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Morphological and molecular identification of a new species of *Truncospora* (Polyporales, Basidiomycota) in North America

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Abstract

Truncospora wisconsinensis sp. nov., a new poroid wood-inhabiting species, is proposed based on a combination of molecular and morphological data. This species demonstrates a unique combination of characters including: annual habit; pileate basidiomata with a white pileus and pore surface; a dimitic hyphal system with non- to slightly dextrinoid, cyanophilous skeletal hyphae; and ellipsoid, truncate, slightly thick-walled, strongly dextrinoid basidiospores. Phylogenetic analyses using ITS and partial *tef1-α* support the position of this new species as a sister clade of *T. ohiensis*.

Key words: *Perenniporia*, Phylogeny, Polypores, Taxonomy

Introduction

The genus *Truncospora* Pilát (1953) is characterized by relatively small, pileate basidiomata (about 1.5–3 cm long, 2.5–3.5 cm wide, and 1–4 cm thick), non-dextrinoid to dextrinoid skeletal hyphae, and truncate, strongly dextrinoid basidiospores (Pilát 1953, Corner 1989, Decock 2011, Zhao & Cui 2013, Spirin *et al.* 2015). The type species of *Truncospora* is *T. ochroleuca* (Berk.) Pilát.

Robledo *et al.* (2009), Zhao & Cui (2013), and Zhao *et al.* (2013) have carried out molecular studies, that indicate that *Truncospora* belongs to the core polyporoid clade, distinct from *Perenniporia* Murrill. Phylogenetic studies employing DNA sequence analyses of the ITS rDNA region (internal transcribed spacer ribosomal DNA) and TEF1 (translation elongation factor 1-α) gene have been employed in phylogenic and diversity studies of the genus, which show that 7 species are recognized in the *T. ohiensis* group and 6 of them are described as new (Spirin *et al.* 2015).

About 11 species are accepted in *Truncospora* worldwide (Pilát 1953, Corner 1989, Decock & Ryvarden 1999, Decock 2011, Zhao & Cui 2013, Spirin *et al.* 2015). Five are reported from the USA: *T. arizonica* Spirin & Vlasák, *T. floridana* Vlasák & Spirin, *T. mexicana* Vlasák, Spirin & Kou, *T. ohiensis* (Berk.) Pilát and *T. tropicalis* Vlasák & Spirin (Gilbertson & Ryvarden 1987, Spirin *et al.* 2015). A fungus matching the concept of *Truncospora* was found by the senior author in the state of Wisconsin, USA. To confirm the affinity of this fungus, morphological as well as phylogenetic analyses were carried out using sequences of ITS rDNA and *tef1-α*.

Materials and methods

Morphological studies.—The specimens studied are deposited at the Farlow Herbarium, Harvard University (FH). Macromorphological descriptions were based on field notes prepared at the time of collection. Color terms followed Petersen (1996). Microscopic measurements were made from slide preparations of dried specimens stained with Cotton Blue and Melzer's reagent by light microscopy following Zhao *et al.* (2015). Sections were studied using an Olympus BX40 compound microscope. In presenting spore size variation, 5% of measurements were excluded from each end of the range and are given in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, IKI = Melzer's reagent, IKI– = both non-amyloid and non-dextrinoid, L = indicates basidiospores

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length (arithmetic average of all basidiospores), W = basidiospores width (arithmetic average of all basidiospores), Q = L/W ratio, n (a/b) = number of basidiospore (a) measured from given number of specimens (b).

TABLE 1. A list of species, specimens and GenBank accession number of sequences used in this study.

