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ORIGINAL ARTICLE

Four new species of *Trichoderma* with hyaline ascospores from central China

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Abstract Collections of *Trichoderma* producing hyaline ascospores from central China were examined. Four new species, *Trichoderma asterineum*, *T. henanense*, *T. odoratum* and *T. pseudobritdaniae*, were discovered, described and illustrated. Their phylogenetic positions were explored based on sequence analyses of the combined RNA polymerase II subunit b (*rpb2*) and translation elongation factor 1 alpha (*tef1*) genes. As a sister of *T. leguminosarum*, *T. asterineum* can be easily recognised by its pale yellow stromata, ochre to brown ostiolar dots surrounded by stellate cracks, green conidia and slow growth. *Trichoderma henanense* is distinctive in pulvinate or discoid, dirty yellow to brownish yellow stromata, brown to dark brown ostiolar dots, small monomorphic ascospores in relatively short asci and white colonies with dense aerial hyphae in cultures. *Trichoderma odoratum* forms an independent lineage as a sister of *T. henanense* and is characterised by yellow to greyish yellow, pulvinate stromata with dark brown or reddish brown projecting ostiolar dots, slow growth, trichoderma- to verticillium-like conidiophores, hyaline conidia and producing a mushroom-like odour in culture. *Trichoderma pseudobritdaniae* is closely associated with but easily separated from *T. britdaniae* in pulvinate, brownish yellow or greyish yellow stromata with dark brown or grey black ostiolar dots, relatively large perithecia, monomorphic ascospores, somewhat low growth rate, trichoderma- to verticillium-like conidiophores and hyaline conidia. Morphological distinctions and sequence divergences between the new species and their close relatives are discussed.

Keywords Hypocreaceae · Phylogeny · *rpb2* · Taxonomy · *tef1*

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Introduction

The genus *Trichoderma* Pers. (Ascomycota, Sordariomycetes, Hypocreales) is cosmopolitan, often grows on decaying wood or exists as soil inhabitants, and occasionally lives as one of the most abundant endophytes in stems of woody plants (Evans et al. 2003; Mahesh et al. 2005; Crozier et al. 2006; Gond et al. 2007; Verma et al. 2007; Gazis and Chaverri 2010), as well as saprophytes and parasites of other fungi (Samuels et al. 2002; Samuels et al. 2006; Druzhinina et al. 2011). *Trichoderma* is noteworthy for its important roles in promoting plant growth (Bae and Knudsen 2005; Vargas-Garcia et al. 2005), producing useful secondary metabolites (Samuels 1996; dos Reis Almeida et al. 2007; Degenkolb et al. 2008; Cheng et al. 2012; Lopes et al. 2012; Mukherjee et al. 2013) and remediating soil contaminated by heavy metals (Chaverri et al. 2003a; Chaverri and Samuels 2003; Harman et al. 2004). In particular, as biocontrol agents of plant pathogens, some have been commercialised (Verma et al. 2007). However, several *Trichoderma* species are threatening to the food industry, mushroom production and human health (Kredics et al. 2003; Wiater et al. 2005; Oda et al. 2009; Schuster and Schmoll 2010; Kim et al. 2012, 2013).

Trichoderma is characterised by perithecia immersed in fleshy stromata of a different colour, shape and size, producing cylindrical asci containing usually 16 disarticulating

hyaline or green part-ascospores which are uniseriately arranged, giving rise to conidiophores with several types of branch patterns on natural substrates or in culture, forming either hyaline or green conidia, and with or without chlamydospores. Monographic treatments of *Trichoderma* species were carried out by Jaklitsch (2009, 2011) and Jaklitsch and Voglmayr (2015). Ascospore colour has provided useful phylogenetic information based on morphology and multi-gene sequence analyses (Chaverri and Samuels 2003; Jaklitsch 2009, 2011). Five clades were established to accommodate species with green ascospores, which are named Ceramicum, Chlorosporum, Harzianum, Spinulosum and Strictipile. Nine clades contain mainly species with hyaline ascospores, i.e. Brevicompactum, Deliquescens, Hypocreanum, Longibrachiatum, Polysporum, Psychrophilum, Semiorbis, Stromaticum and Viride. Recognitions of clades in *Trichoderma* are generally based on phylogenetic analyses; however, the phenotypic features, such as stromatal shape and colour, ascospore colour, ostiole type, colony and conidiophore branch patterns, may be proved to be important. The size of clades varies significantly. Some contain only two or three species, while the largest one includes more than 60 species. For clades with a limited number of taxa, it is relatively easy to recognise morphological features shared by members within individual clades. But for complex clades, such as the Viride clade, morphology among species in a clade varies too much to establish a reasonable concept based solely on phenotypes. Integrated studies of morphology and sequence analysis are, thus, required.

Researches of *Trichoderma* in China have concentrated on its potential application and taxonomy (Chen 2014; Zhu and Zhuang 2014a). In taxonomic studies, the first Chinese record of the genus dates back to 1895 (Patouillard 1895), and 11 species were reported mainly from Southern China (Teng 1934, 1935, 1936, 1963). Later, two more species were described based on the Chinese materials, and five new records for China were added (Liu and Doi 1995; Liu et al. 2002, 2003; Zhang et al. 2007; Jaklitsch et al. 2013). Recently, Zhu and Zhuang (2014a) outlined the high species diversity of *Trichoderma* in China and summarised the 91 species currently known from the country. In the past two years, ten more new species were published, four new combinations were made and 23 species were found new to China upon examinations of collections from 18 provinces (Zhu and Zhuang 2014b, c, 2015a, b; Qin and Zhuang 2016a, b). This study updates our knowledge based on the more recent collections.

When the collections from central China were examined, four new species having hyaline ascospores, namely *T. asterineum*, *T. henanense*, *T. odoratum* and *T. pseudobritdaniae*, are discovered. Their morphology of sexual and asexual states were described, and their phylogenetic positions were ascertained based on sequence analyses of the combined RNA polymerase II subunit b (*rpb2*) and translation elongation factor 1 alpha (*tef1*) genes.

Materials and methods

Specimens and strains

Specimens examined were collected from Henan and Hubei provinces, China. They were deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS) and Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica (HKAS). Strains were obtained from single ascospore isolation.

Morphological study

Dried stromata were rehydrated and longitudinal sections were made with a freezing microtome (YD-1508-III, Yidi Medical Appliance Factory, Zhejiang, China) at a thickness of 8–10 μm. Colonies on cornmeal dextrose agar (CMD), potato dextrose agar (PDA) and synthetic low nutrient agar (SNA) were incubated in 90-mm-diameter Petri dishes with alternating light/darkness (12/12 h) at 25 °C and the radius was measured daily until mycelium was covering the dishes. The morphology of sexual and asexual states was described following Jaklitsch (2009) and Jaklitsch and Voglmayr (2015). Photographs were taken with a Zeiss AxioCam MRc 5 digital camera (Jena, Germany) connected to a Zeiss Imager A2 microscope (Göttingen, Germany) for anatomical structures and a Leica DFC450 digital camera (Wetzlar, Germany) connected to a Leica M125 stereomicroscope (Milton Keynes, UK) for gross morphology. The nomenclature of fungi follows the Melbourne Code (McNeill et al. 2012) and the generic name used is according to Rossman et al. (2013).

DNA extraction, amplification and sequencing

Genomic DNA was extracted from mycelia following the methods of Wang and Zhuang (2004). Fragments of *rpb2* were amplified using the primer pairs fRPB2-5f and fRPB2-7cr (Liu et al. 1999) or 5′-TTGKAAAGAARCGTCTGGAT-3′ and 5′-YRRCATACCTGGTTGTG-3′ (newly designed primers); fragments of *tef1* were amplified using the primer pair EF1-728F (Carbone and Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005). Polymerase chain reaction (PCR) conditions were following Zhu and Zhuang (2015b). The PCR products were purified with the PCR Product Purification Kit (Biocolor BioScience and Technology Co., Shanghai, China) and cycle-sequenced using the primer pairs reported by Jaklitsch (2009) on an ABI 3730xl DNA Sequencer (Applied Biosciences, Foster City, CA, USA) at Beijing

Tianyihuiyuan Bioscience and Technology, China. The sequences used in this study are provided in Table 1.

Phylogenetic analyses

The partition homogeneity test (PHT) was performed with PAUP 4.0b10 (Swofford 2002) to evaluate statistical congruence between the sequence data of *rpb2* and *tef1*. To locate the phylogenetic positions of the four new *Trichoderma* species, 42 combined sequences of *rpb2* and *tef1* were analysed with *Nectria berolinensis* and *N. eustromatica* as outgroup taxa. Among them, ten species having green ascospores and 19 species producing hyaline ascospores were in the named clades. Eleven species were scattered as terminal branches which do not assign to any named clades. The sequences were checked and visually adjusted where necessary using BioEdit 7.0.5 (Hall 1999), alignment was carried out by ClustalX 1.83 and a NEXUS file was subsequently generated (Thompson et al. 1997).

