Morphological and molecular characterization of two graminicolous *Exserohilum* species associated with cultivated rice and early barnyard grass from Sri Lanka

H.S. Ferdinandez, D.S. Manamgoda, D. Udayanga, N. Deshappriya and M.L.A.M.S. Munasinghe

**Highlights**

- *Exserohilum rostratum* and *E. oryzicola* were characterized from cultivated rice and early barnyard grass in Sri Lanka
- Evolutionary relationships were inferred based on multi-locus phylogeny
- Both records are novel plant-fungal associations from Sri Lanka.
Morphological and molecular characterization of two graminicolous *Exserohilum* species associated with cultivated rice and early barnyard grass from Sri Lanka

H.S. Fernandez¹, D.S. Manamgoda²*, D. Udayanga³, N. Deshapriya¹ and M.L.A.M.S. Munasinghe¹

¹Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, 10250, Sri Lanka.  
²Department of Biosystems Technology, Faculty of Technology, University of Sri Jayewardenepura, Pitipana, Homagama, 10200, Sri Lanka.

Received: 28/02/2020; Accepted: 06/10/2020

Abstract: The genus *Exserohilum* (Order Pleosporales, Class Dothideomycetes) comprises plant pathogenic hyphomycetous fungi, associated with poaceous hosts. Although numerous pathogenic species of *Exserohilum* are known globally, only *E. turcicum* and *E. rostratum* have been reported from Sri Lanka. In the present study, samples showing the symptoms of leaf blight of *Oryza sativa* (cultivated rice) and sheath blight of *Echinochloa oryzoides* (early barnyard grass) were collected and causal agents were primarily identified as *Exserohilum* spp. based on morphological characters. Molecular phylogenetic analyses based on three loci namely, nuclear ribosomal internal transcribed spacer (ITS), partial glyceraldehyde 3-phosphate dehydrogenase (GPDH) and translational elongation factor (TEF-1α) were used to infer evolutionary relationships and accurate identification. These isolates from *O. sativa* and *Echinochloa oryzoides* were identified as *Exserohilum rostratum* and *E. oryzicola* respectively. Both records are novel plant-fungal associations from Sri Lanka based on available data. This study suggests the need for morphological and molecular reassessments of emerging and poorly known species of fungi associated with cereals, their wild relatives and other economically important hosts in Sri Lanka.

Keywords: Cereal pathogens; emerging species; hyphomycetes; molecular phylogeny.

INTRODUCTION

The hyphomycetous fungi associated with grass hosts, previously known as “graminicolous *Helminthosporium* species”, include six genera belonging to order Pleosporales namely *Curvularia*, *Bipolaris*, *Exserohilum*, *Drechslera*, *Johnalcornia* and *Porocercospora* (Manamgoda et al., 2012; Amaradasa et al., 2014; Tan et al., 2014; Hernandez-Restrepo et al., 2018). The genus *Exserohilum* which contains a number of plant, human pathogenic and saprobic fungi, has been introduced with the type species, *E. turcicum* (syn. *Helminthosporium turcicum*) (Leonard and Suggs 1974; Passerini, 1876). The sexual morph of *Exserohilum* was previously characterized under *Setosphaeria* (Leonard and Suggs, 1974). Species of this genus are frequently encountered as asexual morphs in nature, although the sexual morphs were often obtained by mating compatible strains (Hernandez-Restrepo et al., 2018).

Recent molecular phylogenetic assessments have resulted in considerable taxonomic refinements of numerous species in the genus *Exserohilum*. For instance, previously known two species, *E. heteropogonica* and *E. inaequale* are now placed in the genus *Curvularia* as *C. heteropogonica* and *C. crassiseptum*, respectively (Alcorn, 1991; Zhang et al., 2004; Hernandez-Restrepo et al., 2018). Based on molecular phylogenetic analysis, six formerly known taxa namely *E. antillanum*, *E. gedarefense*, *E. leptochloae*, *E. longirostratum*, *E. macginnisii* and *E. prolatum* were found to be conspecific with commonly encountered taxon *E. rostratum* (Hernandez-Restrepo et al., 2018). In the same study, *E. curvatum* was synonymized with *E. holmii*, and *E. fusiforme* with *E. oryzicola* (Hernandez-Restrepo et al., 2018).

