Article

Fine Identification and Classification of a Novel Beneficial Talaromyces Fungal Species from Masson Pine Rhizosphere Soil

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Abstract: Rhizosphere fungi have the beneficial functions of promoting plant growth and protecting plants from pests and pathogens. In our preliminary study, rhizosphere fungus JP-NJ4 was obtained from the soil rhizosphere of Pinus massoniana and selected for further analyses to confirm its functions of phosphate solubilization and plant growth promotion. In order to comprehensively investigate the function of this strain, it is necessary to ascertain its taxonomic position. With the help of genealogical concordance phylogenetic species recognition (GCCSR) using five genes/regions (ITS, BenA, CaM, RPB1, and RPB2) as well as macro-morphological and micro-morphological characters, we accurately determined the classification status of strain JP-NJ4. The concatenated phylogenies of five (or four) gene regions and single gene phylogenetic trees (ITS, BenA, CaM, RPB1, and RPB2 genes) all show that strain JP-NJ4 clustered together with Talaromyces brevis and Talaromyces liani, but differ markedly in the genetic distance (in BenA gene) from type strain and multiple collections of T. brevis and T. liani. The morphology of JP-NJ4 largely matches the characteristics of genes Talaromyces, and the rich and specific morphological information provided by its colonies was different from that of T. brevis and T. liani. In addition, strain JP-NJ4 could produce reduced conidiophores consisting of solitary phialides. From molecular and phenotypic data, strain JP-NJ4 was identified as a putative novel Talaromyces fungal species, designated T. nanjingensis.

Keywords: rhizosphere beneficial fungi; Pinus massoniana; genealogical concordance phylogenetic species recognition; one new taxon (Talaromyces nanjingensis sp. nov)

1. Introduction

Rhizosphere fungi play roles in promoting plant growth and protecting plants from pests and pathogens. Phosphate-solubilizing fungi (PSF) are an important group of such fungi. Phosphate-solubilizing microbes in soil include PSF [1] and phosphate-solubilizing bacteria (PSB) [2]. Fungi and bacteria have their own advantages in adaptation in different environments. The variety and quantity of PSB were more than that of PSF [3], and the research studies on them are still in progress. Common PSF include Aspergillus, Penicillium, Trichoderma, and some mycorrhizal fungi. Phosphate-solubilizing fungi can be applied to a variety of crop ecosystems. For example, Aspergillus niger and Penicillium chrysogenum promote the growth and nutrient uptake of groundnut (Arachis hypogaea) [4]. Inoculation with the PSF Aspergillus niger significantly increases growth, root nodulation, and yield of soybean plants [5]. Phosphate-solubilizing fungi can also be applied to forest ecosystems. The fungal suspension and extracellular metabolites of Penicillium

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guanacastense have shown to increase the shoot length and root crown diameter of Pinus massoniana seedlings [6].

Penicillium is one of the most common genera of fungi worldwide. It is widely distributed in nature and primarily functions in breaking down organic matter to provide nutrients for its growth [7,8]. Since Link (1809) introduced the species concept of Penicillium [9] and Dierckx [10] introduced the subgenus classification system of Penicillium, studies on Penicillium have become increasingly popular. At the beginning of the 20th century, an early system of classification and identification based on colony characteristics and conidiophore branching patterns was proposed. The genus Talaromyces was first introduced by Benjamin (1955) as the sexual state of the genus Penicillium [11]. Stolk and Samson (1972) divided Talaromyces into four sections based on differences in their asexual states [12]. Later, more advanced and novel classification schemes based on conidiophore structure, branching pattern, and phialide shape, as well as strain growth characteristics, emerged. Pitt (1979) classified Penicillium into four subgenera: Aspergilloides, Biverticillium, Furcatum, and Penicillium, which contain 10 sections and 21 series. Since then, the modern concept of Penicillium sensu lato has emerged [13].

With the popularization of DNA-based phylogenetic studies of fungi, it has been gradually recognized that the subgenus Biverticillium within the genus Penicillium sensu lato is phylogenetically separate from other subgenera of Penicillium and is closely related to Talaromyces, the previously mentioned sexual morph of Penicillium. The subgenera Aspergilloides, Furcatum, and Penicillium originated from Penicillium sensu lato, together with the genus Eupenicillium, and other species now fall within Penicillium sensu stricto, whereas subgenus Biverticillium is synonymized under the current genus Talaromyces [14,15]. Today, section Talaromyces is not limited to sexual species, but it still contains most of the sexually reproducing species in the genus Talaromyces. Yilmaz et al. (2014) proposed a new sectional classification for the genus Talaromyces, placing the 88 accepted species into seven sections, namely, Bacillispori, Helici, Islandici, Purpurei, Subinflatii, Talaromyces, and Trachyspermi [15]. Talaromyces flavus (Klöcker) Stolk and Samson (= T. verniculatus (P.A. Dang.) C.R. Benj.) has always been the typus of genus in the Talaromyces and Penicillium section Talaromyces through many revisions of the genus Talaromyces [12,13,15]. The current latest concept of species in Talaromyces section Talaromyces is consistent with what Stolk and Samson (1972) described. Stolk and Samson (1972) introduced the Talaromyces section to include species that produce yellow ascomata, which can occasionally be white, creamish, pinkish, or reddish and yellow ascospores. Conidiophores are usually biverticillate-symmetrical, with some species having reduced conidiophores with solitary phialides. Phialides are usually acerose, with a small proportion of species having wider bases [12]. Section Talaromyces species are commonly isolated from soil, indoor environments, humans with talaromycosis and food products. Common species include T. flavus, T. funiculosus, T. macrosporus, T. marneffei, T. pinophilus, and T. purpurogenus.

Micromorphological features such as asexual sporulation structures (e.g., conidiophore) and sexual sporulation structures (e.g., cleistothecium) were of great significance for taxonomy. The branching pattern of conidiophores, namely the type of penicillus, is an important reference index for the traditional classification methods of Penicillium and Talaromyces fungi. The branching pattern generally includes Monoverticillate, Biverticillate, Terverticillate, Quaterverticillate, and Conidiophores with solitary phialides and Divaricate [13,16–18]. Although the classification of Penicillium (and Talaromyces) based on these branching patterns is not completely consistent with the classification status of Penicillium (and Talaromyces) in modern taxonomy, an accurate description of these morphological and structural characteristics is still considered important. The important micromorphology characteristics of Penicillium and Talaromyces fungi include the following: all components of conidiophore (stipes, ramus, ramulus, metula, and phialide) and the sizes, wall texture/orientation, color of conidium, ascocarp, ascus, ascospore, and sclerotium. The penicillus includes four parts: ramus, ramulus, metula, and phialide. Sclerotium is produced only under certain conditions; if there are any, observe and record it.
A breakthrough period in the rapid development of classification systems came with the advent of DNA sequencing technology in the 1990s. The identification of *Penicillium*-group and filamentous fungi began to shift from observation of morphological characteristics to molecular phylogeny. Morphological features are the physical structures with which an organism operates and adapts to its environment, and some features may differ or may be affected by specific factors in the surrounding environment. The effects of medium preparation, inoculation techniques, and culture conditions can be minimized by using strictly standardized protocols [19–21]. Morphological identification still plays an irreplaceable role in the fine identification of strains, and a polyphasic approach using both techniques was finally adopted.

In *Penicillium*, *Talaromyces*, and many other genera of ascomycetes, internal transcribed spacer (ITS) sequences have been used to classify strains into species complexes or sections, as well as for species identification [15,18]. Due to the limitations of species barcoding based on the ITS region, secondary barcodes or identification markers are often required to identify isolated strains to the species level. Secondary barcodes should be easily amplified, able to distinguish closely related species, and come with a complete reference dataset (including representative gene sequences of all species). The following barcodes can generally be used for the identification of *Talaromyces* species. The Internal Transcribed Spacer (ITS) rDNA sequence is accepted as the official barcode for fungi [22]. β-tubulin (BenA) is used for the accurately identification of *Penicillium* species and can also be applied to *Talaromyces* species [15,18]. Trees have been constructed using other DNA barcode markers (Calmodulin (CaM), DNA-dependent RNA polymerase II (beta) largest subunit (RPB1), and DNA-dependent RNA polymerase II (beta) second largest subunit (RPB2)). Among these, CaM, RPB1, and RPB2 exhibit the same potential as BenA and can be used as secondary barcodes for species identification. In recent years, usage of the CaM gene has gradually increased, and its reference dataset has become relatively complete. RPB1 and RPB2 have the added advantage of lacking introns in the amplicon, allowing for robust and easy alignment when used for phylogenetic analysis, but they may be difficult to amplify. At present, the reference dataset for the RPB2 gene of *Talaromyces* species is fairly robust, whereas that for the RPB1 gene is still being improved. During phylogenetic tree construction, in addition to the reference sequences of ex-types, other multiple collections from the same species should be considered to cover possible sequence variations. Comparing ITS, BenA, CaM, RPB1, and RPB2 sequences from a suspected new species with sequences of the same markers in related species can help to determine whether a species is new via genealogical concordance phylogenetic species recognition (GCPSR) [23]. This approach, which involved multigene phylogeny, morphological descriptions using macro-morphological and micro-morphological characters and analysis of extrolites, has been used to develop the polyphasic species concept of filamentous fungi such as *Penicillium* and *Talaromyces*.

In our preliminary study, rhizosphere fungus JP-NJ4 was obtained from Masson pine rhizosphere soil and screened for phosphate solubilization and plant growth promotion [24]. Fungus JP-NJ4 has the potential to be used as an ecofriendly soil amendment for forestry and farming. With the aid of internal transcribed spacer (ITS) sequences, this strain was preliminarily identified as *Penicillium pinophilum* (which is now classified in the genus *Talaromyces* and has been renamed *Talaromyces pinophilus*). However, the variability of ITS sequences is insufficient to distinguish among closely related species [22]. To comprehensively investigate the function of fungus JP-NJ4, the classification status of this strain was investigated further. The identification process for strain JP-NJ4 involved many standard strains (type strains) that are currently stored at the Central Bureau of Fungal Cultures (Centraalbureau Voor Schimmelmicrocultures (CBS)), which is part of the Royal Netherlands Academy of Arts and Sciences and was founded in 1904 by the Association Internationale des Botanistes [25]. Currently, CBS is one of the largest mycological research centers in the world, with more than 60,000 species in cultivation, including the type strains of many filamentous fungus and yeast species. Here, after reviewing the literature and observing the characteristics of fungus JP-NJ4, this strain was identified and described by referring to the standard research method (GCPSR).
recommended in previous international research on filamentous fungal species such as *Penicillium* and *Talaromyces*, etc.

2. Materials and Methods

2.1. Source of the Strain

The strain JP-NJ4 was a phosphate-solubilizing fungus isolated from rhizosphere soil of *Pinus massoniana* (yellow brown soil) in the back mountain of Nanjing Forestry University. The strain is now stored in the China Center for Type Culture Collection (CCTCC) (http://www.cctcc.org). Holotype with the preservation number M 2012167 was stored in a metabolically inactive state by cryopreservation [26,27].

2.2. DNA Extraction, PCR Amplification, and Sequencing of Strain JP-NJ4

Strain JP-NJ4 was cultured on malt extract agar (MEA) culture medium at 25 °C for 7-14 days. Genomic DNA was extracted and purified according to the method of Cubero et al. [28], and the extract was stored at -20 °C. The DNA barcode markers required for the identification of JP-NJ4 strain included the ITS region and *BenA, CaM, RPB1*, and *RPB2* genes [29–38]. The primers needed for the amplification of these genes are shown in Table S1. All primers and polymerase chain reaction (PCR) amplification sequences needed for the experiment were synthesized and sequenced by the Shanghai Sangon Company (http://www.sangon.com).

In this study, a 50.0 μL DNA amplification thermal cycling reaction mixture system was selected, and the formula of 20.0 μL reaction system was also provided. The volumes of the components in the system are as follows: premix Taq™ solution 25.0 μL, DNA template (10 ng/μL) 2.5 μL, forward primer 2.5 μL, reverse primer 2.5 μL and dd H2O 17.5 μL for 50.0 μL system; premix Taq™ solution 10.0 μL, DNA template (10 ng/μL) 1.5 μL, forward primer 1.0 μL, reverse primer 1.0 μL, and dd H2O 6.5 μL for 20.0 μL system. Premix Taq™ (Ex Taq™ Version 2.0 plus dye) is a 2x concentration mixed reagent of DNA polymerase, buffer mixture, and dNTP mixture required for PCR reactions purchased from Takara company (https://takara.company.lookchem.cn). The concentration of the ingredients in the Premix Taq™ solution is as follows: Ex Taq Buffer (2×conc.) with Mg2+ at a concentration of 4 mM (mmol/L); highly efficient amplification DNA polymerase (TaKaRa Ex Taq) at a concentration of 1.25 U/25 μL; the dNTP (deoxy-ribonucleoside triphosphate) Mixture (2×conc.), with a concentration of 0.4 mM (mmol/L) for each base; additional pigment markers (Tartrazine/Xylene Cyanol FF), specific gravity additaments, and stabilizers were included. The reagent is stored at -20 °C. The total amount of DNA template can be 10-100 ng, and it can be added according to the experimental requirements. The concentration of primer prepared in accordance with the operational guidelines is 100 μmol/L, diluted 10 times to 10 μmol/L for use.

