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

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Abstract

Collections of *Trichoderma* were made from southwest China and examined. Four new species producing hyaline ascospores, *T. fructicola*, *T. medogense*, *T. palidulum* and *T. virgineum*, were found, and are described and illustrated. Their phylogenetic positions were allocated based on sequence analyses of the combined RNA polymerase II subunit b and translation elongation factor 1 alpha genes. *Trichoderma fructicola*, appearing as a lone lineage among hyaline-ascospored groups, is diagnostic by cortical tissues of *textura epidermoidea*, remarkable colour change of peridium in KOH, and verticillium- to trichoderma-like conidiophores. As a sister of *T. voglmayrii*, *T. medogense*is similar to *T. voglmayrii* in having yellow-brown to purplish red stromata and trichoderma-like conidiophores, but differs in apapillate ostioles, subcortical tissues of *textura intricata*, narrow phialides, andsmaller conidia. *Trichoderma palidulum*is located in the Viride clade andis distinct from its alliesin stroma and colony morphology. *Trichoderma virgineum* is closely associated with *T. henanense* and *T. odoratum*. The three species are similar in having yellowish stromata, monomorphic ascospores, white colonies produced on three standard media, simple-branched conidiophores, and hyaline conidia for which anew clade is proposed. Morphological distinctions and sequence divergences between the new species and their close relatives are discussed.

Key words – 4 new species – Hypocreales – Morphology – Sequence analysis – Taxonomy

Introduction

Trichoderma Pers. is a hyperdiverse genus with an extraordinarily high number of species. It is cosmopolitan on various substrates in a wide range of geographic distributions (Chaverri & Samuels 2003, Kredics et al. 2010). Some species of the genus are found to be beneficial and applied to agriculture, industry and environmental protection (Harman 2006, Bischof et al. 2016, Saravanakumar & Kathiresan 2014, Adnan et al. 2017). Recognizing its biodiversity and exploring continuously new recourses of the group attract the attention of many researchers from diverse regions of the world.

Trichoderma species are characterized by perithecia immersed in fleshy stromata of different colours, cylindrical asci with hyaline or green ascospores which disarticulate at the septum, conidiophores forming several types of branch patterns, and hyaline or green conidia (Jaklitsch et al. 2006, Qin & Zhuang 2016c). Bissett et al. (2015) listed 254 species of the genus with either cultures or DNA sequence data available. More recently, about 40 taxa have been added to the

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genus (Qin & Zhuang 2016a, 2016b, 2016c, 2017, Chen & Zhuang 2017a,b,c, Sun et al. 2016). Classical approaches alone are difficult to make taxonomic opinions of *Trichoderma* species. In recent years, combined analyses of morphology and DNA sequence data have accelerated taxonomic studies of the genus. As a result, ascospore colour has been shown to provide useful phylogenetic information (Chaverri & Samuels 2003, Jaklitsch 2009, 2011). Almost all green-ascospored species clustered together and divided into five clades, Ceramicum, Chlorosporum, Harzianum, Spinulosum and Strictipile. The hyaline-ascospored groups seem to be paraphyletic (Jaklitsch & Voglmayr 2015), which are mainly in ten clades or groups, i.e. Asterineum, Brevicompactum, Deliquescens, Hypocreanum, Longibrachiatum, Polysporum, Psychrophilum, Semiorbis, Stromaticum and Viride. The Viride clade containing about 70 species is so far the largest clade, while the small clades comprised only two or three taxa, such as Asterineum and Ceramicum (Chaverri & Samuels 2003, Qin & Zhuang 2016c). Although *Trichoderma* is among the genera thoroughly investigated, there are still a number of species with their phylogenetic positions not fully understood (Druzhinina & Kubicek 2005).

During the investigations of *Trichoderma* species in southwest China, four new hyaline-ascospored species were discovered, which are named as *T.fructicola*, *T. medogense*, *T. palidulum* and *T. virgineum*. Their sexual and asexual morphsare described and illustrated, and their phylogenetic positions are determined based on sequence analyses of the combined RNA polymerase II subunit B (RPB2) and translation elongation factor 1 alpha (TEF1) genes.

Materials &methods

Specimens investigated were collected from Tibet Autonomous Region and Yunnan Province, and deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS). Strains were isolated from ascospores (Jaklitsch 2009), and deposited in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences.

The isolates were cultured at 25°C with a 12 h light/dark cycle on Cornmeal Dextrose agar [CMD: 40g cornmeal, 2% (*W*/*V*) dextrose, 2% (*W*/*V*) agar], potato dextrose agar[PDA: 200g potato, 2% (*W*/*V*) dextrose, 2% (*W*/*V*) agar], and synthetic low nutrient agar (SNA, Nirenberg 1976). Radial growth rates of colonies were measured. Morphology of asexual and sexual states was observed following Chaverri & Samuels (2003). Photographs were taken with a Canon G15 digital camera (Tokyo, Japan) and a Leica DFC450 digital camera (Wetzlar, Germany) attached to a Leica M125 stereomicroscope (Milton Keynes, UK) for gross morphology, and a Zeiss AxioCam MRc 5 digital camera (Jena, Germany) attached to a Zeiss Imager A2 microscope (Göttingen, Germany) for anatomical structures.

Mycelium was harvested to extract genomic DNA following the methods of Wang & Zhuang (2004). Fragments of RPB2 and TEF1were amplified with the primer pairs and method reported by Jaklitsch (2009) and sequenced at the Beijing Tianyihuiyuan Bioscience and Technology, China.

The sequences used in phylogenetic analysis were listed in Table 1, including sixty *Trichoderma* species representing fifteen named clades and several independent terminal branches. They were assembled, manually adjusted and aligned using the DNAStar Seqman program 7.1.0 (DNASTAR. Inc., Madison) and ClustalX 1.83 (Thompson et al. 1997).

Maximum parsimony (MP) and Bayesian inference (BI) analyses were conducted to allocate the phylogenetic positions of the new species. MP analysis was carried out using PAUP v. 4.0b10 (Swofford 2002) with following settings: all characters were equally weighted, gaps were treated as missing data, starting trees were obtained by random taxon addition with 1000 replicates, branch-swapping algorithm was tree-bisection-reconnection (TBR), steepest descent option and MulTrees option were not in effect. BI analysis was implemented with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003), and the best-fit model GTR+I+G was selected by MrModelTest v. 2.3 (Nylander 2004) using Akaike information criterion (AIC); Metropoles-coupled Markov chain Monte Carlo (MCMCMC) searches were run for 1000000 generations sampling every 100 generations; the first 2500 trees were discarded as the burn-in phase. The statistic supports were

evaluated by maximum parsimony bootstrap proportion (MPBP) and Bayesian inference posterior probability (BIPP). Trees were viewed in FigTree v. 1.4.3 (Rambaut 2016).

Table 1 *Trichoderma* species used in phylogenetic analyses.

