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Truncospora macrospora sp. nov. (Polyporales) from Southwest China based on morphological and molecular data

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Abstract

A new polypore, *Truncospora macrospora*, is described from Southwest China on the basis of morphological and molecular characters. *T. macrospora* is characterized by an annual habit, pileate basidiocarps with a distinct crust, dimitic hyphal system with dextrinoid skeletal hyphae, and large ellipsoid, truncate, strongly dextrinoid basidiospores (16.5–19.5 × 8.0–9.5 µm). Molecular study based on sequence data from the nuclear ribosomal ITS and LSU regions supported the position of the new species in *Truncospora*, forming monophyletic lineage with strong support (100% BP, 1.00 BPP). *Truncospora* and *Perenniporiella* were proved to be sister-genera and grouped with other genera of *Perenniporia sensu lato* within the core polyporoid clade. An identification key to the species of *Truncospora* worldwide is provided.

Key words: Molecular phylogeny, *Perenniporia*, polypore, taxonomy, wood-inhabiting fungi

Introduction

Truncospora Pilát was established by Pilát (1953), and it is typified by *T. ochroleuca* (Berk.) Pilát. The genus is characterized by relatively small, pileate basidiocarps (about 1.5–3 cm long, 2.5–3.5 cm wide, and 1–4 cm thick), indextrinoid to dextrinoid skeletal hyphae, and truncate, strongly dextrinoid basidiospores (Decock 2011, Zhao *et al*. 2013). During the investigations on wood-inhabiting fungi in Southwest China, an undescribed species matching the concepts of *Truncospora* was found. To confirm the affinity of the new taxon to *Truncospora*, phylogenetic analysis was carried out based on ITS and nLSU sequences.

Materials and methods

Morphological studies.—The studied specimens were deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic routine followed Dai *et al.* (2010). Sections were studied at magnification up to × 1000 using a Nikon E80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements and drawings were made from slide preparations stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. Presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range, and were given in parentheses. In the text the following abbreviations were used: IKI = Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, 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 = number of spores measured from given number of specimens. Special color terms followed Petersen (1996).

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Molecular procedures and phylogenetic analyses.—The fungal taxa used in this study were listed in Table 1. Phire® Plant Direct PCR Kit (Finnzymes) was used to obtain PCR products from dry specimens, according to the manufacturer's instructions. A small piece of dried fungus was lysed in 30 µl Dilution Buffer for DNA extraction. Nuclear ITS region and partial LSU were amplified with the primer pairs ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White *et al.* 1990) and LR0R (ACC CGC TGA ACT TAA GC) and LR7 (TAC TAC CAC CAA GAT CT) (http://www.biology.duke.edu/fungi/mycolab/primers.htm), respectively. The PCR procedure for ITS was as follows: initial denaturation at 98°C for 5 min, followed by 39 cycles at 98°C for 5 s, 58°C for 5 s and 72°C for 5 s, and a final extension of 72°C for 10 min. The only difference of the LSU amplification procedure was its annealing temperature 48°C. DNA sequencing was performed at Beijing Genomics Institute. All newly generated sequences have been submitted to GenBank and were listed in Table 1.

