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Studies in the *Truncospora ohiensis – T. ochroleuca* group (Polyporales, Basidiomycota)

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With 4 figures and 1 table

Abstract: Based on both morphological and phylogenetic analyses, 7 species are recognized in the *Truncospora ohiensis* group and 6 of them are described as new. *T. ohiensis* s.str. occurs in the American North-East; its closest relatives are *T. arizonica*, the long-spored species from the South-West USA, and *T. ornata* from East Asia. *T. atlantica* is another long-spored member of the group, distributed in Macaronesia and the Iberian Peninsula. The dwarf-sized and pale-coloured *T. mexicana* is described from the western coast of the Gulf of Mexico, and its look-alikes, *T. floridana* and *T. tropicalis*, are from the Caribbean. The identification key for the *T. ohiensis – ochroleuca* group is provided.

Key words: polypores, taxonomy, *Perenniporia*.

Introduction

The genus *Truncospora* was established by Pilát (1953) with two species, *T. ochroleuca* (Berk.) Pilát (the genus type) and *T. ohiensis* (Berk.) Pilát, and later it was placed into the synonyms of *Perenniporia* (Ryvarden 1972). Recent DNA-based studies indicated that *Truncospora* should be separated from other genera in the so called "core polyporoid clade" (Robledo et al. 2009, Zhao & Cui 2013). Decock & Ryvarden (1999) and Decock (2011) emphasized some morphological features (i.e., large truncate basidiospores with a germ pore and skeletal hyphae variably branched at their distal parts) as unique to this genus.

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Zhao & Cui (2013) described a new species, *T. macrospora*, and provided a key to the species of *Truncospora* worldwide; *T. ohiensis* was included as a species occurring in both North America and East Asia. However, DNA data and morphological study of specimens collected in North America, Europe and East Asia and labelled as *T. ohiensis* or *T. ochroleuca* showed that 7 closely related species exist. Their descriptions are presented below.

Materials and methods

The specimens from mycological herbaria of Botanical Museum, University of Helsinki (H), New York Botanical Garden (NY), Center for Forest Mycology Research (CFMR), Mycological Herbarium of the Department of Biology, University of West Bohemia (KBI), and Institute of Biology and Soil Science, Russian Academy of Sciences (VLAM), as well as from a private herbarium of the author JV, were studied. Herbarium acronyms are given according to Index Herbariorum (Thiers 2014). Type specimens are deposited in H. Morphological techniques of this study follow Miettinen et al. (2006, 2012). Microscopic structures were studied and measured with Leitz Diaplan microscope (×1250 magnification). Measurements were done in Cotton Blue using phase contrast illumination and oil immersion (with a subjective accuracy of 0.1 μm – Miettinen et al. 2006). The next abbreviations are used in the species descriptions: d/p – dissepiments thickness/pore diameter ratio (a quotient counted from the measurements of 10 subjectively chosen tube dissepiments and equal number of pores), L – mean spore length, W – mean spore width, Q' – spore length/width ratio, Q – mean spore length/width ratio (counted for each specimen measured).

DNA isolation and sequencing: 0.02–0.2 g of the context tissue was disintegrated for 30 s with a steel ball mixer mill MM301 RETSCH at room temperature. DNA was isolated using CTAB/NaCl extraction buffer as described by Murray & Thompson (1980), followed by two times repeated extraction with chloroform, and isopropanol precipitation. Crude DNA was dissolved in 100 μL of sterile water and further purified using Wizard Clean Up kit PROMEGA. 0.5 ul of resulting DNA solution of 50 μL was used as a template for amplification with ITS5 and ITS4 primers (White et al. 1990), or EF983F and EF2218R primers (Matheny et al. 2007) in 25 μL reaction mixture using 55° C annealing temperature. With ITS primers, 30 cycles were used and 0.3 μL of crude amplified DNA was directly applied for sequencing. With EF primers, 35 cycles PCR were used and the amplified DNA band was purified from agarose gel using Machery-Nagel NucleoSpin kit. Sequencing from ITS1 and ITS4 primers, resp. from EF983F, EF2218R and EF1567R primers was performed in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on ABI 3730xl DNA analyzer, using BigDye Terminator 3.1 kit.

Phylogenetic analysis We prepared two datasets for analysis: ITS dataset containing our sequences of 18 *Truncospora* specimens (two of them yielded 2 haplotypes), a sequence of *Perenniporia medulla-panis* (GenBank KJ410710) for rooting the phylogeny, and four similar sequences of *P. ohiensis/ochroleuca* from GenBank. *Tef*1 dataset contained only newly generated sequences and *P. medulla-panis* sequence (GenBank KJ410711). After alignment in MEGA5 (Tamura et al. 2011), ITS dataset yielded 634 characters of which 126 variable and 76 parsimony informative. In the *tef*1 dataset, unaligned 3' tail was removed first (about 30 bp), yielding 1152 aligned characters, 258 variable and 159 parsimony informative. Evolutionary analyses were conducted in MEGA5 using the Maximum Likelihood (ML) method.

Results

The species of the *T. ohiensis* group share all principal features characterizing the genus in general. Skeletal hyphae are dextrinoid and cyanophilous (although those reactions vary a bit from species to species), unbranched along their most length, except distal

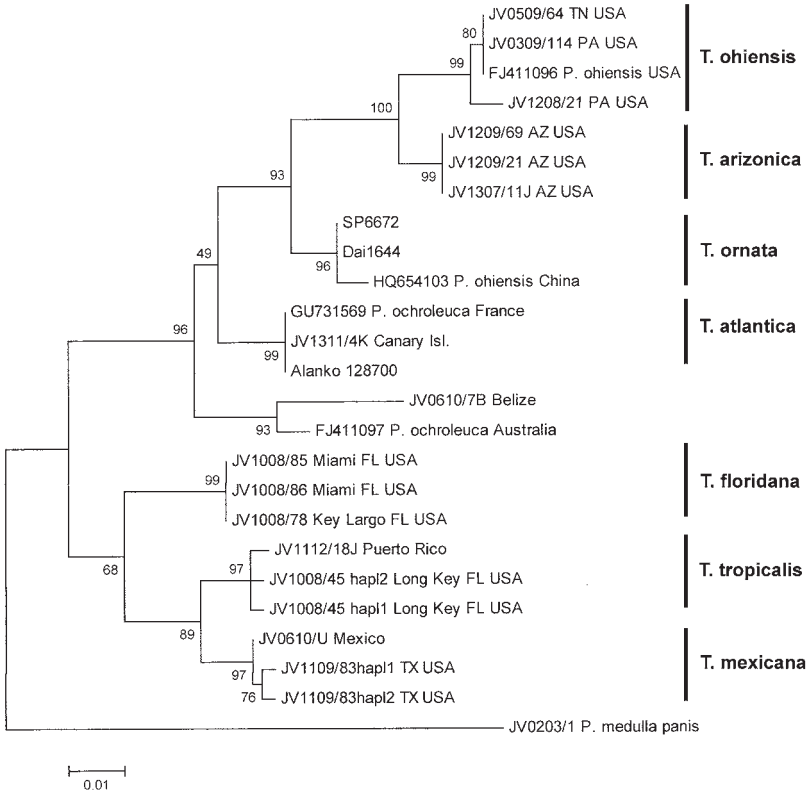

Fig. 1. Phylogenetic relationships of 22 *Truncospora* specimens inferred with ITS rRNA sequences. *Perenniporia medulla-panis* was used to root the tree. Topology from maximum likelihood (ML) analysis. Support values along branches from ML bootstrap (1000 replicates). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. GenBank numbers with assigned names indicate sequences retrieved from GenBank. Other GenBank numbers in "Specimens examined".