Maximum parsimony (MP) analysis was performed via PAUP 4.0b10 using a heuristic search with tree-bisection-reconnection branch swapping, with settings as follows: all characters were treated as unordered and unweighted, gaps treated as missing data, Maxtrees = 1000 and auto-increased. Clade stability was assessed by maximum parsimony bootstrap proportion (MPBP) with 1000 replicates, each with ten replicates of random stepwise addition of taxa.

Bayesian inference (BI) analysis was conducted by MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo (MCMC) algorithm. MrModeltest 2.3 (Nylander 2004) was used to determine the appropriate nucleotide substitution models and select the best-fit model by Akaike information criterion for the investigated dataset. Four MCMC chains (one cold and three heated) were run for 1,000,000 generations, with the trees sampled every 100 generations. The first 25 % of trees were excluded as the burn-in phase of the analyses, and the remaining trees were used for estimating Bayesian inference posterior probability (BIPP) values. Trees were viewed in TreeView 1.6.6 (Page 1996).

Taxonomy

Trichoderma asterineum W.T. Qin & W.Y. Zhuang, **sp. nov.** (Fig. 1) MycoBank: MB 813807

Etymology: The specific epithet refers to the stellate cracks surrounding ostiolar dots of the fungus.

Typification: China. Henan, Jiaozuo, Yuntaishan, 35°24′53″N, 113°22′22″E, alt. 800 m, on twig lying on the ground, 24 Sep 2013, H.D. Zheng, Z.Q. Zeng & Z.X. Zhu 8892 (holotype HMAS 271353, isotype HKAS 95057, ex-type culture HMAS 244996).

Stromata solitary, scattered or aggregated in a group of 2–3, pale yellow, pulvinate or lenticular, often narrowly attached, with rounded or widely free margin, ca. 1.5–3.5 mm diam., 0.7–1.0 mm thick ($n = 8$). Surface convex, comprising a thick chalky or calcareous, white, sometimes pale yellow covering layer, cracked into polygonal plates. Ostiolar dots ochre to brown, densely disposed, numerous, rounded, slightly projecting, convex, minute but distinct, surrounded by stellate cracks. Rehydrated stromata turning orange in 3 % KOH.

In section, cortical tissue of textura angularis mixed with textura intricata, cells hyaline, thin-walled, (3–)3.5–7.5(–8) × (2.5–)3.5–5(–5.5) μm ($n = 30$), hyphae hyaline, thin-walled, (2–)3–5(–6) μm ($n = 30$) wide; subcortical tissue of textura angularis mixed with textura intricata, cells hyaline, thin-walled, (4–)4.5–7.5(–8) × (2.5–)3–5 μm ($n = 30$), hyphae hyaline, thin-walled, 3.5–5(–8) μm ($n = 30$) wide; subperithecial tissue of textura angularis mixed with textura epidermoidea, cells hyaline, thin-walled, (6.5–)8–18(–32) × (5–)7.5–11(–21) μm ($n = 30$); tissue at the base of textura angularis, cells hyaline to light brown, thin-walled, (4–)7–17(–18) × (4–)6–10(–13) μm ($n = 30$). Perithecia subglobose or flask-shaped, crowded, numerous, (171–)197–224(–237) × (118–)124–171(–176) μm ($n = 30$); peridium light yellow in lactic acid, turning pale tan in 3 % KOH, 10–13(–16) μm thick at flanks, (8–)11–16(–18) μm thick at the base ($n = 30$). Ostioles projecting up to 28 μm, (47–)53–68(–74) μm high, 32–40(–45) μm wide at the apex ($n = 30$). Asci cylindrical, (70–)72–77(–79) × 4–5(–5.5) μm, with a stipe 5–10(–11) μm long ($n = 40$). Ascospores hyaline, verruculose or spinulose, dimorphic, distal cells subglobose to slightly ovoid, 3–4(–4.5) × 3–3.5(–4) μm, l/w 1.0–1.3(–1.4); proximal cells globose, subglobose to nearly wedge-shaped, (3–)4–5 × 2.5–3 μm, l/w 1.3–1.7(–1.9) ($n = 50$).

Colony on CMD 4–6 mm in radius after 72 h at 25 °C, 28–33 mm after 25 days. Colony hyaline, dense, with lobed or irregular outline; aerial hyphae inconspicuous. Conidiation noted after 10–15 days, first effuse in minute shrubs, later in numerous minute granules and pustules with granulose or plumose surface, turning pale green from the centre, additional new pustules produced consecutively in distal areas. Conidiophores mostly asymmetrically arranged, densely disposed. Phialides often asymmetric, straight, narrowly lageniform or subulate, less commonly curved or sinuous, divergent in whorls of 2–3(–5), also solitary or paired along conidiophores, (7–)9–16(–22) × 2–3(–4) μm, l/w (2.1–)2.6–6.4(–9.1), (1–)1.5–2.5(–3) μm wide at the base ($n = 50$). Conidia green, mostly ellipsoidal, less commonly subglobose or oblong, smooth, (2.5–)3–4.5(–5.5) × 2–3, l/w 1.0–1.6(–2.1) ($n = 50$). Chlamydospores noted after 2 weeks, terminal or intercalary, smooth, globose, ellipsoidal or fusoid, (5–)6–10(–16) × (5–)6–8(–9), l/w 1.0–1.3(–2.2) ($n = 50$). No distinct odour, no diffusing pigment observed.

Table 1 Materials of *Trichoderma* species used in the phylogenetic analyses

Species	Strain	GenBank accession numbers	
		rpb2	*tef1*
Trichoderma alcalifuscescens (Overton) Jaklitsch & Voglmayr	TFC 00-36	–	FJ860610
	TFC 181548	DQ834462	–
T. asterineum W.T. Qin & W.Y. Zhuang	HMAS 271353	KT224469	KT224465
T. austriacum Jaklitsch	CBS 122494	FJ860525	FJ860619
T. britdaniae (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr	WU 31610	JQ685880	JQ685866
T. ceriunum Bissett, C.P. Kubicek & Szakács	CBS 120637	FJ860532	FJ860629
T. citrinoviride Bissett	S20	KJ665250	KJ665449
T. cuneisporum P. Chaverri & Samuels	GJS 91-93	AF545512	AF534600
T. danicum Jaklitsch	CBS 121273	FJ860534	FJ860634
T. deliquescens (Sopp) Jaklitsch	CBS 121132	–	FJ860644
	CBS 121131	FJ179609	–
T. estonicum P. Chaverri & Samuels	GJS 96-129	AF545514	AF534604
T. euskadiense Jaklitsch & Voglmayr	S377	KJ665269	KJ665492
T. flavipes (Peck) Seifert, Jaklitsch & Voglmayr	GJS 92-102	DQ834461	DQ834454
T. henanense W.T. Qin & W.Y. Zhuang	HMAS 252889	KT224467	KT224464
T. leguminosarum Jaklitsch & Voglmayr	S391	KJ665287	KJ665548
T. luteocrystallinum Jaklitsch	CBS 123828	FJ860544	FJ860646
T. margaretense Jaklitsch	CPK 3127	FJ860529	FJ860625
T. moravicum Jaklitsch	CPK 2489	FJ860549	–
	CBS 120539	–	FJ860651
T. odoratum W.T. Qin & W.Y. Zhuang	HMAS 271354	KT224468	KT224463
T. orientale (Samuels & Petrini) Jaklitsch & Samuels	S187	JQ685884	JQ685868
T. parestonicum Jaklitsch	CBS 120636	FJ860565	FJ860667
T. polysporum (Link) Rifai	CPK 3131	FJ860558	FJ860661
T. pseudobritdaniae W.T. Qin & W.Y. Zhuang	HMAS 271355	KT224466	KT224462
T. psychrophilum Jaklitsch	CPK 2435	FJ860576	FJ860682
T. rhododendri (Jaklitsch) Jaklitsch & Voglmayr	CBS 119288	FJ860578	FJ860685
T. rodmanii (Samuels & P. Chaverri) Jaklitsch & Voglmayr	CBS 121553	FJ860580	FJ860687
T. rossicum Bissett, C.P. Kubicek & Szakács	S334	KJ665335	KJ665700
T. sambuci (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr	WU 29467	FJ860585	FJ860693
T. semiorbis (Berk.) Jaklitsch & Voglmayr	DAOM 167636	AF545522	AF545568
T. sinuosum P. Chaverri & Samuels	CPK 1595	FJ179619	FJ860697
T. spinulosum Fuckel	CBS 121272	FJ860590	FJ860700
T. stellatum (B.S. Lu, Druzhin. & Samuels) Jaklitsch & Voglmayr	GJS 99-222	KJ665349	KJ665741
T. strictipile Bissett	CPK 1601	FJ860594	FJ860704
T. stromaticum Samuels & Pardo-Schulth.	GJS 97-183	HQ342245	AY937418
T. subalpinum Jaklitsch	CPK 3126	FJ860596	FJ860706
T. thelephoricola P. Chaverri & Samuels	CBS 120925	FJ860600	FJ860711
T. tomentosum Bissett	S33	KF134793	KF134801
T. tremelloides Jaklitsch	CBS 120634	FJ860602	FJ860713
T. victoriense (Overton) Jaklitsch & Voglmayr	GJS 99-130	EU338336	EU338331
T. viridarium Jaklitsch, Samuels & Voglmayr	S136	KC285760	KC285658
T. viridialbum Jaklitsch, Samuels & Voglmayr	S250	KC285774	KC285706
Nectria berolinensis (Sacc.) Cooke	CBS 127382	HM534883	HM534872
N. eustromatica Jaklitsch & Voglmayr	CBS 121896	HM534886	HM534875

