New Species of Talaromyces (Fungi) Isolated from Soil in Southwestern China

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Simple Summary: Talaromyces species are distributed all around the world and occur in various environments, e.g., soil, air, living or rotten plants, and indoors. Some of them produce enzymes and pigments of industrial importance, while some cause Talaromycosis. Talaromyces marneffei, a well-known and important human pathogen, is endemic to Southeast Asia and causes high mortality, especially in HIV/AIDS patients and those with other immunodeiciencies. China covers 3 of the 35 global biodiversity hotspots. During the explorations of fungal diversity in soil samples collected at different sites of southwestern China, two new Talaromyces species, T. chongqingensis and T. wushanicus, were discovered based on phylogenetic analyses and morphological comparisons. They are described and illustrated in detail. Six phylogenetic trees of the sections Talaromyces and Trachyspermi were constructed based on three-gene datasets and revealed the phylogenetic positions of the new species. This work provided a better understanding of biodiversity and phylogeny of the genus. The results make the concepts of the two sections of Talaromyces well-established. The discovery will be beneficial for future evaluation of the potential usages and functions of the new species.

Abstract: Southwestern China belongs among the global biodiversity hotspots and the Daba Mountains are recognized as one of the priority conservation areas. During the exploration of fungal biodiversity from soil samples collected from Mount Daba, two species of Talaromyces were discovered as new to science based on phylogenetic analyses and morphological comparisons. Talaromyces chongqingensis sp. nov. is a sister taxon of T. minioluteus and T. minnesotensis in the section Trachyspermi; and T. wushanicus sp. nov., affiliated to the section Talaromyces, is closely related to T. crustii and T. siamensis. The new species differ from their sisters in DNA sequences, growth rates, and morphological characteristics. Descriptions and illustrations of them are provided in detail.

Keywords: Ascomycota; biodiversity hotspot; DNA barcodes; Eurotiales; phylogeny; taxonomy; Trichocomaceae

1. Introduction

Talaromyces C.R. Benj. is a cosmopolitan genus occurring in various environments, e.g., soil, air, living or rotten plants, and indoors. Its beneficial and harmful effects on humans have been well documented. Enzymes and pigments produced by some species of the genus are of industrial importance, such as β-glucosidase produced by T. amestolkiae N. Yilmaz et al. [1] and T. cellulolyticus T. Fujii et al. [2], and red pigments by T. atroroseus N. Yilmaz et al. [3,4]. Talaromycosis caused by several species were also reported [5,6]. Among them, T. marneffei (Segretain et al.) Samson et al., endemic to Southeast Asia, is a well-known and important human pathogen causing high mortality in the absence of proper diagnosis and prompt treatment, especially in HIV/AIDS patients and those with other immunodeficiencies [7].
A total of 170 *Talaromyces* species were accepted and classified into seven sections according to a recent monographic study [8]. Moreover, *T. albisclerotius* B.D. Sun et al., *T. aspriconidius* B.D. Sun et al., *T. aureolinus* L. Wang, *T. bannicus* L. Wang, *T. brevis* B.D. Sun et al., *T. guizhouensis* B.D. Sun et al., *T. penicilloides* L. Wang, *T. putearis* Crous, *T. rufus* B.D. Sun et al., *T. sparsus* L. Wang, *T. tenuis* B.D. Sun et al., and *T. yunnanensis* Doilm and C.F. Liao were later described [9–12]. In the section (sect.) *Trachyspermi* Yaguchi and Udagawa, 30 species are commonly accepted; and in the sect. *Talaromyces*, the largest part of the genus, 75 species have been recognized.

During the explorations of fungal diversity in soil samples collected at different sites of Chongqing and Sichuan in southwestern China, two *Talaromyces* species belonging to the sections *Talaromyces* and *Trachyspermi* were further discovered as new to science based on phylogenetic analyses and morphological comparisons. They are described and illustrated in detail.

### 2. Materials and Methods

#### 2.1. Fungal Materials

Cultures were isolated from soil samples collected from Chongqing and areas nearby in Sichuan Province in October 2020. Dried cultures were deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS), and the living ex-type strains were preserved in the China General Microbiological Culture Collection Center (CGMCC).

#### 2.2. Morphological Observations

Morphological characterization was conducted following the standardized methods [13]. Four standard growth media were used: Czapek yeast autolysate agar (CYA, yeast extract Oxoid, Hampshire, UK), malt extract agar (MEA, Amresco, Solon, OH, USA), yeast extract agar (YES) and potato dextrose agar (PDA). The methods for inoculation, incubation, microscopic examinations, and digital recordings were following our previous studies [14–16].

#### 2.3. DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from the cultures grown on PDA for 7 days using the Plant Genomic DNA Kit (DP305, TIANGEN Biotech, Beijing, China). Polymerase chain reaction (PCR) amplifications of the internal transcribed spacer (ITS), beta-tubulin (BenA), calmodulin (CaM) and RNA polymerase II second largest subunit (RPB2) gene regions were conducted with routine methods [14–16]. The products were purified and subject to sequencing on an ABI 3730 DNA Sequencer (Applied Biosystems, Bedford, MA, USA). Although the ITS region, the recommended standard DNA barcode for fungi, is not sufficient to discriminate the species of this genus, the sequences provided here will be helpful for other researchers in case of need.

