Four new species of *Trichoderma* in the *Harzianum* clade from northern China

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Abstract

The *Harzianum* clade of *Trichoderma* comprises many species, which are associated with a wide variety of substrates. In this study, four new species of *Trichoderma*, namely *T. lentinulae*, *T. vermifimicola*, *T. xixiacum*, and *T. zelobreve*, were encountered from a fruiting body and compost of *Lentinula*, soil, and vermicompost. Their colony and mycelial morphology, including features of asexual states, were described. For each species, their DNA sequences were obtained from three loci, the internal transcribed spacer (ITS) regions of the ribosomal DNA, the gene encoding the second largest nuclear RNA polymerase subunit (RPB2), the translation elongation factor 1-α encoding gene (TEF1-α). The analysis combining sequences of the three gene regions distinguished four new species in the *Harzianum* clade of *Trichoderma*. Among them, *T. lentinulae* and *T. xixiacum* clustered with *T. lixii*, from which these new species differ in having shorter phialides and smaller conidia. Additionally, *T. lentinulae* differs from *T. xixiacum* in forming phialides with inequilateral to a strongly-curved apex, cultural characteristics, and slow growth on PDA. *Trichoderma vermifimicola* is closely related to *T. simmonsii*, but it differs from the latter by producing phialides in verticillate whorls and smaller conidia. *Trichoderma zelobreve* is the sister species of *T. breve* but is distinguished from *T. breve* by producing shorter and narrower phialides, smaller conidia, and by forming concentric zones on agar plates. This study updates our knowledge of species diversity of *Trichoderma*.

Keywords

compost, fungicolous, Hypocreaceae, mycoparasite

* Authors contributed equally to this work

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Introduction

The genus *Trichoderma* Pers., introduced by Persoon (1794), is cosmopolitan, including saprotrophs and mycoparasites in a diversity of ecosystems, such as agricultural fields, prairies, forests, salt marshes, and fungal fruiting body (Gazis and Chaverri 2010; Chaverri et al. 2015; Qiao et al. 2018). Species of this genus have been widely used in the biocontrol of plant pathogens (Chaverri et al. 2015; Degenkolb et al. 2015; Bunbury-Blanchette and Walke 2019) and production of enzymes and bioactive compounds (Sun et al. 2016). Nevertheless, some of them are associated with green mold diseases in the commercial production of mushrooms (Innocenti et al. 2019; Sun et al. 2019a). Morphologically, the asexual-morphs are similar in producing branched tree-like conidiophores with cylindrical to nearly subglobose phialides and ellipsoidal to globose conidia, but their variation is insufficient to differentiate the *Trichoderma* species (Chaverri et al. 2015; Qin and Zhuang 2017; Qiao et al. 2018). Multilocus molecular phylogeny, based on combined sequence data of the internal transcribed spacer (ITS) regions, RNA polymerase II subunit (RPB2), and the translation elongation factor 1-α gene (TEF1-α), enables rapid and accurate identification of the *Trichoderma* species (Druzhinina et al. 2005; Atanasova et al. 2013; Chaverri et al. 2015). Currently, the combination of multi-gene phylogenetic analysis and phenotypic characteristics is extensively applied in species delimitation of *Trichoderma* (du Plessis et al. 2018; Qiao et al. 2018; Innocenti et al. 2019).

*Trichoderma harzianum* Rifai is one of the most well-known *Trichoderma* species, due to its antifungal properties and effective bio-control ability, used to suppress soil-borne plant pathogens (Chaverri et al. 2015; Degenkolb et al. 2015; Bunbury-Blanchette and Walker 2019). As a cosmopolitan and ubiquitous fungus, it has been isolated from diverse substrates, such as soil, plant tissue, and mushrooms (Chaverri et al. 2015; Jaklitsch and Voglmayr 2015; Innocenti et al. 2019; Sun et al. 2019b). Since Chaverri et al. (2015) provided a systematic revision of species in the *Harzianum* clade, numerous new species have been described (Jaklitsch and Voglmayr 2015; Qin and Zhuang 2016a; Sun et al. 2016; Chen and Zhuang 2017b; Qiao et al. 2018). Currently, more than 60 species are placed in the *Harzianum* clade (Jaklitsch and Voglmayr 2015; Qin and Zhuang 2016a, b, 2017; Chen and Zhuang 2017b; Qiao et al. 2018; Phookamsak et al. 2019).

It is estimated that 136 new species of *Trichoderma* have been recognised since 2015 (www.indexfungorum.org 2020), with 84 among these reported from China (Sun et al. 2012; Qin and Zhuang 2016a, b, 2017; Chen and Zhuang 2017a, b; Qiao et al. 2018), which evidenced that China has a high species diversity of *Trichoderma* (Zhu and Zhuang 2015; Jiang et al. 2016). In our survey of *Trichoderma*, eighteen isolates were obtained from soil, mushroom substrates, and vermicompost from northern China. Four new species belonging to the *Harzianum* clade were identified based on morphological features and DNA sequence data at three loci: the genes encoding RNA polymerase II subunit (RBP2) and translation elongation factor 1-α gene (TEF1-α), and the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene.
Materials and methods

Sampling sites and strains isolation

Since *Trichoderma* is easily isolated from soil, mushroom substrates, and earthworm substrates, the soil, mushroom substrates, and earthworm were therefore collected from Yinchuan, Ningxia Hui Autonomous Region, and Chaoyang district, Beijing, China. All the samples were stored at 4 °C before fungal isolation. *Trichoderma* strains were isolated by gradient dilution and the spread plate method or directly from the mushroom substrates. Three dilutions (10⁻¹, 10⁻², and 10⁻³) were prepared with 1 g soil and sterile water, and 100 µl of each dilution was spread on a 9 cm diameter Petri dish of PDA agar with 100 mg/L chloramphenicol added. The plates were then incubated at 25 °C. Each of the individual colonies was transferred to a new PDA dish after 1–3 days and incubated at 25 °C. Dried cultures from the single spore or specimens of new species were deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS) and the ex-type strains were preserved in the China General Microbiological Culture Collection Center (CGMCC).

Morphological analysis

For morphological studies, we used three different media: cornmeal dextrose agar (CMD, Difco, BD Science, USA), PDA (Difco, BD Science, USA), and synthetic low nutrient agar (SNA, Difco, BD Science, USA) (Chaverri et al. 2015). Each strain was first cultured on an SNA plate for 3 days and a small agar piece of 0.5 cm diameter with mycelium was then transferred, respectively, to new CMD, PDA, and SNA plates. Strains were incubated in 9 cm diam with three replicates. Petri dishes at 25 °C with a 12 h natural light and 12 h darkness interval. Colony diameter at 25 °C was measured three days after inoculation, and the time when mycelium entirely covered the surface of the agar plate was also recorded. Micromorphological characters were examined from the cultures of one-week-old colonies on SNA (Chaverri et al. 2015). A Nikon Ellipse 80i light microscope, equipped with differential interference contrast (DIC) optics, was used to capture digital images.

