

First Report of *Apiospora acutiapica* and *Apiospora malaysiana* from Indian Mycobiota: Insights from Morphology and Molecular Phylogeny

Rashmi Dubey^{1,*}, Sayantan Jash¹, Amit D. Pandey¹

¹*Mycological Research Laboratory, Botanical Survey of India, Western Regional Centre, 7 Koregaon Road, Pune - 411 001, Maharashtra, India.*

**Corresponding author Email: dr.rashmidubey@gmail.com*

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ABSTRACT

This paper presents an account of two *Apiospora* species newly recorded from India: *Apiospora acutiapica* (Senan. & K.D. Hyde) X.G. Tian & Tibpromma (2021) and *Apiospora malaysiana* (Crous) Pintos & P. Alvarado (2021). Identification of these taxa is based on detailed morphological comparisons and supported by phylogenetic analyses of ITS sequences. Comprehensive species descriptions, illustrative figures, and phylogenetic data validated their identification as new records for India are provided.

Keywords: Apiosporaceae, Ascomata, Bamboo, Goa, ITS

INTRODUCTION

Apiospora Sacc. is a significant genus within the fungal class Sordariomycetes under family Apiosporaceae K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr, characterized by basauxic, *Arthrinium*-like conidiogenesis (Hyde *et al.*, 2020). The family Apiosporaceae was established to accommodate this genus due to its distinctive conidiogenesis (Hyde *et al.*, 1998) and now includes morphologically similar genera such as *Apiospora*, *Arthrinium* Kunze and *Nigrospora* Zimm. (Wang *et al.*, 2017; Pintos and Alvarado, 2021; Jiang *et al.*, 2022). *Apiospora* is phylogenetically close to *Arthrinium*, with both previously considered different life stages of the same taxon (Ellis, 1965; Crous and Groenewald, 2013; Réblová *et al.*, 2016; Jiang *et al.*, 2019). Although morphologically similar, *Arthrinium* typically produces variable-shaped conidia, whereas, *Apiospora* species often have rounded, lenticular conidia (Pintos and Alvarado, 2021; Li *et al.*, 2023; Liao *et al.*, 2023). Recent molecular analyses confirm that *Arthrinium* s. str. and *Apiospora* have distinct lineages within Apiosporaceae, leading to the reclassification of the most *Arthrinium* species under *Apiospora* (Pintos and Alvarado, 2021).

Currently, *Apiospora* comprises 202 epithets worldwide (Index Fungorum, 2025). Historically, based on morphological characteristics, four species of *Apiospora* and nineteen species of *Arthrinium* were documented from India (Butler and Bisby, 1931; Sarbhoy *et al.*, 1986; Bilgrami *et al.*, 1991; Jamaluddin *et al.*, 2004; Manoharachary *et al.*, 2022).

However, recent studies employing a polyphasic approach, including morphological, molecular, and phylogenetic analyses, have resulted in the reclassification of a substantial number of *Arthrinium* species into the genus *Apiospora* (Pintos and Alvarado, 2021). Based on this study, Jash and Dubey (2025) recently documented 17 species of *Apiospora* reported from India.

As part of a project focused on documenting bambusicolous fungi in Goa, two species of *Apiospora* viz., *Apiospora acutiapica* (Senan. & K.D. Hyde) X.G. Tian & Tibpromma and *Apiospora malaysiana* (Crous) Pintos & P. Alvarado have been identified, which represent new records for Indian mycobiota. This paper provides taxonomic descriptions of these two species, including phylogenetic relationships and geographical distribution.

MATERIALS AND METHODS

Fungal isolation and morphological characterization

Seasonal surveys were conducted in protected areas of Goa and their adjoining regions to assess the diversity of microfungi associated with different parts of bamboo. The geographic coordinates of each collection site were recorded using a Global Positioning System (GPS). Microscopic examination of the vegetative and asexual structures of the fungi was carried out using an Olympus CX41 microscope equipped with DP22 digital camera. Fungal isolates from the bamboo

litter were cultured on Potato Dextrose Agar (PDA) at 28°C and incubated for 10 days for observation. The digital photo plates were made with the help of Adobe Photoshop CS5 (Adobe Inc., California, USA). The specimens were then deposited at the Herbarium of Botanical Survey of India, Western Regional Centre, Pune (BSI).

