Trichoderma (Hypocrea) species with green ascospores from China

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Abstract
Stromata of Trichoderma species having green ascospores were collected in various regions of China. Based on morphology of the sexual and asexual morph, culture characteristics, and sequence analyses of rpb2 and tef1 genes, 17 species with green ascospores were identified. Among them, Trichoderma rosulatum, T. rufobrunneum and T. stipitatum are described as new species, and seven other species are reported for the first time from China. Trichoderma rosulatum produces small bright yellow or pale greenish stromata with dense dark green ostioles and gliocladium-like conidiophores, shows a close relationship to T. thelephorica, and belongs to the Chlorospora clade. Trichoderma rufobrunneum, which typically forms reddish brown stromata, is recognised as a member of the Harzianum clade. Trichoderma stipitatum is characterised by turbinate, pale yellow to nearly orange stromata and verticillium-like conidiophores; it forms a distinct, independent lineage with strong bootstrap support in the phylogenetic trees. The distinctions between the new species and their close relatives are discussed, and their phylogenetic positions are explored.

INTRODUCTION

The sexual morphs of species of Trichoderma have until recently been classified in Hypocrea, a genus now considered to be a later synonym of Trichoderma (Rossman et al. 2013). The stromata of Trichoderma commonly grow on decaying wood and other fungi, rarely on leaves or monocotyledonous substrata. Trichoderma species are economically important, with a wide range of uses, such as biological control of several soil-borne plant pathogens (Samuels 1996, Kovach et al. 2000, Mishra et al. 2000, Cheng et al. 2012), plant growth promotion, induction of plant resistance (Inbar et al. 1994, Gromovich et al. 1998, Yedidia et al. 2001, Hanada et al. 2008), production of industrial enzymes and antibiotics (Reese & Mandels 1989, Russell & Guesdon 1990), bioconversion of domestic wastewater sludge (Molla et al. 2002), and bioremediation (Rigot & Matsumura 2002, Chaverri & Samuels 2003, Harman et al. 2004). In contrast, some species have been identified as causal agents of diseases in immunosuppressed humans (Samuels 1996), and cause economic production losses in commercial mushroom farms (Samuels et al. 2002, Park et al. 2006).

The sexual genus Hypocrea typified by H. rufa has Trichoderma viride as asexual morph (Jaklitsch et al. 2006). It is characterised by perithecia immersed in fleshy stromata and hyaline, 2-celled ascospores disarticulating at the septum within asci (Jaklitsch et al. 2008). Ascospore colour is an important taxonomic character, separating large, phylogenetically distinct groups of species. Species producing green ascospores were monographed by Chaverri & Samuels (2003). Along with the taxonomic treatment of the European green-spored species, Jaklitsch (2009) showed that these species are nested within Trichoderma.

The first record of the sexual morph of Trichoderma from China dates back to 1895 when H. cornu-damae was reported on rotten wood in Sichuan Province (Patouillard 1895). Eleven species were subsequently added (Teng 1934, 1935, 1936, 1963, Tai 1979). After a long break, four new species and six new Chinese records were described and reported (Doi et al. 1984, 1991, Samuels et al. 1998, Liu et al. 2000, 2002, 2003), five more records were further discovered from Taiwan (Wu & Wang 2000, Chang & Wang 2008). Until now, the sexual morphs of 27 species of Trichoderma (as Hypocrea), are known from the country, including eight species possessing green ascospores. Over 40 asexual morphs (recorded as Trichoderma spp.) were found in China, which were mostly isolated from soil, litter and mushrooms (Wen et al. 1992, 1993, Bissett et al. 2003, Zhang & Xu 2004, 2005, Zhao et al. 2004, Zhang et al. 2005, 2007, 2013, Samuels et al. 2006, Sun et al. 2006a, b, 2012, Gao et al. 2007, Yu et al. 2007, 2010, He et al. 2008, 2010, Shao et al. 2008, Wu et al. 2008, Yuan et al. 2008, Jia et al. 2009, Li et al. 2010, 2013, Pan et al. 2010, Jaklitsch et al. 2013).

In this study, all available specimens deposited in the mycological fungarium of the Institute of Microbiology, Chinese Academy of Sciences (HAMAS) and Cryptogamic Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), as well as newly collected materials from Beijing, Anhui, Guangdong, Guangxi, Guizhou, Hainan, Henan, Hunan, Jilin, Liaoning and Yunnan provinces were examined. In addition to morphological observations, ITS nrDNA (ITS) and genes of RNA polymerase II subunit B (rpb2) and transcription elongation factor 1 alpha (tef1) were analysed to aid in species identification. Seventeen species with green ascospores, including three new species and seven new Chinese records were recognised. Distinctions between the new species and closely related fungi and their phylogenetic relationships are discussed.

An important change in the International Code of Nomenclature for algae, fungi and plants (ICN) was made at the International Botanical Congress in Melbourne in July 2011: from 1 January 2013 only one official name is allowed for each pleomorphic
fusus. Following the current Code and the decision made by the International Subcommission on Trichoderma and Hypocrea Taxonomy, the asexual name *Trichoderma* (1794) is recommended as preferable over the sexual name *Hypocrea* (1825) (Rossman et al. 2013). The generic name *Trichoderma* is here used to treat the holomorphic new species described in this paper. However, no name changes are proposed for previously published *Hypocrea* species by different authors.

**MATERIALS AND METHODS**

**Isolates and specimens**

Ascospore isolates were prepared as described by Jaklitsch (2009), except that the suspension of ascospores was transferred to water agar plates (2 %). The specimens examined were deposited in the HMAS and HKAS, and isolates were kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences.

**Growth rate and morphology**

Radial growth rates on potato dextrose agar (PDA), commeal agar (Disco) + 2 % (w/v) dextrose (CMD) and synthetic low nutrient agar (SNA; Nirenberg 1976) were measured, and morphology of sexual and asexual morphs was described with the methods of Jaklitsch (2009). Photographs were taken using a Canon G5 digital camera (Tokyo, Japan) connected to a Zeiss Axioskop 2 Plus microscope (Göttingen, Germany) for anatomical structures and to a Zeiss Stemi 2000C stereomicroscope for gross morphology.

**DNA extraction, PCR amplifications and sequencing**

Mycelium was harvested from colonies grown on PDA 1–3 wk. DNA was extracted using the CTAB method as described by Wang & Zhuang (2004). Complete ITS was amplified using primers ITS5 and ITS4 (White et al. 1990). A 1 kb fragment of the *rpb2* gene was amplified using the primer pair RRPB2-5f and RRPB2-7cr (Liu et al. 1999). A 1.3 kb fragment of the *tef1* gene was amplified using the primer pair EF1728F (Chaverri & Samuels 2003) and TEF1LLErev (Jaklitsch et al. 2005). The *rpb2* gene was amplified using the primer pair EF1728F (Chaverri & Samuels 2003) and TEF1LLErev (Jaklitsch et al. 2005). PCR was performed with an ABI 2720 Thermal Cycler (Gene Co. Ltd., Foster City, California, USA) at Shanghai Majorbio Bio-pharm Technology Co., Ltd., Beijing Branch, China. Sequences generated from this study and those retrieved from GenBank are listed in Table 1.

