655.74<sup>T</sup> = BRIP 11983 | KJ415551  | KJ415396  | KJ415442   |
| *C. nodosa*                   | CPC 28800<sup>T</sup> | MF490816  | MF490838  | MF490859   |
|                              | CPC 28801     | MF490817  | MF490839  | MF490860   |
|                              | CPC 28812     | MF490818  | MF490840  | MF490861   |
| *C. nodulosa*                 | CBS 160.58     | JN601033  | JN600975  | JN601019   |
| *C. oryzae*                   | CBS 169.53<sup>T</sup> | KP406050  | KP645344  | KM196590   |
| *C. ovarticola*               | CBS 470.90<sup>T</sup> | JN192384  | JN600976  | JN601020   |
| *C. pallescens*               | CBS 156.35<sup>T</sup> | KJ922380  | KM083606  | KM196570   |
| *C. pallescens*               | MFLUCC 14-0404 | MF621582  | –         | –          |
| *C. pizoni*                   | CBS 308.67<sup>T</sup> | KJ909774  | KM083617  | KM196594   |
| *C. peritidis*                | CBS 350.90<sup>T</sup> | JN192385  | KJ415394  | JN601021   |
| *C. peteritii*                | BRIP 14642<sup>T</sup> | MH141405  | MH433650  | MH433668   |
| *C. pisi*                     | CBS 190.48<sup>T</sup> | KY905678  | KY905690  | KY905607   |
| *C. platanei*                 | BRIP 27703<sup>T</sup> | MH141406  | MH433651  | MH433669   |
| *C. portulacaee*              | CBS 239.48<sup>T</sup> = BRIP 14541 | KJ415553  | KJ415393  | KJ415440   |
| *C. prasadii*                 | CBS 143.64<sup>T</sup> | KJ22373  | KM061785  | KM230408   |
| *C. protuberata*              | CBS 376.65<sup>T</sup> | KJ22376  | KM083605  | KM196576   |
| *C. pseudobrachyspora*        | CPC 28808<sup>T</sup> | MF490819  | MF490841  | MF490862   |
| *C. pseudolunata*             | UTHSC 09-2092<sup>T</sup> | HE861842  | HF565459  | –          |
| *C. pseudorobusta*            | UTHSC 08-3458  | HE861838  | HF565476  | –          |
| *C. ravenni*                  | BRIP 13165<sup>T</sup> | JN192386  | JN600978  | JN601024   |
| *C. resii*                    | BRIP 4358<sup>T</sup> | MH141407  | MH433637  | HM433670   |
| *C. richardiae*               | BRIP 4371<sup>T</sup> | KJ415555  | KJ415391  | KJ415438   |
| *C. robusta*                  | CBS 624.68<sup>T</sup> | KJ909783  | KM083613  | KM196577   |
| *C. ruhini*                   | CBS 144674<sup>T</sup> | KJX139030  | MG428694  | MG428687   |
| *C. ryleyi*                   | BRIP 12554<sup>T</sup> | KJ415556  | KJ415390  | KJ415437   |
| *C. senegalensis*             | CBS 149.71     | HG779001  | HG779128  | –          |
| *C. sesuvi*                   | Bp-Zj 01<sup>T</sup> | EF175940  | –         | –          |
| *C. shahidchamranensis*       | IRAN 3133C     | MH550084  | MH550083  | –          |
| *C. soli*                     | CBS 222.96<sup>T</sup> | KY905679  | KY905691  | KY905608   |
| *C. sorghina*                 | BRIP 15900<sup>T</sup> | KJ415558  | KJ415388  | KJ415435   |
| *C. subpapendorfii*           | CBS 173.55<sup>T</sup> | JX256433  | JX276445  | JX266599   |
| *C. subpapendorfii*           | CBS 146.63<sup>T</sup> | JX256433  | JX276445  | JX266599   |
| *C. subspicifera*             | CPC 274.52     | JX256433  | JX276445  | JX266599   |
| *C. subulicola*               | CPC 28813     | MF490820  | MF490842  | MF490863   |
| *C. subulicola*               | CPC 28814     | MF490821  | MF490843  | MF490864   |
| *C. subulicola*               | CPC 28815<sup>T</sup> | MF490822  | MF490844  | MF490865   |
| *C. subulicola*               | CPC 28816     | MF490823  | MF490845  | MF490866   |
| *C. verruciformis*            | CBS 537.75<sup>T</sup> | HG779026  | HG779134  | –          |
| *C. verruculosa*              | CBS 150.63<sup>T</sup> | KP400652  | KP645346  | KP735695   |
|                              | CPC 28792     | MF490825  | MF490847  | MF490868   |
|                              | CPC 28809     | MF490824  | MF490846  | MF490867   |
| *C. warraberensis*            | BRIP 14817<sup>T</sup> | MH141409  | MH433653  | MH433672   |
| *Bipolaris drechleri*         | MUS00028      | KF500532  | KM034846  | KM037971   |
| *B. maydis*                   | CBS 136.29<sup>T</sup> | AF071325  | KM034846  | KM037974   |