DNA extraction, PCR and sequencing

Genomic DNA of each strain was extracted from fresh mycelium growing on PDA after 5 days of growth following the rapid “thermolysis” method described in Zhang et al. (2010). For the amplification of ITS, RPB2, and TEF1-α gene fragments, ITS4 and ITS5 for ITS (White et al. 1990), EF1-728F (Carbone and Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005) for TEF1, and RPB2-5F and RPB2-7R for rpb2 (Liu et al. 1999) were used. Each PCR reaction consisted of 12.5 µl T5
Super PCR Mix (containing Taq polymerase, dNTP, and Mg$^{2+}$, Beijing TsingKe Biotech Co. Ltd., Beijing), 1.0 µl of forward primer (10 µM), 1.0 µl of reverse primer (10 µM), 0.5 µl DMSO, 3 µl DNA template and 7 µl double sterilized water. PCR reactions were in Eppendorf Mastercycler, following the protocols described by Sun et al. (2016). PCR products were purified with the PCR product purification kit (TIANGEN Biotech, Beijing, China), and sequencing was carried out in both directions on an ABI 3730 XL DNA sequencer (Applied Biosystems, Foster City, California) with primers used during PCR amplification.

**Phylogenetic analyses**

Preliminary BLAST searches with ITS, RPB2, and TEF1-α gene sequences of the new isolates against NCBI, TrichOKey (Druzhinina and Kopchinski 2006), and TrichoBlast (Kopchinskiy et al. 2005) databases identified species closely related to our isolates. Based on this information, sequences of ITS, RBP2, and TEF1-α of 133 strains, representing 59 species were downloaded from GenBank, following recent publications (Qin and Zhuang 2017; Qiao et al. 2018; Innocenti et al. 2019). Among them, 139 strains are belonging to the *Harzianum* clade, and *Trichoderma ceramicum*, *T. parestonicum*, and *T. estonicum* were chosen to represent the outgroup.

Tree alignment files were generated by using MAFFT version 7.03 with the Q-INS-I strategy (Katoh and Standley 2013). Conserved blocks were selected from the initial alignments with Gblocks 0.91 b (Castresana 2000). The appropriate nucleotide substitution model for each gene was determined by using MrModeltest v2.4 (Nylander 2004). HKY + I + G was estimated as the best-fit model for RPB2, and GTR + I + G was estimated as the best-fit model for TEF1-α and ITS under the output strategy of AIC. The partition homogeneity test ($p = 0.01$) indicated that the individual partitions were not significantly incongruent (Cunningham 1997), thus the aligned sequences of ITS, RPB2, and TEF1-α were combined for analyses. The multi-locus phylogenetic analyses included 1065 characters for RPB2, 587 characters for TEF1-α, and 555 characters for ITS. All characters were weighted equally and gaps were treated as missing characters.

Maximum Likelihood (ML) analyses were performed by RAxML (Stamatakis 2006), using the GTR-GAMMA-I model. The maximum likelihood bootstrap proportions (MLBP) were using 1000 replicates. Bayesian Inference (BI) analyses were conducted with MrBayes v3.2.6 (Ronquist et al. 2012). Metropolis-coupled Markov Chain Monte Carlo (MCMC) searches were calculated for 10,000,000 generations, sampling every 100th generation with the best best-fit model for each gene. Two independent analyses with six chains each (one cold and five heated) were carried out until the average standard deviation of the split frequencies dropped below 0.01. The initial 25% of the generations of MCMC sampling were discarded as burn-in. The refinement of the phylogenetic tree was used for estimating Bayesian
inference posterior probability (PP) values. The Tree was viewed in FigTree v1.4 (Rambaut 2012), values of Maximum likelihood bootstrap proportions (MLBP) greater than 50% and Bayesian inference posterior probabilities (BIPP), greater than 95% at the nodes, are shown along branches. The final alignments and the trees obtained have been deposited in TreeBASE (TreeBASE accession number: 25400).

Results

Phylogeny

The preliminary BLAST searches with ITS, RPB2, and TEF1-α gene sequences of the new isolates suggest our isolates were highly similar to species from *Trichoderma* in the *Harzianum*-complex. Therefore, as the next step phylogenetic analyses were conducted by using a single gene of ITS, RPB2, TEF1-α, and multi-gene dataset of cascaded ITS, RPB2, and TEF1-α, respectively. The phylogenetic trees showed that our isolates were placed in the *Harzianum* clade (Fig. 1, Suppl. material 1: Fig. S1, Suppl. material 2: Fig. S2, Suppl. material 3: Fig. S3). In the phylogenetic tree conducted by a combined matrix of ITS, RPB2, and TEF1-α sequences, isolates of *T. lentinulae*, *T. xixiacum*, and *T. lixii* formed a well-supported clade (MLBP/BIBP = 73%/1.00). Within this clade, isolates of *T. lentinulae* and *T. xixiacum* formed a subclade with maximum support. Isolates of *T. vermifimicola* clustered together with *T. simmonsii* (BIBP = 1.00), both forming a subclade with maximum support (MLBP/BIBP = 100%/1.00, Fig. 1). *Trichoderma zelobreve* and *T. breve*, were distinguished by maximum support to respective clades while forming a highly supported clade (MLBP/BIBP = 100%/1.00, Fig. 1).

The ITS gene could not distinguish our isolates from other species within the *Harzianum* clade (Suppl. material 1: Fig. S1). In the phylogenetic tree resulted from the RPB2 gene, *Trichoderma lentinulae*, *T. xixiacum*, and *T. lixii* formed a highly supported clade (MLBP/BIBP = 100%/1.00), but within this clade, *T. lentinulae*, *T. xixiacum* were not distinguished (Suppl. material 2: Fig. S2). Isolates of *T. vermifimicola* formed a distinct clade (MLBP/BIBP = 100%/1.00) and grouped with *T. simmonsii*, *T. guizhouense*, and *T. rugulosum* but weakly supported (Suppl. material 3: Fig. S3). *Trichoderma zelobreve* and *T. breve* also formed a highly supported clade (MLBP/BIBP = 98%/1.00), but *T. zelobreve* and *T. breve*, were distinguished by maximum support to respective clades while forming a highly supported clade (MLBP/BIBP = 100%/1.00, Suppl. material 2: Fig. S2). In the phylogenetic tree resulted from the TEF1-α gene, *T. zelobreve* and *T. breve* also formed a highly supported clade (MLBP/BIBP = 98%/1.00), but were not distinct from each other (Suppl. material 3: Fig. S3). Isolates of *T. lentinulae*, *T. xixiacum*, *T. vermifimicola*, and *T. simmonsii* clustered together but this clade was not well-supported. Within this clade, isolates of *T. lentinulae* formed a well-supported subclade (MLBP/
Figure 1. Phylogenetic tree based on Maximum Likelihood analysis of a combined ITS, RPB2, and TEF1α sequence dataset. *Trichoderma estonicum*, *Trichoderma parastinicum*, *Trichoderma ceramicum* were chosen as the outgroup. Bootstrap Values higher than 70% from RAxML (BSML) (left) and Bayesian posterior probabilities greater than 0.95 (BYPP) (right) are given above the nodes. T indicates the type; ET indicates the ex-living type. Isolates obtained in this study are in red.
Four new *Trichoderma* spp. (MLBP/BIBP = 91%/1.00). *Trichoderma xixiacum* and *T. vermifimicola* formed a highly supported subclade (MLBP/BIBP = 100%/1.00). Within this group, isolates of *T. vermifimicola* clustered together with well-supported (MLBP/BIBP = 93%/1.00, Suppl. material 3: Fig. S3).

