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Talaromyces peaticola (Aspergillaceae, Eurotiales), a new species from the Zoige wetlands, China

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Abstract

Species in *Talaromyces* section *Trachyspermi* are isolated from a wide range of substrates, including soil, house dust, leaf, wood and fruit from temperate region to tropical areas. During a survey of fungal diversity in Zoige wetlands, three isolates with biverticillate adpressed penicilli and spheroidal conidia belonging to *Talaromyces* are isolated from peat soil. Phylogenetic analyses based on a combined ITS, BenA, CaM and RPB2 sequence data suggests they represent a novel taxon in *Talaromyces* section *Trachyspermi*, namely *Talaromyces peaticola.* In spite of *T. peaticola* has a close affinity to *T. diversus* in phylogeny, it is readily distinguished from the later, resulted from growing slowly on CREA at 25ºC, exudating small clear droplets on MEA, and producing smaller conidia than that of *T. diversus*.

Keywords – 1 new taxon – peatland – Qinghai-Tibetan Plateau – taxonomy

Introduction

The genus *Talaromyces* was initially described by Benjamin (1955) to accommodate sexual morph *Penicillium* species with soft and yellowish ascomata surrounded by multiple layers of interwoven hyphae. Samson et al. (2011) redefined *Talaromyces* by transferring *Penicillium* subgenus *Biverticillium* into *Talaromyces* regarding the phylogenetic analysis of sequence data from the nuclear ribosomal internal transcribed spacer (ITS) and DNA-dependent RNA polymerase II largest subunit (RBP2) genes. Based on a multi-gene phylogeny of a combination of ITS, β-tubulin gene (BenA) and DNA-dependent RNA polymerase II second largest subunit (RPB2), *Talaromyces* has been divided into seven sections, namely as sections *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces* and *Trachyspermi* (Yilmaz et al. 2014). Currently, phylogenetic analyses of the ITS, BenA, CaM, and RPB2 genes are imperative for new species identification of *Talaromyces* (Yilmaz et al. 2014, Chen et al. 2016, Houbraken et al. 2020). The number of species in *Talaromyces* grew rapidly and have now reached more than 170 species (Houbraken et al. 2020).

Species in section *Trachyspermi* differ from other *Talaromyces* by conidiophores producing biverticillate phialides and when ascomata produced, have a creamy white or yellow color (Yilmaz et al. 2014, Chen et al. 2016, Wang et al. 2017). Additionally, they grow restrictedly on Czapek yeast autolysate agar (CYA), yeast extract sucrose agar (YES), and dichloran 18% glycerol agar

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(DG18), and slightly faster on malt extract agar (MEA) (Yilmaz et al. 2014, Chen et al. 2016, Luo et al. 2016, Romero et al. 2016, Wang et al. 2017). Consideration of the phylogeny and morphological features, 27species in Trachyspermi have been accepted by Houbraken et al. (2020) These species are always isolated from a wide range of substrates, including soil, house dust, leaf, wood and fruit from temperate region to tropical area (Chen et al. 2016, Romero et al. 2016, Wang et al. 2016). Their surviving strategy in the low osmotic environment, such as heat and dry-resistance as well, and bioactive compounds were studied comprehensively (Frisvad et al. 2013, Chen et al. 2016, Romero et al. 2016).

Peatlands only cover about 3% of the land surface but currently maintain one-third of global carbon stores (Turetsky et al. 2015). Fungi, with their heterogeneous physiology, metabolic activities, and ecological functions, are recognized as key decomposers of organic matter in these ecosystems (Thormann et al. 2001, Gilbert & Mitchell 2006). Studies of fungi in peatlands mostly focused on the relationship between fungal diversity and environmental factors using sequencing methods (Myers et al. 2012, Asemaninejad et al. 2017). A few culture-dependent studies indicated that *Aspergillaceae* (accounted for 25–30%) are the most frequently isolated fungi from peatlands (Thormann 2006, Wu et al. 2013), while *Talaromyces* accounted for 4.2% of the isolates (Wu et al. 2013).