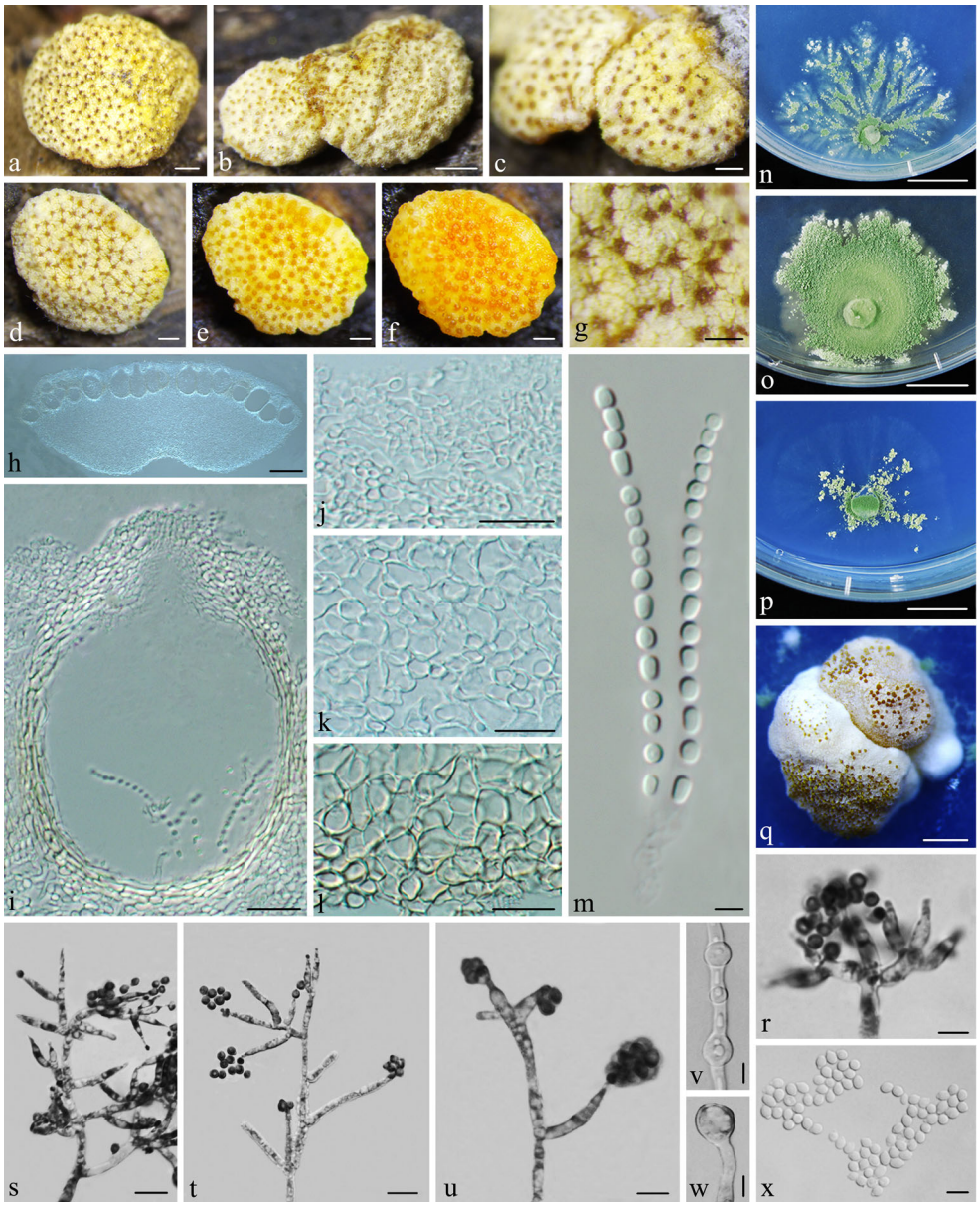

Fig. 1 *Trichoderma asterineum* (holotype HMAS 271353). **a**–**m** Sexual state. **a**–**d** Dry stromata on natural substrates. **e** Mature stroma after rehydration. **f** Mature stroma in 3 % KOH after rehydration. **g** Surface of dry stroma showing ostioles. **h** Longitudinal section of a stroma. **i** Perithecium in section. **j** Cortical and subcortical tissues in section. **k** Subperithecial tissue in section. **l** Stroma base in section. **m** Asci with ascospores. **n**–**x** Asexual state. **n**–**p** Cultures after 25 days at 25 °C (**n** CMD, **o** PDA, **p** SNA). **q** Aggregated young stromata (CMD, 26 days). **r**–**u** Conidiophores and phialides (CMD, 25 days). **v**–**w** Chlamydospores (CMD, 25 days). **x** Conidia (CMD, 25 days). Scale bars: **a**, **c**–**f**, **h** = 200 μm. **b** = 400 μm. **g** = 100 μm. **i** = 30 μm. **j**–**l** = 20 μm. **m**, **r**, **u**, **v**–**x** = 5 μm. **n**–**p** = 10 mm. **q** = 1 mm. **s**, **t** = 10 μm

Colony on PDA 22–26 mm in radius after 25 days at 25 °C. Colonies green, dense, with little mycelium on agar surface. Conidiation noted after 16 days. No distinct odour, no diffusing pigment observed.

Colony on SNA 16–20 mm in radius after 25 days at 25 °C. Colony subhyaline, circular, conidiation noted after 20 days, mostly around the plug, first yellowish green, finally green. No distinct odour, no diffusing pigment observed.

Notes: *Trichoderma asterineum* is most similar to *T. leguminosarum*. Both species share scattered pale yellow stromata with ochre to brown ostiolar dots surrounded by stellate cracks that are unknown in any other species of the genus, slow growth in cultures at 25 °C, and green dense colony with a lobed or irregular outline on CMD (Jaklitsch and Voglmayr 2015). They can be separated from each other by the colour of rehydrated stromata in 3 % KOH, colonies on PDA, growth rates and some other characteristics detailed in Table 2. In our phylogenetic analyses, *T. asterineum* formed a well-supported terminal branch associated with *T. leguminosarum* (Fig. 5). As to sequence divergence, their *rpb2* sequences are similar, while 21-bp differences among 856 bp for *acl1* (unpublished data) and 21-bp differences among 1193 bp for *tef1* were detected, which indicates that the two fungi are not conspecific.

Trichoderma henanense W.T. Qin & W.Y. Zhuang, **sp. nov.** (Fig. 2) MycoBank: MB 813808

Etymology: The specific epithet refers to the locality of the fungus.

Typification: China. Henan, Jiaozuo, Yuntaishan, 35°24′53″N, 113°22′22″E, alt. 800 m, on twig lying on the ground,

Table 2 Major morphological comparison between *Trichoderma asterineum* and *T. leguminosarum*

Character	Species	
	T. leguminosarum (Jaklitsch and Voglmayr 2015)	*T. asterineum* (this study)
Colour of rehydrated stromata in 3 % KOH	Pale red	Orange
Size of asci		
Length (μm)	(53–)58–72(–87)	(70–)72–77(–79)
Width (μm)	(3.8–)4.0–4.5(–5.0)	4–5(–5.5)
Colour of colonies on PDA at 25 °C	Brown, covered by whitish floccules	Green, aerial hyphae inconspicuous
Colony radius after 72 h at 25 °C (mm)		
PDA	19–22 (1 month)	22–26 (25 days)
SNA	34–37 (1 month)	16–20 (25 days)
CMD	4–6 (72 h), 35–37 (19 days)	4–6 (72 h), 28–33 (25 days)
Stromata formed in cultures		
Colour	Ochre to medium brown	Cream to pale yellow
Time	After 18 months at 15 °C (in one instance)	After 14 days on CMD or 20 days on PDA at 25 °C

24 Sep 2013, H.D. Zheng, Z.Q. Zeng & Z.X. Zhu 8889 (holotype HMAS 252889, isotype HKAS 95055, ex-type culture HMAS 244997).

Stromata solitary, scattered or gregarious in small groups, pulvinate, discoid or subturbinate, centrally attached and with margin free, outline rounded, angular or irregular, dirty yellow to brownish yellow, 1.5–3.5 mm diam., 0.9–1.0 mm thick ($n = 20$). Surface usually farinose or granular due to spore deposits, rarely smooth. Ostiolar dots brown to dark brown, distinct, densely distributed. Rehydrated stromata turning brownish red in 3 % KOH.

