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# Phylogenetic Analysis of *Trichoderma* Species Associated with Green Mold Disease on Mushrooms and Two New Pathogens on *Ganoderma sichuanense*

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**Abstract:** Edible and medicinal mushrooms are extensively cultivated and commercially consumed around the world. However, green mold disease (causal agent, *Trichoderma* spp.) has resulted in severe crop losses on mushroom farms worldwide in recent years and has become an obstacle to the development of the *Ganoderma* industry in China. In this study, a new species and a new fungal pathogen on *Ganoderma sichuanense* fruitbodies were identified based on the morphological characteristics and phylogenetic analysis of two genes, the translation elongation factor 1-α (TEF1) and the second-largest subunit of RNA polymerase II (RPB2) genes. The new species, *Trichoderma ganodermatigerum* sp. nov., belongs to the Harzianum clade, and the new fungal pathogen was identified as *Trichoderma koningiopsis*. Furthermore, in order to better understand the interaction between *Trichoderma* and mushrooms, as well as the potential biocontrol value of pathogenic *Trichoderma*, we summarized the *Trichoderma* species and their mushroom hosts as best as possible, and the phylogenetic relationships within mushroom pathogenic *Trichoderma* species were discussed.

**Keywords:** taxonomy; green mold disease; one new taxon; mycoparasites; biological agents



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## 1. Introduction

Mushrooms have been used by humans for millennia and are consumed for their nutritive and medicinal values [1,2]. Most of them are appreciated as delicacies and are extensively cultivated and commercially consumed in many countries. Some mushrooms also have high pharmacological activities, especially *Ganoderma* spp. [3,4]. *Ganoderma sichuanense*, described from China and previously confused with *G. lucidum*, an oriental fungus, has a long history in China, Japan, and other Asian countries for promoting health and longevity [5,6]. The mushroom is famous for its pharmacological effects [7,8] and is widely cultivated in northeastern China. However, *Trichoderma* green mold diseases have increased and pose a serious threat to its production [9–11].

*Trichoderma* has been studied for more than 200 years since it was established by Persoon in 1794 [12], while sharp development occurred in the past few decades, when a large number of taxonomic articles were published [13–26]. At present, similar to *Fusarium*, *Aspergillus*, or *Penicillium*, *Trichoderma* is a species-rich genus [15] and has been segregated into many groups or clades based on the phylogenetic relationships within the genus [27–29]. Moreover, the rapid development of *Trichoderma* is inseparable from its various uses. For example, it can not only be used as a highly efficient producer of plant biomass-degrading enzymes for biofuel and other industries, but also as a very effective biological agent for plant disease management [30–33]. Furthermore, *Trichoderma* has also been an initially produce white and dense mycelia highly similar to mushroom mycelia, which makes it difficult to distinguish them, causing the best period of control to be missed. Thus, it is particularly important to explore the specificity of *Trichoderma* species and the interaction between *Trichoderma* and its host for disease control.

Between 2020 and 2021, during fieldwork at mushroom cultivation bases, we found that green mold disease occurred continuously in *G. sichuanense* production areas in the following provinces of China: Heilongjiang, Jilin, and Shandong, leading to a significant negative effect on the development of fruitbodies. We collected diseased specimens and isolated the pathogens from several bases and identified them based on molecular and morphological characteristics. A new *Trichoderma* species and a new host record were confirmed. In addition, we summarized the *Trichoderma* species reported on mushrooms as best as possible and provided their recorded hosts. The relationships among these species were also discussed by constructing a phylogeny tree with multi-locus data, which is expected to help us know more about the relationships between *Trichoderma* species and their hosts, and to help search for *Trichoderma* species with potential biocontrol value.

## 2. Materials and Methods

### 2.1. Fungal Isolation

Diseased samples of *G. sichuanense* were collected from Jilin, Heilongjiang, and Shandong Provinces, China, and deposited in the Herbarium of Mycology, Jilin Agricultural University (HMJAU). Diseased tissues were cut into small pieces (5 mm × 5 mm × 5 mm) using a sterilized scalpel, immersed in 75 percent alcohol for 45 s before being rinsed three times with sterilized water, and placed onto Potato Dextrose Agar (PDA, BD, USA) plates containing 100 mg/L of streptomycin sulfate (Solarbio, Bejing, China), and then incubated at room temperature. Pure cultures were obtained using single-spore isolates following the method described by Chomnuti et al. [34]. Germinated spores were transferred to fresh PDA plates and incubated at 25 °C for one or two weeks. Living cultures were deposited in the Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University (Changchun, Jilin, China).

### 2.2. Growth Characterization

Colony characteristics, growth rates, and optimum temperatures for growth were determined according to the methods of Jaklitsch [18,19] by using agar media cornmeal dextrose agar (CMD, 40 g cornmeal + 2% (*w*/*v*) dextrose (Genview, Beijing, China) + 2% (*w*/*v*) agar (Genview, Beijing, China)), PDA, and synthetic low nutrient agar (SNA, pH adjusted to 5.5) [35]. Colonies were incubated in 9 cm diameter Petri dishes at 25 °C with alternating 12 h/12 h fluorescent light/darkness and measured daily until the dishes were covered with mycelia. The influence of temperature on growth was studied by growing isolates on PDA, SNA, and CMD at 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C under dark conditions. Each temperature was repeated for five plates, and the experiment was repeated three times.

### 2.3. Morphological Study

The characteristics of asexual states were described following the methods of Jaklitsch [36] and Rifai [37]. Microscopic observations were conducted using a Zeiss Axio Lab A1 light microscope (Göttingen, Germany) (objectives 10, 20, 40, and 100 oil immersion). All measurements and photographs were performed using a Zeiss Imager A2 microscope with an Axiocam 506 color camera and integrated software. Microscopically, the characteristics of 50 conidia and conidiophores from the isolates were observed. The effects of *Trichoderma* on *Ganoderma* morphology were studied using a Hitachi, model SU8010, Field Emission Scanning Electron Microscope (FESEM) at Jilin Agricultural University.

### 2.4. DNA Extraction, PCR, and Sequencing

Mycelia were harvested from three-day-old cultures on PDA for DNA extraction according to the manufacturer's instructions (NuClean Plant Gen DNA Kit, CWBIO, Taizhou, China). Sequences of ITS, TEF1, and RPB2 genes were amplified by polymerase chain reaction (PCR) with the pairs of primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) [38], primers EF1-728F (5′-CATCGAGAAGT TCGAGAAGG-3′) [39] and TEF1-LLErev (5′-GCCATCCTTGGAGATACCAGC-3′) [40], and primers RPB2-5F (5′-GAYGAYMGWGATCAYTTYGG-3′) and RPB2-7CR (5′-CCCATRGCTT GYTTRCCCA-3′) [41], respectively.

PCR was carried out in a 25 μL reaction mixture containing 1 μL of DNA sample, 12.5 μL 2 × SanTaq PCR Mix (Sangon Biotech, Shanghai, China), 1 μL of each primer (10 μM), and 9.5 μL nuclease-free water. The PCR conditions were as follows: initial denaturation at 94 °C for 3 min, then denaturation at 94 °C for 30 s, annealing for 45 s with the corresponding temperatures (56 °C for TEF1, and 55 °C for RPB2), extension at 72 °C for 1 min, followed by 35 cycles, then a final extension at 72 °C for 10 min, using an Applied Biosystems S1000 TM Thermal Cycler machine. PCR products were sent to the Changchun Branch of Sangon Biotech Co., Ltd. (Changchun, China) for paired-end sequencing, and the results were first assembled using BioEdit [42] and then confirmed by BLAST on NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 21 June 2021).

### 2.5. Phylogenetic Analyses

BLASTn searches with the sequences were performed against NCBI to detect the most closely related species (http://www.blast.ncbi.nlm.nih.gov/, accessed on 22 December 2021). Phylogenetic trees were constructed using TEF1 and RPB2 sequences, and phylogenetic analyses were performed with the Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI) methods. New sequences were generated from the new species in this study, along with reference sequences retrieved from GenBank (Table 1). The *Trichoderma* sequences associated with mushroom green mold are listed in Table 2. Multiple alignments of all common sequences and reference sequences were automatically generated using MAFFT V.7.471 [43], with manual improvements made using BioEdit when necessary [42], and converted to nexus and NEX format through the software Aliview [44]. In the analysis, ambiguous areas were excluded and gaps were regarded as missing data.

