Four new species and three new records of helicosporous hyphomycetes from China and their multi-gene phylogenies

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Helicosporous hyphomycetes have the potential to produce a variety of bioactive compounds. However, the strain resources of this fungal group are relatively scarce, which limits their further exploitation and utilization. In this study, based on phylogenetic analyses of combined ITS, LSU, RPB1, and TEF1α sequence data and the morphology from 11 isolates, we introduce four new species of helicosporous hyphomycetes, viz. Helicoma wuzhishanense, Helicosporium hainanense, Helicosporium viridisporum, and Neohelicomyces hainanensis, as well as three new records, viz. Helicoma guttulatum, H. longisporum, and Helicosporium sexuale. Detailed morphological comparisons of the four new species that distinguish them are provided.

KEYWORDS
freshwater fungi, taxonomy, Tubeufiales, woody substrates, saprophytic fungi

Introduction

The most remarkable feature that distinguishes helicosporous hyphomycetes from other fungal groups is that its conidia curve through at least 180° in one plane as they extend in length (Goos, 1986; Zhao et al., 2007; Luo et al., 2017; Lu et al., 2018a,b; Tian et al., 2022). They are distributed in the Dothideomycetes (Capnodiales, Microthyriales, Pleosporales, Tubeufiales, and Venturiales), Leotiomycetes (Helotiales), Orbiliomycetes (Orbilicales), Sordariomycetes (Hypocreales, Lulworthiales, Microascales, Torpedosporales), Agaricomycetes (Agaricales), Atractiellomycetes (Atractiellales), Exobasidiomycetes (Exobasidiales), Tremellomycetes (Tremellales), and Zoopagomycetes (Zoopagales) (Lu and Kang, 2020). Helicosporous fungi are widespread in tropical and temperate regions (Lu et al., 2018b). Most species in this group, which were published more than 10 years ago, were saprophytic on terrestrial woody substrates, and most of them were lacking in DNA molecular data (Goos, 1986; Zhao et al., 2007; Boonmee et al., 2014; Lu et al., 2018b). However, the species of this group discovered in the last decade mainly come from aquatic...
habitats (Lu et al., 2018b; Boonmee et al., 2021; Tian et al., 2022), and almost all newly published helicosporous species have molecular data. The latest comprehensive revision on helicosporous hyphomycetes was carried out by Lu et al. (2018b), who established nine new helicosporous genera based on morphology and phylogeny, viz. *Dematiohelicothecium*, *Dematiohelicospicum*, *Dematiohelicosporium*, *Helicoarctatus*, *Helicothecium*, *Helicotrichococcus*, *Pleurohelicosporium*, *Pseudohelicosporium*, and *Pseudohelicothecium*, and reassessed the taxonomic system of the three earliest described helicosporous hyphomycete genera, viz. *Helicomyces*, *Helicosporium*, and *Helicoma*. For example, in the genus *Helicosporium*, Lu et al. (2018b) redefined its generic concept based on morphological and phylogenetic evidence, and accepted 13 species, including five new species, and excluded 25 species from this genus which were transferred to the genera *Neohelicosporium* and *Helicoma*. In addition, although Lu et al. (2018b) proposed some suggestions on how to classify and identify helicosporous fungi, there are still some species in this group that need more morphological and molecular data to solve their taxonomic status.

The focus of research on helicosporous fungi has been mainly in the field of taxonomy. However, these fungi are not only morphologically fascinating but also a potential source to produce a variety of bioactive secondary metabolites. For example, species of *Helicomyces*, *Helicosporium*, and *Helicoma* have been reported to produce natural products with antibacterial, anticancer, and anti-diabetic activities (Itazaki et al., 1990; Hanada et al., 1996; Ohtsu et al., 2003; Yoshimura et al., 2003; Zenkoh et al., 2003; Dong et al., 2004; Hu et al., 2006; Jiao et al., 2006; Jung et al., 2012; Lee et al., 2013). Furthermore, recent studies have revealed that other helicosporous fungi also show great potential for exploring new active natural products (Qian et al., 2022; Zeng et al., 2022; Zheng et al., 2022). Zheng et al. (2022) reported two novel compounds in *Tubeufia rubra*, one of which reverses multidrug resistance of tumor cell lines to Doxorubicin. Qian et al. (2022) also discovered another two new compounds in *Tubeufia rubra*, and one, namely, Rubrosin-D displayed significant multidrug resistance reversal effects. Zheng et al. (2022) discovered that some alkaloids in *Neohelicosporium hyalosporus* were cytotoxic against human cancer (A549, TCA, and RD) cells.

In order to solve the classification problems related to helicosporous hyphomycetes and enrich the species resources of the fungal group, we have recently collected a large number of specimens of this group from various terrestrial and aquatic environments. In this study, we report on 11 helicosporous hyphomycetes collected from decaying woody substrates from freshwater streams and terrestrial habitats in southern China. The taxa are characterized based on morphological features and phylogenetic analyses. The new species are morphologically and phylogenetically distinct. Detailed descriptions, illustrations, and phylogenetic analyses are provided.

**Materials and methods**

### Sample collection and specimen examination

Submerged decaying wood samples were collected from various sites in freshwater streams and terrestrial environments in Guangxi Zhuang Autonomous Region and Hainan Provinces, China (Figure 1). Techniques in Senanayake et al. (2020) were followed for morphological study and single spore isolation. Morphological characteristics were examined with a stereomicroscope (SMZ 745 Nikon, Japan). Micro-morphological characters were photographed using a Nikon EOS 70D digital camera attached to an ECLIPSE Ni compound microscope (Nikon, Japan). Measurements were made with a Tarosoft (R) Image Frame Work program. Figures were processed and combined using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA).

Herbarium specimens were deposited in the Herbarium of Guizhou Academy of Agriculture Sciences (Herb. GZASAS) and the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (Herb. HKAS). Ex-type living cultures are deposited at Guizhou Culture Collection (GZCC). Facesoffungi database and Index Fungorum numbers are provided (Jayasiri et al., 2015).

### DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from at least 3-week-old living pure cultures grown on PDA at 28°C using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, China), and following the manufacturer’s protocol. The primer pairs of ITS5/ITS4, LR0R/LR5, RP2B-5F/RP2B-7cR, and EF1-983F/EF1-2218R were used to amplify the internal transcribed spacer (ITS) (White et al., 1990), the large subunit ribosomal DNA (LSU) (Vilgalys and Hester, 1990), the RNA polymerase II second largest subunit (RPB2) (Liu et al., 1999), and the translation elongation factor 1-alpha gene (TEF1α) (Rehner and Buckley, 2005) regions, respectively. The ITS, LSU, RPB2, and TEF1α amplification reactions were carried out using the method described by Lu et al. (2017b, 2018a). The PCR products were purified and sequenced with the same primers at Tsingke Biological Technology (Kunming) Co., China.

### Phylogenetic analysis

DNASTAR Lasergene SeqMan Pro v. 7.1.0 (44.1) was used to edit ambiguous bases at both ends of the raw forward and reverse reads and to assemble them. The newly obtained sequences were used as queries to perform BLAST searches against the
nr database to check for contamination, compare species, and create datasets. MAFFT v.7 was used to align the individual datasets (Katoh et al., 2019). Each alignment was trimmed using Trimal (Capella-Gutiérrez et al., 2009). BioEdit was used to check the alignment manually (Hall, 1999).

Four genetic markers, including ITS, LSU, RPB2, and TEF1α, were used for phylogenetic inferences (Table 1). The phylogeny tree was inferred using 147 taxa. IQ-Tree v.2 (Minh et al., 2020) was used to infer maximum likelihood trees (ML) according to the Bayesian information criterion (BIC). Partitioned analyses were carried out for the combined datasets, which were partitioned according to genetic markers. Branch support was estimated from 1,000 ultrafast bootstrap replicates. RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis, 2014) in the CIPRES Science Gateway platform was also used. ModelTest, as implemented in MrMTgui (Nuin, 2007), was used to determine the best-fit evolution model for Bayesian inference analyses using the Akaike Information Criterion (AIC). Bootstrap support was estimated from 1,000 rapid bootstrap replicates. MrBayes v.3.1.2 (Ronquist et al., 2012) was utilized to evaluate the posterior probabilities (PP) by Markov Chain Monte Carlo sampling (MCMC). The number of generations was determined separately for each dataset and is noted in the individual tree legends. The first 25% of the trees were discarded, as they represented the burn-in phase of the analyses, while the remaining were used for calculating PP in the majority rule consensus tree. For all Bayesian inference trees, convergence was declared when the average standard deviation reached 0.01. The trees were figured in the FigTree v1.4.0 program (Rambaut and Drummond, 2008). The approximately unbiased (AU) test, implemented in CONSEL, was used to test the placement of the newly erected family (Shimodaira and Hasegawa, 2001). Topologies with AU test p-values <0.05 were rejected.

Results

Phylogenetic analysis of combined ITS, LSU, RPB2, and TEF1α sequence data

The combined ITS, LSU, RPB2, and TEF1α datasets comprised 11 newly sequenced strains. Multiple genes were concatenated, which comprised 146 taxa and 3313 nucleotide characters, including gaps (ITS: 513 bp; LSU: 843 bp; RPB2: 1045
| Taxa                          | Strain/Voucher No. | GenBank accession no. |   |   |
|------------------------------|--------------------|-----------------------|---|---|
| Acanthohelicospora aurea     | GZCC 16-0060       | KY321323              | KY321326 | KY92600 | MF589911 |
| Acanthohelicospora pinicola  | MFLUCC 10-0116     | KF301526              | KF301534 | KF301555 | – |
| Acanthostigma changmuensis   | MFLUCC 10-0125     | JN865209              | JN865197 | KF301560 | – |
| Acanthostigma perpusillum    | UAMH 7237          | AY916492              | AY856892 | – | – |
| Acanthostigmina multisepatum | ANM 475            | GQ856145              | GQ850492 | – | – |
| Acanthostigmina multisepatum | ANM 665            | GQ856144              | GQ850493 | – | – |
| Aquaphila allicaens          |                       | DQ414096              | DQ41101 | – | – |
| Aquaphila allicaens          | MFLUCC 16-0010      | KX454165              | KX454166 | KY171034 | MF535255 |
| Berklesiaum fusiforme        | MFLUCC 17-1978      | MHS58693              | MHS58820 | MHS50884 | MHS51007 |
| Berklesiaum longisporum      | MFLUCC 17-1999      | MHS58698              | MHS58825 | MHS50889 | MHS51012 |
| Boerlagiomyces maccropora    | MFLUCC 12-0388      | KU144927              | KU764712 | KU872750 | – |
| Botryosphaeria agarae        | MFLUCC 10-0051      | JX646790              | JX646807 | – | – |
| Botryosphaeria dothidea      | CBS 115476          | KF66153               | DQ78051 | DQ787637 | DQ77944 |
| Chlamydotubeufia cylindrica  | MFLUCC 16-1130      | MHS58702              | MHS58830 | MHS50893 | MHS51018 |
| Chlamydotubeufia huaikangplaensis | MFLUCC 10-0926  | JN65210               | JN65198 | – | – |
| Chlamydotubeufia krabiensis  | MFLUCC 16-1134      | KY678767              | KY678759 | KY925989 | MF535261 |
| Dematiotehelicoma pulchrum   | MUCJ 39827          | AY916457              | AY56872 | – | – |
| Dematiotehelicomyces helicosporus | MFLUCC 16-0003    | KX454169              | KX454170 | KY171035 | MF535258 |
| Dematiotehelicomyces helicosporus | MFLUCC 16-0007    | MHS58703              | MHS58831 | MHS50894 | MHS51019 |
| Dematiotehelicomyces helicosporus | MFLUCC 16-0213    | KX454169              | KX454170 | KY171035 | MF535258 |
| Dematiotehelicoporum guttalatum | MFLUCC 17-2011 | MHS58705              | MHS58833 | MHS50896 | MHS51021 |
| Dematiotebuefia chiangraiensis | MFLUCC 10-0115    | JN65220               | JN65188 | KF301551 | – |
| Dictyospora thailandica      | MFLUCC 16-0001      | KY73627               | KY73622 | KY73286 | – |
| Dictyospora thailandica      | MFLUCC 16-0215      | KY73628               | KY73623 | KY73287 | – |
| Helicangiospora lignicola    | MFLUCC 11-0378      | KF01523               | KF01531 | KF01552 | – |
| Helicosortatus aquaticus      | MFLUCC 17-1996      | MHS58707              | MHS58835 | MHS50988 | MHS51024 |
| Helicoderchium aquaticum     | MFLUCC 17-2016      | MHS58709              | MHS58837 | MHS50900 | MHS51026 |
| Helicoderchium aquaticum     | MFLUCC 18-0490      | MHS58710              | MHS58838 | MHS50901 | MHS51027 |
| Helicobyalum aquaticum       | MFLUCC 16-1131      | KY73625               | KY73620 | KY73284 | MF535257 |
| Helicobyalum infundibulum    | MFLUCC 16-1133      | MHS58712              | MHS58840 | MHS50903 | MHS51029 |
| Helicoma ambiens             | UAMH 10533          | AY916451              | AY586916 | – | – |
| Helicoma ambiens             | UAMH 10534          | AY916450              | AY586899 | – | – |
| Helicoma aquaticum           | MFLUCC 17-2025      | MHS58713              | MHS58841 | MHS50904 | MHS51030 |
| Helicoma brunnesporum        | MFLUCC 17-1983      | MHS58714              | MHS58842 | MHS50905 | MHS51031 |
| Helicoma demissi             | NBRC 30667          | AY916455              | AY586897 | – | – |
| Helicoma freycinetiae        | MFLUCC 16-0363      | MH275062              | MH260295 | MH412770 | – |
| Helicoma fusiforme           | MFLUCC 17-1981      | MHS58715              | – | MHS50906 | – |
| Helicoma guttalatum          | GZCC 22-2004        | OP508739              | OP508779 | OP698090 | OP698079 |
| Helicoma guttalatum          | GZCC 22-2024        | OP508733              | OP508773 | OP698084 | OP698073 |
| Helicoma guttalatum          | GZCC 22-2025        | OP508737              | OP508777 | OP698088 | OP698077 |
| Helicoma guttalatum          | MFLUCC 16-0022      | KX454171              | KX454172 | MF535254 | – |
| Helicoma guttalatum          | MFLUCC 21-0152      | OL454546              | OL606150 | OL64521 | OL64527 |
| Helicoma wuzhishanense       | GZCC 22-2003        | OP508732              | OP508772 | OP698083 | OP698072 |
| Helicoma hongkongense        | MFLUCC 17-2005      | MHS58716              | MHS58843 | MHS50907 | MHS51033 |
| Helicoma hydei               | MFLUCC 18-1270      | MH747116              | MH747101 | MH747100 | – |

