

RESEARCH ARTICLE

Morphological and phylogenetic appraisal of *Ophioceras* (Ophiocerales, Magnaporthales)

Hong-Bo Jiang^{1,2,3,4,5}, Kevin D. Hyde^{1,2,3,6,7}, Er-Fu Yang^{2,5,8,9}, Pattana Kakumyan⁴, Ali H. Bahkali⁶, Abdallah M. Elgorban⁶, Samantha C. Karunarathna^{2,5,10}, Rungtiwa Phookamsak^{1,2,5,10*}, Saisamorn Lumyong^{1,8,11*}

1 Faculty of Sciences, Research Center of Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai, Thailand, **2** CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, P.R. China, **3** Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand, **4** School of Science, Mae Fah Luang University, Chiang Rai, Thailand, **5** Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Honghe County, Yunnan, P.R. China, **6** Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, **7** Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Haizhu District, Guangzhou, Guangdong, P.R. China, **8** Faculty of Science, Department of Biology, Chiang Mai University, Chiang Mai, Thailand, **9** Faculty of Science, Master of Science Program in Applied Microbiology (International Program), Chiang Mai University, Chiang Mai, Thailand, **10** CIFOR-ICRAF China Program, World Agroforestry (ICRAF), Kunming, Yunnan, China, **11** Academy of Science, The Royal Society of Thailand, Bangkok, Thailand

* jomjam.rp2@gmail.com (RP); scboi009@gmail.com (SL)



OPEN ACCESS

Citation: Jiang H-B, Hyde KD, Yang E-F, Kakumyan P, Bahkali AH, Elgorban AM, et al. (2021) Morphological and phylogenetic appraisal of *Ophioceras* (Ophiocerales, Magnaporthales). PLoS ONE 16(8): e0253853. <https://doi.org/10.1371/journal.pone.0253853>

Editor: Tamás Papp, University of Szeged, HUNGARY

Received: November 28, 2020

Accepted: June 14, 2021

Published: August 25, 2021

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0253853>

Copyright: © 2021 Jiang et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its [Supporting Information](#) files.

Abstract

Ophioceras is accommodated in the monotypic family Ophiocerales (Magnaporthales, Sordariomycetes), and the genus is delimited based on molecular data. During an ongoing survey of bambusicolous fungi in southwest China, we collected a submerged decaying branch of bamboo from Sichuan Province, China and an *Ophioceras* species occurring on this substrate was observed and isolated. An *Ophioceras* taxon was delimited based on morphological characteristics and combined LSU, RPB1 and ITS sequence analyses and is described as *Ophioceras sichuanense* sp. nov. The species formed a well-supported clade basal to *Ophioceras* (100% ML, 1.00 PP). Based on the updated phylogenetic tree of Magnaporthales, *Ceratosphaerella castillensis* (generic type) and *C. rhizomorpha* formed a clade within *Ophioceras* and morphologically resemble *Ophioceras*. Therefore, *Ceratosphaerella* is synonymized under *Ophioceras*. The phylogenetic relationships of *Ophioceras* are discussed in relation to morphological similarities of genera in Magnaporthales. The generic circumscription of *Ophioceras* is emended.

Introduction

Klaubauf et al. [1] introduced the family Ophiocerales to accommodate *Ophioceras* Sacc. which is typified by *O. dolichostomum* (Berk. & M.A. Curtis) Sacc. The family is recognized as black, immersed to superficial, globose to subglobose, perithecial ascomata with long, periphysate necks, 8-spored, unitunicate, subcylindrical to narrowly fusoid asci with a J-, apical ring,

Funding: The authors are grateful for the support of the Mushroom Research Foundation, Chiang Rai, Thailand (to H-BJ) and Yunnan Provincial Science and Technology Department grant no. 202003AD150004 (to RP and H-BJ under Jianchu Xu). KDH thanks the Foreign Experts Bureau of Yunnan Province, Foreign Talents Program (2018; grant no. YNZ2018002), Impact of Climate Change on Fungal Diversity and Biogeography in the Greater Mekong Subregion (grant no: RDG6130001). RP sincerely acknowledges the CAS President's International Fellowship Initiative (PIFI) for young staff (grant no. Y9215811Q1), the "High-level Talent Support Plan" Young Top Talent Special Project of Yunnan Province and Chiang Mai University for financial support. SCK thanks the CAS President's International Fellowship Initiative (PIFI) young staff under the grant number: 2020FYC0002 and the National Science Foundation of China (NSFC) under the project code 31851110759 for funding. AHB and AME thank the International Scientific Partnership Program ISPP at King Saud University through ISPP#0089. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

and filiform, hyaline to olivaceous, septate ascospores without sheaths [1–3]. Species of Ophiocerales are saprobes on wood and herbaceous plants in aquatic or terrestrial habitats [1–6].

Saccardo [7] introduced *Ophioceras* to accommodate taxa with immersed, sub carbonaceous, globose perithecia, with conical-cylindrical, filiform ostioles, elongate asci and filiform, septate ascospores. The genus initially accommodated *O. bacillatum* (Cooke) Sacc., *O. dolichostomum*, *O. friesii* (Mont.) Sacc., *O. hystrix* (Ces.) Sacc., *O. macrocarpum* (Sacc.) Sacc., *O. longisporum* Sacc. and *O. therryanum* (Sacc. & Roum.) Sacc [7]. *Ophioceras* was accommodated in Magnaporthaceae based on limited molecular data [8–10]. However, the genus was excluded from Magnaporthaceae and accommodated in the separate family Ophiocerales in Magnaporthales based on combined LSU and RPB1 phylogenetic analyses [1]. To date, the asexual morph of *Ophioceras* has not yet been reported [1,2,6], and only 40 epithets are listed under this genus in Index Fungorum [11]. However, only eleven *Ophioceras* species have molecular data in GenBank, and only LSU and ITS sequence data are most available for these species [12].

Ophioceras species are commonly discovered from wood in freshwater and are generally clumped under the name *Ophioceras* sp. [13,14], *O. commune* [15–17] or *O. dolichostomum* [18–21]. Twenty-three *Ophioceras* species are listed in Species Fungorum [22] and accepted in Hyde et al. [3]. *Ophioceras* species occur in disparate streams in different islands and continents and are therefore likely to have separated for millions of years ago, potentially explaining their evolution into distinct species. The number of existing *Ophioceras* species is therefore likely to exceed more than presently known. Hyde et al. [23] has shown that numerous new taxa await description across most under-collected and -studied.

Ceratosphaerella comprises *C. castillensis* (C.L. Sm.) Huhndorf, Greif, Mugambi & A.N. Mill. and *C. rhizomorpha* Huhndorf & Mugambi, introduced by Huhndorf et al. [8]. Phylogenetic analyses of the LSU sequence dataset showed that *Ceratosphaerella* grouped with *Ophioceras* in Magnaporthaceae [8]. Klaubauf et al. [1] only accommodated *Ophioceras* into Ophiocerales, but they did not incorporate *Ceratosphaerella* in their analyses. Thus, *Ceratosphaerella* remains in Magnaporthaceae. To date, there are only two species included in *Ceratosphaerella* [11], and these taxa only have LSU sequence data in GenBank [12].

Ceratosphaerella castillensis has been reported only as a sexual morph, while *C. rhizomorpha* is holomorphic and has a Didymobotryum-like asexual morph [8]. In Luo et al. [2], *Ceratosphaerella* did not group within Magnaporthaceae but clustered with *Ophioceras* in Ophiocerales. However, Luo et al. [2] did not verify the phylogenetic status of *Ceratosphaerella* in Ophiocerales, leading to the uncertain placement of the genus.

This study aims to introduce the novel *Ophioceras* taxon on a submerged bamboo branch in Sichuan Province, China and resolve the congeneric status of *Ceratosphaerella* and *Ophioceras* in Ophiocerales based on a morpho-molecular approach.

Materials and methods

Sample collection, observation and isolation

Decaying branches of bamboo submerged in freshwater were collected in the stream in the Shunan Artificial Bamboo Forest, Sichuan Province, P.R. China in July 2019. Samples were kept in a paper bag for further morphological examination in the laboratory. Pseudostromata visualized on decaying branches of bamboo were observed and examined under a stereo microscope (Motic series SMZ 140) and captured via digital phone camera (iPhone 7, Apple Inc., USA). Microscopic features (e.g., asci, ascospores and paraphyses) were prepared using the squashing mount technique in sterilized distilled water on clean slides for morphological study. Sections of pseudostromatic ascomata, ostiolar necks and peridial structures were by

free-hand sectioning using Gillette razor blades. Melzer's reagent and Indian ink were used to detect the J-/J+ apical ring of the asci and mucilaginous sheath surrounding the ascospores, respectively. Morphological features visualized under a Nikon ECLIPSE Ni compound microscope were photographed using a Canon EOS 600D digital camera. Measurements (n = 10–20) of pseudostromata, locules, peridium, paraphyses, asci and ascospores were carried out using Tarosoft (R) Image Frame Work version 0.9.7. Photographic plate and line drawings of fungal morphologies were edited and combined using Adobe Photoshop CS6 (Adobe Systems Inc., USA).

Single spore isolation based on the spore suspension technique [24] was carried out to obtain a pure fungal culture. Germinated ascospores were transferred to the new potato dextrose agar plates (PDA; Qingdao Daily Water Biotechnology co. LTD. Shandong, P.R. China) under aseptic conditions and grown under normal day/nightlight at room temperature. Culture characteristics (e.g., growth, shape, colour, margin, elevation, consistency) were checked and recorded after one week and four weeks.

The holotypic specimen is conserved in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, P.R. China. The isotype is stored in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). Ex-type living cultures are preserved in the Kunming Institute of Botany Culture Collection (KUMCC) and Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi and Index Fungorum numbers were registered for the new taxon [11,25].

DNA extraction, amplification and sequencing

Fungal genomic DNA was extracted from fresh mycelia using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux[®], P.R. China) following manufacturer's instructions (Hangzhou, P.R. China) and also extracted from fruiting bodies (= pseudostromata) directly using the Forensic DNA Kit (Omega[®], USA) for a duplicated strain. DNA amplification was performed by polymerase chain reaction (PCR). Two gene regions including the internal transcribed spacer (ITS) and 28S large subunit rDNA (LSU), were used to amplify PCR fragments using forward and reverse primer pairs: ITS5/ITS4 [26] and LR0R/LR5 [27], respectively. PCR reactions were conducted in a 25 µL total volume, consisted of 2 µL of DNA template, 1 µL of each forward and reverse primer, 12.5 µL of 2× Power Taq PCR Master Mix (Beijing BioTeke Corporation, P.R. China) and 8.5 µL double-distilled water (ddH₂O). The PCR thermal cycle program for ITS and LSU was set up following Jiang et al. [28]. PCR fragments were purified and sequenced at TsingKe Biological Technology (Beijing) Co., Ltd, P.R. China.

