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Trechispora daweishanensis and *T. xantha spp. nov.* (Hydnodontaceae, Trechisporales) found in Yunnan Province of China

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Abstract

Two wood-inhabiting fungal species, *Trechispora daweishanensis* and *T. xantha spp. nov.* are proposed based on morphological features and molecular evidence. *Trechispora daweishanensis* is characterized by basidiomata with an annual growth habit, a tuberculate hymenophore, a monomitic hyphal system with clamped generative hyphae and ellipsoid, hyaline, thin-walled, smooth basidiospores measuring $3.8-5 \times 2.7-3.5 \mu m$. *Trechispora xantha* is characterized by resupinate, cracked basidiomata with a buff-coloured hymenial surface, a monomitic hyphal system and ellipsoid, hyaline, thin-walled, smooth basidiospores measuring $4.3-5.7 \times 3.2-4 \mu m$. Sequences of ITS and nLSU gene regions of the studied samples were generated. The phylogenetic analysis based on molecular data of ITS+nLSU sequences supported the novelty of these two species. Furthermore, we provide a key to the known species of *Trechispora* in China.

Keywords: Basidiomycetes, phylogeny, taxonomy, Trechisporales, wood-inhabiting fungi

Introduction

Trechispora P. Karst. typified by *T. onusta* P. Karst. (1890: 147) is characterized by resupinate to effused basidiomata; a smooth to hydnoid to poroid hymenophore; ampullaceous septa; short cylindric basidia and smooth to verrucose or aculeate basidiospores (Karsten 1890, Bernicchia & Gorjón 2010). About 50 species are currently known in *Trechispora* worldwide (Liberta 1966, 1973, Larsson 1994, 1995, 1996, Ryvarden 2002, Trichiès & Schultheis 2002, Miettinen & Larsson 2006, Bernicchia & Gorjón 2010, Ordynets *et al.* 2015).

In 2002, Ryvarden, based on his interpretation of morphological characters, changed Hydnodon thelephorus (Lév.) Banker (1913: 297) to Trechispora thelephora (Lév.) Ryvarden (2002: 32). Molecular phylogenetic analyses of corticioid fungi by Larsson (2007) demonstrated that two species of Trechispora, Trechispora farinacea (Pers.) Liberta (1966: 318) and T. hymenocystis (Berk. & Broome) K.H. Larss. (1994: 1167), grouped together and nested within the family Hydnodontaceae. Birkeback et al. (2013) provided molecular evidence that supported the conclusion of Ryvarden (2002) and proposed that Hydnodontaceae needed a new name, and changed it to Trechisporaceae. Other molecular phylogenetic studies suggested that Porpomyces Jülich (1982: 425), Sistotremastrum J. Erikss. (1958: 62), Subulicystidium Parmasto (1968: 120) as well as Trechispora belonged to a well-supported clade in Hydnodontaceae (Telleria et al. 2013). More recently, Liu et al. (2019) using ITS+nLSU sequences of Trechisporales revealed that Porpomyces, Scytinopogon Singer (1945: 139) and Trechispora grouped together and nested within the family Hydnodontaceae; Phookamsak et al. (2019) using the ITS sequence dataset of Trechispora showed that T. echinospora Telleria, M. Dueñas, I. Melo & M.P. Martín (2019: 239) was sister to the clade comprising T. araneosa (Höhn. & Litsch.) K.H. Larss. (1995: 104), T. farinacea, T. hymenocystis and T. mollusca (Pers.) Liberta (1973: 1878). Based on the ITS+nLSU dataset, Xu et al. (2019) introduced a new taxon, T. yunnanensis C.L. Zhao (2019: 256) within Trechispora, which formed a monophyletic lineage and was closely related to T. byssinella (Bourdot) Liberta (1966: 318) and T. laevis K.H. Larss. (1996: 88).

Currently, fourteen species of *Trechispora* have been found in China (Dai 2009, 2011, Yuan & Dai 2012, Xu *et al.* 2019, Wang *et al.* 2020). Recently, we collected two taxa from Yunnan Province that could not be assigned to any described species. We present morphological and molecular phylogenetic evidence that support them as two new species in *Trechispora*.