#### 2.4. Phylogenetic Analyses

The forward and reverse sequences newly generated in this study were assembled using Seqman v. 7.1.0 (DNASTAR Inc., Madison, WI, USA). The assembled sequences were deposited in GenBank. Previously described species from the corresponding sections, which were used for phylogenetic analyses, are listed in Tables 1 and 2. Newly generated sequences of this study are shown in Table 3. For each section, three datasets of BenA, CaM, and RPB2 were compiled. Sequences of each dataset (35 species for sect. *Trachyspermi* and 79 species for sect. *Talaromyces*) were aligned using MAFFT v. 7.221 [17], and then manually edited in BioEdit v. 7.1.10 [18] and MEGA v. 6.0.6 [19]. Maximum likelihood (ML) analyses were performed using RAxML-HPC2 [20] on XSEDE 8.2.12 on CIPRES Science Gateway v. 3.3 [21] with the default GTRCAT model. Bayesian Inference (BI) analyses were performed with MrBayes v. 3.2.5 [22]. Appropriate nucleotide substitution models and parameters were determined by Modeltest v. 3.7 [23]. The consensus trees were viewed in FigTree v. 1.3.1 (Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on
The type species of section Talaromyces served as outgroup taxon of the Trachyspermi tree and vice versa.

Table 1. Previously described Talaromyces species used in phylogenetic analyses of the sect. Trachyspermi.

| Species             | Strain     | Locality                  | Substrate                | ITS          | BenA          | CaM          | RP2          |
|---------------------|------------|---------------------------|--------------------------|--------------|---------------|--------------|--------------|
| T. aerius           | CGMCC 3.18197 T | China: Beijing indoor air| KU866647 KU866835 KU866731 KU866991 |
| T. affinitatimellis | CBS 143840 T | Spain honey               | LT906543 LT906552 LT906549 LT906546 |
| T. albiscerotius    | CBS 141839 T | China: Guizhou soil       | MN864276 MN863345 MN863322 MN863334 |
| T. albobicerticillus| CBS 133440 T | China: Taiwan decaying leaves | HQ605705 KF114778 KJ885258 KM023310 |
| T. amyrossmaniae    | NFCC 1919 T | India decaying fruits of Terminalia bellerica | MH909062 MH909064 MH909068 MH909066 |
| T. assiutensis      | CBS 147.78 T | Egypt soil                | JN899323 KJ865720 KJ885260 KM023305 |
| T. atroroseus       | CBS 133442 T | South Africa house dust  | KF114747 KF114789 KJ775418 KM023288 |
| T. austrocalifornicus| CBS 644.95 T | USA soil                  | JN899357 KJ865732 KJ885261 MN969147 |
| T. basipetosporus   | CBS 143836 T | South Korea decaying leaves | LT906542 LT906563 n.a. LT906545 |
| T. brasilensis      | CBS 142493 T | Brazil honey              | MF278323 LT855560 LT855563 MN969198 |
| T. catalonicus      | CBS 143039 T | Spain herbivore dung      | LT899793 LT898318 LT899775 LT899811 |
| T. clemensii        | PPRI 26753 T | South Africa wood in mine| MK951940 MK951833 MK951906 MN418451 |
| T. convolutus       | CBS 100537 T | Nepal soil                | JN899330 KF114773 MN969316 JN121414 |
| T. diversus         | CBS 320.48 T | USA mouldy leather        | KJ865740 KJ865723 KJ885268 KM023285 |
| T. erythromelis     | CBS 644.80 T | Australia soil            | JN899383 HQ156945 KJ885270 KM023290 |
| T. guatemalensis    | CCF 6215 T | Guatemala soil            | MN322789 MN329687 MN329688 MN329689 |
| T. halophytorum     | KACC 48127 T | South Korea roots of Limonium tetragonum | MH725786 MH729367 MK111426 MK111427 |
| T. halenensis       | CGMCC 3.18012 T | China: Heilongjiang rotten wood | XXK447526 XXK447525 XXK447532 XXK447529 |
| T. minioluteus      | CBS 642.68 T | unknown unknown           | JN899346 MN969409 KJ885273 JF417443 |
| T. minnesotensis    | CBS 142381 T | USA human ear             | LT558966 LT559083 LT795604 LT795605 |
| T. pernambucoensis  | URM 6894 T | Brazil soil               | LR535947 LR535945 LR535946 LR535948 |
| Species                      | Strain       | Locality          | Substrate                      | ITS            | BenA       | CaM       | RPB2       |
|------------------------------|--------------|-------------------|--------------------------------|----------------|------------|-----------|------------|
| T. resinae (Z.T. Qi and H.Z. Kong) Houbraken and X.C. Wang 2020 | CGMCC 3.4387 T | China: Guizhou    | resin of Eucalyptus tereticornis | MT079858       | MN969442   | MT066184  | MN969221   |
| T. rubrifaciens W.W. Gao 2016 | CGMCC 3.17658 T | China: Beijing    | hospital air                    | KR855658       | KR855648   | KR855653  | KR855663   |
| T. solicola Visagie and K. Jacobs 2012 | DAOM 241015 T | South Africa     | soil                           | FJ160264       | GU385731   | KJ885279  | KM023295   |
| T. spelancarum Rodr.-Andr. et al. 2020 | CBS 143844 T | Spain             | sparkling wine                  | LT985890       | LT985901   | LT985906  | LT985911   |
| T. subericola Rodr.-Andr. et al. 2020 | CBS 144322 T | Spain             | sparkling wine                  | LT985888       | LT985899   | LT985904  | LT985909   |
| T. systyatus S.M. Romero et al. 2015 | BAFcult 3419 T | Argentina         | soil                           | KP026917       | KR233838   | KR233837  | n.a.       |
| T. trachyspermus (Shear) Stolk and Samson 1973 | CBS 373.48 T | USA               | unknown                         | JN899354       | KF114803   | KJ885281  | JF417432   |
| T. ucrainicus (Panas.) Udagawa 1966 | CBS 162.67 T | Ukraine           | potato starch                   | JN899394       | KF114771   | KJ885282  | KM023289   |
| T. udagawae Stolk and Samson 1972 | CBS 579.72 T | Japan             | soil                           | JN899350       | KF114796   | KX961260  | MN969148   |
| T. flavus (Klöcker) Stolk and Samson 1972 | CBS 310.38 T | New Zealand       | unknown                         | JN899360       | JX494302   | KF741949  | JF417426   |

