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Four new species of *Talaromyces* from clinical sources

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Short title: Novel species of *Talaromyces*

ABSTRACT

The genus *Talaromyces* constitutes an important group of molds with species that are mainly found in soil, indoor environments and food products. Traditionally, it has been considered, together with *Eupenicillium*, the teleomorphic state of *Penicillium*. However, the taxonomy of these fungi has changed considerably, and *Talaromyces* currently includes sexually and asexually reproducing species. In a previous study of the occurrence of penicillium-like fungi from clinical samples in the USA, we used the combined phylogeny of the internal transcribed spacer (ITS) region of the rDNA and β-tubulin (*BenA*) gene to identify 31 isolates of *Talaromyces*, 85 of *Penicillium* and two of *Rasamsonia*. However, seven isolates of *Talaromyces* were assigned to the corresponding sections but not to any particular species. In this paper, we have resolved the taxonomy of these isolates through a multilocus sequence analysis of the ITS, fragments of the *BenA*, calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) genes, and a detailed phenotypic study. As a result, four new species are described and illustrated, i.e., *T. alveolaris*, *T. georgiensis, T. minnesotensis* and *T. rapidus.*

Keywords: clinical isolates, molecular identification, *Penicillium*, *Talaromyces*, taxonomy.

INTRODUCTION

The genus *Talaromyces* (*Trichocomaceae*, *Eurotiales*) has traditionally been characterized by its sexual morph having gymnothecial or cleistotecial ascomata, unitunicate 8-spored asci, and unicellular ascospores with or without equatorial crests. Their species were commonly associated with the asexual genus *Penicillium*, but also with other related genera such as *Geosmithia*, *Merimbla*, *Paecilomyces* and *Sagenomella*.[1,2] However, based on phylogenetic studies and following the abandonment of the dual nomenclature for pleomorphic fungi, *Penicillium* and *Talaromyces* have been separated into two distinct genera, including both sexually and asexually reproducing species, and Penicillia of the subgenus *Biverticillium* transferred to the latter genus.[3-5]

Although *Penicillium* and *Talaromyces* share many phenotypic features (i.e. micro- and macromorphology), the former is more related phylogenetically to the genus *Aspergillus* than the latter.[4] In addition, the species of *Talaromyces* grow particularly restricted on low water activity media and have a quite different extrolite pattern than those of *Penicillium*.[6,7] It is noteworthy, however, that the identification of these fungi at the species level is currently a complex task. That requires to study morphological and physiological characters (i.e. growth on different culture media at different temperatures and extrolite profiles), as well as sequence data mainly of the β-tubulin (*BenA*) gene.[6,7] Nevertheless, to establish species boundaries or introduce new taxa, multilocus sequence analysis, including the internal transcribed spacer (ITS) region, *BenA* and fragments of the calmodulin (*CaM*) or the DNA-dependent RNA polymerase II largest subunit (*RPB*2), is necessary.[6,7]

Talaromyces currently includes around 110 accepted species, grouped into seven sections, i.e. *Bacillispori, Helici, Islandici, Purpurei, Subinflati, Talaromyces* and *Trachyspermi*.[8,9] Species within the genus have important biotechnological applications,[6] and have been reported to spoil pasteurized fruit juices and other fruit based products.[10,11] The genus also includes clinically relevant species such as *T. marneffei* (formerly *Penicillium marneffei*), which is considered an emerging pathogen that causes fatal systemic mycosis in, mostly, immunosuppressed patients from Southeast Asia, India, and China.[12] Other species, such as *T. amestolkiae*, *T. indigoticus*, *T. piceus, T. purpurogenus, T. radicus, T. ruber, T. rugulosus, T. stollii* and *T. verruculosus* have also, more rarely, been reported to cause human disease.[2,8]

Although numerous penicillium-like fungi are commonly reported in the clinical environment, both the incidence and diversity of these species in clinical samples is poorly documented. A survey was carried out recently on the presence of such fungi in a large set of clinical isolates from a USA reference laboratory. By using the sequences of the ITS region and of the *BenA* gene, we found that most of those isolates belonged to the genera *Penicillium, Talaromyces* and *Rasamsonia*.[13] In that study, we identified 31 isolates of *Talaromyces* recovered from human and animal clinical specimens; however, we were not able to identify seven of the isolates at the species level. The purpose of the present study was to resolve the taxonomy of these unidentified isolates using a polyphasic approach, including further molecular markers and a detailed phenotypic study.

MATERIALS AND METHODS

Isolates and sequences

Seven *Talaromyces* isolates were investigated in the present study (Table 1). They were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA). These specimens were part of a set of 118 clinical isolates of penicilium-like fungi included in Guevara-Suarez et al.[13] which had been assigned to the corresponding sections but not to particular species. Sequences of ex-type strains of all the species of the sections involved (i.e., *Helici, Islandici, Talaromyces* and *Trachyspermi*) and reference strains of *T. aurantiacus* and *T. minioluteus*, mostly reported in different studies,[7-9,14-19] were retrieved from GenBank (Table S1–supplemental material) and included in the phylogenetic analyses.

Morphological characterization

Colony features were studied following Yilmaz et al.[7] Briefly, the isolates were cultured onto malt extract agar (MEA; Difco Inc.),[20] oatmeal agar (OA),[20] Czapek yeast autolysate (CYA),[21] yeast extract sucrose agar (YES),[22] creatine sucrose agar (CREA),[22] and dichloran 18 % glycerol agar (DG18),[23] incubated at 25 °C for 7 d in darkness. Colony diameters were also measured after 7 d at 30 ºC and 37 °C on CYA, MEA, YES and OA. Color notations in colony descriptions are from Kornerup and Wanscher.[24] For ascoma production, OA plates were incubated at 25 ºC for up to four weeks.

Microscopic characters were examined and measured from the isolates after 7 days of growth on MEA at 25°C and mounted on slides with Shear's solution. Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope

with Nomarski differential interference contrast and phase-contrast optics (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital camera.

DNA extraction, amplification and sequencing

DNA was extracted directly from colonies on MEA after 7-14 days at 25 ºC, using the FastDNA® kit protocol (MP Biomedicals, Solon, OH) and for the homogenization step a FastPrep® FP120 cell disrupter (Thermo Savant, Holbrook, NY). We amplified the ITS region, including the 5.8S rDNA gene, and fragments of the *BenA*, *CaM* and *RPB2* genes proposed by Yilmaz et al.[7] for the phylogenetic studies in the genus *Talaromyces*. The primer pairs used were: ITS5/ITS4 for the ITS region,[25] Bt2a/Bt2b for *BenA* for most isolates and T10/Bt2b for one isolate of the section *Islandici*,[26] CMD5/CMD6 for *CaM*,[27] and RPB2-5F/RPB2-7Cr for *RPB2*.[28]

Single band PCR products were purified and sequenced at Macrogen Europe (Macrogen Inc., Amsterdam, the Netherlands) with a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequence assembly and editing were performed using SeqMan v. 7.0.0 (DNASTAR, Madison, WI). GenBank accession numbers for the sequences newly generated in this study are listed in Table 1.

Phylogenetic reconstructions

Sequences from each locus were aligned with MEGA v 6.0 software,[29] using the CLUSTALW algorithm,[30] refined with MUSCLE,[31] and visually adjusted using the same software platform. Phylogenetic analyses were made for each section with the individual locus and combined genes using maximum-likelihood (ML) in

MEGA v. 6.0 and Bayesian inference (BI) under MrBayes version 3.1.2.[32] For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference; support for internal branches was assessed by 1,000 ML sets of data. A bootstrap support (bs) ≥ 70% was considered significant. The phylogenetic reconstruction by BI was carried out using five million Markov chain Monte Carlo (MCMC) generations, with two runs (one cold chain and three heated chains) and samples were stored every 1,000 generations. The 50% majority-rule consensus trees and posterior probability values (pp) were calculated after removing the first 25% of the resulting trees for burn-in. A pp value ≥0.95 was considered significant. The best substitution model for all gene matrices was estimated using jModelTest v.2.1.3.[33,34] Phylogenetic trees were edited for publication in Adobe Illustrator CS3.

RESULTS

We carried out a phylogenetic study for the sections *Helici, Islandici, Talaromyces* and *Trachyspermi*. Phylogenies of each section were performed for the ITS region, *BenA, CaM* and/or *RPB2* loci (according to the availability of sequences of type strains for each section), as well as a concatenation of the three or four mentioned loci. The length, number of phylogenetic informative and variable sites, and substitution models (for ML) for each dataset are summarized in Table 2. The topologies of the trees of ML and BI analyses did not differ, therefore we used ML trees for representing results, with BI posterior probability values marked on relevant branches.