Species	**Strain**	**GenBank accessionnumbers**	
		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. barbatum Samuels	GJS 04-308	HQ342286	HQ342223
T. bavaricum Jaklitsch	CPK 2021	FJ860526	FJ860620
T. brevicompactum G.F. Kraus, C.P. Kubicek & W. Gams	GJS 04-381	EU338317	EU338299
T. britdaniae (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr	WU 31610	JQ685880	JQ685866
T. caribbaeum Samuels & Schroers	GJS 97-3	KJ665246	KJ665443
T. christiani Jaklitsch & Voglmayr	S442	KJ665244	KJ665439
T. citrinoviride Bissett	S20	KJ665250	KJ665449
T. confluens W.T. Qin & W.Y. Zhuang	HMAS 244993	KT001964	KT001959
T. crystalligenum Jaklitsch	CBS 118980	DQ345347	DQ345342
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. flavipes (Peck) Seifert, Jaklitsch & Voglmayr	GJS 92-102	DQ834461	DQ834454
T. fomiticola Jaklitsch	CBS 121136	FJ860538	FJ860639
T. fructicola Y.B. Zhang & W.Y. Zhuang	HMAS 275663	**MG383484***	**MG383490**
T. grande W.T. Qin & W.Y. Zhuang	HMAS 248749	KX066266	KX066254.
T. istrianum Jaklitsch & Voglmayr	S123	KJ665280	KJ665521
T. hamatum (Bonord.) Bainier	Hypo 647	KJ665274	KJ665513
T. henanense W.T. Qin & W.Y. Zhuang	HMAS 252889	KT224467	KT224464
T. hubeiense W.T. Qin & W.Y. Zhuang	HMAS 271352	KT001963	KT001958
T. koningii Oudem.	S227	JN715609	KC285596
T. leguminosarum Jaklitsch & Voglmayr	S391	KJ665287	KJ665548
T. luteocrystallinum Jaklitsch	CBS 123828	FJ860544	FJ860646
T. medogense Y.B. Zhang &W.Y. Zhuang	HMAS 254526	**MG383486**	**MG383491**
	HMAS 275662	**MG383485**	**MG383492**
T. melanomagnum P. Chaverri & Samuels	GJS 99-153	AY391926	AY391985
T. moravicum Jaklitsch	CPK 2489	FJ860549	–
	CBS 120539	–	FJ860651
T. neorufum (Samuels, Dodd &Lieckf.) Jaklitsch & Voglmayr	CBS 119498	FJ860550	FJ860653
T. neorufoides Jaklitsch	CPK 1904	FJ860554	FJ860658
T. odoratum W.T. Qin & W.Y. Zhuang	HMAS 271354	KT224468	KT224463
T. palidulum Y.B. Zhang & W.Y. Zhuang	HMAS 275665	**MG383487**	**MG383493**
	HMAS 254527	**MG383488**	**MG383494**
T. paraviridescens Jaklitsch, Samuels & Voglmayr	S122	KC285764	KC285671
T. parestonicum Jaklitsch	CBS 120636	FJ860565	FJ860667

Table 1 Continued.

Species	Strain	GenBank accessionnumbers	
		RPB2	**TEF1**
T. parareesei Atan., Jaklitsch, Komoń-Zel., C.P. Kubicek & Druzhin.	TUB F-1066	HM182963	GQ354353
T. petersenii Samuels, Dodd & Schroers	CBS 119507	FJ860568	FJ860670
T. phellinicola Jaklitsch	CBS 119283	FJ860569	FJ860672
T. polysporum (Link) Rifai	CPK 3131	FJ860558	FJ860661
T. psychrophilum Jaklitsch	CPK 2435	FJ860576	FJ860682
T. rhododendri (Jaklitsch) Jaklitsch & Voglmayr	CBS 119288	FJ860578	FJ860685
*T. rossicum*Bissett, C.P. Kubicek & Szakács	S334	KJ665335	KJ665700
T. sambuci (Jaklitsch & Voglmayr) Jaklitsch & Voglmayr	WU 29467	FJ860585	FJ860693
T. saturnisporopsis Samuels & Jaklitsch	S19	JQ685885	JQ685869
T. semiorbis (Berk.) Jaklitsch & Voglmayr	DAOM 167636	AF545522	AF545568
T. sinokoningii W.T. Qin & W.Y. Zhuang	HMAS 271397	KU529141	KU529130
T. sinuosum P. Chaverri & Samuels	CPK 1595	FJ179619	FJ860697
T. spinulosum Fuckel	CBS 121272	FJ860590	FJ860700
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. subeffusum Jaklitsch	CBS 120929	FJ860597	FJ860707
T. thelephoricola P. Chaverri & Samuels	CBS 120925	FJ860600	FJ860711
T. tomentosum Bissett	S33	KF134793	KF134801
T. tremelloides Jaklitsch	CBS 120634	FJ860602	FJ860713
T. virgineum Y.B. Zhang & W.Y. Zhuang	HMAS 275664	**MG383489**	**MG383495**
T. viride Pers.	CBS 119325	EU711362	DQ672615
T. voglmayrii Jaklitsch	CBS 117710	DQ086151	DQ086147
T. yui Z.X. Zhu & W.Y. Zhuang	HMAS 266633	KJ634725	KJ634758
T. yunnanense Z.F. Yu & K.Q. Zhang	CBS 121219	GU198274	GU198243
Nectria berolinensis (Sacc.) Cooke	CBS 127382	HM534883	HM534872
N. eustromatica Jaklitsch & Voglmayr	CBS 121896	HM534886	HM534875

*Numbers in boldface indicate the newly submitted sequences.

Results

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 were combined for sequence analyses. The combined dataset contained 64sequences and 2437 characters (1082 characters forRPB2, 1355 characters for TEF1), of which 62sequences represented 60*Trichoderma* species. In the MP analysis, 1248 characters were constant, 171 variable characters were parsimony-uninformative, and 1018 were parsimony-informative. The MP analysis resulted in two most parsimonious trees, and one of them was depicted in Fig.1 (Tree length = 8970, Consistency index= 0.2591, Homoplasy index= 0.7409, Rescaled consistency= 0.1377,Retention index = 0.5314). The BI tree is similar to the MP tree in topology. The phylogenetic tree and DNA sequence alignments are deposited in TreeBASE(no. S21884).

Sixty *Trichoderma* species representing 15named clades, a newly proposed clade, and some independent terminal branches were analyzed. All the investigated species clustered together with

