Species Diversity of Trichoderma Associated With Soil In The Zoige Alpine Wetland of Southwest China

Guiting Tang  
Sichuan Agricultural University - Chengdu Campus

Ying Li  
Sichuan Agricultural University - Chengdu Campus

You Zhou  
Chinese Academy of Tropical Agricultural Sciences

Xiaojuan Zheng  
Sichuan Agricultural University - Chengdu Campus

Xiaoli Chang  
Sichuan Agricultural University - Chengdu Campus

Shirong Zhang  
Sichuan Agricultural University - Chengdu Campus

Guoshu Gong  
(524402678@qq.com)  
Sichuan Agricultural University - Chengdu Campus

Original Article

Keywords: Trichoderma spp., Zoige alpine wetland, Morphology, Phylogeny, Soil fungi

Posted Date: September 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-882568/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background:** The Zoige alpine wetland is one of the most important wetlands in China because of its complex natural environment, abundant ecological resources, and unique climatic conditions. The ecology of soil fungi is poorly understood, and recent comprehensive reports on *Trichoderma* are not available for any region, including the Zoige alpine wetland ecological region in China. Our results may be used as a reference for a greater understanding of soil microorganism at various ecological regions, ecological rehabilitation and reconstruction and as microbial resources.

**Results:** One hundred soil samples were collected from different soil types and soil layers in Zoige alpine wetland ecological regions in 2013. Using the traditional suspension plating method, a total of 80 *Trichoderma* strains were isolated. After a preliminary classification of morphological characteristics and the genes glyceraldehyde-3-phosphate dehydrogenase (*gpd*), 57 strains were representatively selected and eventually identified as seven species via phylogenetic analyses of multilocus sequences based on the genes transcription elongation factor 1 alpha (*tef1*), encoding RNA polymerase II subunit B (*rpb2*) and ATP citrate lyase (*acl1*). Among them, *Trichoderma harzianum* was the dominant species and had the highest isolation frequency (23%) in this zone, while *Trichoderma polysporum* and *Trichoderma pyramidale* were rare species, with isolation frequencies of less than 1%.

**Conclusions:** Our detailed morphological observation and molecular phylogenetic analyses support the recognition of *Trichoderma zoigense* was described for the first time as a new species.

**Background**

As an important member of the soil microflora, soil fungi (along with other microorganisms) participate in the material cycle and energy flow in ecosystems. Fungi play an especially vital role in organic decomposition, carbon and nitrogen storage, biogeochemical cycles, soil stabilization, and plant parasitism (Mueller et al. 2004; Gadd 2007; Hollister et al. 2010; James et al. 2012; Tedersoo et al. 2012), and fungal diversity has been recognized as a key indicator of soil health (Wardle et al. 2006; Singh et al. 2007). Research on the soil ecological environment, especially on the diversity of fungi in some important ecological regions, has recently gained much attention. More specifically, the role of soil microorganisms in promoting the regulatory mechanism of plant communities has become increasingly recognized. Thus, the microbial diversity on the surface and subsurface has remained a major theme in recent ecological research (Nguyen et al. 2016). For instance, in China, fungal flora and diversity in soils have been reported in the Changbai Mountains, three nature reserves in Jiuzhaigou County, and Mount Gongga (Yao et al. 2007; Zhou et al. 2014; Tian et al. 2017).

The genus *Trichoderma*, which includes more than 200 species in various geographical regions and climatic zones around the world (Atanasova et al. 2013; Kredics et al. 2014), is the most common fungi in soil and rotting wood (Nelson 1982; Samuels 1996). Some species of *Trichoderma* have a powerful phosphate-solubilizing ability, whereas other species act as industrial enzymes for the preparation of cellulose, hemicellulase, xylanase, chitinase, protease and antibiotics in agricultural production (Reese & Mandels 1984; Ghisalberti 1998; Hjeljord & Tronsmo 1998; Yedidia et al. 2001; Sivasithamparam & Hanada et al. 2008; Woo et al. 2014). In addition, the genus has been confirmed to be associated with the ability to control plant pathogens, promote plant growth, stimulate plant immunity and remediate soil contaminants by various modes (Kamal et al. 2009; Babu et al. 2014; Cai et al. 2015; Andreolli et al. 2016).

*Hypocrea* and *Trichoderma* were once treated as two separate genera, although studies by the Tulasne brothers indicated that *Hypocrea* is a sexual morph (teleomorph) of *Trichoderma* (Tulasne et al. 1865). Combining *Hypocrea* and *Trichoderma*, Doi et al. summarized previously reported species and revised nearly 50 new species of the genus based primarily on morphological characteristics (Doi 1966; 1968; 1969; 1971; 1972; 1975; 1976; 1978; 1982; 1987; 2001; 2006; Doi et al. 2001). However, distinguishing *Trichoderma* species using traditional morphological methods is both difficult and inaccurate (Blaszczyk et al. 2011). Multiple molecular techniques have been applied for identifying *Trichoderma*; for example the genes encoding RNA polymerase II subunit B (*rpb2*), transcription elongation factor 1 alpha (*tef1*) and ATP citrate lyase (*acl1*) have commonly been used either individually or in combination (Chaverri et al. 2003a, b; Samuels et al. 2006; Jaklitsch et al. 2006a, b, 2008; Jaklitsch 2009; Jaklitsch & Voglmayr, 2015). A combination of phylogenetic analyses of multiple genes and morphological characteristics has been widely used to study fungal diversity, and nearly a hundred new species of this genus have been recorded in various ecological zones worldwide (Chaverri et al. 2001, 2003a; Chaverri & Sarmuuels 2003; Samuels 2006; Hoyo-Carvajal et al. 2009; Jaklitsch 2009, 2011; Sun et al. 2012; Li et al. 2013; Jaklitsch & Voglmayr 2015; Zhang et al. 2015; Zhu & Zhuang 2015).

Wetlands represent a significant land resource and a source of natural resources with a variety of functions, such as forest, cultivated land and sea. These areas are rich in biological diversity in terms of both the ecological landscape and the human living environment. The Zoige alpine wetland is one of the most important wetlands in China because of its complex natural environment, abundant ecological resources, and unique climatic conditions. Although reports have addressed the local soil active organic carbon, vegetation, animal community, gas flux, functional bacteria and microorganism methanogens (Gao et al. 2008; Zhang et al. 2008; Chen et al. 2008, 2009; Dai et al. 2016; Ma et al. 2016a, b; Yuan et al. 2016), the ecology of soil fungi is poorly understood, and recent comprehensive reports on *Trichoderma* are not available.
for any region, including the Zoige alpine wetland ecological region in China. In fact, only Feng et al. (2009) has analyzed the fungal community structure in the soil of this region via a combination of BIOLOG analysis and traditional culture methods. Because morphological and molecular tools are ideal for assessments of the species diversity in all geographical regions, the work described here was designed to investigate the species diversity of the genus Trichoderma in the uniquely ecological environment of the Zoige alpine wetland, with an emphasis on four major soil types (peat soil, meadow soil, subalpine meadow soil and aeolian sandy soil). Our results may be used as a reference for a greater understanding of soil microorganism at various ecological regions, ecological rehabilitation and reconstruction and as microbial resources.

Methods

Study region

The Zoige alpine wetland (32°10′~34°10′N, 101°45′~103°55′E) is located in the northwest part of Sichuan Province in China and the eastern edge of the Qinghai-Tibet Plateau and has an average altitude of 3400 m above sea level and an area of 19600 km². It is a relatively pristine natural area with an annual temperature of 0.6–1.0 ℃ and annual precipitation level of 580–860 mm. The cold, humid weather slows the decomposition of the soil organic matter and facilitates its accumulation in the soil (Sun 1998; Ding et al. 2004; Feng 2009). Peat soil, meadow soil, subalpine meadow soil and aeolian sandy soil are extensively developed and the most common soil types in this area, because of its unique ecological conditions.

Isolates and specimens

A total of 100 soil samples were collected in June 2013 across a range of soil types (peat soil, meadow soil, subalpine meadow soil and aeolian sandy soil) and soil layers (depth 0–10, 10–20, 20–30, 30–50, and 50–100 cm) in the Zoige alpine wetland ecological regions. Global positioning system technology (GPS Map 76; Garmin Ltd, USA) was used to determine the sampling locations. After removal of vegetation debris, approximately 300 g of each soil sample was immediately placed in a sterile plastic bag in a cooler, transported to the laboratory within 48 h and then stored at 4 ℃.

Soil fungi were isolated using the suspension plating method (Mueller et al. 2011). Briefly, suspensions (1 mL) of various dilutions (10⁻¹, 10⁻² and 10⁻³) were placed on 90 mm diameter petri plates and Martin medium was then added and mixed evenly with the suspension. The plates were kept in the dark at 25 ℃ for 5 d, and the colonies of fungi were observed and counted. Three replicates were performed for each concentration. According to the colony characteristics, the purified fungal colonies were transferred onto potato dextrose agar (PDA) and kept in tube slants and glycerol for further taxonomic identification. The specimens were deposited in the Fungal Herbarium of Sichuan Agricultural University, with accession numbers of T1–T80. And the holotype of new species and new record species were deposited in China General Microbiological Culture Collection Center (CGMCC), with accession numbers of CGMCC3.20145 and CGMCC3.20167.

Morphology and growth rate

Cultures were prepared and maintained as described previously (Jaklitsch et al. 2005; Jaklitsch 2009). Cultures used for the study of asexual morph micromorphology were grown on PDA, on CMD (cornmeal agar supplemented with 2% (w/v) D (+)-glucose-monohydrate) containing 0.02% (w/v) streptomycin sulfate (Solarbio, China) and 0.02% (w/v) neomycin sulfate (Solarbio), on SNA (low-nutrient agar, Nirenberg 1976) or occasionally on MEA (2% malt extract, 2% agar-agar) at 20 ℃ or 25 ℃ under a 12 h/12 h light/dark cycle with cool white fluorescent light during the light period.

Fungal colony characteristics were observed on the CMD, PDA, MEA and SNA media and grown under 12 h of white light and 12 h of darkness at 20 ℃ and 25 ℃. Colony textures and the presence or absence of exudates were recorded using a stereomicroscope (OLYMPUS SZX16, Japan). Colony morphologies were observed weekly with a digital camera (Nikon D3100, Japan). Micromorphological characteristics were observed after 3–7 d or 14 d of cultivation, and microscopic observations were performed in 3% KOH. Chlamydospores were measured from 7–30-day-old cultures on CMD or SNA plates under a compound microscope using a 100x objective. The following characteristics of each isolate were measured: length and width of conidia (n = 50), length of phialides (n = 50), width of phialides at the base (n = 50), and width of phialides at the widest point (n = 50). Nomarski differential interference contrast (DIC) was used for observations and measurements, and data were gathered using a Carl Zeiss microscope (Axio Imager Z2, Germany). Colors were determined with Methuen's Handbook of Colour (Kornerup & Wanscher 1981).

