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Talaromyces qii, a New Record of a Rare *Talaromyces* from the Northern Western Ghats, India

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ABSTRACT

In this study, *Talaromyces qii* belonging to the section *Talaromyces* is reported as a new record from India based on the morphology and phylogenetic analyses of four gene datasets *viz*. ITS, *BenA*, *CaM*, and *rpb2*. This is the second report of this rare *Talaromyces* species from across the world. Phylogenetically, the Indian strain *T. qii* (NFCCI 5151) formed a sister lineage to the type species *T. qii* (AS3 15414) due to the sequencing error in the type. The quality assessment of the four gene sequences derived from all type strains of section *Talaromyces* in this study evaded the proposal of a redundant novelty in this section, aligning the Indian strain NFCCI 5151 along with *T. qii* (AS3 15414). Morphology of type strain *T. qii* (AS3 15414) and *T. qii* (NFCCI 5151) are mostly identical, viz. elongated, biverticillate-symmetrical conidiophores, acerose phialides, and ellipsoidal or sub-globose conidia with echinulate ornamentation. However, the Indian strain has longer conidiophores and a larger conidia size than type strain *T. qii* and *T. thailandensis*. This study resolved the phylogeny of a new record of *Talaromyces qii* in the section *Talaromyces* from India through the most modern taxonomic approaches.

Keywords: Ascomycota, *BenA*, *rpb2*, *Talaromyces*, *Trichocomaceae*, India

INTRODUCTION

Talaromyces C.R. Benj., a flagship genus of *Trichocomaceae*, is known for uses in the food industry, medical significance, and the enzymes and soluble pigments important for biotechnological applications. The genus *Talaromyces* was introduced by Benjamin (1955) and described as the teleomorph of *Penicillium*. The *Talaromyces* producing single asci were grouped under *Hamigera* (Stolk and Samson, 1971), while other species producing asci in chains were restricted to *Talaromyces* (Stolk and Samson, 1972). Later, Houbraken and Samson (2011) segregated the thermophilic *Talaromyces* from other *Talaromyces*, dividing it into two genera *viz*., *Rasamsonia* and *Thermomyces* (Houbraken *et al.*, 2012, 2014). Yilmaz *et al.* (2014) used the modern polyphasic approach. They proposed a new sectional classification for the genus delineating 88 *Talaromyces* species into seven sections, namely, section *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces*, and *Trachyspermi*. Presently, *Talaromyces* is a monophyletic genus having eight sections, including the recently assigned section *Tenues* from China (Sun *et al.*, 2020).

The *Talaromyces* typified by *T. flavus* belonging to the section *Talaromyces* was introduced by Stolk and Samson (1972) for species producing yellow ascomata, occasionally white, cream, pink or red in colour with yellow ascospores. Conidiophores of species belonging to this section are typically of the biverticillate-symmetrical type. Rarely species with reduced conidiophores and solitary phialides are seen. Phialides are acerose, with few species having the presence of wider bases (Stolk and Samson, 1972). Visagie *et al.* (2015) introduced five new species of section *Talaromyces* using polyphasic taxonomy. Wang *et al.* (2016) reported two new species of *Talaromyces*, *T. neofusisporus* and *T. qii*, belonging to section *Talaromyces* isolated from plant leaves in Tibet, China. Recently, Guevara-Suarez *et al*. (2020) introduced two new coprophilous *Talaromyces* belonging to this section.

The northern Western Ghats is rich and diverse with fungi, especially with the asexual ascomycetes. The well-protected, pristine natural forests and the warm tropical, humid climate prevailing in these habitats support many fungal species that are novel to science (Rajeshkumar *et al.*, 2012, 2018, 2019a, b; Ashtekar *et al.*, 2022). During the monsoon season (June-July) of 2019, field surveys were conducted to explore the fungal diversity in the natural forests of Tamhini village and adjacent terrain. This paper aims to resolve the taxonomy and phylogeny of a biverticillate penicillium-like *Talaromyces* strain isolated from

the forest soil following the most modern taxonomic approaches in the family *Trichocomaceae*.