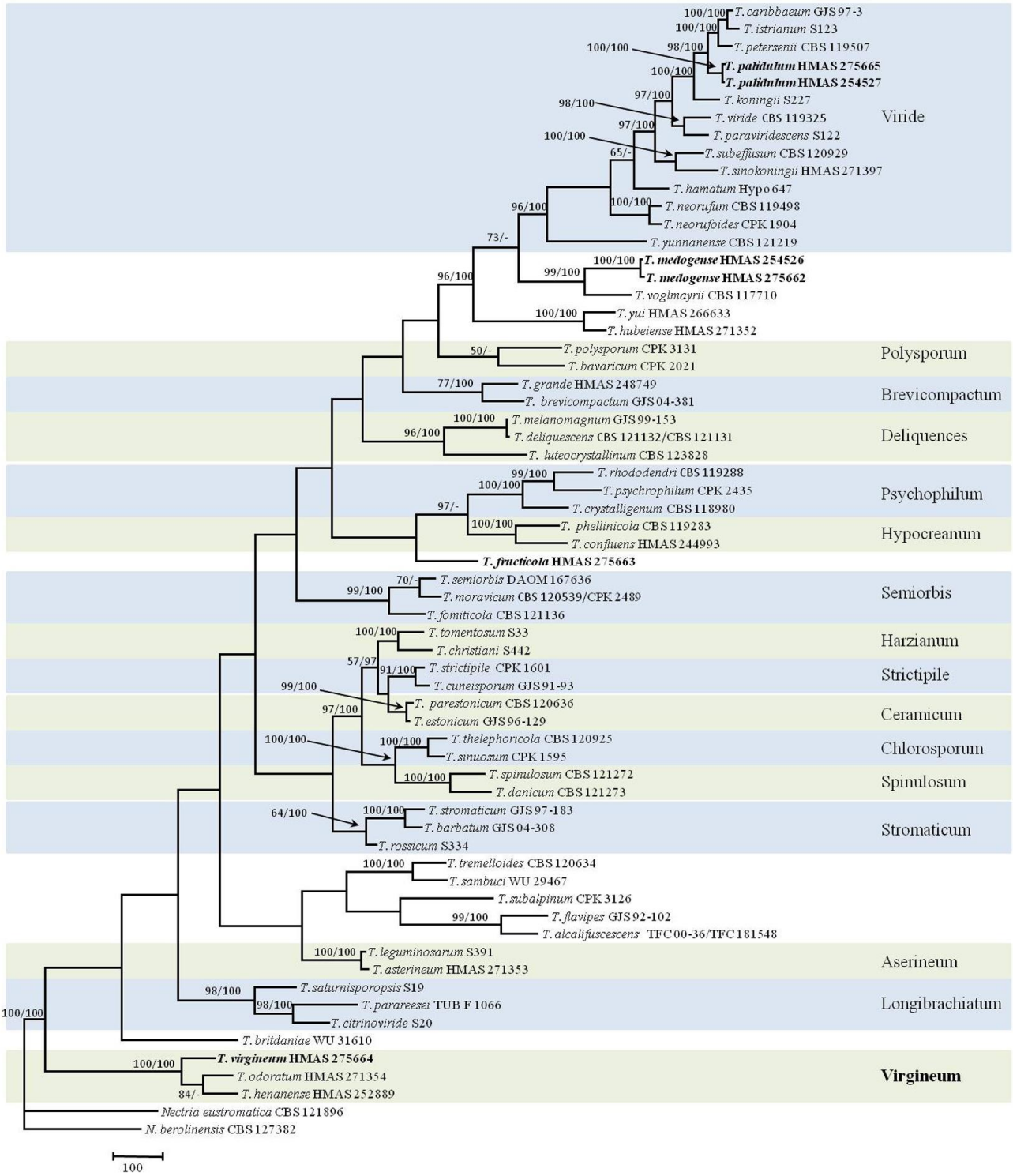

Figure 1 – Maximum parsimony tree of selected *Trichoderma* species inferred from the combined partial sequences of RPB2 and TEF1showing the phylogenetic positions of the new species. MPBP above 50% (left) and BIPP above 90% (right) are indicated at the nodes. New species and clade proposed are in boldface (TreeBASE no. S21884).

high statistical support (Fig.1, MPBP/BIPP = 100%/100%). The analyses revealed the phylogenetic positions of the new species. *Trichoderma fructicola* formed a separate terminal branch, which wasneither close to the Hypocreanum clade nor to the Semiorbis clade. *Trichoderma medogense* became a sister of *T. voglomayrii* (Fig.1, MPBP/BIPP = 99%/100%) which formerly appeared as an

independent terminal branch (Jaklitsch 2011, Jaklitsch & Voglmayr 2015). The collections of *T. palidulum* were located in the Viride clade and associated with *T. caribbaeum*, *T. istrianum*and *T. petersenii* (MPBP/BIPP = 98%/100%).*Trichoderma virgineum* turned out to be most closely related to *T. odoratum* and *T. henanense* receiving very high statistic values (MPBP/ BIPP = 100%/100%).

Taxonomy

TrichodermafructicolaY.B. Zhang & W.Y. Zhuang, sp. nov. Fig. 2

Fungal Names: FN570501; Facesoffungi number: FoF 03915

Etymology – The specific epithet refers to the substrate of the fungus.

Stromata solitary, gregarious or aggregated in small numbers, pulvinate or discoidal, outline circular or elongate to irregular, rounded, broadly attached, pale orange-yellow when fresh, apricot yellow when mature, 0.5–2.5 mm diam., 0.4–0.7 mm thick (n = 35). Surface nearly smooth, the stroma bases surrounded with whitish hyphae. Ostiolar dots dark brown, distinct, densely distributed. Rehydrated stromata deep chrome to orange brown, darkened in 3% KOH.

In section, cortical tissue of *textura epidermoidea*, (12.5–)14.5–26(–29) µm thick (n = 30), cells hyaline to yellowish, thin-walled, (5–)6.5–17.5(–20) × 3–11.5 µm (n = 30), turning orange red in 3% KOH; subcortical tissue of *textura intricata*, hyphae hyaline, thin-walled, 2.0–4.5 µm (n = 32) wide; subperithecial tissues of *textura epidermoidea*, cells hyaline, thin-walled, 6.5–32 × 4.5–13.5 µm (n = 33); tissue at the base of *textura epidermoidea* mixed with *textura intricata*, cells hyaline to light yellow, thin-walled, 4.5–28 × 2.5–8.5 µm (n =30), hyphae hyaline to light yellow, thin-walled, 3–6.5 µm (n =30) wide. Perithecia flask-shaped or globose, 228–286 × 99.5–215.5 µm (n = 30); peridium pale yellow, turning orange red in 3% KOH, 5–14.5 µm thick at flanks, 9–16 µm thick at the base (n = 30). Ostioles 14–39.5(–53.5) µm wide at apex, 50–86(–94.5) µm high (n = 30). Asci cylindrical, 57.5–85 × 3–4.5 µm, with a stipe 4.5–10 µm long (n = 30). Ascospores hyaline, verrucose, cells dimorphic, distal cells globose to ellipsoidal, 1.5–3.5 × 2.5–4 µm, L/W 1–1.6(–1.9), proximal cells ellipsoidal to cylindrical, 3–5 × 1.5–2.5 µm, L/W (1.2–)1.4–2.6 (n = 41).

Colony on CMD 29–33 mm in radius after 72 h at 25 °C, mycelium covering the plate after 9 d. Colony circular, margin well defined; aerial hyphae short and sparsely disposed, longer and denser towards the distal margin. Conidiation noted after 3 d on aerial hyphae. No distinct odor and diffusing pigment observed.

Colony on PDA 27–31 mm in radius after 72 hat 25 °C, mycelium covering the plate after 9 d. Colony nearly circular, zonate; aerial hyphae cottony. Conidiation noted after 1 d on aerial hyphae near the plug. No distinct odor and diffusing pigment observed.

Colony on SNA 8–9 mm in radius after 72 h at 25°C, 25–30 mm after 13 d. Colony irregular, thin, margin diffuse; aerial hyphae loosely disposed. Conidiation noted after 3 d on aerial hyphae. Conidiophores verticillium- to trichoderma-like, branches paired, unpaired, or with up to 4 branches in a whorl around the main axis at an acute or right angle, generally re-branching 1 or 2 times. Phialides subulate or ampulliform, solitary or divergent in whorls of 2–6, 5.5–25.5 × 2–3.5 µm, L/W 1.8–8.7, base 1.5–3.2 µm (n = 43). Conidia pale green, globose, oblong or ellipsoid, 2.5–4.5 × 2–3 µm, L/W 1–2 (n = 30). No distinct odor and diffusing pigment observed.

Material examined – CHINA, Yunnan, Maguan County, 22°50.81'N, 104°31.79'E, alt. 1587 m, on a rotten fruit, 12 August 2016, X.H. Wang, S.H. Li, H.D. Zheng & S.C. Li YN16-88 (HMAS 275663**holotype)**;ex-type culture HMAS247235.

