

RESEARCH ARTICLE



## Eleven new species of *Trichoderma* (Hypocreaceae, Hypocreales) from China

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### ABSTRACT

*Trichoderma* spp. are globally distributed and are considered significant fungal resources. This study presents the discovery of 11 new species of *Trichoderma*: *T. caeruleum*, *T. gongcheniae*, *T. graminicola*, *T. graminis*, *T. hongkuii*, *T. parapeberdyi*, *T. neoguizhouense*, *T. neohongkuii*, *T. parahamatum*, *T. parahongkuii*, and *T. shaanxiensis*. All of these new species were isolated from soils, except for *T. caeruleum*, *T. graminicola*, *T. graminis*, and *T. neohongkuii*, which were found as endophytes in Poaceae plants. The phylogenetic position of these novel species was determined by analysing the concatenated sequences of the second largest nuclear RNA polymerase subunit encoding gene (*rpb2*) and the translation elongation factor 1- $\alpha$  encoding gene (*tef1*). The results of the phylogenetic analysis revealed that each new species formed a distinct lineage: *T. gongcheniae*, *T. graminicola*, *T. graminis*, *T. neoguizhouense*, *T. parapeberdyi*, and *T. shaanxiensis* belong to the *Harzianum* Clade, *T. hongkuii*, *T. parahongkuii*, and *T. neohongkuii* are new members of the *Koningii* Clade, *T. parahamatum* belongs to the *Hamatum* Clade, and *T. caeruleum* does not fall within any of the named clades. The study also provided a detailed description of the morphology and cultural characteristics of the newly discovered *Trichoderma* species. The discovery contributes to the advancement of knowledge about *Trichoderma* species resources in China.

### ARTICLE HISTORY

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### KEYWORDS



Hypocreales; new species;  
phylogenetic analysis;  
taxonomy; *Trichoderma*

## 1. Introduction

The genus *Trichoderma* (Hypocreaceae, Hypocreales, Sordariomycetes, and Ascomycota) was originally established by Persoon in 1974, who described four species, including *T. aureum* Pers., *T. nigrescens* Pers., *T. roseum* Pers., and *T. viride* Pers., based on morphological characterisations. However, subsequent research has shown that these species are unrelated to each other, and only *T. viride* remains classified within *Trichoderma*. Currently, more than 500 species have been reported and recognised (Cai and Druzhinina 2021; Cao et al. 2022; Yu et al. 2022; Zhang et al. 2022; Sousa et al. 2023; Ye et al. 2023; Zhao et al. 2023). *Trichoderma* has evolved into a genus rich in species, primarily due to its economic and ecological significance. Species of this genus have been widely utilised, exerting a significant impact on humans. They are recognised as highly efficient producers of enzymes and antibiotics (Ahamed and Vermette 2008; Stracquadanio et al. 2020; Abdel-Mageed et al. 2022), biocontrol agents

of plant pathogens, and promoters of plant growth (Samuels 1996; Harman et al. 2004; Lorito et al. 2010), as well as bioremediators for certain heavy metal ions (Anand et al. 2006; Mazyar 2010; Tripathi et al. 2013; Zhang et al. 2023). Furthermore, solid-state fermentation of *Trichoderma* enhances phenolic content, antioxidant activity, and antimicrobial properties in plants, making them ideal for use as both a dietary supplement and an antimicrobial agent (Saleh et al. 2018; Abdel Atty et al. 2019). However, despite the widespread benefits, certain *Trichoderma* species are known to cause opportunistic infections in humans (Kuhls et al. 1999; Sandoval-Denis et al. 2014; Dos Santos and Dos Santos 2023) and are responsible for diseases in the commercial production of mushrooms (Savoie and Mata 2003; Komon-Zelazowska et al. 2007).

*Trichoderma* is widely distributed in various ecosystems, commonly found as soil inhabitants, saprophytes, and parasites of other fungi (Jaklitsch and Voglmayr 2015; Jiang et al. 2016). Furthermore, an

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increasing number of *Trichoderma* species have been discovered as endophytes in plant tissues, originating from diverse plants such as wild rice, wild coffee, wheat, rubber, *Theobroma cacao*, and aquatic plants etc. (Zhang et al. 2007; Hanada et al. 2008; Chaverri et al. 2011; Rodríguez et al. 2021; Zheng et al. 2021; Matar et al. 2022). The relationship between endophytic *Trichoderma* and their host plants is not merely a simple symbiotic association; rather, these fungi play a multifaceted role. They can assist plants in enhancing photosynthetic capacity, inducing defence mechanisms, activating stress-sensitive genes, and contributing to nutrient provision (Bae et al. 2011; Rosmana et al. 2015; Harman et al. 2021; Rees et al. 2022).

The identification of the *Trichoderma* genus is increasingly standardised, with numerical standards establishing a similarity threshold for species-level identification (Cai and Druzhinina 2021). Simultaneously, DNA barcodes such as *rpb2* and *tef1* for identification are widely accepted and utilised (Chaverri and Samuels 2003; Li et al. 2013; Zhu and Zhuang 2015; Zheng et al. 2021). In this study, we introduced 11 novel species of *Trichoderma* isolated from soils and living Poaceae plants, collected from five provinces of China. This was based on their morphological characteristics in culture and molecular phylogenetic analyses of *rpb2* and *tef1*.

## 2. Materials and methods

### 2.1. Isolation of strains

Following the methodology outlined by Dou et al. (2019), a total of 618 *Trichoderma* strains were obtained from soils in crop and orchard fields across five provinces in China: Anhui, Guizhou, Yunnan, Shaanxi, and Zhejiang. These strains were isolated using the dilution plating method on modified potato dextrose agar (PDA) with rose bengal (0.02 g/L), chloramphenicol (0.1 g/L), and streptomycin (0.1 g/L) (Dou et al. 2019). Additionally, 33 *Trichoderma* strains were isolated from living Poaceae plants collected from Zhejiang and Yunnan provinces in China (Liu et al. 2021). Fungal endophytes were isolated from surface-sterilised healthy plant tissues. The sterilisation process involved immersion in ethanol (75% v/v) for 30 s, followed by immersion in sodium hypochlorite (1% w/v) for 10 min, and finally rinsed three times in sterile

distilled water. The isolation was performed on malt extract agar (MEA) supplemented with chloramphenicol (0.1 g/L) (Yuan et al. 2010). All *Trichoderma* strains were stored in a 4 °C refrigerator and a –80 °C ultra-low temperature refrigerator at the Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou, China. Furthermore, the holotype and ex-type culture were deposited in the China Guangdong Microbial Culture Collection Center (GDMCC).

### 2.2. Morphological characterization

The morphological observation and growth-rate trials were conducted following the procedures outlined by Zhao et al. (2023). Microscopic preparations were mounted on lactic acid, and at least 30 measurements per structure were documented and examined using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan).

### 2.3. DNA extraction and PCR

Genomic DNA extraction was conducted following the protocol outlined by Jiang et al. (2016). Amplifications of the *rpb2* and *tef1* gene fragments utilised two different primer pairs: EF1/EF2 for *tef1* (O'Donnell et al. 1998) and fRPB2-5F/fRPB2-7cR for *rpb2* (Liu et al. 1999). Polymerase chain reaction (PCR) amplifications were carried out in a total reaction volume of 20 µL, comprising 10 µL of Easy Flash PCR MasterMix (Easy-Do, Hangzhou, China), 0.8 µL of each primer (10 µmol/L), and 0.4 µL genomic DNA (~0.2 µg). The PCR was conducted for 35 cycles with an annealing temperature of 57 °C for *rpb2* and 55 °C for *tef1*. Subsequently, the PCR products were purified and sequenced at Sunya Biotechnology Co., Ltd. (Hangzhou, China).

### 2.4. Phylogenetic analyses

The dataset comprised concatenated sequences of *rpb2* and *tef1*, which were utilised to elucidate and identify the phylogenetic position of new species within *Trichoderma*. Species closely related to our strain were identified by NCBI BLAST searches using *rpb2* and *tef1* sequences (Altschul et al. 1990), and the corresponding sequences were retrieved from the NCBI database for subsequent phylogenetic analysis. The GenBank accession numbers of the retrieved



**Table 1.** Strain numbers and corresponding GenBank accession numbers of sequences used for phylogenetic analyses.

| Species name                                                          | Strain number                              | GenBank accession numbers |                 |
|-----------------------------------------------------------------------|--------------------------------------------|---------------------------|-----------------|
|                                                                       |                                            | <i>rpb2</i>               | <i>tef1</i>     |
| <i>Trichoderma afarasin</i> P. Chaverri & F.B. Rocha                  | DIS 377a <sup>T</sup>                      | FJ442799                  | FJ463322        |
| <i>T. afarasin</i>                                                    | DIS14f                                     | FJ442778                  | FJ463400        |
| <i>T. afarasin</i>                                                    | GJS 99–227                                 | -                         | AF348093        |
| <i>T. afroharzianum</i> P. Chaverri, F.B. Rocha, Degenkolb & Druzhin. | CBS 124620 <sup>ET</sup>                   | FJ442691                  | FJ463301        |
| <i>T. afroharzianum</i>                                               | GJS 04–193                                 | FJ442709                  | FJ463298        |
| <i>T. albofulvopsis</i> W.T. Qin & W.Y. Zhuang                        | 9930 <sup>T</sup>                          | KU529138                  | KU529127        |
| <i>T. amoenum</i> Z.F. Yu & Y.F. Lv                                   | YMF 1.06209 <sup>T</sup>                   | MT052192                  | MT070146        |
| <i>T. amoenum</i>                                                     | YMF 1.06210                                | MT070161                  | MT070145        |
| <i>T. anisohamatum</i> Z.F. Yu & X. Du                                | YMF 1.00333 <sup>T</sup>                   | MH155272                  | MH177912        |
| <i>T. anisohamatum</i>                                                | YMF 1.00215                                | MH262576                  | MH236494        |
| <i>T. arenarium</i> F. Cai, M.Y. Ding & I.S. Druzhinina               | TUCIM 10301 <sup>T</sup>                   | MT242310                  | MT242303        |
| <i>T. arenarium</i>                                                   | TUCIM 10302                                | MT347691                  | MT347687        |
| <i>T. asiaticum</i> Z.F. Yu & X. Du                                   | YMF 1.00168 <sup>T</sup>                   | MH262575                  | MH236492        |
| <i>T. asiaticum</i>                                                   | YMF 1.00352                                | MH158994                  | MH183183        |
| <i>T. atrobrunneum</i> F.B. Rocha, P. Chaverri & Jaklitsch            | CBS 548.92 <sup>T</sup>                    | -                         | AF443942        |
| <i>T. atrobrunneum</i>                                                | GJS 04–67                                  | FJ442724                  | FJ463360        |
| <i>T. atrobrunneum</i>                                                | GJS 05–101                                 | FJ442745                  | FJ463392        |
| <i>T. azevedoi</i> M.C. Valadares-Inglis & P.W. Inglis                | CEN1422 <sup>T</sup>                       | MK696821                  | MK696660        |
| <i>T. azevedoi</i>                                                    | CEN1423                                    | MK696822                  | MK696661        |
| <i>T. bannaense</i> K. Chen & W.Y. Zhuang                             | HMAS 248840 <sup>T</sup>                   | KY687979                  | KY688037        |
| <i>T. bannaense</i>                                                   | HMAS 248865                                | KY688003                  | KY688038        |
| <i>T. breve</i> K. Chen & W.Y. Zhuang                                 | CGMCC 3.18398 <sup>T</sup>                 | KY687983                  | KY688045        |
| <i>T. breve</i>                                                       | HMAS 248845                                | KY687984                  | KY688046        |
| <i>T. caeruleum</i> C. L. Zhang                                       | <b>YNE01446 = GDMCC 3.1021<sup>T</sup></b> | <b>OR779496</b>           | <b>OR779525</b> |
| <i>T. caeruleum</i>                                                   | <b>YNE01429</b>                            | <b>OR779495</b>           | <b>OR779522</b> |
| <i>T. caeruleum</i>                                                   | <b>YNE01447</b>                            | <b>OR779499</b>           | <b>OR779523</b> |
| <i>T. caeruleum</i>                                                   | <b>YNE01448</b>                            | <b>OR779497</b>           | <b>OR779526</b> |
| <i>T. caeruleum</i>                                                   | <b>YNE01460</b>                            | <b>OR779498</b>           | <b>OR779524</b> |
| <i>T. camerunense</i> P. Chaverri & Samuels                           | GJS 99–230 <sup>T</sup>                    | -                         | AF348107        |
| <i>T. camerunense</i>                                                 | Vimi-17.0034                               | MZ675862                  | MZ675908        |
| <i>T. caribbaeum</i> Samuels & Schroers                               | GJS 97–3 <sup>T</sup>                      | KJ665246                  | KJ665443        |
| <i>T. ceraceum</i> P. Chaverri & Samuels                              | GJS 95–159 <sup>T</sup>                    | AF545508                  | AY937437        |
| <i>T. ceraceum</i>                                                    | DAOM 232831                                | -                         | KJ871239        |
| <i>T. ceramicum</i> P. Chaverri & Samuels                             | CBS114576 <sup>T</sup>                     | FJ860531                  | FJ860628        |
| <i>T. cerinum</i> Bissett, C.P. Kubicek & Szakács                     | DAOM 230012 <sup>T</sup>                   | KJ842184                  | KJ871242        |
| <i>T. chlamydosporum</i> K. Chen & W.Y. Zhuang                        | HMAS 248850 <sup>T</sup>                   | KY687989                  | KY688052        |
| <i>T. chlamydosporum</i>                                              | HMAS 248851                                | KY687990                  | KY688053        |
| <i>T. densissimum</i> C.L. Zhang                                      | T32434 = CGMCC 3.24126 <sup>T</sup>        | OP357966                  | OP357971        |
| <i>T. densissimum</i>                                                 | T31818                                     | OP357965                  | OP357967        |
| <i>T. densissimum</i>                                                 | T32465                                     | OP357963                  | OP357972        |
| <i>T. densissimum</i>                                                 | T32353                                     | OP357964                  | OP357970        |
| <i>T. dorotheopsis</i> A.A. Tomah & J.Z. Zhang                        | HZA5 <sup>ET</sup>                         | MH647795                  | MK850827        |
| <i>T. estonicum</i> P. Chaverri & Samuels                             | GJS 96–129 <sup>T</sup>                    | AF545514                  | AY737733        |
| <i>T. evansii</i> Samuels                                             | CBS 123079 <sup>T</sup>                    | EU883558                  | EU883566        |
| <i>T. evansii</i>                                                     | DIS 380a                                   | FJ150785                  | EU856320        |
| <i>T. ganodermatis</i> K. Chen & W.Y. Zhuang                          | HMAS 248856 <sup>T</sup>                   | KY687995                  | KY688060        |
| <i>T. ganodermatis</i>                                                | HMAS 248869                                | KY688007                  | KY688061        |
| <i>T. gongcheniae</i> C. L. Zhang                                     | <b>T33441 = GDMCC 3.1011<sup>T</sup></b>   | <b>OR779489</b>           | <b>OR779518</b> |
| <i>T. gongcheniae</i>                                                 | <b>T33522</b>                              | <b>OR779490</b>           | <b>OR779519</b> |
| <i>T. graminicola</i> C. L. Zhang                                     | <b>YNE00490 = GDMCC 3.1015<sup>T</sup></b> | <b>OR779494</b>           | <b>OR779521</b> |
| <i>T. graminicola</i>                                                 | <b>YNE00489</b>                            | <b>OR779493</b>           | <b>OR779520</b> |
| <i>T. graminis</i> C. L. Zhang                                        | <b>YNE00410 = GDMCC 3.1013<sup>T</sup></b> | <b>OR779491</b>           | <b>OR779514</b> |
| <i>T. graminis</i>                                                    | <b>YNE00430</b>                            | <b>OR779492</b>           | <b>OR779515</b> |
| <i>T. guizhouense</i> Q.R. Li, McKenzie & Yong Wang bis               | CBS 131803 <sup>T</sup>                    | JQ901400                  | JN215484        |

(Continued)

Table 1. (Continued).

