FTA-Kunming Scientific Conference 2021

Reappraisal of *Immotthia* in Dictyosporiaceae, Pleosporales: Introducing *Immotthia bambusae* sp. nov. based on morphology and phylogeny

Rungtiwa Phookamsak[1,2], Hong-Bo Jiang[3]

[1]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R. China; [2]CIFOR-ICRAF China Program, World Agroforestry (ICRAF), Kunming 650201, P.R. China; [3]Mae Fah Luang University, Chiang Rai 57100, Thailand.

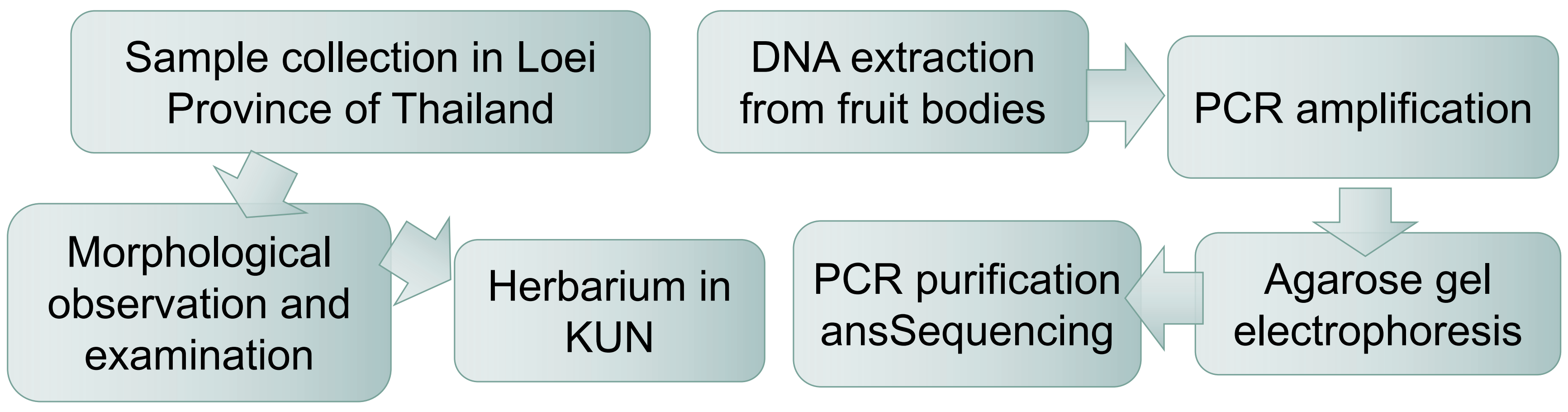
Abstract

Immotthia is a poorly known genus that no DNA sequence data are available to ascertain the proper phylogenetic placement with other bitunicate fungi, and only two species are currently accepted in the genus. The genus is commonly known as an obligate hyperparasite on other fungi (i.e., *Annulohypoxylon*, *Hypoxylon*, and *Pestalopezia*). During our ongoing research study of bambusicolous fungi in Thailand, a fungus associated with stromata of *Hypoxylon* sp. was found on dead bamboo culms in Loei Province, Thailand. Preliminary morphological identification revealed that the fungal collection belongs to *Immotthia*. A novel species, *Immotthia bambusae*, is introduced based on a comparison of morphological characteristics with the type specimen of *I. hypoxylon* (≡ *Amphisphaeria hypoxylon* Ellis & Everh.), a synonym of *I. atrograna* (Cooke & Ellis) M. E. Barr. Multiloci phylogeny of a concatenated ITS, LSU, SSU, and TEF1-a DNA sequence matrix showed that *Immotthia* belongs to Dictyosporiaceae, Pleosporales. Detailed descriptions, illustration, and phylogenetic trees to show the placement of the new taxon are provided.