Molecular phylogeny

The newly generated sequences (ITS and LSU) of fungal strains were initially subjected to the basic local alignment search tool (BLASTn) via the National Center for Biotechnology Information web portal (NCBI; <https://blast.ncbi.nlm.nih.gov>) for discovering closely related fungal taxa. In order to clarify the phylogenetic placement of the new isolate, the representative taxa in Magnaporthales were incorporated with the new taxon to generate the sequence data matrix for further analysis. These representative taxa of Magnaporthales were downloaded from the GenBank database (Table 1) based on recent publications [2,29].

Preliminary single-gene data matrixes were aligned via MAFFT v. 7.452 [30] and improved manually in BioEdit v. 5.0.6 [31]. The single-gene alignments of LSU and ITS data matrixes were prior analyzed by maximum-likelihood (ML) criterion using RAxML v. 7.0.3 [32,33] for checking if there are any conflicts between the tree topologies. The concatenated LSU-ITS and LSU-RPB1-ITS sequence datasets were further analyzed based on maximum-likelihood (ML)

Table 1. Detailed information of fungal taxa used in the phylogenetic analyses. The newly generated sequences are indicated in **blue**, and the ex-type strains are in **bold**.

Species name	Culture collection/ Voucher no.	GenBank accession numbers		
		LSU	RPB1	ITS
<i>Aquafiliformis lignicola</i>	MFLUCC 16–1341	MK835815	/	MK828615
<i>Aquafiliformis lignicola</i>	MFLUCC 18–1338	MK835814	/	MK828614
<i>Bambusicularia brunnea</i>	INA-B-92-45	NG_058671	KM485043	NR_145387
<i>Barretomyces calathea</i>	CBS 129274	MH876639	KM485045	MH865202
<i>Bifusisporella sorghi</i>	URM 7442	NG_067852	MK060159	NR_164042
<i>Budhanggurabania cynodonticola</i>	BRIP 59305	NG_058678	KP162143	NR_137952
<i>Buergenerula spartinae</i>	ATCC 22848	DQ341492	JX134720	JX134666
<i>Bussabanomyces longisporus</i>	CBS 125232	NG_058668	KM485046	NR_145385
<i>Ceratospaeria aquatica</i>	MFLU 18–2323	MK835812	/	MK828612
<i>Ceratospaeria lampadophora</i>	SMH 4822	AY346270	/	/
<i>Ceratospaeria lignicola</i>	MFLU 18–1457	MK835813	/	MK828613
<i>Deightoniella roumegueri</i>	CBS 128780	MH876533	KM485047	MH865092
<i>Falciphora oryzae</i>	CBS 125863	NG_064356	KJ026706	NR_153972
<i>Falciphoriella solaniterrestris</i>	CBS 117.83	NG_058108	KM485058	NR_153995
<i>Gaeumannomyces caricis</i>	CBS 388.81	NG_058109	KM485059	NR_146245
<i>Gaeumannomyces amomi</i>	CMUZE002	DQ341493	/	AY265318
<i>Gaeumannomyces radicola</i>	CBS 296.53	NG_058089	KM009194	NR_146246
<i>Gaeumannomyces tritici</i>	CBS 541.86	DQ341497	/	/
<i>Kohlmeyeriopsis medullaris</i>	CBS 117849	NG_058110	KM485068	NR_154068
<i>Macgarvieomyces borealis</i>	CBS 461.65	NG_058088	KM485070	NR_145384
<i>Macgarvieomyces juncicola</i>	CBS 610.82	KM484970	KM485071	KM484855
<i>Magnaporthiopsis incrustans</i>	M35	JF414892	JF710437	JF414843
<i>Magnaporthiopsis maydis</i>	CBS 133165	KX306614	/	KX306544
<i>Magnaporthiopsis maydis</i>	CBS 662.82A	NG_058111	KM485072	NR_154175
<i>Magnaporthiopsis poae</i>	M47	JF414885	JF710433	JF414836
<i>Muraeriata africana</i>	GKM 1084	EU527995	/	/
<i>Muraeriata collapsa</i>	SMH 4553	EU527996	/	/
<i>Myrmecridium schulzeri</i>	CBS 100.54	EU041826	/	EU041769
<i>Myrmecridium sorbicola</i>	CBS 143433	NG_063957	/	NR_158871
<i>Nakataea oryzae</i>	CBS 252.34	MH867001	KM485078	KM484862
<i>Nakataea oryzae</i>	CBS 288.52	MH868571	KM485080	MH857040
<i>Neocordana malayensis</i>	CBS 144604	NG_066327	/	NR_163364
<i>Neocordana musae</i>	CPC 18127	LN713290	/	NR_154265
<i>Neogaumannomyces bambusicola</i>	MFLUCC 11–0390	NG_059556	/	NR_146247
<i>Neopyricularia commelinicola</i>	CBS 128308	NG_058112	KM485087	NR_154226
<i>Ommidemptus affinis</i>	ATCC 200212	NG_059478	JX134728	NR_154292
<i>Ommidemptus graminis</i>	CBS 138107	MK487734	/	NR_164058
<i>Ophioceras aquaticus</i>	IFRDCC 3091	JQ797433	/	JQ797440
<i>Ophioceras aquaticus</i>	MFLUCC 16–0906	MK835810	/	MK828611
<i>Ophioceras castillensis</i> (as <i>Ceratospaeria castillensis</i>)	SMH 1865	EU527997	/	/
<i>Ophioceras chiangdaoense</i>	CMU 26633	NG_066356	/	/
<i>Ophioceras commune</i>	KUN-HKAS 92569	MH795820	/	MH795815
<i>Ophioceras commune</i>	KUN-HKAS 92587	MH795819	/	MH795814
<i>Ophioceras commune</i>	KUN-HKAS 92590	MK835809	/	MK828610
<i>Ophioceras commune</i>	KUN-HKAS 92640	MH795818	/	MH795813

(Continued)

Table 1. (Continued)

Species name	Culture collection/ Voucher no.	GenBank accession numbers		
		LSU	RPB1	ITS
<i>Ophioceras dolichostomum</i>	CMURp50	DQ341504	/	/
<i>Ophioceras hongkongense</i>	HKUCC 3624	DQ341509	/	/
<i>Ophioceras leptosporum</i>	CBS 894.70	NG_057959	JX134732	NR_111768
<i>Ophioceras rhizomorpha</i> (as <i>Ceratosphaerella rhizomorpha</i>)	GKM 1262	EU527998	/	/
<i>Ophioceras sichuanense</i>	KUN-HKAS 107677	MW057782	/	MW057779
<i>Ophioceras sichuanense</i>	KUMCC 20-0213	MT995046	/	MT995045
<i>Ophioceras submersum</i>	MFLUCC 18-0211	MK835811	/	/
<i>Proxypyricularia zingiberis</i>	CBS 133594	NG_063934	KM485091	AB274434
<i>Pseudohalonestria fagicola</i>	MFLUCC 15-1117	KX426219	/	/
<i>Pseudohalonestria hampshirensis</i>	MFLUCC 15-0774	KX426218	/	/
<i>Pseudohalonestria lignicola</i>	SMH 2440	AY346299	/	/
<i>Pseudohalonestria lutea</i>	CBS 126574	MH875622	/	MH864160
<i>Pseudophialophora eragrostis</i>	CM12m9	KF689638	KF689618	NR_146240
<i>Pseudopyricularia cyperi</i>	CBS 133595	NG_058113	/	NR_137920
<i>Pseudopyricularia kyllingae</i>	CBS 133597	NG_058114	KM485096	NR_155645
<i>Pyricularia ctenantheicola</i>	GR0001	KM484994	KM485098	KM484878
<i>Pyricularia grisea</i>	BR0029	KM484995	KM485100	KM484880
<i>Pyriculariopsis parasitica</i>	HKUCC 5562	DQ341514	/	/
<i>Slopeiomyces cylindrosporus</i>	CBS 609.75	KM485040	KM485158	KM484944
<i>Slopeiomyces cylindrosporus</i>	CBS 610.75	NG_057751	JX134721	NR_120170
<i>Xenopyricularia zizaniicola</i>	CBS 132356	KM485042	KM485160	KM484946

Abbreviations: ATCC: American Type Culture Collection, Virginia, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CMU: Chiang Mai University, Chiang Mai, Thailand; CPC: Culture Collection of Pedro Crous, Netherlands; KUN-HKAS: the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Yunnan, P.R. China; HKUCC: Hong Kong University Culture Collection, Hong Kong, P.R. China; IFRDCC: Fungal Research & Development Centre Culture Collection, P.R. China; KUMCC: Kunming Culture Collection, Yunnan, P.R. China; MFLU: the Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

<https://doi.org/10.1371/journal.pone.0253853.t001>

and Bayesian inference (BI) criteria and the tree topologies of these combined gene datasets were compared for checking the congruence of the tree topologies. The concatenated LSU-ITS and LSU-RPB1-ITS sequence datasets comprise 64 strains of ingroup taxa in Magnaporthales. *Myrmecridium schulzeri* (CBS 100.54) and *M. sorbicola* (CBS 143433) were selected as the outgroup taxa.

Maximum-likelihood (ML) criterion was analyzed by the online tool RAxML-HPC v.8 on XSEDE (8.2.12) via CIPRES Science Gateway v. 3.3 web portal [34]. The ML + thorough bootstrap parameters were set at default values but modified as 1000 replications of bootstraps (-N 1000) and using the GTRGAMMAI model.

The best-fit evolutionary models of nucleotide substitution for LSU, RPB1 and ITS loci were evaluated by MrModeltest 2.3 [35], and the GTR+I+G substitution model under the Akaike Information Criterion (AIC) was the best-fit evolutionary model for each locus. Bayesian inference (BI) analysis was performed by MrBayes v. 3.1.2 [36]. The Markov Chain Monte Carlo sampling (MCMC) sampling method was used to determine posterior probabilities (PP) [37,38]. One million generations of six simultaneous Markov chains were run and sampled every 100th generation. MCMC heated chain was set up with a “temperature” value at 0.15. The burn-in was set to 20% of all sampled trees, meaning that sampled trees beneath the

asymptote (20%) were discarded. Posterior probabilities values were then calculated from the remaining 8000 trees in the majority rule consensus tree.