DNA extraction, PCR amplification, and DNA sequencing

To extract fungal DNA, fresh mycelia were scraped from PDA plates and transferred to 1.5 ml screw-capped microcentrifuge tubes. DNA extraction was done using the HiPurA® Fungal DNA Purification Kit (Himedia, Maharashtra, India) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out using the primers ITS5/ITS4 to amplify internal transcribed spacer regions (ITS) by following the amplification reaction and PCR conditions suggested by White et al. (1990). The PCR product was then purified using HiPurA® PCR Product Purification Kit (Himedia, Maharashtra, India) according to the manufacturer's instructions and visualized using agarose gel electrophoresis. The PCR-purified

DNA was sequenced by Barcode Biosciences in Bangalore, India, using the same PCR primers. The nucleotide sequence data procured were then deposited in GenBank (**Table 1**).

Phylogenetic analysis

The newly generated sequences were visualized in BioEdit (Hall, 1999) and subjected to nucleotide BLAST searches at NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Closely related taxa were retrieved from GenBank based on BLAST closest hit searches and published literature (Pintos and Alvarado, 2021). The sequences were then aligned using MAFFT v. 7.0 (<https://mafft.cbrc.jp/alignment/server/>; Katoh and Standley, 2013) with default settings. Aligned sequences were checked and trimmed manually by using BioEdit (Hall, 1999). The Maximum Likelihood analysis was conducted using the RAXML GUI 2.0 (Edler et al., 2021), employing the GTR+G+I model with 1,000 bootstrap replications. Finally, the phylogenetic trees were observed and edited in iTOL V6 (Letunic and Bork, 2024).

Table 1: Names, strain numbers, reported countries, and GenBank accession numbers of the taxa used in the phylogenetic analyses, along with references

Name of Taxa	Strain Number	Country of Collection	GenBank Accession No. (nrITS)	References
<i>Apiospora acutiapica</i>	KUMCC 20-0210 (T)	China	MT946343	Senanayake et al. (2020)
<i>Apiospora acutiapica</i>	BSI F-BG-107	India	PV767508	Present Study
<i>Apiospora arundinis</i>	CBS 732.71	India	KF144889	Crous and Groenewald (2013)
<i>Apiospora arundinis</i>	CBS 106.12	Germany	KF144883	Crous and Groenewald (2013)
<i>Apiospora hydei</i>	CBS 114990 (T)	China	KF144890	Crous and Groenewald (2013)
<i>Apiospora intestini</i>	CBS 135835 (T)	India	KR011352	Pintos and Alvarado (2021)
<i>Apiospora jatrophae</i>	NFCCI 2599 (T)	India	JQ246355	Sharma et al. (2014)
<i>Apiospora jatrophae</i>	MCC 1037	India	HE981191	Sharma et al. (2014)
<i>Apiospora malaysiana</i>	CBS 102053 (T)	Malaysia	KF144896	Crous and Groenewald (2013)
<i>Apiospora malaysiana</i>	BSI F-BG-214	India	PV767509	Present Study

Name of Taxa	Strain Number	Country of Collection	GenBank Accession No. (nrITS)	References
<i>Apiospora marii</i>	CBS 497.90 (T)	Spain	MH873913	Vu <i>et al.</i> (2019)
<i>Apiospora montagnei</i>	AP301120 (T)	Spain	ON692408	Pintos and Alvarado (2022)
<i>Apiospora mytilomorpha</i>	DAOM 214595 (T)	India	KY494685	Wang <i>et al.</i> (2018)
<i>Apiospora neobambusae</i>	LC7106 (T)	China	KY494718	Wang <i>et al.</i> (2018)
<i>Apiospora sacchari</i>	CBS 212.30	United Kingdom	KF144916	Crous and Groenewald (2013)
<i>Apiospora saccharicola</i>	CBS 191.73	Netherlands	KF144920	Crous and Groenewald (2013)
<i>Apiospora serenensis</i>	SFC20220920-B248	South Korea	OP597860	Lee <i>et al.</i> (2023)
<i>Apiospora sphaerosperma</i>	CBS 142.55	Japan	MH857420	Pintos and Alvarado (2021)
<i>Arthrinium caricicola</i>	CBS 145903 (T)	Germany	MN313782	Crous <i>et al.</i> (2020)
<i>Arthrinium puccinioides</i>	CBS 549.86	Japan	AB220253	Pintos and Alvarado (2021)
<i>Arthrinium sphaerospermum</i>	CBS 146355	Norway	MW208943	Pintos and Alvarado (2021)
<i>Arthrinium sporophleum</i>	AG19067	France	MW208945	Pintos and Alvarado (2021)
<i>Seiridium phylicae</i>	CBS: H-21089 (T)	United Kingdom	NR_120230	Crous <i>et al.</i> (2012)

