



## A new species of *Bipolaris* from *Heliconia rostrata* in India

Singh R<sup>1</sup> and Kumar S<sup>2</sup>

<sup>1</sup>Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, India

<sup>2</sup>Department of Forest Pathology, Kerala Forest Research Institute, Peechi- 680653, Kerala, India

Singh R, Kumar S 2016 – A new species of *Bipolaris* from *Heliconia rostrata* in India. Current Research in Environmental & Applied Mycology 6(3), 231– 237, Doi 10.5943/cream/6/3/11

### Abstract

*Bipolaris rostratae*, a new foliicolous anamorphic fungus discovered on living leaves of *Heliconia rostrata* (*Heliconiaceae*), is described and illustrated. The species was compared with closely related species of *Bipolaris* and similar fungi recorded on *Heliconia* spp. This species is different from other *Bipolaris* spp. reported on *Heliconia* due to its shorter, thinner and less septate conidia. A key is provided to all species of *Bipolaris* reported on *Heliconia*.

**Key words** – fungal diversity – morphotaxonomy – Foliicolous fungi – *Bipolaris* – new species

### Introduction

After several taxonomic refinements, graminicolous *Helminthosporium* were segregated into several genera including *Bipolaris*, *Curvularia*, *Drechslera* and *Exserohilum* (Sivanesan 1987). These genera belong to *Ascomycota*, *Dothideomycetes*, *Pleosporales*, *Pleosporaceae*. These genera can be distinguished on the basis of characters such as conidial shape and size, hilum morphology, origin of the germ tubes from the basal or other conidial cells, and the location and sequence in the development of the conidial septa. Illustrations of different hilum morphologies in graminicolous *Helminthosporium* species were given by Alcorn (1988). The anamorphic genus *Drechslera* (*Pleosporaceae*) was described by Ito (1930), which accommodated previously in subgenus *Cylindro-Helminthosporium*, of graminicolous *Helminthosporium*. These fungi cause disease on many plant hosts (Zhang & Berbee 2001) where they are commonly observed in their asexual state (Zhang & Berbee 2001). *Drechslera* can be differentiated from all other graminicolous helminthosporoid genera by its ability to develop a germ tube from any of the cells in the conidia (Sivanesan 1987, Alcorn 1988). In *Bipolaris* conidia germinate by germ tubes from one or both of the end cells. Hilum morphology can also be used to differentiate *Bipolaris* and *Drechslera*. In *Drechslera* a flat scar without protruding hila exists within the lowest part of the basal cell, whereas in *Bipolaris* it is inconspicuous or very slightly protuberant and is continuous with the conidial wall (Alcorn 1988). Both genera can also be separated on the basis of their sexual morphs; *Drechslera* has been linked to *Pyrenophora* sexual morphs, whereas the sexual morph of *Bipolaris* was *Cochliobolus* (Drechsler 1934, Alcorn 1983). On molecular phylogenetic analyses it has been proved that *Drechslera* and *Bipolaris* are two distinct genera (Berbee et al. 2000).

Another fungus, *Exserohilum* Leonard & Suggs (1974), can be differentiated from other graminicolous helminthosporoid genera by a truncate, strongly protruding hilum with redundant bases, often with an enveloping bubble. The conidia germinate by germ tubes originating from either one or

both of the end cells or other intermediate cells. The sexual morphs of *Exserohilum* have been placed in *Setosphaeria* (Leonard & Suggs 1974).

*Bipolaris* Shoemaker (1959) and *Curvularia* Boedijn (1933) share many morphological similarities (Sivanesan 1987) and both genera have sexual morphs in *Cochliobolus* (Drechsler, 1934). Phylogenetic relationships based on sequences from four gene regions (ITS, GPDH, LSU and EF1- $\alpha$ ) from ex-type strains of *Bipolaris*, *Cochliobolus* and *Curvularia* spp. prove that *Bipolaris* and *Curvularia* are two monophyletic groups which indicate separate generic status (Manamgoda et al. 2012) and these findings are in agreement with previous studies (Shimizu et al. 1998, Berbee et al. 1999, Kodsueb et al. 2006). Although the asexual morphs cluster in two well defined groups (*Bipolaris* and *Curvularia*) based on molecular data and morphology, their sexual morphs are quite similar indicating that the asexual states have evolved and differentiated more rapidly than the sexual morphs (Manamgoda et al. 2012, 2014). *Bipolaris* is characterized by large canoe-shaped conidia, and lack stromata, branched to unbranched conidiophores with polytretic conidiogenous cells and pseudoseptate conidia with scar very slightly protuberant which is continuous with the conidial wall. *Curvularia* species have straight to curved conidia, and usually stromata below the ascumata.

