Three New Species, Two New Records and Four New Collections of Tubeufiaceae from Thailand and China

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Abstract: Tubeufiaceae, a cosmopolitan family with a worldwide distribution, is mostly reported as saprophic on decaying woody materials from both aquatic and terrestrial habitats. The family is commonly found as helicosporous hyphomycetes, while some are chlamydosporous and phragmosporous. In this study, thirteen helicosporous hyphomycetes were collected from Thailand and China. The phylogenetic analyses of combined ITS, LSU, TEF1-α, and RPB2 sequence data placed them in Dematiohelicomyces, Helicoma, Helicotruncatum, Neohelicosporium, Parahelicomyces, and Tubeufia within Tubeufiaceae. Three new species, Tubeufia cocois, Parahelicomyces chiangmaiensis, and Neohelicosporium bambusica, one new host record, Tubeufia laxispora, and one new geographic record, T. longihelicospora, are introduced based on both morphological characteristics and phylogenetic analyses. In addition, Dematiohelicomyces helicosporus, Helicoma guttatum, Helicotruncatum palmigenum, and Tubeufia cylindrothecia are described with detailed descriptions and color photo plates.

Keywords: three new species; two new records; asexual morph; phylogeny; taxonomy; Tubeufia laxispora

1. Introduction

The order Tubeufiales was introduced by Boonmee et al. [1] to accommodate a single family Tubeufiaceae based on phylogenetic evidence. Later, the other two families, Bezerromycetaceae and Wiesneriomycetaceae, were accepted into Tubeufiales by Liu et al. [2] based on phylogenetic analysis and divergence time estimates. Tubeufiaceae currently includes three families viz. Bezerromycetaceae, Tubeufiaceae, and Wiesneriomycetaceae, of which Bezerromycetaceae and Wiesneriomycetaceae are known for only sexual morphs, while Tubeufiaceae has been reported to have both sexual and asexual morphs, and the asexual morph of Tubeufiaceae is characterized by superficial ascomata, pseudoparaphysate hamathecium, and multisepitate, which are hyaline to pale brown cylindrical ascospores [3–10].
while the asexual morph is hyphomycetous, mostly helicosporous, and some are chlamy-
dosporous and phragmosporous [3,11,12]. Tubeufiaceae was recently revised by Lu et al. [3],
providing an updated multi-gene phylogenetic tree for Tubeufiales with 13 new genera in this
family. Currently, the family comprises 46 genera viz: Acantohelicospora, Acanthophiobolus,
Acanthostigma, Acanthotubefia, Aquaphila, Artocarpomyces, Berkleasmium, Bij frontieria,
Boerlagiomyces, Camporesiomyces, Chaetosphaerula, Chlamydotothefia, Dematioshelica,
Dematiophelicospora, Dematiophelicosporum, Dematiotubefia, Dictyospora, Discoat tubefia,
Helicangiopora, Helicoarctatus, Helicodochium, Helicoalamus, Helicomyces, Helicosporium,
Helicotubefia, Kamalomyces, Kevinhydeia, Manohararchiella, Muriipulcheria, Neoa canthhostigma,
Neohelicosporum, Neohelicomyces, Neohelicosporium, Neotubefia, Pleurohelicosporum, Podonec tria,
Pseudohelicosomyces, Pseudohelicicon, Tamhinispora, Thaxteriella, Thaxteriellopsis, and Tubefia [3,4]. Tubeufiaceae is cosmopolitan with a worldwide distribution in both tropical and temperate regions [1,3,5,12].

Previously, the taxonomic placement of Tubeufiaceae was uncertain, thus it has been
discussed by several mycologists. First, Barr [5] accommodated Tubeufiaceae in Pleospo-
rals based on the generic type Tubefia and later it was followed by various mycolo-
gists [6,7,13–15]. Eriksson and Winka [16], and Eriksson [17,18] preferred to accommo-
date Tubeufiaceae within the Dothideales, while Eriksson [19], as well as Lumbsch and
Huhtendorf [20], preferred to accommodate Tubeufiaceae within Dothideomycetes and
Chaetothyriomycetes incertae sedis. Based on 28S rDNA sequence data, Kodsu et al. [21]
preferred to keep Tubeufiaceae in Pleosporales (as the natural placement), which also
follows the ordinal circumscription of Barr [6,7], Sivanesan [13], Rossman, Crane et al. [14],
and Kirk et al. [15]. Species of Tubeufiaceae are commonly reported as saprobes on woody
substrates or submerged decaying wood in terrestrial and aquatic habitats [1–3,10,12,22].
Several studies reported that Tubeufiaceae species are able to produce active secondary
metabolites, which have potential anti-fungal, anti-bacterial, anti-diabetic, and anti-cancer
properties [3,23–25]. Several Tubeufiaceae studies have been carried out based on descrip-
tions, illustrations, and phylogenetic evidence in Asia, especially in China, India, Japan,
and Thailand [1–4,12,26].

In this study, thirteen helicosporous hyphomycetes were collected from Thailand and
China. Phylogenetic analyses of combined ITS, LSU, TEF1-α, and RPB2 sequence data place
them in Dematiosheliconmyces, Helicoma, Helicotubefia, Neohelicosporum, Parahelicosomyces,
and Tubefia. Three new species, Tubefia cocois, Parahelicosomyces chiangmaiensis, and Neoheli-
cosporium bambusicola are introduced with morphological and phylogenetic evidence. One
new host record, Tubefia laxispora, from Cocos nucifera in Thailand, as well as one new
generic record, T. longihelicospora, in China, are introduced. In addition, four known
species, Dematiosheliconmyces helicosporus, Helicoma guttulatum, Helicotubefia palmigenu m,
and Tubefia cylindrotheca are also described. Full descriptions, color photographs, and a
phylogenetic tree to show the placement of nine taxa are provided.

2. Materials and Methods
2.1. Sample Collection, Isolation, and Specimen Examination

Decaying wood, leaves, and culms were collected from Chiang Rai and Chiang Mai
Provinces of Thailand from May 2020 to March 2021 and Yunnan Province of China
in September 2021. Specimens were brought to a mycology laboratory for observation.
Tian et al. [27,28] and Senanayake et al. [29] were followed for the morphological study and
single spore isolation. Morphological characteristics were examined under a stereomicro-
scope (Motic SMZ-171, Wetzlar, Germany). Conidiomata were observed and photographed
using a Nikon ECLIPSE Ni-U compound microscope connected with a Nikon camera series
DS-Ri2. Germinating conidia were transferred aseptically to a potato dextrose agar (PDA)
medium, incubated at 28 °C for 2–4 weeks, and the morphological characteristics of cultures
were recorded.

Herbarium specimens were deposited at the herbarium of the Mae Fah Luang Uni-
versity (MFLU) and Kunming Institute of Botany (HKAS), while the living cultures were
deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi and Index Fungorum numbers were registered as outlined in Index Fungorum [30] and Jayasiri et al. [31].

2.2. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from two-week-old living pure cultures grown on PDA using the Biospin Fungus Genomic DNA extraction Kit (BioFlux, Kun Ming, P.R. China) following the manufacturer’s protocol. DNA was subjected to PCR amplification to amplify the genes ITS, LSU TEF1-α, and RPB2, while internal transcribed spacer (ITS) with the primer pair of ITS4/ITS5 [32], the partial large subunit nuclear rDNA (LSU) with the primer pair of LR0R/LR5 [33], the translation elongation factor 1-alpha gene (TEF1-α) with the primer pair of EF1–983F/EF1–2218R [34], and RNA polymerase II second largest subunit (RPB2) with the primer pair of RPB2–5f/7cR [35]. The PCR was carried out using the method described by Tian et al. [28]. ITS, LSU, and TEF1-α amplification reactions were set using the method described by Cai et al. [36] and Lu et al. [37]. RPB2 amplification reaction was set using the method described by Lu et al. [3]. PCR products were checked and purified in 1% agarose gels and were sequenced at TsingKe Biological Technology (Kunming) Co., China.

2.3. Phylogenetic Analyses

The raw sequences (ITS, LSU, TEF1-α, and RPB2) were spliced using SeqMan and subjected to BLAST in GenBank to find closely related taxa. Sequences of four genes downloaded from NCBI GenBank are listed in Table 1. A single gene sequence alignment was generated with MAFFT v.7.110 online application [38,39] and trimmed using trimAl v 1.2 with the ‘gappyout’ option [40]. Multiple genes were concatenated by Sequence Matrix. Multigene phylogenetic analyses of the concatenated genes were reconstructed from maximum likelihood (ML) and Bayesian inference (BI) analyses. Maximum likelihood was performed using the online RAxML-HPC on XSEDE tool on CIPRES under the GTRGAMMA substitution model and 1000 bootstrap replicates [38,41,42]. Bayesian inference analysis was performed using the MrBayes on XSEDE tool on CIPRES [42]. The best-fit models were selected as GTR+I+G for ITS, LSU, TEF1-α, and RPB2 for the Bayesian posterior probability analysis. Two parallel runs were conducted using the default settings, six simultaneous Markov chains were run for 50,000,000 generations, and trees were sampled every 500th generation. The alignment generated in this study was submitted to TreeBASE (https://treebase.org/treebase-web/home.html, accessed on 10 January 2022) under the submission number ID29068. Trees were visualized with FigTree v1.4.4, and layouts were carried out with Adobe Illustrator CS5 v. 16.0.0.