The DNA amplification thermal cycling programs for each gene is as follows: Standard PCR was selected for general ITS, *BenA*, and *CaM*, with initial denaturing 94 °C for 5 min, cycles 35 of denaturation 94 °C for 45 s, annealing 55 °C (52 °C) for 45 s, elongation 72 °C for 60 s, final elongation 72 °C for 7 min, and rest period 10 °C, ∞. Touch-down PCR was selected for *RPB1*, with 5 cycles of 30 s denaturation at 94 °C, followed by primer annealing for 30 s at 51 °C, and elongation for 1 min at 72 °C; followed by 5 cycles with annealing for 30 s at 49 °C and 30 cycles for 30 s at 47 °C, finalized with an elongation for final 10 min at 72 °C, rest period 10 °C, ∞ (the denaturation and elongation conditions of the second and third cycles are the same as those of the first cycle). Touch-up PCR (= step-up PCR) was selected for *RPB2*, with initial denaturing 94 °C for 5 min, followed by 5 cycles of 45 s denaturation at 94 °C, primer annealing for 45 s at 50 °C (48 °C), and elongation for 1 min at 72 °C; followed by 5 cycles with annealing for 45 s at 52 °C (50 °C) and 30 cycles for 45 s at 55 °C (52 °C), finalized with an elongation for final 7 min at 72 °C, rest period 10 °C, ∞ (the denaturation and elongation conditions of the second and third cycles are the same as those of the first cycle). The values in parentheses refer to alternative reaction conditions.
2.3. Phylogenetic Tree Construction of Strain JP-NJ4

Sequences of five genes from strain JP-NJ4 have been sequenced and deposited in GenBank (Table 1). By conducting a Basic Local Alignment Search Tool (BLAST) search in National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov), the results showed the best matched DNA sequences for each gene/region. In order to make phylogenetic trees, the type strains of *Talaromyces* species were added. For monogenic and polygenic phylogeny, ITS, *BenA*, *CaM*, RPB1, and RPB2 sequence data were compared and aligned using ClustalW software included in the MEGA package version 6.0.6 [39]. All datasets (DNA sequences) were concatenated in MEGA and the BioEdit Sequence Alignment Editor software (Version 7.0.9.0) [40]. The aligned data sets were analysed using both Maximum Likelihood (ML) and Bayesian inference (BI) methods, and ML phylogenetic trees were constructed for each gene/region and concatenated polygenic sequences. According to the results of Akaike Information Criterion (AIC) calculated in MEGA package, the best model for ML phylogenetic tree construction is selected. The ML analysis is performed, and the trees were constructed by calculating the initial tree (constructed by the BioNJ method), selecting the Nearest-Neighbour-Interchange (NNI) option for the following heuristic search. Bootstrap analysis was performed on 1000 repetitions to calculate the support at the node. Bayesian Inference phylogenies were inferred using PhyloSuite v1.2.1 [41]. ModelFinder was used to select the best-fit model (2 parallel runs, 2000000 generations) using Bayesian Information Criterion (BIC) for BI [42]. The sample frequency was set at 100, with 25% of trees removed as burn-in. Bayesian inference posterior probabilities (BIpp) values and bootstrap values are labelled on nodes.

### Table 1. Collection numbers of strains, isolation details and GenBank accession numbers of the five genes/region used for phylogenetic analysis of the strain JP-NJ4.

| Species name | Collection number | Substrate and origin | GenBank accession number |
|--------------|-------------------|----------------------|-------------------------|
| **strain JP-NJ4** | M 2012167 | Rhizosphere soil from *Pinus massoniana*; Nanjing, Jiangsu, China | MW130720 MW147759 MW147760 MW147761 MW147762 |
| **Talaromyces brevis** | CBS 141833 (T) = DTO 349-E7 | Soil; Beijing, China | MN864269 MN863338 MN863315 MN863328 |
| | DTO 307-C1 | Soil; Zonguldak, Turkey | MN864270 MN863339 MN863316 MN863329 |
| | CBS 118436 = DTO 004-D8 | Soil; Maroc | MN864271 MN863340 MN863317 MN863330 |
| **Talaromyces liani** | CBS 225.66 (T) | Soil; China | JN899395 JX091380 JK885257 JN680280 KX961277 |
| | CBS 118434 | Soil in orchid garden; Sanur, Bali, Indonesia | KM066208 KM066139 MK451683= KP453744 |
| | CBS 118885 | Soil of pepper field; Daejeon, Korea | KM066210 KM066138 |
| | NRRL 1009 | Derived from Biourage 368 | MH793030 MH792902 MH792966 |
| | NRRL 1014 = 1009 | USA, Arizona, isol ignotae, KD Butler, 1936. | MH793031 MH792903 MH792967 |
| | NRRL 1015 = 1009 | China, isol ex soil, = CBS 225.66 | MH793032 MH792904 MH792968 MH793095 |
| | NRRL 3380 | China, isol ex soil, = CBS 225.66 | MH793033 MH792905 MH792969 - MH793096 |
| | NRRL 28778 | Brazil, isol ex soil, RW Jackson, 1956. | MH793047 MH792919 MH792983 |
| | NRRL 28834 | India, isol ignotae | MH793048 MH792920 MH792984 - MH793111 |
| | CMV011D7 | Passiflora edulis; South Africa | - MK451201 |

References:

[39] MEGA package version 6.0.6
[40] BioEdit Sequence Alignment Editor software (Version 7.0.9.0)
[41] PhyloSuite v1.2.1
[42] ModelFinder

For a detailed description of each species and its collection details, please refer to the table above.
| **Talaromyces aculeatus** | | | | |
| --- | --- | --- | --- | |
| CBS 289.48 (T) = NRRL2129 | Textile; USA | KF741995 | KF741929 | KF741975 = JX140684 = MH792972 | - | KM023271 |
| CBS 282.92 | Soil in secondary forest; Brazil | KF741981 | KF741914 | KF741946 | - | - |
| CBS 290.65 | Nut; South Africa | KF741982 | KF741915 | KF741948 | - | - |
| CBS 563.92 | Stem of *Dicymbe Altsonii*; French Guiana | KF741986 | KF741920 | KF741963 | - | - |

| **Talaromyces adpressus** | | | | |
| --- | --- | --- | --- | |
| NRRL 6014 | Peanuts; Unknown | MH793039 | MH792911 | MH792975 | - | MH793102 |
| NRRL 62466 | Peanuts; Unknown | MH793088 | MH792961 | MH793025 | - | MH793152 |
| CBS 140620 | Indoor air; China | KU866657 | - | - | - | KU867001 |
| DTO 317-G4 | Indoor air; China | - | KU866844 | KU866741 | - | - |
| CMV011C5 | Soil; South Africa | MK450741 | MK451191 | MK451673 | - | - |

| **Talaromyces aeruginus** | | | | |
| --- | --- | --- | --- | |
| CBS 350.66 (T) | Debris; United Kingdom | AY753346 = NR 147420 | KJ865736 | KJ885285 | JN121657 | JN121502 |

| **Talaromyces albobiverticillius** | | | | |
| --- | --- | --- | --- | |
| CBS 133440 (T) = *Penicillium albobiverticillium* isolate 900890701 | Decaying leaves of a broad-leaved tree; Taiwan | HQ605705 = KF114734 | KF114778 | KJ885258 | KF114753 | KM023310 |
| CBS 133441 | Decaying leaves of a broad-leaved tree; Taiwan | KF114733 | KF114777 | - | KF114755 | - |

| **Talaromyces allahabadensis** | | | | |
| --- | --- | --- | --- | |
| CBS 453.93 (T) = *Crepis zacintha*; Alicante, Spain; Type of *Penicillium zacinthae* | Cultivated soil; Allahabad, India | KF984873 | KF984614 = JX494298 | KF984768 | JN680309 | KF985006 |
| CBS 178.81 | Seed ground; Denmark | KF984863 | KF984612 | KF984767 | - | KF985004 |
| CBS 441.89 | | KF984872 | KF984613 | KF984759 | - | KF985005 |
| CBS 137397 = DTO245E3 | House dust; Mexico | KF984864 | KF984605 | KF984761 | - | KF84998 |
| CBS 137399 = DTO267H6 | House dust; Thailand | KF984866 | KF984607 | KF984762 | - | KF84997 |

| **Talaromyces amestolkiae** | | | | |
| --- | --- | --- | --- | |
| CBS 132696 (T) = DTO179F5 | House dust; South Africa | JX315660 = NR 120179 | JX315623 | JX315660 = JX315650 | JX315679 | JX315698 |
| DTO179E4 | House dust; South Africa | KJ775706 | KJ775199 | JX140685 | - | - |
| DTO179F1 | House dust; South Africa | KJ775707 | KJ775200 | JX140686 | - | - |
| DTO179F6 | House dust; South Africa | KJ775708 | KJ775201 | - | - | - |

| **Talaromyces angelicus** | | | | |
| --- | --- | --- | --- | |
| KACC 46611 (T) = CNU 100013 = DTO303E2 | Dried roots of *Angelica gigas*; P'yongchang, Korea | KF183638 | KF183640 | KJ885259 | - | KX961275 |
| FMR 15489 | Unknown | LT899791 | LT899316 | LT899773 | - | LT899809 |
| FMR 15490 | Unknown | LT899792 | LT898317 | LT899774 | - | LT899810 |

| **Talaromyces apiculatus** | | | | |
| --- | --- | --- | --- | |
| CBS 312.59 (T) | Soil; Japan | JN899375 = NR 121530 | KF741916 = JX091378 | KF741950 | JN680293 | KM023287 |
| CBS 548.73 | Soil; Suriname | KF741985 | KF741919 | KF741962 | - | - |
| CBS 101366 | Soil; Hong Kong, China | KF741977 | KF741910 | KF741932 | - | - |
| Talaromyces argentinensis | NRRL 28750 (T) | Soil; Unknown | MH793045=NR 165525 | MH792917 | MH792981 | - | MH793108 |
|---------------------------|----------------|---------------|---------------------|--------|-------|---|--------|
| NRRL 28758                | Soil; Unknown  | MH793046      | MH792918            | MH792982 | -     | MH793109 |
| CBS 147.78 (T)            | Soil; Egypt    | JN899323      | KJ865720            | KJ885260 | JN680275 | KM023305 |
| CBS 645.80                | Gossypium; India; Type of Talaromyces gossypii | JN899334=NR 147423 | KF114802 | - | JN680317 | - |
| CBS 116554                | Pasteurised canned strawberries; the Netherlands | KM066167 | KM066124 | MK451674 | - | - |
| CBS 118440                | Soil; Fes, Morocco | KM066168 | KM066125 | MK451675 | - | - |
| Talaromyces atricola      | CBS 255.31 (T) | Unknown       | KF984859            | KF984566 | KF984719 | - | KF984948 |
| Talaromyces atroroseus    | CBS 133442 (T) | House dust; South Africa | KF114747=NR 137815 | KF114789 | KF114763 | KM023288 |
| DTO267I1                 | House dust; Thailand | KJ775716 | KJ775209 | - | - | - |
| DTO270D5                 | House dust; Mexico | KJ775734 | KJ775227 | - | - | - |
| DTO270D6                 | House dust; Mexico | KJ775735 | KJ775228 | - | - | - |
| Talaromyces aurantiacus   | CBS 314.59 (T) | Soil; Georgia | JN899380=NR103681.2 | KF741917 | KF741951 | JN680294 | KX961285 |
| Talaromyces australis     | IBT14256 (T)   | Unknown       | KF741991=NR 147431 | KF741922 | KF741971 | - | - |
| IBT14254                 | Unknown        | KF741989      | KF741923            | KF741969 | - | - |
| MDL18159                | Bronchoscopy; USA | MK601840 | MK626507 | - | MK626517 | - |
| Talaromyces austrocalifornicus | CBS 644.95 (T) | Soil; California, USA | JN899357=NR 137079 | JK865732 | JK885261 | JN680316 | - |
| Talaromyces bacillisporus | CBS 296.48 (T) | Leaf; New York, USA | JN899329 | AY753368 | KJ885262 | JN121634 | JF417425 |
| CBS 102389               | Sludge of anaerobic pasteurised organic household waste; Sweden | KM066179 | KM066135 | - | - | - |
| CBS 110774               | Rye bread; the Netherlands | KM066180 | KM066136 | - | - | - |
| CBS 116927               | Soil; the Netherlands | KM066181 | KM066137 | - | - | - |
| Talaromyces bohemicus    | CBS 545.86 (T) | Peloids for balneological purposes; Czech Republic | JN999400=NR 137081 | JK865719 | JK885286 | JN121699 | JN121532 |
| Talaromyces boninensis    | CBS 650.95 (T) | Peloids for balneological purposes; Czech Republic | JN999356=NR 145157 | JK865721 | JK885263 | JN680319 | KM023267 |
| Talaromyces brunneus     | CBS 227.60 (T) | Milled rice imported into Japan; Thailand | JN999365=NR 111688 | JK865722=JX494296 | JK885264 | JN680281 | KM023272 |
| CBS 112002 (T)           | Soil; Nantou County, Taiwan | JN999319=HQ149324=NR 103665.2 | HQ156944 | KF741934=JX140688 | JN899305 | KM023311 |
| ACCC:39162               | Luffa; Beijing; China | KY225703 | KY225714 | - | KY225712 | - |
| ACCC:39164               | Cucumber; Beijing; China | KY225702 | KY225715 | - | KY225711 | - |
| Talaromyces californicus | NRRL 58168 (T) | Air sample; Unknown | MH793056=NR 165527 | MH792928 | MH792992 | - | MH793119 |
| NRRL 58177               | Air sample; Unknown | MH793057 | MH792929 | MH792993 | - | MH793120 |
| NRRL 58207               | Air sample; Unknown | MH793058 | MH792930 | MH792994 | - | MH793121 |
| NRRL 58221               | Air sample; Unknown | MH793059 | MH792931 | MH792995 | - | MH793122 |
| NRRL 58661               | Air sample; Unknown | MH793060 | MH792932 | MH792996 | - | MH793123 |
| **Talaromyces** | **Genus** | **Strain** | **Source** | **Accession Numbers** |
|-----------------|-----------|------------|------------|-----------------------|
| **cecidicola**  | CBS 101419 (T) | = Penicillium cecidicola strain DAOM 233329 = Penicillium cecidicola isolate KAS504 | Cynipid insect galls on Quercus pacifica twigs; Oregon, USA | AY787844 = MH862736 FJ753295 KJ885287 - KM023309 |
| **cellulolyticus** | Y-94 | = FERM: BP-5826 | Unknown; A synonym of Talaromyces pinophilus | AB474749 AB773823 - AB856422 - |
| **chloroloma** | DAOM 241016 (T) = Penicillium sp. CMV-2008a isolate Pen389 = Penicillium sp. CMV-2008a isolate CV389 | Fynbos soil; Western Cape, South Africa | FJ160273 GU385736 KJ885265 - KM023304 |
| **coalescens** | DTO 180-F4 = Penicillium sp. CMV-2008a isolate CV390 = Penicillium sp. CMV-2008a isolate Pen390 | Fynbos soil; South Africa | FJ160272 GU385737 - - - |
| **cinnabarinus** | CBS 267.72 (T) | Soil, Japan | JN899376 = AY753377 KJ885256 JN121625 JN121477 |
| **cnidii** | KACC 46617 (T) = DTO 303-E1 = CNU 100149 | Dried roots of Cnidium officinale; Jecheon, Korea | KF183639 KF183641 KJ885266 - KM023299 |
| **convolutus** | CBS 100537 (T) | Soil; Kathmandu, Nepal | JN899330 = NR 137157 = KF114773 = JN121553 JN121414 |
| **dendriticus** | CBS 104574 (T) | = DTR 189-A5 | Chicken feed (Unga); Nairobi, Kenya | KF984794 KF984659 KF984671 - KF984897 |
| **dendriticus** | DAOM 226674 = Penicillium dendriticum isolate KA5849 | Doryanthes excelsa spathes; Mangrove Mountain, New South Wales, Australia | AY787842 FJ753293 - - - |
| **coelestis** | CBS 660.80 (T) | = Penicillium coelestis isolate KA5849 | Unidentified insect gall on Eucalyptus latifolia | JN899339 JX091391 KF741965 JN121714 KM023286= JN121547 |
| **convolutus** | DAOM 233861 | | - - - |