*Exserohilum* species are encountered as pathogenic fungi of humans and plants and also frequently found as saprobic, endophytic and soil-borne fungi. Human pathogenic *Exserohilum* spp. are generally opportunistic fungi which may also cause life-threatening infections in immune-compromised humans. The most commonly reported human pathogenic species is *E. rostratum*, whereas some cases are attributed to *E. longirostratum* and *E. macginnisii* (McGinnis et al., 1986; De Hoog et al., 2000; Al-Attar et al., 2006). These pathogens have been reported on immune-compromised patients causing skin and corneal infection, invasive diseases, and allergic fungal sinusitis (Adler et al., 2006).

The plant family Poaceae comprises of important cereal crops such as rice, wheat, millet and corn which provide major dietary needs of the human population. Pleosporalean fungal pathogens, bearing brown asexual spores, are often associated with cereal crops, their wild relatives and weeds in the family Poaceae in different life styles including, epiphytes, endophytes, saprophytes or pathogens (Hernandez-Restrepo et al., 2018). Understanding the host associations and host ranges of fungi is important due to the possibilities of host shift of these species from weed hosts to important crops, as observed in many species. For example, *E. fusiforme* (syn. *E. oryzicola*) has originally been identified as pathogenic on the weed, *Echinochloa*...
crus-galli, causing numerous small leaf lesions and later known to cause small linear spots on, cultivated rice plants (Alcorn, 1991).

Majority of Exserohilum species are associated with grasses and important crops in the family Poaceae causing leaf blights of corn and millet, leaf spots and foot rots of wheat and damping-off of sugarcane seedlings (Sivanesan, 1987). The type species of the genus, Exserohilum turcicum, is the causative agent of northern leaf blight of corn which is a widespread foliar disease characterized by oblong, straw-colored to greyish necrotic lesions and causing significant death of foliar tissue. The reduction of effective photosynthetic area of leaves may lead to severe cases of grain yield losses of 20–25 % (Smith et al., 1988).

Although Exserohilum species are widely known emerging fungi on cereal hosts and weeds with worldwide distribution, only two species, E. turcicum and E. rostratum, have been recorded so far from Sri Lanka (Farr and Rossman, 2020). Information on the diversity and DNA sequence data of these common cereal pathogenic fungi is important to establish control measures for emerging fungal diseases (Udayanga, 2019). Therefore, the major aim of this study was to use molecular and morphological data to characterize freshly collected isolates of Exserohilum species associated with rice and associated grass species collected from two selected locations in Sri Lanka.

MATERIALS AND METHODS

Sample collection, isolation and morphological studies

Samples were collected from field surveys carried out in Kegalle and Gampaha districts and all the specimen information (date of collection, collector, locality, host and symptomatology) were recorded and the samples were brought to the laboratory for further processing.

Fresh specimens were observed under stereomicroscope (Optika, LAB 30) and, instances where fungal structures were not visible, they were incubated for another 24 h in a moist chamber. Single spore isolation was done from the sporulating samples to isolate fungi (Chommunti et al., 2011). Pure cultures were prepared on Potato Dextrose Agar (PDA) and stock cultures were maintained on Corn Meal Agar (CMA) slants. To determine colony morphology, cultures were triplicated on several media; PDA, CMA and Malt Extract Agar (MEA), and incubated at 25 °C for 12 h each in light and dark conditions. The color notations were recorded according to the standard color charts (Rayner, 1970). Micro-morphological characters were observed under compound light microscope (Optika, B 290) and measurements of structures were obtained under imaging facility. At least 30 length and width measurements were made from conidia of each isolate. Digital microscopic images were generated to illustrate the morphological characteristics. For all morphological measurements, statistical data (mean, minimum, maximum and standard deviation) were calculated and used in taxonomic descriptions. The specimens collected were dried and preserved as reference herbarium material at the herbarium, University of Sri Jayewardenepura (USJ) and the cultures are maintained at the fungal collection (USJCC) at the Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka.