A first phylogeny concerning all currently accepted species in the section *Talaromyces*, including four unidentified clinical isolates, was performed using

sequences of the ITS, *BenA* and *CaM* genes (Figure 1). The aligned data set was 1372 bp long (ITS 466 bp; *BenA* 404 bp; *CaM* 502 bp). In this section two putative new species could be well delineated. The isolate UTHSC DI16-148 formed an independent branch clearly distinct from the other species of the section, while UTHSC DI16-146 and UTHSC DI16-147 both formed a full-supported clade closely related to *T. aurantiacus.* An additional analysis with the alternative barcode *BenA*, including all available GenBank sequences of *T. aurantiacus* (Figure S1– supplemental material), showed that the clade of the two clinical isolates was phylogenetically distant from the *T. aurantiacus* clade, with a similarity of 97.2% between them, and thus should be considered distinct taxa. The two new phylogenetic species are proposed below as *T. rapidus* and *T. alveolaris*, respectively. The isolate UTHSC DI16-149 matches morphologically and genetically with *T. kabodanensis*.[35]

A second phylogenetic reconstruction was performed for the section *Helici* (Figure 2), using ITS, *BenA* and *RPB2*. *CaM* was not included in the concatenated analysis because we were unable to get a reliable sequence of this locus from the isolate investigated. The aligned data set was 1714 bp long (ITS 465 bp; *BenA* 410 bp; *RPB2* 839 pb). Our phylogeny demonstrated that UTHSC DI16-145 was included in the *T. helicus*-clade,[7] together with *T. boninensis, T. helicus, T. reverso-olivaceus* and *T. varians*. The concatenated analysis showed that our clinical isolate was located between *T. helicus* and *T. varians* in a separate and well-supported branch (91% bs / 0.99 pp), representing a new lineage in the section and described below as *T. georgiensis*. The only species of the section not included in the present analysis was *T. ryukyuensis*, since only ITS sequences

were available for comparison. However, according to the reported phylogeny, *T. ryukyuensis* is closely related to *T. aerugineus, T. bohemicus* and *T. cinnabarinus*, three species that formed a full-supported clade phylogenetically distant from the *T. helicus*-clade. A relevant feature of the species in section *Helici* is their ability to grow at 37 ºC, which was also observed in the new species.

The combined analysis of ITS (462 bp), *BenA* (412 bp), *CaM* (480 bp) and *RPB*2 (754 bp) for the section *Islandici* (Figure 3) allowed for the identification of UTHSC DI16-143 as *T. subaurantiacus*.[8] This clinical isolate exhibited a phylogenetic distance of 0.3% with the ex-type strain of *T. subaurantiacus* (CBS 137383) and, phenotypically, it showed more restricted growth on all culture media and at the various temperatures tested (at 25 ºC on CYA 6-8 mm, MEA 14-15 mm, YES 11-12 mm; at 37 ºC 3-4 mm on CYA) compared with those described in the protologue of the species (at 25 ºC on CYA 16-18 mm, MEA 20-21 mm, YES 17-18 mm; at 37 ºC 7 mm on CYA).[8]

Finally, the phylogenetic relationship of the *Talaromyces* isolate UTHSC DI16-144 with the species of the section *Trachyspermi* was carried out using ITS, *BenA* and *CaM* (Figure 4). The aligned combined data set was 1333 bp long (ITS 477 bp; *BenA* 382 bp; *CaM* 474 bp). This isolate was included in a fully-supported clade with *T. udagawae* and *T. minioluteus*, the latter being the closest species. Considering that *T. minioluteus* has been reported as a species complex by Visagie et al.[14] and to know whether our clinical isolate matches with any of the lineage delineated in the complex, we carried out an additional *BenA* analysis with reliable GenBank sequences of *T. minioluteus*, including those of some species considered synonyms, such as *P. samsonii* (CBS 137.84) and *P. purpurogenum*

var. *rubrisclerotium* (CBS 270.35).[16] In that analysis (Figure S2–supplemental material), as in the combined analysis of the three mentioned loci datasets (Figure 4), our isolate was placed in a single branch distant from the clade where the ex-type strain of the species was included and from the main clade with the rest of *T. minioluteus* isolates, showing a similarity of 95.22% and 94.97% respectively to UTHSC DI16-144. Therefore, this isolate is considered representative of a distinct species and described below as *T. minnesotensis*. Distinctive features of this species were strong acid production on CREA, presence of a soluble red pigment on CYA, restricted growth on MEA at 25 ºC, and absence of growth at 37 ºC.

Taxonomy

Talaromyces alveolaris Guevara-Suarez, Cano & Guarro, sp. nov. — MycoBank MB 820459; Figure 5.

Etymology. Referring to the clinical specimen where the fungus was isolated.

Specimens examined. USA, Utah, human bronchoalveolar lavage, 2010, D.A. Sutton (**holotype** CBS H-22999; ex-type cultures UTHSC DI16–147, CBS 142379, FMR 13963); Texas, human bronchoalveolar lavage, 2010, D.A. Sutton (UTHSC DI16–146, FMR 13962).

Colony diameter in 7 d (mm) — on CYA: 25 ºC 20–22, 30 °C 29–31, 37 °C 20–22; on MEA: 25 ºC 19–24, 30 °C 16–19, 37 ºC 21–23; on YES: 25 ºC 25–28, 30 ºC 29–31, 37 ºC 36–40; on OA: 25 ºC 30–32, 30 ºC 30–35, 37 ºC 18–21; DG18 25 ºC no growth; CREA 25 ºC 8–10.

Colony characters at 25º in 7 d — On CYA, colonies with raised centre, floccose, white, margins entire; reverse greyish brown (8F3) to light orange (5A4);

sporulation sparse; soluble pigment only at 30 ºC and 37 ºC, brownish orange (6C8-6D8) to reddish brown (8E7); exudates hyaline droplets after 14 d. On MEA, colonies flat, velvety, mycelium white becoming pale green (30A3), margins lobate; reverse dark green (30F8) to yellowish green (30B8); sporulation moderate, with inconspicuous conidial masses; exudates and soluble pigments absent. On YES, colonies flat, slightly sulcate, floccose, mycelium light orange (5A5) to white, margins entire; reverse light orange (5A5); sporulation absent; soluble pigments brownish red (8C8) to reddish brown (8F8) only at 30 ºC and 37 ºC; exudates absent. On OA, colonies flat, velvety, white, margins entire; reverse pale orange (5A3); sporulation sparse; soluble pigments and exudates absent. On CREA, weak acid production.

Micromorphology on MEA — Conidiophores biverticillate; stipes smooth-walled, 85–130 × 2–3 µm; metulae two to four, divergent, cylindrical, 11–14 × 2–3 µm; phialides two to four per metulae, acerose, 10–13 × 2–3 µm; conidia smooth-walled, mostly subglobose to somewhat ellipsoidal, 2.5–3 × 2–2.5 µm. Ascomata not observed.

Notes —*Talaromyces alveolaris* is phylogenetically closely related to *T. aurantiacus* and *T. fusiformis*. Morphologically, *T. aurantiacus* mainly differs in having shorter stipes (up to 100 µm long) and cylindrical to ellipsoidal conidia (3–5 × 1.5–2.5 µm), and *T. fusiformis* in its ellipsoidal to fusiform conidia (3–4 × 2–3 µm) and absence of growth on CREA.[7,16]

Talaromyces georgiensis Guevara-Suarez, Sutton & Wiederhold, sp. nov. — MycoBank MB 820460; Figure 6.

Etymology. Referring to the State of Georgia in USA, where the fungus was isolated.

Specimen examined. USA, Georgia, Athens, from animal joint fluid, 2010, D.A. Sutton (**holotype** CBS H-23000; ex-type cultures UTHSC DI16–145, CBS 142380, FMR 14270).

Colony diameter in 7 d (mm) — on CYA: 25 ºC 29–31, 30 °C 48–50, 37 °C 47–50; on MEA: 25 ºC 28–31, 30 °C 40–43, 37 ºC 43–45; on YES 25 ºC 28–30, 30 ºC 38–45, 37 ºC 40–43; on OA: 25 ºC 22–29, 30 ºC 38–40, 37 ºC 40–45; DG18 25 ºC 7–8; CREA 25 ºC 19–21.

Colony characters at 25º C in 7 d — On CYA, colonies with raised centre, flat towards the periphery, velvety, white, margins entire; reverse yellowish white (2A2); sporulation sparse, with conidial masses pale green (30A3); exudates absent; soluble pigments absent. On MEA, colonies cottony, mycelium white becoming greenish grey (29B2), margins entire; reverse greyish yellow (4B3); sporulation moderate, conidial masses pale green (30A3); exudate absent; soluble pigment absent. On YES, colonies with a dome-shaped centre, cottony, white, margins entire; reverse pale yellow (4A3); sporulation absent; exudates and soluble pigments absent. On DG18, colonies flat, floccose, white, margins entire; reverse greyish green (30C5) to white; sporulation absent; exudates and soluble pigments absent. On OA, colonies flat, cottony, mycelium greyish green (28B3), margins entire; reverse yellowish white (2A2); sporulation moderate, conidial masses greenish white (29A2); exudate and soluble pigments absent. On CREA, weak acid production.

Micromorphology on MEA — Conidiophores mostly monoverticillate; stipes rough-walled, somewhat pigmented, 11–15 × 2.5–3µm; metulae two to three, divergent, 12–15 × 2–2.5 µm; phialides two to four per metulae, acerose, 8–13(–20) × 2.5–3 µm; conidia smooth-walled, globose to subglobose, 2.5–4(–4.5) × 2.2–3 µm. Ascomata not observed.

Notes — *Talaromyces georgiensis* can be distinguished easily from its closely related species (i.e. *T. boninensis, T. helicus, T. reverse-olivaceus* and *T. varians*) by its profuse and improved growth at 30-37 ºC than at 25 ºC, by the acid production on CREA and by its rough-walled stipes. The maximum colony diameter reported for the species of the *T. helicus*-clade is 30 mm in 7d at 37 ºC,[7,16] while the novel species can reach 50 mm. In addition, *T. georgiensis*, as well as *T. reverse-olivaceus* and *T. varians*, does not produce the sexual morph, which is present in *T. helicus* and *T. boninensis*.[7,16]

Talaromyces minnesotensis Guevara-Suarez, Cano & D. García, sp. nov. — MycoBank MB 820463; Figure 7.