To identify the optimal growth temperature and differentiate growth rates of the species, 3 representative strains or all strains (≤ 3 in total) for each species were selected to determine the growth rate on CMD at five temperature levels (15 ℃, 20 ℃, 25 ℃, 30 ℃ and 35 ℃) as described previously with minor modifications (Jaklitsch 2009). The strains were pre-grown on PDA for 48 h or 72 h at 25 ℃. For new cultures, 5-mm agar blocks were cut from the margin of the colonies and transferred to fresh medium from the edge of the 9-cm petri dish. The maximum colony radius was measured every day until the plates were entirely covered with mycelium. The growth rate was calculated by linear regression of t
versus \( r \) (\( t \) = time of incubation and \( r \) = radius measured from the edge of the agar plug). Every treatment was repeated twice, with three replicates each time.

**DNA extraction, PCR amplification and sequencing**

Genomic DNA samples of representative isolates of 57 morphotypes, which were chosen according to the morphological and cultural characteristics, were extracted from pure cultures for phylogenetic analyses as described by Barnes et al. (2001). Part of the nuclear rDNA ITS region was amplified by PCR using the primer pair ITS1 5' TCCGTAGGTGAACCTGCGG3 and ITS4 5' TCCTCCGTTATCGATATGC (Tanaka et al. 2009). A 1-kb fragment of RNA polymerase II subunit B (\( rpb2 \)) was amplified using the primer pair fRPB2-5f 5' GAYGAYMGWATCAYTGYGG and fRPB2-7cr 5' CCCATRGCTTGYTTRCCCAT (Liu et al. 1999). A 1.2-kb fragment of translation elongation factor 1 \( \alpha \) (\( tef1 \)) was amplified using the primer pair EF1-728F 5' CATCGAGAAGTTCGAGAAGG (Carbone & Kohn 1999) and TEF1LLErev 5' AACTTGAGGCAATGTGG (Jaklitsch et al. 2005). A 0.9-kb fragment of the larger subunit of ATP citrate lyase (\( acl1 \)) was amplified using the primers acl1-230up 5' AGCCGTCAACGACCCTTCATTGA and acl1-1220low 5' CCTGGCGAAGCATCAGAAGATGG (Gräfenhan et al. 2011). A 0.4-kb fragment of a partial sequence of the glyceraldehyde-3-phosphate dehydrogenase (\( gpd \)) gene region was amplified using the primers GDF1 5' GCCGTCAACGACCCTTCATTGA and GDR1 5' GGGTGGAGTCTGACTTGAGCATGT (Templeton et al. 1992; Vieira et al. 2014). The PCR mixtures (30 \( \mu \)L) contained 1 \( \mu \)L of genomic DNA (approximately 100 ng), 1 \( \mu \)L of each primer (10 mM), 12 \( \mu \)L of sterile deionized water, and 15 \( \mu \)L of 2× PCR MasterMix (TIANGEN Co., China). Amplifications were performed in an Eppendorf PCR amplifier (Mastercycler nexus X2, Germany). PCR products were sequenced with an ABI 3730xl DNA Analyzer by Sangon Biotech (Shanghai, China).

**Phylogenetic analyses**

For approximate identification, all sequences of the 57 strains listed in Table 2 were compared with the NCBI sequence database using the BLAST algorithm. The two markers (ITS and \( gpd \)) sequenced in the present study were analyzed separately. Their closest matches were aligned by ClustalX (Thompson et al. 1997), and a distance tree was built with the neighbor-joining (NJ) algorithm in MEGA v. 6.0 with 1000 bootstrap replicates (Tanaka et al. 2009; Tamura et al. 2011). Combined \( rpb2 \), \( tef1 \) and \( acl1 \) gene sequences were analyzed based on a multilocus dataset. A phylogenetic analysis was performed for the sequences of a total of 101 strains obtained from the present study or other references in previous studies and complemented with GenBank sequences (Jaklitsch 2009; Jaklitsch & Voglmayr 2015) (Table 2).
Table 2
Trichoderma strain included in the multi-gene sequence analysis, with details of clade, strain number, location, and GenBank accessions of the sequences generated

| Species          | Clade                  | Strain             | GenBank accession number | Location | ITS    | TEF    | RPB2  | ACL1  | GPD  |
|------------------|------------------------|--------------------|--------------------------|----------|--------|--------|--------|--------|------|
| *Trichoderma*    |                        |                    |                          |          |        |        |        |        |      |
| *aggressivum*    | Green/harzianum        | CBS 100525         | AF534614 AF545541        |          |        |        |        |        |      |
| *T. alni*        | Green/harzianum        | Hypo 254 = CBS 120633 | EU518651 EU498312 EU498349 KJ664942 |          |        |        |        |        |      |
|                  |                        | C.P.K. 2494        | EU498313 EU498350        |          |        |        |        |        |      |
|                  |                        | C.P.K. 2854        | EU498314 EU498351        |          |        |        |        |        |      |
|                  |                        | C.P.K. 2858        | EU498315                |          |        |        |        |        |      |
|                  |                        | **T16**             | KX632517 KX632574 KX632631 KX632688 KX632745 | China    |        |        |        |        |      |
|                  |                        | **T24**             | KX632518 KX632575 KX632632 KX632689 KX632746 | China    |        |        |        |        |      |
|                  |                        | **T28**             | KX632519 KX632576 KX632633 KX632690 KX632747 | China    |        |        |        |        |      |
|                  |                        | **T36**             | KX632520 KX632577 KX632634 KX632691 KX632748 | China    |        |        |        |        |      |
|                  |                        | **T40**             | KX632521 KX632578 KX632635 KX632692 KX632749 | China    |        |        |        |        |      |
|                  |                        | **T41**             | KX632522 KX632579 KX632636 KX632693 KX632750 | China    |        |        |        |        |      |
|                  |                        | **T53**             | KX632523 KX632580 KX632637 KX632694 KX632751 | China    |        |        |        |        |      |
|                  |                        | **T54**             | KX632524 KX632581 KX632638 KX632695 KX632752 | China    |        |        |        |        |      |
| *T. amazonicum*  | Green/harzianum        | IB 95               | HM142377 HM142368        | Peru     |        |        |        |        |      |
| *T. atrobrunneum*| Green/harzianum        | G.J.S. 90– 254      | AF443943 FJ442735 KJ664942 | Germany  |        |        |        |        |      |
|                  |                        | Hypo 25             | KJ665359                | Austria  |        |        |        |        |      |
|                  |                        | S343                | KJ665383                | Spain    |        |        |        |        |      |
|                  |                        | S447                | KJ665396                | Spain    |        |        |        |        |      |
|                  |                        | Hypo 4              | KJ665365                | Germany  |        |        |        |        |      |
|                  |                        | Hypo 182            | KJ665357 KJ664948       | Germany  |        |        |        |        |      |
|                  |                        | **T39**             | KX632514 KX632571 KX632628 KX632685 KX632742 | China    |        |        |        |        |      |
| *T. brunneoviride*| Green/harzianum        | CBS 120928          | EU518661 EU498318 EU498358 | Austria  |        |        |        |        |      |
|                  |                        | CBS 121130          | EU498316                | Germany  |        |        |        |        |      |
| *T. catoptron*   | Green/harzianum        | G.J.S. 02– 76       | AY737766 AY391963 AY391900 | Sri Lanka|        |        |        |        |      |
| *T. ceraceum*    | Green/harzianum        | G.J.S. 88 – 26      | AY391964 AY391901        | USA      |        |        |        |        |      |
| *T. cerinum*     | Green/harzianum        | CBS 136992 = S357   | KF134797 KF134788 KJ664977 | France   |        |        |        |        |      |

The strains from this study are indicated in bold letters. (T = ex-type). T42, T44, T48 were deposited in China General Microbiological Culture Collection Center (CGMCC), and the rest in the Fungal Herbarium of Sichuan Agricultural University.
| Species          | Clade                | Strain          | GenBank accession number |
|------------------|----------------------|-----------------|--------------------------|
|                  |                      |                 | Location | ITS         | TEF         | RPB2      | ACL1      | GPD |
| *T. cinnamomeum* | Green/harzianum     | G.J.S. 97–230 = CBS 114235 (T) | USA      | –          | –          | AY391918  | KJ664965  | –  |
|                  |                      |                 | G.J.S. 97–237         | USA      | AY737759   | AY391979  | AY391920  | –  |
| *T. citrinoviride* | Longibrachiatum   | CBS 121275 = Hypo 162 | Germany | –          | –          | FJ860586  | KJ64978   | –  |
|                  |                      |                 | C.P.K. 2005          | Austria  | –          | FJ860694  | –          | –  |
| *T. compactum*   | Green/harzianum     | CBS 121218 (T)  | China                | –          | KF134798   | KF134789  | KJ664984  | –  |
| *T. comeum*      | Green/harzianum     | G.J.S. 97–82    | Thailand             | –          | KJ665455   | KJ665252  | KJ664985  | –  |
| *T. dacrymycellum* | Green/harzianum | Hypo 233 = WU 29044 | Germany  | FJ860749   | FJ860633   | FJ860533  | KJ664993  | –  |
| *T. epimyces*    | Green/harzianum     | C.P.K. 1980     | Germany              | EU518662  | EU498319   | EU498359  | KJ664993  | –  |
| *T. guizhouense* | Green/harzianum     | HGUP0039        | China                | –          | JX089585   | –          | –          | –  |
| *T. harzianum*   | Green/harzianum     | CBS 226.95 (T neo) | UK: England | AY605713   | AF534621   | AF545549  | –          | –  |
| T1               | China                | KX632476        | KX632533             | KX632590  | KX632647   | KX632704  |            |    |
| T2               | China                | KX632477        | KX632534             | KX632591  | KX632648   | KX632705  |            |    |
| T3               | China                | KX632478        | KX632535             | KX632592  | KX632649   | KX632706  |            |    |
| T4               | China                | KX632479        | KX632536             | KX632593  | KX632650   | KX632707  |            |    |
| T5               | China                | KX632480        | KX632537             | KX632594  | KX632651   | KX632708  |            |    |
| T6               | China                | KX632481        | KX632538             | KX632595  | KX632652   | KX632709  |            |    |
| T7               | China                | KX632482        | KX632539             | KX632596  | KX632653   | KX632710  |            |    |
| T8               | China                | KX632483        | KX632540             | KX632597  | KX632654   | KX632711  |            |    |
| T9               | China                | KX632484        | KX632541             | KX632598  | KX632655   | KX632712  |            |    |
| T10              | China                | KX632485        | KX632542             | KX632599  | KX632656   | KX632713  |            |    |
| T11              | China                | KX632486        | KX632543             | KX632600  | KX632657   | KX632714  |            |    |
| T12              | China                | KX632487        | KX632544             | KX632601  | KX632658   | KX632715  |            |    |
| T13              | China                | KX632488        | KX632545             | KX632602  | KX632659   | KX632716  |            |    |
| T14              | China                | KX632489        | KX632546             | KX632603  | KX632660   | KX632717  |            |    |
| T15              | China                | KX632490        | KX632547             | KX632604  | KX632661   | KX632718  |            |    |
| T17              | China                | KX632491        | KX632548             | KX632605  | KX632662   | KX632719  |            |    |
| T18              | China                | KX632492        | KX632549             | KX632606  | KX632663   | KX632720  |            |    |
| T19              | China                | KX632493        | KX632550             | KX632607  | KX632664   | KX632721  |            |    |
| T21              | China                | KX632494        | KX632551             | KX632608  | KX632665   | KX632722  |            |    |
| T22              | China                | KX632495        | KX632552             | KX632609  | KX632666   | KX632723  |            |    |
| T23              | China                | KX632496        | KX632553             | KX632610  | KX632667   | KX632724  |            |    |
| T26              | China                | KX632497        | KX632554             | KX632611  | KX632668   | KX632725  |            |    |