In section, cortical tissue of textura angularis, 8–16 μm thick, cells light yellow, thin-walled, (4.5–)6–8.5(–10.5) × (4–)4.5–8(–10) μm ($n = 30$), turning brownish yellow in 3 % KOH; subcortical tissue of textura intricata, hyphae hyaline, thin-walled, (2–)2.5–3 μm ($n = 30$) wide; subperithecial tissue of textura epidermoidea, cells hyaline, thin-walled, 8–19(–24) × 7.5–14(–19) μm ($n = 30$) wide; tissue at the base of textura angularis mixed with textura intricata, cells hyaline, thin-walled, (5–)6–13(–16) × (4–)5–10(–12) μm ($n = 30$), hyphae hyaline, thin-walled, (3.5–)4–5(–6) μm ($n = 30$) wide. Perithecia flask-shaped or subglobose, numerous, (174–)179–200(–216) × (100–)105–158(–197) μm ($n = 30$); peridium hyaline or light yellow in lactic acid, turning pale tan or slightly darkened in 3 % KOH, 8–13 μm thick at flanks ($n = 30$), 11–18(–24) μm thick at the base. Ostioles non-papillate, (40–)42–50(–53) μm high, (18–)21–29(–32) μm wide at the apex ($n = 30$). Asci cylindrical, (54–)57–64 (–67) × 4–4.5 μm, with a stipe 5–8(–9) μm long ($n = 40$). Ascospores hyaline, spinulose or verruculose, cells monomorphic, globose, subglobose or rarely ellipsoidal, sometimes part-ascospores in the ascus base dimorphic, oblong or cuneate; distal cells 2–2.5(–3) × 2–2.5 μm, l/w 1.0–1.2(–1.5); proximal cells (2–)2.5–3(–4) × 2–2.5(–3) μm, l/w (1.0–)1.1–1.5(–1.6) ($n = 50$).

Colony on CMD 29–31 mm in radius after 72 h at 25 °C, mycelium covering the plate after 7 days. Colony hyaline, circular, dense, conspicuously zonate; aerial hyphae dense and short, longer towards the distal margin. Odour slightly fruity, no diffusing pigment observed.

Colony on PDA 19–21 mm in radius after 72 h at 25 °C, mycelium covering the plate after 14 days. Colony whitish, dense and compact, with thin, diffuse margin. Surface downy, farinose to floccose, macroscopically homogeneous with irregularly zonate near the plug. Odour slightly fruity, no diffusing pigment observed.

Colony on SNA 30–32 mm in radius after 72 h at 25 °C, mycelium covering the plate after 7 days. Colony hyaline, radially fan-shaped. Aerial hyphae inconspicuous, appearing empty. Conidiophores sparsely disposed, noted after 10 days, with 1–2 whorls arising from the main axis. Phialides mostly asymmetrically arranged, narrowly subulate or slender, (12–)13–23(–26) × (2–)2.5–3(–3.5) μm, l/w (4.5–)5–9(–9.5), 2–3 μm wide at the base ($n = 50$). Conidia hyaline, subglobose or ellipsoidal, smooth, (2.5–)3–5(–6) × 2–3(–3.5) μm, l/w (1.0–)1.1–2.0(–2.5) ($n = 50$). Chlamydospores distinctly abundant, noted after 8–10 days, terminal and intercalary, globose or ellipsoidal, (3–)4–8(–10) × (3–)4–6(–7) μm, l/w 1.0–1.4(–1.6) ($n = 50$). Autolytic excretions abundant, no distinct odour, no diffusing pigment observed.

Notes: *Trichoderma henanense* is most similar to *T. odoratum* but they represent different species. Both of them form yellowish stromata with brownish ostiolar dots, monomorphic ascospores, white colonies and hyaline conidia. However, *T. odoratum* differs in projecting ostioles, larger perithecia, longer asci, smaller growth rate, larger conidia and the presence of a mushroom-like odour in cultures.

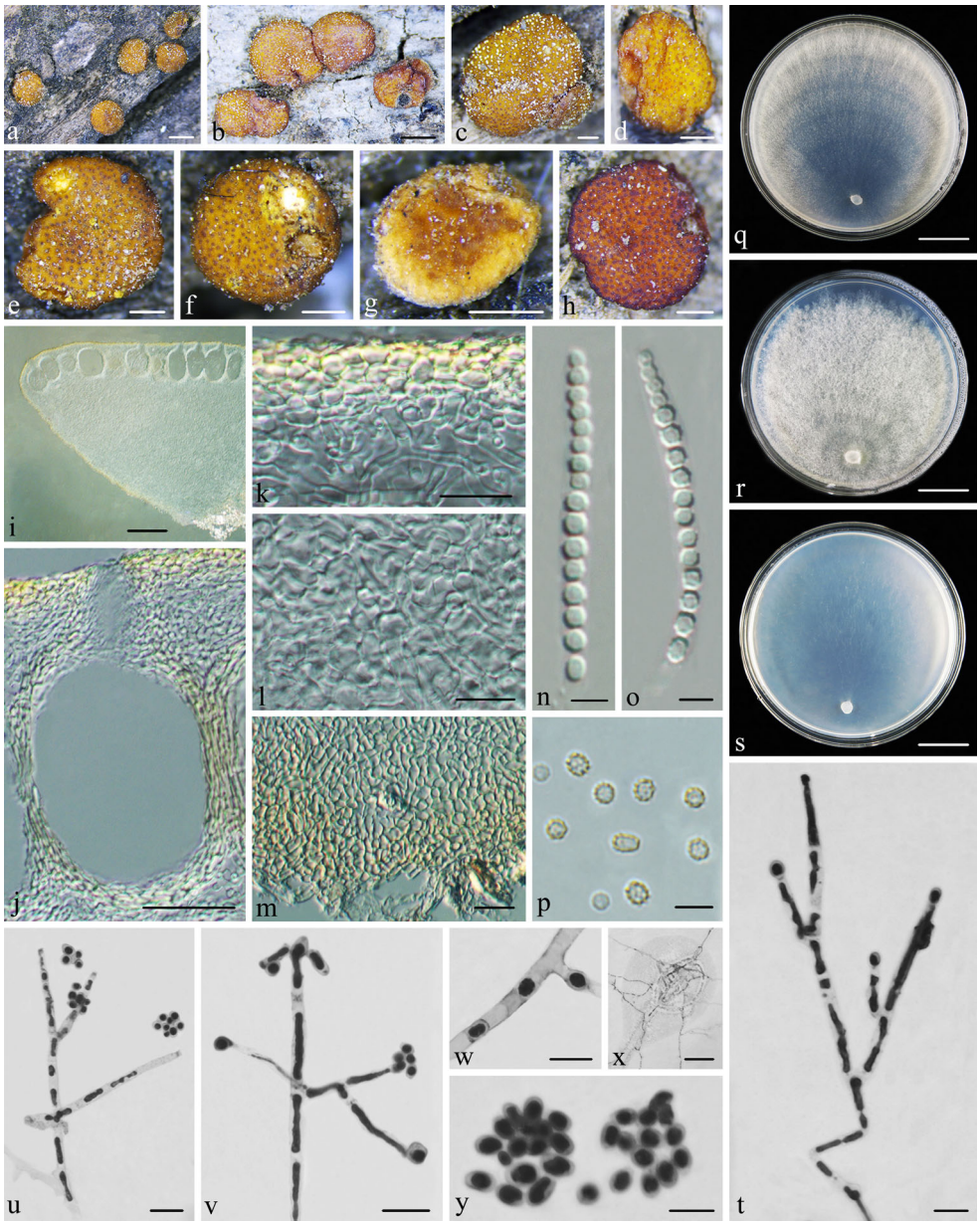

Fig. 2 *Trichoderma henanense* (holotype HMAS 252891). **a**–**p** Sexual state. **a**–**g** Dry stromata on natural substrates (**a**–**f** mature, **g** immature). **h** Mature stroma in 3 % KOH after rehydration. **i** Longitudinal section of a stroma. **j** Perithecium in section. **k** Cortical and subcortical tissues in section. **l** Subperithecial tissue in section. **m** Stroma base in section. **n**–**p** Asci with ascospores. **q**–**y** Asexual state. **q**–**s** Cultures after 13 days at 25 °C (**q** CMD, **r** PDA, **s** SNA). **t**–**v** Conidiophores and phialides (SNA, 13 days). **w** Chlamydospores (SNA, 13 days). **x** Autolytic excretion (SNA, 13 days). **y** Conidia (SNA, 13 days). Scale bars: **a** = 1 mm. **b** = 0.8 mm. **c**–**h** = 400 μm. **i** = 200 μm. **j**, **x** = 50 μm. **k**–**m** = 20 μm. **n**–**p**, **y** = 5 μm. **q**–**s** = 20 mm. **t**–**w** = 10 μm

Detailed morphological distinctions between the two species are shown in Table 3.

Phylogenetically, *T. henanense* formed a branch with *T. odoratum*; however, the sequence similarity of *rpb2* and *tef1* between them was only 93.14 % and 94.67 %, respectively.

Trichoderma odoratum W.T. Qin & W.Y. Zhuang, **sp. nov.** (Fig. 3) MycoBank: MB 813809

Etymology: The specific epithet refers to the distinctive odour produced by the fungus.

Typification: China. Hubei, Shennongjia, Muchengshaoka, 31°27′41″N, 110°30′27″E, alt. 860 m, on twig lying on the ground, 22 Sep 2014, W.T. Qin, K. Chen, H.D. Zheng & Z.Q. Zeng 10035 (holotype HMAS 271354, isotype HKAS 95058, ex-type culture HMAS 244998).

