

Two New Teleomorphic *Talaromyces* Species Discovered From The Estuary Soil In China

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Research

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Abstract

Two new species producing ascospores of *Talaromyces* sect. *Talaromyces* are proposed, namely, *T. haitouensis* (ex-type AS3.160101^T) and *T. zhenhaiensis* (ex-type AS3.16102^T). Morphologically, *T. haitouensis* is featured by moderate growth, green-yellow gymnothecia, orange-brown mycelium, and echinulate ellipsoidal ascospores. *T. zhenhaiensis* is characterized by fast growth, absent sporulation, cream yellow to naphthalene yellow gymnothecia and mycelium, smooth-walled ellipsoidal ascospores with one equatorial ridge. The proposal of the two new taxa is well supported by individual phylogenetic analyses based on individual sequences of *BenA*, *CaM*, *Rpb2* and ITS1-5.8S-ITS2, and the concatenated *BenA-CaM-Rpb2* sequence.

Introduction

Originally, the genus *Talaromyces* C. R. Benjam. was established to accommodate the teleomorphic species producing gymnothecial ascocarps (Benjamin, 1955), but these species had long been studied in the taxonomic schemes of *Penicillium* (e.g., Raper and Thom, 1949; Pitt, 1979). However, the morphological, ecological and molecular characters have implied the common intrinsic properties of the species in *Talaromyces* and Subgen. *Biverticillium* of Pitt different from those of other species in *Penicillium*, and thus recently *Talaromyces* was regarded as the valid genus for these species irrespective of teleomorphs or anamorphs (e.g., Malloch 1985; LoBuglio et al., 1993; Berbe et al., 1995; Wang and Zhuang, 2007; Houbraken and Samson, 2011; Samson et al. 2011; McNeill et al., 2012). In the study of Samson et al. (2011), 71 species were listed in the genus *Talaromyces*. Houbraken et al. (2012) established the genus *Rasamsonia* Houbraken & Frisvad to segregate the thermotolerant and thermophilic species from among *Talaromyces* and *Geosmithia* species. Yilmaz et al. (2014) recognized 88 species and divided *Talaromyces* into 7 sections, namely, sections *Talaromyces*, *Helici*, *Purpurei*, *Trachyspermi*, *Bacillispori*, *Subinflati* and *Islandici*. Sun et al. (2020) added a new section, i.e., sect. *Tenues*. Until 2021, about 175 species has been reported in *Talaromyces*, and as the largest section of the genus, sect. *Talaromyces* encompassed about 76 taxa (Houbraken et al., 2020; Wei et al., 2021).

In this paper, we propose two new species showing teleomorphic states in sect. *Talaromyces*, namely, *T. haitouensis* sp. nov. and *T. zhenhaiensis* sp. nov., using the integration of morphological and molecular phylogenetic approaches.

Materials And Methods

Cultures

The *Talaromyces* cultures were isolated from soil samples by using the improved dilution plating method of Malloch (1981) with 0.1% agar water solution instead of water. Four distinctive isolates were encountered and deposited in the China General Microbiological Culture Collection (CGMCC) as HR1-7 = AS3.16101; ZH3-18 = AS3.16102; WYZ25-10 = AS3.16103; NN72335 = AS3.15693.

Morphological studies

Colony characters were examined in line with the methods and media proposed by Raper and Thom (1949, Czapek agar, Cz), Pitt (1979, Czapek yeast autolysate agar, CYA, yeast extract (Oxoid), Samson et al. (2010, 5% malt extract agar, MEA, malt extract (Oxoid); Oatmeal agar, OA), and Frisvad (1981, yeast extract sucrose agar, YES, yeast extract (Oxoid). The names of colours were referenced to Ridgway (1912). The materials for wet mounts were picked from the colonies on MEA and mounted in 85% lactic acid solution without staining. The microscopic characters were examined and photographed with an Axioplan2 imaging and Axiophot2 universal Microscope (Carl Zeiss Shanghai Co. Ltd.).