(Continued)
| Taxa                        | Strain/Voucher No. | GenBank accession no. |
|----------------------------|--------------------|-----------------------|
|                            |                    | ITS       | LSU     | TEF1α    | RPB2    |
| Helicoma inthanonense      | MFLUCC 11-0003     | JN865211  | JN865199| –        | –       |
| Helicoma khunkornensis     | MFLUCC 10-0119     | JN865203  | JN865191| KF301559| –       |
| Helicoma lindiert           | NBRC 9207          | AY916454  | AY856895| –        | –       |
| Helicoma longisporum       | GZCC 22-2005       | OP508740  | OP508780| OP98091  | OP98080 |
| Helicoma longisporum       | GZCC 22-2026       | OP508738  | OP508778| OP98089  | OP98078 |
| Helicoma longisporum       | MFLUCC 16-0002     | MHS58717  | MHS58844| MHS50908 | MHS51034|
| Helicoma longisporum       | MFLUCC 16-0005     | MHS58718  | –       | MHS50909 | MHS51035|
| Helicoma longisporum       | MFLUCC 16-0211     | MHS58719  | MHS58845| MHS50910 | MHS51036|
| Helicoma longisporum       | MFLUCC 17-1997     | MHS58720  | MHS58846| MHS50911 | MHS51037|
| Helicoma longisporum       | MFLUCC 16-0226     | MHS58721  | MHS58847| MHS50912 | MHS51038|
| Helicoma longisporum       | MFLUCC 18-0491     | MHS58723  | MHS58849| MHS50914 | MHS51040|
| Helicoma longisporum       | MFLUCC 17-1806     | MHS58724  | MHS58850| MHS50915 | –       |
| Helicoma longisporum       | MFLUCC 17-1991     | MHS58725  | MHS58851| MHS50916 | MHS51041|
| Helicoma longisporum       | MFLUCC 17-2001     | MHS58726  | MHS58852| MHS50917 | MHS51042|
| Helicoma longisporum       | MFLUCC 10-0120     | JN865204  | JN865192| KF301558| –       |
| Helicoma longisporum       | HKUCC 9118         | –         | AY849966| –        | –       |
| Helicoma longisporum       | MFLUCC 12-0563     | KU144928  | KU764713| KU782751| –       |
| Helicoma longisporum       | MFLUCC 17-1991     | MHS58725  | MHS58851| MHS50916 | MHS51041|
| Helicoma longisporum       | MFLUCC 17-2001     | MHS58726  | MHS58852| MHS50917 | MHS51042|
| Helicoma longisporum       | MFLUCC 10-0120     | JN865204  | JN865192| KF301558| –       |
| Helicoma longisporum       | MFLUCC 17-1991     | MHS58725  | MHS58851| MHS50916 | MHS51041|
| Helicoma longisporum       | MFLUCC 17-2001     | MHS58726  | MHS58852| MHS50917 | MHS51042|
| Helicoma longisporum       | MFLUCC 10-0120     | JN865204  | JN865192| KF301558| –       |
| Helicoma longisporum       | MFLUCC 17-2001     | MHS58726  | MHS58852| MHS50917 | MHS51042|
| Helicoma longisporum       | MFLUCC 16-1230     | KY763626  | KY763621| KY763285| –       |
| Helicoma longisporum       | GZCC 22-2006       | OP508730  | OP508770| OP98081  | OP98070 |
| Helicoma longisporum       | MFLUCC 16-0226     | KY321324  | KY321327| KY792601| –       |
| Helicoma longisporum       | MFLUCC 16-1233     | –         | KY763624| –        | –       |
| Helicoma longisporum       | BCC 3332           | AY916490  | AY856907| –        | –       |
| Helicoma longisporum       | BCC 3332           | AY916490  | AY856907| –        | –       |
| Helicoma longisporum       | MFLUCC 17-1994     | MHS58735  | MHS58861| MHS50926 | MHS51051|
| Helicoma longisporum       | MFLUCC 17-2006     | MHS58736  | MHS58862| MHS50927 | MHS51052|
| Helicoma longisporum       | MFLUCC 17-2007     | MHS58737  | MHS58863| MHS50928 | MHS51053|
| Helicoma longisporum       | GZCC 22-2007       | OP508731  | OP508771| OP98082  | OP98071 |
| Helicoma longisporum       | MFLUCC 16-1244     | MZ538503  | MZ538537| MZ567082 | MZ567111|
| Helicoma longisporum       | NBRC 9014          | AY916489  | AY856903| –        | –       |
| Helicoma longisporum       | CBS 254.75         | –         | DQ470982| DQ471105| –       |
| Helicoma longisporum       | CBS 269.52         | AY916487  | AY856893| –        | –       |
| Helicoma longisporum       | CBS 941.72         | AY916488  | AY856883| –        | –       |
| Helicoma longisporum       | NBRC 30345         | –         | AY856896| –        | –       |