Introduction

Immotthia was introduced by Barr (1987) with *I. hypoxylon* (Ellis and Everh.) M. E. Barr (≡ *Amphisphaeria hypoxylon* Ellis and Everh.) as the type species. Through examinations of the type material of *I. hypoxylon* and Australian collections of *I. atrograna* (Cooke and Ellis) M. E. Barr (≡ *Sphaeria atrograna* Cooke and Ellis), Jaklitsch et al. (2002) concluded that these two taxa are conspecific. To date, two species are accepted in this genus, viz. *I. atrograna* and *I. atroseptata* (Piroz.) M. E. Barr (Species Fungorum, 2021) based on morphology (Hyde et al., 2017; Doilom et al., 2018). *Immotthia* is characterized by small- to medium-sized, globose to subglobose ascomata, forming on blackened hypostroma, bitunicate, fissitunicate, cylindrical asci, and brown to reddish brown, ellipsoidal to fusiform, 1-septate, smooth or slightly verrucose ascospores (Hyde et al., 2017; Hongsanan et al., 2020). The asexual morph of *Immotthia* has been reported as coelomycetous, identified as *Coniothyrium parasitans* (Berk. and Ravenel) Tassi which formed enteroblastic, phialidic, doliiform to ampulliform, or cylindrical, smooth, hyaline conidiogenous cells bearing brown, ellipsoidal, smooth, and aseptate conidia (Hyde et al., 2017; Hongsanan et al., 2020). However, the link between *Immotthia* and *C. parasitans* has not yet been proven based on DNA sequence analyses. *Immotthia* has been reported as hyperparasites on stromata of *Annulohypoxylon*, *Hypoxylon*, and *Pestalopezia*, or forms compressed ascostromata on decorticated wood (Pirozynski, 1973; Jaklitsch et al., 2002; Akulov and Hayova, 2016; Hyde et al., 2017; Hongsanan et al., 2020). Taxonomic placement of *Immotthia* is variable because of lack of molecular evidence. In the present study, a fresh collection of *Immotthia* is examined and compared with other *Immotthia* species based on morphological characteristics. The new collection is described as a novel species in *Immotthia* and illustrated. Through DNA sequencing of the fresh material, we also resolved the phylogenetic placement of *Immotthia* in Dictyosporiaceae based on maximum likelihood and Bayesian inference analyses.

Materials and Methods



Sample collection in Loei Province of Thailand

DNA extraction from fruit bodies

PCR amplification

Agarose gel electrophoresis

PCR purification ansSequencing

Herbarium in KUN

Morphological observation and examination

Table 1 Primers to amplify fungal nuclear DNA genes will be used in this study.

Target gene	Primers	Nucleotide sequence (5' – 3')	References
ITS1-5.8S-ITS2	ITS1	F: TCCGTAGGTGAACCTGCGG	White et al., 1990
	ITS5	F: GGAAGTAAAAGTCGTAACAAGG	
	ITS4	R: TCCTCCGCTTATTGATATGC	
28S rDNA	LROR	F: ACCCGCTGAACTTAAGC	Vilgalys and Hester, 1990
	LR5	R: TCCTGAGGGAAACTTCG	
18S rDNA	NS1	F: GTAGTCATATGCTTGTCTC	White et al., 1990
	NS4	R: CTTCCGTCAATTCCTTTAAG	
TEF1-α	EF1-983F	F: GCYCCYGGHCAYCGTGAYTTYAT	Rehner, 2001
	EF1-2218R	R: ATGACACCRACRGCRACRGTYTG	