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

Species name	Sample no.	GenBank accession no.	
		ITS	LSU
Abundisporus fuscopurpureus (Pers.) Ryvarden	Cui 8638	JN048771	JN048790
Abundisporus pubertatis (Lloyd) Parmasto	Dai 12140	JN048772	JN048791
Abundisporus violaceus (Wakef.) Ryvarden	MUCL 38617	FJ411100	FJ393867
Basidioradulum radula (Fr.) Nobles	NH 9453	AF347105	AF347105
Bjerkandera adusta (Willd.) P. Karst.	DAOM 215869	DQ060097	AF287848
Boletopsis leucomelaena (Pers.) Fayod	ID 1527	DQ484064	DQ154112
Byssomerulius corium (Pers.) Parmasto	KHL 8593	AY463389	AY586640
Ceraceomyces violascens (Fr.) Jülich	KHL 11169	EU118611	EU118612
Ceriporia viridians (Berk. & Broome) Donk	KHL 8765	AF347109	AF347109
Ceriporiopsis aneirina (Sommerf.) Domański	MUAF 888	EU340895	EU368503
Donkioporia expansa (Desm.) Kotl. & Pouzar	MUCL 35116	FJ411104	FJ393872
Globulicium hiemale (Laurila) Hjortstam	5444b	DQ873595	DQ873595
Gloeoporus dichrous (Fr.) Bres.	KHL 11173	EU118627	EU118627
Gloeoporus pannocinctus (Romell) J. Erikss.	FCUG 2109	AF141612	AF141612
Hornodermoporus latissima (Berk.) Teixeira	Cui 6625	HQ876604	JF706340
Hornodermoporus martia (Berk.) Teixeira	Cui 7992	HQ876603	HQ654114
Hornodermoporus martia	MUCL 41677	FJ411092	FJ393859
Hornodermoporus martia	MUCL 41678	FJ411093	FJ393860
Hyphoderma capitatum J. Erikss. & Å. Strid	KHL 8464	DQ677491	DQ677491
Hyphoderma orphanellum (Bourdot & Galzin) Donk	NH 12208	DQ677500	DQ677500
Hyphodermella corrugata (Fr.) J. Erikss. & Ryvarden	KHL 3663	EU118630	EU118630
Meruliopsis taxicola (Pers.) Bondartsev	Kuljok 00/75	EU118648	EU118648
Microporellus violaceo-cinerascens (Petch) A. David & Rajchenb.	MUCL 45229	FJ411106	FJ393874
Perenniporia medulla-panis (Jacq.) Donk	MUCL 49581	FJ411088	FJ393876
Perenniporia medulla-panis	MUCL 43250	FJ411087	FJ393875
Perenniporia medulla-panis	Cui 3274	JN112792	JN112793
Perenniporia bannaensis B.K. Cui & C.L. Zhao	Cui 8560	JQ291727	JQ291729
Perenniporia bannaensis	Cui 8562	JQ291728	JQ291730
Perenniporia rhizomorpha B.K. Cui, Y.C. Dai & Decock	Cui 7507	HQ654107	HQ654117
Perenniporia rhizomorpha	Dai 7248	JF706330	JF706348
Perenniporia substraminea B.K. Cui & C.L. Zhao	Cui 10177	JQ001852	JQ001844