(Continued)
TABLE 1 (Continued)

| Taxa                      | Strain/Voucher No. | GenBank accession no.         |
|---------------------------|--------------------|--------------------------------|
|                           |                    | ITS   | LSU   | TEF1α | RPB2   |
| *Helicosporium vesicarium*| MFLUCC 17-1795     | MH558739 | MH558864 | MH550930 | MH551055 |
| *Helicosporium viridiflavum* | MFLUCC 17-2336   | MH58738 | –     | MH550929 | MH551054 |
| *Helicosporium viridisporum* | GZCC 22-2008     | OP508736 | OP508776 | OP698087 | OP698076 |
| *Helicotrunatum palmigenum* | KUMCC 21-0474     | OM102542 | OL985959 | OM355488 | OM355492 |
| *Helicotrunatum palmigenum* | NBRC 32663        | AY916480 | AY85689 | –     | –     |
| *Helicotubefia guangxensis* | MFLUCC 17-0040    | MH290018 | MH290023 | MH290028 | MH290033 |
| *Helicotubefia jonesii*   | MFLUCC 17-0043    | MH290020 | MH290025 | MH290030 | MH290035 |
| *Kevinhydea brevistipitata* | MFLUCC 18-1269    | MH747115 | MH747102 | –     | –     |
| *Manoharachariella tectonae* | MFLUCC 12-0170   | KU144935 | KU746705 | KU747262 | –     |
| *Marti pulchra aquatica*  | KUMCC 15-0276     | KY320534 | KY320551 | KY320564 | –     |
| *Marti pulchra aquatica*  | MFLUCC 15-0249    | KY320532 | KY320549 | –     | –     |
| *Neocanthothea fusiforme* | MFLUCC 11-0510    | KF015129 | KF015137 | –     | –     |
| *Neochlamydotubefia fusiformis* | MFLUCC 16-0016   | MH58740 | MH58865 | MH590931 | MH59109 |
| *Neochlamydotubefia khunkornensis* | MFLUCC 10-0118 | JN65202 | JN65190 | KF01564 | –     |
| *Neohelicoma fagacearum*  | MFLUCC 11-0379    | KF015124 | KF015132 | KF015153 | –     |
| *Neohelicosporium aquaticum* | MFLUCC 17-1519   | MF467916 | MF467929 | MF353242 | MF353272 |
| *Neohelicosporium astrictum* | MFLUCC 17-2004   | MF58747 | MH58872 | MH590938 | MH51070 |
| *Neohelicosporium ellipsoideum* | MFLUCC 16-0229  | MH58748 | MH58873 | MH590939 | MH51071 |
| *Neohelicosporium guangxense* | MFLUCC 17-1522  | MF467922 | MF467935 | MF353248 | MF353278 |
| *Neohelicosporium hainanensis* | GZCC 22-2009 | OP508734 | OP508774 | OP698085 | OP698074 |
| *Neohelicosporium hainanensis* | GZCC 22-2027 | OP508735 | OP508775 | OP698086 | OP698075 |
| *Neohelicosporium hyalosporus* | GZCC 16-0086 | MH58745 | MH58870 | MH590936 | MH51064 |
| *Neohelicosporium longisetus* | NCUY 106H1-1-1 | MT939303 | – | – | – |
| *Neohelicosporium pallidus* | CBS 245.49       | –     | GU566745 | –     | –     |
| *Neohelicosporium pallidus* | CBS 27L.52       | AY916461 | AY856887 | –     | –     |
| *Neohelicosporium pallidus* | CBS 962.69       | AY916460 | AY856886 | –     | –     |
| *Neohelicosporium pallidus* | UAMH 10535       | AY916462 | AY856913 | –     | –     |
| *Neohelicosporium pandanicola* | KUMCC 16-0143 | NR_168180 | MH260307 | MH41277 | –     |
| *Neohelicosporium submersus* | MFLUCC 16-1106 | KY20530 | KY20547 | –     | –     |
| *Neohelicosporium aquaticum* | MFLUCC 17-1519 | MF467916 | MF467929 | MF353242 | MF353272 |
| *Neohelicosporium astriculum* | MFLUCC 17-2004 | MF58747 | MH58872 | MH590938 | MH51070 |
| *Neohelicosporium ellipsoideum* | MFLUCC 16-0229 | MH58748 | MH58873 | MH590939 | MH51071 |
| *Neohelicosporium guangxense* | MFLCC 17-1522 | MF467922 | MF467935 | MF353248 | MF353278 |
| *Neohelicosporium hainanensis* | GZCC 16-0076 | MF467923 | MF467936 | MF353249 | MF353279 |
| *Neohelicosporium irregularis* | MFLUCC 17-1796 | MH58752 | MH58877 | MH59043 | MH51075 |
| *Neohelicosporium krabense* | MFLUCC 16-0224 | MH58754 | MH58879 | MH59045 | MH51077 |
| *Neohelicosporium laxispore* | MFLUCC 17-2027 | MH58755 | MH58880 | MH59046 | MH51078 |
| *Neohelicosporium ovoides* | GZCC 16-0064 | MH58756 | MH58881 | MH59047 | MH51079 |
| *Neohelicosporium parvisporum* | MFLUCC 17-1523 | MF467926 | MF467939 | MF353252 | MF353282 |
| *Neohelicosporium thailandicum* | MFLUCC 16-0221 | MF467928 | MF467941 | MF353253 | MF353283 |
| *Neotubefia krabensis* | MFLUCC 16-1125 | MG012031 | MG012024 | MG012010 | MG012017 |
| *Parahelicosporium aquaticus* | MFLUCC 16-0234 | MH58766 | MH58891 | MH59098 | MH51092 |
| *Parahelicosporium chingmaoensis* | MFLUCC 21-0159 | OL697884 | OL606145 | OL64518 | OL64522 |
| *Parahelicosporium talbotii* | MFLUCC 17-2021 | MH58765 | MH58890 | MH59097 | MH51091 |
| *Parahelicosporium yunnanensis* | CGMCC 3.20429 | MZ092717 | MZ841658 | – | OM82000 |