Acknowledgements

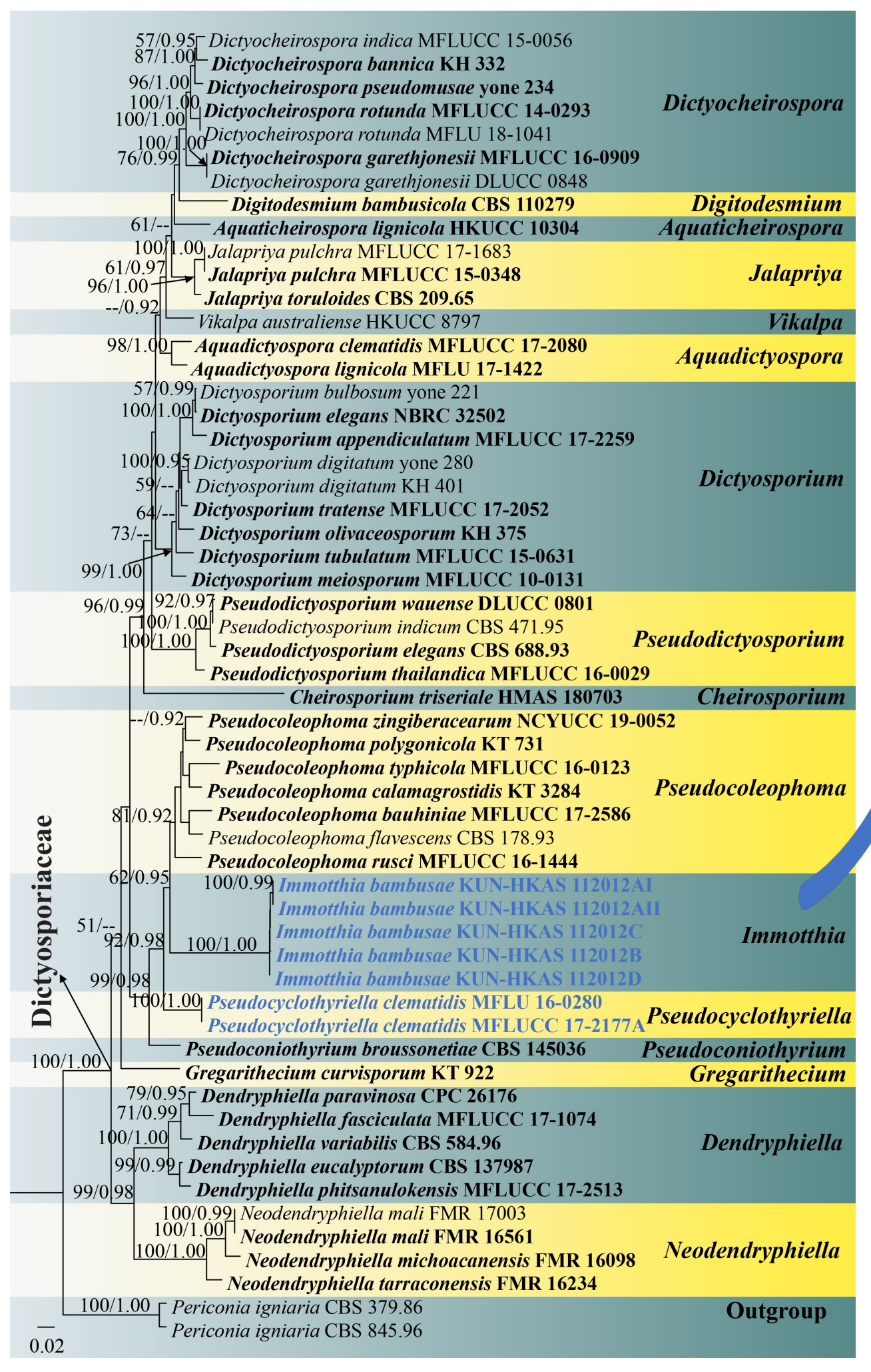
This research was supported by the Key Research Project, Agroforestry Systems for Restoration and Bio-industry Technology Development (grant no. 2017YFC0505101), Ministry of Sciences and Technology of China (grant no. 2017YFC0505100), National Science Foundation of China (NSFC) project code 31850410489 (grant no. Y81I982211), and Chiang Mai University. Rungtiwa Phookamsak sincerely acknowledges the CAS President's International Fellowship Initiative (PIFI) for young staff (grant no. Y9215811Q1). We also thank Shaun Pennycook from Manaaki Whenua - Landcare Research for his assistance in naming the new species in the genus *Immotthia*. Hong-Bo Jiang would like to thank Mae Fah Luang University for a Ph.D scholarship.