Molecular studies

Genomic DNA extraction was conducted according to Wang and Zhuang (2004). The partial β -tubulin gene (*BenA*) sequences were Polymerase chain reactions (PCR) amplified with primers Bt2a or I2 and Bt2b (Glass and Donaldson 1995; Wang and Wang 2013), the partial calmodulin gene (*CaM*) sequences were obtained with the primers AD1, AD2 and Q1, Q2 (Wang 2012); the partial DNA-dependent RNA polymerase II second largest subunit gene (*Rpb2*) sequences were amplified with primers T1, T2 and E1, E2 (Jiang et al. 2018), and the rDNA ITS1-5.8S-ITS2 (ITS) sequences were obtained with the primers ITS5 and ITS4 (White et al. 1990). PCR parameters were set according to Wei et al. (2021). The amplicons of the anticipated length were purified and sequenced in double direction with an ABI 3700 DNA analyzer (Applied Biosystems, Waltham, Massachusetts, USA), the obtained raw sequences were proofread manually with Bioedit 5.0.9 (Hall, 1999), and the edited sequences were deposited in GenBank. Fifty-five strains of 49 taxa of sect *Talaromyces* were included in the individual analysis of *BenA*, *CaM*, *Rpb2*, ITS sequences, and the concatenated *BenA-CaM-Rpb2* sequences, with *T. dendriticus* CBS 660.80 of sect. *Purpurei* as the outgroup. The five sequence matrices were aligned and trimmed with muscle implemented in MEGA version 6 to generate sequence matrices, then analyzed with Maximum Likelihood (ML) method and subjected to 1000 bootstrap replications (Tamura et al., 2013), with substitution model and rates among sites set as K2+G for *BenA*, K2+G+I for *CaM*, K2+G+I for *Rpb2*, T92+G+I for ITS and K2+G+I for *BenA-CaM-Rpb2*. Gaps were treated as partial deletion according to Hall (2013).

Results

PCR amplification generated amplicons of *BenA* about 420 bp or 660 bp, *CaM* about 650 bp, *Rpb2* about 825 bp, and ITS about 560 bp. The trimmed alignments of *BenA*, *CaM*, *Rpb2*, ITS and the combined *BenA-CaM-Rpb2* sequences were 371, 521, 670, 456 and 1562 characters with gaps, respectively.

The phylogenetic trees generated by either the concatenated *BenA-CaM-Rpb2* sequences or the four individual loci show three isolates (AS3.16101; AS3.16102 and AS3.15693) as two distinct species of Sect. *Talaromyces* (Figs. 1, S1–4). One proposed new species, namely, *T. haitouensis* sp. nov. (ex-type AS3.16101^T), together with *T. aspriconidius* and *T. flavus* forms one clade with 79% and 72% bootstrap support in *BenA-CaM-Rpb2* and *BenA* phylograms, respectively. In *CaM* phylogram, *T. haitouensis* and *T.*

flavus are close related with 99% bootstrap support, but *T. aspriconidius* is not in the same clade with them. While in *Rpb2* phylogram, *T. haitouensis* and *T. aspriconidius* form one clade but without bootstrap support, and *T. flavus* forms a separate clade. However the ITS phylogram does not support the close relationship of these three species. Another new species, *T. zhenhaiensis* sp. nov. (ex-type AS3.16102^T), together with *T. stipitatus* and *T. purpureogenus* is consistently clustered in one clade with strong bootstrap support according to *BenA-CaM-Rpb2*, *BenA*, *CaM*, *Rpb2* and ITS sequences.

TAXONOMY

Talaromyces haitouensis L. Wang, sp. nov. — *Fungal Names* FN570868; (Fig. 2)

Etymology. — *haitouensis* (Lat.): named after the locale where the ex-type strain was isolated.

Diagnosis: *T. haitouensis* is characterized by normal growth at 25°C and 37°C, green-yellow gymnothecia, orange-brown mycelium, and echinulate broadly ellipsoidal ascospores.

Type. China: *Jiangsu:* Lianyungang, Haitou Town, Haizhouwan Resort, 34° 55' 57"N, 119° 11' 56"E, 3 m, HMAS 350335, dried culture of ex-type AS3.16101 on MEA, the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; ex type culture AS3.16101^T = HR1-7 from the riverside soil, 5 May 2021, X-Y. Liu; GenBank: ITS = MZ045695, *BenA* = MZ054634, *CaM* = MZ054637, *Rpb2* = MZ054631.