(Continued)
TABLE 1 (Continued)

| Taxa                                           | Strain/Voucher No. | GenBank accession no. | ITS      | LSU    | TEF1α   | RPB2    |
|------------------------------------------------|--------------------|-----------------------|----------|--------|---------|---------|
| Pleurohelicosporium parvisporum                 | MFLUCC 17-1982     | MH558764              | MH558889 | MH550956 | MH551088 |
| Pseudohelicon giganstiporum                    | BCC 3550           | AY916467              | AY856904 | –       | –       |
| Pseudohelicon subglobosum                      | NCUY K3-2-3        | LC316609              | LC316612 | –       | –       |
| Tampinipora indica                             | NFFCI 2924         | KC469282              | KC469283 | –       | –       |
| Tambinipora srinivasanii                       | NFFCI 4323         | MG763746              | MG763745 | –       | –       |
| Thaxteriellopsis lignicola                     | MFLUCC 16-0026     | MH558768              | MH558893 | MH550960 | MH551094 |
| Thaxteriellopsis lignicola                     | MFLUCC 10-0124     | JN865208              | JN865196 | KF301561 | –       |
| Tubeufia bambuicola                            | MFLUCC 17-1803     | MH558771              | MH558896 | MH550963 | MH551097 |
| Tubeufia brevis                                | MFLUCC 17-1799     | MH558772              | MH558897 | MH550964 | MH551098 |
| Tubeufia javanica                              | MFLUCC 12-0545     | KH800034              | KH800036 | KH800037 | –       |
| Tubeufia rubra                                 | GZCC 16-0081       | MH558801              | MH558926 | MH550994 | MH551128 |

New sequences are in bold.

1ANM, A.N. Miller; BBB, Bahía Blanca Biology Herbarium, Argentina; BCC, BIOTEC Culture Collection, Thailand; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CGMCC, the China General Microbiological Culture Collection Center, Beijing, China; GZCC, Guizhou Culture Collection, Guizhou Academy of Agricultural Sciences, Guiyang, China; ICM, Japan Collection of Microorganisms; KUMCC, Culture collection of Kunming Institute of Botany, Kunming, China; MFLU, the Herbarium of Mae Fah Luang University, MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCL, Mycothèque de l’Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NBRC, the NITE Biological Resource Center; NCUY, National Chiai University, Taiwan, China; NFCCI, the National Fungal Culture Collection of India; UHAM, UAMH Center for Global Microfungals Diversity, University of Toronto, Canada; UBC, University of British Columbia, Canada.

bp; TEF1α: 912 bp). The maximum likelihood and Bayesian analysis of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and the IQ-Tree is shown in Figure 2.

Representatives of the sequenced genera (with molecular data) of helicosporous hyphomycetes (Boonmee et al., 2011, 2014; Rajeshkumar and Sharma, 2013; Brahamanage et al., 2017; Doilom et al., 2017; Lu et al., 2017a, 2018a,b; Luo et al., 2017; Phookamsak et al., 2017; Liu et al., 2019; Tian et al., 2022) are included in our phylogenetic analysis (Figure 2). Thirty-six genera are represented by at least one species in Tubeufiaceae. Our 11 isolates are recognized as four new species, viz. Helicoma wuzhishanense, Helicosporium hainanense, H. viridisporum, and Neohelicoomycetes hainanensis, and three new records, viz. Helicoma guttulatum, H. longisporum, and Helicosporium sexuale.

Taxonomy

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

Index Fungorum number: IF 552218; Facesoffungi number: FoF 02358.

Saprobic on submerged decaying wood in a freshwater stream. Sexual morph Undetermined. Asexual morph

Hyphomycetous, helicosporous. Colonies superficial, effuse, gregarious, brown to dark brown. Mycelium mostly immersed, composed of branched, septate, brown hyphae. Conidiophores 120–202 μm (x = 169 ± 5.5 μm, n = 20), macronematous, mononematous, cylindrical, erect, septate, unbranched, pale brown to brown at the apex, dark brown at the base, smooth-walled. Conidiogenous cells 18–37 × 4–6 μm (x = 24 × 5 μm, n = 20), holoblastic, mono- to polyblastic, integrated, terminal, cylindrical, brown, and smooth-walled. Conidia 20–26.5 μm (x = 22 μm, n = 25) in diam., and conidial filament 7.5–9.5 μm (x = 8.5 μm, n = 25) wide and 43–57 μm long (x = 51.5 μm, n = 25), solitary, acrogenous, helicoid, tightly coiled 1–1½ times, guttulate, do not become loose in water, 7–8-septate, straight constricted at the septa, subhyaline to pale brown, tapering toward the flat end, rounded at the apex, conico-truncate at the base, smooth-walled.

Culture characteristics: Conidia germinating on PDA within 12 h; Colonies growing on PDA, reaching 9 mm in 2 weeks at 25°C, circular, with a flat surface, edge undulate, and pale brown to brown in the PDA medium.

Material examined: CHINA, Hainan Province, Yanoda Tropical rainforest scenic area, on submerged decaying wood in a freshwater stream, 23 October 2021, Jian Ma, Y16.2 (GZAAS 22-2004), living culture, GZCC 22-2004; living culture, GZCC 22-2004; Ibid., Y4 (GZAAS 22-2025), living culture, GZCC 22-2025; Hainan Province, Wuzhishan City, Shuimanhe tropical rainforest scenic area in Wuzhishan, on submerged decaying wood in a freshwater stream, 15 August 2021, Jian Ma, WZS34 (GZAAS 22-2024), living culture, GZCC 22-2024.
FIGURE 2 (Continued)
Phylogenetic tree generated from a maximum likelihood analysis based on a concatenated alignment of ITS, LSU, RPB2, and TEF1α sequence data. Bootstrap support values of maximum likelihood (ML) ≥75% and Bayesian posterior probabilities (PP) ≥0.95 are given near the nodes as PP/ML BS. The tree is rooted with 

Botryosphaeria agaves MFLUCC 10-0051 and B. dothidea CBS 115476. Newly generated sequences are in red. Ex-type strains are in bold.
Helicoma guttulatum (GZAAS 22–2004). (a) Colony on decaying wood. (b–d) Conidiophores and conidia. (e–g) Conidiogenous cells. (i) Germinating conidium. (h,j–l) Conidia. (m,n) Colonies on PDA observed from above and below. Scale bars: (b–d) = 20 µm, (e–j,l–l) = 10 µm, and (h) = 5 µm.
GenBank accession numbers: GZCC 22-2004: OP508739 (ITS), OP508779 (LSU), OP698079 (RPB2), and OP698090 (TEF1α); GZCC 22-2025: OP508737 (ITS), OP508777 (LSU), OP698077 (RPB2), and OP698088 (TEF1α); GZCC 22-2024: OP508733 (ITS), OP508773 (LSU), OP698073 (RPB2), and OP698084 (TEF1α).