The strains from this study are indicated in bold letters. (T = ex-type). T42, T44, T48 were deposited in China General Microbiological Culture Collection Center (CGMCC), and the rest in the Fungal Herbarium of Sichuan Agricultural University.
| Species               | Clade                  | Strain      | GenBank accession number | Location | ITS       | TEF       | RPB2       | ACL1       | GPD       |
|-----------------------|------------------------|-------------|--------------------------|----------|-----------|-----------|------------|------------|-----------|
| T. hausknechtii       | Green/harzianum        | Hypo 649 = CBS 133493 (T) | France       | –         | KJ665515  | KJ665276  | KJ665034   |            |           |
| T. helicolixii        | Green/harzianum        | S640 = CBS 133499 (T) | Greece       | –         | KJ665517  | KJ665278  | KJ665036   |            |           |
| T. inhamatum          | Green/harzianum        | CBS 273.78 (T) | Colombia     | –         | AF348099  | FJ442725  |            |            |           |
| T. italicum           | Green/harzianum        | S131 = CBS 132567 (T) | Italy        | –         | KJ665525  | KJ665282  | KJ665045   |            |           |
| T. longibrachiatum    | Longibrachiatum        | CBS 816.68  | USA                      | –         | EU401591  | DQ087242  |            |            |           |
|                       |                        | S328        | Spain                    | –         | JQ685867  | JQ685883  |            |            |           |
| T. parepimyces        | Green/harzianum        | CBS 122769 (T) | Austria     | –         | FJ860664  | FJ860562  | KJ665138   |            |           |
| T. pleuroti           | Green/harzianum        | CBS 124387 (T) | Korea       | –         | HM142382  | HM142372  | –          |            |           |
| T. pleuroticola       | Green/harzianum        | CBS 124383 (T) | Korea       | –         | HM142381  | HM142371  | –          |            |           |
| T. polysporum         | Polysporum             | Hypo 422 = C.P.K. 2461 | Austria     | –         | –         | FJ179613  | KJ665057   | –          |           |
|                       |                        | Hypo 522 = C.P.K. 3131 | Austria     | –         | –         | FJ860661  | JQ685878   | KJ665138   | –          |
| T. priscilae          | Green/harzianum        | S168 = CBS 131487 (T) | Spain       | –         | KJ665691  | KJ665333  | KJ665151   |            |           |
| T. pseudogelatinsum   | Green/harzianum        | CNU N309    | Korea        | –         | HM920202  | HM920173  |            |            |           |

The strains from this study are indicated in bold letters. (T = ex-type). T42, T44, T48 were deposited in China General Microbiological Culture Collection Center (CGMCC), and the rest in the Fungal Herbarium of Sichuan Agricultural University.
| Species              | Clade                        | Strain                  | GenBank accession number |
|----------------------|------------------------------|-------------------------|-------------------------|
|                      |                              |                         | Location | ITS    | TEF    | RPB2   | ACL1   | GPD   |
| *T. pyramidal*       | Green/harzianum              | S73 = CBS 135574 (T)    | Italy     | –      | KJ665699 | KJ665334 | KJ665116 | –     |
|                      |                              | S573                    | Italy     | –      | KJ665698 | –        | –        | –     |
|                      |                              | S533                    | Spain     | –      | KJ665697 | –        | KJ665162 | –     |
|                      |                              | T20                     | China     | KX632513 | KX632570 | KX632627 | KX632684 | KX632741 |
| *T. reesei*          | Longibrachiatum              | QM 6a = CBS 383.78 (T)  | New Guinea | –      | –        | HM182969 | KJ665163 | –     |
| *T. rossicum*        | Stromaticum                  | DAOM 230011 (T)         | Russia    | –      | AY937441 | HQ342288 | –        | –     |
|                      |                              | T27                     | China     | KX632526 | KX632583 | KX632640 | KX632697 | KX632754 |
|                      |                              | T51                     | China     | KX632527 | KX632584 | KX632641 | KX632698 | KX632755 |
|                      |                              | T52                     | China     | KX632528 | KX632585 | KX632642 | KX632699 | KX632756 |
| *T. saturnisporopsis*| Longibrachiatum              | TR 175 = C.P.K. 1356 (T)| USA       | –      | –        | DQ857348 | –        | –     |
| *T. saturnisporum*   | Longibrachiatum              | ATCC 18903 = CBS 330.70 | USA       | –      | EU280044 | DQ087243 | –        | –     |
| *T. simmonsii*       | Green/harzianum              | Hypo 15 = C.P.K. 1596   | Austria   | –      | KJ665706 | –        | –        | –     |
|                      |                              | Hypo 30 = C.P.K. 2391   | Austria   | –      | KJ665707 | –        | KJ665182 | –     |
|                      |                              | S7                      | Italy     | –      | KJ665719 | KJ665337 | KJ665182 | –     |
| *T. stramineum*      | Green/harzianum              | G.J.S.02–84 = CBS 114248 | Sri Lanka | AY737765 | AY391999 | AY391945 | –        | –     |
| *T. stromaticum*     | Stromaticum                  | P.C. 209                | Brazil    | –      | AF534613 | AF545539 | KJ665185 | –     |
| *T. tawa*            | Green/harzianum              | G.J.S. 97–174           | Thailand  | AY737756 | AY392004 | AY391956 | –        | –     |
| *T. tomentosum*      | Green/harzianum              | CBS 120637              | Austria   | FJ860744 | FJ860629 | FJ860532 | KJ665222 | –     |
|                      |                              | DAOM 178713a (T)        | Canada    | –      | AF534630 | AF545557 | –        | –     |
| *T. zoigense*        | Longibrachiatum              | T25                     | China     | KX632529 | KX632586 | KX632643 | KX632700 | KX632757 |
|                      |                              | T43                     | China     | KX632530 | KX632587 | KX632644 | KX632701 | KX632758 |
|                      |                              | T44 (CGMCC 3.20145)     | China     | KX632531 | KX632588 | KX632645 | KX632702 | KX632759 |
|                      |                              | T48 (CGMCC 3.20146)     | China     | KX632532 | KX632589 | KX632646 | KX632703 | KX632760 |
| *Protocrea farinosa* | Outgroup                     | CBS 121551              | Austria   | –      | –        | EU703935 | –        | –     |
|                      |                              | C.P.K. 2472             | Austria   | –      | –        | EU703892 | –        | –     |
| *P. pallida*         | Outgroup                     | CBS 121552              | Denmark   | –      | –        | EU703944 | –        | –     |
|                      |                              | CBS 299.78 (T)          | USA       | –      | –        | EU703900 | –        | –     |

The strains from this study are indicated in bold letters. (T = ex-type). T42, T44, T48 were deposited in China General Microbiological Culture Collection Center (CGMCC), and the rest in the Fungal Herbarium of Sichuan Agricultural University.
Maximum parsimony (MP) analyses of the combined DNA matrix were performed with PAUP* v. 4.0 b10 (Swofford 2002) using 1000 replicates of a heuristic search with the random addition of sequences. All molecular characteristics were unordered and given equal weight, and all gaps were treated as missing data. The stability of clades was evaluated by bootstrap analysis with 1000 replicates. Descriptive tree statistics for parsimony (tree length [TL], consistency index [CI], retention index [RI], related consistency [RC] and homoplasy index [HI]) were calculated.

**Relationship with ecological factors**

The isolation frequency was calculated at the species level using the following formula:

\[
F = \frac{n}{N} \times 100\%
\]

where \(F\) = the isolation frequency (%), \(n\) = the number of species isolated from soil samples, and \(N\) = the number of total soil samples. The relationships between the isolation frequency and soil types and soil layers were subsequently analyzed.