Description: On **CYA** at 25°C after 7 d: Colonies 22–25 mm diam, thin, radially plicate slightly, margins submerged, fimbriate; velutinous and granular due to gymnothecia; gymnothecia limited, Pinard Yellow (R. Pl. IV); sporulation absent or sparse; mycelium Mars Yellow (R. Pl. III) mingled with Orange Vinaceous (R. Pl. XXVII); no exudate and soluble pigment; reverse Morocco Red (R. Pl. I), while Strawberry Pink (R. Pl. I) at margins. On **MEA** at 25°C after 7 d: Colonies 48–51 mm diam, thin, plane, margins submerged, regular; granular due to gymnothecia; gymnothecia abundant, Green Yellow (R. Pl. V); sporulation absent or limited, Olive-Gray (R. Pl. LI); mycelium Green Yellow (R. Pl. V) while white at margins; no exudate, no soluble pigment; reverse Light Danube Green (R. Pl. XXXII) centrally while Cinnamon Buff (R. Pl. XXIX) at peripheries. On **YES** at 25°C after 7 d: Colonies 25–28 mm diam, thin, radially sulcate, margins on agar surface, fimbriate; velutinous and granular due to limited gymnothecia at centres; gymnothecia Pinard Yellow (R. Pl. IV); sporulation absent or sparse, Light Olive Gray (R. Pl. LI); mycelium Pecan Brown to Cacao Brown (R. Pl. XXVIII), while white at margins; no exudate and soluble pigment; reverse Morocco Red (R. Pl. I), while Moral Red (R. Pl. I) at margins. On **OA** at 25°C after 7 d: Colonies 33–35 mm diam, thin, plane, mostly submerged and sparsely granular due to gymnothecia; gymnothecia limited in centres, Light Greenish Yellow (R. Pl. IV); sporulation absent; no exudate and soluble pigment; reverse Sulphur Yellow (R. Pl. V). On **Cz** at 25°C after 7 d: Colonies 14–17 mm diam, slightly thick, umbonate centrally, plane, margins submerged, fimbriate; velutinous and granular due to gymnothecia; gymnothecia limited, Pinard Yellow (R. Pl. IV); sporulation absent; mycelium Orange-Vinaceous to Pale Vinaceous (R. Pl. XXVII), while white at margins; exudate limited, Strawberry Pink (R. Pl. I); soluble pigment absent or limited, light red; reverse Nopal Red (R. Pl. I). On **CYA** at **37°C** after 7 d: Colonies 18–20 mm diam, slightly thick,

irregularly plicate slightly, margins on agar surface, regular; sporulation absent; mycelium Shell Pink (R. Pl. XXVIII); no exudates and soluble pigment; reverse Ochraceous-Salmon (R. Pl. XV). On CYA at **5°C** after 7 d: No growth.

Gymnothecia globose, (400–) 450–480 µm, asci globose, 12.5–13 µm, and ellipsoidal, 15 × 12–13 µm, maturing after 14 d; ascospores broadly ellipsoidal, 5–6 × 4 µm, echinulate. Conidiophores from aerial hyphae, 20–60 × 2–2.5 µm, smooth-walled, penicilli biverticillate, metulae 2–4, 10–13 × 2–2.5 µm, phialides 2–4, 9–12 × 2–2.5 µm, conidia pyriform to ellipsoidal, 2.5–3 × 2–2.5 smooth-walled.

Talaromyces zhenhaiensis L. Wang sp. nov. — *Fungal Names* FN570869; (Fig. 3)

Etymology. —*zhenhaiensis* (Lat.): named after the locale where the ex-type strain was isolated.

Diagnosis. *T. zhenhaiensis* is characterized by fast growth at 25°C and 37°C, naphthalene-yellow gymnothecia and mycelium, and smooth-walled ellipsoidal ascospores with one equatorial ridge.

Type. China: *Zhejiang:* Ningbo, Zhenhai, 29°58'19"N 121°48'19"E, 8 m, HMAS 350336, dried culture of ex-type AS3.16102 on MEA, the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; ex type culture AS3.16102^T = ZH3-18 from mudflat soil, 10 Sep 2019, *F-H Song*; GenBank: ITS = MZ045697, *BenA* = MZ054636, *CaM* = MZ054639, *Rpb2* = MZ054633.