**Figure 1.** Continued.
Taxonomy

*Trichoderma lentinulae* Jing Z. Sun & X.Z. Liu, sp. nov.
MycoBank No: 833233

Fig. 2

Etymology. Latin, *lentinulae*, refers to the host from which the fungus was isolated.

Type. CHINA. Haidian District, Beijing, 39°57’40”N, 116°19’40”E, ca. 27 m elev., from a fruiting body and mushroom spawn of *Lentinula edodes*, 19 Oct 2018, Jing Z. Sun (HMAS 248256, holotype), ex-type culture CGMCC 3.19847.

Description. On CMD after 72 h, colony radius 57–58 mm at 25 °C, covering the plate at 30 °C, 4–5 mm at 35 °C. Colony hyaline, weak, indistinctly radial. Aerial hyphae short, inconspicuous. No diffusing pigment noted, odor indistinct (Fig. 2B). Conidial production noted after 3 days, scant, effuse in aerial hyphae, becoming blue-green after 7 days. Chlamydospores not observed.

On PDA after 72 h, colony radius 45–46 mm at 25 °C, mycelium covering the plate at 30 °C, 11–12 mm at 35 °C. Colony white to yellowish-white, regularly circular, indistinctly zonate; mycelium dense and radial. No diffusing pigment, not distinct odor (Fig. 2A). Conidial production noted after 3 days, starting around the original inoculum, effuse in the aerial hyphae, first white, turning green after 3 d. Chlamydospores unobserved.

On SNA after 72 h, colony radius 51–52 mm at 25 °C, 52–53 mm at 30 °C, 4–5 mm at 35 °C. Colony hyaline, indistinctly zonate; mycelium loose, especially at the margin. Aerial hyphae loose. No diffusing pigment, not distinct odor (Fig. 2C). Conidial production noted after 2 days, starting around the inoculum, effuse in the aerial hyphae. Small pustules formed around the inoculum, first white, turning green after 3 d, with hairs protruding beyond the surface. Conidiophores pyramidal with opposing branches, less frequently solitary, closely-spaced branches, each branch, and the main axis terminating in 2–5 cruciately to nearly verticillately disposed phialides (Fig. 2F, H, I). Phialides ampulliform, typically strongly constricted below the tip, less frequently lageniform and then usually apex and inequilateral to strongly curved, hyaline, (3.5–)4.0–6.0(−6.5) × (2.0–)2.5–3.0(−3.5) μm (\(\bar{x} = 4.5 \times 3.0 \mu m, n = 30\)), length/width ratio (1.5–)2.0–3.0(−5.0) (\(\bar{x} = 2.0, n = 30\)), base 1.0–2.5 μm (\(\bar{x} = 1.5 \mu m\))(Fig. 2G, H, I). Conidia ovoid to globose, smooth, hyaline when young, becoming green to dark green with age, (2.0–)2.5–3.0(−3.5) × (1.5–)2.0–2.5(−3.0) μm (\(\bar{x} = 2.5 \times 2.2 \mu m, n = 50\)), length/width ratio (1.0–)1.1–1.4 (−1.5) (\(\bar{x} = 1.2, n = 50\)) (Fig. 2J). Chlamydospores common, apex or intercalary, ellipsoid or subglobose, (3.5–)5.0–6.5(−7.0) × (3.0–)4.0–5.0(−6.0) μm (\(\bar{x} = 5.5 \times 4.5 \mu m, n = 30\)), length/width ratio (1.0–)1.2–1.5 (−1.7) (\(\bar{x} = 1.2, n = 30\)) (Fig. 2K–M).

Additional specimen examined. CHINA. Haidian District, Beijing, 39°57’40”N, 116°19’40”E, ca. 27 m elev., From a fruiting body and mushroom spawn of *Lentinula edodes*, 19 Oct 2018, Jing Z. Sun, living culture CGMCC 3.19848; Xixia District, Yinchuan, Ningxia Hui Autonomous Region, 38°38’52”N, 106°9’33”E, ca. 1127 m elev., from rhizosphere soil of *Lycium chinensis*, 17 Oct 2018, Jing Z. Sun, living culture CGMCC 3.19699; ibid., living culture CGMCC 3.19670.
Four new *Trichoderma* spp.

**Note.** The species is characterized by tree-like conidiophores, phialides verticillate or in whorls of 3–4, spindle-like to fusiform phialides (4.0–6.0 × 2.5–3.0 µm) and ovoid to subglobose conidia. Differs from *T. lixii* by shorter and wider phialides and smaller conidia. Differs from *Trichoderma xixiacum* by compact, relatively smaller phialides, and the pustules not forming distinctly zonate of pustules on SNA.

**Teleomorph.** Undetermined.

**Figure 2.** *Trichoderma lentinulae* (CGMCC 3.19847). Cultures at 25 °C after 3 days (A on PDA B on CMD C on SNA) D conidiation pustules on CMD after 10 days E conidiation pustules on CMD after 10 d F conidiophores G–I Conidiophores and phialides J conidia K–M chlamydospores. Scale bars: 25 µm (F); 10 µm (G–M).
Trichoderma vermifimicola Jing Z. Sun & X.Z. Liu, sp. nov.
MycoBank No: 833234
Fig. 3

Etymology. Latin, vermifimicola, refers to the habitat of the type species.

Type. CHINA. Yongning, Yinchuan, the Ningxia Hui Autonomous Region, 40°0’41”N, 116°23’37”E, ca. 1678 m elev., from the substrates for earthworm cultivation, 18 Oct 2018, Jing Z. Sun (HMAS 248255, holotype), ex-type culture CGMCC 3.19694.

Description. On CMD after 72 h, colony radius 49–51 mm at 25 °C, 51–52 mm at 30 °C, 4–5 mm at 35 °C. Colony hyaline, irregularly circular, indistinctly zonate; mycelium loose. Aerial hyphae short, inconspicuous. No diffusing pigment, not distinct odor. Conidial production noted after 3 days, starting around the inoculum (Fig. 3B). Small pustules formed at the colony margin, first white, turning blue-green after 7 d, with hairs protruding beyond the surface. Chlamydospores unobserved.

On PDA after 72 h, colony radius 55–58 mm at 25 °C, 55–56 mm at 30 °C, 5–6 mm at 35 °C. Colony white-green to bright green, regularly circular, distinctly zonate; mycelium dense and radial. Aerial hyphae short, inconspicuous. No diffusing pigment, not distinct odor. Conidial production noted after 2 days, starting around the inoculum, effuse in the aerial hyphae, first white, turning green after 2 d (Fig. 3A). Chlamydospores unobserved.