Ex-type isolates were labeled with "<sup>T</sup>".
BIOMIGA, Inc., San Diego, California, USA) was used to extract the genomic DNA. DNA amplification was performed in a 25 μl reaction volume which contained 2.5 μl 10 × PCR buffer, 1 μl of each primer (10 μM), 1 μl template DNA, 0.25 μl Taq DNA polymerase (Promega, Madison, WI, USA) and 18.5 μl ddH₂O. Primers used and thermal cycling programme for PCR amplification of the ITS (ITS4/ITS5), GAPDH (gpd1/gpd2) and tef1 (EF-526F/1567R) genes were followed as described previously (White et al. 1990; Berbee et al. 1999; Schoch et al. 2009; Liang et al. 2018).

Phylogenetic analyses

DNA sequences originated from five strains (GUCC 11000, GUCC 11001, GUCC 11002, GUCC 11003 and GUCC 11005) and reference sequences of ex-type or representative sequences of *Curvularia* species were downloaded from GenBank database (Table 1) with strains of *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker (CBS 136.29) and *B. drechsleri* Manamgoda & Minnis (MUS0028) as outgroup taxa. Alignments for each locus were performed in MAFFT v7.307 online version (Katoh and Standley 2016) and manually verified in MEGA 6.06 (Tamura et al. 2013). Phylogenetic analyses were performed by Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian methods. Sequences were optimised manually to allow maximum alignment and maximum sequence similarity as detailed in Manamgoda et al. (2012). MP analyses were performed in PAUP v. 4.0b10 (Swofford 2003) using the heuristic search option with 1,000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. Five thousand maxtrees were set to build up the phylogenetic tree. The characters in the alignment matrix were ordered according to ITS+GAPDH+tef1 with equal weight, and gaps were treated as missing data. The MP phylogenetic analysis of *Curvularia* ITS sequences included pathogens from China, India and Pakistan and the wrong sequence (KN879930), actually belonging to *Alternaria alternata* (taxon:5599), was selected as the outgroup. The Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Index (HI) were calculated for each tree generated. The resulting PHYLIP file was used to generate the ML tree on the CIPRES Science Gateway (https://www.phylo.org/portal2/login.action) using the RAxML-HPC2 black box with 1000 bootstrap replicates and GTR+GAMMA as the nucleotide substitution model. For Bayesian inference analysis, the best model of evolution (GTR+I+G) was determined using MrModeltest v2 (Nylander 2004). Bayesian inference analysis was done using MrBayes v 3.2.6 (Ronquist et al. 2012). Bayesian analyses were launched with random starting trees for 2 000 000 generations and Markov chains were sampled every 1000 generations. The first 25% resulting trees were discarded as burn-in. Alignment matrices are available in TreeBASE under the study ID 25080.
Koch’s Postulate test

To confirm the pathogenicity of the fungus, five healthy plants of *Cymbopogon citratus* were inoculated with 5 mm diameter mycelial plugs of the five isolates (GUCC 11000, GUCC 11001, GUCC 11002, GUCC 11003 and GUCC 11005) cut from the margins of 10-day-old actively growing cultures; the control was treated with sterile agar plugs. The plants were kept for two days in an illuminating incubator at 28° ± 3°C. Additionally, two plants were sprayed with distilled water and kept as control under the same conditions. Both inoculated (host and detached leaves) and control plants were kept for two days in an illuminating incubator at 28 ± 3°C. After four days of incubation, the inoculated plants and leaves were observed for the development of symptoms (Zhang et al. 2018). Infected leaves were collected and the fungus was re-isolated using PDA medium and the ITS sequence was compared with original strains.