...... *continued on the next page*

Species name	Sample no.	GenBank accession no.	
		ITS	LSU
Perenniporia substraminea	Cui 10191	JQ001853	JQ001845
Perenniporiella chaquenia Robledo & Decock	MUCL 47647	FJ411083	FJ393855
Perenniporiella chaquenia	MUCL 47648	FJ411084	FJ393856
Perenniporiella micropora (Ryvarden) Decock & Ryvarden	MUCL43581	FJ411086	FJ393858
Perenniporiella neofulva (Lloyd) Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852
Perenniporiella pendula Decock & Ryvarden	MUCL 46034	FJ411082	FJ393853
Phanerochaete sordida (P. Karst.) J. Erikss. & Ryvarden	KHL 12054	EU118653	EU118653
Poriodontia subvinosa Parmasto	H 10493	FN907920	FN907920
Pyrofomes demidoffii (Lév.) Kotl. & Pouzar	MUCL 41034	FJ411105	FJ393873
Rickenella mellea (Singer & Clem.) Lamoure	Lamoure 74	U66438	U66438
Trametes elegans (Spreng.) Fr.	Dai 10748	JN048766	JN048785
Trametes hirsuta (Wulfen) Pilát	Cui 7784	JN048768	JN048787
Trametes ochracea (Pers.) Gilb. & Ryvarden	PRM 900601	AY684177	AY855908
Trichaptum abietinum (Dicks.) Ryvarden	FCUG 2581	AF141636	AF141636
Truncospora detrita (Berk.) Decock	MUCL 42649	FJ411099	FJ393866
Truncospora macrospora B.K. Cui & C.L. Zhao	Yuan 3777	JX941574[a]	JX941597[a]
Truncospora macrospora	Cui 8106	JX941573[a]	JX941596[a]
Truncospora ochroleuca (Berk.) Pilát	Cui 8805	JX941575[a]	JX941591[a]
Truncospora ochroleuca	Cui 8818	JX941576[a]	JX941592[a]
Truncospora ochroleuca	Cui 8324	JX941577[a]	JX941593[a]
Truncospora ochroleuca	Cui 8704	JX941578[a]	JX941594[a]
Truncospora ochroleuca	Dai 11490	JX941579[a]	JX941595[a]
Truncospora ochroleuca	Dai 11809	JX941580[a]	JX941598[a]
Truncospora ochroleuca	Cui 8335	JX941581[a]	JX941599[a]
Truncospora ochroleuca	Cui 2780	JX941582[a]	JX941600[a]
Truncospora ochroleuca	Cui 7388	JX941583[a]	JX941601[a]
Truncospora ochroleuca	Cui 5671	JX941584[a]	JX941602[a]
Truncospora ochroleuca	Cui 5673	JX941585[a]	JX941603[a]
Truncospora ochroleuca	Cui 5674	JX941586[a]	JX941604[a]
Truncospora ochroleuca	Cui 8870	JX941587[a]	JX941605[a]
Truncospora ochroleuca	Dai 11711	JX941588[a]	JX941606[a]
Truncospora ochroleuca	Cui 2703	JX941589[a]	JX941607[a]
Truncospora ochroleuca	Dai 11486	HQ654105	JF706349
Truncospora ochroleuca	MUCL 39726	FJ411098	FJ393865
Truncospora ohiensis (Berk.) Pilát	MUCL 41036	FJ411096	FJ393863
Truncospora ohiensis	Cui 5714	HQ654103	HQ654116
Vanderbylia fraxinea (Bull.) D.A. Reid	DP 83	AM269789	AM269853
Vanderbylia fraxinea	Cui 7154	HQ654095	HQ654110
Vanderbylia fraxinea	Cui 8871	JF706329	JF706345
Vanderbylia fraxinea	Cui 8885	HQ876611	JF706344
Vanderbylia robiniophila (Murrill) D.A. Reid	Cui 5644	HQ876609	JF706342
Vanderbylia robiniophila	Cui 7144	HQ876608	JF706341
Vanderbylia robiniophila	Cui 9174	HQ876610	JF706343
Vanderbylia vicina (Lloyd) D.A. Reid	MUCL 44779	FJ411095	FJ393862

[a] Sequences newly generated in this study.

New sequences were aligned with additional sequences from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thomson *et al.* 1997). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimize the alignment. Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13560?x-access-code=9b8f9e3772b61ed90981422ab29cf727&format=html; submission ID 13560).

Maximum parsimony analysis was applied to the combined dataset of ITS and nLSU sequences. *Boletopsis leucomelaena* (Pers.) Fayod, a member of the Thelephorales, was chosen as the outgroup. 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 1,000 random sequence additions. Max-trees were set to 5,000, 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 (MPT) generated.

MrMODELTEST2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for the combined dataset for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites. Four Markov chains were run for 2 runs from random starting trees for 1 million generations, and trees were sampled every 100 generations. The burn-in was set to discard 25% of the trees. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) and Bayesian posterior probabilities (BPP) greater or equal than 75% (MP) and 0.95 (BPP) respectively were considered as significantly supported.

Results

Taxonomy

Truncospora macrospora B.K. Cui & C.L. Zhao, *sp. nov.* (Figs. 1–2)
MycoBank no.: MB 801932

Differs from other *Truncospora* species by annual basidiocarps with a distinct dark brownish crust, a dimitic hyphal system with dextrinoid skeletal hyphae, and larger basidiospores (16.5–19.5 × 8.0–9.5 µm).

Type.—CHINA. Yunnan Prov.: Baoshan, Gaoligong Mountains, alt. 2400 m, on fallen angiosperm branch, 25 October 2009, *Cui 8106* (holotype, BJFC!).

Etymology.—*macrospora* (Lat.) referring to the large basidiospores.

