Trichoderma juruarense sp. nov. (Hypocreaceae) a new fungal species from sediments of the Amazonian Juruá River with agricultural potential

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Abstract

The species of *Trichoderma* are the most common fungi to be used as biocontrol agents, and their metabolic arsenal has a wide variety of applications that places this genus among those with the potential to provide biotechnological products. The ubiquitous nature of *Trichoderma* has favored a rapid increase in the number of described species, and significant efforts have been made towards the taxonomy of *Trichoderma* in order to improve the accuracy of identification. During a study of cultivable microbiota from the Juruá River, Amazon, Brazil. Isolates with morphological characteristics of the genus *Trichoderma* were screened for their capacity to control phytopathogens. A total of five *Trichoderma* isolates were identified using morphological data combined with phylogenetic analysis of the ITS region, and partial sequences of *TEF* and *RPB2*. *Trichoderma juruarens* sp. nov. form a monophyletic clade that is closely related to *T. cyanodichotomus*, a species that occupies an unresolved position in the *Trichoderma* taxonomy. *T. cyanodichotomus* and *T. juruarens* sp. nov. present an intracellular blue-green pigment in potato dextrose agar (PDA) and differ in conidia and chlamydospores sizes. These data support the proposition of a new species, named here as *Trichoderma juruarens*. The holotype of *T. juruarens* INPA0108 presents *in vitro* inhibition against phytopathogens, such as *Colletotrichum siamense* (50%), *Corynespora cassiicola* (43%), *Fusarium decemcellulare* (61%) and *Sclerotium rolfii* (51%), which demonstrates desirable traits that warrant further studies on plant protection. In addition, *T. cyanodichotomus* and *T. juruarens* formed a new clade based on the sequence data of the *RPB2* gene.

Introduction

The abundant biodiversity of the Amazon region is scattered among many distinct environments (upland forest, white-sand savanna, flooded forest, and floodplains). In this scenario, the high precipitation rate favors the deposition and transport of biological material along its flow, making the Amazonian wetlands a valuable source of biodiversity (Junk et al. 2010).

The Juruá River begins in Peru and flows for over 3,280 km inside the Brazilian territory, and is considered the world's most sinuous river. Along the extension of the Juruá River, there are two environmental protection areas (Uacari Sustainable Development Reserve and the Medio Juruá Extractive Reserve) that host diverse ecosystems. Moreover, several indigenous communities live in this region, which is considered a promising place for the establishment of a circular economy, due to a highly diverse natural resource-based economy and strong social organization of local communities (da Silva Abel et al. 2021; Paes et al. 2021).

The Juruá River is the third largest whitewater tributary of the Amazon River and plays an important role in riverside communities, hosting a diverse inventory of fauna and flora species (Del-Rio et al. 2021). Annually, the Juruá River has a flood and ebb period (Colwell 2000), each one taking several months to complete. This phenomenon, also known as flood pulse (Junk et al. 1989), has a direct impact on the local economy, since agriculture, fishing and transportation are all directly influenced by the water level. Inundation of the floodplains along the Juruá River favors many ecological processes, including the exchange of genetically diverse biological material across distinct environments, which is especially favorable for microorganisms.

Within the microbial diversity of the Amazon region, several new species of *Trichoderma* have been identified, such as *Trichoderma stromaticum* (Samuels et al. 2000), *Trichoderma ovalisporum* (Holmes et al. 2004), *Trichoderma theobromicola*, *T. paucisporum* (Samuels et al. 2006b), *Trichoderma martiale* (Hanada et al. 2008, 2009), *T. amazonicum* (Chaverri et al. 2011), *T. strigosellum* (López-Quintero et al. 2013), *T. awajun*, *T. jaklitschii* and *T. peruvianum* (Bustamante et al. 2021). Despite all the *Trichoderma* species listed above originating from the Amazon biome, only *T. stromaticum*, *T. ovalisporum* and *T. martiale* were collected in Brazil, pointing to a gap in information regarding the diversity of *Trichoderma* in the Brazilian Amazon.

Trichoderma has been largely associated with enzyme production, plant protection and growth promotion, but the importance of this genera goes far beyond this, due to the vast amount of possible applications in agriculture and industry in the form of bioremediation, industrial enzymes, food additives, probiotics, antibiotics, pigments, biofuel production and battery components (Kunamneni et al. 2014; Kalsoom et al. 2019; Li et al. 2019, 2021; Shen et al. 2019; Daccò et al. 2020; Ghosh et al. 2020; Venil et al. 2020). Since some *Trichoderma* species can cause losses in mushroom production and infections in immunocompromised humans (Kredics et al. 2021), this makes an accurate species identification particularly important.

The taxonomy of *Trichoderma* has a long history of changes that were instigated by taxonomists who played a crucial role in the development and enhancement of the identification methods of *Trichoderma*. The genus was first introduced more than 200 years ago by Persoon in 1794 (Druzhinina et al. 2006). In 1966, the taxonomy of *Trichoderma* was reviewed based on the species' life cycle (Rifai and Webster 1966), and the taxonomy of *Trichoderma* species was re-shaped (Druzhinina et al. 2006). Rifai revised the genus and proposed nine aggregated species (Rifai 1969) that were later redefined in sections by Bissett (Bissett 1984, 1991a, b, c), which differentiated new *Trichoderma* species within sections, and established 9 aggregates disposed into 4 sections based on morphological traits, resulting in 27 *Trichoderma* species. Less than a decade later, (Kindermann et al. 1998) presented the first ITS-based phylogeny of the whole genus. The first multigene phylogeny was ITS1, ITS2, *TEF* and *ECH42*, (Kullnig-Gradinger et al. 2002), which increased the number of species to 47.

Despite the advances in the identification of *Trichoderma*, the first steps into the “molecular era” revealed the lack of more strict quality control and standardization procedures during species identification, which favored the deposition of sequences with unreliable information. In 2005, the first attempt to standardize the identification of *Trichoderma* was made with the development of an automated method called TrichOKey (Druzhinina and Kubicek 2005; Druzhinina et al. 2005), which is considered the first fungal barcode for *Trichoderma* species, and is based on ITS1 and ITS2 regions. The low resolution of ITS regions for supporting species differentiation led to the development of a more robust method for the identification of *Trichoderma*, in which the most commonly used phylogenetic markers were added. This method is known as TrichoBLAST (Kopchinskiy et al. 2005).

A decade later, the taxonomy of *T. harzianum* species complex was revised and the identity of commercial strains of *T. harzianum* was determined (Chaverri et al. 2015) pointing out the low resolution of nuclear internal transcribed spacers rDNA regions for *Trichoderma* species delimitation. The mentioned study proposed the adoption of the translation elongation factor 1- α (*TEF*), as a more precise secondary barcode for *Trichoderma* species identification. Also in 2015, a list of 254 accepted names for *Trichoderma* species was

published (Bissett et al. 2015). Recently, in an effort to standardize taxonomy procedures for *Trichoderma*, a guideline on molecular identification of *Trichoderma* was published that highlighted the difficulties involved in molecular identification of species from this genus (Cai and Druzhinina 2021).

The development of molecular methods for identification of *Trichoderma* changed the criteria for species delimitation and favored the exponential growth in the number of species. However, accurate molecular identification of *Trichoderma* species can be a challenging process even for experts, and sometimes leads to incorrect species identification (Cai and Druzhinina 2021). Inaccurately identified species can become a source for further misidentification, perpetuating this taxonomical issue. The genus *Trichoderma* presents several invalid species names due to lack of compliance with “The International Code of Nomenclature for algae, fungi, and plants” (Turland et al. 2018) or incomplete deposition in public databases (May et al. 2019). One example is *Trichoderma cyanodichotomus* strain TW21990_1, a potential biocontrol agent that is capable of promoting plant growth (Li et al. 2018; Zhou et al. 2020). Despite its description and available genomic data in public repositories, *T. cyanodichotomus* is still considered an illegitimate name due to failure to comply with all rules of the International Code of Nomenclature for algae, fungi, and plants. *T. cyanodichotomus* was first isolated from sediments from across the Pacific Ocean at Dongting Lake, China, and presented the production of a blue-green pigment as a striking feature. Moreover, the strain TW21990_1, showed production of enzymes of industrial interest, such as cellulase, β -1,3-glucanase, chitinase and protease.

