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RESEARCH ARTICLE

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Trichoderma (*Hypocrea*) species with green ascospores from China

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Key words

Ascomycota
Hypocreales
ITS
morphology
rpb2
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tef1

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. thelephoricola*, 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.

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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, Nsereko et al. 2002, Degenkolb et al. 2008), 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*.

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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, 2001, 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 (HMAS) 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

fungus. 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), cornmeal agar (Difco) + 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 fRPB2-5f and fRPB2-7cr (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). PCR was performed with an ABI 2720 Thermal Cycler (Gene Co. Ltd., Foster City, California, USA) using a 25 µL reaction system consisting of 2.5 µL 10× PCR buffer, 1.5 µL $MgCl_2$ (25 mM), 1.25 µL each primer (10 µM), 0.5 µL dNTP (10 mM each), 1.25 µL template DNA, 0.25 µL *Taq* polymerase (5U / µL) and 16.5 µL ddH_2O. For ITS, PCR conditions were an initial step of 5 min at 95 °C, 30 cycles of 30 s at 94 °C, 30 s at 52 °C, and 30 s at 72 °C, followed by 10 min at 72 °C. For *rpb2* and *tef1*, PCR conditions were an initial step of 5 min at 95 °C; 30 cycles of 1 min at 95 °C, 90 s at 59 °C for *rpb2* or 55 °C for *tef1*, 90 s at 72 °C; followed by 10 min at 72 °C. PCR products were purified with the PCR Product Purification Kit (Biocolor BioScience & Technology Co., Shanghai, China) and sequencing was carried out in both directions with the same primers as in PCR using an ABI 3730 XL DNA Sequencer (Applied Biosciences, Foster City, CA, 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 replicates 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 replicates, 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 *TrichO*Key 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-ascospored species of *Trichoderma* were named, i.e. Ceramica, Chlorospora, Harzianum, Spinulosa, 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

divergences and morphological distinctions of *T. phyllostachydis* and *T. stipitatum*, two new clades, Phyllostachydis and Stipitatum, 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 accessions of sequences used for phylogenetic analyses.

Species	Strain	GenBank accession number[1]		
		ITS	*rpb2*	*tef1*
Hypocrea atrogelatinosa	G.J.S. 95-159	–	AF545508	AF534603
H. costaricensis	P.C. 21	AY737754	AY391921	AY391980
H. danica	CBS 121273	FJ860750	FJ860534	FJ860634
H. lixii	G.J.S. 90-22	AF443915	AY391925	AY391984
	HMAS 244981	**KF923297**	**KF923308**	**KF923284**
H. spinulosa	CBS 121280	FJ860699	FJ860589	FJ860699
H. virescentiflava	P.C. 278	AY737768	AY391959	AY392007
Trichoderma aerugineum (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
	HMAS 266607	**KF923293**	**KF923306**	**KF923280**
T. brunneoviride (as *H. brunneoviridis*)	CBS 120928	EU518661	EU498358	EU498318
T. candidum (as *H. candida*)	P.C. 59	AY737757	AY391899	AY391962
T. catoptron (as *H. catoptron*)	G.J.S. 02-76	AY737766	AY391900	AY391963
	HMAS 252533	–	**KF923310**	**KF923290**
T. ceraceum (as *H. ceracea*)	G.J.S. 88-28	–	AY391901	AY391964
T. ceramicum (as *H. ceramica*)	CBS 114576	FJ860743	FJ860531	FJ860628
T. cerinum	CBS 120637	FJ860744	FJ860532	FJ860629
T. chlorosporum (as *H. chlorospora*)	G.J.S. 88-33	–	AY391903	AY391966
T. chromospermum (as *H. chromosperma*)	G.J.S. 94-68	–	AY391913	AY391974
	HMAS 252535	**KF923304**	**KF923315**	**KF923292**
	HMAS 252536	**KF923296**	**KF923307**	**KF923283**
	HMAS 252537	**KF729993**	**KF730004**	**KF729986**
	HMAS 252538	**KF730000**	**KF730008**	**KF729987**
	HMAS 252539	**KF923303**	**KF923314**	**KF923287**
	HMAS 252540	**KF923299**	**KF923311**	**KF923291**
	HMAS 266603	**KF923300**	–	–
	HMAS 266604	**KF923298**	**KF923309**	**KF923288**
	HMAS 266605	**KF923305**	–	**KF923289**
	HMAS 76657	**KF923294**	–	**KF923281**
T. cinnamomeum (as *H. cinnamomea*)	G.J.S. 97-237	AY737759	AY391920	AY391979
T. crassum (as *H. crassa*)	DAOM 164916	EU280067	AF545542	AF534615
T. cremeum (as *H. cremea*)	G.J.S. 91-125	AY737760	AF545511	AF534598
T. cuneisporum (as *H. cuneispora*)	G.J.S. 91-93	AY737763	AF545512	AF534600
T. dacrymycellum (as *H. dacrymycella*)	WU 29044	FJ860749	FJ860533	FJ860633
T. epimyces (as *H. epimyces*)	C.P.K. 1980	EU518662	EU498359	EU498319
T. estonicum (as *H. estonica*)	G.J.S. 96-129	AY737767	AF545514	AF534604
T. fomiticola (as *H. fomiticola*)	CBS 121136	FJ860755	FJ860538	FJ860639
T. gelatinosum (as *H. gelatinosa*)	G.J.S. 88-17	AY737775	AF545516	AF534579
T. harzianum	CBS 226.95	AY605713	AF545549	AF534621
T. longipile (as *H. longipilosa*)	CBS 120953	FJ860770	FJ860542	FJ860643
T. nigrovirens (as *H. nigrovirens*)	G.J.S. 99-64	AY737777	AF545518	AF534582
T. parepimyces (as *H. parepimyces*)	CBS 122768	FJ860801	FJ860563	FJ860665
T. parestonicum (as *H. parestonica*)	CBS 120636	FJ860803	FJ860565	FJ860667
T. phyllostachydis	HMAS 244842	**KF729997**	**KF730009**	**KF729988**
T. phyllostachydis (as *H. phyllostachydis*)	G.J.S. 92-123	AY737755	AF545513	AF534576
T. rosulatum	HMAS 252548	**KF729995**	**KF730005**	**KF729984**
T. rufobrunneum	HMAS 266614	**KF729998**	**KF730010**	**KF729989**
	HMAS 252547	**KF729999**	**KF730007**	**KF729992**
T. sinuosum (as *H. sinuosa*)	C.P.K. 1595	FJ860838	FJ179619	FJ860697
	G.J.S. 90-88	–	AY391932	AY391990
	HMAS 252541	**KF729996**	**KF730003**	**KF729983**
	HMAS 252542	**KF729994**	**KF730006**	**KF729985**
T. stipitatum	HMAS 266612	**KF730002**	**KF730011**	**KF729990**
	HMAS 266613	**KF730001**	**KF730012**	**KF729991**
T. stramineum (as *H. straminea*)	G.J.S. 02-84	AY737765	AY391945	AY391999
T. strictipile (as *H. strictipilosa*)	C.P.K. 1601	–	FJ860594	FJ860704
	HMAS 252545	**KF923301**	**KF923312**	**KF923285**
T. surrotundum (as *H. surrotunda*)	G.J.S. 88-73	AY737769	AF545540	AF534594
T. tawa (as *H. tawa*)	G.J.S. 97-174	AY737756	AY391956	AY392004
T. thailandicum (as *H. thailandica*)	G.J.S. 97-61	AY737772	AY391957	AY392005
T. thelephoricola (as *H. thelephoricola*)	CBS 120925	FJ860858	FJ860600	FJ860711
T. tropicosinense	HMAS 252546	**KF923302**	**KF923313**	**KF923286**
T. virens (as *H. virens*)	GLi 39	AF099005	AF545558	AF534631
Nectria eustromatica	CBS 121896	HM534896	HM534886	HM534875
N. berolinensis	CBS 127382	HM534893	HM534883	HM534872