| Table 2. Previously described Talaromyces species used in phylogenetic analyses of the sect. Talaromyces. |
|-------------------------------------------------|-------|-----------------|------------------------------|----------------|------------|-----------|------------|
| Species                           | Strain       | Locality          | Substrate                      | ITS            | BenA       | CaM       | RPB2       |
|-----------------------------------|--------------|-------------------|--------------------------------|----------------|------------|-----------|------------|
| T. aculeatus (Raper and Fennell) Samson et al. 2011 | CBS 289.48 T | USA               | textile                        | KF741995       | KF741929   | KF741975  | MH793099   |
| T. adpressus A.J. Chen et al. 2016   | CGMCC 3.18211 T | China: Beijing    | indoor air                     | KU866657       | KU866844   | KU866741  | KU867001   |
| T. alveolaris Guevara-Suarez et al. 2017 | CBS 142379 T | USA               | human bronchoalveolar lavage   | LT558969       | LT559086   | LT795596  | LT795597   |
| T. amazonensis N. Yilmaz et al. 2016 | CBS 140373 T | Colombia          | leaf litter                    | KX011509       | KX011490   | KX011502  | MN969186   |
| T. amestolkiae N. Yilmaz et al. 2012 | CBS 132696 T | South Africa      | house dust                     | JX315660       | JX315623   | KF741937  | JX315698   |
| T. angelicae S.H. Yu et al. 2013   | KACC 46611 T | South Korea       | dried root of Angelica gigas   | KF183638       | KF183640   | KJ885259  | KX961275   |
| T. annesophieae Houtbraken 2017    | CBS 142939 T | Netherlands       | soil                           | MF574592       | MF590098   | MF590104  | MN969199   |
| T. apiculatus Samson et al. 2011   | CBS 312.59 T | Japan             | soil                           | JN899375       | KF741916   | KF741950  | KM023287   |
| T. argentinensis Jurjević and S.W. Peterson 2019 | NRRRL 28750 T | Ghana             | soil                           | MH793045       | MH792917   | MH792981  | MH793108   |
| T. aurantiacus (J.H. Mill. et al.) Samson et al. 2011 | CBS 314.59 T | USA               | soil                           | JN899380       | KF741917   | KF741951  | KX961285   |
| T. aureolimus L. Wang 2021         | CGMCC 3.15865 T | China: Yunnan     | soil                           | MK837953       | MK837937   | MK837945  | MK837961   |
| T. australis Visagie et al. 2015   | CBS 137102 T | Australia         | soil under pasture             | KF741991       | KF741922   | KF741971  | KX961284   |
| Species         | Strain       | Locality            | Substrate     | ITS            | BenA          | CaM           | RPB2          |
|-----------------|--------------|---------------------|---------------|----------------|---------------|---------------|---------------|
| *T. bannicus*   | CGMCC 3.15862 T | China: Yunnan soil  | soil          | MK837955       | MK837939      | MK837947      | MK837963      |
| *T. beijingensis* | CGMCC 3.18200 T | China: Beijing indoor air | soil         | KU866649       | KU866837      | KU866733      | KU866993      |
| *T. c. calidicanius* (J.L. Chen) Samson et al. 2011 | CBS 112002 T | China: Taiwan soil  | soil          | JN899319       | HQ156944      | KF741934      | KM023311      |
| *T. c. californicus* Jurjevic and S.W. Peterson 2019 | NRRL 58168 T | USA air             | air           | MH793056       | MH792928      | MH792992      | MH793119      |
| *T. c. cnidii* S.H. Yu et al. 2013 | KACC 46617 T | South Korea dried roots of *Cnidium* sp. | dried roots of *Cnidium* sp. | KF183639 | KF183641 | KJ885266 | KM023299 |
| *T. c. coprophilus* Guevara-Suarez et al. 2020 | CBS 142756 T | Spain herbivore dung | herbivore dung | LT899794 | LT898319 | LT899776 | LT899812 |
| *T. c. cucurbitiradicus* L. Su and Y.C. Niu 2018 | ACCC 39155 T | China: Beijing endophyte from root of pumpkin (*Cucurbita moschata*) | endophyte from root of pumpkin (*Cucurbita moschata*) | KY053254 | KY053228 | KY053246 | n.a.       |
| *T. d. Takada and Udagawa 1988* | CBS 412.89 T | Japan cultivated soil | soil          | JN899327       | JX494306      | KF741959      | KM023282      |
| *T. dimorphus* K.Z. Jiang and L. Wang 2018 | CGMCC 3.15692 T | China: Hainan forest soil | forest soil   | KY007095       | KY007111      | KY007103      | KY112593      |
| *T. domesticus* Jurjevic and S.W. Peterson 2019 | NRRL 58121 T | USA floor swab      | floor swab    | MH793055       | MH792927      | MH792991      | MH793118      |
| *T. duculueci* (Delacr.) Samson et al. 2011 | CBS 322.48 T | France canvas      | canvas        | JN899342       | JX091384      | KF741955      | JN121491      |
| *T. euchlorocarpius* Yaguchi et al. 1999 | CBM PF1203 T | Japan soil          | soil          | AB176617       | KJ865733      | KJ885271      | KM023303      |
| *T. flavovirens* (Durieu and Mon.) Visagie et al. 2012 | CBS 102801 T | Spain unknown       | unknown       | JN899392       | JX091376      | KF741933      | KX961283      |
| *T. flavus* (Klocker) Stolk and Samson 1972 | CBS 310.38 T | New Zealand unknown | unknown       | JN899360       | JX494302      | KF741949      | JF417426      |
| *T. francae* N. Yilmaz et al. 2016 | CBS 113134 T | Colombia leaf litter | leaf litter   | KX011510       | KX011489      | KX011501      | MN969188      |
| *T. funiculosus* (Thom) Samson et al. 2011 | CBS 272.86 T | India *Lagenaria vulgaris* | *Lagenaria vulgaris* | JN899377 | MN969408 | KF741945 | KM023293   |
| *T. fusiformis* Visagie et al. 2015 | CBS 193.69 T | Netherlands soil    | soil          | KF741979       | KF741912      | KF741942      | MN969156      |
| *T. fuscoviridis* Visagie et al. 2015 | CBS 193.69 T | Netherlands soil    | soil          | KF741979       | KF741912      | KF741942      | MN969156      |
| *T. galapagensis* Samson and Mahoney 1977 | CBS 751.74 T | Ecuador soil under *Maytenus obovata* | soil under *Maytenus obovata* | JN899358 | JX091388 | KF741966 | KX961280 |
| *T. indigoticus* Takada and Udagawa 1993 | CBS 100534 T | Japan soil          | soil          | JN899331       | JX494308      | KF741931      | KX961278      |
| *T. intermedius* (Apinis) Stolk and Samson 1972 | CBS 152.65 T | UK swamp soil       | swamp soil    | JN899332       | JX091387      | KJ885290      | KX961282      |
| *T. kabodanensis* Houbbraken et al. 2016 | CBS 139564 T | Iran hypersaline soil | hypersaline soil | KP851981 | KP851986 | KP851995 | MN969190   |
| *T. kendrickii* Visagie et al. 2015 | CBS 136666 T | Canada forest soil  | forest soil   | KF741987       | KF741921      | KF741967      | MN969158      |
Table 2. Cont.