The present study revealed a new species that forms a monophyletic clade with *T. cyanodichotomus*, strain TW21990_1, named here as *T. juruarensis* and identified using morphological characters such as conidia, phialide and chlamydospore size combined with phylogenetic inference of *RPB2* and *TEF* genes.

Material And Methods

Trichoderma isolation from sediment collection

Trichoderma species were isolated from sediment samples collected along the Juruá River at the following coordinates: INPA 0105 and INPA 0106 (06° 44' 45.1" S, 070° 07' 35.0" W), INPA 0108 (06° 45' 58.8" S, 070° 26' 13.8" W), INPA 0111 (06° 49' 32.8" S, 070° 40' 59.5" W) and INPA 0149 (06° 33' 18.6" S, 068° 59' 22.9" W). The isolation was performed with standard procedures (Siddiquee 2017). The monosporic cultures were prepared using serial dilution; 300 μ L of sterile water was placed in a sporulating plate, 100 μ L of the conidia suspension was added to 900 μ L of sterile water and used for serial dilution until 10^{-7} . The last dilution was streaked onto water agar and a single colony was transferred to a new PDA plate.

Morphological evaluation

The morphological appearance was evaluated in potato dextrose agar (PDA), cornmeal dextrose agar (CMA) and synthetic low nutrient agar (SNA) media (Montoya et al. 2016) to determine the presence of pigments, autolytic activity, concentric rings, mycelium density and appearance, and conidiation color. Microstructures were evaluated using coverslip observation under a microscope (Carl ZEISS Axio Imager v2). The coverslips were previously placed in Petri dishes containing PDA medium. After inoculation, plates with coverslips were incubated for 4 days at 25 °C with photoperiod of 12:12 h light-dark, and observed under a microscope.

Measurements were based on 50 sampled units of the following structures: chlamydospores, microconidia, macroconidia, and phialides (Table 1).

Morphological structures were also evaluated using scanning electron microscopy (SEM). Culture plugs were fixed with Karnovsky's solution, rinsed with sodium cacodylate (0.1 M) for 20 min, post-fixed in osmium tetroxide for 40 min, dehydrated in ethanol series of different concentrations (20%, 30%, 50%, 70%, 90% and 95%), critical point dried, mounted in metal stubs, sputtered with gold and analyzed using conventional and field emission scanning electron microscopy (JSM-IT500HR InTouchScope™, Jeol).

DNA Extraction and Sequencing

DNA extraction followed the CTAB 2% method (Doyle and Doyle 1987) using mycelium grown in potato dextrose broth (PDB) for 4 days at 28 °C under 125 rpm. The DNA concentration was measured using spectrometry (NanoDrop1000) and the integrity was visualized on agarose gel (0.8%). The PCR reactions for the amplification of the ITS region and partial sequences of *TEF* and *RPB2* were prepared using an Easytaq kit (Synapse Biotechnology) with three paired primers: ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATG), which amplifies 550 bp corresponding to the internal transcribed spacer I, 5,8 rDNA and internal transcribed spacer II (White et al. 1990), fRPB2-5f (GAYGAYMGWGATCAYTTYGG) and fRPB2-7cr (CCCATRGCTbTGTYYY), which amplifies 800 bp corresponding to the partial sequence of second largest subunit of RNA polymerase II (Liu et al. 1999), and EF-1αF (ATGGGTAAGGARGACAAGAC) and EF-1αR (GGARGTACCAGTSATCATGTT) primers, which amplifies 500 bp corresponding to the partial sequence of the translation elongation factor gene (O'Donnell et al. 1998; Carbone and Kohn 1999).

The PCR condition for amplification of all loci was initial denaturation at 95 °C for 3 min., 35 cycles of denaturation at 95 °C for 45 sec., annealing at a temperature of 55 °C for 45 sec., extension at 72 °C for 1 min., and final extension at 72°C for 5 min. The PCR products were resolved in agarose gel (1.5%) for confirmation of amplicon length using the 1 kb marker (Invitrogen). The PCR purification was carried out using exoSAP-IT (ThermoFisher; catalog number: 78200.200.UL), according to the manufacturer's recommendations. The sequencing reactions were performed in a volume of 10 µL, containing 2 µL of ultrapure water, 1.5 µL of BigDye buffer, 0.5 µL of BigDye terminator v3.1 (Thermo Fisher), 1 µL of each primer and 5 µL of the purified PCR products. The cycling conditions were 96 °C for 1 minute, followed by 35 cycles at 96 °C for 15 seconds, 50 °C for 15 seconds, and 60 °C for 4 minutes. The sequencing was performed using a genetic analyzer (3500, Thermo Fisher).

Phylogenetic analyses

Consensus sequences of the ITS region and partial gene sequences of *TEF* and *RPB2* were obtained manually based on the alignment of forward and reverse sequences as well as electropherogram analysis. The obtained sequences were deposited in Genbank under accession numbers OL639119-OL639123 (ITS), OL782574-OL782578 (*TEF*) and OL782579-OL782583 (*RPB2*).

The ITS sequences were used to confirm the genus as previously determined by morphological traits. For the identification of the isolates INPA 0105, INPA 0106, INPA 0108, INPA 0111 and INPA 0149 at species level, a dataset based on the concatenation of partial sequences of the genes *TEF* and *RPB2* of closely related

species observed in previous phylogenetic inference totaled 36 species of *Trichoderma* of clade I, 11 species of clade II and 5 species of clade IV, in addition to *Arachnocrea scabrida*, which was used as an outgroup (Table 2). The sequences were aligned in the MAFFT online platform (<https://mafft.cbrc.jp/alignment/software/>) and submitted to maximum-likelihood (ML) analysis in IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016) and Bayesian Inference (BI) in CIPRES (Miller et al. 2011).

BI analysis was based on the model adopted in PAUP* 4 and MrModeltest2 v2 (Nylander 2004). All sites in the loci were considered, and the analysis was performed for ten million generations, with the first 25% of the trees discarded and burned using the MrBayes v 3.7 tool available from CIPRES (<https://www.phylo.org/>). Posterior probability (PP) and tree topology were visualized using FigTree v 1.1.2 (Rambaut 2009). The consensus tree of the ML and BI analyses was generated manually from the topology obtained by FigTree in ML analysis with the bootstrap values, plus the posterior probability values generated by the BI analysis, using the CorelDraw editing package 2020.

For the clade level analysis of the isolates obtained in the study, a phylogenetic inference using the Maximum Likelihood (ML) method was performed with 1,000 replicates in the IQ-TREE platform (<http://iqtree.cibiv.univie.ac.at/>). This was based on the dataset of partial sequences of the *RPB2* gene of representative species of all clades, as reported in the authoritative guidelines on molecular identification of *Trichoderma* (Cai and Druzhinina 2021).

Antagonistic activity

The inhibitory effect was estimated *in vitro* by using paired-cultures of *T. juruarens* against the plant pathogens *Sclerotium rolfii*, *Colletotrichum siamense*, *Fusarium decemcellulare* and *Corynespora cassiicola* (Table 3). *Trichoderma* isolates were grown for 7 days in PDA and 8 mm plugs were used for the paired culture. The plant pathogens were pre-incubated for the antagonism assay according to the growing speed of each pathogen. The only pathogen without an incubation period was *Sclerotium rolfii*, due to its fast-growing characteristics. For *Colletotrichum siamense*, a pre-incubation period of 2 days was adopted. For *Fusarium decemcellulare* and for *Corynespora cassiicola*, 5 days and 3 days were adopted, respectively. Plugs were placed on opposite sides of the plate containing the pre-incubated pathogen, with 5 cm between plugs and 1 cm from the plate's edge. The culture was kept at 25 °C under a photoperiod of 12:12 h light-dark for 7 days. As a control, the plant pathogens were inoculated without the presence of a *Trichoderma* strain. The area of plant pathogen radial growth was determined using ImageJ (Schneider et al. 2012) and the inhibition of plant pathogens by *Trichoderma* was measured based on the relationship between control plates and paired culture using the following formula (Erazo et al. 2021):

$$\text{Inhibition \%} = [(C - T) \div C] \times 100$$

Where, C: control area, and T: area of the pathogen under the influence of a *Trichoderma* isolate.