**References:**
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| Species                          | Isolate/Location                        | GenBank Accession Numbers | Other Accession Numbers |
|----------------------------------|----------------------------------------|---------------------------|-------------------------|
| Talaromyces dendriticum          | Penicillium dendriticum isolate KAS1190 | JX091486, JX091619, JX140692 | -                       |
| Talaromyces caespiticosus        | DTO 183-G3 = CV2026                     | JN899327 = NR 145152, JX494306, KF741959, JN680306 | KM023282                |
| Talaromyces diversus             | CBS 412.89 (T)                          | JX091486, JX091619, JX140692 | -                       |
| Talaromyces flavoconjugatus      | DTO 133-A7 = House dust; Thailand       | KJ775701, KJ775194        | -                       |
| Talaromyces flavovires           | DTO 133-E4 = House dust; Thailand       | KJ775702, KJ775195        | -                       |
| Talaromyces flavovires           | DTO 133-I6 = Lotus dust; Vietnam        | KJ775700, KJ775193        | -                       |
| Talaromyces flavovires           | DTO 244-E6 = House dust; New Zealand    | KJ775712, KJ775205        | -                       |
| Talaromyces domesticus           | NRRL 58121 = Floor swab; Unknown        | MH793055, MH792927, MH792991 | -                       |
| Talaromyces domesticus           | NRRL 62132 = Exposed cloth; Unknown     | MH793066, MH792938, MH793002 | -                       |
| Talaromyces duclauxii            | CBS 322.48 (T) = Canvas; France         | JN899342 = NR 121526, JX091384, KJ775702, KJ775195, JN680315 | JN121643, JN121491      |
| Talaromyces emodensis            | CBS 100536 (T) = Soil; Kathmandu, Nepal | JN899337 = NR 137077, KJ775702, KJ775195, JN680315 | JN121552, JF417445      |
| Talaromyces erythromellis        | CBS 644.80 (T) = Soil from creek bank; New South Wales | JN899383 = HQ156945, KJ775702, KJ775195, JN680315 | JN023290                |
| Talaromyces euchlorocarpius      | PF 1203 (T) = DTO 17618 = CBM-FA-0942  | AB176617, KJ775705, KJ775205 | -                       |
| Talaromyces flavovires           | CBS 102801 (T) = Dead leaves of Quercus ilex; Madrid, Spain | JN899392 = JX091376, KF741933, JX091376 | -                       |
| Talaromyces flavoconjugatus      | DAOM236381 = Leaves of Quercus suber; port de la Selva, Girona, Spain | JX013912, JX013913, KJ775702, KJ775195 | -                       |
| Talaromyces flavoconjugatus      | DAOM236382 = Leaves of Quercus suber; Selva de Mar, Girona, Spain | JX013912, JX013913, KJ775702, KJ775195 | -                       |
| Talaromyces flavoconjugatus      | DAOM236383 = Leaves of Quercus suber; Barraca d’en Rabert, Paau, Girona, Spain | JX013912, JX013913, KJ775702, KJ775195 | -                       |
| Talaromyces flavoconjugatus      | DAOM236384 = Leaves of Quercus suber; Xovar, Alt Palacia, Valencia | JX013912, JX013913, KJ775702, KJ775195 | -                       |
| Talaromyces flavus               | CBS 310.38 (T) = Unknown; New Zealand | JN899360 = JX494302, KF741949 = FJ530982, JN121639, JF417426 | -                       |
| Talaromyces flavus               | CBS 437.62 = Compost; Bonn, Germany     | KM066202, KM066156        | -                       |
| Talaromyces flavoconjugatus      | CBS 113134 (T) = Leaf litter; Colombia | NR 154940, KJ775702, KJ775195 | -                       |
| Talaromyces flavoconjugatus      | DTO 056D9 = Leaf litter; Colombia       | KX011510, KX011489, KX011501 | -                       |
| Talaromyces flavoconjugatus      | CBS 272.86 (T) = Lagenaria vulgaris; India | JN899377 = NR 103678.2, JX091383, KJ775702, KJ775195, JN680288 | KM023293                |
| Talaromyces flavoconjugatus      | CBS 171.91 = Unknown                    | KM066193, KM066162, KM451679, KM450873 | -                       |
| Talaromyces flavoconjugatus      | CBS 883.70 = Unknown                    | KM066196, KM066163, KM451680, KM450874 | -                       |
| Talaromyces flavoconjugatus      | CBS 884.70 = Unknown                    | KM066195, KM066164, KM451681, KM450875 | -                       |
| Talaromyces flavoconjugatus      | CBS 885.71 = Air; Java, Jakarta         | KM066194, KM066165        | -                       |
| Talaromyces flavoconjugatus      | CBS 193.69 (T) = Unknown                | KF741979 = NR 153227, KF741912, KF741942 | -                       |
| Strain | Origin | Type | Accession Numbers |
|--------|--------|------|-------------------|
| NRRL 66370 | Unknown | Shaded soil under *Maytenus obvata*; Galapagos Islands, Isla, Santa Cruz, Ecuador | JN899358 = NR 147426 | JX091388 = KF114770 | KF741966 | JN680321 | KX961280 |
| NRRL 13068 | *Maytenus obvata* | MH793042 | MH792914 | MH792978 | - | MH793105 |
| CBS 53624 (T) = PF 1174 = CBM-FA-0948 | Soil; Hachijojima, Japan | AB176620 | - | - | - | - | - |
| CBS 335.48 (T) | Soil; Sweden | JN899359 = NR 147427 | KJ865725 | KJ885289 | JN680300 | KM023273 |
| CBS 134.67 | Green house soil under *Lycopersicon esculentum*; Wageningen, the Netherlands | KM066176 | KM066133 | - | - | - | - |
| CBS 649.95 = *Talaromyces barcinensis* | Unknown | JN899349 = NR 147427 | JX494308 | KF741931 | JN680323 | KM023273 |
| CBS 100534 (T) | Soil; Japan | JN899331 = NR 137076 | JX494308 | KF741931 | JN680323 | KM023273 |
| CBS 152.65 (T) | Alluvial pasture and swamp soil; Nottingham, England | JN899332 = NR 145154 | JX091387 | KJ885290 | JN680327 | KM023273 |
| CBS 338.48 (T) | Unknown; Cape Town, South Africa | KF984885 | KF984655 = JX494293 | KF984780 | JN121648 | KF985018 = JN121495 |
| CBS 165.81 | Capok fibre; unknown | KF984886 | KF984856 | KF984781 | - | KF985019 |
| CBS 117284 | Wheat flour; the Netherlands | KF984882 | KF984652 | KF984777 | - | KF985015 |
| DI16-149 | Unknown | - | - | LT795598 | - | LT795599 |
| IBT13593 (T) | Unknown | KF741987 = NR 147430 | KF741921 | KF741967 | - | - |
| IBT14128 | Unknown | KF741988 | KF741925 | KF741968 | - | - |
| CBS 100105 | Unknown | KF741976 | KF741909 | KF741930 | - | - |
| CBS 133088 | Unknown | KF741978 | KF741911 | KF741939 | - | - |
| CBS 643.80 (T) | Rye grass (*Lolium*); New Zealand | KF984888 | KF984658 | KF984783 | JN680314 | KF985021 |
| CBS 172.91 | Soil; New Zealand | KF984887 | KF984657 | KF984782 | - | KF985020 |
| NRRL 35823 (T) | Air sample; Unknown | MH793052 = NR 165526 | MH792924 | MH792988 | - | MH793115 |
| NRRL 35826 | Air sample; Unknown | MH793053 | MH792925 | MH792989 | - | MH793116 |
| NRRL 35928 | Air sample; Unknown | MH793054 | MH792926 | MH792990 | - | MH793117 |
| CBS 317.63 (T) | Apple juice; Stellenbosch, South Africa | JN899333 = NR 145155 | JX091382 | KF741952 | JN680296 | KM023292 |
| CBS 117.72 | Cotton fabric; USA | KM066188 | KM066148 | - | - | - |
| CBS 131.87 | Faecal pellet of grasshopper; Malaysia | KM066191 | KM066147 | - | - | - |
| CBS | Tentage; New Guinea | KM066189 | KM066149 | - | - | - |
|------|---------------------|-----------|-----------|-----|-----|-----|
| DTO 077-C5 | Pine apple concentrate; the Netherlands | KM066192 | KM066150 | - | - | - |
| DTO 105-C4 | Unknown | KM066190 | KM066146 | - | - | - |
| BCC 14364 | Unknown | AY753345 | AY753373 | - | - | - |
| AS5.6680 | Unknown | - | - | AY678608 | - | - |
| Talaromyces malicola | NRRL 3724 (T) | Soil under apple tree; Unknown | MH909513 = NR 165531 | MH909406 | MH909459 | - | MH909567 |
| CBS 388.87 (T) | Bamboo rat (*Rhzomyxis sinensis*); Vietnam | JN899344 = NR 103671.2 | JX091389 | KF741958 | JN899298 | KM023283 |
| CBS 108.89 | Unknown | KM066187 | KM066157 | - | - | - |
| CBS 122.89 | Male AIDS patient after travel to Indonesia | KM066183 | KM066161 | - | - | - |
| CBS 135.94 | Haemoculture; Nonthaburi, Thailand | KM066184 | KM066158 | - | - | - |
| CBS 549.77 | Man spleen; unknown | KM066185 | KM066159 | - | - | - |
| CBS 119456 | Male blood; Thailand | KM066186 | KM066160 | - | - | - |
| Talaromyces marmellaei | DTO 077-C5 | Soil from creek bank, New South Wales | JN899338 | KJ865726 | KJ885272 | JN899302 | - |
| CBS 659.80 (T) | Unknown | KX946911 | KX946880 | KX946897 | - | KX946926 |
| CBS 642.68 (T) | Unknown | JN899346 = NR 121527 | KF114799 | KJ885273 | JN121709 | JF417443 |
| CBS 137.84 | Fruit, damaged by insect; Valladolid, Spain | KM066171 | KF114798 | - | JN680273 | - |
| Talaromyces mimosinus | NRRL 13069 = NRRL 13609 (BenA) | Soil from creek bank, New South Wales | JN899338 | KJ865726 | KJ885272 | JN899302 | - |
| CBS 549.77 | Unknown | KX946911 | KX946880 | KX946897 | - | KX946926 |
| CBS 270.35 | Zea mays; Castle Rock, Virginia, USA; Type of *Penicillium purpurogenum var. rubrisclerotium* | KM066172 | KM066129 | - | JN680287 | - |
| CBS 756.96 (T) | Soil; Taiwan | JN899351 = NR 103672.2 | KJ865727 | KJ885274 | JN680322 | KX961276 |
| CBS 261.55 | Clematis; Boskoop, the Netherlands | KM066200 | KM066153 | - | - | - |
| CBS 283.58 | Jute potato bag, treated with copper oxide ammonia; unknown | KM066197 | KM066151 | - | - | - |
| CBS 284.58 | Unknown; the Netherlands | KM066199 | KM066152 | - | - | - |
| CBS 315.61 | Chicken crop; the Netherlands | KM066198 | KM066155 | - | - | - |
| CBS 889.96 | Dung of sheep; Papua New Guinea | KM066201 | KM066154 | - | - | - |
| Talaromyces ouae-annae | CBS 138208 (T) = DTO 269-E8 | House dust; South Africa | KJ775720 = NR 147432 | KJ775213 | KJ775425 | - | KX961281 |
| CBS 138207 = DTO 180-B4 | House dust; South Africa | KJ775710 | KJ775203 | KJ775421 | - | - |
| Talaromyces palmae | CBS 442.88 (T) | Chrysalidocarpus lutescens seed; Wageningen, the Netherlands | JN899396 | HQ156947 | KJ885291 | JN680308 | KM023300 |
| Talaromyces panamensis | CBS 128.89 (T) | Soil; Barro Colorado Island, Panama | JN899362 | HQ156948 = JX091386 | KF741936 = JX140695 | JN899291 | KM023284 |
| Talaromyces paucisporus | PF 1150 (T) = IFM 53616 = CBM-FA-0944 | Soil; Aso-machi, Japan | AB176603 | - | - | - | - |
|------------------------|--------------------------------|------------------------|-----------|---|---|---|---|
| CBS 361.48 (T)         | Unknown                        | KF984792               | KF984668  | KF984680 | - | KF984899 |
| CBS 116872             | Production plant; the Netherlands | KF984788              | KF984660  | KF984678 | - | KF984903 |
| CBS 132063             | Straw used in horse stable; the Netherlands | KF984789              | KF984665  | KF984674 | - | KF984904 |
| CBS 137363             | Pectin; unknown                | KF984787               | KF984664  | KF984677 | - | KF984902 |
| CBS 137377             | House dust; South Africa       | KF984784               | KF984661  | KF984676 | - | KF984900 |
| CBS 361.66 (T)         | PVC; France                    | JN899382 = NR 111691   | JX091381  | KF741964 | JN680313 | KM023291 |
| CBS 173.91             | Unknown; USA                   | KM066206               | KM066141  | - | - | - |
| CBS 225.94             | Unknown; USA                   | KM066204               | KM066145  | - | - | - |
| CBS 269.73             | Unknown; Germany               | KM066207               | KM066144  | KM520392 = MK451686 | - | - |
| CBS 440.89             | *Zea mays*; India              | KM066203               | KM066143  | - | - | - |
| CBS 762.68             | Rhizosphere; India; Type of *Penicillium korosum* | JN899347               | JX494301  | - | - | - |
| CBS 101709             | Soil; Japan                    | KM066205               | KM066142  | KM520391 = MK451685 | - | - |
| DTO183-16             | *Protea repens* infructescense; Struisbaai, South Africa | JX091488              | JX091621  | JX140697 | - | MK450878 |
| NRRL 1060             | Seed; Unknown                  | MH909460               | MH909351  | MH909407 | - | MH909514 |
| NRRL 3503             | Radio set; Unknown             | MH909462               | MH909353  | MH909409 | - | MH909516 |
| NRRL 5200             | Unknown; Type of *Penicillium korosum* | MH909464              | MH909355  | MH909411 | - | MH909518 |
| NRRL 13016             | Dung ball; Unknown             | MH909466               | MH909357  | MH909413 | - | MH909520 |
| NRRL 62103             | Canvas cloth; Unknown          | MH909482               | MH909373  | MH909429 | - | MH909535 |
| NRRL 62172             | Wheat; Unknown                 | MH909492               | MH909383  | MH909439 | - | MH90545 |
| ATCC 11797             | Unknown                        | KU729085               | KU896999  | - | - | - |
| CABI IMI114933         | Unknown; France                | KC962105               | KC992266  | - | - | - |
| Talaromyces pittii     | CBS 139.84 (T)                 | Clay soil under poplar trees; Spain | JN899325 = NR 103667.2 | KJ865728 | KJ885275 | JN680274 | KM023297 |
| Talaromyces pratensis  | NRRL 62170 (T)                 | Unknown                | MH793075 = NR 165529 | MH792948 | MH793012 | - | MH793139 |
| NRRL 13548             | Corn; Unknown                  | MH793044               | MH792916  | MH792980 | - | MH793107 |
| NRRL 62126             | River water; Unknown           | MH793065               | MH792937  | MH793001 | - | MH793128 |
| Talaromyces primulinus | CBS 321.48 (T)                 | Unknown; USA           | JN899317 = NR 145151 | JX494305 | KF741954 | JN680928 | KM023294 |
| Talaromyces proteolyticus | CBS 303.67 (T)                  | Granite soil; Ukraine | JN899387 = NR 103685.2 | KJ865729 | KJ885276 | JN680929 | KM023301 |
| Talaromyces pseudostromaticus | CBS 470.70 (T)                | Feather of *Hylocichla fuscescens*; Minnesota, USA | JN899371 | HQ156950 | KJ885277 | JN899300 | KM023298 |
| Talaromyces ptychoconidium | DAOM 241017 (T) = DTO 180-E7 = CV2808 = *Penicillium* sp. CMV-2008c isolate CV319 | Fynbos soil; Malmesbury, South Africa | FJ160266 | GU385733 | JX140701 | - | KM023278 |
| **Talaromyces purpureus** |  |  |
| --- | --- | --- |
| CBS 475.71 (T) | Soil; France | JN899328 = NR 145153 |
| CBS 286.36 (T) | Parasitic on a culture of *Aspergillus oryzae*; Japan | JN899372 = NR 121529 |
| CBS 184.27 | Soil; Lousiana, USA | JX315665 = MH854924 |
| CBS 122434 | Unknown | JX315663 |
| CBS 132707 = DTO189A1 | Moulded field corn; Wisconsin, USA | JX315661 |