Etymology. Referring to the State of Minnesota in USA, where the fungus was isolated.

Specimen examined. USA, Minnesota, from human ear, 2010, D.A. Sutton (**holotype** CBS H-23001; ex-type cultures UTHSC DI16–144, CBS 142381, FMR 14265).

Colony diameter in 7 d (mm) — on CYA: 25 ºC 24–26, 30 °C 23–25, 37 °C no growth; on MEA: 25 ºC 13–15, 30 °C 19–21, 37 °C no growth; on YES: 25 ºC

21–24, 30 ºC 24–26, 37 °C no growth; on OA:25 ºC 19–20, 30 ºC 17–20, 37 °C no growth; DG18 25 ºC 8–10; CREA 25 ºC 9–12.

Colony characters at 25 ºC in 7 d — On CYA, colonies with a concave centre, radially sulcate to the periphery, velvety, mycelium white to pastel red (9A5), margins entire; reverse reddish brown (9D8); sporulation sparse; exudates orange (5B8) droplets only present at 30 ºC; soluble pigment reddish orange (7B8). On MEA, colonies flat, velvety, mycelium greenish grey (29C2) at the centre, yellowish white (2A3) towards the periphery, margins entire; reverse light orange (5A5); sporulation moderate, with conidial masses greyish green (27B4); exudates and soluble pigments absent. On YES, colonies flat, slightly concentrically sulcate and undulate, white; reverse brownish orange (7C7); sporulation absent; exudates with small clear droplets; soluble pigments absent. On DG18, colonies raised at centre, mycelium white to light yellow (3A4), margins entire; reverse orange (6B8); sporulation moderate; exudates and soluble pigments absent. On OA, colonies flat, velvety, mycelium olive (3D5) to pastel yellow (3A4), margins entire; reverse light yellow (4A5); sporulation abundant, with conidial masses greyish green (30D5); exudates and soluble pigments absent. On CREA, acid production strong.

Micromorphology on MEA — Conidiophores mostly biverticillate; stipes smooth-walled, 90–200 (–250) × 2–3 µm; metulae two to five, divergent, 10–15 × 2–3.5 µm; phialides three to five per metulae, acerose, 10–13(–15) × 2–3 µm; conidia smooth-walled, ellipsoidal, 2.5–3.5 × 2–3 µm. Ascomata not observed.

Notes — *Talaromyces minioluteus*, the species phylogenetically closest to *T. minnesotensis*, as mentioned before, differs in the lack of acid production on CREA and in having a more restricted growth on CYA (17 – 18 mm 7 d at 25 ºC).[7]

Talaromyces udagawae, which is placed in the same clade as *T. minioluteus* and *T. minnesotensis*, can be differentiated easily by the production of ascomata.[7]

Talaromyces rapidus Guevara-Suarez, D. García & Gené, sp. nov. — MycoBank MB 820464; Figure 8.

Etymology. Referring to the fast growth in culture.

Specimen examined. USA, Ohio, human bronchoalveolar lavage, 2011, D.A. Sutton (**holotype** CBS H-23002; ex-type cultures UTHSC DI16–148, CBS 142382, FMR 14293).

Colony diameter in 7 d (mm) — on CYA: 25 ºC 44–46, 30 °C 50–52, 37 °C 20–21; on MEA: 25 ºC 39–42, 30 °C 44–45, 37 ºC 25–27; on YES: 25 ºC 37–39, 30 ºC 45–47, 37 ºC 24–26; on OA 25 ºC 30–37, 30 ºC 40–44, 37 ºC 23–24; DG18 25 ºC 9–11; CREA 25 ºC no growth.

Colony characters at 25 ºC in 7 d — On CYA, colonies raised at centre, concentrically sulcate, floccose, mycelium greyish yellow (3B4) to light green (28B4) fading into white, margins plane, entire; reverse brownish red (9C8) centre fading to white; sporulation abundant, with conidial masses pastel green (28A4); exudates forming small red droplets; soluble pigments absent. On MEA, colonies flat, velvety, mycelium greyish green (29C6) to white, margins entire; reverse high red (10A8) to white; sporulation moderate; exudates and soluble pigments absent. On YES, colonies raised at the centre, with mycelium dark ruby (12F7) to bluish green (25C8), white towards the periphery, velvety, margins low, entire; reverse pastel red (7A4) centre fading to white; sporulation moderate; exudates violet brown droplets (11F7); soluble pigments absent. On DG18, colonies flat, floccose,

white, margins entire; reverse yellowish green (30C8); sporulation absent. On OA, colonies flat, velvety, mycelium greyish green (30E6), margins entire; reverse colourless; sporulation abundant; exudates and soluble pigments absent. On CREA, acid production absent.

Micromorphology on MEA — Conidiophores mostly biverticillate, with a minor proportion having subterminal branches; stipes smooth-walled, 80–130 × 2.5–3 µm; metulae three to five, appressed, cylindrical 13–15 × 2–3 µm; phialides three to four per metulae, acerose almost flask-shaped, 9–13 × 2–3 µm; conidia smooth-walled, ellipsoidal to somewhat fusiform, 2.5–4 × 2–2.5 µm. Ascomata not observed.

Notes — *Talaromyces rapidus* is characterized by rapid growth on practically all media and at all temperatures tested, especially at 30 ºC. Phylogenetically, it forms an independent and distant branch included in an unsupported clade together with the type species of *T. flavovirens, T. cnidii* and *T. siamensis* (Figure 1). Although the analyses of the ITS, *BenA* and *CaM* sequences (including the concatenated) did not resolve the relationship of this species with other species in this section *Talaromyces*, it did allow for the detection of this novel species. Morphologically, *T. rapidus* resembles *T cnidii*, but the latter can be distinguished by the production of a red to yellow diffusible pigment on CYA at 25 ºC, and by its conidiophores with longer stipes (up to 230 µm) bearing divergent metulae.[7] *Talaromyces rapidus* does not produce diffusible pigments in any of the culture media tested, and its conidiophores have stipes up to 130 µm long bearing appressed metulae.

Discussion

The taxonomy of *Talaromyces* was redefined recently on the basis of DNA sequence data, extrolite profiles and other phenotypic features including its morphology, resulting in a modern concept of the genus.[7] However, phylogenetic analyses of the ITS, *BenA, RPB2* and *CaM* genes are imperative for new species identification.[7] In the present study, the multigene phylogeny proposed by Yilmaz et al.[7] allowed us to recognize four new species, and to identify two recently described *Talaromyces*, i.e. *T. kabodanensis* isolated from soil and also from clinical specimens in different countries,[35] and *T. subaurantiacus* recovered from a soil sample in South Africa.[8]

Two of these new species described here belong to the section *Talaromyces*, i.e. *T. alveolaris* and *T. rapidus.* In fact, most of the *Talaromyces* from clinical samples identified in our previous study belong to that section.[13] This is the largest section in the genus and includes nearly 50 species, 13 of them described in the last year from environmental samples.[9,15,16,19] The members of the section *Talaromyces* are closely related phylogenetically, based especially on their ITS sequences, but they also show a very similar morphology. Visagie et al.[15] reported numerous misidentifications in this section, mostly attributed to the similarity of their conidiophores. However, they can be identified easily with the analysis of *BenA* sequences. Recently, Chen et al.[16] described nine new species of *Talaromyces* from indoor environments in China, three of them being assigned to the section *Talaromyces*, i.e. *T. fusiformis, T. adpressus* and *T. beijingensis*. *Talaromyces fusiformis* is closely related to *T. alveolaris* and forms a well-supported clade with *T. aurantiacus* and *T. derxii*. Interestingly, these four species

are able to grow well at human body temperature, which is an important feature when considering their potential to cause disease. It is also noteworthy that *T. aurantiacus* was one of the species found in the set of penicillium-like clinical isolates previously studied, and found from a scalp wound and canine lung tissue.[13] *Talaromyces alveolaris* and *T. rapidus* have been recovered exclusively from the human respiratory tract.

Yilmaz et al.[7] introduced the section *Helici* for seven *Talaromyces* species, mostly isolated from soil, and more recently Chen et al.[16] added two more species from indoor air. The section was characterized by species with biverticillate conidiophores, occasionally consisting of solitary phialides, with stipes generally pigmented, colony reverse on CYA yellowish brown or dark green, usually growing at 37°C, and an absence of acid production on CREA. Our new species *T. georgiensis* shares all these features except the latter one; in fact, it is the only species in the section able to produce acid on CREA. This is phylogenetically related to the species of the *T. helicus*-clade (i.e. *T. boninensis, T. helicus, T. reverse-olivaceus* and *T. varians*),[7.16] and a common feature that distinguishes all them from the other species of the section is the production of conidiophores with pigmented stipes. Although this feature was not mentioned in the despription of *T. reverse-olivaceus,* the stipes are somewhat green coloured in its photomicrographs reported.[16] *Talaromyces georgiensis* can also be identified easily at the molecular level using the ITS barcode and *BenA*, however concatenated analysis with *RPB2* supports a better distinction of species within the section. It is noteworthy that *T. georgiensis* is the first species in the section found from clinical specimens.

