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RESEARCH ARTICLE

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Two New Species and a New Chinese Record of Hypocreaceae as Evidenced by Morphological and Molecular Data

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ABSTRACT

To explore species diversity of Hypocreaceae, collections from Guangdong, Hubei, and Tibet of China were examined and two new species and a new Chinese record were discovered. Morphological characteristics and DNA sequence analyses of the ITS, LSU, EF-1α, and RPB2 regions support their placements in Hypocreaceae and the establishments of the new species. *Hypomyces hubeiensis* sp. nov. is characterized by occurrence on fruitbody of *Agaricus* sp., concentric rings formed on MEA medium, verticillium-like conidiophores, subulate phialides, rod-shaped to narrowly ellipsoidal conidia, and absence of chlamydospores. *Trichoderma subiculoides* sp. nov. is distinguished by effuse to confluent rudimentary stromata lacking of a well-developed flank and not changing color in KOH, subcylindrical asci containing eight ascospores that disarticulate into 16 dimorphic part-ascospores, verticillium-like conidiophores, subcylindrical phialides, and subellipsoidal to rod-shaped conidia. Morphological distinctions between the new species and their close relatives are discussed. *Hypomyces orthosporus* is found for the first time from China.

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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 produced within subiculum 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 Põldmaa 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, septation, 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* Adans, 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

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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], biological 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 ascospored 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 Põldmaa [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 a freezing microtome (YD-1508-III; Jinhua Yidi Medical Appliance Co., Jinhua, China) at a thickness of 6–8 µm. 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 Bio-Technology 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 fRPB2-5F/fRPB2-7cR [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 ddH_2O, 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

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

		GenBank accession numbers		
Species	Herbarium/strain numbers	ITS	LSU	EF-1α
Hypomyces aconidialis	TFC 201215	FN859456	FN859456	FN868774
Hypomyces albidus	CBS 46071	MH860220	MH871987	–
Hypomyces armeniacus	TFC 02862	FN859424	FN859424	FN868742
Hypomyces aurantius	TFC 95171	FN859425	FN859425	FN868743
Hypomyces australasiaticus[T]	TFC 038	NR121428	FN859428	FN868746
Hypomyces australis	TFC 0718	AM779860	AM779860	FN868747
Hypomyces chlorinigenus	KSH 511	KT946843	AF213027	KU041505
Hypomyces completus	KSH 411	KT946842	AF213028	KU041504
Hypomyces corticiicola	CBS 13771	MH860037	MH871817	–
Hypomyces dactylarioides[T]	CBS 14178	NR111430	MH872879	FN868748
Hypomyces ellipsosporus[T]	CBS 69686	NR_155168	–	–
Hypomyces gabonensis[T]	TFC 201156	NR121429	FN859430	FN868749
Hypomyces hubeiensis[T]	HMAS 254597	**MK478467**[a]	**MN044762**	**MK484608**
Hypomyces khaoyaiensis	GJS 01304	FN859431	AJ583483	FN868750
Hypomyces lactifluorum	TAAM 170476	FN859432	EU710768	FN868751
Hypomyces laeticolor[T]	JCM 10758	NR_155202	NG_059815	–
Hypomyces luteovirens	CBS 128483	MH864958	MH876402	–
Hypomyces mycophilus	CBS 17556	MH857567	MH869110	–
Hypomyces odoratus	GAm 329	FN859434	FN859434	FN868753
Hypomyces orthosporus	TFC 97130	–	AF160241	–
	HMAS 279649	**MK478468**	**MN044763**	**MK484609**
Hypomyces peltigericola[T]	CBS 141848	NR_148180	–	–
Hypomyces pseudocorticiicola[T]	JCM 12654	NR_155203	NG_059820	–
Hypomyces robledoi[T]	TFC200717	NR_145022	AM779859	–
Hypomyces rosellus	TFC 201071	FN859443	FN859443	FN868762
Hypomyces samuelsii	TFC 2007-23	FN859451	FN859451	FN868769
Hypomyces semicircularis[T]	CBS 70588	NR_121425	MH873843	FN868735
Hypomyces semitranslucens	CBS 82170/TFC 0323	MH859960	AJ459303	–
Hypomyces sibirinae	CBS 74488	MH862151	AJ459304	–
Hypomyces sinicus[T]	HMAS 251317	NR156252	**MN044986**	**MK484610**
Hypomyces stephanomatis	CBS 44664/GJS 8850	MH858481	AF160243	AF534632
Hypomyces subglobosus[T]	CBS 54386	NR_155169	–	–
Hypomyces subiculosus	TFC 97166	FN859452	AJ459309	FN868770
Hypomyces tremellicola	CBS 44165/TFC 9750	KU382166	U17427	–
Hypomyces tubariicola[T]	CBS 11579	NR_158483	MH872953	–
Hypomyces virescens	GAi 1906	FN859454	FN859454	FN868772
Hypomyces xyloboli[T]	CBS 110280	NR_160212	AJ459299	–
Nectria eustromatica[T]	CBS 121896	NR137579	HM534896	HM534875
Thyronectria berolinensis	CBS 127382	MH864554	MH875990	HM534872