parts located predominantly at the dissepiment edges or in upper layer of tube trama. Basidia in all species studied are rather uniform, clavate-pedunculate, with strongly inflated epibasidial part, 25–35 × 10–14 μm, and basidiospores are unevenly thick-walled, with a germ pore and usually with a large oil-drop. The spore wall thickness is an important character in this group; however, the wall changes depending on age, partly degrading in mature and, especially, in old spores.

Analysis of ITS sequences of *T. ohiensis* coll. from East Asia, Macaronesia, USA, Mexico and Puerto-Rico showed very distinct sequence differences between geographical samples, and ITS based phylogeny produced 7 strongly supported terminal clades (Fig. 1). Dataset of *tef*1 sequences prepared from the same samples also showed considerable sequence differences of ecologically-determined samples and nearly the same topology (Fig. 2), strongly suggesting that 7 species are involved. This was

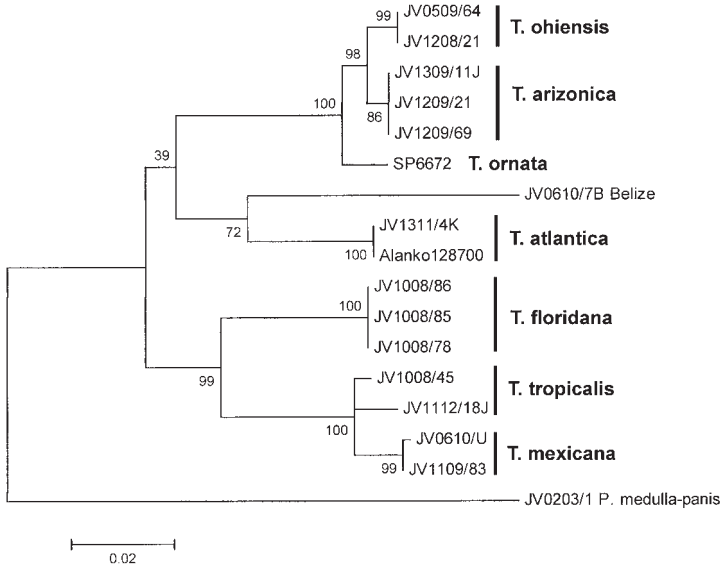

Fig. 2. Phylogenetic relationships of 16 *Truncospora* taxa inferred with *tef*1sequences. Other data as in Fig. 1.

confirmed by painstaking micro- and macroscopic study of many new collections and older herbarium specimens (see below).

In DNA terms, the *T. ohiensis* group is not a monophyletic unity. The members of the *T. ochroleuca* complex split it at least in two subgroups (see Figs 1, 2). One of them consists of *T. arizonica*, *T. ohiensis* and *T. ornata* and thus it encompasses temperate species, while another subgroup includes the subtropical or tropical *T. floridana*, *T. mexicana* and *T. tropicalis*. The Macaronesian *T. atlantica* position is somewhat ambiguous in our phylogenies, quite close to the Australian *T. ochroleuca*; more samples are needed to reveal its phylogenetic relations.

The closely related *T. ochroleuca* group differs in having distinctly wider skeletal hyphae (4–6 μm) in both context and trama; it seems to include many species, too. This group is not yet studied properly, and many older names should be checked for establishing modern species concepts therein.

Truncospora arizonica Spirin & Vlasák, **sp. nov.** Fig. 4

Pileus perennis, consoliformis, superficies nigra, rimosa, poris 3–4 per mm. Systema hypharum dimiticum. Basidiosporae crassitunicatae, ellipsoideae, truncatae, 11.7–15.6 × 7.1–9.5 μm, dextrinoideae, cyanophilae.

Holotype: USA. Arizona: Cochise Co., Portal Area, Chiricahua Mts., *Quercus arizonica*, 6.IX.2012 Vlasák 1209/69 (H).

Mycobank no. MB808453

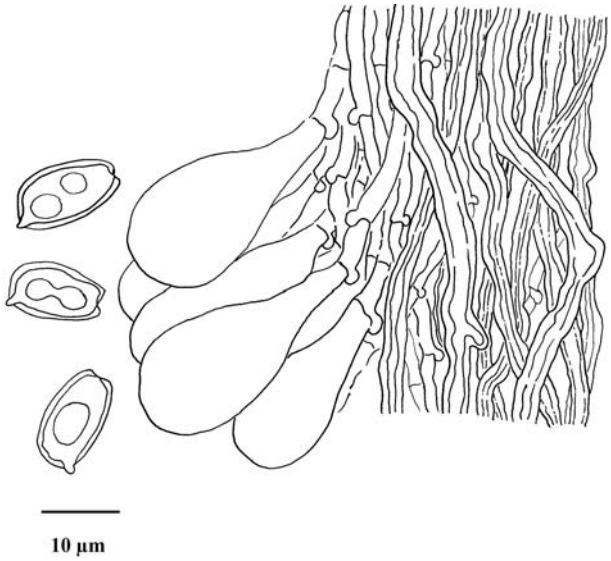

Fig. 3. Hymenium and trama of *T. ohiensis* (isotype).

Basidiocarps perennial, sessile, dimidiate, solitary, 20–30 mm long, 10–20 mm wide. Upper surface first ochraceous to pale brown, in mature basidiocarps black, matt, indistinctly striate, with several unclear annual zones, sometimes longitudinally cracking. Margin sharp but rather thick, even, fertile. Pore surface first pale cream-colored, later pale ochraceous; pores regular, circular, 3–4 per mm, dissepiments normally rather thin, entire (d/p = 1), pubescent under lens. Section: pileal crust brown to black, 0.2–0.8 mm thick; context 1–3 mm thick, ochraceous to brownish; tubes indistinctly stratified, 6–10 mm thick, pale ochraceous to brownish.