Fruitbody.—Basidiocarps annual, pileate, sessile, solitary, corky to wood corky upon drying. Pileus usually semicircular, projecting up to 1.5 cm, 3.5 cm wide, and 0.5 cm thick at base. Pileal surface clay-buff to orange-brown when fresh, reddish brown to greyish brown when drying, with a distinct dark brownish crust, glabrous; margin obtuse. Pore surface cream to cream buff when fresh, becoming buff upon drying; pores regular, more or less round, 3–4 per mm; dissepiments thick, entire. Sterile margin narrow, cream, up to 1 mm wide. Context cinnamon-buff to buff, soft corky, about 1 mm thick. Tubes concolorous with pore surface, woody hard, up to 4 mm thick.

Hyphal structure.—Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae dextrinoid, CB+; hyphae slightly swollen in KOH.

Context.—Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 3.0–4.5 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, unbranched, interwoven, 4.5–6.5 µm in diam.

FIGURE 1. Basidiocarps of *Truncospora macrospora* B.K. Cui & C.L. Zhao sp. nov. from holotype. Scale bars = 1cm.

Tubes.—Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2.0–4.0 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, unbranched, interwoven, 3.0–5.5 µm in diam. Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 17–23 × 4.0–5.0 µm; basidia barrel-shaped, with four sterigmata and a basal clamp connection, 20–29 × 11–15.5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

Spores.—Basidiospores ellipsoid, truncate, hyaline, thick-walled, smooth, strongly dextrinoid, CB+, (16–)16.5–19.5(–20) × (7.5–)8.0–9.5(–10) µm, L = 18.2 µm, W = 8.67 µm, Q = 2.07–2.11 (n = 60/2).

Additional specimen examined.—*Truncospora macrospora*: CHINA. Yunnan Prov.: Baoshan, Gaoligong Mountains, alt. 2450 m, on fallen angiosperm branch, 22 September 2007, *Yuan 3777* (paratype, BJFC!).