[1]Numbers in **bold** indicate newly submitted sequences.

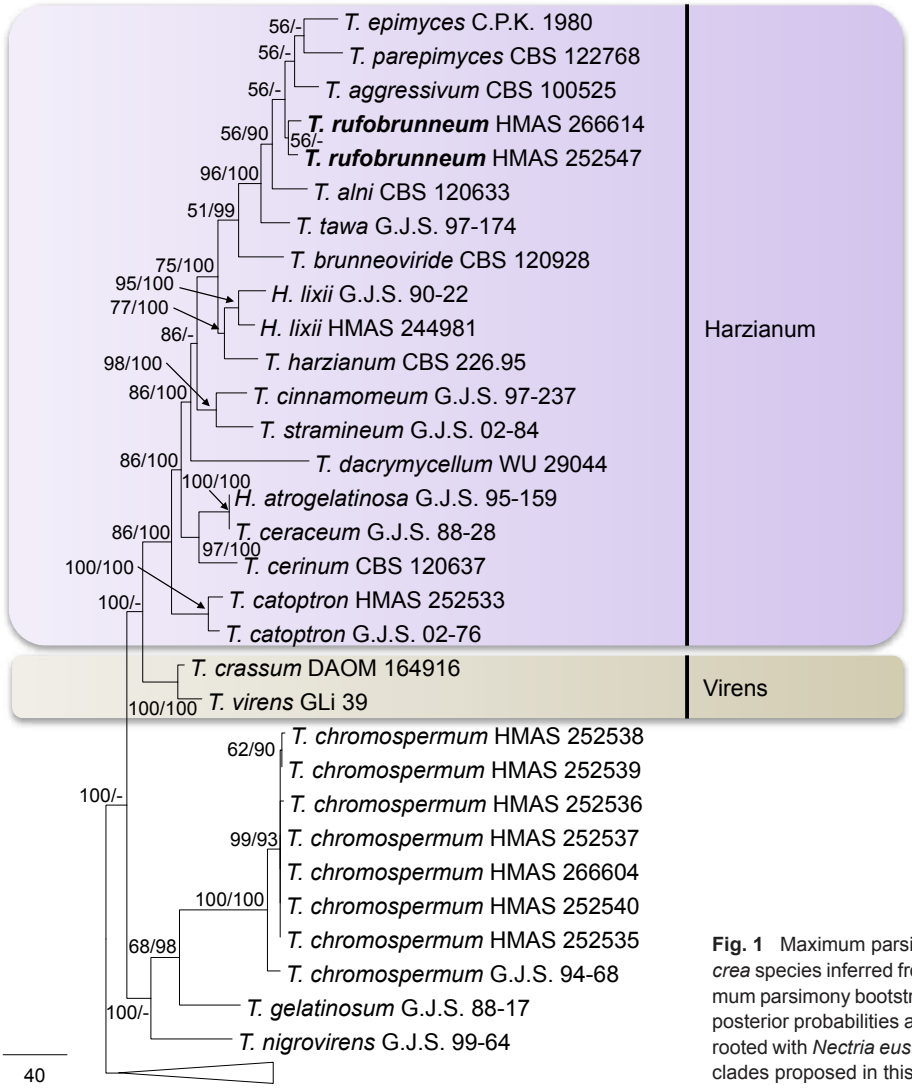
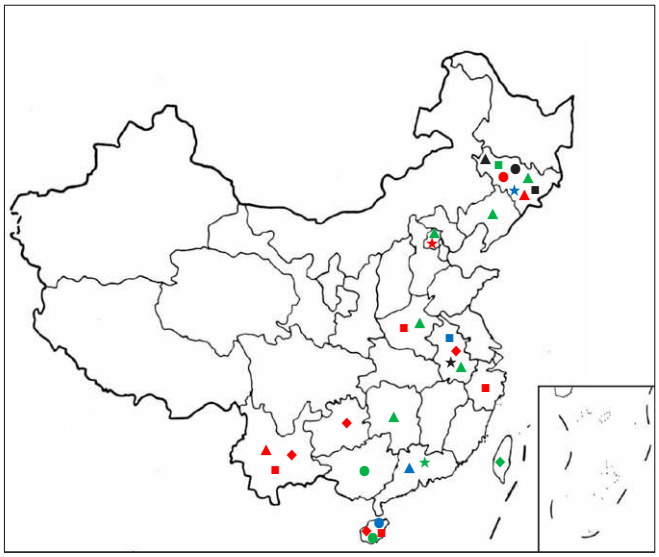


Fig. 1 Maximum parsimony tree of the green-spored *Trichoderma* / *Hypocrea* species inferred from combined *rpb2* and *tef1* partial sequences. Maximum parsimony bootstrap support above 50 % (left) and Bayesian inference posterior probabilities above 90 % (right) are shown at nodes. The tree was rooted with *Nectria eustromatica* and *N. berolinensis*. New species and new clades proposed in this study are indicated in **bold**.

T. rosulatum (HMAS 252548), a member of this clade, was closely related (MPBP = 100 %) to, but distinct from, *T. thelephoricola*. 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*.