DNA extraction, PCR amplification and sequencing

Genomic DNA were extracted from the morphologically identified Exserohilum fungal isolates following the modified Sodium Dodecyl Sulphate (SDS) method as described in Arnold and Lutzoni (2007). The PCR amplifications were carried out in the BIORAD T 100 Thermal cycler according to the protocols described in Manamgoda et al. (2012) with the primer pairs for ITS region with ITS1 and ITS4 (White et al., 1990), GPDH with gpd1 and gpd2 (Berbee et al., 1999) and TEF1-a with EF1-983F and EF1-2218R (Rehner and Buckley, 2005). The PCR products were visualized on 2 % agarose gel electrophoresis. PCR product purification and Sanger sequencing of the successfully amplified samples were carried out in Macrogen Inc, Korea.

Sequence alignment, phylogenetic analyses and species recognition

Raw sequences were assembled on BioEdit v7.0.5 programme for windows. Initial alignments of assembled DNA sequences were accomplished using BioEdit v7.0.5, optimized with MAFFT v. 7 using default settings (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013). Preliminary identification of the isolates was carried out using newly generated ITS, GPDH and TEF1-a sequences with all available ex-type sequences from the GenBank as listed in Table 1.

Phylogenetic analyses were performed in two different criteria; Maximum Parsimony (MP) and Maximum Likelihood (ML) in order to infer evolutionary relationships among closely related species. Sequence data generated in this study were deposited in GenBank (Table 1).

Maximum Parsimony was performed with PAUP v. 4.0b10 (Swofford, 2003). Trees were inferred using the heuristic search option with 1000 random sequence additions. Descriptive tree statistics for parsimony [Tree length (TL), Consistency Index (CI), Retention Index (RI), Rescaled Consistency Index (RC) and Homoplasy Index (HI)] were calculated for trees generated in the parsimony analysis. Maximum likelihood trees were constructed using the RAxML v.7.4.2 Black Box (Stamatakis et al., 2008) in the CIPRES Science Gateway platform (Miller et al., 2010). For the combined dataset all free model parameters were obtained using RAxML with ML estimate of 25 per site rate categories. Phylogenetic trees generated were visualized by FigTree v. 1.4 (Rambaut and Drummond, 2008).