Stromata solitary, scattered or aggregated in small fascicles, yellow or greyish yellow, pulvinate or discoid with rounded margin, 1.5–3 mm diam., 0.5–0.8 mm thick ($n = 15$). Surface usually smooth, occasionally farinose or granular due to spore deposits. Ostiolar dots dark brown or reddish brown, distinct, densely distributed. Rehydrated stromata turning brownish red in 3 % KOH.

In section, cortical tissue of textura angularis, 13–21 μm thick, cells yellow, thin-walled, (4–)5.3–7.9(–12) × (3.7–)4–5.5(–7.9) μm ($n = 30$), turning dark orange red to brownish red in 3 % KOH; subcortical tissue of textura intricata, hyphae hyaline, thin-walled, (1.3–)2–3(–3.7) μm ($n = 30$) wide; subperithecial tissue of textura epidermoidea, cells hyaline, thin-walled, (6–)8–15(–16) × (6–)7.4–11 μm ($n = 30$); tissue at the base of textura angularis mixed with textura intricata,

Table 3 Morphological comparisons of *Trichoderma pseudobritdaniae* with *T. britdaniae* and *T. henanense* with *T. odoratum*

Character		Species			
		T. britdaniae (Jaklitsch and Voglmayr 2012)	*T. pseudobritdaniae* (this study)	*T. henanense* (this study)	*T. odoratum* (this study)
Stromata					
Colour		Light to medium brown, sometimes reddish brown	Brownish yellow or greyish yellow	Dirty yellow to brownish yellow	Yellow or greyish yellow
Colour in 3 % KOH		Orange-red, finally brown	Brownish red	Brownish red	Brownish red
Shape		Scattered or undulate	Pulvinate	Pulvinate or discoid	Pulvinate or discoid
Diam. (mm)		Up to 100	2–3.5(–5)	1.5–3.5	1.5–3
Thickness (mm)		Up to 3	0.7–1.0	0.9–1.0	0.5–0.8
Ostioles					
Colour		Dark brown or reddish brown with light centre	Dark brown or grey black	Brown to dark brown	Dark brown or reddish brown
Projecting (μm)		Not	Not	Not	(8–)16–21
Length (μm)		(46–)54–68(–73)	(53–)55–66	(40–)42–50(–53)	53–66(–74)
Width at the apex (μm)		(11–)15–32(–43)	26–37(–40)	(18–)21–29(–32)	(29–)32–40(–42)
Perithecia					
Colour of peridium		Yellow	Pale yellow brown	Hyaline or light yellow	Yellow
Colour of peridium in 3 % KOH		–	Dark orange	Pale tan or slightly darkened	Dark orange red to brownish red
Length (μm)		(174–)200–235(–255)	(195–)224–263(–283)	(174–)179–200(–216)	184–224(–227)
Width (μm)		(60–)80–125(–165)	(111–)119–142(–171)	(100–)105–158(–197)	(111–)116–145
Asci					
Length (μm)		(52–)60–75(–86)	(62–)68–82 (–89)	(54–)57–64(–67)	(63–)65–74 (–77)
Width (μm)		(3.7–)4.0–5.0(–5.5)	4.2–5(–5.5)	4–4.5	3.4–3.8(–4)
Length of stipe (μm)		(1–)7–16(–23)	(10–)12–29(–31)	5–8(–9)	(11–)12–20
Ascospores					
Colour		Hyaline	Hyaline	Hyaline	Hyaline
Distal	Length (μm)	(2.0–)2.5–3.0(–3.8)	2.8–3.5	2–2.5(–3)	2.4–3.1
	Width (μm)	(2.0–)2.5–3.0(–3.5)	(2.2–)2.5–3.2	2–2.5	2.2–2.8
	l/w	0.9–1.1(–1.4)	1.0–1.5	1.0–1.2(–1.5)	1.0–1.2(–1.3)
Proximal	Length (μm)	(2.0–)2.5–3.0(–3.8)	(2.4–)2.9–3.7(–4.2)	(2–)2.5–3(–4)	2.4–3.8(–4.1)
	Width (μm)	(2.0–)2.5–3.0(–3.5)	2.1–3.2	2–2.5(–3)	2.1–2.7
	l/w	0.9–1.1(–1.4)	1.0–1.5(–1.9)	(1.0–)1.1–1.5(–1.6)	(1.0–)1.1–1.5(–1.6)
Colony radius after 72 h at 25 °C (mm)					
CMD		–	15–18	29–31	19–22
PDA		–	3–5	19–21	5–7
SNA		–	13–15	30–32	19–22
Phialides					
Length (μm)		–	(7–)8.5–18(–20)	(12–)13–23(–26)	(10.5–)11–18 (–21)
Width (μm)		–	(2.5–)2.8–4(–4.5)	(2–)2.5–3(–3.5)	(2–)2.5–3(–4)
l/w		–	(2.1–)2.7–4.7(–7.2)	(4.5–)5–9(–9.5)	(3.3–)3.9–6(–6.6)
Basal width (μm)		–	1.3–3.2(–3.5)	2–3	1.8–2.6(–3.2)
Conidia					
Colour		–	Hyaline	Hyaline	Hyaline
Length (μm)		–	(2.5–)3–7(–7.5)	(2.5–)3–5(–6)	(2.5–)2.8–7.2(–8.7)
Width (μm)		–	(2.5–)3–4(–4.5)	2–3(–3.5)	2.5–3(–3.5)
l/w		–	1.0–1.9(–2.1)	(1.0–)1.1–2.0(–2.5)	(1.0–)1.2–2.9(–3.5)
Chlamydospores					
Length (μm)		–	(4–)5–12	(3–)4–8(–10)	(6.5–)7.2–11.5(–14.4)
Width (μm)		–	4–10	(3–)4–6(–7)	(5.5–)6.5–8.2(–9.5)

Table 3 (continued)

Character	Species				
	T. britdaniae (Jaklitsch and Voglmayr 2012)	*T. pseudobritdaniae* (this study)	*T. henanense* (this study)	*T. odoratum* (this study)
l/w	–	1.0–1.3	1.0–1.4(–1.6)	1.0–1.4(–1.9)
Odour produced on CMD, PDA	no	no	Slightly fruity	Mushroom-like

cells hyaline, thin-walled, (5.3–)7.4–10.5(–11.3) × 5.3–7.9 μm ($n = 30$), hyphae hyaline, thin-walled, (2.8–)3–5.3 μm ($n = 30$) wide. Perithecia subglobose or flask-shaped, 184–224(–227) × (111–)116–145 μm ($n = 30$); peridium yellow in lactic acid, turning dark orange red to brownish red in 3 % KOH, 5.8–8(–11) μm thick at flanks, 6.6–10.5(–13.2) μm thick at the base ($n = 30$). Ostioles projecting by (8–)16–21 μm, 53–66(–74) μm high, (29–)32–40(–42) μm wide at the apex ($n = 30$). Asci cylindrical, (63–)65–74 (–77) × 3.4–3.8(–4) μm, with a stipe (11–)12–20 μm long ($n = 40$). Ascospores hyaline, spinulose or verruculose, cells monomorphic, globose, subglobose or ellipsoidal, sometimes part-ascospores in the ascus base dimorphic, oblong or cuneate; distal cells 2.4–3.1 × 2.2–2.8 μm, l/w 1.0–1.2(–1.3); proximal cells 2.4–3.8(–4.1) × 2.1–2.7 μm, l/w (1.0–)1.1–1.5(–1.6) ($n = 50$).

Colony on CMD 19–22 mm in radius after 72 h at 25 °C, mycelium covering the plate after 10 days. Colony hyaline, thin, circular, dense, with indistinct light/dense and darker/looser concentric zones; denser zones slightly wider. Aerial hyphae apparent toward the downy or floccose distal margin, becoming fertile. No conidiation noted within 20 days at 25 °C. Chlamydospores noted after 12–15 days, terminal or intercalary, smooth, globose or ellipsoidal, (4.8–)5–9 (–11.5) × (4.5–)5–8 μm, l/w 1.0–1.4(–1.8) ($n = 50$). Autolytic excretions rare, coilings numerous, odour mushroom-like, no diffusing pigment noted.

Colony on PDA 5–7 mm in radius after 72 h at 25 °C, 37–39 mm after 20 days. Colony white, not zonate, hyphae densely disposed with a well-defined or slightly wavy margin. Conidiation noted after 7 days, starting on and around the plug in short minute shrubs, spreading, growing to tufts or pustules, white, effuse, farinose, floccose or cottony. Trichoderma-like to verticillium-like conidiophores formed widely spaced on aerial hyphae. Phialides solitary or commonly divergent in whorls of 2–3, lageniform to subulate, commonly slender, (10.5–)11–18(–21) × (2–)2.5–3(–4) μm, l/w (3.3–)3.9–6(–6.6), 1.8–2.6(–3.2) wide at the base ($n = 50$). Conidia hyaline, variable in shape, mostly oblong to cylindrical, also ellipsoidal, sometimes subglobose or oval, smooth, (2.5–)2.8–7.2(–8.7) × 2.5–3(–3.5) μm, l/w (1.0–)1.2–2.9(–3.5) ($n = 50$). Chlamydospores abundant, noted after 8 days, terminal or intercalary, smooth, globose or ellipsoidal, (6.5–)7.2–11.5(–14.4) × (5.5–)6.5–8.2(–9.5) μm, l/w 1.0–1.4(–1.9) ($n = 50$). Odour mushroom-like, no diffusing pigment observed.