During a survey of fungal diversity in Zoige wetlands, three isolates with biverticillate adpressed penicilli and spheroidal rough walled conidia are isolated from peat soil. They are described here as a new taxon of *Talaromyces* based on single and combined ITS, BenA, CaM and the RPB2 gene sequences and cultural features on the recommended media.

Materials & methods

Soil collections and fungal isolation

The cultures are isolated by dilution methods from soil samples collected in Zoige wetlands (33º3′54N, 102º34′23.9E) in Qinghai-Tibetan Plateau, China. Dried cultures are deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS), and the ex-type strains are preserved in the China General Microbiological Culture Collection Center (CGMCC, http://www.cgmcc.net/english/OrderingOfCultures.html).

Morphological observations and growth rate

Macroscopic characters are studied on CYA, CYA supplemented with 5 % NaCl (CYAS), YES, creatine sucrose agar (CREA), DG18, oatmeal agar (OA) and MEA (Samson et al. 2011). Isolates are inoculated at three points on 90 mm Petri dishes and incubated for 7 d at 25°C in darkness. Additionally, CYA plates are incubated at 30 and 37°C, and MEA plates were incubated at 30°C. After 7 d of incubation, colony diameters are recorded. The colony texture, degree of sporulation, front and reverse colony colors, the production of soluble pigments and exudates are observed. Acid production on CREA is indicated by a change in the pH sensitive bromocresol purple dye, from a purple to yellow color in media surrounding colonies. Microscope preparations are made from 1-week old colonies grown on MEA (Yilmaz et al. 2014). A Nikon Ellipse 80i light microscope equipped with differential interference contrast (DIC) optics is used to capture digital images.

DNA extraction, PCR amplification, and sequencing

Isolates are grown on potato dextrose agar (PDA, Oxoid malt) 1 week, and fungal mycelium was scraped off for genomic DNA extraction. Genomic DNA is extracted by using a simple and rapid “thermolysis” method (Zhang et al. 2010) and stored at −20°C. The ITS, BenA, CaM, and RPB2 genes are amplified and sequenced using methods and primers previously described in Yilmaz et al. (2014).

Table 1 Species used in phylogenetic analyses

Species name	Collection number [a]	Accession number			
		BenA	**CaM**	**ITS**	**RPB2**
T. aerius	CBS 140611 [T]	KU866835	KU866731	KU866647	KU866991
T. albobiverticillius	CBS 133440T	KF114778	KJ885258	HQ605705	KM023310
T. albobiverticillius	CBS 133441	KF114777	–	–	–
T. assiutensis	CBS 147.78 [T]	KJ865720	KJ885260	JN899323	KM023305
T. assiutensis	CBS 645.80	KF114802	–	JN899334	–
T. assiutensis	CBS 116554	KM066124	–	KM066167	
T. atroroseus	CBS 133442 [T]	KF114789	KJ775418	KF114747	KM023288
T. atroroseus	DTO 270-D5	KJ775227	–	KJ775734	–
T. atroroseus	DTO 270-D6	KJ775228	–	KJ775735	–
T. austrocalifornicus	CBS 644.95 [T]	KJ865732	KJ885261	JN899357	–
T. convolutus	CBS 100537 [T]	KF114773	–	JN899330	JN121414
T. diversus	CBS 320.48[T]	KJ865723	KJ885268	KJ865740	KM023285
T. diversus	DTO 131-I6	KJ775193	–	KJ775700	–
T. diversus	DTO 133-A7	KJ775194	–	KJ775701	–
T. erythromellis	CBS 644.80 [T]	HQ156945	KJ885270	JN899383	KM023290
T. heiheensis	HMAS 248789[T]	KX447525	KX447532	KX447526	KX447529
T. minioluteus	CBS 642.68 [T]	KF114799	KJ885273	JN899346	JF417443
T. minioluteus	CBS 137.84	KF114798	–	KM066171	–
T. minioluteus	CBS 270.35	KM066129	–	KM066172	–
T. peaticola	**CGMCC 3.18620 [T]**	**MF284705**	**MF284703**	**MF135613**	**MF284704**
T. peaticola	**CGMCC3.18767**	**MF960859**	**MF960861**	**MF960857**	**MF960863**
T. peaticola	**CGMCC3.18768**	**MF960860**	**MF960862**	**MF960858**	**MF960864**
T. purpurogenus	CBS 286.36 [T]	JX315639	KF741947	JN899372	JX315709
T. rubrifaciens	CGMCC 3.17658 [T]	KR855649	KR855653	KR855658	KR855663
T. solicola	CBS 133445 [T]	GU385731	KJ885279	FJ160264	KM023295
T. solicola	CBS 133446	KF114775	–	KF114730	–
T. systylus	BAFCcult 3419 [T]	KR233838	KR233837	KP026917	–
T. trachyspermus	CBS 118438	KM066128	–	KM066166	–
T. trachyspermus	CBS 116556	KM066126	–	KM066170	–
T. trachyspermus	CBS 373.48 [T]	KF114803	KJ885281	JN899354	JF417432
T. ucrainicus	CBS 162.67 [T]	KF114771	KJ885282	JN899394	KM023289
T. ucrainicus	CBS 127.64	–	–	KM066173	–
T. ucrainicus	CBS 583.72A	KM066130	–	KM066174	–
T. udagawae	CBS 579.72 [T]	KF114796	KX961260	JN899350	–