| Species name                                                                      | Strain number                              | GenBank accession numbers |                 |
|-----------------------------------------------------------------------------------|--------------------------------------------|---------------------------|-----------------|
|                                                                                   |                                            | <i>rpb2</i>               | <i>tef1</i>     |
| <i>T. guizhouense</i>                                                             | HGUP 0039                                  | JQ901401                  | JX089585        |
| <i>T. hamatum</i> (Bonord.) Bainier                                               | DAOM 167057 <sup>ET</sup>                  | KJ842139                  | EU279965        |
| <i>T. hamatum</i>                                                                 | TRS121                                     | KP009085                  | KP008953        |
| <i>T. harzianum</i> Rifai                                                         | CBS 226.95 <sup>T</sup>                    | AF545549                  | AF348101        |
| <i>T. harzianum</i>                                                               | TRS55                                      | KP009121                  | KP008803        |
| <i>T. harzianum</i>                                                               | TRS94                                      | KP009120                  | KP008802        |
| <i>T. hongkuii</i> C. L. Zhang                                                    | <b>T32026 = GDMCC 3.1017<sup>T</sup></b>   | <b>OR779477</b>           | <b>OR779504</b> |
| <i>T. hongkuii</i>                                                                | <b>T32028</b>                              | <b>OR779478</b>           | <b>OR779505</b> |
| <i>T. hongkuii</i>                                                                | <b>T32027</b>                              | <b>OR779479</b>           | <b>OR779506</b> |
| <i>T. insigne</i> Z.F. Yu & X. Du                                                 | YMF 1.00207 <sup>T</sup>                   | MH155271                  | MH177911        |
| <i>T. insigne</i>                                                                 | YMF 1.00272                                | MH155274                  | MH177913        |
| <i>T. istrianum</i> Jaklitsch & Voglmayr                                          | CBS 130539 <sup>T</sup>                    | KJ665281                  | KJ665523        |
| <i>T. istrianum</i>                                                               | S123                                       | KJ665280                  | KJ665521        |
| <i>T. koningii</i> Oudem.                                                         | CBS 457.96 <sup>ET</sup>                   | -                         | AF456909        |
| <i>T. koningii</i>                                                                | Hypo 51 = CBS 119500                       | FJ860541                  | KC285594        |
| <i>T. koningii</i>                                                                | S227                                       | JN715609                  | KC285596        |
| <i>T. koningiopsis</i> Samuels, Carm. Suárez & H.C. Evans                         | GJS 93–20 <sup>T</sup>                     | EU241506                  | DQ284966        |
| <i>T. koningiopsis</i>                                                            | DIS 229d = CBS 119069                      | FJ442712                  | DQ284971        |
| <i>T. linzhiense</i> K. Chen & W.Y. Zhuang                                        | HMAS 248846 <sup>T</sup>                   | KY687985                  | KY688047        |
| <i>T. linzhiense</i>                                                              | HMAS 248874                                | KY688011                  | KY688048        |
| <i>T. longifalidicum</i> Q.V. Montoya, L.A. Meirelles, P. Chaverri & A. Rodrigues | LESF552                                    | KT278955                  | KT279020        |
| <i>T. longipile</i> Bissett                                                       | DAOM 177227 <sup>T</sup>                   | AF545550                  | AY937430        |
| <i>T. longipile</i>                                                               | CBS 120953                                 | FJ860542                  | FJ860643        |
| <i>T. neoguizhouense</i> C. L. Zhang                                              | <b>T33324 = GDMCC 3.1012<sup>T</sup></b>   | <b>OR779487</b>           | <b>OR779516</b> |
| <i>T. neoguizhouense</i>                                                          | <b>T33326</b>                              | <b>OR779488</b>           | <b>OR779517</b> |
| <i>T. neohongkuii</i> C. L. Zhang                                                 | <b>YNE00787 = GDMCC 3.1018<sup>T</sup></b> | <b>OR779481</b>           | <b>OR779508</b> |
| <i>T. neohongkuii</i>                                                             | <b>YNE00720</b>                            | <b>OR779480</b>           | <b>OR779507</b> |
| <i>T. neohongkuii</i>                                                             | <b>YNE00948</b>                            | <b>OR779482</b>           | <b>OR779509</b> |
| <i>T. ochroleucum</i> (Berk. & Ravenel) Jaklitsch & Voglmayr                      | CBS 119502 <sup>T</sup>                    | FJ860556                  | FJ860659        |
| <i>T. ovalisporum</i> Samuels & Schroers                                          | DIS 70a = CBS 113299 <sup>T</sup>          | FJ442742                  | AY376037        |
| <i>T. ovalisporum</i>                                                             | DIS 172i                                   | FJ442701                  | DQ288999        |
| <i>T. paradensisissimum</i> C.L. Zhang                                            | T31823 = CGMCC 3.24125 <sup>T</sup>        | OP357962                  | OP357968        |
| <i>T. paradensisissimum</i>                                                       | T31824                                     | OP357961                  | OP357969        |
| <i>T. parahamatum</i> C. L. Zhang                                                 | <b>T30699 = GDMCC 3.1020<sup>T</sup></b>   | <b>OR779474</b>           | <b>OR779501</b> |
| <i>T. parahamatum</i>                                                             | <b>T30064</b>                              | <b>OR779473</b>           | <b>OR779500</b> |
| <i>T. parahongkuii</i> C. L. Zhang                                                | <b>T31356 = GDMCC 3.1019<sup>T</sup></b>   | <b>OR779476</b>           | <b>OR779503</b> |
| <i>T. parahongkuii</i>                                                            | <b>T31355</b>                              | <b>OR779475</b>           | <b>OR779502</b> |
| <i>T. parapeberdyi</i> C. L. Zhang                                                | <b>T30677 = GDMCC 3.1016<sup>T</sup></b>   | <b>OR779483</b>           | <b>OR779510</b> |
| <i>T. parapeberdyi</i>                                                            | <b>T32471</b>                              | <b>OR779484</b>           | <b>OR779511</b> |
| <i>T. peberdyi</i> M.C. Valadares-Inglis & P.W. Inglis                            | CEN1426 <sup>T</sup>                       | MK696825                  | MK696664        |
| <i>T. peberdyi</i>                                                                | CEN1398                                    | MK696794                  | MK696632        |
| <i>T. petersenii</i> Samuels, Dodd & Schroers                                     | GJS 91–99 <sup>T</sup>                     | -                         | AY376039        |
| <i>T. petersenii</i>                                                              | GJS 04–164                                 | FJ442783                  | DQ289004        |
| <i>T. pholiotae</i> Z.J. Cao & W.T. Qin                                           | JZBQH12 <sup>T</sup>                       | ON649972                  | ON649919        |
| <i>T. pholiotae</i>                                                               | JZBQH11                                    | ON649971                  | ON649918        |
| <i>T. phyllostachydis</i> P. Chaverri & Samuels                                   | CBS 114071 <sup>ET</sup>                   | FJ860570                  | FJ860673        |
| <i>T. polypori</i> K. Chen & W.Y. Zhuang                                          | HMAS 248855 <sup>T</sup>                   | KY687994                  | KY688058        |
| <i>T. propepolypori</i> Z.F. Yu & Y.F. Lv                                         | YMF 1.06224 <sup>T</sup>                   | MT052181                  | MT070158        |
| <i>T. propepolypori</i>                                                           | YMF 1.06199                                | MT052182                  | MT070157        |
| <i>T. pseudoasiaticum</i> Z.F. Yu & Y.F. Lv                                       | YMF 1.6178 <sup>T</sup>                    | MT052183                  | MT070155        |
| <i>T. pubescens</i> Bissett                                                       | DAOM 166162 <sup>T</sup>                   | KJ842138                  | EU279963        |
| <i>T. pubescens</i>                                                               | CBS 345.93                                 | -                         | AY665704        |
| <i>T. pyramidale</i> Jaklitsch & P. Chaverri                                      | CBS 135574 <sup>T</sup>                    | KJ665334                  | KJ665699        |
| <i>T. pyramidale</i>                                                              | T20                                        | KX632570                  | KX632627        |

(Continued)

Table 1. (Continued).

| Species name                                               | Strain number                            | GenBank accession numbers |                 |
|------------------------------------------------------------|------------------------------------------|---------------------------|-----------------|
|                                                            |                                          | <i>rpb2</i>               | <i>tef1</i>     |
| <i>T. rifaii</i> F.B. Rocha, P. Chaverri & Samuels         | CBS 130746 = DIS 355B <sup>ET</sup>      | -                         | FJ463324        |
| <i>T. rifaii</i>                                           | DIS 337F                                 | FJ442720                  | FJ463321        |
| <i>T. rifaii</i>                                           | VSL282                                   | -                         | MZ476195        |
| <i>T. rossicum</i> Bissett, C.P. Kubicek & Szakács         | DAOM 230010 <sup>T</sup>                 | HQ400747                  | AY937424        |
| <b><i>T. shaanxiensis</i> C. L. Zhang</b>                  | <b>T32000 = GDMCC 3.1014<sup>T</sup></b> | <b>OR779486</b>           | <b>OR779513</b> |
| <b><i>T. shaanxiensis</i></b>                              | <b>T31999</b>                            | <b>OR779485</b>           | <b>OR779512</b> |
| <i>T. shennongjianum</i> Kai Chen & W.Y. Zhuang            | HMAS 245009 <sup>T</sup>                 | KT735259                  | KT735253        |
| <i>T. simile</i> Z.F. Yu & Y.F. Lv                         | YMF 1.06201 <sup>T</sup>                 | MT052184                  | MT070154        |
| <i>T. simile</i>                                           | YMF1.6180                                | MT052185                  | MT070153        |
| <i>T. strictipile</i> Bissett                              | DAOM 172827 <sup>T</sup>                 | KJ842162                  | AY937451        |
| <i>T. stromaticum</i> Samuels & Pardo-Schulth.             | DAOM 231100 <sup>T</sup>                 | KJ842192                  | KJ871129        |
| <i>T. tibetense</i> Kai Chen & W.Y. Zhuang                 | HMAS 245010 <sup>T</sup>                 | KT735261                  | KT735254        |
| <i>T. tomentosum</i> Bissett                               | DAOM 178713a <sup>T</sup>                | AF545557                  | EU279969        |
| <i>T. tropicosinense</i> (P.G. Liu) Z.X. Zhu & W.Y. Zhuang | HMAS 252,546 <sup>T</sup>                | KF923313                  | KF923286        |
| <i>T. velutinum</i> Bissett, C.P. Kubicek & Szakács        | CPK 298 = DAOM 230013 <sup>T</sup>       | KF134794                  | KJ665769        |
| <i>T. velutinum</i>                                        | LESF132                                  | KT278954                  | KT279019        |
| <i>T. zelobreve</i> Jing Z. Sun & X.Z. Liu                 | CGMCC 3.19695 <sup>T</sup>               | MN605872                  | MN605883        |
| <i>T. zelobreve</i>                                        | CGMCC 3.19696                            | MN605873                  | MN605884        |

Newly-sequenced cultures are highlighted in bold type. T indicates a type culture. ET indicates an epitype culture.

sequences are provided in Table 1. The multiple sequence alignment was conducted using MAFFT (Katoh and Standley 2013), followed by manual adjustments with MEGA7 (Kumar et al. 2018), and the fragments suitable for molecular identification were trimmed according to Cai and Druzhinina (2021). The trimmed sequences were then concatenated using SequenceMatrix v.1.8 (Vaidya et al. 2011).

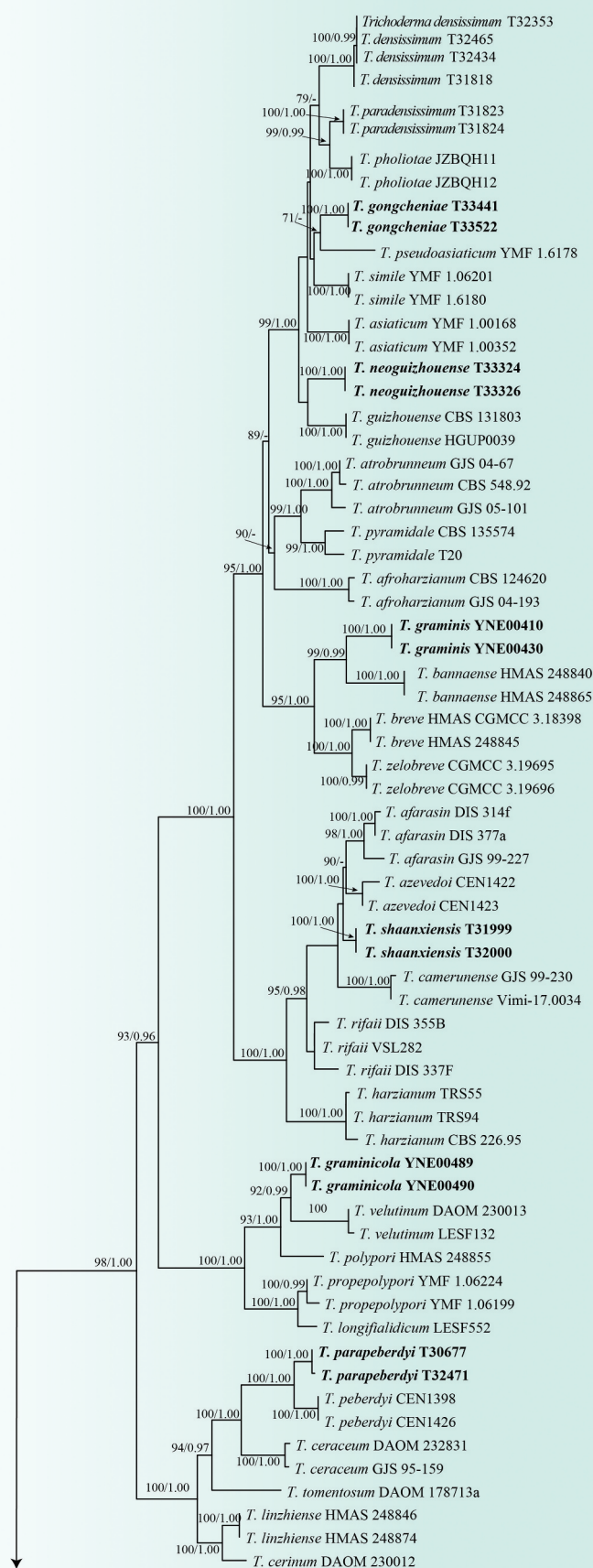
The phylogenetic relationships of taxa were inferred using maximum likelihood (ML) and Bayesian inference (BI) in the PhyloSuite platform (Zhang et al. 2020). ML analysis was performed using IQ-TREE (Lam-Tung et al. 2015). ModelFinder (Kalyaanamoorthy et al. 2017) was employed to select the best-fit partition model (Edge-linked) based on the BIC criterion. The best-fit model according to BIC was TNe+I+G4 for *rpb2* and *tef1*. BI phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under the partition model (2 parallel runs, 2,000,000 generations), within the initial 25% of sampled data discarded as burn-in. ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-linked) based on the Akaike Information Criterion (AIC). The best-fit model according to AIC was SYM+I+G4 for *rpb2* and HKY+F+I+G4 for *tef1*. The phylogenetic tree was visualised in FigTree v1.4.3.

### 3. Results

#### 3.1. Phylogenetic analysis

A total of 126 strains from 63 *Trichoderma* species were included in the phylogenetic analyses, alongside two outgroup species, *T. stromaticum* and *T. rossicum*. Since the ML and BI phylogenetic trees exhibited similar topologies, only the ML tree (Figure 1) is presented. The resulting tree (Figure 1) revealed the classification of six new species in the *Harzianum* Clade, three in the *Koningii* Clade, one in the *Hamatum* Clade, and one in the cluster comprising the *Ceramicum* and *Strictipile* Clade.

In the *Harzianum* clade, *T. gongcheniae* and *T. pseudoasiaticum* were grouped with *T. densissimum*, *T. paradensissimum*, *T. pholiotae*, *T. simile*, *T. asiaticum*, *T. neoguizhouense*, and *T. guizhouense* with strong statistical support (MLBP/BIPP = 99%/1.00). Furthermore, *T. gongcheniae* was found to be related to *T. pseudoasiaticum* with weak statistical support, and *T. neoguizhouense* was associated with *T. guizhouense*, also with weak statistical support. Moreover, *T. graminis* was closely related to *T. bananaense* with strong statistical support (MLBP/BIPP = 99%/0.99). *T. shaanxiensis* was grouped with *T. azevedoi*, *T. afarasin*, and *T. camerunense* with weak statistical support, while *T. graminicola* was closely related to *T. velutinum* with strong statistical support (MLBP/



**Figure 1.** Phylogenetic tree generated by maximum likelihood analysis using the concatenated sequences of the *rpb2* and *tef1* loci of the genus *Trichoderma*. Notes are marked with maximum likelihood bootstrap proportions  $\geq 70\%$  (left) and Bayesian inference posterior probability values  $\geq 0.9$  (right) (MLBP/BIPP). *T. stromaticum* and *T. rossicum* are chosen as outgroups. Newly proposed species are highlighted in bold.