**Table 1.** Strain information and GenBank accession numbers of sequences used for phylogenetic analyses for new species.

| Species | Strains | GenBank Accession Number | | References |
|---|---|---|---|---|
| | | TEF1 | RPB2 | |
| *T. afarasin* | GJS 99-227 | AF348093 | — | [45] |
| *T. afroharzianum* | LESF229 | KT279013 | KT278945 | [46] |
| *T. afroharzianum* | GJS04-186 (T) | FJ463301 | FJ442691 | In GenBank |
| *T. aggregatum* | HMAS248864 | KY688063 | KY688002 | [47] |
| *T. aggressivum* | CBS100525 | AF534614 | AF545541 | [48] |
| *T. aggressivum* | DAOM222156 | AF348098 | FJ442752 | [45] |
| *T. alni* | CPK2494 | EU498313 | EU498350 | [49] |
| *T. alni* | CBS120633 = CPK1982 (T) | EU498312 | EU498349 | [49] |
| *T. alpinum* | HMAS248870 | KY688017 | KY687963 | [47] |
| *T. alpinum* | HMAS248821 (T) | KY688012 | KY687958 | [47] |
| *T. amazonicum* | IB95 | HM142377 | HM142368 | [50] |
| *T. asperellum* | CBS433.97 = TR3 (T) | AF456907 | EU248617 | [51] |
| *T. atrobrunneum* | S3 | KJ665376 | KJ665241 | [20] |
| *T. atrobrunneum* | GJS92-110 (T) | AF443942 | — | [16] |
| *T. atrogelatinosum* | CBS237.63 (T) | — | KJ842201 | In GenBank |
| *T. azevedoi* | CEN1403 | MK696638 | MK696800 | [52] |
| *T. azevedoi* | CEN1422 | MK696660 | MK696821 | [52] |
| *T. bannaense* | HMAS248865 | KY688038 | KY688003 | [47] |
| *T. bannaense* | HMAS248840 (T) | KY688037 | KY687979 | [47] |
| *T. breve* | HMAS248845 | KY688046 | KY687984 | [47] |
| *T. breve* | HMAS248844 (T) | KY688045 | KY687983 | [47] |

**Table 1.** *Cont.*

| Species | Strains | GenBank Accession Number | | References |
|---|---|---|---|---|
| | | TEF1 | RPB2 | |
| *T. brunneoviride* | CBS121130 = CPK2014 | EU498316 | EU498357 | [49] |
| *T. camerunense* | GJS99-231 | AF348108 | — | [45] |
| *T. camerunense* | GJS99-230 (T) | AF348107 | — | [45] |
| *T. catoptron* | GJS02-76 = CBS114232 (T) | AY391963 | AY391900 | [53] |
| *T. christiani* | CBS132572 = S442 (T) | KJ665439 | KJ665244 | [20] |
| *T. cinnamomeum* | GJS97-237 (T) | AY391979 | AY391920 | [53] |
| *T. compactum* | CBS121218 | KF134798 | KF134789 | [54] |
| *T. concentricum* | HMAS248858 | KY688028 | KY687997 | [47] |
| *T. concentricum* | HMAS248833 (T) | KY688027 | KY687971 | [47] |
| *T. endophyticum* | DIS220J | FJ463330 | FJ442690 | [55] |
| *T. endophyticum* | DIS221E | FJ463316 | FJ442775 | In GenBank |
| *T. epimyces* | CPK1980 | EU498319 | EU498359 | [49] |
| *T. epimyces* | CBS120534 = CPK1981 (T) | EU498320 | EU498360 | [49] |
| ***T. ganodermatigerum*** | **CCMJ5245 (T)** | **ON567195** | **ON567189** | **This study** |
| ***T. ganodermatigerum*** | **CCMJ5246** | **ON567196** | **ON567190** | **This study** |
| ***T. ganodermatigerum*** | **CCMJ5247** | **ON567197** | **ON567191** | **This study** |
| ***T. ganodermatigerum*** | **CCMJ5248** | **ON567198** | **ON567192** | **This study** |
| ***T. ganodermatigerum*** | **CCMJ5249** | **ON567199** | **ON567193** | **This study** |
| ***T. ganodermatigerum*** | **CCMJ5250** | **ON567200** | **ON567194** | **This study** |
| *T. guizhouense* | S278 | KF134799 | KF134791 | [54] |
| *T. guizhouense* | S628 | KJ665511 | KJ665273 | [20] |
| *T. harzianum* | GJS05-107 | FJ463329 | FJ442708 | In GenBank |
| *T. harzianum* | GJS04-71 | FJ463396 | FJ442779 | In GenBank |
| *T. harzianum* | Thaum12 | MT081433 | MT118248 | In GenBank |
| *T. harzianum* | CBS226.95 (T) | AF534621 | AF545549 | [48] |
| *T. hausknechtii* | Hypo649 = CBS133493 (T) | KJ665515 | KJ665276 | [20] |
| *T. helicolixii* | S640 = CBS133499 (T) | KJ665517 | KJ665278 | [20] |
| *T. hengshanicum* | HMAS248853 | KY688055 | KY687992 | [47] |
| *T. hengshanicum* | HMAS248852 (T) | KY688054 | KY687991 | [47] |
| *T. hirsutum* | HMAS248859 | KY688030 | KY687998 | [47] |
| *T. hirsutum* | HMAS248834 (T) | KY688029 | KY687972 | [47] |
| *T. ingratum* | HMAS248824 | KY688019 | KY687964 | [47] |
| *T. ingratum* | HMAS248873 | KY688022 | KY688010 | [47] |
| *T. ingratum* | HMAS248822 (T) | KY688018 | KY687973 | [47] |
| *T. inhamatum* | CBS273.78 (T) | AF348099 | FJ442725 | [45] |
| *T. italicum* | S131 = CBS132567 (T) | KJ665525 | KJ665282 | [20] |
| *T. lentiforme* | DIS167C | FJ463309 | FJ442689 | In GenBank |
| *T. lentiforme* | GJS98-6 (T) | AF469195 | — | [16] |
| *T. liberatum* | HMAS248832 | KY688026 | KY687970 | [47] |
| *T. liberatum* | HMAS248831 (T) | KY688025 | KY687969 | [47] |
| *T. linzhiense* | HMAS248874 | KY688048 | KY688011 | [47] |
| *T. linzhiense* | HMAS248846 (T) | KY688047 | KY687985 | [47] |
| *T. lixii* | CBS110080 = GJS97-96 | FJ716622 | KJ665290 | [20] |
| *T. neocrassum* | DAOM164916 = CBS336.93 (T) | AF534615 | AF545542 | [48] |
| *T. neotropicale* | LA11 | HQ022771 | — | [56] |
| *T. peberdyi* | CEN1387 | MK696619 | MK696781 | [52] |
| *T. peberdyi* | CEN1388 | MK696620 | MK696782 | [52] |
| *T. pleuroticola* | T1295 | EU279973 | — | [57] |
| *T. pleuroticola* | CBS124383 (T) | HM142381 | HM142371 | [50] |
| *T. pleuroti* | CBS124387 (T) | HM142382 | HM142372 | [50] |
| *T. polypori* | HMAS248855 | KY688058 | KY687994 | [47] |
| *T. polypori* | HMAS248861 | KY688059 | KY688000 | [47] |
| *T. priscilae* | S129 | KJ665689 | KJ665332 | [20] |
| *T. pseudodensum* | HMAS248829 | KY688024 | KY687968 | [47] |
| *T. pseudodensum* | HMAS248828 (T) | KY688023 | KY687967 | [47] |