Phylogenetic analysis

The ITS sequences of isolates are firstly blasted on NCBI, resulting in 99% similar to *Trachyspermi diversus* ex-type strain CBS 320.48, which belongs to *Talaromyces* section *Trachyspermi*. Then, sequences of ITS, BenA, CaM, and RPB2 of species belong to section *Trachyspermi* determined from recent studies (Yilmaz et al. 2014, Chen et al. 2016, Luo et al. 2016, Romero et al. 2016, Wang et al. 2016, 2017, Houbraken et al. 2020), are downloaded from GenBank (Table 1). A single gene data-set is aligned using MAFFT version 7.03 with the Q-INS-I strategy (Katoh & Standley 2013). The ambiguous areas of alignment are located and removed using the Gblocks version 0.91b software program (Castresana 2000). The appropriate nucleotide substitution model for each gene is tested using the Akaike information criterion (AIC) with MrModeltest v2.3 (Nylander 2004). The 'GTRGAMMAI' model is the best model for ITS, BenA and RPB2 sequences, and the 'GTRGAMMA' model is the best model for CaM sequences. The model for multi-gene analysis is the combination of all models of the single gene. *Talaromyces purpurogenus* in *Talaromyces* section *Talaromyces* is set as outgroup in each analysis.

Combined sequences of the ITS, BenA, CaM, and RPB2 are concatenated using the Sequence Matrix for Windows version 1.7.8 (Vaidya et al. 2011), and missing data are treated as gaps. Single and combined genes are analyzed using maximum likelihood (ML) performed in RAxML (Stamatakis 2006) implemented in raxmlGUI v.1.3 (Silvestro & Michalak 2012) with rapid

bootstrap analysis with 1000 replicates. For Bayesian analyses, the posterior probabilities were determined by Markov chain Monte Carlo sampling (MCMC) in MrBayes v3.2 (Ronquist et al. 2012) based on the best models from MrModeltest. An average standard deviation of < 0.01 for split frequencies is used to suggest a convergence between parallel runs. The first 25% of sampled trees were discarded as 'burn-in'. Trees are figured in FigTree v1.4.2 (Rambaut 2014). Bootstrap values higher than 70% from Paup (BSMP), RAxML (BSML), and Bayesian posterior probabilities (BYPP) greater than 0.95 are indicated in the phylogenetic trees.

