1. Introduction

The family Hypocreaceae typified by Hypocrea Fr. was established by Saccardo [1] and was redefined by Rossman et al. [2] who treated it in a narrow sense and recognized 12 genera. Approximate 17 genera are currently accepted [3–6], including Hypomyces (Fr.) Tul. & C. Tul and Trichoderma Pers., two major genera encompassing the majority of species of the family. The phylogenetic relationship among genera of the group was first revealed by Spatafora and Blackwell [7].

This study is focused on two major genera of the family. For a long time, many fungicolous fungi with light- or bright-colored perithecia were described as Hypomyces which is typified by H. lactifluorum (Schwein.) Tul. & C. Tul. Morphological characteristics and phylogenetic analysis based on sequence of nuclear ribosomal large subunit (LSU) rDNA in the previous work indicated that the genus is not a monophyletic group [8–11]. After the comprehensive studies by Rogerson and Samuels [12–15] and Pöldmaa and collaborators [11,16–22], the generic concept of Hypomyces became clear. Among the 212 names listed in Index Fungorum database, about 77 species are commonly accepted [11,23–29]. Twenty-seven of them have been known from China [27,29–31]. Members of the genus are mainly distributed in temperate and tropical regions and economically important in biomedicine and agriculture [32,33].

Host specificity, color of subicula and perithecia, shape, size, seption, surface ornamentation, and apiculus of ascospores, and type of asexual states are main characters used for identifications of Hypomyces species. The genus grows on Agaricales, Boletales, Helotiales, and Pezizales are highly host-specific, while those occurring on Polyporales may have a slightly wider host range [2,12–16]. For example, H. lithuanicus Heinr.-Norm. lives only on Lactariustor minosus (Schaeff.) Gray, H. hyalinus (Schwein.) Tul. & C. Tul. is restricted to Amanita Adams, and H. melanocarpus Rogerson & Mazzeris is on Tylopilus P. Karst. However, H. australis (Mont.) Höhn., H. rosellus (Alb. & Schwein.) Tul. & C. Tul. and H. tegillum Berk. & M.A. Curtis show the least specialized parasites and even being found on non-fungus substrates, like rotten bark and wood [14].

Trichoderma, the largest genus in the family Hypocreaceae, was originally established by Persoon [34] and typified with T. viride Pers. Since then, number of Trichoderma species increased dramatically. Bissett et al. [35] provided a list of 254 Trichoderma species with DNA sequences or living cultures available. There are more than 340 species
currently recognized in the genus. They occur on rotten wood, bark and leaves, fruitbodies of other fungi, in soil, or within healthy plant tissues as endophytes [36–39]. They are renewable natural resources and play important roles in production of industrial enzymes and antibiotics [40], control of soil-borne plant pathogens [41,42], plant growth promotion [43], induction of plant resistance [44], production of bioactive secondary metabolites [36] and remediation of soil contaminated by heavy metals [45]. Taxonomy of Trichoderma species is mainly based on anatomy of stromata and perithecia, color, shape, size of ascospores, conidia and chlamydospores, type of conidiophores, colony morphology and growth rate, and DNA sequence data [46,47]. The phylogenetic analyses based on translation elongation factor 1-α encoding (EF-1α) and RNA polymerase II subunit 2 (RPB2) regions indicated that the hyaline ascospore species are divided into 11 clades and those of green ones are separated into 7 clades [48–50].

During our survey of hypocrealean species on fungi and plant debris in China, two undescribed taxa are found based on morphological characteristics and DNA sequence analyses of the internal transcribed spacer (ITS), LSU, EF-1α, and RPB2. Differences between the new species and their close relatives are discussed. Hypomyces orthosporus is reported for the first time from China.