On SNA after 72 h, colony radius 48–50 mm at 25 °C, 51–52 mm at 30 °C, 3–4 mm at 35 °C. Colony hyaline, regularly circular, distinctly zonate; mycelium loose, especially at the margin. Aerial hyphae short, inconspicuous. No diffusing pigment, not distinct odor. Conidial production noted after 2 days, starting around the inoculum, effuse in the aerial hyphae. Small pustules formed along with two concentric rings, first white, turning yellow-green after 3 d, with hairs protruding beyond the surface (Fig. 3C). Conidiophores pyramidal with opposing branches, the distance between branches relatively large, each branch terminating in a whorl of 2–3 phialides, phialides sometimes solitary on the main axis (Fig. 3F, H, K); whorls typically cruciate, but often nearly verticillate (Fig. 3K); rarely conidiophores nodose and phialides disposed in more or less botryose clusters (Fig. 3H). Phialides ampulliform to lageniform, often constricted below the tip to form a narrow neck, hyaline, (4.4–)5.0–10.5(–11.2) × (2.0–)2.5–3.0(–3.5) μm (\(\bar{x} = 6.6 \times 2.7 \mu m, n = 30\)), length/width ratio (1.5–)1.8–2.8(–5.3) (\(\bar{x} = 2.4, n = 30\)), base 1.6–2.5 μm (\(\bar{x} = 1.9 \mu m\)) (Fig. 3G, I, K). Conidia ovoid to subglobose, smooth, hyaline when young, becoming green to dark green with age, (2.0–)2.3–2.6(–3.0) × (1.5–)2.0–2.4(–2.8) μm (\(\bar{x} = 2.4 \times 2.2 \mu m, n = 50\)), length/width ratio (1.0–)1.1–1.4(–1.7) (\(\bar{x} = 1.2, n = 50\)) (Fig. 3J). Chlamydospores unobserved. No odor; no diffusing pigment observed.

Additional specimen examined. CHINA. Xixia District, Yinchuan, Ningxia Hui Autonomous Region, 38°38’52”N, 106°9’33”E, ca. 1127 m elev., from rhizosphere soil of Lycium chinois, 17 Oct 2018, Jing Z. Sun, living CGMCC 3.19697.

Teleomorph. Undetermined.
Note. Characterized by tree-like conidiophores, verticillate or in whorls of 3–4, ampulliform to lageniform phialides (5.0–10.5 × 2.5–3.0 µm), ovoid to subglobose conidia (2.4–2.6 × 2.0–2.5 µm). Differs from *Trichoderma simmonsii* by forming loose branches in whorls, relatively longer and thinner phialides, smaller conidia, and the fewer pustules on SNA.
**Trichoderma xixiacum** Jing Z. Sun & X.Z. Liu, sp. nov.
MycoBank No: 833235

Fig. 4

**Etymology.** Latin, *xixiacum*, refers to the type locality.

**Type.** China. Xixia District, Yinchuan, Ningxia Hui Autonomous Region, 38°38′52″N, 106°9′33″E, ca. 1127 m elev., from rhizosphere soil of *Lycium chinois*, 17 Oct 2018, Jing Z. Sun (HMAS 248253, holotype), ex-type culture CGMCC 3.19697.

**Description.** On CMD after 72 h, colony radius 55–56 mm at 25 °C, covering the plate at 30 °C, 9–11 mm at 35 °C. Colony hyaline, indistinctly zonate, mycelia loose. Aerial hyphae short, inconspicuous. No diffusing pigment, not distinct odor (Fig. 4B). Conidial production noted after 3 days, effuse in aerial hyphae, becoming blue-green after 4 days. Chlamydospores unobserved.

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![Figure 4. Trichoderma xixiacum (CGMCC 3.19697). Cultures at 25 °C after 3 d (A on PDA B on CMD C on SNA) D conidiation pustules on CMD after 10 d E conidiation pustules on SNA after 10 d F, G, I conidiophores and phialides H conidia. Scale bars: 10 µm (F, G); 10 µm (H, I).](image-url)
On PDA after 72 h, colony radius 59–60 mm at 25 °C, covering the plate at 30 °C, 7–8 mm at 35 °C. Colony white to yellow-white, regularly circular, indistinctly zonate; mycelium dense and radial. Aerial hyphae conspicuous. No diffusing pigment, not distinct odor (Fig. 4A). Conidial production noted after 3 days, starting around the original inoculum, effuse in the aerial hyphae, first white, turning blue-green after 7 d. Chlamydospores unobserved.

On SNA after 72 h, colony radius 51–52 mm at 25 °C, 52–53 mm at 30 °C, 4–5 mm at 35 °C. Colony hyaline, indistinctly zonate; mycelium loose, especially at the margin. Aerial hyphae short. No diffusing pigment, not distinct odor (Fig. 4C). Conidial production noted after 2 days, starting around the inoculum, effuse in the aerial hyphae. Small pustules formed around the inoculum, first white, turning green after 3 d, with hairs protruding beyond the surface. Conidiophores pyramidal with opposing branches, less frequently solitary, closely-spaced branches, each branch, and the main axis terminating in 2–5 cruciately to nearly verticillately disposed phialides (Fig. 4F, G, I). Phialides ampulliform to lageniform, often constricted below the tip to form a narrow neck, hyaline, (3.2–)3.5–7.0(–9.3) × (2.3–)2.6–3.3(–3.6) µm (\(\bar{x} = 5.0 \times 3.0 \text{ µm}, n = 50\)), length/width ratio (1.2–)1.5–2.5(–4) (\(\bar{x} = 1.8, n = 50\)), base 1.6–2.2 µm (\(\bar{x} = 1.8 \text{ µm}, n = 50\)) (Fig. 4I). Conidia subglobose to globose, smooth, hyaline when young, becoming green to dark green with age, (2.0–)2.3–2.7(–3.0) × (1.6–)2.0–2.6(–3.0) µm (\(\bar{x} = 2.5 \times 2.2 \text{ µm}, n = 50\)), length/width ratio 1.0–1.3(–1.7) (\(\bar{x} = 1.1, n = 50\)) (Fig. 4H). Chlamydospores unobserved. No odor; no diffusing pigment observed.

**Additional specimen examined.** CHINA. Xixia District, Yinchuan, Ningxia Hui Autonomous Region, 38°38′52″N, 106°9′33″E, ca. 1127 m elev., from rhizosphere soil of *Lycium chinoin*, 17 Oct 2018, Jing Z. Sun, living CGMCC 3.19697.

**Teleomorph.** Undetermined.

**Note.** Characterized by tree-like conidiophores, verticillate or in whorls of 3–4, ampulliform to lageniform phialides (3.5–7.0 × 2.6–3.4 µm), subglobose to globose conidia (2.2–2.6 × 2.0–2.4 µm). Differs from *Trichoderma lentinulae* by compact, relatively smaller phialides, and the character of pustules on SNA. Differs from *Trichoderma lixii* by shorter and wider phialides and smaller conidia.

*Trichoderma zelobreve* Jing Z. Sun & X.Z. Liu, sp. nov.

