Curvularia microspora sp. nov. associated with leaf diseases of Hippeastrum striatum in China

Yin Liang¹, Shuang-Fei Ran¹, Jayarama Bhat⁴, Kevin D. Hyde⁵, Yong Wang¹,2, De-Gang Zhao²,3

¹ Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang, Guizhou 550025, China
² Key Laboratory of Plant Resources Conservation and Germplasm Innovation in Mountainous Region, Ministry of Education, Guizhou University, Guiyang, 550025, P. R. China
³ Guizhou Academy of Agricultural Sciences, Guiyang 550006, China
⁴ No. 128/1–J, Azad Housing Society, Curca, Goa Velha, India
⁵ Centre of Excellence in Fungal Research and School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand

Abstract
An undescribed Curvularia sp. was isolated from the leaf spot disease of Barbados Lily (Hippeastrum striatum (Lam.) Moore). Phylogenetic analyses of combined ITS, 28S, GPD1 and TEF1 sequence data place nine strains of this species in the trifolii-clade, but they clustered together as an independent lineage with strong support. This species was morphologically compared with related species in the trifolii-clade. Based on differences in morphology and phylogeny, it is concluded that this species is a new taxon, introduced as Curvularia microspora sp. nov. Pathogenicity testing determined the new species to be pathogenic on H. striatum.

Keywords
China, hyphomycetes, identify, pathogen, taxonomy

Introduction
The genus Curvularia includes pathogens and saprobes of various plants, as well as opportunistic pathogens of humans and animals (Sivanesan 1987, Manamgoda et al. 2011, 2012, da Cunha et al. 2013, Hyde et al. 2014) and has been well-studied in...
recent years. Identification of *Curvularia* spp. was previously mainly based on morphological descriptions and comparisons, however, the use of molecular taxonomy has solved many problems of resolving species (Valente et al. 1999, Mendoza et al. 2001). A multi-gene phylogenetic tree, based on the internal transcribed spacers including the 5.8S nuclear ribosomal DNA gene (ITS), the 5’ end of the nuclear ribosomal large subunit (28S), fragments of the glycerol-3-phosphate dehydrogenase (*GPD1*) and translational elongation factor EF-1 alpha (*TEF1*) gene regions, was provided to identify fresh collections of *Curvularia* from various hosts and geographic locations worldwide (Manamgoda et al. 2015).

In this study, DNA sequences of ITS, 28S, *GPD1* and *TEF1* gene regions were used for phylogenetic analyses to identify a new *Curvularia* species. This was concluded based on the combined morphology and phylogeny. *Curvularia microspora* sp. nov., is introduced here, associated with leaf diseases of *Hippeastrum striatum*.

**Materials and methods**

**Isolation and morphological studies**

All diseased samples were collected from the Medical Plants Herb Garden, in Chongqing City, Nanchuan County, China. This garden is located in a region of subtropical humid monsoon climate and has conserved more than 3000 kinds of medicinal plants. In this study, all fungal strains were isolated by the single-spore technique in order to obtain pure cultures following the method of Chomnunti et al. (2014). Single spores were transferred to potato-dextrose agar (PDA) and incubated at room temperature (28 °C). After several weeks of incubation, the morphological characters were recorded following the methods of Manamgoda et al. (2011, 2012). Conidia and conidiophores were observed using a compound microscope (Nikon Eclipse E600 DIC microscope and a Nikon DS-U2 camera or a Nikon 80i compound microscope fitted with a Canon 450D digital camera). The holotype specimen was deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). Ex-type cultures were also deposited in the culture collection at the Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (GUCC).