Hyphal structure di-trimitic; generative hyphae clamped, skeletal hyphae strongly dextrinoid, distinctly cyanophilous (reactions more or less uniform in all parts of basidiocarps).

Context: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged and occasionally branched, subsolid in upper part, having a distinct lumen in lower layer, (3.0–)3.1–4.9(–5.2) µm in diam. (n = 35/2).

Tubes: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae densely interwoven, thick-walled (wall normally not exceeding 1 µm thick), in some parts easily collapsing, lumen distinct to wide, (2.9–)3.0–4.1(–4.2) µm in diam. (n = 40/2), occasionally branched (terminal branches 1.5–2 µm in diam.), some with adventive septa; generative hyphae thin-walled, 2–3 µm in diam.

Basidiospores thick-walled (spore wall 1–1.5 µm thick), narrowly ellipsoid to ellipsoid, mostly regularly but sometimes obliquely truncate, (11.3–)11.7–15.6(–15.8) × (6.5–)

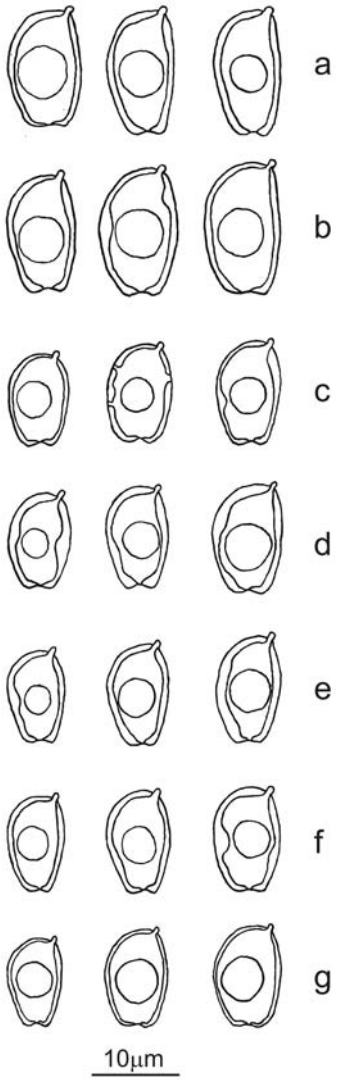

Fig. 4. Basidiospores in the *T. ohiensis* group: a. *T. arizonica* (holotype), b. *T. atlantica* (holotype), c. *T. floridana* (holotype), d. *T. mexicana* (holotype), e. *T. ohiensis* (JV 0108/116), f. *T. ornata* (holotype), g. *T. tropicalis* (holotype).

7.1–9.5(–10.3) µm, L = 13.70, W = 8.11, Q' = (1.3–)1.4–1.9(–2.1), Q = 1.64–1.75 (n = 60/2), strongly dextrinoid, cyanophilous.

REMARKS: *T. arizonica* is a long-spored species in the *T. ohiensis* complex, along with *T. atlantica* (Table 1). According to DNA data, it is closely related to *T. ohiensis* s.str.

Table 1. Spore dimensions in the *Truncspora ohiensis – T. ochroleuca* group.

Species/specimen	L'	L	W'	W	Q'	Q	n
T. arizonica	**(11.3)11.7–15.6(15.8)**	**13.70**	**(6.5)7.1–9.5(10.3)**	**8.11**	**(1.3)1.4–1.9(2.1)**	**1.70**	**60**
Vlasák 1209/69	(12.7)13.1–15.6(15.8)	14.48	(6.7)7.3–9.5(10.3)	8.31	(1.5)1.6–1.9(2.1)	1.75	30
Vlasák 1307/11bJ	(11.3)11.7–14.3(15.1)	12.91	(6.5)7.1–9.2(9.4)	7.91	(1.3)1.4–1.8(1.9)	1.64	30
T. atlantica	**(12.1)12.6–16.0(16.1)**	**14.19**	**(7.2)7.4–10.2(10.3)**	**8.86**	**(1.4)1.5–1.9(2.1)**	**1.61**	**60**
Alanko 128700	(12.1)12.6–16.0(16.1)	14.21	(7.3)8.0–10.2(10.3)	9.03	(1.4)1.5–1.8(2.0)	1.58	30
Väisälä 22	(12.3)12.8–15.6(16.1)	14.17	(7.2)7.4–9.8(10.0)	8.69	(1.4)1.5–1.9(2.1)	1.64	30
T. floridana	**(9.8)9.9–12.0(12.1)**	**11.03**	**(6.0)6.2–8.9(9.0)**	**7.19**	**(1.3)1.4–1.7(1.8)**	**1.54**	**90**
Vlasák 1008/78	(10.0)10.1–11.8(12.1)	11.05	(6.1)6.3–8.3(8.4)	7.25	(1.3)1.4–1.7(1.8)	1.53	30
Vlasák 1008/86	(9.8)9.9–12.0(12.1)	10.76	(6.0)6.2–7.7(8.1)	7.02	(1.3)1.4–1.6(1.7)	1.54	30
West 1936	(10.3)10.6–12.0(12.1)	11.29	(6.2)6.3–8.9(9.0)	7.30	(1.3)1.4–1.7(1.8)	1.56	30
T. mexicana	**(10.4)10.6–13.7(14.0)**	**11.90**	**(6.5)6.6–9.2(9.3)**	**7.68**	**(1.3)1.4–1.7(1.8)**	**1.56**	**90**
JV 0610/U-4 Kout	(11.2)12.0–13.7(14.0)	12.79	(7.2)7.4–9.2(9.3)	8.11	(1.3)1.4–1.7(1.8)	1.58	30
Murrill 183	(10.8)10.9–13.3(13.8)	11.76	(6.5)6.6–8.6(8.9)	7.61	(1.3)1.4–1.7(1.8)	1.55	30
Vlasák 1109/83	(10.4)10.6–12.2(13.6)	11.13	(6.7)6.8–8.1(8.2)	7.32	(1.3)1.4–1.6(1.7)	1.53	30
T. ochroleuca coll.	-	-	-	-	-	-	-
JV0610/7B-Kout	(10.9)11.2–13.2(13.3)	12.39	(6.7)6.8–8.8(9.2)	7.52	(1.3)1.4–1.8(1.9)	1.66	30
Lowe 13123	(12.0)12.2–15.7(15.8)	13.70	(7.1)7.3–10.2(10.3)	8.62	(1.3)1.4–1.8(1.9)	1.81	30
Niemelä 1178	(14.4)14.9–21.8(23.4)	18.46	(8.0)8.4–11.8(12.2)	10.24	(1.5)1.6–2.2(2.4)	1.81	30
Niemelä 5268	(14.6)14.8–17.2(17.6)	15.86	(6.5)7.0–9.0(9.1)	8.03	(1.6)1.7–2.2(2.4)	1.99	30
Niemelä 5647	(10.2)10.8–13.6(14.3)	12.36	(6.3)6.6–9.1(9.7)	7.71	(1.4)1.5–1.9(2.0)	1.61	30
T. ohiensis	**(9.3)9.6–13.2(13.4)**	**11.14**	**(6.2)6.4–9.3(9.8)**	**7.47**	**(1.2)1.3–1.8(1.9)**	**1.50**	**150**
Lea (isolectotype)	(9.5)9.6–11.7(12.2)	10.75	(6.4)6.7–8.5(8.8)	7.41	(1.2)1.3–1.6(1.7)	1.46	30
Vlasák 0108/116c	(9.8)10.0–12.0(12.1)	10.86	(6.2)6.4–8.7(8.9)	7.29	(1.2)1.3–1.7(1.8)	1.50	30
Vlasák 0309/114	(9.3)10.1–12.1(12.2)	10.99	(6.2)6.5–8.7(9.2)	7.32	(1.2)1.3–1.6(1.7)	1.51	30
Vlasák 0509/64	(10.3)10.7–13.2(13.4)	11.92	(7.2)7.3–9.3(9.8)	8.14	(1.2)1.3–1.6(1.7)	1.47	30
Vlasák 1208/21a	(9.7)10.0–12.8(13.2)	11.19	(6.3)6.5–8.0(8.1)	7.20	(1.3)1.4–1.8(1.9)	1.56	30
T. ornata	**(10.1)10.2–12.8(13.0)**	**11.17**	**(6.7)6.8–9.1(9.3)**	**7.73**	**(1.2)1.3–1.6(1.7)**	**1.45**	**60**
Spirin 6672	(10.2)10.3–11.8(12.1)	11.02	(6.7)6.8–9.1(9.3)	7.69	(1.2)1.3–1.6(1.7)	1.44	30
Dai 1644	(10.1)10.2–12.8(13.0)	11.32	(6.9)7.0–9.1(9.2)	7.76	(1.3)1.4–1.6(1.7)	1.46	30
T. tropicalis	**(9.2)9.3–12.2(12.4)**	**10.75**	**(6.1)6.2–9.1(9.2)**	**7.40**	**(1.1)1.3–1.7(1.8)**	**1.47**	**60**
Vlasák 1112/18J	(9.2)10.0–12.2(12.4)	11.21	(6.4)6.8–9.1(9.2)	7.88	(1.2)1.3–1.7(1.8)	1.43	30
Vlasák 1008/45	(9.2)9.3–12.0(12.1)	10.28	(6.1)6.2–8.4(8.8)	6.92	(1.1)1.3–1.7(1.8)	1.50	30
***Truncospora* sp.**	**(10.2)10.3–12.2(12.3)**	**11.19**	**(6.6)7.1–8.3(8.8)**	**7.75**	**(1.3)1.4–1.6(1.7)**	**1.45**	**30**
Ryvarden 5042	(10.2)10.3–12.2(12.3)	11.19	(6.6)7.1–8.3(8.8)	7.75	(1.3)1.4–1.6(1.7)	1.45	30