During a regular visit of the Botanical Garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, India, some *Heliconia rostrata* (Hanging Lobster Claw or False Bird of Paradise) plants showed leaf spot symptoms of various sizes which were caused by a *Bipolaris* species. Upon a critical morphological examination and comparison of its morphotaxonomic features with those of the currently accepted species of this genus, it was considered to represent a novel taxon.

## Materials and Methods

Specimens of living leaves with disease symptoms suggesting the presence of parasitic fungi, were collected at the Botanical garden of the Deen Dayal Upadhyay (DDU) Gorakhpur University, Gorakhpur in 2012. The collected samples were carried to the laboratory and processed by following standard protocols (Hawskworth, 1974, Savile, 1962). The dried and pressed infected leaf samples were placed inside adsorbant paper envelopes along with collection details and then kept in air tight polyethylene bags. Photographs of infection spots on leaf surface were taken by using Sony DSC-5730 camera. Free hand cut sectioning and scrappings were made through infection spots mounted in clear lactophenol cotton-blue mixture for the morphological observation of microscopic structures. Observations were made with an Olympus BX-51 light microscope by using Syntek USB camera. Detailed observations of morphological characters and Line drawings were carried out at different magnification through light microscope (450 $\times$  and 1000 $\times$ ). The measurements of 30 conidia, hila, and conidiophores and conidiogenous cells, with the extremes given in parentheses were done with the help of combination of stage and ocular micrometer. The holotype is deposited in Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India; and an isotype was retained in the herbarium of the Department of Botany, D.D.U. Gorakhpur University (GPU), Gorakhpur. The systematic position of the taxa is given in accordance with following literatures [Ellis (1971, 1976), Cannon and Kirk (2007), Kirk et al. (2008), Seifert et al. (2011), Farr and Rossman (2015), MycoBank ([www.mycobank.org](http://www.mycobank.org); accessed 30 April 2015) and the Index Fungorum ([www.indexfungorum.org](http://www.indexfungorum.org); accessed 30 April 2015)].

## Results

### Taxonomy

***Bipolaris rostratae* Raghv. Singh & Sham. Kumar, sp. nov.**

Figs. 1–3

MycoBank MB 812424

Diagnoses – Differs from other *Bipolaris* spp. reported on *Heliconia* due to its shorter, thinner and less septate conidia.

Etymology – the epithet *rostratae* is derived from name of host species.