The final phylogram presented in this study was visualized in FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). The phylogenetic tree was edited in Microsoft Office PowerPoint 2016 (Microsoft Inc., USA) and converted to jpeg file using Adobe Photoshop CS6 (Adobe Systems Inc., USA). New sequences generated from the present study were registered for GenBank accession numbers (Table 1). The final alignment and phylogram are submitted in TreeBASE submission ID: 28293 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S28293?x-access-code=66338d666c9ae6b7c0a0aa779b50078d&format=html>).

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to Index Fungorum from where they will be made available to the Global Names Index. The unique Index Fungorum number can be resolved, and the associated information viewed through any standard web browser by appending the Index Fungorum number contained in this publication to the prefix www.indexfungorum.org/. The online version of this work is archived and available from the following digital repositories: PubMed Central and LOCKSS.

Compliance with ethical standards

There is no conflict of interest (financial or non-financial) and all authors have agreed to submission of paper. The authors also declare that they have no conflict of interest and confirm that the field studies did not involve endangered or protected species.

Results

Molecular phylogeny

Based on the results from the nucleotide BLAST search tool of LSU sequence, our new strains (KUMCC 20–0213 and KUN-HKAS 107677) are closely related to species of *Ophioceras*, whereas ITS sequence revealed that our new strains are similar to the unidentified fungal endophyte isolate 4583 (86.79% similarity) and other taxa in Magnaporthales. The concatenated LSU-RPB1-ITS dataset included 2594 total characters with gaps (LSU: 1–905 bp, RPB1: 906–1877 bp, ITS: 1878–2594 bp). The best scoring ML tree with the final ML optimization likelihood value of -23570.931644 (ln) was selected to represent the phylogenetic relationships of taxa in Magnaporthales (Fig 1). All free model parameters were estimated using the GTRGAMMAI model, with 1354 distinct alignment patterns and 40.89% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.247576, C = 0.255381, G = 0.292293, T = 0.204750, with substitution rates AC = 1.508478, AG = 2.781197, AT = 1.835341, CG = 0.983246, CT = 6.427316, GT = 1.000000. The Tree-Length = 7.381586 and the gamma distribution shape parameter $\alpha = 0.605644$. The evaluation of Bayesian posterior probabilities (BYPP) from MCMC was carried out with the final average standard deviation of split frequencies reached 0.009301.

The tree topology from ML analysis showed similar results with the BI analysis and comparing LSU-ITS, and LSU-RPB1-ITS phylograms also revealed similarities in overall

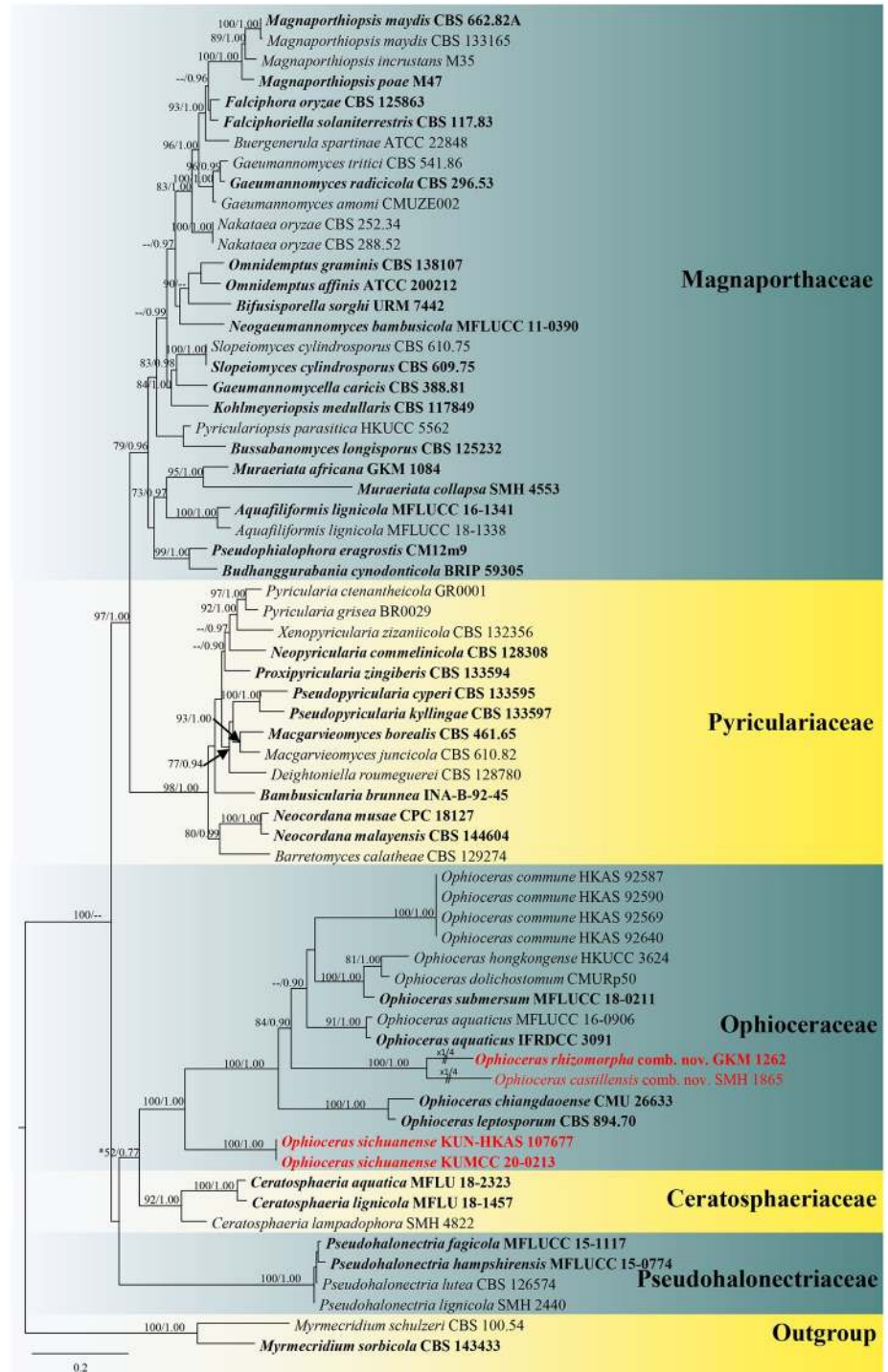


Fig 1. Maximum likelihood tree based on a combined LSU, RPB1 and ITS sequence matrix for taxa in Magnaporthales. Bootstrap support values for ML equal to or higher than 70% and the Bayesian posterior probabilities equal to or higher than 0.90 PP are defined above the nodes as ML/PP. Ex-type strains are in black bold, and new species and new combinations are indicated in red bold.

<https://doi.org/10.1371/journal.pone.0253853.g001>

topologies (Fig 1 and S2 Fig). Thus, we will use the LSU-RPB1-ITS topology for further discussion. Five families of Magnaporthales were included in the presented phylogenetic analyses viz. Ceratosphaeriaceae, Magnaporthaceae, Ophioceraeae, Pseudohalonectriaceae and Pyriculariaceae. These five families formed well-resolved monophyletic clades within Magnaporthales with significant support (greater than 70% ML and 0.95 PP) in our combined gene analyses (Fig 1 and S2 Fig). Ophioceraeae has a close relationship with Ceratosphaeriaceae and Pseudohalonectriaceae. However, the phylogenetic relationships of these three families are not well resolved and pending further clarification.

Phylogenetic analyses of the LSU-RPB1-ITS sequence matrix revealed that the investigated specimen (KUN-HKAS 107677) and its pure culture (KUMCC 20–0213) are grouped together and form an independent lineage basal to *Ophioceras* in Ophioceraeae with high statistical support (100% ML, 1.00 PP; Fig 1). Considering the phylogenetic results and morphology, we propose a novel species, *Ophioceras sichuanense*, occurring on submerged bamboo in Sichuan Province, China.

Ceratosphaeria castillensis (SMH 1865) formed a robust clade with *C. rhizomorpha* (GKM 1262) (100% ML, 1.00 PP; Fig 1) within *Ophioceras* (84% ML, 0.90 PP; Fig 1). *Ceratosphaeria castillensis* (SMH 1865) and *C. rhizomorpha* (GKM 1262) clustered with *Ophioceras aquaticus* (IFRDCC 3091, MFLUCC 16–0906), *O. dolichostomum* (CMURp50), *O. hongkongense* (HKUCC 3624), *O. submersum* (MFLUCC 18–0211) and *O. commune* (KUN-HKAS 92569, KUN-HKAS 92587, KUN-HKAS 92590, KUN-HKAS 92640) in our all analyses (Fig 1, S1 and S2 Figs) and separated distantly from taxa in Magnaporthaceae. Thus, *Ceratosphaerella* is treated as a synonym of *Ophioceras*, the prior introduced genus, in Ophioceraeae.

Taxonomy

Ophioceraeae Klaubauf, Lebrun & Crous, Studies in mycology 79: 103 [1].

Type genus: *Ophioceras* Sacc.

Notes—To date, Ophioceraeae includes a single genus, *Ophioceras*. In the present phylogenetic study, Ophioceraeae formed a stable clade within Magnaporthales and distinguished from other families of Magnaporthales.

Ophioceras Sacc., Sylloge Fungorum 2: 358 (1883), emend. H.B. Jiang, Phookamsak & K.D. Hyde.

Facesoffungi number: FoF01255.

Synonym: *Ceratosphaerella* Huhndorf, Greif, Mugambi & A.N. Mill., Mycologia 100(6): 941 [8].

Type species: *Ophioceras dolichostomum* (Berk. & M.A. Curtis) Sacc.

Saprobic on bamboo, palm, bark or wood, and other herbaceous plants from aquatic or terrestrial environments. **Sexual morph:** *Ascomata* black, perithecial, immersed to superficial, scattered or gregarious, globose to subglobose, or ampulliform, glabrous with ostiolar necks, somewhat forming uni- to multi-loculate pseudostromata. *Pseudostromata if present:* locules immersed in pseudostroma, dark brown to black, subglobose to ampulliform, or irregular in shape, with a long, cylindrical, black, brittle, curved or straight, periphysate neck. *Peridium* composed of several layers, of pseudoparenchymatous cells, arranged in *textura angularis*, inner layers composed of hyaline, elongate cells, with compressed, dark brown to black cells towards the outer layers. *Paraphyses* filiform, hyaline, unbranched, septate, broad at the base, tapering at the tip. *Asci* unitunicate, 8-spored, subcylindrical to acerose or clavate, pedicellate or sessile, with a refractive J-, apical ring. *Ascospores* filiform or narrow fusiform, with rounded ends, slightly curved or sigmoidal, hyaline, pale brown or olivaceous, aseptate or septate, with or without guttulate, lacking a sheath. **Asexual morph:** Hyphomycetous,

Didymobotryum-like. *Colony on substrates* dark brown to black, rhizomorphic-like threads, radiated from central of clustered ascomata on patched subiculum. *Synnemata* formed on rhizomorphic strands, dichotomously branched hyphae, straight or flexuous, branched, lighter brown head, black in mass, with conidiophores at the apical region. *Conidiophores* elongate, septate, with dark brown bands at the septa, verrucose. *Conidiogenous cells* pale brown, cylindrical, tetric, integrated, terminal, verrucose. *Conidia* pale brown, with darker brown at the septa, ellipsoid to cylindrical, 1–3 transverse septa, verrucose [8].