Description. On **CYA** at 25°C after 7 d: Colonies 65–67 mm diam, plane, thin, margins submerged, fimbriate, umbonate centrally; velutinous, overlaid with sparse floccose mycelium; sporulation absent; mycelium Sulphur Yellow (R. Pl. V) to Cartridge Buff (R. Pl. XXX), while white at margins; no exudate; no soluble pigment; reverse Honey Yellow (R. Pl. XXX). On **MEA** at 25°C after 7 d: Colonies 43–45 mm diam, thin, plane, margins on agar surface, fimbriate; velutinous; sporulation absent, mycelium pale yellow, near Massicot Yellow (R. Pl. IV), while white at margins; immature gymnothecia abundant, white to Massicot Yellow (R. Pl. IV); no exudate, no soluble pigment; reverse Pale Orange Yellow (R. Pl. XXX). On **YES** at 25°C after 7 d: Colonies 61–62 mm diam, thin, irregularly sulcate, margins on agar surface, fimbriate; velutinous with sparse floccose mycelium overlaid; no sporulation; mycelium Cartridge Buff (R. Pl. XXX) to Naphthalene Yellow (R. Pl. XVI), white at margins; no exudate and soluble pigment; reverse Raw Sienna (R. Pl. III). On **OA** at 25°C after 7 d: Colonies 37–40 mm diam, thin, plane, margins on agar surface, regular; granular due to abundant gymnothecia, cream yellow to Naphthalene Yellow (R. Pl. XVI); no sporulation; mycelium white; no exudate and soluble pigment; reverse Sulphur Yellow (R. Pl. V). On **Cz** at 25°C after 7 d: Colonies 38–40 mm diam, plane, thin, margins submerged, fimbriate; velutinous; sporulation absent; mycelium pale yellow, near Martius Yellow (R. Pl. IV) to Marguerite Yellow (R. Pl. XXX), while white at margins; immature gymnothecia abundant, white to Marguerite Yellow (R. Pl. XXX); no exudate and soluble pigment; reverse Aniline Yellow (R. Pl. IV). On CYA at **37°C** after 7 d: Colonies 56–60 mm diam, thin, plane, margins on agar surface, regular; velutinous; no sporulation; mycelium white; no exudate and soluble pigment; reverse August Brown (R. Pl. III). On CYA at **5°C** after 7 d: No growth.

Gymnothecia globose, 300–350 µm, asci globose to subglobose, 8–10 µm, borne in short chains, maturing after 14 d; ascospores flattened ellipsoidal, 4–5 × 3 µm, smooth-walled, with one equatorial ridge.

Additional strains examined. China: Hainan, Ledong, Mount Jianfengling, 18°42'36"N 108°49'48"E, 800 m; culture AS3.15693 = NN072335 from soil, 8 Nov 2015, X-Z. Jiang, GenBank: ITS = KY007094, *BenA* = KY007110, *CaM* = KY007102, *Rpb2* = KY112592.

Discussion

The teleomorphs of sect. *Talaromyces* species, when present, usually produce broadly ellipsoidal ascospores with echinulate walls, such as *T. flavus*, *T. liani* and *T. aureolinus*, etc., while some species bears ellipsoidal ascospores with one or several ridges, such as *T. stipitatus* and *T. viridis* (Yilmaz et al., 2014; Wei et al., 2021).

One new taxon proposed here, namely, *T. haitouensis* produces broadly ellipsoidal ascospores with spiny walls. The phylograms inferred from the combined *BenA-CaM-Rpb2*, *BenA* and *CaM* sequences show that it is closely related to *T. flavus* but the phylograms from *Rpb2* and ITS do not (Fig. 1, Figs. S1–4). In micro-morphology, the initials of *T. haitouensis* consist of irregularly swollen cells while those of *T. flavus* comprise the ascogonia encircled with antheridia, and *T. haitouensis* produces much larger gymnothecia, asci and ascospores than those of *T. flavus* (400–480 µm vs. 150–400 µm, 12.5–13 µm or 15 × 12–13 µm vs. 9.5–13.5 × 8–11.5 µm, 5–6 × 4 µm vs. 4–5.5 × 3–3.5 µm, respectively). With respect to colony characters, though they both produce green-yellow ascomata and yellowish to reddish mycelium and absent or sparse sporulation, the new species grows commonly faster than *T. flavus* at 25°C (CYA: 22–25 mm vs. 9–10 mm, MEA: 48–51 mm vs. 31–32 mm, YES: 25–28 mm vs. 24–26 mm, OA: 33–35 mm vs. 30–32 mm) (Yilmaz et al., 2014). *T. aureolinus* also has initials consisting of swollen cells, globose asci and echinulate, broadly ellipsoidal ascospores in similar size, and similar growth rate at 25°C with *T. haitouensis*, but *T. aureolinus* does not grow at 37°C and forms normal colonies on OA, while *T. haitouensis* shows normal growth at 37°C and sparse colonies on OA. Moreover, *T. aureolinus* commonly produces lemon-yellow mycelium on all culture media and much longer stipes (300–450 µm) from surface and substratum but *T. haitouensis* produces orange-brown mycelium on CYA and YES and much shorter stipes (20–60 µm) from aerial hyphae (Wei et al., 2021).