**Table 1.** *Cont.*

| Species | Strains | GenBank Accession Number | | References |
|---|---|---|---|---|
| | | TEF1 | RPB2 | |
| *T. pseudogelatinosum* | TUFC60186 (T) | JQ797397 | JQ797405 | [58] |
| *T. pyramidale* | S573 | KJ665698 | — | [20] |
| *T. pyramidale* | S73 = CBS135574 (T) | KJ665699 | KJ665334 | [20] |
| *T. rifaii* | DIS337F | FJ463321 | FJ442720 | In GenBank |
| *T. rifaii* | DIS355B (T) | FJ463324 | — | In GenBank |
| *T. simmonsii* | GJS90-22 | AY391984 | AY391925 | [53] |
| *T. simmonsii* | GJS92-100 | AF443937 | FJ442710 | [16] |
| *T. simmonsii* | GJS91-138 | AF443935 | FJ442757 | [16] |
| *T. simplex* | HMAS248860 | KY688042 | KY687999 | [47] |
| *T. simplex* | HMAS248842 (T) | KY688041 | KY687981 | [47] |
| *T. solum* | HMAS248848 | KY688050 | KY687987 | [47] |
| *T. solum* | HMAS248847 (T) | KY688049 | KY687986 | [47] |
| *T. spirale* | DAOM183974 | EU280049 | — | [57] |
| *T. spirale* | LESF107 | KT279022 | KT278956 | [46] |
| *T. stramineum* | GJS02-84 = CBS114248 (T) | AY391999 | AY391945 | [53] |
| *T. tawa* | GJS97-174 = CBS114233 (T) | AY392004 | AY391956 | [53] |
| *T. tomentosum* | S33 | KF134801 | KF134793 | [54] |
| *T. tomentosum* | DAOM178713A (T) | AF534630 | AF545557 | [48] |
| *T. velutinum* | DAOM230013 = CPK298 | AY937415 | KF134794 | [59] |
| *T. virens* | DIS162 | FJ463367 | FJ442696 | In GenBank |
| *T. zayuense* | HMAS248836 | KY688032 | KY687975 | [47] |
| *T. zayuense* | HMAS248835 (T) | KY688031 | KY687974 | [47] |

New sequences are shown in bold. The type sequences are marked with (T).

**Table 2.** Isolates and GenBank accession numbers of *Trichoderma* species associated with green mold on mushrooms.

| Species | Host Range | Isolates | GenBank Accession Number | | References |
|---|---|---|---|---|---|
| | | | TEF1 | RPB2 | |
| *T. aggressivum* | *Agaricus bisporus* | CBS100525 | AF534614 | AF545541 | [48] |
| *T. aggressivum f. aggressivum* | *Agaricus bisporus* | GJS99-30 | AF348109 | — | [60] |
| | | DAOM222156 | AF348098 | FJ442752 | [45] |
| *T. aggressivum f. europaeum* | *Agaricus bisporus* | CBS100526 (T) | KP008993 | KP009166 | [45] |
| | — | TRS27 | KP008994 | KP009163 | In GenBank |
| | — | CBS435.95 | KP008998 | KP009169 | In GenBank |
| *T. alni* | *Macrotyphula* cf. *contorta* | CBS120633 | EU498312 | EU498349 | [49] |
| | | CPK2494 | EU498313 | EU498350 | |
| *T. asperellum* | *Pleurotus ostreatus* | T11 (ACCC32725) | MF049065 | — | [61] |
| | *Pleurotus eryngii* | — | — | — | [62] |
| | — | CGMCC6422 | KF425756 | KF425755 | [63] |
| | — | CBS433.97 = TR3 (T) | AF456907 | EU248617 | In GenBank |
| *T. atrobrunneum* | *Ganoderma sichuanense* | CGMCC3.19070 | MH464779 | — | [64] |
| | — | T17-27 | MW232537 | MW232508 | [65] |
| *T. atroviride* | *Pleurotus ostreatus* | CPK3277 | EU918154 | — | [66] |
| | *Ganoderma sichuanense* | 2015005 | — | — | [10] |
| | *Agaricus bisporus* | T33 | — | — | [67] |
| | *Lentinula edodes* | T25 | — | — | [68] |
| | *Pleurotus eryngii* | — | — | — | [69] |
| | — | PARC1011 | MT454114 | MT454130 | [70] |
| | — | PARC1018 | MT454121 | MT454137 | |

**Table 2.** *Cont.*

| Species | Host Range | Isolates | GenBank Accession Number | | References |
|---|---|---|---|---|---|
| | | | TEF1 | RPB2 | |
| | — | DAOM222144 | AF456889 | FJ442754 | [71] |
| | — | Th002 | AB558906 | AB558915 | [72] |
| *T. aureoviride* | *Pleurotus ostreatus* | HMAS266607 | KF923280 | KF923306 | [73] |
| *T. austriacum* | *Peziza* sp. | CBS122494 (T) | FJ860619 | FJ860525 | [19] |
| *T. capillare* | *Agaricus bisporus* | CPK2883 | JN182283 | JN182312 | [74] |
| | | GJS99-3 | JN175584 | JN175529 | |
| *T. catoptron* | *Aphyllophorales* s. l. | GJS02-76 (T) | AY391963 | AY391900 | [53] |
| *T. cerinum* | *Lentinula edodes* | S357 | KF134797 | KF134788 | [75] |
| *T. chromospermum* | black mycelium and black pyrenomycete | GJS95-196 | AY391975 | AY391914 | [53] |
| | | GJS98-73 | AY391976 | AY391915 | |
| | | GJS94-68 = CBS114577 | — | AY391913 | |
| | — | HMAS252537 | KF729986 | KF730004 | [25] |
| | — | HMAS252539 | KF923287 | KF923314 | |
| | — | HMAS252535 | KF923292 | KF923315 | |
| *T. citrinoviride* | *Lentinula edodes* | TAMA0154 | AB807641 | AB807653 | [76] |
| | *Pleurotus ostreatus* | GJS92-8 | JN175595 | JN175544 | [77] |
| | *Pleurotus eryngii* | GJS01-364 | AY225860 | AF545565 | [69] |
| | Polypore mushroom | TAMA0188 | AB807644 | AB807656 | [76] |
| | — | HZA9 | MK850831 | MK962804 | [78] |
| *T. epimyces* | *Polyporus umbellatus* | CPK1980 | EU498319 | EU498359 | [49] |
| | | CBS120534 (T) | EU498320 | EU498360 | |
| *T. erinaceum* | — | DIS7 | DQ109547 | EU248604 | [79] |
| *T. fasciculatum* | *Hypocrea* ascospores | CBS118.72 | — | — | [80] |
| | — | DAOM172827 | AF534628 | AF545555 | [48] |
| *T. fomiticola* | *Fomes fomentarius* | CBS121136 | FJ860639 | FJ860538 | [18] |
| | | CPK3137 | FJ860640 | FJ860539 | |
| *T. ghanense* | *Agaricus bisporus* | NBRC30902 | AB807638 | AB807650 | [76] |
| *T. ganodermatis* | *Ganoderma sichuanense* | HMAS248856 | KY688060 | KY687995 | [47] |
| | | HMAS248869 | KY688061 | KY688007 | [47] |
| ***T. ganodermatigerum*** | ***Ganoderma sichuanense*** | **CCMJ5245(T)** | **ON567195** | **ON567189** | **This study** |
| | | **CCMJ5246** | **ON567196** | **ON567190** | |
| | | **CCMJ5247** | **ON567197** | **ON567191** | |
| | | **CCMJ5248** | **ON567198** | **ON567192** | |
| | | **CCMJ5249** | **ON567199** | **ON567193** | |
| | | **CCMJ5250** | **ON567200** | **ON567194** | |
| *T. ghanense* | *Agaricus bisporus* | NBRC30902 | AB807638 | — | [76] |
| *T. hamatum* | *Agaricus bisporus* | Tham20-3 | — | — | [81] |
| | *Lentinula edodes* | — | — | — | [82] |
| | — | DAOM167057 (T) | EU279965 | AF545548 | [57] |
| | — | Hypo647 = WU31629 | KJ665513 | KJ665274 | [20] |
| | — | Hypo648 = CBS132565 | KJ665514 | KJ665275 | [20] |
| *T. harzianum* | *Pleurotus ostreatus* | KACC40558 | — | — | [66] |
| | *Cyclocybe aegerita* | JB1 | — | — | [73] |
| | *Lentinula edodes* | T50 | — | — | [83] |
| | *Pleurotus eryngii* *Pleurotus ostreatus* | KACC40784 | — | — | [69] |
| | *Agaricus bisporus* | — | — | — | [45] |
| | *Pleurotus ostreatus* | — | — | — | [84] |
| | Polypores/Corticiaceous | — | — | — | [18] |