Species name	Sample no.	Geographic origin	GenBank accession no.		References
			ITS	TEF1	
Perenniporia medulla-panis	JV 0203/1	Czech Republic	KJ410710	KJ410711	Spirin *et al.* 2015
Truncospora arizonica	JV 1209/21	USA, Arizona	KJ410695	KJ410715	Spirin *et al.* 2015
T. arizonica	JV 1209/69	USA, Arizona	KJ410696	KJ410716	Spirin *et al.* 2015
T. arizonica	JV 1307/11J	USA, Arizona	KJ410697	KJ410717	Spirin *et al.* 2015
T. atlantica Spirin & Vlasák	BRFM 1192	France	GU731569	—	Spirin *et al.* 2015
T. atlantica	TAL128700	Spain, Canary Islands	KJ410700	KJ410720	Spirin *et al.* 2015
T. atlantica	JV 1311/4K	Spain	KJ410699	KJ410719	Spirin *et al.* 2015
T. detrita (Berk.) Decock	MUCL 42649	French Guiana	FJ411099	—	Robledo *et al.* 2009
T. floridana	JV 1008/78	USA, Florida	KJ410704	KJ410723	Spirin *et al.* 2015
T. floridana	JV 1008/85	USA, Florida	KJ410705	KJ410724	Spirin *et al.* 2015
T. floridana	JV 1008/86	USA, Florida	KJ410706	KJ410725	Spirin *et al.* 2015
T. macrospora B.K. Cui & C.L. Zhao	Yuan 3777	China, Yunnan	JX941574	—	Zhao & Cui 2013
T. macrospora	Cui 8106	China, Yunnan	JX941573	—	Zhao & Cui 2013
T. mexicana	JV 110983-1	USA, Texas	KJ410708	KJ410727	Spirin *et al.* 2015
T. mexicana	JV 110983-2	USA, Texas	KJ410709	—	Spirin *et al.* 2015
T. mexicana	JV 0610/U	Mexico, Veracruz	KJ410707	KJ410726	Spirin *et al.* 2015
T. wisconsinensis C.L. Zhao & Pfister	CLZ 005	USA, Wisconsin	KP768408	KP784666	In the present study
T. wisconsinensis	CLZ 006	USA, Wisconsin	KP768409	KP784667	In the present study
T. wisconsinensis	CLZ 007	USA, Wisconsin	KP768410	KP784668	In the present study
T. wisconsinensis	CLZ 008	USA, Wisconsin	KP768411	KP784669	In the present study
T. ochroleuca	JV 0610/7B	Belize	KJ410698	KJ410718	Spirin *et al.* 2015
T. ochroleuca	MUCL 39563	Australia	FJ411097	—	Robledo *et al.* 2009
T. ohiensis	JV1208/21	USA, Pennsylvania	KJ410692	KJ410713	Spirin *et al.* 2015
T. ohiensis	JV0309/114	USA, Pennsylvania	KJ410694	—	Spirin *et al.* 2015
T. ohiensis	JV0509/64	USA, Tennessee	KJ410693	KJ410714	Spirin *et al.* 2015
T. ohiensis	MUCL 41036	USA	FJ411096	—	Robledo *et al.* 2009
T. ornata Spirin & Bukharova	Cui 5714	China, Liaoning	KF051056	—	Zhao *et al.* 2013
T. ornata	Dai 1644	China, Jilin	KJ41069	—	Spirin *et al.* 2015
T. ornata	Spirin 6672	Russia, Khabarovsk	KJ410690	KJ410712	Spirin *et al.* 2015
T. tropicalis	JV 1008/45-1	USA, Florida	KJ410702	—	Spirin *et al.* 2015
T. tropicalis	JV 1008/45-2	USA, Florida	KJ410703	KJ410722	Spirin *et al.* 2015
T. tropicalis	JV 1112/18J	Puerto Rico, Rio Grande	KJ410701	KJ410721	Spirin *et al.* 2015