**Results**

*Trichoderma species collection*

A total of 80 strains were obtained from 100 soil samples collected from Zoige alpine wetland ecological regions in China. Details of the strains isolated from soil samples are given in Table 1. All strains were subsequently used for morphological identification, while fifty-seven were used for phylogenetic analysis.
| Isolates | Geographical location | Altitude (m a.s.l.) | Soil types | Soil layers (m) | Species |
|----------|-----------------------|--------------------|------------|----------------|---------|
| T1       | 102°29'05.8", 33°43'17.7" | 3448               | Aeolian sand soil | 0–10 | *Trichoderma harzianum* |
| T2       | 102°56'26.3", 33°36'13.4" | 3446               | Peat soil | 0–10 | *T. harzianum* |
| T3       | 102°42'52.1", 33°31'18.5" | 3461               | Subalpine meadow soil | 10–20 | *T. harzianum* |
| T4       | 102°29'05.8", 33°43'17.7" | 3448               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T5       | 102°29'05.8", 33°43'17.7" | 3448               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T6       | 102°29'05.8", 33°43'17.7" | 3448               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T7       | 102°56'26.3", 33°36'13.4" | 3446               | Peat soil | 0–10 | *T. harzianum* |
| T8       | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. harzianum* |
| T9       | 102°29'04.6", 33°43'17.6" | 3450               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T10      | 102°55'20.3", 33°37'07.8" | 3443               | Peat soil | 0–10 | *T. harzianum* |
| T11      | 102°32'25.4", 33°45'55.8" | 3488               | Peat soil | 0–10 | *T. harzianum* |
| T12      | 102°29'04.6", 33°43'17.6" | 3450               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T13      | 102°49'44.4", 33°38'01.1" | 3446               | Meadow soil | 0–10 | *T. harzianum* |
| T14      | 102°56'37.3", 33°35'12.8" | 3435               | Peat soil | 0–10 | *T. harzianum* |
| T15      | 102°42'52.1", 33°31'18.5" | 3461               | Subalpine meadow soil | 20–30 | *T. harzianum* |
| T16      | 102°29'57.5", 33°23'56.1" | 3452               | Meadow soil | 0–10 | *T. alni* |
| T17      | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. harzianum* |
| T18      | 102°49'44.4", 33°38'01.1" | 3446               | Meadow soil | 0–10 | *T. harzianum* |
| T19      | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. harzianum* |
| T20      | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. pyramidale* |
| T21      | 102°56'26.3", 33°36'13.4" | 3446               | Peat soil | 0–10 | *T. harzianum* |
| T22      | 102°56'37.3", 33°35'12.8" | 3435               | Peat soil | 0–10 | *T. harzianum* |
| T23      | 102°49'44.4", 33°38'01.1" | 3446               | Meadow soil | 10–20 | *T. harzianum* |
| T24      | 102°37'27.9", 33°50'31.1" | 3433               | Subalpine meadow soil | 0–10 | *T. alni* |
| T25      | 102°29'57.5", 33°23'56.1" | 3452               | Meadow soil | 0–10 | *T. zoigense* |
| T26      | 102°32'25.4", 33°45'55.8" | 3488               | Peat soil | 0–10 | *T. harzianum* |
| T27      | 102°49'44.4", 33°38'01.1" | 3446               | Meadow soil | 0–10 | *T. rossicum* |
| T28      | 102°29'57.5", 33°23'56.1" | 3452               | Meadow soil | 0–10 | *T. alni* |
| T29      | 102°42'52.1", 33°31'18.5" | 3461               | Subalpine meadow soil | 0–10 | *T. harzianum* |
| T30      | 102°42'52.1", 33°31'18.5" | 3461               | Subalpine meadow soil | 0–10 | *T. harzianum* |
| T31      | 102°42'52.1", 33°31'18.5" | 3461               | Subalpine meadow soil | 0–10 | *T. harzianum* |
| T32      | 102°29'04.6", 33°43'17.6" | 3450               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T33      | 102°29'05.8", 33°43'17.7" | 3448               | Aeolian sand soil | 0–10 | *T. harzianum* |
| T34      | 102°32'25.4", 33°45'55.8" | 3488               | Peat soil | 0–10 | *T. harzianum* |
| T35      | 102°51'22.1", 33°32'24.6" | 3488               | Peat soil | 0–10 | *T. harzianum* |
| T36      | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. alni* |
| T37      | 102°55'18.5", 33°35'36.0" | 3462               | Peat soil | 0–10 | *T. harzianum* |
| Isolates | Geographical location            | Altitude (m a.s.l.) | Soil types              | Soil layers (m) | Species          |
|----------|---------------------------------|---------------------|-------------------------|----------------|-----------------|
| T38      | 102°56′26.3″, 33°36′13.4″        | 3446                | Peat soil               | 0–10           | T. harzianum    |
| T39      | 102°37′03.3″, 33°57′33.3″        | 3437                | Peat soil               | 0–10           | T. atrobrunneum |
| T40      | 102°56′57.9″, 33°36′29.8″        | 3493                | Subalpine meadow soil   | 0–10           | T. alni         |
| T41      | 102°29′09.9″, 33°26′47.9″        | 3452                | Subalpine meadow soil   | 0–10           | T. alni         |
| T42      | 102°29′26.9″, 33°43′14.3″        | 3462                | Aeolian sand soil       | 0–10           | T. atrobrunneum |
| T43      | 102°29′57.5″, 33°36′29.8″        | 3452                | Meadow soil             | 0–10           | T. zoigense     |
| T44      | 102°29′57.5″, 33°26′47.9″        | 3452                | Meadow soil             | 20–30          | T. zoigense     |
| T45      | 102°49′44.4″, 33°38′01.1″        | 3446                | Meadow soil             | 20–30          | T. harzianum    |
| T46      | 102°42′52.1″, 33°31′18.5″        | 3461                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T47      | 102°42′52.1″, 33°31′18.5″        | 3461                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T48      | 102°52′33.1″, 33°33′55.9″        | 3501                | Subalpine meadow soil   | 0–10           | T. zoigense     |
| T49      | 102°52′33.1″, 33°33′55.9″        | 3501                | Subalpine meadow soil   | 10–20          | T. harzianum    |
| T50      | 102°32′25.4″, 33°45′55.8″        | 3488                | Peat soil               | 50–100         | T. polysporum   |
| T51      | 102°33′21.5″, 33°54′57.6″        | 3426                | Subalpine meadow soil   | 30–50          | T. rossicum     |
| T52      | 102°33′21.5″, 33°54′57.6″        | 3426                | Subalpine meadow soil   | 30–50          | T. rossicum     |
| T53      | 102°55′18.5″, 33°35′36.0″        | 3462                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T54      | 102°29′09.7″, 33°28′02.6″        | 3480                | Subalpine meadow soil   | 0–10           | T. alni         |
| T55      | 102°55′18.5″, 33°35′36.0″        | 3462                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T56      | 102°42′52.1″, 33°31′18.5″        | 3461                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T57      | 102°37′03.3″, 33°57′33.3″        | 3437                | Subalpine meadow soil   | 10–20          | T. rossicum     |
| T58      | 102°29′57.5″, 33°23′56.1″        | 3452                | Subalpine meadow soil   | 0–10           | T. zoigense     |
| T59      | 102°56′37.3″, 33°35′12.8″        | 3435                | Meadow soil             | 0–10           | T. harzianum    |
| T60      | 102°29′57.5″, 33°23′56.1″        | 3452                | Subalpine meadow soil   | 0–10           | T. zoigense     |
| T61      | 102°29′09.9″, 33°26′47.9″        | 3452                | Subalpine meadow soil   | 0–10           | T. alni         |
| T62      | 102°56′57.9″, 33°36′29.8″        | 3493                | Subalpine meadow soil   | 0–10           | T. alni         |
| T63      | 102°37′03.3″, 33°57′33.3″        | 3437                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T64      | 102°49′44.4″, 33°38′01.1″        | 3446                | Subalpine meadow soil   | 0–10           | T. rossicum     |
| T65      | 102°52′33.1″, 33°33′55.9″        | 3501                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T66      | 102°29′57.5″, 33°23′56.1″        | 3452                | Subalpine meadow soil   | 0–10           | T. zoigense     |
| T67      | 102°56′57.9″, 33°36′29.8″        | 3493                | Subalpine meadow soil   | 0–10           | T. alni         |
| T68      | 102°36′51.0″, 33°26′10.7″        | 3531                | Subalpine meadow soil   | 0–10           | T. alni         |
| T69      | 102°37′12.6″, 33°35′10.2″        | 3434                | Subalpine meadow soil   | 0–10           | T. alni         |
| T70      | 102°56′57.9″, 33°36′29.8″        | 3437                | Subalpine meadow soil   | 0–10           | T. alni         |
| T71      | 102°29′57.5″, 33°23′56.1″        | 3452                | Subalpine meadow soil   | 0–10           | T. zoigense     |
| T72      | 102°56′57.9″, 33°23′56.1″        | 3452                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T73      | 102°29′05.8″, 33°43′17.7″        | 3448                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T74      | 102°29′05.8″, 33°43′17.7″        | 3448                | Subalpine meadow soil   | 0–10           | T. harzianum    |
| T75      | 102°37′12.6″, 33°51′02.1″        | 3426                | Subalpine meadow soil   | 30–50          | T. harzianum    |
### Phylogenetic analysis

The ITS region used preliminarily as a species identification criterion was applied to TrichOKey at [www.ISTH.info](http://www.ISTH.info) (Druzhinina et al. 2005). However, the ITS region has a low number of variable sites and long insertions in certain species, thus, it is not suitable for a phylogenetic reconstruction of this group (Samuels et al. 2006). In our study, most fragments of the genes tef1, rpb2 and acl1 were successfully amplified. We also designed a pair of new primers based on the full-length tef1 gene, 5'-GAGAAGTTCGAGAAGGTGAGC-3' and 5'-ATGTCACGGACGGCGGAAAC-3', with which a 1.4-kb fragment was amplified for most isolates.

All samples analyzed in our study were divided into 4 primary clades based on the gpd gene region, including 49 strains from the *Trichoderma harzianum* complex, 3 *Trichoderma rossicum* strains, 1 *Trichoderma polysporum* strain and one unknown species (4 *Trichoderma* sp. strains) (Fig. 1). Maximum parsimony analysis was conducted among 101 strains, with *Protocrea farinosa* (CPK 2472) and *P. pallida* (CBS 299.78) used as outgroup (Table 2). The dataset for the rpb2, tef1 and acl1 genes contained 3403 characteristics, among which 1152 were parsimony-informative, 988 were variable and parsimony-uninformative, and 1263 were constant. The most parsimonious trees are shown in Fig. 2 (tree length = 5054, consistency index = 0.6005, homoplasy index = 0.3995, retention index = 0.8105, rescaled consistency index = 0.4867).

The phylogram showed that 57 stains belonged to the following four clades: Harzianum, Polysporum, Stromaticum and Longibrachiatum. The strains of the first three clades with neighboring named species were well supported by bootstrap values greater than 90%. The Harzianum clade contained *Trichoderma alni*, *Trichoderma atrobrunneum*, *T. harzianum* and *Trichoderma pyramidae* of the *Trichoderma* species complex. The Polysporum clade contained only *T. polysporum*, and the Stromaticum clade contained *T. rossicum*. The Longibrachiatum clade contained four strains of *Trichoderma* sp., T25, T43, T44 and T48, which were clearly separated from any other known taxa of this clade and showed a low bootstrap value (MPBP = 62%) with *T. citrinoviride* and *T. saturnisporum*. We thus regarded it as a new species and named it *Trichoderma zoigense* as described in the next section.

### Growth rates

As shown in Fig. 3, the genus *Trichoderma* from Zoige alpine wetland ecological regions was able to grow in a range from 15–35 °C, and the suitable growth temperature for most species ranged from 20 °C to 30 °C. All seven species identified had normal viability at relatively low temperature (15 °C), and they rarely grew well over 35 °C except for *T. zoigense*. For *T. atrobrunneum*, *T. harzianum* and *T. pyramidae*, the optimum growth temperature on CMD was 25–30 °C. *Trichoderma alni* and *T. rossicum* preferred a cool growth environment, with an optimum temperature of 25 °C, whereas *T. zoigense* was more partial to a hot environment, with an optimum temperature of 30 °C, and it even grew well up to 35 °C. *T. polysporum* was the only slow-growing species that grew with less than 6.0 mm/d between 15–30 °C and did not survive at 35 °C. The above results showed that all species had different growth rates but were not completely differentiated from each other on CMD. These species were roughly divided into four groups based on their optimum growth temperature.

### Relationship with ecological factors

Our results revealed a substantial disparity in the number and distribution of *Trichoderma* species among Zoige alpine wetland ecological regions (Tables 3, 4). Table 3 showed that *T. harzianum* was found in all four soil types, but most isolates of this species were obtained from peat soil. *T. rossicum*, *T. alni* and *T. zoigense* were also present in meadow soil and subalpine meadow soil, whereas *T. atrobrunneum* was found in aeolian sandy soil and peat soil. *T. polysporum* was found only in peat soil.