**Table 2.** *Cont.*

| Species | Host Range | Isolates | GenBank Accession Number | | References |
|---|---|---|---|---|---|
| | | | TEF1 | RPB2 | |
| | *Pleurotus tuoliensis*<br>*Tremella fuciformis*<br>*Flammulina filiformis* | — | — | — | [85] |
| | — | CBS226.95 | AF348101 | AF545549 | [48] |
| | — | Thaum12 | MT081433 | MT118248 | [86] |
| | — | CBS227.95 | AF348100 | — | [45] |
| | — | GJS05-107 | FJ463329 | FJ442708 | In GenBank |
| | — | GJS04-71 | FJ463396 | FJ442779 | In GenBank |
| *T. hengshanicum* | *Ganoderma sichuanense* | 1009 | — | — | [87] |
| | — | HMAS248852 (T) | KY688054 | KY687991 | [47] |
| | — | HMAS248853 | KY688055 | KY687992 | |
| *T. inhamatum* | *Agaricus bisporus* | CBS273.78 (T) | AF348099 | FJ442725 | [81] |
| | *Pleurotus tuoliensis* | — | — | — | [85] |
| *T. koningii* | *Pleurotus eryngii* | — | — | — | [69] |
| | *Agaricus bisporus* | — | — | — | [88] |
| | *Lentinula edodes*<br>*Pleurotus ostreatus*<br>*Pleurotus tuoliensis*<br>*Flammulina filiformis*<br>*Volvariella volvacea*<br>*Hypsizygus marmoreus* | — | — | — | [85] |
| | *Ganoderma sichuanense* | TFl040917 | — | — | [75] |
| | *Tremella fuciformis* | TGy040604 | — | — | |
| | — | 7723 | KJ634753 | KJ634720 | [89] |
| | — | GJS90-18 | DQ289007 | EU248600 | [23] |
| | — | CBS979.70 | AY665703 | EU248601 | In GenBank |
| | — | S22 | KC285595 | KC285749 | [90] |
| ***T. koningiopsis*** | *Phaiius rubrovolvata* | CXYL | MN135988 | MT038997 | [91] |
| | ***Ganoderma sichuanense*** | **CCMJ5253** | **ON567187** | **ON567201** | **This study** |
| | | **CCMJ5254** | **ON567188** | **ON567202** | |
| *T. kunigamense* | *Lentinula edodes* | TAMA193 | AB807645 | AB807657 | [76] |
| *T. leguminosarum* | dark corticiaceous fungus | S391 | KJ665548 | KJ665287 | [20] |
| | | CBS130014 | KJ665551 | KJ665288 | |
| | | S503 | KJ665552 | KJ665289 | |
| *T. lieckfeldtiae* | *Moniliophthora roreri* | GJS00-14 = CBS123049 (T) | EU856326 | EU883562 | [92] |
| *T. longibrachiatum* | *Pleurotus ostreatus* | TUFC61535 = CBS816.68(T) | EU401591 | DQ087242 | [40] |
| | *Agrocybe aegerita* | JB4 | — | — | [73] |
| | *Lentinula edodes* | T57 | — | — | [83] |
| | *Ganoderma sichuanense* | TFl040921 | — | — | [75] |
| | *Pleurotus eryngii* | — | — | — | [93] |
| | *Agaricus bisporus* | — | — | — | [81] |
| | *Pleurotus tuoliensis*<br>*Hypsizygus marmoreus*<br>*Volvariella volvacea* | — | — | — | [85] |

**Table 2.** *Cont.*

| Species | Host Range | Isolates | GenBank Accession Number | | References |
|---|---|---|---|---|---|
| | | | TEF1 | RPB2 | |
| *T. mienum* | *Lentinula edodes* | TUFC61517 | JQ621975 | JQ621965 | [94] |
| *T. orientale* | *Ganoderma applanatum* | LESF516 | KT279041 | KT278976 | [46] |
| | *Ganoderma applanatum* | LESF540 | KT279042 | KT278977 | |
| | *Ganoderma applanatum* | LESF544 | KT279043 | KT278978 | |
| | *Ganoderma applanatum* | TRS707 | KP008888 | KP009202 | |
| *T. oblongisporum* | *Lentinula edodes* | T37 | — | — | [83] |
| | — | DAOM167085 | AF534623 | AF545551 | [48] |
| *T. parareesei* | *Pleurotus eryngii* | TAMA0153 | AB807640 | AB807652 | [76] |
| *T. parestonica* | *Hymenochaete tabacina* | CBS120636 (T) | FJ860667 | FJ860565 | [18] |
| *T. pleuroticola* | *Pleurotus ostreatus* | CBS124383 (T) | HM142381 | HM142371 | [66] |
| | | CPK2885 | EU918161 | EU918141 | |
| | *Pleurotus eryngii* | CAF-TP3 | — | — | [69] |
| | *Lentinula edodes* | T22 | — | — | [83] |
| | *Cyclocybe aegerita* | JB7 | — | — | [73] |
| | — | T1295 | EU279973 | — | [57] |
| *T. pleuroti* | *Pleurotus ostreatus* | KACC44537 | — | — | [69] |
| | *Pleurotus eryngii* var. *ferulae* | — | — | — | [95] |
| | — | CBS124387 (T) | HM142382 | HM142372 | [50] |
| *T. polypori* | *Lentinula edodes* | HMAS248861 | KY688059 | KY688000 | [47] |
| | *Polyporus* sp. | HMAS248855 (T) | KY688058 | KY687994 | |
| *T. polysporum* | *Lentinula edodes* | — | — | — | [96] |
| | — | 8232 | KJ634779 | KJ634746 | [89] |
| | — | 8147 | KJ634771 | KJ634738 | |
| *T. priscilae* | *Crepidotus* sp. | S168 = CBS131487 (T) | KJ665691 | KJ665333 | [20] |
| | *Stereum* sp. | S129 | KJ665689 | KJ665332 | |
| | — | HMAS245002 | KT343760 | KT343764 | In GenBank |
| *T. protopulvinatum* | *Fomitopsis pinicola* | CPK2434 | FJ860677 | FJ860574 | [18] |
| *T. pulvinatum* | *Fomitopsis pinicola* | CBS121279 | FJ860683 | FJ860577 | [18] |
| *T. pseudokoningii* | *Lentinula edodes* | DUCC4021 | KX431217 | — | [77] |
| | *Cyclocybe aegerita* | TGc050619 | — | — | [75] |
| | *Ganoderma sichuanense* | TFl040926 | — | — | |
| | *Pleurotus eryngii* | — | — | — | [97] |
| | *Flammulina filiformis* | — | — | — | [98] |
| | *Pleurotus tuoliensis* *Volvariella volvacea* *Hypsizygus marmoreus* | — | — | — | [85] |
| | — | DAOM167678 | AY865641 | KJ842214 | [99] |
| | — | GJS99-149 | JN175589 | JN175536 | [17] |
| | — | GJSNS19 | JN175588 | JN175535 | |
| *T. pseudolacteum* | *Lentinula edodes* | TUFC61496 | JX238494 | JX238479 | [100] |
| | | TUFC61502 | JX238480 | JX238471 | |
| *T. samuelsii* | *Hymenochaete* sp. | S5 = CBS130537 | JN715651 | JN715599 | [101] |
| | | S42 | JN715652 | JN715598 | |

