

Article



Morphological and Multi-Gene Phylogenetic Analyses Reveal *Pseudotubeufia* gen. nov. and Two New Species in Tubeufiaceae from China

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Abstract: Three helicosporous hyphomycete collections representing two species were obtained from rotting wood found in freshwater and terrestrial habitats in the Guizhou and Guangxi Provinces, China. A new genus *Pseudotubeufia* (Tubeufiaceae, Tubeufiales), comprising *Ps. hyalospora* sp. nov. and *Ps. laxispora* sp. nov., was introduced with morphological characteristic and molecular data. In addition, the molecular evidence showed that *Helicomyces* sp. (G.M. 2020-09-19.1), *H. roseus* (CBS: 102.76), and the new genus *Pseudotubeufia* clustered together with high support based on a multi-gene (LSU, ITS, *tef1* α , and *rpb2*) phylogenetic analysis. Detailed descriptions, illustrations, and notes of the three new collections are provided.

Keywords: three new taxa; asexual morph; taxonomy; Tubeufiales



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1. Introduction

Tubeufia was first introduced by Penzig and Saccardo [1], which included the type species *T. javanica* and two other species (*T. anceps* and *T. coronata*). Based on *Tubeufia*, the family Tubeufiaceae and order Tubeufiales were subsequently established [2,3]. The latest comprehensive study on Tubeufiaceae was carried out by Lu et al. [4]. Currently, there are 46 accepted genera in the family Tubeufiaceae, including Acanthohelicospora, Acanthophiobolus, Acanthostigma, Acanthostigmina, Acanthotubeufia, Aquaphila, Artocarpomyces, Berkleasmium, Bifrontia, Boerlagiomyces, Chaetosphaerulina, Chlamydotubeufia, Dematiohelicoma, Dematiohelicomyces, Dematiohelicosporum, Dematiotubeufia, Dictyospora, Helicangiospora, Helicoarctatus, Helicodochium, Helicohyalinum, Helicoma, Helicomyces, Helicosporium, Helicotruncatum, Helicotubeufia, Kamalomyces, Kevinhydea, Manoharachariella, Muripulchra, Neoacanthostigma, Neochlamydotubeufia, Neohelicoma, Neohelicomyces, Neohelicosporium, Neomanoharachariella, Neotubeufia, Parahelicomyces, Pleurohelicosporium, Podonectria, Pseudohelicoon, Tamhinispora, *Thaxteriella, Thaxteriellopsis, Tubeufia, and Zaanenomyces* [2–16]. Among them, five genera, viz. Acanthophiobolus, Bifrontia, Boerlagiomyces, Podonectria, and Thaxteriella, only have morphological data available, and their systematic evolutionary relationships have not been confirmed by molecular data.

Helicosporous hyphomycetes are asexual fungi that produce various forms of coiled two- or three-dimensional hollow conidia, which is the most common asexual morph in the family Tubeufiaceae [4,17–21]. The classification of helicosporous hyphomycetes has been studied for more than 200 years [22–24]. These fungi are widely distributed in tropical and subtropical regions, mostly acting as saprobes on plant litter, rotten wood, and decaying twigs in freshwater and terrestrial habitats [4,20,21]. However, there have been rare reports of endophytic fungi with coiled conidia [25,26].

In this study, three new collections from the family Tubeufiaceae were obtained during a survey of helicosporous hyphomycetes from the Guizhou and Guangxi Provinces, China. Based on detailed morphological comparisons and multi-gene phylogenetic analyses, we introduced a new genus named *Pseudotubeufia*, which comprises two new species, *Ps. hyalospora* and *Ps. laxispora*.

2. Materials and Methods

2.1. Sample Collection, Specimen Examination, and Isolation

Fresh specimens of submerged rotting wood were collected from May to August 2021 in the Guizhou and Guangxi provinces in southern China. The newly collected samples were processed following the method described by Boonmee et al. [3]. The colonies on the host surfaces were examined and observed with stereomicroscopes (SMZ 745 and SMZ 800N, Nikon, Tokyo, Japan). Their micro-morphological characters were studied using an ECLIPSE Ni compound microscope (Nikon, Tokyo, Japan) and a Canon 90D digital camera. Measurements were made with the Tarosoft (R) Image Frame Work program. Photo-plates were made using Adobe Illustrator CC 2019 (Adobe Systems, San Jose, CA, USA).

Single spores were isolated on potato dextrose agar (PDA) medium and the germinated conidia were aseptically transferred to fresh PDA plates, as described in Senanayake et al. [27]. Fungal colonies growing on the PDA were incubated at 25 °C for 28 or 42 days, and their morphological characteristics, including color and size, were recorded. Dried fungal specimens were deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (Herb. HKAS), Kunming, China, and in the herbarium of the Guizhou Academy of Agriculture Sciences (Herb. GZAAS), Guiyang, China. Ex-type living cultures were deposited at the China General Microbiological Culture Collection Center (CGMCC), Beijing, China, and the Guizhou Culture Collection, China (GZCC). Facesoffungi numbers (FoF) and Index Fungorum numbers were determined according to the guidelines of Jayasiri et al. [28] and the Index Fungorum (2023) [29], respectively.

2.2. DNA Extraction, PCR Amplification, and Sequencing

Fresh fungal mycelia were scraped using the methods described by Lu et al. [30]. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Shanghai, China), according to the manufacturer's protocol. The large subunit of the ribosomal DNA (LSU), the internal transcribed spacer (ITS), the translation elongation factor 1 alpha (*tef1a*), and the RNA polymerase II second largest subunit (*rpb2*) gene regions were amplified using LR0R/LR5, ITS5/ITS4, EF1-983F/EF1-2218R, and fRPB2-5F/fRPB2-7cR primer pairs, respectively [31–34]. PCR amplification was performed in a reaction volume of 50 µL, including 44 µL 1.1 × T3 Supper PCR Mix (Qingke Biotech, Chongqing, China), 2 µL of each forward and reverse primer, and a 2 µL DNA template. The LSU, ITS, *tef1a*, and *rpb2* amplification reactions were carried out according to the following reference method (Table 1) [4,13,30,35–37].