Results

Phylogenetic analysis

The alignment used in the phylogenetic analyses for species delimitation resulted in 1,218 characters including gaps, in which the *TEF* contributed 551 characters and *RPB2* 667 characters. The best model adopted by PAUP*4 for *TEF* and *RPB2* in the BI data was GTR+I+G. For the ML analysis, the best fitting model was TN+F+I+G4 for *TEF* and TIM3e+I+G4 for *RPB2*. All isolates of this study grouped together with high PP and bootstrap values and formed a sister clade with *T. cyanodichotomus*. This data, in conjunction with morphological analysis, supported the proposition of a new fungal species, named here as *Trichoderma juruarens* (Fig. 1).

The phylogram generated by the phylogenetic analysis of partial sequences of the *RPB2* gene from representative species of all known *Trichoderma* clades indicated that all the isolates used in this study grouped with the invalidly named species *Trichoderma cyanodichotomus*, thus forming a distinct monophyletic clade, named here as clade 9 (Fig. 2).

Taxonomy

Trichoderma juruarens, R. Gwinner, T.F. Sousa and G.F. Silva, sp. nov., Fig. 1, Fig. 3; Mycobank: MB842019 accession numbers: ITS = OL639121; *RPB2*= OL782574; *TEF* = OL782580.

Etymology: refers to the Juruá River located in Brazilian Amazon

Holotype: INPA 0108

Macromorphology: Colonies at 25 °C and growing for 72 hours in PDA medium present white mycelium of a cottony appearance in the center and translucent sparse aerial mycelium at the border with low conidia and conidiophore production. No pigment or exudate production was observed. Colony radius after 72 h on PDA. Optimum growth temperature at 30 °C, 69.8–75 mm, 48.4–54.2 mm at 25 °C and 7.4–30.8 mm at 35 °C (Fig. 3). Colonies at 25 °C and growing for 7 days in CMA medium present white mycelium. Most colonies at 25 °C and growing for 7 days in CMA medium present white mycelium of a cottony appearance in the center and border, but can also present homogeneous white cottony mycelium. No pigment or exudate production was observed. Colony radius after 72 h on CMA. Optimum growth temperature at 30 °C 45.9–48.5 mm and 37.3–40.2 mm at 25 °C, absent at 35 °C (Fig. 3). Colonies at 25 °C and growing for 7 days in SNA medium present a homogeneous, translucent, sparse, aerial mycelium. No exudate or pigment production. Colony radius after 72 h on SNA. Optimum growth temperature at 30 °C 35.1–39.3 mm, 27.8–44.9 mm at 25 °C and 14.1–27 mm at 35 °C (Fig. 3).

Micromorphology: conidiophores present smooth-walls containing 2–3 lageniform phialides with a size of 4.33–6.48 µm (M = 5.01) 6.61–7.28 µm (M = 6.31 µm) and with the presence of green globose to obovoid macroconidia measuring 1.72–3.36 µm (M = 1.93 µm) 1.32–2.73 µm (M = 1.53 µm) and microconidia with a length of 0.96–1.54 (M = 1.2) 1.01–1.22 (M = 1.1) (Fig. 3). Presence of chlamydospores with 4.94–7.28 µm (M=6.99 µm) 6.61–7.28 µm (M = 6.31 µm) (Fig. 3).

Sexual stage: Not observed

Distribution: Brazil, Amazon.

Notes: Differs from *T. cyanodichotomus* by its smaller conidia and chlamydospores sizes, as well as low conidiophore production in 7 days growing at 25 °C in PDA media, and absence of abundant blue-green pigmentation under these conditions.

Antagonistic activity

T. juruarensis presented *in vitro* inhibition for all tested phytopathogens (Table 3 and Fig. 4), and it also promoted mean inhibition values ranging from 43% to 61% against plant pathogens. The pattern of inhibition against distinct pathogens suggests that *T. juruarensis* should be studied as a potential biocontrol agent. *T. juruarensis* apparently inhibited the phytopathogens through competition, but further studies are still necessary in order to understand the metabolic response of *T. juruarensis* during the inhibition process.

Discussion

The Amazonian biome hosts a rich fungal biodiversity and, even with numerous reports of new fungi species from this biome, the number of novel species of *Trichoderma* isolated from the Brazilian Amazon is still low when compared to the great diversity of Amazonian species (Ritter et al. 2020). In this paper, a new *Trichoderma* species is described and a new clade is proposed. *T. juruarensis* is closely related to two *Trichoderma* species: *T. cyanodichotomus* and *T. lycogaloides*. *T. cyanodichotomus* is currently under an invalid name due to lack of compliance with “The International Code of Nomenclature for algae, fungi, and plants” (Turland et al. 2018), therefore the identification of closely related species may contribute to a better understanding of *Trichoderma* taxonomy. Previously classified as a species of *Sarawakus*, *S. lycogaloides* was revised several times until it was categorized as a *Trichoderma* species under the name of *T. lycogaloides* (Jaklitsch et al. 2014). The phylogenetic relationship of *T. juruarensis* with the most closely related species reveals a distinct clade of *Trichoderma*.

Phylogenetically, *T. juruarensis* is closely related to *T. cyanodichotomus* with few SNP's (single nucleotide polymorphisms) in the partial sequences of *TEF* and *RPB2* genes in relation to *T. cyanodichotomus*, but isolates related in the present study collected in different points along the river, share the same SNP's. These molecular data, in conjunction with morphological characters such as conidia and chlamydospores sizes, reinforce the proposition of *T. juruarensis* sp. nov.

The taxonomy of the *Trichoderma* genera was recently revisited and a clade system based on *RPB2* sequence data was proposed (Cai and Druzhinina 2021). Herein, we perform a phylogenetic inference using *RPB2* sequences from representative species belonging to all known clades, including for the first time the species *T. cyanodichotomus* and *T. juruarensis* sp. nov.. Surprisingly, both species formed a monophyletic clade and were not grouped with any other species. To provide continuity of this systematization of the clade, we denominate this distinct clade as clade 9. Our data indicate that *T. lycogaloides* formed a sister clade with clade 9 and separate from clade 1 (Fig. 1).

Similarly, to *T. cyanodichotomus*, the blue-green pigment was observed for *T. juruarensis* sp. nov. Nonetheless, this feature seems inconstant and presents an uneven pattern under *in vitro* conditions, while *T. cyanodichotomus* presents a homogeneous production of blue-green pigment in CMA medium (Li et al. 2018).

Trichoderma juruarense sp. nov. showed *in vitro* inhibition of phytopathogens of agricultural importance and may be a promising ally in biological control, as has already been observed in other species of *Trichoderma* (Moutassem et al.; Ghazanfar et al. 2018; Díaz-Gutiérrez et al. 2021). In view of the biotechnological potential of this species, further studies of the whole genome and metabolomics that explore the secondary metabolite arsenal will be the next steps for this new fungal species.

Herein, we presented a novel species of *Trichoderma* that is closely related to an isolate currently under an invalid species name. Therefore, with the aim of a better taxonomic resolution of the genus *Trichoderma*, this work contributes to resolving this taxonomic issue via the first described species belonging to this group under a legitimate name. Furthermore, our data indicate that the monophyletic group composed by *T. juruarense* and *T. cyanodichotomus* is a new *Trichoderma* clade.