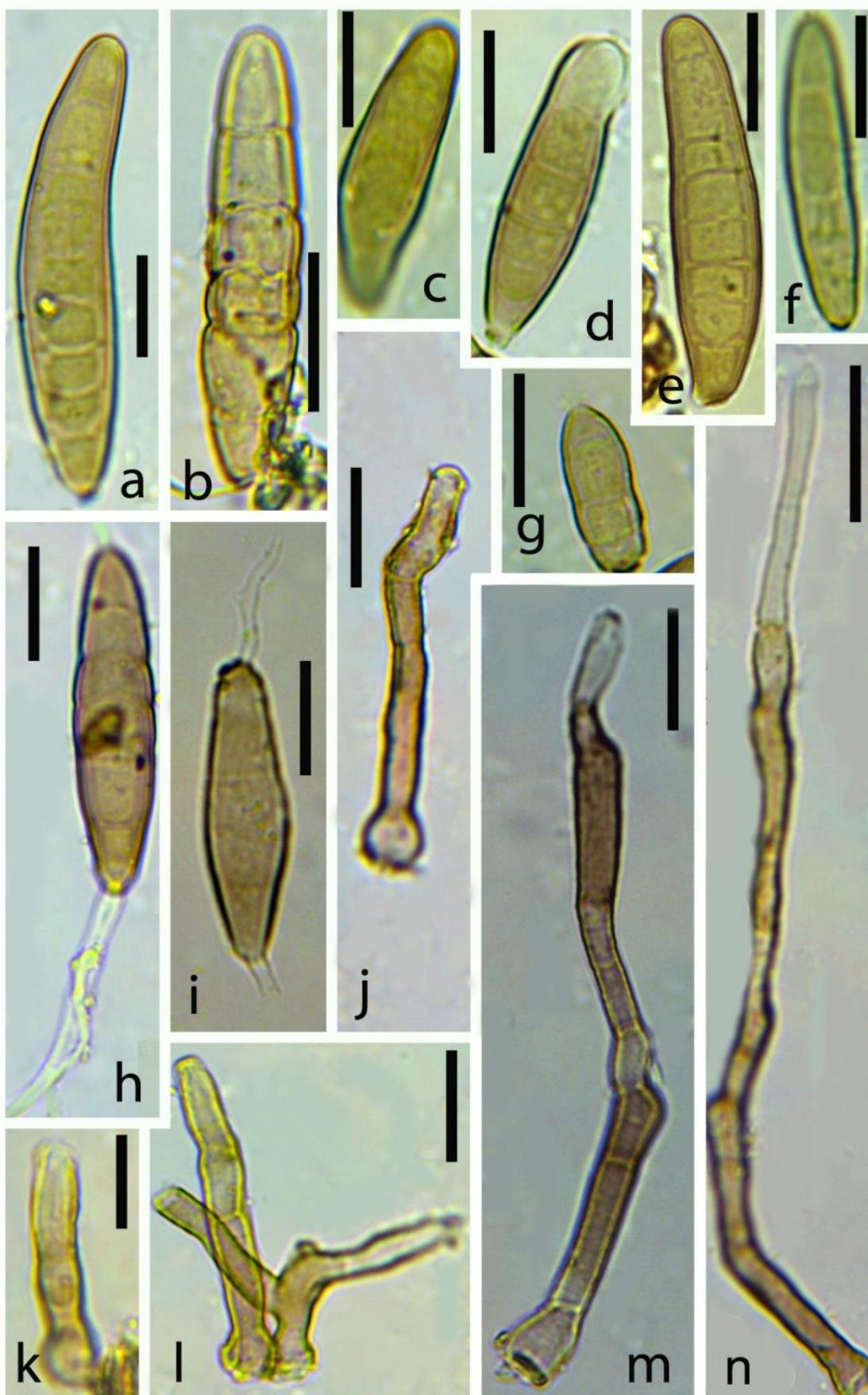
**Fig. 1** *Heliconia rostrata* (AMH 9480, holotype). **a.** Host plant habit. **B.** Early stage of infection. **c–d.** Late stage of infection. Scale bars **b–d** = 20 mm.

*Anamorphic fungus, Hyphomycetes, Foliicolous, Parasitic, Infection spots* amphigenous, initially circular to subcircular, but later irregular and spread on the entire leaf surface, yellowish brown to dark brown in colour. *Colonies* epiphyllous, usually effuse, brown. Mycelium internal. **Sexual morph:** undetermined. **Asexual morph:** Stromata absent. *Conidiophores* macronematous, mononematous, branched to unbranched, cylindrical, erect to procumbent, straight to flexuous, geniculate, smooth, thick-walled, 1–8-septate, mid brown to blackish brown, base bulbous, tip normally swollen, (25–)30–205(–220) × (2.5–)3–6(–8) μm. *Conidiogenous cell* integrated, terminal to intercalary, monotretic to polytretic, conidiogenous loci thickened, 1.5–2.5 μm wide. *Conidia* solitary, simple, acropleurogenous, usually cylindrical to obclavate-cylindrical to fusiform or occasionally orbicular, straight to curved, smooth, thick-walled, mid brown to blackish brown, some conidia constricted at septa, base rounded and apex obtuse, (20–)22–65(–72) × (5–)6–12(–13.5) μm, 2–8-distoseptate, hila thickened, 1.5–2.5 μm wide. Conidia germinating at both ends.