Results

Phylogenetic analyses

First, we compared the DNA sequence identity of ITS, GAPDH and *tef1* gene regions (Table 2). Among our five strains, there was only one base difference. In the ITS gene region, for *C. akatiensis*, the base sequence was identical to our strains; only 1 difference for *C. bothriochloae*; base differences were 8 for *C. akaii*, 9 for *C. deightonii* and 5 for *C. sichuanensis*. Only *C. heteropogonis* had noticeable (25) base differences with our strains. In the GAPDH and *tef1* gene regions, the mutation rate of DNA bases was apparently faster than the ITS region. There were between 9 to 19 base differences in GAPDH and 3 to 8 in *tef1*. This means that in *Curvularia*, GAPDH has a faster

**Table 2.** DNA sequence differences between *Curvularia nanningensis* and related species in three gene regions.

| Species           | Strain number | ITS (1–547 bp) | GAPDH (550–1031bp) | tef1 (1034–1899 bp) |
|-------------------|---------------|----------------|--------------------|--------------------|
| *C. nanningensis* | GUCC11000     | 0              | 1                  | 0                  |
|                   | GUCC11001     | 0              | 0                  | 0                  |
|                   | GUCC11002     | 0              | 1                  | 0                  |
|                   | GUCC11003     | 0              | 1                  | 0                  |
|                   | GUCC11005<sup>T</sup> | 0 | 0 | 0 |
| *C. akaii*        | CBS 317.86    | 8              | 9                  | 4                  |
| *C. akatiensis*   | BRIP 16080<sup>T</sup> | 0 | 10 | 5 |
| *C. bothriochloae*| BRIP 12522<sup>T</sup> | 1 | 19 | 8 |
| *C. deightonii*   | CBS 537.70    | 9              | 13                 | –                  |
| *C. heteropogonis*| CBS 284.91<sup>T</sup> | 25 | 12 | 3 |
| *C. sichuanensis* | HSAUP II.2650-1<sup>T</sup> | 5 | – | – |

<sup>T</sup> = ex-type
Figure 1. Maximum Parsimony (MP) topology of *Curvularia* generated from a combination of ITS, GAPDH and *tef1* sequences. *Bipolaris maydis* (CBS 136.29) and *B. drechsleri* (MUS0028) were used as outgroup taxa. MP and ML above 50% and BPP values above 0.90 were placed close to topological nodes and separated by “/”. The bootstrap values below 50% and BPP values below 0.90 were labelled with “-”. Our main research clade was labelled with green colour.
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Figure 2. Maximum Parsimony (MP) analysis of Curvularia pathogens in China, India and Pakistan based on ITS sequences. Alternaria alternata (taxon:5599) was used as outgroup taxon. Bootstrap values (≥ 50%) of the MP method are shown near the nodes.

evolutionary rate than ITS and tef1 and therefore some mycologists have suggested the use of ITS+GAPDH for phylogenetic analysis and GAPDH as a secondary barcode marker for accurate identification.
The alignment of *Curvularia* combining three gene fragments (ITS, GAPDH and tef1) comprised 116 strains belonging to 104 taxa. In order to accurately identify our strains, phylogenetic analysis included all ex-type and published strains of all *Curvularia* spp. described recently (Hyde et al. 2017; Marin-Felix et al. 2017; Dehdari et al. 2018; Heidari et al. 2018; Hernández-Restrepo et al. 2018; Mehrabi-Koushki et al. 2018; Tán et al. 2018; Jayawardena et al. 2019) which are listed in Table 1. The final alignment comprised 2032 characters (each gene fragment was separated with 2 “N”) including gaps (ITS: 1–600, GAPDH: 603–1162 and tef1: 1165–2032). Among these characters, 2032 are constant, 125 variable characters are parsimony-uninformative and 503 are parsimony-informative. The parameters of the phylogenetic trees are TL = 2590, CI = 0.38, RI = 0.72 and HI = 0.62. In the *Curvularia* phylogenetic tree (Figure 1), all isolates grouped together with 100% (MP and ML) bootstrap support. Our strains (GUCC 11000, 11001, 11002, 11003 and 11005) formed a strongly supported group (MP: 100%; ML: 100%; BPP: 1.00) with a close relationship to *C. akaii*, *C. akaiiensis*, *C. bothriochloae*, *C. deightonii* and *C. heteropogonii* with high bootstrap support (MP: 94%; ML: 97%; BPP: 1.00). In this group, the five examined strains were closer to *C. akaii*, *C. akaiiensis* and *C. bothriochloae* and also showed high bootstrap support (MP: 82% and ML: 94%; BPP: 0.98).

The phylogenetic analysis of the ITS gene region evaluated all new *Curvularia* pathogens recently described from China, India and Pakistan. The aligned matrix consisted of fifty-four ITS sequences and included ex-type sequences of 13 *Curvularia* species (Supplementary Table 1). The phylogenetic tree (Figure 2) indicated that ITS BLAST searches only provided limited value for pathogenic identification. In *Curvularia lunata*, only one sequence WCCL (MG063428) showed a very close relationship with the ex-type strain sequence of *C. lunata* CBS 730.96 (MG722981). The other eight sequences were grouped into two branches, e.g. taxon:5503 (LN879926) which might belong to *C. aeria*, while the other seven formed an independent lineage. ITS sequences did not separate *Curvularia affinis*, *C. asianensis* and *C. fallax* and some of their sequences even clustered with *C. australiensis* HNW99-1 (KT719300). After multi-gene analysis, the phylogenetic distance was shown to be unreliable and may suggest whether they belong perhaps to different species.