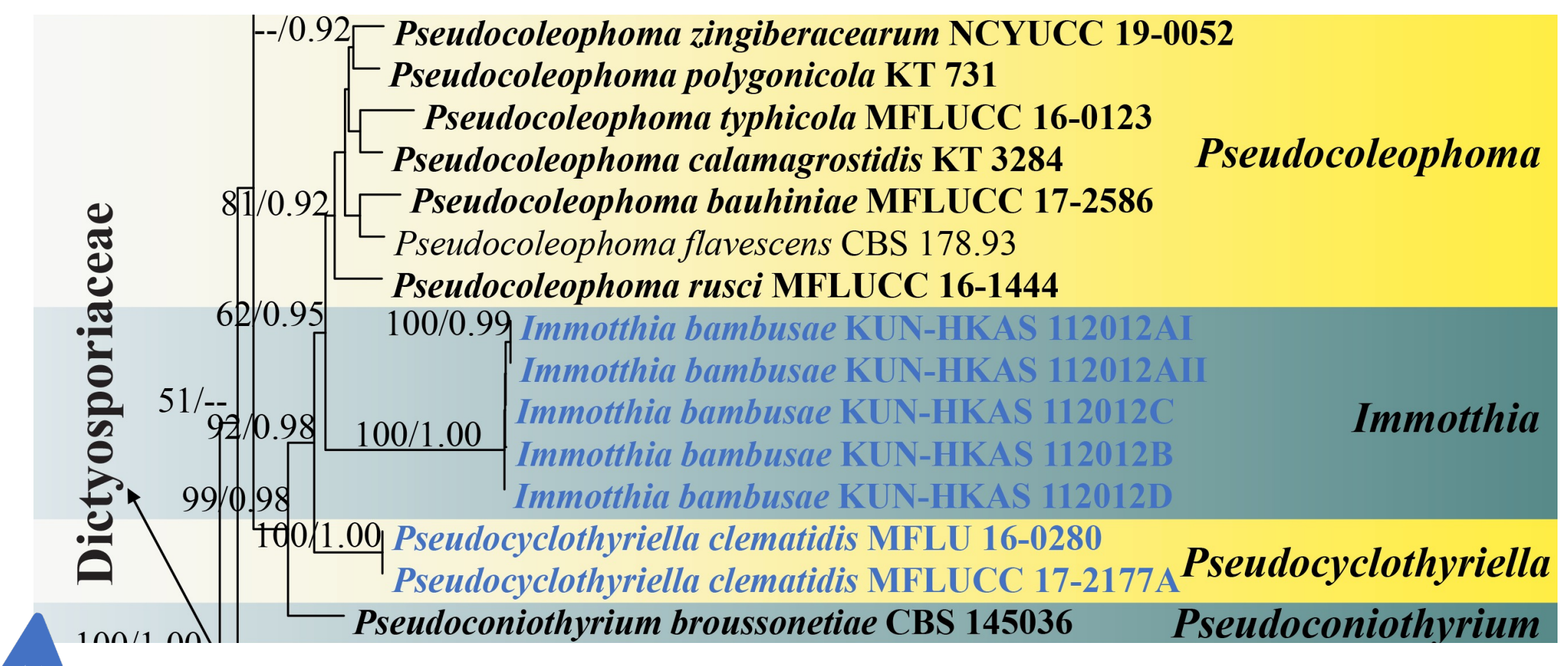
Data Analysis

>>Maximum Likelihood

>>Bayesian Inference

Results





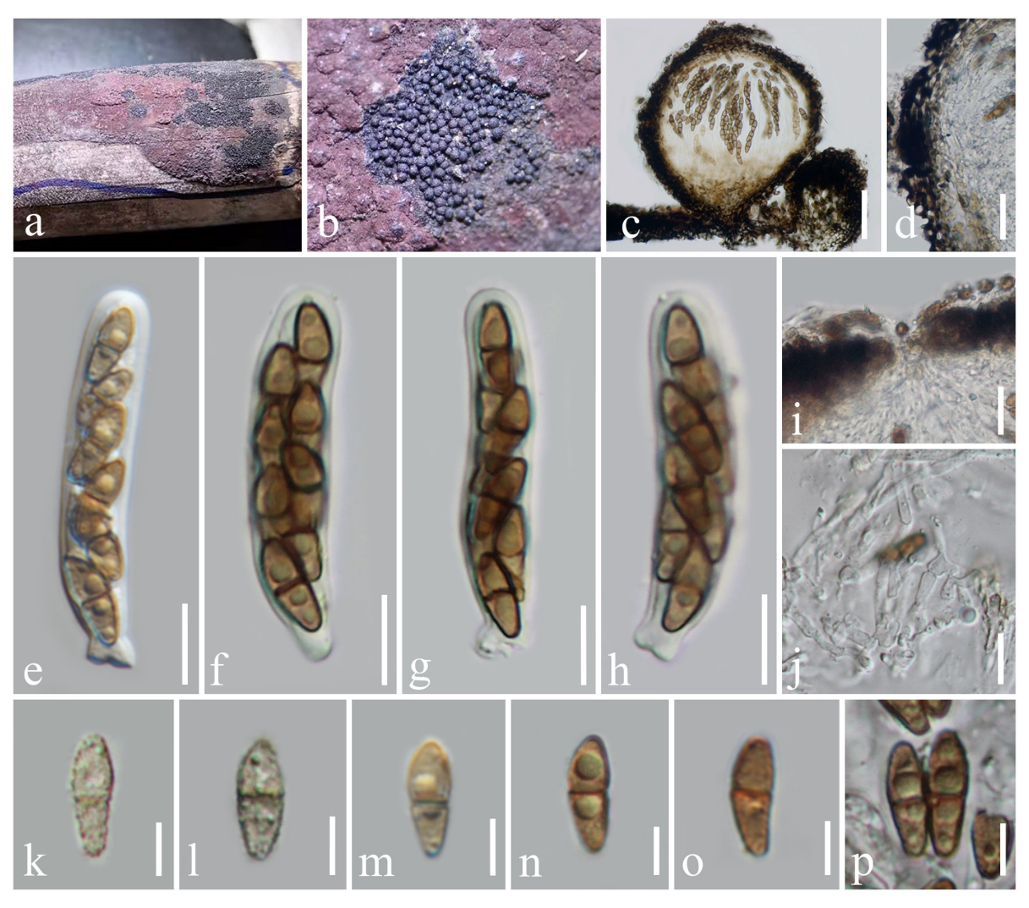


Fig. 1 RAxML tree based on a combined ITS, LSU, SSU and TEF1-α sequence matrix represented the phylogenetic relationships of taxa in Dictyosporiaceae.

Fig. 2 *Immotthia atroseptata* (DAOM 13900; holotype of *Didymosphaeria atroseptata*) and *Immotthia hypoxylon* (NY00830041, holotype of *Amphisphaeria hypoxylon*).

Fig. 3 *Immotthia bambusae* (KUN-HKAS 112012, holotype).

Conclusion

- Phylogenetic placement of *Immotthia* is resolved in to Dictyosporiaceae, Pleosporales for the first time.
- This is the first report of *Immotthia* associated with *Hypoxylon* stromata on bamboo in Thailand.
- *Immotthia* is widely distributed from tropical to temperate regions including Austria, Belgium, China, France, Lithuania, Norway, Poland, Puerto-Rico, Russia, Sweden, Switzerland, Ukraine, USA, and Venezuela (Pirozynski, 1973; Jaklitsch et al., 2002; Akulov and Hayova, 2016; Hyde et al., 2017; Doilom et al., 2018; Farr and Rossman, 2021).
- *Immotthia* does not seem to exhibit a hyperparasitic lifestyle on *Hypoxylon*, but species of this genus were also reported as saprobes on various decayed hardwoods (Jaklitsch et al., 2002).
- *Immotthia bambusae* did not germinate *in vitro*, suggesting that the species has possibly an obligate parasitic life mode, which is in agreement with Jaklitsch et al. (2002).

References

1. Akulov, O. Y., and Hayova, V. P. (2016). *Immotthia atrograna* (Dacampiaceae, Ascomycota), a new for Ukraine fungicolous fungus from Carpathians. Ukr. Bot. J. 73, 84–89.
2. Barr, M. E. (1987). New taxa and combinations in the Loculoascomycetes. Mycotaxon 29, 501–505.