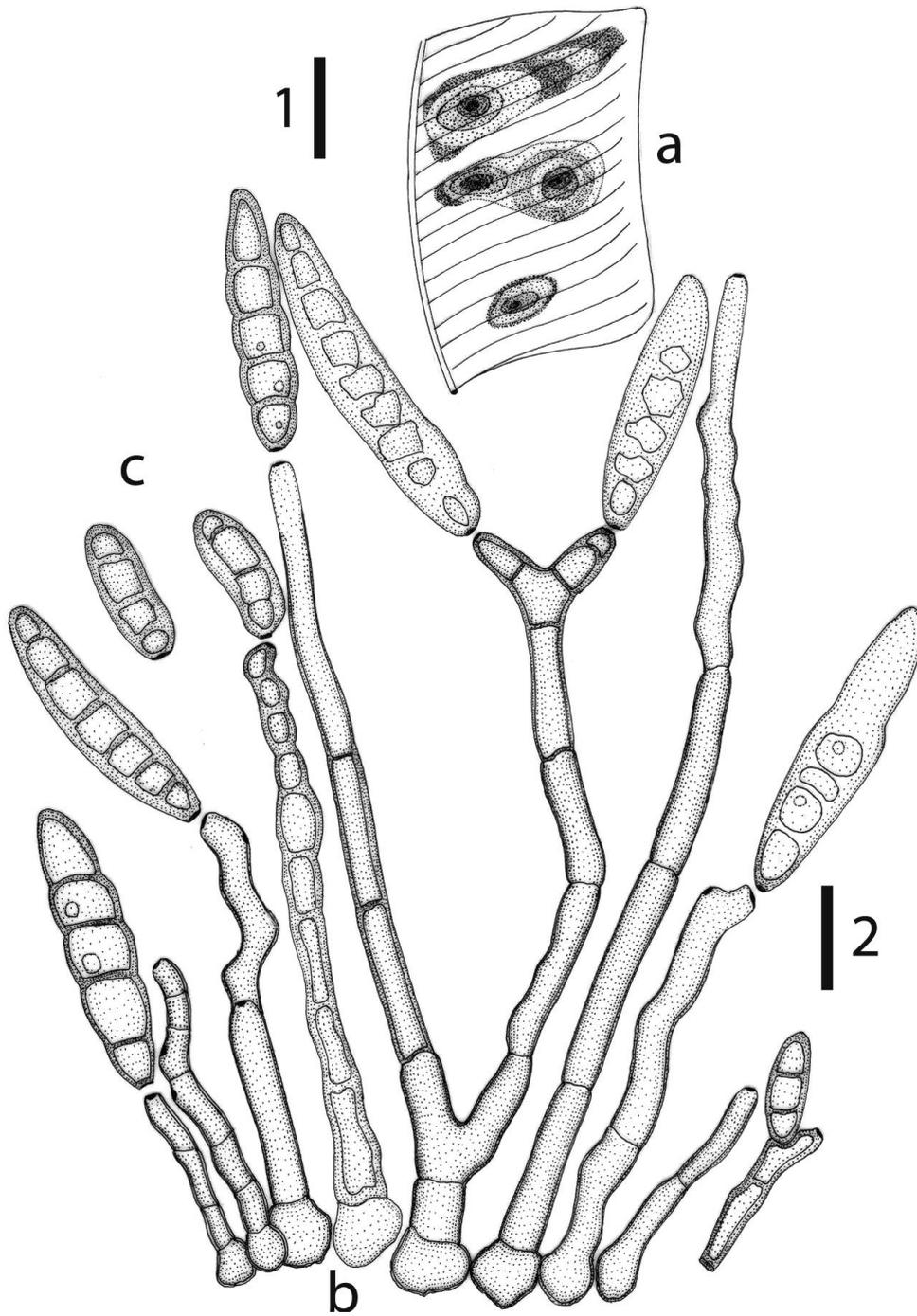
Known distribution – India

Material examined– India, Uttar Pradesh, Gorakhpur, DDU Gorakhpur University, Botanical Garden, on living leaves of *Heliconia rostrata* Ruiz & Pav. (*Heliconiaceae*), 11 February 2012, coll., Raghvendra Singh, AMH 9480 (**holotype**), GPU 604 (**isotype**).

Teleomorph – Not seen.