FIGURE 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species of *Trechispora* and related species in Trechisporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50% and Bayesian posterior probabilities more than 0.95.

Materials and methods

Morphological studies

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, People's Republic of China. Macromorphological descriptions were based on field notes. Petersen (1996) was followed for the special colour terms. Microscopic structures were examined from dried material using a light microscope (Nikon Eclipse E 100). The following abbreviations were used for the micro descriptions: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

Molecular procedures and phylogenetic analyses

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens according to the manufacturer's instructions that were slightly modified by grinding a small piece of dried fungal specimen (about 30 mg) to powder with liquid nitrogen. The powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60

min. After that, 0.4 mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. The mixture was then transferred to an Adsorbing Column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in the AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 100 mL elution buffer was added to the middle of the adsorbed film to elute the genome DNA. The internal transcribed spacer region (ITS) was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). The nuclear large subunit region (LSU) was amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for LSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C 1 min, and 72 °C for 1.5 min; and a final extension of 72 °C for 30 s, 48 °C 1 min, and 72 °C for 1.5 min; and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at the Kunming Tsingke Biological Technology Limited Company, Kunming, Yunnan Province, People's Republic of China. All newly generated sequences were deposited in GenBank (Table 1).

TABLE 1. List of species,	specimens, and	d GenBank	accession	numbers	of sequences	used in this	s study.	The new	species
are in bold.									

Straning more	Sample no.	GenBank access	ion no.	Deferences
Species name		ITS	LSU	References
Dextrinocystis calamicola	Не 5700	MK204534	MK204547	Liu et al. 2019
Fibrodontia alba	TNMF 24944	KC928274	KC928275	Ordynets et al. 2015
F. brevidens	Wu 9807-16	KC928276	KC928277	Yurchenko & Wu 2014
F. gossypina	GEL 5042	DQ249274	AY646100	Unpublished
Hyphodontia floccosa	Berglund 150-02	DQ873618	DQ873617	Larsson et al. 2006
H. subalutacea	GEL 2196	DQ340341	DQ340362	Unpublished
Porpomyces mucidus	Dai 12692	KT157833	KT157838	Wu et al. 2015
P. submucidus	Cui 5183	KT152143	KT152145	Wu et al. 2015
Scytinopogon havencampii	DED 8300	KT253946	KT253947	Desjardin & Perry 2015
S. pallescens	He 5192	_	MK204553	Liu et al. 2019
Sistotremastrum guttuliferum	Не 3338	MK204540	MK204552	Liu et al. 2019
S. niveocremeum	CBS 427.54	MH857380	MH868920	Vu et al. 2019
Subulicystidium acerosus	He 3804	MK204539	MK204543	Liu et al. 2019
S. brachysporum	He 2207	MK204532	MK204549	Liu et al. 2019
S. longisporum	He 2981	_	MK204550	Liu et al. 2019
S. tropicum	He 3968	MK204531	MK204544	Liu et al. 2019
Trechispora alnicola	AFTOL-ID 665	AY635768	AY635768	Unpublished
T. araneosa	KHL 8570	AF347084	AF347084	Larsson et al. 2004
T. bispora	CBS 142.63	MH858241	MH869842	Vu et al. 2019
T. byssinella	UC 2023068	KP814481	_	Unpublished
T. cohaerens	TU 110332	UDB008249	_	Ordynets et al. 2015
T. cohaerens	TU 115568	UDB016421	_	Ordynets et al. 2015
T. confinis	KHL 11064	AF347081	AF347081	Larsson et al. 2004
T. cyatheae	FR-0219442	UDB024014	UDB024014	Ordynets et al. 2015
T. cyatheae	FR-0219443	UDB024015	UDB024015	Ordynets et al. 2015
T. daweishanensis	CLZhao 17860	MW302337	MW293866	Present study
T. daweishanensis	CLZhao 18255	MW302338	MW293867	Present study
T. echinocristallina	FR-0219448	UDB024022	UDB024022	Ordynets et al. 2015
T. echinospora	MA-Fungi 82485	JX392847	JX392848	Telleria et al. 2013