DNA extraction, amplification, sequencing and phylogenetic analyses.—Genomic DNA was extracted from the specimens using the Qiagen DNeasy Plant Mini Kit (cat. no. 69104). 1/10 and 1/100 dilutions of the genomic DNA were used for PCR amplification of the ITS and *tef1-α*. The ITS region was amplified using primer pair ITS5 and ITS4 (White *et al.* 1990). The *tef1-α* gene was amplified with primer pair EF1-983F and EF1-2218R (Rehner & Buckley 2005). All PCR reactions were done in a Peltier Thermal cycler PTC-200 (MJ Research, Watertown, MA, USA) and used Econo*Taq* DNA Polymerase (Lucigen, Middleton, WI, USA). PCR amplification, purification, and sequencing were as previously described in Hansen *et al.* (2005) and Spirin *et al.* (2015). All newly generated sequences have been deposited in GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences generated in this study were aligned with sequences of the ten *Truncospora* species available from GenBank (Table 1) using ClustalX (Thompson *et al.* 1997) and manually adjusted in BioEdit (Hall 1999). In all phylogenetic analyses the sequence of *Perenniporia medulla-panis* (Jacq.) Donk (Table 1) was used as the outgroup to root tree following Spirin *et al.* (2015). The sequence alignment was deposited inTreeBase (submission ID 17104).