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. longipilis* (▲); *T. phyllostachydis* (★); *T. pseudocandidum* (●); *T. rosulatum* (■); *T. rufobrunneum* (▲); *T. sinuosum* (★); *T. stipitatum* (●); *T. strictipile* (■); *T. tropicosinense* (▲).

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-ascospored species and 32 hyaline-ascospored 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.

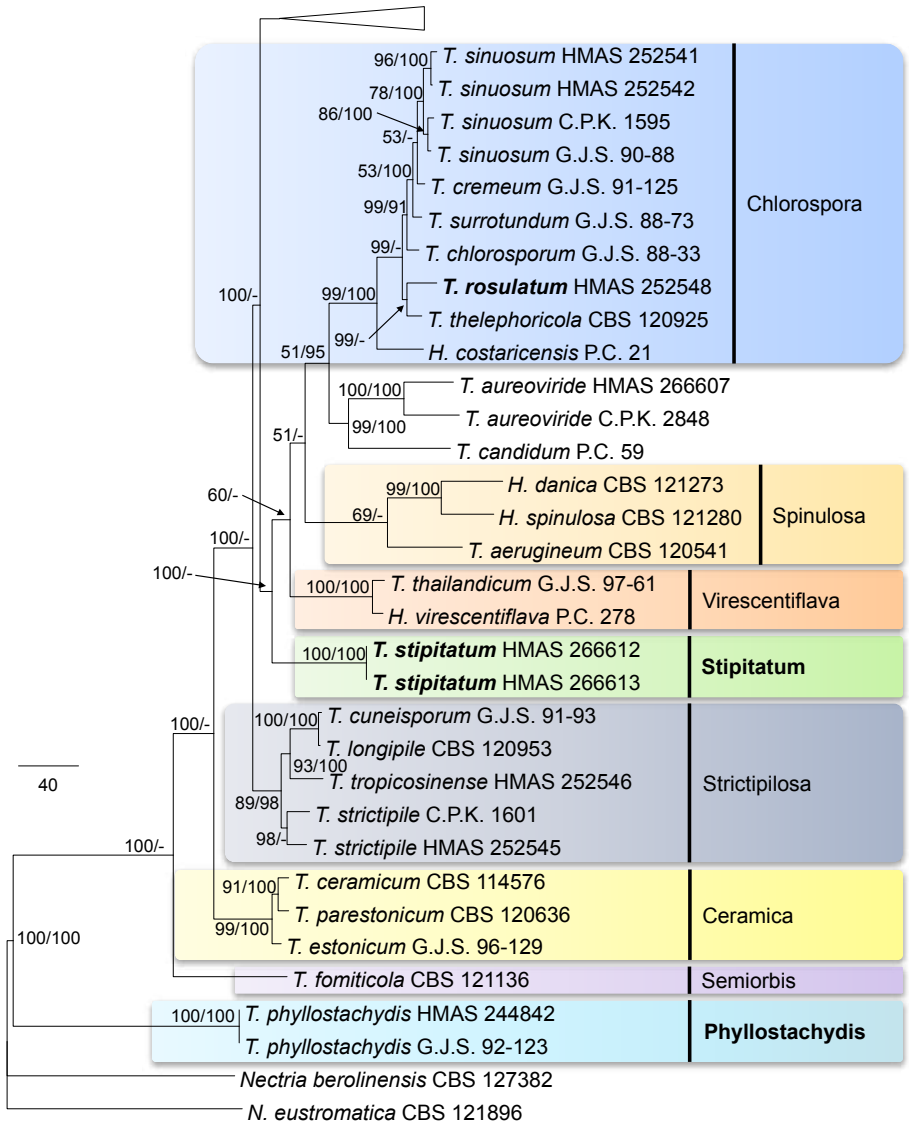


Fig. 1 (cont.)

New species

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

Etymology. The specific epithet refers to the rosulate 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; subperithecial 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, (8–)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, gliocladium-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–)4.5–5(–7.5) (n = 60). *Conidia* light green, smooth, ovoid to ellipsoidal, (3.5–)4–4.5(–6.5) ×