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TABLE 1. (Continued)

Species name	Sample no	GenBank access	ion no.	Dafarancas	
Species name	Sample no.	ITS	LSU	References	
T. farinacea	KHL 8451	AF347082	AF347082	Unpublished	
T. farinacea	KHL 8793	AF347089	AF347089	Larsson et al. 2004	
T. hymenocystis	TL 11112	UDB000778	UDB000778	Ordynets et al. 2015	
T. hymenocystis	KHL 8795	AF347090	AF347090	Unpublished	
T. incisa	EH 24/98	AF347085	_	Unpublished	
T. kavinioides	KGN 981002	AF347086	AF347086	Larsson et al. 2004	
T. laevis	TU 115551	UDB016468	_	Ordynets et al. 2015	
T. mollusca	DLL 2010-077	JQ673209	_	Brazee et al. 2012	
T. mollusca	DLL 2011-186	KJ140681	_	Ordynets et al. 2015	
T. nivea	MA-Fungi 76238	JX392824	JX392825	Telleria et al. 2013	
T. nivea	MA-Fungi 76257	JX392826	JX392827	Telleria et al. 2013	
T. nivea	MA-Fungi 82480	JX392829	JX392830	Telleria et al. 2013	
T. nivea	MA-Fungi 74044	JX392832	JX392833	Telleria et al. 2013	
T. regularis	KHL 10881	AF347087	AF347087	Larsson et al. 2004	
T. rigida	URM 85754	_	MH279999	Unpublished	
T. stevensonii	MA-Fungi 70669	JX392841	JX392842	Telleria et al. 2013	
T. stevensonii	HJM 18087	—	MH290761	Unpublished	
T. stevensonii	KHL 14654	_	MH290762	Unpublished	
T. stevensonii	TU 115499	UDB016467	UDB016467	Ordynets et al. 2015	
T. stellulata	UC 2022880	KP814437	_	Unpublished	
T. stellulata	UC 2023099	KP814451	_	Unpublished	
T. subsphaerospora	KHL 8511	AF347080	AF347080	Larsson et al. 2004	
T. thelephora	URM 85757	_	MH280001	Unpublished	
T. thelephora	URM 85758	_	MH280002	Unpublished	
T. xantha	CLZhao 2632	MW302339	MW293868	Present study	
T. xantha	CLZhao 17781	MW302340	MW293869	Present study	
T. yunnanensis	CLZhao 210	MN654918	MN654921	Xu et al. 2019	
T. yunnanensis	CLZhao 214	MN654919	MN654922	Xu et al. 2019	
T. yunnanensis	CLZhao 215	MN654920	MN654923	Xu et al. 2019	
Tubulicium raphidisporum	He 3191	MK204537	MK204545	Liu et al. 2019	
T. vermiculare	GEL 5015	AJ406424		Langer 2002	
T. vermiferum	KHL 8714	_	AY463477	Larsson et al. 2004	

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 26913). Sequences of *Hyphodontia floccosa* (Bourdot & Galzin) J. Erikss. (1958: 104) and *H. subalutacea* (P. Karst.) J. Erikss. (1958: 104) retrieved from GenBank were used as outgroups in the ITS+nLSU (Fig. 1) analysis following Liu *et al.* (2019). Sequences of *Fibrodontia alba* Yurchenko & Sheng H. Wu (2014: 339) and *F. gossypina* Parmasto (1968: 207) retrieved from GenBank were used as outgroups in the ITS+nLSU (Fig. 2) analysis following Ordynets *et al.* (2015).

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhao & Wu (2017), and the tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency

index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.