Declarations

Author contributions

Gwinner R. concebeu e planejou os experimentos. Sousa, T.F foi responsável pela análise filogenética. Bandeira, I.C foi responsável pela análise morfológica. Sousa, S. B e Castro, G.S foram responsáveis pelos testes de antagonismo e Silva G.F é o líder da pesquisa e orientou o desenvolvimento do estudo, Koolen H.H, Hanada R.E, Faria, J.V supervisionaram os resultados deste trabalho. Todos os autores discutiram e corrigiram o texto e contribuíram para o manuscrito final. All the authors read and approved the final manuscript.

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Conflict of interest: The authors declare no competing interests.

Legal authorizations:

Ministry of Environment - Council for the Management of Genetic Patrimony - (CGEN)

Date availability

Datasets generated and analyzed during the current study are available either in GenBank at NCBI (National Center for Biotechnology Information) or included in this published article (and its supplementary information files).

References

- Atanasova L, Jaklitsch WM, Komoń-Zelazowska M, et al (2010) Clonal Species *Trichoderma parareesei* sp. nov. Likely Resembles the Ancestor of the Cellulase Producer *Hypocrea jecorina* / *T. reesei*. *Appl Environ Microbiol* 76:7259–7267. <https://doi.org/10.1128/AEM.01184-10>
- Bissett J (1984) A revision of the genus *Trichoderma* . I. Section *Longibrachiatum* sect. nov. *Can J Bot* 62:924–931. <https://doi.org/10.1139/b84-131>
- Bissett J (1991a) A revision of the genus *Trichoderma* . II. Infrageneric classification. *Can J Bot* 69:2357–2372. <https://doi.org/10.1139/b91-297>
- Bissett J (1991b) A revision of the genus *Trichoderma* . III. Section *Pachybasium*. *Can J Bot* 69:2373–2417. <https://doi.org/10.1139/b91-298>
- Bissett J (1991c) A revision of the genus *Trichoderma* . IV. Additional notes on section *Longibrachiatum*. *Can J Bot* 69:2418–2420. <https://doi.org/10.1139/b91-299>
- Bissett J, Gams W, Jaklitsch W, Samuels GJ (2015) Accepted *Trichoderma* names in the year 2015. *IMA Fungus* 6:263–295. <https://doi.org/10.5598/ima fungus.2015.06.02.02>
- Bustamante DE, Calderon MS, Leiva S, et al (2021) Three new species of *Trichoderma* in the *Harzianum* and *Longibrachiatum* lineages from Peruvian cacao crop soils based on an integrative approach. *Mycologia* 113:1–17. <https://doi.org/10.1080/00275514.2021.1917243>
- Cai F, Druzhinina IS (2021) In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. *Fungal Divers* 107:1–69. <https://doi.org/10.1007/s13225-020-00464-4>
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553–556. <https://doi.org/10.1080/00275514.1999.12061051>
- Chaverri P, Branco-Rocha F, Jaklitsch W, et al (2015) Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia* 107:558–590. <https://doi.org/10.3852/14-147>

- Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003) *Hypocrea* / *Trichoderma*: species with conidiophore elongations and green conidia. *Mycologia* 95:1100–1140. <https://doi.org/10.1080/15572536.2004.11833023>
- Chaverri P, Gazis RO, Samuels GJ (2011) *Trichoderma amazonicum*, a new endophytic species on *Hevea brasiliensis* and *H. guianensis* from the Amazon basin. *Mycologia* 103:139–151. <https://doi.org/10.3852/10-078>
- Chaverri P, Samuels GJ (2013) Evolution of habitat preference and nutrition mode in a cosmopolitan fungal genus with evidence of interkingdom host jumps and major shifts in ecology. *Evolution (N Y)* 67:2823–2837. <https://doi.org/10.1111/evo.12169>
- Chaverri P, Samuels GJ (2003) *Hypocrea/Trichoderma* (ascomycota, hypocreales, hypocreaceae): species with green ascospores
- Chen K, Zhuang W-Y (2016) *Trichoderma shennongjianum* and *Trichoderma tibetense*, two new soil-inhabiting species in the *Strictipile* clade. *Mycoscience* 57:311–319. <https://doi.org/10.1016/j.myc.2016.04.005>
- Chen K, Zhuang W-Y (2017a) Discovery from a large-scaled survey of *Trichoderma* in soil of China. *Sci Rep* 7:9090. <https://doi.org/10.1038/s41598-017-07807-3>
- Chen K, Zhuang W-Y (2017b) Three New Soil-inhabiting Species of *Trichoderma* in the *Stromaticum* Clade with Test of Their Antagonism to Pathogens. *Curr Microbiol* 74:1049–1060. <https://doi.org/10.1007/s00284-017-1282-2>
- Chernomor O, von Haeseler A, Minh BQ (2016) Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Syst Biol* 65:997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Colwell RK (2000) A barrier runs through it ... or maybe just a river. *Proc Natl Acad Sci* 97:13470–13472. <https://doi.org/10.1073/pnas.250497697>
- da Silva Abel EL, Delgado RC, Vilanova RS, et al (2021) Environmental dynamics of the Juruá watershed in the Amazon. *Environ Dev Sustain* 23:6769–6785. <https://doi.org/10.1007/s10668-020-00890-z>
- Daccò C, Nicola L, Temporiti MEE, et al (2020) *Trichoderma*: Evaluation of Its Degrading Abilities for the Bioremediation of Hydrocarbon Complex Mixtures. *Appl Sci* 10:3152. <https://doi.org/10.3390/app10093152>
- Degenkolb T, Dieckmann R, Nielsen KF, et al (2008) The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. *Micol Prog* 7:177–219. <https://doi.org/10.1007/s11557-008-0563-3>
- Del-Rio G, Mutchler MJ, Costa B, et al (2021) Birds of the Juruá River: extensive várzea forest as a barrier to terra firme birds. *J Ornithol* 162:565–577. <https://doi.org/10.1007/s10336-020-01850-0>
- Díaz-Gutiérrez C, Arroyave C, Llugany M, et al (2021) *Trichoderma asperellum* as a preventive and curative agent to control *Fusarium* wilt in *Stevia rebaudiana*. *Biol Control* 155:104537. <https://doi.org/10.1016/j.biocontrol.2021.104537>

Doyle J, Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue

Druzhinina I, Kubicek CP (2005) Species concepts and biodiversity in *Trichoderma* and *Hypocrea*: from aggregate species to species clusters? *J Zhejiang Univ Sci* 6B:100–112.
<https://doi.org/10.1631/jzus.2005.B0100>

Druzhinina IS, Komoń-Zelazowska M, Ismaiel A, et al (2012) Molecular phylogeny and species delimitation in the section *Longibrachiatum* of *Trichoderma*. *Fungal Genet Biol* 49:358–368.
<https://doi.org/10.1016/j.fgb.2012.02.004>

Druzhinina IS, Kopchinskiy AG, Komoń M, et al (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genet Biol* 42:813–828. <https://doi.org/10.1016/j.fgb.2005.06.007>

Druzhinina IS, Kopchinskiy AG, Kubicek CP (2006) The first 100 *Trichoderma* species characterized by molecular data. *Mycoscience* 47:55–64. <https://doi.org/10.1007/S10267-006-0279-7>

Erazo JG, Palacios SA, Pastor N, et al (2021) Biocontrol mechanisms of *Trichoderma harzianum* ITEM 3636 against peanut brown root rot caused by *Fusarium solani* RC 386. *Biol Control* 164:104774.
<https://doi.org/10.1016/j.biocontrol.2021.104774>

Ghazanfar M, Raza M, Raza W, et al (2018) *Trichoderma* as potential biocontrol agent, its exploitation in agriculture: a review. *esciencepress.net* 109

Ghosh SK, Bera T, Chakrabarty AM (2020) Microbial siderophore – A boon to agricultural sciences. *Biol Control* 144:104214. <https://doi.org/10.1016/j.biocontrol.2020.104214>

Hanada RE, de Jorge Souza T, Pomella AWV, et al (2008) *Trichoderma martiale* sp. nov., a new endophyte from sapwood of *Theobroma cacao* with a potential for biological control. *Mycol Res* 112:1335–1343.
<https://doi.org/10.1016/j.mycres.2008.06.022>