**Taxonomy**

*Curvularia nanningensis* Qian Zhang, K.D. Hyde & Yong Wang bis, sp. nov.

Mycobank No: 829056
Facesoffungi number: FoF 05596
Figure 3A–I

**Diagnosis.** Characterised by the size of conidia.

**Type.** China, Guangxi Province, Nanning City, Guangxi Medicinal Botanical Garden, 22°51’N, 108°19’E, on blighted leaves of *Cymbopogon citratus*, 30 Septem-
Figure 3. *Curvularia nanningensis* (GUCC11005, holotype) A, B diseased symptom C colony on PDA from above D colony on PDA from below E-G conidia and conidiophores H-I conidia. Scale bars: 50 μm (E), 20 μm (F), 10 μm (G-I).

December 2017, Q. Zhang, ZQ0091 (HGUP 11005, holotype, MFLU19-1227, isotype), GUCC 11005 and MFLUCC 19-0092, ex-type.

**Description.** Pathogenic on *Cymbopogon citratus*. Fungus initially producing white to grey lesions with dark borders on all parts of the shoot, later enlarging and coalescing over entire leaf.

Colonies on PDA irregularly circular, with mycelial growth rate = 1.0 cm/day, vegetative hyphae septate, branched, subhyaline to brown, smooth to verruculose, 2–3 μm, anastomosing. *Aerial mycelium* dense, felted, initially pale grey, becoming darkened and greyish-green at maturity, producing black extracellular pigments. On MEA, the colony morphology similar to PDA, with growth rate = 1.35 cm/day. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, arising singly, simple or branched, flexuous, 8–10 septate, geniculate, pale brown to dark brown, paler towards apex, 120–200 × 2–3 μm (av. = 170 × 2.5 μm, n = 30). *Conidiogenous cells* polytretic, sympodial, terminal, sometimes intercalary, cicatrised, with thickened and darkened conidiogenous loci up to 1.0–1.2 μm diam., smooth. *Mature conidia* 3 to rarely 4 septa, acropleurogenous, obovoid, usually straight to curved at the slightly wider, smooth-walled, larger third cell from the base, 24.5–36.0 × 14.0–20.5 μm (av. = 29.5 × 17.5 μm, n = 50), sub-hyaline to pale brown end cells, pale brown to dark brown at intermediate cells, with conspicuous or sometimes slightly protuberant hilum. Germination of conidia bipolar.

**Distribution.** China, Guangxi Province, Nanning City.

**Other material examined.** China, Guangxi Province, Nanning city, Guangxi Medicinal Botanical Garden, on blight leaves of *C. citratus*, 30 September 2017,
With reference to the location, Nanning City where the fungus was isolated.

**Pathogenicity test**

Four days after inoculation, blast symptoms appeared on all inoculated plants, which were similar to symptoms of plants in the field (Figures 3A, B, 4A, B). Non-treated control plants remained healthy without any symptoms (Figure 4C). *Curvularia nanningensis* was re-isolated from the lesions of inoculated plants and the identity of the fungus was confirmed by sequencing the ITS region. Meanwhile, a detached leaf-experiment was also conducted in an illuminated incubator at 28 ± 3°C, where similar symptoms appeared on healthy inoculated leaves of *Cymbopogon citratus* after four days (Figure 4D right), while the control leaf (Figure 4D left) did not show symptoms.