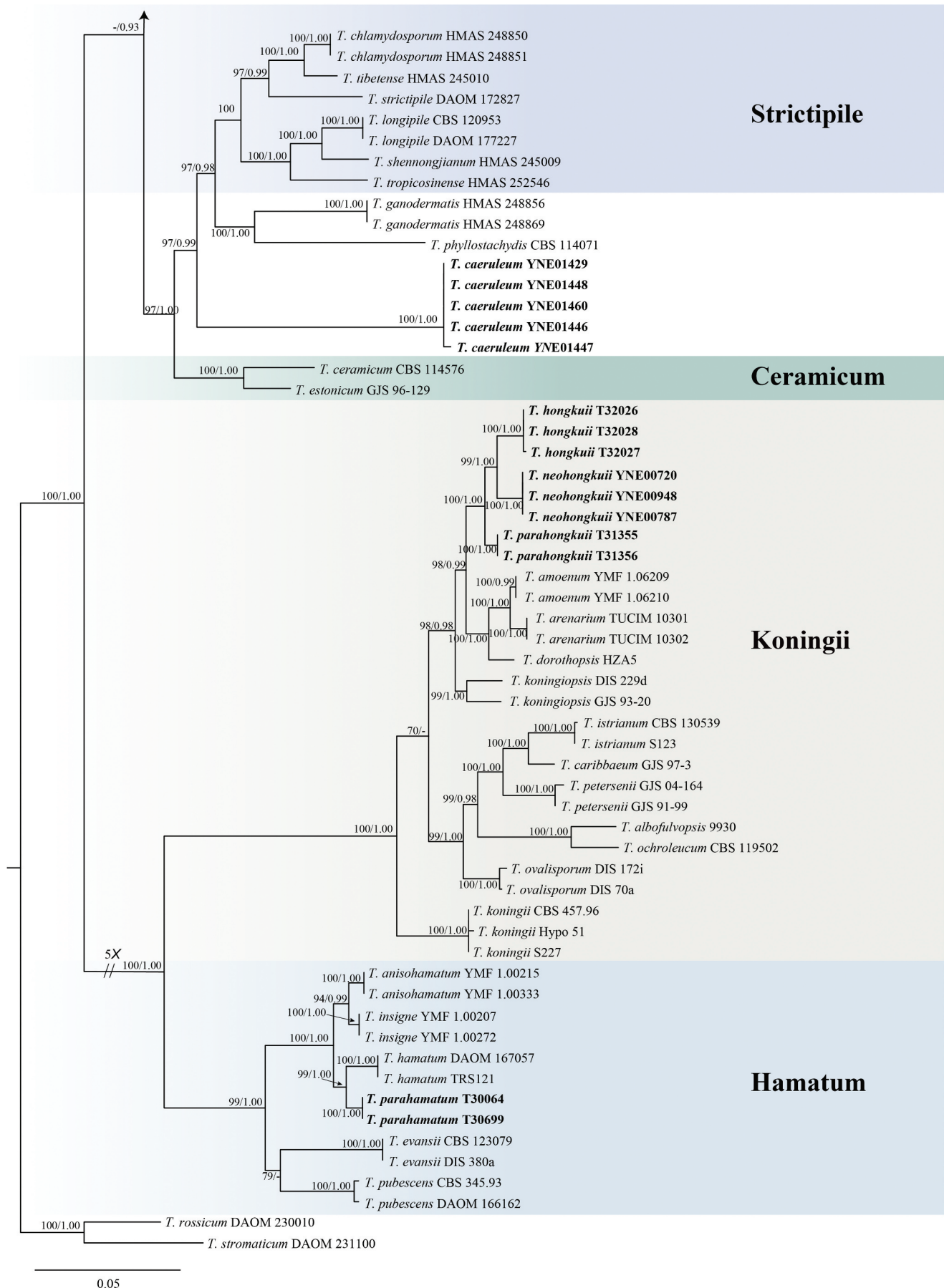


Figure 1. (Continued).

BIPP = 92%/0.99). Furthermore, *T. parapeberdyi* was closely associated with *T. peberdyi* with strong statistical support (MLBP/BIPP = 100%/1.00). In the *Koningii* clade, *T. hongkuii*, *T. neohongkuii*, and *T. parahongkuii* formed a well-supported clade (MLBP/BIPP = 100%/1.00). In the *Hamatum* clade, *T. parahamatum* was closely associated with *T. hamatum* with strong statistical support (MLBP/BIPP = 99%/1.00). Although *T. caeruleum* did not belong to any named clades, it was closely associated with the *Ceramicum* and *Strictipile* clades with strong statistical support (MLBP/BIPP = 97%/1.00).

### 3.2. Taxonomy

***Trichoderma caeruleum*** C. L. Zhang, sp. nov. [Figure 2](#)  
Fungal Names: FN 571773.

**Etymology:** The specific epithet “caeruleum” refers to the blue pigment produced by the fungus.

**Type:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaves of *Oryza rufipogon*, Oct. 2018, X.X. Feng YNE01446 (Holotype GDMCC 3.1021, stored in a metabolically inactive state. Ex-type culture YNE01446).

**Description:** Conidiophores more or less symmetrical, main axis recognisable, branches arising at an angle of less than 90° concerning the main axis. Phialides lageniform, spindly, sometimes slightly swollen in the middle, commonly in whorls of 2–3, or solitary, (9.8–)10.7–22.4(–24.5) × (2.0–)2.1–3.3(–3.7) μm (mean 16.6 × 2.7 μm), phialide length/width ratio (3.1–)3.5–9.0(–9.6) (mean 6.2), base (1.4–)1.6–2.8(–3.3) μm (mean 2.2 μm). Conidia often cylindrical with rounded ends, less ellipsoidal, colourless, 3.7–6.0(–6.3) × (2.9–)2.7–3.8(–4.4) μm (mean 4.8 × 3.3 μm), length/width ratio 1.2–1.9 (mean 1.5). Chlamydospores not observed.

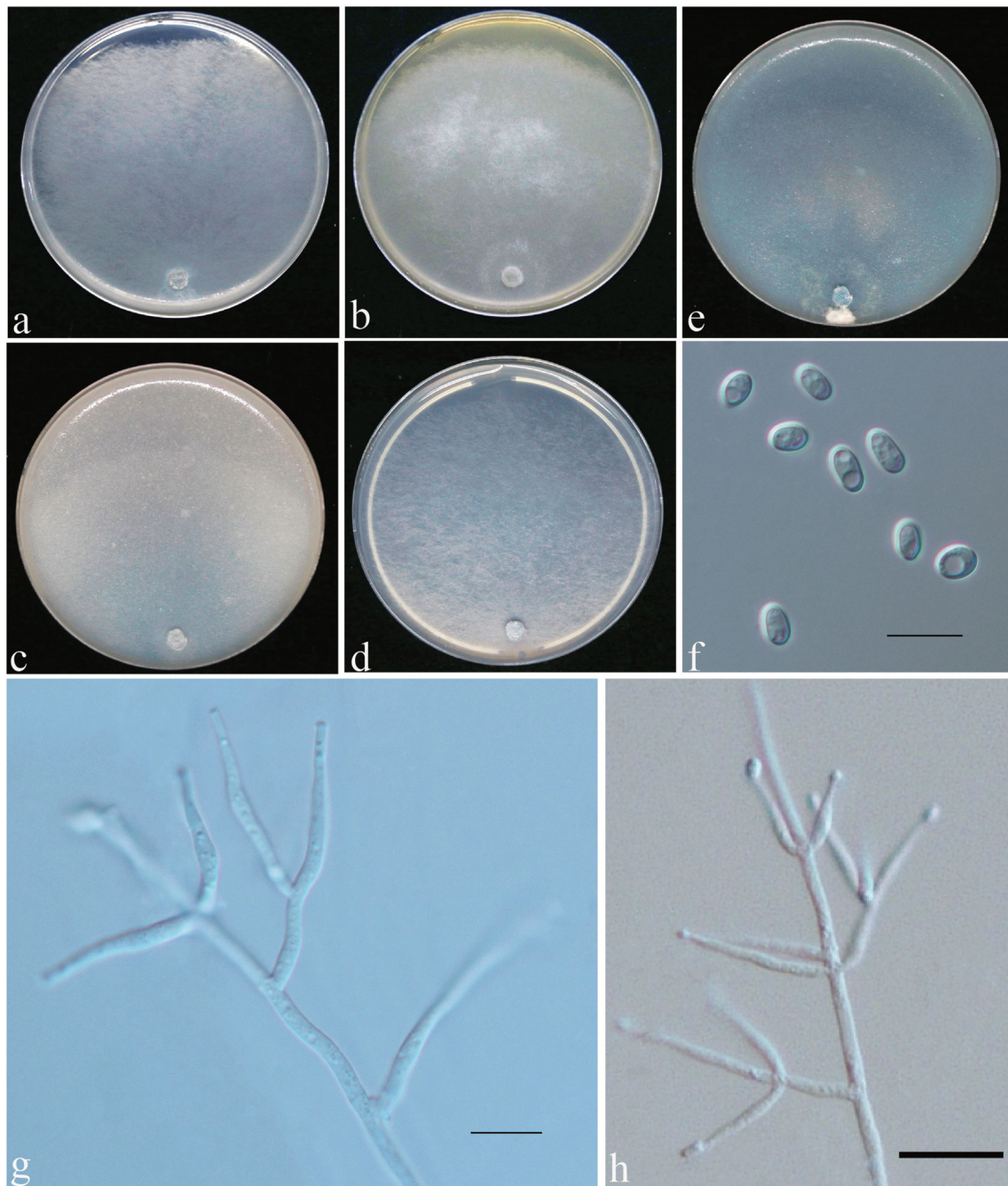
**Culture characteristics:** Lethal at 35 °C, similar growth rates on CMD, MEA, PDA, and SNA. Colony radius on CMD after 72 h: 22–25 mm at 25 °C, 25–27 mm at 30 °C. Covering the plate after 6 days at 25 °C and 7 days at 30 °C. Colonies well-defined, translucent, thin, and flat, with aerial hyphae nearly lacking. Orange pleasant odour apparent. Producing blue pigment around the inoculum after 4 days at 25 °C, covering the plate after 10 days. Colony radius on MEA after 72 h: 30–

31 mm at 25 °C, 25–28 mm at 30 °C. Covering the plate after 6 days at 25 °C and 7 days at 30 °C. Colonies pale white. Aerial hyphae white and sparse. Orange pleasant odour apparent, no diffusing pigment observed. Colony radius on PDA after 72 h: 31–35 mm at 25 °C, 30–31 mm at 30 °C. Covering the plate after 5 days at 25 °C and 7 days at 30 °C. Colonies pale white, conidiation sometimes sparse or absent. Orange pleasant odour apparent, blue pigment detected after 6 days at 30 °C. Colony radius on SNA after 72 h: 35–37 mm at 25 °C, 21–24 mm at 30 °C. Covering the plate after 5 days at 25 °C and 7 days at 30 °C. Colonies similar to that on PDA, white minute pustules noted after 10 days. Orange pleasant odour apparent, no diffusing pigment observed.

**Additional specimens examined:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaves of *Oryza rufipogon*. Oct. 2018, X.X. Feng, living culture YNE01429. China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaves of *Oryza rufipogon*. Oct. 2018, X.X. Feng, living culture YNE01448. China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaves of *Oryza rufipogon*. Oct. 2018, X.X. Feng, living culture YNE01460. China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaves of *Oryza rufipogon*. Oct. 2018, X.X. Feng, living culture YNE01467.

**Notes:** *Trichoderma caeruleum* is characterised by its blue pigment, orange pleasant odour, spindly phialides, rare conidia, and lethality at 35 °C, making it easily identifiable. Phylogenetically, *T. caeruleum* forms a distinct clade and is closely related to *T. phyllostachydis* and *T. ceramicum*, none of which have observed chlamydospores, and all of which are unable to withstand high temperatures, rendering them unable to grow at 35 °C. However, *T. caeruleum* exhibits significant differences in conidia and phialides compared to *T. phyllostachydis* and *T. ceramicum*. Specifically, the conidia of *T. ceramicum* are colourless, whereas *T. phyllostachydis* and *T.*





**Figure 2.** Colonies and microscopic characteristics of *Trichoderma caeruleum* YNE01446 (GDMCC 3.1021). (a – d) colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (e) Colony after 10 d at 25 °C on CMD. (f) Conidia. (g – h) Conidiophores and phialides. Scale bars: f – h = 10 µm.

*ceramicum* have green conidia. Additionally, *T. ceramicum* has larger phialides (10.7–22.4 µm) than both *T. phyllostachydis* (6.5–7.0 µm) and *T. ceramicum* (6.5–8.0 µm). Furthermore, *T. caeruleum* exhibits blue pigment, whereas *T. phyllostachydis* and *T. ceramicum* lack diffusing pigment (Chaverri and Samuels 2003).

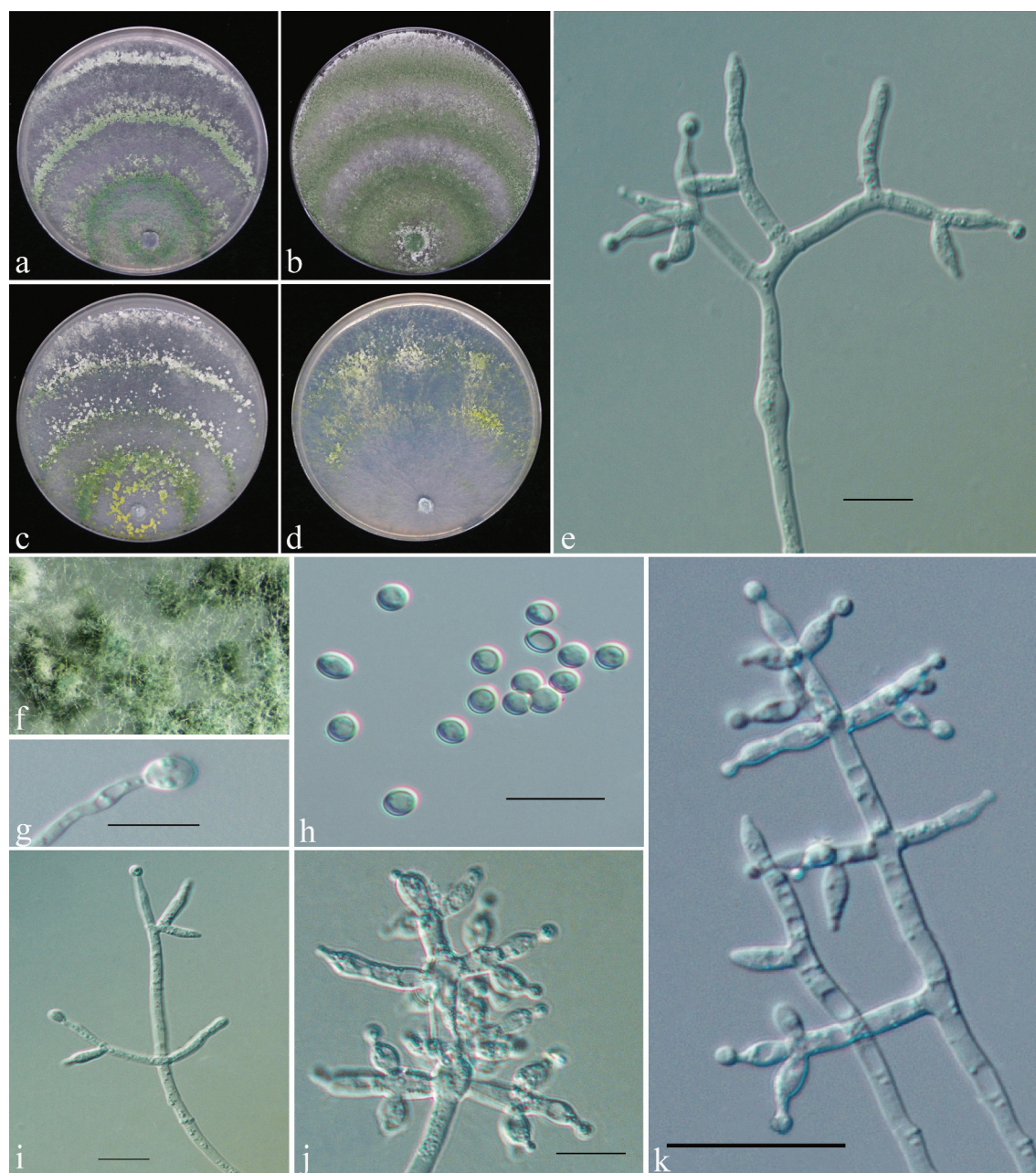
***Trichoderma gongcheniae*** C. L. Zhang, sp. nov.  
Figure 3

*Fungal Names:* FN 571774.

*Etymology:* The specific epithet “gongcheniae” is named after Prof. Gongchen Wang in recognition of her significant contribution to mycology and plant pathology in China.

*Type:* China, Guizhou Province, Tongren City, 27°17' 52.8N, 108°56'48.7"E, 5 m alt., isolated from soil of a rice field. Oct. 2016, J.L. Wang T33441 (Holotype





**Figure 3.** Colonies and microscopic characteristics of *Trichoderma gongcheniae* T33441 (GDMCC 3.1011). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Chlamydospore. (h) Conidia. (e, i – k) Conidiophores and phialides. Scale bars: e, g – k = 10  $\mu$ m.

GDMCC 3.1011, stored in a metabolically inactive state. Ex-type culture T33441).