RESULTS AND DISCUSSION

Molecular Phylogeny

In the present study, two different Exserohilum species from rice and early barnyard grass were accurately identified. The updated backbone phylogenetic tree for the genus Exserohilum presented in Figure 1 includes
| Species          | Strain no. | Host/Substratum | Country | GenBank accessions | Reference(s) |
|------------------|------------|-----------------|---------|-------------------|--------------|
| E. antillanum    | CBS 412.93<sup>ET</sup> | Soil            | Cuba    | MH862427, LT715894, LT883556 | Vu et al., 2019 |
| E. corniculatum  | BRIP 11426<sup>ET</sup>  | Oryza sativa    | Australia | LT837453, LT883533, LT883558 | Hernandez-Restrepo et al., 2018 |
| E. curvatum      | CBS 505.90<sup>ET</sup>  | Sorghum vulgare | Venezuela | KT265252, LT715889, LT883560 | Hernandez-Restrepo et al., 2018 |
| E. fusiforme     | BRIP 16229<sup>ET</sup>  | Echinochloa crus-galli | Australia | KJ415560, KJ415386, KJ415433 | Tan et al., 2014 |
| E. gedarefense   | CBS 297.80<sup>ET</sup>  | Sorghum bicolor | Sudan    | LT631323, LT715895, LT883563 | Hernandez-Restrepo et al., 2018 |
| E. holmii        | CBS 318.64<sup>ET</sup>  | Dactyloctenium aegyptium | Unknown | LT837457, LT883537, LT883565 | Hernandez-Restrepo et al., 2018 |
| E. khartoumensis | CBS 132708<sup>ET</sup>  | Sorghum bicolor var. mayo | Sudan | LT837461, LT715888, LT883569 | Hernandez-Restrepo et al., 2018 |
| E. longirostratum| CBS 128055 | Acacia mellifera subsp. detinens | Namibia | LT837478, LT883549, LT896609 | Hernandez-Restrepo et al., 2018 |
| E. macginnisii   | CBS 325.87<sup>ET</sup>  | Homo sapiens    | USA      | KT265237, LT715898, HE664082 | Hernandez-Restrepo et al., 2018 |
| E. minor         | BRIP 14616<sup>ET</sup>  | Dactyloctenium aegyptium | Australia | LT837470, LT883545, LT883580 | Hernandez-Restrepo et al., 2018 |
| E. monoceras     | BRIP 11542<sup>ET</sup>  | Setaria italica | Australia | LT837473, LT883546, LT896604 | Hernandez-Restrepo et al., 2018 |
| E. neoregeliae   | CBS 132832<sup>ET</sup>  | Neoregelia carolinae | Japan | LT837476, LT715886, LT896607 | Hernandez-Restrepo et al., 2018 |
| E. oryzicola     | CBS 502.90<sup>ET</sup>  | Oryza sativa    | Colombia | HF934949, LT715878, LT896629 | Hernandez-Restrepo et al., 2018 |
|                  | USJCC-0010  | Echinochloa oryzoides | Sri Lanka | MN860001, MN962922, MN962924 | This study |
| E. paspali       | CBS 128057 | Paspalum conjugatum | Brazil | LT837854, LT715857 - | Hernandez-Restrepo et al., 2018 |
| E. pedicellatum  | CBS 322.64<sup>ET</sup>  | Triticum aestivum | USA      | KT265258, LT715902, LT896630 | Hernandez-Restrepo et al., 2018 |
| E. prolata       | CBS 571.73 | Zea mays        | USA      | LT837831, LT715892, LT896646 | Hernandez-Restrepo et al., 2018 |
| E. protrudens    | BRIP 14814<sup>ET</sup>  | Dactyloctenium aegyptium | Australia | KJ415561, LT715880, KJ415432 | Tan et al., 2014; Hernandez-Restrepo et al., 2018 |
| E. rostratum     | BRIP 11416<sup>ET</sup>  | Zea mays        | Australia | LT837466, LT883543, LT883576 | Hernandez-Restrepo et al., 2018 |
|                  | USJCC-0011  | Oryza sativa    | Sri Lanka | MN860002, MN962923 - | This study |
| E. turcicum      | CBS 690.71<sup>ET</sup>  | Zea mays        | Germany | LT837487, LT882581, LT896618 | Hernandez-Restrepo et al., 2018 |

BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; USJCC: University of Sri Jayewardenepura Culture Collection, Sri Lanka. ET: ex-type.
all currently available ex-type or reference sequences of the species. The phylogram is based on multi-locus concatenated alignment of 21 in-group taxa with Bipolaris maydis as the out-group taxon (Hernández-Restrepo et al., 2018). The phylogram consists of 18 taxa of Exserohilum spp. from GenBank and two Exserohilum isolates collected in this study and Curvularia lunata ex-type (CBS 730.96). Maximum parsimony analysis revealed that 1661 characters are constant, 175 variable characters are parsimony–uninformative, while 241 characters are parsimony informative out of 2077 total characters. The analysis generated three compatible parsimonious trees and the best tree with the tree statistics: TL = 708, CI = 0.726, RI = 0.792, RC = 0.575, HI = 0.274, is presented (Figure 1).