Colony on SNA 19–22 mm in radius after 72 h at 25 °C, mycelium covering the plate after 10 days. Colony hyaline, thin, hardly visible, smooth, radially fan-shaped, not zonate, hyphae loosely disposed. No conidiation noted within 20 days at 25 °C. Chlamydospores noted after 10 days. Autolytic activity moderate, excretions minute, coilings numerous, odour mushroom-like, no diffusing pigment noted.

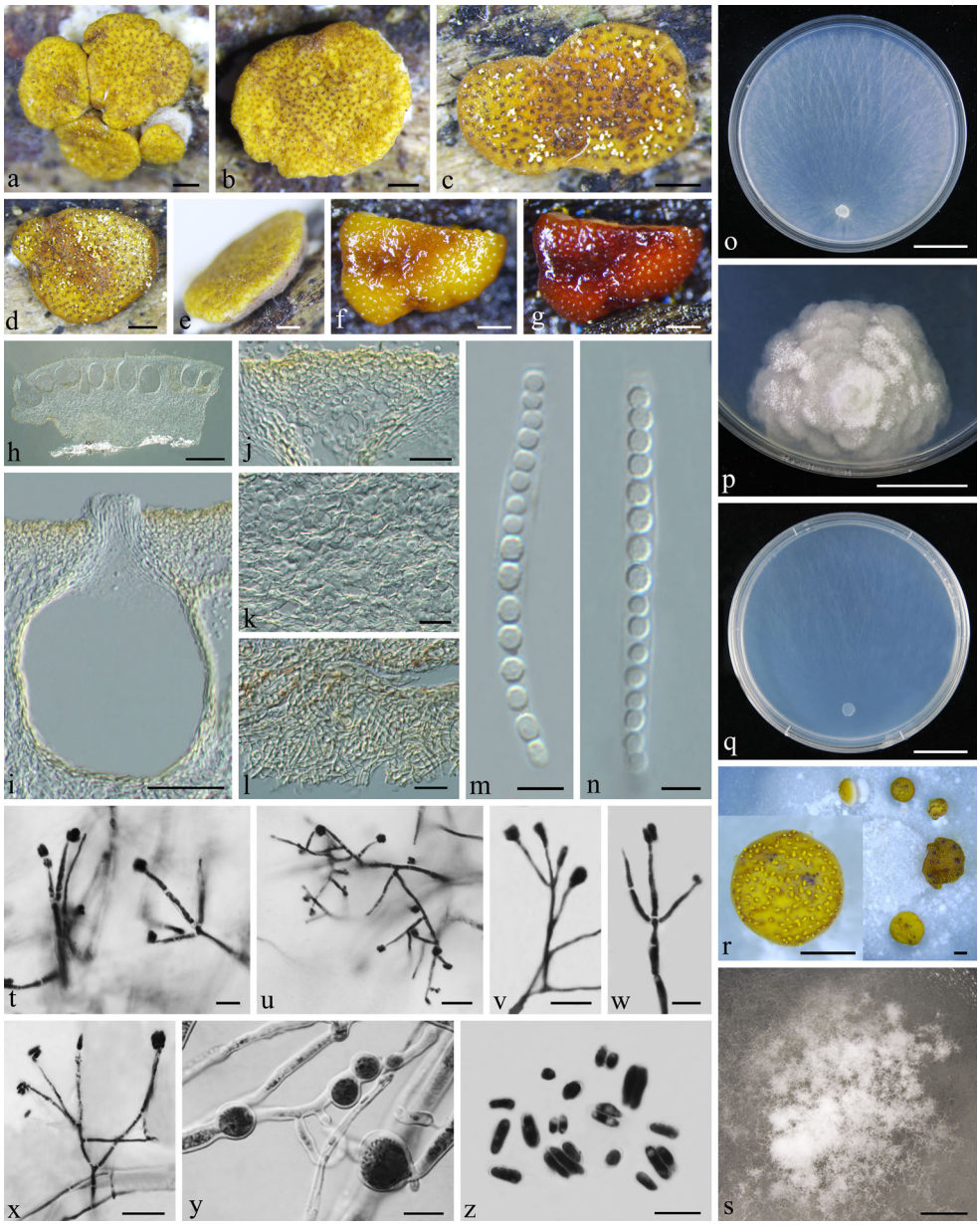

Fig. 3 *Trichoderma odoratum* (holotype HMAS 271354). **a**–**n** Sexual state. **a**–**e** Dry mature stromata on natural substrates. **f** Mature stroma after rehydration. **g** Mature stroma in 3 % KOH after rehydration. **h** Longitudinal section of a stroma. **i** Perithecium in section. **j** Cortical and subcortical tissues in section. **k** Subperithecial tissue in section. **l** Stroma base in section. **m**, **n** Asci with ascospores. **o**–**z** Asexual state. **o**–**q** Cultures after 17 days at 25 °C (**o** CMD, **p** PDA, **q** SNA). **r** Scattered young stromata (PDA, 25 days). **s** Conidiation tufts (PDA, 17 days). **t**–**x** Conidiophores and phialides (PDA, 17 days). **y** Chlamydospores (CMD, 20 days). **z** Conidia (PDA, 17 days). Scale bars: **a**–**g** = 400 μm. **h** = 200 μm. **i** = 50 μm. **j**–**l**, **v**, **x** = 20 μm. **m**, **n** = 5 μm. **o**–**q** = 20 mm. **r** = 0.8 mm. **s** = 1 mm. **t**, **u**, **w**, **y**, **z** = 10 μm

Notes: *Trichoderma odoratum* is most similar to *T. henanense*. See notes under *T. henanense* for comparisons. Notably, yellow to orange aggregated pseudoparenchymatous stromata of *T. odoratum* were formed after 8 days on PDA or after 17 days on SNA at 25 °C. Until now, very few species of *Trichoderma* have been reported producing stromata on artificial media (Table 4).

Trichoderma pseudobritdaniae W.T. Qin & W.Y. Zhuang, **sp. nov.** (Fig. 4) MycoBank: MB 813810

Etymology: The specific epithet refers to its morphological similarity to *Trichoderma britdaniae*.

Typification: China. Henan, Lingbao, Yanzishan, 34°28′ 50″N, 111°05′9″E, alt. 1000 m, on twig lying on the ground, 16 Sep 2013, H.D. Zheng, Z.Q. Zeng & W.T. Qin 8663 (holotype HMAS 271355, isotype HKAS 95056, ex-type culture HMAS 244999).

Stromata solitary, scattered or aggregated in small groups, brownish yellow or greyish yellow, pulvinate, centrally attached and with margin free, outline circular, oblong or irregular, 2–3.5(–5) mm diam., 0.7–1.0 mm thick ($n = 25$). Surface usually smooth, occasionally farinose or granular. Ostiolar dots dark brown or grey black, distinct, densely distributed. Rehydrated stromata turning brownish red in 3 % KOH.

In section, cortical tissue of textura angularis, 15–24 μm thick, cells yellow, walls up to 1.3 μm thick, (5.5–)6.5–10.5(–12.5) × 5–7.5(–8) μm ($n = 30$), turning dark orange in 3 % KOH; subcortical tissue of textura intricata, hyphae hyaline to light yellow, thin-walled, (2.5–)3–4.5(–5) μm ($n = 30$) wide; subperithecial tissue of textura epidermoidea, cells

Table 4 Species of *Trichoderma* producing stromata on artificial media

Species	Colour of ascospores	Reference
Trichoderma americanum (Canham) Jaklitsch & Voglmayr	Hyaline	Overton et al. (2006b)
T. asterineum W.T. Qin & W.Y. Zhuang	Hyaline	This study
T. citrinum (Pers.) Jaklitsch, W. Gams & Voglmayr	Hyaline	Jaklitsch (2011)
T. cuneisporum Chaverri & Samuels	Green	Chaverri and Samuels (2003)
T. hunua (Dingley) Jaklitsch & Voglmayr	Light yellow	Dingley (1957)
T. leguminosarum Jaklitsch & Voglmayr	Hyaline	Jaklitsch and Voglmayr (2015)
T. leucopus Jaklitsch	Hyaline	Jaklitsch (2011)
T. mienum Chang S. Kim, Nakagiri & N. Maek	Green	Kim et al. (2012)
T. nigrovirens Chaverri & Samuels	Green	Chaverri and Samuels (2003)
T. nybergianum (T. Ulvinen & H.L. Chamb.) Jaklitsch & Voglmayr	Hyaline	Jaklitsch (2011)
T. odoratum W.T. Qin & W.Y. Zhuang	Hyaline	This study
T. peltatum (Berk.) Samuels, Jaklitsch & Voglmayr	Hyaline	Samuels and Ismaiel (2011)
T. psychrophilum Jaklitsch	Hyaline	Jaklitsch (2011)
T. pulvinatum (Fuckel) Jaklitsch & Voglmayr	Hyaline	Jaklitsch (2011)
T. spinulosum (Fuckel) Jaklitsch & Voglmayr	Green	Jaklitsch (2009)