2. Materials and methods

2.1. Collections and morphological study

Specimens were collected from Shennongjia Forestry District of Hubei and Chebaling National Nature Reserve of Guangdong and Mainling of Tibet, and deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS). Cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. Methods used by Jaklitsch [46] and Pöldmaa [19] were followed. Test for color change of perithecial wall was made with 3% potassium hydroxide (KOH). To observe anatomic structures of perithecia, longitudinal sections of ascomata were made with 3% potassium hydroxide (KOH). Microscopic examinations and measurements were taken from the sections and squash mounts in lactophenol cotton blue solution using an Olympus BH-2 microscope (Tokyo, Japan). Photographs were taken with a Leica DFC450 digital camera (Leica Camera, Wetzlar, Germany) attached to a Leica M125 stereomicroscope (Leica) for gross morphology and a Zeiss AxioCamMRc 5 digital camera (Carl Zeiss, Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Carl Zeiss) for anatomic structures. Measurements of individual structures were based on 30 units, except when otherwise noted. Cultures were obtained from conidia on subiculum or from fresh ascomata using single ascospore isolation. To determine colony features and growth rates, strains were grown on cornmeal dextrose agar (CMD; Yuanye Biotechnology Co. Ltd., Shanghai, China), malt extract agar (MEA; Oxoid Ltd., Basingstoke, UK), potato dextrose agar (PDA; HuiXing Biochemistry Reagent Ltd Co., Shanghai, China) and synthetic nutrient-poor agar (SNA) [51] in 90 mm plastic Petri dishes at 25°C for 7 or 14 d. For observation of conidiophores and microconidia, cultures were grown on SNA at 25°C with alternating periods of light and darkness (12 h/12 h).

2.2. DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from fresh mycelium following the methods of Wang and Zhuang [52]. Primer pairs, ITS5/ITS4 [53], LR0R/LR5 [9, 54], and EF1-728F/EF1567R [55,56] were used to amplify the sequences of ITS, LSU, and EF-1α regions for Hypomyces species, while EF1-728F/TEF1LLeRev [55,57] and RPB2-5F/rRPB2-7cR [58] were applied to amplify the sequences of EF-1α and RPB2 regions for Trichoderma species. PCR reactions were performed on an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, CA) with a 25 μl reaction system consisting of 12.5 μl Taq MasterMix, 1 μl each primer (10 μM), 1 μl template DNA and 9.5 μl ddH2O, based on the procedures detailed in White et al. [53], Chaverri and Samuels [59], Rehner and Buckley [56], and Liu et al. [58]. DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences).

2.3. Sequence alignment and phylogenetic analyses

Newly generated sequences and those retrieved from GenBank are listed in Table 1 (Hypomyces) and Table 2 (Trichoderma). Nectria eustromatica Jaklitsch & Voglmayr and Thyronectria berolinensis (Sacc.) Seaver were used as outgroup taxa. Sequences were assembled, aligned, and the primer sequences were trimmed with BioEdit version 7.0.5 (Ibis Biosciences, Carlsbad, CA) [60] and converted to NEXUS files by ClustalX version 1.83 (EMBL, Heidelberg, Germany) [61]. Sequences were first subjected to the BLAST searches to determine preliminarily their taxonomic positions. TrichOKEY
was also applied to preliminary identification of Trichoderma. Due to a low number of variable sites and long insertions in certain species of Trichoderma [63], ITS sequences were not incorporated into phylogenetic analyses. The partition homogeneity test of ITS, LSU, and EF-1α sequences of Hypomyces species were performed with PAUP version 4.0b10 (Sinauer Associates, Sunderland, MA) [64]. To confirm the phylogenetic positions of the new species, sequences of these regions were combined and analyzed with maximum parsimony (MP) and maximum likelihood (ML) methods. The MP analysis was performed with PAUP version 4.0b10 [64] using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. Topological confidence of resulted trees was tested by maximum parsimony bootstrap proportion (MPBP) with 1000 replications, each with 10 replicates of random addition of taxa. Four Markov chains were run simultaneously for 1,000,000 generations with the trees sampled every 100 generations. A 50% majority rule consensus tree was computed after excluding the first 2500 trees as “burn-in.” The ML analysis was conducted with IQ-TREE version 1.6.10 (University of Vienna, Vienna, Austria) [65] using the best model for each locus chosen by ModelFinder [66]. Branch support measures were calculated with 1000 bootstrap replicates. Trees were examined via TreeView version 1.6.6 (University of Glasgow, Glasgow, UK) [67]. Maximum likelihood bootstrap (MLBP) and MPBP greater than 50% are shown at the nodes.