**Fig. 2** – *Bipolaris rostratae*, microphotographs (AMH 9480, holotype). **a-g**. Conidia. **h-i**. Germinated conidia at both end. **j-n**. Conidiophores. Scale bars **a-n** = 20  $\mu\text{m}$



**Fig. 3** – *Bipolaris rostratae*, drawings (AMH 9480, holotype). **a.** Infection spot. **b.** Conidiophores. **c.** Conidia. Scale bars **1** = 20 mm, **2** = 20  $\mu\text{m}$ .

### Discussion

A survey of literature indicated that a number of *Bipolaris* spp. have been recorded on *Heliconia* spp. viz., *B. cynodontis* Shoemaker (1959), *B. heliconiae* Alcorn (1996), *B. incurvata* Alcorn (1983a), *B. salviniae* Alcorn (1991) and *B. setariae* Shoemaker (1959).

The conidia of *Bipolaris rostratae* [(20–)22–65(–72)  $\times$  (5–)6–12(–13.5)  $\mu\text{m}$ , 2–8-distoseptate] are shorter, thinner and less septate compared to *B. cynodontis* [(27–)40–80(–100)  $\times$  (10–)12–18(–20)  $\mu\text{m}$ , (3–)7(–9)-distoseptate], *B. heliconiae* [65–150  $\times$  15–19  $\mu\text{m}$ , 7–10-distoseptate], *B. incurvata* [100–150  $\times$  19–22  $\mu\text{m}$ , 8–13-distoseptate], *B. salviniae* [(75–)100–170(–190)  $\times$  (10–)13–17(–19)  $\mu\text{m}$ , (6–)10(–14)-distoseptate] and *B. setariae* [(50–)65–100(–108)  $\times$  (10–)13–16  $\mu\text{m}$ , (5–)8(–10)-distoseptate]. Thus, the fungus *B. rostratae* is designated as new species.

## Identification key to *Bipolaris* species on *Heliconia* spp.

1 Conidia smooth, shorter, thinner and less septate.....	2
1* Conidia smooth or slightly verruculose, longer (>72 µm), thicker (>13.5 µm) and more septate (>8).....	3
2 Conidia (20–)22–65(–72) × (5–)6–12(–13.5) µm, 2–8-distoseptate .....	<i>Bipolaris rostratae</i>
3 Nature of germination in conidia .....	4
4 End cells sometimes swollen to produce a thin, globose vesicle where germ tubes originate .....	5
4* Germ tubes originating from both or one end cells without forming a vesicle .....	6
5 Conidia hyaline when immature, turning olivaceous green, then brown or golden brown when mature, (27–)40–80(–100) × (10–)12–18(–20) µm, (3–)7(–9)-distoseptate.....	<i>B. cynodontis</i>
6. Conidia pale brown, olivaceous brown, golden brown .....	7
7 Conidiophore and conidial size.....	8
8 Conidia less than 150 µm long, conidiophores less than 350 µm long.....	9
8* Conidia length equal to or more than 150 µm, Conidiophores more than 350 µm long .....	10
9. Conidia (50–)65–100(–108) × (10–)13–16 µm, (5–)8(–10)-distoseptate, Conidiophores (96–)145–207(–218) × 4–6 µm .....	<i>B. setariae</i>
10. Conidia less than 20 µm wide.....	11
10* Conidia more than 20 µm wide.....	12
11. Conidia 65–150 × 15–19 µm, 7–10-distoseptate .....	<i>B. heliconiae</i>
11* Conidia (75–)100–170(–190) × (10–)13–17(–19) µm, (6–)10(–14)-distoseptate .....	<i>B. salviniae</i>
12 Conidia 100–150 × 19–22 µm, 8–13-distoseptate .....	<i>B. incurvata</i>

## Acknowledgements

We are thankful to the Head, CAS in Botany, Banaras Hindu University, Varanasi and Director, Kerala Forest Research Institute, Peechi, Kerala for necessary facilities. Thankfulness is also due to both anonymous reviewers for reviewing the manuscript with relevant comments and suggestions. Raghvendra Singh thanks to the University Grant Commission (UGC), Faculty Research Promotion Scheme (FRPS/2014), Govt. of India and Shambhu Kumar extends thanks to the SERB, DST, Govt. of India for financial assistance (SB/YS/LS-288/2013). Authors are indebted to the Curator of AMH, Agharkar Research Institute (ARI), Pune, for accepting the holotype specimens and providing an accession number.