**Description:** Conidiophores pyramidal with opposing branches, the branches generally perpendicular to main axis and each branch terminating in a cruciate whorl of 2–3 phialides. Phialides ampulliform to lageniform, solitary or in whorls of 2–3,  $(5.3\text{--}5.6\text{--}8.4\text{--}9.1) \times (2.7\text{--}2.8\text{--}3.7)$   $\mu$ m (mean  $7.0 \times 3.2$   $\mu$ m), phialide length/width ratio  $(1.5\text{--}1.6\text{--}2.7)$  (mean 2.2), base

$(1.7\text{--}1.8\text{--}2.4)$   $\mu$ m (mean 2.1  $\mu$ m). Conidia subglobose to globose, green,  $(2.7\text{--}2.8\text{--}3.3\text{--}3.4) \times 2.7\text{--}3.1\text{--}3.2)$   $\mu$ m, (mean  $3.1 \times 2.9$   $\mu$ m), length/width ratio 1.0–1.1 (mean 2.2). Chlamydospores commonly single, sometimes terminal and intercalary, globose to subglobose  $5.2\text{--}6.8\text{--}7.0) \times (4.6\text{--}4.8\text{--}6.3\text{--}6.7)$   $\mu$ m (mean  $6.0 \times 5.5$   $\mu$ m), length/width ratio 1.0–1.2 (mean 1.1).

**Culture characteristics:** Optimal growth at 30 °C. Colony radius on CMD after 72 h: 41–43 mm at 25 °C,



58–60 mm at 30 °C, and 44–46 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony hyaline, radial, conidiation forming 3–4 zonates around the inoculums. No diffusing pigment or distinct odour observed. Colony radius on MEA after 72 h: 54–56 mm at 25 °C, mycelium covering the plate at 30 °C, 35–36 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony hyaline, radial, aerial hyphae dense. Green conidiation forms after 2 days, with a semi-circular sporulation area along the inoculation point, and finally 2–3 broad concentric rings. No diffusing pigment or distinct odour observed. Colony radius on PDA after 72 h: 52–53 mm at 25 °C, mycelium covering the plate at 30 °C and 31–32 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony hyaline, radial, aerial hyphae dense. Green conidiation forms after 3 days, around the point of inoculation to margin, conidiation changing from dark green to pale green, forming 3 conspicuously zonates. No diffusing pigment or distinct odour observed. Colony radius on SNA after 72 h: 43–48 mm at 25 °C, 56–60 mm at 30 °C, and 21–25 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony radial, aerial hyphae scant, white conidia noted after 3 days around the inoculum, turning yellow-green after 4 days, indistinct zonate. No diffusing pigment or distinct odour observed.

*Additional specimens examined:* China, Guizhou Province, Liupanshui City, 26°23'58.4"N, 105°00'15.6"E, 1,052 m alt., isolated from soil of a rice field. Oct. 2016, J.L. Wang, living culture T33522.

*Notes:* *Trichoderma gongcheniae* is placed within the *Harzianum* clade and is phylogenetically closely related to *T. pseudoasiaticum*. Morphologically, the two species share similar conidia. However, the size of the phialide of *T. gongcheniae* (5.6–8.4 µm) is smaller than that of *T. pseudoasiaticum* (6.1–9.0 µm). Furthermore, *T. gongcheniae* forms three conspicuously zonate growth patterns on PDA, while *T. pseudoasiaticum* displays indistinct zonation. Additionally, *T. gongcheniae* demonstrates faster growth than *T. pseudoasiaticum* on PDA, CMD, and SNA at 35 °C. After 72 h at 35 °C, the colony radius of *T. gongcheniae* was 31–32 mm on PDA, 44–46 mm on CMD, and 21–25 mm on SNA. In comparison, *T. pseudoasiaticum* exhibited colony radius of 13 mm on PDA, 21 mm on CMD, and 2 mm on SNA after the same 72 h at 35 °C (Zheng et al. 2021).

***Trichoderma graminicola*** C. L. Zhang, sp. nov.

Figure 4

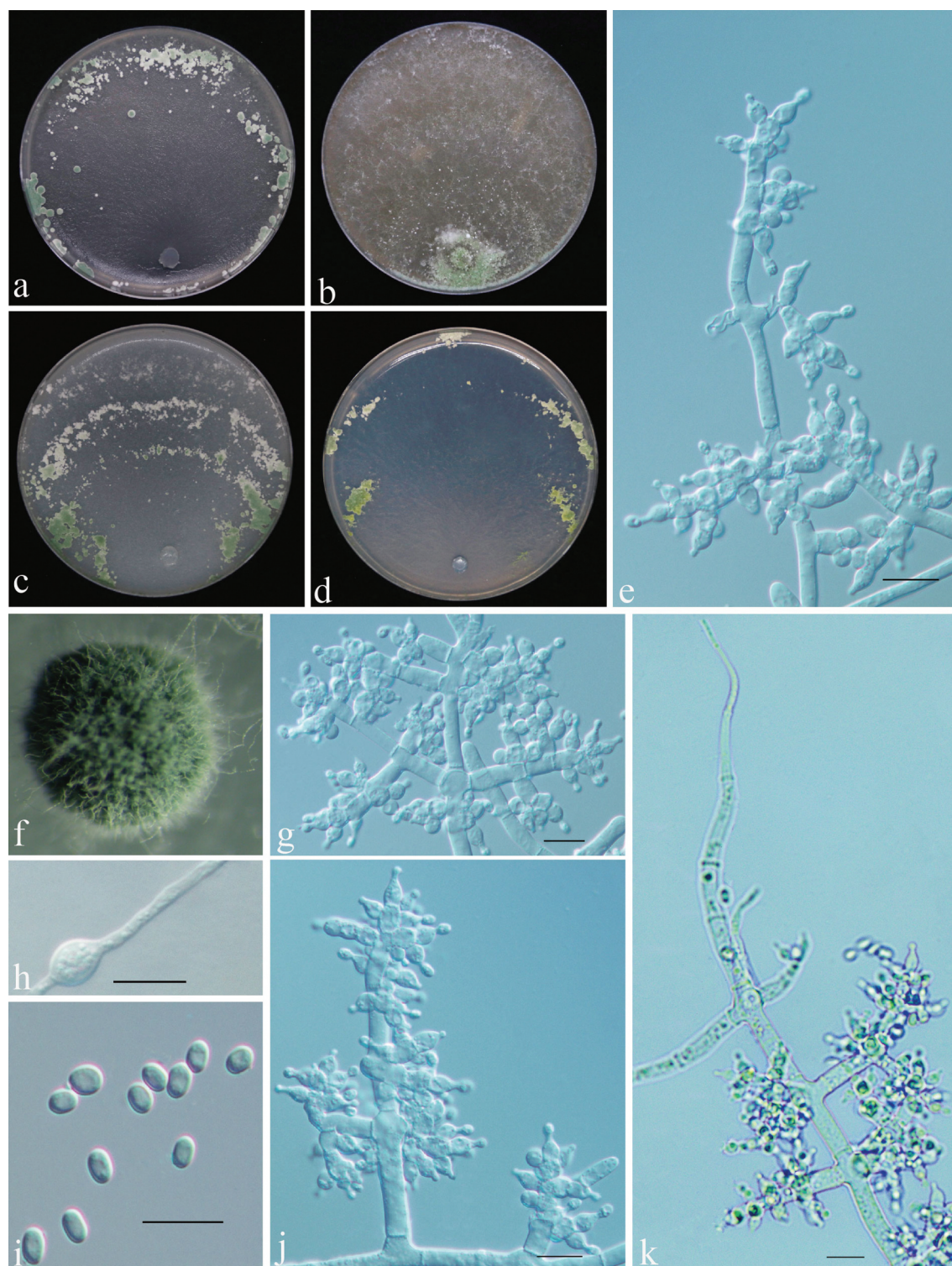
*Fungal Names:* FN 571775.

*Etymology:* Named after the grass hosts from which it was isolated.

*Type:* China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living stems of *Oplismenus undulatifolius*. Aug. 2015, J.J. Chen YNE00490 (Holotype GDMCC 3.1015, stored in a metabolically inactive state. Ex-type culture YNE00490).

*Description:* Main axis relatively broad, gradually narrowing from the base to the termination, terminated by a flexuous or undulate sterile extension. Primary branches mostly paired, occasionally 3–verticillate or solitary. Phialides ampulliform to subglobose, borne mostly in whorls of 3–5 on terminal branches of the conidiophore, or solitary, or paired. (3.9–)4.3–6.9(–6.7) × (2.9–)3.3–4.4 µm (mean 5.6 × 3.9 µm), phialide length/width ratio (1.0–)1.1–1.8 (–2.0) (mean 1.5), base 2.3–3.4(–3.5) µm (mean 2.8 µm). Conidia ovoid to breviter cylindracea, light green, (3.2–)3.3–3.9(–4.1) × (2.0–)2.1–2.6(–2.7) µm (mean 3.6 × 2.4 µm), length/width ratio 1.3–2.1 (mean 1.5). Chlamydospores terminal or intercalary, globose to subglobose, (5.9–)6.2–9.0(–9.2) × (5.3–)5.6–8.7 µm (mean 7.6 × 7.2 µm), length/width ratio 1.0–1.2 (mean 1.1).

*Culture characteristics:* Optimal growth at 30 °C. Colony radius on CMD after 72 h: 42–43 mm at 25 °C, 41–53 mm at 30 °C, and 4–10 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony hyaline, aerial hyphae pale white, loose, rare. Conidiation forms large pustules, spreading in 3–4 indistinct zonates. No distinct odour or diffusing pigment observed. Colony radius on MEA after 72 h: 51–53 mm at 25 °C, 58–61 mm at 30 °C, and 5–11 mm at 35 °C. Covering the plate after 4 days at 25 °C and at 30 °C. Colony hyaline, aerial hyphae pale white, abundant. Conidiation forms minute green pustules on aerial hyphae after 4 days, a few green disks around the inoculums. No distinct odour or diffusing pigment observed. Colony radius on PDA after 72 h: 56–61 mm at 25 °C, covering the plate at 30 °C and 9–10 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony hyaline, radial. Conidiation starts after 4 days, forming in white pustules, turning light green after 6 days, pustules appearing around the margin of the colony,



**Figure 4.** Colonies and microscopic characteristics of *Trichoderma graminicola* YNE00490 (GDMCC 3.1015). (a – d) Colonies after 7 at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (h) Chlamydospore. (i) Conidia. (e, g, j – k) Conidiophores and phialides. Scale bars: e, g – j = 10 µm; k = 20 µm.

sphericity or shape with an irregular outline. No distinct odour or diffusing pigment observed. Colony radius on SNA after 72 h: 46–48 mm at 25 °C, 55–65 mm at 30 °C, and 10–13 mm at 35 °C. Covering

the plate after 5 days at 25 °C and 4 days at 30 °C. Colony hyaline, radial, and aerial hyphae nearly lacking. Conidiation forms large yellow-green pustules, a small number, 2 indistinct zonates.



**Additional specimens examined:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living stems of *Oplismenus undulatifolius*. Aug. 2015, J. J. Chen, living culture YNE00489.

**Notes:** Phylogenetic analyses revealed that *T. graminicola* is closely related to *T. velutinum* with relatively strong supports. Morphologically, they have flexuous or undulate conidiophore extensions and similar phialides. However, *T. graminicola* ( $3.3\text{--}3.9 \times 2.1\text{--}2.6 \mu\text{m}$ ) differs by having larger conidia compared to *T. velutinum* ( $2.8\text{--}4.0 \times 1.6\text{--}2.4 \mu\text{m}$ ). Furthermore, *T. velutinum* lacks aerial mycelium on MEA, while *T. graminicola* exhibits abundant aerial hyphae. *T. velutinum* appears

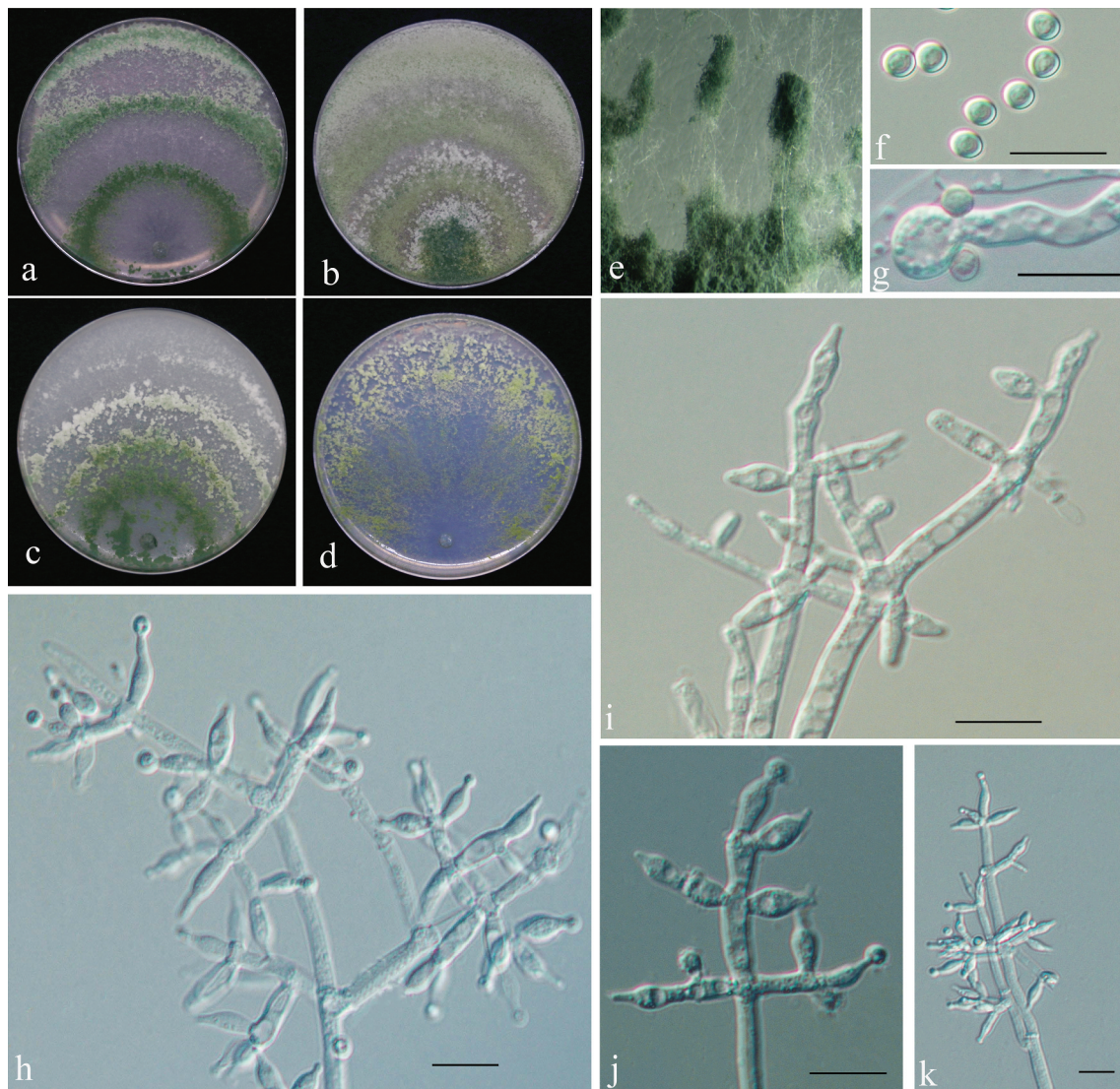
greyish yellow on MEA and produces minute, colourless droplets in the aerial mycelium on PDA, which are not observed in *T. graminicola* (Bissett et al. 2003).

***Trichoderma graminis*** C. L. Zhang, sp. nov. **Figure 5**

**Fungal Names:** FN 571776.

**Etymology:** Named after the grass hosts from which it was isolated.

**Type:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaf sheaths of *Oplismenus undulatifolius*. Aug. 2015, J.J. Chen YNE00410 (Holotype GDMCC 3.1013, stored in a metabolically inactive state. Ex-type culture YNE00410).



**Figure 5.** Colonies and microscopic characteristics of *Trichoderma graminis* YNE00410 (GDMCC 3.1013). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (e) Conidiation pustules on PDA. (f) Conidia. (g) Chlamydospore. (h – k) Conidiophores and phialides. Scale bars: f – k = 10  $\mu\text{m}$ .

**Description:** Conidiophores and branches form a pyramidal structure, with each branch terminating in a cruciate whorl of 2–5 phialides. Phialides lageniform  $(4.7\text{--}5.2\text{--}8.1\text{--}7.9) \times (2.5\text{--}2.7\text{--}3.9\text{--}4.4) \mu\text{m}$  (mean  $6.3 \times 3.3 \mu\text{m}$ ), length/width ratio 1.5–2.5(–2.4) (mean 2.0), base  $(1.5\text{--})1.7\text{--}2.4 \mu\text{m}$  (mean 2.1  $\mu\text{m}$ ). Conidia globose to obovoidal, green,  $(3.0\text{--})3.1\text{--}4.2 \times 2.9\text{--}3.9\text{--}4.0) \mu\text{m}$  (mean  $3.6 \times 3.4 \mu\text{m}$ ), length/width ratio 1.0–1.2 (mean 1.1). Chlamydospores often terminal, globose to subglobose,  $(5.5\text{--})5.6\text{--}7.6\text{--}7.7) \times (5.1\text{--})5.3\text{--}7.1 \mu\text{m}$  (mean  $6.6 \times 6.2 \mu\text{m}$ ), length/width ratio 1.0–1.3 (mean 1.1).