MATERIAL AND METHODS

Isolation

To enrich the indigenous fungal biological resources of National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute (ARI), Pune, surveys were conducted in Tamhini village and adjacent areas of Pune, Maharashtra, during the monsoon (June-July) of 2019. Soil samples collected from different microhabitats were dried under shade overnight for further isolation procedures. Serial dilution technique was followed for isolation of fungi on 2% Water Agar and further sub-cultured to malt extract agar (MEA) media containing Streptomycin sulphate (100 mg/L) (HIMEDIA Laboratories Pvt. Ltd, India). The composition of media used for examining colony characters, the protocols for inoculating and incubating cultures, and microscopic examination were followed as per Yilmaz *et al.* (2014). After inoculating the pure culture on the eight prescribed media, they were incubated in the dark for seven days using a Bio Multi Incubator (Model LH-30-8CT, Japan) at 25±2 °C. The cultures were accessioned and preserved in the NFCCI ARI, Pune, India.

Morphology

Colony characters were recorded after seven days of incubation on various media, including Malt Extract agar (MEA), Czapek Yeast autolysate Agar (CYA), CYA with 5% NaCl (CYAS), Creatine Sucrose agar (CREA), Oatmeal Agar (OA), Czapek's agar (CZ), Dichloran 18% Glycerol agar (DG18), and Yeast Extract Sucrose agar (YES). For media preparation, inoculations, incubation conditions, and microscopic preparations, the recommendations by Visagie *et al.* (2014) were followed. Colour codes and names used in descriptions refer to Kornerup and Wanscher (1967). Microscopic observations were made with an Olympus (Model CX-41, Japan) dissecting microscope and Zeiss (AXIO Imager 2, Germany) compound microscope equipped with Nikon Digital sight DS-Fi1 and AxioCam MRc5 digital cameras driven by AxioVision Rel 4.8 software (AXIO Imager 2, Germany).

DNA extraction, amplification, and phylogenetic analyses

Genomic DNA extraction was done following the modified protocols of rapid salt extraction method by Aljanabi and Martinez (1997). The ITS region was amplified using primer pair ITS5 and ITS4 (White *et al.*, 1990). The partial *BenA* gene was amplified with primer pair Bt2a and Bt2b (Glass and Donaldson, 1995). The partial *CaM* gene was amplified using primer pair CMD5 and CMD6 (Hong *et al.*, 2006). To amplify the *rpb2* gene region, the primer RPB2-5F and RPB2-7cR (Liu *et al.*, 1999) were used. Amplification conditions were set following the protocols given in Ashtekar *et al.* (2022) and Rajeshkumar *et al.* (2019). The PCR products were purified with StrataPrep PCR Purification Kit (Agilent Technologies, TX, USA) and sequenced using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing reactions were run on ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis

Reference sequences of *Talaromyces*, section *Talaromyces* were downloaded from GenBank and aligned in MAFFT v.7.305b (Katoh and Standley, 2013). The aligned sequences were manually edited where required, and consensus sequences were prepared in BioEdit v.7.0.9.0 (Hall, 1999). The phylogeny website tool "ALTER" (Glez-Peña *et al.*, 2010) was used to transfer the alignment file into PHYLIP format for RAxML analyses (Stamatakis *et al.*, 2008). Phylogenetic analysis of the combined aligned data was performed using maximum likelihood (ML) analysis in RAxMLGUI v.1.3 (Silvestro and Michalak, 2012). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMAI substitution model. The Model parameters were estimated to an accuracy of 0.1000000000 log likelihood units. Every 100th tree was saved. RAxML bootstrap support values greater than 70% are given above at the branches. The Bayesian posterior probability clade credibility values greater than 0.90 (BPP; the rounding of values to two decimal proportions) from Bayesian-interference analysis (siMBa) labelled on the nodes (MLBS/BPP). The resulting trees were illustrated with TreeView 1.6.6 (Page, 1996), and the resulting tree layout was created in Microsoft PowerPoint. The DNA sequence datasets generated in this study were deposited in GenBank, and the accession numbers are given in the results.