| **Talaromyces pururogenus** |  |  |
| --- | --- | --- |
| CBS 140.84 (T) | Air under willow tree; Valladolid, Spain | JN899386 = NR 103684.2 |
| CBS 100489 (T) | Root seadling; New South Wales | KF984878 |
| CBS 100488 | Wheat root; New South Wales | KF984877 |
| CBS 100490 | Wheat root; New South Wales | KF984879 |
| CBS 137382 = DTO181D5 | Fynbos soil; South Africa | KF984875 |
| DTO181D4 | Fynbos soil; South Africa | KF984880 |
| DTO181D7 | Fynbos soil; South Africa | KF984881 |

| **Talaromyces rademirici** |  |  |
| --- | --- | --- |
| CBS 122434 | Unknown | JX315663 |
| DTO 180-E9 = Penicillium sp. CMV-2008c isolate Pen319 = Penicillium sp. CMV-2008c isolate CV322 | Fynbos soil; Malmesbury, South Africa | FJ160267 |
| DTO 180-F1 = Penicillium sp. CMV-2008c isolate CV323 | Fynbos soil; Malmesbury, South Africa | GQ414762 |

| **Talaromyces radicus** |  |  |
| --- | --- | --- |
| CBS 100489 (T) | Root seadling; New South Wales | KF984878 |
| CBS 100488 | Wheat root; New South Wales | KF984877 |
| CBS 100490 | Wheat root; New South Wales | KF984879 |
| CBS 137382 = DTO181D5 | Fynbos soil; South Africa | KF984875 |
| DTO181D4 | Fynbos soil; South Africa | KF984880 |
| DTO181D7 | Fynbos soil; South Africa | KF984881 |