**Table 2.** *Cont.*

| Species | Host Range | Isolates | GenBank Accession Number | | References |
|---|---|---|---|---|---|
| | | | TEF1 | RPB2 | |
| *T. songyi* | *Tricholoma matsutake* | TC556<br>TC480 | KX266244<br>KX266243 | KX266250<br>KX266249 | [102] |
| *T. stilbohypoxyli* | *Stilbohypoxylon moelleri* | Hypo256 = CPK1977 | FJ860702 | FJ860592 | [23] |
| *T. stromaticum* | *Agaricus bisporus* | GJS97-181 | AY937447 | HQ342227 | [59] |
| | — | GJS07-88 | HQ342195 | HQ342258 | [103] |
| | — | GJS03-47 | HQ342201 | HQ342264 | |
| | — | GJS00-107 | HQ342202 | HQ342265 | |
| *T. sulphureum* | *Laetiporus sulphureus* | CBS119929<br>CPK1593 | FJ860710<br>FJ860709 | FJ179620<br>FJ860599 | [18] |
| | *Thelephora* sp. | GJS95-135 = CBS114237 | AY392006 | AY391958 | [53] |
| *T. tsugarense* | *Lentinula edodes* | TAMA203 (T) | AB807647 | AB807659 | [76] |
| *T. viride* | *Lentinula edodes* | T13 | — | — | [83] |
| | *Pleurotus ostreatus* | — | — | — | [82] |
| | *Tremella fuciformis* | TGc040905 | — | — | [75] |
| | *Ganoderma sichuanense* | TFl080706 | — | — | [75] |
| | *Flammulina filiformis* | TFj10010 | — | — | [75] |
| | *Cyclocybe aegerita* | TGc040905 | — | — | [75] |
| | *Phallus indusiatus* | TFl080706 | — | — | [75] |
| | *Tremella fuciformis* | TGc040905 | — | — | [75] |
| | *Agaricus bisporus* | — | — | — | [88] |
| | *Pleurotus eryngii* | — | — | | [69] |
| | — | TRS575 | KP008931 | KP009081 | In GenBank |
| | — | LESF115 | KT278989 | KT278921 | [46] |
| *T. virens* | *Agaricus bisporus*<br>*Pleurotus eryngii* | —<br>— | —<br>— | —<br>— | [88] |
| | — | DIS162 | FJ463367 | FJ442696 | In GenBank |
| | — | DIS328A | FJ463363 | FJ442738 | In GenBank |
| *T.* cf. *virens* | *Pleurotus eryngii* | KACC40783 | — | — | [69] |
| | *Pleurotus ostreatus* | TUCIM2558 | KX655776 | — | [104] |
| *T. viridarium* | *Steccherinum ochraceum* | GJS89-142 | AY376049 | EU241495 | [51] |
| | *Nemania* sp. | GJS98-182 | DQ307511 | EU252011 | [23] |
| *Protocrea farinosa*<br>*Protocrea pallida* | —<br>— | CBS121551<br>CBS121552 | EU703889<br>EU703897 | EU703935<br>EU703944 | [105] |

The type sequences are marked with (T), the new sequences are shown in bold.

An MP phylogram was constructed with PAUP 4.0b10 [106] from the combined sequences of TEF1 and RPB2, using 1000 replicates of a heuristic search with random addition of sequences and subsequent tree bisection and reconnection (tbr) branch swapping. Analyses were performed with all characters treated as unordered and unweighted, and gaps treated as missing data. The topological confidence of the resulting trees was tested by maximum parsimony bootstrap proportion (MPBP) with 1000 replications, each with 10 replicates of random addition of taxa. An ML phylogram was constructed with Raxml-gui 2.0 [107] with the sequence after alignment. The ML + Rapid bootstrap program and 1000 repeats of the GTRGAMMAI model were used to evaluate the bootstrap proportion (BP) of each branch for constructing the phylogenetic tree. The BI analysis was conducted using MrBayes 3.2.7 [108] using a Markov Chain Monte Carlo (MCMC) algorithm. Nucleotide substitution models were determined using MrModeltest 2.3 [109]. The best model for combined sequences was HKY + I + G.

## 3. Results

### *3.1. Molecular Phylogeny*

Species recognition: The dataset for the new species phylogenetic analyses included sequences from 100 taxa (Table 1). Multi-locus data were concatenated, which comprised 2321 characters, with TEF1 1293 characters and RPB2 1028 characters. Estimated base frequencies were as follows: A = 0.231650, C = 0.281772, G = 0.234671, and T = 0.251907; substitution rates were as follows: AC = 1.069464, AG = 4.197119, AT = 0.935747, CG = 0.993621, CT = 4.979475, and GT = 1.000000. The MP and ML trees showed similar topologies with high statistical support values. The MP tree was selected as the representative phylogeny. In Bayesian analysis, the average standard deviation of split frequencies at the end of the total MCMC generations was calculated as 0.008946, which is less than 0.01. Most of the tree topologies resulting from three analyses were nearly the same. In the resulting tree (Figure 1), the combined phylogenetic analyses using TEF1-$\alpha$ and RPB2 showed that the six strains of *T. ganodermatigerum* represent phylogenetically distinct species with high statistical supports (MPBP/MLBP/BIBP = 100%/100%/1.0), and clustered together with the species in the Harzianum clade [16]. The new species is most related to the clade that contains *T. amazonicum*, *T. pleuroticola*, *T. hengshanicum*, and *T. pleuroti*. Two collections of CCMJ5253 and CCMJ5254 clustered with *T. koningiopsis* with high support (MPBP/MLBP = 100/100) (Figure 2).

Phylogenetic structure: Some sections could be found among the *Trichoderma* strains associated with mushrooms and are mainly concentrated in the Harzianum clade (Figure 2). *Trichoderma longibrachiatum*, *T. citrinoviride*, *T. pseudokoningii*, and *T. ghanense* are from section *Longibrachiatum*, whose members are best known as producers of cellulose-hydrolyzing enzymes [74,110,111]. *Trichoderma atroviride*, *T. viride*, *T. koningii*, *T. hamatum*, *T. minutisporum*, *T. polysporum*, *T. viride*, and *T. asperellum* are from section *Trichoderma* or the Viride clade [36,111].

The phylogenetic structure according to ecology: Species in the Harzianum clade are commonly fungicolous, living in different types of habitats [112,113]. They are most commonly isolated from soil or found on decomposing plant material where they occur cryptically or parasitize other fungi [18,53,114], and those species are possibly the most common endophytic "species" in wild trees [115,116]. There is usually no apparent host specialization [117]. However, some exceptions to this trend exist. Clade I in the Harzianum clade of the tree is a collection of species with relatively narrow host ranges, or in other words, a strong host preference. *Trichoderma atrobrunneum* was found in soil or on decaying wood, clearly or cryptically parasitizing other fungi. *Trichoderma pleuroti*, just like *T. aggressivum*, has thus far never been isolated from areas outside of mushroom farms [118]. Furthermore, *T. epimyces* has only been reported on *Polyporus umbellatus* [49], and *T. priscilae* has been reported from basidiomes of *Crepidotus* and *Stereum* [20].

Some other species such as *T. atroviride*, *T. asperellum*, *T. harzianum*, and *T. longibrachiatum* were also found in significant proportions in *Agaricus* compost [119]. *Trichoderma stromaticum* and its *Hypocrea* teleomorph are only known from cocoa and are often associated with tissue infected with the basidiomycetous pathogen *Crinipellis perniciosa* [55].

Although some of these pathogenic *Trichoderma* species (e.g., species gathered in or near Clade II) have been explored as biocontrol agents for plant diseases, *T. atroviride*, *T. viride*, *T. koningii*, *T. koningiopsis*, and *T. asperellum* serve as pathogens with broad host ranges on mushrooms. *Trichoderma sulphureum*, *T. protopulvinatum*, *T. pulvinatum*, and *T. austriacum* coalesce into a subclade (Clade III), and each of these species has been reported on a particular fungus [18,19].