RESULTS

Phylogenetic analyses

Based on a Mega BLAST search of the *T. qii* (NFCCI 5151) strain in NCBI's GenBank nucleotide database, the closest hits using *BenA* were *T. qii* (GenBank KP765380; Identities = 373/387 (96%), 9 gaps (2%)), *T. kendrickii* (GenBank KF741921; Identities = 365/393 (93%), 14 gaps (3%)), and *T. francoae* (GenBank KX011489; Identities = 337/362 (93%), 9 gaps (2%)). The Mega BLAST and multigene concatenated phylogenetic analyses further supported the placement of the *Talaromyces* species collected from Mulshi forest, Maharashtra, belonging to the section *Talaromyces*. The phylogenetic relationship between the strain isolated in the current study and the accepted species of the section *Talaromyces*, along with their genetic congruence and phylogenetic consistency, was analyzed and interpreted using single and concatenated sequence datasets based on four gene regions (ITS, *BenA*, *CaM*, and *rpb2*). The length of the data sets was 540 bp, 379 bp, 550 bp, and 851 bp for ITS, *BenA*, *CaM*, and *rpb2* regions, respectively (**Figure 1**). The studied strain aligns with the type species *T. qii* (AS3 15414) with a high support (BS 100), concluding that the isolated strain in the study (NFCCI 5151) is also *T. qii.* The present study thus reports a new record of *T. qii* from the forest soil in Mulshi, Maharashtra. The species is characterized based on morphology and molecular support; the latter based on analyses of combined four gene datasets.

TAXONOMY

Morphology

Talaromyces qii L. Wang *et al.*, 2016. *Scientific Reports,* **6(1)**:1-9 **(Figure 2)**

MycoBank no: MB 811448

Micromorphology: Conidiophores strictly biverticillate. *Stipes* smooth to minutely verruculose towards metuale, (120-) 150-350 (-570) × 2.5-4.0 µm. *Metulae* in verticils of 2-5, symmetrical, verruculose, (7-) 8.5-11 × 2.5-3.5 µm. *Phialides* aceroid, in verticils of 2-6 per metula, smooth or verruculose, 10-11 (-12) × 2.0-3.0 µm. *Conidia* subglobose or ellipsoidal, verruculose, 2.8-3.8 × 2.5-3.2 µm, large-sized conidia 4-6.5 × 3.5-4 µm.

Macromorphology (at 25 ± 2°C after 7 days)

Colonies on **MEA**; slow growing, olive (1E4 to1E5), velutinous, 18-22 mm in diam., margin regular, white (1A1), exudates and soluble pigments absent, reverse light yellow (4A4) to amber yellow (4B6). Colonies on **CYA**; fast-growing, olive (1E4) to olive grey (1E2) center, greyish green (1D3) towards the periphery, velutinous, radially sulcate, 36-50 mm in diam., margin low, white (1A1) with pale turquoise (24A3) tinge, exudate colourless; prominent on the center of colonies, soluble pigments absent, reverse greyish orange (5B4) center, orange white (5A2) towards the periphery. Colonies on **CREA**; slow growing, white (1A1), scanty aerial mycelia, 26-29 mm in diam., acid production present showing yellowish discoloration. Colonies on **CYAS**: fast-growing, greyish green (1C3-1C4), conspicuously radially sulcate, 39-53 mm in diam., margin white (1A1) with pale turquoise (24A3) tinge, exudates, and soluble pigments absent, reverse orange white (5A2) in the center and paler towards the periphery. Colonies on **CZ**; fast-growing, white (1A1) to greyish white (1B1), mycelia scanty, thin, semi-immersed, non-sporulating, 33-44 mm in diam., exudates and soluble pigments absent, colony reverse pale yellow (1A3) to yellowish white (1A2). Colonies on **DG18**; slow growing micro-colonies with semi-immersed, pinkish white (7A2) mycelia, 33-44 mm in diam., exudates and soluble pigments absent, colony reverse white to off white. Colonies on **OA**; slow growing, thin, low, olivaceous (4E4) in the center and olive grey (1E2) towards the periphery, 24-25 mm in diam., exudates and soluble pigments absent, reverse off white. Colonies on **YES**; fast-growing, greyish green (1D4), velutinous to leathery, radially, and longitudinally sulcate, paler towards the margin, 39-53 mm in diam., margin regular, white (1A1) with pale turquoise (24A3) tinge, reverse greyish yellow (4B6) with a conspicuously sulcate pattern.