Another new taxon, i.e., *T. zhenhaiensis* forms a constant clade with *T. stipitatus* in all the phylograms inferred from individual genes and the concatenated *BenA-CaM-Rpb2* sequences with strong bootstrap support (Fig. 1, Figs. S1–4). The morphological differences between them are subtle, but are still tangible, for instance, *T. zhenhaiensis* grows much faster than *T. stipitatus* at 25°C (CYA: 65–67 mm vs. 32–38 mm, YES: 61–62 mm vs. 40–45 mm, OA: 37–40 mm vs. 30–35 mm) and at 37°C (56–60 mm vs. 28–32 mm), and the colour of the mycelium and gymnothecia of the new species is near cream yellow to Naphthalene Yellow, which is lighter than that of *T. stipitatus* which is bright yellow with a greenish tint slightly. Microscopically, though the shapes and dimensions of gymnothecia, asci and ascospores of the

two species are similar, the asci of *T. zhenhaiensis* are larger than those of *T. stipitatus* (asci: 8–10 µm vs. 6–8 µm) (Raper and Thom, 1949; Yilmaz et al., 2014).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Long Wang designed the research. Pei-Jie Han, Jian-Qiu Sun performed experiments and analyzed data. Long Wang wrote and revised the manuscript. The authors read and approved the final manuscript.