hyaline to light yellow, thin-walled, (6.5–)8.5–18(–24) × (5.3–)6–10.5(–13.2) μm ($n = 30$); tissue at the base of textura angularis mixed with textura globulosa, cells hyaline to light yellow, walls up to 1.3 μm thick, (6.5–)7.5–18(–21) × (6.5–)7.5–15.5(–16) μm ($n = 30$). Perithecia flask-shaped, (195–)224–263(–283) × (111–)119–142(–171) μm ($n = 30$); peridium pale yellow brown in lactic acid, turning dark orange in 3 % KOH, 5.3–13.2 μm thick at flanks, 7.9–13.2 μm thick at the base ($n = 30$). Ostioles non-papillate, (53–)55–66 μm high, 26–37(–40) μm wide at the apex ($n = 30$). Asci cylindrical, (62–)68–82(–89) × 4.2–5(–5.5) μm, with a stipe (10–)12–29(–31) μm long ($n = 40$). Ascospores hyaline, spinulose or verruculose, cells monomorphic, globose, subglobose or broad-ellipsoidal, sometimes the basal part-ascospores dimorphic, occasionally oblong to cuneate; distal cells 2.8–3.5 × (2.2–)2.5–3.2 μm, l/w 1.0–1.5; proximal cells (2.4–)2.9–3.7(–4.2) × 2.1–3.2 μm, l/w 1.0–1.5(–1.9) ($n = 50$).

Colony on CMD 15–18 mm in radius after 72 h at 25 °C, 62–65 mm after 12 days. Colony hyaline, sparse, with wavy or irregular outline; aerial hyphae inconspicuous. Conidiation noted on or around the plug after 5 days. No distinct odour, no diffusing pigment observed.

Colony on PDA 3–5 mm in radius after 72 h at 25 °C, 28–34 mm after 12 days. Colony pale creamy, with commonly lobed or irregular outline; aerial hyphae inconspicuous. Conidiation noted on or around the plug after 4–5 days. Conidiophores densely disposed, trichoderma- to verticillium-like. Phialides solitary or divergent in whorls of 2–3(–4), straight, lageniform or subulate, (7–)8.5–18(–20) × (2.5–)2.8–4(–4.5) μm, l/w (2.1–)2.7–4.7(–7.2), 1.3–3.2(–3.5) μm wide at the base ($n = 30$). Conidia hyaline, ellipsoidal or oblong, also subglobose or oval, sometimes cylindrical, smooth, (2.5–)3–7(–7.5) × (2.5–)3–4(–4.5), l/w 1.0–1.9(–2.1) ($n = 50$). Chlamydospores rare and sparsely disposed, noted after 10 days, terminal and intercalary, globose or ellipsoidal, (4–)5–12 × 4–10 μm, l/w 1.0–1.3 ($n = 15$). No distinct odour, no diffusing pigment observed.

Colony on SNA 13–15 mm in radius after 72 h at 25 °C, 60–64 mm after 12 days. Colony hyaline, sparse, with rounded or irregular outline. Aerial hyphae inconspicuous, appearing empty. Conidiation noted on or around the plug after 3 days. No distinct odour, no diffusing pigment observed.

Notes: As a sister of *T. britdaniae*, *T. pseudobritdaniae* also forms small asci and monomorphic ascospores, whereas stromata of *T. britdaniae* look like basidiomata of a corticiaceous fungus, are large, undulate, up to ca. 10 cm long and 3 mm thick when fresh, scattered or forming confluent clusters extending up to 80 cm long, which are much larger than those of *T. pseudobritdaniae*. *Trichoderma britdaniae* forms short orange-brown hyphal protrusions near the stroma base, which is absent in *T. pseudobritdaniae* (Jaklitsch and Voglmayr 2012). Table 3 provides the detailed morphological distinctions between them. As sequences were compared, they shared 92.42 % similarity for *rpb2* and 89.02 % similarity for *tef1*, and should be treated as different species.

Results

The PHT ($p = 0.01$) of *rpb2* and *tef1* sequences indicated that the individual partitions were generally congruent (Cunningham 1997), and, thus, the genes can be combined

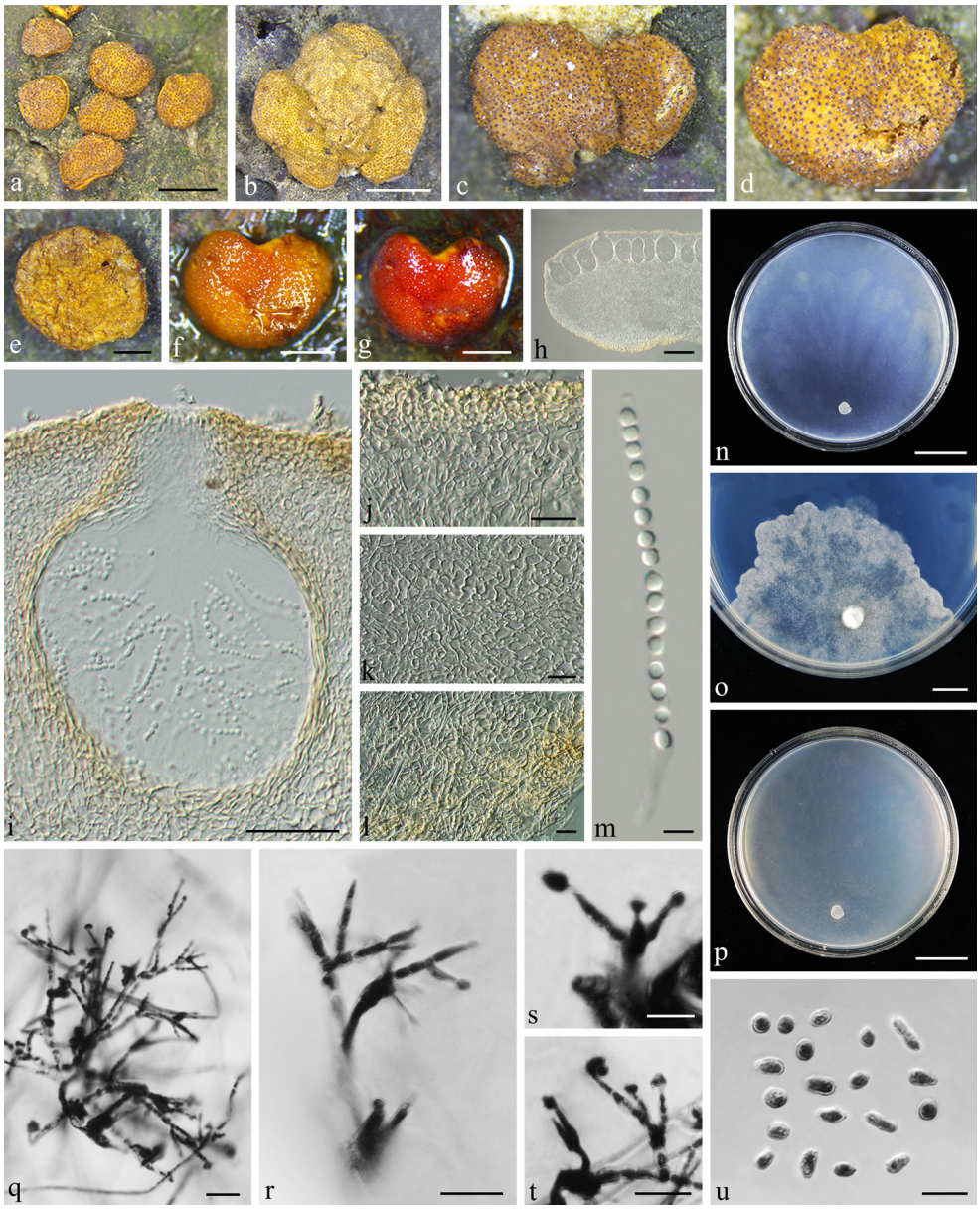

Fig. 4 *Trichoderma pseudobritdaniae* (holotype HMAS 271355). **a**–**m** Sexual state. **a**–**e** Dry stromata on natural substrates (**a**–**d** mature, **e** immature). **f** Mature stroma after rehydration. **g** Mature stroma in 3 % KOH after rehydration. **h** Longitudinal section of a stroma. **i** Perithecium in section. **j** Cortical and subcortical tissues in section. **k** Subperithecial tissue in section. **l** Stroma base in section. **m** Ascus with ascospores. **n**–**u** Asexual state. **n**–**p** Cultures after 11 days at 25 °C (**n** CMD, **o** PDA, **p** SNA). **q**–**t** Conidiophores and phialides (PDA, 10 days). **u** Conidia (PDA, 10 days). Scale bars: **a**, **c**, **d**, **f**, **g** = 1 mm. **b** = 2 mm. **e** = 400 μm. **h** = 200 μm. **i** = 50 μm. **j**–**l** = 20 μm. **m**, **s**, **u** = 10 μm. **n**, **p** = 20 mm. **o** = 10 mm. **q**, **r**, **t** = 20 μm

for analyses. Phylogenetic positions of the four new species were determined by analyses of the combined sequences of *rpb2* and *tef1*. In the MP analysis, the combined dataset contained 42 taxa and 2411 characters, of which 1337 were constant, 200 were variable and parsimony-uninformative, and 874 were parsimony-informative. In the BI analysis, GTR + I + G was selected as the best-fit model.