Locus	Primer	Initial Denaturation	Denaturation	Annealing	Elongation	Final Extension	Hold
LSU	LR0R/LR5	95 °C/3 min	94 °C/30 s	51 °C/50 s 30 cycles	72 °C/1 min	72 °C/7 min	
ITS	ITS5/ITS4	95 °C/3 min	95 °C/30 s	51 °C/1 min 34 cycles	72 °C/45 s	72 °C/10 min	1°C
tef1a	EF1-983F/EF1-2218R	95 °C/3 min	94 °C/30 s	55 °C/50 s 40 cycles	72 °C/1 min	72 °C/7 min	4 C
rpb2	fRPB2-5F/fRPB2-7cR	95 °C/3 min	95 °C/30 s	54 °C/40 s 34 cycles	72 °C/1 min	72 °C/7 min	

Table 1. PCR protocols.

The quality of the PCR products was checked on 1% agarose gel electrophoresis stained with ethidium bromide. The purification and sequencing of the PCR products

were carried out at Tsingke Bio-logical Engineering Technology and Services Co., Ltd. (Chongqing, China).

2.3. Phylogenetic Analyses

The original sequences of our newly obtained strains were checked and assembled using BioEdit v 7.0.5.3 and SeqMan v. 7.0.0 (DNASTAR, Madison, WI) [38,39]. The closest taxa to our strains were determined by blast searches in GenBank (http://www.ncbi.nlm. nih.gov/, accessed on 10 May 2023). The other sequences used in the phylogenetic analysis (Table 2) were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/, accessed on 10 May 2023). The sequence alignments for each locus were performed using the online multiple alignment program MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/, accessed on 10 May 2023) [40], and auto-adjusted by trimAl v1.2 [41]. The multigenic sequences (LSU-ITS- $tef1\alpha$ -rpb2) were merged using the SequenceMatrix-Windows-1.7.8 software, and the sequences were exported to CIPRES for RAxML analyses [42]. The aligned Fasta and Phylip format file was converted to a Nexus format file for Bayesian inference (BI) and Maximum Parsimony (MP) analyses by using AliView v. 1.27 [43]. A phylogenetic tree, which infers phylogenetic relationships, was reconstructed based on a concatenated LSU, ITS, *tef1* α , and *rpb2* dataset using the online CIPRES Science Gateway (https://www.phylo. org/portal2/home.action, accessed on 10 May 2023) to construct the Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian inference (BI), respectively.

Table 2. Taxa used in this study and their GenBank accession numbers.

Tavar	Strain	GenBank Accessions				
Taxon		LSU	ITS	tef1a	rpb2	
Acanthohelicospora aurea	NBRC 7098	AY856894	AY916478	-	-	
Acanthohelicospora guianensis	UAMH 1699	AY856891	AY916479	_	-	
Acanthohelicospora pinicola	MFLUCC 10-0116 T	KF301534	KF301526	KF301555	-	
Acanthohelicospora scopula	ANM 386	GQ850489	GQ856141	_	-	
Acanthostigma chiangmaiensis	MFLUCC 10-0125 T	JN865197	JN865209	KF301560	-	
Acanthostigma perpusillum	UAMH 7237	AY856892	AY916492	_	_	
Acanthostigmina minutum	ANM 238	GQ850487	_	_	-	
Acanthostigmina minutum	ANM 880	GQ850486	_	-	_	
Acanthotubeufia filiforme	ANM 101 ^T	GQ850495	_	_	_	
Acanthotubeufia filiforme	ANM 514	GQ850494	GQ856146	-	-	
Aquaphila albicans	MFLUCC 16-0010	KX454166	KX454165	KY117034	MF535255	
Aquaphila albicans	MFLUCC 16-0020	KX454168	KX454167	-	MF535256	
Berkleasmium aquaticum	MFLUCC 17-0049 T	KY790432	KY790444	KY792608	MF535268	
Berkleasmium fusiforme	MFLUCC 17-1987 T	MH558822	MH558695	MH550886	MH551009	
Berkleasmium guangxiense	MFLUCC 17-0042 T	KY790436	KY790448	KY792612	MF535270	
Berkleasmium latisporum	MFLUCC 16-0019 T	KY790437	KY790449	KY792613	MF535271	
Berkleasmium longisporum	MFLUCC 17-1999 ^T	MH558825	MH558698	MH550889	MH551012	
Berkleasmium thailandicum	MFLUCC 15-1248 T	MH558829	KX454176	KY792614	MH551017	
Boerlagiomyces macrospora	MFLUCC 12-0388	KU764712	KU144927	KU872750	_	
Botryosphaeria agaves	MFLUCC 10-0051	JX646807	JX646790	_	_	
Botryosphaeria dothidea	CBS 115476	DQ678051	KF766151	DQ767637	DQ677944	
Chlamydotubeufia cylindrica	MFLUCC 16-1130 T	MH558830	MH558702	MH550893	MH551018	
Chlamydotubeufia krabiensis	MFLUCC 16-1134	KY678759	KY678767	KY792598	MF535261	
Dematiohelicoma pulchrum	MUCL 39827	AY856872	AY916457	_	_	
Dematiohelicomyces helicosporus	MFLUCC 16-0213 T	KX454170	KX454169	KY117035	MF535258	
Dematiohelicomyces helicosporus	MFLUCC 16-0003	MH558831	MH558703	MH550894	MH551019	
Dematiohelicosporum guttulatum	MFLUCC 17-2011 T	MH558833	MH558705	MH550896	MH551021	
Dematiotubeufia chiangraiensis	MFLUCC 10-0115 T	JN865188	JN865200	KF301551	_	
Dictyospora thailandica	MFLUCC 16-0001 T	KY873622	KY873627	KY873286	MH551023	
Dictyospora thailandica	MFLUCC 18-0641	MH558834	MH558706	MH550897	MH551022	
Dictyospora thailandica	MFLUCC 16-0215	KY873623	KY873628	KY873287	_	
Helicangiospora lignicola	MFLUCC 11-0378 T	KF301531	KF301523	KF301552	_	
Helicoarctatus aquaticus	MFLUCC 17-1996 ^T	MH558835	MH558707	MH550898	MH551024	
Helicodochium aquaticum	MFLUCC 17-2016 T	MH558837	MH558709	MH550900	MH551026	
Helicodochium aquaticum	MFLUCC 18-0490	MH558838	MH558710	MH550901	MH551027	
Helicohvalinum aquaticum	MFLUCC 16-1131 T	KY873620	KY873625	KY873284	MF535257	
Helicohyalinum aquaticum	MFLUCC 16-1133 T	MH558840	MH558712	MH550903	MH551029	
Helicoma outtulatum	MFLUCC 16-0022 T	KX454172	KX454171	MF535254	MH551032	
Helicoma inthanonense	MFLUCC 11-0003 T	IN865199	IN865211	_	_	
Helicoma siamense	MFLUCC 10-0120 T	IN865192	IN865204	KF301558	_	
Helicoma tectonae	MFLUCC 12-0563 T	KU764713	KU144928	KU872751	_	
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Table 2. Cont.