### Table 3

| Isolates | Geographical location | Altitude (m a.s.l.) | Soil types | Soil layers (m) | Species |
|----------|-----------------------|--------------------|------------|----------------|---------|
| T76      | 102°49'44.4", 33°38'01.1" | 3446               | Meadow soil | 30–50          | *T. harzianum* |
| T77      | 102°29'57.5", 33°23'56.1" | 3452               | Meadow soil | 50–100         | *T. harzianum* |
| T78      | 102°51'22.1", 33°32'24.6" | 3444               | Peat soil   | 30–50          | *T. harzianum* |
| T79      | 102°32'25.4", 33°45'55.8" | 3488               | Peat soil   | 50–100         | *T. harzianum* |
| T80      | 102°54'15.2", 33°34'72.2" | 3449               | Peat soil   | 0–10           | *T. harzianum* |
| Species       | Meadow soil | Subalpine meadow soil | Aeolian sand soil | Peat soil | total |
|---------------|-------------|-----------------------|------------------|----------|-------|
| *T. harzianum* | 5           | 6                     | 2                | 10       | 23    |
| *T. rossicum* | 1           | 1                     | 0                | 0        | 2     |
| *T. alni*     | 2           | 6                     | 0                | 1        | 9     |
| *T. zoigense* | 2           | 1                     | 0                | 0        | 3     |
| *T. atrobrunneum* | 0     | 0                     | 1                | 1        | 2     |
| *T. polysporum* | 0         | 0                     | 0                | 1        | 1     |
| *T. pyramidale* | 0         | 0                     | 0                | 1        | 1     |

### Table 4: Isolation frequency of *Trichoderma* species in different soil layers (%)

| Species     | depth (cm) | 0–10 | 10–20 | 20–30 | 30–50 | 50–100 | total |
|-------------|------------|------|-------|-------|-------|--------|-------|
| *T. harzianum* |            | 13   | 3     | 2     | 3     | 2      | 23    |
| *T. rossicum*  |            | 1    | 0     | 0     | 1     | 0      | 2     |
| *T. alni*     |            | 8    | 1     | 0     | 0     | 0      | 9     |
| *T. zoigense* |            | 2    | 0     | 1     | 0     | 0      | 3     |
| *T. atrobrunneum* |         | 2    | 0     | 0     | 0     | 0      | 2     |
| *T. polysporum* |          | 0    | 0     | 0     | 1     | 1      | 1     |
| *T. pyramidale* |           | 1    | 0     | 0     | 0     | 0      | 1     |

With respect to the different soil layers shown in Table 4, *T. harzianum* was widely distributed in the five soil layers at depths of 0–100 cm. *T. rossicum*, *T. alni* and *T. zoigense* were isolated mainly from the soil layers at depths of 0–50 cm. Both *T. atrobrunneum* and *T. pyramidale* were only isolated from depths of 0–10 cm, and *T. polysporum* was found only in the soil layers at depths of 50–100 cm.

Regarding isolation frequency, *T. harzianum* was the most common of the seven species with a 23% isolation frequency, and it was therefore the dominant species in the zone, while the rare species *T. polysporum* and *T. pyramidale* had the lowest isolation frequencies at 1%.

### Discussion

To characterize the biodiversity and establish the species composition of *Trichoderma* associated with soil in the Zoige alpine wetland ecological region of Southwest China, morphological characteristics and multilocus phylogenetic analyses were performed to identify 80 strains as *T. harzianum* (48 strains, 60%), *T. alni* (15 strains, 18.75%), *T. zoigense* (a new species, 8 strains, 10%), *T. rossicum* (4 strains, 5%), *T. atrobrunneum* (3 strains, 3.75%), *T. polysporum* (1 strain, 1.25%) and *T. pyramidale* (1 strain, 1.25%). This is the first comprehensive report on the population structure of *Trichoderma* in the Zoige alpine wetland. A specialized analysis of *Trichoderma* from 100 soil samples shows high richness of the *Trichoderma* species in this region and indicates the presence of latent resources that require further study, such as new species.

Although many studies have focused on the identification of *Trichoderma*, identifying *Trichoderma* species based on only morphological characteristics remains difficult. Amplification of four universal fungal genes, *gpd*, *acl1*, *rpb2* and *tef1*, showed that the *gpd* gene could be used to divide approximately the 57 representative strains into 4 clades, which were exactly aligned with the previous 4 morphological groups. The *gpd* gene was suitable for categorizing large groups but was not useful for the accurate identification of speciation within the *Trichoderma* complex (Druzhinina et al. 2010). In fact, any single gene among *acl1*, *rpb2* and *tef1* can play an important role in the identification of *Trichoderma* species but cannot accurately distinguish *Trichoderma* at the species level. Notably, although the primer pair EF1-728F and TEF1LLRev for *tef1* was useful, it did not always successfully amplify all tested DNA materials. Admittedly, there are many factors affecting PCR amplification, not all of which can be attributed to primers, among which the quality of DNA may also be one of the factors. Phylogenetic studies of many species have proven that the most accurate method of species identification is to combine phylogenetic analysis with morphological phenotypic characteristics. In this study, when the genes *acl1*, *rpb2* and *tef1* were used in multilocus phylogenetic analysis, the phylogenetic relationships among taxa were consistent with those identified in previous studies in which the phylogenetic tree was built based on the genes *rpb2* and *tef1* either singly or in combination (Chaverri & Samuels 2003; Jaklitsch 2009; Jaklitsch & Voglmayr 2015; Zhu & Zhuang, 2015).
We found that the Longibrachiatum clade contained a new species, *T. zoigense*, which was phylogenetically distinct from any other species of *Trichoderma* (Fig. 2) and provided a low level of support for relationships with *T. citrinoviride* (C.P.K. 2005) and *T. saturnisporum* (ATCC 18903) (Fig. 2, MPBP = 62%). Compared to their morphological characteristics of the above two species, *T. zoigense* was difficult to distinguish from *T. citrinoviride* and *T. saturnisporum* by colony and spores. However, *T. zoigense* was able to produce yellow pigment dispersion and a fragrance in all tested media and easily produces chlamydospores (Samuels et al. 1998; Samuels et al. 2006; Jaklitsch 2009; Jaklitsch 2011; Jaklitsch & Voglmayr 2015).

The results of our studies demonstrated significant differences in the abundance and distribution of *Trichoderma* species isolated in the Zoige alpine wetland natural region. *T. harzianum* showed the highest abundance among the species isolated from five soil layers and four soil types, implying that this species had good adaptability and can survive under most environmental conditions. Only *T. polysporum* was isolated at a soil depth of 50–100 cm, indicating that it prefers to live in a low-temperature environment (Domsch et al. 2007). In general, it is assumed that some *Trichoderma* species have stricter requirements for the growth environment and, thus, a narrower range for survival (Chen et al., 2009).

**Conclusion**

**New species**

*Trichoderma zoigense* G.S. Gong & G.T. Tang, *sp. nov*. Figure 4.

MycoBank: MB 821143

Typification: CHINA. SICHUAN PROVINCE: Zoige Alpine Wetland, on soil, 29 June 2013, G.S. Gong T44 (holotype CGMCC3.20145). GenBank: ITS = KX632531; TEF = KX632588; RPB2 = KX632645; ACL1 = KX632702; GDP = KX632799.

**Etymology:** *zoigense* (Latin), the specific epithet in reference to the place where the type was found.

Description: Cultures and anamorph: optimal growth at 25 °C on all four media. On CMD after 72 h, growth is 25–28 mm at 20 °C and 28–31 mm at 25 °C. Colony is dense and has a wavy to crenate margin. Surface becomes distinctly zonate and white to grayish-green but celadon to atrovirens later, and it is granular in the center and distinctly radially downy outside and shows whitish surface hyphae and reverse-diffusing croci to pale brown pigment. Aerial hyphae are numerous to punctate and long, forming radial strands, with white mycelial patches appearing in aged cultures. Autolytic excretions are rare, with no coilings observed. Conidiation noted after 3–4 d at 25 °C, a yellow or greenish color appears after 7 d, conidiation is effuse and in intense tufts, erect conidiophores occur around the plug and on aerial hyphae, and they are mainly concentrated along the colony center, show a white color that turns green, and then finally degenerate, with conidia often adhering in chains. Conidiophores are short and simple with asymmetric branches. Branches produce phialides directly. Phialides are generally solitary along main axes and side branches and sometimes paired in the terminal position of the main axes, sometimes in whorls of 2–3. Phialides are 4.5–10.5 x 2–5 μm (\( \bar{x} = 7.5 \pm 1.5 \times 3 \pm 0.5, n = 50 \)) and 1.5–2.5 μm (\( \bar{x} = 2 \pm 0.2 \)) wide at the base, lageniform or ampulliform, mostly uncinate or slightly curved, less straight, and often distinctly widened in the middle. Conidia are 3–4.5 x 2.3–4 μm (\( \bar{x} = 3.5 \pm 0.3 \times 3 \pm 0.3, n = 50 \)) and initially hyaline, and they turn green and are oblong or ellipsoidal, almost with constricted sides, and smooth, eguttulate or with minute guttules, with indistinct scars.

On PDA, after 72 h, growth is 35–41 mm at 20 °C and 50–55 mm at 25 °C; and mycelium covers the plate after 5 d at 25 °C. Colonies are dense with wavy to crenate margins; and mycelia are conspicuously differentiated in width of the primary and secondary hyphae. Surface becomes distinctly zonate, yellowish-green to prasinous in color and celadon to atrovirens later, and it is farinose to granular in the center, distinctly radially downy outside, with whitish of surface hyphae and reverse-diffusing brilliant yellow to fruit-green pigment. Aerial hyphae are numerous, long and ascend several millimeters, forming radial strands, with white mycelial patches appearing in aged cultures. Autolytic excretions are rare; and no coilings are observed. Odor is indistinct or fragrant. Chlamydospores examined after 7 d at 4.5–9 x 4.5–7.5 μm (\( \bar{x} = 6 \pm 1.1 \times 6 \pm 0.7, n = 50 \)), and they are terminal and intercalary, globose or ellipsoidal, and smooth. Conidiation is noted after 3–4 d and yellow or greenish after 7 d. Conidiophores are short and simple with asymmetric branches. Phialides are similar to CMD. Conidia are greenish, ellipsoidal, and smooth.

On SNA, after 72 h, growth is 13–15 mm at 20 °C and, 16–21 mm at 25 °C; and mycelium covers the plate after 12–13 d at 25 °C. Colony is similar to that on CMD, with a little wave margin, although mycelia are looser and slower on the agar surface. Aerial hyphae are relatively inconspicuous and long along the colony margin. Autolytic activity and coiling are absent or inconspicuous. No diffusing pigment or distinct odor are produced. Conidiation noted after 3–4 d at 25 °C, and many amorphous, loose white or aqua cottony tufts occur, mostly median from the plug outwards, and they are confluent to masses up and white but then turn green. From the inside after 4–5 d, conidiation becoming dense within the tufts, which are loose at their white margins with long, straight or slightly sinuous sterile ends in the periphery. Tufts consisting of a loose reticulum with branches often in right angles give rise to several main axes. Main axes are regular and tree-like, with few or many paired...
or unpaired side branches. Branches are flexuous and phialides are solitary along the main axes and side branches, and they are sometimes paired in terminal position of the main axes, sometimes in whorls of 2–3 that are often cruciform or in pseudo-whorls up to 4. Phialides and conidia are similar to that on CMD.

**New records for China**

Trichoderma atrobrunneum F. B. Rocha et al., Mycologia 107: 571, 2015.

Specimen examined: CHINA. SICHUAN PROVINCE: Zoige Alpine Wetland, on soil, 29 June 2013, G. S. Gong T42 (holotype CGMCC.20167). GenBank: ITS = KX632514; TEF = KX632571; RPB2 = KX632628; ACL1 = KX632685; GPD = KX632742.