### 3. Results

#### 3.1. Phylogenetic analyses

To determine the positions of the Hypomyces collections, the sequences of ITS, LSU, and EF-1α regions of 36 Hypomyces species were analyzed. The PHT (p = .01) indicated that the individual partitions were not highly incongruent [68], thus the three loci were thus combined for phylogenetic analyses. The combined datasets include 2440 characters, of which

| Species | Herbarium/strain numbers | ITS accession numbers | LSU accession numbers | EF-1α accession numbers |
|---------|--------------------------|----------------------|----------------------|-------------------------|
| Hypomyces aconidialis | TFC 201215 | FN859456 | FN859456 | FN868774 |
| Hypomyces albidus | CBS 46071 | MH860220 | MH871987 | – |
| Hypomyces armeniacus | TFC 02862 | FN859424 | FN859424 | FN868742 |
| Hypomyces aurantius | TFC 98171 | FN859425 | FN859425 | FN868743 |
| Hypomyces australasiaticus | TFC 038 | NR121428 | FN859428 | FN868746 |
| Hypomyces australis | TFC 0718 | AM779860 | AM779860 | FN868747 |
| Hypomyces chlorinigenus | KSH 511 | KT946843 | AF213027 | KU041505 |
| Hypomyces complectus | KSH 411 | KT946842 | AF213028 | KU041504 |
| Hypomyces corticiicola | CBS 13771 | MH860037 | MH871817 | – |
| Hypomyces dactylariaoides | CBS 14178 | NR111430 | MH872879 | FN868748 |
| Hypomyces elliptosporus | CBS 69686 | NR, 155168 | – | – |
| Hypomyces gabanonis | TFC 201556 | NR121429 | FN859430 | FN868749 |
| Hypomyces huberiensis | HMAS 254597 | – | – | – |
| Hypomyces khayaensis | GJS 01304 | FN859431 | AJ583483 | FN868750 |
| Hypomyces lactifluorum | TAAM 170476 | FN859432 | EU710768 | FN868751 |
| Hypomyces laeticolor | JCM 10758 | NR, 155202 | NG_059815 | – |
| Hypomyces luteovirens | CBS 128483 | MH864958 | MH874042 | – |
| Hypomyces mycophilus | CBS 17556 | MH857567 | MH860110 | – |
| Hypomyces odotarius | GAm 329 | FN859434 | FN859434 | FN868753 |
| Hypomyces orthosporus | TFC 97130 | – | AF160241 | – |
| Hypomyces peltigericola | CBS 141848 | – | – | – |
| Hypomyces pseudepipolcicola | JCM 12654 | Nr, 155203 | NG_059820 | – |
| Hypomyces rodeloi | TFC200717 | NR, 145022 | AM779859 | – |
| Hypomyces rosetulis | TFC 201701 | FN859443 | FN859443 | FN868762 |
| Hypomyces samuelssii | TFC 2007-23 | FN859451 | FN859451 | FN868769 |
| Hypomyces semicircularis | CBS 70588 | NR, 121423 | MH873843 | FN868735 |
| Hypomyces semitranslucens | CBS 82170/TFC 0323 | LH859900 | AJ493303 | – |
| Hypomyces siberinae | CBS 74488 | MH862151 | AJ493304 | – |
| Hypomyces sinicus | HMAS 279649 | – | – | – |
| Hypomyces stepphanomatis | CBS 44664/GJS 8850 | MN044763 | MN044763 | MK484609 |
| Hypomyces subglobosus | CBS 54386 | – | – | – |
| Hypomyces subiculosus | TFC 97166 | FN859452 | AJ493309 | FN868770 |
| Hypomyces tremellicola | CBS 44165/TFC 9750 | KU382166 | U17427 | – |
| Hypomyces tubariicola | CBS 11579 | NR, 158483 | MH872953 | – |
| Hypomyces virensis | GAi 1906 | FN859454 | FN859454 | FN868772 |
| Hypomyces xyloboli | CBS 151208 | NR, 160212 | AJ493299 | – |
| Nectria eustromatica | CBS 121896 | NR137579 | HM534896 | HM534875 |
| Thryonectria berolinensis | CBS 172382 | MH864554 | MH875990 | HM534872 |