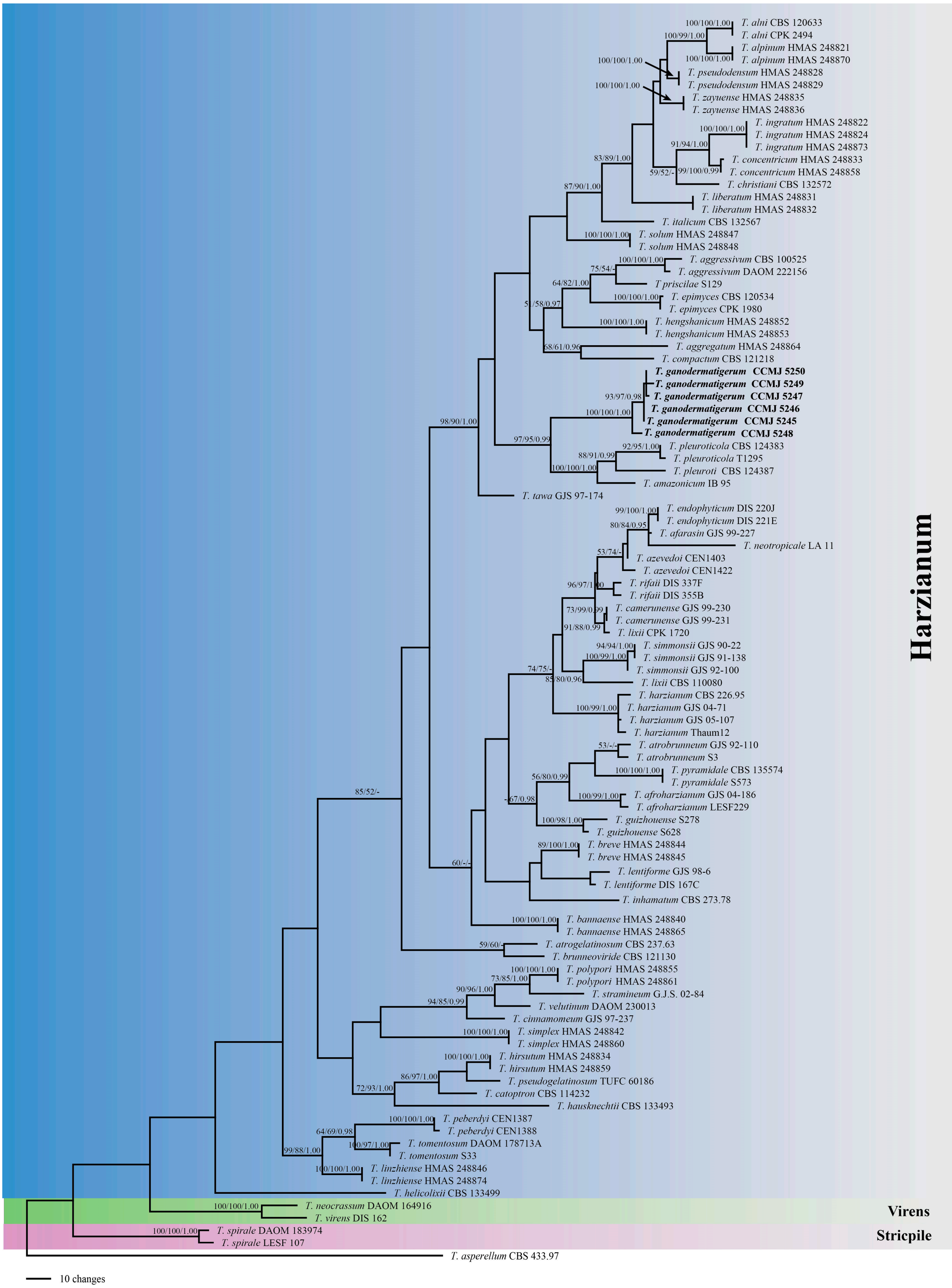


**Figure 1.** Phylogeny of *Trichoderma* using MP analysis based on combined TEF1 and RPB2 sequences. MPBP ≥ 50%, MLBP ≥ 50%, and BIPP ≥ 0.9 are shown on the branches (MPBP/MLBP/BIPP). The sequences in bold are the new species.

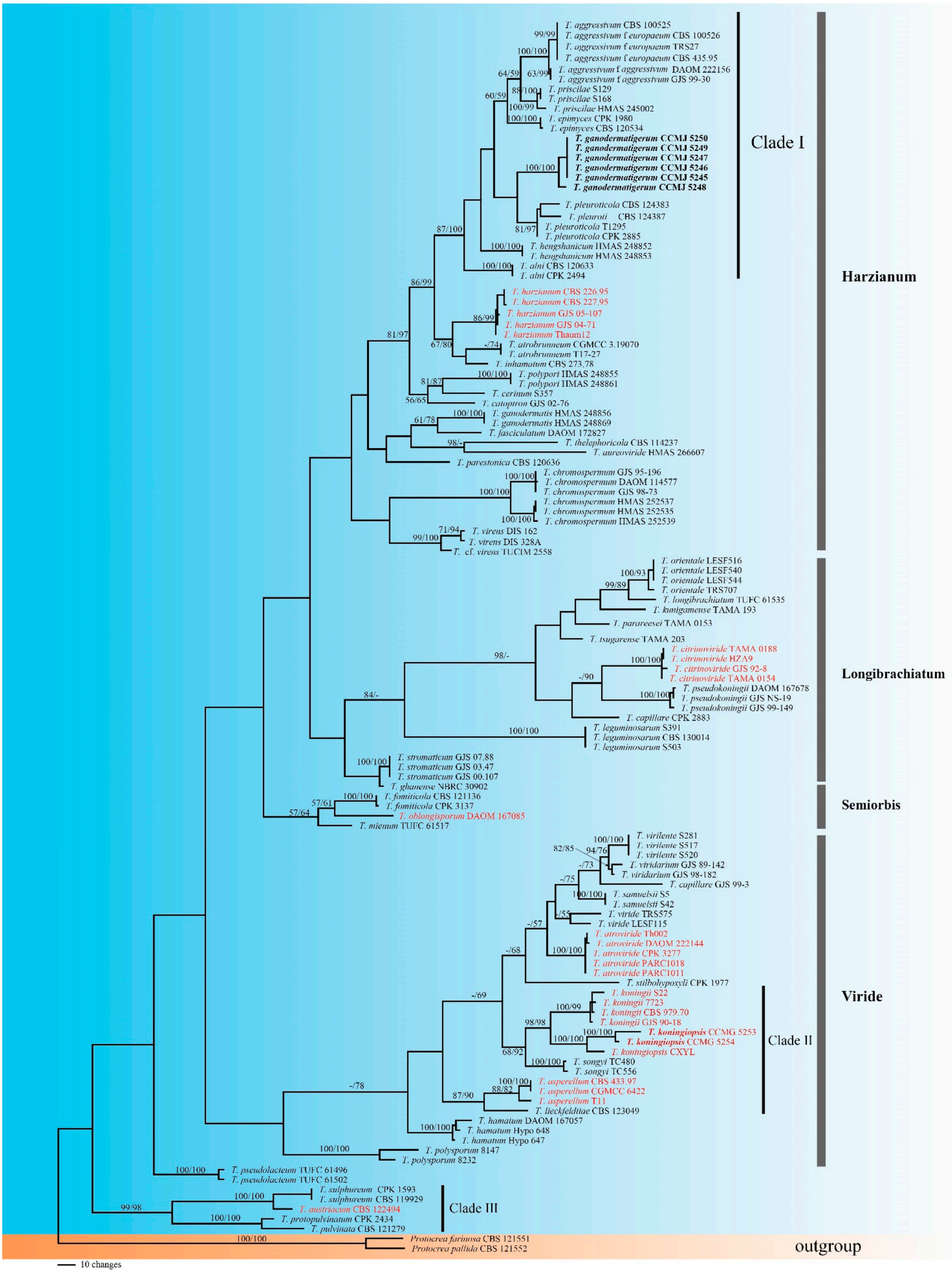


**Figure 2.** Phylogeny of *Trichoderma* associated with mushrooms using MP analysis based on concatenated TEF1 and RPB2 sequences. Branches are labeled with MPBP $\geq$ 50% and MLBP $\geq$ 50%. The biological agents are marked in red, and the new sequences in this study are in bold.