Notes – *Trichoderma fructicola* is diagnostic by the cortical tissues of *textura epidermoidea*, remarkable colour change of peridium in KOH, hyaline ascospores, verticillium- to trichoderma-like conidiophore branching pattern, and occurrence on a rotten fruit. Sequence analyses indicate that the fungus forms a separate phylogenetic lineage. Sequence comparisons (NCBI database: https://www.ncbi.nlm.nih.gov/) reveal that the new species shares 95% similarity with that of *T. strictipile* (54 bp divergences among 1030 bp in the region ofTEF1); it has 92% identity to *T. taxi* but is with 92 bp differences among 1089 bp for the fragment of RPB2.

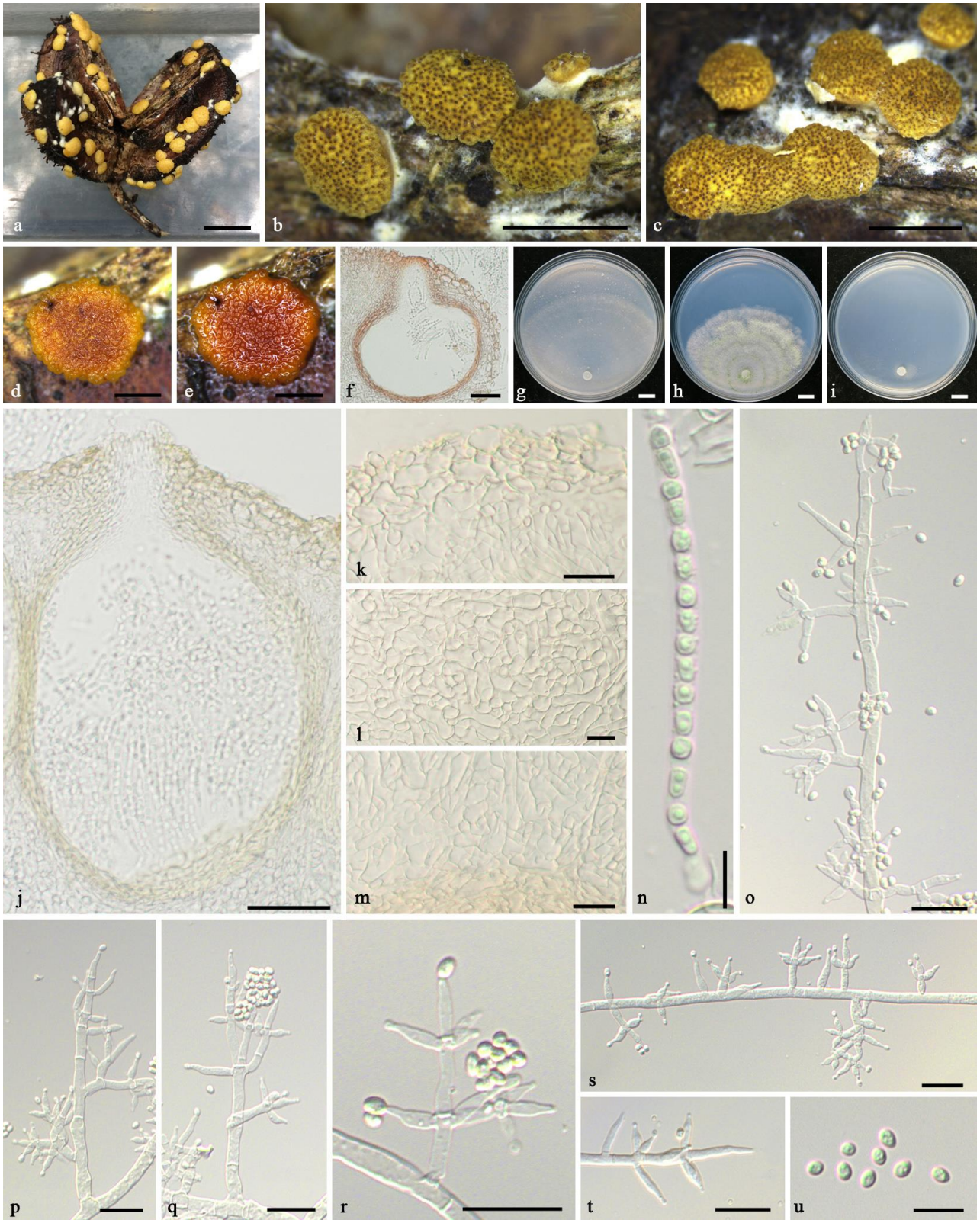

Figure 2 – *Trichoderma fructicola* (HMAS 275663). a Fresh stromata on natural substrate b, c Dry stromata on natural substrate d Mature stroma after rehydration e Rehydrated mature stroma in 3% KOH f Longitudinal section of a perithecium in KOH g–i Cultures after 7 days at 25°C (g CMD, h PDA, i SNA) j Perithecium in section k Cortical and subcortical tissues in section l Subperithecial tissue in section m Stroma base in section n Ascus o–t Conidiophores and phialides (SNA, 7days) u Conidia (PDA, 11 days).–Scale bars: a, g–i = 10 mm, b, c = 2 mm, d, e = 1 mm, f, j = 500 µm, k–m = 200 µm, n, u = 10 µm, o–t = 20 µm.

Trichodermamedogense Y.B. Zhang & W.Y. Zhuang, sp. nov. Fig. 3

Fungal Names: FN570502; Facesoffungi number: FoF 03916

Etymology – The specific epithet refers to the type locality of the fungus.

Stromata solitary or gregarious, pulvinate, discoidal or turbinate, outline circular or variable, centrally attached, margin usually acute and sometimes slightly upwards, yellowish brown, reddish brown or purple red, 0.5–3 mm diam., 0.2–0.5 mm thick (n = 30). Surface smooth. Ostiolar dots inconspicuous. Rehydrated stromata English red, becoming reddish brown in 3% KOH.

In section, cortical tissue of *textura angularis*, 8.5–13.5 µm thick (n = 33), cells orange, thick-walled, 2.5–8.4 × 1.9–5.8 µm (n = 55), no color change in 3% KOH; subcortical tissue of *textura intricata*, hyphae compacted, hyaline to slightly orange, thin-walled, 2.5–5 µm (n = 43) wide; subperithecial tissues of *textura epidermoidea*, cells hyaline, thin-walled, 7.5–20 × 5–14.5 µm (n = 30); tissue at the base of *textura intricata*, hyphae hyaline, thin-walled, 1.5–4 µm (n =34) wide. Perithecia flask-shaped or globose, 107–210.5 × 67.5–210 µm (n = 30); peridium hyaline to orange, 6–11.5 µm thick at flanks, 7.5–15 µm thick at the base (n = 30). Ostioles 12.5–20 µm wide at apex, 37.5–55 µm high (n = 30). Asci cylindrical, 59–77.5 × 3–4.5 µm, with a stipe 3.5–13.5 µm long (n = 30). Ascospores hyaline, verrucose, cells dimorphic, distal cells subglobose to oblong, 2.5–3.5 × 2–2.5 µm, L/W 1–1.5(–1.6), proximal cells oblong to ellipsoidal, 2–3.5 × 1.5–2.5 µm, L/W (1.1–)1.3–1.8(–2) (n = 50).