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

[62] 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*, EF-1α and RPB2 sequences of *Trichoderma* 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 chose 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

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	
		EF-1α	RPB2
Arachnocrea scabrida	BEO 0201	DQ834457	DQ834458
Arachnocrea stipata	TFC 9743	–	EU710770
Hypomyces lactifluorum	TAAM 170476	FN868751	EU710773
Hypomyces rosellus	TFC 201071	FN868762	FN868697
Hypomyces samuelsii	TFC 200723	FN868769	FN868705
Nectria eustromatica^T	CBS 121896	HM534875	HM534886
Protocrea farinosa^T	CBS 121551	EU703889	EU703935
Protocrea illinoensis^T	TFC 9698	EU703905	EU703952
Protocrea pallida^T	CBS 29978	EU703900	EU703948
Thyronectria berolinensis	CBS 127382	HM534872	HM534883
Trichoderma aggressivum^T	DAOM 222156	AF348098	FJ442752
Trichoderma alutaceum^T	CBS 120535	FJ179567	FJ179600
Trichoderma asterineum^T	HMAS 271353	KT224465	KT224469
Trichoderma brevicompactum	TRS 859	KP008906	KP009162
Trichoderma ceramicum^T	CBS 114576	FJ860628	FJ860531
Trichoderma confluens^T	HMAS 244993	KT001959	KT001964
Trichoderma danicum^T	CBS 121273	FJ860634	FJ860534
Trichoderma chlorosporum^T	GJS 981	AY391968	AY391906
Trichoderma deliquescens	GJS 89129	AF534581	AF545517
Trichoderma estonicum	GJS 96129	AF534604	AF545514
Trichoderma foliicola^T	Hypo 645	JQ685862	JQ685876
Trichoderma hainanense^T	HMAS 248837	KY688033	KY687976
Trichoderma henanense^T	HMAS 252889	KT224464	KT224467
Trichoderma hongkongensis^T	HMAS 273832	KX495364	KX980154
Trichoderma hunanense^T	HMAS 248841	KY688039	KY687980
Trichoderma junci^T	CBS 120926	FJ860641	FJ860540
Trichoderma leguminosarum^T	CBS 130014	KJ665551	KJ665288
Trichoderma longibrachiatum^T	CBS 816.68	AY865640	DQ087242
Trichoderma longipile^T	DAOM 177227	AF534622	AF545550
Trichoderma luteocrystallinum^T	CBS 123828	FJ860646	FJ860544
Trichoderma moravicum	CPK 2489/CBS 120539	FJ860651	FJ860549
Trichoderma odoratum^T	HMAS 271354	KT224463	KT224468
Trichoderma orientale	CBS 131488	JQ685868	JQ685884
Trichoderma pseudolacteum	TUFC 61490	–	JX238478
Trichoderma psychrophilum	CPK 2435/HY 8	FJ860682	FJ860576
Trichoderma rhododendri^T	CBS 119288	FJ860685	FJ860578
Trichoderma rodmanii^T	GJS 9188	EU338286	EU338324
Trichoderma rossicum^T	DAOM 230011	AY937441	HQ342288
Trichoderma semiorbis^T	GJS 99108	JN133576	JN133567
Trichoderma sinuosum^T	DAOM:232839	KJ871139	KJ842198
Trichoderma spinulosum^T	CBS 31150	FJ860701	FJ860591
Trichoderma spirale^T	DAOM 183974	EU280049	AF545553
Trichoderma stercorarium	ATCC 62321	FJ860607	EF469103
Trichoderma strictipile^T	DAOM 172827	AF534628	KJ842162
Trichoderma stromaticum^T	GJS 97183	AF534613	AF545539
Trichoderma tomentosum^T	DAOM 178713a	EU279969	AF545557
Trichoderma subiculoides^T	HMAS 254600	**MK484604**[a]	**MK484607**
Trichoderma undatipile^T	HMAS 248854	KY688056	KY687993
Trichoderma viride^T	CBS 119325	DQ672615	EU711362