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Indexturbagin lydel MH2UCC 17-1980 1 MH220025 MH220020 MH220030 MH220035 Kanadomges hambasicala MFLUCC 10-1035 MFE00680 - - - - Kanadomges hambasicala MFLUCC 11-128 MFE00680 - - - - Kanadomges hambasicala MFLUCC 11-128 MF506882 MF506884 MF506886 - Kanadomges hambasicala MFLUCC 13-023 MF506882 MF506884 K7506866 - Kanadomges hambasicala MFLUCC 13-023 MF506882 MF506884 K730564 - Manadmacharricht tectura MFLUCC 15-024 K732054 K730554 - - Murginden aguata MFLUCC 10-016 MH289078 MH289083 - - Nochlommyels aguata MFLUCC 10-017 K732054 K730554 - MH250076 Nochlommyels aguata MFLUCC 10-0397 K732054 K730554 - MH55007 Nochlommyels aguata MFLUCC 10-0397 K732054 K732058 K7320564 -	Helicotruncatum nalmigenum	NBRC 32663	AY856898	AY916480	_	_	
Identifying present MFLUCC 17-0043 MH290025 MH290020 MH290030 MH290035 Kanadomyce bulandicus MFLUCC 11-0128 MF506881 MF506883 MF506885 - Kanadomyce bulandicus MFLUCC 11-0128 MF506881 MF506884 MF506886 - Kennadomyce bulandicus MFLUCC 11-0123 MT506881 MF506884 MF506866 - Kennadomyce bulandicus MFLUCC 11-0120 MT707115 MT747115 MT747102 - - Maripulcina ngatica MFLUCC 11-0510 K7302551 K7320532 - - - Newcantinbastym fasjorma MFLUCC 11-0510 K730257 K730152 - - - Newcantinbastym fasjorma MFLUCC 10-0187 N730547 K7302534 K7302534 - - Neekleicon Ingoscarun MFLUCC 10-1087 K7302547 K7302534 K7302554 - - Neekleicon Ingoscarun MFLUCC 16-0997 MT53057 MF550785 MH550189 MF550895 MF550895 MF550895 MF550189-1 <	Helicotuhenfia hudei	MELUCC 17-1980 T	MH290026	MH290021	MH290031	MH290036	
Earnalomger hambascola MFLU11/2281 ME90680 n	Helicotubeufia ionesii	MELUCC 17-0043 T	MH290025	MH290020	MH290030	MH290035	
	Kamalomuces hamhusicola	MELU 11-0228 T	ME506880	-	-	-	
Earnal compose trailing is a serie of the series	Kamalomyces thailandicus	MFLUCC 11-0158	ME506881	ME506883	ME506885	_	
Keningka herestipitati MFLUCC 19-109 ⁻¹ MH747115 MH747102 - - Muripulcha neutria MFLUCC 10-107 ⁻¹ KV74075 KU147475 KU1872762 - Muripulcha neutria MFLUCC 15-026 ⁻¹ KV320531 KV320534 KV32054 MH550108 Muripulcha neutria MFLUCC 15-026 ⁻¹ KV320571 KV320532 - - Neucathostigna fusifiorme MFLUCC 11-030 ⁻¹ KV32054 K7301529 - - Neuchimogloutueigia fusifiorme MFLUCC 10-010 ⁺¹ K7301537 K7301524 K7301534 - Neuchicomage gauticus MFLUCC 10-019 ⁺¹ NK585100 NK585202 K7301534 K7301533 - Neuchicomages gauticus MFLUCC 10-039 ⁺¹ KV320545 KV320530 - MH550166 Neuchicosportus CZCC 10-008 ⁺¹ MH55870 MH558730 MH558736 MH550936 MH551070 Neuchicosportum astrictum MFLUCC 17-204 ⁺¹ MH558754 MH558749 MH551070 Neuchicosportum fusioporum GZCC 10-064 ⁺¹ MH558754	Kamalomyces thailandicus	MELUCC 13-0233 T	ME506882	ME506884	ME506886	_	
	Keminbudea hrevistinitata	MELUCC 18-1269 T	MH747115	MH747102	-	_	
$\begin{split} \begin{tabular}{lllllllllllllllllllllllllllllllllll$	Manoharachariella tectonae	MELUCC12-0170 T	KU764705	KU144935	KU872762	_	
$\begin{split} & \text{Murrpulchm aquatica} & \text{MFLUCC} 15:039^{-T} & KY320549 & KY320532 & - 0.001 & -0.0000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.000000 & -0.00000000 & -0.000000 & -0.0000000 & -0.0000000 & -0.0000000 & -0.000000000 & -0.0000000000$	Murinulchra aquatica	KUMCC 15-0276	KY320551	KY320534	KY320564	MH551058	
Numerative MHLUCC 11-0510 T KP301537 KP301537 KP301537 - - Neechlamylatulednja fusiormis MHLUCC 10-0118 T MHS5010 MHS5020 KH30153 - Neechlamylatulednja fusiormis MHLUCC 10-0118 T PN865100 PN85502 KF301534 KF301534 - Neechlamylatulednja fusiormis MFLUCC 10-0118 T PN865105 KF301534 KF301535 - Neechlamylatulednja fusiormis MFLUCC 16-0493 T KY32055 KF301533 - MH55066 Neehlcinomyces guantizas MFLUCC 16-0493 T KY320550 MH550730 MH550740 MH5507	Muripulchra aquatica	MELLICC 15-0249 T	KV320549	KY320532	_	_	
Noverhämsgrande inder production in the DCC 16-001 * MH358865 MH358740 MH358931 MH351059 Neechlämgdatubergin kunkernensis NFLUCC 16-001 * PK805190 PK85202 KF301564 Neechlämgdatubergin kunkernensis NFLUCC 16-001 * PK805190 PK85202 KF301564 Neehelicomiges aguaticas NFLUCC 16-008 * KK341174 KV454173 - MH551067 Neehelicomiges granutisporus CZCC 16-008 * MH558870 MH558747 MH550938 MH551068 Neehelicomiges submersus MFLUCC 16-004 * MG07613 MG017612 MG017614 - Neehelicosporium disporum MFUCC 16-004 * MH55879 MH55874 MH550938 MH551077 Neehelicosporum krabiense MFUCC 16-024 * MH558784 MH558754 MH55092 MH551077 Neehelicosporum krabiense MFUCC 16-024 * MH558784 MH558754 MH55092 MH55092 Neehelicosporum krabiense MFUCC 16-024 * MH558789 MH558766 MH55092 MH55092 Neehelicosporium parcisporum MFUCC 16-024 *	Neoacanthostioma fusiforme	MELUCC 11-0510 T	KF301537	KF301529	_	_	