## References

- Alcorn JL. 1983 – On the genera *Cochliobolus* and *Pseudocochliobolus*. *Mycotaxon* 16, 353–379.
- Alcorn JL. 1988 – The taxonomy of “*Helminthosporium*” species. *Annual Review of Phytopathology* 26, 37–56.
- Alcorn JL. 1991 – Combinations and synonymy in *Bipolaris* and *Curvularia*, and a new species of *Exserohilum*. *Mycotaxon* 41(2), 329–343.
- Alcorn JL. 1996 – *Cochliobolus heliconiae* sp. nov. (*Ascomycota*). *Australian Systematic Botany* 9(5), 813–817.
- Alcorn, JL. 1983a – Generic concepts in *Drechslera*, *Bipolaris* and *Exserohilum*. *Mycotaxon* 17, 1–86.
- Berbee M, 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, 964–977.
- Berbee ML, Carmean DA, Winka K. 2000 – Ribosomal DNA and resolution of branching order among the ascomycota: how many nucleotides are enough? *Molecular Phylogenetics and Evolution* 17, 337–344.
- Boedijn, KB. 1933 – Über einige phragmosporen Dematiazen. *Bulletin du Jardin Botanique de Buitenzorg* 13(1), 120–134.

- Cannon PF, Kirk PM. 2007 – Fungal Families of the World. Wallingford, Oxfordshire, UK: CAB International. pp. 456.
- Drechsler C. 1934 – Phytopathological and taxonomical aspects of *Ophilobolus*, *Pyrenophora*, *Helminthosporium* and a new genus *Cochliobolus*. *Phytopathology* 24, 953–981.
- Ellis MB. 1971 – Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England. pp. 608.
- Ellis MB. 1976 – More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England. pp. 507.
- Farr DF, Rossman AY. 2015 – Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved April 30, 2015, from <http://nt.ars-grin.gov/fungaldatabases/>
- Hawksworth DL. 1974 – Mycologist's Handbook. Commonwealth Mycological Institute, Kew. pp. 231.
- Index Fungorum 2015 – Index Fungorum. <http://www.indexfungorum.org>, accessed 30 April 2015.
- Ito S. 1930 – On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals. Supplement of Proceedings of the Imperial Academy of Japan 6, 352–355.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008 – Dictionary of the Fungi. 10th ed. Wallingford, UK: CAB International. pp. 771.
- Kodsueb R, Dhanasekaran V, Aptroot A, Lumyong S, McKenzie EHC, Hyde KD, Jeewon R. 2006 – The family Pleosporaceae: intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. *Mycologia* 98, 571–583.
- Leonard KJ, Suggs EG. 1974 – *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. *Mycologia* 66, 197–281.
- Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD. 2012 – A phylogenetic and taxonomic re-evaluation of the *Bipolaris* – *Cochliobolus* – *Curvularia* complex. *Fungal Diversity* 56, 131–144. <http://dx.doi.org/10.1007/s13225-012-0189-2>
- Manamgoda DS, Rossman AY, Castlebury LA, Crous PW, Madrid H, Chukeatirote E, Hyde KD. 2014 – *Studies in Mycology* 79, 221–288.
- Mycobank 2015 – MycoBank (Fungal databases nomenclature and species banks). [www.mycobank.org](http://www.mycobank.org), accessed 30 April 2015.
- Savile DBO. 1962 – Collection and care of Botanical specimens. Canadian Department of Agriculture, Publication, Research Branch 1113, 1–124.
- Seifert KA, Morgan-Jones G, Gams W, Kendrick WB. 2011 – The Genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. CBS Biodiversity Series no. 9, 997.
- Shimizu K, Tanaka C, Peng YL, Tsuda M. 1998 – Phylogeny of *Bipolaris* inferred from nucleotide sequences of Brn1, a reductase gene involved in melanin biosynthesis. *J. Gen. Appl. Microbiol.* 44, 251–258.
- Shoemaker RA. 1959 – Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from '*Helminthosporium*'. *Canadian Journal of Botany* 37(5), 879–887.
- Sivanesan A. 1987 – Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. CAB International. pp. 261.
- Zhang G, Berbee ML. 2001 – *Pyrenophora* phylogenetics inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 93, 1048–1063.