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Figures

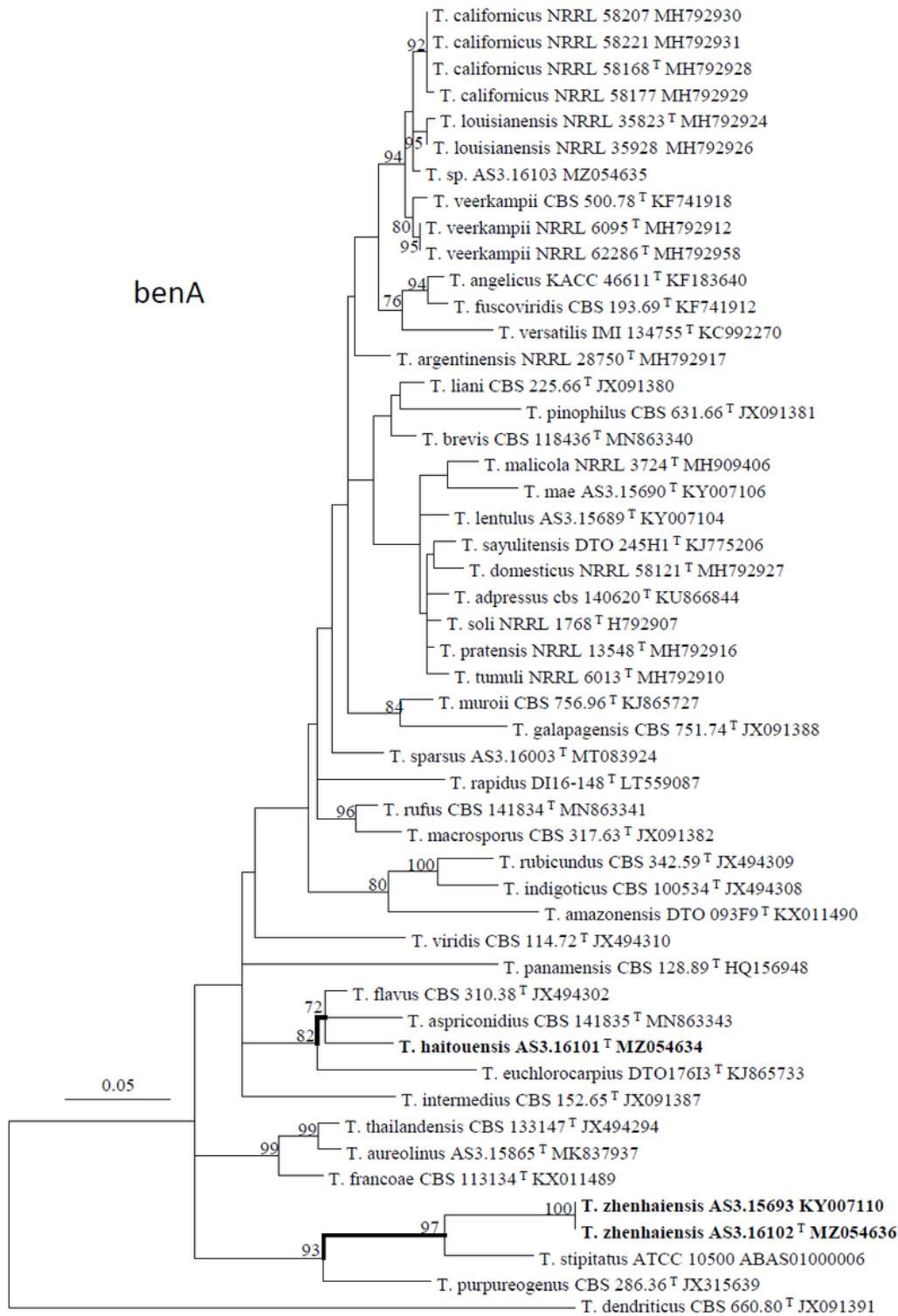


Figure 1

ML phylogram inferred from the concatenated BenA-CaM-Rpb2 sequences. Bootstrap percentages over 70% derived from 1000 replicates are indicated at the nodes. Bar = 0.05 substitutions per nucleotide position.