International problem INTLUCC 10118 INNS500 INN	Neochlamudotubeufia fusiformis	MELUCC 16-0016 T	MH558865	MH558740	MH550931	MH551059	
Nuclearing Nuclear	Neochlamudotubeufia khunkornensis	MELUCC 10 0118 T	INI865190	IN1865202	KE301564	Wil 1551059	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Neohalicoma fagacearum	MELUCC 11 0270 T	KE301532	KE301524	KF301553		
	Neohalicomucae aquaticus	MELUCC 16 0002 T	KY320545	KV220529	KV220561	- MH551066	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Neohelicomyces arandisporus	KUNGC 15 0470 T	K1520545 KY454174	K1520528 KV454172	K1520501	MH551067	
$\begin{aligned} & \text{Partheticonflyces hybrids} & \text{CLCC} 16-0086 & MH2308-00 & MH2308-43 & MH2308-09 & MH2308-09 & MH2308-09 & MH25010-9 & MH2500-9 & MH2500-9 & MH2500-9 & MH2500-9 & MH2500-9 & MH2500-9 & MH250$	Neohelicomyces grunuisporus	C7CC 16 00% T	NA434174	NA434173	- MHEE0026	MH551067	
	Neohelicomyces nyulosporus	GZCC 10-0000	WIG556670 VV220E47	WIE356745 KV220520	MH330936	MHEE1069	
	Neohelicomyces submersus	MFLUCC 17 2004 T	N 1520547	N 1320330	- MHEE0028	MH551000	
	Neohelicosporium fusionemum	MELICC 16 0642 T	MC017612	MC017612	MC017614	WII 1551070	
	Neohelicosporium fusisporum	C7CC 16 0076 T	MG017613 ME467026	MG017612 ME467022	MG017614 MEE2E240	- MEE2E270	
	Neohelicosporium hydiosporum	MELLICC 16 0224 T	MU558870	MH558754	MH550045	MH551077	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Neohelicosporium croideum	$C7CC 16 0064^{T}$	MU559991	MH558754	MH550943	MH551079	
Retailedspiration INTEDCC 17-1523 INTEGV30 INTE	Neohelicosporium parzienorum	MELLICC 17 1522 T	ME467020	ME467026	ME525252	ME525282	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Neotubaufia krabiancia	MELLICC 16 1125 T	MC012024	MC012021	MC012010	MC012017	
$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Darahalicomuces aquaticus	MELUCC 16 0224 T	MU559801	MU559766	MU550058	MH551002	
$ \begin{array}{l l l l l l l l l l l l l l l l l l l $	Parahalicomyces hydosporus	MELUCC 15-0343 T	KV320540	KV320523	10111330938	WII 1551092	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Parabelicomyces indicus	CBS 374 93	AV856885	AV916477	_		
$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Parahelicomyces naludosus	CBS 120503	DO341103	DO341095	_	_	
Paraheliconyces talbbitiMFLUCC 17-2021MH558890MH558765MH550957MH551091Pleurohelicosporium parvisporumMFLUCC 17-1982 TMH558890MH558764MH550956MH551088Pseudohelicon subglobosumBCR 5550AY866904AY916467Pseudohelicon subglobosumBCR CFU30843 TLC316610LC316607Pseudohelicon subglobosumGZCC 22-2011 TOR030831OR030838OR046675OR046682PsedotubeufialaxisporaGZCC 22-2012OR030833OR030830OR046676-PsedotubeufialaxisporaGZCC 22-2011 TOR030833OR030840OR046676-Tamhinispora indicaNFCCI 2924 TKC469283KC469282Tamhinispora indicaNFCCI 4231 TMG763745MG763746Thaxteriellopsis lignicolaMFLUCC 10-0124JN865193JN865205Tubeufia aquaticaMFLUCC 10-0124JN865193JN865208KF301561-Tubeufia aquaticaMFLUCC 17-2024 TMH558896MH558770MH550963MH551095Tubeufia apuaticaMFLUCC 17-1799 TMH558897MH558772MH550965MH551097Tubeufia bambusicolaMFLUCC 17-1998 TMH558897MH558773MH550964MH551099Tubeufia brunneaMFLUCC 16-1247 TKY32058KJ880037-Tubeufia inaequalisMFLUCC 16-1247 TKV85808MH558771MH550965MH551197Tubeufia inaequalisMFLUCC 16-1247 T	Parahelicomyces roseus	KUMCC 15-0411	KY320544	KY320527	KY320560	_	
Pleurohelicosporium paroisporumMFLUCC 17-1982 TMH558889MH558764MH55076MH55076Pseudohelicoon gigantisporumBCC 3550AY856904AY916467Pseudohelicon subglobosumBCRC FU30843 TLC316610LC316607PsedotubeufialaxisporaGZCC 22-2011 TOR030831OR030838OR046675OR046682PsedotubeufialaxisporaGZCC 22-2010 TOR030832OR030839OR046676OR046683PsedotubeufialaxisporaGZCC 22-2010 TOR030833OR030840OR046677-Tamhinispora indicaNFCCI 2924 TKC469283KC469282Tamhinispora srinivasaniiNFCCI 2924 TKC469283KC469285Thaxteriellopsis lignicolaMFLUCC 10-0121JN865193JN865208KF301561-Thaxteriellopsis lignicolaMFLUCC 17-2024 TMH558894MH558769MH550961MH551095Tubeufia adundataMFLUCC 17-1803 TMH558897MH558771MH550963MH551097Tubeufia brunneaMFLUCC 17-199 TMH558897MH558773MH550964MH551117Tubeufia inaequalisMFLUCC 17-199 R TMH558896MH558773MH550984MH551117Tubeufia inaequalisMFLUCC 15-1247 TKY092417KY117033MH551119Tubeufia inaequalisMFLUCC 15-1247 TKY092417KY117033MH551142Tubeufia latisporaMFLUCC 15-1247 TKX454177-MH551144Tubeufia latisporaMFLUCC 15-1247 T <td>Parahelicomyces talhotii</td> <td>MFLUCC 17-2021</td> <td>MH558890</td> <td>MH558765</td> <td>MH550957</td> <td>MH551091</td>	Parahelicomyces talhotii	MFLUCC 17-2021	MH558890	MH558765	MH550957	MH551091	