Colony on CMD 39–53 mm in radius after 72 h at 25 °C, mycelium covering the plate after 5 d. Colony circular, whitish, reverse side yellowish; aerial hyphae distributed homogeneously and intertwined, forming whitish *c.* 0.1–0.5 mm diam. granules after 7 d near the plug. Conidiation noted after 2 d on aerial hyphae. Conidiophores trichoderma-like, branches at an acute or right angle to the main axis, generally re-branching 1 or 2 times. Phialides subulate or ampulliform, solitary or divergent in whorls of 2–6, 7–19 × 2–3.5 µm, L/W 3–6.5(–7), base 1.5–3(–3.3) µm (n = 54). Conidia hyaline, globose to ellipsoid, 2–3.5(–5) × 1.5–2.5 µm, L/W 1–1.6(–2.1) (n = 37). Odor coconut-like; pigment yellowish.

Colony on PDA 45–53 mm in radius after 72 hat 25 °C, mycelium covering the plate after 5 d. Colony circular, slightly zonate, reverse side yellowish; aerial hyphae dense, forming granules after 7 d in the concentric zones. Conidiation noted after 2 d on aerial hyphae near the plug. Odor coconut-like; pigment yellowish.

Colony on SNA 12–19 mm in radius after 72 h at 25°C, covering the plate after 7 d. Colony circular, thin; aerial hyphae loosely disposed. Conidiation noted after 2 d on aerial hyphae. No distinct odor and diffusing pigment produced.

Material examined – CHINA, Tibet Autonomous Region, Mêdog County, 29°19.85'N, 95°18.65'E, alt. 800 m, on rotten twig, 20 September 2016, Y.B. Zhang, Z.Q. Zeng, Z.H. Yu, H.D. Zheng, X.C. Wang & K. Chen 11058 (HMAS254526holotype); ex-type culture HMAS248898; Tibet Autonomous Region, Mêdog County, 29°19.84'N, 95°18.67'E, alt. 800 m, on rotten twig, 20 September 2016, Y.B. Zhang, Z.Q. Zeng, Z.H. Yu, H.D. Zheng, X.C. Wang & K. Chen 11065 (HMAS275662, culture HMAS247234).

Notes – As the sister of *T. voglmayrii*, *T.medogense* forms yellow-brown to purple red stromata and trichoderma-like conidiophores which are similar to *T. voglmayrii*, but ostioles apapillate, and subcotical tissue of *textura intricata* are apparently diagnostic. In addition, the phyalides of *T. voglmayrii* are wider [(5.5–)7.2–12.2(–16.5)× (2.7–)3.2–4.1(–4.7) µm], its conidia are larger [(3–)3.5–6.5(–10.5)× (2.2–)2.6–3.3(–4.2) µm], and crystals producing on CMD medium(Jaklitsch et al. 2005) were not observed in *T.medogense*. As sequence divergences are concerned, 82–86 bp and 151 bp differences among 1073 bp and 1314 bp for RPB2 and TEF1respectivelyare detectedbetween the two fungi. They cannot be conspecific.

Trichodermapalidulum Y.B. Zhang & W.Y. Zhuang, sp. nov. Fig. 4

Fungal Names: FN570503; Facesoffungi number: FoF 03917

Etymology–The specific epithet refers to the somewhat pale-colored colony.

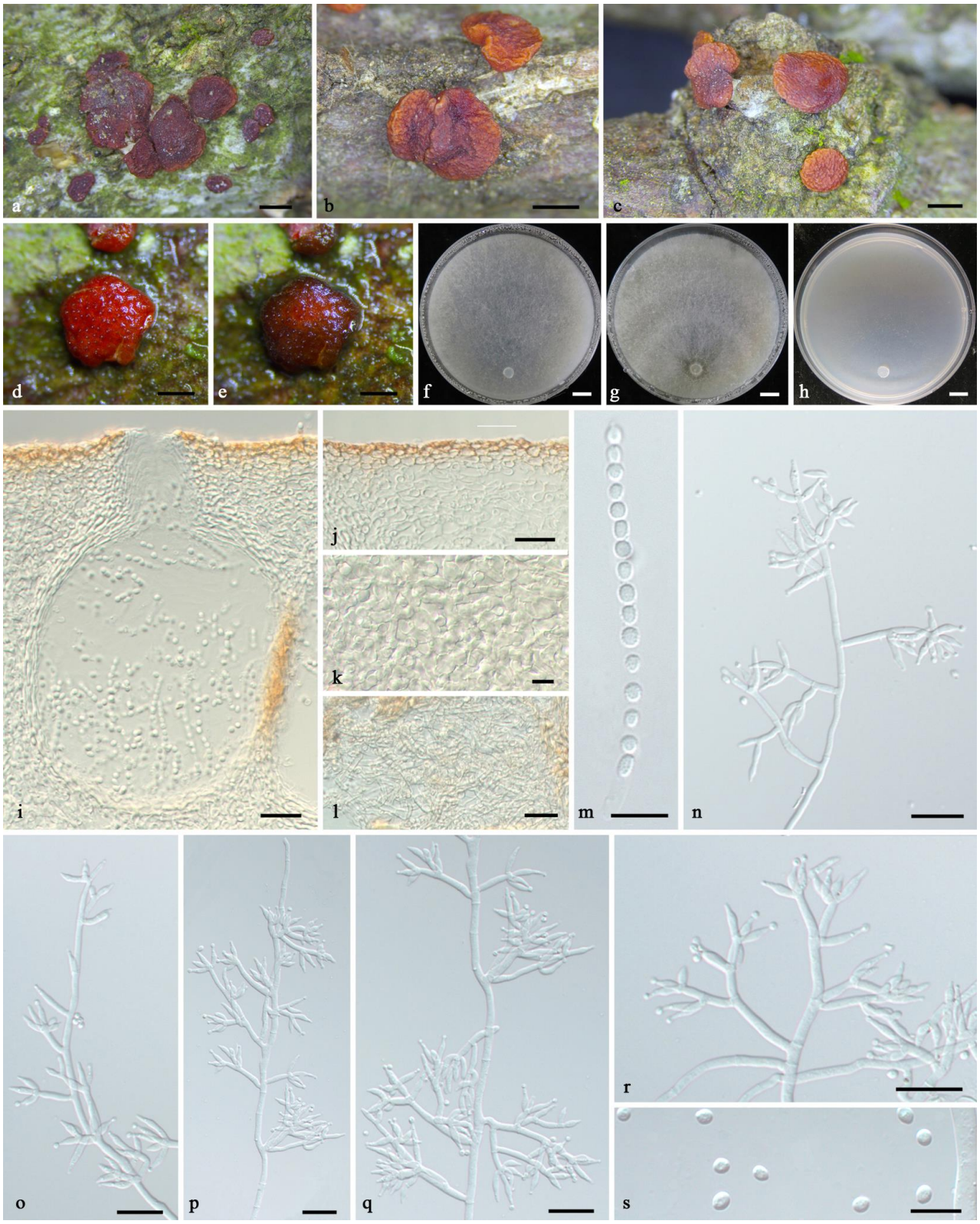

Figure 3 – *Trichoderma medogense*.a–c Stromata on natural substrate d Stroma after rehydration e Rehydrated stroma in 3% KOH f–h Cultures after 7 days at 25°C(f CMD, g PDA, h SNA) i Perithecium in section j Cortical and subcortical tissues in section k Subperithecial tissue in section l Stroma base in section m Ascusn–r Conidiophores and phialides (CMD, 2days) s Conidia (CMD, 6 days). –Photos: a, d, e = HMAS25452, b, c, f–s = HMAS275662. –Scale bars: a–c, f–h = 10 mm, d, e = 500 µm, i–l, n-r = 20 µm,m, s = 10 µm.