**MycoBank No:** 833236

**Fig. 5**

**Etymology.** Greek *zelo*, meaning emulation + *breve*, referred to *Trichoderma breve*.

**Type.** CHINA. Chaoyang District, Beijing, 40°0′41″N, 116°23′37″E, ca. 35 m elev., 19 Oct 2018, isolated from soil, Jing Z. Sun (HMAS 248254, holotype), ex-type culture CGMCC 3.19695.

**Description.** On CMD after 72 h, colony radius covering the plate at 25 °C and 30 °C, 11–12 mm at 35 °C. Colony hyaline, indistinctly radial; Aerial inconspicuous. No diffusing pigment, not distinct odor (Fig. 5B). Conidial production noted after
5 days, starting around the original inoculum. Small pustules formed at the colony margin, first white, olivaceous after 6 d, with hairs protruding beyond the surface. Chlamydospores unobserved.

On PDA after 72 h, colony radius 55–58 mm at 25 °C, covering the plate at 30 °C, 8–9 mm at 35 °C. Colony white to yellow-white; mycelium dense and radial. Aerial conspicuous. No diffusing pigment, not distinct odor (Fig. 5A). Conidial production noted after 3 days, starting around the inoculum, effuse in the aerial hyphae, first white, turning green after 4 d. Chlamydospores unobserved.

On SNA after 72 h, colony radius 62–63 mm at 25 °C, covering the plate at 30 °C, 7–8 mm at 35 °C. Colony hyaline, regularly circular; mycelium loose. Aerial conspicuous. No diffusing pigment, not distinct odor (Fig. 5A). Conidial production noted after 2 days, starting around the inoculum, effuse in the aerial hyphae. Small pustules

Figure 5. *Trichoderma zelobreve* (CGMCC 3.19695). Cultures at 25 °C after 3 days (A on PDA B on CMD C on SNA) D conidiation pustules on CMD after 10 days E conidiation pustules on SNA after 10 d F conidiophores G, I conidiophores and phialides H phialides with conidia J conidia. Scale bars: 25 µm (F); 10 µm (G–J).
formed along with two concentric rings, first white, turning yellow-green after 3 d, with hairs protruding beyond the surface. Conidiophores pyramidal with opposing branches, the distance between branches relatively large (Fig. 5F). Phialides, sometimes solitary, often paired or in whorls of 2–3 (Fig. 5F); whorls typically cruciate but often nearly verticillate; rarely conidiophores nodose and phialides disposed in more or less botryose clusters (Fig. 5G, H). Phialides ampulliform to lageniform, often constricted below the tip to form a narrow neck, hyaline (Fig. 5G, H, I), (3.5–)4.0–6.0(–7.0) × (2.2–)2.6–3.2(–3.5) µm (x̄ = 4.8× 2.9 µm, n = 30), length/width ratio (1.1–)1.4–2.1(–2.5) (x̄ = 1.5, n = 30), base 1.4–2.1 µm (x̄ = 1.7 µm). Conidia ovoid to subglobose, smooth, hyaline when young, becoming green to dark green with age, (2.0–)2.3–2.6(–2.9) × (1.5–)1.8–2.2(–2.5) µm (x̄ = 2.4 × 2.0 µm, n = 30), length/width ratio (0.8–)1.1–1.4(–1.7) (x̄ = 1.2, n = 30) (Fig. 5J). Chlamydospores unobserved.

Additional specimen examined. CHINA. Chaoyang District, Beijing, 40°0'41"N, 116°23'37"E, ca. 35 m elev., isolated from soil, 19 Oct 2018, Jing Z. Sun, living culture CGMCC 3.19696.

Teleomorph. Undetermined.

Note. Characterized by tree-like conidiophores, branches paired or in whorls of 3–4, ampulliform to lageniform (4.0–6.0 × 2.6–3.2 µm), ovoid to subglobose conidia (2.2–2.6 × 1.8–2.2 µm). Differs from Trichoderma breve by shorter phialides and smaller conidia, as well as the cultural characteristics and growth rates.

Discussion

A combination of phylogenetic, morphological, ecological, and biogeographical data has robustly resolved the taxonomy of Trichoderma (Jaklitsch and Voglmayr 2015; Qin and Zhuang 2016a; Sun et al. 2016; Chen and Zhuang 2017b; Qiao et al. 2018). In this study, phylogenetic analysis based on a single gene of ITS could not distinguish species of Trichoderma in the Harzianum clade from each other (Suppl. material 1: Fig. S1), which confirmed that the ITS region is not suitable for species delimitation of Trichoderma (Jaklitsch et al. 2012; Qin et al. 2018). Sequences of RPB2 and TEF1-α were powerful due to their suitable interspecific variations (Jaklitsch and Voglmayr 2015), and these have extensively been used in solving the taxonomy of Trichoderma (Jaklitsch and Voglmayr 2015; Qin and Zhuang 2016a; Chen and Zhuang 2017a, b; Qiao et al. 2018). Despite the phylogenetic analyses based on the single gene of RPB2 and TEF1-α generally revealed the phylogenetic relationship within the Harzianum clade (Suppl. material 1: Fig. S2, Suppl. material 3: Fig S3), but the relationships among T. lentinulae, T. xixiacum, T. vermicificola, T. zelobreve, and their closed taxa were not well distinct. Consideration of the universality and reliability of barcodes for species in the Trichoderma genus (Qiao et al. 2018), combined ITS, RPB2, and TEF1-α dataset was used for phylogenetic analysis in this study, revealing phylogenetic relationship among species in Harzianum clades, and suggesting that T. lentinulae, T. xixiacum, T. vermicificola, and T. zelobreve are distinguishable from each other and species within and outside of Harzianum clade as well.
**Table 1.** Species, strains and their corresponding GenBank accession numbers of sequences used for phylogenetic analyses.