[a]Numbers in bold indicate the newly provided sequences.
^{T}Species 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=.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, Longibrachiatum,

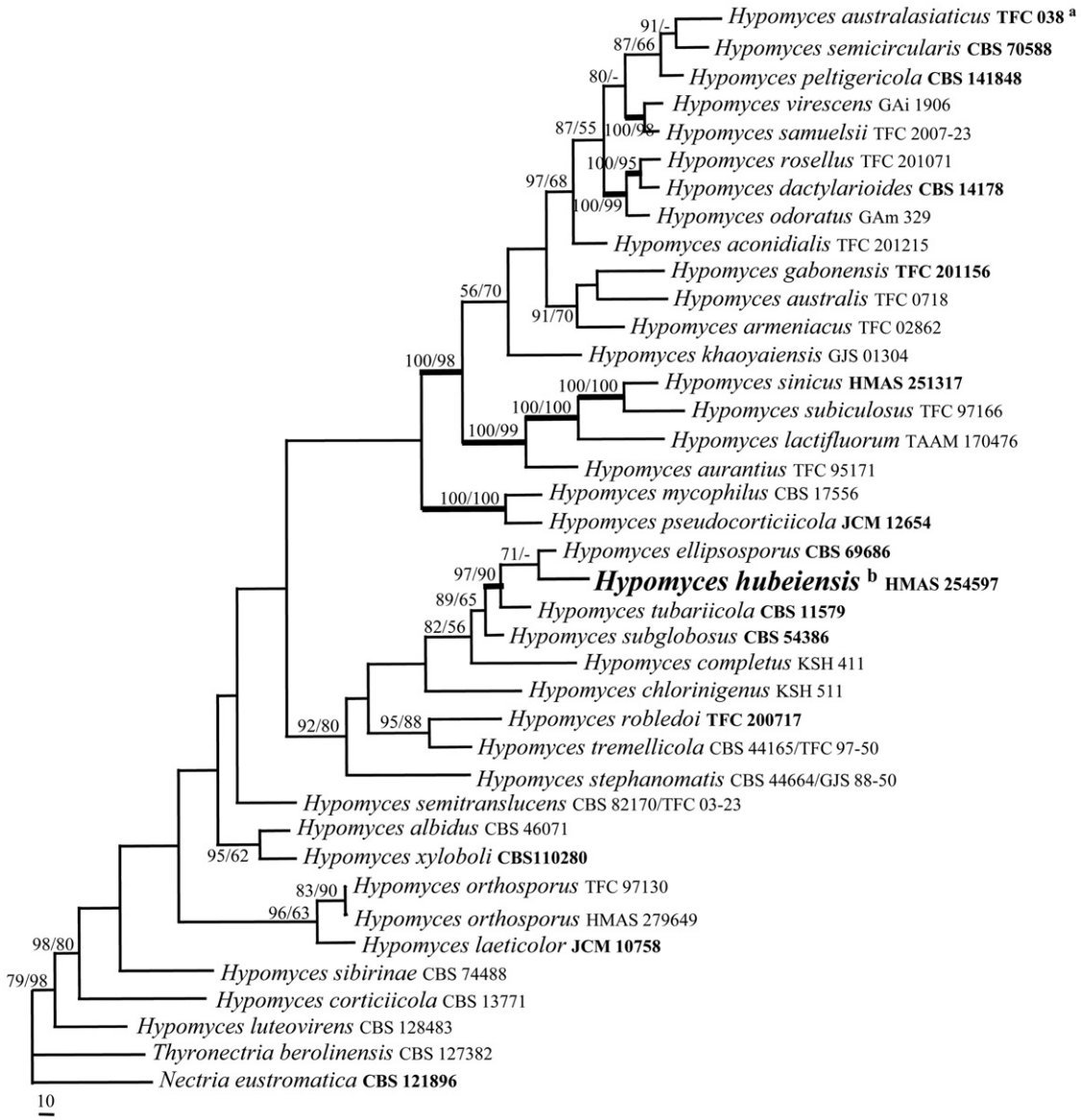

Figure 1. A MP tree generated based on the combined datasets of ITS, LSU and EF-1α sequences of *Hypomyces* species. Supporting values showing at branches: MLBP (left) and MPBP (right). MLBP and MPBP greater than 50% are shown at the nodes. The branch support values ≥90 are indicated by thicker lines. Genbank accession numbers in bold indicate the sequences from ex-type strains. The scale bars indicate number of nucleotide substitutions per site.

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.