Pseudohelicon gigaritisporumBCC 3550AY856904AY916467Pseudohelicon subglobosumBCR FU30843 TLC316610LC316607PsedotubeufialaxisporaGZCC 22-2011 TOR030831OR030838OR046675OR046682PsedotubeufialaxisporaGZCC 22-2012OR030832OR030839OR046676OR046683PsedotubeufialaxisporaGZCC 22-2010 TOR030833OR030840OR046677-Tamhinispora indicaNFCCI 2924 TKC469283KC469282Tamhinispora srinivasaniiNFCCI 4231 TMG763745MG763746Thaxteriellopsis lignicolaMFLUCC 10-0121JN865193JN865205Thaxteriellopsis lignicolaMFLUCC 10-0124JN865196JN865208KF301561-Tubeufia abundataMFLUCC 17-2024 TMH558894MH558779MH550961MH551097Tubeufia banbusicolaMFLUCC 17-1803 TMH55896MH558771MH550963MH551097Tubeufia barbusicolaMFLUCC 17-2022 TMH558897MH558772MH550964MH551097Tubeufia barbusicolaMFLUCC 17-199 TMH558896MH558773MH550965MH551117Tubeufia iavanicaMFLUCC 17-2022 TMH558896MH558773MH550965MH551097Tubeufia iavanicaMFLUCC 17-2023 TMH558896MH558773MH550965MH551097Tubeufia iavanicaMFLUCC 17-2023 TMH558916MH558773MH550965MH551017Tubeufia iavanicaM	Pleurohelicosporium parvisporum	MFLUCC 17-1982 ^T	MH558889	MH558764	MH550956	MH551088	
Pseudohelicoon subglobsumBCRC FU30843 TLC316610LC316607PseudohubufialaxisporaGZCC 22-2011 TOR030831OR030838OR046675OR046682PseudotubufialaxisporaGZCC 22-2012OR030832OR030839OR046676OR046683PseudotubufialaxisporaGZCC 22-2010 TOR030833OR030840OR046677-Tamhinispora sinitoasaniiNFCCI 2924 TKC469283KC469282Tamhinispora sinitoasaniiNFCCI 4231 TMG763745MG763746Thaxteriellopsis lignicolaMFLUCC 10-0121JN865193JN865205Thubufia abundataMFLUCC 10-0124JN865196JN865208KF301561-Tubeufia aquaticaMFLUCC 17-2024 TMH558894MH558769MH550961MH551095Tubeufia bambusicolaMFLUCC 16-1249 TKY320539KY320522KY32056MH551097Tubeufia barunuscolaMFLUCC 17-1799 TMH558896MH558771MH550963MH551097Tubeufia barunuscolaMFLUCC 17-1799 TMH558898MH558772MH550965MH551099Tubeufia iavanicaMFLUCC 17-202 TMH558898MH558773MH550965MH551099Tubeufia iavanicaMFLUCC 17-202 TMH558896MH558771MH550964MH551099Tubeufia iavanicaMFLUCC 17-202 TMH5588916MH558771MH550965MH551099Tubeufia iavanicaMFLUCC 16-0027 TKY092412KY092417KY117033MH551149Tubeufia iavanica </td <td>Pseudohelicoon gigantisporum</td> <td>BCC 3550</td> <td>AY856904</td> <td>AY916467</td> <td>_</td> <td>_</td>	Pseudohelicoon gigantisporum	BCC 3550	AY856904	AY916467	_	_	
Psedotubeufialaxispora $GZCC 22-2011^{T}$ OR030831OR030838OR046675OR046682Psedotubeufialaxispora $GZCC 22-2012$ OR030832OR030839OR046676OR046683Psedotubeufialaxispora $GZCC 22-2010^{T}$ OR030833OR030840OR046677-Tamhinispora indicaNFCCI 2924 TKC469283KC469282Tamhinispora srinicasaniiNFCCI 4231 TMG763745MG763746Thaxteriellopsis lignicolaMFLUCC 10-0121JN865193JN865205Thaxteriellopsis lignicolaMFLUCC 10-0124 TJN865196JN865208KF301561-Tubeufia aquaticaMFLUCC 16-1249 TKY320539KY320522KY320556MH551142Tubeufia aquaticaMFLUCC 17-1709 TMH558896MH558771MH550963MH551097Tubeufia inaequalisMFLUCC 17-1903 TMH558897MH558773MH550964MH551098Tubeufia inaequalisMFLUCC 17-1922 TMH558897MH558773MH550964MH551117Tubeufia inaequalisMFLUCC 17-1928 TKJ880036KJ880034KJ880037-Tubeufia inaequalisMFLUCC 16-0027 TKY092412KY092417KY117033MH551119Tubeufia roseohelicosporaMFLUCC 16-0247 TKY454178KX454177-MH551142Tubeufia roseohelicosporaMFLUCC 16-027 TKY092412KY092417KY117033MH551119Tubeufia roseohelicosporaMFLUCC 15-1247 TKX454177-MH551142Tubeufia	Pseudohelicoon subglobosum	BCRC FU30843 T	LC316610	LC316607	_	_	
PsedotubeufialaxisporaGZCC 22-2012OR030832OR030839OR046676OR046683PsedotubeufialayalosporaGZCC 22-2010 TOR030833OR030840OR046677-Tamhinispora indicaNFCCI 2924 TKC469283KC469282Tamhinispora sinitosaniiNFCCI 4231 TMG763745MG763746Thaxteriellopsis lignicolaMFLUCC 10-0121JN865193JN865205Thaxteriellopsis lignicolaMFLUCC 10-0124JN865196JN865208KF301561-Tubeufia apundataMFLUCC 17-2024 TMH558894MH558769MH550961MH551095Tubeufia apundataMFLUCC 17-1803 TMH558894MH558771MH550963MH551097Tubeufia baruntacaMFLUCC 17-1803 TMH558896MH558772MH550963MH551098Tubeufia brevisMFLUCC 17-1999 TMH558898MH558773MH550965MH551099Tubeufia brunneaMFLUCC 17-1998 TMH558916MH558773MH550965MH551117Tubeufia ipacqualisMFLUCC 12-0545 TKJ880036KJ880034KJ880037-Tubeufia ipacqualisMFLUCC 16-0027 TKY092412KY092417KY117033MH551119Tubeufia roseohelicosporaMFLUCC 16-0027 TKY092412KY092417KY117033MH551142Tubeufia roseohelicosporaMFLUCC 16-0027 TKY092412KY092417KY117033MH551142Tubeufia roseohelicosporaMFLUCC 15-1244 TKX454178KX454177-	Psedotubeufialaxispora	GZCC 22-2011 T	OR030831	OR030838	OR046675	OR046682	
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Zaanenomyces versatilis CPC 41224 T OK663769 OK664730 - -	Zaanenomyces moderatricis-academiae	CPC 41273 T	OK663762	OK664723	-	OK651167	
	Zaanenomyces versatilis	CPC 41224 ^T	OK663769	OK664730	-	-	