Note: Newly generated sequences are in bold, and the type sequences are mentioned as “T” in brackets.

RESULTS

Phylogenetic Analysis: In this study, a total of 23 sequences from the Apiosporaceae, including two sequences of *Apiospora* generated by the authors and *Seiridium phylicae* (CBS: H-21089) as the outgroup taxon, have been selected for the phylogenetic analysis. In the phylogenetic analysis, the final alignment consisted of 687 characters in total, including gaps. The RAxML analysis of the combined dataset yielded the best-scoring tree (Figure 1) with a final ML optimization likelihood value of -3207.215065. The matrix had 310 distinct alignment patterns, with 17.39% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.238320, C = 0.246389, G = 0.241419, and T = 0.273872; substitution rates AC = 0.897256, AG = 1.212115, AT = 0.824094, CG =

0.690963, CT = 2.615148, and GT = 1.000000; and gamma distribution shape parameter $\alpha = 0.268343$.

The phylogram showed that the Indian isolates of *Apiospora acutiapica* and *Apiospora malaysiana* have been grouped inside the clade of respective species with strong bootstrap support (MLbs = 100% in *A. acutiapica* and MLbs = 88% in *A. malaysiana*). Phylogenetic analysis (**Figure 1**) strongly recommends the conspecificity of the Indian isolate of *A. acutiapica* (BSI F-BG-107) with the type sequence of *A. acutiapica* (KUMCC 20-0210) and also that of the Indian isolate of *A. malaysiana* (BSI F-BG-214) with the type sequence of *A. malaysiana* (CBS 102053).