Notes—*Ophioceras* occurs on a wide range of hosts mainly distributed in America, Asia, Africa and Oceania [2,5,8,9,13–17,39–53]. To date, only *O. bambusae* and *O. guttulatum* have been reported from bamboo [51,53,54]. Eleven *Ophioceras* species have been reported from freshwater [2,10], of which nine species were found in China [10,17,41,53]. In this study, *O. sichuanense* is introduced as the second species occurring on submerged decaying branches of bamboo in China.

The genus *Ophioceras* is emended herein to accommodate the genus *Ceratosphaerella* that clustered with other *Ophioceras* species in Ophioceraeae. *Ceratosphaerella* is morphologically different from *Ophioceras* in having clavate asci and hyaline to pale brown, narrow fusiform ascospores, whereas *Ophioceras* has subcylindrical to acerose asci and hyaline to olivaceous, filiform ascospores [1,8]. However, *Ophioceras* resembles *Ceratosphaerella* in the ascomatal morphology and is also supported by phylogenetic analyses. Through ML and BI phylogenetic analyses based on a concatenated LSU-RPB1-ITS sequence matrix (Fig 1), *C. castillensis* (SMH 1865), which was previously treated in Magnaporthaceae, is phylogenetically closely related to *C. rhizomorpha* and clustered within *Ophioceras* in Ophioceraeae. Therefore, we treat *Ceratosphaerella* as a synonym of *Ophioceras* instead of a genus in Ophioceraeae. Using a morpho-phylogenetic approach, *Ophioceras castillensis* comb. nov. and *O. rhizomorpha* comb. nov. are hereby introduced.

Ophioceras sichuanense H.B. Jiang, Phookamsak & K.D. Hyde, *sp. nov.* Fig 2.

[urn:lsid:indexfungorum.org:names:557956].

Facesoffungi number: FoF09404.

Etymology—The specific epithet “*sichuanense*” refers to Sichuan Province, P.R. China, where the species was collected.

Holotype—KUN-HKAS 107677.

Saprobic on decaying branches of bamboo submerged in freshwater. **Sexual morph:** *Pseudostromata* 300–750 μm diam., 230–350 μm high (excluding necks), black, scattered, solitary, semi-immersed to superficial, 1–5-loculate, glabrous, ostiolate, papillate, carbonaceous. *Locules* 150–300 μm diam., 150–250 μm high (excluding necks), immersed within pseudostroma, clustered, subglobose to ampulliform, blackened, with a long black, periphysate neck, up to 1 cm. *Peridium* 20–35 μm wide, thick-walled, composed of several layers, of flattened to broad, pseudoparenchymatous cells, arranged in *textura angularis* to *textura prismatica*, inner layers composed of hyaline cells, outer layers composed of dark brown to black pseudoparenchymatous cells. *Paraphyses* 4–8 μm wide, filiform, hyaline, indistinct septate, unbranched, slightly rough with small guttules, broad at the base, tapering toward the tip. *Asci* 90–115 \times 5–6.5 μm (\bar{x} = 103 \times 5.8 μm , n = 20), 8-spored, unitunicate, cylindrical, sessile to subsessile, with short broad bulb-like at the base, apically rounded with a J-, apical ring. *Ascospores* 80–90 \times 1–1.5 μm (\bar{x} = 85 \times 1.3 μm , n = 15), overlapping, or in parallel, hyaline, filiform, slightly curved to sigmoidal, thin-walled, aseptate, smooth-walled, multi-guttulate. **Asexual morph:** not observed.

Culture characteristics: Ascospores germinated on PDA within 24 hours at room temperature under normal condition. Mycelium superficial to immersed in agar medium, branched,

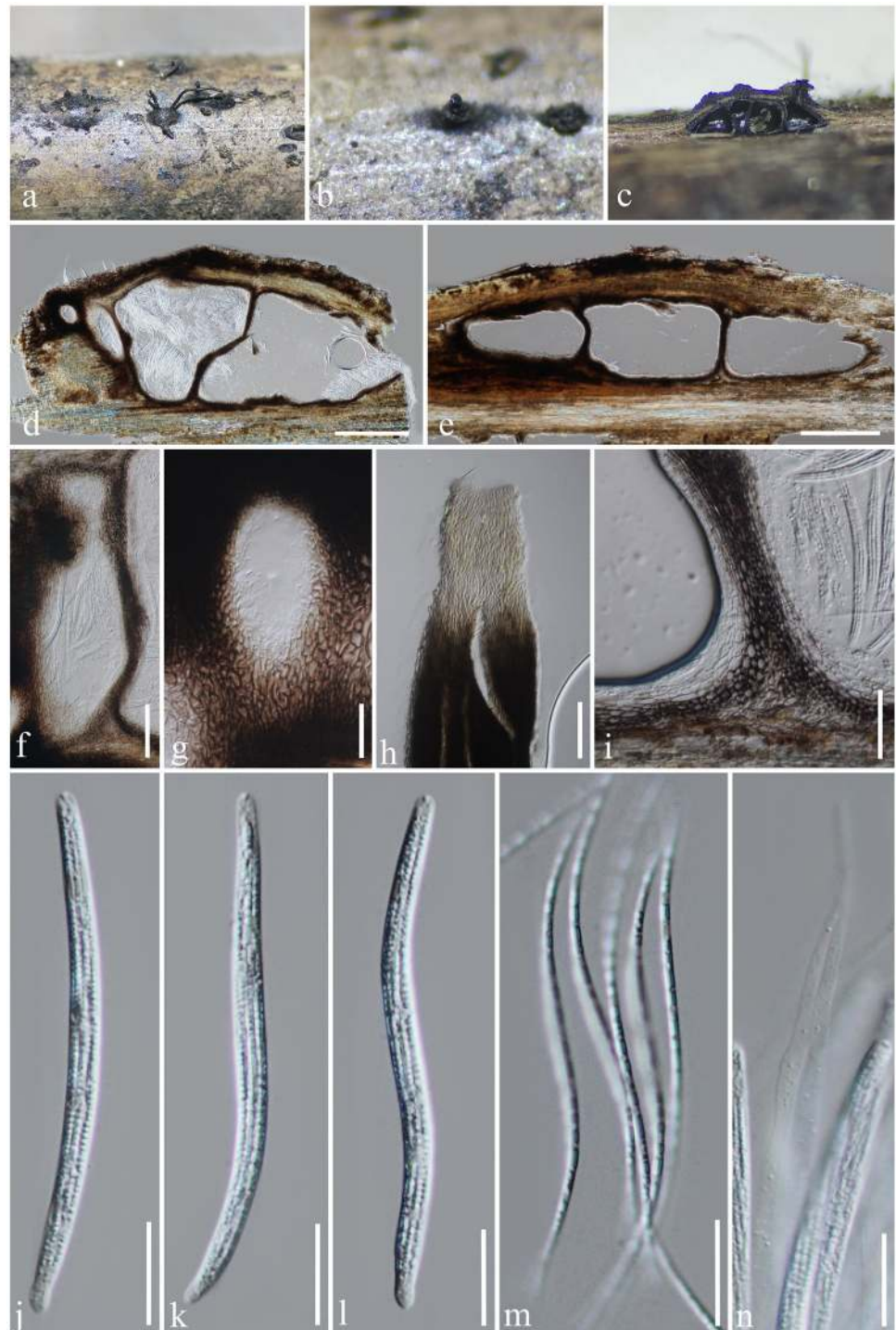


Fig 2. *Ophioceras sichuanense* (KUN-HKAS 107677, holotype). a, b Pseudostromata with long ostiolar necks on host. c–e Section through pseudostromata. f Ascoma. g Ostiole. h Apical part of neck. i Peridium. j–l Asci. m Ascospores. n Paraphyses. Scale bars: d, e = 200 μm, f–h = 50 μm, i = 30 μm, j–n = 20 μm.

<https://doi.org/10.1371/journal.pone.0253853.g002>

septate, smooth hyphae. Colonies growing slowly on PDA, reaching 20 mm in two weeks, cottony, circular, with entire edge, raised, white from above and below.

Material examined: P.R. China, Sichuan Province, Yibin City, Shunan Artificial Bamboo Forest, on submerged decaying branches of bamboo, 25 July 2019, H.B. Jiang & R. Phookamsak, SC011 (KUN-HKAS 107677, holotype), ex-type living culture, KUMCC 20–0213.

GenBank accession numbers: ITS = MT995045, LSU = MT995046, SSU = MT995047 (KUMCC 20–0213; from pure culture); ITS = MW057779, LSU = MW057782, SSU = MW057847, TEF1- α = MW082017 (KUN-HKAS 107677; from fruiting bodies).

Notes—*Ophioceras sichuanense* can be distinguished from other *Ophioceras* species because it forms multi-loculate pseudostromata. *Ophioceras sichuanense* resembles *O. guttulatum*, *O. leptosporum* and *O. tenuisporum*. However, *O. sichuanense* differs from *O. guttulatum* in having smaller asci and ascospores (asci: 90–115 \times 5–6.5 μ m vs. 130–160 \times 14–17 μ m; ascospores: 80–90 \times 1–1.5 μ m vs. 100–128 \times 4–5 μ m) [17]. *Ophioceras sichuanense* resembles *O. leptosporum* and *O. tenuisporum* due to the size ranges of asci and ascospores and extremely long ostiolar necks. However, the species differs from the latter two species in their ascospore septation (Table 2). Although *O. bambusae* and *O. sichuanense* were collected from bamboos, *O. sichuanense* has longer asci and ascospores (asci: 90–115 \times 5–6.5 μ m vs. 90–95 \times 5.5–6.5 μ m; ascospores: 80–90 \times 1–1.5 μ m vs. 75–80 \times 1.5 μ m) [54]. Present phylogenetic analyses show *O. sichuanense* formed a clade basal to *Ophioceras* and close to *O. chiangdaoense* and *O. leptosporum*. *Ophioceras sichuanense* and *O. chiangdaoense* are different in the dimensions of the ostiolar neck, asci and ascospores (Table 2).