Hanada RE, Pomella AWV, Soberanis W, et al (2009) Biocontrol potential of *Trichoderma martiale* against the black-pod disease (*Phytophthora palmivora*) of cacao. *Biol Control* 50:143–149.
<https://doi.org/10.1016/j.biocontrol.2009.04.005>

Holmes KA, Schroers H-J, Thomas SE, et al (2004) Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. *Mycol Prog* 3:199–210.
<https://doi.org/10.1007/s11557-006-0090-z>

Jaklitsch WM (2009) European species of *Hypocrea* Part I. The green-spored species. *Stud Mycol* 63:1–91.
<https://doi.org/10.3114/sim.2009.63.01>

Jaklitsch WM (2011) European species of *Hypocrea* part II: species with hyaline ascospores. *Fungal Divers* 48:1–250. <https://doi.org/10.1007/s13225-011-0088-y>

Jaklitsch WM, Gruber S, Voglmayr H (2008a) *Hypocrea seppoi*, a new stipitate species from Finland. *Karstenia* 48:1–11. <https://doi.org/10.29203/ka.2008.423>

- Jaklitsch WM, Lechat C, Voglmayr H (2014) The rise and fall of *Sarawakus* (Hypocreaceae, Ascomycota). *Mycologia* 106:133–144. <https://doi.org/10.3852/13-117>
- Jaklitsch WM, Pöldmaa K, Samuels GJ (2008b) Reconsideration of *Protocrea* (Hypocreales, Hypocreaceae). *Mycologia* 100:962–984. <https://doi.org/10.3852/08-101>
- Jaklitsch WM, Samuels GJ, Ismaiel A, Voglmayr H (2013) Disentangling the *Trichoderma viridescens* complex. *Persoonia* 31:112–146. <https://doi.org/10.3767/003158513X672234>
- Jaklitsch WM, Voglmayr H (2015) Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. *Stud Mycol* 80:1–87. <https://doi.org/10.1016/j.simyco.2014.11.001>
- Jaklitsch WM, Voglmayr H (2013) New combinations in *Trichoderma* (<I>Hypocreaceae , Hypocreales</I>). *Mycotaxon* 126:143–156. <https://doi.org/10.5248/126.143>
- Junk W, Bayley P, Of RS-C special publication, 1989 U (1989) The flood pulse concept in river-floodplain systems. *cs.ru.nl* 106:110–127
- Junk WJ, Piedade MTF, Parolin P, et al (2010) Ecophysiology, Biodiversity and Sustainable Management of Central Amazonian Floodplain Forests: A Synthesis. In: Junk WJ, Piedade MTF, Wittmann F, et al. (eds). Springer Netherlands, Dordrecht, pp 511–540
- Kalsoom R, Ahmed S, Nadeem M, et al (2019) Biosynthesis and extraction of cellulase produced by *Trichoderma* on agro-wastes. *Int J Environ Sci Technol* 16:921–928. <https://doi.org/10.1007/s13762-018-1717-8>
- Kim CS, Shirouzu T, Nakagiri A, et al (2012) *Trichoderma mienum* sp. nov., isolated from mushroom farms in Japan. *Antonie Van Leeuwenhoek* 102:629–641. <https://doi.org/10.1007/s10482-012-9758-3>
- Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP (1998) Phylogeny of the Genus *Trichoderma* Based on Sequence Analysis of the Internal Transcribed Spacer Region 1 of the rDNA Cluster. *Fungal Genet Biol* 24:298–309. <https://doi.org/10.1006/fgbi.1998.1049>
- Kopchinskiy A, Komoń M, Kubicek CP, Druzhinina IS (2005) Tricho Blast: A Multilocus Database for *Trichoderma* and *Hypocrea* Identifications. *Mycol Res* 109:658–660. <https://doi.org/10.1017/S0953756205233397>
- Kredics L, Naeimi S, Hatvani L, et al (2021) ‘The Good, the Bad and the Ugly’ in the shades of green: the genus *Trichoderma* in the spotlight. *Indian Phytopathol* 74:403–411. <https://doi.org/10.1007/S42360-021-00352-0>
- Kullnig-Gradinger CM, Szakacs G, Kubicek CP (2002) Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. *Mycol Res* 106:757–767. <https://doi.org/10.1017/S0953756202006172>
- Kunamneni A, Plou FJ, Alcalde M, Ballesteros A (2014) *Trichoderma* Enzymes for Food Industries. In: *Biotechnology and Biology of Trichoderma*. Elsevier, pp 339–344

- Li J, Wu Y, Chen K, et al (2018) *Trichoderma cyanodichotomus* sp. nov., a new soil-inhabiting species with a potential for biological control. *Can J Microbiol* 64:1020–1029. <https://doi.org/10.1139/CJM-2018-0224>
- Li L, Zeng X, Chen J, et al (2021) Genome Sequence of the Fungus *Trichoderma asperellum* SM-12F1 Revealing Candidate Functions of Growth Promotion, Biocontrol, and Bioremediation. *PhytoFrontiers*TM 1:239–243. <https://doi.org/10.1094/PHYTOFR-12-20-0052-A>
- Li M-F, Li G-H, Zhang K-Q (2019) Non-Volatile Metabolites from *Trichoderma* spp. *Metabolites* 9:58. <https://doi.org/10.3390/metabo9030058>
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic Relationships Among Ascomycetes: Evidence from an RNA Polymerase II Subunit. *Mol Biol Evol* 16:1799–1808
- López-Quintero CA, Atanasova L, Franco-Molano AE, et al (2013) DNA barcoding survey of *Trichoderma* diversity in soil and litter of the Colombian lowland Amazonian rainforest reveals *Trichoderma strigosellum* sp. nov. and other species. *Antonie Van Leeuwenhoek* 104:657–674. <https://doi.org/10.1007/s10482-013-9975-4>
- May TW, Redhead SA, Bensch K, et al (2019) Chapter F of the International Code of Nomenclature for algae, fungi, and plants as approved by the 11th International Mycological Congress, San Juan, Puerto Rico, July 2018. *IMA Fungus* 10:21. <https://doi.org/10.1186/s43008-019-0019-1>
- Miller MA, Pfeiffer W, Schwartz T (2011) The CIPRES science gateway. In: *Proceedings of the 2011 TeraGrid Conference on Extreme Digital Discovery*. ACM Press, New York, New York, USA, p 1
- Montoya QV, Meirelles LA, Chaverri P, Rodrigues A (2016) Unraveling *Trichoderma* species in the attine ant environment: description of three new taxa. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol* 109:633–651. <https://doi.org/10.1007/s10482-016-0666-9>
- Moutassem D, Belabid L, Protection YB-J of C, 2020 undefined Efficiency of secondary metabolites produced by *Trichoderma* spp. in the biological control of *Fusarium* wilt in chickpea. *jcp.modares.ac.ir* 2020:217–231
- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>
- Nováková A, Kubátová A, Valinová Š, et al (2015) *Trichoderma fassatia*, a new species from the section *Pachybasium* isolated from soil in the Czech Republic. *czechmycology.org* 67:227–231
- Nuankaew K, Sotome K, Lumyong S, Boonlue S (2018) *Trichoderma polyalthiae* sp. nov., an endophytic fungus from *Polyalthia debilis*. *Phytotaxa* 371:273. <https://doi.org/10.11646/phytotaxa.371.5.1>
- Nylander JAA (2004) MrModeltest v2. Progr Distrib by author
- O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc Natl Acad Sci* 95:2044–2049. <https://doi.org/10.1073/pnas.95.5.2044>