*Numbers in bold indicate the newly provided sequences. Species labeled as T indicate the sequences from ex-type strains.

Table 1. List of Hypomyces species, herbarium/strain numbers and GenBank accession numbers of materials used in this study.
Table 2. List of Hypocreaceae species, herbarium/strain numbers and GenBank accession numbers of materials used in this study.

| Species                     | Herbarium/strain numbers | GenBank accession numbers |
|-----------------------------|--------------------------|----------------------------|
| Arachnocrea scabrida       | BEO 0201                 | DQ834457 DQ834458         |
| Arachnocrea stipata        | TFC 9743                 | – EUT10770                |
| Hypomyces lactifluorum     | TAAM 170476              | FN868751 EU701773         |
| Hypomyces roSELLUS         | TFC 201071               | FN868697 FN868697         |
| Hypomyces samuelsi         | TFC 200723               | FN868769 FN868705         |
| Nectria eustromatica       | CBS 121896               | HMs34875 HMS34886         |
| Protocrea farinosa         | CBS 121551               | EU703889 EU703935         |
| Protocrea illinoensis      | TFC 9698                 | EU703905 EU703952         |
| Protocrea pallida          | CBS 29978                | EU703905 EU703952         |
| Thyonectria berolinensis   | CBS 127382               | HMs34872 HMs34883         |
| Trichoderma aggregatum     | DAOM 222156              | AF348098 FJ442752         |
| Trichoderma alutaceum      | CBS 120535               | FJ179567 FJ179600         |
| Trichoderma asteroidi      | HMAS 271353              | KT224465 KT224469         |
| Trichoderma brevicompactum | TRS 859                  | KPOP0890 KPOP0916         |
| Trichoderma ceramicum      | CBS 114576               | FJ860628 FJ860531         |
| Trichoderma confluens      | HMAS 244993              | KT001959 KT001964         |
| Trichoderma danicum        | CBS 121273               | FJ860634 FJ860534         |
| Trichoderma chloropsorum   | GJS 981                  | AY391906 AY391906         |
| Trichoderma delicuens      | GJS 89129                | AFS34581 AFS34551         |
| Trichoderma estonicum      | GJS 96129                | AFS34604 AFS34551         |
| Trichoderma folicoicola    | Hypo 645                 | JQ685802 JQ685876         |
| Trichoderma hainanensis    | HMAS 248837              | KY680303 KY687976         |
| Trichoderma heNanense      | HMAS 252289              | KT224464 KT224467         |
| Trichoderma hongkongensis  | HMAS 273832              | KX45364 KX980154          |
| Trichoderma hunanense      | HMAS 248841              | KY680303 KY687980         |
| Trichoderma junc1          | CBS 120926               | FJ860641 FJ860540         |
| Trichoderma leguminosarum  | CBS 130014               | K665539 K665528           |
| Trichoderma longibrachiati | CBS 816.68               | AY865640 DQ87242          |
| Trichoderma longiPILe      | DAOM 177227              | AFS34622 AFS45550         |
| Trichoderma luteocrystallinum | CBS 123828                | FJ860646 FJ860544         |
| Trichoderma moravicum      | CPK 2489/CBS 120539      | FJ860651 FJ860549         |
| Trichoderma odoradon1      | HMAS 271354              | KT224464 KT224467         |
| Trichoderma orientale      | CBS 131488               | JQ65866 JQ658984          |
| Trichoderma pseudolacteum | TUF 61490                | – JX234878                |
| Trichoderma psychrophilium | CPK 2435/HY 8            | FJ860682 FJ860576         |
| Trichoderma rhododendri    | CBS 119288               | FJ860685 FJ860578         |
| Trichoderma romani1        | GJS 9188                 | EU38324 EU38324           |
| Trichoderma rossicum      | DAOM 230011              | AY973441 HQ942288         |
| Trichoderma semiobiS1      | GJS 99108                | JN135761 JN133567         |
| Trichoderma sinuosum       | DAOM:232839              | KJ871139 KJ82198          |
| Trichoderma spinulosa      | CBS 31150                | FJ860701 FJ860591         |
| Trichoderma spirale        | GJS 89188                | EU38324 EU38324           |
| Trichoderma stercorarium   | ATCC 62321               | FJ860607 EF49103          |
| Trichoderma strictiP1      | DAOM 172827              | AFS34628 KJ842162         |
| Trichoderma stromaticum    | GJS 97183                | AFS34613 AFS45539         |
| Trichoderma tamenosum      | DAOM 178713a             | EU279969 EU279977         |
| Trichoderma subiculoides   | HMAS 254400              | MK448464 MK448467         |
| Trichoderma undatipile     | HMAS 248854              | KY68056 KY687993          |
| Trichoderma vinde1         | CBS 119325               | DQ672615 EUT11362         |