Talaromyces atroroseus, T. diversus, and the novel species *T. minnesotensis* are, to date, the only species in the section *Trachyspermi* isolated from clinical specimens, having been recovered from lung samples.[13] However, considering the absence or the restricted growth at 37 ºC, the pathogenic potential of these fungi in immune competent individuals is probably limited. Species in this section can be distinguished easily by their growth rates on CYA, MEA and CREA.[7,16] Also, *T. minnesotensis* is the only species that shows a strong acid production on CREA. The ITS barcode as well as *BenA*, *CaM* and *RPB2* are good molecular markers for distinguishing the species in section *Trachyspermi*.

The section *Islandici* was re-evaluated recently by Yilmaz et al.[8] and currently includes 19 species. *Talaromyces subaurantiacus,* identified in the present study from a BAL sample, was described as new in the above-mentioned study from a Fynbos soil isolate. Therefore, our strain is only the second identification of this species so far. This clinical isolate shows practically the same phenotypic features as those described in the protologue, with restricted growth on all culture media, especially on CYA, and its ability to grow at 37ºC. The combination of these phenotypic features distinguishes *T. subaurantiacus* from the other species in the section.[8] *Talaromyces columbinus* is another species of the section *Islandici* previously found from a human clinical specimen.[13] Additionally, *T. piceus* has been reported as a causal agent of fungaemia[36] and osteomyelitis,[37] *T. radicus* as the etiologic agent of a fatal infection in a dog,[38] and *T. rugulosus* as responsible for a corneal ulcer.[39] All these species differs from *T. subaurantiacus* mainly by their good growth at 40 ºC;[8,40] *T. subaurantiacus* is unable to grow at this temperature.

The present study expands the species diversity of *Talaromyces* in the clinical setting. Although the pathogenic role of the new species proposed has not been proven, *T. alveolaris*, *T. georgiensis* and *T. rapidus* demonstrate pathogenic potential by their ability to grow at human body temperature. Further studies are necessary, however, to understand both the distribution and the relevance of these new fungi in human and animal disease.

Acknowledgements

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No conflict of interest declared.

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Figure 1. Phylogenetic tree of *Talaromyces* section *Talaromyces*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (466 bp), *BenA* (404 bp) and *CaM* (502 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces dendriticus* CBS 660.80 (Section *Purpurei*). T: type strain, *: isolate identified in the present study. The new species proposed are shown in dark box.

Figure 2. Phylogenetic tree of *Talaromyces* section *Helici*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (465 bp), *BenA* (410 bp) and *RPB2* (839 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces ucrainicus* CBS 162.67 (Section *Trachyspermi*). T: type strain. The new species proposed is shown in dark box.

Figure 3. Phylogenetic tree of *Talaromyces* section *Islandici*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (462 bp), *BenA* (412 bp) *RPB2* (754 bp), and *CaM* (480 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces palmae* CBS 442.88 and *Talaromyces subinflatus* CBS 652.95 (Section *Subinflati*). T: type strain, *: isolate identified in the present study.

Figure 4. Phylogenetic tree of *Talaromyces* section *Trachyspermi*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (477 bp), *BenA*(382 bp) and *CaM* (474) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces purpurogenus* CBS 286.36 (Section *Talaromyces*). T: type strain. The new species proposed is shown in dark box.

Figure 5. Morphological characters of *Talaromyces alveolaris* (UTHSC DI16-147^T). A. Colonies from left to right (top row) CYA, MEA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. B. Colony texture on CYA at 25 ºC after 2-week incubation. C. Colony texture on CYA at 30 ºC after 1-week incubation. C–E Conidiophores. F. Conidia. Scale bars = 10 µm.

Figure 6. Morphological characters of *Talaromyces georgiensis* (UTHSC DI16-145^T). A. Colonies from left to right (top row) MEA, CYA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. B. Colony texture on MEA at 25 ºC after 1-week incubation. C. Conidia. D-E Conidiophores. Scale bars = 10 µm.

Figure 7. Morphological characters of *Talaromyces minnesotensis* (UTHSC DI16-144^T). A. Colonies from left to right (top row) MEA, CYA, YES, DG18 and CREA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and OA. B. Colony texture on MEA at 25 ºC after 1-week incubation. C. Colony texture on YES at 30 ºC after 1-week incubation. D–E. Conidiophores. F. Conidia. Scale bars = 10 µm.

Figure 8. Morphological characters of *Talaromyces rapidus* (UTHSC DI16-148^T). A. Colonies from left to right (top row) MEA, CYA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. B. Colony texture on MEA at 25 ºC after 1-week incubation. C–E Conidiophores. F. Conidia. Scale bars = 10 µm.

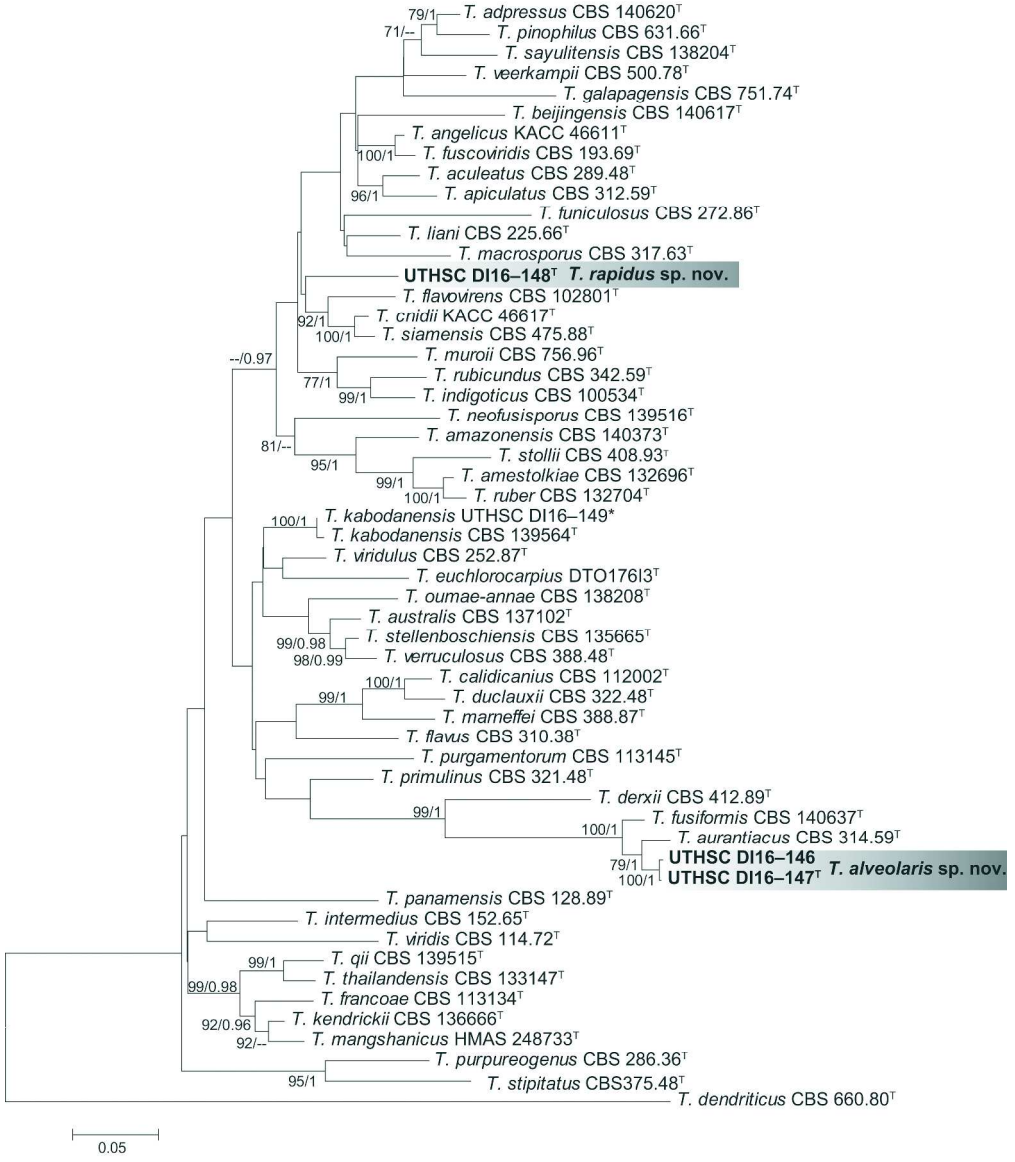
Table 1. *Talaromyces* strains used in this study.

Species	Section	Strain no.	Source	GenBank accession number			
				ITS	*BenA*	CaM	*RPB2*
T. alveolaris	*Talaromyces*	UTHSC DI16-146	BAL	LT558968	LT559085	LT795594	LT795595
		UTHSC DI16-147 = CBS 142379^{T}	BAL	LT558969	LT559086	LT795596	LT795597
T. georgiensis	*Helici*	UTHSC DI16-145 = CBS 142380^{T}	Joint-fluid animal	LT558967	LT559084	-	LT795606
T. kabodanensis	*Talaromyces*	UTHSC DI16-149	BAL	LT558971	LT559088	LT795598	LT795599
T. minnesotensis	*Trachyspermi*	UTHSC DI16-144 = CBS 142381^{T}	Ear	LT558966	LT559083	LT795604	LT795605
T. rapidus	*Talaromyces*	UTHSC DI16-148 = CBS 142382^{T}	BAL	LT558970	LT559087	LT795600	LT795601
T. subaurantiacus	*Islandici*	UTHSC DI16-143	BAL	LT558965	LT559082	LT795602	LT795603

BAL= human bronchoalveolar lavage; ^{T}= Ex-type strain.