| Species | Strain | Locality | Substrate | ITS | BenA | CaM | RPB2 |
|---------|--------|----------|-----------|-----|------|-----|------|
| T. lentulus X.Z. Jiang and L. Wang 2018 | CGMCC 3.15689 T | China: Shandong | soil | KY007088 | KY007104 | KY007096 | KY112586 |
| T. liani (Kamyschko) N. Yilmaz et al. 2014 | CBS 225.66 T | China | soil | JN899395 | JX091380 | KJ885257 | KX961277 |
| T. louisianensis Jurjević and S.W. Peterson 2019 | NRRL 35823 T | USA | air | MH793052 | MH792924 | MH792988 | MH793115 |
| T. macrosporus (Stolk and Samson) Frisvad et al. 1990 | CBS 317.63 T | South Africa | apple juice | JN899333 | JX091382 | KF741952 | KM023292 |
| T. mae X.Z. Jiang and L. Wang 2018 | CGMCC 3.15690 T | China: Shanghai | forest soil | KY007090 | KY007106 | KY007098 | KY112588 |
| T. malicola Jurjević and S.W. Peterson 2019 | NRRL 3724 T | Italy | rhizosphere of an apple tree | MH909513 | MH909406 | MH909459 | MH909567 |
| T. mangshanicus X.C. Wang and W.Y. Zhuang 2016 | CGMCC 3.18013 T | China: Hunan | soil | KX447531 | KX447530 | KX447528 | KX447527 |
| T. marniifera (Segretrain et al.) Samson et al. 2011 | CBS 388.87 T | Vietnam | bamboo rat (Rhizomys sinensis) | JN899344 | JX091389 | KF741958 | KM023283 |
| T. marniifera (Segretrain et al.) Samson et al. 1994 | CBS 756.96 T | China: Taiwan | soil | MN431394 | KJ865727 | KJ885274 | KX961276 |
| T. mycothecae R.N. Barbosa et al. 2018 | CBS 142494 T | Brazil | nest of stingless bee (Melipona scutellaris) | MF278326 | LT855561 | LT855564 | LT855567 |
| T. neofusisporus L. Wang 2016 | CGMCC 3.15415 T | China: Tibet | leaf sample | KP765385 | KP765381 | KP765383 | MN969165 |
| T. ommate-anae Visagie et al. 2014 | CBS 138208 T | South Africa | house dust | KJ775720 | KJ775213 | KJ775425 | KX961281 |
| T. panamensis (Samson et al.) Samson et al. 2011 | CBS 128.89 T | Panama | soil | JN899362 | HQ156948 | KF741936 | KM023284 |
| T. penicillioides L. Wang 2021 | CGMCC 3.15822 T | China: Guizhou | soil | MK837956 | MK837940 | MK837948 | MK837964 |
| T. pinophillus (Hedgc.) Samson et al. 2011 | CBS 631.66 T | France | PVC | JN899382 | JX091381 | KF741964 | KM023291 |
| T. praetensis Jurjević and S.W. Peterson 2019 | NRRL 62170 T | USA | effluent of water treatment plant | MH793075 | MH792948 | MH793012 | MH793139 |
| T. primulinus (Pitt) Samson et al. 2011 | CBS 321.48 T | USA | unknown | JN899317 | JX494305 | KF741954 | KM023294 |
| T. pseudofuniculosus Guevara-Suarez et al. 2020 | CBS 143041 T | Spain | herbivore dung | LT899796 | LT898323 | LT899778 | LT899814 |
| T. purgamentorum N. Yilmaz et al. 2016 | CBS 113145 T | Colombia | leaf litter | KX011504 | KX011487 | KX011500 | MN969189 |
| T. purpureogenus (Stoll) Samson et al. 2011 | CBS 286.36 T | unknown | unknown | JN899372 | JX315639 | KF741947 | JX315709 |
Table 2. Cont.