Note: Newly generated sequences in this study are indicated in blue bold. "T" denotes ex-type strain. "-" as meaning no data available in GenBank.

The maximum likelihood (ML) analysis was carried out with the RAxML-HPC2 tool on XSEDE (8.2.12) using a GTRGAMMA approximation with a rapid bootstrap analysis, followed by 1000 bootstrap replicates [44].

The maximum parsimony (MP) analysis was performed by using PAUP on the XSEDE (4.a168) tool. A heuristic search with 1000 random taxa was added to infer MP trees. The

value of the MaxTrees, which collapsed branches of zero length and saved all the multiple parsimonious trees, was set to 5000. The parsimony score values of the tree length (TL), consistency index (CI), retention index (RI), and homoplasy index (HI) were calculated for the trees generated under different optimum criteria. The clade stability was estimated using a bootstrap analysis with 1000 replicates, and the taxa were added for a random stepwise of each with 10 replicates [45].

The Bayesian inference (BI) analysis was conducted in MrBayes on XSEDE (3.2.7a) [46]. The best-fit substitution model GRT + I + G was determined for the LSU, ITS, *tef1* α , and *rpb2* matrix using MrModeltest 2.3 [47] under the Akaike Information Criterion (AIC). Four simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 1000th generation. The burn-in phase was set at 25% and the remaining trees were used to calculate the posterior probabilities (PP).

The phylogenetic tree and photo-plates were created using FigTree v. 1.4.4., Adobe Illustrator CC 2019 v. 23.1.0 (Adobe Systems, San Jose, CA, USA), and Adobe PhotoShop CC 2019 (Adobe Systems, San Jose, CA, USA).

3. Results

3.1. Phylogenetic Analysis

The partial LSU-ITS-*tef1* α -*rpb2* nucleotide sequences were used to determine the phylogenetic positions of the newly obtained isolates. These sequences were concatenated to generate a sequence matrix consisting of LSU (1–843 bp), ITS (844–1548 bp), *tef1* α (1549–2460 bp), and *rpb2* (2461–3505 bp) regions. The resulting matrix comprised a total of 3505 characters for 105 taxa and two outgroups, *Botryosphaeria agaves* (MFLUCC 10–0051) and *B. dothidea* (CBS 115476). The total characters analyzed in the concatenated dataset were 3505, out of which, 2002 characters were constant, 273 variable characters were parsimony-uninformative, and 1230 characters were parsimony-informative. The ML, MP, and BI analyses of the concatenated LSU-ITS-*tef1* α -*rpb2* dataset yielded similar tree topologies, and the ML tree is shown in Figure 1.

In the phylogenetic analyses (Figure 1), the newly isolated strains GZCC 22–2011 and GZCC 22–2012 clustered together (95% ML/100% MP/1 PP) without a significant branch length, indicating that they are phylogenetically the same species, as *Pseudotubeufia laxispora* sp. nov. *Pseudotubeufia hyalospora* sp. nov. formed a sister clade with *Ps. laxispora* with 91% ML/100% MP/0.97 PP supports. In addition, the three strains of *Pseudotubeufia clustered* with *Helicomyces* sp. (G.M. 2020-09-19.1), *Helicomyces roseus* (CBS 102.76), and *Dematiohelicoma pulchrum* (MUCL 39827) with weak support.

3.2. Taxonomy

Pseudotubeufia J. Ma & Y.Z. Lu, gen. nov.

Index Fungorum number: IF900553; Facesoffungi number: FoF 03700.

Etymology: "*Pseudotubeufia*", referring to the genus morphologically similar to the helicosporous asexual morph of *Tubeufia*.

Saprobic on the decaying wood in a freshwater stream. The sexual morph was undetermined. The asexual morph was helicosporous hyphomycetes. The *colonies* on the substratum were superficial, effuse, gregarious, and white. The *mycelium* were partly immersed, composed of hyaline to pale brown, septate, branched, and smooth hyphae. The *conidiophores* were macronematous, mononematous, erect or procumbent, flexuous, cylindrical, branched or unbranched, septate, hyaline to brown, and smooth-walled. The *conidiogenous* cells were holoblastic, mono- to polyblastic, integrated, sympodial, repeatedly geniculate, intercalary or terminal, irregularly cylindrical, denticulate, hyaline to pale brown, and smooth-walled. The *conidia* were solitary, acropleurogenous, helicoid, rounded at the tip, coiled 2–3 times, became loose in water, indistinctly septate, guttulate, hyaline, and smooth-walled.



Figure 1. Phylogram generated from the maximum likelihood analysis based on a combined of LSU, ITS, *tef1* α , and *rpb2* sequence data. Bootstrap support values of maximum likelihood (ML) and maximum parsimony (MP) equal to or greater than 75%, and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are given near the nodes as ML/MP/PP. *Botryosphaeria agaves* (MFLUCC 10–0051) and *B. dothidea* (CBS 115476) were used as outgroup taxa. The newly generated sequences are shown in red bold.

Type species: Pseudotubeufia hyalospora J. Ma & Y.Z. Lu.