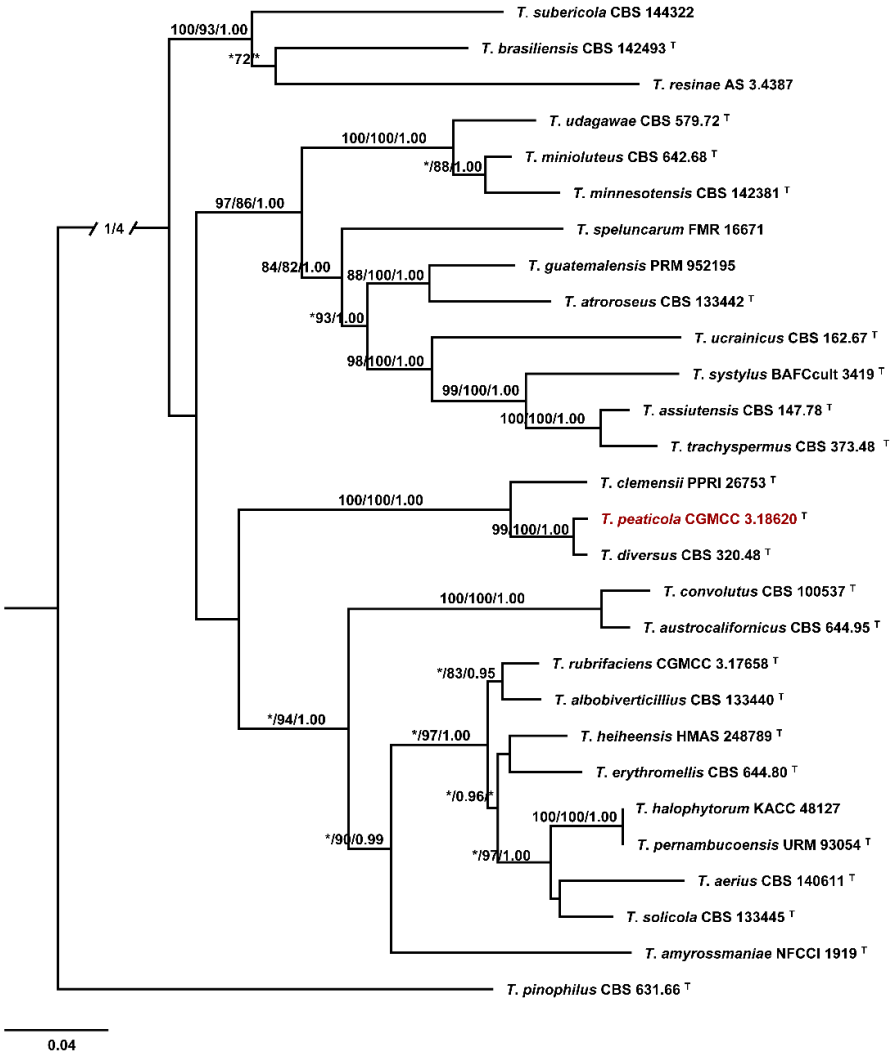

Fig. 1 – Phylogenetic tree generated from ML analysis combined ITS, BenA, CAM and RPB2 sequence data for *Talaromyces* section *Trachyspermi*. *Talaromyces purpurogenus* was chosen as outgroup. Bootstrap values higher than 70% for MP analysis (BSMP) (left) and ML analysis (BSML) (middle) are given above the nodes respectively. Bayesian posterior probabilities greater than 0.95 are indicated (BYPP) (right). * indicates bootstrap values of less than 70% or Bayesian posterior probabilities lower than 0.95 for a lineage. ^{T} indicates the ex-living type.

Results

Phylogenetic analyses

A first phylogeny concerning all currently accepted species in section *Trachyspermi*, including the type stain of our new isolates, was performed by using a sequence data-set of combined ITS (448 bp), BenA (351 bp), CaM (476 bp), and RPB2 (841 bp) genes (Fig. 1). The phylogenetic tree presented our putative new species *Talaromyces peaticola* (CGMCC 3.18620)