**DNA extraction and sequencing**

Fungal cultures were grown on PDA until nearly covering the whole Petri-dish (90 mm) at 28 °C. Fresh fungal mycelia were scraped with sterilised scalpels. A BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) was used to extract fungal genome DNA. DNA Amplification was performed in a 25 μL reaction volume which contained 2.5 μL 10 x PCR buffer, 1 μL of each primer (10 μM), 1 μL template DNA and 0.25 μL Taq DNA polymerase (Promega, Madison, WI, USA). Primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region. The thermal cycling
programme was: 3 min initial denaturation at 95 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 30 s primers annealing at 52 °C, 1 min extension at 72 °C and a total 10 min extension at 72 °C. To amplify the GPD1 gene, the primers gpd1 and gpd2 were used (Berbee et al. 1999). The amplification programme included an initial denaturation step at 96 °C for 2 min, followed by 35 PCR cycles with 1 min at 96 °C, 1 min at 52 °C and 45 s at 72 °C with a final 10 min extension at 72 °C. The TEF1 and 28S regions were amplified using EF-526F/1567R and LR5/LROR primers respectively (Schoch et al. 2009). The 28S amplification programme included an initial denaturation step at 95 °C for 3 min followed by 30 cycles of 40 s denaturation at 94 °C, 50 s primer annealing at 52 °C, 1 min extension at 72 °C. The same PCR reaction was used to amplify TEF1 with the only change being the annealing temperature at 54 °C.

**Phylogenetic analysis**

DNA sequences from these isolates and reference sequences were downloaded from GenBank and analysed by maximum parsimony (MP) and maximum likelihood (ML) (Table 1). Sequences were optimised manually to allow maximum alignment and maximum sequence similarity, as detailed in Manamgoda et al. (2012). The alignment document of four phylogenetic markers has been submitted to TreeBase (https://treebase.org/; Accession number: 21970). A partition homogeneity test (PHT) was performed with 1000 replicates via PAUP v. 4.0b10 (Swofford 2003) to evaluate statistical congruence amongst sequence data of 28S, ITS, GPD1 and TEF1 gene regions. MP analyses were performed in PAUP v. 4.0b10 (Swofford 2003), using the heuristic search option with 1,000 random taxa addition and tree bisection and reconnection (TBR) as the branch swapping algorithm. Maxtrees were set to 10,000. The characters in the alignment document were ordered accordingly: 28S+ITS+GPD1+TEF1, with equal weight and gaps were treated as missing data. The Tree Length (TL), Consistency Indices (CI), Retention Indices (RI), Rescaled Consistency Indices (RC) and Homoplasy Index (HI) were calculated for each tree generated. Maximum likelihood (ML) trees of DNA sequences were obtained by a heuristic search using the TrN + I + G model, which was deduced as the best fit for the data by the likelihood ratio test using the MODELTEST wer3.7 and MrMTgui version 1.01 (Posada and Crandall 1998).

**Pathogenicity test**

Pathogenicity of this species was determined by inoculating healthy leaves of Hippeastrum striatum and Canna indica L. with 5 mm diameter mycelial plugs, cut from the margins of 10-day-old actively growing cultures; the control was treated with sterile agar plugs. Both inoculated and control plants were kept in a moist chamber at 25 °C for 7 days and observed for disease symptom development. Infected leaves were collected and the fungus was re-isolated in PDA medium and compared against the original strains. Control plants were sprayed with sterilised distilled water.
| Species                           | Isolate   | GenBank accession numbers and references |
|----------------------------------|-----------|------------------------------------------|
| Alternaria alternata             | EGS 34.0160 | AF071346 Berbee et al. 1999              |
|                                  |           | –                                         |
|                                   |           | –                                         |
| Curvularia akaii                  | CBS 318.91 | HF934921 Amaradasa et al. 2014           |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. borreriae                      | CBS 859.73 | HE861848 da Cunha et al. 2013            |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. borreriae                      | MFLUCC 11-0442 | KP400638 Manamgoda et al. 2015       |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. gladioli                       | ICMP 6160 | JX256426 Manamgoda et al. 2012           |
|                                  |           | JX256393 Manamgoda et al. 2012           |
|                                  |           | JX276438 Manamgoda et al. 2012           |
| C. gudauskai                      | DAOM 165085 | AF071338 Berbee et al. 1999              |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. heteropogonis                  | CBS 156.35 | JN192379 Manamgoda et al. 2011           |
|                                  |           | JN600992 Manamgoda et al. 2011           |
|                                  |           | JN600978 Manamgoda et al. 2011           |
| C. microspora sp. nov.            | ICMP 1034 | KP400656 Manamgoda et al. 2015           |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. microspora sp. nov.            | ICMP 13910 | KP400655 Manamgoda et al. 2015           |
|                                  |           | –                                         |
|                                   |           | –                                         |
| C. microspora sp. nov.            | ICMP 6149 | JX256434 Manamgoda et al. 2012           |
|                                  |           | JX256402 Manamgoda et al. 2012           |
|                                  |           | JX276457 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | BRIP 15882 | JN192384 Manamgoda et al. 2011           |
|                                  |           | JN600996 Manamgoda et al. 2011           |
|                                  |           | JN600979 Manamgoda et al. 2011           |
| C. microspora sp. nov.            | BRIP 13165 | JX256435 Manamgoda et al. 2012           |
|                                  |           | JX266600 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256436 Manamgoda et al. 2012           |
|                                  |           | JX266595 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256437 Manamgoda et al. 2012           |
|                                  |           | JX266596 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256438 Manamgoda et al. 2012           |
|                                  |           | JX266597 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256439 Manamgoda et al. 2012           |
|                                  |           | JX266598 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256440 Manamgoda et al. 2012           |
|                                  |           | JX266599 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256441 Manamgoda et al. 2012           |
|                                  |           | JX266600 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256442 Manamgoda et al. 2012           |
|                                  |           | JX266601 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256443 Manamgoda et al. 2012           |
|                                  |           | JX266602 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256444 Manamgoda et al. 2012           |
|                                  |           | JX266603 Manamgoda et al. 2012           |
| C. microspora sp. nov.            | ICMP 13910 | JX256445 Manamgoda et al. 2012           |
|                                  |           | JX266604 Manamgoda et al. 2012           |
Curvularia microspora sp. nov. associated with leaf diseases of Hippeastrum striatum...