Notes: Morphologically, *Pseudotubeufia* is the most similar to *Tubeufia* as it has flexuous, cylindrical conidiophores, cylindrical, denticulate, hyaline to pale brown conidiogenous cells, and hyaline helicoid conidia [4]. However, the phylogenetic analysis result showed that *Pseudotubeufia* has a close affinity with the species of *Dematiohelicoma* and *Helicomyces*, and is distant from the group of *Tubeufia* (Figure 1). However, *Dematiohelicoma* can be distinguished from *Pseudotubeufia* by its erect conidiophores and multi-septate, brown to dark brown conidia. *Pseudotubeufia* is also easily distinguished from *Helicomyces* by its repeatedly geniculate conidiogenous cells [4]. Therefore, the new genus *Pseudotubeufia* is introduced to accommodate two species, *Ps. hyalospora* and *Ps. laxispora*.

Pseudotubeufia hyalospora J. Ma & Y.Z. Lu., sp. nov., Figure 2.

Index Fungorum number: IF900554; Facesoffungi number: FoF 14268.

Etymology: The epithet "hyalospora", referring to hyaline helicoid conidia.

Holotype: HKAS 125885.

Saprobic on the decaying wood in a freshwater stream. The sexual morph was undetermined. The asexual morph was helicosporous hyphomycetes. The *colonies* on the substratum were superficial, effuse, gregarious, and white. The *mycelium* were partly immersed, composed of hyaline to pale brown, septate, branched, and smooth hyphae. The *conidiophores* were 31–46 µm long, 3–5.5 µm wide, macronematous, mononematous, procumbent, flexuous, cylindrical, branched, septate, hyaline to pale brown, and smoothwalled. The *conidiogenous* cells were 5.5–27.5 µm long, 3–5 µm wide, holoblastic, mono- to polyblastic, integrated, sympodial, repeatedly geniculate, intercalary or terminal, irregularly cylindrical, denticulate, hyaline to pale brown, and smooth-walled. The *conidia* were solitary, acropleurogenous, helicoid, rounded at the tip, 35–58 µm in diam. and had conidial filaments 4–5.5 µm wide ($\bar{x} = 48 \times 4.5 \mu m$, n = 20), 201–316 µm long, coiled 2–3 times, became loose in water, were indistinctly septate, guttulate, and hyaline.

Culture characteristics: The conidia germinated on the PDA within 10 h. The colonies on the PDA were irregular, with a flat surface, edge undulate, were pale brown to brown from above and below, and reached a 28 mm diam. after 42 days of incubation at 25 $^{\circ}$ C.

Material examined: China, Guizhou Province, Qiandongnan Miao and Dong Autonomous Prefecture, Zhenyuan City, 27°18′ N, 108°21′ E, on rotting wood in a freshwater stream, 1 May 2021, Xing-Juan Xiao, XXJ11.2 (HKAS 125885, holotype; GZAAS 22–2010, isotype), ex-type living cultures, CGMCC, GZCC 22–2010.

Notes: Morphologically, *Ps. hyalospora* is similar to *Ps. laxispora* (HKAS 125868), as it has flexuous, branched conidiophores, repeatedly geniculate conidiogenous cells, and acropleurogenous, guttulate, hyaline helicoid conidia. However, *Pseudotubeufia hyalospora* differs from *Ps. laxispora* (HKAS 125868) in having shorter conidiophores (31–46 µm vs. up to 155 µm), shorter conidiogenous cells (5.5–27.5 µm vs. up to 39 µm), and a different colony morphology in PDA (irregular, undulate edge vs. circular, entire edge). In addition, the phylogenetic analysis result showed that they are a distinct species. In accordance with the recommendations of Jeewon and Hyde [48] for species delimitation, we analyzed the pairwise dissimilarities of the DNA sequences between *Ps. hyalospora* (GZCC 22–2010) and *Ps. laxispora* (GZCC 22–2011) and found 60/905 bp (6.6%) differences in the *tef1a* gene. Therefore, we propose *Pseudotubeufia hyalospora* as a new species.



Figure 2. *Pseudotubeufia hyalospora* (HKAS 125885, holotype). (**a**,**b**) Colonies on the host surface. (**c**–**g**,**i**,**m**,**n**) Conidiophores with attached conidia. (**h**,**j**–**l**,**q**,**r**) Conidiophores and conidiogenous cells. (**o**,**p**,**s**–**u**) Conidia. (**v**) Germinating conidium. (**w**,**x**) Colonies on PDA at 42 days old (from above and below). Scale bars: (**c**–**i**,**m**,**o**,**p**,**s**–**v**) 20 μm, (**j**–**l**,**n**,**q**,**r**) 10 μm.

Pseudotubeufia laxispora J. Ma & Y.Z. Lu, sp. nov., Figure 3.



Figure 3. *Pseudotubeufia laxispora* (HKAS 125868, holotype). (**a**,**b**) Colonies on the host surface. (**c**–**i**) Conidiophores with attached conidia. (**j**,**k**,**m**) Conidiogenous cells. (**l**) Germinating conidium. (**n**–**p**) Conidia. (**q**,**r**) Colonies on PDA at 42 days old (from above and below). Scale bars: (**c**–**i**,**l**,**m**) 20 μ m, (**j**,**k**,**n**–**p**) 10 μ m.

Index Fungorum number: IF900555; Facesoffungi number: FoF 14269. Etymology: The epithet *"laxispora"*, referring to loosely coiled conidia. Holotype: HKAS 125868.