Table 1. Names, culture collection accession numbers, and corresponding GenBank accession numbers of the fungal taxa used in this study.

| Taxa                                | Strain Numbers | GenBank Accession Numbers |
|-------------------------------------|----------------|--------------------------|
|                                     |                | ITS                     | LSU       | TEF1-α   | RPB2   |
| Aquaphila albicans                  | BCC 3543       | DQ341096                | DQ341101  | –         | –       |
| Aquaphila albicans                  | MFLUCC 16–0010 | KX454165                | KX454166  | KY117034 | MF535255 |
| Berkleasmium concinnum              | ILLS 80803     | KY582485                | –         | –         | –       |
| Berkleasmium fusiforme              | MFLUCC 17–1979 | MH558694                | MH558821  | MH550885 | MH551008 |
| Berkleasmium longisporum            | MFLUCC 17–1990 | MH558697                | MH558824  | MH550888 | MH551101 |
| Botryosphaeria agaves               | MFLUCC 10–0051 | JX646790                | JX646807  | JX646855 | –       |
| Botryosphaeria dothidea             | CBS 115476     | –                       | NG_027577 | –         | –       |
| Chlamydotubufia cylindrica          | MFLUCC 16–1130 | MH558702                | MH558830  | MH550893 | MH551018 |
| Chlamydotubufia huaikangplausis     | MFLUCC10–0926  | JN865210                | JN865198  | –         | –       |
| Chlamydotubufia krabiensis          | MFLUCC 16–1134 | KY678767                | KY678759  | KY792958 | MF535261 |
| Taxa                                | Strain Numbers          | GenBank Accession Numbers          |
|-------------------------------------|-------------------------|-----------------------------------|
|                                     | ITS                     | LSU                               | TEF1-α | RPB2 |
| Dematiohelicomyces helicosporus     | MFLUCC 16–0213          | KX454169                          | KX454170 | KY117035 | MF535258 |
| Dematiohelicomyces helicosporus     | MFLUCC 16–0003          | MH558703                          | MH558831 | MH550894 | MH551019 |
| Dematiohelicomyces helicosporus     | MFLUCC 16–0007          | MH558704                          | MH558832 | MH550895 | MH551020 |
| **Dematiohelicomyces helicosporus** | **KUMCC 21–0473**       | **OM331856**                      | **OL985958** | **OM355487** | – |
| Dictyospora thailandica             | MFLUCC 16–0215          | KY873628                          | KY873623 | KY873287 | – |
| Dictyospora thailandica             | MFLUCC 11–0512          | –                                 | –                                 | –                                 | – |
| Helicoarctatus aquaticus            | MFLUCC 17–1996          | MH558707                          | MH558835 | MH550989 | MH551024 |
| Helicodochium aquaticum             | MFLUCC 16–0008          | MH558708                          | MH558836 | MH550999 | MH551025 |
| Helicodochium aquaticum             | MFLUCC 17–2016          | MH558709                          | MH558837 | MH550900 | MH551026 |
| Helicohyalinum aquaticum            | MFLUCC 16–1131          | KY873625                          | KY873620 | KY873284 | MF535257 |
| Helicohyalinum infundibulum         | MFLUCC 16–1133          | MH558712                          | MH558840 | MH550903 | MH551029 |
| Helicoma ambiens                    | UAMH 10533              | AY916451                          | AY856916 | –                  | –                  |
| Helicoma ambiens                    | UAMH 10534              | AY916450                          | AY856869 | –                  | –                  |
| Helicoma aquaticum                  | MFLUCC 17–1983          | MH558714                          | MH558842 | MH550905 | MH551031 |
| Helicoma brunnisporum               | MFLUCC 11–0003          | JN865211                          | JN865199 | –                  | –                  |
| Helicoma dennisii                   | NBRC 30667              | AY916455                          | AY856897 | –                  | –                  |
| Helicoma fusiforme                  | MFLUCC 17–1981          | –                                 | –                                 | MH550906 | –                  |
| Helicoma guttulatum                 | MFLUCC 16–0022          | KX454171                          | KX454172 | MF535254 | MH551032 |
| Helicoma hongkongense               | MFLUCC 17–2005          | MH558716                          | MH558843 | MH550907 | MH551033 |
| Helicoma inthanonense               | MFLUCC 10–0119          | JN865203                          | JN865191 | KF301559 | –                  |
| Helicoma linderi                    | NBRC 9207              | AY916454                          | AY856895 | –                  | –                  |
| Helicoma longisporum                | MFLUCC 16–0002          | MH558717                          | MH558844 | MH550908 | MH551034 |
| Helicoma longisporum                | MFLUCC 16–0005          | MH558718                          | –                  | MH550909 | MH551035 |
| Helicoma longisporum                | MFLUCC 16–0211          | MH558719                          | MH558845 | MH550910 | MH551036 |
| Helicoma longisporum                | MFLUCC 17–1997          | MH558720                          | MH558846 | MH550911 | MH551037 |
| Helicoma miscanthi                  | MFLUCC 11–0375          | KF301525                          | KF301533 | KF301554 | –                  |
| Helicoma muelleri                   | CBS 964.69              | AY916453                          | AY856877 | –                  | –                  |
| Helicoma muelleri                   | UBC F13877              | AY916452                          | AY856917 | –                  | –                  |
| Helicoma multisepulatum             | GZCC 16–0080            | MH558721                          | MH558847 | MH550912 | MH551038 |
| Helicoma nematosporum               | MFLUCC 16–0011          | MH558722                          | MH558848 | MH550913 | MH551039 |
| Helicoma rubriappendiculatum         | MFLUCC 18–0491          | MH558723                          | MH558849 | MH550914 | MH551040 |
| Helicoma rufum                      | MFLUCC 17–1806          | MH558724                          | MH558850 | MH550915 | –                  |
| Helicoma rugosum                    | ANM 196                | GQ856138                          | GQ850482 | –                  | –                  |
| Helicoma rugosum                    | ANM 953                | GQ856139                          | GQ850483 | –                  | –                  |
| Helicoma rugosum                    | ANM 1169               | –                                 | GQ850484 | –                  | –                  |
| Helicoma rugosum                    | JCM 2739              | –                                 | AY856888 | –                  | –                  |
| Helicoma septoconstrictum           | MFLUCC 17–1991          | MH558725                          | MH558851 | MH550916 | MH551041 |
| Helicoma septoconstrictum           | MFLUCC 17–2001          | MH558726                          | MH558852 | MH550917 | MH551042 |
| Helicoma siamense                   | MFLUCC 10–0120          | JN865204                          | JN865192 | KF301558 | –                  |
| Helicoma sp.                        | HKUCC 9118             | –                                 | AY849966 | –                  | –                  |
| Helicoma tectonae                   | MFLUCC 12–0563          | KU144928                          | KU764713 | KU762751 | –                  |
| Helicoma vaccinii                   | CBS 216.90             | AY916486                          | AY856879 | –                  | –                  |
| Helicomyces chilensis               | BCRC FU30842           | LC316604                          | –                  | –                  | –                  |
| Helicomyces colligatus              | MFLUCC 16–1132          | MH558727                          | MH558853 | MH550918 | MH551043 |
| Helicomyces hyalosporus             | GZCC 16–0070           | MH558728                          | MH558854 | MH550919 | MH551044 |
| Helicomyces hyalosporus             | MFLUCC 17–0051          | MH558731                          | MH558857 | MH550922 | MH551047 |
| Helicomyces torquatus               | MFLUCC 16–0217          | MH558732                          | MH558858 | MH550923 | MH551048 |
| Helicosporium flavum                | MFLUCC 16–1230          | KY873626                          | KY873621 | KY873285 | –                  |
| Helicosporium luteosporum           | MFLUCC 16–0226          | KY321324                          | KY321327 | KY792601 | MH551056 |
| Helicosporium vesicarium            | MFLUCC 17–1795          | MH558739                          | MH558864 | MH550930 | MH551055 |
| Helicotruncatum palmigenum          | NBRC 32663             | AY916480                          | AY856898 | –                  | –                  |
| Helicotruncatum palmigenum          | MFLUCC 15–0993          | MT627685                          | MN913690 | –                  | –                  |
| **Helicotubefia guangxiensis**      | **KUMCC 21–0474**       | **OM102542**                      | **OL985959** | **OM355488** | **OM355492** |
| Helicotubefia guangxiensis          | MFLUCC 17–0040          | MH290018                          | MH290023 | MH290028 | MH290033 |
| Taxa                                | Strain Numbers       | GenBank Accession Numbers |
|-------------------------------------|----------------------|---------------------------|
|                                     |                      | ITS | LSU | TEF1-α | RPB2 |
| Helicotubeufia hydei               | MFLUCC 17–1980       | MH290021       | MH290026 | MH290031 | MH290036 |
| Helicotubeufia jonesii             | MFLUCC 17–0043       | MH290020       | MH290025 | MH290030 | MH290035 |
| Muripulchra aquatica              | DLUCC 0571           | KY320531       | KY320548 |          |          |
| Muripulchra aquatica              | KUMCC 15–0245        | KY320533       | KY320550 | KY320563 | MH551057 |
| Muripulchra aquatica              | KUMCC 15–0276        | KY320534       | KY320551 | KY320564 | MH551058 |
| Muripulchra aquatica              | MFLUCC 15–0249       | KY320532       | KY320549 |          |          |
| Neoacanthostigma fusiforme         | MFLUCC 11–0510       | –              | –      |          | –        |
| Neochlamydotubeufia fusiformis     | MFLUCC 16–0016       | MH558740       | MH558865 | MH550931 | MH551059 |
| Neochlamydotubeufia fusiformis     | MFLUCC 16–0214       | MH558741       | MH558866 | MH550932 | MH551060 |
| Neochlamydotubeufia khunkornensis  | MFLUCC 10–0118       | JN865202       | JN865190 | KF301564 | –         |
| Neochlamydotubeufia khunkornensis  | MFLUCC 16–0025       | MH558742       | MH558867 | MH550933 | MH551061 |
| Neohelicomyces aquaticus           | MFLUCC 16–0249       | KY320529       | KY320546 | KY320562 | MH551065 |
| Neohelicomyces aquaticus           | MFLUCC 17–0056       | KY320530       | KY320547 |          | –         |
| Neohelicomyces griseum             | CBS 101688           | AY916140       | Q850084 |          | –         |
| Neohelicomyces griseum             | CBS 113542           | AY916125       | Q850084 |          | –         |
| Neohelicomyces guangxiense         | GZCC 16–0042         | MF467920       | MF467933 | MF535246 | MF535276 |
| Neohelicomyces guangxiense         | MFLUCC 17–0054       | MH558750       | MH558875 | MH550941 | MH551073 |
| Neohelicomyces hyalosporum         | GZCC 16–0063         | MF467923       | MF467936 | MF535249 | MF535279 |
| Neohelicomyces hyalosporum         | GZCC 16–0076         | MF467924       | MF467936 | MF535249 | MF535279 |
| Neohelicomyces irregulare          | MFLUCC 15–1796       | MH558752       | MH558877 | MH550941 | MH551073 |
| Neohelicomyces irregulare          | MFLUCC 18–1708       | MH558753       | MH558878 | MH550943 | MH551076 |
| Neohelicomyces krabense            | MFLUCC 16–0224       | MH558754       | MH558879 | MH550945 | MH551077 |
| Neohelicomyces laxisporum          | MFLUCC 17–2027       | MH558755       | MH558880 | MH550946 | MH551078 |
| Neohelicomyces morganii            | CBS 281.54           | AY916146       | AY856884 |          |          |
| Neohelicomyces ovoideum           | CBS 222.38           | AY916146       | AY856880 |          |          |
| Neohelicomyces ovoideum           | GZCC 16–0064         | MF467924       | MF467937 | MF535250 | MF535280 |
| Neohelicomyces panacheum           | GZCC 16–0066         | MF467925       | MF467936 | MF535250 | MF535280 |
| Neohelicomyces parvisporum         | GZCC 17–2010         | MH558763       | MH558888 | MH550954 | MH551086 |
| Neohelicomyces sp.                 | CBS 189.95           | AY916147       | AY856882 |          |          |
| Neohelicomyces submersum           | HKUCC 10235          | –              | AY849942 |          |          |
| Neohelicomyces taiwanense          | MFLUCC 17–2376       | MT627738       | MN913783 |          |          |
| Neohelicomyces thailandicum       | BCRC FUS0841         | LC316603       | –      |          | –        |
| Neohelicomyces krabensii          | MFLUCC 16–0221       | MF467928       | MF467941 | MF535253 | MF535283 |
| Parahelicomyces aquaticus         | MFLUCC 16–1125       | MG012031       | MG012024 | MG012010 | MG012017 |
| Parahelicomyces chiangmaensis     | MFLUCC 21–0159       | OL606157       | OL606146 | OL964517 | OL964523 |
| Parahelicomyces hyalosporus       | CBS 283.51           | AY916146       | AY856881 | DQ677928 | DQ677981 |
| Parahelicomyces hyalosporus       | KUMCC 15–0411        | KY320527       | KY320544 | KY320563 | MH551057 |
| Parahelicomyces hyalosporus       | MFLUCC 15–0343       | KY320523       | KY320540 |          |          |
| Parahelicomyces indicus           | CBS 374.93           | AY916147       | AY856885 |          |          |
| Parahelicomyces menglunicus       | HKAS 85793           | –              | –      | MK335914 | –         |
| Parahelicomyces paludosus         | CBS 120503           | DQ341095       | DQ341103 |          |          |
| Parahelicomyces quercus           | MFLU 18-2091         | –              | –      | MK360077 | MK349086 |
| Parahelicomyces quercus           | MFLUCC 17-0895       | MK347720       | MK347934 |          |          |
| Taxa | Strain Numbers | GenBank Accession Numbers |
|------|----------------|--------------------------|
|      |                | ITS | LSU | TEF1-α | RPB2     |
| Tubeufia abunata | MFLUCC 17–2024 | MH558769 | MH558894 | MH550961 | MH551095 |
| Tubeufia aquatica | MFLUCC 17–1794 | MH558770 | MH558895 | MH550962 | MH551096 |
| Tubeufia aquatica | MFLUCC 16–1249 | KY320522 | KY320539 | KY320556 | MH551142 |
| Tubeufia brevis | DLUCC 0574 | KY320521 | KY320538 | KY320555 | MH551141 |
| Tubeufia cambesi | MFLUCC 17–1803 | MH558771 | MH558899 | MH550966 | MH551100 |
| Tubeufia cervata | MFLUCC 17–1799 | MH558772 | MH558897 | MH550964 | MH551098 |
| Tubeufia chlamydospora | MFLUCC 16–0223 | MH558775 | MH558900 | MH550967 | MH551101 |
| Tubeufia cocois | MFLUCC 22–0001 | OM102541 | OL985957 | OM355486 | OM355491 |
| Tubeufia cocois | MFLUCC 22–0002 | OM102543 | OL985960 | OM355489 | OM355493 |
| Tubeufia cocois | MFLUCC 22–0003 | OM102544 | OL985961 | OM355490 | OM355494 |
| Tubeufia cylindrothecia | BCC 3559 | – | AY849965 | – | – |
| Tubeufia cylindrothecia | BCC 3585 | AY916482 | AY856908 | – | – |
| Tubeufia cylindrothecia | DLUCC 0572 | KY320520 | KY320537 | KY320554 | – |
| Tubeufia cyathuloides | MFLUCC 16–1253 | KY320519 | KY320536 | KY320553 | – |
| Tubeufia cyanophila | MFLUCC 16–1283 | KY320518 | KY320535 | KY320552 | MH551143 |
| Tubeufia cylindrothecia | MFLUCC 21–0160 | OL545365 | OL606147 | OL964518 | OL964524 |
| Tubeufia cylindrothecia | MFLUCC 17–1792 | MH558776 | MH558901 | MH550968 | MH551102 |
| Tubeufia dictyospora | MFLUCC 17–1805 | MH558778 | MH558903 | MH550970 | – |
| Tubeufia dictyospora | MFLUCC 16–0220 | MH558777 | MH558902 | MH550969 | MH55103 |
| Tubeufia eccentrica | MFLUCC 17–1524 | MH558782 | MH558907 | MH550974 | MH551108 |
| Tubeufia eccentrica | GZCC 16–0035 | MH558779 | MH558904 | MH550971 | MH551105 |
| Tubeufia entadae | MFLU 18–2102 | – | – | – | – |
| Tubeufia geniculata | MFLUCC 16–0236 | – | MH558938 | MH550976 | MH551110 |
| Tubeufia guangxiensis | MFLU 18–2102 | MK347727 | MK347943 | – | – |
| Tubeufia gongylospora | MFLUCC 17–0047 | MH558783 | MH558908 | MH550975 | MH551109 |
| Tubeufia filiformis | MFLUCC 16–1118 | KY921941 | KY921942 | KY171028 | MF535284 |
| Tubeufia filiformis | MFLUCC 16–1128 | – | KY921940 | KY171027 | MF535283 |
| Tubeufia filiformis | MFLUCC 16–0236 | – | MH558938 | MH550976 | MH551110 |
| Tubeufia filiformis | MFLUCC 16–0236 | – | MH558938 | MH550976 | MH551110 |
| Tubeufia geniculata | BCRC FU30849 | LC335817 | – | – | – |
| Tubeufia geniculata | DLUCC 0572 | AY916482 | AY856908 | – | – |
| Tubeufia guangxiensis | MFLUCC 17–0046 | MH558784 | MH558909 | MH550977 | MH551111 |
| Tubeufia hechiensis | MFLUCC 17–0052 | MH558785 | MH558910 | MH550978 | MH551112 |
| Tubeufia hyalospora | MFLUCC 15–1250 | MH558786 | MH558911 | MH550979 | – |
| Tubeufia inaequalis | GZCC 16–0035 | MH558779 | MH558904 | MH550971 | MH551105 |
| Tubeufia inaequalis | MFLUCC 17–1998 | MH558791 | MH558916 | MH550984 | MH551117 |
| Tubeufia inaequalis | BCC 8808 | AY916481 | AY856910 | – | – |
| Tubeufia javanica | MFLUCC 12–0545 | KJ880034 | KJ880036 | KJ880037 | – |
| Tubeufia krabiensis | MFLUCC 16–0228 | MH558792 | MH558917 | MH550985 | MH551118 |
| Tubeufia latispora | MFLUCC 16–0027 | KY921941 | KY921942 | KY171033 | MH551119 |
| Tubeufia latispora | MFLUCC 16–0013 | MH558793 | MH558918 | MH550986 | MH551200 |
| Tubeufia laxispora | MFLUCC 16–0219 | KY921941 | KY921942 | KY171030 | MF535286 |
| Tubeufia laxispora | MFLUCC 16–0232 | KY921941 | KY921942 | KY171029 | MF535287 |
| Tubeufia laxispora | MFLUCC 17–2023 | MH558794 | MH558919 | MH550987 | MH551121 |
| Tubeufia longihelicospora | MFLUCC 21–0163 | OL545455 | OL606148 | OL964519 | OL964525 |
| Tubeufia lilliputana | NBRC 32664 | AY916483 | AY856989 | – | – |
| Tubeufia longihelicospora | MFLUCC 16–0753 | MZ385851 | MZ385865 | MZ387106 | – |
| Tubeufia longihelicospora | MFLUCC 21–0151 | OL606156 | OL606149 | OL964520 | OL964526 |
Table 1. Cont.