Results

Phylogenetic analyses

Nine isolates of Curvularia were sequenced from two plants in Chongqing Municipality, China (seven from Hippeastrum striatum and two from Canna indica). PCR products of approximately 900 bp (28S), 540 bp (ITS), 530 bp (GPD1) and 1200 bp (TEF1) were obtained. In the molecular phylogenetic analyses, the partition homogeneity test ($P = 0.06$) indicated that the individual partitions were not highly incongruent (Cunningham 1997) and thus 28S, ITS, GPD1 and TEF1 sequences were combined for sequence analyses. By alignment with a single gene region and then combination according to the order of 28S, ITS, GPD1 and TEF1, only 2689 characters were obtained, viz. 28S: 1–848, ITS: 849–1330, GPD1: 1331–1771 TEF1: 1772–2689 with 104 parsimony-informative characters and 157 parsimony-uninformative characters. The analysis produced three equally parsimonious trees, one of which (TL = 366, CI = 0.81, RI = 0.82, RC = 0.66 and HI = 0.19) is shown in Figure 1 and the topologies of MP and ML analysis were congruent, thus only MP topology was shown. Phylogenetic analysis confirmed nine strains (GUCC 6272, GUCC 6273, GUCC 6274, GUCC 6275, GUCC 6276, GUCC 6277, GUCC 6278, GUCC 6279 and GUCC 6280) with the same DNA sequences in four phylogenetic markers grouped into an independent clade supported by high bootstrap values (MP: 100%; ML: 99%). These strains were placed in trifolii-clade with strong bootstrap support (MP: 95%; ML: 95%) and had a close relationship with Curvularia gaudauskasii, C. gladioli, C. trifolii, C. borreriae and C. pallescens with a high MP support (MP: 87%), but its ML bootstrap value was lower than 50%.

Taxonomy

Curvularia microspora Y. Liang, K.D. Hyde, J. Bhat & Yong Wang, sp. nov.
MycoBank MB 822544
Figure 2

Diagnosis. Characterised by producing four celled, smaller conidia (4.5–11.5 × 2–6 μm), usually curved at the third cell from the base.

Type. China, Chongqing City, Nanchuan, from leaf spots of Hippeastrum striatum, 28 September 2016, Y. Liang, HGUP 6272, holotype, ex-type living culture GUCC 6272.