Table 2. Overview and details used for phylogenetic analyses.

		Dataset			
		Section *Helici*	**Section *Islandici***	**Section *Talaromyces***	**Section *Trachyspermi***
ITS dataset	Length (bp)	465	462	466	477
	Phylogenetic variable sites	102	102	84	107
	Phylogenetic informative sites	41	56	47	83
	Substitution model (ML)	T92+G	T92+G+I	TN93	T92+G
BenA dataset	Length (bp)	410	412	404	382
	Phylogenetic variable sites	162	179	199	153
	Phylogenetic informative sites	104	128	136	94
	Substitution model (ML)	K2+I	K2+G	K2+G	K2+G
CaM dataset	Length (bp)	-	480	502	483
	Phylogenetic variable sites	-	235	260	236
	Phylogenetic informative sites	-	187	210	168
	Substitution model (ML)	-	K2+G	K2+G	K2+G
RPB2 dataset	Length (bp)	839	754	-	-
	Phylogenetic variable sites	264	267	-	-
	Phylogenetic informative sites	195	218	-	-
	Substitution model (ML)	K2+G	K2+G	-	-
Concatenated data set	Length (bp)	1714	2108	1372	1333
	Phylogenetic variable sites	528	743	543	496
	Phylogeneticin formative sites	340	543	393	345


79/1
71/--
T. adpressus CBS 140620T
T. pinophilus CBS 631.66T
T. sayulitensis CBS 138204T
T. veerkampii CBS 500.78T
T. galapagensis CBS 751.74T
T. beijingensis CBS 140617T
T. angelicus KACC 46611T
100/1
T. fuscoviridis CBS 193.69T
T. aculeatus CBS 289.48T
96/1
T. apiculatus CBS 312.59T
T. funiculosus CBS 272.86T
T. liani CBS 225.66T
T. macrosporus CBS 317.63T
UTHSC DI16–148T T. rapidus sp. nov.
T. flavovirens CBS 102801T
92/1
T. cnidii KACC 46617T
100/1
T. siamensis CBS 475.88T
--/0.97
T. muroii CBS 756.96T
77/1
T. rubicundus CBS 342.59T
99/1
T. indigoticus CBS 100534T
T. neofusisporus CBS 139516T
81/--
T. amazonensis CBS 140373T
95/1
T. stollii CBS 408.93T
99/1
T. amestolkiae CBS 132696T
100/1
T. ruber CBS 132704T
100/1
T. kabodanensis UTHSC DI16–149*
T. kabodanensis CBS 139564T
T. viridulus CBS 252.87T
T. euchlorocarpius DTO176I3T
T. oumae-annae CBS 138208T
T. australis CBS 137102T
99/0.98
T. stellenboschiensis CBS 135665T
98/0.99
T. verruculosus CBS 388.48T
100/1
T. calidicanius CBS 112002T
99/1
T. duclauxii CBS 322.48T
T. marneffei CBS 388.87T
T. flavus CBS 310.38T
T. purgamentorum CBS 113145T
T. primulinus CBS 321.48T
T. derxii CBS 412.89T
99/1
T. fusiformis CBS 140637T
100/1
T. aurantiacus CBS 314.59T
79/1
UTHSC DI16–146
100/1
UTHSC DI16–147T T. alveolaris sp. nov.
T. panamensis CBS 128.89T
T. intermedius CBS 152.65T
T. viridis CBS 114.72T
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T. qii CBS 139515T
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T. thailandensis CBS 133147T
T. francoae CBS 113134T
92/0.96
T. kendrickii CBS 136666T
92/--
T. mangshanicus HMAS 248733T
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T. stipitatus CBS375.48T
T. dendriticus CBS 660.80T
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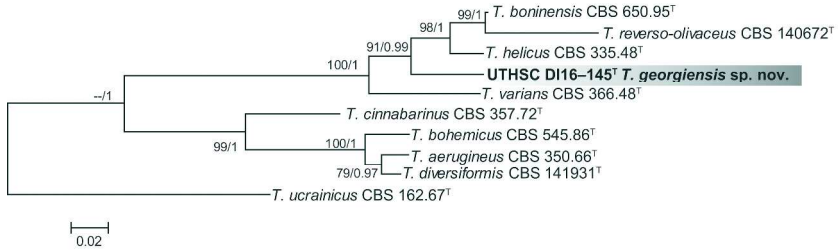

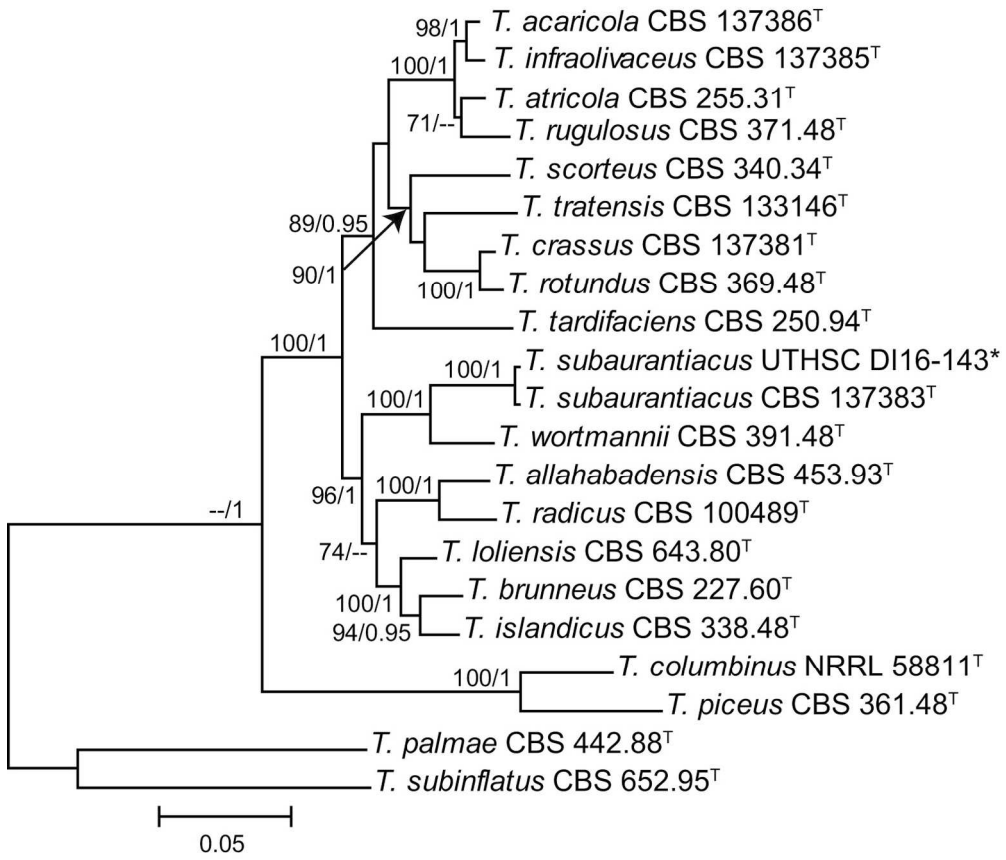
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71/--
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T. scorteus CBS 340.34T
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T. tratensis CBS 133146T
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90/1
100/1
T. rotundus CBS 369.48T
T. tardifaciens CBS 250.94T
100/1
100/1
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100/1
T. wortmannii CBS 391.48T
T. allahabadensis CBS 453.93T
96/1
100/1
--/1
T. radicus CBS 100489T
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T. brunneus CBS 227.60T
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94/0.95
T. islandicus CBS 338.48T
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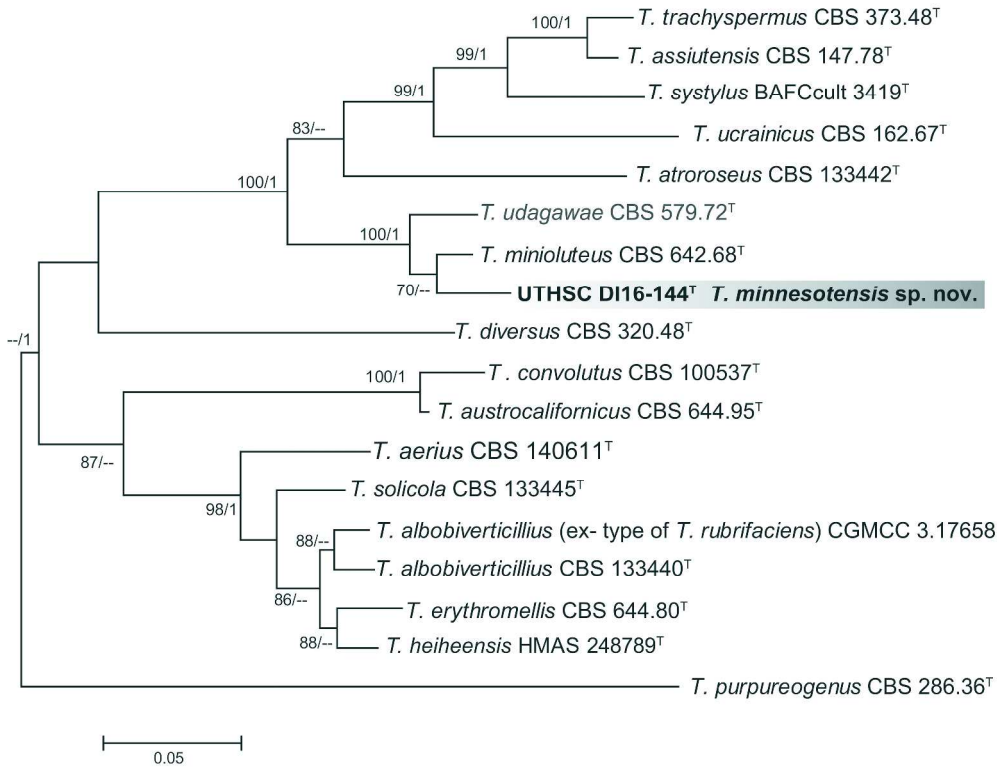
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T. ucrainicus CBS 162.67T
T. atroroseus CBS 133442T
100/1
T. udagawae CBS 579.72T
100/1
T. minioluteus CBS 642.68T
70/--
UTHSC DI16-144T T. minnesotensis sp. nov.
--/1
T. diversus CBS 320.48T
100/1
T. convolutus CBS 100537T
T. austrocalifornicus CBS 644.95T
87/--
T. aerius CBS 140611T
98/1
T. solicola CBS 133445T
88/--
T. albobiverticillius (ex- type of T. rubrifaciens) CGMCC 3.17658
T. albobiverticillius CBS 133440T
86/--
T. erythromellis CBS 644.80T
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T. heiheensis HMAS 248789T
T. purpureogenus CBS 286.36T
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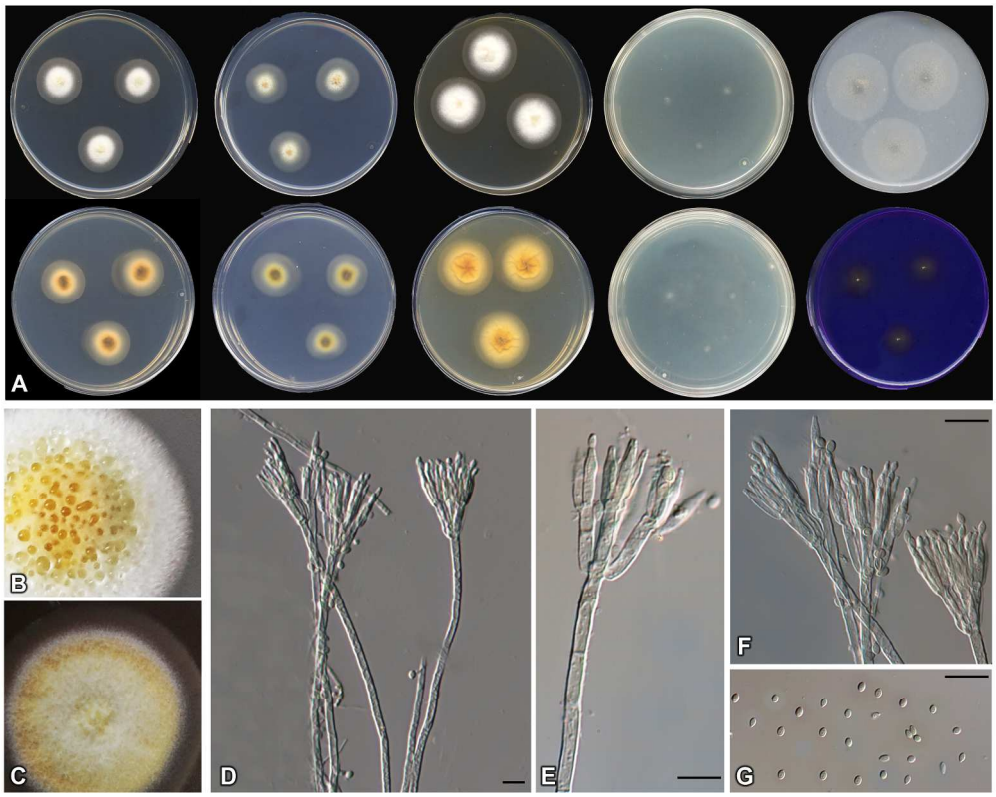