| **Talaromyces ramulosus** |  |  |
| --- | --- | --- |
| DAOM 241660 (T) = CV2837 = CV113 | Soil; Malmesbury, South Africa | EU795706 |
| DTO 181-E3 = CV314 = CV0314 | Mite; Stellenbosch, South Africa | JX091494 |
| DTO 181-F6 = CV394 = CV0394 | *Protea repens* infructescense; Stellenbosch, South Africa | JX091495 |
| DTO 182-A3 = CV735 = CV0735 | *Protea repens* infructescense; Stellenbosch, South Africa | JX091496 |
| DTO 182-A6 = CV787 = CV0787 | Air, Malmesbury; South Africa | JX091497 |
| DTO 183-A7 = CV1426 | *Protea repens* infructescense; | JX091493 |
| **Talaromyces rotundus** | **Cardboard; Norway** | JN899353 | KJ865730 | KJ885278 | - | KM023275 |
|-------------------------|----------------------|----------|----------|----------|---|----------|
| CBS 369.48 (T)          |                      |          |          |          |   |          |
| CBS 132704 (T)          | Air craft fuel tank; United Kingdom | JX315662 = NR 111780 | JX315629 | KF741938 | JX315681 | JX315700 |
| CBS 196.88              | Unknown              | JX315666 = JN899312 | JX315627 | JX315657 | JX680278 = JX315685 | - |
| CBS 237.93              | Unknown              | JX315667 | JX315628 | JX315656 | JX315686 = JN899306 | - |
| CBS 370.48              | Currency paper; Washington, USA | JX315673 | JX315630 | JX315649 | JX315692 | - |
| CBS 868.96              | Unknown              | JX315677 | JX315631 | JX315643 | JX315696 = JN899309 | - |
| **Talaromyces ruber**   |                      |          |          |          |   |          |
| CBS 342.59 (T)          | Roasting potato tubers (Solanum tuberosum), USA | KF984834 | KF984575 = JX494297 | KF984702 | JN680302 | KF984925 |
| CBS 371.48 (T)          | Unknown; Japan; Type of Penicillium echinosporum | KF984858 | KF984574 | KF984701 | - | KF984924 |
| CBS 344.51              | Air sample, beer producing factory; Kaulille, Belgium; Type of Penicillium chrysitis | KF984850 | KF984572 | KF984700 = JX140720 | - | KF984922 |
| **Talaromyces rugulosus** |                      |          |          |          |   |          |
| CBS 137366 = DTO61E8    | Unknown; Japan; Type of Penicillium tardum and Penicillium elongatum | KF984857 | KF984701 | KF984700 = JX140720 | - | KF984922 |
| NRRL 1053              | Decaying twigs; France; Type of Penicillium chrysitis | KF984832 | KF984579 | KF984711 | - | KF984927 |
| **Talaromyces ryukyuensis** | Soil; Naha, Japan | AB176628 = NR147414 | - | - | - | - |
| NHL 2917 (T) = DTO 176-I6 = strain: NHL2917 | House dust; Mexico | KJ775713 | KJ775206 | KJ775422 | - | - |
| CBS 138204 (T) = DTO 245-H1 | House dust; Mexico | KJ775714 | KJ775207 | KJ775423 | - | - |
| CBS 138205 = DTO 245-H2 | House dust; Mexico | KJ775715 | KJ775208 | KJ775424 | - | - |
| NRRL 1064               | Corn; Unknown        | MH793034 | MH792906 | MH792970 | - | MH793097 |
| NRRL 6420               | Corn; Unknown        | MH793041 | MH792913 | MH792977 | - | MH793104 |
| FMR 15842               | Unknown              | LT898325 | - | - | - | - |
| BEOFB2600m              | Unknown; Serbia      | MH630050 | MH780060 | - | - | - |
| BEOFB2601m              | Unknown; Serbia      | MH630051 | MH780061 | - | - | - |
| **Talaromyces sayulitensis** | Military equipment; Japan | KF984892 = NR153234 = KF196908 | KF984856 = KF196851 | KF984684 = KX946895 | KF196953 = KF984916 = KF196961 | - |
| CBS 340.34 (T) = NRRL 1129 | Milled Californian rice; Japan; Type of Talaromyces phialosporus | KF984895 | KF984562 = HQ156949 | KF984683 | JN680282 | KF984917 |
| CBS 233.60              | House dust; Thailand | KF984893 | KF984561 | KF984686 | - | KF984915 |
| **Talaromyces scorteus** | Forest soil; Thailand | JN899385 = NR 103683.2 | JX091379 | KF741960 | - | KM023279 |
| CBS 475.88 (T)          |                      |          |          |          |   |          |
| Species                      | Repository | Isolate Type/Location                                                                 | GenBank Accession Numbers | Notes          |
|-----------------------------|------------|----------------------------------------------------------------------------------------|----------------------------|----------------|
| *Talaromyces solicola*      | CBS 133446 | Soil; Malmesbury, South Africa                                                          | KF114730, KF114775        |                |
|                             | DT0 269-13 | House dust; Thailand                                                                     | KJ775726, KJ775219, KJ775428 |                |
|                             | CBS 133445 (T) | CMV-2008d isolate Soil; Malmesbury, South Africa                  | FJ160264, GU385731, KJ885279 | KM023295      |
|                             | = DAOM 241015 | = *Penicillium* sp.                                                                   |                            |                |
|                             | = CMV-2008d isolate Soil; Malmesbury, South Africa                  |                            |                |
|                             | = Penicillium sp. CV191                                             |                            |                |
|                             | = CMV-2008d isolate Soil; Malmesbury, South Africa                  |                            |                |
| *Talaromyces stipitatus*    | CBS 375.48 (T) | Decaying wood; Louisiana, USA                                                          | JN899348 = NR 147424, KM111288, KF741957, JN680303, KM023280 |                |
|                             | NBRC 100533 | Unknown                                                                                | AB773824                  |                |
|                             | CBS 408.93 (T) | AIDS patient; the Netherlands                                                          | JX315674 = NR 111781, JX315633, JX315646, JX315693, JX315712 |                |
|                             | CBS 169.91  | Unknown substrate; South Africa                                                         | JX315664, JX315634, JX315647, JX315683 |                |
| *Talaromyces stolii*        | CBS 265.93  | Bronchoalveolar lavage of patient after lung transplantation (subclinical); France    | JX315670, JX315635, JX315648, JX315689 |                |
|                             | CBS 581.94  | Unknown                                                                                | JX315675, JX315632, JX315645, JX315694 |                |
| *Talaromyces subinflatus*   | CBS 624.93  | *Ananas camosus* cultivar; Martinique                                                  | JX315676, JX315636, JX315644 = JX965209, JX315644 = JX965209, JX315691 = JX965209, JX965315 |                |
|                             | NRRL 1768   | USA, Georgia, isol ex peanut, *Rj Cole*, 1974.                                         | -                         | MH793098      |
|                             | NRRL 62122  | Unknown                                                                                | -                         | MH793127      |
|                             | NRRL 62160  | Unknown                                                                                | -                         | MH793136      |
|                             | NRRL 62163  | Unknown                                                                                | -                         | MH793137      |
|                             | NRRL 62165  | Soil; Unknown                                                                          | -                         | MH793138      |
|                             | NRRL 62171  | Unknown                                                                                | -                         | MH793140      |
|                             | NRRL 62227  | Corn; Unknown                                                                          | -                         | MH793144      |
| *Talaromyces tardifaciens*  | CBS 250.94 (T) | Paddy soil; Bhaktapur, Nepal                                                        | KC202954 = KF984560, KF984682, JN680283, KF984908 |                |
| *Talaromyces thailandensis* | CBS 133147 (T) | Soil; Thailand                                                                         | JX98041 = NR 147428, JX94294, KF741940, JX98043, KF023307 |                |
| *Talaromyces trachyspermus* | CBS 373.48 (T) | Unknown; USA                                                                          | JN899354 = NR 147425, KF114803, KJ885280, JN121664, JF417432 |                |
|                             | CBS 116556  | Pasteurised canned strawberries; Germany                                                | KM056170, KM056126, MK451694 | -              |
| *Talaromyces tumuli*        | CBS 118437  | Soil; Morocco                                                                          | KM066169, KM066127, MK451695 | -              |
|                             | CBS 118438  | Soil; Morocco                                                                          | KM066166, KM066128, MK451696 | -              |
|                             | CBS 113146 (T) = CBS 133146 (*RPB1*)? | Soil; Trat, Thailand                                                                 | KF984891, KF984859, KF984890, JX98042, KF984911 |                |
|                             | CBS 137400 (T) = DTO 270-F5 | House dust; Mexico                                                                  | KF84889, KF84857, KF84868, - | KF984909      |
| *Talaromyces tratensis*     | CBS 137401 (T) = NRRL1013 | Carbonated beverage; Washington D.C., USA                                           | KF84890, KF84858, KF84869, - | KF984910      |
| *NRRL 62151 (T) = DTO 269-13 | Soil; Unknown |                                                                                       | MH7930701= NR 165528, MH792944, MH793008, - | MH793135      |
| *NRRL 6013*                | Unknown     |                                                                                       | MH793089, MH792910, MH792974, - | MH793101      |
| *NRRL 62469*               | Peanut; Unknown |                                                                                       | MH793089, MH792962, MH793026, - | MH793153      |
| Strain Code | Source Description | Genbank Accession Numbers |
|-------------|-------------------|--------------------------|
| NRRL 62471 | Peanut; Unknown | MH793090 MH792963 MH793027 - MH793154 |
| F-3 | Unknown | MT434004 - - - |
| CBS 162.67 (T) | Unknown | JN899394 = NR 153205 KF114771 KJ885282 JN680277 KM023289 |
| CBS 127.64 | soil treated with cyanamide; Germany; Type of *Talaromyces ohiensis* | KM066173 KF114772 - JN680272 - |
| CBS 583.72A | Soil; Japan | KM066174 KM066130 - - - |
| CBS 583.72C | Soil; Japan | KM066175 KM066131 - - - |
| CBS 127.64 | Soil; Misugimura, Japan | JN899350 = NR 145156 KF114796 KX961260 JN680310 - |
| CBS 100535 (T) | Soil; Taiwan | JN899368 = NR 111689 KJ865731 KJ885283 JN680305 KM023274 |
| CBS 386.48 (T) | Cotton yarn; England | KJ865731 KJ885284 JN680305 KM023274 |
| CBS 500.78 (T) | Unknown | KF741984 = NR 153228 KF741918 KF741961 - KX961279 |
| NRRL 6095 | Unknown | MH793040 MH792912 MH792976 - MH793103 |
| NRRL 62286 | Wheat flour; Unknown | MH793085 MH792958 MH793022 - MH793149 |
| IBT18366 | Unknown | KF741993 KF741928 KF741974 - |
| CMV005D6 | Soil; South Africa | MK450751 MK451043 - - |
| NRRL 1050 (T) = CBS 388.48 | Soil; Texas, USA | KF741994 KF741928 KF741974 - KM023306 |
| NRRL 6795 | Unknown | MH793040 MH792912 MH792976 - MH793103 |
| NRRL 20056 | Wheat flour; Unknown | MH793085 MH792958 MH793022 - MH793149 |
| IBT18366 | Unknown | KF741993 KF741928 KF741974 - |
| AX2101 I | Metallic surface; Para, Brazil | KJ413368 KJ413340 - - KJ476428 |
| NRRL 114.72 (T) = Sagenoma viride | Soil; Australia | AF285782 = MH860406 = NR160136 JX494310 KF741935 JN121571 JN121430 |
| CBS 252.87 (T) | Soil from bank of creek flowing into Little river; New South Wales | JN899314 = NR103663.2 JX091385 KF741943 JN680284 = JN121620 JF417422 |
| CBS 391.48 (T) | Soil; Denmark | KF984829 KF984648 KF984756 JN121669 KF984977 = JF417433 |
| CBS 319.63 | Unknown | KF984828 KF984651 KF984755 - KF984961 |
| CBS 385.48 = NRRL 1048 | coconut matting; Johannesburg, South Africa; Type of *Talaromyces variabilis* | KF196915 KF196853 = JX494295 KF196878 JN680304 KF196975 = KX657552 |
| CBS 895.73 | Unknown; Japan | KF984811 KF984626 KF984737 - KF984982 |
| CBS 137376 = DTO 176-17 | soil; Japan; Type of *Talaromyces sublevisporus* | KF984800 KF984632 KF984724 - KF984979 |
| NRRL 2125 = DTO 276-E7 | Weathering canvas; Panama | KF984797 KF984635 KF984731 - KF984991 |
| HMAS 248732 (T) | China | NR147445 - - - |
| HMAS 248732 | China | KU644580 KU644581 KU644582 - - |
| CBS 138210 (T) = DTO 268-E5 | House dust; Micronesia | KJ775717 KJ775210 KP119162 - KP119164 |
| CBS 138209 = DTO 268-E7 | House dust; Micronesia | KJ775719 = NR 145183 KJ775212 KP119161 - KP119163 |

The result of NCBI standard nucleotide blast is considered preferentially; moreover, the aim of adding type strains genus *Talaromyces* is to make the phylogenetic tree more plentiful. Genus and
species in the columns are represented by bold Italic. T indicates ex type. Sect. Talaromyces; sect. Helici; sect. Purpurei; sect. Trachyspermii; sect. Bacillispori; sect. Subinflati; sect. Islandici.

Table 1 summarised the information of type strains and other related strains, including collection numbers, source and location of strains, and GenBank accession numbers of five genes/regions (ITS barcode and four auxiliary molecular markers: BenA, CaM, RPB1, and RPB2) used for phylogenetic analysis of strain JP-NJ4. According to the information of Samson et al. (2011) and Yilmaz et al. (2014) [14,15], the type strains and other related strains were selected. The current sectional classification information of Talaromyces species was also marked in Table 1.

2.4. Observation on the Morphological Characteristics of Strain JP-NJ4

Important features used to describe the large group of Penicillium and its related fungi are as follows: Macromorphology, including colony texture, mycelium growth and color, shape, color, abundance, and texture of conidia, the presence and color of soluble pigments and exudates, the reverse color of the colony and the acid production of the strain on creatine sucrose agar (CREA) [43], etc. Micromorphology, including asexual sporulation structures (e.g., conidiophore) and sexual sporulation structures (e.g., cleistothecium), etc. To comprehensively investigate the growth of strain JP-NJ4 on different media, we formulated the following supplemented-medium types (see Table S2) from common media, these media can be used to observe other taxonomic characteristics of strains.

Czapek yeast autolysate (CYA) [13] and malt extract agar (MEA) [8] are two standard media recommended for species identification of Penicillium and related filamentous fungi. Czapek’s agar (CZ) [16] CZ is the medium used by Raper and Thom (1949) and Ramirez (1982) in taxonomic studies [44]; this also includes Blakeslee’s Malt extract agar (MEAb) of Blakeslee (1915) [45]; yeast extract sucrose agar (YES) [43]; and YES as the recommended medium for the analysis of species’ extracellular secretions (extrolites). Oatmeal agar (OA) [8] and Hay infusion agar (HAY) medium [46] were also included. Sexual reproduction of fungal strains most often occurs on OA and HAY media, which can provide valuable information for taxonomy. Use oatmeal/flakes for OA and dry straw for HAY. Creatine sucrose agar (CREA) is the production of acid that can be observed by color reactions (ranging from purple to yellow) in CREA, which are often useful for distinguishing closely related species. Dichloran 18% Glycerol agar (DG18) [47] and Czapek Yeast Autolysate agar with 5% NaCl (CYAS) [18] were used. DG18 and CYAS were used to detect the growth rate of the strain under low water activity.

Preparation for macromorphology observation: The strain JP-NJ4 was inoculated in Potato dextrose agar (PDA) medium and cultured at 15 °C for 25 days to collect conidia. Conidia were washed with distilled deionized water (dd H2O) and diluted with a semi-solid agar solution containing 0.2% agar and 0.05% Tween 80 to prepare the conidia suspension, which was stored at 4 °C for standby use [13]. Conidia suspension was extracted with a micropipette (Eppendorf) and inoculated in three points (1 μL per point) [18]. All media were incubated at a constant temperature of 25 °C for 7 days; each formula of medium is shown in Table S2. In addition, the Czapek Yeast Autolysate agar (CYA) was cultured at 30 °C and 37 °C, and Malt Extract agar (MEA) was cultured at 30 °C, and the data were recorded for the species identification of strain JP-NJ4. After 7 and 14 days of strain culture, the criss-cross method was used to measure the colony diameter.

Preparation for the micromorphology observation: Colonies of strain JP-NJ4 cultured on MEA for one to two weeks in a dark environment at 25 °C were used for micromorphology observation, and OA and HAY medium were used when ascomata were not observed on MEA. OA and HAY media are often used for the observation of ascocarp, ascus and ascospore [31,48–50] and may be cultured for up to 3 weeks if required for ascocarp production. Then, the colonies used for micromorphology observation were rinsed with 2mL 0.1 mol/L phosphate-buffered saline (PBS) three times. Lactic acid (60%) was used as the fixative. Since most species produce large amounts of hydrophobic conidia, 70%
ethanol is usually used to flush out excess conidia and prevent air from getting trapped in lactic acid between the slide and cover. The characteristics of strain JP-NJ4 were observed with a compound microscope (Axio Imager M2.0; Zeiss, Germany) equipped with a digital camera (AxioCam HRc; Zeiss, Germany). The colonies were dehydrated in a graded ethanol solution and dried with liquid carbon dioxide at a critical point (EmiTech K850). After gold spraying (Hitachi E-1010), the Micromorphology of strain JP-NJ4 (conidiophore, conidium, ascocarp, ascus, and ascospore) was observed by scanning electron microscope (SEM) (FEI Quanta 200, FEI, USA).

3. Results
3.1. Taxonomy of Strain JP-NJ4

From the molecular and phenotypic data, it can be inferred that the strain JP-NJ4 belongs to Talaromyces. We identified it as a putative new species (new taxon) here [27,51].

Taxonomy

Talaromyces nanjingensis X.R. Sun, X.Q. Wu and W. Wei, sp. nov. (this study).