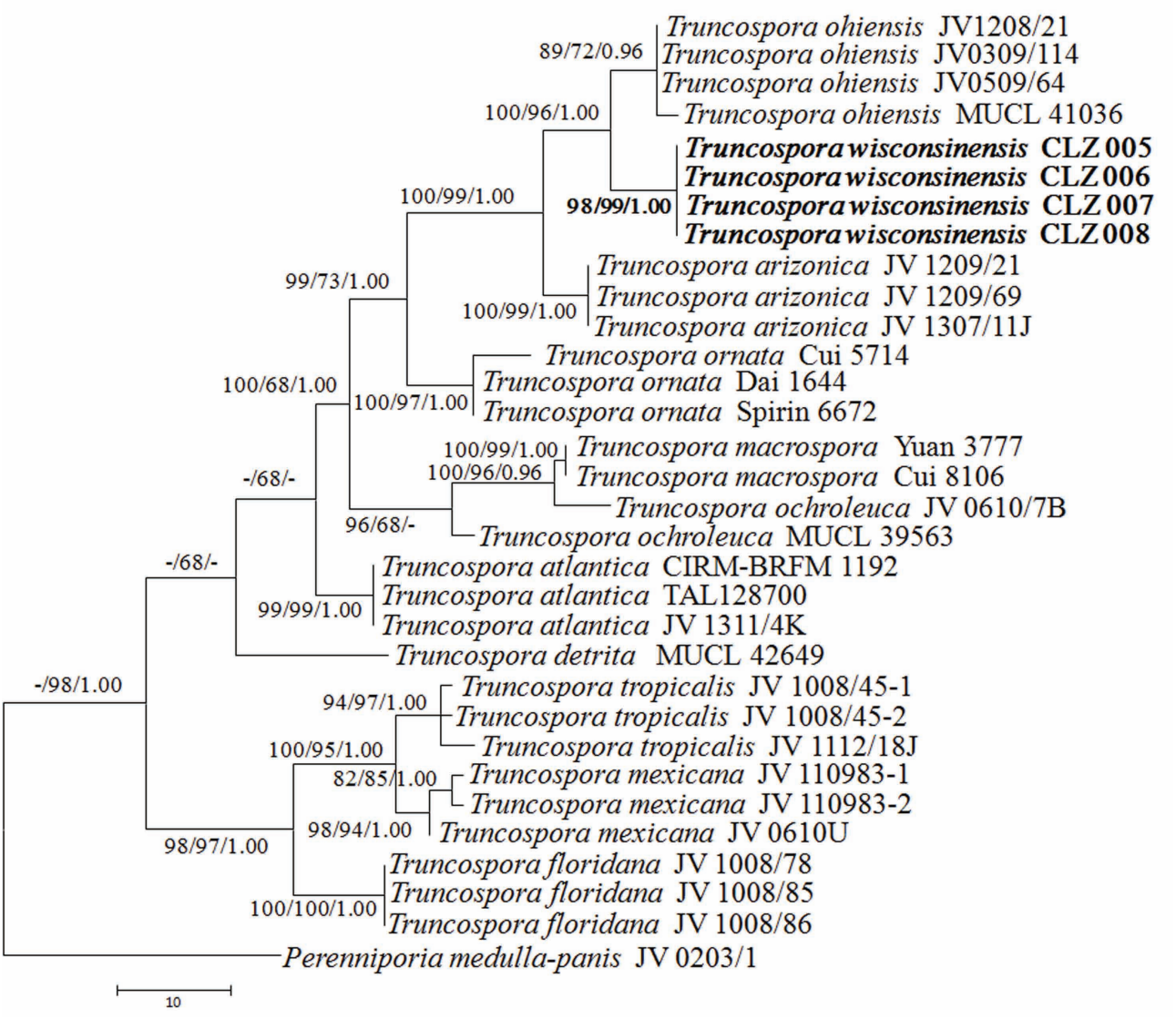

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Truncospora wisconsinensis* and related species based on ITS rDNA sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Approaches to phylogenetic analysis followed Zhao *et al.* (2015). Maximum parsimony (MP) analysis was applied to the dataset and the tree construction procedure 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 1000 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 (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al.* 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). BI was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 1 million generations (ITS) and for 500,000 generations (*tef1-α*). Trees were sampled every 100th generation. The first quarter of the generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 75 % (MP) and 0.95 (BPP) were considered as significantly supported, respectively.

Results

Molecular phylogeny

The ITS dataset included sequences from 32 specimens representing 12 taxa. The dataset had an aligned length of 629 characters, of which 479 characters are constant, 63 parsimony-uninformative and 87 parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 239, CI = 0.749, HI = 0.251, RI = 0.896, RC = 0.671). Best model for ITS alignment estimated and applied in the BI was as follows: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.006038.

Molecular phylogenetic analysis of the ITS sequences (Fig. 1) indicated that four specimens of the undescribed taxon grouped in a single clade, closely related to, but distinct from the *T. ohiensis* clade (100% BS, 96% MP, 1.00 BPP). *Truncospora arizonica* was the sister group to these two species (Fig. 1).

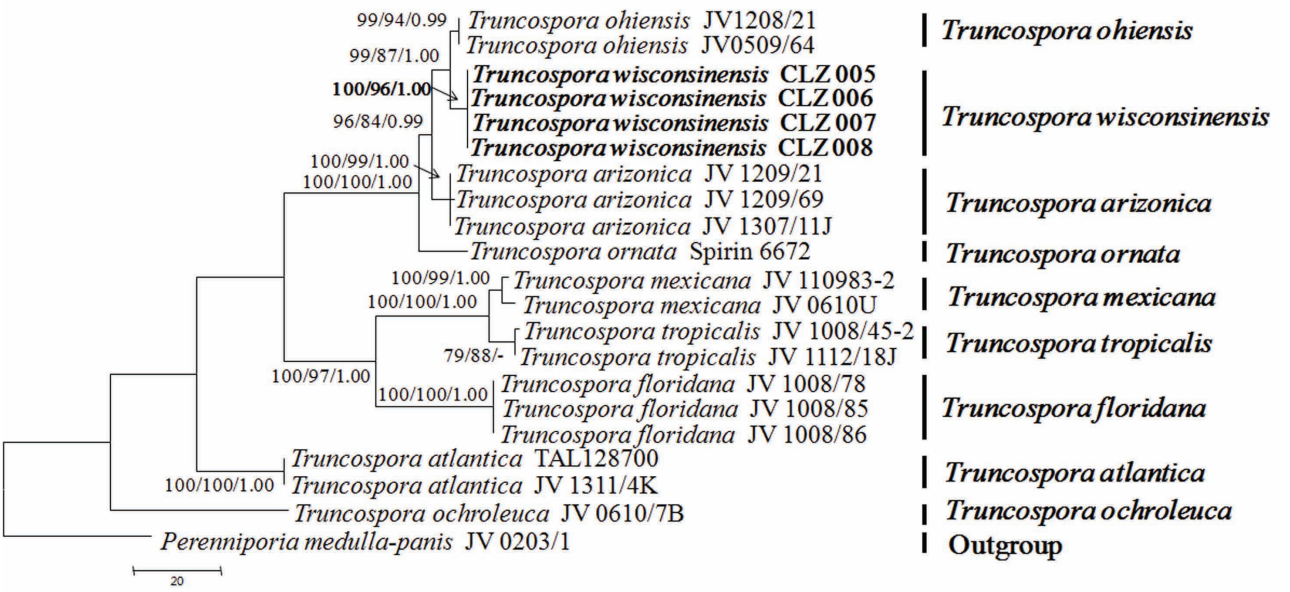

FIGURE 2. Maximum parsimony strict consensus tree illustrating the phylogeny of *Truncospora wisconsinensis* and related species based on *tef1-α* sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Spirin *et al.* (2015).