positioned robustly in section *Trachysperm*. Within section, *T. peaticola* (CGMCC 3.18620), *Talaromyces clemensii* (PPRI 26753), and *T. diversus* (CBS 320.48) formed a clade with strong support (100, BSMP / 100, BSML / 1.00, BYPP). Within this clade, CGMCC 3.18620 and *T. diversus* (CBS 320.48) formed a distinct subclade with strong support (99, BSMP / 100, BSML / 1.00, BYPP) as well, suggesting CGMCC 3.18620 is closely related to *T. diversus*. Consideration of the limited resolution of the ITS in the Trichocomaceae, BenA was proposed as the secondary DNA barcode for *Talaromyces* (Yilmaz et al. 2014). A phylogenetic tree was reconstructed by employing all available sequences BenA of species in section *Trachysperm* (Fig. 2). It presented that all isolates of *T. peaticola* and *T. diversus* formed a clade with strong support (100, BSMP / 100, BSML / 1.00, BYPP). Within this clade, *T. peaticola* formed a distinct subclade with strong support (95, BSMP / 75, BSML / 1.00, BYPP) separated from *T. diversus*, suggesting *T. diversus* is new taxon.

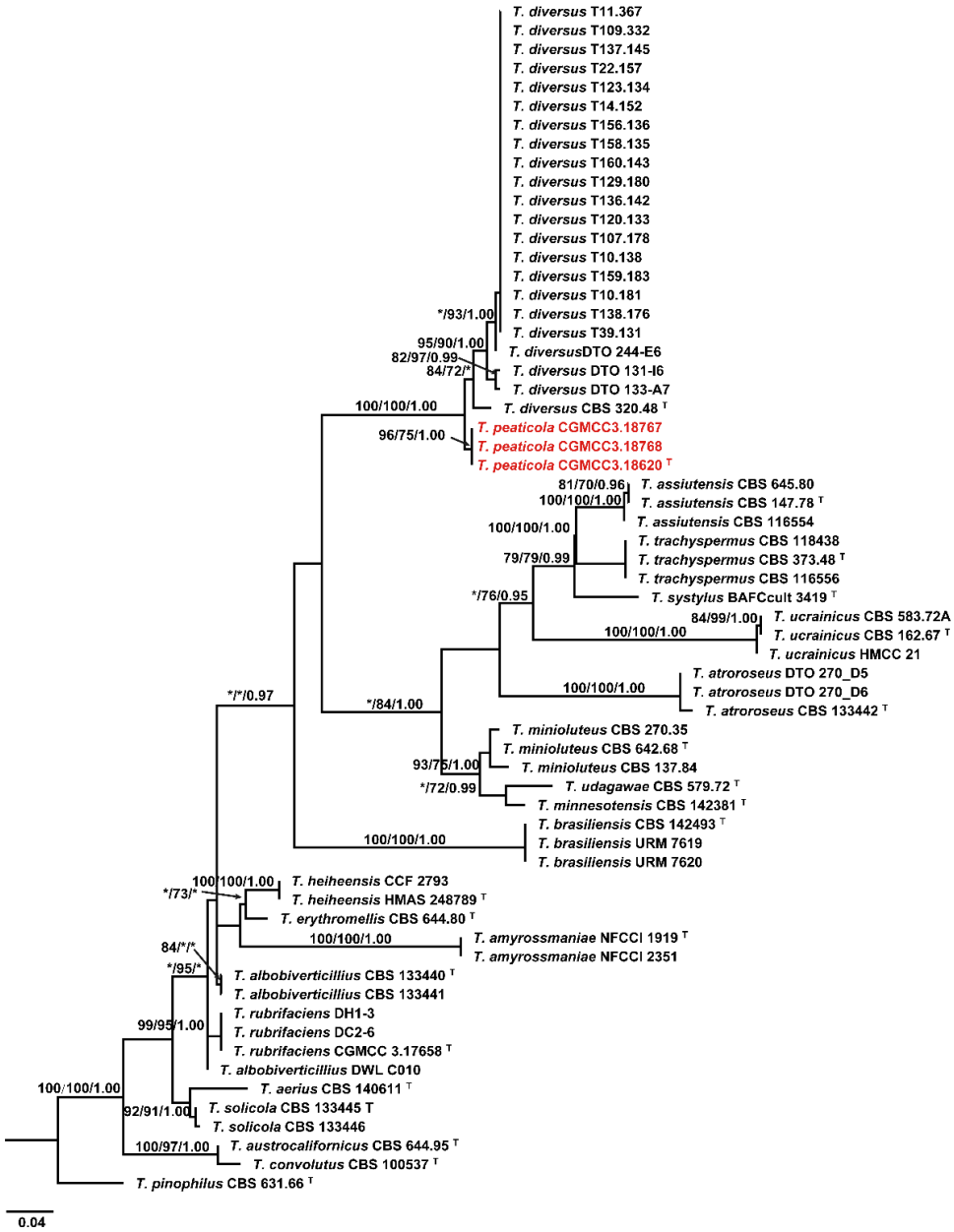

Fig. 2 – Phylogenetic tree generated from ML analysis BenA sequence data for *Talaromyces* section *Trachyspermi*. *Talaromyces purpurogenus* was chosen as an outgroup. Bootstrap values higher than 70% for MP analysis (BSMP) (left) and ML analysis (BSML) (middle) are given above

the nodes respectively. Bayesian posterior probabilities greater than 0.95 are indicated (BYPP) (right). * indicates bootstrap values of less than 70% or Bayesian posterior probabilities lower than 0.95 for a lineage. ^{T} indicates the ex-living type.

Talaromyces peaticola Jian Q. Tian & Jing Z. Sun sp. nov. Fig. 3

Index Fungorum number: IF553909; Facesoffungi number: FoF 09706

Etymology – peaticola, the stem peati- refers to the substrate that the type strain is isolated, the ending -cola means“dweller, inhabit”.

Diagnosis – Colonies slow-growing and concave in centers on CYA at 25°C, extremely slow-growing on CYA at 30°C and 37°C, acid production absents on CREA at 25ºC; conidiophores biverticillate; conidia globose, smooth-walled.