MycoBank (No: MB837590).

Etymology: Latin, `nanjingensis` refers to Nan jing, the name of the city where the species originated.

Typus (Type strain): China, Jiangsu, Nanjing, on the rhizosphere soil from Pinus massoniana, April 11, 2011, W. Wei, deposited in China Center for Type Culture Collection (CCTCC) (Collection number. CCTCC M 2012167) (http://www.cctcc.org). Holotype: CCTCC M 2012167. Culture ex-holotype: CCTCC M 2012167.

Distribution: Area of Nanjing, China.

Habitat: Rhizosphere soil from Pinus massoniana.

ITS barcode: MW130720. (Alternative markers for identification: BenA = MW147759; CaM = MW147760; RPB1 = MW147761; RPB2 = MW147762).

In: Talaromyces section Talaromyces

Colony diam, 7 d (mm): CZ 29-33; CYA 25 °C 25-29; CYA 30 °C 30-37; CYA 37 °C 21-31; MEA 25 °C 31-33; MEA 30 °C 35-41; MEAbl (34-43); OA 38-44; DG18 15-18; CYAS No growth; YES 30-40; CREA 18-24; HAY No growth.

Colony characters: The top and reverse colony morphology of the strain Talaromyces nanjingensis in different media was described. CYA 25 °C, 7 d: top colonies raised at the centre, yellow and margins white; margins low, plane, entire; texture velvety to floccose; sporulation absent to sparse; a small amount of yellow and orange soluble pigments present at 25, 30 and 37 °C; exudates absent; reverse centre pastel yellow (2D4) to pale yellow (1A4); 25 °C, 14 d: top colonies centre pale yellow (1A4) and margins white; the amount of orange exudates present on colonies centre; 30 °C, 14 d: top colonies white, pastel yellow and pinkish-red; a small amount of orange exudates present on colonies centre; 30 °C, 14 d: top colonies centre white and margins yellow (4A5); reverse light orange to light yellow (5A5-4A5); 25 °C, 14 d: formation of yellow ascomata.

DG18 25 °C, 7 d: top colonies slightly raised at the centre, plane; margins low, plane, entire (2
mm); pastel green (28A4) and margins white; texture floccose; sporulation moderately dense, conidia greyish green to dull green (25DS-25E4); soluble pigments absent; exudates absent; reverse centre dark green (28F5) and margins white; 25 °C, 14 d: top colonies centre greyish green (28C5) and margins white, reverse pale light green (1B3) to white. OA 25 °C, 7 d: top colonies raised at the centre, plane, formation of yellow ascomata (abundant at 25 °C, 14 d); margins low, plane, entire (2-3 mm); white and yellow; sporulation absent; soluble pigments absent; exudates absent; reverse pastel yellow (2D4). CREA 25 °C, 7 d: acid production present strong; 25 °C, 14 d: acid production present very strong; mycelia all weak at 7 d and 14 d.

Micromorphology: Conidiophores monoverticillate and biverticillate; it also produces reduced conidiophores consisting of solitary phialides. Stipes smooth-walled, 20-100 × 2.5-3 μm; branches 8-20 μm; metulae two to five, divergent, 7-16 × 2.5-3 μm; phialides acerose, two to five per metulae, 6-8 × 2-3 μm; Conidia smooth, globose to subglobose, 2-3 × 3 μm, sometimes ovoid, 3 × 3-3.5 μm. Ascomata mature after one week of incubation on OA, two weeks of incubation on CZ at 25 °C and on CYA and MEA at 30 °C. Ascomata yellow, globose to subglobose, 300-950 × 300-1000 μm, Asc, which are irregular in shape and size depending on the number of ascospores inside them, 10-12 × 8-10 μm: Ascospores, the shape and size are uniform and stable, broadly ellipsoidal, spiny, 3.5-5 × 2-3 μm.

Distinguishing characters: Talaromyces nanjingensis produces relatively fast-growing colonies (Colony diam (mm)) on MEA (31-33), CYA (25-29) and YES (30-40) at 25 °C (faster at 30 °C, MEA 35-41, CYA 30-37), as well as the fastest-growing colonies on MEAbI (34-43) and OA (38-44) at 25 °C. It produces yellow ascomata on CZ and OA medium with spiny ellipsoidal ascospores, similar to those of T. austrocalifornicus, T. flavovirens, T. flavus, T. macrosporus, T. muroii, T. thailandensis, and T. tratensis. On colony size at 25 °C on CYA and MEA after 7 d (CYA 25-29; MEA 31-33), T. nanjingensis is more similar to T. aculeatus, T. angelicus, T. dendriticus, T. indigoticus, T. panamensis, T. varians, and T. siamensis. According to the phylogenetic tree, T. nanjingensis and T. liani are clustered together. T. nanjingensis produces yellow ascomata, whereas T. brevis and T. liani produce yellow to orange and yellow to orange-red ascomata on OA medium, respectively. Talaromyces nanjingensis, T. brevis and T. liani both have ellipsoidal ascospores. Talaromyces nanjingensis grows more faster and produces more acid on CREA than T. brevis and T. liani.

3.2. Phylogeny-Based Species Identification

With the help of concatenated phylogenetic trees based on five gene regions, including the internal transcribed spacer region, BenA, CaM, RPB1, and RPB2, we investigated the taxonomic position of strain JP-NJ4. Figures 1-3 and Figures S1-S4 show the phylogenetic relationships among strain JP-NJ4 and representative species of Talaromyces. Concatenated phylogenetic trees of five (ITS, BenA, CaM, RPB1, and RPB2) and four (ITS, BenA, CaM, and RPB2) gene regions and individual phylogenetic trees of each gene region were constructed using the maximum-likelihood method. Talaromyces dendriticus (CBS_660.80_T) was chosen as an out-group for Talaromyces section Talaromyces. Trichocoma paradoxa (CBS_788.83_T) was chosen as the out-group for the Talaromyces genus. Bootstrap values obtained from 1000 replications are shown at the nodes of the tree, and bootstrap support lower than 50 is not shown. In the multi-gene phylogenetic analysis (five gene region), strain JP-NJ4 clustered with T. liani (Figure 1) in Talaromyces section Talaromyces (orange area), with bootstrap values of 100% (Blpp = 1). The concatenated phylogeny of five gene region shows that strain JP-NJ4 and T. liani differ in their genetic distance from other species of Talaromyces. Phylogenetically, the results of four genes indicate that strain JP-NJ4 T. nanjingensis is close to T. brevis, with bootstrap values of 93% (Blpp = 1) (Figure 2).

Among the phylogenetic trees obtained from each DNA gene region, that of the ITS region was less clearly resolved; although most species formed monophyletic groups in the strict consensus trees, several had low bootstrap support values. The ITS sequence of strain JP-NJ4 clustered with those of nine other strains of T. liani and three strains of T. brevis (bootstrap = 32%, Blpp = 0.61) (Figure S1). The CaM sequence of strain JP-NJ4 clustered well with two strains of T. brevis (bootstrap = 65%, Blpp = 0.99) (one strain of T. brevis...
was deleted because its sequence was shorter) and seven other strains of *T. liani* (bootstrap = 99%, BIpp = 0.96) (Figure S2). The RPB1 sequence of strain JP-NJ4 clustered with that of the type strain of *T. liani* (CBS_225.66_T) (bootstrap = 85%, BIpp = 0.99) (Figure S3). The RPB2 sequence of strain JP-NJ4 clustered perfectly with three strains of *T. brevis* (bootstrap = 99%, BIpp = 1) (Figure S4). The phylogenetic tree of the CaM gene region shows that strain JP-NJ4 and *T. liani* differed little in their genetic distance from other species of *Talaromyces*. However, the phylogenetic trees of the BenA, RPB1, and RPB2 gene regions show that strain JP-NJ4 and *T. liani* differed markedly in their genetic distance from type strain of *T. liani* and other multiple collections of *T. liani*.

The result of single gene BenA indicate that *T. nanjingensis* and ‘*T. liani*‘ (voucher KUC21412) are clustered together (Bootstrap 88%/ BIpp 1), and both of them have lower bootstrap values (Bootstrap 45%/ BIpp -) with *T. liani* (CBS_118885) and higher bootstrap values (Bootstrap 63%/ BIpp 0.99) with nine other strains of *T. liani* at the node (Figure 3). This indicates that *T. nanjingensis* is still genetically different from its genetic relatives *T. liani* and *T. brevis*. The sequences of *T. nanjingensis* and ‘*T. liani*‘ (voucher KUC21412) were significantly similar in BenA gene. However, *T. nanjingensis* and ‘*T. liani*‘ (voucher KUC21412) differ from *T. liani* and *T. brevis* in BenA gene by more than ten bases, and half of their base arrangement pattern is similar to *T. liani* and the other half is similar to *T. brevis* (Figure S8a). This could mean they should be new species. It also explains the low bootstrap values. This is also due to the continued discovery of new species, filling gaps in the evolutionary trees, and the lack of some transitional species, of which the *T. nanjingensis* is one, which has characteristics common to both *T. liani* and *T. brevis*. *T. nanjingensis* is more similar to *T. brevis* in acid production. *T. liani* (CBS_118885) is the only acid-producing strain of *T. liani* that is genetically closest to *T. nanjingensis*. The phenotypic information of these species may also hint at evolutionary continuity. After a detailed search, we found that *T. liani* strain T2C1 is equivalent to ‘*T. liani*‘ (voucher KUC21412) and ITS sequence was also obtained. ‘*T. liani*‘ (voucher KUC21412) has at least two base differences with *T. nanjingensis*, *T. liani*, and *T. brevis* in the ITS region, and the front-end of the sequence is similar to that of *T. liani* and *T. brevis* (CBS_141833_T). In particular, it has a distinctive differential base A at the end of its sequence (Figure S8b). Therefore, ‘*T. liani*‘ (voucher KUC21412) is also different from *T. nanjingensis*. ‘*T. liani*‘ (voucher KUC21412) is described by Heo et al. as one of the microorganisms selected from intertidal mudflats and abandoned solar salterns that can produce bioactive compounds [52]. The strain voucher KUC21412 was described as ‘*T. liani*‘ (with quotation marks) in this manuscript, as its current species identity may be in some doubt. ‘*T. liani*‘ (voucher KUC21412) is a comparable species, although only ITS (MN518409.1) and BenA sequences (MN531288.1) have been submitted to NCBI, the quality of the sequences is reliable, which proves that the BenA sequence of *T. nanjingensis* is reliable. Although *T. nanjingensis* had little difference with *T. brevis* in ITS, CaM, RPB1, and RPB2 genes, it had great difference with *T. brevis* in BenA gene (Figure S8a). BenA is the secondary barcode with the highest reliability in filamentous fungi; thus, the classification results obtained by using this gene are relatively more referential and accurate [15,18]. According to the Bayesian tree (ITS + BenA + CaM + RPB2 and single gene BenA) and the ML tree with deletion of *T. brevis* and ‘*T. liani*‘ (voucher KUC21412), it can be more obvious that *T. nanjingensis* has a long genetic distance from *T. brevis* and *T. liani* (the small diagrams in Figures 2 and 3).

More obviously, the phylogenetic tree of BenA gene with long sequence version (Figure 3B) was better than that of the BenA gene with short sequence version (Figure 3A) to show the true classification status of strain JP-NJ4. The results showed that strain JP-NJ4 was quite different from *T. brevis* and *T. liani* in phylogeny (Figure 3B). The long sequence version of the phylogenetic tree (Figure 3B) only reduced or lost information on some other species, but this did not affect the accurate identification of JP-NJ4, because this version included *T. brevis* and *T. liani*. New species resources are undoubtedly important, as are innovations in identification methods and fine-delineation of species. Taxonomy of these species of *Talaromyces* are similar to the results obtained by Samson et al. (2011) and
Yilmaz et al. (2014) [14,15]. These phylogenetic results suggest that strain JP-NJ4 is a potential novel species.

Figure 1. Combined phylogeny of the ITS, BenA, CaM, RPB1, and RPB2 gene regions of species from *Talaromyces*. Maximum likelihood tree of strain JP-NJ4 was constructed. *Trichocoma paradoxa* (CBS_788.83_T) was chosen as out-group. Support in nodes is indicated above branches and is
represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+I+G4; best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) +G+I; alignment, 444 (ITS) + 294 (BenA) + 489 (CaM)+ 491 (RPB1) + 677 (RPB2) = 2395 bp. Scale bar: 0.10 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.
Figure 2. Combined phylogeny of the ITS, BenA, CaM, and RPB2 gene regions of species from Talaromyces. Maximum likelihood tree of strain JP-NJ4 was constructed. Trichocoma paradoxa (CBS_788.83_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+I+G4; best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2)+G+I; alignment, 439 (ITS) + 284 (BenA) + 482 (CaM) + 677 (RPB2) = 1882 bp. Scale bar: 0.05 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.
Figure 3. Maximum likelihood phylogeny of BenA gene regions for strain JP-NJ4 and other species classified in Talaromyces sect. Talaromyces. (A) Short sequence version with multiple species, alignment, BenA 316 bp. Best-fit model of Bayesian Inference phylogeny according to BIC: K80 (K2P) +I+G4; best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) +G. Talaromyces dendriticus (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Missing data from the Bayesian inference (BI) analysis is indicated with a hyphen (-) in the tree. (B) Long sequence version with few species, alignment, BenA 391 bp. Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+G4; best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) +G. Talaromyces dendriticus (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Missing data from the Bayesian inference (BI) analysis is indicated with a hyphen (-) in the tree.
3.3. *Species Identification Based on Macromorphology and Micromorphology*

By combining these results with morphological observations, the taxonomic position of strain JP-NJ4 can be further elucidated. The macromorphology of strains, including the morphology and diameter of colonies on specific media, is an important trait for species identification. Based on the preliminary results on CYA and MEA, the macromorphology of the strain may be observed on several other culture media for more accurate identification.