Notes: Helicoma guttulatum was introduced by Hyde et al. (2016) with morphological and phylogenetic evidence. Tian et al. (2022) reported a new collection from Thailand. In this study, three newly obtained isolates clustered with two known strains of *H. guttulatum* (MFLUCC 16-0022 and MFLUCC 21-0152) with high statistical support (100% ML/1.00 PP, Figure 2). We note that there are two isolates (GZCC 22-2004 and GZCC 22-2025) clustered together with high statistical support and were phylogenetically different from the other isolates. However, there are only 5 bp and 12 bp differences in ITS and RPB2 between them and the ex-type strain of *H. guttulatum*. This species has only been previously reported in Thailand. It is the first record of *H. guttulatum* in China and in a terrestrial habitat.

**Helicoma longisporum** Y.Z. Lu, J.K. Liu & K.D. Hyde, Fungal Diversity 92: 178 (2018), Figure 4.

Index Fungorum number: IF 900032;Facesoffungi number: FoF 04715.

*Saprobic* on decaying wood in a freshwater stream. **Sexual morph** Undetermined. **Asexual morph** Hypomycetous, helicosporous. Colonies on the substratum superficial, effuse, gregarious, brown to dark brown. Mycelium partly immersed, brown, septate, branched hyphae, with masses of crowded, glistening conidia. Conidiophores 114–281 × 6–10.5 μm (x = 197.5 × 7 μm, n = 20), macronematous, mononematous, cylindrical, straight, unbranched, septate, part pale brown, smooth-walled. Conidiogenous cells 11–21 × 6.5–10 μm (x = 13.5 × 7.5 μm, n = 20), holoblastic, monoblastic, integrated, intercalary, cylindrical, with denticles, rising laterally from the lower portion of conidiophores as tiny tooth-like protrusions (3–5.5 μm long, 3.5–4.5 μm wide), pale brown, smooth-walled. Conidia 51–70 μm in diam. and conidial filament 6.5–11 μm wide (x = 61 × 9 μm, n = 20), 325–508 μm long, solitary, pleurogenous, helicoid, coiled 2–3 times, becoming loosely coiled in water, rounded at tip, up to 34-septate, constriicted at septa, pale brown to brown, smooth-walled.

**Culture characteristics:** Conidia germinating on water agar and germ tubes produced from conidia within 12 h. Colonies growing on PDA, circular, with a flat surface, edge entire, and pale brown to brown in the PDA medium.

**Material examined:** CHINA, Hainan Province, Yanoda Tropical rainforest scenic area, on submerged decaying wood in a freshwater stream, 23 October 2021, Jian Ma, Y16.3 (GZAAS 22-2005), living culture, GZCC 22-2005; Ibid., Y5 (GZAAS 22-2026), living culture, GZCC 22-2026.

GenBank accession numbers: GZCC 22-2005: OP508740 (ITS), OP508780 (LSU), OP698080 (RPB2), and OP698091 (TEF1α); GZCC 22-2026: OP508778 (ITS), OP508778 (LSU), OP698078 (RPB2), and OP698089 (TEF1α).

Notes: Helicoma longisporum was introduced by Lu et al. (2018b) based on morphology and phylogeny. In this study, two newly obtained isolates are identified as *H. longisporum* based on their identical DNA molecular data, conidiophores, conidiogenous cells, and conidial characteristics (Lu et al., 2018b). This species has only been previously reported in Thailand (Lu et al., 2018b). It is the first record of *H. longisporum* in China.

**Helicoma wuzhishanense** Y.Z. Lu & J.C. Kang, sp. nov.

Index Fungorum number: IF 900032; Facesoffungi number: FoF 13100.

Holotype: GZAAS 22-2003.

Etymology: “wuzhishanense” referring to collecting site.

*Saprobic* on decaying wood in a freshwater stream. **Sexual morph** Undetermined. **Asexual morph** Hypomycetous, helicosporous. Colonies on the substratum superficial, effuse, gregarious, brown to dark brown. Mycelium partly immersed, brown, septate, branched hyphae, with masses of crowded, glistening conidia. Conidiophores 90–130 μm long, 5.5–6.5 μm wide (x = 115 × 6 μm, n = 30), macronematous, mononematous, cylindrical, erect, straight to slightly bent, unbranched, septate, the lower part brown and the upper part pale brown, smooth-walled. Conidiogenous cells 10–13 × 5–6.5 μm (x = 11.5 × 5.5 μm, n = 20), holoblastic, mono- to polyplastic, integrated, intercalary, cylindrical, with denticles, rising laterally from the lower portion of conidiophores as tiny tooth-like protrusions (1.5–3 μm long, 1.5–2.5 μm wide), brown, smooth-walled. Conidia 34–58 μm diam., and conidial filament 2.5–5 μm wide (x = 45 × 4 μm, n = 20), 182–287 μm long, up to 34-septate, solitary, pleurogenous, helicoid, coiled 2½–3½ times, becoming loosely coiled in water, rounded at tip, guttulate, hyaline to pale brown, smooth-walled.

**Culture characteristics:** Conidia germinating on water agar and germ tubes produced from conidia within 12 h. Colonies growing on PDA, circular, with a flat surface, edge entire, reaching 29 mm in 4 weeks at 25°C, pale brown to yellowish in the PDA medium.