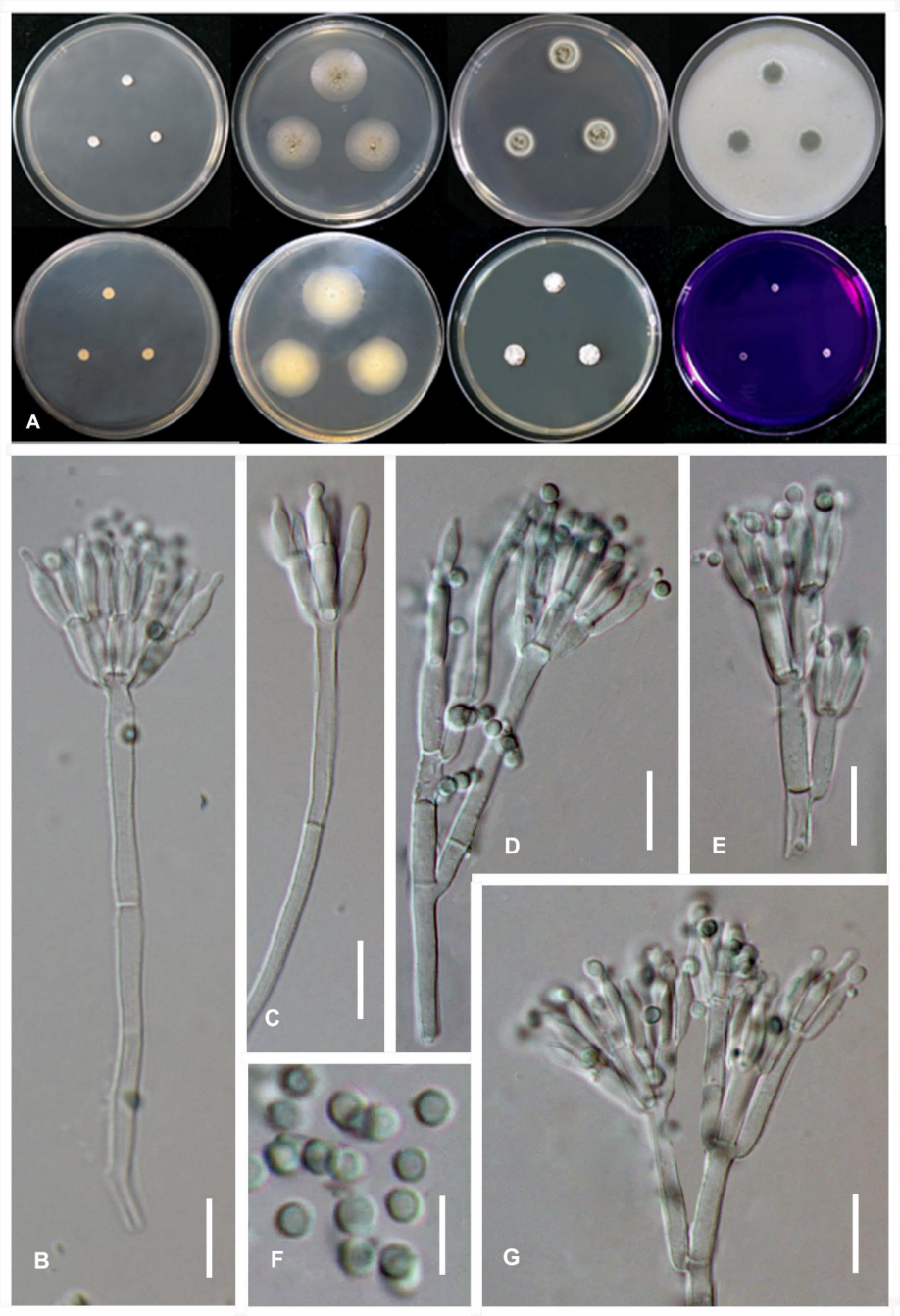

Fig. 3 – Morphological characters of *T. peaticola* (ex-type CGMCC3.18620). A Colonies from left to right (top row) CYA, MEA, YES, and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA. B–G Conidiophores. H Conidia. Scale bars: B–C = 20 μm, D–H = 10 μm.

Colony diam, 7 d, 25ºC (unless stated otherwise): CYA 5.0–6.9 mm; MEA 24.7–24.9 mm; DG18 16.0-16.5 mm; YES 9 -11 mm; CREA 3.3–3.9 mm; OA 11.7–12.5 mm; CYAS 2.9–3.5 mm; CYA 30ºC 4.7–6.6 mm; CYA 37ºC 4.5–4.7 mm; MEA 30 ºC 35.5–37.8 mm.

Colony characters – CYA 25ºC, 7d: colonies slightly raised at center, slightly sulcate; mycelia white; texture velvety; sporulation absent; soluble pigments absent; exudates absent; reverse medium beige (Fig. 3A). MEA 25ºC, 7d: colonies low plane; margins low, plane, entire; mycelia white; sporulation loose; conidia grayish green; texture floccose; soluble pigments absent; exudates small clear droplets; reverse bone white (Fig. 3A). YES 25ºC, 7d: colonies slightly raised at center, sunken at center, sulcate; margin