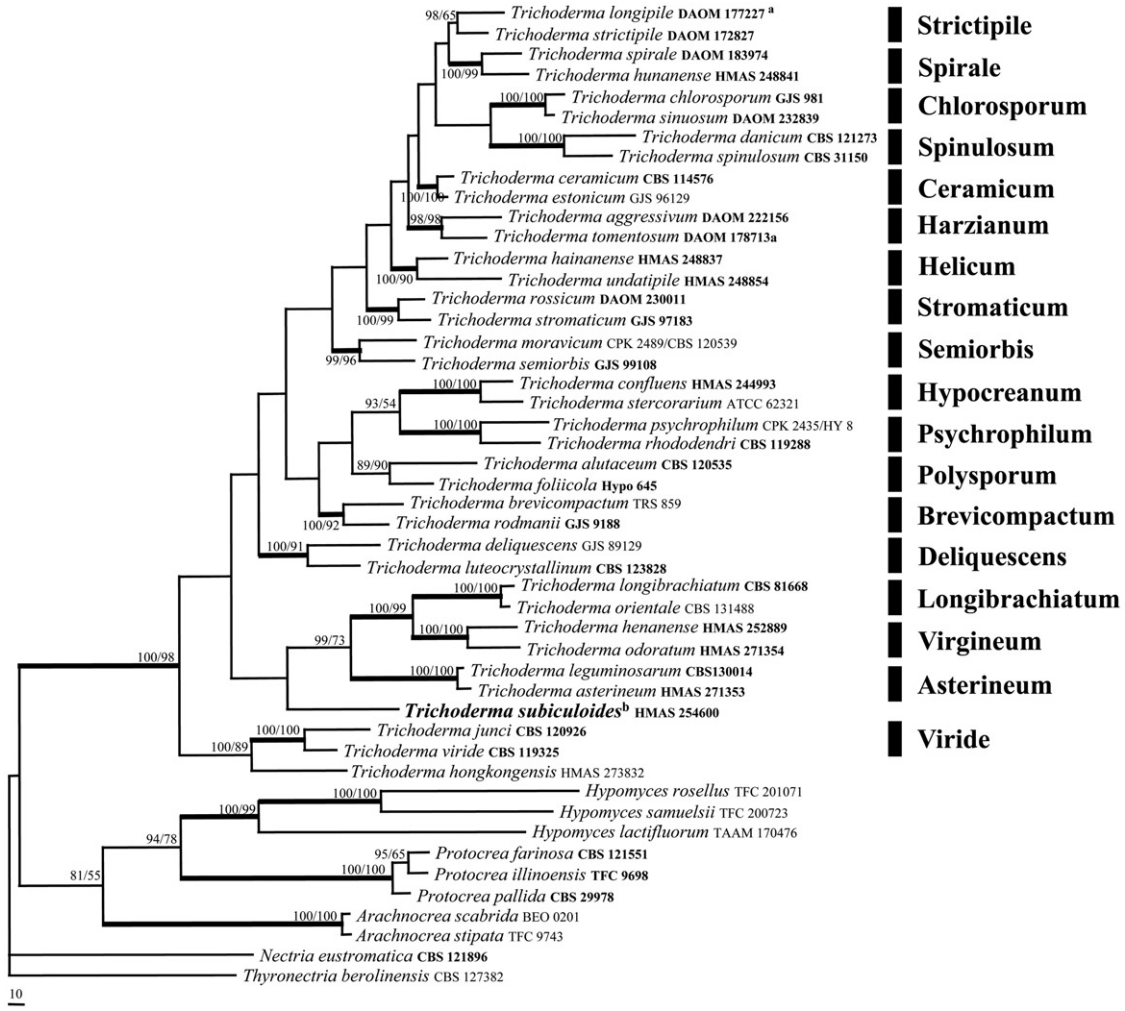

Figure 2. A MP tree generated based on the combined datasets of EF-1α and RPB2 sequences of *Trichoderma* species and relatives. Supporting values showing at branches: MLBP (left) and MPBP (right). MLBP and MPBP greater than 50% are shown at the nodes. The branch support values ≥90 are indicated by thicker lines. Genbank accession numbers in bold indicate the sequences from ex-type strains. The scale bars indicate number of nucleotide substitutions per site.

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 verticillium-like conidiophores. But *H. succineus* differs in occurring on *Pholiota* sp. rather than *Agaricus* sp. and having much larger conidia [(7–)8.8–13.3(–16)×(2.4–)3.3–4.2(–5) µm *vs.* 3–6 × 1–2.3 µm] [15]. *H. tremellicola* grows on *Crepidotus* spp. and has larger conidia [5–9 × 3–4(–5) µm *vs.* 3–6 × 1–2.3 µm] [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 subfusiod, 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

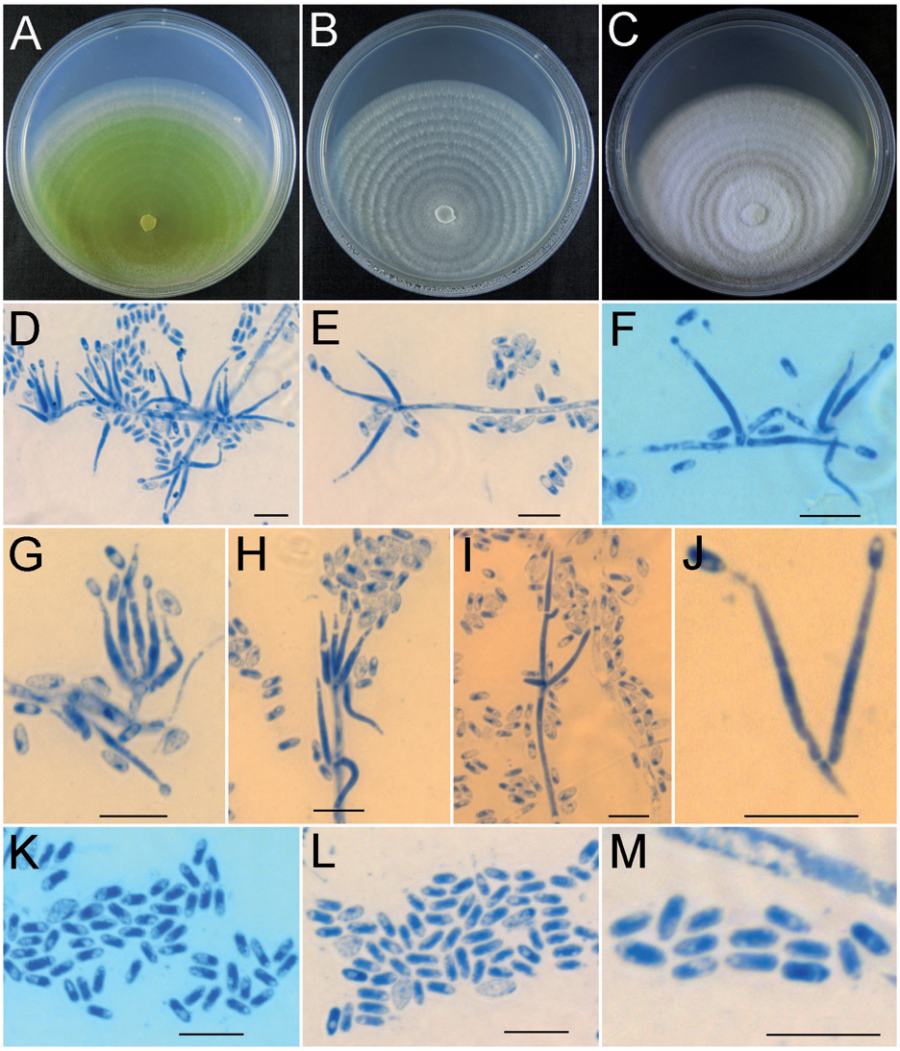

Figure 3. *Hypomyces hubeiensis* (HMAS 254597). (A—C) Cultures after 14 d at 25 °C (A: on CMD, B: on MEA, C: on PDA); (D–I) Conidiophores, phialides, and conidia; (J) Phialides and conidia; (K–M) onidia. Scale bar = 10 µm.