| Taxa                          | Strain Numbers | GenBank Accession Numbers |
|-------------------------------|----------------|--------------------------|
|                               |                | ITS                      | LSU                      | TEF1-α                   | RPB2                     |
| Tubeufia longihelicospora     | KUMCC 21–0814  | OM331690                 | OM331688                 | OM355484                 | –                        |
| Tubeufia longihelicospora     | KUMCC 21–0815  | OM331691                 | OM331705                 | OM355485                 | –                        |
| Tubeufia longiseta           | MFLUCC 15–0188 | KU940133                 | –                        | –                        | –                        |
| Tubeufia mackenziei          | MFLUCC 16–0222 | KY092415                 | KY092410                 | KY117031                 | MF355288                 |
| Tubeufia parvispora          | MFLUCC 17–1992 | MH558796                 | MH558921                 | MH550989                 | MH551123                 |
| Tubeufia parvispora          | MFLUCC 17–2003 | MH558797                 | MH558922                 | MH550990                 | MH551124                 |
| Tubeufia parvispora          | MFLUCC 17–2009 | MH558798                 | MH558923                 | MH550991                 | MH551125                 |
| Tubeufia roseohelicospora    | MFLUCC 16–0230 | MH558799                 | MH558924                 | MH550992                 | MH551126                 |
| Tubeufia roseohelicospora    | MFLUCC 17–1797 | MH558800                 | MH558925                 | MH550993                 | MH551127                 |
| Tubeufia roseohelicospora    | MFLUCC 15–1247 | KY454177                 | KY454178                 | –                        | MH551144                 |
| Tubeufia rubra               | GZCC 16–0083   | MH558802                 | MH558927                 | MH550995                 | MH551129                 |
| Tubeufia rubra               | GZCC 16–0081   | MH558801                 | MH558926                 | MH550994                 | MH551128                 |
| Tubeufia sahyadiensis       | NFCCI 4252     | MH033849                 | MH033850                 | MH033851                 | –                        |
| Tubeufia sessilis            | MFLUCC 16–0021 | MH558803                 | –                        | MH550996                 | MH551130                 |
| Tubeufia sympodihylospora    | MFLUCC 17–0044 | MH558806                 | MH558930                 | MH550999                 | MH551133                 |
| Tubeufia sympodilaxispora   | BCC 3580       | –                        | DQ296554                 | –                        | –                        |
| Tubeufia sympodilaxispora   | GZCC 16–0058   | MH558807                 | MH558931                 | MH551000                 | MH551134                 |
| Tubeufia sympodiaxispora    | MFLUCC 17–0048 | MH558808                 | MH558932                 | MH551001                 | MH551135                 |
| Tubeufia taiwanensis        | BCRC FU30844   | LC316605                 | –                        | –                        | –                        |
| Tubeufia tectonae           | MFLUCC 16–0235 | MH558809                 | MH558933                 | MH551002                 | MH551136                 |
| Tubeufia tectonae           | MFLUCC 17–1985 | MH558810                 | MH558934                 | MH551003                 | MH551137                 |
| Tubeufia tectonae           | MFLUCC 12–0392 | KU144923                 | KU764706                 | KU872763                 | –                        |
| Tubeufia tratensis          | MFLUCC 17–1993 | MH558811                 | MH558935                 | MH551004                 | MH551138                 |
| Tubeufia xylophila          | MFLUCC 17–1520 | MH558813                 | MH558937                 | MH551006                 | MH551140                 |
| Tubeufia xylophila          | GZCC 16–0038   | MH558812                 | MH558936                 | MH551005                 | MH551139                 |