| Species | Strain | Locality | Substrate | ITS          | BenA          | CaM          | RPB2          |
|---------|--------|----------|-----------|--------------|---------------|--------------|---------------|
| T. qii  | L. Wang 2016 CGMCC 3.15414 T | China: Tibet | leaf sample | KP765384     | KP765380      | KP765382     | MN969164      |
| T. rapidus | Guevara-Suarez et al. 2017 CBS 142382 T | USA | human bronchoalveolar lavage | LT558970     | LT559087      | LT795600     | LT795601      |
| T. ruber (Stoll) N. Yilmaz et al. 2012 CBS 132704 T | UK | aircraft fuel tank | JX315662     | JX315629      | KF741938     | JX315700      |
| T. rubicundus (J.H. Mill. et al.) Samson et al. 2011 CBS 342.59 T | USA | soil | JN899384     | JX494309      | KF741956     | KM023296      |
| T. sayulitensis | Visagie et al. 2014 CBS 138204 T | Mexico | house dust | KJ775713     | KJ775206      | KJ775422     | MN969146      |
| T. samensis (Manoch and C. Ramírez) Samson et al. 2011 CBS 475.88 T | Thailand | forest soil | JN899385     | JX091379      | KF741960     | KM023279      |
| T. soli Jurjević and S.W. Peterson 2019 NRRL 62165 T | USA | soil | MH793074     | MH792947      | MH793011     | MH793138      |
| T. sparsus L. Wang 2021 CGMCC 3.16003 T | China: Beijing | soil | MT077182     | MT083924      | MT083925     | MT083926      |
| T. stellenboschensis Visagie and K. Jacobs 2015 CBS 135665 T | South Africa | soil | JX091471     | JX091605      | JX140683     | MN969157      |
| T. stipitatus (Thom) C.R. Benj. 1955 CBS 375.48 T | USA | rotting wood | JN899348     | KM111288      | KF741957     | KM023280      |
| T. stollii N. Yilmaz et al. 2012 CBS 408.93 T | Netherlands | AIDS patient | JX315674     | JX315633      | JX315646     | JX315712      |
| T. striatocandidum (R.F. Castañeda and W. Gams) Houbraken et al. 2020 CBS 550.89 T | Cuba | leaf litter of Pachyanthus poirettii | MN431418     | MN969441      | MN969360     | MT156347      |
| T. thailandensis Manoeh et al. 2013 CBS 133147 T | Thailand | forest soil | JX898041     | JX494294      | KF741940     | KM023307      |
| T. tumali Jurjević and S.W. Peterson 2019 NRRL 62151 T | USA | soil from prairie | MH793071     | MH792944      | MH793008     | MH793135      |
| T. veerkampii Visagie et al. 2015 CBS 500.78 T | Columbia | soil | KF741984     | KF741918      | KF741961     | KX961279      |
| T. verruculosus (Peyronel) Samson et al. 2011 NRRL 1050 T | USA | soil | KF741994     | KF741928      | KF741944     | KM023306      |
| T. versatilis Bridge and Buddie 2013 IMI 134755 T | UK | unknown | MN431395     | MN969412      | MN969319     | MN969161      |
| T. viridis (Stolk and G.F. Orr) Arx 1987 CBS 114.72 T | Australia | soil | AF285782     | JX494310      | KF741935     | JN121430      |
| T. viridulus Samson et al. 2011 CBS 252.87 T | Australia | soil | JN899314     | JX091385      | KF741943     | JF417422      |
| T. xishaensis X.C. Wang et al. 2016 CGMCC 3.17995 T | China: Hainan | soil | KU644580     | KU644581      | KU644582     | MZ361364      |
| T. trachyspermus (Shear) Stolk and Samson 1973 CBS 373.48 T | USA | unknown | JN899354     | KF114803      | KJ885281     | JF417432      |
Table 3. New species and newly generated sequences reported in this study.

| Species             | Strain | Locality     | Substrate | ITS       | BenA       | CaM       | RPB2      |
|---------------------|--------|--------------|-----------|-----------|------------|-----------|-----------|
| T. chongqingensis   | CS26-67 T | China: Chongqing | soil      | MZ358001  | MZ361343   | MZ361350  | MZ361357  |
|                     |        |              |           |           |            |           |           |
| T. wushanicus       | CS17-05 T | China: Chongqing | soil      | MZ356356  | MZ361347   | MZ361354  | MZ361361  |
| X.C. Wang and W.Y. Zhuang sp. nov. |        |              |           |           |            |           |           |
| T. wushanicus       | CS17-04 | China: Chongqing | soil      | MZ356357  | MZ361348   | MZ361355  | MZ361362  |
|                     |        |              |           |           |            |           |           |
| T. wushanicus       | CS17-06 | China: Chongqing | soil      | MZ356358  | MZ361349   | MZ361356  | MZ361363  |
|                     |        |              |           |           |            |           |           |