**Culture characteristics:** Optimal growth at 30 °C. Colony radius on CMD after 72 h: 36–43 mm at 25 °C, 58–60 mm at 30 °C, and 33–34 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony white, radial, aerial hyphae rare. Green and white conidial pustules form after 3 days, finally forming 4–5 indistinct zonate. No distinct odour or diffusing pigment observed. Colony radius on MEA after 72 h: 54–56 mm at 25 °C, 65–67 mm at 30 °C, and 27–28 mm at 35 °C. Covering the plate after 4 days at 25 °C and at 30 °C. Colony well-defined, radial, mycelium white, aerial hyphae dense, abundant. Light green and white conidial pustules form after 3 days, a small green disk around the inoculums, forming 3 broad distinct zonates. No distinct odour or diffusing pigment observed. Colony radius on PDA after 72 h: 60–62 mm at 25 °C, covering the plate at 30 °C and 27–28 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony white, radial, fluffy. Conidiation starts after 3 days, in aggregated green pustules, pustules abundant, spreading in 3 dark green concentric rings. No distinct odour or diffusing pigment observed. Colony radius on SNA after 72 h: 42–44 mm at 25 °C, 61–65 mm at 30 °C, and 25 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony hyaline, radial, Aerial hyphae sparse, scant, inconspicuous. Conidiation starts after 3 days, in aggregated white pustules, turning yellow-green after 4 days. No distinct odour or diffusing pigment observed.

**Additional specimens examined:** Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living leaf sheaths of *Oplismenus undulatifolius*. Aug. 2015, J.J. Chen, living culture YNE00430.

**Notes:** *Trichoderma graminis* is recognised as an endophyte of *Oplismenus undulatifolius*. Phylogenetically, *T. graminis* is closely related to *T.*

*bannaense*; however, its conidia are larger than those of *T. bannaense* ( $2.5\text{--}3.6 \times 2.5\text{--}3.1 \mu\text{m}$ ), and the phialides are relatively smaller than *T. bannaense* ( $7.5\text{--}10.0 \mu\text{m}$ ). Moreover, their conidiation pustules exhibit distinct characteristics. Specifically, the conidial pustules of *T. graminis* form in three concentric rings, whereas those of *T. bannaense* aggregate around the original inoculum (Chen and Zhuang 2017).

***Trichoderma hongkuii*** C. L. Zhang, sp. nov. **Figure 6**  
Fungal Names: FN 571777.

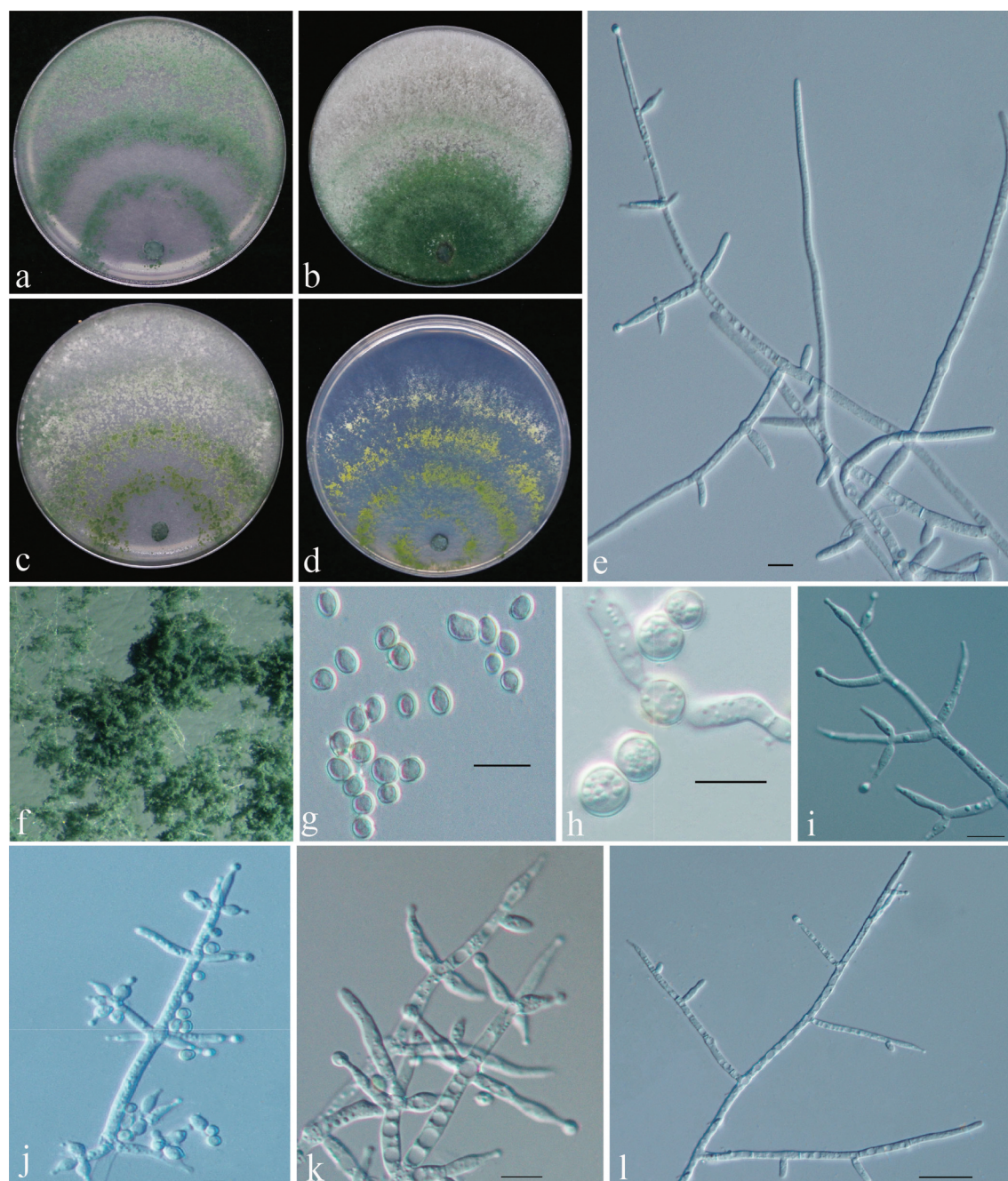
**Etymology:** Named in honour of Prof. Hongkui Chen, a pioneering figure in the fields of Chinese plant pathology and mycology.

**Type:** China, Shaanxi Province, Yan'an City, 36°36' 55.9"N, 109°25'45.5"E, 981 m alt., isolated from soil of a corn field. Jun. 2015, J.L. Wang T32026 (Holotype GDMCC 3.1017, stored in a metabolically inactive state. Ex-type culture T32026).

**Description:** Conidiophores comprising a recognisable main axis, branches pairs or solitary, arising at an angle of slightly less than 90° concerning the main axis, longer branches near the base and short branches or solitary phialides arising near the tip. Phialides solitary or arising in whorls of 2–4 from axis and branches, ampulliform, typically straight, sometimes sinuous to subglobose,  $(5.8\text{--})5.9\text{--}13.2\text{--}14.3) \times (2.6\text{--})2.5\text{--}4.0\text{--}4.2) \mu\text{m}$  (mean  $9.6 \times 3.3 \mu\text{m}$ ), length/width ratio 1.5–4.5(–4.6) (mean 3.0), base  $(1.6\text{--})1.7\text{--}2.8\text{--}3.0) \mu\text{m}$  (mean 2.2  $\mu\text{m}$ ). Conidia subglobose to ellipsoidal, green,  $3.4\text{--}4.1\text{--}4.4) \times 2.7\text{--}3.5\text{--}3.6) \mu\text{m}$  (mean  $3.7 \times 3.1 \mu\text{m}$ ), length/width ratio 1.1–1.4 (mean 1.2). Chlamydospores single, terminal or intercalary, globose to subglobose,  $(5.3\text{--})5.5\text{--}9.2\text{--}9.6) \times (5.3\text{--})5.1\text{--}8.8\text{--}9.1) \mu\text{m}$  (mean  $7.4 \times 7.1 \mu\text{m}$ ), length/width ratio 1.0–1.1 (mean 1.0).

**Culture characteristics:** Colony radius on CMD after 72 h: 51–56 mm at 25 °C, 58–59 mm at 30 °C, and 8–9 mm at 35 °C. Covering the plate after 4 days at 25 °C and at 30 °C. Colony well-defined, aerial hyphae white, abundant. Yellow-green conidia formed after 3 days. Conidial production often in 2–3 concentric rings around the inoculum, near the margin abundant, yellow-green towards the centre and white-grey to white near the margin, cottony pustules. No diffusing pigment or distinctive odour noted. Colony radius on MEA after 72 h: 43–44 mm at 25 °C, 39–40 mm at 30 °C, and 8–10 mm at 35 °C. Covering the plate after 5 days at 25 °C and at 30 °C. Colony





**Figure 6.** Colonies and microscopic characteristics of *Trichoderma hongkuii* T32026 (GDMCC 3.1017). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Conidia. (h) Chlamydospore. (e, i – l) Conidiophores and phialides. Scale bars: e, g – l = 10 µm.

well-defined, aerial hyphae white, fluffy, abundant. Conidiation formed in white minute pustules in the aerial mycelium after 2 days, turning green after 3 days. A large dark green disk around the inoculum. No diffusing pigment or distinctive odour noted. Colony radius on PDA after 72 h: 53–54 mm at 25 °C, 44–46 mm at 30 °C, and 7–10 mm at 35 °C. Covering the plate after 4 days at 25 °C and 5 days at 30 °C.

Colony well-defined, aerial hyphae pale white, abundant. Green conidiation formed after 3 days, in agglomerating minute pustules. Conidia form in 2 conspicuous concentric rings, narrow near the inoculums, far away from the inoculums abundant. No diffusing pigment or distinctive odour noted. Colony radius on SNA after 72 h: 20–25 mm at 25 °C, 20–22 mm at 30 °C, and 3–4 mm at 35 °C. Covering

the plate after 7 days at 25 °C and 13 days at 30 °C. Colony hyaline, radial, aerial hyphae white, less. Conidiation formed after 3 days, yellow-green, abundant, minute cottony pustules in 3–4 indistinct concentric rings. No diffusing pigment or distinctive odour noted.

*Additional specimens examined:* China, Shaanxi Province, Shangluo City, 33°51'44.4"N, 109°51'46.0"E, 738 m alt., isolated from soil of a corn field. Jun. 2015, J.L. Wang, living culture T32027. China, Shaanxi Province, Shangluo City, 33°51'44.4"N, 109°51'46.0"E, 738 m alt., isolated from soil of a corn field. Jun. 2015, J.L. Wang, living culture T32028.

*Notes:* *Trichoderma hongkuii* is phylogenetically classified within the *Koningii* clade and is closely related to *T. neohongkuii* with relatively strong supports. Both *T. hongkuii* and *T. neohongkuii* exhibit a similar branching pattern of conidiophores and share ellipsoidal conidia of similar size. However, *T. hongkuii* forms abundant pustules on CMD, whereas *T. neohongkuii* produces relatively few but large pustules. Furthermore, *T. hongkuii* demonstrates faster growth than *T. neohongkuii* on PDA, with a colony radius after 3 days at 25 °C of 53–54 mm compared to 38–41 mm, and at 30 °C of 44–46 mm compared to 28–31 mm.

***Trichoderma parapeberdyi*** C. L. Zhang, sp. nov.  
[Figure 7](#)

*Fungal Names:* FN 571778.

*Etymology:* Named after its phylogenetic relationship to *T. peberdyi*.

*Type:* China, Anhui Province, Fuyang City, 32°40'08" N, 115°35'45"E, 37 m alt., isolated from the soil of a peach orchard. Aug. 2014, Y. Jiang T30677 (Holotype GDMCC 3.1016, stored in a metabolically inactive state. Ex-type culture T30677).

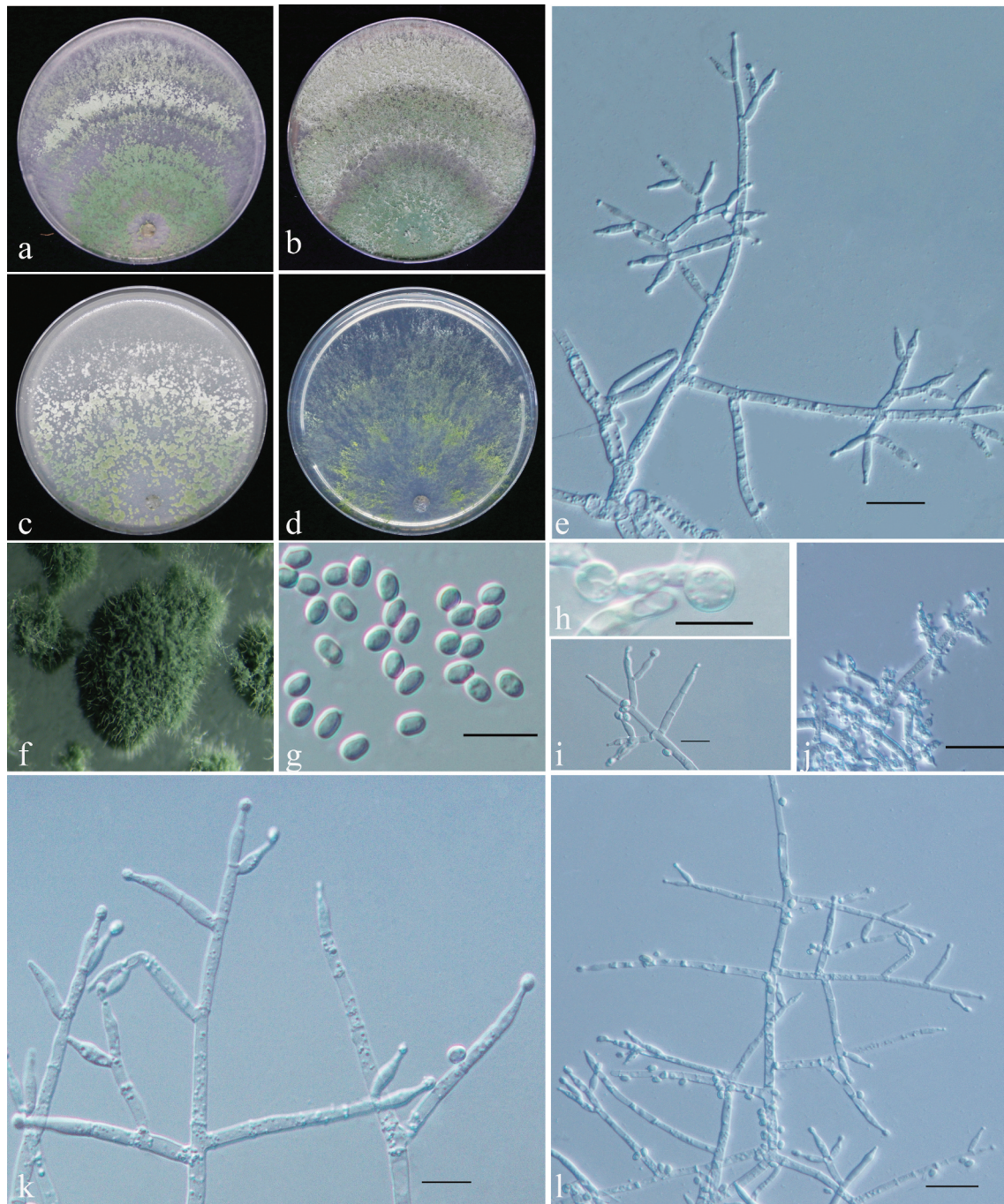
*Description:* Conidiophores pyramidal with misaligned opposing branches. Branches generally perpendicular to the main axis, and each branch terminates in a whorl of 2–3 phialides, or solitary. Phialides ampulliform, (6.6–)7.1–11.7(–12.5) × 2.3–3.6 µm (mean 9.4 × 2.9 µm), length/width ratio (1.9–)2.1–4.4(–4.8) (mean 3.3), base 1.7–2.7 µm (mean 2.2 µm). Conidia ellipsoidal to breviter cylindracea, light green to green, (3.1–)3.2–3.8(–4.0) × (2.0–)2.2–2.7 µm (mean 3.5 × 2.4 µm), length/width ratio (1.3–)1.2–1.7(–1.9) (mean 1.5). Chlamydospores single, terminal or intercalary, globose to subglobose, (5.8–)5.9–8.6(–8.9) × 5.7–8.2(–8.5) µm (mean 7.3 × 7.0 µm), length/width ratio 1.0–1.1 (mean 1.0).