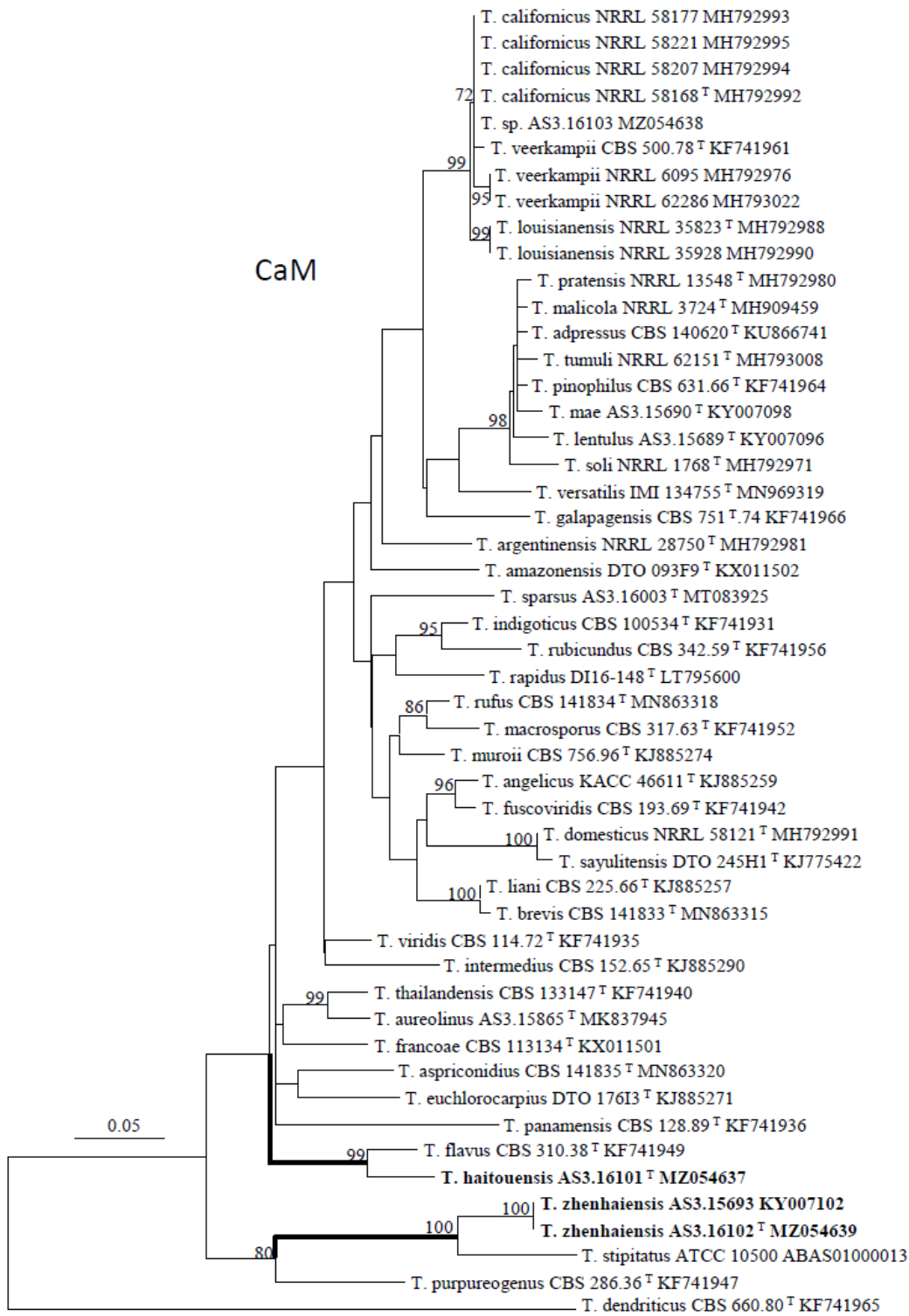


Figure 2

Morphological characters of *T. haitouensis* AS3.1 T incubated at 25 °C for 7 days. a on CYA. b on MEA. c on YES. d on OA. e Initials. f Asci. g, h Ascospores. i Conidiophores. j Conidia. Bar = 10 µm.

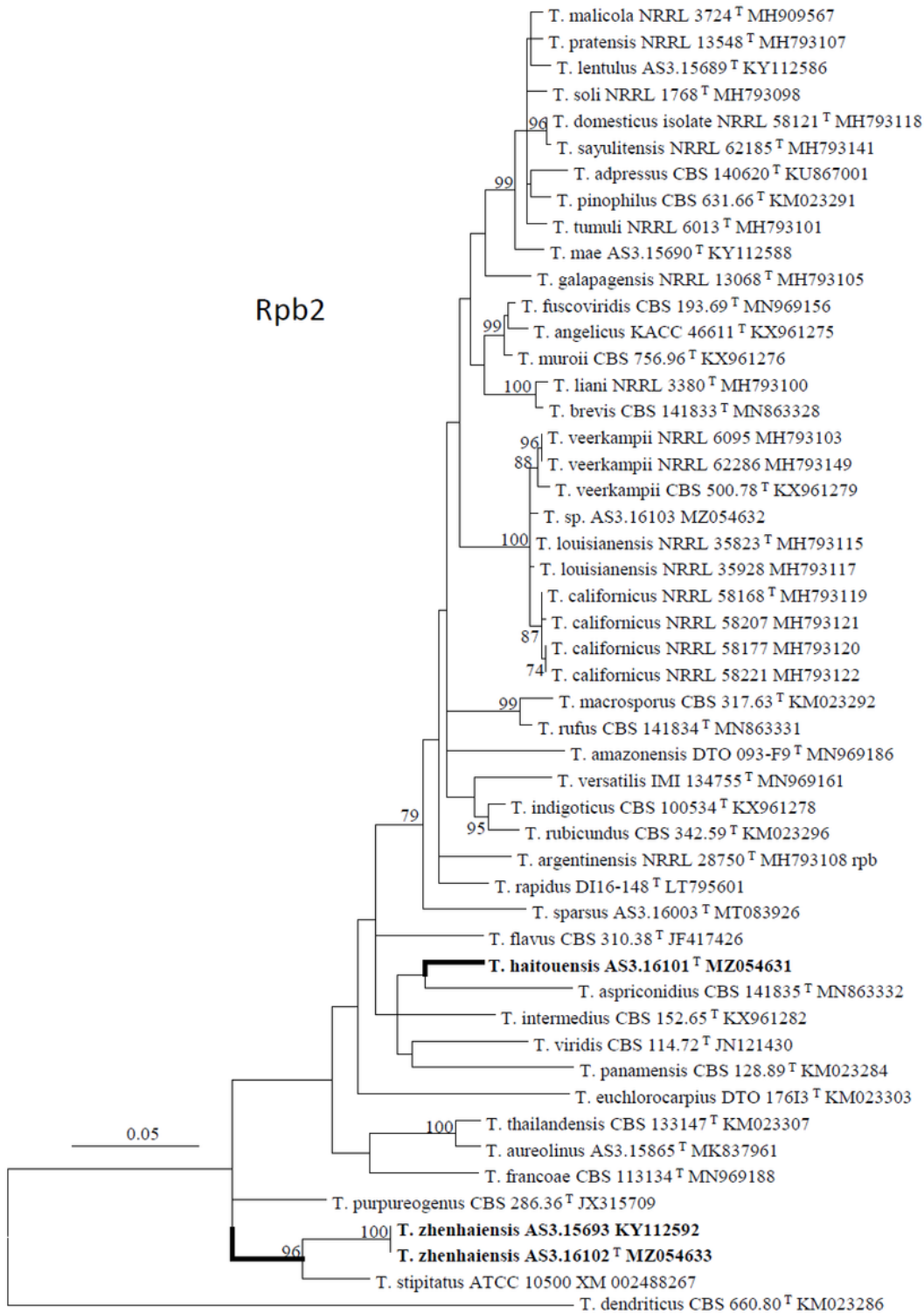


Figure 3

Morphological characters of *T. zhenhaiensis* AS3.1 T incubated at 25 °C for 7 days. a on CYA. b on MEA. c on YES. d on OA. e, g Asci and ascospores. f, h Ascospores. Bar = 10 µm.