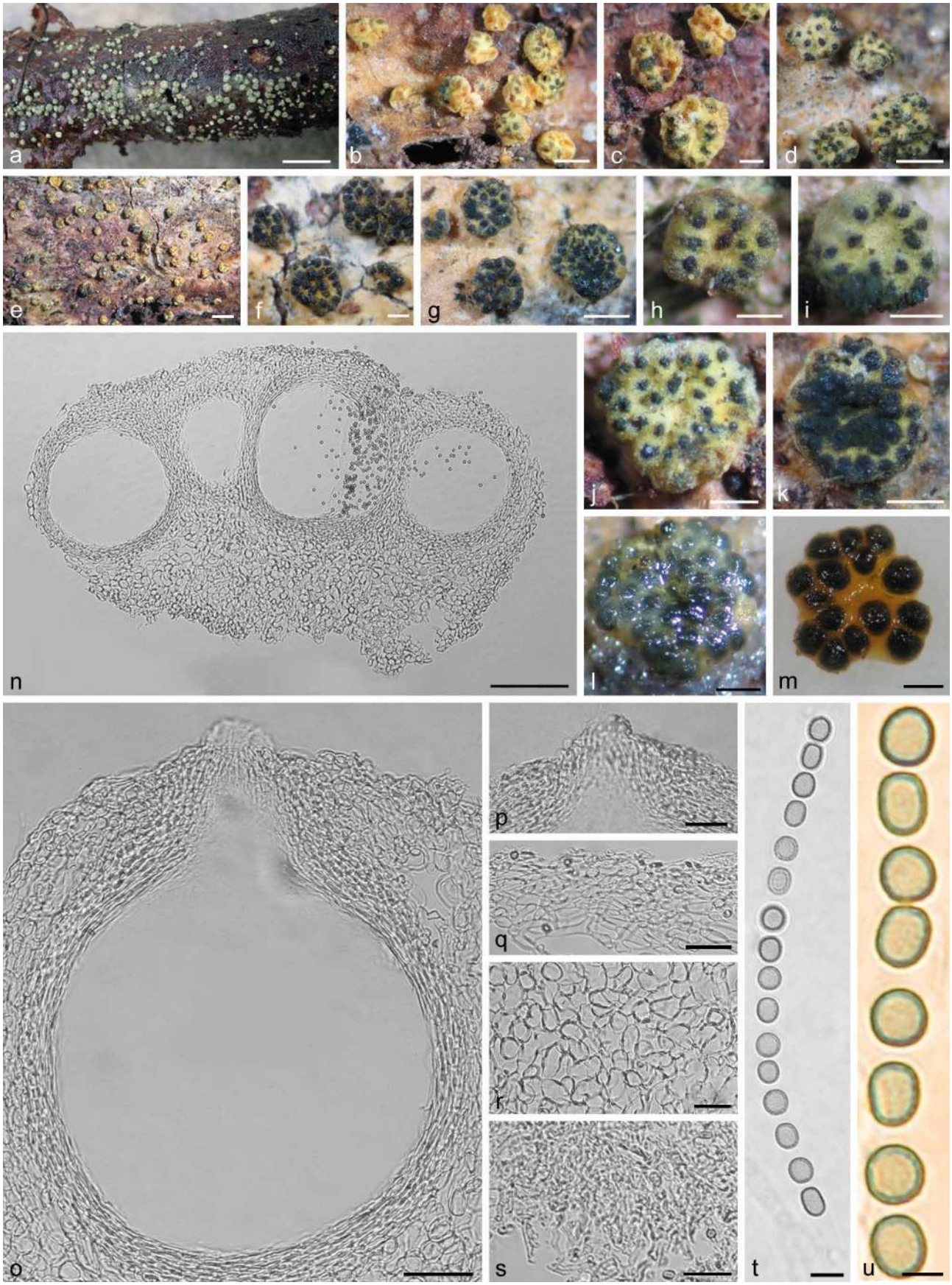


Fig. 2 Sexual morph of *Trichoderma rosulatum* (HMAS 252548). a. Fresh stromata on nature substrate; b. dry stromata on nature substrate; c–k. dry stromata at different development stages (c, d. immature; e–k. mature); l. mature stromata after rehydration; m. mature stroma in 3 % KOH after reconstitution 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.

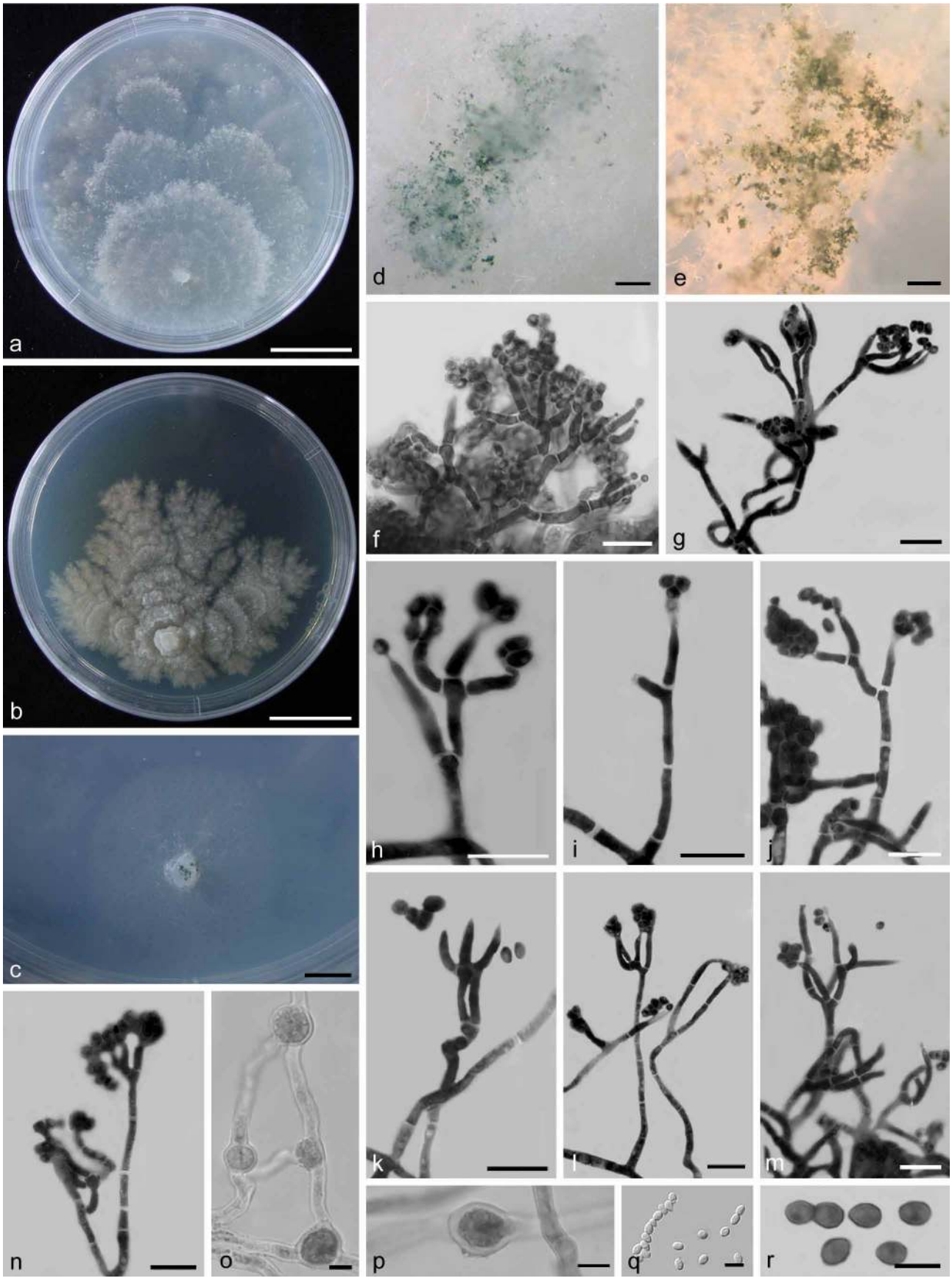


Fig. 3 Cultures and asexual morph of *Trichoderma rosulatum* (HMAS 244906). a–c. Cultures at 25 °C after 27 d (a. on CMD; b. on PDA; c. on SNA); d, e. conidiation tuft (CMD, 40 d); f–n. conidiophores (CMD, 40 d); o, p. chlamydospores (CMD, 40 d); q, r. conidia (CMD, 40 d). — Scale bars: a–c = 20 mm; d = 0.2 mm; e = 0.1 mm; f–n = 10 µm; o–r = 5 µm.