*Culture characteristics:* Optimal growth at 30 °C. Colony radius on CMD after 72 h: 33–39 mm at 25 °C, 42–44 mm at 30 °C, and 1–2 mm at 35 °C. Covering the plate after 5 days at 25 °C and at 30 °C. Colony hyaline, aerial hyphae pale white, abundant. Conidiation forms cottony pustules, spreading uniformly throughout colony. No distinct odour observed. Light yellowish pigment detected at 35 °C. Colony radius on MEA after 72 h: 45–47 mm at 25 °C, 47–49 mm at 30 °C, and 2–3 mm at 35 °C. Covering the plate after 5 days at 25 °C and at 30 °C. Colony well-defined, aerial hyphae pale white, radial, abundant. Conidiation forms white minute pustules after 2 days, turning green after 3 days, pustules forms a large dark green disk around inoculum, with 2 broad greyish green zonates, pustules almost covering the plate. No distinct odour or diffusing pigment observed. Colony radius on PDA after 72 h: 44–48 mm at 25 °C, 51–53 mm at 30 °C, and 4–5 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony similar to that on MEA. Conidiation forms white minute pustules after 3 days, turning green after 4 days, pustules forms a broad green zonates around inoculum, with 3–4 indistinct greyish green zonates. No distinct odour or diffusing pigment observed. Colony radius on SNA after 72 h: 34–38 mm at 25 °C, 37–41 mm at 30 °C, and 5–8 mm at 35 °C. Covering the plate after 5 days at 25 °C and 6 days at 30 °C. Colony hyaline, radial, aerial hyphae white. Conidiation forms white minute pustules after 3 days, turning yellow-green after 3 days, indistinct zonates, radial, uniformly distributed. No distinct odour or diffusing pigment observed.

*Additional specimens examined:* China, Zhejiang Province, Taizhou City, 28°42'16.4"N, 121°28'47.1"E, 23 m alt., isolated from soil of a citrus orchard. Aug. 2014, J.J. Chen, living culture T30064.

*Notes:* Phylogenetically, *T. parapeberdyi* is closely related to *T. peberdyi* with strong supports. Morphologically, they both exhibit the same conidiophores and branching pattern. However, the conidia of *T. peberdyi* are subglobose to ovoid, which significantly differs from the ellipsoidal to breviter cylindracea of *T. parapeberdyi*. The size of conidia of *T. peberdyi* (3.54–4.65 × 2.55–3.33 µm) is larger than that of *T. parapeberdyi* (3.2–3.8 × 2.2–2.7 µm). Additionally, the phialides of *T. peberdyi* (4.91–9.10 µm) are smaller than those of *T.*





**Figure 7.** Colonies and microscopic characteristics of *Trichoderma parapeberdyi* T30677 (GDMCC 3.1016). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Conidia. (h) Chlamydospore. (e, i – l) Conidiophores and phialides. Scale bars: e, g – j, l = 10 µm; k = 20 µm.

*parapeberdyi* (7.1–11.7 µm). Chlamydospores were not observed in *T. peberdyi*. Moreover, the colonies of *T. parapeberdyi* show a slightly yellowish colour on the reverse side of CMD, whereas they were absent in *T. peberdyi*. Additionally, *T. parapeberdyi* exhibits slow growth at 35 °C on PDA, CMD, and

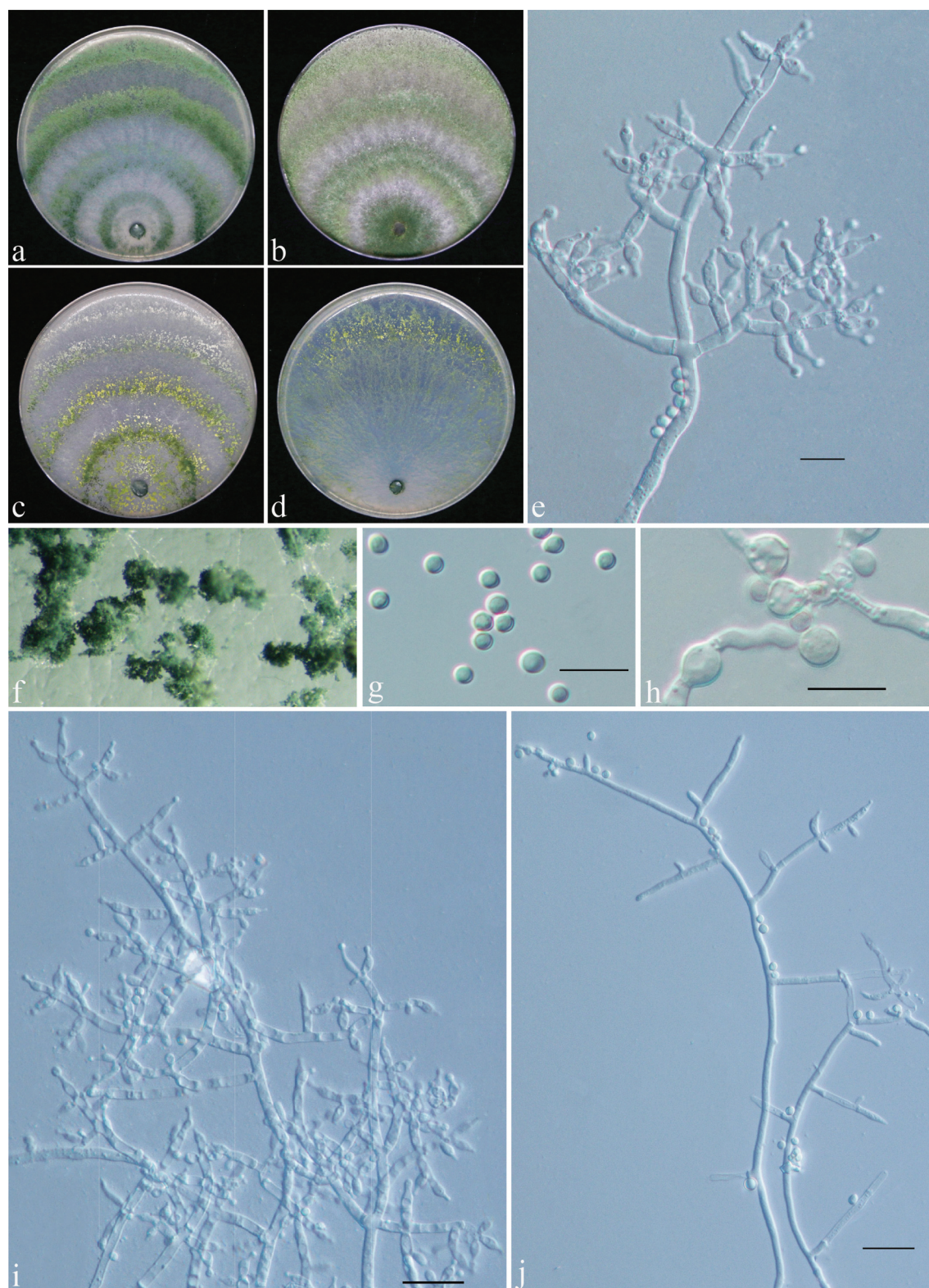
SNA, whereas no growth of *T. peberdyi* was observed (Inglis et al. 2020).

***Trichoderma neoguizhouense*** C. L. Zhang, sp. nov.

**Figure 8**

*Fungal Names:* FN 571779.





**Figure 8.** Colonies and microscopic characteristics of *Trichoderma neoguizhouense* T33324 (GDMCC 3.1012). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Conidia. (h) Chlamydospore. (e, i, j) Conidiophores and phialides. Scale bars: e, g, h = 10  $\mu$ m; i, j = 20  $\mu$ m.



**Etymology:** Named after its morphological similarity to *T. guizhouense*.

**Type:** China, Yunnan Province, Pu'er City, 22°44' 12.1N, 100°29'12.0"E, 1,389 m alt., isolated from soil of an eggplant field. Jun. 2016, J.L. Wang T33324 (Holotype GDMCC 3.1012, stored in a metabolically inactive state. Ex-type culture T33324).

**Description:** Conidiophores pyramidal with opposing branches, less solitary, closely spaced. Branches and main axis often terminate in a cruciate of 3 phialides, less terminating in 2 or solitary phialides. Phialides ampulliform, sometimes constricted below the tip,  $4.6\text{--}8.0(-9.0) \times 2.5\text{--}3.7(-3.6)$   $\mu\text{m}$  (mean  $6.3 \times 3.1$   $\mu\text{m}$ ), length/width ratio  $(1.5\text{--})1.4\text{--}2.7(-3.1)$  (mean 2.1), base  $(1.5\text{--})1.3\text{--}2.6(-3.4)$   $\mu\text{m}$  (mean 2.0  $\mu\text{m}$ ). Conidia subglobose to globose, smooth, green to dark green,  $(2.3\text{--})2.5\text{--}3.2(-3.4) \times (2.3\text{--})2.4\text{--}3.0(-3.3)$   $\mu\text{m}$ , (mean  $2.8 \times 2.7$   $\mu\text{m}$ ), length/width ratio 1.0–1.2 (mean 1.1). Chlamydospores subglobose to globose, terminal and intercalary,  $(4.3\text{--})4.4\text{--}6.3(-6.6) \times 4.2\text{--}6.1(-6.2)$   $\mu\text{m}$ , (mean  $5.4 \times 5.1$   $\mu\text{m}$ ), length/width ratio 1.0–1.2 (mean 1.0).

**Culture characteristics:** Optimal growth at 30 °C. Colony radius on CMD after 72 h: 43–46 mm at 25 °C, 59–61 mm at 30 °C, and 15 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony well-defined, radial, aerial hyphae dense, abundant. Green conidiation forms after 2 days, finally forming 3–4 distinct zonates. Yellow pigment detected after 3 days at 35 °C, no distinctive odour observed. Colony radius on MEA after 72 h: 58–60 mm at 25 °C, covering the plate at 30 °C and 27–33 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony well-defined, radial, aerial hyphae dense, abundant, green conidiation forms after 2 days, a small green disk around inoculum, finally forming 3 broad distinct zonates. Yellow pigment detected after 4 days at 35 °C, and no distinctive odour observed. Colony radius on PDA after 72 h: 58–59 mm at 25 °C, covering the plate at 30 °C and 25–38 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony well-defined, radial, aerial hyphae dense, abundant, green conidiation forms in pustules after 2 days and forming concentric rings around inoculum, finally forming 4 dark green concentric rings. Yellow pigment detected after 2 days at 35 °C, and no distinctive odour observed. Colony radius on SNA after 72 h: 43–45 mm at 25 °C, 59–62 mm at 30 °C, and 23–25 mm at 35 °C. Covering the

plate after 5 days at 25 °C and 4 days at 30 °C. Colony radial, aerial hyphae sparse, scant. Conidiation minute, forming in white minute pustules after 3 days, turning yellow-green after 4 days, spreading uniformly throughout the colony. No distinct odour or diffusing pigment observed.

**Additional specimens examined:** China, Yunnan Province, Pu'er City, 22°44'12.1"N, 100°29'12.0"E, 1,389 m alt., isolated from soil of a rice field. Oct. 2016, J.L. Wang, living culture T33326.

**Notes:** Phylogenetic analyses revealed that *T. neoguizhouense* is closely related to *T. guizhouense*. Morphologically, they both exhibit a high degree of similarity in conidiophore structure as well as the shape and size of conidia. However, *T. neoguizhouense* displays a yellow pigment, whereas *T. guizhouense* produces brown pigmentation on PDA. Additionally, chlamydospores were observed in *T. neoguizhouense*, while not described for *T. guizhouense* (Li et al. 2013).

***Trichoderma neohongkuii*** C. L. Zhang, sp. nov.  
Figure 9

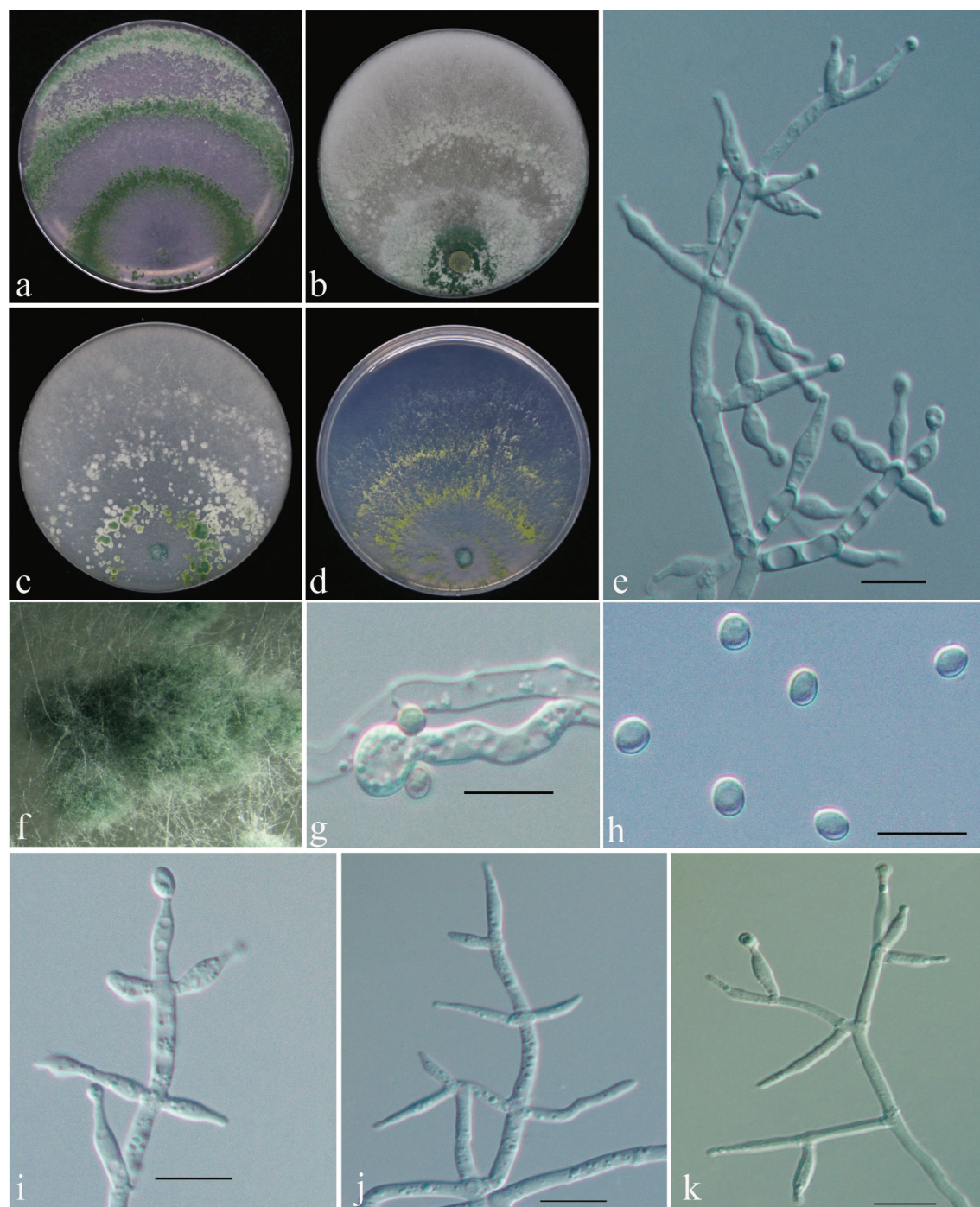
**Fungal Names:** FN 571780.

**Etymology:** Named after its morphological similarity to *T. hongkuii*.

**Type:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living roots of *Thysanolaena latifolia*. Oct. 2016, J.J. Chen YNE00787 (Holotype GDMCC 3.1018, stored in a metabolically inactive state. Ex-type culture YNE00787).

**Description:** Conidiophores tree-type, comprising recognisable main axis with paired or unilateral side branches. Phialides paired or arising in whorls of 2–4 from main axis and branches, ampulliform, cylindrical or slightly swollen in the middle, sometimes with a constricted base,  $(6.1\text{--})5.3\text{--}10.7(-12.3) \times 2.4\text{--}3.5$   $\mu\text{m}$  (mean  $8.0 \times 3.0$   $\mu\text{m}$ ), length/width ratio  $(1.9\text{--})1.7\text{--}3.8(-4.2)$  (mean 2.7), base  $(1.4\text{--})1.5\text{--}2.4$   $\mu\text{m}$  (mean 2.0  $\mu\text{m}$ ). Conidia ellipsoidal, green,  $(3.4\text{--})3.5\text{--}4.3(-4.6) \times 2.7\text{--}3.5(-3.9)$   $\mu\text{m}$  (mean  $3.9 \times 3.1$   $\mu\text{m}$ ), length/width ratio 1.1–1.5 (mean 1.3). Chlamydospores single, terminal or intercalary, globose to subglobose,  $(6.3\text{--})6.5\text{--}9.7(-10.5) \times 6.1\text{--}9.5(-10.3)$   $\mu\text{m}$  (mean  $8.1 \times 7.8$   $\mu\text{m}$ ), length/width ratio 1.0–1.1 (mean 1.0).