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; subperithecial 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.

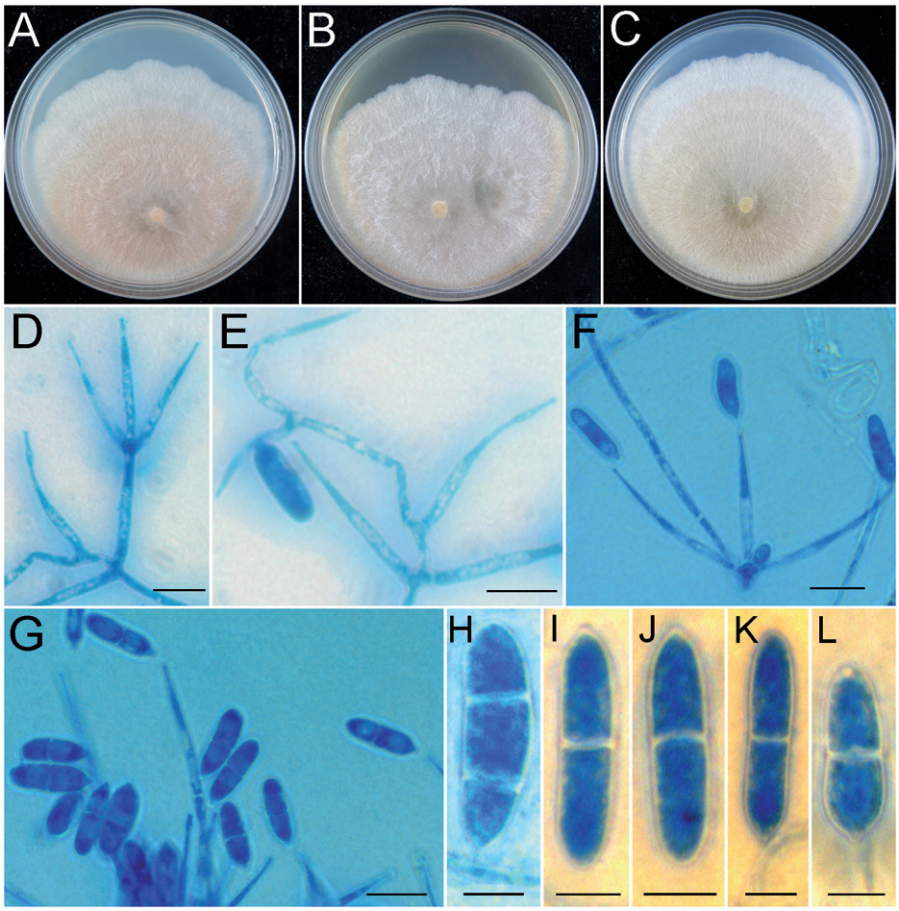

Figure 4. *Hypomyces orthosporus* (HMAS 279649). (A–C) Cultures after 7 d at 25 °C (A: on CMD, B: on MEA, C: on PDA); (D) Conidiophores and phialides; (E–G) Phialides and conidia; (H–L) onidia. Scale bar: D–G = 10 µm; H–L = 5 µm.

Conidiophores arising from aerial mycelium, branched, branches septate, 1–2-verticillate, with the terminal whorl of 2–4 phialides, 15–55 × 2–3.5 µm. Phialides subcylindrical, 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 subiculum-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 perithecia (138–193 × 105–150 µm *vs.* 180–268 × 123–185 µm), ellipsoidal instead of globose, subglobose to nearly wedge-shaped proximal part-ascospores, and the absence of chlamydospores [70]. 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, papulaspora-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