FIGURE 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of two new species and related species in *Trechispora* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50% and Bayesian posterior probabilities more than 0.95. The new species are in bold.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian Inference (BI). BI was calculated with MrBayes 3.2.7a with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2012). Four Markov chains were run for 2 runs from random starting trees, for 180,000 generations (Fig. 1) and 2,500,000 generations (Fig. 2) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (BS) >75%, maximum parsimony bootstrap (BT) >75%, or Bayesian posterior probabilities (BPP) >0.95.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) included sequences from 26 fungal specimens representing 26 species among *Trechispora* and related genera. The dataset had an aligned length of 2517 characters, of which 1642 characters were constant, 232 were variable and parsimony-uninformative, and 643 were parsimony-informative. Maximum parsimony analysis yielded 2 equally parsimonious trees (TL = 2929, CI = 0.5213, HI = 0.4787, RI = 0.4685, RC = 0.2443). the best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: was GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009591 (BI).



FIGURE 3. Basidiomata of *Trechispora daweishanensis*. Bars: A = 0.5 cm, B = 0.5 mm (holotype). Photos by: Tong-Kai Zong.

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences revealed that the two new taxa nested in *Trechispora* and grouped with *T. stellulata* (Bourdot & Galzin) Liberta (1966: 319).

The ITS+nLSU dataset (Fig. 2) included sequences from 46 fungal specimens representing 27 species within *Trechispora*. The dataset had an aligned length of 1727 characters, of which 963 characters were constant, 135 were variable and parsimony-uninformative, and 629 were parsimony-informative. Maximum parsimony analysis yielded 195 equally parsimonious trees (TL = 2645, CI = 0.491, HI = 0.509, RI = 0.646, RC = 0.317). The best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G, lset nst = 6, rates = invgamma; prset

statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009984 (BI).

The phylogenetic tree (Fig. 2) inferred from ITS+nLSU sequences revealed that the two new taxa grouped together and with *T. yunnanensis*, forming a well-supported monophyletic lineage within *Trechispora*.



FIGURE 4. Microscopic structures of *Trechispora daweishanensis* (drawn from the holotype). A: Basidiospores. B: Basidia and basidioles. C: A section of hymenium. Bars: $A = 5 \mu m$, $B-C = 10 \mu m$. Drawings by: Tong-Kai Zong.

Taxonomy

Trechispora daweishanensis C.L. Zhao, *sp. nov.* Figs. 3, 4 MycoBank no.: MB 838158

Holotype:—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°30'10", N 23°42'07", alt. 1500 m, on fallen branch of angiosperm, 1 August 2019, *CLZhao 17860* (SWFC!), GenBank No. (ITS MW302337; nLSU MW293866).

Etymology:—daweishanensis (Lat.): refers to the locality (Daweishan) of the type specimen.



FIGURE 5. Basidiomata of Trechispora xantha. Bars: A = 1 cm, B = 1 mm (holotype). Photos by: Tong-Kai Zong.

Description:—*Basidiomata* annual, adnate, resupinate, without odor or taste when fresh, becoming membranous upon drying, up to 8 cm long, 2.5 cm wide, 30–70 µm thick. *Hymenial surface* tuberculate, white when fresh, turn to white (60) to cream (21:4A2/3) to buff (13:4A4) upon drying. *Subiculum* very thin, white. *Margin* sterile, white (60), 0.3 cm wide.

Hyphal system monomitic; generative hyphae with clamp connections, IKI-, CB-; tissues unchanged in KOH.

Subiculum composed of colorless, thin-walled long, generative hyphae rarely branched, $1-2.5 \mu m$ in diam., ampullate hyphae encrusted with numerous larger crystals.

Subhymenium composed of colorless, thin-walled, shorter generative hyphae rarely branched, 1.5–3.5 μ m in diam., many crystals present; cystidia and cystidoles absent; *basidia* subcylindric to shortly clavate, 9.5–14.5 × 4–7 μ m, four-spored and with a basal clamp connection; basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores ellipsoid, colorless, thin-walled, smooth, with oil drops, IKI–, CB–, $(3.5–)3.8-5(-5.2) \times (2.5–)2.7-3.5(-3.7) \mu m$, L = 4.46 μm , W = 3.10 μm , Q = 1.41–1.44 (n = 60/2).