We selected as many media as possible to observe the strains in more detail. Czapek (CZ) medium was used in early taxonomic studies of *Penicillium* and was selected for comparison with CYA medium. Blakeslee’s MEA, which has been widely used historically, was compared with the MEA culture medium used by the CBS-KNAW Fungal Biodiversity Centre in Utrecht (Table S2). Medium with the addition of hay (HAY) was compared with oatmeal agar for observing the sexual reproduction of fungal strain JP-NJ4. However, strain JP-NJ4 did not grow on this recommended medium.

To clearly observe the colony morphology of strain JP-NJ4 on various culture media, we obtained photographs with black and white background colors. In this paper, we provided colony morphology photographs of strain JP-NJ4 grown at 25 °C for 7 (Figures 4 and S5) and 14 d (Figures 5 and S6) on 10 different media, as well as image data for strain JP-NJ4 grown at 25 °C, 30 °C, and 37 °C in CYA medium (Figure S7).

*Figure 4. Macromorphological characters of strain JP-NJ4 (CCTCC M 2012167) (Inoculation at 25 °C for 7 days). Colonies from left to right: (the top two rows) CZ, CYA, MEA, MEAbl, OA, and the reverse side corresponding to these media; (the bottom two rows) DG18, CYAS, YES, CREA, HAY, and the reverse side corresponding to these media (the background color is black).*
Reverse colonies of *Talaromyces* species on CYA and MEA media commonly produce yellow or red soluble pigments. Numerous species of *Talaromyces*, including *T. albobiverticillius*, *T. amestolkiae*, *T. atroroseus*, *T. cnidii*, *T. coalescens*, *T. marneffei*, *T. minioluteus*, *T. purpurogenus*, *T. ruber*, and *T. stollii* produce soluble red pigments. In *T. nanjingensis* strain JP-NJ4, weak production of yellow and orange soluble pigments was observed on CYA and MEA in colonies grown at 25 °C for 7 d (Figures 4 and S5). The reverse colony color on MEA was similar to those of *T. albobiverticillius*, *T. minioluteus*, and *T. purpurogenus*. Strong and stable red soluble pigment production occurred on MEA in colonies grown at 25 °C for 14 d (Figures 5 and S6). The reverse colony color on MEA in colonies grown at 25 °C for 14 d was similar to those of *T. amestolkiae*, *T. coalescens*, and *T. marneffei* grown on MEA at 25 °C for 7 d. *T. nanjingensis* strain JP-NJ4 could produce acid on CREA at 25 °C for 7 d (present strong, the color reaction showed a marked shift from purple to yellow) and at 25 °C for 14 d (present very strong; the color reaction appears to be intense yellow).

The macromorphologies of *T. nanjingensis* strain JP-NJ4, *T. liani*, and *T. brevis* on various media were obviously different in terms of colony growth rate and mycelia color. In terms of colony growth rate, the difference between the three species on MEA and CREA medium was the greatest. In an ascending order of growth rate, we have MEA medium (25 °C, 7 d), *T. nanjingensis* strain JP-NJ4 (31-33), *T. liani* (35–45), and *T. brevis* (50–51); in addition, we also have CREA medium (25 °C, 7 d), *T. liani* (10-20), *T. brevis* (13–14), and *T. nanjingensis* strain JP-NJ4 (18-24). In terms of mycelia color, on OA medium (25 °C, 7 d), the three species were sequentially *T. nanjingensis* strain JP-NJ4 (white and yellow), *T.
brevis (primrose), and T. liani (white and yellow) according to the color of mycelia from light to dark; on CYA medium (25 °C, 7 d), the order was T. liani (white and pastel yellow), T. brevis (white and flesh) and T. nanjingensis strain JP-NJ4 (yellow and margins white). Other detailed data are shown in Table 2.

Table 2. Morphological comparison of strain JP-NJ4 with Talaromyces liani and Talaromyces brevis.

| Morphological Characters | Species | T. liani (Yilmaz et al. 2014) | Talaromyces strain JP-NJ4 | T. brevis (Sun et al. 2020) |
|--------------------------|---------|------------------------------|--------------------------|-------------------------------|
| Ascomata                 | Present after 25 °C, 7 d on OA and MEA (at 30 °C abundant yellow ascomata) | Present after 25 °C, 14 d on CZ, and 30 °C, 14 d on CYA and MEA | Present after 25 °C, 7 d on OA |
| Growth rate (mm Diam (diameter), 7 d) | Unknown | 29-33 | Unknown |
| MEA colony texture       | Unknown | 29-33 | Unknown |
| Colour of CYA reverse    | Light orange and light yellow (5A5–4A5) | Centre pastel yellow (2D4) to pale yellow (1A4) | Ochreous (44) |
| Soluble pigment          | Absent on CYA (in some isolates yellow) and MEA at 25°C, 7 d | Weak yellow and orange soluble pigments present on CYA and MEA at 25°C, 7 d; Strong red soluble pigments present on MEA at 25°C, 14 d | Absent |
| MEA colony texture       | Velvety and floccose | Velvety to floccose | Floccose |
| Acid production on CREA  | Absent (in some isolates very weak) | Present strong | Present |
| Conidiophore             | Present | Present | Present |
| Conidiophore branching   | Mono- to biverticillate, reduced conidiophores consisting of solitary phialides | Mono- to biverticillate | Mono- to biverticillate |
| Conidium                 | Shape | Ellipsoidal | Globose to subglobose; (sometimes ovoid) | Subglobose to fusiform |
|                         | Size (μm) | 2.5–4.5 | 2–3 | 3–4 |
|                         | Ornamentation | Smooth | Smooth | Smooth |
| Ascomata colour          | Yellow to orange red | Yellow | Yellow to orange |
| Ascomata shape           | Globose to subglobose | Globose to subglobose | Globose to subglobose |
| Ascomata size (μm)       | 150–550 × 150–545 | 300–950 × 300–1000 | 400–550 × 400–550 |
| Asci size (μm)           | 9–13 × 7.5–11 | 10–12 × 8–10 | Unknown |
| Shape                    | Broadly ellipsoidal | Broadly ellipsoidal | Ellipsoidal |
| Size (μm)                | 4–6 × 2.5–4 | 3.5–5 × 2–3 | 3.5–4.5 × 2–4 |
| Ridges                   | Absent | Absent | Absent |
| Ornamentation            | Spiny | Spiny | Spiny |

Talaromyces species generally produce acerose phialides and ellipsoidal to fusiform conidia. T. nanjingensis strain JP-NJ4 produces reduced conidiophores consisting of solitary phialides (Figure 6A), most conidiophores are monovercillate and biverticillate, and conidia are globose to subglobose and sometimes ovoid (Figure 6B-H). With the help of scanning electron microscopy, clearer pictures of conidia can be seen (Figure 6I, J). Talaromyces liani produces ellipsoidal conidia. Talaromyces brevis produces subglobose to fusiform conidia. In addition, some species of Talaromyces produce rough-walled, globose conidia, including T. aculeatus, T. apiculatus, and T. verruculosus (classified in sect. Talaromyces), as well as T. diversus and T. solicola (classified in sect. Trachyspermi).
Figure 6. Micromorphological characters of JP-NJ4 (CCTCC M 2012167) (anamorphic stage) (inoculation for 1–2 wk on MEA). A–D. Conidiophores and conidia, observed by optical microscope (Zeiss). A. Reduced conidiophores consisting of solitary phialides. B. Monoverticillate conidiophores. C. Biverticillate conidiophores. D. Conidia. E–J. Conidiophores and conidia, observed by scanning electron microscope (SEM). E–G. Conidiophores and conidia at different magnification (E. 2500×; F. 4000×; G. 5000×). H. Phialides and conidia (10000×). I–J. Conidia. Scale bars: A= 10 μm, applies to A–D. E = 20 μm; F = 10 μm; G = 10 μm; H = 5 μm; I = 10 μm; J = 5 μm.

Many species of *Talaromyces* have the ability to produce ascomata (ascoma = ascocarp; plural, ascomata) (Figure 7A-D). Generally, ascomata are yellow, but some species produce
green (*T. dixii, T. euchlorocarpus*, and *T. viridis*) or creamish white ascomata (*T. assiutensis* and *T. trachyspermus*). The size, shape, and ornamentation of ascospores can be used to distinguish among species of *Talaromyces*. In most species of *Talaromyces*, ascospores are broadly ellipsoidal and spiny, but *T. bacillisporus* and *T. rotundus* have spiny globose ascospores and *T. tardifaciens* produces smooth globose ascospores. The ascospores of strain JP-NJ4 and *T. liani* are broadly ellipsoidal and spiny, and the ascospores of *T. brevis* are ellipsoidal and spiny. The ascospore sizes of *T. nanjingensis* strain JP-NJ4, *T. brevis*, and *T. liani* differed, at 3.5–5 × 2–3 μm (Figure 7E-I), 3.5–4.5 × 3–4, and 4–6 × 2.5–4 μm, respectively. The ascospores of *T. stipitatus* have single equatorial ridges, whereas those of *T. udagavae* have numerous ornamented ridges, and *T. helicus* has smooth ascospores.

**Figure 7.** Teleomorphic stage of JP-NJ4 (CCTCC M 2012167). (A) Colonies inoculated for 2 wk on CZ (left) and OA (right). B–I. Micromorphological characters of JP-NJ4. (B) Primary ascomata
collected from OA (inoculation for 1 wk), observed by scanning electron microscope (SEM). C–D. A mature ascoma that is releasing asci and ascospores at different magnification ((C) 5×; (D) 20×), observed by optical microscope (Zeiss). (E) An ascus and ascospores (100×). F–I. Ascospores, observed by SEM. Scale bars: B = 2 μm; C = 200 μm; D = 50 μm; E = 10 μm; F = 10 μm; G = 5 μm; H = 3 μm; I = 3 μm.

According to the phylogenetic results, T. nanjingensis strain JP-NJ4 belongs to the genus Talaromyces. The taxonomic status of this strain can be further determined through description of its morphological characters. Talaromyces liani [15] and Talaromyces brevis [53] are the two species most closely related to T. nanjingensis strain JP-NJ4 in terms of molecular phylogeny, and they were selected as the control group for morphological comparison (Table 2). Table 2 contains summaries of the general macro-morphological and micro-morphological characters observed, including the most important characters: growth rates on different media, production of ascomata and soluble pigments, and acid production on creatine sucrose agar.

4. Discussion

Talaromyces species have a cosmopolitan distribution and have been isolated from a wide range of substrates. Soil is their main habitat, but new species have been obtained from indoor air, dust, clinical samples, plants, leaf litter, honey, and pollen [18,54–62]. Talaromyces species have positive impacts in the medical field. The members of this genus can produce a variety of antibiotics and antibacterial substances, such as the rugulosin produced by T. rugulosus [11,63]. Other extrolites of the genus (e.g., erythroskyrine, etc.) have anti-tumor [64], anti-malignant cell proliferation (antiproliferative), and anti-oxidant properties [65]. Talaromyces fungi also have a strong ability to produce enzymes, including that of β-rutinosidase and phosphatase [66,67], endoglucanase and cellulase [68], cellulase [69–71], and others. These fungi have also been investigated for functions in plant disease resistance, such as T. flavus [72–75] and T. pinophilus [76]; moreover, this includes the plant growth promotion of T. pinophilus [77]. In the present study, fungal strain JP-NJ4, which was isolated from the rhizosphere soil of Pinus massoniana, exhibited abilities of phosphate solubilization and plant growth promotion [24], and it was identified as a novel species in genus Talaromyces, section Talaromyces, using the polyphasic approach in this manuscript.

The fungal genera of Penicillium and Talaromyces have many similarities in morphology, such as asexual sporulation structures (e.g., conidiophore), the branching pattern of conidiophores, namely the type of penicillus, and sexual sporulation structures (e.g., cleistothecium). Mistakes are easily made when distinguishing between them. Therefore, we can use molecular methods to conduct preliminary identification of species in these genera. It should be noted, for the modern taxonomic identification of a species, morphological characteristics and molecular phylogenetic results are equally important. Professional recommendations regarding appropriate phylogenetic and morphological data in species delineation are necessary to avoid taxonomic discrepancies [78]. Phylogenetic trees of species are constructed using extensive data obtained through searches and literature review. Normally, the first step is to input a nucleotide sequence obtained through PCR and sequencing technology into the NCBI website for comparison using the nucleotide BLAST. Using the default settings, we obtained 100 sequences that are most similar to the target sequence. The purpose of this step is to roughly determine the genus of the unknown strain. In this paper, BLAST analysis was conducted using ITS, BenA, CaM, RPB1, and RPB2 sequences of strain JP-NJ4.