Description. Symptoms on Hippeastrum striatum: Fructification mostly epiphyllous, disease spot 3–12 mm, subspherical to oblong ovate, brown to dark brown, effuse (Figure 2a, b). Symptoms on Canna indica: Fructification of the fungus was mostly epiphyllous, the large blighted, irregular spots near leaf apex to the whole leaves, greyish-brown (Figure 2c).
Figure 1. The only one parsimonious tree obtained from combined analyses set of ITS, LSU, β-tubulin and tef1 sequence data. MP values (>50 %) resulting from 1000 bootstrap replicates. The tree is rooted with *Alternaria alternata* (EGS 34-0160). The branch of our new *Curvularia* is shown in blue.
Curvularia microspora sp. nov. associated with leaf diseases of Hippeastrum striatum...

Figure 2. Curvularia microspora (HGUP 6272). a–c Leaf diseases symptoms on Hippeastrum rutilum and Canna indica. d–f Conidiophores, conidiogenous loci and conidia g–j Immature and mature conidia k–l Upper (k) and lower (l) surface of colony. Scar bars: d, i (10 μm), e–f = 20μm, g–h, j = (5 μm).

Colonies on PDA, vegetative hyphae septate, branched, subhyaline to brown, smooth to asperulate, 1.5–3 μm, anastomosing. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Conidiophores 10.5–77.5 × 1–3.5 μm (av. = 22.2 × 2.1 μm, n = 30), arising singly, simple or branched, flexuous, septate, geniculate at spore bearing part, pale brown, dark brown, paler towards apex. Percurrent proliferation only observed occasionally. Conidiogenous loci somewhat thickened and darkened, spores up to 0.8–1 μm diam, smooth. Mature conidia always four celled, 4.5–11.5 × 2–6 μm (av. = 8.2 × 3.8 μm, n = 50), smooth-walled, usually curved at the third cell from the base, sometimes straight, navicular, bifurcate, obpyriform, tapering towards rounded ends, pale brown to dark reddish brown. Hilum usually conspicuous or sometimes slightly protuberant.
Habitat and distribution. Isolated from leaf diseases of *H. striatum* and *Canna indica* in China

Etymology. *microspora*, referring to this species producing obviously smaller conidia.

Other material examined. China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6273), living culture GUCC 6273; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6274), living culture GUCC 6274; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6275), living culture GUCC 6275; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6276), living culture GUCC 6276; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6277), living culture GUCC 6277; China, Chongqing City, Nanchuan, from leaf diseases of *H. striatum*, 28 September 2016, Y. Liang (HGUP 6278), living culture GUCC 6278; China, Chongqing City, Nanchuan, from leaf diseases of *Canna indica*, 28 September 2016, Y. Liang (HGUP 6279), living culture GUCC 6279; China, Chongqing City, Nanchuan, from leaf diseases of *C. indica*, 28 September 2016, Y. Liang (HGUP 6280), living culture GUCC 6280.

Pathogenicity test

Test plants (*Hippeastrum striatum*) were inoculated with 5 mm diam mycelial plugs of *Curvularia microspora* with two replicates of each plants and the inoculation experiment was repeated two times (with different sporulation generations). *Hippeastrum striatum* leaves both exhibited brown to dark brown necrotic spots (Figure 3a, b) after 7 days, which were very similar to those of natural infection (Figure 2a, b). The DNA sequencing result (ITS region), after re-isolation, identified this as *C. microspora*. The successful re-isolation of *C. microspora* from the inoculated leaves of *H. striatum* established a credible proof of pathogenicity. All test plants were covered with polyethylene bags for 7 days. However, on *Canna indica*, disease symptoms did not appear again.

Discussion

The nine strains of *Curvularia* had typical characters of the genus., viz. the production of sympodial conidiophores with tretic, terminal and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar (Boedijn 1933). Phylogenetic analyses compared the DNA sequence from four phylogenetic markers with related species in the trifolii-clade: *Curvularia akali*, *C. borrierae*, *C. gladioli*, *C. gaudainskisii*, *C. heteropogonis*, *C. pallescens* and *C. trifolii* (Figure 1, Manamgoda et al. 2012, 2015, Madrid et al. 2014, Jeong et al. 2015, Su et al. 2015). These taxa are morphologically similar in producing a strongly protruding hilum (Madrid et al. 2014). However, the present taxon had bifurcate conidia, which differentiates it from
Curvularia microspora sp. nov. associated with leaf diseases of Hippeastrum striatum...