**Material examined:** CHINA, Hainan Province, Wuzhishan City, Shuimanhe tropical rainforest scenic area in Wuzhishan, on submerged decaying wood in a freshwater stream, 15 August 2021, Jian Ma, WZS23.2 (GZAAS 22-2003), holotype; HKAS 125862, isotype), ex-type living culture, GZCC 22-2003.
FIGURE 4
*Helicoma longisporum* (GZAAS 22-2005). (a,b) Colony on decaying wood. (c,d) Conidiophores with attached conidia. (e,f,j) Conidiogenous cells. (g–i) Conidia. (k) Germinating conidium. (l,m) Colonies on PDA observed from above and below. Scale bars: (c–k) = 20 μm.
FIGURE 5
Helicoma wuzhishanense (GZAAS 22-2003, holotype). (a,b) Colony on decaying wood. (c–f) Conidiophores. (g,h) Conidiogenous cells with attached conidium. (i,j) Conidia. (k) Germinating conidium. (l,m) Colonies on PDA observed from above and below. Scale bars: (c–f, k) = 20 µm, (g–j) = 10 µm.
GenBank accession numbers: OP508732 (ITS), OP508772 (LSU), OP698072 (RPB2), and OP698083 (TEF1α).

Notes: Morphologically, Helicoma wuzhishanense resembles Helicoma rufum, having unbranched, straight to slightly bent, cylindrical conidiophores, and pleurogenous helicoid conidia. However, H. wuzhishanense can be distinguished from H. rufum by its smaller conidiophores (90–130 μm × 5.5–6.5 μm vs. 110–210 μm × 7–8.5 μm) and shorter conidial filament (182–287 μm vs. 240–410 μm) (Lu et al., 2018b). Furthermore, H. rufum produces a reddish brown pigment in the PDA medium in 7 days but H. wuzhishanense lacks this characteristic. Phylogenetically, H. wuzhishanense formed an independent lineage within the genus (Figure 2) and the phylogenetic analysis result supports it as a distinct species.

Helicosporium hainanense Y.Z. Lu & J.C. Kang, sp. nov.

Type species: H. hainanense Y.Z. Lu & J.C. Kang, sp. nov.

Index Fungorum number: IF 558542; Facesoffungi number: FoF 09194.

Holotype: MFLU 21-0104.

Hyphomycetous, helicosporous. Colonies on the substratum superficial, effuse, gregarious, yellow green. Mycelium partly immersed, partly superficial, brown to dark brown, septate, branched hyphae, with masses of crowded, glistening conidia. Conidiophores 118–182 μm long, 2.5–4 μm wide (x = 155 × 3 μm, n = 30), macroconidial, mononematous, cylindrical, unbranched, straight or slightly flexuous, septate, pale brown to dark brown, smooth-walled. Conidiogenous cells holoblastic, mono- to polyblastic, discrete, determinate, rising laterally from the lower portion of the conidiophores as tiny bladder-like protrusions, 2–8.5 μm long, 1.5–3.5 μm diam., each bearing 1–3 tiny conidiogenous loci, hyaline to pale brown, smooth-walled. Conidia 11–13 μm diam. and conidial filament 2–3 μm wide (x = 12 × 2.5 μm, n = 20), 55–60 μm long, solitary, pleurogenous, helicoid, tightly coiled 2¹/₂–3 times, do not become loose in water, tapering toward the rounded ends, indistinctly multi-septate, guttulate, hyaline to yellowish, smooth-walled.

Culture characteristics: Conidia germinating on water agar and germ tubes produced from conidia within 12 h. Colonies growing on PDA, irregular, with a flat surface, edge undulate, reaching 40 mm in 6 weeks at 25°C, brown to dark brown in the PDA medium.

Material examined: CHINA, Guangxi Zhuang Autonomous Region, Liuzhou City, Luzhai County, on submerged decaying wood in a freshwater stream, 4 May 2021, Jian Ma & Yongzhong Lu, LZ15 (GZCC 22-2007 = HKAS 125866), living cultures, GZCC 22-2007.

GenBank accession numbers: OP508731 (ITS), OP508771 (LSU), OP698071 (RPB2), and OP698082 (TEF1α).

Notes: In this study, a new helicosporous hyphomycete (GZCC 22-2007) was phylogenetically grouped with Helicosporium sexuale (MFLUCC 16-1244) and did not show much divergence (Figure 2). We compared their DNA sequences and found that only 5 bp nucleotide differences between them in TEF1α sequence data, whereas their ITS, LSU, and RPB2 sequence data were identical. Therefore, we identify the new isolate GZCC 22-2007 as H. sexuale. Helicosporium sexuale was described as only a sexual morph (Boonmee et al., 2021). Its asexual morph is reported in this study for the first time. This is also the first record of H. sexuale in a freshwater habitat in China.
Helicosporium hainanense (GZAAS 22–2006, holotype). (a,b) Colony on decaying wood. (c–f) Conidiophores and conidia. (g–i) Conidiogenous cells with attached conidia. (j) Germinating conidium. (k–m) Conidia. (n,o) Colonies on PDA observed from above and below. Scale bars: (c–f) = 20 µm, (g–j) = 10 µm, (k–m) = 5 µm.
Helicosporum sexuale (GZAAS 22-2007). (a, b) Colony on decaying wood. (c–h) Conidiophores. (i, j) Conidiogenous cells. (k) Germinating conidium. (l–o) Conidia. (p, q) Colonies on PDA observed from above and below. Scale bars: (c–h) = 20 µm, (i–o) = 10 µm.
Helicosporium viridisporum (GZAS 22-2008, holotype). (a,b) Colony on decaying wood. (c–e,g,i,j) Conidiophores and conidia. (f) Conidiogenous cells. (h) Germinating conidium. (k–n) Conidia. (o,p) Colonies on PDA observed from above and below. Scale bars: (c–f,i,j) = 20 µm, (g,h) = 10 µm, (k–n) = 5 µm.
**Helicosporium viridisporum** Y.Z. Lu & J.C. Kang, sp. nov.

**Figure 8.**

*Index Fungorum number: IF 900030, Facesoffungi number: FoFo 13102.*

*Holotype:* GZAAS 22-2008.