| Species                        | Voucher/ culture Nos. | Origin       | Substrate                | GenBank accession No. |
|--------------------------------|-----------------------|--------------|--------------------------|-----------------------|
| Trichoderma afarasin          | CBS 130755 ET         | Cameroon     | Soil                     | AY027784              |
|                                | DIS 314F              | Cameroon     | Wood                     | FJ442529              |
|                                | GJS 06.98             | Cameroon     | Soil                     | FJ442630              |
| Trichoderma afroharzianum      | CBS 124620 ET         | Peru         | *Moniliophthora roreric* | FJ442265              |
|                                | CBS 466.94            | Netherlands  |                          | KP009262              |
|                                | GJS 04-193            | Cameroon     | Soil                     | FJ442233              |
| Trichoderma aggressivum        | CBS 100525            | UK           | Mushroom compost         | AF057600              |
|                                | DAOAM 222156 ET       | Mushroom compost |                      | AF456924              |
| Trichoderma alba               | CBS 100526            | Ireland      | Mushroom compost         | FJ442607              |
| Trichoderma africicum          | CBS 120633 ET         | UK, England  | *Alnus glutinosa*        | EU518651              |
|                                | CPK 2494              |              |                          | EU518652              |
|                                | HMAS 252890           |              |                          | KT343763              |
| Trichoderma alpinum            | HMAS 248821 ET        | China, Sichuan | Soil                    | KY687906              |
|                                | HMAS 248830           |              |                          | KY687912              |
|                                | HMAS 248870           |              |                          | KY687953              |
| Trichoderma amazonicum         | CBS 126898 ET         | Peru         | *Hevea brasiliensis*     | HM142358              |
|                                | IB95                  |              |                          | HM142359              |
|                                | LA265                 |              |                          | HM142360              |
| Trichoderma aspergillum        | GJS 05-101            |              |                          | FJ442677              |
|                                | GJS 90-254            |              |                          | FJ442745              |
| Trichoderma atrobrunneum       | BMCC LU498            | New Zealand  |                          | MH858272              |
|                                | DAOM 167632           |              |                          | KJ871087              |
| Trichoderma atrobrunneum       | CBS 237.63 ET         | New Zealand  |                          | KJ871083              |
|                                | DAOM 222156 ET       |              |                          | KJ871083              |
| Trichoderma bannaense          | HMAS 248840 ET        | China, Yunan | Soil                     | KY687923              |
|                                | HMAS 248865           |              |                          | KY687948              |
| Trichoderma breve              | HMAS 248844 ET        | China, Beijing | Soil                   | KY687927              |
|                                | HMAS 248845           |              |                          | KY687928              |
| Trichoderma brevicornum        | HMAS 248871 ET        | Soil         |                          | KY687954              |
|                                | HMAS 248872           | Soil         |                          | KY687955              |
| Trichoderma brunneoviride      | CBS 120928            |              |                          | EU518661              |
|                                | CBS 121110 ET         |              |                          | EU518659              |
| Trichoderma cameruneuse        | CBS 137272 ET         | Cameroon     | Soil                     | AY027780              |
|                                | GJS 99 231            |              |                          | AY027783              |
| Trichoderma catoptron          | DAOM 232830           |              |                          | KJ842166              |
|                                | GJS 02 76 ET          | Sri Lanka    | Wood                     | AY737766              |
| Trichoderma cinnamomeum        | CBS 114576            |              |                          | FJ860543              |
|                                | BMCC LU784            | Nepal        |                          | KJ871244              |
| Trichoderma corneum            | CBS 132572 ET         | Cameroon     | Soil                     | AY027780              |
|                                | S93                   |              |                          | KJ665244              |
| Trichoderma cinnamomeum        | GJS 96-128            | Spain        |                          | KJ665245              |
|                                | GJS 97-233            | Spain        |                          | KJ665442              |
| Trichoderma compactum          | GJS 97-237 ET         | USA, Missouri | Decaying wood           | AY737759              |
| Trichoderma concentricum       | CBS 121218            |              |                          | AY941822              |
|                                | HMAS 248833 ET        | China, Hubei | Soil                     | KJ665244              |
| Trichoderma coroncum           | HMAS 248858           |              |                          | KJ665245              |
| Trichoderma endophyticum       | GJS 97-82 ET          | Thailand     |                          | KJ665252              |
|                                | CBS 130729 ET         | Ecuador      | *Theobroma gleri*       | FJ442243              |
|                                | GJS 2014a             |              |                          | FJ67822               |
| Trichoderma epimotoes          | CBS 120534 ET         | Austria      |                          | E518665               |
|                                | CPK 1980              |              |                          | E518665               |
|                                | CPK 2487 ET           |              |                          | E518665               |
| Species                  | Voucher/ culture Nos. | Origin         | Substrate  | GenBank accession No. |
|-------------------------|-----------------------|----------------|------------|-----------------------|
|                         |                       |                |            | ITS                   |
| Trichoderma estonicum   | GJS 96-129            |                |            | AY737767              |
|                         |                       |                |            | AF545514              |
|                         |                       |                |            | AF534604              |
| Trichoderma guizhouense | DAOM 231435           |                |            | EF191296              |
|                         | HGUP0038              |                |            | EJ191321              |
|                         |                       |                |            | JN191311              |
|                         |                       |                |            | JQ201400              |
|                         |                       |                |            | JN215484              |
|                         | S628                  |                |            | KJ665273              |
|                         |                       |                |            | KJ665511              |
| Trichoderma harzianum   | CBS 226.95 ET         | U.K.           | Soil       | AJ222720              |
|                         | CBS 227.95            |                |            | AF057605              |
|                         | GJS 05 107            |                |            | FJ442679              |
|                         | IMI 399823            |                |            | EF113587              |
| Trichoderma hauknechtii | CBS 133493            |                |            | KJ665276              |
|                         |                       |                |            | KJ665515              |
| Trichoderma helicolixii | CBS 13499 ET         | Spain          |            | KJ665278              |
|                         | CBS 135583            |                |            | KJ665516              |
| Trichoderma helicolixii | HMAS 248852 T        | China, Hubei   | Soil       | KY687935              |
|                         | HMAS 248853           |                |            | KY687992              |
|                         |                       |                |            | KY688054              |
| Trichoderma hirustum    | HMAS 248834 T        | China, Hubei   | Soil       | KY687916              |
|                         | HMAS 248859           |                |            | KY687942              |
|                         |                       |                |            | KY688099              |
| Trichoderma huanense    | HMAS 248841 T        | China, Hunan   | Soil       | NR_14571              |
|                         | HMAS 248867           |                |            | KY687950              |
|                         |                       |                |            | KY688005              |
| Trichoderma ingratus    | HMAS 248822 T        | China, Sichuan | Soil       | KY687917              |
|                         | HMAS 248827           |                |            | KY687909              |