3. Results

3.1. Phylogenetic Analysis

The characteristics of datasets used in the phylogenetic analyses are presented in Table 4. Phylogenetic analyses of the section *Trachyspermi* revealed that *T. chongqingensis* always grouped with *T. minioluteus*, *T. minnesotensis*, and *T. udagawae*, having strong statistic supports. In the BenA and CaM analyses (Figures 1 and 2), *T. minioluteus* was the closest sister of the new species; while *T. minioluteus* and *T. minnesotensis* were both closely related to *T. chongqingensis* in the RPB2 tree (Figure 3). In the phylogenetic analysis of section *Talaromyces* based on the BenA dataset, *T. wushanicus* clustered with *T. siamensis* (Figure 4); while *T. cnidii* and *T. siamensis* were closely related to the new species in the CaM and RPB2 analyses (Figures 5 and 6).

Table 4. Detailed characteristics of the datasets.

| Section     | Loci | No. of Seq. | Length of Alignment | Model for BI |
|-------------|------|------------|---------------------|--------------|
| *Trachyspermi* | BenA | 35         | 533                 | TVM+I+G      |
|             | CaM  | 34         | 656                 | SYM+I+G      |
|             | RPB2 | 34         | 920                 | GTR+I+G      |
| *Talaromyces* | BenA | 79         | 490                 | TrN+I+G      |
|             | CaM  | 79         | 565                 | SYM+I+G      |
|             | RPB2 | 78         | 978                 | TVM+I+G      |

Full names of the used models: GTR+I+G (General Time Reversible with Invariant sites and Gamma distribution); SYM+I+G (Symmetrical model with Invariant sites and Gamma distribution); TrN+I+G (Tamura–Nei model with Invariant sites and Gamma distribution); TVM+I+G (Transversion model with Invariant sites and Gamma distribution).
Figure 1. Maximum likelihood phylogeny of *Talaromyces* sect. *Trachyspermi* inferred from the BenA dataset. Bootstrap values $\geq 70\%$ (left) or posterior probability values $\geq 0.95$ (right) are indicated at nodes. Asterisk denotes 100% bootstrap or 1.00 posterior probability.

Figure 2. Maximum likelihood phylogeny of *Talaromyces* sect. *Trachyspermi* inferred from the CaM dataset. Bootstrap values $\geq 70\%$ (left) or posterior probability values $\geq 0.95$ (right) are indicated at nodes. Asterisk denotes 100% bootstrap or 1.00 posterior probability.
3.2. Taxonomy

*Talaromyces chongqingensis* X.C. Wang and W.Y. Zhuang, sp. nov., Figure 7.

Fungal Names: FN570851

Etymology: The specific epithet refers to the type locality.

in Talaromyces sect. Trachyspermi

Typification: China, Chongqing City, Chengkou County, Daba Mountain National Nature Reserve, Gaoguan Town, at the riverside of River Ren, 31°49′40″ N 109°0′24″ E, in soil under a palm tree, 30 October 2020, Xin-Cun Wang, Huan-Di Zheng and Chang Liu, culture, Zhi-Kang Zhang, CS26-67 (holotype HMAS 247849, ex-type strain CGMCC 3.20482).

DNA barcodes: ITS MZ358001, BenA MZ361343, CaM MZ361350, RPB2 MZ361357.

Colony diam: after 7 days at 25 °C (unless stated otherwise): on CYA, 12–13 mm; on CYA at 37 °C, no growth; on CYA at 5 °C, no growth; on MEA, 17–18 mm; on YES 18–19 mm; on PDA, 18–19 mm.

Colonial characteristics:

On CYA at 25 °C, after 7 days: colonies nearly circular, protuberant in centers; margins moderately wide, entire; mycelia white and yellow; texture velutinous; sporulation dense; conidia *en masse* yellowish green to dull green; soluble pigments light brown; exudates small, clear; reverse orange, buff at the margins but dark orange at centers.

On MEA at 25 °C, after 7 days: Colonies irregular, protuberant in centers, pink hyphae growing at centers; margins moderately wide, irregular; mycelia white and yellow; texture floccose; sporulation dense; conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse buff.
Figure 4. Maximum likelihood phylogeny of *Talaromyces* sect. *Talaromyces* inferred from the BenA dataset. Bootstrap values $\geq 70\%$ (left) or posterior probability values $\geq 0.95$ (right) are indicated at nodes. Asterisk denotes 100% bootstrap or 1.00 posterior probability.
Figure 5. Maximum likelihood phylogeny of *Talaromyces* sect. *Talaromyces* inferred from the CaM dataset. Bootstrap values ≥ 70% (left) or posterior probability values ≥ 0.95 (right) are indicated at nodes. Asterisk denotes 100% bootstrap or 1.00 posterior probability.
Figure 6. Maximum likelihood phylogeny of *Talaromyces* sect. *Talaromyces* inferred from the RPB2 dataset. Bootstrap values $\geq 70\%$ (left) or posterior probability values $\geq 0.95$ (right) are indicated at nodes. Asterisk denotes 100% bootstrap or 1.00 posterior probability.
3.2. Taxonomy

*Talaromyces chongqingensis* X.C. Wang and W.Y. Zhuang, sp. nov., Figure 7.

**Figure 7.** *Talaromyces chongqingensis* (CS26-67). (A) Colonies: top row left to right, obverse CYA, MEA, YES, and PDA; bottom row left to right, reverse CYA, MEA, YES, and PDA; (B–F) Conidiophores; (G) Conidia. Bars: (D) 15 µm, applies also to (B,C); (G) 10 µm, applies also to (E,F).