**Phylogenetic analyses**

All sequences were aligned with ClustalX v. 1.8 (Thompson et al. 1997), and the alignments were visually adjusted where necessary with BioEdit v. 7.0.5 (Hall 1999). A partition homogeneity test (PHT) was performed with 1 000 replications via PAUP v. 4.0b10 (Swofford 2002) to evaluate statistical congruence between sequence data of *rpb2* and *tef1* gene regions. *Nectria eustromatica* and *N. berolinensis* were selected as outgroup taxa.

To reveal the phylogenetic affiliation of the sampled fungi, maximum parsimony (MP) analysis was conducted by a heuristic search via PAUP v. 4.0b10 with the following settings: all characters were equally weighted, gaps were treated as missing data, starting trees were obtained by random taxon addition with 1 000 replications, the branch-swapping algorithm was tree-bisection-reconnection (TBR), steepest descent option and MulTrees option were not in effect.

The Bayesian inference (BI) approach to phylogenetic reconstructions (Rannala & Yang 1996, Yang & Rannala 1997) was implemented with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The appropriate models of sequence substitution were tested in the JModelTest v. 0.1.1 (Posada 2008) software package. The Akaike Information Criterion (AIC) was used to select for best fit models after likelihood score calculations were done. The base tree for likelihood calculations was ML-optimized. The model selected for combined *rpb2-tef1* was GTR+I+G. Two concurrent analyses of four chains (one cold and three heated) were both run for 5 million generations sampling every 100th tree until the average standard deviation of the split frequencies dropped below 0.01. The trees obtained before convergence was reached were discarded using the burn-in command, and the remaining trees were used to compute the consensus trees and to estimate Bayesian inference posterior probability (BIPP) values. According to the protocol of Leache & Reeder (2002), BIPP values lower than 0.95 were not considered significant while values below 0.90 were not shown on phylograms. Trees were examined in TreeView v. 1.6.6 (Page 1996). Maximum parsimony bootstrap proportions (MPBP) greater than 50 % and BIPP above 90 % are shown at the nodes.

**RESULTS**

**DNA phylogeny**

In the molecular phylogenetic analyses, the partition homogeneity test (P = 0.01) indicated that the individual partitions were not highly incongruent (Cunningham 1997), and thus *rpb2* and *tef1* sequences were combined for sequence analyses. The combined *rpb2* and *tef1* sequence data have been shown to be reliable in species delimitation of *Trichoderma* (Chaverri & Samuels 2003, Jaklitsch 2009) and were therefore used in this study. As was shown by Samuels et al. (2006), ITS is not suitable for a phylogenetic reconstruction of the group, due to a low number of variable sites and long insertions in certain species. Therefore, ITS sequences were not incorporated into the phylogenetic analyses. But they were useful as species identification criterion applied to *Trichoderma* on the website of www.ISTH.info (Druzhinina et al. 2005).

Maximum parsimony analyses of the combined datasets of 45 species (including three novel ones) produced 126 most parsimonious trees with similar topology. Among the 1 518 characters, 437 were parsimony informative, 133 were variable and parsimony-uninformative, and 948 were constant. Fig. 1 shows one of the trees (tree length = 2 116; consistency index = 0.3965; homoplasy index = 0.6035; retention index = 0.6823; rescaled consistency index = 0.2705).

As determined previously, eight clades containing most green-spored species of *Trichoderma* were named, i.e. Ceramica, Chlorospora, Harzianum, Spolinosa, Strictipilosa, Semiorbis, Virescentiflava and Virens. *Trichoderma aureoviride* and *T. candidum* form an independent lineage with high statistical support, which still remains unnamed (Chaverri & Samuels 2003, Jaklitsch 2009). As shown in our phylogenetic tree (Fig. 1), the green-spored species investigated formed a monophyletic group (100 % MPBP/BIPP), which is basically consistent with the previous study by Jaklitsch (2009). Due to the sequence...
differences and morphological distinctions of *T. phyllostachydis* and *T. stipitatum*, two new clades, Phyllostachydis and *Stipi-

tatum*, are herein proposed.

In our phylogenetic analyses, the Chinese *Trichoderma* species with green ascospores fell in six clades (Fig. 1). For the new taxa, their phylogenetic positions among the known species were explored. Two strains of *T. rufobrunneum*, HMAS 252547 and 266614 were located in the Harzianum clade and clearly separated from any other known taxa of this clade. The two samples of *T. stipitatum*, HMAS 266612 and 266613, constituted a well-supported independent lineage (MPBP/BIPP = 100% / 100%). The Chlorospora clade contained seven species;

Table 1 Materials including strain numbers and GenBank accesses of sequences used for phylogenetic analyses.

| Species | Strain | GenBank accession number | ITS | rpb1 | tef1 |
|---------|--------|--------------------------|-----|------|------|
| Hypocreatastrogleatinosa | G.J.S. 95-159 | – | AF545508 | AF534603 |
| H. costaricensis | P.C. 21 | AY373754 | AY391921 | AY391980 |
| H. danica | CBS 121273 | FJ860769 | FJ860534 | FJ860634 |
| H. liti | G.J.S. 90-22 | AF443915 | AY391925 | AY391984 |
| H. spinosula | CBS 121280 | FJ860699 | FJ860589 | FJ860699 |
| H. virescensflava | P.C. 278 | AY373768 | AY391999 | AY392007 |
| Trichoderma aereum (as *H. aeruginea*) | CBS 120541 | FJ860720 | FJ860516 | FJ860608 |
| T. aggressivum | CBS 100525 | – | AF545541 | AF534614 |
| T. alni (as *H. alni*) | CBS 120633 | EU518651 | EU498349 | EU498312 |
| T. aureoviride (as *H. aureoviridis*) | C.P.K. 2848 | FJ860733 | FJ860523 | FJ860615 |
| T. brunneoviride (as *H. brunneoviridis*) | CBS 120928 | EU518661 | EU498358 | EU498318 |
| T. candidum (as *H. candida*) | P.C. 59 | AY377757 | AY391899 | AY391962 |
| T. catoptron (as *H. catoptron*) | G.J.S. 02-76 | AY377766 | AY391900 | AY391963 |
| T. cerasceum (as *H. cerasceum*) | CBS 125253 | – | KF923310 | KF923290 |
| T. cerasium | CBS 120637 | FJ860744 | FJ860532 | FJ860629 |
| T. chlorosporum (as *H. chlorospora*) | G.J.S. 88-33 | – | AY391903 | AY391966 |
| T. clomosporum (as *H. clomosporum*) | G.J.S. 94-68 | – | AY391913 | AY391974 |
| T. phyllostachydis | CBS 252535 | FJ863004 | FJ863005 | FJ863006 |
| T. rosulatum | CBS 252536 | FJ862997 | KF730009 | KF729988 |
| T. rufobrunneum | CBS 252537 | KF729997 | KF730009 | KF730004 |
| T. stipitatum | CBS 252538 | KF729998 | KF730010 | KF729989 |
| T. thailandicum (as *H. thailandicum*) | CBS 252547 | KF729999 | KF730007 | KF729992 |
| T. tawaka (as *H. tawaka*) | CBS 252548 | KF729996 | KF730003 | KF729983 |
| T. thallicamum (as *H. thallicamum*) | CBS 252549 | KF729994 | KF730006 | KF729985 |
| T. eukamphilephorica (as *H. eukamphilephorica*) | CBS 120925 | FJ860658 | FJ860600 | FJ860711 |
| T. tropicosinense | CBS 252546 | KF923302 | KF923313 | KF923286 |
| T. virescens (as *H. virescens*) | G.J.S. 90-88 | – | AY391932 | AY391990 |
| Nectriaeuromatica | CBS 121896 | HMAS54886 | HMAS54886 | HMAS54875 |
| N. berolinensis | CBS 127382 | HMAS54897 | HMAS54883 | HMAS54872 |