**Etymology:** "viridisporum" referring to the bright lime green conidia in a natural woody substrate.

*Saprobic* on decaying wood in a freshwater stream. **Sexual morph** Undetermined. **Asexual morph** Hyphomycetous, helicosporous. **Colonies** on the substratum superficial, effuse, gregarious, bright lime green. **Mycelium** partly immersed, brown to dark brown, septate, branched hyphae, with masses of crowded, glistening conidia. **Conidiophores** 80–206 µm long, 3–7 µm wide (\( \bar{x} = 146 \times 5 \mu m, n = 30 \)), macronematous, mononematous, erect, setiferous, cylindrical, septate, brown to dark brown, smooth-walled. **Conidigenous cells** holoblastic, polyblastic, discrete, determinate, denticulate, rising laterally from the lower parts of conidiophores as tiny tooth-like protrusions, hyaline to pale brown, smooth-walled. **Conidia** solitary, 12–14 µm in diameter. **Conidial filament** 1–2 µm wide (\( \bar{x} = 13 \times 1.5 \mu m, n = 30 \)), 75–97 µm long, pleurogenous, helicoid, tightly coiled 2–3/3 times, becoming loosely coiled in water, rounded at tip, guttulate, indistinctly multi-septate, hyaline to pale green, smooth-walled.

**Culture characteristics:** **Conidia** germinating on water agar and germ tubes produced from conidia within 12 h. **Colonies** growing on PDA, circular, with a flat surface, edge undulate, reaching 40 mm in 5 weeks at 25°C, brown to dark brown in the PDA medium.

**Material examined:** CHINA, Guangxi Zhuang Autonomous Region, Hechi City, Xiayi Village, on submerged decaying wood in a freshwater stream, 3 May 2021, Jian Ma, YXC2 (GZAAS 22-2009, holotype; HKAS 125863, isotype), ex-type living culture, GZCC 22-2008. **GenBank accession numbers:** OP508736 (ITS), OP508776 (LSU), OP698076 (RPB2), and OP698087 (TEF1α).

Notes: The conidiophores and conidial features of *Neohelicomyces hainanensis* are morphologically similar to those of *N. hyalosporus* but it can be distinguished from *N. hyalosporus* by its shorter conidiophores (137–197 µm vs. 210–290 µm) (Lu et al., 2018b). Its colonies change from white to pink on a natural woody substrate; a feature that other species of the genus do not have. Phylogenetically, *N. hainanensis* shares a sister relationship to *N. pallidus* with high statistical support (97 MLBS/0.99 PP), and the phylogenetic analysis results support it as a distinct species (Figure 2).

**Discussion**

The difficulty in the taxonomic study of helicosporous hyphomycete species is that their morphological characteristics are very similar; it is difficult to distinguish them only by morphological comparison (Linder, 1929; Pirozynski, 1972; Goos, 1985, 1986, 1989; Zhao et al., 2007; Kuo and Goh, 2018; Lu et al., 2018a; Hsieh et al., 2021; Tian et al., 2022). Therefore, polygenic phylogenetic analysis is required to accurately identify them. However, previous studies have mainly focused on the description of morphological characteristics; most of them without obtaining strains and DNA molecular data (Linder, 1929; Pirozynski, 1972; Goos, 1985, 1986, 1989; Zhao et al., 2007). What makes things
Neohelicomyces hainanensis (GZAAS 22-2009, holotype). (a,b) Colony on decaying wood. (c–g) Conidiophores and conidia. (h–j) Conidiogenous cells. (k–n) Conidia. (o) Germinating conidium. (p,q) Colonies on PDA observed from above and below. Scale bars: (c–g) = 20\,\mu m, (h–j,k–n) = 10\,\mu m, (l) = 5\,\mu m.
more complicated is that standards for species identification are not uniform, which creates confusion in this taxonomic system. Some helicosporous fungi have been transferred several times. For example, Moore (1957) treated Drepanospora pannosa as Helicosporium pannosum; Matsushima (1975) classified Drepanospora pannosa, Helicosporium linderi, Helicosporium nematosporum, and Helicosporium serpentinum under Helicosporium pannosum; Goos (1989) treated them as Drepanospora pannosum; Zhao et al. (2007) treated all of them and Helicosporium gigasporum as Helicosporium pannosum. The reason the authors reassessed the taxonomic status of these species is that there were some differences in the morphological characteristics of the conidiophores, conidiogenous cells, and conidia; the authors used different taxonomic principles to identify these species (Moore, 1957; Matsushima, 1975; Goos, 1989; Zhao et al., 2007). In our previous study, we paid attention to the confusion regarding the classification of helicosporous hyphomycete, analyzed the existing problems, and proposed ideas to solve the problems (Lu et al., 2018b). Lu et al. (2018b) provided several examples to show that the morphological characteristics of conidiophores, conidiogenous cells, and conidia, including their color and size, are very important influencing factors that cannot be ignored in distinguishing helicosporous fungi. The key to solve this taxonomic system problem is to obtain more species resources such as molecular data and morphological characteristics, for both newly collected specimens and published specimens with incomplete morphological features. Specimens observed in previously published literature that have molecular data but lack morphological characteristics, and are well preserved, can be borrowed for further morphological research.

In addition, different fungal species with similar morphologies produced distinctly characteristic secondary metabolites. For example, the stromata and ascospores of Annulohypoxylon arceolatum were morphologically similar to those in A. leptiscum. However, they could be distinguished by their unique stromatal HPLC profiles, in which A. arceolatum produced the sole main metabolite viz. urceoline, while A. leptiscum produced large quantities of truncatone A and C (Kuhnert et al., 2017). Annulohypoxylon yangensis was morphologically similar to A. truncatum, but the former produced BNT (1,1′-binaphthalene-4,4′-5,5′-tetrol), whereas the latter produced truncaquenone A and B in large quantities as well as trace truncatone A (Surup et al., 2016; Kuhnert et al., 2017). Kuhnert et al. (2017) provided a good example, using chemotaxonomy to evaluate the taxonomic systems of fungi with similar morphologies. This may be a new way to solve the problem of the taxonomy of helicosporous hyphomycetes by using evidence from chemotaxonomic data together with phylogenetic and morphological data.

In this study, we obtained 11 helicosporous fungal specimens and cultures and introduced four new species and three new records of helicosporous hyphomycetes based on morphological and phylogenetic evidence. We are also carrying out studies on the secondary metabolites of these fungi, and hope to find the characteristic compounds of each genus and solve the classification problem of helicosporous fungi with evidence from chemotaxonomic data in future.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article supplementary material.

Author contributions

Y-ZL and JM conducted the experiments, analyzed the data, and wrote the article. J-CK planned the experiments. X-JX and Y-PX analyzed the data. JM and X-JX conducted the experiments. L-JZ and J-CK revised the article. Y-ZL and J-CK funded the experiments. All authors revised and agreed to the published version of the article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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