Notes: Ex-type strains are indicated by T after the species name. Newly generated sequences are in black bold. The symbol “–” indicates information not available. Abbreviations: ANM, A.N. Miller; BCC, Biotec Culture Collection, Thailand; BCRC, Bioresearch Collection and Research Centre; CBS, Westerdijk Fungal Biodiversity Institute; DLUCC, Culture collection of Dali University; GUCC, Guizhou University Culture Collection; HKAS, the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica; HKUCC, Hong Kong University Culture Collection; JCM, Japan Collection of Microorganisms; KUMCC, Culture collection of Kunming Institute of Botany; MFLU, the herbarium of the Mae Fah Luang University; MFLUCC, Mae Fah Luang University Culture Collection; NBRC, NITE Biological Resource Center; NNCYU, National Chiayi University; NFCCI, National Fungal Culture Collection of India; UAMH, the University of Alberta Microfungus Collection and Herbarium; UBC F, University of British Columbia Herbarium.

3. Results

3.1. Phylogenetic Analyses

The combined ITS, LSU, TEF1-α, and RPB2 dataset comprised thirteen newly sequenced strains, with *Botryosphaeria dothidea* (CBS 115476) and *B. agaves* (MFLUCC 10–0051) as outgroup taxa. Multiple genes were concatenated, which comprised 3425 nucleotide characters, including gaps (ITS: 1–606 bp, LSU: 607–1471 bp, RPB2: 1472–2514 bp, TEF1-α: 2515–3425 bp). The RAxML analysis of the combined dataset yielded the best-scoring tree (Figure 1) with a final ML optimization likelihood value of −50085.613741. The matrix had 1671 distinct alignment patterns, with 26.72% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.244864, C = 0.251768, G = 0.258885, T = 0.244483; substitution rates AC = 1.177463, AG = 5.880242, AT = 2.143397, CG = 0.867985, CT = 9.022451, GT = 1.000000; gamma distribution shape parameter α = 0.224491.
Figure 1. Cont.
Figure 1. Cont.
Figure 1. Phylogenetic tree generated from a maximum likelihood analysis based on a concatenated alignment of ITS, LSU, TEF1-α, and RPB2 sequences data in Tubeufiaceae. The tree is rooted with *Botryosphaeria dothidea* (CBS 115476) and *B. agaves* (MFLUCC 10–0051). Bootstrap support values equal to or higher than 75% ML (left) or posterior probability values equal to or higher than 0.95 Bayesian PP (right) are indicated on the nodes. Newly generated sequences are in red. Ex-type strains are in black/red bold.

Phylogenetic analyses showed that our thirteen collections were placed within Tubeufiaceae viz: *Denatiohelicomyces, Helicoma, Helicotruncatum, Neohelicosporium, Parahelicomyces,* and *Tubeufia.* Eight collections clustered within *Tubeufia;* the new strain *Tubeufia cylindrothecia* (MFLUCC 21–0160) was nested with six strains of *T. cylindrothecia* with strong bootstrap support (98% ML/1.00 PP). Three strains of *Tubeufia cocois* (MFLUCC 22–0001, MFLUCC 22–0002, and MFLUCC 22–0003) clustered together and formed a branch at the basal clades of *T. aquatica* with strong bootstrap support (100% ML/1.00 PP). The new strain *T. laxispora* (MFLUCC 21–0163) nested with four strains of *T. laxispora* with strong bootstrap support (100% ML/1.00 PP), and *T. longihelicospora* (MFLUCC 21–0151, KUMCC 21–0478, and KUMCC 21–0479) clustered together within the same clade as *T. longihelicospora* (MFLUCC 16–0753). *Parahelicomyces chiangmaiense* (MFLUCC 21–0159) formed a single branch at the basal clades of *Parahelicomyces* members with strong support (97% ML). Newly obtained strain *Helicotruncatum palmigenum* (KUMCC 21–0474) nested with two strains of *H. palmigenum* (NMRC 32,663 and MFLUCC 15–0093) strong bootstrap support (100% ML/1.00 PP). *Neohelicosporium bambusicola* (MFLUCC 21–0160) was placed as a sister
taxon to *N. ellipsoideum* (MFLUCC 16–0229) and *N. acrogenisporum* (MFLUCC 17–2019). New strain *Dematiohelicomyces helicosporus* (KUMCC 21–0473) clustered with three strains of *Dematiohelicomyces helicosporus* with strong bootstrap support (100% ML/1.00 PP), and *Helicoma guttulatum* (MFLUCC 21–0152) clustered with its ex-type strain of *H. guttulatum* (MFLUCC 16–0022) with high support (100% ML/1.00 PP).

### 3.2. Taxonomy

#### 3.2.1. Dematiohelicomyces Y.Z. Lu, Boonmee, and K.D. Hyde, Fungal Diversity 92: 159 (2018)

**Type species:** *Dematiohelicomyces helicosporus* (Boonmee, Y.Z. Lu, and K.D. Hyde) Y.Z. Lu

The monotypic genus *Dematiohelicomyces* was introduced by Lu et al. [3], with *D. helicosporus* as the type species based on morphology and phylogeny. *Dematiohelicomyces* are saprobic on submerged decaying wood in a freshwater stream in Thailand. *Dematiohelicomyces* is characterized by short conidiophores that are brown, 0–3-septate, and helicoid conidia, with a spathulate basal end cell. In this paper, *Dematiohelicomyces helicosporus* was collected from submerged decaying wood in a freshwater river in Thailand.

*Dematiohelicomyces helicosporus* (Boonmee, Y.Z. Lu, and K.D. Hyde) Y.Z. Lu, Fungal Diversity 92: 159 (2018) (Figure 2).

≡ *Chlamydotubeufia helicospora* Boonmee, Y.Z. Lu, and K.D. Hyde, Fungal Diversity 80: 123 (2016)

**Index Fungorum**, IF 554824; **Facesoffungi number**, FoF 04701

**Asexual morph** Hyphomycetous, helicosporous. Colonies are superficial, effuse, gregarious, white, and shiny. Mycelium is mostly immersed, composed of branched, septate hyphae, brown, with masses of glistening, crowded conidia. Conidiophores (16.5–)30–65.5(–80.5) × 4–5 µm (x = 47 × 4.5 µm, n = 25) are macronematous, erect, cylindrical, branched, 0–4-septate, hyaline to pale brown, arising as lateral branches from creeping hyphae, and smooth-walled. Conidiogenous cells (9–)14–24.5(–30) × 4–5 µm (x = 19.5 × 4.5 µm, n = 30) are holoblastic, monoblastic, integrated, terminal, cylindrical, truncate at the apex after conidial secession, hyaline, and smooth-walled. Conidia are solitary, acrogenous, helicoid, rounded at tip, with the basal cells broadly spathulate and bearing a flattened attachment scar, guttulate, hyaline, with a (59–)80–139(–158) µm (x = 110 µm, n = 25) diam. and conidial filament 5–6.5 µm (x = 5.5 µm, n = 25) wide in the broadest part, tapering towards the ends, 328–482.5 µm (x = 405.5 µm, n = 25) long, multi-septate, coiled 1–2 1/2 times, tightly to loosely coiled in water, smooth-walled, and contain granules.

Culture characteristics: conidia germinating on PDA within 12 h; colonies growing on PDA, reaching 20 mm in 2 weeks at 28 °C, circular, with a flat surface, edge entire, pale brown to dark brown in PDA medium; mycelium partially immersed, branched, multi-septate, hyaline to pale brown, smooth.