1 Numbers in **bold** indicate newly submitted sequences.
T. rosulatum (HMAS 252548), a member of this clade, was closely related (MPBP = 100 %) to, but distinct from, T. thelephorica. As to the Phyllostachydis clade, the Chinese collection of T. phyllostachydis (HMAS 244842) and the ex-type culture (G.J.S. 92-123) formed a clade with 100 % MPBP/BIPP. The collections of T. chromospermum from China and the ex-type culture from the USA (G.J.S. 94-68) clustered with 100 % MPBP/BIPP. Although the two Chinese isolates of T. sinuosum (HMAS 252541, 252542) and C.P.K. 1595 from Austria and G.J.S. 90-88 from America appear to be the same species, the statistical supports for the branch was not very high (MPBP/BIPP = 78 %/100 %). Trichoderma tropicosinense (HMAS 252546) was located in the Strictipilosa clade, and closely associated with T. longipile and T. strictipile.

Taxonomy
A total of 136 specimens of Trichoderma sexual morphs were collected from 18 provinces of China during 1957–2012. Judging by morphological features and phylogenetic analyses, 49 species were identified, including 17 green-ascospores species and 32 hyaline-ascospores ones. This study focuses on the green-spored species. Among the 17 green-spored species, three species are new to science, and seven are new to China (T. catoptron, T. ceramicum, T. chromospermum, T. cremeum, T. phyllostachydis, T. pseudocandidum and T. sinuosum) and seven were previously reported (T. aureoviride, T. gelatinosum, T. longipile, T. strictipile, H. cupularis, H. lixii and T. tropicosinense). Their distribution in China is shown in Map 1.

Map 1 The distribution of the known Trichoderma species with green ascospores from China. H. cupularis (●); H. cf. flavovirens Berk. (●); H. lixii (●); T. aureoviride (●); T. catoptron (●); T. ceramicum (●); T. chromospermum (●); T. cremeum (●); T. gelatinosum (●); T. longipile (●); T. phyllostachydis (●); T. pseudocandidum (●); T. rosulatum (●); T. rufobrunneum (●); T. sinuosum (●); T. stipitatum (●); T. strictipile (●); T. tropicosinense (●).
New species

Trichoderma rosalatum Z.X. Zhu & W.Y. Zhuang, sp. nov. — MycoBank MB805906; Fig. 2, 3

Etymology: The specific epithet refers to the rosalate colony on CMD.

Stromata gregarious or densely aggregated, pulvinate or turbinate, narrowly attached and margin free, outline circular or angular, white with green ostioles when fresh, bright yellow or pale greenish with dark green ostioles when dry, c. 0.5–1.5(–2) mm diam, 0.4–0.6 mm thick (n = 40); surface flat, concave or with depressed centre, conspicuously coarsely tubercular due to projecting perithecia; ostioles generally distinct, mostly slightly papillate, dark green when mature. Rehydrated stromata larger than dry ones, surface smooth, slightly orange in 3% KOH.

In section, stroma cortical tissues of textura angularis in face view, cells thin-walled, hyaline, (10–)14–17(–20) × (7–)10–13(–15) μm (n = 30); cortical and subcortical tissues in vertical section comprising a textura angularis with thin-walled, hyaline to pale yellowish, angular to globose cells (6–)8–12(–16) × 5–6(–8) μm (n = 40), not changing colour in KOH; subperithecal tissues of textura angularis to textura epidermoidea, cells thin-walled, hyaline, (10–)15–17(–20) × (7–)9–13(–15) μm (n = 40); tissues of stroma basal portion of textura intricata, hyphae thick-walled, hyaline, 2–4.5 μm wide (n = 40). Perithecia flask-shaped or globose, crowded, (160–)197–236 × (134–)170–220 μm (n = 40); ostioles projecting to 26 μm, 38–52(–76) μm wide at apex, (36–)42–65(–80) μm high (n = 20); peridium hyaline, not changing colour in KOH, (6–)10–15(–18) μm thick at the sides, (12–)15–22(–26) μm at the base (n = 40). Asci cylindrical, (84–)88–93(–97) × 5–5.5(–6.5) μm, including a (7–)10–16 μm long stipe (n = 40). Part-ascospores green, becoming brown in KOH, verrucose, dimorphic, distal cell (subglobose-)globose, 4–4.5 × 3.5–4 μm, l/w 1.0–1.1(–1.3); proximal cell ellipsoidal to oblong, 4.5–5(–6.5) × 3–4.5 μm, l/w 1.3–1.6 (n = 60).