Specimen examined: INDIA. Maharashtra State; Pune District, Tamhini village, 624msl, 30° 18° 26' 45" N and 73° 25' 42" E, isolated from soil, 19 June 2019, collected by K.C. Rajeshkumar and N. Ashtekar, Specimen No. RKC NK72, Culture: NFCCI 5151. GenBank accession numbers: OM095473 (ITS), OM249789 (*BenA*), OM287425 (*CaM*), OM249790 (*rpb2*).

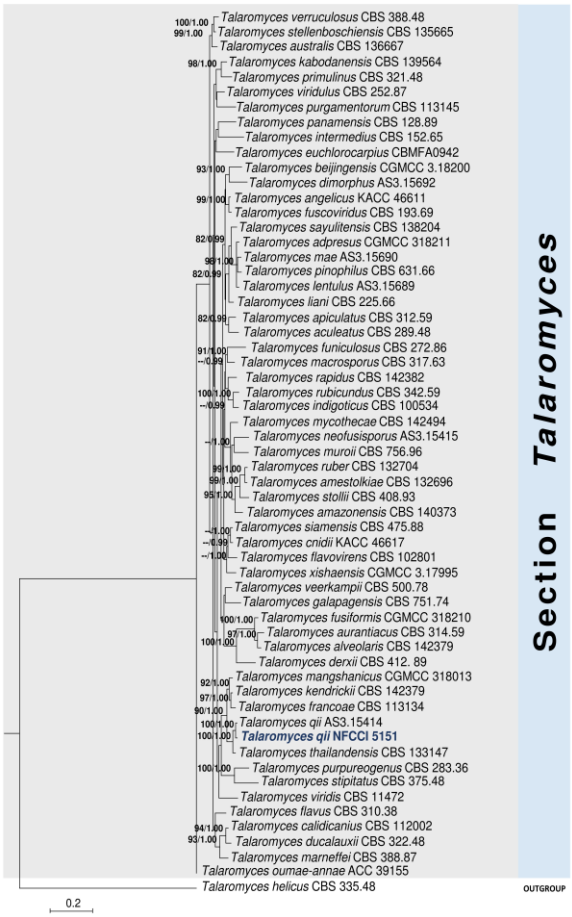

Figure 1: Phylogram generated from a maximum likelihood (ML) analysis based on concatenated datasets of ITS, *BenA*, *CaM*, and *rpb2* sequence data representing the section Talaromyces from genus *Talaromyces* in RAxML v.8.2.12 (Stamatakis 2014). Related sequences for analyses are taken from Sun *et al.* (2020). Fifty-nine strains are included in the combined analyses, which comprise 494, 426, 544, and 836 characters for ITS, *BenA*, *CaM*, and *rpb2*, respectively, after alignment. The tree is rooted to *T. helicus* (CBS 335.48), belonging to the Section Helici. The tree topology of the maximum likelihood analysis is similar to the Bayesian analysis performed in siMBa (Mishra and Thines, 2014). The best-scoring RAxML tree with a final likelihood value of -23437.022988 is presented. The matrix had 1088 distinct alignment patterns, with 24.45% gaps and completely undetermined characters. Estimated base frequencies were as follows: A=0.272837, C=0.272927, G=0.175910, T=0.278326; substitution rates AC=0.829467, AG=4.121432, AT=0.780274, CG=0.740557, CT=3.874825, GT=1.000000; gamma distribution shape parameter α=0.646805. Bootstrap support values for maximum likelihood (MLBS) equal to or greater than 70% and Bayesian posterior probability clade credibility values greater than 0.90 (BPP; the rounding of values to two decimal proportions) from Bayesian-interference analysis (siMBa) labeled on the nodes (MLBS/BPP). The newly recorded species, *T. qii* (NFCCI 5151), is indicated in bold and blue.