from the American North-East and *T. ornata* from East Asia. *T. arizonica* differs from those species in having of thick black crust, rimose in older basidiocarps, and larger pores. Context is also darker than in other members of the group, and contextual skeletal hyphae are wider.

T. arizonica is so far known only from Arizona inhabiting oak wood. Both description and illustration of *Perenniporia ohiensis* given by Gilberston & Ryvarden (1987) refer to this species.

Truncospora atlantica Spirin & Vlasák, **sp. nov.** Fig. 4

Pileus perennis, dimidiatus, superficies cremea vel ochracea, laevis, poris 3–4 per mm. Systema hypharum dimiticum. Basidiosporae crassitunicatae, ellipsoideae, truncatae, 12.6–16.0 × 7.4–10.2 µm, dextrinoideae, cyanophilae.

HOLOTYPE: Spain. Canary Islands: Tenerife, Anaga, *Erica arborea*, 21.XII.2005 Alanko 128700 (H).

Mycobank no. MB808454

BASIDIOCARPS perennial, sessile, dimidiate, solitary, 15–45 mm long, 10–25 mm wide. Upper surface first pale ochraceous, in mature basidiocarps ochraceous to brownish, matt, indistinctly striate, with several unclear annual zones. Margin sharp or blunt, even, fertile. Pore surface first pale cream-colored, later pale ochraceous, sometimes with brownish stains; pores regular, circular, 3–4 per mm, dissepiments thick, entire (d/p = 0.5–1.5), pubescent under lens. Section: no distinct pileal crust; context 1–3 mm thick, cream- to pale wood-colored; tubes one-layered, 4–15 mm thick, cream-colored to pale ochraceous.

HYPHAL STRUCTURE di-trimitic; generative hyphae clamped, skeletal hyphae dextrinoid (reaction distinct), moderately to strongly cyanophilous.

CONTEXT: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged to subparallel, occasionally branched, (2.7–)3.1–5.0(–5.1) µm in diam. (n = 40/2).

TUBES: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae densely interwoven, thick-walled (wall up to 2 µm thick), lumen distinct to capillary, (2.7–) 2.8–4.0(–4.1) µm in diam. (n = 40/2), occasionally branched (terminal branches 1.5–2 µm in diam.); generative hyphae thin-walled, 1.5–3 µm in diam.

BASIDIOSPORES: thick-walled (spore wall 1–2 µm thick), narrowly ellipsoid to ellipsoid, often regularly truncate, (12.1–)12.6–16.0(–16.1) × (7.2–)7.4–10.2(–10.3) µm, L = 14.19, W = 8.86, Q' = (1.4–)1.5–1.9(–2.1), Q = 1.58–1.64 (n = 60/2), moderately to strongly dextrinoid, cyanophilous (staining more or less even in all spores).

REMARKS: *T. atlantica* was first described by Torrend (1910) as *Polyporus ochroleucus* var. *lusitanica*. Later it was reported as *Perenniporia ochroleuca* from several European countries (Jahn 1972, Ryvarden & Gilbertson 1994). However, it differs from *T. ochroleuca* coll. in having distinctly narrower tramal and contextual skeletal hyphae.

T. atlantica is distributed in Macaronesia (Canary Islands and Madeira) and the Iberian Peninsula, and it is probably the only species of *Truncospora* occurring in Europe. It inhabits branches of trees and shrubs, as well as woody fences, poles etc.

Truncospora floridana Vlasák & Spirin, **sp. nov.** Fig. 4

Pileus perennis, perpusillus, superficies cremea vel pallide ferruginea, laevis, poris 6–7 per mm. Systema hypharum dimiticum. Basidiosporae crassitunicatae, ellipsoideae, truncatae, 9.9–12.0 × 6.2–8.3 µm, dextrinoideae, cyanophilae.