Culture characteristics — Colony slow-growing, optimum growth at 25 °C, not growing at 35 °C on all media. On CMD after 72 h 4–6 mm at 15 °C, 14–17 mm at 25 °C, 9–11 mm at 30 °C; mycelium covering the plate after c. 1 mo at 25 °C. Colony conspicuously dense, whitish, up to four floccose zones of irregular outline. Aerial hyphae numerous but loosely disposed, without defined orientation, denser along the rings. No autolytic excretions, no pigment noted within 1 mo. Weak coconut-like odour formed at all temperatures. Conidiation noted after 1 mo, forming in one or two nearly continuous rings around the original inoculum, green, no sterile elongations seen. Conidiophores mostly 1.8–2.5 μm wide, glocladium-like; phialides solitary or in whorls of 2–3(–4), lageniform to subulate, equilateral in the middle of a whorl, otherwise inequilateral, curved, thickest at variable position, 6–16 × 2–2.5 μm, l/w (2–)4–5(–7.5) (n = 60). Conidia light green, smooth, ovoid to ellipsoidal, (3.5–)4–4.5(–6.5) μm.
Fig. 2 Sexual morph of Trichoderma rosulatum (HMAS 252548). a. Fresh stromata on nature substrate; b. dry stromata on nature substrate; c–k. dry stromata at different development stages (c, d. immature; e–k. mature); l. mature stromata after rehydration; m. mature stroma in 3% KOH after reconstitution in water; n. longitudinal section of a stroma; o. perithecium in section; p. structure of ostiole; q. structure of cortical and subcortical tissue; r. structure of subperithecial tissue; s. structure of stroma at basal portion; t. an ascus with part-ascospores; u. part-ascospores. — Scale bars: a = 3 mm; b = 1 mm; c = 0.4 mm; d, f, h–m = 0.2 mm; e, g = 0.5 mm; n = 100 μm; o = 30 μm; p–s = 20 μm; t = 5 μm; u = 3 μm.
Fig. 3 Cultures and asexual morph of *Trichoderma rosulatum* (HMAS 244906). a–c. Cultures at 25 °C after 27 d (a. on CMD; b. on PDA; c. on SNA); d, e. conidiation tuft (CMD, 40 d); f–n. conidiophores (CMD, 40 d); o, p. chlamydospores (CMD, 40 d); q, r. conidia (CMD, 40 d). — Scale bars: a–c = 20 mm; d = 0.2 mm; e = 0.1 mm; f–n = 10 μm; o–r = 5 μm.
Fig. 4 Sexual morph of *Trichoderma rufobrunneum*. a–i. Dry stromata on nature substrate; j. perithecia in section; k. structure of ostiole; l. structure of sub-perithecial tissue; m, n. an ascus with part-ascospores (a–e, j–n. HMAS 252547; f–i. HMAS 266614). — Scale bars: a–c, f = 0.2 mm; d, e, g–i = 0.5 mm; j, k = 30 μm; l = 50 μm; m, n = 10 μm.
2.5–3 μm, l/w 1.3–2.0 (n = 60). *Chlamydospores* observed after 42 d, globose, ellipsoidal or pyriform, terminal and intercalary, 7–12.5 × 7–10.5 μm, l/w 1.0–1.2 (n = 40).

On PDA after 72 h no growth at 15 ºC, 2–3 mm at 25 ºC and 30 ºC; after 2 wk at 25 ºC 32–35 mm; mycelium not reaching the distal margin or covering the plate after more than 2 mo at 25 ºC. Colony irregular to lobed, dense, margin loose and hyaline, surface whitish downy. *Aerial hyphae* inconspicuous, no conidiophores or chlamydospores seen after 2 mo.

On SNA growth extremely slow; after 72 h no growth at 15 ºC, 2–3 mm at 25 ºC and 30 ºC, after 2 wk at 25 ºC 1 cm. Colony hyaline, thin, with circular outline. *Aerial hyphae* scant, forming radial strands. *Conidiophores* scarce, concentrated around the plug.

*Specimen examined.* China, Anhui, Jinzhai, Tiantangzhai, alt. 900–1000 m, on rotten bark, 22 Aug. 2011, S.L. Chen, W.Y. Zhuang, H.D. Zheng & Z.Q. Zeng 7752 (holotype HMAS 252548, culture ex-type HMAS 244906).
Notes — Among the known species of Trichoderma, the stromata of T. rosulatum look much like those of T. sinuosum, H. costaricensis, T. chlorosporum and T. thelephorocolla in bright yellow projecting perithecia, particularly when young. However, T. sinuosum and H. costaricensis differ from T. rosulatum in having larger asci and much larger ascospores (Chaverri & Samuels 2003, Jaklitsch 2009); T. chlorosporum is distinct by the nearly monomorphic and larger ascospores (Chaverri & Samuels 2003); T. thelephorocolla has glaucoid-like conidiophores but differs in perithecial walls turning orange in KOH solution and occurring only on or associated with basidiomata of Steccherinum ochraceum (Jaklitsch 2009). Trichoderma rosulatum is distinguishable by the combination of slow growth and production of abundant chlamydospores in culture (CMD).

Phylogenetic analyses of rpb2 and tef1 partial sequences indicate that T. rosulatum belongs to the Chlorospora clade, where it is distinct from its morphologically similar species. Although T. rosulatum and T. thelephorocolla formed a well-supported terminal branch (Fig. 1, 99 % MPBE), sequence similarity of ITS between them was only 94.2 % with 34 bp different out of 589 bp.

Trichoderma rufobrunneum Z.X. Zhu & W.Y. Zhuang, sp. nov. — MycoBank MB605907; Fig. 4, 5

Etymology. The specific epithet refers to the colour of the ascomata.

Stromata solitary or gregarious, flat pulvinate, outline circular or angular, discoid to turbinate, sometimes undulate, centrally attached, margin or large part of stroma free, dark reddish brown when dry, c. 2–5(–7) mm diam, 1–3 mm thick (n = 10); surface flat, concave or with depressed centre, finely tubercular or rugose when mature; ostioles typically hardly visible, mostly slightly papillate, brown, turning dark green to nearly black when mature. Rehydrated stromata larger than dry ones, surface smooth, and no colour change in 3 % KOH.

In section, cortical layer covering the entire stroma except for the point of attachment, glabrous, 13–26 µm (n = 20) thick, comprising a textura angularis with thick-walled, yellow to orange-brown, angular to oblong to cylindrical, (86–)92–101(–105) × 6–6.5(–7.5) µm, including a 13–18(–20) µm long stipe (n = 40). Part-ascospores green, becoming brown in KOH, verrucose, dimorphic, distal cell subglobose to ellipsoid, (3–)4–5(–6) × (3.5–)4–5(–5.5) µm, l/w 1.0–1.2; proximal cell wedge-shaped to oblong, (4–)4.5–5(–6) × 3–3.5(–4) µm, l/w 1.3–1.5 (n = 40).

Culture characteristics — Growth optimum at 25 °C and no growth at 35 °C on all media. On CMD after 72 h 18–20 mm at 15 °C, 40–50 mm at 25 °C, 24–28 mm at 30 °C; mycelium covering the plate after 3–4 d at 25 °C. Colony outline irregular, margin wavy to lobed. Aerial hyphae and autolistic activity nearly absent. Pustules at first white, becoming green after 3–4 d or later, compact to cottony, pulvinate to hemispherical, 0.5–4 mm diam, 0.5–2 mm high. No pigment, no distinct odour noted. Conidiation noted after 2 d, starting on short shrubs or microtufts close to the proximal margin. Conidiophores 2.8–3.5 µm wide, verticillium-like. Phialides solitary or divergent in whorls of mostly three, lageniform, straight and symmetrical, (6–)8–10(–12.5) × 2–3 µm, l/w (2–)3–4(–5) (n = 60). Conidia green, smooth, subglobose, ellipsoid or ovoid, (2.5–)3–4(–5.5) × 2.5–3(–4) µm, l/w 1.0–1.3 (n = 60). Chlamydospores not observed.

On PDA after 72 h 15–17 mm at 15 °C, 31–35 mm at 25 °C, 18–22 mm at 30 °C, mycelium covering the plate after 3–4 d at 25 °C. Colony flat, dense; surface whitish downy. Aerial hyphae numerous, thin, complexly branched, forming radial strands. Conidiation noted from 2–3 d, green after 3 d, abundant, starting on and around the plug, on short, erect, straight to sinuous conidiophores on surface hyphae of flat central spots or tufts; also on aerial hyphae, with long straight phialides and short branches.