- Overton BE, Stewart EL, Geiser DM (2006) Taxonomy and phylogenetic relationships of nine species of *Hypocrea* with anamorphs assignable to *Trichoderma* section *Hypocreanum*. *Stud Mycol* 56:39–65. <https://doi.org/10.3114/sim.2006.56.02>
- Paes MX, Campos-Silva JV, de Oliveira JAP (2021) Integrating circular economy in urban Amazon. *npj Urban Sustain* 1:29. <https://doi.org/10.1038/s42949-021-00031-z>
- Rambaut A (2009) FigTree. Tree figure drawing tool. ci.nii.ac.jp
- Rifai M (1969) A revision of the genus *Trichoderma*. *ci.nii.ac.jp* 116:1–56
- Rifai M, Webster J (1966) Culture studies on *Hypocrea* and *Trichoderma*. *Trans Br Mycol Soc* 49:289-IN11. [https://doi.org/10.1016/S0007-1536\(66\)80063-3](https://doi.org/10.1016/S0007-1536(66)80063-3)
- Ritter CD, Dunthorn M, Anslan S, et al (2020) Advancing biodiversity assessments with environmental DNA: Long-read technologies help reveal the drivers of Amazonian fungal diversity. *Ecol Evol* 10:7509–7524. <https://doi.org/10.1002/ece3.6477>
- Robbertse B, Strope PK, Chaverri P, et al (2017) Improving taxonomic accuracy for fungi in public sequence databases: applying ‘one name one species’ in well-defined genera with *Trichoderma*/*Hypocrea* as a test case. *Database* 2017:. <https://doi.org/10.1093/database/bax072>
- Samuels GJ (2006) *Trichoderma*: Systematics, the Sexual State, and Ecology. *Phytopathology®* 96:195–206. <https://doi.org/10.1094/PHYTO-96-0195>
- Samuels GJ, Dodd SL, Gams W, et al (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. *Mycologia* 94:146–170. <https://doi.org/10.1080/15572536.2003.11833257>
- Samuels GJ, Dodd SL, Lu B-S, et al (2006a) The *Trichoderma koningii* aggregate species. *Stud Mycol* 56:67–133. <https://doi.org/10.3114/sim.2006.56.03>
- Samuels GJ, Ismaiel A (2011) *Hypocrea peltata*: a mycological Dr Jekyll and Mr Hyde? *Mycologia* 103:616–630. <https://doi.org/10.3852/10-227>
- Samuels GJ, Ismaiel A, Bon M-C, et al (2010) *Trichoderma asperellum* sensu lato consists of two cryptic species. *Mycologia* 102:944–966. <https://doi.org/10.3852/09-243>
- Samuels GJ, Ismaiel A, de Souza J, Chaverri P (2012) *Trichoderma stromaticum* and its overseas relatives. *Mycol Prog* 11:215–254. <https://doi.org/10.1007/s11557-011-0743-4>
- Samuels GJ, Pardo-Schultheiss R, Hebbar KP, et al (2000) *Trichoderma stromaticum* sp. nov., a parasite of the cacao witches broom pathogen. *Mycol Res* 104:760–764. <https://doi.org/10.1017/S0953756299001938>
- Samuels GJ, Suarez C, Solis K, et al (2006b) *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. *Mycol Res* 110:381–392.

<https://doi.org/10.1016/j.mycres.2006.01.009>

Sandoval-Denis M, Sutton DA, Cano-Lira JF, et al (2014) Phylogeny of the Clinically Relevant Species of the Emerging Fungus *Trichoderma* and Their Antifungal Susceptibilities. *J Clin Microbiol* 52:2112–2125. <https://doi.org/10.1128/JCM.00429-14>

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>

Shen S, Xia X, Zhong Y, et al (2019) Implanting Niobium Carbide into *Trichoderma* Spore Carbon: a New Advanced Host for Sulfur Cathodes. *Adv Mater* 31:1900009. <https://doi.org/10.1002/adma.201900009>

Siddiquee S (2017) Practical Handbook of the Biology and Molecular Diversity of *Trichoderma* Species from Tropical Regions. Springer International Publishing, Cham

Turland N, Wiersema J, Barrie F, Greuter W (2018) International Code of Nomenclature for algae, fungi, and plants. Koeltz Botanical Books

Venil CK, Velmurugan P, Dufossé L, et al (2020) Fungal Pigments: Potential Coloring Compounds for Wide Ranging Applications in Textile Dyeing. *J Fungi* 6:68. <https://doi.org/10.3390/jof6020068>

Zhou Y, Wang Y, Chen K, et al (2020) Near-Complete Genomes of Two *Trichoderma* Species: A Resource for Biological Control of Plant Pathogens. *Mol Plant-Microbe Interact* 33:1036–1039. <https://doi.org/10.1094/MPMI-03-20-0076-A>

Zhu ZX, Zhuang WY (2015a) *Trichoderma* (*Hypocrea*) species with green ascospores from China. *Persoonia* 34:113–129. <https://doi.org/10.3767/003158515X686732>

Zhu ZX, Zhuang WY (2015b) Three new species of *Trichoderma* with hyaline ascospores from China. *Mycologia* 107:328–345. <https://doi.org/10.3852/14-141>

Tables

Table 1 Comparison of morphological traits of *Trichoderma juruarens* and closely related species. *M = mean value.

	<i>T. juruarens</i> sp. nov.	<i>T. lycogaloides</i> (Jaklitsch et al. 2014)	<i>T. cyanodichotomus</i> (Li et al. 2018)
Macroconidia	obovoid, smooth, green or yellowish green	oval or subglobose, rarely oblong green, thick-walled, smooth, eguttulate	Macro: ellipsoidal to obovoid, smooth-walled, green or yellowish green
length (µm)	(1.72)1.93–2.70(3.36) (M = 2.31 µm)	(6.5)9.5–13.5(15.0)	3.7–5.5 µm
width (µm)	(1.32)1.53–2.19(2.73) (M=1.84 µm)	(5.8)8.0–11.5(12.5)	3.4–4.6 µm
L/W ratio	(1.01)1.13–1.38(1.68) (M=1.26)		
Microconidia	green, globose	not observed	green, generally globose
length (µm)	0.96–1.54 µm (M=1.2)	-	1.0–2.3 µm
width (µm)	1.01–1.22 µm (M=1.1)	-	1.0–2.2 µm
L/W ratio			
Phialide	lageniform	cylindrical or lageniform	mostly lageniform and bowling pin shaped
length (µm)	(4.33)5.01–6.03(6.48) µm (M=5.45 µm)	22–43 µm	3.7–5.5 µm
width (µm)	(1.18)1.13–1.65(2.15) µm (M=1.47 µm)	4.2–7.5 µm	3.4–4.6 µm
L/W ratio	(2.96)3.4 –4.05(4.02) µm (M=3.76 µm)	3.5–7.6 µm	-
Chlamydospore	subglobose	not observed	subglobose to ellipsoidal, easily deciduous, massively produced on PDA
length (µm)	4.94–7.28 µm (M=6.99 µm)	-	7.3–12.6 µm
width (µm)	6.61–7.28 µm (M = 6.31 µm)	-	5.3–9.7 µm
L/W ratio	0.78–1.53 µm	-	-

Table 2 - Taxa used in the phylogenetic analysis, strain and GenBank accession numbers.