Description: Cultures and anamorph: optimal growth at 25 °C on all media. On CMD, after 72 h, growth is 35–37 mm at 20 °C and 46–53 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelia are loose and thin; hyphae are narrow, sinuous and often form strands on the margin. Aerial hyphae are slight, forming a thin white to green downy fluffy or floccose mat. Light brown or brown pigment is observed, with no distinct odor noted. Conidiophores are pyramidal, often with opposing and somewhat widely spaced branches, with the main axis and each branch terminating in a cruciate, sometimes verticillate, whorl of up to four phialides. Phialides are ampulliform to lageniform and 4.9–7.6 × 2.2–3.0 µm (x̄ = 6 ± 0.7 × 2.5 ± 0.2, n = 50) and 1.5–2.5 µm (x̄ = 1.5 ± 0.3) wide at the base. Conidia are 2.5–4 × 2.5–3.5 µm (x̄ = 3 ± 0.3 × 3 ± 0.2, n = 50), yellow to green, smooth, and circular to ellipsoidal.

On PDA, after 72 h, growth is 41–43 mm at 20 °C and 50–55 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show indistinct zonation. Mycelia are dense, opaque, and thick; hyphae are wide, sinuous and often form strands on the margin. Margin is thick and defined. Aerial hyphae are abundant and form a thick green downy mat. Conidiation forms abundantly within 4 d in broad concentric rings. Chlamydospores examined after 7 d are 5–9 × 5.5–8.5 µm (x̄ = 6.5 ± 0.9 × 6.5 ± 0.9, n = 30), globose when terminal, smooth, and intercalary.

On SNA, after 72 h, growth is 33–35 mm at 20 °C and 38–40 mm at 25 °C; and mycelium covers the plate after 7–8 d at 25 °C. Colonies show distinct zonation. Mycelia are thin and yellow to green; hyphae are wide and sinuous, with indistinct strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight, forming a thin green downy fluffy appearing in the colony. Diffusing pigment observed in a ring, and no distinct odor noted. Conidiation is similar to CMD.

**Accepted species previously reported in China**

Trichoderma alni Jaklitsch, Mycologia 100: 799. 2008.

Description: Cultures and anamorph: Optimum growth at 25 °C on all media; no growth at 35 °C. On CMD, after 72 h, growth of 34–36 mm at 20 °C and 50–51 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelia are loose and thin; hyphae are narrow and sinuous and often form strands on the margin. Aerial hyphae are slight and form a thin white to green downy, fluffy or floccose mat. No diffusing pigment or distinct odor is noted. Conidiophores are hyaline and thick, with side branches on several levels at the base of the elongations that are mostly paired and in right angles with phialides in whorls of 3–5. Phialides are 5.5–11.5 × 2–3.5 µm (x̄ = 8 ± 1.4 × 2.5 ± 0.4, n = 50) and 1.5–2.5 µm (x̄ = 2 ± 0.4) wide at the base, often short and wide, and ampulliform. Conidia are 3–4 × 2.5–3.5 µm (x̄ = 3.5 ± 0.2 × 3 ± 0.2, n = 50), dark green, smooth, and elliptoidal.

On PDA, after 72 h, growth is 33–35 mm at 20 °C and 41–43 mm at 25 °C; and mycelium covers the plate after 6–7 d at 25 °C. Colonies show indistinct zonation. Mycelia are dense, opaque, and thick; hyphae are wide, sinuous and often form strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight, forming a thin white to green downy, fluffy or floccose mat. Chlamydospores examined after 7 d are 6–9.5 × 5–8 µm (x̄ = 7.5 ± 0.9 × 7 ± 0.9, n = 30), globose to oval when terminal, smooth, and few are intercalary.

On SNA, after 72 h, growth is 18–19 mm at 20 °C and 28–32 mm at 25 °C; and mycelium covers the plate after 6–7 d at 25 °C. Colonies show distinct zonation. Mycelia are thin and yellow to green; hyphae are wide and sinuous and show indistinct strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight and form a thin white downy, fluffy or floccose mat appearing in distal parts of the colony. No diffusing pigment or distinct odor noted. Conidiation is similar to CMD.

Trichoderma harzianum Rifai, Mycol. Pap. 116: 38, 1969.

Description: Cultures and anamorph: optimal growth at 25 °C on all media. On CMD, after 72 h, growth is 34–38 mm at 20 °C and 46–53 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelia are loose and thin; hyphae are narrow, sinuous and often form strands on the margin. Aerial hyphae are abundant and radiating and form thick green downy, fluffy or floccose mats. No diffusing pigment, but fragrant odor noted. Conidiophores are pyramidal with opposing branches, with each branch terminating in a cruciate whorl of up to four or five phialides. Phialides are frequently solitary or in a whorl of three or four. Phialides are ampulliform to lageniform and...
often constricted below the tip to form a narrow neck of 4.5–8 × 2–3.5 µm (\(x = 6 \pm 0.8 \times 2.5 \pm 0.3\), \(n = 50\)) and 1–2.5 µm (\(x = 2 \pm 0.3\)) wide at the base. Conidia are subglobose to ovoid, 3–4.5 × 2.5–3.3 µm (\(x = 3.5 \pm 0.3 \times 3 \pm 0.2\), \(n = 50\)), laurel-green to bright green, smooth, and ellipsoidal.

On PDA, after 72 h, growth is 41–43 mm at 20 °C and 50–55 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelia are dense, opaque, and thick; hyphae are wide and sinuous and often form strands on the margin. Margin is thick and ill defined. Aerial hyphae are abundant and radiating and form thick green downy, fluffy or floccose mats. Chlamydospores examined after 7 d are 5.5–9 × 5.5–9.0 µm (\(x = 7 \pm 0.8 \times 7 \pm 0.8\), \(n = 30\)), globose to oval when terminal and smooth and show an almost unobserved intercalary.

On SNA, after 72 h, growth is 33–35 mm at 20 °C and 38–40 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelia are thin and green; hyphae are narrow and sinuous and show indistinct strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight and form a thick downy, fluffy or floccose mat appearing in the colony. No diffusing pigment or, distinct fragrant odor noted. Conidiation similar to CMD.

**Trichoderma polysporum** Rifai, Mycol. Pap. 116: 18, 1969.

Description: Cultures and anamorph: optimal growth at 20 °C on all media, no growth at 35 °C. On CMD, after 72 h, growth is 14–16 mm at 20 °C and 9–12 mm at 25 °C; and mycelium covers the plate after 9–10 d at 20 °C. Colony is hyaline, thin and loose, with little mycelium on the agar surface, and it is indistinctly zonate but becomes zonate by conidiation in white tufts after 4–5 d and grass green to green after 6 d. Aerial hyphae are long and dense and forming little greenish aggregates that are granular to pulvinate. No pigment or odor. Conidiation noted after 4–5 d, and it is white to greenish, with sterile smooth to rough helical elongations in the distal zones from pustules. Conidiphores are hyaline and, thick with side branches on several levels at the base of the elongations that are mostly paired and in right angles with phialides in whorls of 2–5. Phialides are 5–10.5 × 2.5–4 µm (\(x = 7 \pm 1.9 \times 3.5 \pm 0.4\), \(n = 50\)) and 2–4 µm (\(x = 3 \pm 0.5\)) wide at the base, often short and wide and ampulliform. Conidia are 2.5–4 × 2–3 µm (\(x = 3.5 \pm 0.4 \times 2.5 \pm 0.2\), \(n = 50\)), green, smooth, and ellipsoidal.

On PDA, after 72 h, growth is 24–26 mm at 20 °C and 13–16 mm at 25 °C; and mycelium covers the plate after 8–9 days at 20 °C. Colony is densest, distinctly zonate, and grass green to spearmint green; mycelia are conspicuously dense; and surface hyphae form radial strands. Aerial hyphae are long and dense and form greenish aggregates that are granular to pulvinate. No diffusing pigment and odor. Chlamydospores examined after 7 d are 5.5–9 × 5–7.5 µm (\(x = 7 \pm 0.9 \times 6 \pm 0.6\), \(n = 30\)), globose to oval when terminal, and smooth, with an almost unobserved intercalary.

On SNA, growth is approximately 7 mm/d at 20 °C and 5 mm/d at 25 °C; and mycelium covers the plate after 10 d at 20 °C. Colony is hyaline, thin, and loose, with little mycelium on the agar surface, not or indistinctly zonate, but becomes zonate by conidiation in white tufts after 4–5 d; and the margin is downy by long aerial hyphae, which degenerating/dissolving soon.

**Trichoderma pyramidale** W. Jaklitsch & P. Chaverri, Mycologia 107: 581, 2015.

Description: Cultures and anamorph: optimal growth at 25 °C on all media, with little growth at 35 °C. On CMD, after 72 h, growth is 29–32 mm at 20 °C and 48–53 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show distinct zonation. Mycelium is loose and thin; hyphae are narrow, sinuous and often form strands on the margin. Aerial hyphae are slight, form a thin white to green downy, fluffy or floccose mat. Brown pigment is shown, but no distinct odor noted. Conidiophores are hyaline and thick with side branches on several levels at the base of the elongations that are mostly paired and in right angles with phialides in whorls of 3–5. Phialides are 5–9.5 × 2.5–3 µm (\(x = 7 \pm 1.1 \times 3 \pm 0.3\), \(n = 50\)) and 1–2.5 µm (\(x = 1.5 \pm 0.3\)) wide at the base and often short, wide, and ampulliform. Conidia are 2.5–4 × 2.5–3.5 µm (\(x = 3.5 \pm 0.3 \times 3 \pm 0.2\), \(n = 50\)), green, smooth, and ellipsoidal.

On PDA, after 72 h, growth is 41–43 mm at 20 °C and 50–55 mm at 25 °C; and mycelium covers the plate after 5–6 d at 25 °C. Colonies show indistinct zonation. Mycelia are dense, opaque, and thick; hyphae are wide, sinuous and often form strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight and form a thin white to green downy, fluffy or floccose mat. Chlamydospores examined after 7 d are 5.5–10 × 5.5–10 µm (\(x = 7 \pm 0.9 \times 7 \pm 0.9\), \(n = 30\)), globose to oval when terminal or intercalary, and smooth.

On SNA, after 72 h, growth is 33–35 mm at 20 °C and 38–40 mm at 25 °C; and mycelium covers the plate after 7–8 d at 25 °C. Colonies show distinct zonation. Mycelium is thin, yellow to green; hyphae are wide, sinuous, with indistinct strands on the margin. Margin is thin and ill defined. Aerial hyphae are slight and form a thin white downy, fluffy or floccose mat in distal parts of the colony. No diffusing pigment or distinct odor noted. Conidiation similar to CMD.

**Trichoderma rossicum** Bissett et al., Canad. J. Bot. 81: 578, 2003.
Description: Cultures and anamorph: optimal growth at 25 °C on all media. On CMD, growth of 10–11 mm/d at 20 °C and 15–17 mm/d at 25 °C; and mycelium covers the plate after 6–7 d at 20 °C. Colony is dense with a wavy margin, and the surface becomes distinctly zonate. Aerial hyphae are numerous, long, and villiform in the plate. No diffusing pigment or odor. Autolytic activity is variable, and coilings are scarce or inconspicuous. Conidiation noted after 3–4 d at 20 °C. Conidiation is effuse and in intense tufts that are hemispherical or irregular, and they show wide wheel grain banding that is gray green to deep green. Conidiophores radiate from the reticulum and are broad, straight, sinuous or helically twisted, show distally slightly pointed elongations, taper from the main axes to top branches, and present primary branches arranged in pairs or in whorls of 2–3, with secondary branches to solitary. Phialides are 4.5–14 × 2.5–4 µm (x̄ = 7 ± 1.5 × 3.5 ± 0.3, n = 50) and 2–3.5 µm (x̄ = 3 ± 0.4) wide at the base, ampulliform, and in whorls of 3–6. Conidia are 3.5–5.5 × 2.5–4 µm (x̄ = 4.5 ± 0.5 × 3 ± 0.2, n = 50), short cylindrical, and a gray color when single and pea green to yellow green in a group.