On SNA, after 72 h 10–13 mm at 15 °C, 21–25 mm at 25 °C, 15–18 mm at 30 °C. Colony hyaline, thin, similar to CMD. Aerial hyphae scant, forming radial strands.

Specimens examined. CHNA, Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten wood, 24 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8155 (holotype HMAS 252547, culture ex-type HMAS 244907); Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten twig, 23 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8084, HMAS 266614.

Notes — There are four known species with green ascospores and reddish brown stromata in the Harzianum clade, i.e. T. alni, T. brunneoviride, T. epinymyes and T. parepinymyes. Trichoderma rufobrunneum is most similar to T. alni in colour of stromata with inconspicuous ostiolar dots when dry, but the latter differs in smaller ascii, (67–)80–96(–113) × (4.0–)4.5–5.5(–6.5) µm, longer phialides, (8–)9–15(–18) µm, positive reaction of stromata to KOH, and commonly occurring on wood and bark of Alnus glutinosa (Jaklitsch 2009). The phylogenetic analyses based on rpb2 and tef1 reveal that T. rufobrunneum is related to T. alni and T. aggressivum (Fig. 1). However, 36 bp and 27 bp (HMAS 252547, HMAS 266614) differences were found from T. alni and T. aggressivum, respectively.

Trichoderma stipitatum Z.X. Zhu & W.Y. Zhuang, sp. nov. — MycoBank MB605908; Fig. 6, 7

Etymology. The specific epithet refers to the short stipe at the stroma base.

stromata scattered, gregarious or densely crowded, turbinate or short-stipitate, seated on a thick effused whitish subculicium, centrally attached and margin free, white to cream-coloured when young, pale yellow to nearly orange with olivaceous to dark green ostioles at maturity, c. 0.8–1.5 mm diam, 0.6–1.0 mm thick (n = 40); surface smooth, flat to slightly convex when young, finely tubercular and covered by ample dark green spore deposits when mature; ostioles at first diffuse, then distinctly projecting. Rehydrated stromata orange-brown in 3 % KOH (due to the orange-red peridium).

In section, cortical layer glabrous, (18–)26–30(–39) µm (n = 40) thick, of textura angularis; cells thin-walled, pale yellowish, angular to oblong, orange-brown in KOH, 15–23 × 12–17 µm (n = 30) in face view; (8–)10–14(–17) × 5–7(–10) µm (n = 40) in vertical section; subcortical tissues of textura angularis; cells loose, thin-walled, subhyaline to pale yellowish, (4–)8–11–18 (n = 40); subcortical tissues of textura angularis; cells thick-walled, subhyaline to pale yellowish, (10–)15–26–30 (n = 6–9–15–20) µm (n = 30); cells at base of stroma forming at basal portion of textura intricata, hyphae thick-walled, (2–)3–5.5(–6.5) µm wide (n = 40). Perithecia flask-shaped or globose, crowded, 210–289 × 131–171 µm (n = 40); ostioles not emerging through the cortex or projecting to 18–(31) µm, 39–53 µm wide at apex. (54–)62–79(–105) µm high (n = 20); peridium yellow to nearly orange, (4–)5–10(–12) µm thick at the sides, (6–)8–11–14 µm at the base (n = 40). Asci cylindrical, (86–)92–101(–105) × 6–6.5(–7.5) µm, including a 13–18(–20) µm long stipe (n = 40). Part-ascospores green, becoming brown in KOH, verrucose, dimorphic, distal cell subglobose to ellipsoid, (3–)4–5(–6) × (3.5–)4–5(–5.5) µm, l/w 1.0–1.2; proximal cell wedge-shaped to oblong, (4–)4.5–5(–6) × 3–3.5(–4) µm, l/w 1.3–1.5 (n = 40).

Culture characteristics — Growth optimum at 25 °C and no growth at 35 °C on all media. On CMD after 72 h 18–20 mm at 15 °C, 40–50 mm at 25 °C, 24–28 mm at 30 °C; mycelium covering the plate after 3–4 d at 25 °C. Colony outline irregular, margin wavy to lobed. Aerial hyphae and autolistic activity nearly absent. Pustules at first white, becoming green after 3–4 d or later, compact to cottony, pulvinate to hemispherical, 0.5–4 mm diam, 0.5–2 mm high. No pigment, no distinct odour noted. Conidiation noted after 2 d, starting on short shrubs or microtufts close to the proximal margin. Conidiophores 2.8–3.5 µm wide, verticillium-like. Phialides solitary or divergent in whorls of mostly
Fig. 6 Sexual morph of Trichoderma stipitatum (HMAS 266613). a–d, f–i, k. Dry stromata at different development stages (a–c. stroma initials; d–g. immature; h, i, k. mature); e. immature stromata after rehydration; j. whitish subiculum; l, m. mature stromata after rehydration; n. mature stroma in 3% KOH after reconstitution in water; o. longitudinal section of a stroma; p. perithecium in section; q. structure of ostiole; r. structure of cortical and subcortical tissue; s. structure of subperithecial tissue; t. structure of stroma at basal portion; u, v. an ascus with part-ascospores. — Scale bars: a–c, j, m = 0.1 mm; d, h, k = 1 mm; e, g, l, i = 0.5 mm; f, n = 0.2 mm; o = 100 μm; p, s, t = 30 μm; q = 20 μm; r, u, v = 10 μm.
Fig. 7 Cultures and asexual morph of *Trichoderma stipitatum* (HMAS 244908). a–c. Cultures at 25 °C after 10 d (a. on CMD; b. on PDA; c. on SNA); d. culture showing granular pustules on surface (CMD, 6 d); e–q. conidiophores (CMD, 10 d); r–t. conidia (CMD, 10 d). — Scale bars: a–c = 20 mm; d = 10 mm; e = 30 μm; f–q = 10 μm; r–t = 5 μm.
bright yellow, brownish in KOH, (4–)5–10–(12) μm thick at the sides, (9–)12–16–(20) μm at the base (n = 40). Asci cylindrical, (69–)79–89–(97) × 5–6–(7.5) μm, including a 10–18 μm long stipe (n = 40). Part-ascospores green, becoming brown in KOH, verrucose, dimorphic, distal cell subglobose, (3.5–)4–(5.5) × 3.5–4–μm, l/w 1.0–1.1–(1.3); proximal cell wedge-shaped to oblong, 4–5–(6.5) × 3–(4.5) μm, l/w 1.3–1.6 (n = 60).

Culture characteristics — Growth optimum at 25 °C, no growth at 35 °C on all media. On CMD after 72 h 3–5 mm at 15 °C, 18–20 mm at 25 °C, 16–17 mm at 30 °C; mycelium covering the plate after c. 2 wk at 25 °C. Colony circular, with dense small white tufts 0.3–0.5 mm, concentrically zonate; margin wavy. Aerial hyphae inconspicuous, erect, becoming fertile. Pustules formed in concentric rings, first white, turning tandemly and faintly yellow-greenish, with discontinuous surface, densely granular by densely disposed. No autolytic excretions seen, collings inconspicuous. No pigment noticeable within 3 wk. Slight coconut-like odour formed at 25 °C and 30 °C. Conidiation noted after 2–3 d, light (yellowish) green after 13 d, no sterile elongations seen. Conidiospores mostly 4–5.5 μm wide, verticillium-like; phialides solitary or in whorls of 2–3, slender, tapering towards the tip, (10–)14–19–(21) × 2–3 μm, l/w (3–)4.5–6–(7) (n = 60). Conidia light yellowish green, smooth, subglobose, ellipsoidal or ovoid, often attenuated toward one end, 3–5–(6) × 2.5–3–(3.5) μm, l/w 1.2–1.3 (n = 60). Chlamydospores not observed.