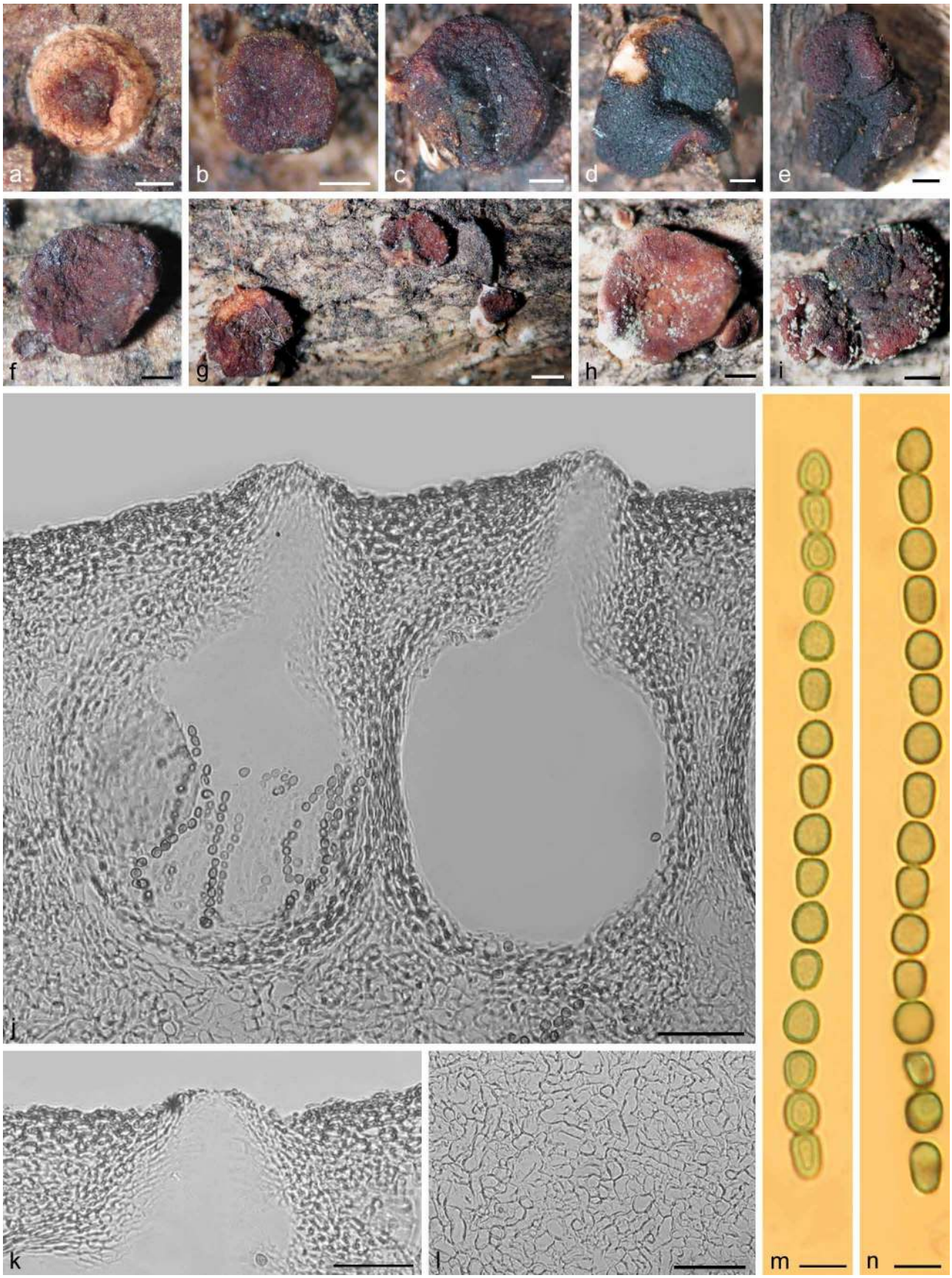


Fig. 4 Sexual morph of *Trichoderma rufobrunneum*. a–i. Dry stromata on nature substrate; j. perithecia in section; k. structure of ostiole; l. structure of subperithecial tissue; m, n. an ascus with part-ascospores (a–e, j–n. HMAS 252547; f–i. HMAS 266614). — Scale bars: a–c, f = 0.2 mm; d, e, g–i = 0.5 mm; j, k = 30 µm; l = 50 µm; m, n = 10 µm.