On PDA, growth is 12–15 mm/d at 20 °C, 12–16 mm/d at 25 °C; and mycelium covers the plate after 4–5 d at 25 °C. Colony is denser with a wavy margin than that on CMD, and the surface is distinctly zonate. Aerial hyphae are numerous, long, and villiform to pulvinate in the plate. No diffusing pigment and odor. Autolytic activity is variable, coilings are scarce or inconspicuous. Chlamydospores examined after 7 d are 6.5–9.5 × 6–9 µm (x̄ = 7 ± 1.0 × 7 ± 0.9, n = 30), terminal and intercalary, globose or ellipsoidal, and smooth.

On SNA, growth is 8–13 mm/d at 20 °C and 8–12 mm/d at 25 °C; and mycelium covers the plate after 6–7 d at 25 °C. Colony is hyaline, thin and dense; and mycelium degenerate rapidly. Aerial hyphae are inconspicuous, autolytic activity is scant, and coilings are distinct. Conidiation noted after approximately 4 d and starts in white fluffy tufts spreading from the center to form concentric zones, and they compact to pustules with a white to greenish color.

Declarations

Acknowledgments

We would like to thank Jia-Yue Wang, Dan-Dan Peng, Meng-Ping Ye, Wen-Jing Xie, Xiao-Bo Qi, Xiao-Fang Sun and Fang-Ling Liu at Sichuan Agricultural University for collecting material and providing methods.

Authors’ contributions

G-TT designed the experiment, analyzed the data, and prepared the writing-original draft. YL and YZ analyzed the data and revised the manuscript. X-JZ performed the micrograph. X-LC revised the manuscript. S-RZ collected the samples and reviewed the manuscript. G-SG designed the experiment and reviewed the manuscript. All authors read and approved the final manuscript.

Funding

This project was supported by the Sichuan Corn Innovation Team of the National Modern Agricultural Technology System.

Availability of data and materials

All DNA sequences generated in this study have been registered to GenBank.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
Author details

1 College of Agronomy & Key Laboratory for Major Crop Diseases, Sichuan Agricultural University, Chengdu 611130, P. R. China. 2 Southeast Chongqing Academy of Agricultural Sciences, Fuling 408099, P. R. China. 3 Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 570100, P. R. China. 4 College of Environmental Sciences, Sichuan Agricultural University, Chengdu 611130, P. R. China.

References

1. Andreolli M, Lampis S, Brignoli P, Vallini G (2016) Trichoderma longibrachiatum Evx1 is a fungal biocatalyst suitable for the remediation of soils contaminated with diesel fuel and polycyclic aromatic hydrocarbons. Environ Sci Pollut Res 23(9):9134–9143
2. Atanasova L, Druzhinina IS, Jaklitsch WM (2013) Two hundred Trichoderma species recognized on the basis of molecular phylogeny. Trichoderma: Boil Applications CABI, Wallingford: 10–42
3. Babu AG, Shim J, Bang KS, Shea PJ, Oh BT (2014) Trichoderma virens PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. J Environ Manage 132:129–134
4. Barnes I, Roux J, Wingfield MJ (2001) Characterization of Seiridium spp. associated with cypress canker based on β-tubulin and Histone sequences. Plant Dis 85:317–321
5. Błaszczyk L, Popiel D, Chełkowski J, Koczyk G, Samuels GJ, Siwulski M (2011) Species diversity of Trichoderma in Poland. J Appl Genetics 52:233–243
6. Bornet B, Goraguer F, Joly G, Branchard M (2002) Genetic diversity in European and Argentinian cultivated potatoes (Solanum tuberosum subsp. tuberosum) detected by inter-simple sequence repeats (ISSRs). Genome 45(3):481–484
7. Cai F, Chen W, Wei Z, Pang G, Li RX, Ran W, Shen QR (2015) Colonization of Trichoderma harzianum strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. Plant Soil 388(1–2):337–350
8. Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3):553–556
9. Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003a) Hypocrea/Trichoderma: species with conidiophore elongations and green conidia. Mycologia 95(6):1100–1140
10. Chaverri P, Castlebury LA, Samuels GJ, Geiser DM (2003b) Multilocus phylogenetic structure within the Trichoderma harzianum/Hypocrea lixi complex. Mol Phylogenet Evol 27(2):302–313
11. Chaverri P, Samuels GJ, Stewart EL (2001) Hypocrea virens sp. nov., the teleomorph of Trichoderma virens. Mycologia 93(6):1113–1124
12. Chaverri P, Samuels GJ (2003) (ascomycota, hypocreales, hypocreaceae): species with green ascospores. In: Hypocrea/Trichoderma. Centraalbureau voor Schimmelcultures, Utrecht
13. Chen H, Gao YH, Yao SP, Ning WU, Wang YF, Peng L (2008) Spatiotemporal variation of methane emissions from alpine wetlands in Zoige Plateau. Acta Ecol Sin 28(7):3425–3437
14. Chen H, Wu N, Gao YH, Wang YF, Luo P, Tian JQ (2009) Spatial variations on methane emissions from Zoige alpine wetlands of Southwest China. Sci Total Environ 407(3):1097–1104
15. Chen JA, Qiu DL, Wang WM, Yang HM, Du FL (2009) Effect of soil ecological environment on survival of Trichoderma. Mod Agric Sci Tec 21:217–218
16. Dai YM, Yan ZY, Jia LL, Zhang SH, Gao LH, Wei XL, Mei ZL, Liu XF (2016) The composition, localization and function of low-temperature-adapted microbial communities involved in methanogenic degradations of cellulose and chitin from Qinghai–Tibetan Plateau wetland soils. J Appl Microbiol 121(1):163–176
17. Devi P, Narayanasamy P, Kamil D, Borah JL (2012) Development of species specific markers for detection of Trichoderma species. Vegetos-An International J Plant Res 25(2):207–217
18. Ding WX, Cai ZC, Wang DX (2004) Preliminary budget of methane emissions from natural wetlands in China. Atmos Environ 38(5):751–759
19. Dodd SL, Crowhurst RN, Rodrigo AG, Samuels GJ, Hill RA, Stewart A (2000) Examination of Trichoderma phylogenies derived from ribosomal DNA sequence data. Mycol Res 104(1):23–34
20. Doi Y (1966) A revision of Hypocreales with cultural observation I. Some Japanese species of Hypocrea and Podostroma. Bull Nat 9:345–357
21. Doi Y (1968) Revision of the Hypocreales with cultural observations II. Hypocrea dichromospora, sp. nov. and its Trichoderma state. Bull Nat 11:185–189
22. Doi Y (1969) Revision of the Hypocreales with cultural observations IV. The genus *Hypocrea* and its allies in Japan (1) General part. Bull Nat (B-rui) 12:693–724

23. Doi Y (1971) Some species of the genus *Hypocrea*. Bull Nat 14:387–400

24. Doi Y (1972) Revision of the Hypocreales with cultural observations IV. The genus *Hypocrea* and its allies in Japan (2) Enumeration of the species. Bull Nat (B-rui) 15:649–751

25. Doi Y (1975) Revision of the Hypocreales with cultural observations VII. The genus *Hypocrea* and its allied genera in South America (1). Bull Nat (B-rui) 1:1–33

26. Doi Y (1976) Revision of the Hypocreales with cultural observation IX. The genus *Hypocrea* and its allied genera in South America (2). Bull Nat (B-rui) 2:119–131

27. Doi Y (1978) Revision of the Hypocreales with cultural observations XI. Additional notes on *Hypocrea* and its allies in Japan (1). Bull Nat (B-rui) 4:19–26

28. Doi Y (1982) Type study on *Hypocrea grandis* Imai and *Chromocrea nigricans* Imai. Bull Nat 8(1):29–33

29. Doi Y (2001) A new species of *Hypocrea* (Ascomycota, Hypocreales) from Mikurajima Island, Japan. Mem Nat 37:113–118

30. Doi Y (2006) Revision of the Hypocreales with cultural observations XIII. The Hypocreaceae of the Sagami Sea maritime forests, Japan. Mem Nat 42:223–232

31. Doi Y, Liu PG, Tamura M (2001) A new species of the Hypocreales (Ascomycota) from Mt. Changbaishan, northeast China. Bull Nat (B-rui) 27:57–63

32. Domsch KH, Gams W, Anderson TH (2007) Compendium of soil fungi, 2nd taxonomically revised edition by Gams W. IHW-Verlag Eching 1–672

33. Druzhinina IS, Kopchinskiy AG, Komor M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet Biol 42(10):813–828

34. Druzhinina IS, Kubicek CP, Komor-Zelazowska M, Mulaw TB, Bissett J (2010) The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. BMC Evol Biol 10(1):94

35. Feng SG, Zhang HX, Wang YF, Bai ZH, Zhuang GQ (2009) Analysis of fungal community structure in the soil of Zoige Alpine Wetland. Acta Ecol Sin 29(5):260–266

36. Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111(1):3–49

37. Gao JQ, Zhang F, Wang CM (2008) Distribution characteristics of soil libile carbon along water table gradient of alpine wetland soil. J Soil Water Conserv 22:126–131

38. Gräfenhan T, Schroes HJ, Nirenberg HI, Seifert KA (2011) An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in Cosmospora, Acremonium, Fusarium, Stilbella, and Volutella. Stud Mycol 68:79–113

39. Hanada RE, Souza TJ, Pomella AWV, Hebbar KP, Pereira JO, Samuels GJ (2008) *Cosmospora, Acremonium, Fusarium, Stilbella, and Volutella*. Stud Mycol 68:79–113

40. Hjeljord L, Tronsmo A (1998) *Theobroma cacao* from sapwood of *Theobroma cacao* with a potential for biological control. Mycol Res 112(11):1335–1343

41. Hollister EB, Schadt CW, Palumbo AV, Ansley RJ, Boutton TW (2010) Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. Soil Biol Biochem 42(10):1816–1824

42. Hoyos-Carvajal L, Orduz S, Bissett J (2009) Genetic and metabolic biodiversity of *Trichoderma* from Colombia and adjacent neotropic regions. Fungal Genet Biol 46(9):615–631

43. Jaklitsch WM (2009) European species of *Hypocrea* Part I. The green-spored species. Stud Mycol 63:1–91

44. Jaklitsch WM (2011) European species of *Hypocrea* part II: species with hyaline ascospores. Fungal Divers 48(1):1–250

45. Jaklitsch WM, Komon M, Kubicek CP, Druzhinina IS (2005) *Hypocrea voglmayrii* sp. nov. from the Austrian Alps represents a new phylogenetic clade in *Hypocrea/Trichoderma*. Mycologia 97:1365–1378

46. Jaklitsch WM, Komon M, Kubicek CP, Druzhinina IS (2006a) *Hypocrea crystalligena* sp. nov., a common European species with a white-spored *Trichoderma anamorph*. Mycologia 98(3):499–513