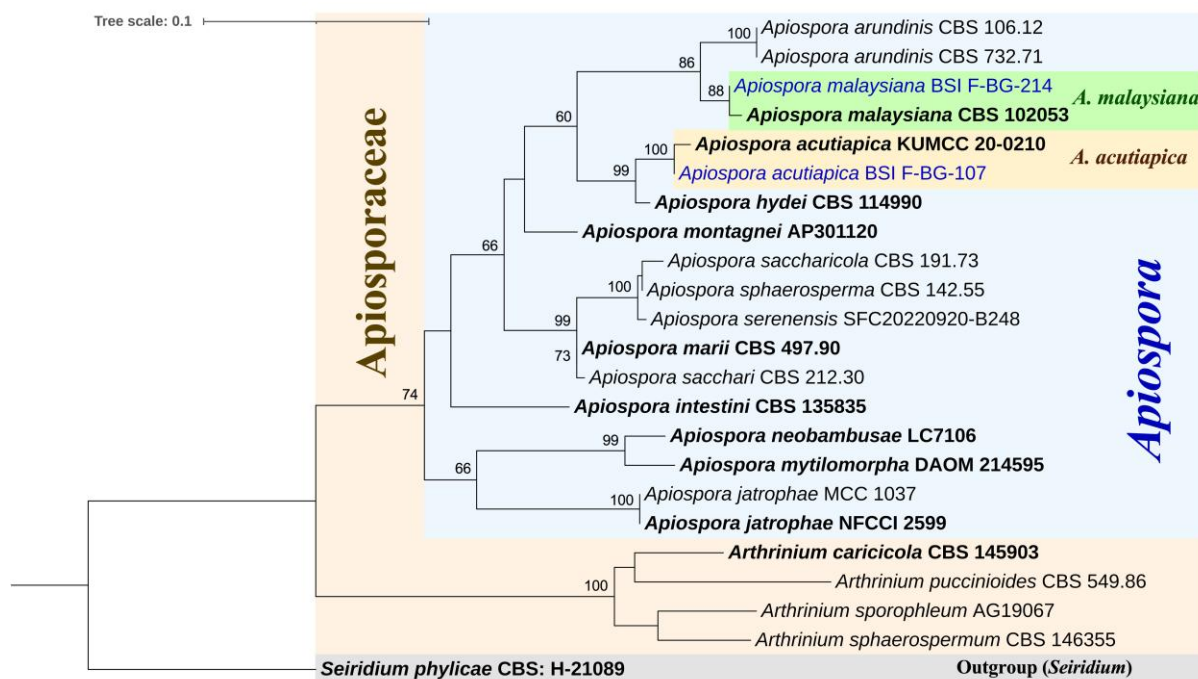


Figure 1: The best phylogram was generated from ITS sequence data representing Apiosporaceae. Bootstrap support values for maximum likelihood (ML) $\geq 60\%$ are provided at the nodes. The isolates for the new records are in blue, whereas type strains are in bold. The scale bar indicates 0.1 substitution changes.

Taxonomic Treatment

Apiospora acutiapica (Senan. & K.D. Hyde) X.G. Tian & Tibpromma, in Tian et al., Life 11(no. 1071): 16 (2021). **Figure 2a-e**

Saprobic on dead culms of bamboo. Colonies compact or widely effused, dark blackish brown, fructifications occasionally erumpent and superficially pulvinate. Hyphae partly superficial, partly immersed, 1.5–2.5 μm diam., hyaline, branched, septate, sparse. **Sexual morph:** undetermined. **Asexual morph:** Conidiomata 380–455 μm , pycnidial, immersed, aggregated, scattered, subglobose, ostiolate, black, coriaceous. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 4.5–7 $\times$ 2–3 μm ($\bar{x}$ = 6.5 $\times$ 2.3 μm , n = 30), holoblastic, erect, basauxic, cylindrical to ampulliform, apex pointed and hyaline, smooth, pale brown. Conidia 7–10.5 $\times$ 8.2–10 μm ($\bar{x}$ = 9 $\times$ 8.5 μm , n = 30), globose in surface view, subglobose to oval in side view, apex and base blunted, smooth-walled, brown to dark brown, with a distinct equatorial slit.

Cultural Characteristics: Colonies grew on PDA at 28°C attenuated 2 cm diam. within 7 days, circular, entire margin, puffy, with abundant aerial mycelia, greyish white in surface view and off-white to yellow in reverse.

Specimen examined: INDIA, Goa, On way from Mhadei to Bondla N 15° 26' 40.74", E 74° 3' 18.29", alt. 17 m, on bamboo culms, 26/02/2022, Rashmi Dubey, BSI F-BG-107.
GenBank: ITS = PV767508

Known Distribution: China (Senanayake et al., 2020)

Apiospora malaysiana (Crous) Pintos & P. Alvarado, Fungal Systematics and Evolution 7: 206 (2021). **Figure 2f-i**

Saprobic on dead culms of bamboo. Colonies effused, dark blackish brown, fructifications occasionally erumpent and superficially pulvinate on host. Hyphae partly superficial, partly immersed, smooth, hyaline, branched, septate, 2–3 μm diam. **Sexual morph:** undetermined. **Asexual morph:** Conidiophores reduced to conidiogenous cells. Conidiogenous cells 4–7 $\times$ 3–5 μm ($\bar{x}$ = 5.5 $\times$ 3.8 μm , n = 30), holoblastic, basauxic, aggregated in clusters on hyphae, doliiform to clavate, smooth, pale brown. Conidia 5–6 $\times$ 3–4 μm (n = 30), globose in surface view, lenticular in side view, smooth, brown, with pale equatorial slit.

Culture characteristics: Colonies grew on PDA at 28°C attenuated 3 cm diam. within 7 days, circular, flat, spreading, entire margin, puffy with aerial

mycelia, dirty white in surface view and off-white to yellow in reverse.

Specimen examined: INDIA, Goa, Mhadei WLS, Glade, N 15° 39' 38.28", E 74° 10' 18.12", alt. 727 m, on bamboo culms, 02/03/2022, *Rashmi Dubey*, BSI F-BG-214.