According to the phylogram generated, two isolates clustered in distinct clades within the genus representing two distinct species. The strain USJCC–0010 isolated from the host Echinochloa oryzoides, grouped as more closely related with Exserohilum fusiforme. Recent phylogenetic assessments of the genus Exserohilum (Hernández-Restrepo et al., 2018) have shown that E. fusiforme is conspecific with closely related E. oryzicola. Therefore the isolate USJCC–0010 was identified as E. oryzicola. Similarly, Hernández-Restrepo et al. (2018) revealed that E. antillanum, E. gedarensis, E. longirostratum, E. macginnisi and E. prolatum are conspecific with E. rostratum. Therefore, the isolate USJCC–0011, which clustered in the broadly classified “rostratum clade” was hereby determined as E. rostratum. Although the aforementioned five species are determined to be one species, E. rostratum, by Hernández-Restrepo et al. (2018) in the phylogeny, sequence variabilities are observed within all three gene loci. Therefore, these species may be segregated in to different taxa if the sampling and gene regions are increased in future studies.

**Taxonomy**

Based on both morphological characteristics and molecular phylogenetic data, updated taxonomic descriptions are provided below with full illustrations, notes on habitats and recorded hosts and geographic distribution.

**Exserohilum oryzicola** Sivan., Transactions of the British Mycological Society 83(2): 325 (1984) (Figure 2) = Exserohilum fusiforme Alcorn, Mycotaxon 41: 337. 1991.

Sheath blight on Echinochloa oryzoides: Linear to irregular, dark brown to brown, elongated lesions. Asexual morph: Hyphae pale brown, branched. Conidiophores (391–) 431–653 (–638) µm long and 8–10 µm wide (av. = 542, SD = 111, n = 8; av. = 9, SD = 1, n = 5), macronematous, simple, septate, thicker than the vegetative hyphae, straight, rarely flexuous, swollen at the base, dark brown, pale brown to hyaline at the upper part. Conidia on CMA (67–) 81–107 (–118) × (12–) 14–18 (–20) µm (av. = 94, SD = 13, n = 30; av. = 16, SD = 2, n = 30), fusiform, straight to slightly curved, pale to dark olivaceous brown, singly or clusters, produced abundantly on CMA, 4–10-distoseptate, pale brown septa. *Hila* strongly protruding.

Colonies characteristics: Colonies on PDA cottony appearance, olivaceous green, concentric growth ring pattern, convex, irregular margin slightly undulated, abundant aerial mycelia, reaching 6.5 cm diam. in 7-d of incubation. Colonies on MEA, dark green and olivaceous
green with mouse grey center, irregular margins, attaining approximately 6.9 cm diam.; on CMA dull green aerial mycelia, convex, approximately 5.8 cm in 7-d.

Type specimen: Colombia, Meta, Villavicencio, on leaves of Oryza sativa, 2nd Nov. 1982, E.A. Urresta (IMI 273194 holotype; CBS 502.90 culture ex-isotype).

Specimens examined: Sri Lanka, Kegalle, Udugama, (N 7°10'23.41801", E 80°17'32.01914"), on sheath of Echinochloa oryzoides, 19th Feb. 2019, H.S. Ferdinandez.

Recorded hosts and geographic distribution: Australia – Echinochloa crus-galli; Colombia – Oryza sativa; Turkey – Oryza sativa (Farr and Rossman, 2020).

Exserohilum rostratum (Drechsler) K.J. Leonard & Suggs, Mycologia 66: 290 (1974) (Figure 3)
Basionym. Helminthosporium rostratum Drechsler, J. Agric. Res. 24: 724. 1923.
= Bipolaris rostrata (Drechsler) Shoemaker, Canad. J. Bot. 37: 883. 1959.
Leaf tip blight on *Oryza sativa*; brown color lesions surrounded by yellow halo. Asexual morph: *Hyphae* pale brown, septate, branched. *Conidiophores* (295–) 341–549 (–656) μm × (4–) 6–8 (–9) μm (av. = 445, SD = 104, n = 8; av. = 7, SD = 1, n = 5), macromonasous, simple, septate, thicker than the vegetative hyphae, straight to flexuous, dark brown, pale brown to hyaline at the upper part. *Conidia* on CMA (45–) 50–66 (–77) × (12–) 15–19 (–21) μm (av. = 58, SD = 8, n = 30; av. = 17, SD = 2, n = 30), fusiform, elongated, curved, ellipsoidal, pale to dark olivaceous brown, basal and apical cells often delimited by a dark septum, pale brown middle septa, singly or clusters, produced abundantly on CMA, 5–8-distoseptate. *Hila* slightly protruding.