Ophioceras castillensis (C.L. Sm.) H.B. Jiang, Phookamsak & K.D. Hyde, *comb. nov.* Fig 3. [urn:lsid:indexfungorum.org:names:557957].

Facesoffungi number: FoF09405.

Basionym: *Ceratosphaeria castillensis* C.L. Sm., Bull. Lab. Nat. Hist. Iowa State Univ. 2: 403 (1893).

Synonym: *Ceratosphaerella castillensis* (C.L. Sm.) Huhndorf, Greif, Mugambi & A.N. Mill., Mycologia 100(6): 944 [8].

Type information: Nicaragua, Castillo Viejo, on bark, Feb–Mar 1893, C.L. Smith, Central American Fungi 13, (isotype, NY).

Detailed description and illustration: see Huhndorf et al. [8].

Known hosts/ habitat and distribution: Saprobic on bark or wood in terrestrial habitat. To date, *Ophioceras castillensis* is only reported from Costa Rica, Nicaragua, and Puerto Rico [8].

Notes—In this study, *Ceratosphaerella castillensis* is transferred to *Ophioceras* as *O. castillensis* based on a concatenated LSU-RPB1-ITS analyses coupled with morphological similarity of the ascomata and ascomatal wall which is typical *Ophioceras*. *Ophioceras castillensis* can be separated from other *Ophioceras* species in forming ascomata on large clusters, superficial on sparse, subicular hyphae and having clavate asci and hyaline to pale brown, fusiform, 3-septate ascospores [8]. Detailed morphological comparison and taxa habitats in *Ophioceras* are described in Table 2.

Ophioceras rhizomorpha (Huhndorf & Mugambi) H.B. Jiang, Phookamsak & K.D. Hyde, *comb. nov.* Fig 4.

[urn:lsid:indexfungorum.org:names:557958].

Facesoffungi number: FoF09406.

Basionym: *Ceratosphaerella rhizomorpha* Huhndorf & Mugambi, Mycologia 100(6): 944 [8].

Type information: Kenya, Kagamega National Park, on decaying wood on the ground, 17 January 2007, G.K. Mugambi, GKM1262 (holotype EA, isotype F).

Detailed description and illustration: see Huhndorf et al. [8].

Table 2. Synopsis of *Ophioceras* species.

Species name	Ascomata	Asci	Ascospores	Habitat	Origin	Host/substrate	References
<i>Ophioceras aquaticus</i>	310–620 µm diam., 1-loculate, superficial to submerged, with a 500–800 µm long neck	85–100 × 9–10 µm, cylindrical	42–68 × 3–4 µm, filiform, slightly acute at each end, falcate, sigmoid, hyaline, 3–5-septate	Submerged	China: Yunnan	Wood	[2, 10]
<i>O. arcuatisporum</i>	313–324 × 252–340 µm, 1-loculate, superficial to immersed, with a long neck, up to 800 µm	276–307 × 15–20 µm, fusoid to narrowly cylindrical	170–239 × 4–7 µm, narrowly fusoid to cylindrical, falcate, hyaline to pale orange, 5–12-septate	Submerged	USA: Minnesota	<i>Typha</i> sp., herbaceous debris, grass	[49]
<i>O. bambusae</i>	1 mm long, 2/3 mm diam., 1-loculate, immersed, with a 2–2.5 mm long neck	90–95 × 5.5–6.5 µm, cylindrical	75–80 × 1.5 µm, filiform, with both blunt ends, curved, hyaline, indistinctly septate	Terrestrial	Indonesia: Java	Bamboo	[54]
<i>O. castillensis</i>	525–650 µm diam., 1-loculate, superficial, with a 250–400 µm long neck	70–90 × 10–14 µm, clavate	29–40 × 4–5.5 µm, narrowly fusiform, slightly curved, hyaline to pale brown, 3-septate	Terrestrial	Nicaragua	Bark and wood	[8]
<i>O. cecropiae</i>	200–250 µm diam., 1-loculate, immersed, with a long neck, up to 2 mm	75–90 × 6.5–7.5 µm, cylindrical to subfusoid	60–70 × 2 µm, filiform, straight to slightly curved, hyaline, septate	Terrestrial	Venezuela	<i>Cecropia</i> sp.	[55]
<i>O. chiangdaoense</i>	200–310 × 170–310 µm, 1-loculate, immersed, with a 93–273 µm long neck	85–125 × 11–17 µm, cylindrical	54–75.5 × 4–5.5 µm, filiform, falcate, fusoid at both ends, hyaline, 3-septate	Terrestrial	Thailand: Chiang Mai	Decaying leaves of <i>Dracaena loureiroi</i>	[9]
<i>O. commune</i>	150–350 × 260–400 µm, 1-loculate, immersed to superficial, with a 375–1660 µm long neck	64–118 × 4–12 µm, cylindrical	50–110 × 2 µm, filiform, arcuate or sigmoidal, hyaline, 3–7-septate	Submerged	Panama: Barro Colorado Island	Wood, herbaceous debris	[2, 49]
<i>O. dolichostomum</i>	500 µm diam., 1-loculate, immersed, with a 1–5 mm long neck	100–130 × 8–12 µm, cylindrical	94–110 × 2–3 µm, filiform, falcate, sigmoid, hyaline, 3–7-septate	Submerged	USA: Florida	Dead wood	[2, 46]
<i>O. filiforme</i>	3–5 mm long, 150–180 µm diam., 1-loculate, immersed, erumpent to superficial, with a long neck	100–120 × 10–13 µm, clavate or fusoid	80–100 × 3–3.5 µm, filiform, hyaline to yellowish, multi-septate	Terrestrial	Indonesia: Java	Rotten leaf sheaths of <i>Amomum</i> sp.	[56]
<i>O. fusiforme</i>	360–500 × 330–450 µm, 1-loculate, immersed to erumpent, with a 250–800 µm long neck	70–112 × 6–12 µm, cylindrical	64–104 × 1.5–3 µm, filiform, fusoid, tapering at both ends, straight to falcate, 3–5-septate	Submerged	USA: Indiana	Decorticated woody debris	[49]
<i>O. guttulatatum</i>	400–600 × 1200–1800 µm, 1-loculate, superficial to immersed, with a 500–1500 µm long neck	130–160 × 14–17 µm, broadly cylindrical	100–128 × 4–5 µm, cylindrical, falcate, pale yellow to hyaline, 3–5-septate	Submerged	China: Hong Kong	Wood	[17]
<i>O. hongkongense</i>	500–640 × 700–800 µm, 1-loculate, superficial to immersed, with a more than 600 µm long neck	100–125 × 12–14 µm, elongated fusoid to broadly cylindrical	72–101 × 3.5–4.5 µm, cylindrical, falcate, tapered at both ends, hyaline, 3–5-septate	Submerged	China: Hong Kong	Wood	[17]
<i>O. indicus</i>	400–650 µm diam., 1-loculate, immersed, with a long neck, up to 1.5 mm	65–90 × 8.5–11.5 µm, cylindrical to subfusoid	60–85 × 2.5–3.5 µm, filiform, tapering towards base, slightly curved, hyaline to subhyaline, 7–10-septate	Terrestrial	India: New Delhi	Dried twigs of <i>Ficus infectoria</i>	[47]
<i>O. leptosporum</i>	250–300 µm diam., 1-loculate, immersed or superficial, with a 1–2 mm long neck	70–95 × 5–6 µm, cylindrical	70–80 × 1–1.5 µm, filiform, apex rounded, base acute, hyaline to faintly tinted, straight to slightly curved or sigmoid, 3–7-septate	Submerged	UK: Exeter	Rotten stems of <i>Umbelliferae</i> sp.	[4]
<i>O. miyazakiense</i>	Data unavailable	Data unavailable	Data unavailable	Terrestrial	Japan: Kyushu	Decaying litter	[57]

(Continued)

Table 2. (Continued)

Species name	Ascomata	Asci	Ascospores	Habitat	Origin	Host/substrate	References
<i>O. palmae</i>	164–320 × 244–288 μm, 1-loculate, partly immersed, with a 180–376 μm long neck	76–96 × 10–14 μm, broadly cylindrical	79–90 × 1.2–2 μm, filiform, tapering at both ends, sigmoid, hyaline, 5-septate	Terrestrial	Philippines: Mt. Makiling	<i>Calamus ornatus</i>	[17]
<i>O. parasiticum</i>	600–800 μm diam.	100–140 × 9–11 μm	48–70 × 2.5–3.3 μm, 3–9-septate	Terrestrial (parasite)	China	Data unavailable	[9]
<i>O. petrakii</i>	600–750 × 555–675 μm, 1-loculate, immersed, with a neck	171–182 × 12–15 μm, cylindrical	152–171 × 3–4 μm, filiform, apex rounded, base acute, slightly curved or sigmoid, hyaline, multi-septate	Terrestrial	India: Karnataka	Dead stems of <i>Vitex negundo</i>	[45]
<i>O. rhizomorpha</i>	500–900 × 500–750 μm, 1-loculate, superficial, with a 300–600 μm long neck	115–145 × 13–16 μm, clavate	39–49 × 3.5–4.5 μm, narrowly fusiform, slightly curved, hyaline to pale brown, 3-septate	Terrestrial	Kenya	Bark or wood	[8]
<i>O. sichuanense</i>	230–350 × 300–750 μm, pseudostromatic, 1–5-loculate, semi-immersed to superficial, with long necks, up to 1 cm	90–115 × 5–6.5 μm, cylindrical	80–90 × 1–1.5 μm, filiform, slightly curved to sigmoidal, hyaline, aseptate	Submerged	China: Sichuan	Decaying branches of bamboo	This study
<i>O. sorghi</i>	300–400 μm diam., 1-loculate, immersed, with a 350–700 μm long neck	85–110 × 12–14 μm, cylindrical to clavate	75–95 × 3–4 μm, filiform, cylindrical, with rounded apex and slightly thinner rounded base, slightly curved, hyaline, 3–12-septate	Terrestrial	Central African Republic: M' Baiki	<i>Sorghum vulgare</i>	[4, 43]
<i>O. submersum</i>	300–400 × 500–600 μm, 1-loculate, immersed, with a long neck	115–137 × 10–11 μm, cylindrical	87–109 × 3–4 μm, filiform, rounded at both ends, slightly curved or sigmoid, hyaline, multi-septate	Submerged	Thailand	Wood	[2]
<i>O. tambopataense</i>	Data unavailable	Data unavailable	Data unavailable	Terrestrial	Peru	Decaying leaf of palm	[57]
<i>O. tenuisporum</i>	240–625 × 260–775 μm, 1-loculate, superficial to partially immersed, with a long neck, up to 20 mm	82–114 × 4–6 μm, cylindrical to narrowly fusoid	66–94 × 1–1.5 μm, filiform, more broadly rounded at one end than the other, curved to sigmoid, hyaline, 3-septate	Submerged	Panama: Barro Colorado Island	Twig	[49]
<i>O. venezuelense</i>	730–890 × 745–868 μm, 1-loculate, partially immersed to superficial, with a 250–800 μm long neck	148–180 × 11–18 μm, cylindrical to narrowly fusoid	130–158 × 2–4 μm, filiform, falcate, more broadly rounded at one end than the other, straight to slightly curved, hyaline, (4)–5–(6)-septate	Submerged	Venezuela: Portuguesa	Wood, herbaceous debris	[49]
<i>O. zaeae</i>	450–650 μm diam., 1-loculate, superficial or partially immersed, with a 350–900 μm long neck	55–65 × 7–8 μm, cylindrical to narrowly fusoid	39–50 × 2.3–2.5 μm, cylindrical to fusoid, with rounded ends, straight or slightly curved, hyaline, 3-septate	Terrestrial	Central African Republic: Boukoko	Dead <i>Zea mays</i>	[4, 42]