Holotype: USA. Florida: Key Largo, John Pennekamp St. Pk., hardwood, 29.VIII.2010 Vlasák 1008/78 (H).

Mycobank no. MB808455

Basidiocarps perennial, sessile, dimidiate, solitary, 12–25 mm long, 8–20 mm wide. Upper surface first almost white to pale cream-colored, in very old basidiocarps brownish to rusty brown, matt, indistinctly striate, azonate. Margin sharp but rather thick, even, fertile. Pore surface first white, later pale cream-colored; pores regular, circular, later elongated, almost invisible by naked eye, (5) 6–7 per mm, dissepiments very thick, entire (d/p = 1.5–2), pubescent under lens. Section: pileal crust first indistinct, in older basidiocarps very thin, dark-brown; context 2–3 mm thick, cream- to pale wood-colored; tubes one-layered, 1–3 mm thick, cream- to pale wood-colored.

Hyphal structure di-trimitic; generative hyphae clamped, skeletal hyphae dextrinoid (reaction weak in context, weak to moderate in tube trama), variably cyanophilous.

Context: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged and occasionally branched, (3.0–)3.1–3.9(–4.1) µm in diam. (n = 40/2).

Tubes: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae densely interwoven, thick-walled (wall normally not exceeding 1 µm thick), lumen distinct, (2.3–)2.4–3.6 (–3.9) µm in diam. (n = 60/3), occasionally branched (terminal branches 1.5–2 µm in diam.), some with adventive septa; generative hyphae thin-walled, 2–3.3 µm in diam.

Basidiospores thick-walled (spore wall 0.8–1.5(2) µm thick), ellipsoid to broadly ellipsoid, often regularly truncate, (9.8–)9.9–12.0(–12.1) × (6.0–)6.2–8.3(–8.4) µm, L = 11.03, W = 7.19, Q' = (1.3–)1.4–1.7(–1.8), Q = 1.53–1.56 (n = 90/3), moderately to strongly dextrinoid, cyanophilous (coloration more or less even in all spores).

Remarks: *T. floridana* produces small-sized basidiocarps. Its pores are smallest in the whole group, poorly visible by the naked eye due to exceptionally thick dissepiments. Moreover, contextual layer of *T. floridana* is as thick as tubes or thicker; this feature distinguishes it from the similarly looking *T. mexicana* and *T. tropicalis*. The latter species, also occurring in South Florida, has wider skeletal hyphae in both context and tubes; in addition, its pores are wider and tube dissepiments are thinner (see below).

T. floridana is so far found only in Florida.

Truncospora mexicana Vlasák, Spirin & Kout, **sp. nov.** Fig. 4

Pileus perennis, perpusillus, superficies cremea vel pallide ochracea, laevis, poris 5–7 per mm. Systema hypharum dimiticum. Basidiosporae crassitunicatae, ellipsoideae, truncatae, 10.6–13.7 × 6.6–9.2 µm, dextrinoideae, cyanophilae.

Holotype: Mexico. Veracruz: Park Zamora, *Casuarina equisetifolia* (fallen branch), 1.X.2006 Kout 0610/U (H, isotype in KBI).

Mycobank no. MB808456

Basidiocarps perennial, sessile, dimidiate, solitary, 5–20 mm long, 3–18 mm wide. Upper surface first almost white or pale cream-colored, in mature basidiocarps pale ochraceous, especially at the margin, matt, indistinctly striate and zonate. Margin sharp but rather thick or blunt, even, fertile. Pore surface first pale cream-colored, later pale ochraceous to pale brownish; pores round to angular, later partly sinuous, 5–7 per mm, dissepiments first entire and thick, then uneven and strongly thinning-out (d/p = 0.25–2.5), pubescent under lens. Section: no distinct pileal crust; context exceptionally thin, 0.1–1(2) mm thick, cream- to pale wood-colored; tubes indistinctly stratified, 3–13 mm thick, cream- to wood-colored.

Hyphal structure: di-trimitic; generative hyphae clamped, skeletal hyphae dextrinoid (reaction weak in context, moderate in tube trama), weakly to moderately cyanophilous.

Context: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged and occasionally branched, some hyphae flexuous and unevenly inflated, (1.9–)2.0–3.5 (–3.6) µm in diam. (n = 40/2).

Tubes: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae loosely interwoven, often flexuous, with thickened walls (wall mostly not exceeding 1 µm thick) and occasional short side branches, lumen wide, (2.2–)2.3–3.3(–3.8) µm in diam. (n = 60/3), regularly branched (terminal branches 1–2 µm in diam.), some with adventive septa; generative hyphae thin-walled, 1.5–2 µm in diam.

Basidiospores: thick-walled (spore wall 1–2 µm thick), ellipsoid to broadly ellipsoid, often regularly truncate, (10.4–)10.6–13.7(–14.0) × (6.5–)6.6–9.2(–9.3) µm, L = 11.90, W = 7.68, Q' = (1.3–)1.4–1.7(–1.8), Q = 1.53–1.58 (n = 90/3), moderately to strongly dextrinoid, cyanophilous (reaction clear and even).

Remarks: *T. mexicana* is similar to *T. floridana* in having dwarf-sized basidiocarps with rather small pores; however, its basidiocarps are more deeply colored, with ochraceous hues, and contextual tissue is very thin. Pores of *T. mexicana* undergo strong changes first being rather regular and thick-walled, and later becoming sinuous, with thinning-out and uneven dissepiments. Moreover, *T. mexicana* has longer spores if compared with *T. floridana* and *T. tropicalis*, but these differences are merely statistical (Table 1). The latter species differs from *T. mexicana* due to larger pores and wider skeletal hyphae in both context and tubes. These species seem to not coincide in their distribution areas.

T. mexicana is known from the western and southern coasts of the Gulf of Mexico and found in South Texas (USA), Veracruz (Mexico), and the westernmost part of Cuba (Pinar del Río Province).

Truncospora ochroleuca (Berk.) Pilát coll.

Polyporus ochroleucus Berk. was described from South-West Australia, and it represents a large-pored (Berkeley 1845) and long-spored (Decock 2011) species. The only ITS sequence of the Australian *T. ochroleuca* available in GenBank (FJ411097) shows no total match with other species in the *T. ohiensis* – *T. ochroleuca* complex. However, several species of the *T. ochroleuca* group may occur in Australia only. ITS sequences obtained by us from some East Asian, African and Central American collections of

T. ochroleuca coll. reveal that several species are hidden under this name (not shown in the phylogram); further studies may elucidate this problem.

Truncospora ohiensis (Berk.) Pilát Figs 3, 4

Lectotype: USA. Ohio: Waynesville, Lea (NY – isolectotype, studied).