### 3.2. Taxonomy

*Trichoderma ganodermatigerum* X.Y. An & Y. Li, sp. nov. Figure 3A–L.

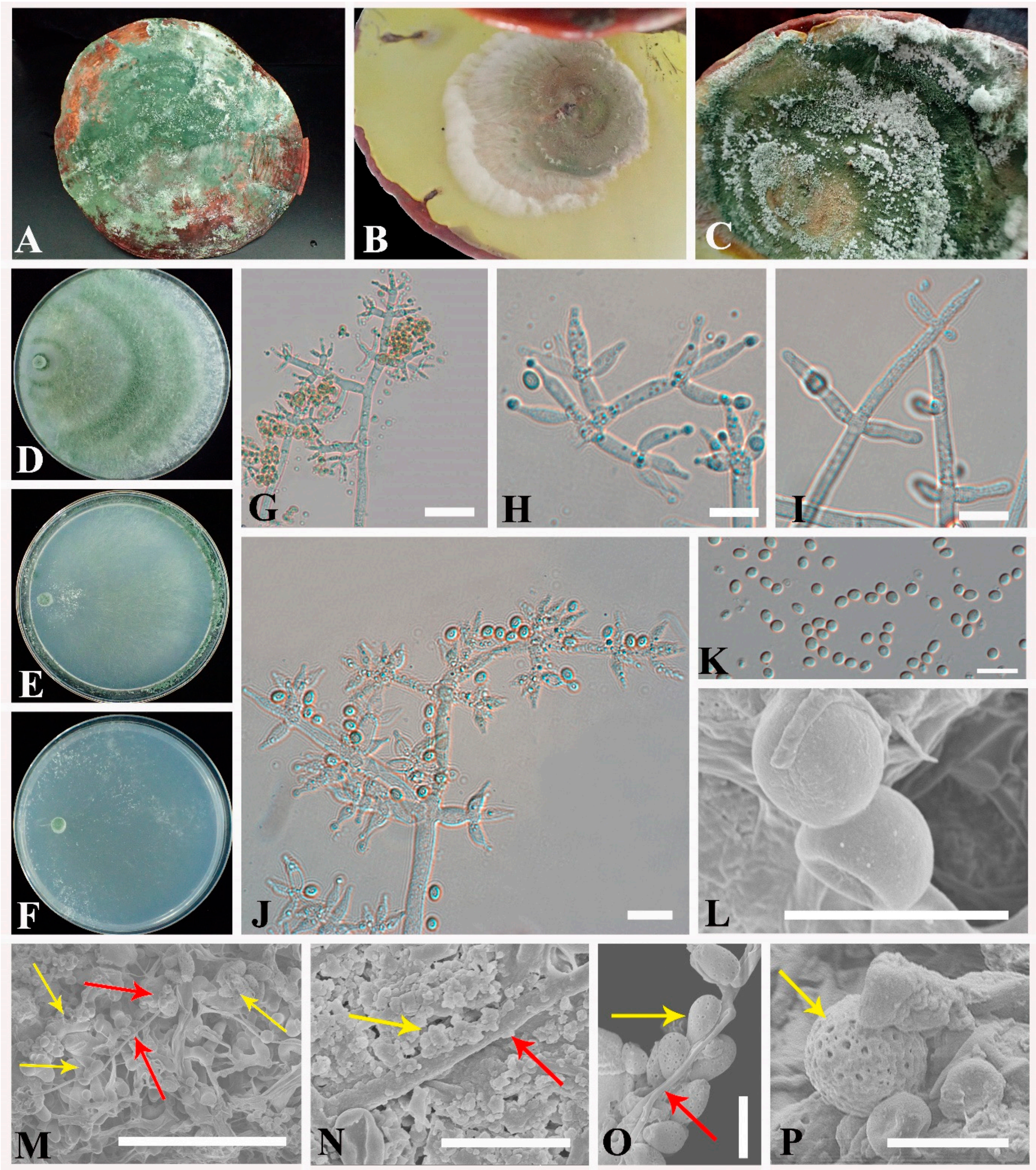


**Figure 3.** Morphological characteristics of *T. ganodermatigerum*. (**A**–**C**) diseased fruitbody; (**D**–**F**) colony on PDA, CMD, and SNA; (**G**–**J**) conidiophores and phialides; (**K**,**L**) conidia; (**M**–**P**) interactions of *G. sichuanense* and *T. ganodermatigerum*; (**M**) *Trichoderma* hyphae and conidia are filled in the *Ganoderma* tissue, causing the tissue to become rough or even depressed; (**N**) *Trichoderma* hyphae covered with *Ganoderma* tissue; (**O**) clinged *Trichoderma* hyphae and healthy *Ganoderma* spores; (**P**) abnormal *Ganoderma* spores in diseased tissue. Bars: G, Q = 20 µm; H–J, M–P = 10 µm; K = 50 µm; L = 5 µm. The yellow arrows indicate the tissues and spores of *G. sichuanense*, and the red arrows indicate the hyphae and spores of *T. ganodermatigerum*.

MycoBank: MB 843898.

Diagnosis: Phylogenetically, *T. ganodermatigerum* formed a distinct clade and is related to *T. amazonicum* (Figure 1). Both *T. amazonicum* and *T. ganodermatigerum* form dense concentric rings, pyramidal branching patterns, and branches toward the tip; mycelium grows slowly or does not grow at 35 °C; conidia globose, smooth, and green. As for *T. amazonicum*, there is no diffusing pigmentation on CMD media and a slightly fruity odor; a brown diffusing pigmentation of the agar is formed in some strains on PDA media [50]. Phylogenetic analysis of TEF1 and RPB2 gene sequences also revealed that *T. ganodermatigerum* was phylogenetically distinct not only from *T. amazonicum* but also from other previously reported *Trichoderma* species.

Etymology: The name refers to the host genus "*Ganoderma*" from which it was isolated.

Typification: CHINA. Jilin Province, Panshi City, Songshan County, from *Ganoderma sichuanense*, alt. 310 m, 126°56′ E, 42°77′ N, 18 August 2021, *Xiaoya An*, HMJAU59014, preserved in Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi of Jilin Agricultural University. Ex-type culture CCMJ5245. Sexual morph: Undetermined. (ITS: ON399102, TEF1: ON567195, and RPB2: ON567189).

Teleomorph: Unknown.

Description: The optimum temperature was 25 °C, and the colony radius on CMD was 7–9 mm at 15 °C, 19–23 mm at 20 °C, 43–52 mm 25 °C, and 32–36 mm at 30 °C, with no growth at 35 °C, and mycelium covering the plate after ten days at 25 °C (Figure 3E). Colony hyaline, thin, and radiating, white in the initial stage, and gradually turned to light green with slight zonate. Mycelia were sparse and delicate, hard to be observed, and aerial hyphae were inconspicuous. Conidiation starting after six days, formed in pustules. Pustules were spreading near the original inoculum or at the edge of the colony, distributed loosely in the plate, white in the initial stage and then turned green. No chlamydospores were observed. No distinct odor and no diffusing pigment were observed.

Colony radius on SNA after 72 h 5–8 mm at 15 °C,13–15 mm at 20 °C, 42–43 mm at 25 °C, and 25–28 mm at 30 °C, and can hardly see the growth at 35 °C. Mycelium covering the plate after six days at 25 °C (Figure 3F). Colony hyaline, thin, irregular, surface mycelium scant. Aerial hyphae are inconspicuous and short. Conidiation starting after three days, formed in loose pustules. Pustules initially white, loose distribution, later turn aggregated and green. No chlamydospores were observed. No distinct odor and no diffusing pigment were observed.