GenBank: ITS = PV767509

Known Distribution: Malaysia (Crous and Groenewald, 2013).

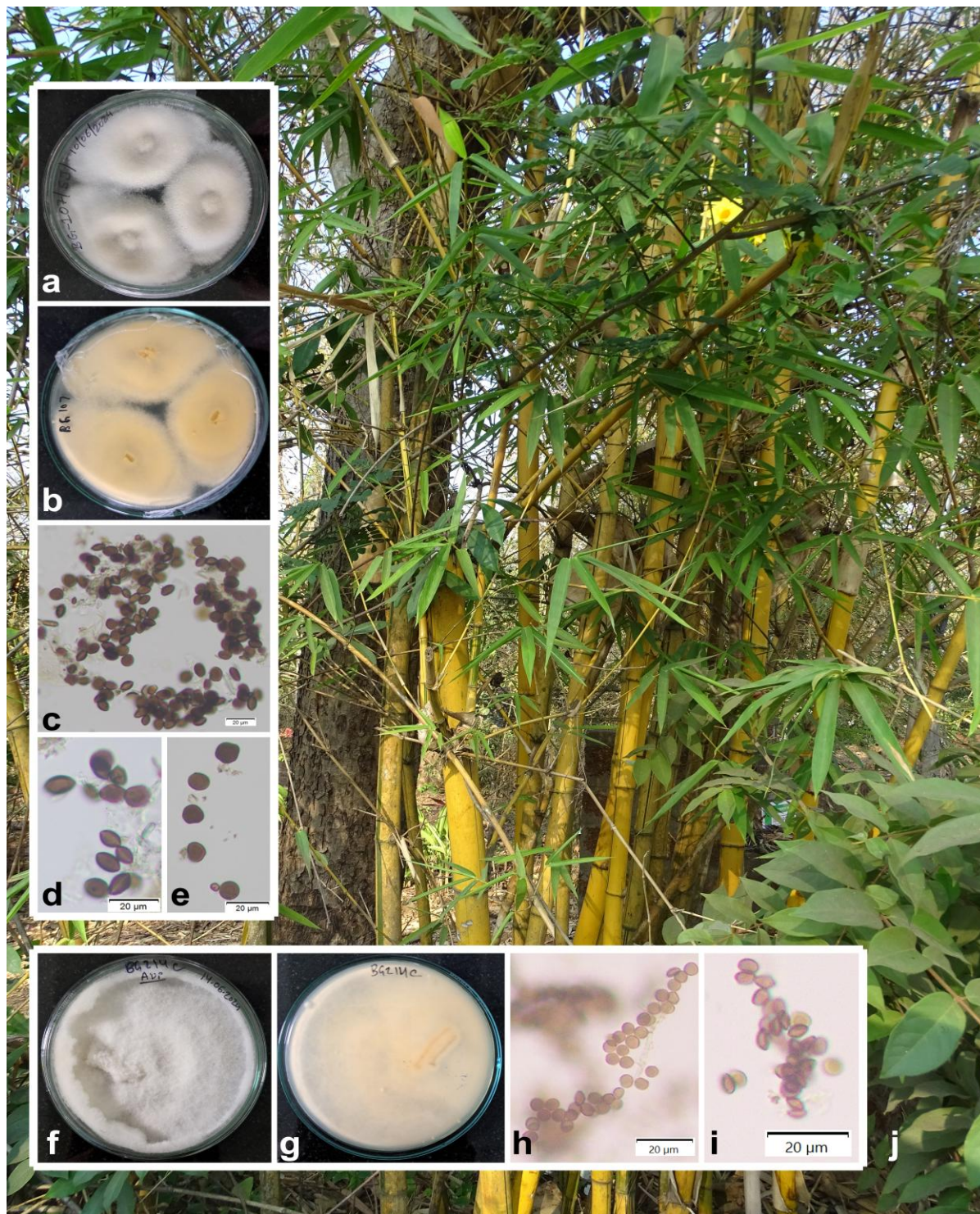


Figure 2: *Apiospora acutiapica* (a-e): **a** Surface view of culture on PDA; **b** Reverse view of culture on PDA; **c** Conidiogenous cells with conidia; **d** Conidia with equatorial slit (side view); **e** Conidia (surface view); *Apiospora malaysiana* (f-i): **f** Surface view of culture on PDA; **g** Reverse view of culture on PDA; **h** Conidiogenous cells with conidia (surface view); **i** Conidia with equatorial slit (side view); **j** Bamboo as a host.