Table 2. Morphological comparison and pathogenicity of Curvularia microspora and related species in trifolii-clade.

| Species name          | Taxonomic references                  | Conidia shape                                                                 | Conidia size range      | Conidio- phores size range | Pathogenicity | Pathogenic reports                                      |
|-----------------------|---------------------------------------|-------------------------------------------------------------------------------|-------------------------|----------------------------|---------------|---------------------------------------------------------|
| Curvularia microspora | This study                            | curved at the third cell from the base, sometimes straight, navicular, bifurcate, obpyriform, tapering towards rounded ends | 4.5–11.5 × 2.0–6.0 μm   | 10.5–77.5 × 1.0–3.5 μm     | Yes           | This study                                              |
| Curvularia akaii      | Tsuda and Ueyama (1985)               |                                                                               | 24–34 × 8.7–13.8 μm     | Yes                        |               | Zhang (2004)                                            |
| Curvularia borreiae   | Ellis (1971)                          |                                                                               | 20–32 × 8–15 μm         | No                         |               |                                                        |
| Curvularia gladioli   | Boerema and Hamers (1989)             |                                                                               | 17.5–37.5 × 6.5–17.5 μm | Yes                        |               | Horita (1995); Torres et al. (2013, 2015)               |
| Curvularia gudauskasii| Morgan-Jones and Karr Jr (1976)       |                                                                               | 27–29 × 15–19 μm        | 62–98 × 5–6 μm              | Yes           | Chinea (2005); Ratón et al. (2012)                      |
| Curvularia heteropogonii | Alcorn (1990)                     |                                                                               | 27–44 × 11–19 μm        | 115–620 × 4–6 μm            | Yes           | Alcorn (1990)                                           |
| Curvularia pallescens | Ellis (1971)                          |                                                                               | 17–32 × 7–12 μm         | Yes                        |               | Berg et al. (1995); Dadwal and Verma (2009); Mabadeje (1969); Rajalakshmy (1976); |
| Curvularia trifolii   | Groves and Skolko (1945)              |                                                                               | 20–34 × 8–14 μm         | Yes                        |               | Falloon (1976); Khadka (2016); Sarwar and Srinath (1965); Sung et al. (2016); Zamorski (1983); |
all other species in the trifolii-clade. Curvularia microspora also has smaller conidia than the related species. A synopsis of the characters in the trifolii-clade is given in Table 2. The phylogenetic analyses (MP and ML) also confirmed these isolates belong to a new taxon with strong bootstrap support (Figure 1).

Curvularia species can cause severe or opportunistic diseases of different plant taxa and are often a threat to agricultural production by reducing yield and quality. In the trifolii-clade, all species except for C. borreriae, have been reported as causing plant disease. This is especially true of C. trifolii and C. pallescens, which cause serious diseases of Agrostis stolonifera and Gloriosa superba respectively (Table 2). Koch’s postulates were performed to show that C. microspora causes leaf spot disease of Hippeastrum striatum (Figure 3), but on Canna indica might only be saprobic or endophytic. Hippeastrum striatum as an economic ornamental plant is grown in some areas of China, thus there is a need to continue investigation on the biology of this species in order to determine whether it can cause serious disease outbreaks.

Acknowledgments

The research is supported by the project of National Natural Science Foundation of China (No. 31560489), National Key Technology Research and Development Programme of the Ministry of Science and Technology of China (2014BAD23B03/03), Genetically Modified Organisms Breeding Major Projects of China [2016ZX08010-003-009], Agriculture Animal and Plant Breeding Projects of Guizhou Province [QNYZZ2013-009], Fundamental Research on Science and Technology, Ministry of Science and Technology of China (2014FY120100), postgraduate education innovation programme of Guizhou Province (ZYRC[2014]004) and Bijie science and technology project No. (2015)39.