The *tef1-α* dataset included sequences from 21 specimens representing 10 taxa. The dataset had an aligned length of 1115 characters, of which 862 characters are constant, 95 parsimony-uninformative and 158 parsimony-informative. MP analysis yielded eight equally parsimonious trees (TL = 396, CI = 0.778, HI = 0.222, RI = 0.870, RC = 0.678). Best model for *tef1-α* alignment was estimated and applied in the BI was as follows: GTR+I+G, lset nst = 6, rates =

invgamma; prset statefreqpr = dirichlet (1,1,1,1). BI resulted in a similar topology with an average standard deviation of split frequencies = 0.004582.

The phylogeny of the *tef1-α* data set (Fig. 2) agreed with the ITS phylogeny. The four specimens of the new taxon formed a highly supported clade (100% BS, 96% MP and 1.00 BPP), with *T. arizonica* and *T. ohiensis* that were closely related.

Taxonomy

Truncospora wisconsinensis C.L. Zhao & Pfister, *sp. nov.* (Figs. 3, 4)
MycoBankno.: MB 816122

Basidiocarps annual, pileate. Pileal surface white when fresh and white to pale whitish upon drying. Pore surface white; pores round. Hyphal system dimitic with subparallel to parallel, unbranched and slightly dextrinoid to non-dextrinoid skeletal hyphae. Basidiospores ellipsoid, hyaline, thick-walled, strongly dextrinoid, strongly cyanophilous, 9–11 × 6–7.5 µm.