Notes — Morphologically, the new species is most similar to T. chromosperum in ascospore shape and size and positive reaction of stromata to KOH; while the latter fungus has pulvinate stromata, much longer phialides ((5.8–)6.9–14.5–(22.5) μm), and relatively large conidia ((3.5–)4.0–6.5–(7.5) × (2.5–)3.7–3.8–(4.0) μm). Similar to T. stipitatum, the stromata of H. substipitata are also seated on a thick effused whitish subiculum; however, the latter obviously differs in its short-cylindrical and larger stromata (2.3–5 mm diam), which do not react to KOH, larger perithecia (231–257–321–(325) × (176–)195–231–(292) μm), much longer asci (184–)190–108–(120) μm) and larger distal and proximal ascospores (4.3–5.0–5.7–(6.0) × (3.5–)3.8–5.0–(6.0) μm; (4.7–)5.2–6.5–(7.0) × (3.0–)3.5–4.2–(5.0) μm; Chaverri & Samuels 2003).

Although T. thelephorica, H. costaricensis, T. sinuosum and T. chlorosporum in the Chlorospora clade are also morphologically similar to T. stipitatum, sequence analyses revealed that they are distantly related, belonging to different clades.

**New records for China**

**Trichoderma catoptron** P. Chaverri & Samuels, Stud. Mycol. 48: 43. 2003

= Hypocrea catoptron Berk. & Broome, J. Linn. Soc., Bot. 14 (no. 74): 112. 1873 (1875).
Specimens examined. CHINA, Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on bark, 24 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8158, HMAS 252533.

**Trichoderma ceramicum** P. Chaverri & Samuels, Stud. Mycol. 48: 47. 2003

= Hypocrea ceramicus Ellis & Everh., N. Amer. Pyrenomyc.: 85. 1892.
Specimens examined. CHINA, Hainan, Ledong, Jianfengling, alt. 1000 m, on twigs, 10 Dec. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3778, HMAS 266598; Hainan, Lingshi, Diaoloushan, alt. 1050 m, on twig, 14 Dec. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3839, HMAS 266598; dinhtrie, Jilin, Jiaohe, Qianjin Forestry Farm, alt. 1000 m, on rotten wood, 24 July 2012, W.Y. Zhuang, Z.X. Zhu & Z.Q. Zeng 8156, HMAS 266605, culture HMAS 252538; Jilin, Jingyuetan, alt. 1100 m, on twig, 5 Nov. 1999, W.Y. Zhuang & Z.H. Yu 3335, HMAS 73226, Zhejiang, Lian’an, Tianmushan, alt. 1100 m, on twig, 9 Sept. 1957, S.Q. Deng 5431, HMAS 26796.

Notes — This is a common species in China. The fungus is characterised by the combination of reddish brown small stromata 0.8–1.7 mm diam and with distinct ostiolar dots, inconspicuous perithecial protuberances, and occurring on decorticated wood and bark.

**Trichoderma chromosperum** P. Chaverri & Samuels, Stud. Mycol. 48: 51. 2003

= Hypocrea chromosperma M.A. Curtis & Peck, Rep. New York State Mus. Nat. Hist.: 56. 1876.
Specimens examined. CHINA, Anhui, Jinzhai, Tianzhangzai, alt. 900–1100 m, on rotten twig, 23 Aug. 2011, S.L. Chen, W.Y. Zhuang, H.D. Zheng & Z.Q. Zeng 7895, HMAS 252534; Zhejiang, Longmen, alt. 800 m, on twig, 27 July 2012, W.Y. Zhuang, Z.X. Zhu, H.D. Zheng & F. Ren 8157, HMAS 252535, culture HMAS 244970; Hunan, Zhangjiajie, Jinxian, alt. 800 m, on twig, 17 Aug. 2010, W.Y. Zhuang, J. Lu & F. Zhao 7555, HMAS 266602; Zhejiang, Lian’an, Tiyueguan, alt. 200–300 m, on rotten wood, 20 July 2012, W.Y. Zhuang, Z.X. Zhu & Z.Q. Zeng, H.D. Zheng & F. Ren 7932, HMAS 252538, culture HMAS 244971; Jilin, Daxinha, Huanglin Farm, alt. 800 m, on twig, 15 Aug. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3493, HMAS 266601; Jilin, Jiaohe, Lafashan, alt. 500 m, on twig, 20 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8019, HMAS 266603, culture HMAS 244972; Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten twig, 23 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8015, HMAS 266604, culture HMAS 244974; Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten twig, 24 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8156, HMAS 266605, culture HMAS 244975; Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten twig, 24 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8160, HMAS 252540, culture HMAS 244976; Liaoning, Anshan, Qianshan, alt. 500 m, on wood, 20 Aug. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3602, HMAS 266600.

Notes — Trichoderma chromosperum is very common in China. It is characterised by pulvinate to somewhat flattened and pale yellow stromata, with obvious ostiolar openings, and glociadium-like conidiophores. This species is often mis-identified as the uncommon T. gelatinosum. The latter differs in having a waxy or gelatinous, pale yellowish to orange and translucent stromata. DNA sequence analyses clustered the
seven Chinese collections with the ex-type culture (G.J.S. 94-68) from USA with 100% statistic support (Fig. 1). Morphology of the Chinese materials matches well the original description of *T. chromospermum* (Chaverri & Samuels 2003).

**Trichoderma cremeum** P. Chaverri & Samuels, Stud. Mycol. 48: 63. 2003

≡ Hypocreopsis cremea P. Chaverri & Samuels, Mycologia 95: 1115. 2003.

Specimen examined. **CHINA**, Guangdong, Xinyi, Daowuiling, alt. 1400 m, on rotten wood associated with lichen, 22 Oct. 1998, W.Y. Zhuang & Z.H. Yu 2795, HMAS 266606.

Notes — Compared with the description of *T. cremeum* by Chaverri & Samuels (2003), the Chinese collection differs slightly in higher stromata 0.8–1.0 mm vs 0.6 mm high, smaller distal part-ascosporae 4–4.5 × 3.5–4 μm vs 5.5–6.0 × 5–5.5 μm, smaller proximal part-ascosporae 4.6–5 × 3–3.5 μm vs 5.5–6 × 4.5–5.5 μm, and association with a lichen. We regard these deviations as infraspecific variations.

**Trichoderma phyllostachydis** P. Chaverri & Samuels, Stud. Mycol. 48: 80. 2003

≡ Hypocreopsis phyllostachydis P. Chaverri & Cand., Mycol. Progr. 3: 33. 2004.