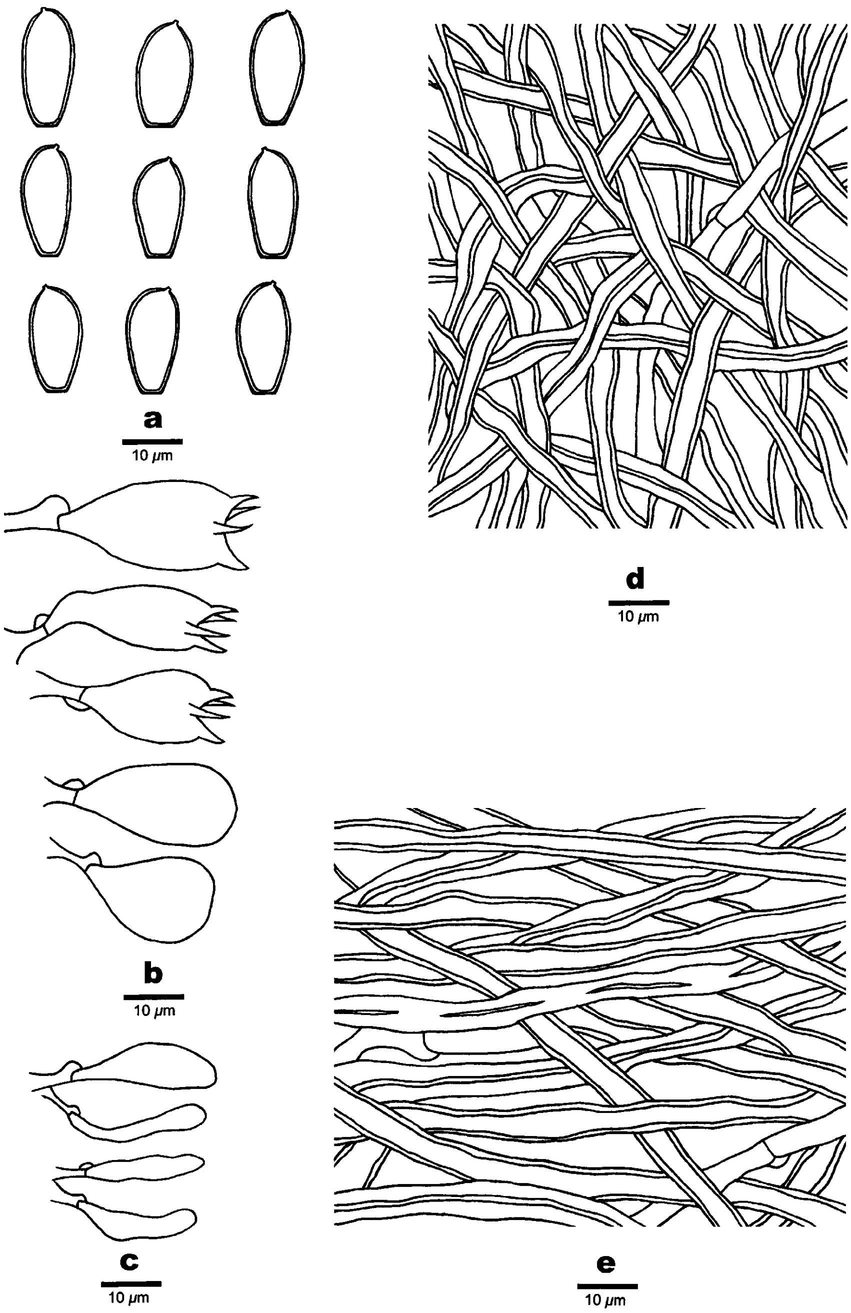

FIGURE 2. Microscopic structures of *Truncospora macrospora* B.K. Cui & C.L. Zhao sp. nov. (drawn from the holotype). a Basidiospores. b Basidia and basidioles. c Cystidioles. d Hyphae from trama. e Hyphae from context.

Molecular phylogeny

The combined ITS + nLSU dataset includes sequences from 75 fungal specimens representing 44 taxa. The dataset has an aligned length of 2089 characters in the dataset of which 1285 characters are constant, 184 are variable and parsimony-uninformative, and 620 are parsimony-informative. Maximum Parsimony analysis yielded 88 equally parsimonious trees (TL = 1402, CI = 0.397, RI = 0.705, RC = 0.280, HI = 0.603), and one of the maximum parsimonious trees was shown in Fig. 3. The best model for the combined ITS + nLSU dataset for Bayesian inference was a GTR+I+G model, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in a same topology with an average standard deviation of split frequencies = 0.007133.