Stromata solitary or gregarious, pulvinate, outline irregular, broadly attached, margin free, yellowish brown, 0.5–1.5 mm diam., 0.2–0.5 mm thick (n = 70). Surface smooth. Ostiolar dots inconspicuous or slightly dark areola. Rehydrated stromata turning reddish brown in 3% KOH.

In section, cortical tissue of *textura angularis*, 13.5–22 µm thick (n = 30), cells slightly orange, thick-walled, 2.5–6 × 2.5–4 µm (n = 30), becoming darkened in 3% KOH; subcortical tissue of *textura intricata*, hyphae hyaline, thin-walled, 1.5–4.5 µm (n = 30) wide; subperithecial tissue of *textura epidermoidea*, cells hyaline, thin-walled, 8.5–27 × 5.5–12 µm (n = 33); tissue at the base of *textura angularis* and *textura intricata*, hyphae hyaline, thin-walled, 2.5–6.5 µm (n =30) wide, cells hyaline, thin-walled, 4.5–11 × 4–8.5 µm (n = 30). Perithecia flask-shaped or globose, (153.5–)174.5–228.5 × (99.5–)112.5–166 µm (n = 30); peridium hyaline, 8.5–14 µm thick at flanks, 9.5–17 µm thick at the base (n = 30). Ostioles 16.5–29 µm wide at apex, 41.5–66.5 µm high (n = 30). Asci cylindrical, 66–82.5 × 3.5–6 µm (n = 31), sessile. Ascospores hyaline, verrucose, cells dimorphic, distal cells globose to subglobose, 2.5–4.5 × 3–4.5 µm, L/W 1–1.3, proximal cells subglobose to ellipsoidal, 3–5 × 2.5–3.5 µm, L/W 1–1.5 (n = 37).

Colony on CMD 36–45 mm in radius after 72 h at 25 °C, mycelium covering the plate after 5 d. Colony circular, greenish; aerial hyphae abundant in the distal part of the colony. Conidiation noted after 3 d on aerial hyphae. Pigment yellowish, no odor formed.

Colony on PDA 37–40 mm in radius after 72 hat 25 °C, mycelium covering the plate after 5 d. Colony nearly circular, slightly zonate, greenish; aerial hyphae short, erect, and downy. Conidiation noted after 3 d on aerial hyphae. Pigment yellowish, no odor formed.

Colony on SNA 8–15 mm in radius after 72 h at 25°C, covering the plate after 28 d. Colony nearly circular, thin; aerial hyphae loosely distributed. Conidiation noted after 3 d on aerial hyphae around the plug. Conidiophores verticillium- to trichoderma-like, branches arising from the main axis at an acute or right angle, mostly re-branching once. Phialides subulate or ampulliform, solitary or divergent in whorls of 2–5, 6.5–14 × 2–3 µm, L/W 2.9–7.2, base 1–2.5 µm (n = 45). Conidia green, subglobose to ellipsoid, 3.5–5 × 2.5–3.5 µm, L/W 1.2–1.5(–1.7) (n = 36). Pigment yellowish, no odor formed.

Material examined – CHINA, Yunnan, Maguan County, 22°56.58'N, 104°32.60'E, alt. 2000 m, on rotten twig, 11 August 2016, X.H. Wang, S.H. Li, H.D. Zheng & S.C. Li YN16-80 (HMAS275665 **holotype**); ex-type culture HMAS247237; Yunnan, Maguan County, 23°6.21'N, 104°19.75'E, alt. 1450 m, on rotten twig, 14 August 2016, X.H. Wang, S.H. Li, H.D. Zheng & S.C. Li YN16-161 (HMAS254527, culture HMAS247238).

Notes – The new species shares common ancestor with *T. petersenii*, *T. istrianum* and *T. caribbaeum* (Fig.1). *Trichoderma petersenii* and *T. istrianum* are easily distinguished from *T. palidulum* by velvety and semi-effused stomata, and larger perithecia [(166–)190–375(–485)× (90–)125–265(–370) µm in *T. petersenii*, (120–)145–205(–225) × (95–)105–180(–220) µm in *T. istrianum*] (Samuels et al. 2006, Jaklitsch & Voglmayr 2015).In *T. caribbaeum*, the stromata do not react to KOH, cortex is thicker (20–35 µm), subcortical tissue is of *textura angularis* instead of *textura intricata*, and the growth is faster than that of the new species (on PDA: 53–56 mm after 72 h, on SNA: 41–42 mm after 72 h) (Samuels et al. 2006). There were also sequence divergences with 59–60 bp differences for TEF1 (95%) and 26–29 bp divergences for RPB2 (98%).

TrichodermavirgineumY.B. Zhang & W.Y. Zhuang, sp. nov. Fig. 5

Fungal Names: FN570504; Facesoffungi number: FoF 03918

Etymology – The specific epithet refers to the white colony of the fungus.

Stromata solitary, gregarious, or aggregated in small numbers, pulvinate or lenticular,outline circular, lobed, or irregular, centrally attached, pale bright yellow when fresh, raw sienna when mature, 1–3.5 mm diam., 0.5–1mm thick (n = 40). Surface nearly smooth. Ostiolar dots dark brown, distinct, densely distributed. Rehydrated stromata turning antique brown in 3% KOH.

In section, cortical tissue of *textura angularis*, 12–18 µm thick (n = 30), cells yellowish brown, thick-walled, 2.5–9.5 × 2–6 µm (n = 30), becoming rufous in 3% KOH; subcortical tissue of *textura intricata*, hyphae hyaline, thin-walled, 2–3.5 µm (n = 39) wide; subperithecial tissue of