*Numbers in bold indicate the newly provided sequences.

+SSpecies labeled as T indicate the sequences from ex-type strains.

1704 were constant, 233 variable and parsimony-uninformative and 503 parsimony-informative. The MP analysis resulted in a single most parsimonious tree (tree length = 2459, CI = 0.4429, HI = 0.5571, RI = 0.5287, RCI = 0.2342). The final matrix was deposited in TreeBASE with accession No. S23771. The MP tree generated is shown in Figure 1. The topology of the ML tree is similar to that of the MP tree. HMAS 254597 clustered with representative species of Hypomyces (MLBP/MPBP = 79%/98%), which confirmed its taxonomic position in the genus.

To place the Trichoderma collection, the sequences of EF-1α and RPB2 regions from 38 species representing 18 clades of Trichoderma, three species of Protocrea, three of Hypomyces and two of Arachnocrea were analyzed by the methods of ML and MP. The PHT (p = 0.01) indicated that the individual partitions were not highly incongruent [68], the two loci were thus combined for phylogenetic analyses. The combined datasets include 1698 characters, of which 1027 were constant, 118 variable and parsimony-uninformative and 553 parsimony-informative. The MP analysis resulted in three most parsimonious trees (tree length = 3602, CI = 0.2984, HI = 0.7016, RI = 0.5171, RCI = 0.1543). The final matrix was deposited in TreeBASE with accession No. S23772. One of the three MP trees generated is shown in Figure 2. The ML tree is of a similar tree topology. HMAS 254600 was shown as a separate lineage associated with Asterineum, Longibrachiatiu,
and Virgineum clades of Trichoderma, and further clustered with other species of the genus forming a highly supported monophyletic group (MLBP/MPBP = 100%/98%), which confirmed its taxonomic position in the genus.

### 3.2. Taxonomy

#### 3.2.1. Hypomyces hubeiensis Z.Q. Zeng & W.Y. Zhuang, sp. nov.

Fungal Names: FN570597.