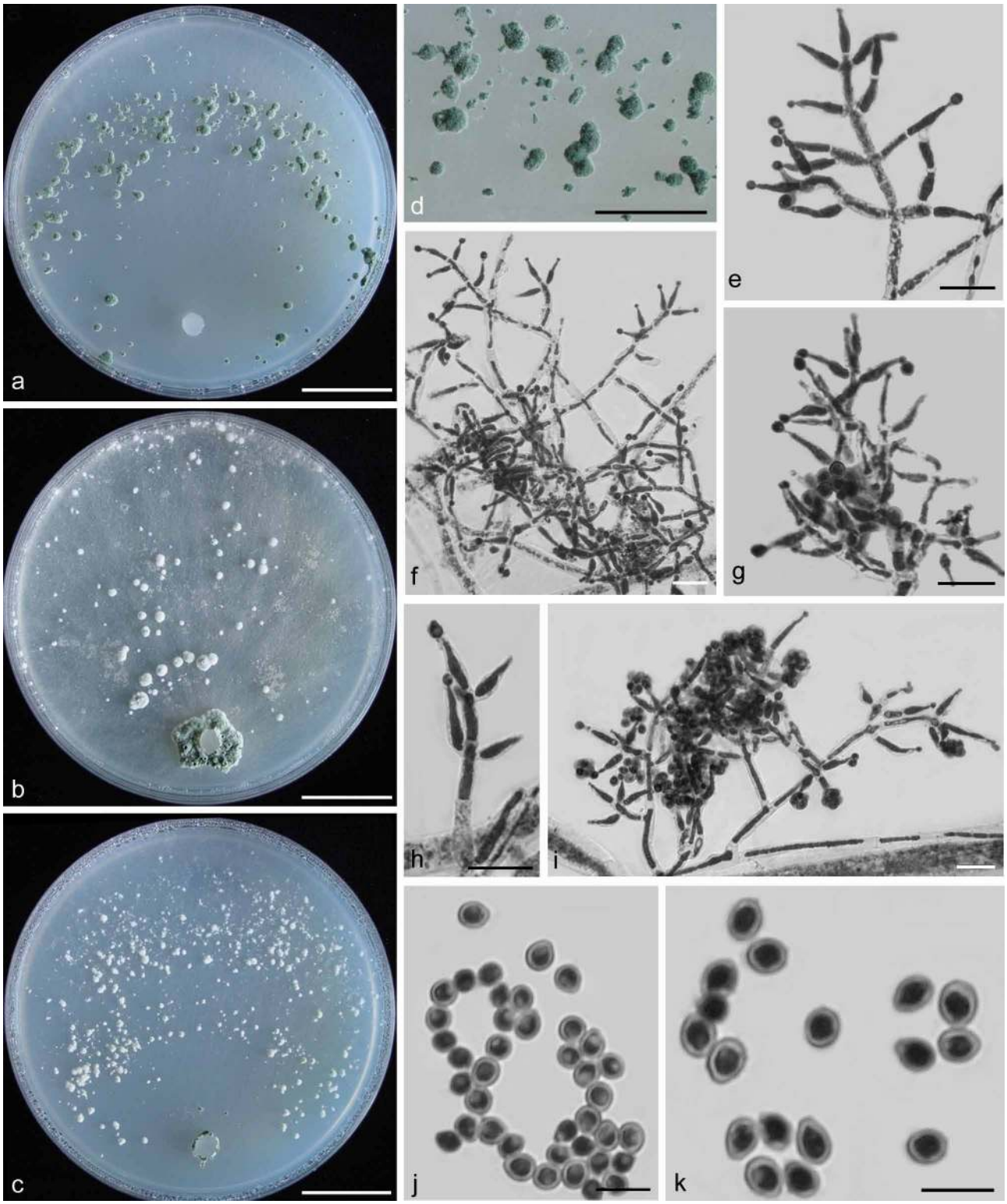


Fig. 5 Cultures and asexual morph of *Trichoderma rufobrunneum* (HMAS 244907). a–c. Cultures at 25 °C after 7 d (a. on CMD; b. on PDA; c. on SNA); d. conidiation tuft on surface (CMD, 7 d); e–i. conidiophores (CMD, 7 d); j, k. conidia (CMD, 7 d). — Scale bars: a–c = 20 mm; d = 10 mm; e–i = 10 µm; j, k = 5 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 °C, 4–6 mm at 25 °C and 30 °C; after 2 wk at 25 °C 32–35 mm; mycelium not reaching the distal margin or covering the plate after more than 2 mo at 25 °C. 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 °C, 2–3 mm at 25 °C and 30 °C, after 2 wk at 25 °C 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. thelephoricola* 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. thelephoricola* has gliocladium-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. thelephoricola* formed a well-supported terminal branch (Fig. 1, 99 % MPBP), 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 MB805907; 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 cells 6–12 × 5–7 µm (n = 40) in face view and in vertical section; subcortical tissue *textura angularis*; cells thin-walled, subhyaline to pale yellowish, 5–9 × 5–7 µm (n = 40); subperithecial tissue a dense *textura epidermoidea* of variable thin-walled hyaline cells, (8–)12–25(–30) × (6–)8–10(–12) µm (n = 40); stroma base *textura intricata*, hyphae thick-walled, (2–)3–5(–6) 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 ellipsoidal, (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 autolytic 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, ellipsoidal 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. China, 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. epimyces* and *T. parepimyces*. *Trichoderma rufobrunneum* is most similar to *T. alni* in colour of stromata with inconspicuous ostiolar dots when dry, but the latter differs in smaller asci, (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 MB805908; 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 subiculum, 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) × (3–)4–7(–10) µm (n = 40); subperithecial tissues of *textura angularis*; cells thin-walled, subhyaline to pale yellowish, (10–)15–26(–30) × (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* globose or flask-shaped, generally crowded or slightly separated, 211–257(–263) × (170–)182–215(–223) µm (n = 40); *ostioles* not emerging through the cortex or projecting to 20(–29) µm, 33–50 µm wide at apex, 41–58(–66) µm high (n = 20); *peridium*