**Culture characteristics:** Colony radius on CMD after 72 h: 49–53 mm at 25 °C, 50–60 mm at 30 °C, and 7–10 mm at 35 °C. Covering the plate after 4 days at



**Figure 9.** Colonies and microscopic characteristics of *Trichoderma neohongkuii* YNE00787 (GDMCC 3.1018). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Chlamydospore. (h) Conidia. (e, i – k) Conidiophores and phialides. Scale bars: e, g – k = 10 µm.

25 °C and at 30 °C. Colony hyaline, radial, mycelium loose, aerial hyphae pale white. Conidiation starts after 3 days, forming large white cottony pustules, turning green after 4–5 days, spreading in 2–3 inconspicuous concentric rings. No odour or pigment noted. Colony radius on MEA after 72 h: 38–41 mm at 25 °C, 36–38 mm at 30 °C, and 13–16 mm at 35 °C. Covering the

plate after 5 days at 25 °C and at 30 °C. Colony well-defined, aerial mycelium cottony, abundant, dense, radiating. Conidia form in aerial mycelium and large white-green cottony pustules. No odour or pigment noted. Colony radius on PDA after 72 h: 38–41 mm at 25 °C, 28–31 mm at 30 °C, and 6–10 mm at 35 °C. Covering the plate after 5 days at 25 °C and 6 days at



30 °C. Colony well-defined, aerial hyphae pale white, radial. Green conidia form minute cottony pustules after 3 days, finally forming in 3 conspicuous narrow concentric rings separated by wider bands of less conidial production. No odour or pigment noted. Colony radius on SNA after 72 h: 24–28 mm at 25 °C, 28–30 mm at 30 °C, and 5–9 mm at 35 °C. Covering the plate after 6 days at 25 °C and 8 days at 30 °C. Colony hyaline, radial, and aerial hyphae rare. Yellow-green conidiation produced after 3 days, forming cottony pustules, indistinct zonation. No odour or pigment noted.

**Additional specimens examined:** China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living roots of *Thysanolaena latifolia*. Oct. 2016, J.J. Chen, living culture YNE00720. China, Yunnan Province, Xishuangbanna Prefecture, Naban River Watershed National Nature Reserve, 22°04'2N, 100°32'00"E, 1,229 m alt., isolated as an endophyte from living roots of *Thysanolaena latifolia*. Oct. 2016, J.J. Chen, living culture YNE00948.

**Notes:** The phylogenetic analyses based on *rpb2* and *tef1* showed that *T. neohongkuii* is closely related to *T. parahongkuii* and *T. hongkuii*, sharing a similar branching pattern of conidiophore. While *T. parahongkuii* and *T. hongkuii* are found in soils, *T. neohongkuii* is an endophyte inhabiting the roots of *Thysanolaena latifolia*. The differences between *T. neohongkuii* and *T. hongkuii* have been previously discussed. Notably, *T. parahongkuii* produces a pleasant fruity odour on CMD and PDA, whereas *T. neohongkuii* does not exhibit this trait. Additionally, *T. parahongkuii* shows no growth at 35 °C on PDA, MEA, CMD, and SNA, while *T. neohongkuii* is characterised by slow growth.

***Trichoderma parahamatum*** C. L. Zhang, sp. nov.

Figure 10

**Fungal Names:** FN 571781.

**Etymology:** Named after its phylogenetic relationship to *T. hamatum*.

**Type:** China, Anhui Province, Xuancheng City, 31°3'29"N, 118°51'21"E, 8 m alt., isolated from soil of a rice field. Aug. 2014, Y. Jiang T30699 (Holotype GDMCC 3.1020, stored in a metabolically inactive state. Ex-type culture T30699).

**Description:** Conidiophore apical elongations sterile, coiled, undulate, or hamate. The main axis and branches conspicuously broad. Phialides ampulliform to

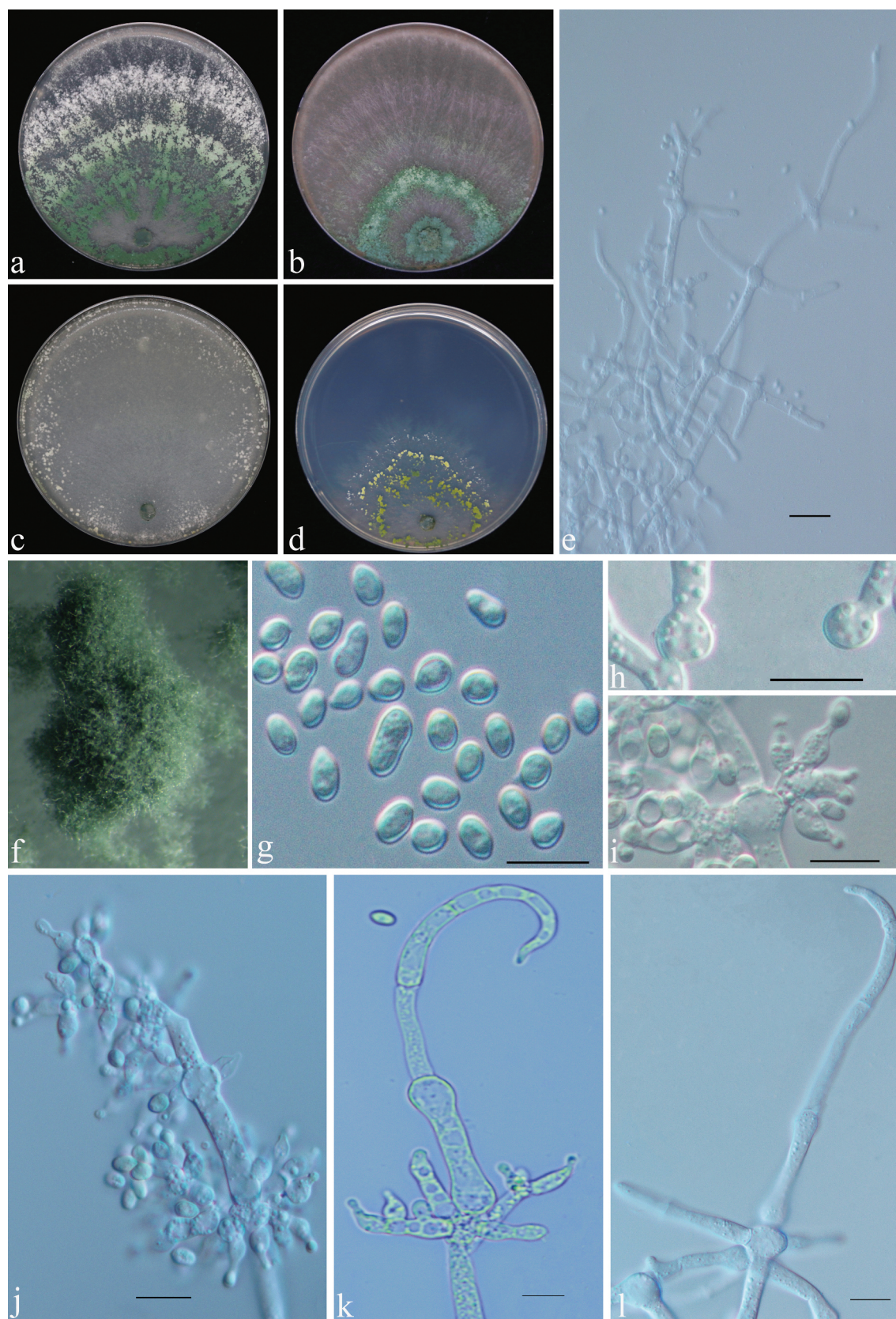
subglobose, short, wide, arising from short lateral branches at the base of elongation, crowded on broad subtending hyphae, 4.4–6.6(–6.9) × (3.1–)3.0–4.2(–4.5) µm (mean 5.5 × 3.6 µm), length/width ratio 1.1–2.0 (–2.2) (mean 1.5), base (1.3–)1.8–3.5(–3.6) µm (mean 2.6 µm). Conidia subglobose, ellipsoidal to breviter cylindracea, green, 3.3–5.5(–7.4) × (2.7–)2.8–3.4(–3.5) µm (mean 4.4 × 3.1 µm), length/width ratio 1.1–1.8(–2.3) (mean 1.4). Chlamydospores subglobose to globose, terminal and intercalary, (5.5–)5.9–11.7 × (5.4–)5.8–11.0 (–11.2) µm (mean 8.8 × 8.4 µm), length/width ratio 1.0–1.2 µm (mean 1.1).

**Culture characteristics:** No growth at 35 °C. Colony radius on CMD after 72 h: 45–47 mm at 25 °C, 50–54 mm at 30 °C. Covering the plate after 5 days at 25 °C and 4 days at 30 °C. Colony pale white, aerial hyphae inconspicuous loose, scant. Conidiation noted in white or light-green cottony pustules, pustules appearing around periphery of colony and Petri dish wall. Pleasant fruity odour detected, no diffusing pigment observed. Colony radius on MEA after 72 h: 40–45 mm at 25 °C, 36–38 mm at 30 °C. Covering the plate after 5 days at 25 °C and 30 °C. Colony hyaline, aerial hyphae radial, abundant. Conidia form in dark green cottony pustules, spreading a small green disk and a concentric ring around inoculum. No distinct odour or diffusing pigment observed. Colony radius on PDA after 72 h: 33–37 mm at 25 °C, 38–44 mm at 30 °C. Covering the plate after 6 days at 25 °C and 30 °C. Colony pale white well-defined, aerial hyphae radial, abundant. Conidiation noted after 4 days in white cottony pustules, turning green after 4 days, radial, indistinct zonation. Pleasant fruity odour sometimes found, and no diffusing pigment observed. Colony radius on SNA after 72 h: 8–15 mm at 25 °C, 21–29 mm at 30 °C. Covering the plate after 14 days at 25 °C and 10 days at 30 °C. Colony pale white, margin lobed, not well-defined, aerial hyphae inconspicuous. Conidiation noted in cottony pustules around inoculum, yellow to yellow-green, indistinct zonation. No distinct odour or diffusing pigment observed.

**Additional specimens examined:** China, Zhejiang Province, Lishui City, 28°42'16.4"N, 121°28'47.1"E, 23 m alt., isolated from soil of a citrus orchard. Oct. 2014, Y. Jiang, living culture T30064.

**Notes:** *Trichoderma parahamatum* is phylogenetically classified within the *Hamatum* clade and is closely related to *T. hamatum* with strong supports. Both *T. parahamatum* and *T. hamatum* exhibit sterile hamate





**Figure 10.** Colonies and microscopic characteristics of *Trichoderma parahamatum* T30699 (GDMCC 3.1020). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Conidia. (h) Chlamydospore. (e, i – l) Conidiophores and phialides. Scale bars: g – j, l = 10 µm; e, k = 20 µm.

elongations, with similar size and shape of phialides. However, *T. parahamatum* occasionally produces a pleasant fruity odour on PDA and CMD, while *T. hamatum* does not exhibit a distinctive odour. Furthermore, *T. hamatum* demonstrates significantly faster growth than *T. parahamatum*, as evidenced by colony radius on PDA after 72 h: 45–52 mm vs. 33–37 mm at 25 °C, and 50–57 mm vs. 38–44 mm at 30 °C; on SNA: 30–47 mm vs. 8–15 mm at 25 °C, and 36–55 mm vs. 21–29 mm at 30 °C (Chaverri et al. 2003).

***Trichoderma parahongkuii* C. L. Zhang, sp. nov.**  
Figure 11

*Fungal Names:* FN 571782.

*Etymology:* Named after its phylogenetic relationship to *T. hongkuii*.

*Type:* China, Zhejiang Province, Lishui City, 28°32'46.8" N, 119°10'04.9"E, 312 m alt., isolated from soil of a waxbe orchard. Oct. 2014, Y. Jiang T31356 (Holotype GDMCC 3.1019, stored in a metabolically inactive state. Ex-type culture T31356).

*Description:* Conidiophores comprise a distinct main axis. Branches paired or solitary, at an angle less than or near 90° concerning the main axis, branches terminating in a single phialide or a whorl of 2–3 phialides. Phialides ampulliform, more or less swollen in the middle,  $5.4\text{--}11.0\text{--}(12.3) \times (2.5\text{--})2.7\text{--}4.1\text{--}(4.4) \mu\text{m}$  (mean  $8.2 \times 3.4 \mu\text{m}$ ), length/width ratio  $1.4\text{--}3.5\text{--}(3.7)$  (mean 2.5), base  $(1.5\text{--})1.6\text{--}2.8\text{--}(2.9) \mu\text{m}$  (mean  $2.2 \mu\text{m}$ ). Conidia ellipsoidal to breviter cylindracea, green,  $(3.3\text{--})3.6\text{--}5.1\text{--}(5.3) \times 2.6\text{--}3.5\text{--}(3.8) \mu\text{m}$  (mean  $4.4 \times 3.1 \mu\text{m}$ ), length/width ratio  $1.1\text{--}1.7$  (mean 1.4). Chlamydospores single, terminal or intercalary, globose to subglobose,  $4.5\text{--}8.3\text{--}(9.0) \times 4.3\text{--}7.8\text{--}(8.3) \mu\text{m}$  (mean  $6.4 \times 6.1 \mu\text{m}$ ), length/width ratio  $1.0\text{--}1.2$  (mean 1.1).

*Culture characteristics:* Optimum growth temperature 25 °C, no growth observed at 35 °C. Colony radius on CMD after 72 h: 60–65 mm at 25 °C, covering the plate at 30 °C. Covering the plate after 4 days at 25 °C. Colony white, aerial mycelium scanty. Conidiation forms in white cottony pustules after 3 days, turning yellow green after 4 days, with indistinct zonation, abundant in the margin. Pleasant fruity odour detected, no diffusing pigment observed. Colony radius on MEA after 72 h: 50–52 mm at 25 °C, 50–51 mm at 30 °C. Covering the plate after 4 days at 25 °C and 30 °C. Colony well-defined, aerial mycelium, abundant, dense, radiating. Abundant dark green conidia form in aerial mycelium and minute pustules, form a disk around inoculum,

nearly covering the plate, a few large yellow-green pustules near inoculum. No distinct odour or diffusing pigment observed. Colony radius on PDA after 72 h: 58–60 mm at 25 °C, covering the plate at 30 °C. Covering the plate after 4 days at 25 °C. Colony hyaline, aerial hyphae scanty and slightly coiling. Conidia form in aerial mycelium and cottony pustules, scant around inoculum, abundant near the margin, and indistinct zonation. Pleasant fruity odour detected, and no diffusing pigment observed. Colony radius on SNA after 72 h: 35–37 mm at 25 °C, 40–41 mm at 30 °C. Covering the plate after 5 days at 25 °C and at 30 °C. Colony hyaline, aerial hyphae radial. Conidia form in large pustules, yellow-green near inoculum, white in distant areas, forming 2–3 concentric rings. No distinct odour or diffusing pigment observed.

*Additional specimens examined:* China, Zhejiang Province, Lishui City, 28°32'46.8"N, 119°10'04.9"E, 312 m alt., isolated from soil of a waxbe orchard. Oct. 2014, Y. Jiang, living culture T31355.

*Notes:* *T. parahongkuii* is characterised by the production of a pleasant fruity odour on both CMD and PDA, with no growth at 35 °C. Phylogenetically, *T. parahongkuii* is closely related to *T. hongkuii* and *T. neohongkuii*. The differences among *T. neohongkuii*, *T. parahongkuii*, and *T. hongkuii* have been previously addressed.