References

Alcorn JL (1990) Additions to Bipolaris, Cochliobolus and Curvularia. Mycotaxon 39: 361–392.
Amaradasa BS, Madrid H, Groenewald JZ, Crous PW, Amundsen K (2014) Porocercospora seminalis gen. et comb. nov., the causal organism of buffalograss false smut. Mycologia 106(1): 77–85. https://doi.org/10.3852/13-147
Berbee ML, Pirseyedi M, Hubbard S (1999) Cochliobolus phylogenetics and the origin of known, highly virulent pathogens inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91(6): 964–977. https://doi.org/10.2307/3761627
Berg D, Garcia JA, Schell WA et al. (1995) Cutaneous infection caused by Curvularia pallescens: A case report and review of the spectrum of disease. Journal of the American Academy of Dermatology 32(2): 375–378. https://doi.org/10.1016/0190-9622(95)90408-5
Boedijn KB (1933) Über einige phragmosporen Dematiazen. Bulletin du Jardin botanique de Buitenzorg 13: 120–134.
Boerema GH, Hamers MEC (1990) Check-list for scientific names of common parasitic fungi. Series 3c: fungi on bulbs: ‘additional crops’ belonging to the Araceae, Begoniaceae, Compositae, Oxalidaceae and Ranunculaceae. Netherlands Journal of Plant Pathology 96(1): 1–23. https://doi.org/10.1007/BF01976398

Chinea A (2005) Las enfermedades de la caña de azúcar en cuba durante las últimas 5 décadas. http://www.inica.minaz.cu/trabajos/40ANIVERSARIO/bio/b06.htm

Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Persoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD (2014) The sooty moulds. Fungal Diversity 66(1): 1–36. https://doi.org/10.1007/s13225-014-0278-5

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14(7): 733–740. https://doi.org/10.1093/oxfordjournals.molbev.a025813

da Cunha KC, Sutton DA, Fothergill AW et al. (2013) In vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus Curvularia. Diagnostic Microbiology and Infectious Disease 76: 168–174. https://doi.org/10.1016/j.diagmicrobio.2013.02.034

Dadwal VS, Verma RK (2009) A new blotch disease (Curvularia pallescens Boedijn) of Gloriosa superba Linn. Journal of Mycology and Plant Pathology 39: 156–157.

Ellis MB (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, 608 pp.

Falloon RF (1976) Curvularia trifoliï as a high-temperature turfgrass pathogen. New Zealand Journal of Agricultural Research 19(2): 243–248. https://doi.org/10.1080/00288233.1976.10426773

Groves JW, Skolko AJ (1945) Notes on seed-borne fungi-III Curvularia. Canadian Journal of Research C 23: 94–104. https://doi.org/10.1139/cjr45c-007

Horita H (1995) Brown spot of Gladiolus caused by Curvularia gladioli in Hokkaido. Annals of the Phytopathological Society of Japan 61(6): 646.

Hyde KD, Nilsson RH, Alias SA et al. (2014) One stop shop: backbones trees for important phytopathogenic genera: I. Fungal Diversity 67(1): 21–125. https://doi.org/10.1007/s13225-014-0298-1

Joeng JS, Thuong NTT, Burm LH (2015) Phylogenetic status of an unrecorded species of Curvularia, C. spicifera, based on current classification system of Curvularia and Bipolaris group using multi loci. Microbiology 43(3): 210–217. https://doi.org/10.5941/MYCO.2015.43.3.210

Khadka RB (2013) First report of Curvularia trifolii causing leaf spot on berseem in Nepal. Plant Diseases. https://doi.org/10.130905135126000

Mabadeje SA (1969) Curvularia leaf spot of maize. Transactions of the British Mycological Society 52(2): 267–271. https://doi.org/10.1016/S0007-1536(69)80039-2

Madrid H, da Cunha KC, Gené J, Dijksterhuis J, Cano J, Sutton DA, Guarro J, Crous PW (2014) Novel Curvularia species from clinical specimens. Persoonia Molecular Phylogeny & Evolution of Fungi 33(1): 48–60. https://doi.org/10.3767/003158514X683538

Manamgoda DS, Cai L, Bahkali AH et al. (2011) Cochliobolus: an overview and current status of species. Fungal Diversity 51(1): 3–42. https://doi.org/10.1007/s13225-011-0139-4
Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivash RG, Tan YP, Hyde KD (2012) A phylogenetic and taxonomic re-evaluation of the Bipolaris–Cochliobolus–Curvularia complex. Fungal Diversity 56(1): 131–144. https://doi.org/10.1007/s13225-012-0189-2