Specimen examined. **CHINA**, Jinzhai, Antu, Songjiang, alt. 400 m, on rotten wood, 22 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8061, HMAS 244842, culture HMAS 244977.

Notes — Our collection is similar to the holotype of the fungus in stroma gross morphology, culture and asexual morph characteristics (pachybasium-like conidiophores), and the identical combined sequence data of *rpb2* and *tef1*; it differs, however, in having larger perithecia (184–236 × 105–157 μm vs 101–121 × 127 × (60–)61–84 (–99) μm) and growing on rotten wood instead of decaying culms of *Phyllostachys bambusoides* (Chaverri & Samuels 2003). This species was previously known only from the type collection in France. The Chinese material extends its distribution to Asia, and reveals an unusually broad range of substrate for the species.

**Trichoderma pseudocandidum** P. Chaverri, Samuels & Minnis, Mycotaxon 109: 246. 2009

≡ Trichoderma candidum P. Chaverri & Samuels, Stud. Mycol. 48: 40. 2003, non *T. candidum* Abk. & Schwein. 1805.

≡ Hypocreopsis candida P. Chaverri & Samuels, Stud. Mycol. 48: 40. 2003.

Specimen examined. **CHINA**, Hainan, Qiongzhong, Limushan, alt. 700 m, on rotten bamboo, 18 Dec. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3959, HMAS 266597.

Notes — Compared with the American material, the Chinese collection is very similar in having flattened and pale greyish yellow to almost white stromata and monomorph part-ascosporae, but differs in having larger ascii 95–105 × 6–6.5 μm vs 74–79 × 4.5–5.2 μm and larger ascospores 4.5–5.2 × 4.5–5.2 μm vs 3.3–3.6 × 3.3–3.5 μm (Chaverri & Samuels 2003). We treat these as infraspecific variations.

**Trichoderma sinuosum** P. Chaverri & Samuels, Stud. Mycol. 48: 81. 2003

≡ Hypocreopsis sinuosa P. Chaverri & Samuels, Stud. Mycol. 48: 81. 2003.

Specimens examined. **CHINA**, Anhui, Jinzhai, Tiantangzhai, alt. 700–900 m, on rotten twig, 22 Aug. 2011, S.L. Chen, W.Y. Zhuang, H.D. Zheng & Z.Q. Zeng *Zeng* 7746, HMAS 252541, culture HMAS 244978; Anhui, Jinzhai, Tiantangzhai, alt. 900–1000 m, on dead twig, 24 Aug. 2011, S.L. Chen, W.Y. Zhuang, H.D. Zheng & Z.Q. Zeng *Zeng* 7862, HMAS 252542, culture HMAS 244979.

Notes — The two specimens of *T. sinuosum* cited above are largely immature but with some mature ascospores observed. Its nearly monomorphic ascospores and sinuous conidiophores are diagnostic. Morphologically, the Chinese collections are similar to those from other regions of the world in colour and size of stromata, shape and size of ascospores, and shape of conidiophores, but differ slightly in shorter ascii (92–108 μm vs (87)–95–113 (–128) μm) (Jaklitsch 2009).

In our phylogenetic tree (Fig. 1), the two strains of *T. sinuosum* from China grouped together with strong bootstrap supports (MPBP/BIPP = 96 %/100 %), and further clustered with collections from Austria (C.P.K. 1595) and America (G.J.S. 90-88) receiving a low support (MPBP = 78 %). Only 16 bp and 11 bp (HMAS 252541), 14 bp and 11 bp (HMAS 252542) differences were found from C.P.K. 1595 and G.J.S. 90-88, respectively. We regard this as infraspecific variation.

**Accepted species previously reported from China**

**Hypocrea lixii** Pat., Rev. Mycol. (Toulouse) 13: 138. 1891

≡ Hypocreopsis nigricens (S.Imai) Yoshim. Doi, Bull. Natl. Sci. Mus. Tokyo 15: 732. 1972.

Specimens examined. **CHINA**, Hainan, Qiongzhong, Limushan, alt. 350 m, on rotten twig, 23 Aug. 2011, S.L. Chen, W.Y. Zhuang, H.D. Zheng & Z.Q. Zeng *Zeng* 7790, culture HMAS 244981; Guizhou, Jiayin, Fajingjiang, alt. 1800 m, on rotten twig, 21 Sept. 1995, P.G. Liu D’95-14, HKAS 29673 (previously filed under *H. nigricens*); Hainan, Ledong, Dingfengling, alt. 1100 m, on twig, 9 Dec. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3746, HMAS 266610; Hainan, Tongzhia, Wuzhishan, alt. 850 m, on bamboo, 17 Dec. 2000, Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3940, HMAS 266611; Yunnan, Mengla, Xishuangbanna, alt. 600 m, on rotten twig, 30 Sept. 1993, Y. Doi D’93-43, HKAS 26206 (previously filed under *H. nigricens*).

Notes — *Hypocrea lixii* and *Trichoderma harzianum* s.str. were first considered as a sexual/asexual connection of a single species (Chaverri & Samuels 2002). But as indicated by Druzhinina et al. (2010), based on phylogenetic analysis of numerous strains under both names, *T. harzianum* and *H. lixii* are not connected but represent two separate species. Isolates that originated from ascospores of *H. lixii* are morphologically indistinguishable from those of *T. harzianum*, including the type. We tentatively treat them as separate taxa.

**Trichoderma tropicosinense** (P.G. Liu) P.G. Liu, Z.X. Zhu & W.Y. Zhuang, comb. nov. — MycoBank MB808310

*Basionym.* *Hypocrea tropicosinensis* P.G. Liu, Mycotaxon 86: 278. 2003.

Specimens examined. **CHINA**, Ji Lin, Jiachao, Qianjin Forest Farm, alt. 450 m, on rotten twig, 23 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8082, HMAS 252546, culture HMAS 244983; Yunnan, Mengla, Cuipingfeng tropical rain forest park, alt. 800 m, on dead bark of Castanopsis, 1 Oct. 1993, P.G. Liu D’93-40 (holotype HKAS 26198).

**Trichoderma aureoviride** Rifai, Mycol. Pap. 116: 34. 1969

≡ Hypocreopsis aureoviridis Plowr. & Cooke, Grevillea 8: 104. 1880.

Specimens examined. **CHINA**, Beijing, Songshan, alt. 300 m, on rotten wood, 8 July 2011, H.D. Zheng & P. Zhao 7613, HMAS 266607, culture HMAS 244980.

**Trichoderma gelatinosum** P. Chaverri & Samuels, Stud. Mycol. 48: 68. 2003

≡ Hypocreopsis gelatinosa (Tode) Fr., Summa Veg. Scand.: 383. 1849.

Specimens examined. **CHINA**, Jinzhai, Antu, Changbaishaan, alt. 1100 m, on rotten bark, 26 July 2012, W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8203, HMAS 266609.
**Trichoderma longipile** Bissett, Canad. J. Bot. 69, 11: 2395. 1992. 1991

= Hypocrea longipila Jaklitsch, Stud. Mycol. 63: 62. 2009.