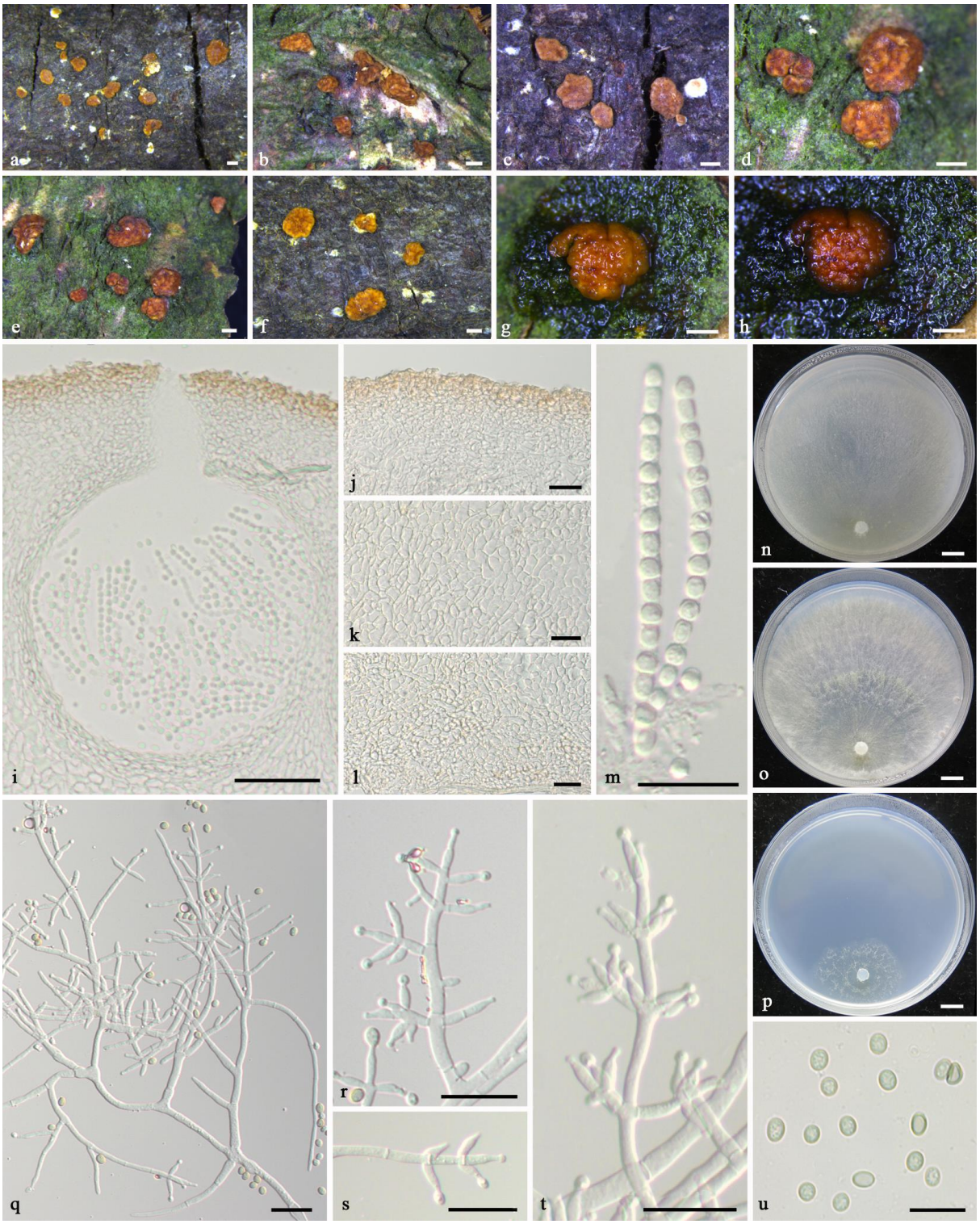

Figure 4– *Trichoderma palidulum*. a–f Dry stromata on natural substrate g Mature stroma after rehydration h Rehydrated mature stroma in 3% KOH i Perithecium in section j Cortical and subcortical tissues in section k Subperithecial tissue in section l Stroma base in section m Asci n–p Cultures after 5 days at 25°C (n CMD, o PDA, p SNA) q–t Conidiophores and phialides (CMD, 3days) u Conidia (SNA, 13 days). –Photos: a, c, i–l, s–t = HMAS254527, b, d–h, m–r, u = HMAS275665. –Scale bars: a–h = 500 µm, i = 50µm, j–m, q–t = 20 µm, n–p = 10 mm, u = 10 µm.

textura epidermoidea, cells hyaline, thin-walled, 4.5–15 × 3–7 µm (n = 30); tissue at the base of *textura epidermoidea* mixed with *textura angularis*, cells hyaline to slightly yellow, thin-walled, 4–10.5 × 3.5–7 µm (n =31). Perithecia flask-shaped or globose, 165–200 × 93–142 µm (n = 30);

peridium pale yellow, slightly darkened in 3% KOH, 6–11.5 µm thick at flanks, 8.5–12.5 µm thick at the base (n = 30). Ostioles 12.5–20 µm wide at apex, 30.5–45 µm high (n = 30). Asci cylindrical, sessile, 41.5–66 × 2–4 µm (n = 30). Ascospores hyaline, spinulose, cells monomorphic, distal cells globose to subglobose, 2–3.5 × 2–3 µm, L/W 1–1.4 (n = 30), proximal cells globose to subglobose, 2–3.5 × 1.5–3 µm, L/W 1–1.5 (n = 30) .

Colony on CMD 48–51 mm in radius after 72 h at 25 °C, mycelium covering the plate after 5 d. Colony circular, thin, with well-defined margin; aerial hyphae rarely. No conidiation observed within 15 d. Chlamydospores noted after 25 d. Odor slightly fruity; no pigment observed.

Colony on PDA 24–30 mm in radius after 72 hat 25 °C, mycelium covering the plate after 25 d. Colony nearly circular, with wavy margin; aerial hyphae intertwined, forming a dense white mat. No conidiation observed within 15 d. Chlamydospores noted after 25 d, abundant, terminal or intercalary, globose or ellipsoidal, 6.5–23.5 × 7.0–22.5 µm (n = 30). Odor slightly fruity; no pigment observed.

Colony on SNA 33–35 mm in radius after 72 h at 25°C, mycelium covering the plate after 10 d. Colony nearly circular, thin, radial; aerial hyphae rarely distributed, becoming cottony near the plate margin. Conidiation noted after 15 d on aerial hyphae. Conidiophores acremonium-like, branches arise from the main axis. Phialides narrowly subulate, mostly solitary, 12–22.5(–26) × 2–3.5 µm, L/W 5–9, base 1.5–3.2 µm (n = 49). Conidia hyaline, oblong or ellipsoid, rarely cylindrical, 3.5–6.5(–8) × 2–3.5 µm, L/W 1–2.6(–2.8) (n = 30). No chlamydospores observed within 25 d. No distinct odor and diffusing pigment formed.

Material examined – CHINA, Yunnan, Maguan County, 22°49.55'N, 104°24.55'E, alt. 1565 m, on rotten twig, 13 August 2016, X.H. Wang, S.H. Li, H.D. Zheng & S.C. Li YN16-110 (HMAS275664 holotype); ex-type culture HMAS247236.

Notes – In Fig.1, *T. virgineum*, *T. henanense* and *T. odoratum* formed a clade which is not closely related to any of the existing clades. These three species are similar in yellowish stromata, monomorphic ascospores, white colonies, simply branched conidiophores and hyaline conidia (Qin & Zhuang 2016c). However, *T. odoratum* differs in projecting, higher and wider ostioles [projecting by (8–)16–21 µm, 53–66(–74) µm high, (29–)32–40(–42) µm wide], longer and wider asci [(62–)68–82(–89) × 4.2–5(–5.5) µm], smaller growth rate and the mushroom-like odor in cultures. Compared with the sequences of RPB2 and TEF1, similarities were 94% and 93% with 68 bp and 84 bp differences among 1073 and 1261 bp respectively. *Trichoderma henanense* has larger cells in cortical, subperithecial and basal tissues [respectively(4.5–)6–8.5(–10.5) × (4–)4.5–8(–10) µm, 8–19(–24) × 7.5–14(–19) µm, and (5–)6–13(–16) × (4–)5–10(–12) µm] than the new species. In its anamorphic state, *T. henanense*grows slower than the new species on CMD and PDA media (19–31 mm on CMD and 19–21 mm on PDA after 72 h at 25°C), produces denser aerial hyphae on CMD and distinctly abundant chlamydospores on SNA. Sequence comparisons indicate there are 61 bp divergences among 1073 bp for RPB2 and 45 bp among 1064 bp for TEF1.