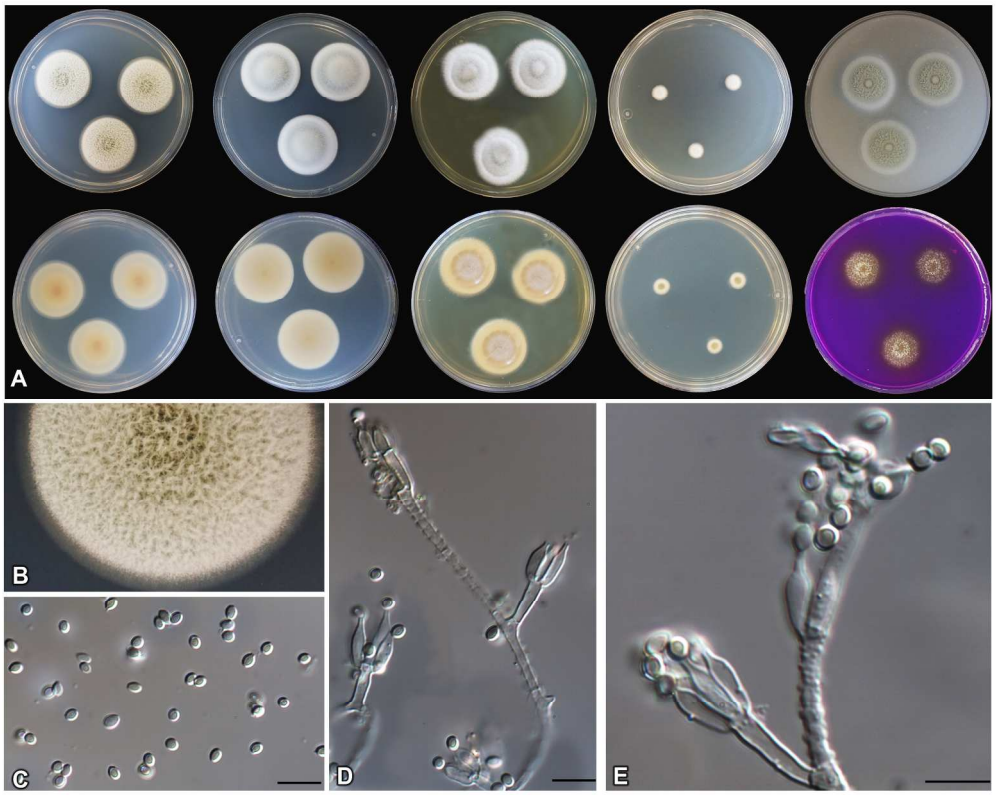

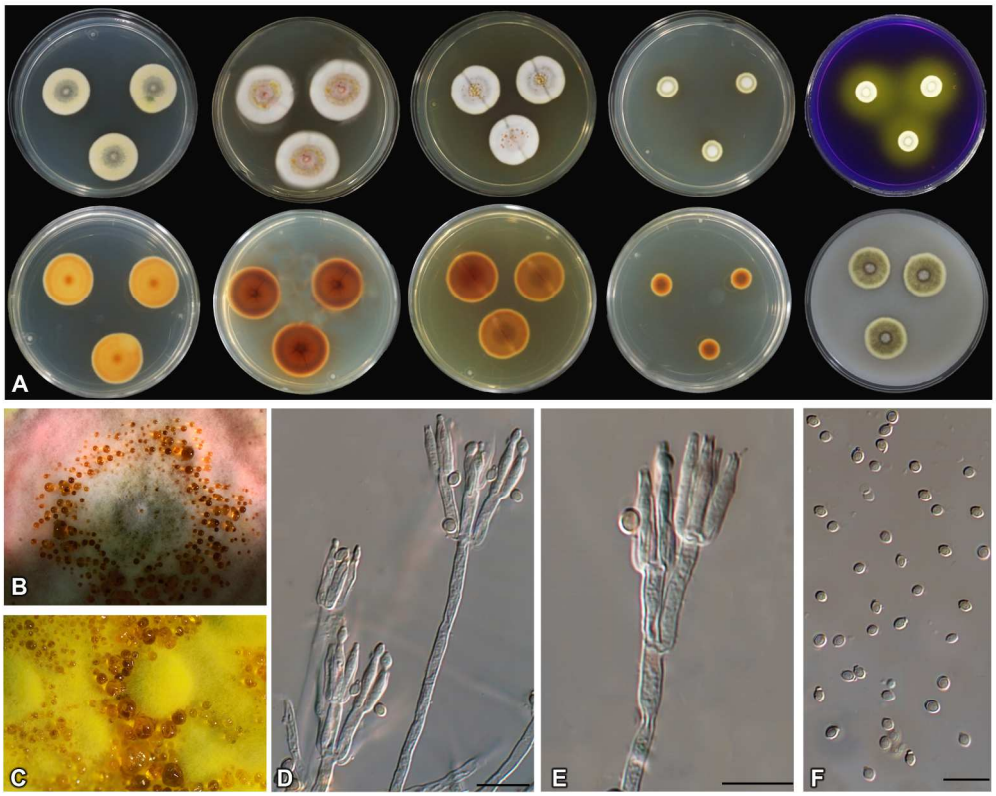

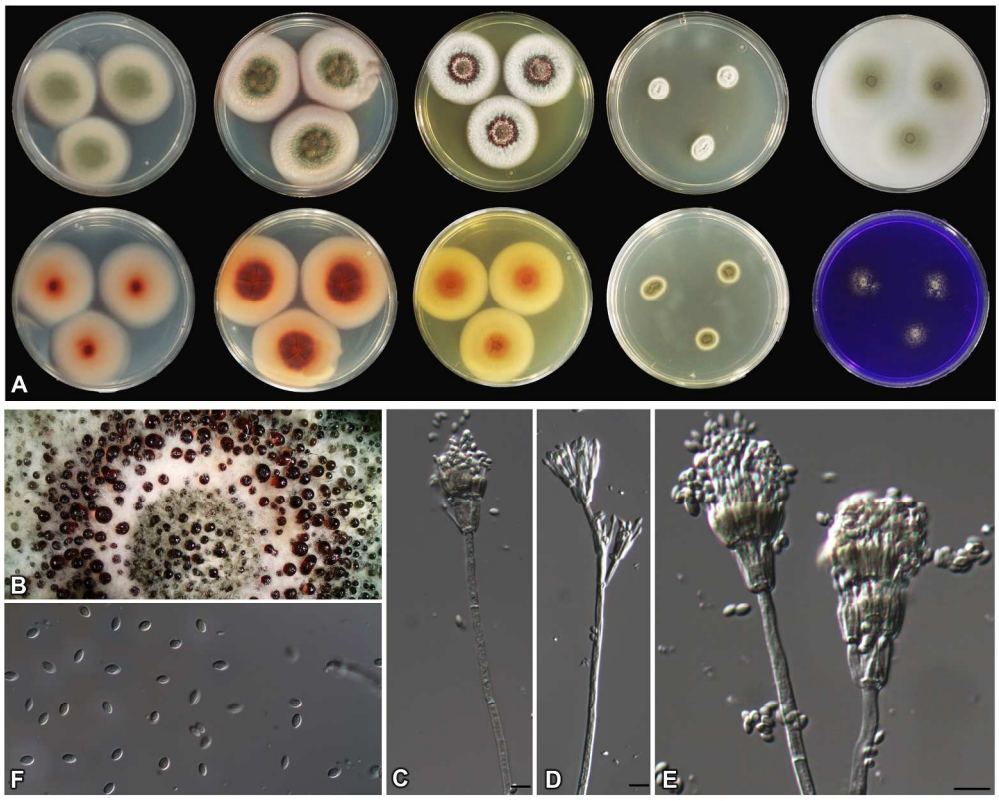

Table S1–supplemental material. GenBank accession numbers of the sequences of the *Talaromyces* species retrieved of NCBI.

Section	Species	ITS	*BenA*	*CaM*	*RPB2*
Islandici	*T. acaricola*	JX091476	JX091610	JX140729	KF984956
	T. allahabadensis	KF984873	KF984614	KF984768	KF985006
	T. atricola	KF984859	KF984566	KF984719	KF984948
	T. brunneus	JN899365	KJ865722	KJ885264	KM023272
	T. columbinus	KJ865739	KF196843	KJ885288	KM023270
	T. crassus	JX091472	JX091608	JX140727	KF984914
	T. islandicus	KF984885	KF984655	KF984780	KF985018
	T. infaolivaceus	JX091481	JX091615	JX140734	KF984949
	T. loliensis	KF984888	KF984658	KF984783	KF985021
	T. piceus	KF984792	KF984668	KF984680	KF984899
	T. radicus	KF984878	KF984599	KF984773	KF985013
	T. rotundus	JN899353	KJ865730	KJ885278	KM023275
	T. rugulosus	KF984834	KF984575	KF984702	KF984925
	T. scorteus	KF984892	KF984565	KF984684	KF984916
	T. subaurantiacus	JX091475	JX091609	JX140728	KF984960
	T. tardifaciens	JN899361	KC202954	KF984682	KF984908
	T. tratensis	KF984891	KF984559	KF984690	KF984911
	T. wortmannii	KF984829	KF984648	KF984756	KF984977
Helici	*T. aerugineus*	AY753346	KJ865736	KJ885285	JN121502
	T. bohemicus	JN899400	KJ865719	KJ885286	JN121532
	T. boninensis	JN899356	KJ865721	KJ885263	KM023276
	T. cinnabarinus	JN899376	AY753377	KJ885256	JN121477
	T. diversiformis	KX961215	KX961216	KX961259	KX961274
	T. helicus	JN899359	KJ865725	KJ885289	KM023273
	T. reverso-olivaceus	KU866646	KU866834	KU866730	KU866990
	T. varians	JN899368	KJ865731	KJ885284	KM023274
Talaromyces	*T. aculeatus*	KF741995	KF741929	KF741975	KM023271
	T. adpressus	KU866657	KU866844	KU866741	KU867001
	T. amazonensis	KX011509	KX011490	KX011502	-
	T. amestolkiae	JX315660	JX315623	KF741937	JX315698
	T. angelicus	KF183638	KF183640	KJ885259	-
	T. apiculatus	JN899375	KF741916	KF741950	KM023287
	T. aurantiacus	JN899380	KF741917	KF741951	-
	T. aurantiacus	-	LT559066	-	-
	T. aurantiacus	-	LT559067	-	-
	T. aurantiacus	-	KC992262	-	-
	T. aurantiacus	-	KX961218	-	-
	T. australis	KF741991	KF741922	KF741971	-
	T. beijingensis	KU866649	KU866837	KU866733	KU866993
	T. calidicanius	JN899319	HQ156944	KF741934	KM023311
	T. cnidii	KF183639	KF183641	KJ885266	KM023299
	T. derxii	JN899327	JX494306	KF741959	KM023282
	T. duclauxii	JN899342	JX091384	KF741955	JN121491
	T. euchlorocarpius	AB176617	KJ865733	KJ885271	KM023303
	T. flavovirens	JN899392	JX091376	KF741933	-
	T. flavus	JN899360	JX494302	KF741949	JF417426
	T. francoae	KX011510	KX011489	KX011501	-
	T. funiculosus	JN899377	JX091383	KF741945	KM023293