Specimen examined. **C** **H** **N** **A**, Guangdong, Xinyi, Dawuling, alt. 1400 m, on rotten wood, 22 Oct. 1998. W.Y. Zhuang & Z.H. Yu 28113, HMAS 252544.

Notes — This species can be easily recognised by incrustation to pale orange-red stromata with a distinct coarsely tubercular surface and large green ascospores. The Chinese material agrees well with the original description of *T. longipile*. The asexual morph of the species was first found in Liao宁 Province (Sun et al. 2006b). This is the first time that its sexual morph is reported.

**Trichoderma strictipile** Bissett, Canad. J. Bot. 69: 2410. 1991

= Hypocrea strictipilosa P. Chaverri & Samuels, Mycologia 95: 1128. 2003.

Specimen examined. **C** **H** **N** **A**, Jilin, Antu, Songjiang, alt. 400 m, on rotten wood, 22 July 2012. W.Y. Zhuang, Z.X. Zhu, Z.Q. Zeng, H.D. Zheng & F. Ren 8056, HMAS 252545, culture HMAS 244982.

Notes — The previous Chinese record of this species was based on asexual morph (Zhang & Xu 2004). The sexual morph is reported here for the first time from China.

**Unconfirmed previous record**

**Hypocrea cupularis** (Fr.) Sacc., Syll. Fung. 2: 535. 1883

Specimens examined. **C** **H** **N** **A**, Guangxi, Longlin, Jiwei, alt. 1200 m, on rotten wood, 5 Nov. 1957, L.W. Xu 757, HMAS 30759; Hainan, Tongzha, Wuzishan, alt. 850 m, on rotten wood, 17 Dec. 2000. Z.H. Yu, W.Y. Zhuang & Y.H. Zhang 3916, HMAS 266608.

Notes — **Hypocrea cupularis** was first reported from China by Teng (1963), as fitting the original description of *H. cupularis* (Saccardo 1883). When Jaklitsch (2009) examined the only authentic specimen in Herb. E. Fries (UPS 133487) labelled *H. cupularis*, the ascomata are neither discoid nor cupulate, which is significantly different from the original description. The species concept of this fungus is dubious.

**Species excluded from China**

**Hypocrea albocornea** Yoshim. Doi, Bull. Natl. Sci. Mus., Tokyo 15: 712. 1972

Notes — The previous Chinese record of *H. albocornea* (Liu et al. 2002) was based on a single collection (HKAS 26273). Re-examination of the specimen indicated that the distal part-ascospores are 3.5–4 × 3.5–4 μm and proximal part-ascospores are 3.5–4 × 3–3.5 μm, which significantly differ from the part-ascospore size of *H. albocornea* (7.2–8.2 × 6.2–7.0 μm and 7.5–8.5 × 5.7–6.5 μm) (Chaverri & Samuels 2003). The correct name for HKAS 26273 is *Trichoderma ceramicum*. The previous record of *H. albocornea* from China was thus based on a misidentification.

**DISCUSSION**

The relationships among 45 *Trichoderma* species with green part-ascospores based on analyses of combined *rpb2* and *tef1* sequence data were explored (Fig. 1). The phylogenetic relationships among taxa are generally consistent with previous studies (Chaverri & Samuels 2003, Jaklitsch 2009). All investigated species formed a well-supported monophyletic group. In addition to the formerly named eight clades (Chaverri & Samuels 2003, Jaklitsch 2009), two new clades, *Phyllostachydis* and *Stipitatum*, are here proposed. The *Phyllostachydis* clade containing *T. phyllostachydis* is phylogenetically distinct from any other species of *Trichoderma* that have green ascospores (Fig. 1). The *Stipitatum* clade, bearing *T. stipitatum* (HMAS 266612 and 266613), is characterised by turbinate, pale yellow to nearly orange-red stromata and verticillium-like conidiophores. It constitutes a lineage with significant bootstrap values (Fig. 1, MPBP/BIPP = 100 %/100 %), and has a low level of support for a relationship with the *Virescentiflava* and *Strictipilosa* clades. Among the species of *Trichoderma* that have green ascospores, the Harzianum clade is the largest (Samuels et al. 2006, Jaklitsch 2009). Jaklitsch (2009) included *T. epimecys*, *T. parepimycys*, *T. alni*, *T. tawa*, *T. bruneoviride*, *T. catoptron*, *T. dacrymcyllum*, *T. cinnamomeum*, *T. stramineum*, *H. atroslategatina*, *H. lixi*, *T. ceraceum*, *T. cinenum* and *T. aggressivum*. In our study, this clade was supported by bootstrap values of 86 % MPBP and 100 % BIPP (Fig. 1). The sexual morphs of most species in this group produce darkly pigmented stromata, except for *T. stramineum* and *T. catoptron*, which have greyish yellow stromata. One of our new species, *T. rufobrunneum*, is located in this clade. It is clearly separated from the other known species in having reddish brown stromata. Although *T. rufobrunneum* is closely related to *T. alni*, it is distinct by having larger ascus, shorter phialides and the negative reaction of stromata to KOH.

The phylogenetic data place *T. rosulatum* in the Chlorospora clade (Fig. 1) along with *T. sinuosum*, *T. cremeum*, *T. surrotundum*, *T. chorisorum*, *T. thelephoricola* and *H. costensis*. All of these species have pale yellow or pale green, semi-translucent stromata, globose to subglobose ascospores and gliocladium / verticillium-like conidiophores. The fungus is related to, but distinct from, *T. thelephoricola* (for details see notes under *T. rosulatum*).

The phylogenetic position of *T. tropicosinense* is explored here for the first time. The Strictipilosa clade containing four taxa was well-supported (Fig. 1). However, distinctions among species can be found in the colour of stromata, which are yellowish in *T. strictipile* and *T. cuneisporum*, somewhat incarnate in *T. longipile*, and greyish brown to blackish brown in *T. tropicosinense*. Furthermore, most species in this group have pachybasium-like asexual morphs, while *T. tropicosinense* is an exception showing gliocladium-like conidiophores (Chaverri & Samuels 2003).

The seven collections of *T. chromospermum* from China and the ex-type culture from the USA (MD) (G.J.S. 94-68) grouped together with high bootstrap support (MPBP/BIPP = 100 %/100 %) (Fig. 1). The Chinese collections agree well with the species as it was described by the original authors (Chaverri & Samuels 2003), indicating that the species is morphologically stable over a wide north temperate distribution. *Trichoderma chromospermum* is common in North America, and this is the first report of the species outside the continent. Interestingly, Jaklitsch (2009) did not record the species from Europe. Despite the fact that *T. chromospermum* is apparently common and widespread in north temperate regions, in nature it is known only from its sexual morph. Even though *T. chromospermum* clustered with *T. gelatinosum* and *T. nigroviride* (100 % MPBP), the grouping is not well-supported (63 % BIPP) (Fig. 1). The stromata of the former two species are pale yellowish to orange, whereas that of the latter are dark green to almost black. Further studies are needed to better define this clade.

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