Material examined: Thailand, Chiang Rai Province, Mae Fah Luang University, on submerged decaying wood, 22 May 2020, R. J. Xu, MD38 (MFLU 21–0184), living culture, KUMCC 21–0473.

Notes: There are some differences between our new isolate (KUMCC 21–0473) and *D. helicosporus* morphologically, such as conidiogenous cells in our new isolate are monoblastic, while in *D. helicosporus* (MFLUCC 16–0003), they are mono- to polyblastic. In addition, the new isolate (KUMCC 21–0473) differs from *D. helicosporus* (MFLUCC 16–0003) in having larger (80–139 vs. 70–100 µm) and shorter conidial filaments (328–482.5 vs. 400–600 µm) [3,43]. However, our phylogenetic results show that the new isolate *D. helicosporus* (KUMCC 21–0473) clusters with three strains of *D. helicosporus* (MFLUCC 16–0003, MFLUCC 16–0007, and MFLUCC 16–0213) with high statistical supports (100% ML/1.00 PP, Figure 1). Therefore, we identify our new isolate as *Dematiohelicomyces helicosporus*. *Chlamydotubeufia helicospora* was collected on decaying wood in a flowing freshwater stream in Uttaradit Province, Thailand [43], and later, based on morphology and phylogeny, Lu et al. [3] synonymized this taxon under *Dematiohelicomyces helicosporus*. In this study,
our new isolate was also collected from a submerged decaying wood in Chiang Rai Province, Thailand, which is a little far from the original collection location, meaning that this species still prefers similar environmental conditions.

3.2.2. Helicoma Corda, Icon. fung. (Prague) 1: 15 (1837)

Index Fungorum: IF 8473
Type species: Helicoma muelleri Corda, Icon.
Helicoma was introduced by Corda [44], with H. muelleri as a type species. Two types of asexual morphs have been observed in Helicoma: the first asexual morphs are characterized by conidiogenous cells that are cylindrical, with denticles, intercalary, arising laterally from the lower portion of conidiophores, and conidia are pleurogenous, tapering towards the apex and rounded at the tip, helicoid, hygroscopic, and become loosely coiled in water [12]. Another asexual morph is characterized by conidia that are acrogenous or acropleurogenous, helicoid, circinate, dry, tapering towards the apex, truncating at the base, coiled \(1/4-3/4\) times, and not becoming loose in the water. There are 97 records listed in Index Fungorum (2021), however, most of them are lacking sequence data in GenBank. The last treatment of Helicoma was provided by Lu et al. [3], and they accepted 57 species within the genus while introducing 10 new species and 11 new combinations. In this study, the new isolate is identified as H. guttulatum based on both phylogenetic analysis and morphological characteristics.

Helicoma guttulatum Y.Z. Lu, Boonmee, and K.D. Hyde, Fungal Diversity 80: 125 (2016) (Figure 3).

Index Fungorum, IF 552218; Facesoffungi, FoF 02358

Saprobic on submerged decaying wood in a freshwater stream. Sexual morph Undetermined. Asexual morph Hyphomycetous, helicosporous. Colonies are superficial, effuse, gregarious, brown to dark brown. Mycelium is mostly immersed, partly superficial, composed of branched, septate, brown hyphae. Conidiophores \((65–)93–156.5 \times 4.5–6 \mu m \quad (x = 125 \times 5 \mu m, n = 20)\) are macronematous, mononematous, cylindrical, septate, erect, unbranched, pale brown to brown at the apex and dark brown at the base, and smooth-walled. Conidiogenous cells \((9–)12–24(–30.5) \times 4–5.5 \mu m \quad (x = 18 \times 4.5 \mu m, n = 20)\) are holoblastic, mono- to polyblastic, integrated, terminal, cylindrical, brown, and smooth-walled. Conidia \(22–26.5 \mu m \quad (x = 24 \mu m, n = 25)\) have a diam. and conidial filament \(7–8.5 \mu m \quad (x = 8 \mu m, n = 25)\) wide and \(49–58 \mu m \quad (x = 53.5 \mu m, n = 25)\) long, are integrated, terminal, helicoid, tightly coiled \(1–11/2\) times, guttulate, do not become loose in the water, 8-septate, straight constricted at the septa, subhyaline to yellowish, rounded at the apex, and smooth-walled.

Culture characteristics: conidia germinated on PDA within 12 h; colonies growing on PDA, reaching 25 mm in 2 weeks at 28 °C, circular, with a flat surface, edge entire, and pale brown to brown in PDA medium; mycelium were partially immersed, branched, multi-septate, hyaline to pale brown, and smooth.

Material examined: Thailand, Chiang Rai Province, Mueang, Nang Lae on submerged decaying wood, 14 August 2020, R. J. Xu, MD106 (MFLU 21–0183), living culture, MFLUCC 21–0152.

Notes: Helicoma guttulatum was introduced by Hyde et al. [43] on submerged decaying wood from a freshwater stream in Thailand. In our phylogenetic analyses, the newly obtained isolate (MFLUCC 21–0152) clustered with the ex-type strain of H. guttulatum (MFLUCC 16–0022) with high statistical support (100% ML/1.00 PP, Figure 1). Morphologically, the new isolate was indistinguishable from the holotype of H. guttulatum [43]. Therefore, we identify the new isolate as Helicoma guttulatum based on morphological and phylogenetic data.
3.2.3. *Helicotruncatum* Y.Z. Lu, J.C. Kang, and K.D. Hyde, Fungal Diversity 92: 220 (2018)

Type species: *Helicotruncatum palmigenum* (Penz. and Sacc.) Y.Z. Lu and K.D. Hyde

The monotypic genus *Helicotruncatum* was established by Lu et al. [3] with *H. palmigenum* as the type species, and it is the only species accepted in the genus [3,12]. *Helicotruncatum palmigenum* was originally placed in *Helicoma*, based on morphological charac-

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**Figure 3.** *Helicoma guttulatum* (MFLU 21–0183). (a) Colony on decaying wood; (b,c) conidiophores and conidia; (d) conidiophore; (e,f) tip of conidiogenous cells; (g,h) conidiogenous cells and conidia; (i–k) conidia; (l) germinated conidium; (m,n) colony cultures on PDA (observe and reverse). Scale bars: (b) = 40 μm, (c,d) = 60 μm, (e–l) = 20 μm.
ters [12,45,46]. Phylogenetic analysis of Lu et al. [3] showed that *H. palmigenum* formed an independent lineage and was distant from *Helicoma*. Morphologically, *H. palmigenum* can be distinguished from other helicosporous hyphomycetes by the distinctively thickened lateral cell wall of the conidiophore and basal cell of the conidium. Thus, Lu et al. [3] introduced a new genus, *Helicotruncatum*, to accommodate *H. palmigenum* based on both phylogeny and morphology. In this paper, *Helicotruncatum palmigenum* was collected from dead *Cocos nucifera* leaves in Thailand.

*Helicotruncatum palmigenum* (Penz. and Sacc.) Y.Z. Lu and K.D. Hyde, Fungal Diversity 92: 220 (2018) (Figure 4).

≡*Helicosporium intermedium* var. *palmigenum* Penz. and Sacc., Malpighia 15(7–9): 249 (1902)
≡*Helicoma palmigenum* (Penz. and Sacc.) Linder, Ann. Mo. bot. Gdn 16: 306 (1929)
≡*Helicoma westoni* Linder [as ‘westoni’], Ann. Mo. bot. Gdn 18: 12 (1931)

Index Fungorum: IF 554860; Facesoffungi number: FoF 04800

*Saprobic* on dead leaves of *Cocos nucifera*. **Sexual morph:** Undetermined. **Asexual morph** Hyphomycetous, helicosporous. Colonies on the substratum are superficial, effuse, gregarious, and velvety black. *Mycelium* is composed of brown, septate hyphae. *Conidiophores* 165.5–283.5 × 6.5–10 µm (*x* = 224.5 × 8.5 µm, *n* = 10) are macronematous, mononematous, cylindrical, stout, septate, erect, unbranched, pale brown to subhyaline at the apex and dark brown at the base, and smooth-walled. *Conidiogenous cells* 22–34.5 × 6–7.5 µm (*x* = 28 × 6.5 µm, *n* = 15) are holoblastic, monoblastic, integrated, determinate, cylindrical, terminal, smooth-walled, and truncate at the apex after conidial secession. *Conidia* 32–44 µm (*x* = 38 µm, *n* = 20) and conidial filament are 10.5–15 µm (*x* = 12.5 µm, *n* = 20) wide, 82–108.5 µm (*x* = 95.5 µm, *n* = 20) long, solitary, terminal, smooth-walled, helicoid, coiled 1½–2⅓ times, do not become loose in the water, septate, not constricted at septa, dilute fuliginously, and the basal cell is truncated with thickened lateral walls.

Culture characteristics: conidia germinating on PDA within 12 h; colonies reaching 40 mm in 2 weeks at 28 °C, irregular, dark brown from above and pale brown from below; mycelium are slow-growing, thin, and effuse brownish grey.

Material examined: Thailand, Chiang Rai Province, on decaying leaves of *Cocos nucifera*, 16 January 2021, X. G. Tian, C6–6, (MFLU 21–0185), living culture, KUMCC 21–0474.

Notes: In the phylogenetic analyses, our new collection KUMCC 21–0474 clusters with two strains of *H. palmigenum* (NBRC 32663, MFLUCC 15–0993) with high statistical supports (100% ML/1.00 PP, Figure 1). Morphologically, our new isolate is almost identical to *H. palmigenum* except for the size of the conidiogenous cells (22–34.5 vs. 17–25 µm long) and the conidia (82–108.5 vs. 50–60 µm long). The nucleotide comparisons show 2 bp and 1 bp of ITS and LSU differences between the new isolate (KUMCC 21–0474) and *H. palmigenum* (NBRC 32663). Thus, we identify the new isolate as *H. palmigenum* based on both phylogenetic analyses and morphological characteristics.