On YES at 25 °C, after 7 days: Colonies nearly circular, strongly protuberant in centers; margins moderately wide, entire; mycelia white and yellow; texture velutinous; sporulation moderately dense; conidia *en masse* pale green; soluble pigments light brown; exudates absent; reverse orange, yellow brown at the margins but dark orange at centers.

On PDA at 25 °C, after 7 days: Colonies nearly circular, plain, slightly protuberant in centers; margins moderately wide, irregular; mycelia white and yellow; texture velutinous; sporulation very dense; conidia *en masse* yellowish green; soluble pigments absent; exudates absent; reverse yellow brown, buff at the margins but orange at centers.

**Micromorphology:** Conidiophores biverticillate; stipes smooth-walled, 90–250 × 2.5–3.0 µm; metulae 4–5, 10–13 × 2.5–3.5 µm; phialides acerose, tapering into very thin neck, 3–5 per metula, 10–13.5 × 2.0–2.5 µm; conidia ellipsoidal to broad fusiform, smooth-walled, 2.5–3.5 × 2.0–2.5 µm.

Additional strains examined: China, Chongqing City, Chengkou County, Daba Mountain National Nature Reserve, Gaoguan Town, at the riverside of River Ren, 31°49′40″ N 109°0′24″ E, in soil under a palm tree, 30 October 2020, Xin-Cun Wang, Huan-Di Zheng and Chang Liu, culture, Zhi-Kang Zhang, CS26-63; *ibid.*, CS26-73; *ibid.*, CS26-75.

**Notes:** This species is phylogenetically close to *T. minioluteus* and *T. minnesotensis*, but differs from them in growth rate on CYA and MEA at 25 °C (Table 5) and pink hyphae present at the centers of colonies on MEA. The sequence data of the four cultures of this fungus are completely identical.
Table 5. Cultural and morphological comparisons of new species and their closely related species.

| Species             | CYA 25 °C (mm) | CYA 37 °C (mm) | MEA (mm) | YES (mm) | Conidia Shape                          | Conidia Wall | Conidia Size (µm) | Reference       |
|---------------------|----------------|----------------|----------|----------|----------------------------------------|--------------|-------------------|-----------------|
| T. chongqingensis   | 12–13          | no growth      | 17–18    | 18–19    | ellipsoidal to broad fusiform          | smooth       | 2.5–3.5 × 2–2.5   | This study      |
| T. minioluteus      | 17–18          | no growth      | 21–22    | 18       | ellipsoidal                             | smooth       | 2.5–4 × 1.5–2.5   | [24]            |
| T. minnesotensis    | 24–26          | no growth      | 13–15    | 21–24    | ellipsoidal                             | smooth       | 2.5–3.5 × 2–3     | [5]             |
| T. udagawae         | 6–8            | no growth      | 10–11    | 8–9      | subglobose to ellipsoidal              | smooth       | 3–4 × 2–3         | [24]            |
| T. cnidii           | 30–35          | 17–20          | 38–43    | 40–45    | ellipsoidal                             | smooth to finely rough | 3–4 × 2–2.5    | [25]            |
| T. siamensis        | 20–22          | 15             | 32–33    | 27–28    | ellipsoidal to fusiform                | smooth to finely rough | 3–4 × 2–3     | [24]            |
| T. wushanicus       | 21–24          | 17–19          | 40–44    | 24–28    | ellipsoidal to broad fusiform          | smooth to finely rough | 3–4 × 2.5–3    | This study      |

Talaromyces wushanicus X.C. Wang and W.Y. Zhuang, sp. nov., Figure 8.