Discussion

Phylogenetic analyses of the 60*Trichoderma* species were carried out based on analyses of the combined sequences ofRPB2 and TEF1to allocate the phylogenetic positions of the four new species. The analyses included representatives of almost all the named clades of the genus and some scattered terminal branches. The resulted tree (Fig.1) is basically congruent with the previous studies(Jaklitsch 2009; Jaklitsch &Voglmayr 2015; Qin & Zhuang 2016c; Zhu & Zhuang 2015a,b), in which 15clades were recognized with five possessing green ascospores, and ten giving rise to hyaline ascospores. The four newly described species are among the hyaline-ascospored groups. One of them belongs to the Viride clade, two are as separate terminal branches, and one clusterswith two knownspecies forming a new clade.

Trichoderma fructicola, featured by cortical tissues of *textura epidermoidea* and occurring ona rotten fruit, isseemingly associated with the Hypocreanum and Psychophilum clades, which

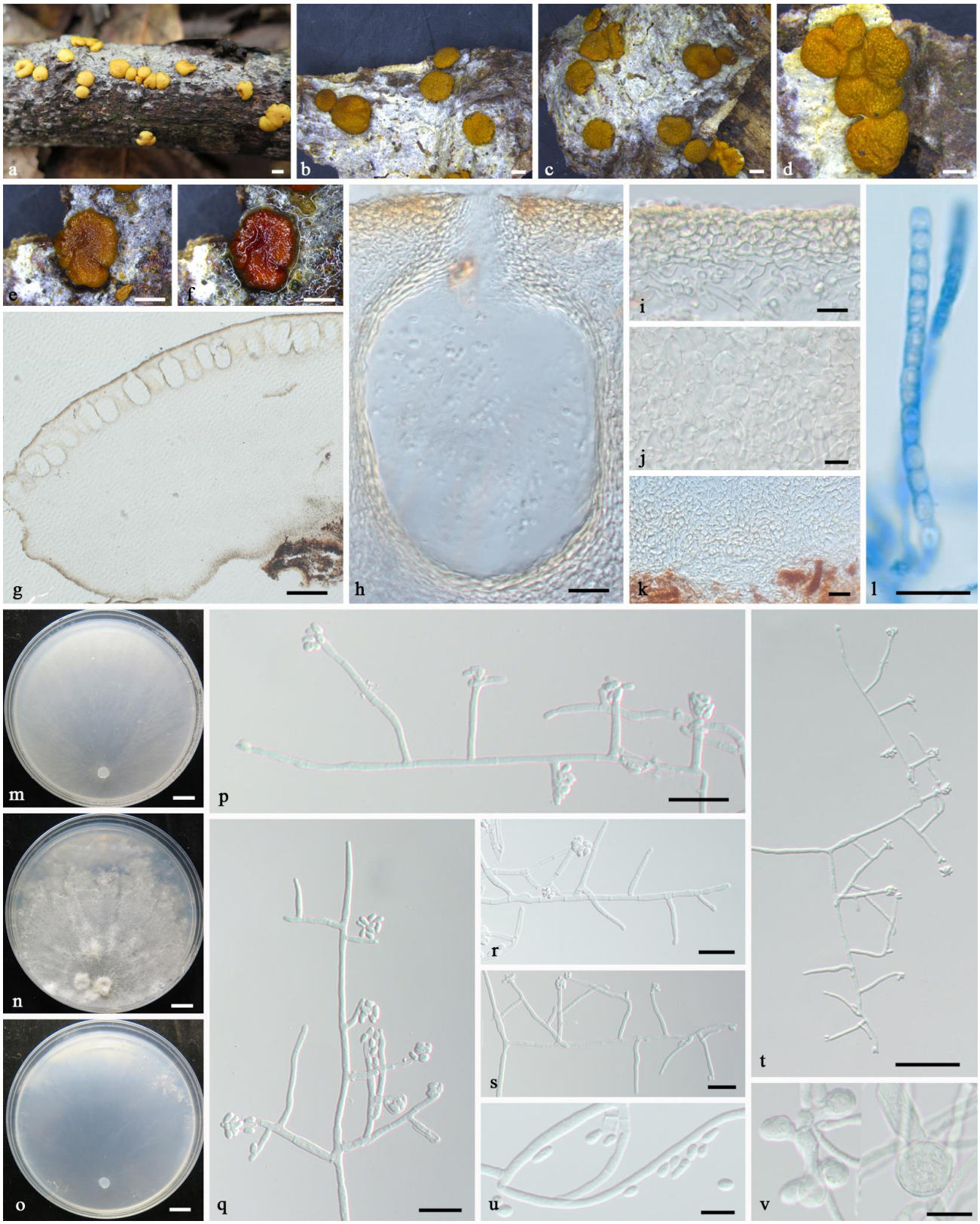

Figure 5– *Trichoderma virgineum*(HMAS275664). a Fresh stromata on natural substrate b–d Dry stromata on natural substrate e Mature stroma after rehydration f Rehydrated mature stroma in 3% KOH g Longitudinal section of stroma h Perithecium in section i Cortical and subcortical tissues in section j Subperithecial tissue in section k Stroma base in section l Ascus m–o Cultures after 30 days at 25°C (m CMD, n PDA, o SNA) p–t Conidiophores and phialides (SNA, 25days) u Conidia (SNA, 25 days) v Chlamydospores (PDA, 25 days). –Scale bars: a= 2 mm, b–f, m–o = 1 mm, g = 200µm, h, k, p–s, u, v = 20 µm, i, j = 10 µm, t = 50 µm.

Nevertheless lacks of sufficient support (Fig.1). *Trichoderma medogense* is closely associated with *T. voglmayrii* (MPBP/BIPP = 100%/100%).They formed a small but highly supported group (MPBP/BIPP = 100%/100%).They both occur at high elevation sites, and have similar stroma color and conidiophore branching pattern. This new fungus is clearly distinguishable in structure of subcortical tissues, shape of phialides, and especially size of conidia. *Trichoderma palidulum* shares some common features with others in the Viride clade, such as brown to rufous stromata with inconspicuous ostiolar dots, hyaline ascospores, and green conidia. In the phylogenetic analyses, it is associated with *T. petersenii*, *T. istrianum* and *T. caribbaeum*, but differs significantly in many other aspects as already mentioned in the notes.

The regional monographic treatments of *Trichoderma* species having hyaline ascospores were carried out by Jaklitsch (2011) and Jaklitsch & Voglmayr (2015). The hyaline-ascospored species of the genus were in ten clades, including the recently added Asterineum clade specialized by ostiolar dots surrounded by stellate cracks. Among the known species, *T. virgineum* shares phenotypic similarity with *T. odoratum* and *T. henanense*, and is closely related to them with high statistic supports(MPBP/BIPP = 100%/100%).These three species are common inyellowish stromata, white colonies on three standard media, monomorphic ascospores, acremonium- or verticillium-like conidiophores, and hyaline conidia. The Virgineum clade is here proposed. Among the existingclades, the Hypocreanum clade forms also simple conidiophore branch patterns and hyaline conidia, however, speciesof that clade have extensively effused stromata and mostlypossess vertically parallel and warted hyphae above stroma surface(Overton et al. 2006). It is remotely related to the Virgineum clade (Fig.1).

Many new species of *Trichoderma* have been published successively based on the materials collected from this country in the past four years (Zhu & Zhuang 2014, 2015a,b, Qin & Zhuang 2016a,b,c, 2017, Chen & Zhuang 2017a,b,c). We believe that more taxa will be discovered in the unexplored regions, which will definitely renew our understanding of species diversity, resources, taxonomy and phylogeny of the genus, and which will certainly broaden our knowledge of application of useful fungal resources.

Acknowledgements

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