Description: On CMD, colony radius 14 mm after 7 d at 25°C, velvet, producing yellowish green pigment in medium, reverse yellowish green; aerial hyphae white, scarce. On MEA, colony radius 13 mm after 7 d at 25°C, velvet, surface white, reverse white; aerial hyphae white, scarce, forming concentric rings. On PDA, colony radius 13 mm after 7 d at 25°C, floccose, surface grey white, reverse light sienna; aerial hyphae white, dense, floccose. Conidiophores arising from aerial hyphae, branched, septate, 1–2–verticillate, with terminal whorl of 2–6 phialides. Phialides subulate, tapering toward apex, smooth, 8–20 × 2–3 μm. Conidia rod-shaped to narrowly ellipsoidal, aseptate, hyaline, smooth, 3–6 × 1–2.3 μm.

Etymology: The specific epithet refers to the type locality.

Holotype: China, Hubei Province, Shennongjia Forestry District, Dajiuhu, on Agaricus sp., September 17 2014, Z.Q. Zeng, W.T. Qin, K. Chen & H.D. Zheng 9791 (HMAS 254597) (Figure 3).

Notes: The new species grows on fruitbodies of Agaricus sp. containing only the asexual state.
Among the known agaricicolous species of Hypomyces, H. hubeiensis is morphologically similar to H. succineus Rogerson & Samuels and H. tremellicola (Ellis & Everh.) Rogerson in forming verticilium-like conidiophores. But H. succineus differs in occurring on Pholiota sp. rather than Agaricus sp. and having much larger conidia \((7–8.8 \times 3.3–4.2(–5) \mu m \text{ vs. } 3–6 \times 1.23 \mu m)\) [15]. H. tremellicola grows on Crepidotus spp. and has larger conidia \([5–9 \times 3–4(–5) \mu m \text{ vs. } 3–6 \times 1.23 \mu m]\) [15].

### 3.2.2. Hypomyces orthosporus K. Põldmaa

Mycotaxon 59: 390 (1996)

= Cladobotryum orthosporum (W. Gams) K. Põldmaa, Mycotaxon 59: 390 (1996)

≡ Sibirina orthospora W. Gams, Persoonia 7: 163 (1973)

On CMD, colony radius 46 mm after 7 d at 25°C, floccose, producing light yellowish brown pigment, reverse brown; aerial hyphae white, scarce. On MEA, colony radius 40 mm after 7 d at 25°C, floccose, producing light yellowish brown pigment, reverse brown; aerial hyphae white, scarce. On PDA, colony radius 42 mm after 7 d at 25°C, floccose, producing light yellowish brown pigment; aerial hyphae white, scarce. Conidiophores arising from aerial mycelium, indefinite in length, 1–2-verticillate, with terminal whorl of 3–10 phialides. Phialides subulate, tapering toward apex, hyaline, smooth, 10–35 μm long, 1–1.8 μm at the base. Conidia subcylindrical, sometimes subfusoid, rarely narrowly ellipsoidal (0–1(–2)-septate, hyaline, smooth, with a rounded tip and a basal hilum, 10–18 × 2.5–5 μm.

Specimen examined: China, Tibet, Nyingchi, Mainling, alt. 2800 m, on fruiting body of a polypore, September 12 2016, H.D. Zheng, Z.Q. Zeng, X.C. Wang, K. Chen & Y.B. Zhang 10736 (HMAS 279649) (Figure 4).

Known distribution: China, Estonia, Finland, and The Netherlands.

Notes: Sibirina orthospora was described by Gams [69] based on the specimen on decaying wood from...
The Netherlands with only asexual state described. The sexual and asexual stage connection of the fungus was established by Poldmaa [16] based on the materials collected from Estonia. The phylogenetic tree based on LSU sequences showed that the Chinese collection (HMAS 279649) associated with that from Estonia (TFC 97-130) receiving high support values (MLBP/MPBP = 83%/90%).

3.2.3. *Trichoderma subiculoides* Z.Q. Zeng & W.Y. Zhuang, sp. nov

Fungal Names: FN570596.