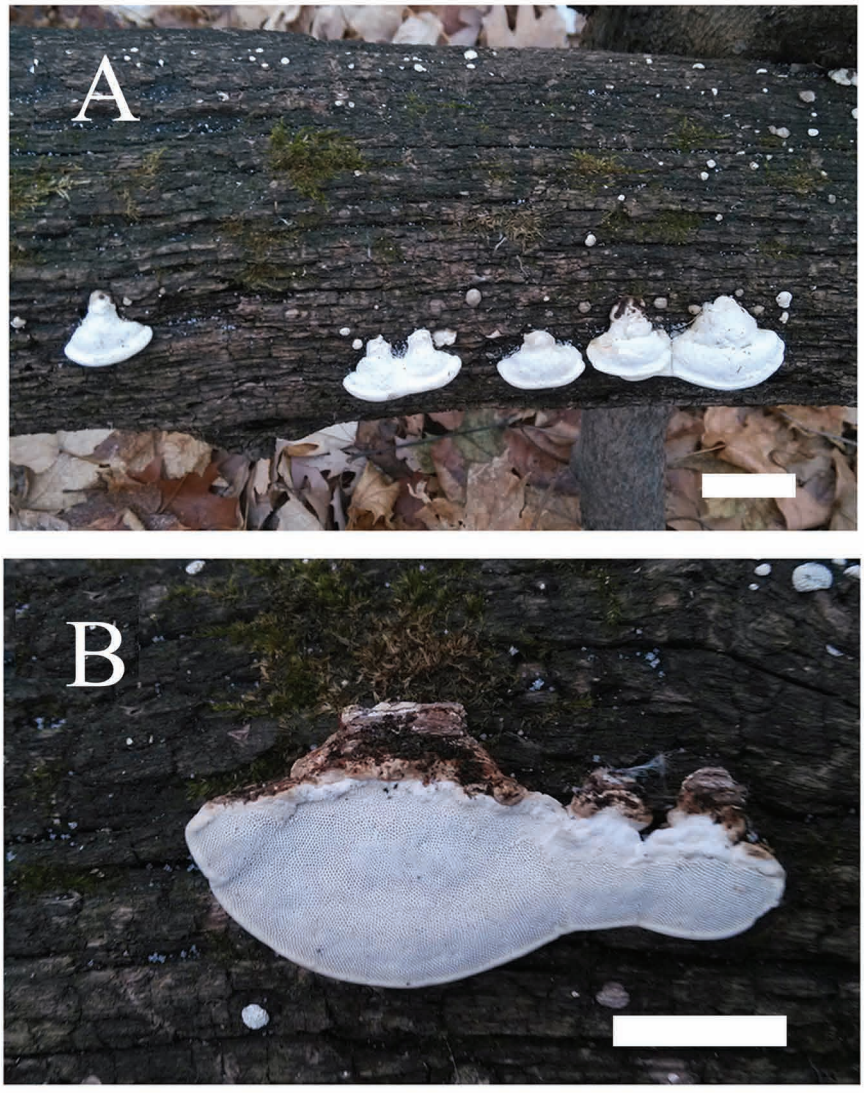

FIGURE 3. Basidiomata of *Truncospora wisconsinensis* (drawn from the holotype). Scale bars: a= 2 cm; b = 1cm.

Type.—**USA**. Wisconsin, Dane County, Madison, Lakeshore Nature Reserve, alt. 263 m, on fallen trunk of *Quercus alba*, 10 December 2014, *CLZhao 005/FH 00290969* (holotype, FH!).

Etymology.—*wisconsinensis* referring to the locality of the type specimens.