	T. fuscoviridis	KF741979	KF741912	KF741942	-
	T. fusiformis	KU866656	KU866843	KU866740	KU867000
	T. galapagensis	JN899358	JX091388	KF741966	-

Talaromyces (cont.)	*T. indigoticus*	JN899331	JX494308	KF741931	-
	T. intermedius	JN899332	JX091387	KJ885290	-
	T. kabodanensis	KP851981	KP851986	KP851995	-
	T. kendrickii	KF741987	KF741921	KF741967	-
	T. liani	JN899395	JX091380	KJ885257	-
	T. macrosporus	JN899333	JX091382	KF741952	KM023292
	T. mangshanicus	KX447531	KX447530	KX447528	KX447527
	T. marneffei	JN899344	JX091389	KF741958	KM023283
	T. muroii	JN899351	KJ865727	KJ885274	-
	T. neofusisporus	KP765385	KP765381	KP765383	-
	T. oumae-annae	KJ775720	KJ775213	KJ775425	-
	T. panamensis	JN899362	HQ156948	KF741936	KM023284
	T. pinophilus	JN899382	JX091381	KF741964	KM023291
	T. primulinus	JN899317	JX494305	KF741954	KM023294
	T. purgamentorum	KX011504	KX011487	KX011500	-
	T. purpurogenus	JN899372	JX315639	KF741947	JX315709
	T. qii	KP765384	KP765380	KP765382	-
	T. ruber	JX315662	JX315629	KF741938	JX315700
	T. rubicundus	JN899384	JX494309	KF741956	KM023296
	T. sayulitensis	KJ775713	KJ775206	KJ775422	-
	T. siamensis	JN899385	JX091379	KF741960	KM023279
	T. stellenboschiensis	JX091471	JX091605	JX140683	-
	T. stipitatus	JN899348	KM111288	KF741957	KM022380
	T. stollii	JX315674	JX315633	JX315646	JX315712
	T. thailandensis	JX898041	JX494294	KF741940	KM023307
	T. veerkampii	KF741984	KF741918	KF741961	-
	T. verruculosus	KF741994	KF741928	KF741944	KM023306
	T. viridis	AF285782	JX494310	KF741935	JN121430
	T. viridulus	JN899314	JX091385	KF741943	JF417422
Trachyspermi	*T. aerius*	KU866647	KU866835	KU866731	KU866991
	T. albobiverticillius	HQ605705	KF114778	KJ885258	KM023310
	T. albobiverticillius (ex-type of *T. rubrifaciens*)	KR855658	KR855648	KR855653	KR855663
	T. assiutensis	JN899323	KJ865720	KJ885260	KM023305
	T. atroroseus	KF114747	KF114789	KJ775418	KM023288
	T. austrocalifornicus	JN899357	KJ865732	KJ885261	-
	T. convolutus	JN899330	KF114773	-	JN121414
	T. diversus	KJ865740	KJ865723	KJ885268	KM023285
	T. erythromellis	JN899383	HQ156945	KJ885270	KM023290
	T. minioluteus	JN899346	KF114799	KJ885273	JF417443
	T. minioluteus	-	KU516404	-	-
	T. minioluteus	-	KU516403	-	-
	T. minioluteus	-	KU516402	-	-
	T. minioluteus	-	KU516401	-	-
	T. minioluteus	-	KU516400	-	-
	T. minioluteus	-	KP330046	-	-
	T. minioluteus	-	KP330045	-	-
	T. minioluteus	-	KP330044	-	-
	T. minioluteus	-	KJ775226	-	-
	T. minioluteus	-	KJ775221	-	-
	T. minioluteus	-	KJ775215	-	-
	T. minioluteus	-	KJ775214	-	-
	T. minioluteus	-	KJ775197	-	-
	T. minioluteus (ex-type of *P. purpurogenum* var. *rubrisclerotium*	-	KM066129	-	-