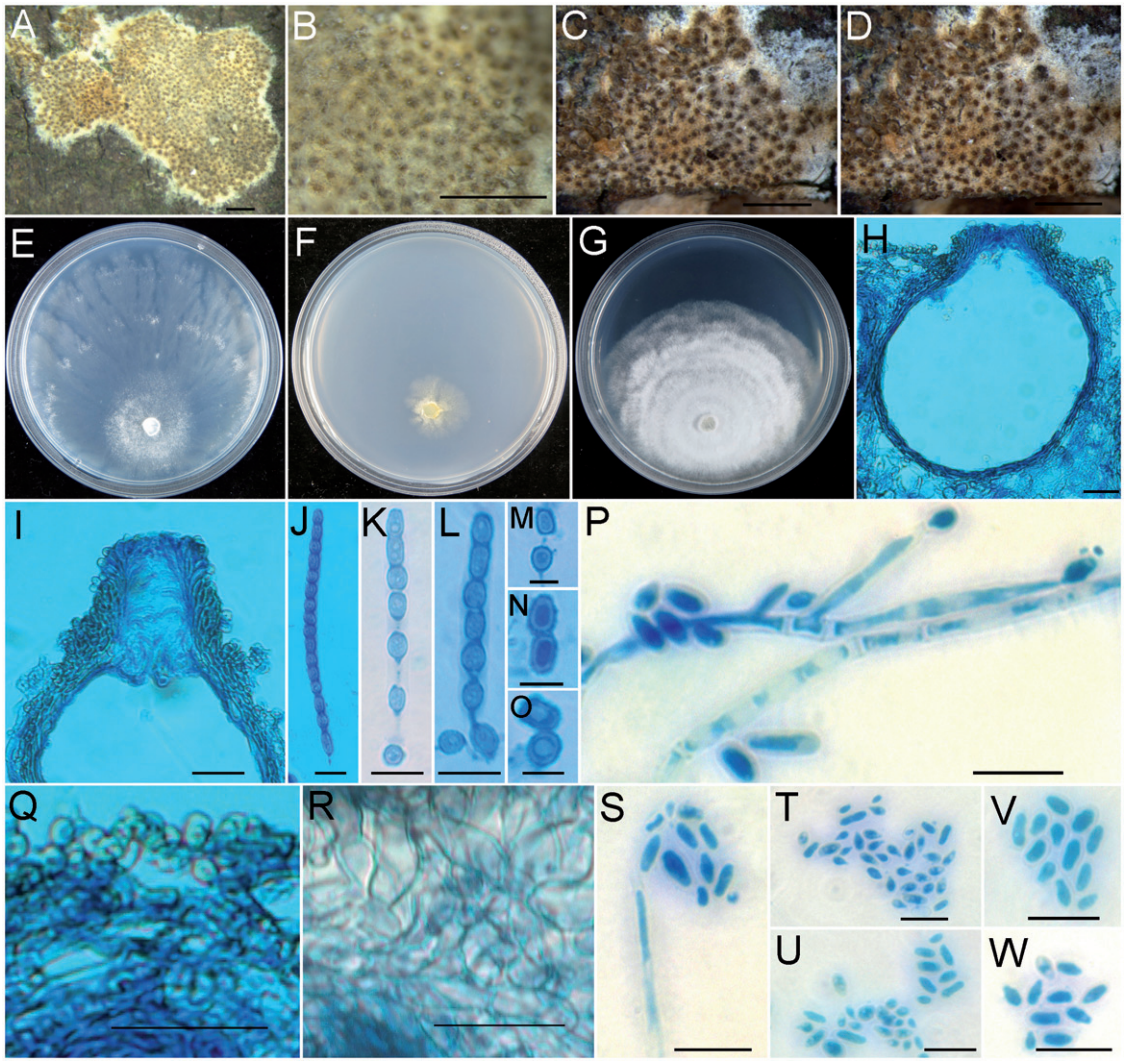

Figure 5. *Trichoderma subiculoides* (HMAS 254600). (A,B) Stroma on nature substrate; (C) Color of stroma after rehydration; (D) Color of rehydrated stroma in 3% KOH; (E–G) Cultures after 14 d at 25 °C (E: on CMD, F: on SNA, G: on PDA); (H) Perithecium in section; (I) Structure of perithecial at upper 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.

asexual states, Rehner and Samuels [9] and Põldmaa 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, turbinate, hemisphericalor 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. alcalifuscescens* (Overton) Jaklitsch & Voglmayr, *T. delicatulum* Jaklitsch and *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

present on the stromatal upper surface and the individual stromata lack of well-developed margin and flanks. Sequence analyses indicated that *T. subiculoides* 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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