3. Doilom, M., Hyde, K. D., Phookamsak, R., Dai, D. Q., Tang, L. Z., Hongsanan, S., et al. (2018). Mycosphere notes 225–274: types and other specimens of some genera of Ascomycota. Mycosphere 9, 647–754.
4. Farr, D. F., and Rossman, A. Y. (2020). Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Available online at: https://nt.ars-grin.gov/ fungaldatabases/ (accessed Jan, 2021).
5. Hongsanan, S., Hyde, K. D., Phookamsak, R., Wanasinghe, D. N., McKenzie, E. H. C., Sarma, V. V., et al. (2020). Refined families of Dothideomycetes: Dothideomycetidae and Pleosporomycetidae. Mycosphere 11, 1553–2107.
6. Hyde, K. D., Norphanphoun, C., Abreu, V. P., Bazzicalupo, A., Chethana, K. W. T., Clericuzio, M., et al. (2017). Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. Fungal Divers. 87, 1–235.
7. Jaklitsch, W. M., Scheuer, C., and Voglmayr, H. (2002). Notes on the genus *Immotthia* (Pleosporales, Ascomycetes), including some type studies. Osterr. Z. Pilzkd. 11, 93–106.
8. Pirozynski, K. A. (1973). Three hyperparasites of ascomycetes. Mycologia 65, 761–767.
9. Rehner, S. (2001). Primers for Elongation Factor 1-Alpha (EF1-Alpha). Available online at: http://ocid.NACSE.ORG/research/deephyphae/ EF1primer.pdf (accessed June 20, 2020).
10. Species Fungorum (2021). Species Fungorum. Available online at: http://www.speciesfungorum.org/Names/Names.asp (assessed Jan, 2021).
11. Vilgalys, R., and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172, 4238–4246.
12. White, T. J., Bruns, T., Lee, S., and Taylor, J. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," in PCR Protocols, A Guide to Methods and Applications, eds M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (San Diego, CA: Academic), 315–322.