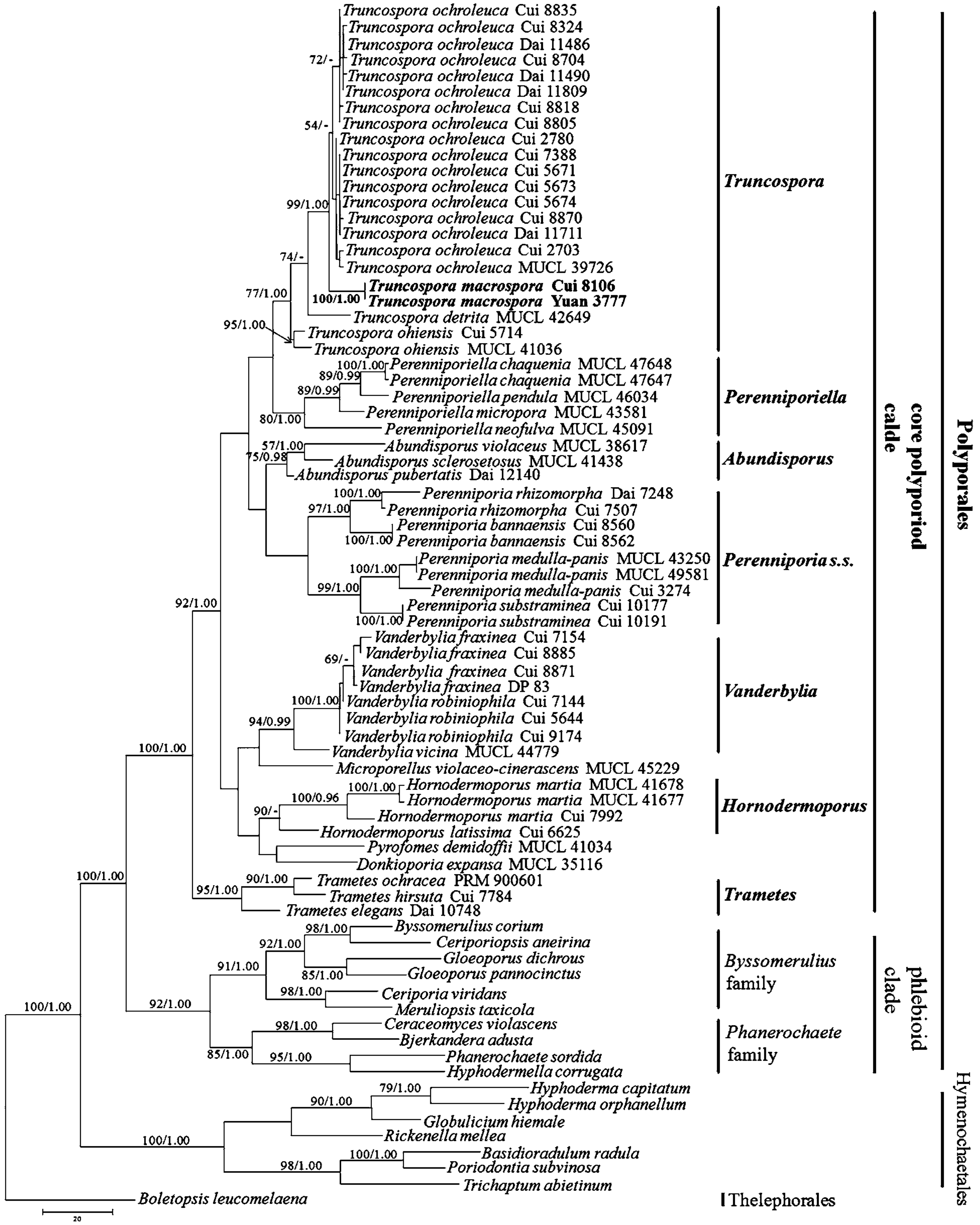

FIGURE 3. Strict consensus tree illustrating the phylogeny of the new species and related species generated by Maximum Parsimony based on combined ITS + LSU sequences. Parsimony bootstrap values (before the slash markers) higher than 50% and Bayesian posterior probabilities (after the slash markers) more than 0.95 were indicated along branches.

Discussion

Morphologically, *Truncospora macrospora* is characterized by its annual basidiocarps with a distinct dark brownish crust, a dimitic hyphal system with dextrinoid skeletal hyphae, and ellipsoid, truncate, strongly dextrinoid and large basidiospores (16.5–19.5 × 8.0–9.5 µm). Phylogenetically, two samples of *T. macrospora* formed a distinct lineage. *T. macrospora* clustered with *T. ochroleuca*, and grouped with *T. detrita* and *T. ohiensis*. Both morphology and rDNA data confirmed that the two samples represent a new species in *Truncospora*.

Until now, five species have been accepted in *Truncospora*. The morphological data for *T. detrita*, *T. macrospora*, *T. oboensis*, *T. ochroleuca* and *T. ohiensis* are listed in Table 2. *T. ochroleuca* may be confused with *T. macrospora* by sharing pileate basidiocarps, similar pore size (2–4 per mm), dextrinoid skeletal hyphae and ellipsoid, truncate, strongly dextrinoid basidiospores; however, *T. ochroleuca* is distinguished from *T. macrospora* by having cream to ochraceous pileus, and smaller basidiospores (14.0–17.0 × 7.0–9.5 µm, Decock 2011). *T. ohiensis* shares pileate basidiocarps, buff pore surface and dextrinoid basidiospores; however, it differs by its perennial basidiocarps, smaller pores (5–7 per mm), and smaller basidiospores (13.0–16.0 × 7.0–10.0 µm, Gilbertson & Ryvarden 1987). *T. detrita* is similar to *T. macrospora* by having pileate basidiocarps, similar pores (4–5 per mm) and strongly dextrinoid basidiospores; however, *T. detrita* has perennial basidiocarps and smaller basidiospores (10.3–12.5 × 6.2–7.8 µm, Decock & Ryvarden 1999). *T. oboensis* Decock differs from *T. macrospora* in having white pileus, and smaller basidiospores (11.0–14.0 × 6.5–8.5 µm, Decock 2011).