***Trichoderma shaanxiensis* C. L. Zhang, sp. nov.**  
Figure 12

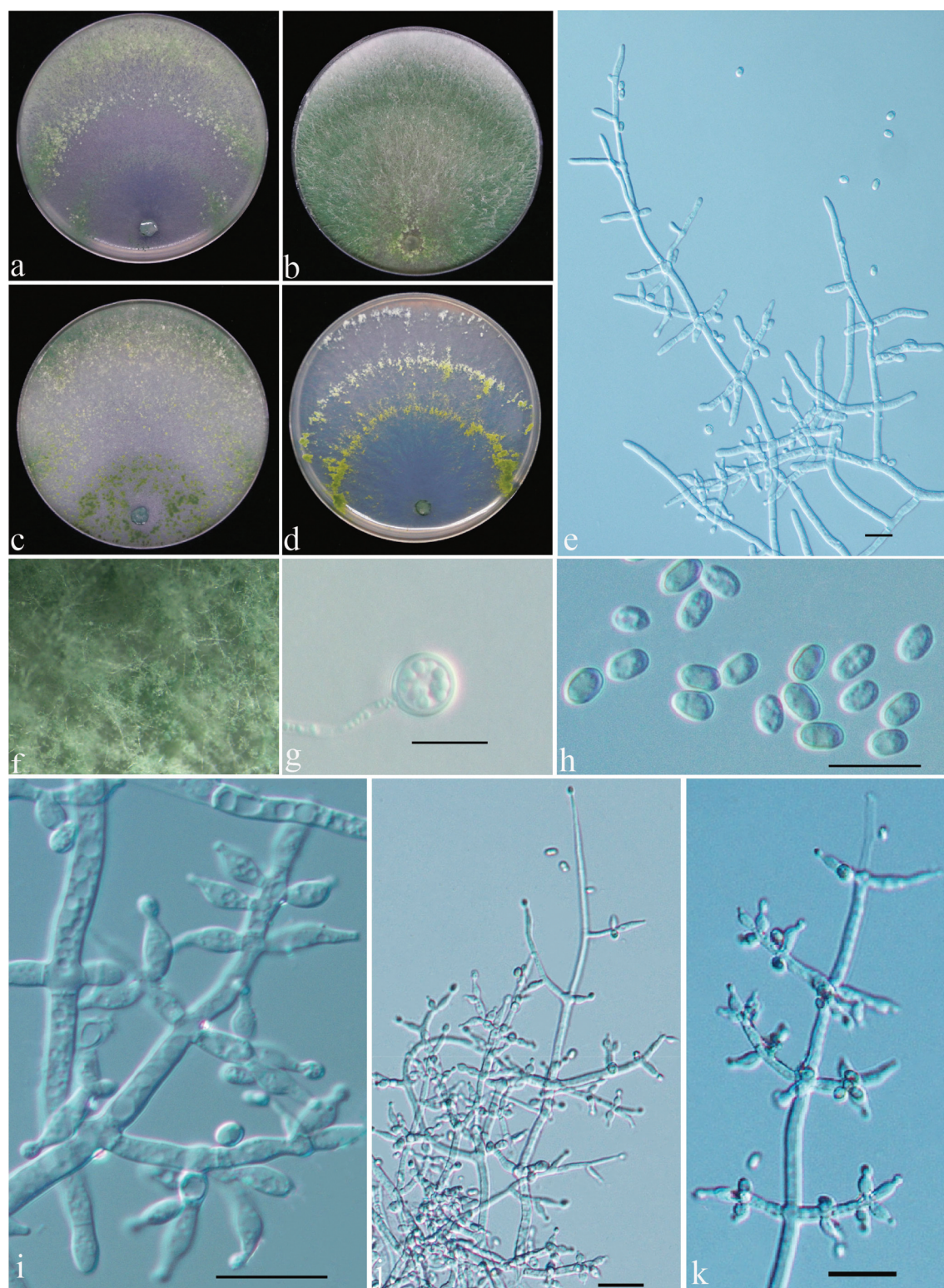
*Fungal Names:* FN 571783.

*Etymology:* Named after its type location, Shaanxi Province.

*Type:* China, Shaanxi Province, Baoji City, 34°23' 54.8"N, 107°10'20.0"E, 827 m alt., isolated from soil of a wheat field. Jun. 2015, J.L. Wang T32000 (Holotype GDMCC 3.1014, stored in a metabolically inactive state. Ex-type culture T32000).

*Description:* Conidiophores pyramidal to tree-type with opposing branches, with a relatively large distance between branches, main axis and branches terminate in a cruciate to slightly verticillate whorl of 2–4 phialides, sometimes with solitary phialides. Phialides ampulliform, sometimes curved,  $5.4\text{--}9.1\text{--}(9.6) \times 2.4\text{--}3.6\text{--}(3.8) \mu\text{m}$  (mean  $7.2 \times 3.0 \mu\text{m}$ ), length/width ratio  $1.6\text{--}3.3\text{--}(3.8)$  (mean 2.4), base  $1.5\text{--}2.5 \mu\text{m}$  (mean  $2.0 \mu\text{m}$ ). Conidia subglobose to globose, green,  $3.0\text{--}3.6 \times 2.9\text{--}3.3 \mu\text{m}$  (mean  $3.3 \times 3.1 \mu\text{m}$ ), length/width ratio  $1.0\text{--}1.2$  (mean 1.1). Chlamydospores commonly terminal, globose to





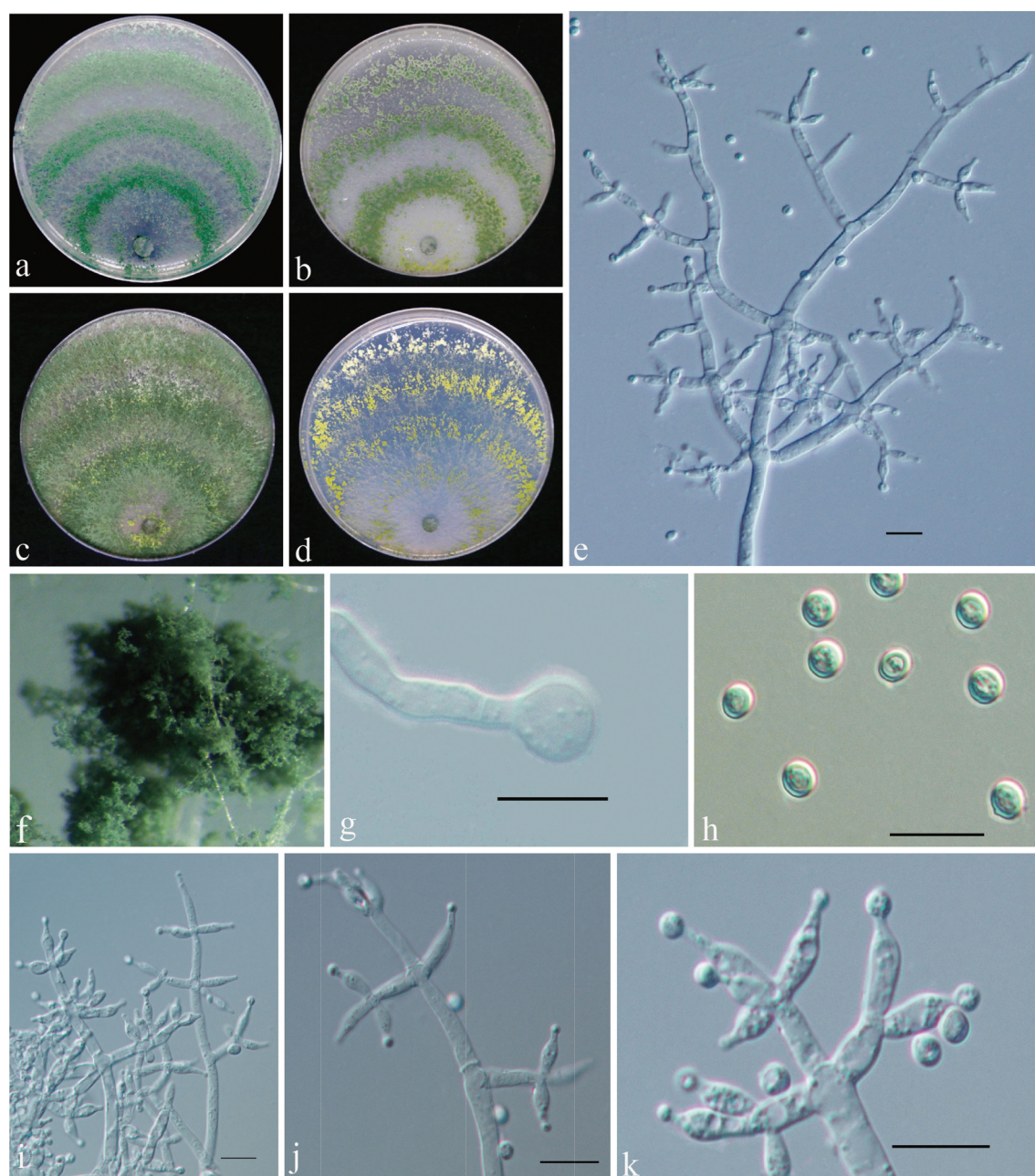
**Figure 11.** Colonies and microscopic characteristics of *Trichoderma parahongkuii* T31356 (GDMCC 3.1019). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Chlamydospore. (h) Conidia. (e, i – k) Conidiophores and phialides. Scale bars: e, g – i, k = 10 µm; j = 20 µm.

subglobose,  $(4.7\text{--}4.8\text{--}8.0\text{--}9.0) \times 4.6\text{--}7.5\text{ }\mu\text{m}$  (mean  $6.4 \times 6.0\text{ }\mu\text{m}$ ), length/width ratio 1.0–1.2 (mean 1.1).

**Culture characteristics:** Optimal growth temperature at 30 °C, slow growth at 35 °C. Colony radius on

CMD after 72 h: 52–63 mm at 25 °C, covering the plate at 30 °C and 17–23 mm at 35 °C. Covering the plate after 4 days at 25 °C. Colony white, radial, fluffy. Light white conidial pustules form after 2 days, aggregated





**Figure 12.** Colonies and microscopic characteristics of *Trichoderma shaanxiensis* T32000 (GDMCC 3.1014). (a – d) Colonies after 7 d at 25 °C on media (a: PDA, b: MEA, c: CMD, d: SNA). (f) Conidiation pustules on PDA. (g) Chlamydospore. (h) Conidia. (e, i – k) Conidiophores and phialides. Scale bars: e, g – k = 10 µm.

at the margin of inoculum, turning green after 2 days, scarcely separated 3 concentric zones, almost covering the plate. No diffusing pigment or distinct odour observed. Colony radius on MEA after 72 h: 53–55 mm at 25 °C, 59–61 mm at 30 °C, and 20–23 mm at 35 °C. Covering the plate after 4 days at 25 °C and 30 °C. Colony white, radial, aerial hyphae dense. Conidiation starts after 2 days, forming in white-green pustules, spreading in 3 broad yellow-green

concentric zones, finally spreading in 3–4 broad concentric rings. No diffusing pigment or distinct odour observed. Colony radius on PDA after 72 h: 46–49 mm at 25 °C, 56–58 mm at 30 °C, and 23–25 mm at 35 °C. Covering the plate after 5 days at 25 °C and 4 days 30 °C. Colony and conidiation similar to that on MEA, but pustules dark green and relatively small, with regular concentric rings. No diffusing pigment or distinct odour observed. Colony radius on SNA after 72 h:

29–31 mm at 25 °C, 43–48 mm at 30 °C, and 9–13 mm at 35 °C. Covering the plate after 6 days at 25 °C and 4 days at 30 °C. Colony white, radial, aerial hyphae rare. Conidiation forms one yellow-green indistinct concentric ring after 3 days, finally forming in 3–4 indistinct concentric rings. No diffusing pigment or distinct odour observed.

*Additional specimens examined:* China, Shaanxi Province, Baoji City, 34°23'54.8"N, 107°10'20.0"E, 827 m alt., isolated from soils of wheat field. Jun. 2015, J.L. Wang, living culture T31999.

*Notes:* Phylogenetic analyses revealed that *T. shaanxiensis* is closely related to *T. azevedoi* and *T. camerunense*, with similar pyramidal conidiophores. However, the conidia of *T. shaanxiensis* ( $3.0\text{--}3.6 \times 2.9\text{--}3.3 \mu\text{m}$ ) are more nearly globose than those of *T. azevedoi* ( $3.54\text{--}4.65 \times 2.55\text{--}3.33 \mu\text{m}$ ). *T. shaanxiensis* also exhibits greater heat resistance compared to *T. azevedoi*, as the latter did not grow at 35 °C on CMD, whereas *T. shaanxiensis* can grow at both 30 °C and 35 °C. Additionally, *T. shaanxiensis* shows faster growth than *T. azevedoi* at 30 °C on PDA, with colony radius of 56–58 mm and 4 mm, respectively (Inglis et al. 2020). Furthermore, *T. camerunense* ( $3.2 \times 5.7 \mu\text{m}$ ) also has shorter phialides than *T. shaanxiensis* ( $5.5 \times 9.1 \mu\text{m}$ ), and *T. camerunense* emits a sweet odour on PDA at 30 °C, a characteristic not observed in *T. shaanxiensis* (Chaverri et al. 2015).

#### 4. Discussion

We examined 27 strains and identified 11 new species within the *Trichoderma* genus, which holds significant importance for understanding the diversity of *Trichoderma* species in China. The 10 new species were classified into the *Harzianum*, *Koningii*, and *Hamatum* clades within the *Trichoderma* genus based on the phylogenetic analysis of combined *rpb2* and *tef1* sequences. However, the new species *T. caeruleum* forms a distinct phylogenetic lineage within the cluster comprised of the *Ceramicum* and *Strictipile* clades. *T. caeruleum* can be easily distinguished by its ability to produce a blue pigment. The occurrence of blue pigments in fungi is exceptionally rare (Chaverri et al. 2003; Santos and Bicas 2021). While *Trichoderma* species typically exhibit colours such as light yellow, yellow, amber, light red, or yellow-green (Brotman et al. 2010), only two

species have been reported to produce blue pigments in *Trichoderma*, namely *T. caerulescens* (= *Hypocrea caerulescens*) on CMD after one month (Jaklitsch et al. 2012) and *T. cyanodichotomus* on CMD and PDA (Li et al. 2018). Given the scarcity of naturally occurring blue pigments *T. caeruleum* presents an intriguing subject for future study.

We identified six new species within the *Harzianum* clade, namely *T. gongcheniae*, *T. graminis*, *T. graminicola*, *T. neoguizhouense*, *T. parapeberdyi*, and *T. shaanxiensis*. The *T. harzianum* species complex, as defined by Rifai (1969), has been further categorised into the *Harzianum* Clade of *Trichoderma* through extensive research. Presently, the *Harzianum* Clade contains the largest number of species within *Trichoderma* (Gams and Meyer 1998; Jaklitsch 2009; Chaverri et al. 2015). As more species join the clade, the relationships among its members have evolved. *T. bannaense*, previously closely related to *T. breve* (Chen and Zhuang 2017), is now associated with the new species *T. graminis*. Similarly, *T. velutinum*, formerly closely related to *T. cerinum* (Bissett et al. 2003), is now associated with the new species *T. graminicola*. Additionally, *T. peberdyi*, previously closely related to *T. ceraceum* (Inglis et al. 2020), is now associated with the new species *T. parapeberdyi*. However, three new species, *T. gongcheniae*, *T. neoguizhouense*, and *T. shaanxiensis*, form a subclade with very low support, the relationships among members of the clade may change in the future with the inclusion of these new species.

Three new species, *T. hongkuii*, *T. neohongkuii*, and *T. parahongkuii*, belong to the *Koningii* clade, which is strongly supported (MLBP/BIPP = 100%/1.00). Their growth was significantly restricted at 35 °C. In the *Koningii* clade, *Trichoderma* species typically exhibited slow growth. For instance, species such as *T. amoenum*, *T. koningiopsis*, *T. ovalisporum*, and *T. koningii* typically reached sizes less than 5 mm at 35 °C (Samuels et al. 2006; Zheng et al. 2021). Additionally, species like *T. arenarium*, *T. caribbaeum*, *T. petersenii*, and *T. istrianum* showed no growth at this temperature (Samuels et al. 2006; Jaklitsch and Voglmayr 2015; Ding et al. 2021). The new species, *T. parahongkuii*, similarly exhibited no growth at 35 °C, while *T. hongkuii* and *T. neohongkuii* displayed particularly slow growth.

The new species *T. parahamatum* was identified as a member of the *Hamatum* clade. Phylogenetic analyses have revealed a close relationship between *T.*



*parahamatum* and *T. hamatum*, with strong bootstrap support (MLBP/BIPP = 99%/1.00). The sterile hamate elongations of *T. parahamatum* closely resembled those of *T. hamatum*, but the two species can be distinguished by differences in odour production and growth rate. Additionally, phylogenetically, *T. parahamatum* is associated with *T. anisohamatum* and *T. insigne*, both of which exhibit no growth at 35 °C (Zheng et al. 2021). However, *T. parahamatum* differs from them due to its pleasant fruity odour and sterile hamate elongations.

In this study, we identified 11 new species, among which four were found as endophytes in Poaceae plants, while the other seven were isolated from soil samples. Poaceae encompasses the primary food crops of humans, making it the most economically important angiosperm family (Huang et al. 2022). The survival and reproduction of Poaceae are positively affected by endophytic fungi (Feng et al. 2021). These four new endophytic *Trichoderma* fungi merit further research. For a long time, *Trichoderma* has been found as soil-inhabiting fungi (Widden 1979; Wuczkowski et al. 2003). An increasing number of soil-inhabiting *Trichoderma* species are being discovered, providing new clues for further exploration of soil microbial diversity. Due to its unique geographical location, Yunnan Province has abundant biodiversity (Myers et al. 2000). Among the 11 new species, five of them were obtained from Yunnan. The discovery of these new species not only enhanced our understanding of microbial diversity in the Yunnan region but also provided important information for the protection of the region's ecosystems.

Among the 11 new species identified in this study, 10 species belong to three clades: *Harzianum*, *Koningii*, and *Hamatum*. These clades encompass economically important species, including *T. afroarizianum*, *T. asperellum*, *T. guizhouense*, *T. hamatum*, *T. harzianum*, *T. koningii*, and *T. koningiopsis*, etc (Zimand et al. 1996; Tsahouridou and Thanassouloupoulos 2002; Tondje et al. 2007; Chaverri et al. 2015; Castrillo et al. 2016; Xu et al. 2020; Han et al. 2023; Lodi et al. 2023). Exploring the potential of these new species as antagonists in plant disease biocontrol and for further research on pathogen eradication is essential.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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