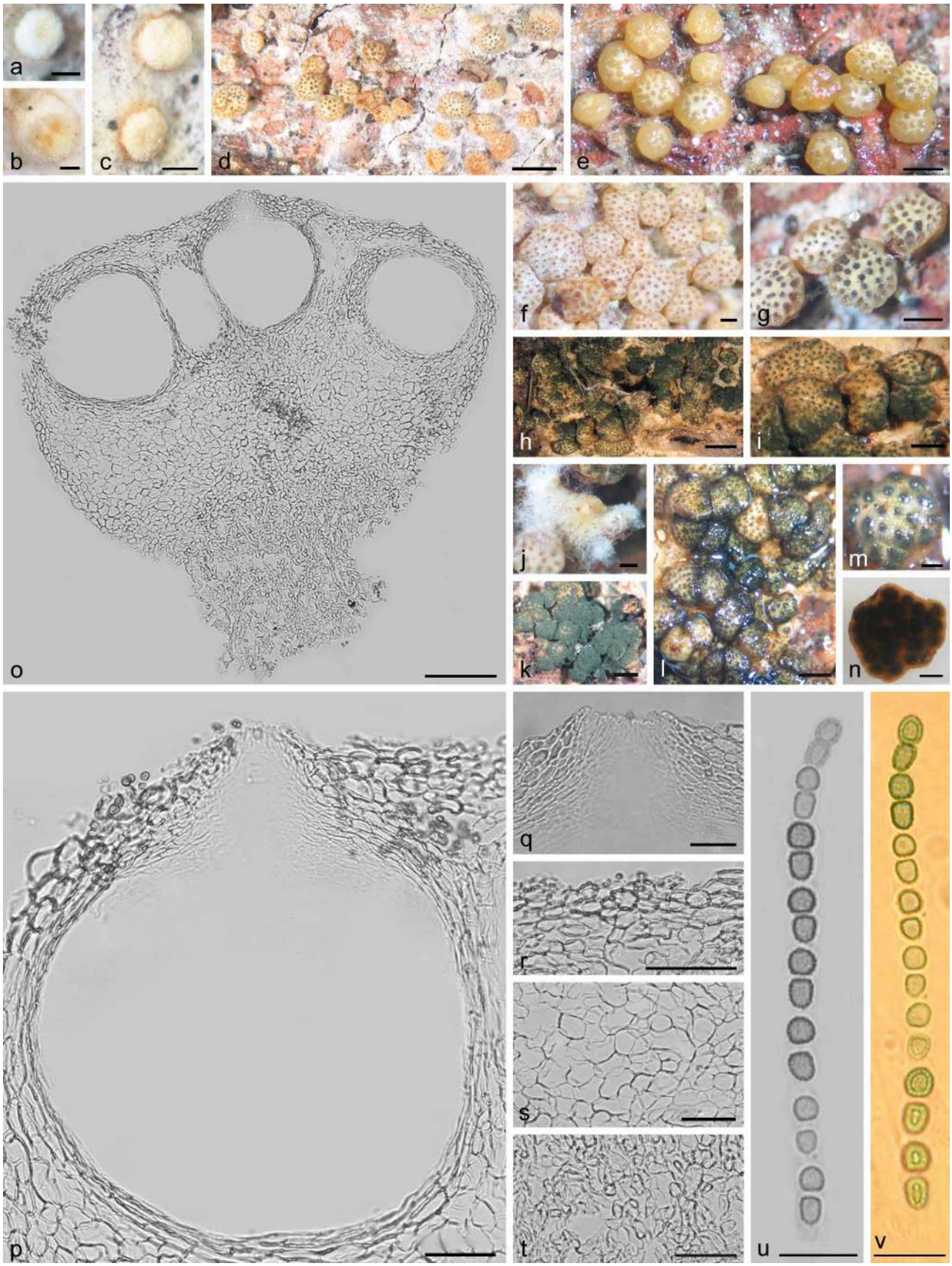


Fig. 6 Sexual morph of *Trichoderma stipitatum* (HMAS 266613). a–d, f–i, k. Dry stromata at different development stages (a–c. stroma initials; d–g. immature; h, i, k. mature); e. immature stromata after rehydration; j. whitish subiculum; l, m. mature stromata after rehydration; n. mature stroma in 3 % 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, i, l = 0.5 mm; f, n = 0.2 mm; o = 100 µm; p, s, t = 30 µm; q = 20 µm; r, u, v = 10 µm.

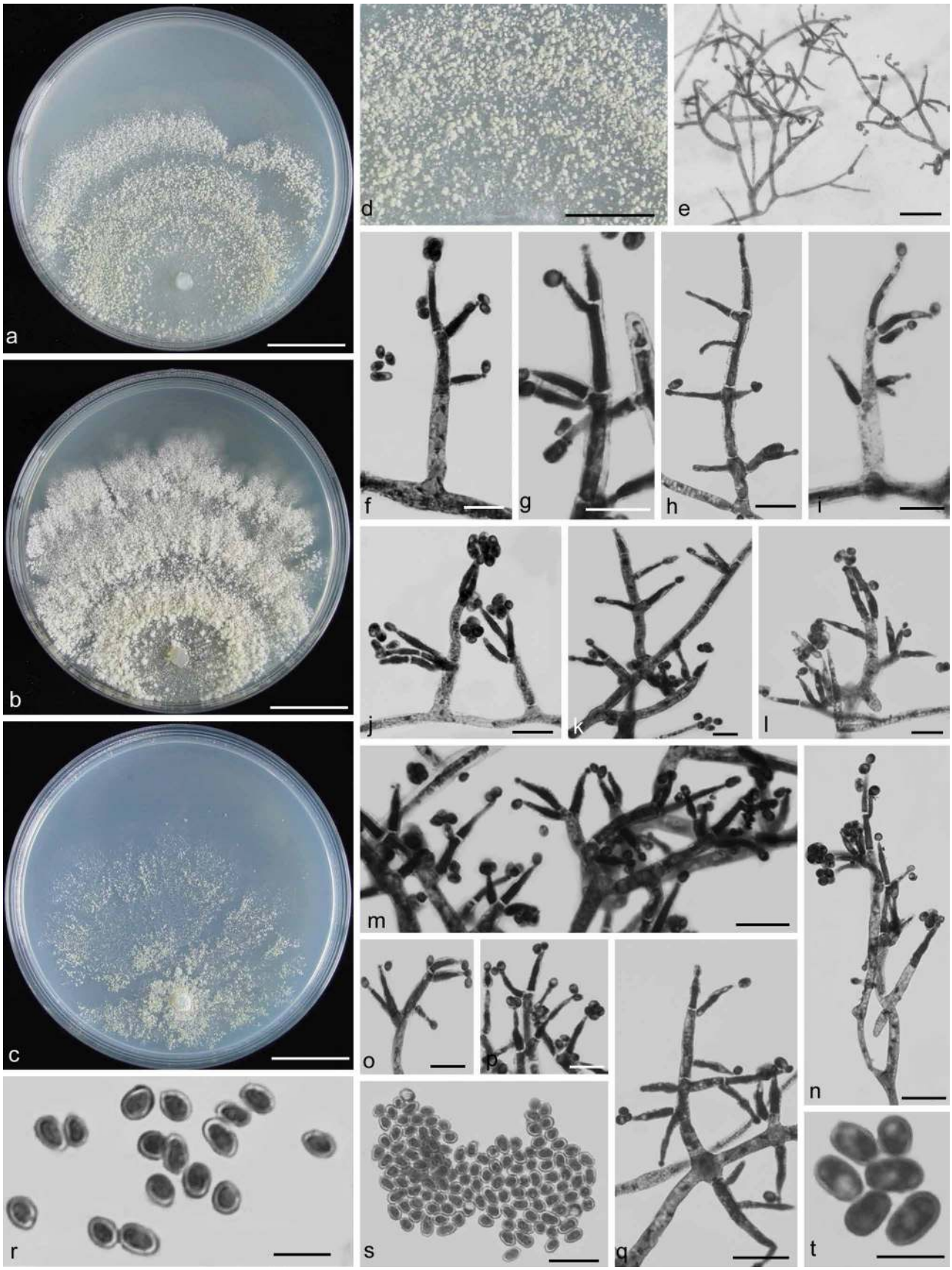


Fig. 7 Cultures and asexual morph of *Trichoderma stipitatum* (HMAS 244908). a–c. Cultures at 25 °C after 10 d (a. on CMD; b. on PDA; c. on SNA); d. culture showing granular pustules on surface (CMD, 6 d); e–q. conidiophores (CMD, 10 d); r–t. conidia (CMD, 10 d). — Scale bars: a–c = 20 mm; d = 10 mm; 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–)4–4.5(–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 tardily and faintly yellow-greenish, with discontinuous surface, densely granular by densely disposed. No autolytic excretions seen, coilings 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. *Conidiophores* 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.

Colony radius on PDA after 72 h 5–7 mm at 15 °C, 22–24 mm at 25 °C, 21–23 mm at 30 °C; mycelium covering the plate after 14 d at 25 °C. Colony flat, dense, conspicuous alternate narrow / wide zones, conidia formed abundantly in concentric rings.

Colony radius on SNA after 72 h 1–3 mm at 15 °C, 9–12 mm at 25 °C, 9–11 mm at 30 °C, not forming concentric rings; mycelium covering the plate after 17 d at 25 °C. Colony irregular, indistinctly zonate. Surface farinose or granulose from the centre and becoming yellow-greenish by conidiation.

Specimens examined. China, Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten bark, 24 July 2012, *W.Y. Zhuang*, *Z.X. Zhu*, *Z.Q. Zeng*, *H.D. Zheng* & *F. Ren* 8152 (holotype HMAS 266613, culture ex-type HMAS 244908); Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten bark, 24 July 2012, *W.Y. Zhuang*, *Z.X. Zhu*, *Z.Q. Zeng*, *H.D. Zheng* & *F. Ren* 8151, HMAS 266612.