*Helicotruncatum palmigenum* was introduced as *Helicoma palmigenum* by Linder [45] on decaying petioles of palms that were collected from Australia, Brazil, China, Indonesia, Japan, Mexico, New Guinea, Seychelles, Thailand, Trinidad, and the USA [3,45,46]. In addition, *Helicotruncatum palmigenum* has been reported on leaves and husks of *Cocos nucifera* [34]. Our new isolate was also collected on dead leaves of *Cocos nucifera* from Thailand.
Figure 4. *Helicotruncatum palmigenum* (MFLU 21–0185). (a,b) Colony on decaying leaves; (c,d) conidiophores, conidiogenous cells, and conidia; (e) conidiophores and conidiogenous cells; (f,g) conidiogenous cells and conidia; (h–m) conidia; (n,o) colony cultures on PDA (observe and reverse). Scale bars: (a) = 500 μm, (b) = 200 μm, (c–e) = 80 μm, (f–m) = 30 μm.

3.2.4. *Neohelicosporium* Y.Z. Lu, J.C. Kang, and K.D. Hyde, Mycological Progress 17: 637 (2017)
Index Fungorum: IF 822045
Type species: *Neohelicosporium parvisporum* Y.Z. Lu, J.C. Kang, and K.D. Hyde
Neohelicosporium was introduced by Lu et al. [10], with five new species. The taxonomic revision of the genus was recently provided by Lu et al. [3]; eight Helicosporium, two Helicoma, and one Tubeufia species were transferred to Neohelicosporium based on both phylogeny and morphology. The genus is characterized by superficial, ellipsoidal to subglobose, ostiolate ascomata, bitunicate, cylindrical, pedicellate ascii and fusiform, straight or slightly curved, multi-septate, guttulate, hyaline, smooth-walled ascospores; macronematous, mononematous, branched or unbranched, septe, pale brown to brown conidiophores, holoblastic, mono- to polyblastic, integrated, sympodial, intercalary or terminal conidiogenous cells with denticles and solitary, acrogenous and/or acropleurogenous, helicoid, multi-septate, guttulate, hyaline to pale brown conidia. Species of the genus are saprobic on decaying woody substrates from both aquatic and terrestrial habitats [47]. In this study, the new species Neohelicosporium bambusicola is introduced based on both phylogenetic analysis and morphological characters.

Neohelicosporium bambusicola X.G. Tian and Tibpromma, sp. nov. (Figure 5).

Index Fungorum number, IF 555045; Facesoffungi number, FoF 10571

Etymology: Referring to the host plant bamboo, on which the fungus was collected.

Saprobic on terrestrial dead culms of bamboo. Sexual morph Undetermined. Asexual morph Hyphomycetous, helicosporous. Colonies on the substratum are superficial, effuse, and white. Mycelium is composed of partly immersed, hyaline to brown, septate, branched hyphae with glistening conidia. Conidiophores 21–76 × 3–5 µm (x = 48.5 × 4 µm, n = 10) are macronematous, mononematous, cylindrical, unbranched or branched, septe, subhyaline to brown, and smooth-walled. Conidiogenous cells 8.5–16 × 3–4.5 µm (x = 12 × 4 µm, n = 15) are holoblastic, mono to ployblastic, integrated, sympodial, terminal or intercalary, cylindrical, truncate at apex after conidial secession, pale brown, smooth-walled. Conidia are solitary, acropleurogenous, helicoid, multi-septate, guttulate, hyaline when young and become brown when mature, smooth-walled, and do not become loose in water, with a 24–30 µm (x = 27 µm, n = 20) diam. and a conidial filament 3–4.5 µm (x = 3.9 µm, n = 20) wide, 100.5–128 µm (x = 114 µm, n = 20) long, and coiled 2–2 times.

Culture characteristics: conidia germinated on PDA within 12 h; colonies on PDA reach 20 mm in 2 weeks at 28 °C, and are superficial, effuse, and brown; mycelium is composed of partly immersed, hyaline to brown, septe, branched, smooth hyphae.

Material examined: Thailand, Chiang Mai Province, on dead culms of bamboo, 16 December 2020, X. G. Tian, U4–10 (MFLU 21–0189 holotype), ex-type culture, MFLUCC 21–0156.

Notes: In the phylogenetic analyses, the new isolate Neohelicosporium bambusicola (MFLUCC 21–0156) formed a distinct lineage sister to N. ellipsoideum (MFLUCC 16–0229) and N. acrogenisporum (MFLUCC 17–2009). Neohelicosporium bambusicola resembles N. ellipsoideum and N. acrogenisporum in having macronematous, mononematous, unbranched or branched, septe conidiophores, holoblastic, mono- to ployblastic conidiogenous cells, and helicoid, septe conidia. However, Neohelicosporium bambusicola is distinct from N. ellipsoideum and N. acrogenisporum as it has shorter and narrower conidiophores (21–76 × 3–5 vs. 50–230 × 5–6 vs. 45–150 × 6–7 µm), smaller conidiogenous cells (8.5–16 × 3–4.5 vs. 15–25 × 5–6 vs. 12–15 × 4–6 µm), and narrower conidia (3–4.5 vs. 5–6 vs. 4.5–7.5 µm) [3]. Pairwise nucleotide comparisons revealed that the new isolate Neohelicosporium bambusicola is different from N. ellipsoideum (MFLUCC 16–0229) in 64/544 bp (11.76%) of the ITS, 9/814 (1.1%) of the LSU, 20/1045 bp (1.91%) of RPB2, and 15/894 bp (1.68%) of TEF1-α, while Neohelicosporium bambusicola is different from N. acrogenisporum (MFLUCC 17–2009) in 2/370 bp (0.54%) of the ITS, 6/831 (0.72%) of the LSU, 27/1045 bp (2.58%) of RPB2, and 16/894 bp (1.79%) of TEF1-α. Both phylogenetic analyses and morphological characteristics support this species as a distinct new species.
and 16/894 bp (1.79%) of TEF1-α. Both phylogenetic analyses and morphological characteristics support this species as a distinct new species.

Figure 5. Neohelicosporium bambusicola (MFLU 21–0189, holotype). (a,b) Colony on culms of bamboo; (c,e–g,i) conidiogenous cells and conidia; (d) conidiophores; (h,k,l) conidia; (j) germinated conidium; (m,n) colony cultures on PDA (observe and reverse). Scale bars: (a) = 500 μm, (b) = 200 μm, (c,d,g–j) = 10 μm.
3.2.5. Parahelicomyces Goh, in Hsieh, and Goh and Kuo, Mycological Progress 20 (2): 182 (2021)

=Pseudohelicomyces Y.Z. Lu, J.K. Liu, and K.D. Hyde

Index Fungorum, IF 554886; Facesoffungi number, FoF 04745

Type species: Parahelicomyces talbotii (Goos) S.Y. Hsieh, Goh, and C.H. Kuo

Parahelicomyces is a well-studied genus, introduced as Pseudohelicomyces by Lu et al. [3] with Pseudohelicomyces talbotii as the type species [3,48]. Pseudohelicomyces was renamed Parahelicomyce by Hsieh et al. [49] because Parahelicomyces was a homonym and illegitimate. Currently, seven species are accepted in the genus, and all the species have sequence data available in the GenBank database. The genus is characterized by superficial, subglobose, ellipsoidal-ovate, coriaceous, ostiolate ascomata, bitunicate, cylindrical, apically thickened and rounded asci, and fusiform, multi-septate, hyaline, smooth-walled ascosporae [3], as well as macronematous, mononematous, hyaline to brown, branched, septate conidiophores, holoblastic, mono- to polyblastic, integrated, intercalary or terminal, determinate or sympodial conidiogenous cells with denticles and pleurogenous or acropleurogenous, helicoid, multi-septate, hyaline to pale brown conidia. Species of the genus Parahelicomyces are found from both terrestrial and freshwater habitats in China, Japan, Mexico, South Africa, and Thailand [3,50]. In this study, we introduced a new Parahelicomyces species from Thailand.

Parahelicomyces chiangmaiensis X.G. Tian and Tibpromma, sp. nov. (Figure 6).

=Index Fungorum, IF 555060; Facesoffungi number, FoF 10570

Etymology: Referring to Chiangrai Province, Thailand, where the fungus was collected.

Saprobi c on a terrestrial woody substrate. Sexual morph Undetermined. Asexual morph Hyphomycetous, helicosporous. Colonies on the substratum are superficial, effuse, gregarious, and white. Mycelium is composed of partly superficial, hyaline to pale brown, branched hyphae, with masses of crowded, glistening conidia. Conidiophores 85–180 × 2.9–3.7 µm (x = 132 × 3 µm, n =10) are macronematous, mononematous, cylindrical, pale brown to brown, paler towards the apex, straight or flexuous, branched, septate, and smooth-walled. Conidiogenous cells 6–10 × 2.5–3.5 µm (x = 8 × 3 µm, n =20) are holoblastic, mono- to polyblastic, integrated, sympodial, terminal or intercalary, cylindrical, with denticles, hyaline to pale brown, and smooth-walled. Conidia have a 21–33.5 µm (x = 27 µm, n = 20) diam. and a conidial filament 2–3 µm (x = 2.5 µm, n = 20) wide, 73–130 µm (x = 101.5 µm, n = 20) long, and coiled 1½–3 times, and are acropleurogenous, solitary, multi-septate, helicoid, rounded at the tip, hyaline to pale brown, guttulate, tightly to loosely coiled in water, and smooth-walled.

Culture characteristics: conidia germinated on PDA within 12 h; colonies adpressed reaching 30 mm in 2 weeks at 28 °C, amd were circular, brown to dark brown, reverse brown, and slow-growing; mycelium was superficial and partially immersed, branched, septate, hyaline to pale brown, and smooth.

Material examined: Thailand, Chiang Mai Province, on the dead terrestrial woody substrate, 16 December 2020, X. G. Tian, U4–8 (MFLU 21–0188 holotype), ex-type culture, MFLUCC 21–0159.