Additional specimen (paratype) examined:—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°30'10", N 23°42'07", alt. 1500 m, on fallen angiosperm branch, 2 August 2019, *CLZhao 18255* (SWFC!), GenBank No. (ITS MW302338; nLSU MW293867).



FIGURE 6. Microscopic structures of *Trechispora xantha* (drawn from the holotype). A: Basidiospores. B: Basidia and basidioles. C: A section of hymenium. Bars: $A = 5 \mu m$, B–C = 10 μm . Drawings by: Tong-Kai Zong.

Trechispora xantha C.L. Zhao, *sp. nov.* Figs. 5, 6 MycoBank no.: MB 838160

Holotype:—CHINA. Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, E 101°20'30", N 22°02'20", alt. 2007 m, on the trunk of *Albizia julibrissin*, 20 August 2017, *CLZhao 2632* (SWFC!), GenBank No. (ITS MW302339; nLSU MW293868).
Etymology:—*xantha* (Lat.): refers to the buff colour of the hymenial surface.

Description:—*Basidiomata* annual, resupinate, without odor or taste when fresh, becoming hard corky up on drying, up to 17 cm long, 3 cm wide, 20–60 µm thick. *Hymenial surface* smooth, cracking, slightly buff (13:4A4) when fresh, becoming darker yellowish (22:4A4) upon drying. *Subiculum* very thin, pale buff (13:4A4). *Margin* narrow, pale buff (13:4A4).

Hyphal system monomitic; *generative hyphae* with clamp connections, IKI–, CB–; tissues unchanged in KOH. *Subiculum* composed of colorless, thin-walled, frequently branched, long generative hyphae, 2–3.5 μm in diam., ampullate hyphae frequently present.

Subhymenial composed of colorless, thin-walled, rarely branched, short generative hyphae, 1.5–3.5 μ m in diam.; cystidia and cystidoles absent; *basidia* subcylindrical to subclavate, 14.5–23 × 4.5–7.5 μ m, four-spored and with a basal clamp connection; basidioles dominant, in shape similar to basidia, but slightly smaller.

Basidiospores ellipsoid, colorless, thin-walled, smooth, with oil drops, IKI–, CB–, $(4-)4.3-5.7(-6) \times (3-)3.2-4(-4.3) \mu m$, L = 4.97 μm , W = 3.64 μm , Q = 1.32–1.41 (n = 60/2).

Additional specimen (paratype) examined:—CHINA. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, E 103°30'10", N 23°42'07", alt. 1500 m, on fallen angiosperm branch, 1 August 2019, *CLZhao 17781* (SWFC!), GenBank No. (ITS MW302340; nLSU MW293869).

Discussion

In the present study, two new species, *Trechispora daweishanensis* and *T. xantha* are described based on phylogenetic analyses and morphological characters.

A phylogeny of corticioid species in the Trechisporales from East Asia revealed that eight genera clustered in the Trechisporal grouped with *Scytinopogon* (Liu *et al.* 2019). In the present study, the two new species clustered in *Trechispora* (Fig. 1) within the Trechisporales and four species of *Trechispora viz., T. alnicola* (Bourdot & Galzin) Liberta (1966: 318), *T. byssinella, T. cyatheae* Ordynets, Langer & K.H. Larss. (2015: 3) and *T. stevensonii* (Berk. & Broome) K.H. Larss. (1995: 115), grouped together. These four species then grouped with both *Scytinopogon* and three more species of *Trechispora* including the two new species (*T. daweishanensis, T. stellulata* and *T. xantha*), which shows that the two genera are closely related and need deeper phylogenetic investigation. Morphologically, *T. stellulata* differs by the arachnoid to byssoid hymenophore with whitish hymenial surface and basidiospores ornamented with obtuse spines (Larsson 1995). Further phylogeny (Fig. 2) within *Trechispora* showed that *T. daweishanensis* is sister to *T. xantha* and then groups with *T. yunnanensis* with strong support (BS = 100%, BP = 100%, BPP = 1), based on the combined ITS+nLSU sequence data. However, morphologically *T. xantha* differs from *T. daweishanensis* by having a smooth hymenophore that cracks, and a buff hymenial surface in contrast to *T. daweishanensis* which has a white (at least when fresh) tuberculate hymenial surface and the presence of larger crystals among subiculum and subhymenium. *Trechispora yunnanensis* differs in having thick-walled, ornamented and larger basidiospores (7–8.5 × 5–5.5 µm, Xu *et al.* 2019).