Genbank accession numbers						Reference
Species	Strain	Clade	ITS	<i>RPB2</i>	<i>TEF</i>	
<i>T. juruarense</i> sp. nov.	INPA 0108 ^T	Clade 9	OL639121	OL782574	OL782580	In this study
<i>T. juruarense</i> sp. nov.	INPA 0105	Clade 9	OL639119	OL782576	OL782581	In this study
<i>T. juruarense</i> sp. nov.	INPA 0106	Clade 9	OL639120	OL782577	OL782579	In this study
<i>T. juruarense</i> sp. nov.	INPA 0111	Clade 9	OL639122	OL782578	OL782582	In this study
<i>T. juruarense</i> sp. nov.	INPA 0149	Clade 9	OL639123	OL782575	OL782583	In this study
<i>Arachnocrea scabrida</i>	BEO 02-01	-	-	DQ834458.1	DQ834457.1	(Overton et al. 2006)
<i>Arachnocrea stipata</i>	-	-	-	EU710770.1	-	(Jaklitsch et al. 2008b)
<i>T. lycogaloides</i>	SL ^T	Clade 1	-	KF134792.1	KF134800.1	(Jaklitsch et al. 2014)
<i>T. tibetense</i>	HMAS 245010 ^T	Clade 1	NR_144881.1	KT735261.1	KT735254.1	(Chen and Zhuang 2016)
<i>T. chlamydosporicum</i>	HMAS 248850 ^T	Clade 1	NR_154577.1	KY687989.1	KY688052.1	(Chen and Zhuang 2017a)
<i>T. strictipile</i>	CBS 347.93 ^T	Clade 1	NR_134337.1	KJ842182.1	AY865644.1	(Druzhinina et al. 2005)
<i>T. tropicosinense</i>	HMAS 252546 ^T	Clade 1	NR_134441.1	KF923313.1	KF923286.1	(Zhu and Zhuang 2015a)
<i>T. longipile</i>	CBS 120953 ^T	Clade 1	NR_134354.1	FJ860542.2	FJ860643.1	(Jaklitsch 2009)
<i>T. shennongjianum</i>	HMAS 245009 ^T	Clade 1	NR_144880.1	KT735259.1	KT735253.1	(Chen and Zhuang 2016)
<i>T. phyllostachydis</i>	GJS 92-123 ^T	Clade 1	AY737755.1	AF545513.1	AY737745.1	(Chaverri et al. 2003)
<i>T. ganodermatis</i>	HMAS 248856 ^T	Clade 1	NR_154581.1	KY687995.1	KY688060.1	(Chen and Zhuang 2017a)

<i>T. crassum</i>	GJS 01-227	Clade 1	MH862406.1	AY481587.1	JN133572.1	(Chaverri and Samuels 2013)
<i>T. chromospermum</i>	GJS 94-67	Clade 1	AY737774.1	AY391912.1	AY737728.1	(Chaverri and Samuels 2003)
<i>T. gliocladium</i>	CBS 130009 ^T	Clade 1	NR_160252.1	KJ665271.1	KJ665502.1	(Jaklitsch and Voglmayr 2015)
<i>T. hainanense</i>	HMAS 248837 ^T	Clade 1	NR_154568.1	KY687976.1	KY688033.1	(Chen and Zhuang 2017a)
<i>T. silvae-virgineae</i>	CBS 120922 ^T	Clade 1	NR_134414.1	FJ860587.1	FJ860696.1	(Jaklitsch 2011)
<i>T. helicum</i>	DAOM 230016	Clade 1	DQ083022.1	KJ842200.1	EU280055.1	(Bissett et al. 2015)
<i>T. byssinum</i>	HMAS 248839	Clade 1	KY687922.1	KY687978.1	KY688036.1	(Chen and Zhuang 2017a)
<i>T. hebeiense</i>	HMAS 248743 ^T	Clade 1	NR_153278.1	KX344439.1	KX344434.1	(Chen and Zhuang 2017b)
<i>T. sichuanense</i>	HMAS 248736	Clade 1	KY014087.1	KX344436.1	KX344427.1	(Chen and Zhuang 2017b)
<i>T. verticillatum</i>	HMAS 248740 ^T	Clade 1	KY014091.1	KX344438.1	KX344431.1	(Chen and Zhuang 2017b)
<i>T. rossicum</i>	DAOM 230011 ^T	Clade 1	HQ342419.1	KJ842191.1	AY937441.1	(Samuels 2006)
<i>T. barbatum</i>	GJS 04-308 ^T	Clade 1	NR_134430.1	HQ342286.1	HQ342223.1	(Samuels et al. 2012)
<i>T. floccosum</i>	GJS 01-238 ^T	Clade 1	NR_137306.1	HQ342281.1	HQ342218.1	(Robbertse et al. 2017)
<i>T. ivoriense</i>	GJS 01-312 ^T	Clade 1	HQ342411.1	HQ342280.1	HQ342217.1	(Bissett et al. 2015)
<i>T. stromaticum</i>	GJS 97-183 ^T	Clade 1	AF097913.1	HQ342245.1	AY937418.1	(Samuels et al. 2000)
<i>T. vermipilum</i>	PPRI 3559 ^T	Clade 1	AF400268.1	HQ342282.1	HQ342219.1	(Kullnig-Gradinger et al. 2002)

<i>T. caesareum</i>	GJS 01-225 ^T	Clade 1	NR_134427.1	HQ342279.1	HQ342216.1	(Robbertse et al. 2017)
<i>T. medusae</i>	GJS 01-171 ^T	Clade 1	NR_134426.1	HQ342277.1	HQ342214.1	(Robbertse et al. 2017)
<i>T. lanuginosum</i>	GJS 01-176 ^T	Clade 1	NR_134429.1	HQ342284.1	HQ342221.1	(Robbertse et al. 2017)
<i>T. spirale</i>	DAOM 183974 ^T	Clade 1	NR_077177.1	AF545553.1	EU280049.1	(Robbertse et al. 2017)
<i>T. pyramidale</i>	CBS 135574 ^T	Clade 1	KX632513.1	KJ665334.1	KJ665699.1	(Jaklitsch and Voglmayr 2015)
<i>T. afroharzianum</i>	GJS 04-02	Clade 1	NR_137304.1	FJ442691.1	FJ463401.1	(Bissett et al. 2015)
<i>T. harzianum</i>	CBS 227.95	Clade 1	AF057605.1	AF545549.1	AF348100.1	(Samuels et al. 2002)
<i>T. inhamatum</i>	CBS 273.78 ^T	Clade 1	NR_134378.1	FJ442725.1	AF348099.1	(Robbertse et al. 2017)
<i>T. afarasin</i>	CBS 130755 ^T	Clade 1	NR_137301.1	FJ442799.1	AF348093.1	(Chaverri et al. 2015)
<i>T. cyanodichotomus</i>	TW21990-1 ^T	Clade 1	KY189312.1	KY707175.1	KY707174.1	(Li et al. 2018)
<i>T. fassatiae</i>	CCF 5000	Clade 2	LN866275.1	LN866276.1	LN866277.1	(Nováková et al. 2015)
<i>T. fertile</i>	DAOM 167161 ^T	Clade 2	NR_134336.1	AF545546.1	KJ871131.1	(Chaverri et al. 2003)
<i>T. fomiticola</i>	CBS 121136 ^T	Clade 2	NR_134391.1	FJ860538.1	FJ860639.1	(Jaklitsch 2009)
<i>T. hunua</i>	CBS 238.63 ^T	Clade 2	NR_160091.1	KJ665279.1	KJ665519.1	(Jaklitsch and Voglmayr 2015)
<i>T. mienum</i>	TUFC 61533 ^T	Clade 2	NR_134433.1	AB856754.1	JQ621978.1	(Kim et al. 2012)
<i>T. moravicum</i>	CBS 120539 ^T	Clade 2	NR_134398.1	FJ860548.1	FJ860651.1	(Jaklitsch 2011)
<i>T. oblongisporum</i>	DAOM 176226 ^T	Clade 2	NR_138437.1	KJ842199.1	KJ871142.1	(Bissett et al. 2015)
<i>T. semiorbis</i>	GJS 99-108 ^T	Clade 2	NR_134423.1	JN133567.1	JN133576.1	(Chaverri and