Truncospora has been usually treated as a synonym of *Perenniporia* Murrill (Ryvarden 1972, 1991, Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994, Dai *et al.* 2002). Decock & Ryvarden (1999) concluded that *P. detrita*, *P. ochroleuca* and *P. ohiensis* formed a morphologically homogeneous alliance, which could be recognized at the genus level, differing from *Perenniporia* by having relatively small, pileate basidiocarps, unbranched skeletal hyphae and truncate, strongly dextrinoid basidiospores, and the name *Truncospora* would be available for it. Recently, phylogenetic analysis based on rDNA sequence data by Robledo *et al.* (2009) and Zhao *et al.* (2013) showed that these three taxa formed a monophyletic clade distinct from *Perenniporia* sensu stricto, and should be recognized at genus level. In the present study (Fig. 3), *Truncospora* forms a monophyletic entity, is sister to *Perenniporiella* Decock & Ryvarden with a lower support less than 75% MP and 0.95 BPP, and then grouped with other genera of *Perenniporia sensu lato* within the core polyporoid clade, and it is distinct from *Perenniporia sensu stricto*. This indicates that *Truncospora* could be recognized as a separate genus in this group.

TABLE 2. Morphological data for *Truncospora* species.

Species	Specimens	Basidiospores (µm)	Average (µm)	Pores/mm
T. detrita	Spruce no.49 (Type)	(10.1–)10.1–11.7(–11.7) ´ (5.5–)5.9–7.8(–7.8)	10.9 ´ 6.8	4–5
T. detrita	MUCL 40753	(10.7–)11.0–12.5(–13.0) ´ (6.8–)7.0–7.8(–8.0)	11.9 ´ 7.3	4–5
T. macrospora	Cui 8106 (Type)	(16.5–)17.5–20.0(–22.0) ´ (8.0–)8.5–10.0(–10.5)	18.7 ´ 9.0	3–4
T. macrospora	Yuan 3777	(16.0–)16.5–19.0(–20.0) ´ (7.5–)8.0–9.5(–10.0)	18.0 ´ 8.5	3–4
T. oboensis	MUCL 53565 (Type)	(10.0–)11.0–14.0(–15.0) ´ (6.5–)6.5–8.5(–9.5)	12.7 ´ 7.7	3–4
T. ochroleuca	Swan River 141 (Type)	(12.0–)14.0–17.0(–18.0) ´ 7.0–9.5	15.4 ´ 8.3	3–4
T. ochroleuca	Gilsenius 721	12.0–17 .0´ 7.0–10.0	—	2–4
T. ochroleuca	Cui 5671	(12.5–)13.0–15.0(–15.5) ´ (7.0–)7.5–9.0(–9.5)	14.5 ´ 8.0	4–5
T. ochroleuca	Cui 8329	(13.0–)13.5–16.0(–17.0) ´ (7.0–)7.5–8.5(–9.0)	15.0 ´ 8.0	3–4
T. ohiensis	RLG 10829	13.0–16 ´ 7.0–10.0	—	5–7
T. ohiensis	Cui 5714	(10.5–)11.0–13.5(–14.0) ´ (6.5–)7.0–8.5(–9.0)	12.5 ´ 7.7	5–7

Key to accepted species of *Truncospora* worldwide

1. Basidiocarps with a distinct crust .... *Truncospora macrospora*
- Basidiocaps without a crust 2
2. Arboriform skeletal hyphae present.......... *Truncospora detrita*
- Arboriform skeletal hyphae absent 3
3. Pileus white, soft and spongy when fresh.......... *Truncospora oboensis*
- Pileus ochraceous to black, soft corky when fresh 4
4. Pores 5–7 per mm, pileus light brown to black .... *Truncospora ohiensis*
- Pores 2–5 per mm, pileus cream to ochraceous.......... *Truncospora ochroleuca*

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