Notes: Phylogenetic analyses of combined LSU, ITS, RPB2, and TEF1-α sequence data showed that our new isolate Parahelicomyces chiangmaiensis (MFLUCC 21–0159) formed an independent lineage within the genus with strong support (97% ML). Parahelicomyces chiangmaiensis is phylogenetically closely related to P. talbotii (MFLUCC 17–2021), however, P. chiangmaiensis can be distinguished from P. talbotii by the size (21–33.5 vs. 7–16 µm diam.) of conidia and the size (6–10 vs. 7–16 µm long) of conidiogenous cells. Parahelicomyces chiangmaiensis is morphologically closely related to P. indicus, however, P. chiangmaiensis can be distinguished from P. indicus by the colour (pale brown to brown vs. dark to yellowish-brown) and size (85–180 × 2.9–3.7 vs. 47–145 × 3–7.5 µm) of the conidiophores [51]. Both phylogenetic analyses and morphological characteristics support Parahelicomyces chiangmaiensis as a distinct new species.
Both phylogenetic analyses and morphological characteristics support **Parahelicomyces chiangmaiensis** as a distinct new species.

**Figure 6.** *Parahelicomyces chiangmaiensis* (MFLU 21–0188, holotype). (a,b) Colony on decaying wood; (c–g) conidiophores, conidiogenous cells, and conidia; (h–m) conidia; (n) germinated conidium; (o) colony cultures on PDA (observe and reverse). **Scale bars:** (a) = 500 µm, (b) = 200 µm, (e,f,k–m) = 20 µm, (c,d,g–j,n) = 10 µm.

3.2.6. **Tubeufia** Penz. and Sacc., Malpighia 11(11–12): 517 (1898)

- Index Fungorum: IF 5635
- Type species: *Tubeufia javanica* Penz. and Sacc., Malpighia 11(11–12): 517 (1898)
Tubeufia, the type genus of Tubeufiaceae, was established by Penzig and Saccardo [52]. Currently, 88 records are listed in the Index Fungorum (2021); however, most of the species are lacking sequence data in the GenBank. While morphologies of Tubeufia species are quite similar, using morphology alone presents difficulties for identification; thus, sequence data are required to resolve taxonomic confusions. The last treatment of Tubeufia was provided by Lu et al. [3], and they introduced seventeen new species and six new combinations in the genus, accepting fifty species in the genus based on both phylogenetic analysis and morphological characters. In this paper, we introduced two novel species, one new record species, and a new isolate of known species in Tubeufia.

**Tubeufia cocois** X.G. Tian and Tibpromma, sp. nov. (Figure 7).

- **Index Fungorum number, IF** 555070; **Facesoffungi number, FoF** 10576
- **Etymology:** Referring to the host plant *Cocos nucifera*, on which the fungus was collected.

**Saprobic** on the decaying leaves of *Cocos nucifera*. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous, helicosporous. **Colonies** on the substratum are superficial, effuse, gregarious, and white to pale brown. **Mycelium** is partly immersed, partly superficial, hyaline to brown, septate, branched, and with glistening conidia. **Conidiophores** 38–123 × 4.5–6 µm (x = 80.5 × 5.5 µm, n = 20) are macronematous, mononematous, straight or slightly flexuous, cylindrical, branched, septate, orange brown to dark brown, paler towards the apex, and smooth-walled. **Conidiogenous cells** 8–17.5 × 4–5.5 µm (x = 13 × 5 µm, n = 25) are holoblastic, mono- to polyblastic, integrated, sympodial, terminal or intercalary, irregular cylindrical, hyaline to pale brown, and smooth-walled, with most of them being denticulate protrusions. **Conidia** are solitary, acropleurogenous, helicoid, rounded at tip, with a 26–32.5 µm (x = 29 µm, n = 20) diam. and a conidial filament 3.5–5 µm (x = 4 µm, n = 20) wide, 116–136 µm (x = 126 µm, n = 20) long, and coiled 2 1/4–2 3/4 times, and they do not become loose in water, are indistinctly multi-septate, guttulate, hyaline when young, pale brown to brown at maturity, and smooth-walled.

**Culture characteristics:** conidia germinated on PDA within 12 h; colonies grow on PDA, reach 30 mm in 2 weeks at 28 °C, are irregular, with a flat surface, edge undulate, and brown to dark brown in PDA medium; mycelium are superficial and partially immersed, branched, septate, hyaline to brown, and smooth.

**Material examined:** Thailand, Chiang Rai Province, on decaying leaves of *Cocos nucifera*, 16 January 2021, X. G. Tian, C6–15 (MFLU 21–0192, holotype), ex-type culture, MFLUCC 22–0001; *ibid*, C6–8 (MFLU 21–0186, paratype), ex-paratype, MFLUCC 22–0002; *ibid*, C6–20 (MFLU 21–0187, paratype), ex-paratype, MFLUCC 22–0003.

**Notes:** *Tubeufia cocois* is introduced as a distinct new species from *Cocos nucifera* in Thailand. In the phylogenetic analyses, three newly obtained strains of *T. cocois* (MFLUCC 22–0001, MFLUCC 22–0002, and MFLUCC 22–0003) clustered together and were sister to three *T. aquatica* strains with strong statistical support values (100% ML/1.00 PP, Figure 1). Morphologically, *T. cocois* can be easily distinguished from *T. aquatica* by the shape and size of the conidiophores, conidiogenous cells, and conidia. *Tubeufia cocois* has branched or unbranched, multi-septate, and longer conidiophores (38–123 vs. 18–40 µm), while the conidiophores of *T. aquatica* are unbranched, 0–1-septate, and shorter than those of *T. cocois*. The conidiogenous cells of *T. cocois* are terminal or intercalary and conidia are acropleurogenous, whereas *T. aquatica* has terminal conidiogenous cells and conidia are acrogenous [53]. Based on pairwise nucleotide comparisons, the new strain (MFLUCC 22-0001) is different from *T. aquatica* (MFLUCC 16–1249) in 16/413 bp (3.87%) of the ITS, 2/845 (0.24%) of the LSU, 23/919 bp (2.5%) of RPB2, and 10/617 bp (1.62%) of TEF1-α.
Figure 7. *Tubeufia cocois* (MFLU 21–0192, holotype). (a) Colony on decaying leaves of *Cocos nucifera*; (b–e) conidiophores and conidiogenous cells; (d) conidia and conidiogenous cells; (f–l) conidia; (m) germinated conidium; (n,o) colony cultures on PDA (observe and reverse). Scale bars: (b–m) = 20 µm.

*Tubeufia laxispora* Y.Z. Lu, Boonmee, and K.D. Hyde, Mycological Progress 16: 409 (2017) (Figure 8).
Figure 8. *Tubeufia laxispora* (MFLU 21–0191). (a,b) Colony on decaying leaves of *Cocos nucifera*; (c–f) conidiogenous cells and conidia; (g–m) conidia; (n) germinated conidium; (o,p) colony cultures on PDA (observe and reverse). **Scale bars:** (c–n) = 20 μm.

Index Fungorum, IF 818987; Facesoffungi number, FoF 02694

*Saprobic* on the decaying leaves of *Cocos nucifera*. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous, helicosporous. **Colonies** on the substratum are superficial, effuse, gregarious, and range from white to brown. **Mycelium** is partly immersed, partly superficial, pale brown, septate, sparsely branched hyphae, with masses of crowded conidia. **Conidiophores** 26–53 × 3.5–5 μm (x = 39.5 × 4 μm, n =15) are hyaline to brown, macronematous, erect, short, and smooth-walled. **Conidiogenous cells** 8–16 × 3–4.5 μm (x = 12 × 4 μm, n =20) are monoblastic, holoblastic, integrated, and each have a single conidium. **Conidia** are solitary, acropleurogenous, helicoid, and rounded at the tip, with a 17.5–29 μm (x = 23 μm, n = 20) diam. and a conidial filament 2–3.5 μm (x = 2.5 μm, n = 20) wide, 63–94.5 μm (x = 78.5 μm, n = 20) long, loosely coiled 1–2½ times in the water, indistinctly multi-septate, hyaline, and smooth-walled.

Culture characteristics: conidia germinating on PDA within 12 h; colonies growing on PDA, reaching 20 mm in 2 weeks at 28 °C—they are circular, with a flat surface, edge undulate, and brown to dark brown in PDA medium; mycelium are superficial and partially immersed, branched, septate, hyaline to brown, and smooth.

Material examined: Thailand, Chiang Rai Province, on decaying leaves of *Cocos nucifera*, 9 March 2021, X. G. Tian, C7–10 (MFLU 21–0191), living culture, MFLUCC 21–0163.
Notes: In our phylogenetic analyses, the newly obtained isolate (MFLUCC 21–0163) clustered with four strains of *T. laxispora* with high statistical supports (100% ML/1.00 PP, Figure 1). Based on pairwise nucleotide comparisons, the new strain (MFLUCC 21–0163) almost overlapped with the ex-type strain of *T. laxispora* (MFLUCC 16–0232), except TEF1-α 1 bp out of 878 bp (<1%). Morphologically, our new isolate fits well with the description of *T. laxispora*, except for the conidial size (17.5–40 µm diam., 63–94.5 µm long vs. 17.5–29 µm diam., 111–182 µm long) [37]. Hence, we identify our new isolate as *T. laxispora*. *Tubeufia laxispora* was described by Lu et al. [37] on submerged wood in Thailand, while our new isolate was collected on decaying leaves of *Cocos nucifera* in Thailand, and this is the first report of *Tubeufia laxispora* associated with a coconut tree from Thailand.

*Tubeufia cylindrothecia* (Seaver) Höhn Sber. Akad. Wiss. Wien, Math. -naturw. Kl., Abt. 1 128: 562 (1919) (Figure 9).