Basidiocarps perennial, sessile, dimidiate, solitary, 20–60 mm long, 10–40 mm wide. Upper surface first pale ochraceous to pale brown, in mature basidiocarps brown to brownish-black, matt, with several unclear annual zones. Margin first sharpened, in older basidiocarps blunt, cream-colored to pale ochraceous, even, fertile. Pore surface white to pale cream-colored; pores regular, circular or elongated, 5–6 per mm, dissepiments thick, entire (d/p = 1–2), pubescent under lens. Section: pileal crust brown to blackish, 0.1–0.3 mm thick; context 1–3 mm thick, pale cream-colored to ochraceous; tubes indistinctly stratified, 1–8(15) mm thick, pale cream-colored to ochraceous.

Hyphal structure: di-trimitic; generative hyphae clamped, skeletal hyphae moderately dextrinoid, moderately to strongly cyanophilous (reactions more or less uniform in all parts of basidiocarps).

Context: Hyphal structure dimitic; skeletal hyphae dominating, loosely interwoven or arranged in parallel bundles, occasionally branched, lumen wide to narrow, (2.1–) 2.3–3.7(–4.0) µm in diam. (n = 70/4).

Tubes: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae densely interwoven, thick-walled (wall normally not exceeding 1 µm thick), in some parts easily collapsing, lumen distinct to wide, (1.8–)2.0–3.1(–3.2) µm in diam. (n = 60/3), occasionally branched (terminal branches 1–1.5 µm in diam.), some with adventive septa; generative hyphae thin-walled, 1–3.5 µm in diam.

Basidiospores thick-walled to very thick-walled (spore wall 1–2.5 µm thick), narrowly ellipsoid to ellipsoid, normally regularly truncate, (9.3–)9.6–13.2(–13.4) × (6.2–) 6.4–9.3(–9.8) µm, L = 11.14, W = 7.47, Q' = (1.2–)1.3–1.8(–1.9), Q = 1.47–1.56 (n = 150/5), strongly dextrinoid, cyanophilous (reaction more or less even).

Remarks: This species was described as *Trametes ohiensis* based on the sole specimen from Ohio (Berkeley 1872); we studied its part (NY), and it agrees well with other specimens collected in the American North-East. *T. ohiensis* has rather big, ungulate basidiocarps finally covered by brownish-black crust with large, unclear zones, while margin usually stays pale-colored (concolorous with pore surface). Its closest relative, *T. arizonica*, is characterized by larger pores with thinner dissepiments and distinctly longer spores, as well as wider skeletal hyphae. Another counterpart, *T. ornata* from East Asia, is microscopically almost identical with *T. ohiensis*, but it produces considerably smaller, conchate basidiocarps (see below). The specimen Ryvarden 5042 from Kenya (O, H, labeled as *Perenniporia ochroleuca*) is morphologically very similar to *T. ohiensis* s.str., and it seems to be another, still undescribed species in this group; this subject deserves a separate study.

T. ohiensis is a widely distributed species in the US North-East and adjacent parts of Canada. It inhabits fallen logs and branches of deciduous trees, as well as "structural timber" (Overholts 1914).

Truncospora ornata Spirin & Bukharova, **sp. nov.** Fig. 4

Pileus perennis, conchatus, superficies pallide ochracea vel cinnamomea, laevis, poris 5–6 per mm. Systema hypharum dimiticum.Basidiosporae crassitunicatae, ellipsoideae, truncatae, 10.2–12.8 × 6.8–9.1 µm, dextrinoideae, cyanophilae.

Holotype: Russia. Khabarovsk Reg.: Khabarovsk Dist., Bol'shoi Khekhtsir Nat. Res., *Fraxinus mandshurica*, 5.IX.2013 Spirin 6672 (H).

Mycobank no. MB808457

Basidiocarps perennial, sessile, dimidiate, solitary, 7–25 mm long, 5–10 mm wide. Upper surface first pale cream-colored or grayish, warted, in mature basidiocarps brown, matt, indistinctly striate, with several unclear annual zones. Margin sharp, even, fertile. Pore surface first white, later pale cream-colored, in senescent basidiocarps brownish; pores regular, circular, 5–6 per mm, dissepiments entire, first think, later thinning-out (d/p = 0.7–2). Section: pileal crust brownish, seen as a thin line ca. 0.1 mm thick; context 1–2 mm thick, cream-colored, sometimes with brownish hues; tubes one-layered or indistinctly stratified, 1–2 mm thick, cream-colored to brownish.

Hyphal structure di-trimitic; generative hyphae clamped, skeletal hyphae strongly dextrinoid, distinctly cyanophilous (reactions more or less uniform in all parts of basidiocarps).

Context: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged and occasionally branched, (2.4–)2.9–3.7(–3.9) µm in diam. (n = 20/1).

Tubes: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae densely interwoven, thick-walled (lumen usually distinct), (1.9–)2.0–3.3(–3.4) µm in diam. (n = 40/2), occasionally branched (terminal branches 1–1.5 µm in diam.), some with adventive septa or occasional inflations up to 5 µm in diam.; generative hyphae thin-walled, 1.5–2.5(3) µm in diam.

Basidiospores thick-walled (spore wall 1–1.5 µm thick), ellipsoid to broadly ellipsoid, mostly regularly but sometimes obliquely truncate, (10.1–)10.2–12.8(–13.0) × (6.7–)6.8–9.1(–9.3) µm, L = 11.17, W = 7.73, Q' = (1.2–)1.3–1.5(–1.6), Q = 1.44–1.46 (n = 60/2), strongly dextrinoid, cyanophilous. Basidiospores detected on pileal surface variably brownish, evidently absorbing a colored matter from underlying hyphae.

Remarks: This species was first collected by L.V.Ljubarsky in Russian Far East and reported as *Fomitopsis ohiensis* by Bondartsev (1953). That time this identification was feasible due to lack of any striking morphological differences between *T. ornata* and *T. ohiensis*. The only peculiar character of *T. ornata* is its dwarf-sized, conchate basidiocarps (versus normally large and ungulate fruitbodies of *T. ohiensis*); however, these species are certainly different according to DNA data (Figs 1, 2). *Polyporus junctus* was described by Lloyd (1924: 1317) from Japan, and now it is considered as a synonym of *T. ochroleuca* (Ryvarden 1990). Its picture published by Lloyd (1924, plate 311, fig. 3028) shows several thick and light-colored caps fusing together – thus, this name would be hardly addressed to *T. ornata*, having small-sized, solitary basidiocarps with brown upper surface. Identity of Lloyd's species versus the East Asian *T. ochroleuca* should be checked separately.

T. ornata is a temperate species, distributed in deciduous and mixed forests of Russian Far East (Khabarovsk Reg. and Primorie) and North-East China but it seems to be

rare. *T. ornata* grows on fallen logs and thick branches of frondose trees (*Fraxinus mandshurica*, *Maakia amurensis*, *Phellodendron amurense*, *Quercus mongolica*) and evidently avoids the man-induced ecosystems.