Colony characteristics: Colonies on PDA dark greenish center and olivaceous green to the periphery, flat, entire margin slightly undulated, sparse aerial mycelia, reaching 4.4 cm diam. after 7-d of incubation. Colonies on MEA dark green and olivaceous green concentric rings, flat, attaining approximately 5.8 cm diam.; on CMA brownish aerial mycelia, flat colony, approximately 7.9 cm in 7-d.

Type specimen: USA, Washington DC, on dry leaves of *Eragrostis major*, Sept. 1921, C. Drechsler BPI 430144 holotype.

Recorded hosts and geographic distribution: Australia – *Areca catechu*, *Cenchrus setigerus*, *Chloris barbata*, *Chrysadiocarpus lutescens*, *Croton sp.*, *Cymbopogon citratus*, *Dactyloctenium aegyptium*, *Dinerea retroflexa*; Barbados – *Cynodon dactylon*; Brazil – *Brachiaria ruziizensis*; China– *Ananas comosus*, *Cynodon × dactylotransvaalensis*; India – *Acacia auriculiformis*, *Eleusine coracana*, *Sorghum vulgare*, *Triticum aestivum*, *Vigna sinensis*; United States: Florida– *Aechmea fasciata*, *Aloe vera*, *Bromelia sp.*, *Caryota mitis*, *Chamaedorea elegans*, *Chamaedorea seifrizii*, Hawaii– *Dendrobium* sp., North Carolina– *Cannabis sativa*, *Cassia obtusifolia*, *Cyprus*, Texas– *Panicum texanum*, Namibia – *Acacia mellifera* subsp. *detinens*, Oman– *Citrus aurantiifolia*; Taiwan– *Bromus inermis*, *Oryza sativa*; Thailand– *Zea mays*; Sri Lanka– *Cocos lacryma* (Farr and Rossman, 2020).

Based on the available sources and databases, we confirm that the two species *Exserohilum oryzae* on *Echinocloa oryzoides* and *Exserohilum rostratum* on *Oryza sativa* are novel plant-fungal association records. This study highlights the potential occurrence of *Exserohilum* associated with rice and associated weeds in Sri Lanka. Further studies in combination with phytopathological surveys incorporated with molecular data could reveal many unknown fungi and fungal-hosts associations, significance in agriculture and biocosecurity. Therefore, this study urges the need for molecular identification and taxonomic studies in Sri Lanka for the control of emerging plant and human pathogens and also to update quarantine measures, pathogen lists for cereal and fiber crops and weeds.

**ACKNOWLEDGMENT**

University of Sri Jayewardenepura is acknowledged for the Research grant ASP/01/RE/SCI/2018/036 to work on the taxonomy and molecular phylogeny of graminicolous hyphomycetes in Sri Lanka. The study is also partially funded by Emory Simmons research award to DSM by Mycological Society of America in 2018.
REFERENCES

Adler, A., Yaniv, I., Samra, Z., Yacobovich, J., Fisher, S., Avrahami, G. and Levy, I. (2006). Exserohilum: an emerging human pathogen. European Journal of Clinical Microbiology and Infectious Diseases 25(4): 247-253.

Al-Attar, A., Williams, C.G. and Redett, R.J. (2006). Rare lower extremity invasive fungal infection in an immunosuppressed patient: Exserohilum longirostratum. Plastic and Reconstructive Surgery 117(3): 44e-47e.

Alcorn, J. L. (1991). New combinations and synonymy in Bipolaris and Curvularia, and a new species of Exserohilum. Mycologia 41: 329-343.