Index Fungorum, IF 340543; Facesoffungi number, FoF 02650

Saprobic on submerged decaying wood. **Sexual morph** See Seaver [54]. **Asexual morph** Colonies on the substratum are superficial, effuse, gregarious, and white to pale brown. Mycelium is partly immersed, partly superficial, hyaline to brown, septate, and with masses of conidia. Conidiophores 57–95 × 4–7 µm (x = 76 × 5.5 µm, n =10) are pale brown, macronematous, mononematous, septate, cylindrical, unbranched, erect, and smooth-walled. Conidiogenous cells 6.5–16 × 3.5–5 µm (x = 11 × 4 µm, n =15) are holoblastic, monoblastic, integrated, smooth, terminal or intercalary, and cylindrical. Conidia are acropleurogenous, 40.5–82 µm (x = 61.5 µm, n = 20) in diam. and with a conidial filament that is 3.5–8 µm (x = 5.5 µm, n = 20) wide, 220–321 µm (x = 270.5 µm, n = 20) long, helicoid, with conidial loosely coiled 1–3½ times in the water, hyaline to brown, indistinctly multisepate, guttulate, and smooth.

Culture characteristics: conidia germinating on PDA within 12 h; colonies growing on PDA, reaching 25 mm in 2 weeks at 28 °C, irregular, with a flat surface, edge undulate, and brown to dark brown in PDA medium; mycelium are partially immersed, branched, septate, hyaline to brown, and smooth.

Material examined: Thailand, Chiang Rai Province, on decaying submerged wood, 11 November 2020, X. G. Tian, W1–10 (MFLU 21–0190), living culture, MFLUCC 21–0160.

Notes: *Tubeufia cylindrothecia* was originally introduced with both sexual and asexual morphs that link to *Helicomyces roseus* based on morphological studies [6,53], while phylogenetic analyses showed that *Tubeufia cylindrothecia* and *Helicomyces roseus* can be recognized as two different species [1,8,53,56]. Luo et al. [53] first reported its asexual morph as collected from a freshwater habitat in China. Our phylogenetic results show that the newly obtained isolate (MFLUCC 21–0160) clustered with six strains of *T. cylindrothecia* with high bootstrap support (98% ML/1.00 PP). Morphologically, our new isolate is almost identical to *T. cylindrothecia*, except for the conidiogenous cells of the new isolate (MFLUCC 21–0160) that are terminal or intercalary, while the conidiogenous cells are terminal in *T. cylindrothecia* (MFLU 16–2547). Thus, based on morphological and molecular data, we identify the new isolate as *Tubeufia cylindrothecia*. 
Figure 9. *Tubeufia cylindrothecia* (MFLU 21–0190). (a,b) Colony on decaying wood; (c,e,f) conidiophores with attached conidia; (d,g) conidiophores and conidiogenous cells; (h–k,m,n) conidia; (l) germinated conidium; (o,p) colony cultures on PDA (observe and reverse). **Scale bars:** (a) = 1000 μm, (b) = 200 μm, (l) = 50 μm, (c–f,h) = 30 μm, (i–k,m,n) = 20 μm, (g) = 10 μm.

*Tubeufia longihelicospora* Boonmee, Promputtha, and K.D. Hyde, in Boonmee et al., Fungal Diversity 111: 133 (2021) (Figure 10).
Figure 10. *Tubeufia longihelicospora* (MFLU 21–0182). (a,b) Colony on decaying wood; (c–f) conidiophores, conidiogenous cells, and conidia; (g,h) stalked sclerotia; (i–m) conidia; (n) germinated conidium; (o,p) colony cultures on PDA (observe and reverse). **Scale bars:** (n) = 40 μm, (c–m) = 20 μm.

Index Fungorum number, IF 558543; Facesoffungi number, FoF 09195

*Saprobic* on submerged decaying wood in a freshwater stream. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous, helicosporous. **Colonies** on the substratum are superficial, effuse, gregarious, and white. **Mycelium** is composed of partly immersed, hyaline to pale brown, septate, branched hyphae with masses of glistening, crowded conidia. Stalked sclerotia are often present and are medium brown, spherical, and muriform. **Conidiophores** (10.5–)13.5–35.5(–55.5) × 3.5–5(–6) μm (x = 24.5 × 4 μm, n = 15) are macronematous, mononematous, arise as lateral branches from creeping hyphae, cylindrical, branched,
0–4-septate, hyaline to pale brown, and smooth-walled. Conidiogenous cells (7–)9–19(–24.5) × 3.5–4.5 μm (x = 14 × 4 μm, n = 15) are holoblastic, monoblastic, integrated, terminal or intercalary, cylindrical, truncate at the apex after conidial secession, hyaline to pale brown, and smooth-walled. Conidia are solitary, acrogenous, holoblastic, helicoid, rounded at the tip, hyaline to pale brown, (40–)55.5–86.5(–100.5) μm (x = 71 μm, n = 15) in diam. and with a conidial filament 6.5–8.5 μm (x = 7.5 μm, n = 15) wide in the broadest part and tapering towards the ends, (99.5–)240.5–355.5 μm (x = 298 μm, n = 15) long, loosely coiled 1–2 times, multi-septate, tightly to loosely coiled in the water, constricted at the septa, guttulate, hyaline to pale brown, rough-walled, and bearing conidiola. Conidiola are globose, unicellular, and rough-walled.

Culture characteristics: conidia germinated on PDA within 12 h; colonies growing on PDA reach 20 mm in 2 weeks at 28 °C, are irregular, with a flat surface, edge undulate, and brown to dark brown in PDA medium; mycelium are superficial and partially immersed, branched, septate, hyaline to brown, and smooth.

Material examined: Thailand, Chiang Rai Province, Mueang, Ban Du, on decaying submerged wood, 15 August 2020, R. J. Xu, MD77 (MFLU 21–0182), living culture, MFLUCC 21–0151; China, Yunnan Province, Xishuangbanna, on decaying submerged wood, 13 September 2021, X. G. Tian, WB12 (HKAS 122173), living culture, KUMCC 21–0814; ibid NWBB9 (HKAS 122169), living culture, KUMCC 21–0815.

Notes: In the phylogenetic analyses, our three new strains (MFLUCC 21–0151, KUMCC 21–0478, and KUMCC 21–0479) are clustered together within the same clade as_T. longihelicospora (MFLUCC 16–0753). Based on pairwise nucleotide comparisons, our three new strains almost overlap with the ex-type strain of _Tubeufia longihelicospora_ (MFLUCC 16–0753). Morphologically, our new isolate (MFLU 21–0182) is almost identical to _Tubeufia longihelicospora_ based on morphological and phylogenetic data. _Tubeufia longihelicospora_ was introduced by Boonmee et al. [57] on a submerged decaying wood in a small freshwater stream in Thailand. Our isolate _Tubeufia longihelicospora_ (MFLUCC 21–0151) was also collected in Thailand, while the other two isolates of _Tubeufia longihelicospora_ (KUMCC 21–0478 and KUMCC 21–0479) were collected in China, which is a new geographical record.

4. Discussion

_Tubeufiaceae_ is an interesting family with diverse morphologies, habitats, and a worldwide distribution [1,3,12,24]. The asexual morph of the _Tubeufiaceae_ species is reported as helicosporous, chlamydosporous, and phragmosporous conidia, of which helicosporous conidia are the most common morphology in _Tubeufiaceae_. However, species with helicosporous conidia are not only found in _Tubeufiaceae_; for example, _Helicoascotaiwania_ also has helicosporous conidia but it is phylogenetically distinct from _Tubeufiaceae_. _Helicoascotaiwania_ is placed in Pleurotheciaceae and Sordariomycetes [38], while _Tubeufiaceae_ is placed in Pleosporales. The interesting finding is that the asexual morphs of some genera were reported with two different morphologies. For example, _Tubeufia_ and _Berkleasmium_ produce both dictyosporous and helicosporous conidia, while _Helicoma_ produces both helicosporous and phragmosporous conidia, confirmed by phylogenetic analyses [3].

The morphology of helicosporous hyphomycetes is quite similar; thus, the phylogenetic analyses are efficient to identify the helicosporous hyphomycetes at the species level [59–61]. With the availability of molecular data, some species were revised and transferred to other genera based on phylogenetic analyses. For example, _Helicomyces roseus_ (BCC 3381) and _Helicoma perelegans_ (ATCC 22621) were transferred to _Tubeufia_ [3]. However, many helicosporous species lack sequence data in the GenBank, and the taxonomy of helicosporous hyphomycetes needs revisions based on phylogenetic analyses. For example, species in the genera of _Tubeufia_, _Helicoma_, and _Helicomyces_ were introduced based on morphological characteristics, but many of them lack sequence data in the GenBank; thus,
further study into herbarium specimens is necessary to resolve taxonomic problems in the three genera.

In this study, nine Tubeufiaceae species were collected from terrestrial and freshwater habitats in Thailand, of which three were introduced as new species, while six were identified as existing species based on phylogenetic analyses and morphological characteristics. The nine species were placed in Dematiohelicomyces, Helicoma, Helicotruncatum, Neohelicosporium, Parahelicomyces, and Tubeufia, respectively, of which the genera Dematiohelicomyces, Helicotruncatum, Neohelicosporium, and Parahelicomyces are well studied, and all species in these genera have sequence data available in the GenBank. Helicoma and Tubeufia were recently revised by Lu et al. [3]. In our phylogenetic analyses, Helicoma and Tubeufia formed well-supported and monophyletics clades within the family. The morphologies of Tubeufia and Helicoma are quite similar; thus, morphology alone is not enough to identify species in Tubeufia and Helicoma, and phylogenetic analyses are necessary. However, earlier studies identified the two genera only based on morphological characteristics, and sequence data of many species are not available in the GenBank, so, it is entirely possible that some species were incorrectly identified; accordingly, fresh collections and molecular data are required to clarify their taxonomic status. Even though Dematiohelicomyces helicosporus, Helicotruncatum palmigenum, Helicoma guttulatum, Neohelicosporium bumbusicola, Tubeufia cylindrothecia, T. laxispora, and T. longihelicospora are known species and were collected again, some species are known as new hosts and new geographical records. In addition, it is also better to provide the full descriptions and color plates of the micro-characteristics of new isolates to understand some fine morphological differences.

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