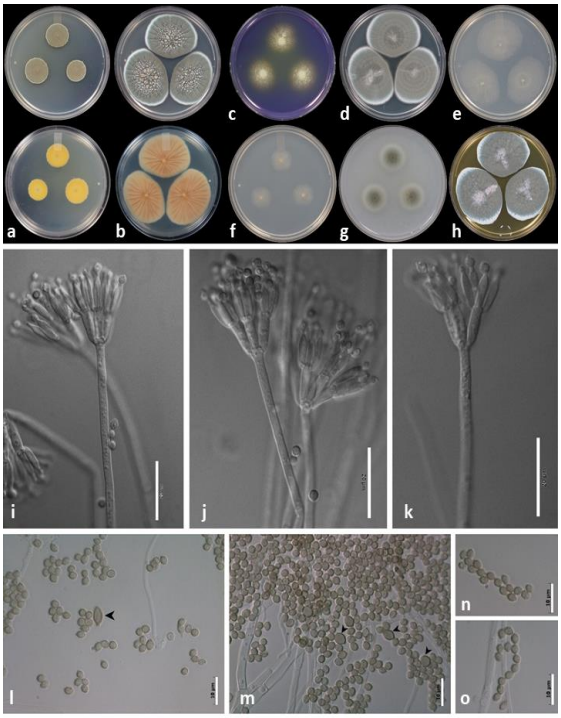

Figure 2: *Talaromyces qii* (NFCCI 5151). a-b, Colonies after 7d at 25±2 ºC on CYA and MEA obverse and reverse; c, CREA obverse; d, CYAS obverse; e, CZA obverse; f, DG18 obverse; g, OA (natural) obverse; h, YES obverse; i-k, Typical biverticillate symmetric conidiophores; l-o, Normal and large-sized conidia (Scale Bar: CPH=20 µm; CDA=10 µm).

Notes: The minor morphological differences of the new record species and closely allied species *T. qii* and *T. thailandensis* are noted. The stipes in *T. qii (NFCCI 5151)* were longer (120-570 µm) when compared to *T. qii* (AS3 15414) *(*150-360 µm) and *T. thailandensis* (200-370 µm). Phialides of *T. qii (NFCCI* 5151*)* were typically aceroid; however, phialides in *T. qii were aceroid to ampulliform and* lanceolate *in T. thailandensis.* Conidial size in *T. qii, (NFCCI* 5151) (2.8-3.8 × 2.5-3.2 µm; large sized conidia 4-6.5 × 3.5-4 µm) is larger compared to *T. thailandensis* (1.7-2.3 × 1.8-2.4 µm) and *T. qii* (3-3.5 µm), however, the shape and ornamentation of conidia was almost identical.

DISCUSSION AND CONCLUSION

In a landmark evolutionary study of the family *Trichocomaceae*, the species of *Penicillium* subgenus *Biverticillium*, that formed a distinct monophyletic clade from genus *Penicillium* were further amended in *Talaromyces* based on four-gene phylogeny (Houbraken and Samson, 2011) in accordance with the ICNafp single name nomenclature (ICNafp; McNeill *et al.*, 2012).

Yilmaz *et al.* (2014) published an extensive monographic account of *Talaromyces* that classified the genus into seven sections and 88 accepted species based on modern taxonomy. Most recently, Houbraken *et al.* (2020) re-evaluated the evolutionary relationships between families and genera of the order *Eurotiales*, including *Talaromyces,* using a nine-gene sequence dataset.