Description: Stromata broadly attached on natural substratum, widely effuse to confluent, rudimentary and somewhat subiculum-like, lacking of a defined margin or flank, whitish to beige when dry, cinnamon brown after rehydration, not changing color in 3% KOH, 3–7 × 2–5 mm, 0.4 mm thick. Ostiolar dots distinct, dirty brownish to light brown when dry, brown to dark brown when rehydrated. In section, cortical tissue of textura globulosa, 5–25 μm thick, cells light yellow, 1.5–5 × 1.5–4.5 μm; subcortical tissue of textura intricata, hyphae hyaline to pale brown, 2–3.5 μm thick; subperithelial tissue of textura epidermoidea, cells hyaline, thin-walled, 5–10 × 3–5 μm. Perithecia globose, subglobose to pyriform, 138–193 × 105–150 μm; peridium 6–12 μm thick at flanks, 15–30 μm thick at the base. Papilla prominent, blunt or truncate, brown, 18–63 μm high, 35–58 μm wide at the base. Asci subcylindrical, 78–115 × 2.8–5 μm. Part-ascospores hyaline, smooth, dimorphic, distal cells broadly ellipsoidal to globose, 3.5–5 × 2–4 μm, l/w 1–2; proximal cells ellipsoidal, 4–5 × 2–4 μm, l/w 1.3–2.

On CMD, colony radius 10 mm after 7 d at 20 °C, 41 mm at 25 °C, no growth at 30 and 35 °C, white, velvet; aerial hyphae scarce, hyaline. On PDA, colony radius 10 mm after 7 d at 20 °C, 26 mm at 25 °C, 8 mm at 30 °C, no growth at 35 °C, white, velvet; aerial hyphae dense, hyaline. On SNA, colony radius 9 mm after 7 d at 20 °C, 5 mm at 25 °C, no growth at 30 and 35 °C, producing cream to pale yellow pigment; aerial hyphae hyaline, scarce.

![Figure 3. *Hypomyces hubeiensis* (HMAS 254597). (A–C) Cultures after 14 d at 25 °C (A: on CMD, B: on MEA, C: on PDA); (D–I) Conidiophores, phialides, and conidia; (J) Phialides and conidia; (K–M) onidia. Scale bar = 10 μm.](image-url)
Conidiophores arising from aerial mycelium, branched, branches septe, 1–2-verticillate, with the terminal whorl of 2–4 phialides, 15–55 × 2–3.5 μm. Phialides subcylindriical, tapering toward apex, smooth, 5–25 × 1.5–3 μm. Conidia subellipsoidal to rod-shaped, hyaline, smooth, 3–9 × 1.5–3 μm. No distinct odor detected.

Etymology: The specific epithet refers to the sub-iculum-like and rudimentary stromata.

Holotype: China, Guangdong Province, Shixing County, Chebaling National Nature Reserve, on rotten branch, 2 November 2015, Z.Q. Zeng, X.C. Wang, K. Chen & Y.B. Zhang 10623 (HMAS 254600) (Figure 5).

Notes: Among the known species of Trichoderma, T. subiculoides is morphologically similar to T. confluens W.T. Qin & W.Y. Zhuang and T. pseudolacteum C.S. Kim & N. Maek. in having effuse to confluent stromata which are broadly attached to substrates [70,71]. However, T. subiculoides differs from T. confluens in stromatal gross morphology and perithecia not changing color in 3% KOH, smaller part-ascospores (distal 3.5–5 × 2–4 μm vs. 5.4–6.5 × 5.0–5.9 μm, proximal 4–5 × 2–4 μm vs. 5.3–6.9 × 4.3–5.2 μm), ellipsoidal to rod-shaped rather than globose to subglobose conidia [71]. The RPB2 sequence of T. subiculoides differs from that of T. confluens by 64 bp divergences in a total length of 751 bp. Trichoderma subiculoides can be easily distinguished from T. pseudolacteum by narrower asci (2.8–5 μm vs. 5.9–7.1 μm wide), smaller part-ascospores (distal 3.5–5 × 2–4 μm vs. 5.4–6.5 × 5.0–5.9 μm, proximal 4–5 × 2–4 μm vs. 5.3–6.9 × 4.3–5.2 μm), ellipsoidal to rod-shaped rather than globose to subglobose conidia [71]. Sequence comparisons revealed that there are 62 bp unmatched loci among 452 bp for partial RPB2 region between the type strains (HMAS 254600 and TUFC 61490).