On PDA, the colony radius was 9–12 mm at 15 °C, 22–28 mm at 20 °C, 38–44 mm at 25 °C, and 30–40 mm at 30 °C, with no growth at 35 °C after 72 h, and mycelium covering the plate after 5–6 days at 25 °C (Figure 3D). The colony was circular, spreading in several concentric rings; aerial hyphae were common, dense, and green; the margin was relatively loose and whitish under the alternative light situations. However, mycelia were aerated and white, and only green appeared near the inoculation site under the condition of total darkness. Conidiation starting after 3–4 days, formed on aerial hyphae, spreading in a circle around the original inoculum. Conidiophores are typically tree-like, straight, or slightly curved, comprising a distinct main axis with side branches paired or unilateral and often terminating in whorls of 3–4 divergent phialides, rarely with a terminal solitary phialide (Figure 3G–J), branches densely disposed, arising at mostly vertical angles upwards, rebranching 1–3 times; the distance between two neighboring branches is (6.6–) 10.0–30.0 (–35.6) µm. Phialides formed paired or in whorls of 3–5, lageniform, spindly, usually arising at an acute angle to the axis, rarely solitary (Figure 3F), (1.1–) 2.8–12.3 (–16) µm× (0.2–) 1.9–3.4 (–3.6) µm, *l/w* ratio (1.6–) 1.7–5.9 (–7.0), (0.2–) 1.4–2.6 (–2.8) µm wide at the base. Conidia one-celled, green, smooth-walled, globose to subglobose, sometimes ellipsoid, (3.4–) 3.6–4.8 (–5.3) µm× (2.9–) 3.2–4.3 (–4.6) µm, *l/w* ratio 1.1–1.5. No chlamydospores were observed. No distinct odor and no diffusing pigment were observed.

Distribution: Jilin, Shandong, and Heilongjiang Provinces, China.

Additional specimen examined: China, Jilin Province, Panshi city, Songshan County, from *Ganoderma sichuanense*, alt. 310 m, 126°56′ E, 42°77′ N, 11 Oct. 2021, *Xiaoya An*, HMJAU59013.

Notes: Fungicolous on the fruiting body of *G. sichuanense* in terrestrial habitats. It produces extremely tree-like main axes and branches and green, globose conidia (Figure 3N). The results of the phylogenetic tree strongly support its status as a new taxon (Figure 1), indicating its affinity to the Harzianum clade [16]. The species was related to *T. amazonicum* and *T. pleuroticola*. Regarding *T. amazonicum*, it is a host-specific endophyte and might have potential for biocontrol of *Hevea* diseases [50]. Phylogenetically, *T. ganodermatigerum* is related to *T. pleuroticola* in the mycoparasite group. Morphologically, both species grow rapidly and form broad concentric rings on PDA. Conidiation formed small pustules, and the green spores cause the colony to change from light to dark green [120]. The difference is that the new species starts with white, aerial mycelia and spores are more spherical or nearly spherical, with obvious green color, while the spores of *T. pleuroticola* are light green, subglobose to broadly ellipsoidal conidia, slightly smaller than *T. ganodermatigerum*, and reported more on *Pleurotus ostreatus*, *Pleurotus eryngii* var. *ferulae*, *Lentinula edodes*, and *Cyclocybe aegerita* [69,73,83,120].

*Trichoderma koningiopsis* Samuels, Carm. Suárez & H.C. Evans 2006.

Description: Fungicolous, colonized the fruiting body of *G. sichuanense*, causing green mold disease and occurring mostly from June to September. It is very difficult to distinguish the mycelium in the early stage, and only scattered spots present under the cap. Then, white mycelium appeared, with radiating growth. The edge of the colony is often accompanied by a yellow or brown line. A large number of green spores were produced in the late stage. Young basidiomes were inoculated with *T. koningiopsis*, which reproduced the original signs; the same pathogen was isolated again from the diseased fruitbody.

On PDA, the colony was radial, first whitish, became dark green with fluffy hyphae after ten days. Aerial hyphae were common and dense, but no concentric rings were observed. Mycelia often appear white in complete darkness, and light stimulates spore production, resulting in a green colony. Conidia formed in pustules, spreading near the original inoculum, white, turning green later. On CMD, mycelium covering the plate after ten days at 25 °C, loose and slim, aerial hyphae were absent. Conidia were formed in pustules, which were only produced at the edge of a colony. On SNA media, concentric rings of light yellow or green appeared, and spores were produced in four days. Conidiophore branches arose at right angles, and primary branches arose singly or in pairs. Conidia were ellipsoidal to oblong-shaped, green, 2.8–7.3 × 2.5–7.0 μm. No chlamydospores, no distinct odor, and no diffusing pigment were observed.

Material examined: CHINA, Jilin Province, on a fruiting body of *Ganoderma*, 4 August 2020; *Xiaoya An*, HMJAU59012, living culture CCMJ5253, CCMJ5254 (ITS: ON385996, ON385947; TEF1: ON567187, ON567188, and RPB2: ON567201, ON567202, respectively).

Notes: *Trichoderma koningiopsis* is found throughout tropical America, as well as East Africa, Europe, Canada, and eastern North America [23]. This species is mainly found in soil, twigs, and decayed leaves, and the sexual type is mostly found in wood. At present, *T. koningiopsis* has been reported to cause green mold of *Phaiius rubrovolvata* [91], and to our knowledge, this is the first time that it has caused green mold on *G. sichuanense*. Our sequences had high similarity to the *T. koningiopsis* sequence after BLAST, and the results of the phylogenetic tree also confirmed the correctness of the classification (Figure 2).

## 4. Discussion

Edible and medicinal mushrooms have become a very important crop and are grown commercially in many countries [1,121], but the production, including the yield and quantity, is challenged by fungal diseases [2,24]. *Trichoderma ganodermatigerum* is a new species of *Trichoderma*. The results from the phylogenetic analyses separate the new species from other closely related and morphologically similar species. The sequences indicate it belongs to the Harzianum clade. To date, more than forty *Trichoderma* species have been reported to be associated with mushroom green mold disease. *Trichoderma atroviride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii*, and *T. viride* are the six most commonly cited species causing disease on edible mushrooms (Table 2), all of which could infect six to eleven species of cultivated mushrooms [61,64,68,73,83,91,119,122,123]. Before this study, there were seven known species that could cause *G. sichuanense* diseases, namely, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii*, *T. viride*, *T. atrobrunneum*, *T. ganodermatis* [47], and *T. hengshanicum* [87], while *T. orientale* can cause disease on *G. applanatum* [124].

*Trichoderma* green mold infection in edible basidiomycetes has a long history [125]. There are many types of interactions between mushrooms and *Trichoderma* [126–129]. Similar to *T. aggressivum*, the causal agent of *Agaricus* green mold disease [130], no obvious biting phenomenon was observed between pathogen and mushroom in this study. Through SEM observation, in the interaction zone between *G. sichuanense* and *T. ganodermatigerum*, the tissue surface of *Ganoderma* became uneven with irregular holes (Figure 3K), the pores on the *Ganoderma* spores became larger, and the double-layer structure was damaged, resulting in spore invagination (Figure 3L), which was similar to the interaction between *Trichoderma* and shiitake [83]. We can at least suspect that the cell-wall-degrading enzymes play an important role in the process according to the symptoms of soft tissue with holes or even oozing liquid of *Ganoderma*. In addition, *T. songyi* could have great biological potential because it is closely related to the biological agents (Figure 2, Clade II).

The application of the *Trichoderma* species as biocontrol agents began in 1934 when Weindling first discovered that *Trichoderma* could be parasitic on the hyphae of *Rhizoctonia solani*, and since then, an increasing amount of research has focused on this field [131]. Because many *Trichoderma* species are symbiotic and fungal parasitoids, they need to produce degradation enzymes or secondary metabolites to obtain nutrients from the host, so they have been developed as biocontrol agents for plant diseases [50,55,112,132,133]. Among the species associated with mushrooms, nine species are used as biological agents already. *Trichoderma koningiopsis*, the new pathogen for *G. sichuanense* in this study, has been a biocontrol agent for a long time [134]. Since *T. ganodermatigerum* can infect cultivated *Ganoderma*, leading to growth stagnation or the cessation of sporulation of *Ganoderma*, it could be a potential biocontrol agent for plant disease. Therefore, the parasitic characteristics and compounds should be further studied.

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