Truncospora tropicalis Vlasák & Spirin, **sp. nov.** Fig. 4

Pileus perennis, perpusillus, superficies cremea vel pallide ochracea, laevis, poris 4–5 per mm. Systema hypharum dimiticum. Basidiosporae leniter crassitunicatae, ellipsoideae, truncatae, 9.3–12.2 × 6.2–9.1 µm, dextrinoideae, cyanophilae.

HOLOTYPE: Puerto Rico. Rio Grande: El Yunque Nat. Forest, hardwood, XII.2011 J.Vlasák Jr. 1112/18J (H).

Mycobank no. MB808458

BASIDIOCARPS perennial, sessile, dimidiate, solitary, 10–20 mm long, 10–15 mm wide. Upper surface first pale cream-colored, in mature basidiocarps pale ochraceous, matt, indistinctly striate and zonate. Margin sharp but rather thick, even, fertile. Pore surface first pale cream-colored, later pale ochraceous; pores angular, later elongated and partly sinuous, in older basidiocarps showing a tendency to radial arrangement, 4–5(6) per mm, dissepiments thin, entire (d/p = 0.3–0.8), pubescent under lens. Section: no distinct pileal crust; context 1–2 mm thick, cream- to pale wood-colored; tubes one-layered, 1.5–5 mm thick, cream- to pale wood-colored.

HYPHAL STRUCTURE di-trimitic; generative hyphae clamped, skeletal hyphae dextrinoid (reaction moderate in context, weak to moderate in tube trama), weakly to moderately cyanophilous.

CONTEXT: Hyphal structure dimitic; skeletal hyphae dominating, irregularly arranged and occasionally branched, (3.1–)3.3–4.9(–5.1) µm in diam. (n = 40/2).

TUBES: Hyphal structure di-trimitic; trama irregular. Skeletal hyphae loosely interwoven, often flexuous, with thickened walls (wall not exceeding 1 µm thick), lumen wide, a few deeply arranged skeletals distinctly thick-walled and with a capillary lumen, (2.8–)2.9–4.2(–4.3) µm in diam. (n = 40/2), regularly branched (terminal branches 1–1.5 µm in diam.), some with adventive septa; generative hyphae thin-walled, 3–4 µm in diam.

BASIDIOSPORES slightly thick-walled (spore wall 0.5–1 µm thick), ellipsoid to broadly ellipsoid, a few spores almost subglobose, often regularly truncate, (9.2–)9.3–12.2 (–12.4) × (6.1–)6.2–9.1(–9.2) µm, L = 10.75, W = 7.40, Q' = (1.1–)1.3–1.7(–1.8), Q = 1.43–1.50 (n = 60/2), weakly to moderately dextrinoid, unevenly cyanophilous (reaction mostly weak, more or less visible in thickest parts of the spore wall only).

REMARKS: *T. tropicalis*, *T. mexicana* and *T. floridana* are pale-colored and dwarf-sized species displaying weak morphological differences from each other. *T. tropicalis* is distinguishable due to larger, thin-walled pores, more or less radially oriented in mature basidiocarps, and relatively wide and loosely arranged tramal skeletals. In addition, spores of *T. tropicalis* have thinnest walls if compared with the rest of species in this group, quickly degrading and thus showing an uneven coloration in Cotton Blue.

T. tropicalis is so far found in South Florida (the Florida Keys), Hispaniola (Dominican Republic) and Puerto Rico; it may be widely distributed in the eastern part of the Caribbean.

Species key for the *T. ohiensis – T. ochroleuca* group

1 Tramal skeletals 4–6 µm wide ..... *T. ochroleuca* coll.
1' Tramal skeletals up to 4 µm wide 2

2 Pores 3–4 per mm, spores regularly reaching 14 µm long 3
2' Pores 4–7 per mm, spores reaching 14 µm long in exceptional cases 4

3 Mature basidiocarps with thick, black crust. Arizona (USA) ..... *T. arizonica*
3' Mature basidiocarps with ochraceous or brownish upper surface; no distinct crust. Macaronesia and the Iberian Peninsula ..... *T. atlantica*

4 Pores 4–5 per mm, in mature basidiocarps distinctly elongated and with a tendency to radial arrangement. Dissepiments rather thin (d/p = 0.3–0.6). Spore wall up 1 µm thick, only partly and weakly cyanophilous. South Florida, Hispaniola, Puerto-Rico ..... *T. tropicalis*
4' Pores 5–7 per mm; no tendency to radial arrangement, dissepiments normally thick. Spore wall 1 µm thick or more, distinctly cyanophilous 5

5 Temperate species 6
5' Subtropical or tropical species (the Caribbean and the Gulf of Mexico) 7

6 Basidiocarps ungulate, 3–10 mm thick. American North-East ..... *T. ohiensis*
6' Basidiocarps conchate, 2–4 mm thick. East Asia ..... *T. ornata*

7 Context equally thick to or thicker than tube layer. Florida ..... *T. floridana*
7' Context distinctly thinner than tube layer. Western and southern coasts of the Gulf of Mexico ..... *T. mexicana*

Specimens examined

T. arizonica. USA. Arizona: Cochise Co., Portal Area, Chiricahua Mts., *Quercus arizonica*, 6.IX.2012 Vlasák 1209/69 (H – holotype, JV) (GenBank KJ410696, KJ410716); Santa Cruz Co., Coronado Nat. Forest, Madera Canyon, *Quercus hypoleucoides*, 5.IX.1988 Gilbertson 16372 (CFMR), *Q. arizonica*, 2.IX.2012 Vlasák 1209/21a (JV, H) (GenBank KJ410695, KJ410715), VII.2013 J. Vlasák Jr. 1307/11b (JV, H) (GenBank KJ410697, KJ410717).

T. atlantica. Portugal. Estremadura: Mata do Bombarral, *Arbutus unedo*, 27.X.1978 Melo & Cardoso 469 (H ex LISU). Madeira: Funchal, Botanical Garden, vineyard support wood, 6.I.1974 Väisälä 22 (H). Spain. Canary Islands: Tenerife, Anaga, *Erica arborea*, 21.XII.2005 Alanko 128700 (H – holotype, JV) (GenBank KJ410700, KJ410720), Myrica faya, 30.XI.2013 Kout 1311/4K (JV) (GenBank KJ410699, KJ410719).

T. floridana. USA. Florida: Gainesville, Upper Sugarfoot Prairie, *Persea borbonia* (decorticated stump), 14.II.1936 West (NY); Key Largo, John Pennekamp St. Pk., hardwood, 29.VIII.2010 Vlasák 1008/78 (H – holotype, JV) (GenBank KJ410704, KJ410723); Miami, Matheson Hammock, hardwood, 30.VIII.2010 Vlasák 1008/85 (JV) (GenBank KJ410705, KJ410724), 1008/86 (JV, H) (GenBank KJ410706, KJ410725).