<https://doi.org/10.1371/journal.pone.0253853.t002>

Known hosts/ habitat and distribution: Saprobic on decaying wood of terrestrial habitat. To date, *Ophioceras rhizomorpha* is only reported from Kenya.

Notes—*Ophioceras rhizomorpha* was reported with a Didymobotryum-like asexual morph on a natural substrate [8]. The species resembles *O. castillensis* in forming ascomata on the large subicular, and having clavate asci and hyaline to pale brown, fusiform, 3-septate ascospores [8]. *Ophiocera rhizomorpha*, however, differs from *O. castillensis* in having larger ascomata, necks, peridia, paraphyses, asci and ascospores [8] (see Table 2).

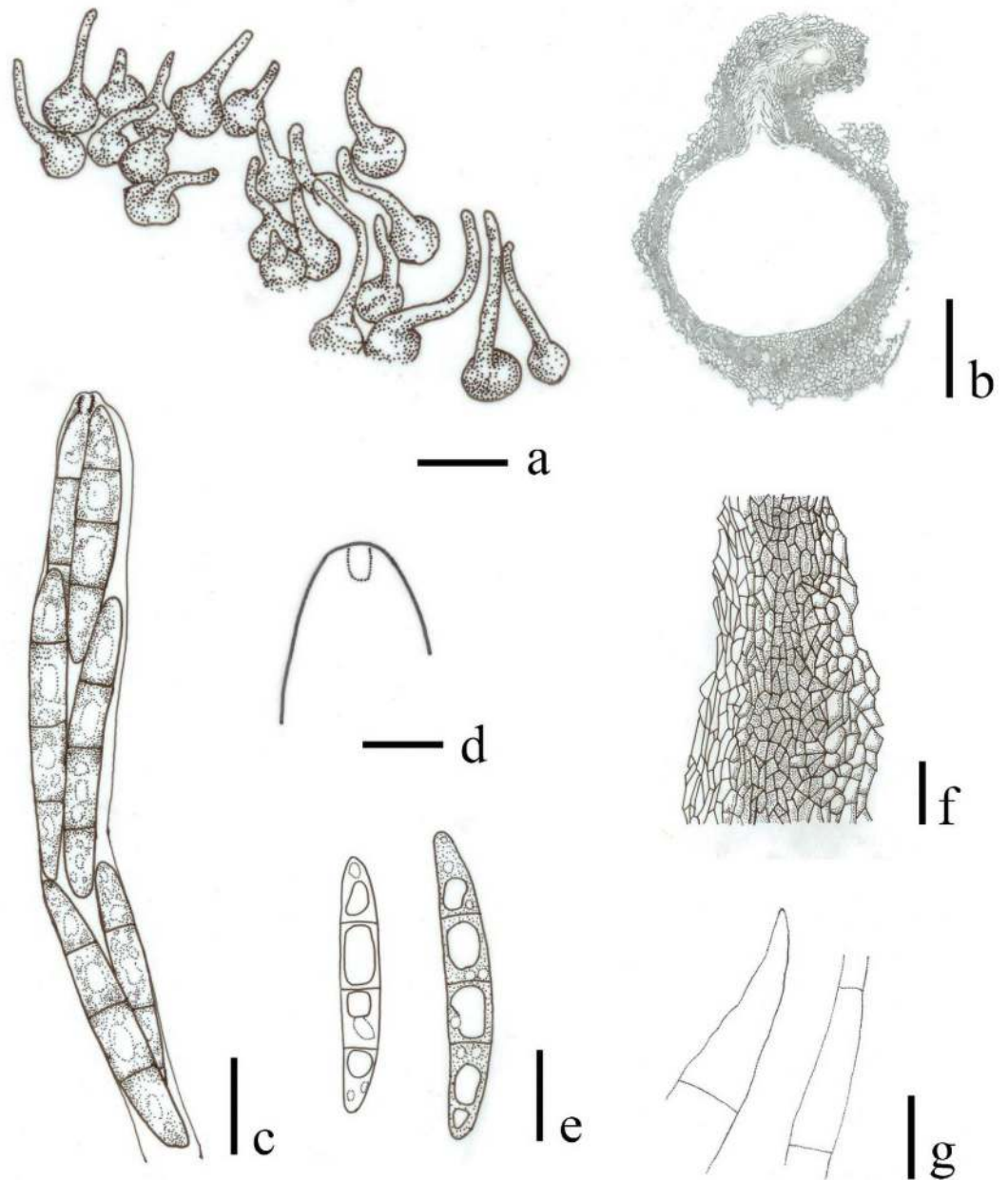


Fig 3. *Ophioceras castillensis* (redrawn from Huhndorf et al. [8], NY isotype). a, b Ascomata. c Ascus. d Apical ring. e Ascospores. f Peridium. g Paraphyses. Scale bars: a = 1 mm, b = 200 μ m, f = 20 μ m, c, e, g = 10 μ m, d = 5 μ m.

<https://doi.org/10.1371/journal.pone.0253853.g003>

Discussion

Ophioceraaceae currently accommodates only *Ophioceras*. Maharachchikumbura et al. [58] accommodated the family in Magnaporthales (Diaporthomycetidae, Sordariomycetes) based on literature review and phylogenetic analysis. Ophioceraaceae has limited taxon sampling, and most taxa in this family lack reliable protein coding genes to clarify phylogenetic affinities. For example, *Ophioceras arcuatisporum* (strains A9-1, A167-1B) has only SSU sequence data

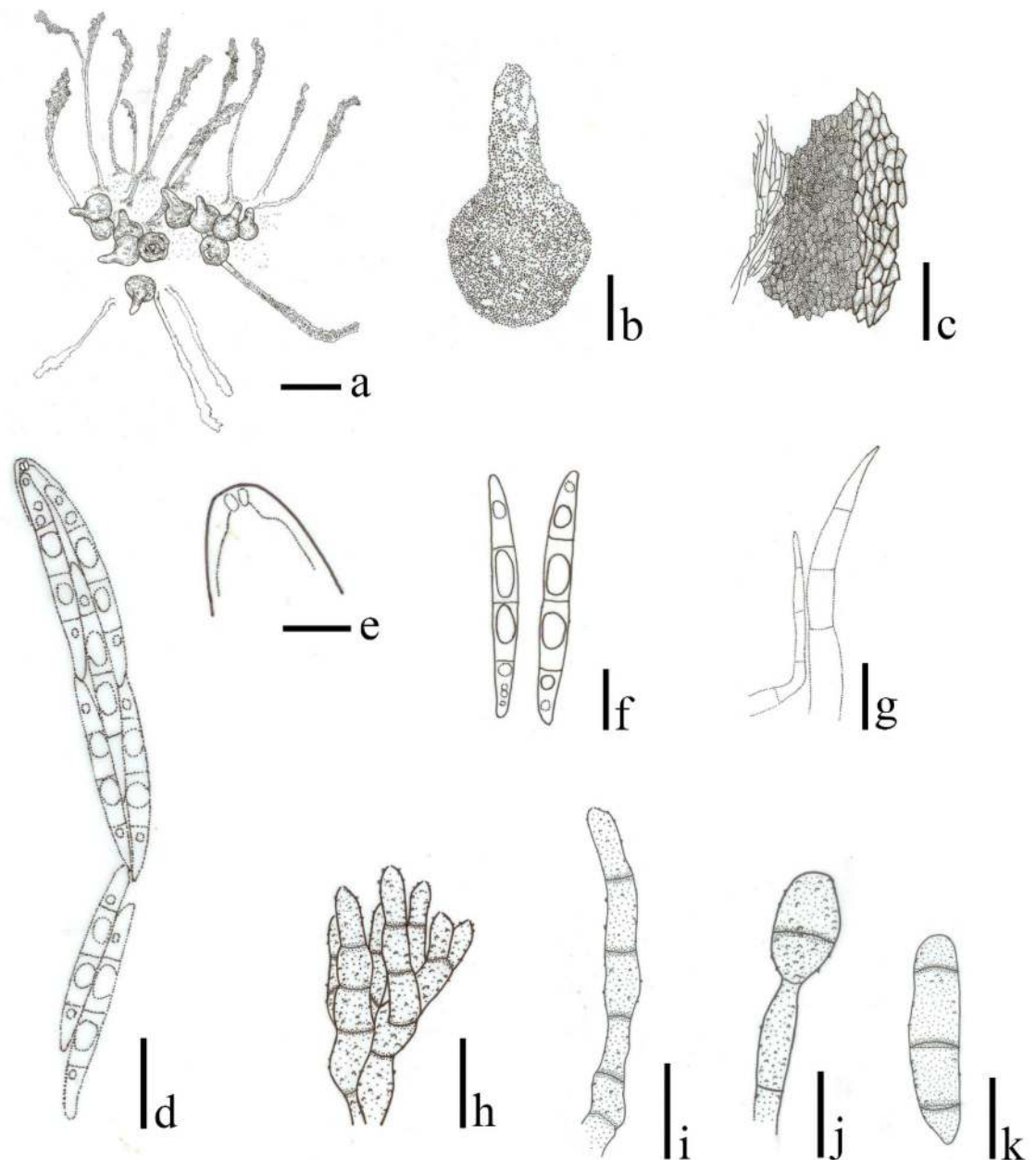


Fig 4. *Ophioceras rhizomorpha* (redrawn from Huhndorf et al. [8], all from Mugambi 1262). a Ascomata and synnemata on the substrate. b Ascoma. c Peridium. d Ascus. e Apical ring. f Hyaline to pale brown ascospores. g Paraphyses. h, i Conidiophores. j Conidiogenous cell bearing conidium. k Conidium. Scale bars: a = 1 mm, b = 300 μ m, c = 50 μ m, g = 30 μ m, d = 20 μ m, i, k = 15 μ m, f, h, j = 10 μ m, e = 5 μ m.