During the process of collecting and collating the sequences needed to build phylogenetic trees, we encountered the following problems. Among the sequences submitted to NCBI, for the same gene from the same strain of the same species, sequences were uploaded under multiple sequence numbers. By conducting BLAST analysis of the sequence and preliminary phylogenetic tree construction, we found that some of the sequences were consistent with the earliest submitted sequences, whereas others did not cluster with
the type strains of their species. This difference may be due to misidentification by later sequence submitters or mislabeling of different strains as the same strain. Therefore, when selecting sequences to construct phylogenetic trees, if two sequences are obtained with differing base compositions, we used the sequences submitted earlier or those referenced in the authoritative literature. Only using validated sequences is also reliable. If the sequences were identical, they were all retained in the tables used to build the phylogenetic tree (Table 1).

The ITS region is the most commonly used molecular marker for fungal identification. In *T. liani*, NRRL 1014 and NRRL 1015 are equivalent to NRRL 1009, and the base sequences of the ITS region and the other four specific genes in the three strains are identical. Therefore, when constructing the ITS phylogenetic tree for strain JP-NJ4, NRRL 1009 was selected to represent all three strains [79]. In addition, nine other *T. liani* strains were added. By conducting sequence alignment analysis of the CaM gene, we found notable differences in the composition and arrangement of the bases in this gene among species in different sections of genus *Talaromyces*. This may result in the deletion of too many bases in order to ensure sequence alignment in the tree constructing of strain JP-NJ4 at the genus level, resulting in loss of information. Therefore, in order to ensure the length of a CaM sequence in tree construction and improve the accuracy of species identification, the CaM gene phylogenetic tree was constructed at the level of section *Talaromyces*. We further determined the taxonomic status of strain JP-NJ4 by evaluating the taxonomic relationships among these highly similar species within the genus *Talaromyces*. In addition, during the Alignment-Align process of ClustalW in MEGA software (Version 6.0 and 7.0), inaccuracies may be introduced into the alignment results when large differences exist among the sequences. Therefore, the best comparison results can be obtained through multiple repeated comparisons.

We also found that the gene sequences of RPB2 from some type strains of *Talaromyces* species could not be retrieved from the NCBI database. By performing comparison and analysis of the RPB2 sequences of other species in genus *Talaromyces*, we found that the gene sequence data of RNA polymerase (RNA polymerase gene, partial cds) downloaded for these type strains included the gene sequence of RPB2. Therefore, these RNA polymerase gene sequences can be used to complement the construction phylogenetic trees based on the RPB2 gene. Moreover, in previous studies of *Penicillium* and *Talaromyces* [14,15,18,31,80], the precedent of using the RNA polymerase gene sequence for constructing a RPB2 phylogenetic tree has been established (e.g., JX315698 *Talaromyces amestolkiae* DTO 179F5_T). Using this method, the taxonomic status of unknown species can be further refined. The sequences used for this analysis include the following: KX961275 *Talaromyces angelicus* Korean Agricultural Culture Collection (KACC) 46611, KX961285 *T. auranitians* CBS 314.59, KX961283 *T. flavovirens* CBS 102801, KX961280 *T. galapagensis* CBS 751.74, KX961278 *T. indigoticus* CBS 100534, KX961282 *T. intermedius* CBS 152.65, KX961276 *T. muroii* CBS 756.96, KX961281 *T. oumae-anuae* CBS 138208, JX315712 *T. stollii* CBS 408.93, and KX961279 *T. veerkampii* CBS 500.78. Some specific genes, such as Translation elongation factor (Tef) and mitochondrial Cytochrome c oxidase 1 (Cox1), have not been universally used in *Talaromyces*, and relatively few sequences for these genes are available from the NCBI database. Currently, although phylogenetic trees of the genus *Talaromyces* constructed from these remain imprecise, the genes have been used for identifying *Penicillium* species [6].

When constructing phylogenetic trees, it is necessary to delete redundant and irrelevant sequences. In the BLAST comparison results, the sequences related to some species did not include the corresponding type strains. In such cases, the sequence information should be validated, as the sequences might have been misidentified (wrongly identified as another species). For example, in Table 1, species marked with a yellow background color did not cluster with the type strains of the corresponding species, and phylogenetic results indicate that these species may be new species—*Talaromyces_stollii* (blue font) (Figure S4). This discrepancy is due to the fact that not all sequences in the NCBI database
have been verified. Therefore, type strains of these species should be added as references for molecular identification and construction of phylogenetic trees. Here, we selected sequences from the type strain of *T. pinophilus* and other related strains, and some sequences of *T. pinophilus* that were not relevant to our study were removed.

In addition, when building phylogenetic trees, if the sequences used to construct the tree are not sufficiently comprehensive, the strain to be identified will only cluster with the sequences of similar species, rather than the sequence of the closest species. This problem occurs because the sequences closest to that of the strain to be identified at the genetic level may not be included in the NCBI-BLAST results due to differences in the length of the uploaded sequences or differences in gene coverage, resulting in an inaccurate phylogenetic tree. Specifically, analysis of NCBI-BLAST results revealed that most sequences included only partial sequences of a gene (not all the bases of the gene). The uploaded gene sequences are inconsistent in length, and each sequence contains a different region of the full-length gene. These differences result in the common phenomenon of sequences that appear most similar in the alignment results not being those that are actually most similar to the destination sequence (i.e., the results are inaccurate).

In summary, in previous international research on filamentous fungal species such as *Penicillium* and *Talaromyces*, the standard research method (GCPSR) was recommended. This polyphasic approach, which involved multigene phylogeny, morphological descriptions using macro-morphological and micro-morphological characters. To build an accurate phylogenetic tree based on NCBI-BLAST sequences, it is essential to refer to sequences provided in the authoritative literature. For the gene sequences of type strains, the selected sequences should be validated or verified. Using ITS and four specific gene sequences in various *Talaromyces* species, we constructed two phylogenetic trees (tree 1: ITS, *BenA*, *CaM*, *RPB1*, and *RPB2* (Figure 1); tree 2: ITS, *BenA*, *CaM*, and *RPB2* (Figure 2)) based on combinations of multiple genes. In the genus *Talaromyces*, combinations of three or four genes are more common, whereas analyses of five genes have been rare. At present, ITS, *BenA*, *CaM*, *RPB1*, and *RPB2* are the most authoritative and reliable genes for the identification of *Talaromyces* species. The preliminary phylogenetic tree construction results indicate that the species most closely related to strain JP-NJ4 is *T. liani*. The concatenated phylogenies of five (or four) gene regions and single gene phylogenetic tree (*BenA*, *RPB1*, and *RPB2* genes) all also show that *T. nanjingensis* strain JP-NJ4 and *T. liani* clustered together but differ markedly in their genetic distance from type strain of *T. liani* and other multiple collections of *T. liani*. The morphology of JP-NJ4 (M 2012167) largely matches the characteristics of *T. liani*, but the rich and specific morphological information provided by its colonies was different from that of *T. liani*. In addition, strain JP-NJ4 could produce reduced conidiophores with solitary phialides. From molecular and phenotypic data, strain JP-NJ4 was identified as a putative novel *Talaromyces* fungal species, designated *T. nanjingensis*. *T. nanjingensis* also can produce yellow, orange, and red soluble pigments in their mycelium, including diffusing pigments, similar to other species of the genus [81,82]. Due to the rich and specific morphological information provided by colonies, additional colony morphology photographs of this strain growing at 25 °C for 14 days on 10 different media were captured. We believe that it is essential to apply this information as part of the general method of strain identification. Future research will focus on the ecological function of *T. nanjingensis* JP-NJ4 and its impacts on the environment in terms of ecological security will also be assessed.

The information of the culture preservation institutions involved is as follows (alphabetically)

**ACCC**: Agricultural Culture Collection of China.

**ATCC**: American Type Culture Collection, Manassas, VA, USA (WDCM 1) http://www.atcc.org/;

**CABI**: Centre for Agriculture and Bioscience International (International Mycological Institute, CABI Genetic Resource Collection).
**CBS:** culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands (WDCM 133) [http://www.cbs.knaw.nl/databases/index.htm](http://www.cbs.knaw.nl/databases/index.htm).

**DTO:** internal culture collection of CBS-KNAW Fungal Biodiversity Centre; IMI, CABI Genetic Resources Collection, Surrey, UK (WDCM 214) [http://www.cabi.org/](http://www.cabi.org/).

**FERM:** (Patent and Bio-Resource Center, National Institute of Advanced Industrial Science and Technology-AIST).

**FMR:** facultad de medicina, Universidad de Oviedo. 33071-Oviedo. Spain. Institute de Investigaciones Biomédicas C.S.I.C., Facultad de Medicina UAM, E-28029 Madrid, Spain.

**HMAS:** Fungarium of Institute of Microbiology.

**IBT:** culture collection of Center for Microbial Biotechnology (CMB) at Department of Systems Biology, Technical University of Denmark (WDCM 758) [http://www.biocentrum.dtu.dk/](http://www.biocentrum.dtu.dk/).

**MUCL:** Mycotheque de l’Universite catholique de Louvain, Leuven, Belgium (WDCM 308).

**NBRC:** Biological Resource Center, NITE.

**NRRL:** ARS Culture Collection, U.S. Department of Agriculture, Peoria, Illinois, USA (WDCM 97) [http://nrrl.ncaur.usda.gov/](http://nrrl.ncaur.usda.gov/).

### Abbreviation

**Notes:** The abbreviations below are listed in the order in which they first appear in the manuscript.

- Genealogical concordance phylogenetic species recognition (GCPSR)
- Internal Transcribed Spacer rDNA area (ITS)
- β-tubulin (BenA)
- Calmodulin (CaM)
- DNA-dependent RNA polymerase II (beta) largest subunit (RPB1)
- DNA-dependent RNA polymerase II (beta) second largest subunit (RPB2)
- Talaromyces (T.)
- Penicillium (P.)
- Phosphate-solubilizing fungi (PSF)
- Phosphate-solubilizing bacteria (PSB)
- Centraalbureau Voor Schimmelcultures (CBS)
- The China Center for Type Culture Collection (CCTCC)
- Malt extract agar (MEA)
- Polymerase chain reaction (PCR)
- Basic Local Alignment Search Tool (BLAST)
- National Center for Biotechnology Information (NCBI)
- Maximum Likelihood (ML)
- Bayesian inference (BI)
- Akaike Information Criterion (AIC)
- Nearest-Neighbour-Interchange (NNI)
- Bayesian Information Criterion (BIC)
- Bayesian inference posterior probabilities (BIpp)
- Czapek stock solution (CSS)
- Trace elements stock solution (TESS)
- Czapek’s agar (CZ)
- Czapek Yeast Autolysate agar (CYA)
- Czapek Yeast Autolysate agar with 5% NaCl (CYAS)
- Blakeslee’s Malt extract agar (MEAbi)
- Dichloran 18% Glycerol agar (DG18)
- Yeast extract sucrose agar (YES)
- Oatmeal agar (OA)
- Creatine sucrose agar (CREA)
Hay infusion agar (HAY)
Potato dextrose agar (PDA)
Phosphate-buffered saline (PBS)
Scanning electron microscope (SEM)
Translation elongation factor (Tef)
Mitochondrial Cytochrome c oxidase 1 (Cox1)

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/jof8020155/s1, Figure S1: Maximum likelihood phylogeny of ITS regions for strain JP-NJ4 and other species classified in *Talaromyces* sect. *Talaromyces*. *Talaromyces dendriticus* (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+I+G; Best-fit model of Maximum likelihood phylogeny according to AIC: Tamura 3-parameter (T92) +G+I; alignment, ITS 467 bp. Scale bar: 0.0020 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.

Figure S2: Maximum likelihood phylogeny of CaM gene regions for strain JP-NJ4 and other species classified in *Talaromyces* sect. *Talaromyces*. *Talaromyces dendriticus* (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+I+G; Best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) +G+I; alignment, CaM 475 bp. Scale bar: 0.05 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.

Figure S3: Maximum likelihood phylogeny of RPBI gene regions for strain JP-NJ4 and other species classified in *Talaromyces* sect. *Talaromyces*. *Talaromyces dendriticus* (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Best-fit model of Bayesian Inference phylogeny according to BIC: SYM+I+G; Best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) + G + I; alignment, RPBI 491 bp. Scale bar: 0.05 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.

Figure S4: Maximum likelihood phylogeny of RP2B gene regions for strain JP-NJ4 and other species classified in *Talaromyces* sect. *Talaromyces*. *Talaromyces dendriticus* (CBS_660.80_T) was chosen as out-group. Support in nodes is indicated above branches and is represented by posterior probabilities (BI analysis) and bootstrap values (ML analysis). Full support (1.00/100%) is indicated with an asterisk (*). Bootstrap values lower than 50 is hidden. Best-fit model of Bayesian Inference phylogeny according to BIC: K80 (K2P) +I+G4; Best-fit model of Maximum likelihood phylogeny according to AIC: Kimura 2-parameter (K2) +G+I; alignment, ITS 467 bp. Scale bar: 0.05 substitutions per nucleotide position. T indicates ex type. The strain with red font is the strain JP-NJ4 to be identified.
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