Morphologically, *T. cohaerens* (Schwein.) Jülich & Stalpers (1980: 257) and *T. confinis* (Bourdot & Galzin) Liberta (1966: 318) are similar to the two new species based on the smooth basidiospores. However, *T. cohaerens* differs in its thick-walled basidiospores (Jülich & Stalpers 1980, Bernicchia & Gorjón 2010); *T. confinis* differs in its smaller basidiospores (3–3.5 × 2–2.5 μ m, Liberta 1966).

The presence of ampullate septa hyphae in *Trechispora* is an important character. Both new species *Trechispora daweishanensis* and *T. xantha* have this character. *Trechispora byssinella*, *T. caucasica* (Parmasto) Liberta (1966: 318) and *T. mollusca* also have ampullate septa hyphae. However, *T. byssinella* differs in its white hymenial surface and narrower basidiospores ($3-4.5 \times 2-2.5 \mu m$, Bernicchia & Gorjón 2010); *T. caucasica* differs in its arachnoid hymenophore with white to greyish hymenial surface and narrowly ellipsoid to reniform basidiospores with a median constriction (Liberta 1966); and *T. mollusca* differs in its poroid hymenophore with a white, rhizomorphic hymenial surface (Liberta 1973).

Most species of *Trechispora* grow on very decayed wood and other debris on the ground (Bernicchia & Gorjón 2010, Dai 2011). *Trechispora* species have been described from boreal and temperate zones, especially in subtropical and tropical areas (Phookamsak *et al.* 2019, Xu *et al.* 2019), and many recently described taxa of wood-rotting fungi were also from these areas (Yurchenko & Wu 2015, Cui *et al.* 2019, Huang *et al.* 2020, Ma *et al.* 2020, Wang *et al.* 2020). The two new species in the present study are also from the subtropics, supporting the possibility of finding more new taxa belonging to the Trechisporales.

Key to the known species of Trechispora in China

1.	Basidiospores smooth	2
1.	Basidiospores aculeate, verrucose or ornamented	5
2.	Ampullate hyphae > 5 µm in width, basidiospores angular	T. subsphaerospora
2.	Ampullate hyphae < 5 µm in width, basidiospores ellipsoid	
3.	Basidiospores thick-walled	T. cohaerens
3.	Basidiospores thin-walled	4
4.	Hymenial surface tuberculate, white	T. daweishanensis
4.	Hymenial surface smooth, buff	T. xantha
5.	Hyphal system dimitic	T. dimitica
5.	Hyphal system monomitic	
6.	Hyphae without ampullate septa	7
6.	Hyphae with ampullate septa	
7.	Basidiospores thin-walled, ovoid to subglobose	T. suberosa
7.	Basidiospores thick-walled, ellipsoid	T. yunnanensis
8.	Sphaerocysts present, hyphae inflated	T. hymenocystis
8.	Sphaerocysts absent, hyphae uninflated	9
9.	Ampullate septa > 6 μ m in width	
9.	Ampullate septa < 6 µm in width	
10.	Basidiospores sparsely vertucose	T. polygonospora
10.	Basidiospores densely aculeate	
11.	Hyphae in subiculum thin-walled, > 3 µm in width	T. candidissima
11.	Hyphae in subiculum thick-walled, < 3 µm in width	T. mollusca
12.	Subhymenium with short celled hyphae	
12.	Subhymenium with long celled hyphae	14
13.	Basidiome thin, ochraceous	T. farinacea
13.	Basidiome thick, dirty white to buff	T. rigida
14.	Basidia < 10 µm in length	T. microspora
14.	Basidia > 10 µm in length	
15.	Basidiospores thin-walled, < 5 µm in length	
15.	Basidiospores thick-walled, $> 5 \ \mu m$ in length	T. praefocata

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