|                         | HMAS 248873           |                |            | KY687956              |
|                         |                       |                |            | KY688010              |
| Trichoderma inhamatum   | CBS 273.78 ET        | Colombia       | Soil       | FJ442680              |
|                         |                       |                |            | FJ442725              |
|                         |                       |                |            | AF348099              |
| Trichoderma italicum    | CBS 132567            |                |            | KJ665282              |
|                         |                       |                |            | KJ665525              |
|                         | S15 ET                |                |            | KJ665283              |
|                         |                       |                |            | KJ665526              |
| Trichoderma lentiforme  | CBS 100542 ET        | French Guiana  | Decorticated wood | AF469189  |
|                         | DIS 253B              |                |            | FJ442619              |
|                         | DIS 94D               |                |            | FJ442749              |
|                         |                       |                |            | FJ463379              |
| Trichoderma lentiniulae | HMAS 248256 T        | China          | Lentinula  | MN594469              |
|                         | CGMCC 3.19848        |                |            | MN605867              |
|                         | CGMCC 3.19849        |                |            | MN605887              |
|                         | CGMCC 3.19699        |                |            | MN605887              |
|                         | CGMCC 3.19670        |                |            | MN605888              |
| Trichoderma liberatum   | HMAS 248831 T        | China, Hubei   | Soil       | KY687913              |
|                         | HMAS 248832           |                |            | KY687914              |
|                         |                       |                |            | KY687970              |
| Trichoderma linzhiense  | HMAS 248846 T        | China, Tibet   | Soil       | KY687929              |
|                         | HMAS 248874           |                |            | KY687957              |
|                         |                       |                |            | KY688011              |
| Trichoderma licii       | CBS 110080 ET        | Thailand       | Decayed Ganoderma | AF443920  |
|                         |                       |                |            | KJ665290              |
|                         |                       |                |            | AF443938              |
| Trichoderma neotropicale| LA11 ET              | Thailand       |            | HQ202467              |
|                         | T51                   |                |            | FJ884180              |
|                         |                       |                |            | FJ967825              |
| Trichoderma parestonicum| CBS 120636 ET        |                |            | FJ860803              |
|                         | Trichoderma parepimyces| CBS 122768    |            | FJ860801              |
|                         |                       |                |            | FJ860563              |
|                         |                       |                |            | FJ860665              |
| Trichoderma perviride   | HMAS 273786          | China, Hubei   | Wood       | KX026962              |
|                         |                       |                |            | KX026954              |
| Trichoderma pinicola    | KACC 4848 ET         | Korea          | root of Pinus densiflora | MH050354  |
|                         |                       |                |            | MH025993              |
|                         |                       |                |            | MH025981              |
|                         | SFC20130926-S014     |                |            | MH025991              |
|                         | SFC20130926-S111     |                |            | MH025992              |
| Trichoderma pleurotus   | CBS 124387 ET        | Korea          | Pleurotus substrate | HM142363  |
|                         |                       |                |            | HM142372              |
|                         |                       |                |            | HM142382              |
| Trichoderma pleuroticoila| CPK 2117            |                |            | EU279975              |
|                         | CBS 124383 ET        | Korea          | Pleurotus substrate | HM142362  |
|                         | GJS 95 ET            |                |            | AF345948              |
|                         |                       |                |            | AF348102              |
| Trichoderma polysporum  | CBS 12438 ET         | Korea          | Pleurotus substrate | HM142371  |
|                         |                       |                |            | AF345948              |
|                         |                       |                |            | AF348102              |
| Trichoderma polysporum  | CBS 12438 ET         | Korea          | Pleurotus substrate | HM142371  |
|                         |                       |                |            | AF345948              |
|                         |                       |                |            | AF348102              |
| Trichoderma polyporus   | HMAS 248855 T        | Hunan          | Soil       | KY687938              |
|                         | HMAS 248861          |                |            | KY687944              |
|                         |                       |                |            | KY688000              |
|                         | S72                  |                |            | KJ665685              |
|                         |                       |                |            | KJ665685              |
| Species                        | Voucher/ culture Nos. | Origin       | Substrate            | GenBank accession No. |
|-------------------------------|-----------------------|--------------|----------------------|-----------------------|
| Trichoderma priscilae         | CBS 131487 ET         | Spain        |                      | KJ665333, KJ665691    |
| Trichoderma pseudodensum      | HMAS 248828 T         | Hubei        | Soil                 | KY687910, KY687967, KY688023 |
| Trichoderma pseudogelatinosum | HMAS 248829           |              |                      | KY687911, KY687968, KY688024 |
| Trichoderma pseudogelatinosum | CNU309 ET             | Japan        | Shiitake mushroom    | HM769754, HM920173, HM920202 |
| Trichoderma purpureum         | HMAS 273787 ET        | China, Hubei |                      | KYX026953, KYX026961, KYX026953 |
| Trichoderma pyramidalae       | CBS 135574 ET         | Italy        | Olea europaea        | KJ665334, KJ665699    |
| Trichoderma rifaii            | CBS 130746            | Ecuador      | Theobroma gleri     | FJ442663, FJ463324    |
| Trichoderma rugulosum         | HMAS 248829, KY687910 | Hubei        | Soil                 | KY687911, KY687967, KY688023 |
| Trichoderma rufobrunneum      | HMAS 248829           | Hubei        | Soil                 | KY687911, KY687967, KY688023 |
| Trichoderma simplex           | CBS 130431            | USA, Maryland| Decaying wood bark   | AF443917, AF442757, AF443935 |
| Trichoderma simplex           | HMAS 248842 T         | China, Guangxi| Soil                | KY687925, KY687981, KY688041 |
| Trichoderma solum             | HMAS 248847           | Hubei        | Soil                 | KY687930, KY687986, KY688049 |
| Trichoderma stramineum        | HMAS 248848 T         | China, Hubei | Soil                 | KY687931, KY687987, KY688050 |
| Trichoderma stramineum        | HMAS 248849           |              |                      | KY687932, KY687988, KY688051 |
| Trichoderma tawa              | CBS 114248 ET         | Sri Lanka    | Decaying wood        | AY737765, AY391945, AY373746 |
| Trichoderma tawa              | TAMA 0425             |              |                      | ABB56609, ABB56748, ABB56675 |
| Trichoderma velutinum         | DAOM 232841           |              |                      | KXJ842187, EU279972    |
| Trichoderma tenue             | HMAS 273785 ET        | China, Hubei | Wood                 | KX026950, KX026952    |
| Trichoderma tomentosum        | DAOM 171918           | Canada, Ontario| Ulmus wood           | AY605715, AY605759    |
| Trichoderma velutinum         | DAOM 234236           |              |                      | EU280083, EU279971    |
| Trichoderma zayuense          | DAOM 230013 ET        | Nepal        | Soil                 | AY937415, AY937415    |
| Trichoderma zylophiformida    | HMAS 273865 T         | China, Heilongjiang| Soil            | KX026956, KX026957    |
| Trichoderma zylophiformida    | CGMCC 3.19850         | China        | Compost              | MN594472, MN605870, MN605881 |
| Trichoderma zylophiformida    | HMAS 248255 T         | China        | Compost              | MN594473, MN605871, MN605882 |
| Trichoderma zylophiformida    | CGMCC 3.19698         | China        | Soil                 | MN594477, MN605875, MN605886 |
| Trichoderma zylophiformida    | HMAS 248836           | China, Tibet | Soil                 | KY687919, KY687974, KY688031 |
| Trichoderma zylophiformida    | HMAS 248836           |              |                      | KY687919, KY687975, KY688032 |
| Trichoderma zylophiformida    | HMAS 248254 T         | China        | Mushroom             | MN594474, MN605872, MN605883 |
| Trichoderma zylophiformida    | CGMCC 3.19696         | China        | Soil                 | MN594475, MN605873, MN605884 |
| Trichoderma zylophiformida    | YMF 1.00268 ET        | China, Yunnan| Soil                 | MH113932, MH158996, MH183181 |