DISCUSSION

The genus *Apiospora* comprises cosmopolitan fungi occurring across a range of substrates including plants, soil, air, and marine environments in diverse climatic zones - tropical, subtropical, temperate, and cold. Members of *Apiospora* function as endophytes, saprobes, or plant pathogens, particularly in the family Poaceae (Pintos and Alvarado, 2021).

This study reports the occurrence of two species of *Apiospora* viz., *A. acutiapica* and *A. malaysiana* on bamboo (Family Poaceae). Review of literature reveals that *A. acutiapica* has been reported from China on dead twigs of *Bambusa bambos* (L.) Voss (Senanayake *et al.*, 2020) while *A. malaysiana* has been reported from Malaysia on *Macaranga hullettii* King ex Hook.f. stem colonised by ants (Crous and Groenewald, 2013). Therefore, this finding adds *A. acutiapica* and *A. malaysiana* to Indian mycobiota and also reports bamboo as a new host for *A. malaysiana*.

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REFERENCES

- Bilgrami, K.S., Jamaluddin, Rizwi, M.A. 1991. *Fungi of India (List and References)*. Today & Tomorrow's Printers and Publishers, New Delhi, India.
- Butler, E.J. and Bisby, G.R. 1931. *The fungi of India*. Imperial Council of Agricultural Research, India Scientific Monographs 1, India.
- Crous, P.W. and Groenewald, J.Z. 2013. A phylogenetic re-evaluation of *Arthrimum*. *IMA Fungus*, **4**(1):133–154; doi: 10.5598/ima fungus.2013.04.01.13.
- Crous, P.W., Schumacher, R.K., Wood, A.R., *et al.*, 2020. The Genera of Fungi–G5: *Arthrimum*, *Ceratosphaeria*, *Dimerosporiopsis*, *Hormodochis*, *Lecanostictopsis*, *Lembosina*, *Neomelanconium*, *Phragmotrichum*, *Pseudomelanconium*, *Rutola*, and *Trullula*. *Fungal Systematics and Evolution*, **5**(1):77–98; doi: 10.3114/fuse.2020.05.04.
- Crous, P.W., Summerell, B.A., Shivas, R.G., *et al.*, 2012. Fungal Planet description sheets: 107–127. *Persoonia*, **28**:138–182; doi: 10.3767/003158512X652633.
- Edler, D., Klein, J., Antonelli, A. *et al.*, 2021. raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, **12**(2):373–377; doi: 10.1111/2041-210X.13512.
- Ellis, M.B. 1965. Dematiaceous Hyphomycetes. VI. *Mycological Papers*, **103**(29):1–46.
- Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**:95–98.
- Hyde, K.D., Fröhlich, J., Taylor, J.E. 1998. Fungi from palms. XXXVI. Reflections on unitunicate ascomycetes with apiospores. *Sydowia*, **50**(1):21–80.
- Hyde, K.D., Norphanphoun, C., Maharachchikumbura, S., *et al.*, 2020. Refined families of Sordariomycetes. *Mycosphere*, **11**(1):305–1059; doi: 10.5943/mycosphere/11/1/7.
- Index Fungorum. 2025. Available at: <https://www.indexfungorum.org/names/Names.asp> (Accessed on August 10, 2025).
- Jamaluddin, Goswami, M.G., Ojha, B.M. 2004. *Fungi of India (1989–2001)*. Scientific Publishers, India.
- Jash, S. and Dubey, R. 2025. *Apiospora* vs. *Arthrimum*: Taxonomic status of Indian species with special reference to their host range and distribution in India. *Kavaka*, **61**(1):40–49; doi: 10.3767/003158515X690269.
- Jiang, H.B., Hyde, K.D., Doilom, M., *et al.*, 2019. *Arthrimum setostromum* (Apiosporaceae, Xylariales), a novel species associated with dead bamboo from Yunnan, China. *Asian Journal of Mycology*, **2**(1):254–268; doi: 10.5943/ajom/2/1/16.
- Jiang, N., Voglmayr, H., Ma, C.Y., *et al.*, 2022. A new *Arthrimum*-like genus