Species from the genus *Talaromyces* have been recorded as saprophytes, endophytes, and human pathogens from different geoclimatic regions and microhabitats across India. The new record established in the current study, *T. qii* (NFCCI 5151) belongs to the section *Talaromyces* and shares morphological characters like biverticillate-symmetric conidiophores and acerose phialides, commonly found in this section. The two species, *T. flavus* and *T. stipitatus,* belonging to the section *Talaromyces* have been reported from India. *T. flavus,* which produces vermiculine, an antiprotozoal antibiotic (Jones *et al.,* 1984) has been isolated from air, soil, textile, animal dung, seeds, roots, and leaves of plants in Delhi, Kerala, Orissa, Tamil Nadu, Uttar Pradesh, Uttarakhand, and West Bengal states of India (Basu, 1951; Benjamin, 1955; Chattopadhyay and Gupta, 1959; Gupta *et al*., 1966; Mukerjee, 1966; Saxena *et al*., 1969). Similarly, *T. stipitatus,* which produces a signature stipitatic acid along with the deadly mycotoxins, duclauxin, talaromycins, and botryodiploidin (Frisvad *et al.,* 1990), has been isolated using soil, leaf, and litter from Gujarat, Karnataka, Kerala, Madhya Pradesh, Uttar Pradesh, Uttarakhand, and West Bengal (Rai and Tewari, 1961, Rai *et al.,* 1969, Sarbhoy, 1965). *T. stipitatus*. Both *T. flavus* and *T. stipitatus* share a weakly coloured secondary metabolite (mycotoxin) known as duclauxin. A potentially pathogenic thermally dimorphic fungus, *Talaromyces marneffei,* causing systemic mycosis in HIV-infected patients was thoroughly studied from Manipur state of India (Singh *et al.,* 1999; Ranjana *et al.,* 2002). However, modern taxonomic tools validated for the taxonomy of order *Eurotiales* are yet to be widely adopted for the strain typing from India. Recently, Rajeshkumar *et al.* (2019a) established a new species, *T. amyrossmaniae*, isolated from decaying fruit and litter of *Terminalia bellerica* belonging to section *Trachyspermi* from the Tamhini village collection based on modern taxonomy. Prior to that, the taxonomy of Indian *Talaromyces* species was primarily based on morphological characters that are often outdated and underestimate the species diversity in these biodiversity hotspots. Authentication of these invaluable biological resources through modern taxonomy will enhance our understanding of the evolution of these species and the presence of range of metabolites for potential future biotechnological applications.

This study reports a new record of *T. qii* (NFCCI 5151) from India belonging to section *Talaromyces* based on morphology and a concatenated phylogenetic analysis using four gene datasets *viz*. ITS, *BenA*, *CaM* and *rpb2*. Phylogenetically, *T. qii* (NFCCI 5151) was aligned as a sister species to the type species of *T. qii* (AS3 15414) and *T. thailandensis* with high statistical support (BS/PP 100). The quality assessment of the four gene sequences of all type strains of section *Talaromyces* in this study evaded the proposal a redundant novelty in this section. The original *BenA* sequence of type species *T. qii* (AS3 15414) submitted to GenBank has a gap of seven bases (CACAGAC) from 482 to 488 that made our strain (NFCCI 5151) as a sister clade due to the sequencing error in the type species in *T. qii* (AS3 15414). Similarly, while establishing *T. qii* (AS3 15414), *RPB2* sequences were not used for phylogenetical interpretation. Morphologically, *T. qii* can be distinguished based on key features such as predominantly biverticillate-symmetrical conidiophores, acerose phialides, and sub-globose conidia. However, the stipes of the Indian strain, *T. qii (NFCCI 5151),* were longer (120-570 µm) when compared to the type, *T. qii* (AS3 15414) *(*150-360 µm) that are amended in the circumscription of the species. A synopsis of the new record with its closely related type species in section *Talaromyces* is given in **Table 1**.

Table 1: Comparative morphology of the *T. qii* and allied species under section *Talaromyces*.

Species	Stipe length (µm)	Conidiophore branching	Phialides	Conidia ornamentation	Conidial shape	Conidia size (µm)	References
T. francoae	125-450	Biverticillate	Ampulliform	Rough	Globose	2.5-4 × 2.5-4	Yilmaz *et al.*, 2016
T. kendrickii	150-500	Biverticillate with a minor proportion monoverticillate	Ampulliform	Rough	Subglobose, rarely ellipsoidal	2.5-3.0 × 2.5-3.0	Visagie *et al.*, 2015
T. mangshanicus	50-250	Biverticillate with a minor proportion monoverticillate or terverticillate	Ampulliform	Echinulate	Subglobose to ellipsoidal	4.5-5.5 × 4-5	Wang *et al.*, 2017
T. qii (NFCCI 5151)	120-570	Biverticillate with a minor proportion monoverticillate	Acerose	Echinulate to Verruculose	Subglobose to ellipsoidal	2.8-3.8 × 2.5-3.2 µm (large-sized conidia 4-6.5 × 3.5-4 µm)	This study
T. qii (AS3 15414)	150-360	Biverticillate	Acerose to ampulliform	Echinulate	Ovoid to subglobose	3-3.5	Wang *et al.*, 2016
T. thailandensis	200-370	Biverticillate	Lanceolate	Smooth	Subglobose to ellipsoidal	1.7-2.3 × 1.8-2.4	Manoch *et al.*, 2013

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