Trichoderma lentinulae was phylogenetically close to *T. xixiacum* and *T. lixii* but represents a taxon (Fig. 1). Morphologically, it differed from *T. xixiacum* in producing less frequently lageniform phialides with inequilateral to a strongly-curved apex. The conidia of *T. lentinulae* are usually more slender (*x* = 2.0), than those of *T. xixiacum* (*x* = 1.8). In addition, the conidia of *T. lentinulae* (length/width ratio, *x* = 1.2) are slightly more slender than *T. xixiacum* (length/width ratio, *x* = 1.1). The two species also differ from each other in their cultural characteristics and growth rates (Figs 2A–C, 4 A–C). *Trichoderma lentinulae* differed from *T. lixii* in producing less fre-
Four new *Trichoderma* spp.

... subsequently lageniform phialide with inequilateral to a strongly-curved apex. Additionally, *T. lentinulae* forms 2–5 apex phialides on the main axis (Fig. 2F, I) in contrast to 2–4 apex phialides of *T. lixii* (Chaverri et al. 2015). *Trichoderma lentinulae* is also clearly distinguished from *T. lixii* (phialides, 6.5–3.5 µm; conidia, 3.0–2.7 µm) (Chaverri et al. 2015) in producing shorter phialides ($\bar{x} = 4.5 \times 3.0$ µm) and smaller conidia ($\bar{x} = 2.5 \times 2.2$ µm). *Trichoderma vermifimicola* was phylogenetically associated with *T. simmonsii* (Fig. 1). Morphologically, it is hard to distinguish *T. vermifimicola* from *T. simmonsii*, because both form similar tree-like conidiophores, ampulliform to lageniform phialides and ovoid to subglobose conidia, but phialide whorls of *T. vermifimicola* were often nearly verticillate rather than cruciate in *T. simmonsii* (Chaverri et al. 2015). Furthermore, *T. simmonsii* grew fast (PDA 25–55 mm, SNA 10–35 mm) at 35 °C than *T. vermifimicola*. Additionally, the length/width ratio phialide of *T. vermifimicola* is larger ($\bar{x} = 2.4$) than that of *T. simmonsii* ($\bar{x} = 1.9$) (Chaverri et al. 2015), and *T. vermifimicola* also produces smaller conidia ($\bar{x} = 2.4 \times 2.2$ µm) (Fig. 3) than *T. simmonsii* (3.0–2.7 µm) (Chaverri et al. 2015). *Trichoderma zelobreve* was closely related to *Trichoderma breve* in the multi-gene phylogenetic analysis (Fig. 1). Morphologically, both fungi have short phialides, however, *T. zelobreve* differs from *T. breve* by producing shorter and narrower phialides (4.0–6.0 × 2.6–3.2 µm) than that of *T. breve* (6.7–10.0 × 2.8–3.9 µm) (Chen and Zhuang 2017a). The conidia of *T. zelobreve* are smaller ($\bar{x} = 2.4 \times 2.0$ µm) than those of *T. breve* ($\bar{x} = 3.0 \times 2.8$ µm). Additionally, *T. zelobreve* does not form a zonate colony on CMD, PDA, and SNA, whereas the colony of *T. breve* presents concentric zones on CMD and PDA and finely concentric zones on SNA (Chen and Zhuang 2017a). In a previous study, the phylogenetic analysis indicated that *T. breve* was a sister taxon of *T. bannaense*, but morphologically more similar to *T. harzianum* (Chen and Zhuang 2017a). Herein, our phylogenetic analyses presented *T. breve* was associated with *T. zelobreve* (Fig. 1), resulted from the little genetic variation of sequences of ITS and TEF1-α between them. The phylogenetic analysis in Chaverri et al. (2015) presented that *T. simmonsii* was associated with *T. camerunense*. In this study, our phylogenetic analysis presented that *T. simmonsii* was phylogenetically closed to *T. vermifimicola*, and *T. camerunense* phylogenetic to *T. rifaii* (Fig 1, Suppl. material 3: Fig S3). In a previous study, these species were recognized as the cryptic species in under *T. harzianum* (Chaverri et al. 2015).

Currently, the *Harzianum* clade contains more than 60 species which were isolated from soil, plant tissues, and other fungi (Jaklitsch and Voglmayr 2015; Qin and Zhuang 2016a; Chen and Zhuang 2017b; Qiao et al. 2018; Sun et al. 2019a, b). Several studies have confirmed that species in this clade are important because of their mycoparasitism (Chaverri et al. 2015; Chen and Zhuang 2017a; Sun et al. 2019). When numerous biological control agents were explored deriving from species in the *Harzianum* clade (Chaverri et al. 2015, several taxa, such as *T. atrobrunneum*** T. pleuroti*, and *T. pleuroticola* were recognized as causing agents of “Green mold” disease of cultivated mushroom (Innocenti et al. 2019; Sun et al. 2019a, b). In this study, *T. lentinulae* was isolated from a fruiting body and the cultivated substrates of *L. edodes*, causing the decay of the host as well. How *T. lentinulae* affect the cultivation of *Lentinula edodes* is worthy of further studies. Since *T. lentinulae* was isolated from mushroom, *T. lentinulae* and *T. vermifimicola*...
were isolated from the mushroom spawn and substrates for earthworm cultivation, *T. xixiacum* and *T. zelobreve* were isolated from soil, confirming that species in the *Harzianum* clade have flexible nutrition modes (Chaverri and Samuels 2013; Zhang et al. 2018). The new species introduced here are not only potential candidates for biological agent exploration, but also improve our understanding of the diversity of *Trichoderma*, especially of the *Harzianum* clade in China.

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Supplementary material I

**Figure S1**

Authors: Xin Gu, Rui Wang, Quan Sun, Bing Wu, Jing-Zu Sun

Data type: phylogenetic tree

Explanation note: Phylogenetic tree based on Maximum Likelihood analysis of ITS sequence dataset. *Trichoderma ceramicum*, *Trichoderma estonicum*, and *Trichoderma parastinicum* were chosen as the outgroup. Bootstrap Values higher than 70% from RAxML (BSML) (left) and Bayesian posterior probabilities greater than 0.95 (BYPP) (right) are given above the nodes. \( ^T \) indicates the type; \( ^{ET} \) indicates the ex-living type. Isolates obtained in this study are in red.

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Supplementary material 2

Figure S2
Authors: Xin Gu, Rui Wang, Quan Sun, Bing Wu, Jing-Zu Sun
Data type: phylogenetic tree
Explanation note: Phylogenetic tree based on Maximum Likelihood analysis of RPB2 sequence dataset. *Trichoderma ceramicum*, *Trichoderma estonicum*, and *Trichoderma parastinicum* were chosen as the outgroup. Bootstrap Values higher than 70% from RAxML (BSML) (left) and Bayesian posterior probabilities greater than 0.95 (BYPP) (right) are given above the nodes. \( T \) indicates the type; \( E \) indicates the ex-living type. Isolates obtained in this study are in red.

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Link: https://doi.org/10.3897/mycokeys.73.51424.suppl2

Supplementary material 3

Figure S3
Authors: Xin Gu, Rui Wang, Quan Sun, Bing Wu, Jing-Zu Sun
Data type: phylogenetic tree
Explanation note: Phylogenetic tree based on Maximum Likelihood analysis of TEF1α sequence dataset. *Trichoderma ceramicum* was chosen as the outgroup. Bootstrap Values higher than 70% from RAxML (BSML) (left) and Bayesian posterior probabilities greater than 0.95 (BYPP) (right) are given above the nodes. \( T \) indicates the type; \( E \) indicates the ex-living type. Isolates obtained in this study are in red.

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