<https://doi.org/10.1371/journal.pone.0253853.g004>

available in GenBank. In preliminary phylogenetic analysis of SSU sequence matrix, the species formed a stable clade within Magnaporthaceae rather than Ophioceraaceae. *Ophioceras arcuatisporum* needs to be re-visited and re-illustrated, incorporating details from molecular data.

Luo et al. [2] performed combined LSU and TEF1- α phylogenetic analyses to investigate the relationships of taxa in Magnaporthales. In their phylogeny, *Ceratosphaeria* grouped with

Pseudohalonectria (Pseudohalonectriaceae) and separated from *Ophioceras* (Ophiocerales). Based on the fact that *Ceratosphaeria* differs from *Pseudohalonectria* in having narrow cylindrical-fusiform to filiform and longer ascospores, Ceratosphaeriaceae was thus introduced as a new family within Magnaporthales to accommodate *Ceratosphaeria* [2]. In the present study, we performed an updated phylogenetic tree based on a concatenated LSU-RPB1-ITS sequences and showed that *Ceratosphaeria* (Ceratosphaeriaceae) clustered with *Ophioceras* (Ophiocerales) with low statistical support, suggesting that gene selection in the data matrix affects the tree topology at the familial levels in Magnaporthales. *Ceratosphaeria* is morphologically similar to *Ceratosphaerella* [8]. Although *Ceratosphaeria* clustered with *Ophioceras* with low statistical support, *Ceratosphaeria* possibly belongs to *Ophiocerales*. However, the phylogenetic status of *Ceratosphaeria* needs to be clarified with more evidence in the future studies.

In the present study, we synonymize *Ceratosphaerella* under *Ophioceras* based on molecular phylogeny coupled with similar ascomatal morphology. Phylogenetic analyses based on the LSU sequence dataset (S1 Fig) and the concatenated LSU-ITS (S2 Fig) and the LSU-RPB1-ITS (Fig 1) sequence datasets have always shown that *O. castillensis* (\equiv *C. castillensis*) and *O. rhizomorpha* (\equiv *C. rhizomorpha*) formed a stable clade within *Ophioceras*. *Ophioceras castillensis*, *O. rhizomorpha* and most *Ophioceras* species lack protein coding genes and other reliable genes to clarify phylogenetic placement as well as limited taxon sampling. The ex-type strain of *O. rhizomorpha* and the reference strain of *O. castillensis* were sequenced only for LSU locus. Hence, more reliable gene loci (e.g., SSU, ITS, RPB1 and TEF1- α) from the ex-type strain of *O. rhizomorpha* should be obtained and the epitype of *O. castillensis* should be designated and incorporated with morpho-molecular based taxonomic treatment. Furthermore, the new collections and sequence data of taxa in *Ophioceras* are required to provide a better taxonomic resolution for robust species delineations in this genus.

Many genera in Magnaporthales have similar morphological characteristics with *Ophioceras* (Table 3). However, these genera are considered distinct genera based on phylogenetic investigations [1,2,8,59–61]. *Pseudohalonectria* (Pseudohalonectriaceae) is also similar to

Table 3. Morphological comparisons of similar genera to *Ophioceras*.

Generic name	Ascomata	Asci	Ascospores	References
<i>Aquafiliformis</i> (Magnaporthaceae)	Globose to subglobose, with long beak	Cylindrical to clavate, with an inconspicuous apical ring	Aseptate, filiform, hyaline	[2]
<i>Ceratosphaeria</i> (Ceratosphaeriaceae)	Globose to pyriform, with a long black or yellow neck	Cylindrical, with a conspicuous, non-amyloid, apical ring	Multi-septate, filiform, hyaline	[2]
<i>Gaeumannomycella</i> (Magnaporthaceae)	Subglobose to elliptical, with a lateral, central cylindrical neck	Cylindrical to elongated clavate, apical ring not observed	0–3-septate, narrowly fusiform, hyaline	[61]
<i>Gaeumannomyces</i> (Magnaporthaceae)	Subglobose to elliptical, with a cylindrical neck	Cylindrical to elongated clavate, with an apical refringent ring	Indistinctly septate, filiform, hyaline to pale brown	[60]
<i>Kohlmeyeriopsis</i> (Magnaporthaceae)	Ellipsoid, with a long cylindrical periphysate neck	Fusoid to cylindrical, with a large apical ring	Indistinctly septate, filamentous, tapering towards the base, hyaline	[1, 63]
<i>Muraeriata</i> (Magnaporthaceae)	Lageniform to globose, with long beak	Cylindrical to ventricose, with a tall, narrow, apical ring	3-septate, narrowly fusiform, ends slightly curved, hyaline	[8]
<i>Neogaeumannomyces</i> (Magnaporthaceae)	Globose to subglobose, with a long, periphysate neck	Cylindrical, with an apical ring	2–3-septate, filiform to long fusiform, hyaline	[59]
<i>Ophioceras</i> (Ophiocerales)	Subglobose to ampulliform, with a long cylindrical periphysate neck	Subcylindrical to narrowly fusoid or clavate asci with a small, refractive, non-amyloid apical ring	Aseptate to multi-septate, filiform, narrowly fusiform, hyaline pale brown or olivaceous	[2, 8, this study]
<i>Slopeiomyces</i> (Magnaporthaceae)	Globose, with periphysate neck bearing hyphae	Clavate, with a non-amyloid apical ring	3–4-septate cylindrical to fusoid, tapering somewhat to base, hyaline	[1]

<https://doi.org/10.1371/journal.pone.0253853.t003>

Ophioceras in its ascomata and asci. However, *Pseudohalonestria* varied in shape of ascospores, such as ellipsoidal, fusiform or filiform [62]. In the present study, *Pseudohalonestria* (Pseudohalonestriaceae) formed an independent lineage separate from other families in Magnaporthales; however, *Pseudohalonestria* could not be resolved at the species level such as in Perera et al. [62]. It may be because molecular data of most taxa in this genus are unavailable in GenBank database. Moreover, some sequences of *P. lignicola* deposited in GenBank are likely to be misidentified [62]. Therefore, sequences of *Pseudohalonestria* species used for phylogenetic analyses are limited.

Supporting information

S1 Fig. RAxML tree generated from an analysis of the LSU matrix of taxa in Magnaporthales.

(TIF)

S2 Fig. RAxML tree generated from an analysis of the LSU-ITS matrix of taxa in Magnaporthales.

(TIF)

Acknowledgments

The authors gracefully thank the Biology Experimental Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences for providing the facilities of molecular laboratory. We also thank Shaun Pennycook from Manaaki Whenua—Landcare Research for his assistance in naming the new species in the genus *Ophioceras*. Austin G. Smith is thanked for the English proofreading of this manuscript. Hong-Bo Jiang would like to thank Mae Fah Luang University for Ph.D scholarship.

Author Contributions

Conceptualization: Hong-Bo Jiang, Kevin D. Hyde, Rungtiwa Phookamsak, Saisamorn Lumyong.

Data curation: Hong-Bo Jiang.

Formal analysis: Hong-Bo Jiang, Rungtiwa Phookamsak.

Funding acquisition: Ali H. Bahkali, Abdallah M. Elgorban, Samantha C. Karunarathna, Rungtiwa Phookamsak, Saisamorn Lumyong.

Investigation: Hong-Bo Jiang, Kevin D. Hyde, Er-Fu Yang, Rungtiwa Phookamsak.

Methodology: Hong-Bo Jiang, Rungtiwa Phookamsak.

Project administration: Rungtiwa Phookamsak.

Supervision: Kevin D. Hyde, Pattana Kakumyan, Rungtiwa Phookamsak, Saisamorn Lumyong.

Writing – original draft: Hong-Bo Jiang, Er-Fu Yang, Rungtiwa Phookamsak.

Writing – review & editing: Kevin D. Hyde, Pattana Kakumyan, Samantha C. Karunarathna, Saisamorn Lumyong.