						Samuels 2013)
<i>T. euskadiense</i>	CBS 130013 ^T	Clade 3	NR_160254.1	KJ665269.1	KJ665492.1	(Jaklitsch and Voglmayr 2015)
<i>T. sinense</i>	DAOM 230004 ^T	Clade 3	NR_134425.1	JN175528.1	AY750889.1	(Druzhinina et al. 2012)
<i>T. konilangbra</i>	GJS 96- 147 ^T	Clade 3	MH862712.1	KJ665284.1	AY937425.1	(Samuels 2006)
<i>T. flagellatum</i>	PPRC- ET58	Clade 3	MH865822.1	JN258688	FJ763184.1	(Druzhinina et al. 2012)
<i>T. parareesei</i>	TUB F- 1066 ^T	Clade 3	MH863773.1	HM182963.1	GQ354353.1	(Atanasova et al. 2010)
<i>T. reesei</i>	CBS 383.78 ^T	Clade 3	Z31016	HM182969.1	DQ025754.2	(Atanasova et al. 2010)
<i>T. orientale</i>	GJS 04- 321	Clade 3	NR_111317.1	JN175522.1	JN175573.1	(Druzhinina et al. 2012)
<i>T. bissettii</i>	CBS 137447 ^T	Clade 3	NR_134442.1	KJ665244.1	HG931266.1	(Sandoval- Denis et al. 2014)
<i>T. longibrachiatum</i>	CBS 816.68 ^T	Clade 3	EU401556.1	DQ087242.1	AY865640.1	(Druzhinina et al. 2005)
<i>T. alcalifuscescens</i>	TFC 00- 36 ^T	Clade 4	FJ860722.1	DQ834462.1	FJ860610.1	(Overton et al. 2006)
<i>T. delicatulum</i>	CBS 120631 ^T	Clade 4	FJ860751.1	FJ860535.1	FJ860636.1	(Jaklitsch 2011)
<i>T. parmastoi</i>	TFC 97- 143 ^T	Clade 4	NR_134405.1	DQ834463.1	FJ860669.1	(Jaklitsch and Voglmayr 2013)
<i>T. peltatum</i>	GJS 08- 207 ^T	Clade 4	NR_134422.1	HQ260610.1	KR135819.1	(Samuels and Ismaiel 2011)
<i>T. polyalthiae</i>	UBZSN2- 1 ^T	Clade 4	MF135132.1	LC373011.1	LC328976.1	(Nuankaew et al. 2018)
<i>T. asperelloides</i>	GJS 04- 116	Clade 5	GU198301.1	GU248411.1	GU248412.1	(Samuels et al. 2010)
<i>T. asperellum</i>	CBS 433.97 ^T	Clade 5	NR_130668.1	EU248617	AY376058.1	(Bissett et al. 2015)
<i>T. atroviride</i>	CBS 142.95 ^T	Clade 5	AY380906.1	EU341801.1	AF456891.1	(Bissett et al. 2015)

<i>T. gamsii</i>	GJS 04-09	Clade 5	DQ315459.1	JN133561.1	DQ307541.1	(Samuels et al. 2006a)
<i>T. hamatum</i>	DAOM 167057 ^T	Clade 5	NR_134371.1	AF545548.1	AF456911.1	(Bissett et al. 2015)
<i>T. koningii</i>	CBS 457.96 ^T	Clade 5	MH862585.1	FJ442761	KC285594.1	(Jaklitsch et al. 2013)
<i>T. ovalisporum</i>	Dis70a ^T	Clade 5	AY380897.1	FJ442742.1	AY376037.1	(Holmes et al. 2004)
<i>T. stilbohypoxyli</i>	CBS 992.97 ^T	Clade 5	DQ313152.1	EU341805	DQ109546.1	(Samuels et al. 2006a)
<i>T. strigosellum</i>	CPK 3604 ^T	Clade 5	NR_134437.1	EU248607.1	JQ425705.1	(López-Quintero et al. 2013)
<i>T. strigosum</i>	CBS 348.93 ^T	Clade 5	NR_103571.1	AF545556	AY376057.1	(López-Quintero et al. 2013)
<i>T. theobromicola</i>	Dis 85f ^T	Clade 5	NR_134359.1	FJ007374	EU856321.1	(Samuels et al. 2006b)
<i>T. viride</i>	CBS 119325 ^T	Clade 5	NR_138441.1	EU711362.2	DQ672615.2	(Jaklitsch et al. 2008b)
<i>T. arundinaceum</i>	GJS 05-180	Clade 6	EU330928.1	EU338303.1	EU338275.1	(Degenkolb et al. 2008)
<i>T. aurantioeffusum</i>	CBS 119284 ^T	Clade 6	NR_134383.1	FJ860520.1	FJ860613.1	(Jaklitsch 2011)
<i>T. brevicompactum</i>	GJS 04-381	Clade 6	EU330941.1	EU338317.1	EU338299.1	(Degenkolb et al. 2008)
<i>T. deliquescent</i>	CBS 121132	Clade 6	NR_134394.1	FJ179609.1	FJ860644.1	(Jaklitsch et al. 2008a)
<i>T. luteocrystallinum</i>	CBS 123828 ^T	Clade 6	NR_134396.1	FJ860544.1	FJ860646.1	(Jaklitsch 2011)
<i>T. margaretense</i>	C.P.K. 3127 ^T	Clade 6	FJ860741.1	FJ860529.1	FJ860625.1	(Jaklitsch 2011)
<i>T. melanomagnum</i>	GJS 99-153 ^T	Clade 6	AY737770.1	AY391926.1	AY737751.1	(Chaverri and Samuels 2003)
<i>T. protrudens</i>	DIS 119F ^T	Clade 6	NR_134373.1	EU338322.1	EU338289.1	(Degenkolb et al. 2008)

<i>T. rodmanii</i>	GJS 91-88 ^T	Clade 6	NR_134374.1	EU338324.1	EU338286.1	(Degenkolb et al. 2008)
<i>T. turrialbense</i>	CBS 112445 ^T	Clade 6	NR_138448.1	EU338321.1	EU338284.1	(Degenkolb et al. 2008)
<i>T. applanatum</i>	HMAS 266666 ^T	Clade 7	NR_134443.1	KJ634726.1	KJ634759.1	(Zhu and Zhuang 2015b)
<i>T. austriacum</i>	CBS 122494 ^T	Clade 7	NR_134384.1	FJ860525.1	FJ860619.1	(Jaklitsch 2011)
<i>T. calamagrostidis</i>	CBS 121133 ^T	Clade 7	NR_134386.1	FJ860528.1	FJ860622.1	(Jaklitsch 2011)
<i>T. ceciliae</i>	CBS 130010 ^T	Clade 7	NR_160253.1	KJ665247.1	KJ665444.1	(Jaklitsch and Voglmayr 2015)
<i>T. citrinum</i>	C.P.K. 960	Clade 7	NR_134368.1	AF545561.1	FJ860631.1	(Bissett et al. 2015)
<i>T. decipiens</i>	CBS 121307 ^T	Clade 7	EF558546.1	KJ665256.1	FJ860635.1	(Jaklitsch and Voglmayr 2015)

Ex-type strains are indicated in “T” after the collection number

Table 3 Percentage of growth inhibition of plant pathogens by *Trichoderma juruarens*.

Pathogen	ID	Collection	Inhibition (%)	SD (%)
<i>Sclerotium rolfsii</i>	2941	INPA	51	31
<i>Colletotrichum siamense</i>	Coll 2N	Embrapa CPAA	50	2.5
<i>Fusarium decemcellulare</i>	307	Embrapa CPAA	61	14
<i>Corynespora cassiicola</i>	2671	INPA	43	11

Figures

Figure 1

Phylogram obtained from maximum-likelihood and Bayesian inference analysis by concatenation of the partial sequences of *TEF* and *RPB2*.

Figure 2

Phylogram obtained using maximum-likelihood (ML) analysis of the partial sequence of *RPB2* gene of representative species of all clades of *Trichoderma*. The new clade 9 contained *Trichoderma juruarens* sp. nov., and *Trichoderma cyanodichotomus* species is greenish-blue colored.

Figure 3

Trichoderma juruarens (INPA 0108). **a** in potato dextrose agar; **b** in cornmeal dextrose agar; **c** in synthetic low nutrient agar; **d** Conidiophore; **e** Anelar mycelium arrangement; **f** Chlamydospores; **g** Microconidia.

Figure 4

a Control **b** Inhibition effect promoted by *Trichoderma juruarens* against *Corynespora cassiicola*, *Fusarium decemcellulare*, *Sclerotium rolfsii*, and *Colletotrichum siamense*

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