4. Discussion

Hypomyces is connected with diverse asexual states, such as mycogone-like, stephanoma-like, papulapora-like, sepedonium-like, verticillium-like, acremonium-like, and cladobotryum-like [2]. Host fungi in combination with types of asexual states are regarded as important taxonomic criteria for species identifications [17]. Asexual states are sometimes even critical to distinguish genera in Hypocreaceae. Due to that H. berkeleyanus Plowr. & Cooke and H. broomeanus Tul. & C. Tul. are of gliocladium-like

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**Figure 4.** Hypomyces orthosporus (HMAS 279649). (A–C) Cultures after 7 d at 25°C (A: on CMD, B: on MEA, C: on PDA); (D) Conidiophores and phialides; (E–G) Phialides and conidia; (H–L) onidia. Scale bar: D–G = 10 μm; H–L = 5 μm.
asexual states, Rehner and Samuels [9] and Pöldmaa et al. [11] excluded them from Hypomyces and transferred these two species to another genus Sphaerostilbella (Henn.) Sacc. & D. Sacc. The taxonomic position of H. hubeiensis is revealed based on the sequence analyses of ITS, LSU, and EF-1α regions (Figure 1) as well as the morphological characters such as the substrate and verticillate conidiophores. However, its complete life cycle and infra-specific variation await future investigation. Some Hypomyces species with asexual states unknown due to their ascospores not germinating in laboratory condition [27]. Establishment of asexual and sexual state connections for these fungi will provide essential information about life cycle of the whole fungus.

Stroma is a vegetative tissue that subtends or surrounds the ascomata [2]. Tissues of the stromata of Trichoderma are composed of textura angularis, textura globulosa, textura intricata, textura prismatica, and textura epidermoidea depending on locations of the tissues. Stromatal anatomy is considered as one of the important morphological characters at generic and species levels for taxonomy of Hypocreaceae [2]. Most species of Trichoderma have pulvinate, disciform, flat, peltate, tubinate, hemispherical or clavate stromata which are usually of a well-defined margin and flanks [2,59,72], while very few species possess rudimentary or subiculum-like stromata, such as T. alcalifuscens (Overton) Jaklitsch & Voglmayr, T. delicatulum Jaklitsch & T. parmastoi (Overton) Jaklitsch & Voglmayr [47]. In Hypocreaceae the genera Arachnocrea Z. Moravec and Protocrea Petch produce subiculum surrounding their perithecia. However, the anatomic structure of T. subiculoides reveals that the fungus has a subiculum-like stroma instead of true subiculum that a cortical layer is

Figure 5. Trichoderma subiculoides (HMAS 254600). (A,B) Stroma on nature substrate; (C) Color of stroma after rehydration; (D) Color of rehydrated stroma in 3% KOH; (E–G) Cultures after 14 d at 25°C (E: on CMD, F: on SNA, G: on PDA); (H) Perithecium in section; (I) Structure of perithecial at upper portion; (J) Ascus with ascospores; (K–O) Part-ascospores; (P) Phialides and conidia; (Q) Cortical and subcortical tissues in section; (R) Subperithecial tissue in section; (S–W) Conidia. Scale bars: A = 1 cm; B–D = 1 mm; H, I = 50 μm; J–P = 10 μm; Q, R = 20 μm; S–W = 10 μm.
present on the stromatal upper surface and the individual stromata lack of well-developed margin and flanks. Sequence analyses indicated that *T. subiculooides* clustered with other *Trichoderma* species receiving high bootstrap supports (MLBP/MPBP = 100%/98%) as a separate lineage and does not belong to any existing clades.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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