All the investigated *Trichoderma* species clustered together, receiving high statistical supports (MPBP/BIPP = 100 %/100 %). The ten green-ascospored species representing the five clades as defined by Jaklitsch (2009) and Jaklitsch and Voglmayr (2015) formed a relatively high-supported group (MPBP/BIPP = 65 %/100 %). The 21 hyaline-ascospored species were distributed in ten recognisable clades. Among them, Brevicompactum, Deliquescens, Hypocreanum, Polysporum, Psychrophilum and Viride clades were closely related (MPBP/BIPP = 80 %/100 %). The rest of the species were attributed to the Longibrachiatum clade (MPBP/BIPP = 99 %/100 %), Semiorbis clade (MPBP/BIPP = 97 %/100 %), Stromaticum clade (MPBP/BIPP = 100 %/100 %), a clade containing *T. asterineum* and *T. leguminosarum* (MPBP/BIPP = 100 %/100 %), and several terminal branches which did not belong to any of the named clades (Fig. 5). *Trichoderma britdaniae* and *T. pseudobritdaniae* (MPBP/BIPP = 93 %/100 %), and *T. henanense* and *T. odoratum* (MPBP/BIPP = 100 %/100 %) appeared as two sister pairs (Fig. 5). These four species showed a close relationship to the Longibrachiatum clade.

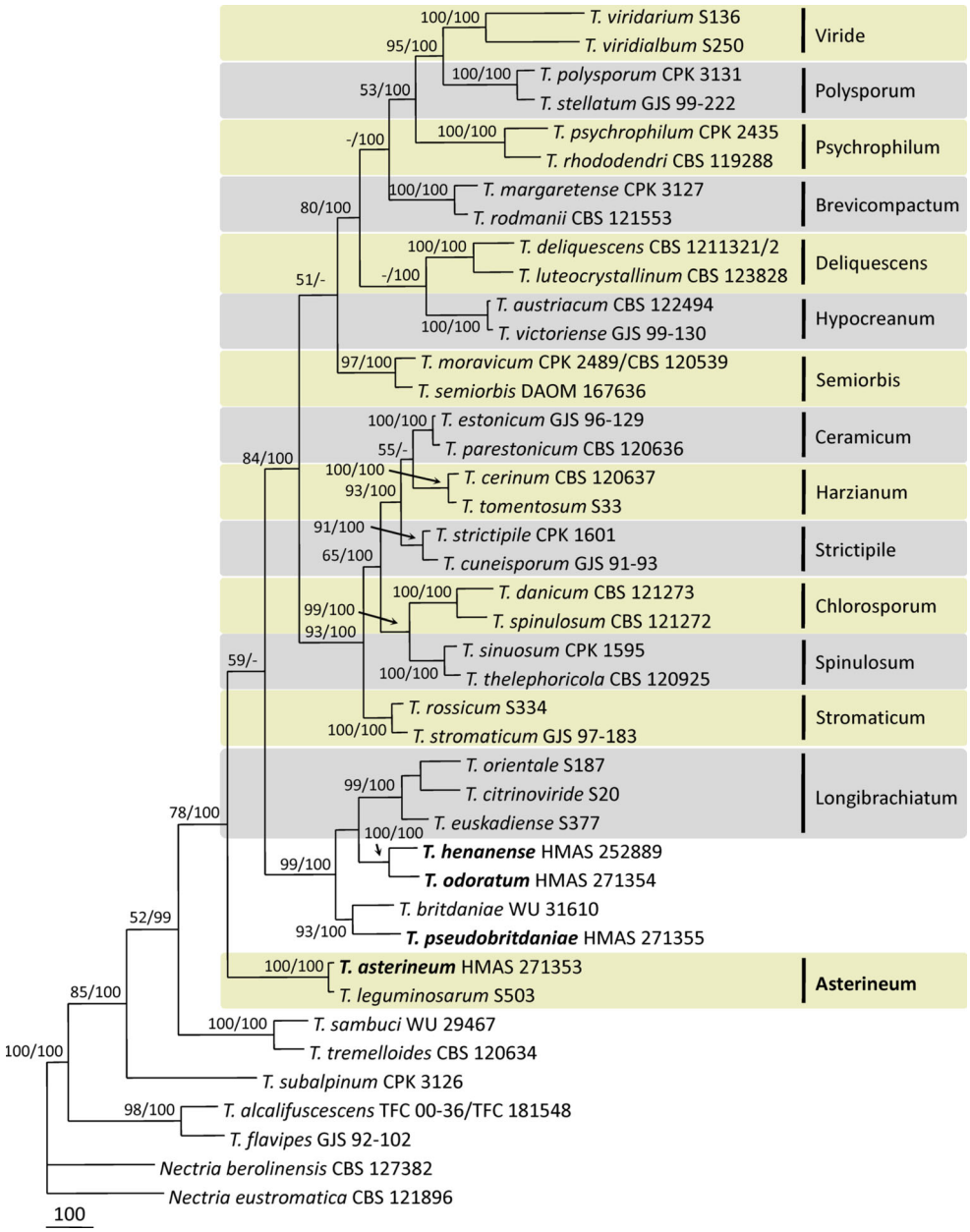

Fig. 5 Maximum parsimony phylogram reconstructed from the combined sequences of *rpb2* and *tef1* (tree length = 5600, CI = 0.3414, HI = 0.6586, RC = 0.1759 and RI = 0.5151), showing the phylogenetic position of the four new *Trichoderma* species and a new clade (in **bold**). MPBP above 50 % (*left*) and BIPP above 90 % (*right*) are given, respectively. TreeBASE S18017

Discussion

The application of molecular tools for fungal taxonomy prompted the researches on morphology-based taxonomy of *Trichoderma*. Till now, more than 260 species have been recognised in the genus (Samuels et al. 1998; Chaverri and Samuels 2003; Samuels et al. 2006; Jaklitsch 2009, 2011; Jaklitsch and Voglmayr 2014, 2015; Zhu and Zhuang 2014b, c, 2015a, b; Bissett et al. 2015; http://www.isth.info/tools/molkey/index.php). As shown in the previous studies, ITS is not suitable for a genus-wide species reconstruction, and the *tef1* introns can only be used in individual clades or certain groups due to their high sequence variability (Samuels et al. 2006). Endochitinase 42, chitinase 18-5, calmodulin- and actin-encoding genes were also tested for a limited number of species, which did not provide a reasonable resolution (Chaverri et al. 2003b; Druzhinina and Kubicek 2005). *rpb2* sequences appeared powerful due to their suitable interspecific variations and, therefore, they were accepted as the main marker for the exploration of phylogeny of the group (Zhu et al. 2014; Jaklitsch and Voglmayr 2015). The *tef1* exons are less variable among species compared with its introns, but capable of providing additional useful information (Chaverri and Samuels 2003; Jaklitsch et al. 2006; Overton et al. 2006a, b). In this study, the combined sequences of *rpb2* and *tef1* regions were chosen to clarify the phylogenetic positions of the new *Trichoderma* species (Fig. 5). The tree topology is

basically congruent with the previous reports (Jaklitsch 2009, 2011; Jaklitsch and Voglmayr 2015).

Trichoderma leguminosarum was first discovered as a separate terminal branch and did not belong in any known clades (Jaklitsch and Voglmayr 2015). In the present work, *T. asterineum* and *T. leguminosarum* clustered together with high statistical supports (Fig. 5, MPBP/BIPP = 100 %/100 %) and share significant morphological characteristics that are unknown in any other hyaline-ascospored species of the genus. Considering the distinctive morphological similarity and close phylogenetic relationship of these two fungi, we name them the Asterineum clade.

Trichoderma henanense, *T. odoratum* and *T. pseudobritdaniae* are associated with but outside the Longibrachiatum clade (Fig. 5). Their densely disposed ostiolar dots and monomorphic ascospores resemble members of the Longibrachiatum clade, but their yellowish stromata, hyaline or yellowish apical fascicle of periphyses in lactic acid and whitish colonies recall species in the Polysporum clade. *Trichoderma henanense* and *T. odoratum* are sisters, as well as *T. britdaniae* and *T. pseudobritdaniae* (Fig. 5). Although taxa of the individual sisters share certain morphological features, the two species in the former sister differ in size of perithecia, asci and conidia, growth rate in cultures and sequence data; and those of the latter sister are hardly confused in stromatal size, perithecia gross morphology and sequence data.

Trichoderma species inhabit commonly in soil and on woody substrates. The genus is widely distributed in world temperate and tropical regions. China is rich in species diversity of the group. We believe that more new taxa will be discovered. For future research, integrated studies of morphology and phylogeny are needed, which will comprehensively deepen our knowledge of species diversity, phylogeny and potential use of *Trichoderma*.

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