Amaradasa, B.S., Madrid, H., Groenewald, J.Z., Crous, P.W. and Amundsen, K. (2014). Porocercospora seminalis gen. et comb. nov., the causal organism of buffalo grass false smut. Mycologia 106(1): 77-85.

Arnold, A. E. and Lutzoni, F. (2007). Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88(3): 541-549.

Berbee, M.L., Pirseyedi, M. and Hubbard, S. (1999). Cochliobolus phylogenetics and the origin of the kingdom fungi and reveals thresholds for fungal species delimitation. Mycologia 91: 964-977.

Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., Ko-Ko, T.W., Hongsanan, S., Jones, E.B.G., Kodsueb, R., Phookamsak, R., Chukeatirote, E. and Bahkali, A.H. (2011). Capnodiaceae. Fungal Diversity 51: 103-134.

De Hoog, G.S., Guarro, J., Gené, J. and Figueras, M.J. (2000). Atlas of clinical fungi (2nd Ed.). Centraalbureau voor Schimmelcultures (CBS), Utrecht.

Farr, D.F. and Rossman, A.Y. (2020). Fungal databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/. 15th January 2020.

Hernandez-Restrepo, M., Madrid, H., Tan, Y.P., Da Cunha, K.C., Gene, J., Guarro, J. and Crous, P.W. (2018). Multi-locus phylogeny and taxonomy of Exserohilum. Persoonia: Molecular Phylogeny and Evolution of Fungi 41: 71.

Katoh, K. and Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772-780.

Leonard, K.J. and Suggs, E.G. (1974). Setosphaeria prolate, the ascigeros state of Exserohilum prolatum. Mycologia 66: 281-297.

McGinnis, M.R., Rinaldi, M.G. and Winn, R.E. (1986). Emerging agents of phaeohyphomycosis: pathogenic species of Bipolaris and Exserohilum. Journal of Clinical Microbiology 24(2): 250-259.

Manamgoda, D.S., Cai, L., McKenzie, E.H., Crous, P.W., Madrid, H., Chukeatirote, E., Shivis, R.G., Tan, Y.P. and Hyde, K.D. (2012). A phylogenetic and taxonomic re-evaluation of the Bipolaris-Cochliobolus-Curvularia complex. Fungal Diversity 56(1): 131-144.

Miller, M.A., Pfeiffer, W. and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), November 14, 2010, New Orleans, Louisiana: 1-8.

Passerini, G. (1876). La nebbia del grano turco. Bolletino del Comizio Agrario Parmense 10: 1-3.

Rambaut, A. and Drummond, A. (2008). FigTree: tree figure drawing tool, version 1.2.2. Institute of Evolutionary Biology, University of Edinburgh, UK.

Rayner, R.W. (1970). A mycological colour chart. Commonwealth Mycological Institute, UK.

Rehner, S.A. and Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EF-1α sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia 97(1): 84-98.

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

Smith, I.M., Dunetz, J., Phillips, D.H., Lelliott, R.A. and Archer, S.A. eds. (1998). European Handbook of Plant Diseases. John Wiley & Sons.

Stamatakis, A., Hoover, P. and Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57: 758-771.

Swofford, D.L. (2003). PAUP*: phylogenetic analysis using parsimony, version 4.0 b10. Sunderland, Massachusetts, USA.

Tan, Y.P., Madrid, H., Crous, P.W. and Shivis, R.G. (2014). Johnalcornia gen. et. comb. nov., and nine new combinations in Curvularia based on molecular phylogenetic analysis. Australasian Plant Pathology 43(6): 589-603.

Udayanga D. (2019). The promise of molecular identification in plant biosecurity, Vidyodaya Current Journal 1(1): 103-113.

Vu, D., Groenewald, M., De Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., Al-Hatmi, A., Groenewald, J.Z., Cardinali, G., Houbraiken, J. and Boekhout, T. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92: 135-154.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J.L. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. New York: Academic Press. 315-322.

Zhang, M., Zhang, T.Y. and Wu, Y.M. (2004). A new name and a new variety in Curvularia. Mycosystema 23: 177-178.