References

1. Klaubauf S, Tharreau D, Fournier E, Groenewald JZ, Crous PW, de Vries RP, et al. Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae). *Stud Mycol*. 2014; 79:85–120. <https://doi.org/10.1016/j.simyco.2014.09.004> PMID: 25492987
2. Luo ZL, Hyde KD, Liu JK, Maharachchikumbura SSN, Jeewon R, Bao DF, et al. Freshwater Sordariomycetes. *Fungal Divers*. 2019; 99:451–660. <https://doi.org/10.1007/s13225-019-00438-1>
3. Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ, Jones EBG, Bundhun D, et al. Refined families of Sordariomycetes. *Mycosphere*. 2020; 11:305–1059.
4. Walker J. *Gaeumannomyces*, *Linocarpon*, *Ophiobolus* and several other genera of scolecospored Ascomycetes and Phialophora conidial states, with a note on hyphopodia. *Mycotaxon*. 1980; 11:1–129.
5. Chen W, Shearer CA, Crane JL. Phylogeny of *Ophioceras* spp. based on morphological and molecular data. *Mycologia*. 1999; 91:84–94. <https://doi.org/10.1080/00275514.1999.12060995>
6. Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H, Kirk PM, et al. Notes for genera: Ascomycota. *Fungal Divers*. 2017; 86:1–594. <https://doi.org/10.1007/s13225-017-0386-0>
7. Saccardo PA. *Sylloge Pyrenomycetum, Vol. II. Sylloge Fungorum*. 1883; 2:1–813.
8. Huhndorf SM, Greif M, Mugambi GK, Miller AN. Two new genera in the Magnaporthaceae, a new addition to *Ceratospheeria* and two new species of *Lentomitella*. *Mycologia*. 2008; 100:940–955. <https://doi.org/10.3852/08-037> PMID: 19202848
9. Thongkantha S, Jeewon R, Vijaykrishna D, Lumyong S, McKenzie EHC, Hyde KD. Molecular phylogeny of Magnaporthaceae (Sordariomycetes) with a new species, *Ophioceras chiangdaoense* from *Draacaena loureiroi* in Thailand. *Fungal Divers*. 2009; 34:157–173.
10. Hu DM, Cai L, Hyde KD. Three new ascomycetes from freshwater in China. *Mycologia*. 2012; 104:1478–1489. <https://doi.org/10.3852/11-430> PMID: 22684292
11. Index Fungorum. <http://www.indexfungorum.org/Names/Names.asp>. 2021 (accessed on 8 April 2021).
12. NCBI: National Center for Biotechnology Information (GenBank). <https://www.ncbi.nlm.nih.gov/nuccore/>. 2020 (accessed on 10 August 2020).
13. Hyde KD, Goh TK. Fungi on submerged wood in the Riviere St Marie-Louis, the Seychelles. *S Afr J Bot*. 1998; 64:330–336. [https://doi.org/10.1016/S0254-6299\(15\)30920-0](https://doi.org/10.1016/S0254-6299(15)30920-0)
14. Hyde KD, Goh TK. Fungi on submerged wood in Lake Barrine, north Queensland, Australia. *Mycol Res*. 1998; 102:739–749. <https://doi.org/10.1017/S0953756297005868>
15. Tsui CKM, Hyde KD, Fukushima K. Fungi on submerged wood in the Koito River, Japan. *Mycoscience*. 2001; 44:55–59. <https://doi.org/10.1007/s10267-002-0083-y>
16. Tsui CKM, Hyde KD, Hodgkiss IJ. Longitudinal and temporal distribution of freshwater ascomycetes and dematiaceous hyphomycetes on submerged wood in the Lam Tsuen River, Hong Kong. *J N Am Benthol Soc*. 2001; 20:533–549.
17. Tsui CKM, Leung YM, Hyde KD, Hodgkiss IJ. Three new *Ophioceras* species (Ascomycetes) from the tropics. *Mycoscience*. 2001; 42:321–326. <https://doi.org/10.1007/BF02461213>
18. Tsui CKM, Hyde KD, Hodgkiss IJ. Biodiversity of fungi on submerged wood in Hong Kong streams. *Aquat Microb Ecol*. 2000; 21:289–298. <https://doi.org/10.3354/ame021289>
19. Ho WH, Hyde KD, Hodgkiss IJ. Fungal communities on submerged wood from streams in Brunei, Hong Kong, and Malaysia. *Mycol Res*. 2001; 105:1492–1501. <https://doi.org/10.1017/S095375620100507X>
20. Ho WH, Yanna, Hyde KD, Hodgkiss IJ. Seasonality and sequential occurrence of fungi on wood submerged in Tai Po Kau Forest Stream, Hong Kong. In: *Fungal Succession* (eds. Hyde K.D. and Jones E. B.G.). *Fungal Divers*. 2002; 10:21–43.
21. Cai L, Zhang KQ, McKenzie EHC, Hyde KD. Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. *Fungal Divers*. 2003; 13:1–12. [https://doi.org/10.1016/S1567-1356\(03\)00107-7](https://doi.org/10.1016/S1567-1356(03)00107-7)
22. Species Fungorum. <http://www.speciesfungorum.org/Names/Names.asp>. 2021 (accessed on 8 April 2021).
23. Hyde KD, Jeewon R, Chen YJ, Bhunjun CS, Calabon MS, Jiang HB, et al. The numbers of fungi: is the descriptive curve flattening? *Fungal Divers*. 2020.
24. Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, et al. Bambusicolous fungi. *Fungal Divers*. 2017; 82:1–105. <https://doi.org/10.1007/s13225-016-0367-8>
25. Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat DJ, Buyck B, Cai L, et al. The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Divers*. 2015; 74:3–18. <https://doi.org/10.1007/s13225-015-0351-8>

26. White TJ, Bruns T, Lee SJWT, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR—Protocols and Applications*. 1990; 18:315–322.
27. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol*. 1990; 172:4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990> PMID: 2376561
28. Jiang HB, Phookamsak R, Xu JC, Karunarathna SC, Mortimer PE, Hyde KD. Taxonomic and phylogenetic characterizations reveal three new species of *Mendogia* (Myriangiaceae, Myriangiales). *Mycol Prog*. 2020; 19:41–51. <https://doi.org/10.1007/s11557-019-01540-z>
29. Hernández-Restrepo M, Bezerra JDP, Tan YP, Wiederhold N, Crous PW, Guarro J, et al. Re-evaluation of *Mycleptodiscus* species and morphologically similar fungi. *Persoonia*. 2019; 42:205–227. <https://doi.org/10.3767/persoonia.2019.42.08> PMID: 31551619
30. Katoh K, Rozewicki J, Yamada KD. Mafft online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform*. 2017. <https://doi.org/10.1093/bib/bbx108> PMID: 28968734
31. Hall T. BioEdit version 5.0.6. North Carolina State University. 2001.
32. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*. 2006; 22:2688–2690. <https://doi.org/10.1093/bioinformatics/btl446> PMID: 16928733
33. Silvestro D, Michalak I. raxmlGUI: a graphical front-end for RAxML. *Org Divers Evol*. 2011; 12:335–337. <https://doi.org/10.1007/s13127-011-0056-0>
34. Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Gateway Computing Environments Workshop 2010 (GCE). 2010; pp.1–8. <https://doi.org/10.1109/GCE.2010.5676129>
35. Nylander J. MrModeltest2 v. 2.3 (Program for selecting DNA substitution models using PAUP*). Evolutionary Biology Centre, Uppsala. 2008.
36. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 2003; 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180> PMID: 12912839
37. Rannala B, Yang Z. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *J Mol Evol*. 1996; 43:304–311. <https://doi.org/10.1007/BF02338839> PMID: 8703097
38. Zhaxybayeva O, Gogarten JP. Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *Genomics*. 2002; 3:1–15.
39. Hu HL, Fournier J, Jeewon R, Bahkali AH, Hyde KD. Revisiting the taxonomy of *Daruvedia bacillata*. *Mycotaxon*. 2010; 114:135–144. <https://doi.org/10.5248/114.135>
40. Ellis JB, Everhart BM. The North American Pyrenomycetes. 1892;1–793.
41. Teng SJ. Notes on Sphaeriales from China. *Sinensia*. 1934; 4:359–449.
42. Saccas AM. À propos de quelques champignons nouveaux parasites et saprophytes sur maïs. *Revue de Pathologie et d'Entomologie Agricole de France*. 1951; 30:161–196.
43. Saccas AM. Les champignons parasites des sorghos (*Sorghum vulgare*) et des penicillaires (*Pennisetum typhoideum*) en Afrique Equatoriale Française. *Agronomie Tropicale*. 1954; 9:263–301.
44. Saccas AM. Étude de la Flore Cryptogamique des Caféiers en Afrique Centrale. 1981;1–522.
45. Tilak ST, Kale SB. Contribution to our knowledge of Ascomycetes of India—XX. *Sydowia*. 1969; 23:17–23.
46. Conway KE, Barr ME. Classification of *Ophioceras dolichostomum*. *Mycotaxon*. 1977; 5:376–380.
47. Lal SP. A new species of *Ophioceras* Sacc. from India. *Kavaka*. 1987; 15:7–8.
48. Hyde KD. Tropical Australian freshwater fungi. IV. *Halosarpehia aquatica* sp. nov., *Garethjonesia lacunosispora* gen. et. sp. nov. and *Ophioceras dolichostomum* (Ascomycetes). *Aust Syst Bot*. 1992; 5:407–414. <https://doi.org/10.1071/SB9920407>
49. Shearer CA, Crane JL, Chen W. Freshwater Ascomycetes: *Ophioceras* species. *Mycologia*. 1999; 91:145–156. <https://doi.org/10.1080/00275514.1999.12061004>
50. Matsushima T. Matsushima Mycological Memoirs 9. *Matsushima*. Mycol Mem. 2001; 10:1–214.
51. Cai L, Ji KF, Hyde KD. Variation between freshwater and terrestrial fungal communities on decaying bamboo culms. *Antonie Van Leeuwenhoek*. 2006; 89:293–301. <https://doi.org/10.1007/s10482-005-9030-1> PMID: 16710640
52. Abdel-Aziz FA. Freshwater fungi from the River Nile, Egypt. *Mycosphere*. 2016; 7:741–756.
53. Farr DF, Rossman AY. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. 2020. <https://nt.ars-grin.gov/fungaldatabases/> (accessed on 1 June 2020).

54. Höhnelt FV. Fragmente zur Mykologie: VI. Mitteilung (Nr. 182 bis 288). Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abteilung I. 1909; 118:275–452.
55. Müller E, Dennis RWG. Fungi venezuelani: VIII: Plectascales, Sphaeriales, Loculoascomycetes. Kew Bulletin. 1965; 19:357–386. <https://doi.org/10.2307/4108161>
56. Hennings P. Fungi monsunenses. Monsunia. 1900; 1:137–174.
57. Matsushima T. Matsushima. Mycol Mem. 2003; 10. CD ROM [214 pp.+ incl. colour plates].
58. Maharachchikumbura SSN, Hyde KD, Jones EBG, McKenzie EHC, Bhat DJ, Dayarathne MC, et al. Fungal Divers. 2016; 79:1–317. <https://doi.org/10.5598/imafungus.2016.07.01.08> PMID: 27433444
59. Liu JK, Hyde KD, Jones EBG, Ariyawansa HA, Bhat DJ, Boonmee S, et al. Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Divers. 2015; 72:1–197. <https://doi.org/10.1007/s13225-015-0324-y>
60. Hernández-Restrepo M, Groenewald JZ, Elliott ML, Canning G, McMillan VE, Crous PW. Take-all or nothing. Stud Mycol. 2016; 83:19–48. <https://doi.org/10.1016/j.simyco.2016.06.002> PMID: 27504028
61. Crous PW, Schumacher RK, Akulov A, Thangavel R, Hernández-Restrepo M, Carnegie AJ, et al. New and interesting fungi. 2. Fungal Syst Evol. 2019; 3:57–134. <https://doi.org/10.3114/fuse.2019.03.06> PMID: 32467898
62. Perera RH, Maharachchikumbura SSN, Ariyawansa HA, Bahkali AH, Jones EBG, Al-Sadi AM, et al. Two new *Pseudohalonectria* species on beech cupules (*Fagus sylvatica*) and a new genus to accommodate *P. suthepensis*. Phytotaxa. 2016; 278:115–131. <https://doi.org/10.11646/phytotaxa.278.2.2>
63. Kohlmeyer J, Volkmann-Kohlmeyer B, Eriksson OE. Fungi on *Juncus roemerianus*. 4. New marine ascomycetes. Mycologia. 1995; 87:532–542. <https://doi.org/10.1080/00275514.1995.12026565>