slow, plane, entire (< 1 mm); mycelia white; texture velvety; sporulation absents to sparse; soluble pigment absent; exudates absent; reverser white (Fig. 3A). DG18 25ºC, 7d: colonies raised at center, sunken in the centre, sulcate; margins low, plane, entire (1–2 mm); mycelia white; texture velvety; sporulation moderately dense to dense; conidia dark green; soluble pigments absent; exudate absent (Fig. 3A). OA 25ºC, 7d: colonies low, plane; margins low, plane, entire; mycelia white; texture floccose; sporulation dense, conidia dark green; soluble pigments absent; exudates absent (Fig. 3A); CREA 25ºC, 7d: restricted growth; acid production absent (Fig. 3A). On MEA 25ºC, 7d conidiophores biverticillate (Fig. 3B-G); stipes smooth-walled, 160–200 × 3–4 μm (Fig. 3D, G); metulae, 3–8, divergent, 7.5–11.5 × 2–3 μm; phialides acerose, per metulae, 7.0–13.5×1.5–2.0 μm (Fig. 3E); conidia smooth, in chain, subglobose, globose, smooth-walled, 1.5–2.5×1.5–2.0 μm (Fig. 3H).

Teleomorph – Undetermined

Known distribution – CHINA. Sichuan Province.