Notes — Morphologically, the new species is most similar to *T. chromospermum* 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 ((84–)90–108(–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. thelephoricola*, *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).

Specimen 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 ceramica* Ellis & Everh., N. Amer. Pyrenomyc.: 85. 1892.

Specimens examined. China, Hainan, Ledong, Jianfengling, alt. 1000 m, on twig, 10 Dec. 2000, *Z.H. Yu*, *W.Y. Zhuang* & *Y.H. Zhang* 3776, HMAS 266598; Hainan, Lingshui, Diaoluoshan, alt. 1050 m, on twig, 14 Dec. 2000, *Z.H. Yu*, *W.Y. Zhuang* & *Y.H. Zhang* 3839, HMAS 266599; Henan, Xinyang, Jigongshan, alt. 250 m, on twig, 13 Nov. 2003, *W.Y. Zhuang*, *C.Y. Liu* & *Y. Nong* 4357, HMAS 252534; Yunnan, Mengla, Xishuangbanna, alt. 600 m, on rotten twig, 30 Sept. 1993, *Y. Doi* D'93-27.51, HKAS 26273; Yunnan, Pingbian, Daweishan, alt. 1000 m, on twig, 5 Nov. 1999, *W.Y. Zhuang* & *Z.H. Yu* 3335, HMAS 73226; Zhejiang, Lin'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 chromospermum P. Chaverri & Samuels, Stud. Mycol. 48: 51. 2003

= *Hypocrea chromosperma* M.A. Curtis & Peck, Rep. New York State Mus. Nat. Hist.: 56. 1878.

Specimens examined. China, Anhui, Jinzhai, Tiantangzhai, alt. 900–1100 m, on rotten twig, 23 Aug. 2011, *S.L. Chen*, *W.Y. Zhuang*, *H.D. Zheng* & *Z.Q. Zeng* 7780, HMAS 252536, culture HMAS 244968; Anhui, Nanjing Normal University, Xianlin campuses, alt. 200 m, on rotten twig, 25 July 2011, *H.D. Zheng* & *Z.Q. Zeng* 7896, HMAS 252537, culture HMAS 244969; Beijing, Donglingshan, alt. 1150 m, on wood, 6 Aug. 2002, *Y.Z. Wang*, HMAS 76657; Henan, Jiaozuo, Yuntaishan, alt. 800 m, on rotten twig, 24 Sept. 2013, *Z.X. Zhu*, *H.D. Zheng* & *Z.Q. Zeng* 8890, HMAS 252535, culture HMAS 244970; Hunan, Zhangjiajie, Jinbianxi, alt. 800 m, on twig, 17 Aug. 2010, *W.Y. Zhuang*, *J. Luo* & *P. Zhao* 7555, HMAS 266602; Jilin, Changchun, Jingyuetan, 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, Dunhua, 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 rotten twig, 22 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* 8085, HMAS 252539, culture HMAS 244973; Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten bark, 24 July 2012, *W.Y. Zhuang*, *Z.X. Zhu*, *Z.Q. Zeng*, *H.D. Zheng* & *F. Ren* 8145, HMAS 266604, culture HMAS 244974; Jilin, Jiaohe, Qianjin Forestry Farm, alt. 450 m, on rotten bark, 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 chromospermum* is very common in China. It is characterised by pulvinate to somewhat flattened and pale yellow stromata, with obvious ostiolar openings, and gliocladium-like conidiophores. This species is often misidentified 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

= *Hypocrea cremea* P. Chaverri & Samuels, Mycologia 95: 1115. 2003.

Specimen examined. China, Guangdong, Xinyi, Dawuling, 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-ascospores 4–4.5 × 3.5–4 µm vs 5.5–6.0 × 5–5.5 µm, smaller proximal part-ascospores 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

= *Hypocrea phyllostachydis* P. Chaverri & Cand., Mycol. Progr. 3: 33. 2004.

Specimen examined. China, 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* 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 substrata 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* Alb. & Schwein. 1805.

= *Hypocrea 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 monomorphic part-ascospores, but differs in having larger asci 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

= *Hypocrea 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* 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* 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 asci (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 intraspecific variation.

Accepted species previously reported from China

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

= *Hypocrea nigricans* (S.Imai) Yoshim. Doi, Bull. Natl. Sci. Mus. Tokyo 15: 732. 1972.

Specimens examined. China, Anhui, Jinzhai, Tiantangzhai, alt. 900–1100 m, on rotten twig, 23 Aug. 2011, *S.L. Chen*, *W.Y. Zhuang*, *H.D. Zheng* & *Z.Q. Zeng* 7790, culture HMAS 244981; Guizhou, Jiangkou, Fanjingshan, alt. 1800 m, on rotten twig, 21 Sept. 1995, *P.G. Liu* D'95-14, HKAS 29673 (previously filed under *H. nigricans*); Hainan, Ledong, Jianfengling, alt. 1100 m, on twig, 9 Dec. 2000, *Z.H. Yu*, *W.Y. Zhuang* & *Y.H. Zhang* 3746, HMAS 266610; Hainan, Tongzha, 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. nigricans*).

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, 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* 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

= *Hypocrea aureoviridis* Plowr. & Cooke, Grevillea 8: 104. 1880.

Specimen 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

= *Hypocrea gelatinosa* (Tode) Fr., Summa Veg. Scand.: 383. 1849.

Specimen examined. China, Jilin, Antu, Changbaishan, 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 longipilosa* Jaklitsch, Stud. Mycol. 63: 62. 2009.

Specimen examined. China, Guangdong, Xinyi, Dawuling, alt. 1400 m, on rotten wood, 22 Oct. 1998, *W.Y. Zhuang* & *Z.H. Yu* 2813, HMAS 252544.

Notes — This species can be easily recognised by incarnate 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 Liaoning 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. China, 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. China, Guangxi, Longlin, Jiuwei, alt. 1200 m, on rotten wood, 5 Nov. 1957, *L.W. Xu* 757, HMAS 30759; Hainan, Tongzha, Wuzhishan, 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 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. epimyces*, *T. parepimyces*, *T. alni*, *T. tawa*, *T. brunneoviride*, *T. catoptron*, *T. dacrymycellum*, *T. cinnamomeum*, *T. stramineum*, *H. atrogelatinosa*, *H. lixii*, *T. ceraceum*, *T. cerinum* 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 asci, 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. chlorosporum*, *T. thelephoricola* and *H. costaricensis*. 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. nigrovirens* (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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