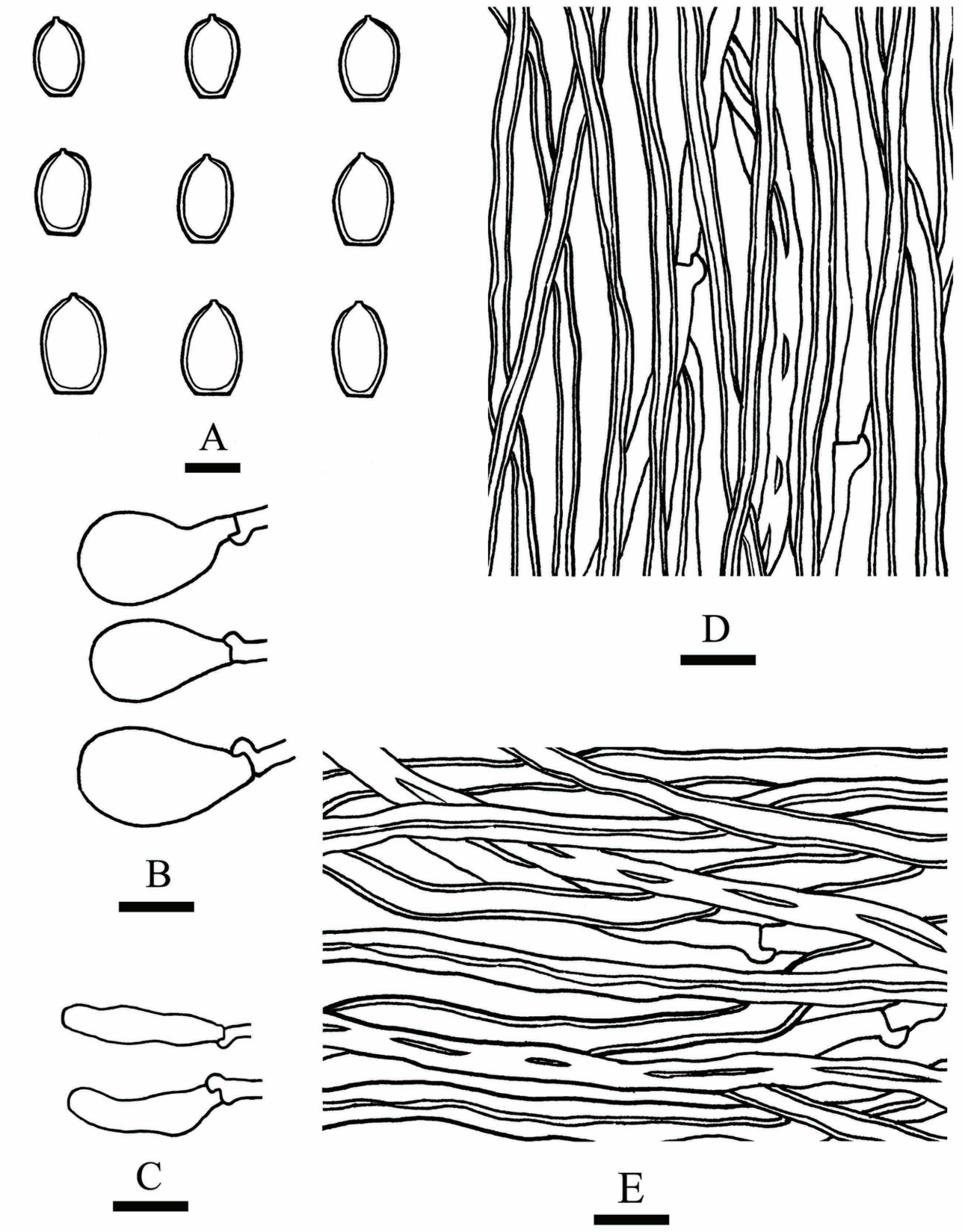

FIGURE 4. Microscopic structures of *Truncospora wisconsinensis* (drawn from the holotype). a Basidiospores. b Basidioles. c Cystidioles. d Hyphae from trama. e Hyphae from context. Bars: a = 5 µm; b–e = 10 µm.

Basidiomata.—Annual, pileate, solitary or gregarious, without odor or taste and corky when fresh, becoming hard corky upon drying. Pilei dimidiate, projecting up to 1.5 cm, 2.5 cm wide, 1.2 cm thick at centre. Pileal surface pure white when fresh and white to pale whitish upon drying (59-60), smooth, gently concentrically sulcate, glabrous,

margin sharp. Pore surface pure white when fresh (60), white to grayish upon drying (59-60); pores round, 3–5 per mm; dissepiments thick, entire. Sterile margin narrow, white, up to 0.5 mm wide (60). Context white to pale cream (21, 60), up to 7 mm thick. Tubes concolorous with pore surface, up to 5 mm long.

Hyphal structure.—Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae non- to slightly dextrinoid, strongly cyanophilous; hyphae unchanged in KOH.

Context trama.—Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2–4 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, unbranched, subparallel to parallel, 3–5.5 µm in diam.

Tube trama.—Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2–3.5 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, unbranched, subparallel to parallel, 2.5–4 µm in diam.

Hymenium.—Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 15–20 × 3.5–5.5 µm; basidia not seen; basidioles predominant, barrel-shaped to pear-shaped; basidiospores ellipsoid, truncate, hyaline, slightly thick-walled, smooth, strongly dextrinoid, strongly cyanophilous, (8–)9–11(–11.5) × (5.5–)6–7.5(–8) µm, L = 9.8 µm, W = 6.58 µm, Q = 1.44–1.58 (n = 120/4).

Additional specimens examined.—USA. Wisconsin, Dane County, Madison, Lakeshore Nature Reserve, alt. 263 m, on fallen trunk of *Quercus alba*, 10 December 2014*, CLZhao 006/FH 00290968*, *CLZhao 007/FH 00290967*, *CLZhao 008/FH 00290966* (paratype, FH!).

Discussion

Truncospora wisconsinensis is characterized by an annual basidiomata with a whitish pileus and pore surface, a dimitic hyphal system with non- to slightly dextrinoid skeletal hyphae, and ellipsoid, truncate, slightly thick-walled, strongly dextrinoid basidiospore. 9–11 × 6–7.5 µm.