47. Jaklitsch WM, Kubicek CP, Druzhinina IS (2008) Three European species of *Hypocrea* with reddish brown stromata and green ascospores. Mycologia 100(5):796–815

48. Jaklitsch WM, Samuels GJ, Dodd SL, Lu BS, Druzhinina IS (2006b) *Hypocrea rufa/Trichoderma viride*: a reassessment, and description of five closely related species with and without warted conidia. Stud Mycol 56:135–177

49. Jaklitsch WM, Voglmayr H (2015) Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. Stud Mycol 80:1–87
50. James AW, Brain CS, Neil JM, Alan CG (2012) Species and organ specificity of fungal endophytes in herbaceous grassland plants. J Ecol 100:1085–1092

51. Kamal S, Ajaykumar M, Rajeshkumar M (2009) Morphological, biochemical and molecular characterization of Trichoderma harzianum isolates for their efficacy as biocontrol agents. J Phytopathol 157(1):51–56

52. Körnerup A, Wanscher JH (1981) Taschenlexikon der Farben: 1440 Farbnuancen und 600 Farbnamen. Muster–Schmidt

53. Kredics L, Hatvani L, Naeimi S, Kormoczi P, Manczinger L, Vagvolgyi C, Irida D (2014) Biodiversity of the genus Hypocreaceae. Stud Mycol 25(24):4876–4882

54. Li QR, Tan P, Jiang YL, Hyde KD, Mckenzie EHC, Bahkali AH, Kang JC, Wang Y (2013) A novel Trichoderma species isolated from soil in Guizhou, T. guizhouense. Mycol Progr 12(2):167–172

55. Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Bio Evol 16(12):1799–1808

56. Ma K, Liu J, Balkovič J (2016a) Changes in soil organic carbon stocks of wetlands on China’s Zoige plateau from 1980 to 2010. Ecol Modell 327:18–28

57. Ma K, Zhang Y, Tang SX, Liu JG (2016b) Spatial distribution of soil organic carbon in the Zoige alpine wetland, northeastern Qinghai–Tibet Plateau. Catena 144:102–108

58. Mueller GM (2011) Biodiversity of fungi: inventory and monitoring methods. Academic Press, New York

59. Mueller GM, Bills GF, Foster MS (2004) Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, Burlington, pp 280–302

60. Nelson EE (1982) Occurrence of Trichoderma in a Douglas-fir soil. Mycologia 74(2):280–284

61. Nguyen NH, Williams LJ, Vincent JB, Stefanski A, Cavender-Bares J, Messier C, Paquette A, Gravel D, Reich PB, Kennedy PG (2016) Ectomycorrhizal fungal diversity and saprotrophic fungal diversity are linked to different tree community attributes in a field-based tree experiment. Mol Ecol 25(16):4032

62. Nirenberg HI (1976) Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium-Sektion Liseola. Mitt Biol Bundesanst Land-u Forstwirtsch Berlin-Dahlem 169:1–117

63. Reese ET, Mandels M (1984) Rolling with the times: production and applications of Trichoderma reesei cellulase. Annu Rep Ferment Processes (United States) 7:1–20

64. Rohif FJ (2000) NT SYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1. Exeter Software Setauket NY

65. Samuels GJ (1996) Trichoderma: a review of biology and systematics of the genus. Mycol Res 100(8):923–935

66. Samuels GJ (2006) Trichoderma: systematical, the sexual state, and ecology. Phytopathology 96(2):195–206

67. Samuels GJ, Dodd SL, Lu BS, Petriani O, Schroers HJ, Druzhinina IS (2006) The Trichoderma koningii aggregate species. Stud Mycol 56:67–133

68. Saumels GJ, Petriani O, Kubls K, Lieckfeldt E, Kubicek CP (1998) The Hypocrea schweinitzii complex and Trichoderma sect. Longibrachiatum. Stud Mycol 41:1–51

69. Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36(2):147–155

70. Sivasithamparam K, Ghisalberti EL (1998) Secondary metabolism in Trichoderma and Gliocladium. Trichoderma and Gliocladium basic biology taxonomy and genetics 1: 139–191

71. Sun GY, Zhang WF, Zhang JJ (1998) The mire and peatland of the hengduan mountainous region. Science Press, Beijing, p 352

72. Sun R, Liu Z, Fu K (2012) Trichoderma biodiversity in China. J Appl Genet 53(3):343–354

73. Swofford DL (2001) PAUP*: phylogenetic analysis using parsimony (*and other methods), v. 4.0b10. Sinauer Associates, Sunderland

74. Templeton MD, Rikkerink EHA, Solon SL, Crowhurst RN (1992) Cloning and molecular characterization of the glyceroldehylde-3-phosphate dehydrogenase-encoding gene and cDNA from the plant pathogenic fungus Glomerella cingulata. Gene 122(1):225 – 230

75. Thompson JD, Gibson TJ, Plewniak F (1997) The CLUSTAL_X windows interface: flexible strategies for multiple seRuence alignment aided by quality analysis tools. Nucleic acids Res 25(24):4876–4882
79. Tian JQ, Wu B, Chen H, Jiang N, Kang XM, Liu XZ (2017) Patterns and drivers of fungal diversity along an altitudinal gradient on Mount Gongga, China. J Soils Sediments: 1–10
80. Vieira WAS, Michereff SJ, Morais MA, Hyde KD, Camara MPS (2014) Endophytic species of Colletotrichum associated with mango in northeastern Brazil. Fungal Divers 67:181–202
81. Wardle DA, Yeates GW, Barker GM, Bonner KI (2006) The influence of plant litter diversity on decomposer abundance and diversity. Soil Biol Biochem 38(5):1052–1062
82. Woo SL, Ruocco M, Vinale F (2014) Trichoderma-based products and their widespread use in agriculture. The Open Mycology Journal 8(1)
83. Yao XM, Lv GZ, Yang H, Zhao ZH, Chen R (2007) Studies of fungal flora in forest soil of Changbai Mountains. J Fungal Res 5(1):43–46
84. Yedidia I, Srivastva AK, Kapulnik Y, Chet I (2001) Effect of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. Plant Soil 235(2):235–242
85. Yuan N, Kang ZJ, Lu SE, Wang YY, Zhang XP, Gu YF (2016) Community structures of the cold-adapted cellulose-degrading bacteria in the Zoige plateau wetland under enrichment culture conditions. J Environ Biol 22(3):0402–0408
86. Zhang GS, Tian JQ, Jiang N, Guo XP, Wang YF, Dong XZ (2008) Methanogen community in Zoige wetland of Tibetan plateau and phenotypic characterization of a dominant uncultured methanogen cluster ZC-I. Environ Microbiol 10(7):1850–1860
87. Zhang GZ, Zhang XJ, Chen K, Wu XQ, Li JS, Yang HT (2015) Trichoderma paratroviride, Chinese new record of Trichoderma. Shandong Science 28:35–40
88. Zhu ZX, Zhuang WY (2015) Trichoderma (Hypocrea) species with green ascospores from China. Persoonia-Molecular Phylogeny Evolution of Fungi 34(1):113–112

Figures
Figure 1

Neighbor-joining tree based on partial gpd gene sequences from 57 Trichoderma isolates. Parsimony bootstrap values of more than 50% are shown at nodes.
Figure 2

Maximum parsimony tree of Trichoderma species inferred from the combined rpb2, tef1 and acl1 partial sequences. Maximum parsimony bootstrap values above 50% are shown at nodes. The tree was rooted with Protocrea farinose and P. pallida. Isolates from this study are shown in red (new species in bold)
Figure 3

Growth rates of 7 species of Trichoderma on CMD given as mm per day at five temperatures. The values were the means of 3–5 experiments, with 1–3 representative isolates per species.
Figure 4

Cultures and asexual morph of Trichoderma zoigense. a–d. Cultures at 20 °C (a. on CMD, 7 d; b. on MEA, 4 d; c. on PDA, 4 d; and d. on SNA, 7 d). e. Conidiation tuft (CMD, 4 d). f–k. Conidiophores and phialides (CMD, 5–7 d). l. Chlamydospores (PDA, 8 d). m. Conidia (CMD, 5 d). Scale bars: e = 2 mm; f–m = 10 μm
Figure 5

Cultures and asexual morph of Trichoderma atrobrunneum. a–d. Cultures at 25 °C (a. on CMD, 7 d; b. on MEA, 4 d; c. on PDA, 15 d; and d. on SNA, 7 d). e. Conidiation tuft (SNA, 7 d). f–i, k, l. Conidiophores and phialides (CMD, 5–7 d). j. Conidia (CMD, 6 d). m. Chlamydospores (PDA, 7 d). Scale bars: e = 2 mm; f–m = 10 μm
Figure 6

Cultures and asexual morph of Trichoderma alni. a–d. Cultures after 7 d at 25 °C (a. on CMD; b. on MEA; c. on PDA; and d. on SNA). e. Coilings of aerial hyphae (PDA, 6 d). f–j, l. Conidiophores and phialides (CMD, 5–7 d). k. Conidiation tuft (PDA, 7 d). m. Conidia (CMD, 6 d). n, o. Chlamydospores (PDA, 7 d). Scale bars: e–j, l–o = 10 μm; k = 2 mm
Figure 7

Cultures and asexual morph of Trichoderma harzianum. a–d. Cultures after 7 d at 20 °C (a. on CMD; b. on MEA; c. on PDA; and d. on SNA). e. Conidiation tuft (CMD, 7 d). f–j. Conidiophores and phialides (CMD, 5–7 d). k. Conidia (CMD, 5 d). l, m. Chlamydospores (PDA, 7 d). Scale bars: e = 2 mm; f–m = 10 μm
**Figure 8**

Cultures and asexual morph of *Trichoderma polysporum*. a–d. Cultures at 20 °C (a. on CMD, 7 d; b. on MEA, 15 d; c. on PDA, 15 d; and d. on SNA, 15 d). i. Conidiation tuft (PDA, 15 d). e–h, j. Conidiophores and phialides (CMD, 5–7 d). k. Chlamydospores (CMD, 7 d). l. Conidia (PDA, 6 d). Scale bars: i = 2 mm; e–h, j = 10 μm
Figure 9

Cultures and asexual morph of Trichoderma pyramidale. a–d. Cultures at 25 °C (a. on CMD, 7 d; b. on MEA, 4 d; c. on PDA, 4 d; and d. on SNA, 4 d). e. Conidiation tuft (PDA, 7 d). f–j. Conidiophores and phialides (CMD, 5–7 d). k. Conidia (CMD, 6 d). l. Chlamydospores (PDA, 7 d). Scale bars: e = 2 mm; f–l = 10 μm
Figure 10

Cultures and asexual morph of Trichoderma rossicum. a–d. Cultures after 7 d at 25 °C (a. on CMD; b. on MEA; c. on PDA; and d. on SNA). e. Conidiation tuft (PDA, 7 d). f–h, j, k. Conidiophores and phialides (CMD, 5–7 d). i. Elongations (CMD, 6 d). l, n. Conidia (CMD, 6 d). m. Chlamydospores (PDA, 7 d). Scale bars: e = 2 mm; f–n = 10 μm