Figure 8. Talaromyces wushanicus (CS17-05). (A) Colonies: top row left to right, obverse CYA, MEA, YES, and PDA; bottom row left to right, reverse CYA, MEA, YES, and PDA; (B–F) Conidiophores; (G) Conidia. Bars: (D) 15 µm, applies also to (B,C); (G) 10 µm, applies also to (E,F).
Fungal Names: FN570852
Etymology: The specific epithet refers to the type locality.
in Talaromyces sect. Talaromyces
Typification: China, Chongqing City, Wushan County, Dachang Town, Yanghe Village, 31°17′33″ N 109°50′44″ E, in soil, 29 October 2020, Xin-Cun Wang, Huan-Di Zheng and Chang Liu, culture, Zhi-Kang Zhang, CS17-05 (holotype HMAS 247848, ex-type strain CGMCC 3.20481).
DNA barcodes: ITS MZ356356, BenA MZ361347, CaM MZ361354, RPB2 MZ361361.
Colony diam: after 7 days at 25 °C (unless stated otherwise): on CYA, 21–24 mm; on CYA at 37 °C, 17–19 mm; on CYA at 5 °C, no growth; on MEA, 40–44 mm; on YES, 24–28 mm; on PDA, 37–38 mm.
Colony characteristics: On CYA 25 °C, 7 days: Colonies nearly circular, protuberant in centers; margins narrow to moderately wide, nearly entire; mycelia white; texture velutinous; sporulation moderately dense; conidia en masse yellowish green; soluble pigments absent; exudates almost absent, sometimes very tiny, red, clear; reverse buff, orange to light brown at centers, but white and pink at periphery.
On CYA at 37 °C, after 7 days: Colonies irregular or nearly circular, protuberant in centers; margins moderately wide, nearly entire; mycelia white; texture velutinous; sporulation moderately dense; conidia en masse dull green to greyish green; soluble pigments absent; exudates absent; reverse buff.
On MEA at 25 °C, after 7 days: Colonies nearly circular, plain; margins wide, entire; mycelia yellow; texture velutinous; sporulation dense; conidia en masse yellowish green; soluble pigments absent; exudates almost absent, sometimes very tiny, hyaline, clear; reverse buff, but yellow to orange in centers.
On YES at 25 °C, after 7 days: Colonies nearly circular, deep, wrinkled, highly protuberant in centers; margins narrow to moderately wide, entire; mycelia white; texture velutinous; sporulation dense; conidia en masse yellowish green to dark green; soluble pigments absent; exudates absent, rarely red and clear; reverse white, yellow brown to light brown, rimose, or deeply concave in centers.
On PDA at 25 °C, after 7 days: Colonies nearly circular, plain, slightly protuberant in centers; margins moderately wide, entire; mycelia white; texture velutinous; sporulation dense; conidia en masse yellowish green; soluble pigments absent; exudates almost absent, sometimes very tiny, hyaline, clear, present at centers; reverse greyish white to greenish white, reddish brown at centers.
Micromorphology: Conidiophores biverticillate, rarely terverticillate; stipes smooth-walled, 85–225 × 2.0–3.0 µm; metulae 5, 9.5–11.5 × 2.5–3.0 µm; phialides acerose, tapering into very thin neck, 3–4 per metula, 10–11 × 2.0–2.5 µm; conidia ellipsoidal to broad fusiform, smooth to finely rough, 3–4 × 2.5–3 µm.
Additional strains examined: China, Chongqing City, Wushan County, Dachang Town, Yanghe Village, 31°17′33″ N 109°50′44″ E, in soil, 29 October 2020, Xin-Cun Wang, Huan-Di Zheng and Chang Liu, culture, Zhi-Kang Zhang, CS17-04; ibid., CS17-06.
Notes: This species is closely related to T. cnidii and T. siamensis in the phylogenetic trees (Figures 4–6), but it differs from T. cnidii in obviously slower growth rate on CYA and YES at 25 °C and from T. siamensis by an obviously faster growth on MEA at 25 °C (Table 5). Sequence comparisons indicate that the isolate CS17-04 has a one-base difference in ITS and a two-base difference in BenA from the other two strains. No morphological diversification was found among the strains.

4. Discussion

Of the 35 global biodiversity hotspots, 3 are located in southwestern China, consisting of Chongqing, Guizhou, Sichuan, Tibet, and Yunnan provinces [26]. Eight hotspot regions in the southwest of China were identified as priority conservation areas, including the Daba Mountains [27] where materials used for this study were gathered. Soil samples for floristic studies of fungi were collected from Chengkou, Wushan, and Wuxi counties in Chongqing and Wanyuan City in Sichuan. Although Talaromyces is a widespread genus
and distributed in more than 27 provinces, cities, or regions of China [14], it has never been reported from the above areas. In recent years, the number of new species of Talaromyces increased dramatically. There were 12 species recorded in Talaromyces sect. Trachyspermi and 36 ones in Talaromyces sect. Talaromyces in 2014 [24]. From 2018 to 2021, 13 additional species were discovered in the former section, and 20 new members were described in the latter. We are witnessing a trend: new fungal species are described at an accelerated rate.

Talaromyces species occur in diversified environments. When the information about the extype strains of more than 100 species in these two sections is gathered (Tables 1 and 2), it is found that soil is commonly the substrate. Fifty or so species were isolated from different kinds of soil, e.g., forest, cultivated, and swamp soil. Plant debris appears to be the second frequent source, which nearly 20 species inhabited. Four species were from humans and one, the well-known T. marneffei, from bamboo rat. Surprisingly, T. pinophilus was discovered on PVC, the third widely used plastic in the world, which is hard to biodegrade.

Among the 30 species accepted in Talaromyces sect. Trachyspermi, 6 were originally reported from China (Table 1). Moreover, 18 of the 75 species known in Talaromyces sect. Talaromyces were described based on the Chinese samples or specimens (Table 2). These data surely demonstrate that China has a high fungal diversity. With more investigations conducted, we expect to discover more new species of this group of fungi.

5. Conclusions

The present work provides a better understanding of biodiversity and phylogeny of the genus. The results make the concepts of the two sections of Talaromyces well-established and more sophisticated. The discovery will be beneficial for future evaluation of the potential usages and functions of the new species.

Author Contributions: Conceptualization, W.-Y.Z. and P.Z.; methodology, Z.-K.Z. and X.-C.W.; software, X.-C.W.; validation, X.-C.W.; formal analysis, X.-C.W. and Z.-K.Z.; investigation, X.-C.W.; resources, W.-Y.Z., P.Z. and X.-H.C.; data curation, X.-C.W.; writing—original draft preparation, X.-C.W.; writing—review and editing, W.-Y.Z. and X.-C.W.; visualization, X.-C.W.; supervision, X.-C.W., P.Z. and W.-Y.Z.; project administration, W.-Y.Z.; funding acquisition, P.Z., W.-Y.Z. and X.-C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by the National Natural Science Foundation of China (31750001) and Key Research Program of Frontier Science, Chinese Academy of Sciences (QYZDY-SSW-SMC029).

Acknowledgments: The authors would like to thank Huan-Di Zheng, Zhao-Qing Zeng, and Chang Liu of Institute of Microbiology, Chinese Academy of Sciences for collecting jointly the samples for this study.

Conflicts of Interest: The authors declare no conflict of interest.

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