	T. minioluteus (ex-type of *P. samsonii*)	-	KF114798	-	-
	T. solicola	FJ160264	GU385731	KJ885279	KM023295
	T. systylus	KP026917	KR233838	KR233837	-
	T. trachyspermus	JN899354	KF114803	KJ885281	JF417432
	T. ucrainicus	JN899394	KF114771	KJ885282	KM023289
	T. udagawae	JN899350	KF114796	KX961260	-

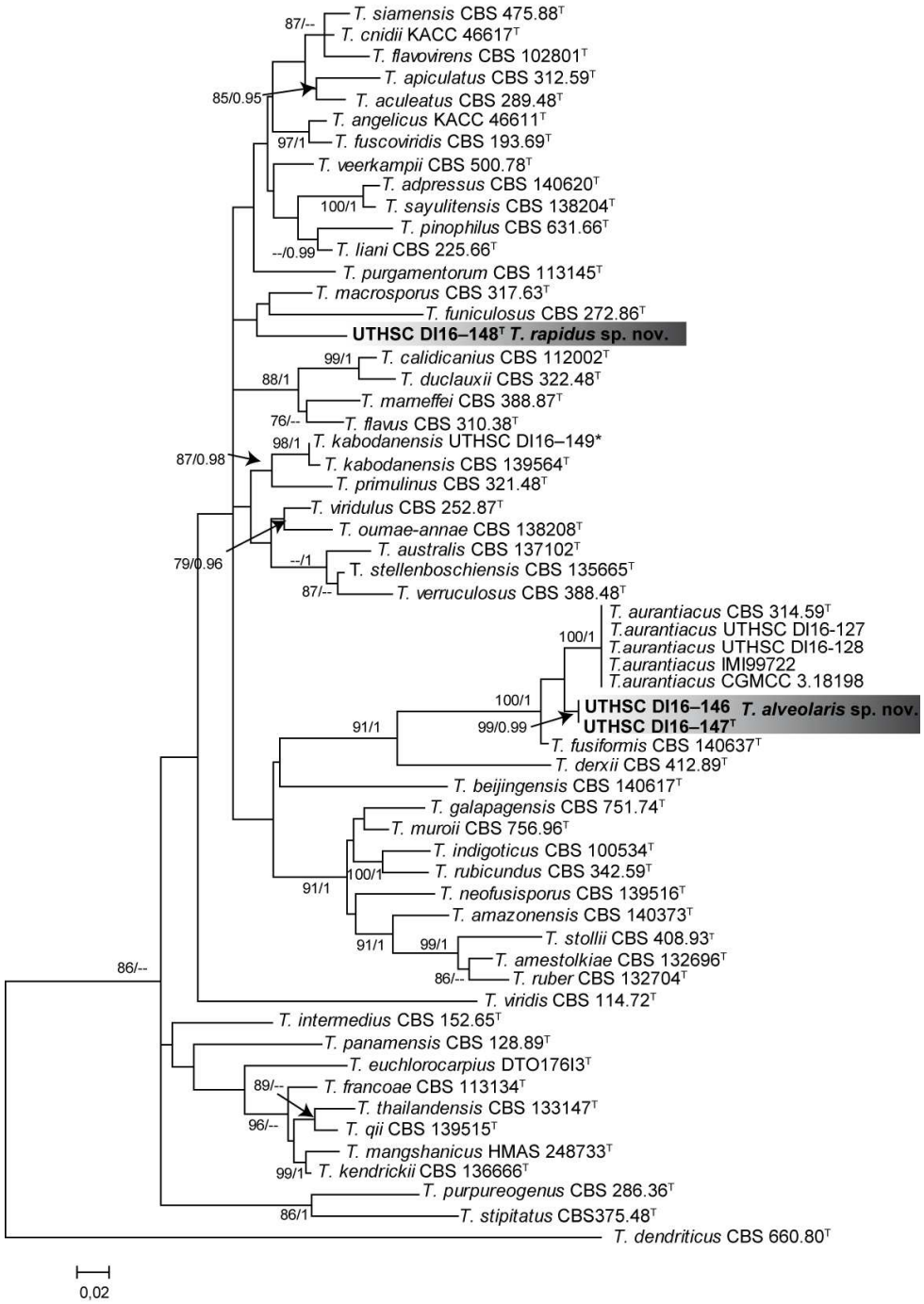

Figure S1. Phylogenetic tree of *Talaromyces* section *Talaromyces*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with *BenA* (404 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces dendriticus* CBS 660.80 (Section *Purpurei*). T: type strain, *: UTHSC isolate identified as *T. kabodanensis*. The new species proposed are shown in dark box.

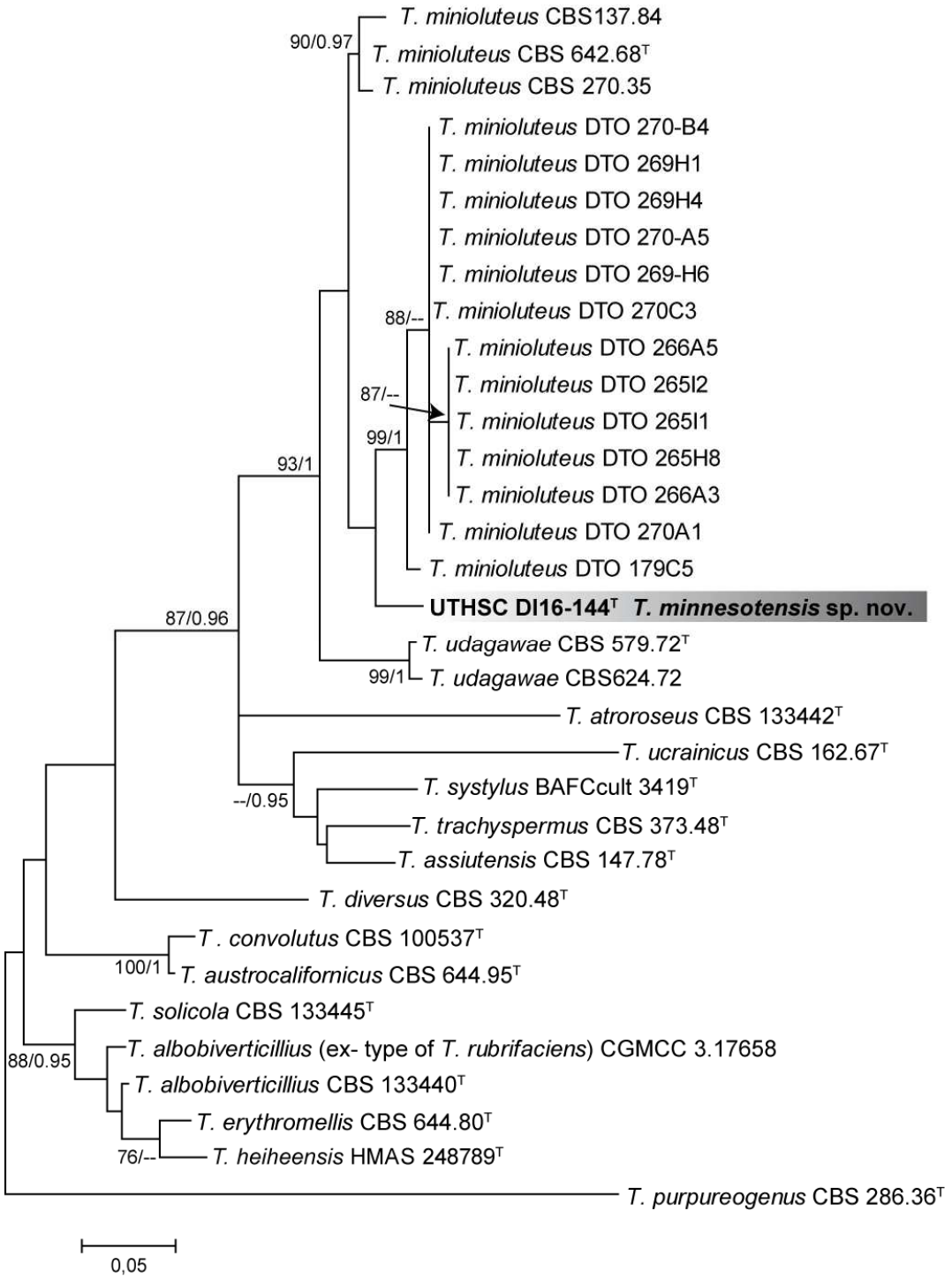

Figure S2. Phylogenetic tree of *Talaromyces* section *Trachyspermi*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the *BenA* (386 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces purpurogenus* CBS 286.36

(Section *Talaromyces*). T: type strain. The new species proposed is shown in dark box.