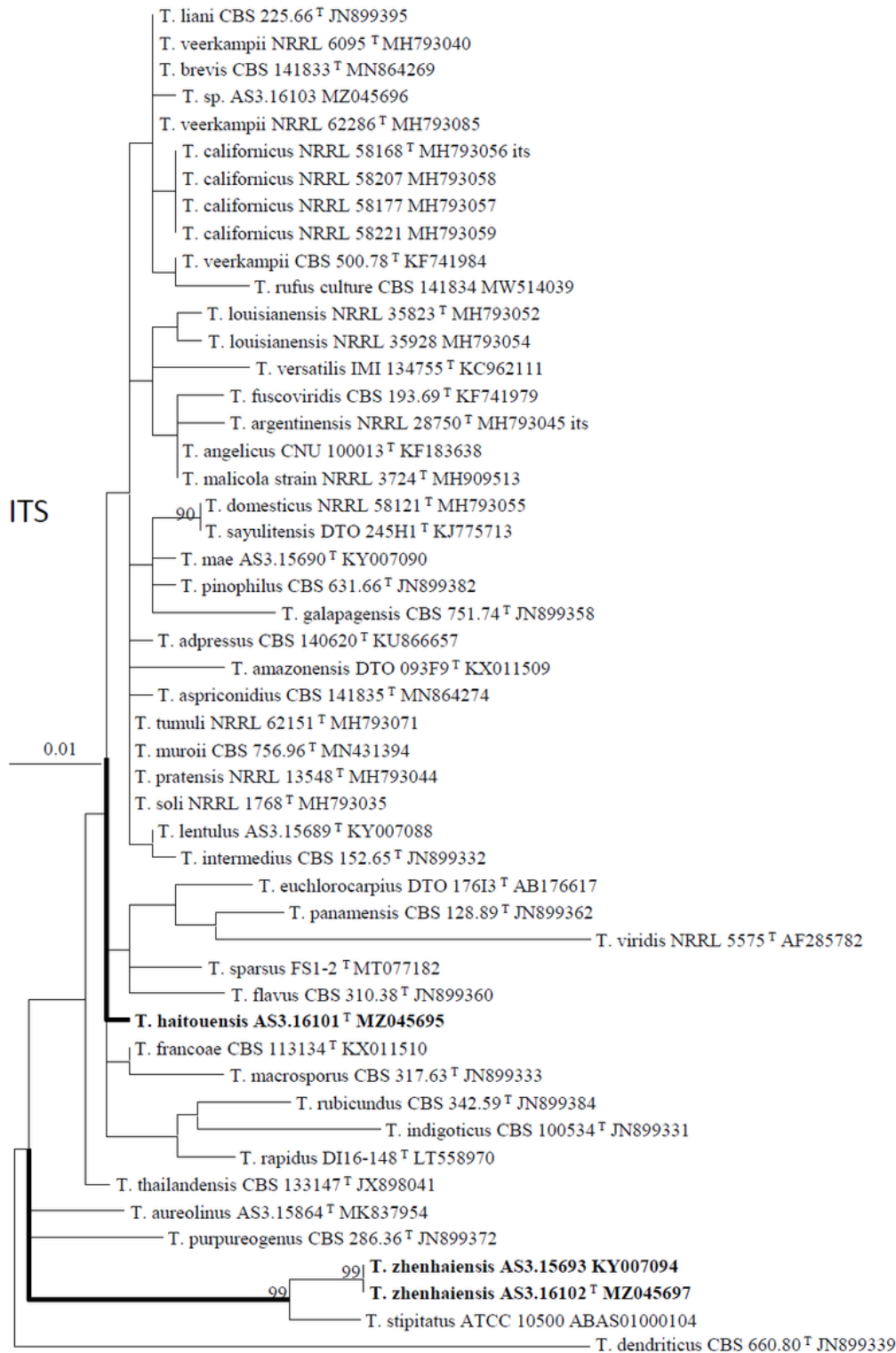


Figure 4

Legend not included with this version.

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