Manamgoda DS, Rossman AY, Castlebury LA, Chukeatirote E, Hyde KD (2015) A taxonomic and phylogenetic re-appraisal of the genus Curvularia (Pleosporaceae): human and plant pathogens. Phytotaxa 212(3): 175–198. https://doi.org/10.11646/phytotaxa.212.3.1

Mendoza L, Ajello L, Taylor JW (2001) The taxonomic status of Lacazia loboi and Rhinosporidium seeberi has been finally resolved with the use of molecular tools. Revista Iberoamericana Micologia 18: 95–98.

Morgan-Jones G, Karr GW Jr (1976) Notes on hyphomycetes part 9 Curvularia gudauskasii new species. Mycotaxon 3: 559–563.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rajalakshmy VK (1976) Leaf spot disease of rubber caused by Curvularia pallescens Boedijn. Current Sciences 45(14): 530.

Ratón TO, Giro ZG, Díaz MS, Pérez SR (2012) In vitro growth inhibition of Curvularia gudauskasii by Bacillus subtilis. Annals of Microbiology 62(2): 545–551. https://doi.org/10.1007/s13213-011-0290-x

Sarwar M, Srinath KV (1965) Occurrence of Curvularia trifolii (Kauff.) Boed. on Khas-Vetiveria zizanoides Stapf. Current Sciences 34(23): 668.

Schoch CL, Sung GH, López-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciufetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossman AY, Lumbsch HT, Lücking R, Büdel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Kohlmeyer J, Volkman-Kohlmeyer B, Mostert L, O’Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Unterreiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Systematic Biology 58(2): 224–239. https://doi.org/10.1093/sysbio/syp020

Sivanesan A (1987) Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs. Mycological Papers 158: 1–261.

Su HY, Udayanga D, Luo ZL et al. (2015) Hyphomycetes from aquatic habitats in Southern China: Species of Curvularia (Pleosporaceae) and Phragmocephala (Melannomataceae). Phytotaxa 226(3): 201–216. https://doi.org/10.11646/phytotaxa.226.3.1

Sung CH, Koo JH, Kim JH et al. (2016) First report of Curvularia leaf blight caused by Curvularia trifolii on Creeping bentgrass in Korea. Weed & Turfgrass Sciences 5(2): 101–104. https://doi.org/10.5660/WTS.2016.5.2.101

Swofford D (2003) PAUP* – Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.
Curvularia microspora sp. nov. associated with leaf diseases of Hippeastrum striatum...

Torres DP, Silva MA, Pinho DB, Pereira OL, Furtado GQ (2013) First report of *Curvularia gladioli* causing a leaf spot on *Gladiolus grandiflorus* in Brazil. Plant Diseases 97(6): 847. https://doi.org/10.1094/PDIS-12-12-1118-PDN

Torres DP, Silva MA, Furtado GQ (2015) Infection process of *Curvularia gladioli*, on gladiolus leaf. Tropical Plant Pathology 40(6): 382–387. https://doi.org/10.1007/s40858-015-0045-5

Tsuda M, Ueyama A (1985) Two new *Pseudocochliobolus* and a new species of *Curvularia*. Transactions of the Mycological Society of Japan 26(3): 321–330.

Valente P, Ramos J, Leoncini O (1999) Sequencing as a tool in yeast molecular taxonomy. Canadian Journal of Microbiology 45(11): 949–958. https://doi.org/10.1139/w99-094

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: Gelfand M, Sninsky JI, White TJ (Eds) PCR protocols: a guide to methods and applications, Academic Press, USA, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zamorski C, Bielska E (1983) *Curvularia trifolii* a new for Poland pathogen of gladiolus. Acta Agrobotanica. 36(1–2): 135–144. https://doi.org/10.5586/aa.1983.011

Zhang M (2004) Taxonomic studies on *Helminthosporium* and *Curvularia* from China and a survey of mitosporic fungal pathogens of main weeds in north (PhD thesis). Shandong Agricultural University, 237 pp.