Phylogenetic analyses demonstrated that *T. wisconsinensis* (Figs. 1, 2) is closely related to *T. ohiensis* and *T. arizonica*. Morphologically, *T. ohiensis* differs from *T. wisconsinensis* in its ochraceous to brownish-black pileus, smaller pores (5–6 per mm), trimitic hyphal system in tubes with branched, densely interwoven skeletal hyphae and large, and very thick-walled basidiospores (9.6–13.2 × 6.4–9.3 µm, Spirin *et al.* 2015). *Truncospora arizonica* is distinguished from *T. wisconsinensis* by its black pileus, thin-walled pore dissepiments, branched, strong dextrinoid skeletal hyphae and larger basidiospores (11.7–15.6 × 7.1–9.5 µm, Spirin *et al.* 2015).

Morphologically, *Truncospora oboensis* Decock has some similarities with *T. wisconsinensis* that includes the annual basidiomata with white pileus and similar pores (3–4 per mm); it differs in its angular pores with thin-walled dissepiments and larger basidiospores (11–14 × 6.5–8.5 µm, Decock 2011). *Truncospora detrita* may be confused with *T. wisconsinensis* since it has similar basidiospores (10.3–12.5 × 6.2–7.8 µm), but *T. detrita* can be distinguished from *T. wisconsinensis* by larger basidiomata and a dark reddish brown crust on the pileus surface (Decock & Ryvarden 1999).

The genus *Truncospora* has been often treated as a synonym of *Perenniporia* (Ryvarden 1972, 1991, Gilbertson & Ryvarden 1987, Ryvarden & Melo 2014). Decock & Ryvarden (1999) concluded that *Perenniporia detrita* (Berk.) Ryvarden, *P. ochroleuca* (Berk.) Pilát and *P. ohiensis* (Berk.) Ryvarden formed a morphologically homogeneous alliance that should be recognized at the genus level for which the name *Truncospora* was available. These taxa differ from *Perenniporia* by having relatively small, pileate basidiomata, and ellipsoid, large, apically truncate and dextrinoid basidiospores. Molecular phylogenetic analysis based on the IST+LSU rDNA gene regions supported *Truncospora* as monophyletic and places the genus as a clade distinct from the *Perenniporia* sensu stricto clade (Zhao & Cui 2013).

In the present study, based on the framework provided by Spirin *et al.* (2015), three additional species, *T. detrita*, *T. macrospora* and *T. wisconsinensis*, are included in the ITS analysis. *Truncospora detrita* forms a monophyletic entity with a low support. Although *T. macrospora* groups with *T. ochroleuca*, the former differs from *T. ochroleuca* by having annual basidiocarps with a distinct dark brownish crust and larger basidiospores (16.5–19.5 × 8–9.5 µm). Morphological characters are very important when delimiting taxa in *Perenniporia* s.l. in previous studies (Ryvarden 1972, 1991, Gilbertson & Ryvarden 1987, Decock & Ryvarden 1999, Decock 2001, Robledo *et al.* 2009, Zhao & Cui 2013). Spirin *et al.* (2015) employed *tefl-α to* study the *T. ohiensis* group; that dataset of *tef1-α* produced 7 strongly supported terminal clades. In the present study, *Truncospora ohiensis* group is easily separated 9 clades based on *tef1-α* sequence, so the *tef1-α* may be a potential marker to apply on *Perenniporia* s.l. complex species in future.

Key to accepted species of *Truncospora* in USA

1. Pores 3–5 per mm 2
   Pores 5–7 per mm 4
2. Pileus light brown to black, basidiospores >12 µm long ..... *Truncospora arizonica*
   Pileus white to ochraceous, basidiospores < 12µm long 3
3. Pores round, dissepiments thick, skeletal hyphae unbranched, parallel ..... *Truncospora wisconsinensis*
   Pores angular, dissepiments thin, skeletal hyphae branched, interwoven ..... *Truncospora tropicalis*
4. Species temperate in distribution; basidiomata brown to black ..... *Truncospora ohiensis*
   Species subtropical or tropical in distribution; basidiomata white to pale cream to ochraceous 5
5. Context thicker than the tube layer, Florida ..... *Truncospora floridana*
   Context distinctly thinner than the tube layer, coastal Gulf of Mexico ..... *Truncospora mexicana*

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