**Discussion**

Phylogenetic analysis based on combined DNA sequences of ITS, GAPDH and tefl showed that our strains were related to three *Curvularia* species named *C. akaii* (Tsuda & Ueyama) Sivan., *C. akaiiensis* Sivan. and *C. bothriochloae* Sivan., Alcorn & R.G. Shivasa. The main morphological characters that discriminate our strains from related species are the size-range of conidia and length of conidiophores. *Curvularia bothriochloae* produced conidia measuring 30–47 × 15–25 μm (Sivanesan et al. 2003) while *C. akaiiensis* produced the smallest conidia (22.5–27.5 × 7.5–15.5 μm). Conidial length of *C. nanningensis* was very close to *C. akaiii* (24–34 μm) (Tsuda and Ueyama 1985) but the conidia of our species were broader than those of *C. akaiii* (8.7–13.8 μm). Conidiophores of *C. nanningensis* were shorter than those of *C. bothriochloae* (360–425 μm) (Alcorn 1990). In the case of *C. sichuanensis* Meng Zhang & T.Y. Zhang, only one ITS sequence AB453881 was available in GenBank for analysis. While examining our sequences, only 4–5 bp differences were revealed in 499 bp characters between *C. nanningensis* and *C. sichuanensis*, thus indicating a close relationship between the two strains based on ITS sequence data and likely between the two species. However, according to Zhang et al. (2007), the conidial width of *C. sichuanensis* (10–15 μm) is smaller than *C. nanningensis* (14–20.5 μm) on PDA. For *C. sichuanensis*, the conidial wall of the median cell is deepened and thickened while *C. nanningensis* obviously does not have these characters. Meanwhile, the hilum of conidia in *C. sichuanensis* is obviously protuberant while *C. nanningensis* lacked this character.

The pathogenicity test based on natural inoculation and detached leaves (Figure 3) confirmed that *Curvularia nanningensis* is a pathogen of *Cymbopogon citratus* blast disease. We previously named our strains as *C. cymbopogonis* following a previous report of the species by Groves and Skolko (1945) as a seed-borne pathogen of *Cymbopogon*.
Curvularia nanningensis sp. nov

Figure 4. Pathogen inoculation and symptom (4 days). A Cymbopogon citratus inoculated and disease symptom B inoculation point and disease symptom C control D detached experiment. Left. Control. Right. Inoculation point and disease symptoms.

Curvularia cymbopogonis is a common pathogen which also causes diseases of sugar-cane, rice, seedlings of itchgrass, Agrostis palustris Huds. and Dactylis glomerata L. (Santamaria et al. 1971; Walker and White 1979; Olufolaji 1996; Yi et al. 2002). A single strain named C. cymbopogonis (CBS 419.78) included in our analyses grouped distant from C. nanningensis but its reliability seems questionable and apparently belongs to a different species (Fig. 1). We further checked the original description of this species (Groves and Skolko 1945) and found that differences in conidial shape mainly resulted from conidial width (C. cymbopogonis: 11–13 μm vs C. nanningensis: 14–20.5 μm). Additionally, Groves and Skolko (1945), Hall and Sivanesan (1972) and Yi et al. (2002) reported that C. cymbopogonis produced 4 to 5-septate conidia, whereas conidia of C. nanningensis only had 3-septa. Curvularia spp. are important pathogens of lemongrass. Morphological studies together with phylogenetic analyses provided evidence that C. nanningensis is a new pathogen distinct from all hitherto reported diseases on lemongrass. Our findings expanded the documented diversity of Cymbopogon
pathogens within the genus *Curvularia* and further clarified the taxonomy of this novel pathogen, *Curvularia nanningensis*.

Moreover, 29 first reports of *Curvularia* diseases on different plants in China, India and Pakistan were found in the literature from 2010 to the present. It is evident that in this vast geographical area, *Curvularia* spp. have maintained a close association with plant diversity and thereby possess a rich fungal diversity that is affected by crops distribution. Among them, six reports only provided morphological data and more than half (16) only referred to ITS sequence data and morphological description (Suppl. Table 1). For unknown reasons, Iftikhar et al. (2016) misidentified the *Curvularia* pathogen with an *Alternaria* sequence (LN879930.1). Our phylogenetic tree, based on 54 reported ITS sequence data of *Curvularia* diseases in these countries (Figure 2), also indicated that this approach is not effective for identifying these pathogens, especially in the case of *C. lunata* as a prevalent species. However, identification of *Curvularia* isolates by multi-gene phylogenetic analyses has withstood scrutiny (Liang et al. 2018; Wang et al. 2018; Zhang et al. 2018). Additionally, nearly all reports, even for severe diseases, are based on a single isolate, which preclude an objective evaluation. We, therefore, propose the following standardised steps as required for the reliable identification of *Curvularia* diseases: 1) collect several isolates from diseased samples, 2) obtain sequences of the ITS, GAPDH and *tef*1 or at least ITS+GAPDH for phylogenetic analysis, 3) perform BLAST searches with sequences originated from ex-type or representative strains in GenBank, and 4) combine morphological comparison and phylogenetic analysis for accurate identification.

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Supplementary material 1

Table S1. Disease occurrence caused by *Curvularia* spp. in China, India and Pakistan

Authors: Qian Zhang, Zai-Fu Yang, Wei Cheng, Nalin N. Wijayawardene, Kevin D. Hyde, Zhuo Chen, Yong Wang

Data type: occurrence

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