Material examined – CHINA. Sichuan Province, Aba Autonomous Prefecture, Hongyuan County, Zoigê wetland, 33º3′54N, 102º34′23.9E, peaty soil, September 15, 2016, Jianqing Tian, ZRT-4 (holotype: HMAS 247296). ex-type living culture: CGMCC3.18620, CGMCC3.18767, CGMCC3.18768. *ibid.* LB1.17020001.

Notes – *Talaromyces peaticola* belongs to *Talaromyces* section *Trachyspermi* are well supported by the phylogenetic analyses of a combination of ITS, BenA, CaM and RPB2 sequence data-set. BenA marker and multi-gene of ITS, BenA, CaM and RPB2 performed well in differing *T. peaticola* from *T. diversus*. It also can be distinguished from other *Talaromyces* species by colony slow-growing on CYA at 25°C, extremely slow-growing on CYA at 30°C and 37°C, acid production absents on CREA at 25ºC; conidiophores biverticillate; conidia globose, smooth-walled. In spite of *T. peaticola* phylogenetically and morphologically closed to *T. diversus*, it could be easily distinguished from *T. diversus* by the later could not grow on CREA at 25ºC and *T. peaticola producing* smaller conidia (Yilmaz et al. 2014).

Discussion

The taxonomy of *Talaromyces* was redefined recently on the basis of DNA sequence data, extrolite profiles and other phenotypic features (Yilmaz et al. 2012, 2014, Chen et al. 2016). The phylogenetic analysis resulted from the combined sequence of ITS, BenA, CaM and RPB2 well distinguish *Talaromyces peaticola* from other *Talaromyces* species in section *Trachyspermi* (Fig. 1), which supported combined ITS, BenA, CaM and RPB2 sequence is imperative for new species identification (Yilmaz et al. 2014, Chen et al. 2016). Additionally, phylogenetic analysis conducted by the single gene of BenA well differs *T. peaticola* from other species within *Talaromyces* section *Trachyspermi*, as well as its sister taxon *T. diversus*, which resulted from a 9 bp difference in BenA locus (448/457). This confirms that BenA, is imperative for new species identification of *Talaromyces* (Yilmaz et al. 2014, Chen et al. 2016). However, both phylogenetic analyses based on the single gene of ITS and RPB2 not well differ *T. peaticola* from *T. diversus* owning the little differences ITS sequence (585/590 bp) and RPB2 sequences (850/852 bp). These results agree with that ITS and RPB2 sequences are insufficiently variable to reliably discriminate species in *Talaromyces* (Skouboe et al. 1999, Yilmaz et al. 2012, Frisvad et al. 2013, Yilmaz et al. 2014). BenA and CaM sequences perform well in species delimitation of Aspergillaceae, especially in

Penicillium, *Aspergillus* and *Talaromyces*, and even some intraspecies (Seifert et al. 2007, Visagie et al. 2014, Yilmaz et al. 2014, Chen et al. 2016).

Colony features on seven standardized media and morphological characters on MEA and are recommended as important phenotypic features in the identification of *Talaromyces* (Visagie et al. 2014, Yilmaz et al. 2014). *Talaromyces peaticola* characteristically displays restricted growth on CYA and CREA and exudates small clear droplets on MEA (Fig. 3). In spite that *T. peaticola* has an affinity to *T. diversus* in all phylogenetic trees (Fig. 1). These two species are readily distinguished from each otherin regard to *T. diversus* could not grow on CREA at 25ºC and exudates small clear droplets on MEA (Yilmaz et al. 2014). Additionally, the conidia of *T. peaticola* are smaller than of *T. diversus*. Therefore, *Talaromyces peaticola* is introduced as a new taxon.

Zoige peatland is the largest alpine peatland, characterized by high moisture and low temperature. Previous studies have reported that species in genus *Talaromyces* are one of the major fungal survivals and frequently isolated from peatland (Gilbert & Mitchell 2006, Wu et al. 2013, Grum-Grzhimaylo et al. 2016, Asemaninejad et al. 2017), the ecological function and metabolic activities need to be further explored.

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