T. mexicana. Cuba. Pinar del Río Prov.: Herradura, dead wood in dense thickets, 7–12.III.1905 Earle & Murrill *183* (NY). Mexico. Veracruz: Zamora Park, *Casuarina equisetifolia* (fallen branch), 1.X.2006 Kout 0610/U (H – holotype, JV, KBI) (GenBank KJ410707, KJ410726). USA. Texas: Cameron Co., Brownsville, Resaca de la Palma St. Park, fallen hardwood branch, IX.2011 Vlasák 1109/83 (JV, H) (GenBank KJ410708, KJ410709, KJ410727).

T. ochroleuca coll. Belize. Cockscomb Basin, Antilope Trail, 30.X.2006 Kout JV0610/7B-Kout (JV, H, KBI) (GenBank KJ410698, KJ410718). Costa Rica. Heredia Prov.: Uvita, hardwood fencepost, 13.VII.1963 Lowe 13123 (NY, CFMR). Jamaica. Castleton Bot. Garden, 14–15.XII.1908 Murrill 126 (NY). Singapore. Nee Soon, Botanical Garden, angiosperm branch, 15.III.2011 Tran &

Skornickova (H ex SING 2011-131) (var. *brevispora* – Corner 1989). Tanzania. Kilimandjaro Reg.: Kilimanjaro, Mandara, *E. arborea*, 27.I.1973 Niemelä 1178 (H). Tanga Reg.: Lushoto, Mazumbai Forest Reserve, dead stem, 8.XII.1989 Niemelä 5268 (H). Iringa Reg.: Mufindi, Ludoga, *Acacia mearnsii*, 4.II.1993 Niemelä 5647 (H).

T. ohiensis. Canada. Ontario: Middlesex Co., London, dead branch and timber wood, XII.1890 Dearness (NY); York Co., Nashville, *Fagus grandifolia*, 25.IX.1957 Cain (NY). USA. Florida: Alachua Co., Gainesville, hardwood, 2.IX.1952 Rhoads (CFMR), *Carya* sp., 13.IX.1952 Rhoads (CFMR). Georgia: Coweta Co., Coweta Watershed, *Robinia pseudoacacia*, 20.IX.1965 Ross 215 (CFMR). Illinois: Piatt Co., Cerro Gordo, rotten wood, 6.VII.1915 Overholts 3210 (NY). Indiana: Putnam Co., Greencastle, *Quercus* sp. (rails and logs), XI.1892 Underwood (NY). Iowa: Fayette, *Quercus* sp. (fence posts), III.1908 Wilson (NY); Winneshiek Co., Decorah, X.1882 Holway 303 (NY). Kansas: Riley Co., Manhattan, dead wood, 24.IX.1951 Rogerson (NY). Michigan: Washtenaw Co., Ann Arbor, dead tree, 20.XI.1893 Pieters (NY). New York: Onondaga Co., La Fayette, hardwood log, 11.X.1961 Gilbertson 3034 (NY); Wayne Co., Clyde, IX.1887 Cook (NY). North Carolina: Buncombe Co., Ashville, fence post, 22.XII.1920 Bache-Wiij (NY); Transylvania Co., Pink Bed Valley, 13–23.VII.1908 Murrill 440 & House (NY). North Dakota: Cass Co., Fargo, *Quercus macrocarpa*, IX.1921 Stevens & Brenckle (NY). Ohio: Butler Co., Ohio, *F. grandifolia*, 26.VII.*1911* Overholts 215 (NY); Erie Co., Milan, 26.VII.1974 Jones 74-741 (NY); Lorain Co., Henrietta Township, *Maclura pomifera* (living tree), 12.X.1949 Bigelow 359 (NY), Vermillion River, III.1951 Bigelow 361 (NY); Preble Co., Fairhaven, dead wood, 23.VI.1939 Overholts (NY); Warren Co., Waynesville (NY – isotype, see above). Oklahoma: Payne Co., Ripley Bluffs, Quercus sp., 16.VIII.1979 Rogerson (NY). Pennsylvania: Bucks Co., Point Pleasant, Quercus sp., 21.VIII.2003 Vlasák 0108/116 (JV), 16.IX.2003 Vlasák 0309/114 (JV, H) (GenBank KJ410694); Montgomery Co., Schwenksville, *R. pseudoacacia*, 31.VIII.2012 Vlasák 1208/21 (JV, H), (GenBank KJ410692, KJ410713). Tennessee: Great Smoky Mts., Cove Hardwood Nat. Trail, *R. pseudoacacia*, 6.IX.2005 Vlasák 0509/64 (JV, H) (GenBank KJ410693, KJ410714). Virginia: Clarke Co., White Post, 16.IX.1910 (anonymous collector) (NY); Giles Co., Mountain Lake, *Castanea dentata*, 8–14.VII.1909 Murrill 370 (NY). West Virginia: Mason Co., Apple Grove, deciduous tree, 12.I.1935 Gould 10 (NY); Monongalia Co., Morgantown, 26.X.1907 Hartley 57 (NY).

T. ornata. China. Jilin: Huadian Co., Dongxing, *Maakia amurensis*, 17.X.1993 Dai 1644 (H) (GenBank KJ410691). Russia. Khabarovsk Reg.: Khabarovsk Dist., Bol'shoi Khekhtsir Nat. Res., *Fraxinus mandshurica*, 5.IX.2013 Spirin 6672 (H – holotype, JV) (GenBank KJ410690, KJ410712). Primorie Reg.: Ussuri Dist., Ussuri Nat. Res., hardwood, 27.VIII.1964 Smekalkina (VLA M-14234), hardwood, 19.VII.1964 Nazarova (VLA M-14236, 14237), 7.VIII.1964 Nazarova (VLA M-14238); Khasan Dist., Kedrovaya Pad' Nat, Res., Quercus sp., 5.IX.1958 Ryffa (VLA M-14235).

T. tropicalis. Dominican Republic. La Vega Prov.: Ebano Verde Res., hardwood, 29.V.1997 Ryvarden 40273 (CFMR DR-763 ex O). Puerto Rico. Rio Grande: El Yunque Nat. Forest, hardwood, XII.2011 J.Vlasák Jr. 1112/18J (H – holotype, JV) (GenBank KJ410701, KJ410721).USA. Florida: Long Key St. Pk., hardwood, VIII.2010 Vlasák 1008/45 (JV, H) (GenBank KJ410702, KJ410703, KJ410722).

Truncospora sp. Kenya. Coast Prov.: Kwale, Makadara, 9.I.1970 Ryvarden 5042 (H ex O 15806).

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