Holotype: Saprobic on dead bamboo culms in a freshwater stream. The sexual morph was undetermined. The asexual morph was helicosporous hyphomycetes. The colonies on the substratum were superficial, effuse, gregarious, and white. The *mycelium* were partly immersed, composed of hyaline to pale brown, septate, and abundantly branched hyphae. The *conidiophores* were 30–155 μm long, 3.5–6.5 μm wide, macronematous, mononematous, procumbent, flexuous, irregular cylindrical, branched, septate, hyaline to pale brown, and smooth-walled. The *conidiogenous* cells were 10–39 µm long, 3.5–6 µm wide, holoblastic, mono- to polyblastic, integrated, sympodial, intercalary or terminal, cylindrical, repeatedly geniculate, hyaline to pale brown, and smooth-walled. The conidia were solitary, acropleurogenous, helicoid, rounded at the tip, 35–56 µm in diam. and had conidial filaments that were 3–6.5 μ m wide (\overline{x} = 45 \times 4.5 μ m, n = 20), 242–327 μ m long, loosely coiled $2^{1}/_{4}-2^{3}/_{4}$ times, became loosely coiled in water, were indistinctly multi-septate, guttulate, hyaline, and smooth-walled; **Paratype** (Figure 4): Saprobic on the decaying wood in a terrestrial habitat. The sexual morph was undetermined. The asexual morph was helicosporous hyphomycetes. The *colonies* on the substratum were superficial, effuse, gregarious, and white. The *mycelium* were partly immersed, composed of hyaline to pale brown, septate, and abundantly branched hyphae. The conidiophores were 21–184 µm long, 3.5–9 µm wide, macronematous, mononematous, erect, flexuous, cylindrical, branched, septate, with the lower part dark brown and the upper part hyaline to pale brown, and smooth-walled. The *conidiogenous* cells were 4.5–33.5 µm long, 3–5.5 µm wide, holoblastic, mono- to polyblastic, integrated, sympodial, with arising tiny bladder-like protrusions, intercalary or terminal, cylindrical, truncate at apex after conidial secession, hyaline to pale brown, and smooth-walled. The conidia were solitary, acropleurogenous, helicoid, rounded at the tip, 36–50.5 μ m in diam. and had conidial filaments that were 3.5–6 μ m wide ($\overline{x} = 42 \times 4.5 \mu$ m, n = 20), 189–231 μ m long, coiled $1^{1}/_{2}$ – $2^{1}/_{2}$ times, became loosely coiled in water, were indistinctly multi-septate, guttulate, hyaline, and smooth-walled.

Culture characteristics: Holotype: The conidia germinated on the PDA within 10 h. The colonies on the PDA were circular, with a flat surface, edge entire, pale brown to brown from above and below, and reached 33 mm in diam. after 42 days of incubation at 25 °C; Paratype: The conidia germinated on the PDA within 10 h. The colonies on the PDA were circular, with a flat surface, edge entire, dark brown from above and below, and reached 22 mm in diam. after 28 days of incubation at 25 °C.

Material examined: China, Guangxi Province, Liuzhou City, Luzhai County, 24°46′ N, 109°53′ E, on dead bamboo culms in a freshwater stream, 4 May 2021, Jian Ma, LZ6.2 (HKAS 125868, holotype; GZAAS 22–2011, isotype), ex-type living cultures, CGMCC, GZCC 22–2011; China, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Sandu City, 25°56′ N, 107°57′ E, on decaying wood in a terrestrial habitat, 12 August 2021, Jingyi Zhang, SD12 (GZAAS 22–2012; paratype), living culture GZCC 22–2012.

Notes: Two collections, HKAS 125868 and GZAAS 22–2012, were obtained from freshwater and terrestrial habitats in southern China. Morphologically, HKAS 125868 has procumbent and hyaline conidiophores, while GZAAS 22–2012 has erect and brown conidiophores. Additionally, GZAAS 22–2012 has smaller conidia compared to HKAS 125868 (189–231 µm vs. 242–327 µm). However, based on pairwise nucleotide comparisons of ITS, LSU, *tef1* α , and *rpb2*, GZCC 22–2011 only differs from GZCC 22–2012 in a few genetic markers (2/469 bp for ITS, 1/824 bp for LSU, 1/916 bp for *tef1* α , and 13/1113 bp for *rpb2*). Furthermore, the phylogenetic analysis did not show any significant differences between these two strains (Figure 1). Therefore, despite their distinct morphology, we introduce these two isolates as one species named *Pseudotubeufia laxispora*.



Figure 4. *Pseudotubeufia laxispora* (GZAAS 22–2012, paratype). (a,b) Colonies on host surface. (c-g,j) Conidiophores with attached conidia. (h,i) Conidiogenous cells. (m–p) Conidia. (q) Germinating conidium. (k,l) Colonies on PDA at 28 days old (from above and below). Scale bars: (c–f) 20 μ m, (g,h,j–q) 10 μ m, (i) 5 μ m.

4. Conclusions

In this study, we introduced a new genus, *Pseudotubeufia*, based on multi-gene phylogenetic analyses and morphological characteristics. Morphologically, the asexual morphs of *Ps. hyalospora* and *Ps. laxispora* (HKAS 125868) are most similar to the species of *Tubeufia*, while *Ps. laxispora* (GZAAS 22–2012) resembles the species of *Parahelicomyces*. However, the multi-gene phylogenetic analyses showed that they did not cluster with *Tubeufia* or *Parahelicomyces*. Instead, they formed a distinct sister clade with the strains *Helicomyces* sp. (G.M. 2020-09-19.1, GenBank: MW276143) and *H. roseus* (CBS: 102.76), with 100% ML/100% MP/1 PP supports (Figure 1).

The ITS sequences of *Ps. hyalospora* and *Ps. laxispora* were searched using BLASTn in NCBI GenBank, and they exhibited the highest similarities to *Helicomyces* sp. (G.M. 2020-09-19.1: 58% query cover, 97.49% similarity and 100% query cover, 97.53% similarity), *Helicomyces roseus* (CBS 102.76: 58% query cover, 97.11% similarity and 100% query cover, 97.35% similarity), and *Tubeufia* sp. (MFLUCC 17–1520 and KUMCC 21–0472: 97% query cover, 84.98% similarity and 99% query cover, 86% similarity), respectively. In order to confirm the phylogenetic positions of the newly isolated strains, we performed singlegene and multi-gene phylogenetic analyses, including all species of the genera *Tubeufia*, *Parahelicomyces*, *Helicomyces*, and other related taxa, and obtained the same conclusion as shown in Figure 1. It is worth noting that *Helicomyces* sp. (G.M. 2020-09-19.1) and *H. roseus* (CBS 102.76) currently lack morphological descriptions and only have molecular data [49]. Their taxonomic positions require further molecular data and morphological descriptions for clarification.

Morphological differences can vary widely, even within the same species of helicosporous hyphomycetes. For instance, two collections (MFLU 16–2544 from decaying wood in China and MFLU 17–1091 from decaying wood in Thailand) have been identified as the same species, namely *Tubeufia aquatica* [4,50]. However, MFLU 16–2544 has larger conidiophores (109.5–189.5 µm) than those of MFLU 17–1091 (18–40 µm). Additionally, the conidiophores of MFLU 16–2544 are multi-septate, branched, and brown to dark brown, while those of MFLU 17–1091 are 0–1-septate, unbranched, and pale brown [4,50]. Similarly, our two collections of *Ps. laxispora* (HKAS 125868 and GZAAS 22–2012) showed significant differences in their conidiophores (Figures 3 and 4). We speculate that such differences may be attributable to variations in their habitats and geographical regions.

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