- of *Amphisphaeriales* in China. *MycKeys*, **92**: 27–43; doi: 10.3897/mycokeys.92.86521.
- Katoh, K. and Standley, D.M. 2013. MAFFT multiple sequence alignment software ver. 7: improvement in performance and usability. *Molecular Biology and Evolution*, **30**(4):772–780.
- Lee, J.W., Seo, C.W., Lee, W., *et al.*, 2023. Diversity and Dynamics of Marine Arenicolous Fungi in Three Seasides of the Korean Peninsula. *J Microbiol.*, **61**(1):63–82; doi: 10.1007/s12275-023-00011-1.
- Letunic, I. and Bork, P. 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic acids research*, **52**(W1):W78–W82; doi: 10.1093/nar/gkac268.
- Li, S., Peng, C., Yuan, R. *et al.*, 2023. Morphological and phylogenetic analyses reveal three new species of *Apiospora* in China. *MycKeys*, **99**:297–317; doi: 10.3897/mycokeys.99.108384.
- Liao, C., Senanayake, I.C., Dong, W., *et al.*, 2023. Taxonomic and phylogenetic updates on *Apiospora*: Introducing four new species from *Wurfbainia villosa* and grasses in China. *Journal of Fungi*, **9**(11):1087; doi: 10.3390/jof9111087.
- Manoharachary, C., Atri, N.S., Prameela Devi, *et al.*, 2022. *Bilgrami's Fungi of India: List and References*. Today & Tomorrow's Printers and Publishers, India.
- Pintos, A. and Alvarado, P. 2021. Phylogenetic delimitation of *Apiospora* and *Arthrimum*. *Fungal Systematics and Evolution*, **7**:197–221; doi: 10.3114/fuse.2021.07.10.
- Pintos, Á. and Alvarado, P. 2022. New studies on *Apiospora* (Amphisphaeriales, Apiosporaceae): epitypification of *Sphaeria apiospora*, proposal of *Ap. marianiae* sp. nov. and description of the asexual morph of *Ap. sichuanensis*. *MycKeys*, **92**:63–78; doi: 10.3897/mycokeys.92.87593.
- Réblová, M., Miller, A.N., Rossman, A.Y., *et al.*, 2016. Recommendations for competing sexual-asexually typified generic names in Sordariomycetes (except Diaporthales, Hypocreales, and Magnaporthales). *IMA Fungus*, **7**(1):131–153; doi: 10.5598/ima fungus.2016.07.01.08.
- Sarbhoj, A.K., Agarwal, D.K., Varshney, J.L. 1986. *Fungi of India (1977–1981)*. Associated Publishing Company, India.
- Senanayake, I.C., Bhat, J.D., Cheewangkoon, R. *et al.*, 2020. Bambusicolous *Arthrimum* Species in Guangdong Province, China. *Frontiers in Microbiology*, **11**:602773; doi: 10.3389/fmicb.2020.602773
- Sharma, R., Kulkarni, G., Sonawane, M.S. *et al.*, 2014. A new endophytic species of *Arthrimum* (Apiosporaceae) from *Jatropha podagrica*. *Mycoscience*, **55**(2):118–123; doi: 10.1016/j.myc.2013.06.004.
- Vu, D., Groenewald, M., De Vries, M., *et al.*, 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; doi: 10.1016/j.simyco.2018.05.001.
- Wang, M., Liu, F., Crous, P.W. and Cai, L. 2017. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia*, **39**(1):118–142; doi: 10.3767/persoonia.2017.39.06.
- Wang, M., Tan, X.M., Liu, F. *et al.*, 2018. Eight new *Arthrimum* species from China. *MycKeys*, **34**:1–24; doi: 10.3897/mycokeys.34.24221.
- White, T.J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and application* (Eds: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J.). Academic Press, San Diego, pp.315–322.